ON THE NATURE OF THE SO-CALLED METHOXYCHELIDONINE*

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The so-called methoxychelidonine, an alkaloid isolated in 1924 from *Chelidonium majus* L. (*Papaveraceae*), is not an individual compound, but a mixture of three related alkaloids (+)-chelamine (*III*), (+)-homochelidonine (*V*) and (+)-chelamidine (*IV*) as it was deduced from high resolution mass spectral, thin-layer chromatographic and high-performance liquid chromatographic (HPLC) analyses of the authentic Gadamer's sample. The content of *III*, *V* and *IV* in "methoxychelidonine" found by HPLC technique was 67, 28, and 5 wt.%, respectively.

The alkaloid methoxychelidonine (*I*) was described in 1924 by Gadamer and Winterfeld[1] as one of the minor alkaloids of *Chelidonium majus* L. (*Papaveraceae*). It was characterized by the composition $C_{21}H_{21}NO_6$ calculated from elemental analysis, melting point 221 °C, optical rotation $[\alpha]_D$ +115.48°, by the presence of one hydroxy, methoxy and *N*-methyl group, and two methylene dioxy groups as well as by some derivatives. The methoxy group was tentatively placed in the position C-4 of the (+)-chelidonine formula (*II*) according to the analogy with narcotine[1,2].

Since that, the alkaloids of *C. majus* were many times studied by a number of authors (cf. for example the reviews[3 – 6]), but methoxychelidonine has nevermore been found with certainty. The identity of an isolate to the Gadamer's methoxychelidonine mentioned in the paper[7] without any characteristic is rather doubtfull. Even so the finding of methoxychelidonine by high-performance liquid chromatography[8] seems to be not sufficiently proved. Nevertheless, the occurrence of methoxychelidonine as an individual alkaloid of *C. majus* is cited in monographies and reviews[3 – 6,9] till now.

In the course of our investigations of the *Chelidonium majus* alkaloids[10 – 12] we have several times observed some mixed crystallizates consisting of (+)-chelamine (*III*), (+)-chelamidine (*IV*) and (+)-homochelidonine (*V*) which possessed the melting points very close to that of methoxychelidonine. Shortly afterwards, we have had an oppor-

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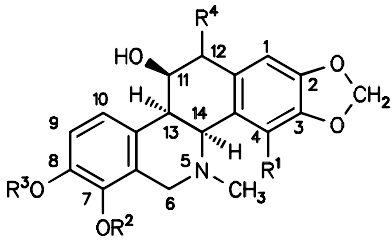
tunity to examine a sample of the original methoxychelidonine from the Gadamer's collection.



I, $R^1 = OCH_3$; $R^2 + R^3 = CH_2$; $R^4 = H$
II, $R^1 = R^4 = H$; $R^2 + R^3 = CH_2$
III, $R^1 = H$; $R^2 + R^3 = CH_2$; $R^4 =$ ····OH
IV, $R^1 = H$; $R^2 = R^3 = CH_3$; $R^4 =$ ····OH
V, $R^1 = R^4 = H$; $R^2 = R^3 = CH_3$

Using the high resolution mass spectrometry, thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC), we have found that the so-called methoxychelidonine is a mixture consisting of three closely related alkaloids (+)-chelamine (*III*), (+)-homochelidonine (*V*) and (+)-chelamidine (*IV*) with a considerable predominance of chelamine. The content of these alkaloids in "methoxychelidonine" found by HPLC was 67, 28, and 5 wt.%, respectively.

Therefore, the name "methoxychelidonine" should be deleted from literature.

In the high resolution electron impact mass spectrum (HR EIMS) of "methoxychelidonine", two series of ions originating from the fragmentation of the molecular ions of chelamine $C_{20}H_{19}NO_6$ (*m/z* 369.1212) and homochelidonine $C_{21}H_{23}NO_5$ (*m/z* 369.1576) were observed, corresponding to the characteristic fragments occurring in the spectra of chelamine and homochelidonine, namely $M - H_2O$, $M - H_2O - CH_3$ and $M - H_2O - CH_3NH_2$. The presence of a small amount of chelamidine in "methoxychelidonine" was verified by TLC in several solvent systems and quantified by HPLC.

Figure 1*a* shows representative high-performance liquid chromatogram of "methoxychelidonine", Fig. 1*b* illustrates chromatogram of the mixture of standards chelamine, chelamidine and homochelidonine. The identity of peaks at Fig. 1*a* was confirmed by the comparison of the retention times of standards and peaks of "methoxychelidonine" and on the basis of chromatogram standards co-injected with the sample analyzed.

It is also worth noting that artificial mixtures of chelamine and homochelidonine, when crystallized together, yielded crystallizates of higher melting points than have had

the starting components. Especially, the melting points increased remarkably by even a small admixture of chelamidine. In this way, it was possible to obtain products with melting points very close to that of the authentic "methoxychelidonine". Similarly, the mixtures of chelamine and chelamidine also showed the higher melting point than that of the pure chelamidine.

The structures of chelamine (*III*) and chelamidine[12] (*IV*) as 12-hydroxychelidonine and 12-hydroxyhomochelidonine, respectively, were deduced[13] from spectral analyses (EIMS, ^{1}H NMR, IR and UV spectra), but neither the spectral data nor the stereochemical conclusions were published till now. The structures suggested were confirmed by total synthesis of both chelamine and chelamidine by Hanaoka et al.[14,15] several years later. HR EIMS of chelamine, chelamidine and homochelidonine, given in the Experimental, are reported for the first time.

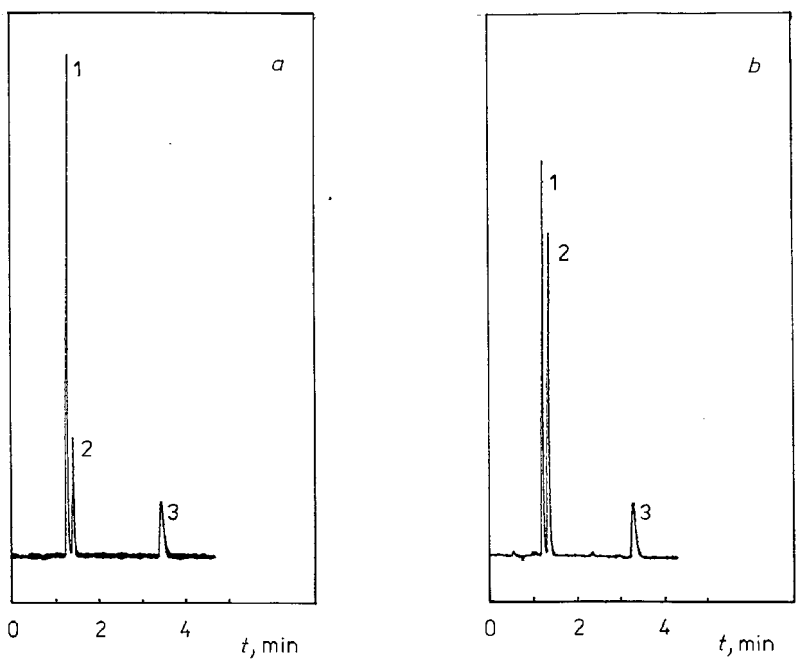


FIG. 1

HPLC analysis (UV detection at 280 nm): *a* of the so-called methoxychelidonine *b* of the model mixture of chelamine, chelamidine and homochelidonine. Peaks: 1 (+)-chelamine (*III*), 2 (+)-chelamidine (*IV*), 3 (+)-homochelidonine (*V*)

EXPERIMENTAL

The melting points were determined on a Mettler apparatus FP 51 and were not corrected. The mass spectra were measured on an AEI-MS 902 spectrometer, ^{1}H NMR measurement (in $CDCl_3$) were performed with a Varian HA-100 (100 MHz) and Tesla 587 A (80 MHz) instruments using hexamethyldisiloxan or tetramethylsilane as internal standards, chemical shifts are given in ppm (δ-scale) and coupling constants (*J*) in Hz. IR spectra (in Nujol) were recorded on a Zeiss UR 10 and UV spectra (in methanol) on a Unicam SP 1800 spectrometer. Liquid chromatography equipment consisted of solvent pump LCP 3001, UV detector LCD 2040 with variable wavelength, linear recorder TZ 4221, Rheodyne 7125 sample injector with sample loop 50 μl. Column 3 × 150 Separon™ SGX C18 (5 μm) Tessek was used. The thin layer chromatography (TLC) was carried out on silica gel G (Merck) using the following systems: cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S1), 6 : 3 : 1 (S2), and 5 : 4 : 1 (S3), benzene–methanol 9 : 1 (S4) and 4 : 1 (S5). The spots were detected with potassium hexaiodoplatinate(IV). Preparative TLC was performed on the thin layer of silicagel (Merck) with the layer thickness 1.5 mm. The solvent system S5 was used.

Characteristics of the Compounds Used

(+)-*Chelamine* (III): some crude fractions obtained formerly[11] were purified by crystallizations of poorly soluble hydrochloride from water; base from methanol, prisms m.p. 201 – 202 °C, $[\alpha]_D^{21}$ +111° ± 2° (*c* 0.3, ethanol). HR EIMS: *m/z* 369.1223 (M^+, calculated for $C_{20}H_{19}NO_6$ 369.1212), 368, 367, 351, 351.1106 (M – H_2O, calculated for $C_{20}H_{17}NO_5$ 351.1107), 336.0887 (M – H_2O – CH_3, calculated for $C_{19}H_{14}NO_5$ 336.0872), 320.0687 (M – H_2O – CH_3NH_2, calculated for $C_{19}H_{12}O_5$ 320.0685). ^{1}H NMR spectrum: 2.24 s, 3 H (NCH_3); 3.24 t, 3.42 d, 3.48 d, 4.07 d, 4 H; 4.01 m, 1 H; 4.74 d, 1 H; 5.92 d, 5.98 d, each 2 H (2 × OCH_2O); 6.62 and 6.96, AB system, *J* = 7; 6.76 s, 2 H. IR spectrum: ν(OH) broad band 3 200 – 3 400 cm^{-1}. UV spectrum: λ_{max}, nm (log ε): 208 (4.73), 240 sh (3.96), 289 (3.92); λ_{min} 259 (3.44). TLC: R_F 0.19 (S1), 0.24 (S2), 0.41 (S3), 0.37 (S4), 0.58 (S5).

(+)-*Chelamidine* (IV): the product isolated some time ago[11] contained small amount of chelamine even after several crystallizations; pure substance was obtained from a mixed fraction of m.p. 239 – 240 °C (consisting of 58% chelamine and 42% chelamidine) by means of several times repeated preparative TLC; prisms m.p. 231 – 232 °C (methanol), $[\alpha]_D^{24}$ +120° ± 2° (*c* 0.3, ethanol). HR EIMS: *m/z* 385.1531 (M^+, calculated for $C_{21}H_{23}NO_6$ 385.1525), 384, 383, 367.1419 (M – H_2O, calculated for $C_{21}H_{21}NO_5$ 367.1420), 336.0995 (M – H_2O – CH_3NH_2, calculated for $C_{20}H_{16}O_5$ 336.0998). ^{1}H NMR spectrum: 2.31 s, 3 H (NCH_3); 3.32 br s, 1 H; 3.48 – 3.67 m, 1 H; 3.49 and 4.24, AB system, *J* = 16.3; 3.87 s, 6 H (2 × OCH_3); ; 2 H; 4.14 – 4.02 m, 1 H; 4.83 d, 1 H; 5.99, 5.97 AB system, *J* = 1.5, (OCH_2O); 6.71 s, 6.95 s, 2 H; 6.86 and 7.05, AB system, *J* = 8.6. IR spectrum: ν(OH) 3 330 cm^{-1}. UV spectrum: λ_{max}, nm (log ε): 208 (4.59), 238 sh (3.88), 284 (3.49), λ_{min} 260 (2.64). TLC: R_F 0.15 (S1), 0.21 (S2), 0.36 (S3), 0.27 (S4), 0.49 (S5).

(+)-*Homochelidonine* (V): large prisms m.p. 187 – 188 °C (ethanol), $[\alpha]_D^{22}$ +128° ± 2° (*c* 1.1, ethanol). HR EIMS: *m/z* 369.1579 (M^+, calculated for $C_{21}H_{23}NO_5$ 369.1576), 368, 367, 351.1476 (M – H_2O, calculated for $C_{21}H_{21}NO_4$ 351.1470), 336.1230 (M – H_2O – CH_3, calculated for $C_{20}H_{18}NO_4$ 336.1236), 320.1055 (M – H_2O – CH_3NH_2, calculated for $C_{20}H_{16}O_4$ 320.1049). ^{1}H NMR spectrum: 2.29 s, 3 H (NCH_3); 2.95 t, *J* = 2.6, 1 H; 3.10 – 3.18 m, 2 H; 3.42, 4.21 AB system, *J* = 16, 3.50 br m, 1 H, 3.86 s, 3.87 s, each 3 H (2 × OCH_3); 4.22 br s, 1 H; 5.91, 5.94 AB system, *J* = 1.4, 2 H (OCH_2O); 6.65 s, 2 H; 6.85, 6.99 AB system, *J* = 8.6, 2 H; 7.71 br s, 1 H (OH). IR spectrum: no absorption band in the region of 3 000 – 4 000 cm^{-1}. UV spectrum: λ_{max}, nm (log ε): 208 (4.73), 240 sh (4.17), 288 (3.80), λ_{min} 259 (3.29). TLC: R_F 0.66 (S1), 0.68 (S2), 0.88 (S3), 0.72 (S4), 0.83 (S5).

"*Methoxychelidonine*": prisms m.p. 222 – 223 °C (ref.[1]: m.p. 221 °C). HR MS: all peaks at *m/z* 369, 351, 336 and 320 are doublets corresponding to those in the spectra of chelamine, and homochelidonine. TLC: in all solvent systems used (S1 – S5) three spots of R_F values identical with those of the reference samples of chelamine, chelamidine and homochelidonine.

High-Performance Liquid Chromatography of "Methoxychelidonine"

The mobile phase consisted of two solvents. *A* contained heptanesulfonic acid (Sigma) 10 mmol/l and triethylamine (Sigma) 0.1 mol/l in redistilled water, pH was adjusted to 2.5 with H_3PO_4. *B* was acetonitrile. Isocratic elution with 33% *B* in *A* was used. Flow rate was 0.5 ml/min, chart speed 0.3 cm/min. The substance eluted were detected at 280 nm. The determination of chelamine, chelamidine and homochelidonine in the mixtures was accomplished by comparison of their peak hights with calibration graphs (regression coefficients 0.9955 for chelamine, 0.9965 for chelamidine, and 0.9988 for homochelidonine).

Preparation of Artificial "Methoxychelidonine"

a) Mixture of 10.8 mg chelamine (with a trace of chelamidine), m.p. 202 °C, and 5.2 mg homochelidonine, m.p. 188 °C, was dissolved in chloroform, evaporated to a small volume and methanol added. The crystals obtained (5.7 mg) had m.p. 211 – 213 °C and consisted, according to HPLC, from 33% chelamine, 63.5% homochelidonine and 3.5% chelamidine. The mother liquors yielded a mixture of individual crystals (5.2 mg) of chelamine (m.p. 201 °C) and of a less pure homochelidonine (m.p. 183 °C).

b) A mixed fraction of chelamine and chelamidine (in the ratio of about 10 : 1), m.p. 207 °C, when crystallized together with homochelidonine by the same way, yielded a crystallizate of m.p. 219 – 220 °C, which contained 40.5% chelamine, 37.8% homochelidonine and 21.7% chelamidine.

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