UNIVERSITY OF EDINBURGH

A THESIS submitted

by

EDMUND GEORGE VINCENT PERCIVAL, B.Sc., Ph.D. (Birm.), F.I.C.,

a candidate to qualify

for the degree

of

DOCTOR OF SCIENCE

April 1938

Title.

"Studies in the Carbohydrate Field"

By hydrolytic cleavage this product (IV), which showed accurate analytical figures for $C_{17}H_{32}O_{10}$, gave rise to crystalline 2 : 3 : 4-trimethyl xylopyranose (V) in a yield of over 80%, and there was isolated as a second component 2 : 3 : 5-trimethyl γ-xylonolactone (VI) which, although distilling as a liquid in a yield of 73%, was recognised by conversion into the crystalline phenylhydrazide of the corresponding 2 : 3 : 5-trimethyl xylonic acid. These crystalline products were compared directly with authentic specimens and their identity confirmed. It follows that, since the 4-position in the latter acid carries a free hydroxyl group, this must have been the point of linking with the 1-position of the adjoining xylopyranose unit (V). Moreover this lactone had been previously investigated by Haworth and Porter (J., 1928, 611), who degraded it to *d*-dimethoxysuccinic acid. Position 5 of the lactone represents the point of junction of the xylose ring which was opened by oxidation to the bionic acid and subsequently methylated to give the terminal CH_2·OMe group in the γ-lactone (VI).

We can therefore apply the constitution (II) to the partly methylated disaccharide which is here shown to be a *dixylopyranose*. It follows that the xylose units in the polysaccharide are of the pyranose type and that xylan can be represented by formula I (with H in place of Me).

Experimental.

Methylation of Xylan.—The methylation of xylan was carried out with the following quantities of reagents, which differ slightly from those previously used (Hampton, Haworth, and Hirst, J., 1929, 1739) : xylan (7·5 g.), potassium hydroxide (500 g. in 650 c.c. of water), and methyl sulphate (430 c.c.). The methyl sulphate was added to the alkaline solution of xylan during 4 hours at room temperature with continuous stirring. Thereafter the temperature was raised to 100° for 1 hour, the mixture was diluted with 2 litres of boiling water, and the liquid filtered through muslin. The crude methylated xylan obtained from two such operations was again methylated by the use of potassium hydroxide (365 g. in 400 c.c. of water) and methyl sulphate (290 c.c.). The white methylated compound was washed with hot water, dissolved in chloroform, dried with magnesium sulphate, filtered through glass wool, and the dimethyl xylan precipitated by an excess of ether. The product had the same constants as those already recorded (Hampton, Haworth, and Hirst, *loc. cit.*).

Degradation of Dimethyl Xylan.—To dimethyl xylan (4 g.), dissolved in glacial acetic acid (44 c.c.) and cooled to 0°, acetic anhydride (40 c.c.) containing concentrated sulphuric acid (1·6 c.c.) was added,

C O N T E N T S

XII	Monomethyl Hexoses, Pt. I. The Constitution of the Supposed 4-Methyl Glucose	J.Chem.Soc., 1935, 873.
XIII	Monomethyl Hexoses, Pt. II. A Revision of the Constitutions of the Supposed 4-Methyl Galactose and 4-Methyl Mannose of Pacsu, and their Formulation as 6-Methyl Galactose and 2-Methyl Mannose Respectively	J.Chem.Soc., 1936, 640.
XIV	The Methylation of Glucosephenylosazone and its Formulation as a Derivative of Fructopyranose	J.Chem.Soc., 1935, 1398.
XV	The Structure of Osazones and the Isolation of a New Hexosazone Anhydride	J.Chem.Soc., 1936, 1770.
XVI	Sugar Osazones and Their Anhydrides	J.Chem.Soc., 1937, 1320.
XVII	Acetylation of Agar	Nature, 1936, 137, 997.
XVIII	Structure of Agar-Agar	Nature, 1937, 139, 512.
XIX	The Acetylation of Agar-Agar and the Isolation of 2:4:6-Trimethyl α-d-Galactose by Hydrolysis	J.Chem.Soc., 1937, 1615.
XX	Investigations on the Configuration of Carbohydrates by Conductivity Measurements in Boric Acid Solution.	J.Chem.Soc., 1937, 1920.

Acknowledgments

I N T R O D U C T I O N

The work presented in this thesis was commenced in October 1931 in the University of Birmingham, where, until July 1933 the author was private research assistant to Professor W.N.Haworth, F.R.S. The first eight papers therefore represent work carried out under his general direction and of which he is the senior author. They are included however because the whole of the experimental work in papers (I) to (IV) was carried out by the candidate and the development of the practical side was largely his responsibility, although invaluable advice was always available during this period from Professor Haworth and Professor E.L.Hirst, F.R.S. In the next four papers on l-ascorbic acid it is difficult to attempt to define the part played by the writer of the thesis, but an attempt has been made in the summary to indicate this as far as possible.

None of the work contained in these papers was embodied in a thesis presented for the degree of Ph.D. in 1932.

The remainder of the thesis contains the results of researches conducted in the Chemistry Department of the University of Edinburgh since October, 1933.

The work described in papers [IX], [X] and [XV] was carried out entirely by the author. The writer's wife carried out the major portion of the experimental work contained in papers [XIV] and [XVI]. For the remaining papers the practical work was conducted by various students during the course of training for the degree of Ph.D. In all cases however the researches were initiated by the candidate and carried out under his direction.

SUMMARY OF PAPERS PRESENTED

[I]

Evidence of Continuous Chains of α-Glucopyranose Units in Starch and Glycogen

Trimethyl amylose was degraded by contact with acetyl bromide in chloroform solution to give derivatives of (a) a monosaccharide, (b) a disaccharide and (c) a trisaccharide. The disaccharide derivative was converted into a monoacetyl hexamethyl biose by removal of the bromine residue, after which oxidation followed by methylation yielded the corresponding octamethyl methyl maltobionate (22.4%). This compound on hydrolysis with mineral acid gave rise to two cleavage fragments, crystalline tetramethyl glucopyranose (95%) and crystalline 2:3:5:6-tetramethyl γ-gluconolactone (80%). The latter was identified both by its melting point and specific rotation as well as

by the physical constants of the crystalline phenylhydrazide of the corresponding acid. Hence it is concluded that only position 4 can be involved in the union of this part of the molecule with the adjoining residue, namely, tetramethyl glucopyranose in the octamethyl bionic ester, so that the 4-position is involved in the union of the contiguous glucopyranose units in amylose.

Parallel experiments with trimethyl glycogen yielded 22.9% of octamethyl methyl maltobionate, the hydrolysis of which yielded almost exactly similar results to those obtained with trimethyl amylose, from which the conclusion is reached that this polysaccharide contains continuous chains of α-glucopyranose units linked together through positions 1 and 4.

[II]

Evidence of the Pyranose Structure of Xylan

By W.N.Haworth and E.G.V.Percival

Dimethyl xylan was degraded by acetolysis and by a procedure similar to that adopted for starch and glycogen a methyl ester of hexamethyl dixylobionic acid (21%) was isolated. This product on hydrolysis yielded crystalline 2:3:4-trimethyl xylopyranose (80%) and 2:3:5-trimethyl γ-xylonolactone (73%) recognised as the crystalline phenylhydrazide. It follows therefore that position 4 in the trimethyl xylonic acid was available for union with position 1 of the adjoining xylose unit which is shown to be pyranose. Position 5 of the lactone represents the point of junction of the xylose ring which was opened by oxidation to the bionic acid and then methylated to give the terminal $-CH_2OMe$ group in the γ-lactone. This evidence therefore supports the view that xylan is chiefly composed of linked xylopyranose units and that positions 1 and 4 are involved in the union of adjacent members. More recent work of Haworth, Hirst and Oliver, (J.Chem. Soc., 1934, 1917), has shown that xylan consists of a chain of 18 or 19 xylopyranose units terminated by an arabofuranose residue.

[III]

The Molecular Structure of Glycogen

By W.N.Haworth and E.G.V.Percival

By the hydrolysis of methylated glycogen crystalline tetramethyl glucopyranose (9%) and 2:3:6-trimethyl glucopyranose (87%) were obtained. The tetramethyl glucopyranose unit was derived from a terminating group from which it can be calculated that, in the specimen examined, the number of glucose units in the methylated polysaccharide was about 12 corresponding to a molecular weight of approximately 2500. Later work on this subject has revealed that some specimens of methylated glycogen derived from rabbit liver appear to consist of chains of 18 glucopyranose units (Bell, Biochem.J., 1936, 30, 1612; Haworth, Hirst and Isherwood, J.Chem.Soc., 1937, 577), but Bell (Biochem.J., 1935, 29, 2031; loc.cit., 1937 31, 1683) has also shown, in agreement with this paper, that specimens of methylated glycogen derived from rabbit liver or horse muscle are composed of chains of 12 glucopyranose units.

[IV]

The Molecular Structure of Inulin

By W.N.Haworth, E.L.Hirst and E.G.V.Percival

Methylated inulin on hydrolysis gave 3:4:6-trimethyl fructofuranose and 1:3:4:6-tetramethyl fructofuranose (3.7%) indicating that methylated inulin is composed of continuous chains of methylated fructofuranose units and not of large rings of such units. From this evidence it is concluded that the minimum average chain length of methylated inulin consists of 30 fructofuranose units with a molecular weight of about 5,000 for the free polysaccharide assuming that no degradation had occurred during acetylation and methylation. This result was in agreement with a previous estimate of the molecular weight of inulin determined ebullioscopically by Drew and Haworth (J.Chem.Soc.,1928, 2670), and has since been confirmed by osmotic pressure measurements (Carter and Record, J.Soc.Chem.Ind., 1936, 218).

[V]

The Structure of Ascorbic Acid

By E.L.Hirst and Co-workers

[VI]

Constitution of Ascorbic Acid

By E.L.Hirst, E.G.V.Percival and F.Smith

[VII]

The Constitution of Ascorbic Acid

By R.W.Herbert, E.L.Hirst, E.G.V.Percival, R.J.W.Reynolds and F.Smith

In this research the candidate was concerned with oxidation and methylation studies in an attempt to arrive at the structure by degradative methods. Oxidation of ascorbic acid by potassium hypochlorite followed by oxidation with nitric acid, methylation and amide formation, yielded the crystalline amide and methyl amide of d-dimethoxy succinic acid which indicated the stereochemical arrangement on the penultimate carbon atom to be HO-C-H and thus supported the assignment of natural ascorbic acid to the l-series.

The strongest evidence for the proposed structure was obtained by methylation of the reactive enolic hydroxyl groups with diazomethane to yield a dimethyl ascorbic acid and then with methyl iodide and silver oxide to yield tetramethyl ascorbic acid. Ozonisation caused fission at the double bond and on hydrolysis oxamide and 3:4-dimethyl l-threonamide (together with a little 3:4-dimethyl l-erythronamide formed by a Walden Inversion) were isolated. The structure of the threonamide was proved by the fact that complete methylation furnished 2:3:4-trimethyl l-threonamide whilst 3:4-dimethyl l-threonamide underwent the Weerman reaction. This observation served to fix the position of the lactone ring at C_4 in the original tetramethyl ascorbic acid and ascorbic acid must accordingly be represented as the enolic form of l-gulofuranolactone.

[VIII]

Synthesis of d- and of l-Ascorbic Acid and of Analogous Substances

By R.G.Ault, D.K.Baird, H.C.Carrington, W.N.Haworth
R.W.Herbert, E.L.Hirst, E.G.V.Percival
F.Smith and M.Stacey

The synthetic work described in this paper required much large scale preparation and the candidate was concerned with the preparation of several kilogrammes of diacetone galactose, its oxidation to diacetone d-galacturonic acid, conversion to potassium d-galacturonate, reduction to l-galactonic acid, conversion to l-galactonolactone and amide formation, followed by the Weerman degradation to l-lyxose, then osazone formation to yield l-xylosazone which was converted to l-xylosone the essential starting point in the synthesis. The isolation of the synthetic ascorbic acid in a crystalline condition was however accomplished after the author had left the Birmingham laboratory.

Having regard to the unsatisfactory position of the so-called compounds of the sugars and alkali hydroxides in 1933, it was determined to attempt to decide whether they were mixtures, substitution compounds of the alkoxide type, or addition compounds, and, if either of the two latter possibilities was correct, to determine which groups in the sugar molecule were involved.

[IX]

Addition Compounds of the Carbohydrates

Part I.

Potassium Hydroxide-Glucose and Related Compounds

By E. G. V. Percival

This paper deals with investigations on compound formation between potassium hydroxide and glucose from which it is concluded that, (a) compound formation takes place between the hydroxyl group of the potential reducing group of the sugar and the potassium hydroxide to form a 1:1-compound, (b) the link between the two derivatives is labile on account of the low yield of methylglucosides pro-

duced on direct methylation, and (c) that cellobiose and maltose form more complex derivatives with potassium hydroxide, the former appearing to combine with two molecules and the latter with three molecules of the alkali hydroxide for every molecule of disaccharide.

[X]

Addition Compounds of the Carbohydrates

Part II.

Potassium Hydroxide-Sucrose

By E. G. V. Percival

This paper on sucrose represents a study on similar lines to the previous one and is of importance because of the use of the addition compounds of sucrose and strontium hydroxide in sugar refining. Evidence was secured that a labile compound of the formula $C_{12}H_{22}O_{11}.3KOH$, appears to be formed between sucrose and potassium hydroxide and furthermore that the three primary alcoholic residues in the disaccharide appear to be involved in the combination since a derivative of 6-methyl glucose was isolated and by oxidation methods a dimethyl fructofuranose which was also isolated was shown to be 1:6-dimethyl fructofuranose.

[XI]

Addition Compounds of the Carbohydrates

Part III.

Potassium Hydroxide Derivatives of Cellobiose, Lactose and Galactose

By E.G.V.Percival and G.G.Ritchie

The above work was extended to galactose, lactose and cellobiose. In the latter case a compound, $C_{12}H_{22}O_{11}.2KOH$ appears to be formed, and methylation studies resulted in the isolation of 6-methyl glucose phenylosazone from the monomethyl methylcellobioside first formed. It is considered in this case, therefore, that the potassium hydroxide residues, are associated with the reducing group of the disaccharide and with one of the primary alcohol residues, probably that located on the non-reducing glucopyranose unit. The case of lactose, however, appears to be anomalous since it appeared possible to prepare a compound containing as many as three molecular proportions of potassium hydroxide to every molecule of lactose. Evidence

was secured that the reducing group and positions 2 and 4 on the galactopyranose residue were concerned with compound formation since no methylated glucose derivatives were isolated but a dimethyl galactose was isolated which formed a monomethyl galactosazone identical with 4-methyl galactosazone, and other experiments such as oxidation and glycoside formation confirmed the view that it was 2:4-dimethyl galactose. No such anomalous behaviour was found for the galactose-potassium hydroxide derivative which appeared to be of a similar structure to the corresponding glucose compound.

Very early in these studies it became clear that any assignment of structure to these addition compounds by the method employed must depend on the isolation and recognition of partially methylated sugars usually as phenylosazones. There being some dubiety as to the constitution of a number of the monomethyl hexoses at this time (1934) it was necessary to remove this doubt.

[XII]

Monomethyl Hexoses

Part I

The Constitution of the Supposed 4-Methyl Glucose

By J.Munro and E.G.V.Percival

Well characterised derivatives of 2, 3, and 6-methyl glucose were known, but it was considered that, owing to the methods by which the structure was assigned, the possibility that the supposed 4-methyl glucose was in fact 5-methyl glucose was not entirely excluded. However the supposed 4-methyl glucose gave tetramethyl glucopyranose and 2:3:4:6-tetramethyl δ-gluconolactone on suitable treatment, conclusive proof that the substance in question was indeed 4-methyl glucose.

[XIII]

Monomethyl Hexoses

Part II

A Revision of the Constitutions of the Supposed 4-Methyl Galactose and 4-Methyl Mannose of Pacsu, and their Formulation as 6-Methyl Galactose and 2-Methyl Mannose Respectively

By J.Munro and E.G.V.Percival

Even more unsatisfactory was the position with regard to the supposed 4-methyl galactose and 4-methyl mannose which are prepared by a similar procedure to the 4-methyl glucose just mentioned. It was proved beyond doubt, by a direct comparison of the free sugar and corresponding osazone and phenylhydrazone, that the galactose derivative was 6-methyl galactose. The supposed 4-methyl mannose, however, gave rise to an osazone of no methoxyl content, viz. glucosazone and the conclusion was reached therefore that the substance examined was not 4- but 2-methyl mannose.

[XIV]

The Methylation of Glucosephenylosazone and its Formulation as a Derivative of Fructopyranose

By (Mrs) E.E.Percival and E.G.V.Percival

There has been much debate as to the cyclic or acyclic structure of sugar osazones. The fact that most of them appear to mutarotate in solution tends to support the former possibility. From the complex mixture of products obtained on the methylation of glucosephenylosazone, a crystalline monomethyl glucosazone was isolated, different from any of the known monomethyl glucosazones and since it was a true osazone being convertible into the osone from which the original monomethyl osazone was regenerated, the structure 5-methyl glucosazone was assigned to it. Recently v.Vargha (Ber., 1936,69, 2098) claims to have synthesised 5-methyl glucose and although the osazone he prepared from this source was too impure for analysis, the melting point quoted (ca.128°) is of the same order as that found for the osazone prepared by direct methylation (117°) and

widely different from the melting point of any other monomethyl glucosazone. There appears to be some discrepancy however in the recorded specific rotations (v.Vargha: $[\alpha]_D^{20°}$ -72°; this paper -49° in alcohol) so that the possibility that the two osazones are isomeric must not yet be excluded.

Reduction of the osone yielded a monomethyl ketose the negative rotation of which, and conversion to a monomethyl methylfructopyranoside and finally to tetramethyl fructopyranose, supported the view that the methyl residue was located in position 5 in the osazone. Thus the possibility of a 1:5- or a 2:5-oxide ring in the original glucosazone was excluded. Repeated methylation of glucosazone failed to introduce more than three methoxyl residues into the molecule, a derivative containing only three methoxyl (OCH_3) residues being obtained although some methylation had taken place on the nitrogen atoms owing to the prolonged methylation treatments (see Paper [XV]). The syrupy product was converted into a syrupy trimethyl fructose which on examination proved to be essentially 3:4:5-trimethyl fructopyranose. Glucosazone is therefore formulated as a derivative of fructopyranose.

[XV]

The Structure of Osazones and the Isolation of a New Hexosazone Anhydride

By E. G. V. Percival

By a simple deacetylation of glucosazone tetra-acetate a very stable hexosazone anhydride was isolated. The same compound was obtained from the corresponding galactose derivative from which it is clear that a Walden Inversion must have taken place in one of the operations. The new anhydride contains only one hydroxyl group since it yielded a monomethyl compound and a mono-acetate, so that three rings must be present in the molecule, of which two are formed by the cyclisation of the phenylhydrazone residues and hydroxyl groups of the sugar chain, and the other is the normal oxide ring which was shown in the previous paper to be present in glucosazone; this observation is considered to strengthen the view that oxide rings are present in osazones.

[XVI]

Sugar Osazones and Their Anhydrides

By (Mrs) E.E.Percival and E.G.V.Percival

This work was extended to the osazones of lactose and maltose. A monoanhydro-lactosazone and two isomeric monoanhydro-maltosazones were prepared by the deacetylation method. All yielded penta-acetyl derivatives and must therefore possess a pyranose ring structure in addition to the pyrazolidine and pyridazine ring, a furanose ring being excluded because of the disaccharide linkage. The monoanhydro-glucosazone and -galactosazone of Diels and Meyer (Ann., 1935, 519, 157) prepared by a different method were shown to yield diacetates so that these derivatives also appear to contain an oxide ring structure.

[XVII]

Acetylation of Agar

By E.G.V.Percival and W.S.Sim

[XVIII]

Structure of Agar-Agar

By E.G.V.Percival, J.Munro and J.C.Somerville

[XIX]

The Acetylation of Agar-Agar and the Isolation of 2:4:6-Trimethyl α-d-Galactose by Hydrolysis

By E.G.V.Percival and J.C.Somerville

This paper contains details of the preliminary work on agar-agar briefly discussed in the two previous notes. It is also shown that the acid material produced on hydrolysing methylated agar with dilute mineral acid is laevulic acid. The major portion of the hydrolysis product however is the previously unknown 2:4:6-trimethyl α-d-galactose. This structure is assigned because (a) the substance formed a dimethyl osazone, (b) complete methylation yielded 2:3:4:6-tetramethyl galactose, (c) oxidation yielded a trimethyl δ-galactonolactone, (d) glycoside

formation with 1% methyl-alcoholic hydrogen chloride regenerated the crystalline 2:4:6-trimethyl methyl-galactopyranoside, (e) the sugar was different from the known 2:3:4-trimethyl galactose of Challinor, Haworth and Hirst, (J.Chem.Soc., 1931, 258) and (f) oxidation failed to produce the 2:3:4-trimethyl mucic acid obtained by these authors. This assignment of structure was confirmed in January 1938 by D.J.Bell working in Cambridge who has synthesised 2:4:6-trimethyl α-d-galactose by a method which leaves no doubt as to its constitution. The synthetic product was identical in every respect with the trimethyl galactose derived from methylated agar.

From the products of hydrolysis of methylated agar with methyl-alcoholic hydrogen chloride a substance which appeared to be a dimethyl methyl-ketoside was isolated. Treatment with aqueous mineral acid yielded laevulic acid indicating the probably source of this substance. Agar itself on mild hydrolysis gives ketose reactions and the isolation of a methylated fragment which appears

to be a ketose derivative strengthens the view that the polysaccharide contains ketose residues. From this preliminary work agar-agar is thought to be composed chiefly of β-galactopyranose units linked at positions 1 and 3 with ketose units at points on the chain (or loop) at positions not as yet determined, and since a dimethyl ketose has been isolated the possibility of loops or cross linkages is apparent. Much more work will be necessary however before an accurate picture of the structure of this complex polysaccharide can be drawn. In none of the agar preparations could the presence of sulphate residues be detected, so that the previously widely held view that such residues played an essential part particularly in the capacity of agar to form a gel must be discounted, since the agar regenerated from acetylated agar readily forms a gel. After this paper was received for publication Neuberg and Schwietzer (Monatshefte, 1937, 71, 46) have shown that by a simple washing process an agar preparation in ca. 90% yield, almost free from sulphur, can be obtained and this substance readily undergoes gelation.

[XX]

Investigations on the Configuration of Carbohydrates by Conductivity Measurements in Boric Acid Solution

By H.T.Macpherson and E.G.V.Percival

The last paper presented in this thesis was the result of work initiated in an attempt to verify the validity or otherwise of the boric acid method of testing the configuration of the α- and β-forms of the sugars. This method was first introduced by Böeseken (Ber., 1913, 46, 2612) and his results for α- and β-d-glucose were confirmed. Owing to the fact that none of the hydroxyl groups here are substituted and the results are interpreted with reference to the hydroxyl groups on C_1 and C_2, it is clear that some element of doubt must arise as to the validity of such conclusions. Experiments with various methylated sugars were instituted therefore and the conclusion was reached that the only hydroxyl groups effective in increasing the conductivity of boric acid are those located on C_1 and C_2. In the case of all the methylated

pyranose derivatives a depression of conductivity was observed corresponding to an increase in viscosity, whereas β-glucose which contains no cis-hydroxyl groups caused an elevation in the conductivity of boric acid solution. It is considered therefore that this initial conductivity may be due to the presence in solution of a small amount of a straight chain aldehydic form since glucose diethyl mercaptal was found to display a strong positive effect, and straight chain compounds such as dulcitol and sorbitol have a strongly positive effect on the conductivity of boric acid solution.

The behaviour of 3:4:6-trimethyl α-d-mannose under these conditions was studied, and was shown to give an initial depression of conductivity which diminished on muta-rotation. In this case the only possibilities of compound formation are with the hydroxyl residues on C_1 and C_2, which thus appear to be trans in the pure α-form. This is opposed to the results of Böeseken on β-d-mannose, but agrees with the accepted views on the structure of mannose. α-l-Rhamnose suffered an elevation of conductivity during mutarotation in boric acid solution which agrees

with the view that the hydroxyl groups on C_1 and C_2 are trans. Furanosides gave interesting effects with boric acid solution, γ-methylgalactoside gave rise to a depression of conductivity, γ-methylglucoside an elevation and α-methylmannofuranoside an abnormally high elevation. An examination of models reveals that, in the latter case four hydroxyl groups are present in close proximity to one another and on the same side of the plane of the ring, in the case of γ-methylglucoside three hydroxyl groups fulfil this condition, and in γ-methylgalactoside only two, and these are in the -CHOH-CH_2OH side chain where free rotation is possible, and, by analogy with ethylene glycol, no elevation of conductivity is to be expected. An accumulation of hydroxyl groups in a rigid structure in close proximity to one another seems to be the factor influencing the abnormally high conductivity of the mannofuranoside and it is considered likely that these four hydroxyl groups are coordinated to one central boron atom.

α-l-Sorbose gives an elevation of conductivity comparable with that given by α-methylmannofuranc

side whereas α-d-fructose only furnishes an elevation of about one third that recorded for sorbose. Coupled with the fact that sorbose appears not to undergo mutarotation, this might suggest that the straight chain keto form is present in solution. Other evidence, however, does not favour such a view.

The cis-hydroxyl residues present in α-d-galactose and α-methylgalactopyranoside at C_3 and C_4, appear to have no, or in the latter case only a slight effect on the conductivity in boric acid solution. If we consider the pyranose ring to exist in the "bed" type of "Sachse-Mohr" strainless ring structure, examination of models reveals that the C-OH bonds on C_3 and C_4 are no longer parallel to one another whilst in the α-form those at C_1 and C_2 are. Cox and his co-workers, (J.Chem.Soc., 1936, 1495) from X-ray measurements of crystalline sugar derivatives conclude however that the pyranose ring does not assume either of the "Sachse-Mohr" forms, but exists as a ring of almost uniplanar carbon atoms with the oxygen atom projecting from this plane by 1/2 to 1 Å. If this model be taken, the cis-hydroxyl groups on C_3 and C_4 are in a more favourable posit-

ion to participate in ring formation than those on C_1 and C_2. The result for α-methylgalactopyranoside, by comparison with that for α-methylglucopyranoside is in the direction which Cox's theory demands, but further work on these lines is desirable if information is to be obtained about the conformation of the pyranose ring in solution.

A possible mechanism for the formation of boric acid complexes with hydroxyl compounds is discussed. It is considered unlikely that anhydride formation occurs, but more probably that owing to the formation of 'hydroxyl bonds' between the substance and boric acid the ease with which hydrogen ions may be lost is increased.

I

POLYSACCHARIDES. PART VIII. EVIDENCE OF CONTINUOUS CHAINS OF α-GLUCOPYRANOSE UNITS IN STARCH AND GLYCOGEN.

BY

WALTER NORMAN HAWORTH

AND

EDMUND GEORGE VINCENT PERCIVAL.

Reprinted from the Journal of the Chemical Society.
June, 1931.

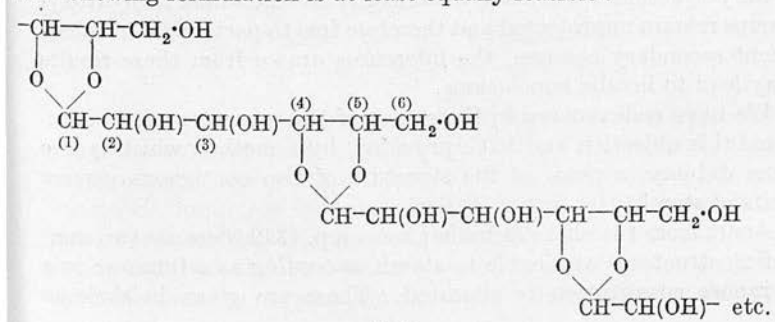
Reprinted from the Journal of the Chemical Society, 1931.

CLXXVIII.—*Polysaccharides. Part VIII. Evidence of Continuous Chains of α-Glucopyranose Units in Starch and Glycogen.*

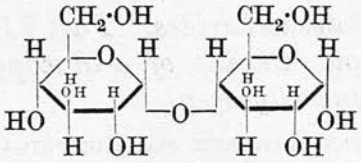
By Walter Norman Haworth and Edmund George Vincent Percival.

The conception, previously advanced, that starch is composed of conjugated maltose residues is the simplest basis on which to formulate this carbohydrate (Haworth, "Constitution of Sugars," Ed. Arnold & Co.). It recognises the significant facts of the hexagonal structure of glucose units in maltose and cellobiose, and also the ease with which these disaccharides are generated from the two polysaccharides. It recognises α-glucose as the essential residue in starch, and β-glucose as the corresponding residue in cellulose. Associated with this conception is the idea that these units are joined entirely by glucosidic linkings, and the wide inference may be drawn that starch and cellulose are analogues of the simplest glucosides.

It was also pointed out that the isolation of 2 : 3 : 6-trimethyl glucose from trimethyl starch and trimethyl cellulose furnishes no evidence of the existence of glucopyranose units in these polysaccharides, since the only inference to be drawn from this result is that positions 1 : 4 : 5 in the glucose units are concerned in the internal structural arrangements of the carbohydrates. Whilst the pyranose or the furanose ring would equally explain this mode of union of glucose residues, yet it may be argued that neither of these cyclic forms is necessary to interpret these simple facts inasmuch as the following formulation is at least equally feasible :



The basis for the recognition of the glucopyranose residue in starch is the experimental observation that starch is degraded to maltose almost quantitatively, combined with the further observation that α-maltose can only be constituted as shown below (Haworth and Peat, J., 1926, 3094).



This expression is structurally identical and stereoisomeric with that allocated to cellobiose (Haworth, Long, and Plant, J., 1927, 2809) and it is this structure which is embodied in the constitution assigned to cellulose.

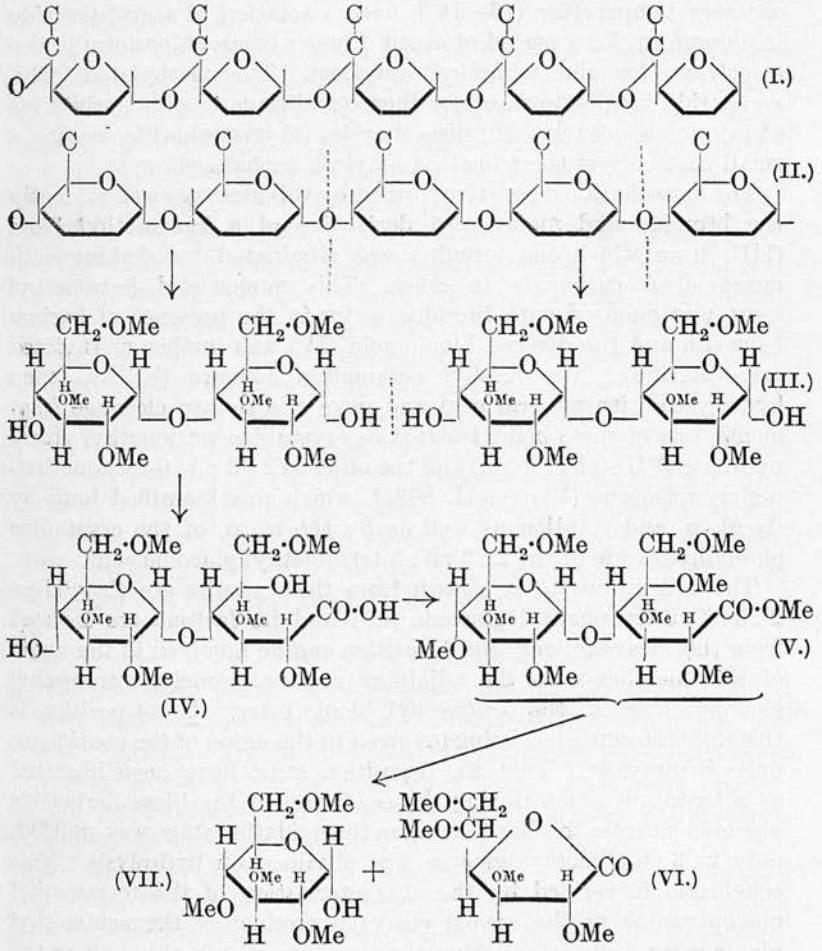
In supporting the continuous chain structure of α-glucopyranose units in starch, one has always been conscious of other factors which may intervene to render this conception untenable. One of the chief difficulties urged against the acceptance of this view is that no valid proof exists that maltose is preformed in starch. It has been suggested on many occasions that maltose may be only a reversion product of the action of enzymes on starch, and in support of this contention reference is made to the ease with which maltose can be synthesised from glucose by enzyme action. The analogy which can be drawn between the behaviour of α-methylglucoside and starch favours, however, the existence of α-glucopyranose units in the polysaccharide, as does also the observation of Karrer that starch is degraded to hepta-acetyl maltosidobromide by the action of acetyl bromide (Karrer and Nägeli, *Helv. Chim. Acta*, 1921, **4**, 263).

At the same time it must be confessed that it is always open to dispute whether an independent proof has been furnished of the precise mode of linking of the constituent hexose units of starch. If the polysaccharide is made to undergo cleavage whilst its hydroxy groups remain unprotected and therefore free to participate in subse quent secondary changes, the inferences drawn from these result may lead to invalid conclusions.

We have endeavoured in the course of the present work to ove come this objection and have provided, by a method which is fr from dubiety, a proof of the structure of two contiguous gluco units of starch.

Apart from the chain formula given on p. 1342 there are two cor peting structures ascribable to starch, according as a furanose or pyranose constitution is allocated. These are given in skelet

form below, and it will be seen that in the furanose formula the mutual linking of units occurs at position 5 in the hexose residue, ring formation occurring at positions 1 : 4. In the pyranose formula the linking of contiguous glucose units is represented at position 4 and the ring closure occurs at positions 1 : 5.



We have previously observed in our experiments with triacetyl starch that no essential constitutional change is introduced during the process of acetylation, since we have regenerated the polysaccharide from this triacetyl derivative and found it to behave exactly as the amylose portion of starch (Haworth, Hirst, and Webb, J., 1928, 2681). Similarly we have developed a procedure by which triacetyl starch is simultaneously deacetylated and methyl-

tracts yielded 12 g. of a light brown syrup (reducing). A portion of the trimethyl amylose was recovered unchanged (1·4 g.).

Oxidation of the Biose Derivative.—To an ice-cold solution of this syrup, there were added barium benzoate (16 g.) and water (450 c.c.) (solid insoluble in water, 1·4 g.). Bromine (1·6 c.c.) was now introduced, and the mixture kept in the dark. After 23 hours, more bromine (0·5 c.c.) was added and the oxidation was complete after 2 days. The barium was precipitated by the addition of sodium sulphate (8 g.) and the bromine was removed by aeration: filtration removed barium sulphate and benzoic acid. The solution was then made slightly alkaline with sodium carbonate and concentrated to 100 c.c. at 50°. Cautious acidification of the cold solution precipitated more benzoic acid, which was removed. The filtrate was again made alkaline and concentrated to 50 c.c. in readiness for methylation.

Methylation of the Mixed Sodium Salts.—Methylation was carried out at an initial temperature of 35°, which was raised finally to 65°, methyl sulphate (25 c.c.) and sodium hydroxide (30%; 67 c.c.) being used in the presence of acetone (200 c.c.). The methylated mixture was cooled to 0° and rendered acid to Congo-red with 5*N*-sulphuric acid in the presence of ice and was extracted six times with chloroform (1200 c.c.). The aqueous layer was rendered alkaline and evaporated to dryness at 60°, and the residue was extracted with boiling 90% alcohol; this extract yielded on evaporation under diminished pressure a mixture of sodium salts. These were dissolved in water and remethylated, together with the residue from the chloroform extraction, by methyl sulphate and alkali. On acidification and extraction of the solution with chloroform there was obtained a viscid syrup (8·5 g.).

Preparation of the Bionic Ester (Methyl Octamethyl Maltobionate).—The above product was now methylated twice with silver oxide (16 g.) and methyl iodide (25 c.c.) and yielded 9·1 g. of a mobile syrup, which was subjected to fractional distillation: (1) b. p. 100—180°/0·05 mm., 4·7 g., $n_D^{15°}$ 1·4410; (2) b. p. 180—210°/0·05 mm., 2·0 g., $n_D^{15°}$ 1·4620; (3) b. p. 210—250°/0·05 mm., 0·2 g., $n_D^{15°}$ 1·4695; residue, 0·3 g.

The second fraction was redistilled and showed the following properties: $[\alpha]_D^{20°}$ + 116°, $n_D^{15°}$ 1·4620 (Found: C, 52·4; H, 8·3; OMe, 56·5; CO_2Me, 12·0. Calc. for $C_{21}H_{40}O_{12}$: C, 52·1; H, 8·3; OMe, 57·6; CO_2Me, 12·2%). These analyses and constants, together with the subsequent behaviour of the product, indicated that it was mainly methyl octamethyl maltobionate (compare Haworth and Peat, J., 1926, 3094). Yield, 22·4% of the theoretical.

Hydrolysis of the Ester.—2·78 G. in 52·8 c.c. of 5% hydrochloric

acid were heated at 95° on the water-bath. Polarimetric readings were taken at intervals : $[\alpha]_D^{20°}$ 115° (initial value); 88·2° (30 mins.); 73° (80 mins.); 66·4° (175 mins.); 61·2° (220 mins.); 60·2° (335 mins.); 55·9° (420 mins.); 55·7° (440 mins.; constant value).

Isolation of Tetramethyl Glucose.—The solution was neutralised with barium carbonate at 50°, air drawn through it for an hour, and the mixture kept at the ordinary temperature during 2 days. The neutral filtered solution was then evaporated at 40° under reduced pressure, and the solid residue extracted ten times with ether (500 c.c.). The ethereal extracts yielded on evaporation a syrup which crystallised spontaneously in colourless needles; these were purified from light petroleum and identified as 2 : 3 : 4 : 6-tetramethyl glucopyranose (yield, 1·35 g.), m. p., and mixed m. p. with an authentic specimen, 86°; $[\alpha]_D^{18°}$ + 91·0° (after 10 mins.), 83·2° (90 mins.) (*c*, 1·04).

Isolation of Tetramethyl γ-Gluconolactone.—The dried barium salts remaining after the ether extraction were dissolved in water, and the solution acidified with *N*-hydrochloric acid. The aqueous solution was then evaporated as before, the solid residue repeatedly extracted with boiling ether, the extracts evaporated, and the residual syrup heated for ½ hour at 100° to complete lactonisation (yield, 1·2 g., *i.e.*, 96% of the theoretical). Distillation in a high vacuum at a bath temperature of 115—120°/0·01 mm. gave a clear mobile liquid, $n_D^{15°}$ 1·4490 (yield, 1·0 g., *i.e.*, 80% of the theoretical). Nucleation of the liquid with a crystal of 2 : 3 : 5 : 6-tetramethyl γ-gluconolactone resulted in complete crystallisation. M. p. and mixed m. p. with an authentic specimen 26° : $[\alpha]_D^{18°}$ + 61·0° (30 mins.) (*c*, 1·1); 58·2° (1 day); $[\alpha]_D^{20°}$ + 57·0° (2 days) (*c*, 1·1). M. p. of the phenylhydrazide 134—136°.

The Action of Acetyl Bromide on Trimethyl Glycogen.—The trimethyl glycogen was prepared from triacetyl glycogen as described by Haworth, Hirst, and Webb (*loc. cit.*). The procedure followed for the acetyl bromide treatment of the methylated polysaccharide was identical with that given above in the case of trimethyl amylose. The yield of methyl octamethyl maltobionate obtained after the oxidation stage, followed by that of remethylation and esterification, was 22·9% of the theoretical, as compared with the yield of 22·4% from trimethyl amylose. This was a colourless syrup distilling at about 190°/0·012 mm., having $n_D^{15°}$ 1·4610, $[\alpha]_D^{20°}$ + 119° (*c*, 0·86) (Found : C, 51·8; H, 8·5; OMe, 57·3. Calc. for $C_{21}H_{40}O_{12}$: C, 52·1; H, 8·3; OMe, 57·6%).

Hydrolysis of the Bionic Ester (*Methyl Octamethyl Maltobionate*).—This process was complete after 2·58 g. of the ester had been heated with 5% hydrochloric acid at 95°. The rate of hydrolysis was

identical with that of the bionic ester from trimethyl amylose and similar polarimetric data were observed. Isolation of the products from this treatment yielded crystalline 2 : 3 : 4 : 6-tetramethyl glucopyranose (1·25 g.) corresponding in amount to 93% of the theoretical, and also crystalline 2 : 3 : 5 : 6-tetramethyl γ-gluconolactone (1 g.) corresponding to 87% of the theoretical. This gave the crystalline phenylhydrazide of 2 : 3 : 5 : 6-tetramethyl gluconic acid, m. p. 135—136° (Haworth and Peat, *loc. cit.*).

The authors are grateful to the Department of Scientific and Industrial Research for a grant in aid of this investigation.

UNIVERSITY OF BIRMINGHAM,
EDGBASTON.

[*Received, March* 31*st*, 1931.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY & SONS, LIMITED, BUNGAY, SUFFOLK.

II

POLYSACCHARIDES. PART IX. EVIDENCE OF THE PYRANOSE STRUCTURE OF XYLAN.

BY

WALTER NORMAN HAWORTH

AND

EDMUND GEORGE VINCENT PERCIVAL.

Reprinted from the Journal of the Chemical Society, November, 1931.

Reprinted from the Journal of the Chemical Society, 1931.

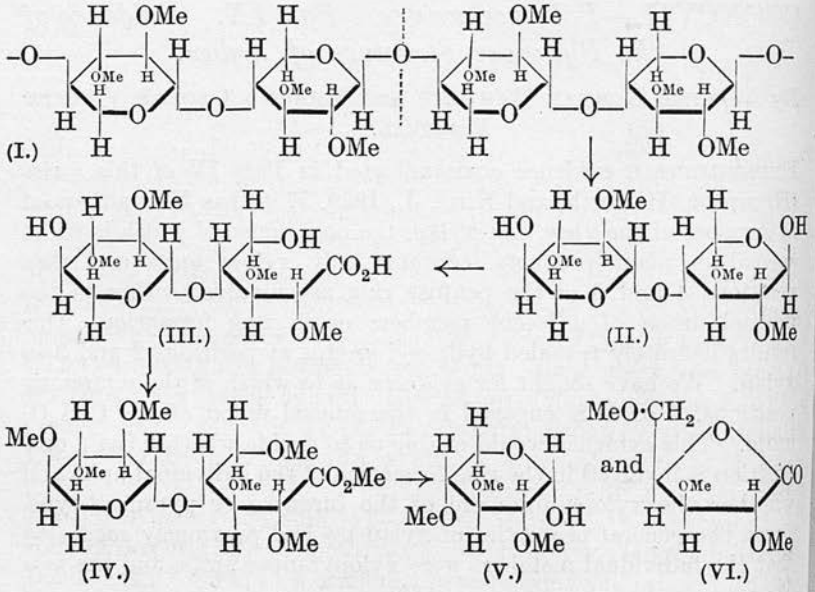
CCCXCVII.—*Polysaccharides. Part IX. Evidence of the Pyranose Structure of Xylan.*

By Walter Norman Haworth and Edmund George Vincent Percival.

Experimental evidence communicated in Part IV of this series (Hampton, Haworth, and Hirst, J., 1929, 1739) has been advanced in support of the view that xylan, the occurrence of which is widespread in plant products, contains only xylose units and that positions 4 and 5 of the pentose ring are involved either in the mutual union of adjacent members or in ring formation. Our results definitely revealed hydroxyl groups at positions 2 and 3 in xylan. We have sought for evidence as to which of the remaining positions, 4 or 5, is engaged in the mutual union of the $C_5H_8O_4$ units. This evidence would enable us to decide whether the 5 or 4 position is involved in the ring formation of the individual units and whether the xylose units are of the furanose or pyranose type. From the general properties of xylan we had previously suggested that the individual members were xylopyranose units, and the new

evidence now communicated leads us finally to this conclusion. For the purpose of the present work we have prepared by a modified procedure a fully methylated specimen of xylan derived from esparto. It was shown in the former paper that methyl groups were introduced into the 2- and 3-positions, and from the present work the formulation of dimethyl xylan can be represented by a repetition of the scheme given below (I).

This specimen of dimethyl xylan has been degraded by acetolysis at 0° during the very short interval of 10 minutes. In this way the complete scission of the polysaccharide to the ultimate C_5 units was averted and we were able by subsequent analysis to recognise among the products a partly methylated *disaccharide* to which, by the sequence of operations herein described, we are able to allocate the constitution (II). Adopting now a procedure which we had applied both to starch and to glycogen (Part VIII) and earlier to maltose and cellobiose (J., 1926, 3094; 1927, 2809), we investigated both the ring structure and the position of the biose union of the substituted disaccharide. The acetolysis products from dimethyl xylan were first deacetylated and then oxidised with bromine water in order to convert the sugars generated by the hydrolysis into the corresponding monobasic acids. Among these was a *bionic acid derivative* (III) from the disaccharide of the dixylose type. The bionic acid was methylated and esterified and yielded a *methyl* ester of *hexamethyl dixylobionic acid* (IV).



By hydrolytic cleavage this product (IV), which showed accurate analytical figures for $C_{17}H_{32}O_{10}$, gave rise to crystalline 2 : 3 : 4-trimethyl xylopyranose (V) in a yield of over 80%, and there was isolated as a second component 2 : 3 : 5-trimethyl γ-xylonolactone (VI) which, although distilling as a liquid in a yield of 73%, was recognised by conversion into the crystalline phenylhydrazide of the corresponding 2 : 3 : 5-trimethyl xylonic acid. These crystalline products were compared directly with authentic specimens and their identity confirmed. It follows that, since the 4-position in the latter acid carries a free hydroxyl group, this must have been the point of linking with the 1-position of the adjoining xylopyranose unit (V). Moreover this lactone had been previously investigated by Haworth and Porter (J., 1928, 611), who degraded it to *d*-dimethoxysuccinic acid. Position 5 of the lactone represents the point of junction of the xylose ring which was opened by oxidation to the bionic acid and subsequently methylated to give the terminal CH_2·OMe group in the γ-lactone (VI).

We can therefore apply the constitution (II) to the partly methylated disaccharide which is here shown to be a *dixylopyranose*. It follows that the xylose units in the polysaccharide are of the pyranose type and that xylan can be represented by formula I (with H in place of Me).

Experimental.

Methylation of Xylan.—The methylation of xylan was carried out with the following quantities of reagents, which differ slightly from those previously used (Hampton, Haworth, and Hirst, J., 1929, 1739): xylan (7·5 g.), potassium hydroxide (500 g. in 650 c.c. of water), and methyl sulphate (430 c.c.). The methyl sulphate was added to the alkaline solution of xylan during 4 hours at room temperature with continuous stirring. Thereafter the temperature was raised to 100° for 1 hour, the mixture was diluted with 2 litres of boiling water, and the liquid filtered through muslin. The crude methylated xylan obtained from two such operations was again methylated by the use of potassium hydroxide (365 g. in 400 c.c. of water) and methyl sulphate (290 c.c.). The white methylated compound was washed with hot water, dissolved in chloroform, dried with magnesium sulphate, filtered through glass wool, and the dimethyl xylan precipitated by an excess of ether. The product had the same constants as those already recorded (Hampton, Haworth, and Hirst, *loc. cit.*).

Degradation of Dimethyl Xylan.—To dimethyl xylan (4 g.), dissolved in glacial acetic acid (44 c.c.) and cooled to 0°, acetic anhydride (40 c.c.) containing concentrated sulphuric acid (1·6 c.c.) was added,

this mixture having previously been cooled to 0°. After remaining for 10 mins. at 0°, the mixture was poured into ice water (300 c.c.) and cautiously neutralised with sodium carbonate (40 g.), followed by barium carbonate. After 5 hours the solution was filtered and extractions with chloroform (800 c.c.) yielded, on evaporation, a viscid yellow syrup.

Deacetylation. The above syrup was dissolved in acetone (50 c.c.) and allowed to react with *N*-sodium hydroxide (50 c.c.) at 15° for 2 hours. The excess of alkali was then neutralised with *N*-hydrochloric acid, and the acetone removed under diminished pressure at 30°.

Oxidation. The above neutral solution was oxidised with bromine (1 c.c.) in the presence of barium benzoate (7 g. in 250 c.c. of water), the halogen being added at 0°, and the oxidation allowed to proceed during 40 hours in the dark. Thereafter the mixture was aerated to remove bromine, filtered from benzoic acid, and the barium salts were precipitated with sodium sulphate (4 g.). After filtration the solution was rendered alkaline and concentrated to 50 c.c. Cautious acidification of the cold solution with dilute sulphuric acid caused the separation of more benzoic acid, which was removed. The filtrate was again rendered alkaline and concentrated for methylation.

Methylation. The above aqueous solution was mixed with acetone (100 c.c.) and treated with 30% sodium hydroxide solution (81 c.c.) and methyl sulphate (33 c.c.) at 35—40° for ½ hour and finally at 55—60°, the reagents being added in $\frac{1}{10}$ portions every 10 minutes in the usual way. The organic acids were liberated by adding dilute sulphuric acid (using Congo-red as indicator) in the presence of ice, and the solution was now extracted with chloroform. The aqueous residues were rendered alkaline, evaporated under diminished pressure, and extracted with boiling 95% alcohol. This extract was evaporated in a vacuum, and the solid material remaining was dissolved in water, added to the syrup obtained from the above chloroform extraction, and submitted to another methylation with the same quantities of reagents as before. This yielded a viscid brown syrup (2·5 g.). Several such preparations of material were carried out and finally the combined syrups were dissolved in methyl iodide and methylated twice by the Purdie method. From 10 g. of dimethyl xylan, the mixed esters resulting from this treatment weighed 5·6 g. A first distillation yielded the following: Fraction 1 (bath temperature 90—135°/0·06 mm.) 2 g., $n_D^{15°}$ 1·4452; fraction 2 (bath temperature 135—170°/0·06 mm.) 0·3 g., $n_D^{15°}$ 1·4561; fraction 3 (170—210°/0·06 mm.) 2·4 g., $n_D^{15°}$ 1·4618; fraction 4 (210—250°/0·06 mm.) 0·4 g., $n_D^{15°}$ 1·4680; residue 0·5 g. Fraction 3 was redistilled and showed b. p. about 170°/0·06 mm., $n_D^{15°}$ 1·4610; $[\alpha]_D^{17°}$ + 10·4°

(*c*, 1·06 in water) (Found : C, 51·2; H, 8·1; OMe, 54·5; CO_2Me, 15·2. $C_{17}H_{32}O_{10}$ requires C, 51·5; H, 8·1; OMe, 54·8; CO_2Me, 14·9%). Redistillation of fractions 2 and 4 yielded a further quantity (0·2 g.) of the same material as fraction 3, so that the combined yields of this ester (IV) amounted to 21% of the theoretical.

Hydrolysis of the Bionic Ester.—The above ester (2·0 g.) was hydrolysed at 100° with 2% hydrochloric acid (60 c.c.) and underwent the following polarimetric changes. $[\alpha]_D^{17°}$ + 10° (initial); + 25° (10 mins.); + 30° (20 mins.); + 31·8° (45 mins., constant).

Isolation of 2 : 3 : 4-*Trimethyl Xylose.*—The acid solution was neutralised with excess of barium carbonate and aerated during 2—3 hours at 50° in contact with animal charcoal. The filtrate, on evaporation under diminished pressure, yielded a mixture of barium salts and a sugar. These residues were dried by distilling from them a mixture of benzene and alcohol, and were extracted repeatedly with dry ether. This extract yielded a syrup which crystallised on nucleation with 2 : 3 : 4-trimethyl xylopyranose. The crude product weighed 0·82 g. (84% of the theoretical yield). On recrystallisation this showed m. p. 90—92°, $[\alpha]_D^{17°}$ + 20·4° (after ½ hour). It was identified as α-2 : 3 : 4-trimethyl xylopyranose by mixed m. p. determination (compare Hampton, Haworth, and Hirst, *loc. cit.*) and by the following analysis (Found : C, 50·2; H, 8·7; OMe, 48·1. Calc. : C, 50·0; H, 8·3; OMe, 48·4%). Among the products derivatives of furfural were detected.

Isolation of 2 : 3 : 5-*Trimethyl* γ-*Xylonolactone.*—The residue of barium salts remaining from the previous extraction was dissolved in water (10 c.c.) and acidified with *N*-hydrochloric acid (10 c.c.), and the aqueous solution was evaporated under diminished pressure. The residue, dried at 100°, was repeatedly extracted with dry ether and gave a pale yellow syrup, which was distilled : b. p. 80—90°/0·05 mm., $n_D^{16°}$ 1·4450, $[\alpha]_D^{17°}$ + 95·2° (after ½ hour); + 90·5° (2½ days); + 75·5° (14 days); 70·0° (20 days) (*c*, 1·05 in water). Yield, 0·7 g. (73% of the theoretical). The phenylhydrazide of the corresponding acid was prepared from this lactone (compare Haworth and Porter, J., 1928, 611) and showed m. p. 88° alone or mixed with an authentic specimen (Found : C, 56·3; H, 7·2; N, 9·6; OMe, 31·1. Calc. : C, 56·4; H, 7·4; N, 9·4; OMe, 31·2%).

The authors acknowledge with thanks a grant from the Department of Scientific and Industrial Research in aid of this work.

UNIVERSITY OF BIRMINGHAM,
EDGBASTON.

[*Received, September 21st,* 1931.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY & SONS, LIMITED,
BUNGAY, SUFFOLK.

III

POLYSACCHARIDES. PART XI. MOLECULAR STRUCTURE OF GLYCOGEN.

BY

W. N. HAWORTH,

AND

E. G. V. PERCIVAL.

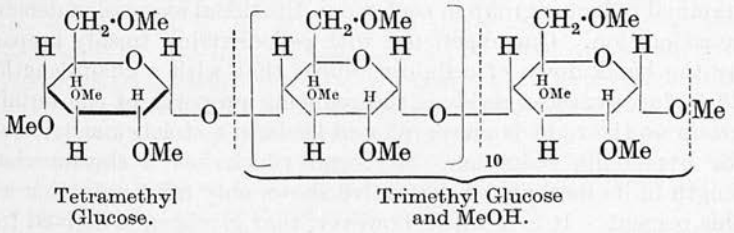
Reprinted from the Journal of the Chemical Society,
August, 1932.

Reprinted from the Journal of the Chemical Society, 1932.

324. *Polysaccharides. Part XI. Molecular Structure of Glycogen.*

By W. N. Haworth and E. G. V. Percival.

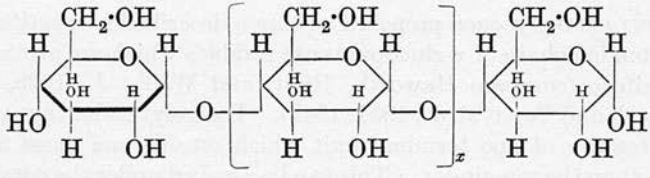
Methylated glycogen prepared as herein described is constituted as an extended chain of α-glucopyranose residues which are arranged as in maltose (compare Haworth, Hirst, and Webb, J., 1929, 2482; Haworth and Percival, J., 1931, 1342). Hydrolytic cleavage reveals the presence of one terminal unit which retains one more methyl group than the remainder. This can be assayed under the conditions described and is recognised as crystalline tetramethyl glucopyranose, which occurs to the extent of 9%.



It follows that the number of glucose residues in the methylated polysaccharide is not more than about twelve, corresponding to a

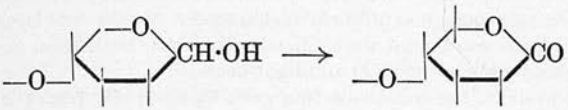
M.W. of approximately 2500, a value which is in agreement with that determined by Rast's method.

The glycogen was prepared from rabbit's liver by British Drug Houses Ltd. and was a good specimen of the commercial product. It was purified by the method previously described (Haworth, Hirst, and Webb, *loc. cit.*) and acetylated under a new procedure, and the glycogen acetate readily underwent methylation in aqueous acetone solution by eight treatments with methyl sulphate. Precautions were taken to test the homogeneity of the specimens of glycogen acetate and of methylated glycogen and no evidence of the presence of smaller degraded fragments was obtained. Under the treatment described, there is therefore nothing to suggest that the glycogen with which we started has suffered any breakdown by conversion into these derivatives. It is, however, clear that natural glycogen contains a minimum number of 12 glucose units in its molecular structure. The process adopted for the isolation of glycogen from livers involves the use of alkali and we have not studied the changes which may be involved in this procedure, although the fact is significant that the glycogen so extracted exhibits a marked stability towards alkali. As ordinarily obtained, the polysaccharide cannot contain many more units than the average value of twelve, and its general properties, such as the comparison of its reducing power with that of starch, suggest that glycogen is not a highly complex polysaccharide. The structure of native glycogen may be deduced from the expression



where the minimum value of x is 10, and in the commercial specimen this minimum is probably not much exceeded. The fate of the terminal reducing group in such a constitutional expression demands consideration. Our experience with cellodextrins, freshly prepared by the break-down of cellulose, shows that with a chain-length of 26 β-glucopyranose residues the reducing property of the terminal group on the right is preserved and leads to a stoicheiometric value for hypoiodite reduction. Glycogen which has a shorter chain-length in its methylated derivative shows only faint reduction with this reagent. It is recalled, however, that glycogen is derived from animal sources by extraction with fairly concentrated alkali, and either *in vivo* or by this subsequent treatment the terminal reducing

group must be affected. This is a problem remaining over for solution, in the cases of cellulose, glycogen and starch. It is possible that the terminal " aldose " component in each is modified by oxidation to the acid (or lactone) group.



EXPERIMENTAL.

The Acetylation of Glycogen.—Haworth, Hirst, and Webb (J., 1929, 2482) had shown that glycogen could be acetylated with pyridine and Ac_2O by heating for 20 hrs. at 80°. A new method has been developed during this investigation which is suitable for the acetylation of large quantities of glycogen, by treatment with the above reagents for only 3 hrs. without heating. This method of acetylation is extremely mild, and it is considered unlikely that degradation of the glycogen macromolecule occurs.

Glycogen (25 g.) was dissolved in H_2O (250 c.c.) and pptd. by the gradual addition of abs. EtOH (1750 c.c.), the glycogen being collected and washed once with EtOH. The dried product was added immediately to a mixture of pyridine (285 c.c.) and Ac_2O (250 c.c.). Heat developed; the mixture was agitated from time to time, nearly the whole of the glycogen dissolving in 1 hr. After 3 hrs., the faintly yellow solution was filtered into cold H_2O, and the white product washed with H_2O, EtOH, and Et_2O. Yield 39 g., *i.e.*, 87%. The " triacetate " was thus obtained as a white powder, sol. in acetone and $CHCl_3$. $[\alpha]_D^{20°} = +170°$ (*c*, 1·0 in $CHCl_3$) (Found: C, 50·0; H, 5·9; CH_3·CO, 44·4. $C_{12}H_{16}O_8$ requires C, 50·0; H, 5·6; CH_3·CO, 44·8%).

This *glycogen acetate* was homogeneous in a general sense, since careful fractional pptn. failed to reveal any differences in the properties of a wide range of fractions. For example, glycogen acetate (32 g.) dissolved in $CHCl_3$ and pptd. with Et_2O and light petroleum as well as by evaporation of the final residual solution yielded six fractions having identical solubilities, iodine values, specific rotations, and acetyl values. The same results were observed in a fractional pptn. of glycogen acetate (36 g.) from $CHCl_3$–acetone by means of EtOH, Et_2O, and light petroleum. Having regard to the large amount of polysaccharide acetate employed in these typical expts., it seems inconceivable that marked differences in molecular aggregation exist.

Simultaneous Deacetylation and Methylation of Glycogen Acetate.—The glycogen acetate (35 g.) was dissolved in acetone (350 c.c.) and treated at 50° during 90 mins. with Me_2SO_4 (170 c.c.) and 30% NaOH aq. (450 c.c.) with vigorous stirring. The reagents were admitted in 1/10th portions every 10 mins., acetone being added from time to time to replace that lost by evaporation. The operation was completed by heating at 80° for 30 mins. to remove acetone; the methylated product, which then separated as a solid, was removed and dissolved in acetone for remethylation. Eight successive methylations were carried out in this way except that two batches, each from 35 g. of the acetate, were combined for the second and subsequent treatments.

The final isolation of the methylated glycogen was accomplished by extracting the solid methylated product with boiling $CHCl_3$, the extract being dried

over $MgSO_4$ and concentrated to a pale yellow glass, which on trituration with light petroleum yielded a fine white powder. This was extracted with boiling Et_2O to remove traces of impurities. Yield from 140 g. of glycogen acetate, 83·5 g., *i.e.*, 82%.

As in the case of glycogen acetate, expts. were instituted to discover whether the methylated glycogen was uniform in character. To this end large amounts of the methylated compound were fractionally pptd. both from acetone and $CHCl_3$ solution by means of Et_2O and light petroleum.

In a typical expt. the initial fraction gave C, 52·7; H, 7·9; OMe, 45·0%, and the final fraction C, 52·5; H, 7·6; OMe, 45·0%; whereas a representative sample of the methylated polysaccharide used in the hydrolysis expts. gave C, 52·9; H, 8·0; OMe, 45·4 (Calc. for $C_9H_{16}O_5$: C, 52·9; H, 7·8; OMe, 45·6%).

The specific rotation of methylated glycogen, $[\alpha]_D^{20°} = +209°$ (c, 1·0 in $CHCl_3$), was observed for all the fractions obtained. Experience has shown that the determination of the specific rotation of methylated polysaccharides, as well as being more convenient, is more sensitive as a control of OMe content than Zeisel estimations.

Refractionation of the final fractions from a number of pptns. having failed to indicate any differences, the conclusion was reached that the methylated glycogen used in these expts. consisted of groups of products of similar molecular size.

The Hydrolysis of Methylated Glycogen.—Powdered methylated glycogen (80 g.) was added to cold HCl aq. (d 1·16; 400 c.c.), contained in a large distillation flask fitted with a Bunsen valve on the side arm. The methylated polysaccharide dissolved in 2 hrs.; the liquid was then cooled to − 15° and saturated with dry HCl. After a further 2 hrs. the liquid was again saturated with HCl and the mixture was kept for a total of 44 hrs. at room temp. The excess of HCl was then removed by aeration, and the solution diluted and neutralised with $BaCO_3$ in the presence of a little charcoal. After filtration the almost colourless liquid, together with the washings (1½ l.), was extracted with $CHCl_3$ (5 l.) in eight operations, the extracts being evaporated at 50°/15 mm. after drying over $MgSO_4$. The aq. solution was treated with an equal vol. of abs. EtOH, which eliminated a large quantity of $BaCl_2$, and the aq.-alc. filtrate was concentrated at 50° under diminished press., the residue being dried with EtOH–C_6H_6.

Examination of the material from the chloroform extract. The syrup (15 g.) was redissolved in $CHCl_3$ (75 c.c.), and light petroleum (b. p. 40—60°; 1500 c.c.) added slowly and with stirring. The petroleum was poured off after 30 mins., and the syrup redissolved in $CHCl_3$ and repptd. in an exactly similar manner, the entire operation being repeated a third time. The light petroleum was removed by distillation to yield a syrup (10 g.), part of which crystallised in the characteristic form of 2 : 3 : 4 : 6-tetramethyl glucose.

Preparation of the glucosides. This mixture was heated at 70° for 8 hrs. with 1% methyl-alc. HCl (150 c.c.), the acid neutralised with Ag_2CO_3, and the filtered solution evaporated to a thin syrup (10·5 g.) (C) and heated at 100°/15 mm. for several hrs. to remove solvent.

Treatment of the solid residues from the aqueous solution. The well-dried residues together with the pptd. $BaCl_2$ were extracted 10 times with boiling $CHCl_3$, the extracts being dried over $MgSO_4$ and concentrated. This treatment yielded a syrup which, on trituration with Et_2O and cooling in the refrigerator,

crystallised to yield 2 : 3 : 6-trimethyl glucose (25 g.). The crystals were removed by filtration and the ethereal solution of syrup was concentrated, the resulting syrup being added to that left over from the light-petroleum extractions of the material from the $CHCl_3$ extract. This syrup (46 g.) was dissolved in $CHCl_3$ (100 c.c.), and the above treatment with light petroleum repeated. The petroleum extracts on concn. yielded a syrup (1·5 g.), which was converted into the methylglucosides as before (1·5 g.) (D).

The isolation of 2 : 3 : 4 : 6-*tetramethyl methylglucopyranoside.* This was achieved by fractional distillation of (C) and (D) in a high vacuum from a Widmer flask fitted with a special fractionating column. Previous expts. (Haworth and Machemer, *Ber.*, 1932, **65**, [*A*], 43) had proved that an almost quantitative separation of the tetra- from the tri-methyl methylglucosides could be obtained.

Syrup C was distilled into the Widmer flask for the first fraction and into a collecting tube for the second.

(I) 7·5 g., b. p. 93°/0·03 mm. Bath temp. 108—110°. $n_D^{12^\circ}$ 1·4462.
(II) 2·5 g., b. p. 93—97°/0·03 mm. Bath temp. 115—120°. $n_D^{12^\circ}$ 1·4540. Still residue, 0·5 g.

Refractionation from the Widmer flask of fraction (1).

(III) 6·40 g., b. p. 87°/0·04 mm. Bath temp. 130°. $n_D^{13^\circ}$ 1·4456. Distilled in 1½ hrs.
(IV) 0·95 g., b. p. 87°/0·04 mm. Bath temp. 140°. $n_D^{13^\circ}$ 1·4460. Distilled in ½ hr.
(V) 0·02 g., b. p. 90°/0·04 mm. Bath temp. 150°. $n_D^{13^\circ}$ 1·4511.
(VI) 0·05 g., b. p. 98°/0·04 mm. Bath temp. 150—160°. $n_D^{13^\circ}$ 1·4575.

After fraction (III) had been collected, fraction (II) was added to the residue in the Widmer flask and the distillation was continued as above. After the collection of fraction (VI), the syrup D was distilled from the Claisen flask into the Widmer apparatus (bath temp. 90—110°/0·04 mm.). The fractional distillation was continued, and the following fractions collected.

(VII) 0·30 g., b. p. 85—90°/0·04 mm. Bath temp. 135°. $n_D^{13^\circ}$ 1·4450, in 1 hr.
(VIII) 0·10 g., b. p. 95—100°/0·04 mm. Bath temp. 140—150°. $n_D^{13^\circ}$ 1·4550.
(IX) 0·02 g., b. p. 95—100°/0·04 mm. Bath temp. 150—160°. $n_D^{13^\circ}$ 1·4570.

From a consideration of the refractive indices, fraction (I) appeared to be in the main tetramethyl methylglucopyranoside, and fraction (II) the corresponding trimethyl derivative. A more detailed examination confirmed this, analyses being carried out for each fraction, the final fraction hydrolysed, and the resulting sugar weighed.

It was found that (III), (IV), and (VII) consisted of tetramethyl methylglucopyranoside (Found : C, 52·75; H, 8·9; OMe, 59·5. $C_{11}H_{22}O_6$ requires C, 52·8; H, 8·9; OMe, 62·0%), whilst the remainder was trimethyl methylglucopyranoside. The total yield of tetramethyl methylglucopyranoside was therefore 7·65 g., so the result, expressed as the percentage of tetramethyl glucose obtained from the trimethyl glycogen, is 9·0%. The expression connecting the number of anhydroglucose residues (x) with the percentage of tetramethyl glucose (y) derived from any fully methylated polysaccharide is $y = 236 \times 100/(204x + 46)$. The shorter expression, $x = 116/y$, gives the approximate result.

By the hydrolysis of 16·0 g. of methylated glycogen in another series of expts., 8·8% of tetramethyl methylglucopyranoside was obtained. This is seen to be in good agreement with the previous result when it is considered that the losses are relatively greater by working with smaller quantities.

The isolation of tetramethyl glucopyranose. Tetramethyl methylglucopyranoside (3 g.) obtained from fractions (III), (IV), and (VII) was hydrolysed during 8 hrs. with 5% HCl aq. (100 c.c.) and gave cryst. tetramethyl glucopyranose (2·65 g.), which was recrystallised from light petroleum and had m. p. 88—89° alone or mixed with an authentic specimen, $[\alpha]_D^{20°}$ + 83° (equilibrium value in H_2O; *c*, 1) (Found: C, 50·5; H, 8·6; OMe, 50·6. $C_{10}H_{20}O_6$ requires C, 50·8; H, 8·5; OMe, 52·5%).

The recovery of trimethyl glucose. The total yield was 73 g., being made up of 25 g. of cryst. material, 46 g. of syrup, and 2 g. as the methylglucoside. This, together with the 7 g. of tetramethyl glucopyranose, constitutes a recovery of 96% of products derived from the original methylated glycogen.

The Reducing Power of Glycogen.—It was observed that slight but definite reduction of Fehling's solution took place when pure glycogen (electrodialysed and pptd. twice with EtOH; ash = 0·15%) was heated at 100° for 10 mins. with the reagent.

The reducing value according to the method of Bergmann and Machemer (*Ber.*, 1930, **63**, 316) was investigated, and the iodine value, *i.e.*, the number of c.c. of *N*/10-I required to oxidise 1 g. of the substance, as the mean of several expts., is given as 1·95, whereas the value for starch, which is non-reducing to Fehling's solution, is *ca.* 0·7 depending on the source. Glycogen is therefore more reducing in character than starch, a fact which is readily explained if glycogen contains fewer anhydroglucose residues than starch.

Estimations of the Molecular Weights (Rast).—These gave the following mean values: Glycogen acetate, 2600; methylated glycogen, 2400. As in other cases, these are to be regarded as minimum values. The results are interpreted merely as an indication that no degradation occurs on methylation of the acetate.

The authors are indebted to the Department of Scientific and Industrial Research for a grant.

UNIVERSITY OF BIRMINGHAM, EDGBASTON. [*Received, August 3rd*, 1932.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY & SONS, LIMITED, BUNGAY, SUFFOLK.

IV

POLYSACCHARIDES. PART XV. THE MOLECULAR STRUCTURE OF INULIN.

BY

W. N. HAWORTH,

E. L. HIRST,

AND

E. G. V. PERCIVAL.

Reprinted from the Journal of the Chemical Society
September, 1932.

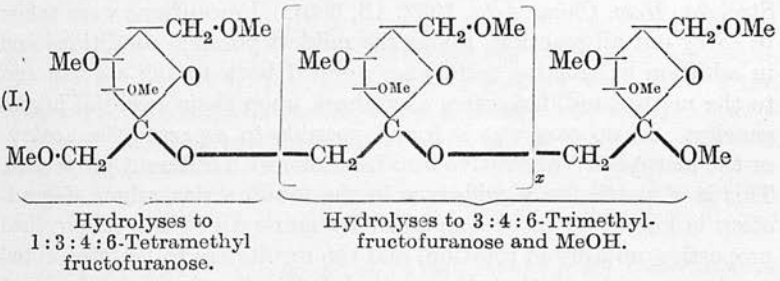
Reprinted from the Journal of the Chemical Society, 1932.

345. *Polysaccharides. Part XV. The Molecular Structure of Inulin.*

By W. N. Haworth, E. L. Hirst, and E. G. V. Percival.

In previous papers of this series it has been shown that evidence concerning the molecular structure of polysaccharides can be obtained by hydrolysing their fully methylated derivatives. The polysaccharides hitherto examined by this method have had a common feature in that they were derivatives of glucose and yielded on hydrolysis the extremely stable substances tetramethyl glucopyranose and 2 : 3 : 6-trimethyl glucopyranose. It is now shown that the same method of enquiry is applicable also to methylated inulin, which on hydrolysis gives labile fructofuranose derivatives. Inasmuch as methylated inulin gives 3 : 4 : 6-trimethyl fructofuranose accompanied by 1 : 3 : 4 : 6-tetramethyl fructofuranose to the extent of 3·7%, it is concluded that methylated inulin is composed of continuous chains of methylated fructofuranose residues united through positions 1 and 2 of the fructofuranose (compare Haworth and Learner, J., 1928, 619).

(I.)



Hydrolyses to 1 : 3 : 4 : 6-Tetramethyl fructofuranose.

Hydrolyses to 3 : 4 : 6-Trimethyl-fructofuranose and MeOH.

The isolation of tetramethyl fructofuranose demonstrates clearly that the macromolecules are not in the form of large rings and establishes the nature of one of the terminal groups of the chain. Under the experimental conditions adopted, it seems extremely unlikely that appreciable degradation of the inulin molecule had taken place during the transformation to the methylated derivative. The inulin macromolecule may be pictured, therefore, as consisting of a chain of fructofuranose units united as shown in (II) and having a minimum average length of 30 fructofuranose residues. From the evidence given, the nature of one of the terminal groups may be deduced and whilst the character of the other is not immediately derivable from the present experiments the instability of inulin in the presence of alkali and the fact that it is not possible to prepare a sample of inulin free from action on Fehling's solution (Drew and Haworth, J., 1928, 2670) are strong indications that the other ter-

[*Reprinted from the Journal of the Society of Chemical Industry, March* 10, 1933, *Vol. LII., No.* 10, "*Chemistry and Industry,*" *pp.* 221–222.]

V

THE STRUCTURE OF ASCORBIC ACID

SIR,—We have shown previously[1] that ascorbic acid (hexuronic acid) gives on oxidation oxalic acid and threonic acid. We have now completed our examination of the configuration of the latter acid and have proved it to be *l*-threonic acid, since on oxidation with nitric acid it gives *d*-tartaric acid. Ascorbic acid is therefore related in stereochemical configuration to *l*-sorbose.

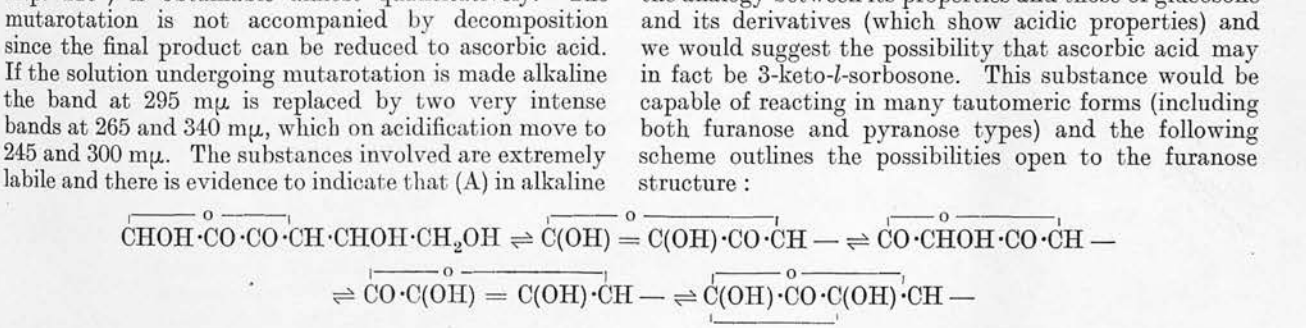
The same threonic acid is obtained by oxidizing with alkaline hypochlorite the highly characteristic product (A) produced from ascorbic acid by the action of aqueous iodine. These observations confirm the view that on reversible oxidation ascorbic acid is transformed into a substance which can react as the structure (I) indicated in our former communication.

(I) $COOH \cdot CO \cdot CO \cdot CHOH \cdot CHOH \cdot CH_2OH \leftarrow COOH \cdot CO \cdot C(OH) = CH \cdot CHOH \cdot CH_2OH$ (II)

Further investigation has revealed that the mode of formation and the properties of (A) are complex. At the moment of formation (A) does not exist as a free acid, but titrates like a lactone. In this condition it does not show selective absorption, has a high positive rotation in water and gives a cryst. phenylhydrazine derivative (red, m.p. 216°). When the aqueous solution of (A) is kept the rotation slowly falls and a moderately intense absorption band makes its appearance at 295 mμ. When equilibrium has been reached ($[\alpha]_D$—6°, approx.) a different phenylhydrazine derivative (yellow, m.p. 210°) is obtainable almost quantitatively. The mutarotation is not accompanied by decomposition since the final product can be reduced to ascorbic acid. If the solution undergoing mutarotation is made alkaline the band at 295 mμ is replaced by two very intense bands at 265 and 340 mμ, which on acidification move to 245 and 300 mμ. The substances involved are extremely labile and there is evidence to indicate that (A) in alkaline solution can undergo self-oxidation and reduction with partial regeneration of ascorbic acid (which has a band at 265 mμ in water, moving to 245 mμ in acid solutions).

The above phenylhydrazine derivatives have the same elementary composition and appear to be derived by the condensation of 2 mol. of phenylhydrazine with 1 mol. of a substance $C_6H_6O_6$. Ascorbic acid gives a cryst. di-(phenylhydrazine) derivative (dark red, m.p. 187°) which is not identical with either of the other two and is derived from $C_6H_8O_6$. It is probable therefore that the oxidation of ascorbic acid by aqueous iodine consists, in effect, of the removal of two hydrogen atoms, and the reversible nature of the change suggests a specially situated group of the type $>CHOH \rightleftharpoons >CO$. [Compare the views of Karrer,[2] who has given analytical evidence in favour of the formula $C_6H_6O_6$ for (A).] The oxidation product gives little furfural with HCl, in strong contrast with ascorbic acid and its methyl ethers.

Structure (I) which conceivably can lactonize and can react as a furanose and as a pyranose sugar and in a variety of enolic modifications remains the basis for interpreting the properties of ascorbic acid, the formula of which must be derived from one of the modifications of (I) by removal of an oxygen atom or by addition of two hydrogen atoms to the lactone. One method (Structure II) which accounted for the chemical and crystallographic data was put forward some time ago. Before suggesting (II) we had carefully considered structures of the furane and pyrane-carboxylic acid type (one of which has recently been advanced by Micheel and Kraft[3]) but we rejected them principally because they were incompatible with the crystallographic and *X*-ray observations. These observations still hold and moreover cyclic or open chain formulæ of this type are difficult to reconcile with the new facts. We consider therefore that attention must now be given to the possibility that ascorbic acid possesses a different type of structure, related to (I), but depending for its acidic properties on an activated –OH group and not on a true –COOH group. In studying ascorbic acid we have been impressed by the analogy between its properties and those of glucosone and its derivatives (which show acidic properties) and we would suggest the possibility that ascorbic acid may in fact be 3-keto-*l*-sorbosone. This substance would be capable of reacting in many tautomeric forms (including both furanose and pyranose types) and the following scheme outlines the possibilities open to the furanose structure :



Consideration of the whole of the available evidence has shown that the properties of ascorbic acid can be adequately interpreted on the basis of the above structure which, in addition, conforms with the stringent requirements of the crystallographic and *X*-ray measurements.[4]

Messrs. R. W. Herbert, E. G. V. Percival, R. J. W. Reynolds, and F. Smith are joint authors with me in this work.

I am, Sir, etc.,

E. L. HIRST

Birmingham University
March 4, 1933

REFERENCES

[1] Cox, Hirst and Reynolds, Nature, 1932, **130,** 888.
[2] Vierteljahrschrift d. Naturforschenden Ges. in Zürich, 1933, **78,** 9.
[3] Nature, 1933, **131,** 274.
[4] Cox, Nature, Aug. 6, 1932.

Harrison & Sons, Ltd., Printers, 44–47, St. Martin's Lane, W.C.2.

(*Reprinted from* NATURE, *Vol.* 131, *page* 617, *April* 29, 1933.)

VI

CONSTITUTION OF ASCORBIC ACID

BY

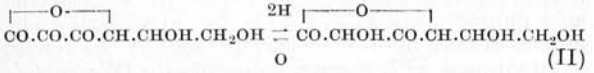
E. L. HIRST, E. G. V. PERCIVAL

AND

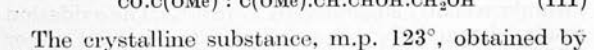
F. SMITH

Chemistry Department, University of Birmingham

WE have now confirmed the accuracy of the structure (I) COOH.CO.CO.CHOH.CHOH.CH_2OH which we had previously assigned to the first (reversible) oxidation product of ascorbic acid on the ground that it yields oxalic acid and trihydroxybutyric acid (*l*-threonic acid) on further oxidation[1]. The above formulation represents an open chain acid, but it is now evident that at the moment of formation the substance behaves as a lactone of (I) and not as the free acid. We have already advanced a constitutional formula for ascorbic acid (represented by (II) and its tautomerides) which shows the relationship between (I) and (II) to be as follows :[2]



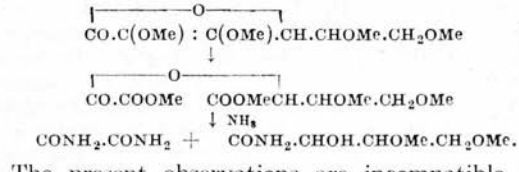
Strong evidence in favour of these views has been obtained from a study of the properties of the methylated derivatives of ascorbic acid. We find that dimethyl ascorbic acid (III), obtained by the action of diazomethane on ascorbic acid, is a neutral substance which reacts with one equivalent of warm $N/10$ alkali without elimination of methyl alcohol. The formation of the sodium salt appears to involve the opening up of the lactone ring in (III). Both methoxy groups are therefore enolic in character (contrast Karrer[3] and Micheel and Kraft[4]). Profound decomposition occurs when the dimethyl derivative is warmed with strong alkali. In this respect the tetramethyl derivative (see below) is much less stable and on continued heating with $N/10$ alkali it breaks down with elimination of at least three molecules of methyl alcohol.



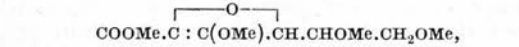
The crystalline substance, m.p. 123°, obtained by Micheel and Kraft[5] by the action of methyl alcoholic ammonia on dimethyl ascorbic acid is apparently formed in an analogous manner by the addition of ammonia to the lactone group of (III). The two

methoxyl groups of dimethyl ascorbic acid are retained in the product, which we find behaves as an amide. It contains one molecule of combined methyl alcohol and has an empirical formula $C_9H_{19}O_7N$ (not $C_8H_{15}O_6N$).

The nature of the ring system (1 : 4) present in dimethyl ascorbic acid has been determined by the degradative oxidation of tetramethyl ascorbic acid, obtained by the action of methyl iodide and silver oxide on the dimethyl derivative. On treatment with ozone the tetramethyl derivative gives rise to a neutral substance (V) which reacts immediately with ammonia giving quantitatively oxamide and 2-hydroxy 3 : 4-dimethoxybutyramide. Detailed examination of the hydroxy dimethoxybutyric acid has revealed that it consists of two isomerides, the main portion (80 per cent) being 3 : 4-dimethyl-threonic acid, which we have characterised by its conversion into 2 : 3 : 4-trimethyl *l*-threonic acid (amide, m.p. 77°, $[\alpha]_D$ + 66° in water). The remainder was found to be 3 : 4-dimethyl *l*-erythronic acid (amide, m.p. 113°, $[\alpha]_D$ — 34° in water). The amides of both hydroxy acids gave sodium isocyanate on treatment with sodium hypochlorite (Weerman's reaction) and are therefore α-hydroxy derivatives. The isolation of the two epimeric acids suggests that at some stage during the series of reactions enolisation has occurred at C_4. The observation is of special interest in that any possibility of such a change taking place in the plant or animal or during the process of isolating ascorbic acid has great significance from the biological point of view.



The present observations are incompatible with the furane carboxylic acid structure for ascorbic acid advocated by Micheel[4] (which we had previously rejected for crystallographic reasons) and with the formulæ recently suggested by Karrer[6]. The oxidation results demand for tetramethyl ascorbic acid either (IV) or the structure



but apart from its inherent improbability on account of the propylene oxide ring, the latter formula is unable to explain the non-acidic character of the newly-formed first oxidation product of ascorbic acid or the behaviour of the methylated derivatives towards alkali and

towards ammonia. On the other hand, all the observations offer strong support to (IV) for tetramethyl ascorbic acid and to (II) and its various tautomeric modifications for free ascorbic acid.

We wish to thank Prof. A. Szent-Györgyi for his kindness in placing at our disposal the ascorbic acid used in this work.

[1] NATURE, **130**, 888 ; 1932.
[2] *J.S.C.I.* (*Chemistry and Industry*), **52**, 221 ; 1933.
[3] *Helv. Chim. Acta*, **16**, 181 ; 1933.
[4] NATURE, **131**, 274, Feb. 25, 1933.
[5] *Z. physiol. Chem.*, **215**, 222; 1933.
[6] *Helv. Chim. Acta*, **16**, 302 1933.

Printed in Great Britain by FISHER, KNIGHT & Co., LTD., St. Albans

VII

THE CONSTITUTION OF ASCORBIC ACID

BY

R. W. HERBERT,

E. L. HIRST,

E. G. V. PERCIVAL,

R. J. W. REYNOLDS,

AND

F. SMITH

Reprinted from the Journal of the Chemical Society,
September, 1933.

Reprinted from the Journal of the Chemical Society, 1933.

299. *The Constitution of Ascorbic Acid.**

By R. W. Herbert, E. L. Hirst, E. G. V. Percival, R. J. W. Reynolds, and F. Smith.

Investigations into oxidation systems of biological interest led Szent-Györgyi (*Biochem. J.*, 1928, **22**, 1387) to the discovery of a crystalline substance, $C_6H_8O_6$, which, widely distributed in plants and animals, possesses chemical and physiological properties of outstanding importance. On account of its acidic character, strong reducing power, and colour reactions, which recalled those given by carbohydrates, the substance was named hexuronic acid, but the present work has demonstrated that it is not in reality a member of the uronic acid class. In view of the fact that all specimens which have been examined possess strong antiscorbutic properties (Svirbely and Szent-Györgyi, *Nature*, 1932, **129**, 576, 690 ; *Biochem. J.*, 1932, **26**, 865; 1933, **27**, 279; Birch, Harris, and Ray, *Nature*, 1933, **131**, 273; Tillmanns, Hirsch, and Vaubel, *Z. Unters. Lebensm.*, 1933, **65**, 145. See also Hirst and Zilva, *Biochem. J.*, 1933, in the press) the name has been changed to ascorbic acid (Haworth and Szent-Györgyi, *Nature*, 1933, **131**, 24). Much evidence has been accumulated concerning the relationship between this substance and the antiscorbutic factor (vitamin C) (for a summary of the evidence, see Szent-Györgyi, *Nature*, 1933, **131**, 225) and the view is held by many workers that ascorbic acid is vitamin C in a pure crystalline condition. The biological problem is, however, one of great complexity and it is

* Summaries of the work now presented have already been published as follows: The General Properties of Ascorbic Acid and Oxidation to Threonic Acid (with R. J. W. Reynolds; *Nature*, 1932, **129**, 576; **130**, 888). Absorption Spectrum of Ascorbic Acid (with R. W. Herbert; *ibid.*, 1932, **129**, 205). Configuration of Ascorbic Acid and Proposal of a Lactone Formula (*J. Soc. Chem. Ind.*, 1933, **52**, 221). Investigation of Methylated Derivatives of Ascorbic Acid (with E. G. V. Percival and F. Smith; *Nature*, 1933, **131**, 617).—E. L. Hirst.

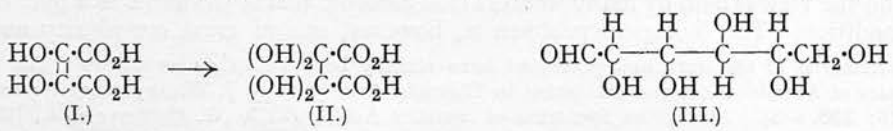
necessary to proceed cautiously before the claim that ascorbic acid is to be regarded as the only antiscorbutic agent can be considered as definitely established.

The present paper deals with the chemical constitution of ascorbic acid and describes work carried out with material derived both from animal (adrenal glands) and from plant (paprica) sources. We confirmed in the first place, using rigorously purified material, m. p. 192°, the early observations of Szent-Györgyi, who assigned the elementary formula $C_6H_8O_6$ (M.W. 176). We found that the substance behaved on titration as a weak organic acid, giving salts of the type $C_6H_7O_6M$. Ascorbic acid is not, therefore, a lactone of an acid $C_6H_{10}O_7$, as was formerly supposed, and on the other hand it cannot be lactonised. It does not exhibit mutarotation in aqueous solutions and the magnitude of the rotation, $[\alpha]_{5780}$ +24°, is not appreciably affected by the acidity of the solution. By contrast the salts display a high positive rotation (*ca.* + 100°) and the value varies greatly with the alkalinity, increasing to over 160° in 2*N*-alkali. These high rotations are not indicative of decomposition, since on acidification the value falls immediately to that of ascorbic acid.

Ascorbic acid is a powerful reducing agent. In cold neutral or acid solution it is attacked immediately by iodine, ozone, silver nitrate, copper acetate, and potassium permanganate. It reduces Fehling's solution vigorously in the cold, and in alkaline solution it is rapidly attacked by gaseous oxygen. Alkaline solutions of ascorbic acid are, however, relatively stable in an inert atmosphere and acidified solutions are only slightly affected by oxygen. The reducing properties are much less evident in non-aqueous media, iodine, for example, being entirely devoid of action upon alcoholic solutions of the acid. It does, however, react with permanganate in acetone. These properties and the ease of reaction with phenylhydrazine, which readily gives a red crystalline derivative, m. p. 187°, point to the presence of at least one carbonyl group capable of enolisation, and this conclusion is supported by the nature of the ultra-violet absorption spectrum, which resembles that given by many labile ketonic substances. The colour reactions with ferric chloride and sodium nitroprusside also indicate the presence of an enolic group, and the work of Karrer, Salomon, Schöpp, and Morf (*Vierteljahrsch., Naturforsch. Ges. Zurich*, 1933, **78**, 9; *Helv. Chim. Acta*, 1933, **16**, 181; *Biochem. Z.*, 1933, **258**, 4) lends further support to this view. Since no colour is given with Schiff's reagent, it is unlikely that a free aldehyde group is present. That five at least of the six carbon atoms are present as an unbranched chain follows from the observation that ascorbic acid yields furfuraldehyde quantitatively on treatment with boiling hydrochloric acid.

Insight into the structure of ascorbic acid was gained from a quantitative study of its behaviour towards oxidising agents. Two well-defined stages mark the course of the oxidation. When the substance is oxidised by iodine in acid solution, two atomic proportions of iodine are required, and two molecules of hydriodic acid are liberated during the reaction, which in effect consists in the addition of two hydroxyl groups to a double bond. The intervention of water is essential, alcoholic iodine being without action. The newly formed product does not display selective absorption and does not yield furfuraldehyde with hydrochloric acid. It is neutral in character and behaves towards water and towards alkalis as the lactone of a monobasic hydroxy-acid. No disintegration of the molecule takes place during this stage of the oxidation inasmuch as the product can be converted quantitatively into ascorbic acid by reducing agents such as hydrogen sulphide or hydriodic acid.

These observations show clearly that there is no free carboxyl group in ascorbic acid and that the acidic properties are due to the presence of an activated –CH·OH group situated next to a carbonyl group. The reactive group would be of the type –C(OH):C(OH)–,

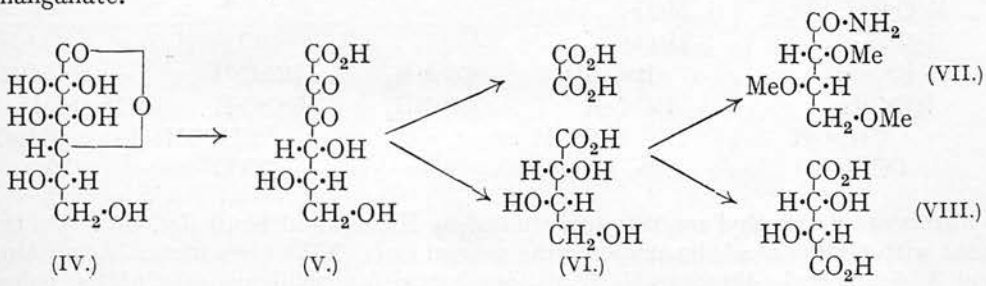


(I.) (II.) (III.)

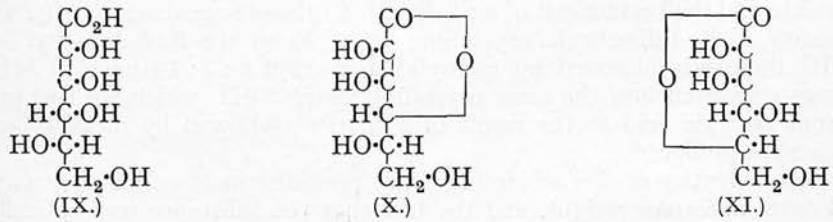
giving on oxidation in the presence of water $-C(OH)_2\cdot C(OH)_2-$. Such a system is present in dihydroxymaleic acid and it is of special interest to find that dihydroxymaleic acid (I)

reacts in acid solution with iodine (2 atoms), giving dihydroxytartaric acid (II). The product can be quantitatively reduced with hydriodic acid. During the oxidation the intense absorption band of dihydroxymaleic acid, which closely resembles that of ascorbic acid, disappears and the product does not show selective absorption. This analogy may be extended in that the two enolic groups in ascorbic acid and in dihydroxymaleic acid react with diazomethane, giving methylated derivatives whose absorption spectra stand in a similar close relationship with those of the parent substances. Since we advocated this type of linking for ascorbic acid in our preliminary note (Hirst, *J. Soc. Chem. Ind., loc. cit.*) the same kind of group has also been suggested by von Euler and Martius (*Arkiv Kemi Min. Geol.*, 1933, **11**, *B*, 1) from analogies with gluco-reductone [CHO·C(OH):CH·OH], which reacts with acid iodine, is acidic in character without possessing a carboxyl group, and shows a strong absorption band in the ultra-violet region.

The first oxidation product of ascorbic acid still possesses reducing power, especially in alkaline solution, and on treatment with alkaline sodium hypoiodite it takes up one atomic proportion of oxygen and is transformed quantitatively into oxalic acid and a trihydroxybutyric acid (VI). The latter substance was recognised in the form of the crystalline amide of its trimethyl derivative, which was proved to be trimethyl *l*-threonamide (VII). The identity of the trihydroxybutyric acid as *l*-threonic acid was further established by its conversion, on oxidation with nitric acid, into *d*-tartaric acid (VIII). The same product (*l*-threonic acid) was obtained when ascorbic acid was oxidised directly with acid permanganate.



These observations demonstrate that ascorbic acid is a derivative of *l*-gulose (III) and that its first oxidation product (which, as we shall show later, has structure IV) must be capable of reacting in the form of structure (V) (2 : 3-diketo-*l*-gulonic acid). The evidence given above indicates that the primary oxidation product is a lactone of (V), the carbonyl groups being in all probability hydrated. Ascorbic acid is the reduced form of this lactone and in view of its enolic character is to be represented as a lactone of the acid (IX) (3-keto-*l*-gulonolactone).

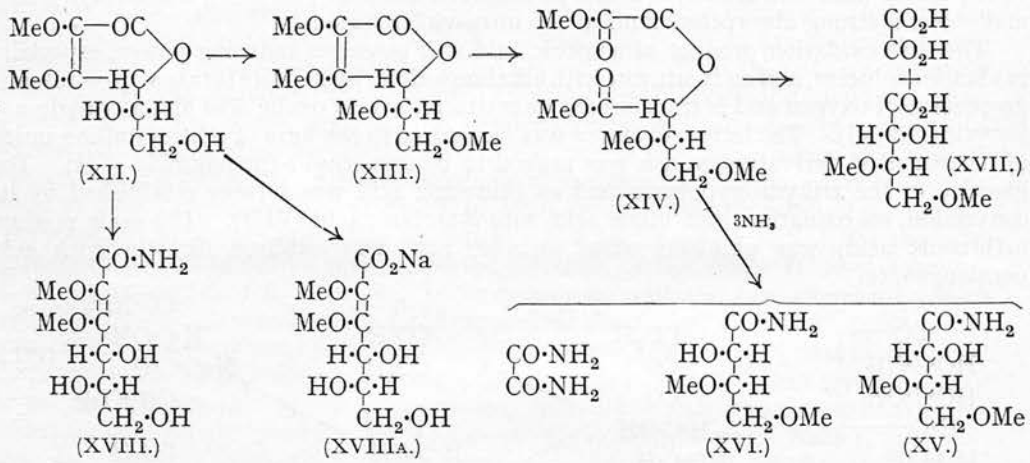


Of the various possible modes of lactonisation, (X) (furanose) and (XI) (pyranose) represent the two most probable types, but the evidence already outlined above does not permit a definite allocation of ring structure to be made, and in view of the unknown effect on ring formation of the active groups at C_2 or C_3 it is impossible to rule out at this stage the formation of a 1 : 6-lactone ring.

The nature of the ring system in ascorbic acid was determined by a study of the methylated derivatives of the acid. By this means complete confirmation was obtained of the accuracy of the views advanced above concerning the stereochemical configuration of the molecule and the nature of the reactive enolic groups. The results enabled a clear decision

to be made in favour of the furanose structure (X) for ascorbic acid, which may therefore be designated 3-keto-*l*-gulofuranolactone.

By the action of diazomethane on ascorbic acid a dimethyl derivative (XII) is readily obtained (Karrer, Salomon, Schöpp, and Morf, *loc. cit.*; Micheel and Kraft, *Z. physiol. Chem.*, 1933, **215**, 222). We have found that both the methoxyl groups so introduced are enolic in origin. In addition there are two other hydroxyl groups which can be methylated by Purdie's reagents, giving tetramethyl ascorbic acid (XIII). This substance reacts easily with ozone, two atoms of oxygen being added with formation of a neutral product (XIV) which we identified as methyl 3 : 4-dimethyl *l*-threonate substituted in position 2 by a methyl oxalate residue. This reaction proceeds similarly to the ozonisation of di-



p-nitrobenzoyl dimethyl ascorbic acid studied by Micheel and Kraft (*loc. cit.*). On treatment with methyl-alcoholic ammonia the neutral ester (XIV) gives immediately oxamide and 3 : 4-dimethyl *l*-threonamide (XV) together with a small quantity of the epimeric 3 : 4-dimethyl erythronamide (XVI). Both amides give rise to sodium *iso*cyanate with sodium hypochlorite (Weerman reaction) and must therefore possess a hydroxyl group in the α-position to the $-CO\cdot NH_2$ group. Reference to our earlier papers on the use of this test will suffice to show that it is of general application in the aliphatic group and quite diagnostic. The criticism of Micheel and Kraft (*J. physiol. Chem.*, 1933, **218**, 280) does not apply, since the only example they have quoted to the contrary is that of mandelamide, which is not a comparable case. Hydrolysis of (XIV) with barium hydroxide gave barium oxalate and the barium salt of 3 : 4-dimethyl *l*-threonic acid, again admixed with a small quantity of 3 : 4-dimethyl *l*-erythronic acid. From the 3 : 4-dimethyl *l*-threonic acid (XVII) there was obtained by methylation methyl 2 : 3 : 4-trimethyl *l*-threonate, and this was converted into the same crystalline amide (VII) which we had previously derived from ascorbic acid as the result of oxidation, followed by methylation of the *l*-threonic acid so produced.

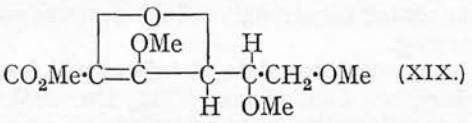
Proof of the identity of this amide (VII) was provided in the following ways. The analytical data, molecular weight, and the fact that the substance was optically active were sufficient to show that it was a 2 : 3 : 4-trimethoxy-*n*-butyramide. Four possibilities then arose, namely, the *d*- and *l*-forms of trimethyl erythronamide and the *d*- and *l*-forms of trimethyl threonamide. Now trimethyl *d*-erythronamide was already known (Avery, Haworth, and Hirst, J., 1927, 2308) and was definitely not identical with (VII). *X*-Ray examination confirmed that (VII) was different in structure from *d* (or *l*)-trimethyl erythronamide. The substance was therefore either *d*- or *l*-trimethyl threonamide, and conclusive proof that it was in fact the *l*-enantiomorph was provided by the observation that the unmethylated acid corresponding to (VII) gave *d*-tartaric acid on oxidation with nitric acid and must therefore be *l*-threonic acid. It is of interest to observe that the sign of the

rotation of (VII) is positive and that in consequence this substance follows the amide rotation rule.

The identity of the 3 : 4-dimethyl *l*-threonic acid was determined by the following considerations. On methylation it gave methyl trimethyl *l*-threonate, showing that the partly methylated derivative was 2 : 3-, 2 : 4-, or 3 : 4-dimethyl *l*-threonic acid. The first two of these were ruled out by the observation that the dimethyl ester yielded an amide which gave a positive Weerman reaction. The substance was therefore 3 : 4-dimethyl *l*-threonic acid, and this view is in agreement with the fact that methylation was accompanied by a large increase in dextrorotation.

The isomeric amide which was also isolated differed from 3 : 4-dimethyl *l*-threonamide and was not the enantiomorph of (XV). Since the analytical data, the optical activity, and positive Weerman reaction sufficed to establish its structure as 2-hydroxy-3 : 4-dimethoxy-*n*-butyramide, it followed that it must be either *d*- or *l*-3 : 4-dimethyl erythronamide. Since inversion of the groups attached to the penultimate carbon atom is most improbable, we designate it 3 : 4-dimethyl *l*-erythronamide. In agreement with this is the fact that the amide is lævorotatory in accordance with the amide rotation rule which appears to hold generally in this series.

The reaction between tetramethyl ascorbic acid (XIII) and ozone involved the addition of two oxygen atoms with formation of a neutral ester (XIV), and the breaking of the bond between the two carbon atoms which were united by a double linkage did not result in the formation of a substance containing a smaller number of carbon atoms. It follows, therefore, that a ring system was present in tetramethyl ascorbic acid and the nature of the reaction leaves open only two possibilities for the structure of (XIII), for which the alternative is the following (XIX) containing a propylene oxide ring. The latter is in-



(XIX.)

herently improbable owing to the strained nature of the ring and furthermore the properties of dimethyl ascorbic acid and the fact that the primary oxidation product of ascorbic acid is a lactone and not an acid provide decisive evidence in favour of (XIII).

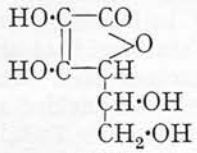
Dimethyl ascorbic acid is a neutral substance which reacts with one equivalent of sodium hydroxide, giving a sodium salt. This reaction was claimed by Karrer (*loc. cit.*) and by Micheel (*loc. cit.*) as proof that one of the methoxyl groups was esteric in character. A closer examination of the reaction has revealed that the sodium salt is formed without elimination of methyl alcohol and proceeds in the cold in a manner similar to the opening of a lactone ring. The formation of a sodium salt from (XIX) would necessarily involve ester hydrolysis and this structure may therefore be discarded.

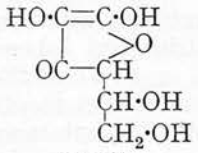
The origin of the small quantity of 3 : 4-dimethyl *l*-erythronic acid which accompanies the 3 : 4-dimethyl *l*-threonic acid is at present obscure. Its formation involves inversion of the groups attached to C_4 of the original ascorbic acid. This may have occurred during the reaction with diazomethane or during the subsequent methylation with silver oxide, or more probably during the treatment of the ozonised product with alkali, epimerisation of α-hydroxy-acids by alkali being of well-known and frequent occurrence. We have considered the possibility that the ascorbic acid used might have contained two isomerides, but have rejected it on the ground that no *i*-tartaric acid could be discovered in a rigorous search for traces of this material amongst the *d*-tartaric acid produced by direct oxidation of *l*-ascorbic acid. It follows that 3 : 4-dimethyl *l*-erythronic acid had been formed by an indirect route involving isomerisation and that *l*-ascorbic acid is configurationally related to *l*-gulose and *l*-sorbose.

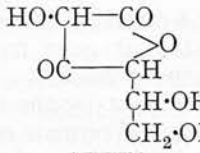
Tetramethyl ascorbic acid has therefore the structure represented by (XIII) and the form of ascorbic acid which reacted with diazomethane must be (XX). It is obvious that various tautomeric modifications of this structure are possible, for example (XXI), (XXII) and (XXIII), and the versatile character of ascorbic acid suggests that it can indeed react

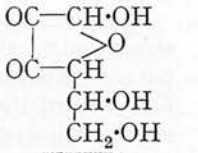
*

in more than one of these forms. Two of those illustrated (XXII) and (XXIII) (3-keto-*l*-sorbosone) should give rise to two stereochemical isomerides and, apart from the fact that


(XX.)

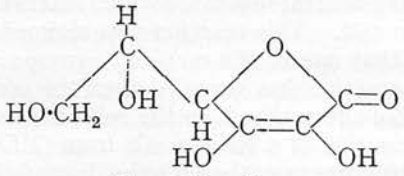

(XXI.)


(XXII.)


(XXIII.)

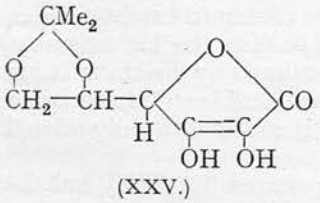
no such modifications of ascorbic acid have been observed, a structure of this type cannot be reconciled with the *X*-ray data of the crystalline material (Cox, *Nature*, 1932, **130**, 205), which demand an extraordinarily flat molecule. The very close similarity between the absorption spectra of ascorbic acid and its dimethyl derivative, taken in conjunction with the similar relationship existing in the dihydroxymaleic acid series, provides a strong indication that in solution, whether in acid, neutral or alkaline media, ascorbic acid is essentially in the condition represented by (XX) (enolic form of 3-keto-*l*-gulofurano-lactone, which, despite the views of Micheel and Kraft, *J. physiol. Chem.*, 1933, **218**, 280, is in no sense equivalent to the 2-keto-isomeride). It is surprising to find that a structure of this type should remain intact in the presence of alkali. The stability of the lactone ring in the free acid would appear to be connected in some way with the ionised condition of the hydroxyl group responsible for salt formation. If a non-ionised ether group replaces the ionised hydroxyl, the lactone ring appears to be very much less stable and opens readily in the cold under the influence of dilute alkali. A search for analogies has revealed that this behaviour is simulated by certain other substances and a detailed investigation, now in progress in these laboratories, of the very remarkable mannosaccharodilactone has indicated that a lactone containing strongly reducing groups may yield a sodium salt without opening of the lactone ring.

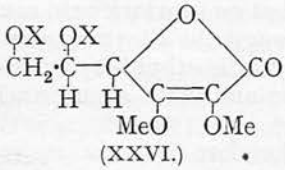
A critical discussion of the *X*-ray data, details of which will be published later by Mr. E. G. Cox (for summary, see Cox, *Nature*, 1932, **130**, 205), reveals that (XX) accounts satisfactorily for the crystallographic properties. Reference to the model, a diagrammatic representation of which is given in (XXIV), shows that of the total of 12 carbon and oxygen atoms all but one can be accommodated in one plane without appreciable valency strain, whilst the remaining carbon (C_5) lies less than 1 Å. above the plane.

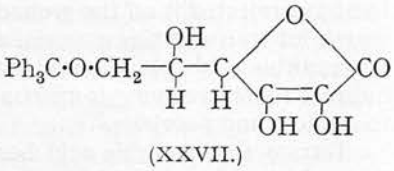

(XXIV.)

l-Ascorbic acid.

Structures may now be assigned to various derivatives of ascorbic acid. Vargha's monoacetone derivative will be represented by structure (XXV) (*Nature*, 1932, **130**, 847), which is transformed by diazomethane into dimethyl monoacetone ascorbic acid (Karrer, Salomon, Schöpp, and Morf, *loc. cit.*). This is in agreement with Karrer's observation that the acetone body retains unimpaired its enolic character. Micheel and Kraft's dimethyl


(XXV.)

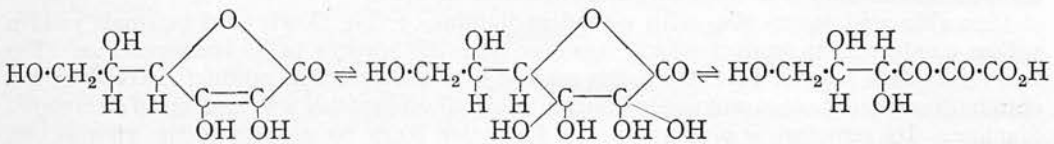

(XXVI.)


(XXVII.)

di-*p*-nitrobenzoyl derivative is (XXVI). The observation of the latter authors that (XXVI) gives oxalic acid and *l*-threonic acid on ozonisation, followed by hydrolysis, is

in exact agreement with the structural views now advocated, although a different explanation, rendered untenable by the present results, was adopted by them. The structure (XXIV) contains a primary alcoholic group on C_6 and so accounts for the formation of a triphenylmethyl derivative (XXVII) (Vargha, *Nature*, 1933, **131**, 363).

It is necessary to discuss in greater detail the properties of the first oxidation product of ascorbic acid. It has already been mentioned that when newly formed this substance is neutral in reaction and possesses no trace of the intense selective absorption characteristic of ascorbic acid. If, however, the aqueous solution of the oxidation product is kept at room temperature, the rotation gradually changes from $+ 56°$ to $- 6°$ in the course of 70 hours, a longer time being required for the attainment of equilibrium if the mineral acid (2 mols.) formed during the oxidation is neutralised. The fall in rotation is accompanied by a gradual development of a weak absorption band at λ 290 mμ, which attains its maximum intensity when the solution has reached equilibrium, in which condition at least 80% of the oxidation product is present as free acid. No disintegration of the molecule has occurred during these changes, since on reduction with hydriodic acid the " equilibrium " product gives rise to ascorbic acid in good yield. As will be seen later, however, with hydrogen sulphide the reduction proceeds only to the extent of about 10%. The freshly prepared oxidation product gives an orange derivative, m. p. 216°, with phenylhydrazine, the analytical data for which indicate that it is obtained by the condensation of a molecule $C_6H_6O_6$ with two molecules of the base. A different phenylhydrazine deriv-



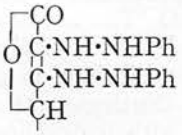
(XXVIII.)

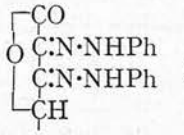
ative (yellow, m. p. 210°), which nevertheless has the same empirical formula, is obtained from the " equilibrium " solution and also from the oxidised product after opening of the lactone ring by salt formation. The rotation of the neutral sodium salt of the oxidation product is $[\alpha]_{5780} - 26°$. The value depends on the p_H of the solution and approaches $- 100°$ in *N*-alkali. These alkaline solutions are yellow and extraordinarily unstable in the presence of oxidising agents, including gaseous oxygen. Some decomposition takes place, even in an inert atmosphere, with formation of oxalic acid and the series of changes which takes place appears to be highly complex. For instance, slightly alkaline solutions of the oxidation product display relatively intense absorption bands at 265 mμ and 340 mμ, which, on acidification of the solution, move to 245 mμ and 300 mμ respectively. Only slight decomposition takes place under these conditions, since the re-acidified solution gives the yellow phenylhydrazine derivative in good yield. Iodine, however, is now taken up to an extent consonant with the idea that the band at 245 mμ is due to ascorbic acid. If this is indeed the case, it appears that amongst the changes which take place in alkaline solution there occurs to a small extent self-oxidation and reduction of the oxidation product with partial regeneration of ascorbic acid. A tentative explanation of these phenomena may be offered on the following lines. The primary oxidation product (XXVIII) would not show selective absorption, but if on opening of the lactone ring one (or both) of the hydrated keto-groups resumes its normal form the carbonyl band at 290 mμ would be expected to appear. It is readily understandable also that the lactone form would be more readily reduced to ascorbic acid than the open-chain form, since the latter, which must lactonise during the regeneration of ascorbic acid, can conceivably react both as a furanose and as a pyranose sugar. The facility with which hydriodic acid effects the lactonisation and reduction is probably due to the fact that the experiment is carried out by evaporating to dryness a solution of the oxidation product containing the requisite amount of reducing agent. The complex behaviour of the oxidation product towards alkalis is normal for reactive keto-hydroxy-compounds of this type.

The possibilities for structural isomerism and stereoisomerism amongst phenylhydrazine

derivatives of ascorbic acid and its reversible oxidation product are so numerous that the allocation of precise structures is a matter of extreme difficulty. The highly coloured nature of the ascorbic acid derivatives renders it doubtful whether a true osazone structure is present. The analytical data point to a condensation product derived from $C_6H_8O_6$ rather than from $C_6H_6O_6$, but with phenylhydrazine compounds it is difficult to discriminate by analysis between formulæ so closely related. On the basis of the formula $C_6H_8O_4(N{\cdot}NHPh)_2$ it is perhaps possible that the structure of the red derivative, m. p. 187°, from ascorbic acid is (XXIX). The orange derivative, m. p. 216°, obtained from the lactone form of the oxidised substance may be a true osazone (XXX). The yellow deriv-

(XXIX.)





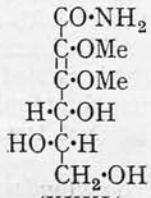
(XXX.)

ative, m. p. 210°, may also be an osazone and in this case condensation probably takes place with the free acid, followed by ring closure. In addition to alternative methods of ring closure, various tautomeric and stereoisomeric modifications are theoretically possible. The various derivatives with phenylhydrazine, *p*-nitrophenylhydrazine, *p*-bromophenylhydrazine, and 2 : 4-dinitrophenylhydrazine form well-defined series of characteristic compounds obtainable in most instances in excellent yield.

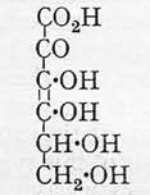
Ascorbic acid reacts also with *o*-tolylenediamine, giving slowly and in small yield a yellow condensation product which, however, does not appear to be homogeneous. The analytical data suggest that it consists mainly of a condensation product derived by the elimination of one molecule of water from one molecule of ascorbic acid and one of *o*-tolylenediamine. Its structure is obscure and its formation lends no support to the view at one time held that ascorbic acid contains two carbonyl groups.

Another derivative of special interest from the point of view of structure is the crystalline substance obtained by Micheel and Kraft by the action of methyl-alcoholic ammonia on dimethyl ascorbic acid. No loss of methyl groups occurs during the reaction, which involves the addition of ammonia. These authors denied the amidic character of the product, but we have found that it gives the usual amide reactions and on the basis of the above structure for dimethyl ascorbic acid its formation can be simply represented as the ordinary addition of ammonia to a lactone, giving a product of structure (XXXI). The reaction is analogous to the opening of the lactone ring by alkali and it is significant that precisely the same type of rotation change occurs during the transformation of dimethyl ascorbic acid into the sodium salt and into the amide. Furthermore, both products are devoid of selective absorption in the ultra-violet region, although dimethyl ascorbic acid has a strong band at λ 230 mμ. The disappearance of the band may be ascribed to various causes, but little stress can be laid on this point, since a slight movement, towards the ultra-violet, of the band at 230 mμ would place it in a region of wave-length (< 200 mμ) where observations are impossible.

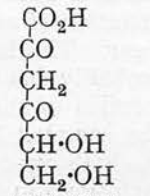
The evidence which has been cited above is inconsistent with the various alternative formulæ which have been suggested for ascorbic acid. The earliest of these (XXXII) was proposed as an attempt to derive a reduced form of the first oxidation product (V) which would satisfy both the chemical and the crystallographic data. It was discarded in favour of a ring structure (Hirst, *J. Soc. Chem. Ind.*, 1932, **52**, 221) as soon as it became evident that the newly formed first oxidation product was a lactone and not an acid.



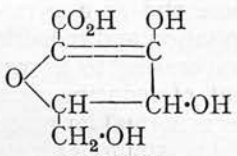
(XXXI.)



(XXXII.)



(XXXIII.)



(XXXIV.)

Formulæ of the type (XXXIII), including various tautomeric ring forms, were proposed by Karrer almost simultaneously with our suggestion of (XXXII). These also suffer from the same disability as regards the presence of a carboxyl group. Moreover, we find that acetylpyruvic acid is not oxidised by iodine in acid solution and on the other hand is very readily hydrolysed by alkali. Recently a synthesis of a substance closely allied to (XXXIII) has been achieved (Fischer and Baer, *Helv. Chim. Acta*, 1933, **16**, 534), but its properties do not favour in any way this type of structure for ascorbic acid. The structure (XXXIV), in which ascorbic acid is represented as a furancarboxylic acid, was later advocated by Micheel and Kraft (*Nature*, 1933, **131**, 274 and *loc. cit.*) and postulated as an alternative to (XXXIII) by Karrer. We had long been aware that this particular formulation served to explain many of the chemical properties of ascorbic acid, but we did not advance it because of its incompatibility with the crystallographic and *X*-ray requirements, which sufficed to exclude it (Cox, *loc. cit.*; Cox and Hirst, *Nature*, 1933, **131**, 402). The first oxidation product derived from a substance of this structure would have a free carboxyl group, the properties of its dimethyl derivative would be quite different from those observed, and ozonisation of the tetramethyl derivative would yield a product giving on hydrolysis oxalic acid and 2 : 4-dimethyl *l*-threonic acid instead of the 3 : 4-dimethyl *l*-threonic acid we actually found. On the other hand the facts reported in Micheel and Kraft's papers find a ready and natural interpretation (which has since been accepted by these authors, *Z. physiol. Chem.*, 1933, **218**, 280) in terms of the structure (XXIV) here advocated for ascorbic acid. It should be mentioned also that the synthetic experiments of Reichstein, Grüssner, and Oppenauer (*Helv. Chim. Acta*, 1933, **16**, 561) offer no support to the furancarboxylic acid structure for ascorbic acid (see Haworth, *J. Soc. Chem. Ind.*, 1933, **52**, 482), but on the contrary a synthesis of ascorbic acid by a similar procedure furnishes evidence of the accuracy of the constitution herein assigned (Haworth and Hirst, *ibid.*, p. 645).

Experimental.

Properties of Ascorbic Acid.—Three samples of ascorbic acid were examined: (a) Crude material from adrenal glands. Yellow powder, m. p. 170—175° after previous softening. $[\alpha]_{5780}$ + 23° in water. After recrystallisation from methyl alcohol–ether–light petroleum it had m. p. 190—191° (decomp.), $[\alpha]_{5780}$ + 24° in water. Spectrophotometric examination indicated that the crude material was very nearly pure (Found: C, 41·0; H, 4·7. Calc. for $C_6H_8O_6$: C, 40·9; H, 4·6%). (b) Material from adrenal glands, prepared at Mayo Clinic, Rochester, U.S.A. Cream-coloured powder, m. p. 182—184°. $[\alpha]_D$ + 24° in water (Found: C, 41·2; H, 4·8%). After recrystallisation this had m. p. 190—191°. (c) Material from paprica. White crystalline powder, m. p. 192°. $[\alpha]_{5780}$ + 24° in water (Found: C, 41·1; H, 4·6%). The properties of this material did not alter on recrystallisation from acetone, dioxan, alcohol, or methyl alcohol–ether–petroleum. The properties of recrystallised ascorbic acid from adrenal glands and from paprica were identical in all respects except that when heated the material of animal origin turned pink (on subsequent cooling the colour disappeared) whereas the paprica material remained colourless up to the m. p. The slight trace of impurity responsible for this could not be removed by crystallisation. The following experiments were mainly carried out with ascorbic acid from paprica, but were duplicated in many instances by similar experiments with material of animal origin. In no case was any difference in behaviour noticed. Pure ascorbic acid has m. p. 192° without previous darkening and rapidly decomposes with effervescence just above the m. p. When heated slowly, it begins to liberate carbon dioxide at 170° without darkening and without melting.

The mean of several analyses gave the figures C, 41·0; H, 4·7%. *M* 170 by *X*-ray analysis, 172 by titration with sodium hydroxide (calculated as monobasic acid), 176 by iodine titration. The substance contained no nitrogen, sulphur, or methoxyl. It reduced Fehling's solution in the cold. Neutral silver nitrate and neutral permanganate were reduced instantaneously. It decolorised aqueous bromine and iodine at once. Alcoholic iodine was not attacked, but it reduced potassium permanganate in acetone solution. It did not restore the colour to Schiff's reagent, nor did it give the naphtharesorcinol test for glycuronic acid. When heated with boiling 12% hydrochloric acid, it readily gave furfuraldehyde, which was estimated in the usual way as the phloroglucide (yield, 87% of the theoretical). It reacted vigorously with carbonates, giving salts. The salts gave with ferric chloride an intense violet colour (fleeting colour only

with the free acid). Alkaline solutions of ascorbic acid gave with sodium nitroprusside a deep blue colour, changing to green and then to red.

The *calcium* salt was prepared by adding a slight excess of calcium carbonate to an aqueous solution of ascorbic acid, filtering the solution, and evaporating it to dryness in a vacuum desiccator. On trituration with alcohol the neutral salt was obtained as a pale yellow powder. $[\alpha]_D^{19^\circ}$ + 91° in water (*c*, 0·3) [Found : Ca, 9·9. $(C_6H_7O_6)_2Ca$ requires Ca, 10·2%].

The *brucine* salt of ascorbic acid was prepared by warming at 70° for 5 minutes an aqueous solution of ascorbic acid with an alcoholic solution containing the calculated amount of brucine. On evaporation a syrup was obtained which soon crystallised. After recrystallisation from hot alcohol it was a cream-coloured crystalline powder, soluble in water and in hot alcohol. M. p. 216—217° (decomp.) (Found : C, 60·6; H, 6·6; OMe, 12·2; N, 5·0. $C_{29}H_{34}O_{10}N_2,C_2H_5{\cdot}OH$ requires C, 60·4; H, 6·5; OMe, 15·1; N, 4·6%).

Rotation of Ascorbic Acid and its Sodium Salt.—$[\alpha]_{5780}^{19^\circ}$ + 24° in water (*c*, 3·0), 25° (*c*, 0·5), 24° (*c*, 1·1) (contrast Karrer, von Euler, and Hellström, *Arkiv Kemi Min. Geol.*, 1933, **11**, *B*, No. 6). No mutarotation. Unless air-free water and hard-glass vessels were used, a gradual rise, followed by a fall in rotation, was observed. This was traced to partial salt formation caused by alkali dissolved from the glass, followed by oxidation. $[\alpha]_D^{17^\circ}$ + 22° in *N*/20-hydrochloric acid or *N*-sulphuric acid. No mutarotation. The sodium salt of ascorbic acid had $[\alpha]_{5780}^{18^\circ}$ + 116° in neutral aqueous solution (rotation calculated on concentration of ascorbic acid), + 130° in *N*/20-sodium hydroxide, + 149° in *N*/7-sodium hydroxide, 155° in *N*/2-sodium hydroxide, 161° in 2*N*-sodium hydroxide (constant for 1 hour; value after acidification + 21°) (all solutions made up in an atmosphere of nitrogen). The alkaline solutions were yellow, the acidified solution colourless. In acid solutions the rotation value remained almost unchanged for some hours when oxygen was passed through the solution; but rapid decomposition took place when oxygen was bubbled through alkaline solutions, oxalic acid being formed.

In 50% aqueous acetic acid ascorbic acid reacts rapidly (10—15 minutes) with ozone, giving the same product (" first oxidation product," $[\alpha]_{5780}$ + 56°) as that produced by oxidation with iodine or chlorine in acid solutions. The continued action of ozone results in decomposition with formation of oxalic acid.

Titration of Ascorbic Acid with Iodine and Chlorine.—0·1000 G. of ascorbic acid, dissolved in water, required 11·4 c.c. of neutral *N*/10-iodine to complete the first stage of the oxidation (calc., 11·4 c.c. for 2 atomic proportions of iodine). After this no more iodine was taken up. The solution, which showed $[\alpha]_{5780}$ + 56° (calc. on concentration of ascorbic acid), was then strongly acid and required, when titrated immediately, 12 c.c. of *N*/10-sodium hydroxide to restore neutrality. Since 2 mols. of hydrogen iodide are liberated during the oxidation (see below), 11·4 c.c. of *N*/10-alkali were required for the hydriodic acid and 0·6 c.c. for the organic acid. The end-point was indefinite and the titration proceeded as for a lactone until in all 17 c.c. of alkali were added (calc. for 2 mols. HI + 1 mol. of a monobasic organic acid, 17·0 c.c.). The end-point was now sharp and any further addition of alkali produced a yellow colour. No oxalic acid was formed during this stage of the oxidation. At the neutral point the rotation was $[\alpha]_{5780}$ — 26°. The oxidation was continued with iodine in alkaline solution. An excess of iodine and sodium hydroxide was added and the solution was kept for 15 minutes at room temperature. An excess of acid was then added, and the remaining iodine titrated with thiosulphate. The iodine used in the second stage of the oxidation was 11·6 c.c. (calc., 11·4 c.c. for 2 atomic proportions). Titration of the excess of acid showed that for the second stage of the oxidation 22·8 c.c. of *N*/10-alkali were required. Now the 11·6 c.c. of iodine utilised as an oxidising agent account for 11·6 c.c. of alkali, leaving 11·2 c.c. of alkali utilised in neutralising the carboxyl groups produced during the oxidation (calc. for two CO_2H groups, 11·4 c.c.). These two groups are additional to the one present as a lactone in the newly formed first oxidation product. At the end of the titration the amount of oxalic acid present in solution was estimated in the usual way as calcium oxalate (yield, 95% of the theoretical quantity for 1 mol. of oxalic acid). Exhaustive blank experiments at each stage of the above procedure proved that there were no interfering factors. The above results are in exact accord with the view that the first oxidation product is a 2 : 3-diketohexonic acid which is oxidised to oxalic acid and trihydroxybutyric acid. The isolation and characterisation of the latter substance are described below. Precisely similar results were obtained when chlorine water, followed by alkaline hypochlorite, was used as oxidising agent.

In another experiment the hydriodic acid formed during the titration with iodine was eliminated by carrying out the titration in the presence of an excess of calcium carbonate (0·100 g. of substance required 11·4 c.c. of *N*/10-iodine. Calc., 11·4 c.c.). We established also that the

titration may be carried out in the presence of an excess of citric acid without alteration of the end-point. An estimation was also made of the quantity of hydriodic acid liberated during the titration. Ascorbic acid (0·0737 g.) was dissolved in water and titrated with an alcoholic solution of iodine (8·0 c.c. of 0·1022 *N*-iodine. Calc., 8·2 c.c.). The presence of water is essential. A slight excess of aqueous silver nitrate was then added to the cold solution. It was easy to filter off the precipitated silver iodide before any interaction took place between the oxidation product and the silver nitrate (Found : 0·2016 g. AgI. Calc. for 2 mols. HI : AgI, 0·1920 g.).

When aqueous solutions of ascorbic acid were titrated with potassium permanganate the reagent was decolorised almost instantaneously until the equivalent of about 1·3 atoms of oxygen had been added. A slower reaction followed, which ended somewhat indefinitely when permanganate equivalent to 2O had been used up. Thereafter the reaction proceeded still more slowly without definite end-point. The arrest at the point corresponding to 1·2O is caused by slight overlapping of the first and the second stage of the oxidation, the primary oxidation product being readily attacked by permanganate. Oxalic acid and carbon dioxide were detected as oxidation products.

FIG. 1.

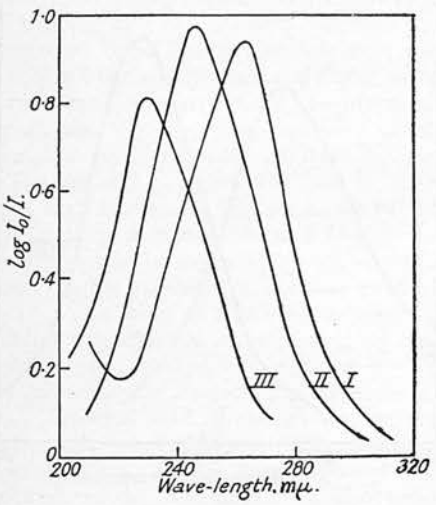


FIG. 2.

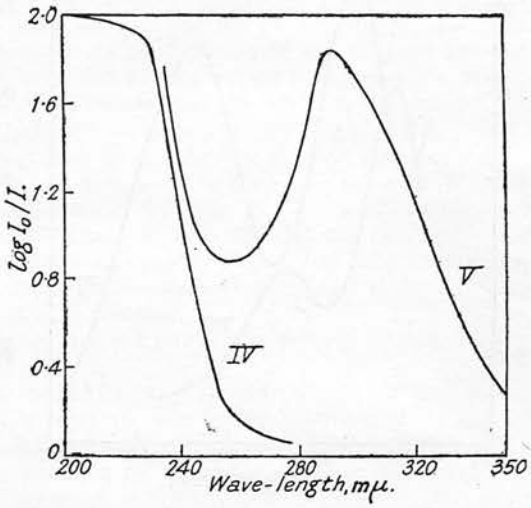


FIG. 1.—I. *Ascorbic acid or its sodium salt in water* (2 *mg. per* 100 *c.c.*); $l = 1$ *cm.* II. *Ascorbic acid in alcohol or acid aqueous solution* (2 *mg. per* 100 *c.c.*); $= 1$ *cm.* III. *Dimethyl ascorbic acid in alcohol* (2·5 *mg. per* 100 *c.c.*); $l = 1$ *cm.*

FIG. 2.—IV. *Oxidation solution of ascorbic acid immediately after preparation* (330 *mg. per* 100 *c.c.*); $l = 1$ *cm.* V. *Oxidation solution of ascorbic acid at equilibrium* (330 *mg. per* 100 *c.c.*); $l = 1$ *cm.*

Absorption Spectrum of Ascorbic Acid.—The observations were obtained by use of a Hilger sector photometer and a Hilger quartz spectrograph. For most of the work cells of 1 cm. length were employed. In aqueous solution ascorbic acid is characterised by a single very intense band with its head at 260—265 mμ. The molecular extinction coefficient is approximately 7000 for solutions containing about 2 mg. per 100 c.c. In stronger solutions (*ca.* 50 mg. per 100 c.c.) wide deviations from Beer's law are encountered. But for concentrations ranging between 0·5 and 2·5 mg. per 100 c.c. Beer's law holds with sufficient exactitude to permit of the use of spectrophotometric measurements for quantitative estimations of concentration. The intensity of the band diminishes rapidly, falling to half value in a few hours (decomposition of ascorbic acid by oxidation).

The band is much more persistent in acidified aqueous solutions ($p_H < 3$). The head is then at λ 245 mμ (ε approx. 7500 for *c* 0·002%). In ethyl alcohol it occurs at λ 245 mμ (ε approx. 7500, for *c* 0·002%). In methyl alcohol the head of the band is at λ 263 mμ (ε 7500 for *c* 0·002%). In this solvent concentrated solutions show wide divergences from Beer's law; *e.g.*, at λ 280 mμ ε is 800 for solutions with *c* 0·02%, 2000 for *c* 0·005%, and 4400 for *c* 0·002%. The sodium salt of ascorbic acid shows in aqueous solution a band at λ 265 mμ, the character of which, including intensity, is exactly similar to that of ascorbic acid in water.

These absorption bands closely resemble those shown by acetylpyruvic acid ($\varepsilon^{c.\ 2\ mg.\ per\ 100\ c.c.}_{\lambda\ 285}$

4000 in water) and dihydroxymaleic acid ($\varepsilon^{c.\ 2\ mg.\ per\ 100\ c.c.}_{\lambda\ 290}$ 6700 in water). Similar but weaker bands are shown by lævulic acid in water ($\varepsilon^{c.\ 0{\cdot}6\ g.\ per\ 100\ c.c.}_{\lambda\ 263}$ 24), glucosone in alkali ($\varepsilon^{c.\ 134\ mg.\ per\ 100\ c.c.}_{\lambda\ 310}$ 13), and acetone in alcohol ($\varepsilon^{c.\ 0{\cdot}1\ g.\ per\ 100\ c.c.}_{\lambda\ 265}$ 15). Selective absorption is not shown by ordinary sugars and sugar derivatives, either of the pyranose or the furanose type, neither is it displayed by hexuronic acids, *e.g.*, galacturonic acid, glycuronic acid, nor by 2-ketogluconic acid. Such substances are highly transparent in solution and display only weak continuous absorption in the ultra-violet, with ε less than 5 at λ 260 mμ. Details of these observations will be given in a separate communication.

Properties of the First Oxidation Product of Ascorbic Acid.—The newly formed first oxidation product has $[\alpha]_{5780}$ + 56°. This value diminishes when the solution is kept. $[\alpha]_{5780}$ + 46° (2 hrs.); 38° (4 hrs.); 30° (6 hrs.); 25° (8 hrs.); 21° (10 hrs.); 6° (20 hrs.); ± 0° (28 hrs.); − 3° (40 hrs.); − 6° (70 hrs., constant value). These observations were made in the presence of the mineral acid formed during the oxidation. When this mineral acid was exactly neutralised, the mutarotation followed a similar course but at a much slower speed. $[\alpha]_{5780}$ + 56°

FIG. 3.

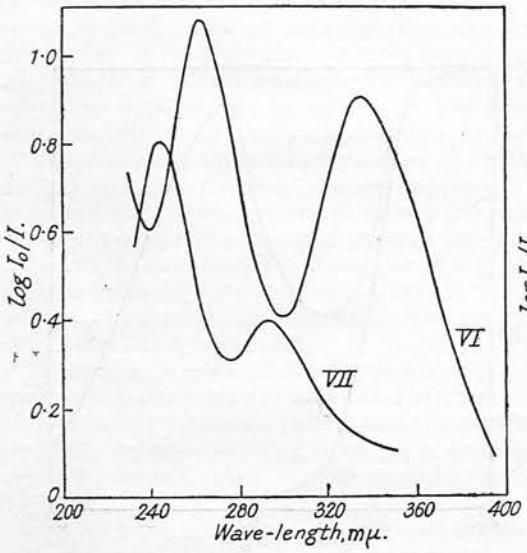


FIG. 4.

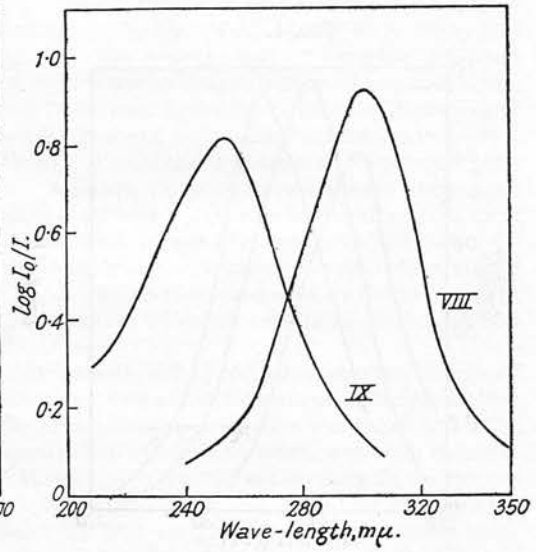


FIG. 3.—VI. *Oxidation solution of ascorbic acid made alkaline* (23·3 *mg. per* 100 *c.c.*); l = 1 *cm.* VII. *Oxidation solution of ascorbic acid made alkaline and immediately acidified* (14 *mg. per* 100 *c.c.*); l = 1 *cm.*

FIG. 4.—VIII. *Dihydroxymaleic acid in alcohol* (1·8 *mg. per* 100 *c.c.*); l = 1 *cm.* IX. *Methyl dimethoxymaleate in alcohol* (2·7 *mg. per* 100 *c.c.*); l = 1 *cm.*

(initial value); 44° (10 hrs.); 38° (20 hrs.); 32° (30 hrs.); 27° (40 hrs.); 19° (60 hrs.); 13° (80 hrs.); 8° (100 hrs.); ± 0° (140 hrs.); − 4° (170 hrs.); − 7° (200 hrs.); − 9° (250 hrs.); − 12° (300 hrs., constant value). The equilibrium solutions can be preserved indefinitely without change in properties. Simultaneously with the decrease in rotation there occurs an increase in the acidity of the solution. At the equilibrium position titration with alkali shows that about 85% of the organic material exists as free acid and only about 15% as lactone. For 0·1000 g. of ascorbic acid a total of 5·6 c.c. of N/10-alkali was required for the organic acid, and of this 4·8 c.c. were used immediately (free acid) and the remainder slowly (lactone). When the titration is carried out quickly on a freshly oxidised solution, only a negligible proportion of the organic substance is titrated as free acid. The equilibrium solution contains no oxalic acid.

The rotation of the sodium salt of the oxidation product depends markedly on the p_H value of the solution. For example, when 1·5 c.c. of N/10-sodium hydroxide were added to a neutral solution (5 c.c.) of the oxidation product from 0·017 g. of ascorbic acid the rotation changed immediately from − 26° to − 100°. This value is probably not the maximum, since rapid mutarotation was observed. $[\alpha]_{5780}$ − 93° (1 min. after addition); − 69° (5 mins.); − 56° (7 mins.); − 46° (10 mins.); − 41° (12 mins.); − 36° (14 mins.); − 32° (16 mins.); − 29° (18 mins.); − 26° (20 mins.); − 24° (26 mins., constant for a few minutes). At this stage no oxalic acid was present in the solution. After acidification the rotation was − 22° and remained

constant indefinitely. When this solution, the alkaline solution with $[\alpha]_{5780}$ — 100°, either of the equilibrium solutions mentioned above ($[\alpha]_{5780}$ — 6° and — 12° respectively), or the neutral sodium salt of the oxidised substance was treated with phenylhydrazine in acetic acid, in every case the same yellow phenylhydrazine derivative, m. p. 210°, was obtained in good yield (see below for details concerning phenylhydrazine derivatives). An entirely different orange phenylhydrazine derivative, m. p. 216°, is given by the newly formed oxidised ascorbic acid.

Alkaline solutions of oxidised ascorbic acid decompose in the presence of oxygen with formation of oxalic acid and other products. For this reason the observations recorded above for alkaline solutions were carried out in an inert atmosphere, but slow decomposition of the oxidation product, again with formation of oxalic acid, took place even under these conditions.

Solutions of oxidised ascorbic acid which had been made slightly alkaline and then acidified always took up iodine corresponding to about 5% of the total quantity of ascorbic acid submitted to oxidation.

Corresponding with these changes there occur changes in the absorption spectrum of the oxidised ascorbic acid. On account of the transparency of chlorides in solution all spectrophotometric work on the oxidised substance was carried out after oxidation by chlorine. The newly formed oxidation product displays no selective absorption and is remarkably transparent in the ultra-violet region even when examined in concentrated solution, general absorption commencing at λ 240 mμ with solutions containing 300 mg. per 100 c.c. (l, 1 cm.). No trace of the intense band due to ascorbic acid is present. As the solution of the oxidised substance approaches the equilibrium condition, a weak band at λ 290 mμ makes its appearance, reaching its maximum intensity when the mutarotation has ceased and the solution has reached equilibrium (see Fig. 2). The condition of the oxidised substance can be judged equally well by absorption measurements, rotation measurements or by titration of the free acid present.

The slightly alkaline solutions of the oxidised substance display two moderately intense bands with heads at λ 265 mμ and λ 340 mμ respectively (see Fig. 3). On acidification of the solution these bands move to λ 245 mμ and λ 295 mμ. A band at λ 245 mμ having the same intensity would be given by an amount of ascorbic acid equal to 5% of that submitted to oxidation. In the alkaline solution the band at λ 265 mμ is fleeting and disappears as decomposition sets in. The band at λ 245 mμ is persistent in acid solution.

After oxidation of ascorbic acid by chlorine water the product gave only a trace of furfuraldehyde when heated with 12% hydrochloric acid.

Regeneration of Ascorbic Acid from the Oxidised Substance.—(a) From freshly oxidised ascorbic acid: Ascorbic acid (0·176 g.) was oxidised in aqueous solution by addition of an alcoholic solution of iodine (0·254 g.). The colourless solution was then evaporated to dryness in a vacuum at room temperature. Separation of iodine commenced when the solution had evaporated to a thick syrup. The solid mixture of crystalline ascorbic acid and iodine was placed in a current of air. The iodine soon disappeared by sublimation, leaving ascorbic acid as a cream-coloured powder identical in all respects with an authentic sample (yield, quantitative). The crude material before recrystallisation had m. p. 180° and iodine titration and spectrophotometric examination showed that it was at least 90% pure.

With freshly oxidised material the reduction may be carried out also by hydrogen sulphide. In this case it is possible to follow the reaction spectrophotometrically. Ascorbic acid was oxidised by the calculated quantity of chlorine water. A photometric test showed that no trace of the ascorbic acid band remained. Hydrogen sulphide was then passed through the solution for 15 minutes. The dissolved sulphide was removed by addition of the minimum quantity of lead acetate, and the lead removed by addition of a little oxalic acid. A portion of the solution appropriately diluted showed the ascorbic acid band at λ 245 mμ. The yield of regenerated ascorbic acid calculated from the spectrophotometric observations was 90—95% of the theoretical. The iodometric titration value was in exact agreement with this.

(b) From the equilibrium solution of the oxidised product: In this case hydrogen sulphide was ineffective. The maximum yield of regenerated product, when the experiment was carried out as previously described, was 6%. The reduction may still be carried out by hydriodic acid. The procedure was exactly as given above except that the solution containing the oxidised product was allowed to reach equilibrium before the evaporation was commenced. An alcoholic iodine solution was used as oxidising agent and the solution finally obtained contained 15% of alcohol by volume ($[\alpha]_D$ + 60°). The presence of the alcohol greatly retarded the mutarotation, equilibrium ($[\alpha]_D$ — 6°) being reached in about 200 hours. Reduction of the oxidation product and separation of iodine took place as before, with the exception that in this case some brown amorphous by-product accompanied the regenerated ascorbic acid. The yield

of the latter judged by (a) iodometric titration, (b) spectrophotometric examination, (c) quantitative *X*-ray examination—all carried out on the crude product—was 75—80%. One recrystallisation from acetone gave pure ascorbic acid.

Phenylhydrazine Derivatives.—I. *Derivatives obtained from ascorbic acid.* (a) With phenylhydrazine : An aqueous solution of ascorbic acid was heated for 30 minutes at 90° with phenylhydrazine (3 mols.) until the solution became red. The water-bath was then allowed to cool slowly. The *product* separated as deep red needles, which when washed with dilute acetic acid, water, and dried in a vacuum had m. p. 187° (decomp.). It was insoluble in water, moderately easily soluble in dilute aqueous sodium hydroxide, giving an orange solution from which the original osazone was precipitated on acidification (Found : C, 60·8; H, 5·6; N, 15·7. $C_{18}H_{20}O_4N_4$ requires C, 60·7; H, 5·6; N, 15·7%. $C_{18}H_{18}O_4N_4$ requires C, 61·0; H, 5·1; N, 15·8%).

If the red solution mentioned above is cooled rapidly, the product obtained is mainly amorphous, m. p. 204° (decomp.), but has the same elementary composition (Found : C, 60·8; H, 5·6; N, 15·7%).

(b) With *p*-nitrophenylhydrazine : The procedure was the same as in (a) and the crystalline *product* obtained was bright red, m. p. 259° (decomp.). M. p. 262° (decomp.) after recrystallisation from alcohol (Found : C, 48·3; H, 4·2; N, 18·8. $C_{18}H_{18}O_8N_6$ requires C, 48·4; H, 4·0; N, 18·8%. $C_{18}H_{16}O_8N_6$ requires C, 48·6; H, 3·6; N, 18·9%).

(c) With *p*-bromophenylhydrazine : The crystalline compound obtained was dark red, m. p. 170° (decomp.) (Found : C, 42·3; H, 3·4; N, 10·4. Calc. for $C_{18}H_{18}O_4N_4Br_2$: C, 42·05; H, 3·5; N, 10·9%. Calc. for $C_{18}H_{16}O_4N_4Br_2$: C, 42·2; H, 3·1; N, 10·9%). This substance was also obtained as a monohydrate.

(d) With 2 : 4-dinitrophenylhydrazine : A methyl-alcoholic solution of ascorbic acid was boiled for 3 hours with a slight excess of 2 : 4-dinitrophenylhydrazine. When the dark red solution was cooled, brownish-red needles separated, m. p. 282° (decomp.) (Found : C, 40·45; H, 2·8; N, 21·4. Calc. for $C_{18}H_{16}O_{12}N_8$: C, 40·3; H, 3·0; N, 20·9%. Calc. for $C_{18}H_{14}O_{12}N_8$: C, 40·4; H, 2·6; N, 21·0%).

II. *Derivatives obtained from oxidised ascorbic acid.* (a) With phenylhydrazine : (i) Ascorbic acid was dissolved in water and oxidised with *N*/10-iodine. The solution was made slightly alkaline with *N*/10-sodium hydroxide and then immediately acidified with acetic acid. It was warmed for 15 minutes at 70° with a slight excess of phenylhydrazine in acetic acid. An orange-yellow precipitate separated. This was washed with dilute acetic acid and water and dried in a vacuum. M. p. 187° (decomp.). After recrystallisation from absolute alcohol it was obtained as yellow needles, m. p. 210° (decomp.) (Found : C, 60·9; H, 5·4; N, 15·6%).

(ii) Ascorbic acid was oxidised with iodine in aqueous solution. A slight excess of phenylhydrazine in acetic acid was added immediately. The solution was warmed for 15 minutes at 70°. A light red compound separated which after recrystallisation from alcohol gave orange silky needles, m. p. 216° (decomp.) (Found : C, 60·8; H, 5·1; N, 15·7%).

(b) With *p*-nitrophenylhydrazine : (i) An oxidised solution of ascorbic acid was used which had been rendered slightly alkaline and then immediately acidified with acetic acid. The product obtained was light red, m. p. 255° (decomp.). Recrystallisation from alcohol–acetone gave light red needles, m. p. 260° (decomp.) (Found : C, 48·5; H, 4·4; N, 18·8%).

(ii) A solution of freshly oxidised ascorbic acid was used. The mineral acid was not removed. The product on recrystallisation from alcohol formed yellow needles, m. p. 246° (decomp.) (Found : C, 48·2; H, 3·9; N, 17·8%).

(c) With *p*-bromophenylhydrazine : (i) The compound obtained after neutralisation of the oxidised ascorbic acid was yellowish-red, darkened at 160°, and melted at 220° (decomp.) (Found : C, 40·6; H, 3·7; N, 10·5. Calc. for $C_{18}H_{16}O_4N_4Br_2,H_2O$: C, 40·7; H, 3·4; N, 10·6%).

(ii) When freshly oxidised ascorbic acid was used, a bright red compound was isolated which after recrystallisation from alcohol gave yellow needles, m. p. 208° (decomp.) (Found : C, 42·3; H, 3·5; N, 8·15%). Repetition of the nitrogen estimation gave N, 8·1%. This substance and also the corresponding derivative with *p*-nitrophenylhydrazine gave values for the nitrogen content which are abnormal when compared with those given by the other derivatives.

(d) With 2 : 4-dinitrophenylhydrazine : (i) An aqueous solution of ascorbic acid was oxidised with iodine. The solution was made slightly alkaline with sodium hydroxide and immediately acidified with hydrochloric acid. A slight excess of 2 : 4-dinitrophenylhydrazine in 2*N*-hydrochloric acid was added, and the solution warmed for 30 minutes at 70°. A light red compound was obtained, m. p. 268° (decomp.) (Found : C, 40·4; H, 2·95; N, 20·7%).

(ii) Ascorbic acid was oxidised with iodine in aqueous solution, and the product treated immediately with 2 : 4-dinitrophenylhydrazine in 2*N*-hydrochloric acid. The mixture was warmed for 30 minutes at 70°. The product, recrystallised from alcohol–acetone, had m. p. 280° (decomp.) (Found : C, 40·95; H, 2·7; N, 20·4%).

The above derivatives can be obtained with equal facility after oxidation of ascorbic acid by chlorine water.

o-Tolylenediamine and Ascorbic Acid.—Ascorbic acid was treated with *o*-tolylenediamine (2 mols.) in aqueous solution. The mixture was warmed at 40° for 10 minutes and then kept at room temperature. After 4 days a yellow precipitate formed. This separated from aqueous solution as a yellow amorphous powder which after recrystallisation from aqueous alcohol was obtained as pale yellow needles, darkening at 110°, m. p. 115° (decomp.) (Found : C, 59·4; H, 6·2; N, 12·7%).

Oxidation of Ascorbic Acid by Potassium Permanganate. Isolation of Methyl Trimethyl Threonate.—I. *Oxidation.* Ascorbic acid (6 g.) (from paprica) was dissolved in water (60 c.c.). 5*N*-Sulphuric acid (30 c.c.) was added, and a normal aqueous solution of potassium permanganate slowly added at room temperature. The oxidation was instantaneous until the equivalent of about one and half atoms of oxygen had been added, after which the reaction proceeded more slowly. More sulphuric acid was now added and oxidation continued. After the addition of the equivalent of two atoms of oxygen the reaction was slow and evolution of carbon dioxide took place as the permanganate solution was added. Some manganese dioxide was deposited, but disappeared on shaking, the solution becoming colourless. In all 205 c.c. of *N*-potassium permanganate (equivalent to 3 atoms of oxygen) and 40 c.c. of 5*N*-sulphuric acid were used.

The solution was now left over-night, the clear liquid made alkaline by addition of concentrated aqueous potassium hydroxide, and the precipitated manganese hydroxide filtered off after treatment with charcoal. The brown liquid was concentrated in a vacuum at 40° to 30 c.c., and the potassium sulphate which crystallised was filtered off. The alkaline solution was now non-reducing.

II. *Methylation of the product.* After the addition of a little acetone this solution was methylated in the usual manner by 50% aqueous potassium hydroxide (95 c.c.) and methyl sulphate (57 c.c.). The solution was cooled over-night and, after removal of the precipitated potassium sulphate, acidified with hydrochloric acid. After concentration and filtration methyl alcohol was added, and the solution concentrated under diminished pressure at 40°. This operation was repeated several times, the precipitated inorganic salts being filtered off from time to time. Finally a yellow syrup containing much inorganic material was obtained.

The syrup was boiled for 4 hours with 3% methyl-alcoholic hydrogen chloride and neutralised (silver carbonate). On evaporation a yellow syrup contaminated with inorganic matter was obtained. Extraction with warm chloroform and evaporation of the solvent gave a mobile syrup (3·0 g.), $n_D^{15°}$ 1·4460. On fractional distillation this gave (a) 0·64 g., b. p. 125°/25 mm. (bath temp.), $n_D^{15°}$ 1·4332, $[\alpha]_{5780}^{18°}$ + 25° in methyl alcohol (*c*, 3·6) (Found : C, 47·3; H, 7·9; CO_2Me, 35%); (b) 0·87 g., b. p. 140—145°/25 mm. (bath temp.), $n_D^{15°}$ 1·4386. The remainder (1·4 g.) was incompletely methylated and did not distil. This distillation results in loss of yield, but is necessary to remove all inorganic matter from the partly methylated ester, which requires further methylation. Previous experience had shown us that the above sequence of operations results in the formation of potassium methyl sulphate during the esterification and if this is not removed serious complications are encountered later on owing to its transformation into methyl sulphate. Fractions (a) and (b) were combined and remethylated with methyl iodide and silver oxide. The product gave on distillation *methyl trimethyl* l-*threonate* as a colourless mobile liquid (1·11 g.), b. p. 120°/13 mm. (bath temp.), $n_D^{15°}$ 1·4275, $[\alpha]_{5780}^{18°}$ + 49° in methyl alcohol (*c*, 2·9), + 31° in water (*c*, 1·3), $d^{15°}$ 1·090 (Found : C, 49·9; H, 8·4; OMe, 63·4; CO_2Me, 32·4. $C_8H_{16}O_5$ requires C, 50·0; H, 8·3; OMe, 64·6; CO_2Me, 30·8%).

Methyl trimethyl *l*-threonate (0·1 g.) was dissolved in dry methyl alcohol (3 c.c.) saturated with ammonia at 0°. After 24 hours at 15° the solvent was evaporated in a desiccator, leaving a colourless syrup which soon crystallised. Recrystallisation from light petroleum (b. p. 40—60°) gave *trimethyl* l-*threonamide* as shining colourless hexagonal plates (yield, almost quantitative), m. p. 78°. $[\alpha]_{5780}^{20°}$ + 44° in water (*c*, 1·0), + 68° in methyl alcohol (*c*, 0·8). A mixed m. p. with trimethyl *d*-erythronamide, m. p. 57°, showed a large depression (Found : C, 47·7; H, 8·5; N, 8·2; OMe, 50·8; *M*, by *X*-ray analysis, 177. $C_7H_{15}O_4N$ requires C, 47·5; H, 8·5; N, 7·9; OMe, 52·5%; *M*, 175).

Oxidation of Ascorbic Acid from Adrenal Glands.—A specimen of ascorbic acid from suprarenal glands (specimen b; see first paragraph) was oxidised with gaseous oxygen in faintly alkaline

solution with a trace of copper as catalyst until one extra carboxyl group per molecule of ascorbic acid had been formed. The oxidation was completed by acid potassium permanganate (2O) and the product, which was worked up as before, was identified in the form of trimethyl *l*-threonamide, m. p. 78°, identical with the above. Mixed m. p. 78°. $[\alpha]_D^{15°}$ + 67° in methyl alcohol (*c*, 0·3) (Found: C, 47·5; H, 8·9; N, 8·2; OMe, 52·6%).

Oxidation of Ascorbic Acid by Iodine and Alkaline Hypoiodite.—To a solution of ascorbic acid (6 g.) in water (30 c.c.), 100 c.c. of an iodine solution (19 g. of crystalline iodine and 16 g. of potassium iodide in 200 c.c. of water) were added until a permanent brown colour was obtained. More iodine solution (20 c.c.) was added, followed by 20% potassium hydroxide solution (10—15 c.c.) until the brown colour was almost discharged. Alternate additions of the reagents were continued in such a way that a slight excess of iodine over alkali always prevailed, so that the last addition of alkali (making 80 c.c. in all) produced a colourless solution. The mixture was maintained at 0° and after 45 minutes it was cautiously acidified with 5*N*-sulphuric acid, the excess of iodine being removed by sulphur dioxide. The amount of iodoform produced was negligible.

Isolation and esterification of the oxidation products. Aeration removed sulphur dioxide and the solution was made neutral with silver carbonate. Shaking with silver sulphate (60 g.) for 3 hours removed all iodides and oxalates, and the liquid was filtered, treated with a slight excess of potassium carbonate to remove silver sulphate, again filtered, and concentrated to 120 c.c. at 40°/20 mm. The potassium sulphate which separated was removed, and the filtrate and washings were united and treated with methyl sulphate (60 c.c.) and 40% potassium hydroxide solution (144 c.c.) in ten portions during 90 minutes, the methylation being conducted in the presence of acetone at 50°. The alkaline solution was cooled and neutralised with 5*N*-sulphuric acid, potassium sulphate removed, and the solution concentrated to 200 c.c. Sulphuric acid was then added until the solution was definitely acid, followed by an equal bulk of methyl alcohol. After standing, the inorganic salt was removed and the acid solution concentrated at 35°/20 mm. to 200 c.c. By neutralisation with barium carbonate, filtration and concentration the partly methylated organic acids were obtained as barium salts mixed with potassium sulphate. This solution was treated with a slight excess of sulphuric acid, filtered, and concentrated, the residue being extracted repeatedly with warm ethyl alcohol. The extract was concentrated and neutralised in aqueous solution with barium carbonate, filtered, and treated with a slight deficiency of sulphuric acid so that some barium salt remained undecomposed. After centrifuging, the solution was taken to dryness under diminished pressure and the residue dried.

Esterification and methylation. The mixture of organic acid and barium salts so obtained was treated with 2½% methyl-alcoholic hydrogen chloride during 6 hours at 70°. The acid was neutralised with silver carbonate, and the alcohol removed. The syrupy esters were incompletely soluble in methyl iodide, therefore two methylations with silver oxide and methyl iodide were conducted in the presence of methyl alcohol at 45° for 5 hours. The product was isolated in the usual way and a third methylation followed, methyl iodide and silver oxide alone being used. A mobile syrup (2·1 g.) was isolated which was subjected to fractional distillation: (A) 0·3 g., b. p. 120—130°/15 mm., $n_D^{15°}$ 1·4252; (B) 1·1 g., b. p. 130—140°/15 mm., $n_D^{15°}$ 1·4291; (C) 0·2 g., b. p. 140—145°/15 mm., $n_D^{15°}$ 1·4325; (D) 0·1 g., b. p. 145—170°/15 mm., $n_D^{15°}$ 1·4370; residue 0·4 g. (all temperatures are bath-temperatures). Fraction B, being incompletely methylated, was subjected to a further two Purdie methylations, followed by distillation at 135°/15 mm. to give a mobile ester showing $[\alpha]_{5780}^{18°}$ + 49° (*c*, 1 in methyl alcohol), $n_D^{16°}$ 1·4270 (Found: C, 49·5; H, 7·9; OMe, 62·4. Calc. for $C_8H_{16}O_5$: C, 50·0; H, 8·3; OMe, 64·8%).

Isolation of trimethoxy l*-threonamide.* The syrup (0·3 g.) was treated with methyl-alcoholic ammonia at 0° for 2 days. On removal of solvent partial crystallisation set in, which was completed on the addition of ether. The crystals were removed, washed, and recrystallised from ethyl alcohol (0·02 g.). M. p. above 255° (decomp.) (Found: OMe, 36·4. Calc. for dimethoxysuccinamide: OMe, 35·2%).

The ethereal extract crystallised in needles which on extraction with boiling light petroleum (b. p. 40—60°) gave the characteristic plates of trimethyl *l*-threonamide (0·06 g.), m. p. 75—76° alone or in admixture with an authentic specimen. $[\alpha]_{5780}$ + 44° in water (*c*, 1) (Found: C, 47·9; H, 8·4; OMe, 50·75. Calc. for $C_7H_{15}O_4N$: C, 47·5; H, 8·5; OMe, 52·5%).

The Oxidation of Ascorbic Acid with Hypochlorite: Conversion of the Oxidation Product into d*-Tartaric Acid.*—Ascorbic acid (10 g.) in water (50 c.c.) was oxidised by the addition of small portions of an alkaline hypochlorite solution (100 c.c. of 15% potassium hydroxide solution containing 8·4 g. of available chlorine) and alternately with 15% potassium hydroxide solution

(140 c.c.) so that a slight excess of chlorine was present over the theoretical quantity (7·8 g.). After the mixture had stood for 30 minutes at 15° and after acidification with sulphuric acid, a trace of sulphur dioxide was sufficient to remove the excess of chlorine. Treatment with silver carbonate, silver sulphate (30 g.), and potassium carbonate in the manner described in the first part yielded a solution containing the potassium salts of the oxidation products together with potassium sulphate, which was evaporated to dryness at 40°/15 mm. The powdered residue was treated with 5*N*-sulphuric acid (30 c.c.) and shaken with absolute alcohol (1 l.) for 2 hours. The residue was re-extracted in the same way after acidification, and the combined alcoholic filtrates were neutralised with barium hydroxide solution and allowed to stand. The clear filtrate was evaporated to dryness, forming a glass, the mixture of barium sulphate and insoluble barium salts was leached with hot water, and the solution of barium salts obtained was mixed with the residue from the alcoholic filtrates. Cautious addition of sulphuric acid followed until only a trace of barium remained in solution. Barium sulphate was removed and the solution was taken to dryness under diminished pressure (yield, 7 g.).

Oxidation with nitric acid. (I) The thick syrup (3·7 g.) obtained by this treatment was oxidised with nitric acid (*d* 1·2; 8 c.c.) at 40—45° for 24 hours. The solution was diluted with water, and water was distilled off continuously for 3 hours. Methyl alcohol was then added and the solution concentrated to a syrup, which was esterified with 3% methyl-alcoholic hydrogen chloride (150 c.c.) for 6 hours at 70°. The esters were isolated after neutralisation with silver carbonate and two methylations with methyl iodide and silver oxide followed, to yield 3·35 g. of a mobile syrup.

Fractional distillation of the esters. After heating at 100° for 3 hours at 15 mm. to remove methyl oxalate, the residue was distilled at 15 mm.

	Bath temp.	Yield, g.	$n_D^{18°}$.	$[\alpha]_{5780}^{19°}$ in MeOH (*c*, 1).
A	100—130°	0·5	1·4180	−14°
B	145—160	1·6	1·4339	+52
C	160—200	0·4	1·4465	+31

Fraction B was mainly methyl *d*-dimethoxysuccinate (Found: C, 47·1; H, 7·0; OMe, 56·3; CO_2Me, 55·2%. Calc. for $C_8H_{14}O_6$: C, 46·6; H, 6·9; OMe, 60·2; CO_2Me, 57·3%).

Isolation of d-*dimethoxysuccinamide.* Fraction B (0·16 g.) was treated with ammonia in dry methyl alcohol. On standing over-night, large crystals separated (0·1 g.), $[\alpha]_{5780}^{19°}$ + 98° (*c*, 1) in water. M. p. 285—290° (decomp.), alone or in admixture with an authentic specimen. A second crop of small crystals had m. p. 285° (decomp.), $[\alpha]_{5780}$ + 89°.

Isolation of d-*dimethoxysuccinomethylamide.* 0·1 G. of fraction B was treated in methyl alcohol (5 c.c.) with methylamine. On concentration feathery crystals of the methylamide separated, m. p. 206° alone or mixed with an authentic specimen prepared from *d*-dimethoxysuccinic ester. $[\alpha]_{5780}^{19°}$ + 137° in water (*c*, 1), m. p. 190° mixed with an authentic sample of *i*-dimethoxysuccinomethylamide.

Oxidation with nitric acid. (II) A syrup (3 g.) prepared as described by means of alkaline potassium hypochlorite was oxidised with nitric acid as before, esterified, methylated, and distilled at 15 mm.

	Bath temp.	Yield, g.	$n_D^{18°}$.	$[\alpha]_{5780}^{25°}$ in MeOH (*c*, 1).
A	100—135°	0·5	1·4200	+ 3°
B	145—155	0·3	1·4340	+50
C	155—165	0·6	1·4355	+55

All three fractions were treated with dry methyl-alcoholic ammonia. A gave oxamide, and *d*-dimethoxysuccinamide, m. p. 280° (decomp.); B gave *d*-dimethoxysuccinamide, m. p. 280° (decomp.); C gave *d*-dimethoxysuccinamide, $[\alpha]_{5780}^{19°}$ + 97° in water (*c*, 1). The total yield of crystalline amide was 0·7 g. Repeated crystallisations after the first crop had been removed yielded no evidence of the presence of any inactive dimethoxysuccinamide.

The Preparation of Dimethyl Ascorbic Acid.—Ascorbic acid (4 g.), dissolved in dry methyl alcohol (30 c.c.), was mixed with dry ether (30 c.c.) and treated at − 5° with diazomethane generated from nitrosomethylurethane (32 c.c.) and 25% methyl-alcoholic potassium hydroxide (48 c.c.). After being kept over-night, the yellow solution on concentration yielded a neutral syrup which ultimately crystallised. Dimethyl ascorbic acid was not oxidised by iodine in neutral or acid solution. It did not reduce Fehling's solution except on prolonged boiling. $[\alpha]_{5780}^{18°}$ + 27° in water (*c*, 1·5) (Found: OMe, 31·0. Calc. for $C_8H_{12}O_6$: OMe, 30·4%).

In alcohol and in water dimethyl ascorbic acid showed an intense absorption band with its head at λ 230 mμ (ε 7000, for an ethyl-alcoholic solution; *c*, 2·5 mg. per 100 c.c.) (see Fig. 1).

Dimethyl ascorbic acid reacts very readily in the cold with *N*/10-sodium hydroxide, one equivalent of alkali being used. The titration resembles that of an acid lactone and is complete in a few minutes at 15°. During the reaction the rotation changes from $[\alpha]_{5780}$ + 27° to $[\alpha]_{5780}$ − 12°. The same change takes place with hot aqueous sodium hydroxide and the product is not affected by continued heating at 90° in *N*/10-alkali. Concentrated alkali effects profound decomposition. The sodium salt does not display selective absorption in the ultra-violet region. On acidification of solutions of the sodium salt ($[\alpha]_{5780}$ − 12°) the rotation remains negative ($[\alpha]_{5780}$ − 6°, constant value). The sodium salt is produced without loss of either of the methoxyl groups of dimethyl ascorbic acid. A weighed quantity (about 100 mg.) of dimethyl ascorbic acid was placed in a small distillation flask together with an excess of *N*/10-sodium hydroxide. The side limb of the flask entered a trap (to retain any liquid which might creep over) and the exit from the trap was a glass tube which led directly into the hydriodic acid in the bulb of a micro-Zeisel apparatus. A current of nitrogen was passed through the apparatus. The flask and trap were kept at 100° with all exposed parts well lagged to prevent undue condensation. The Zeisel apparatus was operated in the usual way. The amount of methyl alcohol which distilled over in the experiments with dimethyl ascorbic acid was negligible (Found : OMe < 2% : experiment continued for 3 hours). During control experiments with methyl dimethoxysuccinate, over 70% of the methyl alcohol liberated on hydrolysis of the ester groups collected in the Zeisel apparatus and was converted into methyl iodide within 2 hours. Confirmation of the above results was obtained by converting dimethyl ascorbic acid into the barium salt by treatment with hot barium hydroxide {Found : OMe, 19·3. $[C_6H_7O_5(OMe)_2]_2Ba$ requires OMe, 21·3%}.

Dimethyl ascorbic acid remained unaltered when heated for 30 minutes at 65° with *N*/10-sulphuric acid. When dimethyl ascorbic acid was heated in a sealed tube at 35° for 12 hours in methyl alcohol saturated with ammonia at 0°, the crystalline substance described by Micheel and Kraft (*loc. cit.*) was formed in small yield and separated on evaporation of most of the solvent. After recrystallisation from methyl alcohol it had m. p. 124°, $[\alpha]_{5780}$ − 24° in 50% aqueous methyl alcohol (*c*, 2·1), in agreement with Micheel and Kraft's observations. We found, however, that this material gave analytical figures in agreement with the formula $C_9H_{19}O_7N$ and not $C_8H_{15}O_6N$ as reported by these authors. Their value for nitrogen is in exact agreement with ours and is much lower than the calculated value for their formula. The substance contains one molecule of combined methyl alcohol, which is lost at 100°. (The methyl alcohol eliminated by heating at 100° was swept forward by a current of carbon dioxide into a micro-Zeisel apparatus and weighed as silver iodide in the usual way. Found : MeOH, 11·6. Calc. for $C_8H_{15}O_6N$,MeOH : MeOH, 12·6%.) The two methyl groups of dimethyl ascorbic acid are retained in the product, which is amidic in character, giving off ammonia on treatment with alkali and reacting as an amide with sodium hypochlorite. It does not show selective absorption in the ultra-violet region (observations carried as far as 200 mμ). It showed unexpected resistance to oxidation by ozone in aqueous acetic acid [Found : C, 42·7; H, 7·6; N (amidic) 5·7; OMe, 35·5. $C_9H_{19}O_7N$ requires C, 42·7; H, 7·5; N, 5·5; OMe, 36·7%].

Tetramethyl Ascorbic Acid.—Dimethyl ascorbic acid (4·5 g.) was treated with silver oxide and methyl iodide in the presence of methyl alcohol at 45° for 5 hours. Three such operations (no methyl alcohol being necessary for the last two) yielded on distillation at 150°/0·06 mm. a syrup (5·0 g.) having $n_D^{17°}$ 1·4690. This syrup gave the correct analytical figures for tetramethyl ascorbic acid. $[\alpha]_{5780}^{20°}$ 0° in water, + 2° in 30% methyl alcohol–water (*c*, 6·0); + 8° in 50% methyl alcohol–water (*c*, 0·8) (Found : C, 51·9; H, 7·2; OMe, 53·1. Calc. for $C_{10}H_{16}O_6$: C, 51·7; H, 6·95; OMe, 53·4%). Tetramethyl ascorbic acid is much less stable towards alkali than the above dimethyl derivative.

Other preparations were carried out in which the yield of the fully methylated product on distillation was not so high owing to the presence of a resinous residue, although it never fell below 50%. The distillates, however, appeared to be identical in all cases except for slight variations in the refractive index ($n_D^{17°}$ 1·4680—1·4690). This may be due to the existence of different enolic modifications. No trace of oxidation was observed during the methylation.

The Ozonisation of Tetramethyl Ascorbic Acid.—(I) *Preparation of a neutral ester.* Tetramethyl ascorbic acid (1·9 g.), dissolved in acetic acid (30 c.c.) and water (6 c.c.), was ozonised during 90 minutes. Removal of solvent was effected at 35—40°/20 mm., methyl alcohol being added in the later stages. A syrup was obtained, a small portion of which was purified for analysis by solution in chloroform and treatment with sodium bicarbonate solution (Found : C, 46·4; H, 6·4; OMe, 44·0. Calc. for $C_{10}H_{16}O_8$: C, 45·5; H, 6·1; OMe, 46·2%).

Hydrolysis of the ester and esterification of the products. The syrup obtained from the previous experiment was dissolved in acetone (10 c.c.) and treated with 0·27*N*-barium hydroxide (120 c.c.). Barium oxalate was immediately precipitated, and removed after warming to 50° and standing for 45 minutes. The solution was then neutralised with *N*-sulphuric acid so that barium still remained in solution; the barium sulphate was removed in a centrifuge, and the solution taken to dryness at 50°/20 mm. The mixture of barium salt and organic acid was esterified during 6 hours with 70 c.c. of 5% methyl-alcoholic hydrogen chloride. The esters were isolated after neutralisation with barium carbonate and extracted from the barium chloride residues with ether (1·2 g.) and fractionally distilled, giving (a) 0·75 g., b. p. 140—150°/20 mm. (bath temp.), $n_D^{16°}$ 1·4395, $[\alpha]_{5780}^{20°}$ + 6° in water (*c*, 1·0) (Found: C, 47·5; H, 8·0; OMe, 49·5; CO_2Me, 35·5. Calc. for $C_7H_{14}O_5$: C, 47·2; H, 7·9; OMe, 52·2; CO_2Me, 33·1%), and (b) 0·1 g., b. p. 150—180°/20 mm. (bath temp.), $n_D^{16°}$ 1·4540. Residue 0·3 g. By treatment of fraction (a) with methyl-alcoholic ammonia an amide was obtained which crystallised spontaneously on removal of the solvent and was readily recrystallised from ethyl acetate (or water), m. p. 113° and mixed with 2 : 4-dimethyl *d*-erythronamide (m. p. 106°), m. p. 85°. $[\alpha]_{5780}^{20°}$ — 34° in water (*c*, 1) (Found: C, 44·4; H, 8·0; OMe, 36·1. Calc. for $C_6H_{13}O_4N$: C, 44·2; H, 8·0; OMe, 38·0%). The amount of crystalline material was, however, not more than 15—20% of the total. After removal of the crystals the syrupy amide which constituted the bulk of the material had $[\alpha]_{5780}^{20°}$ + 29° in water (*c*, 4).

0·025 G. of the above crystalline amide, dissolved in water (0·4 c.c.) and cooled in ice, was treated with 0·11 c.c. of the standard sodium hypochlorite solution described by Weerman (*Rec. trav. chim.*, 1917, **36**, 16). The hypochlorite disappeared more rapidly than in the case of gluconamide and when none remained saturated solutions of semicarbazide hydrochloride and sodium acetate were added. After one minute a copious precipitate of hydrazodicarbonamide came down which had m. p. 255° alone or admixed with an authentic specimen prepared from gluconamide. Control experiments with acetamide and gluconamide emphasised the accuracy of the observations.

The syrup from which the crystals had been removed also gave a strong positive Weerman reaction, the yield of hydrazodicarbonamide being such that almost the whole of it must necessarily have been derived from the 3 : 4-dimethyl *l*-threonamide.

(II). Tetramethyl ascorbic acid (4 g.) was ozonised for 2 hours in acetic acid (60 c.c.) and water (15 c.c.). The subsequent treatment was exactly as described under (I), the ozonisation product being hydrolysed with barium hydroxide and esterified. The amount of oxalate liberated was determined by titration and amounted to only two-thirds of the theoretical quantity (220 c.c. *N*/10-$KMnO_4$). This may have been due to polymerisation during ozonisation or the presence of a different form of the tetramethyl compound due to enolisation. Fractional distillation of the esters gave: (a) 1·67 g., b. p. 140—160°/15 mm. (bath temp.), $n_D^{17°}$ 1·4400, $[\alpha]_{5780}^{20°}$ + 5° in methyl alcohol (*c*, 2·0), — 4° in water (*c*, 1·0); (b) 0·9 g., b. p. 160—200°/15 mm. (bath temp.), $n_D^{17°}$ 1·4730; residue 0·4 g.

Isolation of 3 : 4-*dimethyl* l-*erythronamide.* 0·6 G. of fraction (a) was treated with methyl-alcoholic ammonia. On removal of the solvent partial crystallisation occurred and the amide described above, 3 : 4-dimethyl *l*-erythronamide, m. p. 112°, was isolated. The syrup remaining had $[\alpha]_{5780}^{20°}$ + 25° and both the syrup and the crystalline material gave positive Weerman tests both by the isolation of hydrazodicarbonamide and benzaldehyde semicarbazone.

Conversion of methyl 3 : 4-*dimethyl* l-*threonate into trimethyl* l-*threonamide.* The distillate [fraction (a) above] (1 g.) was methylated three times with methyl iodide and silver oxide and the resulting syrup was distilled, giving 0·8 g., b. p. 135—140°/20 mm. (bath temp.), $n_D^{16°}$ 1·4280, $[\alpha]_{5780}^{18°}$ + 37° in methyl alcohol (*c*, 1·5) (Found: C, 49·5; H, 8·2; OMe, 61·8. Calc. for $C_8H_{16}O_5$: C, 50·0; H, 8·3; OMe, 64·6%).

0·2 G. of this ester yielded on treatment with methyl-alcoholic ammonia the characteristic amide previously described (0·12 g.), m. p. 77° after one recrystallisation and mixed m. p. 76—77° with an authentic specimen, $[\alpha]_{5780}^{20°}$ + 44° in water (*c*, 1). (XVII) is therefore the main constituent of the degradation products.

(III) *Hydrolysis with ammonia.* Tetramethyl ascorbic acid (1·5 g.) was ozonised in acetic acid (30 c.c.) and water (7 c.c.) during 2½ hours. Solvent was removed at 40—50°/20 mm. and the syrup was dissolved in chloroform, the solution being washed with dilute sodium bicarbonate solution and water. Removal of chloroform yielded a syrup, which was treated with methyl-alcoholic ammonia. Oxamide was precipitated almost immediately (0·27 g. or 54% of the theoretical). The solution was concentrated to a syrup having $[\alpha]_{5780}^{20°}$ + 14° in water. Estimation of the amidic nitrogen indicated that 46% of amide was present, but no crystallisation was

observed. The syrup gave a positive Weerman test, the hydrazodicarbonamide isolated having m. p. 257°.

The syrupy amide (0·9 g.) was heated with 8 c.c. of *N*-sodium hydroxide for 1 hour, the alkali neutralised with hydrochloric acid, and the neutral solution taken to dryness. The residue was esterified with 5% methyl-alcoholic hydrogen chloride (50 c.c.) for 6 hours, after which neutralisation with silver carbonate, filtration and concentration were followed by fractional distillation, giving (a) 0·2 g., b. p. 140—155°/25 mm. (bath temp.), $n_D^{15°}$ 1·4410, $[\alpha]_{5780}^{21°}$ + 11° in methyl alcohol; (b) 0·05 g., b. p. 155—180°/25 mm. (bath temp.), $n_D^{15°}$ 1·4500, $[\alpha]_{5780}^{21°}$ + 7° in methyl alcohol; residue 0·4 g.

On treatment with methyl-alcoholic ammonia fraction (a) gave an amide, $[\alpha]_{5780}^{20°}$ + 11°, which crystallised partly on nucleation with 3 : 4-dimethyl *l*-erythronamide, the crystals having m. p. 113°.

The results obtained by this method therefore are similar to those obtained by methods (I) and (II).

The yield of oxamide produced in duplicate experiments varied from 90 to 50% in the same way as the yield of barium oxalate varied when barium hydroxide was employed to hydrolyse the neutral ester. It is only possible to account for these variations by supposing that different varieties of the tautomeric forms possible by enolisation are present in the fully methylated derivative.

A trimethyl derivative of ascorbic acid can be prepared by methylation with methyl sulphate and alkali in an atmosphere of nitrogen. This substance is markedly different from the neutral dimethyl derivative prepared by the action of diazomethane, since the trimethyl derivative is definitely acidic in character.

Properties of Acetylpyruvic Acid.—This substance was prepared by Claisen and Stylos' method (*Ber.*, 1887, **20,** 2188). It did not react with iodine in aqueous acid solution and in alkaline solution it hydrolysed rapidly, giving oxalic acid and acetone (contrast with ascorbic acid). In aqueous solution it gives a strong absorption band at λ 285 mμ; ε 4000 (*c*, 2 mg. per 100 c.c.).

Properties of Dihydroxymaleic Acid.—In aqueous solution dihydroxymaleic acid has a strong band at λ 290 mμ, ε = 6700 (*c*, 2·4 mg. per 100 c.c.) and at λ 300 mμ in alcohol, ε 7500 (*c*, 1·8 mg. per 100 c.c.). The substance reacts with aqueous iodine (2 atomic proportions), giving dihydroxytartaric acid, 2 mols. of hydrogen iodide being liberated in the process (0·092 g. required 9·8 c.c. of *N*/10-iodine, and 10 c.c. of *N*/10-NaOH were required to neutralise the hydriodic acid liberated. Calc. for $C_4H_4O_6,2H_2O$: 10 c.c. of iodine and 10 c.c. of NaOH). The same oxidation can be carried out with chlorine water. Spectrophotometric examination of the oxidised solution showed that the band had disappeared entirely, the product showing no selective absorption (compare ascorbic acid). When the solution (after oxidation with iodine) was evaporated to dryness in a vacuum desiccator, reduction took place with liberation of iodine. The iodine was removed by sublimation in a current of air. The product (yield, quantitative) had all the properties of the original dihydroxymaleic acid. Alcoholic iodine solutions have no action on dihydroxymaleic acid in the absence of water. Anhydrous dihydroxymaleic acid reacted rapidly with diazomethane in ether containing some methyl alcohol, giving methyl dihydroxymaleate, which reacted more slowly, giving the enolic dimethyl ether (Found : OMe, 53·3%, after one treatment with diazomethane). Methyl dihydroxymaleate (prepared by Fenton's method, J., 1894, **65,** 905) reacted with aqueous iodine in the same manner as the free acid. The fully methylated substance was most conveniently obtained by one treatment of the acid with diazomethane, followed by methylation with silver oxide and methyl iodide.

Methyl dimethoxymaleate was a mobile liquid, b. p. 85°/0·03 mm., $n_D^{15°}$ 1·4525 (Found : OMe, 58·0. $C_8H_{12}O_6$ requires OMe, 60·8%). It did not react with neutral or acid iodine and had negligible reducing power even in alkaline solution. In alcoholic solution it showed an intense absorption band at λ 255 mμ, ε 6000 (*c*, 2·7 mg. per 100 c.c.).

The work now described was rendered possible by the kindness of Prof. A. Szent-Györgyi, who generously provided the ascorbic acid, and to him and his collaborator, Dr. Svirbely, we desire to express our thanks and appreciation. Some of the material was prepared in the Biochemical Laboratory of the University of Cambridge. Another supply came from the Mayo Clinic, Rochester, New York, and the remainder from the Medical Chemistry Department of the University of Szeged. It was by the wish of Prof. Szent-Györgyi that the chemical investigation of ascorbic acid was undertaken in Professor W. N. Haworth's laboratories and we wish to

record our gratitude to Professor Haworth for providing facilities for the work and for his constant encouragement and continued interest in its progress. We are indebted also to Mr. E. G. Cox, whose crystallographic and *X*-ray investigations have been of the greatest assistance to us at various stages of the work.

UNIVERSITY OF BIRMINGHAM, EDGBASTON. [*Received, August 5th,* 1933.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY & SONS, LIMITED,
BUNGAY, SUFFOLK.

VIII

SYNTHESIS OF *d*- AND OF *l*-ASCORBIC ACID AND OF ANALOGOUS SUBSTANCES

BY

R. G. AULT,

D. K. BAIRD,

H. C. CARRINGTON,

W. N. HAWORTH,

R. HERBERT,

E. L. HIRST,

E. G. V. PERCIVAL,

F. SMITH,

AND

M. STACEY

Reprinted from the Journal of the Chemical Society,
October, 1933.

Reprinted from the Journal of the Chemical Society, 1933.

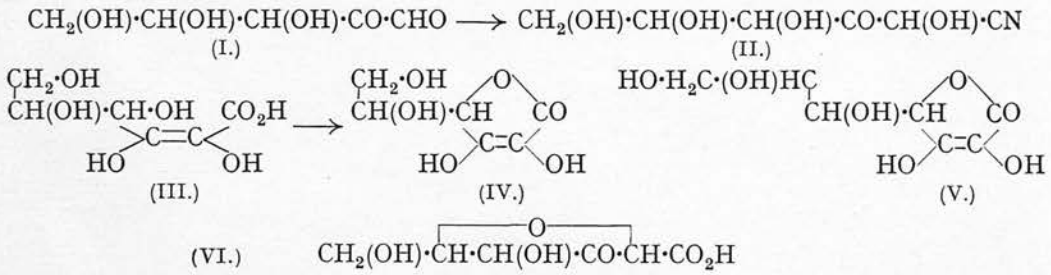
332. *Synthesis of* d- *and of* l-*Ascorbic Acid and of Analogous Substances.*

By R. G. Ault, D. K. Baird, H. C. Carrington, W. N. Haworth, R. Herbert, E. L. Hirst, E. G. V. Percival, F. Smith, and M. Stacey.

The co-operation of the Birmingham Chemical Laboratories with Professor Szent Györgyi of the University of Szeged, Hungary, has resulted in the publication of the detailed proof of the constitution of ascorbic acid by Herbert, Hirst, Percival, Reynolds, and Smith (this

vol., p. 1270) and we are greatly indebted to Professor Szent Györgyi and his collaborators for the facilities provided in the way of materials. The constitutional formula which is now generally accepted was first proposed by E. L. Hirst (*J. Soc. Chem. Ind.*, 1933, **52**, 221; March 10th). The analytical work of Hirst and his co-workers had revealed the groups associated with each carbon atom, and in the latter communication the new lactone ring structure was advanced for the first time. We then embarked upon a series of experiments directed to the synthetic preparation of both *d*- and *l*-ascorbic acid from *d*- and *l*-xylosone. A preliminary account of these syntheses has already been communicated (Haworth and Hirst, *J. Soc. Chem. Ind.*, 1933, **52**, 645; Aug. 4th): the complete account, including also the synthesis of an analogue of ascorbic acid from glucosone, is now submitted (compare Reichstein, Grüssner, and Oppenauer, *Nature*, 1933, Aug. 19th, p. 280).

The provision of the initial materials, namely, the *d*- and *l*-forms of xylosone, in sufficient quantity for the projected syntheses has involved the labour of a team of workers over many months. The chief losses in the yield of the final product occurred during these stages leading to the preparation of the xylosone. Thereafter, by the methods herein described, it has been found possible to isolate the *d*- and *l*-ascorbic acid in a yield of upwards of 70% of the theoretical. We have found that the best conditions to attain this end are to ascend the series from xylosone (I) to the β-keto-nitrile (II) by placing xylosone in contact with potassium cyanide and calcium chloride. The reaction involving the addition of hydrogen cyanide proceeds to completion in the course of a few minutes with the almost simultaneous hydrolysis to the β-keto-acid (III) and the instantaneous elimination of ammonia. The product (III) can exist in many forms, both enolic and keto, and is also capable of lactonisation. This intermediate product (III), although capable of reducing acid iodine solution, does not possess the properties of ascorbic acid and we have for reasons indicated below designated it ψ-ascorbic acid. It exhibits a different absorption spectrum from that of ascorbic acid, and gives rise to a different osazone. Its conversion into ascorbic acid proceeds quantitatively in the presence of 8% aqueous hydrochloric acid at 40—50°. The isolation of the *d*- and *l*-forms of ascorbic acid from the preparation in which either enantiomorph of xylosone has been employed is effected by the removal of such mineral salts as are formed in the process and the product in either case is isolated in good yield and with considerable ease as a highly pure crystalline specimen.



The formulation of natural ascorbic acid (which is the *l*-variety) by the constitutional formula (IV) (Hirst and co-workers, *loc. cit.*) receives strong experimental support from the synthesis which is now communicated.

By an analogous procedure glucosone has given rise to a crystalline product having properties which are almost identical with those of ascorbic acid. Its constitution is represented by (V) on the analogy of the structure which has been determined for ascorbic acid. It may provisionally be claimed to be 3-*keto-glucoheptonofuranolactone*. This structure is dependent upon the similarity in properties to ascorbic acid and the similar procedure involved in its synthesis.

All these synthetic products are being submitted to physiological tests.

A constitutional formula (VI) which we had earlier considered and rejected was at one time advanced by Micheel (*Nature*, 1933, **131**, 274). He has since abandoned this formula in favour of that proposed by Hirst and his co-workers (*loc. cit.*). But in the interim a communication by Reichstein, Grüssner, and Oppenauer (*Helv. Chim. Acta*, 1933, **16**,

561) appeared to give support to this formulation. As was pointed out by one of us (Haworth, *J. Soc. Chem. Ind.*, 1933, **52**, 482), an erroneous interpretation was given to the mechanism of the reaction involved in the synthesis of the *d*-enantiomorph of acetone ascorbic acid, and the Swiss authors have now accepted the interpretation which supports the constitution (IV) for ascorbic acid (private communication from Dr. Reichstein).

Experimental.

Preparation of d- *and* l-*Xylosone.*—As these preparations have not been described in the literature, the following directions are given (compare Fischer, *Ber.*, 1889, **22**, 87).

Finely-powdered dry *d*- or *l*-xylosazone (10 g.) was mixed with 90 c.c. of concentrated hydrochloric acid at 15°, and warmed quickly to 40° (vigorous shaking) until a clear dark red solution was obtained. This was immediately cooled to 25°, kept at this temperature for 10 minutes, and then cooled in a freezing mixture (ice and salt) for 15 minutes. The crystalline phenylhydrazine hydrochloride which separated was removed by filtration through glass wool and washed with a little concentrated hydrochloric acid. The filtrate was diluted with water (1 l.), neutralised with lead carbonate, and filtered. The pale yellow filtrate was cooled to 0° and made just alkaline with barium hydroxide. The lead hydroxide complex of xylosone which was precipitated as a pale yellow compound was washed twice by decantation and collected on a centrifuge. The lead compound was stirred into 60 c.c. of water containing 2·5 g. of sulphuric acid. The excess of acid was neutralised with barium carbonate, charcoal added, and the liquid filtered. The amount of xylosone in solution (estimated as xylosazone) was 0·35 g.

Synthesis of d- *and* l-*ψ-Ascorbic Acid.*—Xylosone (2 g.) in water (350 c.c.) was mixed with an aqueous solution containing calcium chloride (1·6 g.) and potassium cyanide (1·2 g.). A gentle stream of oxygen-free nitrogen was passed continuously through the liquid. There was an immediate evolution of ammonia and the course of the reaction was followed by titration of test samples with *N*/50-iodine in acid solution. The reaction was complete in 20 minutes. The calcium was precipitated with the equivalent of oxalic acid. A few drops of acetic acid were added to ensure that the filtrate was acid. The solution was concentrated at 35° under diminished pressure in an atmosphere of carbon dioxide to a thick syrup containing, as estimated by iodine titration, 1·8 g. of ψ-ascorbic acid. The syrup was extracted with ethyl alcohol, and inorganic material removed by filtration.

The absorption spectrum of the neutral syrup in aqueous solution showed a band at 275 mμ which did not change on acidification (contrast with ascorbic acid). ψ-Ascorbic acid was considerably less stable than ascorbic acid, but was convertible into the latter as indicated below.

A portion of the syrup (0·1 g.) was oxidised with iodine in acid solution, neutralised, then made acid again with acetic acid, and treated at 100° with phenylhydrazine. A yellow crystalline osazone (0·1 g.) was formed, m.p. 210°. *X*-Ray crystallographic examination by Mr. E. G. Cox revealed that it was not identical with the corresponding osazone from ascorbic acid.

Synthesis of d- *and* l-*Ascorbic Acid.*—The syrupy ψ-ascorbic acid (containing 1·8 g. of active material as estimated by the iodine titration) was dissolved in 20 c.c. of 8% hydrochloric acid. This solution was digested for 26 hours at 45—50° in an atmosphere of carbon dioxide. Its absorption spectrum then showed a band at 245 mμ in aqueous acid solution, moving to 265 mμ on formation of the sodium salt. The intensity of the band indicated a concentration of ascorbic acid identical with that estimated by a titration with acid iodine (compare Herbert, Hirst, Percival, Reynolds, and Smith, *loc. cit.*).

The solution was diluted with oxygen-free water, and most, but not quite all, of the hydrochloric acid removed by the addition of lead acetate (not basic). It is essential that the solution should remain acid at this stage. The lead was collected as sulphide, and the pale yellow solution concentrated to a thick syrup under diminished pressure at 35°. The syrup was extracted with dry ethyl alcohol and on the careful addition of dry ether to the alcoholic solution a considerable amount of inorganic material was precipitated. This operation was twice repeated. Evaporation of the solvents yielded a syrup which still contained inorganic impurity, although the whole was completely soluble in a little acetone. Addition of a large excess of dry acetone precipitated more of the mineral impurity. Ether was now added to the acetone solution until a permanent turbidity was observed and the solution was kept at 0° for 3 hours; a little syrup then separated. The decanted solution was concentrated at 30° in an atmosphere of carbon dioxide and gave a colourless crystalline mass of ascorbic acid (1·2 g.), which was pure after being washed with acetone. M.p. 190°. Its absorption spectra in aqueous acid and in neutral solution (*c*, 0·002)

were identical with those of natural ascorbic acid (bands at 245 and at 265 mμ respectively; ε, 7500).

The above process represents the experiments carried out both for the synthesis of the *d*- and the *l*-form of ascorbic acid. The physical properties and analytical data shown by each enantiomorph are as follows :

d-*Ascorbic acid* (synthetic) (Found : C, 40·8; H, 4·9. Calc. for $C_6H_8O_6$: C, 40·9; H, 4·6%). M.p. 190°. $[\alpha]_D^{18°}$ −48°. $[\alpha]_{5780}^{18°}$ −50° (in methyl alcohol; *c*, 0·75), $[\alpha]_{5780}^{18°}$ −24° (in water; *c*, 1).

l-*Ascorbic acid* (natural) (Found : C, 41·0; H, 4·7%). M. p. 190°. $[\alpha]_D^{18°}$ +49°. $[\alpha]_{5780}$ +50° (in methyl alcohol; *c*, 1·0), $[\alpha]_{5780}$ +24° (in water; *c*, 1).

l-*Ascorbic acid* (synthetic), m. p. 190° alone or when mixed with natural *l*-ascorbic acid, was in respect of all its properties indistinguishable from the natural product; its *X*-ray diagram was identical with that of the natural substance, and it had the same crystalline habit and highly characteristic refractive indices (E. G. Cox).

Synthesis of an Analogue of Ascorbic Acid (from Glucosone). 3-*Keto*-d-*glucoheptonofurano-lactone*.—A solution of glucosone (3·5 g.) in 300 c.c. of water was mixed with a solution of calcium chloride (3·2 g.) and potassium cyanide (2·4 g.) in 50 c.c. of boiled-out water. A stream of nitrogen (oxygen-free) was passed through the liquid with the immediate evolution of ammonia. Test samples of the product were titrated with *N*/50-acid iodine, and the reaction was complete in about 10 minutes. By iodine titration the amount of ψ-3-keto-*d*-glucoheptonofuronolactone was estimated to be 3·0 g. An equivalent amount of oxalic acid was now added to remove the calcium, a few drops of acetic acid being added to the filtrate to maintain acidity. This was then concentrated to 20 c.c. in a current of carbon dioxide. A considerable volume of alcohol was now added and after keeping over-night a cream-coloured crystalline powder separated. This consisted chiefly of the ammonium and potassium salts of the ψ-acid, showing an absorption band at 275 mμ which did not change in acid solution. After acidification of this salt and oxidation with iodine it gave rise to a yellow osazone, m. p. 215°, which will be described later. The isolation of 3-*keto*-d-*glucoheptonofuranolactone* from the salt of the ψ-compound was carried out as follows : The crystalline salt was dissolved in 40 c.c. of 8% hydrochloric acid and digested at 50° for 24 hours. The solution now showed absorption bands at 245 mμ in acid and 265 mμ in neutral solution. The hydrochloric acid was removed by the addition of lead acetate (not basic) and the lead in solution was precipitated as the sulphide, the pale yellow filtrate being concentrated under diminished pressure at 30°. At this stage also ammonium chloride was collected. Much of the inorganic matter was eliminated by alcohol–ether precipitation and the product was finally purified by trituration of the syrup with a large volume of dry acetone. Removal of the acetone yielded a crystalline mass, which was recrystallised by solution in acetone containing a few drops of ethyl alcohol and light petroleum. The crystals were larger than those of ascorbic acid and consisted of clusters of rods with pointed ends, m. p. 191° (Found : C, 41·0; H, 4·7. $C_7H_{10}O_7$ requires C, 40·8; H, 4·85%). The yield was 2·5 g. $[\alpha]_{5780}^{18°}$ + 14° (in water; *c*, 1), + 22° (in methyl alcohol; *c*, 1). Iodine titration : 0·052 g. required 5·2 c.c. *N*/10-iodine.

Preparation of l-*Xylosephenylosazone*.—The initial material for this preparation was *d*-galacturonic acid. This is obtainable either by the method already described in the literature from citrus pectic acid (Link and Nedden, *J. Biol. Chem.*, 1931, **94**, 307) or from the diacetone galacturonic acid prepared by Ohle and Berend (*Ber.*, 1925, **58**, 2585). The following series of transformations is then required for the conversion of *d*-galacturonic acid into *l*-galactonic acid, lactone, *l*-galactonamide, *l*-lyxose, *l*-xylosephenylosazone.

Reduction of potassium galacturonate to l-*galactonic acid.* The potassium salt (11 g.) of *d*-galacturonic acid in 250 c.c. of water was vigorously stirred at 15° with 480 g. of freshly prepared sodium amalgam (2·5%), the latter being added in 50 g. lots over a period of 5—6 hours. The alkaline solution was carefully neutralised with 50% sulphuric acid before each fresh addition of sodium amalgam. After keeping over-night, the solution showed no reducing action towards Fehling's solution. The mercury was separated, and the solution neutralised with 50% sulphuric acid and then filtered (charcoal). It was concentrated under diminished pressure to a thick syrup, which was poured into 1500 c.c. of 95% alcohol maintained at 65°. The precipitated sodium sulphate was extracted with hot alcohol and the filtrate and extracts were concentrated to 50 c.c., and any slight excess of sulphuric acid neutralised with barium carbonate. After filtration and complete evaporation the syrup was heated for 2 hours at 70° to complete the lactonisation. A portion of the lactone was crystallised for the purpose of identification, but the remainder was utilised as syrup, dissolved in dry methyl alcohol, and converted into *l*-galactonamide as follows :

Conversion of galactonolactone into l-*galactonamide.* When the methyl-alcoholic solution of the lactone was saturated at 0° with dry ammonia, there was an immediate precipitation of the amide. This was kept for several hours, collected, and recrystallised from ethyl alcohol–water, forming fine rods with pointed ends, m. p. 170°, $[\alpha]_{5780}^{18°}$ — 28° (in water; *c*, 1·0).

Conversion of l-*galactonamide into* l-*lyxose.* The method was that already described for the *d*-series by Weerman (*Rec. trav. chim.*, 1917, **36**, 16; compare Haworth and Hirst, J., 1928, 1221). The lyxose was not isolated but was converted immediately by contact with phenylhydrazine into *l*-xylosephenylosazone, m.p. 158— 160°. Yield from 10 g. of *l*-galactonamide, 4 g. This was purified by repeated crystallisation, the losses being about 50%.

The authors express thanks to the Government Grant Committee of the Royal Society for grants in aid of materials, and to Mr. E. Gordon Cox for his *X*-ray crystallographic examinations.

UNIVERSITY OF BIRMINGHAM, EDGBASTON. [*Received, October 2nd,* 1933.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY & SONS, LIMITED,
BUNGAY, SUFFOLK.

Reprinted from the Journal of the Chemical Society, 1934.

251. *Addition Compounds of the Carbohydrates. Part I. Potassium Hydroxide–Glucose and Related Compounds.*

By Edmund G. V. Percival.

The fact that sugars and polysaccharides appear to form compounds with the hydroxides of the alkali and alkaline-earth metals has been known for many years, but the precise nature of such compounds is still in doubt, research having been directed rather to the elucidation of stoicheiometric relationships between the organic and inorganic constituents than to the problems of structure.

Several workers have attacked the problem from a physicochemical standpoint, regarding the sugars as weak acids. Madsen (*Z. physikal. Chem.*, 1901, **86**, 290) calculated the heat of neutralisation of glucose and sodium hydroxide to be 5340 cals. from determinations of the rate of hydrolysis of ethyl acetate by sodium hydroxide in the presence of glucose, assumed to be a weak monobasic acid. Hirsch and Schlags (*Z. physikal. Chem., A*, 1929, **141**, 387), however, from conductivity measurements, conclude that glucose behaves as a feeble dibasic acid, $K_1 = 7{\cdot}82 \times 10^{-13}$, $K_2 = 1{\cdot}54 \times 10^{-14}$ at 25°.

That compounds appear to be formed between glucose and alkali even in aqueous solution was pointed out by Groot (*Biochem. Z.*, 1924, **146**, 72; 1927, **180**, 340), who observed that the maximum depression of the specific rotation of a glucose solution in the presence of potassium or sodium hydroxide occurred when the constituents were present in approximately molecular proportion. From measurements of the rate of decline of rotation at different concentrations, he concluded that the dissociation constant of glucose as an acid was $K = 8{\cdot}6 \times 10^{-13}$ at 25°, the decline of rotation being accounted for by the initial formation of an unstable compound $C_6H_{11}O_6K$, followed by Lobry de Bruyn–van Eckenstein transformations; but the possibility of the formation of other sugars during these transformations lessens the force of the argument. The cause of the apparent acidity and the point at which it arises in the molecule cannot be decided by experiments along these lines, although Michaelis and Rona (*Biochem. Z.*, 1913, **49**, 232), on the basis of potentiometric measurements of hydrogen-ion concentrations of solutions of alkali hydroxides and sugars, suggest the possibility that the acidity is due to the presence of enolic forms $-CH(OH){:}C(OH)-$. This, however, is open to the objection that such an explanation could only apply to the reducing sugars, and the many compounds which sucrose forms with alkali and alkaline-earth hydroxides cannot therefore be explained on this basis. Such physicochemical results simply serve to show that the sugars examined remove alkali or hydroxyl ions from the solution.

It was decided therefore to institute investigations with the object of deciding at which points in the molecule the inorganic constituents are attached, and the nature of the linkages involved. The isolation of about 80 authenticated compounds from various

carbohydrates and alkalis which appear to contain the metallic hydroxide (or oxide) and sugar in stoicheiometric proportion (see, *e.g.*, Mackenzie and Quin, J., 1929, 951), seems to preclude the possibility that the phenomenon is due to adsorption or to a fortuitous precipitation of the metallic constituent along with the sugar residue. In particular, when both the reacting hydroxide and the sugar are soluble in the reaction medium, simple mixing affording a precipitate of the compound, it would seem to be clearly a case of chemical reaction.

It remains to decide therefore whether the " saccharates " are substitution compounds comparable with sodium ethoxide, or co-ordination compounds in which the inorganic residues are attached to the hydroxyl groups of the sugar molecule by covalent links. For this purpose potassium glucosate was selected for a preliminary examination.

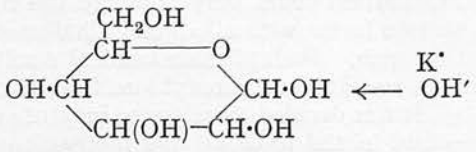
It was assumed by Marchlewski (*Ber.*, 1893, **26**, 2928) that the alkali metal was attached to the reducing group, but this was based on negative and unsatisfactory evidence, *viz.*, his failure to isolate glucosephenylosazone from phenylhydrazine and potassium glucosate. Marchlewski did not describe his experiments in detail, but it may be pointed out that pure phenylhydrazine and pure glucose do not yield the osazone under normal conditions, acidification with acetic acid being necessary.

Hönig and Rosenfeld (*Ber.*, 1877, **10**, 871) claimed to have isolated the compound $C_6H_{11}O_6Na$ from glucose and sodium ethoxide in alcoholic solution. Zemplén and Kunz (*Ber.*, 1923, **56**, 1705) reopened the question and pointed out that attempts to use the compound in synthetic experiments, notably by Skraup and Kremann (*Monatsh.*, 1901, **22**, 1040), had failed. The existence of an alcoholate structure was therefore considered to be doubtful, and Zemplén showed that an addition compound between glucose and sodium ethoxide, $C_6H_{12}O_6,NaOC_2H_5$, appeared to be formed, the presence of the ethoxide residue being established by a qualitative test.

This work has now been repeated and *compounds* of the type $C_6H_{12}O_6,NaOR$ have been isolated from glucose in absolute-alcoholic solution. If a trace of water was present, however, the compounds isolated contained no combined alcohol, which may explain the results of the earlier workers. From potassium hydroxide and glucose, the *compound* $C_6H_{12}O_6,KOH$ may readily be isolated, which is probably similarly constituted to the sodium alkoxide compounds. By analogy, therefore, it appears that potassium glucosate is an addition compound of potassium hydroxide and glucose, and this is supported by the analytical results. No evidence can be secured for the existence of any other compound in the range of concentrations studied. It has been found possible, however, to indicate the probable position of the addendum. By a single treatment of potassium glucosate with pure methyl sulphate under mild conditions, apart from unchanged glucose, crystalline tetra-acetyl β-methylglucoside may be obtained, together with a syrup which is evidently a mixture of this with the α-isomeride. All the methoxyl is glucosidic, there being no evidence of any further substitution of the glucose molecule by methyl groups.

Hence, one is entitled to assume that the reducing group is involved in the combination between sugar and alkali, as might have been expected from general considerations. If the alcoholate formula, $C_6H_{11}O_6K$, were the true one, a more complete conversion into the methylglucosides would have been expected, but the isolation of so much unchanged glucose lends support to the theory that the attachment between glucose and alkali is relatively weak, and is probably to be ascribed to co-ordination.

The fact that α- and β-methylglucosides form no addition compounds with potassium hydroxide, or that the glucosides examined by Mackenzie and Quin (*loc. cit.*) form no derivatives with the alkaline-earth hydroxides, renders it difficult to imagine that the oxygen atom of the reducing group can act as a donor of electrons to the metal atom concerned. If, however, we suppose that the hydrogen atom of that group acts as an acceptor of electrons from the oxygen atom of the alkali hydroxide or alkoxide, the known facts are explained (see inset). This is in agreement with the well-known fact that alkaline hydroxides appear to form very stable monohydrates, which is attributed



to hydration of the negatively charged hydroxyl ion HOH ⟵ OH′, the ion acting as a powerful donor of electrons (Sidgwick, "Electronic Theory of Valency," Oxford, 1927). The suggestion is therefore made that the compounds under review are of this general type.

The reducing disaccharide cellobiose (glucose-β-glucoside) forms an addition compound $C_{12}H_{22}O_{11}$,KOH in which it is clear that the reducing group is involved, since crystalline β-methylcellobioside hepta-acetate may be isolated by suitable treatment with methyl sulphate. The situation is complicated by the fact that cellobiose appears to form in addition $C_{12}H_{22}O_{11}$,2KOH, which implies that at least two positions in the molecule are available for the attachment of potassium hydroxide residues. This has been confirmed by the isolation of a monomethyl methylcellobioside, the structure of which is being investigated to determine the position of the second methyl group.

Even more complex is the case of maltose, which would appear to form a tri- as well as mono- and di-potassium hydroxide derivatives; and a dimethyl methylmaltoside has been isolated confirming this view.

It is not thought probable that during the treatment with methyl sulphate the alkali residue would migrate to a new position in the sugar group, thus causing the introduction of the methyl residue in a position different from that originally concerned. The possibility has, however, been kept in mind, and minimised as far as possible by the avoidance of the presence of water during methylation. It may be pointed out, however, that even if water or other ionising solvents were present, the method would still be expected to indicate those hydroxyl groups of maximal acidity in the sugar molecules examined, because, whether they are closely bound to alkali or not, it is not likely that the centres of acidity will change during the operation.

In the case of disaccharides, therefore, hydroxyl groups other than the reducing group appear to unite with potassium hydroxide. This is to be expected because of the formation of the important and apparently well-marked series of saccharates from sucrose and alkali and alkaline-earth hydroxides. The structure of these more complex addition compounds is being studied in detail.

Experimental.

Typical Preparations of Potassium Hydroxide–Glucose.—(1) *From penta-acetyl glucose.* β-Penta-acetyl glucose (5 g.) was moistened with absolute alcohol (10 c.c.), and a solution of potassium hydroxide (6 g.) in alcohol (60 c.c.) added; after an hour the insoluble product was filtered off, washed first with alcohol and finally with ether, and dried in a vacuum over phosphoric oxide [Found: KOH, by titration with $N/10$-H_2SO_4 to phenolphthalein, 23·6; $C_6H_{12}O_6$, by treatment with alkaline hypoiodite (Bergmann and Machemer, *Ber.*, 1930, **63**, 316), 75·1. $C_6H_{12}O_6$,KOH requires KOH, 23·7; $C_6H_{12}O_6$, 76·3%].

(2) *From glucose.* *d*-Glucose (10 g.) dissolved in water (10 c.c.) was mixed with alcohol (150 c.c.), and alcoholic potassium hydroxide (50 c.c., 8%) added, and the precipitated derivative was treated as in (1) (Found, by foregoing methods: KOH, 24·0; glucose, 75·2%).

Sodium Ethoxide–Glucose.—Ethyl alcohol was dried by distillation over quick-lime and twice over sodium. Glucose (1 g.), dried over phosphoric oxide, was dissolved in dry alcohol (100 c.c.), and a solution of sodium ethoxide in alcohol (0·4 g. Na in 10 c.c.) added. The precipitate was rapidly collected in a funnel protected from moisture and carbon dioxide by means of a soda–lime tube, washed with alcohol, and dried, at first at room temperature over phosphoric oxide in a vacuum and then for 24 hours at 60° under the same conditions [Found: NaOEt (titration), 27·8; OEt (Zeisel), 16·0; $C_6H_{12}O_6$ (Bertrand), 66·9. $C_6H_{12}O_6$,$NaOC_2H_5$ requires NaOEt, 27·4; OEt, 18·1; $C_6H_{12}O_6$, 72·6%]. A similar preparation made from penta-acetyl glucose had similar properties.

Sodium Methoxide–Glucose.—This was prepared from penta-acetyl glucose (5 g.) in admixture with sodium methoxide in methyl alcohol (2 g. Na in 25 c.c.). The precipitate was washed with methyl alcohol, in which it was slightly soluble, and finally with dry ether, and dried as for the ethoxide [Found: NaOMe (titration), 22·7; $C_6H_{12}O_6$ (hypoiodite), 77·4; OMe (Zeisel), 13·0. $C_6H_{12}O_6$,$NaOCH_3$ requires NaOMe, 23·1; OMe, 13·2; $C_6H_{12}O_6$, 76·9%].

The Formation of Potassium Hydroxide–Glucose under Different Conditions.—A rough estimate of the amount of potassium hydroxide taken up by the glucose molecule can be obtained

by treating a known volume of alcoholic potassium hydroxide of known strength with a known volume of a glucose solution in 80% alcohol. By filtration through a Gooch crucible a solution can be obtained, titration of which indicates how much alkali has been withdrawn by the sugar. It is necessary to assume that no significant volume changes have taken place and also that the addition compound is not appreciably soluble in this equilibrium solution. The solid in the crucible is drained and washed once with alcohol to remove adhering liquid; solution in water and titration with standard acid then gives a direct estimate (method 2) of the combined alkali, which, however, is probably less accurate than the value obtained by the indirect method (method 1). Typical results are as follows:

Total concn. of glucose, %		1·01	1·78	2·51	4·32	4·81
Concn. of KOH, *N*	Initial	0·75	0·51	0·30	0·29	0·27
	Final	0·69	0·40	0·16	0·04	0·03
KOH combined, %	Method (1)	33·6	31·8	32·1	32·6	28·7
	,, (2)	31·9	32·5	32·4	30·7	29·6

Since 100 g. of glucose require 31·1 g. of potassium hydroxide to form a 1 : 1 compound, it would appear that such a compound is formed within the range studied.

Formation of More Complex Compounds from Cellobiose and Maltose.—The results with these sugars are tabulated below. For a compound of the type $C_{12}H_{22}O_{11}$,KOH, 100 g. of the disaccharide require 16·4 g. of potassium hydroxide. Evidently in these cases this simple compound may be formed only in dilute solution, but it is possible that small quantities of the more complex derivatives are produced at the same time. In the case of cellobiose the composition approximates to $C_{12}H_{22}O_{11}$,2KOH, and for maltose in the more concentrated alkaline solutions $C_{12}H_{22}O_{11}$,3KOH appears to exist. The variations between the results of the two methods are due to the decomposition, by washing with alcohol, of the higher addition compounds, and illustrate the instability of these more complex derivatives.

		Cellobiose.				Maltose.			
Total concn. of sugar, %		0·88	1·49	1·9	1·8	1·6	0·9	1·5	2·1
Concn. of KOH, *N*	Initial	0·76	0·65	0·15	0·075	0·4	1·0	0·92	0·15
	Final	0·69	0·58	0·080	0·02	0·31	0·92	0·80	0·043
KOH combined, %	Method (1)	32·9	26·0	21·4	17·1	36·2	44·5	45·6	28·5
	,, (2)	23·0	19·5	21·4	16·0	36·0	36·8	37·0	25·1

Potassium Hydroxide–Glucose and Methyl Sulphate.—The dry compound (13 g.) was stirred with dry, neutral methyl sulphate (120 c.c.) for 5 minutes at 45° and for 5 minutes at 70°, the liquid was then removed, the product washed with acetone, and dissolved in hot methyl alcohol (100 c.c.). On cooling, crystals of potassium methyl sulphate separated which were filtered off, and a solution of potassium hydroxide (3 g.) in alcohol (20 c.c.) was added, followed by ether (700 c.c.). By this means most of the potassium glucosate so produced was recovered (9·5 g.), whilst there remained in solution that portion of the starting material which had undergone reaction with the methyl sulphate.

Isolation of tetra-acetyl β-methylglucoside. After acidification with acetic acid and removal of the solvent, the syrup was acetylated by treatment for 2 hours with acetic anhydride (30 c.c.) and anhydrous sodium acetate (5 g.). The mixture was poured into water, the solution neutralised with sodium bicarbonate, and extracted with chloroform. Removal of the solvent (diminished pressure) after drying with sodium sulphate yielded a syrup which, on treatment with alcohol, partly crystallised in the prisms characteristic of tetra-acetyl β-methylglucoside (0·9 g.); this was non-reducing, had m. p. 102° (not depressed on admixture with an authentic specimen prepared from acetobromoglucose), and $[\alpha]_D^{20°} = -20°$ in chloroform (*c*, 2·5) (Found: OMe, 8·5; CH_3·CO, 47·0. Calc. for $C_{15}H_{22}O_{10}$: OMe, 8·6; CH_3·CO, 47·5%).

The non-reducing syrupy residue from which this crystalline derivative had been extracted weighed 3·0 g.; $[\alpha]_D^{20°} = +20°$ in chloroform (*c*, 1) (Found: OMe, 8·6; CH_3·CO, 46·9%). It was assumed therefore to consist of a mixture of α- and β-methylglucoside tetra-acetates, but a further investigation was carried out to see if any substitution whatever had occurred in other parts of the glucose molecule.

Examination of the syrupy glucoside. Deacetylation was carried out with sodium methoxide (Zemplén, *loc. cit.*), and the resulting aqueous solution was hydrolysed by dilute sulphuric acid (6%) at 90° for 7 hours. After neutralisation by means of barium carbonate, the solution was evaporated under diminished pressure, yielding a syrup which was insoluble in absolute alcohol (cf. glucose). To the solution in 80% alcohol, excess of alcoholic potassium hydroxide was

added, and the thick precipitate of potassium hydroxide–glucose was collected and dried (1·5 g. Calc., 1·9 g.). The residual solution was acidified with acetic acid and treated with phenylhydrazine (0·5 g.) at 90° for 30 minutes. On cooling, phenylglucosazone separated (0·1 g.); recrystallised from aqueous alcohol it had m. p. 204°, and contained no methoxyl residue (Zeisel). It followed therefore that the original syrup was only substituted by methoxyl in the reducing group, the formation of the osazone being due to the slight solubility of potassium hydroxide–glucose. No evidence could be obtained of the formation of addition compounds between potassium hydroxide and α- and β-methylglucosides.

Potassium Hydroxide–Cellobiose.—Cellobiose octa-acetate, prepared by the method of Haworth and Hirst (J., 1921, **119**, 193), was deacetylated after Zemplén (*loc. cit.*) to yield the free sugar, of which 2 g. were dissolved in 80% ethyl alcohol (50 c.c.), alcoholic potassium hydroxide (100 c.c., 0·08*N*) being added. The product was washed rapidly with alcohol (20 c.c.) and dried in the usual way [Found : KOH (titration), 13·8; $C_{12}H_{22}O_{11}$ (iodine), 86·0. Calc. for $C_{12}H_{22}O_{11}$,KOH : KOH, 14·1; $C_{12}H_{22}O_{11}$, 85·9%]. The white powder was similar in properties to the glucose derivative.

Reaction with Methyl Sulphate.—The dry product (2 g.) was stirred with dry, neutral methyl sulphate for 8 minutes at 60°. Coagulation ensued, the reagent was removed, the product washed with acetone, and dissolved in hot methyl alcohol (40 c.c.). On cooling, the precipitated potassium methyl sulphate was removed, and unchanged cellobiose was precipitated by the addition of potassium hydroxide (0·5 g.) in alcohol (10 c.c.). The recovered product weighed 0·9 g. After acidification with acetic acid, the solvent was removed (diminished pressure) and the syrup subjected to acetylation by acetic anhydride (10 c.c.) and sodium acetate (1 g.) at 100° for 30 minutes.

Isolation of Hepta-acetyl β-Methylcellobioside.—The acetylation mixture was poured into water, and the solution neutralised with sodium bicarbonate before extraction with chloroform. Removal of the solvent after drying over sodium sulphate yielded a faintly reducing syrup (1·4 g.) which crystallised on treatment with alcohol giving hepta-acetyl β-methylcellobioside (0·7 g.); m. p. 178°, $[\alpha]_D^{15°} = -23·0°$ in chloroform (*c*, 1·6) (Found : C, 50·0; H, 6·0; OMe, 4·7. Calc. for $C_{27}H_{38}O_{18}$: C, 49·9; H, 5·9; OMe, 4·9%). The residual syrup showed $[\alpha]_D^{15°} = -1°$ in chloroform (*c*, 1·1) (Found : OMe, 5·4; CH_3·CO, 45·9. Calc. for $C_{27}H_{38}O_{18}$: CH_3·CO, 46·3%). It would appear therefore to consist of a mixture of α- and β-glycosides.

The use of dilute alcoholic potassium hydroxide appears, then, to favour the production of a compound of the type $C_{12}H_{22}O_{11}$,KOH in which the reducing group and the hydroxide are intimately connected.

When, however, preparations using more concentrated alkali (see table, p. 1163) are treated with methyl sulphate, more complex methylated derivatives may be isolated, in particular a monomethyl methylcellobioside, the structure of which is being investigated. Even more interesting is the case of maltose which can be made to afford methylmaltosides, monomethyl methylmaltosides, and dimethyl methylmaltosides, as might be expected from a consideration of the table on p. 1163.

Thanks are expressed to the Earl of Moray Endowment for a grant in aid of these researches.

King's Buildings, University of Edinburgh. [*Received, May 28th*, 1934.]

Printed in Great Britain by Richard Clay & Sons, Limited,
Bungay, Suffolk.

X

ADDITION COMPOUNDS OF THE CARBOHYDRATES. PART II. POTASSIUM HYDROXIDE-SUCROSE

BY
EDMUND G. V. PERCIVAL

Reprinted from the Journal of the Chemical Society,
May 1935

Reprinted from the Journal of the Chemical Society, 1935.

145. *Addition Compounds of the Carbohydrates. Part II. Potassium Hydroxide–Sucrose.*

By Edmund G. V. Percival.

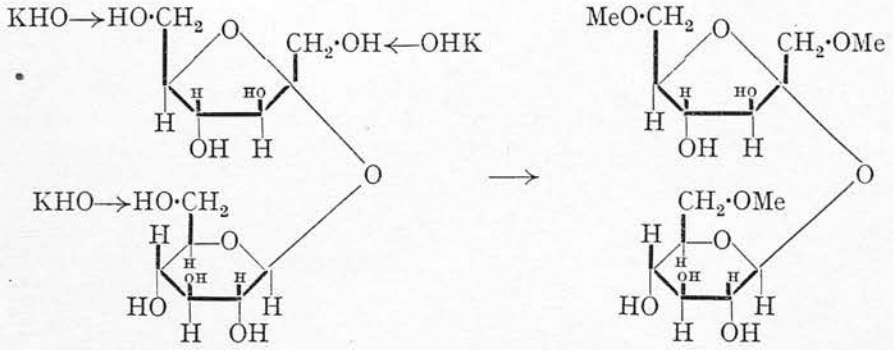
Sucrose forms a large number of compounds with metallic oxides and hydroxides, the compounds with strontium hydroxide indeed forming a basis of sugar refining. From a survey of the literature, it seems clear that, although various molecular proportions of metallic hydroxides may be associated with one molecule of sucrose, no trustworthy evidence exists pointing to the occurrence of compounds containing more than three molecules of the inorganic components as in $C_{12}H_{22}O_{11},3CaO,3H_2O$ (v. Lippmann, 1904, "Chemie der Zuckerarten," 3 Aufl., 1338; Mackenzie and Quin, J., 1929, 951).

Physicochemical methods such as the phase-rule studies of Reinders and Klinkenberg (*Rec. trav. chim.*, 1929, **48**, 1227), the examination of the decline in specific rotation of sucrose solutions in the presence of alkali (Thomsen, *Ber.*, 1881, **14**, 1647), or the conductometric analyses of Hirsch and Schlags (*Z. physikal. Chem.*, 1929, *A*, **141**, 387) are valuable in indicating that compound formation between sucrose and alkalis does occur. The last authors point out that in aqueous solution sucrose behaves as a weak dibasic acid,

$K_1 = 3{\cdot}1 \times 10^{-13}$, $K_2 = 3{\cdot}0 \times 10^{-14}$ at 25°. It is significant that the second stage of ionisation is about twice as great as for glucose, which may account for the fact that the sucrose derivatives are more complex.

In Part I of this series (J., 1934, 1160) it was concluded that the probable formulation of compounds of this type was similar to that of the stable monohydrates of the alkali-metal hydroxides and involved co-ordination between the hydroxyl ion of the hydroxide and the most active hydrogen atoms of the sugar concerned, notably in the case of glucose the mobile hydrogen atom in the reducing group associated with the pyranoid ring formation.

The results of the present investigation are in harmony with these conclusions, although, in the absence of a reducing group, the primary alcoholic residues in sucrose appear to be the centres of acidity and each of the three appears to be associated with the potassium hydroxide in the compound $C_{12}H_{22}O_{11}$,3KOH studied. The basis of this conclusion rests on the isolation, on treatment with methyl sulphate under mild conditions, of a trimethyl sucrose in which the primary alcohol residues alone are substituted.



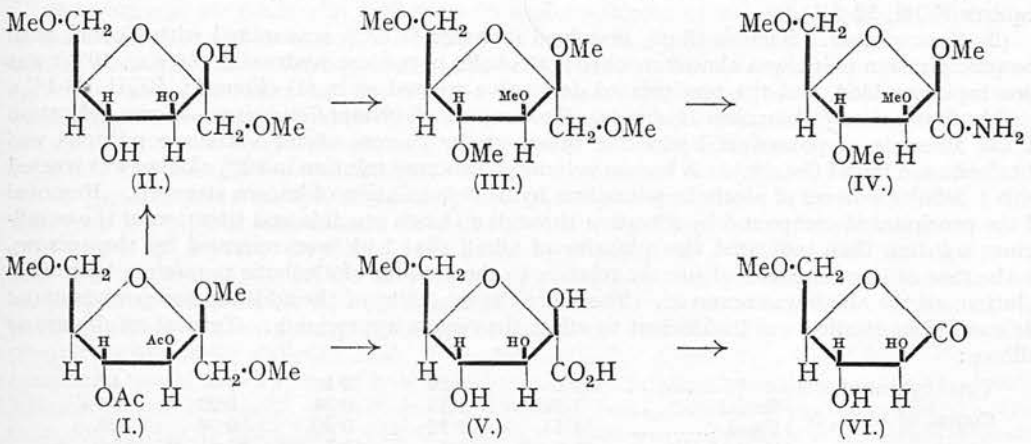
Addition compounds of sucrose and potassium hydroxide were prepared by the action of concentrated alcoholic potassium hydroxide both on sucrose octa-acetate and on an aqueous-alcoholic solution of the free sugar. By the indirect method of analysis (see Part I) it was established that three potassium hydroxide residues were the maximum number which one sucrose molecule would hold in combination, although in ordinary preparations, after washing with alcohol and ether, direct analysis revealed compositions ranging from $C_{12}H_{22}O_{11}$,KOH to $C_{12}H_{22}O_{11}$,3KOH, which explains the isolation by Pfeiffer and Tollens (*Annalen*, 1881, **210**, 296) of a monopotassium derivative after continued washing with alcohol. Whether the tripotassium compound is decomposed completely into the free sugar and potassium hydroxide in contact with alcohol or whether in contact with more dilute alkaline solutions only mono- and di-potassium hydroxide derivatives can exist is not clear, although the latter conclusion would seem more probable.

Products approximating in composition to $C_{12}H_{22}O_{11}$,3KOH were treated with methyl sulphate for a brief period and after removal of unchanged sucrose (90%), followed by acetylation, a glass was obtained which on analysis was shown to be a trimethyl penta-acetyl sucrose. Hydrolysis produced no optical inversion, so it was clear that the resulting methylated fructose was of the furanose type as in the case of the hydrolysis of octamethyl sucrose by Haworth and Law (J., 1916, **109**, 1314). By the formation of the furanosides, followed by acetylation and distillation in a high vacuum, it was found possible to separate these hydrolysis products into a methyl tetra-acetyl methylglucofuranoside and a dimethyl triacetyl methylfructofuranoside.

The former after hydrolysis was recognised as a genuine derivative of glucopyranose, since methylation yielded 2 : 3 : 4 : 6-tetramethyl methylglucoside giving on hydrolysis crystalline 2 : 3 : 4 : 6-tetramethyl glucopyranose, and that the methyl group occupied the primary alcohol residue was proved by the isolation of crystalline 6-methyl glucose phenylosazone.

On account of the positive specific rotation of the dimethyl fructose (II) obtained on

hydrolysis of the fructofuranoside (I) it was suspected that a derivative of fructofuranose was concerned. This would imply the presence of one methoxyl group in the 6-position to prevent reversion to the more stable pyranose form. Complete methylation of (II) to a tetramethyl methylglycoside (III), followed by oxidation with nitric acid (Avery, Haworth, and Hirst, J., 1927, 2313), gave rise to a tetramethyl lactol acid which on appropriate treatment yielded crystalline 2 : 3 : 4 : 6-tetramethyl fructofuronamide (IV) identical with that obtained from tetramethyl fructofuranose, thus establishing the furanose ring and the participation of the primary alcohol residue at the 6-position in the original methylation.



It remained to indicate the position of the second methoxyl residue. Because the free dimethyl sugar failed to form an osazone, substitution in the 1-position was indicated, and this was confirmed as follows. By direct oxidation of the diacetyl dimethyl fructofuranoside under similar conditions to those employed for the tetramethyl derivative, a lactol acid was obtained (V) containing only one methoxyl residue. This was converted into the monomethyl arabonolactone (VI) by treatment with the calculated quantity of acid permanganate (Haworth and Learner, J., 1928, 619), and this lactone was found to have the properties of a γ-lactone, confirming the view that the terminal position is occupied. The position of the second methoxyl group in the dimethyl fructofuranose (II) on the secondary alcohol residues is obviously excluded, since only a monomethyl γ-arabonolactone resulted on oxidation (VI), the methyl group at the first carbon atom having disappeared on oxidation at that point.

Evidence is thus provided that the trimethyl sucrose obtained by direct methylation of $C_{12}H_{22}O_{11}$,3KOH is substituted only at the three primary alcohol groups present in the molecule. If, therefore, it is considered, as in Part I (*loc. cit.*), that the methyl groups have entered at the points of maximum acidity in the sucrose molecule, it is probable that these primary alcohol residues are involved in the addition compounds of sucrose with alkaline hydroxides. Since the association of sugar and alkali is an unstable one as indicated by the large amount of unconverted sucrose which is recovered from the methylation, there is no evidence that the compounds considered are substitution products, although Pfeiffer and Tollens (*loc. cit.*) described a monosodium derivative which was prepared in a similar manner to $C_{12}H_{21}O_{11}Na$ on the basis of a direct analysis. Their results are, however, just as readily interpreted on the theory of the formation of an addition compound.

Lest it should be considered that the methylation of addition compounds of the type under discussion is due merely to the presence of a certain amount of free potassium hydroxide which, reacting with methyl sulphate, makes possible a substitution in the sugar molecule concerned, although the points of maximum acidity might thereby be indicated, mixtures of dry, finely powdered potassium hydroxide and glucose in molecular proportion were subjected to the methylation process under the conditions used when treating the addition compounds. Careful study of the products of the reaction revealed that the highest yield of methylglucoside obtained by this method was never greater than 0·5%

of the weight of glucose taken, as compared with a conversion of 20% when the addition compound $C_6H_{12}O_6$,KOH was treated under the same conditions (Part I, *loc. cit.*).

Experimental.

Typical Preparations of Potassium Hydroxide–Sucrose.—(1) *From octa-acetyl sucrose.* Octa-acetyl sucrose (5 g.) was moistened with absolute alcohol (10 c.c.), and a solution of potassium hydroxide (10 g.) in alcohol (50 c.c.) added. After 2 hours, the insoluble product was filtered off, washed quickly with alcohol and ether, and dried in a vacuum over phosphoric oxide (Found: KOH, by titration with $N/10$-H_2SO_4 to phenolphthalein, 31·0. $C_{12}H_{22}O_{11}$,3KOH requires KOH, 32·9%).

(2) *From sucrose.* Sucrose (5 g.), dissolved in water (7 c.c.), was mixed with alcohol until the precipitation point was almost reached; alcoholic potassium hydroxide (50 c.c., 10%) was then rapidly added, and the precipitated derivative treated as in (1) (Found: KOH, 28·1%).

The Formation of Potassium Hydroxide–Sucrose under Different Conditions.—Some indication of the amounts of potassium hydroxide taken up by sucrose under various conditions was obtained as in Part I (*loc. cit.*). A known volume of a sucrose solution in 80% alcohol was treated with a definite volume of alcoholic potassium hydroxide solution of known strength. Removal of the precipitated compound by filtration through a Gooch crucible and titration of the equilibrium solution then indicated the quantity of alkali that had been removed by the sucrose. In the case of large excesses of sucrose relative to the amount of alcoholic potassium hydroxide solution, all the alkali was removed. Therefore the solubility of the addition compounds under the conditions studied was insufficient to affect the results appreciably. Typical results are as follows:

Total concn. of sucrose, %	1·2	1·25	2·1	4·0	4·5
Concn. of KOH, N { Initial	1·24	1·33	0·96	0·22	0·08
{ Final	1·14	1·22	0·80	0·03	nil.
KOH combined, % { Method (1).....	46·8	49·3	42·7	26·6	10·0
{ ,, (2).....	45·0	45·0	40·8	20·5	10·5

The results are similar to those previously recorded in the case of maltose (see Part I), for, whilst in the more concentrated alkaline solutions the composition approximates to $C_{12}H_{22}O_{11}$,3KOH (100 g. of the disaccharide require 49·2 g. of potassium hydroxide), under less rigorous conditions $C_{12}H_{22}O_{11}$,2KOH and even $C_{12}H_{22}O_{11}$,KOH appear to exist, indicating the instability of the tripotassium derivative. It is at present impossible to decide by analytical methods whether decomposition to a mixture of sucrose and potassium hydroxide or to the lower types of addition compound takes place on washing with alcohol.

Potassium Hydroxide–Sucrose and Methyl Sulphate.—The dry compound (45 g.) prepared as in (2) was stirred with dry, neutral methyl sulphate (90 c.c.) for 10 minutes at 60° and for 5 minutes at 70—75°, at the end of which time the mass coagulated. At this point the flask was cooled, the liquid removed, and the product washed with acetone and dissolved in hot methyl alcohol (200 c.c.). On cooling, potassium methyl sulphate separated, which was filtered off, and excess of alcoholic potassium hydroxide was added, followed by ether (1000 c.c.). The potassium hydroxide derivative so isolated weighed 40 g.

Isolation of a Trimethyl Sucrose Penta-acetate.—After acidification with acetic acid and removal of the solvent, the syrup was acetylated by treatment for 2·5 hours with acetic anhydride (50 c.c.) and anhydrous sodium acetate (8 g.). The mixture was poured into water, neutralised by sodium bicarbonate, and extracted with chloroform. Removal of the solvent after drying with sodium sulphate yielded a non-reducing glass (4·8 g.). $[\alpha]_D^{20°} = +52°$ in acetone (c, 1) (Found: C, 50·3; H, 6·5; OMe, 15·4; CH_3·CO, 37·0. Calc. for $C_{25}H_{38}O_{16}$: C, 50·6; H, 6·4; OMe, 15·6; CH_3·CO, 36·2%).

Deacetylation and Hydrolysis of Trimethyl Sucrose Penta-acetate.—Deacetylation after Zemplén (*Ber.*, 1923, **56**, 1705) yielded a solution (60 c.c.), which was hydrolysed with oxalic acid (1 g.) at 90—100° until a constant rotation was reached (3 hours). $[\alpha]_D^{20°}$ approx. $+20°$ (c, 4). After neutralisation with calcium carbonate, filtration, and concentration, the syrupy mixture of methylated sugars showed $[\alpha]_D^{20°} = +19°$ in water (c, 1) [Found: OMe, 23·2. Calc. for $C_6H_{11}O_5(OMe) + C_6H_{10}O_4(OMe)_2$: OMe, 23·1%].

Alternative Method for isolating the Mixture of Methylated Sugars.—A more rapid method was used with success as follows. Following methylation of the dry potassium hydroxide derivative (70 g.) and the precipitation of the unchanged sucrose, the excess of potassium hydroxide was removed by the passage of carbon dioxide through the alcohol–ether solution. After removal of the precipitated potassium carbonate and evaporation of the solvent, a syrup (5 g.)

was obtained. Hydrolysis under the same conditions as before yielded the mixed methylated sugars. It was sometimes observed that, unless very large quantities of ether were employed to complete the precipitation of the potassium hydroxide addition compounds during the removal of unchanged sucrose, a small quantity of sucrose avoided precipitation, with the result that the syrup had a low methoxyl content (17%) and a low specific rotation after hydrolysis. This appeared to be due to the greater solubility of the derivatives in mixtures of methyl with ethyl alcohol. After hydrolysis, however, further treatment of the mixed sugars in ethyl-alcoholic solution with potassium hydroxide served to remove any glucose or fructose formed by the hydrolysis.

Separation of the Glucose and Fructose Fractions.—Mixtures of methylated sugars prepared as described above of methoxyl content 23% (5 g.) were dissolved in dry methyl alcohol containing hydrogen chloride (0·5 g.) and kept at 15° for 48 hours. Acid was removed with barium carbonate and after filtration the solution was evaporated. Acetylation was carried out by dissolving the product in pyridine (20 c.c.) and treating the solution with a mixture of pyridine (25 c.c.) and acetic anhydride (25 c.c.) at 70° for 20 minutes, followed by standing at 15° for 2 days. The solution was poured into water and extracted with chloroform, from which dissolved pyridine was removed by washing with dilute sulphuric acid. After removal of solvent the syrup (6·5 g.) was subjected to distillation under 0·03 mm. pressure: (1) 2·1 g. (bath temp. 145—160°), $n_D^{20°}$ 1·4470; (2) 0·5 g. (bath temp. 160—170°), $n_D^{20°}$ 1·4475; (3) 2·0 g. (bath temp. 170—180°), $n_D^{20°}$ 1·4510; (4) 0·3 g. (bath temp. 170—200°), $n_D^{16°}$ 1·4550; residue 1·7 g. Redistillation of fractions (1) and (2) appeared to yield a homogeneous product, practically the whole distilling at a bath temp. of 146°/0·04 mm.; $n_D^{18°}$ 1·4475, $[\alpha]_D^{21°}$ +24° in chloroform (*c*, 1) (Found: OMe, 30·6; CH_3·CO, 28·4. Calc. for $C_{13}H_{22}O_8$: OMe, 30·4; CH_3·CO, 28·1%). The compound would thus appear to be a dimethyl methylglycoside diacetate. On the other hand, fraction (3) showed $[\alpha]_D^{21°}$ + 33·0° in chloroform (*c*, 1·5) (Found: OMe, 19·0; CH_3·CO, 38·9. Calc. for $C_{14}H_{22}O_9$: OMe, 18·6; CH_3·CO, 38·6%). This was evidently a monomethyl glycoside triacetate.

Identification of the Fraction of High Methoxyl Content as a Derivative of 1 : 6-*Fructofuranose.*—1·0 G. was deacetylated according to Zemplén (*loc. cit.*) and this was followed by hydrolysis in contact with *N*/10-sulphuric acid for 2 hours at 90°, during which period the rotation only fell slightly but the solution rapidly became strongly reducing. No crystalline osazone could be isolated on heating with phenylhydrazine and acetic acid in the usual manner. Two methylations with methyl sulphate (10 c.c.) and sodium hydroxide (25 c.c., 30%) (Haworth, J., 1915, **107**, 8) followed and the full methoxyl content was introduced by one treatment with methyl iodide (25 c.c.) and silver oxide (4 g.) at 43° during 6 hours. After being worked up in the usual way, the product was distilled and yielded 0·6 g. of a fraction at a bath temp. of 100°/0·03 mm., $n_D^{15°}$ 1·4430, and having all the properties of tetramethyl methylfructofuranoside. This derivative was characterised by its conversion into crystalline 2 : 3 : 4 : 6-tetramethyl fructofuronamide by direct oxidation with nitric acid. The fructofuranoside (0·4 g.) was treated with nitric acid (3 c.c., *d* 1·42) for 90 minutes at 70—90°. When all action had ceased, an excess of water was added and continuous distillation with the addition of water was carried out during 6 hours. The residue was dried with benzene and esterified with methyl-alcoholic hydrogen chloride (20 c.c., 3%) for 3·5 hours. The solution was neutralised with silver oxide and after filtration and removal of the solvent was methylated with silver oxide and methyl iodide. Distillation from a bath at 135°/0·03 mm. yielded a non-reducing ester (0·3 g.), $n_D^{18°}$ 1·4430, which was converted into the amide by contact for 3 days with methyl-alcoholic ammonia. On removal of solvent the characteristic long needles appeared of the 2 : 3 : 4 : 6-tetramethyl fructofuronamide (0·2 g.) described by Avery, Haworth, and Hirst (J., 1927, 2313). $[\alpha]_D^{20°}$ − 81° in water (*c*, 0·5), m. p. 100—101° alone or in admixture with a specimen prepared directly from tetramethyl fructofuranose (Found: OMe, 48·1; N, 5·6. Calc. for $C_{10}H_{19}O_6N$: OMe, 49·8; N, 5·6%).

Direct Oxidation of the Diacetyl Dimethyl Methylfructofuranoside.—A second portion (1·4 g.) was oxidised with nitric acid (5 c.c., *d* 1·42) for 2 hours at 70—95° and the excess of nitric acid was removed by continuous distillation with the addition of water for 24 hours. The reducing syrup obtained appeared to be the monomethyl analogue of the trimethyl lactol acid described above (Found: OMe, 14·4. Calc. for $C_7H_{12}O_7$: OMe, 14·9%).

Oxidation of the Monomethyl Lactol Acid.—A solution of the above syrup in water was acidified with *N*-sulphuric acid (11 c.c.), and the volume made up to 40 c.c. by the addition of water. This solution was titrated with 6·7 c.c. of *N*-barium permanganate. Excess of barium hydroxide was added and after some hours this was neutralised with carbon dioxide.

Filtration yielded a solution, which was evaporated (diminished pressure) to yield a barium salt (0·8 g.) [Found: OMe, 11·1; Ba, 30·3. Calc. for $(C_6H_{11}O_6)_2Ba$: OMe, 12·5; Ba, 27·7%].

Isolation of 5-Methyl γ-Arabonolactone.—Barium was removed by the addition of the calculated quantity of *N*/10-sulphuric acid and the aqueous solution was evaporated under diminished pressure to yield a glass (0·5 g.), which was heated at 90—100° for some hours (Found: C, 44·0; H, 6·4; OMe, 19·3. Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·2; OMe, 19·1%). It showed $[\alpha]_D^{18°}$ + 40° (30 mins.); + 35° (1 day); + 31° (2 days); + 29° (8 days); + 27° (17 days, constant value); in water (*c*, 0·4). This slow hydrolysis is in harmony with the presence of a γ-lactone.

Identification of the Fraction of Low Methoxyl Content as a Derivative of 6-Methyl Glucose.—A portion of the fraction showing $n_D^{20°}$ 1·4510 (0·7 g.) was hydrolysed with *N*/6-sulphuric acid for 2 hours after deacetylation by the method of Zemplén (*loc. cit.*). Neutralisation with barium carbonate, filtration, concentration, and acetylation with pyridine and acetic anhydride yielded a syrup, which, dissolved in acetic acid (1 c.c.), was converted into the acetobromo-compound by contact for 2 hours with acetic acid saturated with hydrogen bromide (1·2 c.c.). The product was mixed with chloroform, and the chloroform solution washed with water and sodium bicarbonate solution, dried, and concentrated to a syrup under diminished pressure. Solution in dry methyl alcohol, followed by shaking with dry silver carbonate for 24 hours, gave rise to a non-reducing syrup, which failed to crystallise, $[\alpha]_D^{18°}$ + 73° in chloroform (*c*, 2·2) (Found: OMe, 17·4. Calc. for $C_{14}H_{22}O_9$: OMe, 18·6%). Nucleation with a specimen of 4-methyl 2 : 3 : 6-triacetyl β-methylglucoside failed to induce crystallisation.

Isolation of 2 : 3 : 4 : 6-Tetramethyl Glucopyranose.—This syrup was methylated once with methyl sulphate (30 c.c.) and sodium hydroxide (70 c.c. of 30%) in the usual way, followed by treatment with silver oxide (5 g.) and methyl iodide (20 c.c.). The syrup obtained was distilled under 0·03 mm. from a bath at 100° and had all the properties of tetramethyl methylglucopyranoside. By hydrolysis for 8 hours with hydrochloric acid (5%), neutralisation with barium carbonate, concentration, and extraction with ether, crystalline 2 : 3 : 4 : 6-tetramethyl glucopyranose was obtained, m. p. 82·3° alone or in admixture with an authentic specimen. $[\alpha]_D^{18°}$ + 83° (equil.) in water (*c*, 0·6) (Found: OMe, 51·8. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%).

The Reaction with Phenylhydrazine and the Isolation of 6-Methyl Glucosazone.—The remainder of the glucose fraction (1·0 g.) was deacetylated and hydrolysed as above to yield the syrupy monomethyl glucose. It was attempted to prepare 2-methyl glucose phenylhydrazone by the method of Brigl and Schinle (*Ber.*, 1929, **62**, 1716). The product, dissolved in methyl alcohol (1 c.c.), was treated with phenylhydrazine (0·6 c.c.) and a drop of glacial acetic acid at 15° for 24 hours. Removal of solvent in a vacuum, followed by nucleation with authentic 2-methyl glucose phenylhydrazone, gave rise to no crystals. A further quantity of phenylhydrazine (2 c.c.), acetic acid (3·0 c.c.), sodium acetate (10 g.), and water (30 c.c.) were added together with sodium bisulphite (1·0 g.). This mixture was heated for 3 hours at 90—100°; on cooling, an osazone separated, which was removed. A further quantity was precipitated on dilution, followed by further heating (8 hours). Total yield of crude product (0·5 g.) (cf. Helferich and Günther, *Ber.*, 1931, **64**, 1276). Several recrystallisations from aqueous pyridine raised the m. p. from 172° to 183—186°, the osazone when pure appearing in pale yellow needles. The m. p. showed no depression in admixture with a specimen of 6-methyl glucosazone prepared by the method of Helferich and Günther (*loc. cit.*), but with 3-methyl glucosazone (m. p. 179°) the m. p. was depressed to 163°. $[\alpha]_D^{20°}$ — 69° in ethyl alcohol (*c*, 0·4) (Found: OMe, 8·1; N, 14·75. Calc. for $C_{19}H_{24}O_4N_4$: OMe, 8·3; N, 15·0%). The properties were thus in accord with those of 6-methyl glucosazone.

The Reaction of a Mixture of Glucose and Potassium Hydroxide with Methyl Sulphate.—Glucose (10 g.) and powdered potassium hydroxide (3 g.) which had been dried in a vacuum over phosphoric oxide were mixed with dry neutral methyl sulphate (50 c.c.) and stirred at 45° (5 mins.) and 70° (5 mins.). After the separation of the excess of glucose and acetylation (see Part I, *loc. cit.*) a syrupy acetate (0·3 g.) was obtained which was still reducing (Found: OMe, 3·0%, corresponding to 0·05 g. of methylglucoside). This was the highest yield obtained in three experiments.

Thanks are expressed to Professor W. N. Haworth, F.R.S., and Dr. E. L. Hirst, F.R.S., for the gift of specimens of a number of the reference compounds used in this investigation and to the Earl of Moray Endowment for a grant in aid of these researches.

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, March 19th*, 1935.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY & SONS, LIMITED, BUNGAY, SUFFOLK.

XI

ADDITION COMPOUNDS OF THE CARBOHYDRATES. PART III. POTASSIUM HYDROXIDE DERIVATIVES OF CELLOBIOSE, LACTOSE, AND GALACTOSE

BY
EDMUND G. V. PERCIVAL
AND
GEOFFREY G. RITCHIE

Reprinted from the Journal of the Chemical Society, December, 1936.

Reprinted from the Journal of the Chemical Society, 1936.

390. *Addition Compounds of the Carbohydrates. Part III. Potassium Hydroxide Derivatives of Cellobiose, Lactose, and Galactose.*

By Edmund G. V. Percival and Geoffrey G. Ritchie.

In Part I (J., 1934, 1160) it was pointed out that certain disaccharides appear to unite with more than one molecular proportion of potassium hydroxide. This has now been confirmed for cellobiose, which in the presence of excess of the reagent forms a *complex*, $C_{12}H_{22}O_{11}$,2KOH, as indicated by titration experiments. Controlled methylation resulted in the isolation of a monomethyl methylcellobioside, from which on hydrolysis and suitable treatment 6-methyl glucosephenylosazone has been isolated. These results can be explained if we consider the reducing group and one of the primary alcoholic residues to be concerned in the union. Since glucose itself only takes up one potassium hydroxide residue, it seems reasonable to suppose that in the cellobiose complex the second potassium hydroxide residue is to be found in the non-reducing glucopyranose unit (I).

Indirect support for this view was afforded by a study of the compounds which lactose forms with potassium hydroxide, since in this case the production or otherwise of methylated galactose derivatives would indicate whether the non-reducing unit were involved or not. Titration experiments showed that lactose combines with more potassium hydroxide than does cellobiose under the same conditions, the results indicating the presence of a mixture of $C_{12}H_{22}O_{11}$,2KOH and $C_{12}H_{22}O_{11}$,3KOH. Methylation under anhydrous conditions, followed by acetylation, gave a non-reducing syrup, from which, by hydrolysis, acetylation and distillation in a high vacuum, a triacetyl dimethyl hexose and a tetra-acetyl monomethyl hexose were isolated. Complete methylation of both these products and the isolation from each in good yield of crystalline tetramethyl galactopyranose anilide, together with the absence of any glucose derivatives, showed that, except for the normal reaction at the reducing group, substitution had taken place exclusively in the galactose portion of the lactose molecule.

The monomethyl galactose, unlike 6-methyl galactose, gave no crystalline phenylhydrazone. On heating with phenylhydrazine, however, a crystalline osazone (yield, 40% of the theoretical) was obtained which contained no methoxyl and was identical with galactosazone. No mucic acid could be isolated on oxidation of the original syrup with nitric acid; therefore it is necessary to assign the methoxyl residue to position 2. Robertson and Lamb (J., 1934, 1321) have recorded a case where the 2-methyl group in 2 : 3-dimethyl galactose is eliminated on osazone formation.

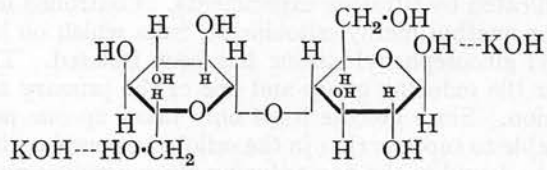
Similarly the dimethyl galactose gave a monomethyl galactosazone (m. p. 150°), and a rigorous search failed to reveal the presence of any dimethyl galactosazone. This monomethyl galactosazone was clearly not identical with 6-methyl galactosazone, m. p. 204° (Munro and Percival, this vol., p. 640), or 3-methyl galactosazone, m. p. 176—179° (Robertson and Lamb, *loc. cit.*), and 5-methyl galactosazone was excluded by the isolation of tetramethyl galactopyranose anilide. Furthermore the osazone was found to be identical with authentic 4-methyl galactosazone* kindly supplied by Professor W. N. Haworth, F.R.S., thus indicating that the dimethyl galactose was 2 : 4-dimethyl galactose, and further experiments confirmed this view. Glycoside formation with methyl-alcoholic hydrogen chloride in the cold followed a different course from that described by Robertson and Lamb (*loc. cit.*) for 2 : 3-dimethyl galactose, since the rotation remained strongly positive, indicating the absence of galactofuranosides, and this was confirmed by titration experiments after Levene, Raymond, and Dillon (*J. Biol. Chem.*, 1932, **95**, 699). Oxidation with nitric acid, followed by esterification and amide formation, yielded no *d*- or *i*-dimethoxysuccinamides, the presence of adjacent methyl groups (*e.g.*, in positions 2 : 3 or 3 : 4) thus being excluded.

From this examination of the products of the controlled methylation of the potassium hydroxide–lactose complexes, it appears that substitution has taken place at the reducing group of the glucopyranose unit and at the hydroxyl groups at positions 2 and 4 in the

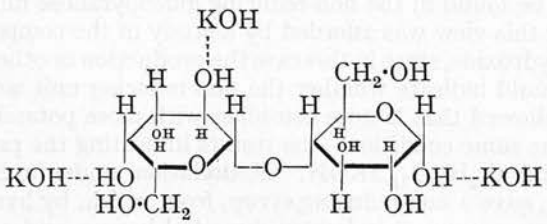
* Private communication.

galactopyranose residue. It is suggested, therefore, that lactose associates itself with potassium hydroxide residues at these points (II).

The difference in the results obtained for cellobiose and lactose is not due to the greater affinity of galactose than glucose for potassium hydroxide, since galactose forms a compound $C_6H_{12}O_6$,KOH, and this formulation is supported by methylation with dry methyl sulphate, no evidence for substitution beyond the methylgalactoside stage being found. While, therefore, it is reasonable to suppose that the tendency is for the reaction to take place in the sugar unit remote from the reducing group, there is no explanation yet avail-



(I.)



(II.)

able for the anomalous behaviour of lactose, since the other disaccharides so far examined, cellobiose and sucrose (Part II, J., 1935, 648), undergo substitution in the primary alcoholic residues.

In Part I (*loc. cit.*) it was suggested that the addition compounds were comparable with the hydrates of the alkali hydroxides and a structure involving co-ordination through hydrogen was proposed. In the light of modern views, however, this is doubtful and an alternative system is provided by the theory of resonance. At this stage, therefore, it does not seem desirable to define the union between the sugars and alkali hydroxides other than by some type of loose combination probably best represented by a dotted line in the manner of the residual valencies of Werner.

Experimental.

Titration Experiments with Cellobiose.—Cellobiose (0·2 g.), prepared by Zemplén's method (*Ber.*, 1923, **56**, 1705) from the octa-acetate, was dissolved in alcohol (6 c.c., 90%), standard alcoholic potassium hydroxide (20 c.c.) added, and the mixture kept for 10 minutes. The precipitate was removed by filtration, and an aliquot portion of the filtrate titrated with standard acid (method 1). The precipitate was drained, washed with the minimum quantity of alcohol, dissolved in water, and titrated (method 2). The results are below :

Total concn. of cellobiose, %	0·57	0·77	0·88
Concn. of KOH, *N* initial	0·839	0·742	0·76
Concn. of KOH, *N* final	0·807	0·697	0·69
KOH combined, % method (1)	32·5	33·2	32·9
KOH combined, % ,, (2)	25·7	25·1	23·0

For $C_{12}H_{22}O_{11}$,2KOH, 100 g. of cellobiose require 32·8 g. of potassium hydroxide.

Typical Preparation of Potassium Hydroxide–Cellobiose.—Cellobiose octa-acetate (20 g.), prepared by Haworth and Hirst's method (J., 1921, **119**, 193), was made into a paste with alcohol (40 c.c.), potassium hydroxide (40 g.) in alcohol (175 c.c.) stirred in, and stirring continued for 2 hours in a closed flask. The insoluble *product* (14 g.) was then filtered off, washed quickly with alcohol and ether, and dried in a vacuum over phosphoric oxide [Found : KOH (titration), 22·1. $C_{12}H_{22}O_{11}$,2KOH requires KOH, 24·7%].

Reaction with Methyl Sulphate.—The method described in Parts I and II (*loc. cit.*) was employed, the dry addition compound (8 g.) being stirred with dry, neutral methyl sulphate for 5 minutes at 35—40° and for 10 minutes at 70°. After removal of the potassium methyl sulphate, and unchanged cellobiose (4·9 g.) by precipitation with alcoholic potassium hydroxide,

the residue on acetylation yielded a syrup (4·7 g.), which partly crystallised on trituration with alcohol. The crystals (1·4 g.), m. p. 178°, $[\alpha]_D^{20°}$ — 22° in chloroform (*c*, 1·5), were hepta-acetyl β-methylcellobioside (Found : C, 50·0; H, 5·9; OMe, 4·5. Calc. for $C_{27}H_{38}O_{18}$: C, 49·9; H, 5·9; OMe, 4·9%).

Examination and Hydrolysis of the Residual Syrup.—The non-reducing syrup (3·2 g.), $[\alpha]_D^{20°}$ + 2° in chloroform (*c*, 5·0), appeared to be a hexa-acetyl monomethyl methylcellobioside (Found : OMe, 8·5; CH_3·CO, 41·4. Calc. for $C_{26}H_{38}O_{17}$: OMe, 9·9; CH_3·CO, 41·5%). It was accordingly deacetylated by Zemplén's method (*loc. cit.*) and hydrolysed in hydrochloric acid (80 c.c., 7%) at 90° until a constant rotation was attained :

Time (minutes)	0	65	125	220	300	380
$[\alpha]_D^{20°}$	+3°	13°	27°	37°	50°	50°

After neutralisation with silver carbonate and filtration, the solvent was removed at 50°/15 mm. to yield a reducing syrup (1·9 g.).

Removal of glucose. Potassium hydroxide (1 g.) in alcohol (15 c.c.) was added to the syrup dissolved in alcohol (20 c.c.). The precipitate (1·2 g.) was filtered off after 15 minutes, dissolved in water, acidified with acetic acid, and treated with phenylhydrazine to yield glucosephenylosazone (0·3 g.), m. p. 206° (Found : OMe, nil).

Isolation of 6-methyl glucosephenylosazone. The filtrate was acidified with acetic acid, alcohol removed under diminished pressure, and the residue dissolved in water (10 c.c.) and heated with phenylhydrazine (1 g.) and glacial acetic acid (1 c.c.) at 90° for 30 minutes. An orange osazone (0·5 g.) was obtained, which was recrystallised from aqueous pyridine; m. p. 180—184°, $[\alpha]_D^{20°}$ — 70° in 50% alcohol–pyridine (*c*, 0·3) (Found : C, 61·2; H, 6·6; OMe, 7·6; N, 14·75. Calc. for $C_{19}H_{24}O_4N_4$: C, 61·3; H, 6·45; OMe, 8·3; N, 15·0%). This osazone agreed in properties with 6-methyl glucosazone (see Part II, *loc. cit.*) and its m. p. was unaltered by authentic 6-methyl glucosazone, but was depressed to 164° by 3-methyl glucosazone (m. p. 179°).

Potassium Hydroxide–Lactose. Titration Experiments.—An approximate estimate of the alkali-combining capacity of lactose was obtained by the titration method previously used. Lactose monohydrate, dissolved in 75% alcohol, was employed and the results are below :

Total concn. of lactose, %		0·72	0·94	1·00	1·01
Concn. of KOH, *N*	initial	0·221	0·427	0·747	0·774
	final	0·181	0·373	0·685	0·704
KOH combined, %	method (1)...	30·9	32·1	35·1	39·2
	,, (2)...	28·1	26·0	34·2	41·6

$C_{12}H_{22}O_{11}$,3KOH requires 49 g. of potassium hydroxide for 100 g. of lactose. Thus it would appear that, in the higher concentrations of alkali examined, some of this higher compound was present; it was found impracticable to use very concentrated alkaline solutions, since brown solutions were thereby produced.

Preparation of Potassium Hydroxide–Lactose.—Lactose octa-acetate (35 g.), prepared by Hudson and Johnson's method (*J. Amer. Chem. Soc.*, 1915, **37**, 1270), was suspended in alcohol (200 c.c.), potassium hydroxide (66 g.) in alcohol (1 l.) added, and the mixture stirred for 2 hours, the insoluble product (20 g.) being isolated as before (Found : KOH, 26·0 $C_{12}H_{22}O_{11}$,3KOH requires KOH, 32·9%).

Reaction with Methyl Sulphate.—The powdered product thus obtained (20 g.) was stirred with methyl sulphate under the conditions obtaining above. Potassium methyl sulphate was removed, followed by the removal of lactose by the addition of potassium hydroxide (7 g.) in alcohol (100 c.c.). Ether was then added to bring about the complete removal of unchanged lactose until the precipitate obtained was no longer reducing. The residual solution was acidified with acetic acid and evaporated under diminished pressure. The product was acetylated at 95° for 1 hour with acetic anhydride (120 c.c.) and anhydrous sodium acetate (23 g.). After the usual treatment a non-reducing syrup (4·4 g.) was obtained (OMe, 12·1%). This was deacetylated by Zemplén's method (*loc. cit.*), and the product hydrolysed with sulphuric acid (1·5*N*) at 95° till a constant rotation was reached :

Time (minutes)	0	150	290	395	435	480
$[\alpha]_D^{20°}$	+18°	34°	41°	45·6°	47·4°	48° (const.)

The solution was neutralised with barium carbonate and evaporated to dryness at 50°/15 mm., yielding a syrup.

Separation of the Products of Hydrolysis.—The syrup was extracted with boiling alcohol (50 c.c.). To the extracts was added an alcoholic solution of potassium hydroxide (2 g. in 40 c.c.), and a precipitate (A) was filtered off rapidly, washed with alcohol, and dried. The addition of dry ether (250 c.c.) to the filtrate deposited another precipitate (B), which was collected and dried. The filtrate was neutralised with acetic acid and evaporated to dryness, and the residue (C) acetylated with acetic anhydride (35 c.c.) and anhydrous sodium acetate (7 g.) at 100° for 1 hour, a brown syrup being obtained by the usual method of isolation. The dry sugar–alkali compounds (A) and (B) were similarly acetylated. (A) Yield, 0·98 g.; OMe, 2·0%. (B) 0·49 g.; OMe 8·1%. (C) 0·83 g.; OMe, 15·5%. An approximate fractionation of the mixture had thus been achieved. Fraction (B) distilled almost completely at 182—183° (bath temp.)/0·04 mm. to yield a colourless reducing syrup (Found : OMe, 8·3. Calc. for $C_{15}H_{22}O_{10}$: OMe, 8·6%). Fraction (C) was distilled at 155—162° (bath temp.)/0·05 mm. (Found : OMe, 16·7. Calc. for $C_{14}H_{22}O_9$: OMe, 18·6%).

Examination of the Monomethyl Hexose Acetate.—Complete methylation. In order to determine whether this was derived from glucose or galactose a portion of the syrup (B) (0·5 g.; OMe, 8·3%), dissolved in acetone (10 c.c.) and water (10 c.c.), was methylated with methyl sulphate (10 c.c.) and aqueous sodium hydroxide (25 c.c., 30%) with the usual precautions for the methylation of a reducing sugar. After isolation of the product two further methylations with methyl iodide (10 c.c.) and silver oxide (2 g.) were conducted and the product was distilled at 113°/0·08 mm. to yield a clear, mobile, non-reducing syrup (0·26 g.). The glucosidic methoxyl residue was removed by heating for 2 hours at 100° in hydrochloric acid (10 c.c., 7%). The solution was neutralised with barium carbonate, alcohol was added to precipitate barium salts, which were filtered off, and the solution was evaporated to dryness under diminished pressure. The residue was extracted three times with ether, and from the filtered extracts on evaporation a syrup (0·2 g.) was obtained. Inoculation with tetramethyl glucopyranose failed to induce crystallisation.

Tetramethyl galactopyranose anilide. The anilide was prepared from the above sugar by boiling the syrup (0·19 g.) under reflux with aniline (0·6 g.) in alcohol (2 c.c.) for 3 hours. White needles (0·14 g.) separated on cooling, which after two recrystallisations from alcohol had m. p. 193°, not depressed in admixture with an authentic specimen. No product corresponding to tetramethyl glucopyranose anilide could be isolated from the mother-liquors.

Attempted phenylhydrazone formation. The method described by Munro and Percival (*loc. cit.*) for the isolation of 6-methyl galactosephenylhydrazone was applied to the deacetylated monomethyl galactose (B), but in spite of various modifications no crystalline phenylhydrazone could be isolated.

Osazone formation. A specimen of the syrupy monomethyl galactose tetra-acetate (0·58 g.) was deacetylated by Zemplén's method and treated with phenylhydrazine acetate. The first crop of osazone (0·17 g.) had m.p. 175°, which was raised to 186—188° by one recrystallisation (Found : OMe, nil). The m. p. was not depressed in admixture with authentic galactosazone. A second crop (0·08 g.), m. p. 186—188°, was obtained (OMe, nil). That this galactosazone could not have been derived from free galactose was shown by repeated failures to isolate mucic acid, from the deacetylated syrup used for osazone formation, by oxidation with nitric acid (*d* 1·15). It is therefore necessary to conclude that the galactosazone was derived from 2-methyl galactose.

Examination of the Dimethyl Hexose Acetate.—The colourless syrup was reducing to Fehling's solution and had $[\alpha]_D^{20°}$ + 59° in chloroform (*c*, 0·8); $n_D^{20°}$ 1·4525° (Found : C, 50·0; H, 6·4; OMe, 16·7. Calc. for $C_{14}H_{22}O_9$: C, 50·3; H, 6·6; OMe, 18·6%).

Complete methylation was carried out as previously described for 2-methyl galactose, and tetramethyl galactopyranose anilide (yield, 46%), m. p. 192°, unchanged in admixture with an authentic specimen, was obtained as before. No glucose derivatives could be detected and it was concluded that the specimen was triacetyl dimethyl galactopyranose.

Osazone formation.—Triacetyl dimethyl galactose (0·27 g.) was deacetylated by Zemplén's method. To the solution of the deacetylated sugar in water (20 c.c.) were added phenylhydrazine (0·5 g.), acetic acid (2 c.c.), sodium acetate (1 g.), and a trace of sodium bisulphite, the mixture being heated at 90—100°. Osazone formation was slow, but after 3 hours a yellow oil (0·07 g.) separated which solidified on standing (OMe, 7·5%). Purification by solution in chloroform, filtration, and precipitation by light petroleum gave a yellow solid (0·05 g.) (OMe, 8·0%), and the solution on evaporation gave a yellow glass (0·01 g.) (Found : OMe, 8·1. Calc. for $C_{19}H_{24}O_4N_4$: OMe, 8·3%). It thus appeared that from a dimethyl sugar a monomethyl osazone was obtained. As this would establish one of the methyl groups in the 2-position

an exhaustive search for the presence of dimethyl osazone was carried out. A further crop of osazone (0·01 g.) had m. p. 150° (OMe, 7·2%). After six recrystallisations from aqueous alcohol a small amount of galactosazone, m. p. 186°, not depressed in admixture with an authentic specimen, was obtained (OMe, nil). This was undoubtedly derived from a small amount (*ca.* 10%) of 2-methyl galactose which could not be completely separated from the initial material. The search for a dimethyl osazone was continued by extraction of the original aqueous solution with chloroform, washing with acetic acid, drying, and evaporation (Found for the glass obtained : OMe, 4%).

Another series of experiments confirmed these results and five crops of osazone were isolated in a total yield of over 80% of the dimethyl galactose used. Four crops each had OMe, 7% and the other, OMe, 5%. The best crystalline fraction (20%) had m. p. 145—150° (OMe, 7·1%).

Identification of the osazone as 4-methyl galactosazone. The main portion of monomethyl osazone on recrystallisation from alcohol had m. p. 147—150° and showed no depression in admixture with an authentic specimen of 4-methyl galactosazone, m. p. 148—150° (Found : OMe, 7·1; N, 14·9. Calc. for $C_{19}H_{24}O_4N_4$: OMe, 8·3; N, 15·0%). No evidence was found for the presence of 6-methyl galactosazone (m. p. 204°).

The Course of Glycoside Formation.—A portion of the dimethyl galactose acetate was deacetylated by Zemplén's method and the aqueous extract of the free sugar was evaporated to dryness (diminished pressure). The product was dissolved in methyl alcohol (10 c.c.) containing 1·9% of hydrochloric acid; the concentration of sugar being 0·94% :

Time (hours)	0	19	43	67
$[\alpha]_D^{18°}$	+49°	34°	33°	33° (const.)

The equilibrium solution was non-reducing. These values differ from those given by Robertson and Lamb (*loc. cit.*) for 2 : 3-dimethyl galactose, the rotation of which under the same conditions fell during 7 days from $[\alpha]_D^{15°}$ + 38° to − 24°.

The semi-quantitative method of Levene, Raymond, and Dillon (*loc. cit.*; see also this vol., p. 643) was employed to follow the course of glycoside formation at room temperature. Methyl-alcoholic hydrogen chloride (0·5%) was employed, the concentration of sugar in the solution being 9 mg./c.c. The reducing values were corrected for the hydrolysis of the pyranoside, which was found to take place to the extent of 13%.

	0·01*N*-$Na_2S_2O_3$, c.c.		Free sugar, %.					
Time (hours).	Before hydrolysis.	After hydrolysis.	Before hydrolysis.	After hydrolysis.	Corrected.	Free sugar, %.	Furanoside, %.	Pyranoside, %.
0	2·20	2·40	100	100	100	100	0	0
1	1·70	2·20	77·5	92	91	77·5	13·5	9
23·5	0	0·70	0	29	16	0	16	84
27·0	0	0·67	0	28	15	0	15	85

If the sugar in question was, as supposed, 2 : 4-dimethyl galactose, then no furanoside should have been formed. The small amount present is accounted for by the presence of 2-methyl galactose in the sample used. Under the experimental conditions which favour furanoside formation the main portion is obtained as pyranoside, and this is evidence for the conclusion that position 4 is occupied by a methyl group.

The Oxidation of Dimethyl Galactose.—Triacetyl dimethyl galactose (0·52 g.) was deacetylated by Zemplén's method, and the aqueous solution of the sugar evaporated under reduced pressure. The product was dissolved in nitric acid (5 c.c., *d* 1·44), and after standing at room temperature for 2 hours was heated at 85—90° for 6 hours. The excess of nitric acid was then removed by distillation at 70° under reduced pressure for 48 hours with the continuous addition of water and the product was subjected to esterification, distillation, and amide formation according to the method described by Herbert, Hirst, *et al.* (J., 1933, 1286). After 4 days, the syrup obtained on evaporation of the solution of the ester in ammoniacal methyl alcohol was examined for both *d*- and *i*-dimethoxysuccinamides with a negative result. This experiment was repeated. The two methyl groups present in the dimethyl galactose could not therefore occupy adjacent positions in the molecule.

Potassium Hydroxide–Galactose. Titration Experiments.—On account of the low solubility of galactose in alcohol and since the presence of water led to the formation of syrupy products it was necessary to use penta-acetyl galactose for the titration experiments. The method had to

be modified, since both deacetylation and compound formation were responsible for removing alkali from solution and furthermore the amount of potassium hydroxide required for deacetylation was not that theoretically required for 5 acetyl groups owing to the catalytic nature of the process of deacetylation by alkali in alcoholic solution (Zemplén, *loc. cit.*). To each of two similar suspensions of galactose penta-acetate (0·2 g.) in absolute alcohol (5 c.c.) a definite quantity of standard alcoholic potassium hydroxide was added. One sample was then filtered, the filtrate titrated, and from the decrease in alkalinity the total amount of potassium hydroxide removed from solution was determined. By titration of the second sample without filtration the quantity of alkali required for deacetylation was determined and by difference the potassium hydroxide engaged in compound formation was found. A second value was obtained by direct titration of the compound as before :

Concn. of KOH, *N* { initial	0·27	0·41	0·61
Concn. of KOH, *N* { final	0·07	0·18	0·41
KOH, g., required to deacetylate 100 g. of penta-acetate	68·5	72·0	69·0
KOH, g., combined with 100 g. of galactose { indirect method	18·0	32·2	27·0
KOH, g., combined with 100 g. of galactose { direct method	25·3	26·1	27·5

Potassium Hydroxide–Galactose.—Galactose penta-acetate (18 g.), suspended in absolute alcohol (200 c.c.), was stirred with potassium hydroxide (45 g.) in alcohol (1 l.) in a closed flask. The *product* (10 g.), isolated in the usual way, was a white deliquescent powder more sensitive to moisture than the compounds previously described (Found : KOH, 21·2. $C_6H_{12}O_6$,KOH requires KOH, 23·7%).

Methylation. The compound (10 g.) was mechanically stirred in a closed flask with neutral methyl sulphate (75 c.c.) for 10 minutes at 34—40° and for 10 minutes at 70°. After the removal of potassium methyl sulphate, potassium hydroxide (8 g.) in alcohol (100 c.c.) was added, and the precipitate removed. Ether (1200 c.c.), added to the filtrate, brought down a further precipitate, which was discarded. The solution was then acidified with acetic acid and evaporated to dryness, and the residue acetylated, finally yielding a non-reducing syrup (4·5 g.) consisting of a mixture of α- and β-tetra-acetyl methylgalactosides; $[\alpha]_D^{18°}$ + 21·7° in chloroform (*c*, 2·3) (Found : OMe, 8·1. Calc. for $C_{15}H_{22}O_{10}$: OMe, 8·6%).

The authors thank the Carnegie Trust for the award of a Teaching Fellowship (E. G. V. P.) and a Scholarship (G. G. R.), and the Earl of Moray Endowment and Imperial Chemical Industries, Ltd., for grants.

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, September 30th*, 1936.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY & SONS, LIMITED, BUNGAY, SUFFOLK.

XII

MONOMETHYL HEXOSES. PART I. THE CONSTITUTION OF THE SUPPOSED 4-METHYL GLUCOSE

BY

JOHN MUNRO

AND

EDMUND G. V. PERCIVAL

Reprinted from the Journal of the Chemical Society,
July, 1935.

Reprinted from the Journal of the Chemical Society, 1935.

198. *Monomethyl Hexoses. Part I. The Constitution of the Supposed 4-Methyl Glucose.*

By John Munro and Edmund G. V. Percival.

During the investigation of the alkali addition compounds of the carbohydrates (J., 1934, 1160) it became clear that the assignment of structure by a methylation method depended on the isolation of derivatives of partly methylated sugars, usually in the form of the crystalline phenylhydrazones or phenylosazones. Whereas well-characterised derivatives of 2-, 3-, and 6-methyl glucose were already available, there was dubiety about the existence of such reference compounds for 4- and 5-methyl glucose, and it was considered desirable to remove all doubt on this point.

In 1925 Pacsu (*Ber.*, **58**, 1455) reported the isolation of 4-methyl glucose and of 4 : 5 : 6-trimethyl glucose by the methylation of acetone compounds of glucose dibenzyl mercaptal. This work was, however, challenged by Schinle (*Ber.*, 1931, **64**, 2361), who revealed that the so-called 4-methyl glucosazone was, in reality, impure glucosazone, an observation which led to the conclusion that the parent monomethyl sugar was 2-methyl glucose identical with that previously described by Hickinbottom (J., 1928, 3140) and Brigl and Schinle (*Ber.*, 1929, **62**, 1716; 1930, **63**, 2887). Schinle followed up these observations (*Ber.*, 1932, **65**, 318) by a study of the 4 : 5 : 6-trimethyl glucose reported by Pacsu, and found it to be a monomethyl glucose yielding an osazone, m. p. 158°. Accordingly this sugar was designated 4-methyl glucose, since the osazone was different from the apparently well-characterised derivatives obtained from 3-, 5- and 6-methyl glucose. Unfortunately, however, Schinle carried out no conclusive experiments to characterise the sugar in question, and the position became indefinite once more when Levene and Raymond (*J. Biol. Chem.*, 1932, **97**, 751) proved by the isolation of 2 : 3 : 4 : 6-tetramethyl glucopyranose by methylation of the supposed 5-methyl glucose of Ohle and v. Vargha (*Ber.*, 1929, **62**, 2435), that the supposed 5-methyl glucose was in reality 6-methyl glucose. Obviously when these new facts came to light Schinle's method of assignment of the 4-position to the methyl group in the sugar under review did not exclude a selection of the 5-position. As was to be expected, Levene and Raymond (*J. Biol. Chem.*, 1932, **97**, 763) re-examined the position and adduced evidence supporting the original conclusion of Schinle (*loc. cit.*), but a survey of this evidence reveals the fact that it too is not conclusive.

Instead of a critical examination of the " trimethyl glucose " of Pacsu, Levene and Raymond synthesised a crystalline compound described as 2 : 3 : 6-triacetyl 4-methyl β-methylglucoside and compared it with the corresponding derivative prepared directly from the sugar in question. The stages of their synthesis are: β-methylglucoside (I) ⟶ 4 : 6-benzylidene β-methylglucoside (II) ⟶ 2 : 3-dibenzoyl 4 : 6-benzylidene β-methylglucoside (III) ⟶ 2 : 3-dibenzoyl β-methylglucoside (IV) ⟶ 2 : 3 : 6-tribenzoyl β-methylglucoside (V) ⟶ 2 : 3 : 6-tribenzoyl 4-methyl β-methylglucoside (VI) ⟶ 4-methyl β-methylglucoside (VII) ⟶ 2 : 3 : 6-triacetyl 4-methyl β-methylglucoside (VIII). Levene and Raymond (*loc. cit.*) accept the view of Ohle and Spencker (*Ber.*, 1928, **61**, 2387) that (II) has a pyranoside form and argue therefore that positions 2 and 3 are available for benzoylation. They consider that, since (VIII) is originally derived from a normal glucoside, it cannot be substituted in position 5. But it is clear that no direct evidence is presented, at any rate after stage (II), that glucopyranosides are concerned. In addition, the possibility of the wandering of acyl groups during the methylation with methyl iodide and silver

oxide was not considered, and though Helferich and Günther (*Ber.*, 1931, **64**, 1276) record that 2 : 3 : 4-tribenzoyl β-methylglucoside passes on methylation into the corresponding 6-methyl derivative, it is well known that acetyl groups migrate during such treatment (Haworth, Hirst, and Teece, J., 1930, 1405; 1931, 2858). Since, although the interpretation of Levene and Raymond was by no means improbable, in our opinion the question at issue was not decisively proved, it was determined to put the matter to a critical test, from which the fact emerges that the 4-methyl glucose of Schinle has indeed that structure.

By a modified method the syrupy methylated glucose of Schinle (*loc. cit.*) was isolated. Complete methylation, followed by hydrolysis, yielded crystalline 2 : 3 : 4 : 6-tetramethyl glucopyranose in good yield, which thus excluded the possibility of the presence of 5-methyl glucose. Oxidation of the monomethyl glucose with bromine water yielded a monomethyl gluconolactone which on account of its rapid hydrolysis in aqueous solution was shown to be a δ-lactone (Haworth, " Constitution of Sugars," London, 1929). The inference is, therefore, that the possibility of the formation of the more stable γ-lactone was ruled out by the presence of a methyl group in the 4-position. Furthermore, complete methylation of the monomethyl δ-gluconolactone yielded 2 : 3 : 4 : 6-tetramethyl δ-gluconolactone identified as the crystalline phenylhydrazide, proving that the original oxidation product was indeed 4-methyl δ-gluconolactone.

In addition, crystalline 2 : 3 : 6-triacetyl 4-methyl β-methylglucoside (Levene and Raymond, *loc. cit.*) was prepared from the monomethyl glucose, and this on deacetylation and methylation, followed by hydrolysis, again yielded 2 : 3 : 4 : 6-tetramethyl glucopyranose. There is thus no room for doubt that the monomethyl glucose is indeed 4-methyl glucose.

Experimental.

Preparation of 4-Methyl Glucose Dibenzyl Mercaptal.—The methods described by Pacsu (*Ber.*, 1924, **57**, 851; 1925, **58**, 1455) and Schinle (*loc. cit.*) were followed except for modifications of detail. Glucose dibenzyl mercaptal (30 g.) was condensed with dry acetone (300 g.) containing concentrated sulphuric acid (6 g.) for 42 hours at 15°. The acetone compound (20 g.) obtained in the form of a syrup after neutralisation and removal of solvent was dissolved in dry ether (130 c.c.) and treated with excess of sodium shavings for 24 hours. After filtration and removal of the ether by distillation the resulting glass was methylated with methyl iodide (35 c.c.) at 40° for 24 hours. The solution on extraction with ether, filtration and evaporation yielded a syrup, which was dissolved in ten times its weight of 90% alcohol and hydrolysed by boiling for 20 minutes with *N*-hydrochloric acid (6 c.c.). On cooling, 2-methyl glucose dibenzyl mercaptal (3·5 g.) crystallised, m. p. 191°, *i.e.*, the " 4 "-methyl glucose dibenzyl mercaptal of Pacsu (*loc. cit.*). The 4-methyl glucose dibenzyl mercaptal, *i.e.*, the trimethyl glucose dibenzyl mercaptal of Pacsu, was obtained by addition of water to the filtrate until a turbid solution was produced, which on standing at 0° gave place to a crystalline precipitate. This was dissolved in alcohol, treated with silver carbonate to remove hydrochloric acid, and decolourised with animal charcoal. Concentration yielded 4-methyl glucose dibenzyl mercaptal (7 g.), m. p. 73°.

Isolation of 4-Methyl Glucose.—For the removal of the mercaptan residue, 4-methyl glucose dibenzyl mercaptal (8 g.) was dissolved in acetone (100 c.c.), and a concentrated acetone solution of mercuric chloride (13 g.) added. After refluxing for an hour, the insoluble $C_6H_5 \cdot CH_2 \cdot S \cdot HgCl$ was filtered off, and the acetone removed by evaporation (diminished pressure). The syrup was dissolved in water, and the solution filtered from a further crop of the insoluble mercury compound. The excess of mercuric chloride was then removed by treatment with hydrogen sulphide, and the hydrochloric acid formed during the reaction was neutralised with silver carbonate. The solution, after filtration, was concentrated to a syrup (2·5 g.), $[\alpha]_D^{20°}$ + 53° (equil.) in water (*c*, 2·1) (Found : OMe, 14·8. Calc. for $C_7H_{14}O_6$: OMe, 16·0%). Treatment with phenylhydrazine and acetic acid yielded an osazone, m. p. 158° after recrystallisation from aqueous alcohol (cf. Pacsu, *loc. cit.*; Schinle, *loc. cit.*).

Preparation and Identification of the Fully Methylated Glucose from 4-Monomethyl Glucose.—Tetra-acetyl 4-methyl glucose. 4-Monomethyl glucose (1 g.) was dissolved in warm pyridine (4·5 c.c.), and acetic anhydride (4·5 c.c.) slowly added. The solution was warmed to 50°, kept at room temperature for 36 hours, poured into ice-water (50 c.c.), and extracted with ether. The ethereal solution was washed, first with dilute sulphuric acid, then with sodium bicarbonate solution, and finally with water. After drying over calcium chloride and removal of solvent a yellow syrup (1·5 g.) was obtained.

Triacetyl 4-Methyl Glucosidyl Bromide.—To the acetyl compound (1·5 g.) dissolved in glacial acetic acid (2 c.c.), glacial acetic acid saturated with hydrogen bromide at 0° (3 c.c.) was added. After 2 hours, cold chloroform (15 c.c.) was added, and the mixture poured into ice-water (40 c.c.). The chloroform solution was washed with sodium bicarbonate solution and water and dried, and the solvent removed at 45° (diminished pressure) to yield a pale yellow syrup (1·15 g.).

2 : 3 : 6-*Triacetyl 4-Methyl β-Methylglucoside.*—The acetobromo-compound (1·15 g.) was dissolved in dry methyl alcohol (20 c.c.), and dry silver carbonate (3 g.) added. The solution was shaken for 12 hours, until no bromine remained in solution. The solution was filtered and evaporated to a thin syrup. This crystallised on standing and the long colourless needles (0·7 g.) were washed free from syrup with alcohol. They showed m. p. 106°, $[\alpha]_D^{20°}$ — 34·0° in chloroform (*c*, 1·2) (Found: OMe, 16·9. Calc. for $C_{14}H_{22}O_9$: OMe, 18·55%) (cf. Levene and Raymond, *J. Biol. Chem.*, 1932, **97**, 763).

Methylation of Triacetyl 4-Methyl β-Methylglucoside.—The triacetyl 4-methyl β-methylglucoside (0·7 g.), dissolved in acetone, was methylated in the usual way (Haworth, J., 1915, **107**, 8) with methyl sulphate (15 c.c.) and sodium hydroxide solution (40 c.c., 30%). The syrup obtained was methylated during 6 hours at 40° in contact with methyl iodide (10 c.c.) and silver oxide (4 g.). After extraction with ether and removal of solvent the syrup yielded on distillation at 0·03 mm. tetramethyl methylglucopyranoside (0·3 g.) at 100° (bath temp.), $n_D^{15°}$ 1·4450.

2 : 3 : 4 : 6-*Tetramethyl Glucopyranose.*—The tetramethyl methylglucoside was hydrolysed with 5% hydrochloric acid (2 c.c.) for 8 hours. After neutralisation with barium carbonate the solution was evaporated. After the addition of alcohol to precipitate most of the barium chloride, and filtration, the solid was extracted three times with boiling ether. The ethereal solution was evaporated, and the syrup extracted with boiling light petroleum (b. p. 60—80°). From this solution the characteristic crystals of 2 : 3 : 4 : 6-tetramethyl glucose were deposited, m. p. 81—82° alone or in admixture with an authentic specimen. It was thus established that Levene and Raymond's (*loc. cit.*) 2 : 3 : 6-triacetyl 4-methyl β-methylglucoside yielded 2 : 3 : 4 : 6-tetramethyl β-methylglucoside on methylation.

Direct Methylation of 4-Methyl Glucose.—The 4-monomethyl glucose (0·35 g.), dissolved in acetone (10 c.c.), was twice methylated as before with methyl sulphate (10 c.c.) and sodium hydroxide solution (20 c.c., 30%). During the first three additions the temperature was maintained at 30° in order to facilitate the initial formation of the glucoside. The product was extracted with chloroform, and the chloroform removed by evaporation. The second methylation was followed by two treatments with methyl iodide (10 c.c.) and silver oxide (5 g.). The resulting syrup (0·15 g.) distilled at 100—110° (bath temp.)/0·03 mm. to yield a mobile colourless liquid (0·07 g.), $n_D^{15°}$ 1·4445. The glucoside was hydrolysed as before to yield 2 : 3 : 4 : 6-tetramethyl glucose, and this was recrystallised twice from light petroleum (b. p. 60—80°) (0·04 g.), m. p. 82—83° alone or in admixture with an authentic specimen (Found: OMe, 51·2. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%).

Oxidation of 4-Methyl Glucose to 4-Methyl δ-Gluconolactone.—4-Methyl glucose (0·8 g.) was oxidised in water (7 c.c.) with bromine (1·5 c.c.) at 35° for 3 days until all reducing action had ceased. The excess of bromine was then removed by aëration, and the solution neutralised with silver carbonate. To obtain the lactone, the silver was precipitated with hydrogen sulphide and the solution after filtration was evaporated to dryness (diminished pressure) at about 80°. $[\alpha]_D^{20°}$ + 54·6° (3 mins.); 47·0° (4 mins.); 39·5° (6 mins.); 37·6° (15 mins.); 35·7° (50 mins.); + 33·9° (280 mins.; constant value) (Found: OMe, 14·0. Calc. for $C_7H_{12}O_6$: OMe, 16·1%).

From the hydrolysis curve it is evident that the lactone belongs to the δ-series and that the methyl group is in the 4-position.

Methylation of 4-Methyl δ-Gluconolactone.—The lactone (0·4 g.) was dissolved in the minimum quantity of methyl alcohol and treated with methyl iodide (10 c.c.) and silver oxide (5 g.) during 20 hours at 40°. This process was repeated four times for shorter periods of 5 hours until the lactone became completely soluble in methyl iodide; a final methylation in absence of methyl alcohol was then carried out. After isolation in the usual way the syrup was distilled at 0·03 mm., the fraction distilling at 110—115° (bath temp.) being collected (0·15 g.). This lactone was digested with phenylhydrazine at 100° for 3 hours and on extraction of the product with an ether–light petroleum mixture crystals of the phenylhydrazide of 2 : 3 : 4 : 6-tetramethyl gluconic acid were isolated, m. p. 114° (cf. Charlton, Haworth, and Peat, J., 1926, 89).

Thanks are expressed to Imperial Chemical Industries, Ltd., and to the Earl of Moray Endowment for grants.

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, March 14th*, 1935.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY & SONS, LIMITED, BUNGAY, SUFFOLK.

XIII

MONOMETHYL HEXOSES. PART II. A REVISION OF THE CONSTITUTIONS OF THE SUPPOSED 4-METHYL GALACTOSE AND 4-METHYL MANNOSE OF PACSU, AND THEIR FORMULATION AS 6-METHYL GALACTOSE AND 2-METHYL MANNOSE RESPECTIVELY

BY

JOHN MUNRO

AND

EDMUND G. V. PERCIVAL

Reprinted from the Journal of the Chemical Society,
May, 1936

Reprinted from the Journal of the Chemical Society, 1936.

147. *Monomethyl Hexoses. Part II. A Revision of the Constitutions of the Supposed 4-Methyl Galactose and 4-Methyl Mannose of Pacsu, and their Formulation as 6-Methyl Galactose and 2-Methyl Mannose respectively.*

By John Munro and Edmund G. V. Percival.

Schinle (*Ber.*, 1931, **64**, 2361) proved that the monomethyl glucose prepared from glucose-dibenzylmercaptal was 2-methyl glucose and not 4-methyl glucose as stated by Pacsu (*Ber.*, 1925, **58**, 1455). Since a monomethyl galactose prepared by a similar method was described as 4-methyl galactose by Pacsu and Löb (*Ber.*, 1929, **62**, 3104), it became of importance to review this case also. The crystalline monomethyl galactose of Pacsu and Löb (*loc. cit.*) was prepared and a preliminary examination of the phenylosazone indicated that it was a true monomethyl galactosazone, thus excluding substitution in position 2. 3-Methyl galactose is excluded by the melting point of its phenylosazone, so that only positions 4, 5, and 6 remain available for the assignment of the methoxyl residue. Complete methylation afforded tetramethyl galactopyranose, isolated as the crystalline anilide, thus excluding substitution in position 5. Proof that position 4 also was unmethylated was obtained by oxidation of the free sugar and the isolation of a monomethyl γ-galactonolactone, easily recognised by its negative specific rotation and slow rate of hydrolysis (Haworth, "Constitution of Sugars," London, 1929). Further confirmation that both positions 4 and 5 were free was secured by following the progress of glycoside formation at 20° according to Levene, Raymond, and Dillon (*J. Biol. Chem.*, 1932, **95**, 699), which indicated that both galactofuranosides and galactopyranosides were formed (see Table II). Close agreement was observed between the physical constants of the free sugar, its phenylhydrazone and phenylosazone with those recorded by Freudenberg and Smeykal (*Ber.*, 1926, **59**, 100) for 6-methyl galactose and its corresponding derivatives, of which the structure appears to be well established by the method of preparation from diacetone galactopyranose. A direct comparison of the compounds concerned confirmed this view (see Table I) and it is considered therefore that the "4"-methyl galactose of Pacsu and Löb (*loc. cit.*) must now be described as 6-methyl galactose.

There is also dubiety about the assignment of the structure of 4-methyl mannose to the sugar isolated by Pacsu and v. Kary (*Ber.*, 1929, **62**, 2811) and an examination of their paper reveals the fact that the osazone prepared from this substance may have been glucosazone. There is no possibility that it is identical with 4-methyl glucosazone, since this has widely different properties (Munro and Percival, J., 1935, 873).

According to Pacsu and v. Kary (*loc. cit.*) a single methylation of diacetone mannose-dibenzylmercaptal, followed by hydrolysis to remove acetone, caused the separation of a crystalline product described as "4"-methyl mannosedibenzylmercaptal, m. p. 188°, $[\alpha]_D^{18°}$ — 106·6° (pyridine), together with a syrup. Many attempts to reproduce this result failed. In every case we obtained with the syrup crystalline products, m. p. 118,° $[\alpha]_D$ — 48°, which were apparently homogeneous but on analysis invariably showed a methoxyl content of about one-third of the theoretical for a monomethyl mannosedibenzylmercaptal. By repeated acetone condensation, methylation, and hydrolysis it was found possible to increase the methoxyl content of the crystals to 80% of the theoretical amount. Con-

TABLE I.

Substance.	M. p.	Rotatory power.	Reference.
(1) " 4 "-Methyl galactose	118°	$[\alpha]_D^{18°}$ +117° ⟶ +68° (after 3 hrs. in water)	(a) Pacsu and Löb, *loc. cit.*
(2) " 4 "-Methyl galactose	118—119	$[\alpha]_D^{20°}$ +120 ⟶ +70 (after 6 hrs. in water)	Present authors according to (a).
(3) 6-Methyl galactose	122—123	$[\alpha]_D^{20°}$ +112 (4 mins.) ⟶ +66 (after 6 hrs. in water)	Present authors according to (b).
(4) 6-Methyl galactose	128	$[\alpha]_{5780}^{20°}$ +114 (5 mins.) ⟶ +77 (after 3 hrs. in water)	(b) Freudenberg and Smeykal, *loc. cit.*
Mixture of (2) and (3)	120		
(5) " 4 "-Methyl galactose phenylosazone	194—195	$[\alpha]_D^{18°}$ +131 (in pyridine)	(a)
(6) " 4 "-Methyl galactose phenylosazone	200	$[\alpha]_D^{20°}$ +144 (in pyridine)	Present authors according to (a).
(7) 6-Methyl galactose phenylosazone	200—201	$[\alpha]_D^{20°}$ +141 (in pyridine)	Present authors according to (b).
(8) 6-Methyl galactose phenylosazone	204—205	$[\alpha]_{5780}^{20°}$ +135 (in pyridine)	(b)
Mixture of (6) and (7)	200		
(9) " 4 "-Methyl galactose phenylhydrazone	179	$[\alpha]_D^{20°}$ +24·4 ⟶ +14·1 (after 24 hrs. in pyridine)	Present authors.
(10) 6-Methyl galactose phenylhydrazone	179	$[\alpha]_D^{20°}$ +23·5 ⟶ +14·8 (after 24 hrs. in pyridine)	Present authors according to (b).
(11) 6-Methyl galactose phenylhydrazone	182—183	$[\alpha]_D^{20°}$ +14·5 (in pyridine)	(b)
Mixture of (9) and (10)	179		
(12) 3-Methyl galactose phenylosazone	176—179		Robertson and Lamb, J., 1934, 1321.

version into the free sugar yielded a product which was still contaminated with free mannose as shown by the isolation of pure mannosephenylhydrazone in quantity corresponding with the amount of mannose (20%) calculated from the analytical results. No methylated phenylhydrazone could be isolated during many attempts and there is thus reason to suppose that the " 4 "-methyl mannosephenylhydrazone (m. p. 179°) of Pacsu and v. Kary was indeed mannosephenylhydrazone (m. p. 183—184°). After removal of the mannosephenylhydrazone, heating produced three crops of an osazone of no methoxyl content, identical with glucosazone.

The results of Pacsu and v. Kary, however, do not depend on a study of the crystalline " 4 "-methyl mannosedibenzylmercaptal but are based on an examination of the sugar obtained on simultaneous removal of acetone and mercaptan residues from diacetone " 4 "-methyl mannosedibenzylmercaptal. The syrup obtained after one methylation, following the removal of the crystalline mixture of low methoxyl content previously described, had a slightly higher methoxyl content (17·5%) than is required for monomethyl mannose, but in this case also crystalline mannosephenylhydrazone was readily isolated. An examination of the osazones produced on heating after removal of the mannosephenylhydrazone revealed that they were specimens of glucosazone contaminated with small amounts of methylated by-products. It is considered, therefore, that the syrup was 2-methyl mannose admixed with mannose and some polymethylated derivatives.

The structures of the acetone compounds of the dibenzylmercaptals of glucose, galactose, and mannose, assigned by Pacsu, require revision in the light of the facts now known and this problem is under investigation.

EXPERIMENTAL.

Preparation of " 4 "-Methyl Galactosedibenzylmercaptal.—The methods described by Pacsu and Löb (*loc. cit.*) were followed for the preparation. Galactosedibenzylmercaptal (20 g.) yielded fine needles of " 4 "-methyl galactosedibenzylmercaptal (7 g.), m. p. 130°, $[\alpha]_D^{20°}$ — 27° in pyridine (*c*, 3·3) (Found: C, 59·5; H, 6·7; OMe, 6·7. Calc. for $C_{21}H_{28}O_5S_2$: C, 59·4; H, 6·6; OMe, 7·3%).

Isolation of " 4 "-Methyl Galactose.—The mercaptan residues were removed from " 4 "-

methyl galactosedibenzylmercaptal (10 g.) as described for 4-methyl glucose (Munro and Percival, *loc. cit.*) to yield a syrup (3·6 g.) which slowly crystallised; m. p. 118° (Found : C, 43·7; H, 7·4; OMe, 13·9. Calc. for $C_7H_{14}O_6$: C, 43·4; H, 7·2; OMe, 16·0%).

" 4 "-Methyl Galactosephenylosazone.—" 4 "-Methyl galactose (0·3 g.), dissolved in water (1·5 c.c.), was heated at 100° for 1 hour with phenylhydrazine (1·5 g.) and acetic acid (0·5 g.). On recrystallisation from alcohol (needles) it showed m. p. 200°, $[\alpha]_D^{20°}$ + 144° in pyridine (*c*, 0·4) (Found : OMe, 7·9; N, 15·1. Calc. for $C_{19}H_{24}O_4N_4$: OMe, 8·3; N, 15·0%).

" 4 "-Methyl Galactosephenylhydrazone.—" 4 "-Methyl galactose (0·2 g.) was dissolved in water (0·5 c.c.), and phenylhydrazine (0·5 g.) added. Crystallisation began after a few hours and was complete in 2 days; m. p. 179° after recrystallisation from methyl alcohol, $[\alpha]_D^{20°}$ + 24·4° (initial value), falling to 14·1° in 24 hours, in pyridine (*c*, 0·8) (Found : OMe, 10·2; N, 9·8. Calc. for $C_{13}H_{20}O_5N_2$: OMe, 10·9; N, 9·85%).

Complete Methylation of " 4 "-Methyl Galactose.—In preparing the fully methylated galactose we employed a series of reactions precisely similar to that for the complete methylation of 4-methyl glucose (J., 1935, 873), namely, tetra-acetyl " 4 "-methyl galactose ⟶ triacetyl " 4 "-methyl galactosidyl bromide ⟶ triacetyl " 4 "-methyl methylgalactoside, in the hope of isolating some intermediate crystalline product, but without success.

Triacetyl " 4 "-methyl methylgalactoside (1·4 g.), dissolved in acetone (30 c.c.), was methylated with methyl sulphate (15 c.c.) and sodium hydroxide solution (40 c.c., 30%). The syrup obtained in the usual way was twice methylated with methyl iodide (10 c.c.) and silver oxide (2·5 g.) during 6 hours and gave on distillation at 115° (bath temp.)/0·04 mm. an oil (0·45 g.), $n_D^{15°}$ 1·4500. The galactoside (0·44 g.) was heated on a water-bath at 80° with 8% hydrochloric acid (4 c.c.) for 2 hours (cf. Irvine and Cameron, J., 1904, **85**, 1071), the acid neutralised with barium carbonate, and the filtered solution evaporated to dryness (diminished pressure). On extraction of the solid with boiling ether, a clear syrup (0·4 g.) of tetramethyl galactose was obtained. This (0·13 g.) was digested with aniline (0·4 g.) and alcohol (1 c.c.) at 100° for 3 hours. 2 : 3 : 4 : 6-Tetramethyl galactose anilide crystallised, on cooling, in long needles (0·1 g.), m. p., after recrystallisation from alcohol, 192—193°, unchanged by an authentic specimen; $[\alpha]_D^{20°}$ — 71° (initial) in acetone (*c*, 0·2) (Found : OMe, 40·8; N, 4·5. Calc. for $C_{16}H_{25}O_5N$: OMe, 39·9; N, 4·5%).

Oxidation of " 4 "-Methyl Galactose to " 4 "-Methyl γ-Galactonolactone.—" 4 "-Methyl galactose (1·5 g.), dissolved in water (10 c.c.), was oxidised with bromine (3 c.c.) at 35° until all reducing action had ceased (48 hours). The excess of bromine was removed by aeration, and the solution neutralised with silver carbonate. The lactone (1·0 g.) was obtained by precipitation of the silver with hydrogen sulphide, filtration, and evaporation to dryness (diminished pressure), followed by heating at 100° in a vacuum (2 hours); $[\alpha]_D^{20°}$ — 43° (10 mins.), — 40° (9 days; const.). 0·0183 G. required 1·32 c.c. 0·01*N*-sodium hydroxide for neutralisation of the free acid and 8·95 c.c. for complete neutralisation (calc., 9·5 c.c.), giving 85% lactone at equilibrium (cf. Freudenberg and Smeykal, *loc. cit.*) (Found : OMe, 14·4. Calc. for $C_7H_{12}O_6$: OMe, 16·1%).

Glycoside Formation with " 4 "-Methyl Galactose at 20°.—The method employed for examining the rate of glycoside formation at room temperature was essentially that described by Levene, Raymond, and Dillon (*loc. cit.*). From a 0·5% methyl-alcoholic hydrogen chloride solution containing " 4 "-methyl galactose (approximately 3 mg. per 0·5 c.c.), two samples of 0·5 c.c. were withdrawn at intervals. One was treated with 0·5 c.c. of 0·4*N*-sodium carbonate and water (3 c.c.), and kept for 15 minutes with 0·3*N*-sodium hydroxide (1 c.c.) and 0·03*N*-iodine (5 c.c.). The excess of iodine, liberated with 5*N*-sulphuric acid (0·2 c.c.), was titrated with 0·01*N*-sodium thiosulphate. The second sample was heated for 10 minutes at 100° with water (2 c.c.) and 0·26*N*-hydrochloric acid (1 c.c.). After immediate cooling, the acid was neutralised with the calculated amount of 0·4*N*-sodium carbonate, and the solution kept for 15 minutes with 0·3*N*-sodium hydroxide (1 c.c.) and 0·03*N*-iodine (5 c.c.). The excess of iodine was determined as before. The difference between these titrations and blank experiments carried out under similar conditions gave the figures for the reducing values. A correction of 21% had to be made on the reducing values obtained after hydrolysis with 0·1*N*-hydrochloric acid owing to the hydrolysis of the " 4 "-methyl methylgalactopyranoside under these conditions, this being determined by a separate experiment.

At the end of 48 hours, the sugar is transformed into a mixture of galactofuranosides and galactopyranosides in approximately equal amounts.

6-*Methyl Galactose.*—The compound was prepared as described by Freudenberg and Smeykal (*loc. cit.*). It was readily obtained crystalline, but repeated crystallisation from alcohol–ether failed to raise the m. p. above 123°. M. p. in admixture with " 4 "-methyl galactose 120°;

TABLE II.

	0·01*N*-$Na_2S_2O_3$, c.c.		Free sugar, %.					
Time.	Before hydrolysis.	After hydrolysis.	Before hydrolysis.	After hydrolysis.	Corrected.	Free sugar, %.	Furanoside, %.	Pyranoside, %.
0	2·13	2·39	100	100	100	100	—	—
15 mins.	1·68	2·46	78·9	102·9	100	78·9	21·1	—
30 ,,	1·60	2·31	75·1	96·6	95·8	75·1	20·7	4·2
1 hr.	1·38	2·24	64·8	93·7	92·1	64·8	27·3	7·9
2 hrs.	1·05	2·10	49·3	87·8	84·8	49·3	35·5	15·2
4 ,,	0·78	1·93	36·6	80·8	76·0	36·6	39·4	24·0
24 ,,	0·20	1·40	9·4	58·6	48·2	9·4	38·8	51·8
48 ,,	0·11	1·52	6·6	63·6	54·5	6·6	47·9	45·5

$[\alpha]_D^{20°}$ + 112° in water (*c*, 1), 4 minutes after dissolution, + 66° after 6 hours (constant). 6-Methyl galactosephenylhydrazone prepared in the usual way and recrystallised four times from methyl alcohol had m. p. 179°, unchanged by " 4 "-methyl galactosephenylhydrazone; $[\alpha]_D^{20°}$ +23·5° in pyridine (*c*, 1), +14·8° (after 24 hrs.; const.). 6-Methyl galactosephenylosazone, recrystallised from alcohol, showed m. p. 200° alone or in admixture with " 4 "-methyl galactosephenylosazone; $[\alpha]_D^{20°}$ +141° in pyridine (*c*, 0·5).

d-*Mannosedibenzylmercaptal.*—The method of preparation employed by Pacsu and v. Kary (*loc. cit.*) was followed, and the product obtained had the properties ascribed to it by those authors; m. p. 126°, $[\alpha]_D^{20°}$ −32·6° in pyridine (*c*, 0·7).

Preparation of the Acetone Compound of Mannosedibenzylmercaptal.—Mannosedibenzylmercaptal was condensed with acetone as described by Pacsu and v. Kary (*loc. cit.*). The acetone compound, after preliminary heating for some hours at 100°/15 mm., was heated for 20 minutes at 110° (bath temp.)/0·07 mm. The product gave $[\alpha]_D^{20°}$ +79° in acetylene tetrachloride (*c*, 2·0) ($[\alpha]_D^{20°}$ +66° in acetylene tetrachloride recorded by Pacsu and v. Kary, *loc. cit.*).

Methylation of the Acetone Compound of Mannosedibenzylmercaptal.—The methods of Pacsu and v. Kary (*loc. cit.*) were followed to yield a syrup, from which the acetone groups were removed as before. The white crystalline product was recrystallised from alcohol; m. p. 118°, and a mixed m. p. with mannosedibenzylmercaptal showed a depression of 1°. $[\alpha]_D^{20°}$ − 48° in pyridine (*c*, 1) (Found : OMe, 2·4. Calc. for $C_{21}H_{28}O_5S_2$: OMe, 7·3%). These results were twice confirmed.

Removal of the Mercaptan Residue and Isolation of the Reducing Sugar.—The mercaptan groups were removed, as described in the case of the galactose derivatives, to yield a reducing syrup which failed to crystallise (Found : OMe, 5·2. Calc. for $C_7H_{14}O_6$: OMe, 16·0%).

Attempted Separation of " 4 "-Methyl Mannose Derivatives.—The glycoside was prepared by the method of Bott, Haworth, and Hirst (J., 1930, 2653) and acetylated with pyridine and acetic anhydride in the usual way. The acetate, however, distilled in one fraction at 185° (bath temp.)/0·07 mm. and analysis showed it to be a mixture of tetra-acetyl methylmannoside and triacetyl monomethyl methylmannoside (Found : OMe, 11·1%).

Increase in the Methoxyl Content of the Partly Methylated Mannosedibenzylmercaptal.—Two consecutive methylations with methyl sulphate and sodium hydroxide without an intermediate hydrolysis to isolate the crystals of the methyl mannosemercaptal failed to produce any increase in the methoxyl content, but if the crystals and syrup formed after the methylation and hydrolysis of the acetone compound of mannosedibenzylmercaptal (1·5 g.) were condensed again with acetone and concentrated sulphuric acid and then remethylated and hydrolysed as before, the crystals (0·5 g.) had a higher methoxyl content. Pure monomethyl mannosedibenzylmercaptal, however, could not be produced by this method; the best sample obtained showed m. p. 117°, $[\alpha]_D^{20°}$ − 54° in pyridine (*c*, 0·5), m. p. on admixture with mannosedibenzylmercaptal 116° (Found : OMe, 5·8. Calc. for $C_{21}H_{28}O_5S_2$: OMe, 7·3%).

Preparation of the Partly Methylated Mannose.—The sugar was obtained as a reducing syrup after removal of the mercaptan residues with mercuric chloride; $[\alpha]_D^{20°}$ + 4·3° in water (*c*, 1·7) (Found : OMe, 11·7. Calc. for $C_7H_{12}O_6$: OMe, 16·0%).

Phenylhydrazone Formation.—The syrup (0·06 g.), dissolved in water (1 c.c.), was mixed with phenylhydrazine (0·5 g.) and glacial acetic acid (0·05 g.). In a few seconds a white crystalline precipitate appeared and was filtered off. After ½ hour a further crop was removed. No more crystals appeared after 2 days. The phenylhydrazone (0·02 g.) was washed with cold

acetone and dried; m. p. 183—184° alone or in admixture with a specimen prepared directly from mannose (Found : OMe, nil; N, 10·3. Calc. for $C_{12}H_{18}O_5N_2$: N, 10·4%).

If it be assumed that the phenylhydrazone formation from mannose is almost quantitative, 0·02 g. of phenylhydrazone would be produced from 0·013 g. of mannose. The monomethyl mannose is therefore present to the extent of 75—80%, which agrees with the previous analytical results.

After 2 days the clear solution was heated for 1 hour at 100° with glacial acetic acid (0·1 c.c.) and a crystal of sodium bisulphite (to retard tar formation). The osazone which formed (0·01 g.) was washed with cold acetone; m. p. 204° alone or in admixture with an authentic specimen of glucosazone (Found : OMe, nil). The filtrate on further heating yielded a second and a third crop of osazone, neither of which contained methoxyl (total yield, 0·02 g.). Since mannose gives a 30% yield of osazone under these conditions, it is clear that the glucosazone now isolated must have been formed from that portion of the syrup containing methoxyl.

Examination of the Syrup " 2 : 3-5 : 6 "-Diacetone " 4 "-Methyl Mannosedibenzylmercaptal.—The acetone compound of mannosedibenzylmercaptal (5 g.) was methylated once as before with methyl sulphate and sodium hydroxide. After extraction the product was hydrolysed with hydrochloric acid, and the crystalline, partly methylated, mannosedibenzylmercaptal obtained. The crystals (0·45 g.) were separated as completely as possible from syrup (1·8 g.) with ether and were identical with the mixtures of low methoxyl content (OMe, 2·8%) previously described; m.p. 117°, $[\alpha]_D^{20°}$ — 48° in pyridine (*c*, 1).

Removal of Mercaptan Residues from the Syrup.—The syrup (1·5 g.) was treated as before with mercuric chloride in acetone to yield a reducing syrup (0·5 g.) having $[\alpha]_D^{20°}$ + 9·6° in water (*c*, 3) (Found : OMe, 17·5. Calc. for $C_7H_{14}O_6$: OMe, 16·0%). The syrup (0·4 g.) on treatment with phenylhydrazine acetate rapidly gave mannosephenylhydrazone (0·1 g.), m. p. 183° (Found : OMe, nil; N, 10·6. Calc. for $C_{12}H_{18}O_5N_2$: N, 10·4%).

Preparation of Osazone.—The filtrate from the hydrazone was subjected to osazone formation. The two crops of osazone (0·07 g.), m. p. 180—185° (OMe, 2%), obtained proved to be glucosazone contaminated with a small amount of methylated material.

The authors thank the Carnegie Trust for the award of a Teaching Fellowship to one of them (E. G. V. P.), and the Earl of Moray Endowment and Imperial Chemical Industries Ltd. for grants.

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, February 24th*, 1936.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY & SONS, LIMITED,
BUNGAY, SUFFOLK.

XIV

THE METHYLATION OF GLUCOSEPHENYLOSAZONE AND ITS FORMULATION AS A DERIVATIVE OF FRUCTOPYRANOSE

BY

(Mrs.) ELIZABETH E. PERCIVAL

AND

EDMUND G. V. PERCIVAL

Reprinted from the Journal of the Chemical Society,
October, 1935.

Reprinted from the Journal of the Chemical Society, 1935.

335. *The Methylation of Glucosephenylosazone and its Formulation as a Derivative of Fructopyranose.*

By (Mrs.) Elizabeth E. Percival and Edmund G. V. Percival.

According to many workers, osazones such as glucosazone, galactosazone (Levene and Laforge, *J. Biol. Chem.*, 1915, **20**, 429), and 3-methyl glucosazone (Anderson, Charlton, and Haworth, J., 1929, 1329) in solution in alcohol or pyridine exhibit mutarotation which, unless the rotational changes are due to decomposition, may indicate the existence of some type of ring structure.

After a single methylation of glucosephenylosazone with methyl sulphate and sodium hydroxide, excess of alkali being avoided, a new crystalline *monomethyl glucosazone* was isolated, which did not agree in physical properties with any of the known monomethyl glucosazones (see Table I). It was a true osazone, since treatment with *p*-nitrobenzaldehyde gave an osone, from which the original osazone was regenerated in five minutes at room temperature by treatment with phenylhydrazine acetate.

Table I.

Glucosazone.	M. p.	$[\alpha]_D$ in alcohol.	Form.	Reference.
3-Methyl	178—179°	− 109° → − 9°	Needles	Anderson, Charlton, and Haworth, J., 1929, 1329.
4-Methyl	158—159	− 33 → − 15	Needles	Pacsu, *Ber.*, 1925, **58**, 1463; Schinle, *Ber.*, 1932, **65**, 315; Munro and Percival, this vol., p. 873.
6-Methyl	184—187	− 69; no mutarotation	Needles	Helferich and Günther, *Ber.*, 1931, **64**, 1276.
New methyl	116—117	− 50 → − 12	Plates (aqueous alcohol) Needles (equilibrium solution in alcohol)	

The evidence presented below gives no reason to doubt that the new compound is the missing 5-methyl glucosazone.

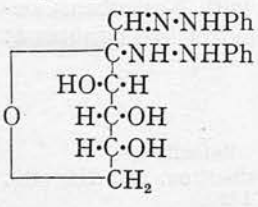
p-Nitrobenzaldehyde, having given a better yield of glucosone from glucosazone than benzaldehyde (Fischer and Armstrong, *Ber.*, 1902, **35**, 3143), was used to prepare the methyl glucosone. The yield was poor (10%) and attempts to improve it by using hydrochloric acid (Fischer, *Ber.*, 1889, **22**, 87) failed. By reduction with zinc dust and acetic acid (Fischer, *loc. cit.*) the corresponding ketose was obtained as a syrup of negative rotation, from which the original osazone could be regenerated in the usual manner. Its properties agreed with those of a monomethyl fructose and the negative rotation indicated its relationship to fructopyranose, so substitution in position 6 was unlikely.

The course of glycoside formation in the cold was followed as described by Levene, Raymond, and Dillon (*J. Biol. Chem.*, 1932, **95**, 699). A comparison of the results (Table III) with those given by fructose (Table II) under parallel conditions showed that, whereas the sugar under review gave 62% of a pyranoside in 24 hours, fructose was exclusively transformed into furanoside. A small constant amount of furanoside appeared to be formed, but this was ascribed to the presence of a 5-methyl aldose produced by a Lobry de Bruyn transformation during the removal of zinc with barium hydroxide. These observations are in harmony with the substitution of methyl in the penultimate hydroxyl group in fructose, such a 5-methyl fructose being capable only of a pyranose formulation. The sugar was accordingly transformed into the pyranoside by heating with methyl-alcoholic hydrogen chloride, and methylation, followed by distillation and hydrolysis, yielded crystalline tetramethyl fructopyranose, indicating that we were dealing with a genuine derivative of fructopyranose.

That 5-methyl glucosazone can be so readily obtained from glucosazone is to be ascribed to the fact that the particular hydroxyl group in question is the most vulnerable to attack by methyl sulphate and alkali. Obviously, whatever rings, if any, exist in the original glucosazone, neither a 1 : 5- nor a 2 : 5-oxide ring is possible.

In order to obtain more evidence on this point glucosazone was methylated by three treatments with methyl sulphate and sodium hydroxide, followed by three with methyl iodide and silver oxide. A red syrup, the methoxyl content of which could not be increased by further methylation, was obtained which gave the analytical figures required for trimethyl glucosazone. Evidently, therefore, there is only one ring in glucosazone, provided that one position is not made unavailable for methylation by steric effects. Repeated attempts to crystallise the syrup failed and it was apparently not identical with the crystalline 3 : 5 : 6-trimethyl glucosazone of Anderson, Charlton, and Haworth (*loc. cit.*) or the 3 : 4 : 6-trimethyl fructosazone of Haworth and Learner (J., 1928, 619). 3 : 4 : 5-Trimethyl fructopyranose has been prepared (Irvine and Patterson, J., 1922, **121**, 2159; for structure see Haworth, Hirst, and Learner, J. 1927, 1040), but there is no record of its phenylosazone.

The syrupy trimethyl glucosazone was converted into a trimethyl fructose by way of the osone as before. Methyl-alcoholic hydrogen chloride reacted slowly at room temperature to form almost exclusively a pyranoside (Table IV), again indicating substitution in position 5 and a free hydroxyl group in position 6. Further methylation of this pyranoside, followed by hydrolysis, again yielded tetramethyl fructopyranose, proving that the sugar was essentially 3 : 4 : 5-trimethyl fructose. Position 6 in the glucosazone is therefore either prevented from undergoing methylation by steric effects, which is improbable, or is concerned with ring formation, and it is considered probable that the osazone contains a pyranose ring and has the annexed structure. Examination of a model of this substance shows that the hydroxyl group on carbon atom 5 is the one most remote from the phenylhydrazine residues, and this may be the reason for the preferential formation of 5-methyl glucosazone.



Although 3-, 4-, and 5-methyl glucosazones may all have the fructopyranose structure, this cannot be the case for 6-methyl glucosazone. The observation of Helferich and Günther (*loc. cit.*) that the purest 6-methyl glucosazone so far obtained exhibits no muta-

rotation in alcohol is in agreement with this, though earlier workers (Kuhn and Ziese, *Ber.*, 1926, **59**, 2314; Ohle and v. Vargha, *Ber.*, 1929, **62**, 2434; Levene and Raymond, *J. Biol. Chem.*, 1932, **97**, 751) had apparently observed mutarotation in this case. The fact that 6-methyl glucosazone is only slowly precipitated (6 hours) during its formation from 6-methyl glucose in the usual way may also have some significance in connexion with its structure.

Experimental.

5-Methyl Glucosazone.—Methyl sulphate (60 c.c.) and 30% sodium hydroxide solution (140 c.c.) were added to glucosazone (20 g.), dissolved in acetone (50 c.c.) and alcohol (125 c.c.), during 2 hours with constant stirring at 50°. The mixture was then maintained at 70° for 10 minutes, diluted with hot water (500 c.c.), neutralised with glacial acetic acid, and kept overnight. The yellow precipitate and brown tarry matter were filtered off and dissolved in boiling alcohol, and water added until precipitation was just maintained on further heating. The tar that separated was removed; the filtrate on cooling deposited a yellow precipitate, which was collected and subjected to further treatment as above. After ten recrystallisations a pale yellow solid was obtained (Found : OMe, 5·0%). This was dissolved in boiling chloroform; unchanged glucosazone crystallised from the cold solution, and the mother-liquor on removal of the solvent under diminished pressure left a product, which was crystallised from aqueous alcohol (Found : OMe, 9·3%). Fractional crystallisation from aqueous alcohol then gave *5-methyl glucosazone* (5 g.) in shining rectangular plates with saw-like edges, m. p. 116—117°, $[\alpha]_D^{20°}$ − 44° in chloroform (*c*, 0·7), − 49° in alcohol (10 mins. after dissolution; *c*, 0·7), − 12° (36 hrs., constant value). This equilibrium solution crystallised in fine needles, m. p. 117° (Found : C, 61·3; H, 6·5; OMe, 7·9; N, 14·8. $C_{19}H_{24}O_4N_4$ requires C, 61·3; H, 6·45; OMe, 8·3; N, 15·0%).

Conversion of Glucosazone into Glucosone.—(1) Glucosazone (1 g.) was dissolved in alcohol (30 c.c.), and water added to produce a turbidity. The vigorously stirred mixture was heated with benzoic acid (0·5 g.) and benzaldehyde (10 c.c.) on a boiling water-bath for 1 hour. After 30 minutes water (50 c.c.) was added. The cooled filtered solution was extracted with ether and evaporated to 20 c.c. at 35° under diminished pressure; on treatment with phenylhydrazine acetate a yellow precipitate of glucosazone appeared after 3 minutes at room temperature (yield, 7%).

(2) Glucosazone (1 g.), in alcohol (100 c.c.), was stirred with benzoic acid (1 g.) and *p*-nitrobenzaldehyde (5 g.) at 90—100° until all the solid had dissolved. Water (150 c.c.) was then added, and the heating continued for 65 minutes, alcohol (50 c.c.) being added after 30 minutes to replace the loss by evaporation. The solution was cooled, filtered (residue A), extracted three times with ether, and evaporated to 20 c.c. at 35° under diminished pressure; treatment with phenylhydrazine acetate gave glucosazone (0·3 g.). The residue (A), similarly treated with *p*-nitrobenzaldehyde (3 g.), yielded glucosazone (0·1 g.), so the conversion was complete to the extent of 40% (cf. Fischer, *loc. cit.*).

Conversion of 5-Methyl Glucosazone into 5-Methyl Glucosone.—By method (2) above, a light yellow syrup of the osone was obtained, which strongly reduced Fehling's solution and on treatment with phenylhydrazine acetate gave a yellow precipitate after 5 minutes in the cold (yield, 10%). On recrystallisation this gave the characteristic shining plates of 5-methyl glucosazone, m. p. 116—117° (Found : OMe, 7·8%).

Reduction of 5-Methyl Glucosone to 5-Methyl Fructose.—5-Methyl glucosazone (5 g.) was converted into 5-methyl glucosone in the above manner. After extraction with ether, in order to avoid decomposition, zinc dust (1 g.) and glacial acetic acid (0·5 c.c.) were added and the solution was evaporated to 80 c.c. This was heated with zinc dust (20 g.) and a few drops of platinic chloride solution on a boiling water-bath with vigorous stirring for 90 minutes during the addition of glacial acetic acid (8 c.c.). A portion (1 c.c.) of the cooled filtered solution failed to give an osazone on treatment with phenylhydrazine acetate in the cold or on heating for 10 minutes, but after 1 hour's heating the osazone came down in the characteristic plates. 2*N*-Barium hydroxide was added to the main bulk until all the zinc was precipitated as zinc hydroxide; the filtrate gave no precipitate with ammonium sulphide. Barium was removed by means of 2*N*-sulphuric acid, and the filtered solution evaporated to dryness at 40°/20 mm. The resulting solid was extracted three times with absolute alcohol (200 c.c.), and the extracts evaporated to dryness, leaving a pale yellow, reducing glass (0·3 g.) consisting of barium acetate mixed with monomethyl fructose. It was considered inadvisable to remove the whole of the

barium because of the danger of decomposition in the presence of a trace of sulphuric acid, but solution in water and addition of more sulphuric acid removed a large part of the inorganic material. The liquid was filtered and evaporated at 40°/20 mm. $[\alpha]_D^{20°}$ — 40° in water (*c*, 0·5) (Found: OMe, 11·8; Ba, 3·8. The assumption that all the barium was present as barium acetate gives $[\alpha]_D^{20°}$ — 50°; OMe, 14·8. Calc. for $C_7H_{14}O_6$: OMe, 16·1%).

Attempted Furanoside Formation.—The method of Levene, Raymond, and Dillon (*loc. cit.*) is by no means quantitative as regards the estimation of fructofuranoside by hydrolysis with 0·1*N*-hydrochloric acid at 100°, since it was found that the monomethyl methylfructopyranoside was hydrolysed to the extent of 15% under the experimental conditions. This is not surprising, since 1 : 3 : 4 : 5-tetramethyl methylfructopyranoside is completely hydrolysed by 0·7*N*-hydrochloric acid during 30 minutes. Further, the effect of acid treatment on the free sugar itself under the conditions obtaining during the hydrolysis is such that a higher reducing value is obtained by the Hagedorn–Jensen ferricyanide method, modified by Hanes (*Biochem. J.*, 1929, **23**, 99), notably to the extent of 25%. The figures recorded for the percentages of pyranoside and furanoside are corrected according to these factors. Table III shows the effect at 20° of 0·5% methyl-alcoholic hydrogen chloride on monomethyl fructose (*ca.* 0·3%). The method employed was to withdraw two samples of 1 c.c. at a time, one being treated with a 20% excess of 0·4*N*-sodium carbonate solution, the volume made up to 5 c.c., 5 c.c. of the standard potassium ferricyanide–sodium carbonate mixture [8·25 g. $K_3Fe(CN)_6$, 10·6 g. Na_2CO_3/litre] added, and the solution heated for 15 minutes at 100°. After cooling for 3 minutes, 5 c.c. of a solution (potassium iodide, 12·5 g., zinc sulphate, 25·0 g., and sodium chloride, 125·0 g./litre) were added, followed by 3 c.c. of 1% acetic acid, the liberated iodine being titrated with 0·015*N*-sodium thiosulphate. The difference between this titre and a blank carried out under the same conditions gave the figure for the reducing value. To the second 1 c.c. portion, 0·4*N*-hydrochloric acid and water were added so that the solution was 0·1*N* with respect to hydrochloric acid, and the solution was heated at 100° for 10 minutes. Sodium carbonate (20% excess) was then added as before, the amounts being adjusted to bring the final volume to 5 c.c. The reducing power was then determined as above. Table II shows parallel experiments with fructose.

TABLE II.

Time.	0·015*N*-Thiosulphate, c.c. Before hydrolysis.	After hydrolysis.	Free sugar, %.	Furanoside, %.	Pyranoside, %.
0	4·2	4·5	100	—	—
40 mins.	0·1	4·5	2	100	—
3 hrs.	—	4·4	—	100	—
5 ,,	0·1	4·6	2	100	—
24 ,,	—	4·5	—	100	—

TABLE III.

Time.	0·015*N*-Thiosulphate, c.c. Before hydrolysis.	After hydrolysis.	Free sugar, %.	Furanoside, %.	Pyranoside, %.
0	5·2	6·5	100	—	—
30 mins.	3·4	5·1	65	14	21
2 hrs.	3·0	4·6	58	14	28
10 ,,	1·8	3·2	35	15	50
24 ,,	1·2	2·5	23	15	62

At the end of 24 hours the solution was still reducing to Fehling's reagent. The table shows that, whereas furanoside formation from fructose is complete after 1 hour, in the case of the sugar under review about 14% of furanoside appears to be formed at once but this figure remains constant while the amount of pyranoside gradually increases. The method is only regarded as semi-quantitative, indicating that in contrast with fructose the sugar forms a pyranoside in preference to a furanoside, an observation which agrees with the structure assigned to the monomethyl osazone.

Preparation of 5-Methyl Methylfructopyranoside and Methylation of the Product.—The sugar (0·25 g.) was dissolved in 3% methyl-alcoholic hydrogen chloride (20 c.c.) and heated at 75° under reflux for 5 hours; reducing action had then ceased. After neutralisation with barium carbonate the methyl-alcoholic solution, to which acetone (20 c.c.) had been added, was methylated twice with methyl sulphate (25 c.c.) and 30% sodium hydroxide solution (70 c.c.). The product was extracted with chloroform, the solvent removed, and the mobile syrup remethylated by two treatments with silver oxide (10 g.) and methyl iodide (30 c.c.). This resulted in the isolation of a syrup, which was distilled at 110° (bath temp.)/0·03 mm. to yield a mobile liquid (0·10 g.), $n_D^{20°}$ 1·4540; this was evidently a fully methylated fructoside.

Isolation of 1 : 3 : 4 : 5-*Tetramethyl Fructose.*—The tetramethyl methylfructoside was hydrolysed during 30 minutes with 3% hydrochloric acid, the solution neutralised with barium carbonate and evaporated to dryness under diminished pressure, and the residue extracted with ether. Removal of the solvent yielded a syrup, which partly crystallised in the square

plates of tetramethyl fructopyranose, m. p. 94—96° after recrystallisation from light petroleum, alone or in admixture with a specimen prepared directly from fructose, $[\alpha]_D^{20°}$ — 109° in water (*c*, 0·5).

Preparation of Trimethyl Glucosazone and Conversion into Trimethyl Fructose.—The tar (4 g.) obtained from the original methylation was found to contain OMe, 14·1%, and was subjected to two further methylations with methyl sulphate (20 c.c.) and 30% sodium hydroxide solution (50 c.c.) at 50° during 2 hours. Water (300 c.c.) was added, and the mixture cooled and filtered. The tarry residue was extracted with chloroform, the chloroform solution washed with water till neutral and dried with sodium sulphate, and the solvent removed under diminished pressure. The red syrup obtained (Found : OMe, 18·2%) was subjected to three methylations with methyl iodide (30 c.c.) and silver oxide (10 g.), which was added in 1 g. portions every 20 minutes. The product was isolated as a red syrup in the usual way (Found : C, 63·3; H, 6·9; OMe, 22·6; N, 14·1. $C_{21}H_{28}O_4N_4$ requires C, 63·0; H, 7·0; OMe, 23·2; N, 14·0%).

The conversion of this *trimethyl glucosazone* (12 g.) into trimethyl glucosone and the reduction to the trimethyl fructose were carried out precisely as described for the monomethyl derivative, with the exception that the sugar was extracted from the mixture with barium acetate by means of boiling chloroform. This yielded a reducing syrup (0·4 g.), $[\alpha]_D^{20°}$ — 43° in methyl alcohol (*c*, 0·4), — 39° in water (*c*, 0·3) (Found : OMe, 42·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Attempted Fructofuranoside Formation.—This was carried out as described for the monomethyl derivative, and the results are in Table IV. Blank experiments showed that the trimethyl methylfructopyranoside was 20% hydrolysed and the reducing value of free sugar increased by 20% under the experimental conditions. The figures recorded are for the relative amounts of the glycoside corrected by these factors.

TABLE IV.

	0·015*N*-Thiosulphate, c.c.				
Time.	Before hydrolysis.	After hydrolysis.	Free sugar, %.	Furanoside, %.	Pyranoside, %.
0	5·3	6·4	100	—	—
1 hr.	4·1	5·5	78	9	13
4 hrs.	3·8	4·9	72	6	23
19 ,,	3·2	4·4	62	8	30
24 ,,	3·1	4·2	58	8	34
48 ,,	2·4	3·4	45	8	47

At the end of 48 hours the solution was still reducing to Fehling's reagent. The results show in a roughly quantitative manner the gradual formation, as the available sugar disappears, of a glycoside which is hydrolysed only with difficulty. The values calculated as furanoside are uniformly low and constant, as in Table III, and are ascribed to impurity.

Preparation of 3 : 4 : 5-*Trimethyl Methylfructopyranoside and Methylation of the Product.*—The sugar (0·3 g.) was dissolved in 3% methyl-alcoholic hydrogen chloride (20 c.c.) and heated at 75° under reflux for 5 hours; reducing action had then ceased. After neutralisation with barium carbonate the methyl-alcoholic solution, to which acetone (20 c.c.) had been added, was methylated once with methyl sulphate and sodium hydroxide and once with silver oxide and methyl iodide. This resulted in the isolation of a syrup, which distilled at 110° (bath temp.)/0·03 mm. to yield a mobile liquid (0·15 g.), $n_D^{20°}$ 1·4520; this was evidently a fully methylated fructoside.

Isolation of 1 : 3 : 4 : 5-*Tetramethyl Fructose.*—This tetramethyl methylfructoside was hydrolysed as in the previous case to yield a syrup, which partly crystallised in the square plates of tetramethyl fructopyranose, m. p. 94—96° alone or in admixture with a specimen prepared directly from fructose.

Thanks are expressed to the Earl of Moray Endowment and to Imperial Chemical Industries, Ltd., for grants.

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, June 25th,* 1935.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY & SONS, LIMITED, BUNGAY, SUFFOLK.

XV

THE STRUCTURE OF OSAZONES AND THE ISOLATION OF A NEW HEXOSAZONE ANHYDRIDE

BY

E. G. V. PERCIVAL

Reprinted from the Journal of the Chemical Society,
December, 1936.

Reprinted from the Journal of the Chemical Society, 1936.

391. *The Structure of Osazones and the Isolation of a New Hexosazone Anhydride.*

By E. G. V. Percival.

In a previous paper (Percival and Percival, J., 1935, 1398) the suggestion was put forward on the basis of methylation experiments that glucosephenylosazone possessed a 2 : 6-oxide ring structure. Engel (*J. Amer. Chem. Soc.*, 1935, **57**, 2419) has since adduced evidence in support of the original acyclic formula of Emil Fischer. He rejects the results obtained in his own methylation experiments as inconclusive, but the highest methoxyl content for the methylated glucosazone he obtained (OMe, 20·7%) approaches the value for a trimethyl derivative, and this is in agreement with our results. His results dealing with the absorption spectra of osazones are of interest, but the conclusion that they disprove a cyclic structure for glucosazone must be accepted with reserve, for it must be pointed out that the effect on the absorption of the group $\cdot O \cdot \overset{\cdot}{C}(CH{:}N \cdot NHPh) \cdot NH \cdot NHPh$ may not differ greatly from that of $\cdot C(CH{:}N \cdot NHPh) \cdot N \cdot NHPh$ owing to the presence of the aromatic nuclei.

Engel (*loc. cit.*), Maurer and Schiedt (*Ber.*, 1935, **68**, 2187), and Wolfrom, Konigsberg, and Soltzberg (*J. Amer. Chem. Soc.*, 1936, **58**, 490) describe a tetra-acetyl glucosephenylosazone, and the last authors a tetra-acetyl galactosephenylosazone. Wolfrom *et al.*

(*loc. cit.*) claim that by using the method of Freudenberg and Harder (*Annalen*, 1923, **433**, 230) for the estimation of the total number of acetyl groups and the method of Kunz and Hudson (*J. Amer. Chem. Soc.*, 1926, **48**, 1982) for the determination of *O*-acetyl residues, they can distinguish between *O*-acetyl and *N*-acetyl groups. Thus β-*d*-glucoseoxime hexa-acetate is shown to possess five *O*-acetyl groups and one *N*-acetyl group. This method on application to the above osazone tetra-acetates indicated that all the acetyl groups were bound through oxygen in agreement with an acyclic structure. These experiments have been repeated, the potentiometric method described by Wolfrom being used both under the conditions employed by Kunz and Hudson (*loc. cit.*) and under those of Wolfrom, Konigsberg, and Soltzberg (*loc. cit.*). The results indicate that only three of the four acetyl groups are removed during the deacetylation in the cold, even when the reagent is in contact with the acetylated osazones for periods much in excess (6 hours) of the 1 hour period used by the latter authors. On the other hand, deacetylation at room temperature indicated the presence of four acetyl groups, and this was confirmed by the method of Freudenberg and Harder (*loc. cit.*). Unless, therefore, one *O*-acetyl group is difficult to remove, the present results appear to indicate that both tetra-acetyl glucosephenylosazone and tetra-acetyl galactosephenylosazone have cyclic structures. Although the structure of tetra-acetyl glucosazone cannot yet be defined with certainty, it has been pointed out by Behrend and Reinsberg (*Annalen*, 1910, **377**, 187) that acetylation on the nitrogen of a true hydrazone in the cold is difficult, whereas phenylhydrazides are easily acetylated, and it is thought probable, therefore, that the *N*-acetyl group is to be found on the phenylhydrazide residue (I).

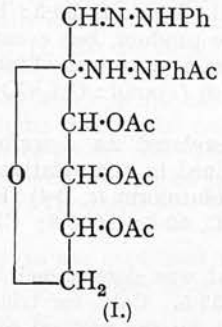
The description of the fully methylated glucosazone as trimethyl glucosazone (Percival and Percival, *loc. cit.*) must now be corrected to trimethyl glucosemethylphenylphenylosazone, since a subsequent estimation of methylimino-groups indicated the presence of one NMe residue per molecule introduced during the prolonged methylation process employed to secure complete methylation.

By deacetylating glucosazone tetra-acetate at room temperature a new derivative was isolated which by analysis and molecular weight determinations was shown to be glucosazone minus two molecules of water, $C_{18}H_{18}O_2N_4$. Diels and Meyer (*Annalen*, 1935, **519**, 157) had previously reported the isolation from glucosazone of a monoanhydroglucosazone, $C_{18}H_{20}O_3N_4$, which they considered to be **3** : 6-anhydroglucosazone. The *dianhydro-hexosazone* described above contained one hydroxyl group only, since it gave rise to a monomethyl ether and a monoacetate. This demands the assumption of the presence of an oxide ring structure, a fact which is rendered the more probable by the existence of such a ring in the acetylated osazones themselves. By the addition of bromine in chloroform this dianhydride immediately gave rise to an insoluble dibromide, which appeared to indicate the presence of a double bond either C:C or C:N. The dianhydrohexosazone was very stable and resisted all attempts to remove the phenylhydrazine residues by means of benzaldehyde, *p*-nitrobenzaldehyde, or concentrated hydrochloric acid; it could in fact be recrystallised from the last reagent.

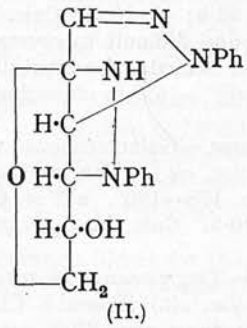
When galactosazone tetra-acetate was deacetylated in the same way, a compound of the same composition was obtained, identical in crystalline form, melting point, and specific rotation with that described above, and mixed melting point determinations appeared to prove their identity. Similarly the monoacetate of the "dianhydrogalactosazone" was identical in all respects with the corresponding compound prepared from glucosazone. It is suggested that on deacetylation of the osazone tetra-acetate (I) ring closure with the elimination of two molecules of water takes place between the hydroxyl groups on C_3 and C_4 and the hydrogen atoms of the imino-groups attached to C_1 and C_2, so that two five-membered rings are formed (II). To explain the formation of the same dianhydride from both glucose and galactose it is necessary to assume either a Walden inversion at C_4 on deacetylation and ring formation in one case (II), or a migration of hydrogen atoms from C_3 and C_4 so that the pyrazoline ring is converted into a pyrazolidine ring system with the production of a double bond between C_3 and C_4 (III). The latter structure appears from inspection of models to be impossible owing to the strained nature of the ring joining C_2 and C_4.

It is noteworthy that Karrer and Pfaeler (*Helv. Chim. Acta*, 1934, 766) by oxidation of glucosazone with periodic acid isolated 4-benzeneazo-1-phenylpyrazol-5-one by fission between C_3 and C_4.

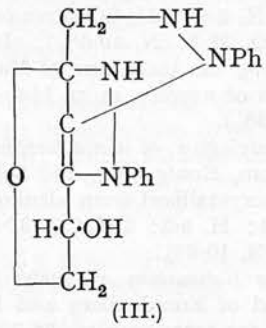
An examination of the possibilities of ring formation for derivatives of fructopyranose



(I.)



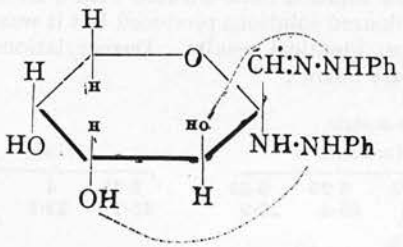
(II.)

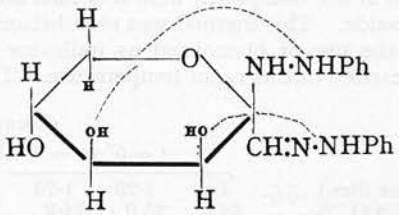


(III.)

and tagatopyranose of the above type reveals the fact that only the α-fructopyranose derivative (IV) and the β-tagatopyranose derivative (V) can yield structures of the type (II) with any ease, and the Walden inversion may take place in the case of either derivative.

(IV.)

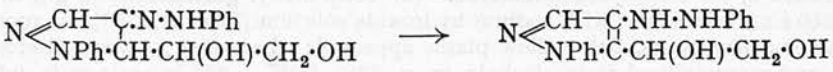




(V.)

From the specific rotations of the acetates of glucosazone and galactosazone it would appear that the former is present in the β- and the latter in the α-form. If the above mechanism is correct, however, unless Walden inversion also takes place on C_2, it must be assumed that the acetylated osazones are not pure α- and β-forms, but mixtures. This is probable both from the magnitude of the specific rotations observed and from the yields of the dianhydro-hexosazone isolated (30—40%).

Diels, Meyer, and Onnen (*Annalen*, 1936, **525**, 94) have now revised the structure proposed for the mono-anhydrides of the osazones and suggest their formulation as pyrazole derivatives by a process similar to (III) in which the 4-pyrazolone derivative (VI) isomerises into the pyrazole structure (VII).

(VI.) 

(VII.)

The basis for this assumption rests on the observation that the monoanhydrides of *l*-arabinosazone and *d*-xylosazone are optical enantiomorphs, and since this must involve the abolition of the asymmetry on C_3, this would be accounted for by the formation of a double bond. Diels, Meyer, and Onnen (*loc. cit.*) have also prepared a dianhydro-maltosazone and have proposed a structure containing both pyrazole and pyridazine rings. So far it is not known whether the monoanhydro-hexazones of Diels possess an oxide ring structure. The fact that they form dibenzoates suggests this possibility, but triacetates have also been isolated. The possibility is not entirely excluded, however, as the authors point out that they have not attempted to discriminate between *O*-acetyl and *N*-acetyl residues.

Owing to the stability of the dianhydro-hexosazone it has not yet been found possible to confirm the structure by degradative experiments and further investigations are proceeding in order to establish the structure with certainty.

Experimental.

Acetylation of Glucosephenylosazone.—The method described by Maurer and Schiedt (*loc. cit.*) was employed, giving an amorphous yellow powder in almost quantitative yield; the method of Engel (*loc. cit.*) gave the same result. M. p. 70°, $[\alpha]_D^{20°}$ − 57° in alcohol (*c*, 0·7) [Found: C, 59·4; H, 5·6; CH_3·CO (Freudenberg), 33·5; N, 10·4. Calc. for $C_{26}H_{30}O_8N_4$: C, 59·3; H, 5·9; CH_3·CO, 32·7; N, 10·6%]. It was found difficult to crystallise the product, but eventually, following the technique of Maurer and Schiedt, the crystalline tetra-acetate was obtained in clumps of needles, m. p. 114—115°, $[\alpha]_D^{20°}$ − 58° in chloroform (*c*, 0·3) (Found: CH_3·CO, 33·1; N, 10·3%).

Acetylation of Galactosephenylosazone.—Galactosazone was acetylated as described by Wolfrom, Konigsberg, and Soltzberg (*loc. cit.*). The product, obtained in quantitative yield, was recrystallised from alcohol; m. p. 178—180°, $[\alpha]_D^{20°}$ + 90° in chloroform (*c*, 0·4) (Found: C, 59·4; H, 5·5; CH_3·CO, 32·5; N, 10·5. Calc. for $C_{26}H_{30}O_8N_4$: C, 59·3; H, 5·9; CH_3·CO, 32·7; N, 10·6%).

The Estimation of Acetyl Groups.—The amount of total acetyl was determined by the method of Freudenberg and Harder (*loc. cit.*) (Found: CH_3·CO, 33·5. Calc. for triacetate, 24·5; for tetra-acetate, 32·7%). Titrations were then carried out by the method of Kunz and Hudson (*loc. cit.*) using a potentiometer and the quinhydrone electrode. The substance (*ca.* 0·2 g.), dissolved in acetone (35 c.c.), was cooled to − 5°, and 0·1*N*-sodium hydroxide added drop by drop, the mixture being kept below 0° for the periods shown in the table. An excess of 0·1*N*-sulphuric acid was then added, and the solution back-titrated with 0·1*N*-sodium hydroxide. This method was used because of the coloured solutions produced but it was found that the use of phenol-red as indicator gave almost identical results. Deacetylations were also carried out at room temperature. The results are below:

Glucosazone Tetra-acetate.

	− 5° to − 0° (Kunz and Hudson).							18°.		
Time (hrs.)	1	1·25	1·75	2·5	2·75	3·25	6·25	2·25	4	18
CH_3·CO, %	24·5	25·0	24·9	25·4	25·4	25·8	27·9	33·7	33·2	34·9

Galactosazone Tetra-acetate.

	− 5° to − 0° (Kunz and Hudson).				18°.
Time (hrs.)	1	1·75	2	2·75	4
CH_3·CO, %	24·1	25·0	25·6	25·6	32·8

Analysis of "Trimethyl Glucosazone."—The "trimethyl glucosazone" described in the previous paper (Percival and Percival, *loc. cit.*) was purified by solution in chloroform and precipitation with a large quantity of light petroleum (b. p. 40—60°), this throwing out a small quantity of red tar, which was discarded. The syrup obtained on evaporation of the bulk of the solution was analysed (Found: C, 63·5; H, 6·9; N, 14·0; OMe, 21·6; NMe, 6·8. $C_{22}H_{30}O_4N_4$ requires C, 63·7; H, 7·3; N, 13·5; OMe, 22·5; NMe, 7·0%).

Preparation of the Dianhydro-hexosazone.—(a) Tetra-acetyl glucosazone (5 g.), dissolved in acetone (250 c.c.), was mixed with sodium hydroxide solution (320 c.c., 1·5%) at room temperature. After a few hours, pale yellow plates appeared; these (1·2 g.) were filtered off after 24 hours, and recrystallised from alcohol; m. p. 238°, $[\alpha]_D^{20°}$ − 88° in acetone (*c*, 0·3) with no mutarotation [Found: C, 67·0; H, 5·6; N, 17·4; *M* (Rast), 319. $C_{18}H_{18}O_2N_4$ requires C, 67·1; H, 5·6; N, 17·4%; *M*, 322].

(b) Tetra-acetyl galactosazone (30 g.) was deacetylated as described above to yield a product (6 g.) similar to that obtained from tetra-acetyl glucosazone, which after recrystallisation had m. p. 238°, not depressed on admixture with the *anhydro-compound* described above, and $[\alpha]_D^{20°}$ − 88° in acetone (*c*, 0·3) (Found: C, 67·1; H, 5·7; N, 17·4%). This result was twice confirmed.

Acetylation of the Dianhydro-hexosazone.—A solution of the above product (1 g.) in acetic anhydride (2 c.c.) and pyridine (5 c.c.) was kept overnight and then poured into water. The solid obtained, recrystallised from aqueous alcohol, formed shining crystals (0·7 g.), m. p. 135°, $[\alpha]_D$ + 108° in chloroform (*c*, 0·5) [Found: C, 65·8; H, 5·5; CH_3·CO, 12·0; N, 15·5; *M* (Rast), 339. $C_{20}H_{20}O_3N_4$ requires C, 65·9; H, 5·5; CH_3·CO, 11·8; N, 15·4%; *M*, 364]. Deacetylation of this *mono-acetyl* derivative yielded yellow needles of the original dianhydride, m. p. 235°, $[\alpha]_D^{20°}$ − 89° in acetone (*c*, 0·4). The same monoacetyl derivative was obtained from the di-

anhydride obtained from tetra-acetyl galactosazone and no differences could be detected by mixed m. p. determinations or in specific rotation.

Methylation of the Dianhydro-hexosazone.—The dianhydride (2 g.), dissolved in acetone (75 c.c.), was methylated at 60° with methyl sulphate (20 c.c.) and sodium hydroxide solution (50 c.c., 30%) in the usual way. The *dianhydro-hexosazone monomethyl ether* which separated was easily recrystallised (2·1 g.) and had m. p. 172°, $[\alpha]_D^{20°}$ — 170° in chloroform (*c*, 0·4) (Found : C, 68·0; H, 6·3; N, 16·3; OMe, 8·9; NMe, nil. $C_{19}H_{20}O_2N_4$ requires C, 67·8; H, 6·0; N, 16·7; OMe, 9·2%).

Isolation of a Dianhydro-hexosazone Dibromide.—The dianhydroglucosazone (0·2 g.) in chloroform (6 c.c.) was treated with a solution of bromine (0·6 g.) in chloroform (5 c.c.). An immediate precipitate (0·28 g.) was obtained, which was filtered off, washed with alcohol, and recrystallised from a large quantity of alcohol on account of its low solubility; m. p. 240° (decomp.) (Found : N, 11·2; Br, 34·2. $C_{18}H_{18}O_2N_4Br_2$ requires N, 11·6; Br, 33·2%).

Thanks are expressed to the Carnegie Trust for the award of a Teaching Fellowship and to the Earl of Moray Endowment and Imperial Chemical Industries, Ltd., for grants.

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, October 1st,* 1936.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY & SONS, LIMITED, BUNGAY, SUFFOLK.

XVI

SUGAR OSAZONES AND THEIR ANHYDRIDES

BY

E. E. PERCIVAL

AND

E. G. V. PERCIVAL

Reprinted from the Journal of the Chemical Society,
July, 1937.

Reprinted from the Journal of the Chemical Society, 1937.

268. *Sugar Osazones and their Anhydrides.*

By E. E. Percival and E. G. V. Percival.

This paper deals with further investigations on the production of anhydro-osazones by the deacetylation of osazone acetates. A monoanhydro-lactosazone and two isomeric monoanhydro-maltosazones are described, all of which yield penta-acetates and therefore possess a pyranose ring structure in addition to the pyrazolidine or pyridazine ring. The monoanhydro-glucosazone and -galactosazone described by Diels and his co-workers also appear to contain an oxide ring, since they yield diacetates on acetylation.

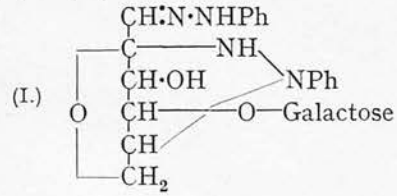
It is pointed out as a result of further work on the differential deacetylation of osazone acetates that this titration method cannot be used as evidence of the cyclic structure or otherwise of such derivatives.

It was shown by one of us (J., 1936, 1770) that the deacetylation of the acetyl derivatives of glucosephenylosazone and galactosephenylosazone gave rise to the same dianhydro-hexosazone, for which a structure was proposed incorporating a pyrazoline, a pyrazolidine, and a pyranose ring. Previously Diels and Meyer (*Annalen*, 1935, **519**, 157) and later Diels, Meyer, and Onnen (*ibid.*, 1936, **525**, 94) had reported the isolation of monoanhydro-glucosazone, -galactosazone, -lactosazone, -xylosazone, -arabinosazone, -cellobiosazone, dianhydro-maltosazone and other similar derivatives by the action of sulphuric acid in alcoholic solution on the osazones. Emil Fischer in his classical paper on osazone formation (*Ber.*, 1887, **20**, 830) had also reported the isolation of anhydrolactosazone by this method.

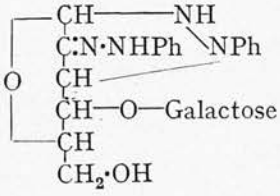
We have now investigated further the possibility of anhydride formation in the sugar osazone series by the deacetylation method for the acetates of the phenylosazones of lactose, maltose, *d*-xylose, *l*-arabinose, and *l*-rhamnose, as well as for certain phenylhydrazones and methylphenylhydrazones, but positive results have been obtained only in the case of the first two substances.

Lactosazone yields an amorphous *hepta-acetate*, which on deacetylation reverts to the same anhydro-derivative (m. p. 232°) as that described by Fischer and by Diels and Meyer (*locc. cit.*). Ultimate analysis reveals the fact that the formula must be represented as $C_{24}H_{32}O_9N_4$, and since it is not identical with lactosazone it appears to be a hydrated anhydride. The corresponding acetyl derivative crystallises with one molecule of benzene and appears to be a *penta-acetyl monoanhydrolactosazone*, the analytical figures not being in agreement with those required by a dianhydrolactosazone tetra- or penta-acetate, or by monoanhydrolactosazone hexa-acetate. It is probable, therefore, that a pyranose ring

structure is present in this monoanhydrolactosazone and it can accordingly be formulated as either (I) or (II).



(I.)



(II.)

It should be noted that the above formulæ are not stereochemical.

Crystalline *maltosazone hepta-acetate* gave two distinct products on deacetylation: (A) $C_{24}H_{30}O_8N_4$, long, lemon-yellow needles, m. p. 245°, $[\alpha]_D^{20°}$ + 57·6° in pyridine (*c*, 0·38), and (B) $C_{24}H_{34}O_{10}N_4$, pointed, yellow plates, m. p. 194°, $[\alpha]_D^{20°}$ + 160° in pyridine (*c*, 0·23). Since by no method of dehydration or hydration attempted was it possible to interconvert (A) and (B), coupled with the evidence of the specific rotations, it is necessary to conclude that the two derivatives are structurally different. The possibility too that (B) is maltosazone hydrate can be ruled out on the basis of the rotational evidence and the fact that on acetylation (B) yields a *penta-acetate*, $C_{34}H_{40}O_{13}N_4$, and not maltosazone hepta-acetate. Acetylation of (A) also yields an amorphous *penta-acetate* of the same composition but with properties markedly different from those of acetylated (B). The simplest explanation available is that (A) and (B) are the isomeric ring forms corresponding to formulæ (I) and (II) suggested for the anhydrolactosazone, but it has not yet been found possible to distinguish between the two forms. It is interesting to note that, although by the deacetylation method glucosazone yields a dianhydrohexosazone and maltosazone and lactosazone monoanhydrides, yet Diels and Meyer (*loc. cit.*) by their method obtained a monoanhydroglucosazone and a dianhydromaltosazone.

In a paper (J., 1935, 1398) a structure for glucosazone was proposed embodying a fructopyranose ring on the basis of methylation experiments. There seems to be no reason why this should not hold for the anhydro-osazones of the disaccharides now considered, but since no experimental proof is yet available for these derivatives it is necessary to suspend judgment on the location of the oxide ring which is undoubtedly present.

The *triacetates* of *d*-xylosazone, *l*-arabinosazone and *l*-rhamnosazone were prepared, but despite repeated attempts crystalline anhydro-osazones could not be obtained by the deacetylation method, although Diels, Meyer, and Onnen (*loc. cit.*) found it possible with acidified alcohol to prepare monoanhydrides of xylosazone and arabinosazone.

Since all the osazone anhydrides prepared by deacetylation appeared to possess an oxide ring structure, it seemed of interest to investigate whether the monoanhydrides of Diels were of the same type. Accordingly his monoanhydro-glucosazone and -galactosazone were prepared and acetylated. The former yielded an amorphous *diacetate*, $[\alpha]_D^{17°}$ — 125°, and a crystalline *diacetyl monoanhydrogalactosazone*, $[\alpha]_D^{17°}$ + 64°, was also isolated. These results would suggest that these monoanhydrides possess an oxide ring structure. This is supported by the observation of Diels, Meyer, and Onnen (*loc. cit.*) that they yield dibenzoates, but these authors also report the isolation of triacetates. The latter observation may be due to acetylation on one of the nitrogen atoms of the hydrazone residue due to the employment of vigorous methods of acetylation. No specific rotations were recorded for these derivatives.

Fructosemethylphenylosazone tetra-acetate on deacetylation yielded the original osazone as would be expected, since no α-hydrogen atom is available for anhydride formation; Diels and Meyer (*loc. cit.*) record a similar observation for their acid alcohol method. Incidental to the preparation of fructosemethylphenylosazone a compound which appears to be a *fructosemethylphenylhydrazone*, m. p. 170°, $[\alpha]_D^{17°}$ — 253°, yielding a *penta-acetate*, m. p. 121°, $[\alpha]_D^{17°}$ + 86·5°, was isolated. Ofner (*Monatsh.*, 1905, **26**, 1165) has described a fructosemethylphenylhydrazone of m. p. 116—120°. It was not found possible to isolate an anhydro-compound from the acetate of glucosephenylhydrazone or from *glucosemethylphenylhydrazone penta-acetate.*

Because of the importance of being able to distinguish between *O*-acetyl and *N*-acetyl groups in this work and because the results in a previous paper (Percival, *loc. cit.*) did not agree with those of Wolfrom, Konigsberg, and Soltzberg (*J. Amer. Chem. Soc.*, 1936, **58**, 490) an extensive survey of the method previously described has been carried out. Repetition of the work on tetra-acetyl galactosazone and tetra-acetyl glucosazone with the conditions of temperature prescribed by Kunz and Hudson (*J. Amer. Chem. Soc.*, 1926, **48**, 1982) indicated the presence of *ca.* 30—31% of *O*-acetyl group, and we are now in complete agreement with Wolfrom and his co-workers on the experimental facts. The reason for the discrepancy was the inaccuracy below 0° of the thermometer used for registering the temperature of the freezing mixture, with the result that the earlier experiments had been conducted at — 15° to — 18° instead of *ca.* — 5°. Whereas at the lower temperature results were obtained, and these are now confirmed, corresponding to the removal of but three acetyl residues, it is apparent that the speed of deacetylation under these conditions will be much reduced, and experiments with octa-acetyl lactose reveal that, although 20 minutes at room temperature is sufficient to eliminate all the acetyl residues, at — 20° only about 90% is removed in 2 hours, so that the agreement may be fortuitous. It must be emphasised, however, that it does not follow that the compounds in question do not contain *N*-acetyl groups, and are therefore acyclic. It is clear that it is not sufficient to compare *O*-acetylated compounds with such derivatives as acetanilide and methylacetanilide, which are untouched during 24 hours with *N*/10-sodium hydroxide at room temperature, since the compounds under review, if cyclic, will be acetylated hydrazides. Accordingly the ease of deacetylation of α-acetylphenylhydrazine, β-acetylphenylhydrazine, αβ-diacetylphenylhydrazine and benzaldehyde-α-acetylphenylhydrazone was studied. The general conclusion emerges that such compounds are hydrolysed much more easily than *N*-acetylated amines, but to a varying extent; *e.g.*, β-acetylphenylhydrazine requires but 2 hours at room temperature under the prescribed conditions to lose 50% of its acetyl residues and diacetylphenylhydrazine loses almost the same proportion in 10 minutes, although benzaldehyde-α-acetylphenylhydrazone only loses 13% and α-acetylphenylhydrazine 7% in 2 hours. It is therefore clear that a sharp differentiation between NH·NAc and OAc is difficult by the method proposed and that the question of the structure of the osazone acetates cannot yet be regarded as settled.

Experimental.

Acetylation of Lactosephenylosazone.—The method described for the acetylation of dianhydrohexosazone (J., 1936, 1773) was employed, giving, in almost quantitative yield, an amorphous yellow powder, which was washed, dried, dissolved in benzene, and precipitated with light petroleum (b. p. 40—60°) to yield a pale yellow solid, m. p. 105—110°, $[\alpha]_D^{20°}$ + 27° in chloroform (*c*, 0·28) (Found: C, 56·6; H, 5·6; CH_3·CO, 35·7; N, 7·1. $C_{38}H_{46}O_{16}N_4$ requires C, 56·0; H, 5·65; CH_3·CO, 37·0; N, 6·9%).

Conversion into Anhydrolactosephenylosazone.—Hepta-acetyl lactosephenylosazone (4 g.), dissolved in acetone (180 c.c.) and water (100 c.c.), was mixed with sodium hydroxide solution (44 c.c., 8%) at room temperature and kept for 21 hours. The resulting solution was neutralised with sulphuric acid and diluted with acetone until the precipitation of sodium sulphate was complete. This was removed by filtration, and the acetone by distillation. Yellow needles were deposited, which were filtered off from the hot solution; a further quantity of needles was deposited from the filtrate on standing. Recrystallisation from hot pyridine–alcohol, followed by the addition of water, gave light yellow, fan-like needles (1 g.), m. p. 231—232° (not depressed by Diels's anhydrolactosazone, m. p. 230°), $[\alpha]_D^{20°}$ — 147° in methyl alcohol (*c*, 0·18) (cf. Diels's anhydrolactosazone, $[\alpha]_D^{20°}$ — 146° in methyl alcohol; *c*, 0·19) (Found: C, 55·2; H, 6·1; N, 11·6. Calc. for $C_{24}H_{32}O_9N_4$: C, 55·4; H, 6·2; N, 10·8%).

Preparation of Anhydrolactosephenylosazone Penta-acetate.—Anhydrolactosephenylosazone (0·4 g.) was acetylated as described for the acetylation of lactosephenylosazone. On pouring into water a yellow precipitate was obtained. Rosettes of shining yellow needles were obtained by solution in warm benzene, followed by the addition of light petroleum (b. p. 40—60°) until turbidity was almost reached (yield 0·5 g.); m. p. 115—117°, $[\alpha]_D^{20°}$ — 102° in acetone (*c*, 0·4) [Found: C, 60·3; H, 5·82; CH_3·CO, 29·0 (titrn.), 28·0 (Freudenberg); N, 7·1. $C_{40}H_{46}O_{13}N_4$ requires C, 60·7; H, 5·9; CH_3·CO, 27·3; N, 7·1%]. Deacetylation of the *penta-acetate* gave the original anhydrolactosazone, m. p. 232°, $[\alpha]_D^{17°}$ — 147° in methyl alcohol (*c*, 0·2).

Acetylation of Maltosephenylosazone.—Maltosephenylosazone was acetylated as described above for lactosephenylosazone. The *product*, obtained in quantitative yield, was recrystallised from alcohol and water to yield rosettes of yellow needles, m. p. 162°, $[\alpha]_D^{20°}$ + 41° in chloroform (*c*, 0·515) (Found: C, 55·7; H, 5·6; CH_3·CO, 37·0; N, 6·8. $C_{38}H_{46}O_6N_4$ requires C, 56·0; H, 5·65; CH_3·CO, 37·0; N, 6·9%).

Preparation of Anhydromaltosephenylosazone.—Hepta-acetyl maltosephenylosazone (9·5 g.) was deacetylated as described above for hepta-acetyl lactosephenylosazone. A yellow precipitate (A) was again deposited in the hot solution and a further quantity of precipitate (B) in the filtrate on standing. The precipitate (A) was recrystallised in the same way as the lactose compound to give long, pale yellow needles (0·3 g.), m. p. 245—246°, mixed m. p. with anhydroglucosephenylosazone (m. p. 230—232°) 224—226°; $[\alpha]_D^{20°}$ + 58° in pyridine (*c*, 0·382) (Found: C, 57·6; H, 6·0; N, 10·9. $C_{24}H_{30}O_8N_4$ requires C, 57·4; H, 6·0; N, 11·2%).

The precipitate (B) was similarly treated to yield bright yellow, pointed plates mixed with a small quantity of the light yellow needles (0·05 g.). It was found that the needles were deposited while the recrystallisation solution was warm and separation was effected by filtration of the hot solution. Any plates adhering to the needles could be removed by washing with alcohol. The addition of a further quantity of water was sometimes necessary to ensure complete deposition of the plates (2·3 g.), m. p. 194°; mixed m. p. with maltosephenylosazone (m. p. 196—199°) 165°; $[\alpha]_D^{20°}$ + 160° in pyridine (*c*, 0·23), + 90° in methyl alcohol (*c*, 0·552), + 92° in 6 : 4 alcohol–pyridine (*c*, 0·3) (Found: C, 53·6; H, 6·3; N, 10·7. $C_{24}H_{34}O_{10}N_4$ requires C, 53·5; H, 6·3; N, 10·4%).

Acetylation of Anhydromaltosephenylosazone.—(a) *Needle form.* The needles (0·27 g.) were acetylated as described above, slight warming being necessary to obtain solution of the crystals. On pouring into water an orange-red precipitate was obtained, which defied all attempts at crystallisation. The best method of purification was solution in benzene, followed by precipitation with light petroleum (b. p. 60—80°) to give a pale fawn, amorphous *penta-acetate* (0·4 g.), $[\alpha]_D^{20°}$ + 90·7° in acetone (*c*, 0·275) [Found: C, 56·7; H, 5·6; CH_3·CO (Freudenberg), 30·0; N, 7·3. $C_{34}H_{40}O_{13}N_4$ requires C, 57·3; H, 5·6; CH_3·CO, 30·2; N, 7·9%]. All attempts to obtain a crystalline anhydro-compound or to regenerate the original material by deacetylation of the above acetate failed.

(b) *Plate form.* The plates (1·3 g.) were acetylated as described above to yield a pale yellow solid. Recrystallisation from alcohol gave an amorphous *penta-acetate* (1·8 g.), m. p. 110—112°, $[\alpha]_D^{20°}$ + 150° in acetone (*c*, 0·29) [Found: C, 56·8; H, 5·5; CH_3·CO, 30·5 (titrn.), 30·8 (Freudenberg); N, 7·5. $C_{34}H_{40}O_{13}N_4$ requires C, 57·3; H, 5·6; CH_3·CO, 30·2; N, 7·9%]. Deacetylation of the above acetate (1 g.) and treatment in the usual manner gave the original pure plates (0·25 g.), m. p. 194°, $[\alpha]_D^{18°}$ + 160° in pyridine (*c*, 0·243).

d-*Xylosazone Triacetate*, l-*Arabinosazone Triacetate, and* l-*Rhamnosazone Triacetate.*—The pure osazone (1 g.) was dissolved in pyridine (5·5 c.c.) and acetic anhydride (2 c.c.) and kept for 36 hours. The solid obtained on pouring into water was recrystallised from aqueous ethyl alcohol.

d-*Xylosazone triacetate* crystallised in clumps of needles, m. p. 116—117°, $[\alpha]_D^{16°}$ − 46° in chloroform (*c*, 0·3) [Found: C, 60·9; H, 5·9; CH_3·CO, 29·9 (Freudenberg), 27·9 (titrn.); N, 12·7. $C_{23}H_{26}O_6N_4$ requires C, 60·7; H, 5·8; CH_3·CO, 28·4; N, 12·3%].

l-*Arabinosazone triacetate* was similar in appearance to the corresponding xylose derivative; it had m. p. 114°, $[\alpha]_D^{16°}$ *ca.* + 5° in chloroform (*c*, 0·3) [Found: C, 61·2; H, 6·1; CH_3·CO, 30·0 (Freudenberg), 28·0 (titrn.); N, 12·5. $C_{23}H_{26}O_6N_4$ requires C, 60·7; H, 5·8; CH_3·CO, 28·4; N, 12·3%].

l-*Rhamnosazone triacetate* was obtained as an amorphous yellow solid, m. p. 75°, $[\alpha]_D^{18°}$ + 52° in chloroform (*c*, 0·4) [Found: C, 61·4; H, 6·0; CH_3·CO (titrn.), 28·4; N, 12·2. $C_{24}H_{28}O_6N_4$ requires C, 61·5; H, 6·0; CH_3·CO, 27·6; N, 12·0%].

Deacetylation of these compounds according to the conditions previously described gave ill-defined brownish-yellow solids which could not be obtained crystalline.

Monoanhydroglucosazone and Monoanhydrogalactosazone Diacetates.—Diels and Meyer's method (*loc. cit.*) was used to prepare monoanhydroglucosazone, m. p. 177°, $[\alpha]_D^{17°}$ − 154° in methyl alcohol (*c*, 0·45) (Found: C, 63·2; H, 6·1; N, 16·3. Calc. for $C_{18}H_{20}O_3N_4$: C, 63·5; H, 5·9; N, 16·5%), and monoanhydrogalactosazone, m. p. 217°, $[\alpha]_D^{20°}$ + 28° in methyl alcohol (*c*, 0·3) (Found: C, 63·1; H, 6·0; N, 16·2. Calc. for $C_{18}H_{20}O_3N_4$: C, 63·5; H, 5·9; N, 16·5%). Both these derivatives (1 g.) were acetylated with pyridine (8 c.c.) and acetic anhydride (3·5 c.c.) during 3 days at room temperature and the acetates were isolated by pouring into water. *Monoanhydroglucosazone diacetate* was obtained as a yellow amorphous powder, m. p. 70°,

$[\alpha]_D^{17°}$ — 125° in chloroform (*c*, 0·3) [Found: C, 62·0; H, 5·8; CH_3·CO, 21·2 (Freudenberg), 21·4 (titrn.); N, 12·8. $C_{22}H_{24}O_5N_4$ requires C, 62·3; H, 5·7; CH_3·CO, 20·3; N, 13·2%]. *Monoanhydrogalactosazone diacetate* crystallised in yellow needles, m. p. 86°, $[\alpha]_D^{18°}$ + 64° in chloroform (*c*, 0·2) [Found: C, 61·9; H, 5·8; CH_3·CO, 21·6 (Freudenberg), 21·0 (titrn.); N, 13·5. $C_{22}H_{24}O_5N_4$ requires C, 62·3; H, 5·7; CH_3·CO, 20·3; N, 13·2%].

Fructosemethylphenylosazone Tetra-acetate.—The instructions of Ofner (*Ber.*, 1904, **37**, 3362) were followed, but the product was invariably the *methylphenylhydrazone* described below. Neuberg's method (*Ber.*, 1902, **35**, 959), however, gave the methylphenylosazone, m. p. 156°, $[\alpha]_D^{17°}$ + 90° in pyridine–alcohol (4 : 6) (*c*, 0·4). Acetylation according to the usual method gave a yellow crystalline acetate in quantitative yield, m. p. 128°, $[\alpha]_D^{17°}$ — 435° in chloroform (*c*, 0·4), — 236° in 95% alcohol (*c*, 0·2) (cf. Engel, *J. Amer. Chem. Soc.*, 1935, **57**, 2419) (Found: C, 60·9; H, 6·2; CH_3·CO, 30·9; N, 10·0. Calc. for $C_{28}H_{34}O_8N_4$: C, 60·6; H, 6·2; CH_3·CO, 31·0; N, 10·1%).

Fructosemethylphenylhydrazone.—Ofner's method (*loc. cit.*) for the preparation of the osazone readily gave a colourless crystalline derivative, which on recrystallisation yielded prisms, m. p. 170°, $[\alpha]_D^{17°}$ — 253° in pyridine–alcohol (4 : 6) (*c*, 0·6) (Found: N, 10·3. $C_{13}H_{20}O_5N_2$ requires N, 9·9%).

Fructosemethylphenylhydrazone Penta-acetate.—The methylphenylhydrazone (1 g.) was kept with acetic anhydride (3 c.c.) and pyridine (6 c.c.); after 2 days the mixture was poured into water, and the solid recrystallised from 50% aqueous alcohol to yield colourless plates of the *penta-acetate*, m. p. 121°, $[\alpha]_D^{17°}$ + 86·5° in chloroform (*c*, 0·9) (Found: C, 56·0; H, 6·1; CH_3·CO, 42·8; N, 6·4. $C_{23}H_{30}O_{10}N_2$ requires C, 55·85; H, 6·1; CH_3·CO, 43·5; N, 5·7%).

Substance.	Temp.	Time (hrs.).	% CH_3·CO.
Galactosazone tetra-acetate (32·5)	−22°	0·5	22·2
	−22	1·33	21·2
	−20 → −14	2·0	23·0
	−20 → −18	2·33	25·0*
	−20 → −10	3·17	26·6
	−23 → −21	3·5	28·3
	−20 → −17	4·0	27·4
	−20 → −18	6·42	29·0
	− 4 → − 6	2·0	29·9*
	− 4 → − 6	3·0	31·9
	+ 17	2·0	33·4*
	+ 17	2·67	34·0
	+ 17	3·0	33·6
	+ 17	4·0	33·5*
Lactose octa-acetate (50·0)	−19 → −18	0·2	24·4
	−19 → −18	0·45	30·0
	−19 → −18	0·83	42·9
	−19 → −18	1·5	43·0
	−19 → −18	2·0	45·9
	−20 → −17	4·67	48·9
	+ 16	0·02	28·0
	+ 16	0·08	39·3
	+ 16	0·25	47·3
	+ 16	4·0	49·2*
Acetanilide (31·9)	+ 18	24·0	0
Methylacetanilide (28·9)	+ 18	24·0	0
Fructosemethylphenylosazone tetra-acetate (31·0)	−19	2·0	27·0
	−10	2·0	30·8
Fructosemethylphenylhydrazone penta-acetate (43·5)	−19	2·0	38·0
	− 5 → + 2	2·5	42·8
Xylosazone tri-acetate (28·4)	−12	2·0	18·0
	+17	2·0	26·0
Arabinosazone tri-acetate (28·4)	−13 → −10	2·0	20·0
	+17	2·0	28·0
"Dianhydro-hexosazone" mono-acetate (11·8)	−20°	2·0	7·0
	−10	2·0	9·0
	+17	7·0	11·9
Lactosazone hepta-acetate (37·0)	−20	2·0	24·1
	+17	2·0	36·0
Maltosazone hepta-acetate (37·0)	−10	2·0	32·0
	+16	2·0	37·0
	+16	4·0	37·3
Glucosephenylhydrazone penta-acetate (44·8)	−10 → −15	2·0	32·0
	+16	3·0	43·4
Glucosemethylphenylhydrazone penta-acetate (43·6)	−17 → −14	0·5	22·3
	−17 → −14	1·0	34·6
	−17 → −14	2·0	37·9
	−17 → −10	4·0	39·8
	−17 → − 8	6·5	42·3
	− 0 → − 5	14·0	40·5
	+16	3·0	43·1
β-Acetylphenylhydrazine (28·7)	−20	2·0	8·0
	−4 → 0	2·0	9·0
	+17	2·0	14·0
	+17	5·0	22·7
	+17	17·0	24·5
α-Acetylphenylhydrazine (28·7)	+16	2·0	1·7
	+16	18·0	8·0
	+16	47·0	18·0
αβ-Diacetylphenylhydrazine (44·7)	−17 → −14	2·0	19·9
	+17	0·08	17·0
	+17	0·16	20·5
	+17	0·5	20·8
	+17	3·5	21·0
	+17	4·0	24·2
	+17	21·0	33·7
	+17	42·0	41·1
Benzaldehyde-α-acetylphenylhydrazone (18·1)	+17	2·0	2·3
	+17	20·0	13·0

Glucosephenylhydrazone Penta-acetate.—This derivative was prepared from glucosephenylhydrazone, m. p. 159°, $[\alpha]_D$ — 82° in water (*c*, 0·5), by the method of Behrend and Reinsberg

XVII

(*Reprinted from* NATURE, *Vol.* 137, *page* 997, *June* 13, 1936.)

Acetylation of Agar

IN view of the recent publication by N. W. Pirie[1] on the acetolysis of agar, it seems advisable to communicate preliminary results from this laboratory, where an investigation on the structure of agar has been proceeding.

Pirie states that agar is difficult to acetylate in comparison with many other polysaccharides. This does not appear to be the case, however, when suitably prepared agar is treated with pyridine and acetic anhydride, as chloroform-soluble agar acetates are formed, the acetyl content of which varies, with the duration of the time of acetylation, from 36 to 43 per cent (as CH_3CO). Deacetylation regenerates agar indistinguishable from the original in its ability to form a gel, and no apparent degradation has therefore occurred during the acetylation process. When a chloroform solution of the acetate is allowed to evaporate, a tough colourless film is obtained.

The low specific rotation of these acetates in chloroform solution ($[\alpha]_D^{15}$ *c.* $-30°$) may be connected with the presence of β-linkages in the molecule, since the hydrolysis of agar gives rise to a solution of positive rotation ($[\alpha]_D^{15}$ *c.* $+30°$).

Experiments on the hydrolysis of agar indicate that the reducing power of the solution after hydrolysis is not wholly accounted for by the presence of *d*-galactose together with the as yet unidentified reducing acid first described by Lüdtke[2]. The *l*-galactose isolated by Pirie accounts to some extent for this discrepancy. Much further work, however, appears to be necessary before even a tentative structure can be proposed for the polysaccharide, and stress must be laid on the necessity of securing a homogeneous starting material.

E. G. V. PERCIVAL.
W. S. SIM.

Chemistry Department,
The University,
Edinburgh.
April 28.

[1] Pirie, *Biochem. J.*, **30**, 369 (1936).
[2] Lüdtke, *Biochem. Z.*, **212**, 419 (1929).

Printed in Great Britain by FISHER, KNIGHT & CO., LTD., St. Albans.

membered ring, or alternatively as a zigzag chain terminated by residues as yet undetermined. It is not yet possible to form an opinion on the 'fine structure' of the agar macro-molecule, whether for example the supposed sulphuric ester residues exist as cross-linkages, or indeed their approximate location. Further work will also be necessary to decide the constitution and mode of union of the acid portion of the hydrolysed product.

These investigations will be discussed elsewhere.

E. G. V. PERCIVAL.
Chemistry Department, J. MUNRO.
University of Edinburgh. J. C. SOMERVILLE.

[1] Percival and Sim, NATURE, **137**, 997 (1936).
[2] Haworth, Raistrick and Stacey, *Biochem. J.*, **29**, 2668 (1935).
[3] Challinor, Haworth and Hirst, *J. Chem. Soc.*, 258 (1931).
[4] Onuki, *Chem. Zentr.*, II, 367 (1933).

Printed in Great Britain by FISHER, KNIGHT & CO., LTD., St. Albans

XIX

THE ACETYLATION AND METHYLATION OF AGAR-AGAR AND THE ISOLATION OF 2:4:6-TRIMETHYL α-*d*-GALACTOSE BY HYDROLYSIS

BY

E. G. V. PERCIVAL

AND

J. CARGILL SOMERVILLE

Reprinted from the Journal of the Chemical Society,
September, 1937.

Reprinted from the Journal of the Chemical Society, 1937.

338. *The Acetylation and Methylation of Agar-agar and the Isolation of 2 : 4 : 6-Trimethyl α-d-Galactose by Hydrolysis.*

By E. G. V. Percival and J. Cargill Somerville.

Methods for the acetylation and methylation of agar are described. Hydrolysis of methylated agar with dilute sulphuric acid yields (A) methyl lævulate, (B) a 2 : 4 : 6-trimethyl methylgalactoside, from which the previously unknown 2 : 4 : 6-trimethyl α-galactose has been isolated, and (C) a fraction which gives strong ketose reactions—as does agar itself on mild hydrolysis—and is considered to be a dimethyl methylketoside.

β-*d*-Galactopyranose units linked either directly together by positions 1 and 3 or by means of an intermediary ketose residue appear to be chiefly concerned in the structure of this complex polysaccharide.

Percival and Sim (*Nature*, 1936, **137**, 997) showed that agar could be acetylated to yield a chloroform-soluble agar acetate which on deacetylation regenerated a substance identical with the original polysaccharide in its ability to form a gel. This was regarded as evidence that no substantial degradation had taken place during acetylation. Later Percival, Munro, and Somerville (*ibid.*, 1937, **139**, 512) described some preliminary results of the hydrolysis of the methylated derivative obtained from the acetate. The details of this work and also the progress made in the identification of the acid portion of the hydrolysis product of agar are now described.

Agar acetate was obtained either as a tough colourless glass from chloroform solution or as a white powder by precipitation from solution by light petroleum. Further acetylation had no effect on its properties, and almost ash-free agar was obtained from it on deacetylation. Deacetylation and methylation yielded a product, soluble in chloroform and acetone, unchanged on further methylation. Fractional precipitation from chloroform solution gave four identical fractions, indicating the homogeneous character of the product.

Hydrolysis with 6% sulphuric acid was complete in four hours, the solution acquiring a positive rotation; at the same time methyl alcohol (3—4%) was evolved, and a brown resin (3%) deposited. No furfural derivatives could be detected. After neutralisation with barium carbonate an estimation of barium in the hydrolytic product gave 6·7%. The barium salt, however, obtained by precipitation with alcohol contained no methoxyl residue. Owing to the solubility of this barium salt it was impossible to separate it quantitatively from the sugars (*ca.* 75%) formed on hydrolysis; therefore glucoside formation and esterification were carried out on the entire hydrolytic product. Distillation in a high vacuum yielded: (A) an optically inactive, mobile ester (16%), (B) a crystalline trimethyl methylgalactoside (65%), and (C) a syrup (14%). The yield of (B) thus isolated from methylated agar was *ca.* 50% of the starting material, and by the hydrolysis of methylated agar with methyl-alcoholic hydrogen chloride a yield of *ca.* 60% was obtained. Assuming that methylated agar, as appears likely, is a true derivative of agar, it follows from this that the polysaccharide is composed of galactose residues to the extent of at least 55%, whereas previous authors (*e.g.*, Lüdtke, *Biochem. Z.*, 1929, **212**, 419) were able to detect only 30—40%.

form (250 c.c.), was precipitated by successive additions of light petroleum (b. p. 60—80°) to yield four fractions:

(A)	0·9 g., $[\alpha]_D^{15°}$	− 92°	in chloroform	(*c*, 0·4);	OMe, 31·0%.
(B)	1·2 g., $[\alpha]_D^{17°}$	− 92°	,, ,,	,,	OMe, 30·8%.
(C)	2·0 g., $[\alpha]_D^{17°}$	− 92°	,, ,,	,,	OMe, 30·9%.
(D)	0·6 g., $[\alpha]_D^{17°}$	− 92·5°	,, ,,	,,	OMe, 30·7%.

The Hydrolysis of Methylated Agar with Sulphuric Acid.—Methylated agar (8 g.) was heated at 95° with sulphuric acid (170 c.c., 6%). The substance gradually dissolved and a brown resin was deposited, which was filtered off (0·25 g.; OMe, nil). A constant rotation ($[\alpha]_D^{20°}$ + 45°) was reached in 4 hours and the hydrolysis was continued during a further hour. The hydrolysate was neutralised with barium carbonate, and the filtered solution evaporated almost to dryness at 50°/10 mm.; alcohol then precipitated a barium salt contaminated by reducing sugars. Solution in water and reprecipitation with alcohol gave a white powder (0·7 g.; OMe, nil). The aqueous-alcoholic residues on evaporation to dryness gave a pale yellow, reducing syrup (6 g.; OMe, 44%) which still contained some of the barium salt. By a modification of the method of Freudenberg and Soff (*loc. cit.*) it was estimated that methylated agar lost 3·5% of methyl alcohol during the hydrolysis.

Simultaneous Esterification and Glucoside Formation.—The neutralised hydrolytic product (7 g.) from another hydrolysis, which still contained the barium salt, was boiled with 5% methyl-alcoholic hydrogen chloride for 6 hours; the solution was neutralised with silver carbonate, filtered, and evaporated at 40°/15 mm. to a non-reducing syrup (5·9 g.), which was fractionally distilled to yield the following fractions: (A) 0·98 g., bath temp. 90—100°/0·01 mm.; (B) 3·8 g., bath temp. 150—160°/0·01 mm.; (C) 0·85 g., bath temp. 180—190°/0·01 mm.; residue, 0·4 g.

Identification of (A) *as methyl lævulate.* The colourless mobile liquid, which gave the iodoform reaction, had $n_D^{15°}$ 1·4250, $[\alpha]_D^{15°}$ ± 0° (Found: C, 54·8; H, 7·9; CO_2Me, 48·0. Calc. for $C_6H_{10}O_3$: C, 55·3; H, 7·8; CO_2Me, 45·3%). The 2 : 4-dinitrophenylhydrazone had m. p. 136°, alone or mixed with the 2 : 4-dinitrophenylhydrazone of methyl lævulate (m. p. 137°) (Found: N, 15·9. Calc. for $C_{12}H_{14}O_6N_4$: N, 16·2%). The *p*-nitrophenylhydrazone had m. p. 136°, alone or mixed with *methyl lævulate* p-*nitrophenylhydrazone* (m. p. 136°) (Found: C, 54·25; H, 5·65; N, 16·2. $C_{12}H_{15}O_4N_3$ requires C, 54·3; H, 5·7; N, 15·85%).

Identification of (B) *as* 2 : 4 : 6-*trimethyl methylgalactoside.* This fraction, which solidified completely during distillation, was recrystallised from light petroleum and had m. p. 62—64°, $[\alpha]_D^{15°}$ + 107° in water (*c*, 0·4) (Found: C, 50·0; H, 8·6; OMe, 51·1. $C_{10}H_{20}O_6$ requires C, 50·8; H, 8·5; OMe, 52·5%). 2 : 4 : 6-*Trimethyl methylgalactoside* may also be isolated as a crystalline *hydrate*, m. p. 37°, $[\alpha]_D^{15°}$ + 101° in water (*c*, 0·4) (Found: C, 47·0; H, 8·6; OMe, 47·1. $C_{10}H_{20}O_6,H_2O$ requires C, 47·2; H, 8·7; OMe, 48·8%).

Complete Methylation and the Isolation of 2 : 3 : 4 : 6-*Tetramethyl Galactose Anilide.*—The crystalline galactoside (1 g.) was methylated in the usual way once with methyl sulphate and sodium hydroxide and once with silver oxide and methyl iodide. After extraction and distillation in a high vacuum the glucosidic residue was removed by heating for 2 hours with hydrochloric acid (7%), the fully methylated sugar being isolated and treated with aniline. Tetramethyl galactopyranose anilide was obtained (0·6 g.), m. p. 192°, unchanged on admixture with an authentic specimen.

The Isolation of 2 : 4 : 6-*Trimethyl α-Galactose.*—Hydrolysis of the trimethyl methylgalactoside (1 g.) during 2 hours with 7% hydrochloric acid, followed by neutralisation with silver carbonate and evaporation, gave a syrup, which crystallised on treatment with ether (0·7 g.). Recrystallisation from ether–light petroleum gave colourless needles, m. p. 104—105°, $[\alpha]_D^{15°}$ + 124° in water (*c*, 0·9), falling to + 93° (equilibrium value). This substance is shown below to be 2 : 4 : 6-*trimethyl α-galactose* (Found: C, 48·2; H, 7·9; OMe, 40·0. $C_9H_{18}O_6$ requires C, 48·7; H, 8·1; OMe, 41·9%).

Osazone Formation and the Isolation of 4 : 6-*Dimethyl Galactosazone.*—The above sugar (0·5 g.) on treatment with phenylhydrazine (1 c.c.) and acetic acid (1 c.c.) and heating at 90° for 2 hours gave rise to yellow needles (0·4 g.), which were recrystallised from aqueous alcohol. They had m. p. 158°, $[\alpha]_D^{20°}$ − 25° in alcohol (*c*, 0·3) (Found: C, 62·3; H, 6·5; OMe, 14·9; N, 15·6. $C_{20}H_{26}O_4N_4$ requires C, 62·1; H, 6·8; OMe, 16·1; N, 14·5%).

Isolation of 2 : 4 : 6-*Trimethyl δ-Galactonolactone and its Crystalline Amide.*—The trimethyl galactose (1 g.) in water (14 c.c.) was treated with bromine (2 c.c.) for 26 hours at 35° and for 22 hours at 18°. Bromine was removed by aeration, the solution neutralised with silver carbonate, and the silver precipitated by hydrogen sulphide. Water was removed at 50°/15 mm. and

the yellow syrup (0·7 g.) was finally heated at 100°/0·01 mm. for 2 hours. It had $[\alpha]_D^{15°}$ + 152° in water (*c*, 0·2) (initial value); + 122° (45 minutes); + 112° (2 hours); + 90° (4 hours); + 50° (16 hours, constant value) (Found: OMe, 40·4; 0·165 g. required 14·9 c.c. of *N*/20-NaOH. Calc. for $C_9H_{16}O_6$: OMe, 42·4%; *N*/20-NaOH, 15·0 c.c.). A crystalline *amide* (0·3 g.) was formed by treating the lactone (0·3 g.) overnight with methyl-alcoholic ammonia, removing the solvent, and recrystallising the product from acetone; it formed lustrous plates, m. p. 167°, $[\alpha]_D^{12°}$ + 74° in water (*c*, 0·3) (Found: C, 46·0; H, 7·8; OMe, 37·6; N, 6·2. $C_9H_{19}O_6N$ requires C, 45·6; H, 8·1; OMe, 39·2; N, 5·9%).

Oxidation of the Lactone with Nitric Acid.—The lactone (2 g.) was oxidised with nitric acid under the conditions laid down by Challinor, Haworth, and Hirst (*loc. cit.*). No crystalline trimethyl mucic ester was obtained, but an ester (0·2 g.) resulted, which was distilled in a high vacuum and converted into an amide (0·05 g.) of zero rotation; this (Found: OMe, 1%) reduced ammoniacal silver nitrate, melted to a red liquid at 165—170°, and was probably impure tartronamide.

Regeneration of 2 : 4 : 6-*Trimethyl Methylgalactoside from* 2 : 4 : 6-*Trimethyl Galactose.*—The sugar in the form of a syrup (0·1 g.) was dissolved in 0·7% methyl-alcoholic hydrogen chloride (10 c.c.), and the change in rotation observed: $[\alpha]_D^{15°}$ + 52° (initial value); + 55° (13 hours); + 82° (26 hours); + 100° (70 hours, constant value). Neutralisation with silver carbonate and removal of solvent gave crystalline 2 : 4 : 6-trimethyl methylgalactoside in quantitative yield.

Examination of Fraction (C).—Redistillation at 190°/0·01 mm. gave a colourless non-reducing syrup, $[\alpha]_D^{15°}$ + 29° in chloroform (*c*, 0·4) [Found: OMe, 39·9; glucosidic OMe, 14·0 (Freudenberg and Soff, *loc. cit.*). $C_9H_{18}O_6$ requires OMe, 41·9%]. The syrup gave a strong Seliwanoff reaction with resorcinol, and the Bredereck reaction with ammonium molybdate. Positive tests were also secured with fructose and trimethyl methylfructofuranoside as controls, but negative results were obtained from galactose, glucose, 2 : 4 : 6-trimethyl galactose, and methyl lævulate. Agar too on mild hydrolysis gave the Bredereck reaction strongly. The syrupy ketoside was treated at 100° with 6% sulphuric acid (2 hours); esterification and distillation then gave more methyl lævulate (30%), indicating at least one probable source of this ester.

When the crystalline 2 : 4 : 6-trimethyl methylgalactoside was hydrolysed with sulphuric acid and submitted to glucoside formation and esterification exactly as for methylated agar, no methyl lævulate could be detected in the final product, so the trimethyl galactose must be discounted as a source of lævulic acid.

The Hydrolysis of Methylated Agar with Methyl-alcoholic Hydrogen Chloride.—Methylated agar (3 g.), dissolved in methyl-alcoholic hydrogen chloride (130 c.c., 5%), was heated at 80° for 6 hours until the rotation was constant. After neutralisation with silver carbonate and evaporation a syrup was isolated which on distillation gave crystalline 2 : 4 : 6-trimethyl methylgalactoside (1·9 g.) at 140—150°/0·03 mm. and a syrup (0·8 g.) at 190°/0·02 mm.; the latter appeared to be identical with the syrup (C), except that the rotation, $[\alpha]_D^{15°}$ + 13° in chloroform (*c*, 0·4), was somewhat lower. It was also found possible to hydrolyse methylated agar with 1% methyl-alcoholic hydrogen chloride.

Thanks are expressed to Imperial Chemical Industries Ltd. and to the Earl of Moray endowment for grants in aid of this research and to the Carnegie Trust for the award of a Teaching Fellowship to E. G. V. P.

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, July 29th,* 1937.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY & SONS, LIMITED,
BUNGAY, SUFFOLK.

XX

INVESTIGATIONS ON THE CONFIGURATION OF CARBOHYDRATES BY CONDUCTIVITY MEASUREMENTS IN BORIC ACID SOLUTION

BY

H. T. MACPHERSON

AND

E. G. V. PERCIVAL

Reprinted from the Journal of the Chemical Society,
December, 1937.

Reprinted from the Journal of the Chemical Society, 1937.

403. *Investigations on the Configuration of Carbohydrates by Conductivity Measurements in Boric Acid Solution.*

By H. T. Macpherson and E. G. V. Percival.

Böeseken's conclusions on the configuration of α- and β-*d*-glucose are confirmed, since conductivity and viscosity experiments with glucose, α-methylglucoside, and various methylated glucose derivatives make it clear that the only hydroxyl groups in glucose having any effect on the conductivity of a boric acid solution are those on C_1 and C_2. By using 3 : 4 : 6-trimethyl α-mannopyranose, the configuration is shown to be *trans*- with respect to the hydroxyl groups on C_1 and C_2, in contradistinction to Böeseken's results for β-mannose.

A striking elevation of conductivity is found for α-methylmannofuranoside, which is attributed to the orientation of the hydroxyl groups, four being situated in close proximity to one another; γ-methylglucoside, where three hydroxyl groups are close to one another, gives a lower elevation, and γ-methylgalactoside a depression. The behaviour of α-*l*-sorbose appears to be anomalous.

In a series of papers during 25 years Böeseken and his co-workers developed a method by means of which the structure of α-*d*-glucose was indicated to be of the *cis*-configuration with reference to the hydroxyl groups on C_1 and C_2. This conclusion was derived from the fact that α-glucose exhibited a higher conductivity than β-glucose in boric acid, and also that during mutarotation the conductivity of the α-glucose–boric acid solution fell, and that of the β-glucose–boric acid rose, to an intermediate equilibrium value (*Ber.*, 1913, **46**, 2612). From these facts Böeseken considered that complex formation occurred between boric acid and the hydroxyl residues on C_1 and C_2 in the case of α-glucose but not β-glucose. Although other methods of determining configuration, such as optical calculations (Freudenberg, Helferich, and Winkler, *Z. physiol. Chem.*, 1932, **209**, 270), *X*-ray studies (Cox *et al.*, J., 1932, 138, *et seq.*), and methods depending on differences of reactivity (Micheel and Micheel, *Ber.*, 1930, **63**, 386; Vavon, *Bull. Soc. chim.*, 1931, **49**, 997, 1011), have been elaborated, it seemed to us that this elegant method, if substantiated, might be of service particularly with regard to the structure of dissolved molecules.

It must be borne in mind, however, that in a molecule such as glucose with five free hydroxyl groups, other possibilities of combination with boric acid exist. Irvine and Steele (J., 1915, **107**, 1221) recognised this and examined methylated glucoses, and on the basis of a considerable elevation in conductivity following an initial depression for tetramethyl glucopyranose and an elevation for tetramethyl methylglucoside in boric acid

solution, concluded that the oxygen atom of the ring combined with water to form an oxonium hydrate, which in turn formed a complex, together with the hydroxyl group on C_1, with boric acid. Böeseken and Couvert (*Rec. trav. chim.*, 1921, **40**, 354) repeated this work on purer specimens and found that, instead of the rapid increase of conductivity in boric acid solution, the initial depression underwent little change. We have now confirmed this result and extended it to α-methylglucopyranoside, 2 : 3 : 6-trimethyl glucopyranose, 2 : 3 : 6-trimethyl methylglucopyranoside, and sucrose. In all these cases diminution of conductivity below the sum of the values for the sugar derivative alone and of the boric acid solution was observed with no appreciable increase on standing. Viscosity determinations indicated that the observed depression ran parallel to the relative viscosity, so the diminution in conductivity was not anomalous. Incidentally, it was shown that Einstein's equation (*Ann. Physik*, 1906, **19**, 289), according to which viscosity is a function of the volume of the dissolved phase for approximately spherical molecules, was obeyed by these substances.

It is clear, therefore, not only that the ring oxygen atom, whether in a pyranose or a furanose sugar, has no effect on the conductivity of a boric acid solution, but also that in the case of glucose, the only hydroxyl groups having a positive effect are those on C_1 and C_2. No increase in conductivity is observed unless both these hydroxyl groups are unsubstituted and in the *cis*-position to one another.

Although the change in conductivity during the mutarotation of glucose is explained, it is to be noted that α-methylglucoside depresses the conductivity of boric acid solution; β-glucose in boric acid solution, however, gives, not the expected depression, but an initial elevation of the same order as the difference between the initial conductivities of the α- and the β-glucose. It may be that this initial conductivity is due to the presence in solution of a small amount of a straight-chain aldehydic form, possibly present as the aldehydrol. Straight-chain polyhydroxy-compounds such as dulcitol and sorbitol have a strongly positive effect on the conductivity of boric acid solution (*ca.* 600×10^{-6} mho* in M/2-solution), and it was found that glucose diethylmercaptal also had a strong positive effect (176 in M/10-solution).

The case of mannose is of interest since the earlier conductivity results indicate β-*d*-mannose to be a *trans*-form on the basis of a rise in conductivity during mutarotation, contrary to the accepted views for the configuration of this sugar. This case is complicated by the difficulty of securing β-mannose sufficiently pure for conductivity determinations, although our result for β-mannose is opposed to that of Böeseken and Couvert (*Rec. trav. chim.*, 1921, **40**, 370) in that we found a fall in conductivity. This result was supported, however, by using 3 : 4 : 6-trimethyl α-*d*-mannose (Bott, Haworth, and Hirst, J., 1930, 1395), in which any possibility of combination with boric acid at positions other than those under investigation is precluded. An initial depression was observed, in harmony with the results for the methylated glucose derivatives, and in agreement with viscosity determinations, but this depression gradually became less, parallel with the mutarotation, which at once confirms our observation on β-mannose and agrees with the present conception of the structure of this sugar. It is noteworthy that the change in conductivity is relatively small (this depends on the proportion of α- and β-forms at equilibrium) and that the relatively high initial value as found for β-glucose is absent.

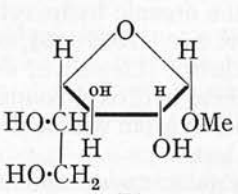
α-*l*-Rhamnose suffered an elevation of conductivity during mutarotation, which agrees with the view that the hydroxyl groups on C_1 and C_2 are *trans*-.

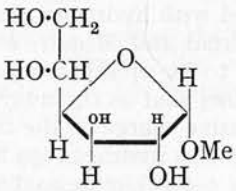
Striking results were obtained with furanosides: γ-methylgalactoside (I) showed a depression of conductivity (12 gemmhos) (cf. α-methylglucopyranoside), but γ-methylglucoside (II) gave an elevation of 110 and α-methylmannofuranoside (III) an elevation of 3350. (These results are for M/2-solutions.) The result for the galactofuranoside reveals that the ·CH(OH)·CH_2·OH group alone has no positive influence on the conductivity in this case. By comparison with ethylene glycol, which shows a depression, this is only to be expected, since this group, being a side chain, is permitted complete

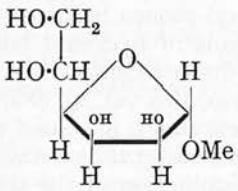
* All conductivities are subsequently given in terms of 10^{-6} mho.

freedom as to the relative positions of the hydroxyl groups, which will naturally incline to repel one another.

An examination of models, however, reveals that the glucofuranoside has a hydroxyl group in close proximity to the $CH(OH)\cdot CH_2\cdot OH$ group, giving three hydroxyl residues near to one another. For the α-methylmannofuranoside we have two hydroxyl groups within easy reach of the $CH(OH)\cdot CH_2\cdot OH$ group and on the same side of the ring plane.


(I.)


(II.)


(III.)

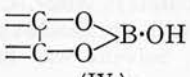
We thus have four hydroxyl groups as in erythritol, but here there is much less freedom of movement. In this case there is an accumulation of hydroxyl groups but the conditions are so complex that it is not yet possible to advance any definite hypothesis to account for the increase in conductivity.

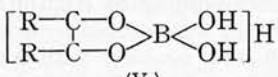
Comparable with the elevation given by α-methylmannofuranoside is that of a α-*l*-sorbose, *viz.*, 2159 gemmhos in M/2-solution (cf. Böeseken and Leefers, *Rec. trav. chim.*, 1935, **54**, 865, who found 230 for M/10-solution). β-*d*-Fructose also gives a considerable elevation (703 in M/2-solution), but an examination of the ring structures for these ketoses fails to reveal the reason for the great difference between them. The fact that sorbose does not mutarotate might suggest that this sugar exists in solution in the keto-form, but other evidence does not favour such a view, and the question must be postponed for further study.

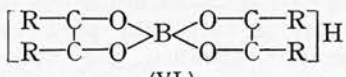
Rotation experiments have been carried out on α- and β-*d*-glucose, 3 : 4 : 6-trimethyl α-mannose, and α-methylmannofuranoside in both aqueous and boric acid solutions. No differences either in the equilibrium values or in the velocity of mutarotation were observed.

Consideration of the results for glycol, glycerol, erythritol, dulcitol, αβ-propylene glycol, and triethylene glycol shows that there is no very considerable increase in the conductivity of boric acid solution until four hydroxyl groups are present. Böeseken (*Rec. trav. chim.*, 1915, **34**, 96) showed that for pentaerythritol the elevation was about 6 times that observed for erythritol, and this appears to be due to the more rigid tetrahedral structure present in the former compound.

There has been some conflict of opinion as to whether or not the conductivity effects of polyhydroxy-compounds with boric acid are dependent on the formation of complexes (IV), as first proposed by van't Hoff, and since adopted by Böeseken and his co-workers. The extensive literature includes papers by Kolthoff (*Rec. trav. chim.*, 1925, **44**, 975), Bancroft and Davis (*J. Physical Chem.*, 1930, **34**, 2479), Henderson and Prentice (J., 1902, **81**, 658), Darmois (*J. Chim. physique*, 1926, **23**, 130, 649), Burgess and Hunter (J., 1929, 2838), Böeseken and Vermaas (*J. Physical Chem.*, 1931, **35**, 1477), Hermans (*Z. anorg. Chem.*, 1925, **142**, 83), and many others. The objections raised are chiefly based on the inability to isolate such complexes in the solid state from the highly conducting aqueous solutions. Nevertheless, Lowry (J., 1929, 2853) described a borotartaric acid, Hermans


(IV.)


(V.)


(VI.)

(*loc. cit.*) a disalicylboric acid, and Brigl and Grüner (*Annalen*, 1932, **495**, 60) a glucose diboric acid, although special conditions have to be used for the isolation of such derivatives. Hermans (*loc. cit.*) and Böeseken and Vermaas (*loc. cit.*) have suggested that the dissociable complexes may possess structures of the type (V) or (VI), according as

one or two molecules of the hydroxy-compound are involved. In aqueous solution, however, it seems unreasonable that definite anhydrides such as this should be formed, and it is much more probable that the cyclic structure containing the boron atom is formed by a co-ordination process. Any such hypothesis must account for the increase in conductivity. By a mechanism analogous to the formation of BF_3,NH_3, boric acid may react with water to form the necessarily weak acid $\overset{+}{H}[\overset{-}{B}(OH)_4]$. If then the hydroxyl groups become associated with hydrogen atoms of the organic hydroxyl residue by means of hydroxyl bonds (Bernal and Megaw, *Proc. Roy. Soc.*, 1935, **151**, *A*, 384), which have recently been shown to be present in pentaerythritol (Llewellyn, Cox, and Goodwin, this vol., p. 883), it follows that as the number of these hydroxyl bonds around the boric acid is increased the negative charge on the central boron atom will be decreased, thus increasing the ease with which the hydrogen ion may be lost.

Examination of the results for α-*d*-galactose and α-methylgalactopyranoside suggests that the *cis*-hydroxyl groups at C_3 and C_4, by comparison with α-methylglucoside, have no (or, in the latter case only a slight) effect on the conductivity of boric acid solution. Böeseken (*Rec. trav. chim.*, 1921, **40**, 558) showed that whereas *cis-cyclo*pentane-1 : 2-diol gave a marked increase in conductivity, yet *cis-cyclo*hexane-1 : 2-diol gave a decrease. This can be understood when it is considered that the *cyclo*pentane ring is flat and the hydroxyl groups are in the same plane, but if the *cyclo*hexane ring is strainless, then the adoption of one or other of the Sachse–Mohr forms results in changes of the relative positions of both *cis*- and *trans*-hydroxyl groups and the alteration of conformation may perhaps account for the altered behaviour with boric acid. This can easily be seen by reference to models, and it is hoped that further investigation will lead to information on the conformation of the pyranose ring in solution.

Experimental.

Conductivity Experiments.

The Pyrex-glass conductivity vessel was specially constructed so that less than 1 c.c. of solution sufficed to cover the plates; the *polished* platinum electrodes were carried by the ground-in stopper, contact with the atmosphere being thereby excluded. Platinised plates were less satisfactory than polished platinum, which gave a sharp minimum for the solutions of low conductivity investigated in these experiments. The bridge, which was calibrated before use, was of the cylindrical type, wound with 3·6 m. of wire and divided into 1000 equal divisions, readings being taken to 0·25 of a scale division. A small induction coil and telephone were used in the usual manner in conjunction with standard variable resistances. The cell constant (0·4378) was determined with N/50-potassium chloride in conjunction with platinised electrodes, and with N/500-potassium chloride with the polished electrodes; the conductivity water used throughout had a specific conductivity of 2×10^{-6} mho. A larger cell of the same type (capacity 2 c.c.) was employed in experiments where relatively large quantities of pure material were available. The measurements were carried out at 25° ± 0·01°, the solutions being made up with water at this temperature; the same apparatus was used throughout, so the results are strictly comparable. From considerations of space, complete lists of the readings are not included, but readings of the variation in conductivity at 5- and 10-minute intervals (unless the reading remained constant) were taken in all cases. κ_1, κ_2, and κ_3 refer respectively to the conductivity (in gemmhos) of an M/2-aqueous solution of the sugar or sugar derivative concerned, of the M/2-boric acid solution employed, and of a solution in M/2-boric acid of the sugar or sugar derivative at the same concentration.

α-d-*Glucose.*—α-*d*-Glucose was prepared according to Hudson and Dale (*J. Amer. Chem. Soc.*, 1917, **39**, 322). The specific conductivity was reduced by grinding with pyridine at room temperature, filtration, and precipitation and washing with alcohol. Solvent was finally removed in a vacuum at 40°: $[\alpha]_D^{20°}$ + 106° in water (*c*, 3·0) (after 2 mins.).

$\kappa_1 = 11{\cdot}5$; $\kappa_2 = 27{\cdot}6$.

Time (mins.)	5	30	60	80	100	120
κ_3	96·3	89·8	86·0	84·7	83·8	83·3 (constant value).

Elevation of conductivity: 59·4 (initial value; by extrapolation); 44·2 (final value).

β-d-*Glucose.*—β-*d*-Glucose, prepared by Behrend's method (*Annalen*, 1907, **353**, 107), was well washed with alcohol to remove pyridine, and the solvent removed as before : $[\alpha]_D^{20°} + 19·5°$ in water (*c*, 1·0) (after 3 mins.).

$\kappa_1 = 8·5$; $\kappa_2 = 28·0$.

Time (mins.)	12	20	30	60	80	100
κ_3	66·6	68·3	70·0	73·1	74·1	75·0 (constant value).

Elevation of conductivity : 26·5 (initial value; by extrptn.); 38·5 (final value).

It will be noted that a difference exists between the final values—expressed as elevations of conductivity—for α- and β-glucose. Böeseken (*loc. cit.*) also found this to be the case. The same " gap " was found to occur if the α- and β-glucose solutions were allowed to come to equilibrium before the addition of boric acid.

α-d-*Galactose.*—Kerfoot's " pure " galactose was found to be almost entirely α-*d*-galactose, and after being washed with 90% ethyl alcohol it gave $[\alpha]_D^{20°} + 137·8°$ in water (*c*, 1·3) (after 3 mins.).

$\kappa_1 = 23·3$; $\kappa_2 = 27·9$.

Time (mins.)	10	40	60	100
κ_3	78·4	74·4	73·1	72·2 (constant value).

Elevation of conductivity : 29·5 (initial; by extrptn.); 21·0 (final).

β-d-*Mannose.*—In aqueous solution six samples of mannose had a high conductivity, not reduced by recrystallisation : $[\alpha]_D^{18°} - 17·0°$ in water (*c*, 3·0) (initial value), + 14·8° (final value).

$\kappa_1 = 78·2$; $\kappa_2 = 28·0$.

Time (mins.)	7	15	30	50
κ_3	105·8	104·9	104·1	103·8 (constant value).

Elevation of conductivity : 0·4 (initial; by extrptn.); − 2·4 (final).

α-l-*Rhamnose.*—Specimen from B.D.H. of high purity : $[\alpha]_D^{18°} - 6·8°$ in water (*c*, 1·0) (after 3 mins.); + 9·0° (final value).

$\kappa_1 = 5·2$; $\kappa_2 = 29·0$.

Time (mins.)	5	100
κ_3	41·8	46·1 (constant value).

Elevation of conductivity : 7·5 (initial; by extrptn.); 11·9 (final).

Sucrose.—$[\alpha]_D^{20°} + 66·4°$ in water (*c*, 3·0).

$\kappa_1 = 7·8$; $\kappa_2 = 28·9$; $\kappa_3 = 27·2$. *Depression of conductivity :* 9·5 (constant value).

α-*Methylglucopyranoside.*—This was prepared according to Patterson and Robertson (J., 1929, 300), and after 5 recrystallisations from absolute alcohol had m. p. 167°, $[\alpha]_D^{20°} + 159°$ in water (*c*, 3·0) (Found : OMe, 15·4. Calc. for $C_7H_{14}O_6$: OMe, 16·0%).

$\kappa_1 = 3·9$; $\kappa_2 = 27·9$; $\kappa_3 = 22·5$. *Depression of conductivity :* 9·3.

α-*Methylgalactopyranoside.*—Prepared as above, this had m. p. 116°, $[\alpha]_D^{20°} + 179°$ in water (*c*, 1·0) (Found : OMe, 15·9. Calc. for $C_7H_{14}O_6$: OMe, 16·0%).

$\kappa_1 = 12·9$; $\kappa_2 = 27·9$; $\kappa_3 = 36·8$. *Depression of conductivity :* 4·0.

2 : 3 : 4 : 6-*Tetramethyl Glucose.*—This sugar was prepared by the method of West and Holden (*J. Amer. Chem. Soc.*, 1934, **56**, 930) and recrystallisation from light petroleum gave a product, m. p. 85—88° (Found : OMe, 51·5. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%); $\kappa_1 = 50$. By 5 distillations at 135° (bath temp.)/0·03 mm., a specimen of the glucopyranose was secured with m. p. 72°, $[\alpha]_D^{20°} + 70·3°$ in water (*c*, 0·5) ⟶ + 79·8° (constant value).

$\kappa_1 = 7·0$; $\kappa_2 = 27·9$; $\kappa_3 = 20·8$. *Depression of conductivity :* 14·1.

Tetramethyl Methylglucopyranoside.—West and Holden's method (*loc. cit.*) was employed, and the syrup distilled twice at 85°/0·04 mm. with a fractionating column; $n_D^{18°}$ 1·4465 (Found : OMe, 60·5. Calc. for $C_{11}H_{22}O_6$: OMe, 62·0%).

$\kappa_1 = 12·2$; $\kappa_2 = 27·6$; $\kappa_3 = 21·5$. *Depression of conductivity :* 18·3.

2 : 3 : 6-*Trimethyl Methylglucopyranoside.*—Starch was methylated according to Haworth, Hirst, and Webb (J., 1928, 2681) until the methoxyl content reached 44%. Hydrolysis by the method of Baird, Haworth, and Hirst (J., 1935, 1201) yielded a mixture of tri- and tetramethyl methylglucosides which were fractionated in a high vacuum, the first runnings being rejected. A colourless syrup, $n_D^{18°}$ 1·4550, was obtained (Found : OMe, 51·5. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%).

$\kappa_1 = 14·1$; $\kappa_2 = 27·6$; $\kappa_3 = 25·5$. *Depression of conductivity :* 16·2.

2 : 3 : 6-*Trimethyl Glucopyranose.*—Hydrolysis of the corresponding methylglucoside by means of aqueous hydrochloric acid, followed by isolation in the usual way, yielded the sugar, after recrystallisation from ether, of m. p. 117° : $[\alpha]_D^{18°}$ + 66° in water (*c*, 1·4) (final value) (Found : OMe, 40·5. Calc. for $C_9H_{18}O_6$: OMe, 41·8%).

$\kappa_1 = 10·4$; $\kappa_2 = 27·9$; $\kappa_3 = 28·8$. *Depression of conductivity :* 9·5.

3 : 4 : 6-*Trimethyl* α-d-*Mannose.*—Bott, Haworth, and Hirst's method (*loc. cit.*) was used. Care was necessary to keep the intermediate "γ"-tetra-acetyl methylmannoside from becoming even faintly acid, the addition of sodium bicarbonate being necessary to prevent this. The product (Found : C, 48·5; H, 8·1; OMe, 41·0. Calc. for $C_9H_{18}O_6$: C, 48·7; H, 8·1; OMe, 41·8%) had m. p. 104°, $[\alpha]_D^{20°}$ + 22° in water (*c*, 0·6) (initial value) falling to + 8·3° (constant value). The same results were obtained in boric acid solution.

$\kappa_1 = 18·0$; $\kappa_2 = 27·0$.

Time (mins.)	6	15	20	40	60	80
κ_3	34·8	36·4	37·2	39·4	40·3	40·9 (equilibrium value)

Depression of conductivity : 12·1 (initial), 4·1 (final).

Glucose Diethylmercaptal.—The product obtained by Fischer's method (*Ber.*, 1894, **27**, 673) was recrystallised repeatedly from water; m. p. 128°.

$\kappa_1 = 11·7$; $\kappa_2 = 5·1$; $\kappa_3 = 193·0$. *Elevation of conductivity :* 176·2.

Ethylene Glycol.—$\kappa_1 = 4·8$; $\kappa_2 = 30·0$; $\kappa_3 = 27·1$. *Depression of conductivity :* 7·7.

Glycerol.—$\kappa_1 = 7·2$; $\kappa_2 = 30·0$; $\kappa_3 = 45·3$. *Elevation of conductivity :* 8·1.

αβ-*Propylene Glycol.*—$\kappa_1 = 16·2$; $\kappa_2 = 30·0$; $\kappa_3 = 33·5$. *Depression of conductivity :* 12·7.

Triethylene Glycol.—$\kappa_1 = 7·6$; $\kappa_2 = 30·0$; $\kappa_3 = 23·1$. *Depression of conductivity :* 14·5.

Erythritol.—$\kappa_1 = 25·0$; $\kappa_2 = 30·0$; $\kappa_3 = 97·0$. *Elevation of conductivity :* 42·0.

Dulcitol.—$\kappa_1 = 35·9$; $\kappa_2 = 30·0$; $\kappa_3 = 660$. *Elevation of conductivity :* 594.

Mannitol.—$\kappa_1 = 12·3$; $\kappa_2 = 29·0$; $\kappa_3 = 667·3$. *Elevation of conductivity :* 626.

γ-*Methylglucoside.*—Fischer's method (*Ber.*, 1914, **47**, 1984) was employed, and the product when distilled at 220° (bath temp.)/0·04 mm. had no action on Fehling's solution; $[\alpha]_D^{18°}$ − 14° in water (*c*, 5·0) (Found : OMe, 15·8. Calc. for $C_7H_{14}O_6$: OMe, 16·0%).

$\kappa_1 = 15·0$; $\kappa_2 = 30·0$; $\kappa_3 = 156·3$. *Elevation of conductivity :* 111·3.

γ-*Methylgalactoside.*—The method of Haworth, Ruell, and Westgarth (J., 1924, **125**, 2468) gave a non-reducing syrup, $[\alpha]_D^{18°}$ − 57·6° in water (*c*, 2·3) (Found : OMe, 15·1%). Comparison of the rate of hydrolysis in N/10-hydrochloric acid at 90° with that for α-methylgalactopyranoside showed that, whereas only 50% of the latter was hydrolysed during 9 hours, the furanoside was completely hydrolysed in 5 hours.

$\kappa_1 = 30·0$; $\kappa_2 = 30·0$; $\kappa_3 = 48·0$. *Depression of conductivity :* 12·0.

α-*Methylmannofuranoside.*—Haworth, Hirst, and Webb's method (J., 1930, 658) yielded a colourless, non-reducing syrup which crystallised immediately on nucleation with a crystal of the substance kindly provided by Professor Haworth; m. p. 119°; $[\alpha]_D^{18°}$ + 113° in water (*c*, 1·5), + 112·2° in M/2-boric acid (*c*, 0·5).

$\kappa_1 = 20·0$; $\kappa_2 = 30·0$; $\kappa_3 = 3400$. *Elevation of conductivity :* 3350.

β-d-*Fructose.*—Commercial fructose was purified by slow recrystallisation from absolute alcohol and washing with acetone; $[\alpha]_D^{17°}$ − 130° in water (*c*, 3·6).

$\kappa_1 = 28{\cdot}6$; $\kappa_2 = 30{\cdot}0$; $\kappa_3 = 762$. *Elevation of conductivity :* 703.

α-l-*Sorbose.*—M. p. 164—165°, $[\alpha]_D^{18°}$ − 43·5° in water (*c*, 1·0).

(i) $\kappa_1 = 24{\cdot}8$; $\kappa_2 = 30{\cdot}0$; $\kappa_3 = 2214$. *Elevation of conductivity :* 2159.

(ii) For M/10-solutions in each case : $\kappa_1 = 9{\cdot}3$; $\kappa_2 = 5{\cdot}1$; $\kappa_3 = 212$. *Elevation of conductivity :* 197·6.

Rotation Experiments.

α-d-*Glucose.*—The values of $[\alpha]_D^{20°}$ at time *t* (mins.) for (i) M/2-aqueous solution and (ii) M/2-solution in M/2-boric acid were :

t	5	30	60	140	220	280	∞
$[\alpha]_D^{20°}$ (i)	+108·2°	94·1°	82·6°	64·4°	57·6°	54·9°	52·7°
$[\alpha]_D^{20°}$ (ii)	+108·3°	94·0°	82·8°	64·8°	57·4°	54·7°	52·6°

Similar experiments were carried out with M/4- and M/8-solutions; the figures in each case showed the same rate of mutarotation in aqueous and boric acid solution and the equilibrium values only varied from 52·3° to 52·6°.

β-d-*Glucose.*—The data, expressed as above, are :

t	3	20	40	60	90	150	240	∞
$[\alpha]_D^{20°}$ (i)	+20·6°	25·7°	30·6°	34·6°	39·2°	44·7°	49·0°	52·2°
$[\alpha]_D^{20°}$ (ii)	+20·8°	25·8°	30·8°	34·8°	39·2°	44·7°	49·2°	52·3°

Viscosity Experiments.

The apparatus used was that described by Hornel and Butler (J., 1936, 1361); it required less than 1 c.c. of liquid for a determination, and the capillary was of such dimensions that approximately 100 seconds were required for water to pass between the fixed points. M/2-Solutions of the substances examined were made up in water and in boric acid solution; a weighed amount of solution was transferred to the apparatus, and its time of flow determined by means of a Venner stop-watch reading to 0·1 sec., the mean of several determinations at 25° ± 0·02° being taken as the result.

The relative viscosity η_r was calculated from $\eta_r = \eta'/\eta = \rho' T'/\rho T$, where η and η' are the viscosity coefficients of two liquids of densities ρ and ρ' and times of fall T and T'. The results are recorded in the following table, in which G denotes the weight (in g.) in the viscometer, and T the mean time of fall (in secs.).

Relative Viscosities.

	M/2-*Aqueous solutions.*			M/2-*Solutions in* M/2-*boric acid.*		
	G.	*T.*	η_r.	*G.*	*T.*	η_r.
(Conductivity water)	0·9445	102·5	1·000	—	—	—
α-*d*-Glucose	0·971	124·2	1·250	0·982	128·9	1·240
β-*d*-Glucose	0·973	124·3	1·250	0·983	129·4	1·245
α-Methylglucopyranoside	0·967	129·7	1·295	0·978	134·2	1·290
3 : 4 : 6-Trimethyl α-mannose	0·967	136·5	1·360	0·977	141·1	1·350
2 : 3 : 6-Trimethyl methylglucopyranoside	0·962	140·7	1·400	0·972	146·0	1·400
2 : 3 : 4 : 6-Tetramethyl glucose	0·962	139·5	1·390	0·973	145·4	1·390
2 : 3 : 4 : 6-Tetramethyl methylglucoside	0·966	142·2	1·420	0·976	148·0	1·415
Boric acid	0·959	106·4	1·050	—	—	—

As shown by the following table, Einstein's equation (*loc. cit.*), which can be modified to $\eta_{sp.}S/c = K$, is approximately obeyed, K having a mean value of 0·44 for the solutions in M/2-boric acid. Here $\eta_{sp.}$ is the specific viscosity, c the weight concentration of dissolved phase, S the density of dissolved phase calculated from a knowledge of the weight of substance taken, the volume of water used, and the weight of solution, and K should be constant for any one class of substance.

It will be noted that the relative viscosity increases with increase in molecular weight; this is analogous to the increasing depression of conductivity on the introduction of an

increasing number of methoxyl groups, and both these results are complementary to the above relation between viscosity and molecular volume.

Comparison of Viscosity with Volume of Dissolved Phase for M/2*-Aqueous Solutions.*

Dissolved phase.	$\eta_{sp.}$	$c.$	$\eta_{sp.}/c.$	$S.$	$\eta_{sp.}S/c.$
α-d-Glucose	0·250	8·25	0·0303	1·552	0·46
β-d-Glucose	0·250	8·25	0·0303	1·552	0·46
α-Methylglucopyranoside	0·295	8·82	0·0335	1·386	0·48
3 : 4 : 6-Trimethyl α-mannose	0·360	9·98	0·0361	1·337	0·48
2 : 3 : 6-Trimethyl methylglucopyranoside	0·400	10·55	0·0379	1·242	0·47
2 : 3 : 4 : 6-Tetramethyl glucose	0·390	10·55	0·0370	1·242	0·46
2 : 3 : 4 : 6-Tetramethyl methylglucoside	0·420	11·10	0·0378	1·275	0·48

Thanks are expressed to the Carnegie Trust for the award of a Teaching Fellowship to one of us (E. G. V. P.), to the Earl of Moray Endowment, and to Imperial Chemical Industries Ltd. for grants.

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, August 4th*, 1937.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY & SONS, LIMITED,
BUNGAY, SUFFOLK.

ACKNOWLEDGMENTS

I wish to thank Professor James Kendall, F.R.S., for his interest and generous provision of facilities for the work carried out in Edinburgh, and the Moray Fund for grants in aid of special apparatus. I am also indebted to the Carnegie Trust for a Teaching Fellowship held since October 1935. My thanks are also due to Professor W.N. Haworth, F.R.S., for introducing me to sugar chemistry and together with Professor E.L.Hirst,F.R.S., for continual counsel during the period spent at Birmingham. To those research students whose names appear on many of the papers and to which they contributed most of the experimental work I express my gratitude for their close co-operation. I have also to thank my wife for her constant encouragement and for her collaboration in papers [XIV] and [XVI].