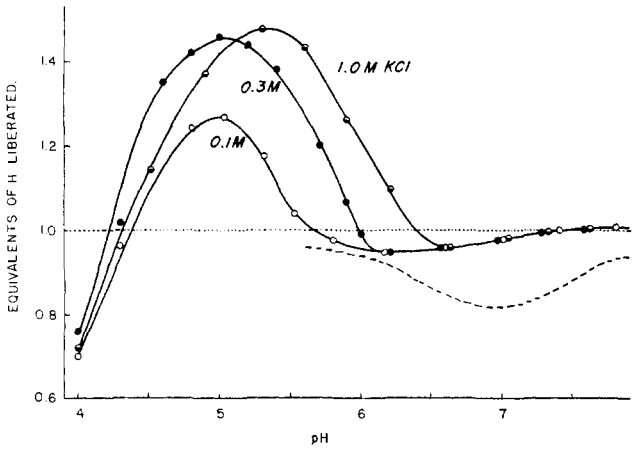

Fig. 2.—The liberation of hydrogen ions during the reaction of PMSF with α-chymotrypsin: dashed line calculated for a change in pK_a from 6.8 to 7.1.

reacting. The values determined by these methods agreed within 1%.

The acylation and deacylation steps in chymotrypsin-catalyzed hydrolyses are pH controlled. It is generally agreed that the simplest explanation requires that the basic form of a dissociable group (assumed to be imidazole) participates directly in the rate-controlling step. A number of investigators[5] have attempted to evaluate the pK_a of this essential group by kinetic means with a variety of acylating agents; they agree on values of 6.6–7.0 for acylation and 6.8–7.4 for deacylation. In all cases the apparent pK_a for deacylation is at least 0.1 pK_a unit greater than that for the acylation step. A change in pK_a from 6.8 to 7.1[3] (*cf.* dashed line in Fig. 2) would have led to the liberation of only 0.82 equivalent of hydrogen ion at pH 7.0, whereas in fact 0.98 equivalent is released. The method used here, actually a difference titration, is capable of an accuracy within 1% and leads to the conclusion that no group in chymotrypsin having an apparent pK_a near 7.0 undergoes a change of more than 0.03 pK_a unit upon sulfonylation of the enzyme.[6] This fact is inconsistent with the postulate that the active-site serine hydroxyl is hydrogen-bonded to an imidazole group in the free enzyme.

The release of "extra" protons below pH 6 (see Fig. 2)[7] shows that the titration curve of the derivative is different from that of chymotrypsin in that pH region and suggests different conformations. If phenylmethanesulfonyl chymotrypsin has a conformation at pH 7–8 differing from that of the native enzyme, this is not reflected in the titration data.

(5) For pertinent references, see ref. 3.

(6) It must be noted, however, that the values above pH 6.2 are roughly consistent with a change in pK_a from 6.0 to 6.1.

(7) The results are not corrected for uptake of hydrogen ions by fluoride at low pH.

(8) (a) National Science Foundation predoctoral fellow; (b) Research Career Development Award grantee of the National Institutes of Health.

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RECEIVED NOVEMBER 15, 1962

INTRAMOLECULAR BIFUNCTIONAL GENERAL BASE–GENERAL ACID-CATALYSIS OF ESTER SOLVOLYSIS[1]

Sir:

Earlier studies have provided support for the view that the ready methanolysis of the C-16 acetate ester grouping in ceveratrum alkaloid derivatives is a base-catalyzed solvolysis[2] which is facilitated by a neighboring hydroxyl group bearing a *cis*-1,3-diaxial relationship to the ester group.[3–8] Furthermore, the solvolysis of 1,3-diaxial hydroxyacetates recently has been shown to be subject to general base–general acid catalysis.[9] Evidence is presented herewith for the argument that the tertiary nitrogen atom of ceveratrum ester alkaloid derivatives participates in an intramolecular basic catalysis of the solvolysis of C-16 esters. The reaction is therefore an instance of *intramolecular bifunctional general base–general acid-catalysis of ester solvolysis.*[10]

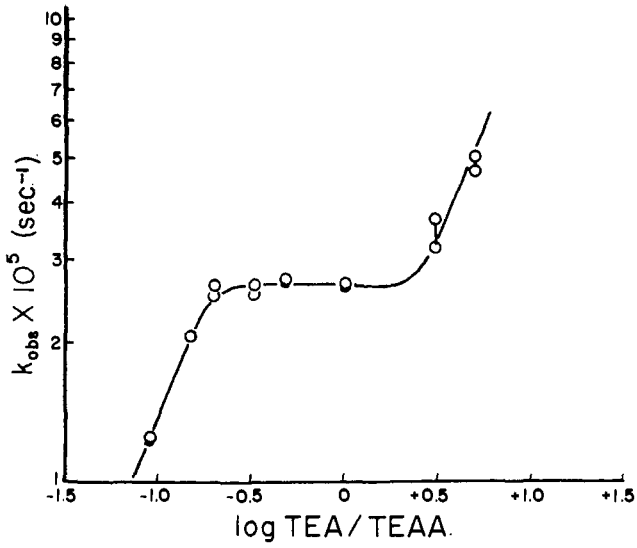


Fig. 1.—Logarithmic plot of the rate of methanolysis of cevadine D-orthoacetate diacetate (III) against the logarithm of the buffer ratio in 0.06 *M* triethylamine/triethylammonium acetate buffers at 25° and ionic strength 0.09.

Acetate esters were methanolyzed in solutions prepared by dissolving each compound in chloroform (10% of the total volume), adding buffer and tetramethylammonium chloride, and diluting to the required volume with 10% aqueous methanol. The rate of production of methyl acetate, the solvolysis product, was determined by direct gas chromatographic analysis of the reaction mixture, as described earlier.[9]

RATES OF ESTER SOLVOLYSIS AT 1:3 TRIETHYLAMINE:TRIETHYLAMMONIUM ACETATE BUFFER (0.12 *M*) AND IONIC STRENGTH 0.09 AT 25°

Compound	k_{obs} (sec.$^{-1}$) (pseudo-first-order)[11]	Ratio of rates
Dehydrocevadine-D-orthoacetate diacetate (I)	1.2×10^{-8}	1
Formamido-ketone from cevadine D-orthoacetate diacetate (II)	4.8×10^{-7}	40
Cevadine D-orthoacetate diacetate (III)	1.2×10^{-5}	1000

(1) This is part VII of a series entitled "Intramolecular Catalysis"; part VI is reference 9.

(2) W. J. Rosenfelder, *J. Chem. Soc.*, 2638 (1954).

(3) (a) S. M. Kupchan and W. S. Johnson, *J. Am. Chem. Soc.*, **78**, 3864 (1956); (b) S. M. Kupchan, W. S. Johnson and S. Rajagopalan, *Tetrahedron*, **7**, 47 (1959).

(4) S. M. Kupchan and C. R. Narayanan, *J. Am. Chem. Soc.*, **81**, 1913 (1959).

(5) S. M. Kupchan, C. I. Ayres, M. Neeman, R. H. Hensler, T. Masamune and S. Rajagopalan, *ibid.*, **82**, 2242 (1960).

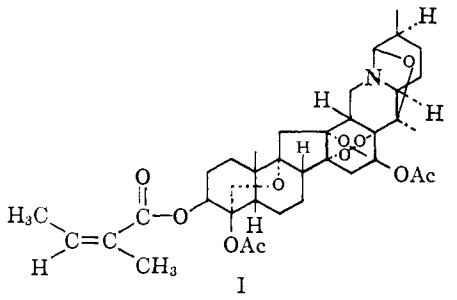
(6) S. M. Kupchan, N. Gruenfeld and N. Katsui, *J. Med. Pharm. Chem.*, **5**, 690 (1962).

(7) *Cf.* H. B. Henbest and B. J. Lovell, *Chemistry and Industry*, 278 (1956); *J. Chem. Soc.*, 1965 (1957).

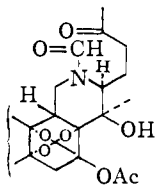
(8) *Cf.* R. West, J. J. Korst and W. S. Johnson, *J. Org. Chem.*, **25**, 1976 (1960).

(9) S. M. Kupchan, S. P. Eriksen and M. Friedman, *J. Am. Chem. Soc.*, **84**, 4159 (1962).

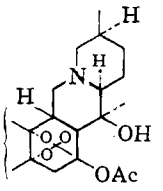
(10) Preliminary polarimetric data in accord with the interpretation discussed herein were presented at the 140th Natl. Meeting of the American Chemical Society, Chicago, September, 1961 (S. M. Kupchan, A. Afonso and P. Slade, Abstracts, p. 88-Q).



I



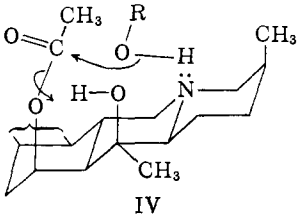
II



III

From the data in the table, it is evident that the conversion of the 16-β-axial acetate ester I into the 1,3-diazial 20-hydroxy-16-acetate II resulted in a 40-fold increase in the rate of solvolysis. Participation of the alkaloid nitrogen in the facilitation of the solvolysis of III is supported by the 25-fold increase in the rate of solvolysis relative to amide II.[12]

Intramolecular base-catalysis of the solvolysis of cevadine D-orthoacetate diacetate (III) (possibly to be regarded as in IV) was confirmed by the experimentally determined buffer ratio-rate profile (Fig. 1). In the central horizontal portion of the curve,



IV

the ring nitrogen is essentially non-protonated and serves as an intramolecular base for the normally general base-catalyzed solvolysis of 1,3-diaxial hydroxyacetates.[9] As the buffer is made more acidic, the rate drops off, decreasing approximately in proportion to the protonation of the ring nitrogen. With higher base concentrations, *intermolecular* general base catalysis plays an increasingly competitive role, adding its effect to that of the *intramolecular* general base. The effect of the external general base in the "high base" region is better indicated by the standard experiments for studying general base catalysis, shown in Fig. 2. Varying the buffer concentration produces no effect if the buffer ratio chosen is in the "intramolecular general base-catalyzed" region (*i.e.*, the flat portion of Fig. 1, TEA/TEAA = 0.5), whereas in the region of external competition (TEA/TEAA = 5.0), a definite intermolecular general base-effect is observed.[13]

Considerable evidence has been accumulated during the past few years to indicate that esters are catalytically hydrolyzed by esteratic enzymes through a double displacement reaction involving an acylated

(11) Control experiments with cevadine D-orthoacetate 4-monoacetate indicated that the 4-acetate group is stable under the reaction conditions, and that the rate of production of methyl acetate from III therefore corresponds to the pseudo-first-order rate constant for the solvolysis of the 16-acetate group.

(12) The very weak basicities of I (*cf.* reference 3b) and of II preclude intramolecular base-catalysis as an important factor in the solvolysis of the latter compounds.

(13) The extrapolated zero buffer values for these two buffer ratios indicate, as expected, that another base (perhaps methoxide from the solvent) is also playing a role.

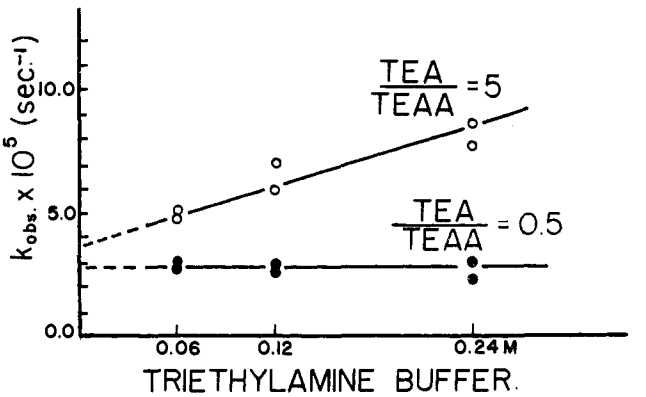


Fig. 2.—Triethylamine catalysis of the methanolysis of cevadine D-orthoacetate diacetate (III) at 25°, ionic strength 0.09, and two buffer ratios, triethylamine/triethylammonium acetate 1:2, ●, and 5:1, O.

enzyme intermediate. The formation of acyl-enzyme takes place after formation of an enzyme-substrate complex, and undoubtedly involves intracomplex participation of specific catalytic groups.[14,15] The deacylation step apparently utilizes the same enzymatic components and one widely accepted mechanism of hydrolytic enzyme action involves intramolecular general base–general acid-catalysis.[16,17] In view of the foregoing, much effort has been expended recently in a search for hydrolytic reactions which proceed *via* first order processes with assistance of an intramolecular nature. One instance of an intramolecular bifunctional general acid-nucleophilic catalysis of ester hydrolysis has been noted.[18] The solvolysis of cevadine orthoacetate diacetate appears to be the first recognized nonenzymatic example of intramolecular bifunctional general base–general acid-catalysis of ester solvolysis and may have considerable significance as an appropriate model for esteratic enzyme action.[19]

We take pleasure in thanking Professors M. L. Bender and T. Higuchi for stimulating discussions.

(14) H. Gutfreund and J. M. Sturtevant, *Biochem. J.*, **63**, 656 (1956).

(15) M. L. Bender, *Chem. Rev.*, **60**, 53 (1960).

(16) M. L. Bender, G. R. Schonbaum, G. A. Hamilton and B. Zerner, *J. Am. Chem. Soc.*, **83**, 1255 (1961).

(17) R. M. Krupka and K. J. Laidler, *ibid.*, **83**, 1458 (1961). *Cf.* especially Fig. 3 with IV of this paper.

(18) H. Morawetz and I. Oreskes, *ibid.*, **80**, 2591 (1958).

(19) This investigation was supported by grants from the National Institutes of Health (H-2275) and the Wisconsin Alumni Research Foundation.

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RECEIVED DECEMBER 19, 1962

PRIMARY PRODUCTS OF DECALIN AUTOXIDATION[1]

Sir:

Although the autoxidation of decalin is a well known reaction,[2] detailed studies of the primary autoxidation products have not been reported. We have

(1) Autoxidation of Decalin. I.

(2) (a) A. Castiglioni, *Gazz. chim. ital.*, **64**, 465 (1934); (*C.A.*, **29**, 30 (1935)); (b) A. C. Cope and G. Holzman, *J. Am. Chem. Soc.*, **72**, 3062 (1950); (c) R. Criegee, *Ber.*, **77B**, 22 (1944); (d) M. S. Eventova and I. A. Yavich, *Vestnik Moskov. Univ., Ser. Mat., Mekhan., Astron., Fiz. i. Khim.*, **14**, No. 2, 149 (1959); (*C.A.*, **54**, 9797*i* (1960)); (e) H. L. Goering and A. C. Olsen, *J. Am. Chem. Soc.*, **75**, 5853 (1953); (f) H. E. Holmquist, H. S. Rothrock, C. W. Theobald and B. E. Englund, *ibid.*, **78**, 5339 (1956); (g) K. I. Ivanov and U. K. Savinova, *Doklady Akad. Nauk S.S.S.R.*, **48**, 32 (1945); (*C.A.*, **40**, 4706^7 (1946)); (h) A. I. Kamneva and A. I. Efimenkova, *Trudy Moskov. Khim.-Teknol. Inst. im. D. I. Mendeleeva*, 1957, No. 25, 38 (1957); (i) H. Kleinfeller, *Angew. Chem.*, **62**, 342 (1950); (j) C. Kroger and K. Struber, *Naturwissenschaften*, **32**, 229 (1944); (k) C. Kroger, K. Struber and C. Umland, *Erdöl u. Kohle*, **1**, 241 (1948); (C.A., **44**, 1683*f* (1950)).