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THE SYNTHESIS OF

2:3:4-TRIMETHYL SACCHARDIAMIDE.

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INTRODUCTION

THE OCCURRENCE OF URONIC ACIDS IN NATURE

As constituents of polyuronides, uronic acids have a frequent and wide occurrence in nature. Much of the carbohydrate material in plants, which includes all pectic materials and plant gums, many plant mucilages, hemicelluloses and gel-forming substances and some microbial polysaccharides, belong to the group. Uronic acid residues have been shown to exist in the animal body where they may be linked either to complex polysaccharides or to proteins.

The uronic acids are reducing sugar acids formed by the oxidation of the terminal carbinol group (C atom 6) of the sugar, a process which apparently occurs readily in both plants and animals. Although a large number of uronic acids are theoretically possible, only three have been found to occur naturally, these being *d*-galacturonic, *d*-glucuronic and *d*-mannuronic acids.

PECTIC SUBSTANCES.

The pectic substances are a group of polyuronides which occurs in practically all plant materials, especially in young tissues and fruits.

All the pectins so far isolated give, on hydrolysis, in various proportions, *l*-arabinose, *d*-galactose, *d*-galacturonic acid and methyl alcohol.[1]

The constitution of the pectic acid[2,3,4,5,6] fraction of pectin has been investigated by many workers who concluded that the acid was of high molecular weight and consisted exclusively of *d*-galacturonic acid.

PLANT GUMS.

As a broad generalization, those substances described as gums usually occur as exudations on the bark of trees, or on fruits, particularly after wounding.

Plant gums always contain either d-galacturonic or d-glucuronic acid and generally at least two of the following sugars: d-galactose, l-arabinose, l-rhamnose, d-mannose and d-xylose.

Investigations into plant gum polyuronides have revealed the presence of glucuronic acid as a constituent of gum arabic,[7,8,9] mesquite gum,[10] damson gum[11,12] and cherry gum.[13,14] Galacturonic acid has been found in gum tragacanth.[15]

MUCILAGES.

Mucilages are secreted by the hairs of many plants, but may be obtained in any quantity only from certain seeds, such as those of mustard, flax, linseed, quince, etc. The structure of polyuronide mucilages[16] has been less thoroughly studied than that of plant gums, but in those investigated, the same general group of sugars have been found together with d-galacturonic acid. Both d-glucuronic and d-galacturonic acids have been reported to be present in mustard seed[17] and cress seed mucilages[18].

A recent investigation into the constitution of the unusual mucilage, slippery elm, which is found in the inner bark of the tree, revealed the presence of galactose, rhamnose and d-galacturonic acid.[19]

GEL-FORMING SUBSTANCES.

From various algae, and in particular from marine algae, mucilaginous or gel-forming carbohydrates, sometimes of polyuronide nature, may be

extracted. These polyuronides have unusual features which clearly distinguish them from the seed mucilages and plant gums.

Two kinds of mucilaginous solution may be extracted from marine algae, fucoidin or fucosan and algin or alginic acid. The former consists solely of calcium sulphate and fucose whilst the latter is built up of chains of d-mannuronic acid[20] units joined by 1:4 β-linkages.[16]

POLYURONIDE HEMICELLULOSES.

Polyuronide hemicelluloses, which seem to occur in two distinct configurational groups, are cell-wall polysaccharides which may be extracted from plant tissues. Many are known to consist of d-glucuronic acid, combined with d-xylose, l-arabinose, or both and to contain no hexose. A few hemicelluloses have been reported to contain d-galacturonic acid, d-glucose, d-mannose or d-galactose.

Polyuronide hemicelluloses extracted from hardwoods are usually composed of mono-methoxy glucuronic acid combined with a series of molecules of d-xylose.[21] The occurrence of d-glucuronic acid has also been reported in the hemicelluloses extracted from maize cobs,[22] cotton seed hulls,[23] hop flower[24] and phormium.[25]

A recent examination on the hemicellulose from Iceland moss was carried out by Granichstadten and Percival.[26] Methylation and hydrolysis yielded a mixture of sugar derivatives containing mainly glucose and small amounts of galactose and mannose and glucuronic acid.

In a study of hemicelluloses extracted from non-lignified sources such as leaves, pods and certain grasses, Buston found them to contain d-galacturonic acid combined with galactose and arabinose.[27]

MICROBIAL POLYSACCHARIDES.

Under this heading must be grouped a large number of bacterial and fungal carbohydrates of very different composition and function. Combined with protein, these polysaccharides are responsible for the immunizing powers of many bacteria, and the structure of the polysaccharide determines the exact or specific immunological response to these organisms. An interesting feature of these substances is that many contain uronic acid groups in the form of acidic polyuronides.

The polysaccharides produced from sucrose by such nitrogen-fixing organisms as Rhizobium radicicolum (clover strain) and Azobacter chroococcum contain glucuronic acid.[28,29] Both probably belong to the same class as the specific polysaccharides of pneumococcus Types II and III.

The characteristics of the polysaccharides of pneumococcus Types I - XXXII have been studied in some detail,[30] nine of them being found to contain uronic acid residues. Hydrolysis of Type I polysaccharide[31] has revealed the presence of galacturonic acid and an amino sugar derivative. Types III and VIII yield glucuronic acid.[32]

MUCOPOLYSACCHARIDES.

The high polymers, of which animal body tissues and fluids are formed, are all products of cellular origin, and it is therefore not surprising that these substances are closely related to those found among the constituent protoplasm of plants and micro-organisms. Stacey[33] classifies these compounds as mucopolysaccharides, some of which contain uronic acid residues, mucoproteins and mucolipids.

TABLE I.[33]

TYPE OF MUCOPOLYSACCHARIDE.	IDENTIFIED PRODUCTS OF ACID HYDROLYSIS OF CARBOHYDRATE PORTION.
1. Containing hexosamine and hexuronic acid.	
(a) Sulphate free.	
(1) Hyaluronic acid.[34] (from vitreous humor, umbilical cord, synovial fluid, ovarian tumor, Group "A" hemolytic streptococcus and skin.)	*N*-acetyl-*d*-glucosamine hexuronic acid (probably *d*-glucuronic acid.) acetic acid
(2) Type I pneumococcus specific polysaccharide.[31]	hexosamine *d*-galacturonic acid
(b) Sulphate containing.	
(1) Heparin.[35] (natural blood anticoagulant occuring in liver, heart and muscle).	sulphuric acid *d*-glucuronic acid *d*-glucosamine (unacetylated)
(2) Chondroitin sulphate.[36] (major constituent of cartilaginous tissue).	sulphuric acid *d*- glucuronic acid *N*-acetyl-chrondrosamine
(3) Mucoitin sulphate.[36] from gastric mucin, funis mucin, vitreous humor, cornea, serum mucoid and ovomucoid.)	sulphuric acid hexuronic acid *N*-acetyl-*d*-glucosamine
(4) Hyaluronic acid sulphate,(from the cornea.)	sulphuric acid hexuronic acid *d*-acetyl-hexosamine

TABLE I (Contd.)

2. Containing hexuronic acid but no hexosamine.

(a) Type II pneumococcus specific polysaccharide[30]	d-glucose l-rhamnose d-glucuronic acid
(b) Type III pneumococcus specific polysaccharide[32]	d-glucose d-glucuronic acid
(c) Type VIII pneumococcus specific polysaccharide[32]	d-glucose d-glucuronic acid

METHODS FOR DETERMINING THE STRUCTURE OF POLYURONIDE MOLECULES

In order to determine the structure, the polyuronide must first be isolated and purified. It must be hydrolysed; the sugars, uronic acid and other groups present must be identified, and their relative positions and types of the glycosidic linkages as well as the types of lactol ring must be determined. The length of the glycosidic chain, or of the branches, and the approximate size of the molecule must be established. When possible X-ray pictures of the material are made.

The acids most often used in hydrolysing polyuronides are sulphuric, hydrochloric and oxalic, but sometimes enzymatic hydrolysis has been found preferable. It has been shown that the ease and extent of hydrolysis may be influenced by the type of glycosidic linkage.

Fractional hydrolysis is often used to advantage. In this method the sugars are split off in successive stages by subjecting the material to successively more severe conditions of hydrolysis and seperating the fission products of each step. Such a method indicates the relative positions of the sugar units in the molecule. It also yields an aldotrionic or aldobionic acid from which can be obtained detailed information concerning these small units.

After the polyuronide has been partially hydrolysed by the mineral acid, the unhydrolysed portion of the molecule which contains the uronic acid, and the free sugars are separated from each other. The method used in this process varies with the polyuronide, the degree of hydrolysis and the catalysing acid. When hydrolysis has been prolonged and the uronic acid is present largely as an aldobionic acid, the solution is neutralised and filtered. After concentration under reduced pressure, the salt of

the aldobionic acid is precipitated by addition of alcohol[10,11,12] and separated from the alcoholic solution of the sugars.

The most valuable means of determining the structure of a polyuronide is by a study of the products obtained by hydrolysis of the completely methylated material, as developed by W.N. Haworth and associates.[6,16] The fact that the variously methylated monosaccharides may be separated quantitatively makes for their easy characterisation. Their relative amounts in the hydrolysate will indicate the extent of branching of the chain both on the basis of the number of end groups and of branching groups.

This method of methylation followed by hydrolysis may lead to the formation of uronic acids in which one or more of the hydroxyl groups are substituted by methoxyl groups. By conversion to a crystalline derivative, these compounds have been identified.

From the methylation and hydrolysis of certain polyuronides containing glucuronic acid, 2:3-dimethyl-d-glucuronoside methyl ester has been isolated. Subsequent hydrolysis to 2:3-dimethyl-d-glucuronic acid followed by bromine oxidation yielded 2:3-dimethyl-d-gluco-saccharic acid which was identified as the crystalline diamide.[9,12,29]

Any investigation of polyuronides by this method of methylation and oxidation would not be complete without knowing the characteristics of the crystalline diamide of fully methylated saccharic acid i.e. the diamide of 2:3:4-trimethyl-d-glucosaccharic acid.

The present investigation is an attempt to synthesise and characterise this compound.

By this method of examination of polyuronides a mixture of diamides of glucosaccharic acid would be obtained. These could be separated by

fractional crystallization and the proportions obtained would indicate the mode of linkage of the glucuronic acid in the polyuronide. As the corresponding diamides of mucic and mannosaccharic acids are known, the method of methylation and oxidation could be used as a general procedure.

THE CONSTITUTION OF CHARACTERISTIC POLYURONIDES

During the last decade, the method of methylation and hydrolysis has been used almost invariably for the critical examination into the structures of polyuronides. With the exception of perhaps, pectic acid and gum arabic, the complete picture of these molecules is far from clear, but the hydrolysis products obtained gives some idea as to their complexity.

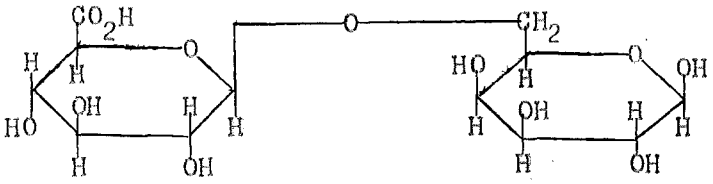
Pectic acid[3,4,5,6] yielded the methyl ester of 2:3 - dimethyl methyl-d-galacturonoside. This result, taken in conjunction with the extraordinarily high stability of pectic acid to acidic reagents and its high positive rotation, indicates that in the pectic acid molecule there is present a series of pyranose α-galacturonic acid residues mutually linked through positions 1 and 4.

By an analgous proof, degraded alginic acid was shown to consist of a series of pyranose β-mannuronic acid units combined through positions 1 and 4.[16,20,37]

The structure of gum arabic is very complex. Partial hydrolysis yielded l-arabinose, l-rhamnose, a disaccharide which was proved to be 3-galactosides-l-arabinose, and a resistant nucleus or core, degraded arabic acid. Further hydrolysis of the degraded polysaccharide resulted in the isolation of an aldobionic acid, which proved to be 6-d-glucuronosido-d-galactose.[8]

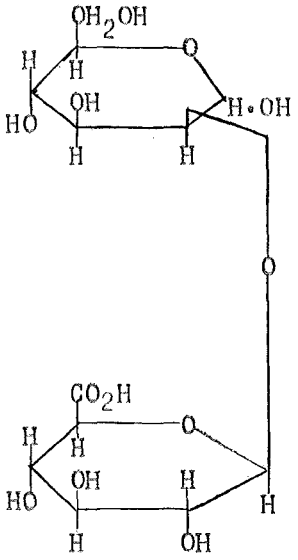
From a study of the products of hydrolysis of methylated arabic acid, Smith[9] was able to draw conclusions as to the positions of the arabinose, rhamnose and galactose units. He showed that the sugars are linked through positions 1 and 3, and 1 and 6, but the isolation of 2:3-dimethyl

ALDOBIONIC ACIDS.



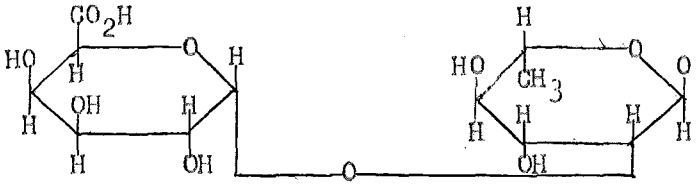
6-d-glycuronosido-d-galactose.

Occurs in gum arabic.



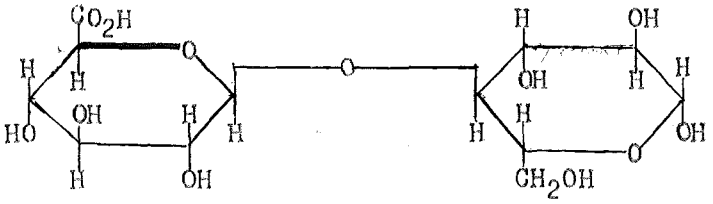
β-d-glycuronosido-
2-d-mannose.

Occurs in damson gum,
cherry gum.



2-d-galacturonido-l-rhamnose.

Occurs in slippery elm mucilage,
flax seed mucilage.



cellobiuronic acid.

Occurs in polysaccharides of pneumococcus
Types III and VIII.

methyl-d-glucuronoside proved that the 1:4-linkage is also present.

The aldobionic acid isolated from damson gum[11,12] and from cherry gum[13,14] has been shown to be 1:2-d-glycuronosido-d-mannose. Although the two gums differ in their chemical constitutions, in the degraded form they are very similar.

Hydrolysis products from methylated tragacanthic acid[15] have been characterised as the trimethyl derivatives of α-methylfucoside and methylxyloside, 3:4-dimethyl methxyloside, the methyl ester of 2:3-dimethyl methylgalactofururonoside and the methyl ester of a monomethyl-β-methylgalactopyruronoside. This clearly indicates the branched character of the molecular complex.

A quantitative examination of the methylated sugars obtained from the slippery elm mucilage[19] led to a structural formula of the carbohydrate portion. The aldobionic acid was proved to be 2-d-galacturonosido-l-rhamnose and identical with the acid obtained from the flax seed mucilage[38].

Some evidence concerning the ring structure and linkage of the component sugars of lucerne seed mucilage[6] has been obtained. Preliminary investigations into the structural configurations of mucilages from the seeds of plantago psyllium,[39] of plantago fastigiata[40] and of plantago lanceolata[41] have been carried out.

Little has been done in studying the size and structure of hemicellulose molecules. The equivalent weights calculated for these bodies[42] often indicate the presence of 16 to 18 units of d-xylose for each uronic acid unit. An investigation,[25] carried out in this laboratory, showed that the hemicellulose extracted from Phormium tenax

(N.Z. flax) is constituted of a main chain containing 9 or 10 xylo-pyranose residues united by 1:4-β-linkages, terminated at the reducing end by a complex, highly branched acid nucleus. Granichstadten and Percival[26] examined Iceland moss hemicellulose, which they showed to be a mixture of polysaccharides made up chiefly of β-glucose units linked through positions 1:2, 1:3, 1:4 and 1:6.

The polysaccharides of pneumococcus Types III and VIII[32] yield cellobiuronic acid when hydrolysed by acid. Methanolysis of Type III[43] gave 2:3:6-trimethyl-d-glucose and the anomeric forms of methyl 2:4-dimethyl-d-glucoside. The cellibiuronic acid units in the polysaccharide are thus linked through position 3 of the d-glucuronic acid residue. That is, the polysaccharide contains alternate 1:3- and 1:4- linkages.

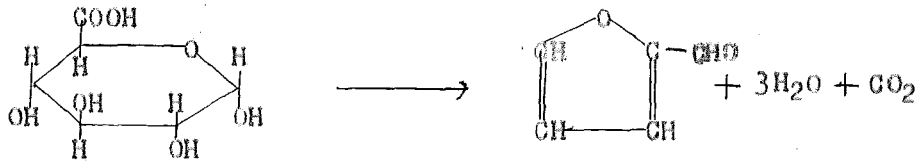
The structures of mucopolysaccharides are far from clear. Bray, Gregory and Stacey[44] isolated and characterised derivatives of glucuronic acid and of chrondrosamine from a methylated degraded chondroitin. It appears that the repeating unit is a trisaccharide and that the structure is a branched chain type.

DERIVATIVES OF GLUCURONIC ACID

Although the presence of uronic acids may have been established by colour tests or by carbon dioxide evolution, these acids form only a few, well-defined, insoluble derivatives with distinguishing characteristics and have consequently remained unidentified in many investigations. However, the identity of naturally occurring uronic acids has now been fully established by comparing the properties of their derivatives with synthetically prepared compounds from sugars of know structure.

The colour test consists of boiling a substance suspected of containing a uronic acid with strong hydrochloric acid solution in the presence of naphthoresorcinol.[45] On extracting with benzene, a purple pigment is produced. This test has now been adopted for quantitative estimations, the intensity of the colour of an amyl alcoholic extract being measured with a photoelectric colorimeter.[46]

The evolution of carbon dioxide and the formation of furfural after boiling uronic acids with 12% hydrochloric acid has also been adopted for the quantitative estimation of the acid.[1] Owing to possible degradation

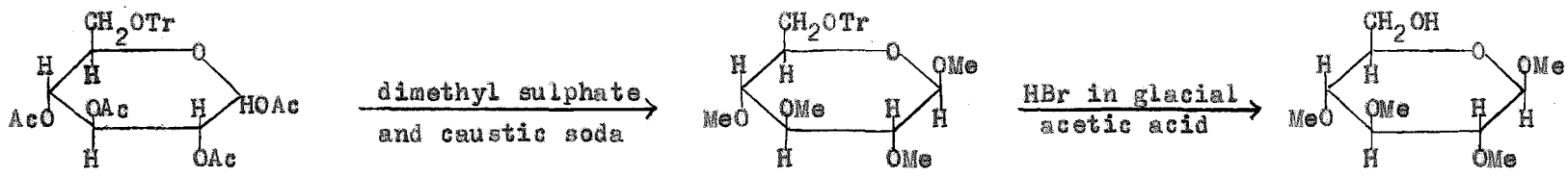


d-glucuronic acid furfural

or reversion products being formed, the furfural is not produced in quantitative yield.

Norman[1] has tabulated the constants for many derivatives of

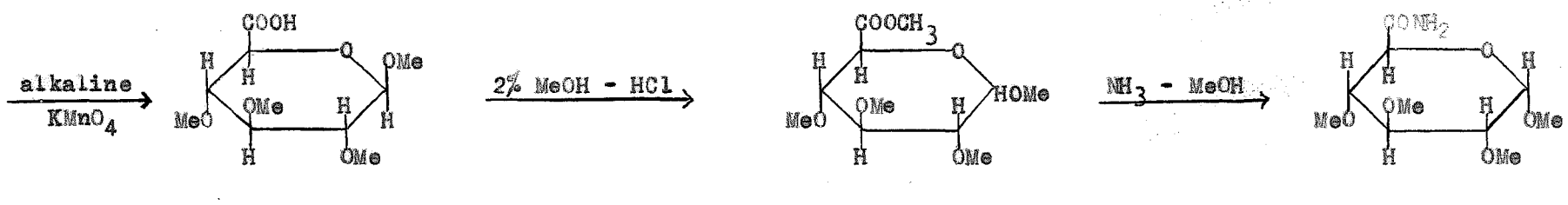
Synthesis of 2:3:4-trimethyl ∝-methylglucuronamide.[53]



6-trityl 1:2:3:4-tetra-acetyl glucoside

6-trityl 2:3:4-trimethyl β-methylglucoside

2:3:4-trimethyl β-methylglucoside

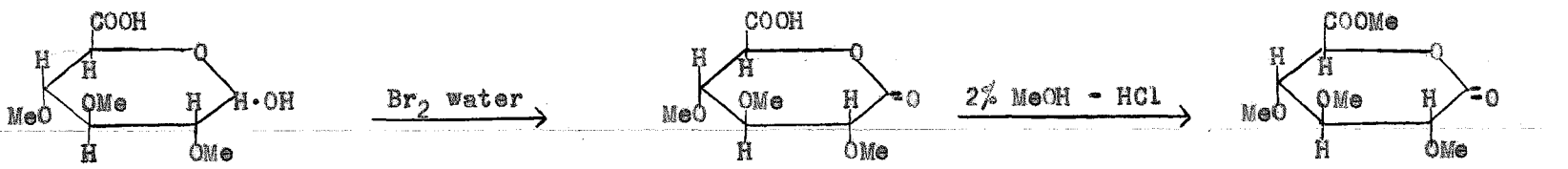


2:3:4-trimethyl β-methylglucuronoside

methyl ester of 2:3:4-trimethyl -methylglucuronoside

amide of 2:3:4-trimethyl ∝-methylglucuronoside

The conversion of the hydrolysis product 2:3:4-trimethyl glucuronic acid to crystalline 2:3:4-trimethyl δ-saccharolactone methyl ester.

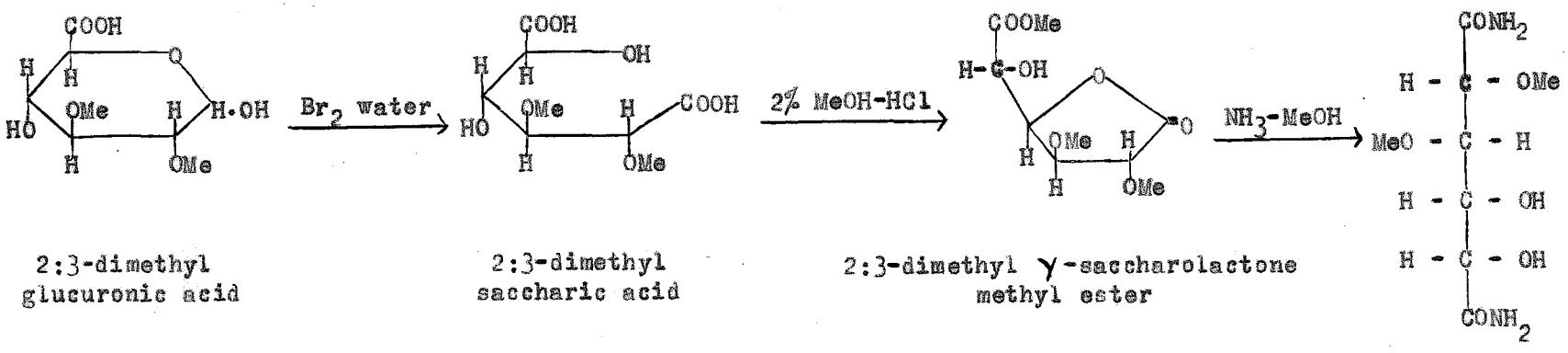


2:3:4-trimethyl glucuronic acid

2:3:4-trimethyl δ-saccharolactone

methyl ester of 2:3:4-trimethyl δ-saccharolactone

The conversion of the hydrolysis product 2:3-dimethyl glucuronic acid to crystalline 2:3-dimethyl saccharamide.[9]



2:3-dimethyl glucuronic acid

2:3-dimethyl saccharic acid

2:3-dimethyl γ-saccharolactone methyl ester

2:3-dimethyl saccharamide

d-glucuronic acid. Such derivatives are the cinchonine and quinine salts, the complex hydrazone and osazone formed by the addition of ρ-bromophenylhydrazine, the oxime of the lactone, the triacetyl glucurone and two of the methyl compounds. Heidelberger and Goebel[47] identified d-glucuronic acid as potassium acid saccharate.

In order to facilitate the identification of the methylated glucuronic acid, which is a hydrolysis product of fully methylated polyuronides, it is necessary to synthesise glucuronic acid derivatives, containing two or more methoxyl groups. Many such compounds have found frequent use in the structural determination of polyuronides during the past few years.

Direct oxidation of the hexoses is not as yet practicable[48] and poor yields of uronic acids result from the oxidation of the glycosides.[49] If, however, all the hydroxyl groups in a particular hexose, with the exception of that on C_6, are suitably protected, oxidation proceeds smoothly and the corresponding uronic acid is produced. Thus, the oxidation of 1:2:3:4-diacetone galactose with potassium permanganate in alkaline solution affords 1:2:3:4-diacetone galacturonic acid.[50] The oxidation of 1:2:3:4-tetra-acetyl glucose with potassium permanganate in glacial acetic acid yields 1:2:3:4-tetra-acetyl glucuronic acid[51] and this, in turn, can be transformed into glucuronic acid. Similarly, oxidation of 1:2-monoacetone 3:5-benzylidene glucose with alkaline potassium permanganate gives 1:2-monoacetone 3:5-benzylidene glucuronic acid.[52]

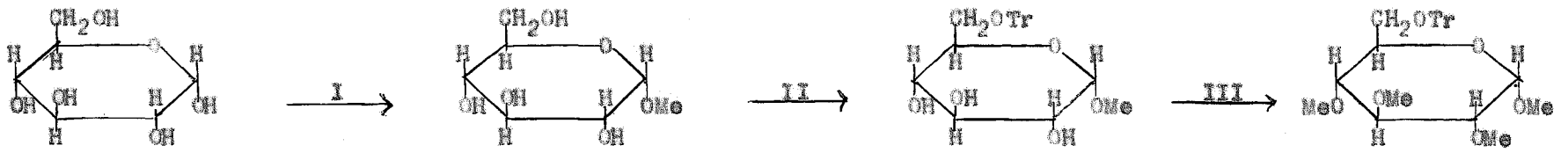
A summary of the synthesis[53] of 2:3:4-trimethyl ∝-methylglucuronamide is shown on the opposite page. Another crystalline compound used for the structural studies of aldobionic acids is the methyl ester of 2:3:4-trimethyl saccharolactone. The characterisation of 2:3-dimethyl glucuronic acid is

performed by its conversion to a crystalline diamide.[9] The method used is tabulated on page 14a.

Although not reported in the literature as products obtained from polyuronides after methanolysis, other methyl derivatives of glucuronic acid have been synthesised. Smith[54] obtained 2:3:5- and 2:4:5-trimethyl saccharo-diamides to prove that the oxidation of glucose with nitric acid gave a mixture of glucosaccharo-3:6- and -1:4- lactones. Owen, Peat and Jones[56] showed that the action of methanol on d-glucuronolactone depended on the temperature. In the cold, methyl β-d-gluco-fururonoside 3:6-lactone was obtained. From this was prepared crystalline 2:5-dimethyl glucofururonamide and 2:3:5-trimethyl glucosaccharolactone methyl ester. Dewar and Fort[55], starting with 4:6-ethylidene β-methylglucoside, isolated 2:4- and 3:4- dimethyl β-methylglucosides. Oxidation of these, with potassium permanganate, would afford the unknown 2:4- and 3:4-dimethyl methylglucuronosides.

T A B L E II.

PRESENT INVESTIGATION



PRESENT INVESTIGATION

A summary of the synthetic methods used for the synthesis of 2:3:4-trimethyl saccharidiamide is set out on the accompanying page. In this section, each of these reactions is discussed in turn from the theoretical and practical viewpoint.

DISCUSSION ON SYNTHETIC METHODS.

GLUCOSIDE FORMATION REACTION I.

The preparation of methyl glycosides by the action of methanolic hydrogen chloride on monosaccharides was first investigated by Fischer.[57]

In 1915, Bourquelot[58] prepared ∝-methyl-d-glucoside by boiling a solution of glucose in methanol containing 0.25% hydrogen chloride for eighty hours, obtaining a yield of about one tenth of the glucose used. Using this method, Hudson[59] prepared methylyxloside and some similar compounds in shorter time.

In the present investigation the method of Patterson and Robertson[60] was used. They obtained a 46% yield of ∝-methyl-d-glucoside by dissolving glucose (one part) in methanol (two parts) containing 3% hydrogen chloride, and allowing the solution to boil under reflux for four and a half hours.

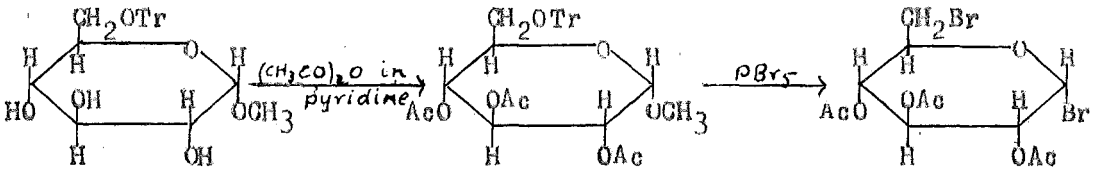
DISCUSSION

No difficulties were experienced in carrying out this reaction. The process was repeated (Reaction V) to ensure complete conversion. This was necessary before oxidation, as C_1 if unprotected, will be affected.

TRITYLATION REACTION II.

Helferich et alia[61] used triphenyl-chloromethane as an organic reagent to prepare crystalline trityl ethers of simple primary and secondary alcohols. Since the reaction was conducted in the presence of an excess of pyridine, this method could be applied successfully to the study of glycosides which are sensitive to acids.

Following this procedure, Helferich and Becker[62] obtained a monotrityl-∝-methyl d-glucoside as a crystalline compound. Treatment with acetic anhydride in pyridine yielded a triacetyl derivative which reacted with excess phosphorous pentabromide to give aceto-bromo-glucose, thus establishing the position of the trityl residue on carbon 6.[63]



6-trityl-∝-methyl d-glucoside

6-trityl 2:3:4-triacetyl ∝-methyl d-glucoside

aceto dibromo glucose

In their study of trityl derivatives of glucose, fructose, and mannose it was observed by Helferich et alia[64,65] that trityl chloride exerted a marked preference for primary hydroxyl groups. The results of Josephson,[66] who worked with di- and tri- saccharides, and of Helferich and Koester,[67] who worked with starch and cellulose, supported this statement. Hockett, Fletcher and Ames[69] pointed out that trityl chloride's preference for esterifying primary hydroxyl groups in presence of secondary ones was due to:-

(1) The relatively high reactivity of the primary hydroxyl group.

(2) The crystallising properties of trityl ethers.

Although no dityityl derivatives of glucose have been reported, recent work proves that trityl chloride may react with secondary hydroxyl groups. This was demonstrated by Hockett and Hudson[68] who obtained trityl derivatives of ∝-methyl-d-xyloside, β-methyl-d-xyloside and β-methyl-d-arabinoside. Other evidence in this direction was furnished by the isolation of tri-trityl derivatives of fructose and of sorbose[70] and of 3:5-ditrityl methylxyloside.[71]

As trityl groups may easily be hydrolysed off, they may be used for blocking hydroxyl groups in order to synthesise sugar derivatives. This has proved an effective method for preparing glycuronosides.

DISCUSSION

In this present investigation, the method of Helferich and Becker, as described in "Polarimetry, Saccharimetry and the Sugars" by Bates, (Circular of National Bureau of Standards C440, 1942, p.512) was used for the tritylation of ∝-methyl d-glucoside. The detailed account ends as follows: "The crude product is recrystallised from 5 parts of alcohol. When air-dried the compound contains 1.5 molecules of alcohol of crystallisation and melts at 80° C. if heated rapidly. Methyl 6-trityl-∝-d-glucopyranoside, when alcohol free, melts at 151° to 152° C. and gives $[\alpha]_D^{16}$ = 86.3° (pyridine)."

The first unusual feature was the formation of crystalline material in the pyridine solution during the reaction time. Jackson, Hockett and Hudson[72] reported a similar phenomenon during the tritylation of methyl-xyloside. Analysis of the crystals showed them to be triphenyl carbinol, which proved that moisture must have been present. As the reaction flask was thoroughly dried before use and was provided with a calcium

chloride tube, the source of moisture must have come from the pyridine or the ∝-methyl d-glucoside. The former possibility was ruled out as the pyridine used had the physical constants of a pure solvent. It was therefore conluded that the drying of the ∝-methyl d-glucoside for 5 hours at 40° C. in a vacuum oven was not sufficient to dehydrate it. Smith, preparatory to making 6-trityl ∝-methylgalactoside, dehydrated ∝-methylgalactoside (a syrup) for several hours in a vacuum at 110° C.

The crude 6-trityl ∝-methylglucoside was taken up in ether but during the process of washing, the solution became a solid mass. This surprising result is explained by a summary of observations given below.

After recrystallisation from alcohol, an analysis of the well formed, colourless, needle-like crystals showed them to have a low trityl content. Further recrystallisations did not alter the value. Similar results were obtained when the process of tritylation was repeated. Both products had a melting point of 128° C., a figure which is not recorded in Bates or the American Chemical Abstracts, but only in the original work of Helferich and Becker.

A methoxyl determination expelled any doubt about the completeness of the tritylation, as the result indicated the presence of 1.5 moles of alcohol of crystallisation, a fact which is in agreement with the work of Helferich and Becker.

Experiments on the compound showed:-

(1) That when the product was recrystallised from alcohol, it contained 1.5 molecules of alcohol of crystallisation.

(2) That after drying the compound in a P_2O_5 drier, the alcohol of crystallisation was still present.

(3) That drying the compound at 70° C. in a vacuum oven also failed to remove the alcohol of crystallisation.

(4) That the compound was

(a) easily soluble in chloroform, acetone, benzol and ethyl acetate.

(b) soluble in comparatively large volumes of methanol and ethanol.

(c) practically insoluble in ether, thus explaining a difficulty referred to earlier.

From these observations, all the difficulties would be overcome if the crude yellow-brown syrup of 6-trityl ∝-methylglucoside was taken up in chloroform, acetone, benzol or ethyl acetate. The latter solvent is undesirable as ethyl acetate of crystallisation has been reported. It was noted that the melting point of the compound containing alcohol of crystallisation changed from 128° C. to 140° C. after recrystallisation from ethyl acetate, a result in conformity with the work of Helferich and Becker.

METHYLATION

Purdie and Irvine[73] showed that their method of methylation using methyl iodide and silver oxide, oxidised sugars unless Cl was protected. Using -methyl d-glucoside they obtained trimethyl methyl-glucoside as the main product, indicating that the primary hydroxyl group was hard to methylate. Even using an excess of reagents, only a small amount of tetramethyl methylglucose was isolated.

The mild experimental conditions is the main advantage of the method. Drastic changes such as degradation, racemisation, Walden inversion or interconversion of glycosides do not take place.

The method of Haworth[74] has often been used to advantage. By this procedure the sugar is dissolved or suspended in water, and dimethyl sulphate (three times theoretical amount) and 30% caustic soda are added over a period of five hours. The reaction, which proceeds best in a slightly alkaline medium, is carried out at 70° C. The excess dimethyl sulphate is then boiled off and the sugar extracted with chloroform.

Musket[75] developed a process of methylation which depends on the methylglycoside being soluble in liquid ammonia. This is subjected to the action of sodium or potassium to form a salt, which, if treated with methyl iodide gives a fully methylated product.

Pacsu and Trister[76] used a combination of Haworth's[74] and Freudenberg and Hixon's[77] methods. The former process was used until the secondary hydroxyl groups were occupied. Then the sodium alcoholate of the carbohydrate was prepared in ether solution and methyl iodide added to complete the methylation.

Fear and Menzies[78] showed that ∝-methylglucoside readily formed a trithallium derivative which reacted with boiling methyl iodide,

giving a trimethyl methylglucoside. This method of methylation has been applied to simple sugars[79,81] and to polysaccharides[80]. Also, 6-trityl ∝-methyl galactoside can be converted directly to 6-trityl 2:3:4-trimethyl ∝-methylgalactoside.[82]

DISCUSSION

The reasons for the choice of Purdie and Irvine's methylation process may be summarised as follows:-

(1) Better yields and fewer methylations are necessary as compared with Haworth's method.

(2) The unavailability of the apparatus prevented the use of Musket's method.

(3) The fact that the primary hydroxyl group was already occupied, ruled out the method of Pacsu and Trister.

(4) Thallous salts are expensive and unstable. For this reason the process of Fear and Menzies was not tried.

The interesting feature noted was that after three or more methylations, the crystalline compound, which was deposited during removal of the solvent, had the composition of 6-trityl di-methyl ∝-methylglucoside. It was thought that the comparatively large trityl group hindered the methylation of the hydroxyl group (on C4) which was nearest it. This fact was borne out by subsequent results.

Six methylations did not completely alkylate the compound. However the partially and fully methylated products were easily seperated as the latter, which was a yellow syrup after removal of the solvent, took three weeks to crystallise into pale yellow hydroscopic crystals. A

portion of these was recrystallised from acetone before an anlysis was carried out.

Helferich and Becker[62] used only two alkylations to methylate fully 6-trityl ∝-methylglucoside. Using smaller quantities of reagents, other workers found it necessary to repeat the process a number of times to obtain the required compound. Thus, Smith[9] used Haworth's method six times and Purdie and Irvine's twice to prepare 6-trityl 2:3:4-trimethyl ∝-methylgalactoside. Five methylations were required to replace the acetyl groups in 6-trityl 1:2:3:4-tetra-acetyl glucose.[53] Luckett and Smith[5] found it necessary to repeat the process of methylation nine times to produce 6-trityl 2:3:5-trimethyl methylgalacto-furanoside.

DETRITYLATION REACTION IV.

The methods of detritylation are based on the fact that trityl groups are hydrolysed in an acid medium.

Helferich and Becker[62] used 1.2% methanolic hydrogen chloride to detritylate 6-trityl 2:3:4-trimethyl ∝-methylglucoside. Although triphenyl carbinol was obtained, the sugar portion could not be isolated. A widely used method due to Helferich and Klein[83] makes use of hydrogen bromide in acetic acid at 0° C. The process adopted by Smith[9] was to saturate an ethereal or chloroform solution of the tritylated sugar with dry hydrogen chloride at 0° C., and to allow the mixture to stand for 2 hours at room temperature to complete the reaction.

DISCUSSION

At this stage the product was divided into two portions, this being done in event of a later reaction being unsuccessful. Smith's method was used successfully for the detritylation of both portions of 6-trityl 2:3:4-trimethyl ∝-methylglucoside. The process was carried out in chloroform, as 6-trityl 2:3:4-trimethyl ∝-methylglucoside proved to be insoluble in ether.

Analysis of the yellow syrup obtained showed that either detritylation was not complete or that the product was not fully methylated. Fractional distillation of a later product proved the latter to be the correct explanation.

OXIDATION REACTIONS VI, IX AND XII.

Three methods of oxidation have been used successfully for the structural determinations and for the synthesis of uronic acids.

Pryde and Williams[84] used nitric acid to determine the structure of glucuronic acid of animal origin. The type of ring structure of the acid can be deduced from the examination of the oxidation products. This process of oxidation when applied to the methyl ester of 2:3:4-trimethyl ∝-methylglucuronoside produced i-xylotrimethoxy glutaric acid and d-dimethoxy succinic acid, which were identified as crystalline diamides. These results prove the ester to have a 1:5 lactone ring.[9] Similarly, 2:3-dimethyl δ-saccharolactone methyl ester was shown to contain a 1:4 lactone ring.[9]

This oxidising agent has been used with some success for the conversion of sugar derivatives to saccharic acids. Thus Smith, Stacey and Wilson[53] used nitric acid to obtain 2:3:4-trimethyl δ-mannonolactone from 2:3:4-trimethyl mannose. Similarly, the γ-lactone of 2:3:5-trimethyl mucic acid may be obtained.[5] However, the severe conditions are not conducive to good yields.

To oxidise C_6 only and thus form a glycuronoside, potassium permanganate has been used. Stacey[51] oxidised tetra-acetyl glucose with potassium permanganate in acetone and acetic acid to obtain an excellent yield of tetra-acetyl d-glucuronic acid. To complete the reaction, he allowed the solution to be stirred slowly for 2 days during which time portions of oxidising agent were added. Using an alkaline medium of potassium hydroxide in water, Smith, Stacey and Wilson[53] employed a similar procedure to obtain 2:3:4-trimethyl ∝-methylmannuronoside in good yield from 2:3:4-trimethyl ∝-methylmannoside. Similarly, Luckett

and Smith[5] prepared 2:3:5-trimethyl β-methylgalacturonoside.

The use of bromine as an oxidising agent has been employed for the conversion of glycuronic acid derivatives or monosaccharide derivatives, having C1 and C6 free, to the corresponding dibasic acids which immediately revert to the lactone. Excess bromine is used and the reaction time controlled by the following facts:-

(1) The temperature at which the process is carried out.

(2) After removal of bromine by aeration, the solution should give a negative Fehling's solution test and react acid to Congo red paper. This denotes that the reaction is complete.

This reagent is less severe than nitric acid and hence is preferable. Smith[9] prepared 2:3:4-trimethyl δ-saccharolactone from 2:3:4-trimethyl glucuronic acid by bromine oxidation. Smith, Stacey and Wilson[53] obtained the corresponding derivative of δ-mannonolactone by the oxidation of 2:3:4-trimethyl mannose and of 2:3:4-trimethyl mannuronic acid. Similarly, 2:3:4-trimethyl δ-galactonolactone has been prepared from 2:3:4-trimethyl galactose.[9] 2:3:5-Trimethyl γ-galactonolactone has also been obtained by bromine oxidation of 2:3:5-trimethyl galactose.[5] The dimethyl glycuronic acids have also been oxidised by this means.[9]

The process by which Maurer and Drefahl[85] oxidised carbohydrates with nitrogen dioxide is beset with many difficulties. It has been shown[86] in this laboratory that sugar nitrates are obtained if the secondary hydroxyl groups are not protected.

DISCUSSION

Reaction VI.

For the conversion of a portion of 2;3:4-trimethyl α-methylglucoside

to 2:3:4-trimethyl ∝-methylglucuronoside, Stacey's method of oxidation was employed. A number of difficulties were encountered, some of which could not be overcome.

When the reaction was completed, the manganese oxides were separated by centrifuging the solution for 1 hour, but even after this time it was obvious that some unused potassium permanganate was present in the bulk phase. After removal of the water, a syrup was left, this being interspersed with permanganate. This was taken up in chloroform and washed several times with dilute sulphuric acid to remove the permanganate. After washing with distilled water, the chloroform layer was evaporated to dryness to give a small yield of yellow syrup. The washings were neutralised to litmus, evaporated to dryness and taken up in chloroform. After washing, removal of the solvent gave another small amount of yellow syrup. Repetition of the procedure produced a similar yield.

These results indicate that this glucuronoside derivative is soluble in water, whereas, those of Stacey show that the corresponding acetyl derivative is not.

Better results might have been obtained had the excess potassium permanganate been reduced with hydrogen peroxide before the solution was centrifuged.

Reaction IX.

No difficulties were encountered when Smith, Stacey and Wilson's oxidation process was used to prepare a second portion of 2:3:4-trimethyl ∝-methylglucuronoside. As potassium hydroxide was present in the

reaction medium, a potassium salt was obtained. This was converted to the methyl ester of 2:3:4-trimethyl ∝-methylglucuronoside by boiling the salt with 2% methanolic - hydrogen chloride. (Reaction X).

Fractional distillation of the syrup, followed by the analysis of the fractions, showed the methyl ester of 2:3-dimethyl ∝-methylglucuronoside to be present. This result is in agreement with a theory previously mentioned, i.e. that trityl groups hinder the methylation of the hydroxyl group on C4. Confirmation of this was given by construction of a model of 6-trityl 2:3-dimethyl ∝-methylglucoside.

Of the three boiling point fractions that were obtained, the first and third were shown to be pure samples of the methyl esters of 2:3:4-trimethyl and 2:3-dimethyl ∝-methylglucuronosides respectively. However, the boiling point of the former was found to be lower than that recorded in the literature. The second fraction had an intermediate methoxyl content so was methylated with Purdie and Irvine's reagents and then put aside.

Reaction XII.

Before 2:3:4-trimethyl ∝-methylglucuronoside methyl ester can be converted to a dicarboxylic acid, the methyl group on C1 must be removed by hydrolysis (Reaction XI).

In strongly acidic solutions, sugars are easily degraded, and it is therefore necessary to use mild conditions for hydrolysing glycosidic linkages. The method widely used is to heat a solution of the glycoside in dilute acid (0.1 N - 1.0 N), on a boiling water bath, until the specific rotation, which is measured at regular intervals, becomes constant.

Having successfully hydrolysed the methyl ester of 2:3:4-trimethyl ∝-methylglucuronoside to 2:3:4-trimethyl glucuronic acid, the dibasic acid was prepared using bromine as the oxidising agent. No difficulties were encountered during this reaction.

ESTERIFICATION REACTIONS VII, X AND XIII.

Diazomethane is an invaluable reagent for the methylation of carboxylic acids or other substances which are strongly or moderately acidic. This yellow gas is employed in the form of an ethereal solution. The reaction is as follows:

$$R{\cdot}COOH + CH_2N_2 \longrightarrow R{\cdot}COOCH_3 + N_2$$

In practice, an excess of diazomethane is employed, and at the end of the reaction the unused material is removed by evaporation of a part or all of the ether. Although the reagent is expensive, it provides a rapid and elegant method for the preparation of a small quantity of an ester in very pure condition.

Because of the more severe conditions, the use of 1% or 2% methanolic hydrogen chloride gives smaller yields. However, this method has been widely adopted for esterifying sugars. It consists of boiling a solution of the sugar in methanolic hydrogen chloride for 6 to 10 hours, neutralising the excess acid and evaporating off the solvent.

DISCUSSION

Reagent VII.

The failure of Stacey's oxidation method to give a good yield of glucuronoside has already been discussed. It was hoped that the small amount obtained could be purified by converting the corresponding methyl ester to a crystalline amide. This ester was obtained as a yellow syrup after boiling the sample of 2:3:4-trimethyl α-methylglucuronoside with 2% methanolic hydrogen chloride and removing the solvent. Analysis of

the syrup showed it to be a mixture of the methyl esters of 2:3-dimethyl and 2:3:4-trimethyl α-methylglucuronosides. However, these were not separated by distillation, it being hoped that both the corresponding amides would be crystalline and could thus be separated. The amide of 2:3-dimethyl α-methylglucuronoside is not reported in the literature.

The potassium salt of 2:3:4-trimethyl α-methylglucuronoside was also esterified with 2% methanolic hydrogen chloride (Reaction X). No difficulties were encountered, both reactions producing good results.

Reaction XIII.

Diazomethane was used to convert 2:3:4-trimethyl δ-saccharolactone to the corresponding methyl ester. Separation of a small portion of the pale yellow crystals on which a melting point was taken, confirmed the results of Smith[9] for this constant. Analysis of the syrup indicated that it was quite pure. Considering these results, and the loss incurred due to decomposition, it was decided not to distil the ester, but to convert it directly to the diamide.

AMIDE FORMATION REACTION VIII AND XIV.

DISCUSSION

Reaction VIII.

After forming the amide of 2:3:4-trimethyl ∝-methylglucuronoside in the prescribed manner, the methanol was removed to give a yellow syrup. Many solvents and their combinations were used in an attempt to crystallise the syrup, but without avail. It seems that the small amount of the amide of 2:3-dimethyl methylglucuronoside that was present, prevented the crystallisation of the known amide of 2:3:4-trimethyl methylglucuronoside. This conclusion explains why 2:3-dimethyl glucuronic acid, a hydrolysis product of certain methylated polyuronides, has always been identified as the crystalline 2:3-dimethyl saccharidiamide.

Reaction XIV.

The usual reaction time of two days proved insufficient to convert 2:3:4-trimethyl δ-saccharolactone methyl ester to 2:3:4-trimethyl saccharidiamide.

The hydroscopic nature of the crystals, and their slow formation, showed them to have the characteristic properties of most uronic acid derivatives. Hence, the suggested method for the identification of the components of a mixture of uronic acids by conversion to their crystalline amides, cannot be adopted as a general procedure.

SUMMARY

1. The synthesis of 2:3:4-trimethyl saccharidiamide from d-glucose has been effected by the following route:
d-glucose ⟶ ∝-methyl d-glucoside ⟶ 6-trityl ∝-methyl d-glucoside ⟶ 6-trityl 2:3:4-trimethyl ∝-methylglucoside ⟶ 2:3:4-trimethyl ∝-methylglucoside ⟶ 2:3:4-trimethyl ∝-methylglucuronoside methyl ester ⟶ 2:3:4-trimethyl glucuronic acid ⟶ 2:3:4-trimethyl δ-saccharolactone ⟶ 2:3:4-trimethyl δ-saccharolactone methyl ester ⟶ 2:3:4-trimethyl saccharidiamide.

2. 6-Trityl ∝-methyl d-glucoside, after recrystallisation from ethyl alcohol, contained 1.5 moles of alcohol of crystallisation. This confirms the results of Helferich and Becker.

3. Methylation of the hydroxyl group on C4 was hindered by the presence of a trityl group on C6. Confirmation of this was given by construction of a model of 6-trityl 2:3-dimethyl ∝-methylglucoside. The isolation of a pure sample of the methyl ester of 2:3-dimethyl ∝-methylglucuronoside also supported the statement.

4. Stacey's method proved impracticable for oxidising 2:3:4-trimethyl ∝-methylglucoside, as the corresponding glucuronoside was soluble in water. Smith, Stacey and Wilson's oxidation method produced the potassium salt of 2:3:4-trimethyl ∝-methylglucuronoside in good yield.

5. In the presence of the amide of 2:3-dimethyl methylglucuronoside which has not been reported, the known amide of 2:3:4-trimethyl ∝-methylglucuronoside could not be crystallised.

6. 2:3:4-Trimethyl saccharidiamide was isolated as a hydroscopic syrup which crystallised only with the greatest difficulty. Hence, it is not a convenient derivative for characterisation of 2:3:4-trimethyl glucuronic acid.

PREPARATION AND PURIFICATION OF CHEMICALS

METHYL ALCOHOL.[88]

Commercial "absolute" methanol (300 mls.) and magnesium turnings (15 gms.) were mixed in a bolt-head flask (3 litres), to which was fitted a double surface reflux condenser. Iodine (3 gms.) dissolved in a little methanol, was added slowly to the reaction flask which was cooled by a stream of water.

When the initial vigorous reaction had subsided, the solution was warmed on a water bath until all the magnesium had been converted to the methylate. A further amount of commerical methanol was then added, and the mixture refluxed on a water bath for 8 hours. After allowing the solution to stand overnight, the methanol was slowly distilled through a long fractionating column packed with glass helices After rejecting the initial fraction, the main portion distilling at 64.8° C. was collected.

Found	$\eta_D^{20} =$	1.3306
c.f	$\eta_D^{20} =$	1.3312 (I.C.T.)

METHYL IODIDE[89]

Methyl alcohol (1080 mls.) and red phosphorus (240 gms.) were mixed in a three litre bolt-head flask fitted with a double surface reflux condenser. Powdered iodine (2400 gms.) was added in small portions over a period of five hours, with frequent shaking. During the addition of iodine, the flask was cooled in a water bath so as to control the vigorous reaction. The mixture was then refluxed

for one hour (bath temperature 50° C.) and allowed to stand overnight.

By immersing the flask in a water-bath controlled at 55° C., the methyl iodide distilled into a receiver immersed in an ice-salt mixture. The distillation was continued until no oily drops remained in the condenser, the residue being discarded. The distillate was washed in turn with equal volumes of 10% aqueous sodium hydroxide, 10% aqueous sodium thiosulphate, and distilled water, thus removing alcohol and free iodine.

After drying overnight over anhydrous calcium chloride, the methyl iodide was redistilled at a rate of one drop per second through a double surface condenser into a dark storage bottle. On completion of the distillation, the bottle was tightly stoppered and kept away from light.

Yield: 2354 gms. (88% theoretical)

Found: B.P. = 42.5° C.

n_D^{21} = 1.5296

c.f. n_D^{21} = 1.5293 (I.C.T.)

TRITYL CHLORIDE[89,90]

A mixture of benzene (thiophene free; 452 mls.) and carbon tetrachloride (B.P., 88 mls.) was dried over anhydrous calcium chloride for 24 hours, and filtered into a one litre bolt-head flask, to which a reflux condenser and calcium chloride tube were attached. A second opening in the neck of the flask was connected by means of a wide rubber tube to an Erlenmeyer flask (250 mls.) containing powdered aluminium chloride, which could then be added without its exposure to the air.

Aluminium chloride was added to the boiling mixture of benzene-carbon tetrachloride at a rate so that the heat of the reaction kept the mixture at this temperature. The reddish-black mixture was then heated on a water bath till the evolution of hydrogen chloride subsided. With constant stirring, the solution, after being cooled, was poured slowly into crushed ice. Benzene (thiophene free, 400 mls. in all) was added from time to time to keep the trityl chloride in solution. After separation of the benzene layer, the aqueous suspension was extracted with a further quantity of benzene (100 mls.). The combined benzene solutions were extracted twice with 20% hydrochloric acid (800 mls. portions) and finally with concentrated hydrochloric acid (500 mls.).

After drying over anhydrous calcium chloride, the greenish-brown benzene extract was transferred to a one litre distilling flask which was heated to 120° C. on a glycerol bath to remove the solvent. The dark brown product was transferred to a round bottom flask (500 mls.) fitted with a calcium chloride tube. After cooling to 40° C., acetyl chloride 5 mls.) was added, and the mixture heated on an oil bath almost to B.P. The flask was cooled rapidly by means of tap water, and then thoroughly shaken. This caused rapid crystallisation of trityl chloride and after immersing the flask in ice-water for several hours, the product was filtered off and washed with ligroin (B.P. 70° - 90° C.) three times with 60 mls. portions. The solvent was removed by placing the crystals in a vacuum dessicator over paraffin shavings.

Yield: 112 gms. (56% theoretical based on aluminium chloride)

Found: M.P. 111° - 112° C.

SILVER OXIDE[91]

A hot filtered barium hydroxide solution (300 gms. $Ba(OH)_2 \cdot 8H_2O$ in 3 litres of distilled water) was added to a hot solution of silver nitrate (300 gms. in 1.5 litres of distilled water) in a five litre flask, and the precipitated silver oxide washed with boiling distilled water on a Buchner funnel till all excess barium hydroxide had been removed. The precipitate was dried on a porous plate and then dried at 60-80° C. for several days, after which the oxide was finely powdered, stored in a dark bottle, and kept away from light.

SILVER CARBONATE[91]

A saturated solution of potassium carbonate (35 gms.) was added to a saturated solution of silver nitrate (85 gms.) The precipitated silver carbonate was filtered, thoroughly washed with cold distilled water, and dried at 100-105° C. The yellow compound was stored in a well-stoppered dark bottle away from light.

DIAZOMETHANE[92]

The reaction was carried out in a distilling flask (100 mls.) with a high side arm connected to a double surface reflux condenser. In the flask was placed 25% methanolic potassium hydroxide (2 mls.) and ether (10 mls.). The flask was provided with a small dropping funnel with the stem extending well below the side arm and in this was added ether (20 mls.), followed by nitrosomethylurethane (1 ml.). The flask was heated gently on a water bath, and the solution was run in from the dropping funnel at about the rate at which ether distilled from the reaction vessel. The diazomethane formed was carried over with the ether and the yellow distillate collected in a flask cooled in an ice

bath. Some additional ether (10 mls.) was used to rinse the dropping funnel and the distillation continued until the condensate became colourless.

The diazomethane in the ethereal solution amounted to about 0.2 gms. or 0.005 mole.

PYRIDINE

Pyridine (pure) was stored in a dark bottle over anhydrous barium oxide and kept away from light. It was filtered before use.

$\eta_D^{21} = 1.5085$

c.f. $\eta_D^{21} = 1.5092$ (I.C.T.)

CHLOROFORM[88]

Chloroform (B.P.) dried over anhydrous sodium sulphate was, after filtering, pure enough for the purposes of this investigation.

$\eta_D^{20} = 1.4465$

c.f. $\eta_D^{20} = 1.4460$ (I.C.T.)

ETHER[88]

Ether (B.P.), after preliminary drying over anhydrous calcium chloride, was stored in a dark bottle over sodium wire.

ACETONE[88]

Acetone (rectified) was stored over anhydrous potassium carbonate.

B.P. = 56.2^0 C

$\eta_D^{20} = 1.3590$

c.f. $\eta_D^{20} = 1.3590$ (I.C.T.)

EXPERIMENTAL

PREPARATION OF ∝-METHYL d-GLUCOSIDE.

∝-Methyl d-glucoside was prepared by the method of Robertson and Patterson.[60]

Dextrose, pure anhydrous (100 grams) was dried in a vacuum oven at 40° C. for five hours.

Found; M.P. = 145° C.

$[\alpha]_D^{16}$ = +52° C. after 2 days (C = 1.0 in water)

M.P. = 146° C.; $[\alpha]_D^{20}$ = +52.7° (Bates[87])

The methyl alcoholic - hydrogen chloride (3% solution) was prepared by generating pure dry hydrogen chloride into a weighed volume (200 grams) of pure anhydrous methyl alcohol until the desired increase in weight was observed.

The anhydrous dextrose (100 grams) and methyl alcoholic - hydrogen chloride (206 grams) were mixed in a one litre bolt-head flask. The solution was then boiled on a water bath under reflux for 4½ hours using a double surface reflux condenser fitted with a calcium chloride tube. Decolourizing animal charcoal (5 grams) was added 15 minutes before the end of this time.

The solution was filtered rapidly on a Buchner funnel and cooled in a bath of ice with occasional stirring. After an hour, the ∝-methyl d-glucoside formed a thick paste, and the crystalline material was filtered off under vacuum. This was washed with a little dry methanol, and then dried on a porous plate for 5 hours at 40° C. in a vacuum oven.

The mother liquor treated with decolourizing animal charcoal and filtered, was concentrated to a syrup. After cooling in a bath of ice

for one hour, a further amount of ∝-methyl d-glucoside crystallised out. This was filtered off and dried as before.

Yield: 65.2 grams (60.4% theoretical)

Found: M.P. = 161° C.

The impure product was recrystallised from methanol (150 mls.).

Yield: 42.5 grams (39.4% theoretical)

Found: M.P. = 165°- 165.5° C.

$[\alpha]_D^{17}$ = + 158.5° (C = 0.5 in water)

OMe = 15.7%

Calculated for $C_7H_{14}O_6$, OMe = 16.0%

Robertson and Patterson[60] report; M.P.= 165°-166°C.; $[\alpha]_D^{17.5}$ = 157.9° (in water)

TRITYLATION OF ∝-METHYL d-GLUCOSIDE

∝-Methyl d-glucoside (20 grams, 0.10 mole) and trityl chloride (30 grams, 0.11 mole) were dissolved in pyridine (160 mls.), and the solution allowed to stand for 24 hours at room temperature in a stoppered flask fitted with a calcium chloride tube. During this time some crystals, (A), formed and these were filtered off.

The solution was then poured into ice cold water and allowed to stand for 1 hour. The syrupy layer, which was separated and rubbed up repeatedly with fresh portions of water, was taken up in ether (200 mls.). The ethereal solution was washed three times with each of the following solutions using 100 ml. portions; acetic acid (10% solution), sodium becarbonate (50 mls. of saturated solution diluted to 100 mls. with distilled water) and finally with distilled water.

During the second washing with bicarbonate solution, the ethereal layer solidified very suddenly.

The solid, after washing with distilled water was taken up in absolute ethyl alcohol (350 mls.) by warming to 40° C. on a water bath. The solution was cooled in an external bath of ice. The white crystalline material (B), which was deposited, was filtered off on a Buchner funnel, washed with ice cold absolute ethyl alcohol and dried for 5 hours at 40° C. in a vacuum oven.

The mother liquor was concentrated under vacuo to 100 mls. This solution was treated as previously, causing a further quantity of crystals (C) to be deposited. These were washed and dried as before.

The mother liquor was again concentrated, this time however, buff crystals (D) were deposited.

Analysis of Fractions

Fraction	Yield	M.P.	Rotation	% Trityl
A	1.5 grams	160-161° C.	$[\alpha]_D^{18} = +38^0$	90.1
B	6.5 grams	137° C.	$[\alpha]_D^{19} = +33.4^0$	53.3
C	13.5 grams	136° C.	$[\alpha]_D^{19} = +33.2^0$	53.0
D	10.0 grams	156° C.	$[\alpha]_D^{18} = +37.7^0$	89.9

Total Yield; 31.5 gms. (70% theoretical)

Calculated for 6-trityl α-methyl d-glucoside, $C_{26}H_{28}O_6$, trityl = 55.7%

Calculated for triphenylcarbinol, $C_{19}H_{16}O$, trityl = 93.1%

Fractions B and C were recrystallised from absolute ethyl alcohol.

Found; M.P. = 130° C.

trityl = 48.0%

$[\alpha]_D^{16} = +66.1^0$ (C = 0.5 in pyridine)

Recrystallisation from absolute ethyl alcohol was carried out a further three times, but the analysis for trityl showed that the results were still low but constant.

After third recrystallisation, trityl = 47.90%

After fourth recrystallisation, trityl = 48.0%

After fifth recrystallisation, trityl = 47.95%

Yield; 18.5 grams (41.1% theoretical)

Found; M.P. = 128-129°C.; $[\alpha]_D^{19} = +67.6^0$ (C = 0.4 in pyridine)

A FURTHER ATTEMPT TO PREPARE 6-TRITYL - α-METHYL- d - GLUCOSIDE.

The large amount of triphenyl carbinol produced during the previous preparation of 6-trityl-α-methyl-d-glucoside, led to the belief that the reaction time of 24 hours was insufficient. Further evidence supporting this view, was that the final product had a low trityl content.

α-Methyl-d-glucoside (25 grams, 0.13 mole) and trityl chloride (36 grams, 0.13 mole) were dissolved in pyridine (200 mls.) and the solution allowed to stand for 9 days at room temperature in a stoppered flask fitted with a calcium chloride tube. During this time crystals (A) were deposited and these were filtered off.

The solution was treated as before, being poured into ice-cold water. The syrup formed was taken up in ether (300 mls.) and the solution washed with acetic acid, sodium bicarbonate solution and distilled water.

Again crystals appeared during the washing. It was therefore evident that ether is not a suitable solvent for the purpose.

After allowing the ether to evaporate off, the solid mass was taken up in absolute alcohol (500 mls.) by warming to 40° C. on a water bath. Crystals (B), which formed when the solution was cooled in an external ice bath, were filtered off, washed with ice-cold alcohol and dried for 5 hours at 40° C. in a vacuum oven.

The mother liquor was concentrated under vacuo at 40° C. and after cooling, more crystals (C) came down. These were filtered off, washed and dried as before.

The filtrate was discarded, it being a yellow syrup similar to that of the first preparation from which triphenyl carbinol was obtained.

Analysis of Fractions.

Fraction	Yield	M.P.	Rotation	% Trityl
A	5 grams	157°-158°C.	-	89.60
B	16 grams	128° C.	$[\alpha]_D^{16}$=+66.9 (C=0.5 in pyridine)	47.90
C	19 grams	131° C.	-	60.5

Total yield: 40 grams (71.2% theoretical).

Calculated for 6-trityl α-methyl d-glucoside, $C_{26}H_{28}O_6$, trityl = 55.7%

Calculated for triphenyl carbinol, $C_{19}H_{16}O$, trityl = 93.1%

Fraction (C) was recrystallised from absolute ethyl alcohol (150 mls.)

Found: M.P. = 128° C.

$[\alpha]_D^{15}$ = +67.0° (C = 0.5 in pyridine)

trityl = 48.1%

A methoxyl determination was carried out on fraction (B).

Found: OMe = 19.1%

Calculated for $C_{26}H_{28}O_6$: OMe = 7.1%

Calculated for $C_{26}H_{28}O_6 \cdot C_3H_9O_{1.5}$: OMe + OEt = 19.5%

trityl = 48.1%

Helferich and Becker[62] report: M.P. = 128°-130° C.; $[\alpha]_D$ = + 72.8°

It is therefore evident that both preparations of 6-trityl α-methyl d-glucoside contained 1.5 molecules of alcohol of crystallisation.

In an attempt to remove the alcohol of crystallisation, a sample (1 gram) was dried in a vacuum drier at 70° C. for 14 hours. The melting point (128° C.) showed that the alcohol was still present.

The same sample was then dried for 8 hours in a P_2O_5 drier, but again, the melting point was 128° C.

Helferich and Becker[62] report: M.P. = 151-152°C.; $[\alpha]_D^{16}$ = +86.3°C. when alcohol free.

METHYLATION OF 6-TRITYL-∝-METHYL-d-GLUCOSIDE.

6-Trityl-∝-methyl-d-glucoside (27 grams, 0.05 mole), containing 1.5 molecules of alcohol of crystallisation, was dissolved in methyl iodide (60 mls., 0.18 mole) in a ground glass stoppered round bottom flask (250 mls.) to which was fitted a double surface reflux condenser with a calcium chloride tube attached. Silver oxide (24 grams, .10 mole) was added hourly in 3 gram portions, by momentarily lifting the ground glass stoppered reflux condenser from the flask.

The methyl iodide was allowed to reflux slowly by keeping the water bath, in which the flask was immersed, at a temperature of 45° C. In order that the reaction be efficient, the flask was shaken vigorously at frequent intervals during the reaction time of 9 hours. The methylation mixture was left overnight to allow colloidal silver to settle out.

The solution was then filtered, the silver residues being exhaustively extracted with hot dry methanol and by refluxing with dry chloroform. The combined filtrate and washings were then evaporated to dryness at 40° C. under vacuum. The product was crystalline, but seemed to be mixed with a small amount of syrup.

After repeating the above process of methylation, a methoxyl determination on the crystalline product was carried out.

Found: OMe = 9.5%

Calculated for 6-trityl 2:3:4-trimethyl ∝-methyl d-glucoside,

$C_{29}H_{34}O_6$: OMe = 25.9%

After a third methylation, a sample was examined.

Found: OMe = 18.0%

After four methylations, the crystalline material constituted only 9 grams of the total product of 24.5 grams.

Examination of crystals.

Found: OMe = 18.2%

M.P. = 95° - 96° C.

$[\alpha]_D^{16}$ = +64.6° (C = 0.3 in chloroform)

Examination of syrup.

Found: OMe = 27.4%

η_D^{25} = 1.5525

This syrup crystallised on standing in a vacuum dessicator for 3 weeks. This product was recrystallised from acetone.

Found: OMe = 25.1%

trityl = 50.7%

M.P. = 83° - 84° C.

Calculated for $C_{29}H_{34}O_6$; OMe = 25.9%

trityl = 50.8%

Helferich and Becker[62] report: M.P. = 82° - 83° C.

After six methylations, 2 grams of crystalline product remained; 22.3 grams of fully methylated material were obtained.

Yield: 24.3 grams (95.1% theoretical)

DETRITYLATION

A solution of 6-trityl 2:3:4-trimethyl ∝-methyl d-glucopyranoside (6.8 grams, 0.014 mole) in chloroform (50 mls.) at 0° C. was saturated with hydrogen chloride, kept for one hour each at 0° C. and 20° C., and then exhaustively extracted with water. The combined aqueous extracts were neutralised with silver carbonate and filtered. The soluble silver was removed by saturating the solution with hydrogen sulphide, aerating and filtering.

Evaporation to dryness under diminished pressure at 35°-40° C. gave a reddish-yellow syrup which was boiled with methanolic-hydrogen chloride (1%, 60 mls.) for 8 hours to effect glycoside formation. Animal charcoal (0.5 gram) was added 15 minutes before the end of the run. The solution was neutralised with silver carbonate, filtered before and after treatment with hydrogen sulphide, and evaporated to dryness under reduced pressure at 35°-40° C. The product, a yellow syrup, contained a few crystals of 6-trityl 2:3:4-trimethyl ∝-methyl d-glucopyranoside which were separated.

Yield: 3.05 grams (90.8% theoretical)

Found: OMe = 45.3%

η_D^{ω} = 1.4625

$[\alpha]_D^{13}$ = +119.5° (C = 0.24 in methanol)

Calculated for 2:3:4-trimethyl ∝-methyl d-glucoside, $C_{10}H_{20}O_6$: OMe = 52.5%

A further amount of 6-trityl 2:3:4-trimethyl ∝-methyl d-glucoside (15.5 grams, 0.032 mole) was detritylated in chloroform (100 mls.) saturated with hydrogen chloride at 0° C. The same procedure as described was used.

Yield: 5.15 grams (67.3% theoretical)

Found: OMe = 46.3%

OXIDATION OF 2:3:4-TRIMETHYL ∝-METHYLGLUCOSIDE BY ACID POTASSIUM PERMANGANATE

To a solution of 2:3:4-trimethyl ∝-methylglucoside (2.7 grams, 0.011 mole) in glacial acetic acid (40 mls.), A.R. potassium permanganate (1 gm.) dissolved in methanol (40 mls.) was added. The liquid was slowly stirred at room temperature for 6 hours and a further amount of potassium permanganate (3.2 gms.) added in small portions during 2 days. Methanol (40 mls.) was added to the solution which was then centrifuged until it was apparently clear. The deposit of manganese ocides was washed with methanol until it no longer gave the naphtharesorcinol test for uronic acids.

A portion of a washing (1 ml.) was mixed with an equal volume of concentrated hydrochloric acid and boiled. Three drops of a 1% solution of naphtho-resorcin in 95% ethyl alcohol were added, the solution being boiled for half a minute. After cooling to about 50° under a tap, the solution was shaken with benzene and a violet colouration in the top benzene layer denoted the presence of glucuronic acid.[45]

The combined liquid and washings were evaporated under diminished pressure at 25° C. to a syrup, which was mixed with manganese oxides. This syrup was taken up in chloroform and filtered. After washing the solution six times with 0.1N sulphuric acid (50 mls. portions) and twice with distilled water (100 mls. portions), it was dried over anhydrous sodium sulphate. Evaporation under diminished pressure at 25° C. gave a yellow syrup (0.32 gms.)

The washings were neutralised with barium hydroxide solution, filtered and evaporated to dryness under diminished pressure at 25° C. The residue was extracted twice with chloroform, which, after washing

with water was dried over anhydrous sodium sulphate and evaporated to a syrup (0.31 gms.)

The above process was repeated producing another 0.30 gms. of yellow syrup.

The product (0.93 gms.) was dissolved in 2% methanolic hydrogen chloride (100 mls.) and boiled under reflux for ten hours to effect ester formation. Animal charcoal (0.5 gm.) was added before the end of the run. The solution was filtered before and after neutralising the excess hydrogen chloride with silver carbonate, and then evaporated to dryness under diminished pressure to a pale yellow syrup.

Yield: 0.85 gms. (28.2% theoretical)

Found: $\eta_D^{21} = 1.4544$

$[\alpha]_D^{14} = +42^0$ (C in water = 0.63)

OMe = 54.9%

Calculated for the methyl ester of 2:3:4-trimethyl α-methyl d-glucuronoside, $C_{11}H_{20}O_7$: OMe = 58.7%

Hirst and Jones[11] report: $[\alpha]_D = +31^0$ (water); $\eta_D^{21} = 1.4471$

Calculated for the methyl ester of 2:3-dimethyl α-methyl d-glucuronoside, $C_{10}H_{18}O_7$: OMe = 49.6%

Smith[9] reports: $[\alpha]_D^{16} = +76^0$ (water); $\eta_D^{18} = 1.4620$

The impure mixture of esters (0.8 gms.) was not distilled, but converted into a mixture of amides by saturating a methanolic solution with dry ammonia at 0^0 C. The solution was left for five days at 0^0 C. and then the solvent was removed under diminished pressure.

Found: $\eta_D^{18} = 1.4627$

$[\alpha]_D^{16} = +105.4^\circ$ (C = 0.21 in ethanol)

OMe = 46.3%

Calculated for 2:3:4-trimethyl α-methylglucuronamide,

$C_{10}H_{19}O_6N$: OMe = 49.8%

Smith[9] reports: $[\alpha]_D^{18} = +139^\circ$ (in water)

Calculated for 2:3-dimethyl α-methylglucuronamide,

$C_9H_{19}O_6N$: OMe = 39.6%

All attempts to crystallise this yellow syrup failed.

OXIDATION OF 2:3:4-TRIMETHYL ∝-METHYLGLUCOSIDE BY ALKALINE POTASSIUM PERMANGANATE

2:3:4-Trimethyl ∝-methylglucoside (4.35 gms., 0.018 mole) was dissolved in water (200 mls.) containing potassium hydroxide (2.18 gms.) To this solution, a solution of potassium permanganate (6.00 gms., 0.038 mole) in water (400 mls.) was added in portions over ten hours. The volume was adjusted to 850 mls., and the oxidation allowed to proceed at room temperature for three days. The mixture was then treated with animal charcoal (1 gm.), warmed to 50° C. on a water bath, filtered and neutralised with carbon dioxide. The filtrate was evaporated to dryness under diminished pressure. Extraction of the residue six times with boiling methyl alcohol gave a colourless glassy solid (5.15 gms.) consisting mainly of the potassium salt of 2:3:4-trimethyl methylglucuronoside. This potassium salt was converted into the corresponding methyl ester by boiling it under reflux for ten hours with 2% methanolic hydrogen chloride (200 mls.) Animal charcoal (1 gm.) was added ten minutes before the end of the run. After filtering, the solution was neutralised with silver carbonate, filtered again and saturated with hydrogen sulphide. Aeration of the solution expelled the excess hydrogen sulphide. Colloidal sulphur was absorbed by the addition of animal charcoal which was filtered off. The filtrate, after evaporation to dryness under diminished pressure gave a pale yellow syrup.

Yield: 4.35 gms. (87.4% theoretical)

This syrup was distilled under high vacuum into three boiling-point fractions.

Fraction I.

Yield: 1.00 grams of colourless syrup.

Found: B.P. = 80^0-90^0 C./0.005 mm.

η_D^{20} = 1.4470

$[\alpha]_D^{15}$ = +31.1° (C = 0.6 in water)

OMe = 57.4%

Calculated for the methyl ester of 2:3:4-trimethyl α-methylglucuronoside, $C_{11}H_{20}O_7$: OMe = 58.7%

Hirst and Jones[11] report: B.P. 140°/0.002 mm.; η_D^{21} = 1.4471

$[\alpha]_D$ = + 31° (water)

Fraction II

Yield: 1.02 grams of colourless syrup.

Found: B.P. = 105°-110° C./0.005 mm.

η_D^{20} = 1.4572

OMe = 53.9%

This fraction was methylated using Purdie's reagents. Methyl (10 mls.) and silver oxide (5 grams) were used, the procedure being identical with that described previously.

Found: OMe = 59.4%

Fraction III

Yield: 0.15 grams of colourless syrup.

Found: B.P. = 150°-160° C./0.01 mm.

η_D^{20} = 1.4616

$[\alpha]_D^{14}$ = + 75.2° (C = 0.55 in water)

OMe = 49.2%

Calculated for the methyl ester of 2:3-dimethyl α-methylglucuronoside $C_{10}H_{18}O_7$: OMe = 49.6%

Smith[9] reports: B.P. = 145°-150° C./0.02 mm.; $[\alpha]_D^{16}$ = +76° (in water);

η_D^{18} = 1.4620

HYDROLYSIS OF THE METHYL ESTER OF 2:3:4-TRIMETHYL ∝-METHYLGLUCURONOSIDE (FRACTION I)

A solution of the methyl ester of 2:3:4-trimethyl methylglucuronoside (0.88 gms., 0.003 mole) in N-hydrochloric acid (20 mls.) was refluxed on the boiling water bath for nine hours. The solution, which had $[\alpha]_D^{20} = +38^\circ$ initially, now had $[\alpha]_D^{20} = +60^\circ$ (constant value). The rotation increased steadily up to $[\alpha]_D^{20} = +57^\circ$ after five hours, after which it remained practically constant. After neutralising the excess hydrochloric acid with silver carbonate, the solution was filtered, treated with hydrogen sulphide, aerated and filtered again. Evaporation to dryness under diminished pressure at 35°-40° C. gave a colourless viscous syrup (0.65 gms.) which was reducing to Fehling's solution, and reacted acid to Congo-red paper.

Yield: 0.67 grams (85.2% theoretical)

Found: $[\alpha]_D^{16} = +54^\circ$ (C = 0.5 in water)

$\eta_D^{18} = 1.4695$

OMe = 38.5%

Calculated for 2:3:4-trimethyl glucuronic acid, $C_9H_{16}O_7$:

OMe = 39.4%

Jones[14] reports: $[\alpha]_D^{20} = +52.4^\circ$ (in water); $\eta_D^{20} = 1.4710$.

OXIDATION OF 2:3:4-TRIMETHYL GLUCURONIC ACID BY BROMINE.

To a solution of the syrupy 2:3:4-trimethyl glucuronic acid (0.65 gms., 0.003 mole) in water (50 mls.), bromine (1.1 mls., excess) was added, and the mixture kept at room temperature for one day and then at 60° C. for eight hours. The excess bromine was removed from the solution by aeration. The solution, which contained some hydrobromic acid, was neutralised with silver carbonate, filtered before and after treatment with hydrogen sulphide and evaporated to dryness under reduced pressure at 25° C. The syrup produced, (0.54 gms.) reacted acid to Congo-red paper, and was non-reducing to Fehling's solution.

The acid was esterified with diazo methane (0.2 gms.) in ether (50 mls.) at 0° C. for twenty hours. Removal of the solvent produced a yellow syrup (0.50 gms.) containing a large proportion of pale yellow crystals, some of which were separated and recrystallised from ethanol.

Yield: 0.50 gms. (73.2% theoretical)

Found: M.P. = 112° C.

η_D^{18} = 1.4538

$[\alpha]_D^{15}$ = +101° (C = 0.24 in ethanol)

OMe = 51.1%

Calculated for the methyl ester of 2:3:4-trimethyl δ-saccharolactone,

$C_{10}H_{16}O_7$: OMe = 50.0%

Hirst and Jones[11] report: M.P. = 110°; $[\alpha]_D^{21}$ = +102° (in ethanol)

η_D^{20} = 1.4600

Hirst and Jones[12] report: η_D^{18} = 1.4545 before distillation of the ester; M.P. = 112° C. after distillation.

Amide Formation.

Because of the loss due to decomposition and charring, and since the sample was already sufficiently pure, the methyl ester of 2:3:4-trimethyl δ-saccharolactone was not distilled but converted to the amide. This was effected by dissolving the methyl ester (0.44 gms.; 0.0018 mole) in methanol (25 mls.), and saturating the solution at 0° C. with ammonia dried through soda-lime and potassium hydroxide. The stoppered solution was left at 0° C. for two days. On removal of the methanol under reduced pressure at 25° C., a viscous, opaque, buff coloured syrup was produced.

Found: OMe = 45.1%

$[\alpha]_D^{13}$ = 79.4° (C = 0.84 in ethanol)

Calculated for 2:3:4-trimethyl saccharidiamide, $C_9H_{18}O_6N_2$:

OMe = 37.2%

This syrup was treated with methanolic ammonia for a further five days.

The resulting yellow syrup was taken up in ethanol which was allowed to evaporate off slowly. After five weeks some hydroscopic crystals, m.p. 165°, were deposited on the side of the crystallising dish. Analysis showed:-

OMe = 37.8%

$[\alpha]_D^{10}$ = 66.7° (C = 1.0 in ethanol)

ANALYTICAL METHODS

QUANTITATIVE DETERMINATION OF TRITYL GROUPS.[93]

The trityl groups in a compound can be determined by their conversion to triphenylcarbinol. A weighed sample is dissolved with careful trituration in the minimum amount of sulphuric acid (sp.gr. 1.84). This solution is poured quickly into distilled water (50 mls.) and allowed to stand for 30 minutes. The triphenylcarbinol is collected on a weighed sintered-glass crucible, washed with distilled water, and dried at 110° C. to constant weight. By using the appropriate factor, the results are expressed as percentage of triphenylmethyl groups.

SEMI-MICRO METHOXYL DETERMINATION.[94]

The methoxyl groups in a compound can be determined by their indirect conversion to silver iodide. When a weighed sample is heated in hydriodic acid, the methoxyl groups are converted to methyl iodide. This is swept by a stream of carbon dioxide, through a trap containing red phosphorus and water to remove hydriodic acid, into alcoholic silver nitrate where silver iodide is precipitated. The silver iodide is collected on a weighed sintered glass crucible, washed with distilled water, and dried at 110° C. to constant weight. By using the appropriate factor, the results are expressed as percentage of methoxyl groups.

A modified form of Zeisel apparatus was used, and the determination carried out according to the usual Zeisel procedure.

BIBLIOGRAPHY.

1.	Norman	"The Biochemistry of Cellulose, The Polyuronides, Lignins, etc. Oxford University Press, London. 1937.			
2.	Dore	J.A.C.S.	1926	48	232.
3.	Henglein and Schneider	Ber.	1936	69	309.
4.	Baur and Link	J.Biol.Chem.	1935	109	293.
5.	Luckett and Smith	J.C.S.	1940	143	1106; 1506.
6.	Hirst	J.C.S.	1942	145	70.
7.	Butler and Cretcher	J.A.C.S.	1929	51	1519.
8.	Challinor, Haworth and Hirst	J.C.S.	1931	134	258.
9.	Smith	J.C.S.	1939	142	744, 1724.
		J.C.S.	1940	143	1035.
	Smith and Jackson	J.C.S.	1940	143	74, 79.
10.	Anderson and Otis	J.A.C.S.	1930	52	4461.
11.	Hirst and Jones	J.C.S.	1938	141	1174.
12.	Hirst and Jones	J.C.S.	1939	142	1482.
13.	Butler and Cretcher	J.A.C.S.	1931	53	4160.
14.	Jones	J.C.S.	1939	142	558.
15.	James and Smith	J.C.S.	1945	148	739.
16.	Peat	Ann.Reports Progress Chem. (Chem. Soc., London)	1941	38	150.
17.	Bailey and Norris	Biochem. J.	1932	26	1609.
18.	Bailey	Biochem. J.	1935	29	2477.
19.	Gill, Hirst and Jones	J.C.S.	1939	142	1469.
		J.C.S.	1946	149	1025.
20.	Nelson and Cretcher	J.A.C.S.	1929	51	1914.

21.	O'Dwyer	Biochem. J.	1934	28	2116.
22.	Angell and Norris	Biochem. J.	1936	30	2155.
23.	Anderson and Kinsmann	J.Biol.Chem.	1931	94	39.
24.	Angell and Norris	Biochem. J.	1936	30	2159.
25.	McIlroy, Holmes and Mauger	J.C.S.	1945	148	796.
26.	Granichstadten and Percival	J.C.S.	1943	146	54.
27.	Buston	Biochem. J.	1934	28	1028.
		Biochem. J.	1935	29	196.
28.	Cooper, Daker and Stacey	Biochem. J.	1938	32	1752.
29.	Schluchterer and Stacey	J.C.S.	1945	148	776.
30.	Brown	J. Immunol.	1939	37	445.
31.	Heidelberger, Goebel and Avery	J.Exptl.Med.	1925	42	701.
32.	Hotchkiss and Goebel	J.Biol.Chem.	1937	121	195.
33.	Stacey	"Advances in Carbohydrate Chemistry", Vol.2. Edited by Pigman and Wolfrom. Academic Press Inc. Publishers. New York. 1946.			
34.	Meyer and Palmer	J.Biol.Chem.	1934	107	629.
35.	Jorpes	"Heparin" Oxford University Press. London. 1939.			
36.	Levene	"Hexosamines and Mucoproteins" Longmans, Green and Co., London. 1926			
37.	Hirst et alia	J.C.S.	1939	142	1880.
38.	Christman, Levene and Tipson	J.Biol.Chem.	1939	128	609.
39.	Anderson and Firman	J.Biol.Chem.	1935	109	437.
40.	Anderson, Gillette and Seeley	J.Biol.Chem.	1941	140	569.
41.	Mullan and Percival	J.C.S.	1940	143	1501.
42.	Anderson et alia	J.Biol.Chem.	1940	135	189.
43.	Reeves and Goebel	J.Biol.Chem.	1941	139	511.

44.	Bray, Gregory and Stacey	Biochem. J.	1944	38	142.
45.	B. Tollens	Ber.	1908	41	1788.
46.	Hanson, Mills and Williams	Biochem. J.	1944	38	274.
47.	Heidelberger and Goebel	J.Biol.Chem.	1927	74	613.
48.	Jolles	Biochem. Z.	1911	34	242.
49.	Bergmann and Wolff	Ber.	1923	56	1060.
50.	Ohle and Behrend	Ber.	1925	58	2585.
51.	Stacey	J.C.S.	1939	142	1529.
52.	Zervas and Sessler	Ber.	1933	66	1326.
53.	Smith, Stacey and Wilson	J.C.S.	1944	147	131.
54.	Smith	J.C.S.	1944	147	571.
55.	Dewar and Fort	J.C.S.	1944	147	492.
56.	Owen, Peat and Jones	J.C.S.	1941	144	339.
57.	Fischer (E.)	Ber	1895	28	1151.
58.	Bourquelot	Amm. Chem.	1915	3	298.
59.	Hudson	J.A.C.S.	1925	47	266.
60.	Patterson and Robertson	J.C.S.	1929	132	300.
61.	Helferich, Speidal and Treldte	Ber.	1923	56B	766.
62.	Helferich and Becker	Ann.	1924	440	1 .
63.	Helferich, Klein and Schafer	Ann.	1926	447	19.
64.	Helferich, Brederick and Modrow	Ann.	1928	465	180.
65.	Helferich and Leete	Ber.	1929	62B	1549.
66.	Josephson	Ann.	1929	472	230.
67.	Helferich and Koester	Ber.	1924	57B	587.
68.	Hockett and Hudson	J.A.C.S.	1931	53	4456.
69.	Hockett, Fletcher and Ames	J.A.C.S.	1941	63	2516.
70.	Zeile andKruckenberg	Ber.	1942	75B	1127.

71.	McIlroy	J.C.S.	1946	149	100.
72.	Jackson, Hockett and Hudson	J.A.C.S.	1934	56	947.
73.	Purdie and Irvine	J.C.S.	1903	83	1021.
74.	Haworth	J.C.S.	1915	107	8.
75.	Musket	J.A.C.S.	1934	56	693, 2449.
76.	Pacsu and Trister	J.A.C.S.	1939	61	2442.
77.	Freudenberg and Hixon	Ber.	1923	56	2119.
78.	Fear and Menzies	J.C.S.	1926	129	937.
79.	Purves and Hudson	J.A.C.S.	1939	59	49, 1170.
80.	Hirst and Jones	J.C.S.	1938	141	496.
81.	Barker, Hirst and Jones	J.C.S.	1938	141	1695.
82.	Haworth,Hirst,Isherwood and Jones	J.C.S.	1939	142	1878.
83.	Helferich and Klein	Ann.	1926	450	219.
84.	Pryde and Williams	Biochem.J.	1933	27	1197.
85.	Maurer and Drefahl	Ber.	1942	75B	1489.
86.	Sewell	M.Sc. Thesis. N.Z.U. 1946.			
87.	Bates	"Polarimetry, Saccharimetry and the Sugars." Circular of the National Bureau of Standards, C440. U.S. Government Printing Office, Washington, 1942.			
88.	Weissberger and Proskauer	"Organic Solvents" Clarendon Press. Oxford. 1934.			
89.	J.B. Cohen	"Practical Organic Chemistry" Macmillan and Co. Ltd. London. 1930.			
90.	Hauser and Boyd Hudson	"Organic Synthesis", Vol. 23. John Wiley and Sons Inc. New York. 194			
91.	J.W. Mellor	"Comprehensive Treatise on Inorg. and Theor. Chem." Vol. III. Longmans, Green and Co. London. 1923.			
92.	Fieser	"Experiments in Organic Chemistry" Heath and Co. Boston. 1935.			
93.	Valentin	Coll. Czechoslav. Chem. Comm. 1931 3 499.			
94.	Browne and Zerban	"Sugar Analysis" Wiley and Sons Inc. London. 1941.			