

ALKALOIDS OF *Zygadenus sibiricus*.

THE STRUCTURE OF VERAZININE

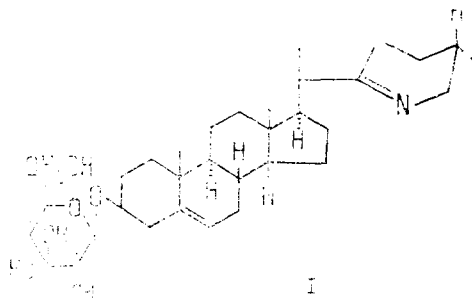
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The alkaloids verazine, veratoylzygadenine, a base with mp 224-226°C, and a new alkaloid verazinine have been isolated from *Zygadenus sibiricus* (L.) A. Gray. It has been established by chemical and spectral methods that verazinine is verazine 3-O-β-D-glycopyranoside.

We have investigated the alkaloid composition of the plant *Zygadenus sibiricus* (L.) A. Gray, growing in the village of Bogdarin Buryat ASSR. The genus *Zygadenus* is represented by approximately 15 species, of which one species is distributed over the southeastern European part of the USSR, in Siberia, and in the Far East (apart from Kamchatka, Sakhalin, and the Kurile Islands) [1]. The epigeal part of the plant in the state of incipient flowering contains a total of 0.44% of alkaloids.

By separating the ethereal fraction of the total alkaloids according to basicities, we isolated verazine, veratoylzygadenine [2-6], and a base with mp 224-226°C, and from the chloroform fraction we obtained a new alkaloid, verazinine, C₃₃H₅₃NO₆ (I), M+559. Verazinine forms a pentaacetyl derivative, M+ 769. The IR spectrum of verazinine has absorption bands at 3430 cm⁻¹ (OH) and 1655 cm⁻¹ (C=N), and a broad absorption band at 1030-1105 cm⁻¹ that is characteristic for glycoalkaloids [7]. The hydrolysis of verazinine gave an aglycone with mp 173-175°C and D-glucose (PC and GLC). The aglycone proved to be identical with verazine (melting point, IR spectrum), a sample of which was kindly given to us by N. V. Bondarenko. Consequently, verazinine is a glycoalkaloid of verazine [2, 3]. The determination of molecular rotation differences according to Klyne's rule [8] showed that in verazinine the D-glucose residue was attached to verazine by a β-glycosidic bond. Thus, verazinine has the structure of 3-O-β-D-glycopyranosylverazine (I).



EXPERIMENTAL

To separate the mixtures and identify the alkaloids we used type KSK silica gel. IR spectra were taken on a UR-20 spectrophotometer in tablets with KBr, PMR spectra on a JNM-4H 100/100 MHz instrument (CDCl₃; TMS was used as internal standard; δ scale), and mass spectra on an MKh-1310 mass spectrometer.

Isolation and Preparation of the Total Alkaloids of the Epigeal Part of *Zygadenus sibiricus*. The air-dry and comminuted plants (3.3 kg) were moistened with a 10% solution of ammonia, charged into a percolator, and covered with chloroform for 2 h, 13 such extractions being made. The concentrated and combined extracts were treated with 5% sulfuric acid, and

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then the acid solution was made alkaline with 25% ammonia and the alkaloids were extracted with ether (4.4 g) and with chloroform (5.8 g).

Veratroylzygadenine, and the Base with mp 224-226°C. The combined ether-soluble material (4.4 g) was dissolved in benzene and separated by means of acetate buffer solution at pH 4.05. The buffer solution was taken in an amount of 10 ml of each fraction, and 10 fractions were obtained. Then the combined alkaloids from the benzene solution were extracted with 5% acetic acid. The acidic benzene solution was made alkaline with ammonia, the solvent was distilled off, and the residue was left in acetone. The acetone deposited crystals with mp 173-177°C, $[\alpha]_D - 98.82^\circ$ (c 0.34; chloroform) which were identified as verazine. IR spectrum, cm^{-1} : 3390 (OH); 3095 (C=CH); 1670 (C=N). Mass spectrum: m/z 397 (M⁺), 382, 368, 354, 327, 259, 164, 149, 150, 125 (100%), 111, 98. PMR spectrum, ppm: singlets at 0.70 (18-CH₃) and 0.99 (19-CH₃); doublets at 0.88 (21-CH₃) and 1.09 (27-CH₃); multiplet at 5.32 (C=CH).

Combined fractions 4-10 were chromatographed on a column of silica gel with elution by benzene and by chloroform-methanol (9:1). The chloroform-methanol (9:1) eluate yielded an alkaloid with mp 260-261°C, $[\alpha]_D - 5.63^\circ$ (c 1.087; chloroform) which was identified as veratroylzygadenine. IR, cm^{-1} : 3480 (OH); 2775 (trans-quinolizidine); 1715, 1260 (ester group); 1525, 1610 (trisubstituted benzene ring). Mass spectrum: m/z 657 (M⁺), 642, 640, 639, 492, 475, 458, 440, 183, 165, 125, 112 (100%), 98. The benzene eluate yielded a base with mp 224-226°C, M⁺ 413.

Verazine. The total chloroform-fraction (5.8 g) was dissolved in chloroform and separated by means of an acetate buffer solution at pH 4.05. The buffer solution was taken in 10-ml portions, and 19 fractions were obtained. The combined fractions 5-8 yielded 0.25 g of verazine with mp 259-261°C (acetone), $[\alpha]_D - 112.58^\circ$ (c 0.49 chloroform). R_f 0.40 in the chloroform-methanol (9:1) system on TLC, silica gel; M⁺ 559.

Pentaacetylverazine. A mixture of 0.14 g of verazine, 2 ml of pyridine, and 1.5 ml of acetic anhydride was left at room temperature for 72 h. After the pyridine and the excess of acetic anhydride had been driven off, the residue was dissolved in 2% sulfuric acid, and the solution was made alkaline with ammonia and was extracted with ether. The residue after the ether had been distilled off was treated with acetone, leading to the isolation of 0.16 g of pentaacetylverazine with R_f 0.90 in the chloroform-methanol (9:1) system on TLC, silica gel; M⁺ 769. IR spectrum, cm^{-1} : 1760, 1235 (O-acetyl), 1670 (N-acetyl).

Hydrolysis of Verazine. A mixture of 0.11 g of verazine, 5 ml of 10% hydrochloric acid solution, and 5 ml of ethanol, was boiled for 3 h. The ethanol was distilled off in vacuum, and the residue was diluted with water, made alkaline with caustic soda solution, and extracted with ether. When the ethereal extract was concentrated, 0.04 g of crystals with mp 173-175°C (ether) deposited, which were identified as verazine (from their IR, PMR, and mass spectra); M⁺ 397. D-Glucose was identified in the hydrolysate with the aid of PC and GLC.

SUMMARY

1. We have begun a study of the alkaloids of Zygadenus sibiricus growing on the territory of the Buryat ASSR. The known alkaloids verazine and veratroylzygadenine, the new alkaloid verazine, and a base with mp 224-226°C have been isolated from the epigeal part of the plant.

2. The structure of verazine has been established as 3-O-β-D-glycopyranosylverazine.

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