ALKALOIDS OF *Papaver nudicaule* SUBSP. *xanthopetalum* (TRAUTV.) FEDDE AND *P. nudicaule* SUBSP. *album* (REGEL) FEDDE FROM THE SECTION *Scapiflora* REICHB.*

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The alkaloidal composition in the two title taxa differs only quantitatively. Both contain amurine as the main alkaloid. *P. nudicaule* ssp. *xanthopetalum* contains muramine, protopine and alkaloid PN1 (probably identical with (±)-nudaurine (*Ia*)) as dominant alkaloids; further were isolated amurensine, epialpinine, cryptopine, mecambridine and alpinigenine. Allocryptopine, papaverrubines A, B, D and G, palmatine and coptisine were detected chromatographically. The quaternary alkaloid fraction afforded *cis*-N-methylstylopinium hydroxide (as the iodide *II*) which was isolated for the first time from *P. nudicaule*. In *P. nudicaule* ssp. *album* the alkaloid PN1, epialpinine, amurensine and muramine represented the dominant alkaloids, accompanied with mecambridine, protopine, cryptopine, allocryptopine and alpinigenine. Among the quaternary alkaloids N-methylstylopinium hydroxide was found.

As a continuation of our previous studies of alkaloids from the *Scapiflora* section of the *Papaver* genus[1] we turned our attention to *P. nudicaule* L., a perennial species with a Mongolian–Siberian area reaching to Central Asia. A strong infraspecific variability has been described among the *P. nudicaule* species[2]. Fedde[3] mentions numerous subspecies and varieties of *P. nudicaule*, many of which are recently considered independent species. Novák[4] classifies these taxa into the *Nudicauliatae* series of the *Scapiflora* REICHB. section. The reported alkaloidal compositions in the hitherto studied taxa denoted *P. nudicaule* differ considerably. Thus, coptisine, sanguinarine, chelerythrine[5] and papaverrubines B, D and F were found[6] in unspecified *P. nudicaule* species. Amurine, amuronine and amuroline were found in *P. nudicaule* var. *amurense*[7]. It is not clear whether the plant investigated was the now individual *P. amurense* N. BUSCH species or a *P. nudicaule* L. cultivar. Amurine, muramine, 13-oxomuramine and protopine are reported[8,9] in *P. nudicaule* var. *croceum* LEDEB.; on the other hand in *P. croceum* we found[1] nudaurine, amurine,

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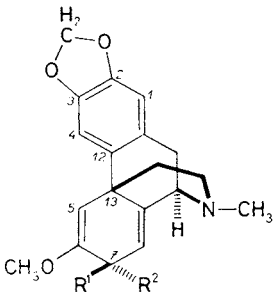
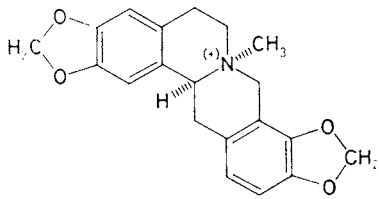
oxysanguinarine, corydine and papaverrubine D. The taxone *P. nudicaule* ssp. *radicatum* FEDDE – now classified as *P. radicatum* ROTTB. – contains sanguinarine, amurine, protopine, berberine, cryptopine, allocryptopine, amurensinine, O-methylthalisopavine and papaverrubine E, B and D (refs[6,10]). Several subspecies of *P. nudicaule* have been studied closely by Maturová and coworkers[11,12]. In the taxone, originally classified as *P. anomalum* FEDDE[11] and later re-classified as *P. nudicaule* L. ssp. *xanthopetalum* (TRAUTV.) FEDDE var. *leiocarpum* (TURZ.) FEDDE[12] (recently *P. leiocarpum* (TURZ.) FEDDE[4]), these authors found[11,12] rhoeadine, cryptopine, amurensine, amurensinine, protopine, oxysanguinarine, glaucamine, mecambridine, sanguinarine and papaverrubines A, D, E and B. They also repeatedly investigated several samples of *P. nudicaule* ssp. *xanthopetalum* (TRAUTV.) FEDDE which, however, differed considerably in the alkaloidal composition[11,12]. From samples of relatively high content of alkaloids (0·38% and 0·34%) they isolated protopine, muramine and oxysanguinarine; in three samples of low alkaloid content (0·12%, 0·16% and 0·11%) they found cryptopine, mecambridine and rhoeadine, respectively, in addition to oxysanguinarine, present in all three samples. It cannot be decided whether the reported differences are caused by infraspecific variability, incorrect botanical classification or inhomogeneity of the plant material.

The present study concerns the alkaloids from two very close taxa *P. nudicaule* ssp. *xanthopetalum* (TRAUTV.) FEDDE with yellow to orange flowers and *P. nudicaule* ssp. *album* (REGEL) FEDDE with white flowers. The alkaloidal composition of the latter subspecies was so far unknown. We focussed our attention on completion and verification of data concerning the presence of tertiary bases and strongly polar quaternary alkaloids, extractable with chloroform as iodides.

The alkaloid fraction of *P. nudicaule* ssp. *xanthopetalum* (0·11% of dry material) afforded amurine as the main constituent. This promorphinane alkaloid is very widespread in species of the *Scapiflora* section and has been found in the studied taxone already previously. Other dominant alkaloids were muramine and another promorphinane alkaloid of the nudarine type, denoted as PN 1. Chromatographic properties as well as the mass, ^{1}H NMR and ^{13}C NMR spectra of the alkaloid PN 1 were identical with those of nudaurine; it had, however, a different melting point and, unlike the laevorotatory nudaurine, was optically inactive. Both PN 1 and nudaurine were oxidized to amurine. The alkaloid PN 1 was chromatographically different from epinudaurine (*Ib*) which is formed together with nudaurine by reduction of amurine[13]. These properties suggest that the compound is (±)-nudaurine. We isolated also cryptopine, epialpinine, alpinigenine, mecambridine and amurensine. Coptisine, palmatine, allocryptopine and papaverrubines A, B, D and G were detected chromatographically. The quaternary alkaloid fraction afforded *cis*-N-methylstylopinium hydroxide (*II*), isolated (as the iodide) for the first time from *P. nudicaule*.



I a, R^1 = OH; R^2 = H
I b, R^1 = H; R^2 = OH



II

Also *P. nudicaule* ssp. *album* had a low alkaloid content (0·10%). As in the preceding case we obtained amurine as the main alkaloid, together with epialpinine, alkaloid PN 1, amurensine and muramine. As minor components we isolated mecambridine, cryptopine, protopine, allocryptopine, alpinigenine and detected chromatographically coptisine, palmatine and papaverrubines A, B, D and G. In the strongly polar quaternary alkaloid fraction we found *cis*-N-methylstylopinium hydroxide and traces of an unspecified alkaloid.

The alkaloidal composition of both subspecies *P. nudicaule* is qualitatively practically identical and we found only quantitative differences. For both taxa the principal morphinane alkaloid amurine is characteristic. The presence of protopine alkaloids, particularly muramine, isopavinane alkaloid amurensine, rhoeadane alkaloid epialpinine and the promorphinane alkaloid PN 1 is significant. Contrary to previous studies[11,12] we did not detect rhoeadine, amurensinine, sanguinarine or oxysanguinarine.

EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorrected. Ultraviolet spectra were taken in methanol on an SP-1800 Pye Unicam spectrophotometer, IR spectra were recorded in Nujol on an IR-75 Specord (Zeiss, Jena G.D.R.) spectrometer. Mass spectra were measured on a Jeol MS D 100 instrument. Proton and ^{13}C NMR spectra were obtained with a Varian XL-200 spectrometer (200·058 MHz for ^1H and 50·309 MHz for ^{13}C) in deuteriochloroform with tetramethylsilane as internal standard. Thin-layer chromatography (TLC) was performed on silica gel LS 5–40 μ (Lachema; gypsum binder), in the systems cyclohexane–diethylamine 9 : 1 (S_1), cyclohexane–chloroform–diethylamine 7 : 2 : 1 (S_2), benzene–acetone–methanol 7 : 2 : 1 (S_3), benzene–diethylamine 19 : 1 (S_4), methanol–water–25% ammonia 15 : 3 : 1 (S_5), methanol–water–25% ammonia 15 : 9 : 1 (S_6), methanol–25% ammonia 200 : 1 (S_7), and on commercial Silufol plates (Kavalier, Czechoslovakia) in methanol–diethylamine 4 : 1 (S_9). Descending paper chromatography (PC) was carried out on a paper Whatman No-1 in 1-butanol. –98% acetic acid–water 10 : 1 : 3 (S_{10}) and ethanol–water 3 : 2 (S_{11}). Spots of fluorescing alkaloids were detected in UV light at 235 and 336 nm; papaverrubines were detected by concentrated

hydrochloric acid fumes, other alkaloids by spraying potassium iodoplatinate (TLC) or Dragendorff reagent (PC). Column chromatography was carried out on silica gel L 100—400 μ (Lachema) in appropriate solvents.

Extraction and Isolation

The plants were cultivated in the Center for Cultivation of Medicinal Plants of the Medical Faculty, Purkyně University, Brno, from the seeds obtained from various botanical gardens and were harvested at the stage of flowering. The dry ground material was extracted with cold methanol. After evaporation of the solvent, the crude mixture of alkaloids was dissolved in acetic acid (0·5 mol l^{-1}) and the solution processed as described in ref.[1] to afford alkaloidal fractions A, B, E and I.

Papaver nudicaule L. ssp. *xanthopetalum* (TRAUTV.) FEDDE

The extraction was performed with whole dried plants (17·26 kg) harvested on June 25th, 1970. Fractionation of the crude total alkaloids afforded fraction A (16·78 g), fraction B (21·1 mg), fraction E (1·42 g) and fraction I (0·64 g). The fraction A was further separated into non-phenolic bases (A_1; 9·04 g) and phenolic bases (A_2; 1·84 g).

Fraction A_1 gave amurine (0·45 g) and muramine (0·63 g) which were purified by crystallization from methanol, and protopine (0·18 g), crystallized from chloroform–ethanol. The remaining amorphous portion of A_1 (3·72 g) was chromatographed on a column of silica gel (200 g; benzene). Elution with benzene and benzene–ether (99 : 1) furnished fractions from which epialpinine (89·8 mg) was separated by crystallization from methanol and methanol–ether. The mother liquor contained traces of alpinigenine and mecambridine. Elution with benzene–ether (1 : 1), ether and ether–chloroform (99 : 1) gave mixtures from which alpinigenine (13·7 mg) was separated. In the mother liquors we detected mecambridine and amurine. Ether–chloroform 4 : 1 and 1 : 1 and chloroform eluted mainly mecambridine along with minor amounts of amurine, protopine and traces of alpinigenine. Mecambridine (25·3 mg) was obtained by crystallization from methanol. Chloroform and chloroform–methanol 99·9 : 0·1 to 99 : 1 eluted predominantly amurine along with small amounts of mecambridine, protopine and cryptopine. Crystallization from methanol gave amurine (0·74 g). Elution with chloroform–methanol 99 : 1 to 95 : 5 led to fractions which upon crystallization from chloroform–ethanol afforded protopine (0·15 g). The presence of allocryptopine, cryptopine and amurine was proven by TLC. Fractions eluted with chloroform–methanol 9 : 1 were crystallized from methanol to give cryptopine (27·8 mg). Chloroform–methanol 4 : 1 and 1 : 1 and pure methanol eluted mixtures which on crystallization from methanol furnished alkaloid PN 1 (0·34 g). The mother liquor contained, in addition to muramine and protopine, traces of cryptopine, allocryptopine and amurensine. The fraction A_2 on crystallization from methanol gave amurensine (0·15 g); in the mother liquors small amounts of protopine, mecambridine and traces of cryptopine were detected in addition to amurensine. Thin-layer chromatography (in systems S_1—S_4) of the mother liquors of fraction A proved the presence of papaverrubines A, B, D and G. In fraction B (5·0 mg) coptisine and palmatine were detected by PC and TLC, fraction E contained almost no alkaloids. The crude fraction I contained great amount of non-alkaloid compounds; after purification, *cis*-N-methylstylopinium iodide (14·5 mg) was obtained by crystallization from methanol.

Papaver nudicaule L. ssp. *album* (REGEL) FEDDE

The extraction was carried out with whole dried plants (1·38 kg) harvested on July 9th, 1969. Fractionation of the crude alkaloid mixture afforded fraction A (0·95 g), traces of fraction B, fraction E (0·30 g) and fraction I (0·11 g).

Crystallization from methanol yielded muramine (19·7 mg) from fraction A. Then, the remaining part of fraction A (0·94 g) was separated into fraction A_1 (0·52 g) and A_2 (0·14 g).

The fraction A_1 which remained amorphous even after repeated purification was chromatographed on a silica gel column (100 g; benzene). Elution with benzene–ether 4 : 1 afforded epialpinine (11·1 mg). Another portion of epialpinine (4·6 mg) was obtained by elution with benzene–ether 1 : 1. In the mother liquors alpinigenine and mecambridine were detected. Fractions eluted with ether and ether–chloroform 95 : 5 remained amorphous (4·2 mg) and contained alpinigenine. Material, eluted with chloroform–methanol 99·9 : 1 to 95 : 5 on crystallization from methanol gave mecambridine (5·1 mg) and amurine (25·3 mg). The mother liquors contained protopine and traces of alpinigenine. Elution with chloroform–methanol 95 : 5 to 9 : 1 afforded fractions from which protopine (4·1 mg), cryptopine (5·0 mg), amurine (5·2 mg) and allocryptopine (2·9 mg) were obtained. The alkaloid PN 1 was detected by TLC. Fractions from chloroform–methanol 4 : 1 furnished protopine (13·1 mg), cryptopine (1·8 mg), allocryptopine (8·3 mg) and alkaloid PN 1 (25·4 mg); traces of amurensine and muramine were detected chromatographically. Papaverrubines A, B, D and G were found by TLC (in S_1-S_4). Crystallization of fraction A_2 from methanol separated amurensine (18·7 mg); mother liquor contained much non-alkaloid material and amurine, together with two further unidentified bases and papaverrubines D and G (according to TLC). Fraction B contained traces of coptisine and palmatine, fraction E only traces of alkaloids. Crystallization of the purified fraction I from methanol yielded *cis*-N-methylstylopinium iodide (2·4 mg), TLC proved traces of another alkaloid of R_F 0·20 (S_5) and 0·30 (S_6).

Characterization of the Alkaloids Isolated

Yields in % of dry material from *P. nudicaule* ssp. *xanthopetalum* and *P. nudicaule* ssp. *album*, respectively, are given in parentheses.

Allocryptopine (traces; 0·0008%): from methanol, needles, m.p. 161–163°C, no depression on admixture with an authentic sample, identity confirmed by UV and IR spectra and TLC.

Alkaloid PN 1 (0·0020%; 0·0018%): from methanol, needles, m.p. 116–117°C, optically inactive. Mass spectrum, *m/z* (intensities): 327 (M^+; 88), 326 (43), 312 (62), 310 (81), 309 (64), 252 (52), 240 (74), 152 (47), 139 (35), 42 (100). UV spectrum: λ_{max} (log ε) 215 nm (4·23), 245 sh (3·95), 294 (3·91); λ_{min} (log ε) 258 nm (3·36); no change upon alkalization. IR spectrum (Nujol): 1 645 cm^{-1}, 3 380 and 3 600 cm^{-1}. ^1H NMR spectrum (deuteriochloroform, acylation shifts after addition of trichloroacetyl isocyanate are given in parentheses after the signal assignment): 1·51 (ddd, J = 12·5, 2·5 Hz, 1 H), 16-CH_2 (+0·24); 1·82 (m, 1 H) 16-CH_2 (+0·14); 2·36 (s, 3 H) N-CH_3 (+0·01); 2·39–2 46 (m, 3 H) 15-CH_2 (+0·3–0·4) and OH; 2·84 (ddd, J = 17·8; 6·3 and 0·8 Hz, 1 H) 10-CH_2 (+0·32); 3·15 (d, J = 17·8 Hz, 1 H) 10-CH_2 (+0·23); 3·38 (d, J = = 6·3 Hz, 1 H) H-9 (+0·62); 3·70 (s, 3 H) OCH_3 (+0·04); 4·64 (d, J = 4 Hz, 1 H) H-7 (+1·3); 5·22 (s, 1 H) H-5 (+0·26); 5·71 (d, J = 4 Hz, 1 H) H-8 (+1·08); 5·88 (AB, 2 H) O—CH_2—O (+0·09), 6·52 (s, 1 H) (+0·09) 6·77 (s, 1 H) (+0·05) H-1, H-4. ^{13}C NMR spectrum (deuteriochloroform, ^1J and ^nJ obtained by gated decoupling by first order analysis): 31·36 tm (^1J = = 127 Hz, ^2J = 3·7 Hz) C-16; 39·66 sm (W = 10 Hz) C-13; 41·72 qs, N—CH_3; 41·92 t (^1J = = 131 Hz) C-10; 46·30 tm (^1J = 134 Hz, W_m = 10 Hz) C-15; 54·35 qs OCH_3; 60·59 dm (^1J = = 145 Hz) C-9; 64·14 ddd (^1J = 146·5, ^3J = 6·1, ^2J = 3·5 Hz) C-7; 100·73 ts (^1J = 173·5 Hz) O—CH_2—O; 101·23 ds (^1J = 154·3), 104·86 ds (^1J = 159·5), 107·25 ds (^1J = 161 Hz), C-8, C-5, C-4; 117·88 dt (^1J = 160, ^3J = 5·3 Hz) C-1; 129·40 sm, 134·62 sm C-11, C-12; 141·14 sm C-14; 145·84 sm, 146·20 sm, 154·01 s, C-2, C-3, C-6.

Alpinigenine (0·00008%; 0·0003%): from methanol, prisms, m.p. 195–197°C. Identified by mixture m.p. and comparison (UV spectrum, TLC) with an authentic sample.

Amurensine (0·00086%; 0·0014%): from methanol, prisms, m.p. 214—215°C. Identical with an authentic sample (UV, IR, ^{1}H NMR spectra, TLC, mixture m.p.).

Amurine (0·069%; 0·0022%): from methanol, prisms, m.p. 215—216°C. Identical with an authentic sample (UV, IR, ^{1}H NMR spectra and TLC).

Cryptopine (0·00016%; 0·0005%): from methanol, prisms, m.p. 220—221°C; identical with an authentic sample (UV, IR spectra TLC).

Epialpinine (0·00049%; 0·0011%): from methanol, needles, m.p. 121—123°C. Its identity was proven by comparison with an authentic sample (UV and IR spectra, TLC, mixture m.p.).

Mecambridine (0·00015%; 0·0004%): from methanol, needles, m.p. 182—183°C. Identical with an authentic sample (UV and IR spectra, TLC, mixture m.p.).

Muramine (0·0036%; 0·0014%): from methanol, needles, m.p. 171—172°C; UV, IR and ^{1}H NMR data agree with those published. The identity confirmed by TLC and mixture melting point.

Protopine (0·0019%; 0·0013%): from chloroform–methanol prisms, m.p. 203—205°C, no depression in mixture m.p. The UV and IR spectra and TLC agree with those of an authentic sample.

cis-N-*Methylstylopinium iodide* (II) (0·00008%; 0·0002%): from methanol, prisms, m.p. 278 to 280°C. Its identity with an authentic sample was proven by UV and IR spectra, TLC, mixture m.p. and PC.

Reduction of Amurine and TLC Comparison with Alkaloid PN 1

Sodium borohydride (50 mg) was added portionwise to amurine (5·0 mg) in methanol (1 ml) and the mixture was refluxed for 1 h. The reaction products were identified by TLC as nudaurine (higher R_F values in S_1-S_4) and epinudaurine (lower R_F values)[13]. Alkaloid PN 1 had the same R_F values as nudaurine.

Oxidation of Alkaloid PN 1 and Comparison with Amurine

Active manganese(IV) oxide (30 mg) was added to a solution of alkaloid PN 1 (10 mg) in chloroform (3 ml). After stirring for 1 h, the excess oxide was removed by filtration, the solvent was evaporated and the dry residue was crystallized from methanol. The obtained product (3 mg) was identical with amurine (mixture m.p., TLC).

R_F Values

In S_1, S_2, S_3 and S_4 alpinigenine 0·24; 0·69; 0·81; 0·96; amurensine 0·08; 0·18; 0·45; 0·54; amurine 0·04; 0·32; 0·62; 0·79; allocryptopine 0·33; 0·68; 0·20; 0·95; alkaloid PN1 0·10; 0·31; 0·24; 0·30; cryptopine 0·27; 0·76; 0·33; 0·95; epialpinine 0·60; 0·87; 0·83; 0·95; epinudaurine 0·07; 0·24; 0·18; 0·25; mecambridine 0·16; 0·45; 0·49; 0·80; muramine 0·21; 0·68; 0·22; 0·95; nudaurine 0·10; 0·31; 0·24; 0·30; papaverrubine A 0·32; 0·67; 0·84; 0·80; papaverrubine B 0·23; 0·57; 0·78; 0·79; papaverrubine D 0·07; 0·21; 0·71; 0·33; papaverrubine G 0·14; 0·33; 0·77; 0·42; protopine 0·50; 0·77; 0·30; 0·95; in S_5, S_6 and S_7 *cis*-N-methylstylopinium iodide 0·17; 0·26; 0·02; in S_9, S_{10} and S_{11} coptisine 0·55; 0·42; 0·08; palmatine 0·29; 0·54; 0·24; *cis*-N-methylstylopinium iodide —; 0·76; 0·75; *trans*-isomer —; 0·59; 0·19.

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