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Furanoflavonoid glycosides from *Pongamia pinnata* fruits☆

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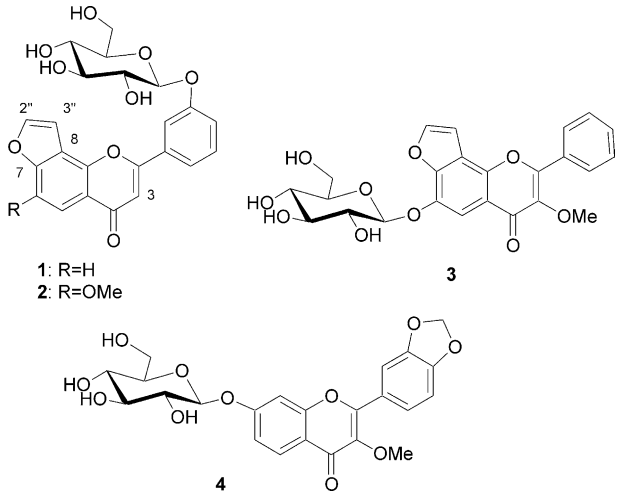
Abstract

Pongamia pinnata fruits afforded three new furanoflavonoid glucosides, pongamosides A-C (**1**–**3**), and a new flavonol glucoside, pongamoside D (**4**). The structures of these compounds were established on the basis of spectroscopic studies. This is the first time that furanoflavone glucosides have been found as naturally occurring compounds.

Keywords: *Pongamia pinnata*; Leguminosae; furanoflavonoid glucosides; flavone glucoside

1. Introduction

Pongamia pinnata Pierre (Leguminosae) is commonly known as Karanja. It is distributed throughout Western Ghats and chiefly found in tidal forests of India (Krishnamurthi, 1969). Different parts of the plant have been used in traditional medicines for bronchitis, whooping cough, rheumatic joints and to quench dipsia in diabetes (Kirtikar et al., 1995). Previous phytochemical examination of this plant indicated the presence of furanoflavones, furanoflavonols, chromenoflavones, flavones, and furanodiketones (Talapatra et al., 1980, 1982; Murty et al., 1944; Rangaswami et al., 1942; Sharma et al., 1973; Pathak et al., 1983; Toshiyuki et al., 1992). In the present communication, we describe the isolation and characterization of three new furanoflavonoid glucosides, pongamosides A-C (**1**–**3**), and a new flavonol glucoside pongamoside D (**4**).



2. Results and discussion

The *n*-BuOH soluble fraction of the EtOH extract of *Pongamia pinnata* fruits afforded four new compounds **1**–**4,** after repeated CC purifications.

Compounds **1**–**4** displayed certain common structural features; a positive Shinoda test (Grayer, 1989), Fiegle test, IR and UV suggested a flavonoid glycoside. The sugar was identified as glucospyranose by acid hydrolysis and co-TLC with authentic sample and NMR data (Table 1) for compounds **1**–**4.** The anomeric protons appeared at δ 5.12 (*d*, $J=6.6$ Hz); δ_C 100.3, 5.09 (*d*,

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Table 1
^{1}H NMR spectral data of **1**, **2**, **3** and **4** in DMSO-d_6

Position	**1** δ (J in Hz)	**2** δ (J in Hz)	**3** δ (J in Hz)	**4** δ (J in Hz)
3	7.10 (*s*)	7.09 (*s*)	–	–
4	–	–	–	–
5	7.99 (*d*, 8.7)	7.39 (*s*)	7.62 (*s*)	7.98 (*d*, 9.0)
6	7.77 (*d*, 8.7)	–	–	7.12 (*dd*, 2.4, 9.0)
8	–	–	–	7.33 (*d*, 2.4)
2′	7.75 (*br s*)	7.73 (*brs*)	8.14 (*m*)	7.57 (*d*, 1.5)
3′	–	–	7.60 (*m*)	–
4′	7.29 (*dd*, 1.5, 7.5)	7.26 (*dd*, 2.1, 7.8)	7.60 (*m*)	–
5′	7.52 (*t*, 7.5)	7.52 (*t*, 7.8)	7.60 (*m*)	7.11 (*d*, 8.4)
6′	7.85 (*d*, 7.5)	7.83 (*d*, 7.8)	8.14 (*m*)	7.65 (*dd*, 1.5, 8.4)
2	8.25 (*d*, 2.1)	8.24 (*d*, 2.1)	8.24 (*d*, 2.1)	–
3	7.58 (*d*, 2.1)	7.58 (*d*, 2.1)	7.52 (*d*, 2.1)	–
3-OMe	–	–	3.85 (*s*)	3.79 (s)
6-OMe	–	4.04 (*s*)	–	–
-OCH_2O-	–	–	–	6.15 (s)
Glc H-1	5.12 (*d*, 6.6)	5.09 (*d*, 6.9)	5.27 (*d*, 6.9)	5.06 (*d*, 7.2)
Glc H-2	3.28–3.56 (*m*)	3.28–3.50 (*m*)	3.30–3.43 (*m*)	3.27–52 (*m*)
Glc H-3	3.28–3.56 (*m*)	3.28–3.50 (*m*)	3.30–3.43 (*m*)	3.27–52 (*m*)
Glc H-4	3.28–3.56 (*m*)	3.28–3.50 (*m*)	3.30–3.43 (*m*)	3.27–52 (*m*)
Glc H-5	3.19 (m)	3.18 (*m*)	3.27 (*m*)	3.19 (m)
Glc H-6a	3.75 (*br d*, 11.1)	3.72 (*br d*, 11.2)	3.69 (*dd*, 3.6, 11.7)	3.70 (*dd*, 4.5, 10.2)
6b	3.28–3.56 (*m*)	3.28–3.50 (*m*)	3.53 (*dd*, 5.7, 11.7)	3.27–52 (*m*)
OH	5.38 (*d*, 4.5)	5.27 (*hump*)	5.45 (*d*, 3.6)	5.43 (*d*, 4.5)
OH	5.12 (*d*, 4.2)	5.12 (*hump*)	5.17 (*brs*)	5.13 (*d*, 4.8)
OH	5.08 (*d*, 5.1)	5.06 (*hump*)	5.06 (*d*, 5.1)	5.07 (*d*, 5.1)
OH	4.66 (*t*, 5.1)	4.66 (*hump*)	4.56 (*d*, 5.7)	4.60 (*t*, 5.4)

J = 6.9 Hz), 5.27 (*d*, J = 6.9 Hz); δ_C 100.8, and 5.06 (*d*, J = 7.2 Hz); δ_C 103.3 for compounds **1**–**4**, respectively, indicating that it was linked to the phenolic OH and have a β-configuration as suggested from coupling constants of anomeric proton signals.

The FAB-mass spectrum of compound **1** contained a peak at m/z 441 $[M+H]^+$ corresponding to the molecular formula of $C_{23}H_{20}O_9$. This conclusion was supported by the elemental analysis and NMR spectra. IR absorptions (ν_{max} 3433, 1625, 1594, 1459, 1353, 1082, 758, 631 cm^{-1}), UV spectrum (λ_{max} 216, 261, 300 nm) and NMR data are indicative of a furanoflavone glycoside nucleus. The ^{1}H and ^{13}C NMR (Table 1 and 2) showed characteristic signals for H-3 at δ 7.10 (1H, *s*), δ_C 104.5, and a furan ring at δ 7.58 (1H, *d*, 2.1 Hz, H-3″), δ_C 107.6 and δ 8.25 (1H, *d*, J = 2.1 Hz, H-2″), δ_C 147.4. The unsubstituted C-5 and C-6 positions are revealed by doublets for H-5 and H-6 at δ 7.99(1H, J = 8.7 Hz) and δ 7.77 (1H, J = 8.7 Hz). H-3″ showed correlation with the H-6, in ^{1}H–^{1}H long range COSY spectrum, indicating that the furan ring was fused in an angular position at C-7 (oxygenated) and C-8. ^{1}H–^{1}H COSY correlations observed for ^{1}H NMR signals at δ 7.75 (1H, *brs*, H-2′), δ 7.29 (1H, *dd*, J = 1.5, 7.5 Hz, H-4′), δ 7.52 (1H, *t*, J = 7.5 Hz, H-5′) and δ 7.85 (1H, d, J = 7.5 Hz, H-6′) indicated ring-B as monosubstituted at C-3′. The glucosidic linkage at C-3′-OH was revealed by nOe between the Glu H-1 (δ 5.12) and H-2′ (δ 7.75), H-4′ (δ 7.29) signals. Furthermore, aglycone was confirmed as pongol by acid hydrolysis of compound **1** and co-TLC with authentic sample, isolated from the same plant. Thus, based on the above information compound **1** was characterized as 3′-*O*-β-D-glucopyranosyl[2″,3″:7,8] furanoflavone, and we named it pongamoside-A. It is the first time that a furanoflavone glucoside has been found as a naturally occurring compound.

The HRFAB-mass spectrum of compound **2**, shows a $[M+H]^+$ peak at m/z 471 consistent with the formula $C_{24}H_{22}O_{10}$, which was supported by the ^{1}H NMR spectrum (Table 1) and an adduct ion at m/z 493 $[M+Na]^+$ in the FAB mass spectrum. The ^{1}H NMR spectrum was similar to that of **1**, suggesting **2** as furanoflavone glycoside. It could be inferred from the molecular weight and ^{1}H signal at δ 4.04 (3H, s) that **2** differed from **1** by one methoxy group. The methoxy group was placed at C-6 because H-5 appeared as singlet at δ 7.39 (1H), and the absence of long range correlation in the ^{1}H–^{1}H COSY experiment between H-3″ at δ 7.58 (1H, *d*, J = 2.1 Hz) with H-6. The four aromatic protons of ring B moiety are superimposed on those of **1**. The ring B protons resonated at δ 7.73 (1H, *brs*, H-2′), 7.26 (1H, *dd*, J = 2.1, 7.8 Hz, H-4′), 7.52 (1H, *t*,

$J=7.8$ Hz, H-5′) and 7.83 (1H, *d*, $J=7.8$ Hz, H-6′) indicating ring-B was monosubstituted at C-3′. Further glycosidation of C-3′ hydroxyl was confirmed by nOe experiment; irradiation of anomeric proton at δ 5.09 (1H, *d*, $J=6.9$ Hz) enhances signals for H-2′ (δ 7.73) and H-4′ (δ 7.26). Thus, on the basis of the above spectral data compound **2** was identified as 6-methoxy-3′-*O*-β-D-glucopyranosyl[2″,3″:7,8] furanoflavone, a new naturally occurring compound, named pongamoside-B.

Compound **3** was assigned the molecular formula $C_{24}H_{22}O_{10}$ (FAB-MS, *m*/*z* 471 $[M+1]^+$). This conclusion was supported by the ^1H and ^{13}C NMR spectra. Compound **3** was recognized as a furanoflavonol glycoside. Analysis of the ^1H and ^{13}C NMR spectra (Tables 1 and 2) reveals the presence of a furan ring fused to ring A, with the characteristic proton doublets at δ 7.52 and 8.24 (1H each, *d*, $J=2.1$ Hz, H-3″ and H-2″); one aromatic proton in ring A at δ 7.62 (*s*, H-5), an unsubstituted ring B, δ 8.14 (2H, *m*, H-2′, 6′) and 7.60 (3H, *m*, H-3′,4′,5′), and one methoxy group at δ 3.85 (s); δ_C 59.6. The latter was located at C-3 based on nOe between the methoxy protons and the H-2′ and H-6′ resonances at δ 8.14 (2H, *m*). Further NMR spectra indicated a glucosidic moiety located at C-6 as revealed by absence of correlation for H-3″ in ^1H-^1H long range COSY experiment and an nOe between the anomeric proton at δ 5.27 and H-5 at δ 7.62. Spectral similarity of the aglycone portion of **3** to that reported in the literature for the 6-methoxy derivative (Kamperdick et al., 1998), along with spectrochemical observations led structure of new compound **3** as 3-methoxy-6-*O*-β-D-glucopyranosyl[2″,3″:7,8] furanoflavone, a new naturally occurring compound, named pongamoside-C.

The molecular formula $C_{23}H_{22}O_{11}$ of compound **4** was determined by FAB-mass spectrometry (*m*/*z* 474 $[M+1]^+$) and is in accord with ^1H and ^{13}C NMR data summarized in Tables 1 and 2. The IR, UV and NMR were characteristics of a flavonol glycoside moiety. Ring-A exhibited an ABX spin system consisting of two doublets at 7.98 (1H, $J=9.0$ Hz, H-5), δ 7.33 (1H, $J=2.4$ Hz, H-8) and a double doublet at δ 7.12 (1H, $J=2.4$, 9.0 Hz, H-6). The nOe correlation of the anomeric proton at δ 5.06 with H-6 (δ 7.12) and H-8 (δ 7.33) suggested the location of the glucose moiety at C-7. Absence of the characteristic flovone singlet for H-3 in the ^1H NMR, the appearance of C-3 at δ 139.9, and one methoxy group at δ 59.4 in ^{13}C NMR, revealed the presence of one methoxy group at C-3. The presence of one methylenedioxy group at C-3′,4′ (δ 6.15, 2H, *s*); δ_C 101.7, and an ABX splitting system consisting of two doublets at δ 7.57 (1H, $J=1.5$ Hz, H-2′), 7.11 (1H, $J=8.4$ Hz, H-5′) and a double doublet at δ 7.65 (1H, $J=1.5$, 8.4 Hz, H-6′) indicated a disubstituted B-ring. Based on the above spectral evidences, compound **4** was characterized as 3-methoxy-3′,4′-methylenedioxy-7-*O*-β-D-glucopyranosyl flavone, and named pongamoside D.

Table 2
^{13}C NMR spectral data of **1**, **3** and **4** in DMSO-d_6

Position	**1** δ_C	**3** δ_C	**4** δ_C
2	150.0[a]	153.8	155.9[a]
3	104.5	141.2[a]	139.9
4	176.8	173.3	172.9
5	120.9	103.3	126.1
6	110.0	140.7[a]	115.3
7	161.6[a]	141.2[a]	161.3[a]
8	118.8	118.8	99.8
9	157.8[a]	144.5	154.2[a]
10	117.0	119.6	118.1
1′	132.3	130.3	123.9
2′	119.9	128.0	108.4
3′	157.6[a]	128.6	149.2[a]
4′	119.9	130.6	147.5[a]
5′	130.3	128.6	107.9
6′	113.6	128.0	123.2
2″	147.4	147.4	–
3″	107.6	104.8	–
3-OMe	–	59.6	59.4
–OCH_2O–	–	–	101.7
Glc-1	100.3	100.8	103.3
Glc-2	73.2	73.2	73.0
Glc-3	76.4	76.4	76.4
Glc-4	69.7	69.3	69.5
Glc-5	77.1	77.2	77.1
Glc-6	60.7	60.4	60.5

[a] Values may be interchanged within the column.

3. Experimental

3.1. General

Melting points were recorded on a Complab melting point apparatus and are uncorrected. IR spectra (KBr) were recorded on a Perkin-Elmer RX-1 spectrophotometer. UV spectra were obtained on a Perkin-Elmer λ-15 UV spectrophotometer. NMR spectra were run on an AVANCE DPX 200 and Bruker DRX 300 spectrometer, FAB MS were carried out on JEOL SX 102/DA-6000 mass spectrometer. Elemental analyses were obtained in a Carlo-Erba-1108 CHN elemental analyzer. Column chromatography was performed using flash silica gel (230–400 mesh). Preparative and analytical HPLC performed on Shimadzu model LC-8A instrument.

3.2. Plant material

The fruits of *P. pinnata* were collected from Lucknow in the month of May 2000, and identified by Botany Division of Central Drug Research Institute. A voucher specimen (No. 6331) is kept in the herbarium of the Institute.

3.3. Extraction and isolation

Air dried and powdered fruits of *P. pinnata* (6 Kg) were extracted at room temperature with EtOH. The EtOH extract (750 g) was then fractionated successively into four fractions: *n*-hexane (360 g), $CHCl_3$ (70 g), *n*-BuOH (50 g), and aqueous (240 g). The *n*-BuOH (45 g) fraction was subjected to column chromatography over flash silica gel, eluted with $CHCl_3$–MeOH mixture of increasing polarity to obtain five fractions (F1-F5). Fraction [F2 (8.0 g), eluted with $CHCl_3$–MeOH; 85:15], was further separated by silica gel chromatography, eluted with $CHCl_3$–MeOH–H_2O (80:19:1) to give nine fractions (F6-F14). Fraction (F7, 40 mg) on a silica gel TLC plate showed a single spot but three spots on a reverse phase TLC plate (RP-18 F254, MeOH: H_2O; 1:1). Therefore, fraction F7 was further purified by reverse phase semi-preparative HPLC (ODS column 20.0 mm× 25cm, using MeOH:H_2O (60:40) 8 ml/min. flow rate, UV; λ 254 nm) to afford compounds **1** (8 mg), **3** (12 mg) and **4** (6 mg). Fraction F-8 (20 mg) was purified on a silica gel column by elution with EtOAc (saturated with water): MeOH (49:1) to afford compound **2** (0.9 mg).

3.4. Pongamoside A (1)

Pale yellow crystals from DMSO; mp 259–260 °C; $[\alpha]_D^{31}$ −33.6° ($c = 0.280$, pyridine). UV (MeOH) λ_{max} nm: 216, 261, 300; IR ν_{max} (KBr) cm^{-1}: 3433, 1625, 1594, 1459, 1353, 1082, 758, 631. ^1H NMR (DMSO-d_6, 300 MHz) and ^{13}C NMR (DMSO-d_6, 50.32 MHz) see Table 1; FAB MS (pos.): m/z 441 $[M+H]^+$. Elemental analysis: Found: C, 62.70%, H, 4.60%. Calc. for $C_{23}H_{20}O_9$: C, 62.73%, H; 4.58%.

3.5. Pongamoside B (2)

Pale yellow solid, due to insufficient amount other data were not recorded. ^1H NMR (DMSO-d_6, 300 MHz) Table 1. HRFABMS, m/z 471.1250 $[M+H]^+$ ($C_{24}H_{22}O_{10}$ requires 471.1291).

3.6. Pongamoside C (3)

White crystals from DMSO; mp 237–238 °C; $[\alpha]_D^{31}$ −32.8° ($c = 0.265$, pyridine). UV (MeOH) λ_{max} nm: 218, 260, 306; IR ν_{max} (KBr) cm^{-1}: 3437, 1620, 1595, 1480, 1381, 1352, 1076, 772. ^1H NMR (DMSO-d_6, 300 MHz) and ^{13}C NMR (DMSO-d_6, 50.32 MHz) see Table 1; FAB MS (pos.): m/z 471 $[M+1]^+$. Elemental analysis: Found: C, 61.31%, H, 4.69%. Calc. for $C_{24}H_{22}O_{10}$: C, 61.28%, H; 4.71%.

3.7. Pongamoside D (4)

White crystals from DMSO; mp 214–215 °C; $[\alpha]_D^{31}$ −56.6° ($c = 0.265$, pyridine). UV (MeOH) λ_{max} nm: 211, 241,306, 344; IR ν_{max} (KBr) cm^{-1}: 3426, 1635, 1595, 1448, 1383, 1351, 1096, 786. ^1H NMR (DMSO-d_6, 300 MHz) and ^{13}C NMR (DMSO-d_6, 50.32 MHz) see Table 1; FAB MS (pos.): m/z 475 $[M+1]^+$. Elemental analysis: Found: C, 58.27%, H, 4.71% calc. for $C_{23}H_{22}O_{11}$: C, 58.23%, H; 4.67%.

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