Anal. Calcd. for $C_{28}H_{41}O_7N$: C, 66.77; H, 8.21; acetyl, 8.55. Found: C, 66.75; H, 8.10; acetyl, 8.42.[10]

Oxidation of Veracevine with Periodic Acid.—Veracevine (201 mg., 254 mg.) was oxidized with periodic acid in 50% aqueous methanol by the procedure of Barton and Eastham.[11] The periodic acid uptake after 24 hours corresponded to 1.81, 1.96 moles for the respective runs.

Oxidation of Veracevine Triacetate with Periodic Acid and Chromic Acid.—Veracevine triacetate (320 mg., 352 mg.) was oxidized with periodic acid as above. The periodic acid uptake after 24 hours corresponded to 1.16, 1.05 moles for the respective runs.

Veracevine triacetate (400 mg., 0.63 mmole) was treated with chromium trioxide (400 mg.) in 50% aqueous acetic acid at 5°. With the aid of an appropriate blank, the consumption of chromic acid after 24 hours was found to be 0.157, 0.123 meq. in successive runs (theor. for one oxygen, 1.26 meq.).

Cevagenine Diacetate.—The following results have been obtained upon analysis of the second compound isolated from the product of room temperature acetylation of cevagenine (m.p. 242–243.5° dec., $[\alpha]^{22}D$ −62° (*c* 1.33, alc.).[10]

Anal. Calcd. for $C_{31}H_{47}O_{10}N$: C, 62.71; H, 7.98; acetyl, 14.50. Found: C, 62.48; H, 8.09; acetyl, 14.70.

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(10) Hydrolysis in the acetyl determination was done with *p*-toluenesulfonic acid; *cf.* J. B. Niederl and V. Niederl, "Micromethods of Quantitative Organic Analysis," John Wiley and Sons, Inc., New York, N. Y., pp. 257–262. This determination and the other microanalyses were carried out by Dr. S. M. Nagy and associates at the Massachusetts Institute of Technology. All samples were dried *in vacuo* at 110°.

(11) D. H. R. Barton and J. G. Eastham, *J. Chem. Soc.*, 424 (1953).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY]

Schoenocaulon Alkaloids. IV. The Isomeric Cevagenine Orthoacetates[1]

BY S. MORRIS KUPCHAN

RECEIVED JULY 30, 1954

Hydrolysis of cevadine orthoacetate diacetate with 5% methanolic potassium hydroxide affords cevagenine D-orthoacetate, $[\alpha]D$ +20°. Treatment of cevagenine D-orthoacetate with mineral acid yields cevagenine C-orthoacetate, $[\alpha]D$ −35°. Acetylation of cevagenine D-orthoacetate with acetic anhydride–pyridine at steam-bath temperature gives cevagenine C-orthoacetate diacetate. Hydrolysis of cevagenine C-orthoacetate diacetate with 20% alcoholic potassium hydroxide gives cevagenine C-orthoacetate. Treatment of cevagenine D-orthoacetate with 20% alcoholic potassium hydroxide gives cevine orthoacetate. The structures of cevagenine C- and D-orthoacetates and the stereochemical implications of these structures are discussed.

No explanation has been advanced for the fact that cevagenine forms an orthoacetate under milder conditions than its isomers (*i.e.*, upon treatment with acetic anhydride–pyridine at steam-bath temperature, cevagenine yields cevagenine orthoacetate diacetate, whereas veracevine and cevine yield triacetates).[2]

It now appears that two isomeric orthoacetates of cevagenine exist. One of these, levorotatory, was first reported by Stoll and Seebeck[3] who obtained it by mild alkaline hydrolysis of cevadine orthoacetate diacetate, followed by extraction with mineral acid. On repetition of their hydrolysis without subsequent treatment with mineral acid, a dextrorotatory isomer has been obtained. Since these isomers are both cevagenine orthoacetates, I propose to distinguish them by the prefixes D- and C-, the former prefix for the dextrorotatory isomer and the latter for the levorotatory isomer.

The levorotatory or C-isomer, $C_{29}H_{43}O_8N$, m.p. 180–190°, $[\alpha]^{24}D$ −35° (*c* 1.58, alc.) was first obtained in this Laboratory by alkaline hydrolysis of cevagenine orthoacetate diacetate with 20% alcoholic potassium hydroxide. This result differs from that of Stoll and Seebeck,[4] who reported the isolation of cevine orthoacetate from the reaction. The assignment of a cevagenine orthoacetate formulation to the product was made on the bases: (1) The infrared spectrum shows carbonyl absorption characteristic of a 6-membered ring ketone (5.84 μ) and the displaced methyl band (7.12 μ) and ether bands (near 8.85 μ) characteristic of orthoacetates.[5] (2) The compound yields one mole of acetic acid upon acid hydrolysis, though the infrared spectrum shows no ester carbonyl absorption.

The physical constants of cevagenine C-orthoacetate showed certain anomalies with respect to the physical constants of the other orthoacetates in the series. The infrared spectrum of cevagenine C-orthoacetate closely resembles that of cevagenine orthoacetate diacetate in the region from 8.50 to 11.50 μ (*cf.* Fig. 1) but differs from the spectra of cevine orthoacetate[3,5] and cevadine orthoacetate diacetate ("anhydrocevadine triacetate"[2c]) (*cf.* Fig. 2). Particularly noteworthy is the presence of a medium intensity band near 11.20 μ in the former pair of compounds, which is absent in the latter pair. Furthermore, the molecular rotation change from cevagenine to cevagenine C-orthoacetate (+57°) approximates the change from cevagenine diacetate[6] to cevagenine orthoacetate diacetate (+77°), but differs markedly from the change from cevine to cevine orthoacetate (+441°). Hence it appeared that the site of the orthoacetate grouping in cevagenine C-orthoacetate is the same as in cevagenine (C-) orthoacetate diacetate but differs from its location in cevine orthoacetate and cevadine orthoacetate diacetate; and that a second cevagenine orthoacetate, related to the

(1) This investigation was supported by a research grant (H-1563 (C3)) from the National Health Institute, of the National Institutes of Health, Public Health Service.

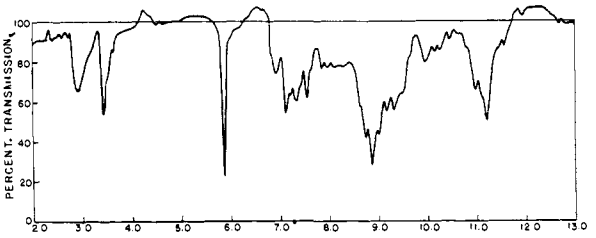
(2) (a) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **35**, 1142 (1952); (b) D. H. R. Barton and C. J. W. Brooks, *Chem. and Ind.*, 1366 (1953); (c) S. M. Kupchan, D. Lavie, C. V. Deliwala and B. Y. A. Andoh, THIS JOURNAL, **75**, 5519 (1953).

(3) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **37**, 824 (1954).

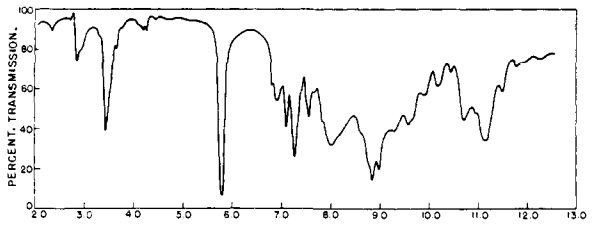
(4) A. Stoll and E. Seebeck, *ibid.*, **36**, 189 (1953).

(5) D. H. R. Barton, C. J. W. Brooks and J. S. Fawcett, *J. Chem. Soc.*, 2137 (1954). I thank Professor Barton for kindly communicating these results to me prior to publication.

(6) Paper III, S. M. Kupchan and D. Lavie, THIS JOURNAL, **77**, 683 (1955).

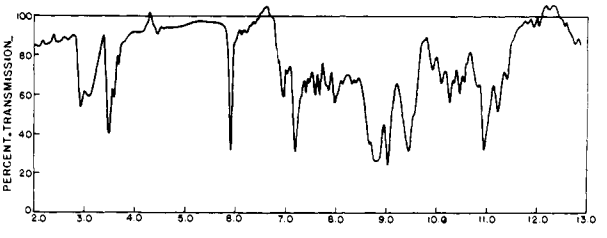


Cevagenine C-orthoacetate

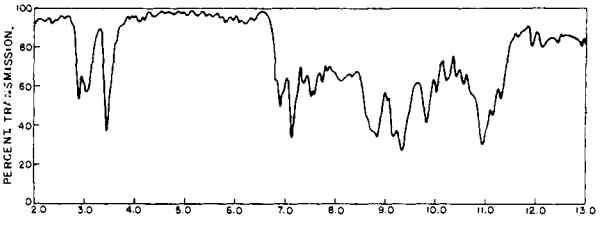


Cevagenine C-orthoacetate diacetate

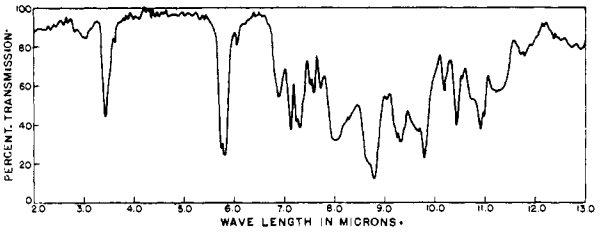
Fig. 1.



Cevagenine D-orthoacetate



Cevine orthoacetate



Cevadine orthoacetate diacetate

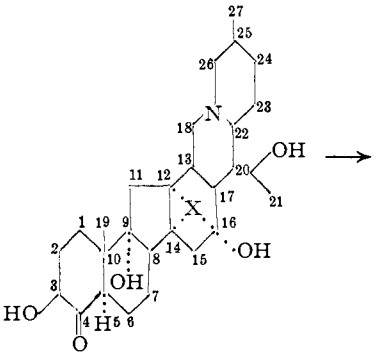
Fig. 2.

orthoacetates of cevine and cevadine, should exist. In fact, Stoll and Seebeck[2a] reported an amorphous product of mild alkaline hydrolysis of cevadine orthoacetate diacetate whose rotation ($[\alpha]^{20}D$ +34.4°, ΔM_D from cevagenine +427°) suggested that it might be the expected isomer.

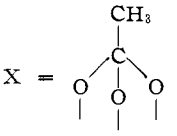
Further investigation of the alkaline hydrolysis of cevadine orthoacetate diacetate with 5% methanolic potassium hydroxide has now led to isolation of two crystalline products. Crystallization from methylene chloride–petroleum ether gave cevagenine D-orthoacetate, $C_{29}H_{43}O_8N$, m.p. 175–185°, $[\alpha]^{24}D$ +20° (*c* 1.89, alc.). After removal of this compound, crystallization of the residual material gave an isomeric material, $C_{29}H_{43}O_8N$, m.p. 185–195°, $[\alpha]^{24}D$ +42° (*c* 1.78, alc.). The infrared spectrum of this second product and its rotation both suggested that this might be a molecular complex of cevagenine D-orthoacetate and cevine orthoacetate ($[\alpha]D$ +62°).[5] The correctness of this suggestion was demonstrated by admixture of equal quantities of cevagenine D-orthoacetate and cevine orthoacetate and crystallization from ether, whereupon the crystalline complex was obtained.

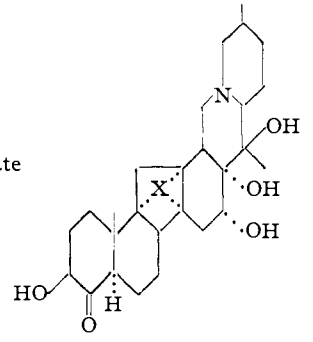
Cevagenine D-orthoacetate was characterized by its characteristic carbonyl band (5.84 μ) and orthoacetate bands (7.12 and 8.85 μ)[5] and by the fact that it yielded one mole of acetic acid on acetyl determination, though its infrared spectrum showed no ester carbonyl absorption. The infrared spectrum of this isomer showed additional bands at 9.40 μ and 10.95 μ, which are present in cevine orthoacetate and cevadine orthoacetate diacetate (*cf.* Fig. 2). The molecular rotation change from cevagenine to cevagenine D-orthoacetate (+367°) approximates the change from cevine to cevine orthoacetate (+441°). Hence it appeared likely that the site of the orthoacetate in cevagenine D-orthoacetate is the same as that in cevine orthoacetate and in cevadine orthoacetate diacetate. Confirmation for this relationship was obtained by treatment of cevagenine D-orthoacetate with 20% alcoholic potassium hydroxide, whereupon cevine orthoacetate was obtained. Treatment of cevagenine D-orthoacetate with mineral acid caused isomerization to cevagenine C-orthoacetate. Acetylation of cevagenine D-orthoacetate with acetic anhydride–pyridine at steam-bath temperature gave cevagenine C-orthoacetate diacetate. The relationships of the orthoacetates are summarized in Chart I.

The cevagenine orthoacetates can best be formulated as



Cevagenine D-orthoacetate

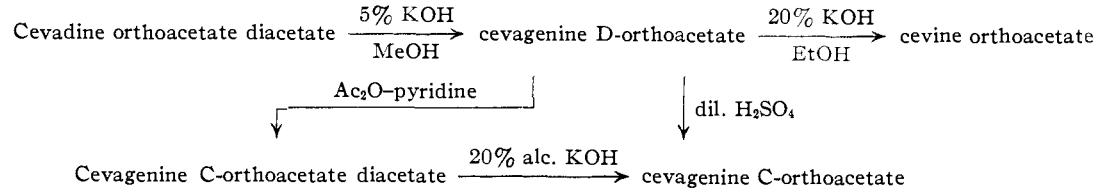




Cevagenine C-orthoacetate

CHART I

RELATIONSHIPS OF THE CEVAGENINE D- AND C-ORTHOACETATES



Of the alkamines in this series, cevagenine alone possesses a free hydroxyl group at C_9.[7] Hence only cevagenine can readily form the C-(or 9,12,14)-orthoacetate upon treatment with acetic anhydride–pyridine. Examination of models of the D- and C-orthoacetates shows that the D-structure is more strained. The greater strain may be attributable to the presence of a 7-membered ring in the D-orthoacetate cage, which is absent from the C-orthoacetate cage. Isomerization of the less stable acid-labile D-orthoacetate to the more stable C-orthoacetate is explicable on this basis. Likewise, the conversion of cevagenine D-orthoacetate into cevagenine C-orthoacetate diacetate by acetic anhydride–pyridine can be assumed to involve prior isomerization of the D-orthoacetate under the influence of acetic anhydride. The stability of cevagenine C-orthoacetate to hot 20% alcoholic potassium hydroxide (the conditions employed to convert cevagenine to cevine and cevagenine D-orthoacetate to cevine orthoacetate) may be attributed to the non-availability of the hydroxyl function at C_9 for closure to the hemiketal.

The structure of cevagenine C-orthoacetate furnishes valuable evidence on the orientation of the hydroxyl groups at C_{12}, C_{14} and C_{17}. The hydroxyl function at C_9 is known to be α-oriented from its ability to form stable hemiketals with the keto group at C_4.[7] Since the hydroxyl groups at C_{12} and C_{14} can form an orthoacetate involving the hydroxyl group at C_9, the hydroxyl groups at C_{12} and C_{14} must also occupy the α-orientation. Furthermore, since the hydroxyl function at C_{17} can form an orthoacetate involving the hydroxyl groups at C_{12} and C_{14}, it, too, must occupy the α-orientation.

Acknowledgment.—I take pleasure in expressing my appreciation to Mrs. Mary Fieser for stimulating discussions.

Experimental

Hydrolysis of Cevagenine C-Orthoacetate Diacetate to Cevagenine C-Orthoacetate.—Cevagenine C-orthoacetate diacetate (3.3 g.) was treated with 20% alcoholic potassium hydroxide (30 ml.) and the mixture was heated under reflux for 40 minutes. Water (50 ml.) was added and the alcohol was evaporated *in vacuo*. The aqueous suspension was extracted with ether (four 35-ml. portions). The ether was washed with water (two 10-ml. portions), dried on anhydrous sodium sulfate, and evaporated to dryness *in vacuo*. The residue was crystallized from acetone; yield 2.1 g. of colorless needles. Recrystallization from acetone gave pure cevagenine C-orthoacetate, m.p. 180–190°, $[\alpha]^{24}D$ −35° (*c* 1.58 alc.).

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

Anal. Calcd. for $C_{29}H_{43}O_8N$: C, 65.27; H, 8.12; acetyl, 8.07. Found: C, 65.15; H, 8.21; acetyl, 8.49.[8]

Hydrolysis of Cevadine Orthoacetate Diacetate to Cevagenine D-Orthoacetate.—Cevadine orthoacetate diacetate (2.0 g.) was treated with 5% methanolic potassium hydroxide (20 ml.) and the solution was heated under reflux for 30 minutes. Water (40 ml.) was added and the aqueous suspension was extracted with chloroform (ten 10-ml. portions). The chloroform solution was washed with water (10 ml.), dried over anhydrous sodium sulfate, and evaporated to dryness *in vacuo*. Crystallization of the residue from methylene chloride–petroleum ether led to the separation of plates (700 mg.). Recrystallization from the same solvents gave pure cevagenine D-orthoacetate containing one mole of methylene chloride of crystallization (500 mg.), m.p. 175–185°, $[\alpha]^{24}D$ +20° (*c* 1.89, alc.). (Attempts to remove the solvent of crystallization by drying *in vacuo* at elevated temperatures led to partial loss of the solvent of crystallization and unsatisfactory analyses of the dried material.)

Anal. (without drying *in vacuo*) Calcd. for $C_{29}H_{43}O_8N \cdot CH_2Cl_2$: C, 58.25; H, 7.35; acetyl, 6.96. Found: C, 58.52; H, 7.50; acetyl, 6.69.

The mother liquor was brought to dryness *in vacuo*. The residue was boiled with ether (30 ml.) and filtered from insoluble solid. Concentration of the ether led to crystallization of the molecular complex of cevagenine D-orthoacetate and cevine orthoacetate (550 mg.). Recrystallization from ether gave colorless needles (470 mg.), m.p. 185–195°, $[\alpha]^{24}D$ + 42° (*c* 1.78 alc.).

Anal. Calcd. for $C_{29}H_{43}O_8N$: C, 65.27; H, 8.12. Found: C, 64.86; H, 8.09.

Cevine orthoacetate (50 mg.) and cevagenine D-orthoacetate (50 mg.) were dissolved in methylene chloride and evaporated to dryness *in vacuo*. Crystallization of the residue from ether afforded the above-mentioned molecular complex (60 mg.); m.p., rotation and infrared spectrum in chloroform identical with the sample described above.

Acid Isomerization of Cevagenine D-Orthoacetate to Cevagenine C-Orthoacetate.—Cevagenine D-orthoacetate (250 mg.) was treated with 5% sulfuric acid (5 ml.) for ten minutes at room temperature. The solution was made alkaline with aqueous ammonia and extracted with chloroform (eight 5-ml. portions). The chloroform was washed with water (5 ml.), dried over anhydrous sodium sulfate, and evaporated to dryness *in vacuo*. Benzene (5 ml.) was added and boiled off to remove last traces of chloroform. The residue crystallized from acetone and gave cevagenine C-orthoacetate (147 mg.), m.p. 180–190°; rotation and infrared spectrum in chloroform identical with those of an authentic sample.

Acetylation of Cevagenine D-Orthoacetate to Cevagenine C-Orthoacetate Diacetate.—Cevagenine D-orthoacetate (300 mg.) was treated with acetic anhydride (4 ml.) and pyridine (4 ml.) and the mixture was heated on the steam-bath for two hours. After cooling, water (5 ml.) was added, and the mixture was allowed to stand at room temperature for one hour. The solvents were evaporated to dryness *in vacuo* and the residue was treated with dilute aqueous ammonia (6 ml.) and extracted with chloroform (nine 5-

(8) Hydrolysis in the acetyl determinations was done with *p*-toluenesulfonic acid; F. J. B. Niederl and V. Niederl, "Micromethods of Quantitative Organic Analysis," John Wiley and Sons, Inc., New York, N. Y., pp. 257–262. This determination and the other microanalyses were carried out by Dr. S. M. Nagy and associates at the Massachusetts Institute of Technology. All samples were dried *in vacuo* at 110° unless otherwise specified.

ml. portions). The chloroform was washed with water (5 ml.), dried over anhydrous sodium sulfate, and brought to dryness *in vacuo*. The residue was dissolved in ether (20 ml.) and the solution was filtered from a small amount of colored insoluble solid. Concentration of the ether led to crystallization of cevagenine C-orthoacetate diacetate (200 mg.); m.p., rotation and infrared spectrum in chloroform identical with those of an authentic sample.

Alkaline Isomerization of Cevagenine D-Orthoacetate to Cevine Orthoacetate.—Cevagenine D-orthoacetate (300 mg.) was treated with 20% alcoholic potassium hydroxide (4 ml.) and the solution was heated under reflux for 30 minutes. Water (4 ml.) was added, and the alcohol was removed *in vacuo*. The aqueous suspension was extracted with chloroform (eight 5-ml. portions). The chloroform was washed, dried over sodium sulfate and evaporated to dryness *in vacuo*. Crystallization of the residue from methanol gave cevine orthoacetate (80 mg.), m.p. 180–190°. The rotation and infrared spectrum in chloroform were identical with those of an authentic sample.

CAMBRIDGE, MASSACHUSETTS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY]

Zygadenus Alkaloids. V. Active Principles of *Zygadenus venenosus*. Zygacine[1]

BY S. MORRIS KUPCHAN, DAVID LAVIE[2] AND RICHARD D. ZONIS

RECEIVED AUGUST 19, 1954

A new ester alkaloid, zygacine, has been isolated from *Zygadenus venenosus*. Zygacine, $C_{29}H_{45}O_8N$, is a monoacetate ester of the alkamine zygadenine. Methanolysis of zygacine yields zygadenine and acetic acid. Acetylation of zygacine and of zygadenine with acetic anhydride at steam-bath temperature affords zygadenine triacetate. Treatment of zygacine, zygadenine or zygadenine triacetate with acetic anhydride and pyridine at steam-bath temperature yields zygadenine tetraacetate. Crystalline acetonides of zygacine and zygadenine have been prepared.

Recent investigations of the alkaloidal constituents of *Zygadenus venenosus* have revealed the occurrence of the esters veratroylzygadenine,[3] vanilloylzygadenine,[3] neogermitrine,[4] germidine,[4] neogermidine,[4] and protoveratridine,[4] and the alkamines zygadenine[3] and germine.[3]

As noted earlier, [4b] chromatography of the hydrophilic alkaloids from *Zygadenus venenosus* yielded neogermidine in the fractions eluted from the column with chloroform. However, more than half of the alkaloidal material was not eluted from the column with chloroform alone, and it was necessary to use chloroform–methanol mixtures to elute the remaining material. The infrared spectra of the successive fractions eluted with gradually increasing percentages of methanol indicated that their compositions were quite similar. Furthermore, these infrared spectra more closely resembled the spectra of the zygadenine esters previously encountered than those of the germine esters. Several attempts were made to separate a crystalline product from the amorphous ester fractions by countercurrent distribution and re-chromatography, but these attempts were unsuccessful.

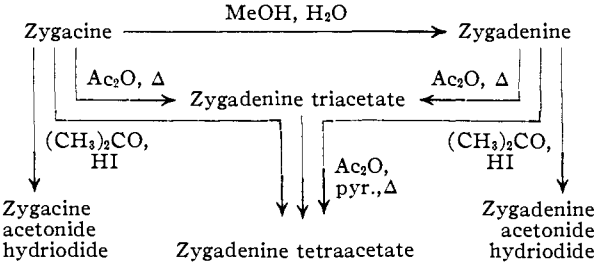
The behavior of the amorphous ester fractions in countercurrent distribution and chromatography suggested substantial homogeneity of the material and this led us to attempt to prepare crystalline salts from the amorphous alkaloid bases. Attempts to prepare a crystalline hydrochloride or hydrobromide were unsuccessful, but upon treatment of the material in acetone with hydriodic acid, a crystalline product (I, $C_{32}H_{49}O_8N \cdot HI$, m.p. 270–271° dec.) was obtained. Further investigation of this product soon revealed that it is an acetonide hydriodide derivative of the ester alkaloid. The free acetonide derivative (II, $C_{32}H_{49}O_8N$, m.p. 253–255° dec., $[\alpha]^{23}D$ +2° (*c* 1.41, chf.)) was liberated by treatment of the salt with dilute ammonium hydroxide. Treatment of the acetonide derivative with dilute hydrochloric acid gave the free ester alkaloid (III, $[\alpha]^{23}D$ −22° (*c* 1.53 chf.).

The new ester alkaloid III afforded analytical values for carbon, hydrogen and nitrogen which agree with the formula $C_{29}H_{45}O_8N$. Acetyl determination revealed the presence of one acetyl group in the molecule. Methanolysis of III yielded zygadenine and acetic acid, which was characterized as its *p*-phenylphenacyl ester. Hence III is a monoacetate ester of zygadenine, for which we propose the name **zygacine.**

Acetylation of zygacine with acetic anhydride at steam-bath temperature gave zygadenine triacetate.[3] The preparation of triacetates from zygadenine and pseudozygadenine also were found to proceed best in the absence of pyridine. Treatment of zygadenine, zygacine or zygadenine triacetate with acetic anhydride and pyridine at steam-bath temperature afforded zygadenine tetraacetate (m.p. 207–209° dec., $[\alpha]^{23}D$ −24° (*c* 1.70 chf.).

CHART I

DERIVATIVES OF ZYGACINE AND ZYGADENINE



Like zygacine, zygadenine failed to form a crystalline derivative when treated in acetone with hydrochloric or hydrobromic acid. Upon treat-

(1) This work was supported (in part) by grants from the National Institutes of Health (RG-2553) and Research Corporation.

(2) On leave of absence from the Weizmann Institute of Science, Rehovot, Israel.

(3) S. M. Kupchan and C. V. Deliwala, THIS JOURNAL, **74**, 2382 (1952); **75**, 1025 (1953).

(4) S. M. Kupchan and C. V. Deliwala, *ibid.*, (a) **74**, 3202 (1952); (b) Paper IV, **76**, 5545 (1954).