MEDICINAL PLANTS

STUDY OF BY-PRODUCTS OBTAINED DURING SANGUIRITRINE PRODUCTION FROM *Macleaya microcapra*

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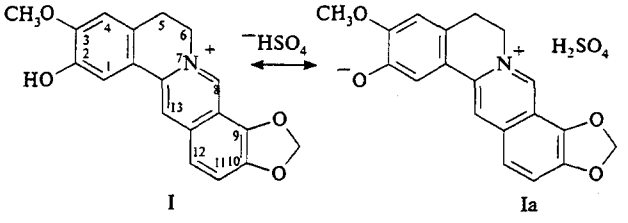
Sanguiritrine is a plant antimicrobial preparation possessing a broad spectrum of action [1, 2]. The active substance is obtained from the above-ground part of *Macleaya microcapra* (Maxim Fedde) and *Macleaya cordata* (Willd. R. Br.) [3, 4] cultured as medicinal plants in Russia and other CIS countries [5 – 13]. The drug comprises a mixture of bisulfates of two tertiary benzo[c]phenanthridine alkaloids, sanguinarine and chelerythrine.

Study of the raw plant materials of *M. microcapra* of various origin and quality showed that the extraction of technical-purity sanguiritrine is accompanied by the coprecipitation of by-products responsible for the deeper coloration of the target technical products. Thin-layer chromatograms on a silica gel exhibited an additional spot having an orange-red color and a lower mobility compared to those of sanguinarine and chelerythrine.

The impurity compound was extracted from the aqueous phase in the form of bisulfate. UV spectrum [λ_{max}, nm (log ε)]: 221.5 (4.42), 241 (4.37), 270 (4.44) 345 (4.41), 456 (3.72). This spectrum is close to the electronic absorption spectra of protoberberine alkaloids. The ^{1}H NMR spectrum is also typical of these alkaloids containing substituents in positions 2, 3, 9, and 10 of the core (CF_3COOH; δ, ppm): 3.39 and 4.96 ($CH_2CH_2N^+$), 7.70 (s, H^1), 7.04 (s, H^4), 8.50 (s, H^{13}), 9.41 (s, H^8), 7.82 (s, H^{11} and H^{12}), 4.07 (s, OCH_3), 6.42 (s, OCH_2O).

One of the substituents in the impurity alkaloids was identified as a hydroxy group. The absence of a clearly pronounced color reaction with sodium bicarbonate indicated that the hydroxy group occurred in position 2 or 9. Direct comparison of the data for this compound and berberubine (9-berberoline chloride, II), possessing a hydroxy group in position 9, showed that compounds I and II have different TLC mobilities, UV, and ^{1}H NMR spectra. The final decision in favor of position 2 was made on the basis of two-dimensional NMR spectra measured in the COSY and NOESY modes.

The NOESY spectra exhibited cross-peaks between unambiguously interpreted strong-field signal at 3.39 ppm, assigned to protons of the methylene group in position 5, and a signal at 7.04 ppm. This fact allowed the latter peak to be attributed to H^4. At the same time, the presence of cross-peaks (in both COSY and NOESY spectra) between the signals of H^4 protons and those of the methoxy group indicated that the latter group is situated at C^3. The other cross-peaks, including those due to the long-range spin-spin interaction observed in the COSY spectra, agree with the structure of dehydrocheilanthifoline bisulfate (I).



I Ia

It should be noted that the positions of signals observed in the ^{1}H NMR spectra of compound I dissolved in CF_3COOH and D_2O are significantly different (see Table 1). An unusually large shift of the H^1 proton signal toward stronger fields ($\Delta\delta = 1.44$ ppm) observed upon going to the D_2O solution is indicative of an increase in the electron density on the H^1 atom as a result of the formation of a bipolar (betaine) structure (Ia) for dehydrocheilanthifoline in this aqueous medium. Table 1 also gives the chemical shifts for the alkaloid berberine (III) having the same molecular skeleton as that of compound I, excluding the hydroxy group. The

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proximity of proton chemical shifts of the compound III in both solutions also indicates that changes in the spectra of compound I are explained by the presence of the hydroxy group.

Dehydrocheilanthifoline was previously detected in *M. cordata* [14, 15] and *Fumaria indica* [16]; the structure of dehydrocheilanthifoline was established by comparing the racemic tetrahydro derivative with the natural cheilanthifoline, which is an optically active compound. No data on the extraction of dehydrocheilanthifoline from *M. microcapra* were reported.

TABLE 1. Characteristics of the ^{1}H NMR Spectra of Dehydrocheilanthifoline Bisulfate (I) and Berberine (III) in CF_3COOH (A) and D_2O (B)

Compound	Chemical shift (δ, ppm) of signals of protons in positions									
	1	4	5	6	8	11	12	13	OCH_2O	OCH_3
I A	7.70	7.04	3.39	4.95	9.41	7.82	7.82	8.50	6.42	4.07
I B	6.26	6.10	2.75	4.45	9.10	7.40	7.10	7.44	6.1 – 6.3	3.50
Δδ	1.44	0.94	0.64	0.50	0.31	0.42	0.72	1.06	0.32, 0.12	0.57
III A	7.18	6.62	3.02	4.62	9.24	7.73	7.63	8.12	5.88, 4.0	3.88
III B	6.86	6.68	3.00	4.65	9.40	7.72	7.49	7.82	5.92, 4.0	3.90
Δδ	0.32	– 0.06	0.02	– 0.03	– 0.16	0.01	0.14	0.30	– 0.04, 0.0	– 0.02

EXPERIMENTAL PART

Dehydrocheilanthifoline was obtained on the VILAR Experimental Plant (Moscow) from *M. microcapra* containing 1.85% total sanguinarine and chelerythrine. A chloroform solution of unpurified alkaloid bases (20 g extracted from 1.3 kg raw material) was washed with water (1 : 4). Then the aqueous solution was acidified with a 10% sulfuric acid to pH 2. After standing for 2 h, brick-red crystals precipitated with a 0.19 – 0.26% yield (as calculated with respect to the sum of unpurified alkaloid bases); m.p., 255 – 260°C (with decomposition).

The UV spectra were measured on a Specord M-40 spectrophotometer (Germany), and the ^{1}H NMR spectra were obtained on a Gemini 200 (Varian, USA) spectrometer using TMS as an internal standard. The melting temperatures were determined on a Boetius heating stage (Germany). The purity of compounds was checked by TLC on Silufol UV-254 plates. The samples were eluted in a chloroform – methanol (10 : 1) system; the spots were visualized by exposure to visible and UV light.

9-Berberoline chloride was obtained by thermal O-demethylation of berberine chloride according to [17]. The product had a melting temperature of 243 – 247°C (with decomp.) and was identified by the ^{1}H NMR spectrum.

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