the mixture was vacuum filtered and the insoluble salts washed with DMF. The combined filtrates were evaporated to small volume *in vacuo* at 100°. Chloroform was added and the mixture refiltered or centrifuged to remove as much solid material as possible. The chloroform filtrate was then washed several times with water. From measurements of the apparent distribution ratios of completely methylated glucose and maltose between chloroform and water (15:1 and 30:1, respectively), it was concluded that washing losses were not large. For convenience, however, an aqueous cyanide extraction to remove traces of silver salts was omitted. After drying the chloroform solution over sodium sulfate and filtering, it was evaporated carefully on a rotary vacuum evaporator using an aspirator and a water bath at a final temperature of 60–70°. Traces of residual solvent did not interfere with gas chromatographic analysis of crude product.

Yields.—No attempt was made to investigate all reaction variables influencing yield. Kuhn reported an 85% yield of completely methylated sucrose isolated by vacuum distillation.[3] From one experiment we obtained 7.90 g. of crude completely methylated glucose from 5.00 g. of glucose. Aliquots of this material were hydrolyzed overnight in 1 *N* hydrochloric acid in sealed tubes on the steam bath. Hypoiodite analysis[13] indicated that the crude product contained 78% of a mixture of methyl tetra-*O*-methyl-D-glucosides; that is, a yield of 88% for the overall methylation reaction. Admittedly, this analysis would include also any incompletely methylated products, but significant amounts of these materials were shown to be absent by gas chromatographic analysis. The hypoiodite method was more satisfactory than colorimetric or copper methods for analysis of tetra-*o*-methylglucose; results of an analysis of a standard sample were in excellent agreement with polarimetric data. DMF did not interfere with the hypoiodite determination.

The infrared spectrum of a crude completely methylated glucose sample (78% purity by hypoiodite analysis) was measured. Absorption in the carbonyl region was so small that oxidation of glucose to gluconic acid could not have been a major factor in the reaction. The amount of absorption could be attributed to traces of side products and DMF which were already known to be present from other analyses.

Gas Chromatographic Analysis of Reaction Products.—The equipment, methods, and polar substrates used (polyesters and polyethers) have been described in a separate publication.[14] Generally, component identifications were based on chromatographic comparison of known and unknown samples, but in certain cases peaks were collected and confirmed by rotational measurements. The isomeric composition of crude reaction products was estimated from peak height measurements of chromatograms run at temperatures high enough to eliminate significant peak broadening.

Rate of Glycosidation.—The rate of glycosidation of D-glucose was followed by measuring the disappearance of reducing power (measured by hypoiodite) as a function of time. Samples of the reaction mixture were withdrawn periodically, filtered, and an aliquot titrated. Although the method was not altogether suitable for precise kinetic work, the results of a typical experiment are shown in Table II.

TABLE II

REACTION OF D-GLUCOSE (0.222 *M*), METHYL IODIDE (3.31 *M*) AND SILVER OXIDE (1.13 *M*) IN DIMETHYLFORMAMIDE AT 21°

Time, Min.	Reducing Function Remaining, %
0	100
6	94
10.5	92
15	88
20	82
25.5	69
31	65
35.5	49
43.5	28
52	20
63.5	8

A plot of these data shows an S-shaped curve that could be expected from a heterogeneous reaction requiring an equilibration before rapid reaction occurs. Results from both glucose and maltose show definitely that glycosidation occurs more rapidly than complete etherification. In an experiment using only glucose and silver oxide at the above concentration levels in DMF, hypoiodite analysis showed that 30 and 45% of the reducing groups were destroyed in 1 and 2 hr., respectively.

(13) E. F. Jansen and L. R. MacDonnell, *Arch. Biochem.*, **8**, 97 (1945).

(14) M. Gee and H. G. Walker, Jr., *Anal. Chem.*, in press.

The Synthesis and Acid Hydrolysis of Methyl α-D-Glucopyranosiduronic Acid[1]

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Methyl α-D-glucopyranosiduronic acid was synthesized by a catalytic air oxidation of methyl α-D-glucopyranoside followed by: (a) conversion of the oxidation product into crystalline methyl α-D-glucopyranosiduronic hydrazide and (b) hydrolysis of the hydrazide to yield barium (methyl α-D-glucopyranosid)uronate. Methyl α-D-glucopyranosiduronic acid and methyl α-D-glucopyranoside were hydrolyzed in *N* sulfuric acid at 70, 80, and 90°. The results of the hydrolysis suggested that the uronoside and neutral glycoside did not hydrolyze *via* identical mechanisms and that the uronoside was not stabilized by a significant inductive effect. Results from a study of the degradation of methyl α-D-glucopyranosiduronic acid support the reaction sequence: hydrolysis of methyl α-D-glucopyranosiduronic acid followed by degradation of D-glucuronic acid.

Methyl α-D-glucopyranoside (methyl α-glucoside) was oxidized by air in the presence of a platinum-carbon catalyst; the acidic products of the oxidation were isolated as their barium salts. Paper chromatographic analysis of the crude products indicated that, in addition to the expected methyl α-D-glucopyranosiduronic acid (methyl α-glucuronide), the oxidation produced several unidentified compounds. Thus, it appears that the catalytic

(1) Based on a thesis submitted in partial fulfillment of the requirements of The Institute of Paper Chemistry for the degree of Doctor of Philosophy from Lawrence College, Appleton, Wis., June, 1961. This work was carried out under the direction of Edgar E. Dickey.

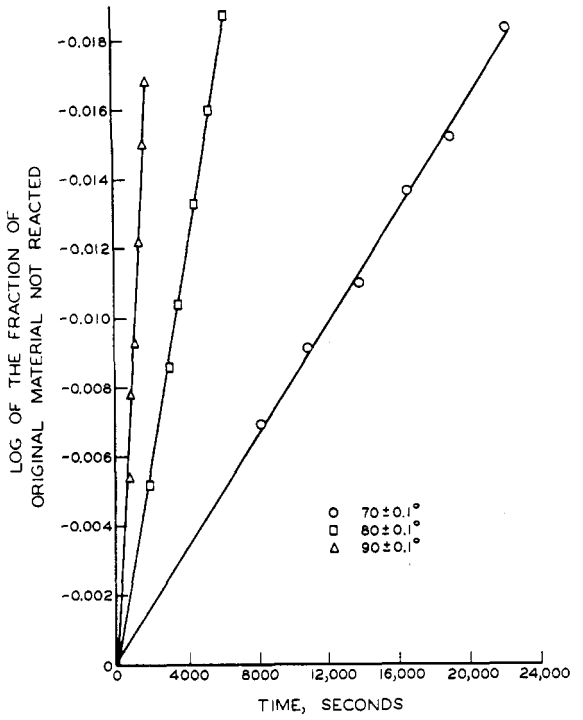
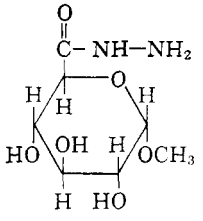


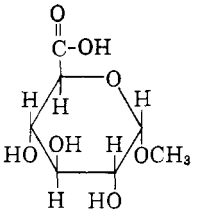
Fig. 1.—Hydrolysis of 0.241 *M* methyl α-glucuronide in *N* sulfuric acid.

air oxidation was not specific for the conversion of the primary alcohol group of methyl α-glucoside to a carboxyl group.

The crude oxidation product was purified by conversion into crystalline methyl α-D-glucopyranosiduronic hydrazide (methyl α-glucuronide hydrazide) (I). Then barium hydroxide was used to remove the hydrazine from the methyl α-glucuronide hydrazide and form pure barium (methyl α-D-glucopyranosid)uronate. This preparation was the first synthesis of pure barium (methyl α-D-glucopyranosid)uronate and it provided the first demonstration of the lability to basic hydrolysis of the hydrazine portion of methyl α-glucuronide hydrazide.



I



II

The initial portions (less than 5% hydrolysis) of the hydrolysis of methyl α-glucuronide (II) in *N* sulfuric acid at 70, 80, and 90° were studied by measurement of the methanol evolved. For comparative purposes, the hydrolysis of methyl α-glucoside was studied under identical conditions. Pseudo-first order rate constants were determined from data plotted as shown in Fig. 1 and are recorded in Tables I and II.

TABLE I

FIRST-ORDER RATE CONSTANTS FOR THE HYDROLYSIS OF 0.241 *M* METHYL α-GLUCURONIDE IN *N* SULFURIC ACID

Temperature	First-Order Rate Constant, Min.$^{-1}$ × 10^3	Correlation Coefficient
70 ± 0.1	0.113 ± 0.0088	−0.998
80 ± 0.1	0.413 ± 0.017	−0.999
90 ± 0.1	1.45 ± 0.16	−0.994

TABLE II

FIRST-ORDER RATE CONSTANTS FOR THE HYDROLYSIS OF 0.257 *M* METHYL α-GLUCOSIDE IN *N* SULFURIC ACID

Temperature	First-Order Rate Constant, Min.$^{-1}$ × 10^3	Correlation Coefficient
70 ± 0.1	0.177 ± 0.0014	−0.999
80 ± 0.1	0.748 ± 0.11	−0.994
90 ± 0.1	3.14 ± 0.21	−0.997

The linear relationship between the logs of the rate constants and reciprocals of absolute temperature enabled energies of activation to be calculated by the Arrhenius equation. The activation energy for methyl α-glucuronide hydrolysis, 31,600 ± 2370 calories per mole (90% confidence limits), is insignificantly different from that which was calculated from Nakano and Rånby's[2] optical rotation data, 30,000 calories per mole. Also exhibiting only insignificant differences are the activation energy for methyl α-glucoside hydrolysis from this investigation, 35,600 ± 927 calories per mole, and a similar value determined by Heidt and Purves,[3] 34,780 ± 360 calories per mole.

This investigation and the work of Nakano and Rånby have both shown that methyl α-glucoside is hydrolyzed at about twice the rate of methyl α-glucuronide. The rate constants for the hydrolysis of methyl α-glucoside from this study were further evaluated by comparing them with the values obtained by several independent investigators. This comparison utilized the relationship between the rate constant and the Hammett acidity function[4] as suggested by Bunton,[5] for the hydrolysis of methyl α-glucoside. The rate constant at 72.9° (the temperature at which all constants were interpolated for the comparison) from this investigation correlates well with similar values obtained by Nakano and Rånby,[2] Bunton,[5] Moelwyn-Hughes,[6] and Heidt and Purves,[3] and tends to enhance the reliability of the rate constants determined in this investigation for the hydrolysis of methyl α-glucuronide.

(2) J. Nakano and B. G. Rånby, paper presented before the Cellulose Division of the American Chemical Society, New York City Meeting, September, 1960.

(3) L. J. Heidt and C. B. Purves, *J. Am. Chem. Soc.*, **66**, 1385 (1944).

(4) M. A. Paul and F. A. Long, *Chem. Rev.*, **57**, 1 (1957).

(5) C. A. Bunton, T. A. Lewis, D. R. Llewellyn, and C. A. Vernon, *J. Chem. Soc.*, 4419 (1955).

(6) E. A. Moelwyn-Hughes, *Trans. Faraday Soc.*, **25**, 81 (1929).

Products of the acid degradation of methyl α-glucuronide and glucuronic acid in *N* sulfuric acid at 90 ± 0.1° were determined as furfural by measurement of optical density at 277 mμ.

Glasstone[7] has presented the following equations for the study of consecutive reactions:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \quad (1)$$

$$c_B = ak_1/k_2 - k_1 [\exp(-k_1t) - \exp(-k_2t)] \quad (2)$$

$$dc_C/dt = k_2c_B \quad (3)$$

In these equations k_1 and k_2 are the two rate constants, a is the concentration of reactant A at the beginning, and c_A, c_B, and c_C are the values for A, B, and C, respectively, after time t.

The degradation of methyl α-glucuronide in dilute acid was assumed to follow equation 1, in which A is the methyl α-glucuronide, B is glucuronic acid, and C represents degradation products as furfural. An indication of the conformity of the methyl α-glucuronide system to the hypothesis was obtained by the use of Glasstone's equations, though the following assumptions appeared necessary: (a) There were no significant rate-influencing interactions between methyl α-glucuronide and glucuronic acid under the conditions of this study. (b) The formation of furfural from glucuronic acid after 1000 seconds approximates a first-order reaction.

From hydrolysis studies k_1 was found to be 2.42×10^{-5} sec.$^{-1}$. From determinations of furfural formation from glucuronic acid k_2 was found to be 1.01×10^{-7} sec.$^{-1}$, and a was known to be 0.241 *M*. Substitution in equations 2 and 3 for the proposed two-step degradation of methyl α-glucuronide in *N* sulfuric acid at 90 ± 0.1° yielded a calculated rate of furfural formation at 1000 seconds of 5.86×10^{-10} moles per second. At 1000 seconds an observed rate of 6.76×10^{-10} moles per second was found.

The agreement, within probable experimental error, of the calculated and observed rates indicates that the pathway proposed by equation 1 describes the system during the portion of the reaction that was studied. This greatly reduces the possibility of significant amounts of direct degradation of methyl α-glucuronide concomitant with hydrolysis. Therefore, the predominant reaction that occurred initially when methyl α-glucuronide was treated with *N* sulfuric acid at 90° was simple glycoside hydrolysis.

Qualitative paper chromatograms provided further support for the proposed reaction pathway. As hydrolysis proceeded, spots on the chromatograms illustrated the decreasing concentration of methyl α-glucuronide and the appearance and increasing concentration of glucuronic acid in the reaction mixture. That nothing else appeared on the chromatogram was taken as evidence that no significant amounts of other carbohydrate materials were formed.

Marchessault and Rånby[8] and Nakano and Rånby[2] described the hydrolysis of the glycosides of uronic acids (uronosides) in terms of an electronic theory and concluded that an inductive effect of the uronic carboxyl group strengthens the glycosidic bond. Hamilton and Thompson[9] described the proposed inductive effect as acting in a manner that would increase repulsion between the uronoside and an attacking hydronium ion. Relationships between the electronic theories of organic reactivity, the influence of substituents on reactants, and activation energies were investigated by Hinshelwood, Laidler, and Timm.[10] They concluded that the changes in reactivity which result from electronic displacements caused by substituents are due primarily to changes in the activation energy. They pointed out that an inductive effect will influence the repulsive energies and bond strengths of the groups involved in a reaction and, thereby, influence the activation energy.

The parallelism between the conclusion of Hinshelwood, Laidler, and Timm and the theories describing the inductive effect in uronoside hydrolysis presented above suggests that if an inductive effect stabilizes methyl α-glucuronide, its activation energy should be larger than that for the hydrolysis of a compound that does not have a substituent capable of producing a stabilizing inductive effect, like methyl α-glucoside. However, the present investigation has shown that the activation energy for the hydrolysis of methyl α-glucuronide was actually *less* than that for hydrolysis of methyl α-glucoside. By the arguments presented above, this result indicates that there was not a significant stabilizing inductive effect acting in the hydrolysis of methyl α-glucuronide.

In the theory of glycoside hydrolysis used by the proponents of the inductive effect, the point that must be influenced by the inductive effect is the oxygen of the glycosidic bond, which lies three atoms away from the carboxyl group. Because the inductive effect in aliphatic systems becomes almost negligible at a distance of three atoms away from the primary electron attractor,[11] the inductive effect as proposed becomes somewhat improbable from the standpoint of distance alone.

The carbon-oxygen (single) bond has a low polarizability,[11] which has been described as a measure of the ease of transferring electronic charge

(7) S. Glasstone, "Textbook of Physical Chemistry," 2d ed., Van Nostrand, New York, 1946, p. 1075.

(8) R. H. Marchessault and B. G. Rånby, *Svensk Papperstidn.*, **62**, 230 (1959).

(9) J. K. Hamilton and N. S. Thompson, *Pulp Paper Mag. Can.*, **61**, 263 (1960).

(10) C. N. Hinshelwood, K. J. Laidler, and E. W. Timm, *J. Chem. Soc.*, 848 (1938).

(11) E. R. Alexander, "Ionic Organic Reactions," John Wiley and Sons, Inc., New York, 1950, p. 318.

from one atom to an adjacent one.[12] Therefore, in addition to distance, the inductive effect might be diminished by the presence of the ring oxygen between the carboxyl and the glycosidic bond.

Calculated constants of activation[13] given in Table III provide the basis for consideration of the differences in hydrolysis behavior of methyl α-glucuronide and methyl α-glucoside. From a comparison of rate constants, activation energies, and entropies of activation it is evident that the lower rate constant for methyl α-glucuronide hydrolysis resulted from the lower entropy of activation. The difference in the entropies of activation of methyl α-glucuronide and methyl α-glucoside suggests that the mechanisms of their hydrolysis differ in some manner, but knowledge of the systems studied is insufficient to provide a basis for description of the hydrolysis differences of the two compounds in terms of specific mechanisms or intermediates.

TABLE III

CALCULATED THERMODYNAMIC CONSTANTS OF ACTIVATION FOR HYDROLYSIS AT 80 ± 0.1°

	Methyl α-Glucuronide	Methyl α-Glucoside
ΔH^*	30,900 cal./mole	34,900 cal./mole
ΔS^*	6.42 e.u.	18.9 e.u.
ΔF^*	28,600 cal./mole	28,200 cal./mole

Results obtained in this investigation have been found to be quite similar to results of a study of the dilute acid hydrolysis of methyl α-D-galactopyranosiduronic acid (methyl α-galacturonide) and methyl α-D-galactopyranoside (methyl α-galactoside).[14] Table IV shows results of the study of Morell and Link[14]; the entropies of activation were calculated by this author from Morell and Link's data. Because the trends in the various hydrolysis constants are similar in these two investigations, the observations concerning inductive effects and mechanisms described in this paper quite possibly could represent definite characteristics of methyl uronoside behavior.

TABLE IV

CONSTANTS FOR HYDROLYSIS AT 80°[14]

	Methyl α-Galacturonide	Methyl α-Galactoside
Rate constant, k	5.42×10^{-3} min.$^{-1}$	8.70×10^{-3} min.$^{-1}$
Activation energy, E	29,000 cal./mole	35,000 cal./mole
ΔS^*	2.78 e.u.	20.7 e.u.

Experimental

Catalytic Air Oxidation.—Fifty-eight grams of methyl α-glucoside was treated by the catalytic air oxidation procedure of Mehltretter.[15] The methyl α-glucoside was put in a 3-l. Morton (creased) flask with 7.6 g. of platinum-Darco G-60 catalyst and 6.3 g. of sodium bicarbonate. The reaction mixture, stirred at about 240 r.p.m., was maintained at 50° by a water bath as air was bubbled into the mixture at about 290 l./hr. Additional sodium bicarbonate was added as needed to control pH between 7.5 and 8.5. When the reaction reached 80% completion, as determined by the amount of bicarbonate added, it was stopped, and the reaction mixture was filtered. Sodium ions were removed on a column of Amberlite IR-120(H)[16] resin. Then the solution was neutralized with barium hydroxide, concentrated to about 250 ml., and placed in the refrigerator for several days during which time insoluble barium salts of impurities precipitated.

Barium ions were removed from small amounts of the above material on Amberlite IR-120(H) resin, and samples of the solution and the precipitate were examined by paper chromatography. The developer used was ethyl acetate–pyridine–water–acetic acid (5:5:3:1). Spots were detected by dipping the sheet in 3% silver nitrate in water–acetone (1:19), spraying with 0.5 *N* ethanolic sodium hydroxide, and finally, after 10 min., dipping in 10% aqueous sodium thiosulfate. Certain impurities were detected with a spray composed of 0.4% 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid followed by a spray of 0.5 *N* ethanolic sodium hydroxide.

After allowing precipitation of impurities to occur for several days (and filtering off the precipitate each day), the oxidation product as barium salts was concentrated to about 100 ml. and run in a fine stream from a pipet into about 500 ml. of rapidly stirred absolute ethanol. The resulting white floc of crude barium salt of methyl α-glucuronide was dried in a vacuum oven at 50°; yield, 73 g. (85.7%).

Anal. Found: CH_3O, 10.8; Ba, 26.5.

Preparation of Methyl α-Glucuronide Hydrazide.—The crude barium salt was dissolved in about 500 ml. of water, and sufficient sulfuric acid was added to precipitate the barium and lower the pH to 2–2.5. After filtration, the solution was stirred with Amberlite IR-120(H) resin to remove the remaining barium, and the solution was then concentrated to about 60 ml. In order to remove water, the sirup was concentrated from about 400 ml. absolute ethanol seven times; yield, 54.6 g. of dry sirup (102.6%, cumulative yield, 87.8%, indicating that some alcohol remained).

The sirup was dissolved in 910 ml. of methanol and was treated with diazomethane (70% excess) at 0° by an adaptation of the method of Hardegger and Spitz.[17] Ethereal diazomethane was prepared by the method of DeBoer.[18] The solution was stirred for 2 hr., then concentrated to a thick sirup, and diluted with methanol to 140 ml.

The hydrazide was prepared by the method of Wolfrom, Kowkabany, and Binkley.[19] The methanolic solution of the ester was added slowly with stirring to a solution of 37.2 ml. of 95+% hydrazine in 118 ml. of methanol at room temperature. Within 15 min. the solution became a mass of crystals. The reaction mixture was allowed to stand for 30 min.: the crystals were filtered off and dried in a vacuum oven at 50°. The yield was 27.5 g. (47.2%, cumulative yield, 41.4%). Methyl α-glucuronide hydrazide was recrystallized several times from water–ethanol; yield, 15.5 g. (56.3%, cumulative yield, 23.4%); m.p. 231.5–232.5°; $[\alpha]_D$ +150° (*c* 0.623, in water). Hardegger and Spitz[17] reported the following constants for methyl α-glucuronide hydrazide prepared in a different manner: m.p. 234°; $[\alpha]_D$ +151° (*c* 1.0, in water).

(12) R. P. Smith and H. Eyring, *J. Am. Chem. Soc.*, **75**, 5183 (1953).

(13) S. Glasstone, K. J. Laidler, and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., New York, 1941, p. 599.

(14) S. Morell and K. P. Link, *J. Biol. Chem.*, **104**, 183 (1934).

(15) C. L. Mehltretter, B. H. Alexander, R. L. Mellies, and C. E. Rist, *J. Am. Chem. Soc.*, **73**, 2424 (1951).

(16) Cation-exchange resin manufactured by Rohm and Haas Co., Philadelphia, Pa.

(17) E. Hardegger and D. Spitz, *Helv. Chim. Acta*, **32**, 2165 (1949).

(18) T. J. DeBoer and H. J. Backer, *Rec. trav. chim.*, **73**, 229 (1954).

(19) M. L. Wolfrom, G. N. Kowkabany, and W. W. Binkley, *J. Am. Chem. Soc.*, **76**, 4011 (1954).

Hydrolysis of Methyl α-Glucuronide Hydrazide with Barium Hydroxide.—Using the general procedure of Fischer and Passmore,[20] 15.5 g. of the hydrazide was added to a boiling solution of 50 g. of crystalline barium hydroxide in 1000 ml. of water, and the mixture was refluxed for 10 min. The hot solution was neutralized to pH 7.5 with *N* sulfuric acid, and the barium sulfate was removed by filtration. The filtrate was allowed to cool and was then shaken for two 0.5-hr. periods with Amberlite IR-120(H) resin to remove liberated hydrazine.

The solution was adjusted to pH 7.6 with aqueous barium hydroxide and was concentrated to 30 ml. of clear, colorless sirup which was run from a pipet into about 200 ml. of stirred absolute ethanol. The resulting white floc, the barium salt of methyl α-glucuronide, was recovered by filtration and dried; yield, 18.3 g. (92%, or an over-all yield, based on the original methyl α-glucoside, 21.5%).

Prior to analysis occluded alcohol was removed from a small sample of an aqueous sirup of the purified product in an abderhalden drier over phosphorus pentoxide at the temperature of boiling acetone (56°).

Anal. Calcd. for $(C_7H_{11}O_7)_2Ba \cdot H_2O$: CH_3O, 10.88; Ba, 24.10. Found: CH_3O, 11.00; Ba, 23.96.

Hydrolysis of Methyl α-Glucuronide.—A portion of the barium salt of methyl α-glucuronide, 8.3626 g., was dissolved in 100 ml. of water and deionized on a column of Amberlite IR-120(H). After concentration of the free acid solution, a weighed portion was titrated potentiometrically. Thus, the solution contained 0.07572 g. of methyl glucuronide per milliliter or per 1.0217 g. of solution. From this master solution, 0.241 *M* solutions of methyl α-glucuronide in *N* sulfuric acid were prepared for each hydrolysis.

A modified hypodermic syringe was used to load the solution for hydrolysis into 2-ml. glass ampoules. The modification involved a stop on the syringe plunger that enabled the syringe to deliver reproducible amounts. Eight or nine ampoules were loaded for each hydrolysis run. The first and last ampoules and one in the middle of the series were tared before loading. Ampoule loads of 2 g. were found to vary by only a few tenths of a milligram. After loading, the ampoules were sealed in a gas-oxygen flame. The flame was small enough not to heat the ampoule contents.

Hydrolyses were conducted in an ethylene glycol bath maintained at 70, 80, or 90 ± 0.1°. Bath temperatures were measured by a calibrated thermometer. The ampoules, weighted with lead collars, were lowered in a basket into the bath. At various times individual ampoules were removed from the bath and quenched by immersion in an ice water mixture.

For the determination of methanol a 10-ml. distilling flask was clamped in an upright position, an ampoule was opened, and its contents were transferred quantitatively to the distilling flask with 1.5 ml. of water by means of a capillary pipet. The acid in the sample was then neutralized by the addition of the calculated amount of sodium hydroxide solution.

The methanol liberated on hydrolysis was distilled from a 10-ml. distilling flask into a 10-ml. volumetric flask slipped over the sidearm of the distilling flask. The distilling flask was heated in a glycerine bath at *ca.* 110°, and the volumetric flask was cooled in ice and water. The contents of the distilling flask were distilled almost to dryness within 2 to 3 hr., the sidearm of the distilling flask was washed with water, and the combined distillate and washings in the volumetric flask were diluted to the mark.

The methanol was determined by the method of Boos[21] without modification. In this method potassium permanganate and phosphoric acid oxidized methanol to formaldehyde, which was treated with 4,5-dihydroxy-2,7-naphthalenedisulfonic acid (chromotropic acid) to give color measured at 580 mμ on the Beckman DU spectrophotometer.

Hydrolysis of Methyl α-Glucoside.—The methyl α-glucoside used in this study was a commercial preparation[22] which had been recrystallized once from methanol and once from ethanol, m.p. 166°; $[\alpha]_D$ +157.5° (*c* 1.14, in water). For methyl α-glucoside Bollenback[23] reported m.p. 167° and $[\alpha]_D$ +158.2° (*c* 1.0, in water). The recrystallization from ethanol was necessary to avoid a large "blank" methanol determination from unhydrolyzed methyl α-glucoside.

The hydrolysis of 0.257 *M* methyl α-glucoside was conducted by the same procedure used for the hydrolysis of methyl α-glucuronide.

Acid Degradation of Methyl α-Glucuronide.—Samples were prepared and treated as has been described for methyl α-glucuronide hydrolysis. After reaction, the ampoules were opened and their contents transferred to 10-ml. volumetric flasks without neutralizing the acid, and the flasks were filled to the mark. Furfural in these solutions was then determined spectrophotometrically at 277 mμ on the Beckman DU spectrophotometer as described by Wolfrom, Schuetz, and Cavalieri.[24]

Acid Degradation of Glucuronic Acid.—A commercial sample of glucuronic acid[25] was used, m.p. 160°; $[\alpha]_D$ +35.7° (*c* 5.06, in water) after mutarotation. For glucuronic acid, Artz and Osman[26] reported m.p. 165° and $[\alpha]_D$ +36°. The concentration of glucuronic acid in the solution for the degradation study was 0.234 *M*. The procedures used were identical with those used in the degradation of methyl α-glucuronide.

Calculations.—The ordinates of Fig. 1 were calculated from methanol evolution data. The methanol found was considered to be equivalent to the amount of methyl α-glucuronide hydrolyzed.

Acknowledgment.—Sincere appreciation is expressed to Dr. B. G. Rånby for a preprint of his paper and for samples of the potassium salts of methyl α- and β-glucuronides for chromatography.

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