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A biflavanone from *Cycas beddomei*

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Abstract

A new biflavanone, 7,7′′-di-*O*-methyltetrahydrohinokiflavone together with tetrahydrohinokiflavone were isolated from the stems of *Cycas beddomei*. The structures were established on the basis of spectral and chemical studies.

Keywords: *Cycas beddomei*; Cycadaceae; Stems; Biflavanones

1. Introduction

The genus *Cycas* (Cycadaceae) is rich in biflavonoids (Kariyone & Sawada, 1958; Geiger & Pfleiderer, 1971; Varshney et al., 1973; Gadek, 1982; Sobha Rani, Venkata Rao, Gunasekar, Blond & Bodo, 1998) and some of the *Cycas* species are extensively used in traditional Indian medicine as stimulants, narcotics, aphrodisiac, expectorants and in the treatment of malignant ulcers (Kirthikar & Basu, 1980). *Cycas beddomei* Dyer is a tall shrub endemic to Tirumala hills, Andhra Pradesh, India (Hooker, 1973). Previous investigation of the leaves of this species has led to the isolation of a new biflavanone, tetrahydrohinokiflavone (**2**) along with amentoflavone (Sobha Rani et al., 1998). In the present communication we report the isolation and characterization of a new biflavanone, 7,7′′-di-*O*-methyltetrahydrohinokiflavone (**1**) together with tetrahydrohinokiflavone (**2**) from the stems of *C. beddomei*.

2. Results and discussion

The positive-ion HRFAB mass spectrum of **1** showed pseudomolecular ion at m/z 571.1595 consistent with the molecular formula $C_{32}H_{26}O_{10}$ (corroborated by ^{13}C-NMR spectrum). The UV spectrum of **1** exhibited absorption maxima at 287 and 333 nm characteristic of a flavanone derivative (Mabry, Markham & Thomas, 1970).

The ^{1}H-NMR spectrum of compound **1** showed two sets of ABX signals of a biflavanone at δ 2.79 (1H, *dd*, J = 17.5, 4.1 Hz), 3.21 (1H, *dd*, J = 17.5, 13.1 Hz) and 5.53 (1H, *dd*, J = 13.1, 4.1 Hz), and 2.80 (1H, *dd*, J = 17.6, 4.4 Hz), 3.28 (1H, *dd*, J = 17.6, 13.5 Hz) and 5.54 (1H, *dd*, J = 13.5, 4.4 Hz). The biflavanone skeleton in **1** was further supported by the presence of two benzylic methine carbon signals at 79.8 (C-2) and 80.5 (C-2′′) ppm, two methylene carbon signals at 43.4 (C-3, C-3′′) ppm and two carbonyl carbon signals at 197.5 (C-4) and 198.3 (C-4′′) ppm. Compound **1** formed a triacetate indicating the presence of three hydroxyl groups in **1**. Two D_2O exchangeable signals at δ 12.12 and 12.05 in its ^{1}H-NMR spectrum were attributed to chelated hydroxyls at 5 and 5′′ positions. The non-chelated hydroxyl at δ 8.57 was placed at C-4′′′ as it showed ^2J correlation with the carbon at 158.9 (C-4′′′) and ^3J correlation with the carbons at 116.2 (C-3′′′ and C-5′′′) ppm in its HMBC spectrum (see Fig. 1). The ^{1}H-NMR spectrum of **1** also showed two methoxy signals at δ 3.84 and 3.86 and from HMBC correlations these

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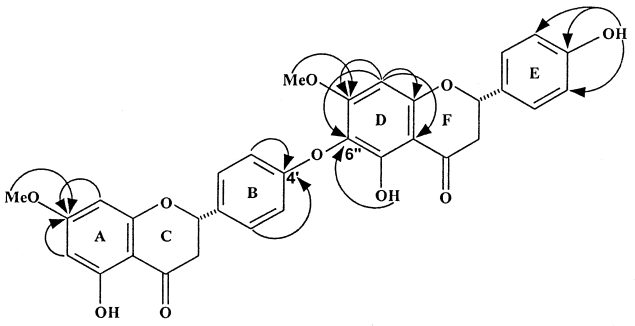
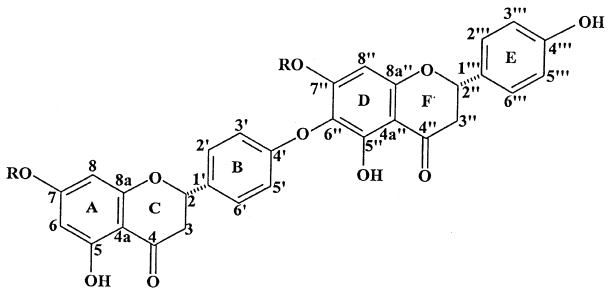


Fig. 1. Major HMBC correlations observed in **1**.

two methoxyls were placed at C-7 and C-7′′ positions as they showed ^{3}J correlations with these carbons at 168.8 and 161.4 ppm, respectively. Two *meta* coupled doublets at δ 6.03 and 6.05 were assigned to C-6 and C-8 protons. Two sets of *ortho* coupled doublets at δ 7.47 (2H, J = 8.7 Hz) and 6.93 (2H, J = 8.7 Hz), and 7.43 (2H, J = 8.5 Hz) and 6.92 (2H, J = 8.5 Hz) were assigned to H-2′, 6′ and H-3′, 5′, and H-2′′′, 6′′′ and H-3′′′, 5′′′, respectively. The presence of a lone aromatic proton singlet at δ 6.33 is consistent with a trisubstituted aromatic D ring and was assigned to either H-6′′ or H-8′′. From HMBC studies this proton was attributed to H-8′′ as it showed ^{2}J correlation with C-7′′ and C-8a′′, and ^{3}J correlation with C-6′′ and C-4a′′.

The foregoing spectral studies suggested that compound **1** could be a biflavanone with an interflavonoid ether linkage between C-4′ of ring B and C-6′′ of ring D. The HMBC spectrum of **1** further confirmed the involvement of C-4′ and C-6′′ in C–O–C linkage as these carbons showed correlations with H-2′, 6′, 3′, 5′, and OH-5′′, H-8′′, respectively. The CD spectrum of **1** exhibited positive and negative maxima at 334 and 288 nm, respectively establishing *S* configuration at 2 and 2′′ positions (Gaffield, 1970). Thus the structure of compound **1** was established as 7,7′′-di-*O*-methyltetrahydrohinokiflavone (**1**).



1 R = Me
2 R = H

3. Experimental

3.1. General

Mps were uncorr. The CD spectrum was recorded in MeOH at 15°C on a Jasco J715 spectropolarimeter. IR spectra were recorded in KBr discs on a Perkin–Elmer 283 double beam spectrophotometer and UV in MeOH on a Shimadzu UV-240 spectrophotometer. ^{1}H and ^{13}C-NMR spectra were determined on a Bruker AC 300 spectrometer using Me_2CO-d_6 and $CDCl_3$ with TMS as int. standard. HMBC spectrum (optimized for 7 Hz) was recorded using standard pulse sequences. EIMS was recorded on a Nermag R10-10 mass spectrometer at 70 eV and HRFAB mass spectrum was obtained on a 700 JEOL mass spectrometer in thioglycerol matrix. CC was performed on Acme silica gel finer than 200 mesh (0.08 mm).

3.2. Plant material

The stems of *C. beddomei* were collected from Tirumala hills, Andhra Pradesh, South India during January, 1997. The herbarium specimen (DG-971) was deposited at the Department of Botany, Sri Venkateswara University, Tirupati.

3.3. Extraction and isolation

Dried and ground stems of *C. beddomei* (2 kg) were successively extracted with *n*-hexane, Me_2CO and MeOH. The Me_2CO extract was defatted with hexane. The residue obtained (500 mg) was CC on silica gel using gradients of C_6H_6 and EtOAc. The C_6H_6 and C_6H_6:EtOAc (9:1) fractions yielded **1** (20 mg) and **2** (50 mg), respectively.

3.4. 7,7′′-Di-O-methyltetrahydrohinokiflavone (**1**)

Colorless needles (Me_2CO), mp. 226–228°C. $[\alpha]_D^{25}$ − 1.45° (MeOH, *c* 1.0). CD (MeOH, *c* 0.12) $[\theta]_{334}$ + 965 (max), $[\theta]_{288}$ − 3263 (max). UV λ_{max}^{MeOH} nm (log ε): 287 (4.40), 333 (3.69). IR ν_{max}^{KBr} cm^{-1}: 3436 (–OH), 2926, 1640 (⟩C=O), 1573, 1509, 1449, 1290, 1159, 1114. ^{1}H-NMR (300 MHz, Me_2CO-d_6) δ: 12.12 (1H, *s*, OH-5), 12.05 (IH, *s*, OH-5′′), 8.57 (1H, *s*, OH-4′′′), 7.47 (2H, *d*, J = 8.7 Hz, H-2′, 6′), 7.43 (2H, *d*, J = 8.5 Hz, H-2′′′, 6′′′), 6.93 (2H, *d*, J = 8.7 Hz, H-3′, 5′), 6.92 (2H, *d*, J = 8.5 Hz, H-3′′′, 5′′′), 6.33 (1H, *s*, H-8′′), 6.05 (1H, *d*, J = 2.3 Hz, H-8), 6.03 (1H, *d*, J = 2.3 Hz, H-6), 5.54 (1H, *dd*, J = 13.5, 4.4 Hz, H-2′′), 5.53 (1H, *dd*, J = 13.1, 4.1 Hz, H-2), 3.86 (3H, *s*, OMe-7′′), 3.84 (3H, *s*, OMe-7), 3.28 (1H, *dd*, J = 17.6, 13.5 Hz, H-3_{ax}′′), 3.21 (1H, *dd*, J = 17.5, 13.1 Hz, H-3_{ax}), 2.80 (1H, *dd*, J = 17.6, 4.4 Hz, H-3_{eq}′′), 2.79 (1H, *dd*, J = 17.5, 4.1 Hz, H-3_{eq}). ^{13}C-NMR (75 MHz, Me_2CO-d_6)

δ: 198.3 (C-4″), 197.5 (C-4), 168.8 (C-7), 165.0 (C-5), 164.1 (C-8a), 161.8 (C-8a″), 161.4 (C-7″), 159.7 (C-4′), 158.9 (C-4‴), 156.9 (C-5″), 132.9 (C-1′), 130.4 (C-1‴), 129.1 (C-2‴, 6‴), 128.8 (C-2′, 6′), 124.7 (C-6″), 116.2 (C-3‴, 5‴), 115.6 (C-3′, 5′), 103.8 (C-4a″), 103.7 (C-4a), 95.5 (C-6), 94.6 (C-8), 92.8 (C-8″), 80.5 (C-2″), 79.8 (C-2), 56.9 (OMe-7), 56.2 (OMe-7″), 43.4 (C-3, 3″). EIMS m/z (rel. int.): 570 $[M]^+$ (25), 450 (4), 404 (10), 391 (13), 285 (25), 269 (14), 193 (31), 167 (84), 120 (40), 43 (100). HRFABMS m/z: $[M+H]^+$ 571.1595 (calcd. 571.1604).

3.5. Acetylation of 1

A mixture of **1** (7 mg), Ac_2O (2 ml) and C_5H_5N (1 ml) was kept at room temperature for 48 h and poured into crushed ice to yield the triacetate of **1** as colorless crystals (9 mg) from $CHCl_3$, mp. 212°. IR ν_{max}^{KBr} cm^{-1}: 2932, 1769, 1681, 1618, 1450, 1370, 1195, 1075. ^{1}H-NMR ($CDCl_3$) δ: 7.50 (2H, *d*, J = 8.7 Hz, H-2′, 6′), 7.43 (2H, *d*, J = 8.5 Hz, H-2‴, 6‴), 7.19 (2H, *d*, J = 8.5 Hz, H-3‴, 5‴), 6.92 (2H, *d*, J = 8.7 Hz, H-3′, 5′), 6.58 (1H, *s*, H-8″), 6.40 (1H, *d*, J = 2.3 Hz, H-8), 6.28 (1H, *d*, J = 2.3 Hz, H-6), 5.50 (1H, *dd*, J = 13.5, 4.4 Hz, H-2″), 5.40 (1H, *dd*, J = 13.1, 4.1 Hz, H-2), 3.81 (6H, *s*, OMe x 2), 3.08 (IH, *dd*, J = 17.6, 13.5 Hz, H-3_{ax}″), 3.0 (1H, *dd*, J = 17.5, 13.1 Hz, H-3_{ax}), 2.78 (1H, *dd*, J = 17.6, 4.4 Hz, H-3_{eq}″), 2.67 (1H, *dd*, J = 17.5, 4.1 Hz, H-3_{eq}), 2.39 (3H, *s*, OAc-5), 2.31 (3H, *s*, OAc-5″), 2.25 (3H, *s*, OAc-4‴).

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