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# TIRUMALIN, A NEW PRENYLATED DIHYDROFLAVONOL FROM *RHYNCHOSIA CYANOSPERMA*

DAMA ADINARAYANA*, DUVVURU GUNASEKAR*, PASUPULATI RAMACHANDRAIAH*
OTTO SELIGMANN† and HILDEBERT WAGNER†

*Department of Chemistry, Sri Venkateswara University, Tirupati, India;
†Institut für Pharmazeutische Arzneimittellehre der Universität München. D-8000 München 2, Karlstrasse, 29, W. Germany

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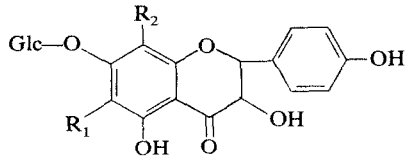
**Key Word Index**—*Rhynchosia cyanosperma*; Leguminosae; prenylated dihydroflavonol; tirumalin; flavonol-*O*-glycosides; rutin; kaempferol-3-rutinoside; pinitol.

**Abstract**—A new dihydroflavonol has been isolated together with the known flavonol-*O*-glycosides, rutin and kaempferol-3-rutinoside, and (+)-pinitol from the leaves of *R. cyanosperma* Benth. The dihydroflavonol was identified as (+)-(2*R*, 3*R*)-8-*C*-prenyltaxifolin-7, 4′-dimethyl ether on the basis of spectroscopic studies and the compound given the trivial name tirumalin.

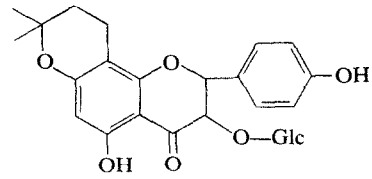
## INTRODUCTION

Chemical investigation of several species of the genus *Rhynchosia* ([1, 2];Adinarayana, D., Gunasekar, D. and Ramachandraiah, P., unpublished results) for their flavonoid constituents indicated the presence of *C*-glycosylated flavones in most of them. However, our present study of *Rhynchosia cyanosperma* (syn. *Cylista tomentosa*) did not show the presence of these compounds and thereby exhibited deviation. Instead we isolated tirumalin (**8**) (named after the collection of the plant material from Tirumala Hills, Andhra Pradesh, India) a new prenylated dihydroflavonol, along with the known 3-rutinosides of kaempferol and quercetin (**1**, **2**) and (+)-pinitol. While prenylation has been often encountered in the case of flavanones and chalkones [3], it is of rare occurrence in the case of dihydroflavonols, with only five such examples being known (Table 1).

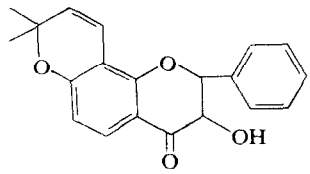
The UV absorption data of the compound were similar to those of a flavanone showing the presence of a free 5-OH and a substituted 7-OH group. The $^1$H NMR spectrum in DMSO showed doublets (J = 11 Hz) at δ 4.50 and 5.09 ppm which are typical for H-3 and H-2 of dihydroflavonols. In this case a coupling (*J* = 6 Hz) between H-3 and the hydroxylic proton could also be observed. The presence of a *C*-linked prenyl residue was inferred from the signals at δ 1.58, 3.10, and 5.08 ppm. This suggested that the structure of tirumalin could be a *C*-prenylated taxifolin derivative containing two methoxyl groups in the molecule. The positions of the different substituents in tirumalin were arrived at by NMR and MS. Thus the MS showed several fragments indicating that the prenyl group was on the A-ring and that one of the methoxyl groups was on the B-ring. From a comparison of the NMR spectra of the dihydroflavonol and its acetate with the



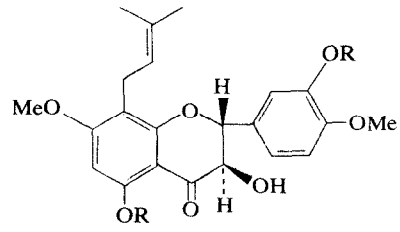
**3** $R_1$ = H; $R_2$ = —$CH_2$—CH=$CMe_2$
**4** $R_1$ = H; $R_2$ = —$CH_2$—$CH_2$—$C(Me_2)$OGlc
**5** $R_1$ = —$CH_2$—$CH_2$—$C(Me_2)$OH; $R_2$ = H



**6**



**7**



**8** R = H
**9** R = Ac

Table 1.

| Compound | Source | Family |
|---|---|---|
| Phellamurin (**3**) | *Phellodendron amurense* [4,5] | Rutaceae |
| Dihydrophelloside (**4**) | *P. sachalinense* and *P. amurense* [6] | |
| Phellavin (**5**) | *P. lavalei* and *P. amurense* [7–9] | |
| | *P.* spp. [7–9] | |
| | *P. sachalinense* [7–9] | |
| Phellodendroside (**6**) | *P. japonicum* [10, 11] | |
| 3-Hydroxyisoloncho-carpin (**7**) | *Lonchocarpus eriocaulinalis* [12] | Leguminosae |

spectra of hesperetin, eriodictyol, and their acetates, the methoxyl group in tirumalin was assigned to position 4′. The shift of the signal for the aromatic singlet of ring A, upon acetylation, indicated that it must be *ortho* to the hydroxyl group. This thus fixes the position of the prenyl residue at C-8. A *trans* orientation of the C-ring methine protons was inferred from the large $J$ value (11–12 Hz) which is typical of diaxial coupling. Therefore the positive optical rotation of tirumalin indicates a 2*R*:3*R* configuration and the structure of this compound is 2*R*:3*R*-8-*C*-γ,γ-dimethylallyl-7,-4′-dimethoxy-3,5,3′-trihydroxyflavanone or (+)-(2*R*, 3*R*)-8-*C*-prenyltaxifolin-7,4′-dimethyl ether.

## EXPERIMENTAL

The shade dried leaves of *R. cyanosperma* (0.8 kg) were Soxhleted successively with hot petrol (bp 60–80°), $C_6H_6$, $Me_2CO$ and MeOH. Petrol extract on concn yielded a yellow residue (0.28 g). The acetone-soluble portion of this residue was triturated with hot petrol. The petrol-solution part on concn yielded a green–yellow solid which crystallized from MeOH as pale yellow needles of **8** (0.160 g, 0.02%). The $Me_2CO$ and MeOH extracts on concn gave a sweet-tasting solid, which separated as crystals from absolute alcohol containing a few drops of HOAc, mp 186–87° (1 g, 0.12%) identified as (+)-pinitol on comparison with an authentic sample. The mother liquors of $Me_2CO$ and MeOH extracts showed the same behaviour on TLC, and were therefore combined and solvent fractionated with petrol, $C_6H_6$, $Et_2O$ and EtOAc. The EtOAc extract was chromatographed on Si gel (170 g, 60–120 mesh) column. The EtOAc–MeOH (3:2) eluates afforded a yellow solid which was separated by PC chromatography [Whatman No. 3 mm, *n*-BuOH–HOAc–$H_2O$, 4:1:5, top layer (BAW)] to yield **1** (0.2 g, 0.025%) and **2** (7.3 g, 0.912%).

*Tirumalin* (**8**). Pale yellow needles, mp 198–99° (MeOH), $[\alpha]_D^{26}$ +51.4° (Py, *c* 0.56) $C_{22}H_{24}O_7$ (400.41). (Found: C, 65.88; H, 6.40. Calc. C, 65.99, H, 6.04%).It gave a violet colour with alc. $FeCl_3$ and a pink colour with Mg–HCl and Zn–HCl suggesting a dihydroflavonol structure. $R_f$ values were 0.87 (PC, BAW, 4:1:5), 0.00 (PC, 15% aq. HOAc) and 0.74 (TLC, Si gel $C_6H_6$–dioxane–HOAc, 90:25:4). UV $\lambda_{max}^{MeOH}$ nm: 292, 345 (ε respectively 11 950, 4979); +$AlCl_3$: 272, 316; +NaOAc: 291, 345. IR (KBr)-$cm^{-1}$: 3430 (OH), 1625 (C=O).NMR (DMSO-$d_6$, TMS int.): δ 6.92 ppm (*s* (*br*); 3H, H-2′, 5′, 6′), 6.23 (*s*, 1H, H-6), 5.79 (*d*, $J$ = 6, 1H, OH-3), 5.09, (*m*, 1H, β-CH=), 5.08, (*d*, $J$ = 11, 1H, H-2), 4.50 (*q*, $J$ = 11 and 6, 1H, H-3), 3.86, 3.80 (*s*, 6 H, OMe), 3.10 (*d*, $J$ = 8, 2H, α-$CH_2$), 1.58 (*m*, 6H, γ-Me), 9.11 (*s* (*br*), 1H, OH-3′), 12.12 (*s* (*br*), 1H, OH-5). MS (EI 70 eV, 100μA, 4 kV; 180°; DI 120°, $10^{-6}$T): *m/e* 400 $M^+$ (42% rel. int.), 383 (7), 371 (18), 344 (8), 247 (11), 235 (52), 219 (21), 191 (24), 179 (100), 166 (20), 164 (76), 151 (6), 137 (29), 43 (34).

*Tirumalin triacetate* (**9**). NMR ($CDCl_3$, TMS int.): δ 7.44 ppm (*q*, $J$ = 2 and 8.5, 1H, H-6′), 7.23 (*d*, $J$ = 2, 1H, H-2′), 7.00 (*d*, $J$ = 8.5, 1H, H-5′), 6.33 (*s*, 1H, H-6), 5.67 (*d*, $J$ = 12, 1H, H-2), 5.29 (*d*, $J$ = 12, 1H, H-3), 5.12 (*t* (*br*) $J$ = 8, 1H, β-CH=), 3.88 (*s*, 6H, OMe), 3.29 (*d*, $J$ = 8, 2H, α-$CH_2$), 2.38 (*s*, 3H, OAc-5), 2.31 (*s*, 3H, OAc-3′), 2.02 (*s*, 3H, OAc-3), 1.65, 1.59 (*s* (*br*), 6H, γ-$CH_3$). MS (EI 70 eV, 100 μA, 4 kV, 200°; DI 160; $10^{-6}$T): *m/e* 526 $M^+$ (4% rel. int.), 484 (100), 442 (19), 424 (39), 409 (20), 382 (16), 314 (11), 290 (15), 286 (13), 248 (20), 233 (41), 219 (50), 206 (65), 195 (31), 191 (28), 179 (70), 166 (68), 164 (34), 137 (18), 43 (47).

*Kaempferol-3-rutinoside* (**1**). Yellow needles (MeOH), mp 190–92°. It gave green colour with alc. $FeCl_3$ and deep red with Mg–HCl. $R_f$s: 0.58 (PC, BAW, 4:1:5), 0.55 (PC, 15% aq. HOAc), 0.64 (TLC, microcrystalline cellulose (E. Merck), 15% aq. HOAc); UV data agreed with lit. [13]. MS of permethyl ether *m/e* (%): 575 [S+60] (1), 515 [S] (2), 501 (4), 392 [OS] (6), 328 [A+H] (100), 189 (16), 188 (31), 157 (10), 142 (27), 135 (13). On acid hydrolysis (7% aq. $H_2SO_4$) **1** gave an aglycone (identified as kaempferol by PC cochromatography with authentic sample) and rhamnose and glucose as sugars. **1** was identified as kaempferol-3-rutinoside.

*Quercetin-3-rutinoside* (**2**). Yellow needles (MeOH), mp 188–90°, identified by direct comparison with an authentic sample (mmp, cochromatography).

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# FLAVONE *O*- AND *C*-GLYCOSIDES OF *RHYNCHOSIA BEDDOMEI*

DAMA ADINARAYANA*, DUVVURU GUNASEKAR*, OTTO SELIGMANN† and HILDEBERT WAGNER†

* Department of Chemistry, Sri Venkateswara University, Tirupati-517502, India;
† Institut für pharmazeutische Arzneimittellehre der Universität München, Karlstraße 29, D-8000 München 2, W. Germany

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**Key Word Index**—*Rhynchosia beddomei;* Leguminosae; 3′,4′-di-*O*-methylluteolin-7-*O*-glucuronide; vitexin; isovitexin; orientin; isoorientin; vicenin-2; lucenin-2; rutin; naringenin; D-inositol.

**Abstract**—A new flavone-*O*-glycoside isolated from the leaves of *Rhynchosia beddomei* has been characterized as 3′,4′-di-*O*-methylluteolin-7-*O*-glucuronide.

## INTRODUCTION

Plants belonging to the genus *Rhynchosia* (tribe Phaseoleae, subfamily Papilionoideae) have not been thoroughly examined for their flavonoid constituents. Besson *et al.* [1] reported the presence of four *C*-glycosylflavones in the leaves of *R.minima*. Based on the absence of flavonoid aglycones before or after acid hydrolysis, they concluded that only *C*-glycosides are present in this plant. In our chemical examination of the leaves of *R.beddomei* (Bak.) we have isolated six flavone-*C*-glycosides, a flavonol-*O*-glycoside, a new flavone-*O*-glycoside and a flavanone.

## RESULTS AND DISCUSSION

Acetone extract of the leaves afforded, after repeated column chromatography employing silica gel, PC and TLC (cellulose and silica gel), four mono-*C*-glycosides (vitexin, isovitexin, orientin and isoorientin) and two di-*C*-glycosides established as vicenin-2 and lucenin-2 by mass spectral study of their permethylated derivatives. Methanol extract of the leaves yielded a cyclitol (identified as D-inositol), a flavanone (established as naringenin) and two *O*-glycosides. One of them was obtained as yellow needles (yield 0.002%) melting at 188–90° and showed identity with rutin. The second compound was obtained as pale yellow crystals (yield 0.004%) $[\alpha]_D^{31} - 160.71°$ (*c* 0.56, Py–$H_2O$, 1:1 v/v) which did not melt below 320°. It gave a brown ferric colour, a positive Molisch's test and pale pink colour with Mg–HCl. It also effervesced with $NaHCO_3$.

A strong IR absorption at 1585 $cm^{-1}$ is characteristic for carboxylate. The flame test gives evidence for the presence of a potassium salt. According to the UV spectra, the flavone nucleus must bear protected OH groups in 7,3′,4′-positions and a free 5-OH group. The aglycone peak at *m/e* 314 with the fragment ions at *m/e* 153 and 162 are in congruence with a 3′,4′-di-*O*-methylluteolin structure. This was confirmed by hydrolysis and comparison of the aglycone with an authentic sample obtained by dehydrogenation of synthetic 3′,4′-di-*O*-methyleriodictyol-7-*O*-neohesperidoside followed by hydrolysis [2]. The per-deuteromethylated glycoside showed a $M^+$ of *m/e* 575. The fragmentation sequence *m/e* 244, 210, 175, 147, 122 and 107 is typical for PDM-hexuronides [3]. All these data are in agreement with the structure of a 3′,4′-di-*O*-methylluteolin-7-*O*-β-D-glucopyranuronide.

## EXPERIMENTAL

Shade-dried leaves of *R.beddomei* (3.2 kg) were extracted successively with petrol (bp 60–80°), $C_6H_6$, $Me_2CO$ and