

Alkaloids from *Veratrum album subsp. lobelianum* (BERNH.)
Suessenguth. XXI.

The Structure Elucidation of Two Novel Esters of Germine

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*Dedicated to Professor Dr. Dr. h. c. mult. Kurt Mothes in honour
of his 70th birthday*

By chemical and physicochemical methods it has been evidenced the novel, amorphous ester alkaloid isolated from *Veratrum album subsp. lobelianum* (BERNH.) *Suessenguth* to be 3-acetyl-15-veratroylgermine. Further novel alkaloid present in this plant was shown to be 15-veratroylgermine. So far, esters of germine with veratric acid have not been reported.

In our preceding paper [1] we reported the isolation of a novel, amorphous ester alkaloid from *Veratrum album subsp. lobelianum* (BERNH.) *Suessenguth*. This alkaloid has been found, together with protoveratrine A, germitetrine and veratroylzygadenine in the ester alkaloid fraction [2]. Acetylveratroylgermine designated originally amorphous alkaloid [1] $[\alpha]_D^{25} +6.6^\circ$ (chloroform) has, according to elemental analysis, molecular formula $C_{38}H_{53}NO_{12}$ (M 715.84). The ultraviolet spectrum of this substance displays maxima at 291 nm ($\log \epsilon$ 3.75), 260 nm ($\log \epsilon$ 4.07) and 218 nm ($\log \epsilon$ 4.35) indicative of substituted benzene ring. The infrared spectrum shows peaks at 1520 and 1610 cm^{-1} (aromatic ring), 1725 cm^{-1} (ester of veratric acid) and 1750 cm^{-1} (ester of acetic acid). Peaks at 2760, 2780, 2815 and 2840 cm^{-1} (Bohlman bands) evidence the presence of transquinolidine skeleton [3] in the molecule of this alkaloid. The NMR spectrum reveals signals at δ 1.00 p.p.m. (singlet, 3H, $C_{(19)}$ angular methyl group), 1.17 (singlet, 3H, $C_{(21)}$ *tert*-methyl group), 1.10 (doublet, $J = 7.0$ cps, 3H, $C_{(25)}$ *sec*-methyl group in axial position [4], 3.87 (singlet, 6H, two methoxy groups), and 2.05 (singlet, 3H, acetyl group).

Upon saponification with methanolic sodium hydroxide acetylveratroylgermine affords acetic acid which was determined as α -naphthylacetamide [1], veratric acid and germine, the latter being identified by its m.p., optical rotation, R_F value and mass spectrum. After standing in aqueous methanol [5] for 24 hours acetylveratroylgermine loses acetic acid to give veratroylgermine.

To the further amorphous alkaloid of $[\alpha]_D^{25} -2.2^\circ$ (chloroform) obtained from the ester alkaloid fraction the molecular formula $C_{36}H_{51}NO_{11}$ (M 673.80) was assigned according to the elemental analysis. The ultraviolet spectrum of this substance is similar to that of acetylveratroylgermine, the maxima being at 292 nm ($\log \epsilon$ 3.81), 262 nm ($\log \epsilon$ 4.13) and 219 nm ($\log \epsilon$ 4.47) indicating thus the presence of veratric acid in the structure of this alkaloid. Similarly, the infrared spectrum

reveals peaks at 1525 and 1620 cm^{-1} (aromatic ring), 1730 cm^{-1} (ester of veratric acid) and 3320–3350 cm^{-1} (hydroxy groups). The NMR spectrum shows signals at δ 0.97 p.p.m. (singlet, 3H, $\text{C}_{(19)}$ methyl group), 1.17 (singlet, 3H, $\text{C}_{(21)}$ methyl group), 1.10 (doublet, $J = 7.0$ cps, 3H, secondary $\text{C}_{(27)}$ methyl group), 3.87 (singlet, 6H, two methoxy groups). Protons due to the acetyl group are not present. When saponified with methanolic potassium hydroxide, this alkaloid furnishes veratric acid and germine, in the mass spectrum of which the fragment of m/e 112, characteristic of cevane type alkaloids [6] is encountered together with the molecular ion peak.

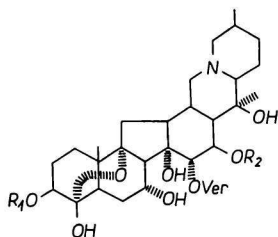
Further compound isolated from this hydrolysis is isogerminine; its ORD curve displays a negative Cotton effect characteristic of $\text{C}_{(4)}$ steroidal ketones [7]. Its mass spectrum reveals — besides the molecular ion peak at m/e 509 — the parent fragment at m/e 112.

Veratric acid was found in the acidic portion after saponification from which it was extracted with ether and purified by sublimation. Its infrared spectrum was superimposable with that of authentic sample and also the mixed melting point does not show any depression.

Veratroylgermine obtained by methanolysis of veratroylacetylgermine was found to be identical with the amorphous substance separated chromatographically from the mixture of ester alkaloids (infrared and NMR spectra, R_F value).

As evident, both alkaloids are esters of germine having the veratric acid attached to the same carbon atom. The point of attachment was inferred as follows. Acetylveratroylgermine does not undergo oxidation with periodic acid and consequently, no α -diol grouping is present. Moreover, neither borate complexes, nor acetonides were formed as is the case with other veratrum alkaloids possessing a *cis*-diol grouping. Basing upon these arguments, one hydroxyl of germine is acylated at $\text{C}_{(15)}$ and the other at $\text{C}_{(3)}$ or $\text{C}_{(4)}$. The consumption of periodic acid for oxidizing veratroylgermine was found to be 1 mole equivalent, thereby evidencing the α -diol grouping in ring A. From the above it is apparent that the hydroxy group at $\text{C}_{(15)}$ is esterified with veratric acid and this alkaloid is to be assigned the structural formula I (15-veratroylgermine).

The position of the acetyl group of acetylveratroylgermine was adduced from the difference of molecular optical rotation between acetylveratroylgermine $[M]_D + 47^\circ$ and veratroylgermine $[M]_D - 15^\circ$. The difference has a positive value ($\Delta[M]_D + 62^\circ$)



- I $R_1 = R_2 = \text{H}$
 II $R_1 = \text{Ac}; R_2 = \text{H}$
 III $R_1 = R_2 = \text{Ac}$

comparable with that reported by *Kupchan* [8] who acylated 3 β -hydroxy group of germine derivatives; acetylveratroylgermine is, therefore, represented by the structural formula *II* (3-acetyl-15-veratroylgermine).

When acetylating acetylveratroylgermine with acetic anhydride in pyridine at room temperature only one hydroxy group becomes acetylated and 3,16-diacetyl-15-veratroylgermine *III* could be isolated. In the NMR spectrum of the latter signals at δ 1.96 and 2.02 p.p.m. (two singlets, 6H, two acetyl groups) appeared. The 0.06 p.p.m. shift of the C₍₂₁₎ methyl group singlet to the higher field indicates that acetylation at C₍₁₆₎ hydroxyl took place. Upon methanolysis at room temperature diacetylveratroylgermine affords 3-acetyl-15-veratroylgermine (*II*) and 15-veratroylgermine (*I*).

Experimental

Melting points were measured on a micro hot-stage apparatus (according to Boëtius) and are corrected. Optical rotations were taken with a Zeiss Winkel polarimeter in ethanol and chloroform, infrared spectra were recorded with a double-beam UR-10 spectrophotometer, ultraviolet spectra and ORD curves were run with a UV/ORD JASCO 5 spectrophotometer. NMR spectra were measured with a Tesla 487 apparatus at 80 MHz in deuteriochloroform, hexamethyldisiloxane being the internal reference, mass spectra with the MCh 1306 apparatus. Whatman 1 paper impregnated with formamide-formic acid (1 : 1) was the carrier for chromatography in the solvent system benzene-chloroform-acetic acid (50 : 50 : 1). Chromatographic spots were visualized with Dragendorff reagent. Thin layer chromatography was performed on alumina Woelm in benzene-ethanol (19 : 1). Detection of spots as above, *R_F* values in this paper refer to paper chromatography. For column chromatography alumina neutral was used.

The ester alkaloid mixture (2 g) from the benzene extract of the plant separated by countercurrent distribution according to previously described process [1] was chromatographed on alumina (60 g, grade IV, 10 ml fractions) with benzene and benzene-ethanol (98 : 2). Benzene eluates 10-29 contain 100 mg of a substance which was rechromatographed in the same way.

The *R_F* value of the amorphous substance thus obtained was found to be 0.55, [α]_D + 6.6° (*c* 0.81, chloroform).

For C₃₈H₅₃NO₁₂ (715.8) calculated: 63.76% C, 7.46% H, 1.95% N; found: 63.63% C, 7.55% H, 1.81% N.

Eluates 40-43 (benzene with 2% ethanol) were evaporated and afforded a further alkaloid (50 mg) of *R_F* 0.14 and [α]_D -2.2° (*c* 0.88, chloroform).

For C₃₆H₅₁NO₁₁ (673.8) calculated: 64.17% C, 7.63% H, 2.07% N; found: 63.98% C, 7.59% H, 1.91% N.

The saponification of 15-veratroylgermine

15-Veratroylgermine (115 mg) was dissolved in methanol (18 ml) and NaHCO₃ (115 mg) in water (10 ml) was added. The solution was refluxed for one hour, then methanol was distilled off under reduced pressure and the residue was diluted with water and extracted with chloroform (10 × 10 ml). Chloroform was recovered and the residue (50 mg) was recrystallized from chloroform to yield crystals (20 mg), m.p. 176°C, [α]_D +3.0° (*c* 0.72, ethanol). In its spectrum molecular ion peak M⁺ at *m/e* 509 and the parent peak at *m/e* 112 are of importance. The water layer remaining after the extraction with chloro-

form was acidified with 0.1 N sulfuric acid and exhaustively extracted with ether. The extract was washed with water, dried with anhydrous sodium sulfate and evaporated to dryness. Yield 14 mg was purified by sublimation to afford 10 mg of substance, m.p. 182°C (veratric acid).

Mother liquors after crystallization were evaporated to dryness (30 mg) and chromatographed on alumina (5 g). Fractions 2 ml each, solvent system chloroform—ethanol 9 : 1, chloroform—ethanol 1 : 1. From the last chloroform—ethanol 1 : 1 eluates crystalline compound (6.5 mg), m.p. 226—227°C was obtained. $M^+ m/e$ 509, parent fragment m/e 112; $[\alpha]_{265}^D + 535^\circ$, $[\alpha]_{298}^D - 230^\circ$, $[\alpha]_{340}^D + 52^\circ$.

The saponification of 3-acetyl-15-veratroylgermine

The amorphous substance (70 mg) was refluxed on a steam bath for 15 minutes with 1% methanolic sodium hydroxide (5 ml) solution and diluted with water (5 ml). Methanol was removed *in vacuo*, water was added and the base was extracted with chloroform. Germine and isogermine were obtained from chloroform as described before. The water layer, containing acetic acid and veratric acid was acidified with 0.1 N sulfuric acid and worked up according to a previously published paper [1].

Acetylation of veratroylacetylgermine

Compound II (30 mg) in pyridine (1 ml) and acetic anhydride (1 ml) was allowed to stand at room temperature for 24 hours. The acetylation mixture was distilled off under reduced pressure, the substance was dissolved in chloroform and purified by filtering through acidic alumina. The amorphous residue thus obtained yielded 21 mg; $[\alpha]_D^{26} - 4.5$ (c 0.72, ethanol).

Methanolysis of 3-acetyl-15-veratroylgermine

The title compound (50 mg) was dissolved in methanol (3.5 ml), then distilled water (1.6 ml) was added and the reaction mixture was kept standing at room temperature for 20 hours. Methanol was distilled off under diminished pressure, the solution was diluted with water (10 ml), made alkaline with ammonia, extracted with chloroform and worked up in usual manner to afford 15-veratroylgermine (35 mg).

Periodic acid oxidation

To veratroylgermine (16 mg) dissolved in 5% acetic acid (1 ml) 0.5% sodium periodate (10 ml) was added and the volume was set to 25 ml with distilled water; this solution was allowed to stand 5 hours at room temperature, then 2 ml were withdrawn and titrated according to [8]. The sodium periodate consumption was as follows: veratroylgermine 0.8 mole equivalents, veratroylzygadenine 1.9 mole equivalents and acetylveratroylgermine none.

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