ALKALOIDS FROM *Stylophorum lasiocarpum* (OLIV.) FEDDE*

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Received July 3, 1990
Accepted August 15, 1990

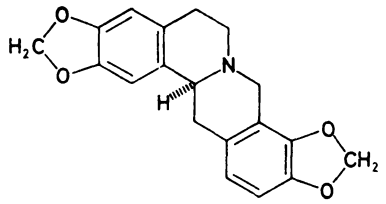
Dedicated to the memory of Professor František Šorm.

From the underground part of *Stylophorum lasiocarpum* (OLIV.) FEDDE (0·58% alkaloids) (−)-stylopine and coptisine (as chloride *IIa*) were isolated as the principal alkaloids. Smaller quantities of sanguinarine, macarpine, chelerythrine, chelirubine, chelilutine (as the respective chlorides *IIIa*, *IIIb*, *IIIc*, *IIId*, *IIIe*), protopine, corysamine (as chloride *IIb*), berberine (as chloride *IIc*) and (−)-*trans*-N-methylstylopinium hydroxide (as iodide *IVa*) were also isolated. Chromatographically were detected small amounts of chelidonine, allocryptopine, cryptopine, scoulerine, isoboldine, magnoflorine and salt of *cis*-N-methylstylopinium. The aerial part (0·16% alkaloids) afforded (−)-stylopine, coptisine and (+)-chelidonine as dominant alkaloids and small amounts of protopine and (−)-*cis*-N-methylstylopinium hydroxide (as iodide *IVb*). Chromatography proved negligible amounts of sanguinarine, chelirubine, cryptopine, scoulerine, isoboldine, corytuberine, corysamine, berberine, magnoflorine and *trans*-N-methylstylopinium iodide. The so far undescribed 8-methoxydihydromacarpine (*VIb*) was prepared.

The genus *Stylophorum* NUTT., tribe *Chelidonieae* REICHB., family *Papaveraceae*, includes (according to Fedde[1]) three species of which one grows in the Atlantic region of North America and the other two in the most eastern part of the Middle-Asian region. All these species contain orange to blood-red latex. Of this genus only alkaloids from the North-American species *S. diphyllum* (MICHX.) NUTT. have been hitherto investigated[2−4]. *S. lasiocarpum* (OLIV.) FEDDE (synonymum *Chelidonium lasiocarpum* OLIV.) is a perennial herb from Central and Eastern China and its root, containing a blood-red latex, is used in Chinese traditional medicine. So far, nothing has been known about alkaloids of this plant.

Our present communication concerns the alkaloid investigation of plants cultivated on Czechoslovak territory and harvested at the stage of flowering and unripe fruits. From the relatively small amount of plant material available we isolated thirteen individual alkaloids and further six, present in only negligible quantities, were identified by chromatography.

* Part XCI in the series Alkaloids of the *Papaveraceae*; Part XC: Collect. Czech. Chem. Commun. *55*, 1812 (1990).



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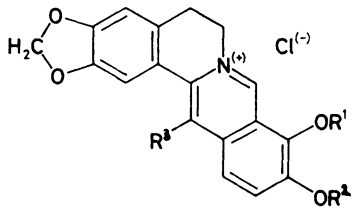
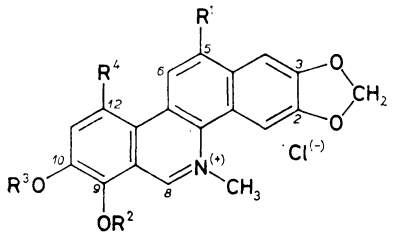
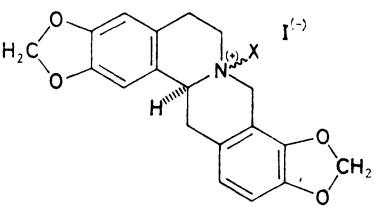


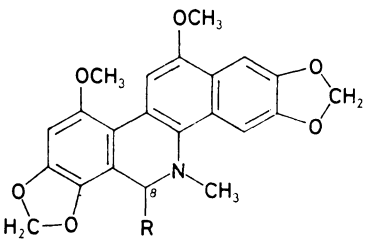
IIa, $R^1 + R^2 = CH_2$; $R^3 = H$
IIb, $R^1 + R^2 = CH_2$; $R^3 = CH_3$
IIc, $R^1 = R^2 = CH_3$; $R^3 = H$



IIIa, $R^1 = R^4 = H$, $R^2 + R^3 = CH_2$
IIIb, $R^1 = R^4 = OCH_3$; $R^2 + R^3 = CH_2$
IIIc, $R^1 = R^4 = H$; $R^2 = R^3 = CH_3$
IIId, $R^1 = H$; $R^2 + R^3 = CH_2$; $R^4 = OCH_3$.
IIIe, $R^1 = H$; $R^2 = R^3 = CH_3$; $R^4 = OCH_3$



IVa, X = $-CH_3$
IVb, X = $\equiv CH_3$



Va, R = CN
Vb, R = OCH_3
Vc, R = OH

From the underground part, i.e. rhizome and roots, we obtained 0·58% of alkaloids. (−)-Stylopine ((−)-tetrahydrocoptisine, *I*; 0·25%) and coptisine (as chloride *IIa*; 0·20%) were the dominant alkaloids; these alkaloids represent also the main constituents of *S. diphyllum*[3,4]. A significant portion of the total alkaloid content were quaternary benzophenanthridine alkaloids (0·082%) which, together with

coptisine, are responsible for the blood-red colour of the latex. Column chromatography of this fraction on acidic alumina, performed as usual in our investigations (see e.g. ref.[5]), separated sanguinarine and macarpine as the principal constituents of this fraction, along with smaller amounts of chelerythrine, chelirubine and chelilutine; all these alkaloids were characterized as the corresponding chlorides (*IIIa*, *IIIb*, *IIIc*, *IIId* and *IIIe*, respectively). The fraction of tertiary bases afforded minor quantities of protopine; the quaternary protoberberine fraction gave, besides coptisine, small amounts of corysamine chloride (*IIb*) and berberine chloride (*IIc*). The fraction of strongly polar alkaloids, separated as iodides (see e.g. ref.[4]) gave negligible amount of (−)-*trans*-N-methylstylopinium iodide (*IVa*). In the mother liquors from the above-mentioned fractions we identified chromatographically minor amounts of chelidonine, allocryptopine, cryptopine, scoulerine, isoboldine, corytuberine, magnoflorine and *cis*-N-methylstylopinium iodide.

The total amount of alkaloids in the aerial part was considerably smaller (0·16%), the dominant alkaloids being again (−)-stylopine (0·082%) and coptisine (as chloride; 0·048%). In contrast to the underground part, we found also (+)-chelidonine (0·016%) among the dominant alkaloids. As minor alkaloids we isolated protopine and (−)-*cis*-N-methylstylopinium hydroxide (obtained as iodide *IVb*). Quaternary benzophenanthridines were represented only by negligible amounts of sanguinarine and chelirubine. In addition, we detected chromatographically small amounts of cryptopine, scoulerine, isoboldine, corytuberine, corysamine, berberine, magnoflorine and *trans*-N-methylstylopinium iodide.

Macarpine, isolated for the first time from *Macleaya microcarpa* (MAXIM.) FEDDE[6], is so far the only one hexa-oxygenated quaternary benzophenanthridine alkaloid found in nature. It has been hitherto found in negligible amounts only in several species of genera *Eschscholtzia*, *Macleaya* and *Stylophorum* (cf.ref.[7]). The underground part of *S. lasiocarpum* is so far the relatively most abundant source of this rare alkaloid. The originally suggested structure of dimethoxysanguinarine with the methoxy groups tentatively placed in positions 5 and 6 (see ref.[8]) has been later revised[9] in favour of the 5,12-dimethoxysanguinarine structure (*IIIb*) and confirmed by synthesis[9,10]. In these studies pseudocyanide (*Va*) or chloride (*IIIb*) of macarpine were used, however, no information about the free base was available. It appeared that, contrary to all other alkaloids (cf.[5]) with tetra- or penta-oxygenated patterns in positions 2, 3, 9, 10 or 2, 3, 9, 10, 12, the macarpine base did not crystallize from ether. Crystallization of the amorphous product from methanol afforded the expected 8-methoxydihydromacarpine (*Vb*, macarpine pseudomethanolate).

The presence of methoxyl in position 8 was confirmed by comparison of mass spectral fragmentation of *Vb* with that of pseudomethanolates of sanguinarine and chelerythrine. For all the three compounds, the most abundant ions are those arising by loss of the methoxy group, analogous to the loss of the CN group in pseudo-

cyanides[11]. This fragmentation is confirmed also by the metastable ion transition. The pseudobase *Vc*, if present in the substance at all, affords only ions of low abundance (*m/z* 409; 0·8%); a metastable transition, corresponding to loss of hydroxyl from the ionized molecule of the pseudobase, was not observed.

The properties of macarpine pseudomethanolate are typical of 8-alkoxydihydro derivatives of other known quaternary benzophenanthridines (see e.g. refs[5,12–14]). The alkoxy group bonded to C-8 has the character of an acetal group and on treatment even with weak or dilute acids this bond is immediately cleaved to give a salt of the quaternary cation *IIIb* whose fully aromatic system is manifested by an intense colouration of the solution.*

These findings show that the constituents of *S. lasiocarpum* do not differ qualitatively from those of the *S. diphyllum* species. Insignificant differences have been found only in the quantitative content and distribution of some alkaloids between the underground and aerial parts. It seems that the combination of stylopine and coptisine as dominant alkaloids with chelidonine and quaternary benzophenanthridines may be considered as a specific chemotaxonomic feature of the *Stylophorum* genus showing its close relation to the *Chelidonium* genus with the only one species *Ch. majus* L.

EXPERIMENTAL

The melting points were determined on a Mettler FP 51 instrument and are uncorrected. Mass spectra were taken on a Jeol MS D 100 spectrometer (ionizing chamber temperature 150°C, high resolution experiments were performed by the "peak match" method, metastable ions were followed by increasing the accelerating voltage at constant analyzer fields). IR spectra were measured in nujol on a Specord 75 IR (Zeiss, Jena) instrument and UV spectra in methanol on a Unicam SP 1800 spectrometer. Thin-layer chromatography (TLC) was performed on silica gel G (Merck). Less polar bases were chromatographed in the systems cyclohexane–diethylamine 9 : 1 (S1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S2) and 6 : 3 : 1 (S3), and benzene–methanol 4 : 1 (S4); strongly polar quaternary alkaloids and corytuberine in the systems methanol–water–25% aqueous ammonia 15 : 3 : 1 (S5), ethanol–water–25% aqueous ammonia 15 : 9 : 1 (S6), and 1-propanol–water–85% formic acid 12 : 7 : 1 (S7). Chromatography on Silufol UV 254 plates (Kavalier, Czechoslovakia) was performed in methanol (S8) (benzophenanthridines), in methanol–diethylamine 4 : 1 (S9) and 1 : 1 (S10) (quaternary protoberberines). Fluorescing alkaloids were detected by UV light, other alkaloids with potassium iodoplatinate.

Extraction and Isolation of Alkaloids

The plants were cultivated at the Center for Cultivation of Medicinal Plants, Medical Faculty, Brno, from the seeds from Göteborg botanical garden and harvested in the first vegetation year

* Even recently, 8-methoxy or 8-ethoxy dihydro derivatives of sanguinarine and chelerythrine are sometimes erroneously described as natural compounds (cf. e.g. ref.[7]). Very probably, all such cases are artifacts because of the more or less acidic reaction of the plant tissues these basic compounds can exist in the plants only in the ionized form *III*.

on October 9, 1989 at the stage of flowering and unripe fruits. The voucher specimen is deposited at our Department. The plants were dried at room temperature. The underground (rhizomes and roots, 50 g) and aerial parts (164 g) were worked up separately.

The dry ground plant material was exhaustively extracted with methanol in a Soxhlet apparatus, methanol was evaporated, the residue extracted with 1% sulfuric acid, filtered and the insoluble material washed several times with water. The combined acid filtrates were made alkaline with sodium carbonate solution and extracted several times with ether (fraction A). The aqueous layer was treated with sodium hydroxide to pH > 13 and again extracted several times with ether (fraction B). To the filtered ethereal fraction B was immediately added solid citric acid which resulted in formation of rich orange crystalline precipitate (citrates of quaternary protoberberines). The alkaline aqueous layer was adjusted to pH 5—6 with 20% sulfuric acid, saturated aqueous potassium iodide solution was added and the mixture was several times extracted with chloroform and chloroform–ethanol (4 : 1) (fraction I) to a negative reaction with Mayer's reagent.

Underground Part

Fraction A was purified by the usual acidobasic procedure. The obtained bases were dissolved in 1% sulfuric acid, the bright red solution was mixed with a solution of potassium cyanide to alkaline reaction and then acidified. The insoluble whitish precipitate was filtered (pseudocyanides of quaternary benzophenanthridines), the filtrate was made alkaline with ammonia and extracted with ether. The obtained bases were crystallized from chloroform–methanol or methanol to give (—)-stylopine (125·5 mg) and protopine (3·2 mg). The remaining amorphous bases (9·1 mg) contained (according to TLC in S1—S4) small amounts of chelidonine, allocryptopine, cryptopine, scoulerine and isoboldine, along with another unidentified alkaloid.

The pseudocyanides of quaternary benzophenanthridines were refluxed in a mixture of chloroform, ethanol and conc. hydrochloric acid (1 : 3 : 1) on a water bath for 1 h, the mixture was diluted with water, the organic solvents were evaporated on a water bath, the aqueous solution of the chlorides was made alkaline with ammonia and extracted several times with ether. The obtained bases were dissolved in benzene containing 1% acetic acid and separated on a column of alumina (Reanal, pre-washed with 0·5% hydrochloric acid[5] and then heated to 220°C for several hours). The elution was performed with benzene containing 1% of acetic acid and a gradient of ethanol (1—5%) and afforded, after conversion into bases, 4·2 mg of chelirubine, 14·3 mg of sanguinarine, 3·1 mg of chelilutine, 11·6 mg of macarpine and 8·2 mg of chelerythrine.

Citrates of the fraction B were crystallized from dilute hydrochloric acid to give coptisine chloride (100·4 mg), corysamine chloride (3·7 mg) and berberine chloride (1·4 mg). Crystallization of fraction I from methanol afforded 0·4 mg of (—)-*trans*-N-methylstylopinium iodide and TLC (systems S5—S7) of the mother liquors revealed the presence of magnoflorine, corytuberine and small amount of *cis*-N-methylstylopinium iodide.

Aerial Part

The purified bases of the fraction A yielded by crystallizations from chloroform–methanol total 134·0 mg of (—)-stylopine. The mother liquors on crystallization from dilute hydrochloric acid gave the sparingly soluble chelidonine hydrochloride (26·4 mg of base). The hydrochlorides remaining in the mother liquors were converted into bases and these were crystallized from methanol to afford another portion (2·5 mg) of stylopine and 2·1 mg of protopine. The amorphous residue (17·7 mg) contained (TLC in S1—S4) small amounts of scoulerine, isoboldine, cryptopine, sanguinarine, traces of chelirubine and two other unidentified alkaloids.

Fraction B, treated as described above, afforded coptisine chloride (79·1 mg). Thin-layer chromatography (in S9, S10) of bases (6·9 mg) in the mother liquor detected corysamine and small amount of berberine. Fraction I on crystallization from methanol gave 5·5 mg of (−)-*cis*-N--methylstylopinium iodide. In the mother liquors, TLC in S5−S7 detected small amounts of magnoflorine and *trans*-N-methylstylopinium iodide.

Characterization of the Alkaloids Isolated

The alkaloids were characterized by their melting points, mixed melting points, optical rotation values, UV and IR spectra (unless stated otherwise) and co-chromatography on thin layers with authentic samples. Yields of bases of the individual alkaloids (unless stated otherwise) in weight % from dry underground and aerial parts are given in parentheses. The presence (or absence) of the alkaloid, as found by TLC, is denoted + (or −).

(−)-*Stylopine* (0·25; 0·082): leaflets, m.p. 206−207°C (chloroform–methanol), no depression with an authentic sample, $[\alpha]_D^{23}$ −305° ± 3° (*c* 0·42, $CHCl_3$).

(+)-*Chelidonine* (+, S1−S4; 0·016): prisms, m.p. 135−136°C (ether or aqueous ethanol), no depression with an authentic specimen, $[\alpha]_D^{23}$ +123° ± 5° (*c* 0·11, methanol).

Sanguinarine (0·029; +, S8): chloride *IIIa*: copper-red needles, m.p. 283−284°C (dilute hydrochloric acid); free base:colourless small prisms, m.p. 271−272°C (ether); pseudomethanolate (8-methoxydihydrosanguinarine): colourless prisms, diffuse m.p. 186−195°C (chloroform–methanol), solidified up to 200°C with change of modification, remelted at 252−255°C.

Macarpine (0·023; −): chloride *IIIb*: crimson-red needles, m.p. 283−285°C (dilute hydrochloric acid), no depression and identical UV spectrum with a synthetic sample (cf. ref.[9]); free base did not crystallize from ether; pseudomethanolate (8-methoxydihydromacarpine) was obtained by crystallization of the base from methanol: colourless leaflets m.p. 178−179°C, resolidified at about 200°C, then remelted at about 223°C; UV spectrum, λ_{max}, nm (log ε): 222 (4·48), 287 (4·38), 346 (4·08); λ_{min} 257 (3·99), 310 (3·92), shoulder 320 (3·97). Mass spectrum, *m/z* (%): 423 ($M^{+\cdot}$, $C_{23}H_{21}NO_7$, 20), 394 (7), 393 (32), 392 ($C_{22}H_{18}NO_6$, 100), 379 (6), 378 (25), 377 (40), 372 (7), 363 (9), 362 (26), 348 (11), 332 (8), 196 (24), 189 (7), 182 (10), 152 (6), 31 (12), 29 (9), 15 (8).

Chelerythrine (0·0164; −): chloride *IIIc*: yellow needles, m.p. 209−210°C (dilute hydrochloric acid); base: small prisms, m.p. 288−289°C (ether); pseudomethanolate (8-methoxydihydrochelerythrine): colourless prisms with diffuse m.p. at 207°C (chloroform–methanol), then resolidified with change of modification and remelted at 240−241°C.

Chelirubine (0·0084; +, S8): chloride *IIId*: deep-purple needles, m.p. 281−282°C (dilute hydrochloric acid).

Chelilutine (0·0062; −): chloride *IIIe*: yellow-orange needles, m.p. 197−198°C (dilute hydrochloric acid); identified by mixture m.p. and chromatography (S8).

Protopine (0·0064; 0·0013): prisms, m.p. 209−210°C (chloroform–methanol).

Coptisine (chloride 0·201; 0·048): chloride *IIa*: orange needles not melting up to 300°C (darkening, decomposition).

Corysamine (chloride 0·0074; +; S9, S10): chloride *IIb*: bronze-coloured leaflets, not melting up to 290°C (darkening, decomposition), identification by chromatography (S9, S10).

Berberine (chloride 0·0028; +; S9, S10): chloride *IIc*: yellow needles, m.p. 206−207°C (dilute hydrochloric acid), identification by mixture m.p. and chromatography (S9, S10).

(−)-*cis*-N-*Methylstylopinium hydroxide* (+, S5−S7; iodide 0·0034): iodide *IVa*: small prisms m.p. 280−281°C (methanol), no depression with an authentic sample, its identity confirmed by chromatography (S5−S7).

(−)-*trans*-N-*Methylstylopinium hydroxide* (iodide 0·0008; +, S5−S7): iodide *IVb*: small prisms m.p. 297−298°C (methanol), no depression with an authentic sample, the identity confirmed by chromatography (S5−S7).

The authors are indebted to Professor N. Takao, Kobe, Japan for the kind gift of synthetic macarpine chloride. Their thanks are also due to Mrs J. Bochořáková for the UV *and* IR *spectral measurements.*

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Translated by M. Tichý.