

FURTHER ALKALOIDS FROM *Glaucium flavum* CR.*

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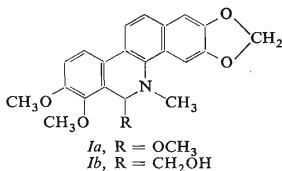
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In addition to alkaloids described earlier corytuberine, bocconoline and coptisine were isolated from *Glaucium flavum* CR., and the presence of magnoflorine was confirmed. An advantageous method of isolation of corytuberine is also described.

We had already studied the alkaloids from *Glaucium flavum* CR. and *G. flavum* ssp. *fulvum* (SMITH) FEDDE many years ago¹⁻³. Recently attention was given to the alkaloids of this species and some of its subspecies by several authors⁴⁻¹⁰ who isolated several additional minor alkaloids of aporphine type, mostly related to glaucine. From the quaternary fraction of bases Gertig and coworkers^{4,5} isolated magnoflorine, a new quaternary alkaloid glauflavine $C_{19}H_{20-22}NO_3^{(+)}$, and an insufficiently characterized alkaloid A. In connection with the recent finding of (-)-stylopine methohydroxide and (-)- β -canadine methohydroxide in *G. corniculatum* CURT.¹¹ we were interested in the question of quaternary alkaloids present in *G. flavum*. In view of the fact that among the alkaloids occurring in *G. flavum* and *G. flavum* ssp. *fulvum* no differences were observed², we now worked up both subspecies together. After the separation of bases which can be extracted with ether after alkalization ("non-quaternary fraction") we isolated the fraction of quaternary alkaloids using the procedure used in our investigations¹², i.e. by extraction with chloroform after addition of potassium iodide. Crystallization from methanol of the iodides obtained enabled the separation of two substances of which one was identical with magnoflorine iodide. The second substance coincided with glauflavine iodide in its melting point (217°C) and very similar UV and IR spectra⁵. However, it was found that this substance does not possess the properties of a quaternary alkaloid and that it is identical with corytuberine hydriodide, as was shown after its conversion to free base by direct comparison with a sample of corytuberine. It seems probable that the presumably quaternary alkaloid glauflavine⁵ was in fact corytuberine. This method seems at present to be the most viable method for the isolation of corytuberine, of which it is known that it cannot be practically extracted with a non-polar solvent in the form of a base, and that it thus escapes attention.

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Another alkaloid which was not yet found in *G. flavum* was isolated in a minute amount from the fraction of non-phenolic tertiary bases from the roots. The substance has the properties of a very weak base. According to mass spectrometry it has the composition C₂₂H₂₁NO₅. In the mass spectrum characteristic ions at *m/e* 379 (M⁺) and 348 (M-31, C₂₁H₁₈NO₄, base peak) were observed. The ions of masses 333 (C₂₀H₁₅NO₄) and 318 (C₁₉H₁₂NO₄) were formed from the ion 348 by gradual splitting off of the methyl groups from vicinal methoxyls. The ion of mass 318 (of ortho-quinone structure) split off one of the carbonyl groups formed, under formation of fragment of *m/e* 290 (C₁₈H₁₂NO₃). From these results it could be inferred that it had a dihydrobenzophenanthridine structure corresponding to chelerythrine pseudomethanolate¹³ (*Ia*) or to bocconoline¹⁴ (*Ib*). The structure *Ia* could be excluded on the basis of the completely different properties of both substances. A direct comparison of the substance isolated from *G. flavum* with authentic bocconoline¹⁵ ("substance C")* confirmed the supposed identity. The alkaloid bocconoline was found so far in *Bocconia cordata* WILLD. only¹⁵.

From the fraction of "non-quaternary" alkaloids we isolated all alkaloids described in our earlier papers^{1,2}, i.e. (+)-glaucine, protopine, allocryptopine, (+)-isocorydine, (+)-corydine, (-)-norchelidonine, (-)-chelidonine, sanguinarine, chelerythrine, chelirubine and isoboldine ("aurotensine", see¹⁷). As a further alkaloid coptisine was isolated from the roots in trace amount.

EXPERIMENTAL

The melting points were determined in a capillary and on a Kofler block and were not corrected. The mass spectra were measured on a AEI-MS 902 mass spectrometer, the IR spectra (in KBr) on an Infracan, Hilger and Watts apparatus, and the UV spectra (in methanol) on a Unicam SP 500 instrument. For thin layer chromatography silica gel G Merck (silica gel to gypsum ratio 5 : 1) with the solvent systems cyclohexane-diethylamine 9 : 1 (S₁), cyclohexane-chloroform-diethylamine 7 : 2 : 1 (S₂), methanol-diethylamine 4 : 1 (S₃) and 1 : 1 (S₄), ethanol-water-25% ammonia 15 : 9 : 1 (S₅), 1-propanol-water-85% formic acid 7 : 2 : 1 (S₆) and 12 : 7 : 1 (S₇) and 1-propanol-water-25% ammonia 40 : 9 : 1 (S₈), and "Silufol UV 254" (Kavalier) with the systems cyclohexane-methanol 3 : 1 (S₉), methanol-diethylamine 4 : 1 (S₁₀) and 1 : 1 (S₁₁)

* For comparison a preparation of "substance C" was used which was kindly donated¹⁶ by Prof. Dr C. Tani, Kobe, Japan.

were used. Paper chromatography was carried out on paper Whatman No 1, by descending manner, in 1-butanol-acetic acid-water 10 : 1 : 3 (S_{12}). Fluorescent spots were detected in the UV light, the others with potassium iodoplatinate or Dragendorff's reagent. The alkaloids which were isolated from this plant earlier^{1,2} were identified by their comparison with authentic samples, on the basis of their physical constants and R_F -values.

Extraction and Isolation of Alkaloids

The plants were cultivated in the Experimental Botanical Garden of the Medical Faculty in Brno from the seeds of the same origin as in paper² (*G. flavum* and *G. flavum* ssp. *fulvum*), harvested in the 2nd and 3rd year of vegetation on October 25th, 1971, and dried at room temperature. The roots (3880 g) and the above-ground parts (8050 g) were worked up separately. The dry ground material was extracted in the cold with several portions of methanol and the fractions *A*, *B*, *I* and *E* were obtained from the extract in the usual manner^{2,12}.

a) *Roots*. Fraction *A* (34.73 g) gave on crystallization from chloroform-ethanol 20.59 g of protopine (total yield, including the portions obtained from fraction AD_1 , was 22.44 g; 0.59%), and the crystallization of mother liquors from dilute hydrochloric acid gave 0.30 g of sanguinarine chloride (0.27 g of base; 0.007%). The remaining bases were separated to fractions AC_1 , AC_2 , AD_1 , and AD_2 (see²). From the bases AC_1 (3.12 g) isocorydine was obtained (total yield 1.86 g; 0.048%) on crystallization from ether and corydine (total yield 0.41 g; 0.010%) was separated in the form of its poorly soluble hydrochloride. From the mother liquors hydrochloride of bocconoline (base 7.2 mg; 0.0002%) was obtained. From the bases AC_2 (0.76 g) isocorydine and corydine were again obtained in the same manner. From the fraction AD_1 crystallization from ether gave 1.85 g of protopine and in the form of non-basic pseudo-cyanides quaternary benzophenanthridine alkaloids were separated (base 0.37 g; 0.010%) which were composed of sanguinarine, chelerythrine, a small amount of chelirubine and a further unidentified base (R_F in S_9 0.89, yellow-orange fluorescence). From the mother liquors, applying the crystallization of hydrochloride, (-)-norchelidonine (1.14 g; 0.030%) and (-)-chelidonine (0.07 g; 0.0017%) were isolated, and crystallization from ether of the residual bases gave allocryptopine (1.36 g; 0.036%). From the fraction AD_2 isoboldine (24.2 mg; 0.0006%) was isolated by crystallization from ethanol. In the amorphous residue of the fraction *A* (0.51 g) the presence of a small amount of some additional unidentified alkaloids was demonstrated in addition to the residues of the above-mentioned alkaloids. From the fraction *B* 1.6 mg of a yellow base were obtained, which was according to its R_F values in S_3 (0.70), S_{11} (0.51) and S_{12} (0.48) identical with coptisine. From the fraction *I*, which was further purified and freed from the majority of non-alkaloidal substances, magnoflorine iodide (0.22 g; 0.006%) and corytuberine hydriodide (33.6 mg of base; 0.001%) were isolated by crystallization from methanol. In the amorphous residue (0.08 g) another base was demonstrated (R_F 0.18 in S_5). From the bases of the fraction *E* (0.12 g) no crystalline substance could be obtained. Thin-layer chromatography proved the presence of some additional unidentified alkaloids in addition to traces of corytuberine, magnoflorine and isoboldine.

b) *Above-ground parts*. From the fraction *A* (94.32 g) the major part of glaucine (46.52 g) was separated first in the form of a poorly soluble hydrochloride (total yield of glaucine including the fraction obtained from *AC*, 62.25 g; 0.77%), and the remaining bases were separated to the fractions *AC*, AD_1 and AD_2 . From the fraction *AC*, and after its further separation to AC_1 and AC_2 an additional portion of glaucine was obtained in the form of its poorly soluble hydrobromide, and from the residues of the fraction AC_1 and AC_2 (+)-isocorydine (16.25 g; 0.20%) and (+)-corydine (0.16 g; 0.002%) were isolated in the above-mentioned manner in addition to a small amount of corytuberine and protopine. On application of the above-mentioned procedure

the fraction AD_1 afforded protopine (7.03 g; 0.087%), (–)-norchelidonine (1.09 g; 0.013%), (–)-chelidonine (0.10 g; 0.001%), allocryptopine (0.05 g; 0.0006%), and a mixture of quaternary benzophenanthridines (0.01 g; 0.0001%), composed predominantly of sanguinarine and chelirubine and small amounts of chelerythrine and an unidentified base (0.89 in S_9 , yellow–orange fluorescence) which was also found in the root. From the fraction AD_2 isoboldine (1.89 g; 0.023%) was isolated. In the amorphous residue (2.79 g) of the fraction A the presence of a larger number of other unidentified alkaloids was found in addition to the residues of the above mentioned alkaloids. In the fraction B the presence of quaternary protoberberines has not been demonstrated. The fraction I after elimination of non-basic substances, when crystallized from methanol gave corytuberine hydriodide (i.e. 0.46 g of the free base; 0.006%) and magnoflorine iodide (0.25 g; 0.003%). In the residue traces of another alkaloid (R_F 0.11 in S_5) were detected. From the fraction E a small amount of corytuberine was also obtained; the residue (0.27 g) was amorphous.

Characterization of Alkaloids

Bocconoline: base from chloroform–methanol, m.p. 221–222°C (capillary) or 215–218°C (Kofler block), undepressed on admixture of an authentic sample¹⁵. It has very weak basic properties and it gives with strong acids colourless salts (in contrast to the isomeric chelerythrine–pseudo-methanolate). The molecular weight determined by mass spectrometry was 379.1421 (for $C_{22}H_{21}NO_5$ calculated 379.1420). The mass spectrum was identical with that of the authentic specimen. UV spectrum, λ_{max} (log ϵ): 230 nm (4.52), 283 (4.61), 320 nm (4.11), λ_{min} 255 nm (4.17), 314 nm (4.10). This is identical with the literature data^{14,15}. R_F values, 0.22 in S_1 and 0.50 in S_2 , were identical with the values of the authentic sample. With concentrated sulfuric acid it gave a light yellow colour, with Erdmann's and Fröhde's reagent a yellow, then yellow-brown coloration, and with conc. nitric acid a redbrown coloration turning to orange-yellow.

Magnoflorine: iodide from methanol had m.p. 249–250°C (capillary) or 260–263°C (Kofler block), undepressed on admixture with an authentic sample¹⁸, $[\alpha]_D^{23}$ 197° \pm 3° (c 0.16, methanol). UV spectrum (see¹⁸) and IR spectrum were identical with the respective spectra of an authentic sample. The same is true of its R_F values in four solvent systems (0.50 in S_5 , 0.65 in S_7 , 0.14 in S_{10} and 0.42 in S_{12}) and of colour reactions.

Corytuberine: from methanol, m.p. 240–242°C (capillary) or 235–238°C (Kofler block), undepressed on admixture of a preparation isolated from *Corydalis cava* tubers¹⁹, $[\alpha]_D^{22}$ 314° \pm 7° (c 0.15, methanol). UV spectrum, λ_{max} (log ϵ), 225 nm (4.58), 272 nm (4.07), 312 nm (3.91), λ_{min} 260 nm (4.00) and 293 nm (3.77), and IR spectrum were identical with the spectra of a reference sample and coincided with the literature data²⁰. The same is true of the R_F values (0.40 in S_4 , 0.77 in S_5 , 0.71 in S_6 , 0.76 in S_7 , 0.41 in S_8 and 0.51 in S_{12}) and colour reactions. Hydriodide from methanol, m.p. 215–217°C (capillary) or 210–214°C (Kofler block).

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