[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY]

Zygadenus Alkaloids. III. Active Principles of *Zygadenus venenosus*. Veratroylzygadenine and Vanilloylzygadenine[1]

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Two new ester alkaloids and the alkamines, germine and zygadenine, have been isolated from *Zygadenus venenosus*. The ester alkaloids possess pharmacological activity resembling that of the known veratrum ester alkaloid, veratridine. Veratroylzygadenine, $C_{36}H_{51}O_{10}N$, is a veratric acid ester of zygadenine. Vanilloylzygadenine, $C_{35}H_{49}O_{10}N$, is a vanillic acid ester of zygadenine. Alkaline hydrolysis of the esters under drastic conditions leads to the formation of an isomer of zygadenine, pseudozygadenine, and treatment of zygadenine under the same conditions also yields pseudozygadenine.

Several species of the plant genus *Zygadenus* have long been known to possess principles which are poisonous to livestock. The growing season of these plants is early, and grazing livestock have frequently gorged themselves on these plants in the spring, when other plants are not available. This has led to considerable livestock poisoning, and the United States Department of Agriculture has issued a number of bulletins to breeders informing them of the habits and management of the plants.[2]

Some 50 years ago, Dr. Reid Hunt studied the pharmacological behavior of crude extracts of *Zygadenus venenosus* (Death Camas), and observed that these extracts possess pharmacological activity resembling that of the veratrum alkaloids.[3] In view of the recent interest in the pharmacology of the veratrum alkaloids[4] and in the clinical use of the hypotensive activity of the tertiary ester alkaloids,[5] it appeared of importance to isolate and study the active principles of *Zygadenus venenosus*.

The earliest investigators[6] of the alkaloidal extracts of *Zygadenus venenosus* did not succeed in isolating any of the components of the mixture in a pure state, and they drew several questionable conclusions on the basis of the solubility behavior and color reactions of their amorphous substances. Heyl and co-workers[7] isolated the first crystalline alkaloid in this series, zygadenine, and they described its physical properties and empirical formula. It appeared unlikely, however, that zygadenine is the most important toxic agent in *Zygadenus venenosus*. In a note appended to the first of Heyl's papers,[7a] Dr. Philip H. Mitchell reported, "Toxicologically, this preparation is quite different from that of the mixed alkaloids. It shows none of the characteristic effects given by the mixture. It kills guinea pigs slowly, and only in comparatively large doses."

The probable presence in *Zygadenus venenosus* of ester alkaloids similar to the tertiary alkamine ester alkaloids of the veratrum series was indicated by the pharmacodynamic properties of the alkaloidal mixtures.[8] In a preliminary note,[9] we have reported the occurrence in the plant of the zygadenine esters veratroylzygadenine and vanilloylzygadenine. The purpose of the present paper is to describe in detail the isolation and characterization of the latter compounds.

Heyl and co-workers[7] used alcohol for the extraction of ground *Zygadenus venenosus*. To prevent possible loss in hypotensive activity due to alcoholysis of ester alkaloids,[10] we chose to use chloroform for the initial extraction. The yield of crude alkaloid obtained by chloroform extraction was equivalent to 0.28% of the weight of the dried plant. Subsequent alcohol extraction yielded an additional 0.22%; the total yield of crude alkaloid was about the same as that obtained by Heyl and Herr (0.49%).[7b]

The chloroform-extracted bases were next subjected to 8-plate countercurrent distribution using benzene and 2 *M* acetate buffer at *pH* 5.5. The alkaloidal material was distributed fairly evenly among the 8 plates; no discrete peaks were obtained. Pharmacological screening of the fractions,[11] however, showed that the major portion of the hypotensive activity resided in the alkaloids of plates 5–8. The distribution was then modified in such a way as to concentrate the bulk of the active material in a single fraction. This was accomplished by means of a modified 8-plate countercurrent distribution using benzene and phosphate buffer at *p*H 7.1. This procedure (see Experimental) afforded a plate-8 fraction which accounted for 25–30% of the weight and the major proportion of the hypotensive activity of the starting material. The remaining alkaloids were separated into the plates-1–7 fraction and the plate-0 fraction.

A crystalline alkaloid (I) separated from an acetone solution of the crude plate-8 fraction. After recrystallization from absolute alcohol, this compound melted at 270–271° (dec.) and showed $[\alpha]^{20}D$ −27° in chloroform. Hydrolysis of I by warming with 0.1 *N* aqueous methanolic alkali for 15 minutes yielded veratric acid and an amorphous

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

(2) U. S. Dept. Agr. Bull. 125 (1915); 1240 (1924); 1376 (1926).

(3) R. Hunt, *Am. J. Physiol.*, **6**, XIX (1902).

(4) O. Krayer and G. Acheson, *Physiol. Rev.*, **26**, 383 (1946); G. L. Maison, E. Gotz and J. W. Stutzman, *J. Pharmacol. Exptl. Therap.*, **103**, 74 (1951).

(5) E. Meilman and O. Krayer, *Circulation*, **1**, 204 (1950); E. D. Fries, J. R. Stanton and F. C. Moister, *J. Pharmacol. Exptl. Therap.*, **98**, 166 (1950).

(6) M. Vejux-Tyrode, *J. Med. Res.*, **11** (new series **6**), 399 (1904); H. B. Slade, *Am. J. Pharmacy*, **77**, 262 (1905).

(7) (a) F. W. Heyl, F. E. Hepner and S. K. Loy, THIS JOURNAL, **35**, 258 (1913); (b) F. W. Heyl and M. E. Herr, *ibid.*, **71**, 1751 (1949).

(8) S. Yaffe and S. M. Kupchan, *Federation Proc.*, **9**, 326 (1950).

(9) S. M. Kupchan and C. V. Deliwala, THIS JOURNAL, **74**, 2382 (1952).

(10) J. Fried, H. L. White and O. Wintersteiner, *ibid.*, **72**, 4621 (1950).

(11) All the pharmacological results reported herein were obtained at the laboratory of Professor Otto Krayer at the Department of Pharmacology of Harvard Medical School. We should like to express our gratitude to Professor Krayer for his invaluable coöperation and inspiration during the entire course of the work.

alkamine fraction which resisted crystallization. Attempts to prepare crystalline hydrochloride or sulfate salts from this material were unsuccessful. Analysis of this amorphous solid gave figures which agreed with the theoretical figures for the zygadenine formula ($C_{27}H_{43}O_7N$).[7b] When zygadenine was similarly treated with 0.1 *N* aqueous methanolic alkali an amorphous solid was obtained whose infrared spectrum was identical with that of the alkamine obtained by hydrolysis of I. The infrared spectra of the amorphous alkamine fractions above contained a band at 5.86 μ which was absent from the zygadenine spectrum. The behavior of zygadenine upon treatment with alkali recalls the alkali induced isomerization of germine, cevine and protoverine[12] and suggests that, like those alkamines, zygadenine is converted by alkaline treatment to an isomer containing a carbonyl group. Several attempts were made to separate crystalline substances from the amorphous alkali transformation product by countercurrent distribution and chromatography, but these attempts were unsuccessful.

Alternative hydrolysis procedures were tried next in an effort to obtain a crystalline alkamine hydrolysis product of ester I. Treatment of the ester under mild alkaline conditions (*e.g.*, with aqueous methanolic ammonia or aqueous methanolic barium hydroxide) failed to cleave the ester, and the starting material was recovered unchanged. Hydrogenolysis of I with lithium aluminum hydride or high pressure catalytic hydrogenation did not yield any crystalline products. When the alkaloid I was hydrolyzed under more drastic conditions, such as by prolonged heating with an alcoholic solution of sodium ethoxide, veratric acid and a new alkamine isomeric with zygadenine was obtained. The new alkamine, for which we propose the name **pseudozygadenine**, melts at 169–171° (dec.), $[\alpha]^{25}D$ −33° in chloroform. Acetylation with acetic anhydride and pyridine gave a triacetate which melted at 235–236° (dec.) and showed $[\alpha]^{23}D$ −33° in chloroform. Treatment of zygadenine under strong alkaline conditions similar to those above also yielded pseudozygadenine. The infrared spectrum of pseudozygadenine contains no band at 5.86 μ, and does not, therefore, account for the carbonyl band present in the spectrum of the amorphous material obtained by mild alkaline treatment of zygadenine. On drastic alkaline treatment, the carbonyl-containing amorphous alkamine was converted to pseudozygadenine. This suggests that a carbonyl-containing zygadenine isomer may be an intermediate in the isomerization of zygadenine to pseudozygadenine. Further work on the isolation of the carbonyl-containing zygadenine isomer is in progress.

Analysis of I afforded figures which are in good agreement with the theoretical values for the formula $C_{36}H_{51}O_{10}N$. This information and the fact that one molar equivalent of veratric acid was obtained on alkaline hydrolysis suggest that I is a veratric acid ester of either zygadenine or pseudozygadenine. Examination of the infrared spectra of I and the acetyl derivatives of zygadenine and pseudozygadenine indicated a closer resemblance of I to the zygadenine ester, particularly in the fingerprint region (9–11 μ).[13] This indicates that I is a veratric acid ester of zygadenine, and we propose the name **veratroylzygadenine** for the compound.

Pharmacological assay[11] of the plate-8 fraction after the removal of veratroylzygadenine showed that it contained the major portion of the hypotensive material originally present in the plate-8 fraction. Further fractionation was effected by chromatographing this active material in chloroform solution on acid-washed alumina. The material eluted with chloroform–7.5% methanol solution crystallized from acetone, yielding a second ester alkaloid (II). Compound II melted at 258–259° (dec.), $[\alpha]^{20}D$ −27.5° in chloroform. Hydrolysis by warming with 0.1 *N* aqueous methanolic alkali (15 minutes) yielded vanillic acid and an amorphous alkamine fraction. Hydrolysis under more drastic conditions, as described above, afforded vanillic acid and pseudozygadenine. Analysis of II gave values for carbon and hydrogen content which agree with the theoretical values for $C_{35}H_{49}O_{10}N$. Methoxyl determination indicated the presence of one methoxyl group in the molecule. Methylation of II with diazomethane yielded veratroylzygadenine. Compound II, then, is a vanillic acid ester of zygadenine and has been named **vanilloylzygadenine**.

When the plate-0 buffer solution of the modified distribution was made alkaline and extracted with chloroform, an amorphous alkaloidal solid was obtained which resisted all attempts at crystallization. However, if the alkaline solution was extracted first with benzene (plate-0 benzene fraction) and then with chloroform, three crystalline compounds could be obtained from the alkaloidal mixture of the plate-0 chloroform fraction. Crystallization of this material from acetone afforded zygadenine and another compound, III, m.p. 265–267°, in very small quantity. (Compound III has subsequently been characterized as protoveratridine.[14]) Zygadenine was separated from III by recrystallization from benzene and was found to melt at 218–220° (dec.),[15] $[\alpha]^{22}D$ −48.5° in chloroform. Analysis of our sample gave results which support the formulation advanced by Heyl and Herr ($C_{27}H_{43}O_7N$).[7b] Conclusive identification of our material was achieved by direct comparison with an authentic specimen of zygadenine.[15] Acetylation with acetic anhydride and pyridine yielded zygadenine triacetate, which melted at 273–275° (dec.) and showed $[\alpha]^{29}D$ − 55° in chloroform.

A considerable proportion of the alkaloidal material of the plate-0 chloroform fraction remained in acetone solution after removal of zygadenine and protoveratridine. The amorphous solid ob-

(12) H. Jaffe and W. A. Jacobs, *J. Biol. Chem.*, **193**, 325 (1951).

(13) We have subsequently noted a similar correspondence of infrared spectra (in the 9–11 μ region) of the naturally-occurring germine triesters, germitrine and neogermitrine (in chloroform), to that of germine pentaacetate.

(14) S. M. Kupchan and C. V. Deliwala, THIS JOURNAL, **74**, 3202 (1952).

(15) Zygadenine has been reported to have a melting point of 201–204° by Heyl and Herr.[7b] However, a redetermination of the melting point of the original sample by Mr. Herr, of the Upjohn Company, Kalamazoo, Michigan, has indicated that the correct melting point is 218–220° (dec.). We wish to thank Mr. Herr for generously supplying an authentic specimen of zygadenine.

tained upon lyophilization of the acetone solution crystallized from chloroform, yielding the chloroform addition compound of germine. After recrystallization from methanol the alkamine was characterized by its rotation, analysis and comparison of its infrared spectrum with that of an authentic sample of germine from *Veratrum viride*, kindly made available by Dr. J. Fried, E. R. Squibb and Sons, New Brunswick, New Jersey.

The two new ester alkaloids and zygadenine were examined[11] for their circulatory action in the anesthetized cat, their effect upon the failing heart in the heart-lung preparation of the dog, and their effect upon the amphibian skeletal muscle. In all three types of experiments the actions of zygadenine were similar to those of cevine, and the actions of veratroylzygadenine and vanilloylzygadenine were similar, qualitatively as well as quantitatively, to the actions of veratridine.

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Experimental

Chloroform Extraction of *Zygadenus venenosus*.—Ground (60-mesh) *Zygadenus venenosus*[16] (16.2 kg., whole plant) was extracted five times with chloroform and ammonium hydroxide by allowing the material to stand in contact with the solvent in percolators and then draining. For each extraction, 1.1 liters of concentrated ammonium hydroxide and 30 liters of chloroform were used. The first extraction was allowed to stand for six hours before draining, the second for 16 hours, and the last three for 20 hours. The chloroform extracts were concentrated *in vacuo* at a temperature of 24–34° to about 900 ml. total volume. On standing overnight, 38 g. of yellow solid separated. The solid was found to be insoluble in 5% tartaric acid solution, and was not investigated further.

The chloroform solution (filtrate) was extracted exhaustively with 250-ml. portions of 5% tartaric acid solution until the acid extract no longer gave a positive test with Mayer reagent. Fourteen extractions were required. (The extractions were complicated by the formation of rather stable emulsions; the emulsions were broken by warming slightly (not above 40°) on the steam-bath and stirring.) The combined tartaric acid extracts were cooled to 10° in an ice–water-bath and made alkaline to *p*H 8 with 20% sodium hydroxide and then to *p*H 10 with 20% sodium carbonate. The milky suspension was exhaustively extracted with 300-ml. portions of chloroform until the chloroform extract no longer gave a positive test with Mayer reagent. (A sample was extracted with 5% tartaric acid and the acid extract was tested.) Fifteen extractions were required.

The chloroform extracts were concentrated *in vacuo* at a temperature of 25–35°. Forty-five grams (0.28% of the dried plant) of tan-brown amorphous residue was obtained.

Ethanol Extraction of Chloroform-extracted Plant.—Ground *Zygadenus venenosus* (16.2 kg., see above), which had been previously extracted with chloroform and ammonium hydroxide, was extracted twice with ethanol, using 32 liters for the first extraction and 20 liters for the second. Both extractions were allowed to stand for two days in contact with ground plant before draining. The extracts were combined and evaporated to sirup-like consistency *in vacuo* at a temperature not exceeding 32°.

Two liters of 5% tartaric acid was added to the sirup-like mass and the suspension was warmed slightly and stirred, and then allowed to settle overnight. The solid was filtered and then repeatedly extracted with 5% tartaric acid with stirring until the extract no longer gave a positive test with Mayer reagent. The combined tartaric acid extracts were cooled to 10° in an ice–water-bath, and made alkaline to *p*H 8 with 20% sodium hydroxide and then to *p*H 10 with 20% sodium carbonate. The milky suspension was exhaustively extracted with chloroform until the chloroform extract no longer gave a positive test with Mayer reagent. The chloroform extracts were combined and concentrated *in vacuo* at a temperature of 25–35°; yield of tan-brown amorphous residue, 35 g. (0.22% of the dried plant).

Fractionation of the Chloroform-extracted Bases by Modified Countercurrent Distribution.—The amorphous bases obtained by chloroform extraction were fractionated by distribution between benzene and phosphate buffer of *p*H 7.1. The distribution was carried out in two separatory funnels. The mixture of bases (6 g.) dissolved in benzene (300 ml.) was added to the first funnel containing an equal volume of buffer and shaken for about two minutes. A small amount of sticky insoluble material separated during shaking which clung to the walls of the separatory funnel and was removed by decanting from it the liquid phases. The layers were separated and the benzene layer was further extracted with seven more 100-ml. portions of buffer and these seven buffer extracts were combined. The benzene layer was dried over sodium sulfate and the benzene was removed *in vacuo*. The residue (1.6 g.) represents the plate-8 fraction of the distribution.

The first buffer layer was extracted with seven 100-ml. portions of benzene and these benzene extracts were combined. The buffer layer (plate-0 fraction) was made alkaline to *p*H 10 at 0–5° with 10% sodium hydroxide and extracted with benzene (two 100-ml. portions) and then with chloroform (four 200-ml. portions). The product recovered from the benzene extract after removal of solvent *in vacuo* weighed 0.42 g. and represents the plate-0 benzene fraction. Concentration of the chloroform extract *in vacuo* left 1.8 g. of colorless amorphous solid, representing the plate-0 chloroform fraction.

The combined seven buffer extracts of the initial extraction were made alkaline to *p*H 10 at 0–5° with 10% sodium hydroxide and extracted with chloroform (six 200-ml. portions). The resulting chloroform extracts were combined with the seven benzene extracts and the solution was dried over sodium sulfate. The solvents were removed *in vacuo*, leaving 1.9 g. of tan solid. This represents plates 1–7 of the distribution.

Isolation of Veratroylzygadenine (I).—The amorphous plate-8 fraction (1.6 g.) was dissolved in acetone (10 ml.). After two days, a colorless, crystalline solid (150 mg.) was filtered off. The crystalline material was found to be very sparingly soluble in most of the common organic solvents and was purified by reprecipitation from dilute acetic acid solution with aqueous ammonia. The solid was dissolved in a mixture of 10% acetic acid (1 ml.) and ethanol (2 ml.) and the slightly turbid solution was filtered. The filtrate was heated to boiling on the steam-bath, and aqueous ammonia was added dropwise to turbidity. Upon cooling, elongated prisms (111 mg.), m.p. 267–268° dec., were obtained. Recrystallization of this material by dissolving in a large volume of hot absolute ethanol (40 ml.) and concentrating the solution to a small volume (7 ml.) yielded clusters of transparent rectangular prisms (90 mg.), m.p. 270–271° (dec.), $[\alpha]^{20}D$ −27° (*c* 2.08 in chf), $\lambda_{max}^{alc.}$ 262, 293 mμ (log ε 4.13, 3.85).

Anal. Calcd. for $C_{36}H_{51}O_{10}N$: C, 65.73; H, 7.82; N, 2.13. Found (after drying *in vacuo* at 110°): C, 65.90; H, 7.86; N, 2.09.

Alkaline Cleavage of Veratroylzygadenine. A. With Aqueous Methanolic Alkali.—Veratroylzygadenine (200 mg.) was added to a solution of 4% sodium hydroxide (2 ml.), methanol (6 ml.) and water (1.5 ml.). The suspension was warmed gently on the steam-bath until a vigorous exothermic reaction began, and in 10 minutes (total time) all of the solid had dissolved. The solution was then heated under reflux for five minutes longer. Water (3 ml.) was then added, and the methanol was removed *in vacuo*. The residual aqueous residue and precipitate were extracted with chloroform (three 25-ml. portions) and the chloroform extract was washed with water (5 ml.) and dried over sodium sulfate. Evaporation to dryness *in vacuo* left an amorphous solid (120 mg.) which resisted all attempts at crystallization.

Anal. Calcd. for $C_{27}H_{43}O_7N$: C, 65.69; H, 8.78; N,

(16) Plant gathered in Washington in June, 1950. We are grateful to Dr. Reed Rollins, Gray Herbarium, Harvard University, for confirming the identity of the plant.

2.84. Found (after drying *in vacuo* at 110°): C, 65.98; H, 9.06; N, 3.06.

The alkaline solution and water washings were combined and acidified to *p*H 2 with 0.1 *N* hydrochloric acid, whereupon a copious precipitate of veratric acid separated. Recrystallization from water afforded colorless needles (40 mg.), m.p. 178–179°. The melting point of this sample was not depressed on admixture of an authentic sample of veratric acid and the infrared spectrum of the sample was found to be identical with that of veratric acid.

B. With Alcoholic Sodium Ethoxide.—Veratroylzygadenine (200 mg.) was added to a solution of sodium (200 mg.) in absolute ethanol (30 ml.) and the mixture was heated under reflux for four hours. The solution was then cooled and brought to *p*H 6.5 by addition of 0.1 *N* hydrochloric acid. Water (3 ml.) was added, and the ethanol was removed *in vacuo*. Aqueous ammonia was now added to make the solution alkaline, and the mixture was extracted with chloroform (three 25-ml. portions). The chloroform extract was washed with water (5 ml.) and dried over sodium sulfate. Evaporation to dryness *in vacuo* left an amorphous solid residue (130 mg.) which crystallized from benzene in the form of needles (75 mg.), m.p. 167–169° (dec.). Recrystallization from ethyl acetate–petroleum ether afforded pseudozygadenine in the form of glistening clusters of needles, m.p. 169–171° dec., $[\alpha]^{25}D$ −33° (*c* 2.00 in chf).

Anal. Calcd. for $C_{27}H_{43}O_7N$: C, 65.69; H, 8.78; N, 2.84. Found (after drying *in vacuo* at 110°): C, 65.46, 65.79; H, 9.10, 8.69; N, 2.95.

The alkaline solution and water washings were worked up as above, and yielded veratric acid (38 mg.).

Pseudozygadenine Triacetate.—A mixture of pseudozygadenine (100 mg.), acetic anhydride (2 ml.) and pyridine (1 ml.) was heated on the steam-bath for two hours. Evaporation of the reagents *in vacuo* at room temperature left an amorphous solid, which was dissolved in cold water (3 ml.). The solution was made alkaline with aqueous ammonia, and extracted with chloroform (three 35-ml. portions). The chloroform extract was washed with water (5 ml.) and dried over sodium sulfate. The residue after evaporation of the solvent *in vacuo* (105 mg.) crystallized readily from ether. Recrystallization from the same solvent yielded pseudozygadenine triacetate (70 mg.) in the form of rhomboids, m.p. 235–236° (dec.), $[\alpha]^{23}D$ −33° (*c* 1.89 in chf).

Anal. Calcd. for $C_{27}H_{40}O_7N(COCH_3)_3$: C, 63.95; H, 7.97; acetyl, 20.89. Found (after drying *in vacuo* at 110°): C, 64.13; H, 8.11; acetyl, 20.69.

Alkaline Isomerization of Zygadenine. A. With Aqueous Methanolic Alkali.—Zygadenine (200 mg.) was added to a solution of 4% sodium hydroxide (2 ml.), methanol (6 ml.) and water (1.5 ml.). The solution was heated under reflux for a period of 15 minutes. The reaction mixture was worked up as described under part A of the account of the alkaline cleavage of veratroylzygadenine. An amorphous solid S (140 mg.) was obtained which resisted all attempts at crystallization. The infrared spectrum of this material was identical with that of the amorphous solid obtained from alkaline cleavage of veratroylzygadenine by heating with aqueous methanolic sodium hydroxide for 15 minutes.

Solid S (100 mg.) was added to a solution of 4% sodium hydroxide (1 ml.), methanol (3 ml.) and water (0.8 ml.). The solution was heated under reflux on the steam-bath for a period of five hours. The reaction mixture was cooled and worked up as above. When the crude solid product was dissolved in benzene (3 ml.) and the solution was seeded with pseudozygadenine, a colorless solid crystallized. Recrystallization from benzene afforded pseudozygadenine (38 mg., m.p. 168–170° (dec.)).

B. With Alcoholic Sodium Ethoxide.—Zygadenine (200 mg.) was treated with alcoholic sodium ethoxide as described above in procedure B for the alkaline cleavage of veratroylzygadenine. The colorless solid obtained upon evaporation of the chloroform extract crystallized readily from benzene to give pseudozygadenine (105 mg.), m.p. 167–169° (dec.). Recrystallization from ethyl acetate–petroleum ether afforded pure pseudozygadenine, identical in all respects with the product obtained by alkaline cleavage of veratroylzygadenine.

Isolation of Vanilloylzygadenine (II).—The acetone solution obtained by filtration of veratroylzygadenine from the plate-8 fraction in acetone was lyophilized. The tan-colored amorphous residue (1.3 g.) was dissolved in chloroform (20 ml.) and passed through a column of 15-mm. diameter containing sulfuric acid-washed alumina (30 g.). Washing of this column with chloroform (200 ml.), chloroform–2% methanol (100 ml.) and chloroform–5% methanol (100 ml.) eluted fractions which resisted crystallization. Further washing with chloroform–7.5% methanol (100 ml.) eluted alkaloidal material (120 mg.) which crystallized from acetone (94 mg., m.p. 255–257° (dec.)). Recrystallization from ethanol afforded heavy rods (65 mg.), m.p. 258–259° (dec.), $[\alpha]^{20}D$ −27.5° (*c* 2.00 in chf); $\lambda_{max}^{alc.}$ 264, 294 mμ (log ε 4.07, 3.83).

Anal. Calcd. for $C_{35}H_{49}O_{10}N$: C, 65.30; H, 7.67; N,

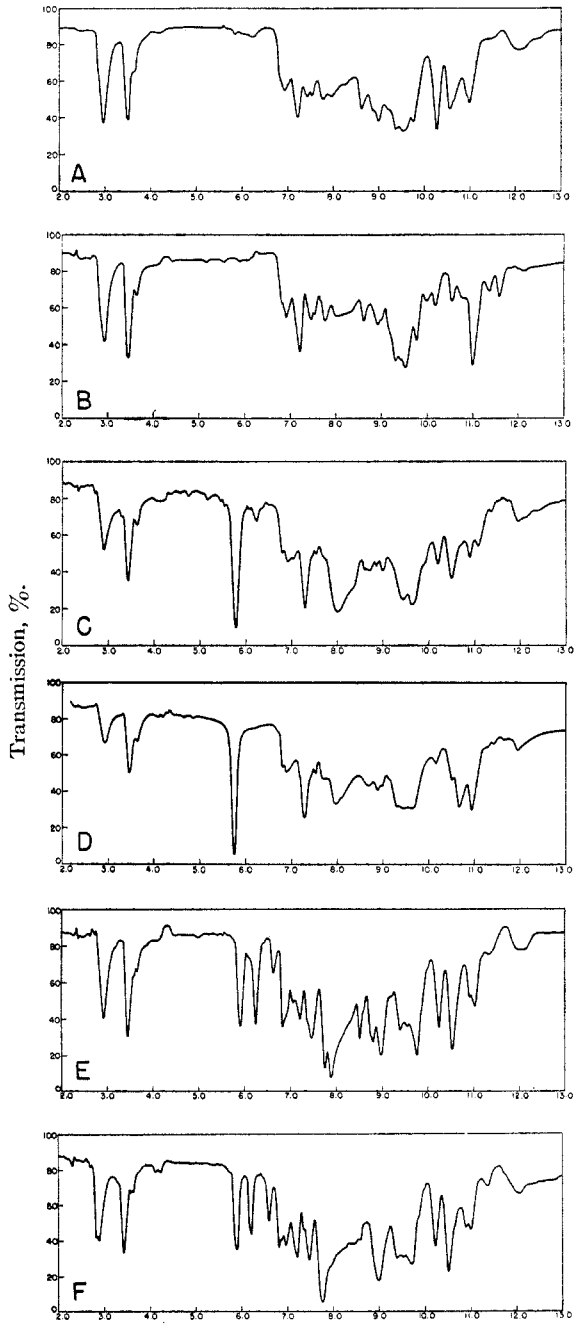

Fig. 1.—Infrared spectra in chloroform: A, zygadenine; B, pseudozygadenine; C, zygadenine triacetate; D, pseudozygadenine triacetate; E, veratroylzygadenine; F, vanilloylzygadenine.

2.18; 1 methoxyl, 4.82. Found (after drying *in vacuo* at 110°): C, 65.35; H, 7.93; N, 2.29; methoxyl, 4.34.

Alkaline Cleavage of Vanilloylzygadenine. A. With Aqueous Methanolic Alkali.—Vanilloylzygadenine (200 mg.) was hydrolyzed with aqueous methanolic sodium hydroxide and the hydrolysis mixture worked up as described under procedure A for the alkaline cleavage of veratroylzygadenine. The amorphous residue (107 mg.) from the chloroform extract was found to have an infrared spectrum identical with that of the solid obtained from veratroylzygadenine by the same procedure.

The alkaline solution and water washings were worked up as above, and afforded vanillic acid (38 mg., m.p. 206–207°). The identity of the acid was demonstrated by mixed melting point and infrared spectral comparisons with an authentic sample of vanillic acid.

B. With Alcoholic Sodium Ethoxide.—Vanilloylzygadenine (200 mg.) was treated with alcoholic sodium ethoxide and the reaction mixture was worked up as described above in procedure B for the alkaline cleavage of veratroylzygadenine. The residue from the chloroform extract crystallized from benzene, yielding pseudozygadenine (96 mg., m.p. 168–170° (dec.)). The alkaline solution and water washings yielded vanillic acid (35 mg., m.p. 205–207°).

Methylation of Vanilloylzygadenine.—An ethereal solution of diazomethane prepared from N-nitrosométhylurea (1 g.) was added to a chloroform solution (15 ml.) of vanilloylzygadenine (100 mg.). After standing at room temperature overnight the solvents and excess diazomethane were evaporated *in vacuo*. The solid residue crystallized from absolute ethanol, yielding veratroylzygadenine (53 mg., m.p. 266–268° (dec.)). The melting point of the product was not depressed on admixture of veratroylzygadenine, and its infrared spectrum was identical with that of veratroylzygadenine. (It is evident from Fig. 1 that there are distinct differences between the spectra of vanilloylzygadenine and veratroylzygadenine.)

Isolation of Zygadenine.—The amorphous plate-0 chloroform fraction (1.8 g.) of the modified countercurrent distribution was dissolved in acetone (10 ml.). After two days, a colorless crystalline solid (320 mg.) was obtained. Recrystallization from benzene afforded clusters of needles (105 mg.), m.p. 218–220° (dec.), $[\alpha]^{22}D$ −48.5° (*c* 1.85 in chf). The mixed melting point with an authentic sample of zygadenine[15] was not depressed, and the infrared spectra of the two samples were identical.

Anal. Calcd. for $C_{27}H_{43}O_7N$: C, 65.69; H, 8.78; N, 2.84. Found (after drying *in vacuo* at 110°): C, 65.85; H, 8.72; N, 3.05.

In the recrystallization of the crude crystalline solid obtained from acetone above, a small quantity of benzene-insoluble solid (III, m.p. 265–267° (dec.)) was obtained. The amounts available were too small for rigorous purification and analysis (see discussion).

Zygadenine Triacetate.—Zygadenine (100 mg.) was acetylated with acetic anhydride and pyridine as described above for the acetylation of pseudozygadenine. The residue obtained from the chloroform extract (95 mg.) crystallized from ether as clusters of small needles, m.p. 267–270° (dec.). Recrystallization from acetone–petroleum ether yielded colorless needles (64 mg.), m.p. 273–275° (dec.), $[\alpha]^{29}D$ −55° (*c* 2.00 in chf).

Anal. Calcd. for $C_{27}H_{40}O_7N(COCH_3)_3$: C, 63.95; H, 7.97; acetyl, 20.89. Found (after drying *in vacuo* at 110°): C, 63.77; H, 8.14; acetyl, 20.63.

Isolation of Germine.—The acetone solution obtained by filtration of zygadenine and III from the plate-0 chloroform fraction in acetone was lyophilized. The solid residue (1.4 g.) was dissolved in chloroform (15 ml.) and the chloroform solution was concentrated to half its original volume by boiling on the steam-bath. Upon cooling, a colorless crystalline solid (200 mg.) separated. Recrystallization from methanol yielded heavy prisms (95 mg.) which began to sinter at 155–165° and melted at 220–225° (dec.), $[\alpha]^{24}D$ 4° (*c* 2.00 in abs. alc.). The infrared spectrum in Nujol was identical with that of an authentic specimen of germine from *Veratrum viride*.

Anal. Calcd. for $C_{27}H_{43}O_8N$: C, 63.63; H, 8.50. Found (after drying *in vacuo* at 110°): C, 63.96; H, 8.62.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY]

The Structure of Umbellulone Dibromide[1]

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Structures for umbellulone dibromide, bromodihydroumbellulone and some of their congeners are proposed on the basis of degradation and ultraviolet and infrared absorption spectroscopy.

When the crude product obtained by treating umbellulone (I) with bromine in carbon tetrachloride solution is distilled, decomposition with evolution of hydrogen bromide occurs, and two bromine-containing products are obtained[2]: a crystalline solid, $C_{10}H_{14}OBr_2$, umbellulone dibromide, and an oil, $C_{10}H_{13}Br$. This communication provides evidence that umbellulone dibromide is 1-methyl-2-bromo-3-keto-4-bromomethyl-4-isopropylcyclopentene (II).[3]

Umbellulone dibromide is not attacked by boiling, dilute nitric acid containing silver nitrate and it does not react with bromine in boiling chloroform. The ultraviolet absorption spectrum (Table I) is that of an α,β-unsaturated ketone,[4] and the infrared absorption (Fig. 1) indicates the presence of both the C=O (5.72 μ) and C=C (6.20 μ) groups.[5] These properties are in accord with the structure II for umbellulone dibromide. In II one of the bromine atoms is unreactive toward silver ion because it is attached to a neopentyl-type system[6] and the second is unreactive on the basis of its vinyl halide character.[7] The transformations and degradation of umbellulone dibromide described below are in accord with the structure II and provide a chain of

(1) This material is taken from the Dissertation of Aaron Oken offered in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Stanford University, where he was du Pont Fellow, 1951-1952.

(2) F. H. Lees, *J. Chem. Soc.*, **85**, 639 (1904).

(3) For early structure assignments see F. Tutin, *ibid.*, **89**, 1104 (1906).

(4) R. B. Woodward, THIS JOURNAL, **64**, 76 (1942); A. E. Gillam and T. F. West, *J. Chem. Soc.*, 815 (1941); 486 (1942).

(5) R. N. Jones, P. Humphries and K. Dobriner, THIS JOURNAL, **72**, 956 (1950); H. M. Randall, R. G. Fowler, J. L. Dangl and N. Fuson, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949, p. 28.

(6) F. C. Whitmore and G. H. Fleming, THIS JOURNAL, **55**, 4161 (1933).

(7) α-Bromobenzalacetophenone does not react with alcoholic silver nitrate solution: N. H. Cromwell, *Chem. Revs.*, **38**, 83 (1946).