[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY]

The Hydrolysis of Esters of Some Substituted Benzoic Acids in Strongly Acid Aqueous Solutions[1]

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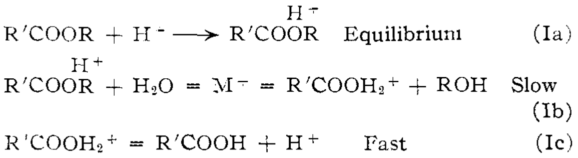
The hydrolysis rates of esters of several substituted benzoic acids have been found to agree with the Zucker–Hammett hypothesis which predicts that rates of acid-catalyzed reactions of uncharged molecules should vary linearly with the concentration of hydrogen ion when there is a water molecule in the transition state but should vary with the h_0 acidity function if the transition state does not include water. For the following esters the rates vary linearly with C_{H^+}: methyl benzoate at 90° in aqueous perchloric acid of from 1 to 4 *M*; α-glyceryl monobenzoate and monoanisoate at 90° in solutions of from 0.04 to 6 *M* aqueous perchloric acid; α-glyceryl esters of benzoic, anisic and 3,4,5-trimethoxybenzoic acids at 50 or 70° in aqueous sulfuric acid of from 1 to 10 *M*. In contrast, the hydrolysis rate of methyl mesitoate at 90° in aqueous solutions of from 1 to 6 *M* perchloric and sulfuric acids varies linearly with the h_0 acidity function, not with C_{H^+}. Since the unhindered esters undoubtedly hydrolyze by the A-2 mechanism with a rate-determining reaction of the conjugate acid of the ester and a water molecule, proportionality of rate and C_{H^+} is the expected result. Methyl mesitoate probably hydrolyzes by the A-1, "unimolecular" mechanism to give an acylium ion; this should lead to the observed proportionality between rate and h_0.

In several recent investigations of the mechanism of acid-catalyzed hydrolysis reactions, experiments on rates of reaction in concentrated aqueous solutions of mineral acids have been used as a diagnostic tool. The method stems from a proposal by Zucker and Hammett[2,3] that for an acid-catalyzed reaction of a neutral molecule to give a transition state which involves only an added proton, the rate will increase in proportion to the H_0 acidity function. If, however, the reaction involves in addition a water molecule in the transition state, it was proposed that the rate will more nearly vary in proportion to concentration of hydrogen ion. To apply this criterion to reactions catalyzed by aqueous solutions of strong acids it is necessary to make studies at acid concentrations above about one molar since only then does the acidity as measured by $-H_0$ differ significantly from log C_{H^+}.

Since precise calculations of the effect of changing media on rates are not yet possible, the chief justification for the use of the Zucker–Hammett hypothesis is the experimental one that it appears to fit a variety of reactions whose mechanisms are fairly well established by other criteria. From this standpoint one of the particularly important reactions to consider is the acid-catalyzed hydrolysis of esters of carboxylic acids, since an extensive series of studies has led to the fairly definite conclusion that the normal mechanism for this reaction is of the A-2 type[4,5] with the further simplification that in not too concentrated solutions of acid only a negligible amount of the ester is transformed into the conjugate acid.



$$R'COOR + H^+ \longrightarrow \overset{H^+}{R'COOR} \quad \text{Equilibrium} \qquad (Ia)$$

$$\overset{H^+}{R'COOR} + H_2O = M^+ = R'COOH_2^+ + ROH \quad \text{Slow} \qquad (Ib)$$

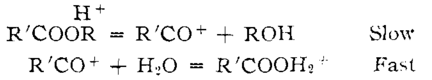
$$R'COOH_2^+ = R'COOH + H^+ \quad \text{Fast} \qquad (Ic)$$

This mechanism leads to the rate law

$$\text{hydrolysis rate} = k_1C_{\text{ester}} = k_2C_{H^+}C_{\text{ester}} \qquad (1)$$

and since the activated complex contains a water molecule the prediction from the Zucker–Hammett hypothesis is that for hydrolysis in concentrated solutions of mineral acids log k_1 will increase linearly with log C_{H^+}, not with $-H_0$. Long, Dunkle and McDevit[6] confirmed this prediction for a γ-lactone by showing that the rate of hydrolysis of γ-butyrolactone in aqueous solutions of up to 4 *M* perchloric and hydrochloric acids did indeed vary with concentration of acid rather than h_0 (where h_0 = – antilog H_0). A similar parallelism of rate and concentration of acid is shown by data for the hydrolysis of methyl acetate in solutions of up to 3.6 molar hydrochloric acid.[7] Finally, there is a very recent study by Bell, Dowding and Noble[8] on the hydrolysis of ethyl acetate and of methyl formate in aqueous hydrochloric acid solutions up to concentrations of ten molar. For these high concentrations the plots of log k_1 *vs.* log C_{H^+} depart significantly from linearity. However, the rate is still much more nearly linear in C_{H^+} than in h_0 and in this sense the expectation from the Zucker–Hammett hypothesis for the A-2 mechanism is confirmed. The results to be reported in this paper for esters of benzoic, 3,4,5-trimethoxybenzoic and anisic acids lead to a similar conclusion.

Even though the A-2 mechanism is normally observed, the possibility exists that esters of certain carboxylic acids may hydrolyze by an A-1 mechanism, characterized by a unimolecular rate-determining step.[5] Specifically, the previously listed slow bimolecular reaction of the conjugate acid of the ester with water, step Ib, might be replaced by



$$\overset{H^+}{R'COOR} = R'CO^+ + ROH \quad \text{Slow} \qquad (Ib')$$

$$R'CO^+ + H_2O = R'COOH_2^+ \quad \text{Fast} \qquad (Ib'')$$

For hydrolysis in dilute aqueous solutions of acids this shift would not change the experimental rate

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[1] Work supported in part by a grant from the Atomic Energy Commission.

(2) L. Zucker and L. P. Hammett, THIS JOURNAL, **61**, 2791 (1939).

(3) L. P. Hammett, "Physical Organic Chemistry," McGraw–Hill Book Co., New York, N. Y., 1940, p. 275.

(4) J. D. E. Day and C. K. Ingold, *Trans. Faraday Soc.*, **37**, 686 (1941).

(5) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 767.

(6) F. A. Long, F. B. Dunkle and W. F. McDevit, *J. Phys. Colloid Chem.*, **55**, 829 (1951).

(7) M. Duboux and A. de Sousa, *Helv. Chim. Acta*, **23**, 1381 (1940).

(8) R. P. Bell, A. L. Dowding and H. A. Noble, *J. Chem. Soc.*, 3106 (1955).

law. However, the Zucker–Hammett hypothesis predicts that for this mechanism the rate should vary with h_0 rather than with concentration of hydrogen ion. Hence the hydrolysis in more concentrated solutions of acid should show a quite different behavior from that of ordinary esters.

A favorable case for a shift to an A-1 mechanism is the hydrolysis of methyl mesitoate (methyl 2,4,6-trimethylbenzoate) where both steric hindrance and relative electron release favor a unimolecular rate step.[5] The work of Treffers and Hammett[9] and of Newman[10] has shown that in pure sulfuric acid, mesitoic acid is largely converted to the acylium ion. Qualitative studies by these same investigators also strongly suggest that in pure sulfuric acid the hydrolysis of methyl mesitoate goes through an acylium ion path. It does not follow that this mechanism will hold in dilute acid solution but this is surely a good possibility.[11] On this basis we have made studies of the hydrolysis of methyl mesitoate in aqueous solutions of both perchloric and sulfuric acid at concentrations of from 1 to 6 molar and have compared the results with those for methyl and α-glyceryl esters of unhindered benzoic acids.

Results

Table I gives data for the hydrolysis of α-glyceryl monobenzoate in aqueous perchloric acid at 90°. The second-order rate coefficient (k_2 as defined in eq. 1) remains constant within experimental error up to perchloric acid concentrations of six molar. Quite evidently the rate is proportional to C_{H^+} and not to h_0. However, the complete absence of any medium effect on k_2 is somewhat surprising. The Brönsted relation for the A-2 mechanism for ester hydrolysis is

$$\log k_2 = \log k_2^\circ + \log \frac{f_{\text{ester}} f_{\mathrm{H}^+} a_{\mathrm{H_2O}}}{f^*_{\mathrm{M}^+}} \qquad (2)$$

where k_2° is a constant, $a_{\mathrm{H_2O}}$ is activity of water and f_{ester}, f_{H^+} and $f^*_{\mathrm{M}^+}$ are activity coefficients of ester, hydrogen ion and transition state, respectively. The observed constancy of k_2 indicates that for this case the Brönsted activity coefficient ratio remains effectively constant.

TABLE I

HYDROLYSIS OF 0.05 M SOLUTIONS OF α-GLYCERYL MONOBENZOATE IN AQUEOUS PERCHLORIC ACID AT 90°

Concn. $HClO_4$, mole/l.	h_0 (25°)	$k_1 \times 10^5$, sec.$^{-1}$	$k_2 \times 10^4$, l. mole^{-1} sec.$^{-1}$
0.0446[a]	0.04	0.68	1.52
.223[a]	.22	3.22	1.44
.223	.22	3.37	1.51
.510	.51	7.2	1.42
.519	.52	8.0	1.52
.926	1.2	13.6	1.47
1.94[b]	4.3	28.2	1.45
3.01[b]	13	44.6	1.48
5.12[b]	150	79	1.53
5.77[b]	370	85	1.47

[a] Analysis by titration of acid produced. [b] Rate coefficient calculated on basis that reaction goes to an equilibrium mixture.

A few experiments were made with the glyceryl benzoate at 50° in solutions of from 5.8 to 9.6 M sulfuric acid. Typical values of k_2 are 1.1×10^{-5} l. mole^{-1} sec.$^{-1}$ for 5.6 M sulfuric acid and $k_2 = 0.4 \times 10^{-5}$ for 9.3 M sulfuric acid. The accuracy of these experiments is low and the only conclusion of significance is that the second-order rate coefficient is again essentially independent of acidity.

Table II gives data for the glyceryl ester of anisic acid at 90° in aqueous solutions of perchloric acid. The principal conclusion is the same as with the benzoate ester: the rate increases linearly with concentration of acid and hence not with h_0. With the anisic ester two fairly extensive series of runs were made with sulfuric acid at 70 and 50°. The accuracy of these studies is lower because the product anisic acid normally precipitated from the reaction mixture but the data do show that at each temperature and for sulfuric acid concentrations of from 1 to 9 molar the second-order rate coefficient k_2 varies by less than a factor of three.

TABLE II

HYDROLYSIS OF α-GLYCERYL MONOANISOATE AT 90°

Concn. $HClO_4$, mole/l.	h_0(25°)	Concn. ester, mole/l.	$k_1 \times 10^5$, sec.$^{-1}$	$k_2 \times 10^4$, l. mole^{-1} sec.$^{-1}$
0.0432[a]	0.04	0.037	0.421	0.98[a]
.172[a]	.17	.037	1.64	.95[a]
.472[a]	.47	.037	4.48	.95[a]
1.05	1.3	.037	8.9	.85[a]
1.93	4.3	.034	15.7	.81
1.94	4.3	.033	15.7	.81
1.92	4.2	.033	15.7	.82
1.95[b]	4.9[b]	.033[b]	19.9[b]	1.02[b]
3.84	33	.027	33.2	0.86
3.87	34	.027	35.0	.90
5.74	360	.024	55.4	.96

[a] Analysis by titration of acid produced. [b] Measurement in 1.95 M H_2SO_4. Rate coefficient calculated on basis that reaction goes to equilibrium. Value of k_2 calculated with assumption that $C_{\mathrm{H}^+} = 1.95\ M$.

Table III gives results for the glyceryl ester of 3,4,5,-trimethoxybenzoic acid in aqueous sulfuric acid at 50°. The calculated second-order rate coefficients remain roughly constant for a wide range of acidity although there is some fluctuation. Actually a small variation in k_2 is not surprising. Some of it might result from a general medium effect, *i.e.*, changes in the Brönsted activity coefficient ratio of eq. 2. Of more importance, at sufficiently high acidities one would expect the rate to become independent of acid concentration simply because all of the ester is transformed into its conjugate acid. The acidity at which this occurs will depend on the base strength of the ester and we have no information on this for any of our esters. As a result all rates have been calculated under the assumption that only negligible quantities of ester were transformed to the conjugate acid. This assumption seems reasonable in view of the approximate constancy of k_2 for solutions as acid as 9 M sulfuric acid.

Table IV lists the data for a few experiments

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(9) H. P. Treffers and L. P. Hammett, THIS JOURNAL, **59**, 1708 (1937).

(10) M. Newman, *ibid.*, **63**, 2431 (1941).

(11) W. M. Schubert, R. E. Zahler and J. Robins [*ibid.*, **77**, 2293 (1955)] have studied the ionization of a related ester, methyl 2,4,6-trimethoxybenzoate, and find that it forms an acylium ion slowly in 60% perchloric acid at 10°.

TABLE III

HYDROLYSIS OF 0.012 M α-GLYCERYL MONO-3,4,5-TRIMETHOXYBENZOATE IN AQUEOUS SULFURIC ACID AT 50°[a]

Concn. H_2SO_4, mole/l.	h_0(25°)	$k_1 \times 10^5$, sec.$^{-1}$	$k_2 \times 10^6$, l. mole^{-1} sec.$^{-1}$ [b]
1.15	1.7	0.340	3.0
1.94	4.9	.58	3.0
2.02	5.4	.62	3.1
3.99	52	1.88	4.7
5.80	340	3.03	5.2
7.78	4300	3.67	4.7
9.56	36000	1.46	1.5
9.68	42000	1.73	1.8

[a] Rate coefficient calculated on assumption that reaction reaches an equilibrium. [b] Second-order rate coefficients calculated by assuming C_{H^+} is given by molarity of H_2SO_4.

with the simpler ester, methyl benzoate, where the rate was followed spectrophotometrically. With this ester, as with the previously discussed ones, the rate increases linearly with concentration of acid and not with the acidity function. A minor point to note is that the rate coefficient for this reaction is only 30% larger than that for the glyceryl benzoate, indicating that the rate is not very dependent on the properties of the alcohol moiety.

TABLE IV

HYDROLYSIS OF METHYL BENZOATE AT 90°

Concn. $HClO_4$, mole/l.	h_0(25°)	Concn. ester $\times 10^4$, mole/l.	$k_1 \times 10^5$, sec.$^{-1}$	$k_2 \times 10^4$, l. mole^{-1} sec.$^{-1}$
0.98	1.3	9.6	18.7	1.91
1.93	4.3	8.8	36.6	1.90
3.82	32	7.0	74.5	1.95

The hydrolysis behavior of all of these ordinary esters is clearly consistent with the Zucker–Hammett hypothesis. The mechanism of the hydrolysis is almost certainly of the A-2 type and hence involves a water molecule in the transition state. The prediction is thus that rate will vary with log C_{H^+} and this is observed. This confirms and extends the conclusions reached by Bell, Dowding and Noble.[8] Actually the present data differ slightly from those for methyl formate and ethyl acetate in that the hydrolyses of these two in aqueous hydrochloric acid exhibited a sizable positive salt effect. With the benzoic esters there appears to be almost no salt effect on the rate coefficient, k_2, in either perchloric or sulfuric acid solutions. However, this is really a minor difference and is probably a consequence of the differences in size and polarity of the two sets of esters.

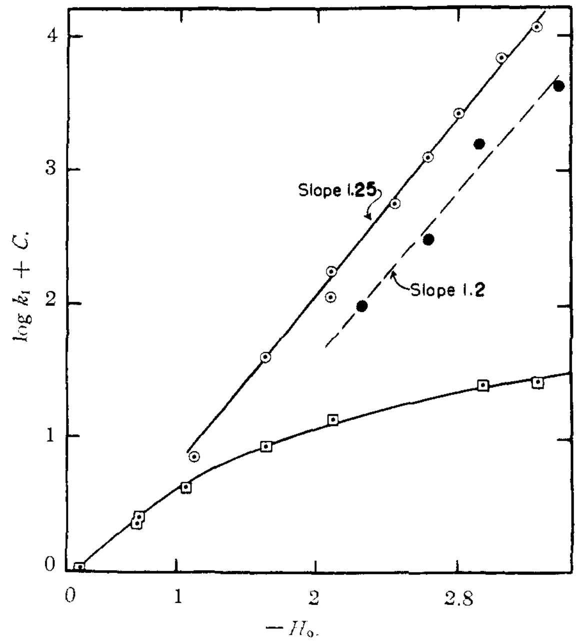

Fig. 1.—Plots of log k_1 vs. $-H_0$ for hydrolysis at 90°: ⊙, log k_1 + 7 for methyl mesitoate in perchloric acid; ●, log k_1 + 7 for methyl mesitoate in sulfuric acid; ⊡, log k_1 + 4.5 for α-glyceryl monobenzoate in perchloric acid.

The data for the hydrolysis of the sterically hindered methyl mesitoate, shown in Table V, are strikingly different from those for the other esters. At low acidities the rate is much less than that of the anisoate or benzoate esters and this is the expected result for a sterically hindered molecule. With increasing acidity the rate increases very rapidly, roughly in proportion to the increase in h_0, so that the rate coefficient k_2 is not at all constant. This difference in behavior is shown more clearly in Fig. 1 where log k_1 is plotted against $-H_0$. The data for the mesitoate ester in aqueous perchloric acid fall on a straight line of slope 1.25; those for reaction in sulfuric show more scatter but appear to fall on a line with a smaller slope of 1.2. The data for the hydrolysis of α-glyceryl monobenzoate are plotted on the same scale and illustrate the very different course of a reaction whose rate actually varies with C_{H^+} rather than h_0.

TABLE V

HYDROLYSIS OF METHYL MESITOATE (CONCN. OF FROM 4 TO 8 × 10^{-4} M) IN AQUEOUS MINERAL ACIDS AT 90°

Concn. $HClO_4$, mole/l.	Concn. H_2SO_4, mole/l,	h_0(25°)	$k_1 \times 10^5$, sec.$^{-1}$	$k_2 \times 10^4$, l. mole^{-1} sec.$^{-1}$
1.00		1.3	0.073	0.0073
1.93		4.3	.42	.022
2.94		12	1.16	.039
2.96		12	1.8	.061
3.90		35	5.6	.14
4.39		62	12.7	.29
4.80		100	26	.54
5.36		200	67	1.25
5.76		360	114	2.0
	3.12	21	1.0	0.034[a]
	4.16	63	3.1	.080[a]
	5.00	140	15.5	.31[a]
	6.14	530	42	.68[a]

[a] Calculated assuming C_{H^+} is given by molarity of H_2SO_4.

The lack of a complete parallelism of rate and h_0 for methyl mesitoate is not surprising since similar results have been found with other systems where rate closely parallels h_0.[12,13] The reason for it can be seen from the Brönsted expression for the dependence of log k_1 for the A-1 mechanism on the medium

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(12) D. McIntyre and F. A. Long, THIS JOURNAL, **76**, 3240 (1954).

(13) V. Gold and J. Hilton, *J. Chem. Soc.*, 843 (1955).

$$\log k_1 = \log \frac{a_{\mathrm{H}^+} f_{\mathrm{ester}}}{f^*_{\mathrm{M}^+}} + \text{constant}$$

If one adds and subtracts $-H_0$ the result is

$$\log k_1 = -H_0 + \log \frac{f_{\mathrm{ester}}}{f^*_{\mathrm{M}^+}} - \log \frac{f_{\mathrm{B}}}{f_{\mathrm{BH}^+}} + \text{constant}$$

where f_{B} and f_{BH^+} are, respectively, the activity coefficient for the indicator used to determine H_0 and for its conjugate acid BH^+. A lack of complete parallelism of rate and h_0 evidently means that the ratio $f_{\mathrm{ester}}/f^*_{\mathrm{M}^+}$ does not vary with medium in quite the same way as does $f_{\mathrm{B}}/f_{\mathrm{BH}^+}$.

Since methyl mesitoate almost certainly hydrolyzes in pure sulfuric acid by an A-1 mechanism with production of an acylium ion, it is a quite reasonable expectation that the same mechanism will hold in dilute acid solutions. This mechanism leads to a transition state which does not contain a water molecule and which in essence is the conjugate acid of the reactant. Hence the prediction from the Zucker–Hammett hypothesis is that rate will depend on the H_0 acidity function and in this sense the variation of the rate coefficients of Table V with acidity is as expected. In view of these results and of the different behavior of the ordinary, unhindered esters, it is reasonable to conclude that the Zucker–Hammett hypothesis is in agreement with the data for ester hydrolysis by both the A-2 and A-1 mechanisms.

An incidental reason for making studies with the benzoic and anisic esters at very high acidities was to see if by chance a change in mechanism would occur as the acidity increased. Given the very different dependence of rate on acidity illustrated in Fig. 1 for reactions which follow the A-2 and A-1 mechanisms there is a possibility that certain esters will hydrolyze by the A-2 mechanism in dilute solutions of acid and by the A-1 in concentrated solutions. This would result in a shift from parallelism of rate with C_{H^+} to a parallelism of rate with h_0. No evidence for this kind of shift has so far been obtained but it might still be of interest to search for it by a study of esters of such acids as 2,6-dimethylbenzoic and 2,4-dimethylbenzoic.

Experimental

α-Glyceryl esters were synthesized according to the method of Fischer and co-workers[14] by the preparation and subsequent esterification of acetone glycerols. The α-glyceryl monobenzoate which was prepared by this method had a m.p. of 38°, close to the value of 36° reported by Fischer. α-Glyceryl monoanisoate and 3,4,5-trimethoxybenzoate have not been reported previously. The former is a white solid, m.p. 83°; analysis: C, 58.45; H, 6.16% compared to calculated C, 58.4 and H, 6.24%. The latter compound has m.p. 94.0°; analysis C, 54.46, H, 6.29% compared to calculated C, 54.54, H, 6.29%. Methyl mesitoate was purchased from Chemicals Procurement Company and the methyl benzoate was Eastman reagent grade. Other chemicals were reagent grade and were used without further purification.

The hydrolysis experiments were performed in a thermostated oil-bath whose temperature was held constant to ±0.04°. Temperatures were measured with a Bureau of Standards calibrated thermometer. For experiments with the glyceryl esters at 70 and 90°, solutions of ester in aqueous acid were sealed in Pyrex tubes, placed in the bath and then withdrawn for analysis at predetermined times. Reaction was stopped by chilling. For all other systems the reactions were carried out in flasks and samples were removed by pipetting. The acid concentrations listed in the tables are for the temperatures of the experiments and were determined by titration. The h_0 values are for 25° and were taken from smoothed plots of the data of Hammett and Deyrup[15] as corrected by Hammett and Paul.[16]

With catalyst acid concentrations of less than 0.3 M, the progress of the hydrolysis of the glyceryl esters was followed by titration of the acid produced. For more concentrated solutions of acid the analysis was made by periodate oxidation using a procedure suggested by Miner and Dalton.[17] In acid solution periodate oxidation is selective for terminal alcohol groups so that glycerol reacts with twice as many equivalents as does the unhydrolyzed glyceryl ester. The actual analytical procedure involved adding a slight excess of standardized periodate solution to an acidified aliquot of the reaction mixture, letting the oxidation proceed to completion, then adding excess potassium iodide and titrating the iodine formed with standardized thiosulfate. Preliminary tests showed that the method is quantitative and of adequate accuracy.

Hydrolysis of the glyceryl esters was carried out using ester concentrations of from 0.01 to 0.1 molar. Preliminary tests in dilute aqueous acid showed that the hydrolysis of all three glyceryl esters was first order in concentration of ester and concentration of hydrogen ion and also that the reactions went to completion. For solutions in more concentrated acid the reactions did not go entirely to completion and the first-order rate coefficients were calculated by assuming the existence of a second-order reverse reaction. This leads to the equation

$$k_1 = \frac{2.303x_e}{(2a - x_e)t} \log \frac{ax_e + x(a - x_e)}{a(x_e - x)} \qquad (3)$$

where a is initial concentration of ester, x and x_e are concentrations reacted at time t and at equilibrium and k_1 is the first-order rate coefficient for the hydrolysis. Actually the use of this equation was not essential since application of the ordinary first-order rate law, assuming a reaction which went to completion, invariably gave k_1 values which agreed with those from the above to within 20%.

For most hydrolyses of glyceryl esters in concentrated solutions of sulfuric acid, the carboxylic acid which was formed precipitated out but the reaction still did not go entirely to completion. Rate coefficients for this case were normally calculated only from data for samples taken before precipitation of the acid, using eq. 3. Actually for all cases the reactions went almost to completion and as a result about the same value of k_1 was obtained by use of eq. 3 as by use of the equation for a first-order irreversible reaction. Even so the estimated accuracy of the experiments with sulfuric acid is only about ±20% compared to ±10% for experiments with perchloric acid.

The hydrolysis of methyl benzoate and methyl mesitoate was followed by spectrophotometric analysis. Ester concentrations were in the order of 10^{-4} molar and as a result all hydrolyses went to completion and could be treated as simple first order. The analytical procedure involved removal of the unreacted ester from the solution and analysis for the benzoic or mesitoic acid. An aliquot of the reaction mixture was added to a solution of a borate buffer, chosen so that the solution had a pH of about 8 after the catalyst acid had been neutralized by the calculated amount of standard sodium hydroxide solution. (This buffer procedure was not necessary with methyl mesitoate, since for this ester the rate of the basic hydrolysis is very low.) The buffered basic solution was extracted several times with methylene chloride to remove the unreacted ester. The aqueous solution was then made slightly acid, diluted to volume and analyzed with a Beckman DK spectrophotometer. Benzoic acid was analyzed at wave length 231 mμ and mesitoic at 235 mμ. The estimated accuracy of these spectrophotometric runs is ±10%.

Preliminary experiments with methyl mesitoate showed that the hydrolysis reaction is followed by a decarboxylation of mesitoic acid,[18-20] the rate of which is about one-

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[14] E. Fischer, M. Bergman, H. Bärwind and E. Pfahler, *Ber.*, **53**, 1589, 1606 (1920).

[15] L. Hammett and A. Deyrup, THIS JOURNAL, **54**, 2721 (1932).

[16] L. P. Hammett and M. A. Paul, *ibid.*, **56**, 827 (1934).

[17] C. S. Miner and N. N. Dalton, "Glycerol," Reinhold Publ. Co., New York, N. Y., 1953, p. 206.

[18] W. M. Schubert, THIS JOURNAL, **71**, 2639 (1949).

[19] W. M. Schubert, J. Donohue and J. D. Gardner, *ibid.*, **76**, 9 (1954).

[20] F. M. Beringer and S. Sands, *ibid.*, **75**, 3319 (1953).

tenth that of the hydrolysis. This somewhat complicates calculation of the rate coefficients for the hydrolysis since it becomes desirable (although not essential, since the decarboxylation is relatively slow) to use a rate law for successive first-order reactions of the type

$$\text{ester} \xrightarrow{k_1} \text{acid} \xrightarrow{k_D} \text{hydrocarbon} + CO_2$$

The applicable equation for calculation of k_1 is

$$C_{\text{acid}} = \frac{ak_1}{k_D - k_1}\left(e^{-k_1 t} - e^{-k_D t}\right)$$

where a is initial concentration of ester. The value of k_D is measured either by following the decarboxylation of the mesitoic acid which remains after the ester has reacted or by separate experiments with the acid. Incidentally, the decarboxylation is acid catalyzed, as is to be expected from the experiments of Schubert[18,19] at higher acidities, and the rate varies with the H_0 acidity function.

ITHACA, N. Y.

[CONTRIBUTION FROM THE NAVAL MEDICAL RESEARCH INSTITUTE]

On Intermolecular and Intramolecular Interactions between Independent Pairs of Binding Sites in Proteins and Other Molecules

BY TERRELL L. HILL

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The Kirkwood–Shumaker suggestion, that matching constellations of dissociable groups on two different protein molecules might lead to a significant intermolecular attractive force, is examined in terms of two models. The general conclusion reached is that in many cases the Kirkwood–Shumaker proposal is a very reasonable one. Two related topics also discussed are: (1) the intermolecular or intramolecular potential of average force between two groups whose charges are not permanent but fluctuate because of binding equilibria with ions in solution; and (2) the effect of pairs of interacting groups on titration curves of proteins and other molecules.

I. Introduction

At the end of their paper on the force between two protein molecules, Kirkwood and Shumaker[1] suggest that "... steric matching of a constellation of basic groups on one molecule with a complementary constellation on the other could conceivably produce a redistribution of protons leading to a strong and specific attraction ...." The primary purpose of this paper is to examine this suggestion quantitatively for perhaps the simplest possible model (described in Section II). Specifically, we assume that matching constellations on two neighboring protein molecules exist, and then calculate the extent to which the protons would, in fact, take advantage of these matching constellations by redistributing themselves in such a way as to produce an attractive force. The most extreme situation would arise when the protons are frozen in the particular distribution corresponding to the maximum possible attractive force between the protein molecules. Roughly speaking, such redistributions of protons would be favored as far as the energy of the systems is concerned, but opposed by configurational entropy considerations. The outcome of this energy–entropy competition is not particularly obvious in advance.

The treatment to be given applies also to intramolecular interactions and therefore to titration curves of proteins and other molecules. The results suggest that the usual discussion of electrostatic effects on titration curves of proteins in terms only of the net charge of the entire molecule may overlook important local electrostatic interactions.

Some of the equations derived below are new only[2] in their manner of derivation (*via* the grand partition function) and in their particular application to the problem outlined above.

II. The Model and General Relations

Figure 1a represents two protein or other molecules close together and with matching constellations of two types of sites or groups, 1 and 2. Both sites of a pair are capable of binding the same kind of ion (or molecule), for example, a proton. The distance between each pair of sites is r. The effects we are interested in here arise only when r is rather small (see Section III); hence, as a first approximation, we neglect (1) interactions between sites on the same protein molecule, (2) second and higher neighbor interactions between sites on different protein molecules, and (3) interactions between the sites shown and other types of sites. Thus we are concerned here with a group of independent pairs of binding sites.

With this model the discussion will apply also to Fig. 1b, in which several equivalent but independent pairs of sites are distributed through a single large molecule.

Because of the assumed independence of pairs, the average properties per pair will not depend on the number of pairs under consideration; hence this number need not be specified. For example, Figs. 1c and 1d, each with only one pair of sites, are included.

The most straightforward way to derive the required equations is to consider each pair of sites as a system in a grand ensemble. The ensemble consists of a very large number of systems (pairs). Let j_1 be the partition function (including the binding energy) of a molecule or ion bound at site 1 of a pair, and similarly let j_2 refer to a molecule or ion bound at site 2. Let $W_{AA}(r)$, $W_{AB}(r)$, $W_{BA}(r)$ and $W_{BB}(r)$ be the free energies[3] of interaction between the sites, separated by a distance r, when both sites are occupied (AA), when site 1 is occupied and site 2 unoccupied (AB), etc., respectively. That is, the

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[1] J. G. Kirkwood and J. B. Shumaker, *Proc. Nat. Acad. Sci.*, **38**, 863 (1952).

[2] See, for example, E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943.

[3] The corresponding energies are $W_{AA} - T(\partial W_{AA}/\partial T)$, etc.