MINOR ALKALOIDS FROM *Stylophorum diphyllum* (MICHX.) NUTT.*

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In addition to known alkaloids in *Stylophorum diphyllum* (MICHX.). NUTT., *i.e.* (−)-stylopine, (+)-chelidonine, coptisine, protopine, sanguinarine, macarpine, chelirubine, and (±)-chelidonine (diphylline) the following minor alkaloids were also isolated: (±)-stylopine, chelerythrine, corysamine, corytuberine, magnoflorine, (−)-β-N-methylstylopinium hydroxide and (−)-α-N-methylstylopinium hydroxide (the last three after conversion to iodides). Allocryptopine, berberine, cryptopine, isoboldine and scoulerine were detected in this material in trace amounts.

Stylophorum diphyllum (MICHX.) NUTT. is a perennial herb from the *Papaveraceae* family, *Chelidonieae* tribe, indigenous to North America. We investigated the alkaloids of this plant earlier[1] and isolated as dominant components from the roots or the aerial parts coptisine, (−)-stylopine and (+)-chelidonine in addition to a smaller amount of protopine, sanguinarine, macarpine, chelirubine and (±)-chelidonine (diphylline[2]). For the alkaloid macarpine, which we isolated for the first time from *Macleaya microcarpa* (MAXIM). FEDDE[3] and for which we assumed the structure of dimethoxysanguinarine[4] with the methoxyl groups located tentatively in the positions 11 and 12, the structure of 10,12-dimethoxysanguinarine (*Ia*) has been demonstrated more recently[5]. In this paper we concentrated on the study of minor alkaloids and especially on the question of the presence of highly polar quaternary alkaloids which usually remain in aqueous phase after conventional isolation procedures. We have found thirteen additional alkaloids of which seven were obtained in crystalline form and additional six were identified chromatographically.

As found earlier, the root of *S. diphyllum* is so far the richest source of the quaternary protoberberine alkaloid coptisine (*IIa*), from which it may be obtained very simply in pure form[1]. Several recent reports can be found concerning the biological activity of quaternary protoberberines, including coptisine (*cf.* review[6,7]). More recently its inhibitory effect on some enzymes (for example[8–11]), its interaction with biopolymers and the inhibition of their biosynthesis[12–14], *etc.* have been

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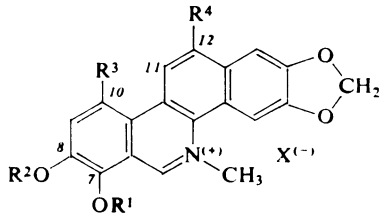
ascertained. In addition to this coptisine is one of the cytotoxic principles of *Chelidonium majus* L.[15], of the antiinflammatory components of the rhizomes of *Coptis japonica* MAKINO[16] and it has a stronger antimicrobial activity than other protoberberines (*cf.*[6]).

In our previous study[1] we observed that the alkaloid fraction from the root and the aerial part of *S. diphyllum* differ both in total content and in the proportion of individual alkaloids, and therefore we worked up these two plant parts separately. From the dry plant material, collected during the flowering and the period of unripe fruits, we isolated the sum of alkaloids in a 0·58% yield from the roots and 0·23% yield from the aerial parts. From the less basic fraction of the root (−)-stylopine and (+)-chelidonine were isolated as the main components, in addition to smaller amounts of protopine, (±)-chelidonine and a mixture of quaternary benzophenanthridines. From this mixture we isolated by column chromatography on alumina (see[17]) a small amount of chelerythrine (*Ib*) (the presence of which in *S. diphyllum* was not known so far), in addition to sanguinarine, macarpine and chelirubine[1]. The main component of the weakly basic fraction from the aerial part was also stylopine, which, however, represented a mixture of (−)- and (±)-forms according to optical rotation values. Both these forms could be separated to a considerable degree on the basis of a lower solubility of the racemate. (±)-Stylopine has not been detected in *S. diphyllum* so far. In contrast to the root, (+)-chelidonine and protopine were present in small amounts only, while (±)-chelidonine and quaternary benzophenanthridines were completely absent. In the mother liquors after the mentioned alkaloids from the root and the aerial part, trace amounts of allocryptopine, corydine, cryptopine, isoboldine and scoulerine were detected chromatographically.

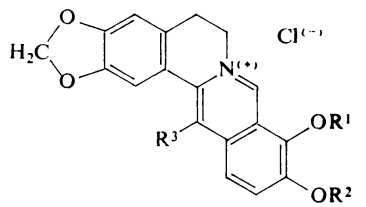
From the strongly basic fraction of quaternary protoberberines from roots and aerial parts, which was converted to chlorides, coptisine chloride (*IIa*) was obtained as the main component, and from the mother liquor a small amount of corysamine chloride (*IIb*) was obtained the presence of which had been detected chromatographically earlier[1,18]. In the mother liquors after crystallization of corysamine chloride trace amounts of berberine (*IIc*) were detected.

Since the aqueous layer after the separation of alkaloids extractable with ether with a weakly or strongly alkaline (pH < 13) reaction still gave a weakly positive reaction to alkaloids with Mayer's reagent, it was adjusted to pH 6−7, additioned with excess potassium iodide and extracted with chloroform in order to obtain highly polar alkaloids (see[19,20]). From the fraction of iodides obtained we isolated (−)-β-N-methylstylopinium iodide (*trans*-B/C rings juncture, *IIIa*) in addition to a smaller amount of (−)-α-N-methylstylopinium iodide (*cis*-B/C rings juncture, *IIIb*). In addition to this magnoflorine (*IV*) was obtained from the root in the form of iodide and its biogenetical precursor corytuberine (*V*) from the aerial part. Magnoflorine and corytuberine represent the first two aporphine alkaloids isolated from *S. diphyllum*. The occurrence of the mentioned three quaternary N-methylated

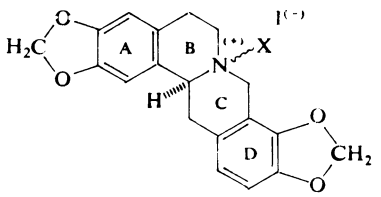
alkaloids is further evidence for the close relationship of *S. diphyllum* and *Ch. majus* in which the same quaternary alkaloids were detected[21]. However, in comparison with *Ch. majus* the content of magnoflorine in *S. diphyllum* is approximately lower by two orders of magnitude.



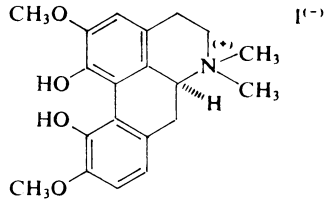
Ia, $R^1 + R^2 = CH_2$, $R^3 = R^4 = OCH_3$
Ib, $R^1 = R^2 = CH_3$, $R^3 = R^4 = H$



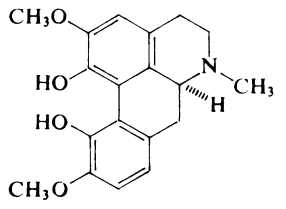
IIa, $R^1 + R^2 = CH_2$, $R^3 = H$
IIb, $R^1 + R^2 = CH_2$, $R^3 = CH_3$
IIc, $R^1 = R^2 = CH_3$, $R^3 = H$



IIIa, ~ X = —CH_3
IIIb, ~ X = ····CH_3



IV



V

Similarly as in *Ch. majus*[18,21] the content and the ratio of individual alkaloids in *S. diphyllum* is also largely dependent on the vegetation period. Together with the above mentioned sample we also investigated the alkaloids of this plant in the period of the vegetational rest, when the aerial part is withered. This sample of the root displayed not only a substantially higher content of alkaloids (1·18%), but it also differed by a considerably increased content of chelidonine, coptisine and protopine, and a decreased content of stylopine, while the presence of quaternary benzophenanthridines could not be detected at all.

EXPERIMENTAL

The melting points up to 280°C were determined on a Mettler FP 51 apparatus, above 280°C on a Kofler block, and they are not corrected. The UV spectra were measured in methanol on a Unicam SP 1800 and the IR spectra in nujol on a Specord 75 IR, Zeiss, Jena, instrument. For thin-layer chromatography (TLC) silica gel G Merck was used with the solvent systems cyclohexane–diethylamine 9 : 1 (S_1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S_2) and 6 : 3 : 1 (S_3), methanol–water–diethylamine 15 : 3 : 1 (S_4), ethanol–water–diethylamine 15 : 9 : 1 (S_5) and propanol–water–formic acid 12 : 7 : 1 (S_6), or Silufol UV 254 (Kavalier) sheets with the systems methanol–diethylamine 4 : 1 (S_7) and 1 : 1 (S_8). Paper chromatography (PC) was carried out on Whatman paper No 1, descending mode, using the systems butanol–water–acetic acid 10 : 3 : 1 (S_9) and ethanol–water 3 : 2 (S_{10}). The spots of fluorescing alkaloids were detected with UV light, while the spots of other alkaloids were visualized with potassium iodoplatinate (TLC) or Dragendorff's reagent (PC).

Extraction and Isolation of Alkaloids

The plants were cultivated at the Centre for the Cultivation of Medicinal Plants, Medical Faculty, Brno, and they were gathered on 22nd June 1981 in the second and third year of vegetation, at the stage of flowering and ripening of the first fruits (sample 1: dry weight of the roots 612 g, of the aerial parts 1 708 g), and — in the case of the roots of three years old plants at the period of the vegetational rest — on the 17th November 1970 (sample 2: dry weight 850 g). Both samples came from the same population. The plant material was dried at room temperature and worked up immediately after in order to prevent possible charges of native alkaloids. The voucher specimen is deposited in our Department. Unless stated otherwise the procedure of the isolation of alkaloids from sample 1 is described in further text.

Root: Dry, ground root (612 g) was extracted in a Soxhlet extractor with methanol. The solvent was removed by distillation and the residue was treated with 1% sulfuric acid. The insoluble matter which still contained a considerable amount of coptisine was extracted repeatedly with hot water and filtered. The combined filtrates were alkalized with sodium carbonate and extracted four times with ether (fraction *A*), the aqueous layer was alkalized with sodium hydroxide to pH < 13 and extracted again four times with ether (fraction *B*). The remaining aqueous layer was adjusted to pH about 6—7 with 20% sulfuric acid, a solution of 50 g of potassium iodide was added and the mixture extracted five times with chloroform (fraction I) until the aqueous layer no longer reacted with Mayer's reagent. When alkaloids from sample 2 were isolated, the procedure for obtaining fraction I was not applied.

Fraction *A* after purification (2·80 g) was crystallized from chloroform–methanol to afford 1·31 g of (—)-stylopine. From the rest of the bases, converted to hydrochlorides, 0·69 g of (+)--chelidonine hydrochloride was obtained by crystallization from methanol. The hydrochlorides from the mother liquors were dissolved in water, alkalized with a sodium cyanide solution and the solution obtained was acidified with dilute hydrochloric acid. The insoluble precipitate of *ps*-cyanides of quaternary benzophenanthridines was filtered off under suction (0·12 g) and the filtrate was separated by extraction with chloroform to a fraction of hydrochlorides extractable with chloroform (*AC*) and non extractable with this solvent (*AD*). From the fraction *AC*, which was converted to bases, crystallization from methanol gave a further fraction of (—)-stylopine (0·08 g). In the amorphous residue (0·07 g) six further alkaloids were detected in addition to stylopine, of which corydine (R_F in S_2 and S_3 0·52 and 0·69, resp., grey-blue spot) was identified by TLC. The fraction *AD* was separated in the conventional manner to non-phenolic (AD_1) and phenolic (AD_2) bases. From fraction AD_1 protopine (0·15 g) and (±)-chelidonine (17 mg) were isolated

by crystallization from chloroform–methanol, and a further amount of (+)-chelidonine (base 0·09 g) was separated from mother liquors in the form of poorly soluble hydrochloride. In the remaining amorphous residue (0·02 g) small amounts of cryptopine (R_F in S_1 and S_2 0·19 and 0·56, red-violet spot) and allocryptopine (R_F in S_1, S_2 and S_3 0·29, 0·61 and 0·79, brown-violet spot) were detected by TLC, in addition to the residues of the above mentioned alkaloids. Fraction AD_2 (15 mg) was amorphous and according to TLC it contained trace amounts of scoulerine (R_F in S_2 and S_3 0·19 and 0·29, brown spot), isoboldine (R_F in S_2 and S_3 0·09 and 0·16, violet-red spot) and a third unidentified alkaloid.

ps-Cyanides of quaternary benzophenanthridines were converted to chlorides by 1 h boiling in chloroform–methanol solution with hydrochloric acid[17]. The bases (0·08 g) were set free from them. Chromatography on acid alumina (see[17]) afforded chelirubine (8·6 mg), sanguinarine (33·2 mg), macarpine (11·3 mg) and chelerythrine (1·4 mg).

Solid citric acid was added to the ethereal solution of the bases of fraction *B*. An orange precipitate of citrates of quaternary protoberberines was thus obtained. Ether was evaporated, the citrates dissolved in boiling water and hydrochloric acid added. Pure coptisine chloride (1·08 g) crystallized out, which was filtered off under suction. The mother liquor was alkalized with sodium hydroxide to pH > 13 and extracted with ether. After conversion to hydrochlorides in the above mentioned manner another 10 mg of coptisine chloride were obtained, and 1·3 mg of corysamine chloride crystallized from mother liquor after prolonged standing. From the mother liquors additional 13 mg of impure bases were recovered which according to TLC contained predominantly corysamine in addition to a smaller amount of coptisine and berberine (R_F in S_7, S_8, S_9 and S_{10} 0·25, 0·70, 0·63 and 0·21).

After evaporation of chloroform fraction I was dissolved in boiling water, active charcoal was added and the mixture filtered, then potassium iodide was added and the mixture extracted repeatedly with chloroform. The residue after evaporation of chloroform (0·26 g) was separated to a non-phenolic (I_1) and a phenolic (I_2) fraction[22]. Crystallization of fraction I_1 from methanol gave first the poorly soluble β-N-methylstylopinium iodide (9·1 mg) and from the mother liquors the better soluble α-N-methylstylopinium iodide (1·1 mg). In the amorphous residue small amounts of two additional alkaloids (R_F 0·04 and 0·12 in S_4) were detected by TLC. From fraction I_2 magnoflorine iodide (7·2 mg) was isolated by crystallization from methanol and two unidentified alkaloids were detected by TLC (R_F 0·46 and 0·65 in S_4) in addition to traces of corytuberine.

Aerial part: The dry aerial part (1 708 g) was further extracted and processed as described above (for the root). From the bases of fraction *A* (3·55 g) 1·26 g of stylopine were obtained by crystallization from chloroform–methanol, m.p. 200–218°C. According to $[\alpha]_D^{21}$ −139° ± 3° (*c* 0·26, chloroform) it was a mixture of 56% of racemate and 44% of the (−)-form. Crystallization of this mixture from chloroform–methanol gave the less soluble (±)-stylopine (0·60 g). From the mother liquors after (±)-stylopine, from the residues of bases of fraction *A* and from fraction *AC* (0·38 g) a total of 1·53 g of the better soluble optically pure (−)-stylopine was obtained, together with 0·63 g of (−)-stylopine with an admixture of the racemate (the total yield of stylopine was 2·76 g, *i.e.* 0·16%, of the aerial part). In the amorphous fraction *AC* (0·20 g) small amounts of allocryptopine and four unidentified alkaloids were detected by TLC in addition to stylopine and protopine. Crystallization of fraction AD_1 (0·54 g) from chloroform–methanol gave protopine (0·18 g), while crystallization of hydrochlorides from water afforded (+)-chelidonine hydrochloride (base: 0·15 g). The amorphous residue of the bases (0·08 g), regenerated from the mother liquors of the hydrochlorides contained according to TLC a negligible amount of allocryptopine and further unidentified alkaloid in addition to chelidonine and protopine. In the amorphous fraction AD_2 (0·05 g) scoulerine, a small amount of isoboldine and an unidenti-

fied alkaloid (R_F 0·47 in S_3) were identified by TLC. From fraction *B* 0·39 g of coptisine chloride and 19 mg of corysamine chloride were obtained in the above mentioned manner.

Fraction I was crystallized from methanol to give 19·6 mg of (−)-β-N-methylstylopinium iodide and the residue was separated into a nonphenolic (I_1) and a phenolic (I_2) fraction. Crystallization of fraction I_1 from methanol gave (−)-α-N-methylstylopinium iodide (5·5 mg), while I_2 gave corytuberine hydriodide (6·2 mg). Using TLC small amounts of two further alkaloids (R_F 0·04 and 0·12 in S_4) were identified in the residue of the fraction I_1 and one unidentified alkaloid (R_F 0·58 in S_4) in the residue of the fraction I_2.The presence of magnoflorine could not be proved.

Characterization of the Isolated Alkaloids

The isolated alkaloids were characterized by melting points, mixed melting points, optical rotation values, UV and IR spectra and co-chromatography on thin layers or paper with authentic samples. The yields of individual alkaloids in mass % of dry plant material, roots and aerial parts of sample 1 and roots of sample 2, are given in brackets.

(−)-*Stylopine* (0·23; 0·12; 0·16): needles, m.p. 203–204°C, $[\alpha]_D^{21}$ −318° ± 3° (*c* 0·26, chloroform). UV spectrum: λ_{max} (log ε) 209 nm (4·62), 287 nm (3·93), shoulder 236 nm (4·02), λ_{min} 257 nm (3·01). With Erdmann's reagent it gave a characteristic green-blue and then blue coloration. R_F in S_1, S_2 and S_3 0·68, 0·83 and 0·95 (orange), respectively.

(±)-*Stylopine* (−; 0·042; −): needles, m.p. 221–222°C, $[\alpha]_D^{24}$ 0° ± 3° (*c* 0·20, chloroform). UV and IR spectrum, R_F values and reaction with Erdmann's reagent were the same as in the case of the (−)-form.

(+)-*Chelidonine* (0·12; 0·008; 0·60): prisms from ether or aqueous ethanol, m.p. 114–116°C (hydrate) or 135–136°C (anhydrous), $[\alpha]_D^{25}$ +115° ± 3° (*c* 0·58, methanol). UV spectrum: λ_{max} (log ε) 210 nm (4·66), 238 nm (4·00), 288 nm (3·91), λ_{min} 230 nm (3·95), 258 nm (3·25); IR spectrum: ν(OH) 3 250, 3 360 and 3 630 cm^{-1}. With Erdmann's reagent it gave a green coloration. R_F in S_1, S_2 and S_3 0·16, 0·52 and 0·86 (brown-yellow).

(±)-*Chelidonine* (0·003; −; 0·006): hexangular leaflets, m.p. 217–218°C (chloroform–ethanol), optically inactive; UV and IR spectrum, R_F values and colour reaction with Erdmann's reagent were the same as in (+)-chelidonine.

Protopine (0·025; 0·011; 0·044): prisms, m.p. 209–210°C (chloroform–ethanol). UV spectrum: λ_{max} (log ε) 209 nm (4·64), 240 nm (3·95), 287 nm (3·90), λ_{min} 231 nm (3·90), 259 nm (3·25); IR spectrum: ν(C=O) 1 660 cm^{-1}. R_F in S_1, S_2 and S_3 0·36, 0·68 and 0·88 (brown-violet).

Sanguinarine (0·0054; −; −): chloride, copper red needles, m.p. 278–281°C (dilute hydrochloric acid). UV spectrum: λ_{max} (log ε) 234 nm (4·45), 284 nm (4·48), 325 nm (4·14), λ_{min} 255 nm (4·14), 311 nm (4·04). R_F in S_9 0·53 (orange).

Macarpine (Ia) (0·0019; −; −): chloride, crimson red needles, m.p. 282–283°C (dilute hydrochloric acid). UV spectrum: λ_{max} (log ε) 222 nm (4·53), 287 nm (4·49), 318 nm (3·97), 346 nm (4·02), shoulder 240 nm (4·34), λ_{min} 256 nm (4·04), 310 nm (3·92), 325 nm (3·93); it was in good agreement with the spectrum of a preparation from *Escholtzia douglasii*[4]. R_F in S_9 0·60 (red).

Chelirubine (0·0014; −; −): chloride, purple red needles, m.p. 284–286°C (dilute hydrochloric acid). UV spectrum: λ_{max} (log ε) 229 nm (4·37), 281 nm (4·33), 340 nm (4·07), 353 nm (3·99), λ_{min} 252 nm (4·08), 314 nm (3·87). R_F in S_9 0·61 (purple).

Chelerythrine (Ib) (0·0002; −; −): chloride, yellow needles, m.p. 207–209°C (dilute hydrochloric acid). UV spectrum: λ_{max} (log ε) 228 nm (4·54), 283 nm (4·65), 320 nm (4·19), λ_{min} 253 nm (4·24), 311 nm (4·17). R_F in S_9 0·67 (yellow).

Coptisine (IIa) (chloride 0·18; 0·023; 0·36): orange needles (water), does not melt up to 350°C (carbonization). UV spectrum: λ_{max} (log ε) 209 nm (4·39), 229 nm (4·46), 244 nm (4·44), 267 nm (4·41), 353 nm (4·34), 460 nm (3·61), λ_{min} 215 nm (4·38), 239 nm (4·43), 254 nm (4·35), 307 nm (3·69), 395 nm (2·64). R_F in S_7, S_8, S_9 and S_{10} 0·58, 0·83, 0·49 and 0·09 (golden-yellow).

Corysamine (IIb) (chloride 0·0002; 0·0011; 0·0018): bronze coloured leaves (water), does not melt up to 350°C (carbonization). UV spectrum, λ_{max} (log ε) 208 nm (4·43), 231 nm (4·50), 268 nm (4·44), 346 nm (4·32), 452 nm (3·67), shoulder 244 nm (4·43), λ_{min} 214 nm (4·42), 254 nm (4·35), 307 nm (3·82), 394 nm (2·97), and the IR spectrum were identical with those of an authentic sample. The same is true of chromatographic data, R_F in S_7, S_8, S_9 and S_{10} 0·12, 0·60, 0·73 and 0·72 (green-yellow).

(—)-β-N-*Methylstylopinium iodide* (IIIa) (0·0015; 0·0012; —): needles from methanol, m.p. 297—298°C, $[\alpha]_D^{24}$ —123° ± 3° (*c* 0·11, methanol). UV spectrum: λ_{max} (log ε) 209 nm (4·46), 287 mm (3·78), shoulder 244 nm (3·81), λ_{min} 262 nm (3·30). R_F in S_4, S_5, S_6, S_9 and S_{10} 0·26, 0·55, 0·71, 0·59 and 0·19.

(—)-α-N-*Methylstylopinium iodide* (IIIb) (0·0002; 0·0003; —): needles from methanol, m.p. 278—280°C, undepressed in admixture with a reference sample. The UV spectrum was identical with that of the β-form, the IR spectrum was identical with that of reference samples of the α-form, but it was not identical with the spectrum of the β-form; the same is true of the R_F values in TLC and paper chromatography, R_f in S_4, S_5, S_6, S_9 and S_{10} 0·21, 0·48, 0·67, 0·76 and 0·75.

Magnoflorine (IV) (iodide 0·0012; —; —): iodide from methanol m.p. 264—265°C, perchlorate trom methanol m.p. 282—284°C, both undepressed in mixtures with reference samples. UV specfrum: λ_{max} (log ε) 226 nm (4·66), 274 nm (3·87), 320 nm (3·77), λ_{min} 263 nm (3·83), 292 nm (3·41); IR spectrum: ν(OH) 3 180 cm^{-1}. R_F in S_4, S_5, S_6, S_9 and S_{10} 0·51, 0·65, 0·53, 0·47 and 0·43.

Corytuberine (V) (—; 0·0004; —): hydriodide from methanol gives prisms, m.p. 212—213°C, undepressed with an authentic sample. UV spectrum: λ_{inax} (log ε) 224 nm (4·72), 272 nm (4·06), 310 nm (3·78), λ_{min} 257 nm (3·97), 291 nm (3·57); IR spectrum: ν(OH) 3 400, 3 520 and 3 590 cm^{-1}. R_F in S_4, S_5 and S_6 0·92, 0·89 and 0·72.

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