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Note

A new prenylated isoflavone from *Tephrosia tinctoria*

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A new prenylated isoflavone, 7-*O*-geranylbiochanin A **1** has been isolated from the roots of *Tephrosia tinctoria*, together with three previously known compounds, 7-*O*-methylglabranin **2**, flemichapparin B **3** and dehydrodeguelin **4**. The structures of the compounds, **1** - **4** have been established by spectroscopic methods, including analysis by 2D NMR spectroscopy.

Keywords: *Tephrosia tinctoria*, Isoflavone

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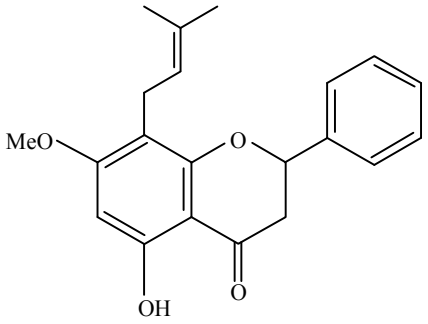
Tephrosia PERS. (Fam: Leguminosae, Sub fam: Papilionoideae) is a large tropical and sub-tropical genus estimated to contain 300 species[1]. The genus *Tephrosia* is known to elaborate a rich variety of flavonoids and isoflavonoids[2]. *Tephrosia tinctoria* Pers. is an erect undershrub widely distributed in Talakona forest of Andhra Pradesh, South India[3]. As there is no record of any phytochemical work on *T. tinctoria*, we have examined the roots of this species and report herein the isolation and structure elucidation of a new prenylated isoflavone, 7-*O*-geranylbiochanin **A 1** together with three known compounds, 7-*O*-methylglabranin **2**, flemichapparin B **3** and dehydrodeguelin **4**.

Results and Discussion

Compound **1**, obtained as pale yellow amorphous powder, showed $[M + H]^+$ peak at m/z 421 in the positive ion FABMS corresponding to the molecular formula $C_{26}H_{28}O_5$. This was corroborated by the ^{13}C NMR spectrum, which showed signals for all the 26 carbons present in the molecule. The UV spectrum showed absorption maxima at 262 and 327 nm, characteristic of an isoflavone derivative[4]. The ^1H NMR spectrum of **1** showed a sharp signal at δ 7.90 (1H, s), typical of the proton at C-2 of an isoflavonoid skeleton. Addition of NaOAc caused no shift in band II UV absorption maximum indicating the absence of a free hydroxyl at C-7. A bathochromic shift of 11 nm in band I UV absorption maximum with $AlCl_3$ and a downfield ^1H NMR signal at δ 12.82 in **1,** suggested the presence of a chelated hydroxyl at C-5. The IR spectrum exhibited absorption bands for chelated hydroxyl (3376 cm^{-1}), conjugated carbonyl (1646 cm^{-1}), olefin (1415 cm^{-1}) and ether (1179 cm^{-1}) functionalities.

The ^1H NMR spectrum of **1** showed two singlet signals at δ 6.12 and 6.29, which correlated to carbons at 99.6 (C-6) and 94.4 ppm (C-8), in the HSQC spectrum, were characteristic of H-6 and H-8 protons of ring A. It also showed two sets of *ortho*-coupled doublets (J = 8.8 Hz) at δ 6.95 and 7.44, integrating for two protons each, correlating with C-3′ and C-5′ (114.0 ppm) and C-2′ and C-6′ signals (130.1 ppm), respectively in the HMBC spectrum (**Figure 1**), were assigned to H-3′, 5′ and H-2′, 6′ protons of ring B. A sharp three-proton singlet at δ 3.82 was attributed to a methoxyl group and was placed at C-4′ as it showed ^3J correlation with this carbon at 159.7 ppm in the HMBC spectrum.

The ^1H NMR spectrum of **1** further showed three olefinic methyl singlets δ (1.57, 1.65, 1.81), two multiplet methylene signals (2.06, 2.08), an oxygenated doublet methylene signal (4.54) and two triplet vinyl proton signals (5.03, 5.234) assignable to either geranyl or neryl moiety. The ^{13}C NMR data, particularly the chemical shifts of the methyl at C-3″ and the C-4″ methylene, aid in distinguishing geranyl from neryl side chain. The chemical shifts at 16.1 and 39.6 ppm observed for methyl and methylene groups, respectively, confirmed the presence of a geranyl side chain[5] in **1.** The geranyl moiety was placed at C-7 as the geranyloxy methylene protons at C-1″ correlated to C-7 of the isoflavone nucleus in the HMBC spectrum. Moreover, the interactions observed in the 2D phase-sensitive NOESY (**Figure 1**) experiment between the methylene protons at C-1″ and H-6 and H-8 aromatic protons unambiguously confirms the presence of an *O*-geranyl moiety at C-7 position. On the basis of the foregoing spectral studies, the structure of **1** was elucidated as 5-hydroxy-4′-methoxy-7-*O*-geranyl isoflavone (or 7-*O*-geranyl-biochanin A). Incidentally, the isolation of **1**



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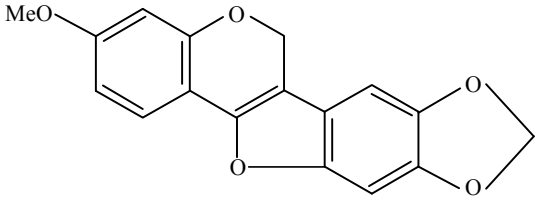

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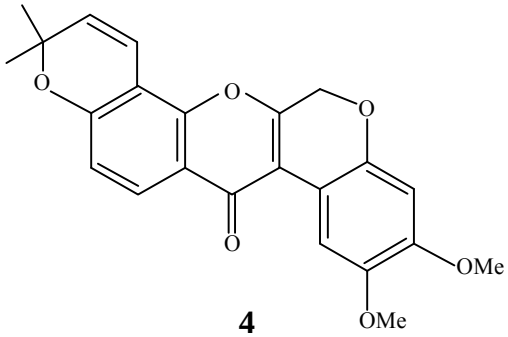

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constitutes the rare occurrence of an *O*-geranylated isoflavone from a natural source.

The structures of known compounds, **2**, **3** and **4** were established by comparison of their physical and spectral data with literature values [6-8].

Experimental Section

General. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded in KBr discs on a Perkin-Elmer 283 double beam spectrophotometer and UV spectra on a Shimadzu UV-240 spectrophotometer. NMR spectra were run on a Bruker instrument equipped with a 5 mm ^{1}H and ^{13}C probe operating at 400 and 100 MHz, respectively, using $CDCl_3$ with TMS as internal standard (chemical shifts in δ, ppm). ^{1}H assignments were made using 2D-COSY and NOESY (mixing time 500 ms) while ^{13}C assignments were made using HSQC and HMBC experiments. FABMS was recorded on VG Autospec - 251M mass spectrometer using *m*-nitrobenzyl alcohol as a matrix. ESITOFMS is recorded on an API Q-STAR PULSA

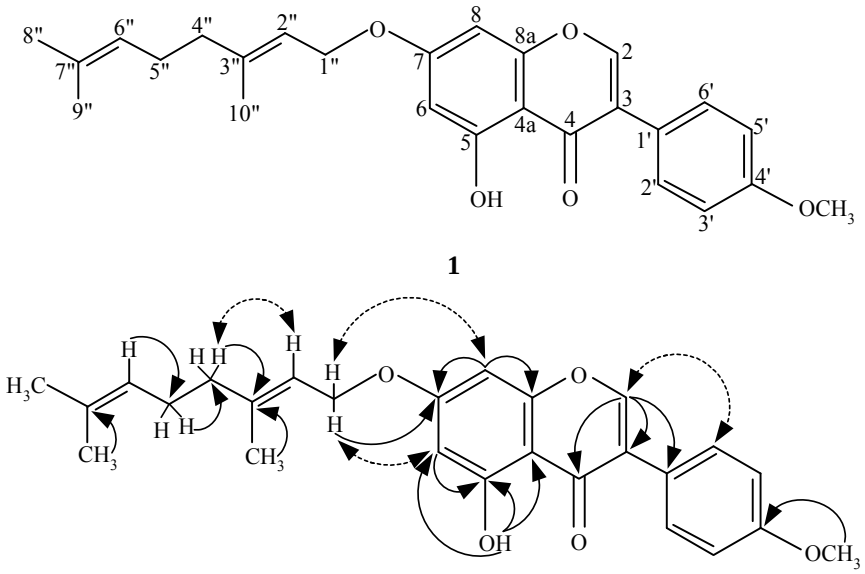

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Figure 1—Significant HMBC (→) and NOESY (↔) Correlations observed in **1**

of Applied Bio-system. Column chromatography (CC) was performed on Acme silica gel finer than 200 mesh (0.08 mm).

Plant material. The roots of *T. tinctoria* were collected from Talakona forest, Andhra Pradesh, South India in October 2003. A voucher specimen (DG-009) has been deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati, India.

Extraction and Isolation.

Air-dried and coarsely powdered roots (3 kg) of *T. tinctoria* were extracted successively with *n*-hexane, Me_2CO and MeOH. The *n*-hexane and Me_2CO extracts were found to be similar on TLC and hence combined and evaporated *in vacuo* to yield a dark brown residue (30 g). It was purified over a silica gel column using *n*-hexane and EtOAc step gradient as eluents. The *n*-hexane-EtOAc, 7:3 and 1:1, eluates yielded **1** (15 mg) and **2** (8.5 mg), respectively. The MeOH extract (20 g) was purified over a silica gel column using *n*-hexane-EtOAc step gradient mixtures. The *n*-hexane-EtOAc, 4:6 and 2:8, eluates yielded **3** (10 mg) and **4** (18 mg), respectively.

7-*O*-geranylbiochanin A 1: Pale yellow amorphous powder ($CHCl_3$), m.p. 116-18ºC; UV (MeOH): 262 (4.5), 327 (3.6); (NaOAc) 262, 330; ($AlCl_3$) 273, 310; (NaOMe) 272, 327 nm; IR (KBr): 3376 (-OH), 2920 (-OMe), 2851, 1646 (>C=O), 1571, 1513, 1415 (C=C), 1291, 1250, 1179 (C-O-C), 1061, 1023, 877, 834, 728 cm^{-1}; ^1H NMR ($CDCl_3$): δ 12.82 (1H, s, OH-5), 7.90 (1H, s, H-2), 7.44 (2H, d, *J* = 8.8 Hz, H-2′, 6′), 6.95 (2H, d, *J* = 8.8 Hz, H-3′, 5′), 6.29 (1H, s, H-8), 6.12 (1H, s, H-6), 5.23 (1H, t, *J* = 6.7 Hz, CH=, H-2″), 5.03 (1H, t, *J* = 6.7 Hz, CH=, H-6″), 4.54 (2H, d, *J* = 6.7 Hz, CH_2, H-1″), 3.82 (3H, s, OMe-4′), 2.08 (2H, m, CH_2, H-5″), 2.06 (2H, m, CH_2, H-4″), 1.81 (3H, s, Me-10″), 1.65 (3H, s, Me-8″), 1.57 (3H, s, Me-9″); ^{13}C NMR ($CDCl_3$): δ 181.1 (C-4), 160.9 (C-7), 160.6 (C-5), 159.7 (C-4′), 155.0 (C-8a), 152.5 (C-2), 138.6 (C-3″), 132.0 (C-7″), 130.1 (C-2′, 6′), 123.6 (C-6″), 123.5 (C-3), 123.1 (C-1′), 120.9 (C-2″), 114.0 (C-3′, 5′), 106.1 (C-4a), 99.6 (C-6), 94.4 (C-8), 67.2 (C-1″), 55.3 (OMe-4′), 39.6 (C-4″), 26.3 (C-5″), 25.5 (C-8″), 17.6 (C-9″), 16.1 (C-10″); FABMS (positive ion mode): m/z 421 $[M + H]^+$, 405, 391, 363, 351, 337, 297, 285, 267, 165, 154, 136, 121, 107, 91, 81; ESITOFMS: m/z 443.1879 $[M + Na]^+$, 421.1977 $[M + H]^+$ (Calcd for $C_{26}H_{29}O_5$: 421.1937).

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