ADDITIONAL ALKALOIDS FROM *Glaucium squamigerum* KAR. ET KIR.*

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A new quaternary alkaloid, (−)-β-N-methylisocorypalminium hydroxide, was isolated from *Glaucium squamigerum* KAR. et KIR. in the form of iodide. In addition to the alkaloids already known to occur in this plant, *i.e.* allocryptopine (the main alkaloid), protopine, chelerythrine, sanguinarine, corydine, coptisine and berberine, additional alkaloids, *i.e.* (−)-chelidonine, (±)-chelidonine (diphylline) and chelirubine were also isolated from the non-quaternary fraction, while from the fraction of quaternary alkaloids (−)-β-N-methylcanadinium iodide (the second main alkaloid), (−)-α-N-methylcanadinium iodide and (−)-β-N-methylstylopinium iodide were isolated after conversion of the salts to iodides. Further the presence of small amounts of stylopine, canadine, scoulerine, corysamine, corytuberine, magnoflorine, α-N-methylstylopinium hydroxide and β-N-methyltetrahydropalmatinium hydroxide were also detected.

Glaucium squamigerum KAR. et KIR. is a biennial to perennial herb[1,2] indigenous to Central Asia. The alkaloids of this plant were investigated for the first time by Platonova and coworkers[3] who isolated corydine in high yield (1·8%) as the main alkaloid in addition to a smaller amount of protopine and allocryptopine. In contrast to this Slavíková[4] isolated allocryptopine and protopine as the dominant alkaloids, and chelerythrine, sanguinarine. corydine, berberine and coptisine as minor alkaloids. Since Slavíková[4] investigated one year old plants, plant samples in the second vegetation year were also later examined[5]. However, the results did not differ practically, so that we were unable to confirm the presence of corydine as the main alkaloid in the population.

In the present study we again investigate alkaloids from *G. squamigerum*, focussing our attention on minor alkaloids and the possible presence of quaternary alkaloids not yet detected in this species. We found that in comparison with other species of the *Glaucium* genus, *G. squamigerum* displays a relatively high content of quaternary alkaloids. After their conversion to iodides, (−)-β-N-methylcanadinium iodide**

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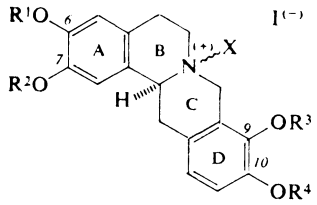
** For symbols α and β see ref.[6].

was isolated from this fraction as the main component (*Ia*; 0·055%). After allocryptopine[4] it represents the second dominant alkaloid of the plant. (−)-α-N-Methylcanadinium iodide (*Ib*), (−)-β-N-methylstylopinium iodide (*Ic*) and a further alkaloid which did not correspond to any known quaternary alkaloid from *Papaveraceae* were isolated in smaller amounts. In the mass spectrum the iodide of this alkaloid was cleaved thermally predominantly to a tertiary base with mass 341·1632 ($C_{20}H_{23}NO_4$ requires 341·1627) and characteristic ions of *m/z* 178, 176, 164 (base peak), 149, 142 (CH_3I), 127 (I) and 121. After labelling with [O—^{2}H]ethanol in the ion source the peaks at masses 341, 178 and 176 shifted by one mass unit upwards. From this it follows that this alkaloid has a structure corresponding either to the methiodide of corypalmine (*Id* or *Ie*) or isocorypalmine (*If* or *Ig*). A direct comparison with reference samples *Id*, *Ie*, *If* and *Ig*, prepared by methylation of the tertiary bases with methyl iodide, melting and mixed melting points determination, optical rotation measurements and the analysis of the UV and the IR spectra, as well as chromatographic data indicated that the alkaloid isolated from *G. squamigerum* was identical with (−)-β-N-methylisocorypalminium iodide (*If*). Thus, this alkaloid was detected as a natural compound for the first time.



Ia, $R^1 + R^2 = CH_2$, $R^3 = R^4 = CH_3$, ~X = — CH_3
Ib, $R^1 + R^2 = CH_2$, $R^3 = R^4 = CH_3$, ~X = ⁞⁞ CH_3
Ic, $R^1 + R^2 = R^3 + R^4 = CH_2$, ~X = — CH_3
Id, $R^1 = H$, $R^2 = R^3 = R^4 = CH_3$, ~X = — CH_3
Ie, $R^1 = H$, $R^2 = R^3 = R^4 = CH_3$, ~X = ⁞⁞ CH_3
If, $R^1 = R^3 = R^4 = CH_3$, $R^2 = H$, ~X = — CH_3
Ig, $R^1 = R^3 = R^4 = CH_3$, $R^2 = H$, ~X = ⁞⁞ CH_3
Ih, $R^1 = R^2 = R^3 = R^4 = CH_3$, ~X = — CH_3

The same as in other tetrahydroprotoberberines, N-methylation of corypalmine and isocorypalmine with methyl iodide also gave preferentially the more stable β-N-methyl derivatives (*Id* or *If*), while the less stable α-isomers (*Ie* or *Ig*) were present in the mother liquors only in small amounts. While α-N-methylcorypalminium iodide (*Ie*) was obtained crystalline, α-N-methylisocorypalminium iodide (*Ig*) remained amorphous. The stereochemical purity of the preparations obtained was checked chromatographically[7].

In the mother liquors after the quaternary alkaloids isolated from *G. squamigerum* trace amounts of magnoflorine, α-N-methylstylopinium iodide and β-N-methyltetrahydropalmatinium iodide (*Ih*) were detected chromatographically. The last mentioned alkaloid was also detected in small amounts in a crude preparation of β-N-methylisocorypalminium iodide from *G. squamigerum* using mass spectrometry (inferred from the presence of the ions *m/z* 355 ($M-CH_3I$) and 190). Small amounts of corytuberine were also detected, which being a highly polar alkaloid, passes into this fraction as hydriodide.

In agreement with ref.[4] allocryptopine constituted the dominant component of tertiary bases. In this case too it represented the dominant alkaloid of the plant (0·11%). Protopine and quaternary benzophenanthridines were isolated in smaller amounts. They were separated in the form of non-basic pseudo-cyanides. When chromatographed on a column of acid alumina (ref.[8]) they were separated to cheleerythrine, sanguinarine and also chelirubine, which was isolated for the first time from this plant. Small yields of (+)-corydine and additional alkaloids, (−)-chelidonine and (±)-chelidonine (diphylline) were obtained from tertiary bases. Their presence in *G. squamigerum* has not yet been known. We isolated (−)-chelidonine as a minor component from some species of *Glaucium* genus (*G. corniculatum* CURT., *G. flavum* CR. and *G. fulvum* SMITH) earlier, while its enantiomer (+)-chelidonine is known as a dominant alkaloid in *Chelidonium majus* L. and *Stylophorum diphyllum* (MICHX.) NUTT. In the residue of the non-phenolic fraction a small amount of canadine and stylopine could be detected, and in the phenolic fraction scoulerine was found. From the fraction of quaternary protoberberine coptisine chloride and berberine chloride were isolated for the first time in crystalline form. In mother liquors a small amount of corysamine was detected chromatographically.

EXPERIMENTAL

The melting points up to 280°C were determined on a Mettler FP 51 apparatus, and above 280°C on a Kofler block and they are not corrected. The mass spectra were measured on an AEI-MS 902 spectrometer, the UV spectra in methanol on a Unicam SP 1800 and the IR spectra in nujol on a Specord 75 IR spectrophotometer, Zeiss, Jena. For thin-layer chromatography (TLC) both Silica gel G Merck with the solvent systems cyclohexane–diethylamine 9 : 1 (S_1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S_2) and 6 : 3 : 1 (S_3), chloroform–ethanol–diethylamine 8 : 1 : 1 (S_4), methanol–water–25% ammonia 15 : 3 : 1 (S_5), ethanol–water–25% ammonia 15 : 9 : 1 (S_6), 1-propanol–water–85% formic acid 12 : 7 : 1 (S_7), and silufol sheets UV 254 (Kavalier) with the systems cyclohexane saturated methanol (S_8), methanol–diethylamine 4 : 1 (S_9) and 1 : 1 (S_{10}) were used. Paper chromatography (PC) was carried out on paper Whatman No 1, descending arrangement, in the systems 1-butanol–water–acetic acid 10 : 3 : 1 (S_{11}) and ethanol–water 3 : 2 (S_{12}). The spots of fluorescing alkaloids were inspected under UV light, while spots of other alkaloids were detected with potassium iodoplatinate (on TLC) or Dragendorff's reagent (on PC).

Extraction and Isolation of Alkaloids

The plants were cultivated in the Centre for the Cultivation of Medicinal Plants, Medical Faculty, Brno, from the seeds obtained from the Botanical Gardens in Moscow and Minsk of the same population as in ref.[4], which were harvested after the first year of vegetation at the stage of flowering and unripe fruits, on June 27th, 1967. The voucher specimen is deposited in our Department. The plants were dried at room temperature.

The dry ground plant material (3 017 g) was extracted in a Soxhlet extractor, the solvent (methanol) evaporated and the residue dissolved in 1% sulfuric acid and filtered. The non-basic components (fraction *L*) were eliminated by extraction with ether, the aqueous phase was alkalized in the conventional manner, with sodium carbonate[9] and extracted with ether (fraction *A*). Further alkalization with sodium hydroxide to pH > 13 and extraction with ether gave fraction *B*. After adjusting the aqueous phase to pH 6—7 and addition of an excess of potassium iodide[9] the mixture was extracted repeatedly with chloroform (fraction *I*: quaternary bases iodides). Since the aqueous phase still gave a weak positive reaction with Mayer's reagent it was alkalized with ammonia and extracted with chloroform (fraction *E*).

Fraction *L* contained a considerable amount of non-alkaloidal, mainly crystalline components (m.p. 221°C) in which, however, non-basic alkaloids dihydrosanguinarine and oxysanguinarine could not be detected. Therefore this material was not further investigated.

From fraction *A* (5·18 g), which was separated to fraction *AC*, AD_1 and AD_2 (as in ref.[10]), crystallization from methanol or chloroform methanol gave allocryptopine (3·28 g) and protopine (0·77 g), and — in the form of non-basic pseudocyanides — a mixture of quaternary benzophenanthridines. The latter were converted to bases (0·31 g) and chromatographed on acid alumina[8] to give chelerythrine (221 mg), sanguinarine (85 mg) and chelirubine (4 mg). From fraction *AC* corydine was obtained in the form of poorly soluble hydrochloride (base 29·3 mg) and in the mother liquors small amounts of canadine and stylopine were detected. From the residue of the non-phenolic fraction AD_1 (±)-chelidonine (13·6 mg) could be separated by crystallization from methanol and from the mother liquors (—)-chelidonine (base 19·9 mg) was isolated as a poorly soluble hydrochloride. Thus 0·15 g of an amorphous residue of fractions *AC* and AD_1 remained in which a further three non-identified alkaloids were detected by TLC in addition to the remains of the mentioned alkaloids. Fraction AD_2 (0·07 g) was amorphous and according to TLC it contained scoulerine and at least five additional unidentified alkaloids.

Fraction *B* was converted to chlorides and crystallized from water to give 22 mg of coptisine chloride, while 2·6 mg of berberine chloride were obtained from the mother liquors. In the mixture of bases (8 mg) regenerated from the mother liquor after crystallization of chlorides corysamine was detected by TLC and PC.

Crystallization from methanol of fraction *I* gave β-N-methylcanadinium iodide (1·67 g) and a smaller amount of α-N-methylcanadinium iodide (0·11 g), β-N-methylisocorypalminium iodide (22·2 mg), β-N-methylstylopinium iodide (8·2 mg) and a crystalline non-alkaloidal substance (85 mg) of m.p. 168°C, which was not further investigated. There remained 0·24 g of the amorphous material in which corytuberine, magnoflorine and a small amount of α-N-methylstylopinium iodide, β-N-methyltetrahydropalmatinium iodide and two unidentified alkaloids were detected by TLC and PC in addition to the remains of the above mentioned alkaloids. Fraction *E* (0·20 g) was amorphous and according to TLC it contained four unidentified alkaloids in addition to the rests of corytuberine.

Characterization of the Isolated Alkaloids

The isolated alkaloids were characterized by melting points, mixed melting points, optical rotation values, UV and IR spectra, sometimes by mass spectra, and by chromatographic comparison

with authentic samples. The yields of individual alkaloids in weight % of the dry plant material are given in brackets.

Allocryptopine (0·11%): prisms, m.p. 161—162°C (methanol); UV spectrum: λ_{max} (log ε) 209 nm (4·69), 231 nm (4·03), 286 nm (3·79), λ_{min} 222 nm (4·0), 258 nm (3·22); IR spectrum: ν(CO) 1 645 cm^{-1}.

Protopine (0·026%): prisms, m.p. 208—209°C (chloroform-methanol); UV spectrum: λ_{max} (log ε) 209 nm (4·71), 240 nm (3·96), 288 nm (3·90), λ_{min} 232 nm (3·95), 261 nm (3·39); IR spectrum: ν(CO) 1 655 cm^{-1}.

(+)-*Corydine* (0·001%): needles, m.p. 148—149°C (ether), $[\alpha]_D^{24}$ +208° ± 3° (*c* 0·11, methanol); UV spectrum: λ_{max} (log ε) 221 nm (4·61), 269 nm (4·16), 305 nm (3·80), λ_{min} 248 nm (3·82), 290 nm (3·70); IR spectrum: ν(OH) 3 180 and 3 370 cm^{-1}. Hydrochloride m.p. 262—263°C (water).

(—)-*Chelidonine* (0·0007%): prisms m.p. 135—136°C (ether), undepressed in admixture with an authentic sample, $[\alpha]_D^{23}$ —114° ± 3° (*c* 0·1, methanol). UV spectrum: λ_{max} (log ε) 208 nm (4·60), 240 nm (3·85), 287 nm (3·80), λ_{min} 227 nm (3·73), 259 nm (3·13). IR spectrum: ν(OH) 3 260, 3 370 and 3 640 cm^{-1}. With Erdmann's reagent it gave a characteristic grass-green coloration.

(±)-*Chelidonine* (0·0005%): hexagonal leaflets, m.p. 217—218°C (ether), undepressed with an authentic sample, UV and IR spectra identical with those of (—)-chelidonine, the same as the colour reactions with Erdmann's reagent.

Chelerythrine (0·007%): chloride from dilute hydrochloric acid, yellow needles, m.p. 210 to 211°C; UV spectrum, λ_{max} (log ε) 209 nm (4·42), 228 nm (4·54), 283 nm (4·65), 320 nm (4·19), λ_{min} 212 nm (4·40), 253 nm (4·24), 311 nm (4·17), as well as the IR spectrum were identical with the spectra of a reference sample. The base set free from a solution of the chloride with ammonia and crystallized from ether had m.p. 265—267°C. The mass spectrum was identical with the spectrum of an authentic sample. The base, crystallized from methanol (6-methoxy-5,6-dihydrochelerythrine*) had m.p. about 207°C under change of its crystal form, which melted then at 240—241°C. The base crystallized from ethanol (6-ethoxy-5,6-dihydrochelerythrine), m.p. 201—206°C, resolidified under change of its crystal form and remelted at 245—246°C.

Sanguinarine (0·003%): chloride from dilute hydrochloric acid, copper red needles, m.p. 282—283°C; the UV spectrum, λ_{max} (log ε) 211 nm (4·36), 235 nm (4·46), 284 nm (4·48), 322 nm (4·11), λ_{min} 216 nm (4·34), 255 nm (4·13), 324 nm (3·99), as well as the IR spectrum were identical with the spectra of reference sample. The base was crystallized from ether, m.p. 261—262°C. The mass spectrum was identical with the spectrum of an authentic sample. The base, when crystallized from methanol gave 6-methoxy-5,6-dihydrosanguinarine, m.p. 195—201°C (unsharp) under change of its crystal form which melted then at 250—251°C. The base crystallized from ethanol (6-ethoxy-5,6-dihydrosanguinarine) had m.p. 201—202°C under change of crystal form and resolidification, and it remelted at 250—251°C.

Chelirubine (0·00013%): chloride from dilute hydrochloric acid, purple red needles, m.p. 281—282°C, undepressed with an authentic sample. UV spectrum: λ_{min} (log ε) 230 nm (4·51), 281 nm (4·45), 342 nm (4·21), shoulder at 352 nm (4·16), λ_{min} 254 nm (4·16), 316 nm (4·92), identical with the spectrum of an authentic sample.

Coptisine chloride (0·00072%): orange needles from dilute hydrochloric acid, which gradually darkened above 250°C under decomposition and which did not melt up to 300°C (the authentic specimen showed the same behaviour). UV spectrum: λ_{max} (log ε) 230 nm (4·30), 242 nm (4·30),

* Numbering according to the Ring Index.

266 nm (4·27), 362 nm (4·27), λ_{min} 236 nm (4·28), 254 nm (4·22), 308 nm (3·63). IR spectrum: identical with a reference samples.

Berberine chloride (0·00009%): yellow needles from dilute hydrochloric acid, m.p. 207—209°C, undepressed on admixture of an authentic sample. The identity was confirmed by chromatographic analysis.

(—)-β-N-*Methylcanadinium iodide* (0·055%): from colourless methanolic solution large orange to garnet red prisms (solvate) crystallized (depending on crystallization conditions) which when dried formed a yellowish powder with m.p. 252—253°C; from water colourless prisms with the same m.p.; $[\alpha]_D^{23}$ —124° ± 3° (*c* 0·23, methanol). UV spectrum: λ_{max} (log ε) 210 nm (4·71), 288 nm (3·98), shoulder at 220 nm (4·54), λ_{min} 260 nm (3·60).

(—)-α-N-*Methylcanadinium iodide* (0·0037%): prisms from methanol, melting at 162—165°C, which solidified at 180—185°C under change of crystal form and remelted unsharply at 218 to 219°C, then resolidified above 230°C and remelted again at 247—249°C; a reference sample behaved in the same manner; $[\alpha]_D^{24}$ —113° ± 2° (*c* 0·31, methanol). The UV spectrum was identical with the spectrum of β-N-methylcanadinium iodide, while the IR spectrum displayed differences in the 750—1 100 cm^{-1} region, in comparison with the spectrum of the β-form.

(—)-β-N-*Methylisocorypalminium iodide* (0·00074%): prisms from methanol, m.p. 175—176°C, undepressed in admixture with a sample prepared from (—)-isocorypalmine, $[\alpha]_D^{24}$ —127° ± 5° (*c* 0·13, methanol). The UV spectrum, λ_{max} (log ε) 209 nm (4·83), 285 nm (3·90), shoulder at 225 nm (4·45), λ_{min} 258 nm (3·43), and the IR spectrum, ν(OH) 3 230 and 3 620 cm^{-1}, were identical with the spectra of an authentic sample. Mass spectrum: *m/z* 341, 178, 176, 164, 149, 142, 127, 121.

(—)-β-N-*Methylstylopinium iodide* (0·00027%): from methanol m.p. 298—302°C, undepressed in admixture with an authentic sample prepared from (—)-stylopine. UV spectrum: λ_{max} (log ε) 209 nm (4·67), 289 nm (3·92), shoulder at 232 nm (3·96), λ_{min} 260 nm (3·10). With Erdmann's reagent it gave a characteristic transitory blue-green and then a dark blue coloration.

Preparation of N-Methylisocorypalminium Iodide

(—)-Isocorypalmine (52·4 mg) isolated from *Bocconia frutescens*[11] was dissolved in 4 ml of hot chloroform, 5 ml of methanol were added, followed by 1 ml of methyl iodide. After three days the mixture was partly concentrated and diluted with ether, giving 59·0 mg of the β-form (79%). After crystallization from methanol m.p. was 177—178°C, $[\alpha]_D^{23}$ —128° ± 3° (*c* 0·15, methanol). The UV and the IR spectrum were identical with those of the preparation isolated from *G. squamigerum* (see above). Evaporation of the mother liquor after the separation of the β-form gave 16 mg of an amorphous residue which contained according to PC predominantly the α-form in addition to the remains of the β-form. However, the α-form could not be brought to crystallization.

Preparation of N-Methylcorypalminium Iodide

(+)-Corypalmine (56·0 mg), isolated from *Corydalis nobilis*[12], was dissolved in 4 ml of boiling chloroform and diluted with 5 ml of methanol and 1 ml of methyl iodide. After three day's standing and partial concentration and addition of ether 63·8 mg of the β-form crystallized out (85% of the theory), which after recrystallization from methanol had m.p. 231—232°C, $[\alpha]_D^{23}$ +131° ± 3° (*c* 0·17, methanol). UV spectrum: λ_{max} (log ε) 209 nm (4·72), 285 nm (3·82), shoulder at 225 nm (4·34), λ_{min} 258 nm (3·38); IR spectrum: ν(OH) 3 160 and 3 200 cm^{-1}. From the mother

liquor the α-form was obtained by further crystallization (5·7 mg), m.p. from methanol 149 to 150°C, the stereochemical purity of which was checked by PC (see below). The amorphous residue of the methiodides (5 mg) represented a mixture of both forms.

R_F Values

In the system S_1, S_2 and S_3, respectively: allocryptopine 0·22, 0·57, 0·64; canadine 0·65, 0·72 and 0·76; chelidonine 0·23, 0·62, 0·66; corydine 0·16, 0·50, 0·56; protopine 0·35, 0·66, 0·72; scoulerine 0·08, 0·23, 0·29; stylopine 0·72, 0·75, 0·78. In the system S_4 corytuberine 0·56, magnoflorine 0·00, β-N-methylcanadinium iodide 0·41, β-N-methylstylopinium iodide 0·03. In the system S_5, S_6, S_7, S_{11} and S_{12}, respectively: corytuberine 0·96, 0·97, 0·77, —, —; magnoflorine 0·38, 0·52, 0·55, —, —; α-N-methylcanadinium iodide 0·17, 0·24, 0·82, 0·83, 0·85; β-N-methylcanadinium iodide 0·19, 0·27, 0·82, 0·68, 0·48; α-N-methylcorypalminium iodide 0·22, 0·28, 0·79, 0·76, 0·87; β-N-methylcorypalminium iodide 0·24, 0·29, 0·83, 0·67, 0·68; α-N-methylisocorypalminium iodide 0·23, 0·27, 0·77, 0·73, 0·87; β-N-methylisocorypalminium iodide 0·26, 0·31, 0·78, 0·63, 0·60; α-N-methylstylopinium iodide 0·15, 0·21, 0·80, 0·76, 0·78; β-N-methylstylopinium iodide 0·17, 0·24, 0·80, 0·61, 0·33; α-N-methyltetrahydropalmatinium iodide 0·16, 0·20, 0·73, 0·81, 0·90; β-N-methyltetrahydropalmatinium iodide 0·17, 0·21, 0·73, 0·71, 0·78. In the system S_8: chelerythrine 0·43, chelirubine 0·62, sanguinarine 0·53. In the system S_9 and S_{10}: berberine 0·40, 0·68; coptisine 0·54, 0·86; corysamine 0·24, 0·59.

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