

Biflavonoids from *Cycas beddomei*¹⁾

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Chemical investigation on the constituents of the cones of *Cycas beddomei* has resulted in the isolation of a new biflavonoid, 2,3-dihydro-4'''-*O*-methyl amentoflavone, along with 2,3,2'',3''-tetrahydro hinokiflavone, 2,3,2'',3''-tetrahydro amentoflavone, 2,3-dihydro amentoflavone. The last two compounds were not reported earlier from this plant. The structure of the new compound was established by detailed analysis of its spectral (mainly 1D and 2D NMR) data.

Key words *Cycas beddomei*; cycadacea; cone; biflavonoid; 2,3-dihydro-4'''-*O*-methyl amentoflavone

Cycas beddomei DYER is a small shrub available in South India.²⁾ Previously, two biflavonoids, amentoflavone and 2,3,2'',3''-tetrahydro hinokiflavone were reported³⁾ from the leaves of the plant. Biflavonoids have recently been shown⁴⁾ to possess significant cytotoxic activity. So, we were interested to examine the chemical constituents of different parts of the plant and isolated a new biflavonoid, 2,3-dihydro-4'''-*O*-methyl amentoflavone (**1**) along with 2,3,2'',3''-tetrahydro hinokiflavone, 2,3,2'',3''-tetrahydro amentoflavone, 2,3-dihydro amentoflavone from the cones. Here we discuss the structure elucidation of the new compound, **1**.

2,3-Dihydro-4'''-*O*-methyl amentoflavone (**1**) was isolated as yellow crystals. Its molecular formula was deduced to be C₃₁H₂₂O₁₀ from its elemental analysis and mass (*m/z* 555, M⁺+1 in LSI-MS) and ¹³C-NMR spectra (indicating the presence of 31 carbons). Its UV spectrum in methanol showed absorption maxima similar to those of 2,3-dihydro amentoflavone⁵⁾ and the IR spectrum indicated the presence of hydroxyl and carbonyl groups. The NMR (1D and 2D) spectra clearly suggested that the new compound **1** is a monomethyl derivative of 2,3-dihydro amentoflavone. The ¹H-NMR spectra revealed the presence of two chelated hydroxyl groups (δ 13.12, 1H, br s and 12.18, 1H, br s) and a methoxyl group (δ 3.82, 3H, s). H-6 and H-8 appeared at δ 5.90 (1H, d, *J*=2.0 Hz) and 5.98 (1H, d, *J*=2.0 Hz) respectively while H-6'' at δ 6.33 (1H, s). The signals at δ 5.44 (1H, dd, *J*=13.0, 3.0 Hz, H-2), 3.21 (1H, dd, *J*=17.0, 13.0 Hz H-3a) and 2.76 (1H, dd, *J*=17.0, 3.0 Hz, H-3b) as well as at δ 6.59 (1H, s, H-3''') indicated the presence of flavanone and flavone system in the molecule.⁵⁾ The ¹H-NMR spectrum also showed an ABX system (δ 7.62, 1H, d, *J*=2.0, H-2'; 7.05, 1H, d, *J*=8.0 Hz, H-5' and 7.42, 1H, dd, *J*=8.0, 2.0 Hz, H-6') and an AA'BB' system (δ 7.77, 2H, d, *J*=8.0 Hz, H-2''' and H-6''' and 6.95, 2H, d, *J*=8.0 Hz, H-3''' and H-5''') associated with the protons of the rings B and E respectively. All these signals are characteristic of the monomethyl derivative of 2,3-dihydro amentoflavone having 3', 8'' interflavonyl linkage. The above assignments of the proton signals were made with the help of ¹H-¹H COSY and NOESY results. The NOESY experiment (Fig. 1) showed the correlation between H-2 and H-3, H-2 and H-6', H-3 and H-2' and H-5' and H-6' for the flavanone unit. On the other hand, for flavone unit H-3''' was correlated with H-2''', H-2''' with H-3''' and H-3''' with

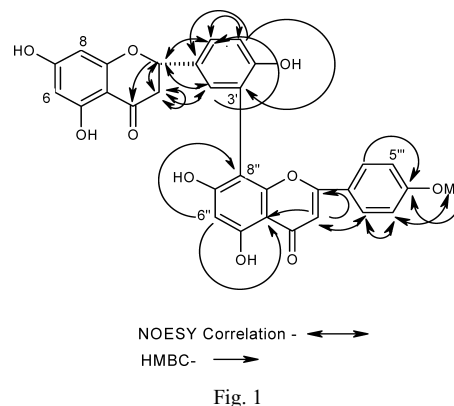
–OMe. These correlation suggested the presence of methoxy group at C-4'''. The ¹³C-NMR spectrum of **1** showed the presence of 31 carbons in the molecule (*vide* Experimental). The positions of these signals supported^{5,6)} the structure of monomethyl derivative of 2,3-dihydro amentoflavone for **1**. The HMBC experiment (Fig. 1) demonstrated that H-5' was related to C-3' (δ 121.2) and C-1' (δ 128.6) while H-2' to C-6' (δ 127.3). In ring D H-6'' showed correlation with C-8'' (δ 105.9) and C-10'' (δ 103.4). The –OMe protons was related to C-4''' (δ 162.5) and the latter was again to H-2'''. The structure of the new biflavonoid **1** was thus clearly established as 2,3-dihydro-4'''-*O*-methyl amentoflavone.

Along with **1** three other known biflavonoids, 2,3,2'',3''-tetrahydro hinokiflavone,³⁾ 2,3,2'',3''-tetrahydro amentoflavone⁷⁾ and 2,3-dihydro amentoflavone⁵⁾ were also isolated. The last two compounds were not reported earlier from the plant. The structures of the known compounds were settled from their spectral data and by comparison of the values with those reported in the literature.

Experimental

General mp uncorr. The spectra were recorded with the following instruments. IR, Perkin-Elmer RX1 FT-IR; NMR, Varian Gemini-200 MHz and LSI-MS: Finnigan-MAT 1020 instrument. The optical rotations were determined with a JASCO DIP-360 polarimeter. Column chromatography was performed with silica gel (BDH 100–200 mesh) and TLC with silica gel GF₂₅₄.

Plant Materials The cones of *C. beddomei* were collected from the Tirumala Hills, Andhra Pradesh in March, 2002 and identified botanically. A voucher specimen (No. *Cycas beddomei* 15202) was deposited in IICT



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herbarium.

Extraction and Isolation The shade dried and powdered cones (2 kg) were extracted with CHCl_3 -MeOH (1 : 1) (3 l) for 3 d at room temperature. The extract was concentrated and extracted with EtOAc-H₂O (4 : 1) (3 × 50 ml). On concentration a gummy residue (42 g) was obtained. The residue was subjected to column chromatography using hexane, CHCl_3 , acetone and MeOH as eluents. The following compounds were obtained according to the increasing order of polarity: 2,3,2'',3''-tetrahydro hinokiflavone (72 mg), 2,3-dihydro-4''-*O*-methyl amentoflavone (**1**) (34 mg), 2,3,2'',3''-tetrahydro amentoflavone (18 mg) and 2,3-dihydro amentoflavone (58 mg). The first compound was eluted with 2% acetone in CHCl_3 and the other three compounds with 5% acetone and 1% MeOH in CHCl_3 . Compound **1** crystallized from MeOH.

2,3-Dihydro-4''-*O*-methyl Amentoflavone (**1**): Yellow crystals, mp 231—232 °C (d), $[\alpha]_D^{25} -0.53^\circ$ ($c=1.33$, MeOH); UV (MeOH): λ_{max} 225, 289, 334 nm (log ϵ : 4.20, 4.16, 4.06); IR (KBr): ν_{max} 3439, 1644, 1460, 1394, 1055, 1014 cm^{-1} , ¹³C-NMR (acetone-*d*₆): δ 80.8 (C-2), 42.3 (C-3), 196.6 (C-4), 168.0 (C-5), 98.4 (C-6), 168.2 (C-7), 98.0 (C-8), 164.5 (C-9), 102.1 (C-10), 128.6 (C-1'), 132.1 (C-2'), 121.2 (C-3'), 157.5 (C-4'), 118.8 (C-5'), 127.3 (C-6'), 163.1 (C-2''), 103.2 (C-3''), 183.4 (C-4''), 161.6 (C-5''), 100.4 (C-6''), 163.7 (C-7''), 105.9 (C-8''), 158.8 (C-9''), 103.4 (C-10''), 124.0 (C-1'''),

128.2 (C-2'''), 114.4 (C-3'''), 162.5 (C-4'''), 56.7 (–OMe); LSI-MS: m/z 555 ($\text{M}^+ + 1$); (Found: C, 76.32; H, 3.91. $\text{C}_{31}\text{H}_{22}\text{O}_{11}$ required: C, 67.15; H, 3.97%).

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References and Notes

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