QUATERNARY ALKALOIDS FROM *Glaucium oxylobum* BOISS. *et* BUSHE*

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From the fraction of quaternary alkaloids of the aerial part of *Glaucium oxylobum* BOISS. *et* BUHSE (−)-*trans*-N-methylcanadinium iodide was isolated as the main alkaloid after conversion to iodides, and, in a smaller amount, (+)-N-methylcorydinium iodide, detected for the first time in *Papaveraceae*, further (−)-*trans*-N-methylstylopinium iodide, magnoflorine iodide and a new quaternary alkaloid, N-methyldomesticinium iodide, isolated for the first time as a natural substance. In the roots, magnoflorine iodide accompanied by (+)-N-methylcorydinium iodide represented the main component of the quaternary fraction. From both plant parts corytuberine was also isolated for the first time. In the tertiary fraction of alkaloids (+)-corydine, protopine and allocryptopine were the major components, accompanied by smaller amounts of sanguinarine, chelerythrine, chelirubine, domesticine, isoboldine and scoulerine. In the fraction of quaternary protoberberines coptisine, berberine and traces of corysamine were detected.

Glaucium oxylobum BOISS. *et* BUHSE (*Papaveraceae*) is an annual or biennial plant, up to 40 cm high, with deep red petals with a black spot at the basis[1-3]. From this species we isolated[4] corydine as the main alkaloid and – to a smaller extent – protopine, allocryptopine, isoboldine ("aurotensine", *cf.*[5]), domesticine, traces of coptisine and berberine, and from the root also sanguinarine, chelerythrine, chelirubine and chelilutine. Later Shafiee and coworkers[6] investigated alkaloids from the species called *G. oxylobum*, of the population Ab-Ali from Iran, and found, in addition to protopine, completely different alkaloids. The dominant component was glaucine and as minor alkaloids O-methylatheroline and predicentrine were isolated. The botanical characteristic of the Ab-Ali population, mentioned in the above cited paper[6] differs considerably from the classical description in literature[1-3]. The authors[6] described the investigated plant as a perennial herb, 50–100 cm high, with yellow petals and a brown spot at their base. In view of the mentioned botanical and chemical differences it does not seem probable that the samples studied by us[4] and by Iranian authors[6] could belong to the same species.

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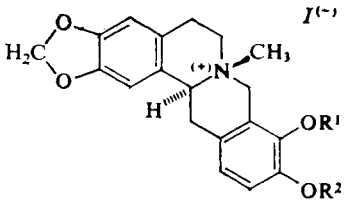
In the present paper we devote out attention to the investigation of the strongly polar alkaloidal fraction which contains in the species of *Glaucium* genus a number of quaternary N-methylated alkaloids from the group of tetrahydroprotoberberines and aporphines[7,8]. In the majority of cases they are accompanied by a considerably polar tertiary alkaloid – corytuberine. The plants studied originated from the same population as in paper[4] and in their botanical characters they agreed with the description in literature[1-3].

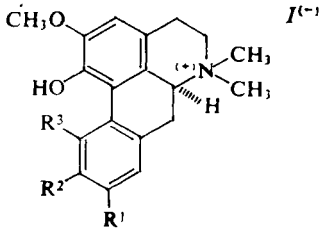
After conversion to iodides we isolated from the quaternary fraction of the aerial part as the main component (–)-*trans*-N-methylcanadinium iodide (*Ia*) in a 0·014% yield. We detected this alkaloid earlier in *G. corniculatum* CURT.[9] and *G. squamigerum* KAR. et KIR.[8]. In *G. oxylobum* it is accompanied by a small amount of (–)-*trans*-N-methylstylopinium iodide (*Ib*). Corresponding *cis* stereoisomers which are biogenetic precursors of protopine and benzophenanthridine alkaloids[10] were not detectable in this plant material, evidently in consequence of their rapid biotransformation. As regards the quantity, (+)-N-methylcorydinium iodide (*IIa*) was isolated from the aerial part as the second main quaternary alkaloid, the occurrence of which in *Papaveraceae* has not been known so far. This alkaloid was isolated for the first time from *Fagara nigrescens* FRIES (*Rutaceae*)[11,12] and later found in several further plants from the *Rutaceae* and *Annonaceae* families[13,14]. In addition, we isolated magnoflorine (*IIb*), corytuberine (*III*) and the iodide of a new quaternary alkaloid, for which we determined the structure of N-methyldomesticinium iodide (*IIc*), in relatively low yields.

In the measurement of the mass spectrum of the iodide of this alkaloid the non-volatile salt undergoes pyrolysis to methine *IV* (M_1 339·1461, $C_{20}H_{21}NO_4$) and a tertiary base (M_2 325). The molecular ion of the aporphine base does not contribute much to the fragmentation pattern. The majority of the characteristic peaks of the spectrum may be attributed to the cleavage ions of the methine: *m/z* 281 ($C_{17}H_{13}O_4$, $M_1 - CH_2N(CH_3)_2$), 266 (281-CH_3), 238 ($C_{15}H_{10}O_3$, 281-CH_3CO) and 58 ($CH_2{=}N(CH_3)_2$). The spectrum contains the peaks of iodine, hydrogen iodide and methyl iodide at masses 127, 128 and 142, respectively. The shift of the molecular peaks M_1 and M_2 and of all the fragments, with the exception of the one with *m/z* 58, in the spectrum of a sample labelled in the ion source with [O—^{2}H]ethanol is in agreement with the presence of a phenolic group in the molecule. The mass spectrum of N-methyldomesticinium iodide (*IIc*) prepared by methylation of domesticine[4] with methyl iodide was identical with the standard sample in all details. The identity of both substances was also confirmed by direct correlation of their melting points, UV and IR spectra and by chromatography.

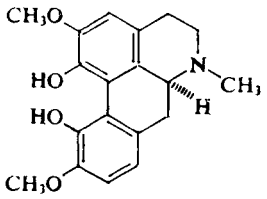
As already observed in other plants of *Papaveraceae*, the quaternary alkaloids from the aerial part of *G. oxylobum* and its roots differed considerably in their composition and mutual ratio. In the quaternary fraction from the root magnoflorine (iodide 0·068% of dry weight) was the major component. It was accompanied



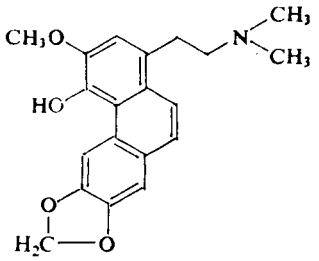
Ia, $R^1 = R^2 = CH_3$
Ib, $R^1 + R^2 = CH_2$



IIa, $R^1 = H$, $R^2 = R^3 = OCH_3$
IIb, $R^1 = H$, $R^2 = OCH_3$, $R^3 = OH$
IIc, $R^1 + R^2 = OCH_2O$, $R^3 = H$



III



IV

by a smaller amount of (+)-N-methylcorydinium iodide (0·026%). In contrast to the presence of N-methylcanadinium salt in the aerial part, its presence in the root could not be detected at all. We also obtained corytuberine from the strongly polar fraction of the root. It seems that the pair of alkaloids corytuberine (*III*) and its quaternary N-methyl derivative magnoflorine (*IIb*) are almost generally distributed not only in *Papaveraceae*, but they also occur in some families of the *Ranales* (*Polycarpicae*) order, as for example *Berberidaceae*. This knowledge on the wide distribution of corytuberine was made possible only after an adequate isolation technique was available, because this considerably polar alkaloid when submitted to the classical extraction of alkaloid bases with non-polar solvents remains in the aqueous phase together with quaternary alkaloids and so escapes attention.

In the fraction of tertiary alkaloids from the aerial part (0·08%) and the root (0·26%) we found the same alkaloids as in our previous study[4]. As main components we isolated from the aerial part corydine, protopine and allocryptopine and from the root corydine and protopine. In small amounts we detected domesticine, isoboldine ("aurotensine"), scoulerine, sanguinarine, chelerythrine and chelirubine and a neglibile amount of quaternary protoberberines, which were a mixture of coptisine, berberine and traces of corysamine. Since all these minor alkaloids were already isolated earlier[4] no special attention has been given to them in this paper.

From the results mentioned it is evident that the population of *G. oxylobum* studied by us is related in their chemical characters to the species *G. fimbrilligerum* Boiss. (see for example ref.[15]) and to some populations of *G. squamigerum* Kar. et Kir.[8,16] with the main alkaloid corydine (all of them are maximally biennial plants). In contrast to this the Iranian population Ab-Ali, named *G. oxylobum*[6] sensu Cullen[17] is evidently a different taxon which is not only botanically but also chemically as well close to the perennial species from the alliance of *G. flavum* (*cf.*[3]), with glaucine as the predominant alkaloid.

EXPERIMENTAL

The melting points up to 280°C were determined on a Mettler FP 51 instrument, and above 280°C on a Kofler block. They were not corrected. The mass spectra were measured on an AEI MS 902 spectrometer, the UV spectra on a Unicam SP 1800 instrument and the IR spectra in nujol on a Specord 75 IR, Zeiss (Jena), or in KBr on a Perkin–Elmer spectrophotometer. For thin-layer chromatography (TLC) both silica gel G Merck was used, with the solvent systems for tertiary alkaloids (cyclohexane–diethylamine 9 : 1, cyclohexane–chloroform–diethylamine 7 : 2 : 1 and 6 : 3 : 1) and for corytuberine and quaternary alkaloids (methanol–water–25% ammonia 15 : 3 : 1 (S_1), ethanol–water–25% ammonia 15 : 9 : 1 (S_2) and 1-propanol–water–85% formic acid (S_3)), and Silufol UV 254 (Kavalier) with the systems methanol–diethylamine 4 : 1 and 1 : 1 for quaternary protoberberines. Paper chromatography (PC) was carried out on paper Whatman No 1 (descending manner) in 1-butanol–water–acetic acid 10 : 3 : 1 and ethanol–water 3 : 2. The spots of fluorescing alkaloids were detected under the UV light, the spots of other alkaloids with potassium iodoplatinate.

Extraction and Isolation of Alkaloids

The plants were cultivated in the Centre for the Cultivation of Medicinal Plants, Medical Faculty, Brno, from the seeds of the same population as in ref.[4] (origin: Tashkent, USSR), harvested at the stage of flowers and unripe fruits on 21st July 1978. The material was dried at room temperature. The voucher specimen is deposited in our department.

Aerial part: The dry, ground aerial part (4 910 g) was extracted with methanol in a Soxhlet extractor and methanol distilled off. The residue was dissolved in 1% sulfuric acid and filtered. The isolation of the alkaloid fractions A, B and I from the acid filtrate was carried out in substantially the same manner as in ref.[18]. Crude bases of the fraction A (5·56 g; 0·11%) were separated in the conventional manner[19] to fraction AC, AD_1 and AD_2. Crystallization of hydrochlorides AC from dilute hydrochloric acid (1 : 10) gave a total of 2·36 g of corydine hydrochloride and from the mother liquors (after conversion to bases) crystallization from methanol gave 0·07 g of protopine. In the amorphous residue of the bases (0·38 g) mainly corydine could be detected by TLC in addition to a small amount of domesticine, protopine and allocryptopine. On crystallization from chloroform–methanol or methanol fraction AD_1 (1·20 g) afforded 0·70 g of protopine and 0·39 g of allocryptopine. In the residual amorphous bases (0·07 g) the following alkaloids could be detected by TLC: residues of protopine, allocryptopine and corydine, and negligible amounts of sanguinarine, chelerythrine and chelirubine. The amorphous fraction AD_2 (0·18 g) contained according to TLC isoboldine, scoulerine, remains of corydine and 4 unidentified alkaloids. According to TLC and PC the yellow fraction B (10·6 mg) was a mixture of coptisine and a smaller amount of berberine. From fraction I (2·59 g) 0·60 g of *trans*-N-methylcanadinium

iodide, 52·2 mg of *trans*-N-methylstylopinium iodide, 37·8 mg of magnoflorine iodide and 1·2 mg of N-methyldomesticinium iodide were obtained by crystallization from methanol. The amorphous residue, containing a considerable amount of non-alkaloidal substances, was purified and separated to iodides of non-phenolic bases (I_1) and phenolic bases (I_2) (*cf.*[20]). From fraction I_1 a further fraction of *trans*-N-methylcanadinium iodide (0·10 g) was obtained by crystallization from methanol, and in the amorphous residue (0·08 g) one unidentified alkaloid could be detected by TLC in addition to the mentioned quaternary alkaloids. N-Methylcorydinium iodide (181·5 mg) and corytuberine hydriodide (32·4 mg) were obtained from fraction I_2 by crystallization from methanol. The amorphous residue (0·28 g) contained according to TLC a mixture of the mentioned quaternary alkaloids and a considerable amount of non-alkaloidal dark ballasts.

Root: The dry, ground root (330 g) was worked up in the same manner as the aerial part of the plant. The crude bases of the fraction A (1·60 g, 0·48%) were separated to fraction AC, AD_1 and AD_2. From fraction AC corydine (yield of base: 0·37 g) was isolated in the above-mentioned manner in the form of a poorly soluble hydrochloride, as well as 0·05 g of protopine. In the amorphous bases the presence of domesticine was detected by TLC. 0·25 g of protopine were isolated from fraction AD_1 and in the amorphous residue sanguinarine was found as the main component in addition to a smaller amount of chelerythrine, chelirubine and allocryptopine. In the amorphous fraction AD_2 (0·03 g) isoboldine and 5 unidentified alkaloids were detected by TLC. The yellow fraction B (6·8 mg) represented according to TLC and PC a mixture of coptisine and a small amount of berberine and corysamine. From fraction I (0·64 g) crystallization from methanol gave 223·0 mg of magnoflorine iodide, 84·6 mg of N-methylcorydinium iodide and 15·1 mg of corytuberine hydriodide. The amorphous residue contained predominantly non-alkaloidal substances and only small amounts of the three mentioned alkaloids.

Characterization of the Isolated Alkaloids

The isolated alkaloids were identified on the basis of their melting points, mixed melting points, or also optical rotation, mass, UV and IR spectra (by comparison with authentic samples), and chromatographically by TLC and PC (the mentioned R_F values are for the systems S_1, S_2 and S_3). The yields of individual alkaloids from the aerial part or root in weight % of the dry plant material are given in brackets.

(−)-trans-N-*Methylcanadinium iodide* (0·014%; −): from hot methanol large orange prisms are formed (solvate) which rapidly weather in air[8] or from water colourless prisms with m.p. 255–256°C, $[\alpha]_D^{23}$ −124° ± 3° (*c* 0·21, methanol). UV and IR spectra and R_F values (0·31, 0·56, 0·80) were identical with those of reference samples[8].

(−)-trans-N-*Methylstylopinium iodide* (0·0011%; −): small prisms, m.p. 297–302°C (methanol), $[\alpha]_D^{23}$ −125° ± 3° (*c* 0·12, methanol). UV and IR spectra and chromatographic data (R_F 0·25, 0·50, 0·80) were identical with those of a reference sample[8].

(+)-N-*Methylcorydinium iodide* (0·0037%; 0·026%): needles (from methanol), m.p. 196–207°C, $[\alpha]_D^{23}$ +152° ± 3° (*c* 0·20, methanol). The mass spectrum was identical with that of a sample prepared by methylation of corydine with methyl iodide. UV spectrum: λ_{max} (log ε) 223 nm (4·81), 267 nm (4·38), 274 nm (4·39), shoulder 302 nm (4·12), λ_{min} 251 nm (4·21), 270 nm (4·37). IR spectrum: ν(OH) 3 400 cm^{-1}. The mentioned values and the chromatographic behaviour (R_F 0·05, 0·17, 0·56) were identical with the values of an authentic specimen.

(+)-*Magnoflorine iodide* (0·0008%; 0·068%): prisms from methanol, m.p. 262–263°C, $[\alpha]_D^{23}$ +183° ± 3° (*c* 0·14, methanol). UV and IR spectra and R_F values (0·48, 0·65 and 0·56) were identical with those of a reference sample.

(±)-*Corytuberine hydriodide* (0·0007%; 0·005%): needles from methanol, m.p. 209—210°C; UV and IR spectra and R_F values (0·80, 0·87 and 0·79) were identical with those of an authentic sample.

(±)-N-*Methyldomesticinium iodide* (0·00002%; —): thin prisms from methanol, m.p. 255 to 256°C, undepressed with an authentic sample. Mass spectrum (*m/z*, composition, abundance): 339 ($C_{20}H_{21}NO_4$, 6·2) 325 (1·2), 324 (1·5), 310 (1·0), 308 (0·8), 295 (0·7), 294 (0·8), 281 ($C_{17}H_{13}O_4$, 3·2), 238 ($C_{15}H_{10}O_3$, 2·7), 210 (0·9), 209 (0·9), 165 ($C_{13}H_9$, 2·1), 163 ($C_{13}H_7$, 1·8) 152 ($C_{12}H_8$, 3·5), 142 (CH_3I, 6·0), 128 (HI, 6·4), 127 (I, 5·0), 58 (C_3H_8N, 100). UV spectrum: λ_{max} (log ε) 224 nm (4·64), 284 nm (3·95), 311 nm (4·06), λ_{min} 263 nm (3·78), 292 nm (3·81), shoulder 321 nm (4·02). IR spectrum (KBr): bands at 760, 800, 830, 840, 860, 870, 885, 930 (O_2CH_2), 1 060, 1 080 and 1 120 cm^{-1} (C—O—C), 1 470, 1 490 and 1 500 cm^{-1} (aromatic cycle), 1 600, 2 850, 2 920, 3 000, 3 230 and 3 410 cm^{-1} (OH). R_F values 0·09, 0·28, 0·68. All spectra and chromatographic properties were identical with the properties of a sample prepared by methylation of domesticine with methyl iodide.

(±)-*Corydine* (0·043%; 0·11%): prisms, m.p. 149—150°C (ether), undepressed on admixture with an authentic sample. UV and IR spectra and the chromatographic properties were identical with the properties of the reference samples.

Protopine (0·016%; 0·091%): prisms, m.p. 208—209°C (chloroform–methanol); identification was carried out by mixed melting point determination and UV and IR spectra and chromatographic data.

Allocryptopine (0·008%; traces): prisms, m.p. 158—159°C (methanol); identification was carried out by mixed-melting point determination and UV and IR spectra and chromatographic data.

Preparation of N-Methyldomesticinium Iodide

(±)-Domesticine (7·9 mg) isolated from *G. oxylobum*[4] was dissolved in 0·5 ml of methanol, 2 ml of ether and 0·2 ml of methyl iodide were added and the mixture was allowed to stand for several hours. The expected methiodide crystallized out (8·3 mg) which was crystallized from methanol, m.p. 256—257°C. The mass, IR and UV spectra and chromatographic behaviour were identical with the properties of the natural alkaloid isolated from *G. oxylobum*.

For the measurement of the IR *and the* UV *spectra and for technical assistance we thank Mrs J. Bochořáková, Department of Medical Chemistry and Biochemistry, Brno.*

Note added in proof.: Recently Soviet authors (Karimova S. U., Israilov I. A.: Khim. Prir. Soedin. *1984*, 259) also isolated from *Glaucium oxylobum* — in agreement with our results — corydine, protopine and allocryptopine as the dominant alkaloids, in addition to some additional minor alkaloids, including domesticine.

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