ALKALOIDS OF *Meconopsis cambrica* (L.) VIG. AND *M. robusta* HOOK. f. et THOMS.*

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Additional alkaloids (+)-roemerine (**3b**), (+)-corytuberine (**3d**), (–)-*N*-methylmecambridinium and alkaloid MC 2 (the last two as iodides) were isolated as minor alkaloidal components from *Meconopsis cambrica* (L.) VIG. (*Papaveraceae*) besides of the dominant known alkaloids (–)-mecambrine (**1**), (–)-mecambridine (**2**), (–)-flavinantine (**4**) and (+)-magnoflorine (**5**), and a small amount of (+)-mecambroline (**3a**). Minute quantities of protopine, allocryptopine, roemeroline, papaverrubine D and C, berberine, coptisine, corysamine and palmatine were identified by thin layer chromatography (TLC). From *M. robusta* HOOK. f. et THOMS., protopine, (–)-amurensinine (**6**) and two incompletely characterized alkaloids MRO 1 and MRO 2 were isolated, and allocryptopine, cryptopine, rhoeadine, 6-methoxy-2-methyl-1,2,3,4-tetrahydro-β-carboline, alkaloid MR 1, coptisine, corysamine, magnoflorine, corytuberine and *N*-methylamurensininium salt were detected on TLC.

Key words: *Meconopsis cambrica*; *Meconopsis robusta*; *Papaveraceae*; Minor alkaloids.

The genus *Meconopsis* (*Papaveraceae*) comprises about fourty monocarpic species indigenous to high mountain regions of Himalayas and China except only one species, *M. cambrica* (L.) VIG., which is polycarpic and native to West Europe. Several *Meconopsis* species have been the subject of our previous alkaloid studies[2–5]. In this communication, we report the isolation and identification of some additional alkaloids from *M. cambrica* (L.) VIG. and *M. robusta* HOOK. f. et THOMS.

M. cambrica is a perennial polycarpic plant occurring in the Pyrenees, Central France and England. Several isoquinoline alkaloids of various structural types have been isolated from this species among which[2,3] (–)mecambrine (**1**), (–)-mecambridine (**2**) and (+)-mecambroline (**3a**) together[5–7] with (–)-flavinantine (**4**) and (+)-magnoflorine (**5**) were found to be the dominant components.

Using methanol extraction and isolation procedure generally adopted in our investigations (cf. for example[8]), we have examined the alkaloids of a population of *M. cambrica* which was cultivated under the climatic conditions of our country. In order to

* Part XCVI in the series Alkaloids of the *Papaveraceae*; Part XCV: see ref.[1].

prevent the chemical changes of the alkaloids sensitive to mineral acids, especially of the proaporphine cyclohexadienones and *N*-demethylrhoeadines, acetic acid was used exclusively in the isolation procedure and all operations were carried out in the cold. Eighteen alkaloids were now identified, thirteen of which were found in *M. cambrica* for the first time. Some of them were briefly mentioned in ref.[5], but no experimental data have been published.

The ether-soluble non-phenolic fraction yielded (–)-mecambrine (**1**) together with lesser amount of (–)-mecambridine (**2**) (cf. refs[2,3]). As a minor component, (+)-roemerine (aporheine, **3b**) was separated from the mother liquors in the form of its sparingly soluble hydrochloride. It was identified by spectral data and other physical constants in direct comparison with a reference sample originating[9] from *Papaver dubium* L. This is the first report on the occurrence of roemerine in *M. cambrica*. Minute quantities of protopine and allocryptopine were also proved for the first time. From the ether-soluble phenolic fraction, small amount of (+)-mecambroline (**3a**) was separated. It appears to be probable that a great majority of the mecambroline sum obtained from *M. cambrica* in the previous studies[2,3] was an artifact of the isolation procedure arising from the acid-catalyzed rearrangement of mecambrine[3]. The genuine (+)-mecambroline is accompanied by a minute quantity of its biosynthetic congener roemeroline, incorporating likely the same (+)-(*S*)-configuration at C-6a (**3c**) as the other aporphines occurring in *M. cambrica*. Enantiomeric (–)-(*R*)-roemeroline is known from *Roemeria refracta* (STEV.) DC. (*Papaveraceae*)[10] and *Stephania sasakii* HAYATA (*Menispermaceae*)[11]. Together with roemeroline, papaverrubine D (porphyroxine) and C (epiporphyroxine) were detected by thin layer chromatography. The occurrence of the papaverrubines (*N*-demethylrhoeadines) in the *Meconopsis* species is of chemosystematic value suggesting a close affinity to the genus *Papaver*.

Sanguinarine as well as the other minor alkaloids isolated from plants of English origin[6,7] were apparently lacking in our plant material.

The ether-insoluble chloroform extract afforded the morphinandienone alkaloid (–)-flavinantine (**4**). The identification was carried out by means of mass, IR and UV spectra as well as by melting point and optical rotation. The IR spectrum exhibited an absorption pattern typical of morphinandienones, with cross-conjugated carbonyl peaks at 1 621, 1 640 and 1 667 cm^{-1}. All these constants named were in full agreement with data found in literature[7,12]. (–)-Flavinantine was reported[12] for the first time from *Croton flavescens* L. (*Euphorbiaceae*) in 1968. It is of some interest to note that the phenolic base E (or MC 1) isolated[3,5] in 1963 from *M. cambrica*, of the composition $C_{19}H_{21}NO_4$, was now stated to be pure flavinantine.

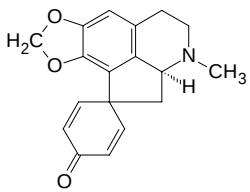
The quaternary protoberberine fraction obtained in a small yield from the aerial parts only was a mixture of coptisine and berberine with traces of corysamine and palmatine. None of these four alkaloids has been found in *M. cambrica* hitherto.

The strongly polar alkaloid fraction extracted from the aqueous layer with chloroform, after potassium iodide has been added, afforded (+)-magnoflorine iodide (**5**). It represented the major alkaloid of the root (0.31% of the dry material). In aerial parts, magnoflorine occurs only in a minute quantity. In the whole plant, it is accompanied with a small amount of its biosynthetic precursor (+)-corytuberine (**3d**) which has not been documented in *M. cambrica* before. Two further quaternary alkaloids were isolated from the roots as iodides in a low yield. One of them was shown to be identical to (–)-*N*-methylmecambridinium iodide by direct comparison with an authentic sample isolated[13] from *Papaver pseudocanescens* M. POP. as well as with the product of the *N*-methylation of (–)-mecambridine. The second compound labelled MC 2 was a quaternary alkaloid likely of the aporphine class. In spite of some physical constants similar to those of (+)-menisperine iodide (*N*-methylisocorydinium iodide), these two alkaloids were not identical. The paucity of material precluded further immediate investigation.

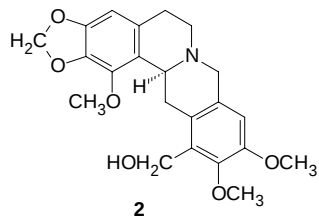
Meconopsis robusta HOOK. f. et THOMS. is a monocarpic species growing in the high mountain regions of Eastern Himalaya. The alkaloids of this plant have been screened only by thin layer chromatography[5] due to the scarcity of the plant material. Protopine was found to be the main alkaloid, and several minor alkaloids have been identified. Now, we have undertaken a re-examination with a relatively larger amount of the plants. However, the alkaloid content of this material was found to be unusually low (0.010 and 0.002% of the dry roots and aerial parts, respectively) so that this work resulted only in the isolation of four crystalline alkaloids two of which could be identified. In both roots and aerial parts, protopine was separated as one of the dominant alkaloidal constituents accompanied with a lesser amount of (–)-amurensinine (**6**). The latter was found in *M. robusta* for the first time, although its distribution in some other Himalayan *Meconopsis* species has been already reported[5]. Furthermore, a third crystalline alkaloid labelled MRO 1 was obtained in an impure state as the major alkaloid of the plant. The attempts at its purification failed because of its rapid decomposition. The chromatographic properties and colour reactions were very similar to those of β-carboline alkaloids found in several *Meconopsis* species[5], *Papaver pavoniunm* FISCH. et MEY.[14] and *P. rhoeas* var. *chelidonioides* O. KUNTZE[15]. The β-carboline derivatives are probably responsible for the known toxicity and hallucinogenic activity of some Himalayan *Meconopsis* species (cf. also refs[5,6]). The strongly polar alkaloid fraction separated as iodides yielded a crystalline compound designated MRO 2, characterized only by m.p. 250 °C, UV spectrum and chromatographic behaviour. In the remaining bases from the roots, following additional alkaloids were identified by thin layer chromatography: allocryptopine, cryptopine, rhoeadine, 6-methoxy-2-methyl-1,2,3,4-tetrahydro--β-carboline, and alkaloid MR 1 (cf. ref.[5]) in the non-quaternary fraction, and coptisine, traces of corysamine, magnoflorine, corytuberine, and *N*-methylamurensininium salt in the strongly polar fractions.

EXPERIMENTAL

The melting points were determined on a Mettler FP 51 instrument and are uncorrected. UV spectra were measured in methanol on a Unicam SP 1800 apparatus and IR spectra on a Specord 75 IR (Zeiss, Jena) in Nujol and on a Bruker IFS 28 FT-IR spectrometer in KBr pellets. Electron impact mass spectra (EIMS) were recorded on an AEI-MS 902 instrument. Thin layer chromatography (TLC) was performed on silica gel (Merck) in systems cyclohexane–diethylamine 9 : 1 (S1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S2), 6 : 3 : 1 (S3) and 4 : 5 : 1 (S4), methanol–water–25% ammonia 15 : 3 : 1 (S5), ethanol–water–25% ammonia 15 : 9 : 1 (S6), 1-propanol–water–85% formic acid 12 : 7 : 1 (S7) and chloroform–methanol 7 : 3 (S8), and on Silufol UV 254 plates (Kavalier, Czech Republic) in systems methanol–diethylamine 4 : 1 (S9) and 1 : 1 (S10). Fluorescing alkaloids were detected in UV light at 254 and 360 nm, the other alkaloids by following spraying with potassium iodoplatinate(IV). Papaverrubines were made visible by treatment with vapours of concentrated hydrochloric acid for 20 min (purple spots).



1



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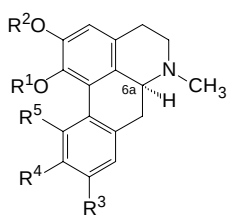
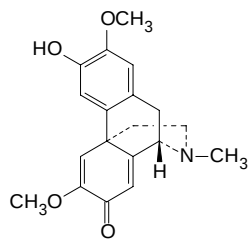


3	R^1	R^2	R^3	R^4	R^5
a	CH_2		H	OH	H
b	CH_2		H	H	H
c	CH_2		OH	H	H
d	H	CH_3	H	OCH_3	OH



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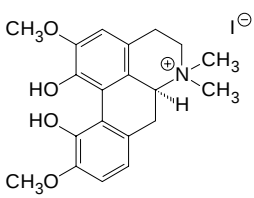

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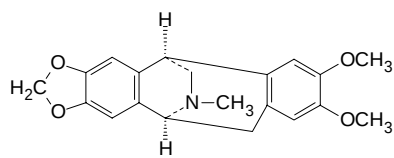

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Extraction and Isolation of Alkaloids

The plants were grown in the Center for Cultivation of Medicinal Plants of the Medical Faculty, Masaryk University, Brno. *Meconopsis cambrica* of the same origin as in refs[2,3] was gathered in the second year of vegetation July 9th, 1973 at the stage of flowering and ripening of the fruits. *M. robusta* was cultivated from the seeds obtained from the Botanical Gardens Graz and Zurich. Several years old plants were harvested at the stage of flowering and unripe fruits in the years 1981–1986. The plants were divided into the roots and aerial parts and dried at room temperature. Herbarium specimens are deposited at our Institute.

Meconopsis cambrica

The air-dried, ground roots (1 307 g) and aerial parts (1 630 g) were extracted separately in a Soxhlet apparatus with methanol. After evaporation of the solvent, the sirupy residue was extracted several times with cold 1% acetic acid until a negative reaction with Mayer's reagent. The acidic solution was filtered, made alkaline with sodium carbonate solution and extracted with ether (fraction A). To the aqueous layer, sodium hydroxide solution was added to pH value above 13 and extracted with ether again (fraction B). Then the aqueous phase was adjusted to pH value about 9 and extracted with chloroform (fraction E). Finally, the aqueous layer was brought to pH value 6–7, the saturated aqueous potassium iodide was added and extracted several times with chloroform and chloroform containing 20 vol.% of ethanol (fraction I). The organic solvents were evaporated and the crude alkaloid extracts of the fraction A, B and E purified by the acido-basic process in the usual manner.

The bases of the fraction A were dissolved in 2% acetic acid and divided into a non-phenolic (A_1) and a phenolic (A_2) portion by partitioning between ether and 5% sodium hydroxide solution. Successive crystallizations of the fraction A_1 from a concentrated ethereal solution gave (–)-mecambrine (2.76 g and 1.98 g, respectively) and (–)-mecambridine (0.36 g and 0.29 g). From the mother liquors, (+)-roemerine was separated in the form of sparingly soluble hydrochloride (base, 11.7 mg and 4.0 mg). In amorphous bases of the fraction A_1 (0.22 g and 0.46 g), small quantities of protopine and allocryptopine were detected by TLC (S1–S4) besides of the residues of mecambrine and mecambridine, which strongly prevailed. The phenolic bases A_2 from the roots and aerial parts (0.07 g and 0.13 g), practically of the same composition (TLC in S1–S4), were combined, dissolved in 5% acetic acid and concentrated aqueous potassium chloride solution was added. Mecambroline hydrochloride crystallized out (base 48.2 mg). TLC of the bases regenerated from the mother liquor in S1–S4 revealed the presence of small amounts of flavinantine, roemeroline (identified by co-chromatography with a reference sample[10]), papaverrubines D and C and a further non-identified alkaloid.

Fraction B from the aerial parts afforded 4.1 mg of bright yellow bases which consisted mainly of berberine and coptisine together with trace amounts of corysamine and palmatine (identification by TLC in S9 and S10). In the fraction B from the roots, the presence of quaternary protoberberines was not proved.

The crude fraction E (0.46 g and 0.35 g), after appropriate purification (cf. ref.[3]) and crystallization from methanol and methanol–ether, respectively, gave (–)-flavinantine (0.24 g and 0.22 g).

Fraction I obtained from the roots when crystallized from methanol yielded (+)-magnoflorine iodide (3.88 g). The non-crystallizable iodides regenerated from the mother liquors were dissolved in hot water, filtered with charcoal and, after cooling, 20% aqueous solution of sodium perchlorate was added. The precipitate of the perchlorates was collected and dried. Successive crystallizations from methanol afforded magnoflorine perchlorate (210.4 mg), perchlorate of alkaloid MC 2 (after conversion to iodide, 4.4 mg) and a crystalline mixture of corytuberine and *N*-methylmecambridinium perchlorates (TLC in S5–S8). The mixture was dissolved in hot water, cooled and aqueous potassium iodide solution was added. By partitioning between chloroform and 5% aqueous sodium hydroxide

solution, *N*-methylmecambridinium iodide (14.1 mg) and corytuberine hydriodide (41.6 mg) were obtained by crystallization of the non-phenolic and phenolic part, respectively, from methanol–ether. Fraction I from the aerial parts, after purification and crystallization from methanol, gave corytuberine hydriodide (33.3 mg) and magnoflorine iodide (20.3 mg).

Characterization of the Alkaloids Isolated

All the known alkaloids were identified by comparison of their physical, spectral and chromatographic data with those of authentic samples (except flavinantine which was not available). The yields of isolated alkaloids in wt.% of the dry roots and aerial parts, respectively, are given in parentheses.

(–)-*Mecambrine* (**1**, 0.204; 0.121): prisms, m.p. 179–180 °C (ether), $[\alpha]_D^{21}$ –94 ± 3° (*c* 0.18, methanol). UV and IR spectra as well as the R_F values (S1–S4) were in accordance with those of an authentic sample[2,3].

(–)-*Mecambridine* (**2**, 0.028; 0.018): needles, m.p. 180–181 °C (ethanol–ether), $[\alpha]_D^{22}$ –254 ± 3° (*c* 0.11, methanol). UV and IR spectra and R_F values in S1–S4 were consistent with those of an reference sample[2,3].

(–)-*Flavinantine* (**4**, 0.018; 0.013): prismatic needles, m.p. 130–132 °C (methanol–ether), very easily soluble in methanol, ethanol and chloroform, insoluble in ether, $[\alpha]_D^{22}$ –14.8 ± 0.5° (*c* 0.66, methanol). UV spectrum, λ_{max}, nm (log ε): 240 (4.18), 288 (3.82); λ_{min}, 225 (3.71). IR spectrum (KBr pellets), cm^{-1}: 1 621, 1 640 and 1 667 (dienone), 3 207 and 3 392 (OH). EIMS, *m/z*: 327.148 (M^+, calculated for $C_{19}H_{21}NO_4$ 327.147), 284 (M – methyl methylene imine). All these constants agreed well with the literature data[7,12,16]. The purity was checked by TLC in S2–S4.

(+)-*Mecambroline* (**3a**, 0.0016 – whole plant): prismatic needles, m.p. 252–253 °C (methanol) and the same m.p. in admixture with an authentic sample[3]. UV and IR spectra as well as the R_F values in S1–S4 confirmed the identity. Methiodide, m.p. 261–262 °C (methanol–ether).

(+)-*Roemerine* (**3b**, 0.0008; 0.0002): base, amorphous solid, $[\alpha]_D^{20}$ + 78 ± 3° (*c* 0.04, methanol); hydrochloride, m.p. 250–251 °C (methanol), without depression with reference sample (m.p. 251–252 °C). Literature[9] gives m.p. 266–267 °C (capillary) and $[\alpha]_D^{22}$ +80 ± 3° (ethanol). The identity was verified by UV and IR spectrum and R_F values in S1–S4.

(+)-*Magnoflorine iodide* (**5**, 0.31; 0.0015): prisms, m.p. 264–265 °C (methanol) and the same mixed m.p. with authentic sample, $[\alpha]_D^{21}$ +196 ± 3° (*c* 0.12, methanol); perchlorate, prisms, m.p. 278–279 °C (methanol). UV and IR spectrum and TLC in S5–S8 were in agreement with those of a reference sample.

(+)-*Corytuberine hydriodide* (**3d**, 0.0032; 0.0024): leaflets, m.p. 212–214 °C (methanol), without depression with an authentic sample. The identity was corroborated by UV and IR spectra and TLC behaviour (S5–S8) in comparison with an authentic sample.

(–)-*N-Methylmecambridinium iodide* (0.001; –): prisms, m.p. 203–205 °C (methanol–ether); mixed m.p., UV and IR spectra as well as R_F values in S5–S8 confirmed the identity with an authentic sample isolated from *Papaver pseudocanescens* or prepared by methylation of (–)-mecambridine with methyl iodide[13].

Alkaloid MC 2 (0.0003; –): iodide, prisms, m.p. 222–224 °C (methanol–ether), rapidly turning gray, in admixture with (+)-*N*-methylisocorydinium iodide[17] (m.p. 226–228 °C) gave a distinct depression (mixed m.p. 211–213 °C); also, not identical to methiodides of mecambrine, mecambroline and flavinantine prepared by *N*-methylation of the terciary compound with methyl iodide (comparison of the melting points and R_F values in S5–S8). Perchlorate, m.p. 276–277 °C, $[\alpha]_D^{20}$ +174 ± 3° (*c* 0.02, methanol). UV spectrum (calculated for the approximative molecular weight 450), λ_{max}, nm (log ε):

218 (4.65), 273 (4.32), 318 (3.81). λ_{min}, nm 257 (4.14), 302 (3.71). R_F values 0.12 and 0.28 in S5 and S8, respectively (*N*-methylisocorydinium iodide, R_F 0.10 and 0.31 in the same solvent systems).

Meconopsis robusta

The dry, ground roots (410 g) and aerial parts (580 g) were separately extracted with methanol in the cold. After evaporation of methanol, the residue was processed in the same manner as was described in the case of *M. cambrica* yielding the fractions A, B, E, and I.

Roots. The fraction A, after purification, was crystallized from a concentrated ether solution to give protopine (2.2 mg), amurensinine (1.3 mg) and alkaloid MRO 1 (12.0 mg; 0.003%) contamined with some mother liquor. When exposed to air, it turns brown and decomposed in a short time. R_F values 0.22 and 0.29 in S1 and S2, respectively (6-methoxy-2-methyl-1,2,3,4-tetrahydro-β-carboline 0.05 and 0.10, and MR 1 0.11 and 0.20, respectively, in the same solvent systems); after detection, deep violet blue spot typical of β-carboline derivatives[5,14,15]. In the remaining amorphous bases (27.5 mg), following additional alkaloids were identified in S1–S4: allocryptopine, cryptopine, rhoeadine, 6-methoxy-2-methyl-1,2,3,4-tetrahydro-β-carboline, and alkaloid MR 1.

The yellow fraction B (citrates) contained coptisine and traces of corysamine (TLC in S9 and S10). The fraction E was almost free of alkaloids. In the fraction I, corytuberine was the main component besides of magnoflorine and traces of *N*-methylamurensininium salt (TLC in S5–S7).

Aerial parts. Purified fraction A on crystallization from ether yielded protopine (2.3 mg) and 11.3 mg of an amorphous residue in which amurensinine and two unidentified alkaloids were detected by TLC in S1–S4. Fractions B and E were almost devoid of alkaloids. The fraction I crystallized from methanol yielded 7.8 mg of crude iodide of alkaloid MRO 2, m.p. 248–250 °C, UV spectrum (calculated for the approximative molecular weight 450), λ_{max}, nm (log ε): 211 (4.93), 222 shoulder (4.67), 242 shoulder (4.32), 292 (4.18); λ_{min} 263 (3.62). TLC: R_F value 0.79 in S5.

Protopine (0.00054; 0.00040): prisms, m.p. 208–209 °C (ether); identification by mixed m.p., UV and IR spectra as well as TLC in S1–S4.

(–)-*Amurensinine* (**6**, 0.00032; +): needles, m.p. 137–138 °C (ether), without change of m.p. in admixture with authentic[5] sample of (–)-amurensinine from *M. rudis* PRAIN. UV spectrum identical with that of an authentic sample. TLC: identical spots in S1–S4 in co-chromatography with a reference sample.

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