Zygadenus Alkaloids. VI.[1] Active Principles of *Zygadenus paniculatus*[2]

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Alkaloidal extracts of *Zygadenus paniculatus* have long been known to possess principles which are poisonous to livestock.[4] Comparative pharmacological studies of the crude extracts of several species of Zygadenus have shown that the extract of *Zygadenus paniculatus* is among the most toxic in the series.[5] In the course of our investigation of the zygadenus alkaloids, we have studied a sample of *Zygadenus paniculatus* and have isolated the ester alkaloids veratroylzygadenine,[6] vanilloylzygadenine,[6] zygacine,[1] neogermitrine[7,8] and neogermidine.[8] We should now like to report briefly on these findings.

In this investigation, a batch of 5.2 kg. of *Zygadenus paniculatus*[9] was processed initially by the procedure reported earlier for *Zygadenus venenosus*.[6] The yield of crude alkaloid obtained by chloroform extraction was equivalent to 0.80% of the weight of the dried plant.

The crude chloroform-extracted alkaloids were first subjected to detailed 8-plate countercurrent distribution using benzene and *M*/15 phosphate buffer at *p*H 7.1 as solvents. Veratroylzygadenine crystallized from acetone solutions of the material recovered from plates 6 to 8. Vanilloylzygadenine crystallized from acetone solutions of the alkaloids in plates 4 and 5.

The filtrates after removal of the crystalline alkaloids from plates 4 to 8 were combined and the amorphous mixture obtained upon evaporation of the solvents was designated as the organophilic fraction. The material recovered from plates 0 to 3 was designated as the hydrophilic fraction. Chromatography of the organophilic fraction yielded neogermitrine. Chromatography of the hydrophilic material led to the isolation of neogermidine and zygacine.

Acknowledgment.—The assistance of Eli Lilly and Company in gathering and extracting *Zygadenus paniculatus* is gratefully acknowledged.

Experimental

Chloroform Extraction of *Zygadenus paniculatus*.—Ground *Zygadenus paniculatus*[9] (5.2 kg., stems and flowers) was extracted with chloroform by the procedure used earlier for *Zygadenus venenosus*[6]; 44.5 g. (0.80% of the dried plant) of crude alkaloid was obtained.

Fractionation of the Chloroform-extracted Bases by Countercurrent Distribution.—The crude amorphous bases (8 g.) were fractionated by 8-plate countercurrent distribution between benzene and *M*/15 phosphate buffer at *p*H 7.1. Crystallization of the alkaloid recovered from plates 6 to 8 from acetone afforded veratroylzygadenine (1.44 g.). Purification by reprecipitation from hot dilute alcoholic acetic acid with aqueous ammonia gave prisms (1.1 g.), m.p. 267–269° dec. The melting point was not depressed by admixture of an authentic specimen of veratroylzygadenine and the infrared spectra of the two samples in chloroform were identical.

Crystallization of the alkaloid recovered from plates 4 and 5 from acetone gave vanilloylzygadenine (480 mg.). Recrystallization from alcohol gave rods (320 mg.), m.p. 257–259° dec. Mixed melting point and infrared spectral comparisons with an authentic sample of vanilloylzygadenine confirmed the identity of this product.

Isolation of Neogermitrine by Chromatography of the Organophilic Fraction.—The filtrates after removal of the crystalline products from plates 4 to 8 above were combined and evaporated to dryness *in vacuo*. The residual alkaloids (2.1 g.) were dissolved in chloroform and chromatographed on Merck sulfuric acid-washed alumina. The most easily eluted fractions crystallized from ether-petroleum ether and yielded neogermitrine (65 mg., m.p. 235–237° dec.). Characterization of the product was accomplished by mixed m.p., rotation and infrared spectral comparisons with an authentic specimen of neogermitrine.

Isolation of Neogermidine and Zygacine by Chromatography of the Hydrophilic Fraction.—The material recovered from plates 0 to 3 (3.3 g.) was dissolved in chloroform and chromatographed on Merck sulfuric acid-washed alumina. The easily eluted alkaloid crystallized from ether. Recrystallization from benzene gave neogermidine (70 mg., m.p. 220–222° dec.); mixed m.p. unchanged by admixture of authentic neogermidine and infrared spectra of two samples identical. The next fractions eluted with chloroform resisted crystallization and showed infrared absorption indicative of germine esters.

The alkaloidal fractions eluted with chloroform–methanol mixtures up to 10% methanol all showed infrared absorption indicative of the presence of zygacine. These fractions were combined in acetone (20 ml.) and treated with hydriodic acid (1.0 ml., Merck reagent). Upon rubbing, zygacine acetonide hydriodide crystallized (1.5 g., m.p. 267–269° dec.). The salt was converted to zygacine acetonide and to zygacine as described earlier.[1] The products were characterized by rotation and infrared spectral comparisons with authentic specimens.

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[1] Paper V, S. M. Kupchan, D. Lavie and R. D. Zonis, THIS JOURNAL, **77**, 689 (1954).

[2] This investigation was supported (in part) by research grants from the National Institutes of Health (RG-2553) and Research Corporation.

[3] Haffkine Institute, Bombay, India.

[4] C. E. Fleming, N. F. Peterson, M. R. Miller and L. H. Wright, *Univ. Nevada Agr. Expt. Sta. Bull.*, **101**, 1 (1921); *cf. C. A.*, **15**, 3867 (1921).

[5] O. A. Bealth, H. F. Eppson, J. H. Draize and R. S. Justice, *Wyo. Agr. Expt. Sta. Bull.*, **194**, 3 (1933); *cf. C. A.*, **28**, 802 (1934).

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

[7] J. Fried, P. Numerof and N. M. Coy, *ibid.*, **74**, 3041 (1952).

[8] S. M. Kupchan and C. V. Deliwala, *ibid.*, **74**, 3202 (1952); **76**, 5545 (1954).

[9] Plant collected in Washington in June, 1950. We are grateful to Dr. Reed Rollins, Gray Herbarium, Harvard University, for confirming the identity of the plant.

Infrared Spectra of Halogenated Acetic Esters[1]

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The effect of α-substituents on the carbonyl stretching frequency in the infrared and Raman spectra has received considerable attention. Rasmussen and Brattain[2] have studied the infrared spectra of a number of aliphatic esters and noted that an alpha electron-donor group causes a shift of the carbonyl stretching band toward longer wave lengths; an electron-withdrawing group causes the reverse effect.[3] Several studies of the Raman

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[1] This paper presents part of a thesis submitted by D. L. Christman to the Graduate School, Purdue University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] R. S. Rasmussen and R. R. Brattain, THIS JOURNAL, **71**, 1073 (1949).

[3] R. H. Gillette, *ibid.*, **58**, 1143 (1936), has shown this same effect by studying the infrared spectra of several halogenated acetic acids. The position of the carbonyl bond in these acids shifts toward shorter wave lengths in the order of CCl_3 > $CHCl_2$ > CH_2Cl CH_2Br > CH_2I.