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ALKALOIDS OF *PAPAVER BRACTEATUM*: PRESENCE OF CODEINE, NEOPINE AND ALPININE*

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Key Word Index—*Papaver bracteatum*; Papaveraceae; alkaloids, codeine; neopine; alpinine.

Papaver bracteatum Lindl. is known to contain at least 5 classes of alkaloids among which only three representatives of the morphinane-type are reported to be present, thebaine [1], oripavine [2] and salutaridine [3]. More abundant are the (pro)-aporphines [2–6], the protopines [3,4,6–8] and the rhoeadines: alpinigenine [9] and the papaverrubines A-F [10]. A low-molecular-weight alkaloid, bractamin ($M^+ = 193$), was reported to be present [2] and was tentatively identified as an isomer of corypalline [6]. In addition a non-alkaloidal substance has been identified as α-thebaol [11].

We now wish to report the presence of at least 8 further alkaloids in capsules of *P. bracteatum*, var. "Arya I" [12] using GC–MS for analysis after preliminary counter-current separation of the total extract. Three of these alkaloids were unequivocally identified as codeine, neopine and alpinine by comparison with reference samples.

GC–MS, TLC and GLC data, obtained from these fractions, are given in Table 1.

As can be seen from Table 1, 2 more morphinane alkaloids, codeine and neopine appear to be present in *P. bracteatum* in minor quantities (see Experimental). Although common in other *Papaver* species, these compounds have previously not been found in *P. bracteatum*. This observation indicates that there is no chemotaxonomic difference between *P. bracteatum* and other *Papaver* species in relation to the presence of codeine. However, there were no indications for the presence of morphine, the next product in the biosynthetic pathway from codeine. The possibility of morphine being present in fractions 3–30 (Table 1) can be discounted, since the system used was specifically designed by Battersby *et al.* [14] for separation and isolation of morphine, codeine and thebaine. Moreover, a separate analysis of a small scale sample, specifically pointed at the isolation of morphine [15], showed no trace of this substance.

The possibility that alpinine is an artefact derived from alpinigenine during the extraction procedure can be excluded by the fact that dilute aqueous acetic acid (which was used as an extractive at room temperature) does not bring about the interconversion of these two compounds [13].

Identification of the 5 other unknown alkaloids will be the subject of further research.

EXPERIMENTAL

General. GC–MS was carried out using a modified Jeol JMS-07 instrument with a double stage jet separator and a 200 × 0·3 cm glass column with He carrier gas (pre-pressure: 0·8 kg/cm^2). The stationary phase was 3% OV-17 on Chromosorb G, AW DMCS, 80–100 mesh. Injector temperature was 250°, the column 235°, the separator 220° and the chamber 255°. Accelerating voltage was 3·0 kV, trap current 300 μA and the electron energy 70 eV. GLC traces were obtained by recording the total ion current at 30 eV. GLC was on the same system, the column temperature being programmed from 220 to 285°, using N_2 as carrier gas. For quantitative measurements, tetrahydrothebaine and cholesterol acetate were used as internal standards. TLC was carried out on 0·25 mm Merck SiO_2 GF 254 plates with toluene–Me_2CO–MeOH–NH_4OH (40:40:30:2). Detection was with iodoplatinate.

Isolation. After removal of seeds, capsules (2·1 kg dry wt) were ground to 1 mm and the powder extracted at room temp. with 15·0 l. 10% HOAc for 16 hr and then 10·0 l. 2% HOAc. Combined extracts were adjusted to pH 9·5 with 6N KOH and extracted 2 × $CHCl_3$. $CHCl_3$ extract gave 22·4 g of a pale yellow substance, which was submitted to countercurrent distribution (Craig type) between EtOAc and 0·5 M Pi buffer (pH 6·9). After 100 transfers, the contents of tubes 51–81 were removed; tubes were refilled with fr. lower phase and another 27 transfers were performed. Based on TLC analysis, the following tubes were combined: 3–8, 9–14, 15–30, 31–36, 37–50, 51–98 and 99–127. After adjusting the pH of the aq phase to 8·5 using Na_2CO_3, phases were extracted with their corre-

* Part 3 in a series; for Part 2 see United Nations Document ST/SOA/SER.J/14. Supported by a part time grant from the University of Utrecht.

Table 1. The separation of alkaloids by countercurrent fractionation of an extract of *Papaver bracteatum*

Compound	Counter current fraction* 3–8	9–14	15–30	31–36	37–50	51–98	99–127
Codeine	–	+	+	+	–	–	–
Neopine	+	+	–	–	–	–	–
Alpinine	–	–	–	–	–	+	+
α-Thebaol	–	–	–	–	+	+	+
Thebaine	+	+	+	++	+++	++++	++

* In addition 5 further compounds were found in fractions 15–127.

sponding upper phase and this subjected to TLC, GLC and GC–MS.

Chromatographical and mass spectral data. Codeine: R_f 0·32, RRT (relative retention time; RRT thebaine = 1·00): 0·61, *m/e* (relative intensity): 300(18%), 299(100%), 298(12%), 284(7%), 282(9%), 280(5%), 242(11%), 229(29%), 214(15%), 188(20%), 162(52%), 149·5(3%), 124(49%), 115·5(10%), 59(44%), 58(13%). *Neopine*: R_f 0·28, RRT 0·62, *m/e* 300(18%), 299(100%), 298(12%), 284(20%), 282(9%), 280(7%), 255(27%), 254(88%), 243(18%), 225(16%), 197(20%), 152(17%), 149·5(6%), 127·5(16%), 59(4%), 58(20%). *Alpinine*: R_f 0·70, RRT 2·08, *m/e* 416(8%), 415(27%), 401(11%), 400(32%), 384(8%), 383(6%), 311(14%), 222(35%), 206(37%), 194(15%), 193(100%). *α-Thebaol*: RRT 0·78, *m/e* 255(18%), 254(99%), 240(18%), 239(100%), 211(36%), 196(21%), 193(9%), 183(16%), 168(25%), 152(20%), 139(35%). *Thebaine*: R_f 0·48, RRT 1·00, *m/e* 312(24%), 311(100%), 297(15%), 296(58%), 282(5%), 280(4%), 267(8%), 254(8%), 242(12%), 239(10%), 165(7%), 155·5(7%), 139·5(14%).

Concentration of alkaloids. The concentration of alkaloids is given in % dry weight of capsule material. Calculations are made by comparison of peak areas in the gas chromatograms of the $CHCl_3$ extract to those obtained from thebaine calibration curves: codeine 0·004, neopine 0·003, alpinine 0·022, α-thebaol 0·053 and thebaine 1·070. The extraction procedure described does not give quantitative alkaloid extraction; small scale thebaine determination in the same starting material gave a percentage of 1·72.

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