Pergamon

PII: S0013-9422(97)00417-2

Phytochemistry, Vol. 47, No. 2, pp. 319–321, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

A BIFLAVONOID FROM *CYCAS BEDDOMEI*

M. SOBHA RANI, C. VENKATA RAO, D. GUNASEKAR,* A. BLOND† and B. BODO†

Department of Chemistry, Sri Venkateswara University, Tirupati 517 502, India; † Laboratoire de Chimie des Substances Naturelles, URA 401 CNRS, Museum National d'Histoire naturelle, 63 rue Buffon, 75005, Paris, France

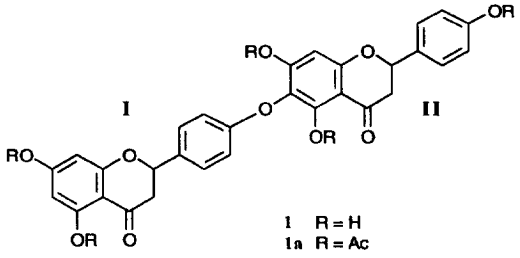
(*Received in revised form* 11 *March* 1997)

Key Word Index—*Cycas beddomei*; Cycadacea; leaves; biflavonoids; tetrahydrohinokiflavone; amentoflavone.

Abstract—A new biflavanone, tetrahydrohinokiflavone, together with amentoflavone has been isolated from the leaves of *Cycas beddomei*. The structures were established on the basis of spectral and chemical evidence.

INTRODUCTION

Cycas beddomei Dyer is a small shrub endemic to the Tirumala Hills, Andhra Pradesh, India [1]. There have been no reports on the chemical constituents of this species. The genus, however, is rich in biflavonoids [2–5]. The present paper describes the isolation and structure elucidation of a new biflavanone, tetrahydrohinokiflavone (**1**), together with amentoflavone from the leaves of *C. beddomei*.



1 R = H
1a R = Ac

RESULTS AND DISCUSSION

Tetrahydrohinokiflavone (**1**) analysed for $C_{30}H_{22}O_{10}$ which is constituent with the presence of a $[M]^+$ at *m/z* 542 in its EI mass spectrum. It gave a deep violet ferric reaction and an orange red colour with $NaBH_4$/HCl characteristic of a flavanone. The UV spectrum of **1** in MeOH exhibited absorption maxima at 289 and 326 (sh) nm which were very similar to those of naringenin [6]. Addition of aluminium chloride and sodium acetate caused band II to shift to 312 and 327 nm, respectively, indicating the presence of the 5,7-dihydroxyflavanone system in the molecule [7]. IR absorption bands were present at 3480 (-OH) and 1648 cm^{-1} (>C = O).

Acetylation of **1** gave a pentaacetate (**1a**) (M^+, 752). The ^1H NMR spectra of **1** and **1a** established the presence of five hydroxyl groups, and 11 aromatic, two benzylic methine and four methylene protons in **1** indicating it to be a biflavanone derivative with an interflavonoid ether linkage. Two of the five hydroxyl protons resonated at δ 12.15 and 12.29, indicating the presence of two chelated hydroxyl groups at the 5 and 5″ positions, respectively. The remaining three non-chelated hydroxyl groups at δ 10.30, 10.10 and 9.0 were assigned to 7,7″ and 4‴ positions, respectively. The presence of two sets of AA′ BB′ doublets at δ 7.47 (2H, *d*, J = 9.0 Hz) and 6.94 (2H, *d*, J = 9.0 Hz); δ 7.42 (2H, *d*, J = 9.0 Hz) and 6.91 (2H, *d*, J = 9.0 Hz) were ascribed to eight aromatic protons of rings B and E, respectively. There were also two superimposable doublet of doublets (J = 13.0, 2.8 Hz) at δ 5.51 integrating for two benzylic methine protons at C-2 and C-2″. The four methylene protons at C-3″ and C-3 were present as two sets of doublet of doublets at δ 3.24 (1H, J = 17.1, 13.0 Hz) and 2.77 (1H, J = 17.1, 2.8 Hz); and δ 3.18 (1H, J = 17.1, 13.0 Hz) and 2.72 (1H, J = 17.1, 2.8 Hz), respectively. The presence of two *meta* coupled doublets (J = 2.2 Hz), each due to one proton, in the upfield aromatic region at δ 5.96 and 5.94 were assigned to the C-8 and C-6 protons of ring A. The only signal at δ 6.15 was assigned to H-8″ or H-6″ which implied that either C-6″ or C-8″ of ring D had to be involved in the interflavonoid ether linkage. A fragment at *m/z* 390 resulting from *retro*-Diels–Alder cleavage of ring C in the EI-mass spectrum of compound **1** further supported the involvement of ring B and D in the interflavonoid ether linkage. The structure of **1** was, therefore, composed of two naringenin units with an ether

* Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR assignments for tetrahydrohinokiflavone (**1**) and naringenin in Me_2CO-d_6

C	Naringenin	Tetrahydrohinokiflavone (**1**) Moiety I	Moiety II
2	80.1	79.6	80.2
3	43.7	43.4*	43.5*
4	197.3	197.0	198.1
4a	103.1	103.2	103.4
5	165.0	165.2	156.7
6	97.0	96.9	124.0
7	168.0	167.3	160.1
8	96.1	95.9	96.2
8a	164.5	164.2	160.8
1′	130.7	133.2	130.6
2′	128.8	128.8	129.6
3′	116.2	115.7	116.2
4′	158.5	159.3	158.7
5′	116.2	115.7	116.2
6′	128.8	128.8	129.6

* Exchangeable assignments.

linkage either between C-4′, and C-6″ or C-4′ and C-8″. Comparison of the ^{13}C NMR data of 31 with naringenin [8] (Table 1) showed that C-6″ in **1** was involved in interflavonoid ether linkage as the resonance of this carbon was shifted downfield by 27.0 ppm and carbons 5″, 7″ and 8a″ (*ortho* and *para* to the ether linked carbon) were shifted upfield by 8.3, 7.9 and 3.7 ppm, respectively. Thus from the foregoing spectral studies the structure of compound **1** was established as 2,3,2″,3″-tetrahydrohinokiflavone.

A final proof of the proposed structure for **1** was obtained by dehydrogenation [9] of **1** which resulted in a biflavone whose physical and spectral data agreed well with those of hinokiflavone [8, 10].

EXPERIMENTAL

General. MPs uncorr.; ^1H and ^{13}C NMR: 300.13 (^1H) and 75.43 (^{13}C) MHz, respectively, in Me_2CO-d_6 and $CDCl_3$ with TMS as int. standard; EIMS: 70 eV (probe); UV and IR MeOH and KBr, respectively; CC: Silica gel finer than 200 mesh (0.08 mm).

Plant material. Fresh leaves of *C. beddomei* were collected from the Tirumala Hills, Andhra Pradesh, India, in January 1994. A voucher specimen was deposited in the Herbarium of the Department of Botany, Sri Venkateswara University.

Extraction and isolation. The shade dried and powdered leaves (5 kg) of *C. beddomei* were successively extracted with *n*-hexane, Me_2CO and MeOH. The concd Me_2CO extract was fractionated into toluene- and EtOAc- soluble parts. Concn of the EtOAc fr. gave a brownish yellow solid (1.1 g) which was purified over a silica gel column using a C_6H_6–EtOAc step gradient. Frs 11–20 and 51–65 eluted with C_6H_6–EtOAc, 9:1 and 1:1, respectively, afforded 2,3,2″,3″-tetrahydrohinokiflavone (**1**) (70 mg) and amentoflavone (130 mg). The identity of amentoflavone was based on a comparison of this physical and spectral data reported [11] in the literature and by co-chromatography with an authentic sample.

2,3,2″,3″-*Tetrahydrohinokiflavone* (**1**). Colourless needles, mp 241–242°; $C_{30}H_{22}O_{10}$; $[\alpha]_D^{28}$ −2.64° (*c* 1.62, MeOH); UV λ_{max}^{MeOh} nm (log ε): 289 (5.87), 326 (sh) (4.38); +$AlCl_3$: 312, 375; +$AlCl_3$/HCl: 310, 372; +NaOAc: 285 (sh) 327; IR ν_{max}^{KBr} cm^{-1}: 3480 (-OH), 3150, 1648 (>C = O), 1600, 1590, 1500, 1460, 1300, 1170; ^1H NMR (Me_2CO-d_6): δ 12.29 (1H, *s*, OH-5″), 12.15 (1H, *s*, OH-5), 10.30 (1H, *s*, OH-7), 10.10 (1H, *s*, OH-7″), 9.00 (1H, *s*, OH-4‴), 7.47 (2H, *d*, *J* = 9.0 Hz, H-2′,6′), 7.42 (2H, *d*, *J* = 9.0 Hz, H-2‴,6‴), 6.94 (2H, *d*, *J* = 9.0 Hz, H-3′,5′), 6.91 (2H, *d*, *J* = 9.0 Hz, H-3‴,5‴), 6.15 (1H, *s*, H-8″), 5.96 (1H, *d*, *J* = 2.2 Hz, H-8), 5.94 (1H, *d*, *J* = 2.2 Hz, H-6), 5.51 (2H, *dd*, *J* = 13.0, 2.8 Hz, H-2,2″), 3.24 (1H, *dd*, *J* = 17.1, 13.0 Hz, H-3″ *trans*), 3.18 (1H, *dd*, *J* = 17.1, 13.0 Hz, H-3 *trans*), 2.77 (1H, *dd*, *J* = 17.1, 2.8 Hz, H-3″ *cis*), 2.72 (1H, *dd*, *J* = 17.1, 2.8 Hz, H-3 *cis*); ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 542 $[M]^+$ (38) 422 (10), 390 (7), 297 (10), 287 (2), 271 (37), 270 (38), 257 (10), 255 (7), 244 (11), 242 (5), 179 (37), 153 (100), 152 (10), 120 (50), 107 (19), 77 (9).

Tetrahydrohinokiflavone pentaacetate (**1a**). Tetrahydrohinokiflavone (**1**) (10 mg) was acetylated with pyridine and Ac_2O in the usual way to yield the pentaacetate derivative **1a** as an amorphous powder (13 mg), mp 156°. IR ν_{max}^{KBr} cm^{-1}. 1760 (>C = O of OAc), 1690, 1610, 1400, 1350, 1180; ^1H NMR ($CDCl_3$): δ 7.44 (2H, *d*, *J* = 9.0 Hz, H-2′,6′), 7.17 (2H, *d*, *J* = 9.0 Hz, H-2‴,6‴), 7.08 (2H, *d*, *J* = 9.0 Hz, H-3′,5′), 7.01 (2H, *d*, *J* = 9 Hz, H-3‴,5‴), 6.85 (1H, *s*, H-8″), 6.75 (1H, *d*, *J* = 2.2 Hz, H-8), 6.50 (1H, *d*, *J* = 2.2 Hz, H-6), 5.45 (2H, *dd*, *J* = 13.0, 2.8 Hz, H-2, 2″), 3.08 (1H, *dd*, *J* = 17.1, 13.0 Hz, H-3″ *trans*), 3.01 (1H, *dd*,

J = 17.1, 13.0 Hz, H-3 *trans*), 2.81 (1H, *dd*, J = 17.1, 2.8 Hz, H-3″ *cis*), 2.72 (1H, *dd*, J = 17.1, 2.8 Hz, H-3 *cis*), 2.36 (3H, *s*, OAc-5″), 2.30 (3H, *s*, OAc-5), 2.26 (3H, *s*, OAc-7″), 2.16 (3H, *s*, OAc-7), 2.12 (3H, *s*, OAc-4‴); EIMS *m/z* (rel. int.): 752 $[M]^+$ (9), 710 (13), 668 (11), 623 (3), 584 (23), 542 (9), 422 (25), 390 (5), 297 (7), 287 (1), 271 (28), 270 (23), 257 (7), 255 (5), 244 (12), 242 (4), 179 (25), 153 (100), 152 (24), 120 (41), 107 (18), 77 (11).

Dehydrogenation of compound **1**. A mixt. of **1** (15 mg), KOAc (0.25 g) and I_2 (50 mg) in glacial HOAc (1.5 ml) was heated under reflux in an oil bath for 2 hr, after which the reaction medium was cooled, poured into ice-cold H_2O, and extracted with EtOAc. The solvent was removed *in vacuo* and a satd soln of $NaHSO_3$ was added to the residue to destroy excess I_2. It was filtered and the yellow residue obtained on crystallisation from MeOH afforded the dehydrogenated product as yellow crystals, mp > 330°, which were identical in all respects with hinokiflavone [8, 10].

Acknowledgements—We are grateful to Dr K. Madhava Chetty (S. V. University) for his help in the collection and identification of plant material.

REFERENCES

1. Hooker, J. D., *Flora of British India*, Vol 5. Periodical Experts, Delhi, 1973, p. 658.
2. Kariyone, T. and Sawada, T., *Journal of the Pharmaceutical Society of Japan*, 1958, **78**, 1013.
3. Geiger, H. and Pfleiderer, W. D. G., *Phytochemistry*, 1971, **10**, 1936.
4. Varshney, A. K., Mah, T., Khan, N. V., Rahman, W., Hwa, C. W., Okigawa, M. and Kawano, N., *Indian Journal of Chemistry*, 1973, **11**, 1209.
5. Gadek, P. A., *Phytochemistry*, 1982, **21**, 889.
6. Mabry, T. J., Markham, K. R. and Thomas, M. B., *The Systematic Identification of Flavonoids*. Springer, New York, 1970, p. 215.
7. Mabry, T. J., Markham, K. R. and Thomas, M. B., *The Systematic Identification of Flavonoids*. Springer, New York, 1970, p. 170.
8. Markham, K. R., Sheppard, C. and Geiger, H., *Phytochemistry*, 1987, **26**, 3335.
9. Goel, R. N., Mahesh, V. B. and Seshadri, T. R., *Proceedings of the Indian Academy of Science*, 1958, **47**, 184.
10. Chen, F. C., Lin, Y. M. and Liang, C. M., *Phytochemistry*, 1974, **13**, 276.
11. Dora, G. and Edwards, J. M., *Journal of Natural Products*, 1991, **54**, 796.