THE ACID HYDROLYSIS OF GLYCOSIDES

II. EFFECT OF SUBSTITUENTS AT C-5

T. E. TIMELL, W. ENTERMAN, F. SPENCER, AND E. J. SOLTES
Department of Forest Chemistry, State University College of Forestry, Syracuse, New York
Department of Chemistry, McGill University, Montreal, Que.
and
The Pulp and Paper Research Institute of Canada, Montreal, Que.

Received March 5, 1965

ABSTRACT

First-order rate coefficients at three temperatures, and energies and entropies of activation, have been determined for the acid-catalyzed hydrolysis of methyl glucopyranosides containing various substituents at C-5 and for glycopyranosiduronic acids with different aglycones. Substitution at C-5 increased the stability towards acids of methyl α- and β-D-glucopyranosides, but there was no correlation between either the polarity or the size of the substituent and the rates of hydrolysis. The operation of either an inductive or a conformational effect alone was accordingly deemed unlikely.

Methyl α- and β-D-glucopyranosiduronic acids and methyl α-D-galactopyranosiduronic acid were only slightly more stable towards acids than the glycoside analogs, while benzyl β-D-glucopyranosiduronic acid was three times more stable. The presence of a methyl ester group at the carboxyl function increased the stability of the glycuronide bond. Isopropyl, *n*-butyl, isobutyl, and neopentyl β-D-glucopyranosiduronic acids were hydrolyzed approximately twice and cyclohexyl β-D-glucopyranosiduronic acid five times as fast as the corresponding glucosides. This appears to be the first time that glycuronides have been found to be hydrolyzed by dilute acid at a higher rate than their glycoside analogs.

The energies and, especially, the entropies of activation were, throughout, lower for the glycuronides than for the glycosides. The difference in entropy suggests that the two classes of compounds are hydrolyzed by different mechanisms.

INTRODUCTION

The mechanism of the acid hydrolysis of glycosides containing a carboxylic acid group at C-5 (glycopyranosiduronic acids, glycuronides) has attracted considerable attention in recent years, partly, no doubt, because polysaccharides with uronic acid residues are common in nature.

The glycuronide linkages have, so far, always been found to be hydrolyzed by moderately concentrated acids at a lower rate than the corresponding glycosidic bonds. Several investigators have attributed this to an inductive effect of the electron-attracting carboxyl group (1–8). Two alternatives are possible in the case of a glycopyranosiduronic acid. Either the polar effect is transmitted via one oxygen and two carbon atoms, which does not appear very likely, or, as has recently been suggested (8), the electrons at the ring oxygen are pulled by the carboxyl groups towards C-5, thus decreasing the ability of the ring oxygen to release electrons to the exocyclic oxygen. It is interesting in this connection to note that xylothiapyranosides are hydrolyzed faster than their oxygen analogs, apparently because of the ability of the less electronegative sulfur to furnish electrons to the glycosidic oxygen (9).

Alternative explanations for the stability of the glycuronide linkage have also been offered. Easty (10) noted that both the energy and the entropy of activation were lower for the acid hydrolysis of methyl α-D-glucopyranosiduronic acid as compared to methyl

α-D-glucopyranoside.[1] This was not considered compatible with the operation of an inductive effect, and Easty concluded that the mechanisms by which the two compounds hydrolyzed probably differed in some unknown manner. McKee and Dickey (11) have recently suggested that the entire uronic acid residue but not the carboxyl group, as such, stabilizes the xylosidic bond in *O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylose. A conformational rather than a polar effect was accordingly assumed to operate.

In a previous paper in this series, the general conditions for the acid-catalyzed hydrolysis of glycosides were discussed, as well as the influence of the nature of the aglycone (12). The present investigation is concerned with the influence on the rate parameters of substituents at C-5, and especially with the acid hydrolysis of glycopyranosiduronic acids. A brief account of some preliminary results has been given elsewhere (13).

RESULTS AND DISCUSSION

Hydrolyses were carried out with 0.05 *M* glycoside solutions in 0.5 *M* aqueous sulfuric acid. The extent of hydrolysis was determined polarimetrically. Kinetic parameters and deviations from the mean were calculated as described previously (12). All compounds studied were chromatographically pure, and all except five were crystalline.

TABLE I

Rate coefficients and kinetic parameters for the hydrolysis of methyl α-D-glucopyranosides with different substituents at C-5 (0.05 *M*) in 0.5 *M* sulfuric acid

Compound	$k \times 10^{-6}$ s^{-1}			*E*, kcal mole^{-1}	$\Delta S^{\ddagger}$ at 60 °C, cal deg^{-1} mole^{-1}
	60 °C	70 °C	80 °C		
Methyl α-D-xyloside	3.06	13.9	57.9	33.4	+14.9
Methyl 6-deoxy-α-D-glucoside	3.22	14.4	61.8	34.6	+18.6
Methyl α-D-glucoside	0.637	2.85	12.6	35.1	+16.9
Methyl α-D-glucuronide	0.572	1.93	7.41	30.2	+2.0
Methyl 6-deoxy-6-chloro-α-D-glucoside	0.092	0.441	1.92	35.7	+14.9
Methyl 6-deoxy-6-iodo-α-D-glucoside	0.099	0.445	1.82	34.2	+10.5
Methyl 6-amino-6-deoxy-α-D-glucoside	0.065	0.284	1.04	33.0	+6.1
Methyl 6-*O*-methyl-α-D-glucoside	0.449	1.90	8.52	34.2	+13.5

Kinetic data for a series of eight methyl α-D-glycopyranosides carrying different substituents at C-5 are presented in Table I. The greater stability of the glucoside as compared to the xyloside is well known (14). The stability towards acids of the 6-iodo- and the 6-amino glucosides has also been noted previously (15, 16). In view of the postulated inductive effect of the carboxyl group, it is of interest to compare the electron affinity of the C-5 substituents with their rate coefficients. Such a comparison is seen in Table II, where the rate constant at 60° and Taft's (17) polar substituent constant σ^* are used as parameters.

There is obviously little or no correlation between the polarity of the C-5 substituents and the rate coefficients, an inverse correlation being expected if the inductive effect alone were operating. The carboxymethyl and the chloromethyl groups, for example, have the

[1]*In Part I of this series (Table III), the rate coefficient reported by D. B. Easty in ref. 10 was erroneously stated to be 6.79 × 10^{-6} s^{-1}, when it was assumed that the original value was given in min^{-1} and 10-logarithms, while actually min^{-1} and natural logarithms had been used. The correct value, 2.95 × 10^{-6} s^{-1} is in excellent agreement with the corresponding value found in these studies, namely 2.85 × 10^{-6} s^{-1}. The authors are thankful to Dr. Alexander Meller, Australian Paper Manufacturers Limited, Melbourne, Australia, for drawing his attention to this error.*

TABLE II

Rate coefficients at 60 °C and polar substituent constants (σ^*) for some C-5-substituted methyl α-D-glucopyranosides

Substituent	$k \times 10^{-6}$ s^{-1}	σ^*
—CH_2CO_2H	0.572	+1.05
—CH_2Cl	0.092	+1.05
—CH_2I	0.099	+0.85
—CH_2OH	0.637	+0.56
—CH_2OCH_3	0.449	+0.52

same σ^* -values, yet the glucuronide was hydrolyzed more than six times faster than the 6-deoxy-6-chloro compound. The glucoside and the 6-*O*-methylglucoside were both hydrolyzed at approximately the same rate as the glucuronide, yet the σ^* -value for the hydroxymethyl and methoxymethyl groups is only about half of that of the carboxymethyl substituent. It is obvious that in these cases an inductive effect is not the only factor involved. The stability of the 6-deoxy-6-amino compound could be at least partly due to the electrostatic shielding effect of the positive ammonium ion present in acid solution.

TABLE III

Rate coefficients and kinetic parameters for the hydrolysis of methyl β-D-glucopyranosides with different substituents at C-5 (0.05 *M*) in 0.5 *M* sulfuric acid

Compound	$k \times 10^{-6}$ s^{-1}			E	$\Delta S\ddagger$
	60 °C	70 °C	80 °C		
Methyl β-D-xyloside	6.56	28.6	121	33.9	+17.9
Methyl β-D-glucoside	1.38	6.25	24.1	32.5	+10.6
Methyl β-D-glucuronide	1.16	4.14	14.4	29.3	+0.8
Methyl 6-*O*-methyl-β-D-glucoside	0.840	3.88	16.1	34.9	+16.8
Methyl 6-*O*-ethyl-β-D-glucoside	1.01	4.22	18.2	34.4	+15.8
Methyl 6-*O*-isopropyl-β-D-glucoside	1.11	5.08	20.6	34.8	+17.1

Corresponding data obtained for a series of six methyl β-D-glucopyranosides are given in Table III. Again, the difference in rate of hydrolysis between the glucuronide and the glucoside is quite small. All three 6-*O*-alkyl glucosides were hydrolyzed more slowly than the glucuronide.

There can be little doubt that some of the present results can be more rationally explained in terms of conformational rather than of polar effects. As first pointed out by Edward (14), the carbonium–oxonium ion present as an intermediate in the hydrolysis will tend to assume a half-chair conformation, a transformation which will be hindered by any substituent at C-5 relative to C-4. Rates of hydrolysis should thus decrease with increasing size of a C-5 substituent. This would explain the well-known increase in stability towards acids in the series pentosides, hexosides, and heptosides. It is evident, however, from the data in Tables I and III that this conformational effect cannot be adduced to explain all of the present results. The C-5 substituents are not of the same size in the 6-deoxy-6-chloro and 6-deoxy-6-iodo compounds, yet their rates of hydrolysis are almost the same. The methyl 6-deoxy-α-D-glucoside is hydrolyzed as rapidly as the methyl α-D-xyloside, which is also unexpected. The increase in rate of hydrolysis is less than the increase in size of the C-5 substituents in the series 6-*O*-methyl, 6-*O*-ethyl, and 6-*O*-isopropyl β-D-glucosides.

With a view of obtaining more information on this point, a number of methyl α- and β-D-glycopyranosiduronic acids were synthesized, and their kinetic parameters were compared with those previously obtained for their glycopyranoside analogs. Similar data were also established for a series of seven β-D-glucopyranosiduronic acids containing different aglycones. The results are presented in Tables IV and V.

TABLE IV

Rate coefficients and kinetic parameters for the hydrolysis of methyl glycopyranosiduronic acids and the corresponding glycopyranosides (0.05 *M*) in 0.5 *M* sulfuric acid

Compound	$k \times 10^{-6}$ s^{-1}			E	$\Delta S‡$
	60 °C	70 °C	80 °C		
Methyl α-D-galactoside	3.04	13.6	56.4	34.0	+16.7
Methyl α-D-galacturonide	3.01	13.3	43.0	31.1	+8.0
Methyl α-D-galacturonide*	2.87	10.7	38.6	30.3	+5.3
Methyl β-D-galactoside	5.87	23.3	94.4	32.0	+12.0
Methyl β-D-galacturonide	0.852	3.50	12.8	32.2	+8.8
Methyl α-D-mannoside	1.45	6.56	28.9	34.7	+17.3
Methyl α-D-mannuronide	3.72	9.10	28.4	31.0	+8.1

*Using the methyl ester as starting material.

TABLE V

Rate coefficients and kinetic parameters for the hydrolysis of six β-D-glucopyranosiduronic acids and the corresponding β-D-glucopyranosides (0.05 *M*) in 0.5 *M* sulfuric acid

Compound	$k \times 10^{-6}$ s^{-1}			E	$\Delta S‡$
	60 °C	70 °C	80 °C		
Isopropyl β-D-glucoside	2.65	11.3	44.5	33.2	+14.0
Isopropyl β-D-glucuronide	4.87	17.7	52.6	28.3	+0.5
n-Butyl β-D-glucoside	1.62	6.98	27.9	33.6	+14.3
n-Butyl β-D-glucuronide	4.20	14.4	49.2	29.3	+3.3
Isobutyl β-D-glucoside	1.90	8.21	34.4	33.8	+15.2
Isobutyl β-D-glucuronide	3.74	14.9	51.4	29.5	+3.7
Neopentyl β-D-glucoside	2.41	10.6	39.9	36.2	+22.9
Neopentyl β-D-glucuronide	4.65	16.9	57.2	30.0	+5.6
Cyclohexyl β-D-glucoside	3.25	13.9	56.4	33.5	+15.3
Cyclohexyl β-D-glucuronide	18.5	63.7	217	28.7	+4.5
Benzyl β-D-glucoside	1.56	6.75	28.3	33.6	+14.2
Benzyl β-D-glucuronide	0.587	2.29	8.44	30.9	+4.1

The methyl α-D-glucuronide and methyl α-D-galacturonide were hydrolyzed at a rate which was only slightly lower than that of the corresponding neutral glycosides, which is in agreement with the previous observations of Morell and Link (18), Nakano and Rånby (7), and Easty (10). The same applies to the methyl β-D-glucuronide, while the methyl β-D-galacturonide was hydrolyzed approximately seven times more slowly than the galactoside. The methyl α-D-mannuronide was hydrolyzed 2.5 times faster than the mannoside.

In the series of β-D-glucuronides, the benzyl β-D-glucuronide was approximately three times more stable than the corresponding glucoside. The isopropyl *n*-butyl, isobutyl, and neopentyl compounds were all hydrolyzed about twice as fast as their glucoside analogs, while the cyclohexyl β-D-glucuronide reacted five times more rapidly than the β-D-glucoside. The fact that certain glucuronides are less stable towards dilute acids than their neutral analogs does not seem to have been observed previously. Nakano and Rånby (7) noted that methyl α- and β-D-glucopyranosiduronic acids were both hydrolyzed more

rapidly in 81% phosphoric acid than the corresponding glucopyranosides. The activation energies were unusually low, however, and it was also found that the acid solutions quickly became colored, indicating the occurrence of secondary reactions.

During the acid hydrolysis of methyl esters of glycuronides the ester groups are rapidly eliminated. When the hydrolysis of methyl (methyl α-D-galactosid) uronate was followed with the aid of thin-layer chromatography, the results showed that ester linkage was hydrolyzed at a much higher rate than the galacturonide bond. The rate coefficients increased gradually during the initial stage of the reaction, indicating that the methyl ester group stabilized the galacturonide. After this period, the rate coefficients became constant and were those of methyl α-D-galacturonide, as can be seen from Table IV. Similar results were obtained with methyl (methyl β-D-galactosid) uronate and methyl (methyl β-D-glucosid) uronate. This is in agreement with the data of Dyer, Glaudemans, Koch, and Marchessault (6), who have shown that ethyl tetrahydro-6-methoxypyran-2-carboxylate is hydrolyzed in 0.001 *N* hydrochloric acid at 30 °C 7.4 times faster than is tetrahydro 6-methoxypyran-2-carboxylic acid. In this case, the extremely labile nature of the 6-methoxy group made it possible to establish quantitatively the effect of the ester group. The electron affinity of an ester group is greater than that of a carbylate ion, and the above investigators attributed the stabilization exerted by the ethyl ester group to an inductive effect. It seems more likely, however, that also in this case conformational factors are involved.

Inspection of the data in Tables I and III shows that nearly all values for the activation energy of the neutral glycosides fall within the range of 33 to 35 kcal $mole^{-1}$. The entropy of activation of the neutral glycosides ranges from +10 to +18 cal deg^{-1} $mole^{-1}$, indicating that the reaction is unimolecular (19). With the single exception of the β-D-galacturonide, all glycuronides and the methyl 6-amino-6-deoxy-α-D-glucoside have energies of activation which are 3 to 5 kcal $mole^{-1}$ lower than those of their neutral analogs, and the entropies of activation are in the range of +0.5 to +8 cal deg^{-1} $mole^{-1}$.

It is clear that the lower rate coefficients of the methyl and benzyl glucuronides and of the methyl galacturonides are entirely due to their lower entropies of activation. Differences in entropies of activation for the acid hydrolysis of esters, acetals, and lactones have previously been found to be associated with differences in reaction mechanisms (20). Whether the present glycosides and glycuronides differ in this respect cannot be decided on the basis of the evidence now available.

The energies of activation of the larger alkyl and the cyclohexyl β-D-glycuronides are 1 to 2 kcal $mole^{-1}$ lower than those of the methyl glycuronides. It is possible that this decrease in activation energy is sufficient to overcome the influence of the low entropy values, thus causing an increase rather than a decrease in rate of hydrolysis. It is interesting to note that these aglycones are all groups with a low electron affinity, while the benzyl group is electron-attracting. Semke, Thompson, and Williams (8) have recently reported that the phenyl, *p*-cresyl, and *p*-chlorophenyl β-D-glucopyranosiduronic acids, all compounds with an electron attracting aglycone, are hydrolyzed 16, 19, and 13 times more slowly, respectively, at 95 °C than the corresponding glycosides. In this case both glucuronides and glucosides had entropies of activation of +10 cal deg^{-1} $mole^{-1}$, and the authors concluded that both classes of compounds were hydrolyzed by the same mechanism.

CONCLUSIONS

The present data indicate that the effect of a carboxyl substituent at C-5 in a glycopyranosiduronic acid, whether deactivating or activating, is probably due to neither polar nor

conformational factors alone. Instead, both effects might be operative, each contributing to a different extent under different conditions. A reaction mechanism other than that by which most neutral glycosides are hydrolyzed might, however, also be involved. In view of their conspicuously low entropies of activation it is, for example, possible that glycopyranosiduronic acids might be at least partially hydrolyzed by a bimolecular reaction mechanism, which would then involve the participation of a mole of water (19, 20).

A more definite rationalization of the present experimental evidence does not appear feasible at the moment. More data for glycopyranosiduronic acids containing aglycones with different steric and polar characteristics would be particularly desirable. Investigations with such compounds are now in progress.

EXPERIMENTAL

Most experimental conditions were the same as in the previous study in this series (12). One-third of the polarimetric measurements were carried out at 436 mμ with a Perkin–Elmer model 141 photoelectric polarimeter.

Thin-layer chromatography was carried out with Silica Gel H and pyridine – ethyl acetate – acetic acid – water (5:5:1:3). One set of chromatograms was sprayed with 72% sulfuric acid and the other with *o*-aminobiphenyl. After hydrolysis for 6 h at 60 °C, methyl (methyl α-D-galactosid) uronate had been completely replaced by methyl α-D-galacturonide. Galacturonic acid was observed after 24 h and onward. Data for the hydrolysis of methyl (methyl α-D-galactosid) uronate at 60 °C are found in Table VI.

TABLE VI

Kinetic data for the hydrolysis of methyl (methyl α-D-galactosid) uronate (0.05 *M*) in 0.5 *M* sulfuric acid at 60 °C

Time, h	0	12	24	30	54	78	96		
α_{405}, degrees	+3.515	3.365	3.145	3.025	2.625	2.340	2.145		
$k \times 10^4$ min^{-1}	—	0.402	0.523	0.571	0.643	0.652	0.669		
Time, h	108	120	126	144	150	168	179	180	∞
α_{405}, degrees	2.075	1.980	1.910	1.815	1.775	1.168	1.660	1.620	1.330
$k \times 10^4$ min^{-1}	0.645	0.649	0.672	0.658	0.664	0.669	0.663	0.677	—

Mean value: 0.656×10^{-4} min^{-1}, 2.52×10^{-6} s^{-1} (excluding the first three values)
Mean deviation: 2.3%

MATERIALS

Methyl 6-Deoxy-β-D-glucopyranoside

Methyl α-D-glucopyranoside was treated with *p*-toluenesulfonyl chloride in pyridine (16, 21), giving methyl 6-*O*-*p*-toluenesulfonyl-α-D-glucopyranoside, m.p. 122–124 °C and $[\alpha]_D$ +95° (*c*, 1.0 in water) (22). The compound was reduced with lithium aluminium hydride in tetrahydrofuran (23, 24) to give methyl 6-deoxy-α-D-glucopyranoside (methyl α-D-quinovoside) with m.p. 96-98° and $[\alpha]_D$ +145° (*c*, 1.0 in water) (25).

Methyl 6-Amino-6-deoxy-α-D-glucopyranoside

This compound was prepared from methyl 6-*O*-*p*-toluenesulfonyl-α-D-glucopyranoside by treatment with methanolic ammonia at 120° (22). The hydrochloride of methyl 6-amino-6-deoxy-α-D-glucopyranoside melted with decomposition at 180–190° and had $[\alpha]_D$ +136° (*c*, 1.0 in water) (16).

Methyl 6-Chloro-6-deoxy-α-D-glucopyranoside

This compound was prepared by a combination of the methods reported by Helferich and his co-workers (26, 27). Methyl 2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-glucopyranoside on treatment with phosphorus pentachloride and subsequent removal of the trityl group with hydrogen bromide in acetic acid gave methyl 2,3,4-tri-*O*-acetyl-6-chloro-6-deoxy-α-D-glucopyranoside. Saponification with aqueous barium hydroxide yielded methyl 6-chloro-6-deoxy-α-D-glucopyranoside with m.p. 108–110° and $[\alpha]_D$ +141° (*c*, 1.0 in water) (25, 26).

Methyl 6-Deoxy-6-iodo-α-D-glucopyranoside

The method of Compton (21) was followed. Methyl 6-*O*-*p*-toluenesulfonyl-α-D-glucopyranoside on acetylation in pyridine gave methyl 2,3,4-tri-*O*-acetyl-6-*O*-*p*-toluenesulfonyl-α-D-glucopyranoside in a yield of 41%, m.p. 169–170° and $[\alpha]_D$ +7.4° (*c*, 3.7 in chloroform). Treatment with sodium iodide in boiling cyclopentanone gave methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-iodo-α-D-glucopyranoside in a yield of 70%, m.p. 148.5–149.5° and

$[\alpha]_D$ +111° (*c*, 1.3 in chloroform) (15, 27). The compound was treated with alcoholic hydrochloric acid and crystallized from ethanol – ethyl acetate to give methyl 6-deoxy-6-iodo-α-D-glucopyranoside in a yield of 50%, m.p. 136–137° and $[\alpha]$ +102° (*c*, 1.0 in water).

Methyl 6-O-Methyl-α-D-glucopyranoside

This compound was prepared essentially as described by Helferich and co-workers (25, 28), namely by methylation, according to the method of Kuhn, Trischmann, and Löw (29), of methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside. The crystalline compound had m.p. 146–149° and $[\alpha]_D$ +143.5° (*c*, 1.0 in water).

Anal. Calcd. for $C_8H_{14}O_6$: OCH_3, 29.8. Found: OCH_3, 29.1.

Methyl 6-O-Methyl-β-D-glucopyranoside

1,2; 3,5-Di-*O*-methylene-D-glucofuranose (14 g, m.p. 57–58°, $[\alpha]_D$ +51° in water) (30) was methylated with methyl iodide (100 ml) and silver oxide for 24 h at room temperature. After addition of chloroform, filtration through Celite, treatment with Darco G-60 charcoal, and concentration, a sirup (13 g) was obtained which could not be induced to crystallize; $[\alpha]_D$ +52° (*c*, 1.4 in water).

Anal. Calcd. for $C_9H_{14}O_6$: OCH_3, 14.2. Found: OCH_3, 14.3.

The 6-*O*-methyl bismethylene glucose was refluxed with *N* sulfuric acid (400 ml) for 5 h. After neutralization with barium carbonate and treatment with Amberlite IR-120 exchange resin, an aqueous solution of the reaction mixture was extracted with chloroform. The water layer was concentrated to a sirup. Crystallization from ethanol gave 6-*O*-methyl-D-glucose (5.5 g), m.p. 141–143.5° and $[\alpha]$ +55° (*c*, 1.0 in water) (31, 32).

6-*O*-Methyl-D-glucose (4.0 g) was acetylated in the usual way with hot acetic anhydride in the presence of anhydrous sodium acetate. Yield of crude, crystalline product: 8.1 g. After recrystallization from absolute ethanol the 1,2,3,4-tetra-*O*-acetyl-6-*O*-methyl-β-D-glucose (5.1 g) had m.p. 92–94° and $[\alpha]_D$ +20° (*c*, 1.0 in chloroform) (32, 33). The compound (5.0 g) was treated with glacial acetic acid (30 ml), saturated at 0° with hydrogen bromide to give the sirupy α-acetobromo compound (6.4 g) which was not characterized.

The 6-*O*-methyl acetobromoglucose (6.0 g) was dissolved in anhydrous methanol (50 ml) and shaken overnight with silver oxide (6.0 g) in the presence of some Drierite and iodine. After filtration through Celite, treatment with charcoal, and concentration, the reaction product was recrystallized from ethyl ether – petroleum ether to give 4.3 g of methyl 2,3,4-tri-*O*-acetyl-6-*O*-methyl-β-D-glucopyranoside, m.p. 103–105° and $[\alpha]_D$ −14.8° (*c*, 1.0 in chloroform) (32, 33). The compound (4.2 g) was deacetylated with anhydrous methanol (150 ml) containing sodium methoxide (1 g). After 2 h at room temperature, sodium ions were eliminated with Amberlite IR-120, and the solution was decolorized with charcoal and concentrated to dryness, yielding 2.9 g of a crude product which was crystallized from ethyl acetate. The methyl 6-*O*-methyl-β-D-glucopyranoside (1.5 g) had m.p. 133–135° and $[\alpha]_D$ −27.8° (*c*, 1.0 in water).

Anal. Calcd. for $C_8H_{16}O_6$: OCH_3; 29.9. Found: OCH_3, 30.5.

Methyl 6-O-Ethyl-β-D-glucopyranoside

Bismethylene glucose (14 g) was treated with ethyl iodide and silver oxide in the presence of dimethyl formamide (29). The sirupy product obtained (9.0 g) gave an infrared diagram with no hydroxyl band. Hydrolysis with *N* sulfuric acid yielded, after crystallization from absolute ethanol, 6-*O*-ethyl-D-glucopyranose (7.9 g), m.p. 163–167° and $[\alpha]_D$ −51.6° (*c*, 1.3 in water). The product (7.5 g) was acetylated with hot acetic anhydride in the presence of anhydrous sodium acetate, giving 1,2,3,4-tetra-*O*-acetyl-6-*O*-ethyl-β-D-glucose (12 g) as a sirup. After deacetylation of a portion of the sirup (8 g) with methanol – sodium methoxide, contaminating glucose was removed by fermentation with baker's yeast. The 6-*O*-ethyl-D-glucopyranose, after crystallization from ethanol (5.0 g) had m.p. 166–168° and $[\alpha]_D$ −53° (*c*, 1.3 in water) (34). Another portion of the crude product (4 g) was converted to the acetobromo compound, which was condensed with methanol in the usual way, giving methyl 2,3,4-tri-*O*-acetyl-6-*O*-ethyl-β-D-glucoside (2.7 g), m.p. 102–102.5° and $[\alpha]_D$ +1.2° (*c*, 1.3 in chloroform). Deacetylation of 1.5 g of this material gave methyl 6-*O*-ethyl-β-D-glucopyranoside (0.8 g) with m.p. 125.5–127.5° and $[\alpha]$ −25.5° (*c*, 1.5 in water).

Methyl 6-O-Isopropyl-β-D-glucopyranoside

Bismethylene glucose (20 g) was dissolved in dimethyl formamide and treated with silver oxide and isopropyl iodide in the usual way, giving the 6-*O*-isopropyl compound (9 g). Hydrolysis with *N* sulfuric acid, removal of glucose with yeast, and crystallization from ethanol gave 6-*O*-isopropyl-D-glucose (4.9 g) m.p. 128–130° and $[\alpha]_D$ +49° (*c*, 1.1 in water) (34). The 1,2,3,4-tetra-*O*-acetyl-6-*O*-isopropyl-β-D-glucose, after recrystallization from ethanol had m.p. 120–121° and $[\alpha]_D$ +16.6° (*c*, 1.0 in chloroform).

Condensation of the acetobromo derivative (3.0 g) with methanol yielded a sirup (2.7 g) which crystallized from ethyl ether – petroleum ether (1.8 g). The methyl 2,3,4-tri-*O*-acetyl-6-*O*-isopropyl-β-D-glucoside had m.p. 57–59° and $[\alpha]_D$ +12.2° (*c*, 1.5 in chloroform). This compound (1.7 g) was deacetylated in the usual way. After crystallization from ethyl acetate – petroleum ether, methyl 6-*O*-isopropyl-β-D-glucopyranoside (1.0 g) had m.p. 83.5–84° and $[\alpha]_D$ −24.1° (*c*, 1.0 in water).

Methyl α-D-Glucopyranosiduronic Acid

Methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (15 g) was oxidized with potassium permanganate (16 g) in a solution consisting of 150 ml each of acetone, glacial acetic acid, and water (35). After 3 days at room temperature, the solution was treated with potassium sulfite and extracted with chloroform to yield

11.3 g of a crude product. Benzoyl groups were eliminated by treatment with methanol and sodium methylate, and the methyl benzoate formed was removed by extraction with chloroform. The crude uronic acid was purified by adsorption on a column containing Dowex 1-X4 (acetate form) exchange resin. Neither the free acid nor the potassium salt of methyl α-D-glucopyranosiduronic acid could be induced to crystallize. The chromatographically pure product had $[\alpha]_D$ +129° (*c*, 1.0 in water).

Anal. Calcd. for $C_7H_{12}O_7$: OCH_3, 14.9. Found: OCH_3, 14.6.

Methyl β-D-Glucopyranosiduronic Acid

Methyl 1,2,3,4-tetra-*O*-acetyl-α-D-glucuronate and the corresponding β-anomer were prepared as described by Bollenback and co-workers (36). The α-compound had m.p. 111–112° and $[\alpha]_D$ +98° (*c*, 1.0 in chloroform). The β-anomer had m.p. 177–178° and $[\alpha]_D$ +8.6° (*c*, 1.0 in chloroform) (37, 38). Methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate was obtained in a yield of 82% with m.p. 104–105.5° and $[\alpha]_D$ +198° (*c*, 1.0 in chloroform) (36, 38).

The acetobromo sugar (18 g) was dissolved in methanol (300 ml), and the solution was shaken in the dark with silver carbonate (20 g) in the presence of Drierite (10 g) and some iodine. After 40 h at room temperature, the solids were removed by filtration, and the methanol solution was decolorized with charcoal and evaporated to dryness. After recrystallization from ethanol, the methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid) uronate (10.5 g) had m.p. 153–155° and $[\alpha]_D$ −30° (*c*, 1.0 in chloroform) (39). A portion of the product (6.2 g) was treated with barium hydroxide (14 g) in water (100 ml) at 60° for 3 h. The reaction mixture was neutralized with carbon dioxide, filtered through Celite, treated with charcoal and Amberlite IR-120, and evaporated to dryness. The methyl β-D-glucopyranosiduronic acid was crystallized from acetone containing some methanol to give 3.1 g, m.p. 68–70° and $[\alpha]_D$ −55.3° (*c*, 1.0 in water).

Anal. Calcd. for $C_7H_{12}O_7$: OCH_3, 14.9. Found: OCH_3, 14.9.

Methyl (Methyl β-D-Glucopyranosid) Uronate

This compound was obtained by treating methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid) uronate with methanol – sodium methylate, followed by re-esterification with diazomethane. It had $[\alpha]_D$ −45° (*c*, 1.0 in water).

Anal. Calcd. for $C_8H_{14}O_7$: OCH_3, 27.9. Found: OCH_3, 26.0.

Methyl α-D-Galactopyranosiduronic Acid

This compound was prepared from D-galacturonic acid in a yield of 50%, essentially as described by Jones and Stacey (40). After two recrystallizations from ethanol, the methyl α-D-galactopyranosiduronic acid sintered at 88–90° and melted at 107–108°. It had $[\alpha]$ +128° (*c*, 1.4 in water) (41).

Methyl (Methyl α-D-Galactopyranosid) Uronate

D-Galacturonic acid (30 g) was boiled under reflux for 15 h with 0.7 *N* methanolic hydrogen chloride (300 ml) in the presence of Drierite (20 g). After neutralization with silver carbonate, treatment with charcoal, and filtration through Celite, evaporation of the solution gave a pale yellow sirup which was dissolved in a minimum amount of boiling methanol. The crystals deposited on cooling to room temperature were recrystallized from hot methanol, giving methyl (methyl α-D-galactopyranosid) uronate (4.0 g) with m.p. and mixed m.p. 147–148 °C and $[\alpha]_D$ +128° (*c*, 2.0 in water) (42–44).

Anal. Calcd. for $C_8H_{14}O_7 \cdot H_2O$: OCH_3, 25.9. Found: OCH_3, 25.9.

Methyl β-D-Galactopyranosiduronic Acid

Methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosid) uronate, prepared from methanol and the acetobromo derivative of D-galacturonic acid, was saponified with barium hydroxide. The resulting methyl β-D-galactopyranosiduronic acid, after crystallization from acetone–chloroform, had m.p. 161–163° and $[\alpha]_D$ −40.2° (*c*, 1.1 in water) (42, 45).

Methyl (Methyl β-D-Galactopyranosid) Uronate

The above compound was obtained on deacetylation of the triacetate derivative with methanol – sodium methylate. It had $[\alpha]_D$ −27.5° (*c*, 1.0 in water).

Methyl α-D-Mannopyranosiduronic Acid

Methyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranosiduronic acid (46) was oxidized with potassium permanganate as described for the corresponding glucoside derivative (35). The methyl α-D-mannopyranosiduronic acid had m.p. 114–115° and $[\alpha]_D$ +57° (*c*, 1.4 in water) (42, 47). The potassium salt had $[\alpha]_D$ +52° (*c*, 1.0 in water) (42).

Anal. Calcd. for $C_7H_{11}O_7K$: K, 15.8. Found: K, 14.9.

Both the mannuronide and the derived mannuronic acid gave only one sharp peak on ion exchange resin chromatography (48).

Cyclohexyl β-D-Glucopyranosiduronic Acid

Methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate (12.1 g) (36) was added to a suspension of silver carbonate (10 g), Drierite (20 g), and a little iodine in cyclohexanol (150 ml). The mixture was shaken in the dark at room temperature for 24 h. The crystalline product was recovered in the usual way. After

recrystallization from 75 ml of absolute ethanol, the long needles of methyl (cyclohexyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid) uronate (10.6 g, 83%) had m.p. 140–141.5° and $[\alpha]_D$ −31.7° (*c*, 1.5 in chloroform) (49). Use of mercuric cyanide instead of silver carbonate, as suggested by Helferich and Berger (49), gave the same crystalline compound in a yield of 70%.

The above compound (10.3 g) was treated with barium hydroxide (20 g) in water (200 ml) at 60° for 3 h. The reaction product was recovered in the usual way and crystallized from ethanol to give cyclohexyl β-D-glucopyranosiduronic acid (4.0 g) with m.p. 168–170° and $[\alpha]$ −59° (*c*, 1.0 in water) (51).

Neopentyl β-D-Glucopyranosiduronic Acid[2]

Methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate (12.0 g) was condensed with neopentyl alcohol (20 g) in benzene (125 ml) in the presence of silver carbonate (10 g), Drierite (20 g), and some iodine. After it was shaken at room temperature for 24 h, the solution was recovered by filtration through Celite. Filtrate and washings (benzene) were evaporated to dryness, leaving a crystalline residue which was recrystallized from ethanol. The methyl (neopentyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid) uronate (11.0 g) had m.p. 153–154° and $[\alpha]_D$ −51.3° (*c*, 3.0 in chloroform). A portion of the product (10.0 g) was treated with barium hydroxide (22 g) in the usual way, giving neopentyl β-D-glucopyranosiduronic acid (5.1 g); m.p. 149–151° and $[\alpha]_D$ −51.3° (*c*, 3.0 in chloroform).

Isopropyl β-D-Glucopyranosiduronic Acid[2]

The methyl (isopropyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid) uronate, obtained as described above in a yield of 71%, had m.p. 143.5–145.5° and $[\alpha]$ −33.4° (*c*, 1.5 in chloroform). The isopropyl β-D-glucopyranosiduronic acid had $[\alpha]$ −45° (*c*, 1.0 in water). It could not be induced to crystallize.

n-Butyl β-D-Glucopyranosiduronic Acid[2]

The methyl (*n*-butyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid) uronate was obtained in a yield of 68% and had m.p. 92–93° and $[\alpha]_D$ −26.1° (*c*, 1.3 in chloroform). The sirupy *n*-butyl β-D-glucopyranosiduronic acid had $[\alpha]_D$ −44° (*c*, 1.0 in water).

Isobutyl β-D-Glucopyranosiduronic Acid[2]

The methyl (isobutyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid) uronate, obtained in a yield of 38%, had m.p. 138.5–139.5° and $[\alpha]_D$ −24.3° (*c*, 1.3 in chloroform). The isobutyl β-D-glucopyranosiduronic acid, which could not be induced to crystallize, had $[\alpha]_D$ −51.8° (*c*, 1.4 in water).

ACKNOWLEDGMENTS

The authors express their gratitude to Mr. N. Roy for the gift of methyl (methyl α-D-galactopyranosid) uronate and to Mr. B. W. Simson for technical assistance. This investigation was supported by a United States Public Health Service Research Grant (AM 07193 MCHA) from the National Institute of Arthritis and Metabolic Diseases, Public Health Service.

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[2]*These compounds are new.*

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