ALKALOIDS FROM *Papaver pinnatifidum* MORIS*

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Oripavine (*Ia*) was isolated from *Papaver pinnatifidum* MORIS (tetraploid, $2n = 28$) of the section *Rhoeadium* SPACH as the major alkaloid. In a low yield, protopine and (+)-isocorydine were obtained together with a novel levorotatory alkaloid *PP 1* of the composition $C_{17}H_{19}NO_3$ and m.p. 179 °C, probably of a normorphinane type. Small amounts of thebaine, allocryptopine, scoulerine, isoboldine, and papaverrubines A, C and D, berberine, and corytuberine were also detected by thin layer chromatography.

Papaver pinnatifidum MORIS is an annual herb from the section *Rhoeadium* SPACH (synonym: *Orthorhoeades* FEDDE) of the *Papaveraceae* family indigenous to the Mediterranean region of South-western Europe and to Morocco[1]. According to its morphological characters, this plant species is closely allied to *P. dubium* L. agg. several taxa of which, native to the Czech Republic and Slovakia, were the subject of our previous alkaloid studies[2–4]. No data on alkaloids of *P. pinnatifidum* were hitherto available.

In the present communication, we report the results of an investigation of *P. pinnatifidum* alkaloids carried out with a plant material which was cultivated under the climatic conditions of this country. The plants were stated to be tetraploid (chromosome number, $2n = 28$) in agreement with literature data[5], and displayed a very low alkaloid content (0.004% of dry plants) similarly as it was found in the majority of the tetraploid taxa of *P. dubium* aggregate[2–4]. Using methanol extraction of the dried whole plants and conventional procedure (cf. for example refs[6,7]), a sum of weakly polar bases was isolated and divided into a nonphenolic and a phenolic fraction by partitioning between ether and diluted sodium hydroxide solution.

The phenolic alkaloid fraction yielded the major alkaloid of the plant as a crystalline product of m.p. 205 °C and of the composition $C_{18}H_{19}NO_3$. Spectral analyses (EIMS, ^{1}H NMR, IR, and UV spectra), optical rotation and thin layer chromatography proved

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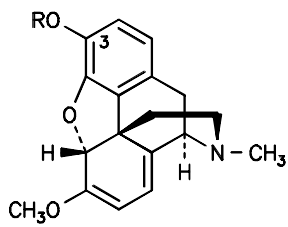
its identity to (–)-oripavine (*Ia*). The identity was confirmed by direct comparison with a referent sample originating from *P. orientale* L. (ref.[7]). The occurrence of oripavine in the *Rhoeadium* section was rather unexpected, since it was known from taxa of the section *Macrantha* ELKAN (synonym: *Oxytona* BERNH.) and it was considered as a specific chemotaxonomic feature of the species *P. orientale* (ref.[8]). At a later date, oripavine was also isolated from a variety of *P. somniferum* L. (section *Papaver* SPACH, synonym: *Mecones* ELKAN) cultivated on Tasmania[9].



I

Ia, R = H *Ib*, R = CH_3

The nonphenolic fraction afforded protopine as a minor constituent of the plant by direct crystallization from methanol. The non-crystallizable both phenolic and nonphenolic bases were combined and submitted to column chromatography on neutral alumina. From the fractions eluted, small amounts of (+)-isocorydine and of a levorotatory base, m.p. 179 °C, were obtained. This base, provisionally designated as alkaloid *PP 1*, is evidently a novel alkaloid. It is of nonphenolic nature, and it has the composition $C_{17}H_{19}NO_3$. IR spectrum indicated the presence of a carbonyl and an imino function. On the basis of IR, UV and mass spectrum, it may be deduced that alkaloid *PP 1* has probably an *N*-demethyl morphinane skeleton, but its complete structure cannot be solved at present due to paucity of material.

In addition to the alkaloids mentioned, minute quantities of thebaine, allocryptopine, scoulerine, isoboldine and papaverrubines D (porfyroxine), C (epiporfyroxine) and A (*N*-demethylisorhoeadine) were identified in the remaining amorphous bases by thin layer chromatography. In the fraction of quaternary protoberberines, traces of berberine were detected, and in strongly polar alkaloid fraction, the presence of a small amount of corytuberine was found.

From the results given, it may be deduced that the plant species *P. pinnatifidum* is not only botanically but also biochemically closely related to *P. dubium* aggregate. Several years ago, it had been proposed[10] to separate this group of taxa into an independent section *Dubia* MIKHEEV which is in its both morphological and chemical characters very well differentiated from the section *Rhoeadium* s. str. It is known that

oripavine is biosynthetized from reticuline by way of thebaine (*Ib*) and following *O*-3-demethylation[9]. The same biochemical pathway is likely to be supposed also in *P. pinnatifidum*. From this point of view, *P. pinnatifidum* may be considered as the most related to the tetraploid taxon *P. albiflorum* PACZ. subsp. *albiflorum* which accumulates thebaine as the principal alkaloid[2], probably because of the lack of an enzyme system capable of thebaine *O*-3-demethylation.

EXPERIMENTAL

The melting points were determined on a Mettler FP 51 apparatus and were uncorrected. UV spectra were recorded in methanol on a Unicam SP 1800 instrument and IR spectra in Nujol (unless stated otherwise) on a Specord 75 IR (Zeiss, Jena) spectrometer. Electron impact mass spectra (EIMS) were taken on a Jeol MS D 100 spectrometer. ^{1}H NMR spectra were measured on a Varian VXR-400 spectrometer (400 MHz) in $CDCl_3$ with tetramethylsilane as an internal standard at 25 °C, and chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Thin layer chromatography (TLC) was performed on silica gel G (Merck), and the following solvent systems were used: cyclohexane–diethylamine 9 : 1 (S1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S2) and 6 : 3 : 1 (S3), benzene–methanol 4 : 1 (S4) and benzene–acetone–methanol 7 : 2 : 1 (S5) for the less polar bases, and methanol–water–25% aqueous ammonia 15 : 3 : 1 (S6), 1-propanol–water–85% formic acid 12 : 7 : 1 (S7) and chloroform–ethanol–diethylamine 8 : 1 : 1 (S8) for the strongly polar alkaloids. Silufol UV 254 plates (Kavalier, The Czech Republic) and systems methanol–diethylamine 4 : 1 (S9) and 1 : 1 (S10) were used for quaternary protoberberines. The spots exhibiting fluorescence were detected in UV light (254 and 360 nm), the other spots with potassium hexaiodoplatinate(IV). The papaverrubines were made visible by 20 min exposure to vapours of concentrated hydrochloric acid (formation of purple spots).

Extraction and Isolation of the Alkaloids

The plants were cultivated at the Centre for Cultivation of Medicinal Plants, Medical Faculty, Masaryk University, Brno, from the seeds obtained from the Botanical Gardens of Basel and Gattersleben. Plants of both origin were identical and agreed with the description in literature[1]. A voucher specimen is deposited at our Institute. The plants were harvested at the stage of unripe fruits in July 1985 and dried at room temperature.

The dried ground whole plants (5.3 kg) were exhaustively extracted in the cold with methanol (total 160 l) in a percolator. Methanol was distilled off, the sirupy residue was extracted several times with cold 1% acetic acid until a negative reaction with Mayer's reagent and the solution filtered. The combined acid filtrates were alkalized with a sodium carbonate solution and extracted several times with ether (fraction *A*). The aqueous layer was treated with sodium hydroxide solution to pH > 13 and again extracted with ether (fraction *B*). Then the aqueous layer was adjusted to pH about 6 with 20% sulfuric acid, saturated aqueous potassium iodide solution was added and the mixture was extracted several times with chloroform and chloroform–methanol (4 : 1) (fraction *I*) to a negative reaction with Mayer's reagent.

Crude fraction *A* was purified by an usual acido-basic procedure and divided into nonphenolic (A_1) and phenolic (A_2) bases (cf. refs[6,7]). Crystallization of the fraction A_1 from methanol yielded protopine (3.9 mg). Oripavine (84.4 mg) was obtained from the fraction A_2 by crystallization from methanol. Small amounts of papaverrubine *A* (in A_1), and D and C (in A_2) were identified using TLC and systems S1, S2, S3, S4, and S5 by comparison with authentic samples.

The remaining amorphous bases of both fractions were combined and separated on a column of aluminium oxide (Reanal, according to Brockmann, activity about II). From the fractions eluted with ether–chloroform (3 : 2), isocorydine was obtained and purified in the form of poorly soluble hydrobromide (7.9 mg) by crystallization from methanol. The fractions eluted with chloroform and chloroform–methanol (1 : 1) contained a mixture of at least eight alkaloids of both phenolic and nonphenolic nature. From the nonphenolic part, crude alkaloid *PP 1* (12.4 mg) was obtained. Several crystallizations from methanol yielded pure product (4.5 mg). In addition to the alkaloids mentioned, the presence of small amounts of thebaine, allocryptopine, scoulerine and isoboldine was proved in remaining amorphous bases by TLC in S1, S2, S3 and S4.

In the fraction *B*, traces of berberine were found by TLC in S9 and S10, and in the fraction *I*, a small amount of corytuberine (in S6, S7 and S8) could be demonstrated.

Characterization of the Alkaloids Isolated

The individual alkaloids were identified by their melting points, mixed melting points, UV and IR spectra, EIMS, and ^{1}H NMR spectra, respectively, and TLC comparison with the referent samples. The yields of isolated alkaloids in wt.% from dry whole plants are given in parentheses.

Oripavine (0.0016): small prisms from methanol, m.p. 204 – 205 °C, mixed m.p. with an authentic sample[7] isolated from *P. orientale* without depression, $[\alpha]_D^{22}$ –217° ± 3° (*c* 0.2, chloroform); literature[9,11] gives m.p. 201 – 203 °C and $[\alpha]_D^{20}$ –216.9° (chloroform). EIMS, *m/z* (composition, %): 297 ($C_{18}H_{19}NO_3$, 100), 296 (33), 282 (19), 266 (14), 254 (20), 241 ($C_{15}H_{13}O_3$, 31), 240 (21), 228 (10), 227 (11), 223 (12), 211 (10), 176 ($C_{11}H_{14}NO$, 22), 139 (11), 115 (C_9H_7, 10), 58 (16), 42 (36); literature[9,11] gives *m/z* 297 only. ^{1}H NMR spectrum: 1.739 ddd, 1 H (*J* = 12.9, 3.5, 3.5); 2.206 ddd, 1 H (*J* = 12.9, 12.9, 5.2); 2.447 s, 3 H (NCH_3); 2.635 ddd, 1 H (*J* = 12.9, 5.2, 1.5); 2.722 ddd, 1 H (*J* = 18.0, 7.0, 0.7); 2.875 ddd, 1 H (*J* = 12.9, 12.9, 3.5); 3.277 dd, 1 H (*J* = 18.0, 1.0); 3.602 dd, 1 H (*J* = 7.0, 1.0); 3.629 s, 3 H (OCH_3); 5.094 d, 1 H (*J* = 6.4); 5.272 s, 1 H; 5.584 d, 1 H (*J* = 6.4); 6.548 ddd, 1 H (*J* = 8.1, 1.0, 0.7); 6.624 d, 1 H (*J* = 8.1). UV spectrum: λ_{max}, nm (log ε): 209 (4.46), 286 (3.93), 227 sh (4.23); λ_{min}: 258 (3.67). IR spectrum was identical to the spectrum of the authentic sample, the same being true of the R_F values on TLC in S1, S2 and S3.

Alkaloid PP1 (0.00014): prisms, m.p. 178 – 179 °C (ether), $[\alpha]_D^{23}$ –135° ± 10° (*c* 0.05, methanol). EIMS, *m/z* (composition, %): 285 ($C_{17}H_{19}NO_3$, 100), 284 (7), 283 (4.6), 270 ($C_{16}H_{16}NO_3$, 10), 257 (8.4), 251 (8.1), 242 ($C_{15}H_{14}O_3$, 29), 241 (22), 228 ($C_{14}H_{14}NO_2$, 38), 226 (15), 215 (23), 213 (34), 212 (22), 199 (17), 198 (17), 182 ($C_{12}H_8NO$, 22), 181 ($C_{13}H_9O$, 15), 154 (17), 128 (11), 115 (C_9H_7, 13); one exchangeable hydrogen atom. UV spectrum: λ_{max}, nm (log ε): 233 (4.34), 276 (4.14), 303 sh (3.77), 316 (3.60); λ_{min}: 259 (4.03). IR spectrum (KBr, cm^{-1}): 1 670 (CO); 3 400 (NH). TLC: R_F 0.18 (S1), 0.30 (S2).

(+)-*Isocorydine* (0.00012): prisms, m.p. 183 – 184 °C (methanol), and the same m.p. in admixture with an authentic sample of (+)-isocorydine, R_F values in S1, S2 and S3 identical to those of an reference sample.

Protopine (0.000074): prisms, m.p. 208 – 209 °C (chloroform–methanol), and the same mixed m.p. with an authentic sample, the identity being confirmed by TLC in S1, S2 and S3.

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REFERENCES

1. Fedde F. in: *Das Pflanzenreich – Regni vegetabilis conspectus* (A. Engler, Ed.), Part IV, Vol. 104. Leipzig 1909.
2. Slavik J., Slavikova L., Dolejs L.: Collect. Czech. Chem. Commun. *46*, 2587 (1981).
3. Slavik J., Slavikova L., Bochorakova J.: Collect. Czech. Chem. Commun. *54*, 1118 (1989).
4. Slavik J., Slavikova L.: Collect. Czech. Chem. Commun. *55*, 1812 (1990).
5. Duke J. A.: CRC Crit. Rev. Toxicol. *1974*, 1.
6. Slavik J., Slavikova L.: Collect. Czech. Chem. Commun. *50*, 1216 (1985).
7. Slavik J., Slavikova L.: Collect. Czech. Chem. Commun. *56*, 1534 (1991).
8. Goldblatt P.: Ann. Missouri Bot. Gard. *61*, 264 (1974).
9. Nielsen B., Roe J., Brochmann-Hanssen E.: Planta Med. *48*, 205 (1983).
10. Chelombitko V. A., Mikheev A. D.: Rastit. Resur. *21*, 316 (1985).
11. Shafiee A., Lalezari I., Assadi F., Khalafi F.: J. Pharm. Sci. *66*, 1050 (1977).

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