

ALKALOIDS FROM *Papaver rhoeas* var. *chelidonioides* O. KUNTZE, *P. confine* JORD., AND *P. dubium* L.*

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Papaver rhoeas var. *chelidonioides* O. KUNTZE (diploid, $2n = 14$) contains rhoeadine (0.027%) and coptisine (0.004%) as the dominant alkaloids, the latter being the yellow principle of the latex. As minor constituents were isolated protopine, papaverrubine A, rhoeagenine, (–)-stylopine, an unidentified alkaloid PCH 1 (m.p. 205°C), 2-methyl-1,2,3,4-tetrahydro- β -carboline and (–)-*trans*-N-methylstylopinium hydroxide. Chromatographic analysis detected isorhoeadine, allocryptopine, cryptopine, papaverrubine C, D and E, scoulerine, isoboldine, berberine, corysamine, magnoflorine and corytuberine. *P. confine* JORD. (tetraploid, $2n = 28$) was found to display a very low alkaloid content (0.003%) and afforded corytuberine and berberine as principal alkaloids, along with minor amounts of protopine, rhoeadine, scoulerine and cryptopine. Chromatography identified isocorydine, corydine, allocryptopine, aporheine, stylopine, sinactine, thebaine, mecambaine, papaverrubines A, E, C and D, coptisine and corysamine. The tertiary alkaloid fraction from ripe capsules of *P. dubium* L. (hexaploid, $2n = 42$; 0.24% of alkaloids) of domestic origin afforded (+)-roemerine (aporheine) as the main constituent (87% of total bases); mecambaine, protopine, rhoeadine and papaverrubines D and C (traces of A and E) were detected. From strongly polar bases N-methylaporheinium hydroxide (as iodide) was isolated and corytuberine detected.

The *Papaver* species of the *Rhoeadium* SPACH (*Orthorhoeades* FEDDE) section, distributed in Czechoslovakia, have been recently the subject of botanical studies leading to revision of their taxonomic value and to characterization of several new taxa¹⁻³. The present communication contributes to the knowledge of alkaloid constituents in three of these taxa: *Papaver rhoeas* var. *chelidonioides* O. KUNTZE and *P. confine* JORD., which were not hitherto studied, and *P. dubium* L. s. str. of domestic origin.

P. rhoeas var. *chelidonioides* O. KUNTZE (1867)⁴ has been described as a variety of the extremely variable and polymorphous species *P. rhoeas* L. which is characteristic by the yellow colour of latex. Its morphology, phenology, ecology or distribution do not differ from those of typical *P. rhoeas* populations with the usual white

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latex¹. Both the forms are diploid ($2n = 14$). The yellow colour is due to a higher content of coptisine (*Ia*) the presence of which we have proven by chromatographic analysis of a herbarium specimen⁵. Recently, we had the opportunity to investigate alkaloids from a greater amount of plant material, cultivated from seeds collected in a natural locality in Central Bohemian Highlands. From the tertiary alkaloid fraction (0.046% of the whole dry plant) we isolated rhoeadine (*Iia*) as the main alkaloid. We also isolated minor amounts of protopine, papaverrubine A (*Iib*), rhoeagenine (*Iic*), (-)-stylopine, an alkaloid of m.p. 205°C (provisionally denoted as PCH 1), and a negligible quantity of an alkaloid melting at 217°C, which was identified as 2-methyl-1,2,3,4-tetrahydro- β -carboline (*III*) by comparison with an authentic sample. After the species *P. pavoninum* FISCH. et MEY.⁶, this is the second case of detection of this little specific indole alkaloid in the *Papaveraceae* family. In the mother liquors we proved chromatographically isorhoeadine (*Iid*), allocryptopine, cryptopine, papaverrubines C, D and E, scoulerine and isoboldine.

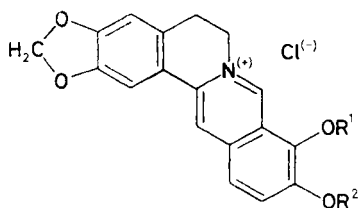
The fraction of quaternary protoberberines was converted into chlorides and crystallized to give coptisine chloride (*Ia*) which represented (after rhoeadine) the second dominant alkaloid of the plant. Chromatography of the mother liquor detected small amounts of berberine and traces of corysamine. The fraction of quaternary alkaloids, extracted with chloroform as iodides, afforded (-)-*trans*-N-methylstylopinium iodide; minor quantities of magnoflorine, corytuberine and two unidentified alkaloids were detected.

Dry ripe capsules were studied separately because capsules of several species of the *Papaver* genus have the highest alkaloid content. In this case, however, we have found an only slightly higher alkaloid content (0.057%) and, compared with rhoeadine (0.052% of dry capsules), a lower content of minor alkaloids whose composition did not differ much from that found for the whole plant.

Compared with the typical form of *P. rhoeas* L., the var. *chelidonioides* contains substantially more coptisine and other minor alkaloids, particularly papaverrubine A and protopine. The indole alkaloid *III* was found in *P. rhoeas* for the first time. These results lead to the conclusion that *P. rhoeas* var. *chelidonioides* represents a well defined chemotype, although its taxonomic value remains doubtful^{1,4}.

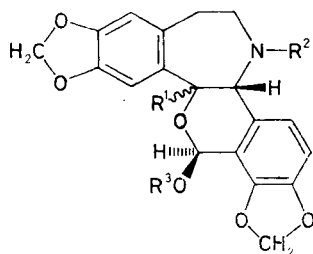
Papaver confine JORD., a taxon with red petals from the group *P. dubium* agg., was until recently not distinguished from the typical *P. dubium* L., in spite of some different morphological features (e.g. shape of buds, capsules and leaves¹⁻³). Moreover, it is a tetraploid ($2n = 28$), in contrast with the hexaploid *P. dubium* ($2n = 42$)¹⁻³. In Czechoslovakia it is distributed only in the warmest regions. We have found marked differences also in the alkaloid profile of both these taxa. The alkaloid content in *P. confine* (0.003%) is unusually low compared with that in *P. dubium* (0.14–0.24%)⁷. From the whole plant we isolated corytuberine (*IVa*) and berberine chloride (*Ib*) as the main alkaloids, along with minor amounts of protopine, rhoeadine, scoulerine and cryptopine. Among the amorphous bases from the mother liquors

we identified chromatographically small amounts of isocorydine, corydine, allocryptopine, aporheine, stylopine, sinactine, thebaine, mecambaine, papaverrubine A, E, C and D, coptisine and corysamine. The content and composition of alkaloids in dry ripened capsules of *P. confine* also did not differ substantially from those for the whole plant, except that thebaine was the principal tertiary base.



I a, $R^1 + R^2 = \text{CH}_2$

I b, $R^1 = R^2 = \text{CH}_3$



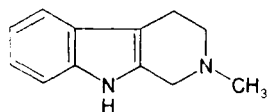
II a, $R^1 = \text{H}$; $R^2 = R^3 = \text{CH}_3$

II b, $R^1 = \text{H}$; $R^2 = \text{H}$; $R^3 = \text{CH}_3$

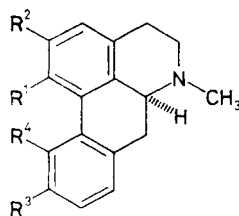
II c, $R^1 = \text{H}$; $R^2 = \text{CH}_3$; $R^3 = \text{H}$

II d, $R^1 = \text{H}$; $R^2 = R^3 = \text{CH}_3$

II e, $R^1 = \text{H}$; $R^2 = \text{CH}_3$; $R^3 = \text{CH}_2\text{CH}_3$



III



IV a, $R^1 = R^4 = \text{OH}$; $R^2 = R^3 = \text{OCH}_3$

IV b, $R^1 + R^2 = \text{O}-\text{CH}_2-\text{O}$; $R^3 = R^4 = \text{H}$

For comparison we also investigated dry ripened capsules of *P. dubium* L. of domestic origin, collected in the same region of Central Bohemian Highlands as the studied population of *P. confine*. It is a hexaploid¹⁻³ ($2n = 42$). As the main alkaloid (0.24%) of the capsules we isolated (+)-roemerine (aporheine⁷, IVb) which represented 87% of the tertiary alkaloid content. In the mother liquors we identified mecambaine as the second dominant alkaloid, along with small amounts of protopine, rhoeadine, papaverrubine D and C, and traces of papaverrubine A and E. The fraction of quaternary protoberberines contained negligible amounts of coptisine. Strongly polar alkaloids were converted into iodides, affording N-methylaporheinium

iodide (0.003%); the mother liquors contained corytuberine as the main constituent. All these alkaloids had been isolated practically in the same ratios from *P. dubium* of foreign origin^{5,7-9}. Obviously, our domestic population of *P. dubium* does not differ in the alkaloid content from other European populations*. From another sample of *P. dubium* of West-European origin we isolated rhoeadine (0.002%) as minor alkaloid (about 1% of total bases), along with aporheine and mecambine. Dubirheine (O-ethylrhoeagenine, *Ile*)⁷ was undoubtedly an artifact formed by transacetalization of rhoeadine during the extraction with ethanol.

It is surprising that the two botanically so closely related taxa such as *P. dubium* L. and *P. confine* JORD. which until recently were even not distinguished, differ so markedly in their alkaloid profile so that this can be used for an additional botanical characterization of both taxa. From the biochemical viewpoint, *P. confine* has no common features with the hexaploid *P. dubium* s. str., however, its dominant alkaloids corytuberine (*Iva*) and berberine (*Ib*) indicate a close relationship with the tetraploids *P. lecoquii* LAMOTTE and *P. albiflorum* subsp. *austromoravicum* KUBÁT of the *P. dubium* agg. group^{1,3}.

EXPERIMENTAL

Melting points were determined on a Mettler FP 51 instrument and are uncorrected. Ultraviolet spectra were measured in methanol on a Unicam SP 1 800 spectrometer, IR spectra in Nujol on a Specord IR (Zeiss, Jena) instrument. Thin-layer chromatography (TLC) was carried out on silica gel G (Merck): tertiary alkaloids in cyclohexane-diethylamine 9 : 1 (S1), cyclohexane-chloroform-diethylamine 7 : 2 : 1 (S2) and 6 : 3 : 1 (S3), papaverrubines in S1, S2, benzene-methanol 4 : 1 (S4) and benzene-acetone-methanol 7 : 2 : 1 (S5), quaternary alkaloids and corytuberine in methanol-water-25% ammonia 15 : 3 : 1 (S6), ethanol-water-25% ammonia 15 : 9 : 1 (S7) and 1-propanol-water-85% formic acid 12 : 7 : 1 (S8). Quaternary protoberberines were chromatographed in the systems S6 and S7 and also on Silufol UV 254 (Kavalier) in methanol-diethylamine 4 : 1 (S9) and 1 : 1 (S10). Fluorescing alkaloids were detected by UV light, the other alkaloids with potassium iodoplatinate. Papaverrubines were detected by treatment with vapours of concentrated hydrochloric acid for 20 min (purple spots).

Extraction and Isolation of Alkaloids

The plants were cultivated in the Centre for Cultivation of Medicinal Plants of the Medical Faculty Purkyně University, Brno, from seeds collected at the natural localities in Central Bohemian Highlands (collected by Dr Kubát). The cultivated plants were harvested at the stage of unripe fruits (end of July and beginning of August) in 1985 and 1986. Ripe capsules were harvested separately and the seeds were removed. The plant material was dried at room temperature and

* A sample of plants collected in Czechoslovakia and studied in ref.¹⁰ designated as *P. dubium* L. was examined by us in 1962 and has been identified unequivocally as *P. rhoeas* L. In spite of our finding some authors still state (see e.g. ref.^{11,12}) that some populations of *P. dubium* may produce rhoeadine as the main alkaloid. All these statements, based on ref.¹⁰, have to be considered invalid because of erroneous determination of the botanical material.

ground. Herbarium specimens are deposited at the Medical Faculty of this University. The dry plant material was exhaustively extracted with cold methanol in a percolator, the methanol was distilled off, the residue was dissolved in 1% acetic acid and the insoluble ballast material was filtered off. The filtrate was made alkaline with sodium carbonate and extracted several times with ether (fraction A), to the aqueous phase sodium hydroxide was added to strongly alkaline reaction ($\text{pH} > 13$) and the mixture was again extracted with ether (fraction B). The ethereal solution of B was immediately treated with solid citric acid (conversion into citrates). The remaining aqueous layer was adjusted to $\text{pH} 4-5$ with 20% sulfuric acid (to $\text{pH} 7$ in the case of *P. confine*), saturated aqueous solution of potassium iodide was added and the mixture was extracted with chloroform or chloroform with 20% of ethanol to negative reaction with Mayer's reagent (fraction I).

Papaver rhoeas var. *chelidonioides* O. KUNTZE

The whole dry plant (830 g) was extracted. The crude fraction A was purified by dissolution in 5% acetic acid, filtration with charcoal, addition of ammonia to the filtrate to alkaline reaction, and extraction with ether. The purified bases of fraction A were crystallized from chloroform-methanol to afford 219.0 mg of rhoeadine. The remaining bases were dissolved in 5% acetic acid and mixed with saturated potassium chloride solution. Extraction with chloroform separated the bases into fractions of hydrochlorides or acetates, extractable (AC) and non-extractable (AD) with chloroform. Systematic crystallization of bases of fraction AC from methanol afforded further 2.7 mg of rhoeadine, 21.0 mg of papaverrubine A, 6.7 mg of (–)-stylopinine and 2.6 mg of alkaloid PCH 1. As shown by TLC, the amorphous residue (55.8 mg) contained, in addition to the already mentioned alkaloids, small amounts of isorhoeadine and papaverrubine E and several unidentified alkaloids. The fraction AD was separated into non-phenolic (AD_1) and phenolic (AD_2) bases by the usual¹⁴ procedure. Crystallization of bases AD_1 from methanol separated protopine (29.1 mg), rhoegenine (19.4 mg) and 2-methyl-1,2,3,4-tetrahydro- β -carboline (1.8 mg). In the amorphous residue (11.6 mg) allocryptopine and cryptopine were identified by TLC. The amorphous phenolic bases AD_2 (10.7 mg) contained scoulerine, isoboldine and papaverrubines C and D.

Citrates of the fraction B were dissolved in water, the solution was filtered, acidified with hydrochloric acid and the crystalline coptisine chloride (*Ia*; 31.3 mg) was collected. The mother liquors contained (according to TLC) small amounts of berberine and corysamine. From fraction I (–)-*trans*-N-methylstylopinium iodide (9.9 mg) was obtained by crystallization from methanol; mother liquors were shown to contain minor amounts of magnoflorine, corytuberine and two unidentified alkaloids.

Dry ripened capsules (4.8 g) gave fraction A by the same procedure as described above; its crystallization from methanol separated rhoeadine (2.5 mg). Protopine, stylopinine and two other alkaloids were found in the mother liquors (0.2 mg of bases). Fraction B consisted practically solely of coptisine (max. 0.01%).

Papaver confine JORD.

Extraction of dry whole plants (4 650 g) and processing as described above afforded 0.62 g of crude bases of fraction A which were twice purified and separated¹⁴ into non-phenolic fractions AC_1 and AD_1 and phenolic fractions AC_2 and AD_2 . Crystallization of fraction AC_1 from methanol gave rhoeadine (4.9 mg) and chromatographic analysis of the amorphous residue (47.1 mg) identified isocorydine, aporheine, stylopinine, papaverrubines A and E, and traces of corydine. In the amorphous fraction AC_2 (10.2 mg) the principal component was isocorydine.

Crystallization of fraction AD₁ from methanol furnished protopine (7.0 mg), cryptopine (2.5 mg) and amorphous bases (9.7 mg) in which TLC identified thebaine as the main constituent, along with some unidentified alkaloids. From the amorphous fraction AD₂ scoulerine (2.6 mg of base) was isolated as the sparingly soluble hydrochloride; amorphous bases (10.3 mg), obtained from the mother liquors, contained papaverrubines C and D.

The citrates of fraction B were converted into chlorides, affording berberine chloride (16.4 mg; 0.00035%); the mother liquors were shown by TLC to contain negligible amounts of coptisine and corysamine. Crystallization of fraction I from methanol afforded corytuberine (25.0 mg) as the free base. Further amount of corytuberine (as hydriodide, 16.7 mg) was isolated after purification of alkaloids from the mother liquors and conversion into iodides. No other alkaloids than corytuberine were detected in the mother liquors.

Dry ripened capsules (42 g) furnished 2.8 mg of crude bases of fraction A (0.007%) in which chromatographic analysis detected thebaine as the main constituent, along with minor amounts of allocryptopine, cryptopine, protopine, rhoeadine and four unidentified alkaloids. Fraction B (3.8 mg) contained berberine and small amount of coptisine; in fraction I (2.0 mg) corytuberine was detected as the only alkaloid.

Papaver dubium L.

Extraction of dry ripened capsules (130 g) afforded 317.9 mg (0.24%) of fraction A. After conversion into hydrochlorides, aporheine hydrochloride (319.4 mg) crystallized from the mixture. Amorphous bases (15.7 mg), recovered from the mother liquors, contained mecambaine as the main constituent and small amounts of protopine, rhoeadine and unidentified alkaloids. Fraction B contained negligible amount of coptisine. Fraction I on crystallization from methanol gave N-methylaporheinium iodide (3.9 mg) and the mother liquors contained mainly corytuberine.

A sample of *P. dubium* (384 g of dry whole plants) of West-European origin was processed analogously. In addition to the main alkaloid aporheine and lesser amount of mecambaine, it afforded rhoeadine (8.2 mg) from fraction A, and N-methylaporheinium iodide (13.0 mg) and corytuberine hydriodide (9.3 mg) (cf. refs^{5,8}) from fraction I.

Characterization of the Alkaloids Isolated

The isolated alkaloids were characterized by melting points, mixed melting points, UV and IR spectra and TLC comparison with authentic specimens and, where necessary, also by optical rotation. Unless stated otherwise, yields of individual alkaloids are given in weight % of dry whole plants in the order *P. rhoeas* var. *chelidonioides*, *P. confine* and dry ripe capsules of *P. dubium*. The presence of alkaloids, detected only by TLC, is denoted +, their absence —.

Aporheine (—; +; 0.21): needles, m.p. 102–103°C (ether–hexane), undepressed on admixture with an authentic compound⁷, identical UV and IR spectra and TLC. Hydrochloride m.p. 266 to 267°C (ethanol).

Berberine (+; 0.00035; —): chloride, yellow needles, m.p. 207–208°C (dilute hydrochloric acid), in all respects identical with an authentic sample.

Coptisine (0.004; +; +): chloride, orange needles (from dilute hydrochloric acid), no melting up to 290°C, only carbonization; identical (spectra and TLC) with an authentic sample.

Corytuberine (+; 0.0008, +, whole plant 0.002%): base, needles, m.p. 239–240°C (ethanol); hydriodide, lustrous leaflets, m.p. 213–214°C; in both cases spectra and TLC identical with those of authentic samples.

Cryptopine (+; 0·00005; -): prisms, m.p. 220–221°C (methanol); identity proven by mixed m.p. and TLC (R_F 0·26 in S1).

N-Methylaporphinium iodide (-; -; 0·003, whole plant 0·003): prisms, m.p. 235–236°C (methanol-ether), identified by mixed m.p. and TLC.

(-)-*trans-N-Methylstylopinium iodide* (0·001; -; -): small aggregates, m.p. 296–298°C (methanol), no depression on admixture with an authentic sample, identical R_F values.

2-Methyl-1,2,3,4-tetrahydro- β -carboline (0·0002; -; -): prisms, m.p. 218–219°C (methanol), identical with an authentic sample⁶ (UV spectrum, TLC: R_F 0·10 in S1, 0·24 in S2, detected as a deep blue-violet spot).

Papaverrubine A (0·0025; +; +): needles, m.p. 219–220°C (chloroform-methanol), the same mixed m.p. with an authentic sample¹⁵, $[\alpha]_D^{23} + 385 \pm 5^\circ$ (c 0·1, chloroform); reported¹⁵ m.p. 223–224°C and $[\alpha]_D^{22} + 356^\circ \pm 10^\circ$ or $406^\circ \pm 2^\circ$ (chloroform). Its UV spectrum, λ_{max} , nm (log ϵ): 208 (4·46), 242 (3·92), 293 (3·96), λ_{min} 227 (3·81), 263 (3·23) and IR spectrum were completely identical with simultaneously run spectra of an authentic sample. Also the TLC parameters were the same (R_F in S1 0·43, in S2 0·56, in S4 0·84 and in S5 0·89). Our sample contained traces of papaverrubine E (R_F in S4 0·58, in S5 0·52).

Protopine (0·0035; 0·00015; +): prisms, m.p. 208–209°C (chloroform-methanol), no mixture m.p. depression, spectral data and TLC identical with an authentic sample.

Rhoeadine (0·027; 0·00011; +; whole plant 0·002): fine needles, m.p. 255–256°C (chloroform-methanol); identified by mixed m.p., UV and IR spectra, and TLC.

Rhoeagenine (0·0023; -; -): m.p. 240–241°C (chloroform-methanol), identical with an authentic sample (mixed m.p., TLC).

Scoulerine (+; 0·00006; -): aggregates, m.p. 195–197°C (ether), no mixture m.p. depression, TLC in S1 (0·10), S2 (0·23) and S3 (0·30) identical with the authentic sample.

(-)-*Stylopine* (0·0008; +; -): needles, m.p. 196–198°C (chloroform-methanol), $[\alpha]_D^{23} - 307^\circ \pm 5^\circ$ (c 0·1, chloroform). Identified by mixture m.p., UV spectrum and R_F values.

Alkaloid PCH 1 (0·0003; -; -): small prisms, m.p. 204–205°C (methanol); UV spectrum, λ_{max} , nm (log ϵ , calculated for M.w. 320): 209 (4·46), 243 (3·70), 293 (3·70), λ_{min} : 229 (3·67) and 264 (2·90). R_F in S1 0·32 (detected as a green-yellow spot), different from the other identified alkaloids.

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