EDUCATUM - Journal of Science, Mathematics and Technology Vol. 1 No.1 (2014) 1 - 9
ISSN 2289-7070

C_{23}-Carbazole Alkaloids From The Bark of *Murraya koenigii* (L.) Spreng

C_{23}-Carbazole Alkaloids Daripada Kulit Kayu Murraya koenigii (L.) Spreng

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Abstract

Murraya koenigii (Rutaceae), locally known as curry leaf tree, is a medicinally important herb of Indian origin and now is widely distributed throughout southern Asia. The bark of Malayan *Murraya koenigii* was investigated for phytochemical content. The isolation of chemical constituents from its hexane extract was carried out by using various chromatographic techniques. Spectroscopic methods, including NMR, IR, UV, MS spectra data, were used for the structural characterization. Four C_{23}-carbazole alkaloids were isolated and identified as mahanimbine, murrayamine-J, murrayazolinol and bicyclomahanimbine.

Keywords carbazole alkaloid , curry leaf , *Murraya koenigii*, Rutaceae, NMR

Abstrak

Murraya koenigii (Rutaceae), nama tempatan dikenali sebagai pokok kari, adalah herba perubatan yang penting yang berasal dari India dan kini telah tersebar secara meluas di seluruh selatan Asia. Kulit *Murraya koenigii* telah dipilih untuk kajian kandungan fitokimia. Pengasingan bahan kimia daripada ekstrak heksana telah dijalankan dengan menggunakan pelbagai teknik kromatografi. Kaedah spektroskopi, termasuk NMR, IR, UV, data spektra MS, telah digunakan untuk pencirian struktur. Empat alkaloid carbazola-C_{23} telah diasingkan dan dikenalpasti sebagai mahanimbina, murrayamina-J, murrayazolinol dan bisiklomahanimbina.

Kata kunci alkaloid carbazola, daun kari, *Murraya koenigii*, NMR, Rutaceae

Introduction

The plant *Murraya koenigii*, commonly known as curry leaf tree, belonging to the family Rutaceae are aromatic shrubs or a small tree of up to 6 m in height. It is native to India and is now distributed throughout Southern Asia (Tachibana *et al*., 2001). Traditionally, it is used in Indian culinary practices (Abu Bakar *et al*., 2007), treatment on rheumatism, traumatic injury, dysentery, diarrhea and snake bite (Dineshkumar *et al*., 2010; Sim & Teh, 2011). The plant is also reported to have antioxidant, antidiabetic, anticarcinogenic, anti-dysenteric, stimulant, hypoglycaemic and antimicrobial activities (Khanum *et al*., 2000; Ningappa *et al*., 2008; Ningappa & Srinivas, 2008; Yadav *et al*., 2002).

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Previous studies on the *Murraya* species included reports of coumarins, terpenoids and many investigations on carbazole alkaloids (Obadiah *et al*., 2012). Carbazole alkaloids are the major constituents of the plant and are known to possess cytotoxic, antioxidative, antimutagenic and anti-inflammatory activities (Adebajo *et al*., 2006; Tachibana *et al*., 2001; Ramsewak *et al*., 1999). Biologically active carbazole alkaloids are also reported to have antimicrobial properties (Ramsewak *et al*., 1999).

In continuation of our studies on carbazole of *Murraya koenigii*, we report the isolation and elucidation of four C_{23}-carbazole alkaloids from the bark of the plant which have been identified as mahanimbine, murrayamine-J, murrayazolinol and bicyclomahanimbine.

Materials and Methods

General Methods

Nuclear Magnetic Resonance spectra (NMR) were recorded using Joel (500 MHz). Deuterated chloroform ($CDCl_3$) was used as the NMR solvent. Chemical shifts (δ) were reported in ppm and coupling constants (*J*) in Hz. Mass spectra (MS) were determined by using the GC-mass spectrometry (GC-MS Agilent 5975 Series). The ultra violet spectrum (UV) were obtained in methanol on a Perkin Elmer uv-visible spectrophotometer and the wavelength of the spectrum was determined in the range of 200 to 1000 nm. The infrared spectra (IR) were recorded on a Nicolet 6700 FTIR spectrophotometer, with methanol as dilution solvent of the sample.

Column chromatography were prepared by using Silica Gel $60F_{254}$, 70-230 mesh ASTM and 230-400 mesh ASTM as stationary phase. Silica Gel $60F_{254}$ containing Gypsum was used as the stationary phase for the preparative thin layer chromatography (TLC). Analytical TLC was performed on commercially precoated aluminium supported silica gel $60F_{254}$ TLC sheets.

Plant Material

The species selected for the current study was *Murraya koenigii (L.)* Spreng (TM1006) which was collected from Jerantut, Pahang in early 2012 and identified by the phytochemical group, Chemistry Department, Faculty of Science and Mathematics, Sultan Idris Education University.

Extraction and Isolation

The dried bark (3.0 kg) of *Murraya koenigii* were extracted with hexane (three times) at room temperature (28ºC). The extracts were combined and were concentrated under reduced pressure to yield a brown syrup of hexane crude (106 g). After evaporation of the solvent, 30 g of the crude extract was subjected to column chromatography over silica gel (gradient solvent system: hexane, CH_2Cl_2 and methanol).

Spectoscopic Characterization

Different spectroscopic methods were used to elucidate the structure of isolated compounds. Among the spectroscopic techniques, UV, IR, 1D-NMR, 2D-NMR and GC-MS were carried out.

Results and Discussion

The bark of *M. koenigii* were extracted with hexane. The fractionation of the hexane extract followed by column chromatography and TLC yielded four C_{23}-carbazole alkaloids (Figure 1) which were identified as mahanimbine (1), murrayamine-J (2), murrayazolinol (3) and bicyclomahanimbine (4) by spectroscopic methods. All compounds showed the similar spectroscopic features with the carbazole alkaloids as published in the literature. The details are as follows:

Mahanimbine ($C_{23}H_{25}NO$), **1**. Colourless crystal, mp 94-95 °C (ethyl acetate). UV λ_{max} (MeOH) nm (log e): 238 (4.39), 288 (3.83). IR ν_{max} cm^{-1} : 3338, 1646, 1610, 1444, 1156, 1121, 782. GC-MS, m/z: 331 (M^+). ^1H and ^{13}C NMR.

Murrayamine-J ($C_{23}H_{23}NO_2$), **2**. Pale yellowish oil. UV λ_{max} (MeOH) nm (log e): 215 (4.25), 248 (4.35), 265 (4.25), 290 (4.33), 310 (4.45). IR ν_{max} cm^{-1} : 3332, 2855, 1675, 1615, 1575, 1156, 782. GC-MS, m/z: 345 (M^+). ^1H and ^{13}C NMR.

Murrayazolinol ($C_{23}H_{25}NO_2$), **3**. brown solid. UV λ_{max} (MeOH) nm (log e): 248 (4.84), 265 (4.82), 305 (4.40). IR ν_{max} cm^{-1} : 3360, 1650, 1620, 1380. GC-MS, m/z: 347 (M^+). ^1H and ^{13}C NMR.

Bicyclomahanimbine ($C_{23}H_{25}NO$), **4**. Brown solid. UV λ_{max} (MeOH) nm (log e): 242 (4.20), 255 (4.35), 265 (4.15), 305 (4.40). IR ν_{max} cm^{-1} : 3455, 2950, 2920, 2840, 1625, 1605, 1350, 1156. MS, m/z: 331 (M^+). ^1H and ^{13}C NMR.

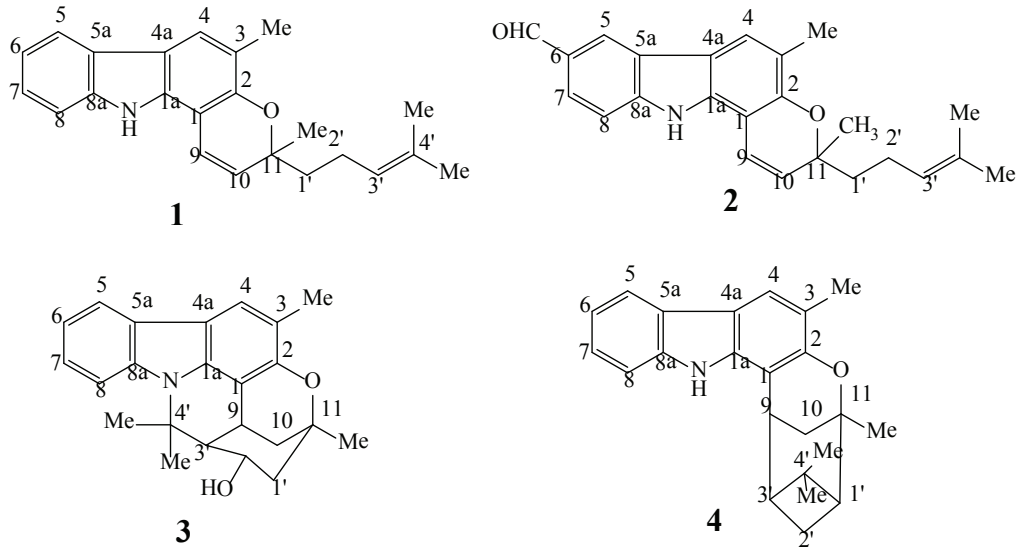

Figure 1 The four compounds yielded from column chromatography

Table 1 ^{1}H NMR [500 MHz, d_H (*J*, Hz)] values of Compound 1,2,3 and 4 in $CDCl_3$

Position	d_H, *J* Hz			
	1$_a$	**2**$_b$	**3**$_c$	**4**$_d$
NH	7.81 (*br s*)	8.35 (*br s*)		7.42 (*br s*)
1				
1a				
2				
3		6.81 (*d*, 8.6 Hz)		
4	7.69 (*s*)	7.83 (*d*, 8.6 Hz)	7.49 (*s*)	7.67 (*s*)
4a				
5	7.94 (*d*, 8.1 Hz)	8.46 (*d*, 1.7 Hz)	7.90 (*d*, 7.5 Hz)	7.92 (*d*, 8.0 Hz)
5a				
6	7.21 (*td*, 7.5, 1.2 Hz)		7.16 (*td*, 6.9, 1.2 Hz)	7.16 (*td*, 8.0, 1.2 Hz)
7	7.33 (*td*, 6.9, 1.2 Hz)	7.89 (*dd*, 8.6,1.7 Hz)	7.25 (*td*, 7.0, 1.2 Hz)	7.28 (*td*, 8.0, 1.2)
8	7.35 (*d*, 7.5 Hz)	7.47 (*d*, 8.6 Hz)	7.47 (*d*, 8.0 Hz)	7.37 (*d*, 7.5 Hz)
8a				
9	6.60 (*d*, 9.8 Hz)	6.66 (*d*, 9.7 Hz)	3.32 (*m*)	3.29 (*d*, 9.2 Hz)
10	5.65 (*d*, 9.7 Hz)	5.71 (*d*, 9.7 Hz)	1.22 (*m*)	2.06 (*m*)
11				
3-Me	2.37 (*s*)		2.36 (*s*)	2.34 (*s*)
6-CHO		10.07 (*s*)		
11-Me	1.48 (*s*)	1.47 (*s*)	1.50 (*s*)	1.44 (*s*)
1’	1.79 (*t*, 8.1)	1.74 (*m*)	1.76 (*dd,* 16.1, 6.9 Hz)	2.50 (*t*, 7.4 Hz)
2’	2.20 (*m*)	2.16 (*m*)	3.83 (*m*)	1.65 (*m*)
3’	5.15 (*tt*, 7.5, 1.2 Hz)	5.10 (*t*, 6.3 Hz)	2.17 (*m*)	2.70 (*t*, 7.5 Hz)
4’				
4’-Me’	1.62 (*s*)	1.58 (*s*)	1.29 (*s*)	0.74 (*s*)
4’-Me’’	1.72 (*s*)	1.66 (*s*)	1.93 (*s*)	1.56 (*s*)

a: Joshi, Kamat, & Gawd (1970); b: Wu, Wang, Wu, Ito, & Furukawa (1996); c: Bhattacharyya, Chatterjee, Roy, & Chakraborty (1989); d: Kureel, Kapil, & Popli (1969)

Compound **1** was obtained as a colourless crystal (mp 94-95°C) (Roy & Chakraborty, 1974). The IR spectrum of compound **1** indicating the presence of N-H (3338 cm^{-1}) C-O and C-H stretching (1121 cm^{-1} and 782 cm^{-1}, respectively). It was readily recognized as

Table 2 ^{13}C NMR [125 MHz, d_C] value of Compound **1,2,3** and **4** in $CDCl_3$

Position	d_C, *J* Hz			
	1$_a$	**2**$_b$	**3**$_c$	**4**$_d$
1	104.2	105.1	105.3	106.4
1a	139.4	136.8	142.4	137.8
2	149.9	152.7	153.3	153.3
3	123.9	111.0	118.5	120.1
4	121.2	121.0	119.7	119.4
4a	116.6	117.2	115.1	124.2
5	119.3	122.9	120.1	119.3
5a	118.4	124.3	127.2	115.8
6	119.4	129.5	119.5	119.3
7	124.2	126.5	123.1	124.0
8	110.4	110.8	113.6	110.4
8a	134.8	143.4	140.7	139.3
9	117.5	116.8	36.9	37.5
10	128.4	129.6	21.5	38.4
11	78.1	78.8	79.5	83.6
3-Me	16.1		15.5	16.8
6-CHO		192.1		
11-Me	25.8	26.2	25.1	27.5
1’	40.7	41.0	36.0	46.5
2’	22.7	22.8	72.2	25.7
3’	118.4	124.0	49.5	37.9
4’	131.6	132.0	60.6	39.5
4’-Me’	17.6	17.7	23.1	18.7
4’-Me’’	25.7	25.8	30.2	35.2

a: Joshi, Kamat, & Gawd (1970); b: Wu, Wang, Wu, Ito, & Furukawa (1996); c: Bhattacharyya, Chatterjee, Roy, & Chakraborty (1989); d: Kureel, Kapil, & Popli (1969)

C_{23}-pyranocarbazole derivative from its preliminary spectral data. Its molecular weight was deduced as $C_{23}H_{25}NO$ by GC-MS at m/z 331 (M^+). Its UV spectrum showed characteristic absorbance for pyranocarbazole moiety with λ_{max} 238 and 288 nm (Roy & Chakraborty, 1974). The ^1H NMR spectrum (Table 1) of compound **1** showed a broad downfield signal at *d* 7.81 assignable to the presence of N-H in the carbazole nuclues. The signals at *d* 7.94 (*d*, *J* = 8.1, H-5), *d* 7.35 (*d*, *J* = 7.5, H-8), *d* 7.33 (*td*, *J* = 6.9, 1.2, H-7) and *d* 7.21 (*td*, *J* = 7.5, 1.2, H-6) of **1** was deduced as an unsubstituted carbazole ring-C (Table 1). Two olefinic protons at *d* 6.60 (*d*, *J* = 9.8, H-9) and *d* 5.65 (*d*, *J* = 9.7, H-10) due to a pyran ring annulated to a carbazole moiety. Finally, the ^1H NMR spectrum contained signals for an aromatic methyl at *d* 2.37 (s), a methyl group at *d* 1.48 (s) and one prenyl group (2-methylpent-2-en-5yl substituent) adjacent to a oxygen in pyran ring of the pyranocarbazole at C-11 with 5.15 (*tt*, *J* = 7.5, 1.2 Hz, H-3’), 2.20 (*m,* H-2’), 1.79 (*t*, *J* = 8.1, H-3’), 1.72 (s, 4’’-Me) and 1.62 (s, 4’-Me). The ^{13}C NMR spectrum (Table 2) indicated the presence of 23 carbons resonances and proven to be a C_{23}-carbazole. The complete assignments of carbon signals

EDUCATUM - Journal of Science, Mathematics and Technology Vol. 1 No.1 (2014) 1 - 9
ISSN 2289-7070

and location of **1** was achieved on the basis of DEPT, ^{1}H-^{1}H COSY , HMQC and HMBC spectra (Figure 2). The structure of compound **1** was identified as mahanimbine (Joshi *et al*. 1970).

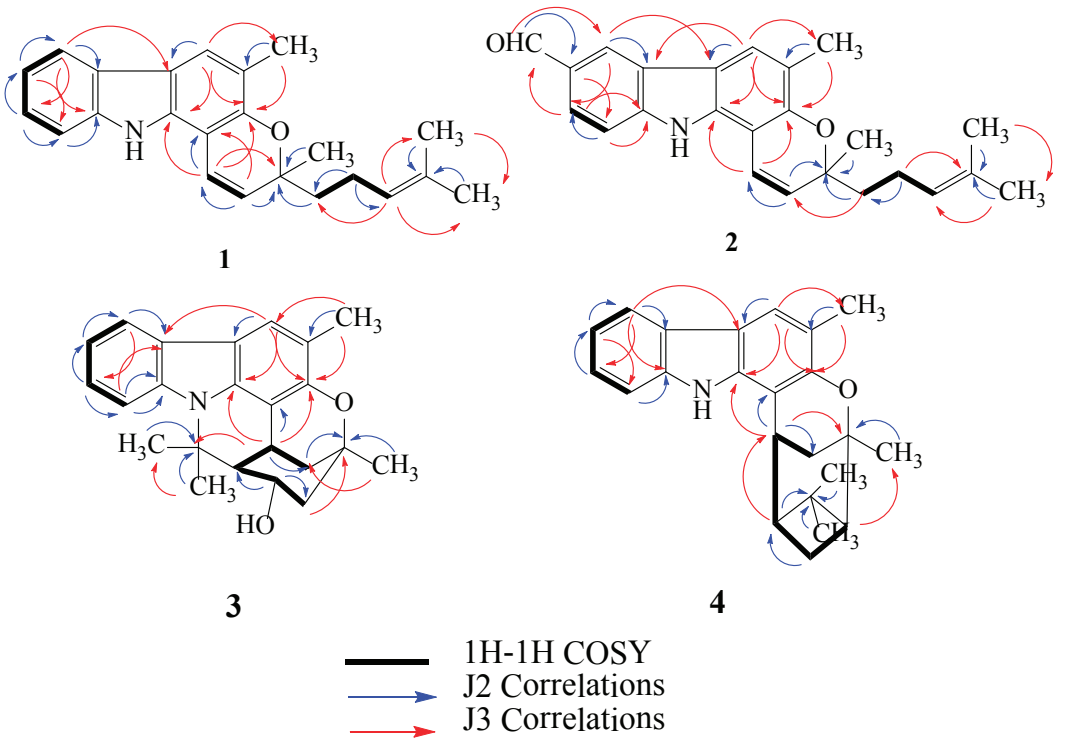

Figure 2 Selected COSY and HMBC correlations in Compound 1, 2, 3, and 4.

Compound **2** was obtained as a pale yellowish oil. This compound was determined to have molecular formula $C_{23}H_{23}NO_2$ by GC-MS. The UV spectrum at 215, 248, 265, 290, 310 nm suggested that this compound was thought to be a 6-oxygenated pyranocarbazole derivatives (Wu *et al*., 1996) . Examination of the aromatic region of the ^{1}H NMR spectrum suggested that compound **2** was also similar to that of **1**, which is a 2-substituted-1-alkoxy-6-formylcarbazole. The ^{1}H NMR spectrum of compound **2** showed a broad downfield signal at *d* 8.35 deduced to the presence of N-H in the carbazole nuclues. A characteristic aldehydic singlet at *d* 10.07 in ^{1}H NMR spectrum together with a strong carbonyl band at 1675 cm^{-1} revealed a formyl group attached to C-6. In the aromatic region of the ^{1}H NMR spectrum, there were sets of ABX mutually-coupled proton system at *d* 8.46 (1H, *d*, J = 1.7, H-5), *d* 7.89 (1H, *dd*, J = 8.6, 1.7, H-7), and *d* 7.47 (1H, *d*, *J* = 8.6, H-8), which were deshielded by an aldehyde substituent at C-6 in the C-ring. An AB type spin system at *d* 6.66 (*d*, *J* = 9.7 Hz), 5.71 (*d*, *J* = 9.7 Hz) for H-9 and H-10, respectively, was affecting an oxygenated substituent at C-2. This gave further evidence for the characteristic of pyranocarbazole skeleton (Wu *et al*., 1996). Conversely, the signals in the high field region contained signals for a methyl group at *d* 1.47 (s) and one prenyl group (2-methylpent-2-

en-5yl substituent) adjacent to an oxygen in pyran ring of the pyranocarbazole at C-11 with 5.10 (*t*, *J* = 6.3 Hz, H-3'), 2.16 (*m,* H-2'), 1.80 (*m*, H-1'), and a long-range coupling with geminal dimethyls at 1.68 (s, 4'-Me) and 1.58 (s, 4'-Me). The ^{13}C NMR spectrum (Table 2) indicated the presence of 23 carbon resonances, a C_{23}-carbazole. The complete assignments of carbon signals and location of substituent on the pyranocarbazole skeleton of **2** was achieved on the basis of DEPT, ^{1}H-^{1}H COSY , HMQC and HMBC spectra (Figure 1). The structure of compound **2** was identified as murrayamine-J (Wu *et al*., 1996).

Compound **3** was obtained as a brown solid. This compound was determined to have molecular formula $C_{23}H_{25}NO_2$ by GC-MS. The UV spectrum at 245, 265 and 305 nm was readily recognized it as C_{23}-pyranocarbazole derivative from its preliminary spectral data (Bhattacharyya *et al*., 1989). This also indicated a similar pyranocarbazole framework. The IR spectrum showed absorption peaks at 3360 cm^{-1} thus confirmed the pyranocarbazole structure with an additional OH group. The ^{1}H NMR spectrum of murrayazolinol exhibited signals for an aromatic methyl group at d 2.17 (H-3), and confirmed an aromatic substitution pattern similar to mahanimbine **1**. The differences between compound **1** and **3** were the additional substitution of a monoterpenoid moiety and the absence of N-H signal in compound **3**. The ^{1}H NMR spectrum of the monoterpenoid moiety of murrayazolinol showed a signal for the carbinyl hydrogen of a secondary alcohol, which appeared as a doublet (*J* = 7.2 Hz) at d 3.80. The ^{13}C NMR spectrum (Table 2) indicated the present of 23 carbons resonances, a C_{23}-carbazole. The complete assignments of carbon signals and location of substituent on the skeleton of **3** was supported on the basis of DEPT, ^{1}H-^{1}H COSY , HMQC and HMBC spectra (Figure 2). Based on the spectroscopic data, structure **3** was assigned to murrayazolinol. (Bhattacharyya *et al*., 1989).

Compound **4** had the molecular formula $C_{23}H_{25}NO$ by GC-MS. The UV spectrum showed characteristic absorbance at 242, 255, 260, and 305 nm for the typical absorption of a 2-hydroxy-3-methylcarbazole chromophore. The IR spectrum of compound **4** indicated the presence of N-H (3455 cm^{-1}), C-CH_3 (2850 cm^{-1}) and C-O (1156 cm^{-1}). The aromatic region of the ^{1}H NMR spectrum resembled that of mahanimbine **1**, indicating a similar aromatic substitution pattern on the carbazole skeleton. However, the monoterpene moiety fused at C-1 and C-2 was different. In consideration of the molecular formula and the absence of olefinic protons present in mahanimbine **1**, bicyclomahanimbine **4** was proposed as a hexacyclic base with a cyclobutane system. The presence of a cyclobutane ring was supported by the downfield shift of the methyl group at 4'-CH_3 to d 0.74 in bicyclomahanimbine when compared to the methyl group of mahanimbine at d 1.62. The ^{13}C NMR spectrum (Table 2) also showed the presence of 23 carbon resonances. The complete assignments of carbon signals and location of substituent on the skeleton of **4** was deduced on the basis of DEPT, ^{1}H-^{1}H COSY , HMQC and HMBC spectra (Figure 2). Based on these spectroscopic data, the structure **4** was assigned to bicyclomahanimbine (Kureel *et al*., 1969).

Conclusions

The chemical constituents of *Murraya koenigii* (TM 1006) collected from Jerantut, Pahang consisted of Four C_{23}-carbazole alkaloids which were isolated and identified as

mahanimbine, murrayamine-J, murrayazolinol and bicyclomahanimbine**.** The spectral data of the compound was also compared with those in the literature.

Acknowledgement

We gratefully acknowledged the generous support and excellent technical assistance of Sultan Idris Education University.

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