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ALKALOIDS OF *UNCARIA CALLOPHYLLA*

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Key Word Index—*Uncaria callophylla*; Rubiaceae; alkaloids; pseudoyohimbine; dihydrocorynantheine; chemotaxonomy.

Abstract—A verified sample of *Uncaria callophylla* was investigated for its alkaloid content. The stems provided mainly pseudoyohimbine and dihydrocorynantheine while the leaves furnished dihydrocorynantheine, gambirine and an unknown dimeric alkaloid probably derived from gambirine and pseudoyohimbine.

INTRODUCTION

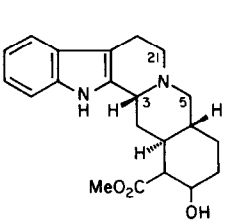
Although the alkaloids of *Uncaria* species have been well studied, there exists in the literature [1–4] considerable discrepancies especially concerning the alkaloids from *Uncaria gambier*, *U. callophylla*, *U. acida* and *U. elliptica*. Most of the problems arise as a result of the difficulties in the identification of this group of taxonomically related species. The recent reclassification by Phillipson *et al.* [1] has helped to resolve some of the problems and the continuing work of Phillipson and co-workers [2–5] has shown that phytochemical characters (e.g. alkaloidal pattern) can be useful for species verification. We have examined the alkaloids of a plant specimen which have been properly verified to be *U. callophylla* and the new results are discussed in relation to previous findings.

RESULTS AND DISCUSSION

The alkaloidal fraction (1.86 g/kg) from the stems of *U. callophylla* after column chromatographic separation gave mainly pseudoyohimbine (**1**) and a smaller amount of dihydrocorynantheine (**2a**), as characterized by their physical and spectroscopic data. From the alkaloidal extract of the leaves (1.78 g/kg) were isolated mainly gambirine (**2b**) and a smaller amount of dihydrocorynantheine (**2a**). A minor amount of an unknown alkaloid was also isolated but it was unstable and was not obtained in sufficient quantities for complete spectral characterization. However, the UV, IR and ^{1}H NMR spectra indicate that the compound was possibly a dimeric alkaloid with gambirine (**2b**) and pseudoyohimbine (**1**) moieties. The presence of gambirine as one of the two moieties of the dimer is suggested by strong bands at ν_{max} 1705 and 1690 cm^{-1} and an olefinic proton singlet at δ7.4, typical of alkaloids possessing the conjugated chromophore MeOOC–C=CHOMe [6, 7]. This is also confirmed by the ^{1}H NMR C_{18}-Me triplet at δ0.87, which is unsymmetrical at 100 MHz [8] but will be more symmetrical at higher frequencies. The aromatic protons of the gambirine fragment show up as two AB doublets, typical of adjacent protons. The broad one-proton singlets at δ4.20 and 4.73 together with the four-proton aromatic multiplet at δ7.0–7.5 probably arise from a pseudoyohimbine unit in the dimer. In fact, the ^{1}H NMR spectrum obtained closely resembles that reported for an unidentified dimeric alkaloid isolated from '*U. gambir*' by Merlini *et al.* [9]. The above data suggest that the dimeric alkaloid is bonded at the 10- or 12-position of gambirine to the 5- or 21-position of pseudoyohimbine.

In view of the fact that the alkaloids obtained by Merlini *et al.* from the leaves of '*U. gambir*' are similar to what we have obtained, it may be concluded that the alkaloids are actually from *U. callophylla*; this is not surprising since these and closely related species of *Uncaria* can only be differentiated with difficulty [1, 3]. The previous investigation [2] of the alkaloidal pattern of *U. callophylla* from different geographical regions of the Far East also indicates that gambirine was the common indole alkaloid and it is likely that this species of *Uncaria* provides the only source of the alkaloid, the name of which has remained a misnomer. Furthermore, *U. callophylla* provides a substantial amount of rutin whereas *U. gambir* gives catechin. It may be noted that the taxonomically related *U. elliptica* is also reported to have a unique alkaloidal content as it is the only known species of the genus to elaborate roxburghines [2, 10].

It is interesting to note that the alkaloidal content of the stems and leaves of *U. callophylla* are different, the stems having major amounts of pseudoyohimbine, which is not found in the leaves. Gambirine was found in the leaves and flowers but not in the stem but dihydrocorynantheine was found in both the leaf and stem. Phillipson *et al.* [11] had previously found that the bark alkaloids in *Mitragyna parvifolia* were different from those of the leaf and they



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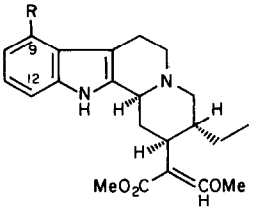

2a R=H
2b R=OH

suggested that alkaloid biogenesis takes place in the roots and further biochemical changes can occur in the leaf. In the case of *U. callophylla*, it may be suggested that hydroxylation of dihydrocorynantheine occurs in the leaf to give 9-hydroxydihydrocorynantheine or gambirine and further oxidative coupling of this with pseudoyohimbine could result in the dimeric indole alkaloid. A study of this alkaloid will be pursued when further samples of this species can be found.

EXPERIMENTAL

Plant material. A sample of *U. callophylla*, Korth. was collected from Puchong, Selangor, Malaysia and was identified by Dr. C. E. Ridsdale of Rijksherbarium, Leiden, The Netherlands and Mr. K. M. Wong of the Forest Research Institute, Kepong, Malaysia.

Analytical methods. Mass spectra were determined on a Kratos MS3074 mass spectrometer at 70 eV and source temp. 100–150°. ^{1}H NMR and ^{13}C NMR spectra were recorded at 100 and 25 MHz respectively, using $CDCl_3$ as solvent and TMS as internal standard. Analytical TLC was carried out using system A: silica gel 60 F_{254} (Merck), $CHCl_3$–EtOH (9:5), and system B: neutral alumina 150 F_{254} (Merck Type T), $CHCl_3$. Alkaloids were visualized by Dragendorff's reagent. Prep. TLC was carried out with 2 mm plates of silica gel 60 F_{254} and CC with silica gel 60 (Mesh 35–75 ASTM).

Extraction and separation of alkaloids. In general, 1 kg of the dried plant material was soaked in EtOH for 48 hr and after removal of the solvent from the extracts, alkaloids were partitioned into dilute HCl (2 M). The crude alkaloids were obtained by $CHCl_3$ extraction after basification with NH_3. Extraction of 1 kg of ground dried stems gave 1.86 g of alkaloids, from which pseudoyohimbine (1.1 g) was crystallized out using $CHCl_3$ as solvent. The mother liquor when chromatographed through silica gel with $CHCl_3$–MeOH (98:2) gave dihydrocorynantheine (0.68 g). Similar extraction of the leaves (1 kg) furnished 1.78 g of crude alkaloids, from which gambirine (0.95 g) was crystallized out using $CHCl_3$ as solvent. Chromatography of the mother liquor on silica gel using $CHCl_3$–MeOH (98:2) gave 0.45 g dihydrocorynantheine and 0.02 g of a dimeric indole alkaloid.

Pseudoyohimbine (**1**). This alkaloid was obtained as colourless needles from $CHCl_3$, mp 268° (lit. [12] 268°); TLC R_f: system A, 0.13; B, 0.1; MS *m/z* (rel. int.): 354 $[M]^+$ (96), 353 $[M-1]^+$ (100), 295 (0.7), 225 (1.3), 197 (0.7), 184 (13), 170 (17), 169 (31), 156 (16); calculated for $C_{21}H_{26}N_2O_3$: 354.206; measured: 354.205; IR ν_{max}^{KBr} cm^{-1}: 3540, 3310, 1730 and 1690 cm^{-1}: UV λ_{max}^{EtOH} nm: 226, 284 and 290. ^{1}H NMR: δ1.35–3.4 (15H, overlapping multiplet), 3.78 (3H, *s*, OMe), 4.25 (1H, *br s*, CHOH), 4.45 (1H, *br s*, C_3-H), 7.2–7.4 (4H, *m*, ArH) and 7.95 (1H, *br s*, NH, exchanged by D_2O); ^{13}C NMR: δ174 (C=O), 135 (C-13), 132 (C-2), 127 (C-8), 121 (C-10), 119 (C-11), 117 (C-9), 111 (C-12), 108 (C-7), 67.0 (C-17), 54.6 (C-3), 52.1 (C-16), 52.0 (C-21), 51.0 (C-5), 50.9 (Me), 40.2 (C-20), 32.5 (C-15), 31.6 (C-14), 31.4 (C-18), 23.0 (C-19), and 16.9 (C-6).

Dihydrocorynantheine (**2a**). This alkaloid was crystallized from Et_2O as colourless rod-shaped crystals, mp 104–105° (lit. [7] 105°); TLC R_f: system A, 0.66; B, 0.64; MS *m/z* (rel. int.): 368 $[M]^+$ (100), 367 $[M-1]^+$ (80), 353 (50), 351 (18), 337 (14), 239 (12), 225 (29), 184 (94), 170 (39), 169 (34), 156 (37) and 59 (6); calculated for $C_{22}H_{28}N_2O_3$: 368.310; measured: 368.305; IR ν_{max}^{KBr} cm^{-1}: 3500, 1700 and 1630; UV λ_{max}^{EtOH} nm: 227, 283 and 291; ^{1}H NMR: δ0.92 (3H, *t*, CH_2Me), 1.35–3.20 (13H, overlapping multiplet), 3.68 (3H, *s*, CO_2Me), 3.73 (3H, *s*, OMe), 7.0–7.5 (4H, *m*, ArH), 7.36 (1H, *s*, olefinic) and 7.95 (1H, *br s*, exchanged by D_2O); ^{13}C NMR: δ169 (C=O), 160 (C-17), 136 (C-13), 135 (C-2), 128 (C-8), 121 (C-10), 119 (C-11), 118 (C-9), 112 (C-16), 111 (C-12), 108 (C-7), 61.6 (C-21), 61.1 (CHOMe), 60.4 (C-3), 53.3 (C-5), 51.4 (COMe), 39.3 (C-20), 38.9 (C-15), 33.2 (C-14), 24.5 (C-19), 22.0 (C-6) and 11.3 (C-18).

Gambirine (**2b**). This alkaloid was recrystallized from $CHCl_3$ as fine colourless needles, mp 165° (lit. [12] 165°); TLC R_f: system A, 0.16; B, 0.08; MS *m/z* (rel. int.): 384 $[M]^+$ (100), 383 $[M-1]^+$ (43), 369 (30), 241 (13), 200 (70), 186 (33), 185 (23) and 172 (27); calculated for $C_{22}H_{28}N_2O_4$: 384.205: measured: 384.200; IR ν_{max}^{KBr} cm^{-1}: 3200–3350, 1700 and 1630; UV λ_{max}^{EtOH} nm: 228, 250 (sh), 287 and 296; ^{1}H NMR: δ0.88 (3H, *t*, CH_2CH_3), 1.80–3.20 (13H, overlapping multiplet), 3.69 (3H, *s*, OMe), 3.77 (3H, *s*, OMe), 6.40 (1H, *dd*, J = 8.2 and 2.1 Hz), 6.92 (1H, *dd*, J = 6.4 and 8.2 Hz), 6.84 (1H, *dd*, J = 6.4 and 2.1 Hz), 7.36 (1H, *s*, olefinic), 8.21 (1H, *br s*, NH, exchanged by D_2O) and 10.34 (1H, *br s*, OH, exchanged by D_2O); ^{13}C NMR (DMSO-d_6): δ173 (C=O), 160 (C-17), 150 (C-9), 138 (C-13), 133 (C-2), 121 (C-11), 116 (C-8), 111 (C-16), 106 (C-10), 103 (C-12), 102.7 (C-7), 61.6 (C-21), 60.4 (C-3), 60.4 (OMe), 52.7 (C-5), 51.4 (OMe), 43.0 (C-20), 38.2 (C-15), 33.2 (C-14), 24.1 (CH_2Me), 23.9 (C-6), and 11.3 (CH_2Me).

Unknown dimeric indole alkaloid. Small amounts of this alkaloid were isolated as a yellow powder, mp > 330°; TLC R_f: system A, 0.30; B, 0.28; UV λ_{max}^{EtOH} nm: 228, 233, 275, 295 and 300; IR ν_{max}^{KBr} cm^{-1}: 3200–3450, 1705, 1640; ^{1}H NMR: δ0.87 (3H, *t*, CH_2CH_3), 1.0–4.1 (*ca* 29H, overlapping multiplet), 3.71 (3H, *s*, OMe), 3.82 (6H, *s*, 2 × OMe), 4.20 (1H, *br s*), 4.73 (1H, *br s*), 6.75 and 6.85 (2H, AB doublets, J = 8 Hz), 7.0–7.5 (4H, *m*), 7.4 (1H, *s*), 7.65 (1H, *br s*, exchanged by D_2O) and 8.29 (1H, *br s*, exchanged by D_2O).

Trace alkaloids and other compounds. Rhynchophylline, isorhynchophylline, mitraphylline and isomitraphylline were detected by TLC to be in the leaf extracts; TLC R_f: system A, 0.33, 0.57, 0.46 and 0.58, respectively; B, 0.11, 0.38, 0.19 and 0.40, respectively. Substantial amounts of crude rutin (20 g/kg) were obtained from the alcoholic leaf extracts.

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