HAPLOSININE – A NEW FURANOQUINOLINE GLYCOALKALOID FROM Haplophyllum perforatum

Kh. A. Rasulova, I. A. Bessonova,
M. R. Yagudaev, and S. Yu. Yunusov

UDC 547.944/945

The new glycoalkaloid haplosinine has been isolated from the epigeal part of Haplophyllum perforatum (M. B.) Kar. et Kir., and its structure has been established on the basis of chemcial transformations and a comparative analysis of its ^{13}C NMR spectra with those of known compounds as haplopine 7-O-[O-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside].

The epigeal part of Haplophyllum perforatum (M. B.) Kar. et Kir., growing in the foothills of Baba-Dag, has previously yielded 12 furanoquinoline alkaloids, including the glycoalkaloids haplopine 7-O-α-L-rhamnopyranoside (glycoperine), its acetyl derivatives, and haplopine 7-O-β-D-glucopyranoside (glucohaplopine) [1-3].

Continuing the separation of the water-soluble fraction of the combined chloroform-extracted alkaloids of this plant on silica gel, we have obtained a crystalline glycoalkaloid more polar than the monosides mentioned above, with mp 227-228°C, which we have called haplosinine (I).

The UV spectrum of (I) had great similarity to the spectra of 7,8-dialkoxy derivatives of dictamine [4]. Its PMR spectrum clearly showed the signals of the protons of a furanoquinoline nucleus at 8.08, 7.65, and 7.82, 7.14 ppm (two pairs of doublets, J = 8.5 and 3.0 Hz, respectively: H-5,6 and the protons of the furan ring) and of two methoxy groups in the form of a six-proton singlet at 4.27 ppm. The presence in the PMR spectrum of haplosinine of signals in the 3.90-5.60 ppm region and of a doublet at 1.54 ppm (J = 6 Hz) from the protons of the carbohydrate moiety, and also the chromatography mobility of the compound, showed the glycosidic nature of the alkaloid isolated.

The acid hydrolysis of haplosinine gave haplopine (II) and L-rhamnose and D-glucose, detected by TLC and PC. The presence of these sugars in a ratio of 1:1 was confirmed by the GLC of their silylated methyl glycosides [5]. These facts showed that (I) was a bioside of haplopine.

TABLE 1. Chemical Shifts of the Carbon Atoms of Hapolosinine (I), Haplopine (II), and Glycoperine (IV) (DMSO–d_6, δ, ppm, 0 – TMS, $\delta_{TMS} = \delta_{DMSO\text{-}d_6} + 39.6$ ppm)

C-Atom	Multiplicity	I	II	IV	C-Atom	Multiplicity	I	II	IV
2a	s	164.02	163.81	163,82	8-OCH_3	q	61,26	60,81	61,11
3a	s	115,64	113 32	115,19	1′	d	100,24		99,97
4	s	157,17	156,94	156,81	2′	d	70,06[b]		70,52
5	d	118,18	117,95	117,77	3′	d	81,35		70,67
6	d	116,63	116,51	116,16	4′	d	70,97		71,94
7	s	143.73	141.41	143,20	5′	d	70,00[b]		70,00
8	s	141.19	139,31	140.96	6′	q	18,16		17,95
8a	s	148,73	150,21	148.66	1″	d	105,03[a]		
4a	s	102,72	101,22	102,34	2″	d	74,33		
2	d	144,32	142.11	143,95	3″	d	76.65		
3	d	105,71[a]	105,40	105,33	4″	d	70,23[b]		
4-OCH_3	q	59.77	59,24	59,47	5″	d	77.02		
					6″	t	61,56		

[a,b] The assignments may be interchanged.

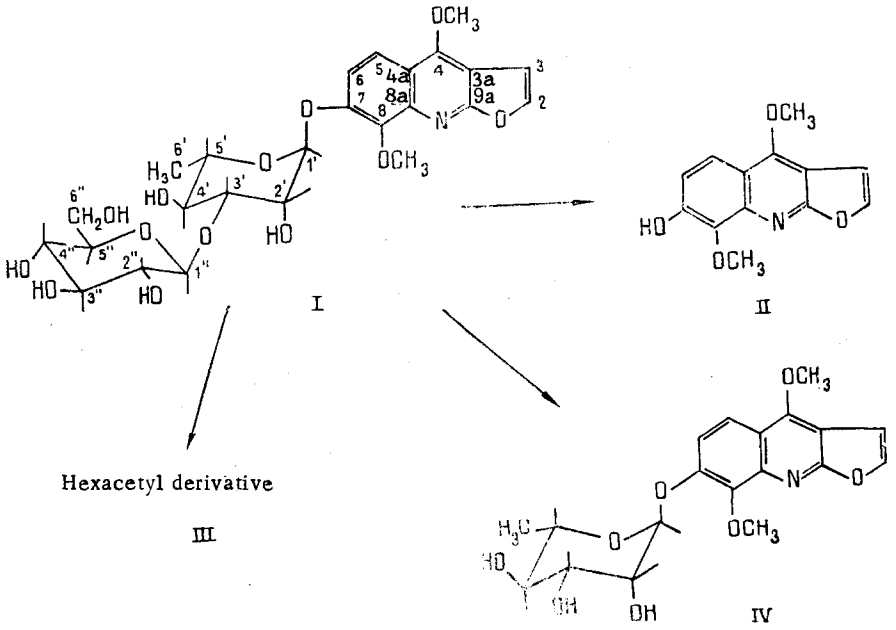
Institute of the Chemistry of Plant Substances, Uzbek SSR Academy of Sciences, Tashkent, All-Union Scientific-Research Institute of Medicinal Plants, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 876-879, November-December, 1987. Original article submitted May 11, 1987.

0009-3130/87/2306-0731$12.50

The order of attachment of the sugars was tentatively established on the basis of the mass spectrum of the hexaacetyl derivative of haplosinine (III) from the peaks of ions present in it with m/z 331 and 231 from tetra-O-acetylglucose (M − 17) and of di-O-acetylrhamnose (M − 17), and was confirmed by enzymatic hydrolysis.

The action on haplosine of snail gastric juice formed D-glucose and a monoglycoside that was identified as glycoperine (IV). Consequently, the terminal sugar in (I) was D-glucose.

The position of attachment of the D-glucose residue to the L-rhamnose residue was found with the aid of a comaprative analysis of the ^{13}C NMR spectra of haplosinine and glycoperine. The assignment of the CSs of the signals of the carbon atoms was made on the basis of the multiplicities of the signals form the spectra of (I), (II), and (IV) obtained under the conditions of incomplete suppression of interaction with protons, and by comparison with literature figures for the CSs of the furanoquinoline alkaloids skimmianine [6], evoxine [7], methyl α-L-rhamnopyranoside and methyl β-D-glucopyranoside, and the bioside multiflorin B [9] (Table 1). It can be seen from the table that no substantial changes take place in the CSs of the carbon atoms on passing from the genin (II) to the monoside (IV) and the bioside (I). The effect of glycosylation at the C-7 atom causes a downfield shift of the signal of this carbon atom ($\Delta\delta$ ~ 2 ppm). An analysis of the CSs of the carbohydrate moieties of (I) and (IV) showed that an appreciable paramagnetic shift ($\delta\Delta$ = +10.6 ppm) was experienced only by the C-3' signal of the L-rhamnose residue. This determines a 1 → 3-bond of the D-glucose residue with the L-rhamnose residue, and this was also confirmed by the β-screening effect of glycosylation on the signals of the C-2' and C-4' atoms of the L-rhamnose residue.



The results of the chemical degradation of (I) also indicated the 1 → 3-linkage of the sugar residues. When haplosine that had been oxidized with sodium periodate was hydrolyzed, L-rhamnose was detected, while its Smith degradation [10] gave glycoperine (IV).

The chemical shifts of the carbon atoms of the carbohydrate residues showed the pyranose form of the monosaccharide residues, the α-configuration of the anomeric center of the L-rhamnose residue, and the β-configuration of the D-glucose residue [8, 11].

The configurations of the glycosidic bonds were also confirmed by the PMR spectrum of haplosinine, in which the L-rhamnose anomeric proton resonated in the form of a doublet at 6.22 ppm (J = 1.5 Hz) and that of the D-glucose residue at 5.48 ppm (J = 7 Hz) [12].

Thus, haplosinine − the first bioside among alkaloids of the quinoline class − has the structure of haplopine 7-O-[O-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside].

EXPERIMENTAL

General Remarks. The separation of the combined alkaloids and their purification was performed on columns filled with silica gel L 100/160 μm and KSK 70-100 μm. The chloroform–methanol (9:1) and (5:1) solvent systems were used.

For thin layer chromatography (TLC), silica gel of type LS 5/40 (Czechoslovakia) containing 13% of gypsum was employed with the following solvent systems: 1) toluene–ethyl acete–acetic acid (5:4:1); 2) chloroform–methanol–dimethylformamide (80:19:1); 3) benzene–methanol (4:1); 4) chloroform–methanol (8:1); 5) butan-1-ol–methanol–water (5:3:1); 6) benzene–chloroform–methanol (5:5:2); 7) ethyl acetate–methanol (8:1); 8) benzene–acetone–water (4:5:1); and 9) ethyl acetate. The alkaloids were revealed in UV light and with the Dragendorff reagent, and the sugars by spraying with o-toluidine salicylate followed by heating at 100-110°C for 2-5 min.

Spectra were measured on Hitachi EPS-3T (ethanol), UR-20 (KBr), and MKh-1310 instruments and, in the case of PMR spectra, on a Tesla-567 NMR spectrometer at ν_0 = 100 MHz (for ^1H, in deuteropyridine, 0 – HMDS) at 24.142 MHz (for ^{13}C, in DMSO-d_6, 0 – TMS). The ^{13}C NMR spectra were obtained under the conditions of complete and incomplete decoupling of C–H interactions.

Isolation of Haplosinine. The extraction of the plant raw material and the treatment of the combined alkaloids has been described previously [1]. Part of the water-soluble fraction of the combined chloroform-extracted alkaloids (50 g) was chromatographed on silica gel. Elution with chloroform–methanol (25:1) gave glucohaplopine [3]. A chloroform–methanol (5:1) fraction contained haplosinine. The repeated rechromatography of these fractions gave chromatographically pure haplosinine (systems 2, 5, and 6). The overall yield calculated on the air-dry weight of the raw material was less than 0.001%.

Haplosinine (I). $C_{25}H_{31}NO_{13}$, mp 227-228°C (from methanol), $[\alpha]_D^{25}$ –74° (c 2.83; pyridine). ν_{max}, cm^{-1}: 3650-3100 (OH), 1630, 1590, 1518, 1495 (aromatic system).

Hydrolysis of Haplosinine. A mixture of 15 mg of haplosinine and 20 ml of acetone containing 1% of hydrochloric acid was left at room temperature for three days. The course of hydrolysis was monitored by TLC. The solution was evaporated to dryness, the residue was dissolved in water, the solution was extracted with chloroform, and evaporation of the extract gave haplopine, mp 203-204°C, identical with an authentic sample according to TLC in systems 1 3, 4, and 7. The aqueous solution was neutralized with sodium carbonate, filtered, evaporated, and analyzed by TLC in systems 5, 6, and 8 on plates impregnated with a 0.3 M solution of sodium dihydrogen phosphate. The presence of L-rhamnose and D-glucose was established.

Enzymatic Hydrolysis of Haplosinine. A solution of 11 mg of haplosinine in 20 ml of water was treated with 3 drops of a snail enzyme preparation and the mixture was left at room temperature for 3 days. Then it was evaporated to dryness in vacuum. The residue was dissolved in ethanol, and the solution was filtered and evaporated. This gave glycoperine, mp 220-221°C. It was identified in systems 1, 2, 3, and 9. The ethanolic mother liquor was analyzed in the way described above. This showed the presence of D-glucose.

Hexaacetylhaplosinine (III). A mixture of 25 mg of haplosinine, 0.5 ml of acetic anhydride, and 1 drop of pyridine was heated in the water bath for 1 min and was then left at room temperature. On the following day the mixture was evaporated and the residue was chromatographed on silica gel. Ethereal eluates yielded compound (III), mp 105°C (petroleum ether–benzene), $[\alpha]_D^{25}$ –68° (c 2.66; pyridine).

Mass spectrum, m/z (%): 805 (M^+, 4), 561 (100), 331 (35), 244 (23), 245 (42), 231 (14), 227 (20), 213 (30), 171 (35), 169 (89), 153 (54), 111 (40).

Periodate Oxidation. A solution of 20 mg of haplosinine in 2 ml of methanol was shaken with a solution of 100 mg of sodium periodate in 2 ml of water, and the mixture was left for 2 days at room temperature. The excess of periodate was decomposed with ethylene glycol. The solution was evaporated to dryness and the residue was treated with ethanol. The ethanolic solution was separated into two equal parts (A and B). Part A was hydrolyzed with 0.1 N sulfuric acid. The reaction mixture was methylated, the residue was dissolved in water, and the solution was extracted with chloroform. The residue after the chloroform had been distilled off was shown by TLC in systems 1, 3, 4, 7, and 9 to contain haplopine. The acid hydrolysate was neutralized with barium carbonate, filtered, and evaporated and the residue was analyzed in a similar manner to that described above. This showed the presence of L-rhamnose.

Smith Degradation of Haplosinine. With shaking, 40 mg of sodium tetrahydroborate was added in portions to part B of the ethanolic extract, and it was left for a day at room tem-

perature. The reaction mixture was acidified and was again left for 2 days. The acid solution was extracted with chloroform. The residue obtained after the chloroform had been distilled off was shown by TLC in systems 2, 3, 7, and 9, to contain glycoperine.

SUMMARY

The first bioside of the furanoquinoline series – haplosinine, which has the structure of haplopine 7-O-[O-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside – has been isolated from the epigeal part of Haplophyllum perforatum.

LITERATURE CITED

1. Kh. A. Abdullaeva, I. A. Bessonova, and S. Yu. Yunusov, Khim. Prir. Soedin., 219 (1978).
2. Kh. A. Abdullaeva, I. A. Bessonova, and S. Yu. Yunusov, Khim. Prir. Soedin., 425 (1977).
3. Kh. A. Abdullaeva, I. A. Bessonova, and S. Yu. Yunusov. Khim. Prir. Soedin., 873 (1979).
4. A. W. Sangster and K. L. Stuart, Chem. Rev., 65, 101 (1965).
5. T. T. Gorovits, Khim. Prir. Soedin., 263 (1970).
6. A. Ahond, F. Picot, P. Potier, C. Poupat, and T. Sevenet, Phytochemistry, 7, 166 (1978).
7. N. M. D. Brown, M. F. Grundon, D. M. Harrison, and S. A. Surgenor, Tetrahedron, 36, 3579 (1980).
8. A. S. Shashkov and O. S. Chizhov, Bioorg. Khim., 2, 437 (1976).
9. K. Yamasaki, R. Kasai, Y. Masaki, M. Okihara, O. Tanaka, H. Oshio, S. Takagi, M. Yamaki, K. Masuda, G. Nonaka, M. Tsuboi, and I. Nishioka, Tetrahedron Lett., 1231 (1977).
10. M. Abdel-Akher, J. K. Hamilton, R. Montgomery, and F. Smith, J. Am. Chem. Soc., 74, 4970 (1952).
11. F. W. Wehrli and T. Nishida, Progr. Chem. Org. Nat. Prod., 36, 174 (1979).
12. C. Altona and C. A. J. Haasnoot, Org. Magn. Reson., 13, 417 (1980).

SEPACONITINE – A NEW ALKALOID FROM Aconitum septentrionale

S. K. Usmanova, V. A. Tel'nov,
M. S. Yunusov, N. D. Abdullaev,
A. I. Shreter, and G. B. Filippova

UDC 547.944/945

The structure of a new alkaloid sepaconitine isolated from the epigeal part of Aconitum septentrionale has been established on the basis of spectral characteristics.

Aconitum septentrionale Koelle (wolfbane monkshood) is a plant that is widely distributed on the territory of the RSFSR [1].

We have investigated the epigeal part of A. septentrionale gathered before the beginning of budding in the Moscow, Yaroslavl', and Vladimir Oblasts in May, 1983.

The total amount of alkaloids in this period was 0.32% of the weight of the air-dry raw material.

Separation of the mixture of bases yielded lappaconitine [2, 3] and a new base with the composition $C_{30}H_{42}N_2O_8$, mp 250-253°C, $[\alpha]_D^{20}$ +25° (c 0.60; chloroform) which we have called sepaconitine (II).

The IR spectrum of (II) contained absorption bands at (cm^{-1}) 1690 (carbonyl of an aromatic acid ester) and at 1596, 1260, 1247, 1170, and 760 (1,2-substituted aromatic ring).

The PMR spectrum of the base contained the signals of the methyl radical of an ethyl group at 1.08 ppm (3 H, t, J = 7 Hz), of three methoxy groups at 3.27, 3.28, and 3.38 ppm (singlets, 3 H each), and a one-proton doublet at 3.73 ppm (J = 5 Hz), together with the signals of four aromatic protons in the weak-field region at 6.55-7.68 ppm.

Institute of the Chemistry of Plant Substances, Uzbek Academy of Sciences, Tashkent, All-Union Scientific-Research Institute of Medicinal Plants, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 879-883, November-December, 1987. Original article submitted May 11, 1987.

 0009-3130/87/2306-0734$12.50