ALKALOIDS OF *Petilium raddeana*.

II. STRUCTURES OF PETISINE AND PETISININE

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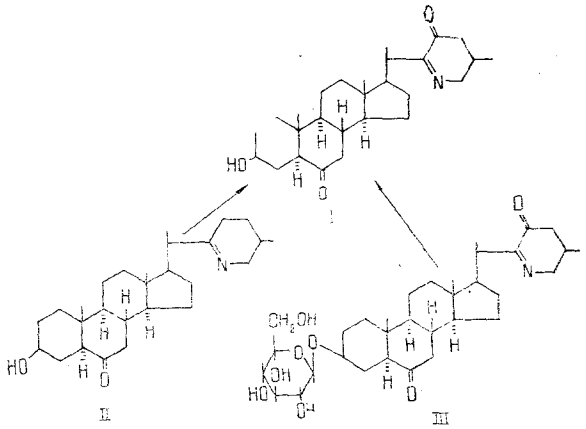
The alkaloids imperialine, imperialone, peumisine, petiline, petilidine, and the new bases petisine (I) and petisinine (III) have been isolated from bulbs of *Petilium raddeana* (Regl.) Vved. On the basis of spectral characteristics and passages to a known compound, the structure of 3β-hydroxy-Δ^{22}(N)-22,26-iminocholestane-6,23-dione has been established for (I) and petisine 3β-glucopyranoside for (III).

The isolation from the epigeal part of *Petilium raddeana* (Regl.) Vved. of imperialine, petiline, petilidine, petilinine, imperialine N-oxide and the new alkaloids petisine and petisidine has been reported previously [1].

Petisine (I) has the composition $C_{27}H_{41}NO_3$, M^+ 427 (mass spectrometrically). In the IR spectra of (I) there are absorption bands at (cm^{-1}) 3400 (OH), 1710 (C=O), and 1628 (C=N). The UV spectrum [λ_{max} 270, 277 nm (log ε 2.74, 2.66)] shows the presence of C=N and C=O chromophores [2]. The mass spectrum has the peaks of ions with m/z 97, 111, 119, 121, 139, 140, 149, 150, 394, 399, 412, and 427 (M^+, 100) which are characteristic for the mass spectrometric fragmentation of 23-oxosolacongestidine and tomatillidine [2-4]. This permits petisine to be assigned to the alkaloids of the verazine group [5, 6]. The PMR spectrum of (I) shows singlets at (ppm) 0.71 (19-CH_3, 18-CH_3) and doublets at 1.0 (21-CH_3) and 1.06 (27-CH_3).

The weak basicity of petisine and the presence in its mass spectrum of an intense peak of an ion with m/z 139 shows that the carbonyl group is present in the azomethine ring F. The C_{24} and C_{26} positions are excluded by virtue of the fact that in either case the signal from the 27-CH_3 protons in the NMR spectrum of petisine would be observed in a relatively weak field (at 1.11 ppm) [4, 7]. Consequently, one carbonyl group is located at C_{23}.

The oxidation of petiline (II) [7] with active manganese dioxide gave a product which proved to be identical with petisine (melting point and IR, mass, and NMR spectra). Thus, in petisine the hydroxy group is located at C_3 and the second carbonyl group at C_6, and the asymmetric centers have the same configuration as in petiline [7]. On the basis of the facts presented above, it may be concluded that petisine has the structure and partial configuration of 3β-hydroxy-Δ^{22}(N)-22,26-iminocholestane-6,23-dione (I).



II III

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Continuing a study of the alkaloid composition of *P. raddeana* we have investigated bulbs collected in June, 1980, in the environs of the village of Saivan, TSSR, at the end of the vegetation stage.

Treatment of the ether-extracted fraction of the combined alkaloids of the bulbs with acetone yielded imperialine [8]. The material from the mother liquor after the separation of the imperialine was dissolved in chloroform and was separated according to basicity by an acetate buffer solution and then 0.2 N acetic and 5% sulfuric acid. When the acetate buffer (pH 3.75) and acetic acid fractions were separated on a column of silica gel, imperialone, peumisine, petiline, and petilidine [7-11] were isolated, while the sulfuric acid fraction gave the new alkaloid petisinine (III) with the composition $C_{33}H_{51}NO_8$, M^+ 589. Petisine was obtained from the acidic chloroform solution after the extraction of the alkaloids by means of buffer solutions. Petisinine forms a tetraacetyl derivative with M^+ 757. The IR spectrum of petisinine has absorption bands at (cm^{-1}) 3430 (OH), 1715(C=O), and 1630 (C=N), and also a broad absorption band at 1000-1100 which is characteristic for glycoalkaloids [6]. The hydrolysis of petisinine gave an aglycone with mp 221-222°C and D-glucose (PC and GLC). The aglycone proved to be identical with petisine (melting point, IR spectrum). Consequently, petisinine is a glycoalkaloid derived from petisine. A determination of molecular rotation differences in accordance with Klyne's rule [12] showed that in petisinine the D-glucose is attached to petisine by a β-glycosidic bond. Thus, petisinine has the structure of petisine 3β-D-glucopyranoside (III).

EXPERIMENTAL

To separate the mixtures and identify the alkaloids we used type KSK silica gel and alumina (Brockmann activity grade II). IR spectra were taken on a UR-20 spectrometer in tablets with KBr, UV spectra on a Hitachi instrument in ethanol, and NMR spectra on a JNM-4H 100/100 MHz instrument ($CDCl_3$ if the solvent is not stated; TMDS was used as internal standard, δ scale), and mass spectra on a MKh-1310 mass spectrometer.

Isolation and Separation of the Combined Alkaloids from the Bulbs of *P. raddeana*. The air-dry and comminuted bulbs (5.8 kg) were moistened with 10% ammonia solution, charged into a percolator, and covered with chloroform for 2 h. Twelve percolations were performed. The concentrated and combined extracts were treated with 5% sulfuric acid, the acid solution was made alkaline with 25% ammonia, and the alkaloids were extracted with ether (50 g) and chloroform (11 g). The total amount of combined alkaloids was 61 g, which corresponds to 1.05% of the weight of the dry plant.

Imperialine, Imperialone, Peumisine, Petiline, Petilidine. The combined ether-extracted alkaloids (50 g) were treated with acetone, which led to the separation of 8 g of imperialine with mp 258-260°C (methanol). The material from the imperialine mother liquor (40 g) was dissolved in chloroform and separated by means of acetate buffer solutions with pH values of 5.57, 4.99, 4.45, 4.05, and 3.75. For each pH value, 100 ml of buffer solution was taken. Then the total alkaloids from the chloroform solution were extracted with 0.2 N acetic acid (100 ml) and with 5% sulfuric acid (100 ml). After this, the acid chloroform residue still gave a positive reaction for alkaloids (solution A).

The combined fractions from the pH 3.75 and 0.2 N acetic acid extracts (15 g) were chromatographed on a column of silica gel with elution by chloroform—methanol—ammonia (95 : 5 : 5) in 20-30-ml fractions. The total number of fractions obtained was 30. Fractions 1-9 yielded imperialone (0.03 g) with mp 228-230°C (acetone), fractions 10-20 yielded peumisine (0.06 g) with mp 265-267°C (ethanol) and petiline (0.1 g) with mp 205-206°C (acetone), and fractions 21-30 gave petilidine (0.12 g) with mp 264-265°C (methanol).

Petisine. The acidic chloroform solution A was made alkaline with 10% ammonia. The residue after the chloroform layer had been distilled off was chromatographed on a column of alumina with elution by benzene—ethyl acetate (1 : 1) in the form of 10-15-ml fractions. Fractions 5-10 of the ten fractions obtained yielded petisine with mp 221-222°C (acetone).

Petisinine. The combined alkaloids from the sulfuric acid fraction (4 g) were chromatographed on a column of silica gel with elution by chloroform—methanol—ammonia (95 : 5 : 5). From fractions 5-10 of the 25 fractions obtained petiline (0.05 g) was isolated, and from fractions 15 and 25 petisinine (0.20 g) with mp 232-234°C (methanol), $[\alpha]_D$ −35° (c 0.48; chloroform).

Oxidation of Petiline. A solution of 0.14 g of petiline in 20 ml of chloroform was treated with 0.75 g of active manganese dioxide, and the mixture was stirred at room temperature for 2 h. Then the solution was filtered and it was chromatographed on a column of alumina with elution by chloroform—ethyl acetate (1 : 1). The residue after the distillation of the eluate was treated with methanol. The resulting product (0.07 g) with mp 220-222°C, $[\alpha]_D$ −34.78° (c 0.46, methanol) was identical with petisine.

Tetraacetylpetisinine. A mixture of 0.06 g of petisinine, 2 ml of pyridine, and 2 ml of acetic anhydride was left at room temperature for 24 h. After the elimination of the pyridine and the excess of acetic anhydride, the residue was dissolved in chloroform and the solution was treated with 2% sulfuric acid. The acid solution was made alkaline with 25% ammonia and extracted with chloroform. After the chloroform had been distilled off, the residue was chromatographed on a column of silica gel and was eluted with benzene—acetone (4 : 1). Fractions with a volume of 6-10-ml were collected. Fractions 4-10 yielded amorphous tetraacetylpetisinine with R_f 0.85 on TLC in silica gel in the chloroform—methanol (9.5 : 0.5) system; M^+ 757.

Hydrolysis of Petisinine. A mixture of 0.03 g of petisinine, 5 ml of 10% hydrochloric acid, 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 ammonia, and extracted with chloroform. The residue after the distillation of the chloroform was chromatographed on a column of alumina. Benzene—ethyl acetate (1 : 1) eluates yielded 0.015 g of an aglycone with mp 220-222°C (acetone), identical with petisine.

SUMMARY

1. The known alkaloids imperialine, imperialone, peumisine, petiline, and petilidine and the new bases petisine and petisinine have been isolated from the bulbs of *Petilium raddeana*.

2. Petisine has the structure of 3β-hydroxy-Δ^{22}N-22,26-iminocholestane-6,23-dione, and petisinine is petisine 3β-D-glucopyranoside.

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