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CYCLIC MONOTERPENOID PYRANOCARBAZOLE ALKALOIDS FROM THE BARK OF *Murraya koenigii* (L.) SPRENG

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Graphical abstract

Abstract

Murraya koenigii, locally known as "kari" tree, was selected for the phytochemical analysis. The extraction and isolation of the carbazole alkaloids from its *n*-hexane extract of the bark was carried out by using various chromatographic techniques. Three cyclic monoterpenoid pyranocarbazole alkaloids namely, murrayazolinol, murrayakoeninol and bicyclomahanimbine, were isolated. Their structures were determined on the basis of 1D-, 2D-NMR, IR, UV and mass spectrometry, and by comparison with the data from literature.

Keywords: Rutaceae, *Murraya koenigii*, pyranocarbazole alkaloids, phytochemicals

Abstrak

Murraya koenigii, tempatan dikenali dengan nama sebagai pokok "kari", telah dipilih untuk analisis fitokimia. Pengekstrakan dan pengasingan alkaloid karbazola daripada ekstrak *n*-heksana kulit kayu telah dijalankan dengan menggunakan pelbagai teknik kromatografi. Tiga alkaloid siklik monoterpenoid piranokarbazola, iaitu murrayazolinol, murrayakoeninol dan bisiklomahanimbina, telah diasingkan. Struktur mereka telah ditentukan berdasarkan 1D-, 2D-NMR, IR, UV dan spektrometri jisim, dan perbandingan dengan data daripada literatur.

Kata kunci: Rutaceae, *Murraya koenigii*, alkaloid piranokarbazola, fitokimia

1.0 INTRODUCTION

Among the 14 species under the genus *Murraya* [1], *Murraya koenigii* (Linn.) Sprengis one of the member belonging to the Rutaceae family found in Malaysia [2, 3]. *M. koenigii* is a medicinal important herb of Indian origin. It has been widely used as natural flavouring in curries and sources [3-5], and ingredient in traditional medicine formulations [4-6]. Phytochemical screening on *M. koenigii* by various researchers showed that alkaloids, carbohydrates, protein, amino acids, saponins, flavonoids and coumarins were present in various parts of the plant [7]. Carbazole alkaloids are the major constituents from the plant were known to possess various biological activities such as antitumor, anti-oxidative, anti-mutagenic, and anti-inflammatory activities [6-9]. Cyclic monoterpenoid pyranocarbazole alkaloid is

one of the groups of carbazole alkaloids which can be isolated from the bark of *M. koenigii* [10-14].

In continuation of our studies on carbazole alkaloids of *M. koenigii* [10], we report the isolation of carbazole alkaloids present in the bark of *M. koenigii*. Three cyclic monoterpenoid pyranocarbazole alkaloids were afforded (Figure 1), *viz.*, murrayazolinol **1**, murrayakoeninol **2** and bicyclomahanimbine **3**. Their structures were elucidated by combination of various spectroscopic methods such as 1D and 2D NMR, IR, UV and mass spectrometry.

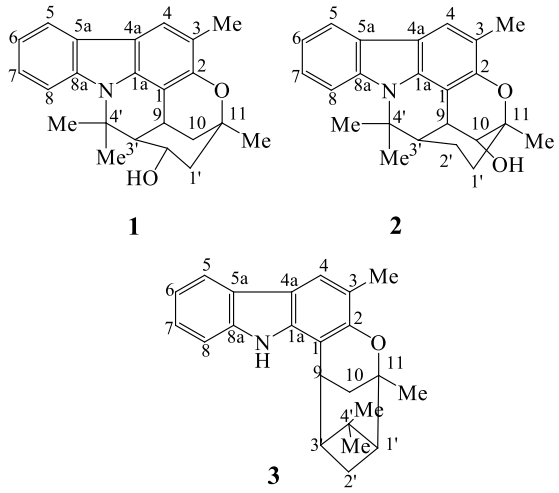

Figure 1 Structures of compound **1**, **2** and **3**

2.0 EXPERIMENTAL

2.1 Plant Materials

The bark of *M. koenigii* (L.) Spreng was collected from Jerantut, Pahang, Malaysia in early 2012. The voucher specimen (TM1006) was identified by the phytochemistry group and deposited at the herbarium of Chemistry Department, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, Malaysia.

2.2 Extraction and Isolation

The dried barks (3.0 kg) of *M. koenigii* were extracted with *n*-hexane and dichloromethane (CH_2Cl_2), three times each, continuously at room temperature. The extracts were combined and were concentrated under reduced pressure to yield brown syrup of *n*-hexane crude extract (106.0 g) and CH_2Cl_2 crude extract (32.0 g). After evaporation of the solvent, 30.0 g of the*n*-hexane crude extract was subjected to column chromatography over silica gel (gradient solvent system: hexane, CH_2Cl_2 and MeOH) to yield a known cyclic monoterpenoid carbazole alkaloid: murrayazolinol [**1**, R_f = 3.4 in CH_2Cl_2:MeOH (99:1)]. The CH_2Cl_2 crude extract (30.0 g) was subjected to column chromatography over silica gel (gradient solvent system: *n*-hexane, CH_2Cl_2 and MeOH) to yield another two known cyclic monoterpenoid carbazoles: murrayakoeninol [**2**, R_f = 3.3 in CH_2Cl_2: MeOH(99:1)] and bicyclomahanimbine [**3**, R_f = 5.1 in Hex:CH_2Cl_2 (1:1)].

2.3 SpectroscopicCharacterization

Different spectroscopic methods were used to elucidate the structure of isolated compounds. Among the spectroscopic techniques UV, IR, ^{1}H-NMR, ^{13}C-NMR, DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC and LC-MS were carried out.

Murrayazolinol, **1**. Brown solid. Melting point,: 289–290°C[11]. UV λ_{max} (MeOH) nm (log ε): 248 (4.84), 265 (4.82), 305 (4.40). IR ν_{max} cm^{-1} : 3360, 1650, 1620, 1380. HRESIMS: calcd for $C_{23}H_{25}NO_2$, *m/z* 348.1964 $[M+H]^+$, found 384.1957. ^{1}H and ^{13}C NMR see Table 1 and Table 2, respectively.

Murrayakoeninol, **2**. Pale yellow oil. UV λ_{max} (MeOH) nm (log ε): 214 (4.50), 275 (4.15), 308 (4.10). IR ν_{max} cm^{-1} : 3405, 1635, 1455, 1330, 1160. HRESIMS: calcd for $C_{23}H_{25}NO_2$, *m/z* 348.1964 $[M+H]^+$, found 384.1943. ^{1}H and ^{13}C NMR see Table 1 and Table 2, respectively

Bicyclomahanimbine, **3**. Brown solid. Melting point,: 145–147°C [13]. UV λ_{max} (MeOH) nm (log ε): 242 (4.20), 255 (4.35), 265 (4.15), 305 (4.40). IR ν_{max} cm^{-1} : 3455, 2850, 1625, 1605, 1350, 1156. HRESIMS: calcd for $C_{23}H_{25}NO$, *m/z* 332.2014 $[M+H]^+$, found 332.2008.^{1}H and ^{13}C NMR see Table 1 and Table 2, respectively.

3.0 RESULTS AND DISCUSSION

The bark of *M. koenigii* collected from Jerantut, Pahang gave three C_{23}-carbazole alkaloids. The fractionation of the *n*-hexane extract of the bark by column chromatography and preparative TLC afforded murrayazolinol**1**. The fractionation of the CH_2Cl_2 extract of the bark by column chromatography and preparative TLC afforded another two compounds: murrayakoeninol**2**, and bicyclomahanimbine**3**. All compounds gave blue to purple colour on spraying with 10% sulphuric acid at 105°C. They also showed similar spectroscopic features with the carbazole alkaloids published in the literature [11-14].

Compound **1** was isolated as a brown solid with melting point at 289–290°C (m.p.: 290°C [11]). Its molecular formula was determined to be $C_{23}H_{25}NO_2$ by HRESIMS. The absorption bands at 248, 265 and 305 nm in the UV spectrum confirmed it to have pyranocarbazole framework [15]. The IR spectrum showed absorption peaks at 3360 cm^{-1}further confirmed the pyranocarbazole skeleton [15] with an additional OH group. The ^{1}H NMR spectrum of **1** showed a signal for an aromatic methyl group at δ_H 2.36 (3-CH_3). In addition, the ^{1}H NMR spectrum also

exhibited signal for a carbinyl hydrogen of a secondary alcohol, which appeared as a multiplet at δ_H 3.83. The ^{13}C NMR spectrum (Table 2) indicated the present of 23 carbons resonances and determined to be a C_{23}-carbazole derivative. The complete assignments of carbon signals and location of substituent on the skeleton of **1** was supported by DEPT, ^1H-^1H COSY, HMQC and HMBC spectra. Based on the spectroscopic data, structure **1** was suggested to be murrayazolinol [11].

Compound **2** with molecular formula $C_{23}H_{25}NO_2$ by its HERSIMS (*m/z*: 348.1943), displayed absorption bands with UV at λ_{max}214, 275 and 308 nm indicating the presence of an oxygenated carbazole chromophore [15]. The ^{13}C NMR spectrum of **2** (Table2) displayed 23 carbon signals which suggested to be a C_{23}-carbazole derivative. The ^1H NMR spectrum (Table 1) showed signals for five aromatic methine protons, of which one appeared as singlet at δ_H 7.50 (H-4), two as doublets at δ_H 7.89 (*J* = 8.0 Hz, H-5) and δ_H7.47 (*J* = 8.1 Hz, H-8) and two as triplet of doublets at δ_H 7.16 (*J* = 8.1, 1.2 Hz, H-6) and δ_H 7.26 (*J* = 8.1, 1.2 Hz, H-7). The HMBC spectrum of **2** suggested the presence of an unsubstituted carbazole ring by showing correlation of the four aromatic methine protons with neighboring carbons resembling those in **1**. The complete assignments for **2** were confirmed by ^1H-^1H COSY and HMBC spectra. The ^1H NMR spectrum showed signal for one aromatic methyl at δ_H 2.23, one methyl attached to a tetra-substituted oxygen bearing group at δ_H1.49 and one *gem*-dimethyl group at δ_H 1.30 and 1.92. The position of OH substituent was further confirmed by the HMBC spectrum with the correlations of the aliphatic methyl protons with mutually coupled methylene protons and the OH bearing carbon. It also showed the correlation between H-10 [δ_H 3.82 (*d*, *J* = 8.3) and C-1 (δ_C 105.4) to give further evidence to confirm the OH group position. Compound **2** was closely resemblance to compound **1** except for the position of OH substituent. Based on the spectroscopic data and comparison with literature, structure **2** was assigned to murrayakoeninol, of which have been isolated previously by Chakraborty research group [12].

Table 1 ^1H NMR [500 MHz, δ_H (*J*, Hz)] of Compound **1**, **2** and **3** in $CDCl_3$

Position	δ_H, *J* Hz					
	1	**[11][a]**	**2**	**[12][b]**	**3**	**[14][c]**
NH					7.42 (*br s*)	8.57 (*br s*)
4	7.49 (*s*)		7.50 (*s*)	7.50 (*s*)	7.67 (*s*)	7.64 (*s*)
5	7.90 (*d*, 7.5 Hz)		7.89 (*d*, 8.0 Hz)	7.86 (*d*, 7.8 Hz)	7.92 (*d*, 8.0 Hz)	7.94 (*d*, 7.7 Hz)
6	7.16 (*td*, 6.9, 1.2 Hz)		7.16 (*td*, 8.1, 1.2 Hz)	7.16 (*t*, 7.8 Hz)	7.16 (*td*, 8.0, 1.2 Hz)	7.18 (*t*, 7.4 Hz)
7	7.25 (*td*, 7.0, 1.2 Hz)		7.26 (*td*, 7.0, 1.2 Hz)	7.27 (*m*)	7.28 (*td*, 8.0, 1.2 Hz)	7.30 (*t*, 7.6 Hz)
8	7.47 (*d*, 8.0 Hz)		7.47 (*d*, 7.8 Hz)	7.48 (*d*, 7.8 Hz)	7.37 (*d*, 7.5 Hz)	7.38 (*d*, 8.0 Hz)
9	3.32 (*m*)		3.32 (*m*)	3.32 (*m*)	3.29 (*d*, 9.2 Hz)	3.30 (*d*, 9.5 Hz)
10	1.22 (*m*)		3.82 (*d*, 8.3 Hz)	3.83 (*d*, 8.3 Hz)	2.06 (*m*)	2.06-2.09 (*m*)
3-Me	2.36 (*s*)		2.23 (*s*)	2.36 (*s*)	2.34 (*s*)	2.36 (*s*)
11-Me	1.50 (*s*)		1.49 (*s*)	1.49 (*s*)	1.44 (*s*)	1.45 (*s*)
1'	1.76 (*dd*, 16.1, 6.9 Hz)		1.53 (*m*)	1.55 (*m*)	2.50 (*t*, 7.4 Hz)	2.51 (*t*, 6.6 Hz)
2'	3.83 (*m*)	3.80 (*m*)	1.74 (*dd*, 16.1, 7.5 Hz)	1.73 (*m*)	1.65 (*m*)	1.64-1.76 (*m*)
3'	2.17 (*m*)		2.18 (*m*)	2.19 (*m*)	2.70 (*t*, 7.5 Hz)	2.71 (*t*, 7.3 Hz)
4'-Me	1.29 (*s*)		1.30 (*s*)	1.29 (*s*)	0.74 (*s*)	0.75 (*s*)
4'-Me	1.93 (*s*)		1.92 (*s*)	1.93 (*s*)	1.56 (*s*)	1.57 (*s*)

All assignments are based on DEPT, COSY, HMQC and HMBC.
[a]Full ^1H NMR spectral data was not available.
[b]^1H NMR spectral data recorded at 600MHz in $CDCl_3$.
[c]^1H NMR spectral data recorded at 500MHzin $CDCl_3$.

Compound **3** was identified as bicyclomahanimbine with molecular formula $C_{23}H_{25}NO$ by its HERSIMS at *m/z* 332.2008. The UV spectrum showed characteristic absorbance at 242, 255, 265, and 305 nm for the typical absorption of a 2-hydroxy-3-methylcarbazole chromophore [14]. The IR spectrum of compound **3** indicating 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 ^1H NMR spectrum (Table 1) resembled that of murrayazolinol **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 bicyclomahanimbine **3** 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 δ_H 0.74 in bicyclomahanimbine when compared to the methyl group of mahanimbine at δ_H 1.62. The ^{13}C NMR spectrum (Table 2) also showed the present of 23 carbons resonances. The complete assignments of carbon signals and location of substituent on the skeleton of **3** was deduced on the basis of DEPT, ^1H-^1H COSY , HMQC and HMBC spectra. Based on these spectroscopic data and comparison with literature, the structure **3** was assigned to bicyclomahanimbine [13, 14].

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

Position	δ_C, *J* Hz					
	1	**[11]**[a]	**2**	**[12]**[b]	**3**	**[14]**[c]
1	105.3		105.4	105.3	106.4	106.3
1a	142.4		142.4	142.3	137.8	137.8
2	153.3		153.3	153.2	153.3	153.4
3	118.5		118.5	118.4	120.1	120.0
4	119.7		119.7	119.6	119.4	119.3
4a	115.1		115.1	114.9	124.2	124.2
5	120.1		120.1	119.9	119.3	119.3
5a	127.2		127.2	127.1	115.8	115.7
6	119.5		119.5	119.4	119.3	119.3
7	123.1		123.1	123.0	124.0	123.9
8	113.6		113.6	113.5	110.4	110.3
8a	140.7		140.7	140.6	139.3	139.2
9	36.9		36.9	36.8	37.5	37.3
10	21.5		72.2	72.1	38.4	38.3
11	79.5		79.5	79.4	83.6	83.5
3-Me	15.5		15.5	15.4	16.8	16.7
11-Me	25.1		25.2	24.9	27.5	27.4
1'	36.0		21.5	21.4	46.5	46.4
2'	72.2		36.0	35.9	25.7	25.6
3'	49.5		49.5	49.4	37.9	37.8
4'	60.6		60.6	60.5	39.5	39.3
4'-Me	23.1		23.1	23.0	18.7	18.6
4'-Me	30.2		30.2	30.1	35.2	35.1

Note: All assignments are based on DEPT, COSY, HMQC and HMBC.
[a] ^{13}C NMR spectral data was not available.
[b] ^{13}CNMR spectral data recorded at 150 MHz in $CDCl_3$.
[c] ^{13}CNMR spectral data recorded at 125 MHz in $CDCl_3$.

4.0 CONCLUSION

In conclusion, the present study was aimed to investigate the chemical constituents of *M. koenigii* (TM 1006) collected from Pahang, Malaysia. Three cyclic monoterpenoid carbazole alkaloids were isolated and identified as murrayazolinol **1**, murrayakoeninol **2** and bicyclomahanimbine **3.** The spectral data of the compounds were also compared with those in the literature.

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