Carbazole Alkaloids from Stem Bark of *Murraya koenigii* (L.) Spreng

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Abstract: *Murraya koenigii* (L.) Spreng (curry patta) has different therapeutic uses and rich source of carbazole alkaloids. Phytochemical studies on the stem bark of *M. koenigii* yielded one new carbazole alkaloid, afifine, along with two known carbazole alkaloids, mahinimbine and girinimbine. These compounds were isolated using chromatographic methods and identified using spectroscopic techniques.

Keywords: Carbazole, afifine, Curry patta.

1. INTRODUCTION

Murraya koenigii (L.) Spreng., commonly known as "Curry patta" belongs to the family Rutaceae [1]. This plant is widely distributed in India, Bangladesh, Malaysia, Sri Lanka and Pakistan [2]. Traditionally, it is used as cooking ingredient and also for the treatment of different diseases such as dysentery, diarrhea and snake bite [3, 4]. *M. koenigii* is known to be the richest source of carbazole alkaloids. The previous investigations resulted in the isolation of different carbazole alkaloids from leaves, stem and roots possessing various biological activities such as anti-tumor, anti-oxidative, anti-mutagenic and anti-inflammatory activities [5, 6, 7]. The aim of the present study is to report the isolation of one new and two known carbazole alkaloids from the stem of this plant.

2. EXPRIMENTAL

2.1. Plant Material

Stem bark of *Murraya koenigii* was collected from University of Karachi Campus and identified by Dr. Rubina Dawar, taxonomist, Botany Department, University of Karachi. The voucher specimen (86445) was deposited at the herbarium of the Department of Botany, University of Karachi, Pakistan.

2.2. General Experimental

Ultra violet (UV) spectra were recorded in methanol in Shimadzu UV-160A, UV visible spectrophotometer. Infrared (IR) spectra were measured on JASCO 302-A Infrared spectrophotometer. Low resolution electron impact mass spectra were recorded on Finnigan-MAT-311A mass spectrometer, coupled with PDP 11/34 computer system. High resolution mass measurements and fast atom bombardment (FAB) mass measurements were carried out on Jeol-JMS-HX 110 mass spectrometer. Glycerol was used as matrix and cesium iodide (CsI) as internal standard in FAB for accurate mass measurements. ^{1}H-NMR (Bruker Aspect AM 600) spectra were recorded at 600MHz and ^{13}C-NMR spectra at 150 MHz on Bruker AM-600 nuclear magnetic resonance spectrometers using $SiMe_4$ as an internal standard. Column chromatography was performed on silica gel (Si 60, 70-230 mesh, E. Merck). Precoated silica gel GF_{254} preparative plates (20×20, 0.5 mm thick; E. Merck) were used for preparative thick layer chromatography. Purity of the samples was also checked on the same precoated plates.

2.3. Extraction and Isolation

The stem bark (3kg) of *M. koenigii* was cut in small pieces and socked in ethanol (95%) for fifteen days at room temperature. The extract was then concentrated under reduced pressure to a gummy mass (268 g) and partitioned with *n*-hexane, ethyl acetate and methanol successively to yield 38.06 g of *n*-hexane extract, 88.3 g of ethyl acetate extract and 46.50 g of methanol extract. The ethyl acetate fraction was then subjected to vacuum liquid chromatography (VLC) on silica gel (Si60, $GF_{254,}$ E. Merck) to yield six fractions: (**a**) Pure hexane, (**b**) hexane: ethyl acetate (1:1), (**c**) pure ethyl acetate (**d**) ethyl acetate: methanol (3:1), (**e**) ethyl acetate: methanol(1:1), and (**f**) pure methanol.

Fraction **b** (3.7 gm) was subjected to column chromatography over silica gel. Column was eluted at gradient solvent system of (*n*-hexane: ethyl acetate; (8.5:1.5) and as a result 46 fractions were obtained. From fraction 27 three compounds were isolated. Two compounds, afifine **I** (0.06 mg) and mahanimibine **II** **(**0.038 mg) were purified through thick layer chromatography using ethyl acetate: *n*-hexane (2:8).

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and girnimbine **III** (0.07 mg) was purified using *n*-hexane: ethyl acetate (6:4).

Afifine **I:** Light brown crystals; UV (MeOH) λ_{max} nm: 236(4.35), 289(3.82); IR (KBr) v_{max} cm^{-1} 3423, 1645, 1458, 1308; EIMS *m/z*: 387.557, 248.0, 276.2, 318.2, 262.1, 125.1, 69.1; ^{1}H and ^{13}C- NMR spectral data are summarized in Table **1**.

Mahaminbine **II** white solid. UV (MeOH) λ_{max} nm: 237 (4.38), 287(3.81). IR (KBr) v_{max} cm^{-1}: 3334, 1643, 1614, 1445, 1153, 1120, 781. EIMS *m/z* 249.96, 263.94, 234.92, 68.04, 54.99.^{1}H and ^{13}C- NMR spectral data are summarized in Table **1**.

Girinimbine **III** white solid. UV (EtOH) λ_{max} nm: 237, 287, 327, 342, 358, 384, IR (KBr) v_{max} cm^{-1}: 3317, 2974, 1642, 1609, 1495. EIMS *m/z*: 248.00, 194.1, 69.0.^{1}H and ^{13}C- NMR spectral data are summarized in Table **1**.

3. RESULTS AND DISCUSSION

Compound **I** was isolated as light brown crystals and molecular formula was established as $C_{27}H_{33}NO$ by HR-FAB-MS showing molecular ion peak *m/z* 387.6120 $[M+H]^{+}$ (calcd. for $C_{27}H_{33}NO$, 387.6110). The UV at λ_{max} 236 nm and IR spectrum at 3423 cm^{-1} corroborated the presence of NH group which indicated

Table 1: ^{13}C and ^{1}NMR Spectral Data of Compound I, II, III

	Compound I		Compound II		Compound III	
Position	**^{13}C-NMR**	**^{1}H-NMR**	**^{13}C-NMR**	**^{1}H-NMR**	**^{13}C NMR**	**^{1}H-NMR**
1	119.63	6.92(d, *J*=10.2)	118.9	6.95(d, *J*=10.2)	105.3	6.88(d, *J*=10.2)
2	128.9	5.75(d, *J*=10.2)	128.8	5.72(d, *J*=10.2)	129.7	5.98(d, *J*=10.2)
3	78.84	-	78.3		76.4	
4	150.54		150.9	-	150.4	-
5	118.7		124.3	-	124.3	-
6	120	7.7(s)	121.7	7.71(s)	121.7	7.74(s)(d, *J*=7.8)
6a	105	-	117.3	-	117.4	
7a	124.4	-	118.0	-	118.2	
7	119.63	7.8(d, *J*=7.8)	119.8	7.93(d, *J*=7.9)	119.5	8.12(d, *J*=7.8)
8	119.83	7.10(t, *J*=7.8)	119.6	7.09(t, *J*=7.2)	119.8	7.29(t, *J*=7.5)
9	121.7	7.25(t, *J*=7.2)	124.8	7.25(t, *J*=7.2)	124.8	7.50(t. *J*=7.2)
10	111.33	7.40(t, *J*=7.8)	111.3	7.40 (d, *J*=8.4)	111.3	7.63(d, *J*=7.8)
10a	140.97	-	136.3	-	136.2	
11a	136.32	-	140.9	-	140.9	
11b	105.7		105.1	-	105.3	-
1'	38.5	1.81(m)	41.5	1.74(m)		
2'	29.2	1.18(d, *J*= 7.8)	23.4	2.31(m)		
3'	39.5	1.27(br s)	125.7	5.1(t, *J*=7.2)		
4'	37.65	1.45(m)	131.8			
5'	23.43	2.19(m)				
6'	125	5.12(t, *J*=7.2)				
7'	131.3	-				
CH_3	16.22	2.29(s)				
OCH_3	26	1.43(s) (C-3)				
CH_3	21.99	0.88(s)				
CH_3	17.55	1.55(s)				
CH_3	25.75	1.62(s)				
3-CH_3			16.24	2.15(s)	16.1	2.27(s)
CH_3			26.15	1.43(s)	27.7	1.52(s)
4'-CH_3			17.55	1.62(s)		
4'-CH_3			25.75	1.82(s)		
11CH_3					27.7	1.49(S)
NH		10.2(s)		10.2(s)		10.2(s)

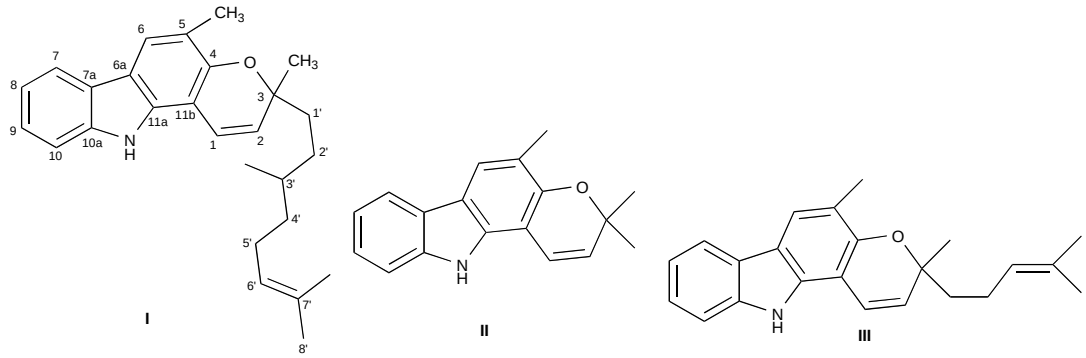

Figure 1: The structure of three carbazole alkaloids isolated from stem of *Murraya koenigii.*

the presence of carbazole nucleus in the molecule. NH group was also confirmed by a singlet at δ 10.2. The ^{1}H- NMR showed two doublets at δ 7.93 (*J*=7.8), δ 7.40 (*J*=7.8), two triplet at δ 7.10 (*J*=7.2), δ 7.45 (*t*, J=7.2) and one singlet at δ 7.7 of one hydrogen each indicating the presence of five aromatic protons in basic structure of carbazole. A singlet of aryl methyl at δ 2.29 was observed. Two proton of six membered oxygen bearing ring gave two doublets at δ 6.95 (d, *J*=10.2) and δ 5.75 (d, *J*=10.2) showing the presence of double bond in the ring [7]. ^{1}H-NMR spectrum presented a similar pattern to that of mahanimbine [8] except the presence of an open side chain in place of prenyl chain which was also confirmed by the HMBC correlations of H-2$^{/}$ to C-1$^{/}$ and H-CH$_3$ to C-3$^{/}$ & C-4$^{/}$. On the basis of all these evidences, the structure of **I** was assigned as 3-(3',7'-dimethyloct-6-en-1yl)-3,5-dimethyl-3,11-dihydropyrano[3,2-a]carbazole (**afifine**).

Compound **II** (Figure **1**) was isolated as white solid. Its molecular formula was determined as $C_{23}H_{25}NO$ by HR-FAB-Ms (Pos) which showed an $[M+H]^+$ ion at *m/z* 331.4510. The UV spectrum was observed at λ_{max} 237 and 287 nm and IR spectrum at 3334 cm^{-1} corroborating the presence of carbazole nucleus in the molecule. The spectral data (MS, UV, IR, ^{1}NMR) of **II** was identical with the previously reported literature values [8] of mahanimibine isolated from this plant.

Compound **III** (Figure **1**) was isolated as white solid and molecular weight was found to be 263.3 *m/z* $[M+H]^+$ (calcd. *m/z* 263.3337) evaluated by the help of by HR-EIMS. The compound **III** was identified as girinimbine by comparing its spectral data (MS, UV, IR, ^{1}NMR) with previously reported literature values [9]. This compound was also earlier isolated from the same plant.

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