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Reactions of Synthesis of Methyl D-Glucuronide

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Reactions of Synthesis of
Methyl d-Glucuronide

by

Edward J. Czalgoszewski

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science
in the Graduate School of Loyola University.

Department of Physiological Chemistry

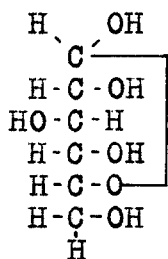
1934

In the course of studies reviewed in this thesis, I have become obligated to certain persons, and it is a pleasure to acknowledge this debt. I am especially grateful to Dr. W. C. Austin, under whose direction these studies were made. The time and effort he has expended in developing in me an understanding of the work and in leading me toward a solution of the problem assigned, are duly appreciated.

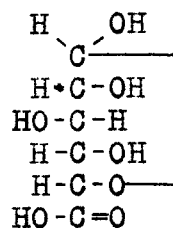
To Drs. W. R. Tweedy and F. L. Humoller, who discussed with me my results and criticized their interpretation---sincere thanks.

I appreciate, also, the aid given me by Mr. C. Vicens in the furtherance of these investigations.

d-Glucuronic acid (II) is a member of the class of aldo-hexuronic acids and is structurally related to d-glucose (I) in the manner shown by the configurations, I and II.



d-Glucose
I



d-Glucuronic Acid
II

This d-glucuronic acid has become, in the course of a half century, a substance of much interest in biochemistry and medicine. As early as 1879, Jaffe (1) after administration of phenol, noticed a tremendous increase in the glucuronic acid content of blood and urine. Later in the same year, Schmiedeberger and Meyer (2) fed camphor to dogs and observed an increased excretion of glucuronic acid. The acid excreted was in combination with phenol and camphor, the indication being, that glucuronic acid was a detoxifying agent which formed, with toxic bodies, physiologically inactive substances for oxidation or excretion. At present, many workers accept the view, postulated by Fischer (3) that the toxic substances combine with glucose, to form glucosides, which are then oxidized in the liver to form the glucuronide excreted. An opposing view held by many and well supported by recent experimental evidence of Pryde

and Williams (4) is that glucuronic acid, either preformed and stored, or manufactured in response to a specific effect of the toxic substance on the organism, forms directly a glucuronide complex with the toxic body, which is then excreted in the urine.

Taking advantage of this physiological property of glucuronic acid, Quick (5), in 1927, fed borneol to dogs and extracted from the urine, the borneol glucuronide, which upon hydrolysis presented the known crystalline acid and lactone. This is at present the most advantageous method of preparing the free acid and lactone.

Of added interest, was the demonstration, by Schmiedeberger (6) in 1891, of glucuronic acid as a product in the hydrolysis of muco- and chondroproteins.

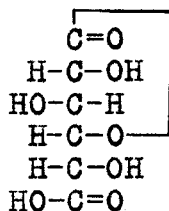
A new interest in uronic acids has been aroused by the isolation of hexuronic acid from Vitamin C containing substances, first by Szent-Gyorgyi (7) and later by other workers; and further by simultaneous demonstration of antiscorbutic potency for this acid by the same workers (8) and King and co-workers (9). There has been, as yet, no published announcement of antiscorbutic activity for synthetic hexuronic acids, and therefore there remains the possibility that glucuronic acid might be the starting point for the preparation of a keto-uronic acid possessing antiscorbutic properties. (There is very good evidence that antiscorbutic hexuronic acid is a keto-uronic acid.)

Glucuronic acid, itself, is inactive as an antiscorbutic agent.

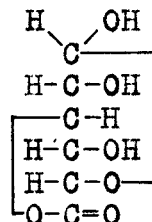
The object of the investigations to be described in this paper was the study of the reactions of formation of the methyl glycoside of d-glucuronic acid. In examination of the literature of the reactions which have been employed to form glycosides of uronic acids in general, it was observed that the studies approached the desired compounds in several directions.

1. Reduction of lactone of di-carboxy acids:

- a. Fischer (10) in 1891 prepared d-glucurone (IV), in very small yield, by reducing the lactone of d-saccharic acid (III) with sodium amalgam, in an acid medium.



Lactone of
d-saccharic acid
III

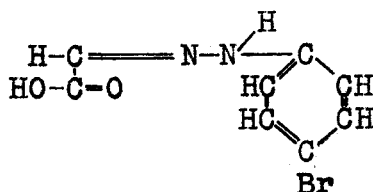
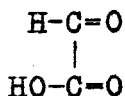
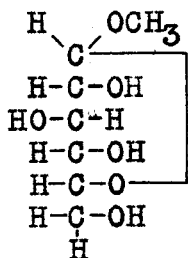


d-Glucuron
IV

2. Oxidation of aldo-hexoses or glycosides of aldo-hexoses:

- a. Jolles (11) using d-glucose (I) as the substrate of action of hydrogen peroxide, obtained a syrup containing d-glucuronic acid (II). This was isolated as the parabromophenyl-hydrazine derivative (V).

In the same publication, Bergmann reported the effect of bromine oxidation of methyl- α -d-glucoside (VIII). Treatment of the syrup with para-bromophenyl-hydrazine resulted in the isolation and purification later of the parabromophenyl-hydrazone of glyoxalic acid (X).

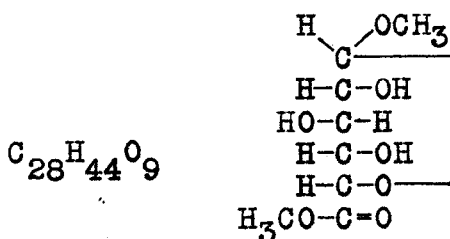


Methyl- α -d-glucoside
VIII

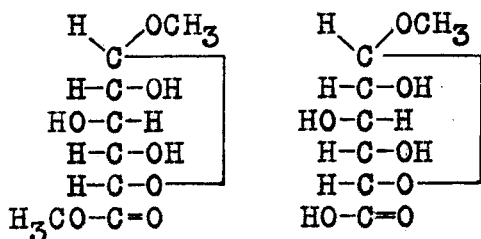
Glyoxalic
acid
IX

Parabromophenyl-hydrazone
of glyoxalic acid
X

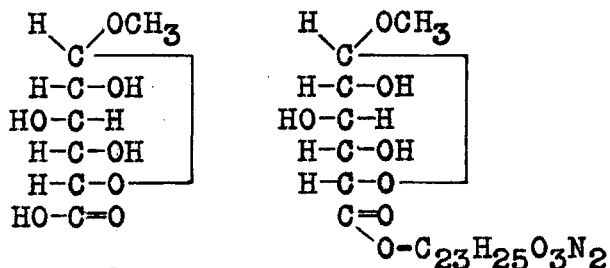
- c. There was isolated in 1911, by Smolenski (13) from the juice of the sugar beet, a glucuronide of beet resinic acid, which upon hydrolysis in methyl alcohol yielded the methyl d-glucuronide methyl ester (XI). This after saponification gave methyl d-glucuronide (XII) which was obtained as a crystalline brucine salt (XIII).



Glucuronide
of beet
resinic acid



Methyl d-
glucuronide
methyl ester
XI

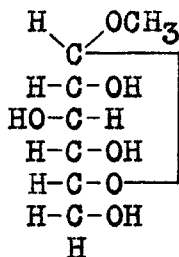


Methyl d-
glucuronide
XII

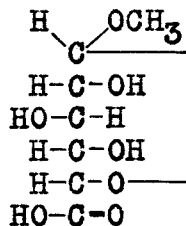
Brucine methyl
d-glucuronide
XIII

In 1924, Smolenski (14) undertook the oxidation of methyl- α -d-glucoside with hydrogen peroxide using ferric hydroxide as catalyst. The

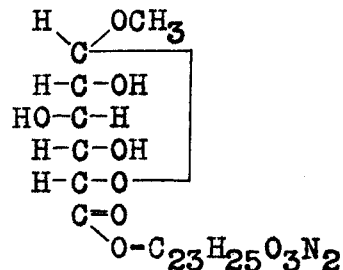
resultant syrup, he reported, contained methyl d-glucuronic acid to the amount of 30% of the theory, which was isolated as the crystalline brucine salt (XIII). In neither of the above papers did Smolenski adequately describe the properties of the brucine salt.



Methyl-d-glucoside
VIII

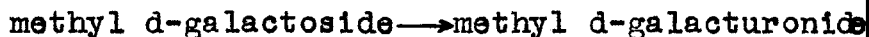


Methyl d-glucuronide
XII



Brucine methyl d-glucuronide
XIII

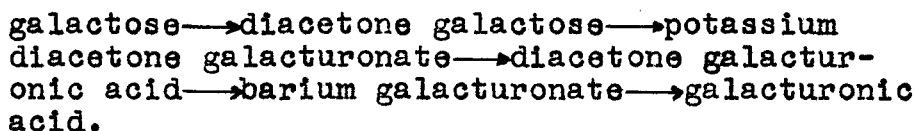
- d. Link (15), in 1934, reported failure to execute the conversion:



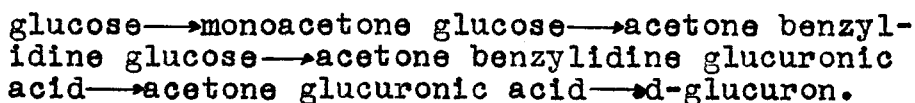
using barium and potassium permanganates, but succeeded by electrolytic oxidation with bromides to the extent of a 3% yield.

3. Oxidation of more complex derivatives of aldohexoses:

- a. Link (15) in 1934, reported the preparation of galacturonic acid by the reactions, in the d-series:

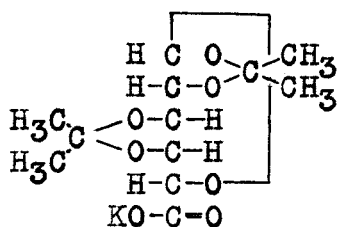


- b. Zervas and Sessler (16), in 1934, prepared d-glucuron from d-glucose by the reactions in the d-series:

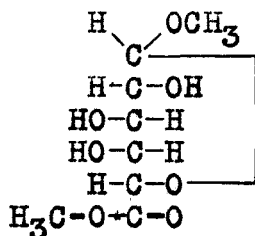


4. Conjugation of Hexuronic acid with methyl alcohol:

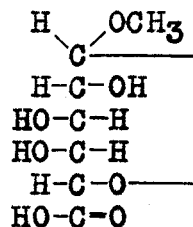
By treating potassium diacetone d-galacturonate, prepared in the course of formation of d-galacturonic acid, with 2% anhydrous acid alcohol, and by subsequent saponification of the methyl d-galacturonide methyl ester, Link (15) in 1934, made the crystalline methyl d-galacturonide.



Potassium diacetone
d-galacturonate
XIV



Methyl d-
galacturonide
methyl ester
XV



Methyl d-galacturonide
XVI

The studies made here were begun prior to the publications of Link (15), described above, and, by coincidence, have taken somewhat the same directions. It was hoped that these studies would lead to a method of preparing glucuronic acid more easily than by the procedure of Quick (5).

Attempts have been made to oxidize methyl- α -d-glucoside to methyl- α -d-glucuronide, under varying conditions. None of the methods tried was adaptable for quantitative preparation of glucuronic acid. The first method employed was suggested by Bornstein (17) in the oxidation of glycerol to glyceric acid by mercuric oxide, and was unsuccessful due to the fact that reduction of the mercuric oxide by methyl- α -d-glucoside does not take place readily at 100° C.

The reaction might possibly proceed with more successful results under conditions of increased temperature and pressure.

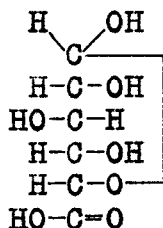
The oxidations with permanganate, suggested by the work of Zervas and Sessler (16) were likewise barren of positive results here. Depending upon the conditions adopted, the oxidation proceeds either too vigorously or not at all, resulting, not in the formation of glucuronic acid (in quantity) but more probably causing a degradation of the hexose molecule to form glyoxalic acid and other fragments. This method may yet be successfully used if conditions can be established which are most suitable for the oxidation.

Due to the fact that Smolenski (14) omitted the mention of some desired measurements, made use of a questionable method to determine quantitatively glucuronic acid, and neglected to give many of the desired properties of the methyl glucuronide and its brucine salt, an oxidation was attempted by his methods. He reported preparation of methyl d-glucuronide in yields as high as 30% using hydrogen peroxide with ferric hydroxide as catalyst. The error he made was in the use of the amount of furfural formed by the action of 12% HCl as an indication of glucuronic acid alone, whereas pentoses and other side products of the oxidation also yield furfural. Our in-

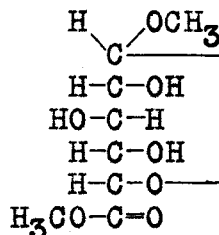
vestigations, using hydrogen peroxide with ferric acetate as catalyst, showed the formation of only a small amount of d-glucuronic acid, which was destroyed by prolonged heating and treatment with hydrogen peroxide.

Many workers have prepared methyl glycosides of reducing sugars by the reaction with methyl alcohol containing dry hydrogen chloride, in adaptations of the method of Fischer (18). The studies given below are, however, the first recorded efforts to effect the synthesis of a methyl hexuronide by reaction of an unsubstituted aldohexuronic acid with methyl alcohol containing dry hydrogen chloride.

d-Glucuronic acid (97.7%) after reaction in methyl alcohol containing 3% hydrogen chloride was isolated as a purified syrupy product. Analysis of this syrup indicated the presence of 15% uncombined reducing sugar acid, and 17% titratable organic acid. Removal of this free acid, as a barium salt insoluble in alcohol, left a fraction containing reducing substances equivalent to 16% d-glucuronic acid, and an estimated content of 83.3% methyl ester.



d-Glucuronic acid
I

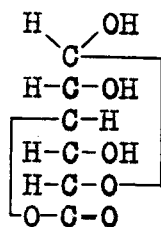


Methyl d-glucuronide
methyl ester
XI

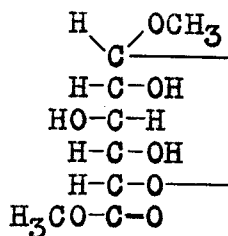
From the action of an alcoholic solution of 1% hydrogen chloride on a mixture of 60% glucuronic acid with 40% lactone (IV) there was prepared a syrup, freed from HCl, having a content of 11% titratable organic acid and 15% uncombined reducing sugar acid. Removal of the free organic acid from this product left a fraction having 16% reducing sugar acid and an estimated content of 92.3% methyl ester (XI). A portion of this product was saponified to yield a new syrup which was 84% acid (XII) and lactone (XVII). This contained 13% reducing sugar acid.

Treatment of one-half of the last syrup with brucine resulted in the formation of a white crystalline brucine compound (or compounds) which may, in future studies, prove to be the brucine salt of methyl d-glucuronide. If the expectations in this direction are realized the brucine salt will be purified carefully for analysis, and determination of its constants. The remainder would then be freed of brucine by standard procedures, for attempts to crystallize the methyl d-glucuronide which it may yield.

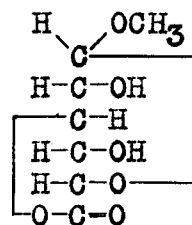
The two types of syrupy products, the first of which appears to contain only relatively pure methyl d-glucuronide (XII) and its lactone (XVII), and the other, the relatively pure methyl d-glucuronide methyl ester (XI), are being stored under conditions which may promote the crystallization of the desired compounds.



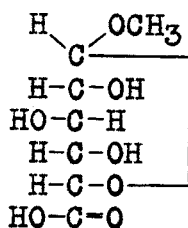
d-Glucuronolactone
IV



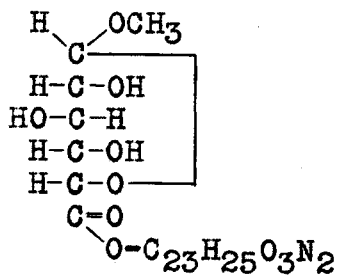
Methyl d-glucuronide
methyl ester
XI



Lactone of methyl
glucuronide
XVII



Methyl d-glucuronide
XII



Brucine methyl
d-glucuronide
XIII

EXPERIMENTAL

The methyl- α -d-glucoside used in these studies was prepared by the directions of Patterson and Robertson (19). The directions of Bornstein (17) for the preparation of glyceric acid by the oxidation of glycerin, were used in the first oxidation study.

To 10 cc. of a 0.5% solution of methyl- α -d-glucoside at 95°-100°C. and saturated with barium hydroxide, was added mercuric oxide in small portions. The mercuric oxide remained unchanged even though only a small fraction of the amount theoretically required for oxidation was used. A filtrate of the reaction mixture gave a negative naphthoresorcin test, by the procedure of Tollens (20), for d-glucuronic acid. The reliability of the conclusions was proven by careful controls with, and without, known amounts of d-glucuronic acid, in the Tollens test. The known d-glucuronic acid was made and purified by the method of Quick (5).

Methyl- α -d-glucoside (5.82 g.) and 8 grams of barium hydroxide were dissolved in water to 250 cc. To this was added 25 cc. of 10% hydrogen peroxide, suggested by the studies of Jolles (11). The system, free from carbon dioxide, was refluxed for three hours at 100°C., aliquot portions being removed every hour and tested for d-glucuronic

acid. Tests were conducted on the supposition of a possible 5% conversion of the glucoside to the acid. Each of the three tests showed less than 5% conversion, comparison being made with the color developed from reaction of 1 mg. of d-glucuronic acid with naphthoresorcin. After the introduction of 25 cc. more of peroxide and refluxing for one hour, a Tollens test on a portion of the reaction mixture indicated only a slight increase in the glucuronic acid content.

A small amount of ferric acetate (U.S.P.) was added to a portion of the reaction product, together with 5 cc. of 10% hydrogen peroxide. This was heated for 15 minutes at 100 C. and then was tested for d-glucuronic acid. All semblance of a violet color disappeared, and was replaced by a strong red-brown coloration.

The larger system was now refluxed to a total of 27 hours, 20 cc. more of peroxide having been added previously. A test with naphthoresorcin indicated no further formation of d-glucuronic acid.

Three grams of methyl- α -d-glucoside was dissolved in 150 cc. of water which contained 2.4 g. of barium hydroxide. Pursuant of the method of Zervas and Sessler (16), 3.6 g. of finely pulverized potassium permanganate was introduced into the system, which was cooled with tap-water. The

mixture was stirred electrically for one-half hour, and then was filtered from insoluble materials. Since the water-clear filtrate, when tested with naphthoresorcin, gave a pale violet color indicative of the presence of d-glucuronic acid, 18 g. of methyl- α -d-glucoside were added to it followed by 14.5 g. of barium hydroxide and dilution to 900 cc. with water. After the introduction of 21 g. finely pulverized potassium permanganate into the solution, the procedure noted above was followed. A portion of the filtrate, tested with naphthoresorcin, gave a red coloration which resembled that resulting from the reaction of glyoxalic acid with naphthoresorcin and HCl. Barium was removed and the reaction mixture was made 0.2N with sulfuric acid, and refluxed for 4.5 hours, to achieve hydrolysis of any glucuronide present. Free sulfuric acid was eliminated by neutralization with barium hydroxide, and the solution was evaporated to a thin syrup, from which potassium sulfate was precipitated with hot 95% ethyl alcohol. Evaporation of the alcoholic extract left a syrup which failed to deposit any crystalline d-glucuronic acid after protracted dessication over calcium chloride, and presented a negative reaction with naphthoresorcin, forming only a deep red color.

The use of barium permanganate by the above method, gave similarly negative results.

To 21.5 g. of methyl- α -d-glucoside in 400 cc. of water, was added 27 g. of barium carbonate. While the system was being stirred mechanically, barium hydroxide, in 0.5 g. lots (sufficient to keep the system just alkaline) and a solution of 10% barium permanganate were introduced at intervals. After the system had accepted the equivalent of 38 g. of permanganate, which is 1.5 times the theory, and 28 g. of barium hydroxide, the reaction was stopped. The solution was neutralized to phenolphthalein with sulfuric acid, filtered and evaporated to a syrup, which when extracted with 90% alcohol gave 5 g. of unaltered methyl- α -d-glucoside and insoluble barium compounds. The latter were dissolved in water and the solution was freed from barium, with sulfuric acid. Any glycosides present were hydrolyzed by refluxing for three hours at 98° C., in 100 cc. of 0.2N H₂SO₄. The sulfuric acid was removed as usual and the solution, positive to Tollens test for d-glucuronic acid, was evaporated to a syrup of 2.05 g. which, after reaction with 2.5 g. benzylphenyl hydrazine in 30 cc. absolute alcohol at 37° C. for 36 hours, formed 0.2 g. of crystalline substance. This, recrystallized from methyl alcohol, yielded 0.08 g. of pale yellow, thread-like crystals of $(\alpha)_D^{20} = + 17.3^\circ$ and M. P. = 157.5° C. A slightly positive color for d-glucuronic acid resulted from reaction of this substance with naphthoresorcin and HCl.

Retreatment of the syrups separated from the above

crystalline material, led to the isolation of crystals unlike those obtained previously. After two recrystallizations from methyl alcohol, there resulted 0.24 g. of a pale yellow substance with M. P. = 105 ° C. and a negative reaction to the Tollens test for d-glucuronic acid. Apparently neither crystalline product isolated here, was the benzylphenyl hydrazone of d-glucuronic acid, which shows $(\alpha)_D^{20} = -26^\circ$ (in methyl alcohol) and M. P. 155 ° C. Nor was either product the glyoxalic acid derivative, which melts at 172 ° C. and forms a deep red color after reaction with naphthoresorcin. Both of the above hydrazones, were described by Bergmann and Wolff (12).

To 1000 cc. of water was added 51.5 g. of methyl- α -d-glucoside and 68 g. of barium carbonate. While the system was heated at 95 ° C., finely pulverized barium permanganate (170 g.) was introduced in 25 portions over a period of 70 minutes. This was 2.5 times the amount required for the oxidation. After the addition of the permanganate, the reaction mixture was stirred well, at 95 ° C., for one hour. Removal of insoluble impurities left a neutral, clarified solution. This was evaporated, and the residual syrup, dissolved to 30 cc. with warm water, was fractionated with 400 cc. of warm 95% ethyl alcohol. The alcohol insoluble fraction was dissolved with water and freed from barium.

Evaporation of solvent, left a syrup which failed to show any crystallization and formed, in the Tollens test, a color characteristic of glyoxalic acid. The alcoholic extracts yielded 17 g. of crystalline methyl- α -d-glucoside.

Reaction of d-Glucuronic Acid and d-Glucuron
with Methyl Alcohol in Presence of
Hydrogen Chloride

Part I Reaction with 97.7% Acid

1. Formation of Methyl d-Glucuronide Methyl Ester

Ten grams of d-glucuronic acid (97.7%) was dissolved to 125 cc. with methyl alcohol containing 3% dry hydrogen chloride. Solution occurred in ten minutes at a temperature of 50 ° C. After refluxing for four hours and after 65 hours at 15 ° C., $(\alpha)_{\text{D}}^{20} = + 38.88^{\circ}$, and reducing sugar acid, determined by the method of Folin-Wu (21) and calculated as glucuronic acid, was 14.7%. After a total refluxing period of 7 hours and reaction for 85 hours at 15 ° C., $(\alpha)_{\text{D}}^{20} = +46.06^{\circ}$, and reducing sugar acid 15.9%. (All reducing values herein recorded, were determined by this method.)

Hydrochloric acid was removed by heating with silver carbonate; soluble silver was precipitated with hydrogen sulfide. Evaporation of the aqueous solution gave a syrup (fraction 1) of 10.45 g. , which showed $(\alpha)_{\text{D}}^{20} = + 34.32^{\circ}$ (in water); reducing value 14.64%; aldose reducing sugar 56.2%; free acid 16.43% and saponification value 106%.

The aldose reducing sugar was determined by the directions of Goebel (22). The discrepancy between the results of this oxidation and the oxidation by the method of Folin-Wu, can be explained by recalling that the reaction of the aldose determination system was strongly alkaline and caused saponification of the methyl ester. Methyl alcohol then reduced the free iodine. The saponification value was determined by allowing excess barium hydroxide to act for 18 hours and then titrating the unutilized hydroxide with standard sulfuric acid. The high value was due, very likely, to the utilization of much of the alkali by the unprotected aldehyde groups present in the syrup. All estimations of ester content (except 1 of part II) will be made by titrations with standard barium hydroxide at room temperature.

2. Purification of the Methyl Ester (Fraction 1)

The above syrup (9.9 g.), dissolved in 100 cc. of water, was treated with 48 cc. barium hydroxide (0.254N) for removal of free acid. Removal of impurities from and subsequent evaporation of the solution left a syrup which was extracted with 400 cc. of warm 90% ethyl alcohol. Examination of the syrup resulting from evaporation of the alcoholic extract showed: $(\alpha)_D^{20} = +29.09^\circ$; reducing value 16.02%; and an estimated content of 83.3% methyl ester.

The acid-free syrup (6.61 g.) was dissolved with 15 cc. methyl alcohol and then treated with 15 cc. ethyl alcohol and 30 cc. ether. The mixture was cooled at 0°C. and an insoluble residue was removed by filtration. The syrup (Fraction 2) remaining after evaporation of the filtrate, was stored for possible crystallization of the methyl d-glucuronide methyl ester. The barium salt of the alcoholic extraction was held for future recovery, as fraction 3.

Part II Reaction with a mixture 60% acid 40% lactone

1. Preparation of Methyl d-Glucuronide Methyl Ester

Twenty grams of d-glucuronic acid (40% lactone) was dissolved to 275 cc. with absolute methyl alcohol containing 1% dry hydrogen chloride. At 20°C., solution occurred in 35 minutes. The changes in rotation and concentration of free d-glucuronic acid are shown in Table 1.

Hydrochloric acid was removed with silver carbonate, and the filtered solution was evaporated to a syrup of 21.78 g. (Fraction 4). Examination of a fraction of this showed: $(\alpha)_D^{20} = +33.79^\circ$ (in water); reducing value 15.24%; aldose reducing sugar 46.9%; free acid 10.65%; and sapanification value 109.2%. These values were determined coincidentally with, and by the methods employed in 1 of Part 1.

Table I Changes During Glycoside Formation from
d-Glucuronic Acid and d-Glucuron

Reaction			
Time hrs.	Temp. °C.	$(\alpha)_{\text{D}}^{20}$	Free Sugar Acid
.3	15°	-16.83°	not observed
20	15°	-16.64°	25%
24	50°	-15.26°	22.75%
48	50°	+19.18°	19.95%
96	50°	+46.95°	11.00%

2. Purification of Fraction 4, Containing Methyl Ester

The above syrup, (21.24 g.) was purified in accordance with the procedures used in the purification of fraction 1 Part I. In all, 67 cc. of barium hydroxide (0.254N) was required. 18.5 g. of syrup (Fraction 5) remaining after fractionation, and evaporation of the alcoholic extract, showed the following values: $(\alpha)_{\text{D}}^{20} = +30.81^{\circ}$; reducing value 16.2%; and an estimated content of 92.32% methyl ester.

For the purpose of removing impurities which might interfere with crystallization, a portion of the acid free syrup (8.15 g. of Fraction 5) was dissolved with 15 cc. methyl alcohol and treated with 15 cc. ethyl alcohol and 45 cc. ether. The mixture was cooled at 0° C. Filtration and subsequent evaporation of the solution left a syrup (Fraction 6), which was stored for crystallization of the methyl d-glucuronide methyl ester.

The alcohol insoluble barium salts (removed from fraction 4, above), after combination with fraction 3

(Part I), was dissolved in water and treated with sulfuric acid for quantitative barium removal. Subsequent to the evaporation of the solution to a red-colored syrup, impurities were removed by precipitation with ether, from alcoholic solution, at -10°C . This left a fairly pure syrup (Fraction 7) containing methyl d-glucuronide methyl ester; this was stored under conditions most conducive to incipient crystallization.

3. Saponification of Methyl d-Glucuronide Methyl Ester

The remaining, non-purified portion of Fraction 5 (10 g.) was dissolved in water and was titrated with barium hydroxide (0.254N) to phenolphthalein at room temperature. After 147 cc. of the alkali was utilized (10 minute end point), barium was removed quantitatively, and the filtered solution was evaporated to a syrup of 9.28 g. (Fraction 8, which upon analysis showed: $(\alpha)_{\text{D}}^{20} = 36.87$; reducing value 13%; and estimated free acid and lactone 84%.

4. Purification of Methyl d-glucuronide (Fraction 8)

Approximately one-half (4.8 g.) of fraction 8, containing the methyl d-glucuronide and its lactone, was dissolved with 10 cc. methyl alcohol and treated with 10 cc. ethyl alcohol and 80 cc. anhydrous ether. Cooling at -10°C . was followed by decantation from impurities, and by rapid

filtration. Evaporation of the solvents left a purified syrup (fraction 9), containing both, the lactone of methyl d-glucuronide and the free acid. The syrup (3.9 g.) was stored for possible isolation of the crystalline materials.

5. Preparation of the Brucine Salt of Methyl d-Glucuronide

A second portion of fraction 8 (4.3 g.) was dissolved in 50 cc. water, kept at 50° C., and 10.5 g. brucine (sufficient to make the system definitely alkaline) was introduced gradually into the solution. After sufficient time for reaction had elapsed, the free brucine was extracted by chloroform. The chloroform insoluble fraction was concentrated under a vacuum, to beginning crystallization. The crystallizing material was stored under conditions conducive to more complete crystallization.

Summary:

1. It has been shown that the oxidation of Methyl- α -d-Glucoside with mercuric oxide, hydrogen peroxide (with and without catalysts), and potassium or barium permanganate did not result in the formation of Methyl-d-Glucuronide. One of the oxidation products, by reaction with benzylphenyl hydrazine, formed a crystalline substance of unknown composition, whose properties differed from those shown by the hydrazones of d-Glucuronic acid and glyoxalic acid. Thus the positive observations of Smolenski have remained unconfirmed.
2. An original investigation of the reactions of d-Glucuronic acid and d-Glucuron with methyl alcohol containing hydrogen chloride has been begun. Several preparations, probably containing methyl d-glucuronide and methyl d-glucuronide methyl ester, have been purified and stored for crystallization. By the reaction of brucine with a portion of one of these fractions, there has been formed a crystalline compound. This is probably the brucine salt of methyl d-glucuronide, a compound which was first prepared by Smolenski from methyl d-glucuronide of other sources. The completion of the study of this brucine salt is reserved for the future.

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