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Isolation and Chemistry of Alkaloids from Plants of the Family Papaveraceae LXVII: *Corydalis cava* (L.) Sch. et K. (*C. tuberosa* DC)

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Abstract □ From *Corydalis cava* (L.) Sch. et K. (*C. tuberosa* DC) (Papaveraceae: genus *Corydalis* Med.), a mixture of alkaloids (0.53%) was isolated. The main alkaloid was (+)-bulbocapnine, and the minor alkaloids were coptisine, (+)-domestine, adlumidiceine, (+)-predicentrine, protopine, (−)-capnoidine, (+)-stylopine, (+)-isoboldine, 8-oxocoptisine, 1,2-methylenedioxy-6a,7-dehydroaporphine-10,11-quinone, and corysamine. In addition, fumaric acid was obtained. Coptisine, adlumidiceine, predicentrine, 8-oxocoptisine, corysamine, 1,2-methylenedioxy-6a,7-dehydroaporphine-10,11-quinone, and isoboldine were found in *C. cava* for the first time, but rhoeadine and papaverrubine alkaloids were not detected. Predicentrine and isoboldine were identified on the basis of the UV, IR, mass, and PMR spectra.

Keyphrases □ *Corydalis cava*—alkaloids isolated, UV, IR, PMR, and mass spectra □ Alkaloids—isolated from *Corydalis cava*, UV, IR, PMR, and mass spectra

During the Middle Ages, the tubers of *Corydalis cava* (L.) Sch. et K. (*C. tuberosa* DC) (Papaveraceae: genus *Corydalis* Med.) (1) were used for headache, neuroses, tremor, pain, and paralysis of the extremities (2). So far, 20 alkaloids have been isolated (3, 4), and Trabert and Schneidewind (5) isolated bulbocapnine as the main alkaloid (97%). The other alkaloids were (+)-stylopine, corydaline, an alkaloid of the formula $C_{18}H_{19}NO_5$, mp 230°, $[\alpha]_D^{20}$ −112.5° (chloroform), and an alkaloid with a melting point of 225–226°. Manske (6) isolated capnoidine, domestine (nantenine), and narcotine, and Blaschke *et al.* (7) detected sinoacutine.

DISCUSSION

As part of a systematic investigation of the alkaloids of the genus *Papaver* and *Corydalis*, *C. cava*, which flowers in abundance in the meadows and woods, was studied using chromatographic methods. In addition to the alkaloids isolated earlier, *i.e.*, (+)-bulbocapnine (53.1%) (3–7), (+)-domestine, protopine, (+)-stylopine, and (−)-capnoidine, the alkaloids coptisine (according to quantity the second main alkaloid), adlumidiceine (8), (+)-predicentrine, (+)-isoboldine, corysamine, 8-oxocoptisine, and 1,2-methylenedioxy-6a,7-dehydroaporphine-10,11-quinone were isolated. The last two substances are new alkaloids which have not been isolated previously.

In the material studied, it was not possible to detect corydine, corytuberine, glaucine, hydrohydrastinine, (+)-canadine, corybulbine, sinoacutine, isocorybulbine, corydaline, dehydrocorydaline, (+)-corypalmine, scoulerine, (+)-isocorypalmine, thalictricavine, corycavamine, corycavidine, corycavine, and narcotine, which had been found previously (3, 4). Neither was it possible to detect the rhoeadine or papaverrubine alkaloids, which are characteristic for the genus *Papaver* (4). An attempt was made to identify some of the earlier isolated alkaloids by comparison of their physical constants. Thus, it was found that the previously isolated (5, 9, 10) alkaloid with a melting point of 230° might be capnoidine (237°), and the previously isolated (9) alkaloid with a melting point of 137° might be domestine (138°).

The structure of the alkaloid called predicentrine (I) (1,9,10-trimethoxy-2-hydroxyaporphine) (11, 12), whose methylation with

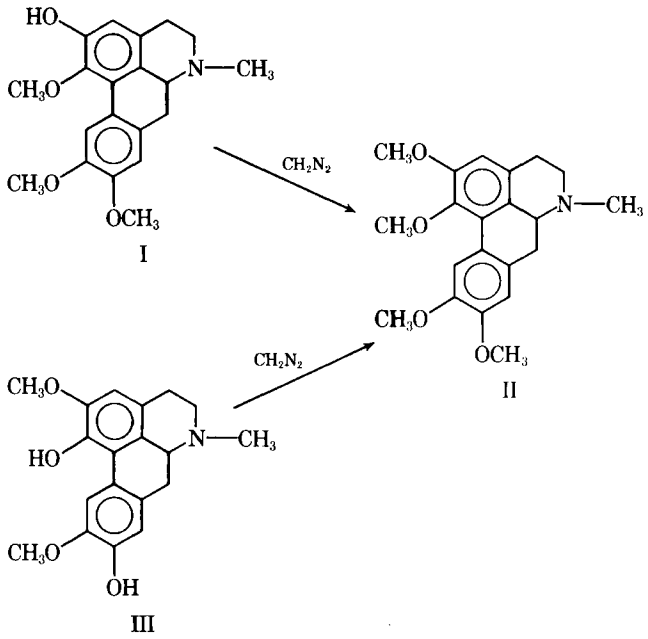
diazomethane afforded glaucine (II) (Scheme I), was elucidated by UV, IR, mass, and PMR spectra; λ_{max} (ethanol) (log ϵ): 218 (4.58), 236 (sh), (4.36), 273 (sh) (4.09), 282 (4.16), 304 (4.14), and 314 (sh) (4.09) nm. Those values are characteristic of the 1,2,9,10-tetrasubstituted aporphine alkaloid (13); the presence of the phenolic hydroxyl was confirmed by the bathochromic shift of the two long wavelength bands due to ionization of the molecule (sodium hydroxide) [λ_{max} (ethanolic sodium hydroxide) (log ϵ): 233 (4.11), 305 (sh) (4.08), and 330 (4.23) nm].

The IR spectrum in chloroform showed a hydroxyl band at 3530 cm^{-1} and aromatic double bond peaks at 1605 and 1585 cm^{-1}. The substitution at C-9 and C-10 also was confirmed by the mass spectrum, where the ion (M − 1) represents a base peak (14). The molecular peak of mass M^+ 341 corresponds to the formula $C_{20}H_{23}NO_4$. In the PMR spectrum of predicentrine (deuterochloroform), the singlet at 2.55 ppm (3H) is attributable to the N—CH_3 group, the singlet at 3.63 (3H) is attributable to the OCH_3 group of ring A, and the singlet at 3.87 (6H) is attributable to two OCH_3 groups at C-9 and C-10 on ring D. The singlets at 6.55 (1H), 6.80 (1H), and 8.03 (1H) correspond to the aromatic protons at C-3, C-8, and C-11, respectively. The broad singlet at 5.65 is attributable to the proton of the hydroxyl group.

The methoxyl bands were separated by using benzene as a solvent. The signals of the methoxyl groups with a free *ortho*-position (at C-9 and C-10) shifted to higher fields (3.42 and 3.45 ppm), whereas the position of the signal of the methoxyl group in ring A practically did not change (3.55). This finding is confirmatory evidence for its position at C-1 and the position of the phenolic hydroxyl group at C-2.

Besides predicentrine (I), another aporphine alkaloid of a phenolic nature was isolated. Its methylation with diazomethane gave glaucine (II). Its UV spectrum [λ_{max} (ethanol) (log ϵ): 219 (4.54), 273 (sh) (4.03), 281 (4.11), 305 (4.16), and 311 (sh) (4.14) nm] was characteristic of a 1,2,9,10-tetrasubstituted aporphine alkaloid. The presence of phenolic hydroxyls was confirmed by the bathochromic shift of both long wavelength bands due to ionization of the molecule (sodium hydroxide) [λ_{max} (ethanolic sodium hydroxide) (log ϵ): 232 (4.20), 299 (sh) (3.95), and 326 (4.07) nm].

The IR spectrum in chloroform showed a hydroxyl frequency at 3530 cm^{-1}. The molecular peak of mass M^+ 327 corresponds to the formula $C_{19}H_{21}NO_4$. The PMR spectrum (deuterochloroform) exhibited the signals of the N—CH_3 group and of two OCH_3 groups. The spectral data showed that this alkaloid is isoboldine (III). Its identity was confirmed by a comparison of its melting point and IR spectrum with those of the authentic sample.



Scheme I

EXPERIMENTAL[1]

Plant Material—The plant material was collected around Hynkov (district of Olomouc, Czechoslovakia) in June 1973. Voucher specimens were identified and authenticated[2].

Extraction, Separation, and Purification of Alkaloids—The dried ground drug (5.8 kg) was extracted (16) with 50 liters of methanol, concentrated *in vacuo* at 36° to ~1.0 liter, diluted with equal volumes of 5% acetic acid, and taken up into light petroleum and then into ether. The acidic aqueous residue was made alkaline with sodium carbonate to pH ~8 and then extracted with ether (Portion A, 27.7 g).

The aqueous residue was made alkaline with sodium hydroxide to pH ~12 and extracted with ether (Portion B, 4.29 g) and then with chloroform (Portion C, 6.0 g). The aqueous phase was acidified with hydrochloric acid to pH ~3, and a saturated solution of potassium iodide was added. After extraction with chloroform, Portion D (2.5 g) was obtained (17), which did not contain any alkaloids.

Workup of Acidic Light Petroleum Extract—The extract was dissolved in 5% acetic acid, refiltered, and taken up into ether to afford fumaric acid (1.25 g), mp 285–287° (in a sealed capillary). Its identity was confirmed with IR spectroscopy. No alkaloids could be detected in the extract.

Workup of Acidic Ether Extract—The extract was dissolved in 5% acetic acid, refiltered, and taken up into ether to give 3.43 g more of fumaric acid. Even in this extract, the alkaloids could not be detected.

Workup of Portion A—Portion A yielded directly 15.14 g of bulbocapnine, mp 197–199° (ethyl acetate and ether), $[\alpha]_D^{25}$ +235 ± 3° (c 0.462, chloroform). After separation of bulbocapnine, the mother liquors were concentrated *in vacuo*, and the residue was dissolved in 500 ml of 1% sulfuric acid. An attempt was made to isolate the benzophenanthridine alkaloids in the form of pseudocyanides (18), but these alkaloids could be neither isolated nor detected. The extract was then made alkaline with ammonium hydroxide and taken up into ether to afford 12.57 g of a mixture of alkaloids. This mixture was chromatographed on a column of aluminum oxide (300 g); 750-ml fractions were collected.

Fraction 1 [ether–light petroleum (50:50)] gave (+)-stylopine (0.09 g), mp 201–203° (ethyl acetate and ether), $[\alpha]_D^{25}$ +313 ± 3° (c 0.984, chloroform), hR_f 63 (Solvent System 1). Fraction 2 [ether–light petroleum (50:50)] yielded domestine (1.08 g), mp 137–139° (ethyl acetate and ether), hR_F 54 (Solvent System 1). Fraction 4 [ether–light petroleum (50:50)] gave capnoidine (0.11 g), mp 235–237°, $[\alpha]_D^{25}$ −115 ± 2° (c 0.652, chloroform), hR_F 28 (Solvent System 1). Fractions 5–14 (ether) gave bulbocapnine (1.86 g), mp 197–199° (ethyl acetate and ether), $[\alpha]_D^{25}$ +235 ± 3° (c 0.832, chloroform), hR_F 33 (Solvent System 1). The mother liquors, after isolation of bulbocapnine from fraction 6, yielded protopine (0.17 g), mp 205–207° (acetone and ether), $[\alpha]_D^{25}$ ± 0° (c 0.902, chloroform).

Fraction 15 [ether–chloroform (90:10)] gave an alkaloid (0.006 g) which formed blue-green crystals, mp 218–220° (acetone and methanol). On the basis of the melting point and the UV and IR spectra, this alkaloid was identical with 1,2-methylenedioxy-6a,7-dehydroaporphine-10,11-quinone[3]; λ_{max} (log ϵ): 229 (4.35), 286 (4.01), 346 (4.20), 405 (3.81), and 639 (3.69) nm. The IR spectrum showed bands in the carbonyl region at 1685, 1650, 1590, and 1580 cm^{-1}. Fractions 16–22 [ether–chloroform (90:10–50:50)] yielded an alkaloid (0.37 g), mp 156–158° (benzene); hydrobromide, mp 200–

[1] Melting points were determined on a Kofler block and are uncorrected. For the measurements, the substances were dried for 1 hr at 100°/Torr. The optical rotation was measured on a polarimeter (Hilger), the UV spectra were measured on a Unicam SP 700, the IR spectra were measured on an Infrascan H-900 (Hilger), the PMR spectra were measured on a Varian T-60 (tetramethylsilane as the internal standard), and the mass spectra were measured on an AEI MS 902 instrument. Preparative column chromatography was carried out on aluminum oxide W 200 neutral (Woelm, Germany) or on aluminum oxide neutral (Reanal, Hungary). TLC was performed on silica gel G plates (Merck, Germany) using cyclohexane–diethylamine (80:20) (Solvent System 1) or methanol–diethylamine (80:20) (Solvent System 2). The alkaloids were detected with Dragendorff reagent or under UV light (protoberberine alkaloids); for the detection of the papaverrubines, hydrochloric acid and heating were used (15).

[2] By Dr. B. Šula, botanist of the Museum in Olomouc. Voucher specimens were deposited at Palacký University.

[3] This substance was prepared by Cava *et al.* (19) by oxidation of bulbocapnine with mercuric chloride.

210° dec. (methanol), $[\alpha]_D^{25}$ +105 ± 3° (c 0.800, chloroform), hR_F 16 (Solvent System 1), which was assigned the structure of predicentrine (I).

Fraction 26 [chloroform–methanol (98:2)] gave the alkaloid isoboldine (III) (0.03 g), mp 122–124° (acetone, no depression with authentic sample), $[\alpha]_D^{25}$ +70 ± 3° (c 0.700, chloroform), hR_F 9 (Solvent System 1). In the presence of air, crystalline isoboldine readily gave a red oxidation product. The mother liquors yielded still another Dragendorff-positive substance of hR_F 3 (Solvent System 1), which could be neither isolated nor identified. Finally, fractions 29–31 [chloroform–methanol (92:8–84:16)] gave adlumidiceine (0.33 g), mp 244–246° (methanol and ether), $[\alpha]_D^{25}$ ±0° (c 0.576, methanol).

Workup of Portion B—Portion B was worked up in the usual manner (20) to afford 4.72 g of coptisine chloride, hR_F 71 (Solvent System 2) and 0.01 g of corysamine chloride, hR_F 29 (Solvent System 2), the identity of which was confirmed by IR.

Workup of Portion C—Portion C (6.0 g) was subjected to column chromatography on aluminum oxide (180 g); 600-ml fractions were collected. Fractions 1 and 2 [ether–light petroleum (50:50)] gave an alkaloid (in traces) which, after transformation into a chloride, was identified by IR as corysamine. Fraction 3 [ether–light petroleum (50:50)] yielded domestine (in traces), and fraction 5 [ether–light petroleum (50:50)] yielded capnoidine. Fractions 6–14 [ether and ether–chloroform (90:10)] gave bulbocapnine (0.04 g).

Fractions 15 and 16 [ether–chloroform (90:10)] yielded an alkaloid (0.01 g), mp 284–286°, which was identified as 8-oxocoptisine [λ_{max} (log ϵ): 228 (4.58), 257 (sh) (4.04), 272 (sh) (3.91), 290 (3.80), 317 (sh) (4.01), 341 (sh) (4.25), 349 (4.28), 377 (4.13), and 398 (sh) (4.01) nm]. In the IR spectrum, the band at 1647 cm^{-1} (potassium bromide) indicated the presence of a carbonyl group. Fraction 28 [chloroform–methanol (92:8)] gave the alkaloid (0.12 g) of hR_F 71 (Solvent System 2) which, after transformation into a chloride, was identified by IR as coptisine.

Acetylation of Predicentrine (I)—Predicentrine (25 mg) was dissolved in 2 ml of anhydrous pyridine, 2 ml of acetic anhydride was added, and the mixture was left standing at room temperature for 24 hr. After removal of the solvent by distillation, the product could not be crystallized from common solvents. In the PMR spectra (deuterochloroform), the singlet at 2.28 ppm (3H) corresponded to the CH_3COO group; the chemical shifts of the signals of the N—CH_3 group (2.47, 3H), OCH_3 groups on ring A (3.62, 3H) and ring D (3.83, 6H), and aromatic protons (6.57, C-3; 6.87, C-8; and 8.10, C-11) were practically consistent with the chemical shifts in the spectrum of predicentrine.

Methylation of Predicentrine (I)—Predicentrine (30 mg) was dissolved in a mixture of methanol and ether (3 ml), an ether solution of diazomethane was added, and the product was left standing for 24 hr at room temperature. After removal of the solvents by distillation, a substance with melting point of 118–120° (ethyl acetate and ether), hR_F 28 (Solvent System 1), was obtained. On the basis of the IR spectrum (chloroform), it was identical with glaucine (II).

Methylation of Isoboldine (III)—Isoboldine (20 mg) was dissolved in a mixture of methanol and ether (3 ml), an ether solution of diazomethane was added, and the mixture was left standing for 24 hr at room temperature. After removal of the solvents by distillation, a substance with a melting point of 118–120° (ethyl acetate and ether), hR_F 28 (Solvent System 1), was obtained. On the basis of the IR spectrum (chloroform), it was identical with glaucine.

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