ALKALOIDS FROM *Corydalis nobilis* (L.) PERS. AND *C. intermedia* (L.) MÉRAT*

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Rhizomes of *Corydalis nobilis* (L.) PERS. (3% of alkaloids) contain (+)-tetrahydropalmatine, (+)-bicuculline and (+)-corytuberine as the main constituents of the tertiary alkaloid fraction. Protopine, (+)-corypalmine and (+)-stylopine, which also belong to the dominant alkaloids, were isolated in lesser amounts. As minor alkaloids were isolated (±)-tetrahydropalmatine, (+)-corydaline, allocryptopine, cryptopine, (−)-scoulerine, (+)-adlumidine, (+)-sinactine, (±)-corlumine, isoboldine, (+)-corybulbine, (±)-stylopine and (−)-isocorypalmine. The fraction of quaternary protoberberine alkaloids afforded coptisine, dehydrocorydaline, palmatine, corysamine, jatrorrhizine and *cis*-N-methylstylopinium hydroxide. Aobamidine (*Z*-adlumidiceine enol lactone), isolated as the principal alkaloid of aerial parts (0·3% of alkaloids), is obviously an artifact arising from bicuculline N-metho salt during the isolation process. Further dominant alkaloids of the tertiary fraction were adlumidine, bicuculline, protopine, (±)-tetrahydropalmatine and (±)-corlumine; as minor alkaloids were isolated corytuberine, scoulerine, corypalmine, cryptopine, isocorypalmine, corybulbine, (+)-corydalidzine, and unidentified alkaloids CN 1 ($C_{23}H_{25}NO_5$, m.p. 211°C) and CN 2 (m.p. 261°C). Quaternary protoberberine fraction afforded coptisine and palmatine. Nineteen of the mentioned alkaloids were isolated from this species for the first time. Tubers of *C. intermedia* (L.) MÉRAT (0·70% of alkaloids) afforded protopine, tetrahydropalmatine and corydaline as the main alkaloids and allocryptopine, canadine, stylopine, palmatine, dehydrocorydaline, berberine, coptisine as minor alkaloids, together with traces of bicuculline and magnoflorine. Dominant alkaloids of the aerial part (0·73% of alkaloids) were bicuculline, bulbocapnine, protopine, stylopine and an unidentified phenolic base, m.p. 258°C. Isoboldine, scoulerine, allocryptopine, corydaline, canadine, coptisine, palmatine and berberine were identified as the minor alkaloids.

As continuation of our previous studies[1,2] on alkaloids of the *Corydalis* species of the *Fumariaceae* family (syn.: *Papaveraceae*, subfamily *Fumarioideae*) we investigated the alkaloids in two other species, *C. nobilis* (L.) PERS. and *C. intermedia* (L.) MÉRAT.

Corydalis nobilis (L.) PERS. of the *Calocapnos Spach* section (syn.: *Capnogorium* (BERNH.) PRANTL et KÜNDIG, *Eucorydalis* subsectio *Ramoso-sibiricae* FEDDE) is a strong perennial herb with a massive flat rhizome, distributed in Western Siberia and Central Asia. Alkaloids of this species were investigated for the first time already

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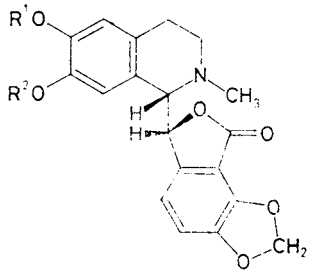
in the end of the 19th century[3]. Six alkaloids were isolated of which the so-called corydalinobiline was probably corytuberine[4]. Later, in 1940 Manske[4] isolated twelve alkaloids from the whole plant: (+)-tetrahydropalmatine, protopine and bicuculline as dominant compounds, and (+)-corlumine, corytuberine, partially racemized stylopine, (+)-isocorypalmine, cryptopine, (±)-tetrahydropalmatine and three unidentified alkaloids F 53, F 54 and F 55 as minor alkaloids. Since that time no study of alkaloids from *C. nobilis* has been reported. Our present communication concerns the study of alkaloids from plants cultivated in Czechoslovakia. We studied separately the rhizomes and aerial parts which differ considerably quantitatively as well as qualitatively in the alkaloid composition, in accord with our earlier studies[1,2] on the *C. cava* and *C. solida* species. From the *C. nobilis* species we isolated twenty eight individual alkaloids nineteen of which were found in this plant for the first time. The plant material used in our study had incomparably higher alkaloid content than found by Manske[4].

The main alkaloids of rhizomes (3% of alkaloids) were (+)-tetrahydropalmatine, (+)-bicuculline (*Ia*) and (+)-corytuberine*; also protopine, (+)-corypalmine and (+)-stylopine were found to be the dominant constituents. From the tertiary base fraction we isolated minor amounts of (±)-tetrahydropalmatine, (+)-corydaline, allocryptopine, cryptopine, (−)-scoulerine, adlumidine and very small amounts of (+)-sinactine, (±)-corlumine (*Ib*), for the first time found as natural alkaloid, isoboldine, (+)-corybulbine, (±)-stylopine and (−)-isocorypalmine. Interestingly, our plant material contained (+)-corypalmine (*IIa*) as the dominant phenolic tetrahydroprotoberberine alkaloid whereas Manske[4] isolated the position isomeric isocorypalmine (*IIb*) as one of the dominant alkaloids and did not find corypalmine at all.

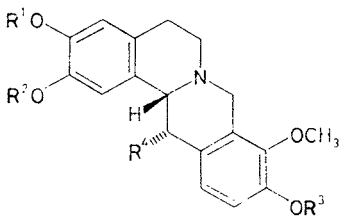
Quaternary protoberberines (about 0·1% of dry rhizomes), whose presence in *C. nobilis* had been hitherto unreported, were represented by coptisine, dehydrocorydaline, palmatine and negligible amounts of jatrorrhizine and corysamine. These alkaloids were isolated as chlorides or iodides and identified by comparison of their physical constants and spectral data with those of authentic samples. From the strongly basic fraction we isolated a non-basic compound, m.p. 200°C, which was identified by physical constants, UV, IR and mass spectra as the adduct of palmatine and chloroform. Its structure was confirmed by direct comparison with the substance prepared from palmatine and chloroform in strongly alkaline medium. This compound is undoubtedly an artifact arising during the isolation procedure. On conversion into iodides, the strongly polar fraction afforded *cis*-N-methylstylopinium iodide.

* In this connection, it is worth notice that the corytuberine base had substantially higher optical rotation ($[\alpha]_{23}^{D}$ +352° (methanol)) than are the currently reported[5,6] values ($[\alpha]_D$ +282° (?) and 286°, respectively, both in methanol). On the other hand, the optical rotation of corytuberine hydriodide and magnoflorine iodide (N-methylcorytuberinium iodide), prepared from corytuberine, agreed with the values given in the literature (e.g., ref.[7]).

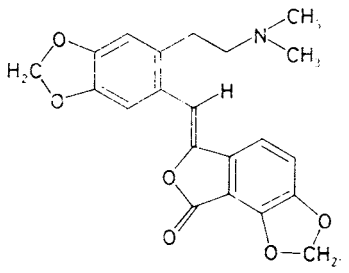
The alkaloid content in the aerial part was approximately ten times lower (0·31%) than in the rhizomes. As the main basic constituent we isolated a yellow alkaloid whose spectra indicated an enol lactone of the secophthalideisoquinoline type. According to the literature (cf. e.g., refs[8-12]), these compounds arise easily from



Ia, R + R^2 = CH_2
Ib, R^1 = R^2 = CH_3



IIa, R^1 = R^4 = H ; R^2 = R^3 = CH_3
IIb, R^1 = R^3 = CH_3 ; R^2 = R^4 = H
IIc, R^1 = R^3 = H ; R^2 = R^4 = CH_3



III

quaternary N-metho salts of phthalideisoquinoline alkaloids in an alkaline medium, the *erythro*-alkaloids affording the *Z*-isomers whereas the *threo*-alkaloids the *E*-isomers. Direct comparison of physical and spectral data of the alkaloid, isolated from *C. nobilis*, has proven its identity with the compound prepared according to ref.[8] from (+)-bicuculline (1*S*,9*R*-*erythro*-isomer, *Ia*). This alkaloid (*III*) named aobamidine was for the first time isolated from *C. ochotensis* (TURCZ.) var. *raddeana*[10]; the *E*-isomer (adlumidiceine enol lactone) was isolated already earlier from *C. sempervirens* (L.) PERS.[8]. According to these facts, the alkaloid from *C. nobilis* is obviously identical with aobamidine (*Z*-adlumidiceine enol lactone) even though both the natural alkaloid and the substance prepared from bicuculline melted considerably higher (210°C) than reported[10] (m.p. 197°C). Very probably, the isolated aobamidine is an artifact formed from the quaternary N-metho salt of bicuculline in the alkaline medium during the isolation. The tertiary base fraction afforded adlumidine, bicuculline and protopine as further dominant constituents and

(±)-tetrahydropalmatine, (±)-corlumine and scoulerine as minor alkaloids. We also isolated small amounts of corypalmine, (+)-corydalidzine (*IIc*), cryptopine, isocorypalmine, corybulbine and an unidentified non-phenolic base denoted as CN 1, m.p. 211°C, probably of the formula $C_{23}H_{25}NO_5$(high resolution mass spectrum), containing one active hydrogen atom. The amount of the compound was too small to allow a closer study. In the amorphous residues we detected chromatographically traces of corydaline, stylopine and allocryptopine. The fraction of quaternary protoberberines (0·002% of dry aerial part) afforded coptisine as the principal constituent, together with small amount of palmatine. From the strongly polar fraction we isolated corytuberine as hydriodide and an alkaloid CN 2 which contained a keto group but no hydroxyl (IR spectrum). Its UV spectrum was not typical and up to 250°C the compound had a too low vapour pressure to allow a mass spectral detection. Above 250°C, the mass spectrum exhibited abundant ions of *m/z* 58 (probably $CH_2{=}N^{(+)}(CH_3)_2$).

Our results confirm that the *C. nobilis* species, the only representative of the *Calocapnos* section, is chemically characterized by the presence of protoberberine, phthalideisoquinoline, aporphine and protopine alkaloids[4]. In analogy to other *Corydalis* species[1,2], tetrahydroprotoberberine alkaloids occur as both enantiomers with the (*R*)-(+)-form usually predominating; however, in rare cases, some of them are present almost exclusively in the (*S*)-(−)-form (scoulerine and isocorypalmine). For the first time we found the phthalideisoquinoline alkaloid corlumine occurring naturally in the racemic form.

Rhizomes of *C. nobilis* may be utilized as the rich source of bicuculline (0·65%) whose biological properties are at present intensively investigated. It is known that bicuculline belongs to the most potent antagonists of γ-aminobutyric acid (GABA) which acts as inhibitory neurotransmitter in the central nervous system and is connected with many physiological functions. Also other phthalideisoquinoline alkaloids have similar effects (see e.g., ref.[12]).

The *C. intermedia* (L.) MÉRAT (syn.: *C. fabacea* (RETZ.) PERS.) species of the *Pes-gallinaceus* IRM. section, a gracile perennial herb with a small solid tuber, is sparsely distributed also on the Czechoslovak territory. Nothing has been known so far about the alkaloids in its Central-European population. So far, only alkaloids in the aerial part (0·4%) of the *C. intermedia* species growing in the Kostroma region (U.S.S.R.) were studied[13]; the species contained protopine, allocryptopine and isoboldine. The small amount of our domestic plant material available made possible only an orientational study. From the tubers (0·70% alkaloids) we separated protopine, tetrahydropalmatine and corydaline as dominant alkaloids of the tertiary alkaloid fraction, and identified chromatographically minor alkaloids allocryptopine, canadine, stylopine and traces of bicuculline. In the quaternary alkaloid fraction we found palmatine and dehydrocorydaline along with small amount of berberine, coptisine and traces of magnoflorine. The aerial part (0·73% of alkaloids) afforded

bicuculline, bulbocapnine, protopine and stylopine as dominant alkaloids and small amount of an unidentified phenolic base, m.p. 258°C. Chromatography detected isoboldine, scoulerine, allocryptopine, corydaline and canadine. The quaternary fraction consisted predominantly of coptisine with small amount of palmatine and berberine.

The described findings show that the Czechoslovak population of the *C. intermedia* species differs considerably in dominant alkaloids from the population distributed in the U.S.S.R. (ref.[13]). On the other hand, it has many common biochemical features with the closely related species *C. solida* (L.) Sw. of our domestic population[2] which also belongs to the *Pes-gallinaceus* section. As the most significant difference between the alkaloid profiles of both species we may consider the presence of bicuculline and bulbocapnine which in the *C. solida* species are not detectable at all or only in traces.

EXPERIMENTAL

The melting points were determined on a Mettler FP 51 apparatus and are uncorrected. Mass spectra were measured on a Jeol MSD 100 instrument, IR spectra in nujol on a Specord 75 IR (Zeiss, Jena) spectrometer and UV spectra in methanol on a Unicam SP 1 800 instrument. Thin-layer chromatography (TLC) was performed on silica gel G (Merck) in the systems: cyclohexane–diethylamine 9 : 1 (S1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S2) and 6 : 3 : 1 (S3), methanol–25% ammonia 200 : 1 (S4), chloroform–ethanol–diethylamine 8 : 1 : 1 (S5), methanol–water–25% ammonia 15 : 3 : 1 (S6), ethanol–water–25% ammonia 15 : 9 : 1 (S7), methanol–water–diethylamine 15 : 3 : 1 (S8) and 1-propanol–water–85% formic acid 12 : 7 : 1 (S9), and on Silufol UV 254 plates (Kavalier, Czechoslovakia) in the systems: methanol–diethylamine 4 : 1 (S10) and 1 : 1 (S11). Fluorescing alkaloids were detected in UV light at 235 and 336 nm and other alkaloids by subsequent spraying with potassium iodoplatinate.

Corydalis nobilis

The plants were cultivated in the Center for Cultivation of Medicinal Plants of the Medical Faculty, J. E. Purkyně University, Brno. Several years old plants were harvested at the stage of flowering and unripe fruits in the late spring of 1978–1980. The rhizomes and aerial parts were separated and dried at room temperature. The herbarium specimen is deposited at this Department.

Extraction and Isolation of the Alkaloids

The ground dried plant material was extracted with methanol in a Soxhlet apparatus. After evaporation of the methanol, the extract was taken up in 1% sulfuric acid and the solution was filtered from insoluble material of lipoid character. The acidic aqueous layer was made alkaline with sodium carbonate and extracted with ether (fraction A), the aqueous phase was adjusted to pH ≥ 13 with 40% aqueous sodium hydroxide and again extracted with ether (fraction B). The aqueous phase was adjusted to pH ~9 with 20% sulfuric acid and extracted with chloroform (fraction E). After neutralization of the aqueous phase with 20% sulfuric acid to pH 7, saturated solution of potassium iodide (75 g) was added. Extraction with chloroform or chloroform with 20% of ethanol to negative reaction with Mayer reagent afforded fraction I.

Rhizomes

The dried material (1 377 g) afforded 43·72 g of crude bases of fraction A which were dissolved in 3·5% hydrochloric acid. The mixture of hydrochlorides that crystallized out was converted back into the bases. Crystallization from methanol gave tetrahydropalmatine (9·82 g), stylopine (0·96 g) and corypalmine (0·20 g). The mother liquor from the crystallized hydrochlorides was then extracted with chloroform and thus separated into the chloroform-extractable (AC) and chloroform-nonextractable (AD) hydrochloride fractions. Both these fractions were further separated into the non-phenolic (AC_1 and AD_1) and phenolic (AC_2 and AD_2) bases[14]. The bases of the fraction AC_1 were crystallized from methanol or chloroform–methanol to give bicuculline (8·69 g), stylopine (0·68 g), tetrahydropalmatine (2·57; separated as the sparingly soluble hydrochloride), corydaline (0·50 g) and sinactine (0·07 g). Small amount (0·08 g) of an amorphous residue was left.

Crystallization of the fraction AD_1 from chloroform–methanol or methanol yielded protopine (3·11 g), bicuculline (0·29 g), cryptopine (0·26 g), adlumidine (0·22 g), allocryptopine (0·21 g) and corlumine (0·05 g). The remaining amorphous impure bases (0·85 g) were separated on a column of alumina (100 g; Reanal, activity 2 according to Brockmann). The chromatography afforded stylopine (0·10 g; benzene–ether 2 : 3), tetrahydropalmatine (0·02 g; ether), two unidentified amorphous alkaloids (0·005 g each; ether; in S1 R_F 0·64 and 0·73; detected as blue-violet spots), bicuculline (0·01 g) and corlumine (0·02 g; ether–chloroform 1 : 1 and 2 : 3), protopine (0·02 g; chloroform), cryptopine (0·01 g; chloroform) and allocryptopine (0·10 g; chloroform). The non-crystalline residues from the chromatographic fractions (0·44 g total) contained, in addition to the mentioned alkaloids, minor amounts of several unidentified alkaloids.

Fraction AC_2 was crystallized from chloroform–methanol and from methanol to give corypalmine (1·47 g), corybulbine (0·06 g) and isocorypalmine (0·03 g). The amorphous residue weighed 0·35 g. Fraction AD_2 on crystallization from methanol afforded corypalmine (0·44 g), the remaining bases were converted into hydrochlorides and crystallized from water, giving scoulerine hydrochloride (0·25 g of the base). The mother liquor from crystallization of hydrochlorides was converted into bases which on crystallization from methanol yielded isoboldine (0·06 g). The residue (0·54 g) was amorphous.

Solid citric acid was added to an ethereal solution of bases of fraction B and the ether was distilled off. The deep orange citrates were dissolved in hot water and hydrochloric acid was added. Coptisine chloride (0·47 g) which crystallized out was collected and the bases regenerated from the mother liquor were converted into citrates. Crystallization from methanol afforded 0·13 g of the sparingly soluble palmatine citrate. The mother liquor was crystallized as chlorides from water, giving 2·0 mg of corysamine chloride. The remaining material (0·08 g of bases) contained (according to TLC): corysamine, palmatine and traces of coptisine and dehydrocorydaline.

Fraction E contained predominantly brown-black nonalkaloidal compounds which were removed by dissolving in 5% sulfuric acid and filtration. The filtrate was mixed with saturated potassium iodide solution and extracted with chloroform. The chloroform was evaporated and the residue crystallized from methanol to give corytuberine hydriodide (0·27 g) and dehydrocorydaline iodide (0·12 g).

Fraction I was stripped of chloroform, leaving a semicrystalline residue which on trituration with methanol afforded 6·66 g of corytuberine base. The methanolic mother liquor was concentrated. Further crystallizations gave palmatine iodide (0·22 g), dehydrocorydaline iodide (0·38 g), an adduct of palmatine with chloroform (0·11 g), jatrorrhizine iodide (0·02 g), coptisine iodide (0·01 g) and *cis*-N-methylstylopinium iodide (9·7 mg). The noncrystalline residue weighed 0·40 g.

Aerial Part

Extraction of dried ground aerial part (1 275 g), using the above-described procedure, afforded crude fraction A. This was dissolved in cold 5% sulfuric acid, leaving a yellow crystalline material (0·27 g) identified as aobamidine sulfate. The filtrate was made alkaline with ammonia and extracted with ether to give 3·88 g of bases of fraction A which were dissolved in 3·5%hydrochloric acid. The separated yellow crystalline aobamidine hydrochloride (0·40 g) was collected and the filtrate was separated into fractions AC and AD by extraction with chloroform; the material of these fractions was further divided into nonphenolic (AC_1 and AD_1, respectively) and phenolic (AC_2 and AD_2, respectively) bases[14]. On crystallization of hydrochlorides from water, the fraction AC_1 afforded (±)-tetrahydropalmatine hydrochloride (0·19 g of base). Bases, recovered from the mother liquor, were crystallized from methanol to give bicuculline (0·33 g) and corlumine (0·13 g). The remainder (0·33 g) was amorphous. Bases of fraction AD_1 were crystallized from chloroform–methanol and methanol, yielding adlumidine (0·36 g), protopine (0·25 g) and cryptopine (0·01 g), bicuculline (0·03 g), alkaloid CN 1 (1·2 mg) and an amorphous residue (0·37 g) in which TLC detected small amount of stylopine and corydaline. Crystallization of fraction AC_2 from the same solvents gave corypalmine (37·2 mg), isocorypalmine (7·6 mg) and corybulbine (5·0 mg), leaving 0·04 g of amorphous bases. Fraction AD_2 on crystallization from 3·5% hydrochloric acid furnished a sparingly soluble hydrochloride from which the liberated bases were crystallized from ether to afford scoulerine (0·08 g) and corydalidzine (0·02 g). Mother liquors from crystallization of the hydrochlorides gave 0·18 g of amorphous bases which contained several other unidentified alkaloids (TLC).

Fraction B was worked up as described above, affording coptisine chloride (27·2 mg). The mother liquors, after conversion into citrates, gave palmatine citrate (3·6 mg). The remaining mother liquors contained (TLC) small amounts of corysamine and dehydrocorydaline.

The very impure fraction E was purified, converted into iodides and crystallized from methanol to furnish 34·3 mg of corytuberine hydriodide. Crystallization of the fraction I from methanol gave 0·09 g corytuberine hydriodide and 0·22 g of alkaloid CN 2. The dark residue (1·90 g) remained amorphous.

Characterization of the Isolated Alkaloids

The individual alkaloids were characterized by melting points and mixture melting points, optical rotation, UV, IR or mass spectra and TLC, in most cases by direct comparison with authentic specimens. The yields of the alkaloids from rhizomes and aerial part of *C. nobilis* are given in parentheses. The presence of alkaloid detected only by TLC is denoted +, its absence −.

(+)-*Tetrahydropalmatine* (0·86%;−): from methanol platelets m.p. 142−143°C, from ether large prisms m.p. 105−106°C; $[\alpha]_D^{23}$ +294° ± 3° (*c* 0·34, methanol). R_F 0·41 (S1), 0·71 (S2).

(±)-*Tetrahydropalmatine* (0·041%, 0·015%): obtained from the total tetrahydropalmatine fraction isolated from rhizomes; after separation of the main part of the (+)-form, the racemate was isolated by crystallizations from methanol as the less soluble component (yield 0·57 g); m.p. 151−152°C (methanol); $[\alpha]_D^{21}$ 0° ± 3° (*c* 0·27, methanol). R_F identical with that of the (+)-form.

(+)-*Bicuculline* (0·65%, 0·028%): compact aggregates, m.p. 196−197°C (chloroform–methanol), $[\alpha]_D^{21}$ +127° ± 3° (*c* 0·40, chloroform). UV spectrum, λ_{max}, nm (log ε): 209 (4·47), 223 (4·39), 297 (3·81), 325 (3·75), sh 232 (4·03), λ_{min} 217 (4·38), 264 (3·41), 310 (3·68). IR spectrum: ν(CO) 1 730 cm^{-1}. R_F 0·18 (S1), 0·55 (S2), 0·77 (S4).

(+)-*Corytuberine* (0·50%; 0·007%): prismatic needles, m.p. 241–242°C (methanol), $[\alpha]_D^{23}$ +352° ± 3° (*c* 0·11, methanol), the optical rotation was repeatedly measured and the $[\alpha]_D$ value found was substantially higher than that reported[5,6]. Mass spectrum, *m/z*: 327·1435 (M^+, $C_{19}H_{21}NO_4$), 312·1236 ($C_{18}H_{18}NO_4$), 282 (M – 43); contains two exchangeable hydrogen atoms; hydriodide lustrous leaflets, m.p. 217–218°C (methanol), $[\alpha]_D^{23}$ +180° ± 3° (*c* 0·11, methanol); UV and IR spectra and R_F values (0·76 in S5, 0·87 in S6, 0·90 in S7, and 0·85 in S8) identical with those of an authentic sample[7]. Methiodide: the base (52·6 mg) was dissolved in hot methanol (40 ml), cooled and mixed with methyl iodide (5 ml). After standing for two weeks, most of the solvents were evaporated. The crystallized product (65·9 mg; yield 87%), m.p. 264 to 265°C, was identical with magnoflorine iodide[7] (UV and IR spectra, R_F and $[\alpha]_D^{23}$ +194° ± 3° (*c* 0·11, methanol)).

Protopine (0·24%; 0·015%): prisms m.p. 208–209°C (chloroform–methanol). R_F 0·40 (S1), 0·66 (S2), 0·83 (S3), 0·64 (S4).

(+)-*Corypalmine* (0·15%; 0·003%): prisms m.p. 223–224°C (chloroform–methanol), $[\alpha]_D^{21}$ +298 ± 3° (*c* 0·24, chloroform). R_F 0·12 (S1), 0·27 (S2), 0·38 (S3).

(+)-*Stylopine* (0·12%; +): needles m.p. 202–203°C (chloroform–methanol), $[\alpha]_D^{23}$ +283° ± 3° (*c* 0·33, chloroform). R_F 0·74 (S1).

(±)-*Stylopine* (0·003%; ?): isolated from mother liquors after crystallization of the (+)-form fyield 0·04 g); needles, m.p. 220–221°C (chloroform–methanol), $[\alpha]_D^{21}$ 0° ± 3° (*c* 0·12, chloroform). R_F 0·74 (S1).

(+)-*Corydaline* (0·036%; +): prisms m.p. 134–135°C (methanol), $[\alpha]_D^{21}$ +312° ± 3° (*c* 0·33, methanol). R_F 0·55 (S1).

Allocryptopine (0·023%; +): prisms m.p. 159–160°C (methanol). R_F 0·28 (S1), 0·58 (S2), 0·83 (S3), 0·52 (S4).

Cryptopine (0·020%; 0·0001%): prisms m.p. 221–222°C (chloroform–methanol). R_F 0·22 (S1), 0·68 (S2), 0·73 (S3), 0·62 (S4).

(–)-*Scoulerine* (0·018%, 0·006%): small aggregates, m.p. 197–198°C (ether); according to the lower value of optical rotation ($[\alpha]_D^{20}$ –315° ± 3° (*c* 0·11, methanol)) than reported[1] ($[\alpha]_D$ –354° in methanol), the substance contained about 10% of racemate. R_F 0·05 (S1), 0·18 (S2), 0·27 (S3).

Adlumidine (0·016%, 0·028%): flat prisms, m.p. 238–239°C (chloroform–methanol), $[\alpha]_D^{22}$ +118° ± 3° (*c* 0·13, chloroform). UV spectrum, λ_{max}, nm (log ε): 208 (4·43), 222 (4·40), 296 (3·81), 325 (3·70), 240 sh (4·02); λ_{min}: 216 (4·36), 264 (3·40), and 310 (3·65). The UV spectrum was identical with that of capnoidine ((–)-adlumidine) and bicuculline, IR spectrum (ν(CO) 1 755 cm^{-1}) was identical with that of capnoidine from *Corydalis cava*[1]. R_F 0·15 (S1), 0·55 (S2), 0·60 (S3), 0·84 (S4).

(+)-*Corlumine* (0·005%, 0·010%): large prisms, m.p. 149–150°C (methanol), $[\alpha]_D^{22}$ 0° ± 3° (*c* 0·30, methanol). High resolution mass spectrum, *m/z*: 206·1173 (100; $C_{12}H_{16}NO_2$), 191·0926 (8·4; $C_{11}H_{13}NO_2$), 190·0857 (12·5; $C_{11}H_{12}NO_2$), 177 (2·9), 148·0764 ($C_9H_{10}NO$), 145·0894 ($C_{10}H_{11}N$), 135 (3·4), 132 (3·0). UV spectrum: λ_{max}, nm (log ε): 210 (4·61), 222 (4·56), 289 (3·67), 326 (3·75), 236 sh (4·31); λ_{min}: 218 (4·55), 265 (3·24), 301 (3·43). IR spectrum: ν(CO) 1 760 cm^{-1}. All these spectral data agree with those reported[15,16]. R_F 0·10 (S1), 0·51 (S2), 0·53 (S3), 0·77 (S4).

(+)-*Sinactine* (0·005%; –): aggregates of needles, m.p. 177–178°C (methanol), $[\alpha]_D^{22}$ +288° ± 3° (*c* 0·22, methanol). High resolution mass spectrum, *m/z*: 339·1459 (32; M^+, $C_{20}H_{21}NO_4$),

338·1400 (23·5; $C_{20}H_{20}NO_4$), 324 (3), 190·0855 (17; $C_{11}H_{12}NO_2$), 176 (3·5), 148·0515 (100; $C_9H_8O_2$). UV spectrum: λ_{max}, nm (log ε) 209 (4·56), 287 (3·70), 235 sh (3·97); λ_{min} 259 (2·97). R_F 0·48 (S1), 0·75 (S4).

Isoboldine (0·004%; −): leaflets, m.p. 126–127°C (methanol). R_F 0·03 (S1), 0·07 (S2), 0·12 (S3).

(+)-*Corybulbine* (0·004%; 0·0004%): prismatic needles, m.p. 225–226°C (chloroform–methanol), $[\alpha]_D^{22}$ +341° ± 3° (*c* 0·11, chloroform). R_F 0·17 (S1), 0·33 (S2), 0·49 (S3).

(−)-*Isocorypalmine* (0·002%; 0·0006%): prisms, m.p. 227–228°C (chloroform–methanol). According to the lower value of optical rotation ($[\alpha]_D^{22}$ −224° ± 10° (*c* 0·04, chloroform)), the material contained about 20% of racemate (reported[2] $[\alpha]_D$ −298° in chloroform). R_F 0·15 (S1), 0·29 (S2), 0·45 (S3).

Aobamidine (+; 0·042%): the base, prepared from the sulfate or hydrochloride, crystallized from ether as tiny lemon-yellow needles, m.p. 209–210°C (reported[10], m.p. 195–197°C). UV spectrum, λ_{max}, nm (log ε): 209 (4·63), 224 (4·42), 300 (4·02), 315 (4·02), 240 sh (4·31); λ_{min}: 221 (4·41), 258 (3·68), and 307 (4·00). IR spectrum: ν(CO) 1 780 cm^{-1}. Both the UV and IR spectra were completely identical with those of the compound prepared from bicuculline methiodide. Sulfate: yellow needles, m.p. 179–182°C (water), hydrochloride: yellow needles, diffuse m.p. 252–272°C (water). R_F 0·04 (S1), 0·37 (S2), 0·41 (S3), 0·63 (S4), 0·67 (chloroform–diethylamine 95 : 5), identical with R_F values for an authentic sample.

(+)-*Corydalidzine* (−; 0·0016%): aggregates, m.p. 202–203°C (methanol), $[\alpha]_D^{22}$ +244° ± 5° (*c* 0·08, chloroform); for the alkaloid isolated from *Corydalis koidzumiana* OHWI reported[17] m.p. 209–210°C (in a vacuum capillary) and $[\alpha]_D^{22}$ +333° (methanol); this shows that the alkaloid from *C. nobilis* is partly racemized. High resolution mass spectrum, *m/z*: 341·1622 (M^+, $C_{20}H_{23}NO_4$), 326·1365 ($C_{19}H_{20}NO_4$), 310·1397 ($C_{19}H_{20}NO_3$), 178 ($C_{10}H_{12}NO_2$) and abundant ions *m/z* 164·0881 ($C_{10}H_{12}O_2$) and 149·0598 ($C_9H_9O_2$). The compound contained two active hydrogen atoms. UV spectrum, λ_{max}, nm (log ε): 208 (4·58), 284 (3·75), 227 sh (4·24); λ_{min}: 255 (3·32). IR spectrum: ν(OH) 3 430 cm^{-1}. All these spectral data were in good accord with those reported[17,18]. R_F 0·05 (S1), 0·12 (S2), 0·23 (S3).

Alkaloid CN 1 (−; 0·0001%): long thin needles, m.p. 210–211°C (methanol). Mass spectrum, *m/z*: 413 ($M^+ + H_2O$), 395·1728 (M^+, $C_{23}H_{25}NO_5$), 350·1386 ($C_{21}H_{20}NO_4$), 207, 206, 192, 191 (most abundant ions), 178 and 150. The alkaloid contained one active hydrogen atom. R_F 0·24 (S1).

Alkaloid CN 2 (−; 0·017%): light yellow prismatic needles, m.p. 260–261°C (methanol). UV spectrum (calculated for M_r 350) λ_{max}, nm (log ε): 212 (4·32), 329 (3·86); λ_{min}: 301 (3·74). IR spectrum, cm^{-1}: 1 660 (ν(CO)), OH band absent. Mass spectrum: up to 250°C the vapour pressure was not sufficient for obtaining the spectrum; above 250°C decomposition, abundant ions of *m/z* 58 (C_3H_8N).

Coptisine (calculated as the base 0·033%; 0·0019%): chloride from water orange needles not melting up to 290°, then carbonization. R_F 0·46 (S6), 0·60 (S10), 0·90 (S11).

Dehydrocorydaline (as base 0·028%; +): iodide orange-yellow needles, m.p. 229–230°C or 217–218°C (methanol), the m.p. varied according to conditions of the crystallization (cf. ref.[2]). R_F 0·61 (S7), 0·36 (S8), 0·15 (S10), 0·25 (S11).

Palmatine (as base 0·025%; 0·0002%): chloride yellow needles, m.p. 201–203°C (water), iodide m.p. 232–233°C (methanol); citrate yellow thin needles, m.p. 214–215°C (methanol). R_F 0·56 (S7), 0·18 (S10), 0·61 (S11). Adduct of the base with chloroform: light yellow prisms, m.p. 199–200°C (methanol), no depression on admixture with an authentic sample prepared

from palmatine. Mass spectrum: above 170°C ions $CHCl_2^+$ due to the liberated chloroform, at higher temperatures abundant ions m/z 352 ($C_{21}H_{22}NO_4$). UV and IR spectra identical with those of an authentic sample (vide infra).

Jatrorrhizine (as base 0·001%; —): iodide orange prisms, m.p. 206—207°C (methanol). R_F 0·80 (S7), 0·75 (S8), 0·49 (S10), 0·51 (S11). Columbamine in the respective systems: R_F 0·64, 0·73, 0·39, and 0·33.

Corysamine (as base 0·0001%; +): chloride bronze leaflets (water), not melting up to 290°C, carbonization. R_F 0·12 (S10), 0·68 (S11).

cis-N-*Methylstylopinium hydroxide* (0·0005%; —): iodide small prisms, m.p. 279—280°C (methanol). R_F 0·18 (S6). 0·43 (S7), 0·23 (S8).

Preparation of Aobamidine from Bicuculline

A) (+)-*Bicuculline* (102·2 mg) was dissolved in hot chloroform (3 ml) and mixed with methanol (3 ml) and methyl iodide (1 ml). After standing for 45 h, the solvents were evaporated leaving the amorphous methiodide in quantitative yield. Bicuculline methiodide (60·2 mg) was dissolved in hot water, the solution was cooled and made alkaline to pH 13—14 with sodium hydroxide solution (essentially as described in ref.[8]). The yellow precipitate was immediately extracted with ether and the ethereal solution was concentrated, affording 5·8 mg (13%) of aobamidine (including further crops from the mother liquor).

B) (+)-*Bicuculline* (103·0 mg) was converted into the methiodide using the above-described procedure. This was dissolved in hot water, cooled and made alkaline (pH 9) with a solution of sodium carbonate. The mixture slowly deposited a yellow precipitate which, after 1·5 h, was taken up in ether. The concentrated ethereal solution afforded total 30·0 mg (28%) of aobamidine as lemon-yellow needles, m.p. 209—210°C (ether or methanol). UV and IR spectra, as well as R_F values, were completely identical with those of a sample isolated from *C. nobilis* (vide supra).

Preparation of Palmatine–Chloroform Adduct

A solution of palmatine chloride (54·5 mg) in water was made alkaline with sodium hydroxide and extracted with chloroform. Concentration of the chloroform solution, followed by addition of methanol, separated 53·6 mg of light yellow prisms, m.p. 199—200°C, insoluble in dilute inorganic or organic acids. UV spectrum, λ_{max}, nm (log ε): 218 (4·23), 278 (4·03), 351 (3·93); λ_{min}: 252 (3·92), 305 (3·67), 380 sh (3·74). The UV and IR spectra were completely identical with those of the compound isolated from *C. nobilis*.

Corydalis intermedia

The plants were collected at a natural locality near Brno at the stage of flowering on April 18, 1987. The tubers and aerial parts were separated and dried at room temperature. In average, one tuber weighed 0·14 g and one aerial part 0·08 g. A voucher specimen is deposited at our Department.

Extraction and Isolation of Alkaloids

Dried ground tubers (2·25 g) and aerial parts (2·23 g) were extracted with cold methanol (7 times). After evaporation of methanol, the residue was processed in the same manner as described for *C. nobilis*, yielding the alkaloid fractions A, B, and I. The individual alkaloids were identified

by their melting points, mixture melting points, TLC and, in case of sufficient amounts, also by UV and IR spectra.

Tubers

Bases of fraction A (12·4 mg; 0·55%) were crystallized from methanol to gave protopine (3·7 mg; 0·16%), m.p. 206—207°C (chloroform–methanol). The mother liquor was converted into hydrochlorides which deposited crystalline tetrahydropalmatine hydrochloride (base 2·7 mg; 0·12%). Bases obtained from the mother liquor on crystallization from methanol gave corydaline (1·6 mg; 0·07%), m.p. 130—132°C. The remaining amorphous bases (4·4 mg) contained (in addition to the mentioned alkaloids) canadine (R_F 0·68 in S1), allocryptopine, minor amounts of stylopine, traces of bicuculline and two unidentified alkaloids (TLC).

In the mixture of yellow quaternary protoberberines (fraction B; 2·8 mg; 0·12%) we identified (TLC) palmatine, dehydrocorydaline, berberine and coptisine, fraction I contained an unidentified alkaloid (R_F 0·18 in S8) along with traces of magnoflorine (R_F 0·47 in S8).

Aerial Part

Fraction A (13·2 mg; 0·59%), isolated from the aerial part, was separated into non-phenolic (A_1) and phenolic (A_2) bases[19]. Crystallization of fraction A_1 from methanol separated stylopine (1·6 mg; 0·07%), m.p. 202—203°C (methanol), protopine (1·7 mg; 0·08%), m.p. 207—208°C (methanol), and bicuculline (3·1 mg; 0·14%), m.p. 194—195°C (chloroform–methanol). In the residual amorphous mixture of bases allocryptopine, canadine and corydaline were identified as minor alkaloids. Fraction A_2 on crystallization from methanol afforded bulbocapnine (1·8 mg; 0·08%), m.p. 201—202°C, R_F 0·22 (S1) and 0·37 (S2), and an unidentified base (0·7 mg; 0·03%), m.p. 257—258°C (methanol), R_F 0·27 (S2). In the mother liquors were detected isoboldine and scoulerine.

Fraction B (golden yellow bases, 1·6 mg; 0·07%) contained (TLC) mainly coptisine along with minor amounts of palmatine and berberine. Only traces of alkaloids were found in fraction I.

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