QUATERNARY ISOQUINOLINE ALKALOIDS AND SOME DITERPENOID ALKALOIDS IN PLANTS OF THE CZECH REPUBLIC

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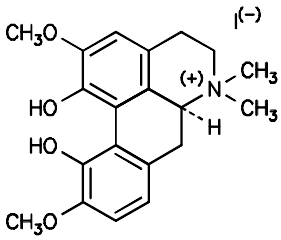
The root bark and the stem bark of *Berberis vulgaris* L. (16.2 and 6.0 wt.% of total alkaloids) yielded berberine (*IIa*), magnoflorine (*I*) and jatrorrhizine (*IIc*) as major quaternary alkaloids along with small quantities of palmatine (*IIb*) and alkaloid *BV 1*. Oxyacanthine and berbamine were separated from the non-quaternary fraction. Isocorydine was the main alkaloid in the leaves. Magnoflorine was also isolated from the stem bark and twigs of *Magnolia* × *soulangeana* SOULANGE–BODIN, *M.* x *speciosa* REICHENB., and *M. kobus* DC., from the tubers of *Aconitum firmum* REICHENB., and from the roots of *Clematis recta* L. (iodide, 0.52 wt.%). Small amounts of magnoflorine were detected in *Magnolia* × *soulangeana* cv. Alexandrina and cv. Norbertiana, in *Aconitum callibotryon* REICHENB., in the roots of *A. vulparia* REINCHENB., and in *Actaea spicata* L. In *Aconitum firmum*, *A. callibotryon*, *A. vulparia*, *Clematis recta* and *Actaea spicata*, the presence of corytuberine was also demonstrated. From the tubers of *Aconitum firmum* (0.97 wt.% of total bases), hypaconitine (*IIIb*) was separated as the major alkaloid accompanied with small amounts of aconitine (*IIIa*), mesaconitine (*IIIc*), and deoxyaconitine (*IIId*). In *A. callibotryon*, mesaconitine and an unidentified alkaloid were found as the dominant basic components together with small quantities of aconitine and hypaconitine.

In the course of our continuing search for quaternary isoquinoline alkaloids and their distribution in domestic or cultivated plants of the Czech Republic[1–3], we have examined further fifteen taxa belonging to five plant families, namely *Berberidaceae*, *Magnoliaceae*, *Helleboraceae*, *Ranunculaceae* and *Aristolochiaceae*. Many genera of these families are well known as rich in alkaloids. However, little attention has been paid to the distribution of quaternary alkaloids because of their strongly polar properties which do not permit their isolation by conventional procedures. The investigation of these specific natural products has made progress first in the last decades, especially on the basis of a simple, fast and effective isolation technique (cf. refs[1–4]). The quaternary isoquinoline alkaloids are considered as a significant chemotaxonomic feature of the phylogenetically old orders such as *Ranunculales* and *Magnoliales* as well as of some others derived from them[4–6]. One of the most widespread quaternary alkaloids is the aporphine derivative magnoflorine (*I*) accompanied in several plants by its tertiary biogenetical precursor corytuberine or by quaternary protoberberines[1–3]. Magnoflorine has been reported to exhibit

anti-inflammatory, curare-like, hypotensive and parasympathomimetic activity[7,8]. Additionally, some non-quaternary diterpenoid alkaloids of two *Aconitum* species were also included in this study.

The genus *Berberis* (*Berberidaceae*) comprises about five hundreds of species from which *B. vulgaris* L. only is native to the Czech Republic, some others being cultivated as ornamental shrubs. Hitherto, over eighty species have been investigated and shown to be rich sources of berberine (*IIa*) and other protoberberine alkaloids. Some of them display remarkable biological activity (cf. for example refs[7,9]). Magnoflorine seems also to be a common element of the alkaloid profile in the genus *Berberis* since it has been isolated from several species when a suitable isolation technique was used.

Alkaloids from *B. vulgaris* of the domestic population have not been extensively studied so far. From a sample gathered on Southern Moravia in early spring, we have isolated total alkaloids in an extremely high yield of 16.2 and 6.0 wt.% of the root bark and the stem bark, respectively, calculated as free bases. The fraction of quaternary non-phenolic protoberberines (cf. ref.[3]) yielded berberine (chloride, 7.57 and 1.97 wt.%) as the major alkaloid together with a small amount of palmatine (*IIb*; citrate, 0.011 wt.% of the root bark). The strongly polar alkaloid fraction extracted with chloroform after conversion into iodides[3,4] afforded jatrorrhizine iodide (*IIc*; 4.31 and 0.67 wt.%) and magnoflorine iodide (1.75 and 2.08 wt.%) by successive crystallizations from methanol. All these four alkaloids mentioned have been previously reported in *B. vulgaris*[10–12], however, no data on the magnoflorine quantity were given[11]. The remarkably high yield from our plant material indicates that magnoflorine belongs to the dominant alkaloids of the plant, the ratio berberine: magnoflorine being 4 : 1 in the root bark and almost 1 : 1 in the stem bark (calculated as free bases). An additional quaternary non-phenolic alkaloid assigned *BV 1* (iodide, m.p. 235 °C) was isolated in a small quantity insufficient for more detailed investigation. The presence of



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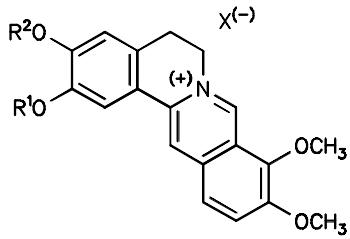


IIa, $R^1 + R^2 = CH_2$; X = Cl
IIb, $R^1 = R^2 = CH_3$; X = citrate
IIc, $R^1 = CH_3$; R^2 = H; X = I
IId, R^1 = H; $R^2 = CH_3$; X = any anion

columbamine (*IId*; cf. refs[10–12]) could not be demonstrated. The fraction of non-quaternary bases (4.35 and 2.04 wt.%) consisted almost exclusively from the bisbenzylisoquinoline alkaloids (+)-oxyacanthine and (+)-berbamine.

The alkaloid content of the leaves collected in late summer was very low (0.008 wt.%). The major alkaloid was isocorydine, for the first time found in *B. vulgaris*, accompanied with minute amounts of oxyacanthine, berbamine and traces of berberine, magnoflorine and some unidentified alkaloids.

The genus *Magnolia* (*Magnoliaceae*) includes approximately 75 species domestic in South-East Asia and in North and Central America. Some drugs originating from various *Magnolia* species are used in Japanese and Chinese traditional medicine[13,14]. The occurrence of magnoflorine and some other specific quaternary alkaloids of isoquinoline and phenyl ethyl amine type[6,13–15] is a chemical marker of the genus *Magnolia*. We have preliminary examined the content of magnoflorine in the stem bark and twigs of six taxa frequently cultivated in the Czech Republic as ornamental shrubs or trees. Magnoflorine was obtained in the highest yield (in wt.% of iodide) from *M.* × *speciosa* REICHENB. (0.13) and *M.* × *soulangeana* SOULANGE–BODIN (0.086), in both taxa being found for the first time, and from *M. kobus* DC. (0.044). In the last named species, literature gives the presence of magnoflorine[13,15] besides of salicifoline[13] and magnocurarine[15]. Only negligible amounts of magnoflorine were detected in the cultivars *M.* × *soulangeana* cv. Alexandrina and cv. Norbertiana. The presence of magnoflorine could not be proved in *M. stellata* (SIEB. et ZUCC.) MAXIM. (ref.[13] gives salicifoline only). In the most taxa examined, magnoflorine was found as a minor component whereas some other quaternary alkaloids prevailed.

In the twigs of *Liriodendron tulipifera* L. (*Magnoliaceae*), only traces of highly polar alkaloids were detected none of which was identical either to magnoflorine (cf. also ref.[15]) or to corytuberine.

The genus *Aconitum* (*Helleboraceae*) consists of about two hundreds of species, five of which are indigenous to the Czech Republic[16,17]. The plants of this genus are chemically characterized by diterpenoid alkaloids which are for the most part highly toxic and are of a limited importance in therapy, pharmacology and toxicology. Some isoquinoline alkaloids of the aporphine class inclusively magnoflorine (cf. ref.[18]) are also distributed in the *Aconitum* species. Very little was known on the alkaloids of our domestic populations. We have screened two species from the *A. napellus* L. aggregate[16,17], viz, *A. firmum* REICHENB. (synonym: *A. napellus* subsp. *firmum* (REICHENB.) GAYER) and *A. callibotryon* REICHENB. (synonym: *A. napellus* subsp. *hians* (REICHENB.) GAYER), in addition to *A. vulparia* REICHENB. (synonym: *A. lycoctonum* L.).

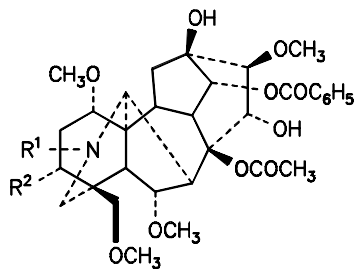
In *A. firmum*, a species endemic to the Carpathians[16,17], only aconitine (*IIIa*) has been reported[19], however, the identity to aconitine seems to be not sufficiently proved. The tubers of *A. firmum* collected in the Moravskoslezské Beskydy Mountains yielded

0.97 wt.% of total non-quaternary alkaloids which afforded hypaconitine (*IIIb*) as the major constituent (more than 90 wt.% of the total bases). The identity was corroborated by m.p., mixed m.p., UV, IR and mass spectrum (M^+, *m/z* 615) as well as by chromatographic behaviour in direct comparison with an authentic sample. Only small quantities of aconitine (*IIIa*), mesaconitine (*IIIc*) and deoxyaconitine (*IIId*) together with some unidentified trace alkaloids were found. Therefore, *A. firmum* appears to be one of the richest sources of hypaconitine known so far. From the strongly polar fraction obtained in the form of iodides, magnioflorine iodide (0.005 wt.% of the tubers) was separated by crystallization from methanol along with a small amount of an unidentified alkaloid (iodide, m.p. 277 °C). The presence of corytuberine was also proved.

A. callibotryon is an endemit of the Czech Massif[16,17]. A small sample collected in the Jeseniky Mountains gives 0.64, 0.62 and 0.16 wt.% of non-quaternary alkaloids from the roots, tubers and aerial parts, respectively. The composition of the alkaloid mixture from all parts of the plant was very similar. The major alkaloid was mesaconitine (*IIIc*) together with an unidentified alkaloid. Small amounts of aconitine (*IIIa*), hypaconitine (*IIIb*) and traces of some additional alkaloids were found as minor components. It should be noted here that the population from the eastern limit of distribution studied in this paper significantly differs in its alkaloid profile from the populations of the west regions in which aconitine strongly prevailed[20]. In the polar alkaloid fraction, small amounts of magnoflorine and corytuberine were detected.

It follows from this that none of both species examined here can be used as a source for aconitine production.

The root of *A. vulparia* was rich in weakly polar alkaloids (3.4 wt.%). Small quantity of corytuberine and traces of magnoflorine (cf. ref.[18]) were detected in the strongly polar fraction.



IIIa, $R^1 = C_2H_5$; $R^2 = OH$
IIIb, $R^1 = CH_3$; $R^2 = H$
IIIc, $R^1 = CH_3$; $R^2 = OH$
IIId, $R^1 = C_2H_5$; $R^2 = H$

The occurrence of magnoflorine as a constant component of *Clematis recta* L. (*Ranunculaceae*)[2] was verified by analysis of another sample. The roots of *C. recta* collected on a natural locality of South-East Moravia furnished magnoflorine in a relatively high yield (iodide, 0.52 wt.%). Small amounts of corytuberine and of an unidentified alkaloid were also demonstrated.

Small quantities of magnoflorine together with traces of corytuberine were found in the roots and aerial parts of *Actaea spicata* L. (*Helleboraceae*). In the roots of *Anemone sylvestris* L. (*Ranunculaceae*) and of *Asarum europaeum* L. (*Aristolochiaceae*), only trace amounts of polar alkaloids were detected, but none of them was identical with magnoflorine or corytuberine.

EXPERIMENTAL

The melting points were determined on a Mettler FP 51 apparatus and were uncorrected. Electron impact mass spectra (EIMS) were measured on a Jeol MS D 100 spectrometer. IR spectra were recorded in Nujol on a Specord 75 IR (Zeiss, Jena) spectrometer and UV spectra in methanol on a Unicam SP 1800 instrument. Thin layer chromatography (TLC) was carried out on silica gel G (Merck) in the following solvent systems: cyclohexane–diethylamine 9 : 1 (S1), cyclohexane–chloroform–diethylamine 8 : 1 : 1 (S2), 7 : 2 : 1 (S3) and 6 : 3 : 1 (S4), cyclohexane–ethanol–diethylamine 8 : 1 : 1 (S5), benzene–methanol 9 : 1 (S6), methanol–25% aqueous ammonia 200 : 1 (S7), chloroform–ethanol–diethylamine 8 : 1 : 1 (S8), methanol–water–25% aqueous ammonia 15 : 3 : 1 (S9), ethanol–water–25% aqueous ammonia 15 : 9 : 1 (S10) and 1-propanol–water–85% formic acid 12 : 7 : 1 (S11). Silufol UV 254 plates (Kavalier, The Czech Republic) and systems methanol–diethylamine 4 : 1 (S12) and 1 : 1 (S13) were used for quaternary protoberberines. The spots exhibiting fluorescence were detected in UV light (254 and 360 nm), the other spots by subsequent spraying with potassium hexaiodoplatinate(IV) or Dragendorff's reagent.

Methods

The plant material was collected at the stage of flowering and unripe fruits (if not stated otherwise), and dried at room temperature. The voucher specimens are deposited at our Institute. In the most cases, the dry and ground plant material was extracted with methanol in Soxhlet apparatus, methanol was distilled off and the sirupy residue was extracted several times with cold 1% sulfuric acid to a negative reaction with Mayer's reagent and filtered. The combined filtrates were alkalized with a sodium carbonate solution and extracted several times with ether (fraction A). The aqueous layer was then treated with sodium hydroxide solution to pH value above 13 and extracted with ether again (fraction B). To the filtered ethereal solution of fraction B, solid citric acid was immediately added. Then the aqueous layer was adjusted to pH 6–7 with 20% sulfuric acid, saturated aqueous potassium iodide solution was added and the mixture was extracted several times with chloroform and chloroform–ethanol (4 : 1), respectively, until negative reaction with Mayer's reagent. The crude alkaloid fractions obtained after evaporation of the solvents were purified in the conventional manner. The alkaloids isolated were identified by their m.p. and mixed m.p., optical rotation, UV, IR and mass spectra, respectively, and TLC behaviour in several solvent systems by comparison with the authentic samples (spectral data, cf. refs[1–3]).

In the following text, the data and locality of the collection of the plants are given in brackets.

Analyses of Plants

Berberis vulgaris (collected 11. 4. 1984, beginning of the vegetation period, Brno). Root bark (100 g) and stem bark (100 g), respectively: fraction A (4.35 and 2.04 g) crystallized from dilute sulfuric acid gave oxyacanthine sulfate; base (2.35 and 0.42 g), prisms, m.p. 216–217 °C (ether), $[\alpha]_D^{19}$ +286° ± 1° (*c* 0.24, chloroform); literature[21] gives m.p. 212–214 °C, $[\alpha]_D^{29}$ +285.6° (chloroform). Fom the bases regenerated from the mother liquor after oxyacanthine sulfate, berbamine was separated as sparingly soluble nitrate; base (1.90 and 1.42 g), prisms, m.p. 155–156 °C (ether–benzene), $[\alpha]_D^{19}$ +110° ± 2° (*c* 0.37, chloroform); literature[21] gives m.p. 156 °C, $[\alpha]_D^{32}$ +109.7°. In the amorphous bases (0.03 and 0.11 g) obtained from the mother liquor after berbamine nitrate, small quantities of four unidentified bases (in S3–S5, S7) were detected. Fraction B (citrates) on crystallization from dilute hydrochloric acid gave berberine chloride (7.57 and 1.96 g), yellow needles, m.p. 209–210 °C. The bases obtained from mother liquor, after conversion to citrates, afforded palmatine citrate (10.7 mg, root bark), long thin yellow needles, m.p. 201–202 °C (ethanol). Crystallization of the fraction I (iodides) from methanol yielded the less soluble magnoflorine iodide (1.80 and 2.08 g), colourless prisms, m.p. 266–267 °C, and the more soluble jatrorrhizine iodide (4.31 and 0.67 g), orange coloured prismatic needles, m.p. 207–208 °C (the lower melting form, cf. ref.[3]) or 228–229 °C (the higher melting form). From the mother liquor, iodide of alkaloid *BV 1* (5.5 mg from the root bark), colourless prisms, m.p. 234–235 °C (methanol), was obtained. UV spectrum, λ_{max} nm (log ε) 209 (4.67), 221 (4.68), 256 (4.93), 316 (4.22), λ_{min} 213 (4.62), 237 (4.56), 280 (3.83) (calculated for the approximative molecular weight 450); R_F 0.10 in S9. Direct comparison with menisperine iodide (isocorydine methiodide, m.p. 233 °C) excluded their identity. The presence of columbamine could not be detected (TLC in S9–S13).

Leaves: (100 g; 19.9.1984, Brno): in the fraction A (7.8 mg), isocorydine was identified as the major alkaloid by TLC (in S3–S5 and S7) besides of a smaller amount of oxyacanthine, berbamine and four unidentified alkaloids. Fraction B (below 1 mg) contained berberine (TLC in S9–S13), whereas in the fraction I trace amount of magnoflorine was detected in S9–S11).

Magnolia species (collected 21. 5. 1984, Brno). *M. kobus* (168 g; twigs, bark and leaves): A (83.6 mg); I (1.18 g): crystallization from methanol yielded magnoflorine iodide (28.0 mg), m.p. 264–265 °C; in the mother liquor, three other alkaloids, R_F in S9 0.11, 0.21 (major alkaloid) and 0.67 were found. *M.* × *soulangeana* (112 g; twigs, bark, leaves and unripe fruits): A (24.0 mg); I (0.56 g): magnoflorine iodide (96.5 mg), m.p. 264–265 °C; in the mother liquor, alkaloids of R_F value in S9 0.11 (major alkaloid), 0.21, 0.28, 0.67 were detected. *M.* × *soulangeana* cv. Alexandrina (27 g; leaves and bark): A (5.2 mg); I (21.0 mg): alkaloids of R_F value 0.21 (main alkaloid), 0.52 (magnoflorine, traces). *M.* × *soulangeana* cv. Norbertiana (91 g; twigs, leaves, unripe fruits): A (5.9 mg); I (0.15 g): alkaloids of R_F value in S9 0.11, 0.21, 0.28 (major alkaloid), 0.52 (magnoflorine), 0.54, 0.67. *M.* × *speciosa* (52 g; leaves and bark): A (5.6 mg); I (0.68 g): magnoflorine iodide (66.5 mg), m.p. 263–264 °C; other alkaloids, R_F in S9 0.11, 0.21, 0.67. *M. stellata* (35 g; leaves, bark): A (25.7 mg); I (0.10 g): alkaloids of R_F value in S9 0.11, 0.21 and 0.31 (major alkaloids), 0.54 , 0.67; magnoflorine was not detected (S9–S11).

Liriodendron tulipifera (collected 13.7.1984, Southern Moravia; twigs and unripe fruits, 67 g): A (35.0 mg), I (64.7 mg, mostly non-alkaloidal), alkaloids of R_F value in S9 0.61 (traces) and others not distinctly separated.

Aconitum firmum (collected August 1947, Moravskoslezske Beskydy Mountains). *1*. Dried tubers (100 g) were extracted with ethanol in the cold few weaks after collection. After removal of the solvent under reduced pressure, the sirupy residue was taken up in water, filtered and defatted with light petroleum before being made alkaline with sodium carbonate solution and extracted with ether. The crude bases obtained (fraction A) were purified by usual acido-basic process and crystallized from a concentrated ethereal solution to give hypaconitine (0.71 g), m.p. 197–198 °C (ether or ethanol). EIMS, *m/z*: 616 (MH^+), 615 (M^+), 600 (M – CH_3), 598 (M – OH), 555 (M – CH_3COOH),

524 (M – CH_3COOH – OCH_3), 105 (C_6H_5COOH), two exchangeable hydrogen atoms. IR spectrum: ν (cm^{-1}): 1 708 and 1 740 (carbonyl groups), 3 480 (hydroxy group). UV spectrum: λ_{max} nm (log ε) 202 (4.14), 231 (4.37), λ_{min} 211 (4.02). In the amorphous bases (0.22 g) obtained from the mother liquor, hypaconitine strongly prevailed (TLC in S1–S5). Only small amounts of aconitine, mesaconitine, deoxyaconitine and four unidentified trace alkaloids were detected. *2*. Dried powdered tubers (100 g) of the same collection worked up in 1991 were extracted with methanol in Soxhlet apparatus. After separation of the fraction A (0.97 g of bases of the same composition as in the experiment *1*), fraction I was extracted from the aqueous phase. Crystallization from methanol afforded magnoflorine iodide (4.5 mg), m.p. 265–266 °C, in all respects identical to a reference sample, and iodide of another alkaloid (1.7 mg), m.p. 276–277 °C, R_F 0.14 in S9. The presence of corytuberine (TLC in S8–S11) was also demonstrated in the mother liquor.

A. callibotryon (collected 31. 7. 1986, Hruby Jesenik Mountains): roots, tubers and aerial parts (10.3 g, 24.3 g, and 47.6 g, respectively) separately extracted with methanol in the cold yielded 66.0, 149.6 and 76.4 mg (0.64, 0.62 and 0.16 wt.%) of the fraction A. Mesaconitine and an unidentified base were detected as the major alkaloids (TLC in S1–S5) together with a small amount of aconitine, traces of hypaconitine and of some other bases. Small quantities of magnoflorine and corytuberine were found in the fraction I (S8–S11).

A. vulparia (collected 24. 6. 1991, Southern Moravia). Root (2.13 g): fraction A, 72.8 mg; corytuberine and traces of magnoflorine were identified (TLC, S8–S11) in the fraction I.

Clematis recta (collected 15. 7. 1990, Hustopece near Brno; root, 54 g): fraction I crystallized from methanol yielded 282.0 mg of magnoflorine iodide, m.p. 264–265 °C, identification by mixed melting point, IR spectrum, ν (cm^{-1}): 3 570 (OH), and R_F values in S8–S11.

Actaea spicata (collected 5. 7. 1986, Brno; 10.4 g of the roots and 5.2 g of the aerial parts). Fraction A: 1.0 and 1.0 mg, respectively; in the fraction I, small quantities of magnoflorine and traces of corytuberine were identified (TLC in S9–S11).

Anemone sylvestris (collected 11. 6. 1990, Southern Moravia; roots, 2 g) and *Asarum europaeum* (28. 9. 1990, Brno; 33 g of the roots): in the fractions I, only traces of alkaloids were detected, none of which was identical to magnoflorine or corytuberine (TLC in S9–S11).

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