STRUCTURE OF HAPLOSIDINE

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A new glycoalkaloid, haplosidine, has been isolated from the epigeal part of the plant Haplophyllum perforatum, and its structure has been established as 7-[O-β-D-glucopyranosyl-(1 → 3)-2'-O-acetyl-α-L-rhamnopyranosyloxy]-4,8-dimethoxyfuranoquinoline.

We have previously reported the determination of the structures of the new minor furanoquinoline glycoalkaloids glucohaplopine [1] and haplosinine [2] isolated from the combined alkaloids of the epigeal part of Haplophyllum perforatum. These substances are readily soluble in water, because of which losses of them in the process of extracting the total alkaloids from aqueous acid solutions with organic solvents are possible. With the aim of checking this, we repeated the extraction of the plant raw material with methanol and subjected the evaporated methanolic extract to separation (without first obtaining the total alkaloids from it) into ethereal, chloroform, ethyl-acetate, ethanolic, and aqueous fractions. From the last fraction, by chromatography on silica gel, we isolated skimmianine, haplamine, glycoperine, a new base with mp 158-160°C, which we have called haplosidine (I), and haplosinine (II). The yield of haplosidine was 0.01% and that of haplosinine 0.003% of the weight of the dry raw material, while in the separation of the combined alkaloids obtained by treating a methanolic extract with acid (I) was isolated in trace amounts, and the yield of (II) was less than 0.001% [2].

The optically active alkaloid (I) was readily soluble in water and methanol, less readily in ethanol, and sparingly in chloroform, and was insoluble in ether and hexane. It crystallized from the mixed solvents methanol–ethyl acetate, methanol–hexane, and ethanol–hexane. Its UV spectrum almost coincided with that of 7,8-dialkoxy-substituted derivatives of 4-methoxyfuranoquinoline and of the glycoalkaloids of this series (glycoperine [3], haplosinine [2]). These facts, and also its chromatographic mobility, showed the glycosidic nature of haplosidine.

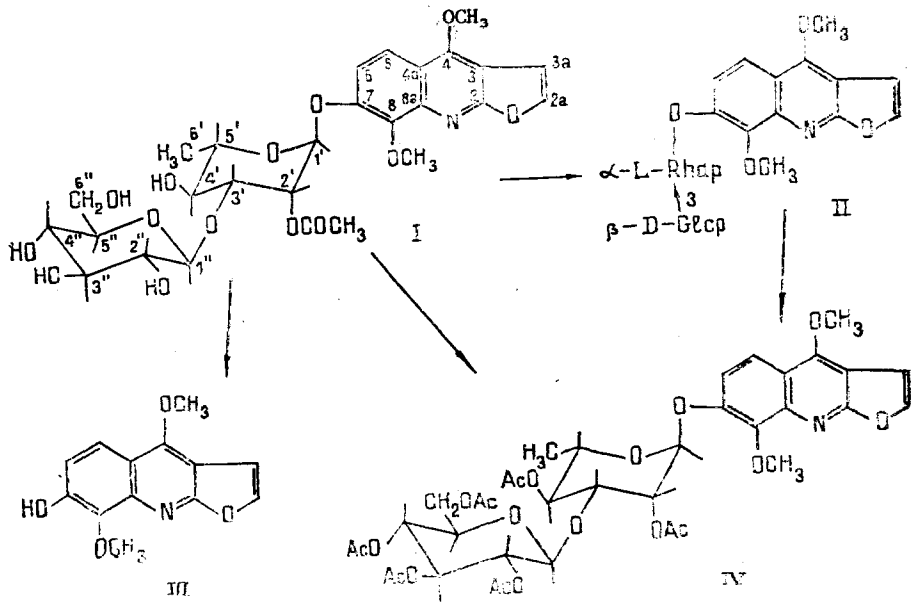

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TABLE 1. Chemical Shifts of the Carbon Atoms of Haplosidine (I) (DMSO-d_6, δ, ppm; 0 – TMS; $\delta_{TMS} = \delta_{DMSO\text{-}d_6} + 39.6$ ppm)

C atom	Multiplicity	I	C atom	Multiplicity	I
2a	s	163,90	1′	d	96,97
3a	s	115,80	2′	d	71,80
4	s	157,00	3′	d	78,30
5	d	117,96	4′	d	71,05
6	d	116,50	5′	d	69,71
7	s	143,80	6′	q	17,78
8	s	141,00	1″	d	104,90
8a	s	148,00	2″	d	74,04
4a	s	102,65	3″	d	76,43
2	d	144,25	4″	d	70,08
3	d	105,50	5″	d	76,87
4-OCH_3	q	59,62	6″	t	61,25
8-OCH_3	q	61,42	CO	s	170,47
			CH_3	q	21,00

The acid hydrolysis of (I) gave haplopine (III), and L-rhamnose and D-glucose were detected in the hydrolysate by TLC and PC. The IR spectra of (I) contained absorption bands at 3650-3100 cm^{-1} (OH groups), 1735, 1245 (ester group), and 1645, 1550, 1530, 1490 cm^{-1} (aromatic system). In the PMR spectrum of haplosidine (deuteropyridine), the protons of the furanoquinoline fragment gave signals at 8.08 and 7.62 ppm (d, 1 H each, J = 8.5 Hz; H-5 and H-6); 7.82 and 7.16 ppm (d, 1 H each; the protons of a furan ring); 4.30 and 4.27 ppm (s, 3 H each, two methoxy groups); the signals of the carbohydrate moiety were found at 6.19 ppm (2 H, the signals of the anomeric and gem-acetyl protons of an acetyl-L-rhamnose residue), 5.45 ppm (d, 1 H, J = 7 Hz; the anomeric proton of a β-D-glucose residue); 2.04 ppm (s, 3 H; the protons of an acetyl group); and 1.57 ppm (d, 3 H, J = 6 Hz; the CH_3 group of a rhamnose residue). The remaining protons of the carbohydrate moiety appeared in the form of multiplets at 5.10-3.90 ppm. These facts indicated that the carbohydrate moiety of haplosidine contained one acetyl group (see scheme on previous page).

When (I) was acetylated, the peracetyl derivative (IV) was obtained, which proved to be identical with the hexaacetyl derivative of haplosinine [2]. The alkaline saponification of haplosidine gave a deacetyl derivative identical with haplosinine according to a mixed melting point, TLC, and IR spectra. The results of these transformations showed that haplosidine was an acetyl derivative of haplosinine.

The position of the acetyl substituent in haplosidine was determined by comparing its ^{13}C NMR spectrum (Table 1) with that of haplosinine [2]. The assignment of the CSs of the signals of the carbon atoms was made in the way described in [2].

The results of a comparison of the ^{13}C NMR spectra of (I) and (II) showed that in the spectrum of haplosidine there was a downfield shift of the C-2′ signal ($\Delta\delta$ = +1.8 ppm) and an upfield shift of the C-1′ and C-3′ signals ($\Delta\delta$ = −3.05 and −3.27 ppm, respectively), which corresponds to the magnitudes and directions of the α- and β-contributions of an acetoxy group [4]. The chemical shifts of the signals of the other carbon atoms of the L-rhamnose and D-glucose residues in the spectrum of the glycoalkaloid with the acetoxy group had similar values to those for its deacetyl derivative (the deviations $\Delta\delta$ amounted to 0.08-0.32 ppm).

The facts stated above unambiguously show that the acetyl group in (I) was located in the 2′ position. Consequently, haplosidine has the structure of 7-[O-β-D-glucopyranosyl-(1 → 3)-2′-O-acetyl-α-L-rhamnopyranosyloxy]-4,8-dimethoxyfuranoquinoline.

EXPERIMENTAL

For general observations, see [2]. The separation and purification of the alkaloids was performed on column filled with silica gels L 100/160 μm and KSK 70-100 μm. The following solvent systems were used: chloroform–methanol (25:1 and 15:1); hexane–acetone (1:1); chloroform–acetone (1:1); benzene–acetone (6:1); and ethyl acetate–methanol (25:1).

Isolation of the Alkaloids. The dry leaves (1.5 kg) were extracted with methanol. The evaporated extract was treated with ether (giving 39 g of extract), chloroform (13 g), ethyl acetate (8 g), and ethanol (112 g). The water-soluble residue was chromatographed on silica

gel. The chloroform eluates yielded skimmianine (0.2 g) and haplamine (0.5 g); chloroform–methanol eluates gave glycoperine (0.5 g); the chloroform–methanol (25:1) fractions contained haplosidine; and the chloroform–methanol (15:1) fraction contained haplosinine. The repeated rechromatography of these fractions on silica gel using the above-mentioned systems gave the individual alkaloids haplosamine and haplosinine.

Haplosidine (I). $C_{27}H_{33}NO_{14}$, mp 158-160°C, $[\alpha]_D^{25}$ −77° (c 2.66, pyridine). UV spectrum, $\lambda_{max}^{ethanol}$: 249, 320, 333 nm (log ε 4.87, 3.85, 3.77). λ_{min} 275 (log ε 3.25).

Hydrolysis of Haplosidine. A solution of 15 mg of haplosidine in 20 ml of ethanol containing 1% of hydrochloric acid was left at room temperature for 2 days. Then it was evaporated to dryness, the residue was dissolved in water, and this solution was extracted with chloroform, the distillation of which then gave haplopine, with mp 203-204°C. It was shown to be identical with an authentic sample of IR spectroscopy and TLC. In the aqueous solution, as described in [2], L-rhamnose and D-glucose were identified by TLC and PC.

Hexaacetylhaplosinine (IV) from (I). A mixture of haplosidine (25 mg), acetic anhydride (0.5 ml), and pyridine (1 drop) was left at room temperature. On the following day, the mixture was evaporated and the residue was chromatographed on silica gel. Ethereal eluates yielded (IV) in the form of an oil which crystallized from a mixture of benzene and petroleum ether with mp 105°C and was identified from a mixed melting point, TLC, and mass spectroscopy as the hexaacetyl derivative of haplosinine [2].

Haplosinine (II) from (I). A solution of 30 mg of haplosidine in 1 ml of absolute methanol was treated with 0.5 ml of a freshly prepared 0.1 N solution of sodium methanolate, and the mixture was left in the cold. On the following day, the precipitate that had deposited was filtered off with suction and was washed with methanol. The combined ethanolic solutions were evaporated in vacuum. The residue was dissolved in 0.5 ml of water. When the aqueous solution was extracted with chloroform, a precipitate deposited which was chromatographed on silica gel. Chloroform–methanol (15:1) eluates yielded crystals of (II) with mp 227-228°C identical with haplosinine according to a mixed melting point, TLC, and IR spectroscopy.

CONCLUSIONS

A new glycoalkaloid, haplosidine, has been isolated from Haplophyllum perforatum, and its structure has been established as 7-[O,β-D-glucopyranosyl-(1 → 3)-2'-O-acetyl-α-L-rhamnopyranosyloxy]-4,8-dimethoxyfuranoquinoline.

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