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by

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Title.

# STUDIES ON THE POLYSACCHARIDES OF ICELAND MOSS ( CETRARIA ISLANDICA ).

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CONTENTS

## GENERAL INTRODUCTION

Cetraria Islandica, Iceland Moss, despite the suggestion of its common name belongs to that interesting plant class the lichens, which are formed by the interdependent growth (symbiosis) of unicellular or filamentous algae with the mycelium of a fungus. In general it is thought that the alga supplies carbohydrates by photosynthesis, while the fungus provides other requirements for growth and water storage. Lichens grow extremely slowly and show great resistance to climatic extremes particularly drought, thanks to their power of withstanding desiccation while retaining their vitality; they can thus often fulfil the role of pioneers of vegetation on otherwise bare areas. In certain, if limited regions lichens are of great economic importance. They cover the soil of the subarctic tundra to the practical exclusion of all other vegetation. Lapp tribes depend for their livelihood on reindeer, which subsist on the easily hydrolysable polysaccharides contained in the lichens.

The habitat of Iceland Moss in particular is Iceland, but it is also found in other areas including the British Isles. Like most of the other members of its class it contains lichenin (1,2,3) to the extent of 50-60% of its dry weight. This polysaccharide is available to the human body, but the bitter taste of the accompanying lichen acids presents difficulties to direct consumption without previous treatment.

Berzelius/

Berzelius (4) investigated the problem of utilization of this vast, hitherto untapped source of glucose. Owing to the property of the lichenin of Iceland Moss decoctions to reduce surface tension, use can be made of these as a washing agent (5); other patents deal with its utilization as a colloid stabilizer for Latex (6).

Iceland Moss is the only lichen included in the British Pharmacopeia, probably because its content of cetrarin acts as soothing agent against chest complaints.

The water-soluble polysaccharides lichenin and isolichenin and the lichen acids comprise in all some 80% of the total dry matter of the tissues of cetraria islandica and after their removal there remains a residue, mainly carbohydrate in nature consisting of cellulose, hemicelluloses etc(7).

The first part of this thesis is devoted to an investigation of the property of lichenin to form alkali addition compounds, while in the second part the hemicelluloses are investigated more fully than has been done hitherto.

At this point it may be permitted to add a few remarks of a rather biological nature.

Since it is impossible at present to draw a complete picture of the alkali soluble fraction of polysaccharides contained in Iceland Moss (the "polyuronide hemicelluloses"), which are suggested (7) to have the dual function of reserve substances and cell wall constituents, interpretation and evaluation of/

their biological role in the plant will have to take into account some such considerations as the following:

(1) The present day method- generally applied - of separating the fractions obtained by extraction with cold alkali by means of graded addition of alcohol is unsatisfactory, being largely based on the physical properties of these substances in the colloidal state (8). Two hemicellulose fractions from two different plants, both classified as "A" were however shown to consist of different chemical units (8). We shall refer to that later in the introduction to part II.

(2) Hemicellulose fractions thus separated may be derived from the fungus- as well from the alga- cell walls (7).

(3) Lichens have, as mentioned already an extremely slow growth. All investigation results will represent averages over all age groups. It is well known that percentages of certain polysaccharides fractions in plants vary both with the season and with the age of the plant; pectin is one example of the former. The chemical properties of hemicelluloses were found to be similarly affected (9) .

(4) The metabolism of one and the same type of lichen may vary according to geographical position and the prevailing climatic conditions.

Thus we are forced to the conclusion that from the point of view of their biochemical interpretation all data obtained on hemicelluloses by the present day technique will have to be regarded as representing/

presenting mean values over a large number of variations and combinations .

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# PART I.

## THE ADDITION COMPOUNDS OF LICHENIN AND POTASSIUM HYDROXIDE.

## INTRODUCTION.

Lichenin is easily prepared from Iceland Moss by extraction with boiling water after the cold-water-soluble carbohydrates and lichen acids have been removed.

The similarity of the repeating unit in lichenin and cellulose was established by the isolation of cellobiose octaacetate by means of acetolysis (1), by obtaining a quantitative yield of glucose from the polysaccharide by the action of certain enzymes from barley malt (2), and by the isolation of 2,3,6-trimethyl glucose in yields similar to those obtained from cellulose by the same procedure (3). Although both polysaccharides therefore appear to be made up of 1,4- $\beta$-glucosido glucose units (4), they differ sharply in their physical properties, in that lichenin easily forms colloidal solutions in water which can be precipitated by addition of alcohol, and is less resistant to hydrolytic agents and enzymes (5). Certain differences in the optical rotation of lichenin and cellulose derivatives, and differences in the x-ray pattern point to a dissimilarity in the macromolecular structure of the two polysaccharides; on the other hand, evidence as to the similarity of their basic structures is presented by Ott (22), who found the x-ray pattern of lichenin to be identical with that of disaggregation derivatives of cellulose like oxycellu-

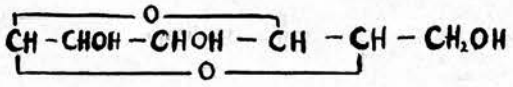
lose/

lose and hydrocellulose. Karrer (23) believed he could furnish further proof of their analogous properties; he claimed that lichenin and cellulose give rise to acetates of identical rotation, yield sodium hydroxide addition compounds of the same composition, that both are practically non-reducing, and are only stained by iodine when suspended and not when dissolved in water.

Because of its relationship with cellulose, the problem of the lichenin structure has received a great deal of attention during the last 18 years. It could reasonably be hoped that its elucidation would contribute to the understanding of the make up of the cellulose molecule and those of other allied polysaccharides.

In the early days of what is now termed "high-polymer chemistry" various means of approaching the subject were tested by trial and error and many papers which appeared then have now historical value only.

In 1925 Pringsheim and co-workers (6,41,42) obtained on glycerolysis of lichenin a non-crystalline, cold-water-soluble substance, which they termed "lichosan", considering it to be a glucose anhydride:-



a suggestion which they backed up by cryoscopic molecular weight determinations of its acetyl derivatives, etc. They observed that in water it reassociated quickly to form a gel, which, after precipitation/

cipitation with alcohol, appeared to be more or less identical with the original lichenin. By comparing these results with those obtained on cellulose they hoped to solve the question, whether two polysaccharides made up of possibly identical repeating units can differ as much as do lichenin and cellulose merely owing to the different engagement of the residual valencies of the participating basic units.

Since Pringsheim's principal experimental basis of investigation of his lichosan was molecular weight determination by cryoscopic methods, the paper subsequently published by Hess and Schultze (7) is of importance. Hess obtained results from cellulose in copper tetrammin solutions from which it follows, that cellulose behaves in these solutions as if it consisted of chemically independent molecules of the size $C_6H_{10}O_5$. Cellulose, upon removal from such solution again displays the well known property of being insoluble in neutral solvents. Its chemical properties, as well those of its acetyl and methyl derivatives were also identical with the starting material. This together with the minimum of molecular size $(C_6H_{10}O_5)_1$ indicated by the cryoscopic measurement, led him to believe, that the cellulose structure embodies rather a quantitative than a fundamental difference to that of the water soluble and easily crystallisable carbohydrates. Cryoscopic values for cellulose acetate in glacial acetic acid gave results with wide variations which occasionally indi-

cated/

cated an even lower molecular weight than that of a basic $C_6H_{10}O_5$ unit. This was found to be due to an enhanced air absorption of acetic acid-cellulose acetate solutions. Elimination of air gave results in agreement with one anhydro-glucose. However , even with exclusion of air, after several days of constant (minimum) molecular weight, reaggregation caused decrease of depression, indicating increase of molecular weight. He concluded therefore, that cryoscopic molecular weight values of polysaccharides which point to multiples of $C_6H_{10}O_5$ molecules give no information as to the structure and he contended, that "there is no need to give different names to the undissolved (high polymer) substance and the monomolecularly dispersed particles derived from it". His observations led him to investigate, whether such drastic measures as glycerolysis need be used to obtain cryoscopic results from lichenin corresponding to one anhydro-glucose. Hess goes on to say that lichenin acetate and cellulose acetate differ in rotation; obviously the two polysaccharides are not identical. The behaviour of the lichenin acetate solution during cryoscopic measurements in air was the same as that of cellulose. His general conclusions as to the make-up of the polysaccharide are the same as those expressed on cellulose. Concerning the observation of Pringsheim, that lichosan displays greater solubility in cold water than lichenin, he suggests that this is due to the presence of decomposition products from the glycerolysis process. In his

his opinion, x-ray analysis is not capable of proving the identity or otherwise of lichenin and cellulose.

Bergmann (8),following up Pringsheim's experiments, obtained a hexosan, which had different properties from those ascribed to lichosan, and which he therefore termed "licho-hexosan". His general attitude to the make-up of polysaccharides rejects "the tendency to search for an independent low molecular elementary molecule of identical structure with the polysaccharide" because he believed (9), that "any difference of two substances goes parallel with differences in structure and configuration"in high polymers as well as in other substances.

Hess (10) summarises his view on the subject by saying that Bergmann's licho-hexosan was nothing more than impure lichenin, that Pringsheim had never been able to put forth any compelling evidence as to the uniformity of his products; that the action of the solvent was the crux of the matter:- in low concentrations molecular dispersion takes place by breaking up the forces between the molecules in the same way as happens with all other substances, with the one remarkable difference that a great tendency for reaggregation exists.

A few years later Berner (11) claimed, that the action of glycerine on lichenin comprises two phenomena; partly lichenin is not disaggregated (or only slightly) but merely dissolved by glycerine, which is in turn adsorbed, causing enhanced water solubility/

solubility; partly, lichenin is disaggregated to cold-water-soluble, non-reducing products("lichosan") which however contain chemically fixed glycerine ("glycerine glucosides").

Thus we see that the protracted discussion between Pringsheim, Bergmann and Hess, with its theoretical digressions (based upon much laborious experimental work ) had in fact led to no real advance in the understanding of the make-up of the lichenin molecule.

With the aid of viscosimetric measurements Staudinger approaches the subject from a different angle:

(1) For dissolved colloidal particles the following equation holds true (12), within certain limits of viscosity and concentration:

$$\frac{\eta_{sp.}}{c_{gm}} = K_m \cdot \text{Molecular Weight}$$

where $\eta_{sp.}$ is the (specific) viscosity of a mono-molar solution and $c_{gm}$ is the concentration in basic mols per litre of the colloid; $K_m$ is the " viscosity-molecular weight constant".

A colloid (13) may either be of macromolecular structure, then, by definition, all its atoms are linked through (primary) covalencies (example: cellulose) or, it may be of micellular structure, then, according to Staudinger's definition the smaller molecules of which it is made up are attached to each other by the (weaker) van der Waal's forces (example : soap) .

(2) /

(2) Whether a colloid is the one or the other can be decided by comparing the specific viscosity of its solutions of equal (mono-molar) concentrations in chemically different solvents. If similar viscosity is obtained, this points then to a macromolecular structure (14), because one could expect that a micellular colloid, less stable would be easily destroyed, because only the weak van der Waal's forces keep the small molecules within the colloid together.

(3) When the macromolecular structure has thus been established, the molecular weight of the substance and its polymer homologues (their derivatives respectively) is found by one of the usual physical methods (osmometric, cryoscopic, ultracentrifugal).

(4) Values for $K_m$ (the only unknown in the equation) are then obtained by viscosimetric measurements. Thus information is gained as to the length and therefore the form of the molecule;

(a) polymer homologues of linear (fibre) colloids will- in equal concentrations and at low viscosity- display increased specific viscosity with increasing molecular weight.

$$\eta_{sp.} = K_m \cdot P \cdot c \quad (15) ,$$

where P is the "degree of polymerisation".

(b) Polymer homologues of sphero - colloids will yield under the same conditions viscosity values that are independent from their molecular weight.

(c) Furthermore, for long chain (linear) molecules

$$\frac{\eta_{sp.}}{c} = \text{constant}$$

holds/

holds true only in solutions of low viscosity; at higher concentrations the viscosity increases by far more rapidly.

(d) Substances of this type also show "structural viscosity" (16) i.e. the rate of flow in capillaries is not proportional to the pressure; the viscosity decreases with pressure.

Staudinger (17) was able to show that lichenin is a macromolecule such as cellulose, starch etc. The $K_m$ has not been determined; but he believes that the greater solubility of lichenin in comparison with cellulose makes it likely, that it is composed of molecules in string form which are curved in the form of maeanders similar to starch. $K_m$ values must therefore be expected to be smaller than those of cellulose derivatives. He estimates the minimum molecular weight of the lichenin molecule to be within the range of 10.000. As pointed out above, complete information necessitates the investigation of the polymer homologues of lichenin as well. It was found impossible (in analogy to starch) to prepare homologue-polymer lichenin-nitrates; they <u>all</u> showed lower viscosity than the original lichenin. It is concluded, that lichenin is disaggregated during nitration.

In a recently published paper(18) to which we have no access, Staudinger amplifies his views on lichenin as follows:- lichenin is not homogeneous but, treated in a number of ways yields two lichenins. They/

They, as well as their derivatives exhibit different rotatory power. By osmotic measurements the lichenins and their acetates show the same degree of polymerisation. If the acetates are saponified, the products show the original rotation of the lichenin. The nitrated lichenin however is disaggregated to a third of the polymerisation degree of the starting material.

Carter and Records (19) osmometric molecular weight determinations agree in general with the order of magnitude of Staudinger's result. Lichenin - methyl derivatives obtained from the acetate have molecular weights of about 10.000 to 14.000, indicating a chain length of 52 - 68 hexose units. Acetylated and directly methylated lichenin samples have molecular weights in the region of 30.000 (chain length 127-162). One lichenin acetate sample falling out of this range with a molecular weight of 118.000. Endgroup assay according to Haworth points to a minimum chain length of about 80. Carter thinks therefore, that "in the case of lichenin the physical and chemical units are possibly identical" in contrast to starch, where the chain length (or rather molecular size) obtained by osmometric measurements (starch 300-600 ) is several times higher than that of the chemically determined minimum chain length ( starch 24 ).

The measurements are carried out at various concentrations because in the case of these long chain molecules deviations from the van't Hoff's law occurr, (i.e. $\pi/c$ increases with increasing c ) which necessitate/

necessitate extrapolation to zero concentration where ideal conditions may be assumed to hold. This indicates that no increase of aggregation takes place at higher concentrations, which would cause the contrary, a fall in the rate of increase of $\pi/c$ . The forces responsible for the particle size of polysaccharides like lichenin are therefore of a more permanent nature ("macromolecules") than those encountered in the micellae of soap e.g.

It is worth emphasising however , that no strict conclusion as to the particle size of the native lichenin can be drawn from those figures, all of which were obtained on lichenin derivatives; these may have been disaggregated to an unknown extent during preparation.

Very recently Hess and Lauridsen (20) investigated the lichenin problem again, this time by means of an "improved" <u>end group assay</u> method on the lines of experiments carried out by them on cellulose. Hess states that the short comings of the usual procedure are :

(1) Methylation of the acetate gives no clue as to the molecular size of the native polysaccharide, because the acetate may be disaggregated.

(2) Separation of tetramethyl from trimethyl methylglucosides by distillation through a Widmer flask only effects imcomplete separation.

(3) Were it only a matter of estimating in the various fractions the amount of tetramethyl methylglucosides mixed with trimethyl methylglucosides, calculation

with the aid of refractive indices would produce correct results. The simultaneous presence however of lower methylated glucosides makes this impossible. For reasons (2) and (3) the amount of "end group" obtained is too low, thus chain lengths inferred too long. Therefore methylation is carried out directly on the polysaccharide in nitrogen athmosphere. Then a chemical method is used to effect complete separation of the "end group" from the other glucosides. The latter are esterified to yield phosphoric esters, the barium salts of which are insoluble in ether and petroleum ether, which are good solvents for tetramethyl methylglucosides.

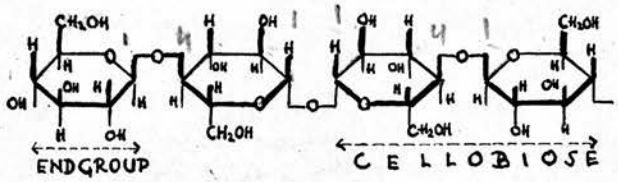
With the aid of this method the authors obtained in one methylation methylated lichenin products of 41-43% OMe, which was increased to 45.5% by Purdie's method. Endgroup estimation then yielded values corresponding to a chain length ("polymerisation degree") of 114 to 116. This result again is well within the range of Staudinger's and Carter's findings.

No dimethyl methylglucoside fraction was obtained. It follows that a branched chain or rather cross linkages as suggested for starch (21) can be excluded with certainty.

On cellulose these authors agreed with Haworth in obtaining no endgroup on exclusion of air during methylation.

That cellulose and lichenin are not chemically identical/

identical substances is further shown by these authors by the difference of rotation of their acetyl and methyl derivatives. Hess then puts forward the speculative suggestion that lichenin possesses 1:1 and 4:4 linkages,




a formula which would explain complete cleavage to cellobiose.

He then compares the polymerisation degree of lichenin with that of starch, (as apparently obtained by him ) which he puts at 52 and speculates - on the assumption of an unbranched chain - about the inconclusive relation between solubility and chain length of cellulose (insoluble), starch (conditionally soluble ) and lichenin (easily soluble in water ). Starch he says, with a materially shorter chain, is less soluble than the longer chain lichenin. At this point it may perhaps be permissible for us to say, that experience with synthetic resins shows, that cross linkages account for decreased solubility. Polystyrene, a thermoplastic resin, soluble in organic solvents, becomes insoluble when polymerised in the presence of a small amount of ("polyfunctional") divinylbenzene (24) because it is believed , that thus cross linkages have been introduced. Also Haworth (25) suggests that the solubility difference of lichenin and cellulose is due to the many cross linkages in cellulose.

To/

To round up the picture of the work that has been done on lichenin with the object of contributing to the elucidation of its structure, there remains to mention that Schmidt (26) believes to have detected COOH-groups in native lichenin and likewise in cellulose.

With regard to lichenin alkali addition compounds, which are the object of our investigation, very little information is recorded. Karrer (27) mentions the existence of such a compound and stresses the difficulty of obtaining compounds of constant composition.

Sodium and potassium atoms were found to be introduced into lichenin, cellulose, starch and inulin by means of the alkali metal in a medium of liquid ammonia (28). The results appeared to indicate that one repeating unit reacts with one alkali atom within a few minutes. On attempting to introduce more than one alkali atom per $C_6H_{10}O_5$ , the reaction proceeded in a different way and much more slowly.

The products obtained from cellulose by a similar, more elaborate method were investigated by Scherer and Hussey (29). They also found that , while finally a trisodium compound of cellulose was obtained, the reaction with one OH-group per repeating unit proceeded much more quickly.

Since the action of an alkali metal in ammonia is a drastic one, a supposed engagement of residual valencies of the primary alcoholic groups in native cellulose/

cellulose may well have been broken; therefore the existence of a trisodium cellulose is not inconsistent with Heddle's (30) result, which we shall discuss later.

Furthermore Schorigin and Makarowa (31) found, that about two out of the three sodium atoms per anhydro glucose can readily be removed on washing the compound with absolute alcohol within two hours. Similarly methylation of trisodium cellulose by means of methyliodide yielded always products only approximating mono-methyl-cellulose.

Because alkali compounds are precursors of the xanthate reaction Lieser's (32) results in that field deserve our interest. He proved that cellulose reacts in position $C_2$. Comparative work on starch (33), lichenin(and also other soluble polysaccharides) shows that both react similarly, but that lesser alkali concentrations are needed than in the case of cellulose. Xanthate formation increases with increasing alkali concentration to an optimum of a 2N alkali concentration. The optimal composition points to $2\ C_6H_{10}O_5 : CS_2$ . The higher xanthation of lichenin is interpreted by Lieser as pointing to a different construction of its molecule as compared with cellulose and starch.

We have gained information about <u>lichenin alkali addition compounds</u> with the aid of the <u>method</u> initiated by Percival and applied by him and coworkers to a number of mono-saccharides, glucosides, di-and polysaccharides/

polysaccharides.

Generally speaking, the addition compounds are formed by the action of cold aqueous alkali upon the saccharide (for the sake of uniformity and in view of its greater reactivity potassium hydroxide is usually employed (30), or by the action of alcoholic alkali solutions upon the acetylated compound, in which case simultaneous deacetylation and compound formation takes place. Solubility in alcohol and water and the physical state of the sugar and its addition compound respectively, are factors determining the choice of procedure.

The amount of alkali attached at various alkali concentrations is estimated by means of direct titration of the compound, after washing with a suitable minimum quantity of alcohol to remove adherent (i.e. non-combined) alkali (direct method ) (36), or by determining the alkali removed from a potassium hydroxide solution of known strength by the saccharide or its acetylated derivative (indirect method ) (36). If , in the latter case alcoholic potassium hydroxide solution is employed the amount of alkali used up for deacetylation has to be determined separately, since, owing to the catalytic nature of this reaction less alkali than stoechiometrically needed is required (30).

In order to determine the point of fixation of alkali the stable $OCH_3$-group is introduced by means of a mild treatment with dry neutral dimethylsulphate, ranging from 5 to 15 minutes duration at a temperature not/

not exceeding 75°. To allow of a clearer interpretation of the results, absence of water is desirable, minimizing the possibility of migration of the OMe group to a position previously not occupied by potassium hydroxide. However, even if water or other ionizing agents are present, the method is considered to indicate the hydroxyl groups of maximal acidity (unless these are shielded by cross linkages or otherwise within the molecule ) because, whether they are closely bound to alkali or not, it is not likely that the centres of acidity will change during the operation. (34). Percival (35) proved that methylation of these addition compounds is not merely due to the presence of the potassium hydroxide reacting with dimethyl sulphate. He, for instance, carried out a test methylation under standard conditions using however an equimolar mixture of glucose, dry potassium hydroxide and dimethyl sulphate and obtained only 2½% of the yield resulting from methylation of the addition compound.

The mild methylation is followed, where feasible, by separation of the methylated from the unchanged material and in the case of di- or polysaccharides by hydrolysis and conversion of the partly methylated hexose into suitable reference compounds with a view to determine the point or points of fixation of the methyl group in the molecule.

Using the described method Percival (34) showed that in glucose the reducing group is exclusively/

vely engaged in addition compound formation and titration experiments revealed, that within the range of an alkali concentration (in the equilibrium mixture) of 0.3 - 0.7N a compound of the composition 1 glucose: 1 potassium hydroxide is formed.

In the case of cellobiose (34) titration indicated that addition in low (alcoholic ) alkali-concentration in the region of 0.02N-0.08N the formation of a compound $(C_6H_{10}O_5)$.KOH is favoured, while at higher concentrations of 0.7 Normality $(C_6H_{10}O_5)$.2KOH appears to be formed, without excluding however the possibility that small quantities of the more complex derivative are formed at low concentration also. Mild methylation of an addition compound formed at low concentration yielded only methyl-cellobioside proving that here too , only the reducing group combines with alkali. If alcoholic potash of greater strength (2.8 N) is employed, subsequent mild methylation yields $\beta$ -methylcellobioside and 6-methyl osazone can after appropriate treatment; at higher concentrations therefore $C_6$ also is involved in the potassium hydroxide addition. Since glucose itself takes up only one potassium hydroxide residue, the author considered it reasonable to suppose, that the second potassium hydroxide is attached at $C_6$ of the non-reducing glucopyranose unit.

Maltose (34) was shown to combine with one molecule potassium hydroxide at low alkali concentrations, with two molecules at the comparatively low/

low alkali equilibrium concentration of 0.04 N, and with three molecules at 0.3 N . The formation of a tri - addition compound at that low alkali concentration seems to indicate maltose to be a highly "acidic" sugar, a result in agreement with physical measurements (37). Heddle (30) prepared an addition compound with 0.3 N potassium hydroxide, obtained after methylation maltosides and separated in the high vacuum two fractions of derivatives : one was a dimethyl triacetyl glucose, the methyl groups being attached to $C_2$ and $C_6$, which was shown by (a) the isolation of 6-methylglucosazone and (b) a negative Weerman test. The second fraction, approximating in OMe content to a monomethyl tetraacetyl glucose yielded glucosazone and gave no Weerman reaction. It was therefore 2-methyl tetraacetyl glucose. Maltose therefore combines with alkali at the reducing group and $C_2$ and $C_6$ of one hexose residue. By analogy to lactose (see later) it is considered plausible to assume, that the non-reducing glucose fragment is the doubly substituted one. The isolation of a monomethyl glucose derivative which was shown to be $C_2$ substituted, but not of a $C_6$ substituted monomethyl derivative seems to show attachment to $C_2$ to be stronger than to $C_6$.

Percival (35) found that sucrose, in analogy with maltose takes up three molecules of potassium hydroxide with Normality increasing to 3 N potassium hydroxide. These are attached to the primary alcoholic/

alcoholic groups exclusively. This was shown by the isolation of two fractions of derivatives through distillation in the high vacuum after the usual mild treatment with dimethyl sulphate, followed by hydrolysis and glucoside- (fructoside- respectively) formation. One was a monomethyl triacetyl methyl-glucoside which yielded a 6-methyl glucosazone, the other fraction proved to be a derivative of 1:6 dimethyl fructofuranose. Its relevant properties being (a) no osazone formation indicating that position $C_1$ is occupied; (b) formation of a 5-methyl arabonolactone with a slow hydrolysis constant in agreement with a $\gamma$-lactone; therefore $C_6$ was considered to be the other occupied position in the fructose part of the molecule, preventing the formation of a pyranose ring.

The attachement of potassium hydroxide to lactose (36) is different from what one might have expected by analogy with the previously investigated disaccharides; two (or three) potassium hydroxide molecules combine on the postions $C_1$ of the glucose fragment and $C_2$ and $C_4$ of the galactose fragment. Percival and Ritchie proved this as follows: the partly methylated lactose obtained by the standard method was separated from the unchanged product by precipitating the latter through addition of alcoholic potassium hydroxide. Acetylation, followed by hydrolysis yielded a mixture of glucose, mono- and dimethylgalactose derivatives. Only the galactose derivatives were found to be partly methylated.

The/

The monomethyl galactose derivative yielded no crystalline phenylhydrazone, but however gave an osazone free from OMe; it did not give rise to mucic acid. The OMe residue was therefore assigned to $C_2$. The dimethyl galactose derivative gave a monomethyl osazone identical with 4-methyl galactosazone.

With a view of finding an explanation for this anomalous behaviour of lactose, (contrasting with substitution in the primary alcoholic residues of cellobiose, sucrose and maltose) galactose (36) was investigated. It combines with one molecule potassium hydroxide within the range of 0.07 - 0.4 N . It was found that only the reducing group combines with potassium hydroxide. The presence of galactose in the lactose molecule offers therefore no clue for the behaviour of the latter .

In $\alpha$ - and $\beta$-methylglucosides (30) $C_6$ was found by Heddle and Percival to be involved in addition compound formation.

The same authors investigated the behaviour of amylose. This polysaccharide forms compounds approximating to the composition $C_6H_{10}O_5$ . 1 KOH by treating amylose acetate with an excess of alcoholic potassium hydroxide. From the product resulting from mild methylation under anhydrous conditions practically all the partly methylated material could be extracted with hot methyl alcohol. The small OMe content of the methyl alcohol insoluble residue was shown to account for an OMe group attached to $C_2$. Similarly only

glucosazone/

glucosazone could be isolated from the soluble part, which had an OMe content of 8.1% . This result therefore indicates attachement to $C_2$ exclusively. The absence of any $C_6$ substituted molecules is particularly interesting in the light of the recent findings of Hirst and Young (38,39), who demonstrated, that primary alcohol groups are engaged in cross linkages within the starch molecule.

While such has not been demonstrated yet for cellulose, it was shown, that here too no $C_6$ positions are involved in alkali addition compound formation. Potash cellulose was prepared with 35% aqueous potassium hydroxide following the method of Percival, Cuthbertson and Hibbert (40). Analysis showed the complex to be of the composition $C_6H_{10}O_5$ . 1 KOH . Upon mild methylation a material of 5 - 9% OMe was obtained (pointing to a mono-substitution of up to 50% of the glucose fragments). Hydrolysis with cold concentrated hydrochloric acid, saturated with hydrochloric acid gas, followed by selective fermentation with yeast yielded mono-methyl glucose from which an unmethylated osazone was obtained. Here again therefore substitution on $C_2$ only has taken place.

None of the investigators of alkali addition compounds is able to define the manner in which the alkali is attached. For a detailed review of opinions expressed on the various possibilities the reader is referred to Heddle's thesis, introduction (Edin.1938).

From/

From the ease with which alkali is again removed through a simple washing process and from the low yields generally obtained on methylation it ensues, that the combination is a loose one. Percival and Ritchie (36) therefore indicate the link between sugar and alkali with a dotted line .

# EXPERIMENTAL.

General remarks: (1) all boiling points mentioned refer to bath temperatures; (2) all melting points were taken with the Reichert microscope.

## ISOLATION AND PURIFICATION OF LICHENIN.

Iceland Moss (5 kg.) was steeped in a cold solution of 2% sodium carbonate (180 litres) for two days in order to extract the lichen acids (43). The moss, after complete removal of the alkaline solution, was extracted with approximately 90 litres of boiling water. The hot colloidal brown solution was decanted and on cooling a gelatimous precipitate settled down. It was found impracticable to effect separation from the rest of the solution either by filtering or centrifuging; but on the addition of alcohol (to the extent of about 40% of the volume) a considerable amount of crude lichenin could be separated by filtration through muslin under suction. By treating the brown jelly with chlorine water for 24 hours its colour changed to grey. Repeated addition of water and decantation through muslin until the solution was nearly neutral served to remove the chlorine. Water (made alkaline with a little sodium hydroxide ) was added and the jelly heated until colloidal dispersion was achieved. Alcohol was then added to the warm solution, and after cooling acetic acid was added until[a] neutral flocculent suspension was obtained; decantation and dissolution in a minimum amount of hot water, followed by sedimen -

tation/

tation of the jelly at 0° was repeated 4 times until the solution gave only a faint blue colour with iodine (44), an indication that isolichenin was virtually absent. By this treatment a light grey, fairly uniform product was obtained, which was filtered and then covered with alcohol. Moisture was removed by repeated renewal of alcohol, followed by ether. The almost white precipitate was then finally dried in the vacuum desiccator over phosphoric oxide.
Yield:- 50-100 g.
Found :- moisture, average 5.8% , ash 0.9-1.5% , iodine number (Bergmann & Machemer ,46) : 0.9 -1.4 , (control experiment, found: starch, soluble 1.95 , glucose 106 ).

## A TYPICAL ACETYLATION OF LICHENIN.

Lichenin (10 g.) was dissolved in boiling water (made slightly alkaline with sodium hydroxide), whence it was precipitated with alcohol and decanted; the precipitate was covered with alcohol for 12 hours and the alcohol renewed once. Lichenin was thus coagulated so that it could be collected without difficulty on a Buchner filter.
The damp material (still containing a considerable amount of water) was then transferred to a flask fitted with a mechanical stirrer and covered with 200 c.c. pyridine. Acetic anhydride (150 c.c.) was added in five batches at intervals of about 10 minutes under mechanical stirring, which was continued for the next/

next 3 hours at 100°. The solution was then kept for 48 hours at room temperature. After this period it was filtered through glass wool and poured into a large volume of water with stirring. The fibrous precipitate was then collected on muslin and thereafter washed in running water for 24 hours. The product (13.8 g.) was finally dried over phosphoric oxide at 45°/12 mm.

Found :- $CH_3CO$, 38.4% $[\alpha]_D^{16}$ -16.1°(c, 0.31 in a solution of 90% chloroform + 10% methyl alcohol ), (Karrer,(45) lichenin acetate: $[\alpha]_D^{20}$ -23.8° ) .

METHOD EMPLOYED FOR THE PRELIMINARY INVESTIGATION OF THE ALKALI COMBINING CAPACITY OF LICHENIN.

The combined alkali was determined by simultaneous deacetylation and compound formation in a solution of absolute-alcoholic potassium hydroxide.

Example:-

Two weighed quantities of lichenin (0.2055 g. and 0.2050 g.) were suspended in absolute alcoholic potassium hydroxide (20 c.c.) (0.1116 N) and the mixture allowed to stand for 2½ hours.

(1) The contents of the first flask were then analysed for the amount of alkali used in deacetylation and compound formation together as follows: The precipitated lichenin potassium hydroxide compound was removed by filtration through a Gooch crucible and adherent liquid squeezed out; the filtrate was titrated/

titrated against standard N/10 sulphuric acid (phenolphtalein as indicator). The residue after washing with absolute alcohol (5 c.c.) was transferred to a flask containing water, dissolved by short boiling, standard N/10 sulphuric acid added in excess, carbon-dioxide removed by boiling and the correct value obtained by back titration with standard N/10 alkali ("direct result").

(2) To the content of the second flask water, and phenolphthalein were added and titrated with standard N/10 sulphuric acid, and in this way the amount of alkali required for deacetylation was determined.

Results:-

The initial Normality was 0.1116 N

∴ the initial weight of potassium hydroxide present in 20 c.c. was 0.1252 g.

The final Normality was 0.0409 N and

∴ the final weight of potassium hydroxide was 0.0459 g.

(a) Weight of potassium hydroxide not used in deacetylation (titration 2) was 0.088 g.,

∴ weight of potassium hydroxide required for deacetylation was 0.1225 g. - 0.088 g. i.e. 0.0372 g.,

∴ 100 g. lichenin acetate will require for deacetylation 18.14 g. potassium hydroxide.

(b) Now amount of alkali used up in deacetylation and compound formation was 0.1252 g. - 0.0459 g., i.e. 0.0793 g.,

∴ 100 g. lichenin acetate require for deacetylation and compound formation 35.58 g. potassium hydroxide,

∴/

∴ 100 g. lichenin acetate require for compound formation 38.58 g. - 18.14 g. i.e. 20.44 g. potassium hydroxide. However 100 g. lichenin acetate correspond to 59 g. lichenin,

∴ 100 g. lichenin require for compound formation

$\frac{20.44 \text{ times } 100}{59}$ i.e. 34.64 g. potassium hydroxide.

(c) From the direct titration of the precipitate after washing with 5 c.c. absolute alcohol we find that 100 g. lichenin react with 19.10 g. potassium hydroxide.

Similar experiments were conducted over a wide range of alkali concentrations and the results will be found in the table and diagram on page 54 .

## EXPERIMENT I.

### PREPARATION OF LICHENIN-POTASSIUM HYDROXIDE ADDITION COMPOUNDS.

The lichenin acetate fibre (25.2 g.), containing 5.8% moisture, was ground in a mortar to a more or less homogeneous powder, suspended in absolute alcohol (100 c.c.) and to this 2 N-absolute potassium hydroxide (320 c.c.) was added; the material was left in contact for 3 hours with mechanical stirring, then filtered and washed with absolute alcohol (500 c.c.) and ether (50 c.c.) . The product was dried in a vacuum desiccator, fitted with a soda lime tube.

Yield :- 24 g. of a greyish powder.

Found /

Found:- potassium hydroxide content, by direct titration of a small sample against litmus : 27% ; (calculated for a 1:1 addition compound $C_6H_{10}O_5.KOH$, potassium hydroxide, 25.7% ).

METHYLATION OF LICHENIN-POTASSIUM HYDROXIDE.

(According to Heddle's and Percival's methylation of the starch-alkali addition compound , 30 ).

To the finely powdered lichenin-potassium hydroxide compound (24 g.) contained in a flask fitted with a stirrer was added dry dimethylsulphate (250 c.c.), previously neutralised with anhydrous potassium carbonate. The flask was surrounded by a water bath, which was maintained at 65° for 15 minutes and then quickly raised to 75° for 7½ minutes. The contents were vigorously stirred all the time. On increasing the temperature to 75° sudden "clogging" of the contents of the flask could be observed; at the end of this treatment the material was found to be acid towards litmus. The dimethylsulphate was filtered off and the partly methylated lichenin repeatedly washed with acetone on the filter and finally covered with acetone. The grey flocculent precipitate was of a slightly gelatinous appearance and had the tendency to become sticky when left in contact with moist air.

In order to assess the quantity of methyl groups introduced, a small sample (1 g.) was acetylated in the usual manner in pyridine with acetic anhydride./

anhydride.

Found:- $CH_3CO$, 37.66% ; OMe, 5.32% ;
iodine number (46); 15, when calculated on the basis of (deacetylated)lichenin present: 24.

Calculated for $(C_6H_7O_4).(CH_3CO)_2.(OCH_3)_1$ :
$CH_3CO$, 33.1% and OMe, 11.9% .

HYDROLYSIS OF THE PARTLY METHYLATED LICHENIN .

The above product was treated with 8% sulphuric acid (160 c.c.) at 100° for 4 hours.

Neutralisation was carried out with an excess of barium carbonate, and the brown, neutral solution was kept at 100° for an hour, the barium salts were filtered off and the solution taken to dryness at 45°/12 mm.

ACETYLATION OF THE HYDROLYSIS PRODUCTS.

To the material obtained on hydrolysis, (mixed with inorganic salts) acetic anhydride (45 c.c.) and anhydrous sodium acetate (7.5 g.) were added. This mixture was kept at 100° for 2 hours, set aside for 24 hours and then poured into water; the aqueous solution was extracted with chloroform; the chloroform solution was thoroughly extracted with dilute hydrochloric acid, followed by a sodium bicarbonate solution and water and finally dried over anhydrous sodium sulphate.

Yield :- 14.7 g.

Attempts to distil in high vacuum led to cracking/

cracking, showing that acetylation was not complete. The distillation was therefore interrupted and complete acetylation effected in pyridine (150 c.c.) and acetic anhydride( 100 c.c.). The mixture was kept at 100° for 1½ hours, put aside at room temperature for 48 hours with occasional shaking, finally poured into a large volume of water and worked up as usual.

DISTILLATION IN THE HIGH VACUUM AND FRACTIONATION.

Distillation at 160°-190°/0.02 mm. yielded a yellow syrup (8.25 g.); the residue in the flask was charred.

Redistillation from a flask with a relatively high neck yielded the following fractions:

fr. I b.p. 162°/0.05-0.02mm., 0.34 g. , $n_D^{14}$ 1.4650, OMe 9.1%

fr. II b.p. 155°-170°/0.01 mm., 1.66 g. $n_D^{17}$ 1.4595 , OMe 8.0% ,(last drop, 6.8% )

fr. III b.p. 165 - 170°/0.01 mm., 3.87 g., $n_D^{14}$ 1.4582, OMe 4.88%, (last drop OMe 3.5% )

fr. IV b.p. 170° -190°/0.01 mm., 2.38 g. , last drop OMe 2.0% .

Calculated for monomethyl tetraacetyl glucose $C_{15}H_{22}O_{10}$, OMe, 8.57% ,

for dimethyl triacetyl glucose $C_{14}H_{22}O_9$ , OMe, 18.6%.

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On the following pages attempts are described to prepare derivatives from the above fractions in order to identify the position in the glucose molecule, at which substitution by the methyl group had taken place.

## UNSUCCESSFUL ATTEMPT TO OBTAIN CRYSTALLINE 2-METHYL β-METHYLGLUCOSIDE FROM FRACTION I.

### Acetobromo compound formation.

Fraction I (0.34 g.) $[\alpha]_D^{\pi}$ +9.4° (c, 0.84 in chloroform ) was dissolved in 1 c.c. glacial acetic acid and glacial acetic acid saturated with hydrobromic acid was added (1.5 c.c.) and the mixture kept at 0° for 3 hours; after this period chloroform (10 c.c.) was added and the solution poured onto ice; after adding more chloroform the solution was washed with water in the separating funnel, followed twice with a sodium bicarbonate solution and finally with water; it was then dried over anhydrous sodium sulphate and the solvent removed at 35°/12 mm.

### Glucoside formation .

The resulting syrup was dissolved in 20 c.c. absolute methyl alcohol, treated with silver carbonate (1 g.) with constant shaking for 24 hours, the silver salts were filtered off and the solvent was removed under reduced pressure .

Yield:- 0.205 g.

### Deacetylation acc. to Zemplén (47).

The /

The syrup (0.205 g.) was dissolved in absolute methyl alcohol (2 c.c.) and 0.08 c.c. of an 0.09 N-sodium methylate solution added; this solution was kept at 100° for 5 minutes and then the solvent removed at 40°/12 mm.

The resulting syrup was taken up in about 5 c.c. hot ethyl acetate, treated with charcoal, filtered and put aside in the ice box. Crystals however failed to appear even on standing for several months.

UNSUCCESSFUL ATTEMPT TO OBTAIN CRYSTALLINE 2-METHYL GLUCOSEPHENYLHYDRAZONE FROM FRACTION II.

Deacetylation.

A modification of Zemplén's method (48) was used in asmuch as higher stoechiometric proportions of sodium methylate were found to be necessary than employed by Zemplén.

Fraction II (0.38 g.), $[\alpha]_D^{15}$ +5.7° (c, 0.9 in chloroform ) was dissolved in absolute methyl alcohol (1.6 c.c.) a N/10 - sodium methylate solution (0.4 c.c.) added and shaken mechanically for 3 hours. The clear solution was then taken to dryness under reduced pressure.

Hydrazone formation.

To the above syrup were added water (1 c.c.), phenylhydrazine (0.3 c.c.), three drops of glacial acetic acid and the mixture put aside in the icebox. Crystals however failed to appear.

## OSAZONE FORMATION FROM FRACTION II.

Deacetylation (according to Zemplén, 49)

A methyl-alcoholic solution (10 c.c.) of sodium (0.2 g.) was added to a chloroform solution (15 c.c.) of the syrup (0.75 g., fraction II) and kept at low temperature with occasional shaking. At the end of 3 hours the solution was acidified with acetic acid and the deacetylated product extracted with water in the usual manner. Thereafter , the solvents were removed under reduced pressure.

The syrups thus obtained were divided into two equal parts :

Osazone formation, experiment (a).

Half of the available amount of the deacetylated sugar (0.2 g.) was dissolved in 20 c.c. of water, phenylhydrazine (0.8 c.c.), glacial acetic acid (1 c.c.) and a little sodium bisulphite were added and the solution kept at 100° for 1 hour. On slow cooling a brown precipitate separated.

Yield:- 0.115 g.

Found :- OMe, 1.98%

Osazone formation, experiment (b).

To the remaining half of the deacetylated sugar (0.20g.) in 20 c.c. of water, phenylhydrazine (0.5 c.c.), acetic acid(0.8 c.c.) and a little sodium bisulphite were added and the solution kept at 100° for 1 hour. On slow cooling a precipitate separated.

Yield :- 0.091 g.

Separation of glucosazone from the methylated osazone.

This precipitated osazone was treated with chloroform at 60° for 4 minutes and then put aside at room temperature for 12 hours (according to Heddle and Percival, 53).

Upon filtration a yellow precipitate was obtained.

Yield:- 0.043 g.

After recrystallisation from aqueous pyridine light yellow needles (0.014 g.) were obtained; after several further recrystallisations ; m.p. 194° .

Authentic glucosazone : m.p. 196° ;

mixed m.p. 194°

The filtrate, a dark brown chloroform solution was reduced to 1 c.c. at room temperature under reduced pressure; on pouring into 25 c.c. petroleum ether (b.p. 60°-80°) a dark brown, amorphous precipitate was obtained; attempts at crystallisation were not successful.

Yield:- 0.02 g.

Found :- OMe 4.75% .

(Calculated for monomethyl glucosazone $C_{19}H_{24}O_4N_4$ ,

OMe 8.3% ) .

ACETONE COMPOUND FORMATION FROM FRACTION III AND FRACTIONATION OF THE RESULTING PRODUCTS IN THE HIGH VACUUM.

This procedure was employed in order to gain information as to the presence or otherwise of 3-methyl glucose in the hydrolysis mixture of the partly/

partly methylated lichenin.

Deacetylation (using 3 times the amount of sodium methylate indicated in Zemplén's paper, 48).

Fraction III (3.87 g.), (average OMe 4.88%) was dissolved in absolute methyl alcohol (15.48 c.c.) and 0.165 N-sodium methylate solution (7.02 c.c.) was added; the solution was kept at 45° for 1½ minutes, shaken mechanically for 3 hours at 15° and taken completely to dryness at reduced pressure by treating repeatedly with a mixture of alcohol and benzene.

Acetone compound formation.

The deacetylated product (approx. 2 g.) was treated with 50 c.c. dry acetone and 1.4 c.c. concentrated sulphuric acid (density 1.84) with constant shaking for 3 days; practically all the material was found to be dissolved. The resulting brown solution was neutralised with an excess of anhydrous sodium carbonate, filtered and taken to dryness under reduced pressure in the presence of a small amount of barium carbonate. The resulting product was taken up in a mixture of chloroform and acetone, filtered free from barium salts, taken to dryness and then dissolved in cold water. After standing for 24 hours a considerable amount of oily matter was filtered off and discarded.

The yellow aqueous solution was taken to dryness and again taken up in a smaller amount of water and filtered. This process of purification was repeated several times. Complete removal of the

less/

less water-soluble matter could however not be achieved.
Yield of crude acetone compounds: 1.84 g.

Distillation in the high vacuum (in the presence of a little barium carbonate ) yielded the following result:

Fraction I (1st drop : b.p. 122°/0.06 mm.); the main volume distils at 128°/0.04 mm. The bath temperature was finally allowed to rise to 145°/0.12 mm.
Yield:- 0.65 g. of a colourless oil , which deposited crystals over night; OMe 3.3% ; average, $n_D^{14}$ 1.4652 , last drop, $n_D^{14}$ 1.4685 .

Fraction II, b.p. 150°/0.14 mm. , 0.25 g. of a pale yellow oil.

Fraction III, b.p. 170°/0.07 mm.- 185°/0.13 mm., 0.3 g. OMe 11.54% , $n_D^{14}$ 1.4740 .

Sudden decomposition and cracking was observed above 180° .

Total distilled: 1.20 g.; the residue in the flask was charred.

Fraction I (OMe 3.3%):

The crystals were found to be easily soluble in hot petroleum ether (b.p. 40°-60°), while the syrupy part appeared to be only slightly soluble in this solvent. After two recrystallisations from petroleum ether the colourless crystals had m.p. 110°-112°; mixed with an authentic sample of diacetone glucose (m.p. 112°) , mixed m.p. 110° .

Fraction/

Fraction III deposited a few crystals over -night, which proved (by mixed m.p. ) to be also diacetone glucose; it was faintly reducing to Fehling's solution.
OMe: 11.54% (value corrected, by substracting 0.2% from the result of the estimation. In a control experiment diacetone glucose yielded 0.4% methoxyl).
Calculated for monomethyl monoacetone glucose, OMe 13.24%.

Separation of diacetone glucose from monoacetone monomethyl glucose could be effected to a certain extent by means of hot petroleum ether (b.p. 40°-60°), in which (as mentioned above) diacetone glucose is easily soluble, while there remains a yellow syrup , which is only very slightly soluble in hot petroleum ether.

A further separation was then attempted by taking the syrup to dryness, covering with petroleum ether and allowing air to bubble through a capillary; thus a temporary emulsifying effect is produced; the layers separate again and the petroleum ether is decanted. After this treatment the oil was dissolved in a mixture of hot chloroform and petroleum ether and any flocculent impurities were filtered off; finally the solvents were removed under reduced pressure.

UNSUCCESSFUL ATTEMPT TO OBTAIN CRYSTALLINE 6-METHYL MONOACETONE GLUCOSE. ( 50 )

The above oil (0.25 g.) was taken up in chloroform, the solution washed twice with water, dried over anhydrous sodium sulphate and taken to dryness. The syrup was taken up in a few c.c. of dry ether, and petroleum/

petroleum ether (b.p. 40°-60°) was added to turbidity and the mixture put aside in the icebox. The solution however failed to yield crystals on standing for a considerable time.

This syrup (after removal of the solvent) was then united with the mother liquors, obtained from acetone fractions I and II, after removal of diacetone glucose in the manner as described above.
Yield of this combined yellow syrup: 0.255 g.
Found:- OMe,(corrected for the acetone group) 11.7%.
(Calculated for monomethyl monoacetone glucose, OMe 13.24% ).
No reducing action towards Fehling's solution could be observed. $[\alpha]_D^{15}$ -2.5°(c, 0.78 in chloroform) .
(6-methyl-1,2-isopropylidene glucose, according to Levene,(50) has $[\alpha]_D^{20}$ -6.0° in chloroform ).

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## EXPERIMENT II.

The acetylation of lichenin, the preparation of lichenin-potassium hydroxide and the subsequent methylation were carried out as already described in experiment I, except for the following modifications :

(a) Before forming the addition compound, the lichenin acetate was dried at 45°/12 mm. (in experiment I it was dried over phosphoric oxide at room temperature and retained 5.8% moisture).

(b) Before methylation the lichenin addition compound was dried at 30°/12 mm.

## ACETYLATION.

20 g. lichenin yielded 32g. lichenin acetate.

## PREPARATION OF LICHENIN-POTASSIUM HYDROXIDE.

Lichenin acetate (29 g.) was suspended in absolute alcohol (100 c.c.), 2 N-absolute-alcoholic potash (520 g.c.) was added and put aside for 3 hours with occasional shaking. The addition compound was filtered through a Buchner filter (the excess liquid squeezed out of the precipitate), washed with absolute alcohol (80 c.c.) and ether (50 c.c.) and then dried at 30°/12 mm. Finally the material was ground in a mortar to a fine powder and kept over phosphoric oxide.

Yield:- about 21 g.

Found:- By direct titration of a small sample dissolved in water, potassium hydroxide content: 23.0% .

(Calculated for a 1:1 addition compound $C_6H_{10}O_5.KOH$ potassium hydroxide: 25.7% ).

## METHYLATION.

The lichenin-potassium hydroxide, obtained as described above was methylated exactly as described in experiment I (page 33).

In order to assess the number of methyl groups introduced, a small sample (1 g.) was acetylated in the usual manner in pyridine and acetic anhydride.

Found:- OMe 11.35% .

(Calculated for monomethyl diacetyl lichenin $(C_6H_7O_4)(CH_3CO)_2(OCH_3)_1$ , OMe 11.9% ).

## HYDROLYSIS OF THE PARTLY METHYLATED LICHENIN.

Approximately half of the product resulting from the methylation (of 21 g. of the addition compound) was hydrolysed with 8% sulphuric acid (100 c.c.) at 100° for 4 hours. The brown solution was neutralised with barium carbonate in the usual manner, filtered and taken to dryness at 45°/12 mm.; by thorough extraction with boiling alcohol the brown syrup could be freed from inorganic material. Treatment with an alcohol/benzene mixture served to remove moisture. Yield:- 8.0 g.

## ACETONE COMPOUND FORMATION.

The above material(8.0 g.) was suspended in a mixture of dry acetone (750 c.c.) and sulphuric acid (density 1.84) (21 c.c.) and agitated mechanically for 4 days. After this period complete dissolution was found to have taken place; the dark brown solution was neutralised with anhydrous sodium carbonate, filtered and taken to dryness under reduced pressure in the presence of a little barium carbonate.

The dark brown viscous syrup thus obtained was treated with a mixture of water and petroleum ether; the greater part (2.68 g.) was soluble in water, while a brown oily material (1.16 g.) dissolved in petroleum ether (b.p. 60°- 80°).

FRACTIONATION OF THE WATER-SOLUBLE PART BY DISTILLATION IN THE HIGH VACUUM .

The water soluble part, on evaporation to dryness under reduced pressure yielded a light yellow, reducing syrup(2.68 g.). It was distilled from a flask with a rather high neck in the presence of a little barium carbonate:

Fraction I , b.p. 140°/0.04 mm.-150°/0.07 mm., 0.45 g. of a yellow syrup, $n_D^{14}$ 1.4679 . The first two drops of this fraction: OMe, 15.84%; reducing to Fehling's solution.

(The properties of 3-methyl-1,2-5,6-diisopropylidene glucose (51,52) are: b.p. 105°/0.3 mm. , $n_D^{14}$ 1.4518, OMe, 11.3% ).

Fraction II , b.p. 170°-200°/0.08 mm., 0.34 g. of a yellow viscous syrup, slightly reducing to Fehling's solution; $n_D^{14}$ 1.4708 , OMe 19.27% .

(Calculated for monomethyl monoacetone glucose, OMe 13.24%.)

When the temperature reached 170° the content of the flask suddenly decomposed and the pressure increased; after a minute or two the vacuum improved again and the last two drops of fraction II distilled. The residue in the flask was charred; total amount distilled: 0.79 g.

FRACTIONATION OF THE PETROLEUM ETHER-SOLUBLE PORTION BY DISTILLATION IN THE HIGH VACUUM.

The petroleum ether-soluble portion, after removal of the solvent was found to be a brown mobile oil (1.16 g.). Distillation in the high vacuum yielded:

Fraction I, b.p. 95°-115°/0.03 mm., 0.06 g. , $n_D^{14}$ 1.4919 , OMe 37.75% .

(3-methyl diacetone glucose gives $n_D^{14}$ 1.4518 )

The highly mobile yellow oil crystallised spontaneously in the receiver.

Fraction II, b.p. 130°/0.05 mm.-200°/0.08 mm., 0.30 g., $n_D^{13}$ 1.5229 .

The residue in the flask was charred.

The conclusion was reached (see discussion) that no 3-methyl diacetone glucose was present in the mixture of acetone compounds.

The refractive indices and OMe contents of the above fractions indicated, that the petroleum ether-soluble portion resulting from the acetone compound formation does not contain glucose acetone condensation compounds; it was therefore discarded.

HYDROLYSIS AND OSAZONE FORMATION.

Fraction I (obtained on distillation of the water-soluble portion of the acetone compounds) (0.45 g.) was hydrolysed by treating the syrup with 50% glacial acetic acid (4 c.c.) for 2 hours at 100°. After this period the brown solution was strongly reducing to, Fehling's/

Fehling's solution.

Osazone formation.

To the solution were added water (3 c.c.), phenylhydrazine (0.9 c.c.), sodium acetate and a little sodium bisulphite. After heating at 100° for 30 minutes and allowing to cool slowly, a yellow precipitate and a tar separated over-night. The tarry patches were separated as far as possible from the solid material.

Yield of the solid precipitate :- 0.08 g.

Found:- OMe, nil (micro determination).

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Fraction II (0.34 g.) was hydrolysed in the same manner as described for fr. I, in 50% glacial acetic acid (2 c.c.); the resulting syrup was strongly reducing to Fehling's solution.

Osazone formation.

To the above solution were added water (2 c.c.), phenylhydrazine (0.68 g.), sodium acetate and a little sodium bisulphite. After heating for 30 minutes at 100° and allowing to cool slowly a precipitate, mixed with some tarry matter separated over-night. The crude osazone was washed repeatedly with water containing a little acetic acid and finally with water.

Yield:- 0.062 g.

Found:- OMe, 1.34% .

Calculated for monomethyl glucosazone $C_{19}H_{24}O_4N_4$ ,

OMe 8.3% .

## SEPARATION OF THE SUBSTITUTED GLUCOSAZONE FROM GLUCOSAZONE.

The osazone (0.062 g.) was treated with chloroform at 60° for 4 minutes (53) and at room temperature for 12 hours. Filtration yielded a yellow precipitate. (0.019 g.) ; found OMe nil .

The volume of the filtrate was reduced at room temperature to about 1 c.c.; on pouring into petroleum ether (b.p. 40°-60°) a brown precipitate was obtained, which was washed with more petroleum ether on the filter.

Yield:- 0.043 g.

In an attempt to crystallise the precipitate, it was dissolved in hot aqueous methyl alcohol, treated with charcoal and put aside at 0°. Crystals however failed to appear.

## HYDROLYSIS OF THE PARTLY METHYLATED LICHENIN FOLLOWED BY ACETYLATION.

Hydrolysis of the remaining half of the partly methylated lichenin was carried out exactly as described before (page45) with 8% sulphuric acid and the material was worked up as usual.

Acetylation of the hydrolysis products was carried out in pyridine (150 c.c.) and acetic anhydride. The mixture was kept at 100° for 1½ hours, allowed to stand for 48 hours and worked up in the usual way.

Yield:- 5.1 g.

FRACTIONATION OF THE ACETYLATED GLUCOSE DERIVATIVES BY DISTILLATION.

The brown syrup (5.1 g.) was distilled and yielded 3.57 g. of a yellow syrup, b.p. 138°/0.13 mm - 200°/0.07 mm. The residue in the flask was charred. Two fractions were separated on redistillation from a flask with a rather high neck. Separate refractionation of each of these fractions yielded the following result :

Fraction I i.e. 1st drop b.p. 125°/0.02 mm., OMe 9.34%, (distilled from a small flask ).

Fraction II, b.p. 145°-175°/0.03 mm., OMe 8.4%, 1.18 g

Fraction III, b.p. 175°-185°/0.02 mm., OMe 8.18%, 1.22 g.

Fraction IV , b.p. 185°-200°/0.02 mm., OMe 5.28%, 1.17 g. (The last drop: OMe 4.36% )

(Calculated for dimethyl triacetyl glucose, OMe 18.6% , for monomethyl tetraacetyl glucose OMe 8.57%.)

DEACETYLATION AND OSAZONE FORMATION.

Fractions II, III and IV were each dissolved in absolute methyl alcohol (5.2 c.c.) and 0.165 N-sodium methylate (3.9 c.c.) was added. After 12 hours the solutions were taken to dryness.

Osazone formation.

Each of the syrups resulting from the deacetylated fractions was dissolved in water (11 c.c.), filtered and sodium acetate, a little sodium bisulphite and/

and phenylhydrazine (1.08 c.c.) in glacial acetic acid (0.63 c.c.) was added; the mixtures were kept at 95° for 45 minutes and then allowed to cool slowly; a yellow precipitate together with some tarry matter separated in each of the fractions. After 12 hours the precipitates were filtered and separated as far as possible from the tar. Each of the precipitates was then suspended three times in water (25 c.c. )(acidified with a few drops of acetic acid ), filtered and finally washed with water (25 c.c.).

Result:-

Osazone from fr.II, 0.042 g., OMe 3.99% (micro estimation), the tar formed along with this precipitate had OMe 5.55%.

Osazone from fr. III, 0.235 g., OMe nil (microestimation) (the tar formed along with this precipitate had OMe 4.7%).

Osazone from fr. IV, 0.144 g. had OMe 4.5% (micro-estimation ),
(the tar formed along with this precipitate had OMe 3.9%).

Separation of the methylated osazones from glucosazone (53).

Each of the osazones was treated with chloroform for 4 minutes at 60° and at room temperature for 12 hours and was then filtered.

Result:

0.042 g. osazone (fr.II) yielded 0.010 g. of chloroform insoluble material;
0.235 g. osazone (fr.III) yielded 0,098 g. and
0.144 g. osazone (fr. IV) yielded 0.089 g. of chloroform insoluble material.

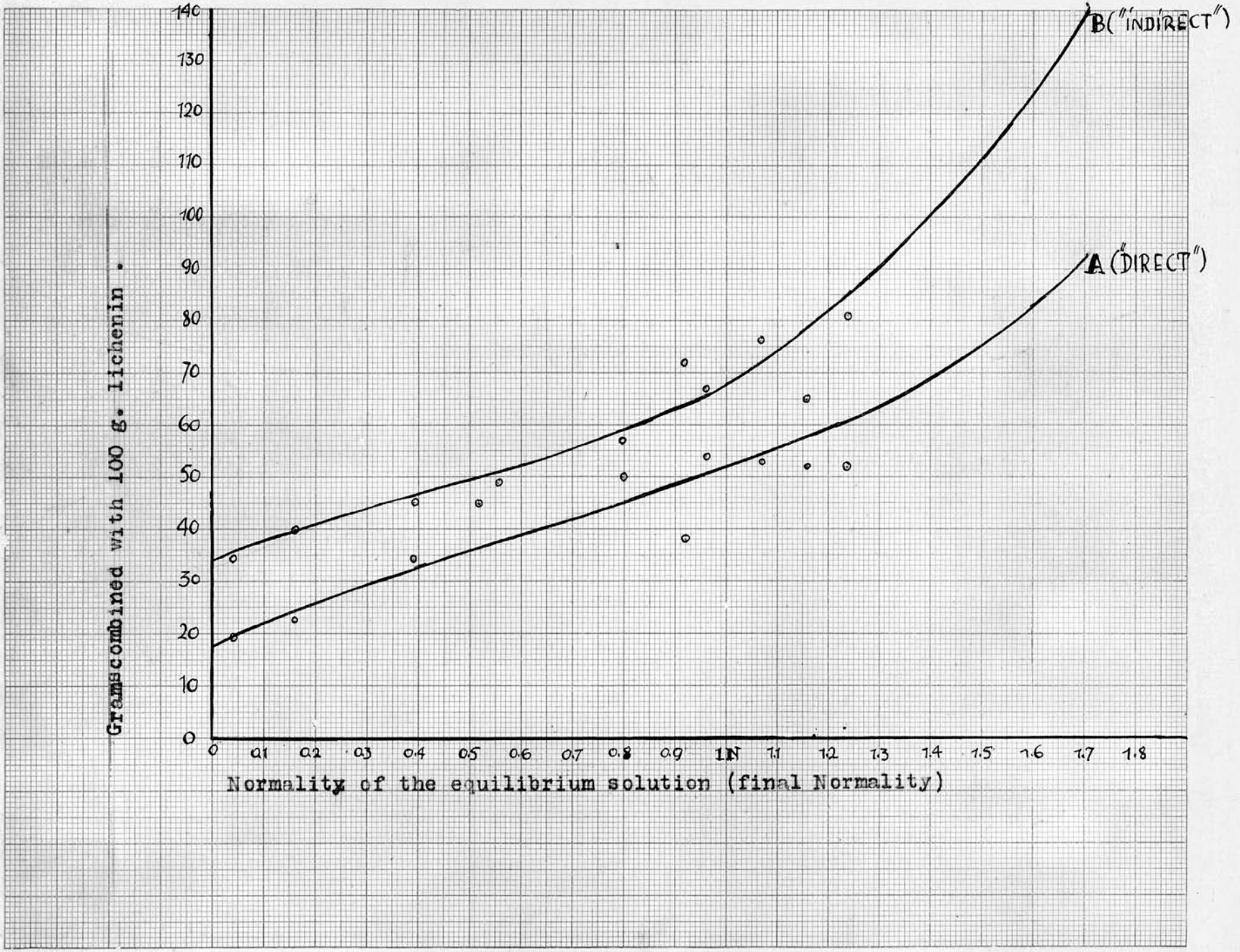
The chloroform-insoluble portions were shown to be mainly glucosazone; after two recrystallisations they had m.p. 196°, not depressed on admixture with an authentic sample of glucosazone.

The chloroform filtrates were reduced to small volume by aeration at room temperature and then poured into light petroleum ether. Dark brown precipitates resulted; attempts however to crystallise them from aqueous methyl alcohol were unsuccessful.

## DISCUSSION.

Taking into account the experiences with other polysaccharides (30), it was decided to prepare the lichenin-potassium hydroxide addition compounds from the acetate by simultaneous deacetylation and compound formation in an absolute-alcoholic medium. The alkali combining capacity of lichenin was investigated over a wide range of alkali concentrations. The method, described in detail in the experimental section, involves briefly: (a) The estimation of alkali required for deacetylation. In absolute-alcoholic medium this is partly a catalytic process, in which decreasing amounts of alkali were found to be required with decreasing alkali concentrations. A few results, apparently not conforming with this rule are believed to be due to the varying time allowed, which sometimes exceeded considerably the minimum of 2½ hours thought to be sufficient for deacetylation (30); thus ethyl acetate, produced in the course of the catalytic deacetylation (47-49) might have been hydrolysed to a varying extent by the alkali, depending on the period of contact. (b) The estimation by titration of the amount of alkali removed from the solution by the addition compound (indirect method), and: (c) The estimation of the amount of alkali combined with the lichenin after washing the latter with a small amount of absolute alcohol (direct method).

Washing/


B("INDIRECT")
A("DIRECT")
Gramscombined with 100 g. lichenin.
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1N 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8
Normality of the equilibrium solution (final Normality)

## T A B L E.

THE ALKALI COMBINING CAPACITY OF L I C H E N I N .

| Conc. of KOH Initial Normality. | Final | KOH in g. required to deacetylate 100 g. lichenin acetate, | KOH in g. in combination with 100 g. l i c h e n i n; indirect method. | direct |
|---|---|---|---|---|
| 0.1116 | 0.0409 | 18.14 | 34.64 | 19.10 |
| 0.221 | 0.166 | 43.84 | 39.81 | 22.48 |
| 0.650 | 0.396 | 58.40 | 45.42 | 34.54 |
| 0.812 | 0.519 | 55.35 | 45.27 | -- |
| 0.910 | 0.556 | 54.95 | 48.85 | 36.73 |
| 0.951 | 0.803 | 81.04 | 56.76 | 49.05 |
| 1.109 | 0.921 | 48.85 | 72.13 | 38.18 |
| 1.250 | 0.957 | 60.08 | 67.07 | 53.78 |
| 1.410 | 1.077 | 56.91 | 76.54 | 53.28 |
| 1.420 | 1.160 | 51.40 | 65.00 | 51.87 |
| 1.625 | 1.240 | 55.52 | 80.51 | 52.73 |
| 2.214 | 1.705 | 55.99 | 142.20 | 93.48 |

Calculated for $C_6H_{10}O_5$.KOH , 100 g. lichenin would combine with 34.6 g. potassium hydroxide;
calculated for $C_6H_{10}O_5$. 2KOH , 100 g. lichenin would combine with 69.1 g. potassium hydroxide.

Washing experiments with alkali cellulose (40) yielded results indicating that a certain amount of alkali, considered by those authors to be merely "physically adsorbed" alkali, was removed easily in the first of a series of consecutive washings, while further washings appeared to remove a small amount of "chemically fixed" alkali. These experiments in an aqueous medium, could be carried out at by far higher alkali concentrations than ours, where the solubility of potassium hydroxide was the limiting factor. Water could not be used because of the solubility of lichenin in this medium. From the results obtained on lichenin (table and diagram) it will be seen that such alkali, as is needed stoechiometrically for a compound $C_6H_{10}O_5$.KOH appears to be removed with the same ease as alkali present in excess of that required for a 1:1 compound. Thus at first sight, the curves do not seem to indicate, that lichenin and potassium hydroxide react in definite stoechiometric proportions. Reference, however, has already been made to the instability of such compounds. Inspection of curve (B)(indirect method) will show that extrapolation to zero alkali concentration points to an alkali combining capacity of lichenin in very good agreement with a compound $C_6H_{10}O_5$.KOH , indicating 100 g. lichenin to combine with 34 g. potassium hydroxide ; ( calculated for $C_6H_{10}O_5$.KOH : 100 g. lichenin combine with 34.6 g. potassium hydroxide.) This result was considered to be significant. The conclusion/

conclusion was drawn :
(a) that lichenin in its fibrous form easily adsorbs alkali mechanically,
(b) that the alkali addition compound is of a very unstable nature; both factors being responsible for the smooth and more or less parallel curves (A) and (B);
(c) that lichenin forms a compound of the composition $C_6H_{10}O_5.KOH$ . As to the formation of more complex compounds no conclusions could be drawn from these curves.

After methylation (experiment I) under the usual, mild anhydrous conditions of a lichenin alkali compound approximating to the composition $C_6H_{10}O_5.KOH$, it was found that substitution by a methyl group had taken place in about 44% yield (calculated on the basis of a monomethyl lichenin). The product was hydrolysed, acetylated and fractionated in a high vacuum. Separation of the unmethylated from the methylated glucose derivatives could thus be achieved to a certain extent. From the OMe content of fr. I (9.1%) it was concluded that a certain amount of dimethyl glucose derivatives was present in addition to monomethyl tetraacetyl glucose. Fraction II had OMe 8.0% (calculated for $C_{15}H_{22}O_{10}$, OMe 8.57%) and on deacetylation and osazone formation gave an osazone containing but 2% of methoxyl. Separation of the methylated osazone from glucosazone was effected according to Heddle and Percival(53); a yield of glucosazone amounting to 21% by weight of the starting material

material was obtained. Now, the optimal yield under these conditions is about 50-55% by weight of glucosazone from glucose, while the yield from 2-methyl glucose is generally considered to be smaller (54). From the OMe content of fr. II the presence of as much as 43% glucose, which would be required to account for the above yield, were the glucosazone derived from glucose alone, can be excluded. The conclusion was therefore drawn, that 2-methyl glucose was present in the starting material, in addition to other monomethyl glucoses. No crystalline 2-methyl glucose derivatives e.g. the phenylhydrazone could be isolated from fraction II and an attempt to prepare 2-methyl β-methylglucoside from fraction I was also unsuccessful.

In view of the failure to obtain a crystalline monomethyl glucosazone the following method was employed to decide, whether 3- or 6-methyl glucose or both were also present. These are the only other possibilities since $C_4$ is involved in the linkage to adjacent units and $C_5$ is involved in the pyranose ring. By forming the respective acetone compounds the possible three monomethyl glucoses must be expected to yield :

(a) 2-methyl-5,6-isopropylidene glucose,

(b) 6-methyl-1,2-isopropylidene glucose and

(c) 3-methyl-1,2-5,6-diisopropylidene glucose .

In case of the presence of the latter it could, because of its relatively low boiling point, be separated from the/

the other compounds. Distillation of the acetone compounds prepared from fraction III yielded fractions, the boiling points and refractive indices of which were considerably higher than those of 3-methyl diacetone glucose ( 51,52; b.p. 105°/0.3 mm., $n_D^{14}$ 1.4518 )

Confirmation of the absence of 3-methyl glucose in the hydrolysis mixture of the partly methylated lichenin was obtained also in experiment II. Methylation of a carefully dried compound approximating the composition $C_6H_{10}O_5$.KOH effected substitution in practically 100% yield. Fractional distillation of the acetone compounds prepared from one half of the available hydrolysed material yielded essentially the same result as obtained in experiment I. The presence of compounds with a free reducing group (2-methyl monoacetone glucose and dimethyl glucose ) was considered to account for the cracking in the distilling flask, which began in both experiments at a temperature of 170°-180°/0.07 mm.

The acetates prepared from the other half of the mixture of hydrolysis products of experiment II were fractionally distilled:

| | | | |
|---|---|---|---|
| Fr. I i.e. 1st drop | | OMe | 9.34% , |
| fr. II | 1.18 g. | OMe | 8.4 % , |
| fr. III | 1.22 g. | OMe | 8.18% , and |
| fr. IV | 1.17 g. | OMe | 5.28% . |

Calculated for dimethyl triacetyl glucose OMe 18.6% ; for monomethyl tetraacetyl glucose OMe 8.57%. Deacetylation and the preparation of osazones followed. Glucosazone/

Glucosazone was again obtained after suitable treatmetn from fr. II (of the acetyl derivatives). The high OMe content of this fraction indicated the virtual absence of pentaacetyl glucose. Proof and confirmation was therefore afforded of the presence of 2-methyl glucose in the hydrolysis mixture of the partly methylated lichenin.

On the other hand, fraction IV had so low a methoxyl content (5.28%) that the conclusion could be drawn that any dimethyl glucose derivatives were absent. The osazone prepared from this fraction after deacetylation, showed OMe 4.5% which, in view of the absence of 3-methyl glucose in the hydrolysis mixture must be accounted for by the presence of 6-methyl glucose.

The solid osazone obtained from fr. III had OMe nil. It appears therefore that some fractionation of the monomethyl glucoses had been effected; the deacetylated fr. III consisting apparently mainly of 2-methyl glucose, while the deacetylated fr. IV contained in addition 6-methyl glucose.

It is concluded therefore, that lichenin combines with potassium hydroxide to form a compound in which alkali is attached at positions $C_2$ and $C_6$ of the $\beta$-glucose units. If every glucopyranose unit were so substituted we should expect the addition compound to be $(C_6H_{10}O_5 . 2\,KOH)_X$ and to isolate mainly dimethyl glucoses on methylation. This is contrary to the evidence and, so far as it goes it seems/

seems likely that either $C_2$ or $C_6$ are concerned with addition compound formation for any one glucose unit, with $C_2$ probably preponderating. In a few cases both are probably substituted, but the structure of the dimethyl glucose resulting on hydrolysis could not be investigated because of the poor yield.

It is of interest to compare this result with those previously reported for amylose (30) and cellulose (30), and for their building units maltose (34) and cellobiose (34). Addition compound formation between amylose and potassium hydroxide was shown by a similar method to involve $C_2$ and no evidence for substitution on $C_6$ was found, although for maltose positions $C_2$ and $C_6$ as well as the reducing group were concerned. In the case of cellobiose only $C_6$ and the reducing group were involved, whereas in cellulose it was proved that the addition compound formation was associated only with $C_2$. The conclusion drawn from these experiments was, that in cellulose, and possibly in amylose, addition compound formation on the primary alcohol residues was hindered in some way, probably as a result of these residues taking part in cross linkages between the chains. In starch it has indeed been shown by Hirst and Young (38) that the "chemical molecules" of 24 -30 anhydroglucose units are indeed joined by the reducing group to an adjacent chain through $C_6$ by primary valencies. This would only explain the failure to substitute one $C_6$ in 25 or so units, but it is difficult to escape the feeling that there is some connexion between these observations.

In/

In lichenin however the results appear to be similar to those recorded for cellobiose, the structural unit. This result is in agreement with the general picture of the lichenin molecule, as it was drawn by various workers like Hess (20) and Carter and Record (19), namely that of a polysaccharide in chain form, "the chemical and physical units of which are possibly identical" (19), meaning that endgroup assay and osmometric measurement yield identical results implying the absence of any cross linkages from one basic unit chain to another.

## SUMMARY.


1. Titration experiments with lichenin acetate showed, that lichenin would combine with one molecular proportion of potassium hydroxide.
2. Mild methylation of a lichenin -potassium hydroxide addition compound $(C_6H_{10}O_5 . KOH)_x$ followed by hydrolysis, acetylation and fractional distillation yielded a small amount of dimethyl glucose derivatives.
3. Another fraction containing mainly monomethyl tetraacetyl glucose gave rise after suitable treatment to an osazone (OMe 2%).
4. Glucosazone was separated from the methylated osazone and was shown on the strength of its yield to be accounted for by the presence of 2-methyl tetraacetyl glucose in the above fraction.
5. After deacetylation of another of the acetyl derivative fractions, the acetone compounds were prepared and shown by means of fractional distillation not to contain 3-methyl diacetone glucose.
6. In another experiment, half of the available hydrolysis products obtained after the mild methylation of a lichenin - potassium hydroxide addition compound, were converted into the corresponding acetone compounds and fractionally distilled. Again the absence of 3-methyl glucose in the hydrolysis mixture was confirmed.

7. The other half of the hydrolysis products was acetylated, fractionally distilled and the osazones prepared from the deacetylated fractions.
8. Again glucosazone was separated from the methylated osazones.
9. Consideration of the methoxyl contents of the acetyl fractions and that of the osazones prepared from them, together with the yields of glucosazone, led to the conclusion that 2-methyl glucose and 6-methyl glucose and in addition, a small amount of dimethyl glucose are the glucose ethers present in the hydrolysis mixture.
10. It follows, that in the lichenin-potassium hydroxide addition compound one glucopyranose unit combines with one potassium hydroxide residue through either position $C_2$ or position $C_6$, and that in addition small amounts of the repeating units react in a more complex manner.

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# PART II.

## THE HEMICELLULOSES OF ICELAND MOSS.

## INTRODUCTION.

In the structural fabric of plants "true cellulose" (identical with cotton cellulose) (1) is rarely met with as such; in general it is found to be associated with other cell wall constituents:- lignin and polysaccharides of a less resistant nature- the so-called hemicelluloses.

By means of the alternate treatment of a cellulosic material with chlorine and hot sodium sulphite, by the method of Cross and Bevan, an insoluble fraction is obtained which represents "natural cellulose" i.e. "true cellulose"plus the intimately associated hemicellulose fraction ("cellulosans"); while in the solution are the incrusting substances. These latter are lignin and hemicelluloses not associated with the cellulosic fraction, and generally seem to contain uronic acid groups ("polyuronide hemicelluloses").

Exact chemical data on hemicelluloses are lacking to such an extent , that it is difficult to differentiate between their molecular structure and that of other chemically related substances of plant origin such as the gums, mucilages, pectic substances, gel forming polysaccharides, etc. While in the latter field a beginning has been made in the configurational study of the various sugars and sugar acids involved, no attempt to elucidate the structure of polyuronide hemicelluloses by /

hemicelluloses by means of complete methylation has yet been recorded.

The name hemicellulose is misleading, if regarded as implying some relationship to cellulose as was indeed intended by its originator Schultze (2), who was the first to isolate these carbohydrate fractions from a number of plant materials by extraction with dilute alkali and precipitation with acid. Today it should perhaps be taken as merely pointing to a physiological association with cellulosic materials. Norman (3) defines them as "those cell wall polysaccharides, which may be extracted from plant tissues by treatment with dilute alkali, hot or cold, but not with water, and which may be hydrolysed to constituent sugars and sugar acid units by boiling with hot dilute mineral acids." This rather empirical definition therefore excludes water soluble hexosans like galactans (4,5). Most hemicelluloses however are reported to be water-soluble once they have been removed (by means of alkali for instance) from the plant material. This remarkable feature was explained tentatively (6) by assuming that in the plant they are not free, but are associated chemically or combined with some other cell wall constituent, such as lignin for instance.

Usually alkali of 4% strength is employed to extract hemicelluloses. It is however believed, that with this treatment a certain amount of "cellulosan" material is removed along with incrusting hemicelluloses (7)./

loses (7).

In general it may be said , that attempts to isolate, separate and purify a mixture of these various polysaccharides of complex nature meet with great difficulties. Chemical or physical methods for quick investigation during these processes are very limited and inconclusive. The colour tests which are sometimes applied in an attempt to follow the fate of the hexose- (and/or ) -pentose-uronic acid containing molecule during isolation and purification are not always reliable.

During the extensive researches on various plant materials carried out in recent years with the object of obtaining information on the type of units contained in the hemicellulose polysaccharides and their percentage composition, the attention of the various workers concerned was mainly directed to the solution of two problems: (a) How extraction and purification of hemicelluloses could best be achieved with the least drastic methods; and: (b) What conditions had to be observed in order to obtain homogeneous fractions of hemicelluloses.

Most workers attempt to fractionate the crude hemicellulose by means of the graded addition of alcohol or acetone. O'Dwyer (8) investigated the hemicelluloses of beechwood and separated two fractions: one, termed (A) by acidification of the crude extract, the other, (B) , by addition of alcohol to the filtrate. These two precipitates differed in appearance as/

as well as in composition and physical properties. Hemicellulose (A) was more resistant to acid hydrolysis than the (B) fraction and was shown to be composed mainly of xylose together with 11% of glucuronic acid. Fraction (B) contained some arabinose and galactose together with a large amount of galacturonic acid. The preparations were dried gradually with alcohol of increasing strength, which was considered likely to remove any lignin precipitated with the hemicellulose. This however, was found subsequently not to be entirely correct. The fractions themselves were not homogeneous. The acetyl-derivatives were separable into several fractions of different rotations and solubilities. Similarly hemicelluloses from oat straw and rye straw were separated (9). Hägglund (10) investigated hemicelluloses obtained during the pulping process of spruce wood; in view however of the drastic measures involved, studies of that type must be regarded of lesser value in throwing light on the original composition in the plant (11).

Prior to the isolation of hemicelluloses from wheat bran, Norris and Preece (12) removed pectic substances with hot dilute ammonium oxalate; subsequently the product was treated with boiling dilute alcoholic sodium hydroxide in order to free the material completely from lignin. Later it was shown by Preece (13), that such treatment has a degradative effect upon hemicelluloses, indicated by significant reductions in the furfuraldehyde yields (14). The crude/

crude hemicellulose obtained with cold 4% alkali (the standard procedure) was divided into fractions:(A) , by addition of a slight excess of glacial acetic acid to the alkaline extract. (B), by addition of half a volume of acetone to the filtrate of (A) and (C), by the addition of more acetone to the filtrate of (B). All these fractions, dissolved in alkali, were then further purified by addition of Fehling's solution. A blue gelatinous hemicellulose - copper complex was thus precipitated (fractions "$A_1$,$B_1$,$C_1$"), filtered off, decomposed by suspension in dilute hydrochloric acid, reprecipitated and washed free from salts. From the respective filtrates of the copper complex further fractions were obtained on addition of acetone (fractions "$A_2$, $B_2$, $C_2$"). Along these lines hemicelluloses from many materials like wheat bran, maize cobs and box wood have been fractionated (15 - 19).

Due to the difficulties outlined already, one can not state definitely, whether these fractions represent separate polysaccharides or whether they are mixtures (20). It appears that hemicelluloses have an optimal $p_H$ for precipitation(21). Precipitates collected on either side of this value have the same composition but are obtained in lower yield. If precipitation is not carried out at the optimal $p_H$, it could be expected, that the incompletely precipitated part of one fraction would then reappear in the next.

Later hemicellulose fractions generally show an increasing uronic acid content and the uronic acid/

acid part of the molecule, because of its electric charge, may well have a dominating effect on the solubility. A higher uronic acid content may be associated with shorter chain length or smaller molecular size (22). If the structure of hemicelluloses is one of relatively simple chain molecules such as six or twelve or more pentose or hexose groups to one hexuronic acid group, then separation on a physical basis might be accompanied by clear differences in the properties of the various components. On the other hand, if their structure is one of large chain molecules, straight or branched, with varying numbers of recurrent units, such as ten, twenty or thirty repetitions of six or twelve sugar groups linked to one hexuronic acid group, then physical separation would not be accompanied by any significant differences in composition.

Bearing in mind the various points raised in regard to fractionation procedures, there is doubt, whether any real advantage is gained by separation (20).

A relatively small range of sugars and sugar acids appears to take part in the hemicellulose molecule. As a rule, hydrolysis mixtures were found to contain two or more of the following : glucose, galactose, mannose, xylose, arabinose, glucuronic and galacturonic acid and /or a methoxy uronic acid.

In addition , acid hydrolysis yields a dark insoluble residue, which Anderson (23,24), believing it/

it to be originally part of the molecule, termed body "X". Others hold that this might be lignin. The possibility must also be considered, that it is derived from furfural, which is known to condense to dark insoluble substances under the influence of mineral acids.

It is only by prolonged treatment with more concentrated mineral acids that hemicelluloses will yield simple uronic acid components; otherwise aldobionic acids (often of varying composition), containing one or more sugars in addition to the uronic acid group, are obtained.

Apart from those materials already quoted, hemicelluloses of many other lignified tissues (mesquite wood, oat hulls, oak wood) have been investigated and were generally found to be of xylan - polyuronide nature (25 - 30).

Previous treatment (rate of drying), age of the plant, and the type of wood (heart or sap wood) were found to be factors influencing the composition of the hemicelluloses (31).

Hemicelluloses also accurr in non-lignified tissue like hop flower (32), alfalfa hay (33) and various grasses (34,35). Sometimes these seem to be of a different kind, apparently containing more units of the galactose-arabinose series.

This leads us to the theory, long entertained by biochemists (36), that the pentose sugars arise in nature from the corresponding hexoses by a process, which involves oxidation to a uronic acid, followed by/

by decarboxylation. Thus the xylose in polyuronide hemicelluloses in lignified tissues would be formed by decarboxylation of d-glucuronic acid; and decarboxylation of d-galacturonic acid would produce l-arabinose (and not d-arabinose), which indeed is found to be associated in nature with d-galactose. A number of prominent workers are at present investigating this problem on pectic substances, gums and mucilages, which afford special opportunities for this kind of study and about which considerably more detailed information has been made available in recent years. Taking into account the type of the linkages involved, the conclusion has been reached, that in none of the substances investigated could the pentose (arabinose) have arisen intramolecularly from the hexose units (galactose).

In the field of hemicelluloses research in xylan has progressed far enough to support the refutation of the hypothesis, that xylose is directly derived from glucose (in cellulose): l-arabofuranose was found to be present as terminal end groups of the xylopyranose chains.

Xylan was isolated from esparto cellulose (37) after removal of lignin, by boiling with 12% aqueous potassium hydroxide. Direct methylation in 45% sodium hydroxide with dimethyl sulphate yielded methylxylan, which on hydrolysis appeared to give rise exclusively to 2:3-dimethyl xylose. The question, whether position 4 or 5 is engaged in chain formation, which was thus left/

left undecided, was settled subsequently by a paper published by Haworth and Percival (38).

The fully methylated dimethyl xylan was degraded by acetolysis to give rise to a partly methylated disaccharide. Deacetylation and oxidation with bromine yielded a partly methylated bionic acid; hydrolytic cleavage yielded 2:3:4-trimethyl xylopyranose and 2:3:5-trimethyl $\gamma$-xylonolactone. Since the acid portion of the bionic acid was thus shown to carry a free hydroxyl group on $C_4$, the conclusion was reached, that the linkage in xylan is 1:4 and the participating xylose units are of the pyranose type.

Subsequent careful investigation of xylan (39), methylated in a slightly modified way, by means of fractionation with petroleum ether proved, that the product was indeed homogeneous. Hydrolysis and careful fractionation of the pentoside derivatives thus obtained led however to the startling result, that 6% consisted of 2:3:5-trimethyl methyl l-arabofuranoside, the identity of which was confirmed by oxidative methods. Identical results were obtained with esparto celluloses of different origin.

In the xylan molecule thus 18 - 20 xylopyranose units appear to be associated with one arabofuranose unit. Viscosity measurements pointed however to a chain length of 75 - 80 units.

Considering the fact that invariably about 5% of monomethyl xylose was obtained and dismissing the possibility that this might be due to incomplete methylation, there remain two possibilities as to the

structure of the xylan macromolecule. Either the basic (18 membered) chains are joined to each other with the reducing xylose end groups (situated at the end of the chain opposite to the terminal arabinose group ) linked to a xylose unit situated within the neighbouring chain (a type of cross linkage similar to that later demonstrated in starch (41)); or the long chains of xylopyranose, possibly linked to each other in the form of a loop, carry at intervals of about 18 xylose residues 1 arabofuranose side chain.

Thanks to the fact, that the end group is of furanose type, it was possible to decide the question in favour of the former possibility (40). The furanoside link of the arabinose end group (more susceptible to hydrolytic agents) was broken by means of a carefully chosen selective agent (aqueous oxalic acid ), which left the rest of the molecule unimpaired. Subsequently, complete methylation and hydrolysis furnished xylopyranose end groups equal in amount to the arabinose end groups obtained from the original xylan. Thus proof was afforded, that the removal of the arabinose residue from xylan had caused no degradation of the basic chain units. The fact that the monomethyl xylose isolated proved to be 2-methyl xylose suggests, that $C_3$ is engaged in the cross linkages from one basic chain unit to another, thus forming the xylan molecule of the size of 75 - 80 units.

Also pure galactans and mannans appear to exist in certain plant materials (12), however it is doubtful/

doubtful whether their role is the same as that of the polyuronide hemicelluloses. A mannan of Ivory Nut, believed to have the function of a "reserve substance" was shown to consist of mannose units linked through positions 1 and 4 (43 - 45).

The present investigation is concerned with the polyuronide hemicellulose fraction of Iceland Moss and in particular with the type of linkage of the basic units within the polysaccharide. Buston and Chambers (46) surveyed this fraction with regard to the type of participating units and it is their paper which has been consulted with respect to the isolation and purification of the material. Ulander and Tollens (47), earlier workers in this field (1906) reported the presence of glucose, mannose and galactose therein (an observation confirmed by Hesse (48)). Salkowski (49), after hydrolysis of the water -extracted plant with dilute acid obtained a resistant xylan-free ashless material, which he showed was not lignin. Poulsson (50) believed pentosan to be present to the extent of 3%.

Buston and Chambers assessed the relative amount of extractible substances in Iceland Moss. They found the dry moss to contain 5.7% of cold-water-soluble substances (yielding some carbondioxide and furfuraldehyde ), 8.5% lichen acids (extracted with hot alcohol), 64% lichenin (extracted with hot water ), 3.2% of hemicelluloses, extracted with 4% alkali and 10.8%/

10.8% of a residue which, from its high furfuraldehyde and carbon dioxide yield was concluded still to contain hemicelluloses (possibly of the "cellulosan" type), resistant to the action of cold 4% alkali. Increasing the strength of the alkali to 17% seemed to remove this hemicellulose fraction also. The residue was regarded to be more or less pure cellulose.

No pectin was found to be present.

The hemicellulose fraction was obtained by extraction of the cell residue (after complete removal of the water and alcohol-soluble material) with cold 4% sodium hydroxide. The extracts were slightly acidified with acetic acid, but failed to yield a precipitate. Therefore, the authors comment, "hemicelluloses of the (A) type appear to be absent". Addition of half a volume acetone precipitated all the hemicelluloses (termed"B" according to Norris and Preece, 12 ). By means of the copper method this fraction was divided into ($B_1$) and ($B_2$). The relative yields between one and another experiment varied considerably; for instance: 200 g. lichen residue yielded in one experiment 16 g. fraction ($B_1$) and 8.8.g. fraction ($B_2$), in another: 300 g. lichen residue yielded 31 g. ($B_1$) and 5 g.($B_2$) .

Further extractions with 17% sodium hydroxide yielded some more hemicelluloses, the only hydrolytic breakdown products of which were found to be mannose, galactose and galacturonic acid.

Hemicellulose fraction ($B_1$) was non-reducing;

it/

it contained 9.7% uronic anhydride and no pentosan, 39.6% anhydromannose and 44.3% anhydrogalactose. No other sugar was found to be present. Partial hydrolysis with 1.4% sulphuric acid yielded only galactose.

Analysis of fraction ($B_2$) showed : on the average 8% uronic anhydride, no pentoses, roughly 20% mannose and 62% galactose. Again no other sugar was found to be present.

It will be seen later , that the author of this thesis has found the hemicelluloses of his Iceland Moss sample to be made up of glucose to the extent of about 85%; galactose and mannose being present in relatively small amounts .

Buston and Chambers invariably obtained on hydrolysis an insoluble body, varying in amount according to the pretreatment of the isolated hemicellulose. Reference has already been made to similar substances obtained by other workers.

Buston and Chambers comment on the notable difference - the absence of any pentose constituent - between these polyuronide hemicelluloses and those generally obtained from the cell walls of higher plants, which are regarded to be of definite structural character. Hemicelluloses of seeds, possibly of "reserve carbohydrate" character have also been shown to consist of hexose + hexuronic acid only. The suggestion is advanced, that in Iceland Moss hemicelluloses combine the functions both of structural and reservecarbohydrates, a suggestion admittedly not very plausible in view of the large amount of other glucose reserves present in the lichen in the form of lichenin.

# EXPERIMENTAL.

General remarks : (1) All boiling points mentioned refer to bath temperatures; (2) all melting points were taken with the Reichert microscope.

## ISOLATION AND PURIFICATION OF THE HEMICELLULOSES.

The figures for the large scale preparation of hemicellulose were calculated on the basis of 1 kg. anhydrous starting material.(The moisture content of the Iceland Moss sample used was 15%)

Iceland Moss (1 kg. dry weight) was extracted, twice with a 1.5% sodium carbonate solution (10 litres) in the cold in order to remove the lichen acids ( 47.), and 20 times with boiling water (15 litres each time for 4 hours ). The complete removal of all water-soluble polysaccharides (of lichenin in particular) was confirmed by a negative Fehling's reaction of a sample of the extracting water, after boiling with hydrochloric acid. The lichen residue was dried at 70°/12 mm.; 366 g. of a dark horny substance were obtained. Two extractions in the cold with 4% sodium hydroxide (total 16.1 litres) served to extract the bulk of the hemicelluloses. The dark colloidal solution could be separated to a certain extent from the moss residue by filtering under suction through muslin; thus 8.3 litres of the sodium hydroxide solution were recovered, while the moss residue, a swollen gelatinous mass retained a little less than 50% of the liquid. The extraction of the hemicelluloses in this manner was therefore/

therefore by no means quantitative.

To the solution (8.3 litres), made slightly acid with glacial acetic acid, alcohol (9.16 litres) was added, which precipitated completely the crude hemicelluloses. These were obtained in the form of a brown jelly by decanting and centrifuging. It was observed that the settling of the precipitate over night was an indication that sufficient alcohol had been added for complete precipitation. The material was redissolved in 4% sodium hydroxide (5.14 litres) under gentle heating and undissolved impurities removed from the cooled solution by centrifuging. Then a quarter volume of Fehling's solution was added under stirring, followed by alcohol (5 litres), and a copious bluish-green hemicellulose-copper-complex was precipitated which could be filtered through muslin. Addition of 2N - hydrochloric acid (1 litre) caused the copper compound to decompose. Care was taken to ensure, that alkali adsorbed within the gelatinous precipitate was completely neutralised. By adding alcohol (2.5 litres) to the acid suspension the purified hemicelluloses were precipitated and collected on a Buchner filter. They were dissolved in boiling water (4.8 litres) containing a little sodium hydroxide. The solution was cooled and made faintly acid with glacial acetic acid. By adding alcohol (3.44 litres) the bulk (about 89%) of the hemicelluloses ("hemicellulose I") was precipitated and removed by centrifuging; the addition to the filtrate of more alcohol (2.5/

(2.5 litres) was required to ensure complete precipitation ("hemicellulose II"; about 11% of the total yield). This division into two fractions was purely arbitrary. The products were covered with alcohol, which was renewed twice. For practical reasons (see later under ACETYLATION) the bulk of the material was not dried any further and therefore the yields were not ascertained directly. For the purpose of investigating the "free" hemicelluloses a small amount was dried in the desiccator over phosphoric oxide, after it had been covered repeatedly with alcohol followed by ether.

The products were powders of light beige colour; hemicellulose II was lighter than hemicellulose I .

Found :- moisture 8-10% , ash 0.6% ,
iodine number 4-6 .

An indication of the yields can be obtained from the amounts of hemicellulose acetate obtained from 1 kg. (dry) Iceland Moss :

hemicellulose I acetate ..50 g. ,
hemicellulose II acetate.. 5.5 g.

## ANALYSIS OF THE "FREE" HEMICELLULOSE .

(a) Rotation in 2% sodium hydroxide.

The solution was too dark to permit a reading.

(b) Equivalent weight.

A known weight of hemicellulose I (0.9012 g.) was allowed to stand over-night in a known volume

(50 /

(50 c.c. N/20 ) of standard sodium hydroxide solution. Titration with standard acid showed , that no free acid groups were present.

(c) OMe: hemicellulose I + II , OMe 0.28% .

(d) Uronic acid.

The method used was that of Dickson, Otterson and Link (52), except for the inclusion of an aniline trap(53) to keep back any furfural which might distil over.

Found :- Hemicellulose I, 4.95% uronic acid, hemicellulose II, 8.5 % uronic acid.

(e) Pentosan.

The method used was that described by Marshall and Norris (54). The amount of apparent pentosan was calculated from the weight of furfural phloroglucide using the appropriate factors (55).

Found :- hemicellulose I + II , yield of furfural 2% of the starting material, which would correspond to 3.6% pentosan. This result was corrected for furfural derived from uronic acid (56) and the conclusion reached that no pentose is present in the hemicelluloses.

(f) Hydrolysis with oxalic acid could not be effected. On heating the hemicelluloses with concentrated oxalic acid a suspension of whitish swollen particles was obtained, together with a small amount of black, apparently non-gelatinous matter. This non-homogeneousness is remarkable; the dark matter is perhaps identical with the insoluble matter obtained on hydrolysis with mineral acids.

(g)/

(g) A typical hydrolysis with 3.7% sulphuric acid.

Hemicellulose I (4.00 g.) was heated on the boiling water bath with 3.7% sulphuric acid (100 c.c.) for 3½ hours. 0.663 g. of insoluble matter was filtered off. The filtrate was neutralised with an excess of barium carbonate in the presence of charcoal and kept for 1 hour at 100° and then filtered and taken to dryness at 45°/12 mm. The resulting viscous syrup was taken up in about 8 c.c. of water and poured into absolute alcohol (2.2 litres); the resulting white flocculent precipitate (0.3281 g.) was collected on a Gooch filter. In a number of similar experiments the data of this precipitate varied widely; it was concluded that it is probably the salt of a complex aldobionic acid.

The alcoholic filtrate was concentrated to a glass (2.72 g.) under reduced pressure.

Estimation of the hexoses present in this glass.

Control experiments.

(1) Mannose (0.052 g.) and glucose (0.376 g.) were dissolved in water (15 c.c.) and alcohol (15 c.c.). Methylphenylhydrazine (0.75 g.) and glacial acetic acid (1.5 c.c.) were added and the mixture kept at 0°. No precipitate resulted. A parallel experiment furnished an identical result.

(2) Estimation of galactose : galactose (0.038 g.), mannose (0.048 g.) and glucose (0.632 g.) were dissolved in water (22 c.c.) and alcohol (22 c.c.) and methyl-

phenyl/

phenylhydrazine (1.1 c.c.) in glacial acetic acid (2.2 c.c.) was added. After 48 hours at 0° the precipitate was filtered and washed with 3 c.c. alcohol and dried at 105°. The result obtained indicated 2.9% galactose , while the mixture had actually contained 5.2% galactose.

In a parallel experiment a yield of galactose-methylphenylhydrazone was obtained indicating 3.2% galactose, while the mixture had actually contained 5.1% galactose.

(3) Estimation of mannose : mannose (0.4000 g.) and galactose (0.7120 g.) were dissolved in water (11 c.c.) and a solution of phenylhydrazine (0.8 c.c.), glacial acetic acid (0.8 c.c.) and water (2 c.c.) was added. After 48 hours at 0° the precipitate was filtered, washed with ice water (200 c.c.), followed by alcohol (10 c.c.) and ether (10 c.c.) and finally dried at 105°. A yield of mannosephenylhydrazone was obtained indicating 34.5% mannose, while the mixture had actually contained 35.6% mannose . Several parallel experiments gave similar results.

(4) Estimation of glucose : to an aqueous solution (60 c.c.) containing mannose (0.035 g.), galactose (0.028 g.) and glucose (0.490 g.), a solution of phenylhydrazine (1.25 c.c.) in glacial acetic acid (1.25 c.c.) and water (0.5 c.c.) was added; furthermore anhydrous sodium acetate (1 g.) and sodium bisulphite (0.05 g.). The mixture was kept at 100° for 1 hour. The precipitate was filtered after 24 hours, washed with slightly acetic water , followed with water (total

30 /

30 c.c.) and dried at 110°.
Yield:- 0.281 g. osazone i.e. 57 weight% of the amount of glucose (0.490 g.) present in the artificial mixture.

In a parallel experiment a yield of osazone was obtained corresponding to 52 weight% of the amount of glucose present in the adtificial mixture. (Mean-value of these experiments: 55 weight%)

The estimation of the hexoses in the glass was carried out according to the methods and results of these control experiments .

Found :- galactose: 6.8 , 8.4%
mannose : 2.5 , 3.5%
glucose : 92.0 , 99.5%

(h) Hydrolysis with 15% sulphuric acid.

Hemicellulose I (7.30 g.) was treated with 15% sulphuric acid (125 c.c.) at 100° for 24 hours; a dark insoluble matter (0.43 g.) was filtered off and the filtrate was neutralised with the calculated amount of barium hydroxide solution in the warmth in the presence of charcoal. A part of the water was evaporated under reduced pressure, the solution filtered and taken to dryness. The viscous syrup was taken up in about 5 c.c. water and poured into absolute alcohol (2.5 litres ); a white flocculent precipitate (0.95 g.) could be separated by centri-fuging and was collected on a Gooch filter, washed with/

with a little absolute alcohol, and ether and dried over phosphoric oxide.

Found:- Ash as sulphate, 68.3 , 69.9% .

(Calculated for $C_6H_9O_7.Ba/2$ 44.6% )

$[\alpha]_D^{18}$ +9.9° (c,1.1 in water )

(barium salt of glucuronic acid acc. to Hirst and Jones (57) $[\alpha]_D^{18}$ +15.0° ).

To the precipitate (0.15 g.) dissolved in water (4 c.c.) p-bromo phenylhydrazine acetate was added and the mixture heated for 30 minutes at 100°; a yellow precipitate resulted (57) .

A precipitate of mucic acid crystals could not be obtained by treating the unknown barium uronate (0.15 g.) in water (3 c.c.) with 1o N-nitric acid (5 c.c.) at 60° for several hours until the volume was reduced to 1 c.c.; water was then added (10 c.c.) and the mixture put aside at room temperature. Treating galactose under the same conditions a good yield of mucic acid crystals was obtained.

Correlating the available data it was concluded that the precipitate was in all probability the barium salt of glucuronic acid, mixed with inpurities. If we apply a correction of 23% (the percentage of ash found above the calculated amount required for the barium salt of glucuronic acid ) to the weight of material used for the polarimetric observation we arrive at a figure of $[\alpha]_D^{18}$ + 13.0° .

All experimental data on the following pages refer to hemicellulose I.

ATTEMPTED DIRECT METHYLATION OF THE "FREE" HEMICELLULOSE.
(According to Hirst's method of methylating xylan (39)).

Hemicellulose (3.3 g., dried over phosphoric oxide), was suspended in water (294 c.c.); potassium hydroxide (240 g.) was added and the resulting heat helped to dissolve the hemicellulose; a brown colloidal solution was obtained. Dimethylsulphate (266 c.c.) was added in one eighth portions every half hour for 4 hours while the contents of the flask were vigorously stirred mechanically at room temperature. Foaming of the solution was prevented by the addition of a few drops of capryl alcohol. When all dimethylsulphate had been added the temperature was raised to 100° for 1 hour. Still hot , the solution was filtered. The precipitate was washed with alcohol, dried and then extracted with chloroform in a Soxhlet apparatus. After drying over anhydrous sodium sulphate the solvent was distilled off under reduced pressure to yield 0.3 g. of a fibrous product. Found:- OMe 16.1% .

In order to check whether the washing of the precipitate with alcohol had caused the removal of some partly methylated hemicellulose, this alcoholic filtrate, taken to dryness after neutralisation, was dialysed through cellophane; after considerable time the dialysate was taken to dryness, yielding 0.1 g.

of/

of partly methylated material.

It has been observed, that the drying of the hemicellulose (even under the described careful conditions) caused it to be less easily soluble in alkali, than samples, which had been kept under alcohol instead. With a sample of the latter the described experiment of direct methylation was therefore repeated. Similar unsatisfactory results however were obtained.

It was therefore decided to adopt the method of simultaneous deacetylation and methylation.

A TYPICAL ACETYLATION OF THE HEMICELLULOSE.

Hemicellulose (kept under moist alcohol ) (estimated: about 10 g.) was suspended in pyridine (150 c.c.); acetic anhydride (100 c.c.) was added in five batches at intervals of about 10 minutes with mechanical stirring, which was continued for the next 3 hours while the flask was kept at 100°. When complete dissolution was achieved, the flask was set aside for 48 hours at room temperature with occasional shaking. After that period the solution was filtered through glass wool and poured into a large volume of water with stirring. The brown fibrous precipitate was then collected on muslin and thereafter washed in running water for 24 hours. Because the solubility of the acetate in acetone appeared to be impaired by previous drying, the product was merely freed from supernatant liquid on a Buchner filter.

Yield/

Yield:- 14 g. (dry weight)
Found:- $CH_3CO$ 41-43.5% ; owing to the dark solution the reading was inaccurate: $[\alpha]_D^{18}$ -10.0° (c, 0.1 ) , iodine number (according to Bergmann and Machemer) 2.7 , calculated for the acetate ; 4.5 calculated for the (deacetylated) hemicellulose present.

An experiment with a small sample of the acetate revealed that it was not homogeneous. By means of the graded addition of light petroleum to its solution in chloroform , it could be separated into numerous fractions. It was however not intended to fractionate it on a large scale.

A TYPICAL METHYLATION OF THE HEMICELLULOSE ACETATE .

Hemicellulose acetate, containing 90% moisture (dry weight 30 g.) was dissolved with mechanical stirring in boiling acetone (1200 c.c.) to a clear brown solution. Vigorous stirring was continued while dimethylsulphate (600 c.c.) and 30% sodium hydroxide (1500 c.c.) were added simultaneously in one tenth portions every 10 minutes at 40°-45°. After all the additions the temperature was raised slowly to remove the acetone and the solution was finally heated at 75°-80° for 1 hour. A brown solid separated along with some sodium sulphate. The whole was filtered hot; if filtration was rapid the solid remained hard and brittle; washing with boiling water served to remove/

the bulk of the sodium sulphate from the filter.

The total of the available acetate was thus methylated in batches of 30 g., twice. Then two batches were united and again subjected to two methylations as described above.

The product was dried at 45°/12 mm.

Yield:- 64 g. from 110 g. hemicellulose acetate.

## FRACTIONATION OF THE METHYLATED PRODUCT.

The methylated hemicellulose (64 g.) was dissolved in chloroform (1500 c.c.), dried over anhydrous sodium sulphate and filtered.

To the solution, thus freed from inorganic salts, light petroleum (b.p. 60°-80°) was added with vigorous stirring. After the addition of 8.800 c.c. the solution was centrifuged and the solid fraction (A) (14.06 g.) dried at 45°/12 mm. More petroleum ether (1500 c.c.) was added to the remaining solution and the solid portion (fraction B, 15.76 g.) again removed and dried. The addition of another 1200 c.c. precipitated fraction C (9.84 g.) and further 1140 c.c. served to separate fraction D (5.62 g.); the remaining solution was taken to dryness under reduced pressure to yield fraction E (18.72 g.).

Found:- fr. A OMe 32.0%

fr. B OMe 41.05% , $[\alpha]_D^{72}$ +11.0° (c, 0.45 in chloroform)

fr. C OMe 41.05% , " + 1.5 (c, 1.2)

fr, D OMe 39.86% , " + 3.5 (c, 0.97)

fr. E OMe 38.8 % , " -2.0 (c, 0.48).

Results, similar within limits, were obtained in another two experiments on a smaller scale.

When dry, the fractions B - E were celluloid like elastic substances of varying shades of yellow; fraction A was of a dark grey colour. When precipitated with petroleum ether from a very concentrated chloroform solution and dried, they showed remarkable physical differences: B was obtained in the form of light yellow granules, C and D were nearly colourless adherent fibres, and E was obtained as an orange "resin".

Twofold remethylation on the previous lines yielded the following results:

Found :- C and D (combined) OMe 41.45%,
E OMe 38.94%.

The fractions B, CD and E were investigated in regard to their constituent units as follows :

HYDROLYSIS OF B AND FRACTIONATION IN THE HIGH VACUUM .

The methylated compound (15.31 g.) was refluxed on the water bath with 4% methyl-alcoholic hydrogen chloride (320 c.c.) for 22 hours; the cooled solution was neutralised with silver carbonate and the filtrate evaporated under reduced pressure. On attempting to distil in the high vacuum the hydrolysis appeared not to be complete and was therefore repeated with 5% methyl-alcoholic hydrogen chloride.

Yield:- 16.54 g., $[\alpha]_D^{13}$ +48.8° (c, 0.95 in methyl alcohol ).

Distillation and fractionation in the high vacuum.

The glucosides (16.54 g.) were distilled from an ordinary distilling flask to yield 15.52 g. The residue in the flask was charred. Three fractions were separated on redistillation:

The first fr., b.p. 93°-110°/0.01 mm.,
the second fr., b.p. 110°-127°/0.01 mm., and
the third fr., b.p. 127°-150°/0.01 mm.

Each of these fractions was redistilled from a flask fitted with a vacuum jacketed fractionating column with a spiral, to yield the following 8 fractions:

BI 0.36 g., b.p. 126°-141°/0.005 mm., $n_D^{12}$ 1.4492 ,
OMe 57.28%

BII 1.68 g., b.p. 141°-153°/0.005 mm., $n_D^{12}$ 1.4560

BIII 3.24 g., b.p. 153°-160°/0.005 mm., $n_D^{12}$ 1.4622 ,
OMe 50.42%

BIV 7.18 g., b.p. 150°-155°/0.01 mm., $n_D^{12}$ 1.4610

BV 1.14 g., b.p. 158°-173°/0.01 mm., $n_D^{12}$ 1.4615

B VI 0.90 g., b.p. 165°-175°/0.01 mm., $n_D^{12}$ 1.4654

B VII 0.10g.,b.p. 175°-195°/0.01 mm., $n_D^{12}$ 1.4701,
OMe 45.64%

B VIII 1.00 g., the residue $n_D^{12}$ 1.4838 .

HYDROLYSIS OF CD AND FRACTIONATION IN THE HIGH VACUUM.

The methylated compound (17.09 g., OMe 41.45%) was treated with 4% methyl-alcoholic hydrogen chloride for 30 hours as described for B,and the solution was worked up as before. The hydrolysis was repeated with 5% methyl-alcohlic hydrogen chloride for 45 hours.
Yield:- 15.49 g., $[\alpha]_D^{\eta}$ + 96.5° (c, 0.58 in methyl alcohol ).

Distillation and fractionation in the high vacuum.

The glucosides (15.49 g.) were distilled from an ordinary distilling flask to yield 13.70 g. The residue in the flask was charred. Three fractions were separated on redistillation :

The first fr., b.p. 115°-130°/0.03 mm.,
the second fr., b.p. 130°-145°/0.01 mm., and
the third fr., b.p. 145°-180°/0.01 mm.

Each of these fractions was distilled from a flask fitted with a vacuum jacketed fractionating column with a spiral, to yield the following 7 fractions:

CD I 1.20 g., b.p. 132°-145°/0.01 mm., $n_D^{17}$ 1.4538

CD II 1.79 g., b.p. 145°-155°/0.01 mm., $n_D^{17}$ 1.4580 ,
OMe 51.74%

CD III 3.64 g., b.p. 155°-165°/0.01 mm., $n_D^{17}$ 1.4598

CD IV 3.93 g., b.p. 145°-175°/0.01 mm., $n_D^{17}$ 1.4600

CD V 1.28 g., b.p. 160°-170°/0.03 mm., $n_D^{15}$ 1.4621

CD VI 0.37 g., b.p. 170°-195°/0.03 mm., $n_D^{15}$ 1.4621,
OMe 47.78%

CD VII 1.03 g., the residue $n_D^{16}$ 1.4729 .

HYDROLYSIS OF E AND FRACTIONATION IN THE HIGH VACUUM.

The methylated compound (17.14 g., OMe 38.94%) was treated three times with boiling 5% methyl-alcoholic hydrogen chloride for 45 hours and the solution was worked up as before.

Yield:- 18.47 g., $[\alpha]_D^{13}$ +94.0° ( c, 0.38 in methyl alcohol ).

Distillation and fractionation in the high vacuum.

The glucosides (18.47 g.) were distilled from an ordinary distilling flask to yield 16.66 g. The residue in the flask was charred. Three fractions were separated on redistillation :

The first fr., b.p. 130°/0.1 mm. - 118°/0.03 mm.,
the second fr., b.p. 132°/0.01 mm.- 142°/0.02 mm.,,
and the third fr. b.p. 140°/0.01 mm.- 150°/0.01 mm.

Each of these fractions was again distilled from the same type of flask used for B and CD, to yield the following 7 fractions :

| | | | |
|---|---|---|---|
| E I | 0.28 g., b.p. 130°-155°/0.03 mm., | $n_D^{14}$ | 1.4560 , |
| | | OMe | 51.61% |
| E II | 3.10 g., b.p. 150°-160°/0.03 mm., | $n_D^{14}$ | 1.4601 |
| E III | 6.68 g., b.p. 159°-167°/0.03 mm., | $n_D^{14}$ | 1.4609 , |
| | | OMe | 48.97% |
| E IV | 3.64 g., b.p. 159°-167°/0.03 mm., | $n_D^{15}$ | 1.4618 |
| E V | 1.75 g., b.p. 167°-175°/0.03 mm., | $n_D^{15}$ | 1.4639 |
| E VI | 0.20 g., b.p. 175°-195°/0.03 mm., | $n_D^{15}$ | 1.4680 , |
| | | OMe | 48.31% |
| E VII | 0.76 g., the residue | $n_D^{14}$ | 1.4828 . |

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THE STUDY OF THE MAIN (MIDDLE) FRACTIONS.

From the refractive indices it was concluded that the large middle fractions of B,CD and E (OMe in agreement with trimethyl methylglucosides) were essentially/

essentially of homogeneous character.

In many instances identical operations were carried out for fractions B, CD and E; in these cases therefore a detailed account of the procedure adopted is given for fraction B only .

COMPLETE METHYLATION, HYDROLYSIS AND ANILIDE FORMATION FROM B III .

Fraction B III (0.61 g., $n_D^{14}$ 1.4622, OMe 50.42%) was fully methylated by Purdie's method and the product (0.57 g.) distilled to give a colourless mobile oil (0.5 g.), b.p. 85°-90°/0.01 mm., $n_D^{16}$ 1.4472 , OMe 60.98%. (Calculated for $C_{11}H_{22}O_6$ , OMe 62.0% ).

Hydrolysis of the fully methylated oil.

The oil (0.50 g.) was hydrolysed with N-sulphuric acid (15 c.c.) at 100° for 7 hours. The solution was neutralised with barium carbonate and kept at 100° for 1 hour to remove any bicarbonate. The filtrate was taken to dryness at 45°/12 mm. and the resulting solid extracted exhaustively with boiling anhydrous ether, to give a viscous syrup (0.35 g.), $[\alpha]_D^{17}$ +85.1° (c, 0.36 in water ). On nucleation with a crystal of authentic tetramethyl glucose crystallisation ensued over night.

Anilide formation.

The syrup (0.24 g.) was dissolved in absolute alcohol (5 c.c.), freshly distilled aniline (0.13 c.c.) added, and the mixture boiled under reflux for two hours/

hours. On cooling crystals separated which were recrystallised twice from dry ether/petroleum ether (b.p. 40°-60°) to yield 0.03 g.
Found :- m.p. 136°-138°, not depressed on admixture with an authentic sample of tetramethyl d-glucose anilide.

C, 61.68 ; H 8.18 ; OMe 39.6 ; N 4.38% .
Calculated for $C_{16}H_{25}O_5N$ :
C, 61.71 ; H 8.09 ; OMe 39.87; N 4.49%.

COMPLETE METHYLATION, HYDROLYSIS AND ANILIDE FORMATION FROM CD VI + E II (COMBINED).

In order to establish , that the main (middle) fractions of CD and E consist of glucose as well (a result to be expected from the analysis of the "free" hemicellulose), two "representative" fractions were combined for practical reasons and subjected to the same treatment as described for fr. B III .

Fractions CD VI (0.25 g.) and E II (0.25 g.) were fully methylated by Purdie's method and the product distilled in the high vacuum to give a colourless mobile oil (0.36 g.), b.p. 100°/0.03 mm. -110°/0.03 mm., $n_D^{16}$ 1.4466 , OMe 61.0% .
(Calculated for $C_{11}H_{22}O_6$, OMe 62.0% )

Hydrolysis of the fully methylated oil.

The oil (0.36 g.) was hydrolysed with N-sulphuric acid and the mixture worked up as described for fr. B III . A viscous syrup (0.28 g.) resulted.

Anilide formation.

The syrup (0.28 g.) was dissolved in absolute alcohol (1.5 c.c.), freshly distilled aniline (0.14 c.c.) added, and the mixture boiled under reflux for two hours. On cooling crystals were obtained in good yield, which were recrystallised from dry ether/petroleum ether (b.p.40°-60°) to yield 0.04 g.; m.p. 138°, not depressed on admixture with an authentic sample of tetramethyl glucose anilide.

## HYDROLYSIS OF THE MIDDLE FRACTIONS AND INVESTIGATION OF THE RESULTING TRIMETHYL SUGARS .

### HYDROLYSIS OF FR. B IV

Fraction B IV (3.81 g.) was dissolved in N- sulphuric acid (120 c.c.); in order to remove a small amount of insoluble impurities, which were found to be present in all other fractions as well, the cold solution was treated with charcoal and filtered. The hydrolysis was then carried out at 100° for 14 hours and followed polarimetrically :

initial, $[\alpha]_D^{12}$ + 62.6° (c, 3.1 , 2 dm tube )
after 3½ hours " + 57.0°
after 9 hours " + 51.2°
after 14 hours " + 45.0° .

The solution was neutralised with barium carbonate in the presence of charcoal and kept at 100° for 1 hour, filtered, taken to dryness at 45°/12 mm. and dried thoroughly with an alcohol/benzene mixture. The solid/

solid was extracted with boiling anhydrous ether to yield 3.0 g. of a colourless viscous syrup.

This syrup, dissolved in a little anhydrous ether began to deposit crystals over-night on standing at 0°. After two weeks these were filtered off rapidly, washed with a small amount of anhydrous ether and dried over phosphoric oxide in the desiccator.

Yield of the crystalline material : 54% of the original syrup.

Behaviour of the crystalline material in 2% methyl-alcoholic hydrogen chloride.

The rotatory power of the crystals (0.016 g.), twice recrystallised from anhydrous ether, dissolved in 2% methyl-alcoholic hydrogen chloride (15 c.c.) was followed at room temperature :

initial, $[\alpha]_D^{16}$ + 75.0° (c, 0.1 , 2 dm tube )
after 2½ hours " + 60.9°
after 9 hours " + 46.8°
after 24 hours " + 37.5° (constant value).

Behaviour of the non-crystalline, syrupy portion in 2% methyl-alcoholic hydrogen chloride.

The syrup (0.094 g.) was dissolved in 2% methyl-alcoholic hydrogen chloride (15 c.c.);

initial $[\alpha]_D^{14}$ + 82.9° (c, 0.62 , 2 dm tube )
after 15 hours $[\alpha]_D^{14}$ + 46.2° (constant value ) .

The crystals, twice recrystallised, showed

$[\alpha]_D^{14}$ + 103.4° → +66.9° (equilibrium reached after 14 hours ) , (c, 0.16 in water ) .

m.p. 100° , sintering at 95° ;

mixed/

mixed m.p.: these crystals (from B IV) mixed with crystals from E III (m.p.109°) : m.p. 90° .

Behaviour of the hydrolysed fr. B IV + V (combined) in 2% methyl-alcoholic hydrogen chloride.

$[\alpha]_D^{18}$ +71.3° → +31.3° (constant value after 24 hours) (c , 0.47 ).

## HYDROLYSIS OF FR. CD III.

Fraction CD III was hydrolysed with N-sulphuric acid (as described for fr. B IV) for 18 hours.
The hydrolysis was followed polarimetrically :

| | | |
|---|---|---|
| initial, | $[\alpha]_D^{14}$ | +82.8° (c, 3.3) |
| after 4½ hours | " | +89.5° |
| after 6½ hours | " | +89.1° |
| after 10 hours | " | +80.1° |
| after 13½ hours | " | +75.3° |
| after 18 hours | " | +69.6° . |

The solution was worked up as usual. The resulting syrup, dissolved in a little dry ether began to deposit crystals over-night in the ice box. After two weeks these were filtered off rapidly, washed with a small amount of anhydrous ether and dried over phosphoric oxide in the desiccator. Yield of the crystalline material 66% of the original syrup. The crystals were recrystallised twice.

Behaviour of this crystalline precipitate in 2% methyl-alcoholic hydrogen chloride.

initial, $[\alpha]_D^{16}$ + 73.9° (c, 0.26 )

after 26 hours $[\alpha]_D^{16} + 11.5^\circ$ (constant value ).

Behaviour of the non-crystalline, syrupy portion in 2% methyl-alcoholic hydrogen chloride.

Initial, $[\alpha]_D^{14} + 73.6^\circ$ (c, 0.57)
after 2½ hours " $+54.3^\circ$
after 6½ hours " $+40.3^\circ$
after 32 hours " $+33.3^\circ$ (constant value ).

The crystals , recrystallised twice, showed :
m.p. 101° , after sintering at 95° ;
mixed m.p. of these crystals (from CD III), mixed with crystals from B IV (m.p. 100°) : m.p. 95°.

Behaviour of the hydrolysed fr. CD IV + V (combined) in 2% methyl-alcoholic hydrogen chloride.

$[\alpha]_D^{18} + 67.5^\circ \rightarrow +22.5^\circ$ (constant value after 24 hours) (c, 0.53 ).

## HYDROLYSIS OF FR. E III .

Fraction E III was hydrolysed with N-sulphuric acid ( as described for fr. B IV) for 18 hours; the hydrolysis was followed polarimetrically :
initial, $[\alpha]_D^{12} + 81.9^\circ$ (c, 3.3.)
after 4½ hours " $+ 90.7^\circ$
after 10 hours " $+ 85.0^\circ$
after 18 hours " $+ 71.3^\circ$ .

The solution was worked up as usual. On taking the ethereal solution of the syrup to dryness it crystallised at once. Yield of the crystalline precipitate nearly/

nearly 100% of the original syrup. The crystals were recrystallised twice from anhydrous ether..

Observation of the inversion of sign in 2% methyl-alcoholic hydrogen chloride .

Initial, $[\alpha]_D^{14}$ + 66.0° (c, 0.16 , 2 dm tube )
after 2 hours + 36.0°
after 6 hours -15.0°
after 26 hours -24.0° (constant value ).

The crystals showed : $[\alpha]_D^{13}$ + 101.6° → +71.0° (equilibrium after 9 hours ) (c, 0.7 in water , 2 dm tube ); m.p. 109°, not depressed on admixture with an authentic sample of 2:3:6- trimethyl d-glucose .
On admixture with crystals from CD III (m.p. 101°) m.p. 95° .

Behaviour of the hydrolysed fr. E III+IV (combined) in 2% methyl-alcoholic hydrogen chloride.

$[\alpha]_D^{18}$ 99.3° → +18.7° (constant value after 24 hours), (c, 0.53 ) .

Behaviour of authentic 2:3:6-trimethyl d-glucose in 2% methyl-alcoholic hydrogen chloride.

$[\alpha]_D^{18}$ + 65.6° → -36.4° (constant value after 24 hours) (c, 0.40 ) .

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## THE INVESTIGATION OF THE MAIN (MIDDLE) FRACTIONS IN RESPECT OF THE POSITION $C_6$.

The method of Oldham and Rutherford (58) was employed: the tosyl derivatives were formed from the trimethyl methylglucosides; a tosyl group in position $C_6$, but not in other positions, can be replaced after suitable treatment by an iodine atom, which is finally estimated as silver iodide. Therefore, if position $C_6$ is occupied by an OMe group no silver iodide is obtained.

### FRACTION B IV .

### Preparation of monotosyl-trimethyl methylglucosides from the mixture of trimethyl methylglucosides B IV.

Fraction B IV (1.007 g.) - after purification by dissolving in water, treating with charcoal, filtering and taking to dryness- was dissolved in pyridine (1.25 c.c.) and p-toluene sulphonyl chloride (1.2 g.) was added. After 48 hours at room temperature water was added and the solution extracted with benzene. The benzene solution was thoroughly washed with dilute hydrochloric acid, followed by aqueous sodium bicarbonate and water and finally dried over anhydrous sodium sulphate.

Yield :- 1.4 g. of a light yellow syrup.

### Attempted preparation of a 6-iodo derivative.

The above syrup (0.980 g.), dissolved in

dry/

dry acetone (10 c.c.) was heated in a sealed tube with anhydrous sodium iodide (1.0 g.) at 100° for 2 hours. The contents of the tube were filtered and the precipitated sodium salt of p-toluene sulphonic acid was well washed with dry acetone.
Yield of the precipitate: 0.064 g., indicating that 13.1% of the starting material had an unsubstituted position $C_6$.
(Calculated yield from 0.980 g. of 2:3:4-trimethyl-6-tosyl methylglucoside is 0.487 g. sodium p-toluene sulphonate)

The acetone solution was taken to dryness under reduced pressure, the solid taken up in a mixture of chloroform and water and excess iodine removed with sodium thiosulphate; after thorough extraction of the aqueous layer the chloroform solution was dried over anhydrous sodium sulphate. Evaporation of the solvent yielded a light yellow syrup.
Yield:- 0.920 g.

Substitution of the iodine atom in position $C_6$ by the nitro group.

To the above syrup (0.6071 g.) were added methylcyanide (10 c.c.) and silver nitrate (0.61 g.) and the solution was boiled under reflux for 2 hours. The solvent was then completely removed at 100°/12 mm. and the solid repeatedly extracted with boiling benzene; by careful decantation through a filter, the loss of inorganic material could be avoided. The mixture of silver salts was dried at 100° and then treated with a/

a boiling mixture of fuming nitric acid/ water 1:1. Water was then added up to the volume of about 400 c.c. and after vigorous boiling for 30 minutes the liquid was filtered and the silver iodide collected on a Gooch filter, washed and dried.

Yield:- 0.0514 g. silver iodide, indicating that 12.4% of the starting material had an unsubstituted position $C_6$.

(Calculated yield from 0.6071 g. of 2:3:4-trimethyl-6-iodo methylglucoside : 0.4123 g. silver iodide.)

A parallel experiment gave a result in agreement with this figure.

## FRACTION CD IV

This fraction was investigated as described for B IV.

### Preparation of the tosyl derivatives.

Fraction CD IV (1.16 g.) yielded 1.24 g. of the corresponding mixture of tosyl derivatives.

### Attempted preparation of a 6-iodo derivative.

After suitable treatment the above material (1.24 g.) yielded 1.203 g. of a syrup.

### Substitution of the iodine atom at position $C_6$ by the nitro group.

The above syrup (1.203 g.) yielded after suitable treatment 0.0718 g. silver iodide, indicating that 8.8% of the starting material had an unsubstituted position $C_6$.

(Calculated/

(Calculated yield from 1.203 g. of 2:3:4-trimethyl-6-iodo methylglucoside : 0.8170 g. silver iodide.)

## FRACTION E IV

This fraction also was investigated as described for B IV.

Preparation of the tosyl derivatives.

Fraction E IV (1.02 g.) yielded 1.17 g. of the corresponding mixture of tosyl derivatives.

Attempted preparation of a 6-iodo derivative.

After suitable treatment the above material (1.17 g.) yielded 1.030 g. of a syrup.

Substitution of the iodine atom at position $C_6$ by the nitro group.

The above syrup (1.030 g.) yielded after suitable treatment 0.0391 g. silver iodide, indicating that 5.6% of the starting material had an unsubstituted position $C_6$.
(Calculated yield from 1.030 g. of 2:3:4-trimethyl-6-iodo methyl glucoside : 0.6996 g. silver iodide.)

## CONTROL EXPERIMENT.

3-tosyl-2:4:6 trimethyl methylgalactoside (0.1270 g.) was subjected to the treatment as described for fr. B IV under the heading "Attempted preparation of a 6-iodo derivative". No sodium p-toluene sulphonate was obtained and after suitable treatment as described for fr. B IV under the heading "Substitution of the/

the iodine atom at position $C_6$ by the nitro group" no silver iodide precipitate could be obtained.

## THE INVESTIGATION OF THE MAIN (MIDDLE) FRACTIONS IN RESPECT OF POSITION $C_2$.

The hydrolysed fractions were oxidised to the corresponding mixtures of trimethyl gluconic acids and these converted into lactones. A few minutes after distillation these were treated with methyl-alcoholic ammonia; thus it could be expected that only a negligible amount of ammonium salts would be formed, since a reversion to an acid/lactone equilibrium had been prevented. With the corresponding amides the Weerman reaction was carried out in a standardised manner and from the yields of hydrazodicarbonamide information could be gained as to the amount of trimethyl hexoses, with an unsubstituted position $C_2$, present in the starting material.

### FRACTION B III

Preparation of the corresponding mixture of lactones.

The hydrolysed fr. B III (0.743 g.) was dissolved in water (3 c.c.) and treated with liquid bromine (1 c.c.) at roomtemperature for 48 hours. After this period the solution was non-reducing to Fehling's solution . The excess of bromine was removed by aeration , the solution neutralised with silver carbonate/

carbonate and the silver ions removed with hydrogen sulphide. The excess of hydrogen sulphide was removed by aeration and the solution finally concentrated to a syrup. It was exhaustively extracted with ether, filtered and the solvent evaporated. The syrup was heated for two hours at 100°/15 mm. to effect lactonisation; it distilled in the high vacuum between 128° - 170°/0.03 mm. to give a colourless syrup (0.538 g.), $n_D^{17}$ 1.4682, OMe 42.3% .
(Calculated for $C_9H_{16}O_6$ OMe 42.3% )

Hydrolysis of the mixture of lactones from fr. B III .

0.1086 g. were dissolved in water (10 c.c.) and the hydrolysis at room temperature was followed polarimetrically:

| | | |
|---|---|---|
| after 10 minutes | $[\alpha]_D^{16}$ | + 57.0° (c, 1.0 ) |
| after 30 minutes | " | + 54.3° |
| after 6 hours | " | + 47.8° |
| after 22 hours | " | + 45.1° |
| after 10 days | " | + 39.5° (constant value). |

Titration of the mixture of lactones with N/20 sodium hydroxide.

2 c.c. of the above solution were titrated 15 minutes after solution with 0.0468 N-sodium hydroxide against phenophthalein. After the addition of 0.73 c.c. the red colour would fade only on gently heating.
A total of 2.3 c.c. 0.0468 N-sodium hydroxide was required for neutralisation.
(Calculated for $C_9H_{16}O_6$ : 2.16 c.c. are required ).

Preparation of the corresponding mixture of amides.

The syrupy mixture of lactones (0.4 g.) was treated with dry concentrated methyl-alcoholic ammonia (5 c.c.) at 0° for 2 days. The solvent was then removed under reduced pressure to yield a syrup, $[\alpha]_D^{19}$ +53.8° (c, 0.2 in methyl alcohol ).

Weerman tests (control experiments).

(a) d-Gluconamide (0.2037 g.) was dissolved in water (5 c.c.), sodium hypochlorite solution (3.5 c.c.) added and the mixture was kept at 0° for 3 hours. The excess of hypochlorite was destroyed with sodium thiosulphate , anhydrous sodium acetate ( 2 g.) added and after dissolution semicarbazide hydrochloride (0.4 g.) was added to the filtered solution. After 12 hours the precipitate was collected quantitatively on a filter, washed with water (2 c.c.) and dried over phosphoric oxide.

Yield:- 0.0881 g. hydrazodicarbonamide (m.p. 256°) i.e. 71.4% of the theory.

(Calculated yield from 0.2037 g. gluconamide is 0.1232 g. hydrazodicarbonamide )

(b) 0.1989 g. gluconamide yielded under the same conditions 0.0863 g. hydrazodicarbonamide i.e. 71.7% of the theory.

(The low solubility of hydrazodicarbonamide was confirmed: the above precipitate (0.0863 g.) was suspended in 5 c.c. of water, filtered and dried. Yield :- 0.0815 g. hydrazodicarbonamide; loss 6% ).

Weerman /

Weerman tests with the mixture of trimethyl gluconamides from fr. B III.

(a) 0.1276 g. yielded under the conditions described above 0.0252 g. hydrazodicarbonamide (m.p. 256°). Calculation : practical yield to be expected according to the control experiments from 0.1276 g. 3:4:6-trimethyl gluconamide is 0.0452 g. hydrazodicarbonamide (i.e. 71.4% of the theory ); therefore a yield of 0.0252 g., amounting to 55.7% of 0.0452 is taken to indicate that 55.7% of fr. B III has an unsubstituted position $C_2$.

(b) 0.2082 g. yielded under the same conditions 0.0373 g. hydrazodicarbonamide. Since the practical yield to be expected from 0.2082 g. 3:4:6-trimethyl gluconamide is 0.0739 g. hydrazodicarbonamide, the result is taken to indicate that 50.4% of fr. B III has an unsubstituted position $C_2$.

## FRACTION CD IV + V (COMBINED)

Preparation of the corresponding mixture of lactones.

The hydrolysed fr. CD IV + V (combined) (1.34 g.) was oxidised with bromine and thereafter treated as described for fr. B III. The resulting syrup was distilled in the high vacuum, b.p. 120°-175°/0.03 mm. Yield : 0.95 g. of a colourless syrup $n_D^{16}$ 1.4580 OMe 39.8% .

(Calculated for $C_9H_{16}O_6$ , OMe 42.3% )

Hydrolysis of the mixture of lactones.

0.1326 g./

0.1326 g. were dissolved in 10 c.c. water and the hydrolysis was followed polarimetrically:

| | | |
|---|---|---|
| after 7 minutes | $[\alpha]_D^{20}$ | + 66.3° (c, 1.36 in water) |
| after 45 minutes | " | + 61.8° |
| after 4 hours | " | + 49.7° |
| after 8 hours | " | + 46.0° |
| after 2 days | " | + 43.7° |
| after 17 days | " | + 37.7° (constant value). |

Titration of the mixture of lactones with N/20 sodium hydroxide.

2 c.c. of the above solution were titrated (10 minutes after solution ) against phenolphthalein. After the addition of 1.25 c.c. 0.0468 N-sodium hydroxide neutralisation was slow. After gentle heating a total of 2.92 c.c. 0.0468 N-sodium hydroxide was required.

(Calculated for $C_9H_{16}O_6$ : 2.58 c.c. are required )

Preparation of the corresponding mixture of amides.

After suitable treatment of the syrupy mixture of lactones (0.6 g.), as described for fr. B III a syrup was obtained; $[\alpha]_D^{19}$ +45.0°(c, 0.4 in methyl alcohol ).

Weerman tests with the mixture of trimethyl gluconamides from fr. CD IV + V (combined ).

(a) 0.2055 g. yielded under the conditions described for gluconamide 0.0356 g. hydrazodicarbonamide (m.p. 256°) i.e. 48.8% of the practical yield which is to be expected from 0.2055 g. 3:4:6-trimethyl gluconamide, which is 0.0729 g. hydrazodicarbonamide (i.e. 71.4% /

71.4% of the theory). This result is therefore taken to indicate that 48.8% of fr. CD IV + V has an unsubstituted position $C_2$.

(b) 0.2686 g. yielded under the same conditions 0.0498 g. hydrazodicarbonamide. The practical yield to be expected from 0.2686 g. 3:4:6-trimethyl gluconamide is 0.0953 g. hydrazodicarbonamide. This result is therefore taken to indicate that 52.2% of fr. CD IV + V has an unsubstituted position $C_2$.

## FRACTION E III + IV (COMBINED).

Preparation of the corresponding mixture of lactones.

The hydrolysed fr. E III + IV (combined) (1.62 g.) was oxidised with bromine and treated as described for fr. B III. The resulting syrup was distilled in the high vacuum: b.p. 110°-160°/0.07 mm. Yield :- 1.28 g., $n_D^{16}$ 1.4618 , OMe 40.5% (Calculated for $C_9H_{16}O_6$, OMe 42.3% ).

Hydrolysis of the mixture of lactones.

0.1341 g. were dissolved in water (10 c.c.)

| | | |
|---|---|---|
| after 4 minutes | $[\alpha]_D^{20}$ | +66.3° (c, 1.34 ) |
| after 30 minutes | " | +62.6° |
| after 2 hours | " | +55.9° |
| after 8 hours | " | +49.8° |
| after 2 days | " | +46.2° |
| after 17 days | " | +38.7° (constant value). |

Titration of the mixture of lactones with N/20 sodium hydroxide.

2 c.c./

2 c.c. of the above solution were titrated (15 minutes after solution ). After the addition of 1.25 c.c. the red colour would fade only after gentle heating. A total of 2.85 c.c. 0.0468 N-sodium hydroxide was required for neutralisation.
(Calculated for $C_9H_{16}O_6$ : 2.61 c.c. are required )

Preparation of the corresponding mixture of amides.

After suitable treatment of the syrupy mixture of lactones (1.20 g.) as described for fr. B III, a syrup was obtained; $[\alpha]_D^{19}$ +45.0° (c, 0.6 in methyl alcohol ).

Weerman tests with the mixture of trimethyl gluconamides from fr. E III + IV (combined ).

(a) 0.2349 g. yielded under the conditions described for gluconamide 0.0298 g. hydrazodicarbonamide (m.p. 256°), i.e. 35.7% of the practical yield to be expected from 0.2349 g. 3:4:6-trimethyl gluconamide, which is 0.0833 g. hydrazodicarbonamide (71.4% of the theory) . This result is therefore taken to indicate that 35.7% of fr. CD III + IV has an unsubstituted position $C_2$.

(b) 0.2314 g. yielded under the same conditions 0.0305 g. hydrazodicarbonamide. The practical yield to be expected from 0.2314 g. 3:4:6-trimethyl gluconamide is 0.0821 g. hydrazodicarbonamide. This result is therefore taken to indicate that 37.1% of fr. E III + IV has an unsubstituted position $C_2$ .

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## THE STUDY OF FRACTION BI .

Refractionation in the high vacuum.

Fraction B I (0.36 g., OMe 57.28%, $n_D^{12}$ 1.4492) was divided into two fractions by distillation :

fr. B I(a) 0.11 g., b.p. 85°-88°/0.04 mm., $n_D^{16}$ 1.4470, $[\alpha]_D^{n}$ 62.7°(c, 0.32 in water)

fr. B I(b) 0.15 g., b.p. 95°-100°/0.04 mm., $n_D^{16}$ 1.4481 , OMe 59.15% .

(Calculated for a tetramethyl methylhexoside, OMe 62.0%).

Hydrolysis of fr. B I(a) .

Fraction B I(a) (0.11 g.) was hydrolysed with N-sulphuric acid at 100° for 7 hours. The solution was neutralised and worked up as usual.

Yield:- 0.07 g.

Nucleation of this syrup was unsuccessful with tetramethyl d-glucose as well as with tetramethyl d- galactose.

Hydrolysis of fr. B I(b).

Fraction B I(b) (0.15 g.) was hydrolysed as described for fr. B I(a).

Yield:- 0.07 g.

Nucleation of this syrup with tetramethyl d-glucose was unsuccessful.

Anilide formation from the hydrolysed fr, B I(a).

To the syrup (0.07 g.) in absolute alcohol (2 c.c.) freshly distilled aniline (0.05 c.c.) was added and the solution refluxed for two hours. On cooling /

cooling long needles separated over-night, which were recrystallised twice from a mixture of dry ether/petroleum ether (b.p. 40°-60°) .

Found :- m.p. 198°-200°

Admixed with an authentic sample of tetramethyl d-galactose anilide (m.p. 198°), mixed m.p. 193° .

Admixed with an authentic sample of tetramethyl l-galactose anilide (m.p. 193°), mixed m.p. 193°.

Anilide formation from the hydrolysed fr. B I(b).

The syrup (0.07 g.) was treated as described above.

Yield :- 0.03 g. of the crude anilide.

The melting point was taken of a sample which had not been recrystallised but had only been washed with a little absolute alcohol; m.p. 196° ,

$[\alpha]_D^{16}$ 0° (c, 0.1 in acetone ).

Found :- C, 61.52 ; H, 7.88%

Calculated for tetramethyl hexose anilide

C, 61.71 ; H, 8.09% .

## THE STUDY OF FRACTION CD I.

Refractionation in the high vacuum.

Fraction CD I (1.20 g., $n_D^{17}$ 1.4538 ) was divided into two fractions by distillation from a vacuum jacketed flask :

fr. CD I(a) 0.44 g., b.p. 142°-146°/0.02 mm., $n_D^{15}$ 1.4558 , OMe 50.95% , and

fr. CD I(b) , the residue.

Refractionation of fr. CD I(a) in the high vacuum.

Fraction CD I(a) was distilled from an ordinary distilling flask; the first two drops : b.p. 92°/0.02 mm. $n_D^{14}$ v1.4577 , OMe 49.5% . (Calculated for trimethyl methylglucoside OMe 52.54%).

It was therefore concluded, that no tetramethyl glucoside was present in fr. CD I .

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In order to ascertain, whether a part of fr. CD I was composed of an ester, all sub-fractions of CD I were again united .

Attempted hydrolysis.

The reunited fr. CD I (0.159 g.) was treated with 0.087 N-potassium hydroxide (39.08 c.c.) at 100° for 2½ hours. After that period the solution was titrated with standard N/10 sulphuric acid, using methyl orange as indicator.

Found :- 33.70 c.c. of 0.1 N-sulphuric acid were required for neutralisation.

Potassium hydroxide had been employed, equivalent to 33.99 c.c. of 0.1 N-sulphuric acid.

The difference : 0.29 c.c. 0.1 N-potassium hydroxide had been used up.

It was concluded that no ester was present in fr. CD I .

## D I S C U S S I O N .

Following the method for the isolation and purification of the hemicelluloses from Iceland Moss layed down in Buston and Chamber's paper (46), a mixture of polysaccharides was obtained in the form of nearly white powders. The non-homogeneous character of this material could be shown by the fractionation of the purified hemicelluloses by means of the graded addition of alcohol to the alkaline solution. Thus at least two fractions of different uronic acid content could be separated ("hemicellulose I" , 4.95% uronic acid and"hemicellulose II" , 8.54% uronic acid). Similarly the acetate and methyl derivatives could be divided into numerous fractions. An analysis of the arbitrarily separated fraction termed "hemicellulose I", amounting to 89% of the total hemicelluloses isolated, showed a surprising difference from Buston and Chamber's findings as regards the participating units. In our sample galactose and mannose were found to account for not more than 12-18% of the hexose units, the rest (82-88%) being glucose. Pentoses were shown to be absent. The above mentioned authors claimed their hemicellulose from Iceland Moss to be made up from mannose and galactose units only, in addition to galacturonic acid. Reference has already been made to the various influences, which may be responsible for a differing composition of polysaccharides of botanically identical origin. As regards the reliability/

bility of our analysis it emerges from the numerous control experiments, that the accuracy of the estimation of mannose is not less than say 2-3%, while the estimation of galactose is probably slightly less accurate. Glucose was estimated as osazone by comparing the results obtained on artificial mixtures of similar composition, a procedure admittedly only applicable to a case where glucose is present in such overwhelming proportions.

The uronic acid component was definitely shown not to contain galacturonic acid ( by the unsuccessful attempt to obtain mucic acid ) and the conclusion was drawn from the experimental data, that it is probably d-glucuronic acid. In addition, hydrolysis with sulphuric acid yielded an insoluble dark body (amounting to 6-16% of the starting material according to the strength of the acid). As mentioned briefly in the introduction, such substances are often encountered during the acid hydrolysis of certain polysaccharides.

In order to obtain information as to the type of linkage of the participating units in the mixture of the hemicellulose polysaccharides ("hemicellulose I"), the classical method of complete methylation followed by hydrolysis and the attempted separation of the fragments was employed. Direct methylation having proved unsuccessful, complete methylation of the acetate was found to be easily effected under the described experimental conditions.

By/

By means of the graded addition of light petroleum ether to the chloroform solution five fractions (A - E) of the methylated compound, showing different rotatory power, were separated arbitrarily. Fraction (A) contained a considerable amount of products of low OMe content and was not investigated any further. Remethylation of fractions CD (combined) and E caused only a slight increase of their OMe content. It was only after long and repeated treatment with 5% methyl-alcoholic hydrogen chloride that complete hydrolysis of the fully methylated fractions (OMe 41%) B, CD, and E could be achieved. The absence of any acid derivatives after careful fractionation of the resulting products in the high vacuum showed, that during this relatively drastic treatment uronic acid derivatives had been destroyed. Similar observations have been made previously (65, 66). From the OMe contents and refractive indices certain conclusions could be drawn. The large middle fractions (trimethyl methylglucosides) from B, amounting to something like 85% of the distilled glucosides of this fraction were considered to be essentially of homogeneous character; and so were those (85%) of CD and those (96%) of E. By the isolation of 2:3:4:6-tetramethyl glucose anilide from the fully methylated middle fractions it could be shown in all three instances that glucopyranose was the repeating unit, a result in agreement with the analysis of the "free" hemicelluloses. Hydrolysis of such "representative" fractions/

fractions yielded in the case of B and CD a syrup which crystallised partly. The melting points and the behaviour of the crystalline material in 2% methyl-alcoholic hydrogen chloride suggested however, that it consisted of a mixture of trimethyl glucoses in which 2:3:6-trimethyl glucose was present. The latter is the only one of the 4 possible trimethyl-d-glucose varieties having an unmethylated position $C_4$; thus in 2% methyl-alcoholic hydrogen chloride transformation to the furanoside, indicated by a downward trend of rotation value, can take place (59). Recrystallisation of the crystals obtained on hydrolysis of a middle fraction from E yielded pure 2:3:6-trimethyl-d-glucose, the identity of which was confirmed by a mixed melting point determination.

In order to estimate the percentage composition of the main (middle) fractions the following methods were employed:

The amount of 2:3:4-trimethyl methylglucoside, being the only trimethyl methylglucoside with a free position $C_6$, was estimated by the method of Oldham and Rutherford (58). By forming the p-toluene sulphonate of the mixed methylglucosides and treating the mixture with sodium iodide in acetone, the p-toluene sulphonyl group at $C_6$ is replaced by iodine, which is ultimately estimated as silver iodide. This method must be considered an accurate one, having been applied, for instance, to the estimation of the amount of 2:3-dimethyl glucose present in the hydrolysis mixture of a fully methylated starch.(60).

An/

An approximation to the content of 3:4:6-trimethyl glucose, the only trimethyl glucose variety with an unmethylated position $C_2$, was arrived at by means of the Weerman reaction. Fairly accurate conclusions from the yields of hydrazodicarbonamide can however only be drawn under the condition, that the amide does not contain appreciable amounts of the corresponding ammonium salts; precautions were therefore taken to prevent as far as possible the establishment of a lactone/acid equilibrium after the distillation of the lactones. It may be noted, that Barker and Hirst (59), who employed this method for a quantitative estimation, considered a yield of 40% of the theory of hydrazodicarbonamide, obtainable from an hexonamide derivative with a free position $C_2$, to be the standard practical yield, while the results of our control experiments with gluconamide indicate it to be in the region of 71.4% of the theory. The hydrolysis of the lactones in water had been followed polarimetrically. In every case a quick downward change within 6 hours was followed by a slow downward change extending over several days, confirming the presence of a $\gamma$-lactone; this is in conformity with the view that 2:3:6-trimethyl methylglucoside was present in each of the fractions investigated.

The amount of 2:3:6-trimethyl glucose in the hydrolysed fractions was calculated approximately from the magnitude of the rotational change of the trimethyl glucose mixture in 2% methyl-alcoholic hydrogen chloride, by/

by comparing it with the behaviour of an authentic sample of 2:3:6 trimethyl-d-glucose (59). Example: The magnitude of the rotational change of 2:3:6-trimethyl glucose was found to be 102° (page 102)(from $[\alpha]_D^{18}$ 65.6° to → -36.4° ). Now, the equilibrium value of the hydrolysed fraction E III+IV in 2% methyl-alcoholic hydrogen chloride was found to be 18.7°, that is 55.1° above the equilibrium value of the 2:3:6 variety. If we assume that approximately every degree above -36.4° represents 1% of the mixture to be composed of a trimethyl glucose other than 2:3:6-trimethyl glucose, this result can be taken to indicate that 44.9% of fr. E III+IV is composed of 2:3:6-trimethyl glucose. This procedure is only justified under the assumption that the rotational equilibrium is indeed proportional to the %age composition of the sugar mixture. To confirm this view it would be necessary to observe the rotational change of artificial mixtures of the trimethyl sugars concerned, which unfortunately were not at our disposal. Furthermore, the equilibrium rotations in 2% methyl-alcoholic hydrogen chloride of 2:3:4- and 3:4:6-trimethyl d-glucose, which have not been recorded yet, need to be known. The equilibrium value of 2:4:6-trimethyl d-glucose has been recorded as $[\alpha]_D^{20}$ +68.4° (61). In our calculation it has been assumed, that the equilibrium value of the above mentioned two trimethyl glucose varieties is of the same order.

It follows from these considerations that the amount of 2:3:4-trimethyl glucose could be estimated accurately/

accurately, that the accuracy of the estimation of 3:4:6-trimethyl glucose was probably within several percent and that the estimated amount of 2:3:6-trimethyl glucose represents an approximation. Further investigations therefore, may cause minor alterations of the figures, alterations however, which will not affect the general picture of the composition of the main fractions arrived at in this investigation. The results are tabulated below:

| trimethyl glucose variety | in middle fractions of B | | CD | | E | |
|---|---|---|---|---|---|---|
| | (fr. B IV (page 104 ) | | (fr. CD IV) (page 105 ) | | (fr. E IV (page 106 ) | |
| 2:3:4 | 13% | | 8.8% | | 5.6% | |
| | ( fr. B III) (page 110 ) | | ( fr. CD IV+V) (page 112 ) | | (fr. E III+IV) (page 113 ) | |
| 3:4:6 | 50.4% | 55.7% | 48.8% | 52.2% | 35.7% | 37.1% |
| | meanvalue 53.0% | | 50.5% | | 36.4% | |
| | (fr. B II+IV) (page 100 ) | | (fr. CD IV+V ) (page 101 ) | | (fr. E III+IV) (page 102 ) | |
| 2:3:6 | 32.3% | | 41.1% | | 44.9% | |
| total: | 98.3% | | 100.4% | | 86.9% | |

The conclusion was reached that the hydrolysed middle fractions from B consisted of glucopyranose units linked through positions 1:6 (13%), 1:2 (53%) and 1:4 (32.3% )/

(32%) , while the corresponding figures for CD are :- 1:6 (8.8%), 1:2 (50.5%) and 1:4 (41.1%) and for E :- 1:6 (5.6%), 1:2 (36.4%) and 1:4 (44.9%). No proof for the absence of 2:4:6-trimethyl glucose could be given, but it is unlikely that this variety is present to any considerable extent.

In addition, evidence of the presence of a tetramethyl dl-galactose "endgroup" to the extent of about 2.5% of the B-fractions could be presented,(but not in CD and E ). This was shown by hydrolysis and anilide formation from fr. B I . On admixture with tetramethyl d-galactose anilide a small depression of the melting point was observed and none on admixture with an authentic sample of tetramethyl l-galactose anilde; the anilide was found to be optically inactive. This observation is very unusual and seems to show that the terminal groups in some instances are composed of d-galactopyranose units and in others of the l-form , presumably in equal proportions. Another recorded observation of the isolation of dl-galactose derivatives is the isolation of heptaacetyl dl- galactose from agar by Pirie (64). This does not involve endgroups however, the l-galactose being derived from the 3:6-anhydro-l-galactose units of the molecule.

If no other endgroup is present, this result corresponds to an average chain length of ca. 50 units for B (cf. 80 for lichenin )(19).

B, CD and E yielded also a small amount of lower methylated glucosides (Ome approximating to dimethyl/

dimethyl methylglycosides), amounting to not more than 8-10% of those fractions; they have not been investigated.

From the non-reducing character of the mixture of polysaccharides and from the negative rotation of the acetyl derivatives and the negative (or small positive ) rotation of the methyl derivatives, it would seem that there is a preponderance of $\beta$ -linkages in the molecules, since they are chiefly composed of d-glucose and carbon 1 must be concerned with the linkages in every case.

The results can be interpreted in at least two different ways. Firstly, "hemicelluloseI" could be a mixture of at least three polysaccharides, which differ mainly by reason of the different linkages between the glucose residues (1:4 , 1:2 , 1:6 ). Secondly a mixture of polysaccharides may be present, in each of which a mixture of linkages appears. The latter possibility is thought to be the most likely one, but in the present state of our knowledge it is impossible to decide definitely between them.

Hitherto the linkage of glucose units in a glucose-composed polysaccharide has been found to be uniform; the prevalent 1:4-type of linkage in starch, cellulose, glycogen and lichenin, the 1:3-type in laminarin (61) and the 1:6-type in the dextran produced by Leuconostoc Dextranicum from sucrose (63), for instance. Indeed in the early days of polysaccharide chemistry starch and cellulose were considered/

sidered to be typical examples of the polysaccharide structure in general. Later it has been shown on pectic substances, gums and mucilages etc., that most polysaccharides isolated consisted of two or more types of units linked to each other in different ways. A mannan isolated from yeast( 62 ) provides an example of a polysaccharide, which is made up of one type of unit only (mannose ) linked in three different ways (1:2 , 1:3 and 1:6 ). Thus in the light of modern research our results are perhaps not surprising, although it is the first time, that the isolation of a mixture of predominantly glucose containing polysaccharides with at least three different types of linkages of the participating units has been recorded.

## SUMMARY.

1. A mixture of hemicellulose polysaccharides was obtained by extracting Iceland Moss with 4% alkali followed by purification by the copper method.

2. At least two fractions of different uronic acid content could be separated ("hemicellulose I", 89% of the total, containing 5% uronic acid and "hemicellulose II", 11% of the total containing 8.5% uronic acid.

3. Hydrolysis of "hemicellulose I" with 3.7% sulphuric acid yielded an insoluble residue (16%), a syrup (73%) and the barium salts of complex aldobionic acids (11%).

4. Analysis of th syrup showed : 7.6% galactose, 3% mannose and 89.4% glucose.

5. Hydrolysis of "hemicellulose I" with 15% sulphuric acid yielded an insduble residue (6%) and a syrup; on pouring the latter into absolute alcohol a precipitate (13% of the starting material) was obtained which contained probably barium d-glucuronate.

6. Methylation of "hemicellulose I"-acetate followed by fractionation with petroleum ether yielded five fractions; B - E (78% of the methylated material) of a methoxy content between 39 and 41%, while one fraction, A (22%) contained partly methylated products.

7./

7. Hydrolysis and fractionation of the fractions B (24.5% of the methylated products), CD (24%) and E (29%) yielded 7-8 fractions each of a mixture of methylglucosides.

8. About 85% of the B-fractions, 94% of the CD-fractions and 96% of the E-fractions were mixtures of trimethyl methylglucosides and the large middle fractions were considered to be essentially homogeneous. No acid derivatives were found in any of the distilled fractions.

9. By obtaining tetramethyl glucose anilide from a fully methylated "representative" middle fraction it was shown for B, CD and E that d-glucopyranose was the repeating unit.

10. Hydrolysis of such "representative" fractions yielded mixtures of partly crystalline trimethyl glucoses in which 2:3:6-trimethyl glucose was present.

11. (a) The amount of 2:3:4-trimethyl glucose present in the middle fractions of B, CD and E was estimated by forming the 6-iodo derivative from the mixture of the respective tosyl derivatives.
(b) The amount of 3:4:6-trimethyl glucose was estimated by the Weerman reaction.
(c) The amount of 2:3:6-trimethyl glucose was estimated roughly by comparing the behaviour of the hydrolysed fractions in 2% methyl-alcoholic hydrogen chloride with that of an authentic sample of 2:3:6-trimethyl glucose.

The/

The results indicated the type of linkage of the glucose units to be for those middle fractions in B 1:6 (13%), 1:2(53.0%), 1:4 (32.3%), in CD 1:6 (8.8%), 1:2(50.5%), 1:4 (41.1%) and in E 1:6 (5.6%), 1:2(36.4%), 1:4 (44.9%) .

12. By isolating tetramethyl dl-galactose anilide from fraction B I the presence of an "endgroup" could be demonstrated which, if no other endgroup is present would correspond to a chain length of 50 units for B.

13. The results are tentatively interpreted as indicating that"hemicellulose I" is probably a mixture of polysaccharides, consisting to the extent of about 85% of β-glucose units linked through positions either 1:4 or 1:2 or 1:6.

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