Laxanol, a new 2,5-diaryl-3,4-dihydroxymethyltetrahydrofuran type lignan from *Justicia laxa*

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The unsymmetrical 2,5-diaryl-tetrahydrofuran type lignan, 2-(3′-methoxy-4′-hydroxyphenyl)-3,4-dihydroxymethyl-5-(3′′-methoxy-5′′-hydroxyphenyl)-tetrahydrofuran, laxanol (**1**), was obtained from *Justicia laxa* via high-throughput natural product chemistry methods as a mass-limited sample and its structure was elucidated by capillary scale NMR and HR-/LR-ESIMS analyses.

Keywords: *Justicia laxa*, 2,5-diaryl-tetrahydrofuran type lignan, high-throughput natural product chemistry, CapNMR probe, laxanol

Justicia (Acanthaceae) is a genus with 600 species worldwide of shrubs or herbs.[1] This genus is a rich source of different types of lignans and their derivatives such as arylnaphthalide lignan,[2-6] *p*-quinone-lignan,[7] podophyllotoxin,[8,9] secolignan,[10] *β*-apolignan,[11] and furanoid lignan.[12,13] The lignans from *Justicia* were found to possess anti-tumor,[14-16] antiplatelet,[3,17] anti-inflammatory,[18] antiviral,[19] anti-depressant,[20,21] hepatoprotective[22] and piscidal[9] activity and cytotoxicity.[4,5,20,21] However, *Justicia laxa* L. has so far not been chemically and pharmacologically investigated. In the course of a project directed toward the discovery of novel anticancer agents from plants, the *Justicia* library was generated and analysed via our standard high-throughput natural product chemistry approach.[23,24] The lignan (**1**), laxanol, which was located in the preparative HPLC fraction 4, showed primary inhibition of a tumor cell line. (Sequoia Sciences, Inc. internal communication. The purified lignan **1** was tested against a tumor cell line, but was found inactive.) The mass-limited sample of **1** (70 μg) was purified using semi-preparative HPLC, and its structure was elucidated primarily using the advanced capillary NMR probe techniques.[23,24]

The molecular weight of compound **1** and its chemical formula of $C_{20}H_{24}O_7$ were deduced from the positive mode high-resolution ESI mass spectrum (HR-ESIMS), which showed the $[M + Na]^+$ ion peak at *m/z* 399.1417 ($C_{20}H_{24}O_7Na$ requires 399.1419). The ^{1}H, COSY and HSQC NMR spectra of **1** revealed the presence of two non-equivalent methoxy groups at δ3.84 (3H, s, δ_c: 56.3, 3'-OMe) and δ3.81 (3H, s, δ_c: 56.0, 3''-OMe), two hydroxymethylene groups of $–C_3–CH_2OH$ [δ3.29 (1H, dd, J = 11.2, 4.3 Hz) and 3.21 (1H, dd, J = 11.2, 5.9 Hz), δ_c: 62.5] and $–C_4–CH_2OH$ [δ4.24 (1H, dd, J = 8.9, 2.4 Hz) and 3.92 (1H, dd, J = 8.9, 7.8 Hz), δ_c: 71.6], two methines bearing oxygen at δ4.61 (1H, d, J = 7.3 Hz, H-2, δ_c: 84.9) and δ4.47 (1H, d, J = 8.2 Hz, H-5, δ_c: 77.5), two methines at δ1.88 (1H, m, H-3, δ_c: 53.9) and δ2.53 (1H, m, H-4, δ_c: 50.8). In the lowfield part of the ^{1}H NMR spectrum, the six aromatic proton signals were assigned to one 1,3,4-trisubstituted [δ6.91 (1H, d, J = 1.5, H-2'), 6.75 (1H, d, J = 8.2, H-5'), 6.79 (1H, dd, J = 8.2, 1.5, H-6')] and one 1,3,5-trisubstituted [δ6.73 (1H, brs, H-2''), 6.72 (1H, brs, H-4''), 6.86 (1H, brs, H-6'')] benzene ring. The above functionalities, showed that **1** was an unsymmetrical 2,5-diaryl-3,4-dihydroxymethyltetrahydrofuran type of lignan.[25,26] The substitution patterns of the two aromatic rings were confirmed by the correlations (Fig. 1) in the HMBC spectrum. Hence, the structure of **1** was established as 2-(3'-methoxy-4'-hydroxyphenyl)-3,4-dihydroxymethyl-5-(3''-methoxy-5''-hydroxyphenyl)-tetrahydrofuran. The relative *trans* configuration between the methine protons at C-2 and C-3; C-3 and C-4; C-4 and C-5 in the tetrahydrofuran ring was deduced by NOESY experiments (Fig. 2). Clear cross-peaks were observed between H-2 at δ4.61 and H-4 at δ2.53, and between H-4 and the hydroxymethylene protons at δ3.29/3.21 ($-C_3-CH_2OH$). There was no NOE cross-peak between H-2 and H-5, requiring these two protons be *trans*-oriented; otherwise, they would have strong NOE effects.[27,28] NOE correlations between H-3 and H-1'/H-6', as well as between H-4 and H-1''/H-6'' were also observed, indicating that the vicinal hydroxymethylene and aryl groups were *trans*-oriented and that the rotation of the single bonds between C-2 (5) and C-1'(1'') were not restricted. Since only a limited amount of **1** was obtained by the unconventional isolation and purification procedures,[23,24,29-31] its optical rotation and the absolute stereochemistry were not determined.

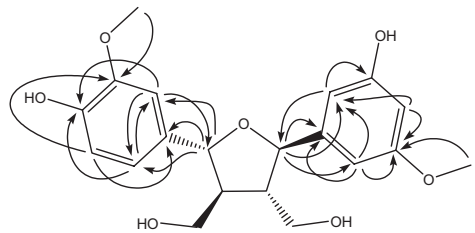

Fig.1 Key HMBC correlations to construct the substitution patterns of the two aromatic rings.

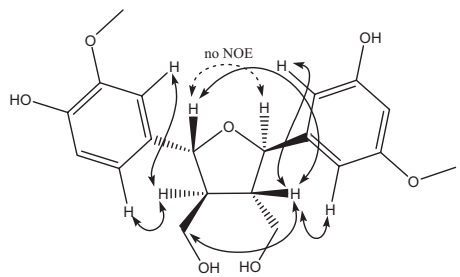

Fig.2 Key observed NOE correlations to confirm the relative stereochemistry of the tetrahydrofuran ring.

2,5-Diaryl-3,4-dihydroxymethyltetrahydrofuran type lignans and their derivatives have been previously isolated from a few plants such as *Urtica dioica*,[25] *Astragalus zahlbruckneri*,[26] *Acanthus ilicifolius*,[28] *Glechoma hederacea*,[32] *Phagnalon rupestre*,[33] *Epimedium sagittatum*,[34] and *Thymus longiflorus*.[35] The 1,3,4- and 1,3,5-trisubstituted aromatic rings in the structure of **1** were only found once in cestrumoside, a norlignan glucoside isolated from *Cestrum diurnum*.[36]

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Table 1 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for **1** (in CD_3OD)

No.	δ_H (mult, *J* in Hz)	δ_c[a]
2	4.61 (1H, d, *J* = 7.3)	84.9
3	1.88 (1H, m)	53.9
3-CH_2OH	3.29 (1H, dd, *J* = 11.2, 4.3)	
	3.21 (1H, dd, *J* = 11.2, 5.9)	62.5
4	2.53 (1H, m)	50.8
4-CH_2OH	4.24 (1H, dd, *J* = 8.9, 2.4)	
	3.92 (1H, dd, *J* = 8.9, 7.8)	71.6
5	4.47 (1H, d, *J* = 8.2)	77.5
1'		134.2
2'	6.91 (1H, d, *J* = 1.5)	111.3
3'		148.1
3'-OMe	3.84 (3H, s)	56.3
4'		146.6
5'	6.75 (1H, d, *J* = 8.2)	115.9
6'	6.79 (1H, dd, *J* = 8.2, 1.5)	120.3
1"		135.3
2"	6.73 (1H, brs)	120.7
3"		148.1
3"-OMe	3.81 (3H, s)	56.0
4"	6.72 (1H, brs)	116.2
5"		146.4
6"	6.86 (1H, brs)	111.7

[a]Assignments were made by a combination of 1D and 2D NMR

Experimental

General procedure

For instrumentation and general procedures, see refs 23, 24, 29–31.

Plant material

The whole plant of *J. laxa* was collected from Ekorado State, Gabon in the Spring of 2000. Plant samples were dried in Gabon, and shipped to Sequoia Sciences for processing. The plant was identified by John Stone (Missouri Botanical Garden Herbarium, St. Louis, MO). A voucher specimen (No. 970) was deposited at the Herbarium of Missouri Botanical Garden.

Extraction and isolation

The whole plant including roots (*ca* 50 g) was extracted with EtOH/EtOAc (50:50) followed by H_2O/MeOH (30:70) to afford 990 mg and 4.5 g dry organic and aqueous extracts, respectively. The entire organic extract was loaded on the Flash Master II automated chromatographic system using our standard elution gradient to generate the Flash Fractions.[23,24] The Flash Fraction 3 (EtOAc, neat) totaled 43 mg; all of it was fractionated by preparative C_{18} HPLC from 45 to 100% acetonitrile in H_2O collecting 40 1-min fractions. Compound **1** resided in preparative HPLC fraction 4, which exhibited primary inhibition of a tumor cell line. Review of the HPLC-ELSD-MS data acquired on all of the preparative fractions from the Flash Fraction 3 suggested that preparative HPLC fraction 4 contained compounds with molecular weights less than 600 daltons which were readily isolated using reversed-phase chromatography. The initial mobile phase gradient applied to isolating compound **1** from HPLC fraction 4 was based on the elution profile observed during the preparative HPLC separation that created the fraction. A semi-preparative HPLC method [Keystone BetaMax Neutral C18 (8 × 250 mm i.d., 5 µm)] was developed which resulted in a linear gradient of acetonitrile in H_2O acidified with 0.05% TFA from 5 to 9% over 4.0 min, followed by a linear gradient of acetonitrile from 9 to 14% over 55.0 min, then followed by a linear gradient of acetonitrile from 14 to 18% over 2.0 min, and finally followed by a linear gradient of acetonitrile from 18 to 31% over 35.0 min, to afford **1** (70 µg, t_R = 32.9 min). The quantity was estimated using HPLC/ELSD as previously described.[23] NMR data for the structure elucidation were acquired on a Bruker Avance 600 MHz NMR system (Bruker Instruments, Rheinstetten, Germany) equipped with a 5 µl capillary scale NMR probe: CapNMR probe (MRM/Protasis, Savoy, IL), having a 1.5 µl active volume.[23] For the new compound **1**, (*ca* 70 µg) was diluted with 6.5 µl CD_3OD and 5 µl were loaded manually into the probe, from which *ca* 16 µg were in the active volume (1.5 µl). Data acquisition for ^{1}H NMR: Number of scans (NS) = 64, 5 min.; for ^{1}H–^{1}H COSY: NS = 4, 32 min; for NOESY: NS = 16, mixing time of 300 ms, 2 h; for HSQC: NS = 128, 128 increments, 5 h; for HMBC: NS = 200, 128 increments, 8 h acquisition time, HMBC long-range coupling delay optimised at 63 ms.

Laxanol (**1**): ^{1}H NMR and ^{13}C NMR data see Table 1. ESIMS *m/z* 375[M–H]$^-$, 751[2M–H]$^-$, 399[M + Na]$^+$, 775[2M + Na]$^+$. HR-ESIMS *m/z* 399.1417 [M + Na]$^+$ (calcd for $C_{20}H_{24}O_7Na$, 399.1419).

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