The Hydrolyses of α-D-Ribose and α-D-Glucose 1-Phosphate[1,2]

C. A. BUNTON AND E. HUMERES[3]

University of California at Santa Barbara, Santa Barbara, California 93106

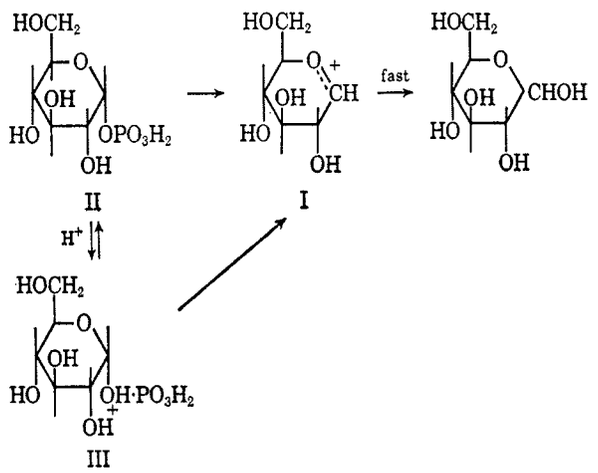
Received September 1, 1968

α-D-Ribofuranose 1-phosphate is several hundred times as reactive as α-D-glucopyranose 1-phosphate in both the acid-catalyzed hydrolysis and the hydrolysis of the undissociated phosphate. The activation energy for the acid hydrolysis is 6 kcal mol^{-1} lower for the ribose phosphate. Hydrolysis of the ribose phosphate monoanion makes no contribution to the over-all reaction. The catalytic efficiencies of the mineral acids for the hydrolysis of glucose 1-phosphate are $HCl > HClO_4 \sim H_2SO_4$, and the positive salt effects of sodium chloride are considerably greater than those of sodium perchlorate.

Hydrolyses of monoalkyl phosphates by the A1 and SN1 mechanisms are observed only in favorable cases. The hydrolyses of α-D-glucose 1-,[4] isopropyl,[5] and *t*-butyl[6] phosphate follow these mechanisms, and carbonium ions are intermediates in the hydrolyses of some allylic phosphates to rearranged products.[7]

Three distinct reaction mechanisms have been observed in the hydrolysis of α-glucose 1-phosphate.[4] In moderately concentrated acid and at a pH in which the phosphate is present as the undissociated acid the rate-limiting step is formation of a glucosyl 1-cation (I), from the undissociated phosphate (II), or from its conjugate acid (III) (Scheme I).[8] (At pH ~4 hydrolysis of the monoanion can be observed with phosphorus–oxygen fission.[4])

SCHEME I

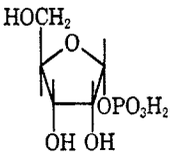


The aim of the present work was to study the effects of acids and salts upon the hydrolysis of glycosidic phosphates as part of a general study of electrolyte effects upon acid hydrolysis.[9] We were also interested in determining the relative reactivities and the kinetic parameters for the hydrolysis of a furanose and a pyranose phosphate, because Wright and Khorana had shown qualitatively that β-D-ribofuranose 1-phosphate was more reactive in acid than β-D-ribopyranose 1-phosphate.[10]

Although relations between rate constant and acidity function or hydrogen ion concentration are no longer considered to be rigorous mechanistic tests,[11,12] it is none the less useful to examine them in terms of known reaction mechanisms of particular classes of substrates and for various acids, using the protonation of primary amines as one measure of acidity.

Experimental Section

Materials.—α-D-Ribofuranose 1-phosphate (as the cyclohexylamine salt), and α-D-glucopyranose 1-phosphate (as the potassium salt) were obtained from Sigma. The optical rotations were for glucose 1-phosphate $[\alpha]_D$ 74.5° (lit.[13] $[\alpha]_D$ 78–79.7°), and for ribose 1-phosphate $[\alpha]_D$ 39.8° (lit.[10] $[\alpha]_D$ 40.3°). α-D-Ribose 1-phosphate has the furanose structure IV.[10,14]



IV

Deuteriosulfuric acid was diluted with 99.8% D_2O. Potassium hydrogen phthalate (0.05 *M*) was used as buffer for pH 3–6 and sodium hydrogen carbonate (0.05 *M*) was used at higher pH. The pH was measured at 25° and corrected to the reaction temperature.[15]

Kinetics.—The hydrolysis was followed polarimetrically, using a visual polarimeter and a jacketed 20-cm cell, or by measuring inorganic phosphate colorimetrically using a Gilford spectrophotometer. The general procedures have been described,[4] except that the inorganic phosphate was determined by the Lowry–Lopez method.[16] For high acid concentrations the electrolyte concentration after neutralization was high and hydrolysis of ribose 1-phosphate continued slowly while the color was developing; therefore we took a series of readings over a period of time as recommended by the original authors.[16] For the runs with perchloric acid the potassium salt of glucose 1-phosphate was treated with Dowex 50W-X8 to convert it into the free acid. When the formation of inorganic phosphate was followed, stoppered flasks were used at the lower and sealed tubes at the higher temperatures.

In general polarimetry was used for the acid hydrolysis of glu-

(1) This work was presented in part at the 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968, Abstracts C.37.

(2) Support of this work by the National Institute of Arthritis and Metabolic Diseases of the U. S. Public Health Service is gratefully acknowledged.

(3) University of Chile–University of California Cooperative Program Fellow.

(4) C. A. Bunton, D. R. Llewellyn, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc.*, 3588 (1958).

(5) L. Kugel and M. Halmann, *J. Org. Chem.*, **32**, 642 (1967).

(6) A. Lapidot, D. Samuel, and M. W. Broday, *J. Chem. Soc.*, 637 (1964).

(7) P. Valenzuela and O. Cori, *Tetrahedron Lett.*, 3089 (1967); W. Ritterdorf and F. Cramer, *Tetrahedron*, **24**, 43 (1968).

(8) Protonation of the ring oxygen atom followed by ring opening is another possible mechanism, but it was considered to be less probable than that shown.[4] In III the proton could be located on one of the phosphoryl oxygen atoms.

(9) C. A. Bunton, J. H. Crabtree, and L. Robinson, *J. Amer. Chem. Soc.*, **90**, 1258 (1968).

(10) R. S. Wright and H. G. Khorana, *ibid.*, **78**, 811 (1956).

(11) (a) J. F. Bunnett, *ibid.*, **83**, 4956 (1961), and accompanying papers; (b) J. F. Bunnett and F. R. Olsen, *Can. J. Chem.*, **44**, 1899, 1917 (1967).

(12) E. M. Arnett and G. W. Mach, *J. Amer. Chem. Soc.*, **88**, 1177 (1966).

(13) J. M. Ashby, H. B. Clarke, E. M. Crook, and S. P. Datta, *Biochem. J.*, **59**, 203 (1955).

(14) H. M. Kalckar, *J. Biol. Chem.*, **167**, 477 (1947).

(15) S. Stene, *Rec. Trav. Chim. Pays-Bas*, **49**, 1133 (1930).

(16) O. M. Lowry and J. A. Lopez, *J. Biol. Chem.*, **162**, 421 (1946).

cose 1-phosphate, and the Lowry–Lopez method for the hydrolysis of ribose 1-phosphate with substrate concentrations of 0.05 and 1.5×10^{-3} M, respectively. The integrated first-order rate constants, k_ψ (sec^{-1}), were calculated graphically, and had an average error of ±3% for glucose 1-phosphate based on the linearity of the plots; for ribose 1-phosphate in the more concentrated acid the error was *ca.* ±10%, and the reaction was too fast to be followed at high acid concentration by conventional methods. Reactions were followed for at least 2 half-lives.

Dissociation Constants.—For α-D-glucopyranose 1-phosphate pK_1 = 1.23 at 30°,[17] and pK_2 (extrapolated to 82°) = 6.72.[18] For α-D-ribofuranose 1-phosphate we obtained pK_2 = 6.55 at 23° by potentiometric titration. (This value is almost identical with that for glucose 1-phosphate at this temperature[18].) The value of pK_1 = 1.5 at 25° was determined by pH measurement, as described elsewhere.[18] These pK_1 values are not very accurate, in part because of the difficulty of determining pK values in this range, but also because of the rapid hydrolysis, especially of ribose 1-phosphate.

Results

The values of k_ψ for the hydrolysis of ribose 1-phosphate at 82.0° and pH >1 are given in Table I. A plot of log k_ψ against pH (Figure 1) is linear with slope −1. The values of k_ψ for the acid hydrolysis of ribose 1-phosphate are in Table II, and in Table III we give the corresponding rate constants for the acid hydrolysis of glucose 1-phosphate. These new values for the hydrolysis of glucose 1-phosphate cover a wider range of acid than that used earlier,[4] and include values in hydrochloric acid. Where comparison can be made the agreement between the two sets of values is good.

TABLE I

HYDROLYSIS OF α-D-RIBOSE 1-PHOSPHATE AT pH 1–8[a]

pH	$10^4 k_\psi$, sec^{-1}
1.00	0.48[b]
1.00	111[c]
2.00	10.1[c]
3.35	0.82[c]
3.26	68.3
4.16	7.00
5.20	0.89
6.06	0.17
7.50	0.012

[a] At 82.0° unless specified. [b] At 0°. [c] At 45.1°.

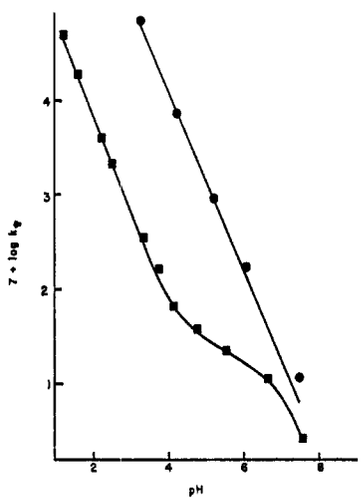


Figure 1.—Relation between log k_ψ and pH for the hydrolysis of ribose 1-phosphate (●) and glucose 1-phosphate (■) at 82.0°.

TABLE II

HYDROLYSIS OF α-D-RIBOSE 1-PHOSPHATE IN STRONG ACID[a]

Acid	C_{H^+}, M	$10^4 k_\psi$, sec^{-1}
HCl	0.10	111[b]
HCl	0.80	3.17
HCl	1.60	9.30
HCl	2.40	25.2
HCl	3.00	42.8
H_2SO_4	0.10	0.48
H_2SO_4	0.49	1.97
H_2SO_4	0.49	63.6[c]
H_2SO_4	1.46	7.87
H_2SO_4	2.50	25.6
H_2SO_4	2.94	41.3
H_2SO_4	3.50	49.8
$HClO_4$	0.50	54.7[c]
$HClO_4$	0.50	1.25
$HClO_4$	0.81	3.25
$HClO_4$	1.50	9.17
$HClO_4$	1.50	28.1[d]
$HClO_4$	1.50	80.0[e]
$HClO_4$	1.50	18.3[f]
$HClO_4$	1.50	55.5[g]
$HClO_4$	1.50	32.3[h]
$HClO_4$	1.50	48.7[i]
$HClO_4$	2.50	30.0
$HClO_4$	2.68	32.5

[a] At 0° unless specified. [b] At 45.1°. [c] At 25.0°. [d] At 7.0°. [e] At 15.0°. [f] 2 M $NaClO_4$. [g] 4 M $NaClO_4$. [h] 1.85 M NaCl. [i] 2.5 M NaCl.

TABLE III

ACID HYDROLYSIS OF α-D-GLUCOSE 1-PHOSPHATE[a]

Acid	C_{H^+}, M	$10^4 k_\psi$, sec^{-1}
HCl	0.96	3.75
HCl	1.98	15.2
HCl	2.86	37.5
HCl	4.00	85.3
HCl	5.41	245
H_2SO_4	3.00	35.8
H_2SO_4	3.00[b]	62.8
H_2SO_4	4.00	74.2
H_2SO_4	5.20	167
H_2SO_4	5.60	228
$HClO_4$	1.47	1.45[c]
$HClO_4$	1.47	7.40
$HClO_4$	1.47	140[d]
$HClO_4$	1.92	11.7[e]
$HClO_4$	1.92	27.0[f]
$HClO_4$	1.92	51.4[g]
$HClO_4$	1.92	33.9[h]
$HClO_4$	1.92	56.8[i]
$HClO_4$	4.40	103
$HClO_4$	4.86	143
$HClO_4$	5.40	275

[a] At 25.0° unless specified. [b] D_2SO_4 in D_2O. [c] At 15.8°. [d] At 45.1°. [e] Ref 4. [f] 2 M $NaClO_4$. [g] 4 M $NaClO_4$. [h] 2 N NaCl. [i] 3 M NaCl.

The activation parameters for the acid hydrolysis for ribose 1-phosphate are ΔH^* = 22 kcal mol^{-1}, ΔS^* = 7.4 eu, and for glucose 1-phosphate ΔH^* = 27.9 kcal mol^{-1}, ΔS^* = 14.9 eu (calculated for 1 M reactants at 25°). We could not calculate meaningful activation parameters for the spontaneous hydrolysis of ribose 1-phosphate because we do not know the

(17) C. F. Cori, S. B. Colowick, and G. T. Cori, *J. Biol. Chem.*, **121**, 465 (1937).

(18) C. A. Bunton, E. J. Fendler, E. Humeres, and K.-U. Yang, *J. Org. Chem.*, **32**, 2806 (1967).

temperature effect upon pK_1, and have no hope of measuring it at high temperature. At pH 3.26, where the monoanion is the bulk species (but is unreactive), E = 25.5 kcal mol^{-1} and log A = 16.4 (the value of log A is corrected for the amount of substrate present as the undissociated acid on the assumption that the pK_1 value does not change in going from 25 to 45°). For the spontaneous hydrolysis of glucose 1-phosphate E = 31 kcal mol^{-1}, and log A = 16.6.[4] For both compounds the value of E is not corrected for the heat of ionization, but the error should be approximately the same for both compounds, showing that the higher rate of the spontaneous hydrolysis of ribose 1-phosphate is caused by the lower activation energy.

Discussion

Variations of Rate Constant with pH.—For the hydrolyses of glucose 1-, isopropyl, and *t*-butyl phosphate at pH >1 the acid-catalyzed hydrolysis is relatively unimportant,[4-6] and the over-all rate is given by

$$v = k_N[ROPO_3H_2] + k_M[ROPO_3H^-]$$

where k_N and k_M are the first-order rate constants for hydrolysis of the undissociated phosphate and its monoanion, respectively, and there is a well-marked contribution from the hydrolysis of the monoanion at pH >5. For the hydrolysis of ribose 1-phosphate there is almost no contribution from this reaction because a plot of log k_ψ against pH has unit slope (Figure 1). The rate constants for hydrolyses of monoanions of alkyl phosphate are not very sensitive to the nature of the alkyl group,[19] and the apparent absence of any hydrolysis of the ribose 1-phosphate monoanion arises simply because it is obscured by the rapid hydrolysis of the undissociated phosphate.

At pH <1 the reaction of these glycosidic phosphates becomes faster than expected in terms of the reactivity of the undissociated acids because of the incursion of acid-catalyzed hydrolysis.

Acid-Catalyzed Hydrolysis.—A considerable body of evidence suggests that the acid hydrolysis of ribose 1-phosphate follows an A1 mechanism. For example the positive values of the entropy of activation are characteristic of A1 hydrolyses.[20] For the acid hydrolysis of glucose 1-phosphate at 25.0° plots of log k_ψ against Hammett's acidity function, $-H_0'$,[21] are linear with slopes of 1.0 for HCl, 0.90 for $HClO_4$, and 0.72 for H_2SO_4. (From an earlier examination of this hydrolysis over a limited acid concentration it had been concluded that the slope was 0.94.)[4] For the acid hydrolysis of ribose 1-phosphate at 0° the corresponding slopes are 1.13 for HCl, 1.05 for $HClO_4$, and 0.85 for H_2SO_4. (Because of the speed of this hydrolysis only a limited range of acidities was examined, and these slopes cannot be determined accurately.) These results are characteristic of A1 hydrolyses,[22] although relations between rate constant and acidity function are now considered to be only qualitatively significant.[11,12]

Following Bunnett and Olsen's treatment,[11b] we find that for the hydrolysis of glucose 1-phosphate plots of log $k_\psi + H_0'$ against log $C_{H^+} + H_0'$ give ϕ values of 0.15 for $HClO_4$, and 0.40 for H_2SO_4. However, these plots do not give a common intercept and that for hydrochloric acid is curved. The ϕ values for perchloric and sulfuric acid differ slightly from those given earlier,[11b] because we have now examined a wider range of acidities. (This treatment was used at acid concentration such that $k_\psi \gg k_N$, and the contribution of the hydrolysis of the undissociated phosphate could be neglected.) Bunnett has already commented on the problem of applying his linear free energy relations to phosphate ester hydrolysis.[11a]

The deuterium solvent isotope effect can be used to distinguish between A1 and A2 mechanisms, particularly when substrates of similar structure are involved.[23,24] The original value of k_{H_2O}/k_{D_2O} = 0.66 for the hydrolysis of glucose 1-phosphate was measured, in 3 M perchloric acid, using only 75% of D_2O,[4] and is therefore too high, and our present value of k_{H_2O}/k_{D_2O} = 0.57 in 3 M sulfuric acid, using fully deuterated acid and solvent, is much more in line with expectation.[23,24] It is considerably lower than the values of k_{H_2O}/k_{D_2O} = 0.9 and 0.76 for the A2 hydrolysis of ethyl phosphate in perchloric and sulfuric acids at 100°, and slightly lower than those for the presumed A1 hydrolysis of isopropyl phosphate under the same conditions,[5] but part of the difference between the values for glucose 1- and isopropyl phosphate could be caused by the temperature difference and the use of undeuterated perchloric and sulfuric acid in the hydrolysis of isopropyl phosphate, because both these effects make k_{H_2O}/k_{D_2O} closer to unity. However, the value of k_{H_2O}/k_{D_2O} = 0.56 for the A1 hydrolysis of *t*-butyl phosphate[5] is very close to our value for glucose 1-phosphate. These values of k_{H_2O}/k_{D_2O} for the A1 hydrolysis of phosphate esters are therefore closer to unity than those generally observed for hydrolyses of substrates such as acetals and carboxylic esters, where frequently $k_{H_2O}/k_{D_2O} < 0.5$.[23-25]

Catalytic Effects of the Mineral Acids.—Halogen acids are much better catalysts than sulfuric or perchloric acid where the halide ion can act as a nucleophile, as for example, in the decomposition of methyl phosphate in aqueous hydrochloric acid,[26] and as would be expected there are no striking differences between the catalytic efficiencies of the mineral acids in the hydrolysis of glucose 1- and ribose 1-phosphate (Tables II and III). (For the hydrolysis of ribose 1-phosphate this result could be caused by the small acidity range studied, and the following discussion is therefore based largely on the behavior of glucose 1-phosphate.)

For A1 acid hydrolyses of carboxylic esters perchloric was a better catalyst than hydrochloric or sulfuric acids, but the opposite was found for A2 hydrolyses.[9] In order to illustrate the order of effectiveness of these mineral acids for the A1 hydrolysis of glucose 1-phos-

(19) J. R. Cox and O. B. Ramsay, *Chem. Rev.*, **64**, 343 (1964).

(20) F. A. Long, J. G. Pritchard, and F. E. Stafford, *J. Amer. Chem. Soc.*, **79**, 2362 (1957); L. L. Schaleger and F. A. Long, *Advan. Phys. Org. Chem.*, **1**, 1 (1963).

(21) M. A. Paul and F. A. Long, *Chem. Rev.*, **57**, 1 (1957); M. J. Jorgenson and D. R. Hartter, *J. Amer. Chem. Soc.*, **86**, 878 (1963); K. Yates and H. Wai, *ibid.*, **86**, 5408 (1964).

(22) F. A. Long and M. A. Paul, *Chem. Rev.*, **57**, 935 (1957).

(23) J. G. Pritchard and F. A. Long, *J. Amer. Chem. Soc.*, **78**, 6008 (1956); **80**, 4162 (1958).

(24) C. A. Bunton and V. J. Shiner, *ibid.*, **83**, 3207 (1961).

(25) K. B. Wiberg, *Chem. Rev.*, **55**, 713 (1955).

(26) C. A. Bunton, D. Kellerman, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc., B*, 292 (1966).

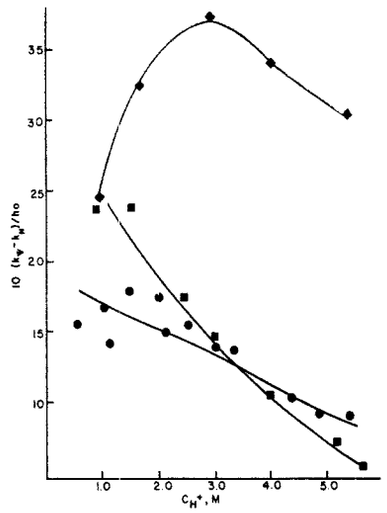


Figure 2.—Effects of acids upon the second-order rate constants $(k_\psi - k_N)/h_0$ for the hydrolysis of glucose 1-phosphate: ●, $HClO_4$; ■, H_2SO_4; ◆, HCl.

phate we plot $(k_\psi - k_N)/h_0$ against C_{H^+} at 25° (Figure 2). The approximate value of k_N, estimated from earlier work,[4] is 0.09×10^{-5} sec^{-1} at 25°, and the contribution of k_N is negligible except at the lower acid concentrations. It is probable that the acids affect the values of k_N differently, because of differences in electrolytic effects, and the differences between the values of $(k_\psi - k_N)/h_0$ at low acid concentrations may be reflecting differences in the values of k_N. The limited values of k_ψ/h_0 for ribose 1-phosphate follow the general patterns found for glucose 1-phosphate. The points are scattered, because of errors in both k_ψ and h_0, but they show that on this criterion hydrochloric acid is a better catalyst than sulfuric or perchloric acid. Long and McIntyre found that hydrochloric acid was a better catalyst than perchloric for A1 acetal hydrolysis,[27] and they showed that part of the effect was caused by changes in the initial state stability. (They did not examine hydrolysis in sulfuric acid.) For the A1 hydrolyses of α-methyl glucosides at 80° Timell found a similar pattern to that which we observe and plots of log k_ψ against $-H_0'$ had slopes of 1.05 for HCl, 0.8 for H_2SO_4, and 0.9 for $HClO_4$.[28]

The acid orders for the A1 hydrolyses of acetals, glucosides and glycosidic phosphates therefore differ from those found for A1 hydrolyses of carboxylic esters, and are somewhat similar to those found for $A_{Ac}2$ hydrolyses.[9] The acid order for A1 and A2 hydrolyses depends to some extent upon salt effects on the initial state,[9,27] but it also appears to depend upon the extent to which the transition state contains strongly hydrogen bonding centers, as in $A_{Ac}2$ hydrolyses, as compared with A1 hydrolyses of, for example, carboxylic esters where the transition state has considerable carbonium ion character.[9] With increasing substrate reactivity and increasing carbonium ion stability, the structure of the transition state for an A1 hydrolysis should become similar to that of the conjugate acid, and less like the carbonium ion,[29] and there is considerable evidence from Fife's work that the transition state for acetal hydrolysis does not have marked carbonium ion character, but has a structure closer to that of the conjugate acid.[30]

For the A1 hydrolysis of isopropyl phosphate in aqueous perchloric acid a plot of log k_ψ against $-H_0'$ has a slope of 1.03[5] and for the A1 hydrolysis of *t*-butyl phosphate in aqueous perchloric acid a plot of log k_ψ against $-H_0'$ has as lope of 1.0, but the ionic strength was kept constant at 3.0,[6] and therefore it is difficult to compare this slope with those for the other hydrolyses. Our results show that observation of a linear relation between k and h_0' may depend fortuitously upon the choice of the catalyzing acid.[9,27,28]

Kinetic Salt Effects.—The slopes of plots of log k_ψ against salt concentration are approximately 0.23 for NaCl, and 0.18 for $NaClO_4$ for the hydrolysis of glucose 1-phosphate in 2 *M* perchloric acid at 25° and 0.28 for NaCl and 0.18 for $NaClO_4$ for the hydrolysis of ribose 1-phosphate in 1.5 *M* perchloric acid at 0°. For both compounds these plots curve upward. These slopes are similar to those found for A1 hydrolyses of carboxylic esters, and are much larger than those for $A_{Ac}2$ ester hydrolysis.[9] However, the salt order is different, because for $A_{Al}1$ hydrolysis perchlorates are more effective than chlorides, but the opposite is true for $A_{Ac}2$ hydrolyses. The salt order for the A1 hydrolysis of glucose 1-phosphate is that already observed for A1 acetal hydrolysis,[27] again illustrating the kinetic similarity between the hydrolysis of acetals and the glycosidic phosphates.

Structural Effects.—Ribose 1-phosphate is much more reactive than glucose 1-phosphate in both the acid and spontaneous hydrolyses, and the reactivity differences are greater than those found for SN1 reactions of cyclopentyl and cyclohexyl compounds.[31] For the glycosidic phosphates the relative reactivities are 200–400 and for solvolyses of the tosylates the corresponding value is ~30.[31]

In the carbocyclic systems the rate differences can be interpreted in terms of the strain in going from the chair conformation in a cyclohexyl compound to the half-chair conformation in the carbonium ionlike transition state as compared with the cyclopentyl system.[31] These effects should also be at work in the acid and spontaneous hydrolyses of glucose 1- and ribose 1-phosphates, and in glycosidic hydrolyses, because of the strain in going to the half-chair conformation for a hexose.[32]

Additional effects involved in the hydrolyses of the glycosidic derivatives are as follows.

(1) The overlap of the unshared electrons of the ethereal oxygen with the vacant p orbital at the forming carbonium ion center should be more effective in the ribose than in the glucose phosphate. This overlap is of considerable importance in stabilizing the transition state for hydrolyses of acetals and glycosides and related compounds.[4,32]

(2) Repulsions between carbon–oxygen dipoles make the differences in stability between α and β anomers in pyranosides less than expected on conformational grounds based on analogies with carbocyclic com-

(27) F. A. Long and D. McIntyre, *J. Amer. Chem. Soc.*, **76**, 3243 (1954).
(28) T. E. Timell, *Can. J. Chem.*, **42**, 1456 (1964).
(29) G. S. Hammond, *J. Amer. Chem. Soc.*, **77**, 334 (1955).
(30) T. M. Fife and L. K. Jao, *J. Org. Chem.*, **30**, 1492 (1965); T. H. Fife, *J. Amer. Chem. Soc.*, **89**, 3228 (1967).
(31) C. S. Foote, *J. Amer. Chem. Soc.*, **86**, 1853 (1964); P. v. R. Schleyer, *ibid.*, **86**, 1854 (1964), and references cited.
(32) J. N. Bemiller, *Advan. Carbohydate Chem.*, **22**, 25 (1967).

pounds.[32,33] These particular dipolar repulsions should disappear with departure of the C_1 leaving group, and this factor could be partly responsible for the greater reactivity of methyl pyranosides which have an equatorial rather than an axial methoxy group.[32] The anomeric stabilization of the α over the β anomer should be less important in the furanosides than in the pyranosides, and therefore part of the greater reactivity of α-ribofuranose over α-glucopyranose 1-phosphate could be caused by the differences in the dipolar repulsions in the ground states.

In glycoside hydrolysis the furanoside is approximately 200 times as reactive as the corresponding pyranoside, at 95°.[32] For the acid hydrolysis of ribose 1- and glucose 1-phosphates the relative reactivities are ~400 at 25° in 1.5 *M* acid and 200 for the spontaneous hydrolysis at pH 2.2 at 82°. Because of differences in the activation energies for these reactions the relative reactivities depend on temperature, but none the less they show the similarity of structural effects upon the rates of glycoside and phosphate ester hydrolysis.

The entropy of activation for the acid hydrolysis of ribose 1-phosphate is 7.5 eu less positive than for glucose 1-phosphate (see Results). The entropy differences are very large for the acid hydrolyses of alkyl furanosides and pyranosides, where the values have led some workers to suggest that A2 mechanisms are involved in the hydrolysis of the furanosides,[34,35] although it is generally believed that both sets of compounds are hydrolyzed by A1 mechanisms.[32]

An A2 mechanism cannot be involved in the acid hydrolysis of α-ribose 1-phosphate, because it would be much too slow to be observed under our reaction conditions,[4] and we believe that the differences in activation entropy arise because of differences in the ground state entropies. The furanose ring, like a cyclopentane, should be "entropy rich" because of the low energy barriers to small conformational changes,[33] but much of this freedom will be lost in the transition state because of the rigidity imposed by delocalization of charge into the ring oxygen atom. The pyranose ring is more rigid, and the loss of entropy due to changes in the flexibility of the ring in going to the transition state should be less serious here. The more effective overlap in the furanosides should make the activation energy lower, and these effects should also be important in glycosidic hydrolysis.

Registry No.—II, 59-56-3; IV, 18646-11-2.

(33) C. T. Bishop and F. P. Cooper, *Can. J. Chem.*, **41**, 2743 (1963).

(34) W. G. Overend, C. W. Rees, and J. S. Sequeria, *J. Chem. Soc.*, 3429 (1962).

(35) B. Capon and D. Thacker, *J. Amer. Chem. Soc.*, **87**, 4199 (1965).

A Nuclear Magnetic Resonance Method for Distinguishing α-Amino Acids from β and γ Isomers

RONALD G. WEBB,[1] MALCOLM W. HASKELL,[1] AND CHARLES H. STAMMER[2]

Department of Chemistry, University of Georgia, Athens, Georgia 30601

Received August 8, 1968

The methyl ester hydrochlorides and N-trityl methyl esters of 24 amino acids have been prepared and their nmr spectra determined. The methyl ester peak of each α-N-trityl ester appeared 0.27–0.97 ppm upfield of the corresponding peak in the untritylated amino ester. Methyl ester peaks in the β and γ relationship to the N-trityl function were shifted only 0.03–0.20 ppm upfield. This difference can be used diagnostically for adjacent amine and ester functions. A discussion of the size of this effect as a function of structure is presented.

In an earlier report[3] we described the preliminary results of an nmr method for distinguishing α-amino acids from isomers having amino groups β or γ to the carboxyl function. It was established that the methyl proton signal of a methyl ester function shifted upfield some 0.2–0.9 ppm when an α-amino group was tritylated. This report extends this work to 24 amino acids and more firmly establishes the necessary spacial relationships between the nitrogen substituent and the ester function.

Table I shows the results of this investigation. Clearly, α-amino ester peaks undergo a much larger diamagnetic shift[4] than β- and γ-amino ester peaks; compare amino acids **1–21** with **22–24**. The more distant ester functions shift upfield from 0.03 for γ-aminobutyric acid (**23**) to 0.21 ppm for anthranilic acid (**24**) including the β-ester group of aspartic acid (**6**), glutamic acid (**13**) and those of the hydroxyaspartic esters (**4** and **8**). Actually, anthranilic ester is a special case in which the benzene ring forces the β-amino group to be in the same plane as the ester function. The shielding effect of the N-trityl group was therefore enhanced because of its greater proximity to the ester. The largest diamagnetic shift of β-carbomethoxyl protons in a free-rotating[5] system was that of β-alanine (0.10 ppm).

The α-amino acid esters can be arranged in two distinct groups—those showing upfields shifts of 0.25–0.27 ppm and those having shifts of 0.59–0.97 ppm. The first and smaller group consists of glycine, sarcosine and proline.[6] All of the remaining esters fall into the

(1) Abstracted from the Master of Science Thesis (1967) and the Ph.D. Dissertation (1968) submitted to the graduate school of the University of Georgia by M. W. Haskell and R. G. Webb, respectively.

(2) To whom inquiries about this paper should be sent.

(3) C. H. Stammer and R. G. Webb, *Tetrahedron Lett.*, 4895 (1966).

(4) The upfield shift was due to tritylation and not just a neutralization of charge on the amino group or to a change in solvent (D_2O to $CDCl_3$). When alanine methyl ester hydrochloride was converted into the free amino ester in $CDCl_3$ a shift of only 0.10 ppm occurred. The methyl ester peak positions of leucine methyl ester hydrochloride were identical when measured in D_2O and $CDCl_3$.

(5) The α-hydroxy-β-amino esters (**4** and **8**) can not be considered entirely free-rotating systems due to H bonding.

(6) An impure sample of N-methylalanine ester also showed a $\Delta\nu$ of 0.30 ppm on tritylation.