ALKALOIDS FROM *Papaver albiflorum* SUBSP. *austromoravicum* KUBÁT, *P. albiflorum* PACZ. SUBSP. *albiflorum*, AND *P. lecoquii* LAMOTTE*

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From *Papaver albiflorum* subsp. *austromoravicum* KUBÁT berberine and (—)-α-canadine methohydroxide (in the form of iodide) were isolated as the main alkaloids, and allocryptopine, corytuberine and small amounts of protopine, rhoeadine and canadine as the minor components. Negligible amounts of mecambrine, thebaine, aporheine (?), corydine, isocorydine, scoulerine and traces of papaverrubines A, C, D and E, coptisine, corysamine, magnoflorine, α-stylopine methohydroxide and aporheine methohydroxide were also detected. *P. albiflorum* PACZ. subsp. *albiflorum* contains thebaine almost exclusively, accompanied by trace amounts of berberine, corytuberine, protopine (?), thebaine methohydroxide and aporheine methohydroxide. From *P. lecoquii* LAMOTTE berberine and corytuberine were isolated as the main alkaloids, as well as small amounts of protopine, a new alkaloid (—)-hexahydromecambrine-B, allocryptopine, rhoeadine and cryptopine. The presence of isocorydine and trace amounts of canadine, stylopine, mecambrine, papaverrubines A, C, D and E, corydine, scoulerine, coptisine, magnoflorine and α-stylopine methohydroxide was also detected.

Papaver albiflorum PACZ. and *P. lecoquii* LAMOTTE are annual species from the section *Orthorhoeades* FEDDE (*Rhoeadium Spach*) formerly designated in botanical literature as *P. dubium* subsp. *albiflorum* (BOISS.) DOST. and *P. dubium* subsp. *lecoquii* (LAMOTTE) FEDDE (*cf.*[1]). *P. albiflorum* with white petals is an East European species, while *P. lecoquii* with red petals is a West European species, the area of occurrence of which takes in the territory of Czechoslovakia. In the populations of *P. albiflorum* occurring very rarely in Czechoslovakia in the warmest areas of south Moravia and south Slovakia two subspecies could be distinguished recently by Kubát[2]: *P. albiflorum* PACZ. subsp. *albiflorum* with a colourless latex growing in south Slovakia, and *P. albiflorum* subsp. *austromoravicum* KUBÁT with a yellow latex, occurring both in south Moravia and south Slovakia. Both subspecies also differ in some morphological characters. Years ago[1] we investigated the alkaloids from *P. lecoquii* and *P. albiflorum* (subsp. *austromoravicum*) preliminarily and we isolated

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berberine from both species as the main alkaloid, giving the yellow colour to the latex, in addition to a negligible amount of allocryptopine. Presently we had the opportunity to work up substantially larger amounts of plant material, enabling us to study the minor alkaloidal components as well. Both taxons display an extremely low content of alkaloids (below 0·01%).

From *P. albiflorum* subsp. *austromoravicum* of the same population as in the preceding study[1] we again isolated berberine (0·003%) as the main alkaloid. The second dominant alkaloid is a quaternary base identical with (−)-α-canadine methohydroxide (0·002%). Among minor alkaloids we isolated allocryptopine, corytuberine, protopine, rhoeadine and canadine, and we detected in the residue of the "non-quaternary" fraction the presence of trace amount of mecambrine, thebaine, aporheine (?), corydine, isocorydine, scoulerine, papaverrubines A, C, D, and E, coptisine and corysamine. In the mother liquors after crystallization of canadine methiodide we detected negligible amounts of magnoflorine, α-stylopine methohydroxide and aporheine methohydroxide. The population with pink to red flowers, found recently in a few localities in south Slovakia[2], coincided completely with the typical white-flowered population according to thin-layer chromatographic analysis of herbarium specimens*, so that these chemical characters also confirmed affinity to the subsp. *austromoravicum.*

P. albiflorum subsp. *albiflorum* occurs in East Europe in the Danube area. We had the opportunity to study preliminarily the alkaloids from a herbarium specimen of plants from Slovakia. These specimens displayed a substantially higher content of the sum of alkaloids (0·12%) than the subsp. *austromoravicum*, almost exclusively the tertiary ones. Practically the only component was a base which we identified as thebaine. In the mass spectrum typical ions occurred of m/z 311·1528 (M^+, for $C_{19}H_{21}NO_3$ calculated 311·1521), 296 ($C_{18}H_{18}NO_3$) and 255 ($C_{16}H_{15}O_3$). The substance did not contain active hydrogen. The mass spectrum was identical with that of thebaine. Using TLC we detected trace amounts of berberine, protopine (?), corytuberine, aporheine methohydroxide and thebaine methohydroxide. This subspecies with its alkaloids differs quite substantially from the subsp. *austromoravicum*, so that the biochemical characters mentioned can be used for the completion of the botanical characterization of both taxons.

In agreement with earlier studies[1,3] we isolated from the West European population of *P. lecoquii* berberine (0·003%) as the main alkaloid, but in contradistinction to *P. albiflorum* subsp. *austromoravicum* in *P. lecoquii* the second main alkaloid is corytuberine (0·002%). Among the minor alkaloids we isolated protopine and a very small amount of allocryptopine, rhoeadine, cryptopine and a further alkaloid in crys-

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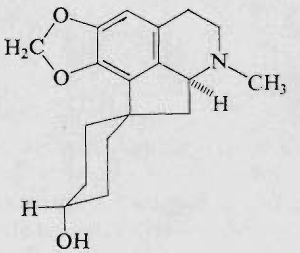
talline state. The latter was identified on the basis of spectral data and TLC as (*S*)-(−)-hexahydromecambrine-B (*I*, OH equatorial), by direct comparison with an authentic sample which was prepared from (*S*)-(−)-mecambrine[4]. This alkaloid was found in this material as a natural component for the first time. It is a stereoisomer of (*S*)-(−)-N-methyllitsericine (hexahydromecambrine-A) with the hydroxyl group in axial position[4]. In the mass spectrum of this new alkaloid there is a molecular ion at m/z 301 (45) and also characteristic ions at m/z 300 ($C_{18}H_{22}NO_3$, 100), 258 ($C_{16}H_{18}O_3$, 49), 240 ($C_{16}H_{16}O_2$, 17) and 203 ($C_{12}H_{13}NO_2$, 12). After labelling with [O-^{2}H]ethanol in the ion source the molecular peak and the peak at mass 258 was shifted upwards by one mass unit. According to the ratio $M-1/M$, the splitting off of $CH_2{=}NCH_3$, the combination of $CH_2{=}NCH_3$ and water, and the elimination of the $C_6H_{10}O$ particle its mass spectrum corresponded to the spectra of proaporphine alkaloids of the cyclohexanol type and it was identical with the spectrum of hexahydromecambrine-B (ref.[5]). In the mother liquors after the alkaloids mentioned we detected by TLC the presence of isocorydine and of trace amounts of canadine, stylopine, mecambrine, papaverrubine A, C, D and E, corydine, scoulerine, and in the quaternary fraction also traces of magnoflorine and α-stylopine methohydroxide.



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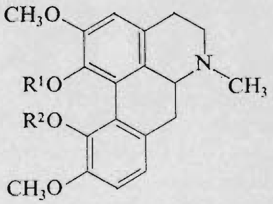

IIa, $R^1 = CH_3$, $R^2 = H$
IIb, $R^1 = H$, $R^2 = CH_3$

In addition to this we had the opportunity of making a preliminary analysis of the alkaloidal components of herbarium specimens of *P. lecoquii* from the territory of Czechoslovakia using thin-layer chromatography. This species was recently[2] discovered for the first time on Czechoslovak territory, at two localities in the Czech Central Mountains. We found that no substantial differences exist between our population and the West European with respect to the composition of the alkaloids, with the exception of one typical character: isocorydine (*IIa*) which belongs among dominant alkaloids in the West European population and which is completely replaced by the closely related corydine (*IIb*) in our population.

From these findings it follows that *P. albiflorum* subsp. *austromoravicum* and *P. lecoquii* are closely related not only botanically, but biochemically as well – on the basis of their main alkaloid berberine and the majority of minor alkaloids[1].In spite of this there exist characteristic differences especially with respect to the relative representation of minor alkaloids. While in the tertiary fraction of *P. albiflorum* subsp. *austromoravicum* allocryptopine is the main component, in *P. lecoquii* it is protopine. The second main alkaloid of *P. albiflorum* subsp. *austromoravicum* is (−)-α-canadine methohydroxide, but in *P. lecoquii* it is corytuberine. By its main alkaloid – thebaine – *P. albiflorum* subsp. *austromoravicum* evidently assumes a rather exceptional position in the *Orthorhoeades* section.

EXPERIMENTAL

The melting points were determined on a Mettler FP 51 apparatus and they were not corrected. The mass spectra were measured on a AEI-MS 902 spectrometer, and the UV spectra on a Unicam SP 1800 spectrophotometer, in methanol. For thin-layer chromatography both silica gel G Merck was used with the solvent systems cyclohexane–diethylamine 9 : 1 (S_1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S_2) and 5 : 4 : 1 (S_3), methanol–25% ammonia 200 : 1 (S_4), benzene–methanol 4 : 1 (S_5), benzene–acetone–methanol 7 : 2 : 1 (S_6), chloroform–ethanol–diethylamine 8 : 1 : 1 (S_7), ethanol–water–25% ammonia 15 : 9 : 1 (S_8) and methanol–water–25% ammonia 15 : 3 : 1 (S_9), and Silufol UV 254 (Kavalier) with the systems methanol–diethylamine 4 : 1 (S_{10}) and 1 : 1 (S_{11}). The spots of fluorescing alkaloids were detected in the UV light, the spots of papaverrubines by 25 min exposure to conc. hydrochloric acid fumes (formation of purple spots) and the spots of other alkaloids with potassium iodoplatinate.

Extraction and Isolation of Alkaloids

P. albiflorum subsp. *austromoravicum* (the origin of the seeds was the same as in ref.[1], *i.e.* the natural locality of Hustopeče near Brno) and *P. lecoquii* (origin of the seeds: botanical gardens in Oxford and Bonn) were cultivated in the Centre for the cultivation of medicinal plants, Medical Faculty, Brno, and harvested over several years in July at the stage of unripe fruits. The plants were dried at room temperature. The samples of other taxons were collected at natural localities in Czechoslovakia (collection by Dr K. Kubát): *P. albiflorum* subsp. *albiflorum* near Nitra, red-flowered population of *P. albiflorum* subsp. *austromoravicum* near Šahy and *P. lecoquii* at two localities in the Czech Central Mountains. The dry, ground plants were extracted with cold methanol, which was then distilled off and the extract dissolved in cold 1% acetic acid. The insoluble components were filtered off. From the filtrate the alkaloidal fractions *A*, *B*, *I* and *E* were isolated in the usual manner[6].

P. albiflorum subsp. *austromoravicum*

For the isolation of the alkaloids 7·00 kg of dry whole plants were used. Fraction *A* (310 mg, 0·0044%) was separated by the procedure used in our studies[6] to a fraction of hydrochlorides extractable with chloroform (*AC*) and non-extractable with it (*AD*), and both fractions were further separated to non-phenolic (AC_1 and AD_1) and phenolic (AC_2 and AD_2) fractions. Crystallization of fraction AC_1 from methanol gave 1·4 mg of rhoeadine (0·00002%) and from the mother liquors canadine was separated in the form of hydrochloride. The base prepared from it (2·1 mg, 0·00003%) remained amorphous and owing to its small amount it was identified merely by thin-layer chromatography, in comparison with an authentic specimen. When crystallized from ethanol and methanol fraction AD_1 afforded 60 mg of allocryptopine (0·0009%) and 5 mg of protopine (0·00007%). In the amorphous residues of fractions AC_1 (70 mg) and AD_1 (97 mg) which contained a considerable amount of non-alkaloidal substances, the presence of negligible amounts of mecambrine, thebaine, papaverrubines *A*, *C*, *D* and *E*, aporheine (?) and at least 5 further unidentified alkaloids could be detected by TLC in addition to the residues of the above-mentioned more important alkaloids. In the amorphous fractions AC_2 (5 mg) and AD_2 (30 mg) small amounts of corydine, isocorydine, scoulerine and 4 unidentified alkaloids could be proved by TLC.

On crystallization from dilute hydrochloric acid (1 : 10) fraction *B* gave 236 mg of berberine chloride (0·0034%) and when the mother liquors were alkalized with sodium hydroxide and extracted with ether 20 mg of yellow bases were obtained which contained neglibible amounts of coptisine and corysamine in addition to berberine. From fraction *I* 125 mg of (—)-α-canadine methiodide (0·0018%) and 51·4 mg of corytuberine hydriodide (0·0007%) were obtained by further purification and crystallization from methanol-ether. In the mother liquors small amounts of magnoflorine and α-stylopine methohydroxide, as well as traces of aporheine methohydroxide could be detected by TLC in addition to the residues of both above-mentioned alkaloids. Fraction *E* contained traces of alkaloids only. Crystallization from methanol gave a non-alkaloidal substance (50 mg) melting at 212—213°C.

From the herbarium specimen of the red-flowered population (4·52 g) the alkaloidal fractions *A*, *B* and *I* were obtained in a yield below 0·01%. In fraction *A* the presence of allocryptopine as the main component and traces of protopine were detected, while fraction *B* consisted of berberine and in fraction *I* canadine methohydroxide and a small amount of corytuberine were detected by TLC.

P. albiflorum subsp. *albiflorum*

Using the above-described procedure alkaloidal fractions *A*, *B* and *I* were isolated from a herbarium specimen (7·45 g). From fraction *A* (9 mg, 0·12%) 7·5 mg of thebaine (0·10%) were isolated on crystallization from ether, and in the mother liquors a trace amount of another alkaloid was detected by TLC in addition to thebaine. According to its R_F the other alkaloid could be identical with protopine. Fraction *B* (0·9 mg) contained traces of berberine only and fraction *I* contained according to TLC traces of corytuberine, aporheine methohydroxide and thebaine methohydroxide.

P. lecoquii

The whole dry plant (10·54 kg) was extracted and fractionated. Fraction *A* (250 mg, 0·0024%) was further fractionated[6] to fractions AC_1, AC_2, AD_1 and AD_2. From fraction AC_1 rhoeadine (8·2 mg, 0·00008%) was obtained on crystallization from ether and methanol. Fraction AD_1 was crystallized from ether to give 17·6 mg of hexahydromecambrine-B (0·00017%), and further crystallization from ether and methanol gave protopine (49·4 mg, 0·00047%), allocryptopine (8·5 mg, 0·00008%) and a crystalline mixture (8·0 mg) with m.p. 197—205°C, composed of approximately equal amounts of protopine and cryptopine (identification by TLC). In the amorphous residues of fractions AC_1 and AD_1 (a total of 45·8 mg) small amounts of canadine, mecambrine, stylopine and papaverrubines *A*, *C*, *S* and *E* were detected by TLC in addition to the mentioned alkaloids. In the phenolic bases AC_2 (45·1 mg) isocorydine was the main component according to TLC, in addition to a negligible amount of corydine, and in fraction AD_2 (65·1 mg) traces of scoulerine were detected in addition to several further unidentified alkaloids.

When crystallized from dilute hydrochloric acid, fraction *B* gave 0·30 g of berberine chloride (0·003%) and in the mother liquors traces of coptisine were detected. Fraction *I* afforded 0·23 g of corytuberine hydriodide (0·0022%) on crystallization from methanol–ether. In the mother liquors a negligible amount of magnoflorine and α-stylopine methohydroxide were detected by TLC. Fraction *E* was practically free of alkaloids.

From two herbarium specimens collected on the territory of Czechoslovakia, in the Czech Central Mountains, (5·92 g and 8·39 g) only a negligible amount of alkaloids was isolated (below 0·01%). Their composition was equal for both localities. In fraction *A* corydine was detected by TLC as the main component in addition to 3 further alkaloids. Fraction *B* was composed of berberine which represents the main alkaloid of the plant. The amount of alkaloids in fraction *I* was below the limit of detection sensitivity.

Characterization of the Alkaloids Isolated

The isolated alkaloids were characterized by their melting points, mixed melting points, or also optical rotation value, UV and IR spectra and R_F values, in direct comparison with authentic samples.

Berberine: chloride, yellow needles, m.p. 211—212°C (water), undepressed in admixture with an authentic sample; UV spectrum: λ_{max} (log ε) 231 nm (4·30), 265 nm (4·30), 350 nm (4·22), 424 nm (3·36), λ_{min} 250 nm (4·08), 305 nm (3·14), 383 nm (2·97). (±)-Tetrahydroberberine, prepared on reduction with sodium borohydride, m.p. 169—170°C (methanol), mixed melting point with an authentic sample was undepressed.

(—)-α-*Canadine methiodide*: from methanol–ether, leaflets, m.p. 163—164°C, solidifying from 168°C to 189°C, partly melting at 205—210°C, again solidifying and remelting at 248 to 254°C; the authentic sample displayed the same behaviour, either alone or in admixture with the former sample; $[\alpha]_D^{22}$ $-125° \pm 2°$ (*c* 0·57, methanol). UV spectrum: λ_{max} (log ε) 209 nm (4·74), 287 nm (3·92), shoulder at 220 nm (4·44), λ_{min} 259 nm (3·56).

Corytuberine: hydriodide, leaflets, m.p. 211—212°C (methanol–ether), undepressed on admixture of an authentic specimen; UV spectrum: λ_{max} (log ε) 224 nm (4·72), 272 (4·11), 312 nm (3·91), λ_{min} 260 nm (4·06), 292 nm (3·81).

Allocryptopine: prismatic needles, m.p. 159—160°C (methanol), undepressed on admixture of an authentic specimen.

Protopine: prisms, m.p. 208—209°C (chloroform–methanol), undepressed on admixture of an authentic sample.

(—)-*Hexahydromecambrine*-B: prisms, m.p. 266—267°C (methanol), undepressed on admixture of a sample prepared from (—)-mecambrine[4], $[\alpha]_D^{25}$ $-44° \pm 3°$ (*c* 0·09, methanol); UV spectrum: λ_{max} (log ε) 211 nm (4·50), 287 nm (3·63), shoulder at 241 nm (3·61), λ_{min} 260 nm (3·13).

Rhoeadine: needles, m.p. 254—255°C (chloroform–methanol), undepressed on admixture of an authentic sample. Their R_F values were also identical.

Thebaine: prisms, m.p. 189°C (ether), undepressed on admixture of an authentic specimen (m.p. 190—191°C). The mass spectrum and the R_F values of both samples were also identical.

R_F Values

In systems S_1, S_2, S_3 and S_4, respectively: allocryptopine 0·27, 0·54, 0·85, 0·59; aporheine 0·60, 0·71, —, 0·84; canadine 0·59, 0·84, —, 0·86; corydine 0·19, 0·46, 0·70, —; cryptopine 0·20, 0·54, —, 0·68; hexahydromecambrine-B 0·09, 0·24, —, 0·80; isocorydine 0·24, 0·52, 0·79, —; mecambrine 0·21, 0·48, —, —; protopine 0·39, 0·66, 0·90, 0·71; rhoeadine 0·51, 0·72, —, —; scoulerine 0·04, 0·12, 0·42, —; stylopine 0·66, 0·86, —, —; thebaine 0·34, 0·57, —, 0·64. In systems S_1, S_5 and S_6: papaverrubine A 0·32, 0·76, 0·83; papaverrubine C 0·11, 0·43, 0·54; papaverrubine D 0·05, 0·39, 0·46; papaverrubine E 0·32, 0·37, 0·34. In S_7: corytuberine 0·62. In systems S_8 and S_9: aporheine methiodide 0·29, 0·10; canadine methidide 0·58, 0·31; corytuberine 0·80, 0·75; magnoflorine 0·65, 0·47; α-stylopine methiodide 0·39, 0·26; thebaine methiodide 0·25, 0·08. In S_{10} and S_{11}: berberine 0·29, 0·65; coptisine 0·57, 0·85; corysamine 0·16, 0·54.

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