# THE ACTION OF HYDROXYLAMINE HYDROCHLORIDE IN PYRIDINE ON METHYL-β-D-GLUCOSIDE TETRANITRATE

A Thesis

by

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# GENERAL INTRODUCTION

In a Ph.D. thesis submitted in 1946 G.H. Segall described a reaction between hydroxylamine hydrochloride in pyridine and cellulose trinitrate in which a stable cellulose "mono-oxime dinitrate" was formed. A gas, predominantly nitrous oxide but containing some nitrogen, was evolved. Segall also found that cellulose trinitrate reacted with free hydroxylamine in pyridine to yield one mole of nitrogen and a cellulose "dinitrate". His attempts to discover whether the "labile" nitrate group occupied the second, third or sixth positions in the glucose units of the cellulose trinitrate led to indecisive results. In these circumstances Hayward reverted to a simpler, crystalline compound, methyl-β-D-glucopyranoside tetranitrate, for his study of the denitrating action of the free base hydroxylamine in pyridine. Hayward in his Ph.D. thesis (1949) reported that 1.26 moles of nitrogen gas was evolved from each mole of the methyl-β-D-glucoside tetranitrate and a syrupy mixture of methylglucoside di- and trinitrates was formed. The reaction seemed to follow the same mechanism as with cellulose trinitrate, but it was not specific for any one nitrate group.

The present investigation was initiated to study the action of hydroxylamine hydrochloride in pyridine on methyl-β-D-glucoside tetranitrate, and parallels the work of Hayward on

the system employing hydroxylamine as a free base.

It was observed that a slow reaction accompanied by the evolution of a gas, composed of about 70% nitrous oxide and 30% nitrogen, occurred and was not quite complete after two days. The carbohydrate products consisted of a complex mixture of partially nitrated methylglucosides and completely denitrated polyoxime derivatives in almost equal amounts. Separation of some crystalline compounds was successfully accomplished by means of column chromatography. As in the case of Dr. Hayward's work, the reaction with methyl-β-D-glucoside tetranitrate appeared more complex than with cellulose trinitrate, and the former substance could not be regarded as analogous in this respect to the latter.

# HISTORICAL INTRODUCTION

The literature concerned with the alkaline decomposition of simple and polynitrate esters was fully reviewed in the thesis by G.H. Segall (1) in 1946. The thesis by L.D. Hayward (2) adequately summarized the known reactions of the simple sugar nitrates up to the end of 1949. Hence in this Introduction, to avoid unnecessary repetition, only those portions of the above surveys directly pertaining to the present investigation have been described.

The reaction of alkali with organic nitrate esters was early shown to be quite complicated. Normal saponification was accompanied usually by profound decomposition producing oxidation products of the original alcohol as well as nitrite salts. This redox reaction, which was not observed in the hydrolyses of the analogous organic halides or nitrites, was obviously related to the high positive valence of the nitrate nitrogen atom. In the presence of reducing agents normal saponification could be accomplished. The five general reaction paths which alkaline denitrations were observed to follow were summarized in this manner (1):

1. Normal saponification

$$RONO_2 + KOH \longrightarrow ROH + KNO_3$$

2. Carbon-carbon bond formation

$$RNO_3 + R\overset{O}{\overset{\|}{C}}CH_2\overset{O}{\overset{\|}{C}}OR'' \xrightarrow{NaOEt} R\overset{O}{\overset{\|}{C}}CHR\overset{O}{\overset{\|}{C}}OR'' + NaNO_3 + EtOH$$

3. Ether formation

$$RNO_3 + KOH(\text{ethanolic}) \longrightarrow ROC_2H_5 + KNO_3$$

4. Redox reaction

$$RCHONO_2R' \longrightarrow R\overset{O}{\overset{\|}{C}}R' + HNO_2$$

5. Olefin formation

$$RR'CHCH_2ONO_2 \longrightarrow RR'C{=}CH_2 + HNO_3$$

More than one of these mechanisms was often found in operation in a single reaction (3). Such factors as the particular nitrate ester, solvent, and alkali affected the final products isolated.

Olefin formation and carbon-carbon bond formation have not been observed in alkaline hydrolyses of carbohydrate and related nitrates, and therefore these two mechanisms will not be described further.

Nef (4) in 1899 pointed out that alkyl nitrates in alcoholic alkali could react according to the remaining three schemes. Methyl nitrate with 2 moles of alcoholic potassium hydroxide at 30-40° gave dimethyl ether and no nitrite, while benzyl nitrate with sodium ethylate in ethanol produced nearly

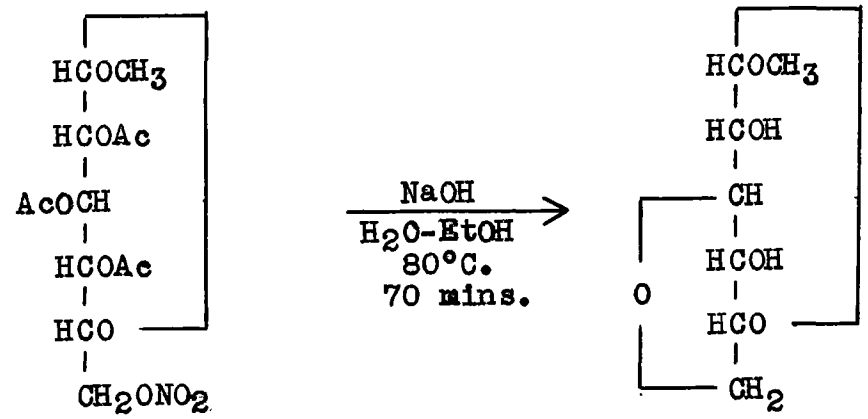
quantitative yields of nitrite and the decomposition products of benzaldehyde in alkali. Ethyl and higher aliphatic nitrates yielded mixtures of ethers, aldehyde decomposition products and in some cases the original alcohols. The extent of the straightforward hydrolysis to the alcohol, though small, increased with the stability of the nitrate toward alkali.

Gladding and Purves (5) observed that the reaction of alkali with some glucose and methylglucoside mononitrate-acetates yielded anhydro derivatives (intramolecular ethers) when suitably situated free, or potentially free, hydroxyl groups were present in the carbohydrate molecule. Normal hydrolysis resulted, with difficulty, when such hydroxyl groups were blocked by methyl ether groups. Thus methyl-2,3,4-triacetyl-α-D-glucoside-6-nitrate (I) when treated with aqueous-ethanolic sodium hydroxide, at 75-80° for 70 minutes, or with sodium methylate in methanol for 48 days at room temperature, was converted in 77-88% yield to methyl-3,6-anhydro-α-D-glucoside (II). Two per cent of the nitrate groups were reduced to nitrite. However, with methyl-2,3,4-trimethyl-β-D-glucoside-6-nitrate (III), denitration required heating at 60° for 24 hours with sodium hydroxide in aqueous methanol. Methyl-2,3,4-trimethyl-β-D-glucoside (IV) was obtained in 75% yield. Twenty per cent of the original nitrate groups were reduced to nitrite and 25% of the methylated glucoside was decomposed to a discolored tar. Methyl-3,4-6-triacetyl-β-D-glucoside-2-nitrate (V) when treated with sodium hydroxide in aqueous dioxane gave

an 84% yield of a clear, colorless, nitrate-free syrup, which appeared to be a mixture of anhydro methylhexosides (VI). Only 2.3% of the nitrate groups were reduced to nitrite.

Sodium methylate in absolute methanol reacted with 2,3,4,6-tetraacetyl-α-glucosyl nitrate (VII) to give methyl-β-D-glucoside in 28% yield with probably an equal amount of glucosan 1,5 <β> 1,6 (VIII). When the hydrolysis was carried out with sodium hydroxide in aqueous dioxane the glucosan was isolated in 33% yield as the trimethyl derivative. The nitrite produced in the last reaction corresponded to 4.5% of the original nitrate groups.

Gladding and Purves concluded that the preferred alkaline cleavage of the nitrate groups in the mononitrates studied took place in the sense $R_1R_2CH-O-NO_2$ leading to the quick expulsion of the elements of nitric acid in a substantially unreduced condition. The mechanism probably involved the momentary existence of a carbonium ion as well as Walden inversion when the nitrate group was attached to an asymmetric carbon atom. This method of elimination depended upon the facile production of methylglucoside or anhydro structures since, when methyl ether groups blocked anhydro ring formation, normal hydrolysis was the main reaction. This reaction resulted only with more drastic conditions and scission in the sense $RCH_2O-NO_2$ was assumed to occur. A considerably greater conversion to nitrite was observed in this case, showing that the direct hydrolysis of "blocked" nitrate groups was slow enough

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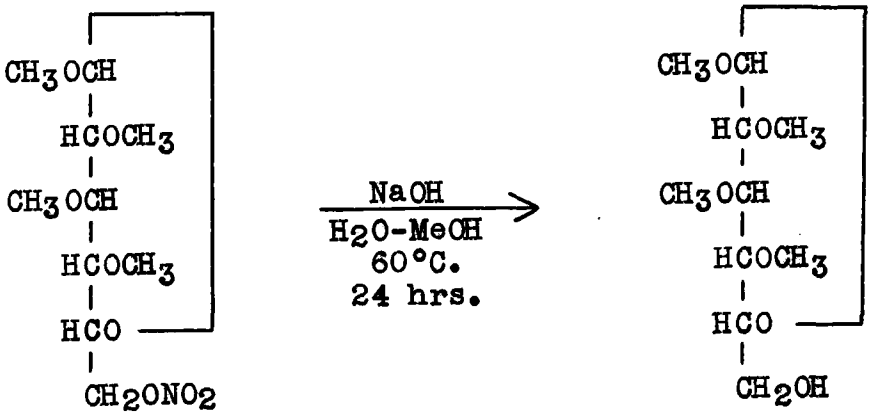


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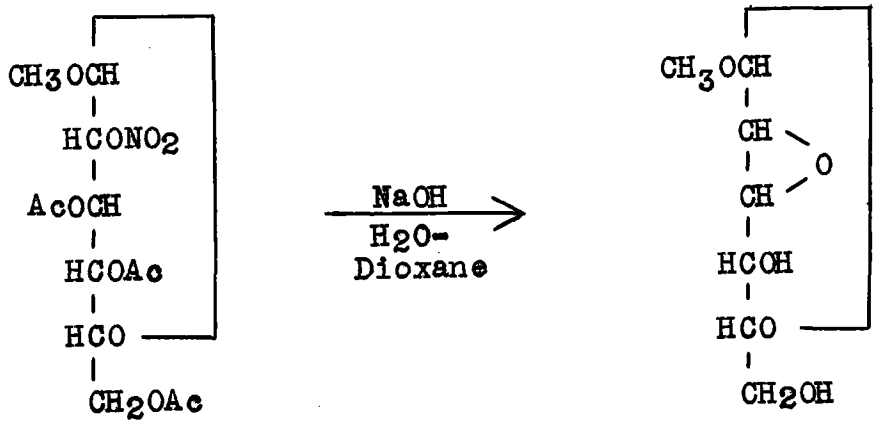


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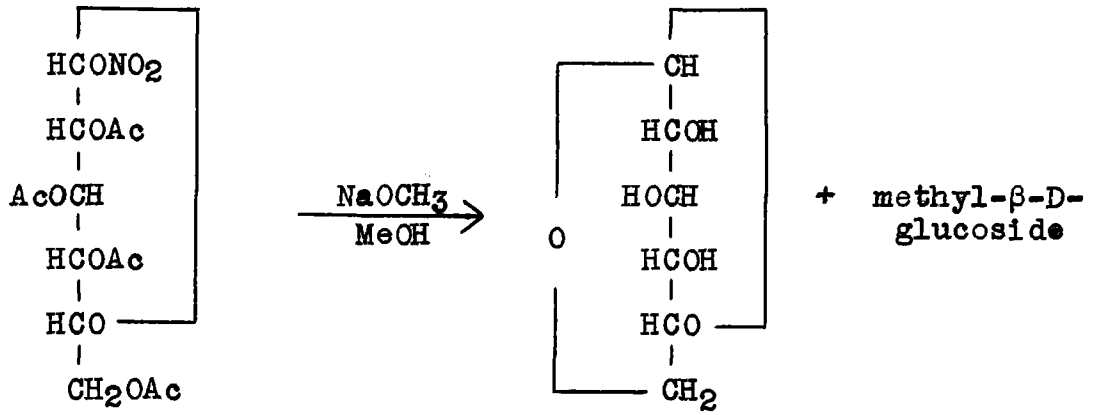
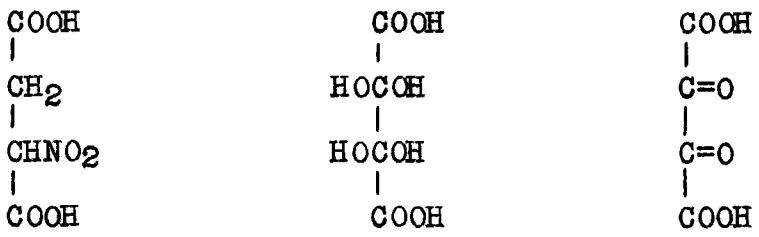



VII VIII

to render the still slower side reaction $RCH_2ONO_2 \longrightarrow RCHO + HNO_2$ of considerable importance. It was pointed out that the behaviour of these mononitrates was very similar to that observed with the corresponding p-toluenesulfonyl, sulfonyl and halide derivatives.

Lachman (6) concluded that the denitration of nitromalic acid (IX) occurred both by normal hydrolysis and by the redox cleavage, and that these reactions proceeded at independent rates. In alkaline solution normal hydrolysis was catalysed to a greater extent than the oxidation reaction, the size of the difference depending upon the solvent. In acid solution the redox cleavage predominated. Lachman (6) had previously shown that dinitrotartaric acid in acid solution yielded quantitatively nitrite and dihydroxytartaric acid (X), which could be considered to be the dihydrate of the diketone (XI). In basic solution nitrite was again produced quantitatively, but



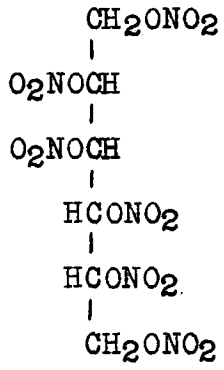
IX X XI

the dihydroxytartaric acid was decomposed into tartronic and oxalic acids. Normal hydrolysis of the nitrotartaric acid was achieved only with concentrated nitric acid containing the stoichiometric amount of water.

Nef (4) observed that alcoholic potassium hydroxide and glycol dinitrate reacted to give potassium nitrite and glycolic acid, which was presumably formed by an intramolecular Cannizzaro reaction from the initial product, glyoxal. It was suggested that a keto-dialdehyde was an intermediate in the reaction of glyceryl trinitrate and alcoholic potassium hydroxide, the potassium salts of acetic, formic, oxalic, mesoxalic, and nitrous acids being actually isolated (4)(7))8). Berl and Delpy (8) found α, α'-glyceryl dinitrate was also produced. The ratio of nitrate to nitrite reported by these workers was 1:2.37. The action of strong alkali on fully nitrated sugars and related polynitrates was reported to result in complex decompositions yielding considerable amounts of nitrite salts (9).

In 1863 Tichanowitsch (10) found that mannitol hexanitrate (XII) when treated in ether with dry ammonia gas yielded a mixture of products from which he isolated a crystalline pentanitrate (XIII), as well as what appeared to be an anhydromannitol tetranitrate and an anhydromannitol tetramine. Wigner (11) in 1903 reported that the pentanitrate was obtained in improved yield using pyridine instead of ammonia in ether. In the pyridine reaction no lower nitrates or amino compounds were detected. Wigner also obtained a pentanitrate of dulcitol from the corresponding hexanitrate by the action of pyridine. Hayward (12) in 1951 reported a 73% yield of the mannitol pentanitrate using dry pyridine and the hexanitrate. The pure pentanitrate was not attacked by pyridine in similar conditions. Hayward proved the substance to be the 1,2,3,5,6-pentanitrate (XIII) when methylation followed by hydrogenolysis of the nitrate groups gave the known 4-methyl-D-mannitol (XIV). Hayward also noted that during the partial denitration in pyridine brown nitrogen dioxide gas was evolved. It was not ascertained whether the nitrogen dioxide was a direct product of the reaction, or whether nitric oxide was formed and then oxidized on contact with air.

The conversion of nitrocellulose to cellulose was of great interest for both commercial and theoretical reasons and several detailed reviews are available (9)(13)(14). Denitrations with aqueous alkalies, ammonia or alkali carbonates resulted in a deep-seated decomposition of the cellulose. The



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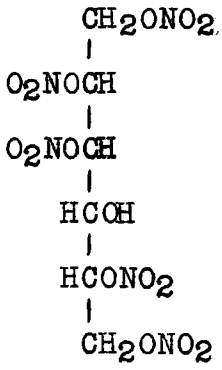

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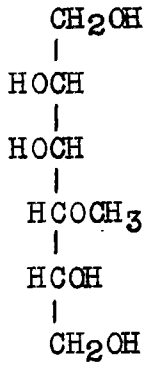


XIV

organic products isolated included carbon dioxide, formic, oxalic and other organic acids. Inorganic nitrates and nitrites, ammonia, cyanide and nitrogen were also identified. Acid hydrolysis of nitrocellulose resulted in degradation of the macromolecule and did not give complete denitration. In the commercial manufacture of nitrofilm and nitrosilk, denitration was achieved by carrying out the denitration process in a reducing medium (15)(16).

Wolfrom and co-workers (17) in 1947 described a method of simultaneous denitration and acetylation of nitrate esters by treating a solution of the nitrate in acetic anhydride with zinc dust and a suitable promoter. The most satisfactory promoter found was dry hydrogen chloride gas; anhydrous pyridine was also used, but it was said to give smaller yields. Cellulose nitrate, although successfully denitrated and acetylated by this process, was badly degraded. In 1951 Wolfrom, Bower and Maher (18) reported a direct acetolysis, applicable to nitrate esters of small carbohydrate units, was

accomplished by the use of a sulfuric acid - acetic anhydride medium.

The decomposition of cellulose nitrate by pyridine at room temperature was studied by Angeli (19) and Giannini (20). Analysis of the carbohydrate products indicated that the cellulose was degraded considerably and highly oxidized. Since the degree of denitration was not great, much of what denitration occurred was probably by the redox mechanism. Gladding and Purves (5) found that pure dry pyridine caused a vigorous decomposition of dissolved, stabilized guncotton at steam-bath temperature. Nitrogen dioxide was evolved as a volatile pyridine complex that readily crystallized above the solution on cooling.

Segall (1) concluded from the study of the published results on the alkaline denitration of nitrocellulose that "no definite evidence was obtained for a possible variation in the reactivities of the three nitrate groups in each glucose unit or of the relative importance of the various mechanisms of nitrate cleavage; except that redox cleavage probably occurred to a great extent".

Segall, interested in the relative reactivities of the nitrate groups, studied the reaction of cellulose trinitrate with pyridine in the presence of hydroxylamine, methoxyamine, and the corresponding hydrochlorides. Since much of the denitration of nitrocellulose in pyridine was known to

occur by the redox reaction, it was thought that the above reagents would react with, and thus protect, the carbonyl groups as they were formed. This action might then be expected to prevent secondary decomposition of the oxidized glucose residues, and permit the isolation of a slightly degraded partially nitrated cellulose derivative.

Segall found that the reaction of cellulose trinitrate with a large excess of hydroxylamine in pyridine solution at room temperature was rapid and exothermic. One mole of nitrogen gas was evolved per glucose residue. The analysis of the carbohydrate product, obtained in 98% yield, indicated 1.7 nitrate and 0.08 oxime groups per anhydroglucose unit. Chain degradation was slight. Renitration almost to the trinitrate, methylation and acetylation to derivatives analysing for monomethyl and monoacetyl dinitrates confirmed the fact that the product was approximately a dinitrate. The results of iodination, with sodium iodide in acetone, showed that the nitrate group removed was of a secondary nature. The "dinitrate" was stable for long periods of time in pyridine, pyridine-hydroxylamine, and pyridine - methoxyamine hydrochloride. Segall claimed that this dinitrate was the first cellulose nitrate to be reported as being stable to pyridine. These results suggested that the instability of cellulose trinitrate in pyridine-hydroxylamine solution was due to a specific nitrate group in a definite position in the glucose residue. Attempts to reduce the monomethyl-dinitrate to a monomethyl cel-

lulose and to determine the structures of the glucose methyl ethers which would be obtained after hydrolysis were unsuccessful. This failure made it impossible to determine the location of the nitrate groups removed from cellulose by pyridine-hydroxylamine.

Methoxyamine in pyridine yielded the same "dinitrate" but there was no noticeable evolution of nitrogen gas in this reaction.

Segall also observed that if cellulose trinitrate were treated with an excess of hydroxylamine hydrochloride in pyridine solution a reaction occurred which was slower than the corresponding reaction with free hydroxylamine. A gas containing over 85% nitrous oxide, the remainder being nitrogen, was evolved. Analysis of the fibrous product isolated in 85% yield showed a ratio of 1.7 nitrate to 1.0 oxime groups per anhydroglucose unit. Substitution of methoxyamine hydrochloride gave a fibrous product in 93% yield with the same value for the ratio of nitrate and methyloxime groups. Reductive acetylation of the "oxime dinitrate" yielded a nitrate-free product with the correct nitrogen analysis for an oxycellulose mono-oxime triacetate. Iodination with sodium iodide in acetone of the "methyloxime dinitrate" failed to give clean-cut results but the analysis of the degraded product suggested that the methyloxime group was a ketoxime. In support of this evidence it was found that the methyloxime group was very stable to hydrolysis, a property characteristic of methylated ketoximes.

Because the cellulose dinitrate formed with the free base in pyridine was recovered unchanged from a solution of methoxyamine hydrochloride in pyridine, Segall suggested that the same nitrate group was attacked by both the free hydroxylamine and its hydrochloride.

Segall also postulated that the denitration in the hydroxylamine reaction was a result of the labile hydrogen in the hydroxylamine molecule and that the reaction proceeded according to the following equations:

$$-\overset{|}{\underset{|}{C}}-O-NO_2 + \xrightarrow[\text{Pyridine}]{NH_2OH} -\overset{|}{\underset{|}{C}}-OH + NO_2NHOH$$

$$NO_2NHOH + NH_2OH \longrightarrow N_2 + HNO_2 + 2H_2O$$

The intermediate nitrohydroxylaminic acid was probably quickly reduced to nitrogen gas by the large excess of hydroxylamine present. Segall assumed that the lack of gas evolution in the methoxyamine reaction was because of the weaker reducing action of this base.

Angeli (21)(22)(23) had carried out an extensive study of the reactions between methyl and ethyl nitrates and hydroxylamine. This worker found a non-oxidative denitration was achieved and the disodium salt of the unstable nitrohydroxylaminic acid was formed by the interaction of ethyl nitrate, sodium ethylate and hydroxylamine:

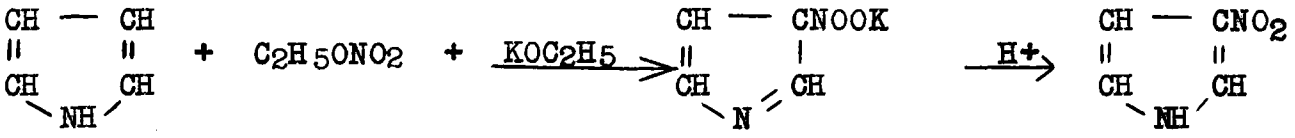
$$C_2H_5ONO_2 + NH_2OH \longrightarrow NO_2NHOH + C_2H_5OH$$

$$NO_2NHOH + 2\ NaOC_2H_5 \longrightarrow O \leftarrow \overset{\overset{ONa}{|}}{N} = N\text{-}ONa + 2C_2H_5OH$$

Analogous reactions giving nitro compounds from ethyl nitrate and substances containing active hydrogen have been reported (24):

$$C_2H_5ONO_2 + KOC_2H_5 + C_6H_5CH_2CO_2C_2H_5 \longrightarrow$$

$$2C_2H_5OH + C_6H_5C(NOOK)CO_2C_2H_5$$



On the other hand the reaction between cellulose trinitrate and hydroxylamine hydrochloride was thought to have occurred almost exclusively by the elimination or oxidation mechanism:

$$HC\text{-}ONO_2 \xrightarrow[NH_2OH\cdot HCl]{\text{Pyridine}} C{=}O + HNO_2$$

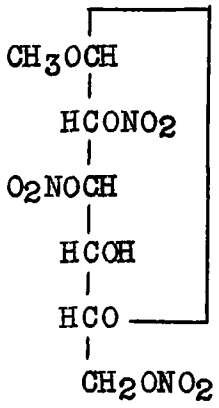
The liberated nitrous acid then reacted with the excess hydroxylamine hydrochloride in the following manner:

$$HNO_2 + NH_2OH\cdot HCl \longrightarrow N_2O + 2\ H_2O + HCl \qquad (25).$$

In this case the labile hydrogen present in the free hydroxylamine was assumed to have become more strongly bound in the hydrochloride. The original purpose of Segall's research was probably realized in this reaction since the denitration was presumably due solely to the action of the pyridine, while the hydroxylamine hydrochloride reacted with, and thus stabilized, the ketone derivative first formed.

L.D. Hayward (2) studied the interaction of hydroxylamine in pyridine with methyl-β-D-glucoside-2,3,4,6-tetranitrate in an effort to throw more light on Segall's results with cellulose trinitrate. Hayward found that a similar denitration proceeded quite vigorously, with the evolution of 1.26 moles of pure nitrogen. The reaction was halted after several hours by pouring the mixture into water. Ether extraction following neutralization yielded a syrup in greater than 80% yield which was shown to contain methyl-β-D-glucoside-2,3,6-trinitrate (XV) (53%), methyl-β-D-glucoside-3,6-dinitrate (XVI) (33%) and unidentified methyl-β-D-glucoside trinitrate (14%). The known 3,6-dinitrate (XVI) was isolated in crystalline form directly from the ether extract while the methylglucoside-2,3,6-trinitrate was methylated, hydrogenated, and acetylated, and identified as the known crystalline methyl-4-methyl-2,3,6-triacetyl-β-D-glucoside (XVII). Although the research added much to knowledge concerning the methyl glucoside tetranitrate, it completely failed to explain Segall's results because about 70% of the nitrate groups were removed from the 4th position, which was not

available for nitration in cellulose. In this particular instance, therefore, methylglucoside proved an unreliable model of the cellulose repeating unit.



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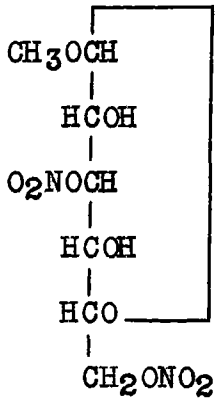

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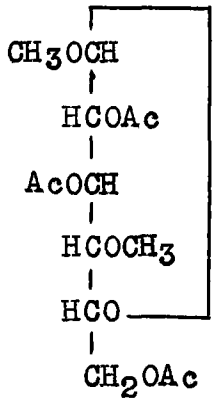


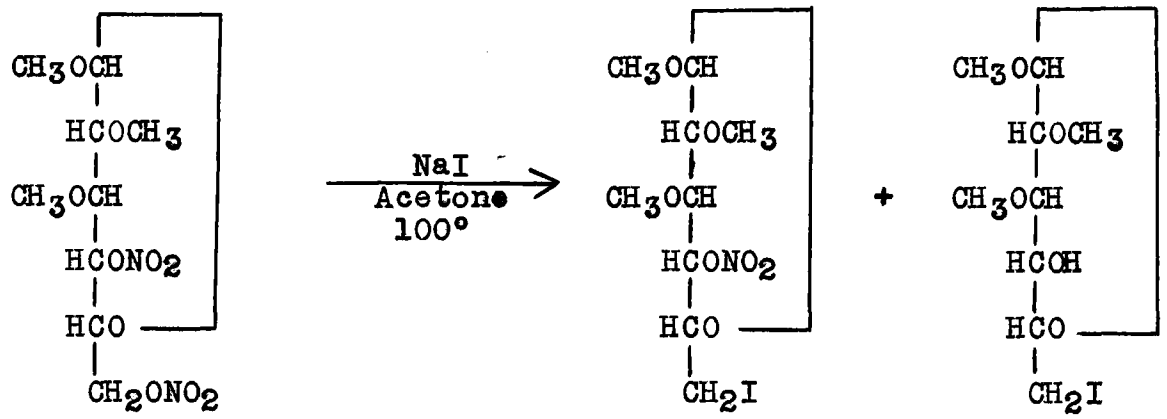
XVII

Hayward also reported that pure pyridine alone reacted readily with methyl-α- and β-D-glucoside tetranitrates. The solutions assumed a bright red color after a few minutes at room temperature, but no gas evolution was discernible. After 12 hours, when the mixture was poured into water, neutralized and extracted with ether, 53.8% of the original tetra-

nitrate was recovered. The products remaining in the aqueous solution were not investigated.

The reaction of certain methylglucoside nitrates with sodium iodide in acetone at 100° was found to result in denitration of some secondary nitrate groups, as well as in the well-known replacement of primary nitrate groups by iodine atoms. Rutherford and Oldham (26) reported that the action of this reagent on methyl-2,3-dimethyl-β-D-glucoside-4,6-dinitrate (XVIII) yielded not only the expected methyl-6-iodo-2,3-dimethyl-β-D-glucoside-4-nitrate (XIX) but also methyl-6-iodo-2,3-dimethyl-β-D-glucoside (XX). The latter compound was usually the major product. Irvine and Rutherford (27) however obtained yields as high as 70% of crude methyl-6-iodo-2,3-dimethyl-α-D-glucoside-4-nitrate from the corresponding 4,6-dinitrate. The same reagent with methyl-4,6-ethylidene-β-D-glucoside-2,3-dinitrate (XXI) was reported by several workers (28)(29) to cause the replacement of the nitrate in position 2 by an hydroxyl group. Dewar and Fort (30) found that methyl-β-D-glucoside-2,3,4,6-tetranitrate under these conditions yielded a mixture of 2- (XXII) and 3- (XXIII) mononitrate-6-iodo compounds, the 2-nitrate being produced in greater amount. The 6-iodo group was readily converted to nitrate when treated with silver nitrate in acetonitrile solution.

To sum up, the literature concerning the reactivity of nitrate groups in cellulose and methylglucosides clearly showed that differently located groups behaved in different

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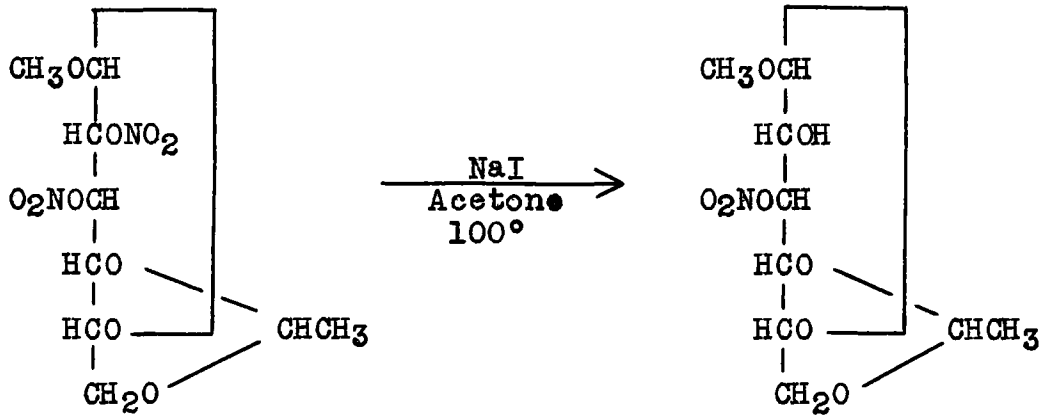


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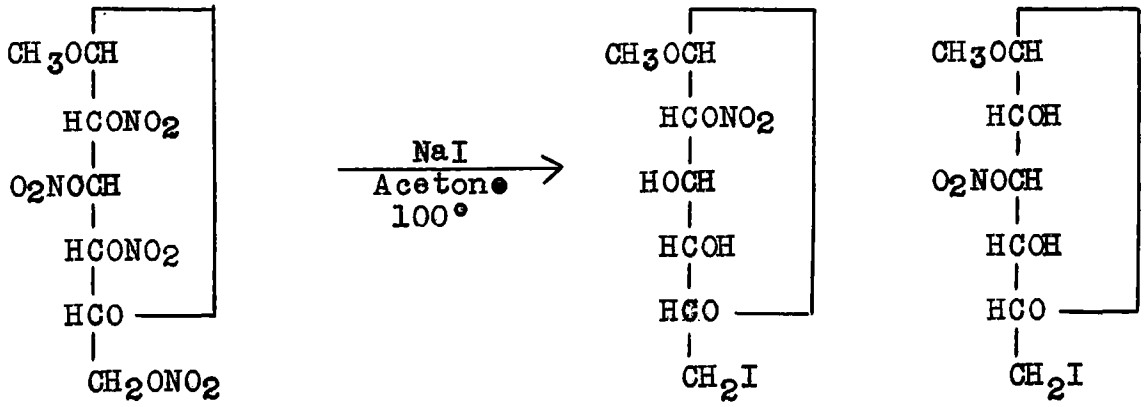



XXII XXIII

ways toward hydroxylamine in pyridine, to pyridine alone and to sodium iodide in a ketone solvent. Apart from the selective iodination of primary nitrate groups brought about by the last-named reagent, it appeared impossible to foretell the course of the reaction in any given case. No work other than that of Segall was found concerning the action of hydroxylamine hydrochloride in pyridine on nitrates of the carbohydrate series.

## DISCUSSION OF RESULTS

A preliminary experiment confirmed the expectation that methyl-β-D-glucoside-2,3,4,6-tetranitrate reacted with hydroxylamine hydrochloride in pyridine. A gradual evolution of a gas was observed, and the color of the solution changed through yellow to green within a short time.

Polarimetric observations of the solution revealed that at 20°C. the originally positive optical rotation of methyl-β-D-glucoside tetranitrate, of perhaps $\left[\alpha\right]_D^{20}$ + 5°, fell approximately according to the first-order rate expression and to a minimum levorotation of $\left[\alpha\right]_D^{20}$ - 9.75° after nine hours (Figure 1, Table I). After twenty-two hours, however, the rotation had slowly risen again to a less negative value. Color formation made it impossible to obtain accurate readings after this time. The shape of the optical rotation plot suggested that a selective reaction might be occurring initially, but on working up the mixture after ten hours a considerable amount of the unreacted tetranitrate was discovered. The significance of the observed change in rotation has remained obscure.

A study of the gaseous product, which in these experiments was liberated over dry mercury in the Toricellian vacuum of a Lunge nitrometer, yielded some significant information. The volume was determined at regular intervals after transferring the liberated gas to the burette portion of the

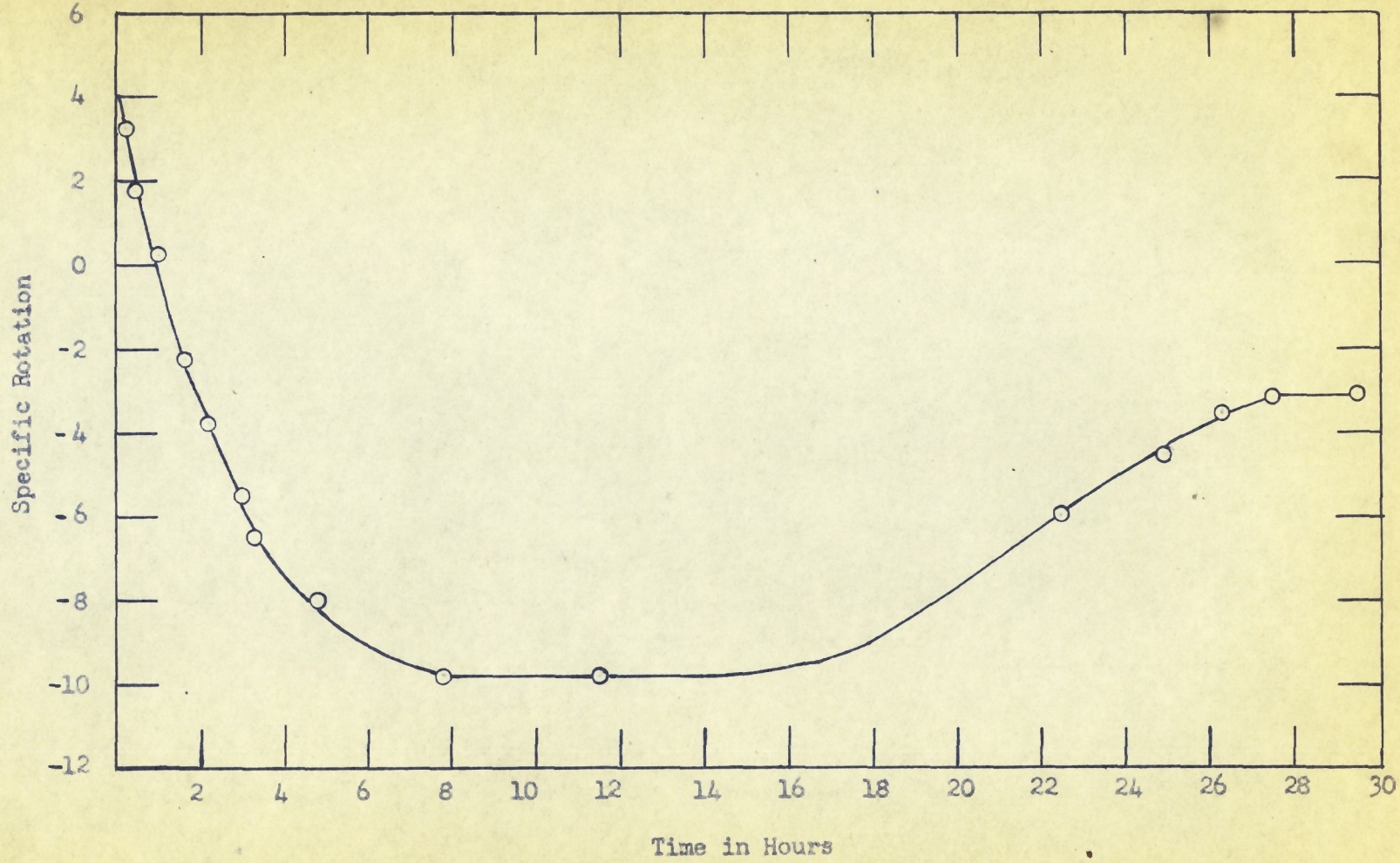



FIGURE 1. Change in Specific Rotation of 1.00 g. of Methyl-β-D-glucoside-tetranitrate in 25 ml. of Pyridine containing 5.00 g. of Hydroxylamine Hydrochloride.

apparatus. The analysis of the gas was carried out in a Fisher Technical Model Orsat Apparatus, and the molecular weight was determined by the gas density method (31). In the Orsat analysis, after a preliminary examination had indicated nitrous oxide, the gas was first passed through a pipet containing ethanol until the volume became constant, in order to remove the nitrous oxide and the pyridine vapor. The same procedure was also employed by Segall (1). A further relatively small decrease in volume resulting on passage through the remaining pipets was attributed to ethanol vapor alone. Orsat and molecular weight analyses showed that the reaction gas contained approximately 70% nitrous oxide, the remainder being nitrogen. The analyses were nearly constant for gases analyzed at different reaction times.

An initial discrepancy between the Orsat analysis and the experimentally determined molecular weight was traced to an inefficient "Dry Ice" trap which did not condense the pyridine vapor. When the gas was assumed to be a mixture of nitrous oxide and nitrogen saturated with pyridine, the molecular weight of 40.8 calculated from the Orsat analysis (Table II) agreed quite well with the value of 41.1 found experimentally. To evaluate accurately the effect of pyridine vapor, a molecular weight determination was performed on a gas sample eleven days after the evolution was complete and no Dry Ice trap was used. Both the density and the Orsat determinations then gave the identical molecular weight of 40.4, the assumption again being

made that the gas was a mixture of nitrous oxide and nitrogen saturated with pyridine (see Table V). The actual value in this experiment corresponded to 38% nitrogen and only about 62% of nitrous oxide. Since double the quantities of reactants were used and the gas studied was evolved in the first ten hours, this experiment also provided evidence that the solubility of nitrous oxide in pyridine affected the composition. An Orsat analysis of the gas liberated between 10 and 29.5 hours from the same reaction showed a higher than usual percentage of nitrous oxide.

When the molecular weight apparatus possessed an efficient trap and in addition a calcium chloride drying tube, the observed value of 39.2 (Table IV) was identical with that calculated for a mixture containing 70% nitrous oxide and 30% nitrogen. The value calculated from the Orsat analysis (Table II) was 39.5. Because of the undetermined effect of the high solubility of nitrous oxide in pyridine, this estimate was believed to be the minimum value possible for nitrous oxide. One experiment was carried out in an effort to gain a rough estimate of the nitrous oxide remaining dissolved in the reaction solution and it was found that at least one-third of an original 137.8 ml. of commercial nitrous oxide could be absorbed irreversibly by a solution of 11.8 g. of hydroxylamine hydrochloride in 59.7 g. of pyridine.

In the Orsat analysis of a sample of commercial nitrous oxide, Segall found that 8% was not absorbed by the pipet

containing ethanol. In his calculations of the composition of the reaction gas he corrected for this amount on the assumption that it was all nitrous oxide. Although Segall's observation with commercial nitrous oxide was confirmed in the present research, analysis by reduction with hydrogen (Table III), as well as by molecular weight determinations, indicated that several per cent of nitrogen was actually present. If this were the true explanation of Segall's observation, the value of 85% nitrous oxide for his reaction gas from cellulose trinitrate would be slightly erroneous. The molecular weights obtained by Segall, however, agreed with the Orsat calculations.

Several rate of gas evolution plots are reproduced in Figure 2. It proved impossible to obtain accurately reproducible results and the differences were assumed to be due to the high and uncontrolled solubility of nitrous oxide in pyridine. In the same way, Segall attributed variations in the total volume of gas from the cellulose trinitrate reaction to the nitrous oxide remaining dissolved in the solution. In further support of this view it was found that with double the usual quantities of reactants the volume of gas liberated in the first ten hours was considerably less than expected (Curve C, Figure 2), and as mentioned earlier the gas itself contained a higher than usual percentage of nitrogen (Table V).

The gas evolution data indicated that the most rapid reaction took place in the first fourteen to eighteen hours, the rate of evolution gradually slowing down after that time.

Measurable quantities were still being given off, however, after forty-eight hours. Total volumes corresponding to 1.2 to 1.5 moles per mole of glucoside were obtained after the longer reaction time. It was interesting to note that the minimum specific rotation (Fig. 1) occurred before the end of the rapid evolution of gas (Fig. 2).

Attention was next directed to the isolation of the carbohydrate products from the methylglucoside tetranitrate - hydroxylamine hydrochloride - pyridine reaction. Since a considerable amount of nitrogen had been detected, together with the nitrous oxide expected from the parallel work by Segall, it was anticipated that some normal hydrolysis as well as the redox cleavage of nitrate groups was occurring. The ratio of the two could hardly be estimated from the gas composition, because of the indeterminate solubility of nitrous oxide in pyridine, even if the doubtful assumption was made that the nitrogen was solely produced by the hydrolysis, and the nitrous oxide by the redox reaction.

Hayward (2) diluted the methyl-β-D-glucoside tetranitrate - hydroxylamine - pyridine solution with water, neutralized the mixture with dilute sulfuric acid, and extracted his product with ether. When this procedure was applied, after the present reaction had proceeded for twenty-one hours, the neutralization caused the precipitation of 8% of unchanged tetranitrate, while after forty-four hours the amount was less than 2%. Hayward in contrast had observed that all the tetra-

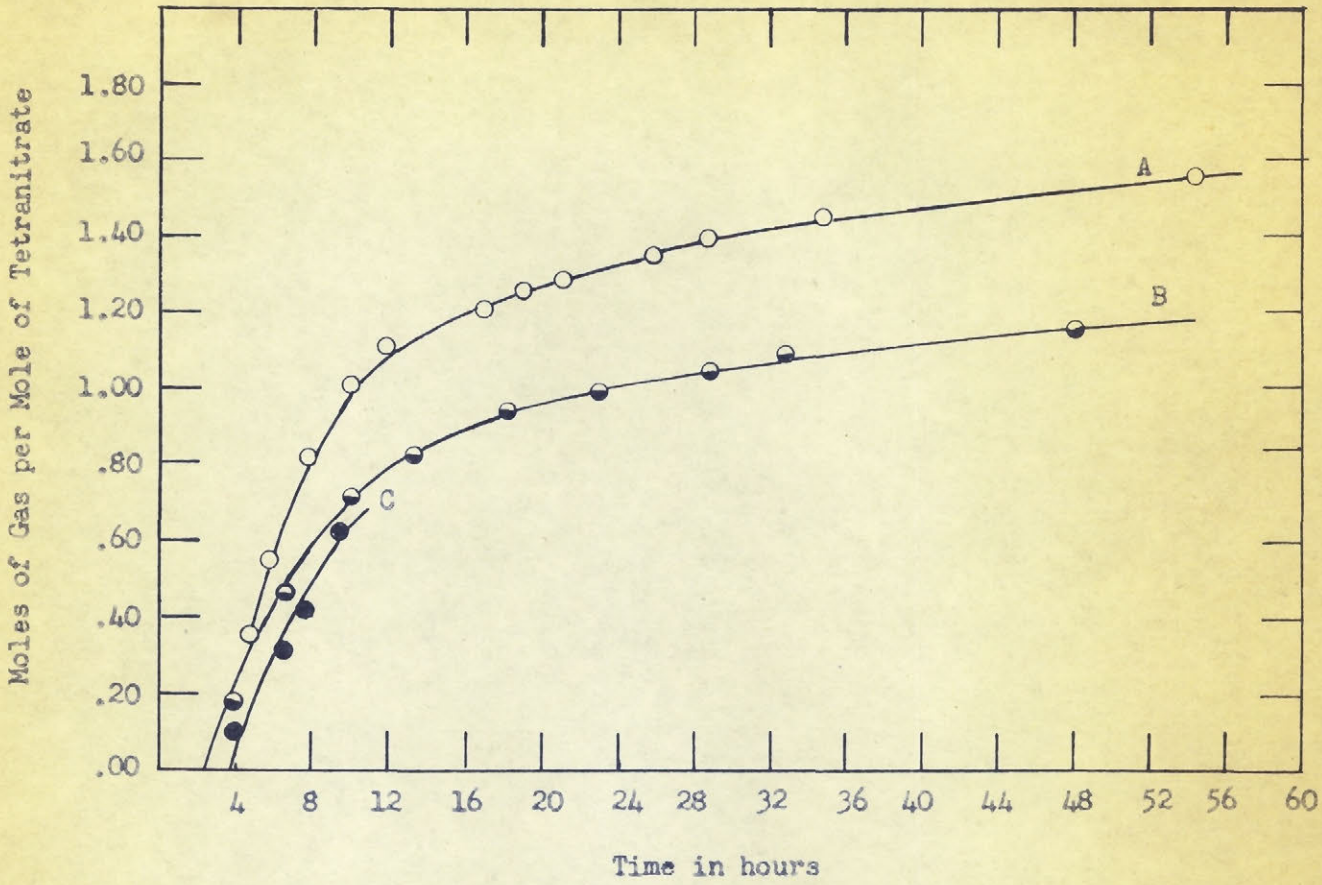



FIGURE 2. Rate of Gas Evolution from Methyl-β-D-glucoside Tetranitrate-hydroxylamine hydrochloride-pyridine

Plot A ○ The gas was evolved from 2.00 g. of methyl-β-D-glucoside tetranitrate, 12.00 g. of hydroxylamine hydrochloride and 50 ml. of pyridine.

Plot B ◒ The gas was evolved from 1.98 g. of methyl-β-D-glucoside tetranitrate, 11.31 g. of hydroxylamine hydrochloride and 60 ml. of pyridine.

Plot C ● The gas was evolved from 4.01 g. of methyl-β-D-glucoside tetranitrate, 23.25 g. of hydroxylamine hydrochloride, and 110 ml. of pyridine.

nitrate was consumed within one hour, when free hydroxylamine replaced the hydrochloride in the reaction mixture.

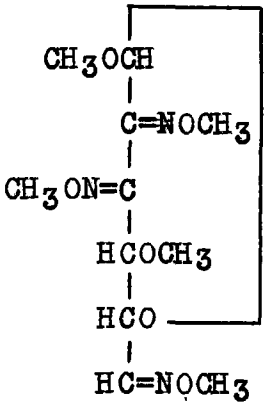
Ether extraction of the filtrate from the tetranitrate precipitated after forty-four hours yielded a yellow, viscous syrup (Syrup A) in an amount that accounted for about 45% of the original methoxyl content. The results of methoxyl, total nitrogen and nitrate nitrogen analyses, as well as of nearly quantitative hydrogenolysis to methyl-β-D-glucopyranoside, showed that no change other than partial denitration had occurred. Syrup A was very similar in both analysis and appearance to the product Hayward first isolated from the tetranitrate, free hydroxylamine, and pyridine. The remaining half of the products, which were presumed to be oximes, could not be removed from the neutralized aqueous solution by ether, even on continuous extraction for several days. Re-extraction with butanol, however, yielded a dark brown syrup which also contained some inorganic material. This product was not investigated because the following more clean-cut method of isolation was found a short time later.

When a large excess of chloroform was added to the pyridine reaction mixture, most of the excess hydroxylamine hydrochloride was precipitated. The chloroform solution after filtration still gave a strong chloride test. Although the residue from the evaporation of this solution was extracted with several solvents in an attempt to isolate the water-soluble portion, the results were unsuccessful, and the removal of the

contaminating chloride ion from the original chloroform solution was then investigated. Silver carbonate or oxide was used for this purpose, but although these compounds removed all chloride ion from the solution and were known to destroy hydroxylamine (32), silver ion invariably remained in the chloroform solution. A brown-colored silver salt with a methoxyl content as high as 7.5% appeared at the interface when the filtered chloroform solution was extracted with water. On evaporation of the filtered aqueous extract a similar yellow-brown precipitate (methoxyl content about 6.5%) formed. Complete evaporation yielded a syrup which contained some silver nitrate as well as carbohydrate material. Re-treatment of this syrup in pyridine-chloroform converted all of the organic material to a silver derivative. The nitrogen contents of the silver compounds were in the range from 12 to 14% and the diphenylamine test for the nitrate group was positive. The chloroform extract on evaporation also yielded a small amount of dark silver salt as well as a syrup which resembled Syrup A in yield and in its ability to give methyl-β-D-glucoside on hydrogenolysis.

Methylation, with silver oxide and methyl iodide, of several preparations of the silver derivatives having methoxyl contents greater than 5%, gave dark-brown, nitrate-free syrups with approximately the same nitrogen and methoxyl values. Remethylation of these combined syrups increased the methoxyl contents only slightly and the ratio of methoxyl groups (34.4%)

to nitrogen atoms (12.3%) was greater than unity. In a methyl-polyketo glucoside polyoxime, with the type of structure shown (XXIV), there would be at least one more methoxyl than nitro-



XXIV

gen per molecule. Hydrolysis of the remethylated product with fuming hydrochloric acid as described by Semper and Lichtenstadt (33) for ketoxime methyl ethers, however, yielded a very dark product from which a small amount of a crystalline compound, probably ammonium chloride, was isolated. Structure (XXIV), on the contrary, would have given methoxyamine hydrochloride $NH_2OCH_3 \cdot HCl$.

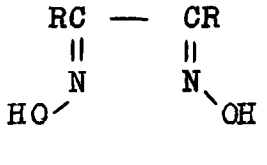
These experiments seemed clearly to indicate the presence of non-nitrate, more acidic nitrogen atoms in the water-soluble products from the methyl-β-D-glucoside tetranitrate - hydroxylamine hydrochloride - pyridine reaction. The high nitrogen contents of the silver salts and their methylated derivatives, as well as the definite lack of nitrate in the latter, were strong evidence that more than one nitrate

group in the methylglucoside tetranitrate molecule had reacted by the redox mechanism. Simple oximes are amphoteric, being both weakly basic and weakly acidic. Silver derivatives of many oximes, as for example benzaldoxime (34), oximinoacetone (35) and 1,2-dioximinopropane (36), have been reported. In the laboratory it was found that on the addition of silver nitrate to an aqueous solution of sodium dimethylglyoximate a nearly-white precipitate formed immediately. The free dimethylglyoxime in ethanol however did not appear to react under similar conditions. This knowledge lent some support to the view that the silver salts isolated from the hydroxylamine hydrochloride reaction were mainly oxime derivatives. It was possible that inorganic salts such as the di-silver derivative of hyponitrous acid (25) AgON=NOAg might also have been present.

The application of dioximes to analytical chemistry, especially to the determination of nickel, has been extensive and an excellent review is available (37). In an attempt to obtain an analogous nickel derivative from the present reaction, a nickel salt was added to the neutralized (pH 7), ether-extracted aqueous solution from which Syrup A had been removed. A brown product was isolated in yields as high as 26%, based on the methoxyl content. The ratio of methoxyl groups to nickel to nitrogen atoms was 1 to 1 to greater than 3.5. Nitrate nitrogen was approximately zero, and no inorganic anions were detected. The base molecular weight (356) calculated from the methoxyl content was unexplainably higher

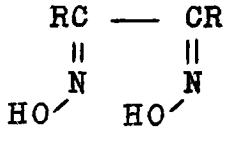
than that (303) of a mono-nickel derivative of even the tetra-oxime which might theoretically be derived from methyl-β-D-glucopyranoside (c.f. structure XXIV). It was known that hydroxylamine might co-ordinate with nickel salts as a neutral component, as for example in $\left[Ni(NH_2OH)_6\right]SO_4$ (38), but such a possibility seemed unlikely in the present case.

Three different isomers are theoretically possible for a symmetrical o-dioxime, and four for an unsymmetrical o-dioxime. It was shown that the anti or α-dioximes (XXV) form stable red co-ordination complexes in which one nickel atom is bound to two molecules of dioxime.



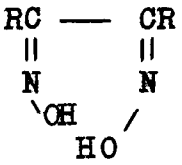
anti

XXV



amphi

XXVI



syn

XXVII

δ or Amphi-dioximes (XXVI) give yellow or green-yellow compounds in which one molecule of dioxime is attached to one nickel atom, the hydrogen atoms of both oxime groups being replaced by the metal. These are reported (37) to pass into the red, stable dioxime salts on contact with acids. The syn-dioximes (β-form) (XXVII) are completely incapable of forming compounds with nickel. The methoxyl-to-nickel ratio in the nickel salts isolated in the present work related

them to the amphi-dioximes. Treatment with dilute acid, however, did not appear to have any effect other than that of slow solution.

The principal conclusion from the consideration of the nickel derivatives was that a complete denitration by the redox mechanism affecting at least 26% of the tetranitrate, had occurred. If, as the nitrogen contents indicated, tri- and tetra-oximes were formed, the water-soluble reaction products might be expected to consist of a complex mixture of several different polyoximes and their stereoisomers.

At this point in the investigation, a procedure was discovered to isolate the water-soluble supposed oxime products in the uncombined state. All chloride ion was readily removed from the filtered chloroform solution of the reaction products by extraction with water. Evaporation of the chloroform layer yielded a clear glass (Syrup B) which represented a methoxyl recovery of 70%. Analysis showed 13.3% nitrogen of which 9.7% was nitrate. No pure product was isolated from the aqueous extract by complete evaporation and subsequent extraction with solvents. In an attempt to remove the hydroxylamine hydrochloride impurity in this extract by the action of a strongly basic ion exchange resin, it was discovered that the carbohydrate products were sufficiently acidic to be absorbed. The original aqueous extract was then poured through a column of the basic Amberlite IRA-410 exchanger. The pyridine, not being absorbed, was easily removed from the resin by washing

with water. Glacial acetic acid liberated the carbohydrate product as a chloride-free, amorphous, brown material (Syrup C), in yields of up to 15% based on the methoxyl content. Autohydrolysis of the glycosidic methyl group seemed to occur slowly on complete drying. No nitrate nitrogen was present and the ratio of nitrogen atoms to methoxyl groups was about four to one. Syrup C reduced Fehling's solution, could be titrated with sodium hydroxide, and yielded water-insoluble nickel and silver salts.

Since Syrups B and C together accounted for only about 85% of the original methoxyl groups, a search was made for the remainder in the excess hydroxylamine hydrochloride precipitated from the pyridine reaction mixture by the addition of chloroform. The ethanol mother liquors from the recrystallization of the hydrochloride gave a positive nitrate test, had a small levorotation, and therefore probably contained the rest of the product.

No crystalline compounds had yet been isolated, and the analyses of all the syrupy products indicated that they were probably mixtures. Since the classical method of determining the composition of a mixture requires the isolation and identification of pure components, effort was directed to the preparation and separation of crystalline derivatives from the syrups.

Previous workers had shown that nitrate ester groups

in carbohydrates did not display the migratory tendencies of carboxylic ester groups (39), that complete methylation of partially nitrated methylglucosides by methyl iodide and silver oxide could be achieved without loss of nitrate groups, and that methyl ether groups were stable to procedures of reductive denitration (40)(29)(41). As Hayward showed (2), a knowledge of the structures of the crystalline methyl-β-D-glucoside methyl ethers (or their acetates) formed in the above series of reactions would therefore reveal the identities of the methylglucoside nitrates originally present.

Methylation of Syrup A was completed in one eight-hour treatment with silver oxide and methyl iodide, as shown by analysis and by remethylation of the syrupy product (Syrup A-1). Hydrogenolysis of Syrup A-1 was nearly quantitative and the nitrate-free product (Syrup A-2) had a methoxyl content approximating that of a methylmonomethyl glucoside. On oxidation of Syrup A-2 with sodium metaperiodate, considerable oxidant was consumed, and a very small quantity of formic acid was produced. Acetylation of Syrup A-2 yielded a syrupy product from which by chromatography on alumina the known crystalline methyl-4-methyl-β-D-glucoside-2,3,6-triacetate was isolated in greater than 25% yield. A syrupy fraction with a methoxyl content intermediate between those for acetylated methyl monomethyl and methyl dimethyl glucosides was also obtained, as well as a trace of another crystalline substance which will be described in detail later under Syrup B-1. The above evi-

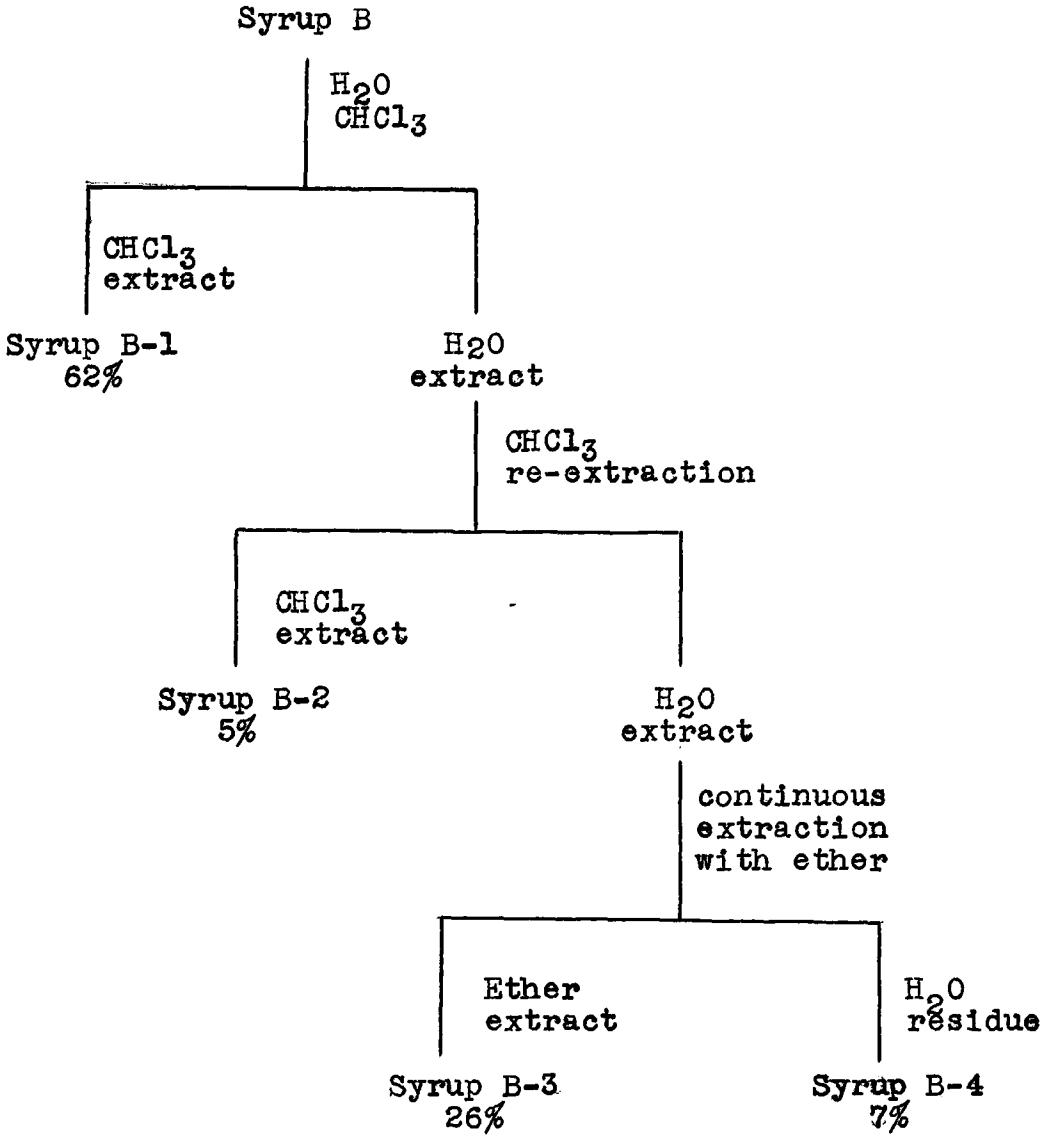
dence indicated that Syrup A was quite similar to the product isolated by Hayward (2) from the tetranitrate-free hydroxylamine reaction.

Syrup B was divided into four fractions on the basis of its solubility in chloroform, water and diethyl ether (Figure 3). The addition of both chloroform and water resulted in complete dissolution of Syrup B. Syrup B-1 obtained on evaporation of the chloroform extract was the largest fraction, amounting to 62% of Syrup B. The aqueous extract, following re-extraction with chloroform to give a second small fraction (Syrup B-2), was continuously extracted with ether for several days until a negative test for nitrate was obtained from the water solution. The ether extract on evaporation yielded a golden-brown syrup which on complete drying readily became amorphous (Syrup B-3). The aqueous solution contained a fourth fraction (Syrup B-4) which was also amorphous after drying in vacuo.

Syrup B-1 was found by analysis, and hydrogenolysis to methyl-β-D-glucoside, to be similar in composition to Syrup A. On submitting Syrup B-1 to the same series of reactions undergone by Syrup A, i.e., methylation, denitration, acetylation and chromatography, methyl-4-methyl-β-D-glucoside-2,3,6-triacetate was isolated in 38% yield. Syrupy fractions were also obtained, one of which had a methoxyl content (25.8%) indicating it was possibly a mixture of methyl monomethyltriacetyl and methyl dimethyldiacetyl-β-D-glucosides. By combining a small crystalline fraction with the sample isolated previously from Syrup A, a sufficient quantity of a second

FIGURE 3

Division of Syrup B into Four Fractions



substance was made available to allow the determination of the melting point, 141.5-142.5°, methoxyl content (24.6%), nitrogen content (0.0%), and the specific rotation $[\alpha]_D^{20}$ -40.7°. The methoxyl value was in agreement with that of 24.5% required for a mole-for-mole ratio in the above mixture of mono- and dimethyl acetates, and just below that of 24.8% for a methyl monomethyl-β-D-glucoside monoacetate. Hayward, it is interesting to note, isolated a crystalline product the analysis of which suggested that it was a methyl monomethyl glucoside triacetate, but which was in fact a mixture of unknown composition. An attempt to acetylate methyl-2,4-dimethyl-β-D-glucoside with acetic anhydride in pyridine yielded the hitherto unknown 3,6-diacetate only as a colorless syrup, unsuited for comparison with the above substance. No description of other methyl dimethyl-β-D-glucoside diacetates was found in the literature.

Syrup B-2, which had methoxyl and total nitrogen contents similar to those of Syrup B-1, was acetylated and then chromatographed on alumina, employing the flowing technique. The results are summarized in Tables VII and VIII. One crystalline compound was isolated from the anhydrous benzene effluent in about 10% yield. The analysis and physical constants for this substance agreed with those reported by Bell and Synge (41) for methyl-4-acetyl-β-D-glucoside-2,3,6-trinitrate. The separation of this derivative therefore only confirmed what the earlier isolation of methyl-4-methyl-β-D-glucoside-2,3,6-triacetate had already indicated.

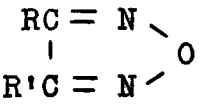
On similarly chromatographing a syrup obtained on acetylation of the known methyl-β-D-glucoside-3,6-dinitrate with acetic anhydride and pyridine, it was found that ethanol-benzene was required to elute the crystalline diacetate (29) from the alumina column. Seeding of the corresponding fraction derived from the acetylated Syrup B-2 (Fraction 2, Table VII), with the above crystals did not cause crystallization and left the identity of the unknown product in doubt.

It is probable that direct chromatographing on alumina of Syrups A and B-1, or of their acetylated derivatives, could have been utilized to separate and identify the reaction products, instead of the methylation and denitration techniques, which Hayward had first used. The advantage of the latter procedure was that the series of methylglucoside methyl ethers is much more completely characterized in the literature.

Syrup B-3, the second largest (26%, Figure 3) of the four fractions from Syrup B, was known from its analysis to be mainly oxime in nature. Of a total nitrogen content of 16%, more than 13% was non-nitrate and the ratio of nitrogen atoms to methoxyl groups was 2.7 to 1. Syrup B-3 was reducing to Fehling's solution and formed water-insoluble nickel and silver salts. The nickel derivative blackened on drying, and its methoxyl content was lower than the nickel derivatives isolated earlier, indicating that some decomposition had probably occurred.

No means were found to separate Syrup B-3 into its

components directly, and the acetylated product was prepared in the hope that it would be soluble in less polar solvents, and might therefore lend itself more readily than the original to chromatography. Pyridine and acetic anhydride were employed for the acetylation. Since the original reaction of methyl-glucoside tetranitrate occurred in pyridine solution, this reagent would probably exert no further action on the oximes themselves. The combined action with acetic anhydride, however, might easily increase the complexity of the mixture. Acetylation of an aldoxime may yield not only the oxime acetate but also, by dehydration, the nitrile. Many aliphatic aldoximes in fact were dehydrated under all conditions (42). Hauser and Jordan (43) found that tertiary amines, including pyridine, converted some aromatic acetyl-β-aldoximes to nitrile quantitatively, while the α-aldoxime acetates remained unaffected. Hauser and Sullivan (44) prepared the acetyl derivatives of the easily dehydrated β-isomers with acetic anhydride at room temperature, the crystalline acetates separating on cooling the solution after five minutes. The dehydrating action of acetic anhydride was used to convert o-dioximes to furazans (XXVIII) (45). It was of course possible that heterocyclic rings were already present in Syrup B-3. Beckmann rearrangements also might be found to occur in the present case.



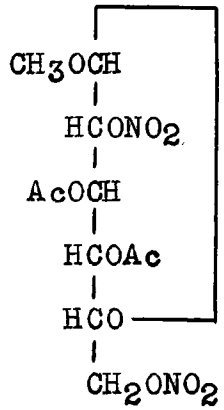
XXVIII

Addition of acetic anhydride to the pyridine solution of Syrup B-3 caused an almost immediate darkening even at 0°. The product was isolated as two fractions, a dark-brown Syrup (B-3a) extracted from the aqueous mixture by chloroform, and a dark water-soluble syrup, which did not crystallize and was not studied. The action of dry benzene subdivided Syrup B-3a into a brown benzene-soluble syrup (B-3b), and a dark-brown benzene-insoluble powder which was no longer soluble in chloroform. Analysis, after reprecipitation from acetone solution by ether, showed a ratio of about four nitrogen atoms per methoxyl group in a molecular weight (calculated from the methoxyl content) of about 370. This nitrate-free amorphous product was not investigated further.

Syrup B-3b did not crystallize and so was chromatographed on a Florisil column employing the flowing technique (Table IX). Dry benzene, the first eluent, removed approximately one-half of the recoverable product, all portions of which gave a strong nitrate test with the diphenylamine reagent. One crystalline compound (Compound A) was isolated from the benzene effluent. Further elution with one per cent ethanol, in benzene or ethylene dichloride, resulted in a further fractionation and removal of the second half of the recoverable material. The column when illuminated with ultraviolet light then displayed four distinctly different zones of fluorescence. The lowest zone contained a second crystalline substance (Compound B) which gave a negative test for nitrate. The remainder of the

product removed by the above solvent mixture gave either no, or very little, color with the diphenylamine reagent indicating that no nitrate was present. The analyses of Compounds A and B and of the two fractions immediately following them off the column, as well as of a syrup representing the total ethanol-benzene effluent, are recorded in Table X.

The nitrogen and methoxyl contents, and also the melting point, of Compound A agreed with those reported by Dewar, Fort and McArthur (30) for methyl-3,4-diacetyl-β-D-glucoside-2,6-dinitrate (XXIX) but the specific rotation, $[\alpha]_D^{20}$ + 32.2° in chloroform was considerably higher than that of $[\alpha]_D^{13}$ + 5.8° reported by these authors. It was believed however that the two compounds were identical. Since the over-all yield of Compound A was well below 1%, the amount was insufficient to allow recrystallization to optical purity.



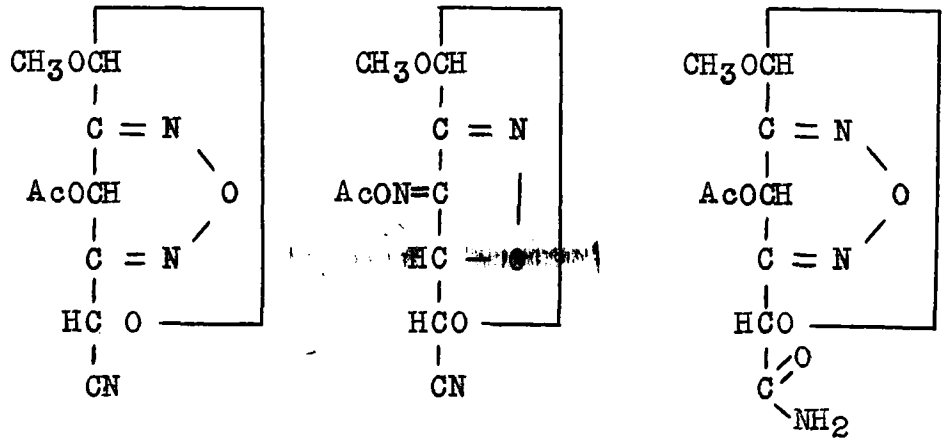

XXIX

It was interesting to note that although Compound A was easily removed by benzene from both alumina and Florisil

methyl-2,4-diacetyl-β-D-glucoside-3,6-dinitrate was not. And, furthermore, Compound A was not detected in the chromatographing of acetylated Syrup B-2. The syrupy fraction following Compound A in the chromatogram had similar methoxyl and total nitrogen contents.

Analysis of Compound B indicated a ratio of three nitrogen atoms to one methoxyl group in a molecular weight of about 260, and no nitrate groups were present. The specific rotation, $[\alpha]_D^{20}$ - 167°, was much more levorotatory than would be expected for a simple methyl-β-D-glucopyramoside derivative. The analysis of the syrup from the fluorescing band directly following Compound B was almost identical, but the specific rotation $[\alpha]_D^{20}$ -67.5 was markedly different. Because of the low molecular weight (260) calculated from the methoxyl contents it seemed likely that there could be little acetyl substitution and the presence of another ring structure, as well as the pyranoside ring, was suspected. The only types of structures which even approximately fitted the analytical data were XXX, XXXI and XXXII. Unfortunately, the yield of compound B was so small that no attempt to determine its structure was possible. The analysis of the syrup, which represented all of the ethanol-benzene effluent from one chromatogram, was similar to, but not identical with, that of Compound B.

Chromatography of Syrup B-3b thus revealed a complex mixture but it was impossible to assess what proportion of the complexity resulted from the acetylation. The isolation of



M.W. 239 M.W. 239 M.W. 257

XXX XXXI XXXII

one crystalline nitrate-free substance (Compound B) with high methoxyl and nitrogen contents was conclusive proof that a complete removal of the nitrate groups in methyl-β-D-glucoside tetranitrate had occurred.

Syrup B-4, a small fraction, (Figure 3) was not studied extensively. Both Syrup B-4 and Syrup C were acidic, reducing to Fehling's solution, nitrate-free, and both had similar nitrogen and methoxyl contents. Acetylation of Syrup C with acetic anhydride in pyridine yielded a chloroform-soluble fraction (Syrup C-1), and a water-soluble fraction (Syrup C-2), as had been observed for Syrup B-3. The methoxyl content of the water-soluble Syrup C-2 was low (4%) and this observation was assumed to be related to the decrease in methoxyl content of Syrup C itself on drying. Only a small portion of Syrup C-1 was soluble in benzene, and this frac-

tion was not investigated. The benzene-insoluble residue (Syrup C-3) was a dark-brown glass with a methoxyl-to-nitrogen ratio of one to four in a molecular weight of about 360 (calculated from the methoxyl value). The analysis of Syrup C-3 was thus similar to that of the amorphous, benzene-insoluble fraction from Syrup B-3a. Syrup C-3 however was not obtained in the same amorphous, powdered form.

Methylation of Syrup C with silver oxide and methyl iodide yielded a brown syrup with a methoxyl content (27.8%) considerably below that of 34% possessed by the syrup obtained earlier by methylation of the silver salts. The ratio of methoxyl groups to nitrogen atoms in the methylated Syrup C was unity. Since both N-methyl and O-methyl ethers of oximes are known (33) the methylated product might easily have been a more complicated mixture than the original Syrup C.

An attempted purification, by chromatography on alumina, of the very dark benzoyl derivative of Syrup C was unsuccessful. It was hoped that by benzoylation a more benzene-soluble derivative of Syrup C might be obtained which could then be separated into its components.

Although the production of oximes in the methylglucoside - hydroxylamine hydrochloride - pyridine reaction seemed to have been conclusively demonstrated, it was felt that one final experiment would complete the evidence. Segall (1) observed that cellulose trinitrate on interaction with methoxyl-

amine hydrochloride in pyridine yielded a substance analyzing for a cellulose "methyloxime dinitrate". The similar reaction with methyl-β-D-glucoside tetranitrate was therefore carried out and resulted in a good yield of a clear, dark-brown, viscous, chloroform-soluble syrup (Syrup D) with a high methoxyl content (34.5%). The ratio of methoxyl to non-nitrate nitrogen was 1.35 to 1. The methoxyamine hydrochloride had therefore produced methyloximes. The low nitrate-nitrogen content (3.4%) of Syrup D indicated that the redox mechanism predominated in this case.

A few experiments were also made to elucidate the effect of pyridine alone on methyl-β-D-glucoside tetranitrate, previous information being restricted to Hayward's observation (2) that only 53.8% of the tetranitrate could be recovered after 12 hours. The quantity remaining after forty-four hours, the reaction time generally used in the present investigation, was found to be 20%, or considerably more than was recovered from the hydroxylamine hydrochloride - pyridine reagent after that length of time. Investigation of the remaining carbohydrate products showed that all were water-soluble, and one portion had a methoxyl content of 5.4% and a total nitrogen content of 10%. Crystalline pyridinium nitrate (46) was isolated, some having formed on the walls of the flask above the solution during the reaction. Nitric acid had therefore been liberated as such, as Gladding and Purves (5) had found for some glucose mononitrates after treatment with alkali.

Hayward (47) also found a crystalline compound, with the same melting point and properties as pyridinium nitrate, while working up the methyl-α-D-glucoside tetranitrate - pyridine reaction, but did not report this observation in his thesis.

A trace of pyridinium nitrate was also obtained on one occasion from the tetranitrate - hydroxylamine hydrochloride - pyridine reaction. In this instance the reaction mixture after dilution with chloroform, filtration and evaporation was partially dissolved in ether. Some pyridinium nitrate crystallized from the ether solution on standing for several months. It was also noted that, on working up the reaction mixture by treatment of the diluted chloroform solution with silver carbonate, silver nitrate was formed. The isolation of pyridinium and silver nitrates was interpreted to mean that a direct action of the pyridine on the methylglucoside tetranitrate was not entirely suppressed, even in the presence of the large excess of hydroxylamine hydrochloride.

The products of the methyl-β-D-glucoside tetranitrate - hydroxylamine hydrochloride - pyridine reaction were far too complex to permit complete identification and therefore no over-all mechanism could be suggested. The decomposition yielding nitrogen gas and a mixture of methyl-β-D-glucoside dinitrates and trinitrates, probably identical with that formed in the reaction of the tetranitrate with free hydroxylamine in pyridine, accounted for at least 55% of the products by weight, representing a methoxyl recovery of 47%. The general equation

for this denitration was presumably that proposed by both Segall and Hayward, by analogy to the reaction studied by Angeli (21) of hydroxylamine with methyl and ethyl nitrates:

$$\text{HCONO}_2 \xrightarrow[\text{Pyridine}]{\text{NH}_2\text{OH}} \text{HCOH} + \text{NO}_2\text{NHOH} \quad .$$

It was postulated, by Segall and Hayward, that the nitrohydroxylaminic acid thus formed reacted with a second mole of hydroxylamine to yield nitrous acid, nitrogen and water:

$$\text{NO}_2\text{NHOH} + \text{NH}_2\text{OH} \longrightarrow \text{HNO}_2 + \text{N}_2 + 2\,\text{H}_2\text{O} \quad .$$

No proof of the existence of nitrous acid among the reaction products from the action of hydroxylamine - pyridine on cellulose trinitrate or methyl-β-D-glucoside tetranitrate was presented however.

The above mechanism, to apply to the hydroxylamine hydrochloride - methyl-β-D-glucoside tetranitrate reaction, would require that there be some essentially free hydroxylamine in the solution. On the assumption that there existed a rivalry between the pyridine and the hydroxylamine for the hydrogen chloride molecules, the presence of the free hydroxylamine could be explained by the following equation:

$$\text{NH}_2\text{OH}\cdot\text{HCl} + \text{C}_5\text{H}_5\text{N} \longrightarrow \text{NH}_2\text{OH} + \text{C}_5\text{H}_5\text{N}\cdot\text{HCl}$$

As Hayward (2) pointed out, free hydroxylamine in pyridine should be a strongly reducing medium, if it was analogous in its be-

haviour to hydroxylamine in potassium hydroxide solution. This solution is comparable to sodium sulfide in its reducing power (48) and like sodium sulfide, might readily reduce nitrate ester groups.

The second main denitration occurred by the redox mechanism, the usual path followed by alkaline denitrations. The carbohydrate products were polyoximes which were mainly, if not entirely, nitrate-free. Thus it would appear that when one oxime group was formed in a molecule of methylglucoside tetranitrate the remaining three nitrate groups were rendered very sensitive to the reagent. Since the rate of denitration of methyl-β-D-glucoside tetranitrate by pyridine alone was roughly comparable to the rate of the hydroxylamine hydrochloride - pyridine reaction, the pyridine was perhaps the active denitrating agent. In this event the hydroxylamine hydrochloride condensed with the ketone derivatives formed. The following equations, originally proposed by Segall to account for the products of the cellulose trinitrate - hydroxylamine hydrochloride - pyridine reaction, were then assumed to be applicable to the methylglucoside tetranitrate - hydroxylamine hydrochloride reaction as well.

$$\text{HCONO}_2 \xrightarrow[\text{Pyridine}]{\text{NH}_2\text{OH}\cdot\text{HCl}} \text{C=O} + \text{HNO}_2$$

$$\text{HNO}_2 + \text{NH}_2\text{OH}\cdot\text{HCl} \longrightarrow \text{N}_2\text{O} + 2\text{H}_2\text{O} + \text{HCl}$$

A third reaction involving the direct cleavage of the elements of nitric acid, (or of nitrate ion), by the pyridine alone probably occurred to a small but unknown extent, by a mechanism that remained obscure. This reaction, together with the redox mechanism, would account for the remaining 45% by weight of the products. As already mentioned, the first decomposition, leading to a mixture whose composition was close to that of a methylglucoside trinitrate, would yield about 0.5 mole of nitrogen gas from the approximately 50% of the starting material decomposed in this way. Most of the remaining half, however, appeared to yield tri- or tetra-oximes by the second mechanism and presumably 0.5 x 3 to 0.5 x 4 moles of nitrous oxide was evolved. On this basis the facts that at least 1.5 moles of the mixed gases were recovered from each mole of methylglucoside tetranitrate, and that at least 70% of the mixture was nitrous oxide, received a plausible explanation.

# EXPERIMENTAL PROCEDURES

## Special Precautions

Great care was exercised at all times because of the well-known explosive character of nitrated carbohydrate derivatives. The products from 8 g. of methyl-β-D-glucoside tetranitrate were the largest quantities ever combined for isolation, and this was done only after many experiments limited to 2 g. quantities had shown the probability of an explosion under these conditions to be small. The nitrations were usually carried out with 5 g. of methyl-β-D-glucoside. However, near the conclusion of the work quantities as large as 15 g. were used without mishap.

All evaporations of solutions were performed in ground-glass apparatus, under reduced pressure, at bath temperatures below 50°. Syrupy samples for analysis were dried to constant weight at room temperature, at pressures below 1 mm., and in the presence of phosphorus pentoxide. Solid products were dried in an Abderhalden pistol *in vacuo* at the temperature of boiling acetone.

All melting points, unless otherwise stated, were uncorrected.

## Analytical Methods

### Nitrogen Determination

Total nitrogen was determined by the micro-Kjeldahl method as modified by Gunning to include nitrate nitrogen (49). The weighing of syrupy samples presented a problem in this determination. Generally, the syrup was weighed into a thin-walled glass cup which could be broken easily once inside the digestion tube. If a cup too heavy to shatter was used the digestion tube was often cracked because of bumping during the heating. Complete solution in the sulfuric acid was also more difficult to achieve in the latter case. On the other hand with thinner cups, when weighing the sample it was often difficult to transfer viscous syrups without breaking the glass container.

Nitrate nitrogen was determined by Elving and McElroy's semimicro modification of the du Pont nitrometer (50). A smaller apparatus, similar in design to the Elving and McElroy model, which was constructed in this Laboratory by G.M. Moulds, was also used.

### Methoxyl Determination

Methoxyl determinations were carried out by the Vieböck and Schwappach method as described by Clark (51). It was found more convenient however to prepare a stock solution of bromine in glacial acetic acid - potassium acetate solution, according to the directions of Hoffman and Wolfrom (52). The apparatus described in Gattermann's handbook was also used (53).

Oxidation with Periodate

An adaptation of the procedures described by several authors (54)(55)(56)(57) as devised by Hayward (2), was used. In this method a single 100 mg. to 200 mg. sample sufficed to reveal the polarimetric change and the final amount of periodate consumed, as well as the formic acid and formaldehyde produced.

Nickel

Nickel was determined both directly as the monoxide by combustion at 800° and as the insoluble dimethylglyoxime complex. With the substances used direct heating of the nickel salts in an open vessel caused an explosion which resulted in the loss of much material. By wrapping the salt in a wet filter paper before heating this loss could be eliminated. In the dimethylglyoxime determination the nickel salt (.05-.07 g.) was treated with 1 ml. of nitric acid, in which it dissolved readily, and the solution was evaporated on a steam bath. Because the residue was not completely soluble in water 3 ml. of concentrated hydrochloric acid and 1 ml. of nitric acid were added and the mixture was heated on a steam bath till solution occurred. Water (50 ml.) was then added and the procedure described by Willard and Diehl (58) followed. A 5% aqueous solution of sodium dimethylglyoximate was used in place of the free dimethylglyoxime in ethanol.

## Materials

### 100% Nitric Acid

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

### Methyl-β-D-Glucopyranoside-2,3,4,6-tetranitrate

Methyl-β-D-glucoside was prepared by the method described by Raymond and Schroeder (59) in an over-all yield of 17% from anhydrous glucose. The glucoside was obtained as the hemihydrate m.p. 105-107°, $[\alpha]_D^{20}$ - 32.7° (water, C = 4.73), after recrystallization from alcohol-ether. Raymond and Schroeder (59) reported m.p. 104-105° (corr.), and $[\alpha]_D^{20}$ - 32° ($H_2O$, C = 5.72).

Nitration was performed as described by Brissaud (60) using a mixture containing acetic acid, acetic anhydride, and 100% nitric acid (1:1:2). The yield of crude tetranitrate isolated by filtration, after pouring the reaction mixture into a large excess of ice water, was almost 100%. The dried product, after recrystallization from ethanol, and then benzene-petroleum ether (b.p. 80-100°) melted at 117-118°, and had the specific rotation $[\alpha]_D^{20}$ + 10° ($CHCl_3$, C = 2.27).

Bell and Synge (39) reported m.p. 116-118°, $[\alpha]_D^{20}$ + 9.35° ($CHCl_3$, C = 4).

Palladized Charcoal Catalyst

The palladium on charcoal catalyst used for the hydrogenolysis of nitrate groups was prepared as described by Hartung (61). The hydrogen, a pure commercial electrolytic product, was transferred directly from the storage tank to the Adams Low-Pressure Hydrogenator.

Reagents and Solvents

Reagent grade pyridine, purchased from the Nichols Chemical Co. Ltd. and British Drug Houses Ltd., dried over barium oxide and distilling in the range 115-115.3, was used in all experiments. The hydroxylamine hydrochloride was a commercial "C.P." grade product. Impure hydroxylamine hydrochloride recovered from experiments was recrystallized twice from ethanol before reuse.

Both commercial "C.P." silver oxide and the freshly prepared oxide (62) were used in methylations with no apparent difference. The chloroform was a constant boiling fraction 60.5-61.2° obtained on distilling the technical grade material. Acetic acid, acetic anhydride, methyl iodide and ether were purified by standard procedures (63)(64). Because many products were isolated as syrups and could not be readily purified, it was essential that all solvents be distilled to ensure that they left no residue on evaporation.

## Reaction of Hydroxylamine Hydrochloride with Methyl-β-D-glucoside Tetranitrate in Pyridine

### Change in Optical Rotation

The change in optical rotation was observed after 1.000 g. of methyl-β-D-glucoside tetranitrate was dissolved in a pyridine solution containing 4.999 g. of hydroxylamine hydrochloride, and the total volume made up to 25 ml. with pyridine. These amounts corresponded to a molar ratio of nitrate to hydroxylamine hydrochloride to pyridine of 1:27:116.5. Polarimetric observations were made in a 1 dm. tube at 20°C. and are reproduced in Table I and in Figure 1. Because considerable color was produced in the reaction, it was impossible to obtain accurate readings after about 20 hours. If a longer tube, or if 6 g. of hydroxylamine hydrochloride were used this period of time became considerably shorter. (Table I, page 56)

### The Rate of Gas Evolution

The determinations of the rate of gas evolution were carried out under the Toricellian vacuum of a Lunge Nitrometer, using clean dry mercury as the confining liquid. In each experiment 2.0 g. of tetranitrate was added to approximately 12 g. of hydroxylamine hydrochloride in 50 to 60 ml. of anhydrous pyridine. It was observed that the total volume of gas obtained as well as the volumes at specific times varied appreciably depending upon factors such as total volume of pyridine, the lengths of time between readings, the frequency and violence of shaking the reaction vessel, and the magnitude of the vacuum applied.

TABLE I

OPTICAL ROTATION OF METHYL-β-D-GLUCOSIDE TETRANITRATE IN PYRIDINE-HYDROXYLAMINE HYDROCHLORIDE

| Time (hrs.) | α (obs.) | $[\alpha]_D^{20}$ |
|---|---|---|
| 0.25 | .13 | 3.25 |
| 0.5 | .07 | 1.75 |
| 1.0 | .01 | 0.25 |
| 1.6 | -.09 | -2.25 |
| 2.16 | -.15 | -3.75 |
| 3.0 | -.22 | -5.50 |
| 3.3 | -.26 | -6.50 |
| 4.9 | -.32 | -8.00 |
| 7.9 | -.39 | -9.75 |
| 11.5 | -.39 | -9.75 |
| 22.5 | -.24(a) | -6.0 |
| 25.0 | -.18(a) | -4.5 |
| 26.4 | -.14(a) | -3.5 |
| 27.5 | -.13(a) | -3.2 |
| 29.5 | -.13(a) | -3.2 |

(a) The solution was colored sufficiently so that accurate observations were impossible. However there was no doubt that the rotation had risen again after having been constant for several hours.

Curves of the same general outline were always obtained. Three plots, corrected for the calculated volume of pyridine assumed to be present (65), are reproduced in Figure 2. Measurable quantities of gas were still being evolved after 48 hours at which time values ranging from 1.2 to 1.5 moles per mole of glucoside were observed.

A blank experiment, omitting the tetranitrate, showed that under these conditions the volume of gas given off from 60 ml. of pyridine and 12.0 g. of hydroxylamine hydrochloride was less than 0.3 ml.

The following experiment was performed in an effort to gain a rough estimate of the solubility of nitrous oxide in the reaction solution. Commercial nitrous oxide (137.8 ml.) was added to the gas burette of the Lunge nitrometer, the bulb of which contained a solution of 11.8 g. of hydroxylamine hydrochloride in 59.7 g. of pyridine. The gas was then placed in contact with the pyridine solution at atmospheric pressure for 17 hours, at which time the volume unabsorbed was 90.5 ml. After removal of this gas from the reaction vessel and application of the Toricellian vacuum to the solution for 10 days, the total residual volume of gas was 91.7 ml. That is 33.5% of the original nitrous oxide was dissolved in an irreversible way.

Analysis of the Evolved Gas

On mixing the evolved gas with air no brown color was produced and in consequence little or no nitric oxide was present. The quantitative analysis of the gas was carried out in a Fisher Technical Model Orsat apparatus fitted with pipets containing potassium hydroxide, potassium pyrogallate, acid cuprous sulfate-β-naphthol (66) and ethanol, for absorption of carbon dioxide, oxygen, carbon monoxide, and nitrous oxide, respectively. Five per cent sulfuric acid solution was used as the confining liquid. In a preliminary experiment it was shown that the gas

was somewhat soluble in the first three of the above-mentioned reagents and was highly soluble in ethanol, or had solubilities indicative of nitrous oxide. The solubility of nitrous oxide was known to be 2.77 ml. per 1 ml. of ethanol at 26°C. (67). This fact was utilized in analyzing quantitatively for nitrous oxide. To remove all the nitrous oxide a measured volume of the reaction gas was first passed in and out of the alcohol pipet until no further absorption occurred, and then was passed through each of the other three reagents. The remaining gas was assumed to be nitrogen. The results for commercial nitrous oxide and the reaction gas are recorded in Table II.

TABLE II

ANALYSIS OF COMMERCIAL NITROUS OXIDE AND THE GAS FROM THE METHYL-β-D-GLUCOSIDE TETRANITRATE - HYDROXYLAMINE HYDROCHLORIDE - PYRIDINE REACTION (a)

| | |
|---|---|
| Initial Volume of Commercial Nitrous Oxide | 99.8 ml. |
| Initial Volume of Reaction Gas | 98.5 ml. |

| Absorbent | Residual Volume (ml.) Commercial Gas | Reaction Gas |
|---|---|---|
| Ethanol | 10.0 | 30.5 |
| Potassium Hydroxide | 9.9 | 30.2 |
| Potassium Pyrogallate | 8.6 | 27.4 |
| Acid Cuprous Sulfate-β-Naphthol | 8.6 | 27.3 |
| Potassium Hydroxide (repass) | 8.4 | 27.3 |

(a) Methyl-β-D-glucoside tetranitrate (2.097 g.) and 12.062 g. of hydroxylamine hydrochloride in 60 ml. of anhydrous pyridine for 26 hours.

Nitrous oxide is reduced by hydrogen in the presence of a glowing platinum wire to yield nitrogen and water. The commercial nitrous oxide was treated with hydrogen in the slow combustion pipet of the Orsat apparatus, and the results of four consecutive determinations are shown in Table III. The percentage of nitrous oxide was calculated from the following equation:

$$\% N_2O = \frac{V_{N_2O} + V_{H_2} - V_{final}}{V_{N_2O}} \times 100$$

where $V_{N_2O}$ was the initial volume of nitrous oxide,
$V_{H_2}$ the initial volume of hydrogen
and $V_{final}$ the final volume after combustion.

TABLE III

ANALYSIS OF COMMERCIAL NITROUS OXIDE BY COMBUSTION WITH HYDROGEN

| | Experiment | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| $V_{N_2O}$ | 42.1 | 52.1 | 55.7 | 58.25 |
| $V_{H_2}$ | 49.2 | 65.0 | 65.0 | 75.15 |
| $V_{final}$ | 50.8 | 68.5 | 69.7 | 78.8 |
| $V_{N_2O} + V_{H_2} - V_{final}$ | 40.5 | 48.6 | 51.0 | 54.6 |
| % Nitrous Oxide | 96.0 | 93.4 | 91.6 | 93.9 |
| Mean % Nitrous Oxide | 94.0 | | | |

The molecular weights of the gases were determined in a gas density apparatus similar to that described by Daniels, Mathews and Williams (31). The reaction gas was passed from the nitrometer burette through a calcium chloride tube and a "Dry Ice" trap into a previously counterpoised and evacuated bulb of known volume. After recording the pressure and temperature of the gas, the bulb was closed by means of a stopcock, wiped with a moist chamois cloth, and allowed to stand at room temperature for ten minutes. The counterpoise was a second bulb of similar dimensions containing air at atmospheric pressure but otherwise treated in the same manner, and the weight of the gas sample was determined by difference. The molecular weight M was calculated from the equation:

$$M = \frac{g \cdot T \cdot R}{p \cdot V}$$

where g was the sample weight in grams,
T the absolute temperature,
R the universal gas constant, 82.07,
p the pressure in atmospheres
and V the volume in ml.

Results of the determinations on commercial nitrous oxide and the gas evolved from the methyl-β-D-glucoside tetranitrate - hydroxylamine hydrochloride - pyridine mixture are recorded in Table IV.

The molecular weight of the reaction gas was calculated as follows from the Orsat analysis (Table II) on the assumption that the gas was a mixture of nitrous oxide and nitrogen.

TABLE IV

GAS MOLECULAR WEIGHT DETERMINATIONS

| | Commercial Nitrous Oxide | Reaction Gas [a] | Commercial Nitrogen |
|---|---|---|---|
| Volume (V) | 153.5 ml. | 153.5 ml. | 153.5 ml. |
| Pressure (p) | 550.0 mm. | 265.0 mm. | 608.0 mm. |
| Temperature (T) | 297.2°K. | 297.8°K. | 298.9°K. |
| Weight of Sample (g.) | 0.1953 g. | 0.0860 g. | 0.1405 g. |
| Molecular Weight (M) | 42.9 | 39.2 | 28.07 |

(a) From methyl-β-D-glucoside tetranitrate (2.00 g.) and 12.00 g. of hydroxylamine hydrochloride in 50 ml. of pyridine after 73 hours.

---

Calculations -

1. The molecular weight assuming the gas was saturated with pyridine vapor:

$$M = \frac{27.3}{98.5} \cdot 28.0 + \frac{2.6^{(a)}}{98.5} \cdot 91.0 + \frac{68.6^{(b)}}{98.5} \cdot 44.0 = \underline{40.85}$$

2. The molecular weight assuming all pyridine was removed:

$$M = \frac{27.3}{95.5^{(c)}} \cdot 28.0 + \frac{68.6^{(b)}}{95.5^{(c)}} \cdot 44.0 = \underline{39.5}$$

(a) Calculated from the vapor pressure of pyridine at 299.1°K. (65).

(b) and (c), page 62.

Calculations (cont'd)

(b) The volume of nitrous oxide was calculated by subtracting the final residual volume plus the calculated volume of pyridine vapor, from the original volume of gas.

(c) The measured total volume was corrected for the calculated volume of pyridine vapor present when the Orsat analysis was carried out.

In the first determinations no calcium chloride tube was used in the molecular weight apparatus. The values obtained were 41.1 and 41.2 in two different experiments, whereas the value calculated from the Orsat analysis (Table II), on the assumption that the gas was saturated with pyridine vapor, was 40.85. For the determination of the reaction gas recorded in Table IV, the original Dry Ice trap was replaced by a second smaller and presumably more efficient one, and in addition the gas was passed through a calcium chloride drying tube.

The Orsat analysis and the molecular weight of the gas from another experiment which was designed to establish the effect of pyridine vapor, are recorded in Table V. The gas density determination was performed 11 days after the evolution had taken place, and no Dry Ice was used. Analysis of the gas evolved in the same reaction, between 10 and 25 hours, showed that of an original 92.8 ml. of gas all but 17.0 ml. was absorbed by the Orsat reagents. This result corresponded to a mixture of 81% nitrous oxide and 19% nitrogen.

The actual molecular weight of the commercial nitrous oxide was not definitely established. A second determination

TABLE V

THE ANALYSIS AND MOLECULAR WEIGHT OF THE GAS FROM METHYL-β-D-GLUCOSIDE TETRANITRATE - HYDROXYLAMINE HYDROCHLORIDE - PYRIDINE AFTER 10 HOURS (a)

Orsat Analysis

| | |
|---|---|
| Original Volume | 62.6 ml. |
| Volume after Ethanol | 22.2 ml. |
| Final Volume (after passage through the other pipets) | 20.0 ml. |

Molecular Weight (experimental)

| | |
|---|---|
| Volume (v) | 153.5 ml. |
| Pressure (p) | 293.6 mm. |
| Temperature (T) | 297.3°K. |
| Weight of Sample (g.) | 0.0982 g. |
| Molecular Weight (M) | 40.4 |

Molecular Weight

(Calc'd from Orsat Values)(b)

$$M = \frac{20.0}{62.6} \cdot 28.0 + \frac{41.0}{62.6} \cdot 44.0 + \frac{1.6^{(c)}}{62.6} \cdot 91.0$$

$$M = 40.4$$

(a) The gas was evolved in 10 hours from 4.01 g. of methyl-β-D-glucoside tetranitrate and 23.25 g. of hydroxylamine hydrochloride in 110 ml. of pyridine contained in the Lunge nitrometer.

(b) Calculated on the assumption that the gas was a mixture of nitrous oxide and nitrogen saturated with pyridine vapor.

(c) The calculated volume of pyridine vapor in 62.6 ml. of gas at 297.3°K. was 1.6 ml.

gave the value 42.2. Several other attempts yielded only lower values. The discrepancies were attributed to the rubber hose connection from the cylinder, which must have allowed air to enter. Unfortunately the remaining gas was accidentally lost so that this point was not cleared up. Correct values for commercial nitrogen were realized in three different determinations so that there was no doubt about the accuracy obtainable from the molecular weight apparatus.

## Isolation of the Carbohydrate Products

The same general reaction conditions were maintained in all experiments. A solution containing 12.0 g. of hydroxylamine hydrochloride (previously dried at 100° for one hour) in 50 ml. of freshly distilled pyridine was added to 2.00 g. of methyl-β-D-glucoside tetranitrate in a 125 ml. Erlenmeyer flask. This was a molar ratio of 1 of nitrate to 32.2 of hydrochloride to 116.5 of pyridine. A one-holed rubber stopper connected to a calcium chloride drying tube protected the reaction from outside moisture. The reaction time was varied from 10 to 48 hours during the course of the research and several different procedures of isolation were attempted. Usually each 2 g. run was worked up separately. However, after the results for a particular method were fully known, and larger quantities of products were required, several runs would be combined to speed the isolations. The yields reported are based on 2 g. of tetranitrate unless otherwise stated.

Isolation involving Ether Extraction
After Sulfuric Acid Neutralization
(Syrup A)

The reaction mixture was poured into 150 ml. of distilled water. The aqueous mixture was rendered just acid to Congo Red indicator by the addition of dilute sulfuric acid (4-6N) and allowed to stand overnight at 5°C. Filtration removed some unreacted tetranitrate -- at 21 hours as much as 0.15 g. was recovered, while after 44 hours 0.03 g. was obtained. The filtrate was extracted with two 100 ml. and two 50 ml. volumes of ether. The combined extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated to give a light yellow viscous syrup (Syrup A). Yield on the average was 0.76 g. after 44 hours. The analyses of samples of Syrup A isolated at different reaction times were approximately constant.

Anal.: Calc'd for methylglucoside trinitrate, $C_7H_{11}O_{12}N_3$:
$OCH_3$, 9.42%; N, 12.8%.
Found: $OCH_3$, 9.34, 9.45%; N (micro-Kjeldahl), 11.9, 12.0%; N (nitrate), 11.3, 11.5%.

Syrup A did not crystallize after standing in a desiccator with phosphorus pentoxide for several months.

Continuous ether extraction after the manual ether extraction of the aqueous solution yielded less than 0.03 g. This material in one experiment appeared to partially crystallize, but attempts to separate the crystalline substance from the syrupy contaminant were unsuccessful.

Extraction with four 100 ml. volumes of n-butanol, directly following the manual ether extraction of the product from a 21-hour run, yielded 0.35 g. of a dark brown syrup with the composition $OCH_3$, 10.1, 10.6%, and N, 14.1, 14.8%. This substance on burning left an ash, did not crystallize and was not studied further.

Four grams of nickel nitrate hexahydrate was added to the aqueous solution from a 44-hour reaction, after extraction with ether and neutralization with dilute ammonium hydroxide. The brown precipitate (0.36 g.) contained methoxyl group, nickel and non-nitrate nitrogen in the ratios 1:1.02:3.75.

Anal.: $OCH_3$, 8.41, 8.48%; N (micro-Kjeldahl), 15.0, 15.0%.
N (nitrate), 0.0%; Ni, 16.1, 16.5%.

In a second 44-hour experiment, 7.5 ml. of a 10% solution of nickel sulfate hexahydrate was added and the resulting brown flocculent precipitate weighed 0.51 g.

Anal.: $OCH_3$, 8.65, 8.70% N (micro-Kjeldahl), 13.9, 14.0%;
Ni, 16.0, 16.6%.

The molar ratios of $OCH_3$ : Ni : N were 1:0.99:3.56. On treatment with dilute sulfuric acid the material slowly dissolved.

The maximum total recovery of methoxyl group achieved by ether extraction combined with nickel salt precipitation was 73%.

Isolation by Removal of Hydroxylamine Hydrochloride with Silver Oxide and Carbonate

After removal of the relatively large amount of excess hydroxylamine hydrochloride precipitated on addition of chloroform (250 ml.) to the reaction medium, 5 g. to 10 g. of silver carbonate or oxide was added and the mixture stirred until a negative test for chloride ion was given. Similar results were obtained with both silver salts, either with slow addition over a period of 2 to 4 hours, or by complete addition at one time. In every case the hydroxylamine was decomposed and silver ion was introduced into the chloroform solution.

After filtration, the green chloroform solution was extracted with six 50 ml. volumes of water. A brown silver salt which collected at the interface between the two layers was removed by filtration. Previous centrifugation, which caused the salt to form into a cake, facilitated its removal. In one 23-hour experiment the yield of this salt was 0.10 g., $OCH_3$ 7.5%. During the evaporation of the combined aqueous extracts 0.24 g. of a yellow powder, $OCH_3$, 6.5%, precipitated. In the same experiment after filtration and complete evaporation a levorotatory syrup (0.5 g., $OCH_3$ 2.5%), which darkened on drying, was obtained. This syrup gave positive tests for silver and nitrate, and crystals of silver nitrate formed in the dry product. Another treatment with silver oxide of the syrup in chloroform-pyridine solution yielded a further 0.10 g. of solid product. The final residue on complete evaporation was mainly silver nitrate.

The chloroform extract from the same 23-hour experiment on evaporation yielded 0.78 g. of an ether-soluble syrup, as well as 0.06 g. of a dark silver salt which was removed from the flask by solution in pyridine. Hydrogenolysis of an unweighed portion of the syrup, by the procedure described in detail later under the hydrogenolysis of Syrup A, yielded crude methyl-β-D-glucoside.

It was found that the methoxyl contents of the silver products precipitated during the aqueous extraction and subsequent evaporation of the filtered aqueous solution were in the range 5.5 to 7.5%, and the nitrogen contents were 12 to 13%. Products with methoxyl values as low as 2% were obtained on retreatment of the water soluble syrup. In an ash determination on one salt ($OCH_3$, 4.25%, N, 13.3%) a 39.5% residue was left.

Isolation by Dilution with Chloroform, and Aqueous Extraction.
(Syrups B and C)

Chloroform (250 ml.) was added to the reaction solution and the mixture was left overnight at 5°C. After filtration of the precipitated hydroxylamine hydrochloride (10.0 g.) the solution was extracted with four volumes of water, amounting altogether to 100-200 ml., until a negative chloride test was obtained for the chloroform layer with alcoholic silver nitrate reagent. On complete evaporation at reduced pressure the chloroform solution yielded 1.17 to 1.18 g. (44-hour reaction time) of a viscous green syrup (Syrup B). This material on long dry-

ing turned into a brown glass.

Anal.: $OCH_3$, 10.0, 10.1%; N (micro-Kjeldahl) 13.3, 13.3%; N (nitrometer), 9.6, 9.9%.

The combined aqueous extracts were passed through a column, 18 by 3.5 cm., of Amberlite IRA-410 cation exchange resin, a product of Rohm and Haas Company. The resin was previously activated by treatment with 10% sodium hydroxide followed by distilled water, which was added until the aqueous effluent was neutral. After the aqueous extract was on the column, distilled water was added until no further pyridine was removed, and then the column was allowed to drain. A negative test for both nitrate and chloride ions was obtained from the effluent. The upper portion of the column, which retained the acidic substances, became lighter in color than the original brown Amberlite resin, and a narrow red band formed at the junction between the two zones. Gas from the decomposed hydroxylamine hydrochloride tended to disrupt the top part of the column if the diameter of the tube were small.

The dried Amberlite resin was transferred to a beaker. Acetic acid (80 to 100 ml.) was added, the mixture stirred for an hour and filtered. Another 80-100 ml. of acetic acid was added to the resin and the extraction process repeated. The resin was washed with 100 ml. of ethanol in some experiments and with an equal volume of water in others. It was observed that dilute acetic acid (15% or less) failed to remove the car-

bohydrate material from the resin. The combined extracts, which gave a negative chloride test, were evaporated at reduced pressure to yield 0.25 to 0.28 g. (reaction time 40-48 hours) of a light brown syrup (Syrup C) which on complete drying became mainly amorphous. Methoxyl contents varying from 10.4 to 7.5% were found. The methoxyl value of one sample dropped from 8.8 to 7.5% during storage for four weeks in a desiccator over phosphorus pentoxide. Most values determined shortly after isolation were above 9.0%. Nitrogen analyses varied from 15.3 to 18.0%. The product with the highest methoxyl content observed gave the following figures: $OCH_3$, 10.4, 10.4%; N (micro-Kjeldahl), 15.3, 15.4%; N (nitrometer), 0.0%. The product with the lowest methoxyl content gave this analysis: $OCH_3$, 7.59, 7.55%; N (micro-Kjeldahl), 18.4, 17.7%. Syrup C reduced Fehling's solution and formed water-insoluble nickel or silver salts. It gave a dark red-brown color with ferric chloride. A neutralization equivalent of approximately 300 was obtained for one sample.

The total recovery of methoxyl by this method was greater than 85%. At least a portion of the remaining 15% had been inadvertently removed with the excess hydroxylamine hydrochloride, for the concentrated ethanolic mother liquors from the purification of the recovered hydrochloride gave a positive nitrate test and were levorotatory.

## Experiments on the Isolated Syrups A, B, and C, and the Silver Salts

### Hydrogenolysis of Syrup A

Syrup A (0.44 g.) in ethanol solution was hydrogenated, according to Kuhn's procedure (68), in the Adams Low-Pressure Hydrogenator, using palladized-charcoal catalyst, until a negative nitrate test with the diphenylamine reagent was obtained. The solution after filtration was evaporated at reduced pressure to a yellow syrup weighing 0.28 g. which crystallized readily without seeding. The theoretical yield of methyl-β-D-glucoside hemihydrate calculated from the methoxyl content of Syrup A was 0.27 g. Following recrystallization of the crude product from ethanol, a mixed melting point with authentic methyl-β-D-glucoside (m.p. 105-108°) showed no depression.

A similar hydrogenolysis of 0.429 g. of methyl-β-D-glucoside tetranitrate gave 0.229 g. or greater than 98% of theory of crude methyl-β-D-glucoside.

### Methylation of Syrup A

Syrup A (2.68 g., 21-hour reaction time) was dissolved in 75 ml. of methyl iodide. After the addition of 10 g. of dry silver oxide and 4 g. of powdered Drierite, the mixture was heated under reflux on a steam bath for 8 hours, then filtered and the solids washed on the funnel, with boiling chloroform. Evaporation of the combined filtrates yielded 2.68 g. of a light-yellow mobile syrup (Syrup A-1). Remethylation of a portion of Syrup A-1 in similar conditions did not increase the

methoxyl content.

Anal.: Calc'd for methyl-methyl-β-D-glucoside-trinitrate

$C_8H_{13}O_{12}N_3$: $OCH_3$, 18.1%; N, 12.2%.

Found: $OCH_3$, 18.3, 18.1%; N, 11.1, 11.1%.

The product from a 44-hour reaction after three similar methylations had the same nitrogen and methoxyl contents.

Hydrogenolysis of Syrup A-1

Syrup A-1 (0.47 g. (see above) ) was hydrogenated in alcohol solution as previously described for Syrup A. The pressure became constant in less than one hour and the solution then gave a negative test for nitrate. After removal of the catalyst by filtration, the solution was evaporated to give 0.31 g. of a viscous yellow syrup (Syrup A-2).

Anal.: $OCH_3$, 29.4, 29.4%; N (micro-Kjeldahl) 0.61, 0.46%.

Oxidation of Syrup A-2 (0.1456 g.) with 0.14 N sodium metaperiodate solution was found to consume $0.63 \times 10^{-3}$ mole of oxidant. If a molecular weight of 208 were assumed for Syrup A-2, then that amount of periodate corresponded to a consumption of 0.90 mole per mole of sugar. The rotation of the oxidizing solution reached a constant value $\left[\alpha\right]_D^{20}$ - 80.6° within less than 45 minutes. Since a portion of Syrup A-2 was water-insoluble and separated on the walls of the reaction flask, the above specific rotation value was probably low in magnitude. Specific rotations found by Hayward after oxidation in sodium meta-

periodate solution of approximately the same normality were $[\alpha]_D^{20}$ -125.8° for methyl-4-methyl-β-D-glucoside, and $[\alpha]_D^{20}$ -122.1° for methyl-β-D-glucoside. Titration to the initial pH required 0.36 x $10^{-4}$ mole of base. This corresponded to .05 mole of formic acid per mole of sugar, based on a molecular weight of 208. A precipitate with dimedon, m.p. 170-173°, weighed 0.1355 g. The significance of this product was unknown since dimedon itself melted at 148-149°, while the pure formaldehyde-dimedon compound was reported (57) to melt at 189-190°.

Chromatography of Acetylated Syrup A-2

A portion of Syrup A-2 which was previously extracted with chloroform during attempts to achieve crystallization, was acetylated with an excess of acetic anhydride in pyridine solution. The syrupy product (0.45 g.) was chromatographed by the flowing technique, using a glass column 2.2 cm. in diameter containing 50 g. of alumina (Brockmann (69) Grade III). The column was packed by the "wet" method (70), the adsorbent being sprinkled in slowly with vigorous tapping of the glass tube, which contained sufficient benzene to immerse completely the added solids. A solution of the acetylated syrup in a few ml. of benzene was then placed on the column. Development was first accomplished with anhydrous benzene. When the pure solvent removed no more material, addition of a small percentage of ethanol resulted in the removal of another fraction. Pure ethanol served as the final developer. Fractions approximately 50 ml. in volume were collected, evaporated under reduced pressure, and weighed. The

results are summarized in Table VI.

TABLE VI

CHROMATOGRAPHY OF ACETYLATED SYRUP A-2

| Fraction | Solvent | Volume (ml.) | Weight Recovered (g.) | Remarks |
|---|---|---|---|---|
| 1 | Anhydrous benzene | 400 | 0.04 | syrup |
| 2 | Anhydrous benzene | 500 | 0.12 | crystalline |
| 3 | 2% Ethanol-benzene | 60 | 0.21 | syrup |
| 4 | 2% Ethanol-benzene | 60 | 0.02 | syrup with trace of crystals |
| 5 | Ethanol | 100 | 0.00 | |
| | | Yield: | 0.39 | |
| | | Recovery: | 87%. | |

The crystalline substance in Fraction 2 after recrystallization from ether-pentane melted at 108-109° and had a specific rotation of $[\alpha]_D^{20}$ - 34.8° (chloroform, c = 2, l = 2).

Anal.: Calc'd. for methyl-4-methyl-2,3,6-triacetyl-β-D-glucoside, $C_{14}H_{22}O_9$: $OCH_3$, 18.6%.

Found: $OCH_3$: 18.4, 18.5%.

A mixed melting point with methyl-4-methyl-2,3,6-triacetyl-β-D-glucoside, prepared by acetylation of authentic methyl-4-methyl-β-D-glucoside *, was unchanged. The reported

* Kindly supplied by Dr. L.D. Hayward

constants for the triacetate are m.p. 107.5-108.5°, $[\alpha]_D^{20}$ -34.8° (2), m.p. 107-108°, $[\alpha]_D^{20}$ - 32.8° (71), m.p. 106°, $[\alpha]_D^{20}$ - 34.0°, (72) and m.p. 105-106°, $[\alpha]_D^{20}$ - 34.9° (41).

Fraction 3 did not crystallize and had a methoxyl content of 23.0%.

A few mg. of a crystalline substance, m.p. 137-140°, was isolated from Fraction 4 and is described under the methylated, denitrated, acetylated Syrup B-1.

Methylation of Silver Salts

Several preparations of silver salts, the isolation of which from the hydroxylamine hydrochloride - methylglucoside tetranitrate reaction was described earlier, were methylated.

The silver salt (0.69 g., $OCH_3$ 5.7%, N, 12.0%) 35 ml. of methyl iodide, 5 g. of silver oxide and 4 g. of Drierite were heated at reflux temperature for 12 hours. The cooled mixture was filtered, and the solid residue was washed on the funnel with boiling chloroform. The combined filtrates on evaporation yielded 0.35 g. of a dark-brown viscous syrup which gave a negative test for nitrate with the diphenylamine reagent.

Anal.: $OCH_3$, 32.8, 32.5%; N (micro-Kjeldahl), 13.5, 13.3%.

Several such products, with approximately the same methoxyl and nitrogen contents, were combined, and remethylated under similar conditions. The resulting dark-brown, tough syrup gave the foll-

owing analysis: $OCH_3$, 33.4, 34.4%; N (micro-Kjeldahl), 12.3, 12.1%.

### Hydrolysis of the Remethylated Silver Salts

Fuming hydrochloric acid (12 ml.), prepared by passing dry hydrogen chloride gas into concentrated hydrochloric acid at 0°, was added to 0.67 g. of the above remethylated syrup. Solution occurred gradually. After 10 days in a tightly stoppered flask the dark-brown reaction mixture was evaporated to yield a dark residue which smelled somewhat like burning sugar. This residue was extracted with two 25 ml. volumes of ethanol. After addition of 75 ml. of ether to the second extract, a small amount of black precipitate was removed by filtration, and the filtrate was evaporated. The residue was a dark syrup containing a small quantity of a white crystalline substance which, following recrystallization from hot ethanol, gave a strong chloride test, did not melt below 210° and when heated on a spatula appeared to volatilize directly.

### Division of Syrup B into Four Fractions by Means of Different Solvents

The following experimental procedure is summarized in Figure 3. Syrup B (1.17 g.) was dissolved by the addition of both chloroform (30 ml.) and water (15 ml.). The blue chloroform solution after further extraction with four 10 ml. volumes of water was evaporated to give 0.73 to 0.76 g. of a viscous light-colored syrup (Syrup B-1).

Anal.: $OCH_3$, 8.70, 8.65%; N (micro-Kjeldahl), 11.8, 11.4%.

The combined yellow aqueous extracts were then extracted four times with 10 ml. volumes of chloroform. The chloroform back-extracts on evaporation yielded 0.06 to 0.07 g. of a light-colored viscous syrup (Syrup B-2).

Anal.: $OCH_3$, 9.65, 9.70%; N (micro-Kjeldahl), 11.7, 11.6%.

The aqueous residue was continuously extracted with peroxide-free ether until the water layer gave a negative nitrate test. The ether extract contained 0.27 to 0.30 g. of a golden syrup (Syrup B-3) which on complete drying in vacuo was usually obtained in an amorphous, porous condition. This product reduced Fehling's solution, formed water-insoluble yellow silver, and brown nickel, derivatives, and gave a dark red-brown color with ferric chloride solution.

Anal.: $OCH_3$, 13.3, 13.1%; N (micro-Kjeldahl), 16.2, 16.1%;
N (nitrometer), 2.3, 2.5%.

Samples (0.05 to 0.07 g.) of Syrup B-3 easily deflagrated on contact with strong sulfuric acid; gas was evolved and the mixture blackened. The volume of nitric oxide produced in the nitrometer after deflagration was small. Successful nitrate-nitrogen determinations were made by adding the sulfuric acid slowly to the finely-powdered sample.

The ether-extracted aqueous solution on evaporation yielded 0.08 to 0.09 g. of a brown, nitrate-free syrup (B-4), which reduced Fehling's solution, and yielded insoluble nickel and silver derivatives. The analyses of Syrup B-4 varied in a

similar fashion to those of Syrup C. The sample with the highest observed methoxyl content gave the following values - $OCH_3$, 10.2, 10.2%, N (micro-Kjeldahl), 15.5, 15.6%.

It was found that variations in the volumes of solvents in these extraction procedures resulted in relative amounts of Syrups B-1, 2, 3 and 4 that were different from those noted above.

Isolation of Crystalline Material from Syrup B-1

Hydrogenation of 0.63 g. of Syrup B-1 with conditions similar to those described for the hydrogenolysis of Syrup A, yielded 0.37 g. of a crude crystalline product, which after recrystallization from ethanol caused no depression in melting point on admixture with authentic methyl-β-D-glucoside.

Methylation of 4.90 g. of Syrup B-1 by heating under reflux for 24 hours with 40 ml. of methyl iodide, 20 g. of silver oxide and 10 g. of powdered Drierite yielded 4.92 g. of product (Syrup B-1a) with a methoxyl content of 21.2, 21.3%. Hydrogenolysis of Syrup B-1a in ethanol solution with a palladium on charcoal catalyst was not quickly accomplished owing to poisoning of the catalyst. Several filtrations followed by additions of fresh catalyst were necessary before the reaction proceeded smoothly. The reduced product isolated on evaporation of the filtered ethanol solution was dissolved in 30 ml. of anhydrous pyridine. Acetic anhydride (20 ml.) was added and the resulting clear solution allowed to stand at 0°C. for 2 days. The solution was then poured into ice-water and extracted with chloroform. The chloroform extract after washings with dilute sodium bicarbonate solution and water, and evapora-

tion at reduced pressure, yielded 3.97 g. of a viscous light-colored syrup (Syrup B-1b) with a methoxyl content of 22.0%.

Syrup B-1b (3.90 g.) was chromatographed employing the flowing technique on a column 2.2 cm. in diameter containing 135 g. of alumina (Brockmann Grade III). The experimental procedure was similar to that described previously in the chromatography of acetylated Syrup A-2. Anhydrous benzene (3000 ml.) was passed through the column until no further material was being eluted. About 1.5 g. of a crystalline product was obtained on evaporation of the combined benzene effluent, which was found to be methyl-4-methyl-2,3,6-triacetyl-β-D-glucoside. Ethylene dichloride (10%) in benzene removed no appreciable quantity, but 1% ethanol-benzene (730 ml.) took off 0.17 g. of a light-brown syrup with a methoxyl content of 25.5, 25.8%. A third fraction, 0.61 g. of a brown syrup containing a small amount of crystalline material, was obtained by combining the material removed by 300 ml. of 10% ethanol-benzene and 100 ml. of pure ethanol. The crystals were separated by dissolving the syrupy contaminant in ethyl acetate. Several recrystallizations from ethyl acetate - pentane yielded 0.03 g. of an apparently pure substance as white needles which melted sharply at 141.5-142.5°, and rotated $\left[\alpha\right]_D^{20}$ - 40.7° (chloroform, c = 1.53, l = 2).

Anal.: Calc'd for a methyl-monomethyl-triacetyl-β-D-glucoside, $C_{14}H_{22}O_9$: $OCH_3$, 18.6%. Calc'd for a methyl-dimethyl-diacetyl-β-D-glucoside, $C_{13}H_{22}O_8$: $OCH_3$, 30.4%. Calc'd for an equimolar mixture of the above two com-

pounds: $OCH_3$, 24.5%.

Found: $OCH_3$, 24.6, 24.6%; N (micro-Kjeldahl), 0.0%

The reliability of these methoxyl values was specially checked by control analyses of pure methyl-β-D-glucoside tetranitrate and of pure methyl-4-acetyl-β-D-glucoside-trinitrate in the same apparati with the same hydrogen iodide-phenol mixtures.

A few mg. of methyl-2,4-dimethyl-β-D-glucoside * was acetylated with acetic anhydride and pyridine. The product isolated by chloroform extraction after pouring into water was a clear syrup which did not crystallize.

Chromatography of Acetylated Syrup B-2

Syrup B-2, 0.69 g., was acetylated in a mixture of 20 ml. of anhydrous pyridine and 12 ml. of acetic anhydride at 0°. After 39 hours the light-colored solution was poured slowly into ice water. The mixture was stirred for several hours and then extracted with chloroform. The chloroform extract was washed with dilute sodium bicarbonate solution, and with water, and evaporated to yield 0.73 g. of a light-colored viscous syrup. This syrup was chromatographed on a column 1.8 cm. in diameter containing 40 g. of alumina (Brockmann grade III) as described earlier for Syrups A-3 and B-1b. The results are summarized in Table VII.

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* Kindly supplied by Dr. L.D. Hayward

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TABLE VII

CHROMATOGRAM OF ACETYLATED SYRUP B-2

| Fraction | Solvent | Volume (ml.) | Weight of Material (g.) |
|---|---|---|---|
| 1 | Benzene | 250 | 0.37 |
| 2 | 2% Ethanol-benzene | 250 | 0.16 |
| 3 | 20% Ethanol-benzene | 275 | 0.02 |
| 4 | 10% $H_2O$-ethanol | 150 | 0.05 |
| | | Yield: | 0.60 |
| | | Recovery: | 82% |

None of these fractions crystallized and the largest, Fraction 1, was therefore rechromatographed, the results being recorded in Table VIII.

Fractions 2 and 3 of the rechromatographed syrup partially crystallized. The syrupy contaminant was removed by partial solution in ethanol, and the remaining crystals, after several recrystallizations from ethanol-water, weighed 0.06 g., with m.p. 95 to 96° (corr.), $[\alpha]_D^{20}$ + 0.3° ( c = 1.64, l = 2, chloroform). The values reported by Bell and Synge (41) for methyl-4-acetyl-β-D-glucoside-2,3,6-trinitrate were m.p. 93-94°, $[\alpha]_D^{19}$ + 0.4° (c = 3, l = 2, chloroform).

Anal.: Calc'd for methyl-4-acetyl-β-D-glucoside-2,3,6-trinitrate, $C_9H_{13}O_{13}N_3$: $OCH_3$, 8.36%; N, 11.3%.

Found: $OCH_3$, 8.45, 8.50%; N (micro-Kjeldahl), 10.1, 10.0%.

TABLE VIII

RECHROMATOGRAM OF ACETYLATED SYRUP B-2 (a)

| Fraction | Solvent | Volume (ml.) | Weight (g.) | Remarks |
|---|---|---|---|---|
| 1 | Benzene | 30 | 0.007 | syrup |
| 2 | Benzene | 32 | 0.170 | partially crystallized |
| 3 | Benzene | 34 | 0.018 | partially crystallized |
| 4 | Benzene | 34 | 0.012 | syrup |
| 5 | Benzene | 30 | 0.005 | syrup |
| 6 | Benzene | 34 | 0.003 | syrup |
| 7 | Benzene | 50 | 0.006 | syrup |
| 8 | 10% Ethanol-benzene | 100 | 0.057 | syrup |

(a) 0.37 g. of syrup on a column 1.8 cm. in diameter containing 30 g. of alumina.

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The nitrogen values were lower than expected, but insufficient material remained to permit investigation of the cause.

A sample of methyl-β-D-glucoside-3,6-dinitrate * was acetylated by the procedure for Syrup B-2. Since the product did not crystallize readily, it was chromatographed by the flowing technique on an alumina column. The crystalline diacetate was not removed by pure benzene, but required several per cent of ethanol in the benzene. Seeding of Fraction 2, Table VII, with methyl-2,4-diacetyl-β-D-glucoside-3,6-dinitrate (29) did not result in crystallization.

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* Kindly supplied by Dr. L.D. Hayward

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Experiments on Syrup B-3

During an early experiment in which the chloroform re-extraction, of the methylglucoside tetranitrate - hydroxylamine hydrochloride reaction mixture, giving rise to Syrup B-2 was omitted, the ether-extractable syrup (0.37 g.) after drying was extracted with chloroform to yield a fraction (0.10 g.) which after standing for several months partially crystallized. The crystalline material was not successfully purified from the surrounding syrup. Syrup B-3 itself however was never induced to crystallize, either by the use of solvents or on complete drying.

Acetylation of Syrup B-3

The following example was typical. Acetic anhydride (10 ml.) was added over a period of one hour to a solution of 0.74 g. of Syrup B-3 in pyridine at 0°. After 2 days the dark-brown mixture was poured slowly into 100 ml. of ice water with stirring. The acetylated product separated as a flocculent dark-brown precipitate which coagulated to form a sticky syrup once the stirring was halted. After several hours the mixture was extracted with 100 ml. of chloroform in 5 portions. Most of the brown color was taken into the organic solution. The chloroform extract after washings with dilute sodium bicarbonate and then water, was evaporated to yield 0.76 g. of a dark-brown product (Syrup B-3a). The chloroform-extracted aqueous solution on evaporation yielded a dark-brown product (0.18 g.) which could not be crystallized and was not investigated further.

Syrup B-3a by treatment with benzene was divided into a benzene-soluble fraction (Syrup B-3b) and a brown, mainly amorphous, benzene-insoluble fraction. In one experiment 0.76 g. of Syrup B-3a yielded 0.20 g. of the amorphous product, while in another 1.43 g. gave 0.31 g. This amorphous substance was also insoluble in chloroform, ether and water, while it was slightly soluble in ethanol and easily soluble in acetone. Reprecipitation from acetone solution with ether yielded a lighter brown product.

Anal.: $OCH_3$, 8.33, 8.42%; N (micro-Kjeldahl), 17.2, 17.4%; N (nitrate), 0.0%.

Chromatography of Syrup B-3b

Syrup B-3b was chromatographed on columns of both alumina and Florisil (magnesium trisilicate, the product of the Floridin Co., Inc.) using the flowing technique. Florisil proved more satisfactory because the recovery of the organic material was greater than from an alumina column. The florisil column was packed dry according to the procedure recommended by Wolfrom and Binkley (73), and then wetted with benzene. The sample of Syrup B-3b, dissolved in a few ml. of anhydrous benzene, was then added to the top of the tube. The results of a typical chromatogram are reproduced in Table IX. Fluorescence in ultraviolet light was utilized to follow the separation of different bands on the column.

TABLE IX

CHROMATOGRAM OF SYRUP B-3b ON FLORISIL[a]

| Fraction | Volume (ml.) | Total Volume (ml.) | Weight | mgm./ml. | Remarks |
|---|---|---|---|---|---|
| Solvent: | Anhydrous Benzene | | | | |
| 1 | 94 | 94 | .006 | 6.4 | Colorless, syrup. |
| 2 | 53 | 147 | .005 | 9.4 | do |
| 3 | 57 | 204 | .004 | 7.0 | do |
| 4 | 76 | 280 | .005 | 6.6 | do |
| 5 | 49 | 329 | .003 | 6.1 | do |
| 6 | 59 | 388 | .005 | 8.3 | do |
| 7 | 70 | 458 | .005 | 7.2 | do |
| 8 | 60 | 518 | .004 | 6.6 | do |
| 9 | 58 | 576 | .004 | 6.9 | do |
| 10 | 87 | 663 | .004 | 4.6 | do |
| 11 | 103 | 766 | .010 | 9.7 | do |
| 12 | 80 | 846 | .013 | 16.2 | do |
| 13 | 57 | 903 | .010 | 17.5 | do |
| 14 | 79 | 982 | .017 | 21.5 | do |
| 15 | 60 | 1042 | .015 | 25.0 | do |
| 16 | 75 | 1117 | .018 | 24.0 | do |
| 17 | 85 | 1202 | .019 | 22.4 | Syrup with some crystals, colorless. |
| 18 | 68 | 1270 | .017 | 25.0 | do |
| 19 | 87 | 1357 | .013 | 14.9 | do |
| 20 | 83 | 1440 | .012 | 14.5 | Mainly crystalline |
| 21 | 98 | 1538 | .011 | 11.2 | do |
| 22 | 92 | 1630 | .009 | 9.8 | do |
| 23 | 89 | 1719 | .007 | 7.8 | do |
| 24 | 94 | 1813 | .006 | 6.4 | do |
| 25 | 102 | 1915 | .007 | 6.8 | do |
| 26 | 196 | 2111 | .013 | 6.6 | do |
| 27 | 112 | 2223 | .008 | 7.1 | Syrup |
| 28 | 110 | 2333 | .006 | 5.4 | do |
| 29 | 120 | 2453 | .007 | 5.8 | do |
| 30 | 204 | 2657 | .009 | 4.4 | do |
| Solvent: | 50% Ethylene dichloride - benzene | | | | |
| 31 | 206 | 2863 | .009 | 4.4 | do |
| 32 | 100 | 2963 | .009 | 9.0 | do |

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TABLE IX (cont'd)

CHROMATOGRAM OF SYRUP B-3b ON FLORISIL(a)

| Fraction | Volume (ml.) | Total Volume (ml.) | Weight | mgm./ml. | Remarks |
|---|---|---|---|---|---|
| Solvent: Ethylene dichloride | | | | | |
| 33 | 120 | 3083 | .026 | 21.6 | Syrup |
| 34 | 100 | 3183 | .031 | 31.0 | do |
| 35 | 100 | 3283 | .018 | 18.0 | do |
| 36 | 200 | 3483 | .010 | 5.0 | do |
| 37 | 100 | 3583 | .006 | 6.0 | do |
| 38 | 200 | 3783 | .009 | 4.5 | do |
| 39 | 100 | 3883 | .003 | 3.0 | do |
| 40 | 98 | 3981 | .004 | 4.0 | do |
| Solvent: 0.4% Ethanol - ethylene dichloride | | | | | |
| 41 | 198 | 4179 | .005 | 2.5 | do |
| 42 | 200 | 4379 | .003 | 1.5 | do |
| Solvent: 1% Ethanol - ethylene dichloride | | | | | |
| 43 | 85 | 4464 | .005 | 5.8 | do |
| 44 | 94 | 4559 | .008 | 8.4 | Partly crystalline, |
| 45 | 84 | 4643 | .071 | 84.5 | brown |
| 46 | 110 | 4753 | .044 | 40.0 | do |
| 47 | 81 | 4834 | .027 | 33.4 | Brown syrup |
| 48 | 82 | 4916 | .033 | 40.0 | do |
| 49 | 81 | 4997 | .028 | 34.6 | do |
| 50 | 191 | 5188 | .041 | 21.4 | do |
| 51 | 112 | 5300 | .020 | 17.8 | do |
| 52 | 84 | 5384 | .014 | 16.6 | do |
| 53 | 100 | 5484 | .014 | 14.0 | do |
| 54 | 87 | 5571 | .008 | 9.2 | do |
| 55 | 102 | 5673 | .007 | 7.0 | do |
| 56 | 80 | 5753 | .005 | 6.2 | do |
| 57 | 87 | 5840 | .006 | 6.9 | do |
| 58 | 115 | 5955 | .010 | 8.7 | do |
| 59 | 90 | 6045 | .001 | 0 | do |
| 60 | 88 | 6133 | .007 | 7.9 | do |

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TABLE IX (cont'd)

CHROMATOGRAM OF SYRUP B-3b ON FLORISIL(a)

| Fraction | Volume (ml.) | Total Volume (ml.) | Weight | mgm./ml. | Remarks |
|---|---|---|---|---|---|
| Solvent: 1% Ethanol - ethylene dichloride | | | | | |
| 61 | 115 | 6248 | .008 | 6.9 | Brown syrup |
| 62 | 92 | 6340 | .006 | 6.5 | do |
| 63 | 289 | 6629 | .018 | 6.2 | do |
| Solvent: 4% Ethanol - ethylene dichloride | | | | | |
| 64 | 200 | 6829 | .027 | 14.5 | do |
| 65 | 115 | 6944 | .039 | 34.0 | do |
| 66 | 210 | 7154 | .024 | 11.4 | do |
| | | | Yield: | .82 g. (73%) | |

(a) 1.12 g. of Syrup B-3b and 90 g. of Florisil in a column 2.2 cm. in diameter were used.

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Benzene was the first developing solvent used. The colorless material removed by this liquid gave a strong nitrate test with the diphenylamine reagent, and contained one crystalline compound (Compound A) which was isolated from Fractions 17 to 26. The purification was not too complete as considerable syrupy product was also present in these fractions. Ethylene dichloride was the next solvent utilized. In other experiments it was found that ethylene dichloride removed the same amount of material as benzene, but was more rapid in its action.

The addition of 1% ethanol in either benzene or ethylene dichloride caused the movement of brown color down the column. At the time that the first color left the bottom of the column, ultraviolet light showed four distinct bands of fluorescence. The material removed from the column now contained no nitrate groups. One crystalline compound (Compound B) was isolated from the ethanol - ethylene dichloride effluent, (Fractions 44, 45, 46) but was accompanied by considerable quantities of brown syrup. The final few fractions (Fractions 61-66)contained inorganic material, presumably owing to chemical combination with the adsorbent. This phenomenon was also observed with alumina. The analyses and specific rotations of some of the fractions isolated on chromatographing Syrup B-3b are recorded in Table X.

Compound A

The yield of pure compound A from 40 g. of original tetranitrate was less than 50 mg. The compound was isolated from impure fractions by dissolving the syrupy contaminant in ethanol. Compound A was soluble in chloroform, benzene, ethanol, methanol, and acetone but insoluble in water and pentane. Recrystallization was readily accomplished from ethanol-pentane giving needles melting at 127-128.5°, but the use of benzene-pentane yielded circular clusters of needles melting as low as 80-108.5° in one case. Recrystallization of this low-melting product from ethanol - pentane raised the melting point again, but on evaporation of the ethanol liquors an appreciable amount of non-crystalline product was obtained. The specific rotation recorded in Table X was mea-

TABLE X

ANALYSES AND SPECIFIC ROTATIONS OF FRACTIONS FROM SYRUP B-3b

| Sample | % $OCH_3$ | % N (micro-Kjeldahl) | $[\alpha]_D^{20}$ $CHCl_3$ (l = 2) |
|---|---|---|---|
| Combined fractions (37 - 42) from Table IX[a] | 9.20<br>8.94 | 6.90<br>.... | |
| Compound A[a] | 8.78<br>8.45 | 6.84<br>6.75 | + 32.2[b] |
| Combined Fractions (47 - 50) from Table IX[c] | 11.8<br>11.8 | 14.4<br>14.2 | - 67.5 |
| Compound B[c] | 11.9<br>11.8 | 14.0<br>.... | -167[d] |
| Syrup[e] | 11.2<br>11.0 | 13.9<br>13.7 | |

(a) Diphenylamine test positive.

(b) Concentration was 2.14 g. per 100 ml.

(c) Diphenylamine test negative.

(d) Concentration was 0.724 g. per 100 ml.

(e) A syrupy fraction, 0.28 g., removed, when chromatographing a B-3b syrup (0.76 g.) on 20 g. of Florisil, by 30 ml. of 7% ethanol-benzene, after pure benzene no longer removed material from the column.

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sured with low-melting material recrystallized from benzene - pentane and was probably not the value for pure Compound A. Analysis of this substance (Table X) indicated that there were

probably 2 nitrogen atoms to 1 methoxyl group in a molecular weight of about 360. It appeared that the micro-Kjeldahl method gave a low nitrogen value for the compound. Insufficient material, however, was available to confirm the nitrogen content and to ascertain if all of the nitrogen was nitrate. These data nevertheless agreed quite closely with those reported by Dewar, Fort and McArthur (30) for methyl-3,4-diacetyl-β-D-glucoside-2,6-dinitrate, m.p. 128-129°, $[\alpha]_D^{13°}$ + 5.8° (chloroform, l = 2, c = 5.932. The theoretical methoxyl content was 8.4%, and the nitrogen content 7.6%.

The observed lowering of the melting point of Compound A on recrystallization from benzene - pentane was at first attributed to oxime isomerism. However on reconsideration of the evidence it was concluded, on the assumption that Compound A and methyl-3,4-diacetyl-β-D-glucoside-2,6-dinitrate were identical, that traces of sodium hydroxide in the benzene were responsible. The solvent, which had been stored over sodium, was not redistilled before use in the recrystallization.

Compound B

The quantity of pure Compound B actually obtained from 32 g. of tetranitrate was less than 30 mg. Rechromatography of the crude fractions on Florisil was usually necessary before recrystallizing. Compound B was soluble in chloroform, benzene, acetone, ethyl acetate, methanol and ethanol, but insoluble in water, pentane and carbon tetrachloride. The substance crystallized as needles m.p. 106-107° from ethylacetate-pentane,

methanol-water and benzene-pentane. Some lower melting material (m.p. 100-102°) was added to the purified product to have sufficient for 2 methoxyl and 1 nitrogen determinations. The analyses (see Table X) indicated three nitrogen atoms per methoxyl group in a molecular weight of about 260. The actual nitrogen value obtained was probably low. No nitrate nitrogen was present.

Nickel Derivative of Syrup B-3

Five millilitres of a 10% aqueous solution of nickel sulfate hexahydrate was added to a solution of 0.304 g. of Syrup B-3 in water, made basic to pH 8 with dilute ammonium hydroxide. A brown precipitate weighing 0.10 g. separatedimmediately and addition of more of the nickel solution to the filtrate caused no further precipitation. While being dried on a sintered-glass funnel with suction, the originally brown nickel salt turned black. The methoxyl content was 7.93, 7.89%. Nickel and nitrogen analyses were not carried out because the color and the methoxyl value indicated some degradation had occurred.

Nickel Derivative of Syrup B-4

To an aqueous solution of Syrup B-4 (0.46 g.), neutralized with dilute ammonium hydroxide, was added 0.6 g. of nickel nitrate hexahydrate in 20 ml. of water. The resulting brown precipitate was recovered, washed well with water, and dried on the funnel under suction. This derivative, which had become black during the drying process, weighed 0.47 g. and had a methoxyl content of 5.30%. The analysis of this substance was

not completed because it was assumed degradation had occurred while drying.

## Experiments on Syrup C

### Acetylation

Syrup C, 0.90 g., was acetylated with 10 ml. of acetic anhydride and 25 ml. of pyridine at 0° for 5 days. The dark brown solution was poured into 150 ml. of ice water with stirring and the aqueous mixture thus formed was extracted with 150 ml. of chloroform. The chloroform extract was washed with dilute sodium bicarbonate solution and water and was then evaporated to yield 0.49 g. of a dark brown syrup (Syrup C-1). On evaporation of the aqueous solution 0.21 g. of a clear brown syrup (Syrup C-2), with $OCH_3$, 3.77, 3.88%, and N (micro-Kjeldahl) 16.6, 17.1%, remained. Syrup C-1, 0.49 g., on direct extraction with 25 ml. of benzene yielded .08 g. of a yellow syrup which was not studied. The ratio of nitrogen atoms to methoxyl groups in the dark-brown, benzene-insoluble residue (Syrup C-3) (0.41 g.) was 4.2 to 1.

Anal.: $OCH_3$ 8.45, 8.58%; N (micro-Kjeldahl), 16.0, 16.2%.

### Benzoylation of Syrup C

Benzoylation of Syrup C, 0.14 g., with 6 ml. of benzoyl chloride and 10 ml. of dry pyridine for 2 days at room temperature yielded 0.35 g. of a dark syrup. An attempted purification by chromatography on an alumina column was unsuccessful.

Methylation of Syrup C

Methyl iodide, 70 ml., 10 g. of silver oxide and 10 g. of powdered Drierite were added to a solution of 1.95 g. of Syrup C in 15 ml. of dry methanol. The mixture was heated under reflux for 16 hours, filtered and evaporated. Methyl iodide, 40 ml., was added to the residue. The solution was decanted from the portion which did not dissolve, was mixed with 10 g. of silver oxide and 5 g. of Drierite, and heated under reflux for another 20 hours. The product after a third methylation was a brown sticky syrup.

Anal.: $OCH_3$, 27.8, 27.8%; N (micro-Kjeldahl), 12.1, 12.5%.

Isolation of Pyridinium Nitrate

The reaction solution, from the gas analysis which was described in Table V, was diluted with 900 ml. of chloroform after 29.5 hours. The precipitated hydroxylamine hydrochloride was removed by filtration and the filtrate evaporated to a volume of about 100 ml. Ether, 500 ml., was added and the two layers which resulted were separated. On standing for several weeks the ether layer deposited a dark syrup. The ether was decanted and a portion (300 ml.) was kept in a cork-stoppered Erlenmeyer flask at 5° for over 8 months. At the end of this time several long crystalline needles had settled out together with more of the dark syrup. The crystals, which on recrystallization from ethanol-pentane melted at 117-118°, gave a positive nitrate test, and were very soluble in water, somewhat soluble in ethanol, but

insoluble in chloroform. Addition of dilute sodium hydroxide produced the odor of pyridine. A mixed melting point with pyridinium nitrate (46) prepared by the interaction of pyridine and nitric acid in chloroform solution, was unchanged.

Reaction of Methoxyamine Hydrochloride in Pyridine with Methyl-β-D-glucoside Tetranitrate

A mixture of methyl-β-D-glucoside tetranitrate (2.00 g.), 12.0 g. of methoxyamine hydrochloride and 50 ml. of pyridine were allowed to react for 5 days. The methoxyamine hydrochloride did not completely dissolve and no appreciable volume of gas was evolved. After dilution of the brown reaction mixture with 300 ml. of chloroform, filtration removed 10.5 g. of the unreacted hydrochloride. The filtrate was extracted with water (200 ml.) until a negative test for chloride ion was obtained and was then evaporated to yield 1.30 g. of a clear dark red-brown syrup (Syrup D).

Anal.: $OCH_3$, 33.6, 34.5%; N (micro-Kjeldahl), 14.7, 14.7%;
N (nitrate), 3.1, 3.4%.

The ratio of methoxyl groups to non-nitrate nitrogen atoms was 1.35:1

The Action of Dry Pyridine on Methyl-β-D-glucoside Tetranitrate

Anhydrous pyridine, 50 ml., was added to 2.00 g. of methyl-β-D-glucoside tetranitrate in a 125 ml. Erlenmeyer flask, which was fitted with a one-holed rubber stopper attached to a

calcium chloride drying tube. The nitrate dissolved quickly. The color of the solution turned first to orange and then to dark red, within the first half-hour. As the reaction proceeded a few mg. of a white, crystalline substance formed around the top of the flask. No gas evolution was obvious. At the end of 40 hours the crystalline compound, m.p. 113-117°, was removed and then the red-colored solution was poured into 250 ml. of chloroform. A flocculent precipitate formed immediately. After standing overnight at about 5° C. the mixture was filtered. The precipitate, which had become gummy on removal of the chloroform, was insoluble in ethanol but soluble in water and was dissolved from the sintered-glass funnel by water. This aqueous solution on evaporation yielded 0.21 g. of a dark-brown syrup which gave a positive test for nitrate with the diphenylamine reagent.

Anal.: $OCH_3$, 5.2, 5.4%; N, 9.8, 10.0%.

The chloroform filtrate was extracted four times with 50 ml. volumes of water. The combined aqueous extracts on evaporation yielded 1.31 g. of a dark-brown mixture which, although mainly a syrup, contained a considerable quantity of long crystalline needles, which were identical with the volatile substance which had crystallized above the reaction solution. A mixed melting point, after recrystallization from ethanol-pentane, with pyridinium nitrate was not depressed. The total yield of this substance was not ascertained because of the inability to separate more than a small fraction from the dark contaminating

syrup.

The chloroform solution was evaporated at reduced pressure, water being added in order that all the remaining pyridine might be removed by azeotropic distillation. Chloroform (60 ml.) and water (50 ml.) were added to the residue to effect complete solution. The chloroform layer, after two more extractions with 25 ml. volumes of water, yielded 0.40 g. of a light brown mainly crystalline product which after two recrystallizations from ethanol melted at 116-118° and caused no depression in a mixed melting point with authentic methyl-β-D-glucoside tetranitrate. The remaining aqueous extract on evaporation yielded 0.08 g. of a brown amorphous material which gave a positive nitrate test.

## SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. Methyl-β-D-glucoside tetranitrate was found to react slowly with a large excess of hydroxylamine hydrochloride in anhydrous pyridine. After a period of forty-four hours approximately 2% of the original tetranitrate remained.

2. The gas evolved in the reaction was found to be a mixture composed of about 70% nitrous oxide and 30% nitrogen. Data on the amount and rate of gas evolution were not accurately reproducible, presumably because varying amounts of nitrous oxide remained dissolved in the reaction solution. At least 1.5 moles of gas per mole of glucoside was produced.

3. Two principal mechanisms affecting roughly equal numbers of molecules were shown to be in operation. The first resulted in a conversion of nitrate to hydroxyl groups with the evolution of nitrogen gas, while the second was believed to involve oxidation to an intermediate carbonyl derivative which reacted with the excess hydroxylamine hydrochloride to yield the observed oxime products. Nitrous oxide was a by-product in this case. There was no definite evidence that denitration by both mechanisms could occur in the same tetranitrate molecule.

4. The more easily isolatable one-half of the products was proven to be a mixture of methyl-β-D-glucoside di- and trinitrates, which was probably identical with that obtained by

Hayward from the closely related reaction between methyl-β-D-glucoside tetranitrate, free hydroxylamine and pyridine. Crystalline methyl-4-acetyl-β-D-glucoside-2,3,6-trinitrate and a crystalline compound believed to be methyl-3,4-diacetyl-β-D-glucoside-2,6-dinitrate were both obtained through chromatography. Following methylation, denitration and acetylation, crystalline methyl-4-methyl-β-D-glucoside-2,3,6-triacetate could be isolated in an over-all yield of at least 19%. A second crystalline substance, with a methoxyl content identical with the value calculated for a molar mixture of a methyl monomethyl-β-D-glucoside triacetate and a methyl dimethyl-β-D-glucoside diacetate was also isolated in very low yield.

5. The second half of the original product was shown to be in all probability a complex mixture of nearly, or completely, nitrate-free, water-soluble, acidic polyoximes in which the methyl-glycosidic group remained intact. The oximes were first isolated as water-insoluble, amorphous silver and nickel salts. One crystalline derivative with three non-nitrate nitrogen atoms per methoxyl group was obtained by acetylation and chromatography, but in too low a yield to permit determination of its structure. Methoxyl and total nitrogen analyses were carried out on various syrupy and amorphous fractions of the supposed polyoximes and their acetyl and methyl ether derivatives. Ratios of glycosidic methyl groups to nitrogen atoms as high as one to four were found in several instances, indicating that tetra-oximes had been formed. No definite evi-

dence was obtained that mono- or dioximes were present.

6. It was shown that methoxyamine hydrochloride in pyridine reacted with methyl-β-D-glucoside tetranitrate, predominantly by the oxidative mechanism, to yield a syrupy methyloxime with a low nitrate-nitrogen content and with a ratio of methoxyl to non-nitrate nitrogen of about 4 to 3. This work confirmed the presence of the methoxyamine, or methyloxime, group in the product.

7. Crystalline pyridinium nitrate was isolated from the reaction of methyl-β-D-glucoside tetranitrate with pyridine alone, and also with pyridine and hydroxylamine hydrochloride. Denitration by a third mechanism involving the cleavage of nitric acid therefore also occurred.

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