ISOLATION AND DETERMINATION OF THE STRUCTURE OF 20-(2-METHYL-1-PYRROLIN-5-YL)-4-PREGNEN-3-ONE*

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Dedicated to Professor F. Šantavý on the occasion of his 60th birthday.

From the above-ground part of *Veratrum album* subsp. *lobelianum* BERNH. SUESSENGUTH a new alkaloid has been isolated in addition to the already isolated veratroylzygadenine and veracintine [20-(2-methyl-1-pyrrolin-5-yl)-5-pregnen-3β-ol], to which the structure of 20-(2-methyl-1-pyrrolin-5-yl)-4-pregnen-3-one has been assigned on the basis of its spectra and correlation with veracintine.

Using extraction with benzene a mixture of alkaloids was isolated from the above-ground parts of *Veratrum album* subsp. *lobelianum* BERNH. SUESSENGUTH, which were separated on a column of alumina. In addition to the already known alkaloids veratroylzygadenine and veracintine[1,2] we obtained another substance $C_{26}H_{39}NO$.

In addition to the molecular peak the mass spectrum contains as the main peak that of the fragment *m/e* 82(C_5H_8N) which, similarly as in the case of veracintine[1], confirms that the substituted pyrroline ring is present in the structure of this alkaloid. The spectrum displays further characteristic peaks at *m/e* 366 ($M-CH_3$), 353, 338, a strong peak *m/e* 110 ($C_7H_{12}N$) and 83 (C_5H_9N).

The PMR spectrum shows 3 protons at δ 1·17 p.p.m. (s) of the methyl group $C_{(19)}$ shifted to lower fields, because a conjugated carbonyl group is present in ring A. The singlet at δ 0·72 is in agreement with the data given by Zürcher[3] for the $C_{(18)}$ methyl group in 5α, 14α-androstane. The other 3 protons appear as a doublet centered around δ 0·72 p.p.m. (J = 7·0 Hz) and belong to the secondary methyl group on $C_{(20)}$, singlet of 3 protons at 2·02 p.p.m. belongs to the methyl group on $C_{(25)}$ in the vicinity of the double bond. The multiplet at δ 4·1 may be assigned to the proton on $C_{(22)}$ and the singlet at δ 5·68 p.p.m. to the vinyl proton on $C_{(4)}$. In the IR spectrum bands are present belonging to the conjugated carbonyl group and the azomethine double bond. The UV absorption and strongly positive molar optical rotation indi-

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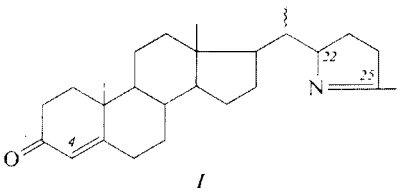
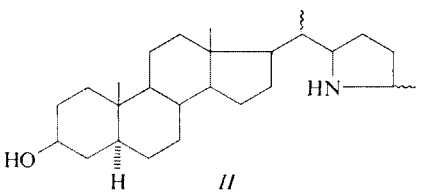
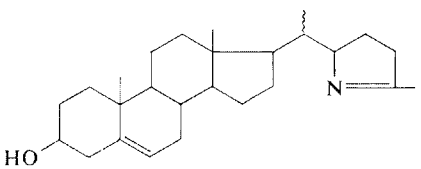
cates that a conjugated 3-oxo group is present in the structure of this alkaloid. The complex Cotton effect of the ORD curve of this base is in agreement with the idea that the substance has the character of a Δ^4-3-ketosteroid.



I



II



III

Reduction of alkaloid *I* with sodium borohydride gave a mixture of bases from which a substance could be isolated by column chromatography on alumina which was identical with tetrahydroveracintine *II*. The reference tetrahydroveracintine was prepared by catalytic hydrogenation of dihydroveracintine[1] in acetic acid. Oppenauer oxidation of veracintine (*III*) gave a conjugated ketone which was identical with the alkaloid isolated in this paper. On the basis of the above enumerated facts we assign the new alkaloid the structure of 20-(2-methyl-1-pyrrolin-5-yl)-4-pregnen-3-one (*I*).

EXPERIMENTAL

The melting points were determined on a Kofler block, optical rotation was measured on a Bendix-Ericsson 143 D apparatus; the UV spectra were recorded with a JASCO UV/ORD spectrophotometer, the mass spectra on a AEI-MS 902 spectrometer. Proton magnetic resonance was measured on a TESLA 487-B instrument with tetramethylsilane as internal standard. Thin layer chromatography was carried out on plates with basic and neutral alumina Woelm (TLC) dried at room temperature for 24 hours. For development the following solvent mixtures were employed: S_1 benzene–ethanol 19 : 1, S_2 benzene–ethanol 9 : 1.

Isolation

Twenty-six kilograms of ground above-ground part of *Veratrum album* (the plants were collected at the end of May 1972 on Kľak, Malá Fatra Mountains) were swelled in a 7·5% $NaHCO_3$ solution (20 l), allowed to stand for one hour, and extracted with 100 l of benzene. After 24 hours' extraction the benzene is filtered and the material on the filter extracted another 3 times with 70 l portions of benzene. From the combined benzene extracts alkaloids were extracted with 5%

tartaric acid solution. The tartaric acid extract was cooled, alkalized with ammonium hydroxide to pH 10 and the alkaloids were again extracted with benzene (5 times 4 l portions). The benzene extract was washed with water, filtered, concentrated and dried. The oily residue weighed 35 g (mixture of alkaloids).

Ten grams of the mixture of alkaloids were chromatographed on 300 g of alumina Merck, act. III. Fractions of 25 ml volume were collected. Fractions 1—80 were eluted with benzene, fractions 81—125 with a benzene–ether 1 : 1 mixture, fractions 126—133 with chloroform, fractions 134—141 with a chloroform–ethanol mixture 98 : 2, fractions 142—153 with the same mixture in the 1 : 1 ratio, and fractions 154—161 with ethanol.

From fractions 85—88 a residue weighing 130 mg was obtained after evaporation of the solvents, which was rechromatographed on alumina (neutral, Reanal, act. II). Elution with ether (1 ml fractions) gave in fractions 12—14 20 mg of an alkaloid of R_F 0·84 (S_1), m.p. 153—157°C (ether), $[\alpha]_D^{24}$ +107° (*c* 0·64, chloroform), $[\alpha]_D^{24}$ +121° (*c* 0·35, ethanol). Mass spectrum, *m/e*: 381·3020 (molecular peak, for $C_{26}H_{39}NO$ calculated 381·3031), 366, 353, 338, 300, 110, 83, 82 (base peak). IR spectrum: 1620 and 1680 cm^{-1} (conjugated ketone), 1650 (C=N). UV spectrum: λ_{max} 240 nm (log ε 4·34). PMR spectrum, p.p.m.: 1·17 (s), 0·72 (d), 0·72 (s), 2·02 (s), 4·1 (m), 5·68 (s).

From fractions 90—118 veracintine (600 mg) was obtained, m.p. 198°C, $[\alpha]_D^{24}$ +9·5° (*c* 0·93, chloroform), R_F 0·50 (S_1). IR spectrum: 1058 and 3636 cm^{-1} (OH), 1655 cm^{-1} (C=N). PMR spectrum, p.p.m.: 0·99 (s), 0·69 (s), 0·69 (d), 5·36 (dd).

Fractions 146—153 contained 680 mg of veratroylzygadenine, m.p. 268—269°C, $[\alpha]_D^{24}$ −29·03° (*c* 0·93, chloroform), R_F 0·16 (S_2). UV spectrum: λ_{max} 291 (log ε 3·75), 260 nm (log ε 4·07) and 218 nm (log ε 4·35). IR spectrum: 1520, 1610 cm^{-1} (aromatic ring), 1725 cm^{-1} (ester), 2760, 2780, 2815 and 2840 cm^{-1} (*trans*-quinolizidine).

Reduction of Alkaloid *I* with $NaBH_4$

Sodium borohydride (12 mg) is added to 21 mg of compound *I* in 3 ml of methanol and 1 ml of ethyl acetate and the solution is allowed to stand at room temperature for 16 hours. It is diluted with water, acidified with 5% sulfuric acid, alkalized with ammonia and extracted with chloroform. Chloroform is distilled off and the residue, representing a mixture of 6 substances, is chromatographed on 1·5 g of neutral alumina (Reanal, act. IV). Elution with benzene–ethanol 199 : 1 gave 3 mg of a compound with R_F 0·21 (S_1). Mass spectrum, *m/e*: 387, 84 (base peak).

Catalytic Hydrogenation of Dihydroveracintine in Acetic Acid

Dihydroveracintine (40 mg) in 8 ml of acetic acid was hydrogenated on 20 mg of PtO_2 at room temperature for 14 hours. The mixture was filtered, diluted with water, alkalized to pH 9 and extracted 6 times with 10 ml portions of chloroform. The chloroform extract was washed with water and distilled off. The residue weighed 37·7 mg and it was chromatographed on neutral alumina (Reanal, act. IV) with benzene. The product obtained had m.p. 247°C, $[\alpha]_D^{24}$ +19·5° (*c* 0·58, ethanol), R_F 0·21 (S_1). Mass spectrum; *m/e*. 387 (molecular ion), 84 (base peak).

Oppenauer Oxidation of Veracintine

210 mg of aluminum phenolate were added to a solution of 63 mg of veracintine in 25 ml of toluene and 3·5 ml of cyclohexane, from which 13 ml of solvent were distilled off, and the mixture was refluxed for 5 hours. After cooling it was extracted with 5% acetic acid. The combined acid extracts were alkalized with ammonia and extracted with chloroform. Chloroform was distilled

off and the residue (47·7 mg) was chromatographed on neutral alumina (Reanal, act. II) collecting 1 ml fractions. Elution with chloroform gave a single substance, m.p. 154–157°C, $[\alpha]_D^{24}$ +120° (*c* 0·3, ethanol), R_F 0·84 (S_1). Mass spectrum, *m/e*: 381 (molecular peak), 83, 82. UV spectrum: λ_{max} 240 nm (log ε 4·5). IR spectrum: 1620 and 1680 cm^{-1} (conjugated carbonyl group), 1650 cm^{-1} (C=N).

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