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Siamenol, a New Carbazole Alkaloid from *Murraya siamensis*[1]

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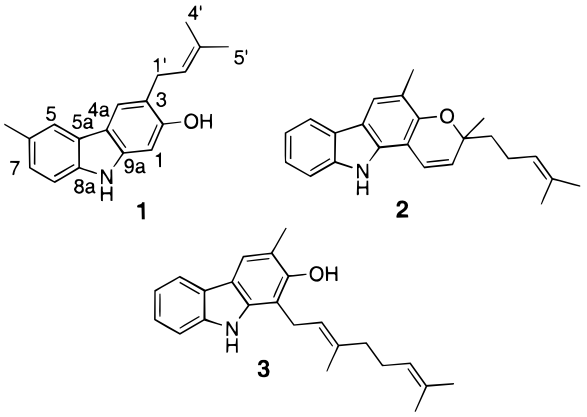
A new carbazole alkaloid, siamenol (**1**), and two known alkaloids, mahanimbine (**2**) and mahanimbilol (**3**), have been isolated from the organic extract of *Murraya siamensis*. The novel compound exhibited HIV-inhibitory activity.

Murraya L. (Rutaceae) is a genus of shrubs or small trees from Southern Asia.[2] The main constituents of this genus include carbazole alkaloids,[3] coumarins,[4,5] and flavonoids.[6,7] Previous chemical studies of *Murraya siamensis* Craib yielded seven carbazole alkaloids and a coumarin.[8] Several biological properties have been reported for carbazole alkaloids including antibiotic,[9] cytotoxic,[10] and antiviral activities,[11] however, not specifically anti-HIV activity.

An organic extract of *M. siamensis* collected in Thailand was active in the XTT-tetrazolium anti-HIV assay.[12] Bioassay-guided fractionation of this extract led to a new carbazole alkaloid, siamenol (**1**), which was isolated along with the known compounds mahanimbine (**2**)[13] and mahanimbilol (**3**).[14]



Compound **1** was a pale yellow solid with a molecular formula of $C_{18}H_{19}NO$, as determined by high-resolution FABMS (*m/z* 265.1472, Δ 0.6 mmu). The UV spectrum (MeOH) λ_{max} 216, 238, 261, 308, 328 (sh) suggested the presence of a carbazole skeleton.[3]

A standard battery of NMR experiments, including COSY, HSQC, and HMBC, led to assignments for all signals in the ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum in CD_3OD contained signals for 17 of the 19 protons, indicated by the HRMS, suggesting two exchangeable protons, one of which was present on the nitrogen of the carbazole system. Five of these signals were attributable to aromatic protons: one ABC system (δ 7.01, dd, J = 8.5, 1.0 Hz; 7.17, d, J = 8.5 Hz; and 7.63, d, J = 1.0 Hz) indicative of a 1,2,4-trisubstituted phenyl ring, and two singlets (δ 6.81 and 7.62) on the other phenyl ring of the carbazole system. The presence of a *meta*-coupled downfield doublet at δ 7.63 assignable to H-5 suggested that this ring had a substituent at C-6 and not at C-7. Also, the presence of two singlets, one of them characteristic for H-4 (δ 7.62),[3] indicated that two additional substituents were present at C-3 and C-2. Finally the ^{1}H NMR spectrum contained signals for an aromatic methyl at δ 2.42 and a prenyl group [δ 3.42 (d, J = 7 Hz, 2H), 5.43 (tq, J = 7, 1.5 Hz, 1H), and 1.75 (brs, 6H)].

The ^{13}C NMR spectrum contained signals for 18 carbons. Twelve signals were accounted for by the carbazole ring, including one at δ 154.1, indicating an oxygenated carbon. Also present were the five carbons of the prenyl side chain (δ 131.1, 124.0, 28.5, 24.9, and 16.7) and a single aromatic methyl group (δ 20.4). Since there was only one oxygenated carbon signal, the signal at δ 154.1 was consistent with the presence of a hydroxyl group and accounted for the second exchangeable proton. The relative upfield shift of the carbon at δ 96.0 indicated shielding by two heteroatoms,[15] suggesting that the hydroxyl is located at C-2. The HMBC correlations (see Table 1) between δ 154.1 and H-1, H-4, and H-1′ confirmed the placement of the hydroxyl group at C-2, while correlations between C-3 (δ 120.5) and H-1′ and H-1 sited the prenyl group at C-3. The aromatic methyl group (δ 2.42, 20.4) was located at C-6 on the basis of HMBC correlations between δ 2.42 ($ArCH_3$) and δ 127.4 (C-6) and δ 124.7 (C-7), as well as correlations from δ 20.4 to H-5 and H-7, to give the gross structure of siamenol (**1**) as 3-hydroxy-6-methyl-2-prenylcarbazole.

Siamenol (**1**) showed anti-HIV activity (EC_{50} = 2.6 μg/mL), reaching 50−60% maximum protection in the XTT-tetrazolium assay. The known alkaloids were also tested: mahanimbilol (**3**) was less active (EC_{50} = 8.6 μg/mL; IC_{50} = 23.0 μg/mL) than siamenol (**1**), and mahanimbine (**2**) was inactive.

The spectral data for mahanimbine (**2**)[13] and mahanimbilol (**3**)[14] were in agreement with those reported in the literature.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Varian Inova Unity 500 MHz spectrometer in MeOH-d_4 or $CDCl_3$ as solvent. The mass spectra were obtained with a JEOL SX102 mass spectrometer. UV spectra were recorded on a Beckman DU 640 spectrophotometer, and IR spectra on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. HPLC separations were performed on a Rainin system using a cyano column (Dynamax, 4.6 mm × 10 cm, hexane−IPA 80:20 or 95:5, flow rate 1.5 mL/min, UV detection at 225 nm).

Plant Material. Aerial parts (flowers, leaves, and twigs) of *M. siamensis* Craib were collected in the Pukae Botanical Garden, Thailand, in March 1987, by D. D. Soejarto under contract to the National Cancer Institute. A voucher specimen

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Table 1. NMR Assignments of Siamenol (**1**)[a]

position	C δ_C mult	H δ_H mult (J in Hz)	HMBC
1	96.0 d	6.81 s	
2	154.1 s		H-1, H-4, H-1′
3	120.5 s		H-1, H-1′
4	119.7 d	7.62 s	H-1′
4a	116.1 s		H-1
5a	123.9 s		H-4
5	118.5 d	7.63 d (1.0)	H-6, H-7
6	127.4 s		H-8, $ArCH_3$
7	124.7 d	7.01 dd (8.5, 1.0)	H-5, H-8, $ArCH_3$
8	109.7 d	7.17 d (8.5)	H-7
8a	138.5 s		H-5, H-7
9a	140.4 s		H-1, H-4
1′	28.5 t	3.42 d (7.0)	H-4
2′	124.0 d	5.43 tq (7.0, 1.5)	H-1′, H-4′, H-5′
3′	131.1 s		H-1′, H-4′, H-5′
4′	24.9 q	1.75 s	H-5′
5′	16.7 q	1.75 s	H-4′
$ArCH_3$	20.4 q	2.42 s	H-5, H-7

[a] Spectra recorded in MeOH-d_4.

(Q660-5834) has been deposited at the Field Museum, Chicago, IL. The taxonomy was determined by J. S. Burley.

Isolation of Compounds. A 1.47 g portion of organic extract of *M. siamensis* was subjected to the following solvent−solvent partitioning scheme. The extract was dissolved in 100 mL of 90% MeOH and partitioned with hexane (3 × 100 mL). The MeOH concentration was adjusted with H_2O to 60% and partitioned with MeOtBu−hexane (9:1; 3 × 100 mL). The MeOH was removed under reduced pressure, 100 mL of H_2O was added, and the mixture was partitioned with EtOAc (3 × 100 mL). The aqueous fraction was lyophilized, and the solvent from the other three fractions was removed under reduced pressure.

The anti-HIV activity was concentrated in hexane and MeOtBu fractions. Both fractions were subjected to gel permeation on Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1), and similar, active fractions from both columns were combined and were purified by vacuum-liquid chromatography[16,17] (C18, 60 Å, EM Science) using a step gradient of MeOH−H_2O [1:1 MeOH−H_2O (100 mL); 7:3 MeOH−H_2O (100 mL); 9:1 MeOH−H_2O (100 mL); MeOH (100 mL); and 9:1 MeOH−CH_2Cl_2 (100 mL) plus CH_2Cl_2 (150 mL)] to give five active fractions (A−E). Three of them (A−C) (85.7 mg, 6% extract) contained mainly **2**. A 5.0 mg aliquot of fraction D was purified by HPLC (Dynamax CN, 10 mm, 4.6 × 100 mm, 8:2 hexane−IPA) to give **2** (2.1 mg) and **3** (0.5 mg, 0.43% extract). HPLC of fraction E using the same column but 95:5 hexane−IPA afforded **1** (6.3 mg, 0.43% extract).

Siamenol (1): pale yellow solid; UV (MeOH) λ_{max} (log ϵ) 216 (4.03), 238 (4.11), 261 (3.73), 308 (3.71), 328 (sh) (3.40) nm; IR (NaCl) ν 3410, 3364, 2916, 2855, 1639, 1560, 1467, 1291, 1203, 1031, 798 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.73 (1H, s, H-5), 7.70 (1H, s, H-4), 7.68 (1H, s, NH), 7.18 (1H, brd, J = 8.0 Hz, H-7), 7.12 (1H, d, J = 8.0 Hz, H-8), 6.74 (1H, s, H-1), 5.39 (1H, t, J = 7 Hz, H-2′), 3.52 (2H, d, J = 7 Hz, H-1′), 2.49 (3H, s, $ArCH_3$), 1.82 (3H, s, CH_3), 1.79 (3H, s, CH_3); ^1H and ^{13}C NMR in CD_3-OD, see Table 1; HRFAB (glycerol) m/z 265.1472, calcd for $C_{18}H_{19}NO$, 265.1466; FAB (glycerol) m/z 265 $[M]^+$ (52), 264 $[M + H - H_2]^+$ (98), 210 $[M - C_4H_7]^+$ (74), 185 (100); EIMS m/z 265 $[M]^+$ (46), 210 (100).

Mahanimbine (2): ^{13}C NMR (CD_3OD) 150.2 (s, C-2), 139.8 (s, C-8a), 135.2 (s, C-9a), 131.9 (s, C-7′), 128.7 (d, C-2′), 124.51 (d, C-7)*, 124.48 (d, C-6′)*, 124.2 (s, C-5a), 121.5 (d, C-4), 119.7 (d, C-6), 119.5 (d, C-5), 118.7 (s, C-3), 117.8 (d, C-1′), 116.9 (s, C-4a), 110.7 (d, C-8), 104.5 (s, C-1), 78.4 (s, C-3′), 41.1 (t, C-4′), 26.1 (q, C-10′), 26.0 (q, C-8′), 23.0 (t, C-5′), 17.9 (q, C-9′), 16.4 (q, $ArCH_3$) (* may be interchanged).

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