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IMIDAZOLE ALKALOIDS FROM *CYNOMETRA HANKEI*

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Key Word Index—*Cynometra hankei*; *Cynometra mannei*; Leguminosae; imidazole alkaloids; cynometrine; N_1-demethyl cynometrine; cynodine; N_1-demethyl cynodine.

Abstract—Two new and two known imidazole alkaloids, N_1-demethyl cynometrine, N_1-demethyl cynodine, cynometrine and cynodine, respectively, have been isolated from the stem bark and seed of *Cynometra hankei*. The structures of the new alkaloids were elucidated by chemical evidence and spectral data. No alkaloids could be detected in *C. mannei* stem bark.

INTRODUCTION

Cynometra hankei and *C. mannei* are two of several species of *Cynometra* indigenous to west tropical Africa [1]. Both species occur in the Douala–Edea Forest Reserve of west Cameroon, *C. hankei* as an important and common element of the forest canopy [2] and *C. mannei* as a relatively rare species restricted to poorly drained areas. Neither species has previously been investigated but two other west African species, *C. ananta* and *C. lujae*, have been reported [3–6] to contain a number of imidazole alkaloids of which anantine (**1**), cynometrine (**2**) and isocynometrine (**3**) are typical examples. In this paper we report the results of an examination of the stem bark and seeds of *C. hankei* and the stem bark of *C. mannei*.

RESULTS AND DISCUSSION

Four alkaloids were isolated from a MeOH extract of *C. hankei* stem bark by initial partitioning into $CHCl_3$ under alkaline conditions and subsequent column chromatography of the $CHCl_3$-soluble fraction. Two of the alkaloids were isolated as crystalline compounds in significant amounts and the other two as brown amorphous solids in much smaller quantities.

One of the major alkaloids was identified as cynometrine (**2**) by comparison of spectral data (UV, IR, ^1H NMR, ^{13}C NMR, MS) and mp with that published [3, 4]. The second major alkaloid had UV and IR spectra similar to **2**. Accurate mass measurement of the M^+ indicated an empirical formula $C_{15}H_{17}N_3O_2$, 14 mass units less than **2**. The ^1H NMR spectrum differed from that of **2** only by the absence of a signal for the N_1-methyl substituent (δ 3.46 in **2**) and by a deshielding of δ 0.31 in the resonance position of the H-5 proton. The ^{13}C NMR spectrum also lacked the N_1-methyl resonance at *ca* δ 33 (cf. **2**). These data suggest that this alkaloid was the N_1-demethyl derivative of **2**, which has not previously been reported. This conclusion was supported by the mass spectrum which showed the major ion for the imidazole system at m/z 94 (**4**) rather than at m/z 108 (**5**). The identity of the new alkaloid as N_1-demethyl cynometrine (**6**) was confirmed by careful methylation with an equimolar amount of methyl iodide which yielded a product identical to **2** in all respects.

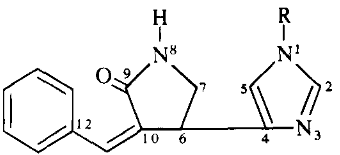
The first of the minor alkaloids eluted gave an empirical formula of $C_{23}H_{23}N_3O_3$. The UV spectrum had bands at longer wavelength indicating an additional chromophore and the IR showed loss of the OH substituent but the presence of a second carbonyl. The ^1H NMR spectrum gave signals for two N-methyl groups, identical to **2**. Significant differences in the ^1H NMR spectrum occurred in the aromatic region where five additional protons were observed, three between δ 7.25 and 7.60 and two further downfield between δ 7.80 and 8.0. The H-11 signal was deshielded by δ 1.5. The mass spectrum exhibited a fragmentation pattern similar to that of **2** but with an additional ion at m/z 105 (C_7H_5O) indicative of a benzoic acid moiety. These data all suggest that this alkaloid was cynodine (**7**), which has been reported to occur in *C. ananta* but for which few data have been published [3, 4]. This was confirmed by alkaline hydrolysis which gave a product with TLC characteristics identical to **2**.

The final alkaloid gave an empirical formula $C_{22}H_{21}N_3O_3$, 14 mass units less than **7**. Spectral data obtained for this alkaloid showed it to differ from **7** only by the absence of the N_1-methyl substituent (^1H NMR and MS) permitting its characterization as N_1-demethyl cynodine (**8**). Careful methylation gave a product with identical TLC characteristics to **7**.

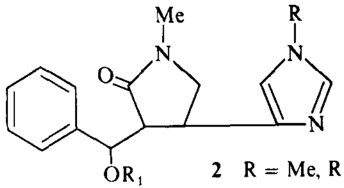
Analysis of the seeds of *C. hankei* revealed the presence of only trace amounts of alkaloids. These were separated by column chromatography and identified as **2**, **6** and **7** by comparison with material isolated from the stem bark. Whilst alkaloid concentrations were much lower in the seeds, extraction of the alkaloids was simpler, not requiring the alkaline partitioning necessary with stem bark extracts. The necessity for using relatively harsh conditions to extract the bark alkaloids is probably related to the extremely high concentrations of tannins in the bark (*ca* 20%; G. M. Choo, personal communication).

By contrast, an examination of the stem bark of *C. mannei* failed to reveal the presence of alkaloids.

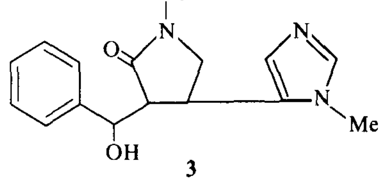
Imidazole alkaloids of the type reported here, in which the third nitrogen of the histidine precursor is retained



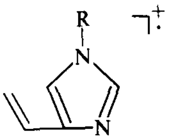
1 R = Me
9 R = H



2 R = Me, R_1 = H
6 R = R_1 = H
7 R = Me, R_1 = COC_6H_5
8 R = H, R_1 = COC_6H_5



3



4 R = H, *m/z* 94
5 R = Me, *m/z* 108

and incorporated into a lactam ring (cf. pilocarpine [7]), are at present known only from *Cynometra* in the Leguminosae. Their distribution within the genus may be of chemotaxonomic value. Of the three species so far reported to contain them only *C. lujae* [5, 6] has yielded alkaloids of the *iso*-series, such as **3**. The isolation of **6** and **8**, reported here, are the second record of N_1-demethyl alkaloids. Nor-anantine (N_1-demethyl anantine **9**) occurs in *C. lujae* [5].

EXPERIMENTAL

Mps are uncorr. UV spectra were run in EtOH, IR spectra as KCl discs. ^{1}H NMR spectra were obtained at 90 MHz in $CDCl_3$ with TMS as int. standard. ^{13}C NMR spectra were obtained at 25.1 MHz in $CDCl_3$ using the FT mode. EIMS were recorded at 70 eV (probe).

Plant material. Stem bark and seeds of *Cynometra hankei* Harms. were collected in the Douala–Edea Forest Reserve, west Cameroon, in the spring of 1979. Stem bark of *C. mannei* Oliv. was collected in the same area in the spring of 1980. Vouchers have been deposited at the Herbarium, Royal Botanic Gardens, Kew (P. G. Waterman and D. McKey 852 for *C. hankei*, D. McKey and J. S. Gartlan 206 for *C. mannei*).

Isolation of alkaloids from C. hankei *stem bark.* Ground stem bark (340 g) was extrd separately and successively with petrol (bp 40–60°), $CHCl_3$ and MeOH. An aliquot (50%) of the MeOH extract was concd and NH_4OH added. Liquid–liquid extraction of the alkaline soln with $CHCl_3$ gave a $CHCl_3$-soluble fraction containing four alkaloids. CC of this fraction over Al_2O_3 gave, on elution with $CHCl_3$, **7** (15 mg) as a brown gum. Further elution with $CHCl_3$–MeOH (49:1) gave **2** (117 mg) followed by **6** (185 mg). Between the bands of **2** and **6** a mixture of **2** and a further alkaloid were eluted. Prep. TLC of the mixture on Al_2O_3 ($CHCl_3$–MeOH, 49:1) gave **8** (R_f 0.48) as a brown amorphous solid (20 mg).

Cynometrine (**2**). Needles from $CHCl_3$, mp 211° (lit. [4], mp 213°), $[\alpha]_D^{20}$ −27.1° (*c* 0.1, $CHCl_3$). Found: M^+ 285.1477; $C_{16}H_{19}N_3O_2$ requires 285.1477. UV λ_{max} nm: 252, 258, 264. IR ν_{max} cm^{-1}: 3150, 3110, 1690. ^{1}H NMR: δ 2.87 (3 H, *s*, N_8-Me), 3.00–3.50 (4 H, *m*, H-6, H-7, H-10), 3.46 (3 H, *s*, N_1-Me), 5.08 (1 H, *d*, *J* = 7 Hz, H-11), 5.96 (1 H, *d*, *J* = 1 Hz, H-5), 7.15–7.42 (6 H, *m*, H-2, H-13–H-17). ^{13}C NMR: δ 29.58 (*q*, N_8-Me), 33.10 (*q*, N_1-Me), 33.86 (*d*, C-6), 52.87 (*t*, C-7), 54.28 (*d*, C-10), 74.98 (*d*, C-11), 116.48 (*d*, C-5), 127.47–127.96 (3 × *d*, C-13–C-17), 137.01 (*d*, C-2), 141.10 (*s*, C-12), 141.72 (*s*, C-4), 174.93 (*s*, C-9). EIMS *m/z* (rel. int.): 285 $[M]^+$ (63), 267 $[M - H_2O]^+$ (9), 209 (27), 179 (69), 151 (37), 136 (100), 131 (39), 122 (53), 108 (73).

N_1-*Demethyl cynometrine* (**6**). Needles from $CHCl_3$, mp 204° $[\alpha]_D^{20}$ −56.2° (*c* 0.1, $CHCl_3$). Found: M^+ 271.1335; $C_{15}H_{17}N_3O_2$ requires 271.1321. UV λ_{max} nm: 252, 258, 264. IR ν_{max} cm^{-1}: 3220, 3100, 1670, 1500. ^{1}H NMR: δ 2.88 (3 H, *s*, N_8-Me), 3.00–3.50 (4 H, *m*, H-6, H-7, H-10), 5.12 (1 H, *d*, *J* = 6 Hz, H-11), 6.27 (1 H, *d*, *J* = 1 Hz, H-5), 7.13–7.40 (5 H, *m*, H-13–H-17), 7.44 (1 H, *d*, *J* = 1 Hz, H-2). ^{13}C NMR: δ 29.79 (*q*, N_8-Me), 33.55 (*d*, C-6), 55.15 (*t*, C-7), 56.61 (*d*, C-10), 75.11 (*d*, C-11), 116.24 (*d*, C-5), 128.20, 129.04, 129.35 (3 × *d*, C-13–C-17), 136.75 (*d*, C-2), 142.64 (*s*, C-4 and C-12), 176.55 (*s*, C-9). EIMS *m/z* (rel. int.): 271 $[M]^+$ (65), 253 $[M - H_2O]^+$ (21), 195 (12), 178 (45), 165 (100), 137 (23), 131 (34), 122 (97), 94 (80).

Methylation of **6**. **6** (40 mg) was refluxed for 24 hr in dry Me_2CO (40 ml) with MeI (20 mg) and fused Na_2CO_3 (0.5 g). The reaction mixture was filtered and on concn and recrystallization from $CHCl_3$ gave **2** (15 mg), identical in all respects (TLC, mp, UV, IR, ^{1}H NMR, EIMS) with **2** isolated from this species.

Cynodine (**7**). Amorphous brown gum with no mp. Found: M^+ 389.1716; $C_{23}H_{23}N_3O_3$ requires 389.1739. UV λ_{max} nm: 260, 273, 281. IR ν_{max} cm^{-1}: 1720, 1680, 1265. ^{1}H NMR: δ 2.77 (3 H, *s*, N_8-Me), 3.00–4.00 (4 H, *m*, H-6, H-7, H-10), 3.56 (3 H, *s*, N_1-Me), 6.58 (1 H, *d*, *J* = 6 Hz, H-11), 6.65 (1 H, *d*, *J* = 1 Hz, H-5), 7.25–7.60 (9 H, *m*, H-2, H-13–H-17, H-3′–H-5′), 7.90 (2 H, *dd*, J_1 = 8 Hz, J_2 = 2 Hz, H-2′, H-6′). EIMS *m/z* (rel. int.): 389 $[M]^+$ (41), 284 $[M - C_6H_5CO]^+$ (30), 268 (56), 267 (46), 210 (54), 209 (34), 179 (40), 150 (39), 137 (100), 136 (70), 135 (77), 133 (68), 131 (30), 122 (59), 108 (31), 105 (79).

Saponification of **7**. **7** (5 mg) was refluxed with 20% alcoholic KOH for 1 hr. Normal work up of the reaction mixture gave a single product identical on TLC (three systems) with **2**.

N_1-*Demethyl cynodine* (**8**). Amorphous brown gum with no mp. Found: M^+ 375.1590; $C_{22}H_{21}N_3O_3$ requires 375.1583. UV λ_{max} nm: 258, 264, 274, 281. IR ν_{max} cm^{-1}: 3250, 3150, 1730, 1690, 1275. ^{1}H NMR: δ 2.76 (3 H, *s*, N_8-Me), 3.00–3.80 (4 H, *m*, H-6, H-7, H-10), 6.63 (1 H, *d*, *J* = 5 Hz, H-11), 6.87 (1 H, *d*, *J* = 1 Hz, H-5), 7.20–7.50 (8 H, *m*, H-13–H17, H-3′–H-5′), 7.55 (1 H, *d*, *J* = 1 Hz, H-2), 7.89 (2 H, *dd*, J_1 = 8 Hz, J_2 = 2 Hz, H-2′, H-6′). EIMS *m/z* (rel. int.): 375 $[M]^+$ (15), 270 $[M - C_6H_5CO]^+$ (24), 253 (73), 195 (34), 165 (68), 164 (63), 137 (20), 131 (42), 123 (91), 122 (73), 105 (100), 94 (50).

Methylation of **8** (15 mg) using the method described previously gave a product identical to **7** by TLC (three systems).

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