ALKALOIDS OF *Haplophyllum perforatum*. II

Kh. A. Abdullaeva, I. A. Bessonova, and S. Yu. Yunusov

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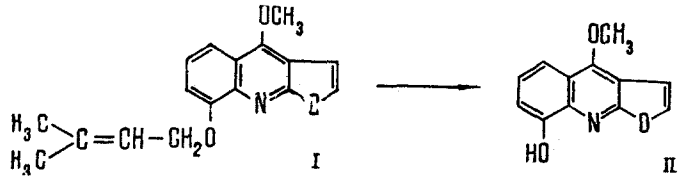
Continuing a systematic study of the alkaloids of *Haplophyllum perforatum* (M.B.) Kar. et Kir. [1], we have investigated the epigeal part collected by S. A. Khamidkhodzhaev on the northern slopes of the Babadag range, where the plant forms large thickets. Skimmianine has previously been isolated from the leaves of this plant collected in this place [2].

A methanolic extract of the epigeal part was obtained, and it was separated into acidic, basic, and neutral fractions. The alkaloids proved to be in the basic fraction. The neutral fraction, unlike that from the epigeal part of plants obtained from the Dzhungarian Ala-Tau [1], contained almost no alkaloids. From the mixture of bases of the epigeal part collected in the budding period we isolated skimmianine, evoxine, haplamine, 7-isopentenyloxy-γ-fagarine, and glycoperine, identified by direct comparison with samples obtained from this plant from other growth sites [1, 3, 4], and the new alkaloid haplofidine (I). Previously, for (I) on the basis of spectral characteristics we proposed an 8-isopentenyloxydictammine structure [5]. The proposed structure was confirmed by the formation of robustine (II) on the saponification of haplofidine in an acid medium, and also by the partial synthesis of (I) by consending robustine with 4-chloro-2-methylbut-2-ene.



I II

S. Yu. Yunusov and G. P. Sidyakin have established that in the leaves of *H. perforatum* collected in the Kitab region of the Kashkadar'ya oblast the amount of evoxine decreases during the growth of the plant and the amount of skimmianine increases [3].

In order to determine the dynamics of the accumulation of alkaloids in *H. perforatum* growing in the Babadag and to find new bases, we also investigated the epigeal part in the budding and flowering periods.

The amounts of alkaloids in the epigeal part of *H. perforatum* were as follows (% on the weight of the dry raw material: the symbol + denotes an alkaloid content less than 0.005%):

Phase of development	Total alkaloids	Evoxine	7-Isopentenyloxy γ-fagarine	Methylevoxine	Evodine	Skimmianine	Glycoperine	Haplofidine	Haplamine	Haplopine
Budding	1,04	0,39	0,01	+	0,01	0,03			+	
Flowering	0,87	0,04	+	+		0,17	0.02	+	+	0,01
Fruit-bearing	1,06	+	+			0,32	0,11	0,02	+	

It can be seen from the figures given that a high content of alkaloids in the epigeal part is found throughout the period of vegetation of the plant and falls only slightly in the flowering period. However, as the plant develops considerable qualitative and quantitative changes take place in the individual components. The budding period is characterized by the maximum amount of evoxine and its analogs — 7-isopentenyloxy-γ-fagarine, methylevoxine, and evodine — in the epigeal part and by the absence of glycoperine and haplofidine. The largest number of alkaloids is found in the flowering period. In the latter period —

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fruit-bearing — the amounts of evoxine and of 7-isopentenyloxy-γ-fagarine fall to traces, and methylevoxine and evodine cannot be detected among the alkaloids even chromatographically while the amounts of skimmianine, glycoperine and haplofidine rise to their maximum values. The role of intermediate compound in the biochemical transformations of the furanoquinoline alkaloids of similar structure is apparently played by haplopine [6], appreciable amounts of which are found only in the flowering period. It is interesting that the amount of haplamine, the only one of these substances not belonging to the furanoquinoline series, in the epigeal part of the plant changes little during the growth of the plant.

From the glycoperine mother liquors, by repeated chromatography on alumina and silica gel, we obtained a new alkaloid, triacetylglycoperine, the structure of which was established by a study of its spectra and by direct comparison with an authentic sample obtained from glycoperine [7]. In various solvent systems, triacetylglycoperine has the same R_f values on TLC as skimmianine, which makes it difficult to detect in a mixture of bases containing skimmianine.

In a similar manner, the skimmianine mother liquors yielded monoacetylglycoperine, mp 120-121°C, mol. wt. 433 (mass spectrometry) and a mixture of crystals of mono- and diacetylglycoperines, mol. wts. 475 and 433, respectively (mass spectrometry). This is shown by the spectral characteristics of these substances and by the formation of triacetylglycoperine when they were acetylated.

EXPERIMENTAL

For general observations, see [1]. For TLC we used the following solvent systems: 1) toluene—ethyl acetate—formic acid (5:4:1); 2) benzene—methanol (4:1); 3) chloroform—methanol (9:1); 4) ethyl acetate; 5) ethyl acetate—methanol (8:1); and 6) hexane—ethyl acetate (4:1).

Isolation and Separation of the Total Alkaloids. The dry comminuted epigeal part of the plant (420 g) collected in the fruit-bearing period on August 25, 1973, was extracted with methanol until the alkaloids had been dissolved out completely. The evaporated methanolic extract was distributed between ether (A) and acid (B). The ethereal solution a was shaken with 10% sulfuric acid (C) and then with a 4% solution of caustic soda. After alkalinization of the combined acid solutions B and C the alkaloids were extracted with ether (3.18 g) and chloroform (1.28 g, basic fraction). The alkaline solution (acid fraction) contained no alkaloids. Distillation of the ether from solution A yielded a neutral fraction.

The treatment of the ether-soluble bases (3.18 g) with acetone led to the separation of skimmianine (0.83 g). The mother liquor was chromatographed on alumina. The ethereal eluate yielded haplofidine (70 mg) and 7-isopentenyloxy-γ-fagarine (5 mg), the chloroform eluates yielded skimmianine (0.43 g), and the chloroform—methanol eluates yielded evoxine (4 mg). Treatment of the chloroform-soluble bases (1.28 g) with acetone yielded glycoperine (0.47 g). The mother liquor was chromatographed on a column of alumina. Ethereal eluates yielded haplofidine (10 mg) and skimmianine (66 mg), and the methanolic eluates yielded haplamine (5 mg).

Column chromatography of the neutral fraction (7.8 g) on alumina with gradient elution (ether, chloroform, methanol) yielded haplamine (5 mg).

In a similar manner, from the epigeal part (463 g) collected in the budding period on April 26, 1974 we obtained the combined ether-soluble (4.16 g) and chloroform-soluble (0.7 g) alkaloids. Treatment of the combined ether-soluble alkaloids with acetone yielded evoxine (0.2 g). The mother solution was chromatographed on alumina. Ethereal eluates yielded 7-isopentenyloxy-γ-fagarine (50 mg) and skimmianine (0.15 g); chloroform eluates yielded a mixture of crystals (0.21 g); and chloroform—methanol eluates gave evoxine (1.55 g). The mixture of crystals (0.21 g) was chromatographed on silica gel with elution by ethyl acetate. The first eluates gave evodine (66 mg) and the subsequent ones gave methylevoxine (6 mg). The combined chloroform-soluble alkaloids (0.7 g) were chromatographed on alumina. Ether—chloroform and chloroform eluates yielded evoxine (50 mg) ethanolic extracts yielded haplamine (5 mg).

Extraction with methanol of the dry epigeal part (53 kg) collected in the flowering period on June 11, 1974 gave the ether-soluble alkaloids in crystalline form (76.47 g) and

the chloroform-soluble alkaloids in semicrystalline form (385.34 g). Acetone treatment of the ether-soluble alkaloids led to the isolation of skimmianine (20 g) and chromatography of the mother liquor on alumina gave haplofidine (20 mg), 7-isopentenyloxy-γ-fagarine (50 mg), skimmianine (6.01 g), methylevoxine (20 mg), evoxine (21.2 g), haplopine (5.01 g), and haplamine (60 mg). From the combined chloroform-soluble alkaloids (385.34 g), by treatment with acetone we isolated a crystalline mixture (79 g), which was boiled with chloroform under reflux. The chloroform solution was filtered from the insoluble residue (15.24 g) and passed through a column of alumina. The chloroform eluates, on evaporation, deposited crystals of skimmianine (62.12 g). Elution with chloroform–methanol gave evoxine (8 mg), and methanol yielded haplopine (7 mg) and glycoperine (0.01 g). After the separation of the skimmianine from the chloroform eluates, the evaporated chloroform solution was twice chromatographed on a column of silica gel. The first ethereal eluates yielded haplopine (0.01 g) and the subsequent ones a mixture of crystals with mp 80°C (0.075 g) and monoacetylglycoperines (8 mg). Treatment of the insoluble residue (15.24 g) with hot benzene gave glycoperine (7.86 g). The benzene solution was evaporated, and chromatography of the residue yielded skimmianine, a noncrystallizing fraction, evoxine (5 mg) and glycoperine (2.1 g). The noncrystallizing fraction was twice chromatographed on silica gel. Skimmianine (50 mg) and triacetylglycoperine (70 mg) were isolated from the ethereal eluates. The separation of the combined chloroform material is continuing.

Methylevoxine and evodine were identified by direct comparison with authentic samples [1, 8].

Evoxine, mp 155-156°C (acetone).

7-Isopentenyloxy-γ-fagarine, mp 105-106°C (ethyl acetate).

Methylevoxine, mp 85-86°C (ether–hexane); $[\alpha]_D^{24}$ −15.3° (c 1.7; ethanol).

Evodine, mp 151-152°C (acetone).

Skimmianine, mp 176-177°C (methanol).

Glycoperine, mp 224-225°C (methanol).

Haplamine, mp 201-202°C (decomp.; ethanol).

Haplopine, mp 204-205°C (methanol).

Haplofidine, colorless needles, mp 111-112°C (ether). In UV light in system 1 it had a deep blue fluorescence and in systems 2, 4, and 6 a pale blue fluorescence. It is revealed by the Dragendorff reagent.

Hydrolysis of Haplofidine. Haplofidine (20 mg) was dissolved in 1 ml of concentrated hydrochloric acid. After half an hour, crystals of a hydrochloride with mp 199°C (ethanol) deposited. The addition to an aqueous suspension of the hydrochloride of a 5% solution of ammonia formed a base with mp 147-148°C (ethanol) which, from its melting point, TLC behavior (systems 1, 4, and 6), and IR spectrum, was identical with robustine (II) [9].

Synthesis of Haplofidine. A mixture of robustine (20 mg), 4-chloro-2-methylbut-2-ene (0.5 ml), and anhydrous potassium carbonate (0.3 g) was boiled in dry acetone (25 ml) for 40 h. The residue obtained after the distillation of the acetone from the filtrate, was dissolved in ether. The ethereal solution was washed with 4% caustic soda to eliminate unchanged robustine and was evaporated. The residue was chromatographed on silica gel. Hexane–ethyl acetate (5:1) eluates yielded robustine, and then crystals with mp 110-111°C which were identical with haplofidine.

Triacetylglycoperine, mp 181-182°C (benzene–petroleum ether), $[\alpha]_D$ −91° (c 0.4; ethanol). Mass spectrum: m/e (%) 517 (5), 273 (41), 245 (93), 227 (52), 171 (30), 153 (100), 111 (80).

Mixture of Mono- and Diacetylglycoperines, mp 80°C, mass spectrum: m/e (%) 475 (4), 433 (5), 245 (93), 227 (100).

By acetylating the mixture (40 mg) with acetic anhydride (1 ml) in pyridine (3 drops) at room temperature, crystals were obtained with mp 181-182°C (benzene–petroleum ether), which were identified by their melting point, TLC behavior (systems 1-5), and IR, mass, and NMR spectra as triacetylglycoperine.

Monoacetylglycoperine, mp 120-121°C (ether–acetone). UV spectrum, $\lambda_{max}^{ethanol}$, nm: 244, 250, 321 (log ε 4.51, 4.64, 3.83); λ_{min}, nm: 220, 271 (log ε 4.09, 4.61). IR spectrum, cm^{-1}: 3420 (OH), 1748 ($OCOCH_3$). Mass spectrum: m/e (%) 433 (3), 245 (100), 227 (60), 189 (9).

The acetylation of monoacetylglycoperine (3 mg) similarly gave crystals with mp 180-181°C, which were identified as triacetylglycoperine.

SUMMARY

From the epigeal part of *Haplophyllum perforatum* growing on the northern slopes of the Babadag range we have isolated the known alkaloids evoxine, 7-isopentenyloxy-γ-fagarine. methylevoxine, evodine, skimmianine, glycoperine, haplamine, and haplopine and the new alkaloids haplofidine, triacetylglyoperidine and monoacetylglycoperidine. Diacetylglycoperine has been detected in the mixture of bases.

It has been established that in the epigeal part, with the growth of the plant the amount of evoxine and its analogs decreases and the amounts of skimmianine, glycoperine, and haplofidine increase. The greatest number of alkaloids is found in the flowering period.

It has been shown that haplofidine has the structure of 8-isopentenyloxy-4-methoxyfuranoquinoline, and triacetylglycoperine that of 4,8-dimethoxy-7-α-L-triacetylrhamnosyloxyfuranoquinoline.

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