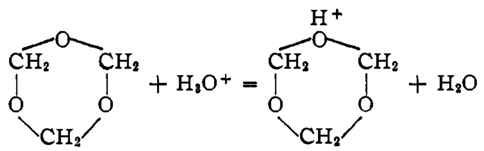
toward simple basic indicators that accompanies the addition of 2 *M* $NaClO_4$, shown by Harbottle's data, is quite accurately reflected in an approximately sixfold increase in the reaction rate; a similar correlation (in 2 *M* $HClO_4$ + 4 *M* $NaClO_4$) has been observed by Long and Purchase in the hydrolysis of β-propiolactone.[6] One will note also the significant spread at 6 *M* concentrations among the three "strong" acids, $HClO_4$, H_2SO_4 and HCl, the order of acid strength being plainly manifested by both the indicator acidity and the reaction rate.

These new results confirm and extend the correlation between log *k* and H_0 previously noted.[8] They are consistent with the supposition that the mechanism of the reaction depends on a rapid equilibrium between trioxane and its conjugate acid ion



followed by a rate-determining step consisting of internal rearrangement and decomposition of the ion. The reaction thus appears to be an unusually satisfactory one for the study of acid catalysis, and should be particularly valuable for the investigation of acidity in non-aqueous media.

The author takes pleasure in acknowledging his indebtedness to Professor F. A. Long of Cornell University for helpful suggestions concerning the dilatometric technique.

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(8) Ref. 4. The rate constants of Walker and Chadwick used in Fig. 1 of that note were expressed in sec.$^{-1}$; the present data indicate that the correlation line there drawn between log *k* and H_0 should be displaced upward by about 0.08 logarithmic unit.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF OKLAHOMA]

Acid Dissociation Exponents of Rutin and Xanthorhamnin[1]

BY WILLIAM L. HOWARD[2] AND SIMON H. WENDER

Rutin is a polybasic acid with four dissociable groups measurable in aqueous solution of pH up to 12.5, while xanthorhamnin behaves as a dibasic acid in this range. Dissociation exponents are: for rutin, pK_1 7.40, pK_2 8.70, pK_3 12.6, pK_4 14.2; for xanthorhamnin, pK_1 8.57, pK_2 10.9.

During the investigation of the isolation and purification of flavonoid compounds by means of ion exchange procedures, it became desirable to know the dissociation behavior of some of these substances. Because of their pharmaceutical interest, relative water solubility, and availability, rutin and xanthorhamnin were chosen for this study.

Experimental

Method.—Weighed samples of the acids were titrated in accurately known volumes of water solution under carbon dioxide-free conditions maintained by passing nitrogen over soda lime and then through the solutions. After the addition of each increment of base, the pH was determined with a Beckman pH meter, Model H, using a type E glass electrode to minimize alkaline error. The data were interpreted by the method of Simms[3] and by means of a method outlined by Glasstone.[4] The measurements were made at room temperature which had a day to day variation from 22 to 24° but the temperature of the solutions remained constant to within 0.5° during a single titration.

For the determination of K_1 and K_2, accurately weighed samples of rutin of the order of 0.35 g. were titrated with 0.426 *N* NaOH in volumes of solution of the order of 500 ml. Xanthorhamnin samples of about 0.30 g. were titrated similarly in volumes of about 50 ml. To determine K_3 and K_4 of rutin, 0.35-g. samples were half-neutralized and titrated with 1.80 *N* KOH in 50-ml. volumes.

At low pH, the hydroxyl ion concentration could be taken as its activity, calculated from the pH and K_w, with negligible error. At higher pH values, it was determined by direct titration of water in the evaluation of K_1 and K_2. In evaluating K_3 and K_4 of rutin, the ionic strength was of the order of 0.1, so the hydroxyl ion concentration was determined by titrating a solution of sodium sulfate of ionic strength made arbitrarily to 0.1.

Activity coefficients were calculated by means of the Debye–Hückel theory for appreciable concentrations by the equation $\log f = -Az^2\sqrt{\mu}/(1 + \sqrt{\mu})$, as given by Glasstone.[5] The ionic strength was determined using the approximations mentioned by Glasstone,[4] in interpreting the data for K_1 and K_2. In the calculations leading to K_3 and K_4 of rutin, the ionic strength was determined by making use of the known composition of the solutions, with the concentrations of the rutin anions being determined from the known values of pK_3' and pK_4' and utilizing the conditions for electroneutrality of the solutions.

In employing the method of Simms, pK' values were calculated from the average values of pG'. The appropriate activity coefficients at $p\mathrm{H} = pK'$ were then determined and utilized to calculate pK from the equation $pK = pK' - \log(f_b/f_a)$ where f_b and f_a are the activity coefficients of the basic and acid forms, respectively, of the substance involved in the equilibrium. In a similar manner, values of pK_3' and pK_4' of rutin, obtained from Fig. 3, were converted to pK_3 and pK_4.

Preparation of the Materials.—Rutin N.F. IX (S. B. Penick and Co., New York) was recrystallized from water–alcohol solution and air-dried, then dried for several hours at 110°. By the method of Porter, *et al.*,[6] the product was found to contain 94.5% rutin, a composition corresponding to that of rutin dihydrate.

Xanthorhamnin (S. B. Penick and Co.) was freed of a reddish impurity, which has the properties of an acid of lower equivalent weight, by recrystallizing several times from a mixture of ethyl and isopropyl alcohols. The bright yellow crystals were air-dried, then dried for several hours at 110°. Titration of this product indicated a molecular weight of 770, that of anhydrous xanthorhamnin.

Results.—Values of *X* and *Y* appropriate to the equation $Y = XK_1 + K_1K_2$ given by Glasstone[4] were calculated from the titration data and plotted to give the straight lines from which K_1 and K_2 can be determined. The data for the first two dissociations of rutin are shown in Fig. 1 and those for xanthorhamnin in Fig. 2.

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(1) This research was supported in part by a grant-in-aid from The Office of Naval Research (Project NR-059 226).

(2) Biological Department, Army Chemical Corps., Camp Detrick, Frederick, Maryland.

(3) H. S. Simms, THIS JOURNAL, **48**, 1239 (1926).

(4) S. Glasstone, "An Introduction to Electrochemistry," D. Van Nostrand Co., Inc., New York, N. Y., 1942, p. 325.

(5) S. Glasstone, ref. 4, p. 146.

(6) W. L. Porter, B. A. Brice, M. J. Copley and J. F. Couch, U. S. Dept. Agr., Circular AIC-159 (1947).

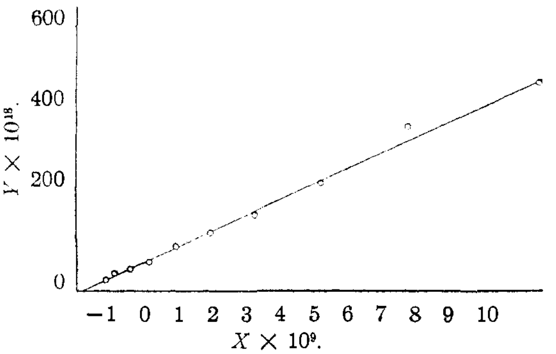

Fig. 1.—Rutin.

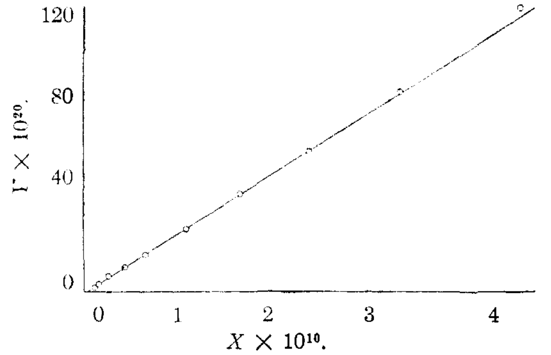

Fig. 2.—Xanthorhamnin.

Preliminary estimates indicated that pK_3 and pK_4 of rutin are at least two logarithmic units greater than pK_2, and therefore the dissociation of the first and second groups is practically complete before the third and fourth are appreciably ionized. Thus, the first two groups and the last two can be treated with negligible error as two independent dibasic acids. From the titration data for half-neutralized rutin, values of X' and Y' were calculated in the same manner as X and Y, but neglecting activity coefficients, and these are plotted in Fig. 3 to give a straight line from which K_3' and K_4' can be determined.

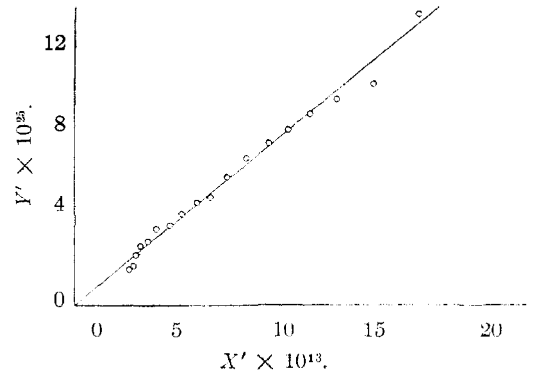

Fig. 3.—Determination of K_3' and K_4' of rutin.

In Table I are collected the values of the dissociation exponents calculated from the illustrative data and other sets of similar data.

Xanthorhamnin was titrated to pH 12.5, but K_3, if it exists, is so small that a third dissociation of the xanthorhamnin anion is not definitely revealed by these methods.

Discussion

Rutin has four phenolic hydroxyl groups potentially capable of dissociating a hydrogen ion, and since four dissociations are realized, it is reasonable to implicate these groups. The ratios $K_1:K_2$ and $K_3:K_4$ are about 25, approaching the value 4 calculated by Adams[7] for independent dissociation of equivalent groups of a polybasic acid. Thus the first dissociation exerts only a moderate effect on the second, and similarly for the third on the fourth, but the first two dissociations considerably diminish the ease of ionization of the last two groups.

The dissociations of only two of the three phenolic hydroxyl groups of xanthorhamnin are realized in the pH range studied and, since the ratio $K_1:K_2$ is of the order of 100, the first dissociation renders the second much more difficult. The effect may extend to the third group, thus accounting for the failure to detect a third dissociation under the above conditions. This behavior, in contrast to that of rutin, is striking in view of the structural similarity of the two substances.

The values of pK_3 and pK_4 of rutin are reported to only one decimal place because of the approximations involved in calculating the activity coefficients of the anions of three and four charges.

TABLE I

DISSOCIATION EXPONENTS AT 23 ± 1°

Column I, calculations by method of Glasstone; Column II by method of Simms; Column III, average of values in Columns I and II.

	Rutin I	Rutin II	Rutin III	Xanthorhamnin I	Xanthorhamnin II	Xanthorhamnin III
pK_1	7.43	7.44		8.59	8.53	
	7.35	7.37	7.40	8.62	8.54	8.57
pK_2	8.74	8.72		10.78	11.13	
	8.74	8.60	8.70	10.80	11.01	10.9
pK_3	12.7					
	12.5		12.6			
pK_4	14.1					
	14.2		14.2			

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[7] E. Q. Adams, THIS JOURNAL, **38**, 1503 (1916).