Two New Carbazole Alkaloids from *Murraya koenigii*

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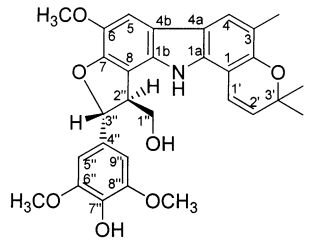
Two new carbazole alkaloids named murrayanine (**1**) and 8,8″-biskoenigine (**2**) were isolated from *Murraya koenigii*. The structure elucidations for **1** and **2** were carried out on the basis of 1D and 2D NMR experiments. Compound **1** was a novel carbazole alkaloid with a rare phenylpropanyl substitution. Compound **2** was a symmetrical dimer of the carbazole alkaloid koenigine and showed antiosteoporotic activity in the CAT-B model with IC_{50} 1.3 μg/mL. The synthesis of **2** from koenigine was carried out through oxidative coupling using a solid state reaction.

Plants of the genus *Murraya* (Rutaceae) are shrubs mainly distributed in Southern Asia.[1] The leaves and bark are used as folk medicines for analgesia and local anesthesia and for the treatment of eczema, rheumatism, and dropsy.[2] The ethanol extract of *M. koenigii* displayed cytotoxic activity against cultured KB cells.[3] In our investigations of the active principles of *M. koenigii*, two novel carbazole alkaloids named murrayanine (**1**) and 8,8″-biskoenigine (**2**) were isolated.



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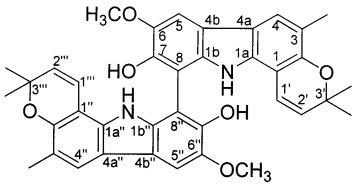

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Compounds **1** and **2** give negative reactions with Dragendorff's alkaloid reagent, but produced blue-violet spots with concentrated sulfuric acid on silica gel TLC, which is a characteristic of carbazole alkaloids.[4,5]

Compound **1** was obtained as a brown powder. The HREIMS gave the $[M]^+$ peak at *m*/*z* 517.2142, corresponding to the molecular formula $C_{30}H_{31}NO_7$. The IR spectrum showed absorption at ν_{max} 3440 and 3410 cm^{-1}, indicating the presence of −OH and −NH groups,[6] which was confirmed by the presence of two broad singlets at δ 4.75 and 9.73 in the ^1H NMR spectrum.[7] Inspection of the NMR data (^1H, ^{13}C, DEPT, HMQC, and HMBC) indicated the presence of a koenigine moiety[1,4,8−13] and an 11-carbon residue. The latter was confirmed by the EIMS fragment ion at *m*/*z* 210 (M − 307) and the NMR data. The $^1H-^1H$ COSY spectrum indicated the presence of the fragment $-OCH_2-CHR_1-CHR_2-O-$, which was supported by HMBC correlations between H-3″ and C-2″, C-1″ (Figure 1). The appearance of an isolated two-spin system at δ_H 6.81 (2H, d, J = 1.9 Hz) indicated a symmetrically substituted aromatic residue.[14−16] Methoxyl substitutions at C-6″ and C-8″ were revealed by analysis of NMR data, while the C-7″ hydroxyl was determined with the aid of EIMS fragmentation and NMR data.[17−19]

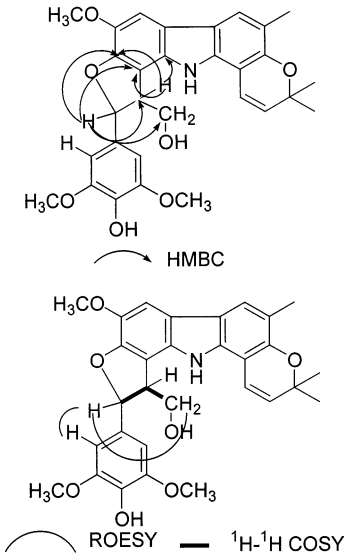

Figure 1. Selected $^1H-^1H$ COSY, HMBC, and ROESY correlations for compound **1**.

The obvious difference between the NMR data of **1** and koenigine[1,4,8−13] was at C-8; the methine (δ_C 97.9, d, δ_H 5.72, 1H, s) in koenigine was replaced by a quaternary carbon (δ_C 110.9s), indicating that **1** had carbon substitution at C-8.[7,9,12,13] This correlation was supported by $^1H-^{13}C$ long-range correlations between the proton at δ 3.88 (H-2″) and the carbons at δ 148.3 (C-7), 110.9 (C-8), and 131.8 (C-1b), and between the methylene protons at δ 4.02 (2H, d, J = 6.0 Hz, H-1″) and the carbon at δ 110.9 (C-8). The connection between C-7 and C-3″ via an oxygen was determined by the $^1H-^{13}C$ long-range correlation between δ_H 5.46 (H-3″) and the carbon at δ 148.3 (C-7) (Figure 1).

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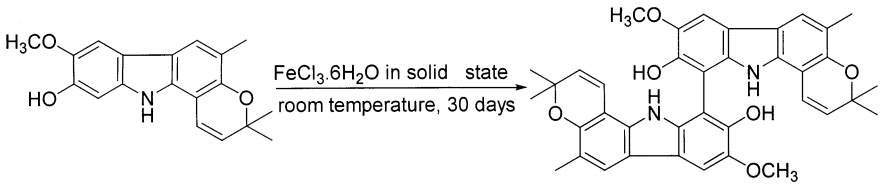

Figure 2. Synthesis of **2**.

The relative configurations of C-2″ and C-3″ were determined to be *trans* on the basis of the coupling constants (J = 7.8 Hz) of H-3″ with H-2″ and the ROESY correlations between the protons at δ 4.02 (H-1″) and 5.46 (H-3″) (Figure 1).[20−24] Thus, the structure of **1**, named murrayanine, was elucidated to be as shown.

Compound **2** was isolated as a brown gum. The HREIMS gave the $[M]^+$ peak at m/z 616.2585, corresponding to the molecular formula $C_{38}H_{36}N_2O_6$. The ^{1}H and ^{13}C NMR signals were half of that expected on the basis of HREIMS data, suggesting that **2** was a completely symmetrical dimer.[9] The EIMS data suggested that koenigine[4] was the monomer of **2**. The UV and ^{1}H and ^{13}C NMR spectra of **2** were similar to those of koenigine,[1,4,8−13] indicating that **2** was a dimer of koenigine.[4,7,9] The NMR signals assigned to the C-8 methine (δ_H 7.43, s, δ_C 97.9, d) in koenigine were replaced by δ 105.0 s in **2**, revealing the linkage between C-8 and C-8″, which was further supported by upfield shifts of 2.0 and 0.2 ppm observed for C-7 and C-8a, respectively. Thus, compound **2**, named 8, 8″-biskoenigine,[25] was elucidated to be as shown.

To confirm the assigned structure, **2** was synthesized from koenigine through an oxidative coupling reaction, with $FeCl_3$, in the solid state (Figure 2).[26−28] This reaction provided a new method for the synthesis of binary carbazole alkaloids for bioactivity research.

Carbazole alkaloids **2** and koenigine were tested using antibacterial (enolpyruvyl transferase, EPT), antifungal (*Candida albicans*, *C. glabrata*, *Aspergillus fumigatus*, YNG), anticancer (cell division cycle 25, CDC25), antithrombus (plasminogen activator inhibitor, PAI), and antiosteoporosis (cathepsin B, CAT-B, caronic anhydrase II, CA-II) bioassays.[29] Compound **2** showed antiosteoporotic activity in the CAT-B model with a IC_{50} of 1.3 μg/mL.

Experimental Section

General Experimental Procedures. IR spectra were measured on a Perkin-Elmer-577 spectrophotometer. MS were performed on an Autospec-3000 spectrometer at 70 eV. The NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers.

Plant Material. The aerial part of *M. koenigii* was collected in Xishuangbanna, Yunnan Province, P. R. China, in April 1999 and identified by Prof. D. D. Tao of Kunming Institute of Botany. A voucher specimen (No. H98041705) is deposited in the Kunming Institute of Botany, Kunming, China.

Extraction and Isolation. The air-dried plant materials (2.5 kg) were extracted with 95% EtOH at ambient temperature for two weeks. The EtOH extract was partitioned between H_2O and $CHCl_3$. An aliquot (100 g) of the $CHCl_3$-soluble portion was subjected to repeated column chromatography over silica gel and eluted with a gradient of petroleum ether−EtOAc from 9:1 to 4:6 (v/v) to afford 10 fractions. Fraction 6 (10.2 g) was further subjected to repeated column chromatography over silica gel (eluted with $CHCl_3$−EtOAc, 9:1, 8:2, 7:3, 6:4, v/v) and Sephadex LH-20 (eluted with MeOH) to afford koenigine (560 mg) and **2** (52 mg). Fraction 8 (1.2 g) was further purified by column chromatography over silica gel eluted with $CHCl_3$−Me_2CO (85:15, v/v) and Sephadex LH-20 eluted with MeOH to yield **1** (7.5 mg).

Compound (1): brown powder; $[\alpha]^{25}_D$ +8.0 (c 0.74, CH_3OH); UV (CH_3OH) λ_{max} 206, 225, 238, 300, 342 nm; IR (KBr) ν_{max} 3440, 3410, 2927, 2853, 1733, 999, 907 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 500 MHz) δ 9.71 (1H, s, NH), 7.59 (1H, s, H-4), 7.47 (1H, s, H-5), 6.81 (1H, d, J = 1.9 Hz, H-9″, 5″), 6.77 (1H, d, J = 9.6 Hz, H-1′), 5.72 (1H, d, J = 9.6 Hz, H-2′), 5.46 (1H, d, J = 7.8 Hz, H-3″), 4.02 (2H, d, J = 6.0 Hz, H-1″), 3.90 (3H, s, OMe-6), 3.88 (1H, dd, J = 7.8, 6.0, H-2″), 3.80 (6H, s, MeO-8″, 6″), 2.26 (3H, s, Me-3), 1.41 (3H, s, Me-3′), 1.40 (3H, s, Me-3′); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 148.7 (s, C-2), 148.3 (s, C-7), 148.2 (s, C-8″, 6″), 140.2 (s, C-6), 136.2 (s, C-7″), 135.5 (s, C-1a), 132.5 (s, C-4″), 131.8 (s, C-1b), 129.2 (d, C-2′), 120.3 (d, C-4), 118.0 (d, C-1′), 117.8 (s, C-4b), 117.5 (s, C-3), 117.2 (s, C-4a), 110.9 (s, C-8), 104.9 (d C-1), 104.2 (d, C-9″, 5″), 103.7 (d, C-5), 87.7 (d, C-3″), 75.7 (s, C-3′), 63.8 (t, C-1″), 56.6 (q, OMe-6), 56.1 (q, OMe-8″, 6″), 54.0 (d, C-2″), 27.3 (q, Me-3′), 27.1 (q, Me-3′), 15.6 (q, Me-3); EIMS m/z (%) 517 (M^+, 100), 502 (10), 499 (15), 484 (25), 418 (30), 396 (17), 368 (10), 210 (10); HREIMS m/z 517.2142 (calcd for $C_{30}H_{31}NO_7$, 517.2100).

Compound (2):[25] brown gum; $[\alpha]^{24}_D$ +139.6 (c 1.00, $CHCl_3$); UV (CH_3OH) λ_{max} 225, 301, 343 nm; IR (KBr) ν_{max} 3387, 2934, 2875, 1671, 1458, 1102, 1076, 999, 907 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 500 MHz) δ 9.56 (1H, s, NH), 7.66 (1H, s, H-4), 7.61 (1H, s, H-5), 6.74 (1H, d, J = 9.8 Hz, H-1′), 5.56 (1H, d, J = 9.8 Hz, H-2′), 4.05 (3H, s, OMe-6), 2.31 (3H, s, Me-3), 1.41 (3H, s, Me-3′), 1.40 (3H, s, Me-3′); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 149.0 (s, C-2), 144.7 (s, C-7), 143.5 (s, C-6), 136.1 (s, C-1a), 135.9 (s, C-1b), 129.2 (d, C-2′), 120.5 (d, C-4), 119.2 (d, C-1′), 118.8 (s, C-4b), 117.5 (s, C-3), 115.4 (s, C-4a), 105.7 (d, C-1), 105.0 (s, C-8), 102.3 (d, C-5), 76.0 (s, C-3′), 57.1 (q, MeO-6), 27.9 (q, Me-3′), 27.2 (q, Me-3′), 16.3 (q, Me-3); EIMS m/z (%) 616 (M^+, 100), 601 (56), 308 (10), 293 (36); HREIMS m/z 616.2585 (calcd for $C_{38}H_{36}N_2O_6$, 616.2573).

Synthesis of Compound 2. A mixture of koenigine (60.9 mg, 0.1 mmol) and $FeCl_3$·$6H_2O$ (reagent I, 270 mg, 1 mmol) was finely pulverized by agate mortar and pestle and placed in a test tube and kept at room temperature for 30 days. The reaction mixture was quenched with diluted HCl (10 mL) and extracted with $CHCl_3$ (7 mL, three times). The $CHCl_3$ extract was subjected to column chromatography over silica gel to produce **2** in 30% yield. The product was identical to natural compound **2** in all respects (Figure 2).

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