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Note

A new biflavonoid from *Cycas beddomei*†

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Chemical investigation on the constituents of the cones of *Cycas beddomei* has been carried out which results in the isolation of a new biflavonoid, 2″,3″-dihydrohinokiflavone, along with 2,3,2″,3″-tetrahydrohinokiflavone, 2,3-dihydroamentoflavone, 2,3,2″,3″-tetrahydroamentoflavone, 2,3-dihydro-4‴-*O*-methyl-amentoflavone, and pinoresinol. The structure of the new compound has been established by detailed analysis of its spectral (mainly 1D and 2D NMR) data.

Keywords: *Cycas beddomei*, Cycadaceae, cones, biflavonoid, 2″,3″-dihydrohinokiflavone

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Cycads are woody-stemmed plants with leaves resembling those of ferns and palms. They are members of a class of plants known as *gymnosperms* which do not bear flowers but rather bear cones or "naked" seeds that develop on the individual cone scales. *Cycas* (Cycadaceae) is the major genus of cycads, and are rich source of biflavonoids such as amentoflavone and hinokiflavone derivatives[1-3], *Cycas beddomei* Dyer is a tall shrub endemic to Tirumala hills, Andhra Pradesh, India[4]. Earlier studies of this species have led to the isolation of different biflavonoids [1-3]. The biological activities of some of the isolated biflavonoids are well investigated. For instance, amentoflavone and its derivatives possess inhibitory effects on lipid peroxidation[5] and hinokiflavone inhibits the expression of the Epstein-Barr virus (EBV) genes[6]. They are also known to possess antituberculosis[7], antifungal[8], antibacterial[9], antiviral[10], anti HIV[11] and antimalarial activities[12]. The present investigation on *C. beddomei* has afforded a new biflavonoid, 2″,3″-dihydrohinokiflavone **1** along with 2,3,2″,3″-tetrahydrohinokiflavone[1], 2,3-dihydro amentoflavone[13], 2,3,2″,3″-tetrahydroamentoflavone[14], 2,3-dihydro-4‴-*O*-methyl amentoflavone[3], and pinoresinol[15]. The structure elucidation of the new compound **1** (**Figure 1**) has been discussed in this note.

2″,3″-Dihydrohinokiflavone **1** was obtained as yellow crystals. Its molecular formula was deduced to be $C_{30}H_{20}O_{10}$ from its elemental analysis and MS ([$M^{+\cdot}$+1] at *m/z* 541 in LSIMS) and ^{13}C NMR spectrum (indicating the presence of 30 carbons). The UV absorption maxima of **1** at 294 and 324 nm similar to (-)-naringenin **2** (ref. 17) are typical for a flavone derivative. The IR spectrum of **1** showed absorption bands at 3440, 1662, 1454, 1394, 1055 and 1014 cm^{-1} and revealed the presence of chelated hydroxyl and carbonyl functionalities as well as aromatic moiety. The ^{1}H NMR spectrum of **1** showed two sets of AA′BB′ pattern splittings (δ 7.98, d, *J* = 8.0 Hz and 7.00, d, *J* = 8.0 Hz; 7.44, d, *J* = 8.0 Hz and 6.94, d, *J* = 8.0 Hz) integrating for two protons each. The spectrum also indicated the presence of five hydroxyl groups (two chelated hydroxy protons at *δ* 13.10, 12.05 and three other phenolic hydroxyls at δ 10-8). In the aromatic region H-6 and H-8 showed the characteristic signals at δ 5.90 (d, *J* = 2.2 Hz) and two additional signals appeared at δ 6.71 (s) and 6.62 (s). A direct comparison of the ^{1}H NMR spectrum of **1** with that of hinokiflavone **3**, a known biflavonoid isolated from the same plant, clearly showed in **3** the disappearance of three aliphatic protons which appeared in **1** at δ 5.54, 3.21 and 2.90. All the other signals of two compounds (**1** and **3**) were almost similar, suggesting that the former is a mono dehydro derivative of the latter. 2,3-Dihydro hinokiflavone is a known compound[17], however, the ^{1}H NMR spectrum of penta methylated 2,3-dihydrohinokiflavone showed the signals at the most downfield region at δ 7.78 (2H, d, *J* = 8.0 Hz) for H-2‴ and H-6‴ (ref.18). The downfield shift (δ 7.98, d, *J* = 8.0 Hz) of the two protons of **1** suggested that a double bond in this compound could be placed in the first moiety at ring C. It is reported[19] that generally I or II-B ring protons will be shifted more downfield near unsaturation of C ring and I-B protons are more shifted (~ δ 8.02, 2H, d, *J* = Hz, H-2′,6′) than II-B protons (~ δ 7.74, 2H, d, *J* = Hz, H-2‴,6‴) when they are near to unsaturation of C ring. The NOESY experiment on **1** showed the

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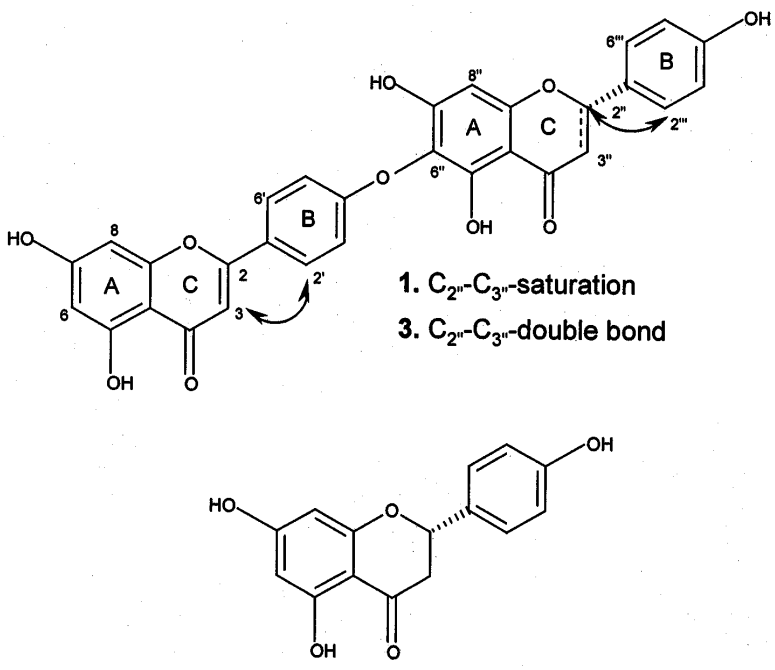

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Fig. 1—Chemical structures of 2′′,3′′-dihydrohinokiflavone (**1**) and hinokiflavone (**2**)
↔NOE Correlation

correlations of H-2′′′,6′′′ with H-2′′ and H-2′,6′ with H-3 suggesting 2′′,3′′ saturation in the compound.

The ^{13}C NMR spectrum of **1** presented signals for thirty carbons (vide Experimental). The spectrum showed peaks at δ 197.0 and δ 183.5 for flavone and flavanone moiety and at δ 79.7 and 43.5 for hydrogenated γ-pyrone. From the ^{1}H NMR spectrum the structure of the compound was settled as a binaringenin derivative with ether linkage. Appearance of ^{13}C NMR signal for oxygenated C′′-6 at δ 126.5 also confirmed it to be a hinokiflavone[19]. In ^{13}C NMR spectrum C-1′ of **1** appeared at δ 123.3 (–9.9 ppm compared to C-1′ of 2,3,2′′,3′′-tetrahydrohinokiflavone) which again suggested[1] the presence of the double bond between C-2 and C-3 in compound **1**. Considering all these spectral data the structure of **1** was thus conformed as a new hinokiflavone derivative, 2′′,3′′-dihydrohinokiflavone.

The CD spectrum of **1** with absorption maxima at 325 and 292 nm was similar to that (-)-naringenin **2** having α -configuration of the 4-hydrophenyl ring[20]. Thus, this spectrum clearly indicated the α-configuration for the C- 2′′ substituent of **1**.

Experimental Section

Melting points were measured in Buchi-510 instrument and are uncorrected. Spectra were recorded with the following instruments: UV, GBC Cintra 10e spectrophotometer; IR, Perkin-Elmer spectrophoto-meter; ^{1}H and ^{13}C NMR, Varian 300 MHz; LSIMS, Micromass Quattro LC. Optical rotation was determined with a JASCO DIP 360 digital polarimeter and CD spectrum with JASCO J-715 CD spectrophotometer. Column chromatography was performed over silica gel (BDH 100-200 mesh) and TLC with silica gel GF_{254}. The visualization of the spots in TLC plates was carried out either in UV light or exposing the plates to iodine vapors or spraying with 10% sulfuric acid in methanol and subsequently heating on hot plate.

Plant material

The cones of *Cycas beddomei* were collected from the forest of Tirumala Hills, Chittoor district, Andhra Pradesh in the month of February, 2003 and were botanically identified. A voucher specimen (No. IICT-15202) has been preserved in the herbarium of our institution.

Extraction of the plant material and separation of phytoconstituents

The shade dried and powdered cones (2 kg) were extracted with $CHCl_3$-MeOH (1:1) (3 L) for 3 days at room temperature. The extract was concentrated and extracted with EtOAc-H_2O (4:1) (3 × 50 mL). On

concentration a gummy residue (42 g) was obtained. A part (0.5 g) of this gummy residue was preserved and the rest was subjected to column chromatography over silica gel (100-200 mesh). The column was eluted with solvents of increasing polarity using $CHCl_3$ and mixtures of $CHCl_3$, acetone and MeOH. The eluents were collected in fractions of 500 mL each and concentrated. Following the TLC analysis, the eluents of similar profiles were combined to give fractions which were rechromatographed and eluted with mixtures of $CHCl_3$ and MeOH. The following compounds were obtained according to the increasing order of polarity: 2,3,2′′,3′′-tetrahydrohinokiflavone (25 mg), 2,3-dihydroamentoflavone (106 mg), 2,3,2′′,3′′-tetrahydroamentoflavone (26 mg), 2,3-dihydro-4′′′-*O*-methylamentoflavone (23 mg), 2′′,3′′-dihydrohinokiflavone (**1**, 31 mg), and pinoresinol (13 mg). Compound **1** crystallized from MeOH.

2′′,3′′ Dihydrohinokiflavone 1: Yellow crystals; M.p. 300°C (d); $[\alpha]_D^{25}$–0.15 (*c* 0.06 in MeOH); UV (MeOH): λ_{max} 294, 324 nm (logε 4.10, 3.96); CD (MeOH)**:** λ_{max} 292, 325nm (Δε +1.78, –8.86); IR (KBr): ν_{max} 3440, 1662, 1454, 1394, 1055, 1014 cm^{-1}; ^{1}H NMR (300 MHz, d_6-acetone): δ 13.10 (1H, s, 5′′-OH), 12.05 (1H, s, 5-OH), 9.70 (1H, brs, 7-OH), 9.30 (1H, brs, 7′′-OH), 8.90 (1H, brs, 4′′′-OH), 7.98 (2H, d, *J* = 8.0 Hz, H-2′, 6′), 7.44 (2H, d, *J* = 8.0 Hz, H-2′′′, 6′′′), 7.00 (2H, d, *J* = 8.0 Hz, H-3′, 5′), 6.94 (2H, d, *J* = 8.0 Hz, H-3′′′, 5′′′), 6.71 (1H, s, H-3), 6.62 (1H, s, H-8′′), 5.90 (2H, d, *J* = 2.2 Hz, H-6, 8), 5.54 (H, dd, *J* = 13.0, 2.8 Hz, H-2′′), 3.21 (1H, dd, *J* = 17.0, 13.0 Hz, H-3′′β), 2.90 (1H, dd, *J* = 17.0, 2.8 Hz, H-3′′α); ^{13}C NMR (75 MHz, d_6-acetone): δ 197.0 (C-4′′), 183.5 (C-4), 167.4 (C-7), 165.6 (C-2), 165.3 (C-5), 164.2 (C-9), 162.1 (C-4′′′), 159.1 (C-4′), 158.0 (C-7′′), 155.2 (C-5′′), 154.8 (C-9′′), 133.5 (C-1′′′), 129.4 (C-2′), 129.4 (C-6′), 128.7 (C-6′′′), 128.7 (C-2′′′), 126.5 (C-6′′), 123.3 (C-1′), 116.9 (C-5′′′), 116.9 (C-3′′′), 116.0 (C-5′), 116.0 (C-3′), 105.9 (C-10), 103.8 (C-3), 103.2 (C-10′′), 96.9 (C-6), 95.9 (C-8′′), 95.4 (C-8), 79.7 (C-2′′), 43.5 (C-3′′); LSIMS: *m/z* 541 ($M^{+\cdot}$+1); Anal. Calcd. for $C_{30}H_{20}O_{10}$: C, 66.67; H, 3.73%. Found: C, 66.61; H, 3.66%.

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