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The alkaloids skimmianine, dubinidine, foliosidine, graveoline, and compounds (I) and (II) have been isolated from the epigeal part of the plant *Haplophyllum foliosum* Vved. (family Rutaceae). On the basis of the spectral characteristics of (I) and its O-isopropylidene derivative it has been established that the compound is the alkaloid edulinine. Substance (II) was identified as ferulic acid by direct comparison with an authentic sample. This is the first time that edulinine and ferulic acid have been detected in plants of the genus *Haplophyllum*.

We have continued a study of the alkaloid composition of the plant *Haplophyllum foliosum* Vved. (family Rutaceae) growing in the environs of the village of Alimtai, Tadzhik SSR. Fifteen alkaloids of the quinoline group have been isolated from this plant [1].

The air-dry epigeal part of *H. foliosum* collected in the incipient flowering phase was extracted with methanol. The extract was separated between acid and chloroform. The acid solution yielded ether-soluble and chloroform-soluble bases (0.2% on the weight of the dry plant) which were further separated into phenolic and nonphenolic fractions. The ether-soluble nonphenolic material yielded skimmianine, dubinidine, foliosidine, and graveoline, and a base with 141-142°C (from acetone) (I).

The IR spectrum of (I) contains absorption bands of active hydrogen (3425 and 3215 cm^{-1}) of an amide carbonyl group (1630 cm^{-1}), and of an orthosubstituted benzene ring (765 cm^{-1}). The UV spectrum of (I) is typical for 4-methoxy-2-quinolones: λ_{max} (cm^{-1}) (log ϵ), 230 (4.44) 245 shoulder (4.02), 267 inflection (3.74), 275 (3.68), 285 (3.82), 316 inflection (3.72), 326 (3.82) 337 shoulder (3.69); λ_{min} (cm^{-1}) (log ϵ), 256 (3.58), 280 (3.81), 295 (3.37). On acidification, the absorption curve did not change, which is also characteristic of 2-quinolones [2].

The PMR spectrum of (I) contained signals at (ppm) 2.25 and 2.63 (doublet, 1 H, $J = 7.6$ Hz and multiplet, 3 H; the H_5 and $H_{6,7,8}$ aromatic protons, respectively): 5.00 (doublet, 1 H, $J = 3$ Hz, disappears on deuterium exchange; OH); 6.11 and 6.32 (singlets, 3 H each; OCH_3 and NCH_3); 6.44 (X); 6.93 (A); 7.33 (B) (multiplet, 1 H each; after deuterium exchange - quartets with $J_{\text{AX}} = 2$ Hz, $J_{\text{BX}} = 13$ Hz; CH-O and benzyl protons) 8.74 (singlet, 6 H, gem-diethyl group) The characteristics given above are close to those for edulinine, in which at C_3 of a 4-methoxy-1-methyl-2-quinoline nucleus there is the substituent $\text{CH}_2\text{CH}(\text{OH})\text{C}(\text{OH})(\text{CH}_3)_2$ [3, 4].

The mass spectrum of (I) contains all the peaks observed in the spectrum of edulinine [m/e (%), 276 (0.8), 273 (2.9), 258 (2), 232 (100), 203 (58.3), 188 (66.7), 160 (8.3), 59 (18.8)], with the exception of the peak of the molecular ion, which has a very low intensity in the spectrum of edulinine [4].

In order to establish the identity of (I) as edulinine, we obtained and characterized its O-isopropylidene derivative with mp 91-92°C (from hexane) (II). The mass spectrum of (II) contained the peak of the molecular ion with m/e 331 (5.4%), confirming the molecular weight (29) of the initial base, and the peaks of ions with m/e (%) 316 (100), 274 (22.6), 273 (90.3), 256 (16.1), 256 (19), 232 (6.4), and 59 (32.2) arising as the result of the decomposition of the dioxolane ring [5] and also the peaks of ions with m/e 203 (40.3%) and 188 (22.6%).

The NMR spectrum of (II) differs from the spectrum of (I) by the absence of the signals of OH groups and the presence of the signal of an isopropylidene group: 2.16 ppm (doublet, 1 H, $J = 8.2$ Hz, H_5); 2.70 (multiplet, 3 H; $H_{6,7,8}$); 5.78 (X); 6.99 (A), and 7.40 (B) (quartets, 1 H each, $J_{\text{AX}} = 8.7$ Hz, $J_{\text{BX}} = 3.7$ Hz, $J_{\text{AB}} = 13.3$ Hz; CH-O and benzyl protons); 6.04 and 6.36

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ppm (singlets, 3 H each; OCH₃ and NCH₃); 8.86 and 8.81 ppm (doublet, 6 H, J = 1.7 Hz, and singlet, 6 H, respectively; two gem-diethyl groups). These facts unambiguously show that (I) is edulinine, which has been found previously in *Casimiroa edulis* [3, 4], *Citrus macroptera* [6], and *Eriostemon trachyphyllus* [7] (family Rutaceae) and has also been obtained from platydesmine [8].

From the aqueous mother liquor after the extraction of the phenolic bases, a substance was isolated with mp 168-169°C (from water), M⁺ 194 (III). The spectral characteristics of (III) proved to be close to those of ferulic acid [9]. The identity of these substances was confirmed by a direct comparison of samples.

This is the first time that edulinine and ferulic acid have been detected in plants of the genus *Haplophyllum*.

EXPERIMENTAL

UV spectra were taken on an EPS-3T spectrometer (Hitachi) (ethanol), IR spectra on a UR-20 (KBr), and the NMR spectrum of (I) on a JNM-4H-100/100 MHz instrument and of (II) on a JNM-C-60/60 MHz instrument (CDCl₃, τ scale), and mass spectra on an MKh-1303 spectrometer.

For TLC (KSK silica gel with 5% of gypsum) we used the solvent system toluene-ethyl acetate-formic acid (5:4:1).

Isolation of the Alkaloids. The raw material (66 g) was extracted with methanol. After elimination of the methanol, 10% sulfuric acid was added to the extract to give it a weakly acidic reaction, and it was then treated with chloroform. The acid solution was washed with ether, made alkaline with concentrated ammonia, and exhaustively extracted with ether and with chloroform. When the ethereal extract was concentrated, dubinidine (7.4 g) with mp 131-132°C (from acetone) and skimmianine (11.6 g) with mp 175-176°C (from ethanol) separated out successively.

The mother liquor was treated with 4% caustic soda solution and evaporated, and the residue (60 g) was chromatographed on a column of alumina. The first fractions of the eluates yielded skimmianine (0.5 g) and the last fractions yielded graveoline (0.5 g), mp 188-189°C (from ethanol), and edulinine (0.06 g).

The alkaline solution was washed with chloroform (A), saturated with ammonium chloride, treated with ether and with chloroform, acidified with concentrated hydrochloric acid, and extracted with chloroform; the evaporation of the last extract yielded ferulic acid (2.6 g). When the chloroform solution (A) was concentrated, foliosidine (3 g) separated out, with mp 141-142°C (from acetone).

The dubinidine, skimmianine, graveoline, and foliosidine were identified by direct comparison with authentic samples.

O-Isopropylidene Derivative of Edulinine (II). A solution of 0.03 g of edulinine in 100 ml of acetone was treated with one drop of concentrated sulfuric acid. On the following day the acid solution was neutralized with anhydrous sodium carbonate and filtered. The residue after the distillation of the acetone was chromatographed on a column of alumina. The first ethereal eluates yielded (II) (0.035 g).

SUMMARY

The alkaloid edulinine and ferulic acid have been isolated from plants of the genus *Haplophyllum* for the first time. For the identification of the edulinine its O-isopropylidene derivative was obtained and characterized.

LITERATURE CITED

1. S. Yu. Yunusov, Alkaloids [in Russian], Tashkent (1974), p. 176.
2. A. W. Sangster and L. K. Stuart, Chem. Rev., 65, 97 (1965).
3. J. Iriarte, F. A. Kincl, J. Rosenkranz, and F. Sondheimer, J. Chem. Soc., 4170 (1956).
4. T. P. Toubé, J. W. Murphy, and A. D. Cross, Tetrahedron, 23, 2061 (1967).
5. J. A. McClosky and M. J. McClelland, J. Am. Chem. Soc., 87, 5090 (1965); Ya. V. Rashkes, I. A. Bessonova, and S. Yu. Yunusov, Khim. Prir. Soedin., 336 (1972).
6. S. R. Johns, J. A. Lamberton, and A. A. Siomis, Aust. J. Chem., 23, 419 (1970).
7. E. V. Lassak and J. T. Pinhey, Aust. J. Chem., 22, 2175 (1969).

8. D. S. Boyd and M. F. Grondon, J. Chem. Soc., (C), 556 (1970).
9. F. Santavi, Ultraviolet, Infrared, and Proton Magnetic Resonance Spectra of Simple Aromatic Compounds Substituted by Hydroxyl, Methoxyl, and Methyleneedioxy Groups, Prague (1973), pp. 59, 95, 120.

DYNAMICS OF THE ACCUMULATION OF ALKALOIDS IN THE EPIGEAL PART OF

Aconitum leucostomum

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The dynamics of the accumulation of alkaloids in the epigeal part of *Aconitum leucostomum* Worosch. from various growth sites according to the vegetation periods have been studied. It has been shown that in all cases in the early period of development of the plant diterpene bases predominate in the total material, and in the fruit-bearing period isoquinoline bases.

Continuing a study of *Aconitum leucostomum* Worosch. [1], we have investigated the alkaloid composition of its epigeal part from various growth sites according to vegetation periods. Let us consider a sample collected in the Santash Pass, Terskei-Alatau range. With the growth of the plant, the amount of combined alkaloids fell sharply (%):

Vegetation period and data of collection (1977)	Total alkaloids on the weight of the air-dry plant	Diterpene alkaloids	Isoquinoline alkaloids
Rosette leaf phase, May 12	0.87	82	18
Budding, June 30	0.30	76	21
Flowering, July 10	0.18	68	30
Fruit-bearing, August 20	0.09	35	57

When the combined alkaloids were separated and their qualitative and quantitative composition was studied by the GLC method it was established that in the early vegetation period the combined alkaloids consisted mainly of diterpene derivatives and in the later periods the isoquinoline alkaloids became predominating in amount.

In the early vegetation period the plant contained lappaconitrine [2], lappaconidine [3], and an alkaloid with the composition $C_{20}H_{23}N_4$ (I) with mp 140-150°C, which, on the basis of spectral characteristics, and also by a direct comparison with an authentic sample, was identified as the aporphine alkaloid corydine [4].

In the budding period we isolated the following alkaloids: lappaconitine, corydine, lappaconidine, an unidentified base with the composition $C_{32}H_{44}N_2O_9$ (II), and a substance with the composition $C_{20}H_{25}NO_3$ (III), which was identified as the benzylisoquinoline alkaloid 0-methylarmepavine [5] on the basis of the spectral characteristics and also by a direct comparison with an authentic sample.

In the flowering period, in addition to the alkaloids, isolated in the budding period, we obtained a base with the composition $C_{19}H_{23}NO_3$ (IV). The UV spectrum of the base had maxima at λ_{max} 223 and 284 nm ($\log \epsilon$ 4.20, 3.69). In the mass spectrum of (IV) the peaks of ions with m/e 192 (100%) and 121 (25%) were observed, which are characteristic for benzyltetrahydroisoquinoline alkaloids with methoxy and hydroxy groups in the isoquinoline and a methoxy group in the benzyl parts of the molecule [6].

The NMR spectrum of (V) contained the signals of the protons of a N-methyl group (2.38 ppm, 3 H, singlet), and of two methoxy groups (3.63 and 3.78 ppm, 3 H each, singlets). Two-proton doublets at 6.37 and 6.91 ppm ($J = 8$ Hz) were assigned to two equivalent pairs of ortho

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