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THE HYDROLYSIS OF α- AND β-HEXOPYRANOSYL PHOSPHATES*

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ABSTRACT

The hydrolysis of the anomeric pairs of the aldopyranosyl phosphates of D-glucose, D-mannose, D-galactose, and L-fucose was studied over the pH range of 1–6. The hydrolysis of the acidic "neutral" species is strongly influenced by the structure of the pyranosyl group. The presence of axial hydroxyl groups, or of the electron-donating methyl group on C-5 (as in L-fucose), greatly enhances the rate of hydrolysis relative to that of D-glucopyranosyl phosphate. In contrast, the hydrolysis of the monoanionic species is mainly influenced by the anomeric configuration, the β anomers of the pyranosyl phosphates being hydrolyzed 3–7 times as fast as the α anomers.

INTRODUCTION

The general features of the pH–rate profile for the hydrolysis of glycopyranosyl phosphates have been established by Desjobert[1], and the mechanisms of bond cleavage at various pH values, by Bunton *et al.*[2,3]. There have, however, been no systematic studies reported of the effects of structural changes, such as between epimers and anomeric pairs.

In recent studies on the conformation of glycopyranosyl phosphates in solution, a systematic difference was observed[4] between the $pK_{a'2}$ values of α and β anomers. In all cases studied, the β anomer was the stronger acid[4,5] by 0.2 to 0.4 pH unit. As the pK_{a2} values determine the proportion of each ionic species present at pH values between 2 and 9, the present study was undertaken to determine the apparent and intrinsic rate-constants for the hydrolysis of the anomeric pairs of glycopyranosyl phosphates of D-glucose, D-galactose, D-mannose, and L-fucose. The relative labilities of these compounds is of interest, because of their role in biological systems, and because structural variations within the group would be expected to have significant effects on reactivity.

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RESULTS AND DISCUSSION

The pK_a values at 25° for the glycopyranosyl phosphates studied are listed in Table I. Within each anomeric pair of glycopyranosyl phosphates, the β anomer is the stronger acid. The rate constants observed for hydrolysis, assuming pseudo-first-order kinetics, are listed in Table II.

Study of the hydrolysis of glycopyranosyl phosphates is complicated by several factors. The phosphate group can exist in several forms, depending on the pH, and the rate of hydrolysis is determined by the species present under the experimental conditions and by the hydrolytic constant for each species. Four species must be considered (see Scheme 1): the dianion (**4**), the monoanion (**3**), the neutral species (**2**), and, at low pH values, the conjugate acid (**1**). Calculation of the proportion of each species present requires knowledge of the pK_a value for each equilibrium. The

TABLE I

pK_a VALUES FOR GLYCOPYRANOSYL PHOSPHATES AT 25°

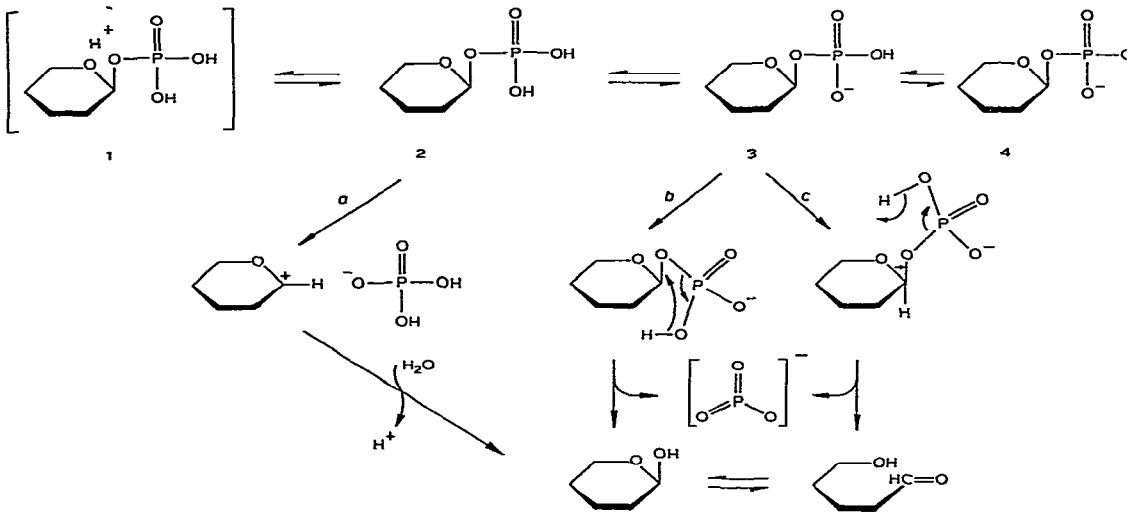
Compound	$pK_{a\,1}$	$pK_{a'2}$
α-L-Fuc	1 3	6 25
β-L-Fuc	1.2	5.85
α-D-Gal	1.3	6 21
β-D-Gal	1.1	5.70
α-D-Glc	1.4	6 08
β-D-Glc	1 3	5 88
α-D-Man	1.3	6.08
β-D-Man	1.1	5.70

TABLE II

OBSERVED RATE-CONSTANTS[a] FOR HYDROLYSIS OF GLYCOPYRANOSYL PHOSPHATES AT 82°, AS A FUNCTION OF pH

Compound	*pH*					
	1	*2*	*3*	*4*	*5*	*6*
α-L-Fuc	1820	158	21	7 0	1.0	0.19
β-L-Fuc	2230	245	30	8.5	1.8	0 56
α-D-Gal	669	30	4 0	1.2	0 34	0 12
β-D-Gal	1438	50	12.0	3.3	1.2	0.48
α-D-Glc	348	20	3.1	0.40	0 30	0.15
β-D-Glc	565	40	7.1	2 3	1.0	0.33
α-D-Man	360	18	1.5	0.37	0 30	0 20
β-D-Man	460	52	6 6	2.9	1.0	0.77

[a]Values are given as observed rate-constants (k_0) × 10^5 sec^{-1}, and are accurate to within ±3%.



Scheme 1. The various ionic species of glycopyranosyl phosphate present at different pH values: conjugate acid (**1**), neutral species (**2**), monoanion (**3**), and dianion (**4**). [Pathway *a* illustrates C–O bond-cleavage; pathways *b* and *c* show two mechanisms for P–O bond-cleavage.]

hydrolysis is further complicated by the existence of two mechanisms for phosphate-bond cleavage, involving either the C–O bond (Scheme 1a) or the P–O bond (Scheme 1b,c). The extent to which each process occurs depends on the pH of the experiment and the structure of the phosphoric ester[2,3].

In simple phosphoric esters, P–O bond-cleavage dominates when the monoanion is the principal form present[2,3,6,7]. At lower pH values, two different reactions occur, involving the hydrolysis of the neutral species and of the conjugate acid. The concentration of each of these species is difficult to determine, as the pK_{a1} value can only be estimated, and the pK_a value of the conjugate acid cannot be determined. However, at pH 1, methyl phosphate hydrolyzes with C–O bond-cleavage, and, in 7M perchloric acid, where the conjugate acid should be present in substantial proportion, both P–O and C–O bond-cleavage occur[2].

In studying the effect of pH on the rate of hydrolysis for α-D-glucopyranosyl phosphate, Bunton *et al.*[2] observed that, unlike the behavior of methyl phosphate, the rate is proportional to the hydrogen-ion concentration over the range of pH 5 to pH 1. This profile suggested that hydrolysis with P–O bond-cleavage of the monoanion occurs at pH values >5, but at pH < 5, is dominated by another mechanism. In acidic solutions, with pH values ranging from 5 to 2, hydrolysis with C–O bond-cleavage was shown to be due to the neutral species **2**. A linear dependence of the rate of hydrolysis on the pH eliminates the possibility of involvement of the conjugate acid, because the rate for this species would be proportional[3] to the square of the hydrogen-ion concentration.

As stated by Bunton *et al.*[3], the observed rate, k_0, in the range pH 2–8 will be the sum of the rates for each species present:

$$k_0 = k_n(C_n/C_p) + k_m(C_m/C_p) + k_d(C_d/C_p) + \ldots \qquad (1)$$

TABLE III

RATE CONSTANTS FOR HYDROLYSIS OF THE NEUTRAL SPECIES **4** (k_n) AND THE MONOANION SPECIES **3** (k_m) OF GLYCOPYRANOSYL PHOSPHATES

Compound	k_n ($\times 10^3$)	β/α	*Relative rate*	k_m ($\times 10^6$)	β/α	*Relative rate*
α-L-Fuc	6.9	1.9	9.4	2.4	5.3	1.0
β-L-Fuc	12 8		19.7	12.9		5.5
α-D-Gal	1 3	2 3	1 8	1.7	6.7	0.7
β-D-Gal	3.1		4.2	11.1		4.8
α-D-Glc	0.73	2 3	1 0	2 3	3.0	1.0
β-D-Glc	1.7		2.3	7.0		3.0
α-D-Man	0.77	4.1	1.1	3.1	5.7	1.3
β-D-Man	3.2		4.3	17 8		7.7

where k_n, k_m, and k_d are the specific rate-constants of the neutral, monoanion, and dianion species, respectively; and the ratios C_n/C_p, C_m/C_p, and C_d/C_p are the mole fractions of each species. As glycopyranosyl phosphates are stable at pH 8, the term for the reaction of the dianionic species may be neglected. If pK_{a1} and pK_{a2} are known, the relative amount of each species may be evaluated at any pH value; and the rate constant for each species may be calculated.

Hydrolysis of the neutral species **2**. — At pH 2, the hydrolysis of α-D-glucopyranosyl phosphate has been shown[2,3] to be due almost entirely to the reaction of the neutral species. On this basis, using the method of Bunton *et al.*[3], the value of k_n may be calculated by dividing the observed rate, k_0, by the proportion of the neutral species present (see equation *1*). At pH 6, the proportion of the neutral species is very small (the mole fraction is 5×10^{-5} for a typical glycosyl phosphate), so that the rate is almost exclusively due to the reaction of the monoanion. The rate constant k_m may be calculated by subtracting the small contribution due to the neutral species from the observed rate and dividing the result by the proportion of the monoanion present. This k_m value may then be used to calculate a corrected k_n value. The values of k_m and the corrected k_n values for various glycopyranosyl phosphates are listed in Table III.

The rate constant k_n for the neutral species **2** is dependent on the anomeric configuration. In all cases studied, the β anomer is hydrolyzed 2 to 4 times as fast as the α anomer. Similar differences in rates have been observed[8] for the anomeric methyl pyranosides of D-glucose, D-mannose, D-galactose, D-xylose, 6-deoxy-L-mannose, and L-arabinose. Two explanations for the higher rate of hydrolysis have been given. One is based on the greater accessibility of the β-glycosidic oxygen atom to protonation[9]. The second attributes[10] the higher reactivity of the equatorial bond to the unfavorable, electronic interaction between the β-glycosidic oxygen atom and the ring-oxygen atom, which brings the nonbonding orbitals of the ring-oxygen atom into closer proximity to an equatorial than to an axial group, thus creating a

TABLE IV

RATE CONSTANTS [k_n ($\times 10^3$) sec^{-1}] FOR THE HYDROLYSIS OF THE NEUTRAL SPECIES (4) OF GLYCOPYRANOSYL PHOSPHATES AT pH 1–3

Compound	*pH*		
	1	*2*	*3*
α-L-Fuc	24.4	6.9	7.1
β-L-Fuc	31.8	12.8	12.3
α-D-Gal	90	13	13
β-D-Gal	22.1	3.1	60
α-D-Glc	4.4	073	0.80
β-D-Glc	7.6	17	22
α-D-Man	48	0.77	0.41
β-D-Man	70	32	2.7

slightly higher ground-state energy for the β anomer. As hydrolysis of both anomers presumably proceeds through energetically similar transition-states, the energy of activation for the equatorial group (β anomer) would be less. Because hydrolysis of the neutral species **2** of the glycosyl phosphates does not[3] appear to require protonation of the anomeric, or ring, oxygen atoms, an explanation based on steric or other structural differences appears to be most likely.

The conclusion of Bunton *et al.*[3] that hydrolysis in the pH range of 2 to 5 principally involves the neutral species **2** in an SN1 process is supported by the data obtained in the present studies. As shown in Table IV, values of k_n at pH 2 and 3 are similar, whereas, at pH 1, where substantial proportions of conjugate acids are present, the values of k_n are greatly increased. Were the conjugate acid involved in the hydrolysis over the pH range 2–5, the values of k_n would be expected, as the pH is decreased, to increase more rapidly than the proportion of neutral species present, as is observed at pH 1.

The rates of hydrolysis of the α anomers of D-mannosyl, D-galactosyl, and L-fucosyl phosphate relative to that of α-D-glucopyranosyl phosphate indicate that the structure of the pyranosyl group is important. The relative rates of hydrolysis of the neutral species are given in Table III. In the fucose derivatives, the methyl group on C-5 enhances electron donation into the pyranosyl ring, thereby stabilizing the carbonium ion at C-1 and increasing the rate of hydrolysis, as proposed[8] for the methyl glycosides. The smaller methyl group may lessen steric hindrance, thereby also facilitating formation of the transition state[8]. The presence of an axially attached hydroxyl group at C-2 of D-mannopyranose and C-4 of D-galactopyranose generally tends to destabilize the pyranosyl group relative to the equatorially attached hydroxyl groups of D-glucopyranose[11]. The effect for the α anomers of the glycosyl phosphates is quite small, particularly with the *manno* epimer, which is hydrolyzed only slightly faster than the *gluco* derivative. In this case, in the development of a half-chair conformation in the transition state, the destabilizing effect of the axial 2-hydroxyl

group is opposed by the requirement that the 2- and 3-hydroxyl groups come closer together, thus making the process more difficult. In the *galacto* isomer, the axial hydroxyl group at C-4 is moved out of the axis of the ring as the half-chair is formed, thus facilitating the process. In the comparison of the α and β anomers, the inversion of relative rate between D-mannose and D-galactose may be explained by the Δ-2 effect of Reeves[11]. The β anomer of mannose has an axial hydroxyl group at C-2 bisecting the angle between the oxygen atom on C-1 and the ring-oxygen atom. Such an arrangement results in maximum repulsion between the oxygen atoms on C-1 and C-2, and compounds having this configuration, such as β-D-mannopyranosyl phosphate, are destabilized.

Hydrolysis of the monoanion 3. — The relative rates and the rate constant (k_m) for hydrolysis of the monoanion species 3 are listed in Table III. Again, an anomeric difference is apparent, with the β anomer being hydrolyzed 3 to 7 times as fast as the α anomer. In addition, the monoanions fall into an order different from that observed for the neutral species. There is a three-fold difference in rate between β-D-glucopyranosyl phosphate, the most stable β anomer, and α-D-mannopyranosyl phosphate, the most labile α anomer, indicating that a principal factor in the hydrolysis of the monoanionic species is the anomeric form, rather than the pyranosyl group. The only exception is the β-D-*manno* derivative, which is hydrolyzed much faster than the other β sugars, probably due to the Δ-2 effect[11], which destabilizes the ground state.

It appears that, in the hydrolysis of monoanions, there is an anomeric effect[10] that causes the β anomer to be the less stable. In the mechanism proposed by Westheimer and co-workers[6,12] for hydrolysis of the monoanion 3, the rate-limiting step is protonation of the glycosidic oxygen atom. Both electronic and steric factors would be expected to affect the ease with which this can be accomplished. For all of the anomeric pairs of glycosyl phosphates examined, the ^{13}C-n.m.r. spectra show[4] that the β-anomeric carbon atom resonates downfield from the α-anomeric, indicating that the β-anomeric carbon atom is deshielded relative to the α-anomeric atom. X-Ray crystallographic studies[13] have shown that the glycosidic bond of methyl glycosides and disaccharides is longer in the β than in the α anomers. Both the downfield resonance and the greater bond-length may reflect electron withdrawal from C-1, probably to the glycosidic oxygen atom. Increased electron-density at the glycosidic oxygen atom would facilitate protonation and intramolecular proton-transfer, causing the β anomer to be the more labile, as is observed.

The latter, namely, intramolecular proton-transfer, might also explain the greater acid strength of the β-monoanion relative to that of the α anomer. Generally, for the β-monoanion to be a stronger acid, electron withdrawal from the oxygen atoms of the phosphate group would be required. To achieve this, it would be necessary to withdraw electrons from the phosphate group into the ring system, thus increasing, rather than decreasing, the shielding at C-1, and causing an upfield shift, rather than the downfield shift observed. However, if intramolecular loss of a proton to the

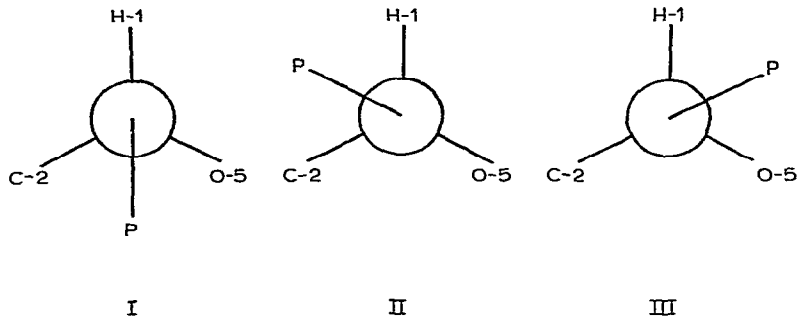


Fig. 1. "Newman" projections, showing rotamers around the C–O bond of glycopyranosyl phosphates.

glycosidic, or the ring, oxygen atom occurs readily, the phosphate monoanion would appear to have a lower pK_a, value.

Consideration should be given to an additional steric factor that might account for the greater lability of the β anomers, namely, the statistical probability of intramolecular proton-transfer, which will be controlled by the frequency with which the acidic hydroxyl group of the phosphate is properly positioned for proton transfer to the acceptor oxygen atom. If this intramolecular transfer involves the glycosidic oxygen atom exclusively, and the electronic effects are ignored, then the α and β anomers have the same probability that transfer will occur. However, if intramolecular transfer involves the ring-oxygen atom, the statistical factors favor the β anomer. It appears that transfer to the ring oxygen atom would be sterically easier than transfer to the glycosidic oxygen atom. Some evidence for the disposition of the phosphate group relative to the ring-oxygen atom has been obtained[4] from ^{13}C- and ^{1}H-n.m.r. spectroscopy. Three stable rotamers of the phosphate group about the glycosidic bond exist (see Fig. 1), and the mole fraction percentages of these rotamers have been computed[4]. In the β anomer, the proton of the phosphate group can be transferred to the π orbitals of the ring-oxygen atom in either rotamer I or III, whereas, in the α anomer, this transfer can occur only in rotamer III. The sum of the mole fractions of rotamers I and III for the β anomers is always greater than the mole fraction of rotamer III for the corresponding α anomers, but there is never a two-fold difference in populations to explain the observed differences (of, at least, a factor of two) in the rates of hydrolysis of the anomeric pairs, indicating that electronic factors may also be involved.

In summary, the rates of hydrolysis of the neutral species of glycopyranosyl phosphates is influenced by the structure of the pyranosyl group and by the anomeric configuration. The replacement of 5-(hydroxymethyl) by a 5-methyl group causes the greatest rate-enhancement. In the monoanion, the effects of changes in the structure of the pyranosyl group are minor, and the anomeric form of a glycopyranosyl phosphate strongly influences its stability, with the β anomer being the more labile in all cases examined.

EXPERIMENTAL

Materials and methods. — α-D-Glucopyranosyl phosphate (potassium salt) and α-D-galactopyranosyl phosphate (potassium salt) were purchased from Sigma. α-D-Mannopyranosyl phosphate was synthesized by the method of MacDonald[14]. α- and β-L-Fucopyranosyl phosphates, as well as the β-D-pyranosyl phosphates of D-galactose, D-glucose, and D-mannose, were synthesized by a modification of the method of Shibaev *et al.*[15].

In hydrolysis studies, solutions of 50mM potassium hydrogenphthalate were used throughout the pH range studied. Standard M hydrochloric acid or M sodium hydroxide was added to adjust the pH to that desired. In a typical experiment, 0.2M glycopyranosyl phosphate (700 μL) was added to start the hydrolysis. At appropriate intervals, 50-μL aliquots were removed, and analyzed for reducing sugar[16]. Total-hydrolysis data were obtained by using 700 μL of 2M hydrochloric acid, and heating for 10 min in a boiling-water bath. Rate constants were obtained from plots of $\log(A_T - A_t)/A_T$ *vs.* time, where A_T is the value of the absorbance for the total-hydrolysis sample, and A_t is the absorbance value at time t.

Values of pK_a· at 25° were obtained by titration of 0.1M solutions of the glycosyl phosphate salts with 1.00M hydrochloric acid. The pK_{a1}· values could be estimated to within ±0.1 pH unit, whereas pK_{a2}· values are accurate to within ±0.01 pH unit. The values reported are corrected for dilution. The pK_a· values at 82° were calculated by using the equation of Harned and Embree[17], and are 0.17 pH unit higher than those measured at 25°.

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REFERENCES

1 P. Desjobert, *Bull. Soc. Chim. Biol.*, 33 (1951) 42–49.
2 C. A. Bunton, D R. Lewellyn, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc., C*, (1958) 3574–3587.
3 C. A. Bunton, D. R. Lewellyn, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc., C*, (1958) 3588–3594.
4 J. V. O'Connor, H. A. Nunez, and R. Barker, *Biochemistry*, in press.
5 E. J. Behrman, *Carbohydr. Res.*, 36 (1974) 231–232.
6 J. Kumamoto and F. H. Westheimer, *J. Am. Chem. Soc*, 77 (1955) 2515–2518.
7 P. A. T. Swoboda and E. M. Crook, *Biochem. J.*, 59 (1955) xxiv–xxv.
8 J. N. BeMiller, *Adv. Carbohydr. Chem.*, 22 (1967) 25–108.
9 J. A. Mills, *Adv. Carbohydr. Chem.*, 10 (1955) 1–53.
10 J. T. Edward, *Chem. Ind. (London)*, (1955) 1102–1104.
11 R. E. Reeves, *Adv. Carbohydr. Chem*, 6 (1951) 107–134.
12 W. W. Butcher and F. H. Westheimer, *J. Am. Chem. Soc.*, 77 (1955) 2420–2424.
13 R. H. Marchessault, F. Brisse, and S. Perez, *Abstr. Pap. Joint Conf. CIC–ACS, 2nd, Montreal, Canada*, (1977) 16.
14 D. L. MacDonald, *J. Org. Chem.*, 27 (1962) 1107–1109.
15 V. N. Shibaev, G. P. Eliseeva, Yu. Yu. Kusov, V. A. Petrenko, S. S. Mishchenko, and N. K. Kochetkov, *Izv. Akad. Nauk SSSR, Ser. Khim*, 11 (1976) 2584–2587.
16 J. E. Hodge and B. T. Hofreiter, *Methods Carbohydr. Chem.*, 1 (1962) 386–388.
17 H. S. Harned and N. D. Embree, *J. Am. Chem. Soc.*, 56 (1934) 1050–1053.