Phytochemistry, 1981, Vol. 20, pp. 323–325. © Pergamon Press Ltd. Printed in England. 0031-9422/81/0201-0323 $02.00/0

ALKALOIDS FROM *PYCNARRHENA LONGIFOLIA**

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(*Revised received* 14 *May* 1980)

Key Word Index—*Pycnarrhena longifolia*; Menispermaceae; isoquinoline alkaloids; obaberine; homoaromoline; aromoline; daphnoline; limacine; krukovine; magnoflorine; pycnarrhine.

Abstract—From the root and stem of *Pycnarrhena longifolia* the following alkaloids were isolated and identified: obaberine, homoaromoline, aromoline, daphnoline, limacine, krukovine, magnoflorine and pycnarrhine. Pycnarrhine is a simple isoquinoline derivative which has not previously been reported as a natural product.

INTRODUCTION

In a previous publication [1] we reported on the alkaloids present in *Pycnarrhena novoguineensis*. In continuation of our studies of Indonesian medicinal plants we now report on a number of alkaloids isolated and identified from *P. longifolia*.

For the large scale extraction two methods were considered, one in which the alkaloids are extracted as their bases (method I, see Experimental) and one in which the alkaloids are extracted as their salts (method II, see Experimental). Comparison of the two methods showed that similar tertiary alkaloid fractions are obtained. However, only in method II, are the quaternary alkaloids extracted. Hence this method, II, was preferred for the large scale extraction. Comparison of the alkaloid fractions obtained from the roots and stem showed that they have a similar alkaloid composition. The percentage of alkaloid present in the root and stem are 4 and 1.2% respectively, calculated as the alkaloid chlorides from the dry wt of plant material. The leaves contained very low alkaloid concentrations.

For the large scale extraction the roots and stem were combined and extracted according to method II. The alkaloids were fractionated into toluene, chloroform and aqueous soluble fractions. The identification of the alkaloids isolated from the toluene and the aqueous fractions will be reported here. The structural elucidation of the alkaloids in the chloroform fraction is still in progress.

RESULTS AND DISCUSSION

Using column chromatography a number of alkaloids were isolated from the toluene fraction and by means of their spectral data (UV, MS, ^{1}H NMR and $[\alpha]_D^{20}$) the main alkaloids were identified as homoaromoline (**1a**) [2], limacine (**2a**) [3], aromoline (**1b**) [3] and krukovine (**2b**) [4]. Limacine (R.R.-configuration) gave similar R_f values as fangchinoline (S.S.-configuration) in the TLC system S1–S3.

A minor alkaloid was identified as obaberine (**1c**) [3] from its spectral data (UV, MS, ^{1}H NMR and $[\alpha]_d^{20}$). In TLC systems S1–S3 it gave similar R_f values to *O*-methylated homoaromoline.

A second minor alkaloid had a MW of 580 and the mass spectral fragmentation pattern was typical of an oxyacanthane type of alkaloid. Comparison of the ^{1}H NMR spectrum with that of aromoline showed a great deal of similarities, however, only one *N*-Me group was observed in the spectrum of the unknown alkaloid. *N*-methylation of the alkaloid was performed with formaldehyde followed by reduction with sodium borohydride, which yielded an alkaloid with a similar TLC behaviour, MS and ^{1}H NMR spectrum as aromoline. The alkaloid is therefore nor-aromoline. Based on the shift of the *N*-Me group (2.65 ppm) in the ^{1}H NMR, this group is thought to be in the *N*-2 position. This means that the alkaloid is identical to daphnoline (**1d**) [5, 6].

In the quaternary alkaloid fraction two main alkaloids were found, which could be isolated by means of chromatography. One of the alkaloids is identified by comparison of its spectral data (UV, MS, ^{1}H NMR, ^{13}C NMR) and chromatographic comparison (S4,S5) with a reference sample, as magnoflorine (**3**).

The other quaternary alkaloid had a characteristic yellow colour under basic conditions. The mass spectrum showed three major ions, at *m/e* 193, 192 and 150. Peak matching gave the formulae $C_{11}H_{15}NO_2$, $C_{11}H_{14}NO_2$ and $C_9H_{10}O_2$ for these ions. The ^{1}H NMR spectrum showed the presence of two Me groups, and three one proton singlets in the aromatic region. Based on these data, structure **4** was assigned to this alkaloid. The positions of the OH and the OMe are derived from the melting point [7], UV spectrum [8] and ^{13}C NMR [9]. To our knowledge this is the first time that this compound has been isolated from a plant.

In order to confirm the identification the alkaloid was synthesized according to the method described by Brossi *et al.* [10]. The starting material 6,7-dimethoxy-3,4-dihydroisoquinoline was kindly provided by Dr. Teitel

* Part 4 in the series "Studies on Indonesian Medicinal Plants". For Part 3 see Siwon, J., van Essen, F., Verpoorte, R. and Svendsen, A. B. *Planta Med.* (in press).

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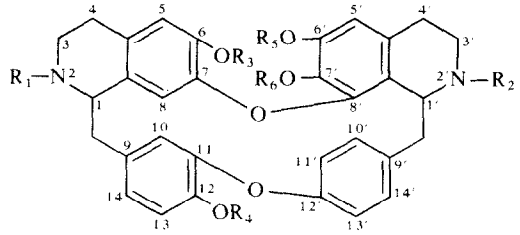
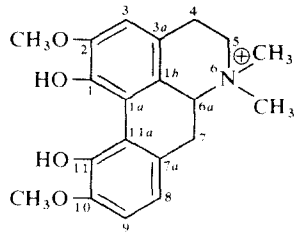
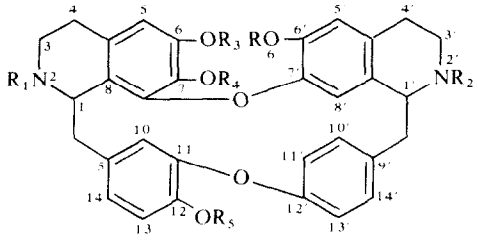


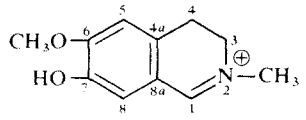
1a Homoaromoline (1-R, 1′-S), $R_1 = R_2 = R_3 = R_4 = R_5 = CH_3$, $R_6 = H$
1b Aromoline (1-R, 1′-S), $R_1 = R_2 = R_3 = R_5 = CH_3$, $R_4 = R_6 = H$
1c Obaberine (1-R, 1′-S), $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = CH_3$
1d Daphnoline (1-R, 1′-S), $R_1 = R_3 = R_5 = CH_3$, $R_2 = R_4 = R_6 = H$



3 Magnoflorine



2a Limacine (1-R, 1′-R), $R_1 = R_2 = R_3 = R_5 = R_6 = CH_3$, $R_4 = H$
2b Krukovine (1-R, 1′-R), $R_1 = R_2 = R_3 = R_6 = CH_3$, $R_4 = R_5 = H$



4 Pycnarrhine

(Hoffman-La Roche Inc., Nutley, New Jersey, USA). Comparison of the spectral data and chromatographic comparison (S5) of the isolated and the synthesized alkaloid proved that they were identical.

Summarizing the results, 6 bisbenzylisoquinoline alkaloids were isolated, four of which had the oxyacanthane skeleton and for these alkaloids the R.S. configuration was determined. Two alkaloids had a berbamane skeleton with a R,R-configuration. In the quaternary alkaloid fraction the ubiquitous magnoflorine was identified and the alkaloid 7-hydroxy-6-methoxy-2-methyl-3,4-dihydroisoquinoline, for which we propose the trivial name pycnarrhine.

EXPERIMENTAL

Plant material. The plant material was collected in Pantai Popoh, on Java, Indonesia. The plant was identified as *Pycnarrhena longifolia* (Decne. ex Miq.) Beccari by Dr. L. L. Foreman (Kew Gardens, London). A voucher specimen is kept in our laboratories.

Apparatus. UV spectra were recorded in MeOH. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Jeol PS100 apparatus in the Fourier transform mode. Some ^{1}H NMR spectra were recorded in the continuous wave mode. The tertiary alkaloids were measured in $CDCl_3$, the quaternary alkaloids in CD_3OD. Shifts are presented in δ-values relative to TMS. MS were obtained with an AEI MS902 or a LKB9000 mass spectrometer, using a direct inlet system and an ionization energy of 70 eV. $[\alpha]_D^{20}$ is recorded in $CHCl_3$.

Extraction methods. Method I. Ground leaves (5 g) (roots or stems) were wetted with 10% $NaHCO_3$ soln. Subsequently the plant material was macerated with toluene–EtOH (8:17) for 3 days. The plant material was filtered off and the extract taken to dryness. The residue was dissolved in toluene and extracted exhaustively with 1% aq. HOAc. The pH of the combined HOAc extracts was adjusted to 8 with 10% aq. $NaHCO_3$ soln and this solution was extracted exhaustively with toluene. *Method II.* Ground leaves (5 g) (roots or stem) were macerated for 3 days in 1% aq. HOAc. The plant material was filtered off. To the filtrate 4 M HCl was added until the pH was *ca* 1–2, subsequently 0.1 M Mayer's reagent was added until no more ppt formed. The ppt was collected by centrifugation, dissolved in an Me_2CO–MeOH–H_2O (6:2:1) and passed through an Amberlite IRA Cl^- ion exchange column. The eluate from the column was taken to dryness, yielding the alkaloid chlorides.

Large-scale extraction. Ground stem and roots (3 kg) were extracted according to method II. The alkaloid chlorides were dissolved in dist. H_2O and, after basification (pH 8) with Na_2CO_3, extracted ×10 with toluene, followed by exhaustive extraction with $CHCl_3$. The toluene, $CHCl_3$ and H_2O fractions were each taken to dryness.

TLC. For the separation and identification of the alkaloids the following TLC systems were used: S1 $CHCl_3$–cyclohexane–Et_2NH (10:8:3); S2 toluene–EtOAc–Et_2NH (7:2:1); S3 EtOAc–*iso*-PrOH–4 M NH_4OH (9:7:1); S4 $CHCl_3$–MeOH–conc NH_4OH (14:5:1); S5 MeOH–H_2O–conc NH_4OH (8:1:1). All solvent systems were used in combination with ready made plates (Si gel Si60 F254, Merck) in saturated chromatography chambers. Detection of alkaloids was made with UV light (254 and 366 nm), iodoplatinate reagent or 0.2 M $FeCl_3$ in 30% perchloric acid and heat.

Column chromatography. The toluene fraction was first separated on a 50 cm × 2.5 cm glass column filled with Sephadex LH20, using toluene–EtOH (1 + 1) as eluant. The fractions obtained were further separated by means of adsorption chromatography on Si gel 60 ready made columns from Merck (size C) using toluene satd with conc. NH_4OH–EtOH (4:1). Aromoline and krukovine were not separated very well by this method. These alkaloids were therefore eventually separated with EtOAc–*iso*-PrOH–4 M NH_4OH (9:7:1) with a ready-made Si gel Si60 (size B, Merck) column. The aq. fraction, containing

the quaternary alkaloids was separated on a ready-made Si gel Si60 (size C, Merck) using MeOH–H_2O–NH_4OH (8:1:1).

Characterization of the alkaloids. Obaberine (**1c**). UV max 280 nm (MeOH). MS (300°) *m/e* 623 (14), 622 (40) (M^+), 621 (15), 515 (3), 397 (8), 396 (29), 381 (13), 199 (5), 198.5 (29), 198 (100), 175 (28) and 174 (22). $[\alpha]_D^{20} = +60°$ ($c = 0.02$). ^{1}H NMR (FT-mode) δ 2.6 (*s*, 2′-*N*-methyl), 2.69 (*s*, 2-*N*-methyl), 3.19 (*s*, 7′-*O*-methyl), 3.63 (*s*, 6-*O*-methyl), 3.79 (*s*, 6′-*O*-methyl), 3.89 (*s*, 12-*O*-methyl) and 5.46–7.43 ppm (aromatic protons). *Limacine* (**2a**). Mp 173–174° (decomp.). UV max 284 nm (MeOH). MS (135°) *m/e* 609 (22), 608 (44) (M^+), 607 (24), 471 (1), 382 (23), 381 (70), 367 (26), 192 (60), 191.5 (20), 191 (100), 174 (13) and 168 (14). ^{1}H NMR δ 2.32 (*s*, 2-*N*-methyl), 2.61 (*s*, 2′-*N*-methyl), 3.33 (*s*, 6′-*O*-methyl), 3.76 (*s*, 6-*O*-methyl), 3.92 (*s*, 12-*O*-methyl) and 6.05–7.40 ppm aromatic protons. $[\alpha]_D^{20} = -190°$ ($c = 0.28$). *Homoaromaline* (**1a**). Mp 230° (decomp.). UV max 284 nm. MS (135°) *m/e* 609 (14), 608 (28) (M^+), 501 (4), 382 (18), 381 (63), 367 (28), 192 (18), 191.5 (20), 191 (100), 175 (18) and 168 (15). ^{1}H NMR δ 2.41 (*s*, 2-*N*-methyl), 2.55 (*s*, 2′-*N*-methyl), 3.61 (*s*, 6-*N*-methyl), 3.76 (*s*, 6′-*O*-methyl), 3.89 (*s*, 12-*O*-methyl) and 5.54–7.40 ppm aromatic protons. $[\alpha]_D^{20} = +324°$ ($c = 0.24$). *Aromaline* (**1b**). UV max 283 nm. MS (135°) *m/e* 595 (13), 594 (26) (M^+), 593 (17), 487 (2), 382 (23), 381 (51), 368 (11), 367 (30), 192 (29), 191.5 (19), 191 (100), 190 (16), 176 (11), 175 (17), 174 (29) and 168 (23). ^{1}H NMR (FT-mode) δ 2.55 (*s*, 2′-*N*-methyl), 2.62 (*s*, 2-*N*-methyl), 3.60 (*s*, 6-*O*-methyl), 3.82 (*s*, 6′-*O*-methyl) and 5.59–7.40 ppm (aromatic protons). $[\alpha]_D^{20} = +229°$ ($c = 0.22$). *Dahpnoline* (**1d**). UV max 285 nm. MS (350°) *m/e* 581 (38), 580.2531 (79) (M^+) ($C_{35}H_{36}N_2O_6$ = 580.2573), 579 (56), 367 (31), 353 (13) and 184 (100). ^{1}H NMR (FT-mode) δ 2.65 (*s*, 2-*N*-methyl), 3.63 (*s*, 6-*O*-methyl), 3.83 (6′-*O*-methyl) and 5.59–7.56 ppm, aromatic protons. $[\alpha]_D^{20} = +217°$ ($c = 0.04$).

N-methylation of daphnoline. Daphnoline (10 mg) was dissolved in 0.6 ml MeOH and cooled to 5°. 35% aq. HCHO soln (0.2 ml) was added and the mixture stirred for 30 min at 5°. To the reaction mixture 50 mg $NaBH_4$ was added in small portions. The reaction mixture was then taken to dryness under N_2 and the residue extracted with Et_2O and dried (Na_2SO_4). The Et_2O was evapd yielding a reaction product which has spectral data (UV, MS, ^{1}H NMR) and chromatographic properties similar to aromoline.

Krukovine (**2b**). Mp 173–178° (decomp). UV max 285 nm. MS (350°) *m/e* 595 (15), 594.2704 (36) (M^+) ($C_{36}H_{38}N_2O_6$ = 594.2730), 593 (20), 471 (1), 382 (13), 381 (46), 367 (16), 192 (100), 191.5 (22), 190 (32), 175 (46), 174 (24) and 167 (27). ^{1}H NMR (FT-mode) δ 2.31 (*s*, 2-*N*-methyl), 2.61 (*s*, 2′-*N*-methyl), 3.36 (*s*, 6′-*O*-methyl), 3.76 (*s*, 6-*O*-methyl) and 6.01–7.45 ppm aromatic protons. $[\alpha]_D^{20} = -134°$ ($c = 0.17$).

Magnoflorine (**3**). UV max 268, 278 and 324 nm (ammoniacal MeOH) and 269 and 303 nm after acidifying with 4 M HCl. MS (170°) *m/e* 342 (0.4), 341 (1.7), 327 (0.7), 326 (0.3), 59 (36) and 58 (100). ^{1}H NMR (base) shows characteristic signals at 2.75 (*s*, *N*-methyl), 3.23 (*s*, *N*-methyl), 3.76 (*s*, *O*-methyl), 3.82 (*s*, *O*-methyl), 6.46 (*s*), 6.44, 6.52, 6.64 and 6.71 ppm (AB doublets). ^{13}C NMR (11): 24.63 (*t*, C-4), 31.61 (*t*, C-7), 43.53 (*q*, *N*-methyl), 53.87 (*q*, *N*-methyl), 55.99 (*q*, *O*-methyl), 56.33 (*q*, *O*-methyl), 62.19 (*t*, C-5), 70.86 (*d*, C-6a), 109.45 (*d*, C-3*), 110.60 (*d*, C-9*), 116.09 (*s*, C-1a† and C-1b†), 117.22 (*d*, C-8), 121.04 (*s*, C-11a†), 123.22 (*s*, C-3a†), 126.01 (*s*, C-7a†), 149.31 (*s*, C-1‡), 150.16 (*s*, C-11‡), 151.52 (*s*, C-10‡) and 152.80 ppm (*s*, C-2‡). (Carbons marked with *, †, ‡ may be interchanged.) *Pycnarrhine* (**4**). Mp 185–187°. The alkaloid has a yellow colour under basic conditions. UV max 265 and 325 nm (ammoniacal MeOH) and 254, 310 and 355 nm after acidifying with 4 M HCl. MS (150°) *m/e* 194 (7), 193.1095 (60) ($C_{11}H_{15}NO_2$ – 193.1103), 192.1023 (66) ($C_{11}H_{14}NO_2$ = 192.1024), 191 (6), 190 (12), 177 (16), 151 (12), 150.0681 (100) ($C_9H_{10}O_2$ = 150.0681) and 135 (8). ^{1}H NMR (FT-mode) δ 3.70 (*s*, *N*-methyl), 4.01 (*s*, *O*-methyl), 7.08 (*s*, H-5*), 7.17 (*s*, H-8*) and 8.78 ppm (*s*, H-1). ^{13}C NMR (D_2O) (8): 25.29 (*t*, C-4), 47.50 (*N*-methyl), 49.24 (*t*, C-3), 57.18 (*Q*-methyl), 112.08 (*d*, C-5), 117.71 (*s*, C-8a), 119.21 (*d*, C-8), 132.34 (*s*, C-4a), 145.16 (*s*, C-7), 156.56 (*s*, C-6) and 165.27 (*d*, C-1).

The synthesis of 7-hydroxy-6-methoxy-2-methyl-3,4-dihydroisoquinoline (pycnarrhine) was performed according to ref. [10]. 6,7-Dimethoxy-3,4-dihydroisoquinoline (1.84 g) and 10 ml 48% HBr were heated while stirring to 95° for 6 hr. After cooling the reaction mixture was concentrated under vacuum. The residue was dissolved in 85 ml MeOH. To the soln, 3 ml MeI was added. After 16 hr at room temp. the soln was concentrated. The reaction product was purified by means of column chromatography as described for the quaternary alkaloids. The spectral data (UV, MS, ^{1}H NMR, ^{13}C NMR) and the chromatographic behaviour (S5) of the synthesized alkaloid were identical to those of the alkaloid isolated from the plant.

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