ALKALOIDS OF THE TWO *Hypecoum* L. SPECIES

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Alkaloids from *Hypecoum procumbens* L. and *H. leptocarpum* HOOK. F. *et* THOMS. were investigated. Protopine was the dominant alkaloid in both species. From *H. procumbens* chelerythrine and corydine were newly isolated in addition to the earlier detected alkaloids allocryptopine, sanguinarine, coptisine, and isocorydine. From *H. leptocarpum* allocryptopine, isocorydine, and corydine were isolated for the first time, in addition to the earlier described alkaloids protopine, sanguinarine, chelerythrine ,and coptisine. Cryptopine was detected chromatographically. From the fraction of strongly polar alkaloids of both species magnoflorine, (—)-*trans*-N-methylstylopinium hydroxide, and in small amounts a new secoberbine alkaloid of oxohypecorinine structure, procumbine (*I*), and two further alkaloids of unsolved structure were isolated in the form of iodides.

The genus *Hypecoum* of the subfamily *Hypecoideae*, *Papaveraceae* family, has been recently classified as the independent family *Hypecoaceae*[1]. It comprizes small annual plants, growing in the Mediterranean region, in Central Asia and Pakistan, and the area of occurrence of some species reaches as far as North China. About 15 species of this genus are known, and the content of alkaloids was investigated in 9 of them[2-13]. The alkaloidal spectrum of all the species is relatively monotonous; protopine is distinctly dominant alkaloid.

In this work we focussed on the study of the alkaloids from *H. procumbens* L. and *H. leptocarpum* HOOK. F. *et* THOMS. In earlier studies minor alkaloids (allocryptopine, sanguinarine and traces of coptisine and chelirubine[4]) were detected in *H. procumbens* in addition to the dominant protopine. Recently, Gözler *et al.* described the presence of scoulerine, norsanguinarine, oxosanguinarine, glaucine, isocorydine, hypecorinine, corydalisol and the new alkaloid turkiyenine[6,10] in this species. Protopine was also the main component of the basic fraction of *H. leptocarpum*; furthermore sanguinarine, chelerythrine, chelirubine, and coptisine[4] were also detected. Our present study of both species was focussed on the completion of the knowledge on the occurrence of minor components and especially on the checking of the presence of strongly polar quaternary alkaloids, extractable in the form of iodides into chloroform.

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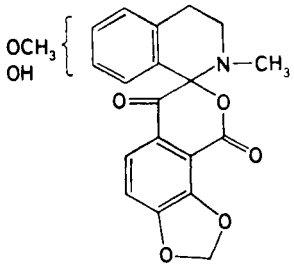
In *H. procumbens* the content of alkaloids was 0·24%. From the fraction of tertiary bases we isolated as the dominant alkaloid protopine (0·22%), accompanied by a small amounts of allocryptopine, sanguinarine and isocorydine. Newly we isolated a small amounts of chelerythrine and corydine, and chromatographically we detected the presence of a further three unidentified bases. Scoulerine and glaucine[6] could not be detected in our plant material. From the fraction of strongly polar quaternary alkaloids, separated in the form of iodides, we isolated for the first time from the *Hypecoum* genus magnoflorine and (−)-*trans*-N-methylstylopinium iodide. We further isolated in low yield an orange-red base with m.p. 191−192°C to which we gave the name procumbine. The structure determination of this substance will be published elsewhere[14]. On the basis of mass and NMR spectra we assigned the substance the structure of oxohypecorinine type (*I*). A further alkaloid, HP 1, isolated from this fraction in negligible yield, we characterized only by its melting point, UV, IR and mass spectra.



I

From *H. leptocarpum* we isolated 0·36% of total alkaloids. Here too, protopine was dominant (0·19%). It was accompanied by sanguinarine, chelerythrine, and traces of coptisine. We isolated allocryptopine and isocorydine for the first time from this species, and we detected the presence of cryptopine. The chloroform extract of the iodides of quaternary alkaloids was a significant part of the alkaloidal fraction from *H. leptocarpum*. Its main component was the yellow alkaloid HL 1 with m.p. 246−248°C and the composition $C_{20}H_{19}INO_6$. Its structure will be published in our subsequent paper[14]. We further isolated from the quaternary fraction magnoflorine, (−)-*trans*-N-methylstylopinium iodide and a small amount of an alkaloid identical with procumbine from *H. procumbens*. By column chromatography on silica gel we separated a negligible amount of a further amorphous alkaloid HL 2, which we have characterized provisionally by UV and IR spectra.

These results confirm that the tertiary fraction of the alkaloids of both species is characterized by the predominance of protopine bases of which protopine represents the dominant alkaloid, accompanied by alkaloids of the protoberberine,

benzophenanthridine and aporphine type. The quaternary alkaloids magnoflorine and (−)-*trans*-N-methylstylopinium hydroxide were detected in the genus *Hypecoum* for the first time. Secoberbine alkaloid procumbine is the fifth alkaloid of this structural type isolated from the *Hypecoum* genus and its presence in both species is a further indication that secoberbine alkaloids may be considered as a characteristic chemotaxonomical feature of the *Hypecoum* genus.

EXPERIMENTAL

The melting points and mixed melting points were determined on a Kofler block, the values presented are uncorrected. The UV spectra were measured in methanol on a UNICAM SP 1800 spectrophotometer. The IR spectra were recorded in Nujol on a Specord IR-75 (Zeiss, Jena) instrument. Thin-layer chromatography (TLC) was carried out on thin layers of silica gel LS 5—40 μ (Lachema, Brno), with gypsum as binder, using the following systems: cyclohexane–diethylamine 9 : 1 (S_1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S_2), benzene–acetone–methanol 7 : 2 : 1 (S_3), methanol–water–25% ammonia 15 : 3 : 1 (S_4), ethanol–water–25% ammonia 15 : 9 : 1 (S_5), methanol–25% ammonia 200 : 1 (S_6), chloroform–ethanol–diethylamine 7 : 2 : 1 (S_7). For commercial Silufol sheets (Kavalier, Votice) the systems methanol–cyclohexane 1 : 3 (S_8) and methanol–diethylamine 4 : 1 (S_9) were used. Descending paper chromatography (PC) was carried out on paper Whatman No 1 in the systems: 1-butanol–water–98% acetic acid 10 : 3 : 1 (S_{10}) and ethanol–water 3 : 2 (S_{11}). For detection fluorescence in UV light was used (253 and 336 nm) and also spraying with potassium iodoplatinate (TLC) or Dragendorff's reagent (PC). Column chromatography was carried out on silica gel L 80—160 μ (Lachema, Brno) or on modified alumina (Reanal)[16].

Extraction and Isolation of Alkaloids

The plant material of both species was cultivated in the Centre for the Cultivation of Medicinal Plants, Medical Faculty in Brno, from seeds obtained from various botanical gardens, and it was harvested at the time of flowering, in July 1976 (*H. procumbens*) and in July 1978—1981 (*H. leptocarpum*). The herbarium samples of both species are deposited at the Department of Medical Chemistry of the Medical Faculty in Brno. The dry ground material was extracted with methanol in a Soxhlet extractor. After evaporation of methanol the extract was dissolved in sulfuric acid (0·1 mol l^{-1}) and the isolated sum of alkaloids fractionated in the conventional manner[15].

Hypecoum procumbens L.

From 6·11 kg of dry whole plant 14·11 g of bases of fraction A were obtained (0·23% of the dry weight), from which crystallization from methanol gave protopine (13·17 g) and allocryptopine (0·50 g). Quaternary benzophenanthridines were then isolated in the form of pseudocyanides, which were separated chromatographically on a column of alumina[16]. Sanguinarine (2·3 mg) and trace amounts of chelerythrine were thus obtained. From the mother liquor which was purified and crystallized 5·5 mg of isocorydine and corydine (3·2 mg) were isolated. In the remaining mother liquor the presence of a further three unidentified bases could be detected, with R_F values 0·15; 0·17; 0·19 (S_1), or 0·37; 0·30; 0·26 (S_2). In fraction B the presence of trace amount of coptisine could be detected by PC. From fraction E and I crystallization from methanol gave (−)-*trans*-N-methylstylopinium iodide (0·44 g), procumbine (0·23 g), alkaloid HP 1 (2·8 mg), and magnoflorine (33·0 mg). In the mother liquors the presence of a further 3 to 4 components was detected by TLC.

From 130·0 g of capsules of *H. procumbens* protopine (23·0 mg), allocryptopine (1·5 mg), *trans*-N-methylstylopinium iodide (6·0 mg) could be isolated and further three bases detected by TLC.

Hypecoum leptocarpum HOOK. F. *et* THOMS.

The dry whole plant (3·9 kg) was extracted. Fraction A (6·86 g, 0·43%) gave after crystallization from chloroform–ethanol protopine (7·46 g) and allocryptopine (1·32 g). The benzophenanthridines isolated in the form of pseudocyanides were separated on a column of alumina[16] to give sanguinarine (18·0 mg) and chelerythrine (23·0 mg). After purification fraction A was crystallized from methanol to give isocorydine (3·5 mg) and corydine (2·7 mg). In the mother liquor traces of cryptopine and three further unidentified bases could be detected by TLC, with the following R_F values: 0·13; 0·16; 0·19 (S_1) and 0·33; 0·37; 0·41 (S_2). From fraction B crystallization in the form of chloride gave coptisine (0·8 mg).

From fraction I crystallization from methanol gave (–)-*trans*-N-methylstylopinium iodide (1·13 g), alkaloid HL 1 (3·88 g) and magnoflorine iodide (15·0 mg). The remaining part of fraction I (0·92 g) was chromatographically separated on a silica gel column (30 g) with chloroform. Trace amounts of protopine and 3·0 mg of amorphous alkaloid HL 2 were obtained. Elution with chloroform–methanol (99 : 1) gave 3·3 mg of an alkaloid which according to TLC and m.p. was identical with procumbine isolated from *H. procumbens*. Chloroform–methanol mixture (97 : 3) eluted 48 mg of alkaloid HL 1 (totally 3·92 g). Elution with chloroform–methanol (95 : 5) gave fractions containing in addition to alkaloid HL 1 an unidentified component with R_F 0·14 (S_5), which, however, could not be obtained in a pure state. Chloroform–methanol mixtures (9 : 1 to 1 : 1) eluted mixed fractions of alkaloid HL 1 and (–)-*trans*-N-methylstylopinium iodide, from which crystallization from methanol gave 0·07 g of (–)-*trans*-N-methylstylopinium iodide (totally 1·20 g).

Characterization of the Isolated Alkaloids

The isolated alkaloids were identified on the basis of melting points, mixed melting points, UV and IR spectra, optical rotation and comparison with authentic samples in TLC and PC. The yields of individual alkaloids in % of dry weight of *H. procumbens* or *H. leptocarpum* respectively, are given in brackets.

Alkaloid HL 1 (–; 0·10%): yellow crystals, m.p. 246–248°C. Mass and NMR spectra[14]; UV spectrum (methanol): λ_{max} nm (log ε) 222–224 (4·32), 247 (4·21), 304 (3·85), 364–366 (3·92); λ_{min} nm (log ε) 238 (4·17), 270 (3·39), 322 (3·63). IR spectrum (Nujol): 1 630, 1 670 cm^{-1}.

Alkaloid HL 2 (–; 0·00007%): amorphous, yellow. UV spectrum (methanol): λ_{max} nm 220, 250, 310, 376–378; λ_{min} nm 236–238, 274.

Alkaloid HP 1 (0·00005%; –): prisms, m.p. 220–227°C (methanol). Mass spectrum, *m/z*: 337 (M^+), 310, 190. UV spectrum (methanol): λ_{max} nm (log ε) 218 (3·75), 246 (3·60), 206–308 (3·24), 352–354 (3·23); λ_{min} nm (log ε) 232–233 (3·55), 270–272 (2·78), 330 (3·15). IR spectrum (Nujol): 1 625 and 1 650 cm^{-1}, undistinct absorption at 3 300–3 400 cm^{-1}.

Allocryptopine (0·008%; 0·034%): needles from methanol, m.p. 161–163°C, undepressed in admixture with an authentic sample. UV, IR and TLC in agreement with an authentic specimen.

Chelerythrine (traces; 0·0006%): chloride, yellow needles, m.p. 210–211°C, mixed melting point with an authentic preparation 211°C. Identity confirmed by IR and UV spectra and TLC

Coptisine (traces; 0·000026%): chloride, orange needles, m.p. > 350°C, does not melt in admixture with authentic sample. Reduction with zinc in hydrochloric acid gave tetrahydro derivative, m.p. 218–219°C, undepressed in admixture with an authentic sample of (±)-stylopine.

Corydine (0·00005%; 0·00007%): from methanol, m.p. 147–149°C, undepressed in admixture with an authentic sample. The identity was confirmed by comparison of its UV, IR and TLC data with those of an authentic sample.

Isocorydine (0·00005%; 0·00009%): prisms from methanol, m.p. 182–183°C, undepressed in admixture with an authentic sample. The UV and IR spectra and TLC data identical with data those of an authentic sample.

Magnoflorine iodide (0·0005%; 0·0003%): prisms, m.p. 263–264°C (methanol), mixed melting point with an authentic sample 264–265°C. UV, IR and TLC data in agreement with an authentic sample.

(–)-*trans*-N-*methylstylopinium iodide* (0·007%; 0·031%): prisms, m.p. 297–299°C (methanol), undepressed in admixture with an authentic sample. $[\alpha]_D^{20}$ −120° (*c* 0·45, methanol), UV, IR and PC in agreement with a reference sample.

Procumbine (0·004%; 0·00008%): orange-red needles, m.p. 191–192°C. For the mass and NMR spectrum see ref.[14]. UV spectrum (methanol): λ_{max} nm (log ε) 222 (4·35), 240–250 sh (4·19), 310 (3·96), 370 (3·96); λ_{min} nm (log ε) 280 (3·92), 340 (3·85). IR spectrum (Nujol): 1 625 and 1 650 cm^{-1}. For $C_{20}H_{17}NO_7$ calculated: 62·66% C, 4·43% H, 3·65% N; found: 62·66% C, 4·31% H, 3·61% N.

Protopine (0·22%; 0·19%): prisms from methanol, m.p. 207–208°C, undepressed in admixture with an authentic sample. The UV, and IR spectra and TLC data identical with those of an authentic sample.

Sanguinarine (0·00003%; 0·0005%): chloride, red needles m.p. 282–283°C, undepressed in admixture with an authentic preparation. Identified by comparison of its UV, IR, and TLC data with those of an authentic sample.

R_F Values

In systems S_1, S_2 and S_3: allocryptopine (0·22; 0·50 and 0·04), corydine (0·16; 0·38; —), cryptopine (0·25; 0·54; 0·30), isocorydine (0·16; 0·42; —), protopine (0·32; 0·62; 0·36). In S_4, S_5, S_6, S_7: allocryptopine (0·70; —; 0·34; —); alkaloid HL 1 (0·60; 0·52; 0·20; 0·22), alkaloid HL 2 (0·74; 0·86; 0·28; 0·80), alkaloid HP 1 (0·62; —; 0·24; —), *trans*-N-methylstylopinium iodide (—; 0·12; —; —), procumbine (0·58; 0·50; 0·30; 0·74), protopine (0·75; —; 0·52; —). In S_8: sanguinarine (0·44), chelerythrine (0·17). In S_9, S_{10}, S_{11}: alkaloid HL 1 (—; 0·61; —), coptisine (0·55; 0·40; 0·07), *trans*-N-methylstylopinium iodide (—; 0·55; 0·17).

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