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The Alkaloids of *Veratrum fimbriatum* Gray

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RECEIVED MAY 18, 1953

A chemical investigation of *Veratrum fimbriatum* Gray has yielded two new hypotensively active germine esters, germanitrine ($C_{39}H_{59}O_{11}N$), and germinitrine ($C_{39}H_{57}O_{11}N$), as well as neogermitrine, jervine and pseudojervine. In addition, the alkaloidal ester, veratroylzygadenine, previously isolated from *Zygadenus venenosus* Wats was found to be present in this species of veratrum. On hydrolysis, the triester germanitrine affords germine, acetic acid, tiglic acid and (*l*)-α-methylbutyric acid. On methanolysis, germanitrine readily loses a labile acetyl group yielding germanidine ($C_{37}H_{57}O_{10}N$). The hydrolysis of the triester germinitrine, yielded germine, acetic acid, tiglic acid and angelic acid.

Germanitrine and germinitrine,[1] two new hypotensively active germine ester alkaloids, have been isolated from the hitherto uninvestigated species, *Veratrum fimbriatum* Gray. In addition, the isolation procedure yielded the known veratrum alkaloids, neogermitrine, jervine and pseudojervine, as well as veratroylzygadenine, an ester alkaloid previously isolated from *Zygadenus venenosus* Wats.[3]

The extraction procedure employed in this investigation was essentially the same as that reported previously in our work on *Veratrum viride* Ait.[4] The total "amorphous bases" thus obtained were subjected to a preliminary fractionation by an 8-plate countercurrent distribution using benzene–2 *M* acetate buffer *p*H 5.5 as the solvent system. The results of the distribution showed the largest portion of the material to be in tubes 0–1 with the remainder distributed fairly evenly in the other tubes. Pharmacological screening[5] for hypotensive activity revealed high activity in tubes 0–1 and tubes 2–5.

The material recovered from tubes 0–1 was redistributed on a 24-plate countercurrent machine with 0.5 *N* sodium acetate buffer *p*H 5.0—benzene–cyclohexane 40:60 as the solvent system. On careful fractional crystallization of the material recovered from tubes 4–14 from acetone–water, two new alkaloids were obtained which after characterization were named **germanitrine** and **germinitrine.** Alkaline hydrolysis of germanitrine yielded germine, acetic acid, (*l*)-α-methylbutyric acid and tiglic acid. On the basis of the analytical data and the hydrolysis products the empirical formula $C_{39}H_{59}O_{11}N$ was established. On methanolysis, germanitrine was converted to a diester, germanidine ($C_{37}H_{57}O_{10}N$), by the loss of the labile acetyl group.

Alkaline hydrolysis of germinitrine afforded germine, acetic acid, tiglic acid and angelic acid. On the basis of the analytical data and the hydrolysis products, the empirical formula $C_{39}H_{57}O_{11}N$ was established.

Germanitrine and germinitrine represent the first naturally occurring germine esters in which one or more of the acid conjugates is angelic or tiglic acid, the *cis–trans* isomers of α-methylcrotonic acid. The possibility that the less stable isomer, angelic acid, was the only isomer occurring in germinitrine and germanitrine and that the tiglic acid isolated was the result of isomerization during the saponification was considered, especially in view of the discrepancies reported for cevadine.[6] The uniformity of results obtained on duplication of our initial saponification and the failure of either angelic or tiglic acid to isomerize under the conditions of hydrolysis used in our experiments indicates that the acids isolated are the true conjugates.

The infrared spectra of germanitrine and germinitrine, in addition to showing similarities common to the known germine triesters, show weak absorption at 6.17 μ which may be attributed to the ethylenic bonds of tiglic and angelic acids. As would be expected, the strong carbonyl band of these esters has been shifted slightly toward the longer wave lengths due to conjugation of the ethylenic bond with the ester carbonyl. This shift is best exhibited in the spectrum of germanidine where two sharp bands are evident, one occurring at 5.80 μ due to the α-methylbutyric acid ester carbonyl and one at 5.89 μ due to the tiglic acid ester carbonyl.

The material recovered from tubes 2–5 of the original 8-plate separation was redistributed on a 24-plate countercurrent machine using 2 *M* acetate buffer *p*H 5.5 and benzene. The triester, neogermitrine, was obtained by crystallizing the material recovered from tubes 6–10 from acetone–water. On dissolving the material recovered from tubes 11–17 in acetone, an alkaloid was obtained which on alkaline hydrolysis yielded veratric acid and an amorphous alkamine. The physical constants, hydrolysis products and characteristic infrared spectrum were in close agreement with those recently reported for veratroylzygadenine.[3] On comparison with an authentic sample kindly supplied by Dr. S. M. Kupchan, the two were found to be identical. The occurrence of this zygadenine conjugate in the veratrum species, along with the recently reported isolation of germine esters from *Zygadenus venenosus*,[11] establishes a close chemical

(1) A preliminary report on this investigation appeared in a previous communication.[2]

(2) M. W. Klohs, M. D. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek, THIS JOURNAL, **74**, 4473 (1952).

(3) S. M. Kupchan and C. V. Deliwala, *ibid.*, **75**, 1025 (1953).

(4) M. W. Klohs, R. Arons, M. D. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek, *ibid.*, **74**, 5107 (1952).

(5) The pharmacological studies were carried out in the Pharmacology Department, Boston University School of Medicine, Boston Mass., under the direction of Dr. G. L. Maison. We should like to express our gratitude to Dr. Maison for his invaluable coöperation during the course of this work.

(6) Cevadine, a constituent of *Veratrum sabidilla* and a monoester, has been variously reported to yield tiglic acid[7] or angelic acid[8-10] on hydrolysis.

(7) C. R. Wright and A. P. Luft, *J. Chem. Soc.*, **33**, 338 (1878).

(8) F. B. Ahrens, *Ber.*, **23**, 2700 (1890).

(9) E. Bosetti, *Arch. Pharm.*, **221**, 81 (1883).

(10) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **35**, 1270 (1952).

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

relationship between these two distinct genera of the *liliaceae* family.

The hypotensively inactive fractions A and B obtained in the initial fractionation of the crude plant extract yielded substantial quantities of jervine and pseudojervine, respectively, by direct crystallization. The well-known veratrum alkaloids isorubijervine, rubijervine, veratramine and veratrosine, if present, occurred only in trace amounts.

Pharmacology.—The hypotensive activity[12,13] of germanitrine, germanidine, germinitrine and veratroylzygadenine was found to be 0.12 μg. [0.11–0.14], 0.77 μg. [0.46–2.3], 0.41 μg. [0.36–0.49] and 1.1 μg. [0.95–1.4], respectively.

Experimental[14]

Extraction of the Roots and Rhizomes of *Veratrum fimbriatum* Gray.—Ground dried roots and rhizomes of *Veratrum fimbriatum* (24.5 kg.), collected in northern California during the summer of 1950, were extracted in the same manner as previously reported in our investigation of *Veratrum viride* Ait.[4] As in this previous work, three main fractions corresponding to fraction A, fraction B and the "amorphous bases" were obtained: fraction A, 130 g. (as sulfate ppt.); fraction B, 50.5 g.; the amorphous bases, 453 g.

Preliminary Fractionation of the Amorphous Bases by an 8-Plate Countercurrent Distribution.—The amorphous bases (30 g.) were distributed using Craig's fundamental procedure employing eight 3-l. separatory funnels. Benzene–2 *M* acetate buffer *p*H 5.5, 1500 ml. in each phase, was used as the solvent system.[15] On completion of the distribution, the contents of each funnel were adjusted to *p*H 7.2 with 6 *N* sodium hydroxide; the temperature was maintained below 20° by the addition of ice. The layers were separated and the aqueous phases extracted with chloroform (250-ml. portions) until a negative test was obtained with Wagner reagent. The benzene and chloroform extracts of each funnel were combined and washed once with water (150 ml.). The extracts were then dried over sodium sulfate, evaporated to dryness *in vacuo* and the weight of the material obtained from each funnel was recorded. The following tubes were then combined: tubes 0–1 (14.0 g.), tubes 2–5 (6.85 g.), tubes 6–8 (6.55 g.).

The Isolation of Germanitrine and Germinitrine.—The material from tubes 0–1 (8.5 g.) was redistributed on a 24-plate countercurrent machine using benzene–cyclohexane 40:60 and 0.5 *N* sodium acetate buffer *p*H 5.0 as the solvent system. The material was recovered from each tube by the procedure described above. The results when plotted showed a main peak at tube 9. The material recovered from tubes 4–14 (3.43 g.) was dissolved in acetone. On diluting the solution with water, germanitrine (0.64 g.) was obtained as heavy needles. On further dilution and standing, germinitrine (0.30 g.) separated as heavy irregular prisms.

Germanitrine.—Germanitrine was recrystallized several times from dilute acetone, m.p. 228–229°, $[\alpha]^{24}D$ −61 ± 2° (*c* 1.0 in pyridine), 0.0 ± 2° (*c* 1.15 in $CHCl_3$). For analysis the sample was dried at 110° (2 mm.) to constant weight.

Anal. Calcd. for $C_{39}H_{59}O_{11}N$: C, 65.25; H, 8.28; N, 1.95; equiv. wt., 717.87. Found: C, 65.30; H, 8.26; N, 1.99; equiv. wt., 721.[16]

In a volatile acid determination, 12.0 mg. required 4.68 ml. of 0.00959 *N* $Na_2S_2O_3$ or 2.66 equivalents.

Germanitrine Picrate.—Germanitrine (46 mg.) was dissolved in a minimum amount of 5% acetic acid and a saturated aqueous solution of picric acid was added until no further precipitate was formed. The resulting powder was recrystallized twice from dilute acetone yielding irregular plates, m.p. 240–241° dec. For analysis the sample was dried at 110° (2 mm.) to constant weight.

Anal. Calcd. for $C_{39}H_{59}O_{11}N \cdot HOC_6H_2(NO_2)_3$: C, 57.07; H, 6.60. Found: C, 56.68; H, 6.52.

Hydrolytic Cleavage of Germanitrine to Germine, Acetic Acid, (*l*)-α-Methylbutyric Acid and Tiglic Acid.—Germanitrine (0.5 g.) was refluxed for one hour with 1 *N* methanolic sodium hydroxide (10 ml.). At the end of this time, the solution was cooled, diluted with an equal volume of water and extracted six times with equal volumes of chloroform. The chloroform extracts were dried over sodium sulfate and evaporated *in vacuo* to dryness. The residue was dissolved in methanol (15 ml.) and concentrated by boiling to 4 ml. Germine (127 mg.) separated as heavy prisms. After several recrystallizations from methanol, the sample melted at 221.5–223°, $[\alpha]^{25}D$ 4.5 ± 2 (*c* 1.0 in 95% ethanol). For analysis the sample was dried to constant weight at 130° (2 mm.).

Anal. Calcd. for $C_{27}H_{43}O_3N$: C, 63.63; H, 8.50. Found: C, 63.78; H, 8.07.

Germine Pentaacetate.—The alkamine (100 mg.) was converted to the pentaacetyl derivative according to a method previously described.[17] Germine pentaacetate crystallized as prisms from chloroform–ether, m.p. 257.5–258.5° dec., $[\alpha]^{24}D$ −88.8 ± 2 (*c* 1.07 in pyridine). A mixed melting point with an authentic sample of germine pentaacetate gave no depression.

The Identification of Acetic, (*l*)-α-Methylbutyric and Tiglic Acids.—After extraction of the alkamine, the acids were recovered from the aqueous layer and converted to their *p*-phenylphenacyl esters by the method previously reported.[4] The *p*-phenylphenacyl esters were separated by chromatography on a silicic acid–Celite column using benzene–petroleum ether (69–74°) 50:50 as the developing solvent.[18] Four main bands were visible under ultraviolet light. The material in each band was recovered by sectioning and eluting the extruded column. The lowest band yielded unreacted *p*-phenylphenacyl bromide, m.p. 125°. Immediately above it, material was obtained which was crystallized from dilute alcohol yielding needles, m.p. 68.5–69.5°. The infrared spectra of this substance and that of *p*-phenylphenacyl α-methylbutyrate were identical. For analysis the sample was dried at 25° (2 mm.) to constant weight.

Anal. Calcd. for $C_{19}H_{20}O_3$: C, 77.01; H, 6.80. Found: C, 77.10; H, 6.88.

The next fraction was crystallized from dilute alcohol yielding feathery needles, m.p. 104.0–104.5°.[19] The infrared spectra of this substance and that of an authentic sample of *p*-phenylphenacyl tiglate were identical. A mixed melting point showed no depression. For analysis, the sample was dried at 25° (2 mm.) to constant weight.

Anal. Calcd. for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16. Found: C, 77.66; H, 6.40.

The topmost band yielded material which crystallized as needles from dilute alcohol, m.p. 109.5–110.5°. A mixed melting point with a sample of *p*-phenylphenacyl acetate gave no depression. The infrared spectra were identical. For analysis the compound was dried at 80° (2 mm.) to constant weight.

Anal. Calcd. for $C_{16}H_{14}O_3$: C, 75.58; H, 5.55. Found: C, 75.55; H, 5.73.

Determination of Optical Configuration of α-Methylbutyric Acid.—Germanitrine (249 mg.) was hydrolyzed by refluxing for one hour in a solution composed of 3.5 ml. of 0.3 *N* sodium hydroxide in methanol plus 3.5 ml. of water. The methanol was distilled off *in vacuo* and the remaining water solution was acidified with 2 ml. of 25% *p*-toluenesulfonic acid. The acids were then steam distilled into an ice-cooled receiver *in vacuo*[20] (total volume of distillate and

(12) E. D. Swiss and G. L. Maison, *Federation Proc.*, **1**, 395 (1952).

(13) Expressed as micrograms per kilogram of anesthetized dog per minute required for a ten-minute intravenous infusion to lower the mean arterial blood pressure 30% when administered according to the method of G. L. Maison and J. W. Stutzman. The bracketed numbers express the 95% confidence limits.

(14) All melting points are corrected. The melting points of the alkaloids and their derivatives were determined in evacuated capillaries.

(15) In all countercurrent distributions, the lower phase was the moving phase.

(16) All equivalent weights were determined by titration with perchloric acid in glacial acetic acid.

(17) Josef Fried, H. L. White and O. Wintersteiner, THIS JOURNAL, **72**, 4621 (1950).

(18) J. G. Kirchner, A. H. Prater and A. J. Haagen-Smit, *Ind. Eng. Chem., Anal. Ed.*, **18**, 31 (1946).

(19) S. W. Pelletier and William L. McLeish THIS JOURNAL, **74**, 6292 (1952).

(20) A. Elek and R. A. Harte, *Ind. Eng. Chem.*, **8**, 267 (1936).

washings, 20 ml.). A 1-ml. aliquot required 2.15 ml. of 0.0207 *N* sodium hydroxide for neutralization or 2.6 equivalents of volatile acid. The optical rotation of the distillate determined in a 2 dm. tube was −7.5°. This is equivalent to 29.8 mg. of (*l*)-α-methylbutyric acid, $[\alpha]^{24}D$ −25°. Calcd. for germanitrine 35.2 mg.

Methanolytic Degradation of Germanitrine to Germanidine.—Germanitrine (0.5 g.) was dissolved in methanol (20 ml.) and allowed to stand for 60 hours. The methanol was then removed *in vacuo* and the residue was crystallized from dilute acetone yielding flat needles (0.22 g.), m.p. 221–222°, $[\alpha]^{24}D$ −30.3 ± 2° (*c* 1.5 in 95% EtOH), −4.1 ± 2° (*c* 1.0 in pyridine). For analysis the sample was dried to constant weight at 110° (2 mm.).

Anal. Calcd. for $C_{37}H_{57}O_{10}N$: C, 65.75; H, 8.73; equiv. wt., 675.84. Found: C, 65.66; H, 8.61; equiv. wt., 672.

In a volatile acid determination 10.06 mg. required 3.15 ml. of 0.009316 *N* $Na_2S_2O_3$ or 1.97 equivalents of acid.

Germinitrine.—Germinitrine was recrystallized from dilute acetone to a constant melting point, yielding irregular prisms, m.p. 175°, $[\alpha]^{24}D$ −36.0 ± 2° (*c* 1.12 in pyridine), +7.8° (*c* 1.35 in $CHCl_3$).

For analysis the sample was dried to constant weight at 130° (2 mm.).

Anal. Calcd. for $C_{39}H_{57}O_{11}N$: C, 65.43; H, 8.03; N, 1.96; equiv. wt., 715.85. Found: C, 65.35; H, 8.27; N, 1.61; equiv. wt., 722.

In a volatile acid determination 14.47 mg. required 4.84 ml. of 0.01 *N* $Na_2S_2O_3$ or 2.32 equivalents of acid.

Germinitrine Picrate.—The picrate was prepared by the same method used for germanitrine picrate yielding plates, m.p. 238° dec. For analysis the sample was dried to constant weight at 110° (2 mm.).

Anal. Calcd. for $C_{39}H_{57}O_{11}N \cdot HOC_6H_2(NO_2)_3$: C, 57.19; H, 6.40. Found: C, 57.17; H, 6.66.

Hydrolytic Cleavage of Germinitrine to Germine, Acetic Acid, Angelic Acid and Tiglic Acid.—Germinitrine (0.5 g.) was subjected to hydrolysis under the same conditions used for the hydrolysis of germanitrine.

Germine (150 mg.) was obtained on crystallization from methanol, m.p. 218.5–220°, $[\alpha]^{24}D$ 4.6 ± 2° (*c* 1.0 in 95% EtOH).

For analysis the compound was dried to constant weight at 140° (2 mm.).

Anal. Calcd. for $C_{27}H_{43}O_8N$: C, 63.63; H, 8.50. Found: C, 63.57; H, 8.60.

Germine Pentaacetate.—Germine (50 mg.) was converted to the pentaacetyl derivative, m.p. 257.5–258.5°, $[\alpha]^{24}D$ −87.9 ± 2° (*c* 1.0 in pyridine). A mixed melting point with an authentic sample gave no depression.

The Identification of Acetic, Angelic and Tiglic Acids.—The acids resulting from the hydrolysis of germinitrine were converted to their *p*-phenylphenacyl esters and separated chromatographically in the same manner as that described above for germanitrine.

The material from the zone immediately above the unreacted *p*-phenylphenacyl bromide was crystallized from dilute alcohol, yielding platelets, m.p. 88.6°.[19] The infrared spectra of this substance and that of an authentic sample of *p*-phenylphenacyl angelate were identical. A mixed melting point showed no depression. For analysis the sample was dried to constant weight at 56° (2 mm.).

Anal. Calcd. for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16. Found: C, 77.89; H, 6.35.

The zone above the *p*-phenylphenacyl angelate yielded material which crystallized as feathery needles from dilute alcohol, m.p. 103.2–104.0°. The infrared spectra of this substance and *p*-phenylphenacyl tiglate were identical.[21] A mixed melting point showed no depression. For analysis the sample was dried to constant weight at room temperature.

Anal. Calcd. for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16. Found: C, 77.48; H, 6.24.

The topmost band yielded *p*-phenylphenacyl acetate, m.p. 110.2–110.6°. The infrared spectrum of this sample was identical with that of an authentic sample of *p*-phenylphenacyl acetate. A mixed melting point showed no depression. For analysis the sample was dried to constant weight at room temperature.

Anal. Calcd. for $C_{16}H_{14}O_3$: C, 75.58; H, 5.55. Found: C, 75.57; H, 5.69.

Attempted Isomerization of Tiglic and Angelic Acids.—In separate experiments, 100 mg. each of tiglic and angelic acid were subjected to the conditions used in the hydrolysis of germanitrine and germinitrine. In each case the acids recovered upon acidification and ether extraction of the aqueous layer exhibited no melting point depression indicating that no appreciable isomerization had occurred.

The Isolation of Veratroylzygadenine and Neogermitrine.—The material from tubes 2–5 (8.5 g.) of the 8-plate countercurrent distribution was redistributed on a 24-plate countercurrent machine using benzene–2 *M* acetate buffer, *p*H 5.5 as the solvent system.

Veratroylzygadenine.—The material from tubes 11–17 (2.04 g.) was dissolved in acetone. On standing, a white powder separated (0.38 g.) which when recrystallized from absolute ethanol yielded platelets, m.p. 264–265°, $[\alpha]^{24}D$ +34 ± 2° (*c* 0.97 in pyridine), −27.6 ± 2° (*c* 0.87 in $CHCl_3$). For analysis the sample was dried at 110° (2 mm.) to constant weight.

Anal. Calcd. for $C_{36}H_{51}O_{10}N$: C, 65.73; H, 7.82. Found: C, 65.62; H, 7.79.

On admixture with an authentic sample of veratroylzygadenine obtained from *Zygadenus venenosus*, no melting point depression was observed. The infrared spectra of the two samples were identical.

Neogermitrine.—The material (2.41 g.) from tubes 6–10 was crystallized from dilute acetone yielding clusters of needles (1.12 g.), m.p. 234°, $[\alpha]^{24}D$ −77.3 ± 2° (*c* 1.0 in pyridine). The sample was identified as neogermitrine by comparison with an authentic sample.

Isolation of Jervine from Fraction A.—Fifty-eight grams of this fraction was processed for jervine according to the method of Jacobs and Craig[22] yielding jervine (36 g.), m.p. 244–245°, $[\alpha]^{24}D$ −149 ± 2° (*c* 1.0 in 95% EtOH). The identity was confirmed by comparison with an authentic sample.

Isolation of Pseudojervine from Fraction B.—Ten grams of fraction B was dissolved in boiling methanol (70 ml.). A fine white powder settled out yielding 5.2 g. of crude pseudojervine. On further purification by the method of Jacobs and Craig,[23] a sample of pure pseudojervine was obtained, m.p. 301° dec., $[\alpha]^{24}D$ −131 ± 2° (*c* 1.0 in EtOH–$CHCl_3$ 1:3). The identity was confirmed by comparison with an authentic sample. Attempts to obtain crystalline fractions from the mother liquors by chromatography on an alumina column were unsuccessful.

Acknowledgment.—The authors are indebted to Dr. Adalbert Elek for the microanalyses; to M. Robinson, C. H. Stimmel and B. Crepeau for the optical rotations, infrared spectra and equivalent weight determinations, and to H. DeVorkin for the pilot plant extraction of the ground roots and rhizomes of *Veratrum fimbriatum*.

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(21) A comparison of the infrared spectra of the *p*-phenylphenacyl esters of these two isomeric acids shows *p*-phenylphenacyl tiglate to have strong absorption at 7.97 μ and moderate absorption at 9.2 μ. These bands are absent in the spectrum of *p*-phenylphenacyl angelate.

(22) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **160**, 555 (1945).

(23) W. A. Jacobs and L. C. Craig, *ibid.*, **155**, 565 (1944).