Intramolecular Catalysis. IX.[1] Intramolecular General Base–General Acid Catalysis of Ester Solvolysis[2,3]

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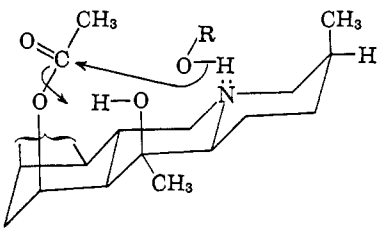
Abstract: Evidence is presented for the argument that the tertiary nitrogen atom of ceveratrum ester alkaloid derivatives participates in an intramolecular basic catalysis of the solvolysis of C-16 esters, and that the reaction is therefore an instance of *intramolecular bifunctional general base–general acid catalysis* of ester solvolysis. The rate of the bifunctionally catalyzed solvolysis of III was found to be 1000 times faster than that of the unassisted 16β-axial acetate ester V. Conversion of the ester V into the 1,3-diaxial-20-hydroxy-16-acetate VII resulted in a 40-fold increase in rate of solvolysis. Participation of the alkaloid nitrogen in the facilitation of solvolysis of III is supported by the 25-fold increase in the rate of solvolysis relative to the formamido ketone VII. Intramolecular base catalysis of the solvolysis of III and VI was confirmed by the experimentally determined buffer ratio–rate profile, and by the results obtained upon variation of buffer concentration at different buffer ratios. The conversion of cevadine diacetate (VI) into cevadine orthoacetate diacetate (III) resulted in a threefold decrease in the rate of solvolysis. The rate change is discussed in the light of an earlier proposal that a chair → twist-boat conformation of the D ring takes place upon formation of the orthoacetate.

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[4] which is facilitated by a neighboring hydroxyl group bearing a *cis*-1,3-diaxial relationship to the ester group.[5-10] Furthermore, the solvolysis of 1,3-diaxial hydroxyacetates recently has been shown to be subject to general base–general acid catalysis.[1,11] 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.*[12]

Examination of models indicated that the tertiary nitrogen atom of ceveratrum ester alkaloids could participate in an intramolecular general base catalysis of the solvolysis of C-16 esters, possibly as depicted in I. To evaluate whether the tertiary nitrogen atom *does* indeed participate in an intramolecular catalysis,



I

studies of the methanolysis of a series of derivatives of cevadine orthoacetate diacetate (III) were undertaken. Cevadine orthoacetate diacetate (III) was selected as the most suitable ceveratrum alkaloidal ester for solvolysis studies, because of the previously observed sensitivity and selectivity of methanolysis of the compound's C-16 acetate ester, to yield cevadine orthoacetate 4-monoacetate (IV).[5,13] Furthermore, III could be converted, by methods developed earlier in this laboratory, to dehydrocevadine orthoacetate diacetate (V) (in essence, a compound without either basic or hydroxyl functions, in view of the very weak observed basicity of such bridged carbinolamine ethers[5b]) and the formamido ketone VII (a compound which contains the *cis*-1,3-diaxial hydroxyacetate system but lacks basic nitrogen).

Cevadine orthoacetate diacetate (III) was prepared by acetylation of cevadine (II)[14] with acetic anhydride–perchloric acid.[13] Oxidation of III with N-bromosuccinimide in chloroform solution gave dehydrocevadine orthoacetate (V). Oxidation of V with chromic acid in pyridine yielded the formamido ketone from cevadine orthoacetate diacetate (VII). Cevadine diacetate (VI) was prepared by acetylation of cevadine with acetic anhydride in pyridine.[13]

Acetate esters were methanolyzed in solutions prepared by dissolving each compound in chloroform (10 or 40% of the final volume[15]), adding buffer and tetra-

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(2) The investigations which form the subject of this paper were first outlined in part in a preliminary communication: S. M. Kupchan, S. P. Eriksen, and Y.-T. Shen, *ibid.*, **85**, 350 (1963).

(3) This work was supported in part by Public Health Service Research Grant HE-02275, from the National Heart Institute.

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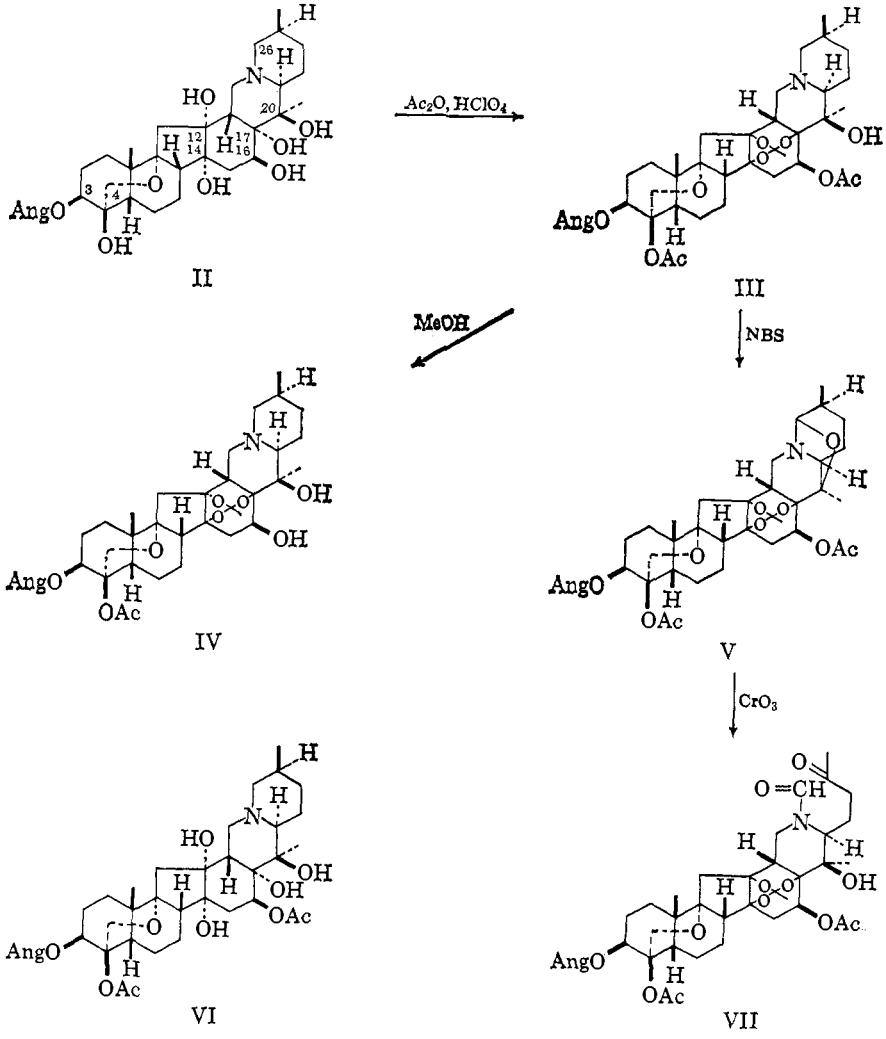
(15) The rates of solvolysis reported in ref. 2 were all determined in the 40% chloroform rather than the 10% chloroform solutions inadvertently indicated there.



Ang = angeloyl

methylammonium chloride, and diluting to the required volume with 9:1 methanol–water. The rate of production of methyl acetate, the solvolysis product, was determined by direct gas chromatographic analysis of the reaction mixture, as described earlier.[1]

From the data in Table I, it is evident that no detectable methyl acetate was produced after attempted solvolysis of cevadine orthoacetate 4-monoacetate (IV) under the usual reaction conditions. Hence the C-4 acetate group is stable under these conditions, and the rate of production of methyl acetate corresponds to the pseudo-first-order rate constant for the solvolysis of the C-16 acetate group of each ester alkaloid derivative. The data indicate that the conversion of the C-16 β-axial acetate ester V into the 1,3-diaxial 20-hydroxy-16-acetate VII resulted in a 40-fold increase in the rate of solvolysis. This 40-fold increase is about one order of magnitude less than the increase noted for conversion of coprostanol acetate into coprostane-3β,5β-diol 3-monoacetate,[1] but the difference in the rate enhancement is attributable to marked differences in structure and rigidity of the respective environments. 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 VII.

Intramolecular general base catalysis of the solvolysis of cevadine orthoacetate diacetate (III) and of cevadine diacetate (VI) was confirmed by the experimentally determined buffer ratio–rate profile (Figure 1).[16] The shape of the curve and theoretical considerations

Table I. Rates of Ester Solvolysis at 1:3 Triethylamine–Triethylammonium Acetate Buffer (0.12 *M*) and Ionic Strength 0.09 *M* at 25°

Compound	k_{obsd}, sec.$^{-1a}$	k_{obsd}, sec.$^{-1b}$	Ratio of rates
Dehydrocevadine orthoacetate diacetate (V)	1.2×10^{-8}	...	1
Formamido ketone from cevadine orthoacetate diacetate (VII)	4.8×10^{-7}	...	40
Cevadine orthoacetate diacetate (III)	1.2×10^{-5}	2.5×10^{-5}	1000
Cevadine diacetate (VI)	...	7.5×10^{-5}	3000
Cevadine orthoacetate 4-monoacetate (IV)	0	...	...

[a] Data obtained in solutions in which chloroform constituted 40% of the total volume. [b] Data obtained in solutions in which chloroform constituted 10% of the total volume.

support the view that the rate equation has at least two terms

$$\text{rate} = k_{obsd}[M] = k_1[E][M] + k_2[E][M][B]$$

(16) Examination of molecular models indicates that intramolecular nucleophilic attack by nitrogen is precluded on the basis of the nitrogen–carbonyl carbon interatomic distance.

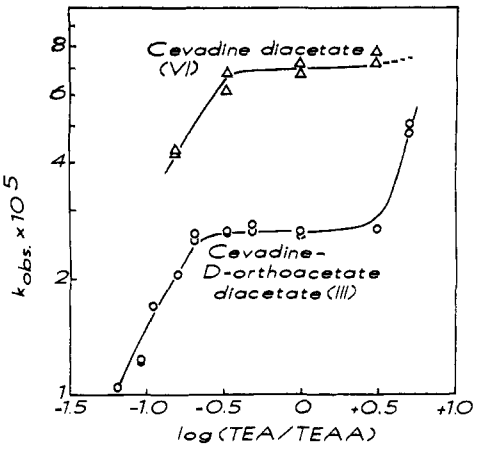


Figure 1. Logarithmic plot of the rate of methanolysis of cevadine orthoacetate diacetate (III) (○) and cevadine diacetate (VI) (△) against logarithm of buffer ratio in 0.06 *M* triethylamine–triethylammonium acetate buffers at 25° and ionic strength 0.09 (solvent system: chloroform, 10% of final volume, diluted with methanol–water, 9:1).

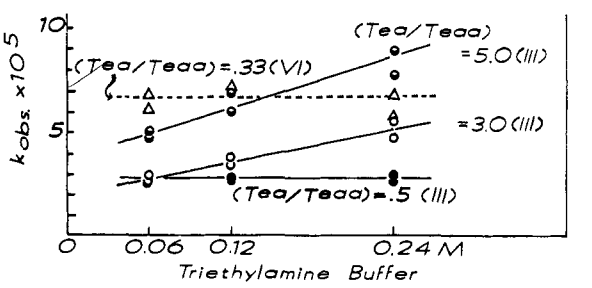
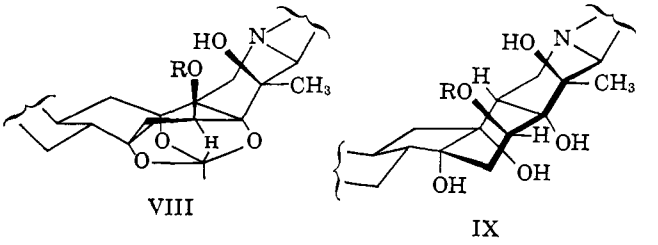


Figure 2. Triethylamine catalysis of the methanolysis of cevadine orthoacetate diacetate (III) at 25°, ionic strength 0.09, and buffer ratios, triethylamine–triethylammonium acetate 1:2 (●), 3:1 (○), 5:1 (◒), and cevadine diacetate (VI) at 1:3 (△) (solvent system: chloroform, 10% of final volume, diluted with methanol–water, 9:1).

where [M] is the concentration of methanol, [E] is the concentration of ester alkaloid, and [B] is the concentration of external base (in this case, triethylamine). The first term represents internal, and the second term external base catalysis. In the central, flat portion of the curve the contribution of the first term predominates; the ring nitrogen is essentially nonprotonated and serves as an intramolecular base for the normally general base catalyzed solvolysis of 1,3-diaxial hydroxyacetates. As the buffer ratio changes, there is essentially no rate change in solvolysis; the ring nitrogen is the only base participating in this buffer ratio range until the added base concentration becomes sufficiently high to compete. As the buffer is made more acidic, the curve shows that the rate decreases approximately in proportion to the protonation of ring nitrogen, although other undetermined catalytic effects may become increasingly important. In the higher base region, the external base, triethylamine, plays an important role in intermolecular general base catalysis. The rate increase in the "high base" region shows both the competitive and additive effect of the external base.

In the "intramolecular general base catalyzed" region, that is, the horizontal portion of the curve, there is no apparent change in rate with variation of the buffer concentration. Thus, with a triethylamine (TEA)–triethylammonium acetate (TEAA) ratio of 1:2 (Figure 2), the participation of the external base (TEA) appears to be nearly negligible in effect when compared with the effect of the internal tertiary amine. In the higher base region, the rate of solvolysis increases with the increase in concentration of buffer (TEA–TEAA = 5:1; 3:1), as one would expect for an intermolecular general base catalyzed reaction.

The results obtained in the study of the solvolysis of cevadine diacetate (VI) indicate that the mechanism of solvolysis is similar to that of cevadine orthoacetate diacetate (III). The buffer ratio–rate profile (Figure 1) shows a general shape similar to that observed for III, with a decrease in rate with increasing acidity and a flat portion which is not affected by varying buffer ratio. Varying the buffer concentration in the "plateau" region, as expected, led to no apparent change in rate of solvolysis. It is noteworthy, however, that cevadine diacetate (VI) is solvolyzed approximately three times as fast as cevadine orthoacetate diacetate (III). A recent study of the nuclear magnetic resonance spectra of veratrum alkaloids yielded results which accorded best with the view that a chair → twist-boat conformation of the D ring takes place upon formation of the orthoacetate.[17] This is illustrated in drawings VIII (from the Dreiding model of III) and IX (from the Dreiding model of VI). Examination of molecular



models indicates that in IX, the C-16 acetate and C-20 hydroxyl groups are aligned in a parallel conformation, whereas in VIII, the corresponding groups are oriented at an angle of approximately 20° with each other. The slow rate of solvolysis of cevadine orthoacetate diacetate (III), relative to cevadine diacetate (VII), may be attributable to a less effective 1,3-interaction between the hydroxyl and acetate groups resulting from the distortion from coplanarity of the two groups in the twist-boat conformation.

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 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.[18,19] The deacylation step apparently utiilzes the same enzymatic components, and one widely accepted mechanism of hydrolytic enzyme action involves intramolecular general base–general acid catalysis.[20–22] In view of the foregoing, much effort has recently been expended in search for hydrolytic reactions which

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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.[23] The solvolysis of the C-16 acetate ester in ceveratrum alkaloid derivatives 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.

Experimental Section

Melting points are corrected for stem exposure. Specific rotations at the sodium D line were determined on a Zeiss Winkel polarimeter. Methanolyses were run at 25 ± 0.1° unless otherwise specified. Paper chromatography was conducted by the descending technique on Whatman No. 4 paper.

Cevadine Orthoacetate 4,16-Diacetate (III). This compound was prepared according to the method of Stoll and Seebeck,[13] m.p. 271–272° dec., $[\alpha]^{24}D$ +90° (*c* 1.02, chloroform).

Cevadine Orthoacetate 4-Monoacetate (IV). This compound was prepared by the methanolysis of III according to the method previously described.[13] The monoacetate IV was crystallized from aqueous acetone as colorless needles, m.p. 272–276° dec., $[\alpha]^{25}D$ +70° (*c* 1.03, chloroform).

Cevadine Diacetate (VI). This compound was prepared by acetylation of cevadine with acetic anhydride and pyridine according to the method previously described.[13] Recrystallization of the crude product from ether–petroleum ether yielded colorless needles, m.p. 258–260° dec., $[\alpha]^{23}D$ −13° (*c* 2.17, 95% alcohol).

Dehydrocevadine Orthoacetate 4,16-Diacetate (V). Cevadine orthoacetate diacetate (3 g.) in chloroform (50 ml.) was treated with a solution of N-bromosuccinimide (1 g.) in chloroform (100 ml.), and the mixture was allowed to stand at room temperature for 5 min. The mixture was washed with cold, dilute ammonium hydroxide, then with water, and evaporated to dryness. Crystallization of the residue from acetone afforded colorless needles (0.9 g.), m.p. 249–253° dec. Purification was effected by adsorption of the product in benzene on Woelm neutral alumina and elution with chloroform and benzene mixtures. Fractions 6–9 afforded a solid residue (0.7 g.). Recrystallization twice from acetone gave V (0.2 g.), m.p. 259–260°, $[\alpha]^{26}D$ +42° (*c* 0.98, chloroform). *Anal.* Calcd. for $C_{38}H_{51}NO_{11}$: C, 65.40; H, 7.37; N, 2.01. Found: C, 65.17; H, 7.59; N, 2.35.

Formamido Ketone from Cevadine Orthoacetate 4,16-Diacetate (VII). Dehydrocevadine orthoacetate diacetate (0.7 g.) in pyridine (5.6 ml.) was added to a complex of chromium trioxide (1.2 g.) and pyridine (8 ml.). The mixture was allowed to stand at room temperature for 40 hr. Water (20 ml.), chloroform (25 ml.), and dilute ammonia (3 ml., 1:1) were added, and the mixture was shaken and filtered through a bed of Filtercel. The chloroform layer was washed with water and dried over sodium sulfate. The chloroform solution was filtered and evaporated to dryness. The residue was chromatographed through 6 g. of neutral alumina with chloroform and benzene mixtures. Fractions 2–8 afforded 105 mg. of solid, m.p. 284–288° dec. Recrystallization from acetone and petroleum ether yielded 65 mg., m.p. 287–288°, $[\alpha]^{26}D$ +71° (*c* 1.03, chloroform). *Anal.* Calcd. for $C_{38}H_{51}NO_{13}$: C, 62.53; H, 7.04; N, 1.92. Found: C, 62.18; H, 7.27; N, 1.77.

Kinetic Measurements. Acetate ester alkaloids were methanolyzed as 0.01 *M* solutions prepared by dissolving each compound in the required volume of chloroform, adding the desired buffer and tetramethylammonium chloride solution in methanol–water (9:1) to adjust the ionic strength to 0.09 *M*, and diluting to the required volume with methanol–water (9:1). Solutions in which chloroform constituted 40% of the final volume were used for compounds III, V, and VII in the comparison of rates of solvolysis, because of the low solubility of compounds V and VII in methanol–water solution. The remaining methanolyses were run in solutions in which chloroform constituted 10% of the final volume. The buffers were prepared as follows. Solutions of triethylamine (2.5 g.) in methanol–water (9:1, 50 ml.) and of acetic acid (1.484 g.) in methanol–water (9:1; 50 ml.) were prepared. To the triethylamine solution (5 ml.) was added the acetic acid solution in appropriate volumes to make each of the buffers defined in terms of the ratio of triethylamine to triethylammonium acetate concentration. The reaction mixtures were equilibrated at 25° in a water bath. Aliquots were taken at intervals, and the production of methyl acetate, the solvolysis product, was determined by direct gas chromatographic analysis of the reaction mixture, using a Wilkens Hy-Fi A600 gas chromatograph with a hydrogen flame detector. Identification of peaks and calibration of peak areas were carried out by injecting standard solutions of methyl acetate in the same solvent system. True first-order behavior was observed in all cases; rate constants observed for solutions up to 0.02 *M* were constant within experimental error. No acetic acid was detected in these systems. We suggest that there was little (if any) acetic acid formed, because the methyl acetate recovered was usually approximately equal to the theoretical expectation. The chromatographic column consisted of 5 in. of 10% Nujol on Fluoropak 80 and 5 ft. of 20% glycerine on 60–80 mesh Gaschrom A. The column temperature was maintained at 65–70°, and the over-all accuracy of the analytical procedure is estimated at ±5%.

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

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