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by

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Title.

Studies on Sulphuric Esters of Carbohydrates.

STUDIES ON SULPHURIC ESTERS OF CARBOHYDRATES

Contents

General Introduction.

A great many polysaccharides are known to occur in nature in the form of sulphuric acid esters. To give but two classes of such compounds we have

(a) some of the gel-forming carbohydrates from various algae, particularly marine algae.

(b) many of the gluco-proteins are protein derivatives of complex substances which are essentially sulphuric acid esters.

In the former class the sulphuric acid esters is usually soluble and can be extracted from the algae by treatment with cold or hot water. In some cases extraction is carried out by cold dilute acids or by cold dilute alkali.

Only a few of these gel-forming carbohydrates have been subjected to any detailed investigations. In the few cases that have been the subject of research the results are of an extremely vague nature and rather inconclusive due to the experimental difficulties involved. A brief summary of the work on some of these compounds follows.

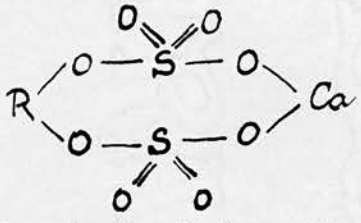
Fucoidin:- When certain marine algae such as Laminaria spp. are extracted with dilute acid two polysaccharides are obtained. We are only concerned with/

with the one named by Kylin, Fucoidin; (1) the other is alginic acid which is sulphur free.

Hoagland and Lieb (2) and Haas (3) and co-workers (6-8) have also isolated the calcium salt of acid carbohydrate sulphates from the aqueous extract of various algae.

When fucoidin solution is treated with ammonium oxalate solution a precipitate is formed which is found to be calcium oxalate. Hence the calcium present in fucoidin is ionised. On hydrolysis with dilute acid fucoidin yielded fucose and calcium sulphate. The sulphate present in fucoidin must be in organic combination since it cannot be precipitated by the addition of barium chloride. Hydrolysis, however, liberates the sulphate in the compound and by treatment of the hydrolysate with barium chloride a determination of the sulphate present in the polysaccharide can be made. On the incineration of the polysaccharide in a weighed crucible an ash is left which is found to be calcium sulphate. This ash must be treated with nitric acid to oxidise all the ash to sulphate before weighing it to calculate[1] the sulphate present in the ash. It is found that in the case of fucoidin, the ratio of sulphate, in the hydrolysed liquid to the sulphate in the ash is 2:1.

We have then that fucoidin contains sulphur in organic combination and that calcium present in fucoidin is ionised. On incineration the calcium and sulphur of the polysaccharide is left as an ash of calcium sulphate. Finally, the ratio of sulphate obtained by hydrolysis to the sulphate obtained as an ash is as 2:1. Haas (3) has concluded therefore, that these esters can be considered to be the calcium salt of esters of the type



The excess sulphate derived from the molecule on heating would escape as sulphuric acid according to the following equation:

$$2H_2O + R\cdot(SO_4)_2Ca = CaSO_4 + H_2SO_4 + R\begin{matrix}OH\\OH\end{matrix}$$

The method employed to deduce that fucoidin is an ethereal sulphate has been covered in some detail since it gives the general prodedure adopted for similar compounds considered to be sulphuric acid esters. Nelson and Cretcher (9), working on the carbohydrate ester from Macrocystis, ~~found~~ by isolating and estimating the fucose and by sulphate analysis, reached the conclusion that their preparation was best represented by a polymer of methylpentose monosulphate/

monosulphate. They give the probable composition as $(R - O - SO_2 - OH)_4$. Gulbrand, Heen and Öy (10) in a recent paper consider that fucoidin isolated from Laminaria digitata has the formula: (R R' O SO_2OMe) where R is fucose, R' is unknown and Me may be K, Na, Ca or Mg.

By the same means extracts of seaweeds in a great many cases have been shown to be carbohydrate esters of sulphuric acid. These polysaccharides in the form of ethereal sulphates possess the property of giving a viscous solution with water in the cold which, after heating, sets to a rigid gel. The most important is perhaps mucilage of carrageen (chondrus crispus).

Carrageen mucilage is obtained by the extraction of Irish Moss. It was said by Haas and Hill (11) to consist of the calcium salts of two sulphate esters of a fucoidin type which were partially separated by their different solubilities in water. Extraction by hot or cold water gave such a partial separation. Butler (12) found that the ash content of each of these sulphuric acid esters was fairly high and, besides calcium sulphate, contained an appreciable amount of potassium sulphate. The ratio of sulphate in the hydrolysed liquid to that in the ash was found to be more nearly 3:1 than the 2:1/

2:1 ratio given by a simple ester of the fucoidin type. However, since the calcium of the polysaccharide sulphuric esters is ionised, Butler was able to dialyse the compound against potassium chloride successfully, to yield the potassium salts free from calcium, and also to regenerate the calcium salts using the potassium carbohydrate sulphuric esters. By this means Butler was able to show that the assumption that the original extracts were pure calcium salts was wrong since the regenerated calcium compound, to be regarded as pure, differed greatly in analysis from the products of the extractions. The ratio of sulphate in the hydrolysis solution to that in the ash was found to be 2:1 in the case of the potassium and regenerated calcium polysaccharide sulphuric acid esters. The constitution of the pure potassium salts was deduced to be probably represented by:

$[R(OSO_2OK)_2]_n$ or $[R(OSO_2OK)_3]_n$

and the polysaccharide may occur naturally in the seaweed as a mixture of the calcium and potassium salts, and the acid.

An interesting point in the study of the mucilage of carrageen is that no conditions of hydrolysis with acid or with alkali could be found which effected the separation of the sulphate residue without

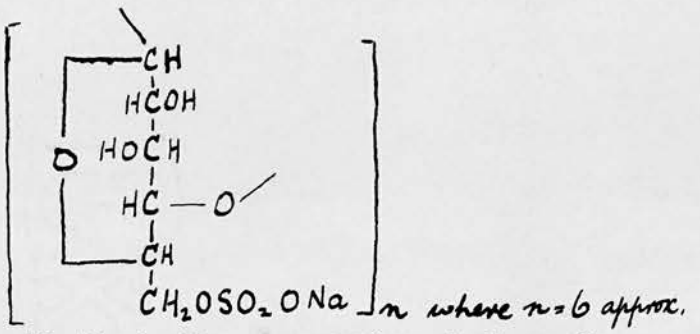
involving the complete breakdown of the carbohydrate part of the molecule.

It has been found that both galactose (11) and glucose units are present in mucilage of carrageen (12). Mucilage of carrageen finds a practical use in the thickening of certain food preparations and also as a clarifying and emulsifying agent.

Hassid has obtained an ethereal sulphate from the marine alga, Irideae laminaricdes (13 and 14). Hydrolysis yields galactose as the only sugar unit present in the carbohydrate molecule. The ash content, which is fairly high, is composed of sodium sulphate and a 2:1 ratio for sulphate in hydrolysis to sulphate in the ash indicates a simple ethereal sulphate with a formula of:

$[(C_6H_9O_4)\ OS_2ONa]_n$ Mild hydrolysis with dilute acid or baryta solution yields a soluble galactan, free from the ethereal sulphate group. Hassid, from results of methylation and acetylation of the sodium monosulphate galactan, has postulated certain positions for the linkages in the galactan. The tentative formula suggested is that of a 1:4 galactan of the normal pyranose type, the sixth carbon atom of the galactan being substituted by the ethereal sulphate group. He has also estimated the molecular weight to/

to be best suited by six of these units per molecule (14). The formula indicated by Hassid's results may be written



but it cannot be said that the experimental work justifies it.

As an example of class b (p.1), the case of the blood-anticoagulant Heparin may be cited. The extraction and purification of heparin from autolysed ox liver was described by Scott and Charles (15). The substance contained nitrogen and sulphur, formed a hydrochloride and its anticoagulation activity was destroyed by ~~the~~ dilute acids. Jorpes (16) has shown that the sulphate present is partly precipitable by barium chloride in acid solution, and is partly in organic combination. The total sulphate could be precipitated after hydrolysis with hydrochloric acid. This indicates the presence of an ethereal sulphate. By hydrolysis, Jorpes has established that heparin contains hexuronic acid, acetyl and a hexosamine, thought at first to be galactosamine. These properties suggested a chondroitin sulphuric acid structure for heparin. Jorpes however, showed that/

that heparin could not be replaced by normal chondroitin sulphuric acid as an anticoagulant. Bergström, (17) by a series of experiments, found that the sulphuric esters prepared by the action of chlorosulphonic acid on cellulose, chitine, starch, glycogen, gum arabic, pectic acid, yeast nucleic acid and chondroitin sulphuric acid all possess anticoagulating activity although only to a slight degree as compared with that of heparin. Later, Jorpes and Bergström (18) obtained glucosamine by boiling heparin with concentrated hydrochloric acid whereas, chondroitin sulphuric acid, under similar conditions, gave chondrosamine. The suggestion was advanced that heparin is not a chondroitin sulphuric acid, but a mucoitin sulphuric acid. These workers were able to isolate, not one brucine salt, but a mixture of brucine salts, which differed in their solubilities sufficiently, to separate them by fractional crystallisation. The more soluble brucine salts were separated from those less soluble and investigated. The more soluble fraction from the mixture of brucine salts as compared with the less soluble fraction showed the following properties: it contained less sulphur as determined by sulphate analysis, less anticoagulation activity and a higher rate of hydrolysis.

These workers were able to go a step further by analysing the fractions obtained, for sulphate. The less soluble brucine salt has at least a tri-sulphate content, while the more soluble fraction gives a sulphate content best accounted for by a mixture of di- and mono-sulphuric esters.

It was concluded that heparin is not a definite chemical compound but a mucoitin polysulphuric ester.

It was at first thought, that agar was an ethereal sulphate of a polysaccharide. Neuberg and Schwietzer (19) showed however, that agar could be separated by a simple extraction with cold water, into two fractions; the soluble portion (10%) contained the sulphate residues and could not be induced to form a gel, while the insoluble portion (90%) was found to be free from sulphur and to possess the same power of gelatinisation as untreated agar. The essential part of agar is therefore, the insoluble portion of Neuberg and Schwietzer and cannot be an ethereal sulphate. Neuberg and Schwietzer (19) have prepared ethereal sulphates from agar by treatment with chlorosulphonic acid. The product obtained contained bound sulphate (17.9%), and showed anticoagulation activity comparable with that of heparin. The original agar extract showed no heparin effect.

It has been noticed that the conditions for hydrolysing/

hydrolysing ethereal sulphates in order to eliminate the bound sulphate from the polysaccharide without the complete decomposition of the main portion of the molecule, show extreme variation. In the simple galactan ethereal sulphate obtained by Hassid from Iridiae laminarioides (13) the ethereal sulphate could be removed to leave the galactan, by means of very dilute acid or baryta solution. In the case of heparin the base of the compound, glucosamine, was isolated after boiling with concentrated hydrochloric acid (18). Most important of all was the behaviour of mucilage of carrageen. In this case no conditions of acid or alkali hydrolysis freed the compound of its bound sulphate, without a complete breakdown of the carbohydrate part of the molecule. It was this fact that caused the following research to be initiated, and also, that in no case can it be claimed with certainty that the position of the sulphuric acid residue in these complex carbohydrates is known, although most workers consider the primary alcoholic residue (when present) to be involved: there is no experimental evidence to justify this view. The object was to take simple carbohydrates and introduce sulphate groupings into the molecule to form ethereal sulphates and to study the action of dilute acids and alkalis/

alkalis on these sulphuric acid esters. Since glucose and galactose units are usually found in the products of hydrolysis of ethereal sulphates, these sugars were chosen as the starting material for the research. The previous researches on glucose sulphates by Ohle and Neuberg, Levene and Soda will be discussed later in this thesis.

Bibliography for General Introduction.

1.	Kylin	Z. physiol. Chem., 1915, 94, 337.
2.	Hoagland & Lieb	J. biol. Chem., 1915, 23, 287.
3.	Haas	Biochem. J., 1921, 15, 469.
4.	Russell-Wells	Biochem. J., 1922, 16, 578.
5.	Haas & Russell-Wells	Biochem. J., 1923, 17, 696.
6.	Haas & Russell-Wells	Biochem. J., 1925, 19, 1915.
7.	Haas & Russell-Wells	Biochem. J., 1929, 43, 425.
8.	Bird & Haas	Biochem. J., 1931, 25, 403.
9.	Nelson & Cretcher	J. biol. Chem., 1931, 94, 147.
10.	Gulbrand, Heen & Öy	Z. physiol. Chem., 1937, 247, 189.
11.	Haas & Hill	Ann. appl. Biol., 1921, 7, 352.
12.	Butler	Biochem. J., 1934, 28, 759.
13.	Hassid	J. Amer. Chem. Soc., 1933, 55, 4163.
14.	Hassid	J. Amer. Chem. Soc., 1935, 57, 2046.
15.	Scott & Charles	J. biol. Chem., 1933, 102, 425.
16.	Jorper	Naturwiss., 1935, 23, 196.
17.	Bergström	Z. physiol. Chem., 1936, 238, 163.
18.	Jorpes & Bergström	Z. physiol. Chem., 1936, 244, 253.
19.	Neuberg & Schwietzer	Monatshefte, 1937, 71(1), 46.

PART I

Investigation of Glucose Sulphates

Glucose Sulphates

The first work on glucose sulphates was carried out by Claësson (1) who isolated tri- and tetra-glucose sulphuric acids, but nothing was decided about the structure of these compounds. In 1921, Neuberg and Ohle (2) reported the presence of sulphur in organic combination in agar, and, although it has since been shown that this is not an essential constituent of the main carbohydrate portion (3, 4), this observation seems to have been the starting point of Ohle's work on the glucose sulphates. His first paper on the subject (5) appeared in 1922, in which he used the method previously carried out by Neuberg and Liebermann (6) of reacting on glucose in pyridine suspension at -10° with chlorosulphonic acid. He acetylated this reactant mixture to yield a tetra-acetyl glucose monosulphate (A) from which subsequently the barium salt and the brucine salt (m.p. 184° $[\alpha]_D$ -4.07 → -6.28 (12 hours)) of the monosulphate were obtained. A similar treatment of 2:3:4:6 tetra-acetyl glucose gave a tetraacetyl glucose monosulphate (B) of which the pyridine salt was different from that obtained directly from glucose. This appeared to indicate, that the sulphate residue in the first place was not present on the reducing group, although, by the action of silver sulphate on acetobromoglucose the/

the product (B) was not obtained. These results illustrate the difficulty of deciding the location of the sulphate group in (A). One of the obvious difficulties is, that in the preparation of (B) the migration of acetyl groups, which has since been well established, might play a part. T. Soda (7) has described a method of preparing both glucose and sucrose monosulphates without the isolation of the acetyl derivatives and his method was adapted for many of the preparations in this thesis. Brucine glucose monosulphate in his case had a m.p. 183° and $[\alpha]_D$, -5.6° (final value). An important paper by Ohle followed in the same year (8), the results of which it is now possible to interpret, partially at any rate, since the structure of glucose diacetone and glucose monoacetone has been proved to be, in the first case, 1:2 - 5:6 diacetone glucofuranose, and the second 1:2 monoacetone glucofuranose by Anderson, Charlton and Haworth (9). By the usual procedure, Ohle prepared diacetone glucose monosulphate, isolated as the pyridine and brucine salts, and by recrystallisation from alcohol of the pyridine salt, pyridine monoacetone glucose sulphate was obtained which he then converted into the barium salt, which crystallised with five molecules of alcohol. By acetonisation, the/

the monoacetone derivative was reconverted to the original diacetone compound. Monoacetone glucose was treated with chlorosulphonic acid to yield what is described as a 6-sulphate from which, after treatment at 37° with $\frac{N}{10}$ - sulphuric acid to remove the acetone residue, a brucine salt identical with that described by Soda (7) and prepared directly from glucose was obtained. It is, therefore, highly probable, according to Ohle, that ordinary glucose monosulphate is glucose-6-sulphate. Whilst it is clear from the evidence that it cannot be glucose-3-sulphate from the fact that its salts are different from those prepared from glucose diacetone (although it is rather remarkable that the vigorous reagent, chlorosulphonic acid, does not hydrolyse the isoprppylidene group on C_5 and C_6, since even on recrystallisation from alcohol the pyridine salt of the glucose diacetone sulphate undergoes decomposition), the choice of position 5 is still open and, if a ring change to a pyranose structure were to occur during the sulphurylation of the monoacetone glucose, position 4 is not excluded.

Levene and Meyer (10) also made diacetone glucose-3-sulphate and monoacetone glucose sulphate, using sulphuryl chloride. (These authors state that in this case the sulphate group may be located either on C_5 or C_6). The velocity of hydrolysis of these

two/

two monosulphates, were determined in sealed tubes, at 75° in $\frac{N}{10}$ - hydrochloric acid solution and shown to be a monomolecular reaction. In the first case, K = 0.0006, and in the second, K = 0.0004. No further work on velocity of hydrolysis was done until 1934, when Soda and Nagai (11) published a paper which, incidentally, was not abstracted until 1937, under the title of "A Rough Estimation of Glucose Polysulphates in their Mixture" as follows: 'Such an estimate may be made from the hydrolysis velocity coefficient' With such a title and abstract, the paper seemed unimportant to the author of this thesis. Recently, however, the paper has been secured, and after some difficulty a translation was found. It appears that Soda believes that polysulphates are formed in small quantity during the reaction of chlorosulphonic acid and glucose in pyridine, and these have a greater hydrolysis velocity constant than the monosulphate, which he describes incidentally as the 6-sulphate. The experiments were carried out at 78 and 80° (in the vapours of alcohol and benzene respectively) in $\frac{N}{10}$ -sulphuric acid, and the mean values of K, which were determined by titration (not in sealed tubes) were ca. 0.0004. It was on the idea of Levene and Meyer (10) however, that the work on hydrolysis velocity constants described in this thesis was undertaken. Soda (12) describes the preparation/

preparation of various salts of glucose monosulphate and speculates as to their structure as the result of mutarotation studies. He finds that the barium, calcium, and sodium salts mutarotate downwards, whereas the potassium and magnesium salts mutarotate upwards. The first three are therefore described as mainly α, and the last two as β forms. From the fact that potassium chloride forms no addition compound with glucose, whereas sodium chloride, barium chloride, and calcium chloride do, and assuming that these salts are coordinated to the ring oxygen atom, Soda suggests that the sulphate group is brought nearer to the molecular hydrogen atom linked to C through oxygen, in the latter case, than in the former. Soda considers that this will favour the production of the α-form. His argument is however, not very clear, since the β-form might equally well be favoured in such circumstances. An inspection of models throws little light on the sub ject since the points of repulsion and attraction due to electronic forces are difficult to predict in such a complex system. The most profitable method of deciding this question, especially since a barium atom can be introduced, would seem to be X-ray analysis which should also throw light on the location of the ethereal sulphate groups.

Preparation of crude Barium Glucose Sulphate.

To glucose (10 g.), which had been previously dried in a vacuum desiccator, suspended in pyridine, also dried beforehand with sodium hydroxide, was added, with constant stirring at -10°, a solution of chlorosulphonic acid (3.4 c.c.) in alcohol-free chloroform (25 c.c.), over a period of 20 minutes. The mixture was allowed to stand overnight, when it separated into two layers, the lower layer being syrupy. The pyridine was distilled off under reduced pressure at 40°, and the resulting syrup was dissolved in cold water. To this aqueous solution barium carbonate and lead oxide were added, and the mixture was shaken for at least 8 hours. It was then filtered and the filtrate was washed four times with ether to remove the last of the pyridine. Excess of a solution of silver sulphate was added to the aqueous solution, the excess being precipitated with sulphuretted hydrogen, the excess of which was removed by aeration. The elimination of chloride was performed in the presence of barium carbonate. The solution (ca. 2000 c.c.) was concentrated at 40°/15 mm. to 20 c.c. when, after filtration/

filtration through a layer of charcoal, it was poured into 2000 c.c. of alcohol to precipitate the barium salt of glucose sulphate $[\alpha]_D^{17}$ +45.6°, (c, 4.60 in water).

Yield 8.45 g.

The exact quantities of aqueous solution to alcohol must be found by trial; if the aqueous solution is too concentrated a syrup is formed, whereas if the solution is too dilute a sol is produced.

The amorphous barium salt was found to be reducing to Fehling's solution.

Analysis:

Found: Ba, 11.52 S(hydrol.), 4.95 S(fusion),5.07

Calc. for barium glucose monosulphate, $(C_6H_{19}O_9S)_2Ba$:

Ba, 20.95 S, 9.76%.

Brucine glucose sulphate.

The crude barium glucose sulphate was converted into brucine glucose sulphate by adding, in the cold, an aqueous solution of barium glucose sulphate (8 g.) to an aqueous solution of brucine sulphate (7 g.), and allowing the mixture to stand overnight at room temperature. The precipitated barium sulphate was filtered off, and the filtrate concentrated at 40°/15 mm. until crystallisation set in. The/

The crystals were filtered off, and further yields were obtained by further concentration. Finally, the remaining aqueous solution was treated with acetone until an opalescence was observed and the solution, on being left overnight in a refrigerator, yielded a further crop of crystals. The brucine glucose sulphate was refluxed three times with alcohol to eliminate free brucine which is always carried down during crystallisation, and the resulting residue was recrystallised from water, and was obtained in clusters of fine needles. The yield was increased by treatment with acetone as before

Yield, 9.48 g.

As obtained from water, brucine glucose sulphate was a colourless, crystalline compound, which reduced Fehling's solution, contained no barium, contained combined sulphur and gave a red coloration with nitric acid m.p. 209° (decomp.) $[\alpha]_D^{16°}$, -4.42 (after 5 mins.) c. 2.01

$[\alpha]_D^{16°}$, -5.82 (after 24 hours) in water, c. 2.01.

The percentage of sulphur in the compound was found by two methods:

(1)

(1) Hydrolysis of the compound by concentrated hydrochloric acid, followed by precipitation with barium chloride

(2) An alkaline fusion (13) to give sodium sulphate which was treated with barium chloride.

Analysis: C, 53.5 H, 5.7 N, 4.53 S(hydrolysis), 4.81 S(fusion), 5.02.

Calc. for brucine glucose monosulphate $C_{29}H_{38}O_{13}N_2S$: C, 53.2 H, 5.8 N, 4.28 S, 4.89%

Barium glucose mono-sulphate

The pure barium glucose monosulphate was prepared from pure brucine glucose monosulphate (8.5 g.) by dissolving the latter in water and adding an excess of saturated barium hydroxide solution. The excess of barium hydroxide was removed by passing carbon dioxide through the solution, whereupon the precipitated barium carbonate and brucine were filtered off immediately. Although the barium of the barium glucose sulphate is ionised, as shown by the addition of sulphuric acid to give barium sulphate, carbon dioxide does not precipitate the carbonate. The filtrate was concentrated at 40° under diminished pressure and the barium glucose sulphate was precipitated/

precipitated by pouring the aqueous solution into alcohol. The correct concentration was found as already indicated $[\alpha]_D^{14^\circ}$ + 33.4° (c, 2.36 in water)

Yield, 2.85 g.

The amorphous white solid was deliquescent. It was found to reduce Fehling's solution.

Analysis:

Found: Ba, 20.88 S(hydrol.), 9.54

Calc. for barium glucose monosulphate, $(C_6H_{11}O_9S)_2Ba$:

Ba, 20.95 S, 9.76 %

Hydrolysis Constant of Barium glucose monosulphate

The hydrolysis of barium glucose monosulphate with acid was attempted first, by heating a known weight of the sulphuric acid ester in a measured volume of standard acid, in flasks fitted with condensers protected by soda-lime tubes. The speed of the hydrolysis was followed by titrating 2 c.c. portions, taken at certain time intervals, with a dilute standard sodium hydroxide solution. The results of a typical determination are given below in Table I. The method was considered as unsatisfactory due to the fact, that the drainage factor, when the volume of the acid solution decreased, assumed

proportions/

proportions inadequately compensated for, even when a correction was applied by blank experiments. The final method adopted was to dissolve a weighed sample of the barium glucose sulphate in 100 c.c. of a solution of barium chloride in standard hydrochloric acid. 10 C.c. of this solution were introduced into tubes and these were then sealed. After a tube had been immersed in boiling water for a definite time interval, it was cooled rapidly, opened, and the deposit of barium sulphate weighed as quickly as possible. Thus, the hydrolysis could be followed by weighing the precipitated barium sulphate.

Hydrolysis Constant by Open-tube Method.

Barium glucose monosulphate (0.6056 g.) was dissolved in 0.2777 N-sulphuric acid (30 c.c.) and introduced into a flask fitted with a reflux condenser which had a soda-lime tube affixed to the open end. A similar flask which contained 0.2777 N-sulphuric acid (30 c.c.), complete with condenser, was placed at the side of the first. At the completion of the time intervals given in Table I both flasks were immersed in cold water and 2 c.c. portions extracted from each and titrated against 0.0185 N-sodium hydroxide using brom-thymol-blue indicator. The readings of the volumes of alkali

in/

in the case of the blank experiment were subtracted from the corresponding readings for the actual hydrolysis experiment and the corrected value calculated for the normality. The increase in normality was converted into the weight of sulphuric acid liberated. This gave the values of x in the table. The total value of sulphuric acid available from complete hydrolysis of the barium glucose monosulphate (0.6056 g.) gave the value of a.

The hydrolysis constant was calculated from the formula for a monomolecular reaction:

$$K = \frac{1}{t} \log \frac{a}{(a - x)}$$

where a is the total amount of sulphuric acid obtained by complete hydrolysis and x is the value for the sulphuric acid obtained by the hydrolysis for a time t.

TABLE I /

TABLE I

t	N	x	a - x	$K = \frac{1}{t} \log \frac{a}{a-x}$
30	0.0171	0.02514	0.06542	.004707
90	0.0339	0.04983	0.04073	.003855
150	0.0442	0.06497	0.02559	.003660
210	0.0459	0.06746	0.02310	.002826
270	0.0494	0.07261	0.01795	.002603
330	0.0517	0.07600	0.01456	.002405
390	0.0583	0.08570	0.00486	.003256
480	0.0611	0.0890	0.00076	.004326

N = increase in normality

a = 0.09056

Hydrolysis constant by sealed tube method

Barium glucose monosulphate (0.5232 g.) was dissolved in 0.1012 N-hydrochloric acid containing 3% of barium chloride (100 c.c. solution). 10 C.c. portions of this solution were introduced into tubes which were then sealed and heated at 100° for the time intervals in minutes indicated in Table II. Each tube at the end of its time interval was cooled as rapidly as possible and the deposit of barium sulphate weighed.

The hydrolysis constant was calculated from:

$$K = \frac{1}{t} \log \frac{a}{(a - x)}$$

where a is the weight in g. of barium sulphate that would be precipitated from 10 c.c. of the solution if the hydrolysis were complete and x is the weight in g. of barium sulphate deposited in time t.

TABLE II /

TABLE II

t	x	a - x	$K = \frac{1}{t} \log \frac{a}{a - x}$
180	0.0203	0.01699	(.001895)
240	0.0229	0.01439	(.001723)
300	0.0255	0.01179	.001666
360	0.0263	0.01099	.001474
420	0.0293	0.00799	.001593
480	0.0308	0.00649	.001619
600	0.0334	0.00389	.001636
720	0.0348	0.00249	.001632

(a = .03729)

Mean value of K_{100} = 0.001623, omitting the values given in brackets.

Alkaline Hydrolysis of Barium Glucose Monosulphate.

When barium glucose monosulphate was heated at 100° with 0.1-N carbonate-free sodium hydroxide solution, there was an almost instantaneous deposition of the total barium and sulphur content as barium sulphate. The hydrolysis to give the sulphate was accompanied by caramelisation. The discoloration due to decomposition of the barium glucose monosulphate was found to occur even at as low a temperature as 40°, when 15-20 minutes were required for the complete removal of barium and sulphur.

The results were similar when 0.1 N-baryta solution was used.

In one experiment barium glucose monosulphate (1.236 g.) was heated at 100° for five minutes with 0.1 N-sodium hydroxide (38 c.c.) which had been previously freed from carbonate. The hydrolysis was carried out in a flask fitted with a condenser, carrying a soda-lime tube. The solution was then filtered through an ashless filter paper and the contents of the paper, after being washed with hot hydrochloric acid solution, were incinerated to give an ash (0.430 g.).

Found: Ash 34.8

Calc./

Calc.: for barium glucose monosulphate $(C_6H_{11}O_9S)_2Ba$:

Ash, 35.6%

The filtrate was neutralised with acetic acid, phenyl hydrazine hydrochloride (1.29) and sodium acetate (1.6 g.) were added to this solution and the mixture was heated at 90° for several hours. No osazone was formed.

In another experiment, barium glucose monosulphate (1.506 g.) was heated at 100° for 10 minutes with 0.1 N-baryta solution (50 c.c.). The deposit was filtered off on to an ashless filter paper where it was washed with hot hydrochloric acid several times and then incinerated and weighed. (1.076 g.)

Found: Ash, 71.4

Calc. for barium glucose monosulphate $(C_6H_{11}O_9S)_2Ba$:

Ash, 35.6%

The filtrate was treated with carbon dioxide to eliminate excess barium hydroxide and to the filtered solution phenylhydrazine hydrochloride-sodium acetate mixture was added.

No osazone was obtained.

Methylation of Barium Glucose Monosulphate.

Barium glucose monosulphate (1 g.) was dissolved in water (5 c.c.) and acetone (15 c.c.) was added to the aqueous solution which was contained/

contained in a wide test-tube. Keeping reactants at 45°, 30% sodium hydroxide (1.5 c.c.) and methyl sulphate (1 c.c.) were added to the well-stirred mixture every 10 minutes for three additions. The sodium hydroxide additions were then increased to 4.5 c.c. to the 1 c.c. of methyl sulphate for another three additions at 10 minute intervals. The temperature was raised to 70° until all the acetone had been evolved, and the solution was extracted with chloroform. The chloroform extract, which was pale yellow in colour, was evaporated to dryness at 40°/15 mm. to a syrup. Thus syrup had a methoxyl content of 43.7%, was non-reducing and contained no sulphur as determined by a sodium fusion.

Yield 0.12 g.

Further Glucose Preparations.

Experiments were now carried out using greater proportions of chlorosulphonic acid than the theoretical proportion for the preparation of glucose monosulphate.

In these experiments the conditions used were the same as for the preparation of the crude barium glucose monosulphate (p. 18)the pure brucine glucose monosulphate (p. 19) and finally, the pure barium glucose monosulphate (p. 21).

In/

In the first experiment glucose (10 g.) was suspended in pyridine (75 c.c.) and to this mixture was added a solution of chlorosulphonic acid (6.8 c.c.) in alcohol-free chloroform (30 c.c.). The results from this preparation are given below:-

Crude barium glucose sulphate

Yield 13.80 g.

Analysis showed the presence of 13.0% barium.

$[\alpha]_D^{16°}$, 37.7 (c, .46 in water).

Pure brucine glucose sulphate

(from crude barium glucose sulphate (12 g.)).

Yield 13.34 g.

$[\alpha]_D^{15°}$, -3.84 after 5 minutes

$[\alpha]_D^{15°}$, -6.22 after 24 hours (c, .21 in water).

Analysis:

Found: C, 53.1 H 6.0 N 4.9

Calc. for brucine glucose monosulphate, $C_{29}H_{38}O_{13}SN_2$:

C, 53.2 H 5.8 N 4.9 %

The rotation of the brucine compound, and the analysis figures showed that, even with the higher proportion of chlorosulphonic acid, the monosulphate was produced. This was verified by preparing the pure barium glucose monosulphate from the brucine salt.

Pure barium glucose monosulphate from brucine salt (12 g.)

Yield 4.85 g.

$[\alpha]_D^{15°}$, +32.4° (c, 1.60 in water).

Analysis:

Found Ba, 21.9 S, 9.9

Calc. for barium glucose monosulphate, $(C_6H_{11}O_9S)_2$ Ba:

Ba, 21.0% S, 9.8%

In the second of these experiments, glucose (10 g.) was treated with chlorosulphonic acid (13.6 c.c.).

Crude barium glucose sulphate

Yield 28.04

$[\alpha]_D^{16°}$, +32.8 (c, 1.41 in water).

Analysis showed the presence of 27.4% barium.

Brucine glucose sulphate

(from crude barium salt (10 g.)).

Yield 16.05 g.

$[\alpha]_D^{16°}$, -7.42 after 5 minutes.

-12.86 after 24 hours (c, .12 in water).

Calc. for brucine glucose monosulphate, $C_{29}H_{38}O_{13}N_2S$:

C, 53.2 H, 5.8 N, 4.9

Calc. for brucine glucose disulphate, $C_{52}H_{64}O_{20}N_4S_2$:

C, 55.8 H, 5.7 N, 5.0 %

Since there is only small difference in the value of the percentages of carbon, hydrogen and nitrogen in the mono-ester and the di-ester it was concluded that the treatment detailed (p.20) for the purification of brucine salts would be just as efficient in this case, and that the pure barium glucose sulphate would be/

be obtained by treatment with barium hydroxide.

Pure barium glucose sulphate

(from brucine salt (14. g.)). Yield 5.88 g. $[\alpha]_D^{16}$, +28.4° (c, 1.4 in water)

Analysis:

Found Ba, 29.4 S(hydrol.) 13.1

Calc. for barium glucose monosulphate, $(C_6H_{11}O_9S)_2Ba$:

Ba, 21.0 S 9.8

Calc. for barium glucose disulphate, $C_6H_{10}O_{12}S_2Ba$:

Ba, 28.5 S, 13.54%

The results of the analysis show that the barium glucose sulphate obtained by using three molecular proportions of chlorosulphonic acid is best represented by the formula for the disulphate. However, these results could easily have been obtained by a mixture of mono-, di-, and higher sulphuric acid esters.

Acid hydrolysis.

The barium glucose sulphate (0.4106 g.) prepared by adding three molecular proportions of chlorosulphonic acid to one molecular proportion of glucose was dissolved in 0.1012 N-hydrochloric acid containing barium chloride (3%) to make a solution (100 c.c.). 10 C.c. portions of this solution were hydrolysed in sealed tubes, as has already been described for barium glucose monosulphate (p.26), and the results are tabulated below.

TABLE /

TABLE III.

t	x	a - x	$K = \frac{1}{t} \log \frac{a}{a-x}$
60	0.0103	0.0102	0.003981
120	0.0121	0.0084	0.003229
180	0.0134	0.0071	0.002542
240	0.0151	0.0054	0.003040
300	0.0173	0.0032	0.002667
360	0.0177	0.0028	0.002402
420	0.0183	0.0022	0.002282
		a = 0.0205	

It was observed that the hydrolysis gave fairly wide divergences for the individual values of the hydrolysis constant. It was concluded that the salt used was a mixture of sulphuric acid esters.

It is evident that acid hydrolysis, in this case, is more rapid, than for barium glucose monosulphate (K_{100} = ca. 0.0016).

Alkaline hydrolysis.

When a sample of the barium glucose sulphates was hydrolysed with $\frac{N}{10}$ -sodium hydroxide or $\frac{N}{10}$ - baryta solution it followed the same course as barium glucose monosulphate.

DISCUSSION

The properties of the derivatives of glucose monosulphate are given in the Table IV below, together with results of previous workers as indicated.

TABLE IV

	Brucine Salts	Acc. to Ohle (5)	Acc. to Soda(7)
$[\alpha]_D$	-4.42→ -5.82°	-4.07→ -6.28°	-5.6 (final value)
m.p.	209°	184°	183°
	Barium salts		
$[\alpha]_D$	+ 33.4°	—	+ 32.6°

Acid Hydrolysis. Since the hydrolysis velocity constant in 0.1N hydrochloric acid were determined at 100°, direct comparison with the results of Levene and Meyer (10), is difficult. It is sufficient to say however, that the glucose sulphates did not give very good velocity constants for the first few time intervals, as calculated for a monomolecular reaction, which may have been due to the presence of a very small proportion of polysulphates as Soda suggests. This may be caused by the fact that a uniform suspension of glucose in pyridine is impossible to achieve. At the same time, the mean value of K is probably not widely inaccurate. The mean values for all the substances studied by the sealed tube method are included in Table V .

Here, (A) is the product from direct treatment of galactose, and (B) is the product obtained by hydrolysis of the diacetone compound with acetic acid.

TABLE V

	K_{100} (mean) × 10^3
Barium glucose monosulphate	1.6
Bariu m galactose monosulphate(A)	1.5
Barium galactose monosulphate(B)	1.3
Barium diacetone galactose mono-sulphate.	1.4
Barium α-methyl glucoside mono-sulphate.	1.1
Barium α-methyl galactoside mono-sulphate.	1.0

It is at once clear, that no great variation in the rate of hydrolysis with 0.1N-acid arise for these different substances so that the method is to be discounted as a means of differentiating between different hexose sulphates.

Alkaline Hydrolysis. When barium glucose sulphate is treated with 0.1N sodium hydroxide solution at 100°, extensive caramelisation takes place, and all the sulphate radical is removed in a few minutes. Since the resulting solution gives an immediate precipitation of iodoform with iodine in the cold, it is concluded that extensive decomposition sets in with the production of some substance containing a CH_3CO - group. W.L. Evans and his co-workers in a series of papers (14) have studied the effect of alkalis on reducing sugars and postulate the formation of enolic forms followed by oxidative fission to various products, including pyruvic aldehyde and lactic acid. Both these substances react with alkaline hypoiodite in the cold with the formation of iodoform.

In/

In the case of glucose sulphate this breakdown of the molecule seems to be accelerated, possibly due to the fact that the sulphate residue is one of the foci of attack. It is noteworthy that no osazone can be obtained from the reaction mixture. As expected from these results, attempts at methylation using di-methyl sulphate and sodium hydroxide yielded only a very small quantity of a sulphur-free partially methylated glucose.

Structure of Glucose Sulphate.

From the results of Ohle (5) in his experiments with 2:3:4:6 tetraacetyl glucose and chlorosulphonic acid it would seem that the reducing group is not involved in the normal preparation of glucose sulphate, but his results with acetobromglucose and silver sulphate are difficult to understand. The fact that glucose sulphate is reducing to Fehling's solution is obviously no proof that C_1 is free, since hot alkali decomposes these compounds so readily. However, the salts of glucose monosulphate mutarotate in aqueous solution, and this seems to indicate the presence of a free reducing group. A determination of the "iodine number" in the cold, according to the method of Bergmann and Machemer (15), indicated the presence of a free reducing group. The results for different preparations/

preparations of glucose sulphate are given in the Table VI.

TABLE VI

Substance	"Iodine Number" (as the equivalent of 1 g. of substance in c.c. of 0.1N-iodine.	
Barium glucose mono-sulphate	calc.	64.02
(a) Barium glucose mono-sulphate.	found	54.36
(b) Barium glucose sulphates.	found	8.74

10 g. glucose treated with 3.4 (or 6.8) c.c. chloro-sulphonic acid gave (a).
10 g. glucose treated with 13.6 c.c. chlorosulphonic acid gave (b).

Although a smell of iodoform was apparent, this could not have been due to the insufficient removal of the ethyl alcohol since a check was carried out using this substance. It is possible that the immediate breakdown, noticed in contact with hot alkali, had set in with cold alkaline hypoiodite during 30 minutes, and although the iodoform was present in insufficient quantity to be precipitated, the results must be taken with some reserve. It would appear, however, that when the molecular preportion of chloro-sulphonic acid has increased to 3 molecular proportions, substitution on the reducing group takes place, but

that/

that under normal experimental conditions (one molecular weight of chlorosulphonic acid), the monosulphate produced, still contains a full reducing group.

SUMMARY

1. Glucose has been treated in pyridine suspension with varying proportions of chlorosulphonic acid in chloroform.

2. The barium glucose monosulphate described by Ohle (5) and Soda (7) has been isolated and purified via the brucine salt, the properties of which, while not identical, agree generally with the results of earlier workers.

3. Determination of the rate of hydrolysis of this monosulphate shows that the reaction is best - but not perfectly - described as a monomolecular reaction where K_{100} = ca. 0·0016 in $\frac{N}{10}$ -hydrochloric acid.

4. Removal of the sulphate residue by alkali has been shown to be extremely rapid and the sugar residue seems to be broken up completely in 5 minutes with $\frac{N}{10}$ -alkali at 100°.

5. The "iodine number" shows that the reducing group is free in barium glucose monosulphate.

6. Products obtained by using larger proportions of chlorosulphonic acid have lower iodine numbers and high barium contents indicating that substitution in the reducing group can take place in these products which appear to be mixtures of poly-sulphates

Bibliography for Glucose Section.

1.	Claësson	J. prakt. Chem., 1879, 20 (2), 17.
2.	Neuberg & Ohle	Biochem. Z., 1921, 125, 311.
3.	Percival & Somerville	J. Chem. Soc., 1937, 1615.
4.	Neuberg & Schwietzer	Monatshefte, 1937, 71, 46.
5.	Ohle	Biochem. Z., 1922, 131, 601.
6.	Neuberg & Liebermann	Biochem. Z., 1921, 121, 326.
7.	Soda	Biochem. Z., 1923, 135, 621.
8.	Ohle	Biochem. Z., 1923, 136, 428.
9.	Anderson, Charlton & Haworth	J. Chem. Soc., 1929, 1329.
10.	Levene & Meyer	J. biol. Chem., 1922, 53, 437.
11.	Soda and Nagai	J. Chem. Soc. Japan, 1935, 56, 1258.
12.	Soda	Bull. Chem. Soc. Japan, 1933, 8,(2), 37.
13.	Cumming and Kay	1916, 2nd Ed., p. 356.
14.	Evans and co-workers	See e.g. J.A.C.S., 1928, 50, 2543.
15.	Bergmann & Machemer	Ber., 1930, 63, 316.

PART II

Investigation of Galactose Sulphates

Galactose Sulphates

The only reference to the preparation of galactose sulphates appears to be due to Akatmatzu (1) who obtained a tetrasulphate by the action of chlorosulphonic acid on galactose in pyridine solution. It has been repeated by Bergström (2). Since diacetone galactose contains a primary alcoholic residue on C_6, and since this substance is relatively stable, since it has a pyranose structure, (since, for example toluenesulphonation of this group is easily carried out (Freudenberg and Hixon (3)) a method seemed available to prepare firstly 1:2:3:4 diacetone galactopyranose 6-sulphates, and hence, by removal of the acetone residues with dilute acetic acid (cf. the corresponding case for the preparation of galactose-6-p-toluenesulphonate by Ohle and Thiel (4)) to proceed to the galactose-6-sulphates, and compare the properties of these salts with the product obtained by direct treatment of galactose and chlorosulphonic acid.

Diacetone galactose.

To Acetone (2000 c.c.), which had been previously dried over calcium chloride for 24 hours, was added slowly and with cooling, concentrated sulphuric acid (56 c.c.), and the mixture well shaken. Galactose (80 g.), dried in a vacuum desiccator, was added in portions to the acetone mixture and the suspension was shaken vigorously for 8 hours. The undissolved galactose was filtered off, and the filtrate was immediately neutralised with anhydrous sodium carbonate. The neutralisation was accompanied by a decided lightening in colour of the yellow acetone solution. The acetone was now distilled off at 40° / 15 mm. and the resulting pale yellow syrup was dissolved in cold water. This was filtered through cotton wool which freed the aqueous solution from oily acetone condensation products. The aqueous solution, after testing to make sure that it was neutral, was taken to dryness at 100° under diminished pressure and the resulting syrup was distilled in a high vacuum (.01 mm.) in the presence of barium carbonate at 160° (bath temp.). The diacetone galactose so obtained was a colourless, extremely viscous syrup.

Yield, 49 g.

Recovered galactose 30 g.

Barium diacetone galactose sulphate.

Diacetone galactose (10 g.) was dissolved in dry pyridine (50 c.c.) and a solution of chlorosulphonic acid (2.6 c.c.) in alcohol-free chloroform (10 c.c.), was added slowly with stirring. The temperature was maintained at -10° during the addition. Barium-carbonate was added immediately to the reaction mixture which was then allowed to stand overnight. Pyridine was then distilled off from the product in the presence of barium carbonate. The elimination of the chloride was completed as has already been described (p. 18). When the aqueous solution of barium diacetone galactose sulphate so obtained was concentrated at 40°/15 mm. instead of precipitating in alcohol, the syrup which resulted from complete distillation to dryness, was dissolved in the minimum of chloroform and this solution was filtered. The barium diacetone galactose sulphate was precipitated by pouring the filtrate into light petroleum (b.p. 60/80°). It showed/

showed

$[\alpha]_D^{14^\circ}$, -35.7 (c, 7.3 in water)

$[\alpha]_D^{14^\circ}$, -42.4 (c, 8.2 in chloroform)

Yield, 4.64 g.

The white amorphous barium diacetone galactose sulphate was deliquescent, non-reducing to Fehling's solution, gave the iodoform reaction after hydrolysis, contained ionised Barium, and combined sulphur.

Analysis:

Found: Ba, 16.82 S(hydrol.), 7.62

Calc. for barium diacetone galactose mono-sulphate, $(C_{12}H_{19}O_9S)_2Ba$.

Ba, 16.85 S, 7.85%.

Hydrolysis constant of barium diacetone galactose monosulphate.

The determination of the hydrolysis constant was by hydrolysis in sealed tubes as described for barium glucose monosulphate (p. 26).

Barium diacetone galactose monosulphate (0.4986 g.) was dissolved in 0.1012 N-hydrochloric acid (100 c.c.) which contained barium chloride (3%). Into glass tubes were introduced 10 c.c. portions of this solution and the tubes were then sealed. After heating at 100° for the time interval indicated in Table VII, a tube was cooled rapidly and the weight of barium sulphate deposited determined - this gave value of x in/

in the table. The hydrolysis constant was calculated from the formula $K = \frac{1}{t} \log \frac{a}{(a-x)}$ where the symbols have the same significance as they had in the determination for barium glucose monosulphate.

TABLE VII

t	x	a - x	$K = \frac{1}{t} \log \frac{a}{a-x}$
120	0.0136	0.02482	0.001581
180	0.0185	0.01992	0.001584
240	0.0218	0.01662	0.001517
300	0.0257	0.01273	0.001489
360	0.0272	0.01122	0.001485
420	0.0288	0.00962	0.001432
480	0.0305	0.00792	0.001429
540	0.0319	0.00652	0.001430
600	0.0331	0.00532	0.001431

$a = 0.03842$

Mean value of K_{100} approaches 0.00143

Alkaline hydrolysis of diacetone galactose monosulphate

On heating at 100° with 0.1N-sodium hydroxide or 0.1N-baryta solution no discoloration of a solution of the salt was observed and the amount of barium sulphate deposited too small to be determined.

Even heating for several hours at 100° with alkali as concentrated as 2N-sodium hydroxide failed to deposit any barium sulphate or to discolour the solution.

Attempted Preparation of Barium diacetone-galactose monosulphate from barium galactose monosulphate.

Barium galactose monosulphate (2 g.) as prepared by direct action of chlorosulphonic acid on galactose under conditions already given (p. 16) was added to a mixture of dried acetone (60 c.c.) containing analar sulphuric acid (0.6 - 0.7 c.c.). This sulphuric acid had been added cautiously to the acetone, thoroughly mixed and the mixture cooled before the addition of the barium galactose monosulphate.

The mixture was shaken by a mechanical shaker for 8 hours, and the solution immediately shaken with barium carbonate until neutral. The mixture was evaporated to dryness at 30°/15 mm. in the presence of barium carbonate. Water (100 c.c.) was added to the residue and after filtration the aqueous solution/

solution was concentrated under reduced pressure at 35°. The aqueous solution (20 c.c.) was filtered free from barium carbonate and evaporated to dryness at 35°/15 mm. There was left a clear syrup which did not contain barium or sulphur. It was non-reducing to Fehling's solution until it had been hydrolysed.

$[\alpha]_D^{14°}$, -52° (c, .02 in water).

Diacetone galactose has all of the above properties $[\alpha]_D$, -54°. The product was evidently diacetone galactose.

Barium Galactose 6-Sulphate from Barium Diacetone Galactose 6-Sulphate.

Barium diacetone galactose 6-sulphate (10 g.) was heated at 100° for three hours with 1% acetic acid. A sample of the solution gave, after hydrolysis, a negative iodoform reaction showing that all the acetone had been removed, and was reducing to Fehling's solution. The solution was evaporated at 35°/15 mm. to dryness, the residue dissolved in a little water, filtered, and the filtrate taken to dryness under the same conditions. The residue was dissolved in water and at suitable concentration poured into alcohol to precipitate the crude barium galactose 6-sulphate $[\alpha]_D^{14°}$, 40° (c, 3.60 in water).

Yield, 9.86 g.

Analysis:

Found: Ba, 17.5 S(hydrol.) 7.5

Calc. for barium galactose 6-sulphate $(C_6H_{11}O_9S)_2Ba$:

Ba, 21.0 S, 9.8%.

The crude barium galactose 6-sulphate (9 g.) was dissolved in water and added to a cold aqueous solution of brucine sulphate (10 g.) and the resulting mixture allowed to stand overnight. The solution obtained by filtration was concentrated at 40° under reduced/

reduced pressure and then washed several times with chloroform to remove any free brucine. The solution was poured into acetone and brucine galactose 6-sulphate was obtained as an amorphous powder.

$[\alpha]_D^{14°}$, 15° after 5 minutes

$[\alpha]_D^{14°}$, 25° after 24 hours (c, 0.20 in water)

Yield 8.83 g.

Analysis:

Found: C, 52.2 H, 5.8 N, 4.5

Calc. for brucine galactose 6-sulphate, $C_{29}H_{38}O_{13}N_2S$:

C 53.2 H, 5.9 N, 4.3 %

When a sample of brucine galactose 6-sulphate was crystallised from water by adding just enough acetone to obtain an opalescence with a warm concentrated aqueous solution of brucine galactose 6-sulphate, and cooling, it did so in clear cubes. These crystals on exposure to the air lost their crystalline form and became floury in appearance.

Analysis:

Found: C, 50.4 H, 5.9 N, 4.2%

The crystals were assumed to contain water of crystallisation, and, by trial of the calculated values for carbon, hydrogen and nitrogen of possible hydrates of brucine galactose 6-sulphate, the results of the analysis were found to be best suited by a formula of the dihydrate.

Analysis:/

Analysis:

Found: C, 50.4 H, 5.9 N, 4.2

Calc. for dihydrate of brucine galactose 6-sulphate, $C_{29}H_{42}O_{15}N_2S$: C, 50.5 H, 5.9 N, 4.1%.

If the assumption that a hydrate was formed on crystallising from water is correct, then the product obtained by precipitation in acetone is essentially the anhydrous brucine galactose 6-sulphate.

Brucine galactose 6-sulphate (6 g.) was treated with excess saturated barium hydroxide solution and the barium galactose 6-sulphate was obtained just as barium glucose monosulphate was obtained from brucine glucose monosulphate (p. 21) $[\alpha]_D^{14^\circ}$ +66.67 (c, 0.19 in water)

Yield 2.41 g.

Barium galactose 6-sulphate was a deliquescent amorphous white powder. It reduced Fehling's solution, contained ionised barium, and combined sulphur.

Analysis:

Found: Ba, 20.8 S(hydrol.), 9.4

Calc. for barium galactose 6-sulphate $(C_6H_{11}O_9S)_2Ba$:

Ba 21.0 S, 9.8%.

Hydrolysis Constant of Barium Galactose 6-Sulphate.

Barium galactose 6-sulphate (0.5242 g.) was dissolved/

dissolved in 100 c.c. of 0.1012 N-hydrochloric acid which contained 3% barium chloride. 10 C.c. portions of this solution were heated at 100° in sealed tubes, as in the determination of the constant for barium glucose monosulphate (p.26), for the time interval shown in table VIII. The hydrolysis constant was calculated from $K = \frac{1}{t} \log \frac{a}{(a - x)}$, where a, x, and t have their usual significance.

TABLE VIII

t	x	a - x	$K = \frac{1}{t} \log \frac{a}{a-x}$
120	0.0127	0.02465	(0.001500)
180	0.0165	0.02085	(0.001401)
240	0.0191	0.01825	0.001300
300	0.0221	0.01525	0.001299
360	0.0235	0.01385	0.001200
420	0.0267	0.01065	0.001365
480	0.0285	0.00885	0.001303
540	0.0299	0.00745	0.001296
600	0.0312	0.00615	0.001305

a = 0.03735

Mean value of K_{100} = 0.001295, omitting the values given in brackets.

Preparation of Barium Galactose Monosulphate.

Barium galactose sulphate was prepared by the procedure already described for barium glucose sulphate (M.18). In one experiment, chlorosulphonic acid (3.4 c.c.) in alcohol-free chloroform (25 c.c.) when added at -10° to dried galactose (10 g.) suspended in dry pyridine (75 c.c.) gave a crude barium galactose sulphate $[\alpha]_D^{16°}$, + 59° (c, .36 in water). The product was a white hygroscopic amorphous solid, which reduced Fehling's splution.

Yield, 10.45 g.

Analysis:

Found: Ba, 14.6%

This crude product (10 g.) with brucine sulphate (13 g.) in cold aqueous solution, under the same conditions as for the preparation of brucine glucose sulphate (p. 19) yielded a brucine salt which, after purification with hot alcohol, crystallised from water in rosettes of needles. $[\alpha]_D^{17°}$, -4.64 after 5 minutes, $[\alpha]_D^{17°}$, -8.22 after 24 hours (c, 0.21 in water).

Yield 15 g.

Analysis:

Found: C, 52.17, H, 6.03 N, 4.30 S(hydrol.) 4.6 S(fusion)4.8.

Calc. for brucinegalactose monosulphate, $C_{29}H_{38}O_{13}N_2S$:

C, 53.2 H, 5.8 N, 4.28 S, 4.89%.

The pure galactose monosulphate was obtained from the pure brucine galactose monosulphate (13 g.) by addition of excess saturated barium hydroxide solution. The excess barium hydroxide was precipitated as barium carbonate on passing carbon dioxide and the brucine, barium sulphate and barium carbonate filtered off from the aqueous solution. This latter was washed several times with chloroform to dissolve out any free brucine and the aqueous solution, after concentration, at 40°/15 mm., was poured into alcohol at a suitable concentration to give the amorphous barium galactose monosulphate $[\alpha]_D^{15°}$, + 48.2° (c, .43 in water).

Yield, 5.2 g.

Barium galactose monosulphate so obtained reduced Fehling's solution, contained barium in the ionic state, and deposited barium sulphate upon hydrolysis.

Analysis:

Found: Ba, 21.10 S(hydrol.), 9.34

Calc. for barium galactose monosulphate $(C_6H_{11}O_9S)_2Ba$:

Ba, 20.95 S, 9.76%.

Hydrolysis Constant of Barium Galactose Monosulphate.

In the determination of the hydrolysis constant, barium galactose monosulphate (0.5082 g.) was dissolved/

dissolved in 0.1012N -hydrochloric acid containing 3% barium chloride to make 100 c.c. of solution. 10 C.c. portions of this solution were heated at 100° in sealed tubes as in the method adopted for barium glucose monosulphate (p.26). The hydrolysis constant was again calculated from the equation for a monomolecular reaction : $K = \frac{1}{t} \log \frac{a}{a - x}$ where t, a, and x have their usual significance. The results were placed in tabular form as in Table IX.

TABLE IX

t	x	a - x	$K = \frac{1}{t} \log \frac{a}{a - x}$
180	0.0187	0.01751	0.001753
240	0.0219	0.01431	0.001680
300	0.0246	0.01161	0.001646
360	0.0257	0.01051	0.001492
420	0.0280	0.00821	0.001535
480	0.0295	0.00671	0.001525
600	0.0318	0.00441	0.001524
720	0.0334	0.00281	0.001542

a = 0.03621

Mean values of K_{100} = 0.001524, omitting the values given in brackets.

Alkaline Hydrolysis of Barium Galactose Monosulphate.

The results of hydrolysis with 0.1N -sodium hydroxide and with 0.1N -baryta solution on barium galactose monosulphate/

monosulphate were the same as for the alkaline hydrolysis of barium glucose monosulphate. At 100° there was an almost immediate deposition of the total sulphate in the compound with a decomposition of the galactose part of the molecule.

In the experiment barium galactose monosulphate (1.602 g.) was hydrolysed by carbonate-free 0.1N - sodium hydroxide by heating at 100° for five minutes. The solution was coloured due to decomposition. The solution was made slightly acid with hydrochloric acid and a cold solution of barium chloride was then added. The deposited sulphate was incinerated, to give an ash (0.558 g.).

Found: Ash, 34.8

Calc. for barium galactose monosulphate, $(C_6H_{11}O_9S)_2Ba$:

Ash, 35.6%

The total barium was therefore deposited by hydrolysis with 0.1N -sodium hydroxide.

In a second experiment, barium galactose monosulphate (0.982 g.) was hydrolysed at 100° with 0.1N-baryta solution. The deposited material was filtered off, incinerated and weighed (0.734 g.).

Found: Ash, 74.7.

Calc. for barium galactose monosulphate, $(C_6H_{11}O_9S)_2Ba$:

Ash, 35.6% (this is only half possible value if excess barium is present).

The/

The filtrate from the above hydrolysis was saturated with carbon dioxide, and to the neutral solution so obtained phenylhydrazine hydrochloride (0.9 g.) and sodium acetate (1.2 g.) were added. This mixture was heated at 90° for several hours without the formation of an osazone.

Methylation of Barium Galactose Monosulphate.

Barium galactose monosulphate (1 g.) were dissolved in water (5 c.c.) and acetone (15 c.c.) and treated by 30% sodium-hydroxide and dimethyl sulphate as already described in the case of barium glucose monosulphate (p.29).

The chloroform extract of the result of this methylation was evaporated at 35°/15 mm. to a pale yellow syrup which contained methoxyl (OMe 27.6%) but did not contain sulphur as shown by a sodium fusion.

Yield 0.18 g.

Further Galactose Preparations.

In the experiments to be described, the same conditions as were used in the sulphurylation of glucose, were followed.

The first preparation was that obtained by using galactose (10 g.) in pyridine (75 c.c.) reacting with chlorosulphonic acid (68 c.c.) dissolved in chloroform (25 c.c.). The following galactose/

galactose sulphate derivatives were obtained:

Crude barium compound

Yield 13.11 g. $[\alpha]_D^{14°}$, +46.2° (c, 0.50 in water).

Analysis showed the presence of 21.2% barium.

Brucine galactose sulphates

(from the crude barium compound)

Yield 14.80 g. (12 g.) $[\alpha]_D^{14°}$, -12.34° after 5 minutes,

-12.36° after 24 hours.

(c, 0.140 in water).

Pure barium galactose sulphates

(from the pure brucine salts)

Yield 3.84 (11 g.) $[\alpha]_D^{15°}$, +38.6° (c, 0.62 in water).

Analysis:

Found: Ba, 30.1 S, 13.2

Calc. for barium galactose monosulphate $(C_6H_{11}O_9S)_2Ba$:

Ba, 21.0 S, 9.8

Calc. for barium galactose disulphate $C_6H_{10}O_9S_2Ba$:

Ba, 28.5 S, 13.5%

The analysis figures show evidence of poly-sulphate formation and in particular the disulphate seems to be favoured.

Acid hydrolysis:

The barium galactose sulphates (0.4225 g.) were dissolved in 100 c.c. of 0.1012N -hydrochloric acid,/

acid, which contained barium chloride (3%). The results of hydrolysis in sealed tubes at 100° are given in Table XI.

TABLE X.

t	x	a - x	$K = \frac{1}{t} \log \frac{a}{a-x}$
120	0.0265	0.01668	0.003443
180	0.0318	0.01138	0.003218
255	0.0343	0.00988	0.002512
315	0.0368	0.00638	0.002637
435	0.0402	0.00298	0.002669
495	0.0408	0.00238	0.002080
555	0.0411	0.00208	0.002373
		a = 0.04318	

The values of the constant vary widely, but the underlined values, show that for these time intervals the hydrolysis was proceeding as a monomolecular reaction and the value obtained here may indeed be due to the disulphate.

In any case, acid hydrolysis of higher sulphates of galactose is more rapid than for galactose monosulphate.

When three molecular proportions (13.6 c.c.) of chlorosulphonic acid are used for one molecular proportion/

proportion of galactose, we obtained the following preparations by the procedure adopted for the corresponding derivatives of galactose monosulphate.

Crude barium galactose sulphate

Yield 21.63 g. (from 10 g. galactose).

$[\alpha]_D^{15°}$ + 22.5° (c, 4.2 in water).

Analysis showed 28.3% barium.

Brucine galactose sulphates

Yield 18.40 g. (from 10 g. crude salts).

$[\alpha]_D^{15°}$ -15.6° after 5 minutes

-15.6° after 24 hours (c, 0.18 in water).

Pure barium galactose sulphates

Yield 3.25 g.(from 17 g. brucine salts).

$[\alpha]_D^{14°}$ + 21.8° (c, .41 in water).

Analysis;

Found: Ba, 32.3 S, 15.2

Calc. for barium galactose disulphates, $C_6H_{10}O_{12}S_2Ba$:

Ba, 28.5 S, 13.5

Calc. for barium galactose trisulphates, $(C_6H_9O_{15}S_3)_2Ba_3$:

Ba, 49.4 S, 23.0%

Acid Hydrolysis.

On treating the compound with 0.1012 N- hydrochloric acid in sealed tubes, no constant for the hydrolysis velocity was obtained. Values ranging from:

$K_{100} = 0.007374$ to $K_{100} = 0.002410$

for the/

for the time intervals t = 60 (mins.) and t = 420 (mins.) respectively, and showed that the hydrolysis does not proceed as a monomolecular reaction.

It was concluded that a mixture of sulphuric acid esters was present.

Alkaline hydrolysis.

When the ethereal sulphates formed from galactose by using the higher proportions of chlorosulphonic acid were treated with 0.1 N- sodium hydroxide or 0.1N -baryta solution, results exactly the same as for barium galactose monosulphate were obtained. The sulphates were decomposed to give the total amount of barium and sulphur, deposited as barium sulphate, almost instantaneously at 100° with dilute alkali.

Galactose Sulphates

Discussion.

Direct Preparation from Galactose.

The properties of the derivative of this galactose monosulphate are given in the following Table XI.

TABLE XI.

(a)	Brucine galactose monosulphate	$[\alpha]_D$	-4.6 → -8.2
(b)	Brucine galactose polysulphates	$[\alpha]_D$	-12.34
(a)	Barium galactose monosulphate	$[\alpha]_D$	+48.2 % Ba, 21.1.
(b)	Barium galactose polysulphates	$[\alpha]_D$	+38.6 % Ba, 30.1

10 g. galactose with 3.4 c.c. chlorosulphonic acid gave (a)
10 g. galactose with 6.8 c.c. chlorosulphonic acid gave (b)

Acid Hydrolysis.

The individual values of the velocity constants for the hydrolysis in $\frac{N}{10}$ acid solution (p.54) are closer than those determined for the corresponding glucose derivative but the mean value K_{100} = ca 1.5×10^{-3} is not widely different. Alkaline hydrolysis followed the same route, and methylation of galactose monosulphate gave a sulphur free, partially methylated galactose in poor yield.

Diacetone Galactose-6-Sulphate.

Owing to the fact that diacetone galactose is completely soluble in pyridine the reaction proceeded more smoothly. The product was found to be soluble in chloroform and gave good analytical figures.

Analysis:

Found: Ba, 16.8 S (hydrol.) 7.6

Calc. for barium diacetone galactose monosulphate $(C_{12}H_{19}O_9S)_2$ Ba:

Ba, 16.9 S, 7.8%

The acid hydrolysis constant K_{100} = ca. 1.4×10^{-3} in 0.1N -hydrochloric acid is not very different from that of the other galactose monosulphate described, but this substance was not hydrolysed by heating for 3 hours with 2N -sodium hydroxide solution. No caramelisation occurred so it must be concluded either that the sulphate group when located on C_6 is very stable or that the presence of the reducing group or a hydroxyl group is necessary before alkali can remove the sulphate groups readily.

Barium Galactose-6-Sulphate.

This substance was secured by hydrolysing the diacetone compound with 1% acetic acid. The barium and brucine salts were completely different in properties from those of the galactose monosulphate prepared in the usual way as the comparison below shows.

	Barium galactose 6-sulphate	'ordinary' barium galactose monosulphate
Brucine salts	$[\alpha]_D$ 15° → 25°	$[\alpha]_D$ -4.64 → -8.22.
Barium salts	$[\alpha]_D$ +66.7°	$[\alpha]_D$ +48.2

It/

It is clear therefore, that the monosulphate prepared directly from galactose is <u>not</u> the 6-sulphate, unless the unlikely migration of the sulphate residue takes place on deacetonisation with acetic acid.

The rate of hydrolysis under the same conditions as before gave K_{100} = ca. $1{\cdot}3 \times 10^{-3}$ i.e. slightly lower than for the diacetone compound and lower than the directly prepared galactose sulphate.

Since mutarotation occurs in the case of the brucine salt substitution on C_1 also seems to be excluded. This work cannot throw any light however, as to whether C_2, C_3 or C_4 are involved.

It will be remembered that Hassid claimed that the galactan sulphuric ester isolated from 'Irideae Laminarioides' was relatively stable to alkali, since he was able to methylate the compound and still retain the ethereal sulphate on his methylated compound. He deduced that the sulphuric acid group was to be found on C_6 but alkaline hydrolysis of barium galactose-6-sulphate showed that, as in all the other cases involving reducing sugars, and in contrast to barium diacetone galactose-6-sulphate, that the sulphate group was removed within 10 minutes at 100° with 0.1N -sodium hydroxide.

It would therefore appear, that when the ethereal sulphate is attached to a polysaccharide chain great stability to hydrolysis with alkali is inferred as in the case of diacetone galactose-6-sulphate.

SUMMARY.

1. The barium and brucine salts were prepared as for the corresponding glucose derivatives, and their constants recorded.

2.(a) The rate of hydrolysis with $\frac{N}{10}$ -acid and

(b) hydrolysis with alkali has been studied.

From (a) no great difference in velocity constant from that found for the glucose sulphates was observed, and from (b) the rapid breakdown with alkali was very similar.

3. Barium diacetone galactopyranose-6-sulphate was prepared and shown to have a hydrolysis velocity constant with $\frac{N}{10}$ -acid of the same order as the values previously obtained for glucose and galactose sulphates, but the compound was very stable to alkali. It was therefore concluded that the presence of hydroxy groups or a reducing group was necessary for the alkaline hydrolysis, or that the sulphate group on the 6-position in galactose was very stable.

4. Galactose-6-sulphate was obtained by hydrolysing the diacetone compound. The brucine and barium salts were found to be quite different in physical properties from the corresponding derivative obtained from the direct treatment

of/

of galactose with chlorosulphonic acid.

5. Although the velocity of hydrolysis of the sulphate group in the case of $\frac{N}{10}$ -acid was slightly different from that of the directly prepared derivative, no distinction could be drawn in the rate of alkaline hydrolysis. It follows therefore, that in barium galactose-6-sulphate the sulphuric acid ester group is not stable per se, although the results in 3. above might have led to this conclusion.
6. It is concluded that 'ordinary' galactose monosulphate must have the sulphate group on C_2, C_3 or C_4.

Bibliography for Galactose Section.

1. Akatmatzu — Biochem. Z., 1923, 142, 181.
2. Bergström — Z. physiol. Chem., 1936, 238, 163.
3. Freudenberg and Hixon — Ber., 1923, 56, 2123.
4. Ohle & Thiel — Ber., 1933, 66, 525.

PART III

Investigation of Glucoside and Galactoside Sulphates

Glucoside and Galactoside Sulphates

Ohle (1) prepared sodium tetraacetyl-β-methyl glucoside monosulphate and its brucine salt. Helferich, Löwe, Nippe and Riedel (2) prepared barium α-methyl glucoside monosulphate using sulphuryl chloride, as well as the corresponding β-glucoside monosulphates. The use of sulphuryl chloride appears to be complicated by the fact that chlorine also enters the glucoside molecule. It was necessary that a more detailed investigation of the properties of such substances should be undertaken for at least two reasons. Firstly, the polysaccharide ethereal sulphates can be considered as glucoside sulphates in the sense that no full reducing group is available, and since alkaline hydrolysis of the reducing hexose sulphates has been shown to be very rapid (p. 28) the possibility exists that the stability of the polysaccharide sulphates such as that of carrageen and the galactan sulphate of Hassid may be due to the absence of a free reducing group. Secondly, the absence of a reducing group would render possible a study of the products of hydrolysis with alkali without the disruption of the molecule which has been found to attend such treatment of glucose and galactose sulphates.

Preparation of α-Methyl Glucoside.

Dried glucose (100 g.) was heated under reflux with methyl alcohol (200 g.) which had been previously dried with magnesium, and which contained 3% of hydrogen chloride until no reduction of Fehling's solution was observed. (5 - 6 hours). The solution was cooled and α-methyl glucoside was obtained. By concentration and cooling the mother liquor, further quantities of α-methyl glucoside were obtained. The α-methyl glucoside was crystallised from methyl alcohol. $[\alpha]_D^{14°}$, 158° (c, 0.206 in water).
m.p. 165-166°.
Analysis:
Found: OMe, 14.9
Calc. for α-methyl glucoside, $C_7H_{14}O_6$:
OMe, 16%

Barium α-Methyl Glucoside Monosulphate.

α-Methyl glucoside (10 g.) in pyridine (100 c.c.) was treated with chlorosulphonic acid (6.8 c.c.) in chloroform (20 c.c.) under the same conditions as for glucose (p. 18).

The final aqueous solution was added to alcohol as described for barium glucose monosulphate (p. 19).

The/

The amorphous white powder so obtained was non-reducing to Fehling's solution until hydrolysed, and contained ionised barium.

Yield, 9.50 g.

Analysis:

Found: OMe, 10.6 Ba 18.9

Calc. for barium α-methyl glucoside monosulphate, $(C_7H_{13}O_9S)_2$ Ba:

OMe, 9.1 Ba 19.6%

The results of the analysis of the product indicated that free α-methyl glucoside was present with the barium α-methyl glucoside monosulphate. The solid was extracted with hot alcohol several times in a continuous extraction apparatus in order to dissolve out any free α-methyl glucoside but leave barium α-methyl glucoside monosulphate. The resulting product was dried. $[\alpha]_D^{15°}$ +90.11 (c, 0.44 in water).

Analysis:

Found: OMe 8.9 Ba 19.1

Calc. for barium α-methyl glucoside monosulphate, $(C_7H_{13}O_9S)_2$ Ba:

OMe 9.1 Ba 19.6%

<u>Hydrolysis Constant of Barium α-Methyl Glucoside Monosulphate</u> /

Hydrolysis Constant of Barium α-Methyl Glucoside Monosulphate.

Barium α-methyl glucoside monosulphate (0.5524 g.) was dissolved in 100 c.c. of 0.1012 N-hydrochloric acid which contained 3% barium chloride. The solution was heated in sealed tubes at 100° as detailed previously for Barium Glucose Monosulphate (p. 26) and the hydrolysis constant K was determined by the formula:

$$K = \frac{1}{t} \log \frac{a}{a - x}$$ where t was the time in minutes, a was the total weight of barium sulphate that would have been deposited if the hydrolysis were complete, and x was the weight of barium sulphate obtained after hydrolysing for a time t.

TABLE XII

t	x	a - x	$K = \frac{1}{t} \log \frac{a}{a - x}$
120	0.0097	0.02802	0.001076
180	0.0134	0.02432	0.001059
240	0.0169	0.02082	0.001075
300	0.0201	0.01762	0.001103
420	0.0244	0.01332	0.001076
480	0.0249	0.01282	0.000976
540	0.0277	0.01002	0.001056

a = 0.03772

Mean value of K_{100} = 0.00106

Alkaline Hydrolysis of Barium α-Methyl Glucoside Sulphate.

When barium α-methyl glucoside sulphate in solution was heated at 100° for six hours with enough solid barium hydroxide to give a normal solution there was complete deposition, as barium sulphate, of the total sulphur in the compound. This hydrolysis was unaccompanied by any coloration of the solution.

Barium α-methyl glucoside sulphate (1.5 g.) was dissolved in water (50 c.c.) and solid barium hydroxide (15.8 g.) added. The mixture was heated at 100° for 6 hours in a flask fitted with a condenser carrying a soda-lime tube. The mixture was then filtered. A sample of the filtrate, hydrolysed with concentrated hydrochloric acid, failed to show any deposited barium sulphate. The filtrate was then saturated with carbon dioxide and filtered free from the precipitated barium carbonate. The solution was evaporated to dryness at 30°/15 mm. and was extracted with alcohol and the alcoholic solution evaporated to dryness at 30°/15 mm. The resulting solid was dissolved in alcohol, filtered, and then evaporated to dryness at 30° under reduced pressure. A crystalline solid was obtained as very fine needles on the sides of the flask and on leaving for a fortnight crystallisation spread throughout the syrup. These crystals melted at

95-96°/

95-96°, and were deliquescent. They reduced Fehling's solution, and gave positive 'ketose' reactions with the Bredereck and Seliwanoff tests respectively. The solid contained no sulphur as determined by a sodium fusion.

Yield, 1.5 g.

$[\alpha]_D^{16}$, +55.9° (c, 0.340 in water).

Barium α-Methyl Galactoside Monosulphate.

α-Methyl galactoside was prepared as for glucoside (p.40) OMe 14.4%. $[\alpha]_D$ +189°

α-Methyl galactoside (10 g.) in pyridine (100 c.c.) was treated with chlorosulphonic acid (6.8 c.c.) in alcohol-free chloroform (20 c.c.) under the same conditions as for barium glucose monosulphate (p. 18).

The final aqueous solution was added to alcohol to precipitate a white amorphous compound which was non-reducing to Fehling's solution after hydrolysis with acid. It also contained barium precipitable with sulphuric acid.

Yield 4.86 g.

Analysis:

Found: OMe 11.3 Ba 19.1

Calc. for barium α-methyl galactoside monosulphate, $(C_7H_{13}O_9S)_2Ba$:

OMe 9.1 Ba 19.6%

The solid was treated in a continuous extraction apparatus with hot alcohol which dissolved out α-methyl galactoside but left the barium α-methyl galactoside monosulphate. $[\alpha]_{D}^{17°}$ +142° (c. .502 g in water).

Analysis:

Found: OMe 8.6 Ba 19.2

Calc. for barium α-methyl galactoside monosulphate, $(C_7H_{13}O_9S)_2Ba$:

OMe 9.1 Ba 19.6%

Hydrolysis Constant of α-Methyl Galactoside Monosulphate.

By exactly the same procedure as for the determination of hydrolysis constant of barium glucose monosulphate (p. 26) that of barium α-methyl galactoside monosulphate was obtained. 10 C.c. portions of a solution of barium α-methyl galactoside (0.5348 g.) in 100 c.c. of 0.1012 N-hydrochloric acid were heated in sealed tubes at 100°. The results are given in table XIII. The hydrolysis constant K was calculated from $K = \frac{1}{t} \log \frac{a}{a - x}$ where these symbols have their usual significance.

TABLE XIII

t	x	a - x	$K = \frac{1}{t} \log \frac{a}{a - x}$
120	0.0095	0.02702	0.001090
180	0.0129	0.02362	0.001052
240	0.0155	0.02102	0.001000
300	0.0181	0.01842	0.000991
360	0.0201	0.01642	0.000965
420	0.0225	0.01402	0.000990
480	0.0243	0.01222	0.000989
540	0.0259	0.01062	0.000993

a = 0.03652

Mean value of K_{100} = 0.00101

Barium α-Methyl Glucoside and α-Methyl Galactoside Sulphates.

Discussion.

Barium α-methyl glucoside sulphate.

Properties:- $[\alpha]_D^{16^\circ}$, + 90.11

Analysis: Found: OMe, 8.9 Ba, 19.1

"Calc. for α-methyl glucoside sulphate $(C_7H_{13}O_9S)_2$ Ba: OMe, 9.1 Ba, 19.6

The properties of the barium α-methyl glucoside sulphate were similar to those ascribed to the so-called 6-sulphate of Helferich, Löwe, Nippe and Riedel (2) although the specific rotation is higher than that quoted by these authors($[\alpha]_D = +81.2^\circ$).

The rate of removal of the sulphate residue from this compound by $\frac{N}{10}$-acid was of the same order as that determined for barium glucose monosulphate, (K_{100} = ca 1.6×10^{-3}), barium galactose monosulphate (K_{100} = ca. 1.5×10^{-3}), barium galactose diacetone-6-sulphate (K_{100} = ca. 1.4×10^{-3}), and barium galactose-6-sulphate (K_{100} = ca. 1.3×10^{-3}). Hydrolysis with $\frac{N}{10}$-alkali proceeded quite rapidly so that the absence of the reducing group in the case of diacetone-6-sulphates was apparently not the operating factor. It has been shown that the product of hydrolysis/

hydrolysis with $\frac{N}{10}$-barium hydroxide solution is certainly not α-methyl glucoside, since a crystalline substance m.p. 95-96°, $[\alpha]_D$ = 50° suspected to be an anhydro-compound is obtained, but this cannot be settled until sufficient of the pure compound has been secured for analysis. Owing to a curtailment of the hours due to the war, it has not been possible to do this. It is well known that, by alkaline hydrolysis of p-toluensulphonyl derivatives of sugars, anhydro compounds are obtained (see e.g. Ohle and Thiel (3): on the other hand, a mass of literature on Walden inversion in such cases is available (4)). It is clear however, that, whether Walden inversion or anhydro-ring formation has taken place, a future study of this crystalline substance should indicate where the sulphuric ester residue originally resided.

Barium α-Methyl Galactoside Sulphate.

Again the velocity constant K_{100} = ca. $1{\cdot}0 \times 10^{-3}$ for acid hydrolysis is similar to that for all the other cases. Alkaline hydrolysis proceeds in a manner like that of the glucoside compound but a detailed study of this reaction must be deferred.

SUMMARY.

1. Barium α-methyl glucoside sulphate was prepared. Hydrolysis with $\frac{N}{10}$-acid gave a hydrolysis velocity coefficient $K_{100} = 0.00106$. Alkaline hydrolysis with $\frac{N}{10}$-alkali yielded a crystalline compound which unfortunately was insufficient for analysis.

2. Barium α-methyl galactoside sulphate was prepared, and its properties studied. With $\frac{N}{10}$-acid it gave a hydrolysis velocity coefficient $K_{100} = 0.00101$.

Bibliography for Glucoside Section.

1.	Ohle	Biochem. Z., 1922, 131, 501.
2.	Helferich, Löwe, Nippe & Riedel	Z. physiol. Chem., 1923, 128, 141.
3.	Ohle & Thiel	Ber., 1933, 66, 525.
4.	Irvine & Robertson	Rec. trav. chim., 1938, 57, 575.

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