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

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RECEIVED AUGUST 19, 1954

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]

BY E. T. MCBEE AND D. L. CHRISTMAN

RECEIVED OCTOBER 1, 1954

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 $CCl_3 > CHCl_2 > CH_2Cl\ CH_2Br > CH_2I$.

spectra of esters[4] with α-halo substituents also indicate that the carbonyl stretching frequencies of the halo esters are higher than those of the non-halogenated esters. It was expected that fluorine in the α-position would cause a marked shift, and recent studies of fluorinated esters have substantiated this expectation.[5] Thus far, no studies have been reported for esters containing several different halogen atoms in the α-position. Such data are furnished in the present investigation (Table I). Since any factor causing a decrease in the ionic character of a carbonyl bond should also cause a shortening of the C–O linkage of the ester, such stretching frequencies also are recorded.

TABLE I

INFRARED SPECTRA OF HALOGENATED ACETIC ESTERS

Ester	(C=O), cm.$^{-1}$	(C—O), cm.$^{-1}$	(C—Cl),[e] cm.$^{-1}$	(C—Br), cm.$^{-1}$
CH_3COOEt	1740	1236, 1245[b]	..	..
$CH_2BrCOOEt$	1740	1281	..	553
$CHBr_2COOEt$	1753, 1739	1268, 1278	..	583, 598[f]
$CH_2ClCOOEt$	1753	1288, 1311	697	..
$CHIFCOOEt$	1754	1275	..	..
$CHBrFCOOEt$	1754	1284	..	594[f]
$CHClFCOOEt$	1754	1276, 1290	731	..
$CHCl_2COOEt$	1758	1268–1302	719	..
$CHBrClCOOEt$	1758, 1742	1265–1297	703	582[f,g]
CCl_3COOEt	1770[c], 1761[d]	1310[c], 1266[d]	..	..
$CH_2FCOOEt$	1778, 1750	1290[a]	..	..
CHF_2COOEt	1770	1301	..	..
$CClF_2COOEt$	1770	1317[a]	731	..
CF_3COOEt	1789	1319[a]	..	

[a] Due to interference by C-F absorption, this value is estimated. [b] Shoulder. [c] H. M. Randell, R. G. Fowler, N. Fuson and J. R. Dangl, "Infrared Determination of Organic Structure," D. Van Nostrand, Co., Inc., New York, N. Y., 1949. [d] Ref. 6; R. R. Hampton and J. E. Newell, *Anal. Chem.*, 21, 914 (1949). [e] Compare with values for CH_3Cl (712 cm.$^{-1}$), CH_2Cl_2 (704 cm.$^{-1}$) and CCl_2F_2 (737 cm.$^{-1}$). [f] Broad absorption band. [g] Estimated.

In ethyl fluoroacetate, ethyl difluoroacetate, ethyl chlorofluoroacetate, ethyl bromofluoroacetate and ethyl iodofluoroacetate, a series of esters is available in which a hydrogen atom is replaced successively by fluorine. chlorine, bromine and iodine. The expected effect of a second halogen would be a shift of the carbonyl stretching band to higher frequencies, the order of the effect being $F > Cl > Br > I$. Such an effect, however, was observed only with a second fluorine substituent. The replacement of hydrogen by halogen atoms other than fluorine caused a *decrease* in the carbonyl band frequency and, surprisingly, chlorine, bromine and iodine all caused a shift of about the same magnitude.

The C–O band at 7.6–8.1 μ, on the other hand, was successively shifted to shorter wave lengths. Ethyl fluoroacetate, ethyl dibromoacetate and ethyl bromochloroacetate showed the presence of two absorption peaks in the carbonyl region.[6] Bender[7] observed this phenomenon with ethyl fluoroacetate and attributed it to a dimer, which would also explain the high boiling point of the ester.

The shift of the stretching frequency of the C–Cl band in ethyl chloroacetate to longer frequencies in the polyhalogen esters implies a shorter bond in the latter esters. The same observation is made for the C–Br band in ethyl bromoacetate as compared to polyhalogen esters.

Acknowledgment.—Financial support by the Ethyl Corporation is gratefully acknowledged. The authors are indebted to Mrs. L. H. Walsh for determining the infrared spectra.

Experimental

Infrared Spectra.—Infrared spectra were determined by use of a Perkin-Elmer model C-21 double beam recording infrared spectrometer for pure liquids in capillary cells or in sealed cells of 0.0125 or 0.029 mm. thickness. The wave length scale was accurate to within ±2 cm.$^{-1}$

Esters.—The esters used in this study were purified by rectification from a standard Todd distilling apparatus. The physical properties of the esters are given in Table II.

TABLE II

PHYSICAL CONSTANTS OF ACETIC ESTERS

Ester	B.p., °C. (mm.) Obsd.	Lit.	n^{20}D Obsd.	Lit.
CH_3COOEt	78	78[d]	1.3718	1.3718[d]
$CH_2BrCOOEt$	154	56–57 (15)[e]	1.4499	1.4489[e]
$CHCl_2COOEt$	73 (30)	158.7[h]	1.4380	1.43860[c]
$CHBr_2COOEt$	65 (5)	121 (74)[g]	1.4973	1.50167[b,g]
$CH_2ClCOOEt$	142.5	142.8[h]	1.4211	1.42162[f]
$CHIFCOOEt$	70 (14)	103 (30)[i]	1.4820	....
$CHBrFCOOEt$	68 (34)	154[j]	1.4275	....
$CHClFCOOEt$	130	128–130[k]	1.3953	1.3927[a]
$CHBrClCOOEt$	66 (12)	174[l]	1.4703	....
$CH_2FCOOEt$	46 (35)	114[d]	1.3750	1.3747[d]
CHF_2COOEt	98.5	98.5[d]	1.3465	1.3463[d]
$CClF_2COOEt$	95.5	97[m]	1.3578	....
CF_3COOEt	60.5	60.5[d]	1.3079	1.3073[d]

[a] At 25°. [b] At 12.5°. [c] W. Perkin, *J. Chem. Soc.*, **65**, 423 (1894). [d] From ref. 6. [e] M. S. Kharasch, P. S. Skell and P. Fisher, THIS JOURNAL, **70**, 1058 (1948). [f] A. Karvonen, *Ann. Acad. Sci. Fennicae*, **A10**, No. 4, 20 (1916). [g] W. Perkin, *J. Chem. Soc.*, **65**, 430 (1894). [h] From ref. 4c. [i] F. Swarts, *Chem. Zentr.*, **80**, I, 13 (1913). [j] F. Swarts, *Mem. couronnés acad. roy. Belg.*, **61**, 1 (1901). [k] J. A. Young and P. Tarrant, THIS JOURNAL, **71**, 2432 (1949). [l] H. Crompton and P. M. Triffitt, *J. Chem. Soc.*, **119**, 1874 (1921). [m] F. Swarts, *Rec. trav. chim.*, **27**, 132 (1908).

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(4) (a) O. Burkard and L. Kahovec, *Monatsh.*, **71**, 333 (1938); (b) L. Kahovec and K. W. F. Kohlrausch, *ibid.*, **74**, 115 (1942); (c) H. Cheng, *Z. physik. Chem.*, **B24**, 293 (1934); (d) R. E. Chernizkaya and J. R. Syrkin, *Compt. rend. acad. sci. URSS*, **55**, 513 (1947.) Chernizkaya and Syrkin[4d] suggest that this α-substitution of halogen decreases the ionic character of the carbonyl bond.

(5) (a) M. Hauptschein, C. S. Stokes and E. A. Nodiff, THIS JOURNAL, **74**, 4005 (1952); (b) G. Rappaport, M. Hauptschein, J. F. O'Brien and R. Filler, *ibid.*, **75**, 2695 (1953); (c) R. Filler, *ibid.*, **76**, 1376 (1954).

(6) It has been suggested by the Referee that in an inert solvent at high dilution these split bands may disappear assuming the phenomena is due to dimer formation.

(7) M. L. Bender, THIS JOURNAL, **75**, 5986 (1953).

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Reactions of Nitrate Esters. III. Evidence for Nitrogen–Oxygen Cleavage in Reductions with Hydrazine and Alkaline Hydrosulfides

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RECEIVED SEPTEMBER 3, 1954

In the reduction of nitrate esters to nitrite ion and the parent alcohols by means of alkaline hydrosulfides[2] or hydrazine,[3] it was suggested that the initial step is a nucleophilic displacement on the nitrate nitrogen atom, *viz.*

$$B: + R\text{-}O\text{-}NO_2 \longrightarrow RO^- + B{:}NO_2^+$$

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(1) Bureau of Mines, Pittsburgh, Pennsylvania.

(2) R. T. Merrow, S. J. Cristol and R. W. Van Dolah, THIS JOURNAL, **75**, 4259 (1953).

(3) R. T. Merrow and R. W. Van Dolah, *ibid.*, **76**, 4522 (1954).