131°. Vacuum sublimation (85–90°, 0.1 mm.) gave a 92% recovery of pure 1-formamido-1,2,2-trimethylcyclohexane, m.p. 134.5–135°.

Anal. Calcd. for $C_{10}H_{19}NO$: C, 70.96; H, 11.31; N, 8.28. Found: C, 70.67; H, 11.03; N, 8.53.

(b) **1-Methylamino-1,2,2-trimethylcyclohexane.**—Lithium aluminum hydride reduction of 5 g. of the formamido compound in ether at reflux for 16 hr. gave 4.4 g. of the free base as an oil, $n^{25}D$ 1.4658. Conversion to the hydrochloride in ethereal hydrogen chloride and recrystallization from 130 ml. of methyl isobutyl ketone gave pure material, m.p. 230–232°.

Anal. Calcd. for $C_{10}H_{22}ClN$: C, 62.64; H, 11.57; N, 7.31; Cl, 18.49. Found: C, 62.98; H, 11.31; N, 7.69; Cl, 18.85.

V. Aliphatic Amines. 3-Methylamino-2,2,3-trimethylbutane Hydrochloride (31). (a) **3-Formamido-2,2,3-trimethylbutane.**—2,2,3-Trimethylbutene-1 (10 g.) was converted to the formamido compound *via* the Ritter reaction using 5.5 g. of sodium cyanide in 13 ml. of glacial acetic acid and 25 ml. of sulfuric acid in 13 ml. of glacial acetic acid for 2 hr. at 40–50°. After quenching with ice the product was extracted with ether and the ether evaporated to give 12 g. of white solid, m.p. 135–140°. The corresponding alcohol (2,3,3-trimethyl-2-butanol) may also be used as a starting material.

(b) **3-Methylamino-2,2,3-trimethylbutane Hydrochloride.**—Eleven grams of 3-formamido-2,2,3-trimethylbutane was reduced with lithium aluminum hydride in refluxing ether for 5 hr. The product was isolated as the hydrochloride (10.7 g.) which was recrystallized from 40 ml. of 2-propanol (6.1 g.), m.p. 238–240°.

Anal. Calcd. for $C_8H_{20}ClN$: C, 57.98; H, 12.17. Found: C, 57.92; H, 12.38.

2,2-Dimethyl-3-methylaminobutane (30).[23]—Pinacolone (20 g.) was reductively aminated with methylamine (8.7 g.) in methanol using Raney nickel catalyst (2 g.) with 196 kg. of hydrogen/cm.^{2} for 8 hr. After removal of the catalyst the free base was isolated by extraction with ether and purified by distillation, yielding 4.7 g., b.p. 108–111°, $n^{25}D$ 1.4110; hydrochloride salt, m.p. 177–183°.

Anal. Calcd. for $C_7H_{18}ClN$: Cl, 23.4. Found: Cl, 23.3.

(23) H. Albers and S. Lange, *Chem. Ber.*, **85**, 278 (1952).

Veratrum Alkaloids. XLIX.[1] The Structures and Configurations of Sabine and Sabadine[2,3]

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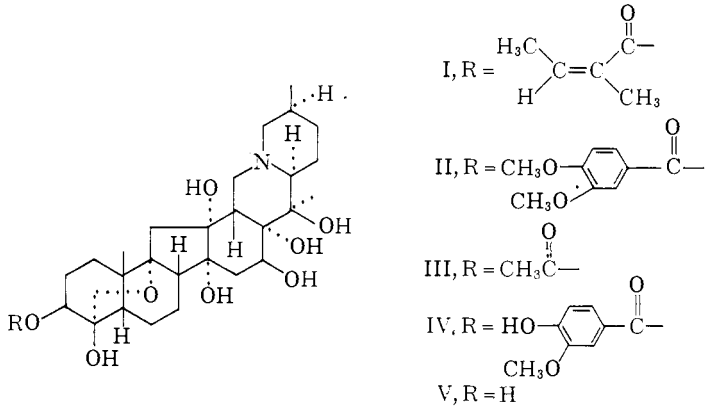
Received April 12, 1962

Evidence is presented for assignment of structure and configuration VI to sabine and VII to its monoacetate ester, sabadine. Elementary analyses of a series of synthetic crystalline ester derivatives strongly support a $C_{27}H_{43}O_7N$ formula for sabine. Room temperature acetylation of sabadine with acetic

anhydride–pyridine yields sabine triacetate (XI). Acetylation with acetic anhydride-perchloric acid affords sabine orthoacetate triacetate (XII), which on alkaline hydrolysis gives sabine orthoacetate (XIII). Methanolysis of XII gives sabine orthoacetate diacetate (XIV) which is oxidized by chromic acid to dehydrosabine orthoacetate diacetate (XV). Alkaline treatment of XV gives the α,β-unsaturated ketone XVI. Tiglation of sabadine under mild conditions affords a monotiglate (XVII), which yields sabine orthoacetate monotiglate diacetate (XVIII) upon acetylation with acetic anhydride-perchloric acid. Osmium tetroxide–periodic acid oxidation of XVIII yields a mixture of sabine orthoacetate diacetate (XX) and sabine orthoacetate monoacetate (XIX). Partial acetylation of sabine orthoacetate yields XX, and chromic acid oxidation of XX affords ketone XXIII. Exposure to XXIII to dilute alkali results in gradual autoxidation to a product with ultraviolet absorption characteristic of a 3,4-diosphenol. The evidence in support of assignment of the acetyl group of sabadine (VII) to C_3 is discussed. Pharmacological studies of sabadine are summarized.

The seeds of *Schoenocaulon officinale* Gray, commonly called "Sabadilla seed," yield a mixture of strongly insecticidal alkaloids, "veratrine." The principal alkaloidal constituents of veratrine are cevadine (I),[5] veratridine (II),[6] cevacine (III),[6] vanilloylveracevine (IV),[6] and veracevine (V).[7,8] In addition to the foregoing, isolation



(1) Part XLVIII in the series: S. M. Kupchan, J. H. Zimmerman and A. Afonso, *Lloydia*, **24**, 1 (1961).

(2) This investigation was supported in part by research grants (H-2275 (C4, C5)) from the National Institutes of Health.

(3) Part of the work was presented at the I.U.P.A.C. Symposium on the Chemistry of Natural Products, Sydney, Australia, August, 1960.

(4) Wisconsin Alumni Research Foundation Predoctoral Fellow, 1957–1959; National Science Foundation Cooperative Graduate Fellow, 1959–1960; recipient of the 1960 Lunsford Richardson Pharmacy Award for a paper including part of this work.

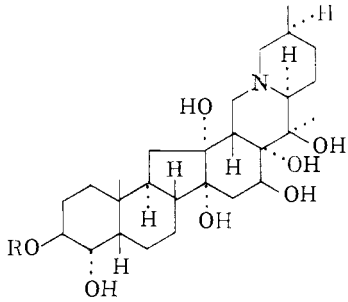
(5) S. M. Kupchan and A. Afonso, *J. Am. Pharm. Assoc.*, **49**, 242 (1960).

(6) S. M. Kupchan, *J. Pharm. Sci.*, **50**, 273 (1961).

(7) D. H. R. Barton, O. Jeger, V. Prelog and R. B. Woodward, *Experientia*, **10**, 81 (1954).

(8) S. M. Kupchan, W. S. Johnson and S. Rajagopalan, *Tetrahedron*, **7**, 47 (1959).

of a series of less well-characterized minor alkaloidal constituents of *S. officinale* has been reported.[9,10] Of the minor alkaloids, sabadine and its alkamine, sabine, have received most attention, and the present report offers evidence for assignment of structure and configuration VI to sabine and VII to sabadine.



VI, R = H

VII, R = $CH_3C(=O)-$

Sabadine was first isolated from the seeds of *S. officinale* in 1891 by E. Merck.[11] The alkaloid, m.p. 238–240°, was assigned the empirical formula $C_{29}H_{51}O_8N$. In 1955, Auterhoff and Schwartz[9] purified Merck's original sample of sabadine, and retained the name for a higher melting material (m.p. 258–260°, $[\alpha]^{20}_D$ −9.5°) to which was assigned the molecular formula $C_{27}H_{43}O_8N$. Some three years later, Auterhoff and Möhrle[12] reported the isolation of a new alkamine, neosabadine ($[\alpha]^{20}_D$ −33°) from Sabadilla seed. Sabadine and neosabadine were reported to be quite similar in their properties except for the presence of a carbonyl band in the infrared spectrum of sabadine and its absence from the spectrum of neosabadine. The same authors showed in 1959 that sabadine is a monoacetate ester of neosabadine, and they assigned to sabadine the molecular formula $C_{27}H_{43}O_8N(CH_3CO)$.[13]

In 1951, Hennig, Higuchi and Parks reported the isolation of an ester alkaloid, sabatine, from the hydrophilic alkaloid fraction of the seed of *S. officinale*.[14] Sabatine was reported to be the monoacetate ester of a new alkamine, sabine. Mitchner and Parks in 1959 proposed the molecular formula $C_{29}H_{47-49}O_8N$ for sabatine, and $C_{27}H_{45-47}O_7N$ for sabine.[15]

(9) H. Auterhoff and F. Schwartz, *Arch. Pharm.*, **288**, 549 (1955).
(10) Z. J. Vejdelek, K. Macek and B. Kakac, *Coll. Czechoslov. Chem. Commun.*, **21**, 995 (1956).
(11) E. Merck, *Arch. Pharm.*, **229**, 164 (1891).
(12) H. Auterhoff and H. Möhrle, *ibid.*, **291**, 288 (1958).
(13) H. Möhrle and H. Auterhoff, *ibid.*, **292**, 337 (1959).
(14) A. J. Hennig, T. Higuchi and L. M. Parks, *J. Am. Pharm. Assoc.*, **40**, 168 (1951).
(15) H. Mitchner and L. M. Parks, *ibid.*, **48**, 303 (1959).

The reported physical constants of sabine, neosabadine, sabadine and sabatine are listed in Table I. It is evident that the melting

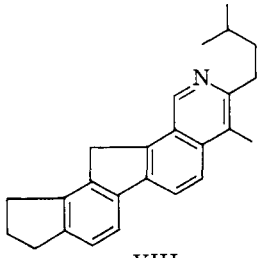
TABLE I

COMPARISON OF PHYSICAL CONSTANTS

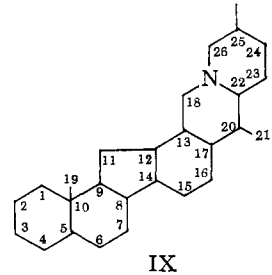
Alkaloid	$[\alpha]_D$, ethanol	M.p., °C.
Sabine[15]	−33°	173–176 dec.
Neosabadine[12]	−33°	amorphous
Sabadine[9, 12, 13]	−9.5°	256–258 dec.
Sabatine[14, 15]	−11.3°	256–258 dec.

points and optical rotations of sabine and neosabadine are very similar; the same relationship applies to sabatine and sabadine. In view of this similarity in physical properties and the apparent absence of the masked α-ketol system in these alkaloids (see below), it seemed likely that the respective alkamines and monoacetate esters might be identical. This hypothesis was tested by comparison of paper chromatographic behavior and infrared spectra of authentic samples obtained from each research group.[16] Indeed, sabatine was found to be identical with sabadine, and neosabadine was found to be identical with sabine. On historical grounds, the names sabadine and sabine (for the ester and alkamine respectively) deserve preference.

Upon selenium dehydrogenation, sabine yielded a product with ultraviolet absorption identical with that of cevanthridine (VIII), a selenium dehydrogenation product of cevine and related alkaloids.[12] On this basis, it was concluded that sabine possesses the cevane



VIII

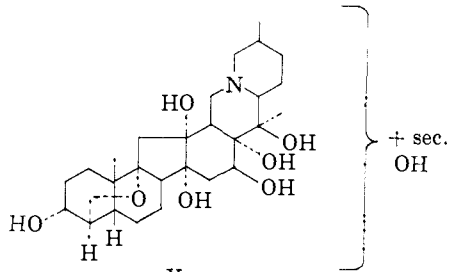


IX

(IX) ring system. The failure of sabine to give the characteristic cevine color reaction with sulfuric acid and to form a potassium salt were interpreted to indicate the absence of a masked α-ketol system.[12] The latter conclusion was advanced independently on the basis of the failure of the alkamine to isomerize under alkaline hydrolytic conditions or to reduce triphenyltetrazolium chloride reagent.[15]

(16) We thank Professor H. Auterhoff for samples of sabadine and neosabadine, and Professor L. M. Parks and Dr. H. Mitchner for samples of sabatine and sabine.

On the basis of dehydrogenation, hydrogenation and acetylation experiments and a proposed $C_{27}H_{43}O_8N$ molecular formula, Auterhoff and Möhrle[12] proposed partial structure X for sabine. The C_4–C_9 ether linkage was assigned in view of the apparent absence of a hemiketal hydroxyl group and by analogy to the cevine structure. In addition, treatment of the alkamine with hydrogen in the presence of platinum was reported to yield a "dihydro" compound (with un-



X

satisfactory elemental analysis) without concomitant change in the end absorption in the ultraviolet spectrum expected for the disappearance of an unsaturated linkage. Acetylation of sabine with acetic anhydride and pyridine either at room temperature or at steam bath temperature was reported to yield the same amorphous triacetate, indicative of the presence of three secondary hydroxyl groups. Cevine[17] and veracevine,[18] on the other hand, each give a diacetate upon low temperature acetylation and a triacetate at steam bath temperature, and it has been assumed that the hemiketal hydroxy of C_4 is the difficultly acylable one. "Dihydroneosabadine" also was reported to form a triacetate under both sets of conditions; this result was interpreted to signify that hydrogenation opened the ether linkage in such a manner as to give rise to the tertiary C_9-hydroxyl group. Assignment of hydroxyl groups at C_{12}, C_{14} and C_{17} was based on perchloric acid-catalyzed acetylation of sabine, which yielded crystalline sabine orthoacetate triacetate perchlorate. The latter salt liberated an amorphous base upon treatment with ammonia. Alkaline hydrolysis of sabine orthoacetate triacetate with methanolic potassium hydroxide gave amorphous sabine orthoacetate. Evidence advanced for the orthoacetate structure was absence of a carbonyl band in the infrared spectrum and formation of one mole of acetic acid upon strong acid hydrolysis (*cf.* references 19, 20). Location of

(17) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 886, footnote 17.

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

(19) D. H. R. Barton, C. J. W. Brooks and J. S. Fawcett, *J. Chem. Soc.*, 2137 (1954).

(20) S. M. Kupchan, *J. Am. Chem. Soc.*, **77**, 686 (1955).

hydroxyl groups at C_3, C_{16} and C_{20} appears to have been based solely upon biogenetic analogy to cevine.

At the outset of our work, considerable ambiguity existed as to the correct molecular formula of sabine. The sabine ester derivatives described by Auterhoff and Möhrle[12] had not been obtained in crystalline form, and no elementary analyses were reported for the latter compounds. Consequently, the proposed molecular formula, $C_{27}H_{43}O_8N$, was clearly not unequivocal. Mitchner and Parks,[15] on the other hand, proposed the empirical formula $C_{27}H_{45-47}O_7N$ for sabine on the basis of careful combustion analyses and molecular weight determinations of crystalline sabine and sabadine. The latter assignment was in good agreement with the original 1891 proposal of the formula $C_{29}H_{51}O_8N$ for sabadine.[11] The results of the present investigation strongly support a $C_{27}H_{45}O_7N$ formula for sabine. This molecular formula was confirmed by elementary analysis of the series of crystalline, paper chromatographically-homogeneous derivatives of sabine discussed below.

The $C_{27}H_{45}O_7N$ molecular formula for sabine, previously recorded chemical properties of the alkaloid, and biogenetic analogy have made possible the development of reasonable hypothetical structures for sabine and sabadine. Our corroborative evidence accumulated in favor of assignment of structure VI to sabine and structure VII to sabadine will be the subject of the remainder of the discussion.

The products of acetylation of sabadine under various conditions and their structural implications indicate that rings C, D, and E of sabine are identical with those of veracevine. Treatment of sabadine (VII) with acetic anhydride and pyridine at room temperature for ten hours and purification of the product on acid-washed alumina gave sabine 3,4,16-triacetate (XI), m.p. 221–222° dec. Treatment of sabadine (VII) with acetic anhydride in the presence of perchloric acid yielded sabine orthoacetate triacetate perchlorate.[12] Liberation of the free base with ammonia and subsequent crystallization from ether gave sabine orthoacetate triacetate (XII), m.p. 255–257°. Hydrolysis of XII with 0.02 *N* methanolic potassium hydroxide under reflux for three hours yielded sabine orthoacetate (XIII), m.p. 172–175°. The infrared spectrum of XIII in chloroform showed absorption bands at 7.12 and 8.87 μ characteristic of orthoacetates in the cevine series.[20] The molecular rotation change from sabine to sabine orthoacetate (+383°) approximates the change from cevine to cevine-D-orthoacetate (+441°); the molecular rotation change from cevagenine to cevagenine-C-orthoacetate is +57°.[20] These facts strongly support the view that sabine orthoacetate is a D-

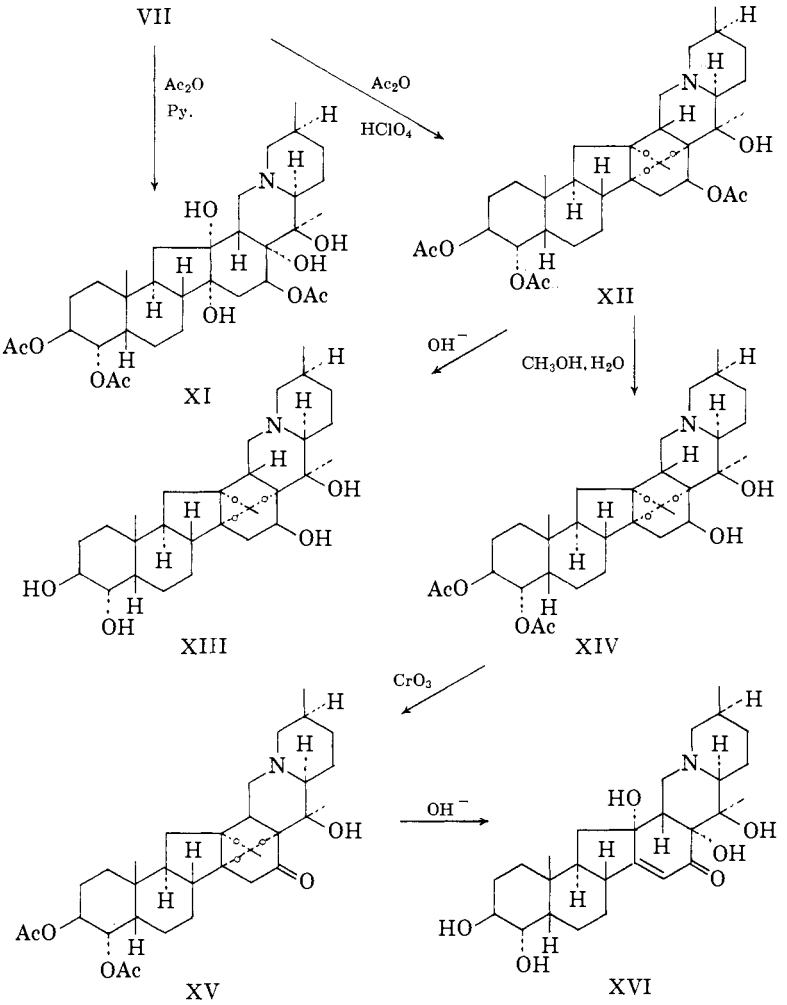
orthoacetate (C_{12},C_{14},C_{17} derivative) and not an isomeric C-orthoacetate which would require the presence of a C_9-hydroxyl group in sabine. Therefore, assignment of tertiary hydroxyl groups to C_{12}, C_{14}, and C_{17} is confirmed.

One characteristic reaction of cevine derivatives is the ready methanolysis of compounds acetylated at C_{16}, and this ease of methanolysis has been attributed to an intramolecular base catalysis coupled with a 1,3-diaxial interaction of the C_{16}-β-acetate with the axial hydroxyl group at C_{20}.[8,21] When sabine orthoacetate triacetate (XII) was treated with methanol-water for twenty hours at room temperature, sabine orthoacetate 3,4-diacetate (XIV), m.p. 274–275° dec., was obtained in good yield. On oxidation of XIV with chromic acid in acetic acid, 16-dehydrosabine orthoacetate diacetate (XV), m.p. 255–258° dec., was obtained. Confirmation of the location of the ketone function at C_{16} was obtained by treatment of XV with $N/50$ methanolic potassium hydroxide under conditions analogous to those used for the hydrolysis of sabine orthoacetate triacetate to sabine orthoacetate. Under those conditions, an α,β-unsaturated ketone, possibly of structure XVI, was obtained. The unsaturated ketone showed an ultraviolet absorption maximum at 238 mμ similar to the peak shown by the product of *very mild* hydrolysis of 16-dehydrocevadine D-orthoacetate monoacetate and attributed to loss of the orthoacetate grouping through β-elimination at C_{14}.[22] The latter reactions strongly support assignment of hydroxyl groups at C_{16} and C_{20}.

The formation of the D-orthoacetate, methanolysis of the C_{16} ester, and β-elimination in 16-dehydrosabine orthoacetate diacetate all support the view that the C, D, E ring structure of sabine is identical to that of veracevine (V). From considerations discussed so far, a *cis* relationship of the C_{16} and C_{20} hydroxyl groups is required to explain the facilitation of methanolysis. The C_{12}, C_{14} and C_{17} hydroxyl groups must also be *cis* to each other in view of the formation of the orthoacetate cage structure. Mitchner and Parks[15] reported that sabine demonstrated a periodic acid consumption of two moles, a value paralleling that for veracevine. The periodic acid consumption of veracevine has been attributed to the cleavage of the C_{12},C_{14}-*vic*-glycol and of the C_3,C_4 masked α-ketol groups. The C_{16},C_{17},C_{20} triol system of veracevine is resistant to periodic acid oxidation, presumably due to the *trans* diaxial juxtaposition of each of the

(21) S. M. Kupchan, A. Afonso and P. Slade, abstracts of papers presented at 140th Meeting of the American Chemical Society, Chicago, September 1961, p. 88-Q.

(22) D. H. R. Barton, C. J. W. Brooks and P. De Mayo, *J. Chem. Soc.*, 3950 (1954).



glycol pairs. If this triol system of sabine did not have the same stereochemical relationship as in veracevine, then the formation of the D-orthoacetate (C_{12},C_{14},C_{17}) would have to block more than one periodic acid-sensitive glycol group. In fact, sabine orthoacetate consumed one mole equivalent of periodic acid, an indication that the orthoacetate has blocked only one oxidizable *vic*-glycol. Therefore the three hydroxyl groups involved in orthoacetate formation (C_{12},-C_{14},C_{17}) must be *trans* to the C_{16},C_{20} hydroxyl groups. These five hydroxyl groups were initially assigned the same absolute configuration as veracevine on the basis of similarity in optical rotation of the

respective sabine and veracevine derivatives. Strong support for the view that the configurations of rings C, D and E in sabine and cevine (or veracevine) are identical follows from the striking similarity of the optical rotatory dispersion curves of 16-dehydrosabine orthoacetate diacetate (XV) and 16-dehydrocevine orthoacetate diacetate[22] (Fig. 1).

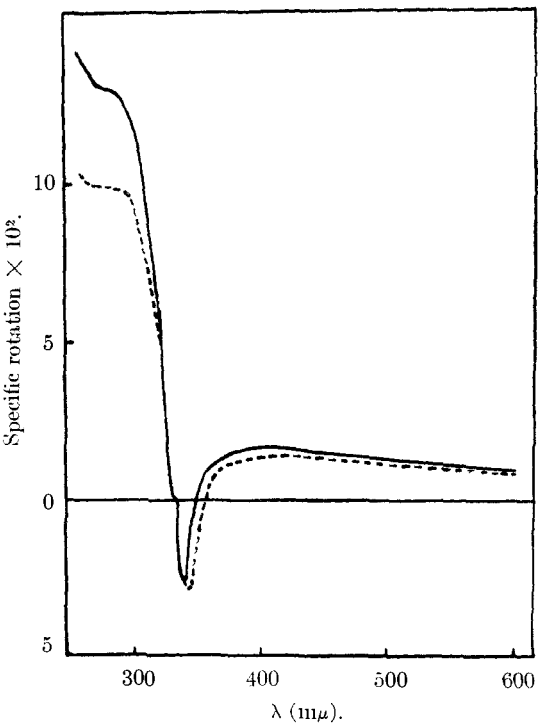


Fig. 1.—Rotatory dispersion curves (dioxane solution) of 16-dehydrosabine orthoacetate 3,4-diacetate (XV), – – – –, and 16-dehydrocevine orthoacetate 3,4-diacetate, ———.

The foregoing considerations provide a basis for assigning locations and configurations to five of the seven oxygen atoms of sabine. Only the assignment of two hydroxyl groups remained, and this proved to be our most difficult task.

The transformation of sabine to sabine triacetate (XI) by acetylation with acetic anhydride-pyridine at room temperature indicates the presence of three secondary hydroxyl groups.[12] In view of the previous assignment of only one secondary hydroxyl group (at C_{16}), the two remaining hydroxyl groups are secondary. Furthermore the

sabine molecule possesses two periodic acid-sensitive *vic*-glycol groupings. One *vic*-glycol system has been assigned at C_{12},C_{14} on the basis of the formation of sabine orthoacetate (XIII). The tertiary nature of the hydroxyl groups at C_{12},C_{14} is confirmed by the observation that sabine triacetate (XI) consumes one mole equivalent of periodic acid while sabine orthoacetate triacetate (XII) is not attacked by the latter reagent. It is apparent that none of the five hydroxyl groups assigned to locations in the ring C, D, E portion of the structure is a partner for the second *vic*-glycol system. C_{11} is eliminated as a possible site for attachment of a hydroxyl group since sabine orthoacetate (XIII) possesses one periodic acid-sensitive grouping. A C_{15},C_{16} glycol structure is precluded by the apparent absence of a diosphenol (expected $\lambda_{max.} > 270$ mμ) among the products obtained upon treatment of 16-dehydrosabine orthoacetate diacetate (XV) with alkali. Hence, the two remaining secondary hydroxyl groups were assumed to constitute the second *vic*-glycol group. The presence of only a 5.80 μ band in the carbonyl region of the infrared spectrum of the periodic acid oxidation product of sabine orthoacetate (XIII) constitutes support for the glycol system. (The periodic acid oxidation product from cevine orthoacetate shows additional absorption at 5.62 μ, a band characteristic of the γ-lactone formed on oxidation of the masked α-ketol group.[18,19])

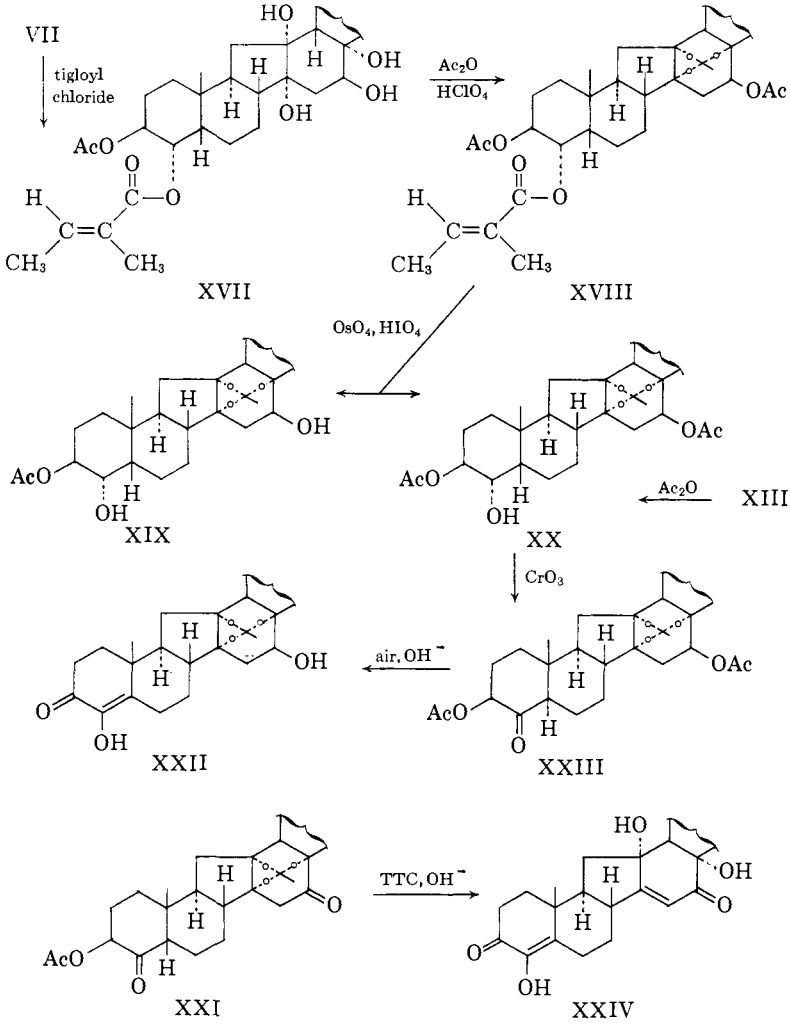
In view of the foregoing considerations, the C_4,C_9 ether linkage (see X) proposed by Auterhoff and Möhrle[12] is not supported by experimental findings. The disecondary nature of the glycol also precludes C_9 as a site for attachment of an oxygen function. Further evidence for absence of an oxygen function from C_9 was obtained by comparison of the elimination products formed from 16-dehydrosabine orthoacetate diacetate (XV) and 16-dehydrocevine orthoacetate diacetate upon treatment with $N/50$ methanolic potassium hydroxide. Whereas the cevine derivative gave rise to a polyunsaturated compound[22] (probably phenolic) having $\lambda_{max.}$ 296 mμ, the sabine derivative yielded only the monounsaturated compound XVI with $\lambda_{max.}$ 238 mμ when treated under identical conditions. Further elimination would require the presence of a vinylogous β-alkoxyl (or hydroxyl) group either at C_9 (as in cevine) or at C_7. Conversely, the apparent absence of elimination past the α,β-unsaturated ketone stage renders C_7 and C_9 improbable sites for attachment of a hydroxyl or alkoxyl group. The only other site for location of the disecondary glycol, apart from ring A, is at C_{23},C_{24}, and the latter location can be discounted on the basis of lead tetraacetate oxidation data.[12] If this were the site of the glycol, the α-amino aldehyde formed as the primary

lead tetraacetate oxidation product of ring F would be expected to undergo further oxidation (*cf.* reference 19). Since the lead tetraacetate consumption by sabine was found to be the same as that of cevine (*ca.* 2 mole equivalents[12]), C_{23},C_{24} can be excluded as a likely site for the glycol group. All the aforementioned facts support assignment of the second (disecondary) *vic*-glycol group to ring A of sabine.

From the biogenetic point of view, the C_3,C_4 location for the disecondary glycol appeared most probable, since all the known cevera-trum alkaloids possess oxygen functions at C_3 and C_4, and nowhere else in ring A. The sequel relates the evidence in support of location of the glycol system at C_3,C_4 and for assignment of the configurations shown in structure VI. Preliminary analytical scale experiments on the partial acetylation of sabadine (VII) indicated that the principal product was a diacetate. The diacetate showed the same R_f as the principal product formed by room temperature methanolysis of sabine triacetate (XI) and therefore, in all probability, was the 3,4-diacetate. The latter preliminary studies suggested that the (presumed) C_4-hydroxyl group might be more readily acylable than the C_{16} hydroxyl group in sabadine. Tiglation of VII under mild conditions afforded a monotiglate, presumably XVII. Acetylation (perchloric acid catalyzed) of the monotiglate gave sabine orthoacetate monotiglate diacetate, assigned structure XVIII. Oxidation of XVIII with osmium tetroxide-periodic acid, known to effect smooth cleavage of tiglate esters,[23] yielded an intractable mixture with two major components. When the mixture was allowed to stand at room temperature in methanol–water, the component with higher R_f was rapidly methanolyzed to the lower R_f component. The latter behavior supported the hypothesis that the higher R_f component was sabine orthoacetate 3,16-diacetate (XX) and the lower R_f component sabine orthoacetate 3-acetate (XIX). The hypothesis received further support from the behavior of the mixture upon chromic acid oxidation followed by treatment with triphenyltetrazolium chloride solution. The ultraviolet spectrum of the product obtained from the reaction sequence showed absorption maxima at 245 and 272 mμ in neutral solution. Addition of alkali gave a solution with maxima at 242 and 312 mμ, and the 312 mμ band had a lower intensity than the 272 mμ band of the neutral solution. The shift of the 272 mμ band to 312 mμ in alkaline solution, accompanied by a decrease in intensity,

(23) S. M. Kupchan, A. D. J. Balon and E. Fujita, *J. Org. Chem.*, in press.

is attributable to a 3,4-diosphenol system,[24,25] probably ascribable to the presence of both XXII and XXIV. The 242 mμ band is attributed to the α,β-unsaturated ketone in ring D of XXIV.



The preparation of the pure 4-ketone was ultimately achieved by an alternate route. Partial acetylation of sabine orthoacetate with acetic anhydride at room temperature, followed by preparative paper chromatographic separation of the products, afforded sabine ortho-

(24) E. Sundt, O. Jeger and V. Prelog, *Chemistry and Industry*, 1365 (1953).
(25) D. H. R. Barton and J. F. Eastham, *J. Chem. Soc.*, 424 (1953).

acetate 3,16-diacetate (XX), m.p. 248–251° dec. The paper chromatographic behavior of the latter compound indicated its identity with the higher R_f component of the mixture obtained by the abortive tiglate route. Oxidation of XX with chromic acid gave XXIII, m.p. 212–214°, which showed a positive test for the α-ketol group with TTC reagent. The optical rotatory dispersion curve of XXIII resembled that of coprostan-4-one[25] and differed markedly from the curve of cholestan-4-one[26] (see Fig. 2).[27] When a solution of the

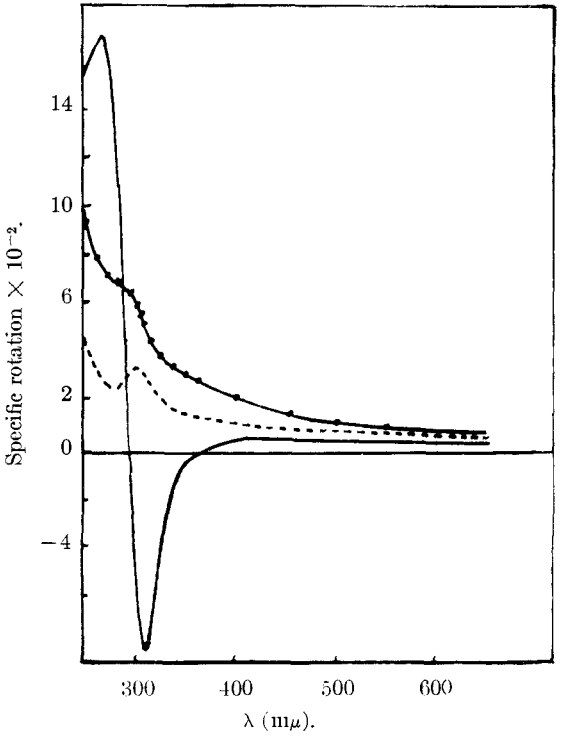


Fig. 2.—Rotatory dispersion curves (methanol solution) of 4-dehydrosabine orthoacetate 3,16-diacetate (XXIII), ●-●-●-, coprostan-4-one,[27] – – – –, and cholestan-4-one,[26] ———.

ketone XXIII in dilute alkali was allowed to stand at room temperature, the solution developed increasing ultraviolet absorption at 320

(26) C. Djerassi, *Bull. Soc. Chim. France*, 741 (1957).

(27) The difference in rotatory dispersion amplitude for XXIII relative to coprostan-4-one is in good agreement with the generalization that axial α-acetoxy ketones show an increase in the rotatory dispersion amplitude of at least 100 per cent; *cf.* C. Djerassi, O. Halpern, V. Halpern, O. Schindler and C. Tamm, *Helv. Chim. Acta*, **41**, 250 (1958); C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p. 113.

mμ; acidification caused a shift of the maximum to 275 mμ with accompanying increase in intensity. The latter absorption properties are characteristic of the C_3,C_4-diosphenol from cevagenine,[24,25] and constitute additional support for assignment of the ring A disecondary glycol system of sabine to C_3,C_4. The autoxidation under the same conditions of cevagenine D-orthoacetate, cevagenine C-orthoacetate, isogermine to products showing similar ultraviolet absorption was also noted.

All the experimental data except the reported yield of a "dihydro"-derivative upon "hydrogenation" of sabine[12] strongly support assignment of structure VI to sabine. In our hands, treatment of sabine with hydrogen in the presence of Adams catalyst led to no hydrogen consumption, although a product of higher melting point than the starting material was obtained. The demonstration of a positive silver nitrate test suggested that the product might be either sabine hydrochloride or a mixture of salts of sabine. This hypothesis was confirmed by recovery of crystalline sabine after alkaline treatment of the "hydrogenation" product. Thus sabine was not reduced, as was expected on the basis of assigned structure VI.

Sabine has fourteen asymmetric centers: C_3, C_4, C_5, C_8, C_9, C_{10}, C_{12}, C_{13}, C_{14}, C_{16}, C_{17}, C_{20}, C_{22} and C_{25}. The close parallel of the reactions of sabine and cevine, and the striking similarity of the optical rotatory dispersion curves of the respective 16-ketones (Fig. 1) strongly support assignment of similar configuration at C_{12}, C_{13}, C_{14}, C_{16}, C_{17}, C_{20}, C_{22} and C_{25}. The configurations at C_3, C_8, C_9 and C_{10} of sabine are assumed to be as indicated in VI on the basis of biogenetic analogy to veracevine and all other naturally-occurring C_{27} steroids. The similarity of the optical rotatory dispersion curve of the 4-ketone XXIII to that of coprostan-4-one constitutes support for assignment of A/B-*cis* configuration to the ketone. Since the A/B-*cis* isomer is generally the *less* stable epimer of 4-ketosteroid derivatives,[28] it is reasonable to assume that the precursorial alcohol XX, and sabine as well, have an A/B-*cis* configuration. There remains only the hydroxyl group at C_4, and the α-orientation is preferred for the C_4-hydroxyl group on the basis of its apparent *trans* relationship to the C_3-hydroxyl. Thus, repeated attempts to prepare an acetonide from sabine orthoacetate (XIII), using perchloric acid and cupric sulfate as catalysts, were unsuccessful. Sabine orthoacetate gave a negative test (for *cis* glycols)[29] with potassium tetramethyl osmate. Upon

(28) *Cf.* S. M. Kupchan, S. McLean, G. W. A. Milne and P. Slade, *J. Org. Chem.*, **27**, 147 (1962).

(29) R. Criegee, E. Höger, G. Huber, P. Kruck, F. Marktscheffel and H. Schellenberger, *Ann.*, **599**, 81 (1956).

treatment with potassium triacetyl osmate,[29] sabine orthoacetate gave color reactions typical for *trans*-glycols. Assignment of *trans* configuration to the C_3, C_4 glycol agrees with earlier observations by Auterhoff and Möhrle concerning the lead tetraacetate consumption of sabine.[12] The latter authors reported that the rates of consumption of the oxidant indicate the presence in sabine of one rapidly-oxidized glycol group (probably at C_{12}, C_{14}) and one difficultly-oxidized glycol group (now to be regarded as the *trans* C_3,C_4 diol).

Sabadine, like sabine, gives on acetylation with acetic anhydride and pyridine at room temperature sabine triacetate (XI). Therefore the acetyl group blocks a secondary hydroxyl group. Furthermore, periodic acid oxidation of sabadine indicates that the acetyl group blocks one glycol grouping[15] and thus the acetyl group is assigned to one of the hydroxyl groups of the ring A glycol. On biogenetic grounds, C_3 is preferred location, since all the characterized naturally occurring monoesters of polyhydroxy veratrum alkamines have the acyl moiety at C_3.[6] The foregoing considerations support the sabine 3-acetate structure (VII) for sabadine.

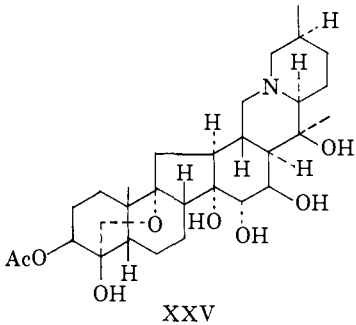
Sabadine was examined by Professor Otto Krayer and Dr. Werner E. Flacke at Harvard Medical School for pharmacological activity in isolated skeletal muscle (6 expts.) and in the anesthetized cat (3 expts.) (*cf.* reference 14). In excised frog sartorius muscle, suspended in modified McLean–Hastings solution and stimulated directly with supramaximal single shocks,[30] sabadine in concentrations from 2×10^{-5} (w./v.) to 2×10^{-4} caused an after-contraction similar to that seen after veratridine. This "veratrine response," recorded isometrically, was, initially after addition of drug or at low concentrations, distinctly separated from the initial twitch by a short dip in tension. Eventually, twitch and after-contraction were fused into one prolonged contraction. The duration of this contraction was only about 30 to 40% of that seen with an optimal concentration of veratridine (1×10^{-7}, w./v.). Twitch tension was increased moderately after sabadine. However, tension developed during optimal tetanic stimulation was not altered with concentrations causing an after-contraction. Increasing the concentration of Ca^{++} in the bathing fluid to six times normal abolished the after-contraction fully and reversibly. Repeated stimulation at a rate of 1/sec. also abolished the after-contraction.

In the anesthetized cat (sodium pentobarbital, 40 mg./kg.), sabadine given intravenously in single injections and in doses from 0.02 mg./kg. to 1 mg./kg. caused a fall in arterial blood pressure and heart

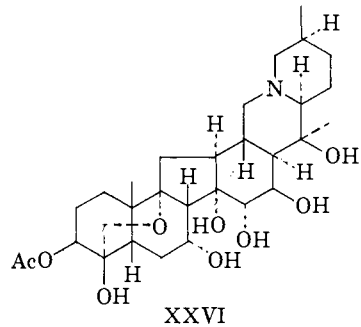
(30) O. Krayer and H. W. George, *J. Pharmacol. Exptl. Therap.*, **103**, 249 (1951).

rate and a marked slowing of the respiration. Maximally, the blood pressure was reduced to 20% of the pre-injection value and the heart rate to 25%. The hypotension and bradycardia were attenuated by atropine sulfate (2 mg./kg.), the latter considerably more than the former. All effects were completely abolished after denervation of the presso-receptor structures (bilateral vagotomy and denervation of the carotid sinus). Sabadine causes reflex hypotension and bradycardia, and reflex respiratory slowing. It thus resembles closely most other naturally occurring veratrum ester alkaloids.[31] Like other veratrum esters, sabadine exerts these effects *via* receptors in the carotid sinus area and in the chest. Its potency in causing hypotension is about 1/10 that of veratridine (II). The potency in skeletal muscle is only about 1/200 to 1/400 that of veratridine.

An interesting and curious contrast exists between the pharmacological properties of sabadine and those of the other known cevera-trum 3-monoacetate esters, cevacine (III),[6,32] zygacine (XXV),[6,33]



XXV



XXVI

and germine 3-monoacetate (XXVI).[18] Of the four monoacetate esters, only sabadine caused a "veratrine response" of the type known from veratrine and veratridine, *i.e.*, a prolonged after-contraction. Cevacine, zygacine and germine monoacetate, on the other hand, caused an increased tension development after a single stimulation, with only moderate prolongation of the contraction, *i.e.*, an increased twitch. At no time or concentration was there any indication of a separate after-contraction. Zygacine and cevacine were about 50 to 100 times more potent than germine monoacetate in muscle. It has been shown for germine monoacetate that the bioelectrical phenomenon underlying the increase in tension and the prolongation is a

(31) J. M. Benforado, W. Flacke, C. R. Swaine, and W. Mosimann, *J. Pharmacal. Exptl. Therap.*, **130**, 311 (1960).

(32) S. M. Kupchan, D. Lavie, C. V. Deliwala, and B. Y. A. Andoh, *J. Am. Chem. Soc.*, **75**, 5519 (1953).

(33) S. M. Kupchan, D. Lavie, and R. D. Zonis, *ibid.*, **77**, 689 (1955).

short burst of repetitive action potentials of the same type as that seen with veratridine. The only difference between the "increased twitch" and the after-contraction lies in the time course of the repetitive firing. The two phenomena therefore have been termed veratrine responses of the brief and prolonged type, respectively.[34]

Of the four monoacetate esters, sabadine alone caused the triad of hypertension, bradycardia and respiratory slowing or arrest in the anesthetized cat. Cevacine, zygacine and germine monoacetate did not cause hypotension and bradycardia in doses below 1 mg./kg. (Cevacine and zygacine were seen after one injection each to cause a moderate hypotension and bradycardia in a dose of 1 mg./kg. However, the effect was accompanied by severe motor excitation and twitching movements, and the effect may not have been a specific cardiovascular effect. Further studies were not possible because of lack of alkaloids.) Instead of a hypotension, the substances caused a hypertension which was accentuated after interruption of the afferent nerves from pressoreceptors (vagotomy and denervation of both carotid sinus). Zygacine and cevacine were about 10 times more potent than germine monoacetate in their action on blood pressure. For germine monoacetate evidence has been presented which suggests that the hypertension is due to an effect upon unidentified peripheral sensory receptors.[34] Thus, all veratrum ester alkaloids appear to affect sensory receptors; they differ in the type of receptors affected most strongly.

Experimental[35]

Paper Chromatography.—The procedure and solvent systems used were similar to those described by Levine and Fischbach.[36] The solvent systems used were: (A) *n*-butyl acetate, 1-butanol, formic acid (25:5:1); (B) 1-butanol, *n*-butyl acetate, water, pyridine (6:3:10:2); (C) 1-butanol, pyridine, water (4:1:5); (D) 2,2,4-trimethylpentane, methyl isobutyl ketone, pyridine (10:5:1); (E) *n*-butyl acetate, 1-butanol, formic acid, water (100:1:1.5:0.5). Small-scale (10 mg.) exploratory experiments were carried out and evaluated by paper chromatographic analysis before the preparative-scale reactions to be described were attempted. The solvent system used is indicated under each experiment. The

(34) W. Flacke, *Arch. Exper. Path. u. Pharmakol.*, **240**, 369 (1961).

(35) Melting points are corrected for stem exposure. Values of $[\alpha]_D$ have been approximated to the nearest degree. Ultraviolet absorption spectra were determined in 95% ethanol on a Cary recording spectrophotometer (Model 11 MS). Infrared spectra were determined in chloroform solution (unless otherwise specified) on a Baird double beam infrared recording spectrophotometer. Paper chromatography was conducted by the descending technique on Whatman No. 1 paper. Microanalyses were carried out by Dr. S. M. Nagy and his associates at the Massachusetts Institute of Technology on samples dried at 110° under reduced pressure. We thank Professor Carl Djerassi for the optical rotatory dispersion measurements.

(36) J. Levine and H. Fischbach, *J. Am. Pharm. Assoc.*, **44**, 543 (1955); **46**, 191 (1957).

same solvent system was also employed to follow the progress of any separation by column chromatography.

Comparison of Sabadine and Sabatine, Sabine and Neosabadine.—Solution and potassium bromide pellet spectra of authentic samples of sabadine and sabatine were identical. The solution spectra of authentic samples of sabine and neosabadine were also identical. Paper chromatographic behavior of the respective samples was identical with solvent systems A, B and C. Sabine and neosabadine also showed identical R_f values with solvent system C using paper impregnated with pH 3.5 buffer containing 4% boric acid.

Isolation of Sabadine (VII).—Sabadilla seed (S. B. Penick and Co., granulated, 9 kg.) was extracted according to the procedure of Poetsch and Parks[37] to obtain hydrophilic alkaloidal fraction "D" (70 g.). Fraction "D" was separated into two portions by a modified nine-plate countercurrent distribution between chloroform and pH 5.0 phosphate buffer (0.5 *M*). Buffer (1000 ml.) was placed in each of three 4-liter separatory funnels, a solution of fraction "D" in chloroform (1000 ml.) was placed in plate 0; the distribution was accomplished by using 8 more portions of chloroform. The contents of the three funnels were rendered alkaline (to pH 10) with 5 *N* potassium hydroxide and exhaustively extracted with chloroform. The combined chloroform extract was evaporated to dryness to yield a yellowish-orange solid (48.9 g.). This residue was submitted to a second twelve-plate distribution between pH 7.3 phosphate buffer (0.5 *M*, 300 ml. in each funnel) and chloroform (300 ml. in each funnel). After completion of the countercurrent distribution, the aqueous phase of each funnel was basified (to pH 10) with 5 *N* potassium hydroxide and exhaustively extracted with chloroform. The chloroform extract of each individual plate was evaporated to dryness under reduced pressure. An aliquot of each fraction was tested for sabadine content by paper chromatographic analysis with solvent system B. Material obtained from plates 6, 7, 8 (8.77 g.) consisted mainly of sabadine; alkaloids obtained from plates 5, 9, 10 (9.13 g.) contained only a minor quantity of sabadine; extracts from remaining plates (0–4, 11) were devoid of sabadine. The alkaloids from plates 6, 7, 8 were dissolved in chloroform, and the chloroform solution was filtered and reduced to a small volume. Ether was added cautiously until precipitation appeared and the flocculent brownish precipitate was removed by filtration. The filtrate, when clear, was evaporated to dryness under reduced pressure. The amorphous solid was dissolved in chloroform (25 ml.), and colorless needles separated (4.76 g.) on standing after seeding with authentic sabadine. The crystals were redissolved in acetone, the acetone solution was filtered and evaporated to dryness under reduced pressure. The amorphous residue was dissolved in boiling ether (25 ml.) and the solution was kept at room temperature. Colorless prisms (4.355 g.), $[\alpha]^{25}D$ −11° (*c* 1.93, ethanol) were obtained. In view of the indefinite melting point of sabadine (softening at 170–190°, dec. above 200°) the infrared spectrum, the optical rotation and the R_f value (solvent system B) were used as criteria of purity and identity.

Hydrogenation of Sabine (VI) and Sabadine (VII).—Sabine was hydrogenated following the procedure of Auterhoff and Möhrle.[12] A solution of sabine (46.8 mg. recrystallized from ether) in 95% alcohol (5 ml.) was treated with hydrogen in the presence of Adams catalyst (23.4 mg.) in a microhydrogenation apparatus at room temperature and atmospheric pressure. No hydrogen uptake was observed within 20 hr. The catalyst was removed by filtration and the solution

(37) C. E. Poetsch and L. M. Parks, *J. Am. Pharm. Assoc.*, **38**, 522 (1949).

was evaporated to dryness under reduced pressure. The residue was dissolved in acetone, the acetone solution was evaporated to dryness under reduced pressure at steam bath temperature and ether was added to the amorphous solid. Crystallization in the form of large prisms (25 mg.) occurred within 5 days; infrared spectrum (KBr pellet, was identical with that of sabine (starting material). The mother liquor material resisted crystallization, was more ether-insoluble and had an infrared spectrum different from that of sabine.

Sabine (VI, 100 mg.) was hydrogenated in the presence of Adams catalyst (50 mg.). After 24 hr. at room temperature and atmospheric pressure the catalyst was removed by filtration and fresh Adams catalyst (50 mg.) was added. The hydrogenation was continued for another 14 hr. The alcohol solution was reduced to a small volume (0.5 ml.) and ether was added dropwise. Microcrystalline solid (A, 43 mg.) sintering at 200°, m.p. 240–280° dec., separated. The water soluble product gave a precipitate with silver nitrate solution; this redissolved on addition of dilute ammonia. The infrared spectrum of A was not identical with that of sabine hydrochloride (m.p. 293–295° dec.) prepared by treatment of a solution of sabine in absolute ethanol with anhydrous HCl. A suspension of the mother liquor residue (53 mg.) in water (5 ml.) was rendered basic with 2 *N* ammonium hydroxide (to pH 9) and extracted with chloroform (6 × 10 ml.). The chloroform extract, dried over sodium sulfate, was evaporated to dryness and the residue was crystallized from anhydrous ether. The prisms obtained (26 mg.) showed an infrared spectrum identical with that of sabine. The microcrystalline hydrogenation product (A, 20 mg.) was treated in an identical manner to give crystalline sabine (11 mg.).

A solution of sabadine (VII, 100 mg., crystallized from chloroform) in absolute ethanol was hydrogenated in the presence of Raney nickel (W-2) catalyst for 24 hr. at room temperature and atmospheric pressure. The catalyst was removed by filtration and the ethanol solution was evaporated to dryness. The greenish product (94 mg., m.p. 276–280° dec.) was insoluble in chloroform, acetone and ether, and had an R_f value (solvent system B) identical to that of sabadine. The latter product (an 85 mg. portion), was treated with dilute ammonia (to pH 9) and the aqueous mixture was repeatedly extracted with chloroform; the aqueous layer retained a greenish flocculent precipitate. The combined chloroform extract was evaporated to dryness to give a resin (74 mg., m.p. 160–180°) having an infrared spectrum identical to that of sabadine. Crystallization from chloroform yielded needles (58 mg.), identified as sabadine.

Sabine Triacetate (XI).—A solution of sabadine (VII, 250 mg.) in pyridine (2 ml.) was treated with acetic anhydride (2 ml.) and allowed to stand at room temperature for 10 hr. The excess acetic anhydride was decomposed by dropwise addition of methanol (4 ml.) to the reaction mixture cooled to 0°. After 1 hr., the solution was evaporated to dryness under reduced pressure. The residue was redissolved in benzene and the solution was re-evaporated to dryness in order to remove residual pyridine. The residue was then treated with water (10 ml.), 2 *N* ammonium hydroxide (to pH 8.5–9), and the alkaline solution was extracted with chloroform (7 × 10 ml.). The chloroform extract was dried over sodium sulfate and evaporated to dryness to yield a resin (312 mg.). The product, found impure by paper chromatographic analysis (solvent system A), was dissolved in benzene and chromatographed on Merck acid-washed alumina (6 g.). Gradient elution with benzene to benzene-chloroform (1:3) yielded minor product of high R_f (40 mg.). Further gradient elution with chloroform-benzene (3:1)

to chloroform-methanol (98:2) afforded paper chromatographically homogeneous material (220 mg.). The yellowish product was redissolved in chloroform and the solution evaporated to dryness under reduced pressure at steam bath temperature. Boiling ether (5 ml.) was added to the powdery residue, and colorless needles (191 mg.) separated on standing. Two recrystallizations in the same manner yielded sabine triacetate (119 mg.) sintering at 165°, m.p. 221–222° dec., $[\alpha]^{25}D$ + 9° (*c* 1.14, $CHCl_3$).

Anal. Calcd. for $C_{33}H_{51}NO_{10}$: C, 63.74; H, 8.27. Found: C, 63.72; H, 8.52; volatile acid, 2.96 mole equivalents.

Periodic Acid Oxidation.—Sabine triacetate (7.3 mg.) was dissolved in 5% acetic acid (1 ml.); 0.05 *M* periodic acid (3 ml.) and *t*-butanol (1 ml.) were added. The solution was kept at room temperature and the residual amount of periodic acid in aliquots (1 ml.) and blank was determined after 1 and 2 hr. Saturated sodium bicarbonate (1 ml.), 0.08 *N* sodium arsenite (1 ml.) and a crystal of potassium iodide were added to aliquot. The sample and blank solutions were kept at room temperature for 15 min. and the excess sodium arsenite was determined with standard 0.02 *N* iodine solution. Sabine triacetate showed a periodic acid consumption of 0.80 mole equivalent.

Paper Chromatography.—Solvent system A was used.

Sabine Orthoacetate Triacetate (XII).—A suspension of sabadine (VII, 500 mg.) in acetic anhydride (4 ml.), cooled in an ice bath, was treated with perchloric acid (0.16 ml. 60%) and agitated to effect solution. After 20 hr. at room temperature, the solution was cooled in an ice bath and excess acetic anhydride was destroyed by cautious addition of methanol (6 ml.). Two hours later the yellowish solution was evaporated to dryness under reduced pressure. The product was redissolved in methanol and this solution was re-evaporated to dryness until all acetic acid was removed. Further addition of methanol to the residue led to the formation of colorless needles (458 mg.). Recrystallization of XII perchlorate (280 mg.) was achieved by dissolution in acetone, evaporation to dryness under reduced pressure, and addition of boiling methanol (5 ml.). Colorless needles (185 mg.), m.p. 244–245° dec., separated.

A solution of XII perchlorate (450 mg.) in 5% acetic acid (5 ml.) was made alkaline (to pH 9) with 2 *N* ammonia and extracted with chloroform (8 × 10 ml.). The combined chloroform extract was dried over sodium sulfate and evaporated to dryness under reduced pressure to obtain a fluffy amorphous residue. Crystallization from anhydrous ether (15 ml.) gave needles (305 mg.). Two recrystallizations of a portion of the aforementioned crystals (150 mg.) from chloroform-ether in the same manner yielded colorless needles (52 mg.), m.p. 255–257° dec., $[\alpha]^{25}D$ + 94° (*c* 0.94, chf.).

Anal. Calcd. for $C_{35}H_{51}NO_{10}$: C, 65.09; H, 7.94. Found: C, 65.01; H, 8.11; volatile acid, 3.64 mole equivalents.

Paper Chromatography.—Solvent systems A and D were used.

Periodic Acid Oxidation.—The periodic acid consumption, determined in the same manner as for sabine triacetate, was 0.05 mole equivalent.

Sabine Orthoacetate (XIII).—A solution of XII (200 mg.) in methanolic potassium hydroxide (50 ml., 0.02 *N*) was heated under reflux for 3 hr. The solution then was evaporated to dryness under reduced pressure. Distilled water (10 ml.) was added to the residue; the aqueous mixture was extracted with chloroform (6 × 10 ml.). The chloroform extract was dried over sodium sulfate and evaporated to dryness. Crystallization from anhydrous ether yielded needles (125

mg.). Two recrystallizations, carried out by dissolving crystals in chloroform, evaporating chloroform solution to dryness under reduced pressure at 100°, and dissolving the amorphous residue in ether, yielded XIII (54 mg.), m.p. 172–175°, which resolidifies and melts again 305–310° dec., $[\alpha]^{25}D$ + 42° (*c* 0.96, ethanol), $[\alpha]^{25}D$ + 25° (*c* 0.97, chf.). XIII showed high intensity infrared absorption bands at 7.12, 8.87, 9.42 and 11.00 μ.

Anal. Calcd. for $C_{29}H_{45}NO_7$: C, 67.02; H, 8.73. Found: C, 66.83; H, 8.60; volatile acid, 1.04 mole equivalents.

Paper Chromatography.—Solvent system A was used.

Periodic Acid Oxidation.—Sabine orthoacetate (XIII, 6.2 mg.) was dissolved in 5% acetic acid (1 ml.); 0.05 *M* periodic acid (3 ml.) and distilled water (1 ml.) were added. The solution was kept at room temperature and the residual quantity of periodic acid in 1 ml. aliquots and blank was determined at intervals.[18] The periodic acid consumption was 1.1 mole equivalents after 1 and 2 hr. The remainder of the oxidation mixture (3 ml.) was treated with saturated sodium bicarbonate solution (3 ml.), 0.08 *N* sodium arsenite (3 ml.) and a crystal of potassium iodide. After 15 min. at room temperature, the solution was adjusted to pH 9 with 2 *N* ammonia and extracted with chloroform (5 × 5 ml.). The chloroform extract was reduced to a small volume, and ether was added until precipitation occurred; the flocculent precipitate was removed by filtration and the filtrate was evaporated to dryness. The amorphous residue showed a single absorption band at 5.80 μ in the carbonyl region of the infrared spectrum.

Sabine Orthoacetate 3,4-Diacetate (XIV).—A solution of sabine orthoacetate triacetate (XII, 468 mg.) in methanol-water (5:1, 108 ml.) was kept at room temperature for 18 hr. The solution was evaporated to small volume (10 ml.), made alkaline (to pH 8.5–9) with 2 *N* ammonia and extracted with chloroform (5 × 20 ml.). The combined chloroform extract was dried over sodium sulfate and evaporated to dryness. The resin (503 mg.) was dissolved in benzene and chromatographed on acid-washed alumina (10 g.). Elution with benzene-chloroform (1:1, 50 ml.) yielded a mixture of XII and XIV. Further gradient elution with chloroform-benzene (from 50% chloroform to pure chloroform) gave a homogeneous product (318 mg.). This material was dissolved in chloroform, the solution was filtered through Celite and evaporated to dryness under reduced pressure at steam bath temperature to give a fluffy solid. Boiling ether was added and yellowish needles (229 mg., m.p. 268–271° dec.) separated. Two recrystallizations from chloroform-ether in the same manner gave colorless needles (135 mg.), m.p. 274–275° dec., $[\alpha]^{25}D$ + 72° (*c* 0.96, chf.).

Anal. Calcd. for $C_{33}H_{49}NO_9$: C, 65.64; H, 8.18. Found: C, 65.59; H, 8.13; volatile acid, 2.84 mole equivalents.

Paper Chromatography.—Solvent system D was used.

16-Dehydrosabine Orthoacetate Diacetate (XV).—A solution of sabine orthoacetate diacetate (XIV, 11.0 mg.) in glacial acetic acid (3 ml.) was treated with a solution of chromic anhydride in acetic acid containing 0.2% water (2 ml., 0.05 *N*). The oxidation was allowed to proceed at room temperature and 1 ml. aliquots were withdrawn after reaction periods of 1 and 2 hr. The chromic acid in the oxidation mixture and blank was reduced with 10% potasium iodide solution (2 ml.) and the released iodine was determined with standard 0.01 *N* sodium thiosulfate. The chromic acid consumption at both time intervals was equivalent to 85% of the theoretical amount of reagent required for the oxidation of one secondary hydroxyl group.

Sabine orthoacetate diacetate (XIV, 252 mg.) was treated with 0.05 *N* chromic acid (45 ml.). The oxidation was allowed to proceed at room temperature for 2.5 hr. The reaction mixture was treated with 10% sodium bisulfite solution (2 ml.) at 0°, made alkaline with 5 *N* NaOH (to pH 8.5) and extracted with chloroform (8 × 75 ml.). The combined chloroform extract was washed with water (5 ml.), dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure. Crystallization from chloroform-absolute ethanol yielded needles (171 mg.). A portion of the product (90 mg.) was recrystallized twice from chloroform-ethanol yielding velvety crystals (53 mg.), m.p. 255–258° dec., $[\alpha]^{25}D$ + 57° (*c* 0.79, chf.).

Anal. Calcd. for $C_{33}H_{47}NO_9$: C, 65.86; H, 7.87. Found: C, 65.58, H, 7.79; volatile acid, 2.71 mole equivalents.

Paper Chromatography.—Solvent system D was used.

Alkaline Treatment of 16-Dehydrosabine Orthoacetate Diacetate (XV).—A solution of 16-dehydrosabine orthoacetate diacetate (XV, 4.0 mg.) in 0.02 *N* methanolic potassium hydroxide (1 ml.) was heated under reflux for 3 hr. The solution was evaporated under reduced pressure to small volume, diluted with distilled water (5 ml.) and extracted with chloroform (6 × 5 ml.). The chloroform extract was dried over sodium sulfate and evaporated to dryness under reduced pressure. The resinous residue was dissolved in 95% ethanol and the solution was filtered. The product showed a single ultraviolet absorption maximum at 238 mμ. Treatment of 16-dehydrocevine orthoacetate diacetate[22] (m.p. 276–277° dec.) under identical conditions yielded product with single ultraviolet absorption maximum at 296 mμ.

Paper Chromatography.—The R_f value of the elimination product of dehydrosabine orthoacetate diacetate is intermediate between that of sabine and sabine orthoacetate (solvent system B).

Sabine Orthoacetate Monotiglate Diacetate (XVIII).—To a solution of sabadine (2 g.) in pyridine (20 ml.) at room temperature was added tigloyl chloride (1.4 ml.) in small portions, and the solution was allowed to stand at room temperature for 5 hr. The reaction mixture was cooled in an ice bath, treated with 10% sodium carbonate solution to pH 9, and extracted with chloroform (6 × 50 ml.). The extract was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure. The residue was dissolved in benzene (50 ml.) and evaporated to dryness to remove residual pyridine. The amorphous residue was treated with acetic anhydride (20 ml.) and perchloric acid (0.7 ml., 60%) in an ice bath. After 15 hr. at room temperature, the reaction mixture was cooled in an ice bath and excess acetic anhydride was destroyed by cautious addition of methanol (25 ml.). After 1 hr., the solution was evaporated to dryness under reduced pressure, the residue was dissolved in water (20 ml.), and the solution was treated with 2 *N* ammonium hydroxide to pH 9. Extraction with chloroform yielded 2.2 g. of solid residue. Paper chromatographic analysis (solvent system E) showed the presence of three spots. The solid was dissolved in benzene and chromatographed on Merck acid washed alumina (40 g.). Elution with benzene and benzene-chloroform (20:1) afforded a mixture of two compounds (400 mg.). Further elution with chloroform-benzene (1:9) and chloroform yielded paper chromatographically homogeneous material (330 mg.). Crystallization from ether-petroleum ether yielded 190 mg. of crystalline product. Two recrystallizations from the same solvent gave colorless needles, m.p. 257–259° dec., $[\alpha]^{25}D$ + 97° (*c* 1.97, chf.). The ultraviolet spectrum of the product showed λ_{max}. 217 mμ

(ϵ 12,900), indicative of the presence of *one* tiglate residue in the molecule.[38]

Anal. Calcd. for $C_{38}H_{55}NO_{10}$: C, 66.54; H, 8.08. Found: C, 66.70; H, 8.18; volatile acid, 3.68 mole equivalents.

Treatment of Sabine Orthoacetate Monotiglate Diacetate (XVIII) with Osmium Tetroxide–Periodic Acid.—A mixture of sabine orthoacetate monotiglate diacetate (XVIII, 140 mg.), water (3 ml.), purified dioxane (14 ml.) and osmium tetroxide (14 mg.) was stirred for 15 min., during which time the mixture became dark brown. Finely powdered periodic acid (18 mg.) was added to the mixture over a period of 30 min., and the reaction was stirred for an additional 3 hr. The reaction mixture (now pale yellow) was poured into 0.1 *N* sodium arsenite solution (130 ml.) and the solution was made alkaline (pH 8) with 10% sodium carbonate solution. After 15 min. the alkaline solution was extracted with chloroform (5 × 50 ml.). Evaporation of the solvent gave a solid residue (130 mg.) which was crystallized from acetone-petroleum ether. Two recrystallizations from acetone yielded colorless needles (70 mg.), m.p. 190–205° (mixture A). Paper chromatographic analysis (solvent system D) showed the two-component nature of the material. Attempts at separation by chromatography on a variety of adsorbents were unsuccessful.

A solution of mixture A (2 mg.) in methanol-water (5:1, 1 ml.) was kept at room temperature for 18 hr. After removal of solvent, the residual product was examined by paper chromatography (solvent system D). The results indicated that the component of higher R_f had been methanolyzed almost completely to the second component, of lower R_f.

A solution of mixture A (75 mg.) in glacial acetic acid (1 ml.) was treated with a solution of chromic anhydride in acetic acid containing 0.2% water (19 ml., 0.05 *N*). The oxidation was allowed to proceed at room temperature for 5 hr. The reaction mixture was worked up as described for XV. The product resisted all attempts at purification. The chromic acid oxidation product (5 mg.) was treated with a solution of triphenyltetrazolium chloride in ethanol (1%, 1 ml.) and 0.2 *N* potassium hydroxide in methanol (1 ml.). The reaction mixture showed a gradually increasing red color after 5 min. After 30 min., the solution was concentrated to a small volume and diluted with an equal volume of water. The mixture was extracted with chloroform and the chloroform solution was extracted with 2% hydrochloric acid. The clear aqueous extract was treated with 5 *N* sodium hydroxide solution to pH 8 and the alkaline solution was extracted with chloroform. Evaporation of the solvent left a residue (5 mg.) which showed maxima in the ultraviolet at 245 and 272 mμ. Addition of alkali caused shifts of the 245 mμ band to 242 mμ, and the 272 mμ band to 312 mμ.

Sabine Orthoacetate 3,16-Diacetate (XX).—A suspension of sabine orthoacetate (XIII, 530 mg.) in acetic anhydride (20 ml.) was cooled in an ice bath and shaken to effect solution. The solution was then allowed to stand at room temperature for 16 hr. Excess acetic anhydride was decomposed by cautious addition of methanol (30 ml.) to the cooled reaction mixture. After 1 hr., the solution was evaporated to dryness under reduced pressure. The residue was treated with water (20 ml.) and 2 *N* ammonium hydroxide to pH 9 and the alkaline solution was extracted with chloroform (5 × 20 ml.). The chloroform extract was dried over sodium sulfate and evaporated to dryness to leave an amorphous residue (700 mg.). The product was found to be inhomogeneous by paper chromatography with solvent system D and was purified by preparative scale paper

(38) *Cf.* S. M. Kupchan and A. Afonso, *J. Org. Chem.*, **25**, 2217 (1960).

chromatography. The solid was dissolved in acetone (6 ml.) and the solution was streaked across 18 sheets of Whatman No. 4 paper (18 × 30 cm.). After the papers were impregnated with pH 3.5 buffer, chromatographic resolution with solvent system D was effected. Spraying with a chloroform solution of bromphenol blue indicated the presence of five major components. Four of the five showed R_f's characteristic of sabine orthoacetate triacetate (XII), sabine orthoacetate 3,4-diacetate (XIV), sabine orthoacetate 3,16-diacetate (XX) and sabine orthoacetate 3-monoacetate (XIX). The R_f value of sabine orthoacetate 3,16-diacetate (XX) was slightly lower than that of sabine orthoacetate 3,4-diacetate (XIV). The band characterized as XX was cut from the papers and extracted with chloroform continuously for 8 hr. in a Soxhlet apparatus. The chloroform extract was evaporated to dryness and the residue was treated with chloroform and 2% ammonium hydroxide (30 ml.) to remove indicator and acidic materials. The chloroform was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness to yield a resin (59 mg.). Further extraction of the filter paper band with 3% methanol in chloroform for 8 hr. and workup as above yielded additional resin (89 mg.). The resinous materials were found to be paper chromatographically identical and homogeneous, whereupon they were combined in ether. Crystallization from ether gave 113 mg. of product, m.p. 245–250°. Two recrystallizations from ether afforded colorless plates (80 mg.), m.p. 248–251°, $[\alpha]^{25}D + 105°$ (c 0.72, chf.).

Anal. Calcd. for $C_{33}H_{49}NO_9$: C, 65.65; H, 8.18. Found: C, 65.41; H, 8.23.

A solution of XX (2 mg.) in methanol-water (5:1, 1 ml.) was kept at room temperature for 18 hr. After removal of solvent by evaporation under reduced pressure, the residue was subjected to paper chromatography (solvent system D). The R_f value of the product was identical with that of the low R_f compound obtained *via* the monotiglate (presumably XIX).

4-Dehydrosabine Orthoacetate 3,16-Diacetate (XXIII).—A solution of sabine orthoacetate 3,16-diacetate (XX, 82 mg.) in glacial acetic acid (1 ml.) was treated with a solution of chromic anhydride in acetic acid containing 0.2% water (8 ml., 0.05 *N*). The oxidation was allowed to proceed at room temperature for 5 hr. The excess reagent was decomposed by addition of 10% sodium bisulfite solution (0.4 ml.) while cooling in the ice bath, and the mixture was treated with 5 *N* sodium hydroxide followed by 2 *N* ammonium hydroxide to pH 7. The neutral solution was extracted with chloroform (4 × 50 ml.). The combined chloroform extract was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure. The amorphous residue (80 mg.) was crystallized from ethyl acetate to yield prisms (10 mg.). Two recrystallizations from ether gave colorless prisms (5 mg.), m.p. 212–214°. The product gave a positive test with triphenyltetrazolium reagent.

Anal. Calcd. for $C_{33}H_{47}NO_9$: C, 65.87; H, 7.87. Found: C, 65.92; H, 8.03.

Autoxidation of 4-Dehydrosabine Orthoacetate 3,16-Diacetate (XXIII) in Alkaline Solution.—The ultraviolet spectrum of a solution of 4-dehydrosabine orthoacetate diacetate (XXIII, 1 mg.) in 0.2 *N* alcoholic potassium hydroxide (10 ml.) was measured after periods of 5, 20 and 60 min., and 20 hr. after preparation. The λ_{max} at 320 mμ increased gradually over the observed period to an ϵ of 5,800 after 20 hr. When the solution ws acidified with concentrated hydrochloric acid the position of the maximum at once changed to 275 mμ and the ϵ value to 7,400. The tabulated results were obtained by similar (20 hour) treatment of the compounds named:

Compound	λ_{max}^{alk}	λ_{max}^{acid}
Cevagenine D-orthoacetate[26]	316 mμ (ε, 3000)	274 (ε, 5000)
Cevagenine C-orthoacetate[26]	316 mμ (ε, 3000)	275 (ε, 5000)
Isogermine[18]	320 mμ (ε, 3000)	275 (ε, 3800)
Cholestane-3β,7α-diol-4-one[28]	320 mμ (ε, 6300)	278 (ε, 8100)

Color Reactions of Sabadine (VII) and Sabine Orthoacetate (XIX) with Potassium Tetramethyl Osmate and Potassium Triacetyl Osmate.[29]—Sabadine (2 mg.) was added to a solution of potassium tetramethyl osmate (2 mg.) in methanol (0.5 ml.). The blue color of the osmate solution was changed immediately to yellow-green and then to brown. Treatment of sabine orthoacetate (2 mg.) in the same manner caused no change in the color of the osmate solution for 2 days.

Sabadine (2 mg.) was added to the cobalt-blue solution prepared by dissolution of potassium tetramethyl osmate (2 mg.) in glacial acetic acid (0.5 ml.). The solution changed in color immediately to a dark red-violet which was unchanged after addition of potassium acetate (10 mg.). Treatment of sabine orthoacetate (2 mg.) in the same manner led to a gradual change in color of the solution to a dark red-violet. Addition of potassium acetate (10 mg.) caused the color to change back to deep blue.

Degradation of Corticosteroids. VII. The Synthesis of 7-Membered Ring-A Enol-Lactones[1]

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Received February 1, 1962

Treatment of Δ^4-3-keto steroids with perbenzoic acid in the presence of perchloric acid gave 3-oxo-3a-oxa-enol lactones. The antiestrogenic activity of several lactones was evaluated.

Steroidal enol lactones of the type (II) were required for studies concerning the degradation of corticosteroids.[3–5] The Baeyer–Villiger oxidation[6,7] is frequently employed for the conversion of ali-

(1) This work was supported by grants from the American Cancer Society, Inc., P-102 and P-103.

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