Juspurpurin, an Unusual Secolignan Glycoside from *Justicia purpurea*

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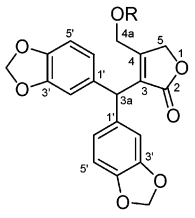
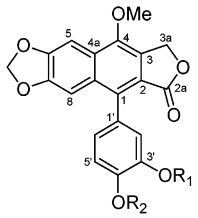
An unusual secolignan glycoside, juspurpurin (**1**), and a new arylnaphthalene glycoside, justalakonin (**2**), together with eight known lignans were isolated from the whole plants of *Justicia purpurea*. Compound **1** is the first glycoside of a rare group of secolignans. The structures of the new compounds **1** and **2** were established using 1D and 2D NMR (^{1}H−^{1}H COSY, HMQC, and HMBC) spectral data and by chemical transformations.

Justicia purpurea L. (Acanthaceae) is a tall, straggling narrow-leaved herb with pale pink flowers and is native to India. The roots of this plant are used as a remedy for insanity and other mental disorders.[1] No previous phytochemical investigations have been reported on this species. Lignans are the major group of compounds that were reported from the genus *Justicia*, and several of these lignans displayed antitumor,[2] antiplatelet,[3] antiviral,[4] and antidepressant[5] activities. As a part of our continuing phytochemical investigations on Indian *Justicia* species,[6] we describe herein the isolation and structure elucidation of an unusual secolignan glycoside, juspurpurin (**1**), and a new arylnaphthalene glycoside, justalakonin (**2**), along with eight known compounds, namely, sesamin,[7] jusmicranthin methyl ether,[8] jusmicranthin,[9] taiwanin E methyl ether,[10] justicidin B,[10] xanthoxylol,[11] neesiinoside A,[12] and cleistanthin B,[13] from a methanol extract of the entire plant of *J. purpurea*. The structures of the known compounds were confirmed by comparison with authentic samples or from literature data.



1 R = Glc
1a R = H



2 R_1 = Glc; R_2 = H
2a R_1 = R_2 = H
2b R_1 = Glc; R_2 = CH_3
2c R_1 = H; R_2 = CH_3

Compound **1** was obtained as a colorless oil, $[\alpha]^{25}_D$ −17.5° (*c* 0.75, acetone). Its molecular formula, $C_{26}H_{26}O_{12}$, was inferred from the ^{13}C NMR and HRFABMS data (*m/z* 531.1508 $[M + H]^+$). The ^{1}H NMR spectral data of **1** showed signals for two symmetrically substituted phenyl units with an ABX system, characteristic of 1,2,4-trisubstituted phenyl units (*δ* 6.78, 2H, d, *J* = 8.0 Hz; 6.72, 2H, d, *J* = 1.5 Hz and 6.67, 2H, dd, *J* = 8.0, 1.5 Hz), two methylene groups (*δ* 4.94 and 4.99, each 1H, d, *J* = 17.9 Hz; 4.38 and 4.45, each 1H, d, *J* = 15.4 Hz), a benzylic methine proton (*δ* 5.22, 1H, s), and two equivalent methylenedioxy groups (*δ* 5.97, 4H, m). In addition, the ^{1}H NMR spectrum also showed a series of signals between *δ* 3.14 and 3.79 (6H), and an anomeric proton at *δ* 4.18 (d, *J* = 7.9 Hz) revealed the presence of one sugar unit. The ^{13}C NMR spectrum showed 19 signals, of which six aromatic signals and one methylenedioxy carbon were attributed to two symmetrically trisubstituted phenyl units. The remaining 12 carbon signals could be derived for a carbonyl carbon, a benzylic methine, two methylenes, two quaternary carbons, and six aliphatic sugar carbons. In the HMBC spectrum (Figure 1), the connectivities observed from H-3a to C-2′/C-6′ as well as to C-2/C-4 indicated a partial secolignan skeleton for compound **1**. A comparison of the ^{13}C NMR data of **1** with literature data revealed that it is closely related to two *β*-apolignans,[14] namely, 1,4-dihydrotaiwanin C and jusneesiinol, except for the lack of a C−C bond between C-4 and C-4a present in these *β*-apolignans.[14] The presence of an extra deshielded methylene carbon at *δ* 64.4 in comparison with these *β*-apolignans suggested that **1** should have a secolignan skeleton like compounds in the peperomin series.[15−18] Acid hydrolysis of **1** gave an aglycon (**1a**) and a sugar identified as glucose by descending paper chromatography. The position of the glucose unit on C-4a was confirmed by the HMBC correlations from H-1″ to C-4a. The *β*-configuration of the sugar unit was evident from the coupling constant of the anomeric proton H-1″ (*J* = 7.9 Hz) observed in the ^{1}H NMR spectrum. Full assignments of the ^{1}H and ^{13}C NMR signals were concluded from the detailed analysis of DEPT, COSY, HMQC, and HMBC spectra (Table 1). From these data, the structure of compound **1** was established as 3-[bis(3,4-methylenedioxyphenyl)methyl]-4-(*β*-D-glucopyranosyloxy-methyl)-2(5*H*)-furanone, a new secolignan glycoside, named juspurpurin. A literature survey revealed that secolignans are a rare unusual group of lignans, and to the best of our knowledge this is the first report of the occurrence of a secolignan

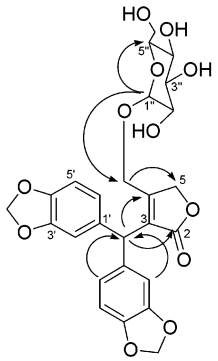


Figure 1. Selected HMBC connectivities for **1**.

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Table 1. NMR Data of Juspurpurin (**1**)[a]

position	δ_C	δ_H (J Hz)	HMBC (H to C)
2	174.0		
3	129.3		
3a	47.0	5.22 s	C-2, C-4, C-1′, C-2′, C-6′
4	160.7		
4a	64.4	4.38 d (15.4)	C-5, C-1″, C-3
		4.45 d (15.4)	
5	71.5	4.94 d (17.9)	C-2, C-3, C-4
		4.99 d (17.9)	
1′	135.5		
2′	110.0	6.72 d (1.5)	C-3a, C-6′
	110.2[b]	6.74 d (1.5)[b]	
3′	148.8		
4′	147.4		
5′	108.8	6.78 d (8.0)	C-1′, C-3′
6′	122.8	6.67 dd (8.0,1.5)	C-3a, C-4′, C-2′
1″	104.0	4.18 d (7.9)	C-4a, C-5″
2″	74.6	3.16 m	
3″	77.4	3.22 m	C-4″, C-5″
4″	71.3	3.31−3.36 m	
5″	77.8	3.31−3.36 m	
6″	62.7	3.65 m	
		3.78 m	
OCH_2O	102.0	5.97 m	C-3′, C-4′

[a] Recorded in d_6-acetone at 500 and 125 MHz. [b] Additional signals are due to hindered rotation; see refs 13, 19.

glycoside from a natural source. Further, this is the first secolignan with a "retro" orientation of the butyrolactone ring.

Compound **2** was isolated as an amorphous powder, with a molecular composition of $C_{26}H_{24}O_{12}$, as determined by its HRFABMS (*m*/*z* 528.1265, M^+) and ^{13}C NMR data. The ^1H NMR spectral data contained five aromatic protons constituted by two singlets at δ 7.52 and 6.88 (1H each) and an ABX pattern of a 1,2,4-trisubstituted phenyl unit (δ 7.02, 1H, s, 6.90, 1H, d, J = 8.0 Hz and 6.75, 1H, dd, J = 8.0, 1.6 Hz), a lactone methylene (δ 5.65, 2H, s), a methylenedioxy group (δ 6.16, 2H, s), and an aromatic methoxyl (δ 4.08, 3H, s). These signals were suggestive of the presence of a 1-arylnaphthalene unit in **2**. In addition, the ^1H NMR spectrum showed the presence of sugar signals. The presence of one anomeric proton at δ 4.69 (d, J = 7.5 Hz) revealed that **2** contains only one sugar unit. Acid hydrolysis of **2** gave an aglycon (**2a**) and a sugar, identified as glucose. A perusal of the above data in comparison with a congeneric lignan revealed that **2** is similar to taiwanin E methyl ether[10] except for the presence of a hydroxyl group and a glycosyloxy substituent instead of a methylenedioxy group on the pendant ring. The placement of glucose on C-3′ was evident from the long-range HMBC correlations, chemical conversions, and NOE data. Methylation of **2** followed by hydrolysis gave **2c**, which did not correspond to the known lignan, 4′-demethylchinensinaphthol methyl ether,[3] indicating the position of glucose at C-3′. In the NOE spectrum of **2c**, the irradiation of the methoxyl protons located at δ 3.99 (C-4) showed a positive NOE (3.35%) for H-5. Similarly, irradiation of the methoxyl on the pendant ring located at δ 3.87 (C-4′) showed a positive NOE (12.80%) for H-5′, leading to the placement of the methoxyl at C-4′ in **2c** and, consequently, the hydroxyl in **2** could be placed at C-4′. The pyranoside form of the glucose unit was supported from the observed HMBC correlation between H-1″ and C-5″. The configuration of the glucose unit was determined as β on the basis of the coupling constant (J = 7.5 Hz) of the anomeric proton. Thus, **2** was characterized as 1-(3′-β-D-glucosyloxy-4′-hydroxyphenyl)-3-hydroxymethyl-4-methoxy-6,7-methylenedioxy-2-naphthoic acid lactone, a new arylnaphthalene glycoside, named justalakonin.

Experimental Section

General Experimental Procedures. Melting points were measured on a Buchi-540 melting point apparatus and are uncorrected. Optical rotations were measured with a Jasco P-1020 polarimeter. IR spectra were obtained on a Perkin-Elmer BX FT-IR spectrometer. NMR spectra were run on a Bruker DRX-500 spectrometer. EIMS were recorded on a Hewlett-Packard 5989 B instrument and FABMS on a JEOL SX 102/DA-6000 mass spectrometer. Medium-pressure liquid chromatography (MPLC) was carried out on a Buchi 687 Gradient Former with a Buchi 688 chromatographic pump. MCI gel [CHP 20, 75−150 μm] was used for reversed-phase columns, and silica gel 60 (Merck, 100−200 mesh) was used for final purifications.

Plant Material. The entire plant was collected from the Talakona forests of Tirumala Hills, Chittoor District, India, in September 1999 and authenticated as *Justicia purpurea* by Research and Specimen Cell, National Institute of Science Communication, CSIR, New Delhi. Voucher specimens are on deposit at NISCOM (NISCOM Field No. 1831) and the Department of Chemistry, Sri Venkateswara University, Tirupati, India.

Extraction and Isolation. The shade-dried and milled plant material (5.5 kg) was extracted with MeOH (5 × 10 L) at room temperature. The MeOH extract was concentrated under reduced pressure and fractionated between EtOAc and MeOH using a Soxhlet apparatus. The EtOAc fraction (36 g) on silica gel chromatography using mixtures of petroleum ether−EtOAc (19:1, 9:1, 4:1, and 2:1) gave sesamin[7] (0.20 g), jusmicranthin methyl ether[8] (0.91 g), and taiwanin E methyl ether[10] (0.25 g). Column chromatography of the MeOH-soluble portion (105 g) on MCI gel and elution with a stepwise gradient mixture of H_2O−MeOH (7:3, 3:2, 1:1, 2:3, 3:7, 1:9, and finally with MeOH) resulted in four fractions (A−D). Fraction A was subjected to MPLC using an ODS column with a gradient H_2O−MeOH system (4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4, and finally with MeOH) for elution to give justalakonin (**2**, 0.36 g) and neesiinoside A[12] (1.50 g). Similarly, fraction B yielded juspurpurin (**1**, 50 mg) and cleistanthin B[13] (30 mg). Fractions C and D were subjected to silica gel column chromatography using a gradient solvent system of petroleum ether−EtOAc (4:1 to 1:1), affording justicidin B[10] (0.20 g), jusmicranthin[9] (40 mg), and xanthoxylol[11] (40 mg).

Juspurpurin (1): colorless oil, $[\alpha]^{25}_D$ −17.5° (*c* 0.75, acetone); IR (KBr) ν_{max} 3401, 1748, 930 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 531 $[M + H]^+$ (63), 530 $[M]^+$ (61), 460 (43), 307 (40), 154 (100), 136 (67); HRFABMS *m*/*z* 531.1508 (calcd for $C_{26}H_{27}O_{12}$ $[M + H]^+$, 531.1503).

Hydrolysis of 1. To a solution of **1** (10 mg) in MeOH (0.2 mL) was added H_2SO_4 (8%, 0.1 mL) at room temperature, and the resulting mixture was refluxed for 4 h. After this period, the reaction mixture was poured into water and extracted with $CHCl_3$. The organic layer was evaporated and purified by silica gel column to give **1a** (5 mg) as a colorless oil. Compound **1a**: ^1H NMR ($CDCl_3$, 500 MHz) δ 6.73 (2H, d, J = 8.0 Hz, H-5′), 6.59 (2H, s, H-2′), 6.55 (2H, m, H-6′), 5.94 (4H, s, OCH_2O), 5.18 (1H, s, H-3a), 4.89 (2H, s, H-5), 4.13 (2H, s, H-4a); EIMS *m*/*z* 368 $[M]^+$ (75), 305 (45), 263 (51), 139 (65), 115(61), 63 (100). The aqueous layer was concentrated and used for the sugar identification.

Justalakonin (2): amorphous solid; mp 205−207 °C; $[\alpha]^{25}_D$ −31.0° (*c* 0.25, MeOH); IR (KBr) ν_{max} 3341, 1760, 940 cm^{-1}; ^1H NMR (d_6-DMSO, 500 MHz) δ 7.52 (1H, s, H-5), 7.02 (1H, s, H-2′), 6.88 (6.93)* (1H, s, H-8), 6.90 (6.91)* (1H, d, J = 8.0 Hz, H-5′), 6.75 (6.76)* (1H, dd, J = 8.0, 1.6 Hz, H-6′), 6.16 (6.17)* (2H, s, OCH_2O), 5.65 (5.66)* (2H, s, CH_2-3a), 4.69 (4.70)* (1H, d, J = 7.5 Hz, H-1″), 4.08 (3H, s, OCH_3), 3.53 (1H, m, H_a-6″), 3.24−3.41 (3H, m, H-2″, H-3″, H_b-6″), 3.16 (2H, m, H-4″, H-5″) (*additional signals are due to hindered rotation[13,19]); ^{13}C NMR (d_6-DMSO, 125 MHz) δ 168.9 (C-2a), 149.6 (C-6), 148.6 (C-7), 147.60 (147.63)* (C-4), 146.5 (146.9)* (C-4′), 144.8 (144.9)* (C-3′), 133.6 (133.7)* (C-1), 131.1 (131.2)* (C-8a), 126.5 (126.6)* (C-4a), 125.6 (125.8)* (C-1′), 125.2

(125.3)* (C-2), 124.9 (125.1)* (C-6′), 119.3 (119.4)* (C-3), 118.6 (C-2′), 115.5 (115.6)* (C-5′), 102.9 (C-8), 102.8 (C-1″), 102.2 (−OCH_2O-), 98.0 (C-5), 77.0 (C-5″), 75.8 (C-3″), 73.3 (73.4)* (C-2″), 69.6 (69.7)* (C-4″), 66.5 (C-3a), 60.6 (C-6″), 59.4 (OCH_3) (*additional signals are due to hindered rotation[13,19]); FABMS *m*/*z* 529 $[M + H]^+$ (41), 528 $[M]^+$ (51), 367 (100), 154 (86), 136 (70); HRFABMS *m*/*z* 528.1265 (calcd for $C_{26}H_{24}O_{12}$ $[M]^+$, 528.1268).

Hydrolysis of 2. Using the procedure described for compound **1a**, **2** was hydrolyzed to give **2a** as a colorless solid (mp 274−276 °C): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.54 (1H, s, H-5), 7.00 (1H, s, H-2′), 6.93 (1H, d, J = 8.0 Hz, H-5′), 6.77 (1H, s, H-8), 6.60 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 6.15 (2H, s, OCH_2O), 5.60 (2H, s, H-3a), 4.15 (3H, s, OCH_3); EIMS *m*/*z* 366 $[M]^+$ (90), 323 (17), 305(11), 276 (18), 58 (30), 44 (100).

Methylation of 2. A mixture of **2** (50 mg), methyl iodide (0.2 mL), K_2CO_3 (50 mg), and acetone (2 mL) was heated under reflux for 4 h. After this period, K_2CO_3 was filtered and the residue obtained was purified over a silica gel column to give **2b** (30 mg) as a semisolid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (7.54)* (1H, s, H-5), 7.09 (7.07)* (1H, d, J = 8.3 Hz, H-5′), 7.00 (1H, s, H-8), 6.90 (1H, s, H-2′), 6.83 (1H, m, H-6′), 6.17 (6.18)* (2H, s, OCH_2O), 5.67 (5.68)* (2H, s, H-3a), 5.00 (4.99)* (1H, d, J = 8.0 Hz, H-1″), 4.09, 3.85 (each 3H, s, 2×OCH_3), 3.14−3.54 (6H, m, H-2″, H-3″, H-4″, H-5″, H-6″) (*additional signals are due to hindered rotation[13,19]).

Hydrolysis of 2b. Using the procedure described for **1a**, **2b** was hydrolyzed to give **2c** as a semisolid: ^{1}H NMR (500 MHz, $CDCl_3$+ d_6-DMSO) δ 7.45 (1H, s, H-5), 6.96 (1H, s, H-8), 6.88 (1H, d, J = 8.1 Hz, H-5′), 6.76 (1H, d, J = 1.9 Hz, H-2′), 6.67 (1H, dd, J = 8.1, 1.9 Hz, H-6′), 5.98 (5.97)* (2H, s, OCH_2O), 5.41 (2H, s, H-3a), 3.99, 3.87 (each 3H, s, 2×OCH_3) (*additional signals are due to hindered rotation[13,19]); EIMS *m*/*z* 380 $[M^+]$ (100), 277 (50), 163 (53), and 63 (62).

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