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GARUGANIN III, A MACROCYCLIC BIPHENYL ETHER FROM *GARUGA PINNATA*

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Key Word Index—*Garuga pinnata*; Burseraceae; macrocyclic biphenyl ether; garuganin III.

Abstract—The structure of garuganin III, a novel macrocyclic biphenyl ether isolated from *Garuga pinnata*, was established by spectroscopic analyses, including ^{1}H NMR and ^{13}C NMR decoupling experiments and chemical transformations.

INTRODUCTION

Garuga pinnata is used in indegenous medicine to cure asthma, opacity of the cornea, etc. [1]. In an earlier article [2] we reported the isolation and structure of garuganin I (**1a**) from the leaves and stem bark of this plant. The structure of garuganin I was confirmed by X-ray studies. Several other compounds were isolated from this plant one of these being garuganin III. In this paper we present the isolation and structure elucidation of garuganin III (**2a**) on the basis of spectral and chemical evidence. The data are compared with those of garuganin I to deduce the structure.

RESULTS AND DISCUSSION

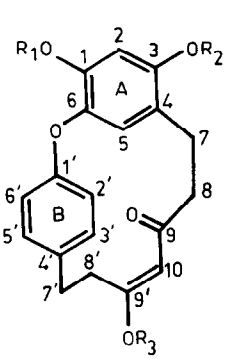
A hot *n*-hexane extract of *G. pinnata* leaves on CC over silica gel yielded several fractions using EtOAc–*n*-hexane (1:9 and 1:3) for elution, which showed the presence of two compounds (in varying amounts) other than chlorophyll. The compound with higher R_f value was found to be garuganin I (**1a**) and the other was garuganin III (**2a**). These two compounds were separated by several fractional crystallizations from solvents to give crystals of garuganin III and the mother liquor was rich in garuganin I. Garuganin III was finally recrystallized from methanol to give needles, mp 176–177°.

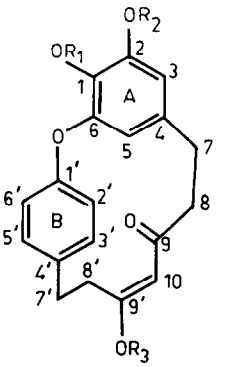
A hot *n*-hexane extract of stem bark on CC over silica gel yielded a fraction containing large amounts of garuganin III (**2a**) after elution with EtOAc–*n*-hexane (1:3) which was purified by several fractional recrystallizations from ether and, finally, from methanol.

The elemental and mass spectral analysis, $C_{22}H_{24}O_5$ ($[M]^+$ *m/z* 368) showed garuganin III (**2a**) to be an isomer of garuganin I (**1a**). The IR spectrum showed the presence of an α,β unsaturated carbonyl group (ν_{max} cm^{-1}: 1670), aromatic rings (ν_{max} cm^{-1}: 1590, 1570 and 1500) and aromatic ether (ν_{max} cm^{-1}: 1270, 1245 and 1210). The UV spectrum indicated the presence of a β-methoxy-α,β-unsaturated carboxyl group (λ_{max}^{MeOH} nm 275.)

The ^{1}H NMR spectrum (Table 1) of **2a** showed signals at δ2.74 (2H, *m*), 2.84 (4H, *m*) and 3.1 (2H, *t*). These were assigned to four methylene groups. The signals for three methoxyl groups were located at δ3.45, 3.84 and 3.94 (3H each, *s*). The double doublets at δ6.95 and 7.27 (2H each) were ascribed to protons of a *para*-substituted benzene ring in view of the coupling constants (J = 8 and 2 Hz). The signal at δ5.20 (1H, *s*) corresponded to the proton attached to the α-carbon (C-10) of the α,β-unsaturated carbonyl group. Two broad doublets at δ4.9 (H-5) and 6.28 (H-3) were assigned to protons of a tetrasubstituted benzene ring. These two doublets (having *meta*-coupling, J = 1.5 Hz) were broad due to the presence of long range benzylic coupling. Furthermore, the proton at δ4.9 (H-5) was heavily shielded due to the anisotropic effect of the carbonyl group.

The proton noise decoupled and proton off-resonance decoupled ^{13}C NMR spectra (Table 2) of **2a** suggested the presence of a carbon skeleton similar to that of garuganin I (**1a**). It had four methylene groups (at δ44.30, 31.16, 28.58, 26.97, *t*), three methoxyl groups (at δ61.15, 56.15, 55.62, *q*). There were signals for seven methine groups (doublets) at δ131.17 (for two), 123.45 (for two), 106.22, 105.33 and 102.10. The singlets at δ198.57 and 173.78 were assigned to the carbonyl (C-9) and β-carbon (C-9′) of the





1a $R_1 = R_2 = R_3 = Me$ **2a** $R_1 = R_2 = R_3 = Me$

1b $R_1 = R_2 = Me$, $R_3 = H$ **2b** $R_1 = R_2 = Me$, $R_3 = H$

1c $R_1 = R_2 = R_3 = H$ **2c** $R_1 = R_2 = R_3 = H$

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Table 1. ^{1}H NMR chemical shifts in ppm relative to TMS*

	1a	1b	1c	2a	2b	2c
H-5	5.32 (1H, *s*)	5.64 (1H, *s*)	5.58 (1H, *s*)	4.90 (1H, *br d*, $J=1.5$)	5.26 (1H, *br d*, $J=1.5$)	5.24 (1H, *br*)
H-2	6.45 (1H, *s*)	6.50 (1H, *s*)	6.44 (1H, *s*)	—	—	—
H-3	—	—	—	6.28 (1H, *br d*, $J=1.5$)	6.30 (1H, *br d*, $J=1.5$)	6.38 (1H, *br*)
H-7	2.45 (2H, *m*)	2.32 (2H, *m*)	2.36 (2H, *m*)	2.84 (2H, *m*)	2.32 (2H, *m*)	2.36 (2H, *m*)
H-8	2.76 (2H, *m*)	2.83 (2H, *m*)	2.74 (2H, *m*)	2.84 (2H, *m*)	2.91 (2H, *m*)	2.74 (2H, *m*)
H-10	5.26 (1H, *s*)	4.94 (1H, *s*)	5.09 (1H, *s*)	5.20 (1H, *s*)	4.93 (1H, *s*)	4.98 (1H, *s*)
H-2′	6.86 (1H, *dd*, $J=8, 2$)	6.95 (1H, *dd*, $J=8, 2$)	6.87 (1H, *dd*, $J=8, 1$)	6.95 (1H, *dd*, $J=8, 2$)	6.96 (1H, *dd*, $J=8, 2$)	7.00 (1H, *dd*, $J=8, 2$)
H-3′	6.98 (1H, *dd*, $J=8, 2$)	7.15 (1H, *dd*, $J=8, 2$)	7.20 (1H, *dd*, $J=8, 1$)	7.27 (1H, *dd*, $J=8, 2$)	7.21 (1H, *dd*, $J=8, 2$)	7.20 (1H, *dd*, $J=8, 2$)
H-5′	7.36 (1H, *dd*, $J=8, 2$)	7.15 (1H, *dd*, $J=8, 2$)	7.20 (1H, *dd*, $J=8, 1$)	7.27 (1H, *dd*, $J=8, 2$)	7.21 (1H, *dd*, $J=8, 2$)	7.20 (1H, *dd*, $J=8, 2$)
H-6′	6.91 (1H, *dd*, $J=8, 2$)	6.95 (1H, *dd*, $J=8, 2$)	6.87 (1H, *dd*, $J=8, 1$)	6.95 (1H, *dd*, $J=8, 2$)	6.96 (1H, *dd*, $J=8, 2$)	7.00 (1H, *dd*, $J=8, 2$)
H-7′	2.97 (2H, *m*)	2.42 (H, *t*, $J=6.5$)	2.47 (2H, *t*, $J=6.5$)	2.74 (2H, *m*)	2.44 (2H, *m*)	2.47 (2H, *m*)
H-8′	2.18 (1H, *m*) 4.01 (1H, *m*)	3.01 (2H, *t*, $J=6.5$)	3.02 (2H, *t*, $J=6.5$)	3.10 (2H, *t*, $J=6.5$)	3.03 (2H, *m*)	3.02 (2H, *m*)
–OMe	3.63 (3H, *s*)	—	—	3.45 (3H, *s*)	—	—
	3.76 (3H, *s*)	3.80 (3H, *s*)	—	3.84 (3H, *s*)	3.86 (3H, *s*)	—
	3.95 (3H, *s*)	3.97 (3H, *s*)	—	3.94 (3H, *s*)	3.96 (3H, *s*)	—
–OH	—		7.80 (1H, *br*)	—	15.10 (1H, *br*)	7.80 (1H, *br*)
		15.20 (1H, *br*)	8.11 (1H, *br*)			8.00 (1H, *br*)

*All assignments were performed by double resonance experiments. J values are in Hz.

Table 2. ^{13}C NMR chemical shifts (in δ-values)

	1a	2a
C-1	146.25 (*s*)	135.62 (*s*)
C-2	101 (*d*)	154.71 (*s*)
C-3	151.2 (*s*)	105.33 (*d*)
C-4	129.95 (*d*)	136.95 (*s*)
C-5	117.2 (*d*)	106.22 (*d*)
C-6	137.7 (*s*)	153.19 (*s*)
C-1′	156 (*s*)	155.24 (*s*)
C-2′, C-6′	124.2, 122.0 (*d*)	123.45 (*d*, for two)
C-3′, C-5′	130.74, 131.0 (*d*)	131.17 (*d*, for two)
C-4′	145.71 (*s*)	137.44 (*s*)
C-7	44.30 (*t*)	44.30 (*t*)
C-7′	33.2 (*t*)	31.16 (*t*)
C-8	32.9 (*t*)	28.58 (*t*)
C-8′	19.1 (*t*)	26.97 (*t*)
OMe-3	56.42 (*q*, for two)	61.15 (*q*)
	55.1 (*q*)	56.15 (*q*)
		55.62 (*q*)
C-9	196.92 (*s*)	198.57 (*s*)
C-9′	172.63 (*s*)	173.78 (*s*)
C-10	97.61 (*d*)	102.10 (*d*)

α,β-unsaturated carbonyl. The singlets at δ155.24, 154.71, 153.19, 137.44, 136.95 and 135.62 were ascribed to the substituted aromatic carbons. These assignments were made by application of chemical shift rules.

Garuganin III was hydrolysed by acid to give a single product (**2b**). Elemental analysis and mass spectral data (m/z 354, $C_{21}H_{22}O_5$) indicated the loss of a methylene group on hydrolysis. The IR spectrum showed bands at ν_{max} cm^{-1}: 1615 (probably due to a hydrogen bonded α,β-unsaturated carbonyl group), 1590, 1570, 1510 (aromatic stretching) and 1220 (aromatic ether). The ^{1}H NMR spectrum (Table 1) of **2b** showed four multiplets at δ2.32 (H-7), 2.44 (H-7′), 2.91 (H-8) and 3.03 (H-8′) and two singlets for two methoxyl groups. The three signals at δ4.93, 5.26 and 6.3 were assigned to H-10, H-5 and H-3, respectively. It was observed that, on hydrolysis, there was a downfield shift of H-5. This could be the result of rotation introduced around the carbonyl group during the reaction. The four aromatic protons of the *para*-substituted benzene ring were located at δ6.96 and 7.21. This spectrum was very similar to that of the ^{1}H NMR spectrum of the hydrolysed product of garuganin I (**1b**) except for the signals at δ5.26 and 6.30 which were doublets (J = 1.5 Hz) due to *meta*-coupling.

Garuganin III was demethylated with boron tribromide to give **2c**, mp 190–191°, $C_{19}H_{18}O_5$ ([M]$^+$ m/z 326) indicating loss of three methylene groups from **2a**. The ^{1}H NMR spectrum of **2c** was very similar to that of **2b** except that the signals for the methoxyl groups were absent.

The ^{13}C NMR and ^{1}H NMR spectra of garuganin III and its derivatives were compared with those of garuganin I (Tables 1 and 2). From this comparison it was concluded that their basic skeleton is almost same. The structure of garuganin III is different from that of garuganin I only in the substitution pattern of ring A, as proposed in structure **2a**.

EXPERIMENTAL

Mps are uncorr. NMR spectra were recorded at 100 MHz using TMS as int. standard.

Garuganin III (**2a**). Colourless needles, mp 176–177°. UV λ_{max}^{MeOH} nm: 215, 250 and 275. IR ν_{max}^{Nujol} cm^{-1}: 1670, 1590, 1570, 1500, 1270, 1245, 1210, 1030, 890, 880 and 810. (Found: C, 71.99; H, 6.57. $C_{22}H_{24}O_5$ requires: C, 71.74; H, 6.51%.)

Hydrolysis. A MeOH soln of **2a** (12 mg in 5 ml MeOH) containing a drop of conc. HCl was refluxed for 4 hr at 100°. The solvent was removed under red. pres. and HCl was removed under vacuum. The single product (**2b**) obtained was recrystallized from MeOH, mp 149–150°. UV λ_{max}^{MeOH} nm: 220 and 273 $\lambda_{max}^{MeOH+AlCl_3}$ nm: 220 and 300; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 220, 300. IR ν_{max}^{Nujol} cm^{-1}: 3450, 1615, 1590, 1570, 1510, 1380 and 1220.

Demethylation. Compound **2a** (25 mg) was dissolved in dry $CHCl_3$ (15 ml). The mixture was cooled to 0° and BBr_3 (1.5 ml) added dropwise with stirring. The resulting mixture was stirred for 5 hr and allowed to warm to room temp. The solvent was evaporated followed by coevaporation with MeOH (3 × 10 ml) to remove B as methyl borate. The reddish residue obtained was dried under vacuum. Further purification was done by recrystallization from MeOH giving brownish needles (15 mg) of **2c**. Mp 190–191°. UV λ_{max}^{MeOH} nm: 219, 278 and 285. IR $\nu_{max}^{Nujol+CHCl_3}$ cm^{-1}: 3560, 3380, 1610, 1590, 1540, 1460, 1455, 1380, 1220, 1020, 930 and 860.

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STRUCTURE REVISION OF THE COUMARIN, CEYLANTIN

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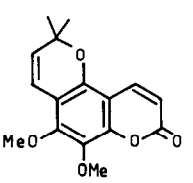
Key Word Index—*Atalantia ceylanica*; Rutaceae; 5,8-dimethoxy-6,7-pyranocoumarin.

Abstract—Ceylantin, isolated from *Atalantia ceylanica*, has been found to be identical with racemosin, isolated from *A. racemosa*, and the revised 5,8-dimethoxy-6,7-pyranocoumarin structure confirmed by nuclear Overhauser experiments.

A $C_{16}H_{16}O_5$ coumarin, ceylantin, mp 126–127° was recently isolated from the heartwood of *Atalantia ceylanica* [1]. The ^{1}H NMR spectrum revealed a pyranocoumarin nucleus and two methoxyl groups but irradiation of H-4 was reported to have no effect on the intensity of either methoxyl signal thereby precluding attachment at C-5 for one of them. Ceylantin was accordingly formulated as the angular pyranocoumarin (**1**).

In 1978, racemosin, mp 125–126°, having the same molecular formula as ceylantin, was isolated from the stem and leaves of *A. racemosa* [2]. The linear pyranocoumarin structure **2** deduced from spectroscopic evidence, was supported by a synthesis. The close similarity in physicochemical properties and botanical origin of ceylantin and racemosin led us to suspect that they might possess the same structure. Direct comparison [mmp, TLC, IR, UV (λ_{max} 222, 262 (sh), 270, 325 nm), ^{1}H and ^{13}C NMR] has now confirmed their identity. Moreover, nuclear Overhauser experiments have revealed that each possesses structure **2**. Separate irradiation of the methoxyl signals at δ3.82 and 3.92 has shown, in each case, that only the former gives an increase in the integrated intensity of both the H-4 and H-4′ signals, by 7% and 7.2%, respectively, consonant with a C-5 methoxyl group on a linear pyranocoumarin.

It has been asserted that trioxygenated coumarins are rare in the Rutaceae and when present the oxygenation is specifically 5,7,8 in *Ruta* and 6,7,8 in *Zanthoxylum* [1].



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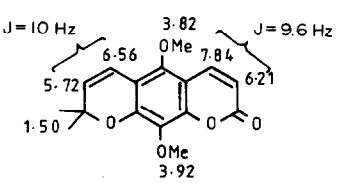

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