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The Reactivity of the Hydroxyl Groups of Methyl β-D-Glucopyranoside in the Koenigs-Knorr Reaction

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THE REACTIVITY OF THE HYDROXYL GROUPS OF
METHYL β-D-GLUCOPYRANOSIDE IN THE
KOENIGS-KNORR REACTION

A thesis submitted by

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SUMMARY

The reactivity of the hydroxyl groups of methyl β-D-glucoside, in the Koenigs-Knorr reaction, was studied by condensing it with α-acetobromo-D-glucose-C^{14}. The reactivities were based on the yields of the four β-linked disaccharide products. The order of the reactivity was found to be, 6-hydroxyl >> 3-hydroxyl > 4-hydroxyl > 2-hydroxyl, with relative yields of 6.4:1.7:1.2:1.0. The 4-hydroxyl was found to be much less reactive than the primary 6-hydroxyl; but it was of the same order of reactivity as the other two secondary hydroxyls. Apparently, the difficulties normally encountered in the synthesis of disaccharides in the Koenigs-Knorr reaction with the β(1 → 4)-linkage are due to steric hindrance of the 4-hydroxyl by the adjacent blocking groups rather than an inherent lack of reactivity.

The α-acetobromo-D-glucose-C^{14} was synthesized from D-glucose which was uniformly labeled with carbon-14 in order to facilitate the analysis of the reaction products. The methyl β-D-glucoside was unlabeled to avoid isotope effects on the hydroxyl groups. The Koenigs-Knorr reactions were carried out at 30°C. for seventy-two hours with silver oxide as an acid acceptor, iodine as a catalyst, and Drierite as a desiccant. Two of the reaction mixtures contained equimolar amounts of the two reactants, and it was found that by varying the solvent from pure p-dioxane to a 50:50 mixture of p-dioxane and nitromethane (by volume), the yield in the reaction was considerably reduced in the latter case. The ratio of products was not appreciably affected by this change in solvent.

Two other reactions contained a fivefold excess of the bromosugar. In this case, the major products of the reaction were higher molecular weight materials rather than the disaccharides. The ratios of the disaccharide products produced were therefore found to vary somewhat from the ratios found in the equimolar reactions. Varying the solvent from pure p-dioxane to a 50:50 mixture of p-dioxane and nitromethane (by volume) caused a large increase in the overall yield in the reaction, with most of the increase being in the higher molecular weight materials.

The reaction products were separated chromatographically, and were then analyzed with the Van Slyke technique to determine the amount of radioactivity in the samples. Two of the products, methyl β-cellobioside and methyl β-sophoroside, had identical R_f values on the chromatograms and were therefore analyzed together.

A large-scale reaction (a one hundred times scale-up) was carried out with a composition identical to that of the equimolar labeled reactions in pure p-dioxane. The products of a part of this reaction mixture were isolated as sirups by heavy paper chromatography and their specific optical rotations were determined. The negative values obtained, which were close to the values for the pure compounds, showed that the chief products of this Koenigs-Knorr reaction were the β-linked disaccharides. The two disaccharides, methyl β-gentiobioside and methyl β-laminaribioside, were identified as their heptaacetates from this large-scale reaction mixture. The other two disaccharides, methyl β-cellobioside and methyl β-sophoroside, were identified by the isotope dilution analysis of one of the labeled reaction mixtures.

A new compound, methyl β-sophoroside, was synthesized. It was characterized as the β-glycoside from its method of preparation, its negative specific optical rotation, its carbon-hydrogen analysis, and its nuclear magnetic resonance spectrum.

The results of this work are consistent with the general view that secondary hydroxyls are considerably less reactive than primary hydroxyls in the Koenigs-Knorr reaction.

INTRODUCTION AND HISTORICAL REVIEW

KOENIGS-KNORR REACTION

In general terms, the Koenigs-Knorr reaction is the reaction of a halogeno-sugar with an alcohol to yield a glycoside. If the alcohol is a liquid, the reaction is generally a solvolysis with the alcohol being in large excess. Koenigs and Knorr (1) originally found that α-acetobromo-D-glucose (I)* reacted with methanol to yield methyl tetra-O-acetyl-β-D-glucoside (II) as shown in Equation (1):

I   II
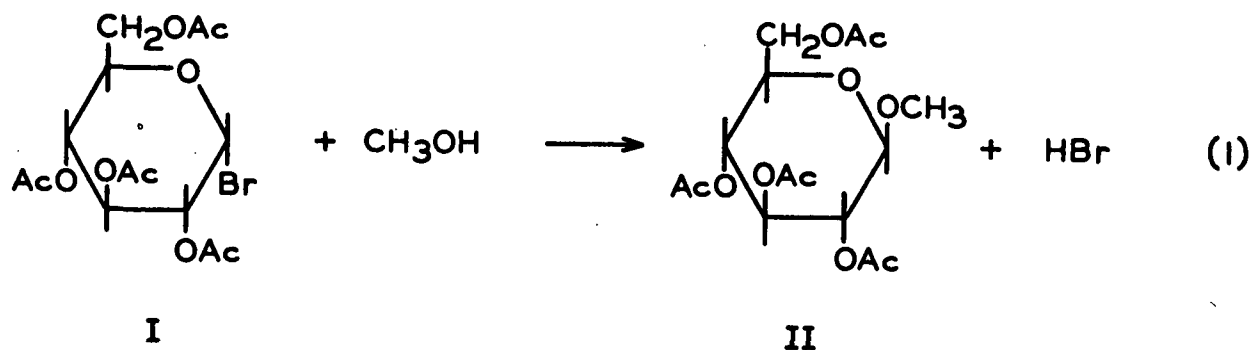
However, if the alcohol is a solid, then an inert solvent is required, and the reaction ceases to be a solvolysis. This is the case in the synthesis of oligosaccharides using the Koenigs-Knorr reaction as shown in Equation (2), in which α-acetobromo-D-glucose reacts with 1,2,3,4-tetra-O-acetyl-β-D-glucose (III) to form β-gentiobiose octaacetate (IV).

III   IV

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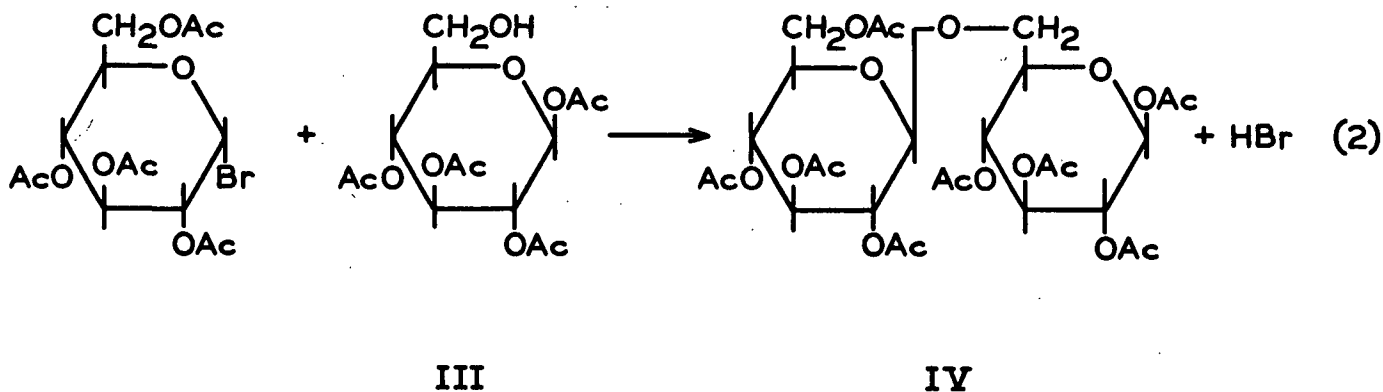
*All references in this work are to the pyranose form of the sugars, unless otherwise stated.

The bromosugars are the most common halogeno-compounds used, since they are quite reactive and yet are relatively stable. The chlorides are somewhat less reactive, although they have been used successfully, while the fluorides are quite unreactive. The iodides are generally too unstable to be of practical use.

The elimination of hydrogen bromide in Equations (1) and (2) may cause two problems: transesterification and transglycosidation.

Transesterification involves the migration of the acyl group from one hydroxyl to a neighboring one or to the solvent. In the case of Reaction (1), acyl migration to the solvent would produce methyl acetate. However, in oligo-saccharide syntheses, the migration of acyl groups to neighboring free hydroxyls is most critical, since the position of the free hydroxyl determines the product. Acyl migration is catalyzed by either alkaline or acidic conditions. Hence, the production of hydrogen bromide may cause transesterification. Schroeder, et al. (2) showed that in the solvolysis of α-acetobromo-D-glucose with various alcohols, hydrogen bromide caused significant deacetylation of the acetylated alkylglucosides.

Transglycosidation can occur in either Reaction (1) or (2). In Reaction (1), hydrogen bromide may cause anomerization of the glycosidic product to form a mixture of α- and β-anomers. It has been shown that methyl β-D-glucoside undergoes anomerization if it is refluxed in methanolic hydrogen chloride.

In Reaction (2), anomerization of the disaccharide linkage may occur yielding a mixture of both the α- and β-linked oligosaccharides.

An acid acceptor is generally employed to remove the hydrogen bromide as quickly as it is formed. Koenigs and Knorr (1) used silver carbonate, pyridine, and silver nitrate. However, many silver and mercury compounds have been used

with varying degrees of success. Silver oxide and silver carbonate have been used most often as acid acceptors.

The silver salts generally yield the β-glycoside, indicating a Walden inversion occurring at the C-1 position in the α-halogeno-sugars. However, there have been reports (3-7) of the formation of measurable amounts of the α-glycosides, especially when mercury compounds were employed as acid acceptors. Schroeder (2) has shown that for the primary alcoholysis of α-acetobromo-D-glucose without the presence of an acid acceptor, up to 34% of the α-glycoside is formed. He concluded that the reaction is probably of the SN1 type in which a carbonium ion is formed by the departure of the bromide group, while the alcohol attacks from the β-side due to the steric hindrance of the large departing bromide group on the α-side, yielding predominantly the β-product. However, as the size of the alcohol molecule increases, it encounters more steric hindrance on the β-side of the bromosugar. This allows more time for the departure of the bromide ion, which in turn increases the chances of alcohol attack from the α-side. He also showed that secondary alcohols react by the SN2 mechanism and that in this case only the β-product was possible.

When high yields are desired in the Koenigs-Knorr reaction, a desiccant is necessary to insure anhydrous conditions. Water will react readily with halogeno-sugars substituting a hydroxyl for the bromide group. Since water is formed in the reaction of the acid acceptor with the halogeno-acid, it must be continuously removed. In solvolysis reactions, where there is a large excess of the alcohol, water does not present such a big problem. However, in oligosaccharide syntheses, where the alcoholic component may be only in slight excess, water can enter into vigorous competition with the alcoholic component for the halogeno-sugar. Levene and Wolfrom (8) used anhydrous sodium sulfate and Schlubach and Schröter (9) used

anhydrous copper sulfate in oligosaccharide syntheses. The yields were then increased to about 25% of the theoretical. Helferich, et al. (10) increased the yields in oligosaccharide syntheses up to 59% with the introduction of calcium chloride as a desiccant. Finally, when Kreider and Evans (11,12) introduced "Drierite" (a hemihydrate of calcium sulfate), Reynolds and Evans (13) were able to increase the yield of β-gentiobiose octaacetate to 74% of the theoretical. Drierite is now one of the most commonly employed desiccants in the Koenigs-Knorr reaction.

Iodine is often employed as a catalyst in the Koenigs-Knorr reaction. Helferich, et al. (10) first reported its catalytic effect in this reaction. It is not well understood how iodine acts as a catalyst. It has been reported (14) that silver oxide causes the decomposition of α-acetobromo-D-glucose under the conditions of the Koenigs-Knorr reaction, and iodine seems to inhibit this decomposition. Iodine also tends to decrease the yield of glycoside. However, the decomposition of the halogeno-sugar is inhibited more than the glycoside formation, and, hence, the net effect is an increase in glycoside production.

Finally, the solvents which are employed in the Koenigs-Knorr synthesis of oligosaccharides are generally inert, nonpolar solvents such as chloroform, benzene, dioxane, and ether. If the alcoholic reactant is a simple alcohol, it is generally used as the solvent and the reaction becomes a solvolysis of the halogeno-sugar. However, it is often necessary to use a certain amount of an inert solvent even in a solvolysis, since the halogeno-sugar may not be readily soluble in the alcohol. This is particularly true in the case of preparative scale reactions. In the case of dilute solutions, an inert solvent is not necessary (2).

THE KOENIGS-KNORR REACTION IN DISACCHARIDE SYNTHESIS

If another sugar unit containing a free hydroxyl group is substituted for the simple alcohol, the product of this condensation is a disaccharide as shown in Equation (2). In this reaction, α-acetobromo-D-glucose reacts with 1,2,3,4-tetra-O-acetyl-β-D-glucose to yield β-gentiobiose octaacetate.

Fischer and Delbrück (15) first reported the synthesis of a disaccharide, β,β-trehalose, using the Koenigs-Knorr reaction. However, it was obtained only as a by-product in a 1% yield. It was not until the work of Reynolds and Evans (13) that substantial yields of disaccharides were obtained in the Koenigs-Knorr reaction. It has been found that the yields for the condensation of the primary hydroxyl groups are much higher than for the secondary hydroxyls in disaccharide syntheses.

KOENIGS-KNORR REACTION IN OLIGOSACCHARIDE SYNTHESIS

Haq and Whelan (16) were able to polymerize the bifunctional compound, 2,3,4-tri-O-acetyl-α-D-glucosyl bromide (V) under the conditions of the Koenigs-Knorr reaction as shown in Equation (3).

$$+ \ HBr \quad (3)$$
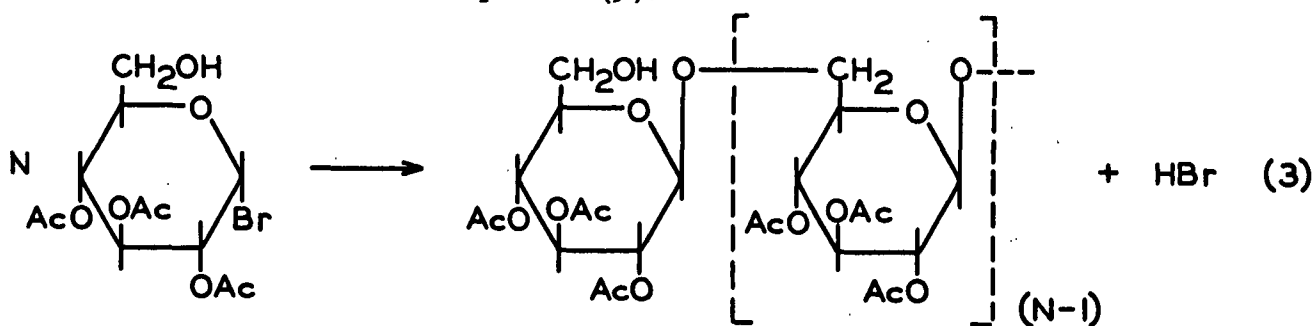
V

In this case, both the hydroxyl and the bromide groups are on the same sugar unit. They obtained a series of oligosaccharides (gentiodextrins) up to nine units in length. Solubility appears to be the determining factor in chain length limitation.

Wadsworth (17) tried a similar polymerization using the bifunctional compound 2,3,6-tri-O-benzoyl-α-D-glucosyl bromide (VI). He hoped that this compound would polymerize to form the cellodextrin series of β(1 → 4)-linked oligosaccharides.
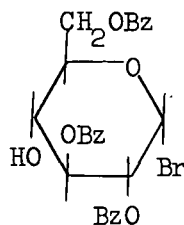
VI

However, he had very little success. He obtained only a trace of cellobiose in one of his condensations. He attributed this to the inherent lack of reactivity of the 4-hydroxyl compared with the 6-hydroxyl in Haq and Whelan's compound (V).

REACTIVITY OF HYDROXYL GROUPS

It has been generally accepted that primary hydroxyls are more reactive than secondary hydroxyls in the Koenigs-Knorr reaction. Schroeder (2) has shown that the rates of primary alcoholyses of α-acetobromo-D-glucose are much higher than for secondary alcoholyses. Also, in the preceding section, it was mentioned that the yields of disaccharides with a secondary hydroxyl linkage are generally much lower than those with a primary hydroxyl linkage.

Sugihara (18) reviewed the general area of the relative reactivities of hydroxyl groups in carbohydrates and concluded that the selective reactivity of the hydroxyl groups in carbohydrates is often a consequence of their steric arrangement in the molecule. Neighboring groups may also have a large effect on the reactivity of a particular hydroxyl group. However, the greater reactivity of the primary hydroxyls over the secondary hydroxyls is generally indicated, except that in alkaline media, the 2-hydroxyl may approach the reactivity of the primary hydroxyl. This is due to its greater acidity, since it is next to an acetal grouping.

Many studies have been made (19-27) of the relative reactivities of the hydroxyl groups in cellulose. These studies indicate that the 6- and 2-hydroxyls are the most reactive under both acidic acetylation conditions and alkaline methylation conditions. Surprisingly enough, the 2-hydroxyl is sometimes more reactive than the 6-hydroxyl in cellulose.

de Belder, et al. (27) determined the relative rates for the methylation of the hydroxyl groups of methyl β-D-glucoside in alkaline dimethyl sulfate. They found that the ratio of the rates for the individual hydroxyl groups, k_6:k_2:k_3:k_4 was 8:8:2:1. So far as this author can determine, no specific studies have been made of the reactivity of the hydroxyl groups of carbohydrates under the neutral conditions of the Koenigs-Knorr reaction.

Schroeder (2) has considered the rates of alcoholysis of α-acetobromo-D-glucose with both primary and secondary alcohols. He found that primary alcohols reacted by the SN1 mechanism and that the rate of reaction was much higher than for secondary alcohols, which apparently reacted by the SN2 mechanism. There may be an analogy between simple alcohols and carbohydrates, except that carbohydrates have more complications from steric hindrance, neighboring group effects, and solvent interactions.

It is, however, quite obvious that the 4-hydroxyl seems to be particularly unreactive. Wadsworth (17) was unable to obtain significant reaction of his bifunctional compound, 2,3,6-tri-O-benzoyl-α-D-glucosyl bromide, which has a free 4-hydroxyl. Many attempts have been made to synthesize disaccharides with the β(1 → 4)-linkage (17,28-30), but in most cases the yields were extremely low or nonexistent. Jones and Curtis (31) argued that steric hindrance due to the presence of the hemiacetal ring and other substituted groups as well as the inherent lesser reactivity of secondary hydroxyls, were the main reasons for the

difficulties encountered in using the Koenigs-Knorr reaction to synthesize di-
saccharides with secondary hydroxyl linkages. They argued further that use of
an acyclic derivative of the alcoholic sugar unit should decrease the steric
hindrance due to the ring. They obtained a 35% yield of lactose (1→4-linked
galactose-glucose) by condensing α-acetobromo-D-galactose with 2,3:5,6-di-O-
isopropylidene-D-glucose diethyl acetal (VII), which is an acyclic derivative
of D-glucose, with a free 4-hydroxyl.
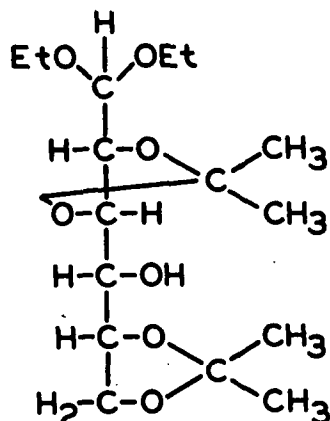
VII

Finally, another method designed to increase the reactivity of the secondary
hydroxyls has been reported by Gilbert, et al. (32). They were able to form the
sodio- derivative of 1,2,3,6-tetra-O-acetyl-β-D-glucose (VIII) and to condense
it with α-acetobromo-D-glucose in the molten state as shown in Equation (4).

VIII　　　　　**β-CELLOBIOSE OCTAACETATE**

$$(4)$$
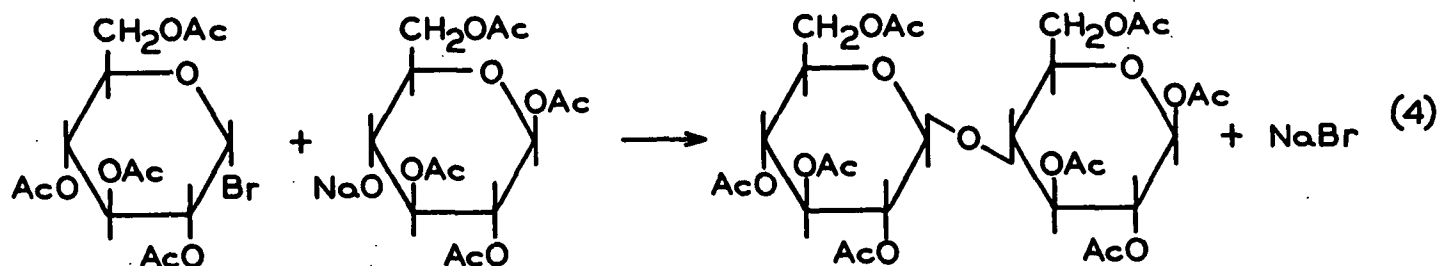
They were able to obtain a 40% yield of β-cellobiose octaacetate. This reaction
might be considered a modified Koenigs-Knorr reaction.

EXPERIMENTAL PROCEDURES AND RESULTS

PREPARATION OF COMPOUNDS

2,3,4,6-TETRA-O-ACETYL-α-D-GLUCOPYRANOSYL BROMIDE-C^{14}

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide-C^{14} (α-acetobromo-D-glucose-C^{14}) was prepared essentially by the method reported by Barczai-Martos and Korosy (33). D-glucose-C^{14} (uniformly labeled)* was acetylated in acetic anhydride with perchloric acid as the catalyst, and then brominated in the re-action mixture by the liberation of hydrogen bromide from phosphorous tribromide with water. The crystalline product obtained was not the expected α-acetobromo-D-glucose-C^{14}. Apparently, the bromination step was unsuccessful. The entire mixture was then rebrominated by the method of Fischer and Fischer (34,35) using a saturated solution of hydrogen bromide in glacial acetic acid. Two recrystal-lizations from absolute ether resulted in a yield of α-acetobromo-D-glucose-C^{14} of 52% of the theoretical. The needlelike crystals melted at 88-89°C., and had a specific optical rotation $[\alpha]_D^{22°C.}$ = +197° (chloroform). Literature values are 88-89°C., $[\alpha]_D$ = +198° (chloroform) (35). The product was stored in a desiccator over sodium hydroxide pellets.

METHYL β-D-GLUCOSIDE

Methyl β-D-glucoside was prepared according to the method of Schroeder and Green (36) for the production of alkyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosides. This method involves the Koenigs-Knorr methanolysis of α-acetobromo-D-glucose in chloroform solution using Drierite as the desiccant, mercuric oxide (yellow) as

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*Obtained from Nuclear-Chicago, Inc., Des Plaines, Illinois.

the acid acceptor, and mercuric bromide as the catalyst. Subsequent catalytic deacetylation of the acetylated methyl β-D-glucoside using sodium methoxide in methanol yielded the product.

The product was purified by dissolution in one normal sodium hydroxide solution and heating at 80°C. for one hour on a steam bath. This treatment was to remove any D-glucose impurity in the sample. (See p. 21 for more details.) The solution was cooled and deionized in a column of Amberlite MB-3 mixed bed resin. The purified product was recrystallized twice from n-propanol containing a small amount of water and isolated in a yield of 40% of the theoretical. The platelike crystals melted at 108.5-110.5°C., and had a specific optical rotation $[\alpha]_D^{25°C.}$ = -32.7° (water). Literature values vary from 105-111°C., $[\alpha]_D$ = -32° to -34° (water).

METHYL β-CELLOBIOSIDE

The synthesis of methyl β-cellobioside was carried out in three steps. The first step was the conversion of β-cellobiose octaacetate to α-acetobromocellobiose according to the method of Fischer and Zemplen (37) in which β-cellobiose octaacetate was reacted with a saturated solution of hydrogen bromide in glacial acetic acid at 0°C. The α-acetobromocellobiose was isolated in 30% yield, melting with decomposition at 174.5-175.5°C., and with a specific optical rotation $[\alpha]_D^{24°C.}$ = +95° (chloroform). Literature values are 182°C., $[\alpha]_D$ = +94.5° (chloroform) (38).

The α-acetobromocellobiose was converted by the Koenigs-Knorr methanolysis to methyl hepta-O-acetyl-β-cellobioside according to the method of Schroeder and Green (36). The product was isolated in 55% yield, melting at 184-186°C., and having a specific optical rotation $[\alpha]_D^{24°C.}$ = -23.5° (chloroform). Literature values are 187°C., $[\alpha]_D^{20°C.}$ = -24° (chloroform) (38).

The final step was the catalytic deacetylation of methyl hepta-O-acetyl-β-cellobioside in a methanolic solution of sodium methoxide. The methyl β-cellobioside was recrystallized from methyl alcohol as needlelike crystals in a 60% yield. The product melted at 190-192°C. (after drying in a vacuum oven at 70°C.), and had a specific optical rotation $[\alpha]_D^{26°C.} = -17.5°$ (water). Literature values are 193°C., $[\alpha]_D^{20°C.} = -18.8°$ (water) (39).

A detailed synthetic pathway is presented in Appendix I.

METHYL β-LAMINARIBIOSIDE

Methyl β-laminaribioside was synthesized in seven steps according to the method of Bachli and Percival (40). This method involves the Koenigs-Knorr condensation of 1,2:5,6-di-O-isopropylidene-D-glucofuranose with α-acetobromo-D-glucose to give the substituted β(1 → 3)-linked disaccharide. Subsequent removal of the blocking groups and separation of the products by column chromatography yielded pure laminaribiose. The laminaribiose was then acetylated, subjected to a normal Koenigs-Knorr methanolysis, and deacetylated to give the final product, methyl β-laminaribioside. The overall yield of methyl β-laminaribioside was only 0.25% of the theoretical based on the initial amount of 1,2:5,6-di-O-isopropylidene-D-glucofuranose. The crystalline methyl β-laminaribioside melted at 159.5-161.5°C., and had a specific optical rotation $[\alpha]_D^{23°C.} = -27°$ (water). Literature values are 166-167°C., $[\alpha]_D^{19°C.} = -28°$ (water) (40).

A detailed synthetic pathway is presented in Appendix I.

METHYL β-GENTIOBIOSIDE

Methyl β-gentiobioside was synthesized in six steps. The first three steps, which involve the synthesis of β-gentiobiose octaacetate, were carried out according

to the method of Helferich (41) as modified by Reynolds and Evans (13). This method involved the tritylation and acetylation of D-glucose in pyridine solution followed by the removal of the O-trityl groups to give 1,2,3,4-tetra-O-acetyl-β-D-glucose. This compound was then condensed with α-acetobromo-D-glucose in a normal Koenigs-Knorr reaction to give β-gentiobiose octaacetate.

β-Gentiobiose octaacetate was converted to α-acetobromogentiobiose by the method of Fischer and Zemplen (37). The bromide was then subjected to a Koenigs-Knorr methanolysis using the modification of Schroeder and Green (36) to yield the desired product, methyl β-gentiobioside. The overall yield based on the initial amount of D-glucose was approximately 1% of the theoretical. The product melted at 95-97°C., and had a specific optical rotation $[\alpha]_D^{23°C.} = -35°$ (water). Literature values are 98°C., $[\alpha]_D^{20°C.} = -36°$ (water) (42).

A detailed synthetic pathway is presented in Appendix I.

METHYL β-SOPHOROSIDE

Since methyl β-sophoroside is a new compound, it was necessary to both synthesize and characterize it. α-Acetobromosophorose, which was first prepared by Freudenberg and Soff (43), was synthesized according to the method of Coxon and Fletcher (44). This was the starting material for the synthesis of methyl β-sophoroside.

A normal Koenigs-Knorr methanolysis of α-acetobromosophorose was carried out according to the method of Schroeder and Green (36). The crude product of the methanolysis, which was presumably methyl hepta-O-acetyl-β-sophoroside, was deacetylated. The deacetylated product (presumably methyl β-sophoroside) was crystallized from 95% ethanol.

The white, fibrous crystals which were isolated, melted at 194.5-195.5°C., and had a specific optical rotation $[\alpha]_D^{27°C.}$ = -36.03° (water) after three re-crystallizations.

Carbon and Hydrogen Determination

A sample of the crystals presumed to be methyl β-sophoroside was sent to Geller Laboratories* for a carbon and hydrogen analysis. The calculated results for $C_{13}H_{24}O_{11}$ are: C, 43.81; H, 6.79. Found: C, 42.87; H, 6.84.

It was suspected that methyl β-sophoroside is a hydrate, since it would only crystallize in the presence of water. If a hemihydrate is assumed, the calculated results for $C_{13}H_{24}O_{11} \cdot 1/2H_2O$ are: C, 42.73; H, 6.89. The data obtained are in good agreement with these results. The crystals were dried at 105°C. under vacuum for two days, but no loss of weight was detectable.

Infrared and Nuclear Magnetic Resonance Spectra

The infrared and nuclear magnetic resonance spectra are presented in Appendix VI. The infrared spectrum indicated that the methyl β-sophoroside is a hydrate. The nuclear magnetic resonance spectrum indicated that the methyl β-sophoroside was the compound with the β-methyl linkage rather than the α-methyl linkage.

METHYL HEPTA-O-ACETYL-β-SOPHOROSIDE

Methyl hepta-O-acetyl-β-sophoroside was prepared as described above by the Koenigs-Knorr methanolysis of α-acetobromosophorose. The long, needlelike crystals melted at 126-128°C., $[\alpha]_D^{22°C.}$ = -1.2° (chloroform) after three recrystallizations from absolute ethanol. This compound has not been reported previously in the literature.

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*Geller Laboratories, P. O. Box 6400, Charleston, W. Virginia 25302.

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Carbon and Hydrogen Determination

A sample of methyl hepta-O-acetyl-β-sophoroside was sent to Geller Laboratories for carbon and hydrogen analysis. Found: C, 49.67; H, 5.84. Calculated results are: C, 49.90; H, 5.75.

PURIFICATION OF SOLVENTS

p-DIOXANE

Reagent-grade p-dioxane was purified by the method reported by Fieser (45). p-Dioxane was refluxed with hydrochloric acid during which time a slow stream of nitrogen was bubbled through to entrain acetaldehyde. The mixture was shaken with solid potassium hydroxide until no more dissolved and a second layer had separated. The p-dioxane was then refluxed with sodium, distilled over sodium, and stored in a dark bottle over sodium.

NITROMETHANE

Reagent-grade nitromethane was purified by shaking with phosphorous pentoxide, and fractional distillation twice from Drierite through a 40-cm. Vigreux column. The fraction boiling at 100.5-101.5°C. was retained and stored in a dark bottle which was sealed with "Parafilm."*

METHANOL

Reagent-grade methanol was purified by a modification of the method of Herold and Wolf (46). The modification has been described by Schroeder (2). It involves the reaction of magnesium turnings with the methanol using iodine as a catalyst. After reflux, the methanol was distilled and stored in a bottle sealed with Parafilm.

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*Parafilm is a product of Marathon, Division of American Can Company, Menasha, Wisconsin.

CHLOROFORM

Reagent-grade chloroform was purified according to a modification of the method of Reynolds and Evans (13). Chloroform was shaken with 12% sulfuric acid, separated, neutralized with sodium bicarbonate solution, washed with water and then dried over calcium chloride. The chloroform was then distilled from Drierite and stored over Drierite in a dark bottle.

LABELED REACTION PROCEDURES

PREPARATION OF STARTING MATERIALS

Methyl β-D-glucoside, which was synthesized as described on p. 11, was ground to a fine powder and heated at 100°C. in a vacuum oven to constant weight to drive off the water of crystallization. The loss in weight after 72 hours was 4.51%. The theoretical amount of water in methyl β-D-glucoside hemihydrate is 4.43%. The sample was then assumed to be anhydrous and was stored in a desiccator over phosphorous pentoxide.

α-Acetobromo-D-glucose-C^{14} was synthesized as described on p. 11. The specific activity of this compound was determined in duplicate using the second method of Van Slyke, et al. (47,48). The calculations involved are presented in Appendix II. The value obtained was $(2.13 \pm 0.062) \times 10^6$ disintegrations per minute per millimole.

Drierite (10-20 mesh) was ground in a mortar and pestle and then screened. The fraction passing the 40-mesh screen but retained by the 50-mesh screen was used as the desiccant. The screened sample was heated at 240°C. for two hours in an oven, and then cooled and stored in a desiccator over Drierite.

Commercial reagent-grade silver oxide, which was stored in a dark desiccator over phosphorous pentoxide, was employed as an acid acceptor.

Commercial reagent-grade iodine was used as the catalyst and it was stored in a desiccator over Drierite.

The solvents, p-dioxane and nitromethane, were purified as described on p. 16.

COMPOSITION OF REACTIONS

All of the reagents were weighed out on an analytical balance which was situated in a dry-box. The dry-box was maintained at a relative humidity of around 0% using phosphorous pentoxide as a desiccant. Each reaction ampule was made by sealing one end of a 20-cm. piece of 11-mm. glass tubing.

The solid reagents, Drierite, silver oxide, and iodine were weighed directly into the reaction ampules. The two reactants, methyl β-D-glucoside and α-aceto-bromo-D-glucose-C^{14}, were weighed out into 10-ml. volumetric flasks. The methyl β-D-glucoside was dissolved in p-dioxane so as to give a concentration of 0.100 g. per 2 ml. Four solutions of α-acetobromo-D-glucose-C^{14} were prepared. Two were prepared in p-dioxane to concentrations of 0.212 g. per 2 ml. The other two were prepared in nitromethane to the same concentrations. The compositions of the reactions are given in Table I.

The amounts of the various reactants were calculated on the basis of the work done by Reynolds and Evans (13) on the Koenigs-Knorr synthesis of β-gentiobiose octaacetate and also on preliminary experimental work done in this thesis.

TABLE I

COMPOSITION OF LABELED REACTION MIXTURES

Reaction	1	2	3	4
MBGL[a], g.	0.100	0.100	0.100	0.100
TAGB-C^{14}[b], g.	0.212	0.212	1.060	1.060
Silver oxide, g.	0.262	0.262	0.262	0.262
Iodine, g.	0.026	0.026	0.026	0.026
Drierite, g.	2.000	2.000	2.000	2.000
p-Dioxane, ml.	4.0	2.0	4.0	2.0
Nitromethane, ml.	--	2.0	--	2.0
Glass beads[c]	10	10	10	10

[a]Methyl β-D-glucoside.
[b]α-Acetobromo-D-glucose-C^{14}.
[c]Glass beads (2-mm.) added to facilitate mixing.

REACTION PROCEDURE

Since the reactions are heterogeneous, it was necessary to keep the reaction mixtures in a constant state of agitation to prevent localized formation of pockets of acidity. To this end, the constant temperature bath and mixing device illustrated in Fig. 1 was constructed in the Institute workshop.

As soon as the 2-ml. aliquots of the two reactants were pipetted into the reaction ampules, the ampules were sealed with an oxygen flame and shaken. The ampules were then wrapped in foil to exclude light and placed on the mixing device as shown in Fig. 1. The 29 r.p.m. motor turned the ampules end over end at such a rate as to keep the contents continuously mixing with a tumbling action. The glass beads in the ampules insured that the solids did not become caked at one end.

All of the reactions were continued for 72 hours at 30°C.

MOTOR 29 R.P.M.

REGULATOR

KNIFE
HEATER

CHAIN
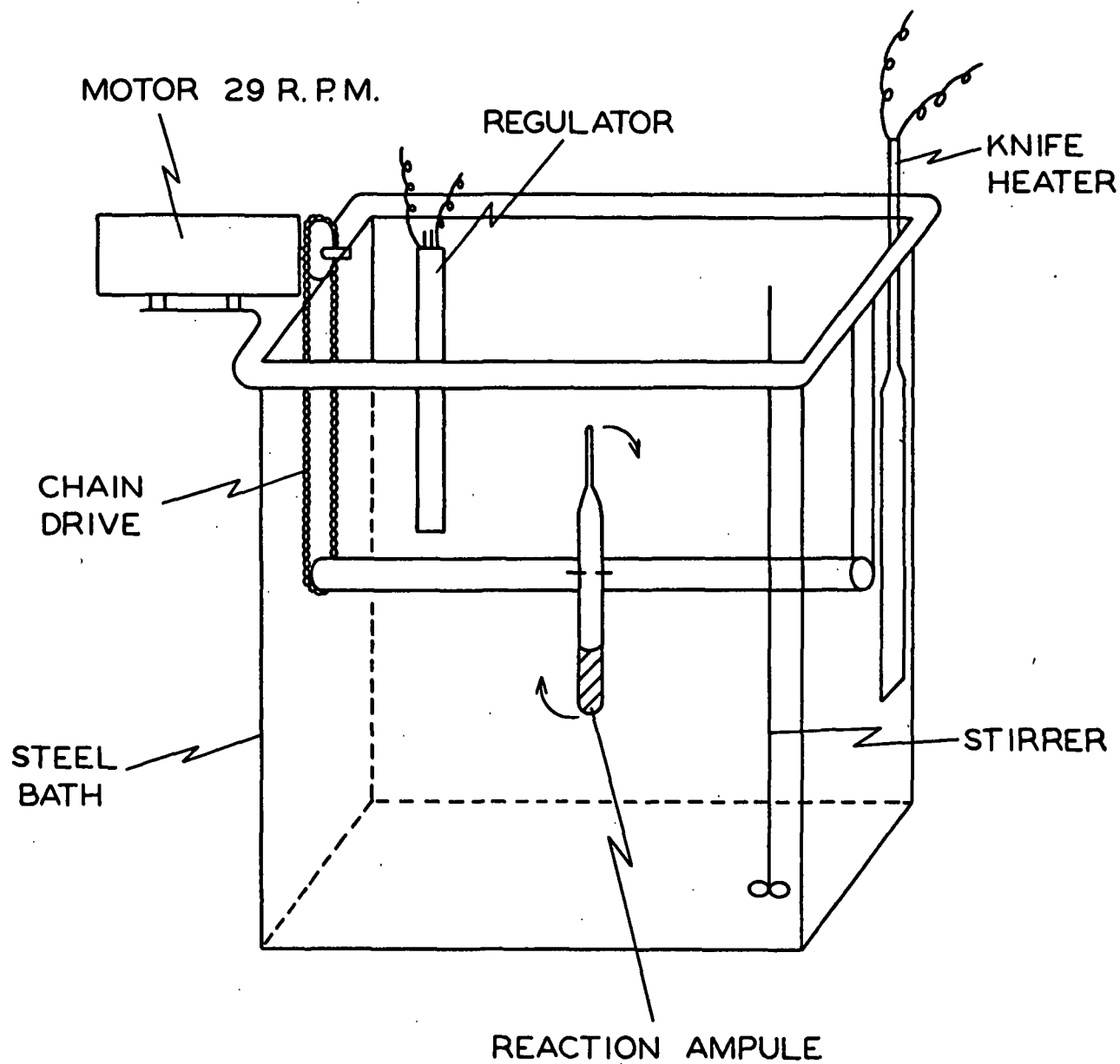DRIVE

STEEL
BATH

STIRRER

REACTION AMPULE

Figure 1. Constant Temperature Bath and Mixing Device

WORK-UP PROCEDURE FOR REACTIONS

After a reaction time of 72 hours, the reactions were quenched by breaking
the ampules and pouring the contents into 100 ml. of 50% acetone-water. The
acetone-water mixture was stirred for 12 hours to insure that any unreacted
α-acetobromo-D-glucose-C^{14} had been converted to 2,3,4,6-tetra-O-acetyl-β-D-
glucose. The mixture was filtered and concentrated to dryness under reduced
pressure. The mixture was then taken up in 100 ml. of hot methanol, cooled,
filtered to remove the insoluble silver salts, and then concentrated to dryness
again under reduced pressure.

Each reaction was treated with one normal sodium hydroxide solution at 90°C.
for one hour on a steam bath. Reactions 1 and 2 were treated with 5 ml. of the
sodium hydroxide, and Reactions 3 and 4 were treated with 10 ml. of the sodium
hydroxide solution. This treatment effected complete deacetylation of the
products, and complete destruction of the reducing products (mainly D-glucose)
to form saccharinic acids. This treatment was thought to have little effect on
the alkali stable glycosidic products of the reaction. Brooks (49) found that
random cleavage of the glycosidic bond in 10% sodium hydroxide solution at 140°C.
was only 0.3% in five hours. However, the final yields of products (see Table II)
showed that there was indeed a loss in some part of the reaction procedure.

The excess sodium hydroxide and the saccharinic acids were removed by
passing the cooled reaction mixtures through a column (50 x 1 cm.) of Amberlite
MB-3 mixed-bed ion-exchange resin. The columns were washed with 200 ml. of
distilled water to insure complete removal of the deionized products. The final
few milliliters of the effluent gave a negative test using the Molisch reaction.
The aqueous solutions were concentrated to dryness under reduced pressure and
then dissolved and reconcentrated from methanol several times to remove all traces

of water. The mixtures were finally concentrated to constant weight under
reduced pressure. The yields are presented in Table II.

TABLE II

TOTAL OBSERVED AND ESTIMATED YIELDS

Reaction	Observed[a] Yield, g.[b]	Estimated[c] Yield, g.	Recovery,[d] %
1	0.1131 0.1076	0.1290	93.5
2	0.0845 0.0845	0.1089	77.5
3	0.0882 0.0843	0.1306	65.9
4	0.1242 0.1270	0.1719	72.3

[a]Results for duplicate reactions.
[b]Yield includes unreacted methyl β-D-glucoside.
[c]Theoretical estimate from the radioactive counting data in Appendix
 III based on the supposition that no methyl β-D-glucoside was
 destroyed in the reaction.
[d]Calculated from the difference between the observed and estimated
 yields.

Apparently, there was a considerable loss of material over that which would
be predicted from the radioactive data obtained later in this work (p. 65). This
was particularly true in Reactions 2, 3, and 4. It was difficult to assign these
losses to a particular step in the reaction procedure. However, since the final
ratios of products were reasonably constant, the losses most likely occurred in
the work-up procedure as mechanical losses. Three other possible losses might
occur in (1) the absorption of products on the ion-exchange resin; (2) the
alkaline treatment, in conjunction with residual silver salts may have caused
oxidation of the products and the starting material; and (3) some oxidation of
either the starting material (methyl β-D-glucoside) and/or the disaccharide

products with silver oxide during the reaction could have occurred. However, these three sources would probably cause preferential losses of each product, and would tend to alter the ratios of products. The constancy of the ratios is good evidence that preferential removal of the products did not occur.

BLANK REACTIONS

The four reactions were repeated exactly as described above except that the methyl β-D-glucoside was omitted. The four reactions were worked up and then paper chromatography showed only a small amount of residual D-glucose in the mixtures. In other words, no alkali stable products were formed in any silver-induced decomposition of α-acetobromo-D-glucose.

ANALYSIS OF REACTION MIXTURES

PAPER CHROMATOGRAPHY

The solvent system chosen was tert-amyl alcohol:n-propanol:water, 4:1:1 (50). This solvent gave good separation of the disaccharide products in seven days. None of the solvent systems tested would give adequate separation of the two products methyl β-cellobioside and methyl β-sophoroside. It was necessary to use isotope dilution analysis to determine the yields of these two products.

An ultramicroburet* was employed to spot the chromatograms. It was calibrated over the range to be used by weighing the amount of mercury ejected over this range. It was found that the instrument delivered 310 microliters for a reading of 250 microliters. The results were subsequently corrected for this discrepancy.

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*Manufactured by Gilmont, New York City, New York.

Whatman No. 1 chromatography paper was employed in this procedure. The
paper was spotted as illustrated in Fig. 2. The 250-microliter aliquots of
the reaction mixtures were spotted 0.5 microliter at a time. This necessitated
spotting each circle twenty times by the following method: each circle was
spotted with 0.5 microliter starting at one end of the sheet and moving along
in succession. By the time one row was completed, the spots at the beginning
of the row were dry and the spotting could be repeated. This process was repeated
twenty times for each chromatogram. Duplicate chromatograms were spotted for
each of the eight reactions analyzed.
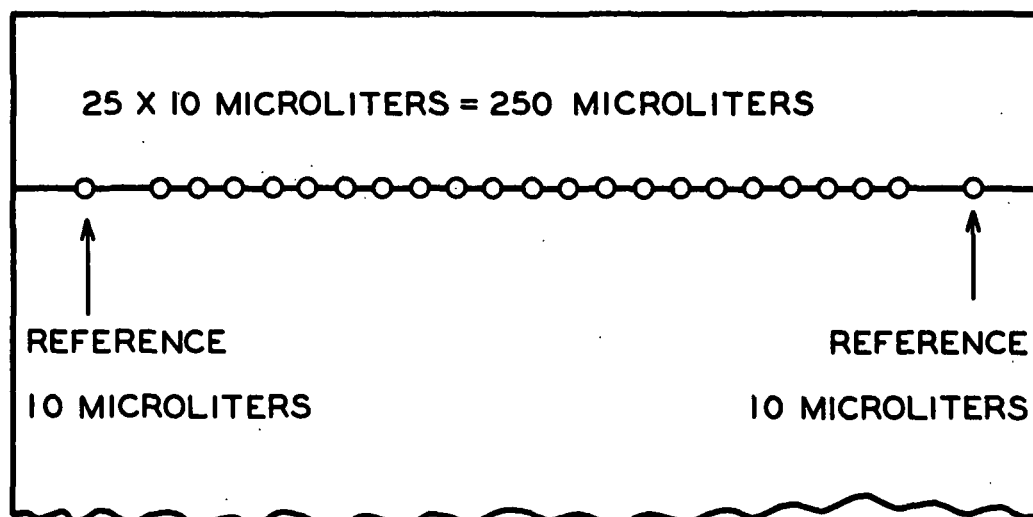
Figure 2. Spotting Technique for Quantitative Chromatography

The chromatograms were irrigated for seven days in the solvent system,
4:1:1. After drying the chromatograms, the reference strips on either side of
the main bands in Fig. 2 were developed using the silver nitrate/sodium hydroxide/
sodium thiosulfate dip. In this way, the location of the main bands could be
found without developing them. This was necessary, since development of the
bands would dissolve the radioactive products and yield only an inactive, visible
band of metallic silver.

-25-

VAN SLYKE MACROANALYSES

.The analysis of the separated reaction products was carried out using a
method similar to that employed by Schneider (51).

Elution of Chromatograms

The located bands of the reaction products were cut from the paper sheet
and eluted with water as shown in Fig. 3. In this way, all of the radioactive
material was moved down the strip to the end without actually running it off
the sheet. This procedure was repeated once for each band. It was found that
all of the radioactive product was located within one centimeter of the bottom
of the strip after two elutions. A one-centimeter strip cut from the bottom of
the sheet contained all of the radioactive product from that band.
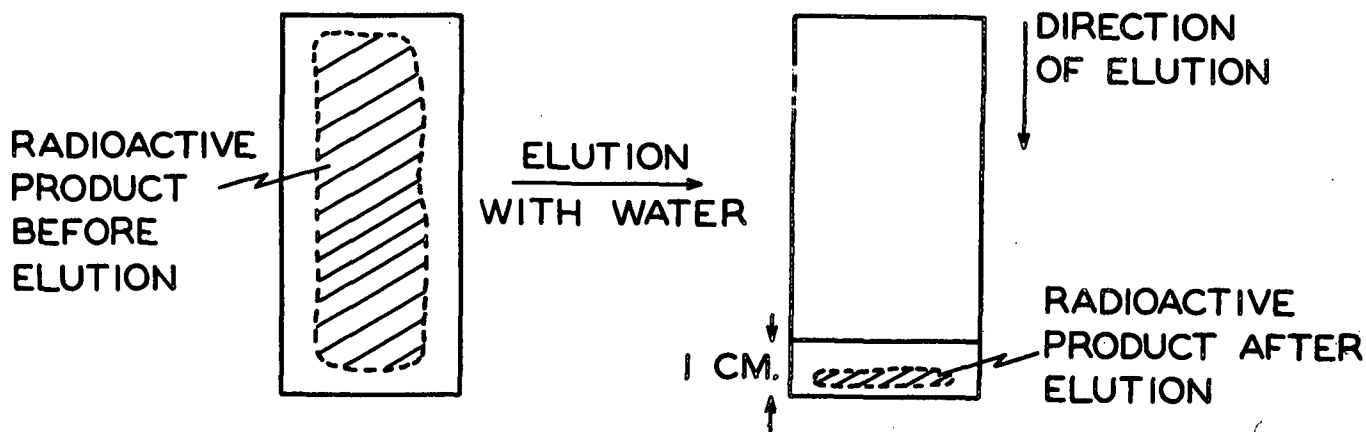
Figure 3. Elution of Radioactive Materials with Water

Manometric Determination of Total Carbon and Its Radioactivity

The one-centimeter strips were subjected to the wet combustion technique as
described by Van Slyke, et al. (47,48). In this procedure, the samples are
chemically oxidized to carbon dioxide in the Thomas-Van Slyke manometric
apparatus (see Appendix II). The carbon dioxide is then separated from the
other gases by absorption in an alkaline hydrazine solution, liberated again

with lactic acid, measured manometrically, and finally transferred to a Bernstein-Ballentine proportional counting tube (52) for counting the radioactivity* in the sample. A more detailed description of the Thomas-Van Slyke manometric system is given in Appendix II. Since the size of the sample in the Van Slyke technique is limited to approximately 15 mg. of carbon, maximum, it was necessary to run two and occasionally three wet combustions for each sample and to add the results.

Calibration of Bernstein-Ballentine Proportional Counting Tubes

Three characteristics of the proportional counting tubes must be determined: the optimum operating voltage; the counting efficiency; and the fraction of the total volume which is enclosed by the silvered surface of the tube.

It is found that, as the voltage through the tube is increased, more and more disintegrations in the sample are recorded until over a 200-300 volt region, there is a plateau where there is very little increase in recorded disintegrations for small voltage increases. This plateau occurred from 3600 to 3900 volts for the tubes used in this study. All of the disintegrations in the sample are recorded in this region. At lower voltages, many disintegrations are not recorded, while at higher voltages, a spurious increase in the number of recorded disintegrations is observed.

The optimum operating voltage is then taken as the midpoint of the plateau on the counts per minute versus voltage curve.

Van Slyke, et al. (48) found that for 100-ml. tubes containing up to 7 mg. of carbon, as carbon dioxide, 98% of the disintegrations occurring were recorded. This efficiency dropped to approximately 93% for samples containing 15 mg. of carbon as carbon dioxide. They therefore determined the variation of the efficiency factor with sample size. These values were employed in this work.

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*The tubes were attached to a Nuclear Chicago Model 182 scaling unit for counting.

Finally, the silvered volume fraction was determined by filling the tubes with toluene to the various levels and weighing.

The calibration characteristics and further details are presented in Appendix II.

Reaction Results

The average yields, which were calculated from the radioactive counting data (see Appendix III), are presented in Table III. Each value is the average of duplicate chromatographic analyses.

TABLE III

PERCENT YIELDS[a] IN THE REACTIONS BASED ON THE
REACTION OF METHYL β-D-GLUCOSIDE

Product	Reaction 1	Reaction 2	Reaction 3	Reaction 4
Methyl β-gentiobioside	18.9	6.1	8.8	10.4
	19.4	6.8	9.8	10.0
Methyl β-cellobioside + methyl β-sophoroside	7.1	2.5	4.1	3.7
	7.2	2.7	3.7	3.5
Methyl β-laminaribioside	4.9	1.6	2.2	3.4
	4.7	1.8	2.2	3.4
Higher molecular weight material[b]	2.3	0.2	10.6	33.9
	1.9	0.3	10.7	37.1

[a] Calculated from radioactive counting data in Appendix III.
[b] Calculated on the assumption that all of the higher molecular weight material is trisaccharide.

The ratios of the three disaccharide regions were then calculated from the ratios of the radioactivity data in Appendix III. The average ratios for the four reactions are presented in Table IV.

TABLE IV

AVERAGE RATIOS OF DISACCHARIDE PRODUCTS IN THE REACTIONS

Reaction	MBG[a] :	MBC[b] + MBS[c] :	MBL[d]
1	4.0	1.5	1.0
2	3.8	1.5	1.0
3	4.3	1.8	1.0
4	2.9	1.1	1.0

---

[a] Methyl β-gentiobioside.
[b] Methyl β-cellobioside.
[c] Methyl β-sophoroside.
[d] Methyl β-laminaribioside.

Isotope Dilution Analysis

Since the chromatographic separation of methyl β-cellobioside and methyl β-sophoroside was unsuccessful, isotope dilution analysis was employed to determine the ratio of these two products in the reaction mixture.

Isotope dilution analysis is generally employed to determine the amount of a radioactive compound of known specific activity in a mixture. A known amount of the inactive form of the compound is added to the mixture in large excess. Some of the compound is then isolated by crystallization, derivatization, or any convenient method available, and the new specific activity is determined. It can then be shown that the amount, x, of the radioactive compound in the original mixture is given by

$$x = yB/(C-B),$$

where y is the amount of inactive material added, C is the original specific activity of the compound in the mixture, and B is the final specific activity of the isolated, purified product. A more detailed description of this method and a sample calculation is given in Appendix IV.

Reaction 1 was repeated and then divided into 1-ml. aliquots. Two of the aliquots were diluted with inactive methyl β-sophoroside and the other two were diluted with inactive methyl β-cellobioside. The products were then isolated by crystallization and were found to be pure and have a constant specific activity after three recrystallizations. Mixed melting points were used as criteria for purity. The results are presented in Table V.

Two 250-microliter aliquots of Reaction 1 were spotted on Whatman No. 1 paper and were separated and analyzed as described earlier in this section (p. 24-26). The total activity in the mixture of methyl β-sophoroside and methyl β-cellobioside was thus determined. The amount of this activity due to each component was then determined from the isotope dilution results. The average yields of the two compounds are given in Table VI. It should be observed that the sum of the two yields does not add up to 100%. Some of this difference might be accounted for in the accuracy of the technique. Olson (53) has found that the overall accuracy of the isotope dilution analysis is within ± 2%. Impurities and possibly small amounts of the α-linked disaccharides might also account for this low summative analysis.

These results were applied to Reactions 1 and 2, since the ratios of products (see Table IV) were quite close and, hence, it was thought to be justifiable to assume that the two compounds, methyl β-cellobioside and methyl β-sophoroside, were in the same proportions in both reactions. The results were also applied to Reactions 3 and 4, although the justification for doing so is in doubt, because of the apparent difference in the observed ratios of the reactivities. The recalculated ratios are given in Table VII.

TABLE V

ISOTOPE DILUTION ANALYSIS RESULTS FOR REACTION 1

Sample	Specific Activity, 10^{-4} dis./min./mM		Melting Points, °C.			Mole Ratio of Inactive to Active Material
	After 2nd Recrystallization	After 3rd Recrystallization	Isolated Product	Pure Product	Mixed	
MBS[a] I	0.98 1.06	0.98 0.98	194.5-195.5	194.5-195.5	194.5-195.0	218:1
MBS II	1.78 1.89	1.68 1.72	194.5-195.5	194.5-195.5	194.5-195.5	120:1
MBC[b] I	2.02 2.00	2.00 --	187.5-188.5	188.0-189.0	187.5-188.5	107:1
MBC II	2.01	--	187.0-188.5	188.0-189.0	187.5-188.5	129:1

[a] Methyl β-sophoroside.
[b] Methyl β-cellobioside.

TABLE VI

AVERAGE YIELDS OF METHYL β-SOPHOROSIDE AND METHYL β-CELLOBIOSIDE FROM ISOTOPE DILUTION ANALYSES

Sample	Average Yield, mg.	% in Mixture
MBS[a]	0.824	41.4
MBC[b]	0.978	49.4

[a] Methyl β-sophoroside.
[b] Methyl β-cellobioside.

TABLE VII

AVERAGE RATIOS OF DISACCHARIDE PRODUCTS USING ISOTOPE DILUTION RESULTS TO SEPARATE MBS[a] AND MBC[b]

Reaction	MBG[c]	MBL[d]	MBC	MBS
1	6.6	1.7	1.2	1.0
2	6.2	1.6	1.2	1.0
3	6.0	1.4	1.2	1.0
4	7.1	2.4	1.2	1.0

[a] Methyl β-sophoroside.
[b] Methyl β-cellobioside.
[c] Methyl β-gentiobioside.
[d] Methyl β-laminaribioside.

LARGE-SCALE REACTION

STARTING MATERIALS

The starting materials were identical to those used in the labeled Reaction 1 (p. 19) except that inactive α-acetobromo-D-glucose was employed instead of the radioactive material.

COMPOSITION OF REACTION

The amounts of the reactants were calculated to be exactly one hundred times the amounts for Reaction 1 (p. 19). The composition is presented in Table VIII. The reagents were weighed out on an analytical balance in a dry atmosphere.

TABLE VIII

COMPOSITION OF LARGE-SCALE REACTION

α-Acetobromo-D-glucose	21.20 g.
Methyl β-D-glucoside	10.00 g.
Silver oxide	26.20 g.
Iodine	2.62 g.
Drierite	200.0 g.
Dioxane	400.0 ml.

REACTION PROCEDURE

The reaction was carried out in a 1000-ml. round-bottomed, foil-covered flask in a constant temperature bath at 30°C. for 72 hours. The reaction mixture was stirred constantly with an electric stirrer motor.

The reaction was quenched in a water-acetone mixture and worked up in the same way as described earlier in this section (p. 21). It was necessary, however, to repeat the sodium hydroxide treatment to remove all of the residual D-glucose in the final mixture. The final deionized reaction mixture was concentrated to a thick sirup and then diluted to 25 ml. with methanol in a volumetric flask.

Chromatography

Whatman No. 3MM chromatography paper was employed to separate the products of this large-scale reaction mixture. Twenty 8 x 22-inch sheets were streaked with

200 microliters each of the reaction mixture. The concentration of the solution was approximately 0.5 mg. per microliter, and so 100 mg. was streaked on each sheet. The chromatograms were irrigated for 48 hours in the solvent, ethyl acetate:pyridine:water (8:2:1). The bands were located by developing a 1.5-cm. strip down the center of each chromatogram with the silver nitrate/sodium hydroxide dip. The bands were then combined so that there were forty strips each of methyl β-gentiobioside, methyl β-sophoroside, and methyl β-cellobioside (together), and methyl β-laminaribioside.

The strips were then eluted with water, according to the method of Bearce (54), ten at a time, by interleaving wicks of Whatman No. 1 paper as shown in Fig. 4. The strips of Whatman No. 3MM interleaved with Whatman No. 1 were held together by wrapping "Parafilm" around the main body and securing with rubber bands. This also prevented excessive evaporation during the elution.
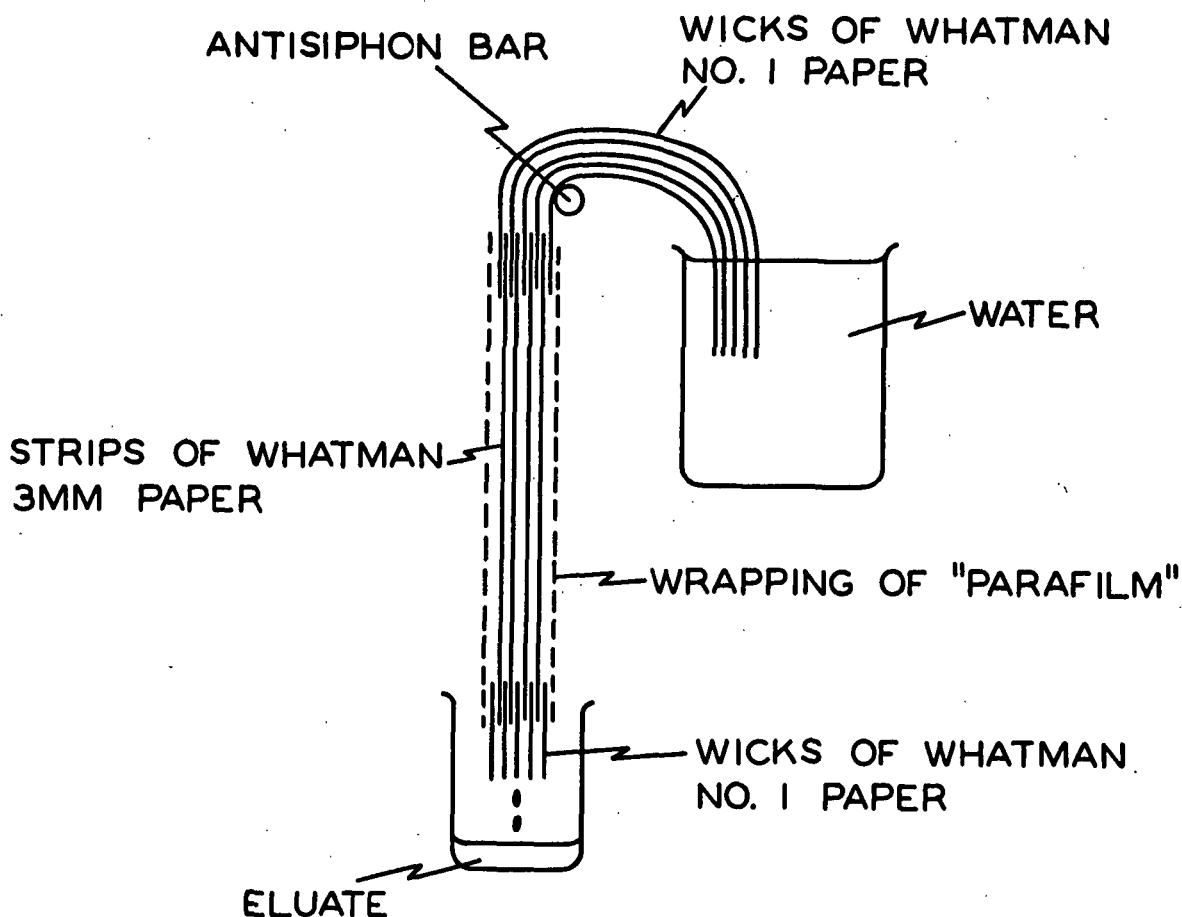

Figure 4. Elution of Heavy Paper Chromatograms

The eluates for each of the three disaccharide regions were concentrated to small volumes at reduced pressure and then filtered through a thin layer of activated charcoal over a layer of "Celite" filter-aid in a microfilter funnel. The water-clear solutions were concentrated to thick sirups on a rotating evaporator under reduced pressure in tared weighing bottles. The sirups were then evaporated to constant weight in a vacuum oven at 50°C. The entire samples were then diluted to exact volumes in volumetric flasks, and the specific optical rotations were determined on each one. The results are given in Table IX.

TABLE IX

YIELDS AND SPECIFIC OPTICAL ROTATIONS FOR THE
PRODUCTS OF THE LARGE-SCALE REACTION

Fraction	Yield[a]	Specific Rotation Found on Sirups, $[\alpha]_D^{22°C.}$ (water)	Concn., g./100 ml.	Specific Rotation of Pure Compounds, $[\alpha]_D$ (water)
MBG[b]	411.9	-21.3°	8.24	-36° (42)
MBS[c] + MBC[d]	148.8	-21.9°	2.98	-36°, -19° ([f],39)
MBL[e]	96.3	-21.2°	1.93	-27° (40)

---

[a] Yield of sirup isolated from 20 chromatograms.
[b] Methyl β-gentiobioside.
[c] Methyl β-sophoroside.
[d] Methyl β-cellobioside.
[e] Methyl β-laminaribioside.
[f] This work.

Identification of Products

The two fractions, presumably consisting of methyl β-gentiobioside and methyl β-laminaribioside, were acetylated by the method reported by Wolfrom, et al. (55) as modified by Olson (53). This procedure involved the acetylation in acetic anhydride with sodium acetate as a catalyst. The acetates crystallized spontaneously from the acetylation mixtures in good yields.

The two acetates were isolated and purified by recrystallization from ethanol. Methyl hepta-O-acetyl-β-gentiobioside melted at 136.5-137.5°C., $[\alpha]_D^{22°C.} = -17.6°$ (chloroform). Literature values are 82°C. and 150-151°C., $[\alpha]_D^{20°C.} = -18.9°$ (chloroform) (42,56). Methyl hepta-O-acetyl-β-laminaribioside melted at 177.0-177.5°C., $[\alpha]_D^{22°C.} = -45.2°$ (chloroform). Literature values are 164-165°C. and 179-202°C., $[\alpha]_D^{20°C.} = -45°$ (chloroform) (57). Apparently, the melting points for these compounds are in doubt, but the specific optical rotations seem to be in accord.

Since the fraction containing methyl β-cellobioside and methyl β-sophoroside could not be separated, acetylation and identification of these acetates was not attempted. It is thought that the isotope dilution analysis of the small-scale Reaction 1 is a good qualitative identification of these two products.

DISCUSSION OF RESULTS

CHARACTERIZATION OF METHYL β-SOPHOROSIDE

Since methyl β-sophoroside is a new compound, it was necessary to character-
ize it as closely as possible. The data presented in the Experimental Procedures
and Results section (p. 15) indicate that the compound is certainly a methyl
glycoside, but do not show conclusively which anomer is involved. The following
argument indicates that it is indeed the β-anomer.

Since the unknown compound (presumably methyl β-sophoroside) was synthesized
by the Koenigs-Knorr methanolysis of α-acetobromosophorose, it can be concluded
that the compound is indeed a derivative of sophorose. This sophorose derivative
was then used in the isotope dilution analysis of Reaction 1. Subsequent isola-
tion of the compound from the reaction mixture and purification by recrystalliza-
tion, yielded a radioactive product of constant specific activity which was
identical with the original sophorose derivative. This shows that the same
sophorose derivative was present in Reaction 1 in the radioactive form.

Reaction 1 involved the condensation of α-acetobromo-D-glucose-C^{14} and methyl
β-D-glucoside. Hence, any condensation product would contain a methyl β-D-gluco-
side residue. Since methyl β-sophoroside contains a methyl β-D-glucoside residue,
the unknown sophorose derivative must be methyl β-sophoroside.

HUDSON'S RULES OF ISOROTATION

An attempt was made to predict the specific optical rotation of methyl
hepta-O-acetyl-β-sophoroside using the known specific optical rotation of methyl
hepta-O-acetyl-α-sophoroside (43). Hudson's rules of isorotation predict that the
specific optical rotation of methyl hepta-O-acetyl-β-sophoroside should be approx-
imately -32°. (The calculations are presented in Appendix V.) The synthesis of

methyl hepta-O-acetyl-β-sophoroside (Experimental Procedures and Results section, p. 15) yielded pure crystals which had a specific optical rotation $[\alpha]_D^{22°C.}$ = -1.2° (c = 13, chloroform). This value is not consistent with the Hudson's rules prediction of -32°.

Korytnyk (58) has critically examined Hudson's rotational correlations and has concluded that for aglycones of low or moderate polarizability, marked alterations in structure at Positions 3 and 5 have little effect on the A value for the aglycone. However, the A values of all aglycones are affected by changes at Position 2. This is due to the vicinal interaction of the aglycone on Position 1 and the 2 position. Korytnyk has modified Hudson's approach by dividing the disaccharide molecule as shown in Fig. 5 for β-cellobiose octaacetate. The contribution of the nonreducing ring, $B_{Ac}^{II} + A_M$, to the optical rotation is calculated as the molecular rotation of β-cellobiose octaacetate minus the molecular rotation of β-D-glucose pentaacetate. This contribution should be constant for any given series of disaccharide derivatives.
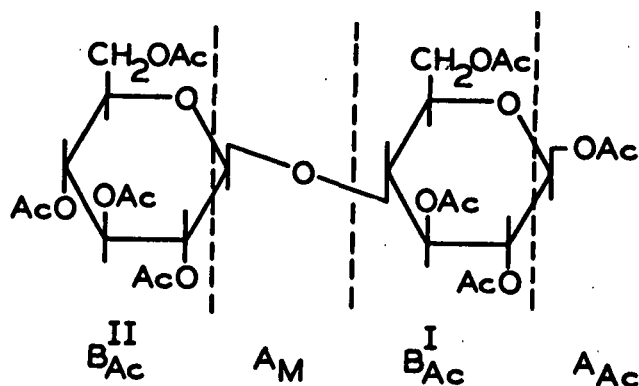

Figure 5. Korytnyk's Division of a Disaccharide Molecule (57)

Hudson's exact relationship:

$$[M_x]^{di} = B_x^{di} \pm A_x^{di} \qquad (5)$$

becomes:

$$[M_x]^{di} = B_{Ac}^{II} + A_M + B_{Ac}^{I} \pm A_{Ac} \qquad (6)$$

which reduces to:

$$[M_x]^{di} = B_{Ac}^{di} + B_x^{mono} - B_{Ac}^{mono} \pm A_x^{mono} \qquad (7)$$

$[M_x]$ is the molecular rotation of the disaccharide with aglycone $\underline{x}$, the subscript $\underline{Ac}$ refers to the fully acetylated derivative, the superscripts indicate mono- or disaccharide, and the positive or negative sign refers to the α- or β-amoner, respectively. Using Equation (7), the values for the molecular rotations of the methyl β-glycosides of the four β-linked glucose-glucose disaccharides were calculated and compared to the known values. The resulting specific optical rotations are compared in Table X.

TABLE X

CALCULATED[a] AND REPORTED ROTATIONS FOR THE
HEPTAACETATES OF FOUR DISACCHARIDE METHYL β-GLYCOSIDES

Disaccharide (heptaacetates)	Glucose-Glucose Linkage, β-	Calculated,[a] $[\alpha]_D$	Reported, $[\alpha]_D$
Methyl β-gentiobioside	1 → 6	-18.3°	-18.0° (42)
Methyl β-cellobioside	1 → 4	-28.1°	-26.0° (39)
Methyl β-laminaribioside	1 → 3	-46.0°	-45.0° (40)
Methyl β-sophoroside	1 → 2	-20.3°	-1.2°[b]

---

[a] Calculated from Equation (7).
[b] This work.

The agreement between the reported values and the values calculated from Equation (7) is remarkably good except for methyl β-sophoroside heptaacetate. This result confirms the vicinal interaction of the aglycone and the glucosyl unit on Position 2.

LABELED REACTIONS

A summary of the average ratios of the reactivities of the hydroxyl groups
of methyl β-D-glucoside is presented in Table XI. The average yields of the
four disaccharide products are presented in Table XII.

TABLE XI

THE REACTIVITY OF METHYL β-D-GLUCOSIDE

| Reaction | Conditions[a] | | Ratio[b] of Yields for | | | |
	Molar Ratio of Reactants[c]	Solvent	6-OH :	3-OH :	4-OH :	2-OH
1	1:1	p-Dioxane	6.6	1.7	1.2	1.0
2	1:1	p-Dioxane/ nitromethane[d]	6.2	1.6	1.2	1.0
3	5:1	p-Dioxane	6.0	1.4	1.2	1.0
4	5:1	p-Dioxane/ nitromethane[d]	7.1	2.4	1.2	1.0

[a] All other conditions held constant (see Table I).
[b] Calculated from the radioactive counting data in Appendix III.
[c] Molar ratio of α-acetobromo-D-glucose-C^{14} to methyl β-D-glucoside in the initial reaction mixture.
[d] Mixed solvent: dioxane/nitromethane, 1:1 by volume.

The most obvious fact that is apparent from the values in Table XI is the
large difference in the reactivity of the primary hydroxyl group compared with
the three secondary hydroxyls. This was to be expected, since it has been
generally accepted that the primary hydroxyl reacts more readily than the secon-
dary hydroxyls in the Koenigs-Knorr reaction. However, the order of reactivity
of the secondary hydroxyls is somewhat surprising. Sugihara (18) found that the
2-hydroxyl was generally very close in reactivity to the 6-hydroxyl, both in
alkaline and acidic media. The Koenigs-Knorr reaction is carried out under
virtually neutral conditions and it is apparent from the results of this work

that the 2-hydroxyl is not so reactive under these conditions. The results for methyl β-D-glucoside in Table XI indicate that the 2-hydroxyl is the least reactive of the secondary hydroxyls and is only about one-sixth as reactive as the 6-hydroxyl. de Belder, et al. (27) studied the alkaline methylation of methyl β-D-glucoside. They found that the 2-hydroxyl was equal in reactivity to the 6-hydroxyl. Apparently, the anomeric methyl group in methyl β-D-glucoside offers considerable steric interference to the 2-hydroxyl in the neutral Koenigs-Knorr reaction.

TABLE XII

PERCENT YIELDS[a] IN LABELED REACTIONS

	Reaction[b] 1	Reaction[c] 2	Reaction[d] 3	Reaction[e] 4
Total reaction, %	32.6	10.8	25.7	52.4
Total trisaccharides[f], %	2.1	0.2	10.7	35.5
Total disaccharides, %	30.5	10.6	15.0	16.9
Methyl β-gentiobioside, %	19.2	6.5	9.3	10.2
Methyl β-laminaribioside, %	4.8	1.7	2.2	3.4
Methyl β-cellobioside[g], %	3.6	1.3	1.9	1.8
Methyl β-sophoroside[g], %	2.9	1.1	1.6	1.5

[a] Yields based on the original amount of methyl β-D-glucoside.
[b] Conditions: molar ratio of reactants, 1:1; solvent: p-dioxane.
[c] Conditions: molar ratio of reactants, 1:1; solvent: p-dioxane/nitromethane, 1:1 by volume.
[d] Conditions: molar ratio of reactants, 5:1; solvent: p-dioxane.
[e] Conditions: molar ratio of reactants, 5:1; solvent: p-dioxane/nitromethane, 1:1 by volume.
[f] Calculated on the assumption that all of the higher molecular weight material is trisaccharide.
[g] Calculated from isotope dilution analysis data.

The most interesting result of the reactivities of the three secondary hydroxyls is that the 4-hydroxyl appears to be of the same order of reactivity as the 2- and 3-hydroxyls. Most of the work up until now has indicated that the 4-hydroxyl is particularly unreactive. The synthesis of oligosaccharides with the (1 → 4)-linkage has not been very successful (28-30). Wadsworth (17) concluded that the reason that his bifunctional compound would not polymerize was that the 4-hydroxyl was inherently too unreactive. This conclusion is probably true for substituted compounds because of the effect of neighboring groups. Wadsworth's compound was substituted in the 2-, 3-, and 6-positions with bulky O-benzoyl groups, and hence the 4-hydroxyl must have encountered considerable steric hindrance. The present work shows that the 4-hydroxyl is not inherently unreactive in an unsubstituted compound.

A virtually constant ratio is found for the reactivities of the hydroxyls in Reactions 1, 2, and 3 with an average value of 6.3:1.6:1.2:1.0 for the reactivities of the 6-position:3-position:4-position:2-position. Reaction 4 appears to be somewhat different.

In Reactions 1 and 2, the disaccharides are the main products of the reaction. There is very little production of higher molecular weight material. The good agreement in the ratio of products is therefore to be expected. The use of the more polar solvent, nitromethane, in Reaction 2 has little effect on the ratio of products, but it does affect the rate of the reaction. The rate is drastically reduced by the addition of nitromethane.

Reactions 3 and 4 cannot be compared directly with Reactions 1 and 2. In this case, the disaccharides are only intermediates in the production of the higher molecular weight materials. It should not be expected then that the ratios of the reactivities of the hydroxyls would be the same as in Reactions 1 and 2.

It is probably fortuitous that the ratios are as close as they are. The large production of trisaccharides would be expected to alter the ratio of disaccharides, since the disaccharides with a free primary hydroxyl, such as methyl β-cellobioside, methyl β-sophoroside, and methyl β-laminaribioside, would be the first to react further to produce trisaccharides. This would tend to decrease the relative amounts of these three disaccharides compared with the methyl β-gentiobiosdde. This is apparent in Reaction 4 where the ratio has been altered in favor of methyl β-gentiobioside.

The order of reactivity, 6-hydroxyl >> 3-hydroxyl > 4-hydroxyl > 2-hydroxyl, is consistent with the steric arrangement of the hydroxyl groups in the methyl β-D-glucoside. The 6-hydroxyl is the most available one sterically and at the same time is a primary hydroxyl group. Schroeder (2) showed that the rate of reaction of primary alcohols with α-acetobromo-D-glucose was much greater than that of secondary alcohols. Of the three secondary hydroxyls, the 3-hydroxyl is the least sterically hindered. It is not surprising, therefore, that it is second in order of reactivity. The 4-hydroxyl encounters some interference from the bulky group on Position 5 and the 2-hydroxyl is well blocked by the anomeric methyl group.

EFFECT OF SOLVENT

The constancy of the reactivity ratios in Table XI for Reactions 1 and 2 shows that the solvent has little or no effect on the ratios of the reactivities of the hydroxyl groups. However, Table XII shows the considerable effect of the solvent on the yields of the various products. Since the data are rather limited, it is difficult to draw any concrete conclusions about this effect, but some speculation on the probable causes is valid.

Reactions 1 and 2 reveal a solvent effect quite different from that in Reactions 3 and 4. Reaction 2 shows a large drop in reaction rate when the more polar solvent, nitromethane, is substituted for one half of the dioxane in Reaction 1. On the other hand, Reaction 4 exhibits a large increase in reaction rate over Reaction 3 when nitromethane is added to the system. The only difference between these two reaction pairs is that the reactants, methyl β-D-glucoside and α-acetobromo-D-glucose-C^{14}, were equimolar in Reactions 1 and 2; while in Reactions 3 and 4, α-acetobromo-D-glucose-C^{14} was in fivefold excess.

In the case of Reactions 3 and 4, it is relatively simple to attribute the increase in reaction rate to the increase in dielectric constant of the solvent. However, in Reaction 2, the opposite effect is observed. It can only be suggested that the solvent, nitromethane, is interfering with the reactivity of the hydroxyl groups in some way, perhaps by physically blocking the polar hydroxyl groups by forming a solvent cage around some of the methyl β-D-glucoside molecules.

It should be remembered that, in these reactions, the concentration of α-acetobromo-D-glucose-C^{14} varies from 5 to 25 weight percent. These solutions are extremely concentrated and hence it is very difficult to predict solvent or other kinetic effects. In the case of Reactions 3 and 4, where the concentration of α-acetobromo-D-glucose is 25%, interactions between individual molecules of α-acetobromo-D-glucose is probably quite significant. Also, the bromosugar, being quite polar itself, may contribute greatly to the polarity of the solvent.

EFFECT OF CONCENTRATION OF α-ACETOBROMO-D-GLUCOSE-C^{14}

Comparing Reactions 1 and 3, the only difference between the two is the excess bromosugar in Reaction 3. Similarly for Reactions 2 and 4, the bromosugar is in excess in Reaction 4. In both cases, there is a large increase in

the production of higher molecular weight material. This might be expected, since addition of a large excess of the bromosugar would tend to increase the probability of any disaccharides already formed to react further to form tri-, tetra-, and even pentasaccharides. These higher molecular weight materials would have the general formula
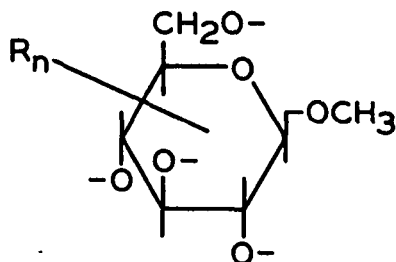
where R is a glucosyl unit linked either α- or β-, and $\underline{n}$ is 2,3, or 4. Since condensation of two molecules of the bulky α-acetobromo-D-glucose-C^{14} with one molecule of methyl β-D-glucoside would give a trisaccharide which would be very sterically hindered, further reaction to form a tetra- and pentasaccharide would be less likely. Hence, the assumption was made in this work that only trisaccharides were formed.

LARGE-SCALE REACTION

A large-scale reaction was carried out primarily for two reasons: (1) to determine if any α-linked products were formed in this condensation; and (2) to identify the reaction products. The large-scale reaction was identical in composition with Reaction 1 except that all of the amounts of the reactants were one hundred times those in Reaction 1. The results obtained for the large-scale reaction are summarized in Table XIII. (See also Table IX of the Experimental Procedures and Results section, p. 34.)

The ratio of yields of the disaccharides compares well with that found for Reaction 1 of 4.0:1.5:1.0. The comparison of the two reactions is then considered justifiable.

TABLE XIII

DATA FOR LARGE-SCALE REACTION MIXTURE

Disaccharide	Ratio[a] of Yields	Specific Rotation Found on Sirups, $[\alpha]_D^{22°C.}$ (water)	Specific Rotation of Pure Compounds, $[\alpha]_D$ (water)
MBG[b]	4.3	-21.3°	-36° (42)
MBS[c] + MBC[d]	1.5	-21.9°	-36°, -19° ([f],39)
MBL[e]	1.0	-21.2°	-27° (40)

[a] Ratios of yields of sirups from chromatograms at constant weight.
[b] Methyl β-gentiobioside.
[c] Methyl β-sophoroside.
[d] Methyl β-cellobioside.
[e] Methyl β-laminaribioside.
[f] This work.

The specific optical rotations, which were determined on the products isolated from chromatograms without purification, are all quite negative in value. If they are compared with the values for the pure compounds, it is apparent that they are somewhat low. There are two probable reasons for these low values. The specific optical rotation is inversely proportional to the concentration of the material. Since the rotations were determined on the sirups, a low value might be expected, because it is not possible to remove all of the solvent from a sirup. Hence, the true concentration of the material is not known. The second reason is that these rotations were determined directly on the unpurified materials as isolated from the paper chromatograms. Any impurities from the paper or chromatographic solvents would result in a low reading of the specific optical rotation. The closeness of the observed values, under these conditions, to the values for the pure compounds is considered to be good evidence that the products are chiefly the β-linked di-saccharides. Also, since the α-linked disaccharides tend to have a high positive specific optical rotation, [e.g., methyl α-maltoside, $[\alpha]_D = +80°$ (water) (56)] any

significant amount of these compounds would tend to reduce the values obtained for the reaction products. In the chromatograms of Reaction 4, the spot for methyl β-gentiobioside did appear to consist of two very closely following spots; the slower moving part of the spot had the same R_f as methyl β-gentiobioside. Speculation that the faster-moving "half spot" might be methyl β-isomaltoside [α(1 → 6)-linked glucose-glucose] is valid. However, since this compound was not available and its R_f in the solvent used was unknown, it was not pursued further.

Finally, the two disaccharides, methyl β-gentiobioside and methyl β-laminaribioside, were identified in the large-scale reaction mixture from their hepta-acetates. Since it was not possible to separate the other two, methyl β-cellobio-side and methyl β-sophoroside, it was not possible to identify them in the large-scale reaction mixture. However, the fact that in the isotope dilution analysis of Reaction 1 the two inactive compounds that were used became radio-active upon reisolation, is considered to be reasonable qualitative evidence that methyl β-cellobioside and methyl β-sophoroside were present in Reaction 1. There is no reason to believe that the large-scale reaction would be any different.

CONCLUSIONS

The order of reactivity of the hydroxyl groups of methyl β-D-glucoside in the Koenigs-Knorr reaction is 6-hydroxyl >> 3-hydroxyl > 4-hydroxyl > 2-hydroxyl. The ratios of these reactivities are reasonably constant for reactions in which the reactants are equimolar for two different solvents. This ratio has an average value of 6.4:1.7:1.2:1.0. It is not constant for reactions in which the bromo-sugar is in large excess over the methyl β-D-glucoside.

The order of reactivity of the three secondary hydroxyls, 3-hydroxyl > 4-hydroxyl > 2-hydroxyl, is consistent with their steric arrangement in the ring. The 4-hydroxyl is inherently less reactive than the 6-hydroxyl, but is of the same order of reactivity as the 3-hydroxyl and 2-hydroxyl. In the synthesis of disaccharides from substituted sugar units in the Koenigs-Knorr reaction, the apparent low reactivity of the 4-hydroxyl is probably due to the influence of neighboring blocking groups.

The solvent has a marked effect on the reaction rate. At low concentrations of the bromosugar (4.6 weight percent), addition of nitromethane considerably retards the rate of reaction. At high concentrations of the bromosugar (23.0 weight percent), addition of nitromethane greatly increases the rate of reaction.

Addition of a fivefold excess of the bromosugar over the methyl β-D-glucoside causes a large increase in the reaction rate of the disaccharide products to form higher molecular weight materials. These materials were assumed to be mainly tri-saccharides containing methyl β-D-glucoside substituted in two positions with glucosyl groups.

The disaccharide products of this reaction were mainly the β-linked glucose-glucose compounds.

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APPENDIX I

SYNTHETIC PATHWAYS FOR PRODUCTION OF
DISACCHARIDE REFERENCE COMPOUNDS

This appendix contains the detailed synthetic pathways which were followed

in the synthesis of the four disaccharide glycosides.

SYNTHESIS OF METHYL β-CELLOBIOSIDE

STEP 1. FISCHER AND ZEMPLEN (37)

β-CELLOBIOSE OCTAACETATE   α-ACETOBROMOCELLOBIOSE

STEP 2. SCHROEDER AND GREEN (36)

METHYL β-CELLOBIOSIDE
HEPTAACETATE

STEP 3

METHYL β-CELLOBIOSIDE
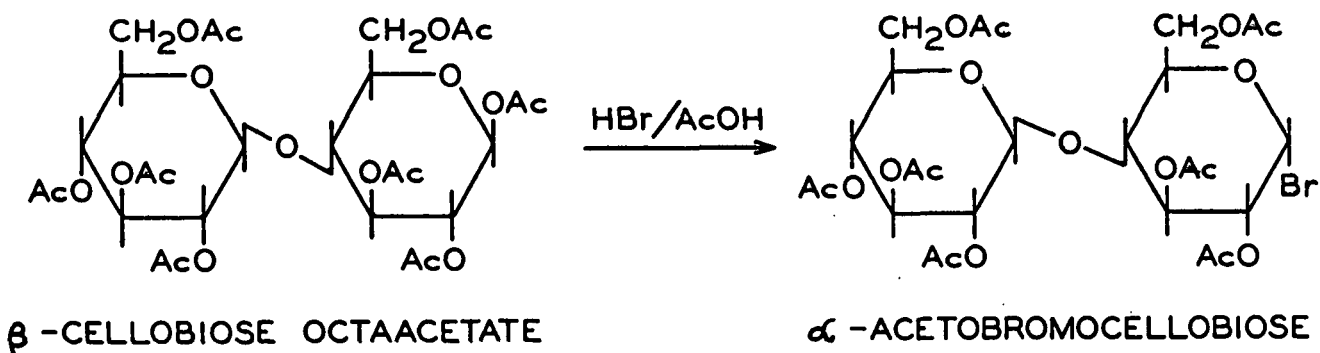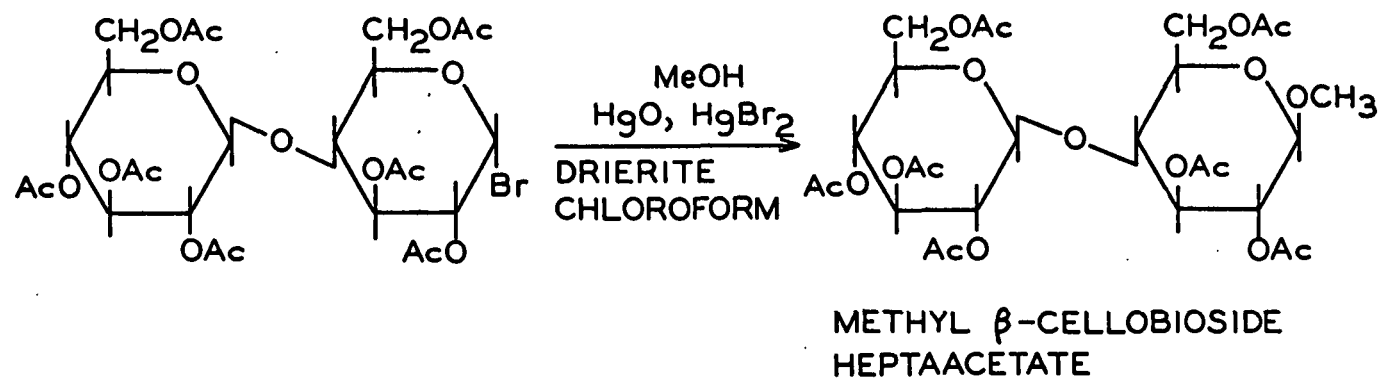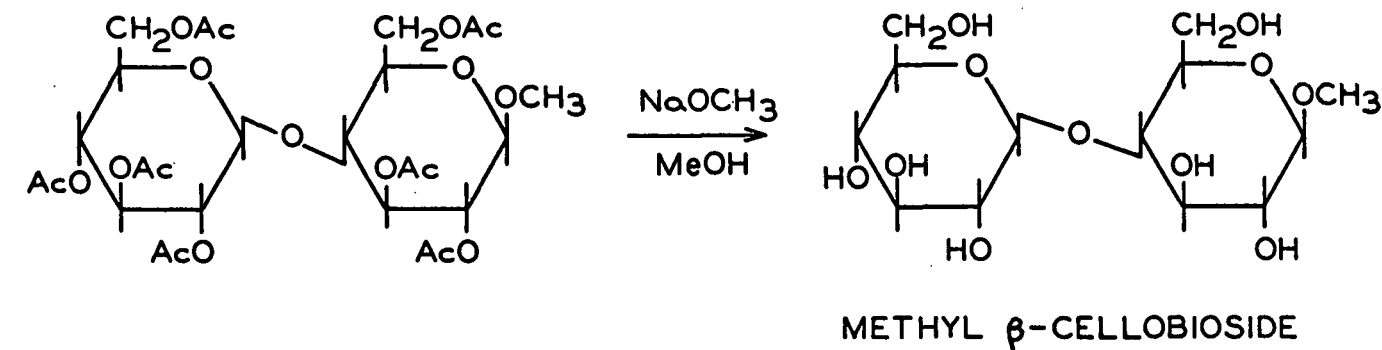
SYNTHESIS OF METHYL β-LAMINARIBIOSIDE

BACHLI AND PERCIVAL (40)

Step 1

D-GLUCOSE   ACETONE

$ZnCl_2$ / H_3PO_4

1, 2:5,6-DI-O-ISOPROPYLIDENE-D-GLUCOFURANOSE

Step 2

Ag_2CO_3, I_2 / DRIERITE / BENZENE

Step 3

$NaOCH_3$/MeOH / 0.1 N OXALIC ACID

α-LAMINARIBIOSE

Step 4

α,β-LAMINARIBIOSE OCTAACETATES

Step 5

α-ACETOBROMOLAMINARIBIOSE

Step 6

METHYL β-LAMINARIBIOSIDE
HEPTAACETATE

Step 7

METHYL β-LAMINARIBIOSIDE
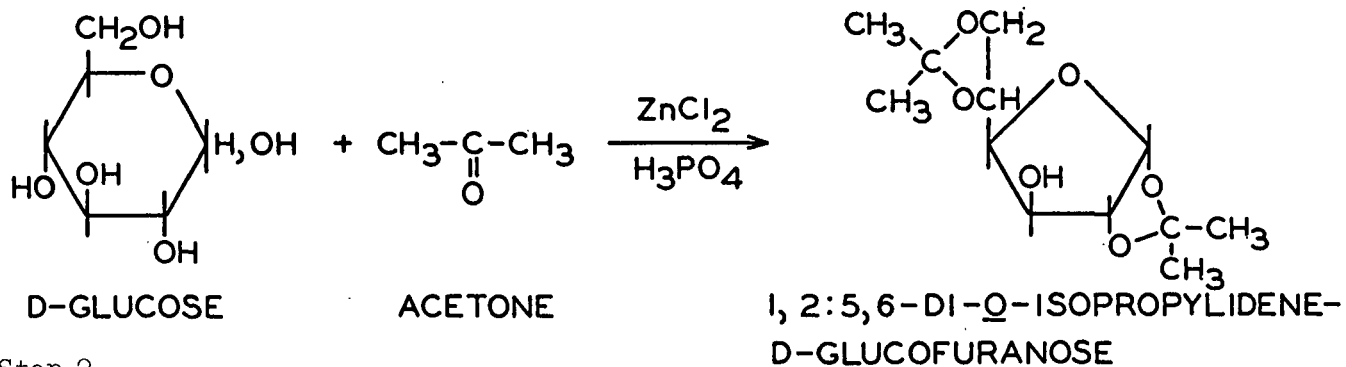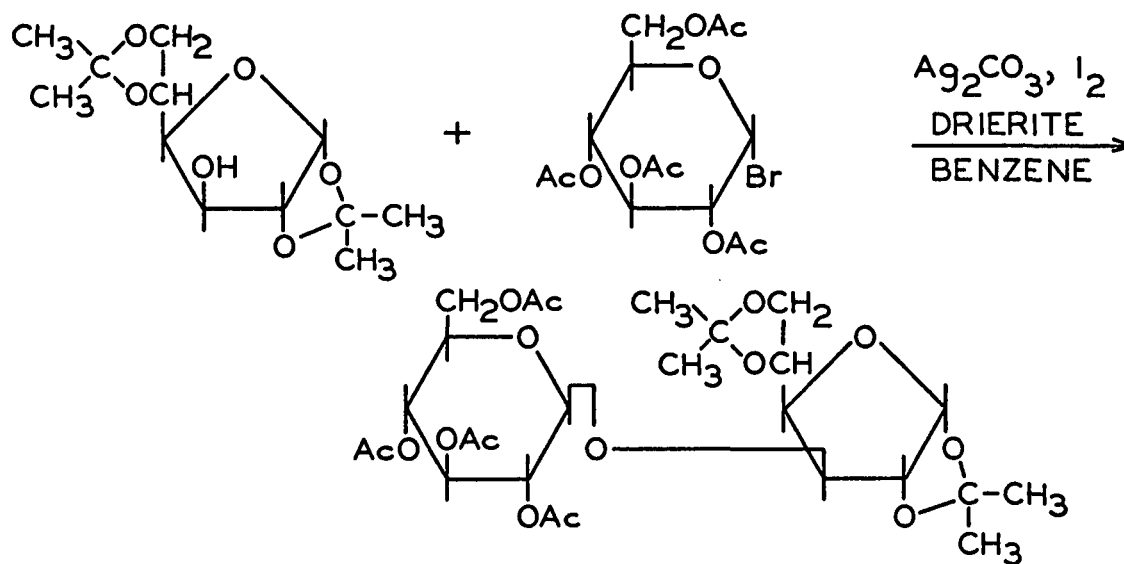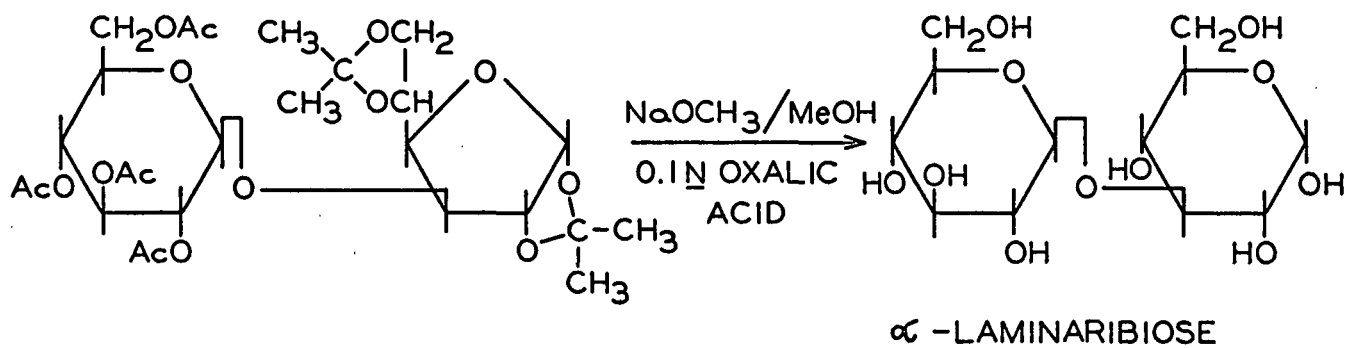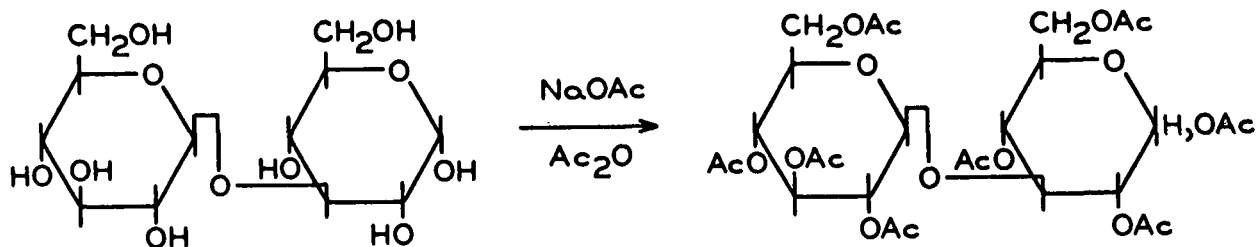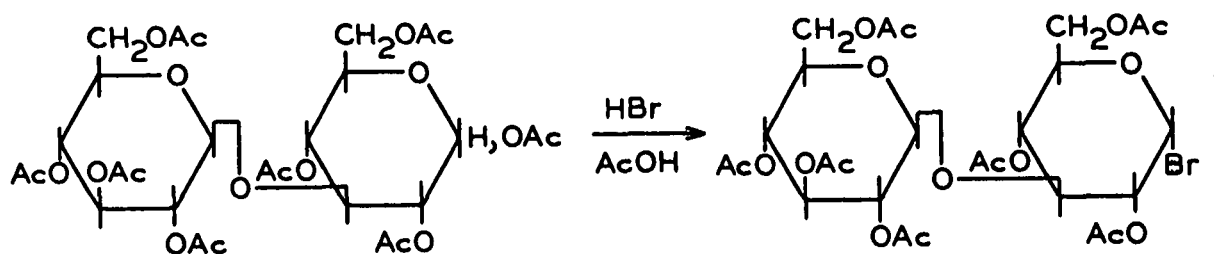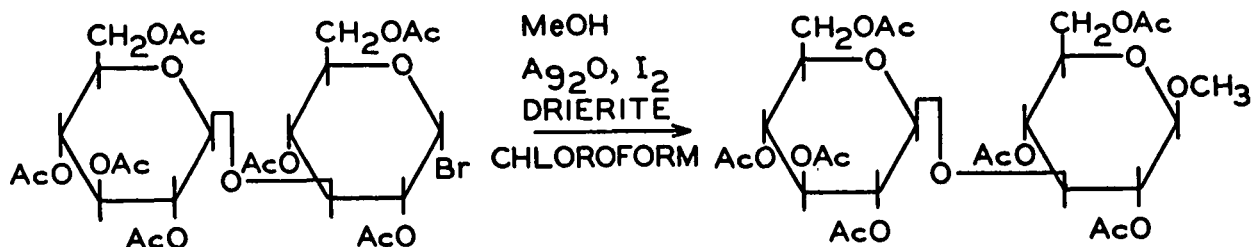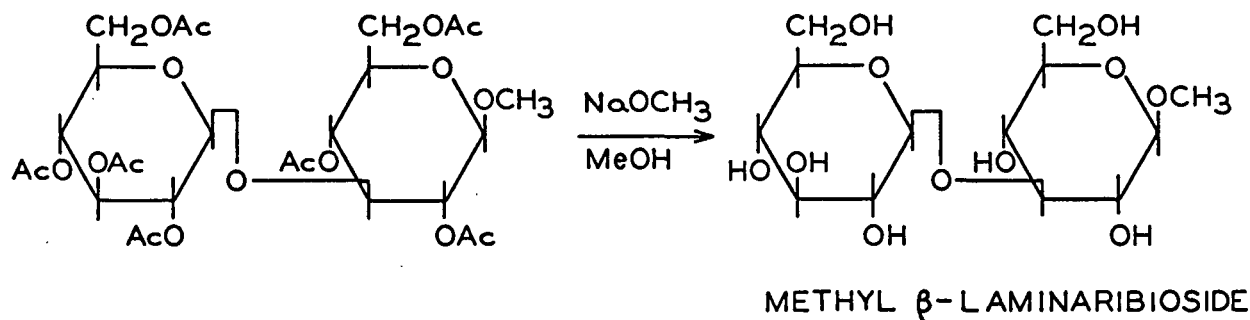
SYNTHESIS OF METHYL β-GENTIOBIOSIDE
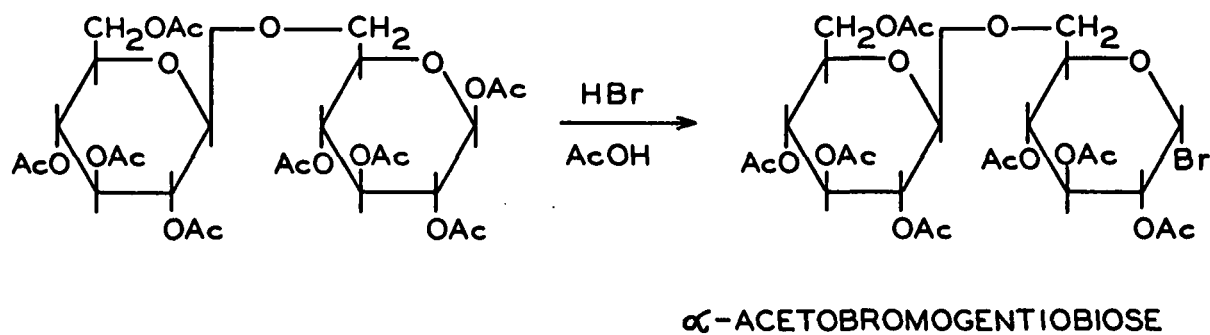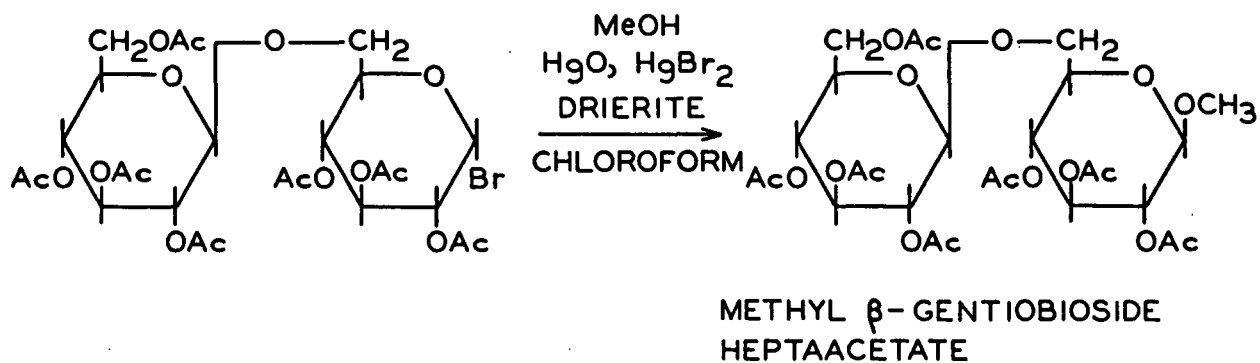
HELFERICH (41); REYNOLDS AND EVANS (13)

Step 1
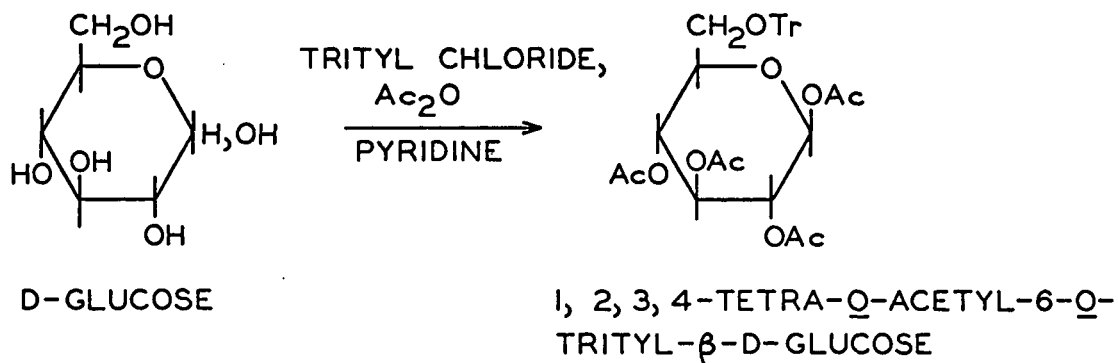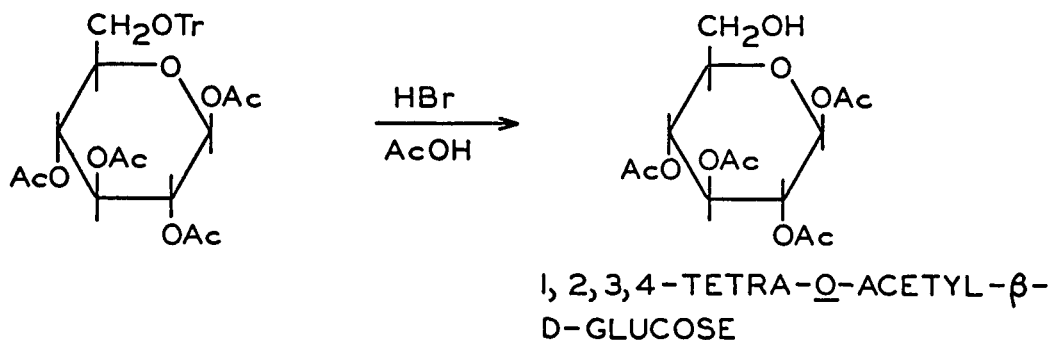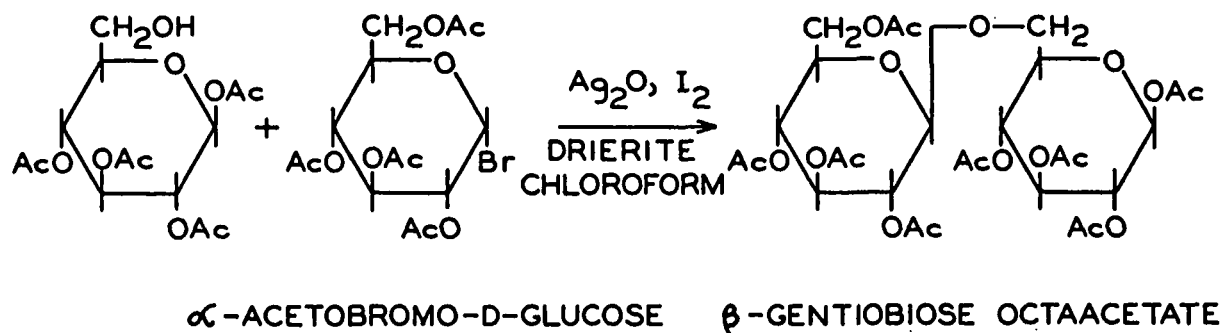
D-GLUCOSE
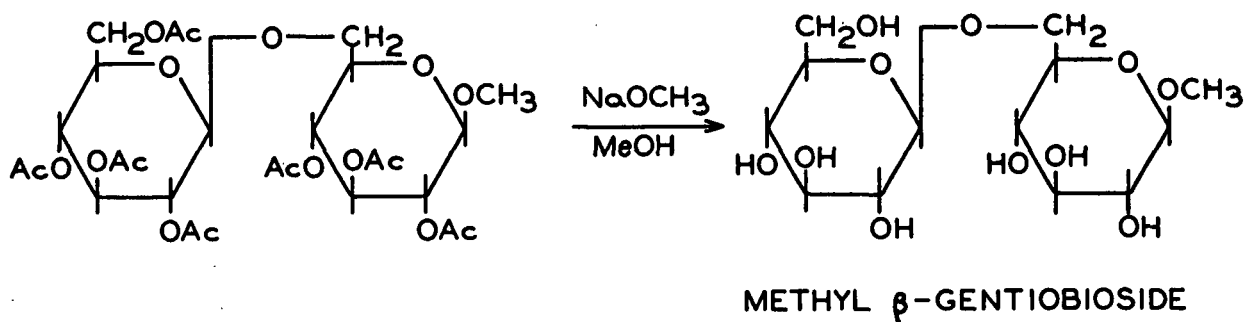
1, 2, 3, 4-TETRA-O-ACETYL-6-O-
TRITYL-β-D-GLUCOSE

Step 2

1, 2, 3, 4-TETRA-O-ACETYL-β-
D-GLUCOSE

Step 3

α-ACETOBROMO-D-GLUCOSE   β-GENTIOBIOSE OCTAACETATE

STEP 4.   FISCHER AND ZEMPLEN (37)

α-ACETOBROMOGENTIOBIOSE

STEP 5.   SCHROEDER AND GREEN (36)

METHYL β-GENTIOBIOSIDE
HEPTAACETATE

Step 6

METHYL β-GENTIOBIOSIDE
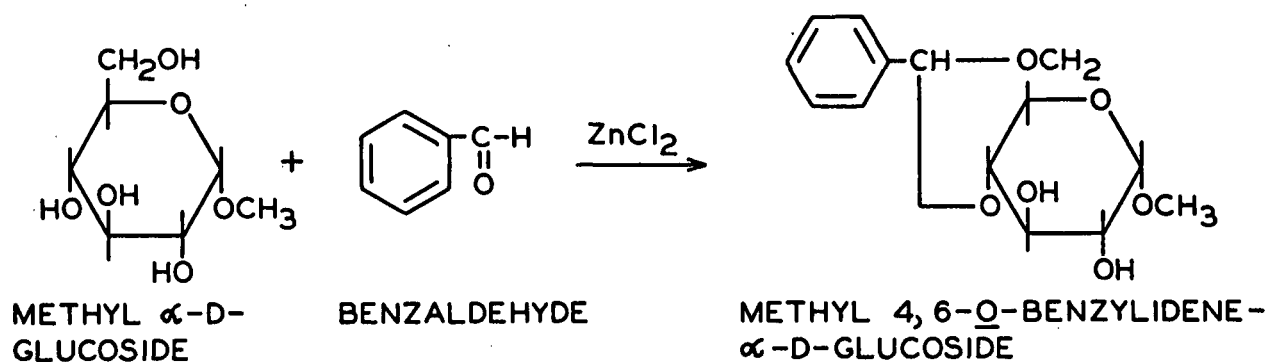
SYNTHESIS OF METHYL β-SOPHOROSIDE

COXON AND FLETCHER (44)

Step 1

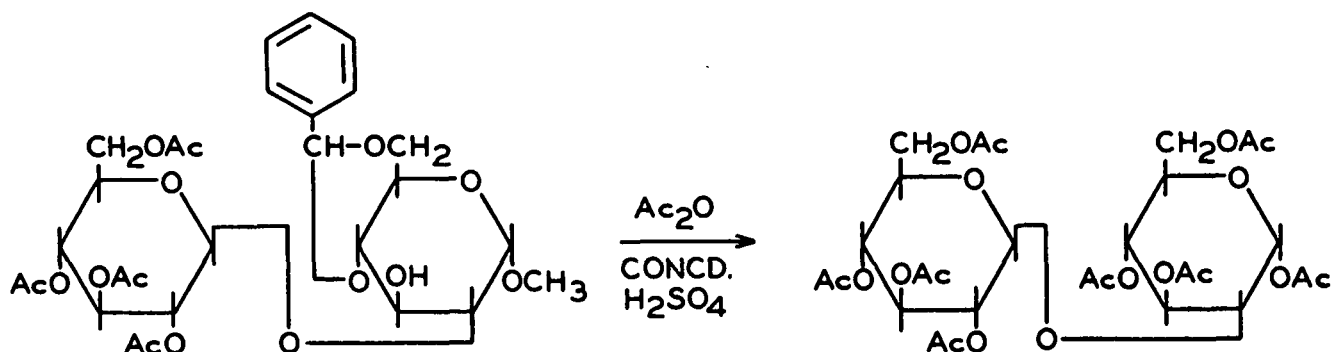METHYL α-D-GLUCOSIDE   BENZALDEHYDE   METHYL 4,6-O-BENZYLIDENE-α-D-GLUCOSIDE
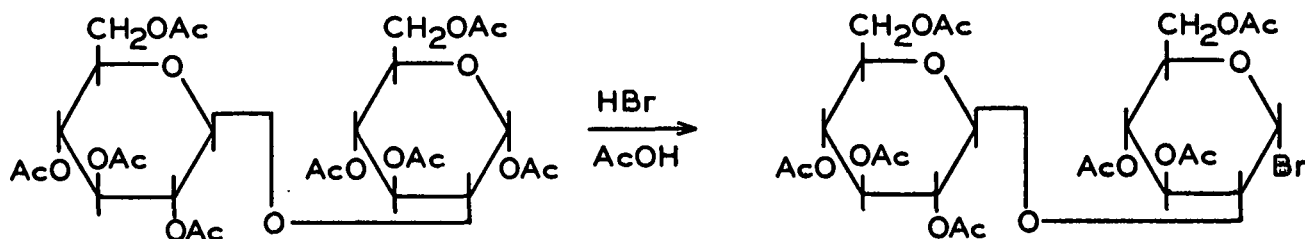
Step 2
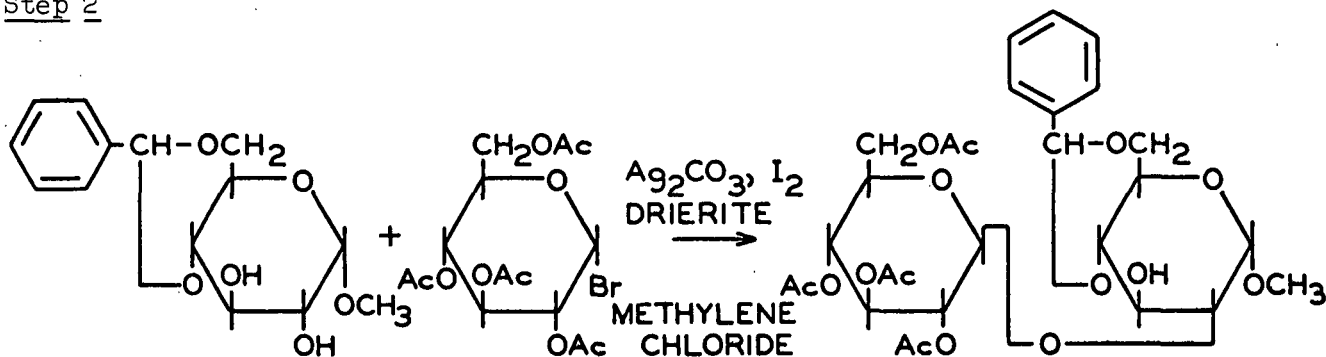
Step 3
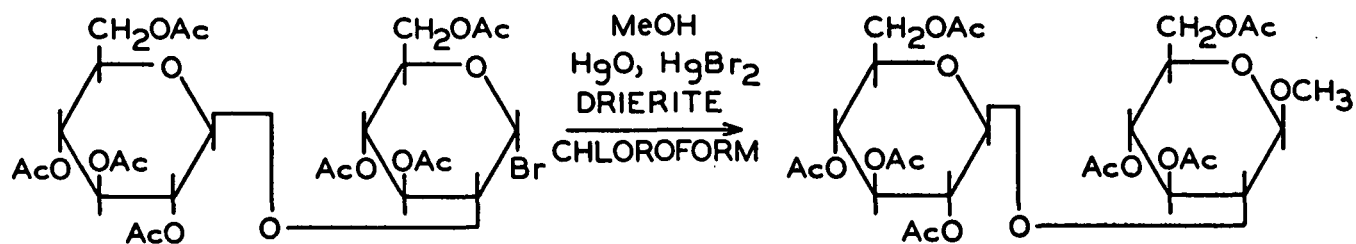

α-SOPHOROSE OCTAACETATE

Step 4
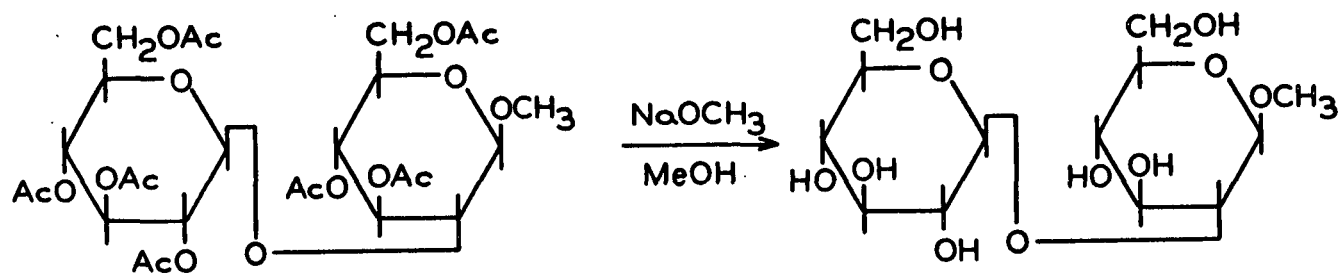

α-ACETOBROMOSOPHOROSE

STEP 5. SCHROEDER AND GREEN (36)

METHYL β-SOPHOROSIDE
HEPTAACETATE

Step 6

METHYL β-SOPHOROSIDE

APPENDIX II

MEASUREMENT OF RADIOACTIVITY

VAN SLYKE MANOMETRIC APPARATUS

A schematic diagram of the Thomas-Van Slyke manometric apparatus is presented in Fig. 6.
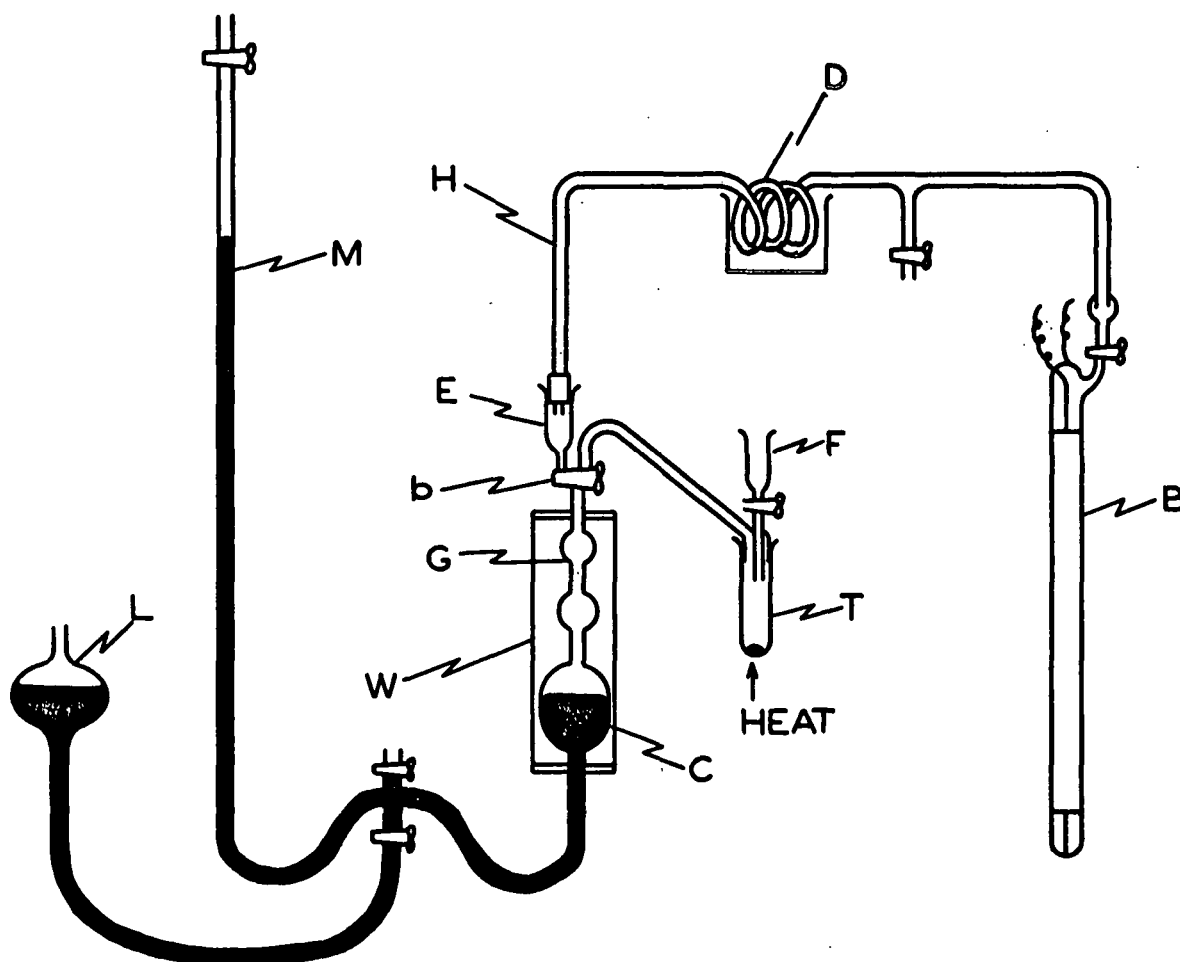

Figure 6. Van Slyke Manometric Apparatus

The sample is placed in tube T and the apparatus is evacuated by repeatedly lowering the mercury level in C with b open to T, and then closing b to T. The air is then forced out of C by raising L and opening b to E. It is necessary to

repeat this operation six to eight times. Two milliliters of hydrazine-sodium hydroxide solution are introduced into C through E and the level is lowered to G. Valve b is now open to T and the combustion is carried out after the combustion fluid is added through F. The carbon dioxide is absorbed in the hydrazine-sodium hydroxide solution by pumping the mercury level in C up and down, and the other gases are then expelled through E. The carbon dioxide is liberated by addition of lactic acid and its partial pressure is measured with manometer M and the temperature in the water jacket W surrounding the gas buret is recorded. With the factors supplied by Van Slyke and Folch (47), the amount of carbon in the sample can be calculated, after correction for the partial pressure of water vapor and a blank run.

TRANSFER OF CARBON DIOXIDE TO BERNSTEIN-BALLENTINE TUBE

After connecting H to E (in Fig. 6) and evacuating system H-D-B to below 3 mm. mercury, the carbon dioxide in C is allowed to flow into B. Any water vapor in the gas is frozen out as the gas passes through D which is immersed in a bath of ethanol-dry ice. The gas is helped over into B by pumping several times with the mercury in C, and then is flushed out of H and D into B with methane. After bringing B to atmospheric pressure with methane, it is removed for counting.

The Bernstein-Ballentine tubes were attached to a Nuclear-Chicago model 182 scaling unit for counting.

CALIBRATION OF BERNSTEIN-BALLENTINE TUBES

Each Bernstein-Ballentine proportional counting tube was calibrated before use. First of all, the effective counting volume was determined. This is the fraction of the total volume of the tube which is enclosed by the silvered surface. This was done by first filling the tube to the bottom of the silvered area with toluene

and weighing ($\underline{V}_1$); the tube was then filled to the top of the silvered area and weighed ($\underline{V}_2$); and finally, was filled completely and weighed ($\underline{V}_t$). The fraction of the tube used in counting, $\underline{V}_r$, is equal to ($\underline{V}_2$ - $\underline{V}_1$)/$\underline{V}_t$.

A radioactive sample of carbon dioxide was then introduced into the tube, and the tube was connected to the scaler in order to determine the optimum counting voltage. A plot of voltage versus counts per minute was drawn and the mid-point of the plateau was taken as the optimum counting voltage for the particular tube. Below this value, all of the disintegrations occurring in the sample are not recorded; and above this plateau, a spuriously large count is recorded. A typical calibration plot is given in Fig. 7.
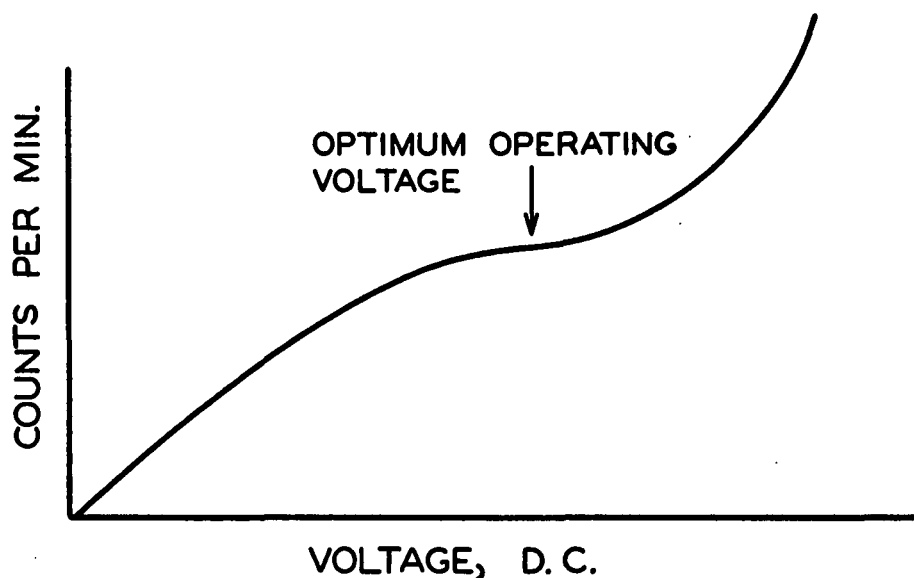

Figure 7. Typical Calibration Curve of a Bernstein-Ballentine Tube

CALCULATION OF SPECIFIC ACTIVITY

The following is a sample calculation of the specific activity of the α-acetobromo-D-glucose-C^{14} used in this study. An explanation follows each step.

Sample weight	=	7.27 mg.	Weighed on semimicro-balance
p_1	=	509.5 mm.	Pressure from manometer of Van Slyke apparatus with the volume of carbon dioxide adjusted to 10 ml.
p_2	=	71.2 mm.	Partial pressure of water vapor
		438.3 mm.	
c	=	20.3 mm.	Correction of $p_1 - p_2$ for blank run
P_{CO_2}	=	418.0 mm.	Corrected partial pressure of carbon dioxide
Temperature	=	25.5°C.	Temperature in water jacket
Factor	=	0.006784	Conversion factor (47)
Mg. carbon found	=	2.84	P_{CO_2} x factor
Known mg. carbon	=	2.97	Calculated from sample weight
Carbon found	=	95.6%	

Radioactive counting: tube no. L465

V_r	=	0.843	Fraction of total volume of tube enclosed by silvered surface
E	=	0.98	Counting efficiency (48)
Volts, d.c.	=	3700	Optimum operating voltage
Time	=	10 min.	Counting time
Total counts	=	313,247	Total counts in 10 minutes
Counts per minute	=	31,325 ± 92.4	
Background	=	146 ± 1.4	Counts recorded for tube charged with methane at atmospheric pressure
Net counts/minute	=	31,179 ± 92.4	
Disintegrations/min.	=	37,740 ± 112	Net counts per minute divided by $(V_r$ x $E)$

Specific activity:

Dis./min./mg. carbon $\qquad$ = 12,707 $\pm$ 38

Dis./min./mM. $\qquad$ = 2.13 x 10^6 $\pm$ 6200

APPENDIX III

COUNTING DATA FOR RADIOACTIVE REACTIONS

This appendix contains the original radioactive counting data obtained in the Van Slyke analysis of Reactions 1, 2, 3, and 4. The reactions are designated as they were in the original entries:

Reaction 1 = 100A, 100B
Reaction 2 = 101A, 101B
Reaction 3 = 103A, 103B
Reaction 4 = 104A, 104B

The counting data are presented in Tables XIV and XV. A sample calculation follows: Sample 100AI-2-B

p_1	=	487.0 mm.	Pressure of carbon dioxide with volume of 46.00 ml.
p_0	=	19.3 mm.	Value obtained for blank run
P_{CO_2}	=	467.7 mm.	Corrected partial pressure of carbon dioxide
Temperature	=	23.0°C.	
Factor	=	0.03132	Factor supplied by Van Slyke and Folch (47)
Mg. carbon	=	14.6	P_{CO_2} x factor

Radioactive counting: Tube H69 at 3800 volts

V_r	=	0.835	Fraction of total volume of tube enclosed by silvered surface
E	=	0.936	Counting efficiency for 14.6 mg. carbon (48)
Time	=	54 min.	Time for counting the sample
Total count	=	130,583	

Counts per min.	=	2418 $\pm$ 11.1	
Background	=	142 $\pm$ .1.4	Background count for tube charged with methane at atmospheric pressure
Net counts per min.	=	2276 $\pm$ 11.1	
Total activity of sample, disintegrations per min.	=	2912 $\pm$ 14	$\dfrac{\text{Net counts per min.}}{(\underline{V}_{\underline{r}} \times \underline{E})}$
Specific activity, dis. per min. per m$\underline{M}$.	=	2.13×10^6	Equal to the specific activity of the α-aceto-bromo-D-glucose-C^{14}
Yield of radioactive product, m$\underline{M}$.	=	1.365×10^{-3}	$\dfrac{\text{total activity}}{\text{specific activity}}$
Mg. radioactive product	=	0.486	$\dfrac{\text{m}\underline{M}.}{\text{molecular weight}}$

TABLE XIV

RADIOACTIVE COUNTING DATA FOR REACTIONS 100A, 100B, 101A, AND 101B[e]

Reaction	MBG[a]	Disaccharides, dis./min. MBS[b] + MBC[c]	MBL[d]	Higher Molecular Weight Material, dis./min.	Total, dis./min.
100A	12137	4637	3295	3090	23159
	13558	4967	3374	3187	25086
100B	12157	4636	3128	2609	22530
	14271	5189	3275	2583	25318
101A	4164	1525	1015	375	7079
	4123	1808	1170	300	7401
101B	4547	1846	1259	320	7972
	4645	1851	1149	440	8085

[a] Methyl β-gentiobioside.
[b] Methyl β-sophoroside.
[c] Methyl β-cellobioside.
[d] Methyl β-laminaribioside.
[e] All of the data are in duplicate.

TABLE XV

RADIOACTIVE COUNTING DATA FOR REACTIONS 103A,
103B, 104A, AND 104B[e]

Reaction	MBG[a]	Disaccharides, dis./min. MBS[b] + MBC[c]	MBL[d]	Higher Molecular Weight Material, dis./min.	Total, dis./min.
103A	6008	2949	1365	14446	24768
	5939	2670	1661	14476	24746
103B	6441	2422	1442	14077	24382
	6840	2557	1496	14914	26007
104A	6889	2468	2494	46141	57992
	7189	2546	2159	45918	57812
104B	6638	2428	2219	50526	61811
	6942	2291	2390	50326	61949

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[a] Methyl β-gentiobioside.
[b] Methyl β-sophoroside.
[c] Methyl β-cellobioside.
[d] Methyl β-laminaribioside.
[e] All of the data are in duplicate.

APPENDIX IV

ISOTOPE DILUTION ANALYSIS

A mixture contains $\underline{x}$ g. of a radioactive component $\underline{A}$ of specific activity $\underline{C}$. If $\underline{y}$ g. of the inactive form of $\underline{A}$ are added ($\underline{y}$ should greatly exceed the expected value of $\underline{x}$) and then $\underline{A}$ is isolated and purified, the specific activity of $\underline{A}$ will have been lowered to a new value $\underline{B}$. The total activity of $\underline{A}$ in the original reaction mixture is $x\underline{C}$. The total activity of $\underline{A}$ in the diluted mixture is $(\underline{x} + \underline{y})\underline{B}$. Since there was no loss of radioactivity in the dilution step, it follows that

$$xC = (x + y)B \tag{8}.$$

Solving (8) for $\underline{x}$, we obtain

$$x = yB/(C - B) \tag{9}.$$

Hence, the amount of $\underline{A}$ in the original reaction mixture can be calculated by determining only the final specific activity of the diluted $\underline{A}$, since the amount $\underline{y}$ and $\underline{C}$ are known.

The only two quantitative steps in this procedure are the dilution step and the final determination of the specific activity of the diluted product. The product can be isolated by any means and it is only necessary to isolate a few milligrams.

A sample calculation using this technique follows:

Isotope dilution analysis of Reaction 1 with methyl β-sophoroside (MBS):

Reaction mixture	= 1.0 ml. (one-fifth of the total)
Specific activity of MBS, $\underline{C}$	= 2.13×10^6 dis./min./m$\underline{M}$
Amount of inactive MBS added, $\underline{y}$	= 182.2 mg.

Specific activity of the
reisolated, purified MBS, $\underline{B}$

$= 9.8 \times 10^3$ dis./min./m$\underline{M}$

Yield of MBS in one-fifth of
original reaction mixture, $\underline{x}$

$= \underline{y}\underline{B}/(\underline{C} - \underline{B})$

$= 182.2 \times 9.8 \times 10^3/(2.13 \times 10^6 - 9.8 \times 10^3)$

$= 0.845$ mg.

Yield of MBS in entire
reaction mixture

$= 4.23$ mg.

Total activity of the mixture of
MBS and MBC* in the reaction mixture

$= 59000$ dis./min.

Amount of MBS + MBC* in the
reaction mixture

$= 59000/2.13 \times 10^6$

$= 2.77 \times 10^{-2}$ m$\underline{M}$

$= 9.95$ mg.

Therefore, the amount of MBS in
the mixture of MBS and MBC*

$= 4.23/9.95$

$= 42.5\%$

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*Methyl β-cellobioside.

APPENDIX V

HUDSON'S RULES OF ISOMOLECULAR ROTATION

If the disaccharide molecule is divided in the way shown in Fig. 8, then Hudson's rules can be applied as follows:
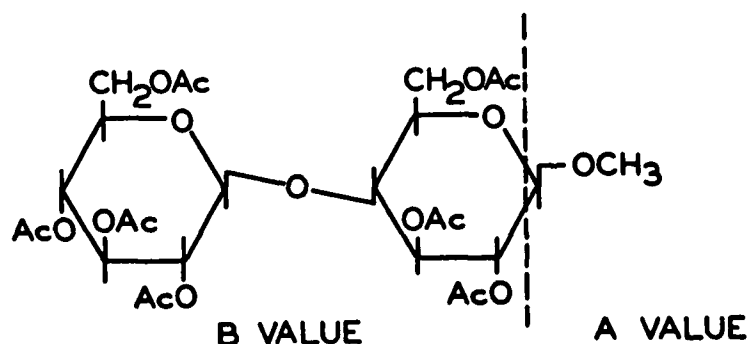

Figure 8. Division of a Disaccharide Molecule
According to Hudson's Rules

Consider first, the two heptaacetates of methyl α- and methyl β-cellobioside:

Methyl α-cellobioside heptaacetate   [M]   =   +36,400

Methyl β-cellobioside heptaacetate   [M]   =   -16,900

where [M] is the molecular rotation.

According to Hudson's rules, for the α-anomer

$$A + B = +36,400$$

and for the β-anomer, $-A + B = -16,900$

$$2B = +19,500$$

Therefore, $B = +9,750$

and $A = \underline{+26,650}$

This A value can then be used in considering the two heptaacetates of methyl α- and methyl β-sophoroside:

Methyl α-sophoroside heptaacetate   [M]   =   +32,500

Methyl β-sophoroside heptaacetate   [M]   =   ?

For the α-anomer, $\qquad$ A + B $\quad$ = $\qquad$ +32,500

Using the A value obtained from the cellobiosides:

$$B = +32,500 - 26,650$$

$$= + 5,850$$

Hence, for the β-anomer, $\qquad$ [M] $\quad$ = $\qquad$ -A + B
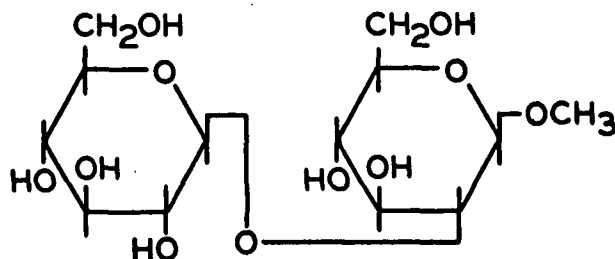
$$= -26,650 + 5,850$$

$$= -20,800$$

therefore, $\qquad$ $[\alpha]_D$ = -32°

Methyl β-sophoroside heptaacetate was prepared and its specific optical rotation was found to be $[\alpha]_D$ = -1.2° (chloroform). In other words, the Hudson's rules prediction was much too high.

APPENDIX VI

SUMMARY OF PROPERTIES OF METHYL β-SOPHOROSIDE

Structure:

Crystals: white, fibrous needles; crystallize easily from 95% ethanol

Melting point: 194.5 - 195.5°C. (corrected)

Specific optical rotation: $[\alpha]_D^{27°C.} = -36.03°$ ($\underline{c}$ = 1.693, water)

Carbon, hydrogen analysis:

C, 42.87; H, 6.84; O (by difference), 50.29

calculated for $C_{13}H_{24}O_{11}$:

C, 43.81; H, 6.79; O, 49.40

calculated for $C_{13}H_{24}O_{11} \cdot 1/2H_2O$:

C, 42.73; H, 6.89; O, 50.38

Infrared spectrum:[1]

The infrared spectrum illustrated in Fig. 9 indicates that the methyl β-sophoroside is a hydrate. The absorption at around 1640 cm.$^{-1}$ is typical of hydrates.

Nuclear Magnetic Resonance spectrum:[2]

The NMR spectrum for methyl β-sophoroside is illustrated in Fig. 10. According to Varian Associates:

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[1] Courtesy of Mr. Lowell Sell, Analytical Group, The Institute of Paper Chemistry.
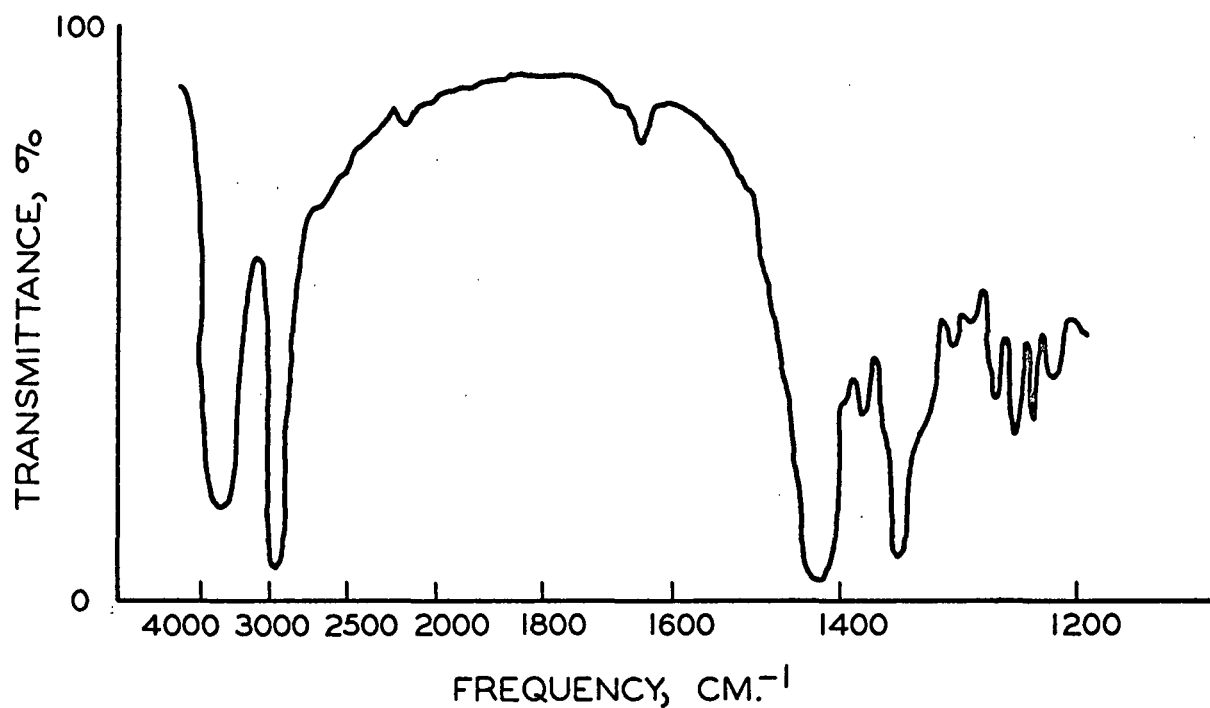[2] Courtesy of Varian Associates, Pittsburgh, Pennsylvania.
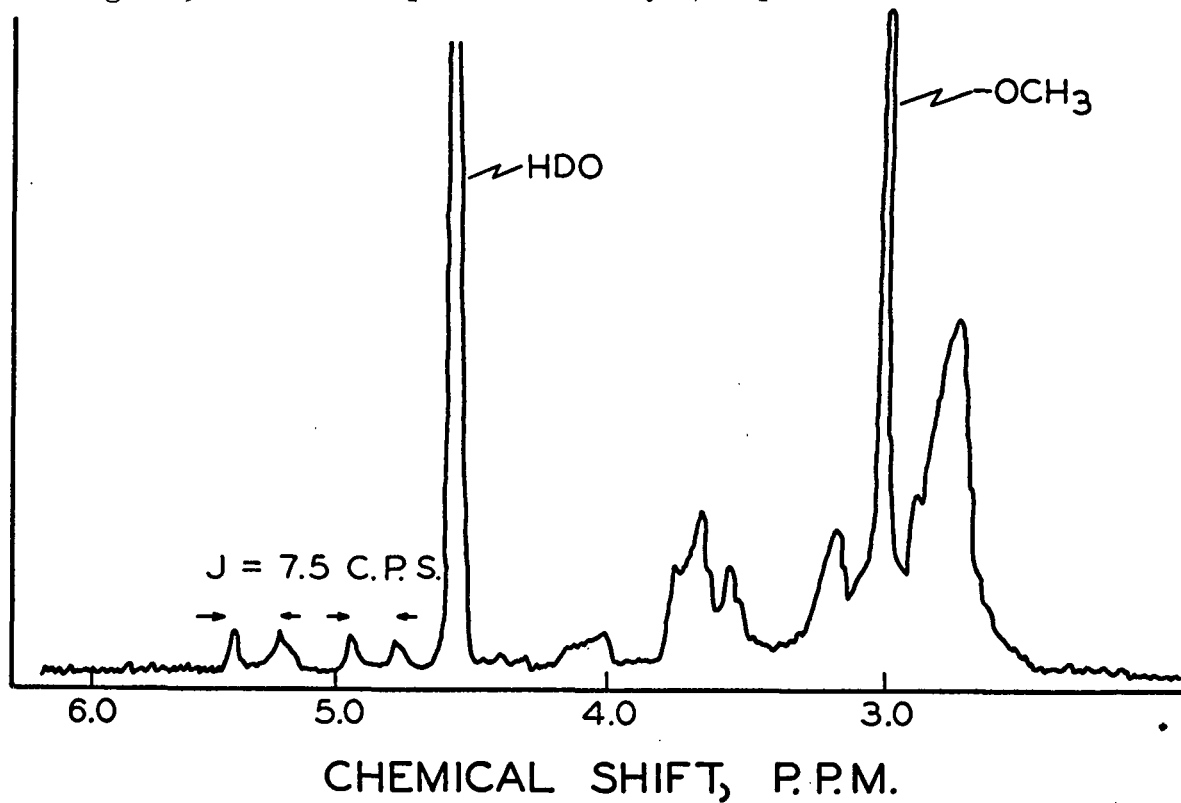
Figure 9.   Infrared Spectrum of Methyl β-Sophoroside

Figure 10.   Nuclear Magnetic Resonance Spectrum
of Methyl β-Sophoroside

We have obtained the 60 and 100 Mc./s. NMR spectra of your sophoroside sample in D_2O solution, and I believe the evidence supports your proposed β-linkage.

The lowest field absorption, (corresponding to two protons), consists essentially of a pair of doublets. The latter undoubtedly arise from the C-1 and C-1' hydrogens with a structure of $-C\big\langle\begin{smallmatrix}O-\\O-\end{smallmatrix}$.

At 60 Mc./s. and room temperature the pair of doublets is partially obscured by the strong HDO absorption; but this may be overcome by observing the sample at 75°C. and shifting the HDO peak upfield.

Comparison of the observed chemical shifts with those of model compounds may enable you to unambiguously assign the C-1 and C-1' protons. In any event the coupling with C-2 in both doublets is about 7.5 c./s., which indicates a similar axial-axial interaction in both rings.

Van der Veen (59) has studied the NMR spectra of various mono- and oligo-saccharides in D_2O. He has found that the chemical shifts for the β-linked disaccharides for the C-1' protons are of the order of 5.5 p.p.m. with the J coupling constant of 7.0-7.7. The chemical shifts for the C-1 protons are a little lower (5.35) but the J coupling constant values are also of the order of 7.5 c.p.s., for the β-anomers. The α-anomers have a chemical shift for the C-1 proton of around 4.8 p.p.m., but the J coupling constant values are of the order of 3.0-3.5 c.p.s. In Fig. 10 both of the doublets have a J coupling constant value of around 7.5 c.p.s., even though the chemical shift for one of the doublets is around 4.8 p.p.m. This is considered to be good evidence of the presence of the β-anomer of methyl sophoroside.