ALKALOIDS OF *Papaver argemone* L. AND *Papaver pavoninum* FISCH. et MEY. FROM THE *Argemonorhoeades* FEDDE SECTION*

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Dedicated to Prof. Jiří Slavík on the occasion of his 65th birthday.

Papaver argemone L. and *Papaver pavoninum* FISCH. et MEY. species of the *Argemonorhoeades* FEDDE (*Papaveraceae*) section were studied. A very low content of alkaloids was found in both species (less than 0·05%). *P. argemone* contains corytuberine and its quaternary N-methyl derivative magnoflorine as the dominant bases. As minor constituents were isolated: protopine, isocorydine, scoulerine, and alkaloids PAR 1, PAR 2, PAR 3. Chromatographic analysis detected allocryptopine, cryptopine, coptisine and traces of rhoeadine, papaverrubines C, D and E, and more than 6 unidentified bases. *P. pavoninum* gave N^2-methyl-1,2,3,4-tetrahydro-β-carboline as the dominant alkaloid, along with minor amounts of the tertiary bases protopine, allocryptopine, corydine, isocorydine and corytuberine. Of quaternary bases, coptisine, magnoflorine and an unidentified alkaloid PP 1 were isolated. The presence of many other, considerably labile bases has been proven in both species.

The section *Argemonorhoeades* FEDDE (*Argemonidium* SPACH) of the *Papaver* L. (*Papaveraceae*) genus includes six annual species distributed mainly in Central and South Europe and also in part of the Armenian-Iranian mediterranean region[1]. As concerns the alkaloid distribution, no unequivocal characterization of this section existed so far. All species are reported to contain an unusually low alkaloid content but data on the individual alkaloids vary considerably[2]. With the exception of coptisine, no quaternary alkaloid has been found in any species.

In this communication we investigate the alkaloid content in *P. argemone* L. and *P. pavoninum* FISCH. et MEY. species. In a previous study of *P. argemone*, Preininger[3] reported 0·15% of alkaloids of which the main constituents were rhoeadine and protopine, accompanied with isorhoeadine, rhoeagenine, oxysanguinarine and papaverrubines D and E. Diametrically different results were obtained by Slavík[4] who isolated only 0·012% of unspecified bases of which 8 were of nonphenolic and 6 of phenolic character. He did not prove the presence of rhoeadine or rhoeagenine

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but identified traces of papaverrubines A, D and E and coptisine. Similarly, in a chromatographic study, Vent[5] has proven the presence of 17 alkaloids in the plant, neither of which was identical with rhoeadine or rhoeagenine. The yield of bases isolated in our present work was also very low (0·009%). The main constituents were the tertiary alkaloid corytuberine and its quaternary N-methyl derivative magnoflorine, both isolated from the fraction of strongly polar bases[6]. The tertiary alkaloid fraction did not contain any dominant base and represented an amorphous mixture of more than 10 constituents. From the fraction of nonphenolic tertiary bases we obtained small amount of protopine and of the unidentified alkaloid PAR 1 (m.p. 193–195°C). Allocryptopine, cryptopine, papaverrubines C, D and E, and traces of rhoeadine and 6 other unidentified bases were detected by chromatography. The portion of phenolic alkaloids afforded scoulerine and negligible amount of phenolic base PAR 2 (m.p. 252–255°C). In the fraction B we identified coptisine. Besides the above-mentioned alkaloids corytuberine and magnoflorine, the fraction of strongly polar alkaloids afforded another amorphous quaternary alkaloid PAR 3 the small amount of which allowed only a UV-spectral characterization.

Also the data on alkaloid content of the *P. pavoninum* species differ considerably. Whereas Platonova[7] isolated allocryptopine, protopine and roemeridine, Šantavý[8] found rhoeadine, protopine and papaverrubines A and E. Slavík[4] has proven the presence of protopine, allocryptopine, an alkaloid chromatographically similar to roemeridine, papaverrubines A, D and E, and 7 other bases. Similarly, Sadykov[9] described protopine and allocryptopine as the dominant bases in this taxone. According to the recent investigations of Chelombitko[2], *P. pavoninum* contains allocryptopine, cheilanthiofoline, scoulerine and 4 unidenitified bases. In our present study we isolated 0·0153% of bases. As the dominant alkaloid of the tertiary fraction we obtained the indole alkaloid N^2-methyl-1,2,3,4-tetrahydro-β-carboline[10] (0·0007%), accompanied by protopine, allocryptopine, corydine, isocorydine and other more than 8 amorphous bases, not identical with the accessible standard alkaloids. We confirmed chromatographically the presence of cheilanthifoline and coptisine, however, rhoeadine, roemeridine or any of papaverrubines were not found. Fraction of strongly polar bases afforded corytuberine, magnoflorine and small amount of iodide of another quaternary alkaloid PP 1. Character of the UV spectrum indicates that the compound is a quaternary phenolic protoberberine[11], not identical with columbamine, berberrubine or jatrorrhizine. Its IR spectrum exhibits characteristic bands at 1 730 and 3 440 cm^{-1} and in the mass spectrum ions of m/z 337 and 148 predominate. Reduction with zinc in hydrochloric acid afforded an unidentified tetrahydroprotoberberine. Because of small amount of this alkaloid, a more detailed structural investigation was not possible.

Our results confirm that the studied two taxones of the *Argemonorhoeades* section of *Papaver* genus have very low content, but a great variety, of alkaloids. Only some of them belong to compounds typical for the *Papaver* genus (protopine, allocrypto-

pine, corydine, isocorydine), many other, however, differ in their chromatographical behaviour from alkaloids usually present in this genus. Often these compounds are very unstable and decompose during the isolation.

Our findings correlate well with the results of other authors[2,4,5,7,9] but are at variance with the data of Preininger[3] and Šantavý[8] who obviously worked with an erroneously determined botanical material, as already suggested by Vent[5]. In both species we have found for the first time magnoflorine and corytuberine which are widespread in species of some other families, particularly of the *Ranales* order[12].

EXPERIMENTAL

Melting points and mixed melting points were determined on a Kofler block and are uncorrected. UV spectra were measured in methanol on an SP-1800 Pye Unicam spectrophotometer. IR spectra were recorded in Nujol on an IR-75 Specord (Zeiss, Jena) instrument. Mass spectra were obtained with a Jeol MS 100 spectrometer. Thin-layer chromatography (TLC) was performed on silica gel LS 5—40 μ (Lachema) with gypsum binder in cyclohexane–diethylamine 9 : 1 (S1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S2), benzene–acetone–methanol 7 : 2 : 1 (S3), cyclohexane–chloroform–diethylamine 6 : 3 : 1 (S4), methanol–water–25% ammonia 15 : 3 : 1 (S5), methanol–water–25% ammonia 15 : 9 : 1 (S6), and on Silufol ready-made plates (Kavalier) in methanol–diethylamine 4 : 1 (S7). Descending paper chromatography (PC) was performed on a Whatmann No. 1 paper in 1-butanol–98% acetic acid–water 10 : 1 : 3 (S8) and ethanol–water 3 : 2 (S9). Fluorescing alkaloids were detected in UV light at 235 and 336 nm, papaverrubines with concentrated hydrochloric acid vapours, other alkaloids by spraying with potassium iodoplatinate (TLC) or Dragendorff's reagent (PC). Column chromatography was performed on silica gel L 100—400 μ (Lachema), the weight of the adsorbent being 200—300 times higher than that of the separated mixture. Benzene was used as the starting solvent and the eluent polarity was increased by successive addition of diethyl ether, chloroform and methanol.

Extraction and Isolation

The plants were cultivated in the Centre for Cultivation of Medicinal Plants of the Medical Faculty, Purkyně University, Brno, from seeds obtained from various botanical gardens and were harvested at the stage of flowering. Herbarium specimens are deposited at the Department of Medical Chemistry and Biochemistry, Purkyně University, Brno.

The dry ground material (whole plants) was moistened with 0·5M-Na_2CO_3 and extracted with cold methanol. After distilling off the solvent, the crude extract was dissolved in 0·5M acetic acid and separated into the alkaloidal fractions A, B, E and I, as described previously[6].

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The extraction was performed with 10·1 kg of plant material (harvested in July 1984—1986). Fractionation of the crude mixture of bases afforded amorphous fractions A (0·86 g), B (14·4 mg), E (0·71 g), and I (3·4 g). Fraction A was further separated into predominantly non-phenolic part A_1 (0·42 g) and a phenolic part A_2 (0·28 g). Both fractions were chromatographed on a column of silica gel. Fraction A_1 on elution with benzene afforded negligible amount of unidentified compounds that turned white on spraying with potassium iodoplatinate. Elution with diethyl ether afforded a non-alkaloidal compound (1·0 mg), m.p. 140—143°C. Chloroform–methanol

(48 : 1) eluted isocorydine (1·5 mg; crystallized from methanol). Fractions, obtained by elution with chloroform–methanol (48 : 1 — 4 : 1) contained predominantly protopine; after its separation by crystallization from chloroform–ethanol (1·3 mg) the mother liquors contained cryptopine, allocryptopine, traces of rhoeadine, papaverrubines C, D and E, and three other unidentified alkaloids. Elution with pure methanol afforded small amount of alkaloid PAR 1 (1·1 mg; m.p. 193°C); some other, unidentified bases were detected. Fraction A_2 already during the TLC chromatography exhibited several spots of obviously labile alkaloids whose amount and number varied according to the conditions. Some of these compounds were isolated by column chromatography in benzene and chloroform–benzene mixtures, however, their lability prevented characterization or identifications. The main base of this fraction, scoulerine (2·1 mg), was eluted with chloroform–methanol 24 : 1. Elution with chloroform–methanol 19 : 1 gave base PAR 2, m.p. 252—256°C, which was only partially characterized because of the small amount available. In fraction B coptisine was found by TLC and PC, fraction E contained practically no alkaloids, except traces of corytuberine. Crystallization of fraction I from methanol afforded corytuberine hydriodide (349·3 mg) and magnoflorine (97·3 mg). After separation of the mentioned alkaloids, the mother liquors gave small amount of amorphous alkaloid PAR 3 (0·5 mg), characterized only by its UV spectrum.

Papaver pavoninum FISCH. et MEY.

Methanolic extract of dried plant material (21·0 kg; harvested in July 1984—1986) was separated into fractions A (3·65 g), B (110·2 mg), E (5·11 g), I (2·01 g). Fraction A was further separated into parts A_1 (1·85 g) and A_2 (1·37 g), both amorphous. The alkaloid constituents of A_1 were fractionated by chromatography on a column of silica gel (100 g) in benzene. Elution with benzene gave small amount of unidentified non-alkaloidal compounds, elution with chloroform and chloroform–methanol mixtures (100 : 1 — 50 : 1) furnished mixtures of 3—4 bases of which corydine (1·7 mg) and isocorydine (2·2 mg) were identified and isolated as hydrochlorides. Chloroform–methanol mixtures (50 : 1 — 1 : 1) eluted fractions, containing mainly protopine, which on crystallization from methanol afforded protopine (18·2 mg) and allocryptopine (3·4 mg). Mother liquors were shown (TLC) to contain other unidentified alkaloids. Chloroform–methanol (19 : 1 — 4 : 1) fractions consisted predominantly of N^2-methyl-1,2,3,4-tetrahydro-β-carboline which was purified by repeated crystallization from methanol (152·8 mg). Three other alkaloids were detected in the mother liquors. Fraction A_2 consisted of more than six bases of which cheilanthifoline was identified chromatographically. Since these bases were highly unstable, their separation and characterization was not possible. Fraction B afforded coptisine (12·1 mg), fraction E contained mostly non-alkaloidal and ballast compounds and was not processed further. Fraction I on crystallization from methanol furnished magnoflorine iodide (12·1 mg) and alkaloid PP 1 (5·6 mg) as orange needles.

Characterization of the Alkaloids Isolated

Yields in % of dry material from *P. argemone* and *P. pavoninum*, respectively, are given in parentheses.

Allocryptopine (TLC; 0·000016%), prisms, m.p. 161—163°C (methanol), identical with an authentic sample (mixture m.p., UV, IR spectra, TLC).

Coptisine (TLC, 0·000058%), chloride, orange needles not melting up to 350°C. Reduction with zinc in hydrochloric acid gave the tetrahydro derivative, m.p. 218—219°C, undepressed on admixture with an authentic specimen of (±)-stylopine.

Corydine (TLC, 0·000008%), hydrochloride, m.p. 253—254°C, identical with an authentic sample (mixture m.p., UV and IR spectra).

Corytuberine hydriodide (0·0035%, 0·000058%), needles, m.p. 215—216°C (methanol). Identical (mixture m.p., UV and IR spectra) with an authentic sample.

Isocorydine (0·000015%, 0·00001%), prisms, m.p. 182—183°C (methanol). IR and UV spectra as well as R_F values identical with those of authentic sample.

Magnoflorine iodide (0·00096%, 0·000017%), prisms, m.p. 257—260°C (methanol), identical (mixture m.p., IR and UV spectra) with an authentic sample.

N^2-*Methyl*-1,2,3,4-*tetrahydro*-β-*carboline* (—, 0·00072%), prisms, m.p. 265°C (methanol). For identification and spectral data see ref.[10].

Alkaloid PAR 1 (0·000011%, —), prisms, m.p. 193—195°C (methanol); UV spectrum (methanol): λ_{max}(nm) 226, 280, λ_{min}(nm) 256.

Alkaloid PAR 2 (0·000011%, —), prisms, m.p. 252—256°C (methanol); UV spectrum (methanol): λ_{max}(nm) 225, 292, 310; λ_{min}(nm) 250; (methanol, after addition of NaOH): λ_{max}(nm) 230, 315 sh, 350; λ_{min}(nm) 268.

Alkaloid PAR 3 (0·000015%; —), amorphous; UV spectrum (methanol): λ_{max}(nm) 222, 273; λ_{min}(nm) 268.

Alkaloid PP 1 (—, 0·000027%), orange needles from methanol, decomposition at 250°C; UV spectrum (methanol): λ_{max}(nm) 222, 239, 248—254 sh, 272, 352 sh, 360, 468; λ_{min}(nm) 240, 305, 390; (methanol after addition of NaOH): λ_{max}(nm) 222, 238—242 sh, 265, 342, 380; λ_{min}(nm) 252, 305, 357. IR spectrum (Nujol): 1 615, 1 730, 3 440 cm^{-1}. Mass spectrum (*m/z*): 337 ($C_{19}H_{15}NO_5$), 148 ($C_9H_8O_2$), 142 (CH_3I). Reduction of 1 mg of PP 1 with zinc in hydrochloric acid afforded 0·5 mg of an amorphous base of tertiary character, R_F 0·20 (S1), 0·58 (S2); UV spectrum (methanol): λ_{max}(nm) 202, 286—288; λ_{min}(nm) 260. IR spectrum (Nujol): 2 330, 3 450 cm^{-1}.

Protopine (0·000013%; 0·000087%), prisms, m.p. 208—209°C (chloroform–ethanol); identical with authentic compound (IR and UV spectra, m.p. and TLC).

Scoulerine (0·000021%,), needles, m.p. 192—193°C (methanol); hydrochloride m.p. 245 to 247°C. Identity with an authentic sample was proven by IR and UV spectra and TLC.

R_F Values

In S1, S2, S3: allocryptopine 0·34, 0·68, 0·21; corydine 0·14, 0·54, 0·36; isocorydine 0·16, 0·58, 0·40; protopine 0·36, 0·72, 0·28; in S2, S3, S4: N^2-methyl-1,2,3,4-tetrahydro-β-carboline 0·14, —, 0·22; PAR 1 0·02, 0·13, 0·18; PAR 2 0·12, 0·22, 0·26; scoulerine 0·10, —, 0·18; in S7, S8, S9: coptisine 0·54, 0·42, 0·08; in S5, S6: corytuberine 0·76, 0·85; magnoflorine 0·40, 0·49; PAR 3 0·06, 0·03; PP 1 0·45, 0·52.

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