

activity of 45.6 dpm./ μ mole, while that in the norbelladine experiment was 24.4 dpm./ μ mole.

The results show unequivocally that both 2-C¹⁴-tyrosine and 1-C¹⁴-norbelladine (I) give rise to haemanthamine (II, R = OH) labeled specifically at the starred position. There is good evidence that the slightly low figure for the dimethone derivative results from formation of traces of formaldehyde-yielding products by side reactions during the Kolbe step. Thus, for reasons discussed earlier,¹ our results demonstrate the derivation from tyrosine of that part of the haemanthamine molecule (II, R = OH) drawn with heavy bonds. The higher incorporation of activity from norbelladine suggests, but does not rigorously prove, that it is incorporated intact into haemanthamine. However, the possibility is not eliminated that norbelladine may be degraded first to a C₆-C₂ fragment which is then incorporated and experiments are in progress to test this.

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A NON-ENZYMATIC OLEFINIC HYDRATION UNDER NEUTRAL CONDITIONS. THE KINETICS OF THE HYDRATION OF FUMARIC ACID MONOANION

Sir:

The classical hydration of an olefin involves a hydronium ion-catalyzed reaction.¹ The hydration of olefins conjugated with carbonyl groups, specifically benzalacetone and fumaric acid, has been shown to be catalyzed by both hydronium ions^{2,3} and hydroxide ions.^{4,5} On the other hand, the hydration of fumaric acid to L-malic acid by the enzyme fumarase occurs in a pH region around neutrality and the pH-rate profile for this enzymatically-catalyzed reaction can be most simply interpreted as involving two catalytic groups, an acid and a base, on the enzyme.⁶ It was therefore of interest to determine whether a non-enzymatic hydration of an olefin could occur near neutrality with the involvement of both acidic and basic catalysts. This communication reports the observation of such a reaction in the hydration of the fumaric acid monoanion.

The kinetics of the hydration of fumaric acid to malic acid in aqueous solution were studied over the pH range⁷ 0 to 6 at 175°. First order kinetics

(1) R. H. Boyd, R. W. Taft, Jr., A. P. Wolf and D. R. Christman, *J. Am. Chem. Soc.*, **82**, 4729 (1960).

(2) D. S. Noyce and W. I. Reed, *ibid.*, **80**, 5539 (1958).

(3) L. T. Rozelle and R. A. Alberty, *J. Phys. Chem.*, **61**, 1637 (1957).

(4) L. E. Erickson and R. A. Alberty, *ibid.*, **63**, 705 (1959). In fumarate dianion the carbon-carbon double bond would not be expected to be fully conjugated with the carboxylate groups.

(5) D. S. Noyce and W. L. Reed, *J. Am. Chem. Soc.*, **81**, 624 (1959).

(6) R. A. Alberty, *J. Cell. Comp. Physiol.*, **47**, Supp. 1, 245 (1956); R. A. Alberty, W. G. Miller and H. F. Fisher, *J. Am. Chem. Soc.*, **79**, 3973 (1957).

(7) The concentration of hydrogen ion was controlled with hydrochloric acid solutions or acetic acid buffers, and was calculated from knowledge of the room temperature concentrations, the specific volume

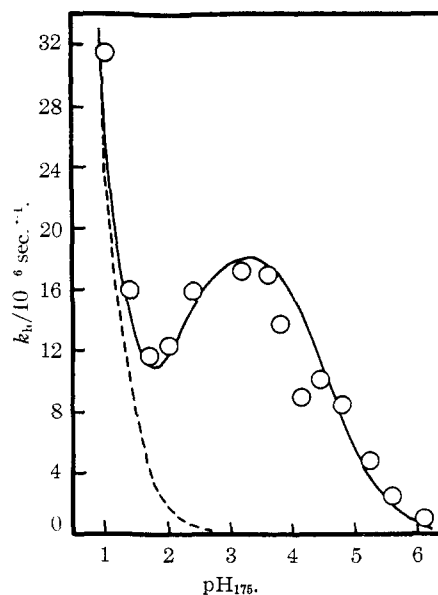


Fig. 1.—The hydration of fumaric acid in aqueous solution at 175°; see text for explanation of the smooth lines.

are observed, and the reaction is reversible.¹⁰ From the kinetic and equilibrium measurements both k_h and k_d , the first-order rate constants for hydration and dehydration, can be evaluated. The variation of k_h with pH is shown in Fig. 1; k_d is a similar function of pH. The results cannot be interpreted in terms of a hydronium ion-catalyzed reaction of the un-ionized acid³ plus a hydroxide ion-catalyzed reaction of the doubly-ionized molecule.⁴ The reaction can be accounted for simply by assuming that the monoanionic forms of the acids undergo an "uncatalyzed" reaction, so that the rate of hydration can be written

$$V_h = k_h^0[\text{H}_2\text{F}][\text{H}^+] + k_h'[\text{HF}^-] + k_h''[\text{F}^{2-}][\text{OH}^-] \quad (1)$$

and a similar equation can be given for the dehydration reaction.¹¹ The relationship between k_h and the hydronium ion concentration can be derived easily from Eq. 1. The smooth curve in Fig. 1 is a calculated one based upon Eq. 1 and assigned values of the rate constants and the first and second dissociation constants of fumaric acid.¹² The dashed line in Fig. 1 shows the curve to be expected if $k_h^{-1} = 0$.

of water at 175° (1.120),⁸ and the pK_a' of acetic acid at 175° (5.40).⁹ The ionic strength was maintained at 0.10 M (at room temperature), except at the lowest pH, by the addition of potassium chloride.

(8) M. G.-A. Hirn, *Ann. chim. phys.*, **10**, 32 (1867).

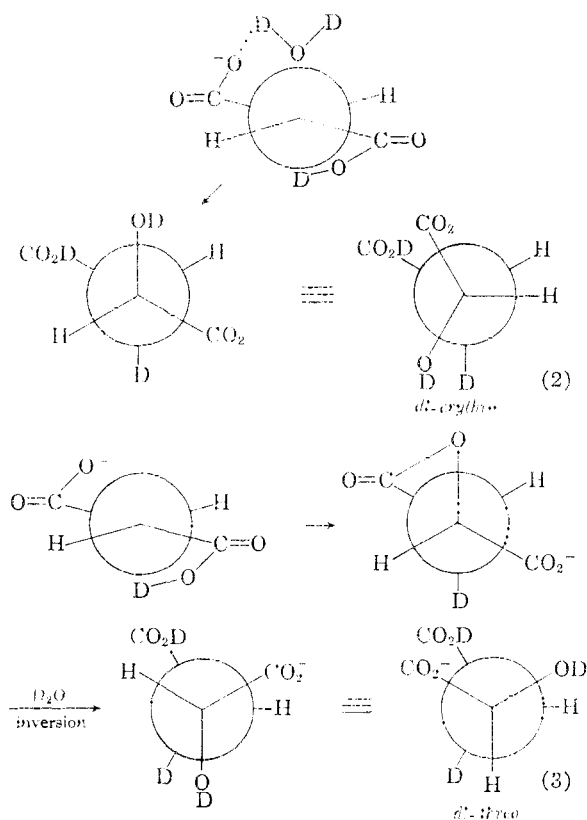
(9) A. A. Noyes, A. C. Melcher, H. C. Cooper, G. W. Eastman and Y. Kato, *J. Am. Chem. Soc.*, **80**, 335 (1958).

(10) The reaction was carried out in Pyrex ampules, which were placed in a constant-temperature aluminum block and removed at appropriate intervals; the rate was followed by spectrophotometric analysis at 270 m μ . Preliminary experiments indicated that the presence of air in the samples caused erratic results, so all samples were degassed.

(11) There appears to be a slight contribution to the rate through a route involving catalysis by the acetic acid buffer; however, at the total acetic acid concentration employed in most of this work (0.1 M), this effect is no greater than the experimental error of the measurements.

(12) These values were employed in the construction of the smooth curve in Fig. 1: $k_h^0 = 3.3 \times 10^{-4}$ l./mole-sec.; $k_h' = 2.0 \times 10^{-6}$ sec.⁻¹; $pK_{1F} = 2.0$; $pK_{2F} = 4.6$. The corresponding constants for the dehydration reaction are: $k_d^0 = 1.1 \times 10^{-4}$ l./mole-sec.; $k_d' = 1.1 \times 10^{-5}$ sec.⁻¹; $pK_{1M} = 2.2$; $pK_{2M} = 4.8$. The hydroxide ion-catalyzed reaction is negligible below pH 7.

Neither the un-ionized nor the doubly-ionized form of the acid appears to undergo an uncatalyzed reaction, while the singly-ionized form, containing both a $-\text{COOH}$ and a $-\text{COO}^-$ group in the molecule, does. Therefore these two groups may be necessary for this uncatalyzed reaction to occur. One may postulate either that the presence of these groups in the molecule allows the spontaneous reaction to proceed (a substituent effect), or alternatively that the carboxylic acid and carboxylate ion groups actively participate in the reaction. The latter possibility seems the more likely one.¹³ Two mechanisms may be written in which these groups act in concert. One involves an intramolecular general acid-general base catalysis, the elements of water being included in the transition state (eq. 2). The other mechanism can be described as a general acid-nucleophile catalyzed reaction, with the carboxylate ion attacking carbon to form an intermediate lactone, which then hydrolyzes to give malic acid (eq. 3).



Equations 2 and 3 imply the formation of stereochemically different products when the hydration is carried out in deuterium oxide: *dl*-erythro-malate (trans addition) and *dl*-threo-malate (over-all cis addition),¹⁴ respectively. However, it has not been possible to observe the stereochemistry predicted by either Eq. 2 or 3, even though the nuclear magnetic resonance spectra of both of these acids

(13) At pH 4.5 the rate is increased by a factor of 3×10^4 over that to be expected if the monoanion does not undergo reaction.

(14) H. N. K. Rørdam, *J. Chem. Soc.*, 2931 (1932); A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed., Chap. 12, J. Wiley and Sons, Inc., New York, N. Y., 1961.

have been recorded,^{15,16} since the monodeuteriomalic acid formed at pH 4.0 and 175° apparently exchanges its methylene proton with the solvent to yield 3,3-dideuterio-malic acid. Therefore it is not possible to decide whether Eq. 2 or 3 describes the course of the hydration reaction, or indeed to show that the reaction is stereospecific.

The reaction described in this communication appears to be the first observed non-enzymatic olefinic hydration under neutral conditions (at 175° neutrality is at pH 5.77). The rate of the reaction is far too small to permit a serious comparison with that of the fumarase-catalyzed hydration of fumaric acid. However, its occurrence demonstrates a new mechanism of olefin hydration and provides some support for a hypothesis of enzymatic catalysis involving the simultaneous functioning of both an acidic and a basic group.

(15) R. A. Albery and P. Bender, *J. Am. Chem. Soc.*, **81**, 542 (1959).

(16) F. A. L. Anet, *ibid.*, **82**, 994 (1960).

(17) Alfred P. Sloan Foundation Research Fellow.

(18) National Institutes of Health Postdoctoral Research Fellow.

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STUDIES ON d-ORBITAL CONJUGATION. I. THE ABSENCE OF A THROUGH-CONJUGATION EFFECT IN SOME SULFONE CARBANIONS

Sir:

In a classic theoretical paper on the conjugative properties of sulfones Koch and Moffitt¹ pointed out that a carbon 2-p orbital may adopt two extreme orientations with respect to an adjacent sulfone group, *viz.*, it may lie in the C-S-C plane ("Case II" conjugation) or perpendicular to that plane ("Case I" conjugation). They concluded, from a treatment involving chiefly symmetry arguments, that Case I conjugation would be better than Case II, and also that either Case I or Case II conjugation would be weak unless *both* substituents on the sulfone group were unsaturated. This treatment has been criticized by Jaffé,² who with an explicit consideration of the form of the d orbitals has concluded that potentially conjugating groups attached to a d-orbital element, far from reinforcing each other's conjugation, will in fact compete for the d-orbital. We have examined this point and wish to report that in a clear example of Case I conjugation there is no evidence for Koch and Moffitt's predicted through-conjugation effect.

The species selected for examination were the enolate ions derived from the disulfone esters I-IV. The ester group guarantees that the carbanion will be sp^2 -p hybridized, which need not otherwise be true,³ and the geometry of the cyclic anions derived from II and IV is such that only Case I conjugation is possible. Compound I

(1) H. P. Koch and W. E. Moffitt, *Trans. Faraday Soc.*, **47**, 7 (1951). This work has been discussed by G. Cilento, *Chem. Rev.*, **60**, 147 (1960).

(2) H. H. Jaffé, *J. Phys. Chem.*, **58**, 185 (1954).

(3) Cf. W. E. Doering and L. K. Levy, *J. Am. Chem. Soc.*, **77**, 509 (1955); H. E. Zimmerman and B. S. Thyagarajan, *ibid.*, **82**, 2505 (1960); D. J. Cram, W. D. Nielsen and B. Rickborn, *ibid.*, **82**, 6415 (1960); E. J. Corey and E. T. Kaiser, *ibid.*, **83**, 490 (1961).