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Carbazole Alkaloids from Roots of Murraya Koenigii (Rutaceae)

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Abstract. The study on petroleum ether extract of the roots of *Murraya koenigii* (curry leaf) which involves extraction, separations by using various chromatographic methods and structural determination by spectroscopic techniques have afforded two compounds; 3-methylcarbazole and murrayafoline A. The structure of the compounds were also confirmed by comparison with the previous works.

Abstrak. Kajian terhadap ekstrak petroleum eter akar pokok *Murraya koenigii* (daun kari) yang melibatkan pengekstrakan, pengasingan menggunakan pelbagai kaedah kromatografi dan pengenalpastian struktur dengan teknik spektroskopi telah menghasilkan dua komponen; 3-methylcarbazole dan murrayafoline A. Struktur sebatian yang dipencilkan juga telah dibuat perbandingan dengan hasil kajian lepas.

Key Words : *Murraya koenigii* , Rutaceae, Carbazole Alkaloids

Introduction

Murraya koenigii is a member of Rutaceae, which is a large plant family and represented by about 150 genera and 1,600 species. About 60 species of the plants are known including two Malaysian species; *M. paniculata* and *M. koenigii*. *M. koenigii* has been widely used as natural flavouring in curries and souces[1,2], and ingredient in traditional medicine formulations [3]. We have previously described the isolation of some carbazole alkaloids from stem bark of the plant[4]. We now report the isolation of two other carbazole alkaloids, 3-methylcarbazole(1) and murrayafoline A (2) from the roots of *M. koenigii*. The structure elucidation of the compounds were carried out using spectroscopic methods.

Experimental

General

Melting point were determined on Kohfler melting point apparatus and were uncorrected. The IR spectra were recorded using KBr disc on Perkin Elmer FTIR Spectrophotometer model 1650. ^{1}H and ^{13}C NMR spectra were obtained on JEOL Spectrometer at 270 MHz and 67.5 MHz, respectively with tetramethylsilane (TMS) as the internal standard. Mass spectra were recorded on an AEI-MS 12 instrument . The column chromatography was carried on silica gel (Merck 9385) and Merck silica gel 60 PF_{254} was used for analytical TLC analysis.

Plant material

The roots of *Murraya koenigii* used in this study was obtained from Banting Selangor in 1999. Sample collected was air dried at room temperature. Voucher specimen (sample number RK 2954A) was deposited in the herberium of Department of Biology, UPM.

Extraction and Isolation

Air dried roots (1.1 kg) was ground into fine powder and extracted by continuously soaking with petroleum ether, followed by chloroform and methanol. The petroleum ether extract was concentrated under reduce pressure to give solid residue using rotary evaporator. The crude petroleum ether extract (10 g) was subjected to vaccum column chromatography over silica gel and eluted with mixtures of petroleum ether, petroleum ether/$CHCl_3$, $CHCl_3$ and finally $CHCl_3$/MeOH to give 27 fractions of 250 ml each. Fraction 9 yielded white crystals which was recrystallized with the mixture of ethyl acetate and hexane to give 3-methylcarbazole (1) (8.3 mg). Fraction 12 of column chromatography yielded murrayafoline A (2), which appeared as brown viscous oil.

3-methylcarbazole (1) : white needles, m.p. = 205 - 208 °C. IR ν_{max} (cm^{-1}, KBr disc); 3407(N-H), 2915, 1607, 1495, 1475, 1461, 1335, 1243, 807, 749, 729, 592, 573, 454. MS m/z (% intensity); 181 (M^+, 100), 180 (78), 179(6), 178(5), 153(3), 152 (8), 90 (23), 77 (7), 76(4), 63(3), 51(1). ^{1}H NMR, δ ppm (270 MHz, $CDCl_3$); 8.04 (*d*, J = 7.91 Hz, H-5), 7.94 (*s*, N-H), 7.87 (*s*, H-4), 7.40 (*d*, J = 8.16 Hz, H-1 and H-8), 7.32 (*d*, J = 8.16 Hz, H-2), 7.21 (*m*, H-6 and H-7), 2.53 (*s*, CH_3-3). ^{13}C NMR, δ ppm (67.5 MHz, $CDCl_3$); 139.8 (C-1a), 137.7 (C-8a), 128.7 (C-5a), 127.1 (C-1), 125.6 (C-7), 123.5 (C-4a), 123.2 (C-3), 120.2 (C-4, C-5), 119.2 (C-2), 110.5 (C-8), 110.2 (C-6), 21.4 (C-9).

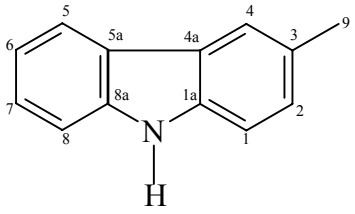
Murrayafoline A (2) : brown viscous oil, IR ν_{max} (cm^{-1} , KBr disc); 3420(N-H), 3056, 2919, 2853, 1640, 1590, 1504, 1454, 1395, 1335, 1307, 1281, 1263, 1232, 1189, 1137(C-O), 1107, 1039, 1014, 945, 830, 767, 749, 671. MS m/z (% intensity); 211 (M^+, 100), 197(9), 196 (74), 180(8), 169(8), 168 (67), 167 (61), 166 (19), 152(6), 140(9), 139 (12), 115(6), 105(8), 84 (10), 63(4), 51(3). ^{1}H NMR, δ ppm (270 MHz, $CDCl_3$); 8.11 (*s*, N-H), 7.98 (*d,* 7.81 Hz, H-5), 7.45 (*s*, H-4), 7.31 (*d*, 6.35 Hz, H-8), 7.15 (*m*, H-6 and H-7), 6.68 (*s*, H-2), 3.89 (*s*, 1-OMe), 2.49 (*s*, CH_3-3). ^{13}C NMR, δ ppm (67.5 MHz, $CDCl_3$); 145.3 (C-1), 139.4 (C-8a), 129.4 (C-1a), 127.9 (C-3), 125.4 (C-8), 124.3 (C-5a), 123.5 (C-4a), 120.4 (C-5), 119.0 (C-6), 112.5 (C-4), 110.9 (C-7), 107.6 (C-2), 55.4 (C-9), 21.9 (C-10).

Results and Discussion

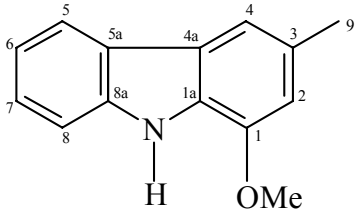
Two carbazole alkaloids were isolated from the petroleum ether extract of the roots of *Murraya koenigii* for the first time from this plant. The compounds were characterized by using spectroscopic techniques including IR, NMR and MS. Compound(1) was isolated from fraction 9 of the column chromatography of crude petroleum ether extract of the roots of the plant sample. The compound was recrystallized with the mixture of ethyl acetate and hexane to give 3-methylcarbazole (1) as white solid (8.3 mg), m.p. 205-208°C (literature [5], m.p. 207° C) with R_f of 0.68 ($CHCl_3$). The MS of the compound indicates the molecular ion at m/z 181, which fits the molecular formula $C_{13}H_{11}N$. The IR spectrum of this compound showed the absorptions due to NH group (sharp peak at 3407 cm^{-1}), aromatic residue (1607, 1495 and 1475 cm^{-1}) and substituted benzene ring (807, 749 and 729 cm^{-1}).

The ^{1}H NMR spectrum of the compound revealed characteristic features of carbazole skeleton, closely resemble those of products described previously[6]. The coupling patterns of aromatic protons indicate the presence of an *ortho*-substituted benzene ring together with an ABX-type aromatic ring. Two doublets, each integrated for one proton at δ 8.04 (J 7.91 Hz) and δ 7.40 (J 8.16 Hz) were due to H-5 and H-8, respectively. A multiplet centered at δ 7.21, integrated for two protons were attributed to H-6 and H-7. Another pair of doublets occur at δ 7.40 (J 8.16 Hz) and δ 7.32 (J 8.16 Hz) were due to H-1 and H-2 of ring C. The remaining aromatic proton H-4 resonates as singlet at δ 7.87. The presence of a low field broad resonance at δ 7.94 was attributed to the NH group, whereas a three-proton singlet peak at δ 2.53 corresponds to an aromatic methyl group. The assignments of H-4 and H-2 was accomplished through COSY technique, where methyl aromatic was observed to correlate with H-4 and H-2. In addition to these correlations, H-5 also showed cross peaks with H-6 and H-7.

The assignments of the compound was further supported by its ^{13}C NMR spectrum . The DEPT ^{13}C NMR indicated the presence of 12 peaks comprising 8 protonated carbons and 5 unprotonated carbons, which due to overlapping of one signal at 120.2 ppm for two carbons (C_4 and C_5). All assignments of ^{1}H and ^{13}C NMR spectra are summerized in Table 1. On the basis of the spectroscopic data mentioned above, the compound was elucidated as 3-methylcarbazole (1), which was isolated earlier from *Clausena heptaphylla* and *Glycosmis pentaphylla* [5].



3-Methylcarbazole (1)



Murrayafoline A (2)

The compound (2) was isolated as brown viscous oils from fraction 12 of the vacuum column chromatography of the petroleum ether extract. The TLC analysis of the compound gave a single spot under UV light with R_f 0.54 ($CHCl_3$). The IR spectrum exhibits absorptions of NH group at 3420 cm^{-1}, C-O stretching at 1232 and 1137 cm^{-1}, aromatic

Table 1 : Chemical Shifts (δ) of ^{1}H and ^{13}C NMR of 3-methylcarbazole (1) and murrayafoline A (2)

Carbon Positions	3-methylcarbazole			Murrayafoline A	
	δ ^{1}H (J)	δ ^{13}C	COSY corelation	δ ^{1}H (J)	δ ^{13}C
1	7.40 (*d*, 8.16 Hz)	127.1		-	145.3
1a	-	139.8		-	129.4
2	7.32 (*d*, 8.16 Hz)	119.2	H-4, H-9	6.68 (*s*)	107.6
3	-	123.2		-	127.9
4	7.87 (*s*)	120.2	H-2, H-9	7.45 (*s*)	112.5
4a	-	123.5		-	123.4
5	8.04 (*d*, 7.91 Hz)	120.2	H-6, H-7	7.98 (*d*, 7.81 Hz)	120.4
5a	-	128.7		-	124.3
6	7.21 (*m*)	110.2	H-5	7.15 (*m*)	119.0
7	7.21 (*m*)	125.6	H-5	7.15 (*m*)	110.9
8	7.40 (*d*, 8.16 Hz)	110.5		7.31 (*d*, 6.35 Hz)	125.4
8a	-	137.7		-	139.4
9	2.53 (*s*)	21.4	H-2, H-4	2.49 (*s*)	21.9
OMe		-		3.89 (*s*)	55.4
NH	7.94 (*s*)	-		8.11 (*s*)	-

residue at 1590, 1504 and 1454 cm^{-1} and substituted benzene at 830 and 749 cm^{-1}. The MS of this compound showed molecular ion peak at m/z 211, which corresponds to molecular formula $C_{14}H_{13}NO$. The peak at m/z 196 is most likely due to removal of a methyl group from molecular structure.

The ^{1}H NMR spectrum confirmed the presence of NH group at δ 8.11 and one methoxy group at δ 3.89. Another three protons singlet was observed at δ 2.49 due to the presence of a methyl aromatic at position 3 of ring C. The aromatic region of ^{1}H NMR spectrum showed the characteristic features appropriate of carbazole skeleton. Two doublets, each integrated for one proton at δ 7.98 (J 7.81 Hz) and 7.31 (J 6.35 Hz) were due to H-5 and H-8, respectively. Two singlet signals occurred at δ 6.68 and 7.45 were attributed to H-2 and H-4 of ring C. A multiplet centered at δ 7.15 integrated for two protons were due to the presence of H-6 and H-7 of ring A.

The ^{13}C NMR spectrum of this compound gave 14 signals for each of 14 carbons present in this molecule. The complete assignments of ^{1}H and ^{13}C NMR spectra were listed in Table 1. The structure of this compound was assigned as murrayafoline A (2), which was reported previously as low melting solid isolated from *Murraya euchrestifolia* [7].

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