ALKALOIDS FROM *Papaver oreophilum* F. J. RUPR.*

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In addition to the known alkaloids from *Papaver oreophilum* F. J. RUPR., *i.e.* oridine, mecambridine, isocorydine, protopine, oreodine, rhoeadine, nuciferine, isooridine (oridine-2), oridine-3, alkaloid PO—5 (R—K, alborine) and trace alkaloids rhoeagenine, oreogenine, isorhoeadine, papaverrubines A, D and E, sanguinarine, N-methyloridine and coptisine, we isolated from the aerial part or from the roots the following further alkaloids: allocryptopine and — from the quaternary fraction — menisperine and magnoflorine (3·6%), which is the main alkaloid in the roots. Further corydine and trace amounts of papaverrubine C, chelirubine, berberine, corysamine and probably O-methyloridine and O,N-dimethyloridine were also detected. It was shown that oridine-3 was identical with the hydrochloride of isooridine.

Papaver oreophilum F. J. RUPR. is a perennial plant from the section *Pilosa* PRANTL., growing in the lower areas of the Caucasus. The alkaloids of this species have already been investigated several times by Pfeifer and coworkers[1-4] and Maturová and coworkers[5,6]. While both groups isolated oridine[5] (oreoline[2]), isocorydine, protopine and rhoeadine as dominant alkaloids, Pfeifer and coworkers[1] obtained as the main alkaloid "oreophiline", identical[5,7] with mecambridine, described earlier. Maturová and coworkers[5,6] have not found this alkaloid in *P. oreophilum*, nor the rhoeadane alkaloid oreodine[1] either. Among the minor alkaloids nuciferine, oxysanguinarine, oreonone (oreocyclohexadienone. 1-methoxy-13-oxoallocryptopine), oridine-2, oridine-3, isorhoeadine, alkaloid R—K (PO—5, alborine)[5,6,8] and further rhoeagenine, oreogenine, N-methyloridine (N-methyloreoline) and papaverrubine F (N-demethyloreodine) were isolated, and the presence of thebaine, sanguinarine, coptisine[5,6] and papaverrubines A, B, D and E (ref.[1,9]) was detected. Although the two mentioned groups of workers described altogether 24 alkaloids in this plant, nothing was known so far on the presence of these highly polar quaternary alkaloids, which remain in the aqueous phase even under alkaline reaction.

We investigated the alkaloids from the aerial part and the roots of the plant and succeeded in isolating or chromatographically identifying 29 alkaloids, of which 9 were found in this species for the first time. From the aerial parts we isolated

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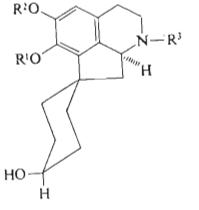
0·35% (Pfeifer and Mann[4] claim a much lower yield – 0·081%) and from the roots 0·63% of a sum of non-quaternary fraction of alkaloids. The main alkaloid of this fraction from the aerial parts was mecambridine[7], followed by the proaporphine alkaloid oridine (*Ia*, see[10,11]), protopine, isocorydine, oreodine and rhoeadine, which all belong to dominant alkaloids. In the same fraction from the roots oridine highly predominated, accompanied by a smaller amount of oridine-2, while the other alkaloids mentioned were present in very small amounts only. Among minor alkaloids we isolated from both the aerial parts and the roots allocryptopine, found in this plant for the first time, further nuciferine and N-methyloridine (*Ib*, from the root only), and we detected by TLC the presence of small amounts of rhoeagenine, oreogenine, sanguinarine, isorhoeadine, papaverrubines A, D, and E and coptisine. In addition to this, TLC showed the presence of corydine and trace amounts of chelirubine, papaverrubine C, corysamine and probably also O-methyloridine (*Ic*) and O,N-dimethyloridine (*Id*). The presence of these alkaloids in *P. oreophilum* has not been proved so far.



Ia: $R^1 = R^3 = H$, $R^2 = CH_3$
Ib: $R^1 = H$, $R^2 = R^3 = CH_3$
Ic: $R^1 = R^2 = CH_3$, $R^3 = H$
Id: $R^1 = R^2 = R^3 = CH_3$
Ie: $R_1 = CH_3$, $R^2 = R^3 = H$

Oridine-2 was identified by direct comparison with an authentic sample[5,6]. Recently[12] we demonstrated that this alkaloid had structure *Ie* and proposed for it the name isooridine. From the roots we isolated a small amount of a substance which according to its physical constants was undoubtedly identical with oridine-3 (ref.[5,6]). We found that it was not a base, but the hydrochloride of an alkaloid the mass spectrum of which was practically identical with the spectra of oridine and isooridine. Direct comparison with isooridine hydrochloride (mixture melting point, UV and IR spectra and R_F values) showed that both substances were identical.

We obtained the fraction of quaternary alkaloids after conversion to iodides and extraction with chloroform (for a more detailed description see[13]). From this frac-

tion, obtained from the aerial parts we isolated menisperine iodide (which is merely a minor alkaloidal component of the aerial parts – 0·022%) in addition to the iodide of alkaloid PO–5 (aiborine). However, the roots are unusually rich in quaternary alkaloids, and we isolated from them as the main component magnoflorine iodide[14] in an extremely high yield (3·58%) in addition to a smaller amount of menisperine iodide (0·37%). These two alkaloids, the presence of which in *P. oreophilum* has not yet been known, represent generally the main alkaloids of this species. In spite of the fact that magnoflorine is an unusually widely distributed alkaloid in the order *Polycarpicae* resp. *Ranales*, the root of *P. oreophilum* is its richest known source.

Similar to Pfeifer and coworkers[1–4] the presence of oreonone and thebaine[5,8] could not be proved in our plant material by us either. The absence of two of the dominant alkaloids, *i.e.* mecambridine and oreodine, in the population investigated by Maturová and coworkers[5,6] seems to indicate the probable existence of different chemotypes in *P. oreophilum*. In its composition of alkaloids this species assumes a rather isolated position in the section *Pilosa* in which two main biochemically different groups may be distinguished[15]: the first group is characterized by the presence of morphinanedienone alkaloid amurine (or together with glaucine and (+)-roemerine), where *P. heldreichii* BOISS., *P. pilosum* SIBTH. et SMITH, *P. spicatum* BOISS. et BALL. (incl. *P. feddei* SCHWARZ and *P. pannosum* SCHWARZ) and others belong while the other one, with the dominant alkaloids rhoeadine and protopine, comprises *P. atlanticum* BALL, *P. rupifragum* BOISS. et REUT., *P. lateritium* C. KOCH and others. These two biochemical groups correspond to a considerable extent to the classification into the section *Pilosa* PRANTL and *Pseudo-pilosa* M. POP. (*cf.*[15]). In contrast to this *P. oreophilum* has very close biochemical relationship with the species *P. lisae* N. BUSCH. from the section *Scapiflora* REICHB., which also contains in the "non-quaternary" fraction oridine, mecambridine, protopine and isocorydine as the dominant alkaloids[16].

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. The IR spectra were measured on a Zeiss UR-10 spectrophotometer in nujol and the UV spectra on a Unicam SP 1800 spectrophotometer in methanol. Thin-layer chromatography (TLC) was carried out on silica gel G (Merck) in cyclohexane–diethylamine 9 : 1 (S_1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S_2), benzene–ethyl acetate–diethylamine 5 : 4 : 1 (S_3), methanol–water–25% ammonia 15 : 3 : 1 (S_4), methanol–25% ammonia 200 : 1 (S_5), benzene–methanol 4 : 1 (S_6), and on Silufol UV 254 (Kavalier) in methanol–diethylamine 4 : 1 (S_7) and methanol–cyclohexane (S_8). For paper chromatography Whatman No 1 paper was used, descending mode, in the systems 1-butanol–acetic acid–water 10 : 1 : 3 (S_9) and ethanol–water 3 : 2 (S_{10}). The spots of fluorescing alkaloids were detected in UV light, for the detection of papaverrubines conc. hydrochloric acid fumes were used, while the spots of other alkaloids were made visible with potassium iodoplatinate.

Extraction and Isolation of Alkaloids

The plants were cultivated in the Experimental Garden of the Medical Faculty in Brno from the seeds kindly donated by the Botanical Gardens in Wroclaw and Poznan. Several years old specimens were harvested at the stage of flowering and unripe fruits, on June 30th, 1967. The aerial parts and the roots were separated and dried at room temperature. The dry, ground material (5·00 kg of the aerial parts and 1·68 kg of roots) was extracted exhaustively with methanol in the cold, and methanol was distilled off, the syrupy extract diluted with 1% acetic acid and filtered. Alkaloid fractions *A*, *B*, *E* and *I* were obtained from the filtrate in the usual manner[17,18].

Alkaloids from the Aerial Parts

The crude fraction *A* (14·60 g) was separated[17] to fractions *ACa*, *ACb*, AD_1 and AD_2. Crystallization of the bases *ACa* (5·73 g) from ether and methanol gave 2·48 g of mecambridine, 0·38 g rhoeadine, 0·43 g isocorydine, 0·69 g oreodine, 0·05 g protopine and 0·62 g of mixed fractions which were separated chromatographically on alumina to afford 0·14 g of rhoeadine, traces of isorhoeadine, 0·09 g nuciferine, 0·16 g oreodine, 0·09g protopine, 0·22 g isccorydine and 0·35 g mecambridine. In the remaining 0·26 g of amorphous bases the presence of trace amounts of allocryptopine, corydine, oreogenine, rhoeagenine, oridine and N-methyloridine was detected by TLC in addition to the above mentioned alkaloids. From fraction *ACb* (1·03 g) 0·01 g oreodine, 0·26 g isocorydine, 0·07 g protopine, 0·12 g mecambridine and 0·96 g of an amorphous residue of a predominantly non-alkaloidal nature were isolated by crystallization from methanol. Using TLC the presence of allocryptopine, oridine, corydine and rhocadine was also detected. In fraction *ACa* resp. *ACb* the presence of papaverrubine E, D and A was also proved by TLC. Crystallization of fraction AD_1 (1·05 g) from a mixture of chloroform and methanol gave 0·76 g of protopine and 0·03 g of allocryptopine. In the remaining 0·03 g of the amorphous residue the two alkaloids mentioned were accompanied by the remains of mecambridine and rhoeadine. TLC also detected sanguinarine and chelirubine. According to TLC the amorphous fraction AD_2 (0·83 g) contained mecambridine, oridine and N-methyloridine in addition to a majority of non-alkaloidal substances.

In fraction *B* (12·1 mg) coptisine and traces of corysamine were detected by TLC on Silufol and by paper chromatography. Crystallization of fraction *E* (9·05 g) from a chloroform–methanol mixture afforded 2·86 g of the crystalline mixture of oridine and isooridine, from which repeated crystallizations from methanol gave 2·26 g of oridine and 0·11 g of isooridine.

From fraction *I* (4·50 g) 1·11 g of menisperine iodide and 0·58 g of the iodide of alkaloid PO—5 (alborine iodide) were obtained by crystallization from methanol. In addition to the residues of these two alkaloids the mother liquors also contained non-alkaloidal substances.

Alkaloids from Roots

Fraction *A* (3·10 g) was separated[17] to fraction *AC*, AD_1 and AD_2. From fraction *AC* (1·50 g) 0·02 g of nuciferine, 0·01 g of rhoeadine, 0·02 g of oreodine, 0·12 g of isocorydine, 0·02 g of protopine, 0·02 g of allocryptopine and 0·03 g of mecambridine were isolated by chromatographic separation on alumina and crystallization of the fractions obtained from methanol. In the amorphous fractions N-methyloridine and small amounts of rhoeagenine and oreogenine were found in addition to non-alkaloidal substances. Using preparative TLC and recrystallization from methanol 0·10 g of N-methyloridine was obtained. In the fraction *AC* the presence of papaverrubine C, D, and E was demonstrated by TLC. From the bases AD_1 (0·13 g) a mixture of protopine and allocryptopine (0·01 g) was obtained by crystallization from chloroform–ethanol,

and in the amorphous residue isocorydine, mecambridine. oridine and sanguinarine were detected by TLC in addition to the two alkaloids mentioned. The amorphous fraction AD_2 (0·06 g) contained trace amounts of mecambridine and oridine.

In fraction *B* (5·0 mg) berberine and coptisine were detected chromatographically. The crude fraction *E* (16·73 g) was washed with chloroform. Crystallization of the non soluble residue from methanol gave 0·62 g of isooridine and 0·30 g of the mixture of oridine hydrochloride and isooridine hydrochloride ("oridine-3"). Crystallization of the purified bases from methanol afforded 4·69 g of oridine. According to TLC the amorphous brown-red residue contained in addition to oridine and its decomposition products (R_F 0·90 in S_2) also N-methyloridine and trace amounts of O,N-dimethyloridine and O-methyloridine.

After extraction of fraction *E* and acidification with acetic acid and addition of potassium iodide solution a precipitate of magnoflorine iodide was formed in the aqueous phase, which was filtered off. The filtrate was extracted several times with chloroform or chloroform with 20% of ethanol (fraction *I*). From both fractions totally 59·96 g of magnoflorine iodide and 6·21 g of menisperine iodide were obtained by crystallization from methanol.

Characterization of the Isolated Alkaloids

The alkaloids isolated from *P. oreophilum* were identified on the basis of their melting points, mixture melting points, specific rotation values, UV and IR or also mass spectra and R_F values (comparing with authentic specimens). The yields of the individual alkaloids from the aerial parts or from roots are given in brackets.

Magnoflorine iodide (—, 3·58%): prisms of m.p. 260—262°C (methanol), undepressed on admixture of an authentic preparation from *Eschscholtzia californica*[19].

Menisperine iodide (0·022%, 0·37%): prismatic needles, m.p. 239—241°C (methanol), undepressed with authentic (+)-isocorydine methiodide.

Oridine (0·045%, 0·28%): needles, m.p. 236—237°C (methanol), undepressed with an authentic sample[5], $[\alpha]_D^{25}$ —82° ±3° (*c* 0·34, methanol). The mass spectrum was identical with the spectrum of oridine. For the UV, IR, ^{1}H-NMR and ^{13}C-NMR spectra see ref.[12]. Oridine hydrochloride, m.p. 210—214°C (methanol–ether).

Isooridine (oridine-2; 0·002%, 0·037%): prisms of m.p. 167—168°C (methanol), with a preparation of oridine-2 (ref.[5]), $[\alpha]_D^{25}$ —57° ± 3° (*c* 0·55, methanol). The mass spectrum did not practically differ from the spectrum of oridine, but the IR spectra were not identical. ^{1}H-NMR and ^{13}C-NMR spectra and the CD curve were as in ref.[12]. UV spectrum, λ_{max} (log ε): shoulder at 231 nm (3·89), 288 nm (3·46), λ_{min} 260 nm (2·60). It was identical with the spectrum of oridine. The R_F values of isooridine were identical with those of oridine in all the systems applied. Isooridine hydrochloride, prepared by acidification of a chloroform solution of the base with hydrochloric acid in methanol, had m.p. 255—258°C (chloroform).

Mecambridine (0·059%, 0·0018%): needles of m.p. 182—184°C (methanol), undepressed in admixture with a preparation from *Meconopsis cambrica*[20,21], the UV, IR and chromatographic data were also identical. UV spectrum: λ_{max} (log ε) 228 nm (4·14), 288 nm (3·74), λ_{min} 257 nm (2·80).

Isocorydine (0·018%, 0·0072%): m.p. 189°C (methanol), undepressed in admixture with an authentic preparation. The UV and IR spectra and the R_F values confirmed the identity.

Protopine (0·019%, 0·0012%): prisms, m.p. 205—206°C (chloroform–methanol), undepressed with an authentic sample, and with identical R_F values.

Oreodine (0·016%, 0·0012%): needles in clusters, m.p. 184—185°C (methanol), undepressed with an authentic sample[1]. UV spectrum: λ_{max} (log ε) 237 nm (3·96), 289 nm (3·72), λ_{min} 226 nm (3·96), 260 nm (3·13).

Rhoeadine (0·010%, 0·0006%): needles, m.p. 251—253°C (methanol), undepressed with an authentic specimen.

Nuciferine (0·002%, 0·0012%): prisms, m.p. 167°C (methanol), mixture melting point and other constants in agreement with an authentic sample.

Allocryptopine (0·0006%, 0·0012%): prisms, m.p. 158—159°C, undepressed in admixture with an authentic preparation. The IR and UV spectra and the TLC data also identical.

Alkaloid PO—5 *iodide* (alborine iodide; 0·012%, —): yellow needles, m.p. >360°C, UV spectrum: λ_{max} (log ε) 246 nm (4·34), 263 nm (4·33), 292 nm (4·51), 336 nm (4·20), λ_{min} 243 nm (4·30), 254 nm (4·28), 272 nm (4·25), 318 nm (4·20), identical with an authentic specimen.

N-*Methyloridine* (—, 0·006%): needles from methanol, m.p. 189—191°C. UV spectrum: λ_{max} (log ε) 219 nm (3·73), 287 nm (3·38), λ_{min} 257 nm (2·68).

Preparation of Methyl Derivatives of Oridine

N-*Methyloridine*: 0·05 g of oridine were refluxed with 5 ml of 85% formic acid and 5 ml of 37% aqueous formaldehyde in methanol for 4 h. After evaporation of the solvents the residue was dissolved in water, the solution alkalized with ammonia and extracted with chloroform. Crystallization of the residue from methanol gave needles of m.p. 189—191°C.

O-*Methyloridine*: 0·05 g of oridine in methanol were mixed with 5 ml of an ethereal diazomethane solution and allowed to stand in a refrigerator for 4 days. After evaporation of the solvents an amorphous product was obtained which was characterized by its R_F values in systems S_1—S_5. The R_F values in S_3 were in good agreement with the values given in ref.[10] for the same compound and system.

O,N-*Dimethyloridine* was prepared analogously by methylation of N-methyloridine with diazomethane. It had m.p. 181—184°C; for the mass and ^{1}H-NMR spectra see ref.[12].

Identity of "Oridine-3" with Isooridine Hydrochloride

From crude "oridine-3" (—, 0·019%) a fraction was separated by crystallization from methanol, having m.p. 212—218°C, which was identical with oridine hydrochloride, and another with m.p. 258—262°C (Maturová and coworkers[5,6] give m.p. 259—261°C for oridine-3), which was microcrystalline and easily soluble in water. The solution gave positive reaction for $Cl^{(-)}$. A mixture with isooridine hydrochloride melted without depression. The mass, IR and UV spectra of both substances were identical, as also were their R_F values. In the IR spectrum of oridine-3 a characteristic group of bands at 2400—2800 cm^{-1} is present (addition salts of the type A.HX) and a sharp band at 3540 cm^{-1} (OH).

R_F Values

In system S_1, S_2 and S_3, respectively: allocryptopine 0·22, 0·61, 0·82; corydine 0·12, 0·49, 0·67; isocorydine 0·16, 0·52, 0·70; isorhoeadine 0·47, 0·78, 0·89; mecambridine 0·13, 0·27, 0·76; nuciferine 0·44, 0·71, 0·79; oreodine 0·40, 0·66, 0·86; oreogenine 0·12, 0·35, 0·82; protopine 0·32, 0·65, 0·87; rhoeadine 0·43, 0·75, 0·89; rhoeagenine 0·19, 0·41, 0·85. In system S_1, S_2, S_3, S_4 and

S_5, resp.: O,N-dimethyloridine 0·13, 0·31, 0·66, 0·05, 0·02; isooridine 0·02, 0·05, 0·37, 0·47, 0·29; N-methyloridine 0·05, 0·13, 0·52, 0·76, 0·61; O-methyloridine 0·00, 0·00, 0·42, 0·63, 0·63; oridine 0·02, 0·05, 0·37, 0·47, 0·29. In system S_1, S_2 and S_6: papaverrubine A 0·35, 0·64, 0·72; papaverrubine C 0·12, 0·41, 0·65; papaverrubine D 0·07, 0·21, 0·50; papaverrubine E 0·35, 0·64, 0·35. In system S_4 and S_5: alkaloid PO—5 (alborine) 0·18, 0·04; magnoflorine 0·30, 0·13; menisperine 0·02, 0·01. In system S_7, S_9 and S_{10}: berberine 0·28, 0·59, 0·18; coptisine 0·55, 0·43, 0·07; corysamine 0·14, 0·70, 0·32. In system S_8: chelirubine 0·77, sanguinarine 0·70.

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