A New Coumestan from *Tephrosia calophylla*

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A new coumestan, tephcalostan (1) has been isolated from the whole plant of *Tephrosia calophylla* Bedd. together with two known flavonoids, 7-*O*-methylglabranin (2) and kaempferol 3-*O*-β-D-glucopyranoside (3). The structure of tephcalostan was elucidated as 5′-(*R*)-8,9-methylenedioxy-5′-isopropenyl-4′,5′-dihydrofurano-[2′,3′:2,3]coumestan by extensive one-and two-dimensional (1D- and 2D-)-NMR techniques including ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond connectivity (HMBC) and nuclear Overhauser enhancement spectroscopy (NOESY) experiments.

Key words *Tephrosia calophylla*; Leguminosae; coumestan; flavonoids

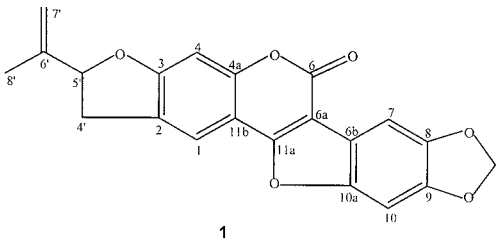
Tephrosia Pers. (Leguminosae, Papilionoideae) is a large tropical and sub-tropical genus estimated to contain 300 species.[1] *Tephrosia calophylla* Bedd. is a perennial undershrub found widely in Talakona forest of Andhra Pradesh, South India.[2] The genus *Tephrosia* is known to elaborate a rich variety of flavonoids and isoflavonoids.[3] Phytochemical investigation of the whole plant of this hitherto uninvestigated species has led to the isolation of a new coumestan, tephcalostan (**1**) together with two known flavonoids, 7-*O*-methylglabranin (**2**) and kaempferol-3-*O*-β-D-glucopyranoside (**3**).

Results and Discussion

Tephcalostan **1**, obtained as colourless needles, showed $[M+H]^+$ peak at *m*/*z* 363.0945 in its positive electrospray ionisation time of flight mass spectrum (ESI-TOF-MS) consistent with the molecular formula $C_{21}H_{14}O_6$. It was supported by ^{13}C-NMR spectrum and distortionless enhancement by polarisation transfer (DEPT, 90 and 135) experiments, which showed 21 carbon resonances consisting of one methyl, three methylene, five methine and twelve quaternary carbons. The UV absorption maxima at 248, 313 and 355 nm were typical of a coumestan skeleton.[4] The IR spectrum of **1** exhibited strong absorption bands attributable to δ-lactone carbonyl (1728 cm^{-1}), benzene ring (1634, 1580 cm^{-1}) and a methylenedioxy group (1038, 940 cm^{-1}).

The ^{1}H-NMR spectrum of **1** exhibited signals for four aromatic protons (δ 7.67, s; 7.42, s; 7.06, s; 6.87, s), a methylenedioxy group (δ 6.02) and an isopropenyldihydrofuran moiety, discernable from two diastereotropic proton signals (δ 3.09, 1H, dd, J=15.8, 7.8 Hz; 3.42, 1H, dd, J=16.4, 9.3 Hz), an oxygenated methine proton signal (δ 5.29, 1H, t, J= 8.6 Hz), two olefinic proton signals (δ 5.10, 1H, br s; 4.94, 1H, br s) and a methyl signal (δ 1.76, 3H, s). Based on heteronuclear multiple bond connectivity (HMBC) correlations (Fig. 1) the aromatic protons at δ 7.67, 6.87, 7.42 and 7.06 were assigned to H-1, H-4, H-7, and H-10. Thus from the foregoing spectral studies, two possible structures (**1**, **1a**) could be assigned for tephcalostan.

A distinction between two possible structures (**1**, **1a**) for tephcalostan was made by HMBC and nuclear Overhauser enhancement spectroscopy (NOESY) studies. The methylenedioxy group at δ 6.02 was placed at C-8/C-9 positions of ring-B as these protons showed HMBC correlations with C-8 (δ 145.9) and C-9 (δ 147.1) which further showed cross correlations with two *para* related aromatic protons at δ 7.42 (H-7) and 7.06 (H-10) in its HMBC spectrum (Fig. 1). The isopropenyldihydrofuran moiety was placed at C-2/C-3 (linear attachment) of ring-A based on HMBC correlations of oxygenated methine proton (δ 5.29) and methylene protons (δ 3.09 and 3.42) of dihydrofuran moiety with C-2 (δ 124.9) and C-3 (δ 162.8), and C-1 (δ 116.8), C-2 (δ 124.9) and C-3 (δ 162.8), respectively. Two strong nuclear Overhauser effect



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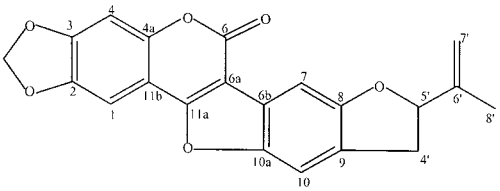

1a

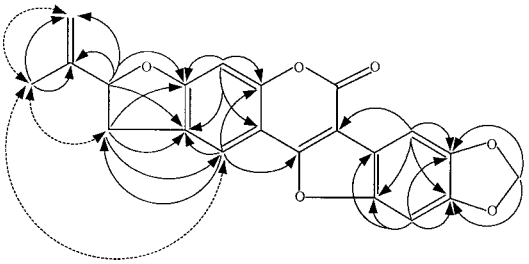

Fig. 1. HMBC (⟶) and NOESY (⇠⇢) Correlations Observed in **1**

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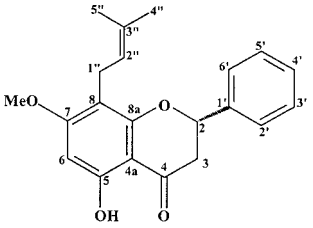

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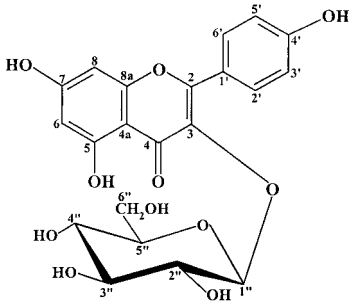

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Fig. 2. Structures for Compounds **2** and **3**

(NOE) correlations between C-4′ methylene protons (δ 3.09 and 3.42) of dihydrofuran moiety with H-1 (δ 7.67) of ring-A and C-8′ methyl protons (δ 1.76) of isopropenyl moiety supporting the attachment of isopropenyldihydrofuran moiety to ring-A. The linear fusion of isopropenyldihydrofuran moiety with ring-A was also evidenced by ^2J and ^3J correlations of the *para* related protons, H-1 (δ 7.67) and H-4 (δ 6.87) with C-2 (δ 124.9) and C-3 (δ 162.8) in its HMBC spectrum (Fig. 1). The foregoing spectral data agreed well with structure **1** for tephcalostan, thus eliminating structure **1a**. The isopropenyl substituent in tephcalostan was linked to C-5′ of dihydrofuran moiety as H-5′ (δ 5.29) showed HMBC correlations with C-6′ (δ 142.8) and C-7′ (δ 112.9) which further showed cross correlations with C-8′ methyl protons (δ 1.76). The ^{1}H and ^{13}C-chemical shift values of **1** were unambiguously assigned based on ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear single quantum coherence (HSQC) experiments. The absolute configuration at C-5′ of isopropenyl moiety in **1** was determined by comparing the sign of the circular dichroism (CD) Cotton effect given by the osmate ester of **1** with that of natural rotenone[5] containing isopropenyl side chain as in **1**. A negative Cotton effect at 477 nm indicated that **1** had *R* configuration at C-5′. Thus, tephcalostan was characterized as 5′-(*R*)-8,9-methylenedioxy-5′-isopropenyl-4′,5′-dihydrofurano[2′,3′:2,3]-coumestan (**1**).

The structures of known compounds, **2** and **3** (Fig. 2) were established by comparison of their spectral data with the literature values.[6,7]

Experimental

General Procedures Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured in MeOH at 25 °C on a Perkin-Elmer 241 polarimeter. IR spectra were recorded in KBr discs on a Perkin-Elmer 283 double beam spectrophotometer and UV spectra on a Shimadzu UV-240 spectrophotometer. The CD spectrum was recorded in CH_2Cl_2 at 25 °C on a JASCO J 715 spectropolarimeter. ^{1}H- and ^{13}C-NMR spectra were determined on a Bruker Avance 400 spectrometer using $CDCl_3$ and DMSO-d_6 with tetramethylsilane (TMS) as internal standard. ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY spectra were recorded using standard pulse sequences. ESI-TOF-MS and ESI tandem mass spectrometry (ESI-MS/MS) were recorded on a API Q-STAR PULSA of Applied Bio-system. Electron impact (EI)-MS were obtained on a Nermag R10-10 mass spectrometer at 70 eV by direct inlet probe. Chemical ionization (CI)-MS was obtained on a 700 JEOL mass spectrometer by direct inlet probe using CH_4 as the ionizing gas at 500 °C. Column chromatography (CC) was performed on Acme silica gel finer than 200 mesh (0.08 mm).

Plant Material The whole plant of *T. calophylla* Bedd. was collected from Talakona forest, Andhra Pradesh, South India in October 1998. A voucher specimen (DG-983) has been deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati, India.

Extraction and Isolation The air-dried and powdered whole plant of *T. calophylla* (10 kg) was successively extracted with *n*-hexane, Me_2CO and MeOH. The *n*-hexane extract on purification over a silica gel column using *n*-hexane as eluent yielded **2** (20 mg). The Me_2CO extract on purification over a silica gel column using *n*-hexane–EtOAc step gradient (7 : 3, 1 : 9) yielded **1** (36 mg) and **3** (15 mg), respectively.

Tephcalostan (**1**): Colourless needles ($CHCl_3$), mp 251—252 °C. $[\alpha]_D^{25}$ −52° (c=0.001, MeOH). UV λ_{max} (MeOH) nm (log ε): 248 (2.6), 313 (2.4), 355 (2.8). IR (KBr) ν_{max} cm^{-1}: 1728 (δ-lactone >C=O), 1634, 1580, 1469, 1348, 1257, 1158, 1038, 940. ^{1}H-NMR ($CDCl_3$) δ: 7.67 (1H, s, H-1), 7.42 (1H, s, H-7), 7.06 (1H, s, H-10), 6.87 (1H, s, H-4), 6.02 (2H, s, –OCH_2O–), 5.29 (1H, t, J=8.6 Hz, H-5′), 5.10 (1H, br s, H-7′a), 4.94 (1H, br s, H-7′b), 3.42 (1H, dd, J=16.4, 9.3 Hz, H-4′a), 3.09 (1H, dd, J=15.8, 7.8 Hz, H-4′b), 1.76 (3H, s, Me-8′). ^{13}C-NMR ($CDCl_3$) δ: 162.8 (s, C-3), 160.2 (s, C-11a), 158.5 (s, C-6), 154.5 (s, C-4a), 150.2 (s, C-10a), 147.1 (s, C-9), 145.9 (s, C-8), 142.8 (s, C-6′), 124.9 (s, C-2), 116.9 (s, C-6b), 116.8 (d, C-1), 112.9 (t, C-7′), 105.9 (s, C-11b), 103.1 (s, C-6a), 101.8 (t, –OCH_2O–), 99.9 (d, C-7), 98.4 (d, C-4), 93.8 (d, C-10), 87.4 (d, C-5′), 33.5 (t, C-4′), 17.1 (q, C-8′). ESI-MS/MS (positive ion mode) *m/z* (%): 363.1 $[M+H]^+$ (20.3), 348.0 $[M+H-CH_3]^+$ (4.2), 335.1 $[M+H-CO]^+$ (10.1), 333.0 $[M+H-CH_2O]^+$ (94.1), 320.0 $[M+H-CH_3-CO]^+$ (17.5), 319.1 $[M+H-CH_3-CHO]^+$ (4.2), 318.1 $[M+H-CH_3-CH_2O]^+$ (100), 317.1 $[M+H-OCH_2O]^+$ (9.0), 295.0 $[M+H-C_5H_8]^+$ (4.2), 292.0 $[M+H-CH_3-CO-CO]^+$ (2.1). ESI-TOF-MS *m/z*: 363.0945 $[M+H]^+$ (Calcd for $C_{21}H_{14}O_6$+H: requires 363.0868).

7-*O*-Methylglabranin (**2**): Colourless needles ($CHCl_3$), mp 124—125 °C $[\alpha]_D^{25}$ −20.9° (c=0.25, MeOH). UV λ_{max} (MeOH) nm (log ε): 292 (4.2), 345 (sh) (3.4); (NaOAc) 292, 332 (sh); ($AlCl_3$) 312, 368; ($AlCl_3$+HCl) 312, 367. IR (KBr) ν_{max} cm^{-1}: 3444 (OH), 1637 (>C=O), 1618, 1584, 1491, 1385, 1274, 1193, 1095, 987, 829, 778. ^{1}H-NMR ($CDCl_3$) δ: 12.04 (OH-5), 7.35 (5H, m, H-2′, 3′, 4′, 5′, 6′), 6.04 (1H, s, H-6), 5.23 (1H, dd, J=13.0, 3.0 Hz, H-2), 5.05 (1H, t, J=8.0 Hz, β-CH=), 3.88 (3H, s, OMe-7), 3.15 (2H, d, J=8.0 Hz, α-CH_2), 3.09 (1H, dd, J=17.2, 13.0 Hz, H-3_{ax}), 2.91 (1H, dd, J=17.2, 3.0 Hz, H-3_{eq}), 1.88 (6H, s, =CMe_2). ^{13}C-NMR ($CDCl_3$) δ: 195.8 (C-4), 163.8 (C-8a), 161.3 (C-7), 159.6 (C-5), 134.1 (C-1′), 130.7 (C-3″), 130.1 (C-2′, 3′, 5′, 6′), 125.6 (C-4′), 123.0 (C-2″), 105.0 (C-8), 102.0 (C-4a), 98.5 (C-6), 79.0 (C-2), 56.2 (7-OMe), 42.5 (C-3), 26.5 (C-4″), 22.0 (C-1″), 17.0 (C-5″). EI-MS *m/z* (%) 338 $[M]^+$ (100), 323 (61), 295 (25), 283 (16), 270 (27), 234 $[A_1]^+$ (5), 219 (78), 206 $[A_1^+-CO]$ (17), 191 (42), 179 (33), 104 $[B_3]^+$ (15), 91 (14).

Kaempferol 3-*O*-β-D-Glucopyranoside (**3**): Yellow needles (MeOH), mp 177—178 °C. $[\alpha]_D^{25}$ −40.5° (c=0.10, MeOH). UV λ_{max} (MeOH) nm (log ε): 267 (4.55), 350 (4.20); (NaOMe) 275, 325, 402; (NaOAc) 275, 312 (sh), 284; ($AlCl_3$) 275, 398; ($AlCl_3$+HCl) 278, 398. IR (KBr) ν_{max} cm^{-1}: 3420 (OH), 2924, 1657 (>C=O), 1609, 1507, 1361, 1289, 1205, 968, 894. ^{1}H-NMR (DMSO-d_6) δ: 12.60 (1H, s, OH-5), 10.40 (2H, s, OH-7, 4′), 8.03 (2H, d, J=8.9 Hz, H-2′, 6′), 6.87 (2H, d, J=8.9 Hz, H-3′, 5′), 6.42 (1H, d, J=2.0 Hz, H-8), 6.19 (1H, d, J=2.0 Hz, H-6), 5.45 (1H, d, J=7.3 Hz, H-1″), 2.90—3.57 (6H, m, sugar protons). ^{13}C-NMR (DMSO-d_6) δ: 177.5 (C-4), 164.3 (C-7), 161.2 (C-5), 160.0 (C-4′), 156.4 (C-8a), 156.2 (C-2), 133.2 (C-3), 130.9 (C-2′, 6′), 120.9 (C-1′), 115.1 (C-3′, 5′), 104.0 (C-4a), 100.9 (C-1″), 98.7 (C-6), 93.7 (C-8), 77.5 (C-3″), 76.4 (C-5″), 74.2 (C-2″), 69.9 (C-4″), 60.9 (C-6″). CI-MS *m/z* (%) 449 $[M+H]^+$ (16), 287 $[M+H-162]^+$+ (100), 180 (54), 162 (9), 145 (4), 121 (2).

Preparation and CD Determination of the Osmate Ester–Pyridine Complex of 1 A solution of **1** (0.40 mg, 1.2 μmol) in CH_2Cl_2 (65 μl) containing 25 μmol of C_5H_5N was treated with OsO_4 (1.4 μmol in 10 μl of CH_2Cl_2) for about 30 min at room temperature. The mixture was diluted with CH_2Cl_2 to a final volume of 2.8 ml. The CD spectrum of the resulting

osmate ester–pyridine complex was recorded over the range 400—600 nm. CD (25 °C): $[\theta]_{477}$ −9973.1.

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