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ALKALOIDS OF *Petilium eduardi.* PETINE AND PETINE N-OXIDE

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Two new alkaloids – petine and petine N-oxide – have been isolated from the bulbs of Petilium eduardi. Their structures have been established on the basis of spectral characteristics and chemical transformations.

We have continued an investigation of the alkaloids of the bulbs of *Petilium eduardi* [1] gathered in Babatag, Surkhandar'ya Oblast, in the period of the withering of the epigeal part of the plant. When the total alkaloids were separated, five known compounds were isolated – imperialine, edpetilidine, eduardine, imperialone, and petiline [2] – and the new alkaloids petine $C_{27}H_{45}NO_3$ (1), and petine N-oxide $C_{27}H_{45}NO_4$ (2). This is the first time that petiline has been isolated from this plant.

Petine is a tertiary saturated base. The PMR spectrum of (1) showed absorption bands at (cm^{-1}) 3445 (OH), 2860-2940 ($-CH_2$, $-CH_3$) and 2780 (trans-quinolizidine) and the finger-print region of the spectrum (1) was similar to that of the C-nor,D-homosteroid alkaloid korselidine [3]. In the mass spectrum of (1) we observed the main peaks of ions with *m/z* M^+ 431, $(M - 15)^+$, $M - 18)^+$, 388, 380, 364, 358, 236, 180, 164, 162, 156, 155, 154, 150, 140, 125, 124, 113, 112(100%), 98, which are characteristic for C-nor, D-homosteroids of the cevine group [4, 5]. The PMR spectrum of petine showed the signals of the protons of two tertiary methyl groups and one secondary methyl group, and multiplets of gem-protons of two hydroxy groups (Table 1).

On oxidation with chromic anhydride, petine formed a diketone – petinedione (3). The IR spectrum of (3) showed an intense absorption band at 1715 cm^{-1} of carbonyl groups, while the narrow band of a tertiary hydroxyl remained at 3470 cm^{-1}. Consequently, petine contains two secondary and one tertiary hydroxyls.

The mass spectrum of (3) contained the main peaks of ions with *m/z*: M^+427, $(M - 15)^+$, 410, 384, 382, 356, 355, 354, 164, 162, 156, 155, 154, 150, 140, 125, 124, 113, 112 (100%), 111, 98. The PMR spectrum of (3) showed the signals of tertiary and secondary methyl protons at (ppm) 0.88 (s, 19-CH_3), 1.02 (s, 21-CH_3), and 0.78 (d, J = 7 Hz, 27-CH_3).

Thus, petine and its oxidation products contained two tertiary and one secondary methyl groups and they possessed a cevanine skeleton [6].

With the variation in the substituents the chemical shifts (CSs) of the 19-CH_3 protons changed by 6 Hz in (3), while no appreciable changes due to the presence of carbonyl groups were observed in the CSs of the 21-CH_3 and 27-CH_3 protons. Consequently, in petine the secondary hydroxy groups could be present only in rings A, B, and C [7, 8].

In the IR spectrum of petine, a trans-quinolizidine band showed that rings E and F were trans-linked [9]. By comparing the CSs of the signals of the 19-CH_3 and 21-CH_3 protons of (1) and (3) with those of dihydroimperialine and imperialone [10] it was possible to conclude that rings A/B and B/C were trans-linked, and C/D and D/E cis-linked, as in imperialine [11], while the secondary hydroxy groups were present at C-3 and C-6. Ring C was excluded for a hydroxy group because of the absence of the absorption of a carbonyl group in a 5-membered ring in the IR spectrum of petinedione (3). The presence in the mass spectra of (1) and (3) of the peaks of ions with *m/z* 154, 155, and 156, formed from the cleavage of the C-13-C-18 and C-17-C-20 bonds of ring E, and also the appearance of a signal from the 21-CH_3 protons in the form of a singlet, together with its CS, showed that the tertiary hydroxy group was present at C-20 and had the β-axial orientation [4, 5].

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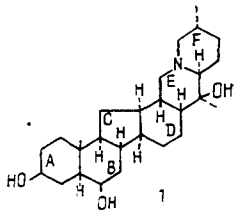
TABLE 1. Chemical Shifts of the Protons (δ, ppm) and Spin–Spin Coupling Constants (J, Hz)

Substance	19-CH3 s	21-CH3 s	27-CH3 d	3-H m	6-H m
Petine	0.94	1.01	0.77 J=7	3.56 W1/2=24	3.76 W1/2=8
Dihydro-imperialine	0.93	1.00	1.01 J=7	3.56 W1/2=24	3.75 W1/2=8
Isoverticine	1.03	1.03	1.09 J=7	3.63 W1/2=24	3.85 W1/2=8
Korselidine	0.95	1.02	0.80 J=6	3.41 W1/2=24	3.73 W1/2=8

A comparison of the $W_{1/2}$ values of the signals of the gem-protons to the hydroxy groups and of the CS of the 19-CH_3 group with those of dihydroimperialine, isoverticine, and korselidine showed that the secondary hydroxy groups were present at C-3 with the β-equatorial orientation and at C-6 with the β-axial orientation. It follows from the CS values that the 21-CH_3 and 27-CH_3 groups were oriented α-equatorially [3, 10, 12, 13].

Thus, petine and isoverticine differ by the configurations of the C-17 and C-25 asymmetric centers; i.e., isoverticine rings D/E are trans-linked and the 27-methyl group has the β-axial orientation, while in petine rings D/E are cis-linked and the 27-methyl group has the α-equatorial orientation. The difference of 9 Hz in the CSs of 19-CH_3 in the isoverticine and petine is apparently due to the different linkages of the D/E rings [8].

With respect to the elementary composition of its functional groups and its heterocyclic skeleton, petine is close in structure to korselidine. However, the melting points, solubilities, and IR spectra, and R_f values of these bases do not agree and, apparently, korselidine and petine differ in the linkage of rings C/D or D/E, which has not been established for korselidine. Consequently, petine belongs to the imperialine group and has the structure and configuration of 3β,6β,20β-trihydroxy-(27α-CH_3)-cevanine (1).



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In the IR spectrum of alkaloid (2) there were absorption bands at (cm^{-1}) 3410 (OH), 2885-2950, 1460 (–CH_2, –CH_3), 970, 937, and 930 (N-oxide), [14]. The mass spectrum of (2) contained the peaks of ions with *m/z* M^+ 447, 431$(M - 16)^+$, 430$(M - 17)^+$, 429$(M - 18)^+$, 413, 414, 415, 416, 388, 386, 374, 358, 344, 236, 180, 164, 162, 156, 155, 154, 150, 140, 125, 124, 113, 112 (100%), 98, which are characteristic for C-nor,D-homosteroid alkaloids of the imperialine group [4, 5]. The PMR spectrum of (2) exhibited singlets at (ppm) 0.93 (19-CH_3) and 1.01 (21-CH_3), a doublet at 0.85 (CH_3-27, J = 7 Hz), and multiplets at 3.60 (3-H, $W_{1/2}$ = 24 Hz) and 3.80 (6-H $W_{1/2}$ = 8 Hz). The low intensity of the molecular peak of (2), differing by 16 mass units from the molecular ion of petine (1), and also the presence of peaks of ions with *m/z* $(M - 17)^+$ and $(M - 18)^+$ and the absence of a Bohlmann band in the IR spectrum of (2) [9] and its high solubility in ethanol, methanol, and water permitted the assumption that base (2) was the N-oxide derivative of petine (1). The reduction of (2) with zinc in hydrochloric acid gave a substance identical with petine (1). The oxidation of petine with hydrogen peroxide produced a N-oxide identical with (2). Consequently, the compound was petine N-oxide.

EXPERIMENTAL

We used type KSK silica gel for column chromatography (125-250 μm) and for thin layer chromatography (50-80 μm), with the solvent system chloroform–methanol (10:1).

IR spectra were taken on a UR-20 spectrophotometer in KBr, mass spectra on a MKh-1310 instrument at an ionizing potential of 60-70 B and temperatures of 100-170°C, and PMR spectra on a Tesla BS-567A instrument at 100 MHz (in $CDCl_3$ with HMDS as internal standard).

Isolation of the Total Alkaloids. The ground dry bulbs of *P. eduardi* (10.5 kg) were wetted with a 10% solution of ammonia and extracted with chloroform. The chloroform extract was concentrated to small volume, and the alkaloids were extracted with 5% sulfuric acid. The acid solution of the bases was washed with ether and, after it had been made alkaline with ammonia, the alkaloids were transferred into ether (50.88 g) and then into chloroform (21.11 g). The total yield of alkaloids was 72.99 g (0.73% on the dry weight of the plant).

Separation of the Total Alkaloids. On standing, the ethereal fraction of the total material deposited crystals of imperialine (7.81 g), and then 19.83 g of the material from the mother solution after the separation of imperialine was dissolved in benzene and was separated according to basicity by means of acetate buffers at pH 5.57, 4.99, 4.45, 4.05, and 3.72. The buffer solutions were collected in 25-ml portions, and then, after being made alkaline with ammonia, were extracted with chloroform. Concentration and drying yielded 15 fractions of alkaloids, three at each pH value.

These fractions were individually separated on a silica gel column with elution by chloroform–methanol (100:1).

The fractions obtained at pH 5.57, 4.99, and 4.45 yielded the following alkaloids: imperialine with mp 264-267°C (0.89 g), edpetilidine with mp 224-226°C (0.09 g) and eduardine with mp 248-250°C (0.02 g).

From the fractions extracted at pH 4.05 and 3.72 we isolated imperialone, with mp 229-232°C (0.01 g) and petiline, with mp 202-203°C (0.02 g). IR spectrum: 3400 (OH), 1715 (C=O), 2870-2950 cm^{-1}. Mass spectrum: M^+ 413, 398, 358, 356, 165, 164, 151, 150, 125 (100%). PMR spectrum (ppm): 0.64 (s, 18-CH_3), 0.69 (s, 19-CH_3), 0.85 (d, 21-CH_3), and 1.03 (d, 27-CH_3).

The mother solution from the fraction with pH 5.57 was separated on a column of silica gel with elution by chloroform–methanol (100:1). The eluate was collected in 10-15 ml fractions, of which 35 were obtained. Fractions 9-24 yielded 0.08 g of petine N-oxide with mp 241-243°C (acetone), M^+ 447, R_f 0.50.

The mother solution from the fraction with pH 4.99 was passed through a column of silica gel. Elution was performed with chloroform–methanol (100:2 and 100:5). This gave 50 fractions. Fractions 20-35 yield 0.05 g of petine with mp 145-147GC (hexane–acetone), M^+ 431, R_f 0.40.

All the monoalkaloids isolated were idenified by direct comparison with authentic samples.

Preparation of Petinedione (3). A solution of 0.11 g of petine in 3 ml of acetic acid was treated with 2 ml of a 3% solution of chromium trioxide in acetic cid, and the mixture was heated at 70-80°C for 30 min. The residual acid was evaporated in vacuum, and a solution of the residue was made alkaline with a solution of sodium carbonate and was extracted with chloroform. This gave the diketone of petine – petinedione (3) – with mp 210-212°C (acetone), M^+ 47, R_f 0.60.

Oxidation of Petine (1) with Hydrogen Peroxide. A solution of 0.02 g of petine in 2 ml of ethanol was treated with 3 ml of 30% hydrogen peroxide and the mixture was left at room temperature for two days. Then the ethanol was distilled off, and the residue was made alkaline and extracted with chloroform. After the chloroform had been distilled off, the new residue was treated with acetone. This gave crystals with mp 240-243°, identical with petine N-oxide (2).

Reduction of Petine N-oxide (2). A few granules of metallic zinc were added to a solution of 0.05 g of petine (1) in 5 ml of 5% of hydrochloric acid, and the mixture was kept at room temperature for two days. This acid solution was made alkaline with ammonia and extracted with chloroform. After the chloroform had been distilled off, the residue was treated with a mixture of acetone and ethyl acetate. This gave crystals with mp 145-150°C, identical with petine (1).

REFERENCES

1. R. Shakirov, R. N. Nuriddinov, and S. Yu. Yunusov, Khim. Prir. Soedin., 429 (1965).
2. R. Shakirov and S. Yu. Yunusov, Khim. Prir. Soedin., 3 (1980).
3. K. Samikov, R. Shakirov, and S. Yu. Yunusov, Khim. Prir. Soedin., 34 (1989).
4. R. N. Nuriddinov, R. Shakirov, and S. Yu. Yunusov, Khim. Prir. Soedin., 316 (1967).
5. H. Budzikiewicz, Tetrahedron, **20**, 2267 (1964).
6. IUPAC-IUB Tentative Rules for Nomenclature of Nomenclature of Steroids, J. Org. Chem., **34**, 1517 (1969).
7. R. N. Nuriddinov, A. I. Saidkhodzhaev, M. R. Yagudaev, and S. Yu. Yunusov, Khim. Prir. Soedin., 333 (1968).
8. R. F. Zürcher, Helv. Chem. Acta, **46**, 2054 (1963).
9. F. Bohlmann, Ber., **91**, 2157 (1958).
10. R. N. Nuriddinov and S. Yu. Yunusov, Khim. Prir. Soedin., 458 (1971).
11. S. Ito, Y. Fukasawa, and M. Miyashita, Tetrahedron Lett., **36**, 3161 (1976).

12. K. Kaneko, N. Naruse, K. Haruki, and H. Mituhashi, Chem. Pharm. Bull., **28**, 1345 (1980).
13. S. Ito, I. B. Stothers, and S. M. Kupchan, Tetrahedron, **20**, 913 (1964).
14. K. Nakanishi, Infrared Absorption Spectroscopy. Practical, Holden-Day, San Francisco (1962).