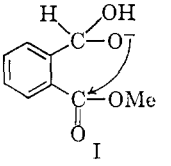
THE HYDROLYSIS OF METHYL *o*-FORMYLBENZOATE. PARTICIPATION OF THE NEIGHBORING ALDEHYDE GROUP IN THE HYDROXIDE ION AND MORPHOLINE-CATALYZED REACTIONS[1]

Sir:

The carboxylate ion, imidazole, carboxylic acid, carboxamide, and aromatic and aliphatic hydroxyl groups can participate in intramolecular catalyses which may serve as models for the intracomplex reactions in enzymic catalysis.[2] Recently Newman and Hishida[3] explained the exceptional hydrolytic reactivity of certain methyl substituted-*o*-benzoylbenzoates in terms of the initial attack of hydroxide ion on the keto group of the substrate. We have investigated the hydrolytic reactions of the similar compound, methyl *o*-formylbenzoate, using hydroxide ion and morpholine as catalysts. Both reactions are exceedingly facile; $k_2^{OH^-} = 2000\ M^{-1}\text{sec.}^{-1}$ ($t_{1/2}$ at pH 8.5 = 115 seconds)[4]; the morpholine catalysis is kinetically a complex reaction involving an intermediate formed in appreciable concentration (Fig. 1). These are the fastest nonenzymatic hydrolyses of *methyl* esters known in aqueous solution at 25°, and presumably involve the participation of the *o*-formyl group in the hydrolytic process.

The rate constant for the alkaline hydrolysis of methyl *o*-formylbenzoate may be calculated to be 1.3×10^{-2} mole^{-1}sec.$^{-1}$, using the ratio of rate constants of the *o*-nitro- and *p*-nitrobenzoate esters, and the rate constant of the *p*-formylbenzoate ester (corrected to aqueous solution). Since the experimental rate constant is over 10^5 faster than that calculated for the compound solely on the basis of its substituent (electronic and steric) effect, the direct involvement of the formyl group as suggested by Newman and Hishida[3] must be operative. The formation and subsequent intramolecular reaction of the intermediate I, containing an alkoxide ion in a position directly adjacent to an ester grouping, presumably will account for the enhanced reactivity found here. The reaction



I

of I is suggestive of the intra-complex reaction of seroxide ion (the anion of the serine hydroxyl group) in the catalytic steps of the enzyme chymotrypsin.[5]

Since nucleophiles other than hydroxide ion can add to carbonyl groups, such reagents were tested as catalysts for this reaction. Of the bases and/or nucleophiles tested, imidazole, diethanolamine, carbonate, bicarbonate, monohydrogen phosphate, dihydrogen phosphate, and azide ions were without significant effect; N-methylhydroxylamine, hydroxylamine, bisulfite ion, and Tris gave complex side reactions; while morpholine was a strong catalyst for hydrolysis.

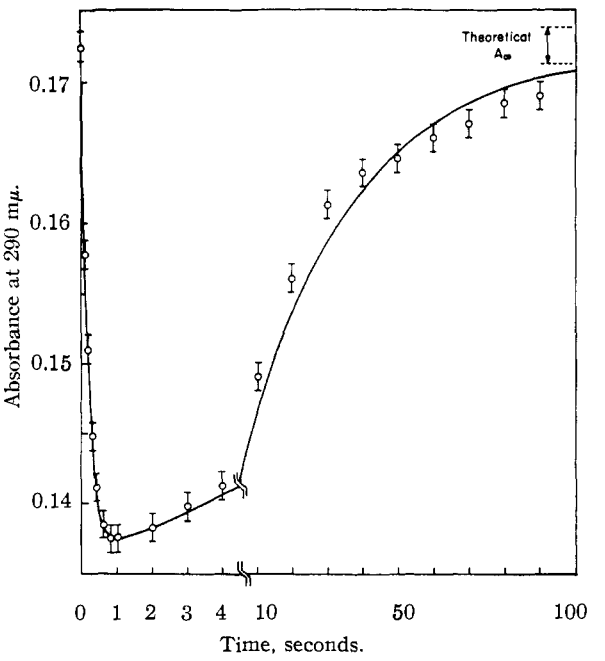


Fig. 1.—The morpholine-catalyzed hydrolysis of methyl *o*-formylbenzoate; [morpholine] = 0.242 *M*; μ = 1.0; *T* 25.0°; pH 8.74; measurements Φ taken on a spectrophotometer equipped with a stopped-flow mixing device (*cf.* F. J. Kezdy and M. L. Bender, *Biochemistry*, in press (1962)); solid line, calculated curve (see text).

The morpholine catalysis was shown spectrophotometrically to produce *o*-formylbenzoate. It was possible to observe (at the isosbestic point of the reactant and product) the formation and decomposition of an unstable intermediate (Fig. 1). Two simple possibilities may explain the behavior in Fig. 1; Equation 2 which postulates the reversible formation of the intermediate B, or a similar system in which both reactions are irreversible.

$$A \underset{k_2}{\overset{k_1[M]}{\rightleftharpoons}} B \xrightarrow{k_3} C \qquad (2)$$

Equation 2 can explain the dependence of the decay of the intermediate on morpholine concentration approaching a saturation value while two irreversible steps cannot. The reaction is not general base-catalyzed; therefore morpholine acts as a nucleophile in step k_1, a conclusion supported by the observation of an intermediate of *lower* extinction coefficient (presumably an adduct of morpholine and the aldehyde group). Equation 2 has been tested by catalyses using four morpholine concentrations from 0.25 to 0.025 *M*. Using the general kinetic solution for Eq. 2,[6] a consistent

(1) This research was supported by a grant from the National Science Foundation.

(2) M. L. Bender, *Chem. Revs.*, **60**, 53 (1960).

(3) M. S. Newman and S. Hishida, *J. Am. Chem. Soc.*, **84**, 3582 (1962). The authors are grateful for the opportunity of seeing this manuscript before publication.

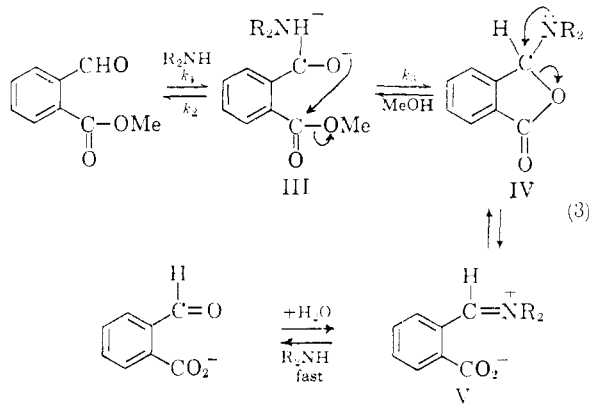
(4) Aqueous solution at 25°. Excellent pseudo first-order plots were obtained in 11 runs from pH 5.3 to 9.3.

(5) M. L. Bender, *J. Am. Chem. Soc.*, **84**, 2582 (1962).

(6) The general solution of Eq. 2 is of the form $B = Q(e^{-\alpha t} - e^{-\beta t})$ where β and α, the apparent rate constant of the decay of the intermediate, are functions of k_1, *M*, k_2 and k_3. See S. W. Benson, "The Foundations of Chemical Kinetics," McGraw-Hill Book Co., New York, 1960, p. 41. Kinetically Eq. 2 cannot be distinguished from $C \overset{k_3[M]}{\longleftarrow} A \underset{k_2}{\overset{k_1[M]}{\rightleftharpoons}} B$, but mechanistically this scheme does not seem probable.

set of data is found, yielding $k_1 = 21.5\ M^{-1}$, $k_2 = 0.24$ and $k_3 = 0.033$ sec.$^{-1}$ at μ 1.0 and pH 8.7. These data yield the calculated solid line in Fig. 1, in good agreement with experiment.[7] The calculated and experimental curves for the other data are in reasonable agreement.

On the basis of the above evidence mechanism (3) for the morpholine catalysis can be postulated.[8,9]



The morpholine catalysis (like the hydroxide ion catalysis) is, on this basis, an example of nucleophilic catalysis of ester hydrolysis in which the nucleophile does not react directly with the ester linkage to form an unstable intermediate (the usual type of nucleophilic catalysis) but rather reacts with a neighboring group to form an unstable intermediate which leads eventually to the hydrolytic products and the regeneration of the catalyst. A similar example in the hydrolysis of a keto-substituted phosphoric acid ester is consistent with this interpretation.[10]

(7) The theoretical curve was calculated using an Applied Dynamics AD-232PB analog computer by Dr. Kenneth A. Connors of the School of Pharmacy, University of Wisconsin. It is assumed in this computation that the intermediate has an extinction coefficient at 290 mμ of approximately two-thirds that of the reactant.

(8) The constant k_1 is increased and k_3 is decreased by increasing μ, as predicted by eq. 3.

(9) Compound III is analogous to an intermediate in Schiff base formation: E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 832 (1962).

(10) F. Ramirez, B. Hansen and N. B. Desai, *ibid.*, **84**, 4588 (1962).

(11) Alfred P. Sloan Foundation Research Fellow.

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(13) The authors acknowledge the assistance of Drs. F. J. Kezdy and G. E. Clement with the morpholine kinetics.

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THE STRUCTURE OF CYCLOBUXINE

Sir:

The medicinal properties of *Buxus sempervirens* L. have been known since ancient times; extracts of the plant have been used in the treatment of a wide variety of diseases, including malaria and venereal disease.[1] More recently, an alkaloidal extract of the plant has been reported to possess antitubercular properties.[2] Earlier studies have indicated the multicomponent nature of the alkaloid extract.[1,3-6] Evidence is presented herewith for assignment of structure I to an alkaloid isolated from the acetone-insoluble portion of the strong base fraction, for which we propose the name *cyclobuxine*.[7] Cyclobuxine represents a novel type of steroidal alkaloid; it appears to be the first recognized to contain a cyclopropane ring and the first with a substitution pattern at C-4 and C-14 which is intermediate in the biogenetic scheme, between lanosterol- and cholesterol-type steroids.

Cyclobuxine (I), $C_{25}H_{42}ON_2$, m.p. 245–247° dec., $[\alpha]^{23}D$ + 98°,[8] shows λ_{max} 6.09, 11.20 μ[8] (terminal methylene) and n.m.r. peaks[9] 5.20 and 5.43 (2H, doublets, $J < 1$ c./s.; terminal methylene), 5.92 (1H, octuplet, J's 3, 7, 9.5 c./s.; CH_2–C*H*OH–CH), 7.53 and 7.57 (6H, two NCH_3), 8.87 and 9.03 (6H, two tertiary CH_3), 8.92 (3H, doublet, J 6 c./s.; one sec. CH_3) and 9.72 and 9.95 τ (2H, doublets, J 4 c./s.; cyclopropyl methylene). Cyclobuxine was converted to several crystalline derivatives: *e.g.*, the dihydrobromide, $C_{25}H_{44}ON_2Br_2$, m.p. 288–292° dec.; the N,N′-dimethyl derivative,[3] $C_{27}H_{46}ON_2$, m.p. 204–205° dec., $[\alpha]^{25}D$ + 99°, n.m.r. peaks at 5.07, 5.38, 5.98, 8.87, 9.03, 9.72, 9.97 (as for cyclobuxine), 5.60 (1H, OH), 7.68 and 7.77 (12H, two $N(CH_3)_2$) and 9.13 τ (3H, doublet, J 6.5 c./s.; sec. CH_3 near $N(CH_3)_2$); the N,N′-dimethyl-O-acetate,[3] $C_{29}H_{48}O_2N_2$, m.p. 173–175°, $[\alpha]^{25}D$ + 69°, λ_{max} 5.81 μ, n.m.r. peaks at 4.95 (1H, octuplet; CH_2-C*H*OAc-CH), 8.03 (3H, CH_3COO–); the triacetate,[3] $C_{31}H_{48}O_4N_2$, m.p. 256–258° dec., $[\alpha]^{24}D$ − 12°, λ_{max} 5.78, 6.14, 11.08μ; the dihydro derivative,[3] $C_{25}H_{44}ON_2$, m.p. 208–209°, $[\alpha]^{25}D$ + 46°, infrared showed no bands at 6.09 or 11.20 μ, n.m.r. peaks at 9.22 (3H, doublet, J 7 c./s.; new sec. CH_3), 9.43 and 9.73 τ (2H, cyclopropyl methylene).

The gross skeletal structure was indicated by the structures suggested for the products of selenium dehydrogenation: an 8-methyl-1,2-cyclopentenophenanthrene (II), $C_{21}H_{22}$, m.p. 145–147°, trinitrobenzene complex, $C_{27}H_{25}O_6N_3$, m.p. 165–168°; the corresponding 5-methyl-1,2-cyclopentenoanthracene (III), $C_{21}H_{22}$, m.p. 110–112°, trinitrobenzene complex, $C_{27}H_{25}O_6N_3$, m.p. 168–169°; and two naphthalenes, IV, $C_{21}H_{28}$, m.p. 134–135°, trinitrobenzene complex, $C_{27}H_{31}O_6N_3$, m.p. 143–145°; and V, $C_{22}H_{28}$, m.p. 111–117°, trinitrobenzene complex, $C_{28}H_{31}O_6N_3$, m.p. 139–141°. The hydrocarbons were characterized by analysis, infrared, ultraviolet,

(1) E. Schlittler, K. Heusler and W. Friedrich, *Helv. Chim. Acta*, **32**, 2209 (1949).

(2) L. E. Weller, C. T. Redemann, R. V. Gottshall, J. M. Roberts, E. H. Lucas, and H. M. Sell, *Antibiotics and Chemotherapy*, **3**, 603 (1953); Merck & Co., Inc., British Patent 782,469 (1957).

(3) K. Heusler and E. Schlittler, *Helv. Chim. Acta*, **32**, 2226 (1949).

(4) W. Friedrich and E. Schlittler, *ibid.*, **33**, 873 (1950).

(5) E. Schlittler and W. Friedrich, *ibid.*, **33**, 878 (1950).

(6) K. S. Brown, Jr., and S. M. Kupchan, *J. Chromatography*, **9**, 71 (1962).

(7) Cyclobuxine is "III," the alkaloid of R_f 0.76 in Fig. 2 of reference 6. It is most probably the same as "Alkaloid A" of reference 3; although no comparison sample of "A" is available, the physical constants of cyclobuxine and its derivatives correspond closely to those of "A" and the respective derivatives.

(8) All rotations and infrared spectra are in chloroform.

(9) All n.m.r. spectra were determined on a Varian Associates recording spectrometer (A-60) at 60 Mc. in deuterated chloroform or carbon tetrachloride. Chemical shifts are reported in τ values (p.p.m.) [G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958)]. We thank Mr. Roy Matsuo and Mr. Arnold Krubsack for these determinations.