

On the other hand, a unit or near unit slope in the Hammett plot is not a *sufficient* basis on which to assign the A-1 mechanism. This was first shown a number of years ago.^{39,40} Since then, the entire Zucker-Hammett hypothesis has been undergoing considerable

(38) N. C. Deno and C. Perizzolo, *J. Am. Chem. Soc.*, **79**, 1345 (1957).

(39) It was also suggested that a general catalysis mechanism of one or more bimolecular proton transfer steps might well give an approximately linear Hammett plot.⁴⁰

(40) W. M. Schubert and R. E. Zahler, *J. Am. Chem. Soc.*, **76**, 1 (1954); W. M. Schubert and H. Burkett, *ibid.*, **78**, 64 (1956); W. M. Schubert and P. C. Myhre, *ibid.*, **80**, 1755 (1958).

reappraisal.^{5,6,33,38,40-42} One definite conclusion emerges, and that is that generally much more than a study of the acidity dependence of a reaction catalyzed in strong acids is necessary to establish unambiguously its mechanism.

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(41) J. F. Bunnett, *ibid.*, **83**, 4956, 4968, 4973, 4978 (1961).

(42) L. Melander and P. C. Myhre, *Arkiv Kemi*, **13**, 507 (1959).

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Kinetics of the Acid-catalyzed Hydrolysis of 1-Methoxy-3,5-dihydroxybenzene and 1,3,5-Trimethoxybenzene

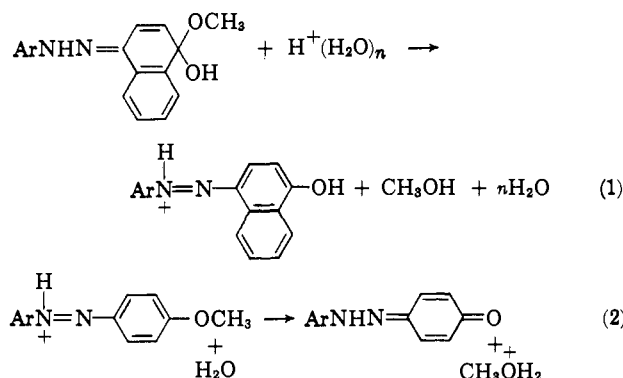
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First-order rate constants for the hydrolysis of 1-methoxy-3,5-dihydroxybenzene and 1,3,5-trimethoxybenzene in strong perchloric acid solutions have been determined. Methanol containing the natural abundance of O¹⁸ has been isolated from a hydrolysis in O¹⁸-enriched acid, showing that Ar-O rather than CH₃-O cleavage occurs. The large increase in *k*_{obs} with increased mineral acid percentage, continuing on after equilibrium protonation to the carbon conjugate acid is extensive, is difficult to reconcile with a one-proton process. Analysis of the results indicates that both a one-proton and two-proton process contribute to the total rate in the lowest acid percentages used and that the two-proton process becomes predominant in higher acid percentages. Clear-cut mechanistic assignments based on the data at hand are not possible, however.

In a spectrophotometric study of the acid-catalyzed decarbonylation of 2,4,6-trimethoxybenzaldehyde, it was observed that the ultraviolet spectrum of the product of this reaction, 1,3,5-trimethoxybenzene, suffered slow change in moderately strong mineral acids and became identical with the spectrum of phloroglucinol.¹ It appeared from this that ethers of phloroglucinol would be suitable substrates for a kinetic study of ether hydrolysis. No detailed studies of the acidity dependence of the rates of ether hydrolysis reactions had been published at the time work on the problem commenced (see, e.g., ref. 2 and 3), and as a first objective it appeared desirable to study the effect of mineral acid acidity on the rate of hydrolysis of 1-methoxy-3,5-dihydroxybenzene and 1,3,5-trimethoxybenzene. Since the extent of equilibrium carbon protonation of these ethers (eq. 3) is extensive in the perchloric acid solutions in which hydrolysis rates were measured, it also was necessary to study the acidity dependence of this equilibrium.⁴

After this work was begun, Bunnett and Buncl published the results of an extensive kinetic study of the acid-catalyzed hydrolysis of 4-(*p*-sulfophenylazo)-1-methoxynaphthalene and 4-(*p*-sulfophenylazo)-1-anisole,⁵ reactions that are similar to the hydrolysis of phloroglucinol ethers. The hydrolysis of the naphthyl ether was assigned a mechanistic path proceeding *via* nucleophilic attack of water on the aromatic 1-position of the conjugate acid followed by the rate-controlling step represented by eq. 1. The assignment was based on the magnitude of the *w*-value⁶ and the recovery of O¹⁸-enriched naphthol from a hydrolysis carried out in O¹⁸-enriched acid.^{5a} The hydrolysis of the structurally very similar 4-(*p*-sulfophenylazo)-1-anisole was tentatively assigned a different path, involving rate-control-



ling attack of water on the methyl group of the conjugate acid, eq. 2.^{5a} More recently, however, as the result of further work including O¹⁸-exchange experiments, the hydrolysis of 4-(*p*-sulfophenylazo)-1-anisole has been assigned the same mechanistic path as the hydrolysis of the naphthyl analog.^{5b}

Rate constants for the hydrolysis of diethyl ether in 0.2–5.65 molar perchloric acid at 120° have been reported by Koskikallio and Whalley.⁷ They concluded that the reaction proceeded by an A-2 mechanism (rate-controlling attack of water on the conjugate acid). Log *k*_{obs} increased faster than log [H₃O⁺], in contradiction to the Zucker-Hammett hypothesis,⁸ and the plot of log *k*_{obs} against $-H_0$ (*H*₀ values at room temperature) was approximately linear, with slope ~0.5.

Experimental

Materials.—The preparation of materials used is described in the preceding paper.^{4b}

Kinetic Method.—The decline in the spectrum of perchloric acid solutions about 10⁻⁴ molar in ether was followed by the method previously described, using a Beckman DU instrument fitted with a constant temperature water-bath.⁹ Temperature was maintained to within ±0.03° by means of a Sargent Thermometer unit.

Hydrolysis in O¹⁸-Enriched Acid.—A solution of 1.4 g. of 1,3,5-trimethoxybenzene in 100 ml. of 38% hydrochloric acid contain-

(1) H. Burkett, W. M. Schubert, F. Schulz, R. B. Murphy and R. Talbot, *J. Am. Chem. Soc.*, **81**, 3923 (1959).

(2) R. L. Burwell, Jr., *Chem. Rev.*, **54**, 616 (1954).

(3) F. A. Long and M. A. Paul, *ibid.*, **57**, 935 (1957).

(4) (a) W. M. Schubert and R. H. Quacchia, *J. Am. Chem. Soc.*, **84**, 3778 (1962); (b) **85**, 1278 (1963).

(5) (a) J. F. Bunnett and E. Buncl, *ibid.*, **83**, 1117 (1961); (b) J. F. Bunnett, E. Buncl and K. V. Nahabedian, *ibid.*, **84**, 4136 (1962).

(6) (a) J. F. Bunnett, *ibid.*, **83**, 4956 (1961); (b) **83**, 4968 (1961).

(7) J. Koskikallio and E. Whalley, *Can. J. Chem.*, **37**, 788 (1959).

(8) L. Zucker and L. P. Hammett, *J. Am. Chem. Soc.*, **61**, 2791 (1959).

(9) W. M. Schubert and R. E. Zahler, *ibid.*, **76**, 1 (1954).

ing 1.7 atom per cent of excess O^{18} was stirred at room temperature for 24 hours (ca. nine half-lives). The solution was cooled in an ice-bath while 40 g. of solid potassium carbonate was added in small portions. The mixture was pressure filtered and the filtrate treated in the same manner with another 35 g. of potassium carbonate. The mixture was again pressure filtered and the filtrate distilled through a modified Claisen head. The first 3 ml. of distillate were redistilled through a Young column.¹⁰ A total of 0.6 ml. (60% yield) of methanol was collected, b.p. 64°, n_D^{25} 1.3274 (reported¹¹ n_D^{25} 1.3276). A mass spectral analysis was made by Mr. B. J. Nist using a Consolidated type 21-103 mass spectrometer at an ionization voltage of 7 v. The ratio of the 32 to 34 peak heights corresponded to that of a normal sample of methanol. The *maximum* amount of excess O^{18} in the methanol recovered from hydrolysis was 0.1 atom per cent. The same result was obtained on another sample of methanol recovered from a duplicate hydrolysis. A 70% yield of pure phloroglucinol was obtained from a hydrolysis carried out on 0.5 g. of 1,3,5-trimethoxybenzene in 50 ml. of 63.5% $HClO_4$.

Results

Hydrolysis of 1-Methoxy-3,5-dihydroxybenzene.—Phloroglucinol monomethyl ether was found to hydrolyze readily in 50–70% perchloric acid. This was accompanied by a decrease in the intensity of the ultraviolet spectrum in the region of the peaks of the conjugate acid.

The spectra of the monoether and phloroglucinol are similar in all acid percentages.⁴ The optimum wave length to follow the reaction proved to be between 255 and 260 $m\mu$, where the measured optical density change over the entire reaction was from 0.4 to 0.6 unit. A complicating factor was the slow decomposition of the phloroglucinol product. This was manifest by a slow increase in the optical density of the solution after the initial decline over 4–5 half-lives. Because of this, calculated rather than directly observed D_∞ values were used in the first-order plots.¹² The calculated D_∞ values, which were about 5–15% lower than the observed (measured after 7 half-lives), were those calculated for fresh solutions of phloroglucinol at the same concentration and temperature as the initial ether. The first-order plots of $\log(D - D_\infty)$ against time were linear over a goodly portion of the reaction, from 0.7 half-life in the lowest perchloric acid percentage to 2 half-lives in the highest acid percentage, and then curved upward, as is to be expected. The pertinent data are given in Table I.

The Hydrolysis of 1,3,5-Trimethoxybenzene.—If the hydrolysis of 1,3,5-trimethoxybenzene in strong perchloric acid were sufficiently slower than that of the diether and the monoether, then the rate of formation of phloroglucinol would be equal to the rate of hydrolysis of triether to diether and simple first-order kinetics should apply. Unfortunately, such a treatment is not entirely valid since the hydrolysis reactions of di- and monoether are not sufficiently more rapid than the hydrolysis of the triether. For example, the rate constants in 52.2% perchloric acid at 60° for tri-, di- and monoether are 1.2×10^{-4} , approximately 15×10^{-4} and 7.8×10^{-4} sec.⁻¹, respectively. Ideally, a treatment in terms of three successive first-order reactions (or four successive reactions, if one includes the slow decomposition of phloroglucinol) should be applied. An attempt was made to do this with the aid of a 709 computer, but it soon became apparent that the return did not warrant the investment of time and money in such a procedure. Fortunately, errors introduced by the second and third steps of hydrolysis could be minimized by a judicious choice of wave lengths at

(10) F. Schneider, "Quantitative Organic Microanalysis," J. Wiley and Sons, Inc., New York, N. Y., 1947, p. 31.

(11) I. M. Heilbron, "Dictionary of Organic Compounds," Vol. III, Oxford University Press, New York, N. Y., 1953, p. 291.

(12) A treatment in terms of successive reactions could not be applied because the slow decomposition of phloroglucinol was not cleanly any particular order.

TABLE I
DATA FOR THE HYDROLYSIS OF 1-METHOXY-3,5-DIHYDROXYBENZENE IN PERCHLORIC ACID AT 50.0°

$HClO_4$, %	$\frac{[SH^+]}{[S]}$	$k_{obs} \times 10^4$, sec. ⁻¹ ^b	Half-lives ^c	D_0 ^d	Calcd. D_∞ ^e
50.0	1.07	1.88	0.7	0.670	0.261
52.1	2.19	2.78	0.9	.800	.289
54.0	4.07	4.02	1	.670	.209
54.2	4.37	4.43	1	.602	.190
55.7	7.59	5.65	1	.705	.193
57.9	19.95	7.86	1	.740	.187
58.0	20.90	8.91	1	.600	.159
60.0	51.3	12.6	1.3	.740	.164
60.3	58.9	14.0	1.3	.600	.175
61.2	91.2	16.0	1.5	.700	.154
63.5	295	24.9	2	.790	.163
63.5 ^f	295	16.3	2
63.5 ^g	295	10.2	2
63.5 ^h	295	3.80	2
63.5 ⁱ	295	1.31	2

^a Room temperature values, either measured directly, or extrapolated with the aid of the relationship $-d \log ([SH^+]/[S])/dH_0 = 0.98$.⁴ ^b Average of two or more runs, maximum deviation $\pm 1.5\%$. ^c Number of half-lives for which $\log(D - D_\infty)$ vs. time was linear. ^d Observed initial optical density at 260 $m\mu$ for one run. ^e Optical density of phloroglucinol at 260 $m\mu$ at 50.0° and at the same concentration as initial ether. These values, used in the first-order plots, are 5–15% lower than the observed D_∞ after 7 half-lives. ^{f–i} At 45°, 40°, 30° and 20°, respectively; $E_A = 18.5 \pm 0.2$ kcal./mole, ΔS^\ddagger at 25° = -15.2 ± 0.7 e.u.

which to follow the decline in spectrum. At the wave lengths of choice (252–255 $m\mu$), the intensity of the spectrum was much greater for the triether than for diether, monoether and phloroglucinol. In this region of the spectrum, the relative intensities for the latter three compounds are similar and do not change a great deal with acid percentage.¹³ For example, in 52.2% perchloric acid at 60°, the ϵ -values at 255 $m\mu$ are approximately 13,000, 2,400, 2,200 and 1,000 for triether, diether, monoether and phloroglucinol, respectively.¹⁴ This means that although there might be a slight build-up during a run of the concentrations of di- and monoether, the effect this build-up could have on the actual physical measurement used (*i.e.*, $D - D_\infty$), is considerably minimized. In short, the values of k_{obs} obtained (Table II) are at least an approximate measure of the first-order rate constants for cleavage of triether to diether and are considered good enough to at least discern trends in the acidity dependence of the hydrolysis.

TABLE II
VALUES OF k_{obs} AND $[SH^+]/[S]$ FOR 1,3,5-TRIMETHOXYBENZENE

$HClO_4$, %	$\frac{[SH^+]}{[S]}$	$k_{obs} \times 10^4$, sec. ⁻¹ , 90° ^c	$HClO_4$, %	$\frac{[SH^+]}{[S]}$	$k_{obs} \times 10^4$, sec. ⁻¹ , 60° ^c
44.7	0.085 ^a	1.20	52.4	2.18 ^a	1.20
48.1	.372 ^b	4.55	54.2	4.41 ^a	2.55
50.2	.847 ^a	9.59	57.9	37.2 ^b	8.10
52.4	2.18 ^a	19.9	60.0	100 ^b	17.5
54.2	4.41 ^a	36.0	61.2	214 ^b	30.4

^a Directly measured, at room temperature.⁴ ^b Extrapolated room temperature values, using $-d \log ([SH^+]/[S])/dH_0 = 1.26$.⁴ ^c Average of two or more runs, duplicable to $\pm 2\%$. The accuracy is less (see Results section).

(13) Although the basicities of the compounds are approximately the same, the relative intensities changed somewhat at between 44 and 53% perchloric acid because the acidity dependence of the protonation is somewhat different for each of the three compounds.⁴ In higher acid percentages, protonation is extensive, and thus the relative intensities changed very little.

(14) The wave lengths chosen are on the side of the sharp peak of the triether, and this could be a source of increased random error in the readings of the optical density. However, such error was minimized by keeping the wave length and slit width settings constant throughout a kinetic run.

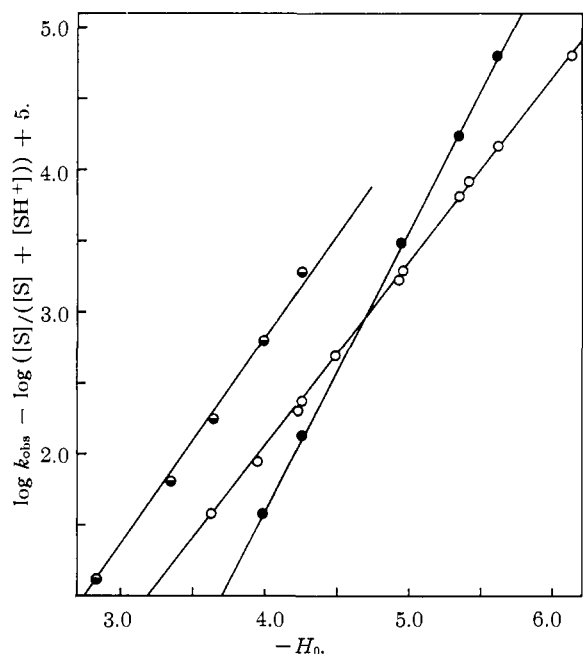


Fig. 1.— $\log k_{\text{obs}} - \log ([S]/([S] + [SH^+] + 5))$ vs. $-H_0$: \circ , 1-methoxy-3,5-dihydroxybenzene at 50° ; \bullet , 1,3,5-trimethoxybenzene at 90° ; \bullet , 1,3,5-trimethoxybenzene at 60° .

The plots of $\log (D - D_\infty)$ against time were linear for one to two half-lives. The slope of the initial linear portion was taken as a measure of the rate constant for cleavage of triether to diether. In 52.2% perchloric acid at 60° , treatment of the data in terms of three successive first-order reactions (using the known ϵ data, the rate constant for monoether hydrolysis and the approximate rate constant for diether hydrolysis) gave a value of the first-order rate constant for hydrolysis of triether to diether that was within 3% of the value obtained from the direct plot of $\log (D - D_\infty)$ against time.

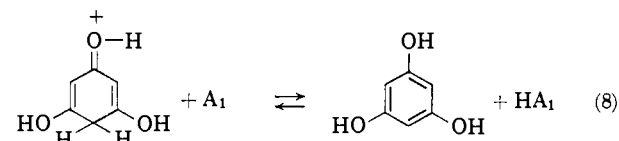
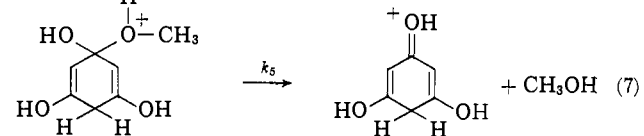
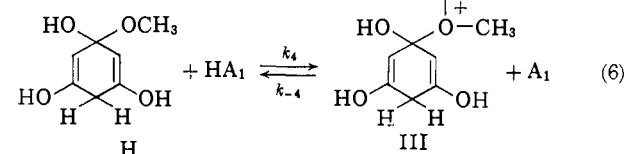
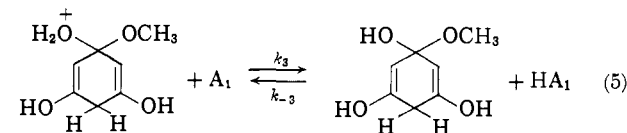
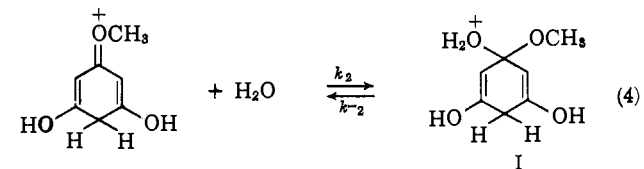
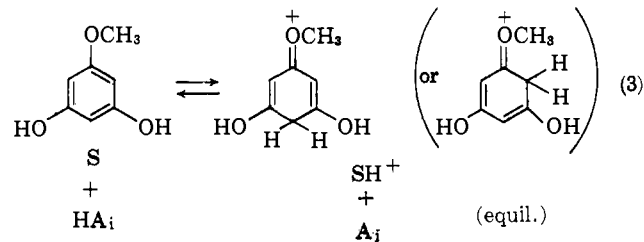
Discussion

The data at hand for an attempt to elucidate the mechanism of the hydrolysis of the methyl ethers of phloroglucinol include: (1) fairly accurate first-order rate constants for 1-methoxy-3,5-dihydroxybenzene in 50–63.5% perchloric acid at 50° (Table I) and approximate values of first-order rate constants for the hydrolysis of 1,3,5-trimethoxybenzene in 44.7 to 54.7% perchloric acid at 90° and in 52.4 to 61% perchloric acid at 60° (Table II); (2) accurate measurements in the same media of the extent of equilibrium carbon protonation of the ethers (eq. 3)⁴; (3) knowledge from O^{18} experiments that the Ar–O rather than the CH_3 –O bond is cleaved; and (4) some activation energy data.

The hydrolysis rates show an unusually steep dependence on the acidity of the medium. Even after the reversible protonation to the carbon conjugate acid (eq. 3) is extensive, the rates continue to rise rapidly as the perchloric acid percentage is increased. Thus, k_{obs} for 1-methoxy-3,5-dihydroxybenzene increases about 13-fold between 50.0 and 63.5% perchloric acid, whereas the percentage of conjugate acid increases from 52 to 99.7%, a factor of only two (Table I). In the case of trimethoxybenzene, even though the values of k_{obs} may be only approximate (see Results section), it can definitely be concluded that the hydrolysis of this compound also shows a very steep acidity dependence. Thus, between 44.7 and 54.2% perchloric acid, k_{obs} at 60° increases by a factor of 52, whereas the percentage of conjugate acid increases from 8 to 80%, a factor of 10 (Table II). The acidity dependence is even steeper

in the higher acid percentages, in which the degree of conjugate acid formation is considerable. Although protonation is already 69% complete in 52.4% perchloric acid, k_{obs} increases further by a factor of 200 between 52.4 and 61.2% perchloric acid (Table II).

It is interesting to note that the Hammett plots of $\log k_{\text{obs}} - \log ([S]/([S] + [SH^+] + 5))$ against $-H_0$ are approximately linear for the three sets of data (Fig. 1). The plot for 1-methoxy-3,5-dihydroxybenzene (data from Table III) has a slope of 1.30. The corresponding plots for 1,3,5-trimethoxybenzene (data from Table IV), for which the k_{obs} data are less accurate, have an approximate slope of 1.5 (line curves upward) for the rate data in 44.7–54.2% perchloric acid at 90° , and a slope of 2.0 for the rate data in 52.4–61.2% perchloric acid at 60° . The approximate linearity of these plots is not to be taken as meaning that these hydrolysis reactions proceed by a Hammett, or A-1, mechanism involving a single proton. Indeed, the largeness of the slopes could be construed as meaning that such a mechanism is unlikely. It should be pointed out that the values of $\log ([S]/([S] + [SH^+] + 5))$ and H_0 used are room temperature values, whereas k_{obs} was determined at an elevated temperature. However, it has been observed that the degree of equilibrium protonation to SH^+ increases slightly with temperature, although no quantitative estimate of the temperature effect has yet been made. A temperature correction on $\log ([S]/([S] + [SH^+] + 5))$ would lead to an even greater slope for the lines of Fig. 1.



(15) See footnote 37 of the paper preceding this one.^{4b}

The steep acidity dependence of the ether hydrolysis rates, as illustrated in Fig. 1, is suggestive of a two-proton process. On the other hand, a one-proton process cannot be entirely ruled out on the sole basis of the acidity dependence of the reaction (*cf.* the Discussion in the preceding paper^{4b}).

One-proton Process.—From a mechanistic standpoint, the most reasonable path for a one-proton hydrolysis is that represented by eq. 3–8. This path is consistent with known equilibrium carbon protonation, and the fact that Ar–O rather than O–CH₃ cleavage occurs. In these equations, charges on solvent acids, HA_i, and their conjugate bases, A_i, are left unspecified.

This path is essentially the reverse of that assigned by Wiberg and Saegbarth, on the basis of an O¹⁸ experiment, to the acid-catalyzed methylation of 1-naphthol.¹⁶ It also is very similar to the path assigned by Bunnett and Buncl to the hydrolysis of 4-(*p*-sulfo-phenylazo)-1-methoxynaphthalene.⁵ It is reasonable to assume that the fifth step, eq. 7, of the above reaction scheme is irreversible under the reaction conditions, since the stoichiometric concentration of methanol in the solution never exceeds 10⁻⁴ molar and reaction 8 is a rapid equilibrium.⁴ This assumption, coupled with the steady state assumption for the intermediates I, II and III and the knowledge that the first step, eq. 3, is an equilibrium, can be used in the derivation of the general rate expression, eq. 9, which applies regardless of whether the second, third, fourth or fifth step is rate-controlling. In eq. 9, the symbols *f* refer to activity coefficients and *v* to velocities.

$$k_{obs} = \frac{[SH^+]}{[S] + [SH^+]} \frac{k_2 a_{H_2O} f_{SH^-} / f_{tr^+}}{1 + A + B + C} = \frac{[S]}{[S] + [SH^+]} \text{const.} \times \frac{[H_3O^+] f_S f_{H_3O^+} / f_{tr^+}}{1 + A + B + C} \quad (9)$$

where $A = v_{-2}/v_3$, $B = Av_{-3}/v_4$ and $C = Bv_{-4}/v_5$.

Attempts to rationalize the rapid increase in rate with acid percentage, manifest in Fig. 1, by considering various steps of the above reaction scheme as rate controlling have been ungratifying. As a first alternative, consider the second step, eq. 4, rate controlling (*i.e.*, unity large relative to $A + B + C$) or the fifth step rate controlling (C large). Under either condition, the general rate expression, eq. 9, reduces to the form of eq. 10 or 11. Equations 10 and 11 are the familiar ones considered by Zucker and Hammett for the enolization of acetophenone.^{8,17}

$$k_{obs} = \text{const.} \frac{[SH^+] a_{H_2O}}{[S] + [SH^+]} \frac{f_{SH^+}}{f_{tr^+} \text{ or } f_S} \quad (10)$$

$$k_{obs} = \text{const.} \frac{[S][H_3O^+]}{[S] + [SH^+]} \frac{f_S f_{H_3O^+}}{f_{tr^+} \text{ or } f_S} \quad (11)$$

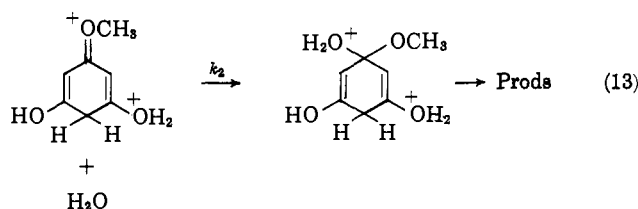
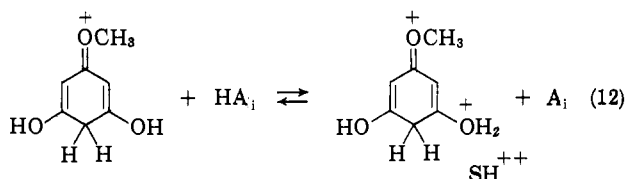
Both the quantity $[SH^+] a_{H_2O} / ([S] + [SH^+])$ of eq. 10 and the quantity $[S][H_3O^+] / ([S] + [SH^+])$ of eq. 11 decrease considerably for both 1-methoxy-3,5-dihydroxybenzene and 1,3,5-trimethoxybenzene over the range of mineral acid used, whereas k_{obs} increases considerably. For the hydrolysis of 1-methoxy-3,5-dihydroxybenzene, the increase in k_{obs} over the mineral acid range used would require the activity coefficient ratio of eq. 10 to increase 44-fold or the activity coefficient ratio of eq. 11 to increase 1200-fold. Even greater increases in the appropriate activity coefficient ratios would be required to fit the data for 1,3,5-trimethoxybenzene to eq. 10 or 11. Assuming either the third step (eq. 5) or the fourth step (eq. 6) rate-controlling, or assuming various steps of the one-proton reaction scheme as competing rate-controlling steps does not improve matters.

(16) K. B. Wiberg and K. A. Saegbarth, *J. Org. Chem.*, **25**, 832 (1960).

(17) For the enolization of acetophenone, Zucker and Hammett used $[S]$ rather than $[S] + [SH^+]$ in the denominator.⁸ In that instance, $[S] \gg [SH^+]$ and hence $[S] + [SH^+] \cong [S]$.

For the data to be consistent with a one-proton process, even including one in which the transition state does not contain a molecule of water (however difficult to conceive this is) would require an unprecedented increase with increasing mineral acid percentage of activity coefficient ratios that historically were treated as being relatively insensitive to medium. However, in view of recent developments in the field of acid-base catalysis in strong mineral acids (see Discussion in ref. 4 and references therein) such dramatic changes in ratios of activity coefficients of like charged species cannot be ruled out with certainty. In short, while a one-proton process for the hydrolysis of mono- or triether over the acid range investigated seems unlikely, it cannot be ruled out as a possibility. However, it is to be emphasized that k_{obs} increases with acid percentage much more than does the actually measured degree of protonation of S.

Two-proton Processes.—The appreciable contribution of a two-proton process to the over-all hydrolysis is qualitatively consistent with the fact that k_{obs} continues to increase considerably as the acidity of the medium is increased beyond that in which the substrate is already largely in the form of SH⁺.¹⁸ The path represented by eq. 12 and 13, or some variation thereof,¹⁹ is conceivable, although from a purely "electron-pushing" point of view the one-proton path of eq. 3–8 seems more reasonable.



For this process, the rate eq. 14 and 15 can be derived. These are the equivalents for a two-proton process of the Zucker–Hammett rate equations, 10 and 11. In eq. 14, h_+ is the Hammett acidity function, in antilogarithmic form, toward a positively charged base, and $f_{BH_2^+} / f_{BH^+}$ is the activity coefficient ratio for the indicator base.

$$k_{obs} = \text{const.} \frac{[SH^+] a_{H_2O}}{[S] + [SH^+]} h_+ \frac{f_{SH^+} f_{BH_2^+}}{f_{tr^+} f_{BH^+}} \quad (14)$$

$$k_{obs} = \text{const.}' \frac{[SH^+][H_3O^+]}{[S] + [SH^+]} \frac{f_{H_3O^+} f_{SH^+}}{f_{tr^+}} \quad (15)$$

In eq. 14 or 15 one does not know how the values of the ratio of the activity coefficients of the similarly constituted species vary with medium. In applying either of these equations to the data then, one is forced to try as a mere working hypothesis that the activity coefficient ratio remains constant. At any rate, eq. 15, under the working hypothesis that $f_{H_3O^+} f_{SH^+} / f_{tr^+}$ is a constant (the equivalent for a two-proton process, of the Zucker–Hammett hypothesis⁸), predicts that even for a two-proton process, k_{obs} should not increase with acid percentage nearly as much as it actually does. This can be seen by inspection of Tables III and IV, where $\log k_{obs} - \log ([SH^+][S] + [SH^+])$ increases much more than $\log M$.

(18) The ultraviolet spectra of phloroglucinol and its ethers reveal no detectable amount of diprotonated species (see Fig. 1 of preceding paper^{4b}).

(19) *E.g.*, the second proton may be introduced later in the process.

TABLE III
 DATA FOR THE HYDROLYSIS OF 1-METHOXY-3,5-DIHYDROXYBENZENE AT 50°

HClO ₄ , %	-H ₀ ^a	log M	-log a _{H₂O} ^b	-log $\frac{[SH^+]^c}{[S] + [SH^+]}$	log k _{obs} + 5	VI + V ^d	-log $\frac{[S]^e}{[S] + [SH^+]}$	VI + VII ^e
50.0	3.64	0.843	0.49	0.29	1.27	1.56	0.32	1.59
52.2	3.95	.870	.57	.16	1.44	1.60	.51	1.95
54.0	4.23	.892	.66	.10	1.60	1.70	.71	2.31
54.2	4.26	.899	.67	.09	1.65	1.74	.73	2.38
55.7	4.50	.912	.75	.05	1.75	1.80	.94	2.69
57.9	4.93	.936	.88	.02	1.90	1.92	1.32	3.22
58.0	4.95	.937	.88	.02	1.95	1.97	1.34	3.29
60.0	5.35	.959	1.02	.01	2.10	2.11	1.72	3.82
60.3	5.41	.962	1.05	.01	2.14	2.15	1.78	3.92
61.2	5.61	.974	1.14	~0	2.20	2.21	1.97	4.17
63.5	6.13	.988	1.30	~0	2.39	2.39	2.42	4.81

^a Room temperature values; those below 55% are values of Paul and Long²⁰; those above 55% are the slightly modified values by Bonner and Lockhardt.²¹ ^b Values of Robinson and Baker.²² ^c See footnotes, Table I. ^d $\log k_{obs} - \log ([SH^+]/([S] + [SH^+])) + 5$. ^e $\log k_{obs} - \log ([S]/([S] + [SH^+])) + 5$.

 TABLE IV
 DATA FOR THE HYDROLYSIS OF 1,3,5-TRIMETHOXYBENZENE AT 60° AND 90°

HClO ₄ , %	-H ₀ ^a	log M	-log a _{H₂O} ^b	90°		90°		VI + VIII ^e
				-log $\frac{[SH^+]^c}{[S] + [SH^+]}$	log k _{obs} + 5	VI + V ^d	-log $\frac{[S]^e}{[S] + [SH^+]}$	
44.7	2.84	0.78	0.33	1.11	1.08	2.19	0.04	1.12
48.1	3.35	.83	.43	0.57	1.66	2.23	.14	1.80
50.2	3.66	.85	.50	.34	1.98	2.32	.27	2.25
52.4	3.99	.88	.57	.16	2.30	2.46	.50	2.80
54.2	4.26	.90	.67	.09	2.56	2.65	.73	3.29
60°								
52.4	3.99	0.88	0.57	0.16	1.08	1.24	0.50	1.58
54.2	4.26	.90	.67	.09	1.41	1.50	0.73	2.14
57.9	4.93	.94	.88	.01	1.91	1.92	1.58	3.49
60.0	5.35	.96	1.02	~0	2.24	2.24	2.00	4.24
61.2	5.61	.97	1.12	~0	2.48	2.48	2.33	4.81

^a See footnote a, Table III. ^b Values of ref. 22. ^c See footnotes a and b, Table II. ^d See footnote e, Table III. ^e See footnote d Table III.

A test of eq. 14 was made under the mere working hypothesis that $h_+ f_{SH^+} f_{BH_2^{++}} / f_{tr} f_{BH^+}$ varies as h_0 .²³ Plots of $\log k_{obs} - \log ([SH^+]/([S] + [SH^+]))$

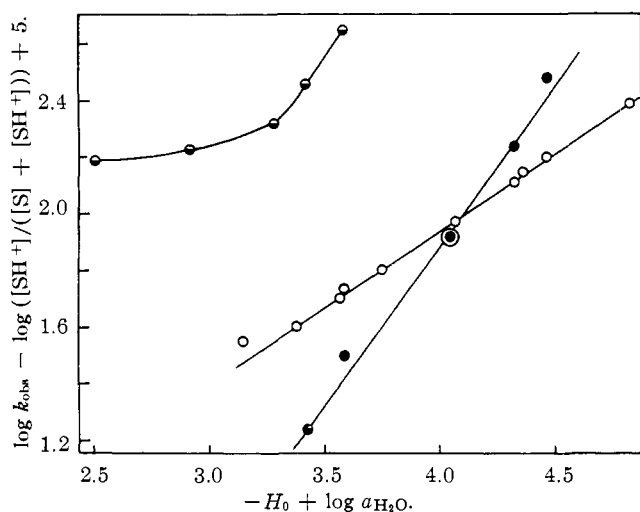


Fig. 2.— $\log k_{obs} - \log ([SH^+]/([S] + [SH^+]))$ vs. $\log a_{H_2O} - H_0$; O, 1-methoxy-3,5-dihydroxybenzene at 50°; ●, 1,3,5-trimethoxybenzene at 90°; ●, 1,3,5-trimethoxybenzene at 60°.

against $\log a_{H_2O} - H_0$ are shown in Fig. 2. It is to be pointed out that the values of $[SH^+]/([S] + [SH^+])$

(20) M. A. Paul and F. A. Long, *Chem. Rev.*, **57**, 1 (1957).

(21) T. G. Bonner and J. C. Lockhardt, *J. Chem. Soc.*, 3840 (1957).

(22) R. A. Robinson and O. J. Baker, *Trans. Roy. Soc. New Zealand*, **76**, 250 (1946).

(23) There is some precedent for the notion that h_+ may vary approximately as h_0 ; see, e.g., M. W. Fuller and W. M. Schubert, *J. Am. Chem. Soc.*, **85**, 108 (1963).

are not based on any assumption that the first protonation follows h_0 (cf. footnote a of Table I and footnotes a and b of Table II). The plot for 1-methoxy-3,5-dihydroxybenzene is linear and of slope 0.56, except for the first point. For 1,3,5-trimethoxybenzene in 44.7 to 54.2% perchloric acid at 90° the line starts out relatively flat and then curves sharply upward. For the triether in 52.4 to 61.2% perchloric acid the plot is approximately linear with slope 1.16. The plots are at least consistent with an appreciable contribution of a two-proton process to the over-all rate. The appearance of the curve for 1,3,5-trimethoxybenzene in the lower acid percentages is strongly suggestive of a major contribution of a one-proton process in this region. That is, the relatively small initial slope and upward curvature of the line, as compared to the large slope and linearity of the plot in the higher acid percentages, is consistent with the overlap of a one- and two-proton process in the lower acid regions and the predominance of the two-proton process in the upper acid regions. Also consistent with this conclusion is the upward curvature of the H_0 plot for an assumed one-proton process, Fig. 1, for the triether in the lower acid percentages.

Of course, if the one-proton process makes only a partial contribution to the total rate in the lower acid percentages, one would expect its contribution to be swamped out by the two-proton process in the high strength acids. This is because the concentration of the reactive species in the one proton process, SH^+ , remains effectively constant once equilibrium protonation to SH^+ is nearly complete. On the other hand, the rate of the two-proton process should continue to rise steeply, since $[SH_2^{++}]$ is small and thus $[SH_2^{++}]/[SH^+]$ increases sharply with acidity.

Finally, a plot of $\log k_{\text{obs}} - \log ([\text{SH}^+]/([\text{S} + [\text{SH}^+])) + H_0$ against $\log a_{\text{H}_2\text{O}}$ for 1-methoxy-3,5-dihydroxybenzene was fairly linear. The slope of the line is the w -value based on a two-proton process.^{6,24} Its value, +2.0, is within the range assigned for reactions in which water acts as a nucleophile in the rate-controlling step.^{6b} It is, however, less than for the hydrolysis of 4-(*p*-sulfophenyl)-1-methoxynaphthalene (w was +4.5 in 1-8 molar hydrochloric acid and +8.2 in 1-4 molar perchloric acid) or of 4-(*p*-sulfophenylazo)-1-anisole (w was +3.1 in 1-9 molar hydrochloric acid).^{5,6} The corresponding w -plot for 1,3,5-trimethoxybenzene was badly curved for the results at 90°, and the points were

(24) In order for a "true" w -value to be obtained, it is necessary that the second protonation follow H_0 .

widely scattered for the results at 60°, the slopes being generally positive.

Activation Energy and Entropy.—The rate of hydrolysis of 1-methoxy-3,5-dihydroxybenzene was measured at five temperatures in 63.5% perchloric acid (Table I). The high acid percentage was chosen in order that the ground state be essentially carbon-conjugate acid, SH^+ . The value of E_A obtained is 18.5 ± 0.2 kcal./mole and ΔS^\ddagger at 25° is -15.2 ± 0.7 e.u. A large negative ΔS^\ddagger is considered to be characteristic of reactions classified as A-2,³ but it is not known whether this should apply also to a two-proton process.

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Interaction and Association of Bases and Nucleosides in Aqueous Solutions¹

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The molal osmotic coefficients (ϕ) of aqueous purine, uridine and cytidine in the concentration range of 0.1 to 1.0 molal, and inosine and caffeine at 0.1 molal, have been determined by thermoelectric measurements of vapor pressure lowering at 25°. The activity coefficients of purine, uridine and cytidine were calculated. The data indicate that these solutes associate extensively in solution and that the association process does not proceed simply to the dimer stage, but continues to form higher polymers. The results are consistent with a set of association processes which have equal equilibrium constants for all successive steps. Equilibrium constants at 25° and standard free energies for association of purine, uridine and cytidine were found to be 2.1, 0.6, 0.9 molal⁻¹ and -440, 290, 80 cal./mole, respectively. The interaction of one base with another base was examined by measuring the increase of solubility of adenine-C¹⁴ or thymine-C¹⁴ in the presence of varying concentrations of a variety of interactants at 25.5° and 38°. The solubility of adenine or thymine was increased by the addition of soluble purine, nucleosides, pyrimidine or phenol, and was essentially unchanged by the addition of cyclohexanol, adonitol or urea. The total base-C¹⁴ in solution was assumed to be composed of the free base in solution and the water-soluble base-interactant complex. Equilibrium constants for these association processes have been estimated, and they agree semiquantitatively with the results from measurements of ϕ . The tendency for association and interaction of the free bases and nucleosides in solution can be arranged in the series: purine-purine > purine-pyrimidine > pyrimidine-pyrimidine. The relationship of this finding to the vertical-stacking interaction of the bases in nucleic acids is discussed.

Introduction

In our laboratories, research has been conducted on factors contributing to the formation and stabilization of helices of nucleic acids.⁴ It is felt that the attraction and the specificity in the formation of helices by strands of nucleic acids must reside in the interaction of the nitrogenous bases on the polynucleotide chains. Thus it is pertinent to ask whether solutes interact in aqueous solutions of the free bases and nucleosides, and if so, to what extent, with what degree of specificity and with what mechanism? In order to answer these questions, the thermodynamic properties and solution behavior of certain bases and nucleosides in water have been studied. Uncharged compounds at neutral $p\text{H}$, such as bases and nucleosides, were chosen in order to avoid the complication due to charge effects.

In the first part of this paper, the osmotic coefficients and the activity coefficients of purine (0.05 to 1.1 m), uridine (0.1 to 0.7 m), cytidine (0.1 to 0.7 m), caffeine (0.1 m) and inosine (0.1 m) at 25° have been determined. The data indicate a high degree of association of the bases and nucleosides in solution. Apparent

equilibrium constants for this association are evaluated for purine, uridine and cytidine. In the second part of the paper, the solubilities of adenine and thymine in water at 25.5° in the presence of bases (purine and pyrimidine), nucleosides (uridine and cytidine) and of other compounds of interest are studied. The solubilities of adenine and thymine are greatly enhanced in the presence of these bases and nucleosides, demonstrating that there are interactions between adenine and thymine with these compounds.

Experimental

Materials.—Commercially available compounds of the highest degree of purity were used without further purification. The following A grade (unless specified otherwise) compounds were obtained from California Corporation for Biochemical Research, Los Angeles: adenine, adenine-8-C¹⁴, thymine, thymine-2-C¹⁴, adonitol, inosine, caffeine (U.S.P. C grade), purine (nitrogen 46.57%, calculated purity 99.85%,⁵ containing 0.3% chloride as stated by the manufacturer), cytidine (nitrogen 17.46%⁸) and uridine (nitrogen 11.28%, calculated purity 98.4%⁸). Pyrimidine and adenine were obtained from Nutritional Biochemical Corporation. Some crystalline thymine from Sigma Co., St. Louis, Mo., as well as some crystalline purine from Cyclo Chemical Corporation were also used. Phenol (chromatographic, 88%) was obtained from Mallinckrodt Co., and cyclohexanol (reagent grade) was obtained from Fisher Scientific Co. All other chemicals were reagent grade. Distilled, de-ionized water was used.

Adenine-C¹⁴ and thymine-C¹⁴ for solubility studies were prepared by dissolving proper amounts of unlabeled base and base-C¹⁴ together to give the desired specific activity. Specific activities were determined from counts and optical density measurement (Beckman DK-2 or Cary-11 spectrophotometer). The

(5) This is calculated on the basis that no other nitrogenous impurities are present.

(6) The nitrogen content exceeds that of the theoretical value of 17.27% based on the chemical formula.

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