QUATERNARY ALKALOIDS OF SOME SPECIES OF THE *Papaveraceae* FAMILY*

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Quaternary alkaloids of the following species of *Papaveraceae* were studied. From *Hunnemannia fumariaefolia* Sweet cyclanoline, escholidine and alkaloid HF3 were isolated, from *Papaver atlanticum* Ball magnoflorine, from *P. caucasicum* Marsch.-Bieb., *P. dubium* L. and *P. litwinowii* Fedde ex Bornm. aporheine methohydroxide, and from *P. pseudo-orientale* (Fedde) Medw. a new alkaloid, isothebaine methohydroxide were isolated. In *P. confine* Jord. magnoflorine was found and in two chemotypes of *P. rhoeas* L. β-stylopine methohydroxide and aporheine methohydroxide could be proved.

The alkaloids from *Papaveraceae* belong to an intensively investigated group of natural substances. The large majority of information concerns, however, "non-quaternary" bases only, that are extractable at alkaline reaction with non-polar solvents. Less is known about quaternary highly polar alkaloids that under current isolation procedures remain in the aqueous phase, thus escaping in the majority of cases the attention of investigators. Information on the occurrence of this type of quaternary alkaloids in *Papaveraceae* began to appear in literature about 15 years ago when magnoflorine was detected in opium[1,2], californidine in *Eschscholtzia californica*[3], escholamin ein *E. californica oregana*[4], remrefine in *Roemeria refracta*[5] and others. In our laboratory we have found quaternary alkaloids in 48 species of *Papaveraceae* so far and in the preceding papers of this series we have described 21 natural quaternary alkaloids (for a review see ref.[6]). In this paper we present the results of the study of several additional species of *Papaveraceae*. In all instances we obtained quaternary alkaloids after conversion to iodides by extraction with chloroform[4] and elimination of the "non-quaternary" fraction of bases.

The alkaloids from *Hunnemannia fumariaefolia* Sweet were studied some time ago[7] and from their quaternary fraction escholidine (α-form, *B*/*C cis*; *1b*, X = ClO_4)[8] was isolated in the form of its perchlorate. Now we have had a much larger amount of plant material at our disposal, which enabled us to isolate two additional alkaloids

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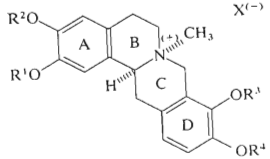
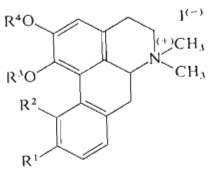
in the form of iodides from the quaternary fraction in addition to escholidine. One of them is identical with (−)-cyclanoline iodide ((−)-α-scoulerine methiodide, *B/C cis*; *Ia*, X = I), the second alkaloid, HF3, could not be studied in greater detail owing to too small quantity. Cyclanoline represents the main component of the quaternary fraction of the aerial part and the root of this plant (0·031% or 0·038%, resp.).

The alkaloids from *Papaver atlanticum* BALL, section *Pilosa* PRANTL, have also been studied several times[9–15], but nothing is known so far about quaternary alkaloids in them. From a small sample of root we now have isolated magnoflorine (*IIc*) in the form of its iodide (0·61% of the root) in a considerable yield, while in the aerial parts of the plant quaternary alkaloids are present in traces only. We also isolated magnoflorine from two other species of the section *Pilosa*, *i.e.* from *P. rupifragum* BOISS. et REUT. and in an extremely high yield from *P. oreophilum* RUPR. (3·58% in roots), where it is accompanied by menisperine (*IId*) (0·37% in roots).



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



IIa, $R^1 = R^2 = H$, $R^3 + R^4 = CH_2$
IIb, $R^1 = R^3 = H$, $R^2 = OCH_3$, $R^4 = CH_3$
IIc, $R^1 = OCH_3$, $R^2 = OH$, $R^3 = H$, $R^4 = CH_3$
IId, $R^1 = OCH_3$, $R^2 = OH$, $R^3 = R^4 = CH_3$

From the iodides of the quaternary fraction from *P. caucasicum* MARSCH.-BIEB. we isolated aporheine methiodide (*IIa*) (0·010%), which is a dextrorotatory enantiomer of (−)-roemerine methiodide found in *Roemeria refracta* (STEV.) DC. and named remrefidine[16]. Aporheine methiodide was also isolated recently from *P. fugax* POIR. by Soviet authors[17]. Both mentioned *Papaver* species belong to the section *Miltantha* BERNH. and they are so closely related that they are sometimes considered as lower taxonomic units of the same species.

Aporheine methohydroxide was also isolated from two related species *P. dubium* L. and *P. litwinowiii* FEDDE ex BORNM. from the section *Orthorhoeades* FEDDE in a 0·003% or 0·010% yield, respectively. In both these species the corresponding tertiary base aporheine ((+)-roemerine[18]), is the main alkaloid. In the quaternary fraction of *P. dubium* we also detected a small amount of magnoflorine.

From the population of *P. pseudo-orientale* (FEDDE) MEDW., characterized by the dominant alkaloid isothebaine, we isolated from the iodides of the quaternary

fraction a new alkaloid which was identified on the basis of its UV and IR spectra, melting point and mixture melting point, specific rotation and TLC as isothebaine methiodide (*IIb*). This represents the first instance of the finding of this substance in natural material.

Nothing was known either about the presence of quaternary alkaloids from the *Bocconia* genus. From *B. frutescens* L. we recently isolated (−)-α-canadine methiodide (*B*/*C cis*; *Ic*, X = I). The highest content of this alkaloid (0·020%) was found in the leaves.

In addition to the species mentioned we also had the opportunity of carrying out an orienting TLC study of the alkaloid spectrum of herbarium specimens* of the species *P. confine* Jord. newly found in Czechoslovakia and of two chemotypes of *P. rhoeas* L. from North Bohemia, which did not differ morphologically from the typical *P. rhoeas*. Both species belong to the section *Orthorhoeades*. In the quaternary fraction of the species *P. confine* we identified magnoflorine in addition to another as yet unidentified alkaloid. Since no literature data are available concerning the alkaloids of this species, the finding of rhoeadine as the main alkaloid and of a small amount of coptisine in the "non-quaternary" fraction of the bases deserves mention. This is a species with unusually low content of total alkaloids (<0·02%). One of the chemotypes of *P. rhoeas* was typical by its intensively yellow latex and it corresponded to Kuntze's description[19,20] of the taxon *P. rhoeas* var. *chelidonioides* O. Ktze., while the other had a white latex, turning pink in air. Both these chemotypes contained β-stylopine methohydroxide in the quaternary fraction, which was already found in *P. rhoeas* earlier[21,22], as well as aporheine methohydroxide. The composition of the "non-quaternary" fraction, in which rhoeadine and aporheine were found as dominant alkaloids in approximately equal amounts, in addition to a small amount of protopine, mecambrine, coptisine, berberine and other alkaloids, characterized the chemical type that forms a transition between the typical *P. rhoeas* and *P. dubium*. The chemical race "*P. rhoeas* var. *chelidonioides*" contained in contrast to other populations of *P. rhoeas* a substantially higher content of coptisine (0·02%), which is undoubtedly responsible for the yellow colour of the latex. The presence of aporheine, aporheine methohydroxide or mecambrine has not so far been detected in the populations of the typical *P. rhoeas*.

EXPERIMENTAL

The melting points were determined on a Mettler FP 51 instrument, the IR spectra (in KBr and Nujol) on a Unicam SP 1000 Infrared Spectrophotometer, and the UV spectra (in methanol) on a Unicam SP 1800 apparatus. Thin layer chromatography was carried out on silica gel G (Merck) using the following solvent systems: ethanol–water–25% ammonia 15 : 9 : 1 (S_1), me-

* For the kind donation of the plant material we thank Dr K. Kubát, District National Science Museum, Litoměřice.

thanol–water–25% ammonia 15 : 3 : 1 (S_2), 1-propanol–water–80% formic acid 7 : 2 : 1 (S_3) and 1-propanol–water–25% ammonia 40 : 9 : 1 (S_4). The spots were detected with potassium iodoplatinate.

Extraction and Isolation of Alkaloids

P. confine and two samples of *P. rhoeas* were collected in a natural locality in the surroundings of Litoměřice (collection by Dr K. Kubát), other plants were cultivated in the Experimental Garden of the Medical Faculty in Brno from seeds obtained from various botanical gardens. They were collected at the time of unripe fruits. The plant material was dried at room temperature and then ground. The extraction and the isolation of the alkaloids was carried out in all instances in the same manner. Dry, ground plant material was extracted with methanol, methanol was distilled off and the residue triturated and dissolved in cold 2% sulfuric acid (in the case of *H. fumariaefolia*) or 1% acetic acid (in the case of other samples). After filtration off of the insoluble matter, alkaloidal fractions *A*, *B* and *E* were obtained from the aqueous filtrate in the usual manner[23]. The alkaline aqueous layer was then adjusted with 20% sulfuric acid to pH 6—7, an excess of aqueous potassium iodide was added and fraction *I* (iodides of quaternary alkaloids) was then obtained by repeated extraction with chloroform or chloroform containing 20% ethanol.

Hunnemannia fumariaefolia

The above-ground parts of the plant used for extraction weighed 7·50 kg and the roots 3·77 kg (the yields from the above-ground parts are given first in brackets, followed by the yields from the roots). From the fraction *A* and *E* hunnemanine (46·5 g, 0·62%; 9·30 g, 0·25%), protopine (9·14 g, 0·122%; 22·59 g, 0·60%), allocryptopine (4·28 g, 0·057%; 6·76 g, 0·18%), (—)-scoulerine (0·91 g, 0·012%; —, —), chelerythrine (62 mg, 0·00083%; 71·7 mg, 0·0019%), sanguinarine (11 mg, 0·00015%; 62·5 mg, 0·0017%), chelilutine (4 mg, 0·00005% —, —) and traces of chelirubine were isolated in the same manner as in ref.[7]. Fraction *B* (10·6 mg, 0·00014%; 16 mg, 0·0004%) was obtained in the form of crystalline chloride composed predominantly of coptisine in addition to a small amount of berberine and traces of corysamine.

From fraction *I* (6·30 g; 3·30 g) cyclanoline iodide (2·35 g, 0·031%; 1·45 g, 0·038%) was obtained by crystallization from water or methanol. The amorphous residue of the iodides was converted to perchlorates by precipitation of the aqueous solution of iodides with 20% sodium perchlorate solution. On crystallization of the precipitate from methanol escholidine perchlorate (0·66 g, 0·009%; 0·104 g, 0·0028%) and the perchlorate of alkaloid HF3 (6 mg, 0·00008%; 7·5 mg, 0·0002%) were isolated.

Papaver atlanticum

For the isolation of the alkaloids 50 g of the aerial parts and 50 g of the roots were used. In fraction *I* of the aerial parts the presence of alkaloids could not be proved. From the chloroform extract of fraction *I* of the roots 306·3 mg of magnoflorine iodide (0·61%) crystallized out.

P. caucasicum

Fraction *I* obtained from 500 g of plant material afforded 50 mg of aporheine methiodide (0·010%) on crystallization from water.

P. dubium

Fraction *I* (0·11 g) was obtained from 384 g of plant material. Crystallization from methanol gave aporheine methiodide (13 mg, 0·0034%). In the mother liquors a small amount of magno-

florine could be detected by TLC in S_1, S_2 and S_3 (violet fluorescing spot under UV light), further corytuberine (R_F 0·81 in S_1, 0·88 in S_2 and 0·54 in S_3) and an unidentified alkaloid (R_F 0·33 in S_1 and 0·54 in S_3).

P. litwinowii

From 3·57 kg of dry plant 3·17 g of fraction *A* were isolated from which aporheine was separated as the main alkaloid. From fraction *I* 0·35 g of aporheine methiodide (0·010%) was obtained by crystallization from methanol.

P. pseudo-orientale

Dry capsules freed of seeds (150 g) and the roots (220 g) of several years old specimens (the yields from capsules and the roots, respectively, are given in brackets) were submitted to extraction. From fraction *A* (1·74 g, 1·16%; 2·04 g, 0·93%) isothebaine was isolated as the main alkaloid. Further significant alkaloids were orientalidine, mecambridine, and salutaridine in addition to several additional minor alkaloids. Fraction *B* contained trace amounts of coptisine and palmatine only (identification by TLC). From fraction *I* (0·14 g; 0·22 g) isothebaine methiodide (60 mg, 0·040%; 150 mg, 0·068%) was obtained on crystallization from methanol. In the mother liquors alkaloid PO-5 (alborine) and an unidentified alkaloid (R_F 0·20 in S_1, 0·12 in S_2, 0·35 in S_3 and 0·14 in S_4) were found.

P. confine

From a herbarium specimen (3·58 g) fraction A (<0·02%) was isolated in which rhoeadine was identified as the main alkaloid by thin-layer chromatography. In fraction *B* traces of coptisine and in fraction *I* magnoflorine (TLC, in S_1, S_2 and S_3) and a small amount of an unidentified alkaloid were found.

P. rhoeas

Two herbarium specimens (sample I and II) were analysed. Sample I (4·84 g) with a yellow latex ("*P. rhoeas* var. *chelidonioides*") and sample II (1·50 g) with a white latex turning pink contained 0·07% and 0·13% of bases of fraction *A*, respectively, in which rhoeadine and aporheine could be detected by thin-layer chromatography in addition to a small amount of protopine and mecambrine. In fraction *B* (0·02% or traces) coptisine and a small amount of berberine were detected. In fraction *I* of both samples β-stylopine methiodide and aporheine methiodide were identified by thin-layer chromatography in systems S_1, S_2 and S_3.

Characterization of the Alkaloids

Cyclanoline iodide: needles from water, m.p. 166—167°C, undepressed in admixture with a preparation isolated from the root of Argemone platyceras[24]; UV spectrum: λ_{max} (log ε) 286 nm (3·82), λ_{min} 257 nm (2·94). IR spectrum and R_F values (0·47 in S_1, 0·49 in S_2 and 0·75 in S_3) were identical with the values of the standard.

Escholidine perchlorate: prisms from methanol, m.p. 281—283°C, undepressed in admixture with an authentic sample[8]. The identity was confirmed by IR spectra, UV spectra, R_F values (0·55 in S_1, 0·43 in S_2 and 0·78 in S_3) and characteristic colour reactions.

Alkaloid HF3 *perchlorate*: from methanol m.p. 291—292°C, UV spectrum: λ_{max} 235 nm and 288 nm, λ_{min} 228 nm and 259 nm. Its iodide decomposed rapidly in air under yellowing and gradual darkening. R_F 0·65 in S_1 and 0·56 in S_2.

Magnoflorine iodide: prisms from methanol, m.p. 264—265°C, undepressed on admixture with an authentic specimen. The IR and UV spectra of both samples were also identical, as well as the R_F values (0·53 in S_1, 0·42 in S_2 and 0·62 in S_3).

Aporheine methiodide: small needles from water or methanol, m.p. 236—237°C, undepressed on admixture with a preparation prepared on methylation of aporheine with methyl iodide, $[\alpha]_D^{25}$ +49° ± 3° (*c* 0·23, methanol). The IR and UV spectra, λ_{max} (log ε) 210 nm (4·50), 271 nm (4·24), 314 nm (3·66), λ_{min} 255 nm (4·03), 300 nm (2·50) were identical with those of a reference sample. The same is true of the R_F values (0·14 in S_1, 0·03 in S_2 and 0·72 v S_3). Preparation of aporheine methiodide: 45·5 mg of aporheine were dissolved in a mixture of 1 ml of methanol and 3 ml of diethyl ether and 0·5 ml of methyl iodide. After about 15 h standing 67·0 mg of a product melting at 236—238°C crystallized out, $[\alpha]_D^{25}$ +49° ± 2° (*c* 0·34, methanol).

Isothebaine methiodide: needles from methanol, m.p. 254—256°C, undepressed on admixture with a sample prepared on methylation of isothebaine with methyl iodide, $[\alpha]_D^{21}$ +194° ± 3° (*c* 0·51, methanol). IR spectrum, ν(OH) 3250, 3420 and 3500 cm^{-1}, UV spectrum, λ_{max} (log ε) 223 nm (4·65), 273 nm (4·13), 300 nm (3·98), λ_{min} 252 nm (3·82), 287 nm (3·88), and the R_F values (0·11 in S_1, 0·06 in S_2, 0·38 in S_3, and 0·10 in S_4) were identical with the spectra and the values of reference sample. Isothebaine methiodide: 0·20 g of isothebaine in 3 ml of methanol and 0·2 ml of methyl iodide was refluxed for 4 h. After cooling a product crystallized out that was crystallized twice from methanol. The needles obtained (96 mg) had m.p. 254—256°C and $[\alpha]_D^{21}$ +194° ± 3° (*c* 0·90, methanol).

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