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ISOLATION AND STRUCTURE OF A NEW MACROCYCLIC, 15-MEMBERED BIPHENYL ETHER—GARUGANIN-I FROM *GARUGA PINNATA*

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Abstract—A new macrocyclic compound, named as garuganin-I, having unique structural features has been isolated from the n-hexane extract of the leaves and the stem bark of *Garuga pinnata* Roxb. The chemical and spectroscopic evidence, including ^{13}C-NMR indicate structure I for garuganin-I.

Garuga pinnata Roxb belongs to the family Burseraceae. It is used in indigenous medicine to cure several disorders. The juice of the leaves mixed with honey is given for asthma, the juice of the stem is used as a remedy for opacity of the cornea and the decoction of the roots is used for pulmonary infections.[1] The aqueous and ethanolic extracts of the leaves were found to possess very high anti-inflammatory and anti-allergic activities.[2] Hardly any reports on the chemical investigations of this plant are available in the literature except for the report on the isolation of a biflavanoid, amentoflavone, from the leaves by Ansari *et al.*[3] This encouraged us to undertake a systematic investigation of this plant.

In the present paper we are reporting the isolation of a new crystalline macrocyclic compound, named garuganin-I and its structure determination on the basis of its spectral and chemical evidence. Besides this compound, waxes, tannins and four more new crystalline compounds named as garuganin-II (m.p. 184–185°, $C_{22}H_{24}O_5$), garuganin-III (m.p. 176–177°, $C_{22}H_{24}O_5$), garuganin-IV (m.p. 191–192°, $C_{21}H_{22}O_4$) and a minor amount of garuganin-V has been isolated from stem bark and the leaves. Garuganin-II and garuganin-III are isomers of garuganin-I and have been found to be similar in several aspects and structure elucidation of these compounds is in progress.

Isolation

The hot n-hexane extract of the leaves on column chromatography over silica gel yielded several fractions containing chlorophyll, which on several further fractional crystallisations from MeOH yielded colourless crystalline garuganin-I. The hot n-hexane extract of the stem bark on column chromatography over silica gel, in a n-hexane–EtOAc (9:1) solvent system yielded a fraction containing the major amount of garuganin-I, which was purified by repeated fractional crystallisations, m.p. 165–166° (yield from leaves 0.0005%, from stem bark 0.00045%).

Structure

The elemental analysis and MS data established the molecular formula as $C_{22}H_{24}O_5$ for garuganin-I. Its IR spectrum showed the presence of an α,β-unsaturated CO (1680 cm^{-1}) aromatic ring(s) (1610, 1520 and 1500 cm^{-1}) and aromatic ethers (1260 and 1220 cm^{-1}). The UV spectrum indicated the presence of a β-methoxy-α,β-unsaturated CO (λ_{max} 263 nm).

The most helpful information about the structure was obtained from the proton noise decoupled and proton off-resonance decoupled ^{13}C-NMR spectra. The assignments were made by application of chemical shift rules. The signals observed indicated the presence of four methylene groups (triplets) at 44.30, 33.19, 32.90 and 19.06 ppm, three OMe groups (quartets) at 56.42 (for two OMe), 55.10 ppm, seven methine groups (doublets) at 131.00, 130.74, 124.21, 122.08, 117.19, 100.93 and 97.61 ppm. The doublet at 97.61 ppm and singlets at 172.63 and 196.92 ppm were assigned to the α-(C-10), β-(C-9′) and CO(C-9) of the α,β-unsaturated CO respectively. The remaining six doublets were due to the unsubstituted aromatic carbons. The singlets at 155.98, 151.14, 146.25, 145.71, 137.65 and 129.95 ppm were assigned to the substituted aromatic carbons. Thus the presence of two benzene rings was revealed.

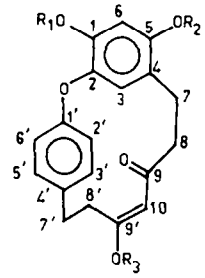
The ^{1}H-NMR spectrum (Table 1) of **1a** showed a complex second order splitting pattern in the region δ 2.01–3.10 (7H) and a multiplet at δ 4.01 (1H). These signals were assigned to four methylene groups, which were arranged as two separate ethylene (—CH_2—CH_2—) groups, with the help of double irradiation experiments. The complex splitting pattern also suggests that these groups are having restricted rotation and the heavily deshielded proton at δ 4.01 (H-8′) probably lies in the deshielding region of an aromatic ring. Irradiation at δ 2.95 (H-7′) resulted in the enhancement of the intensities of the signals at δ 6.86 and 6.91 (H-3′, H-5′) indicating the existence of a long range benzylic coupling. The signals for three OMe groups were located at δ 3.69, 3.76 and 3.95 (s, 3H each). Four aromatic protons were present at δ 6.86, 6.91 (H-3′, H-5′) and at δ 6.98, 7.36 (H-2′, H-6′). From the coupling constant values these were assigned to a *para* substituted benzene ring in which both the pairs of *ortho* protons are deshielded to different extents arising out of a particular orientation of the benzene rings, giving rise to an ABCD system.[4] The signal at δ 5.26 was due to a proton attached to the α-carbon (C-10) of the α,β-unsaturated CO. Two signals at δ 5.32 (s, slightly broad, H-3) and 6.45 (H-6) were assigned to protons of the tetrasubstituted benzene ring. The proton (H-3) is heavily shielded probably due to the anisotropic effect of the CO group and has a long range benzylic coupling.

Garuganin-I was hydrolysed by dil acid to give a single product (**1b**), m.p. 149–150°. The MS data showed highest mass ion at *m/e* 354 ($C_{21}H_{22}O_5$), indicating loss of a methylene group from the original

Table 1. ^{1}H-NMR spectral data of garuganin-I (**1a**) and its derivatives (**1b**, **1c**)[a]

Proton no.	Compound (ppm)		
	1a	**1b**	**1c**
H-3	5.32 (s, 1H)	5.64 (s, 1H)	5.58 (s, 1H)
H-6	6.45 (s, 1H)	6.50 (s, 1H)	6.44 (s, 1H)
H-7	2.45 (m, 2H)	2.32 (m, 2H)	2.36 (m, 2H)
H-8	2.76 (m, 2H)	2.83 (m, 2H)	2.74 (m, 2H)
H-10	5.26 (s, 1H)	4.94 (s, 1H)	5.09 (s, 1H)
H-2′	6.86 (dd, J = 8.2, 1H)	6.95 (dd, J = 8.2, 1H)	6.87 (dd, J = 8.1, 1H)
H-3′	6.98 (dd, J = 8.2, 1H)	7.15 (dd, J = 8.2, 1H)	7.20 (dd, J = 8.1, 1H)
H-5′	7.36 (dd, J = 8.2, 1H)	7.15 (dd, J = 8.2, 1H)	7.20 (dd, J = 8.1, 1H)
H-6′	6.91 (dd, J = 8.2, 1H)	6.95 (dd, J = 8.2, 1H)	6.87 (dd, J = 8.1, 1H)
H-7′	2.97 (m, 2H)	2.42 (t, J = 6.5, 2H)	2.47 (t, J = 6.5, 2H)
H-8′	2.18 (m, 1H)	3.01 (t, J = 6.5, 2H)	3.02 (t, J = 6.5, 2H)
	4.01 (m, 1H)		
OMe	3.69 (s, 3H)	3.80 (s, 3H)	—
	3.76 (s, 3H)	3.97 (s, 3H)	—
	3.95 (s, 3H)	—	—
OH	—	15.20 (br, 1H)	7.80 (br, 1H)
			8.00 (br, 1H)

[a] All assignments were performed by double resonance experiments. J values are in Hz.

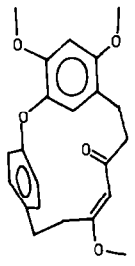


1a : $R_1 = R_2 = R_3 = CH_3$

1b : $R_1 = R_2 = CH_3$, $R_3 = H$

1c : $R_1 = R_2 = R_3 = H$

compound. The IR spectrum showed bands at 1615 cm^{-1} (probably due to a H-bonded α,β-unsaturated CO group), 1610, 1580 and 1500 cm^{-1} (aromatic stretching), 1245 and 1220 cm^{-1} (aromatic ether).



I

The ^{1}H-NMR spectrum (Table 1) of **1b** showed two triplets for an A_2B_2 system at δ 2.42 (H-7′) and 3.01 (H-8′), two multiplets at δ 2.32 (H-7) and 2.83 (H-8) and two singlets for two OMe groups. The three singlets at δ 4.94, 5.64 and 6.50 were assigned to H-10, H-3 and H-6 respectively. It is observed that on hydrolysis there is a down-field shift of H-3 and up-field shift of H-10. Similar shifts are also seen in the NMR spectrum of the demethylated product (**1c**). This could be as a result of varying degrees of rotation introduced around the CO group during the reactions. The four aromatic protons of the *para* substituted benzene ring were located at δ 6.95 and 7.15 and a D_2O exchangeable signal for OH group appeared at δ 15.20.

Since the MF of the hydrolysed product is less by a —CH_2— group it could be concluded that garuganin-I has an easily hydrolysable OMe group. **1b** was not soluble in bicarbonate solution and did not give methyl ester with CH_2N_2 confirming the absence of methyl ester grouping in garuganin-I. The UV spectral studies of **1b** with $AlCl_3$ and HCl shift reagents indicated the presence of an *ortho* keto phenolic or enolic OH group (hence the *ortho* keto phenolic or enolic ether group in garuganin-I). The presence of the *ortho* keto phenolic or enolic group in **1b** was further suggested by the appearance of a keto group at 1615 cm^{-1} and the absence of an OH band in the IR spectrum (due to strong H-bonding).[5] The presence of a D_2O exchangeable proton at δ 15.20 further confirmed the presence of a strongly H-bonded OH group.

Garuganin-I on demethylation with BBr_3 gave a single product (**1c**), m.p. 225–226°. The MS spectral data gave the molecular formula as $C_{19}H_{18}O_5$ (M^+ 326), indicating the loss of three methylene groups from garuganin-I. The ^{1}H-NMR of **1c** was similar to that of **1b** except that the signals for OMe were absent and two D_2O exchangeable peaks at δ 7.80 and 8.00 were observed. The UV spectrum was nearly identical to the spectrum of **1b**.

Out of the five oxygens present in the molecule one oxygen was present as a part of the CO group and three more were present as methyl ethers. The remaining oxygen was resistant to ether cleavage by BBr_3. This indicated that it may be present as an aryl aryl ether (Ar—O—Ar) and its presence was also suggested by the chemical shifts observed for aromatic protons.

The assembly of the structural features discussed above for garuganin-I leads to structure **1a**. The stereo

view(I) for the structure was obtained from the X-ray studies carried out by V. Pattabi *et al.* with the sample provided by us. The details of which are being communicated separately by her.

EXPERIMENTAL

M.ps are uncorrected, ^{13}C-NMR and ^{1}H-NMR of reaction products were recorded on Varian XL-100 FT and double irradiation studies were recorded on Bruker 270 MHz instruments. $CDCl_3$ was used as solvent for NMR except for the demethylated product, where acetone-d_6 was used and TMS was used as internal standard. TLC was carried out on ACME silica gel and ACME silica gel (100–200 mesh) was used for column chromatography which was activated by heating at 120° for 4 hr.

G. pinnata leaves and stem bark were collected from Borivili National Park, Bombay. The leaves were collected during June and July before trees were attacked by galls. The stem bark was collected during April and May. The plant material was shade dried and pulverised to a coarse powder and extracted exhaustively in Soxhlet apparatus with a hot n-hexane–EtOAc–MeOH solvent system in increasing order of polarity. Garuganin-I was obtained from n-hexane–EtOAc (9 : 1) fraction of the column chromatography from leaf extract as a mixture with chlorophyll and as mixture with other garuganins from the stem bark extract. Several fractional crystallisations from MeOH yielded pure garuganin-I.

Garuganin-I

M.p. 165–166°. (Calc: C, 71.74; H, 6.15. Found: C, 71.99; H, 6.57%.) IR (Nujol): 1680, 1610, 1590, 1520, 1500, 1260, 1220, 1030, 875, 860 and 810 cm^{-1}; UV: λ_{max}^{MeOH} 207, 263 and 298 nm.

Hydrolysis of garuganin-I. The methanolic soln of garuganin-I (10 mg) containing a drop of conc HCl was refluxed for 4 hr in a water bath. The solvent and HCl was removed under vacuum. The residue obtained was recrystallised from MeOH. Single product **1b** was obtained, m.p. 149–150°; IR (Nujol): 1615, 1610, 1580, 1500, 1245 and 1220 cm^{-1}; UV: λ_{max}^{MeOH} 217, 283 nm; $\lambda_{max}^{MeOH} + AlCl_3$ 217, 300 nm; $\lambda_{max}^{MeOH} + AlCl_3 + HCl$ 217, 300 nm.

Demethylation of garuganin-I. To the ice-cooled $CHCl_3$ soln of the compound (15 mg), BBr_3 (1 ml) was added dropwise through a dropping funnel while the mixture was being stirred. The resulting mixture was stirred for 4 hr as the ice bath was allowed to warm to room temp. The solvent was evaporated in a water bath in a fume cupboard to dryness, which was followed by co-evaporation with MeOH (3 × 8 ml), which removed all the boron as methyl borate. The reddish brown residue **1c** obtained showed single spot. This was recrystallised from MeOH, m.p. 225–226°; IR (Nujol): 3450, 3200, 1620, 1600, 1510, 1275, 1200 and 875 cm^{-1}; UV: λ_{max}^{MeOH} 217, 285 nm; $\lambda_{max}^{MeOH} + AlCl_3 + HCl$ 217, 302 nm. The ^{1}H-NMR was recorded to 15 δ.

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