[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

Acid and Alkaline Hydrolysis Rates and Heats of Activation of Some *o*- and *p*-Nitrophenyl Glycosides[1]

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The rates of hydrolysis and heats of activation of six *o*- and *p*-nitrophenyl glycosides have been determined in acid and alkali. At 65° these glycosides are hydrolyzed 90 to 360 times as rapidly in 0.1 *N* sodium hydroxide as in 0.1 *N* hydrochloric acid, the differences in rate being greatest for the ortho isomers. The relative rates of alkaline hydrolysis cannot be related to aglycon acidity. While the heats of activation of the *p*-glycosides are essentially comparable in acid and alkali, those of the ortho isomers are significantly lower in alkali.

In accordance with the general alkali-sensitivity of the phenyl and substituted phenyl β-D-glucosides,[2,3] *o*-nitrophenyl β-D-glucopyranoside (I) and *p*-nitrophenyl β-D-glucopyranoside (II) are alkali-sensitive.[4,5] Of several substituted phenyl β-D-glucosides studied by Montgomery, Richtmyer and Hudson,[2] I and II were the most rapidly hydrolyzed. Since quantitative information for comparison of the rates of acid and alkaline hydrolysis of this type of glycoside was lacking, the glycosides of *o*-nitrophenol (VII) and *p*-nitrophenol (VIII) were selected as typical representatives, and the rates of hydrolysis and heats of activation in acid and alkali of I, II, *o*-nitrophenyl β-D-galactopyranoside (III), *p*-nitrophenyl β-D-galactopyranoside (IV), *o*-nitrophenyl α-L-arabinopyranoside (V) and *p*-nitrophenyl α-L-arabinopyranoside (VI) were determined. The nitrophenyl glycosides are colorless, but their aglycons, VII and VIII, are yellow in alkaline solution, having absorption maxima at 420 mμ. Thus, the rate of liberation of the aglycon could be followed colorimetrically.

First-order hydrolysis rates, k, of each glycoside in 0.1 *N* hydrochloric acid at 65 and 85°, and in 0.1 *N* sodium hydroxide at 45 and 65° are given in Table I with their probable errors.[6] Probable errors averaged 2.0% of k for all determinations, and in only two cases, the alkaline hydrolyses of II and V at 65°, were they more than 3.1% of k, indicating satisfactory fits of the data to the calculated slopes. It should be stressed that fits of the data to first-order plots do not indicate the true order of reaction in alkaline hydrolysis, as sodium hydroxide (0.100 *M*) was present in over 500 times the concentration of glycoside (0.0001865 to 0.0001975 *M*).

Heats of activation ΔH_a were calculated from reaction rates at two temperatures and are given in Table I. It is apparent that the *p*-glycosides (with the possible exception of VI) have essentially comparable heats of activation for acid and alkaline hydrolysis. However, the heats of activation for the *o*-glycosides are considerably less in alkali than in acid. Heats of activation cannot be correlated with reaction rates, especially the values obtained for acid hydrolysis, indicating differences in frequency factor for the glycosides studied.

TABLE I

HYDROLYSIS RATES[a] AND HEATS OF ACTIVATION OF NITROPHENYL GLYCOSIDES IN 0.1 *N* HYDROCHLORIC ACID AND 0.1 *N* SODIUM HYDROXIDE

Glycoside	$k \times 10^4$, min.$^{-1}$, 0.1 *N* HCl 65°	85°	$k \times 10^4$, min.$^{-1}$, 0.1 *N* NaOH 45°	65°	ΔH^a, cal./mole 0.1 *N* HCl	0.1 *N* NaOH
I	1.41 ± 0.032[b]	10.5 ± 0.22	47.3 ± 0.70	362 ± 8.5	24,200	21,800
II	0.42 ± 0.011	3.51 ± 0.062	7.40 ± 0.35	82.0 ± 2.4	25,500	25,800
III	3.23 ± 0.027	26.8 ± 0.58	98.6 ± 0.72	634 ± 6.5	25,500	19,900
IV	0.90 ± 0.020	8.73 ± 0.22	7.32 ± 0.24	82.8 ± 0.84	27,400	25,900
V	14.7 ± 0.20	123 ± 1.6	928 ± 46	5240 ± 13	25,500	18,500
VI	3.88 ± 0.053	42.1 ± 0.98	42.1 ± 0.43	423 ± 3.8	28,700	24,700

[a] As is customary in carbohydrate studies, hydrolysis rates are expressed in Briggsian logarithms and minutes. [b] Probable error P_k.

Table II compares rates of hydrolysis of *o*- and *p*-glycosides at 65°, and rates of hydrolysis in acid and alkali. In both acid and alkali the *o*-glycoside hydrolyzes more rapidly than its *para* isomer. The ratios are larger in alkali, particularly for the nitrophenyl arabinosides. Because of differences in ΔH_a for the various glycosides, comparisons of hydrolysis rates are made at the same temperature, as k_{ortho}/k_{para} varies with temperature. Comparison of rates in alkali and acid indicates that the

TABLE II

COMPARISON OF HYDROLYSIS RATES OF NITROPHENYL GLYCOSIDES AT 65°

Glycoside			k_{ortho}/k_{para} 0.1 *N* HCl	0.1 *N* NaOH	$k_{0.1\ N\ NaOH}/k_{0.1\ N\ HCl}$
Glucoside	I	ortho	3.4	4.4	260
	II	para			200
Galactoside	III	ortho	3.6	7.7	200
	IV	para			92
Arabinoside	V	ortho	3.8	12	360
	VI	para			110

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(3) W. W. Pigman and R. M. Goepp, Jr., "Chemistry of the Carbohydrates," Academic Press, Inc., New York, N. Y., 1948, p. 200.

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

(5) W. F. Goebel and O. T. Avery, *J. Exptl. Med.*, **50**, 520 (1929).

(6) H. Margenau and G. M. Murphy, "Mathematics of Physics and Chemistry," D. Van Nostrand Co., Inc., New York, N. Y., 1943, p. 502.

nitrophenyl glycosides are hydrolyzed from 90 to 360 times as rapidly in alkali as in acid of the same concentration, the difference in rate being most pronounced for the ortho isomers.

The relative rates of alkaline hydrolysis of these glycosides cannot be related to the acidity of their aglycones according to the suggestion of Fisher, Hawkins and Hibbert,[7] for VII ($k_a^{25} = 6.2 \times 10^{-8}$) and VIII ($k_a^{25} = 7.0 \times 10^{-8}$) have nearly the same dissociation constant, and it would be expected that the ortho and para compounds would hydrolyze at nearly equal rates.

Experimental

The compounds studied were prepared by catalytic deacetylation of the corresponding acetylated glycosides with barium methoxide,[8] except in the case of I, where the method of Glaser and Wulwek[4] using methanolic ammonia gave the best results. The acetylated glycosides were prepared by condensation of the corresponding polyacetylglycosyl bromide with the phenol in aqueous acetone solution.[4,9] Two new glycosides, *o*-nitrophenyl and *p*-nitrophenyl α-L-arabinopyranoside were prepared.

Preparation of *o*-Nitrophenyl α-L-Arabinopyranoside (V).—To a solution of 10.5 g. of *o*-nitrophenol (VII) and 4.2 g. of sodium hydroxide in 105 ml. of water was added a solution of 18.4 g. of triacetyl β-L-arabinosyl bromide[10] in 155 ml. of acetone. After the mixture stood five hours at room temperature (25°), it was concentrated *in vacuo* (50° bath). A sirup was obtained which could not be crystallized from the usual solvents and solvent combinations. The crude sirup was suspended in 200 ml. of water and extracted with one 200-ml. and two 100-ml. portions of ether. The ether extracts were combined and extracted with three 100-ml. portions cold (5°) 2% sodium carbonate solution, then with three 100-ml. portions cold water. The ether solution thus obtained was dried with anhydrous sodium sulfate and concentrated to a sirup *in vacuo* (50° bath). Crystallization was again attempted using a number of solvents, but was unsuccessful. The sirup was dried one hour (25°, 3 mm.) and taken up in 100 ml. of dry methanol. Two ml. of 0.4 *N* barium methoxide in dry methanol was added and the mixture was allowed to stand overnight at 5°. Crystals appeared and were filtered and washed with ether. The filtrate was concentrated *in vacuo* (50°-bath) until no more crystalline material could be obtained. The crystals were combined, recrystallized twice from 95% ethanol, and washed each time with a small quantity of ether; 2.28 g. of colorless, filamentous needles was obtained, m.p. 139–139.5°, $[\alpha]^{25}D$ −48.9 (*c* 0.2660, water).

Anal. Calcd. for $C_{11}H_{13}O_7N$: C, 48.7; H, 4.83. Found: C, 48.7; H, 4.95.

Preparation of *p*-Nitrophenyl α-L-Arabinopyranoside (VI).—The procedure for the preparation of V was employed with the substitution of *p*-nitrophenol (VIII) for *o*-nitrophenol. A yield of 0.86 g. of colorless, fine needles was obtained, m.p. 201–202°, $[\alpha]^{25}D$ −22.4 (*c* 0.2460, water).

Anal. Calcd. for $C_{11}H_{13}O_7N$: C, 48.7; H, 4.83. Found: C, 48.6; H, 4.89.

Determination of the Hydrolysis Rates of I–VI in 0.1 *N* Hydrochloric Acid at 65 and 85°.—A thermostatically controlled water-bath accurate to ±0.2° was used. The following solutions of nitrophenyl glycosides in water were prepared: I (monohydrate) 476 mg./l. (0.001492 *M*); II–IV 476 mg./l. (0.001580 *M*); V and VI 428 mg./l. (0.001580 *M*). Fifty ml. of glycoside solution in an 125-ml. erlenmeyer flask and about 75 ml. of 0.200 *N* hydrochloric acid in a 100-ml. volumetric flask were heated at bath temperature for 15 minutes. Both flasks were stoppered during the heating period. Then, 50.0 ml. of acid was pipetted into the flask containing the glycoside solution, mixing being accomplished by the jet of acid entering from the pipet. This flask was kept securely stoppered during the determination except for brief periods when samples were removed. Five to six 10.00-ml. samples were removed at approximate intervals (usually every 15 minutes) and placed in 50-ml. erlenmeyer flasks containing 5.00 ml. of 0.200 *N* sodium hydroxide and 1.00 ml. of 1 *M* Clark and Lubs *p*H 10.0 buffer. Flasks were swirled to ensure adequate mixing, and their contents were poured into matched 18-mm. Pyrex culture tubes. The optical density of each sample was determined at 420 mμ in an Evelyn colorimeter against a blank composed of 5.00 ml. of 0.200 *N* NaOH, 5.00 ml. of 0.200 *N* HCl, 1.00 ml. of the buffer and 5.00 ml. of glycoside solution. No attempt was made to correct for changes in volume at the temperatures of hydrolysis.

Logarithms of glycoside concentration were plotted against time in minutes by the method of least squares, and the linear relationship characteristic of first-order reactions was established.

Determination of the Hydrolysis Rates of I–VI in 0.1 *N* Sodium Hydroxide at 45 and 65°.—Glycoside solutions had the following composition: I (monohydrate) 119 mg./l. (0.0003730 *M*); II–IV 119 mg./l. (0.0003950 *M*); V and VI 107 mg./l. (0.0003950 *M*). The procedure was the same as in the determination of acid hydrolysis rates except for the use of 50.0 ml. of 0.200 *N* sodium hydroxide in the hydrolyzing mixture and the use of 5.00 ml. of 0.200 *N* HCl instead of 0.200 *N* NaOH in the mixture in the sample flasks. Samples were taken at shorter intervals (one to ten minutes).

Determination of the Curves for Concentration of VII and VIII *versus* Optical Density.—*o*-Nitrophenol (VII) and *p*-nitrophenol (VIII) do not adhere to Beer's law under the conditions of these experiments. The optical density at 420 mμ of solutions of varying amounts of VII and VIII plus equivalent molar quantities of D-galactose in a mixture of 5.00 ml. of water, 5.00 ml. of 0.200 *N* HCl, 5.00 ml. of 0.200 *N* NaOH and 1.00 ml. of 1 *M* Clark and Lubs *p*H 10.0 buffer was determined in the matched tubes used in the hydrolysis rate studies. The blank was composed of the same mixture with no phenol and D-galactose. Optical density was plotted against phenol concentration in mg./l.

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