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The Synthesis of Aryl-D-glucopyranosiduronic Acids[1]

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Several methyl (aryl tri-*O*-acetyl-β-D-glucopyranosid)-uronates have been prepared by the fusion of methyl tetra-*O*-acetyl-β-D-glucopyranuronate with phenols in the presence of acid catalysts. Methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate has been prepared by fusion and by several other methods. Both phenyl α-D-glucopyranosiduronic acid and the corresponding acetylated methyl ester have been prepared and their identities established. Various aspects of the reactions of glucuronic acid and glucuronolactone with alcohols in the presence of acids and bases are reported. The anomeric acetates of 2-hydroxyethyl glucuronate have been prepared and identified.

Among the numerous derivatives of glucuronic acid the aryl glucosiduronic acids are particularly important since many of them are of biological origin.[2] The chemical synthesis of such compounds has been studied by Tsou and Seligman[3,4] who have prepared phenyl[3] and 2-naphthyl[4] β-D-glucopyranosiduronic acids by catalytic oxidation of the corresponding glucosides using the procedure of Marsh.[5] The same authors reported the synthesis of phenyl[3] and 2-naphthyl[4] β-D-glucofuranosidurono-γ-lactones (as their diacetates) using the well known fusion technique of Helferich and Schmitz-Hillebrecht.[6] We wish, in passing, to note that the synthesis of the latter of these compounds in these laboratories[7] by an identical but independent procedure confirms the results of Tsou and Seligman in this regard.

The commercial availability of glucuronolactone[8] makes this substance particularly attractive as a starting point for the synthesis of aryl glucosiduronic acids and the present research was aimed at exploring this subject. The following methods, for the most part general for the preparation of aryl glucosides, were studied as a means of making methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate.

(1) Treatment of phenol with methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate in the presence of silver carbonate[9] (31% yield).

(2) Treatment of potassium phenolate with methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate in ethanol[10] (75% yield).

(3) Fusing phenol with methyl tetra-*O*-acetyl-β-D-glucopyranuronate in the presence of either *p*-toluenesulfonic acid or zinc chloride[7,11] (55 and 30% yields).

(4) Fusing phenol with methyl tetra-*O*-acetyl-α-D-glucopyranuronate in the presence of zinc chloride[11] (5% yield).

(5) Treatment of phenol with methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate in the presence of quinoline[12] (23% yield).

The yield figures are for the pure β-anomer. Procedure (3) (*p*-toluenesulfonic acid) was found preferable from both the standpoint of yield and convenience. This procedure was applied successfully to the preparation of numerous other esterified aryl β-D-glucopyranosiduronic acids. Under the conditions used the reaction was not entirely general. For instance, crystalline esterified β-D-glucopyranosiduronic acids could not be isolated from fusions with *o*-nitrophenol, *o*-chlorophenol or salicylaldehyde. The crystalline esterified conjugates of *o*-nitrophenol and *o*-chlorophenol were eventually obtained by using the procedure of Mannich[13] as modified by Glaser and Wulwek.[14] With hydroquinone and resorcinol, which might be expected to give diglycosides,[15,16] sirups were obtained.

The melting points of some of the esterified conjugates prepared chemically (notably where aryl is phenyl, *o*-tolyl and *m*-tolyl) are some 10 degrees higher than those prepared from biological uronic acids. The elemental analyses of the chemically prepared compounds indicate that no radical change has occurred during synthesis. The possibility is that the compounds herein reported represent more stable dimorphs. This is emphasized because of the agreement of rotations and also because of experience with initial preparations of the esterified phenyl β-D-glucopyranosiduronic acid. The first preparations of the compound had melting points in the 117–118° range. Repeated recrystallizations from ethanol failed to increase the constant. It was only after substitution of isopropyl alcohol that the higher melting point (126.5–127.5°) was established. The higher melting point is retained on recrystallization from either ethyl or isopropyl alcohol.

Most of the methyl (aryl tri-*O*-acetyl-β-D-glucopyranosid)-uronates reported here have been prepared previously from the biologically available uronic acids.[17] A comparison of physical constants may be made in Table I. New compounds have been obtained from catechol, naphthols and methyl gentisate. During the preparation of this manuscript biosynthesis of 1- and 2-naphthyl β-D-glucopyranosiduronic acids was reported.[19] A comparison of physical constants recorded by the English

(1) Part of this work was presented before the Division of Sugar Chemistry at the 124th National Meeting of the American Chemical Society, Chicago, Ill., September, 1953.

(2) H. G. Bray, *Advances in Carbohydrate Chem.*, **8**, 251 (1953).

(3) K.-C. Tsou and A. M. Seligman, THIS JOURNAL, **75**, 1042 (1953).

(4) K.-C. Tsou and A. M. Seligman, *ibid.*, **74**, 5605 (1952).

(5) C. A. Marsh, *J. Chem. Soc.*, 1578 (1952).

(6) B. Helferich and E. Schmitz-Hillebrecht, *Ber.*, **66**, 378 (1933).

(7) G. N. Bollenback and Elizabeth M. Osman, unpublished work.

(8) Marketed as Glucurone by Corn Products Refining Company, New York, N. Y.

(9) N. M. Carter, *Ber.*, **63**, 586 (1930).

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(11) Edna M. Montgomery, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **64**, 690 (1942).

(12) E. Fischer and L. v. Mechel, *Ber.*, **49**, 2813 (1916).

(13) C. Mannich, *Ann.*, **394**, 225 (1912).

(14) E. Glaser and W. Wulwek, *Biochem. Z.*, **145**, 514 (1924).

(15) B. Helferich and W. Reischel, *Ann.*, **533**, 278 (1938).

(16) A. Robertson and R. B. Waters, *J. Chem. Soc.*, 2729 (1930).

(17) I. A. Kamil, J. N. Smith and R. T. Williams, *Biochem. J.*, **50**, 235 (1951).

TABLE I

COMPARISON OF PHYSICAL CONSTANTS OF METHYL (ARYL TRI-*O*-ACETYL-β-D-GLUCOPYRANOSID)-URONATES PREPARED CHEMICALLY WITH THOSE OBTAINED FROM BIOLOGICALLY PREPARED URONIC ACIDS

Aglycone	M.p., °C. Chem.	Bio.	$[\alpha]^{20-26}D$ (*c* 1, $CHCl_3$) Chem.	Bio.	Anal. (chem. syn.), % Calcd. C	H	Obsd. C	H
Phenyl	126.5–127.5	116[18]	−35.6	−33.0	55.61	5.36	55.65	5.44
							55.40	5.34
o-Chlorophenyl	151–152	151–152[17]	−64.8	−65.0	...	..	...	..
p-Chlorophenyl	152–154	151–152[17]	−32.2	−32.9	...	..	...	..
o-Bromophenyl	137–140	141–143[17]	−64.1	−64.6	...	..	...	..
p-Bromophenyl	162–163.5	157–158[17]	−29.5	−28.0	...	..	...	..
o-Iodophenyl	160–162	161–162[17]	−63.6	−63.3	...	..	...	..
o-Tolyl	138–140	131[17]	−40.6	−46.0	56.60	5.66	56.38	5.45
m-Tolyl	113–115	93–94[17]	−32.8	−25.6	56.60	5.66	56.87	5.57
p-Tolyl	137–138	140[17]	−31.3	−36.0	...	..	...	..
o-Hydroxyphenyl	136–137	.....	−33.4	....	53.52	5.16	53.41	4.92
1-Naphthyl	126–127; 157–159	160[19]	−75.5	−79.0	60.00	5.22	60.11	5.12
2-Naphthyl	188–190	127–128[19]	−29.4	−35.0	60.00	5.22	59.99	5.11
Methyl gentisate	134–136	.....	−26.3	....	52.07	4.96	52.29	4.88
o-Nitrophenyl	175–176	172[20]	+19.0	+18.5	...	..	...	..

workers with those obtained by us may be made in Table I. The lower melting form we obtained from α-naphthol was very labile; we have not been able to transform the higher melting form to the lower melting form. The derivative obtained by us from β-naphthol gave on hydrolysis the known 2-naphthyl β-D-glucopyranosiduronic acid.

From reactions with phenol (procedures 3, 4 and 5 above) a second compound was isolated with melting point 114–115° and $[\alpha]^{25}D$ +157.50 (*c* 1, $CHCl_3$). The dextrorotation and elementary analysis of this compound suggested that it was the α-anomer. Catalytic oxidation of phenyl α-D-glucopyranoside followed by esterification with diazomethane and acetylation gave authentic methyl (phenyl tri-*O*-acetyl-α-D-glucopyranosid)-uronate. The infrared spectra of the compound as obtained by oxidation of the glucoside and as obtained from the fusion mixture are identical. The free phenyl α-D-glucopyranosiduronic acid which was obtained crystalline by oxidation of the glucoside is, so far as we know, the first aryl α-D-glucopyranosiduronic acid to be reported.

In Table II are listed the molecular rotations of the acetylated phenyl glucopyranosides and the acetylated methyl esters of the phenyl glucopyranosiduronic acids. By applying Hudson's rule of isorotation[21] the 2A and 2B values for both sets of anomers were calculated. The 2A values (effect of phenyl at carbon one) agree reasonably well in the two series. The significance of the 2B values must necessarily await isolation of other aryl α-D-glucopyranosiduronic acid derivatives.

Free phenyl β-D-glucopyranosiduronic acid[18,22,23] was obtained in crystalline form by de-esterification of the acetylated methyl ester with barium methoxide. This procedure is quite satisfactory on a small scale (1–2 g. ester) but large scale de-esterification (25–50 g.) gives a product which is rather difficult to purify. The only other β-D-glucopyranosiduronic acid that has been isolated by de-esterification is that of β-naphthol.[25]

TABLE II

RULES OF ISOROTATION APPLIED TO PHENYL GLUCOSIDES AND PHENYL GLUCOPYRANOSIDURONIC ACIDS

	$[M]_D$	2A ($M\alpha - M\beta$)	2B ($M\alpha + M\beta$)
Phenyl α-D-glucopyranoside[7]	+46,300	+64,700	+27,900
Phenyl β-D-glucopyranoside[7]	−18,400		
Phenyl α-D-glucopyranosiduronic acid[24]	+41,470	+65,905	+17,035
Phenyl β-D-glucopyranosiduronic acid[24]	−24,435		
Phenyl tetra-*O*-acetyl-α-D-glucopyranoside[11]	+71,500	+81,040	+61,960
Phenyl tetra-*O*-acetyl-β-D-glucopyranoside[7]	− 9,540		
Methyl (phenyl tri-*O*-acetyl-α-D-glucopyranosid)-uronate[24]	+64,575	+79,170	+49,980
Methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate[24]	−14,595		

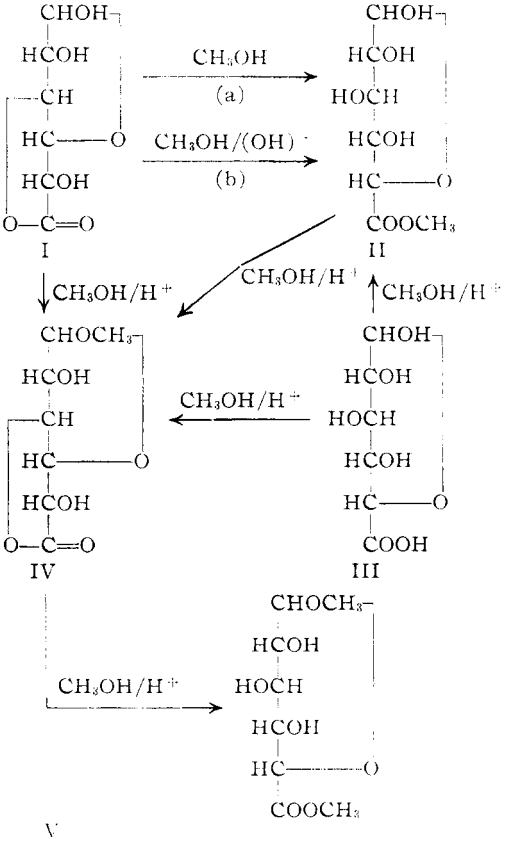
The procedures used for obtaining aryl D-glucopyranosiduronic acids necessitated easy availability of the starting materials methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate and the acetylated methyl glucuronates. The latter are essential to the preparation of the acetobromo compound. Methyl glucuronate has been prepared by the reaction of silver glucuronate with methyl iodide[26] and by refluxing glucuronolactone with methanol for 3 days[27] (a, I → II). Both procedures are lengthy. The procedure of Jansen and Jang[28] for the preparation of methyl galacturonate by the acid-catalyzed reaction of galacturonic acid with methanol leads to extensive glycosidation and lactonization when applied to glucuronic acid (III → IV). Eventually it was found that glucuronolactone could be esterified with methanol in the presence of base catalysts (b, I → II). Concurrent with use of this reaction a similar procedure was published by Touster and

(18) D. V. Parke and R. T. Williams, *Biochem. J.*, **48**, 621 (1951).
(19) E. D. S. Corner, F. S. Billett and L. Young, *ibid.*, **56**, 270 (1954).
(20) D. Robinson, J. N. Smith and R. T. Williams, *ibid.*, **50**, 221 (1951).
(21) C. S. Hudson, THIS JOURNAL, **31**, 66 (1909).
(22) J. W. Porteous and R. T. Williams, *Biochem. J.*, **44**, 46 (1949).
(23) G. A. Garton, D. Robinson and R. T. Williams, *ibid.*, **45**, 65 (1949).
(24) This paper.
(25) M. Berenbom and L. Young, *Biochem. J.*, **49**, 165 (1951).
(26) W. F. Goebel and F. H. Babers, *J. Biol. Chem.*, **106**, 63 (1934).
(27) W. F. Goebel and F. H. Babers, *ibid.*, **111**, 347 (1935).
(28) E. F. Jansen and Rosie Jang, THIS JOURNAL, **68**, 1475 (1946).

Reynolds.[29] The differences in procedures are not significant. However, it is shown here that the base catalyst may be sodium methoxide, sodium hydroxide, triethylamine, tetraethylammonium hydroxide or anion-exchange resin. The latter is especially useful for small scale preparations. Base-catalyzed esterification of glucuronolactone was unsuccessful when the alcohols were ethanol, benzyl alcohol or benzyl cellosolve (ethylene glycol monobenzyl ether). Sirupy esters were obtained using methyl cellosolve (ethylene glycol monomethyl ether) and ethylene glycol. Both esters were identified as crystalline acetates. Only *one* acetate of each ester was isolated. With ethylene glycol there is a possibility of obtaining a mono- or diester. The crystalline acetate isolated was shown to be that of a monoester by reaction of silver glucuronate with ethylene iodohydrin and subsequent acetylation. A crystalline product so obtained was identical with the acetylated product isolated from the lactone–glycol reaction. From the silver glucuronate–ethylene iodohydrin reaction mixture the anomeric ester was also isolated as a crystalline acetate.

CHART 1

SUMMARY OF REACTIONS OF GLUCURONIC ACID DERIVATIVES WITH METHANOL



Several catalysts for acetylation of methyl glucuronate were surveyed for highest possible yield. The yield of mixed acetates with pyridine was 75%; with cold perchloric acid 85%. As reported by Goebel and Babers[26] the acetylated β-form is sufficiently insoluble in cold solvents to give no difficulty in isolation; the α-anomer is much more troublesome. The isolation of the latter was improved by using benzene–hexane as crystallizing medium. Yields of ester acetates are consistently between 75–85% based on starting lactone.

Various methods were tried for the preparation of methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate. Especially disappointing was the inability to apply the short procedure of Eàrczai-Martos and Körösy[30] which would circumvent isolation of intermediate acetates. The preferred procedure is the original (HBr in acetic acid) of Goebel and Babers.[26]

Experimental

Methyl Tetraacetyl Glucopyranuronates. (1) **Acid-catalyzed Acetylation.**—Glucuronolactone (17.6 g., 0.1 mole) was added to 100 ml. of methanol which contained 0.15 g.[31] of sodium methoxide. The mixture was stirred at room temperature for 30 minutes at the end of which time all glucuronolactone had dissolved. After another 30 minutes the methanol was removed under reduced pressure (water pump, 10–12 mm., bath temperature 40°). The sirup[32] was dissolved in 68 ml. of acetic anhydride and a mixture of 0.3 ml. of perchloric acid in 10 ml. of acetic anhydride was added dropwise at a rate such that the reaction temperature never exceeded 40°. After the reaction mixture had stood overnight at room temperature an additional 0.1 ml. of perchloric acid was added and the solution stored in the refrigerator. Overnight 9.95 g. of crystalline material separated. It was isolated by filtration followed by ether wash. The crystals were recrystallized once from hot ethanol—yield 9.6 g. of methyl tetra-*O*-acetyl-β-D-glucopyranuronate; m.p. 176.5–178°; $[\alpha]^{23}D$ +7.4° (*c* 2, $CHCl_3$); literature values[26] m.p. 178°; $[\alpha]^{24}D$ +8.7° (*c* 1, $CHCl_3$).

The mother liquor from the reaction was poured onto 300 g. of crushed ice and neutralized with sodium bicarbonate. After removal of excess sodium bicarbonate on a filter the cake and filtrate were extracted thoroughly with chloroform. The chloroform extract was dried with anhydrous sodium sulfate and concentrated to a sirup which was taken up in hot isopropyl alcohol. On cooling a 21.5-g. crop of crude crystalline material separated; $[\alpha]^{23}D$ + 82° (*c* 2, $CHCl_3$). Over-all yield (crude) was 83% of theory. The crude could be recrystallized best from benzene–hexane to gain at least partial separation of anomers.

(2) **Pyridine-catalyzed Acetylation.**—Sodium hydroxide (1.1 g.) was dissolved in 3 l. of methanol and 400 g. (2.27 moles) of glucuronolactone was added in 100-g. increments. (Lactone should dissolve almost immediately; additional base should be added if *p*H drops below 8.0.) The mixture was stirred 1 hr. at room temperature and methanol was then removed under reduced pressure (water pump, 10–12 mm., bath temperature below 50°). Final methanol removal was accomplished with a vacuum pump. Acetylation was effected as above using 1 l. of pyridine and 1.5 l. of acetic anhydride. On standing in the refrigerator overnight 365 g. of methyl tetra-*O*-acetyl-β-D-glucopyranuronate crystallized from the reaction mixture. An additional 25 g. of β-isomer was obtained on concentration of the mixture.

By processing mother liquors as above 242 g. of methyl tetra-*O*-acetyl-α-D-glucopyranuronate was obtained. In general, yields are in the neighborhood of 70%.

Sodium hydroxide may be replaced by 40 ml. of *N* triethylamine in CH_3OH, 4 g. of tetraethylammonium hydroxide or 40 ml. of *N* sodium methoxide.

Acetoxyethyl Tetraacetyl Glucopyranuronates.—Fifteen grams (0.085 mole) of glucuronolactone was added to 75 ml. of ethylene glycol containing 1.5 ml. of *N* sodium methoxide. Solution was instantaneous. After refrigeration over-

(29) O. Touster and V. H. Reynolds, *J. Biol. Chem.*, **197**, 863 (1952).

(30) M. Bàrczai-Martos and F. Körösy, *Nature*, **165**, 369 (1950).

(31) Since glucuronolactone often contains glucuronic acid as a contaminant, it may be necessary to use more sodium methoxide to obtain the degree of alkalinity required.

(32) If there is any uncertainty about the completeness of methyl ester formation this sirup should be taken up in absolute ethanol and refrigerated. Unreacted glucuronolactone will crystallize.

night the base was neutralized with sulfuric acid and the solution concentrated at 0.3 mm. (bath at 37–45°) to a very thick sirup. The sirup was dissolved in 100 ml. of pyridine and 100 ml. of acetic anhydride was added keeping solution temperature below 20°. After refrigeration overnight solvents were removed under reduced pressure (water pump) and the residue evaporated under reduced pressure several times with 50-ml. portions of toluene. After addition of ethanol to the final sirup crystallization occurred, m.p. 135–138° (10.0 g., 26.3%). After several recrystallizations from ethanol or isopropyl alcohol the acetylated ester melted at 139–140°; $[\alpha]^{25}D$ +12.5° (*c* 1, $CHCl_3$). *Anal.*[33] Calcd. for $C_{19}H_{24}O_{13}$ (monoester): C, 48.21; H, 5.35. Found: C, 48.41; H, 5.23.

Ten grams of ethylene iodohydrin[34] and 1.89 g. (6.3 mmoles) of silver glucuronate[23] were stirred together for 2 hrs. at room temperature. The precipitate was collected on a filter and washed with ether to remove as much excess ethylene iodohydrin as possible. The gummy residue was triturated with water and then filtered free of silver iodide. An excess of solid barium carbonate was added to the aqueous solution to neutralize any free glucuronic acid. After removal of excess barium carbonate by filtration the filtrate was lyophilized and the residual sirup was triturated with ethanol. A small amount of insoluble barium salts separated and was removed by filtration. The filtrate was concentrated to a sirup which was acetylated with 10 ml. of a 1:1 pyridine–acetic anhydride mixture for 24 hr. at room temperature. After processing in the usual manner the 2-acetoxyethyl tetra-*O*-acetyl-β-D-glucopyranuronate crystallized from isopropyl alcohol. The yield was 0.62 g. (22%); m.p. 138.5–140°; $[\alpha]^{25}D$ +12.3° (*c* 0.7, $CHCl_3$). Mixed melting point with the compound prepared above gave no depression. *Anal.*[33] Calcd. for $C_{18}H_{24}O_{13}$: C, 48.21; H, 5.35. Found: C, 48.48, 48.33; H, 5.57, 5.47. After concentration and refrigeration of the mother liquor 0.53 g. (19%) of 2-acetoxyethyl tetra-*O*-acetyl-α-D-glucopyranuronate was obtained. This material was recrystallized from isopropyl alcohol to constant m.p. 82–84.5°; $[\alpha]^{25}D$ +101.5° (*c* 0.5, $CHCl_3$). *Anal.*[33] Calcd. for $C_{18}H_{24}O_{13}$: C, 48.21; H, 5.35. Found: C, 48.49, 48.60; H, 5.51, 5.41.

β-Methoxyethyl Tetraacetyl Glucopyranuronate.—The base-catalyzed esterification reaction was repeated using 80 mg. of sodium hydroxide in 150 ml. of methyl cellosolve (ethylene glycol monomethyl ether) and 10.0 g. (0.057 mole) of glucuronolactone. After all glucuronolactone was in solution (30 min.) excess methyl cellosolve was removed by distillation under reduced pressure. Unreacted lactone (3.8 g.) was removed by refrigeration of an ethanol solution of the crude ester. Acetylation of crude ester (acetic anhydride–pyridine) yielded 4.7 g. (32%) of β-methoxyethyl tetra-*O*-acetyl-β(?)-D-glucopyranuronate from ethanol or isopropyl alcohol as clusters of fine needles. Recrystallization to constant melting point gave m.p. 89–91°; $[\alpha]^{25}D$ +55.0° (*c* 1, $CHCl_3$). *Anal.*[33] Calcd. for $C_{17}H_{24}O_{12}$: C, 48.60; H, 5.72. Found: C, 48.83; H, 6.00.

The esterification reaction failed when the alcohol was ethanol, benzyl alcohol or benzyl cellosolve.

Methyl (Tri-*O*-acetyl-α-D-glucopyranosyl Bromide)-uronate.—Fifty grams (0.133 mole) of methyl tetra-*O*-acetyl-α(or β)-D-glucopyranuronate was dissolved in 200 ml. of 30% hydrobromic acid in acetic acid (Eastman Kodak #1161) and the mixture, after solution, allowed to stand in the refrigerator overnight. Solvent was removed under reduced pressure (water pump, 10–12 mm., bath temperature, 40°) and the residue dissolved in 100 ml. of chloroform. The chloroform solution was extracted with cold saturated aqueous sodium bicarbonate, then water. After drying the chloroform solution (sodium sulfate), solvent was removed under diminished pressure. The residual sirup was dissolved in 150 ml. of absolute ethanol (from which crystals sometimes separate immediately), carbon treated, filtered, and the filtrate refrigerated; first crop 45.0 g. (85%), m.p. 106–107°, $[\alpha]^{25}D$ +197° (*c* 1, $CHCl_3$); literature values[27] m.p. 104–105°, $[\alpha]^{24}D$ +198° (*c* 0.5, $CHCl_3$).

Small additional crops may be obtained from the mother liquor. If the first crop melts low one recrystallization at room temperature from ethanol will suffice to give a high melting compound. Such material can be kept bottled under refrigeration for a period of 6–8 weeks. When decomposition is noted the material can be dissolved in ethanol, carbon treated in the presence of a little calcium carbonate, filtered and crystallized to a product of the original stability.

Methyl (Phenyl Tri-*O*-acetyl-β-D-glucopyranosid)-uronate. Procedure I. (Reaction of phenol with methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate in the presence of silver carbonate).—Ten grams (0.106 mole) of phenol and 4 g. (0.01 mole) of methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate were dissolved in 50 ml. of benzene. Two grams of silver carbonate was added and the mixture stirred overnight at room temperature. The silver salts were collected on a filter and washed with hot benzene. The filtrates were combined and washed with 2 *N* potassium hydroxide then water. The benzene layer was dried (Drierite), filtered and evaporated at room temperature to a solid residue. The residue was dissolved in boiling isopropyl alcohol and allowed to crystallize at room temperature. The methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate crystallized as short colorless needles; yield 1.3 g. (31%), m.p. 126.5–127.5°, $[\alpha]^{25}D$ −35.5° (*c* 1, $CHCl_3$); literature values[18] m.p. 116°, $[\alpha]^{22}D$ −33° (*c* 1, $CHCl_3$).

Procedure II. (Reaction of potassium phenolate with methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate).—Potassium phenolate (160 mg., 1.2 mmoles) and methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate (400 mg., 1.0 mmole) were shaken with absolute ethanol (10 ml.). The solution turned brown immediately and deposited a white substance when allowed to stand at room temperature over a period of 2 months. The precipitate was collected on a filter and discarded. The filtrate was concentrated to a mixture of sirup and crystals. The mixture was triturated with ethanol and the crystals were isolated by filtration. The crystals so obtained melted at 116–118°. After recrystallization from isopropyl alcohol the melting point was 125–126° (yield 310 mg., 75%).

Procedure III. (Fusion of phenol with methyl tetra-*O*-acetyl-β-D-glucopyranuronate.)—These procedures are essentially those given by Montgomery, Richtmyer and Hudson.[11]

(a) **With *p*-Toluenesulfonic Acid as Catalyst.**—Fifty-five grams (0.146 mole) of methyl tetra-*O*-acetyl-β-D-glucopyranuronate and 55.0 g. (0.58 mole) of phenol were fused in the presence of 0.72 g. of *p*-toluenesulfonic acid monohydrate on a steam-bath for 1.5 hr. under reduced pressure (10–12 mm.). The dark melt was then dissolved in 500 ml. of benzene. The benzene solution was washed with 2 *N* potassium hydroxide followed by water. The benzene layer was then dried (Drierite), filtered and concentrated to a crystalline mass. Recrystallization from boiling isopropyl alcohol gave 35.0 g. (58%) of product, m.p. 124–125°.

(b) **With Zinc Chloride as Catalyst.**—Twenty-five grams (0.067 mole) of methyl tetra-*O*-acetyl-β-D-glucopyranuronate, 27.5 g. (0.29 mole) of phenol and 6.8 g. of fused zinc chloride dissolved in 25 ml. of 95:5 = acetic acid:acetic anhydride were heated at 100° for 15 min., then at 120–125° for 20 min. under reduced pressure (10–12 mm.). The melt was dissolved in 300 ml. of benzene. The benzene solution was washed with 2 *N* potassium hydroxide, then water. The benzene solution was dried with Drierite, filtered and concentrated under reduced pressure (water pump) to a solid residue. The residue was dissolved in 100 ml. of warm isopropyl alcohol and after standing at room temperature overnight deposited 8.86 g. (32%) of methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate, m.p. 123–125°.

Crude α-anomer (4.0 g., 15%) was isolated by taking additional crops (five) from the concentrated mother liquors.

These procedures were also used with other phenols.

Yields of *pure* products are given below. In general, crude yields using *p*-toluenesulfonic acid were between 50–90%; one recrystallization was sufficient to give a very pure product. Purification was usually effected by recrystallizing at room temperature from isopropyl alcohol.

The product obtained from catechol, methyl (*o*-hydroxyphenyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate, crystallized as small chunky prisms from isopropyl alcohol or ethanol; m.p. 136–137°, $[\alpha]^{25}D$ −33.4° (*c* 1, $CHCl_3$). *Anal.*[33] Calcd. for $C_{19}H_{22}O_{11}$: C, 53.52; H, 5.16. Found: C, 53.41; H, 4.92.

Methyl (methyl gentisyl tri-*O*-acetyl-β-D-glucopyranosid)-

(33) Analyses were performed by Clark Micro Analytical Lab., Urbana, Ill. and Micro-Tech Laboratories, Skokie, Ill.

(34) J. N. Street and H. Adkins, THIS JOURNAL, **50**, 162 (1928).

Phenol	Yield, % p-Toluenesulfonic acid	Zinc chloride
o-Bromophenol	34.0	..
p-Bromophenol	47.5	28.8
p-Chlorophenol	39.0	28.8
o-Iodophenol	55.0	..
o-Cresol	48.1	32.6
m-Cresol	44.2	31.2
p-Cresol	52.1	27.5
Catechol	23.0	..
Methyl gentisate	45.0	30.3
β-Naphthol	33.0	15.0

uronate crystallized from isopropyl alcohol in the form of clusters of short needles, m.p. 134–136°, $[\alpha]^{25}D$ −26.3° (*c* 1, $CHCl_3$). *Anal.*[33] Calcd. for $C_{20}H_{24}O_{13}$: C, 52.07; H, 4.96. Found: C, 52.29; H, 4.88.

Methyl (2-naphthyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate formed long needles from large volumes of isopropyl alcohol or ethanol; m.p. 188–190°, $[\alpha]^{26}D$ −29.4° (*c* 1, $CHCl_3$). *Anal.*[33] Calcd. for $C_{23}H_{24}O_{10}$: C, 60.00; H, 5.22. Found: C, 59.99; H, 5.11.

Methyl (1-naphthyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate crystallized initially as long colorless needles from ethanol, m.p. 126–127°. After recrystallization from ethanol, isopropyl alcohol or benzene–Skelly B the compound melted at 157–159°, occasionally softening at 127°; $[\alpha]^{25}D$ −75.5° (*c* 1, $CHCl_3$). *Anal.*[33] Calcd. for $C_{23}H_{24}O_{10}$: C, 60.00; H, 5.22. Found: C, 60.11; H, 5.12.

Procedure IV. (Fusion of phenol with methyl tetra-*O*-acetyl-α-D-glucopyranuronate in presence of zinc chloride).—Methyl tetra-*O*-acetyl-α-D-glucopyranuronate (2.75 g., 7.3 mmoles), phenol (3.0 g., 31.9 mmoles) and zinc chloride (0.750 g. dissolved in 2.4 ml. of 95:5 = acetic acid:acetic anhydride) were heated at 110–120° under reduced pressure (10–12 mm.) for 1 hr. The mixture was processed as above to give 0.24 g. (8.0%) of crystalline product. Recrystallization from isopropyl alcohol at room temperature gave 0.15 g. of methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate, m.p. 124.5–126°.

Procedure V. (Reaction of phenol with methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate in the presence of quinoline.)—(a) A mixture of 10 g. (2.51 mmoles) of methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate, 32 g. (0.34 mole) of phenol and 4 g. (0.031 mole) of quinoline was heated on a steam-bath for 2 hr. After cooling to room temperature the mixture was dissolved in 150 ml. of ethyl ether. The ether solution was washed successively with 50 ml. of *N* sulfuric acid, ten times with 50-ml. portions of water, 50 ml. of *N* potassium hydroxide, and finally water. After drying (Drierite) the ether solution was filtered and evaporated to leave a semi-crystalline residue. The residue was dissolved in hot carbon tetrachloride. After cooling, 1.1 g. (10.5%) of methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate crystallized, m.p. 125–126°.

(b) The reaction was repeated using a minimum of toluene (26 ml.) to effect solution at room temperature. The mixture was then heated at 100° for 4 hr.; yield 2.4 g. (23.3%), m.p. 124–126°.

(c) When the procedure involving toluene was run at room temperature with stirring for 21 days only 0.025 g. of product was isolated.

(d) In the presence of quinoline and silver oxide alone (but *not* in the absence of silver oxide) consistent yields in the order of 20% of methyl tetra-*O*-acetyl-β-D-glucopyranuronate were obtained. This particular phase of the reaction is being further investigated.

Isolation of Methyl (Phenyl Tri-*O*-acetyl-α-D-glucopyranosid)-uronate.—The mother liquors from reactions Va and Vb (involving phenol) were concentrated to dryness and the residues recrystallized several times from isopropyl alcohol. The pure α-isomer melted at 114–115°; $[\alpha]^{25}D$ +157.5° (*c* 1, $CHCl_3$). From reaction Va was obtained 65 mg. and from Vb 800 mg. of pure product.

The same material was also isolated from the mother liquors of reaction IIIb (1.5 g.). *Anal.*[33] Calcd. for $C_{19}H_{22}O_{10}$: C, 55.61; H, 5.36. Found: C, 55.90; H, 4.90.

Conversion of Methyl (Phenyl Tri-*O*-acetyl-β-D-glucopyranosid)-uronate to Methyl (Phenyl Tri-*O*-acetyl-α-D-glucopyranosid)-uronate.—Ten grams (0.024 mole) of methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate, 9.2 g. (0.098 mole) of phenol and 2.5 g. of zinc chloride in 23 ml. of 95:5 = acetic acid:acetic anhydride were fused together for 1.5 hr. at 120–130° under reduced pressure (10–12 mm.). The melt was dissolved in 100 ml. of benzene and the benzene solution washed successively with water, 2 *N* potassium hydroxide and water. The benzene layer was then dried with Drierite, filtered and concentrated in an air stream. The residue was dissolved in 30 ml. of hot isopropyl alcohol and crude α-anomer (2.9 g.) obtained in three crops. A small amount (0.5 g.) of β-anomer was obtained by slow crystallization at room temperature of the crude from dilute solutions in isopropyl alcohol. Repeated recrystallizations of material obtained from the mother liquor gave 1.5 g. of pure methyl (phenyl tri-*O*-acetyl-α-D-glucopyranosid)-uronate, m.p. 114–115°.

Methyl (Phenyl Tri-*O*-acetyl-α-D-glucopyranosid)-uronate. Two grams (7.3 mmoles) of phenyl α-D-glucoside (monohydrate), 0.6 g. of platinum black (Baker) were stirred with oxygen ebullition in 300 ml. of distilled water for 1.25 hr. on a steam-bath. During the oxidation a total of 0.79 g. (9.5 mmoles) of sodium bicarbonate was added to maintain *p*H 8–9. Catalyst was removed by centrifugation and after concentration to 50 ml. the centrifugate was passed through a 10-ml. bed of Nalcite HCR cation-exchange resin. The eluate was carbon treated, filtered and concentrated at the water pump to a thick yellow sirup. The sirup was dissolved in ethyl acetate and the phenyl α-D-glucopyranosiduronic acid crystallized therefrom. Repetition of this oxidation showed an average yield, based on oxidized starting material, of about 18%.

The free acid crystallized as prisms from moist ethyl acetate, m.p. 80–85° with softening at 73°. Recrystallization from ethyl acetate failed to improve the melting point. A Karl Fischer analysis indicated the presence of 1 mole of water. After drying over phosphorus pentoxide for 20 hr. at 60° the m.p. was 147–149° with softening at 146°. In this form the acid was very hygroscopic and began to cake immediately after exposure to air. Moisture apparently was absorbed to the extent of 0.5 mole. The melting point of the hemihydrate is the same (147–149°) as the phosphorus pentoxide dried material. The hemihydrate crystallized as needles from ethyl acetate with retention of melting point (147–149°); $[\alpha]^{25}D$ +153.6° (*c* 1, H_2O). *Anal.*[33] Calcd. for $C_{12}H_{14}O_7 \cdot 0.5H_2O$: C, 51.60; H, 5.37. Found: C, 51.61, 51.35; H, 5.60, 5.65.

A 0.55-g. (1.97 mmoles) sample of phenyl α-D-glucopyranosiduronic acid hemihydrate was dissolved in 40 ml. of methanol and treated with diazomethane. The solution was concentrated to a thick yellow sirup which was acetylated with 10 ml. of 1:1 pyridine–acetic anhydride. After processing in the usual manner the crude crystalline methyl (phenyl tri-*O*-acetyl-α-D-glucopyranosid)-uronate was recrystallized several times from isopropyl alcohol to give 0.36 g. (44%); m.p. 110–112°, $[\alpha]^{25}D$ +163° (*c* 1.27, $CHCl_3$). *Anal.*[33] Calcd. for $C_{19}H_{22}O_{10}$: C, 55.61; H, 5.36. Found: C, 55.96, 55.89; H, 5.56, 5.45. A mixed melting point with material obtained by the fusion process (m.p. 114–115°) was 110–115°.

Methyl (*o*-Nitrophenyl Tri-*O*-acetyl-β-D-glucopyranosid)-uronate.—Methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate (3.97 g., 0.01 mole) and *o*-nitrophenol (6.95 g., 0.05 mole) were dissolved in 81 ml. of acetone. Nine milliliters of 5 *N* potassium hydroxide was added rapidly and the solution was allowed to stand at room temperature for 2 days. The dark solution was diluted with 3 volumes of chloroform and the solution shaken with water. The chloroform solution was then extracted with 2 *N* potassium hydroxide followed by water. The chloroform layer was dried (Drierite), filtered and evaporated to dryness at room temperature. The dried crystalline residue weighed 1.55 g. (34%), m.p. 165–174°. After two recrystallizations from acetone the product had a melting point 175–176°, $[\alpha]^{24}D$ +19.0° (*c* 1, $CHCl_3$); literature values[20] m.p. 172°, $[\alpha]^{20}D$ +18.5° (*c* 1, $CHCl_3$).

Methyl (*o*-Chlorophenyl Tri-*O*-acetyl-β-D-glucopyranosid)-uronate.—The acetobromo compound (3.97 g., 0.01 mole) and *o*-chlorophenol (1.54 ml., 0.015 mole) were dissolved in 40 ml. of acetone. Seven milliliters of water was added and, after cooling the solution in an ice-bath, 3 ml. of 5 *N* potassium hydroxide was added. The mixture was allowed to

stand at 5° for 5 days. It was then processed as above. The crude product weighed 1.4 g. Recrystallization from ethanol at room temperature gave 0.55 g. of unreacted aceto-bromo compound. The mother liquor was diluted with water and refrigerated for 3 days. The product was collected on a filter and recrystallized from ethanol at room temperature to give 0.5 g. (11%) of methyl (*o*-chlorophenyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate; m.p. 151–152°, $[\alpha]^{24}D$ −62.8° (*c* 1, $CHCl_3$); literature values[17] m.p. 151–152°, $[\alpha]^{20}D$ −65.0° (*c* 1, $CHCl_3$).

Phenyl β-D-Glucopyranosiduronic Acid.—Three hundred milligrams (0.73 mmole) of methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate was dissolved in 20 ml. of methanol containing 0.3 ml. of 0.5 *N* barium methoxide. The solution was refrigerated for 3 days. Barium was removed by addition of the calculated amount of dilute sulfuric acid. The mixture was filtered and the filtrate was concentrated to a crystalline residue (150 mg., 75%). The residue was suspended in boiling benzene and enough ethanol added to obtain a homogeneous solution. The acid obtained on cooling had a sharp melting point 163–164° with a preliminary softening at 120°. The preliminary melt could be eliminated by recrystallizing from boiling ethanol–benzene or, better, from ethyl acetate. Found: $[\alpha]^{25}D$ −90.0° (*c* 1, H_2O), literature values[24] m.p. 161–162°, $[\alpha]^{18}D$ −90.5° (*c* 1.6, H_2O).

2-Naphthyl β-D-Glucopyranosiduronic Acid.—One gram (0.022 mole) of methyl (2-naphthyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate was suspended in 50 ml. of methanol. A homogeneous system was obtained on addition of 0.5 ml. of 0.5 *N* barium methoxide. The solution was refrigerated for 24 hr. and barium was then removed as barium sulfate by addition of the calculated amount of sulfuric acid. The salt was removed by filtration and the filtrate was evaporated to dryness in an air stream. The residue was crystallized from water to give 360 mg. (52.0%) of 2-naphthyl β-D-glucopyranosiduronic acid; m.p. 151.5–152°, $[\alpha]^{24}D$ −100° (*c* 1, ethanol); literature values[25] m.p. 149–150°, $[\alpha]^{22}D$ −97° (ethanol).

Methyl (Methyl Gentisyl β-D-Glucopyranosid)-uronate.—Two hundred mg. (0.42 mmole) of methyl (methyl gentisyl tri-*O*-acetyl-β-D-glucopyranosid)-uronate was dissolved in 20 ml. of methanol and 0.2 ml. of 0.5 *N* barium methoxide was added to the solution. The solution was refrigerated overnight and then treated with a calculated amount of sulfuric acid. Barium sulfate was removed by filtration and the filtrate concentrated to crystals. The crude material (120 mg., 82%) was recrystallized from ethanol at room temperature to constant melting point 180–181.5°, $[\alpha]^{28}D$ −83.4 (*c* 1.4, CH_3OH). *Anal.*[33] Calcd. for $C_{14}H_{18}O_{10}$: C, 50.28; H, 5.03; CH_3O, 17.32. Found: C, 50.60; H, 5.15; CH_3O, 17.16.

Methyl Glucofuranosidurono-γ-lactones from Glucuronic Acid and Methyl Glucuronate (III → IV, II → IV, Chart 1).—Twenty-five grams (0.129 mole) of glucuronic acid was stirred in 500 ml. of methanol with 12 g. of Nalcite HCR cation-exchange resin at room temperature for 24 hr. Analysis of the liquor by Schoorl's method indicated 13% reducibles as dextrose.[35] After filtration and washing resin with methanol, combined liquors were concentrated under reduced pressure and the resulting light yellow sirup crystallized spontaneously on addition of ethanol; yield 15.9 g. (65%) in two crops. The combined crops were recrystallized from ethanol to give 9.7 g. of prisms; m.p. 138–140°, $[\alpha]^{25}D$ −55.0° (*c* 1, H_2O) and giving no depression of melting point with methyl β-D-glucofuranosidurono-γ-lactone.[35–37] Needles obtained from mother liquor, m.p. 148°; $[\alpha]^{25}D$ +147.0° (*c* 1, H_2O) gave no depression of melting point with methyl α-D-glucofuranosidurono-γ-lactone.[35,36] When the reaction was interrupted at 16 hr. a 50% yield of crystalline methyl α- and methyl β-D-glucofuranosidurono-γ-lactone was obtained. Acetylation of the sirup obtained from mother liquors yielded 5% of methyl tetra-*O*-acetyl-β-D-glucopyranuronate; m.p. 176°, $[\alpha]^{25}D$ +8.50° (*c* 1, $CHCl_3$) (III → II, Chart 1).

Methyl glucuronate (11.8 g., 0.0567 mole) was similarly treated with methanol in the presence of Nalcite HCR; 3.6 g. of methyl α- and methyl β-D-glucofuranosidurono-γ-lactones (33%) were isolated in crystalline form. As a control, methyl glucuronate from the same batch was acetylated with pyridine–acetic anhydride and the crystalline acetylated esters were isolated in 90% yield.

Reaction IV → V is included in Chart 1 to complete the known reactions in this series.[37]

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ARGO, ILLINOIS

[CONTRIBUTION FROM THE CEREAL CROPS SECTION, NORTHERN UTILIZATION RESEARCH BRANCH[1]]

Preparation of Panose by the Action of NRRL B-512 Dextransucrase on a Sucrose–Maltose Mixture[2]

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A new method is presented for the preparation of panose involving the action of culture filtrates of *Leuconostoc mesenteroides* NRRL B-512, containing dextransucrase, upon a sucrose–maltose mixture. A preliminary study of the effects of variations in carbohydrate and enzyme concentration and in reaction temperature provided a basis for selection of suitable conditions for limiting the reaction mainly to panose formation. The most important variable was found to be the ratio of maltose to sucrose, while the total carbohydrate concentration had a smaller but significant effect. The preparative procedure developed included separation of the synthesized panose from the yeast-treated reaction mixture by chromatography on a carbon–Celite[3] column. The resulting product crystallized readily and recrystallization did not change its properties.

Panose, a 4-α-isomaltopyranosyl-D-glucose, was crystallized first by Pan[4] as a product of the transglucosylation action of an *Aspergillus niger* culture filtrate on maltose. From a preparative standpoint this method had the disadvantage that the culture filtrate had to be freshly prepared in order to avoid marked decreases in the yield of panose. We present here an alternative method for preparation of panose which avoids this difficulty and which yields a crystalline product that does not require recrystallization.

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(2) Presented at the Midwest Regional Meeting of the American Chemical Society, Omaha, Nebr., November, 1954.

(3) Mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

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