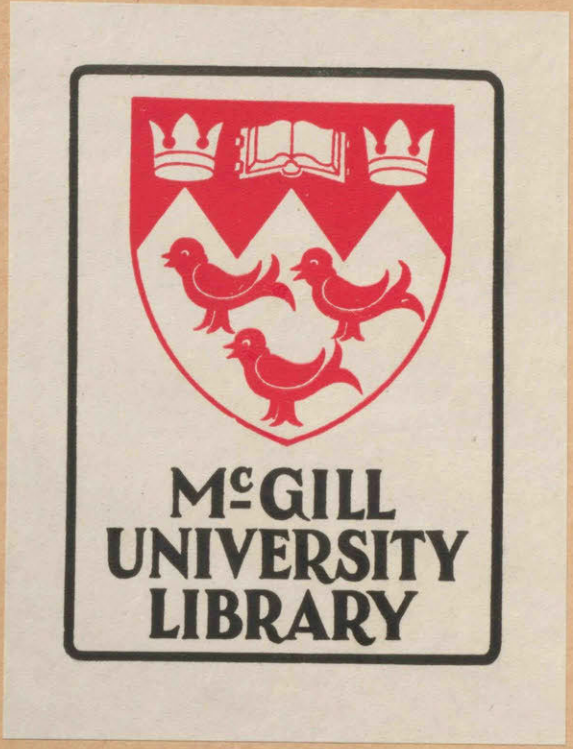

THE DENITRATION OF SUGAR NITRATES

A Thesis

by

Lloyl Douglas Hayward

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requirements for the degree
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Grateful acknowledgements are also made to The National Research Council of Canada for the awards of two studentships, and to The Department of Veterans Affairs and to The Defence Research Board of Canada for maintenance grants.

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GENERAL INTRODUCTION

In the course of a confidential War Research by another worker, it was found that a large excess of free hydroxylamine dissolved in pyridine caused the removal of approximately one mole of nitrate group per glucose unit from cellulose trinitrate, the nitrogen being nearly quantitatively recovered as such in the gaseous phase. Experimental evidence indicated that the removal of the nitrate group left an hydroxyl group in the cellulose portion and that this hydroxyl group was probably of a secondary alcohol type. All attempts to identify the secondary alcohol position from which the nitrate group had been removed were unsuccessful.

In the present research the hydroxylamine - pyridine reaction was applied to crystalline, fully-nitrated methyl-α and β-glucosides in order to locate the nitrate group or groups attacked by the reagent. The methylglucoside nitrates were employed in preference to the nitrated reducing sugars, since the former were closer analogues of the non-reducing glucose units comprising the cellulose macromolecule. It was not expected that the course of the reaction with the monosaccharide nitrates would be entirely analogous to that observed with the cellulose nitrate, since the former compounds had an additional secondary nitrate group in the fourth position instead of the glycosidic linkage characteristic

of the same position in cellulose. In addition, the reaction of the cellulose derivative would very probably be influenced by the unique properties of its macromolecular structure and it was therefore expected that differences in the rate of reaction would be observed.

In the investigation, emphasis was placed on establishing the nature of the partial denitration rather than on interpreting the chemical mechanism. For this reason the conditions employed were selected on the basis of previous work with cellulose nitrate and were then maintained in the subsequent studies. Little attempt was made to investigate the effect of varying these conditions. A vigorous reaction occurred with the methylglucoside tetranitrates, gas was evolved and the carbohydrate products consisted of sirupy mixtures of the corresponding methylglucoside di- and trinitrates. These products were laboriously separated and identified by means of crystalline derivatives.

It was found that the nitrate group in the fourth position (secondary alcohol) of the glucoside took part in the reaction and this fact permitted an easy preparation of crystalline methyl-4-methyl-β-D-glucoside. It is suggested that this compound, which possesses ether linkages at positions one and four, may prove to be valuable as a model substance for future studies of cellulose reactions.

HISTORICAL INTRODUCTION

The nitric acid esters of the sugars were first studied in connection with the technical problem of stabilizing cellulose nitrate.

In the half century following the discovery of nitrocellulose by Schönbein in 1847 (1), the industrial use of this nitrate expanded rapidly with the production of guncotton, nitrosilk, films and lacquers. The early manufacture of the nitrate was attended with an element of hazard and in particular there were several serious explosions in magazines used for storing guncotton. A study of the causes of, and of methods for preventing, such spontaneous decomposition showed at least two factors to be of importance. The first factor was that a small amount of sulfuric acid from the nitrating bath was retained in the nitrate, and the second, that amorphous, gummy organic substances readily giving off nitrous acid could be extracted from the nitrate (2).

The first of these findings led Abel (3) to introduce the now standard process of disintegrating the nitrated fibre on Hollander machines and of washing the product thoroughly with warm water. By this treatment the sulfuric acid content was greatly, but not completely, reduced, and stability was increased. Little seems to have been done concerning the second factor until 1898, when Will and Lenze (4) argued

that the strongly acid conditions in the nitrating bath, with unavoidable high local concentrations, together with temperature gradients, might bring about partial hydrolysis of the cellulose as well as nitration. The partially or completely nitrated fragments of the cellulose might account both for the more soluble portions extractable from nitrocellulose and for the slow spontaneous decomposition. To test this theory, these authors prepared the fully-nitrated esters of the common sugars and sugar derivatives known at that time and made a study of the heat stability of the products.

For the preparations, the crystalline sugars were dissolved in concentrated nitric acid at 0° and the nitrates were precipitated by the addition of ice-cold concentrated sulfuric acid. The sugar nitrates usually settled out as oils which were then separated and triturated with ice-water and finally crystallized from alcohol. With the exceptions of the nitrates of glucose, xylose and the polysaccharides, crystalline products were obtained and their physical properties were recorded.

These substances were found to be insoluble in cold water but were partially decomposed and rendered soluble on long boiling. This property would explain, at least in part, the success of the Abel process. Although the nitrates were generally stable to 135° in a simple heat test, a gradation of stability occurred from the more stable biose

to the less stable pentose derivatives. The glycoside nitrates were always more stable than those of the corresponding reducing sugars. Recently Brissaud and co-workers extended this study and prepared the crystalline α- and β-D-glucose pentanitrates (5) and the heptanitrate of methyl-β-cellobioside (6). These authors also re-examined the relative stability of the esters in relation to their structure (7).

The first use of nitric acid esters in the field of sugar synthesis was that by Koenigs and Knorr in 1901 (8). Colley in 1873 had shown that the action of fuming nitric acid on acetochloroglucose produced a crystalline sugar derivative which he termed "acetonitrose" (9). Koenigs and Knorr followed up this study and found that acetobromoglucose could be converted into the 1-nitrate derivative by fuming nitric acid in chloroform solution. The resulting 1-nitrate tetraacetate could also be obtained directly from pentaacetylglucose by nitration. This glucose mononitrate tetraacetate was identical with "acetonitrose" and could be substituted for α-acetobromoglucose in the preparation of glucosides by condensation with silver carbonate (or pyridine) and the appropriate alcohol. The glucoside obtained in each case was found to have the β-configuration.

No other partially nitrated sugar derivatives were reported until 1925, when Oldham published a paper entitled "Transformation of the Sugar Nitrates" (10). This author pointed

out that the nitrates were generally crystalline compounds that were easily characterized, and he suggested they would find extensive use in sugar syntheses. In this publication Oldham initiated the large task of preparing, by unequivocable methods, all the partially methylated glucose derivatives which were obtained as mixtures from the standard methylation-hydrolysis technique of investigating polysaccharides. The series of syntheses was carried on by many workers and was finally completed by Dewar and Fort in 1944 (11)(12). Throughout this work the partially substituted nitric acid esters were used extensively as intermediates.

In the earlier synthetical work, acetyl and benzoyl radicals were frequently used for temporary substitution of hydroxyl groups in the sugar derivatives. However, it was discovered that these acyl groups could migrate from one hydroxyl to another in the course of so mild a reaction as methylation with silver oxide and methyl iodide (13)(14)(15), and hence the exact structure of products prepared by these procedures became uncertain. In contrast, no case of wandering of a nitrate groups in partially substituted sugars has been detected (16) and this property was therefore a great advantage in syntheses requiring specific substitution. In addition, nitrate groups were as readily introduced or removed as other acyl groups when reasonable attention was given to experimental conditions.

The methylglucoside nitrates were usually employed rather than those of the glucoses. In the former compounds the masking of the highly reactive glycosidic group permitted treatments which distinguished between the secondary and the primary hydroxyl groups (17) and in some instances between different secondary hydroxyls (18)(19). The methyl aglycon was found to be quite stable to direct nitration.

The methyl-β-D-glucosides were readily obtained by way of the Koenigs-Knorr Synthesis, and since they showed generally a greater tendency to crystallize and had higher melting points than the α-isomerides (20) they were employed more frequently in syntheses. When the compounds had been substituted with nitrate groups, submitted to other reactions and then completely denitrated, the desired sugar derivative was readily obtained by acid hydrolysis of the glycosidic methyl ether group.

The known nitrate esters of methyl-β-D-glucopyranoside are listed in Table I, together with their melting points and specific rotations.

Introduction of the nitrate groups was accomplished by direct nitration of the appropriate sugar derivative, usually in anhydrous chloroform solution at 0° with fuming nitric acid. In some instances a dehydrating agent such as phosphorous pentoxide (23) or acetic anhydride (6) was also present.

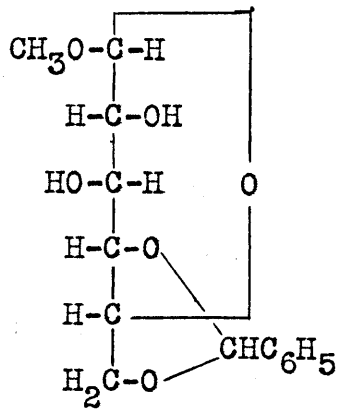
TABLE I

The Nitric Acid Esters of Methyl-β-D-glucopyranoside

Position of Nitrate Group	m. p.	$[\alpha]_D$	Solvent	Reference
3	104 - 106°	-16.8°	EtOH	(11)
6	< 25°	.....	....	(10)
2,3	96 - 98°	-20.5°	$CHCl_3$	(21)
2,6	< 25°	-11.6°	$CHCl_3$	(18)
3,4	116 - 118°	13.9°	MeOH	(19)
3,6	144 - 147°	- 7.8°	Acetone	(18)(11)
4,6	147 - 149°	- 5.3°	MeOH	(21)
2,3,6	<25°	.....	.....	(22)
2,3,4,6	116 - 118°	9.35°	$CHCl_3$	(21)

An objection to the use of nitric acid was found by Oldham (24) in the nitration of methyl 4,6-benzylidine-β-D-glucoside (I).

In this case simultaneous introduction of a nitrate group occurred in the aromatic nucleus and the nitrobenzylidine (Formula II) residue thus produced could not subsequently be removed. Consequently the benzylidine group, a valuable substituent used for selectively blocking the 4- and 6-hydroxyl groups in a synthesis, could not be employed in



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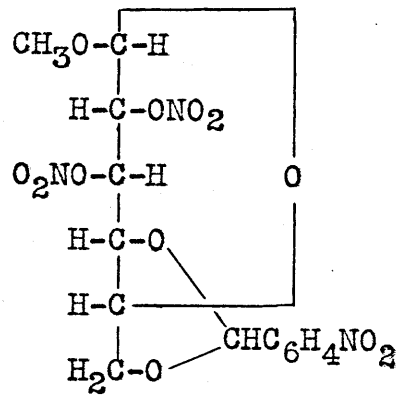

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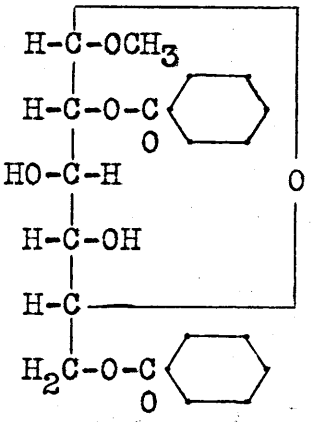

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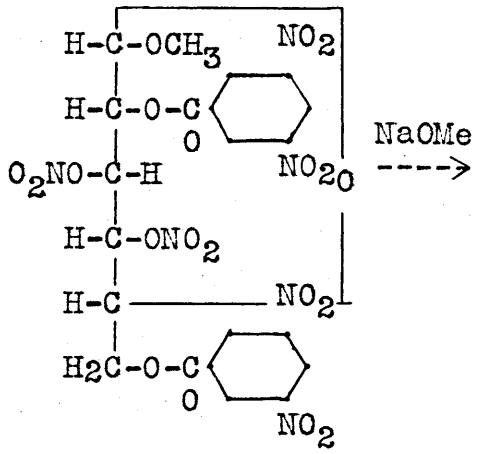

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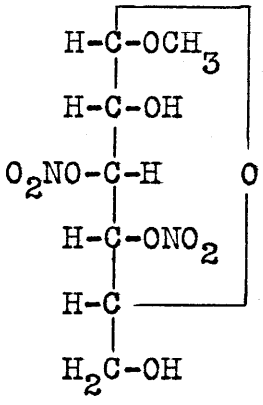

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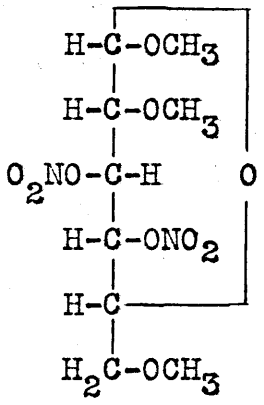

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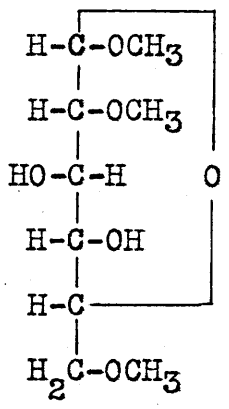


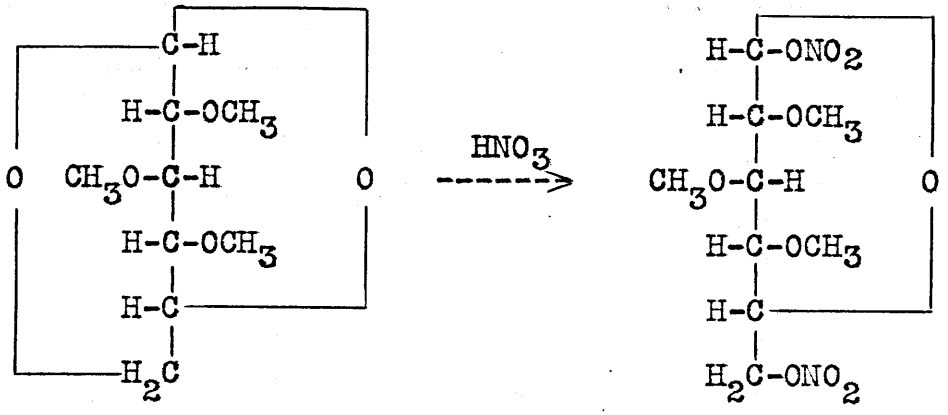
VII

connection with nitration. However, Dewar and Fort (11) avoided this difficulty by employing the ethylidine group to block the same two positions; but another difficulty then occurred in that the fuming nitric acid-chloroform reagent nitrated even in the positions occupied by the ethylidene group. The latter objection was finally overcome by using as the nitrating agent a solution of pure nitrogen pentoxide in anhydrous chloroform; under these conditions the ethylidene group remained in situ and nitration occurred only at positions 2- and 3- of the glucoside molecule.

Recently in the synthesis of methyl-2,6-dimethyl-α-D-glucopyranoside (VII), Reeves (25) found that direct nitration of the dibenzoylated methylglucoside (III) resulted also in nitration of the benzoyl groups. No difficulty however was encountered in hydrolysing the resulting di(m-dinitro-)-benzoate (IV) with sodium methylate; the nitrate groups on the sugar molecule, however, were left intact (Formula V). Methylation and reductive denitration completed the synthesis.

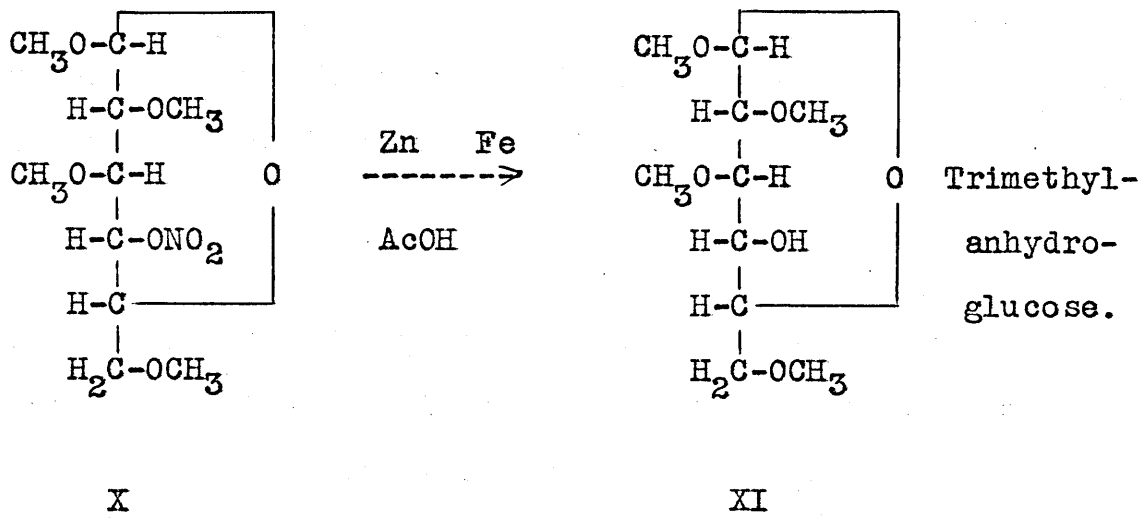
Direct nitration of trimethylglucosan $<1,5>\beta<1,6>$ (VIII) resulted in opening of the $<1,6>$ anhydro ring and nitrate substitution on the freed hydroxyl groups to give 2,3,4-trimethyl-α-glucosyl-1,6-dinitrate (IX)(10)(26). Nitration of partially acetylated (23) or methylated (27) derivatives of the methylglucosides showed the acetyl and methyl ether

groups to be stable to the nitrating medium; other ether radicals such as **triphenylmethyl** (trityl) (23)(28) and α-**acetoxyethyl** (19) were readily replaced by nitrate.



VIII IX

Of the different methods of denitration developed for the sugar nitrates, that of boiling a glacial acetic acid solution of the nitrate with excess of zinc and iron dust has been most frequently employed (10)(18)(22). In the cases cited removal of nitrate appeared to be complete and the sugar hydroxyl group was regenerated with no change in configuration. However, Irvine and Rutherford (27) reported the formation of some trimethylanhydroglucose when methyl-2,3,6-trimethyl-β-D-glucoside-4-nitrate (X) was reduced with hot acetic acid and iron powder.



X XI

The anhydroglucose derivative was not isolated but its presence was inferred from the low value of the Zeisel methoxyl estimation obtained for the methyl-2,3,6-trimethyl-β-D-glucoside (XI).

Other complete denitration methods included treatment of the nitrate with an alcoholic solution of an alkali sulfide (16)(21) and shaking with liquid zinc amalgam (18). Hoffmann and coworkers (29) developed a method in which denitration and simultaneous acetylation occurred when the nitrate was treated with zinc dust and acetic anhydride containing a controlled amount of hydrogen chloride or pyridine. The simplest and most recent method however, included the use of palladium catalyst supported on charcoal or on calcium carbonate (30). Kuhn was able in this way to achieve complete denitration by hydrogenolysis at pressures ranging from 300 to 1500 p. s. i. Ethanol and dioxane were employed as solvents. With the calcium carbonate-supported catalyst and a sugar nitrate, reduction

to the sugar was complete but undesirable calcium compounds were formed from which it was difficult to separate the sugar. The reaction proposed was:

$$2RONO_2 + 5H_2 \longrightarrow 2ROH + N_2 + 4H_2O$$

In support of this equation, Kuhn found a pressure drop corresponding to 2 moles of hydrogen absorbed per nitrate group. This was the theoretical value since, although 2 1/2 moles of hydrogen were actually consumed, 1/2 mole of nitrogen was produced per mole of nitrate. He also obtained nearly quantitative yields of the denitrated sugars. The success of the hydrogenation depended on the avoidance of catalysts and conditions that reduced the nitrogen to ammonia, which decomposed some of the original nitrate in undesired ways. The very active palladium-charcoal catalyst caused reduction in this way at higher pressures.

Alkaline hydrolysis of the nitric acid esters was carefully avoided in synthetic work. It was known that the action of alkalies, especially potassium or sodium hydroxides, on aliphatic nitrates was not one of simple saponification, regenerating the alcohol and forming sodium nitrate, but was a profound decomposition yielding also sodium nitrite and oxidation products of the aliphatic group. The extensive literature on this decomposition has been reviewed in detail by Kenyon and Gray (31) and others (32) and will be only

briefly mentioned here.

In the sugar group, Gladding and Purves (26) reported that the action of alkali on some mononitrate-acetates of glucoses and methylglucosides led to the formation of anhydro derivatives when suitably situated free hydroxyl or potentially free hydroxyl groups existed in the sugar molecule. Ordinary hydrolysis took place, although with difficulty, when such hydroxyl groups were 'blocked' by methyl ether groups. The formation of alkoxyl derivatives was reported when the nitrate group was located in the reducing position of the sugar molecule and the alkali was present in alcoholic medium. Anhydro products were also obtained in the latter case. Other studies of the action of strong alkalies have been reported for the nitrate derivatives of the sugar alcohols and related poly-nitrates (31); in each case the reaction was complex and yielded anomalous results.

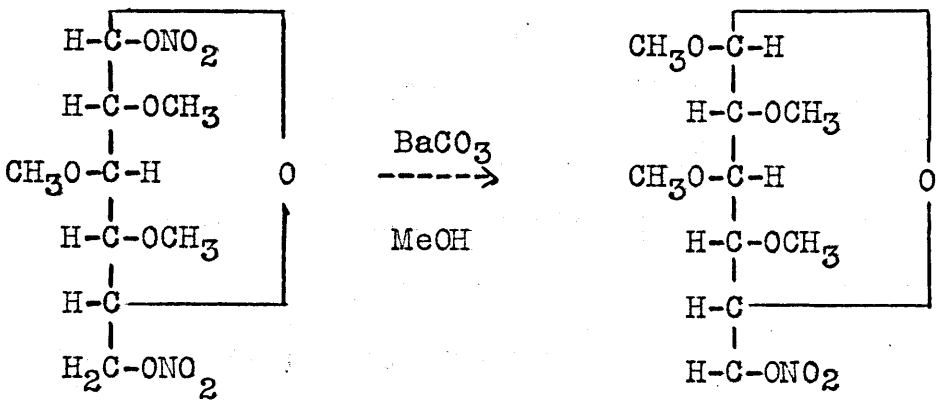
The conversion of cellulose nitrate to cellulose has been studied frequently for commercial as well as theoretical reasons and several detailed reviews are available (31)(33)(34). Denitration of nitrocellulose with aqueous alkalies and alkaline earths, as well as by ammonia and alkali carbonates, proved completely unsuitable since a deep-seated decomposition of the cellulose resulted. The reduction products of nitric acid: nitrite, ammonia and nitrogen, were obtained as well

as oxidation products of the carbohydrate portion, such as carbon dioxide, formic, oxalic and other organic acids.

Acid hydrolysis of nitrocellulose likewise gave no satisfactory product; degradation of the cellulose molecule always occurred as shown by the considerable amounts of reducing constituents in the hydrolysate, and denitration was never complete. More success was achieved by carrying out the alkaline hydrolysis in reducing media and a technical process for the manufacture of nitrosilk and nitrofilm was based upon this reaction (34). For this purpose aqueous or alcoholic solutions of the sulfides of ammonia or alkali metals were found most suitable (33); however the process always involved some degradation of the cellulose chains and failed to remove the last one-half to one per cent of nitrogen from the products.

Sherer and Saul (35)(36) recently made the interesting observation that a solution of sodium acetylide in liquid ammonia replaced one of the nitrate groups in a 'cellulose dinitrate' by the acetylene radical. This reaction was accompanied by a slow conversion of the other nitrate group to an amino group by the sodium amide formed by a secondary reaction of sodium acetylide with the solvent. These publications appear to be the first to describe a reaction which may prove to be specific for one or other of the nitrate groups in the second, third or sixth positions of the anhydroglucose units in nitrocellulose.

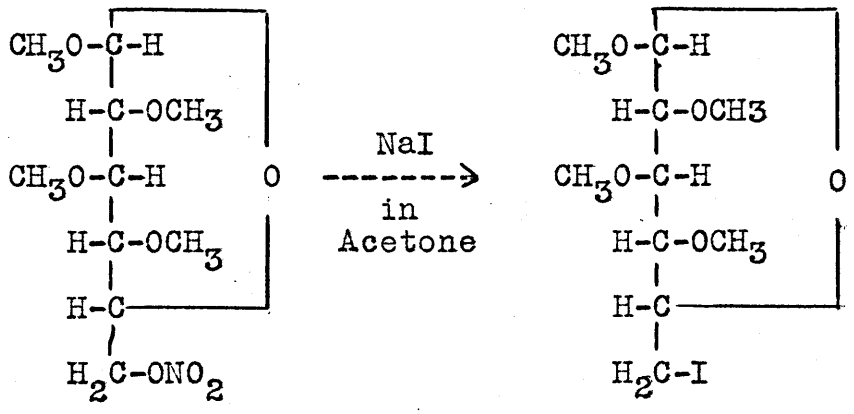
Selective removal or replacement of nitrate groups in carbohydrate polynitrates is known to be greatly influenced by the position of the reacting group or groups in the sugar molecule. The nitrate group in the reducing position of glucose was shown by Koenigs and Knorr (8) to be sufficiently labile to be smoothly exchanged for the methyl radical, when the sugar nitrate was heated in methanol solution with silver or barium carbonate or pyridine. Oldham (10) and later Gladding and Purves (26) obtained methyl-2,3,4-trimethyl-β-D-glucopyranoside-6-nitrate (XII) by boiling 2,3,4-trimethyl-α-glucosyl-1,6-dinitrate (IX) with methanol and barium carbonate.



IX XII

Replacement of nitrate groups in the sixth (primary) position of glucose by iodine was accomplished by heating with sodium iodide in a ketonic solvent at 100° (17)(27)(37) This reaction was first reported by Oldham in 1925 (10) when he prepared methyl-2,3,4-trimethyl-β-D-glucoside iodohydrin (XIV) from the corresponding 6-nitrate derivative (XIII) after

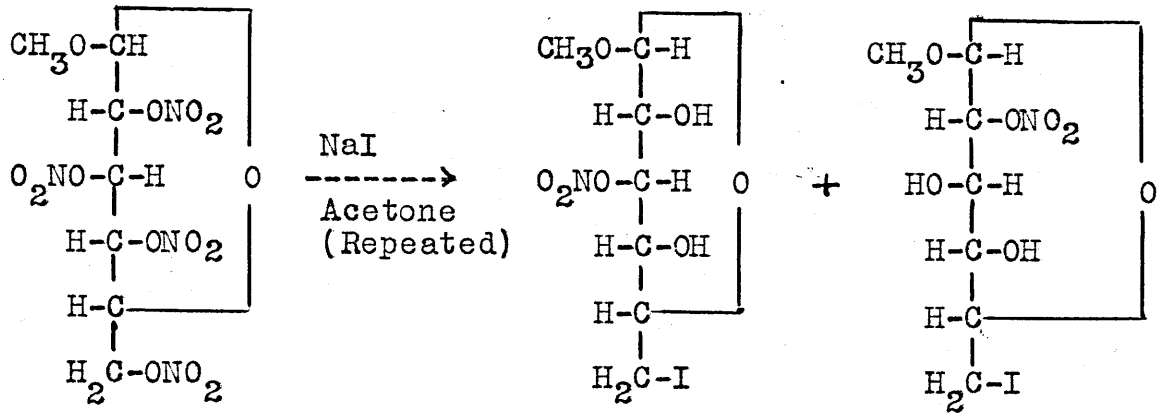
heating 6 hours in acetone with sodium iodide in a sealed tube.



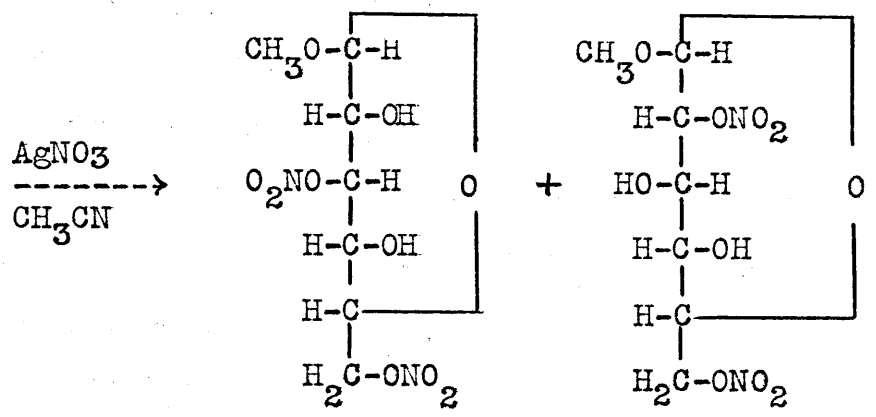
XIII XIV

Oldham and Rutherford (17) showed that longer treatment with sodium iodide in acetone, of a sugar di- or trinitrate caused removal of more than one nitrate group, but iodo substitution occurred only in position six. Dewar, Fort and McArthur (18) were able to prepare 2- and 3-mononitrate-6-iodo compounds in admixture (XVI), by the repeated action of sodium iodide in acetone on methyl-β-D-glucoside tetranitrate (XV). The 6-iodo compounds, when treated with silver nitrate in acetonitrile, gave the 2,6- and 3,6-dinitrates (XVII) which were separated and identified.

The selective removal of nitrate groups from mannitol hexanitrate (XVIII) was reported by Tichanowitsch as early as 1863 (38). When ammonia gas was introduced into an ethereal solution of the hexanitrate a very dark, viscous layer separated and evolution of nitrogen occurred. The nearly colorless

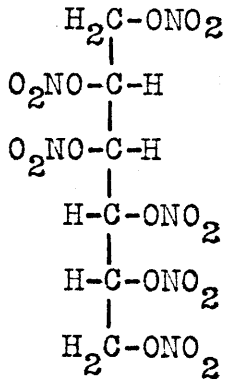


XV XVI

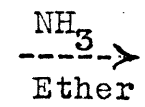


XVII

supernatant liquid yielded a solid which proved to be a mannitol pentanitrate, together with a sirupy substance which appeared to be an anhydromannitol tetranitrate. From the dark viscous layer, a third product was isolated which in behaviour and analysis approximated to an anhydromannitol tetramine. Wigner in 1903 (39) found that the pentanitrate was obtained in better yield by treating mannitol hexanitrate with alcoholic pyridine. In this reaction no lower nitrates



XVIII



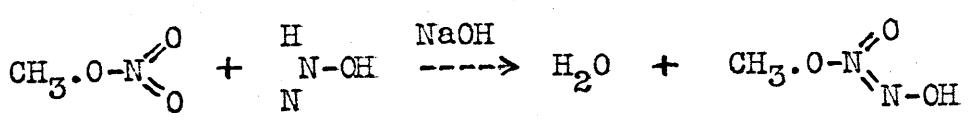
Mannitol pentanitrate
+
Anhydromannitol tetranitrate
+
Anhydromannitol tetramine

or amino compounds were detected and the yield of pentanitrate amounted to 80 - 90% of theory. Wigner also obtained a pentanitrate of dulcitol from the corresponding hexanitrate by the same reaction. Mannitol pentanitrate was also prepared in admixture with the hexanitrate by direct nitration of the sugar alcohol with a sulfuric - nitric acid mixture. The pharmacological action of mannitol and dulcitol pentanitrates was studied (40). Although analyses of these crystalline products established their composition no information as to the identity of the actual nitrate group involved in the partial denitration appeared in the literature.

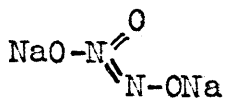
The action of weak organic bases on nitrocellulose was studied by Walter (41) who noted the effects of dimethylaniline, phenylhydrazine, o- and p-toluidine, naphthylamine and other similar compounds. This work, however, was only of a qualitative nature and no analyses of the products were recorded. It was reported by Becker and Hunold (47) that degradation

of nitrocellulose in the presence of diphenylamine and traces of copper salts produced oxidized and nitrated derivatives of the amine. Angeli (42) and later Giannini (43) investigated the action of pyridine on different cellulose nitrates, and the latter author included analyses of the gaseous products which were evolved over several months. In these reports the information about the cellulosic products was too meager to reveal the number of nitrate groups concerned. Gladding and Purves (26) found that pure, dry pyridine caused a vigorous decomposition of dissolved, stabilized guncotton at steam-bath temperature. Nitrogen dioxide was evolved in the decomposition as a volatile pyridine complex that readily crystallized on cooling.

An extensive study of the reaction between methyl and ethyl nitrates and the free base hydroxylamine was undertaken by Angeli (44)(45)(46). It was found that addition of methyl nitrate (XIX) to a solution of hydroxylamine (XX) and sodium hydroxide in methanol caused the precipitation of a white powder which appeared to be the disodium salt of the unstable nitrohydroxylaminic acid, $H_2N_2O_3$. The reaction was postulated to follow the equation.



XIX XX XXI

The sodium nitrohydroxylaminate, 

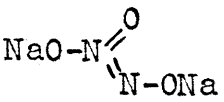
XXII

was thought to be formed by displacement of both the methyl group and hydrogen in methyl nitrohydroxylaminate (XXI) by sodium atoms.

...---...

The preceding survey of the literature seemed to indicate that smooth cleavage of nitric ester groups in carbohydrate polynitrates, to regenerate the alcoholic groups in an unaltered state could best be carried out in media which were both alkaline and reducing. Also it appeared likely that under sufficiently mild denitrating conditions the differences in reactivity exhibited by nitrate groups in the primary and secondary positions of the sugar molecule, and in different secondary positions, might be exploited to allow of selective denitration.

A solution of hydroxylamine in pyridine appeared to meet the above requirements for a mild denitrating reagent. Evidence provided from a War Research project by another workers supported this conjecture.

The sodium nitrohydroxylaminate, 

XXII

was thought to be formed by displacement of both the methyl groups and hydrogen in methyl nitrohydroxylaminate (XXI) by sodium atoms.

The preceding survey of the literature seemed to indicate that smooth cleavage of nitric ester groups in carbohydrate polynitrates, to regenerate the alcoholic groups in an unaltered state could best be carried out in media which were both alkaline and reducing. Also it appeared likely that under sufficiently mild denitrating conditions the differences in reactivity exhibited by nitrate groups in the primary and secondary positions of the sugar molecule, and in different secondary positions, might be exploited to allow of selective denitration.

A solution of hydroxylamine in pyridine appeared to meet the above requirements for a mild denitrating reagent. Evidence provided from a War Research project by another worker supported this conjecture.

The reducing agent is provided in this system by the strong proton donor, hydroxylamine, while pyridine is not only an alkaline medium, but acts efficiently as a solvent for the water-insoluble, fully-nitrated sugars.

The object of the present research was to explore the action of pyridine - hydroxylamine on the tetranitrates of the methyl-D-glucopyranosides. These compounds were chosen for study because of the data available regarding their well-characterized, partially denitrated derivatives. The identity of the expected products could therefore be confirmed by conversion, through methylation and complete denitration, to one or other of the known methyl-glucoside methyl ethers. Other advantages such as comparative stability and crystalline character have already been discussed.

DISCUSSION OF RESULTS

Preliminary experiments on the stability of the crystalline methylglucoside tetranitrates to anhydrous pyridine indicated that, although highly colored products were rapidly formed, no gas was evolved, and the unchanged tetranitrates could be recovered in yields exceeding 50% after being dissolved in the pyridine for sixteen hours at room temperature (Table V). In a similar experiment with methyl-β-D-glucoside tetranitrate and an alcohol solution of free hydroxylamine, evolution of gas from the colorless solution proceeded steadily at room temperature, but the unaltered tetranitrate could be recovered in yields exceeding 75% after twelve hours, when the solution was poured into water and the nitrate was separated with ether. A gaseous product was also evolved from the hydroxylamine-alcohol solution in the absence of the nitrate and was therefore attributed to a slow decomposition of the base. The solubility of methyl-α-D-glucoside tetranitrate in aqueous alcohol permitted the recovery of 37% of the unchanged nitrate by crystallization after the alcohol - nitrate - hydroxylamine solution was poured into water.

In contrast to the foregoing, when methyl-α-D-glucoside tetranitrate was treated with a solution of free hydroxylamine in anhydrous pyridine, a vigorous exothermic reaction took place

almost immediately and large volumes of a colorless gas were evolved. The solution became only slightly yellow after fourteen days, and no unchanged tetranitrate could be recovered, even after ten minutes' reaction time. The product obtained after four and one half hours, by pouring the solution into water and extracting with chloroform, was a sirup having nitrogen and methoxyl contents intermediate between the theoretical values for methylglucoside di- and trinitrates and indicating that the product was a mixture of such substances. The yield of the sirup amounted to 77% of that calculated for a mixture of methylglucoside di- and trinitrates having an average nitrate substitution of 2.70 moles per mole. Methyl-α-D-glucoside tetranitrate, which is more readily available than the β-isomer, was used for the initial detailed study of the pyridine - hydroxylamine reaction. A series of the partially denitrated products, isolated at reaction times extending from ten minutes to twelve hours, all proved to be sirups with very similar methoxyl, total nitrogen and nitrate nitrogen contents as well as similar refractive indices as shown in Table II. These results indicated rapid partial denitration to a uniform product that was almost stable to the conditions of the experiment.

The failure to obtain crystalline compounds at this stage made it desireable to ascertain whether or not the carbohydrate portion of the products was still based exclusively on methyl-α-D-glucopyranoside. The removal of nitrate groups by the

TABLE II

Partial Denitration of Methyl-α-glucoside Tetranitrate with Hydroxylamine - Pyridine

Sample No.	Reaction Time (mins.)	% N (Micro Kjeldahl)	η_D^{20}	% N (Dupont)	% OCH_3
1	10	11.7	1.492	10.93	8.70, 8.65
2	20	11.6	1.494		
3	30	11.6	1.491		
4	60	11.9	1.491		
5	120	11.7	1.493	10.95	8.79, 8.74
6	240	11.5	1.493		
7	360	11.5	1.492		
8	720	11.4	1.492		

catalytic hydrogenolysis method of Kuhn (30) was studied with this end in view and his claim that methyl-α and β-glucoside tetranitrates were almost quantitatively denitrated at room temperature and low pressure to the crystalline methylglucosides was confirmed. The reaction was complete in thirty minutes, as indicated by the attainment of constancy of the hydrogen pressure and the consumption of hydrogen with the α-compound (7.8 moles of hydrogen per mole) agreed with the equation proposed by Kuhn:

$$2RONO_2 + 5H_2 \longrightarrow 2ROH + N_2 + 4H_2O$$

In a similar experiment with the β-tetranitrate, the glucoside

was recovered in 96% yield and the consumption of hydrogen amounted to 7.6 moles per mole.

Under the same conditions, the partially denitrated methyl-α-D-glucoside tetranitrate from the pyridine hydroxylamine reaction absorbed 0.016 moles of hydrogen per gram and gave crystalline methyl-α-D-glucoside in 82% yield based on the nitrogen content removed. These results indicated that no change other than that of denitration, occurred in the methyl glucoside structure in the main reaction although the possibility of small side reactions, of course, was not entirely excluded.

All attempts to isolate crystalline di- or trinitrates from the partial denitration of methyl-α-D-glucoside tetranitrate failed, and the same lack of success was encountered when the products were methylated, benzoylated or acetylated. These substituted products were also obtained only in sirup form after hydrogenolysis of all nitrate groups. Work on the α-series was then abandoned in favor of the β-glucosides, which were known to have a greater ease of crystallization and whose derivatives were more readily characterized (20).

Cyclohexyl-β-D-glucoside was chosen as a suitable compound because it crystallized readily and the cyclohexyl group, in view of its aliphatic character, resisted nitration, Moreover, the work of Richtmeyer (48) showed that the cyclohexyl ether

link was not cleaved by hydrogenation with palladium under the conditions used in the hydrogenolysis. An objection to an aromatic aglycon was the fact, reported by Oldham and others (24)(11)(25), that such substituents were readily nitrated in the conditions required for nitration of the unsubstituted hydroxyl groups.

Cyclohexyl-β-D-glucoside was prepared in good yield from acetobromoglucose, but attempts to prepare the corresponding tetranitrate led to hydrolysis of the cyclohexyl group and to the production of β-glucose pentanitrate (5). This approach, therefore, had to be abandoned in favor of the use of methyl-β-D-glucoside-2,3,4,6-tetranitrate (XV), a well-characterized and crystalline compound (6)(21).

The methyl-β-D-glucoside hemihydrate used in the earlier experiments was prepared by the Koenigs and Knorr procedure and was obtained in high yield in pure crystalline form. In 1948, during the course of the present work, a procedure was reported by Raymond and Schroeder (49) in which the glucoside was obtained in a more convenient manner by direct methylation of anhydrous glucose with methanolic hydrogen chloride and subsequent separation of the methyl-β-D-glucoside from the α-isomeride by means of its crystalline potassium acetate complex. This procedure was used for subsequent preparations and the somewhat lower yields of the glucoside obtained were more than compensated for in savings in time

and costly reagents.

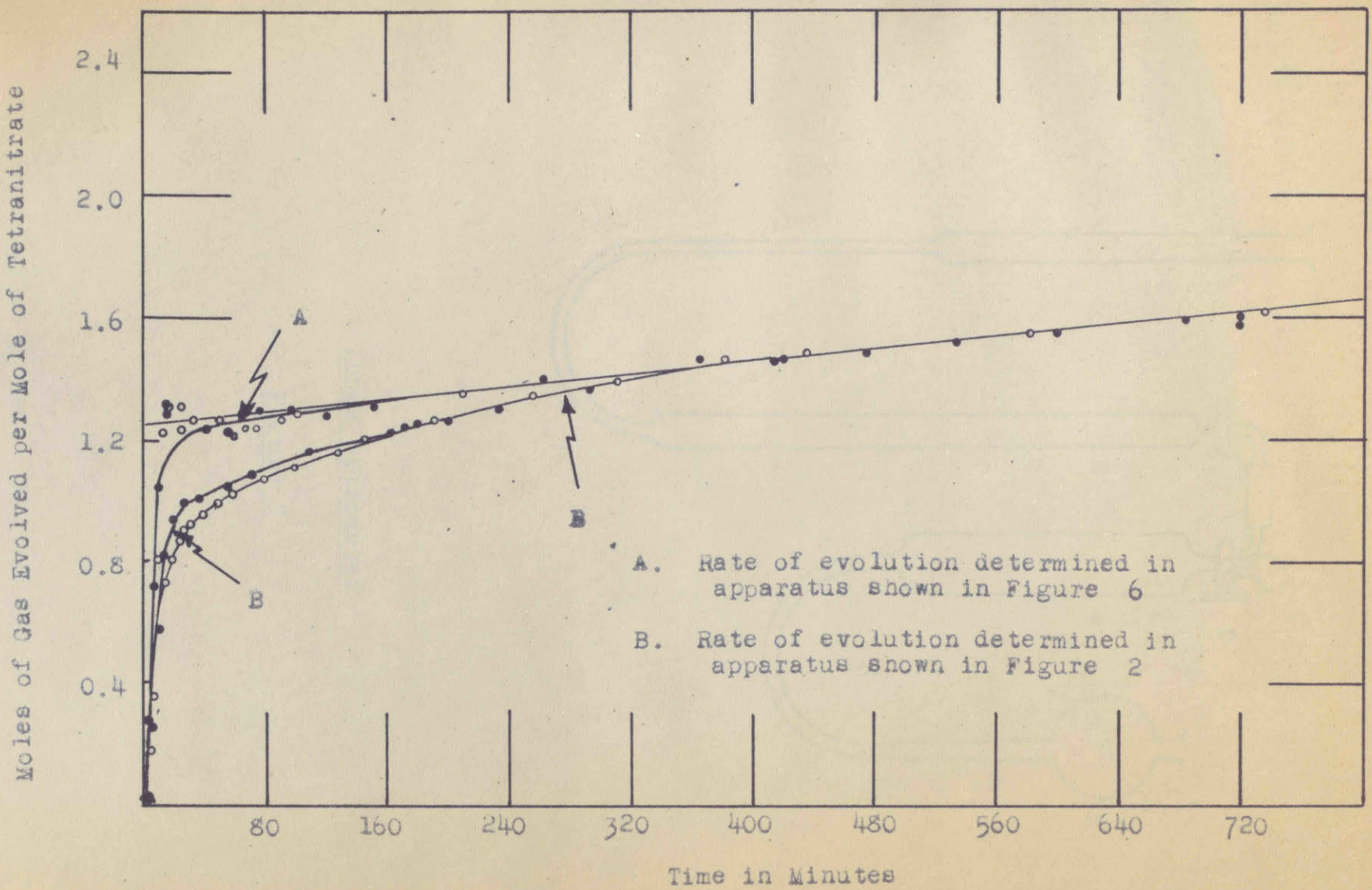
The nitration technique employing 100% nitric acid and phosphorous pentoxide, previously used with methyl-α-D-glucoside, gave low yields of the β-tetranitrate. This result was attributed to the low solubility of the product in the nitrating mixture. Satisfactory yields were obtained with a nitric acid - acetic anhydride - acetic acid medium. Reaction of the methyl-β-D-glucoside tetranitrate with hydroxylamine in pyridine led to a sirupy product very similar to that obtained with the corresponding α-compound but in somewhat higher yield, (83% of that calculated from the nitrogen content). Hydrogenation of a portion of this sirup gave a 90% yield of crystalline methyl-β-D-glucoside hemihydrate.

The study of the partial denitration was next extended to the gas evolved. Methyl-β-D-glucoside tetranitrate was treated with ten parts of a cold 12.5% solution of free hydroxylamine in pyridine in a closed system, as described in the Experimental Part, and the course of the reaction was followed by measuring the volume of the gas evolved over a period of twelve hours. The temperature of the reaction mixture rapidly increased to about 70° (Table VI), but when the observed volume measurements were corrected for the high vapor pressure of pyridine and for temperature, the plot of

volume (moles of gas per mole of tetranitrate, Curve A, Figure 1) against time, rose rapidly in the first twenty minutes, broke sharply, and then became almost exactly liniar. The scattering of points at the break in the curve was attributed to the inaccuracy of temperature and gas volume readings at the high temperature prevailing in the closed system at that time. The results indicated a very rapid initial reaction, which was virtually complete in the first twenty to thirty minutes, followed by a slower reaction continuing beyond twelve hours at a uniform rate.

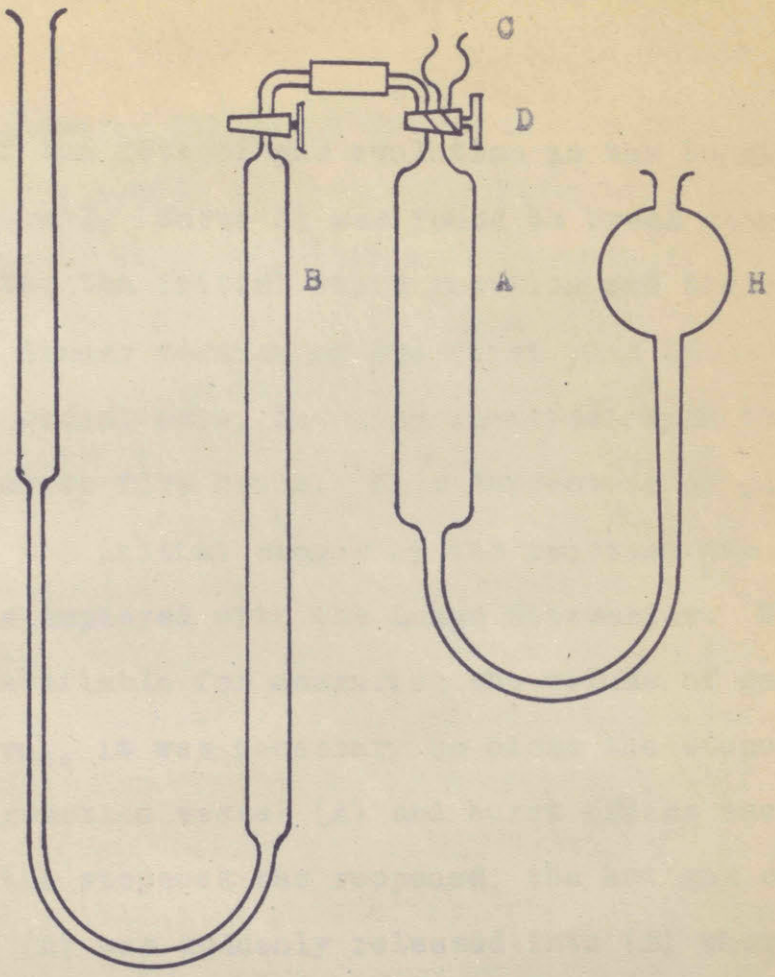
The high temperature reached by the reaction during the first phase, with the maintenance of a linear rate of gas evolution thereafter, indicated that the course of the reaction was almost independent of the temperature between the limits of 25 and 70°.

For the analysis of the gas evolved it was necessary to collect a sample free from air and this was accomplished by carrying out the reaction in the Toricellian vacuum of the Lunge Nitrometer (Figure 2). The rate of gas evolution was also followed in order to note the effect of the presence of mercury in the reaction mixture and at the same time to ascertain if nitric oxide was produced. In the preceding rate experiment, nitric oxide if produced, would have reacted with the air originally present in the system according to the equation:



The Rate of Gas Evolution from Methyl-β-D-glucoside Tetranitrate in Hydroxylamine-Pyridine

Figure 1



Lunge Nitrometer

Figure 2

$$2NO + O_2 \longrightarrow 2NO_2$$

A difference in volume readings could, therefore, be expected with the Lunge Nitrometer if nitric oxide were present in the gas evolved.

The plot of the rate of gas evolution in the Lunge Nitrometer (Figure 1, Curve B) was found to break somewhat less sharply after the initial rapid reaction and thereafter to approach the linear portion of the first plot (Curve A, Figure 1) at a gradual rate, becoming identical with the latter after four to five hours. This depression of plot B below plot A in the initial stages of the reaction was traced to the technique employed with the Lunge Nitrometer. With only one buret available for measuring the volume of gas at each time interval, it was necessary to close the stopcock connecting the reaction vessel (A) and buret (B) at each reading. When the stopcock was reopened, the hot gas confined over mercury in (A) was suddenly released into (B) where a condition of lower pressure prevailed. The rapid cooling in this operation caused a smaller apparent volume, both from the decrease in pressure of the gas and from condensation of the high concentration of pyridine vapor originally present. The evidence to support this explanation was the fact that the plots became identical when the temperature of the reaction mixture came to equilibrium with the surroundings. This coincidence indicated the absence of nitric oxide and the failure of

mercury to affect the course of the reaction. Both plots were shown to be reproducible by duplicating the experiments.

The gas evolved after twelve hours was analysed in conventional fashion by noting any decrease in volume occasioned by passages through a series of absorbing solutions. Carbon dioxide and higher oxides of nitrogen were definitely absent, and an absorption of 9% in acid cuprous sulfate-β-naphthol solution was attributed to the pyridine vapor known to be present, instead of to carbon monoxide. A molecular weight determination by the vapor density method carried out on the residual gas, gave the value 28.8, which agreed reasonably well with a similar determination made on a sample of commercial nitrogen gas. Since nitrous oxide, the remaining possibility, has a molecular weight of 44, its presence in substantial amount was eliminated by this result. At the same time, the extreme solubility of nitrous oxide in pyridine, together with the lack of a good method for estimating this gas in the presence of a large excess of nitrogen, made it possible that small amounts were formed but escaped detection.

The linear later portion of Curve A (Figure 1) indicated a secondary reaction continuing at a uniform rate. To check the assumption that this rate might correspond to the well-known decomposition of hydroxylamine in alkaline solution (50), duplicate blank determinations were carried out with the pyridine-hydroxylamine reagent. A colorless gas was slowly evolved

but not in sufficient quantity (Table IX) to account for the final slope of the reaction plot unless the assumption was made that the reaction products from sugar nitrates catalyzed the decomposition. Some evidence for such catalytic action has been reported in the literature for other nitrates (51)(52).

The data accumulated at this stage in the investigation indicated that a rapid removal of one nitrate group from the methylglucoside tetranitrate was followed by a much slower removal of a second group. This hypothesis was suggested by the way that the reactivity of nitrate groups depended upon their position in the glucoside molecule, as illustrated by several workers (17)(18). The possibility of anhydro sugar derivatives being formed in the reaction was indicated by the work of Gladding and Purves (23) but recovery of the nitrogen-free crystalline glucosides in high yield from the partially denitrated tetranitrates rules out such products. Similarly the possibilities of a Walden inversion or of substitution by amine units occurring during the partial denitration were answered in the negative by the same evidence. The composition of the sirupy reaction product was therefore restricted to a mixture of partly nitrated methylglucopyranosides, and the object of the research was reduced to locating the hydroxyl groups set free in the partial denitration.

Samples of the partially denitrated methyl-β-D-glucoside

tetranitrate (Sirup 1) were accumulated from 5g. portions of the tetranitrate with control of temperature (25 to 35°) and time of reaction (two hours) to ensure a uniform product. Preparations on a larger scale were prohibited by the difficulty of controlling the vigorous reaction, and two hours was chosen since the rate plot showed that secondary reaction products would then be in small proportion only. A shorter reaction time seemed unsuitable since the aberration in the plot in the neighborhood of the break (Figure 1, Curve A), made the determination of the exact volume of gas evolved uncertain. The sirup was dried to constant weight and analysed for total nitrogen and methoxyl.

The reading from the rate plot at two hours reaction time showed that 1.30 moles of nitrogen was evolved per mole of tetranitrate. When the assumption was made that one half of the nitrogen came from the tetranitrate and the other half from the hydroxylamine, the removal of 1.3 atoms of nitrate group per mole of tetranitrate was indicated. The remaining nitrogen content of Sirup 1 was therefore given by:

$$\frac{(4.00 - 1.30)(14)(100)}{(374 - (45)(1.30))} = 12.0\%$$

The total nitrogen content of Sirup 1 was N, 11.7, 11.9%. In a similar manner the methoxyl content of Sirup 1 was calculated:

$$\% \ OCH_3 = \frac{(31)(100)}{(374 - (45)(1.30))} = 9.84\%$$

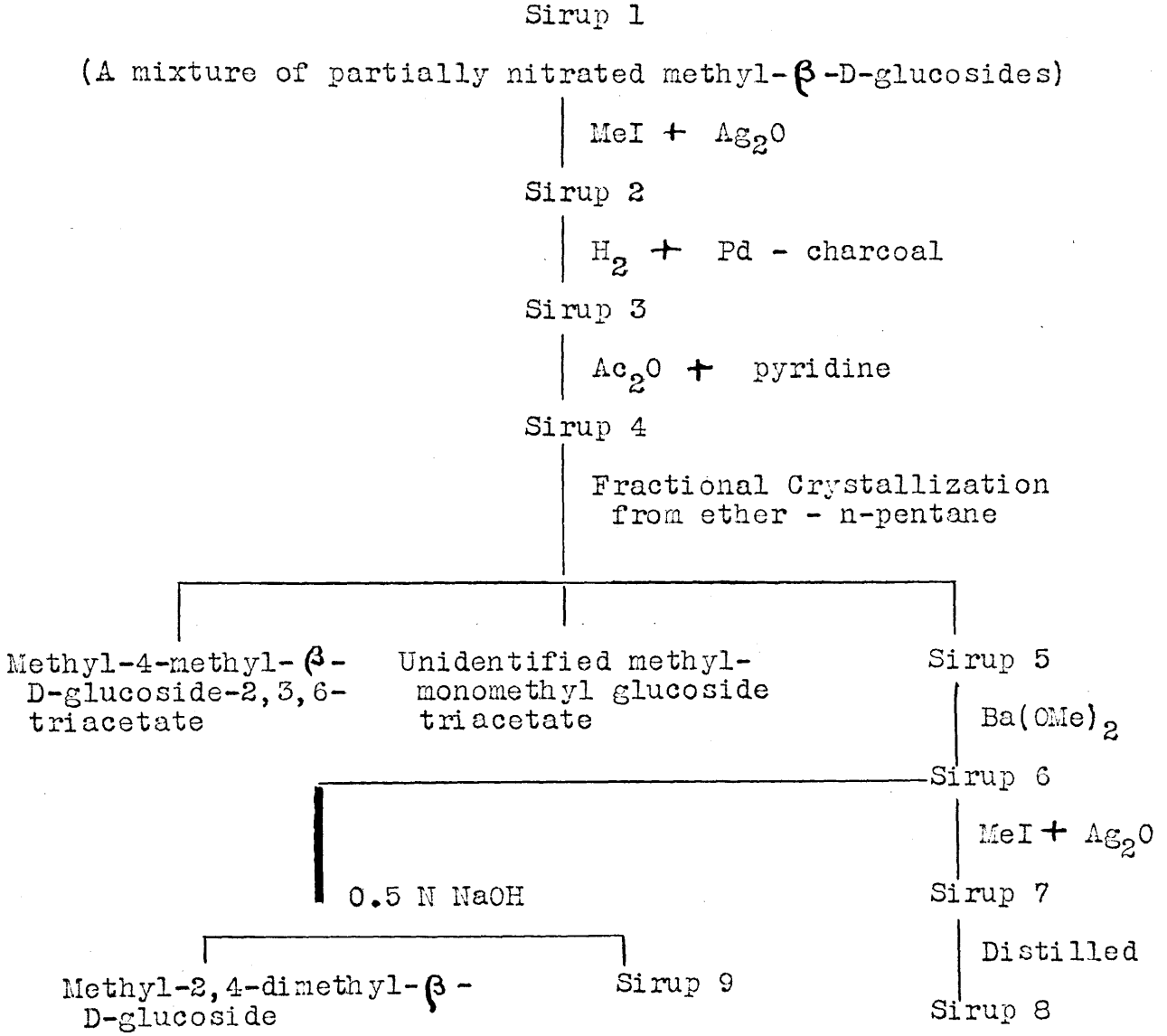
and the values found by analysis were: OCH_3, 9.19, 9.11%.

The first preparations of Sirup 1 were clear, pale yellow glasses and were successively methylated, hydrogenated and acetylated as outlined in Figure 3 (Series I). The products at each stage were sirups and the properties and analyses of these materials are recorded in Table X. The purpose of these operations was to obtain the components of Sirup 1 as specifically substituted derivatives in a form in which they could be separated and identified. The methylation technique was selected in preference to other etherification or esterification procedures since substitution with methyl ether groups, by means of methyl iodide and silver oxide was shown by Dewar and Fort (11) and others (21)(22) to cause no loss of nitrate groups when applied to methyl-β-D-glucoside di- and trinitrates. The methylation was found by these authors to be quantitative when repeated (if necessary) and the degree of substitution could be readily determined by the standard nitrogen and methoxyl analyses. In addition, the methyl ether groups were reported to be stable to reductive denitration procedures.

Methylation of Sirup 1 was completed in one eight-hour treatment with methyl iodide and silver oxide as shown by

Isolation of Crystalline Derivatives of the Products from the Partial Denitration Reaction

Series I[a]

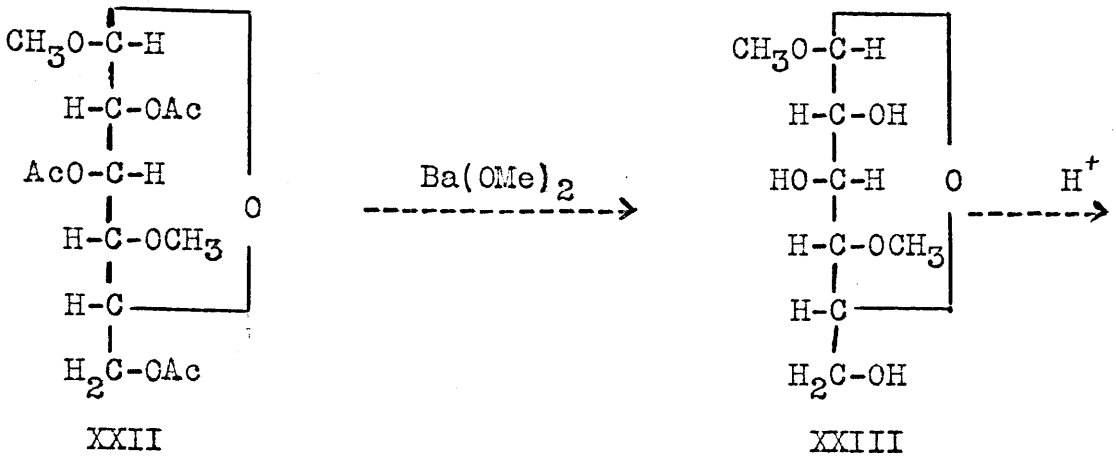


(a) For analyses and properties of the products see Table X.

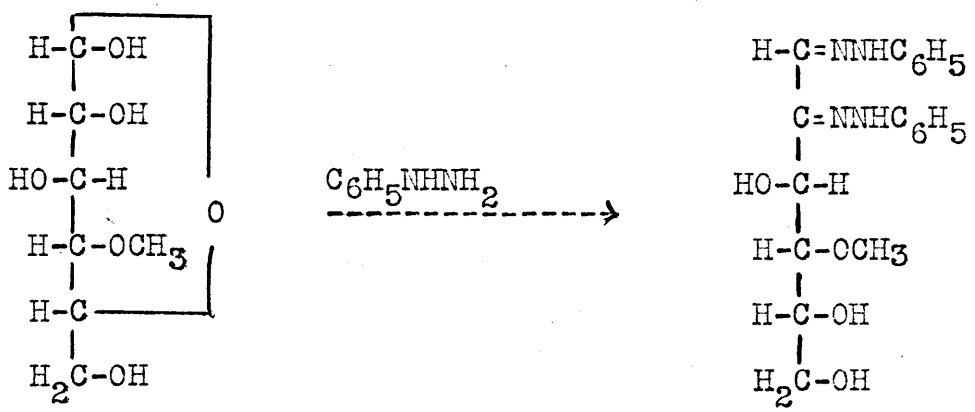
Figure 3

analysis and by remethylation of the sirupy product, Sirup 2 (Figure 3). The methoxyl and nitrogen substitutions of Sirup 2 were calculated and indicated no significant loss of nitrate groups. Hydrogenolysis of Sirup 2 was similarly nearly quantitative and the nitrate-free, colorless, sirupy product (Sirup 3) had a methoxyl content intermediate between those calculated for a methylmonomethyl glucoside and a methyldimethyl glucoside. Acetylation of Sirup 3 also yielded a sirupy product (Sirup 4) which was then fractionally crystallized from ether - n-pentane and yielded two crystalline compounds and Sirup 5.

The major crystalline product (31% of Sirup 4) was identified as methyl-4-methyl-β-D-glucoside-2,3,6-triacetate (XXII) from comparison of its physical constants with those of the known, crystalline methylmonomethyl-β-D-glucoside triacetates (Table III) and by deacetylation to methyl-4-methyl-β-D-glucoside (XXIII), now reported crystalline for the first time. The structure of the glucoside was limited to either methyl-2- or 4-methyl glucoside by the fact that neither formaldehyde nor formic acid was produced, and only one mole of oxidant was consumed, in an oxidation with aqueous sodium metaperiodate. Acid hydrolysis yielded 4-methyl-D-glucose (XXIV) from which the known crystalline 4-methylglucose phenylosazone (XXV) was prepared in 59% yield. The mutarotation of XXIV was followed and an equilibrium value of $[\alpha]_D^{20}$, $+56.2^\circ$ in aqueous solution was found.



XXII XXIII



XXIV XXV

TABLE III

Triacetates of Methylmonomethyl-β-D-glucopyranosides

Position of Methyl	m. p.	$[\alpha]_D^{20}$ ($CHCl_3$)	Reference
2	74 - 75°	+ 6.3°	(53)
3	90 - 90.5°	-34.8°	(54)
	91.5°	-36.4°	(12)
4	107 - 108°	-32.8°	(55)
	106°	-34.0°	(56)
	105 - 106°	-34.9°	(22)
6	107 - 108°	-12.4°	(57)(58)

Analytical data for the second crystalline fraction (13% of Sirup 4) showed that it also was a methylmonomethyl glucoside triacetate. The product melted at 76 - 78° and rotated $[\alpha]_D^{20}$ -34.8° ($CHCl_3$, c = 16.4 , l = 2) (Table X) and these physical constants did not agree with those of any of the known methyl--monomethyl-β-D-glucoside triacetates (Table III). The triacetate was obtained in larger quantities from a second series of experiments (Series II, Figure 4) and attempts to prove its structure were renewed. Deacetylation of the crystalline substance with barium methylate at 0°, or with sodium methylate at 20°, yielded Sirup 33 which agreed in methoxyl content with a methylmonomethyl glucoside. Periodate oxidation of Sirup 33 gave anomalous results; 0.58 moles of periodate were consumed per mole of the methylmonomethyl glucoside, and formic acid, but no formaldehyde was detected in the oxidized solution. Only a methyl-6-monomethylhexopyranoside would yield formic acid under these conditions, but the corresponding consumption of periodate would be 2.0 moles instead of the 0.58 moles observed. It was concluded that the sample was a mixture of two and possibly three monomethyl position isomers. The action of aqueous sodium hydroxide for twenty hours at room temperature did not affect Sirup 33 and repeated benzoylation with benzoyl chloride in pyridine yielded a sirupy product.

An attempt was made to destroy the oxidizable component

of Sirup 33 with periodate and then to crystallize and separate the non-oxidized portions. When oxidation was complete as shown by the optical rotation reaching a constant value the solution contained two products, of which the first, a very deliquescent crystalline material, insoluble in boiling chloroform, gave a negative test for aldehyde and when burned left a large ash. It appeared to be sodium formate contaminated with an optically active sirup. The second and larger product, Sirup 34, was soluble in chloroform and reduced Fehling's solution but could not be crystallized.

Treatment of methyl-β-D-glucoside tetraacetate with a solution of titanium tetrachloride in anhydrous chloroform was shown by Piel and Purves (59) to lead to an equilibrium containing 90% of the corresponding α-compound. This procedure was applied to the crystalline methylmonomethyl-β-D-glucoside triacetate with a view to causing it to undergo the isomerisation and hence of obtaining an identifiable crystalline compound in the α-series. The product of the reaction in this case was again a sirup and was not investigated further.

Sirup 5 (56% of Sirup 4) was isolated from the mother liquor after removal of the crystalline triacetates (Figure 3) and had methoxyl and acetyl contents approximating to those of a mixture of methyldimethylglucoside diacetate (75%) and methylmonomethylglucoside triacetate (25%). The acetate was

was treated with barium methylate in anhydrous methanol solution for twelve hours at 0°. The product, Sirup 6, distributed itself between water and chloroform but was not fractionated, an observation which would indicate a good degree of homogeneity. A portion of Sirup 6 was methylated twice, but the methoxyl content could not be increased to that of a pentamethylglucose and when completely methylated the product (Sirup 7) distilled in a narrow range of temperature. When treated with sodium methylate in anhydrous methanol and chloroform at 20°, Sirup 6 showed no change in rotation during forty eight hours.

It seemed probable that Sirup 6 contained an acetyl group resistant to the catalytic action of the methoxide at the temperatures employed. Evidence in support of this conjecture was provided in the reports of several authors. Helferich and Lang (54) found that heating methyl-3-methyl-β-D-glucoside triacetate under reflux with sodium methylate failed to remove one acetyl group, while the action of normal aqueous sodium hydroxide at room temperature caused complete deacetylation. Similar difficulty in removing a residual benzoyl group from the 3-methylglucoside with potassium methylate was reported by Sundberg et al (60) and treatment with aqueous caustic was necessary to effect complete removal. Dewar and Fort (11) reported that with methyl-2,4-dimethyl-3-acetyl-β-D-glucoside-6-nitrate and sodium methylate, six days were required for complete deacetylation at room temperature.

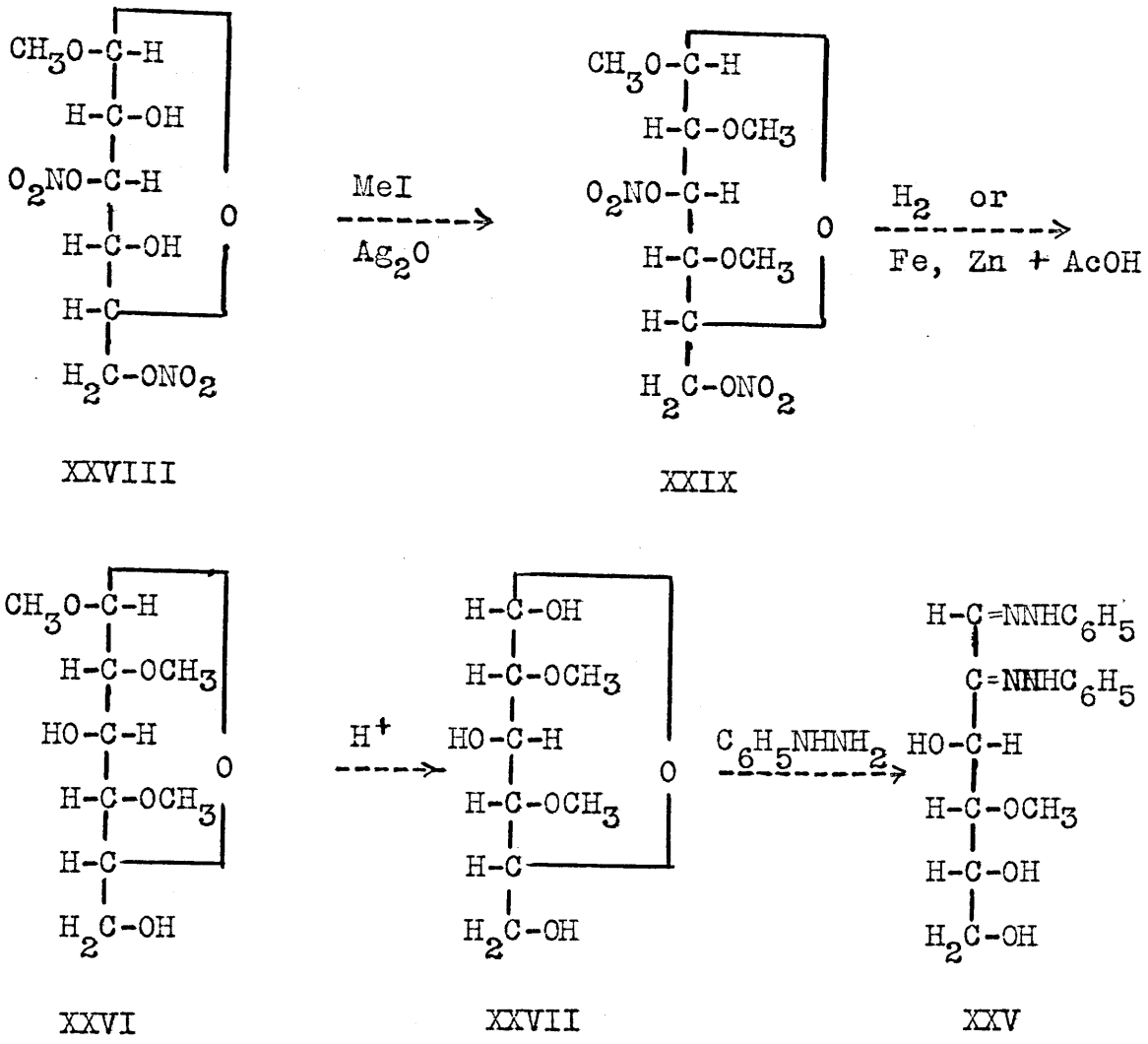
When treated with 0.5 N aqueous sodium hydroxide according to Helferich and Lang (59), Sirup 6 yielded crystalline methyl-2,4-dimethyl-β-D-glucoside (XXVI). The properties of the glucoside agreed with those reported by Dewar, Fort and McArthur (11) and others (61), and the compound did not react with aqueous periodate solution during four hours at 20°. No unsubstituted 1,2 glycol groups were present and none are present in the 2,4-dimethyl derivative (XXVI). Acid hydrolysis yielded the sirupy 2,4-dimethyl glucose (XXVII)(11) and the mutarotation of this compound was observed for the first time. The action of phenylhydrazine hydrochloride and sodium acetate on an aqueous solution of the dimethyl glucose produced 4-methyl-glucose phenylosazone (XXV) in small yield, as reported by Adams, Reeves and Goebel (61) and by Dewar and Fort (11).

After standing over phosphorous pentoxide for two months the original partly denitrated product, Sirup 1, partially crystallized. The crystalline nitrate was isolated and identified as methyl-β-D-glucoside-3,6-dinitrate (XXVIII) by methylation and hydrogenation of the sirupy dimethyl dinitrate (XXIX) to the known crystalline methyl-2,4-dimethyl-β-D-glucoside (XXVI). The specific rotation of the 3,6-dinitrate (XXVIII) $[\alpha]_D^{20}$-7.2°, agreed with that reported by Dewar, Fort and McArthur (18), but the melting point was found to be ten degrees lower than the reported value. The properties of

methyl-2,4-dimethyl-β-D-glucoside-3,6-dinitrate were in close agreement with those reported by these authors. The structure of the dinitrate was proved to be 3,6 by conversion to the methyl derivative (XXIX), and denitration of the latter to the known methyl-2,4-dimethyl-β-D-glucoside (XXVI).

In a second series of experiments (Series II, Figure 4) the crystalline dinitrate (XXVIII) was separated from a chloroform solution of Sirup 1 by aqueous extraction. The residual sirup recovered from the chloroform residues by evaporation, approximated in composition to a mixture of methyl-glucoside trinitrates (Sirup 10). It was successively methylated and hydrogenated and crystalline methyl-4-methyl-β-D-glucoside (XXIII) was isolated by fractional crystallization of the nitrate-free hydrogenation product (Sirup 13). The residual Sirup 14 then analysed for a methylmonomethyl glucoside and when acetylated (Sirup 15) the unidentified methylmonomethylglucoside triacetate of Series I was obtained once more. No dimethyl-glucoside could be found in the residues.

A third series of reactions (Series III, Figure 5) was carried out by crystallizing the dinitrate (XXVIII) from Sirup 1 in chloroform solution and then acetylating the residual sirup (Sirup 17). A crystalline fraction, Product 19, and a sirupy fraction, Sirup 20, were obtained by fractional crystallization of the acetylated sirup (Sirup 18). Attempted separation of pure crystalline glucoside nitrate - acetates



XXVIII XXIX XXVI XXVII XXV

from Product 19 by fractional crystallization was unsuccessful and the crystalline material recovered was deacetylated with sodium methylate. A second small quantity of methyl-β-D-glucoside dinitrate (XXVIII) was separated from the deacetylated sirup (Sirup 22) and the remaining sirup was then methylated, hydrogenated and acetylated to give methyl-4-methyl-β-D-glucoside triacetate (XXII) and a non-crystallizable

Isolation of Crystalline Derivatives of the Products from the Partial Denitration Reaction

Series II

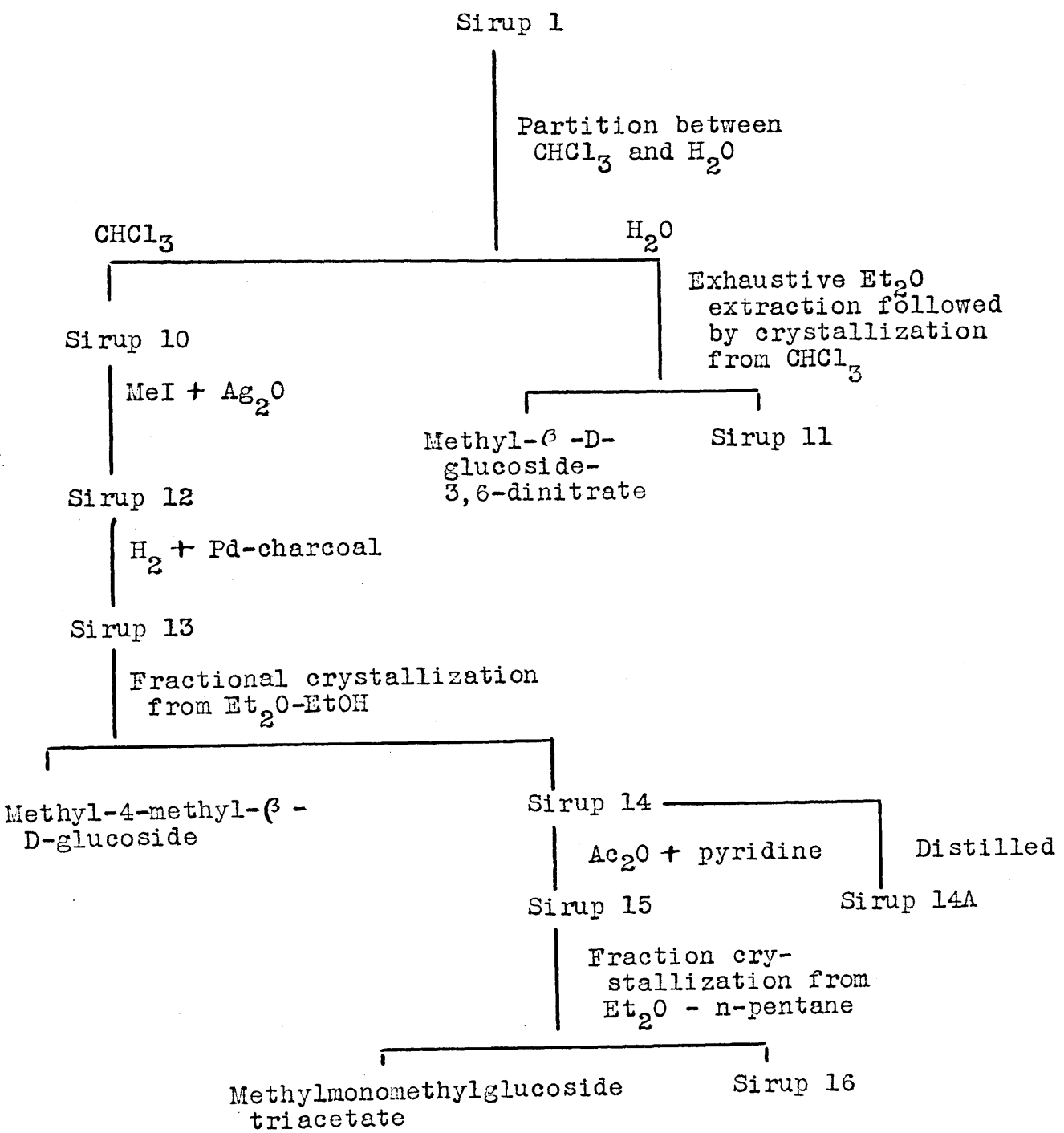


Figure 4

Isolation of Crystalline Derivatives of the Products from the Partial Denitration Reaction

Series III

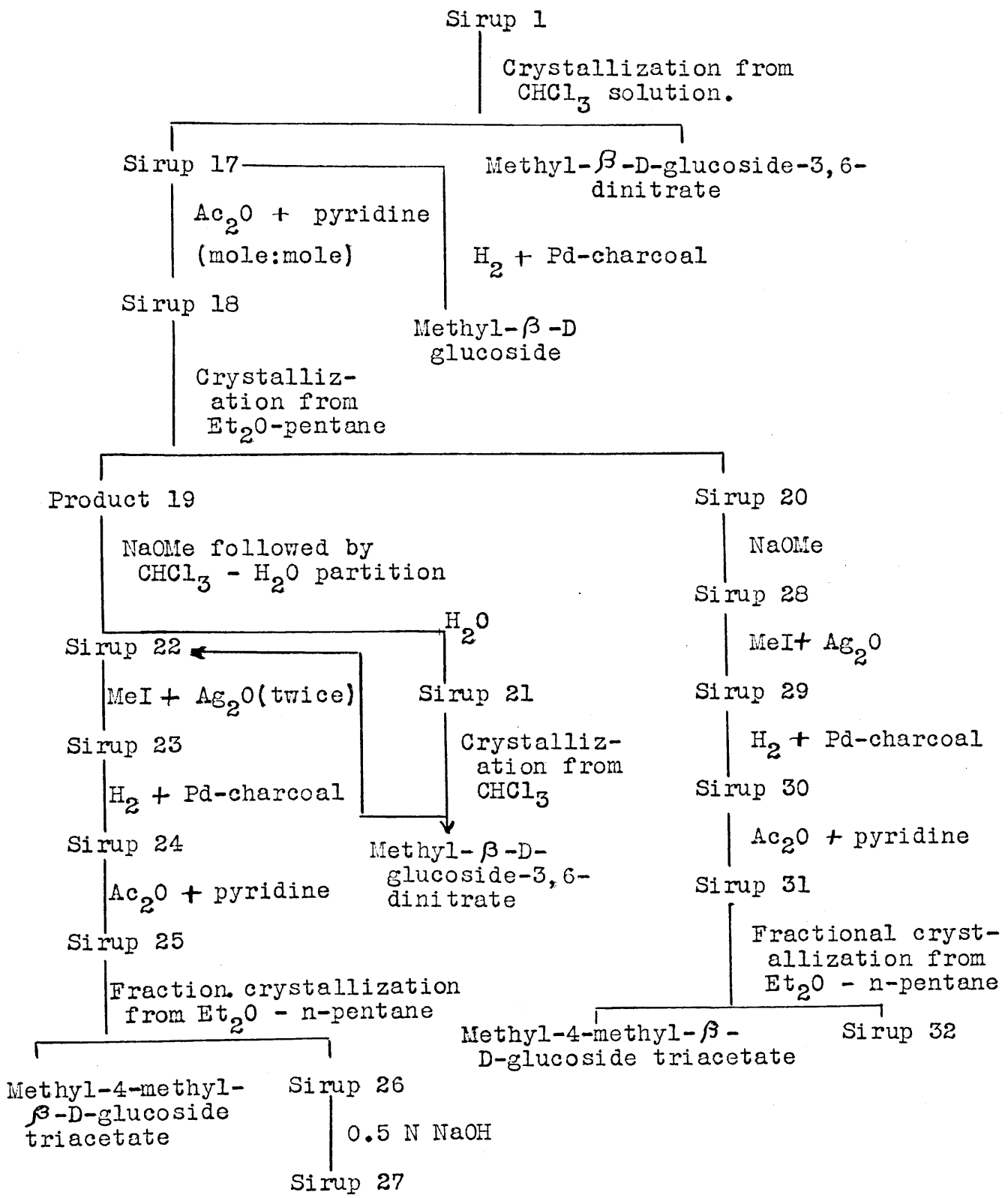


Figure 5

sirup (Sirup 26). The same series of reactions applied to Sirup 20 yielded a second, smaller amount of methyl-4-methyl-β-D-glucoside triacetate (XXII) and no other crystalline product.

A review of the three series of isolations and of the products obtained in each case, together with the information gathered from the studies on the formation of gaseous nitrogen permitted several calculations to be made concerning the composition of Sirup 1. These calculations are summarized in Table IV for purposes of comparison and evaluation. It is felt that the close agreement of the calculated and observed values permits a statement of the composition of the partially denitrated product as quoted in the last line of the table within the limits of $\pm 5\%$. It may also be worthy of note that the individual analyses of Sirups 3 and 2 of Series I agree within this limit to the composition quoted.

Since one of the trinitrates (14% of Sirup 1) could not be completely identified, any mechanism proposed for the original partial denitration must be in the nature of a conjecture. On this basis it is assumed that a reaction analogous to the one suggested by Angeli (44) (45)(46) for hydroxylamine on methyl or ethyl nitrates, may occur with the nitrate group in the 4 position of methylglucoside tetranitrate. The initial reaction

TABLE IV

The Composition of Sirup 1

Source of Data	Analyses % OCH_3	Analyses % N	Degree of Nitrate Substn.	Components	% Composition
Gas Evolution Experiments	9.84[a]	12.0[a]	2.70[d]	Methylglucoside dinitrate	30[f]
				Methylglucoside trinitrate	70[f]
Analysis of Sirup 1	9.15[b]	11.8[b]	2.64[d]	Methylglucoside dinitrate	36[f]
				Methylglucoside trinitrate	64[f]
Crystalline Products Isolated in Series I	9.81[c]	11.8[c]	2.67[e]	Methyl-β-D-glucoside-3,6-dinitrate[h,i]	33[g]
				Methyl-β-D-glucoside-2,3,6-trinitrate[h,i]	53[g]
				Methylglucoside trinitrate (unidentified)[h]	14[g]

(a) Calculation was discussed previously in text, p. 33

(b) Mean of duplicate determinations.

(c) Calculated from nitrate substitution, Column 4, by formula:
$\% N = \frac{1400x}{194 \quad 45x}$ where x is the degree of substitution.

(d) Calculated from formula in (c).

(e) Calculated from % Composition, Column 6.

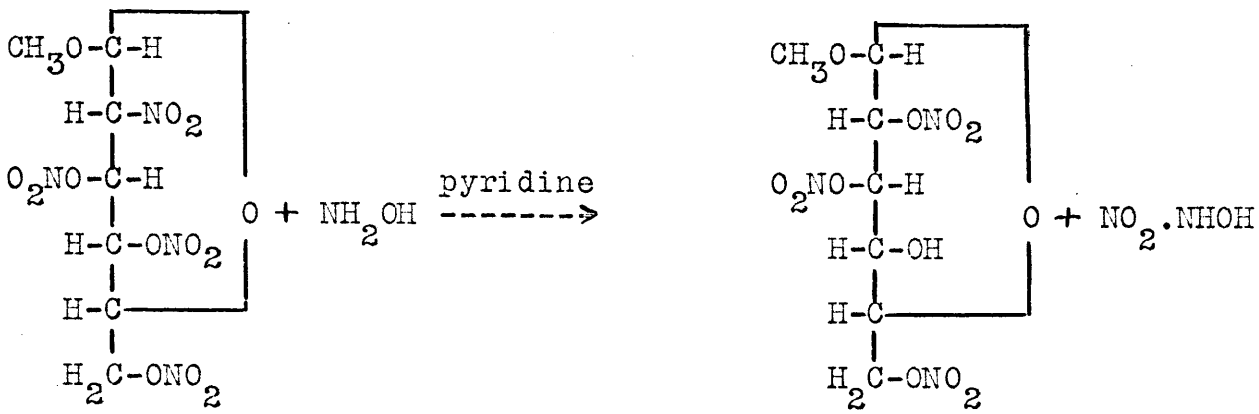
(f) Calculated from degree of substitution, Column 4, by formula:
$3y + 2(100 - y) = 100x$, where y is the % trinitrate and x is the degree of substitution.

(g) Calculated from the actual weights of crystalline derivatives isolated.

(h) The presence of these components was confirmed in Series II.

(i) The presence of these components was confirmed in Series III.

products would then be methyl-β-D-glucoside-2,3,6-trinitrate (XXX) and nitrohydroxylaminic acid.

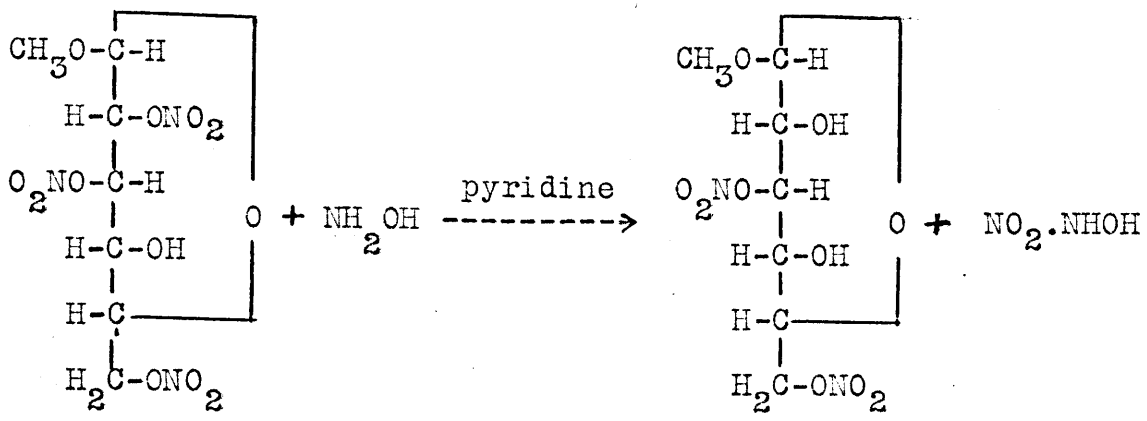


XV XXX

The unstable nitrohydroxylaminic acid then reacts with a second mole of hydroxylamine to give nitrous acid, nitrogen and water:

$$NO_2.NHOH + NH_2OH \longrightarrow HNO_2 + N_2 + 2H_2O$$

Segall (32) employed the same assumption to explain rather similar results with cellulose trinitrate. The secondary, slower reaction would then correspond to the attack of excess hydroxylamine on the nitrate group in the 2 position of the glucoside molecule to give the observed methyl-β-D-glucoside-3,6-dinitrate (XXVIII), and a second mole of nitrogen by decomposition of the nitrohydroxylaminic acid formed.



XXX XXVIII

Concerning the third product of the reaction, it might be conjectured that this resulted from a slow attack of pyridine upon the nitrates in a side reaction, resulting in a compound of such character that crystalline methyl-glucoside was regenerated upon hydrogenation. Some evidence for such a reaction was provided by the effect of pyridine on mannitol hexanitrate reported by Wigner (39).

Experimental

Special Precautions

Throughout the experimental work the explosive character and high reactivity of nitrated derivatives (6) were taken into account. Sample weights were restricted to the minimum which could be conveniently handled in each operation; for most reactions this amounted to 5 g. or less. In nitrations where the yields exceeded 5 g, the products were separated into quantities of that order immediately after isolation and were stored in segregated containers with care to avoid exposure to light and shock or abrasion. Distillation of carbohydrate nitrates was not attempted.

All evaporations of solvents were conducted in ground glass apparatus under reduced pressure at bath temperatures below 50°. Samples for analysis were dried at room temperature to constant weight in vacuo.

A. Materials

100% Nitric Acid

The nitric acid used in all nitrations was prepared by distilling a mixture of concentrated sulfuric and fuming nitric acids (1:2) under reduced pressure in an all-glass still designed in this laboratory by Dr. G. D. Thorn. The distillate was colorless, miscible without turbidity with

chloroform and titrated against standard alkali as 99.3 to 100.8% acid.

Methyl-α-D-glucopyranoside-2,3,4,6-tetranitrate

The nitration was conducted in a 1-liter, 3-necked flask equipped with a stirrer passing through a mercury seal in the middle neck and operated by a high-speed motor. One side neck carried a flask and glass adapter designed for the slow addition of solids without exposure of the contents to the atmosphere, and the other neck a calcium chloride drying tube. All connections were made with ground glass joints. The flask was cooled in a brine bath at - 10°; 370 ml. of nitric acid was added and 20 g. of phosphorous pentoxide was slowly introduced from the addition flask. The mixture was stirred for twenty minutes to effect complete solution and then 35 g. (0.18 mole) of dried, finely powdered methyl-α-D-glucoside (m. p. 165 - 166°, $[\alpha]_D^{20}$ +158.0 in H_2O, c = 1.000) was introduced through the addition flask during ten minutes. Vigorous stirring was continued throughout this time and for thirty minutes after the last addition of the glucoside. At this point stirring was stopped and the contents of the flask were poured slowly into 2 l. of mechanically stirred cracked ice and water. A white, amorphous solid separated which rapidly changed to a heavy, colorless oil as the ice melted. The aqueous mixture was extracted with chloroform and the chloroform solution was washed with bicarbonate solution,

then with water, dried over sodium sulfate and evaporated under reduced pressure. A semi-crystalline solid (63.0 g.) remained which on recrystallization from ethanol after nucleation yielded 56.0 g. (86% of theory) of lustrous, quadratic plates melting at 48.5 - 49.0°, $[\alpha]_D^{20}$ +136.5° (alcohol, c = 1.1, l = 2).

Calcd. for $C_7H_{10}O_{14}N_4$: OCH_3, 8.29; N, 14.9%

Found: OCH_3, 8.25, 8.30; N, 14.8, 14.9%.

The reported constants for methyl-α-D-glucopyranoside tetranitrate are: m. p. 48.5° and $[\alpha]_D^{20}$ +134.6° (alcohol, c = 4.1)(6).

Methyl-β-D-glucopyranoside-2,3,4,6-tetranitrate

Methyl-β-D-glucoside was prepared from glucose by two different procedures. The first, originated by Koenigs and Knorr (8), was modified according to Kreider and Evans (62) and Levene and Tipson (63). The overall yield from commercial glucose monohydrate (Cerelose) via the penta-acetate and acetobromoglucose amounted to 62% of theory. The second and much more convenient procedure reported recently by Raymond and Schroeder (49) gave the glucoside in an overall yield of 18% from anhydrous glucose. The glucoside was obtained as the crystalline hemihydrate by both procedures and in this form melted at 105 - 107° after

recrystallization from alcohol-ether and had a specific rotation of $[\alpha]_D^{20}$ -32.2° in water (c = 4.35, l = 2). Raymond and Schroeder (49) reported m. p. 104 - 106° (corr.) and $[\alpha]_D^{20}$ -32° in water (c = 5.72) for the hemihydrate.

Direct nitration of methyl-β-D-glucoside with nitric acid - phosphoric anhydride gave 50% yields or less of the tetranitrate probably because of the slight solubility of the latter compound in the nitrating liquor. Better yields (80 - 85%) were obtained by use of the nitrating mixture acetic anhydride - acetic acid - 100% nitric acid as described by Brissaud (6). The apparatus and procedure were as described for the α-compound. The tetranitrate was recrystallized from aqueous methanol as colorless broken prisms; m. p. 116 - 117°, $[\alpha]_D^{20}$ +9.35° ($CHCl_3$, c = 4.00, l = 2). Bell and Synge (21) reported m. p. 116 - 118°, $[\alpha]_D^{20}$ +9.35° ($CHCl_3$, c = 4, l = 2).

Anal: Calcd. for $C_7H_{10}O_{14}N_4$: OCH_3, 8.29; N, 14.96%

Found: OCH_3, 8.30, 8.29; N, 14.7, 14.7%.

Cyclohexyl-β-D-glucoside

Acetobromoglucose, 25.06 g. (0.0609 mole) prepared according to Redeman and Niemann (65) was treated with 30.5 g (0.305 mole) of cyclohexanol and 28.3 g (0.122 mole) of silver oxide in anhydrous benzene solution. This method, originated by Fischer and Helferich (66) was modified as proposed by

Krieder and Evans (62) by use of Drierite as an internal desiccant. The yield of the pure tetraacetate amounted to 20.5 g. or 79% of theory. This product formed beautiful long needles melting at 120.5 - 121.0°. Deacetylation was carried out by the method of Levene and Tipson (63) using catalytic amounts of barium methylate in a methanol solution of the tetraacetate at 0°. The yield of pure crystalline cyclohexyl-β-D-glucoside was 82% of theory. The fine white crystals melted at 134 - 135°. Richtmeyer (48) reported 133 - 135° for the melting point.

Attempted Nitration of Cyclohexyl-β-D-glucoside

Nine grams of the dry, finely-powdered cyclohexyl-β-D-glucoside was nitrated as previously described for the α-methyl compound. The chloroform solution when dried, concentrated under reduced pressure and treated with petroleum ether (b. p. 30 - 60°) to turbidity deposited exceedingly fine white crystals which were recovered on a filter by suction. A bright yellow oil separated from the filtrate and could not be crystallized. The crude crystalline product weighed 3.0 g (20% of theory), melted at 86 - 88° and was stable to 100° (capillary tube). Recrystallization from aqueous ethanol gave tiny crystals melting at 110 - 111°.

Calcd. for $C_{12}H_{18}O_{14}N_4$: N, 12.6%

Found: N, 17.1, 16.9, 17.0%

The product agreed in nitrogen content and melting point for β -D-glucose pentanitrate (5).

Anal: Calcd. for $C_6H_7O_{16}N_5$: N, 17.28%

The recovery of an oil suggested that hydrolysis of the cyclohexyl group had occurred during the nitration.

Crystalline Hydroxylamine

Free hydroxylamine was prepared in improved yield by modification of the Hurd and Brownstein procedure (67). A higher temperature (50 - 60°) was maintained during the addition of sodium butylate to hydroxylamine hydrochloride suspended in butanol. This slight change permitted a more rapid addition of the butoxide (twenty five minutes as against one to one and one half hours) without impairing the acidic conditions necessary in the reaction mixture.

The yield of crystalline product after washing with ice-cold anhydrous ether and drying briefly in vacuo amounted to 65 - 70% of theory. Hurd and Brownstein reported a consistent yield of 50%.

The free base appeared to be extremely sensitive to moisture, heat, metals and traces of alkali. Exposure to the atmosphere was avoided as much as possible and all operations involving the crystalline material were conducted

with acid - washed glass apparatus in a room maintained at 5 to 10°.

The crystalline product was prepared as required and was immediately dissolved in anhydrous pyridine previously cooled to 0°. The solution was stored at 0° not more than forty eight hours before use. Gas evolution from the solution was very slight at the low temperature of storage. The hydroxylamine tended to crystallize from the solution but was readily redissolved on warming to room temperature with shaking.

Palladized Charcoal Catalyst

The palladium on charcoal catalyst for the hydrogenolysis of nitrate groups was prepared by the method of Hartung (68). When thoroughly dry it ignited spontaneously in air and required care in handling to avoid ignition of the solvent and nitrate. The same catalyst could be used for three or four experiments and was regenerated by washing on a filter with water, ethanol and anhydrous ether and drying in vacuo.

The hydrogen, a pure, commercial, electrolytic product, was used directly from the storage tank in the Adams Low-pressure Hydrogenator.

Reagents and Solvents

Pyridine, methyl iodide, acetic anhydride, benzoyl chloride

and phenylhydrazine were purified before use by standard procedures (69)(70) and were stored in groundglass - stoppered containers. Silver oxide of "C. P." quality was dried for two hours in a vacuum oven at 50 - 60° before use. For the present work the freshly prepared oxide seemed to offer no advantages over the commercial variety. Barium methylate in absolute methanol solution was freshly prepared according to Isbell (71) and was standardized by direct titration with 0.1 N acid, using phenolphthalein as the indicator.

Specially purified solvents are noted in the text. Other reagents and solvents were of the usual "C. P.", "U. S. P." or "Reagent" quality.

B. Analytical Methods

Nitrogen

Total nitrogen in nitrated derivatives was determined by the micro-Kjeldahl method as modified by Gunning (72). Nitrate nitrogen was determined by Elving and McElroy's semi-micro modification of the du Pont nitrometer method (73).

Analysis for osazone nitrogen was carried out by the Friedrich modification of the micro-Kjeldahl method (74); amino nitrogen was determined by the usual micro-Kjeldahl procedure (74).

Methoxyl

The Viebock and Schwappach estimation for methoxyl, as described by Clark (74) was used; the method when applied to mixtures, was checked in each case by control determinations made on a sample of pure methyl- . -D-glucoside.

Acetyl

Saponification with alcoholic sodium hydroxide as recommended by Clark (74) was employed. Control determinations were made on a sample of pure cellobiose octaacetate.

Oxidations with Periodate

The procedures described by several authors (75)(76)(77)(78) were adapted and combined so that a single 100 mg. to 200 mg. sample revealed the polarimetric change, the final amount of periodate consumed, formic acid, and formaldehyde produced.

The sample was dissolved in 5 or 6 ml. of water in a 25 ml. volumetric flask. Fifteen ml. of a 0.15 M solution of sodium metaperiodate was added and the solution was quickly made up to the mark with distilled water, shaken vigorously, and a 2 dm. polarimeter tube of 16 ml. capacity was filled and used to follow the reaction polarimetrically. All operations to this point were conducted in a room at 20°.

An aliquot (5 ml.) of the solution was taken from the volumetric flask as soon as possible after the mixing and the initial pH was determined with a calibrated Bechman pH-meter (Industrial Model).

The change in rotation was plotted against the time elapsed from the first contact of the periodate solution with the sample (77). When the rotation became constant (one to two hours), three 2 ml. aliquots were withdrawn from the polarimeter tube, diluted to 10 ml., and mixed with 1.5 g. of sodium bicarbonate. Ten ml. of 0.1 N sodium arsenite and one ml. of 20% potassium iodide solutions were added. After standing ten to fifteen minutes at room temperature the solutions were titrated with standard 0.1 N. iodine solution. From the iodine titre and a suitable blank, the amount of periodate consumed was calculated. This estimation is described in detail by Jackson (75).

A 5 ml. aliquot of the oxidized solution was removed from the polarimeter tube and was titrated to the initial pH with 0.02 N barium hydroxide solution, and with the aid of the Bechman pH-meter. The amount of formic acid formed in the reaction was calculated from the alkali titre (76). This value was usually low, probably because a very rapid change in pH during the first five to ten minutes made it difficult to determine the exact initial value. The formic acid determination was definite however, in at least a qualitative sense, since no change in pH could be detected when the compounds examined lacked a 1,2,3-trihydroxy grouping, as shown by the amount of periodate consumed.

For the formaldehyde determination the three 2 ml. aliquots after titration with iodine were combined and treated with 2 ml. of an 8% alcoholic solution of dimethyl-dihydroresorcinol (Dimedon) and allowed to stand for several days at 5 to 10°. The amount of formaldehyde produced in the oxidation was calculated from the weight of the formaldehyde - dimedon complex precipitated according to Reeves (78). Absence of a precipitate after a week at 5 to 10° was considered a negative test for formaldehyde.

C. Experiments on the Methyl-α- and β -D-glucoside Tetranitrates

The Action of Dry Pyridine on the Tetranitrates

Samples of the crystalline tetranitrates (2.00 g.) in small glass-stoppered bottles were separately treated with 20 ml. of cold anhydrous pyridine; the bottles were stoppered, shaken and allowed to stand at room temperature. The nitrates dissolved almost immediately to give colorless clear solutions which acquired a brilliant red color in ten to fifteen minutes. No gas evolution was observed. After several hours the solutions were poured into water, the aqueous mixtures were made acid to Congo Red with dilute sulfuric acid, and were extracted with ether. The ether extracts were washed, dried and evaporated. The crystalline residues were recrystallized from alcohol and their

identities were confirmed from their melting points and mixed melting points with the corresponding methyl-α- and β-D-glucoside tetranitrates. The data from these experiments are recorded in Table V.

TABLE V

The Action of Dry Pyridine on the Tetranitrates of the Methyl-D-glucosides

Tetranitrate	Time of Reaction (Hours)	Weight of Tetranitrate Recovered	% Recovery
α	4.5	1.528	76.4
β	12	1.075	53.8
α	16	1.192	59.6

The Action of Alcoholic Hydroxylamine on the Tetranitrates

Methyl-α-D-glucoside tetranitrate, 0.500 g., contained in a glass-stoppered bottle, was treated with 1.0 g. of crystalline hydroxylamine dissolved in 20 ml. of absolute ethanol. The tetranitrate dissolved after shaking to give a clear, colorless solution which evolved a colorless gas at a steady rate. After standing for sixteen hours at room temperature the solution was poured into water and the aqueous mixture was seeded and set aside to crystallize. After twenty-four hours the crystalline precipitate was

recovered on a filter, dried and weighed. Yield, 0.182 g. The product proved to be unreacted tetranitrate (36.4%) melting at 48.5 - 49.0° alone or in admixture with an authentic specimen.

Methyl-β-D-glucoside tetranitrate, 2.00 g., was treated with 20 ml. of a 13% solution of hydroxylamine in absolute alcohol. The tetranitrate did not dissolve and evolution of a colorless gas commenced almost immediately and continued steadily at room temperature. After twelve hours the mixture was poured into water and worked up exactly as described for the pyridine experiments. The yield of recovered methyl-(β-D-glucoside tetranitrate amounted to 1.537 g. or 76.9%. The ethanol - hydroxylamine solution, when allowed to stand at room temperature, evolved a colorless gas in the same manner when no nitrate was added.

Catalytic Reduction of the Tetranitrates

The hydrogenations were carried out in a roun-bottomed, 125 ml. pyrex flask fitted to the Adam's Low Pressure apparatus by a rubber stopper previously boiled in caustic solution to remove any sulfur. Palladized charcoal catalyst, 2.0 g., was added to a solution of the tetranitrate in 5.0 ml. of dioxane and 40 ml. of 95% ethanol. Hydrogenation at an initial pressure of 30 to 40 p. s. i. and at room temperature was complete in thirty minutes, as indicated by the pressure becoming constant. The solutions at this stage gave no blue

coloration with diphenylamine reagent (79). The catalyst was removed by filtration, was washed and the filtrate and washings were evaporated. The residues readily crystallized and after recrystallization from alcohol had the melting point of the corresponding methylglucoside. The mixed melting points showed no depression. The volume of hydrogen absorbed was calculated from the pressure change and volume of the apparatus.

The α-tetranitrate absorbed 7.8 moles of hydrogen per mole and the glucoside was recovered in 95% yield; the β-compound absorbed 7.6 moles of hydrogen per mole and was recovered as the crystalline hemihydrate in 96% yield. The equation proposed by Kuhn (30),

$$2RONO_2 + 5H_2 \longrightarrow 2ROH + N_2 + 4H_2O$$

required the absorption of 8.0 moles of hydrogen per mole of tetranitrate.

D. The Action of Hydroxylamine in Pyridine Solution on Methyl-α-D-glucoside Tetranitrate

Methyl-α-D-glucoside Tetranitrate

Two grams of the tetranitrate was treated at room temperature with 20 ml. of an ice-cold hydroxylamine - pyridine solution containing 0.125 g. of free hydroxylamine per ml. The tetranitrate dissolved completely and almost

immediately. After five minutes, evolution of a colorless gas commenced, the temperature increased to about 50° and the solution became pale yellow in color. The first vigorous reaction was over in thirty minutes and thereafter the gas evolution continued at a lower rate. After standing for four and one half hours the solution was poured with stirring into 250 ml. of water. A heavy oil separated almost immediately. The aqueous mixture was extracted with chloroform and the chloroform extract was washed with 2 N hydrochloric acid, then with water, dried, and evaporated under reduced pressure. A pale yellow sirup (1.3 g.) remained which could not be induced to crystallize.

Anal: Calcd. for methylglucoside trinitrate, $C_7H_{11}O_{12}N_3$:

OCH_3, 9.43%; N, 12.76%

Found: OCH_3, 8.70, 8.65%; N, 11.7, 11.9%.

These analyses were consistent with a mixture of tri- and di-nitrates. The sirup was very readily soluble in acetone, ether and chloroform; soluble in methanol, ethanol and benzene; insoluble in water.

The Rate of Partial Denitration of Methyl-α-D-glucoside Tetranitrate

One gram portions of the methylglucoside tetranitrate were separately treated with 10 ml. of the pyridine-hydroxylamine solution at room temperature. At intervals these

reaction mixtures were poured into water and worked up as previously described. The results of the analyses are shown in Table II.

Hydrogenolysis of Partially Denitrated Methyl-α-D-glucoside Tetranitrate

The sirups obtained from the experiment on the rate of denitration were dissolved in ether and combined. The ether was evaporated and the residue dried in vacuo over calcium chloride. A solution of 1.11 g. of this sirup in ethanol and dioxane was hydrogenated as described for the methylglucoside tetranitrates. The pressure became constant after twenty minutes, 0.018 moles of hydrogen was absorbed, and a crystalline product, 0.55 g., was recovered. The crude material was nitrate-free as shown by the diphenylamine reagent and melted at 163 - 164 °. After recrystallization from ethanol - ether a mixed melting point of this compound with pure methyl-α-D-glucoside showed no depression.

Resumé of Experiments on Partially Denitrated Methyl-α-D-glucoside Tetranitrate

The partially denitrated methyl-α-D-glucoside tetranitrate was prepared in amounts of 5 to 10 g., and separate portions of this material were methylated (methyl iodide and silver oxide), benzoylated (benzoyl chloride in pyridine) and acetylated (acetic anhydride in pyridine). The respective

products were all sirups and could not be induced to crystallize, either from organic solvents or by being kept over phosphorous pentoxide for periods of up to two years.

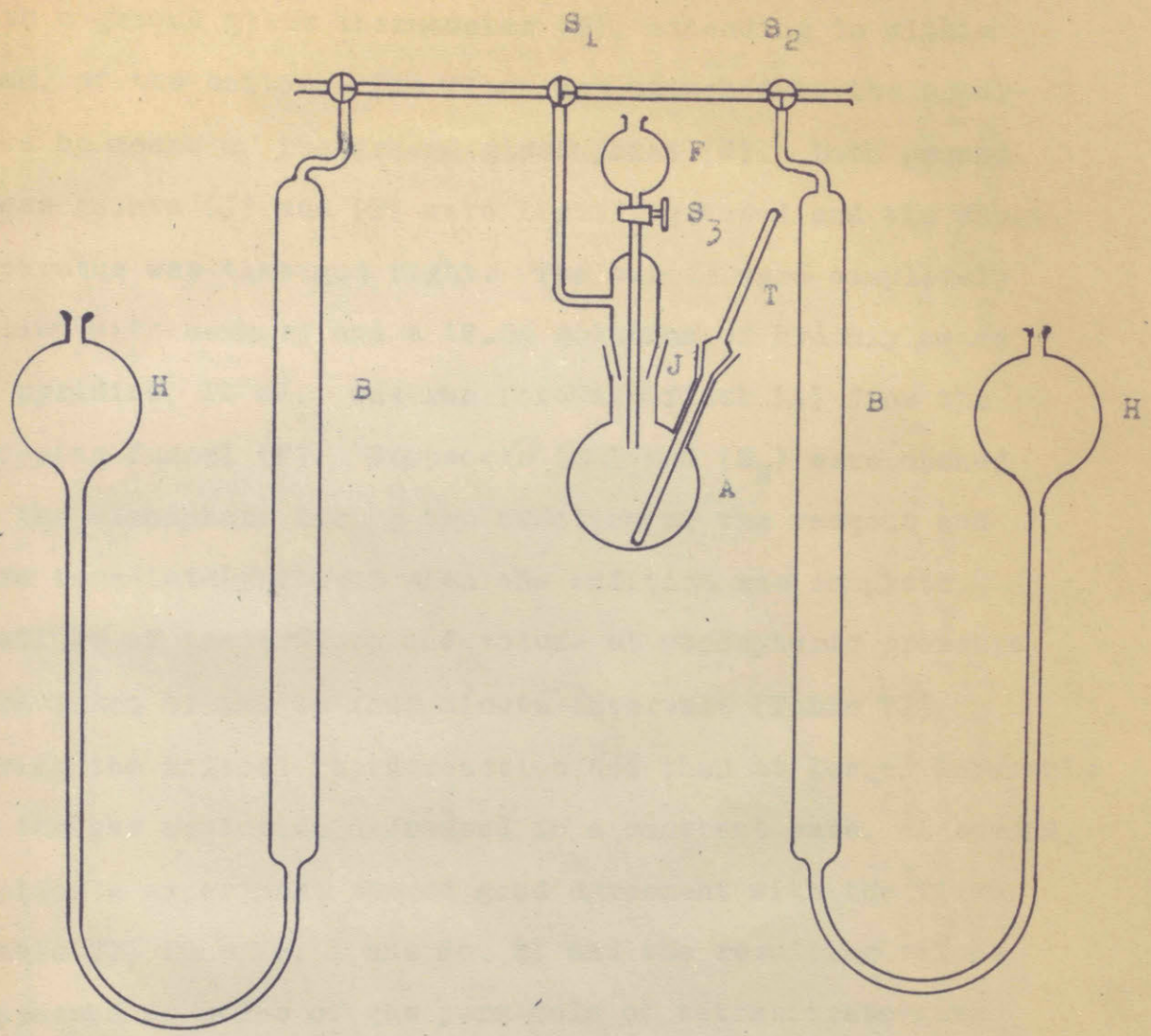
The methylated and benzoylated, sirupy nitrates were separately hydrogenated by the procedures previously described and yielded nitrate-free, glassy materials which also failed to crystallize. Attempts to distil the methylated, nitrate-free product below 1 mm. pressure resulted in charring and decomposition of the sample.

Work on the α - series of compounds was abandoned at this stage in favor of the β - series, from which crystalline products were obtained.

E Hydroxylamine - Pyridine and Methyl-β-D-glucoside Tetranitrate

The Rate of Gas Evolution

The reaction of tetranitrate with hydroxylamine in anhydrous pyridine solution was carried out in the apparatus shown in Figure 6. The two gas burets (B) were read at atmospheric pressure alternately, the gas being directed into the left buret by means of the three-way stopcock (S_1) while the right buret was being read, and vice-versa.



Apparatus for Measuring the Rate of Gas Evolution from Methyl-β-D-glucoside Tetranitrate in Hydroxylamine - Pyridine

Figure 6

Methyl-β-D-glucoside tetranitrate, 1.00 g., was placed in the reaction flask (A) and the flask was fitted with a ground glass thermometer (T), extending to within 1 mm. of the bottom. The flask was attached to the apparatus by means of the ground glass joint (J). Both ground glass joints (J) and (T) were lightly greased and the whole apparatus was then gas tight. The burets were completely filled with mercury and a 12.5% solution of hydroxylamine in pyridine, 10 ml., was run into the flask (A) from the dropping funnel (F). Stopcocks (S_1) and (S_2) were opened to the atmosphere during the addition of the reagent and were immediately closed when the addition was complete. Readings of temperature and volume at atmospheric pressure were taken at two to four minute intervals (Table VI) during the initial rapid reaction and then at longer intervals as the gas evolution decreased to a constant rate. A second, duplicate experiment showed good agreement with the first (Table VI, Runs No. 1 and No. 2) and the resulting values expressed as moles of gas perm mole of tetranitrate gave the plot in Figure 1. Extrapolation of the linear portion of the plot to zero time indicated an initial rapid evolution of 1.26 moles of gas.

Analysis of the Evolved Gas

Samples of the gas evolved from methyl-β-D-glucoside tetranitrate were collected in the absence of air by carrying

TABLE VI

Gas Evolution from One Gram of Methyl-β-D-glucoside Tetranitrate in Hydroxylamine - Pyridine

Run No. 1				Run No. 2			
Time (min.)	Volume (ml.)	Temperature (°C)[a]	Moles per Mole[b]	Time (min.)	Volume (ml.)	Temperature (°C)[a]	Moles per Mole[b]
0	0	25	0	0	0	24.5	0
4	18.5	34	0.27	2	16.6	24.5	0.25
6	58.3	57	0.71	4	36.0	30.0	0.53
8	98.6	69	1.05	6	39.1	40.0	0.55
10	105.8	61	1.24	8	68.6	62.0	0.80
12	105.4	54	1.32	10	104.9	62.0	1.23
14	98.2	48	1.29	12	100.6	55.0	1.26
16	96.5	44	1.30	16	96.5	45.0	1.31
20	90.7	38	1.27	20	91.7	38.0	1.30
24	86.4	34	1.24	24	90.0	34.0	1.31
32	86.9	30	1.27	32	87.1	29.0	1.30
40	82.8	28	1.23	40	83.0	27.0	1.25
56	81.6	26	1.22	48	83.8	26.0	1.27
76	86.4	25	1.30	58	79.9	25.5	1.21
96	86.2	25	1.30	66	80.9	25.0	1.23
120	84.7	25	1.28	74	80.9	25.0	1.23
150	86.9	25	1.31	90	82.6	25.0	1.26
262	92.6	25	1.40	120	84.0	25.0	1.28
366	96.0	25	1.46	210	88.8	24.0	1.35
420	96.5	25	1.46	380	96.0	24.0	1.46
600	101.3	25	1.54				
720	103.0	25	1.57				

(a) Recorded for solution and gas from thermometer (T), Figure 1.
(b) Calculated from $n = Vp/RT$. p was corrected for the vapor pressure of pyridine. The vapor pressure of pyridine was calculated from the formula developed by van der Meulen and Mann (80): $\log p = 6.8827 - 1281.3/(t + 205)$, where p was expressed in millimeters of mercury and t in degrees Centigrade.

out the partial denitration in the reaction vessel (A) of the Lunge Nitrometer shown in Figure 2.

Dry, finely powdered methyl-β-D-glucoside tetranitrate, 2.01 g., was introduced into cup (C) attached to the gas reaction vessel (A). The reaction vessel and buret (B) were completely filled with mercury. Twenty ml. of an ice-cold, colorless 12.5% solution of hydroxylamine in pyridine was added to the cup in small portions. The nitrate quickly dissolved and was washed into (A) by momentarily opening the two-way stopcock (D); the reservoir (H) was lowered to produce a Toricellian vacuum in (A), care being taken to prevent the entrance of air into the reaction vessel. The introduction of the reactants to (A) required less than one minute, and no gas evolution occurred before the addition was complete. The pressure in (A) was maintained a few centimeters below that of the atmosphere by lowering (H). At intervals the colorless gas generated was transferred to the gas buret (B) and its volume was measured at the ambient temperature and pressure. The observed volumes when corrected and expressed as moles of gas per mole of tetranitrate were plotted against time (Figure 1, Curve B) as in the previous experiment.

Analysis of the gas evolved after twelve hours was carried out in a Standard Orsat apparatus fitted with

TABLE VII

Analysis of Gas Evolved from Methyl-β-D-glucoside Tetranitrate and Hydroxylamine - Pyridine

(Initial volume: 100.0 ml.)

Solution.	Residual Volume (ml.)
Potassium Hydroxide	100.0; 100.0; 100.0
Potassium Pyrogallate	100.0; 100.0; 100.0
Acid Cuprous Sulfate--Naphthol	95.6; 94.9; 91.5
	90.8; 90.3; 90.3
	90.0; 90.0.
Potassium Hydroxide (Repass)	91.4; 91.4; 91.4

Analysis of a Sample of Commercial Nitrogen Gas

(Initial volume: 98.9 ml.)

Potassium Hydroxide	98.9;	98.8;	98.9;	98.8
Potassium Pyrogallate	98.8;	98.7;	98.7;	
Acid Cuprous Sulfate--Napthol	98.2;	97.8;	97.2;	97.7;
	97.6;	97.7.		
Potassium Hydroxide (Repass)	98.0;	98.2;	98.6;	98.8;
	98.8.			

pipets containing potassium hydroxide, potassium pyrogallate and acid cuprous sulphate-β-naphthol reagents (81) for the

absorption of carbon dioxide, oxygen and carbon monoxide respectively. Clean, dry mercury was used as the confining liquid. An analysis of a sample of dry, commercial nitrogen gas was made in the same apparatus to serve as a blank. The residual volumes of gas at atmospheric pressure after each pass through the various solutions are recorded in Table VII.

The molecular weight of the gas remaining unabsorbed after being passed through the various solutions (Table VII) was determined in the apparatus described by Daniels, Mathews and Williams (82). The residual gas was transferred from the Orsat apparatus through a calcium chloride tube into a previously counterpoised and evacuated glass bulb of known volume. The pressure and temperature of the gas in the bulb were recorded and the bulb was then closed by means of a stopcock, wiped with a moist chamois cloth and allowed to stand at room temperature for ten minutes. The bulb containing the gas was then counterpoised with a second bulb of similar dimensions previously treated in the same manner, and the weight of the gas sample was determined by difference. The molecular weight was calculated from the equation:

$$M \quad \frac{g.T.R.}{p.V}$$

where M is the molecular weight, g the sample weight, T

the absolute temperature, R the universal gas constant, p the pressure and V the volume. A similar determination was carried out on the residual gas from the sample of commercial nitrogen. Table VIII summarizes the observed values.

TABLE VIII

Molecular Weight Determinations

(from partial denitration reaction)

Residual gas (after analysis)

Volume: 153.5 ml.

Pressure: 260.1 mm.

Temperature: 25.8°C; 298.8°K

Weight of Sample : 0.0618 g.

$$\text{M.W.} \quad \frac{(0.0618)(82.07)(298.8)(760.0)}{(260.1)(153.5)} \qquad 28.8$$

Residual gas from Commercial Nitrogen Sample (after analysis)

Volume: 153.5 ml.

Pressure: 327.0 mm.

Temperature: 26.5°C; 299.5°K

Weight of Sample: 0.0753 g.

$$\text{M.W.} \quad \frac{(0.0753)(82.07)(299.5)(760)}{(327.0)(153.5)} \qquad 28.02$$

The Rate of Gas Evolution from Hydroxylamine in Pyridine

Measurements of the rate of gas evolution from 10 ml. of the pyridine - hydroxylamine solution at room temperature were carried out in duplicate in the Lunge Nitrometer, Figure 2, exactly as described above. The volumes observed after various times are recorded in Table IX. The total volume of gas after twelve hours (2.5 to 3.0 ml.) amounted tl less than 3% of that evolved in the partial denitration reaction (Table VI) and hence was considered to be negligible.

Isolation of the Products from the Partial Denitration Reaction

Partially Denitrated Methyl-β-D-glucoside Tetranitrate (Sirup 1)

The mixture studied was prepared by treating 5 g. samples of methyl-β-D-glucoside tetranitrate for two hours at room temperature with 50 ml. volumes of a 12.5% solution of free hydroxylamine in pyridine. When large quantities of Sirup 1 were required in subsequent operations, the product was obtained by repetition of the following procedure.

The dry, crystalline tetranitrate was weighed into a 125 ml. Erlenmeyer flask fitted with a two-hole rubber stopper carrying a thermometer and calcium chloride drying tube. The ice-cold hydroxylamine - pyridine reagent was added and the flask was stoppered and swirled until the

TABLE IX

Gas Evolution from Hydroxylamine - Pyridine Reagent at 25°

Time (min.)	Volume (ml.)		Moles x 10^4(a)	
	Run 1	Run 2	Run 1	Run 2
60	....	0.07	....	0.03
90	0.07	0.19	0.03	0.07
180	0.43	....	0.17	....
300	1.51	....	0.59	....
420	1.99	....	0.77	....
510	....	1.99	....	0.78
630	2.95	2.35	1.15	0.92
720	....	2.47	....	0.96
735	3.43	....	1.34	....

(a) Corrected for the vapor pressure of pyridine: See Table VI, footnote (b)

nitrate dissolved. Care was taken to prevent contact of the pyridine solution with the rubber stopper. Gas evolution commenced almost immediately and after ten minutes a white precipitate formed; this precipitate redissolved completely in the next thirty to forty minutes. The temperature of the reaction mixture was maintained at 25 to 35 ° throughout

by external cooling. After two hours the clear, pale yellow solution, still evolving gas, was poured into 500 ml. of water. The turbid aqueous mixture was rendered just acid to Congo Red by the addition of 4 N sulfuric acid (163 ml.) and was then extracted with one 200 ml., and four 100 ml. portions of ether. The ether extracts were combined and washed with three 100 ml. portions of water, were dried over anhydrous sodium sulfate and evaporated to give, on the average, 3.50 g. of a golden-yellow, extremely viscous sirup (Sirup 1).

Anal: Calcd. for methylglucoside trinitrate, $C_7H_{11}O_{12}N_3$:

OCH_3, 9.42%; N, 12.8%.

Found: OCH_3, 9.11, 9.19%; N, 11.7, 11.9%.

The aqueous solution, after the above manual extraction with ether, was combined with the aqueous washings in a 1-liter liquid - liquid extractor and the faintly acid mixture was continuously extracted with ether for seven days until fresh ether extracts gave a negative nitrate test with diphenylamine reagent. Evaporation of the dried ether extracts yielded 0.17 g. of a brown, evil-smelling liquid which decomposed to a black tar on standing.

Crystalline Derivatives from Sirup 1 (Series I)

The following series of reactions (Series I) is summarized in Figure 3; the analyses and properties of the products isolated at each stage are recorded in Table X. The sirupy products reported could not be crystallized from organic solvents or by prolonged drying.

Sirup 1, 3.00 g., was dissolved in 75 ml. of methyl iodide and the solution was heated under reflux for eight hours on the steam bath with 10 g. of dry silver oxide and 4 g. of powdered Drierite (anhydrous calcium sulfate). The cooled methylation mixture was filtered and the solids were washed with boiling chloroform. Evaporation of the combined filtrate and washings yielded 3.38 g. of a mobile, yellow sirup (Sirup 2). Remethylation of a portion of Sirup 2 under the same conditions did not alter the methoxyl content of the product.

Sirup 2, 3.38 g., was hydrogenated in alcohol solution, using 2.0 g. of palladized charcoal catalyst, as previously described for the methylglucoside tetranitrates. The pressure became constant in twenty minutes and 0.016 moles of hydrogen was absorbed per gram. After removal of the catalyst by filtration, the nitrate-free alcohol solution was evaporated to leave a colorless glass (Sirup 3);

yield, 2.14 g.

Sirup 3, 7.15 g., from four repetitions of the preceding steps, was dissolved in 196 ml. of anhydrous pyridine, 19.6 ml. of acetic anhydride was added and the clear solution was allowed to stand overnight at room temperature. Sixty ml. of water was then added to the pale yellow solution and the aqueous pyridine mixture was worked up as described by Levene and Raymond (55) to yield 8.00 g. of a clear, colorless sirup (Sirup 4). A solution of this sirup in 55 ml. of anhydrous ether was cooled to 0 and treated with n-pentane (ca. 5 ml.) until just turbid. After standing one hour at 0°, 2.078 g. of clusters of long needles that had separated were recovered on a sintered-glass filter. The filtrate was again cooled and treated with n-pentane and a second crop, (Crop 2, Figure 7), was similarly recovered after a longer period of standing at 0°. Successive treatments in this manner yielded a total of seven crops, the last crop separating over a period of three to four weeks. Attempts to recover further crops of crystals resulted only in precipitation of a sirup and eventually the ether - n-pentane solution was evaporated to give 3.861 g. of a tough, viscous sirup, (Sirup 5, Figure 7).

Crop 1 was recrystallized from anhydrous ether to give Fraction 1 (1.317 g.) melting sharply at 107 - 108°. The

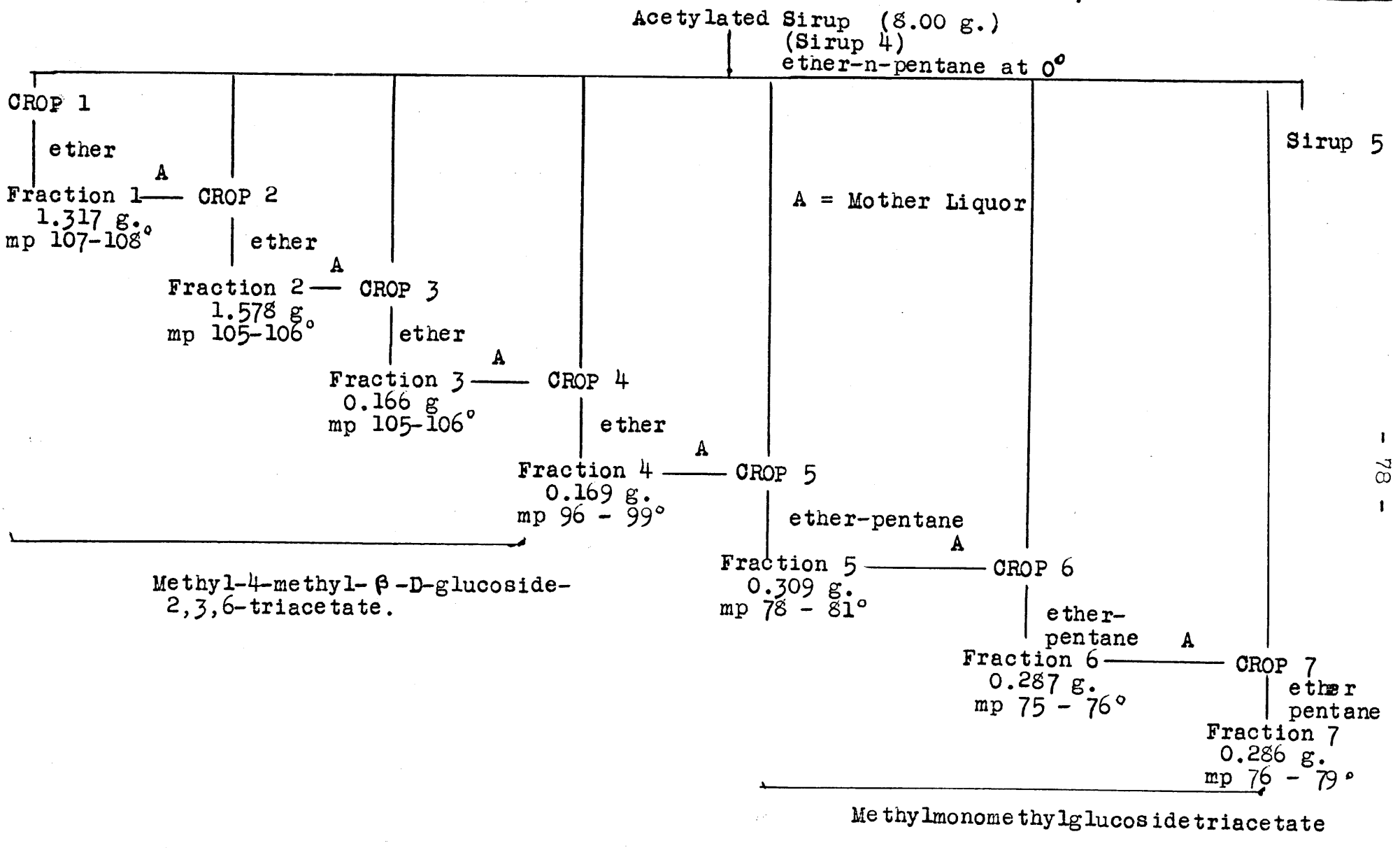

Fractional Crystallization of Acetylated Methyl Ethers of Methyl-β-glucoside (Series I)
Acetylated Sirup (8.00 g.)
(Sirup 4)
ether-n-pentane at 0°
CROP 1
ether
A
Fraction 1 — CROP 2
1.317 g.
mp 107-108°
ether
A
Fraction 2 — CROP 3
1.578 g.
mp 105-106°
ether
A
Fraction 3 — CROP 4
0.166 g
mp 105-106°
ether
A
Fraction 4 — CROP 5
0.169 g.
mp 96 - 99°
Methyl-4-methyl-β-D-glucoside-
2,3,6-triacetate.
A = Mother Liquor
Sirup 5
ether-pentane
A
Fraction 5 — CROP 6
0.309 g.
mp 78 - 81°
ether-
pentane
A
Fraction 6 — CROP 7
0.287 g.
mp 75 - 76°
ether
pentane
Fraction 7
0.286 g.
mp 76 - 79°
Methylmonomethylglucosidetriacetate
78

Figure 7

mother liquor from Fraction 1 was combined with Crop 2 and Fraction 2 was recrystallized after concentration, cooling and seeding. Repetition of this procedure yielded a total of seven crystalline fractions. Crops 5 to 7 were each recrystallized from ether - n-pentane, since the fractions succeeding Fraction 4 were found to be too readily soluble in ether alone. Figure 7 includes the yields and melting points of the fractions obtained.

Fractions 1 to 4 were combined and recrystallized from ether to a constant melting point. The pure product, 2.109 g., formed long colorless needles and its physical constants and analysis (Table X) indicated it was methyl-4-methyl-β-D-glucoside-2,3,6,-triacetate (55)(22). The proof of identity of this compound is given in a later section. Fractions 5 to 7 were combined and recrystallized to constant melting point from alcohol - n-pentane, and the pure product gave the analyses (Table X) required for a methylmonomethylglucoside triacetate. However the physical constants did not agree with any of those reported for the isomeric methylmonomethyl-β-D-glucoside triacetates (Table III). Further investigation of this product is reported in a later section.

Sirup 5 (Figure 7) 3.725 g., was deacetylated with methanolic barium methylate at 0 as described by Levene and Tipson (63). The sirupy product (Sirup 6), 2.949 g., was

dissolved in 40 ml. of water and the solution was extracted three times with 10 ml. volumes of chloroform. The attempted partition proved to be unsuccessful, since the uncrystallized products recovered from the two solvents by evaporation (Sirup 6, A and B, Table X) were nearly identical in methoxyl content, index of refraction and specific rotation. A sample of the recombined fractions, 0.459 g., was methylated with methyl iodide and silver oxide as previously described. The sirupy product (Sirup 7), 0.503 g., did not change in methoxyl content on remethylation. An 0.332 g. sample distilled at 125 - 130°/0.5 mm. but the non-reducing, colorless distillate (Sirup 8), 0.250 g., did not crystallize.

A second sample of Sirup 6, 0.551 g., was dissolved in 15.00 ml. of 0.5 N sodium hydroxide and the clear solution was allowed to stand for twenty hours at room temperature. At the end of that time, a volume of 0.5 N sulfuric acid exactly equivalent to the alkali added, as determined by a previous titration with phenolphthalein, was run into the solution from a buret and the aqueous solution was then evaporated to dryness. The crystalline residue was extracted with boiling, anhydrous acetone and evaporation of the extracts left a colorless, crystalline product, 0.551 g., melting at 99 - 113°. Recrystallization from alcohol - petroleum ether (b. p. 60 - 70°) yielded 0.283 g. of methyl-2,4-dimethyl-β-D-glucoside. The proof of identity of this compound is

TABLE X

Analyses and Properties of Products Isolated in Series I

Product	% OCH_3	Optical Rotation $[\alpha]_D^{20}$	Solvent		m. p.
Sirup 2[a]	21.8 21.5				
Sirup 3[a]	32.5 32.7				
Sirup 4[b]					
Sirup 5[c]	26.8 26.2				
Sirup 6 (A and B)	32.9 32.7	-19.7	H_2O	1.4668	
Sirup 7	47.4 47.5	-18.4	H_2O	1.4482	
Sirup 8	50.1 49.6	-20.4	H_2O	1.4473	b.p. 125 - 130° at 0.5 mm.
Methyl-4-methyl-β-D-glucoside triacetate	18.5 18.7	-34.8	$CHCl_3$		107.5 - 108.5°
Methyl-monomethyl-glucoside triacetate[d]	18.7 18.5	-34.5	$CHCl_3$		76.0 - 77.5°
Methyl-2,4-dimethyl-β-D-glucoside	40.0 41.0	-17.7	Acetone		123.5 - 124.0°

(a) Nitrogen, 10.7, 10.7%. Diphenylamine test positive.

(b) Diphenylamine test negative.

(c) Acetyl, 29.7, 29.5%

(d) Acetyl, 36.7, 37.7%; Nitrogen, 0.0, 0.0%

reported in a later section. From the mother liquor, 0.221 g. of an ether-soluble sirup (Sirup 9) was recovered.

Crystalline Derivatives from Sirup 1 (Series II).

A sample of Sirup 1 partially crystallized after standing for two months in a desiccator over phosphorous pentoxide. The residual sirup dissolved readily in cold chloroform and the residual crystalline material was recrystallized to constant melting point from chloroform. The large, colorless prisms melted at 134 - 135° and had a specific rotation $[\alpha]_D^{20}$ -7.2° (acetone, c = 1.52, l = 1), were reducing and were identified as methyl-β-D-glucoside-3,6-dinitrate (see p. 93).

Anal: Calcd. for methylglucoside dinitrate, $C_7H_{12}O_{10}N_2$:

OCH_3, 10.9%; N, 9.86%

Found: OCH_3, 10.9, 10.3%; N, 9.85, 9.84%.

The crystalline dinitrate was found to be soluble in water and a more complete separation from Sirup 1 was achieved by the following procedure; the operations described as summarized in Figure 4.

Sirup 1, 26.48 g., was dissolved in 500 ml. of chloroform and the chloroform solution was extracted four times with 200 ml. volumes of water. The chloroform solution was then dried over anhydrous sodium sulfate and evaporated. The

sirupy residue (Sirup 10) when thoroughly dry weighed 17.66 g. The aqueous extracts were combined in a liquid - liquid extractor and were continuously extracted with ether for twenty four hours. At the end of that time fresh ether extracts gave no color with diphenylamine reagent. The combined ether extracts were then dried and evaporated to leave 8.56 g. of a semi-crystalline residue. Recrystallization from chloroform gave 3.14 g. of methyl-β-D-glucoside-3,6-dinitrate. The chloroform mother liquor, when evaporated, left 5.23 g. of yellow sirup (Sirup 11).

Sirup 10, 17.19 g., was methylated with methyl iodide and silver oxide and the dark red sirupy product was purified by dissolving it in ether and washing the ether solution with water. The aqueous extracts retained the colored material, a portion of which was isolated by evaporation of the solvent and then appeared as a black tar with a very pungent odor. The ether solution after extraction was dried and evaporated to leave 14.73 g. of a straw-colored sirup (Sirup 12). This material had a methoxyl content approximating to that of methylmonomethylglucoside trinitrate:

Anal: Calcd: OCH_3, 18.08%

Found: OCH_3, 17.1, 17.5%

Sirup 12, 14.15 g., on hydrogenation absorbed 0.0195 moles of hydrogen per gram; the colorless, viscous product weighed

8.27 g. (Sirup 13). This sirup was dissolved in alcohol and ether was added just to turbidity; after standing for one hour at room temperature, colorless crystals of methyl-4-methyl-β-D-glucoside formed in the solution. The crystalline glucoside was recovered by filtration (total yield in three crops, 3.93 g.) and was identified by optical rotation, melting point and mixed melting point with the sample obtained by deacetylation of the corresponding triacetate obtained in Series I. The details of the identification of this compound are given in a later section.

From the alcohol - ether mother liquor, 4.34 g. of a colorless glass (Sirup 14) was obtained on evaporation. A portion of Sirup 14, 0.520 g., was distilled at 130 - 140°/0.13 mm. to give 0.318 g. of a pale yellow uncrystallized distillate (Sirup 14A). A second sample of Sirup 14, 2.19 g., was acetylated as described in Series I to give 3.28 g. of Sirup 15 of which 2.05 g. was crystallized from ether - n-pentane. The crystalline product agreed in rotation, melting point and mixed melting point with the unidentified methylmonomethyl-glucoside triacetate isolated in Series I. The remainder of the acetylated product was recovered from the mother liquor as colorless sirup.

Crystalline Derivatives from Sirup 1 (Series III)

The procedures followed in this series are summarized in

Figure 5.

Sirup 1, 28.0 g., was dissolved in chloroform and methyl-β-D-glucoside-3,6-dinitrate was crystallized in three crops by successive concentration, cooling and seeding. The total yield of the dinitrate obtained at this stage amounted to 1.45 g. The chloroform mother liquor was evaporated and 26.5 g. of Sirup 17 was recovered. A sample of this Sirup 17 was hydrogenated to give crystalline methyl-β-D-glucoside in 96.5% yield calculated from the nitrogen analysis. The remainder of Sirup 17, 23.16 g., was acetylated with an excess of an equimolar mixture of acetic anhydride and pyridine and Sirup 18 was recovered by the usual procedure.

Sirup 18 was dissolved in ether, the solution was cooled and n-pentane was added to turbidity. A total of 13.28 g. of mixed, crystalline glucoside nitrate - acetates (Product 19) separated in three crops; evaporation of the mother liquor left 9.92 g. of Sirup 20. Fractional crystallization of Product 19 was not successful since the melting points of the fractions varied from 80 to 90° and the nitrogen content of the first fraction: N, 10.0, 10.0, 9.97% was intermediate between the theoretical values for methylglucoside diacetate dinitrate (Calcd.: N, 7.60%) and methylglucoside monoacetate trinitrate (Calcd.: N, 11.3%). The crystalline fractions were recombined to recover 12.71 g. of Product 19.

Product 19 was dissolved in anhydrous chloroform, 125 ml., and the solution was treated at 20 with 125 ml. of absolute methanol containing 0.163 g. of sodium. The deacetylation was followed polarimetrically and when the rotation became constant after six hours, 0.400 ml. of glacial acetic acid was added and the yellow solution was evaporated to dryness. The residue was extracted with four 20 - ml. volumes of chloroform, and the combined chloroform extracts were extracted, in turn, ten times with 20 - ml. portions of water. Evaporation of the combined aqueous extracts yielded 2.27 g. of Sirup 21, which, when dissolved in chloroform, cooled and seeded, deposited 0.236 g. of crystalline methyl-β-D-glucoside-3,6-dinitrate. The chloroform mother liquor was combined with the washed and dried chloroform extracts and Sirup 22, 10.07 g., was obtained on evaporation.

Sirup 22 was methylated twice with methyl iodide and silver oxide and the product was hydrogenated and acetylated as described in Series I. Fractional crystallization of the nitrate-free, acetylated product, Sirup 25, yielded 1.96 g. of methyl-4-methyl-β-D-glucoside triacetate, and 4.30 g. of Sirup 26 was recovered from the ether - pentane mother liquor. Four grams of Sirup 26 was treated with 0.5 N sodium hydroxide solution and Sirup 27 was worked up as described in Series I but could not be induced to crystallize.

Sirup 20 was successively deacetylated (sodium methylate), methylated, hydrogenated and acetylated by the procedures previously described. Fractional crystallization of the acetylated product, Sirup 31, yielded 0.438 g. of methyl-4-methyl-β-D-glucoside triacetate and 5.53 g. of Sirup 32 was recovered from the ether - pentane mother liquor.

Identification of the Crystalline Derivatives from the Partial Denitration Reaction

Methyl-4-methyl-β-D-glucopyranoside

Crystalline methyl-4-methyl-β-D-glucoside-2,3,6-triacetate, 2.11 g., dissolved in 50 ml. of anhydrous methanol, was mixed at 0° with 2.7 ml. of 0.2 N barium methylate in anhydrous methanol (63). After being allowed to stand overnight at 0°, the cold solution was saturated with gaseous carbon dioxide, was diluted with water, and was then heated under reflux on the steam bath for five minutes. The hot solution was filtered with suction and the precipitate of barium carbonate was washed with boiling methanol. The combined filtrate and washings were evaporated to dryness and the pale yellow residue crystallized in long silk-like needles, 1.34 g., after drying in a vacuum desiccator over phosphoric anhydride for forty-eight hours. After repeated recrystallization from ethyl acetate, the methyl-4-methyl-β-D-glucoside melted

at 101.0 - 101.5°, and had the specific rotations $[\alpha]_D^{20}$ -21.0° (water, c = 2.00, l = 2) and $[\alpha]_D^{20}$ -17.4° (methanol, c = 3.44, l = 2). Further recrystallization did not alter these constants.

Anal: Calcd. for methylmonomethylglucoside, $C_8H_{16}O_6$:

OCH_3, 29.8%

Found: OCH_3, 29.4, 29.3%.

The rotations observed when a sample was oxidized with 0.15 M sodium metaperiodate are listed in Table XI, together with the molecular rotations calculated from the formula;

$[M]_D^{20} = [\alpha]_D^{20} \times$ molecular weight.

No formic acid or formaldehyde was formed and 0.99 moles of periodate was consumed per mole of glucoside. A 1,2 glycol group, not involving a primary hydroxyl unit was therefore present.

For comparative purposes methyl-β-D-glucoside was oxidized by the same procedure. A consumption of 1.98 moles of periodate per mole of glucoside was found in accourdance with previous results (77). Formic acid was produced but no formaldehyde.

The changes in molecular rotation with time are plotted in Figure 8. The constant value for the 4-methyl compound

was $[M]_D^{20} - 26.2 \times 10^3$; that for methyl-β-D-glucoside, $[M]_D^{20} - 23.7 \times 10^3$ was in close agreement with the result, $[M]_D^{20} - 23.4 \times 10^3$ reported by Jackson and Hudson (77).

4-methyl-D-glucose

Methyl-4-methyl-α-D-glucoside, 1,105 g., was hydrolysed with 25.0 ml. of 1 N sulfuric acid on the steam bath and the reaction was followed polarimetrically. The specific rotation changed from $[\alpha]_D^{20} +13.5^\circ$ (two hours) to the constant value, $[\alpha]_D^{20} +57.0^\circ$ (eleven hours). The acid in the hydrolysate was removed as barium sulfate and the clear filtrate was evaporated under reduced pressure. The sirupy residue was repeatedly extracted with boiling ethanol and the combined ethanol extracts, totaling 45 ml., were cooled and treated with ether. A precipitate of a small amount of inorganic material was removed by filtration and evaporation of the ethanol - ether filtrated yielded 1.043 g. of a colorless glass which readily reduced hot Fehling's solution and exhibited mutarotation (Table X).

Anal: Calcd. for monomethylglucose, $C_7H_{14}O_6$: OCH_3, 15.98%
Found: OCH_3, 15.8, 16.0%.

4-methyl-D-glucose Phenylosazone

A solution of 1 0.160 g of 4-methylglucose in 2.5 ml. of water was heated on the steam bath for one hour with 0.35 ml. of phenylhydrazine and 0.3 ml. of glacial acetic acid.

TABLE XI

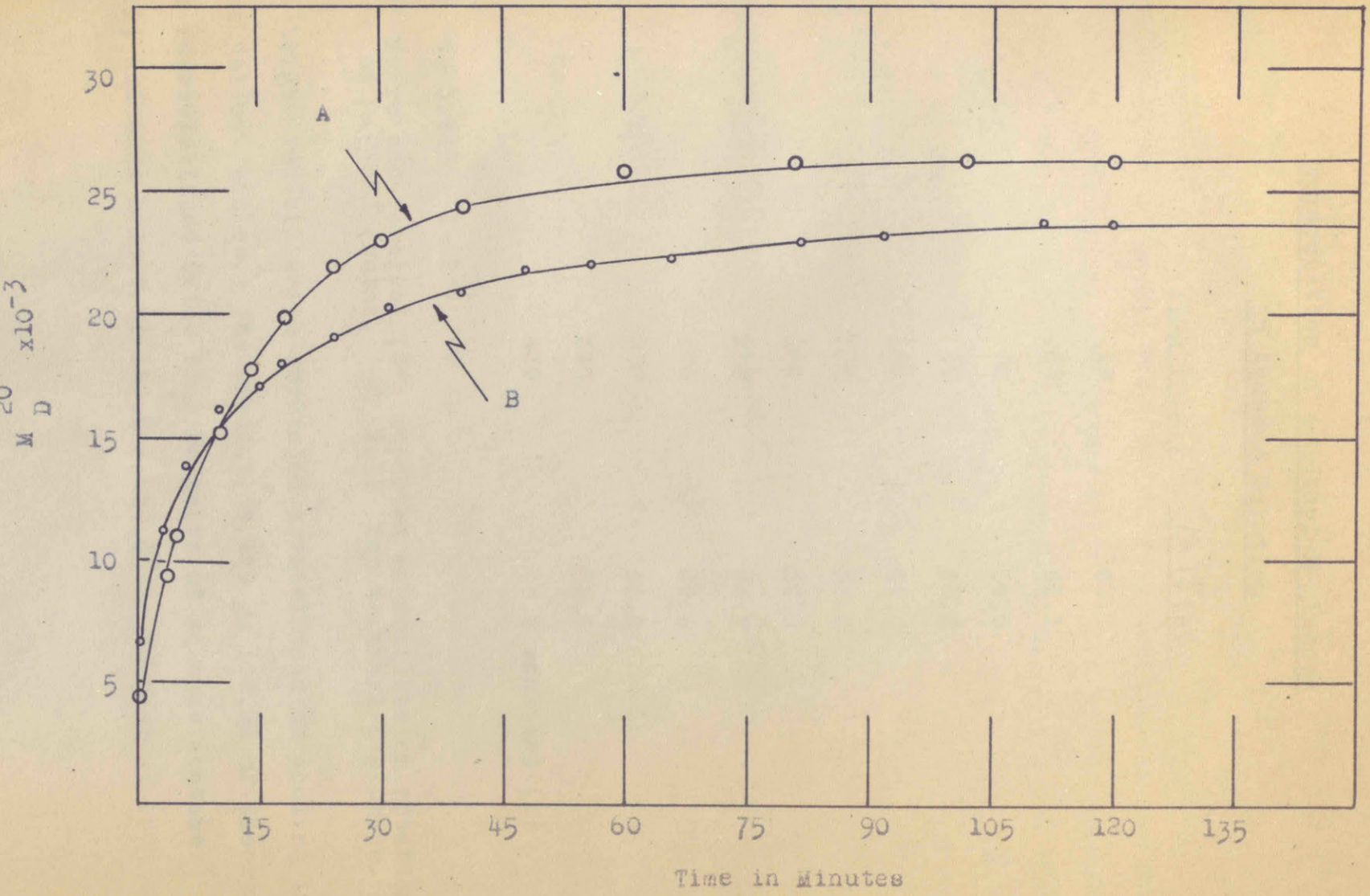
Change in Rotation During the Oxidation of Methyl-4-Methyl-β-D-glucoside with Aqueous Periodate [a]

Time (min.)	α_D^{20} (b)	$[M]_D^{20} \times 10^{-3}$
0		-4.4[c]
4	-0.72	-9.4
5	-0.84	-10.9
10	-1.17	-15.2
14	-1.36	-17.7
18	-1.53	-19.9
24	-1.69	-21.8
30	-1.78	-23.0
40	-1.87	-24.4
60	-1.98	-25.8
80	-2.01	-26.2
100	-2.01	-26.2
120	-2.01	-26.2

(a) All operations were carried out in a room at 20°; 0.200 g. of the glucoside was treated with 15.00 ml. of 0.1458 M sodium metaperiodate and the solution was made up to 25 ml. in a volumetric flask.

(b) $c = 0.800$, $l = 2$

(c) Calculated from the observed specific rotation in water: $[\alpha]_D^{20}$ -21.0° ($c = 2.00$, $l = 2$).



Oxidation of Methyl-β-D-glucoside (A) and Metnyl-4-metnyl-β-D-glucoside (B) with 0.15 M Sodium Metaperiodate at 20°

Figure 8

TABLE XII

Mutarotation of 4-methyl-D-glucose in Aqueous Solution

Time (min.)	$[\alpha]_D^{20}$ (a)
19	62.1
28	61.1
60	59.5
90	58.2
120	57.7
150	57.2
180	56.9
210	56.7
278	56.5
330	56.2
390	56.2
439	56.2 constant (b)

(a) $c = 3.846$, $l = 2$

(b) Munro and Percival (56) reported an equilibrium rotation of $[\alpha]_D^{20}$ 53° (water, $c = 2.1$) for 4-methyl-D-glucose.

The bright red oil which separated crystallized on cooling in fine yellow yeedles. The product, 0.179 g. (58.5% of theory), was recrystallized twice from 30 parts of aqueous acetone (1:1) and then melted at 157.5 - 159.0°. Further

recrystallization did not alter the melting point. The melting point of 4-methylglucose phenylosazone has been reported as 158° (56) and 157 - 158° (11).

Methyl-2,4-dimethyl-β-D-glucoside

Two grams of methyl-β-D-glucoside-3,6-dinitrate was twice methylated with methyl iodide and silver oxide by the procedure previously described. The sirupy product, 2.154 g., had n_D^{20} 1.4623 and $[\alpha]_D^{20}$ -7.9° ($CHCl_3$, c = 5.36, l = 2). The constants reported by Dewar and Fort (11) for methyl-2,4-dimethyl-β-D-glucoside-3,6-dinitrate were n_D^{20} 1.4645; $[\alpha]_D^{20}$ -7.1° ($CHCl_3$, c = 4.2, l = 2).

Anal: Calcd. for methyldimethylglucoside dinitrate, $C_9H_{16}O_{10}N_2$:

OCH_3, 29.8%; N, 8.97%.

Found: OCH_3, 29.2, 28.9%; N, 8.68, 8.68%.

The methylated dinitrate, 1.34 g., was dissolved in 25 ml. of ethanol and hydrogenated in the presence of 1.5 g. of palladized charcoal by the usual procedure. The pressure became constant in thirty minutes and 3.4 moles of hydrogen was absorbed per mole of dinitrate. The nitrate-free ethanol solution was filtered and evaporated and the residue, 0.969 g., crystallized in fine needles on cooling. After recrystallization from carbon tetrachloride the product weighed 0.887 g. (93% of theory), melted at 123 - 124° and rotated $[\alpha]_D^{20}$ -17.7° (acetone,

c= 3.30, l =2); $[\alpha]_D^{26}$ -26.5° (water, c= 3.26 l= 2). The constants reported for methyl-2,4-dimethyl-β-D-glucoside were, m. p. 124° $[\alpha]_D^{16}$ -16.6° (acetone, c= 4.16, l= 2) by Dewar and Fort (11); m. p. 122 - 124°, $[\alpha]_D^{29}$ -18.6° (acetone, c= 1.4) by Adams, Reeves and Goebel (61). A second melting point of 107 - 108° for the freshly remelted product was found as reported by Adams, Reeves and Goebel.

Anal: Calcd. for methyldimethylglucoside, $C_9H_{18}O_6$:

OCH_3, 41.9%.

Found: OCH_3, 40.0, 41.0%.

No change in rotation or pH occurred when the glucoside was treated with aqueous sodium metaperiodate at 20° for four hours. No periodate was consumed and no formaldehyde could be detected in the final solution. No unsubstituted 1,2-glycol group was present.

A sample of methyl-2,4-dimethyl-β-D-glucoside-3,6-dinitrate, 0.530 g., was denitrated with glacial acetic acid and a mixture of zinc and iron powder as described by Dewar and Fort (11). The crystalline dimethyl compound recovered, 0.166 g. (44% of theory), melted at 122 - 124° after recrystallization from carbon tetrachloride or from alcohol - petroleum ether (b. p. 60 - 80°). A mixed melting point with the product prepared by hydrogenolysis of the dinitrate showed no depression.

2,4-Dimethyl-D-glucose

Methyl-2,4-dimethyl-β-D-glucoside, 0.808 g., was

hydrolysed with 1 N sulfuric acid as described for the 4-methyl compound. The sirupy product, 0.768 g, readily reduced Fehling's solution and exhibited mutarotation in aqueous solution (c = 2.88, l = 2); $[\alpha]_D^{20}$ +59.2° (eleven minutes) changing to $[\alpha]_D^{20}$ +62.2° in two hours and remaining constant for a further twenty-four hours.

Anal: Calcd. for dimethylglucose, $C_8H_{16}O_6$: 29.8%.
Found: OCH_3, 29.3, 29.0%.

The dimethylglucose, 0.721 g. was dissolved in 10 ml. of water and 2.0 g. of phenylhydrazine hydrochloride, 3.0 g. of sodium acetate and 3 drops of a saturated sodium bisulfite solution were added. The solution was heated on the steam bath for two and one half hours. A red sirup settled out during the heating but did not crystallize when seeded or after standing at 0° for a week. The colored product was extracted with ether and the ether solution was washed with 4 N acetic acid solution. When cooled and treated with n-pentane, 4-methylglucose phenylosazone crystallized from the ether solution in fine yellow needles; yield, 0.050 g. After three recrystallizations from aqueous acetone the osazone melted at 157.0 - 158.0°. A mixed melting point with 4-methyl glucosazone prepared from 4-methyl-D-glucose was not depressed. The preparation of 4-methylglucose phenylosazone from 2,4-dimethylglucose has been previously reported (11)(61)

Experiments on the Unidentified Methylmonomethyl-glucoside Triacetate

(a) Deacetylation of the Crystalline Triacetate with Sodium Methylate

A 1.64 g. sample of the crystalline triacetate, m. p. 76 - 78°, and $[\alpha]_D^{20}$-34.6° in chloroform (c = 16.4, l = 1), was deacetylated with sodium methylate in chloroform - methanol solution by the procedure described in Series III. The rotation changed from α_D^{20}-3.36° (four minutes) to a constant value of α_D^{20}-1.96° in ninety-six minutes. The sirupy product, 1.025 g. (Sirup 33) could not be crystallized, and rotated $[\alpha]_D^{20}$-22.1° (MeOH, C = 4.00, l = 2) and $[\alpha]_D^{20}$-22.9° (water, c = 4.00, l = 2).

Anal: Calcd. for methylmonomethylglucoside, $C_8H_{16}O_6$:

OCH_3, 29.8%.

Found: OCH_3, 28.3, 28.4%.

The observed rotation of 0.194 g. of Sirup 33 in 25 ml. of sodium metaperiodate solution changed from α_D^{20}-0.54° (three minutes) to a constant value of α_D^{20}-1.14° (sixty minutes). The starting material (0.0010 moles as methyl-monomethyl glucoside) consumed 0.000556 moles of periodate with liberation of 0.000012 moles of formic acid and no formaldehyde could be detected.

In a second oxidation, 0.349 g. of Sirup 33 was treated with 25 ml. of 0.14 M sodium metaperiodate solution and when the rotation became constant, the solution was neutralized to phenolphthalein with 0.02 N barium hydroxide solution. A flocculent, colorless precipitate was removed by filtration and the clear filtrate was concentrated to ca 25 ml. under reduced pressure in the presence of solid barium carbonate. At this stage the aqueous concentrate was filtered and the evaporation was continued to dryness. The dried, semi-crystalline residue was extracted with absolute alcohol and evaporation of the filtered extracts left 0.348 g. of a crystalline product. This material was in turn extracted with boiling chloroform (50 ml. in several portions) and the undissolved crystalline product when dried weighed 0.131 g. and did not reduce hot Fehling's solution; it rotated $[\alpha]_D^{20}$ -15° (water, c = 1.33, l = 1) and was deliquescent, rapidly changing to a yellow sirup on exposure to the atmosphere.

The dried chloroform extracts when evaporated, left 0.162 g. of a yellow sirup (Sirup 34) which reduced Fehling's solution but did not color the Fuchsin - aldehyde reagent.

(b) Deacetylation of Sirup 33 with Sodium Hydroxide

Sirup 33, 0.198 g., was treated with sodium hydroxide solution by the procedure described in Series I. The product, Sirup 35, was recovered and could not be induced to crystallize

from organic solvents as by prolonged drying. This material was benzoylated by the procedure described by Oldham (20) and yielded Sirup 36. Re-benzoylation of Sirup 36 did not alter its unsatisfactory physical character; yield, 0.362 g.

(c) The Action of Titanium Tetrachloride on the Methyl-monomethylglucoside Triacetate

The crystalline triacetate, 0.217 g., was dissolved in 10 ml. of absolute chloroform and 0.060 ml. of titanium tetrachloride dissolved in 1.0 ml. of absolute chloroform was added (59). The mixture was heated under reflux for seventy-five minutes on a steambath with protection against moist air. On the first addition of the titanium tetrachloride, a colorless precipitate had formed which rapidly dissolved on heating to give a bright yellow solution, and this, in turn, slowly darkened to an orange color. The cooled solution was poured into 100 ml. of ice-water in a separatory funnel and the mixture became colorless when shaken. The chloroform solution was separated and washed with 10% potassium bicarbonate solution and dried over calcium chloride. Evaporation of the chloroform gave Sirup 37, which could not be induced to crystallize.

SUMMARY

The methylglucoside tetranitrates, although relatively stable to hydroxylamine in alcohol or to pyridine alone, reacted rapidly and vigorously with an anhydrous pyridine solution of hydroxylamine, as shown by an increase in temperature and by the evolution of about 1.26 moles of nitrogen gas per mole of tetranitrate. Analysis of the sirupy carbohydrate products indicated that about 1.25 nitrate groups were removed from each molecule of tetranitrate within the first ten or twenty minutes, but little further change occurred during the next twelve hours. The recovery of the crystalline methylglucosides in high yields after complete denitration of the sirupy products by hydrogenolysis, indicated that removal of nitrate groups in the initial partial denitration regenerated free hydroxyl groups without Walden inversion or other changes that would have altered the basic methylglucoside structure.

The sirupy mixture of reaction products from methyl-β-D-glucoside tetranitrate was separated by three different methods; (a) methylation - denitration - acetylation and fractional crystallization, (b) fractional crystallization - methylation and denitration, (c) fraction crystallization - acetylation - fractional crystallization - deacetylation - methylation - denitration and reacetylation. The final result was that the

crude product was shown to consist of 33% methyl-β-D-glucoside-3,6-dinitrate, 53% methyl-β-D-glucoside-2,3,6-trinitrate and 14% of unidentified methylglucoside trinitrate. The structures of the two former compounds were confirmed by preparing known crystalline derivatives and by oxidizing their crystalline methylated and completely denitrated products with sodium periodate. In the course of this work methyl-4-methyl-β-D-glucoside was obtained in crystalline form for the first time.

The unidentified methylglucoside trinitrate was isolated as a crystalline methylmonomethylglucoside triacetate; several unsuccessful attempts to identify the latter compound were described.

Throughout the isolation experiments, no trace of unreacted methyl-β-D-glucoside tetranitrate or of methylglucoside mononitrates could be detected in the products.

CLAIMS TO ORIGINAL RESEARCH

1. Methyl-α-and -β-glucoside tetranitrate was proved to be partially denitrated by the action of pyridine containing a large excess of hydroxylamine. The product was practically stable in the excess reagent and consisted entirely, or almost entirely of the methylglucoside nitrated to an average level of 2.67 nitrate groups per mole.

2. The course of the rapid exothermic reaction was followed by noting the rate at which gas was evolved. This gas was shown to be at least 90% nitrogen, and about 1.26 moles were evolved per mole of methylglucoside tetranitrate.

3. The partial denitration of methyl-β-D-glucoside tetranitrate yielded a product whose components were shown to be: methyl-β-D-glucoside-3,6-dinitrate (33%), methyl-β-D-glucoside-2,3,6-trinitrate (53%) and a methylglucoside trinitrate or mixture of trinitrates (14%) of undetermined structure. At least 86% of the denitration therefore affected secondary nitrate groups, as opposed to that occupying the primary or sixth position in the methylglucoside.

4. The structures of methyl-β-D-glucoside-3,6-dinitrate and

methyl-β-D-glucoside-2,3,6-trinitrate were established by oxidizing their methylated and denitrated, crystalline derivatives with periodate, and by the preparation of known, crystalline compounds from the resulting partly methylated methylglucosides.

5. Methyl-4-methyl-β-D-glucoside was prepared for the first time in pure crystalline form and its physical constants were recorded. The new synthesis also rendered this compound easily accessible for the first time.

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