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CARBAZOLE ALKALOIDS FROM *MURRAYA KOENIGII*

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Key Word Index—*Murraya koenigii*; Rutaceae; curry leaf plant; roots; 9-carbethoxy- and 9-formyl-3-methylcarbazoles; cytotoxicity.

Abstract—Two new alkaloids, 9-carbethoxy-3-methylcarbazole and 9-formyl-3-methylcarbazole, and a known metabolite, 3-methyl-carbazole were isolated from the roots of *Murraya koenigii*. All three compounds were identified by detailed spectral analyses including 2D NMR studies and their structures confirmed by synthesis. Of the two new metabolites, the 9-formyl compound displayed weak cytotoxicity against both mouse melanoma B16 and adriamycin-resistant P388 mouse leukemia cell lines. © 1997 Elsevier Science Ltd

INTRODUCTION

Since the first discovery of a carbazole alkaloid from a plant source, *viz.*, that of girinimbine from *Murraya koenigii* [1], this species has proved to be a rich source of carbazole alkaloids [2]; the genus *Murraya* still continues to be the subject of chemical investigations in search of new metabolites [3]. The chloroform extract of the roots of *M. koenigii* furnished, after repeated chromatographic separations, three carbazole alkaloids, designated Mk1, Mk2 and Mk3 in order of increasing polarities. The structural elucidation of these isolates by detailed spectral analyses and their synthesis are described herein. The results of screening the new metabolites for cytotoxic and antibacterial activity are also presented.

RESULTS AND DISCUSSIONS

Mk2, the alkaloid of intermediate polarity, was identified by thorough spectral studies as 3-methylcarbazole (**2**), whose first natural occurrence had earlier been reported from two *Clausena* species [4, 5]. The identify was confirmed by comparison with a synthetic sample [6]. The least polar alkaloid, Mk1, mp 122–123°, analysed for $C_{16}H_{15}NO_2$, which was further corroborated by its high resolution mass spectral data ($[M]^+$ *m/z* 253.1102). Its IR spectrum exhibited a significant band at 1723 cm^{-1}, indicating the presence of an aryl or α,β-unsaturated ester group or an equivalent moiety.

The ^1H NMR spectrum of Mk1 (Table 1) showed signals for an ethoxycarbonyl group (δ 1.56, 3H, *t* and 4.59, 2H, *q*, $J = 7$ Hz) and an aromatic methyl (δ 2.53, 3H, *s*). The presence of both an ethoxycarbonyl and an aromatic methyl group in Mk1 was also discernible from its ^{13}C NMR data (δ 152.4, *s*, 62.9, *t* and 14.5, *q*:CO_2Et; 21.2, *q*:Ar—CH_3), the multiplicities being determined by a DEPT 135 spectrum. The calculated double bond equivalent of 10 and the appearance of seven distinct aromatic proton signals in the range of δ 7.3–8.3 indicated a tricyclic aromatic structure bearing one methyl and a carbethoxy group. The mass spectrum of Mk1 gave the base peak at *m/z* 180, which was reminiscent of a methylcarbazole nucleus [4]. This was reinforced by the fact that two of the aromatic protons resonated at quite downfield, *viz.*, δ 8.18 and 8.30 (1H, *d* each, $J = 8$ Hz), which corresponded to the two *peri*-protons, i.e. H-1 and H-8 of a carbazole nucleus [7].

Both the IR and ^1H NMR spectra of Mk1 lacked any band/signal for an NH-group, which required the placement of one of the substituents at 9(*N*). In the case of *N*-methylcarbazole, the ^1H and ^{13}C NMR chemical shifts of the methyl group are δ 3.92 [8] and 28.8 [9], respectively. These values were quite different from those observed for Mk1. This confirmed 9(*N*) as the site for the carbethoxy group in this alkaloid. Biogenetic considerations [10] strongly suggested C-3 to be the most likely site for the methyl. The chemical shift (δ 2.53) of the methyl group did indeed parallel those in other naturally occurring 3-methylcarbazoles, like 3-methylcarbazole itself (δ 2.55, present study), glycomaurrol (δ 2.52) [11] and eustifolines A–C (δ 2.51/2.52) [12], thus strongly favouring the 3-methyl

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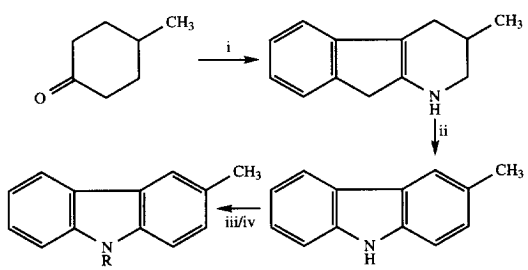
Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR assignments of **1** and **2** (in $CDCl_3$; δ from TMS)

	1				**2**			
H/C	δ_H*	*m*	δ_C	*m*	δ_H*	*m*	δ_C	*m*
1	8.18	*d* (8.5)	115.9	*d*	7.32	*d* (8)	110.2	*d*
2	7.29	*dd* (8.5, 2)	128.3	*d*	7.25	*dd* (9, 1.5)	127.1	*d*
3	—	—	132.8	*s*	—	—	128.6	*s*
4	7.77	*dd* (1.7, 0.8)	119.7	*d*	7.89	*br d* (1.5)	120.2	*d*
4a	—	—	125.9	*s*	—	—	123.4	*s*
4b	—	—	125.8	*s*	—	—	123.1	*s*
5	7.95	*br d* (7.5)	119.5	*d*	8.06	*dd* (8, 0.8)	120.2	*d*
6	7.35	*td* (7.5, 1)	123.1	*d*	7.22	*ddd* (8, 3.5, 4.5)	119.1	*d*
7	7.46	*ddd* (8, 7.5, 1.5)	127.0	*d*	7.40	*m*	125.6	*d*
8	8.30	*d* (8)	116.2	*d*	7.40	*m*	110.5	*d*
8a	—	—	138.5	*s*	—	—	139.7	*s*
NH	—	—	—	—	7.95	*br*	—	—
9a	—	—	136.3	*s*	—	—	137.6	*s*
3-CH_3	2.52	*s*	21.2	*q*	2.55	*s*	21.4	*q*
CO_2Et	—	—	152.4	*s*	—	—	—	—
CO_2CH_2	4.59	*q* (7)	62.9	*t*	—	—	—	—
CH_2CH_3	1.56	*t* (7)	14.5	*q*	—	—	—	—

* *J* in Hz in parentheses.

substitution. Additional support for this assignment was provided by a similarity in the UV absorption spectra of Mk1 and a synthetic sample of *N*-carbethoxycarbazole (*vide* Experimental). The slight bathochromic shifts of (2–6) nm experienced by Mk1 was evidently due to the 3-methyl group. Mk1 thus appeared to be 9-carbethyoxy-3-methylcarbazole (**1**). This structure was commensurate with the ^{13}C NMR data of **1** (Table 1) and was further confirmed by comparing it (mp, mmp and co-TLC) with a synthetic sample. The latter was prepared in good yield by treating 3-methylcarbazole in THF in cold with NaH and then with $ClCO_2Et$ (Scheme 1).

The complete ^1H and ^{13}C NMR assignments of both Mk1 and Mk2, presented in Table 1, were accomplished by a combined analysis of their ^1H-^1H COSY, HMQC and HMBC spectra, and by the selective ^1H-decoupling (*vide* Experimental) of H-5 and H-7,8 of Mk2.



iii: Mk1 (**1**) Mk2 (**2**)
iv: Mk3 (**3**)

i: $PhNHNH_2/C_6H_6/PCL_3$; ii: Pd–C/o–$C_6H_4Cl_2$
iii: NaH/THF, 0-5°; $ClCO_2Et$; or mesitylene, ;
iv: 98% HCO_2H

Scheme 1. Synthesis of alkaloids **1–3**.

The most polar metabolite, Mk3 analysed for $C_{14}H_{11}NO$, also corroborated by the EI mass spectrum ($[M]^+$ *m/z*209). Its UV spectrum and IR carbonyl absorption (1696 cm^{-1}) resembled those of a synthetic sample of 9-formylcarbazole (IR: 1700 cm^{-1}; [13]). Indeed, an analysis of ^1H, ^{13}C, HMQC, HMBC and ^1H-^1H COSY spectra, recorded in pyridine-d_5 at 80°, and the EI mass spectrum easily identified it as 9-formyl-3-methylcarbazole (**3**) and made possible its complete ^1H and ^{13}C NMR assignments (Table 2). The structure **3** was further confirmed by its synthesis from 3-methylcarbazole by treatment with 98% for-

Table 2. ^1H (400 MHz) and ^{13}C (100 MHz) NMR assignments of **3** (in pyridine-d_5 at 80°; δ from TMS)

H/C	δ_H	*m* (*J* in Hz)	δ_C	*m*
1	*ca* 8.3	*br*	113–115	*br*
2	7.33	*dq** (8, 1.0)	128.9	*d*
3	—		134.3	*s*
4	7.85	*quintet*† (1.0)	120.9	*d*
4a	—		126.8	*s*
4b	—		126.6	*s*
5	8.07	*ddd* (7.5, 1.5, 1.0)	120.7	*d*
6	7.45	*td* (7.5, 1.0)	124.6	*d*
7	7.51	*td* (7.5, 1.5)	127.6	*d*
8	*ca* 8.3	*br*	113–115	*br*
8a	—		138.5	*s*‡
9a	—		136.4	*s*‡
3-CH_3	2.48	*br s*	21.3	*q*
9CHO	9.89	*s*	158.4	*d*

* A juxtaposition of two *dd*s.
† Partial overlap of (three lines each of) two *dd*s.
‡ Quaternary carbons assigned from HMBC spectra.

mic acid—an efficient reagent for the *N*-formylation of diaryl, heteroaryl and weakly nucleophilic amines [14].

Interestingly, the appearance of two signals for the formyl group in both ^{1}H (400 MHz, $CDCl_3$, 50°: δ 9.62 and 9.63; intensity ratio: 9:11) and ^{13}C NMR (100 MHz, $CDCl_3$, 25°: δ 157.14 and 157.28, coalescing to δ 157.2 at 50°) spectra necessitates the existence of two rotamers arising out of restricted rotation around the *N*—CHO bond. It also explains the appearance of the NMR signals for H-1, H-8, C-1 and C-8 as broad.

Prompted by the well known [15] anti-cancer activity of vincristine, both Mk1 and Mk3 were screened for cytotoxicity against mouse melanoma B16 cells and adriamycin-resistant P388 mouse leukemia cells following the method of Alley *et al.* [16]. Only Mk3 (**3**) exhibited weak inhibitory activity against the growth of both the cell lines at a dose of 25 μg ml^{-1}, where mitomycin C and doxorubicin were used as standard drugs (*vide* Experimental).

Because of the reported [17] antibacterial properties of the benzo[*b*]carbazoles kinamycins A–D, both Mk1 and Mk3 were also tested against *Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus*, *Micrococcus luteus*, *Mycobacterium smegmatis*, *Escherichia coli* and *Klebsiella pneumoniae*. But neither of the new metabolites exhibited any significant antibacterial activity.

Although alkaloids bearing a *N*-formyl group (e.g. vincristine [18], sativanine-F [19], nummularine-T [20]) or a *N*-carbethoxy group (e.g. kamaline [21]) have earlier been reported from natural sources, there exists, to the best of our knowledge, no report on the isolation or even occurrence of the *N*-formyl and *N*-carbethoxy derivatives of the carbazole nucleus from plants, if not in nature itself.

Regarding the biogenesis of the two new metabolites, a report [22] describing the formation of hydroperoxide-dependent, peroxidase-catalysed oxidation of *N*-methylcarbazole in ^{18}O-enriched medium is suggestive. Thus, the formyl group in Mk3, and, hence, the carboxylic acid moiety—the progenitor of the carbethoxy group in Mk1—may have originated from an appropriate enzyme-catalysed oxidation of *N*,3-dimethylcarbazole. That no *N*-(hydroxymethyl)carbazole was obtained from M.K. is not surprising, since it was demonstrated in the said report that the hydroxymethyl and formyl carbazoles are formed by two different mechanisms and that the former is not an intermediate for the latter. However, no *N*-methylcarbazole has yet been reported from plants, and the reasons for the oxidation of the *N*-methyl group in preference to that of the C-methyl group in *N*,3-dimethyl-carbazole are unknown.

EXPERIMENTAL

General. Mps are uncorr. UV and IR spectra were recorded in EtOH and as KBr pellets, respectively. ^{1}H and ^{13}C NMR spectra, both 1D and 2D, were recorded at 400 MHz (using TMS as int. standard) and 100 MHz, respectively. Low resolution MS were determined by GC-MS, and both low and high resolution MS were also recorded from Kitasato University, Tokyo, Japan. In all the cases, silica gel (60–120 mesh; Qualigens, India) was used for CC and silica gel G (E. Merck, India) for TLC. The spots in TLC were visualised by spraying with 1% $KMnO_4$ in aq. NaOH, followed by brief heating at 110°. Petrol refers to petroleum ether, bp 60–80°.

Plant material. Roots of *M. koenigii* (Linn.) Spreng. were purchased from a local supplier in 1993 and identified by Dr S.R. Das, Survey Officer, Central Council for Research in Ayurveda and Siddha, Calcutta.

Extraction and isolation. Air-dried, milled roots (2.5 kg) were extracted with $CHCl_3$ (3 × 8 l) by percolation at room temp. For the sepn of the acidic and phenolic parts, the cond $CHCl_3$ extract was thoroughly shaken successively with 5% aq. $NaHCO_3$ (× 3) and 2% aq. NaOH (× 3). Each of the two aq. layers was separated, acidified with 2*N* HCl and extracted with $CHCl_3$ (× 3). The residues from these extracts, albeit showing a few spots in TLC, were meagre in amounts and were, therefore, set aside. The oily residue (3.7 g) obtained from the remaining $CHCl_3$ extract (i.e. basic and neutral frs) was chromatographed over silica gel (75 g) and eluted successively with petrol, petrol–EtOAc, EtOAc and finally, with EtOAc–MeOH (19:1). Only petrol–EtOAc (9:1) eluates showed the presence of distinct spots on TLC. This fr. (0.12 g) was rechromatographed, when only petrol–EtOAc (97:3) and (93:7) eluates furnished TLC-positive residues. The residue (65 mg) obtained from the first fr. was recrystallised from petrol–CH_2Cl_2 to furnish a solid, mp 114°, which appeared to be homogeneous, R_f 0.5, on TLC in petrol–benzene (1:1). A TLC examination in petrol–$CHCl_3$ (1:1), however, demonstrated that this fr. contained two components which were sepd by prep. TLC on 0.5 mm thick layers using petrol–$CHCl_3$ (1:1) as developing system. After usual work-up, the upper band (R_f 0.65) furnished Mk1 (**1**, 12 mg), while the lower band (R_f 0.5) afforded Mk2 (**2**, 30 mg). On removal of solvent, the petrol–EtOAc (93:7) eluates produced Mk3 (**3**, 29 mg).

9-Carbethoxy-3-methylcarbazole (**1**). Colourless needles from MeOH. R_f 0.85 in benzene. (Found: C, 75.83; H, 5.94; N, 5.51. $C_{16}H_{15}NO_2$ requires: C, 75.89; H, 5.92; N, 5.53%). UV λ_{max} nm: 229, 250–252, 262 278 (sh), 287, 304, 315. IR ν_{max} cm^{-1}: 1717 (*N*-CO_2Et), 1482, 1442, 1373, 1340, 1320, 1300, 1250, 1224, 1044, 800, 700. MS 70 eV *m/z* (rel. int.): 253 [M]$^+$ (71), 193 (44), 181 (55), 180 (100), 152 (18). HR MS 70 eV *m/z*: 253.1102 [M]$^+$; $C_{16}H_{15}NO_2$ requires: 253.1103.

3-Methylcarbazole (**2**). White shining plates from petrol–CH_2Cl_2, mp 204–206° (lit. [23] mp 206–207°). R_f 0.75 in benzene. Identical with synthetic sample [6] by direct comparisons (mp, mmp and co-TLC). ^{1}H-Decoupling experiments: δ 8.06 (*dd*; H-5)* changed δ 7.22 (*ddd*; H-6) to *dd* (J = 3.5, 4.5 Hz); δ 7.40 (*m*; H-7,8)* changed δ 7.22 to *d* (J = 8 Hz).

9-Formyl-3-methylcarbazole (**3**). Oily residue, solidifying to an off-white solid, mp. 58–60°, when kept in fridge for *ca* 1 week. R_f 0.5 in benzene. (Found: C, 80.32; H, 5.29; N, 6.66. $C_{14}H_{11}NO$ requires: C, 80.38; H, 5.26; N, 6.70%). UV λ_{max} nm: 222, 267, 291 (sh), 315. IR ν_{max} cm^{-1}: 1696 (*N*-CHO), 1588, 1488, 1452, 1359, 1317, 1222, 1148, 808, 747. MS 70 ev *m/z* (rel. int.): 209 $[M]^+$ (77), 181 (57), 180 (100).

Preparation of **1**. 3-Methylcarbazole [6] (362 mg; 2 mM) was added, with stirring, to a suspension of NaH (240 mg; 6 mM) in dry THF (5 ml) at 0–5°. $ClCO_2Et$ (0.5 ml; 5 mM) was then added and the reaction mixt. stirred at room temp. for 3 hr. The soln was then poured into crushed ice and extracted with EtOAc (3 × 20 ml). The residue (457 mg; 90%) obtained from the EtOAc extract was recrystallised from MeOH to furnish **1** in 86% yield (435 mg), mp 120–122°. It was identical to naturally occurring **1** (mp, mmp and co-TLC)

Preparation of **3**. A synthetic sample of **2** (91 mg; 0.5 mM) was treated with 98% HCO_2H (2 ml), heated at 100° for 30 min, then poured into H_2O, neutralised with solid $NaHCO_3$ and extracted with EtOAc (3 × 20 ml). The residue (96 mg; 92%) from the EtOAc extract was purified by a short CC to furnish **3** as a colourless oil (85 mg; 82%). When kept in fridge for a week, it solidified, mp 57–60°. Identical to naturally occurring **3**.

9-Carbethoxycarbazole. Prepd in a similar manner to **1** to furnish 9-carbethoxycarbazole as colourless needles, mp 78–80° (petrol–CH_2Cl_2) (lit. [24] mp 76–77°) in 88% yield. UV λ_{max} nm: 227, 250–252, 260, 283, 298, 310. IR ν_{max} cm^{-1}: 1732 (*N*—CO_2Et). ^1H NMR (400 MHz, $CDCl_3$): δ 1.57 (3H, *t*, $J = 7$ Hz) and 4.61 (2H, *q*, $J = 7$ Hz, $CO_2CH_2CH_3$), 7.37 (2H, *t*, $J = 7.5$ Hz, H-3 and H-6), 7.48 (2H, *t*, $J = 8$ Hz, H-2 and H-7), 7.99 (2H, *d*, $J = 7.5$ Hz, H-4 and H-5), 8.32 (2H, *d*, $J = 8$ Hz, H-1 and H-8). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.49 (CH_3), 63.07 (CH_2), 116.28 (C-1 and C-8), 119.60 (C-4 and C-5), 123.23 (C-3 and C-6), 125.89 (C-4a and C-4b), 127.14 (C-2 and C-7), 138.29 (C-8a and C-9a), 152.45 (CO_2Et). HRFABMS (*m*-NBA) (+ve mode) *m/z*: 239.0947 $[M]^+$.

9-Formylcarbazole. Prepd by treating carbazole with 98% HCO_2H in a similar manner to **3**. Obtained as white needles, mp 94–96° (petrol) (lit. [13] mp 94°) in 86% yield. UV λ_{max} nm: 220, 264, 287 (sh), 310.

Screening for cytotoxicity. Both **1** and **3** were tested for cytotoxicity at doses of 0.39, 1.56, 6.3 and 25 μg ml^{-1}. Only at 25 μg ml^{-1}, the inhibitions of cell growth were found to be 6.5% for **1** and 86.4% for **3** against B16 cells and 47.2% for **3** against P388 cells. The observed cytotoxicities (IC_{50} for **1** and **3**: > 25 μg ml^{-1}) against P388 cells were weak compared with those shown by the standard anti-tumour agents, mitomycin C (IC_{50}: 38 ng ml^{-1}) and doxorubicin (IC_{50}: 0.85 μg ml^{-1}).

Screening for antibacterial activity. Antibacterial properties of both **1** and **3** were tested against the seven stated bacteria using the agar dilution method and heart infusion agar (pH 7.0). n all the cases, the MIC values were found to be > 50 μg ml^{-1}.

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