pretation in terms of this mechanism. Thus the requirement for a *ligand* water molecule in the mechanism can explain the decline in catalytic activity at high $[Cl^-]$, *i.e.*, on going to $RuCl_6^{3-}$. On the other hand, the decrease in catalytic activity at low Cl^- concentrations ($<2\ M$) presumably is associated with the predominance in this region of the lower (*i.e.*, neutral and cationic) chloro-complexes and is attributable to the substitution-inertness of these complexes since the proposed rate-determining formation of an Ru(III)-acetylene complex is essentially a substitutional reaction. This is consistent with Fine's[2] observation that the anionic chloro-complexes of Ru(III) are substitution-labile and equilibrate rapidly with their solution environment, while the lower cationic ones do so only very slowly.

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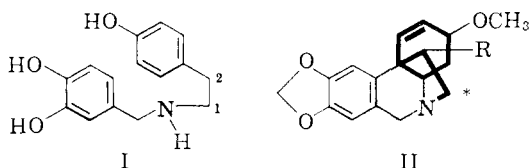UNIVERSITY OF BRITISH COLUMBIA   B. R. JAMES
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BIOSYNTHESIS IN THE AMARYLLIDACEAE. TYROSINE AND NORBELLADINE AS PRECURSORS OF HAEMANTHAMINE

Sir:

The incorporation of $2\text{-}C^{14}$-tyrosine into lycorine,[1,2] norpluvine,[1] and galanthamine[2] by several plants of the *Amaryllidaceae* has been reported. It has been proposed[3] that these alkaloids are synthesized in the plant through the oxidative coupling of phenolic intermediates related to norbelladine (I) and experiments *in vivo* have shown the

incorporation of activity from norbelladine and several of its partially methylated derivatives into galanthamine,[2] galanthine,[4] haemanthamine [4] lycorine,[5] and norpluviine.[5]

At present the position of the radioactive atom in the alkaloids derived from precursors labeled in the carbon skeleton has been determined by degradation only in the cases of lycorine derived from $2\text{-}C^{14}$-tyrosine[1] and lycorine derived from $1\text{-}C^{14}$-norbelladine[5] (I): these two precursors were fed separately to "Twink" double Narcissus plants. We now report the isolation and degradation of radioactive haemanthamine (II, R $-$ OH) isolated from the same feeding experiments. The

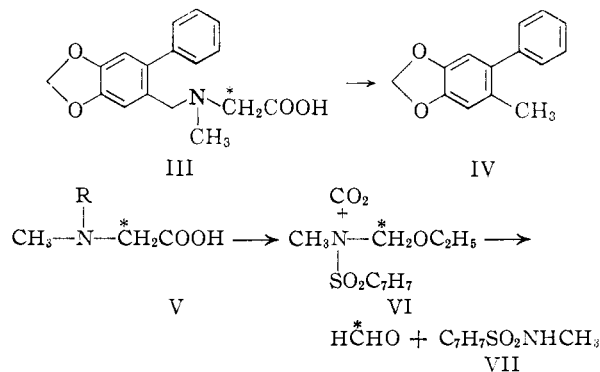(1) A. R. Battersby, R. Binks and W. C. Wildman, *Proc. Chem. Soc.*, 410 (1960).

(2) D. H. R. Barton and G. W. Kirby, *ibid.*, 392 (1960).

(3) D. H. R. Barton and T. Cohen in "Festschrift A. Stoll," Birkhäuser, Basel, 1957, p. 117.

(4) D. H. R. Barton, G. W. Kirby, J. B. Taylor and G. M. Thomas, *Proc. Chem. Soc.*, 254 (1961).

(5) A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales and W. C. Wildman, *ibid.*, 243 (1961).

cultivation of the bulbs and isolation of lycorine and norpluviine have been reported in our earlier work.[1,5] The alkaloidal fraction remaining after the isolation of lycorine and norpluviine was fractionated on alumina to give radioactive caranine and haemanthamine (II, R = OH) [0.079% and 0.078% incorporation, respectively, from tyrosine; 0.28% and 0.15% incorporation, respectively, from norbelladine]. The degradation of haemanth-

amine to the acid (III) and methanol has been reported earlier.[6] The methanol was converted into its 3,5-dinitrobenzoate. Hydrogenolysis of III afforded the oily biphenyl (IV) (*Anal.* Calcd. for $C_{14}H_{12}O_2$: C, 79.22; H, 5.70. Found: C, 79.05; H, 5.95) and sarcosine (V, R = H) which was isolated as the N-tosyl derivative (V, R = tosyl), m.p. 150–151°.[7] Kolbe electrolysis[8] of the latter afforded carbon dioxide (collected as barium carbonate) and N-ethoxymethyl-N-methyl-*p*-toluenesulfonamide (VI), $n^{28}D$ 1.5125. (*Anal.* Calcd. for $C_{11}H_{17}NO_3S$: C, 54.29; H, 7.04; N, 5.76; N-CH$_3$, 6.18; OC$_2$H$_5$, 18.52. Found: C, 54.49; H, 7.13; N, 5.88; N-CH$_3$, 6.42; OC$_2$H$_5$, 17.75. Hydrolysis of VI in dilute ethanolic hydrochloric acid in the presence of excess dimedone reagent gave the formaldehyde derivative, m.p. 190–190.5°, and N-methyl-*p*-toluenesulfonamide (VII), m.p. 78–80°. The relative activities of compounds II–VII are listed in the table. Haemanthamine used in the tyrosine experiment had a specific

TABLE I

Compound	Tyrosine-fed	Norbelladine-fed
Haemanthamine (II, R = OH)	1.00^a	1.00
Oxohaemanthamine (II, R = O)	1.00	0.99
2-Methyl-4,5-methylenedioxy-biphenyl (IV)	<0.01	.01
Methyl 3,5-dinitrobenzoate	(.00)	.00
N-Tosylsarcosine (V, R = tosyl)	.98	.99
Barium carbonate	.00	< .01
N-Methyl-*p*-toluenesulfonamide (VII)	(< .01)	.00
Formaldehyde dimethone	.92	.87

^{a} The two parenthetical figures were obtained by combustion of the samples to barium carbonate, counted as an infinitely thick disc. The rest were obtained by scintillation counting in toluene–(2,5-diphenyloxazole–1,4'-bis-2-(5-phenyloxazolyl)-benzene solution.

(6) H. M. Fales and W. C. Wildman, *J. Am. Chem. Soc.*, **82**, 197 (1960).

(7) E. Fischer and M. Bergmann, *Ann.*, **398**, 118 (1913).

(8) *Cf.* R. P. Linstead, B. R. S. Shepard and B. C. L. Weedon, *J. Chem. Soc.*, 2854 (1951).

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

The results show unequivocally that both 2-C^{14}-tyrosine and 1-C^{14}-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 C_6–C_2 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 range[7] 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
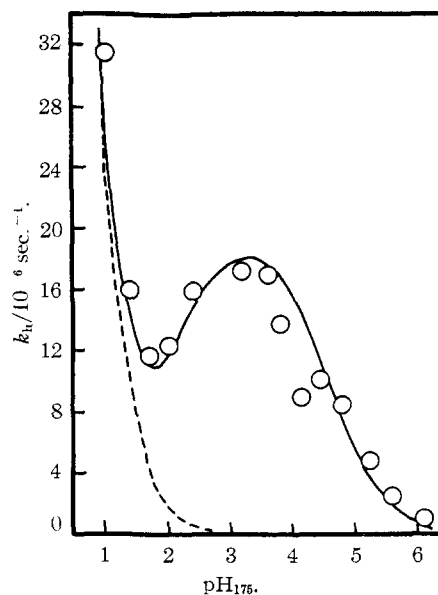
Fig. 1.—The hydration of fumaric acid in aqueous solution at 175°; see text for explanation of the smooth lines.

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 acid[8] 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

$$V_h = k_h^0[H_2F][H^+] + k_h'[HF^-] + k_h''[F^-][OH^-] \quad (1)$$

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.*, **30**, 335 (1908).

(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 mμ. 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 × 10^{-4} l./mole-sec.; k_h' = 2.0 × 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 × 10^{-4} l./mole-sec.; k_d' = 1.1 × 10^{-6} sec.$^{-1}$; pK_{1M} = 2.2; pK_{2M} = 4.8.   The hydroxide ion-catalyzed reaction is negligible below pH 7.