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Chemical Constituents from the Roots of Malayan *Murraya Koenigii* (Rutaceae)

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The purpose of this study is to isolate and identify alkaloid compounds from the roots of Malayan *Murraya koenigii*. This plant has been reported to be rich in carbazole alkaloid. The hexane and dichloromethane crude extracts (roots) were separated using thin layer and column chromatography techniques. Four carbazole alkaloids were afforded and identified as girinimbine **1**, murrayafoline-A **2**, mahanimbine **3** and murrayanine **4**. Their structures were elucidated based on spectroscopic analysis such as UV, MS, IR, and NMR (1D; ^{1}H, ^{13}C, DEPT and 2D; HMQC, HMBC, COSY) and by comparison with the published data.

Key words: *Murraya koenigii*; carbazole alkaloid; girinimbine; murrayafoline; mahanimbine; murrayanine; structures; spectroscopic analysis

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Murraya koenigii is a member of Rutaceae family, which is a large plant family and represented by about 150 genera and 1600 species. About 60 species of the plants are known including two Malaysian species; *M. paniculata* and *M. koenigii* [13]. Various parts of *M. koenigii* have been used in traditional or folk medicine for the treatment of rheumatism, traumatic injury, and snake bite and it has been reported to have antioxidant, anti-diabetic and anti-dysenteric activities [6].

Carbazole alkaloids represent a new and exciting variant in the number of existing indolent alkaloids which yielded several important drugs. A rich and rewarding source of carbazole alkaloids has been found in the curry leaf plant *M. koenigii* (Rutaceae). All parts of this plant including roots, stems, leaves and fruits yielded carbazole alkaloids. It has been reported that carbazole alkaloids possess various biological activities such as anti-tumor, anti-oxidative and anti-mutagenic activities [8].

Previous studies have shown that most of the carbazole alkaloids are found in *M. koenigii*. Mahanimbine and girinimbine most found in barks, roots, and leaves while murrayafoline-A was also found in roots [1–4,14]. The aim of our study was to isolate and elucidate carbazole alkaloids from the roots of Malayan *M. koenigii*. In our research, we gained complete data of NMR to support the data from the previous researchers. We reported the isolation of four known pyranocarbazole type alkaloids from the roots of *M. koenigii* collected from Pahang, Malaysia, which have been identified as girinimbine **1**, murrayafoline-**A 2**, mahanimbine **3** and murrayanine **4**. Their structures were elucidated by using spectroscopic analysis including NMR, UV, IR and MS and also by comparison with published data.

MATERIALS AND METHODS

General

Merck silica gel 60 (70–230 and 230–400 mesh) were used for column chromatography separation, aluminium supported silica gel 60 F_{254} for thin layer chromatography (TLC), and silica gel 60 F_{254} with gypsum for preparative thin layer chromatography (PTLC). NMR spectra were recorded on JEOL ECX (500 MHz) using $CDCl_3$ as a solvent.

Plant Materials

The roots of *M. koenigii* (2.3 kg) was used in this study were obtained from Felda Residence Tekam, Jerantut, Pahang (February 2012). The collected sample was ground after air dried for a week.

Extraction

Air dried roots were ground and extracted by continuously soaking for three days with hexane at room temperature and gave a deep yellowish extract. The solvent of hexane was filtered and removed by using rotary evaporator. This procedure was repeated three times. After dried, the samples were moistened with 25% ammonia solution and left for two h; this was to aggregate the nitrogen-containing compounds from *M. koenigii* roots. It was then re-extracted with dichloromethane and methanol to obtain dichloromethane and methanol crude extracts.

Isolation and Purification

Isolation of alkaloids was performed by using chromatographic techniques such as column chromatography (CC) and PTLC. The crude extract of *M. koenigii* was subjected to CC over silica gel and eluted with increasing polarity solvent system of hexane, dichloromethane and methanol. Fractions which had the same pattern shown in TLC were grouped into a series of fractions. Further isolation and purification were continued until the pure compound was obtained.

RESULTS AND DISCUSSION

The crude hexane and dichloromethane from the roots of *M. koenigii* collected from Pahang were separated by using thin layer and column chromatography techniques which yielded two pyranocarbazole; girinimbine **1**, mahanimbine **3** and two tricyclic carbazole alkaloids; murrayafoline-**A 2** and murrayanine **4** (Figure 1). All these compounds were characterized by using spectroscopic techniques including UV, IR, NMR and MS, also involved comparison with data from the literature.

Compound **1** gave white crystals appearance with melting point 173.0–175.2ºC. The IR spectrum showed the presence of N-H group at 3360.62 cm^{-1} and C-O at 1114.35 cm^{-1}. The mass spectrum of compound **1** showed a molecular ion peak at m/z 264.14 [M^+] corresponding to molecular formula $C_{18}H_{17}NO$. The ^1H NMR spectrum (Table 1) showed three signals at δ 2.33 (*s*, H-3-CH_3), δ 1.49 (*s*, H-12, H-13) and δ 7.67 (*s*, H-4) indicated the presence of four methyl groups. The spectrum also displayed four doublet signals at δ 7.38 (*d*, *J* = 8.00, H-8), δ 7.91 (*d*, *J* = 7.50, H-5), δ 6.64 (*d*, *J* = 9.80, H-9) and δ 5.70 (*d*, *J* = 9.80, H-10). The triplet signals were observed at δ 7.17 (*t*, *J* = 8.10, H-6) and δ 7.30 (*t*, *J* = 8.10, H-7) respectively which confirmed the unsubstituted of ring A. The COSY spectrum showed the correlations between H-5, H-6, H-7, H-8 and another correlation between H-9 and H-10.

Analysis of ^{13}C NMR and DEPT showed the presence of 18 carbons which consisted of eight quaternary carbons; 104.6 (C-1), 135.0 (C-1a), 150.0 (C-2), 118.8 (C-3), 116.9 (C-4a), 124.1 (C-5a), 139.6 (C-8a) and 76.0 (C-11), three methyl carbons; 27.8 (C-12, C-13) and 16.3 (C-14), and seven methine carbons; 121.3 (C-4), 119.5 (C-5), 119.7 (C-6), 124.4 (C-7), 110.6 (C-8), 117.4 (C-9) and 129.6 (C-10). The UV spectrum showed the absorbance of pyranocarbazole characteristics at λ_{max} 204, 237 and 286 nm by Pretsch *et al*. [14]. The complete assignments of proton and carbon signals and location of the substituent on the skeleton of compound **1** were supported by HMQC and HMBC spectra. The structure of **1** was identified as Girinimbine that was also supported by Ahmad [2].

Compound **2** was obtained as the yellowish oil and analyzed as $C_{14}H_{13}NO$. The MS spectrum of compound **2** indicated the presence of molecular ion peak at m/z 212.11. The IR spectrum showed a sharp peak at 3352.17 cm^{-1} due to the stretching of N-H and C-O at 1055.01 cm^{-1}. The UV spectrum showed the absorption peak at 223, 242 and 291 nm which indicates the carbazole derivatives [12].

The ^1H NMR spectrum showed one methoxy signal at δ 4.03 (*s*) was attached to C-1 (145.38). A singlet signal was also observed at δ 2.56 (*s*)

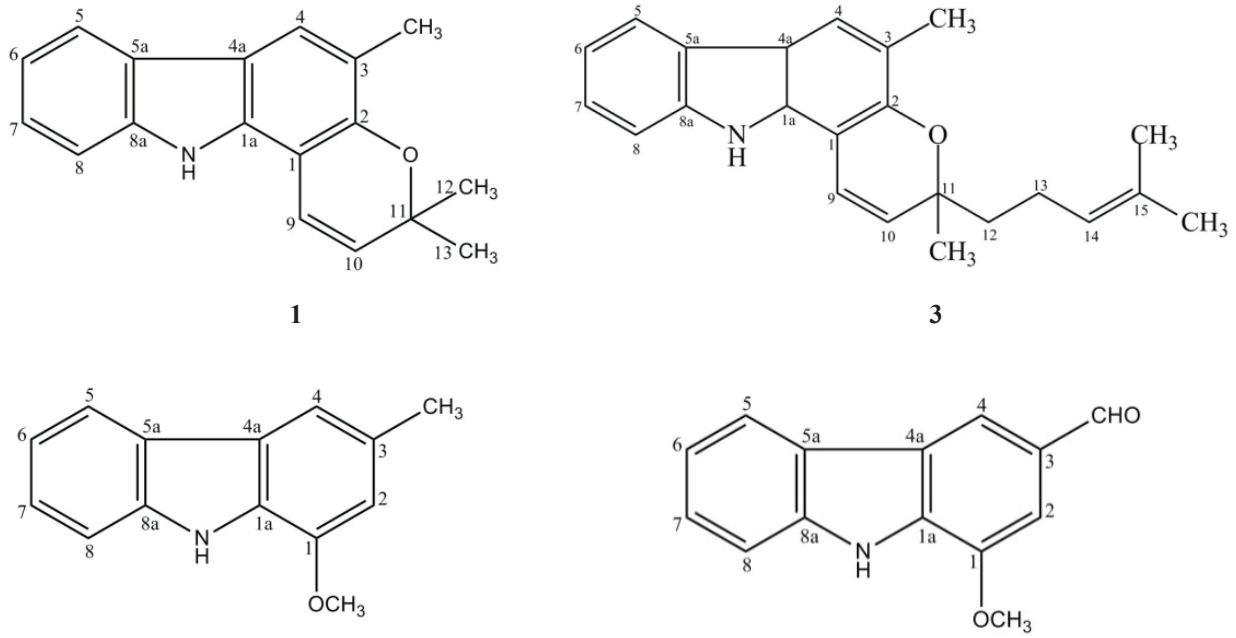

Figure 1. Structures of compound **1–4**.

due to the presence of a methyl group attached to carbon 3 of aromatic ring C (22.01). The spectrum also displayed two doublet signals at δ 8.04 (*d*, *J* = 8.00, H-5), δ 7.44 (*d*, *J* = 8.05, H-8) and two triplet signals at δ 7.43 (*t*, *J* = 8.05, H-7) and δ 7.22 (*t*, *J* = 8.00, H-6) which confirmed that ring A is unsubstituted.

The ^{13}C NMR displayed 14 signals corresponding to fourteen carbons. The position of seven quaternary carbons appeared at 145.38 (C-1), 129.51 (C-1a), 107.71 (C-2), 128.03 (C-3), 123.58 (C-4a), 124.27 (C-5a), 139.51 (C-8a); one methyl carbon: 22.01 (3-CH3); one methoxy carbon: 55.53 (1-OCH3) and five methine carbons: 112.57 (C-4), 120.51 (C-5), 119.21 (C-6), 125.55 (C-7), 110.97 (C-8). The position of methoxy was confirmed at carbon 1 by the long-range correlation between δ 4.03 (OCH_3) and 145.38 (C-1) in HMBC spectra. Comparison of the empirical data with literature confirmed that compound **2** is murrayafoline-A as indicated by Sukari *et al*. in 2001 [13].

Compound **3** was obtained as a colourless crystal (melting point 94.0–95.0ºC). The mass spectral data of **3** gave a molecular formula $C_{23}H_{25}NO$ with m/z 332.26 $[M+H]^+$. The IR spectrum displayed broad signal for N-H (3361.50 cm^{-1}) and strong signal of C-O at 1027.43 cm^{-1}. The ^1H NMR spectrum showed five signals in the aromatic region. Two doublet signals displayed at δ 7.94 (*d*, *J* = 8.05) and δ 7.37 (*d*, *J* = 6.30) were due to the presence of H-5 and H-8, respectively. Two triplet signals appeared at δ 7.21 (*t*, *J* = 8.00, H-6) and δ 7.33 (*t*, *J* = 8.00, H-7) were integrated for two protons attached to C-6 (119.53) and C-7 (124.27) and another signal at δ 7.70 (*s*, H-4) was assigned to proton attached to C-4 (118.47). The present of two methylene signals at δ 1.80 (H-12), δ 2.21 (H-13); one olefinic methine at δ 5.15 (H-14) and two methyl protons at δ 1.62 (15-CH_3) and 1.48 (15-CH_3) at higher field indicated the presence of prenyl group. This prenyl group was attached to C-11 (77.36). The attachment of phenyl group was confirmed by HMBC spectra due to a correlation between H-12 and H-13 with C-11.

The ^{13}C NMR spectrum showed the presence of 23 carbon atoms contain nine quaternary carbons; 104.27 (C-1), 134.91 (C-1a), 149.95 (C-2), 118.47 (C-3), 116.66 (C-4a), 123.96 (C-5a), 139.50 (C-8a), 77.36 (C-11) and 131.75 (C-15), eight olefinic

carbons; 121.25 (C-4), 119.35 (C-5), 119.53 (C-6), 124.27 (C-7), 110.47 (C-8), 117.57 (C-9), 128.54 (C-10) and 124.27 (C-14), two methylene carbons; 40.83 (C-12), 22.83 (C-13) and four methyl carbons; 16.17 (3-CH_3), 25.89 (11-CH_3), 17.66 (15-CH_3) and 25.76 (15-CH_3). All the analysis data are compared with data from the literature and compound **3** is assigned as mahanimbine [14].

Murrayanine **4** was obtained as a brownish oil. The MS spectrum of this compound showed the presence of molecular ion peak at m/z 225.80 corresponding to the molecular formula $C_{14}H_{11}NO_2$. The IR spectrum showed a sharp peak at 3360.67 cm^{-1}, 1032.09 cm^{-1} and 1588.21 cm^{-1} due to the stretching of N-H, C-O and C=O groups, respectively. The ^{1}H NMR data of this compound was nearly identical with compound **2** except for the presence of carbonyl (CHO) group at 192.02. The position of carbonyl group was confirmed at carbon 3 by the HMBC correlation between proton at δ 10.05 (CHO) and 130.16 (C-3).

The ^{13}C NMR spectrum gave 14 signals corresponding to 14 carbons present in this compound. There are six quaternary carbons; 146.13 (C-1), 134.14 (C-1a), 130.16 (C-3), 123.69 (C-4a), 123.66 (C-5a), 139.48 (C-8a), six methine carbons; 103.52 (C-2), 120.51 (C-4), 120.75 (C-5, C-6), 126.68 (C-7), 111.58 (C-8), one methoxy carbon; 55.84 (1-OCH_3) and one aldehyde carbon; 192.02 (3-CHO). This compound **4** was assigned as murrayanine based on comparison with literature [3]. The complete data of ^{1}H and ^{13}C NMR for all compounds were tabulated in Table 1 and 2, respectively.

Table 1. ^{1}H NMR spectral data of compound **1**, **2**, **3** and **4**.

Position	^{1}H $CDCl_3$ (J, Hz)			
	1	**2**	**3**	**4**
C-1				
C-1a				
C-2		6.75 (*s*)		7.45 (*s*)
C-3				
C-4	7.67 (*s*)	7.50 (*s*)	7.70 (*s*)	8.18 (*s*)
C-4a				
C-5	7.91 (*d*, 7.50)	8.04 (*d*, 8.00)	7.94 (*d*, 8.05)	8.10 (*d*, 7.15)
C-5a				
C-6	7.17 (*t*, 8.10)	7.22 (*t*, 8.00)	7.21 (*t*, 8.00)	7.32 (*t*, 6.90)
C-7	7.30 (*t*, 8.10)	7.43 (*t*, 8.05)	7.33 (*t*, 8.00)	7.48 (*t*, 6.90)
C-8	7.38 (*d*, 8.00)	7.44 (*d*, 8.05)	7.37 (*d*, 6.30)	7.51 (*d*, 6.85)
C-8a				
C-9	6.64 (*d*, 9.80)		6.61 (*d*, 9.75)	
C-10	5.70 (*d*, 9.80)		5.65 (*d*, 9.70)	
C-11				
C-12	1.49 (*s*)		1.80 (*t*, 8.30)	
C-13	1.49 (*s*)		2.21 (**m**)	
C-14			5.15 (*t*,1.15)	
C-15				
N-H	7.88 (*s*)	8.17 (*s*)	7.82 (*s*)	8.69 (*s*)
1-OCH_3		4.03 (*s*)		4.05 (*s*)
3-CHO				10.05 (*s*)
3-CH_3	2.33 (*s*)	2.56 (*s*)	2.37 (*s*)	
11- CH_3			1.70 (*s*)	
15- CH_3			1.62 (*s*)	
15- CH_3			1.48 (*s*)	

Table 2. ^{13}C NMR Spectral Data of Compound **1**, **2**, **3** and **4**

Position	13C (δ, $CDCl_3$)							
	1	**[7]**	**2**	**[1]**	**3**	**[4]**	**4**	**[8]**
C-1	104.6	104.4	145.38	145.3	104.27	104.2	146.13	146.1
C-1a	135.0	139.4	129.51	129.4	134.91	139.4	134.14	139.4
C-2	150.0	149.7	107.71	107.6	149.95	149.9	103.52	103.5
C-3	118.8	116.7	128.03	127.9	118.47	123.9	130.16	130.2
C-4	121.3	121.1	112.57	112.5	121.25	121.2	120.51	120.4
C-4a	116.9	119.4	123.58	123.4	116.66	116.6	123.69	123.6
C-5	119.5	119.2	120.51	120.4	119.35	119.3	120.75	120.7
C-5a	124.1	118.6	124.27	124.3	123.96	118.4	123.66	123.6
C-6	119.7	110.4	119.21	119.0	119.53	119.4	120.75	120.7
C-7	124.4	123.9	125.55	110.9	124.27	124.2	126.68	111.5
C-8	110.6	124.2	110.97	125.4	110.47	110.4	111.58	126.6
C-8a	139.6	134.8	139.51	139.4	139.50	134.8	139.48	134.1
C-9	117.4	129.3			117.57	117.5		
C-10	129.6	117.2			128.54	128.4		
C-11	76.0	75.8			77.36	78.1		
C-12	27.8	27.6			40.83	40.7		
C-13	27.8	27.6			22.83	22.7		
C-14					124.27	118.4		
C-15					131.75	131.6		
N-H								
1-OCH_3			55.53	55.4			55.84	55.8
3-CHO							192.02	191.9
3- CH_3	16.3	16.0	22.01	21.9	16.17	16.1		
11- CH_3					25.89	25.8		
15- CH_3					17.66	17.6		
15- CH_3					25.76	25.7		

CONCLUSION

The present study was aimed to study the chemical constituents of Malayan *Murraya koenigii*. Analysis of all spectral data obtained and compared to the previous researcher yielded four known carbazole alkaloids, girinimbine **1**, murrayafoline-**A 2**, mahanimbine **3** and murrayanine **4**.

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