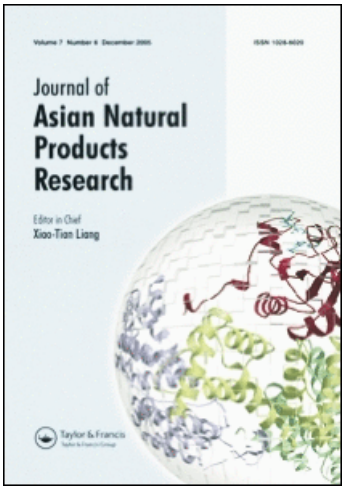

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Two new isoflavonoids from the roots of *Dalbergia congesta* (Grah)

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A systematic examination of the roots of *Dalbergia congesta*, yielded a new oligomeric isoflavonoid (**1**), a new tetramethoxy isoflavone (**2**) along with two known compounds, an isoflavone dalspinin (**3**) and a benzophenone, cearoin (**4**). On the basis of chemical and spectral evidences, compounds **1** and **2** were determined to be 5,7-dihydroxy-6,4′-dimethoxy-6′[2″-hydroxy-2″(2‴,5‴-dimethoxy neoflavonyl) ethenyl] isoflavone (dalcongestin) and 5,7-dihydroxy-2′,3′,5′,6′-tetramethoxy isoflavone, respectively.

Keywords: *Dalbergia congesta*; Leguminosae; Oligomeric isoflavonoids; Flavonoids

1. Introduction

The genus *Dalbergia* (Leguminosae) has been widely investigated for its phytochemical constituents and medicinal properties [1–5]. *Dalbergia congesta* (Grah) is a woody climber of erect shrub. It is available in the Indo-China regions and Burma. There is no previous report on the phytochemical investigation on *D. congesta*. We have systematically examined its roots and herein we report the isolation and characterization of an oligomeric isoflavonoid, dalcongestin (**1**), a tetramethoxy isoflavone (**2**), dalspinin (**3**) and cearoin (**4**). The structure for compound **1** was determined by 2D-NMR techniques. This is the first report of the natural occurrence of compounds **1** and **2**.

2. Results and discussion

Compound **1**, obtained as a pale yellow amorphous solid (m.p. 162–163°C), analysed for $C_{36}H_{28}O_{11}$ by HREIMS, *m/z* 636.6082, $[M]^+$ and NMR data. It gave dark green colour with

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neutral ferric chloride and pink colour with Na–Hg/HCl (Wolfrom test for isoflavones) [6]. The UV absorptions at 268, 295 sh and at 237 and 350 nm indicated the presence of an isoflavone and a neoflavone (4-phenyl chromone) units, respectively. Use of diagnostic shift reagents [7] in the UV spectrum revealed the presence of a chelated hydroxyl group at C-5, an unsubstituted hydroxyl group at C-7 and also the absence of ortho-dihydroxy groups in both the rings A and B of compound **1**. The ^{1}H NMR spectrum of **1** showed three one-proton singlets at δ 13.12 (chelated hydroxyl), 7.89 (H-2 of isoflavone) and 6.53 (H-8 of isoflavone) indicating **1** to be a 5,7-dihydroxy isoflavone with a substitution at C-6. Two one-proton doublets at δ 6.95 and 7.10 and a one-proton double doublet at δ 7.01 showed the ABX pattern in ring B of **1**, which was further observed in the ^{1}H–^{1}H COSY spectrum. The HREIMS fragment at *m/z* 183 $(A_1 + H)^+$ indicated the presence of a methoxyl group and two hydroxyl groups in ring-A. The UV absorptions at 237 and 350 nm, characteristic of a neoflavone, the presence of a lactone carbonyl at 1690 cm^{-1} in the IR spectrum, the presence of two aromatic protons at δ 6.25 and 6.90 and a five-proton multiplet at δ 7.40–7.55 (unsubstituted phenyl ring) in the ^{1}H NMR spectrum suggested the presence of a tri-substituted neoflavone unit in **1**.

The ^{1}H NMR spectrum also showed two hydroxyl groups at δ 6.55 and 5.53, which disappeared on D_2O exchange. A one-proton singlet at δ 6.99 suggested the presence of an olefinic proton in **1**. The IR spectrum showed a medium band at 828 cm^{-1} and a band at 1624 cm^{-1} indicating the presence of a tri-substituted alkene group conjugated with aromatic rings. Closer inspection of the ^{1}H, ^{13}C NMR and DEPT spectra of **1** revealed the presence of four methoxyl signals (two overlapping) and eleven methine carbons. The methine signal, upfield at δ 99.58 confirmed the presence of a stilbene-like olefinic bond in **1** (figure 1).

The HMQC spectrum showed that the proton resonating at δ 6.99 showed correlation to the olefinic carbon resonating at δ 99.58 but did not show any connectivity to any carbon in the isoflavone or neoflavone units, further confirming the presence of an olefinic bond in **1**. The absence of any olefinic proton correlated to C-2″ in the HMQC NMR spectrum and the

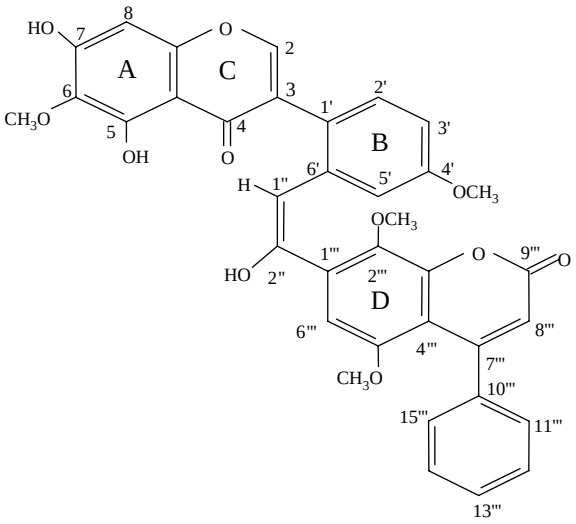

Figure 1. Structure of **1**.

downfield shift of the C-2″ carbon at δ 142.46 (indicating the hydroxyl substitution at C-2″) showed that one of the hydroxyl groups was attached as a hydroxy–ethenyl linkage between the isoflavone and the neoflavone units giving rise to an oligomeric isoflavonoid. Absence of *meta* coupling of H-2′ confirmed the substitution at C-6′ of the isoflavone unit. The overlapping signals of C-5 and C-8 of neoflavone unit at δ 150.15 in ^{13}C NMR and the presence of a lone D-ring proton at δ 6.90 in the ^{1}H NMR spectrum confirmed the substitution at C-1‴ of neoflavone unit. The fragments at *m/z* 455 and 383 showed the presence of a methoxyl group in ring-B, an olefinic linkage between C-6′ of isoflavone and C-1‴ of neoflavone and also the presence of a dimethoxylated neoflavone in the compound. The HMBC correlation can be seen in figure 2. Hence the structure of **1**, was thus assigned as 5,7-dihydroxy-6,4′-dimethoxy-6′[2″-hydroxy-2″(2‴,5‴-dimethoxy neoflavonyl) ethenyl] isoflavone (dalcongestin) (figure 1).

Compound **2** was isolated as an amorphous colorless solid (m.p. 156–157°C). The HREIMS of **2** showed the presence of $[M-H]^+$ ion at *m/z* 373 corresponding to $C_{19}H_{17}O_8$. The UV absorptions at 265 (band II) and 296 sh nm (band I) suggested the presence of an isoflavone skeleton. The fragment at *m/z* 153 arising out of the RDA cleavage of **2**, indicated the presence of two hydroxyl groups in ring-A. The ^{1}H NMR spectrum of **2** showed four one-proton singlets at δ 13.33, 7.87, 6.51 and 6.62, which were consistent with a 5, 7-dihydroxy-substitution pattern in ring-A. Four three-proton singlets indicated the presence of four methoxyl groups in **2**. ^{13}C NMR and DEPT spectral studies revealed the presence of four aromatic methine carbons. HMQC spectrum was used to confirm the assignments due to protonated carbons. The mass spectral fragments due to the RDA cleavage of **2** at *m/z* 221 (B^+) confirmed the tetramethoxy-substitution in ring-B. The methoxyl substitution at C-6′ was confirmed by the presence of a peak at *m/z* 342, corresponding to the formation of $[M-31]^+$. The structure of **2** was thus identified as 5,7-dihydroxy-2′,3′,5′,6′-tetramethoxy isoflavone (figure 3).

The other two known compounds, 5,7-dihydroxy-6-methoxy- 3′,4′-methylenedioxy isoflavone, dalspinin, **3** and 2,5-dihydroxy-4-methoxy benzophenone, cearoin, **4**, were identified by comparison of their physical and spectral data with the literature values for cearoin [8,9] and dalspinin [10–12], respectively.

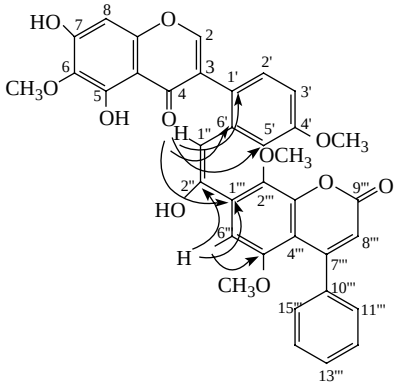

Figure 2. Some key HMBC correlations observed for **1**.

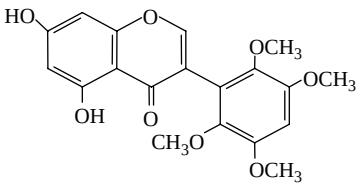

Figure 3. Structure of **2**.

3. Experimental

3.1 General experimental procedure

Melting points were determined on an electrical melting-point apparatus and are uncorrected. UV and IR spectra were recorded with a Shimadzu-UV-240 spectrophotometer and Perkin–Elmer 1600 FTIR spectrophotometer, respectively. 1D and 2D NMR experiments were recorded in $CDCl_3$ (TMS, $\delta = 0$) at 300 K on Bruker AMX 400 MHz operating at 400 MHz for proton and 112.6 MHz for carbon, respectively. FABMS and EIMS were recorded on Shimadzu mass spectrometer. Column chromatography was carried out on Si gel (60–120 mesh, Fischer, India). Fractions were monitored using TLC (on Merck Si gel F254 plates-1 mm thick).

3.2 Plant material

The roots of *Dalbergia congesta* were collected in September 1999 from the forests of Palakkad district of Kerala, South India and the authenticity of the plant was confirmed by Dr. V. Renuka, Scientist, Kerala Forest Research Institute, Peechi, Kerala. A voucher specimen of the sample is kept in GRI for future reference.

3.3 Extraction and isolation

The air-dried and powdered roots of *D. congesta* (0.68 kg) were extracted with petroleum-ether (60–80°C) and 5% aqueous alcohol under hot condition (6 × 6 h) and the solvents were evaporated *in vacuo*. The crude solid obtained on evaporation of the petroleum ether extracts (1.1 g) was chromatographed over a silica gel column built in petroleum ether. Elution of the column with petroleum ether–ethyl acetate mixtures of increasing polarity yielded seven pooled fractions. Fractions 2,3,5 and 6 obtained by elution with petroleum ether: EtOAc mixtures of polarity 9.3:0.7, 9:1, 4:1 and 3:1 gave **4** (5 mg), **3** (20 mg), **1** (38 mg) and **2** (20 mg) respectively.

3.3.1 Compound 1. Pale yellow solid, m.p.162–163°C; TLC (silica gel), detection, I_2 vapors and UV light, Pet. ether/EtOAc 7:3 R_f 0.55, C_6H_6/Me_2CO 9:1, R_f 0.40 and chloroform R_f 0.44 UV λ_{max} (MeOH) 237, 268, 295 sh, 350, (MeOH + $AlCl_3$) 274, (MeOH + $AlCl_3$ + HCl) 274, (MeOH + NaOAc) 274, (MeOH + NaOAc + H_3BO_3) 274 and (MeOH + NaOMe) 276 nm; IR ν_{max} (KBr) 3570, 3373, 1690, 1624, 1620, 1517, 1460, 1366, 1149, 858, 828 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): Isoflavone unit: δ 6.53 (1H, s, H-8), 6.95 (1H, d, $J = 8$ Hz, H-2′), 7.01 (1H, dd, $J = 8$, 1.8 Hz, H-3′), 7.10 (1H, d, $J = 1.8$ Hz,

Table 1. ^{13}C NMR chemical shifts of compound **1**.

Carbons	δ_C	*Carbons*	δ_C
2	152.97	1‴	112.56
3	123.28	2‴	150.15
4	181.26	3‴	156.31
5	155.32	4‴	112.61
6	130.39	5‴	150.15
7	155.16	6‴	110.51
8	93.25	7‴	157.50
9	153.38	8‴	112.36
10	106.36	9‴	161.03
1′	123.28	10‴	135.56
2′	111.27	11‴	128.31
3′	121.28	12‴	128.82
4′	149.32	13‴	129.59
5′	112.62	14‴	128.82
6′	142.46	15‴	128.31
6-OMe	60.68	2‴-OMe	55.97
4′-OMe	56.45	5‴-OMe	55.97
C-1″	99.58	C-2″	142.46

H-5′), 7.89 (1H, s, H-2) and 13.12 (1H, s, 5-OH); Neoflavone unit: δ 6.25 (1H, s, H-8‴), 6.90 (1H, s, H-6‴), 7.40–7.55 (5H, m, H-11‴-H-15‴), 4.03, 3.99, 3.93, 3.92 (each 3H, s, OMe protons); 6.99 (1H, s, H-1″), 6.55 and 5.53 (D_2O exchangeable). ^{13}C-NMR (100 MHz, $CDCl_3$)-see table 1. HREIMS, *m/z* (relative intensity, %): 636.6082 (40) (calcd for $C_{36}H_{28}O_{11}$, 636.6062); 559 (33); 454 (5); 383 (100); 367 (15); 345 (24); 327 (15); 302 (29); 269 (67); 241 (65); 226 (100); 208 (6); 182 (6); 154 (22); 139 (31) and 121 (9).

3.3.2 Compound 2. Amorphous colorless solid, m.p. 156–157°C, TLC (silica gel), detection, I_2 vapors and UV light, Pet. ether/EtOAc 7:3, R_f 0.39, C_6H_6/Me_2CO 9:1, R_f 0.36 and chloroform R_f 0.34 UV λ_{max} (MeOH) 265, 296 sh, (MeOH + $AlCl_3$) 276, (MeOH + $AlCl_3$ + HCl) 276, (MeOH + NaOAc) 270, (MeOH + NaOAc + H_3BO_3) 270 and (MeOH + NaOMe) 273 nm; IR ν_{max} (KBr) 3340, 1626, 1548, 1520, 1456, 1372, 878 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.03, 3.93, 3.85, 3.78 (each 3H, s, OMe protons), 6.51 (1H, s, H-8), 6.62 (1H, s, H-6), 6.87 (1H, s, H-4′), 7.87 (1H, s, H-2) and 13.33 (1H, s, 5-OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.25 (C-4, C═O),155.00 (C-7), 154.90 (C-2), 153.44 (C-9), 152.52 (C-5), 150.16 (C-3′ and C-5′ overlapping), 143.12 (C-2′ and C-6′ overlapping), 121.28 (C-3), 119.29 (C-1′), 115.17 (C-4′), 110.72 (C-10), 98.19 (C-6), 93.15 (C-8) and 60.84, 56.80, 56.60, 56.17 (OMe carbons). EIMS, *m/z* (relative intensity, %): 373.3396 (13) (calcd for $C_{19}H_{17}O_8$, 373.3353); 342 (6); 306 (50); 288 (31); 221(31); 153 (100); 135 (100); 107 (64); 89 (51) and 57 (51).

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