THE OXIDATION OF \( \alpha \)-METHYL \( \beta \)-GLUCOSIDE

WITH NITROGEN DIOXIDE
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THEORETICAL

THE URONIC ACIDS:

The initial step in the present investigation was the preparation of the \( \alpha \)-methyl-d-glucuronoside (ii) by the oxidation of \( \alpha \)-methyl-d-glucoside (i) with an equilibrium mixture of nitrogen dioxide and nitrogen tetroxide in chloroform and carbon tetrachloride.

\[
\begin{array}{c}
\text{H} - \text{C} - \text{OCH}_3 \\
\text{H} - \text{C} - \text{OH} \\
\text{H} - \text{O} - \text{C} - \text{H} \\
\text{H} - \text{C} - \text{OH} \\
\text{CH}_2\text{OH}
\end{array}
\hspace{1cm}
\begin{array}{c}
\text{H} - \text{C} - \text{OCH}_3 \\
\text{H} - \text{C} - \text{OH} \\
\text{H} - \text{O} - \text{C} - \text{H} \\
\text{H} - \text{C} - \text{OH} \\
\text{COOH}
\end{array}
\hspace{1cm}
\begin{array}{c}
\text{H} - \text{C} - \text{OH} \\
\text{H} - \text{C} - \text{OH} \\
\text{H} - \text{O} - \text{C} - \text{H} \\
\text{H} - \text{C} - \text{OH} \\
\text{COOH}
\end{array}
\]

The uronic acids in general will be discussed below, especially d-glucuronic acid (iii) which can not be purchased and hence must be synthesised or isolated from natural products.
Uronic acid groups are frequently found as constituent units of certain cell-wall polysaccharides, plant gums, and some microbial products. The uronic acids are reducing monocarboxylic sugar acids formed by the oxidation of the terminal carbinol group (atom 6) of the sugar, a process which apparently occurs readily in both plants and animals. A large number of uronic acids are theoretically possible - for example, there are sixteen isomeric aldohexuronic acids - but only three have been found to occur naturally: d-galacturonic, d-glucuronic, and d-mannuronic acids. Each of these three has now been synthesised in both stereoisomeric forms (d and l), and in addition, other uronic acids not known to occur in natural products have been prepared by similar means.

Interest in the uronic acids was much stimulated by the belief that vitamin C was a hexuronic acid, but it is now known that this is not the case. The discovery that certain immunologically important polysaccharides with specific properties contain uronic acid groups, apparently in the form of aldobionic acids, has also focussed attention on this group. Aldobionic acids are disaccharide sugar acids formed by linkage of a sugar with a uronic acid unit. They have been found in the acid hydrolysis products of
plant gums and some have been synthesised.

*d*-Glucuronic acid has a very widespread occurrence in the plant and animal world, being a constituent of the plant gums. *d*-Glucuronic acid is found in the type specific polysaccharide of Type III pneumococcus in combination with glucose as, β-<i>d</i>-glucuronosido-<i>4</i>-<i>d</i>-glucose (Heidelberger and Goebel) and in combination with galactose (β-<i>d</i>-glucuronosido-<i>6</i>-<i>d</i>-galactose), which is a partial hydrolysis product of gum arabic (Challinor, Haworth and Hirst). Levene and co-workers have established the presence of glucuronic acid as a constituent of the carbohydrate portion of the mucoproteins. *d*-Glucuronic acid containing an ether-linked methyl group occurs in mesquite gum (Anderson and Otis). It probably is the monomethyl uronic acid that occurs in many hemicelluloses (O'Dwyer).

*d*-Galacturonic acid has been obtained from pectin and has been shown to be present in certain hemicelluloses and mucilages. *d*-Mannuronic acid has been found as a poly-mannuronide in the polysaccharides of various marine algae, being first prepared from an alginic acid by hydrolysis (Schoeffel and Link).

No polyuronide is known that contains two different uronic acids. Polyuronides may be defined as polysaccharides that contain one or more uronic acid units in their molecular
The uronic acids form few, well-defined, insoluble derivatives with distinguishing characteristics. This difficulty accounts for the fact that the uronic acid has remained unidentified in many investigations although its presence has been established by colour tests or carbon dioxide evolution. Two crystalline forms of d-galacturonic acid are known (\( \alpha \) and \( \beta \)), as is the case with mannuronic acid, but glucuronic acid crystallizes as the \( \beta \) form. The \( \alpha \) form has not yet been obtained crystalline. Glucuronic and mannuronic acids readily form lactones that are crystallizable. Indeed, mannanurono-lactone is more stable than the free acid, which may only be obtained with difficulty and is markedly hygroscopic and difficult to handle.

In the galactose series no crystallizable lactone has been obtained from galacturonic or the synthetically prepared alluronic acids. The free acids or their lactones have been used for the purpose of identification. (Schoeffel and Link\(^5\); Nieman and Link\(^6\).) Methylated and acetylated products have also been obtained.

The identity of the naturally occurring uronic acids has now been fully established by comparing their
properties and those of derivatives with synthetically prepared acids from sugars of known structure. In this way it has been shown that the normal pyranose ring (1,5) structure is present. Evidence based upon kinetics of hydrolysis is offered for a pyranoside ring in \( \alpha -d \)-methylgalacturonoside.

The method of Heidelberger and Goebel has been used to identify d-glucuronic and d-mannuronic acids without isolating the free acid. d-Glucuronic acid by this method yields d-saccharic acid, which is identified as the potassium acid saccharate, while d-mannuronic acid yields d-mannosaccharic acid which can be identified as the diamide. Since both d- and l-galacturonic acids give mucic acid, this method cannot be used for the complete identification of galacturonic acid. However, since l-galacturonic acid has not been observed in nature, the formation of mucic acid by this method is very strong evidence of the presence of d-galacturonic acid.

Studies of the methylation products have also supplied evidence for the identification of these acids (Hirst and Jones\(^7\); Jones;\(^8\) Smith\(^9\)). After complete methylation and hydrolysis of the aldobionic or polyuronic acid it is often possible to isolate the methylated uronide as the lactone, or methyl ester. The acid may also be oxidised to the methylated dibasic acid and identified as
such. After methylation and hydrolysis of pectic and alginic acids followed by bromine oxidation of the products the 2:3-dimethyldibasic acid is obtained. If the dimethyldibasic acid is oxidised by periodic acid and bromine, pectic acid yields dextro-dimethoxysuccinic acid while alginic acid yields meso-dimethoxysuccinic acid; thus galacturonic and mannuronic acids may be differentiated.

Stacey (1945) from the methylation and hydrolysis of the capsular polysaccharide Rhizobium radiciculum isolated 2:3-dimethyl-d-glucuronoside methyl ester, and subsequent hydrolysis to 2:3-dimethyl-d-glucuronic acid followed by bromine oxidation yielded 2:3-dimethyl-d-glucosaccharic acid which was identified as the crystalline diamide.

Any investigation of polyuronides by this method of methylation and oxidation would hence not be complete without the preparation of the crystalline diamide of the fully methylated saccharic acid, i.e. the diamide of 2:3:4-trimethyl-d-glucosaccharic acid, in order to identify completely the fully methylated glucuronic acid obtained.

The synthesis of this diamide comprised the final stage of the present investigation.

d-GLUCURONIC ACID:

\[ \text{d-Glucuronic acid was first obtained by reduction} \]
of saccharolactone with sodium amalgam and dilute sulphuric acid (Fischer and Piloty\textsuperscript{11}). Saccharolactone is readily prepared from the parent acid on heating.

\[
\begin{array}{c}
\text{CO} \\
\text{H - C - OH} \\
\text{HO - C - H} \\
\text{H - C} \\
\text{H - C - OH} \\
\text{COOH}
\end{array} 
\quad \rightarrow 
\begin{array}{c}
\text{CHOH} \\
\text{H - C - OH} \\
\text{HO - C - H} \\
\text{H - C} \\
\text{H - C - OH} \\
\text{COOH}
\end{array}
\]

\(\gamma\)-Saccharolactone \quad d\text{-Glucuronic Acid}

Stacey\textsuperscript{12} has devised synthetic methods for the preparation of d-glucuronic acid from d-glucose and d-galacturonic acid from d-galactose.

The glucose is tritylated and acetylated when the 1:2:3:4-tetra-acetyl 6-trityl-\(\beta\)-d-glucose separates on crystallization. Detritylation is effected by the method of Helferich and Klein\textsuperscript{13}.

1:2:3:4-Tetra-acetyl-\(\beta\)-d-glucose is oxidised by potassium permanganate in glacial acetic acid solution to 1:2:3:4-tetra-acetyl-d-glucuronic acid. The latter substance, which need not be isolated is deacetylated and converted to barium glucuronate by barium hydroxide in aqueous solution. After purification and removal of the barium
the lactone (glucurone) of d-glucuronic acid crystallizes. From glucose 20% yields of glucurone were obtained.

Maurer and Drefahl \(^{14}\) prepared \(\alpha\)-methyl-d-glucuronic acid by oxidation of \(\alpha\)-methyl-d-glucoside with nitrogen dioxide in chloroform. On hydrolysis with dilute acid and saponification with barium hydroxide barium glucuronate was obtained as an amorphous powder.

They did not however, isolate any crystalline derivative of \(\alpha\)-d-glucuronic acid. This is one of the aims of the present investigation.

The occurrence of d-glucuronic acid in polyuronides has already been mentioned. The first satisfactory preparation of the acid from a plant source was by hydrolysis of gum arabic (Weinmann \(^{15}\)). The biological methods of Quick \(^{16}\) and of Williams \(^{17}\) yield excellent results, glucurone being readily isolated by their methods.

Owen, Peat and Jones \(^{18}\) have made the interesting discovery that when glucurone is treated with cold methyl alcoholic hydrogen chloride it is converted to the furanose form (the methyl ester of methyl gluco-fururanoside) whereas when hot methyl alcoholic hydrogen chloride is used the methyl ester of methyl gluco-pyuranoside results. This ease of interconversion has an important bearing on the interpretation of methylation and hydrolytic studies of polyuronides.
The initial major step in the present investigation was as stated (Page 1), the oxidation of α-methyl-d-glucoside to α-methyl d-glucuronoside by an equilibrium mixture of nitrogen dioxide in chloroform and in carbon tetrachloride.

The main yield from the oxidation however, appeared to be nitrated α-methyl d-glucuronoside with a small amount of α-methyl d-glucuronoside. Hence sugar nitrates will be discussed below.

As the next step after oxidation in the present investigation was methylation, methylation methods will be discussed as well as oxidation methods. Nitration and de-nitration will be discussed in the sugar nitrate section.
SUGAR NITRATES:

Although the sugar nitrates are, as a rule, definitely crystalline and easily adaptable to synthetic operations, little use was made of them after they were studied by Will and Lenze 19 (1898) and Koenigs and Knorr 20 (1901) until Oldham 21 (1926) studied sugar nitrate transformations. Most of the earlier work was carried out with nitrate groups occupying carbon atoms 1 and 6 of the sugar molecule (Oldham 21; Irvine and Rutherford 22).

Bell and Synge 23 attempted to prepare a glucose derivative substituted in positions 1,2,3 and 6 by some radical e.g., the nitrate group, which does not display the migratory tendencies of carboxylic acyl groups and, unlike toluenesulphonyl radicals, can be removed without danger of Walden inversion. They succeeded in synthesising β-methyl glucoside - 2:3:6 trinitrate 24 which is useful for synthetic work involving substitution of glucose residues at position 4, and they also synthesised 2:6-dimethyl glucose 25 by use of the sugar nitrates and the ease of removal of nitrate groups. No instance of nitrate group migration has been reported.

Dewar 26 also studied the sugar nitrates and succeeded in synthesising many glucose derivatives, e.g., 2:4 dimethyl-β-methyl glucoside, by means of this group of compounds.
Will and Lenzé 19 (1898) prepared glucose penta-
nitrate by dissolving glucose in ice-cold fuming nitric
acid and adding concentrated ice-cold sulphuric acid to
the solution, showing that all the hydroxyl groups are re-
placeable by a nitrate group.

\[
\begin{align*}
\text{H} - \text{C} & \quad \text{OH} \\
\text{H} - \text{C} & \quad \text{OH} \\
\text{HO-C} & \quad \text{H} \quad \text{O} \\
\text{H} - \text{C} & \quad \text{OH} \\
\text{H} - \text{C} & \quad \text{OH} \\
\text{CH}_2\text{OH}
\end{align*}
\]

\[
\begin{align*}
\text{Fuming HNO}_3 & \quad \text{and CHCl}_3 \text{at } 0^\circ\text{C.} \\
\text{NO}_2\cdot\text{O-} & \quad \text{C} \quad \text{H} \\
\text{H} - \text{C} & \quad \text{O}_2\text{NO}_2
\end{align*}
\]

\( \alpha - \text{d-Glucose} \quad \text{Glucose pentanitrate} \)

Nitration of sugars is usually effected by fuming
nitric acid in chloroform at 0°C, or by nitrogen pentoxide
in chloroform. The nitrate groups appear to be stable to
acid. The nitrate group on carbon atom 1 can be replaced
by a methoxyl group on boiling with methanol in the pre-
sence of barium carbonate. The nitrate group on carbon
atom 2 can be removed preferentially by heating with sodium
iodide and acetone for several hours. The nitrate group on
C atom 6 when similarly treated yields the corresponding
iodohydrin derivative. Other denitrating agents used are
sodium sulphide-sodium hydroxide, sodium amalgam, and a
mixture of zinc and iron powders in glacial acetic acid.
The last reagent is most commonly used and all three are used to remove the nitrate group on any C atom.

Denitration by means of iron and zinc and glacial acetic acid appears to cause anhydro sugar formation in some cases (Irvine and Rutherford 22).

Methylation of nitrated sugars by Purdie's reagents has been used extensively as removal of the nitrate groups by any of the general methods yields the required methylated derivative (Dewar 26).
CHART 1

\[
\begin{align*}
\text{CH}_3\text{O}-\text{C}-\text{H} & \quad \xrightarrow{\text{HNO}_3} \quad \text{CH}_3\text{O}-\text{C}-\text{H} \\
\text{H} & \quad \xrightarrow{\text{MeOH-HCl}} \quad \text{H} \\
\text{H} & \quad \xrightarrow{\text{COOCH}_3(1)} \quad \text{COOH (1)} \\
\text{H} & \quad \xrightarrow{\text{MeOH-NH}_3} \quad \text{COOCH}_3(2) \\
\text{COOH} & \quad \xrightarrow{\text{MeOH-HCl}} \quad \text{COOCH}_3(3) \\
\text{CH}_3\text{O}-\text{C}-\text{H} & \quad \xrightarrow{\text{H} \to \text{C}=\text{O}} \quad \text{H} \\
\text{H} & \quad \xrightarrow{\text{H}=\text{C}=\text{OCH}_3} \quad \text{H} \\
\text{COOH} & \quad \xrightarrow{\text{MeOH-HCl}} \quad \text{COOCH}_3(4) \\
\text{CH}_3\text{O}-\text{C}-\text{H} & \quad \xrightarrow{\text{H} \to \text{C}=\text{OCH}_3} \quad \text{H} \\
\text{H} & \quad \xrightarrow{\text{MeOH-NH}_3} \quad \text{COOCH}_3(5) \\
\text{COOCH}_3(6) & \quad \xrightarrow{\text{MeOH-NH}_3} \quad \text{COOCH}_3(7) \\
\text{CH}_3\text{O}-\text{C}-\text{H} & \quad \xrightarrow{\text{H} \to \text{C}=\text{OCH}_3} \quad \text{H} \\
\text{H} & \quad \xrightarrow{\text{MeOH-NH}_3} \quad \text{COOCH}_3(8) \\
\text{COOCH}_3(9) & \quad \xrightarrow{\text{MeOH-NH}_3} \quad \text{COOCH}_3(10) \\
\text{H} & \quad \xrightarrow{\text{MeOH-NH}_3} \quad \text{COOCH}_3(11) \\
\text{H} & \quad \xrightarrow{\text{MeOH-NH}_3} \quad \text{COOCH}_3(12)
\end{align*}
\]
METHODS OF OXIDATION

NITRIC ACID OXIDATION:

Methylation followed by nitric acid oxidation has been used by Pryde and Williams 27 for determining the structure of glucuronic acid of animal origin. Examination of the oxidation products serves to prove the ring structure of the acid, i.e. whether a pyranose (1:5) or a furanose (1:4) ring.

Oxidation of the methyl ester of 2:3:4 trimethyl α-methyl d-glucuronoside (1) with nitric acid furnishes evidence of its structure, since 1-xylotrimethoxy glutaric acid (5) and d-dimethoxy succinic acid (8) are obtained in addition to 2:3:4-trimethyl α-saccharolactone (2). Esterification and distillation separates these products as their methyl esters (Smith 9; Chart 1, (6), (9), and (3) respectively).

The first two have been identified by the isolation of their crystalline amides, (7) and (10) respectively. The diamide of the last product, 2:3:4-trimethyl d-glucosaccharadiamide (4) was the required final product in the present investigation.

Although 2:3:4-trimethyl α-saccharolactone methyl ester (3) is a crystalline compound, its preparation by distillation of a small quantity of material under high
vacuum is not advisable in some investigations due to the unavoidable loss by decomposition; so that the direct preparation and determination of the physical constants of the crystalline diamide would be preferable. Synthesis of this diamide was one of the aims of the present investigation. The oxidation with nitric acid has been set out in Chart 1.

Nitric acid oxidation therefore represents an alternative method to the one intended to be used in the present investigation, namely, oxidation by aqueous bromine.

**BROMINE OXIDATION:**

Oxidation by aqueous bromine has also been used in converting uronic acid methylated derivatives to the corresponding dicarboxylic acid derivatives (Smith 9; Stacey28).

Unlike nitric acid oxidation which first hydrolyses the methyl group off carbon atom 1, bromine oxidation of glycosides must be preceded by hydrolysis of this methyl group. However, bromine oxidation does not cause the breaking up of the molecule at the point of attachment of the ring as does nitric acid and hence is preferable, larger yields of the dicarboxylic acid being more easily obtained.

Bromine oxidation was the method by which it was proposed to oxidise 2:3:4-trimethyl α-methyl d-glucuronoside in the present investigation after first hydrolysing this compound with dilute mineral acid to the trimethyl d-glucuronic acid.
NITROGEN DIOXIDE OXIDATION:

Nitrogen dioxide oxidation of carbohydrates was first applied to the sugars by Maurer and Drefahl \(^1^4\) (1942) and to cellulose by Yackel and Kenyon \(^2^9\) (1942).

The nitrogen dioxide may be either gaseous or in an indifferent organic solvent, e.g., chloroform, carbon tetrachloride, or glacial acetic acid. Care is taken in the choice of the solvent, or occasionally a violent reaction may ensue. Most work in organic chemistry with nitrogen dioxide deals with nitrating rarely in oxidation.

Maurer and Drefahl \(^1^4\) prepared glyoxylic, glucuronic, mucic, and \(\alpha\)-methyl galacturonic acids by nitrogen dioxide oxidation.

\(\alpha\)-Methyl glucoside and nitrogen dioxide in chloroform gave \(\alpha\)-methyl d-glucuronoside isolated finally as barium d-glucuronate. They obtained a barium content of 26.1\% (26.2\% theoretical), and a rotation \(\left[\alpha\right]_D = +17.3^\circ\) (reported in the literature +17.5\(^\circ\) Ehrlich and Rehorst \(^3^4\)). They did not obtain a crystalline derivative. This was one of the aims of the present investigation.

Yackel and Kenyon \(^2^9\) found that under suitable conditions cellulose may be readily oxidised by gaseous nitrogen dioxide to produce a new type of oxidised cellulose which did not show the degradation produced by other means. The nitrogen dioxide apparently selectively attacks the
the primary hydroxyl groups of cellulose as shown by the constant carboxyl content obtained. This is in interesting contrast to the action of periodic acid which attacks preferentially the secondary hydroxyl groups (Jackson and Hudson 30). A later experiment by Maurer and Reiff 31 (1943) showed that prolonged action of nitrogen dioxide gave some degradation of the cellulose.
METHYLATION METHODS

DIMETHYL SULPHATE AND SODIUM HYDROXIDE:

Methylation with dimethyl sulphate and 30% commercial sodium hydroxide was carried out in the present investigation using the method of Haworth in which the above reagents are added to the substance undergoing methylation in such proportions (1:3 respectively) that the liquid is slightly alkaline throughout the process. At the completion of the reaction, after boiling off excess dimethyl sulphate, the solution is neutralised with 5N sulphuric acid at 0°C. The solution is then made slightly alkaline when methylation uronic acids and the solution is extracted with chloroform to separate the sodium salt of the uronic acid from the aqueous solution. The sodium sulphate which crystallizes out on neutralization is usually filtered off before the chloroform extraction, and this sulphate residue taken up in water is then also extracted with chloroform. The chloroform extracts are dried, and the chloroform removed by distillation, giving the sodium salt of the methylated uronic acid.
Purdie Methylation:

This method which was used several times in the present investigation is normally used only on glycosides due to the oxidative action of silver oxide on the reducing hydroxyl group. This more expensive method consists in refluxing the glycoside of the sugar dissolved in a suitable solvent or by itself with methyl iodide and silver oxide at 45°C for 6 hours usually. The silver oxide is added in portions at intervals to increase the effectiveness of its action. It is suggested that an unstable intermediate silver alcoholate is formed.

In the original method of Purdie and Irvine the glycoside (1 mole) was reacted with methyl iodide (10 moles) and silver oxide (5 moles). These proportions have now been considerably reduced owing to the fact that excess of these reagents is usually provided by methyl iodide (10 moles) and silver oxide (3 moles, or a minimum of \(\frac{1}{5}\) mole of silver oxide per hydroxyl group).
CHART III

PRESENT INVESTIGATION:

\[
\begin{align*}
\text{H} & \quad \text{C} \quad \text{O} \quad \text{CH}_3 \\
\text{H} & \quad \text{C} \quad \text{O} \\
\text{HO} & \quad \text{C} \quad \text{H} \\
\text{H} & \quad \text{C} \\
\text{H} & \\
\text{CH}_2\text{OH} & \quad \text{(I)}
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{C} \quad \text{OCH}_3 \\
\text{H} & \quad \text{C} \quad \text{OH} \\
\text{NO}_2 \cdot \text{O} & \quad \text{C} \quad \text{H} \\
\text{H} & \quad \text{C} \\
\text{C} & \quad \text{O} \\
\text{O} & \quad \text{COOH} & \quad \text{(VIII)}
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{C} \quad \text{OCH}_3 \\
\text{H} & \quad \text{C} \quad \text{NO}_2 \\
\text{NO}_2 \cdot \text{O} & \quad \text{C} \quad \text{H} \\
\text{H} & \quad \text{C} \\
\text{C} & \quad \text{O} \\
\text{O} & \quad \text{COOCH}_3 & \quad \text{(XI)}
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{C} \quad \text{OCH}_3 \\
\text{H} & \quad \text{C} \quad \text{OH} \\
\text{CH}_3 & \quad \text{O} \quad \text{H} \\
\text{H} & \quad \text{C} \\
\text{H} & \\
\text{COOCH}_3 & \quad \text{(XIII)}
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{C} \quad \text{OCH}_3 \\
\text{H} & \quad \text{C} \quad \text{OH} \\
\text{HO} & \quad \text{C} \quad \text{H} \\
\text{H} & \quad \text{C} \\
\text{H} & \\
\text{COOCH}_3 & \quad \text{(XII)}
\end{align*}
\]
DISCUSSION

PRESENT INVESTIGATION:

The steps in the present investigation have been set out in Charts II and III.

The preliminary step in the present investigation was the methylation of d-glucose (I) with methanolic-hydrogen chloride to form \( \alpha \)-methyl d-glucoside (II) to protect the hydroxyl group on position 1 from oxidation.

\( \alpha \)-Methyl d-glucoside (II) was oxidised by nitrogen dioxide in chloroform and in carbon tetrachloride following the procedure of Maurer and Drefahl. It was found necessary to shake the mixtures longer than the twenty hours specified by these two workers in order to complete the oxidation.

The product, a colourless viscous syrup, was in both cases strongly acid in solution and non-reducing. The rotation of this free acid, supposedly \( \alpha \)-methyl d-glucuronoside (III) in water was \( [\alpha]_D^{20^\circ} = +99.3^\circ \), c.f. \( [\alpha]_D^{14} = 100^\circ-120^\circ \) (Maurer and Drefahl). This syrup slowly crystallized after keeping several months. The oxidation was thought to have been successful and the \( \alpha \)-methyl d-glucuronoside was converted to the barium salt (IV). After several purifications the barium content was reduced to 27.0% (theoretical 24.92%). This compound has not been reported in the literature except by Maurer and Drefahl.
who did not characterise it but converted it by dilute mineral acid to the known barium glucuronate.

In a preliminary trial experiment a smaller sample of L-methyl d-glucoside was completely oxidised in twenty hours on shaking with nitrogen dioxide in chloroform. The L-methyl d-glucuronoside was isolated and converted to the barium salt which was converted in acid solution to the known barium d-glucuronate. The barium content (27.2%) and the rotation of this compound ($[\alpha]_{D}^{20^\circ} = +17.54^\circ$ in water) agreed fairly well with the reported values; c.f. Ba $= 26.2\%$ (theoretical) and $[\alpha]_{D}^{20^\circ} = +17.45^\circ$ in water (Erlich and Rehorst 34). The oxidation had apparently been successful in this trial experiment.

Methylation of the barium salt from the main experiment with dimethyl sulphate and sodium hydroxide by the method of Haworth 32, and extraction of the slightly alkaline solution with chloroform gave only a very small quantity of impure material of methoxyyl value 4.3%. Extraction of the acid solution likewise failed.

The chloroform insoluble portion of the methylation mixture yielded a product containing much sodium sulphate formed in the methylation. Pure dry methanol was found to be the only pure solvent which would extract the sugar material in the sulphate, but the solubility of sodium sulphate in dry methanol was found to be considerable. This caused much difficulty in removing the sulphate.
As the product contained 62% ash, mainly sulphate, when a methoxyl determination was attempted a black precipitate of silver sulphide made the determination impossible.

The product, since it was insoluble in ether and chloroform had evidently a low methoxyl value and it was remethylated for a longer time with dimethyl sulphate and sodium hydroxide. Extraction as before yielded a colourless syrupy product (0.5 grams) containing crystalline material. It gave a negative test for a sulphate. The methoxyl content of this syrupy material was 50.6%; c.f. sodium salt of 2:3:4-trimethyl \( \alpha \)-methyl d-glucuronoside (V) OMe = 45.6% (theor.) c.f. Methyl ester of 2:3:4-trimethyl \( \alpha \)-methyl d-glucuronoside (VI) OMe = 58.7% (theoretical).

The refractive index of the syrupy product was

\[
\eta^\circ_{20^0} = 1.4486 \quad \text{c.f. methyl ester of 2:3:4-trimethyl} \\
\alpha \text{-methyl glucuronoside,} \quad \eta^\circ_{20^0} = 1.4467 \quad \text{Smith 9).}
\]

The product was esterified by methanic hydrogen chloride to give a colourless syrupy material (VI) (OMe = 57.5%, \( \eta^\circ_{19^0} = 1.4479 \)) which was treated with methanic ammonia to convert it to the known crystalline \( 1:2:3:4 \)-tetramethyl d-glucuronamide (VII). The product obtained was a colourless syrup which contained small crystals. Addition of ice-cold ether caused slightly more crystallization, but the crystals were too small to separate from the adhering syrup. A rotation on this substance gave
Thus the oxidation had apparently been successful but only a small portion of the fully methylated material had been obtained by the chloroform extraction.

The chloroform insoluble portion of the methylation mixture yielded a product containing much sodium sulphate. Methods suggested to eliminate the sodium sulphate included:

1. Chloroform or ether extraction of the sugar material.
   Method failed as the product was not soluble in these solvents.

2. Attempting to crystallize the sodium sulphate from solution by freezing in salt and ice. This was not very successful.

3. Distillation of the sugar material under high vacuum. Causes loss due to decomposition, and was not tried.


5. Repeated extraction and filtration with dry methanol. This was the method used.

6. An organic cation and anion exchanger is suggested as the ideal material to remove the sodium sulphate.

The impure product was methylated with Purdie's reagents which introduced added impurities in the form of

\[
\begin{align*}
\lambda^{15}_\circ &= +135.90 \text{ c.f. 1:2:3:4-tetramethyl d-glucuronamide} \\
\lambda^{20}_\circ &= +137.50 \text{ (Smith)}
\end{align*}
\]

Thus the oxidation had apparently been successful but only a small portion of the fully methylated material had been obtained by the chloroform extraction.

The chloroform insoluble portion of the methylation mixture yielded a product containing much sodium sulphate. Methods suggested to eliminate the sodium sulphate included:

1. Chloroform or ether extraction of the sugar material.
   Method failed as the product was not soluble in these solvents.

2. Attempting to crystallize the sodium sulphate from solution by freezing in salt and ice. This was not very successful.

3. Distillation of the sugar material under high vacuum. Causes loss due to decomposition, and was not tried.


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silver iodide and sodium iodide. The sulphate content made a methoxyl determination impossible, the product being still insoluble in chloroform or ether.

After three Purdie methylations in an attempt to render the material chloroform soluble, the product gave a methoxyl value of 19.14% (ash-free basis; ash 19.0%). A fourth Purdie methylation gave a methoxyl value of 19.3% (ash-free basis), and the fifth Purdie methylation (24 hours) gave a methoxyl value of 17.40% (ash-free basis). All the methylations were carried out in the presence of methanol and it was thought at first that the sulphate and methanol had reduced the effectiveness of the methylations.

Methylation in methanol with silver oxide and methyl iodide has been used quite often, in fact, one of the first methylations using these reagents was done in methanol by Purdie 33, who introduced four methyl groups in one methylation.

By the fifth Purdie methylation the ash consisted of colloidal silver and silver oxide (mainly). No test for a sulphate or sodium iodide was obtained. The ignited residue consisted solely of black silver oxide and it is suggested that the ash as weighed represents a fairly accurate estimate of the actual inorganic material present in the compound. The sample taken after the fifth Purdie methylation was a representative one of 50 milligrams and from this sample both the ash and the methoxyl value were determined. The sample for the methoxyl was thoroughly
dried, then dried in a weighed capsule, weighed with the capsule inside a closed weighing bottle, and the methoxyl content determined as carefully as possible.

Thus it would appear that the compound had reached a constant methoxyl value after seven methylations and would seem to be fully methylated. The product was neutral and non-reducing, and hence must have carried a methoxyl group on carbon atoms 1 and 6.

This would point to the hydroxyl groups on carbon atoms 2, 3 and 4 being somehow blocked by a substituent group, thus causing the failure of the methylations. It was then thought that the oxidation with nitrogen dioxide had caused nitration, the usual function of nitrogen dioxide in Organic Chemistry, as well as oxidation. Anhydro-sugar formation and lactone formation were ruled out by the low methoxyl values.

Maurer and Drefahl 14 evidently had noted the presence of nitrous acid after the oxidation as they had neutralized it with urea. The oxidation of the primary alcohol group on carbon atom 6 produces water which could combine with the nitrogen dioxide to give nitric and nitrous acids. If the nitric acid formed nitrated the secondary alcohol groups more water would be produced, hence more nitric acid, and so on. The reaction should therefore be carried out in the presence of an inert dehydrating agent, e.g. anhydrous sodium sulphate.
Nitration of sugars is usually effected by fuming nitric acid in chloroform at 0°C for 10-15 minutes, or by nitrogen pentoxide in chloroform. Thus, ideal conditions for nitration were available as the \( \alpha \)-methyl d-glucoside was added to the \( \text{NO}_2-\text{CHCl}_3 \) solution standing in an ice-bath to eliminate loss of the nitrogen dioxide from solution. If the oxidation started immediately and produced water, some nitration would occur in the cold solution. The nitric acid formed would be fairly concentrated as the solution was saturated with nitrogen dioxide to a red-brown colour.

It has been calculated that the amount of nitric acid formed would be capable of nitrating nearly all of the sample taken for oxidation. The longer time of shaking required compared to Maurer and Drefahl would suggest that these workers used a larger volume of chloroform to absorb their nitrogen dioxide, and hence more nitrogen dioxide. No quantities of chloroform or nitrogen dioxide were specified in their paper.

That nitric acid must have been present in the present investigation was shown by the evolution of gas with urea on the aqueous solutions after oxidation and distillation of the remaining nitrogen dioxide. Nitric acid decomposes into nitric acid on standing giving nitric oxide and water as well. Hence the small gas evolutions noted
are no indication of the amount of nitric acid present.

The product from the fifth Purdie methylation gave definite spot tests for nitrate with diphenylamine and sulphuric acid and with brucine and sulphuric acid. The methoxyl value (17.40%) (ash-free basis) coincided with the calculated methoxyl value for the methyl ester of \( \alpha \)-methyl d-glucuronomoside - 2:3:4 trinitrate (17.36%). Some of the product when heated in a sealed melting point tube exploded vigorously, this being a characteristic of the higher sugar nitrates, e.g. tetranitrates (Dewar and McArthur 35).

The product was purified by the passage of dry hydrogen sulphide which removed all the colloidal silver, but difficulty was experienced in removing all the silver iodide formed in the methylations. Repeated filtrations and absorption on animal charcoal lowered the ash content to 9.0%. A micro nitrogen analysis on this material showed only 2% nitrogen, but it is suggested that as the sample was impure the analysis of a few milligrams might be only approximate. From this analysis it would appear that the material was not the trinitrate (N=11.7%; theoretical) but nearer the mononitrate (N=5.24%; theoretical).

The analysis showed however, that the material had definitely been nitrated to some extent.

The nitrated methyl ester of \( \alpha \)-methyl d-glucuronomoside formed a hard glass when dried. This material
formed rosettes of long needles from methanol with some adhering syrupy material. Some crystalline material was obtained on washing and drying on a porcelain plate which gave a positive test for uronic acid and had m.p. 158-159° C. Some of this methyl ester was treated with alcoholic ammonia to convert it to the corresponding amide which partly crystallized in small needles, the syrupy material drying to a hard glass. The amide gave a rotation of twice that of the ester which seems to be a common occurrence. After drying on a porcelain plate, it gave m.p. 208° C.

The nitrated methyl ester of L-methyl d-glucuronoside was denitrated by the method of Dewar 26, using a mixture of iron and zinc powders in glacial acetic acid. The product isolated in small yield by means of chloroform gave no test for nitrogen, while a sample of the nitrated material similarly tested gave a positive test.

The denitrated material (0.036 grams) was methylated three times with Purdie's reagents and the methylated product isolated by means of chloroform. The product (0.16 grams) was impure and was purified by ether extractions and filtrations. The product, a syrup, was still impure and gave a methoxyl value of 42.5% and a refractive index \( \eta^18^0_D = 1.4601 \). Product was neutral and non-reducing.
c.f. Methyl ester of 2:3:4-trimethyl \( \alpha \)-methyl d-glucurono-
side, \( \text{C}_{11}\text{H}_{20}\text{O}_7 \): \( \Delta \text{Me} = 58.7\% \) (theoretical)
\[ \eta_{D}^{190} = 1.4470 \] (Smith\(^9\))

c.f. Methyl ester of 2:3-dimethyl \( \alpha \)-methyl d-glucuronoside
\( \text{C}_{10}\text{H}_{18}\text{O}_7 \): \( \Delta \text{Me} = 49.6\% \); \[ \eta_{D}^{180} = 1.4600 \] (Smith \(^{40}\))

The methoxyl content had evidently increased from
17.4\% to 42.5\% by methylation after denitration.

If oxidation had caused nitration the barium
\( \alpha \)-methyl d-glucuronate should also be nitrated and
the barium content considerably lower than that obtained in
the early stages of the investigation. As barium salts are
notoriously impure and difficult to purify, the supposed
barium \( \alpha \)-methyl d-glucuronate (IV) was further examined
and purified by filtration of the aqueous solution and pre-
cipitation by alcohol. Three values below the theoretical
barium content for barium \( \alpha \)-methyl d-glucuronate (24.91\%)
were obtained, the final value being 17.36\%. Barium \( \alpha 
\)-methyl d-glucuronate - 2:3:4-trinitrate (IX) requires
16.72\% Ba.

This suggests that mainly the trinitrate had been
formed in the oxidation. Both the barium salt and the free
acid, \( \alpha \)-methyl d-glucuronoside gave definite tests for
nitrogen.

It is suggested that the oxidations in chloroform
and carbon tetrachloride of \( \alpha \)-methyl d-glucoside (II) by
nitrogen dioxide yielded mainly $\alpha$-methyl d-glucuronoside-2:3:4 trinitrate (VIII) which, on treatment with barium hydroxide yielded the corresponding nitrated barium salt (IX). Dimethyl sulphate and sodium hydroxide gave merely the sodium salt of $\alpha$-methyl d-glucuronoside -2:3:4 trinitrate (X) which, on treatment with methyl iodide and silver oxide gave the corresponding methyl ester (XI). Denitration gave $\alpha$-methyl d-glucuronoside methyl ester (XII) which, on further methylation, gave 2:3-dimethyl $\alpha$-methyl d-glucuronoside methyl ester (XIII).

Formation of the trinitrate would explain:-

1. Failure of methylation and constant methoxyl obtained and agreement with trinitrate calculated methoxyl value.

2. Low barium contents obtained for supposed barium salt of $\alpha$-methyl d-glucuronoside. Agreement with calculated trinitrate value.

3. Why a crystalline compound was obtained instead of usual methylated glucuronoside syrups.

4. Explosive action of product heated in a sealed melting point tube.

The reason for the production of nitrated $\alpha$-methyl d-glucuronoside in the large scale oxidation is thought to be due to the increased time of reaction needed to complete the oxidation which was probably due to the less
efficient shaking of the larger sample as compared to the smaller one used in the trial experiment. It is possible that the samples obtained in the preliminary experiment and by Maurer and Drefahl \(^4\) may have also been nitrated, but as no crystalline derivatives were obtained, this could not be proved definitely. The method of isolation and treatment to convert the free acid to barium d-glucuronate may possibly have removed the nitrate groups, though there is no evidence for this.
1. The small-scale oxidation of $\alpha$-methyl d-glucoside with nitrogen dioxide in chloroform according to the procedure of Maurer and Drefahl yielded $\alpha$-methyl d-glucuronoside which was isolated as barium d-glucuronate. This confirms the results obtained by Maurer and Drefahl.

2. Oxidation of a larger sample of $\alpha$-methyl d-glucoside with nitrogen dioxide in chloroform and in carbon tetra-chloride for forty hours yielded mainly a compound believed to be the barium salt of nitrated $\alpha$-methyl d-glucuronoside together with a small amount of barium $\alpha$-methyl d-glucuronate.

3. Methylation of the mixed barium salts gave the sodium salt of 2:3:4-trimethyl $\alpha$-methyl d-glucuronoside in small yield, which was esterified to the corresponding methyl ester and converted to the known 1:2:3:4-tetramethyl d-glucuronamide. The main yield consisted of partially methylated "nitrated" material.

4. Further treatment of the "nitrated" material with the usual methylating agents failed to increase the methoxyl content. Denitration and subsequent methylation of this material raised the methoxyl content considerably.

5. The production of $\alpha$-methyl d-glucuronoside by the nitrogen dioxide oxidation of $\alpha$-methyl d-glucoside is
thus substantiated and its identity established for the first time by conversion to the known 1:2:3:4-tetramethyl d-glucuronamide.
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EXPERIMENTAL

PREPARATION OF \( \alpha \)-METHYL D-GLUCOSIDE:

\( \alpha \)-Methyl d-glucoside was prepared by the method of Robertson and Patterson \(^{36} \) in fair yield.

Dextrose, pure anhydrous (160 grams) was dried in a vacuum drier to 30-40\(^\circ\) C. for five hours. It had m.p. 146\(^\circ\) C. The anhydrous glucose was then placed in a 1 litre bolthead flask and methyl alcohol (320 grams, carefully dried over lime and distilled from magnesium and iodine) containing 3% dry hydrogen chloride was added. The solution was then boiled on the water bath under reflux for 4\(\frac{1}{2} \) 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 an external bath of ice with occasional stirring. After an hour the \( \alpha \)-methyl d-glucoside formed a thick paste, and the crystalline material was filtered off under vacuum. After being washed with a little dry methanol, it was dried on a porous plate.

Yield: 61 grams of m.p. 163\(^\circ\) C (35.5% theoretical). From this crude yield after recrystallization from methanol (150 cc.), 40 grams of pure \( \alpha \)-methyl d-glucoside were obtained of m.p. 165-166\(^\circ\) and rotation \( [\alpha]_D^{24.5\circ} = \)
154.5° in water ($c = 1.0$).

Yield: 40 grams (23.3% theoretical)

c.f. Robertson and Patterson, m.p. 165-166° C,
$[\alpha]_{D}^{25°} = +157.9°$ in water.
OXIDATION OF \textit{\textbf{M}}ETHYL \textit{\textbf{d}}-GLUCOSIDE \textit{\textbf{B}}Y \textit{\textbf{N}}ITROGEN \textit{\textbf{D}}IOXIDE

The oxidation was carried out exactly according to the method described by Maurer and Drefsahl\textsuperscript{14}, except for the time of reaction. The term nitrogen dioxide refers to the equilibrium mixture of nitrogen dioxide with its dimer nitrogen tetroxide.

Nitrogen dioxide was prepared by slowly heating dry lead nitrate in a pyrex round bottomed flask (750 cc.) on a sand-bath in a fume cupboard.

\[ 2 \text{Pb (NO}_3\text{)}_2 \rightarrow 2\text{PbO} + 4\text{NO}_2 + \text{O}_2 \]

If the heating is too rapid nitric oxide (NO) formed by the decomposition of the nitrogen dioxide will be swept through the apparatus without recombining to form nitrogen dioxide and the condensed liquid will consist of a mixture of liquid nitrogen dioxide (yellow) and liquid nitrogen sesquioxide (N\textsubscript{2}O\textsubscript{3}) which is blue. None of the preparations of liquid nitrogen dioxide showed the slightest trace of a blue colour. The lead nitrate is heated on a platinum foil preferably as lead monoxide combines with the pyrex flask.

The equilibrium mixture of nitrogen dioxide and nitrogen tetroxide was passed through a phosphorus pentoxide drying tube and condensed in two U-tubes cooled in a
mixture of salt and ice. The oxygen formed in the decomposition passed through the apparatus.

The clear brownish yellow liquid condensed mainly in the first U-tube, and when sufficient liquid had been collected, c.a. 10 c.c., the reaction flask was disconnected and that connection closed. The condensed liquid was redistilled slowly to effect purification into the second U-tube cooled in ice, and then distilled into 200 c.c. of chloroform contained in a reagent bottle (250 c.c.) in an ice-bath to eliminate loss due to evaporation.

On the chloroform solution assuming a red-brown colour, the nitrogen dioxide bubbling was stopped and α-methyl d-glucoside (10.85 grams) was added to the cooled solution. The stoppered and securely wired bottle was placed in a mechanical revolving shaker and shaken for 40 hours. The α-methyl d-glucoside slowly reacted with the solution becoming more glutinous. The colour of the brown solution slowly became greenish, and the glucoside finally all changed into a finely divided syrup which, when shaken, was seen to consist of small globules. The increased time of shaking as compared to Maurer and Drefahl was necessary to convert completely the glucoside to the syrupy material.

After forty hours the nitrogen dioxide was removed by evacuation in a 250 c.c. Claisen flask, and repeated
vacuum evaporation with fresh solvent. When all the
nitrogen dioxide had been evaporated off, the clear colour-
less syrup was dissolved in a minimum amount of water, and
freed of nitrous acid by the addition of small amounts of
urea in about 2 milligram portions. As no ebullition of
carbon dioxide and nitrogen was noted on three such addi-
tions, it was concluded that no nitrous acid was present.

The aqueous solution reacted strongly acid to
Congo red paper and was non-reducing to Fehling's solution.
Boiling with acid made the product reducing.

The colourless syrupy free acid gave \( [\mathcal{A}]_{D}^{20^\circ} \)
= + 99.3\(^\circ\) in water (\( C = 0.85 \)), c.f. \( [\mathcal{A}]_{B} = + 100^\circ \)
120\(^\circ\) (Maurer and Drefahl 14). This syrup slowly crystall-
ized after some months in a desiccator forming flat plates.
Found m.p. 78\(^\circ\) - 98\(^\circ\) C. The agreement with Maurer and
Drefahl in specific rotation thus seems doubtful, they
having obtained a syrupy product on isolation as above also.

The crystalline material was extremely hygroscopic
forming a brownish syrup when exposed to the air for only
1-2 minutes. The golden crystalline material was dissolved
in a few c.c's. of cold absolute ethyl alcohol and a white
insoluble residue possibly unoxidised \( \mathcal{A} \)-methyl d-glucos-
side filtered off (filtration done inside a desiccator).
Addition of ice-cold ether to the alcohol filtrate caused
crystallization, colourless plates being obtained, m.p.
\[ \text{96}^\circ - 108^\circ \text{ C. (probably a mixture of nitrated and un-} \\
\text{nitrated} \ \alpha - \text{methyl d-glucuronoside).} \]

The above procedure was repeated in every detail for another sample of \( \alpha - \text{methyl d-glucoside} \) (10.13 grams) in carbon tetrachloride, the reaction taking 45 hours to complete. In this case, on adding the urea to the colourless aqueous solution, evolution of gas was noticed, and urea (c.a. 0.03 grams) was added till no more gas was evolved. The product presumed to be \( \alpha - \text{methyl d-glucuronoside} \) was as before, strongly acid and non-reducing.

**PREPARATION OF THE BARIUM SALT:**

To the aqueous solution of the free acid obtained above was added a saturated freshly filtered solution of barium hydroxide until the solution was slightly alkaline to litmus, and the solution heated at 60\(^\circ\) C. for 3 hours in a flask fitted with a soda-lime tube to avoid neutralization of the baryta by atmospheric carbon dioxide. Excess barium hydroxide was removed by bubbling carbon dioxide through the solution till neutral to litmus, followed by filtration of the barium carbonate. Care was taken to prevent the solution becoming acidic and thus removing the methyl group on C atom 1. The residue from the filtration was washed thoroughly with hot water and the combined filtrate and washings evaporated to dryness in vacuo at 40\(^\circ\) C. in a
weighed 125 c.c. Claisen flask, using a dropping funnel to run in more solution as required. The solution became syrupy and, on further evaporation, a hard golden brown glass was formed.

Total yield: 24.98 grams (63.8% theoretical).

This hard glass was extracted with hot absolute ethyl alcohol to remove any unoxidised α-methyl d-glucoside. On addition of the alcohol, a yellowish amorphous powder was formed in the flask and this was re-extracted with hot alcohol portions (25 c.c.). Ether could not be used for the extractions as α-methyl d-glucoside is insoluble in it. A portion of this material gave a barium content of 29.5%. The sample was dissolved in a minimum amount of water, filtered, and the barium salt re-precipitated by the addition of absolute alcohol. The barium salt was filtered through a glass sintered crucible and dried thoroughly in a vacuum desiccator. Found: Ba = 27.0%

\[ [\alpha]_D^{19^0} = +51.3^0 \text{ in water (C = 0.27)} \].

C.f. Barium α-methyl d-glucuronate, Ba = 24.91% (calculated). Rotation unknown.

Later, on it being suspected that the barium salt had been nitrated in the oxidation with nitrogen dioxide, the barium salt was further purified by filtration and re-precipitation four times.

Found: Ba = 17.4% (final value), \[ [\alpha]_D^{25^0} = +62.2^0 \text{ in water in a micro-polarimeter tube (C = 0.65)} \].
c.f. Barium methyl glucuronate - 2:3:4 trinitrate
Ba = 16.72% (calculated). Rotation unknown.

The barium contents were determined by a semi-
micro method using a muffle furnace (Pregl 47). The car-
bonaceous material was first burnt off to eliminate reduction
of the barium sulphate formed in the estimation. The ig-
nited barium sulphate was weighed, and reignited with a drop
or two of sulphuric acid to convert any sulphide to sulphate,
and weighed. The ash contents were determined by ignition
using the same apparatus.

In a preliminary trial experiment, $\alpha$-methyl
d-glucoside (5 grams) was oxidised in chloroform (100 c.c.)
by nitrogen dioxide exactly as above, except that the sample
was completely oxidised after twenty hours, no glucoside re-
mainig. This quicker time of reaction was possibly due to
the more efficient shaking of the 100 c.c. solution in the
250 c.c. reagent bottle as compared to the 200 c.c. solution
in the bottle in the main experiment, and also to the smaller
size of the sample. The amount of nitrogen dioxide is also
critical in respect to the time of reaction and although
approximately twice the quantity of the reagents was used in
the main experiment compared to the trial run the time of
reaction required was also twice that in the trial experiment.

The oxidised material, apparently the free acid,
was isolated in the trial experiment as above and converted
to barium $\alpha$-methyl d-glucuronate which was not isolated,
but was converted in acid solution to the known barium d-
glucuronate which was reducing to Fehling's solution. The
barium d-glucuronate was purified by filtration and alcohol
precipitation several times.

Found: Ba = 27.2%, $\left[\alpha\right]_{D}^{20} = +17.54^\circ$ in
water (C = 0.83) no mutarotation shown.
c.f. Barium d-glucuronate, Ba = 26.2% (calculated).
Amorphous. $\left[\alpha\right]_{D}^{20} = +17.45^\circ$ in water (C = 3.7)
Ehrlich and Rehorst $^{34}$, no mutarotation found.

As the trial run had apparently been successful, the
main experiment was proceeded with, the methyl group on
C atom 1 being left intact.

The following tests served to identify the glucur-
onic acid. Both the free acids and the barium salts gave
good tests.

**NAPHTHO-RESORCIN TEST:**

The barium salt (c.a. 1-2 m.gms.) was placed in a
test tube in 1 c.c. of water and acidified with dilute sul-
phuric acid dropwise (Congo red paper.). The aqueous
uronic acid solution was then mixed with an equal volume of
concentrated hydrochloric acid and boiled. A few drops of
a 1% solution of naphtho-resorcin in 95% alcohol were added
the solution being boiled for $\frac{1}{2}$ minute. After cooling to
about 50° (tap) the solution was shaken with benzene or
ether and a violet colouration in the top ether or benzene
layer denoted a uronic acid 37.

**BASIC LEAD AND ACETATE TEST:**

This was a specific test for glucuronic acid described by Ehrlich 38. A solution of the \( \alpha \)-methyl d-glucuronoside gave a white precipitate with basic lead acetate solution, dissolving in excess, and on heating, a yellowish precipitate was formed. d-Galacturonic acid gives a dark red-brown precipitate on heating, while d-mannuronic acid gives a buff precipitate.

As the barium salt was insoluble in methanol and in acetone, it was not possible to use the Purdie methylation method without first partly methylating with dimethyl sulphate and sodium hydroxide following the procedure of Haworth 32.

**METHYLATION OF BARIUM SALT USING DIMETHYL SULPHATE AND SODIUM HYDROXIDE:**

The barium salt (22.38 grams) was methylated with dimethyl sulphate (120 c.c.) and 30% sodium hydroxide (320 c.c.) in the presence of 50% aqueous acetone 100 c.c.) at 35-40\(^\circ\) C. in a 2 litre bolthead flask surrounded by a water bath. Dimethyl sulphate (10 c.c) and sodium hydroxide (30 c.c) were added from two dropping funnels at 15 minutes intervals
in twelve equal portions, the rate of addition of the sodium hydroxide portion being twice that of the dimethyl sulphate (10 minutes).

Throughout the methylation the liquid was agitated vigorously by a mechanical stirrer. At the end of 3 hours the temperature of the bath was raised to 90-100°C for 45 minutes to boil off excess dimethyl sulphate. The methylation was carried out in a fume cupboard.

The solution, cooled in ice to 0°C, was neutralized to Congo red paper with 5N sulphuric acid, then made just alkaline with sodium hydroxide. The solution was filtered through a linen cloth to remove the precipitated sodium and barium sulphates, and the filtrate extracted with four 100 c.c. portions of chloroform. The chloroform layer was separated from the aqueous layer and the combined chloroform extracts dried overnight with anhydrous magnesium sulphate.

The solvent was removed from the filtered solution by distillation under vacuum leaving a very small residue of brownish material (0.1 grams). The material gave a positive flame test for sodium and a methoxyl content of 4.3%. It was obviously impure and not fully methylated.

c.f. Sodium salt of α-methyl glucuronoside, OMe = 13.5% (calculated); and sodium salt of 2:3:4-trimethyl α-methyl d-glucuronoside, OMe = 45.58% (Calculated).

The methoxyl content was determined using a
semi-micro modification of the original Zeisel apparatus
(Hewitt and Moore 48, Perkin 49).

The methylated material was tested for reducing action with Fehling's solution and gave no trace of reducing action.

The sulphate residues from the filtration contained sugar material as shown by the positive Molisch test obtained and by the colour of the residues. They were extracted with 90% acetone (100% acetone not removing the sugar material) according to the method of Smith 39. The combined acetone washings were evaporated to dryness under reduced pressure at 40° C when sodium sulphate crystallized out. The dried residue was re-extracted with 90% acetone to remove some of the sulphate.

The chloroform insoluble aqueous portion of the methylation mixture C.a. 400 c.c. was evaporated to dryness under vacuum at 40° C, and the dry residue extracted with 90% acetone, and the extracts filtered through a Whatman 44 (used in most filtrations) without suction to eliminate some of the sulphate. This was not very successful, and pure dry methanol was found to be the only pure solvent which would extract all the sugar material in the sulphate, but at the same time, much sodium sulphate also dissolved in the methanol.

A sample of the filtered acetone extract was
thoroughly dried in a vacuum drier at 40° C. for 7 hours. As this sample still contained much sulphate the ash content was determined. Found: ash = 62%.

When a methoxyl determination was attempted a black precipitate of silver sulphide made the determination impossible.

The acetone extract was next extracted with dry methanol, extracts filtered, and evaporated to dryness. Found: ash = 50%

The methylated product was soluble in water, acetone-water, methanol, and insoluble in ether, acetone or chloroform. The product was non-reducing.

The product, since it was insoluble in ether or chloroform had evidently a low methoxyl value and it was remethylated for a longer time with dimethyl sulphate and sodium hydroxide.

SECOND DIMETHYL SULPHATE METHYLATION:

The impure product (17 grams) containing about 8-9 grams of carbohydrate material was methylated as before using dimethyl sulphate (60 c.c.) and sodium hydroxide (180 c.c. of a 30% solution) in the presence of 50% acetone (100 c.c) at 35-40° C. The reagents were added over 3 hours at the end of which the reaction was continued with vigorous stirring for a further 5 hours, making 3 hours altogether. The dimethyl sulphate was boiled off at 100° C for a further
half hour. The solution was neutralized as above, and the filtered slightly alkaline solution N/10 sodium hydroxide) extracted with chloroform (600 c.c.) and the combined chloroform extracts dried over anhydrous magnesium sulphate overnight.

The chloroform was removed from the extracts by distillation giving a syrupy colourless product (0.5 grams) containing crystalline material. This was thought to be a mixture of the sodium salt and the methyl ester of 2:3:4-trimethyl α-methyl d-glucuronoside. The syrupy product was thoroughly dried in a vacuum oven at 40° C. for 30 hours and in a vacuum desiccator but still remained syrupy.

Found: OMe = 50.6%  
\[ \alpha \]_D^{190} = +106.0° in water (C = 0.60)  
\[ \alpha \]_D^{200} = 1.4486

c.f. Sodium salt of 2:3:4-trimethyl α-methyl d-glucuronoside, C_{10}H_{17}O_7 Na: OMe = 45.58% (Calculated) Rotation unknown.

c.f. Methyl ester of 2:3:4-trimethyl α-methyl d-glucuronoside, C_{11}H_{20}O_7: OMe = 58.7% (Calculated), \( n_D^{200} = 1.4467 \) (Smith 9)  
\[ \alpha \]_D^{180} = +87° in water (Smith 9).

The syrupy product was neutral and non-reducing, giving a positive test for sodium and a negative test for sulphate. The solution used for the rotation came down mainly as a crystalline colourless product on distilling off the water. The product was then esterified by methanolic-
hydrogen chloride to convert the sodium salt to the ester.

**ESTERIFICATION OF SODIUM SALT USING METHANOLIC HYDROGEN CHLORIDE:**

The mixture, apparently of the sodium salt and the methyl ester of 2:3:4-trimethyl α-methyl d-glucuronic acid (0.29 grams) was esterified by boiling with 3% methanolic-hydrogen chloride (100 c.c.) in a 250 c.c. flask fitted with a ground in condenser with a calcium chloride tube attached. After 4 hours the solution was cooled, neutralized with silver carbonate, filtered and evaporated to dryness giving a syrupy product. Extraction with dry ether, filtration and evaporation of the ether yielded a colourless syrupy material containing a small amount of impurity, probably sodium chloride.

Found: OMe = 57.1%, 57.5%; \( \eta_{D}^{190} = 1.4470 \)

Methyl ester of 2:3:4-trimethyl α-methyl d-glucuronic acid \( \text{C}_{11} \text{H}_{20} \text{O}_{7} \): OMe = 58.7% (Calculated) \( \eta_{D}^{190} = 1.4470 \) (Smith 9). Mobile, colourless liquid.

The product was a mobile, colourless syrup, neutral and non-reducing. Yield: 0.25 grams.

**AMIDE FORMATION:**

The methyl ester (0.25 grams) in methanol was filtered to remove a small amount of insoluble material. The solution (c.a.4 c.c.) in a 50 c.c. conical flask was
saturated with dry ammonia for 15-20 minutes at 0° C, and the solution left at 0° C. for 3 days. On removal of the methanol in a vacuum desiccator, minute crystals were noticed, and addition of ice-cold ether caused slight crystallization but the crystals could not be separated from the adhering syrup. The difficulty in crystallization was probably due to small amounts of impurity. A rotation was carried out on the syrupy material.

Found: \[ \alpha^\circ_{D} = +135.9^\circ \] in water (C = 0.16)

This value was checked by an independent worker. c.f. 1:2:3:4-Tetramethyl glucuronamide, C\textsubscript{10}H\textsubscript{19}O\textsubscript{6}N: m.p.183° C [\[ \alpha^\circ_{D} = +137.5^\circ \] in water (Smith 9).}
CHLOROFORM INSOLUBLE PORTION:

The aqueous solution from the second methylation light brown in colour, which was probably due to decomposition products formed at the high temperature (100° C) required to boil off the excess dimethyl sulphate, was taken to dryness and the dried residue extracted with methanol, followed by filtration and concentration of the extracts to a small volume c.a. 20 c.c. As the product was insoluble in chloroform and still evidently of a low methoxyl content, it was methylated in methanol by the Purdie method. A methoxyl determination was impossible due to the high sulphate content. The sulphate residues from the filtration after neutralization of the methylation mixture were thoroughly washed with dry methanol to extract the carbohydrate material, but at the same time some sodium sulphate also dissolved. The concentrated main product and the concentrated washings consisting of crystalline sodium sulphate and the carbohydrate material in methanol (40 c.c.) were methylated. The carbohydrate material was non-reducing to Fehling's solution and insoluble in pure methyl iodide.

PURDIE METHYLATION:

The product obtained as above (15 grams) containing 6-7 grams of carbohydrate material in methanol (40 c.c.)
was methylated by methyl iodide (40 c.c.) and silver oxide (12 grams). The silver oxide was added in 1.5 gram portions hourly by momentarily lifting the ground-glass stoppered reflux condenser from the flask. The bath temperature was 45-46° C and the flask (200 c.c.) and condenser were vigorously shaken at intervals to allow the silver oxide to react more efficiently. The methylation mixture was left overnight, with a calcium chloride tube attached, to allow colloidal silver to settle out.

The solution was then filtered, the silver residues exhaustively extracted with hot dry methanol, and the combined filtrate and washings evaporated to dryness at 40° C under vacuum. The impure product gave positive tests for sodium, sulphate and iodide, while silver iodide which had passed through the finest filter papers was also present.

Great difficulty was found in eliminating the sodium sulphate and iodide as the methylated product was insoluble in ether and in chloroform and hence could not be extracted with chloroform in the standard manner. An earlier attempt to crystallize out the sodium sulphate by freezing the solution was not very successful. Repeated extraction and filtration was the only method found practicable and this was not very successful if the residues were washed too much with methanol due to the dissolution of the sulphate.

In an attempt to render the material chloroform
soluble the product was remethylated with methyl iodide (40 c.c.) and silver oxide (12 grams) as above for 12 hours. After the third Purdie methylation the product gave no test for a sulphate, an iodide test being positive. A sample was tested.

Found: OMe = 19.1% (ash-free basis)

Ash = 19.0%

On a fourth methylation exactly as above for 12 hours, a sample was also examined.

Found: OMe = 19.3% (ash-free basis)

Ash = 26.0%

A fifth Purdie methylation was unsuccessful in making the product chloroform soluble.

Found: OMe = 17.4% (ash-free basis)

Ash = 20.5%

c.f. Methyl ester of 2:3:4-trimethyl α-methyl d-glucuronoside, C₁₁H₂₀O₇: OMe = 58.7 (calculated)

c.f. Methyl ester of α-methyl d-glucuronoside - 2:3:4 trinitrate C₈H₁₁O₁₃N₃: OMe = 17.37% (Calculated)

All the methylations were carried out in the presence of methanol.

The ash consisted of colloidal silver and silver iodide (mainly) by the fifth methylation. No test for an iodide or sulphate was obtained.

A representative sample of 50 m.gms. was taken and after thorough drying in a vacuum oven at 40° C for 20 hours
smaller samples were taken for the ash and methoxyl determinations from this. The sample for the methoxyl determination was dried for several hours in a vacuum desiccator in a weighed capsule and when thoroughly dry, the sample and capsule were weighed in a ground glass stoppered weighing bottle. These precautions were necessary as the sample was quite hygroscopic. The product (3.1 grams) was a reddish colour and on thorough drying, it formed a hard glass. It was neutral and non-reducing. The reddish colour was caused by free iodine formed by the photochemical decomposition of methyl iodide.

Thus it appeared that the compound had reached a constant methoxyl value after seven methylations as shown by the methoxyl values 19.1%, 19.3%, 17.4%, (corrected for ash). Since the product was neutral and non-reducing, it must have carried a methoxyl group on carbon atoms 1 and 6. This would mean that the hydroxyl groups on carbon atoms 2, 3, and 4 were somehow blocked by a substituent group, thus causing the methoxyl content to remain constant.

As it was suspected that the oxidation with nitrogen dioxide had also caused nitration, all the compounds obtained were tested for nitrogen.

**SPOT TESTS FOR NITRATE**

The following spot tests were carried out on the compound obtained.
1. **BROWN RING TEST:** A pin-head sized crystal of ferrous sulphate was placed on the spot plate together with a drop of the test solution, and a drop of concentrated sulphuric acid was allowed to run in at the side of the drop. In the presence of a nitrate a brown ring was formed round the ferrous sulphate crystal. A blank test using distilled water, ferrous sulphate and concentrated sulphuric acid gave no ring.

2. **DIPHENYLAMINE TEST:** About \( \frac{1}{2} \) c.c. of a strongly acid (sulphuric) diphenylamine solution was placed on the spot plate, and a drop of the test solution dropped in the middle. Where the two liquids mixed, a blue ring formed, the depth of colour depending on the nitrate content. A blank gave no colour.

3. **BRUCINE TEST:** A few drops of a freshly prepared 0.02% brucine solution in sulphuric acid were mixed on the spot plate with a few drops of the test solution. In the presence of a nitrate, a red colour appeared which on standing, changed to yellowish red.

The acid used was pure concentrated sulphuric acid which was diluted to sp. gr. 1.4 and heated carefully till boiling to completely free it from nitric and nitrous acids. The acid was then used for all the tests.

The product from the fifth Purdie methylation gave positive tests with all these reagents. The barium salt
which had been thought to be barium \( \alpha \)-methyl d-glucuronate gave a positive test, as did the supposed \( \alpha \)-methyl d-glucuronoside. It therefore appeared that in the initial oxidation nitration had occurred to a large extent giving a main yield of \( \alpha \)-methyl d-glucuronoside - 2:3:4 trinitrate which was converted to the barium salt and methylated. This would explain the failure of the methylations. Some of the material had evidently not been nitrated as this was the portion extracted by chloroform after the second dimethyl sulphate methylation. The latter gave no test for nitrogen when examined.

**EXAMINATION OF NITRATED MATERIAL:**

The product obtained after seven methylations was purified by passing hydrogen sulphide into a methanolic solution of the material and filtering off the precipitated silver sulphide. The silver residue was washed thoroughly with dry methanol and the combined filtrate and washings aerated to remove excess hydrogen sulphide. The solution was then concentrated to a small volume when small crystals were seen to form. The material was thoroughly dried and a sample taken for an ash determination.

**Found:** ash = 15.0%

This ash was now entirely silver iodide as shown by passing more hydrogen sulphide when no more sulphide was formed. The silver iodide was found difficult to remove
passing through the finest filter papers. After three filtrations and a filtration using a small piece of animal charcoal to absorb the inorganic material, the ash content when determined on a representative sample was 9.0%. A determination micro nitrogen on this material showed only 2% nitrogen, but as the sample was impure, the analysis of a few milligrams might be only approximate.

c.f. $\alpha$-methyl glucuronoside - 2:3:4 trinitrate,
$\text{C}_8\text{H}_1\text{O}_{13}\text{N}_3$: $N = 11.76\%$ (Calculated).

c.f. $\alpha$-methyl glucuronoside mononitrate,
$\text{C}_8\text{H}_1\text{O}_9\text{N}$: $N = 5.24\%$ (Calculated)

From this it would appear that the material was not the trinitrate but nearer the mononitrate. The material had definitely been nitrated however, and that the trinitrate was formed at least before methylation was borne out by the barium percentages obtained on examination of the supposed barium $\alpha$-methyl $d$-glucuronate - three values below that of the calculated unnitrated value and the final value close to that of the trinitrate (found: 17.4%, calculated 16.72%).

The nitrate groups may have been removed by the sodium hydroxide during the first two methylations or during the Purdie methylations, but unless anhydro sugar formation occurred at the same time, the methoxyl content should have been higher than 17-19%. The nitrate group removal is however, unlikely.
The nitrated material crystallized in long needles from methanol on concentrating the solution and cooling at 0° C for 2 days. Some of this material was dried on a porous plate after being washed with methanol and ether to remove adhering syrup. The rotation of this nitrated material was also determined.

Found: m.p. 158-159° C, \([\alpha]_{D}^{210} = +17.2°\) in methanol (C = 0.67). No mutarotation shown.

Some of the nitrated product when heated in a sealed melting point tube exploded quite vigorously, this being a characteristic of nitrates of sugars, especially of the higher nitrates (Dewar and McArthur 35).

**AMIDE FORMATION:**

The nitrated product (0.25 grams) in methanol was saturated with ammonia, dried through two drying towers, after 10-15 minutes at 0° C. The stoppered solution was left at 0° C for two days. On removal of some of the methanol in a vacuum desiccator and on standing, short needles were formed, while the syrupy material dried to a hard glass. Some of this material was dried on a porous plate. Yield = 0.21 grams

Found: m.p. 208° C; \([\alpha]_{D}^{1950} = + 35.3°\) in methanol (C = 0.25). No change in the rotation was observed after 12 hours.
Found: OMe = 12.79%
c.f. $\alpha$-methyl d-glucuronamide-2:3:4 trinitrate,
$C_7H_10O_2N_3$: OMe = 9.45% (Calculated)
c.f. $\alpha$-methyl d-glucuronamide mononitrate,
$C_7H_12O_9N_2$: OMe = 11.57% (Calculated)
c.f. $\alpha$-methyl d-glucuronamide, $C_7H_13O_6N$: OMe = 15.0% (Calculated)
c.f. 2:4 anhydride $\alpha$-methyl d-glucuronamide-3 mononitrate
$C_7H_10O_8N_2$: OMe = 12.4% (Calculated)

DENITRATION OF NITRATED MATERIAL:

The nitrated material (1.01 grams) in glacial acetic acid (12 c.c.) was denitrated by adding an excess of a mixture of zinc and iron powders according to the method of Dewar 26. The reaction vessel (100 c.c. conical flask) was gently heated and a vigorous reaction ensued. Gentle heating was continued when necessary for 20 minutes until a test drop gave no blue colour with diphenylamine in concentrated sulphuric acid. Isolation of the carbohydrate material was effected by filtration (the residues being well washed with hot chloroform), adding the filtrate and washings to a concentrated solution of potassium carbonate containing slightly more carbonate than that equivalent to the amount of acetic acid used, and extracting the aqueous mixture with chloroform.

On extraction with chloroform, an emulsion was formed, but the addition of a few drops of methanol reduced
the surface tension of the globules formed and facilitated separation into two layers. The solution was extracted 12 times with chloroform (250 c.c.) and the chloroform extracts dried over anhydrous sodium sulphate overnight. The solvent was distilled off at 30-40° C under diminished pressure giving a syrupy product.

Yield: 0.10 grams

An attempt to extract more denitrated material from the acetate mixture was unsuccessful as much potassium acetate also dissolved up in the solvents used. Methanol, acetone, ethyl acetate and mixed solvents would not extract the carbohydrate portion alone.

The denitrated material was slightly impure (acetate), and was neutral and non-reducing containing no nitrogen.

**METHYLATION OF DENITRATED MATERIAL:**

The denitrated product (0.086 grams) was methylated with methyl iodide (10 c.c) and silver oxide (5 grams) for 10 hours at 45° C. The silver oxide was added hourly in 0.5 gram portions, with vigorous shaking throughout the methylation at intervals.

The solution was filtered, the silver residues exhaustively extracted with hot chloroform, and the combined filtrate and extracts concentrated to a golden coloured syrup.
The product probably contained a little silver iodide.

Yield: 0.18 grams

The product was remethylated as above using the same quantities of reagents giving a syrupy material.

Found: OMe = 33.1%, \( \eta_D^{200} = 1.4572 \)
c.f. 2:3:4-trimethyl \( \alpha \)-methyl d-glucuronoside methyl ester, \( C_{11}H_{20}O_7 \): OMe = 58.7% (Calculated) \( \eta_D^{200} = 1.4467 \) (Smith 9).
c.f. 2:3-Dimethyl \( \alpha \)-methyl d-glucuronoside methyl ester, 
\( C_{10}H_{18}O_7 \): OMe = 49.6%; \( \eta_D^{180} = 1.4600 \) (Smith 40).

The product was remethylated for a third time exactly as above giving a golden syrup. This contained potassium acetate and mainly silver iodide as shown by the yield.

Yield: 0.16 grams

Found: OMe = 42.5%, \( \eta_D^{180} = 1.4601 \)

It was not thought advisable to proceed with this small amount of material further, as it was found difficult to purify without loss. However, the increase in methoxyl content indicates that the denitration procedure had liberated additional hydroxyl groups. The methoxyl content had increased from 17.4% to 42.5%.
PURIFICATION AND PREPARATION OF CHEMICALS

METHYL ALCOHOL:

Commercial methyl alcohol (400 c.c.) previously dried over lime, and magnesium turnings (10 grams) were placed in a 3 litre round-bottomed flask. Iodine (4 grams) dissolved in a little methyl alcohol, was slowly added to the above mixture, the flask being kept cool when necessary.

After the reaction had subsided, the mixture was warmed until all the magnesium had been converted into the methyleate. A further amount (1600 c.c.) of commercial methyl alcohol was added and the mixture refluxed for 8 hours on a water bath. A double surface reflux condenser fitted with a calcium chloride tube was used. The methyl alcohol was distilled through a long fractionating column containing glass helices, and collection of the constant boiling fraction (D.P. 64.6°C.) yielded pure anhydrous methyl alcohol. The refractive index was determined on the sample.

Found: \( n_{D}^{18} = 1.3300 \)

c.f. \( n_{D}^{18} = 1.33001 \) (Landolt-Bornstein)
PREPARATION OF PURE METHYL IODIDE:

Methyl alcohol (72 grams, excess) and red phosphorous (20 grams, excess) were placed in a 2-litre flask fitted with a double surface reflux condenser. Powdered iodine (200g=2.5 mole) was slowly added over an hour with frequent shaking, the condenser being detached from the flask momentarily during each addition. The flask was cooled in cold water when necessary, and the contents refluxed for an hour and allowed to stand overnight.

The methyl iodide was then distilled off on a water bath into a receiver containing water and cooled in salt and ice. The distillation was continued till no oily drops were seen in the condenser, the residue being discarded. The distillate was shaken up with water to remove alcohol, then with dilute sodium hydroxide to remove free iodine and hydrogen iodide and finally with water again. The methyl iodide (lower layer) was separated, dried over calcium chloride and distilled, the fraction D.P. 41.5-43°C being collected and stored in a dark bottle.

Yield: 170 grams 78%

Found: \( \eta^2_{D}^{210} = 1.5299 \)

c.f. \( \eta^2_{D}^{210} = 1.5293 \) (Gladstone 45)
ETHER:
Ether B.P. (3 litres) after preliminary drying over calcium chloride was stored in a dark bottle over sodium wire 50.

ACETONE:
Acetone B.P. (2 litres) previously dried over calcium chloride, was dried over anhydrous potassium carbonate and distilled through a long fractionating column the fraction B.P. 56.2°C being collected and stored in a dark bottle. 50.

Found: $n_D^{20} = 1.3596$

$\eta_D^{20} = 1.3590$ (International Critical Tables 51)

CHLOROFORM:
Chloroform B.P. dried over anhydrous sodium sulphate was, after filtering, used for most purposes 50.

SILVER OXIDE:
A hot filtered barium hydroxide solution (100 grams Ba(OH)$_2$·8H$_2$O in 1 litre of water) was added to a hot solution of silver nitrate (100 grams AgNO$_3$ in 500 c.c. water) in a 3-litre flask, and the precipitated silver oxide washed with boiling 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. The oxide was fiely
powdered and kept in a desiccator till ready for use 46.

**SILVER CARBONATE:**

Silver nitrate (85 grams) was dissolved in a minimum amount of water and a saturated solution of potassium carbonate (35 grams) was added. The precipitated silver oxide was filtered, thoroughly washed with cold water, and dried at 100-105°. The yellow carbonate was kept in a dark bottle in a desiccator 46.
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