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TWO NEW BIOACTIVE CARBAZOLE ALKALOIDS FROM THE ROOT BARK OF *CLAUSENA HARMANDIANA*

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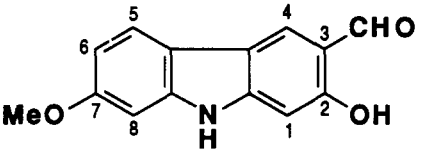
Previous chemical work on the root bark of *Clausena harmandiana* Pierre (Rutaceae), a species reputedly used in folk medicine for stomach ache and fever, revealed the presence of five coumarins and one carbazole (heptaphylline) (1). Further investigation of this plant resulted in the isolation of two novel carbazole alkaloids, 2-hydroxy-3-formyl-7-methoxycarbazole [**1**] and 7-methoxyheptaphylline [**2**]. The two isomers, lansine (2-hydroxy-3-formyl-6-methoxycarbazole) and 6-methoxyheptaphylline, have been previously reported from *Clausena lansium* (Lour.) Skeels (2) and *Clausena indica* Oliv. (3), respectively, and subsequently synthesized (4). The 8-methoxy isomers have also been synthesized (5,6). In this paper we describe the structural elucidation and bioactivity of these two new 7-methoxycarbazole alkaloids.



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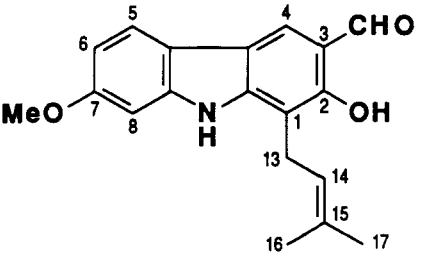

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Our previous study of this species (1) described the isolation of components from the hexane extract; this study focuses on two compounds isolated from the $CHCl_3$ extract. Examination of the nmr, ir, and uv spectral data of **1** and **2** showed the presence of CHO, OH, NH, OMe, and aromatic protons. In addition, the ^{1}H-nmr spectrum of **2** showed the presence of two methyl singlets (1.63 and 1.79 ppm), a doublet for two benzylic methylene protons (3.53 ppm, $J = 6.3$ Hz), and a vinylic triplet for one proton (5.29 ppm) indicative of a γ,γ-dimethyl allyl residue. The mass and ^{1}H-nmr spectra of **1** and **2** indicated the same molecular weight and functional groups as lansine and 6-methoxyheptaphylline, respectively.

To confirm the position of the functional groups, ^{13}C- and ^{1}H-nmr spectra were analyzed. Examination of the ^{1}H-nmr coupling pattern of the A ring protons of compound **1** showed two doublets, one with ortho (7.92 ppm, $J =$ 8.8 Hz) and one with meta coupling (6.93 ppm, $J = 2.2$ Hz), and a doublet of doublets with ortho and meta coupling (6.78 ppm), thus indicating that a functional group must be positioned at C-6 or C-7. Two aromatic singlets suggested para positioning of protons in the C ring with functional groups at C-2 and C-3. The downfield chemical shift of the hydroxyl proton indicated that it is chelated to CHO and so the OH and CHO must be at C-2 and C-3. The ^{13}C-

nmr spectrum (Table 1) showed two doublets centered at 96.3 and 95.4 ppm suggesting that the two carbons are flanked by heteroatoms. The methoxy group (55.32 ppm) must be at C-7 and the OH group at C-2 (and thus the CHO at C-3) in order to shield C-1 and C-8 so far upfield. The doublet at 95.4 showed fine 3-bond coupling to the carbon at 108.3 ppm so the signals at 95.4 ppm could be assigned to C-8, 108.3 to C-6, and 96.3 to C-1. Selective decoupling of the proton doublet at 6.93 ppm (d, $J = 2.2$ Hz) with the concomitant collapse of the carbon doublet at 95.3 ppm is further evidence in support of structure **1**. The chemical shifts of the A-ring carbons corresponded to those reported for 7-methoxypyridocarbazole instead of 6-methoxypyridocarbazole (7).

TABLE 1. ^{13}C-nmr (50 MHz) Spectra of Compounds **1** and **2** in DMSO-d_6.

Carbon	Compound	
	1	**2**
1	96.32 d	108.98 s
2	158.49 s	156.33 s
3	115.63 s	114.72 s
4	123.26 d	124.29 d
5	120.59 d	120.52 d
6	108.30 d	108.48 d
7	159.31 s	158.45 s
8	95.40 d	95.57 d
9	142.06 s	142.31 s
10	145.95 s	144.78 s
11[a]	116.39 s	116.98 s
12[a]	117.09 s	116.63 s
13		22.63 t
14		121.58 d
15		131.73 s
16		17.92 q
17		25.44 q
3-CHO	192.70 d	196.00 d
7-OMe	55.32 q	55.25 q

[a]Assignments may be interchanged.

The great similarity of the spectral data of compound **2** confirms its structure. The ^{1}H-nmr spectrum showed four aromatic protons; three of the protons are coupled in the same manner as compound **1**, and the other proton is deshielded by the formyl group and resonates at 8.18 ppm as a singlet. The uv spectrum was indicative of a methoxylated 3-formylcarbazole (8), and so the deshielded proton singlet is positioned on C-4 with the formyl group on C-3. An OH group that is chelated to the CHO group resonated at 11.56 ppm and is positioned on C-2. The γ,γ-dimethyl allyl moiety is positioned at C-1 as shown by the lack of an upfield aromatic singlet that was present in compound **1** and assigned as H-1.

The ^{13}C-nmr spectrum of compound **2** (Table 1) exhibited a doublet at 95.57 ppm with fine 3-bond coupling to a 108.48 ppm doublet. The upfield signal was assigned to C-8 (95.57 ppm) by analogy with compound **1**, and the methoxy group is thus positioned on C-7. The doublet at 108.48 ppm was assigned to C-6 based on the fine 3-bond coupling to C-8. Compound **1** showed a doublet at 96.32 ppm for C-1, but due to the presence of the γ,γ-dimethyl allyl group in compound **2** at C-1, it resonates at 108.96 ppm as a singlet.

By comparison of the ^{1}H- and ^{13}C-nmr spectral data (2, 3, 6, and Table 1) of the previously known methoxy isomers with the two compounds isolated from *C. harmandiana*, we suggest the structures of the new compounds **1** and **2** as additions to the rare group of C-7 oxygenated carbazole alkaloids.

Compounds **1** and **2** were tested for biological activity in several bioassays including the brine shrimp lethality assay (9), and cytotoxicities in murine leukemia (9PS), human nasopharyngeal carcinoma (9KB and KBMRI), human lung carcinoma (A-549), and human colon adenocarcinoma (HT-29). Compound **1** (2-hydroxy-3-formyl-7-methoxycarbazole) was found to be toxic to brine shrimp at an LC_{50} value of 35.1 ppm with a 95% confidence interval of 21.2–51.4 ppm. Compound **2** was found to have an LC_{50} value >500 ppm. Both compounds were inactive on 9PS with

ED_{50} values >10 μg/ml, but slightly active in 9KB, KBMRI, A-549, and HT-29 cell lines, with compound **2** showing stronger activity (Table 2).

same solvent system afforded a bright yellow compound in pooled fractions 20–25 (20 ml each). Recrystallization from Me_2CO yielded pure needles of 2-hydroxy-3-formyl-7-methoxycarbazole [**1**].

TABLE 2. Biological Activity.[a]

Compound	Brine shrimp LC_{50} (ppm)	9KB ED_{50} (μg/ml)	KBMRI ED_{50} (μg/ml)	A-549 ED_{50} (μg/ml)	HT-29 ED_{50} (μg/ml)
1	35 (51/21)[b]	5.70	4.48	2.74	4.00
2	>500	3.01	2.87	2.16	1.36

[a]Both compounds showed 9PS ED_{50} values >10 μg/ml.
[b]95% confidence levels in parentheses.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-nmr spectra were taken in $CDCl_3$ and DMSO-d_6; ^{13}C-nmr spectra were taken in DMSO-d_6; chemical shifts are given in ppm downfield from TMS; coupling constants are given in Hz. The nmr spectra were obtained with a Chemagnetics A-200 operating at 200 MHz for ^{1}H nmr and 50 MHz for ^{13}C nmr. Mass spectra were run on a JEOL DX 300. Ir and uv spectra were obtained with Perkin-Elmer 283 and Beckman DU-7 spectrophotometers, respectively. Melting points were measured on a Mel-Temp hot stage and are uncorrected. The brine shrimp lethality assay was performed as previously described (9). Cytotoxicity tests were conducted by the Purdue Cell Culture Laboratories following standard protocols.

PLANT MATERIAL.—The root bark was collected in the Kalasinth Province in northeastern Thailand during April 1982. Voucher specimens were identified and deposited at the Botany Section, Botany and Weed Science Division, Department of Agriculture, Ministry of Agriculture and cooperatives, Bangken, Bangkok, Thailand.

EXTRACTION AND ISOLATION.—The dried, powdered root bark of *C. harmandiana* (100 g) was extracted by refluxing first with 500 ml of hexane for 17 h followed by 500 ml of $CHCl_3$ for 17 h. The hexane extract was previously investigated by our group (1). The $CHCl_3$ extract was reduced in vacuo to a gummy residue (5 g) and subjected to separation by chromatography on a 2.5 × 37 cm column containing 60 g of Si gel G 60 (230–400 mesh ASTM). The column was eluted with $CHCl_3$, and 90 fractions (F_1–F_{90}) of 25 ml each were collected. Further chromatography of the combined fractions F_5–F_{10} in C_6H_6 on the Si gel column led to a bright yellow compound from the pooled fractions 15–20 (10 ml each). Recrystallization from Me_2CO yielded pure 7-methoxyheptaphylline [**2**]. Chromatography of the combined fractions F_{12}–F_{14} in the

2-HYDROXY-3-FORMYL-7-METHOXYCARBAZOLE [**1**].—Compound **1** (30.8 mg, 0.03% yield): mp 226–227° (recrystallized from Me_2CO); eims *m/z* (% rel. int.) $[M]^+$ 241 (100%), 240 (11), 226 (71), 198 (28); ir (KBr) 3280, 1640 cm^{-1}; uv (MeOH) λ max (log ε) 224 (4.26), 240 (4.26), 290 sh (4.47), 300 (4.58), and 338 (3.89) nm; ^{1}H nmr (DMSO, 200 MHz) ppm 3.81 (3H, s, OMe), 6.78 (1H, dd, J = 8.8, J = 2.2, H-6), 6.84 (1H, s, H-1), 6.93 (1H, d, J = 2.2, H-8), 7.92 (1H, d, J = 8.8, H-5), 8.30 (1H, s, H-4), 10.11 (1H, s, CHO), 10.9 (1H, s, NH), 11.4 (1H, s, OH); ^{13}C nmr see Table 1.

7-METHOXYHEPTAPHYLLINE [**2**].—Compound **2** (18.9 mg, 0.02% yield): mp 164–166° (recrystallized from Me_2CO); eims *m/z* (% rel. int.) $[M]^+$ 309 (100%), 294 (15), 254 (75), 253 (24); ir (KBr) 3350, 1619 cm^{-1}; uv (MeOH) λ max (log ε) 222 (4.27), 238 (4.30), 244 (4.30), 254 (4.28), 288 sh (4.33), 302 (4.54), 340 (3.87) nm; ^{1}H nmr (DMSO, 200 MHz) ppm 1.63 (3H, s, 16-Me), 1.79 (3H, s, 17-Me), 3.53 (2H, d, J = 6.3, H-13), 3.83 (3H, s, OMe), 5.29 (1H, t, J = 6.3, H-14), 6.81 (1H, dd, J = 8.2, J = 2, H-7), 6.99 (1H, d, J = 2, H-5), 7.89 (1H, d, J = 8.2, H-8), 8.18 (1H, s, H-4), 9.88 (1H, s, CHO), 11.43 (1H, s, NH), 11.56 (1H, s, OH); ^{13}C nmr see Table 1.

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