Pergamon

PII: S0031-9422(97)01052-2

Phytochemistry, Vol. 48, No. 8, pp. 1453–1455, 1998

Printed in Great Britain
0031-9422/98/$—see front matter

C-PRENYLATED DIHYDROFLAVONOL FROM *RHYNCHOSIA DENSIFLORA*

KARUMANCHI V. RAO† and DUVVURU GUNASEKAR*

Natural Products Division, Department of Chemistry, Sri Venkateswara University, Tirupati—517 502, India

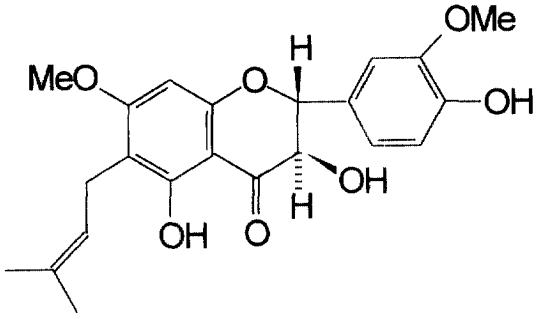
(*Received in revised form* 3 *September* 1997)

Key Word Index—*Rhynchosia densiflora*; Leguminosae; leaves; *C*-prenylated dihydroflavonol; (+)-(2*R*:3*R*)-6-*C*-prenyltaxifolin 7,3′-dimethylether.

Abstract—A new *C*-prenylated dihydroflavonol has been isolated from the leaves of *Rhynchosia densiflora* and characterized as (+)-(2*R*:3*R*)-6-*C*-prenyltaxifolin 7,3′-dimethylether.

INTRODUCTION

Rhynchosia densiflora (Roth) DC (Leguminosae) is a slender twiner found throughout the Annamalai, Pulney and Nilgiri hills of Tamilnadu, South India [1]. As part of a phytochemical study of the genus *Rhynchosia* [2], we have examined *R. densiflora*, a plant which has not been investigated previously for its chemical constituents. In the present paper we describe the isolation and structural determination of a new *C*-prenylated dihydroflavonol (**1**).



1

RESULTS AND DISCUSSION

Compound **1** was obtained as colorless needles and gave a positive ferric chloride test. The high-resolution EI-mass spectrum displayed $[M]^+$ at *m/z* 400.1501, which corresponds to the molecular formula $C_{22}H_{24}O_7$ (Calc. 400.1515) is in accord with ^1H and ^{13}C NMR data, summarized in the Experimental section. The UV methanol spectrum exhibited a maximum at 290 nm and an inflection at 340 nm, which are consistent with that of dihydroflavonol [3]. The IR spectrum showed vibration bands at 3432 (chelated OH), 1635 (conjugated C=O), 1355 (gem dimethyl) and other bands at 1545 and 1470 cm^{-1}, assignable to an aromatic ring. The ^1H NMR spectrum exhibited the typical AB system due to H-2 and H-3 of a dihydroflavonol [4, 5] at δ 5.03 (J = 12.0 Hz) and 4.54 (J = 12.0 Hz), respectively. The doublet at δ 5.80 (J = 6.0 Hz) (exchangeable on deuteration) by coupling with H-3, was assigned to the hydroxyl group at the C-3 position. These assignments were confirmed by the ^{13}C NMR spectrum, which showed three ring C carbon signals at δ 84.85 (C-2), 73.30 (C-3) and 198.50 (C-4).

Apart from H-2, H-3 and OH-3, the ^1H NMR spectrum of **1** gave signals (typical) of a *C*-linked prenyl residue [4, 5] at δ 5.05 (J = 8.0 Hz, vinyl proton), 3.15 (J = 8.0 Hz, allylic proton) and 1.57 (vinyl methyls). This was supported by signals at δ 130.20 (C-3″), 122.35 (C-2″), 25.45 (C-4″), 21.30 (C-1″) and 17.65 (C-5″) in the ^{13}C NMR spectrum. The fragments at *m/z* 235, 219 and 191 formed after retro-Diels-Alder cleavage, suggested that **1** has a prenyl group in the A ring rather than in ring B. The other fragments at *m/z* 166 and 137 resulting from ring B indicated that one hydroxyl and one methoxyl group were present in ring

* Author to whom correspondence should be addressed.

† Present address: Biotechnology Division, Dr. Reddy's Research Foundation, Bollaram Road, Miyapur, Hyderabad—500 050, India. Fax: 91-40-3045438/3045007; e-mail: drf@hd1.vsnl.net.in

B and this was confirmed by the ^{1}H NMR and ^{13}C NMR spectral analysis. Thus, the appearance of one H-2′ doublet (δ 7.21) downfield (0.20 ppm) from the H-6′ double doublet (δ 7.01, J = 8.5 and 2.5 Hz) supported a 3′-methoxyl and 4′-hydroxyl substitution pattern for the B-ring, while chemical shifts of C-3′ and C-4′, resonating at δ 148.40 and 148.65, were in agreement with literature values [6]. Two three-proton singlets at δ 3.79 and 3.87 showed the presence of two methoxyl groups. The singlet at δ 3.79 was assigned to the 7-position based on the fact that there was no bathochromic shift of the UV absorption maximum with NaOAc. A broad signal at δ 12.40 (exchangeable on deuteration) indicated the presence of a chelated hydroxyl at C-5.

The presence of three hydroxyl groups in **1** was confirmed by formation of a triacetate, which did not respond positively to the ferric chloride test and which had the molecular formula $C_{28}H_{30}O_{10}$, assigned from the $[M]^+$ at m/z 526. Found: C, 63.91; H, 5.66. Calc.: $C_{28}H_{30}O_{10}$ requires: C, 63.87; H, 5.74%.

An aromatic singlet at δ 6.15 was assigned to the C-8 proton on the basis of a positive Gibbs test [7] and it was concluded that the prenyl moiety must be attached at C-6. 6-C-Prenylation was confirmed by the ^{13}C NMR spectrum, which showed a downfield shift of C-6 by 12.2 ppm in comparison with the spectrum of taxifolin 7,3′-dimethylether [8]. The spectral analysis clearly indicated that **1** is isomeric with scariosin [9]. The structural assignment was further evidenced by the presence of a 5-OH signal at δ 12.40, which is a downfield shift of 0.32 ppm from that of scariosin. This is consistent with the general observation that the signal for the hydrogen bonded hydroxyl group in 6-C-prenylated flavonoids is shifted further downfield (0.25–0.30 ppm) than those of the corresponding 6-nonprenylated flavonoids [10–12]. Positive optical rotation and *trans* diaxial coupling indicated a 2*R*:3*R* configuration [13] in **1** and thus the structure of **1** was established as (+)-(2*R*:3*R*)-6-*C*-prenyltaxifolin 7,3′-dimethylether.

EXPERIMENTAL

General

Mps are uncorr. Mass spectra were obtained in EI mode at 70 eV. IR spectra were run in KBr. All NMR experiments were performed on a Nicolet NT 300 WB or JEOL-FX-90Q spectrometer equipped with 5 mm ^{1}H and ^{13}C probes operating at 300.06 and 75.45, or 90 and 22.5 MHz, respectively. Samples were run in Me_2CO-d_6 or DMSO-d_6. TMS as int. standard, chemical shifts (ppm) and J (Hz).

Plant material

Leaves of *R. densiflora* were collected from Nilgiri hills of Tamilnadu, South India. A voucher specimen has been deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati.

Extraction and isolation

The air-dried and powdered leaves (600 g) were exhaustively extracted with hexane. The concd hexane extract (4.2 g) was treated with MeOH and MeOH concentrate triturated with light petrol until no more fatty material was separated. The residue (1.7 g) was subjected to CC over silica gel and eluted with petrol, petrol–C_6H_6, C_6H_6–$CHCl_3$, $CHCl_3$, $CHCl_3$–EtOAc and EtOAc. A total of 60 frs of ca 50 ml each were collected and the course of the column was followed by TLC on silica gel (precoated) in C_6H_6–EtOAc (4:1). The spots were visualized by spraying with diazotized sulphanilic acid. Fractions 31–48, on concentration furnished a pale yellow solid (120 mg) which on crystallization from MeOH gave 80 mg of **1**.

Compound 1. Colorless needles, mp 182–183°, $[\alpha]_D^{25}$ +26.45° (MeOH, *c* 0.5). UV λ_{max} nm (log ε): 290 (4.09), 340 sh (3.57): +$AlCl_3$ nm: 270, 314; +NaOAc nm: 290, 344. IR ν_{max} cm^{-1}: 3432, 2975, 2906, 1635, 1545, 1470, 1355, 1265, 1160, 980, 820. ^{1}H NMR: δ 12.40 (1H, *br s*, OH-5, exchangeable), 9.09 (1H, *br s*, OH-4′, exchangeable), 7.21 (1H, *d*, J = 2.5 Hz, H-2′), 7.01 (1H, *d d*, J = 8.5 Hz and 2.5 Hz, H-6′), 6.97 (1H, *d*, J = 8.5 Hz, H-5′), 6.15 (1H, *s*, H-8), 5.80 (1H, *d*, J = 6.0 Hz, OH-3, exchangeable), 5.05 (1H, *t*, J = 8.0 Hz, β-C<u>H</u>=), 5.03 (1H, *d*, J = 12.0 Hz, H-2), 4.54 (1H, *d d*, J = 12.0 and 6.0 Hz, H-3), 3.87 (3H, *s*, OMe-3′), 3.79 (3H, *s*, OMe-7), 3.15 (2H, *d*, J = 8.0 Hz, α-CH_2), 1.57 (6H, *m*, =C(Me)$_2$). ^{13}C NMR: δ 198.50 (C-4), 165.45 (C-7), 160.60 (C-5), 160.50 (C-9), 148.65 (C-4′), 148.40 (C-3′), 130.20 (C-3″), 129.60 (C-1′), 122.35 (C-2″), 122.15 (C-6′), 115.80 (C-5′), 112.65 (C-2′), 108.20 (C-6), 102.10 (C-10), 94.80 (C-8), 84.85 (C-2), 73.30 (C-3), 56.20 (2 × OMe), 25.45 (C-4″), 21.30 (C-1″), 17.65 (C-5″). HRMS m/z: 400.1501 [M^+] (Calc. for $C_{22}H_{24}O_7$, 400.1515). EIMS m/z (rel. int): 400 [M^+] (35), 383 (7), 371 (24), 345 (14), 247 (14), 235 (47), 219 (21), 191 (26), 179 (100), 166 (18), 137 (32).

The triacetate of **1**. Acetylation of **1** (15 mg) with Ac_2O in pyridine yielded the triacetate (10 mg) as colorless crystals, mp 101–102°. ^{1}H NMR: δ 7.25–7.42 (2H, *m*, H-6′ and H-2′), 7.06 (1H, *d*, J = 8.5 Hz, H-5′), 6.36 (1H, *s*, H-8), 5.64 (1H, *d*, J = 12.0 Hz, H-2), 5.28 (1H, *d*, J = 12.0 Hz, H-3), 5.16 (1H, *t*, J = 8.0 Hz, β-C<u>H</u> =), 3.90 (6H, *s*, 7-OMe and 3′-OMe), 3.27 (2H, *d*, J = 8.0 Hz, α-CH_2), 2.42–2.29 (9H, *m*, 3 × OAc), 1.60 (6H, *s*, =C(Me)$_2$). EIMS m/z (rel. int): 526 [M^+] (7), 484 (100), 442 (19), 424 (38), 409 (23), 382 (17), 314 (11), 290 (16), 286 (12), 248 (17), 233 (39), 219 (54), 206 (66), 195 (29), 191 (22), 179 (73), 166 (69), 164 (33), 137 (18).

Acknowledgements—The authors thank Dr A. V. Rama Rao, Former Director of Indian Institute of

Chemical Technology, Hyderabad, India, for providing the spectral data.

REFERENCES

1. Gamble, J. S., *Flora of the Presidency of Madras*, Vol. I, Botanical Survey of India, Calcutta, 1967, p. 265.
2. Rao, K. V., Sreeramulu, K. and Gunasekar D., *Indian J. Nat. Prod.*, 1991, **7**, 3.
3. Mabry, T. J., Markham, K. R. and Thomas, M. B., *The Systematic Identification of Flavonoids*, Springer-Verlag, New York, 1970, p. 166.
4. Barnes, C. S., *Tetrahedron Letters*, 1963, 281.
5. Mabry, T. J., Markham, K. R. and Thomas, M. B., *The Systematic Identification of Flavonoids*, Springer-Verlag, New York, 1970, p. 267.
6. Agarwal, P. K. ed. *Carbon-13 NMR of Flavonoids*, Elsevier, Amsterdam, 1989.
7. King, F. E., King, T. J. and Manning, L. C., *J. Chem. Soc.*, 1957, 563.
8. Balza, F. and Towers, G. H. N., *Phytochemistry*, 1984, **23**, 2333.
9. Ali Nia, M., Rao, K. V., Sreeramulu, K. and Gunasekar D., *J. Nat. Prod.*, 1992, **55**, 1152.
10. Fukai, T. and Nomura, T., *Heterocycles*, 1990, **31**, 1861.
11. Fukai, T. and Nomura, T., *Heterocycles*, 1991, **32**, 499.
12. Fukai, T. and Nomura, T., *Heterocycles*, 1993, **34**, 329.
13. Chan, R. S., Ingold, C. K. and Prelog, V., *Experentia*, 1956, **12**, 81.