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KOENOLINE, A FURTHER CYTOTOXIC CARBAZOLE ALKALOID FROM *MURRAYA KOENIGII**

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Key Word Index—*Murraya koenigii*; *M. siamensis*; Rutaceae; carbazole alkaloids; koenoline; 1-methoxy-3-hydroxymethylcarbazole; murrayanine; cytotoxic activity.

Abstract—Koenoline, a carbazole alkaloid, has been isolated from the root bark of *Murraya koenigii* for the first time as a natural product. Its structure was established as 1-methoxy-3-hydroxymethylcarbazole by analysis of spectroscopic data and was confirmed by partial synthesis from murrayanine isolated from *M. siamensis* roots. Koenoline exhibited cytotoxic activity against the KB cell-culture test system.

INTRODUCTION

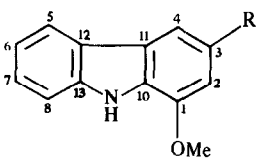
Murraya koenigii is a tree whose leaves are commonly used in India for flavoring foodstuffs [1]. Various plant parts of this species have afforded carbazole alkaloids, embracing the C_{13}-, C_{18}- and C_{23}-types [2, 3]. As part of a continuing search for plant-derived antineoplastic agents, it was found that a chloroform-soluble extract of the root bark of *M. koenigii* displayed significant *in vitro* activity against cultured KB cells, when tested according to standard protocols [4, 5]. While it has been shown previously that the *M. koenigii* constituents girinimbine and mahanimbine exhibit cytotoxic activity toward cultured KB cells [3], we report here the isolation and characterization of koenoline (**1**), a further cytotoxic carbazole alkaloid from *M. koenigii*, which has been obtained for the first time as a natural product.

RESULTS AND DISCUSSION

The chloroform extract of the root bark of *M. koenigii* yielded the carbazole alkaloid **1**, mp 130°, with a molecular formula of $C_{14}H_{13}NO_2$, obtained by high-resolution mass spectrometry. The UV spectrum of this isolate was characteristic of a 1-methoxycarbazole derivative [3], with absorption maxima recorded at 323, 289, 251 and 241 nm. In its IR spectrum, maxima occurred characteristic of NH (3445 cm^{-1}), OH (3235 cm^{-1}) and aromatic (1585 and 1500 cm^{-1}) functionalities. The negative colour reaction obtained after spraying **1** on TLC with ferric chloride [6] suggested that no phenolic groups were present in the molecule.

In the ^1H NMR spectrum of **1**, two sharp singlets resonated at δ4.84 (benzylic methylene) and δ4.01 (OMe), while two broad singlets that occurred at δ8.34 (NH) and δ1.75 (OH) proved to be exchangeable with D_2O. The position of the substituents in **1** in the carbazole ring system could be deduced by analysis of the coupling constants of the aromatic protons. Two signals at δ7.23 (H-6) and δ7.42 (H-7), both exhibiting one *meta*- and two *ortho*-couplings, suggested that one aromatic ring was unsubstituted. However, the resonances at δ7.46 (H-8) and δ8.04 (H-5) showed only *ortho*-coupling, instead of both *ortho*- and *meta*-coupling. The two broad singlets at δ7.66 and δ6.95 were considered to represent protons affixed to the 1-methoxy-substituted carbazole aromatic ring and were therefore assigned to H-4 and H-2, respectively. Decoupling experiments confirmed these assignments.

Compound **1** was therefore suspected as being a carbazole alkaloid with a methoxy group at C-1 and a benzylic alcohol function at C-3. Confirmation of this structure was provided by the ^{13}C NMR spectrum of **1**, obtained in $CDCl_3$ at 90.8 MHz (Table 1), which showed in addition to the signals at δ66.2 (CH_2OH) and δ55.3 (OMe), twelve signals between δ105.5 and δ145.5. Using the Attached Proton Test (APT) experiment [7], six quaternary carbons (C-1, C-3, C-10, C-11, C-12, C-13) could be easily differentiated from the unsubstituted carbons (C-2, C-4, C-5, C-6, C-7, C-8) of the carbazole nucleus. The ^{13}C NMR data of **1** were assigned with reference to previous studies by Gribble *et al.* [8] and Ahond *et al.* [9], and, where necessary, shift calculations were performed using increment values [10] (Table 1).



1 R = CH_2OH
2 R = CH_2OAc
3 R = CHO

*Part 40 in the series "Plant Anticancer Agents". For Part 39 see Wong, S.-M., Oshima, Y., Pezzuto, J. M., Fong, H. H. S. and Farnsworth, N. R., *J. Pharm. Sci.* (submitted).

Table 1. ^{13}C NMR spectral data of carbazole (1)* and koenoline (2)

Carbon	1	2
1	111.0	145.5
2	125.6	105.5
3	118.6	132.6
4	120.1	111.5†
5	120.1	120.3
6	118.6	119.2
7	125.6	125.5
8	111.0	110.9†
10	139.9	129.2
11	122.6	123.8‡
12	122.6	123.3‡
13	139.9	139.3
OMe	—	55.3
CH_2OH	—	66.2

*Data taken from ref. [9].
†‡These values may be interchanged.

On acetylation, 1 furnished a monoacetate (2), mp 110°, which showed in its ^{1}H NMR spectrum an additional singlet at δ2.13 (OAc) and a 0.43 ppm downfield shift of the benzylic methylene group signal compared to 1. The structure of 1 was confirmed by sodium borohydride reduction of murrayanine (3), obtained from both *M. koenigii* root bark, and in greater quantity, from *M. siamensis* roots. This reaction afforded a crystalline product that was identical to 1 in all respects (mp, UV, IR, ^{1}H NMR, EI mass spectrum, TLC, HPLC). Compound 1 was therefore identified as 1-methoxy-3-hydroxymethylcarbazole, and has been accorded the trivial name koenoline to reflect, respectively, its species of origin, its alcoholic substituent and its alkaloidal nature.

Koenoline (1) has been obtained previously by synthesis [11], but it has not been isolated before as a natural product. One reason for this may be the labile nature of this compound, as evidenced by our observation of the partial conversion of 1 to 3 on standing at room temperature. However, the present isolation of 1 from a plant in the same genus that is known to accumulate 1-methoxy-3-methylcarbazole, murrayanine and mukoeic acid, gives strong support to the concept of *in vivo* oxidation of carbazole alkaloids [2, 3, 12].

Murraya koenigii was originally selected for study because of the prospect of isolating similar dimeric carbazole alkaloids to those recently obtained from *M. euchrestifolia* [13, 14], which, by analogy to the dimeric indole alkaloids of *Catharanthus roseus* [15], might be expected to be more active as antineoplastic agents than carbazole alkaloid monomers. While no dimeric compounds were obtained in this investigation, koenoline (1) did prove to be a further KB-active cytotoxic constituent of *M. koenigii*.

EXPERIMENTAL

Mps are uncorr. UV and IR spectra were recorded in MeOH and as KBr pellets, respectively. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS as int. standard. TLC and prep. TLC were conducted on precoated silica gel plates (Merck) of 250 μm and 500 μm thickness, respectively, and the spots were located under UV or by spraying with 60% v/v H_2SO_4 and heating. HPLC separations were carried out using a μ-Bondapak-C_{18} column (30 cm × 3.9 mm, i.d.; Water's Associates), 254 nm, flow rate 0.8 ml/min.

Plant material. M. koenigii (L.) Spreng. root bark was collected in January, 1984, in Sri Lanka by one of us (D.D.S.), with the assistance of Dr. S. Balasubramaniam, of the University of Peradeniya, Sri Lanka. Voucher specimens are in deposit at the John G. Searle Herbarium, Field Museum of Natural History, Chicago, and at the herbarium of the University of Peradeniya. The root sample of *M. siamensis* Craib was collected by D.D.S. in Thailand in January, 1984, and a voucher specimen has been deposited at the Field Museum of Natural History, Chicago.

Extraction and fractionation. M. koenigii root bark (100 g) was percolated overnight with 4 × 1 l. $CHCl_3$. The combined $CHCl_3$ extracts were evapd to dryness to yield 9.4 g of a residue, which was fractionated over silica gel by CC using $CHCl_3$–Me_2CO (19:1). Murrayanine (3) crystallized on standing in fractions 25–28 and was recrystallized (14 mg, 0.014% w/w) from $CHCl_3$–Me_2CO. Fractions 67–75 contained koenoline (1), which was purified (18 mg, 0.018% w/w) by prep. TLC in $CHCl_3$–Me_2CO (9:1) (R_f 0.50). A larger amount of murrayanine (93 mg, 0.011% w/w) was isolated in a similar manner from *M. siamensis* roots (870 g).

Koenoline (1). Mp 130°; UV λ_{max} nm (log ε): 335 (3.58), 323 (3.61), 289 (4.04), 279 (3.86), 258 (4.38), 251 (4.22), 241 (4.71), 225 (4.56); IR ν_{max} cm^{-1}: 3445, 3235, 1585, 1500, 1450, 1390, 1335, 1310, 1280, 1260, 1225, 1130, 1100, 1035, 1010, 995, 940, 830, 735; ^{1}H NMR (360 MHz): δ1.75 (1H, *br s*, exchangeable with D_2O, OH), 4.01 (3H, *s*, OMe), 4.84 (2H, *s*, Ar–C$\underline{H}_2$OH), 6.95 (1H, *br s*, H-2), 7.23 (1H, *ddd*, J = 8.0, 7.0, 1.3 Hz, H-6), 7.42 (1H, *ddd*, J = 8.0, 7.0, 1.2 Hz, H-7), 7.46 (1H, *d*, J = 8.0 Hz, H-8), 7.66 (1H, *br s*, H-4), 8.04 (1H, *d*, J = 8.0 Hz, H-5) and 8.34 ppm (1H, *br s*, exchangeable with D_2O, NH); ^{13}C NMR: see Table 1; EIMS (70 eV) *m/z* (rel. int.): 227 $[M]^+$ (100), 212 (27), 210 (75), 198 (25), 183 (23), 167 (45), 154 (30), 139 (14), 127 (15), 113 (7), 99 (14) and 77 (14); mass measurement: 227.0944, calc. for $C_{14}H_{13}NO_2$, 227.0946; TLC: R_f 0.20 ($CHCl_3$–Me_2CO, 19:1); HPLC: k' 0.69 (MeOH–H_2O, 7:3).

Koenoline (1, 4 mg) was reacted with pyridine–Ac_2O (1:1, 0.5 ml). After 5 days the reaction was stopped by adding H_2O and the ppted product, koenoline acetate (2, 3 mg), exhibited the following data, after crystallization from pyridine–H_2O: mp 110°; UV λ_{max} nm (log ε): 336 (3.58), 323 (3.60), 290 (3.93), 280 (3.84), 259 (4.40), 252 (4.59), 242 (4.65), 226 (4.51); IR ν_{max} cm^{-1}: 3390, 1720, 1585, 1505, 1455, 1360, 1340, 1315, 1250, 1140, 1108, 1043, 1015, 945, 833, 770, 730; ^{1}H NMR (200 MHz): δ2.13 (3H, *s*, OAc), 4.04 (3H, *s*, OMe), 5.27 (2H, *s*, Ar–C$\underline{H}_2$OAc), 6.92 (1H, *d*, J = 1.2 Hz, H-2), 7.18–7.25 (1H, *m*, H-6), 7.35–7.49 (2H, *m*, H-7, H-8), 7.71 (1H, *br s*, H-4), 8.05 (1H, *d*, J = 8 Hz, H-5) and 8.30 ppm (1H, *br s*, exchangeable with D_2O, NH); EIMS (70 eV) *m/z* (rel. int.): 269 ($[M]^+$, 63), 227 (6), 210 (100), 198 (8), 183 (3), 167 (27), 154 (8), 139 (6) and 127 (4).

Murrayanine (3). When isolated from both *M. koenigii* and *M. siamensis*, murrayanine (3), mp 165°, TLC: R_f 0.60 ($CHCl_3$–Me_2CO, 19:1), HPLC: k' 1.48 (MeOH–H_2O, 7:3) exhibited spectral data (UV, ^{1}H NMR, EIMS) consistent with lit. values [2, 3, 11]. Reduction of 3 (64 mg) in 8 ml MeOH was conducted with 70 mg $NaBH_4$ dissolved in MeOH (25 ml). After 24 hr the reaction mixture was evapd to dryness and the residue taken up in $CHCl_3$ (10 ml). After filtering, the crude reaction product was purified by CC over silica gel eluting with $CHCl_3$–Me_2CO (19:1), to afford 20 mg of 1, the identity of which

was confirmed by mp, UV, IR, ^{1}H NMR, EIMS, TLC and HPLC. Conversely, the aerial oxidation of **1** to **3** was apparent in *ca* 10% yield, by storage at room temp. for 1 week, as assessed by spectral and chromatographic data.

Cytotoxic activity. The $CHCl_3$ extract of *M. koenigii* root bark, koenoline (**1**) and murrayanine (**3**) exhibited ED_{50} values in the KB cell-culture test system [4, 5] of 6.0 μg/ml, 4.0 μg/ml and 26 μg/ml, respectively.

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