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THE ACID HYDROLYSIS OF GLYCOSIDES

V. EFFECT OF THE NATURE OF THE AGLYCON ON THE HYDROLYSIS OF GLYCOSIDURONIC ACIDS (GLYCURONIDES)

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INTRODUCTION

A carboxyl group at C-5 in the glycose moiety of a glycoside has long been known to stabilize the glycosidic linkage towards acids. Recently[1], it was observed that D-glucopyranosiduronic acids ("D-glucopyranuronides") containing an aglycon having a low electron-affinity are hydrolyzed more rapidly by acids than are the corresponding D-glucopyranosides. It was the object of the present investigation to adduce more evidence on this point. Several β-D-glucopyranosiduronic acids were accordingly synthesized, and their kinetic characteristics were established and compared with those of the corresponding β-D-glucopyranosides.

RESULTS

Fourteen β-D-glucopyranosiduronic acids were synthesized, most of them for the first time, by conventional methods. All intermediate peracetates, and all but three of the final β-D-glucopyranuronides, were obtained crystalline. Four β-D-glucopyranosides were also prepared. Hydrolysis was conducted as described earlier[1-3] in aqueous sulfuric acid at 60, 70, 80, and 90°; pseudo-first-order rate coefficients, and energies and entropies of activation, were determined from polarimetric measurements[1,2].

It is known that glycopyranosiduronic acids suffer decarboxylation when they are heated in acidic, aqueous solutions[4,5], a fact that renders measurements of the present type subject to considerable doubt. Easty[6] studied the acid-catalyzed hydrolysis of methyl α-D-glucopyranoside and methyl α-D-glucopyranosiduronic acid by determining the methanol produced on hydrolysis of these compounds. In the present series of investigations, similar measurements were made polarimetrically under the same conditions[1,2]. Comparison between the two sets of data revealed that all of the kinetic data were practically identical, indicating the accuracy of the polarimetric method, provided that measurements are made at the initial stage of the hydrolysis. Use of the Guggenheim[7] and Swinbourne[8] procedures, which do not require knowledge of the final optical rotation of the solution, also gave results in agreement with those afforded by the conventional method.

The kinetic data are presented in Table I, which also includes the ratio between the rate coefficients at 60° of the glucuronides and corresponding glucosides[1,2]. New data for four glucopyranosides are summarized in Table II. Standard deviations for the rate coefficients were, with a few exceptions, in the range of 1 to 4%; energies of activation were accurate within ±0.2 kcal. mole^{-1}.

TABLE I

RATE COEFFICIENTS AND KINETIC PARAMETERS FOR THE HYDROLYSIS OF β-D-GLUCOPYRANOSIDURONIC ACIDS IN 0.5M SULFURIC ACID

Aglycon	*$k \times 10^6$, sec^{-1}*			*E, kcal mole^{-1}*	*$\Delta S^\ddagger$ at 60° cal. deg.$^{-1}$ mole^{-1}*	*Ratio*[a]
	60°	*70°*	*80°*			
Ethyl	3.91	13.8	45.9	28.8	+1.5	2.54
Propyl	4.54	16.4	54.3	29.0	+2.5	2.50
Isopropyl	18.8	63.2	205.0	27.9	+2.1	7.10
Butyl	4.61	16.3	53.5	28.7	+1.5	2.84
Hexyl	4.80	16.9	54.0	28.3	+0.4	—
(2-Hydroxyethyl)	0.614	2.35	8.34	30.5	+3.0	0.355
(2-Methoxyethyl)	0.65	2.28	7.56	28.7	−2.3	0.355
(2-Chloroethyl)	0.183	0.716	2.62	31.1	+2.5	0.089
(3-Chloropropyl)	0.791	2.84	9.94	29.6	+0.8	—
(Carboxymethyl)	0.317	1.19	4.92	32.1	+6.4	0.084
(2-Carboxyethyl)	—	1.41	—	—	—	0.310[b]
Cyclopentyl	16.8	57.5	179.6	27.7	+1.1	5.42
Cyclohexyl	17.3	59.8	183.0	27.6	+0.8	5.32
Phenyl	0.57	2.37	9.23	32.6	+9.0	—

[a] $k_{\text{glucuronide}}/k_{\text{glucoside}}$ at 60°. [b] At 70°.

It had previously been found[1] that methyl α- and β-D-glucopyranosiduronic acid are hydrolyzed at rates that are only slightly lower than those of methyl α- and β-D-glucopyranoside. In the present series, ethyl, propyl, butyl, and hexyl glucuronides were all hydrolyzed at approximately the same rate as the isobutyl and neopentyl compounds studied previously[1], and 2 to 3 times faster than their glycoside analogs. The isopropyl, cyclopentyl, and cyclohexyl D-glucuronides exhibited rates that were four

TABLE II

RATE COEFFICIENTS AND KINETIC PARAMETERS FOR THE HYDROLYSIS OF FOUR D-GLUCOPYRANOSIDES IN 0.5M SULFURIC ACID

Aglycon	*$k \times 10^6$, sec^{-1}*			*E, kcal mole^{-1}*	*$\Delta S^\ddagger$ at 60°, cal. deg.$^{-1}$ mole^{-1}*
	60°	*70°*	*80°*		
(2-Chloroethyl)	2.06	8.61	33.7	32.7	+11.9
(Carboxymethyl)	—	14.2	53.1	31.7	+10.1
(Carboxyethyl)	—	4.58	—	—	—
Cyclopentyl[a]	—	10.6	39.7	33.1	+13.5

[a] $k = 152.6$ at 90°.

times those of the above glucuronides, and that were considerably greater than those of the corresponding glucosides. The (2-hydroxyethyl), (2-methoxyethyl), (3-chloropropyl), and phenyl glucuronides were hydrolyzed much more slowly. The (carboxymethyl), (2-carboxyethyl), and, especially, the (2-chloroethyl) glucuronides were even more stable towards-acid.

The energies of activation were all within the range of 28 to 32 kcal. mole^{-1}, and were lower for the more labile and higher for the more stable glucuronides. Most of the entropies of activation lay between +0.5 and +2.5 cal.deg.$^{-1}$mole^{-1} at 60°.

DISCUSSION

The unusual stability of many glucopyranosiduronic acids and of all aldobiouronic acids towards acid has been attributed by several investigators[9-14] to an inductive effect of the (electron-attracting) carboxyl group, whereas others[1,6,15] have objected to this interpretation, without, however, suggesting any alternative explanation. It is clear from the data in Table I that the polarity of the aglycon influences the rate of hydrolysis of glucopyranosiduronic acids. If ln (k/k_0) is plotted against σ^*, where k is the rate coefficient of a glucuronide, k_0 is the rate coefficient of methyl β-D-glucopyranosiduronic acid, and σ^* is Taft's[16] polar substituent constant, the relationships shown in Fig. 1 are obtained. Evidently, the glucopyranosiduronic acids are hydrolyzed at rates that are inversely proportional to the electron affinity of the aglycon. A corresponding plot for glucopyranosides (see Fig. 2) shows that the polarity of the aglycon does not influence the rate of hydrolysis of these compounds.

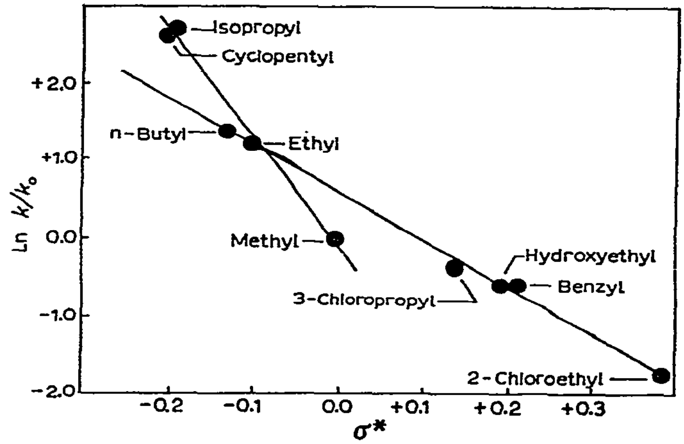

Fig. 1. Relation between the electron affinity of the aglycon and the rate of hydrolysis of β-D-glucopyranosiduronic acids.

The carboxymethyl group has a polar substituent constant of[16] +1.05. Inspection of Fig. 1 reveals that the expected rate of hydrolysis of the (carboxymethyl) β-D-glucopyranosiduronic acid should be approximately one hundredth of that actually observed. Capon[17] has shown that (*o*-carboxyphenyl) β-D-glucopyranoside is hydrolyzed at about 10^4 times the rate for the *p*-analog at a pH of 3.5, and attributed this finding to intramolecular catalysis by the *o*-carboxyl group. Similar arguments may be invoked in the present case to explain the high rate of hydrolysis of the (carboxymethyl) β-D-glucuronide, although the carboxyl group in this compound is probably less favorably situated. In the strongly acidic solution used here, the interaction would probably involve general acid-catalysis *via* the undissociated carboxyl group.

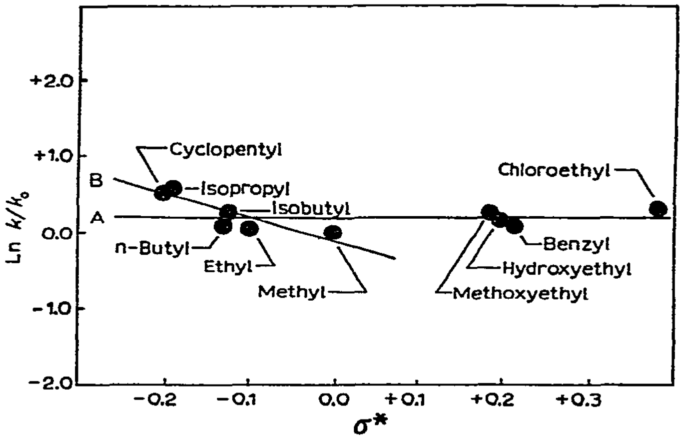

Fig. 2. Relation between the electron affinity of the aglycon and the rate of hydrolysis of β-D-glucopyranosides.

Another factor, besides the inductive effect of the aglycon, is apparent from Figs. 1 and 2, where a linear relationship is indicated between σ^* and ln (k/k_0) for the methyl, ethyl, and isopropyl glucuronides and glycosides. Evidently, the hydrolysis is facilitated by the substitution, in the aglycon, of alkyl groups for hydrogen atoms on the carbon atom adjacent to the glycosidic oxygen atom, probably because of repulsive interaction with the glycose residue. Substitution of all three hydrogen atoms would result in alkyl–oxygen fission and a much enhanced rate of hydrolysis[2,18]. Increase in bulk at other positions of the aglycon has little effect on the rate of hydrolysis[2].

Comparison of the data in Table I with those in Table II, and with those reported previously for other glucopyranosides[1,2,19], shows that energies of activation of the β-D-glucopyranosiduronic acids are 4 to 5 kcal.mole^{-1} lower than those of the corresponding β-D-glucopyranosides. Similarly, as observed by several investigators[1,6,20], albeit not by all[13], entropies of activation are consistently lower for the glucuronides, suggesting that the latter are more ordered in the transition state than are the glucosides.

The electron-attracting nature of the carboxyl group at C-5 undoubtedly lowers the electron density at the ring-oxygen atom, resulting in a destabilization of the intermediate carbonium ion, and hence a decrease in the rate of hydrolysis. Any conformational effect of the bulky carboxyl group would act in the same direction. As emphasized by Semke, Thompson, and Williams[13], the inductive effect of the carboxyl group will depend on the electron density prevailing at the actual site of reaction. Substitution, in the ring, of sulfur for (the more electronegative) oxygen results in an enhanced rate of hydrolysis[21,22]. This effect has been attributed by Whistler and Van Es[21] to the greater ability of the sulfur to release electrons to the glycosidic oxygen atom, thus increasing the extent of protonation.

It is possible that the heterolysis step in the hydrolysis of glycopyranosiduronic acids having aglycons of low electron-affinity is facilitated by interaction between the carboxyl group on C-5 and the glycosidic oxygen atom, resulting in an intramolecular, general-acid catalysis of the type suggested by Capon[17]. An electron-attracting aglycon would lower the electron density at the glycosidic oxygen atom, thus impeding protonation, and, perhaps, would also cause the carboxyl group to interact with the ring-oxygen atom, which would then become less effective in stabilizing the intermediate, carbonium-oxonium ion. These factors could be sufficiently significant to overcome the increased ease of heterolysis brought about by the flow of electrons towards the aglycon, resulting in a lessened overall rate of hydrolysis.

As may be seen from Table I, the effect of the nature of the aglycon is pronounced. At 60°, isopropyl β-D-glucopyranosiduronic acid, for example, is hydrolyzed 100 times faster than the (2-chloroethyl) analog. The stability of cyclohexyl β-D-glucopyranosiduronic acid is almost one thirtieth of that of the benzyl[1] and phenyl analogs.

EXPERIMENTAL

All melting points are corrected. The purity of the three noncrystalline compounds was checked by paper, and by gas–liquid, chromatography.

Synthesis of methyl (2-chloroethyl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate. — Drierite (30 g), freshly prepared[23] silver carbonate (60 g), 2-chloroethanol (100 g), dry benzene (200 g), and a small amount of iodine were stirred for one hour at room temperature with exclusion of moisture. Methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate[24], m.p. 105–106°, $[\alpha]_D^{25}$ +198° (chloroform), (40 g) was added, and stirring was continued for 36 h at room temperature. After the suspension had been filtered through Celite, and the solid had been washed with benzene, the filtrate and washings were combined, washed with water (to remove residual 2-chloroethanol), dried, filtered, and concentrated to a syrup that was dissolved in warm isopropyl alcohol (450 ml). The solution was cooled, to give crystals, 22.7 g (58%), m.p. 141–142°, $[\alpha]_D^{25}$ −23.9° (*c* 2.0, chloroform).

All other *O*-acetylated glycuronides were obtained crystalline in the same way. The yields, melting points, specific rotations, and analytical data are summarized in Table III.

Synthesis of 2-chloroethyl β-D-glucopyranosiduronic acid. — The above acetate (15 g) was suspended in anhydrous methanol (250 ml), and 0.1M methanolic sodium methoxide (250 ml) was added. The solution was kept at room temperature for 2 h, most of the methanol was removed, and water (250 ml) was added to saponify the methyl ester. Aqueous sodium hydroxide (30 ml) was added, and the solution was kept for 15 h at 4°. Sodium ions were removed with a cation-exchange resin, and the

TABLE III

EXPERIMENTAL AND ANALYTICAL DATA FOR THE METHYL (2,3,4-TRI-*O*-ACETYL-β-D-GLUCOPYRANOSID)-URONATES

Aglycon	*Yield, %*	*M.p., degrees*	$[\alpha]_D$, *degrees*	*Carbon, %*		*Hydrogen, %*	
				Calc.	*Found*	*Calc.*	*Found*
Ethyl	82	102–103	−21.0				
Propyl	82	108–109	−30.0	51.60	51.09	6.42	6.41
Isopropyl[25]	77	141–143	−36.7	51.06	50.44	6.42	6.38
Butyl[1]	77	89–90	−27.6	52.30	51.09	6.71	6.70
Hexyl	71	84–85	−31.1	54.53	53.86	7.23	7.33
(2-Hydroxyethyl)	86	162.5–163.5	−22.3	47.62	47.33	5.86	5.84
(2-Methoxyethyl)	81	105.5–106.5	−29.2	48.98	48.28	6.16	6.20
(2-Chloroethyl)[a,26]	58	141–142	−23.9	45.40	44.95	5.43	5.45
(3-Chloropropyl)[b]	54	128–129	−11.0	46.78	46.16	5.64	5.80
(Carboxymethyl)[c]	25	100–102	−49.3	47.29	47.01	5.46	5.47
(Carboxyethyl)[c]	61	86.5–87.5	−22.1	48.57	48.64	5.76	5.92
Cyclopentyl	80	132–132.5	−46.5				
Cyclohexyl[1,25]	83	140–141	−36.2				
Phenyl[13]	12	125–126.5	−29.6				

[a] Cl, Calc.: 8.94. Found: 9.16. [b] Cl, Calc.: 8.63. Found: 8.53. [c] Methyl ester.

solution was filtered, and concentrated to a syrup which was dissolved in ethyl acetate containing 10% of methanol. Crystals (5 g) formed at room temperature; m.p. 153–154°, $[\alpha]_D^{25}$ −47.5° (*c* 2.0, water).

The other β-D-glucopyranosiduronic acids were prepared in the same way; most of them were crystallized from ethyl acetate containing a small proportion of

TABLE IV

EXPERIMENTAL AND ANALYTICAL DATA FOR THE β-D-GLUCOPYRANOSIDURONIC ACIDS

Aglycon	*M.p., degrees*	$[\alpha]_D$, *degrees*	*Carbon, %*		*Hydrogen, %*	
			Calc.	*Found*	*Calc.*	*Found*
Ethyl	160–161	−64.4				
Propyl	125–127	−64.9	45.76	45.43	6.83	6.86
Isopropyl[1]	134–135	−66.6	45.76	45.00	6.83	6.85
Butyl[1]	102–104	−60.3	47.99	47.16	7.25	7.22
Hexyl	110–111	−55.3	51.79	51.02	7.97	8.05
(2-Hydroxyethyl)	87–89	−50.5	40.34	39.80	5.92	6.68
(2-Methoxyethyl)		−50.4				
(2-Chloroethyl)[a]	153–154	−47.5	37.44	37.51	5.10	5.34
(3-Chloropropyl)[b]	108–109	−54.1	39.93	40.72	5.59	5.71
(Carboxymethyl)		−65.9	38.00	38.97	4.80	5.39
(2-Carboxyethyl)		−31.0				
Cyclopentyl	124–126	−68.2				
Cyclohexyl[1,25]	164–165	−62.2				
Phenyl[13]	162–163	−88.1				

[a] Cl, Calc.: 13.82. Found: 13.70. [b] Cl, Calc.: 13.10. Found: 12.56.

ethanol or isopropyl alcohol. Melting points, specific rotations, and analytical data are listed in Table IV.

Synthesis of β-D-glucopyranosides. — The three β-D-glucopyranosides were prepared as described elsewhere[1,2]. Experimental and analytical data are presented in Table V. None of the glucosides were obtained crystalline.

TABLE V

EXPERIMENTAL AND ANALYTICAL DATA FOR THE 2,3,4,6-TETRA-*O*-ACETYL-β-D-GLUCOPYRANOSIDES AND THE β-D-GLUCOPYRANOSIDES

Aglycon	*Peracetate*			*Glucoside*
	Yield, %	*M.p., degrees*	$[\alpha]_D$, *degrees*	$[\alpha]_D$, *degrees*
(2-Chloroethyl)[2,27]	68	116–117	−10.6	−26.3
(Carboxymethyl)[2,28]	42	74–76[a]	−29.4[a]	−33.6
(2-Carboxyethyl)[2]	61	90–95[a]	−10.1[a]	−29.0

[a] Methyl ester.

Kinetic measurements. — Hydrolyses were conducted in 0.05M aqueous sulfuric acid at 60, 70, 80, or 90°, either in sealed ampoules or in a polarimeter tube; the concentration of sugar was 0.05M. Experiments with various concentrations of glycosides gave identical rate-coefficients, showing that the reaction was pseudo-first-order in the presence of an excess of acid. The extent of hydrolysis was determined, usually within the first 10% of conversion, with a Perkin–Elmer Model 141 photoelectric polarimeter. Owing to decomposition of the sugars, all data obtained beyond a conversion of 70% had to be discarded. Rate coefficients, energies and entropies of activation, and standard deviations were calculated with an IBM 1620 computer.

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SUMMARY

Rate coefficients and kinetic parameters have been determined for the acid-catalyzed hydrolysis of fourteen β-D-glucopyranosiduronic acids containing aglycons of different electron-affinity. Energies and entropies of activation of all of these compounds were lower than those of the corresponding β-D-glucopyranosides. The glucopyranosiduronic acids were hydrolyzed at rates that were inversely proportional to the electron affinity of the aglycon, whereas the rates of hydrolysis of the glucopyranosides were independent of the polarity of the aglycon. (Carboxymethyl) β-D-glucopyranosiduronic acid was hydrolyzed at one hundredth of the rate expected.

The phenomena are discussed, assuming a catalytic interaction between the carboxyl groups and the glycosidic oxygen atom. Substitution, in the aglycon, of alkyl groups for hydrogen atoms at the carbon atom adjacent to the glycosidic oxygen atom increased the rate of hydrolysis both of the glucopyranosiduronic acids and of the glucopyranosides.

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