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Macrocyclic diarylheptanoids from *Garuga pinnata*

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Abstract

Three macrocyclic diarylheptanoids, 6′-hydroxygaruganin V (**1**), 9′-desmethylgarugamblin I (**2**) and 1,9′-didesmethylgaruganin III (**3**) were isolated from the petroleum ether and dichloromethane extracts of the stem bark of *Garuga pinnata*. The structures of these compounds were established by extensive spectroscopic studies, including high field NMR and MS measurements.

Keywords: *Garuga pinnata*; Burseraceae; Diarylheptanoids

1. Introduction

Garuga pinnata Roxb. (Bengali name- Silbhadi, Nibhadi, Paharijya, Dabudabi; Family- Burseraceae) is a small tree that grows mostly in mountainous districts and semi evergreen forests of Bangladesh, India, Malaysia and the Philippines (Huq and Hasan, 1987). It is used in indigenous medicines to treat various diseases, including asthma, opacity of cornea and pulmonary infections (Chopra et al., 1959). Previous chemical studies with *G. pinnata* revealed occurrences of several triterpenoids (Venkatraman et al., 1994) and biphenyl ether and biphenyl types macrocyclic diarylheptanoids (Venkatraman et al., 1993). As a part of our continuing studies of medicinal plants of Bangladesh, we investigated *G. pinnata* and we, herein, report the isolation and structure elucidation of three new macrocyclic diarylheptanoids, (**1**–**3**), in addition to the ubiquitous β-sitosterol (Morales et al., 2003) and 21-hydroxydammar-5,24-diene-3-one (Fattorusso et al., 1985).

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2. Results and discussion

Extensive chromatographic separation and purification of the petroleum ether and dichloromethane extracts of the stem bark of *G. pinnata* provided three new macrocyclic diarylheptanoids identified as 6′-hydroxygaruganin V (**1**), 9′-desmethylgarugamblin I (**2**) and 1,9′-didesmethylgaruganin III (**3**) in addition to β-sitosterol (Morales et al., 2003) and 21-hydroxydammar-5,24-diene-3-one (Fattorusso et al., 1985). The structures of the isolated compounds were deduced by extensive NMR and mass spectral analyses and by comparison with related compounds.

The HREIMS of compound **1** showed the molecular ion peak at *m*/*z* 354.1514 corresponding to the molecular formula, $C_{21}H_{22}O_5$. This was 16 atomic mass unit higher than that of garuganin V (**4**, $C_{21}H_{22}O_4$) (Venkatraman et al., 1993). The ^{1}H NMR spectrum of compound **1** (Table 1) was almost identical to that of garuganin V (**4**) suggesting a close structural similarity between these two compounds. However, it showed the presence of two hydroxyl group signals at δ 5.74 and 7.37 instead of one hydroxyl resonance observed in garuganin V (**4**). The ^{1}H NMR spectral

Table 1
^{1}H and ^{13}C NMR spectral data for macrocyclic diarylheptanoids **1**–**3** in $CDCl_3$

Position	**1**		**2**		**3**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	151.6	–	146.6		149.0	–
1-OMe	–	–	56.2	3.95 *s*	–	–
2	116.5	6.83 *d* (8.0)	111.7	6.82 *d* (8.0)	154.3	–
2-OMe	–	–	–	–	61.3	4.05 *s*
3	129.8	7.02 *dd* (8.0, 2.0)	121.2	6.65 *dd* (8.0, 2.0)	108.3	6.36 *d* (1.2)
4	131.3	–	133.8	–	137.1	–
5	133.3	7.08 *d* (2.0)	113.2	5.62 *d* (2.0)	106.2	5.15 *d* (1.2)
6	124.2	–	150.9	–	153.8	–
7	28.9	2.91 *m*	27.5	2.92 *m*	27.9	2.87 *m*
	–	3.32 *m*	–	2.92 *m*	–	2.87 *m*
8	28.8	2.66 *m*	38.0	2.32 *m*	37.7	2.34 *m*
	–	2.91 *m*	–	2.32 *m*	–	2.34 *m*
9	174.0	–	197.6	–	196.8	–
9-OMe	55.6	3.58 *s*	–	–	–	–
10	103.4	5.74 *s*	103.1	4.94 *s*	103.1	4.93 *s*
1′	140.8	–	154.3	–	154.4	–
1′-OMe	61.5	3.69 *s*	–	–	–	–
2′	124.2	–	123.3	7.00 *d* (8.0)	123.1	6.98 *d* (8.2)
3′	127.5	6.52 *d* (2.0)	130.6	7.18 *d* (8.0)	130.6	7.18 *d* (8.2)
4′	136.9	–	136.7	–	136.9	–
5′	114.0	6.77 *d* (2.0)	130.6	7.18 *d* (8.0)	130.6	7.18 *d* (8.2)
6′	148.3	–	123.3	7.00 *d* (8.0)	123.1	6.98 *d* (8.2)
7′	28.7	2.45 *m*	32.2	3.04 *t* (7.0)	32.2	3.04 *t* (6.6)
	–	4.36 *m*	–	3.04 *t* (7.0)	–	3.04 *t* (6.6)
8′	42.9	2.91 *m*	39.5	2.47 *t* (7.0)	39.4	2.46 *t* (6.6)
	–	3.14 *m*	–	2.47 *t* (7.0)	–	2.46 *t* (6.6)
9′	200.0	–	189.2	–	188.8	–
OH	–	5.74 *s*	–	–	–	–
		7.37 *s*				

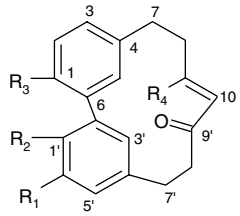
^{1}H NMR: **1**, 400 MHz; **2** and **3**, 600 MHz; ^{13}C NMR: **1**, 100 MHz; **2** and **3**, 150 MHz.

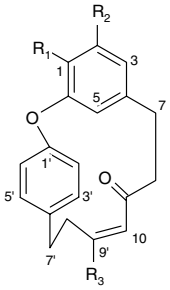
data of **1** revealed a complex second order splitting pattern in the region of δ 2.45–4.36 (8H), which were assigned to four methylene group protons (Table 1). The resonances for the methoxyl groups were observed at δ 3.58 and 3.69. A singlet at δ 5.74 (1H) was ascribed to the proton attached to the α-carbon (C-10) of the α,β-unsaturated carbonyl group. The aromatic region of the spectrum extended from δ 6.52 to 7.08 that integrated for a total of 5 protons. The doublet and a double doublet centered at δ 6.83 (1H) and 7.02 (1H) were assigned to H-2 and H-3, respectively. The meta coupled ($J = 2.0$ Hz) resonances at δ 6.52, 6.77 and 7.08 (each 1H) were attributed to the protons H-3′, H-5′ and H-5, respectively. The methylene protons appeared as well resolved signals at δ 2.45, 2.66, 2.91 (3H), 3.14, 3.32 and 4.36. With the help of ^{1}H–^{1}H COSY and HSQC experiments the methylene protons pair and their respective attached carbons were assigned as 2.45 and 4.36 (δc 28.7), 2.66 and 2.91 (δc 28.8), 2.91 and 3.14 (δc 42.9) and 2.91 and 3.32 (δc 28.9). These signals were eventually attributed to C-7′, C-8, C-8′ and C-7 methylene groups, respectively. Two broad singlets of one proton intensity at δ 5.74 and 7.37 were demonstrative of the phenolic hydroxyl protons at C-6′ and C-1, respectively. The ^{13}C NMR spectrum of compound **1** (Table 1) exhibited signals for four methylene carbons at δ 28.7, 28.8, 28.9 and 42.9 and two methoxyl groups at δ 55.6 and 61.5 at C-9 and C-1′, respectively. The signals at δ 103.4 and at 174.0 and 200.0 were ascribed to the α (C-10), β (C-9) and carbonyl carbon (C-9′) of the α,β-unsaturated carbonyl group. The remaining five signals at δ 114.0, 116.5, 127.5, 129.8 and 133.3 were assigned to the unsubstituted aromatic carbons. The signals at δ 124.2 (2C), 131.3, 136.9, 140.8, 148.3 and 151.6 could be attributed to the substituted aromatic carbons. From the above spectral data and by comparing these values with those published for related compound garuganin V (**4**) (Venkatraman et al., 1993), compound **1** was characterized as 6′-hydroxygaruganin V (**1**).

The molecular formula of compound **2** was deduced as $C_{20}H_{20}O_4$ from ESIMS data at m/z 325 for $[M+H]^+$. Again this was 14 atomic mass unit lower than that of garugamblin I (**5**, $C_{21}H_{22}O_4$) (Keserue and Nogradi, 1993). The ^{1}H NMR spectral data of compound **2** (Table 1) was similar to that of garugamblin I (**5**), indicating a close structural similarity between **2** and **5**. However, compound **2** revealed the presence of one methoxyl group signal instead of two observed in garugamblin I (**5**). The ^{1}H NMR spectrum of compound **2** showed a complex second order splitting of signals at δ 2.32 (2H, *m*), 2.47 (2H, *t*, $J = 7.0$ Hz), 2.92 (2H, *m*) and 3.04 (2H, *t*, $J = 7.0$ Hz) which were assigned to the C-8, C-8′, C-7 and C-7′ methylene group protons,

respectively. The signal for the lone methoxyl group in compound **2** was observed at δ 3.95 (3H, *s*). A singlet at δ 4.94 (1H) corresponded to the proton attached to the α-carbon (C-10) of the α,β-unsaturated carbonyl group, while the resonances at δ 7.00 and 7.18 (2H each, doublet) were assigned to the *para*-substituted benzene ring protons in view of the chemical shifts and coupling constant ($J = 8.0$ Hz). The double doublet centered at δ 6.65 ($J = 8.0$ and 2.0 Hz) was assigned to H-3, while the doublets at δ 5.62 ($J = 2.0$ Hz) and 6.82 ($J = 8.0$ Hz) were demonstrative of the trisubstituted benzene ring protons, H-5 and H-2, respectively. The ^{13}C NMR spectrum of compound **2** (Table 1) suggested the presence of a carbon skeleton similar to that of garugamblin I (**5**). Thus, it showed four methylene groups at δ 39.5, 38.0, 32.2, 27.5 and a methoxyl group at δ 56.2. The ^{13}C spectrum also exhibited signals for eight methines at δ 130.6 (2C), 123.3 (2C), 121.2, 113.2, 117.7 and 103.1. The singlets at δ 197.6 and 189.2 were assigned to the carbonyl (C-9) and β-carbon (C-9′) of the α,β-unsaturated carbonyl group. The resonances at δ 154.3, 150.9, 146.6, 136.7 and 133.8 were attributed to the substituted aromatic carbons, C-1′, C-6, C-1, C-4′ and C-4, respectively. The assignments of the proton and carbon resonances in compound **2** were made by careful analyses of the ^{1}H–^{1}H COSY, HSQC and HMBC spectral data. In the HSQC spectrum the methoxyl group signal at δ 3.95 showed correlation to a carbon at δ 56.2, while it revealed a ^{3}J interaction with δ_C 146.6 in the HMBC experiment. The latter also demonstrated connectivities from H-2 (δ 6.82), H-3 (δ 6.65) and H-5 (δ 5.62). This confirmed the placement of the methoxyl group at C-1. Comparison of the ^{13}C and ^{1}H NMR spectral data of compound **2** and garugamblin I (**5**) indicated one less methoxyl group in compound **2** than garugamblin I (**5**) (Keserue and Nogradi, 1993). On this basis, the structure of compound **2** was resolved as 9′-desmethylgarugamblin I.

The HREIMS of compound **3** displayed the molecular ion peak at 340.1308 that analyzed for $C_{20}H_{20}O_5$. This was 28 atomic mass unit lower than that of garugannin III ($C_{22}H_{24}O_5$, **6**) (Mishra et al., 1985). The ^{1}H NMR spectral data of compound **3** (Table 1) was similar to that of garuganin III (**6**) suggesting a close structural similarity between these two structural analogs. However, the ^{1}H NMR spectrum of compound **3** revealed the presence of one methoxyl signal at δ 4.05 instead of three methoxyl resonances observed in garuganin III (**6**) (Table 1). The ^{13}C NMR of compound **3** (Table 1) suggested the presence of a carbon skeleton similar to that of garuganin III (**6**) (Mishra et al., 1985). It exhibited four methylene groups at δ 27.9, 32.2, 37.7 and 39.4, which were assigned to the C-7, C-7′, C-8 and C-8′, respectively. The ^{13}C NMR signal at δ 149.0 could be assigned to the oxygenated quaternary carbon, C-1. The spectrum also displayed signals for seven methines at δ 130.6 (C-3′ and C-5′), 123.1 (C-2′ and C-6′), 108.3 (C-3), 106.2 (C-5) and 103.1 (C-10). The singlets at δ 196.8 and 188.4 were assigned to the carbonyl (C-9) and β-carbon (C-9′) of the α,β-unsaturated carbonyl group, respectively. The resonances at δ 154.8, 154.3, 153.8, 148.5, 137.1 and 136.9 could be accounted for the substituted aromatic carbons, C-1′, C-2, C-6, C-1, C-4 and C-4′, respectively. The assignments of the proton and carbon resonances in compound **3** were made by careful analysis of the HSQC and HMBC spectral data. The methoxyl group at δ 4.05 demonstrated HSQC correlation to δ 61.3 and a HMBC connectivity to 154.3 (C-2). On this basis and by comparing these values with those reported for related compound, garuganin III (**6**) (Mishra et al., 1985), compound **3** was identified as 1, 9′-didesmethylgarugamblin III.





1. $R_1 = R_3 = OH$; $R_2 = R_4 = OMe$

2. $R_1 = OMe$; $R_2 = H$; $R_3 = OH$

4. $R_1 = H$; $R_3 = OH$; $R_2 = R_4 = OMe$

3. $R_1 = R_3 = OH$; $R_2 = OMe$

5. $R_1 = R_3 = OMe$; $R_2 = H$

6. $R_1 = R_2 = R_3 = OMe$

3. Experimental

3.1. General experimental procedures

NMR spectra were acquired using the Ultra shield Bruker DPX 300 or 400 or 600 NMR instrument. The spectra were recorded in $CDCl_3$ and the chemical shifts are reported in ppm with respect to TMS or residual non deutarated solvent signals. Mass spectra were obtained on a JEOL DX 300 spectrometer.

3.2. Plant material

Stem bark of *G. pinnata* was collected from National Botanical Garden, Dhaka, in April, 2002. It was identified by Dr. Mahbuba Khanam, Principal Scientific Officer, Bangladesh National Herbarium, Dhaka, where a voucher specimen has been deposited (DACB no. 30,734) representing this collection. The bark was first sun dried and then ground to a coarse powder using a grinding machine.

3.3. Extraction and isolation

The powdered bark (500 g) of *G. pinnata* was successively extracted with petroleum ether (2.25 l), dichloromethane (1.75 l) and methanol (1.75 l) in a Soxhlet apparatus. A por-

tion of the petroleum ether extract (1.5 gm) was subjected to column chromatography (CC) over Sephadex LH- 20 (Lipophilic) and the column was eluted with *n*-hexane–dichloromethane–methanol (2:5:1) mixtures (700 ml) to give a total of 70 fractions, each 10 ml. Compound **1** (12 mg) was isolated from the column fractions 16–20 as white crystalline mass by PTLC over silica gel F_{254} (mobile phase – 25% ethyl acetate in petroleum ether, thickness of plates – 0.5 mm). An aliquot of the dichloromethane extract (2 gm) was chromatographed over silica gel (Kiesel gel 60H) and the vacuum liquid chromatography (VLC) column was eluted with petroleum ether, ethyl acetate and methanol mixtures of increasing polarities to give a total of 14 fractions, each 100 ml. Compound **2** (10 mg) was isolated as white gum from fraction-7 by preparative thin layer chromatography over silica gel F_{254}, using 7.5% ethyl acetate in toluene as the mobile phase. Similar purification of fraction-9 using toluene–ethyl acetate (87.5:12.5) provided compound **3** as gummy mass (5.3 mg). β-sitosterol (21 mg) was isolated as colorless mass from fraction-5 by PTLC over silica gel F_{254} using toluene–ethyl acetate (90:10) as the developing solvents where as 21-hydroxydammar-5,24-diene-3-one (15 mg) was obtained as yellowish gum through PTLC of fraction 10 over silica gel F_{254}, using toluene–ethyl acetate (75:25) as the mobile phase.

3.3.1. 6′-Hydroxygaruganin V (*1*)

White crystalline mass; $[\alpha]_{20}^{D} - 10.9$ (*c*. 1.0; $CHCl_3$); for ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* (rel. int.): 355 ($[M+H]^+$, 22), 354 (75), 255 (10), 241 (27), 240 (18), 99 (100); HREIMS *m/z* 354.1514 $[M]^+$(Calc. for $C_{21}H_{22}O_5$, 354.1467).

3.3.2. 9′-Desmethylgarugamblin I (*2*)

White gum; $[\alpha]_{20}^{D} + 6.43$ (*c*. 0.28; $CHCl_3$); for ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z*: 325 ($[M+H]^+$, 60), 324 (100), 281 (16), 240 (33), 239 (32), 211 (13), 120 (15), 90 (16), 85 (12), 77 (12); ESIMS *m/z* 325 $[M+H]^+$ (Calc. for $C_{20}H_{20}O_4$, 324.1361).

3.3.3. 1,9′-Didesmethylgaruganin III (*3*)

Gummy mass; $[\alpha]_{20}^{D}$; −1.0 (*c*. 0.5; $CHCl_3$); for ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z*: 341 ($[M+H]^+$, 50), 340 (100), 256 (16), 255 (37), 241 (36), 128 (11), 121 (11), 107 (16), 91 (11), 77 (10); HREIMS *m/z* 340.1308 $[M]^+$(Calc. for $C_{20}H_{20}O_5$, 340.1311).

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