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A New Arylnaphthalide Lignan from *Justicia prostrata* Gamble

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A new arylnaphthalide lignan glycosides, 4-O-β-D-apiofuranosyl-(1'''→6'')-β-D-glucopyranosyldiphyllin (**1**), together with three known compounds, procumphthalide-A, cilinaphthalide-A, justicidinoside-B, have been isolated from the whole plant of *Justicia prostrata*. The use of IR, UV, one and two-dimensional NMR spectroscopy and chemical methods were applied for the identification of these compounds.

Key Words: *Justicia prostrata*, Acanthaceae, Arylnaphthalide lignan.

INTRODUCTION

Justicia Linn. (Acanthaceae) species are medicinally important, used in indigenous system of medicine as laxative, diaphoretic, diuretic, emetic, emmenagogue, expectorant, anthelmintic, asthma, cough, rheumatism and backache[1]. A number of arylnaphthalene lignans have been isolated from different *Justicia* species, many of which exhibit diverse biological activities including antitumor, antiviral, insecticidal, cardiotonic, analgesic, antiinflammatory and central nervous system depression and stimulation[2-4]. *Justicia prostrata* Gamble has been reported to possess antiinflammatory[5] and antiulcerogenic[6] activity. In a continued search for novel metabolites from plants, a new arylnaphthalide lignan glycosides, 4-O-β-D-apiofuranosyl-(1'''→6'')-β-D-glucopyranosyldiphyllin (**1**), together with three known compounds, procumphthalide A (**2**), cilinaphthalide A (**3**), justicidinoside B (**4**) were isolated from the whole plants of *Justicia prostrata* Gamble.

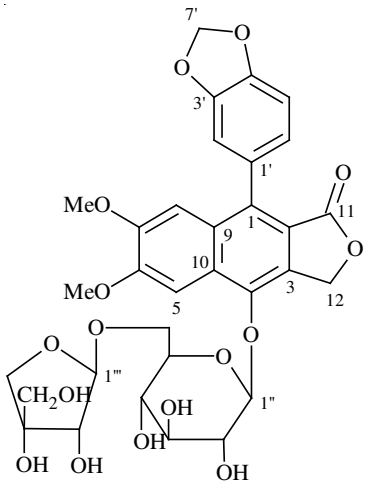
EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument at working frequencies 500 and 125 MHz in $CDCl_3$ and CD_3OD at 25 ºC with TMS as standard. Two-dimensional spectra were measured using standard methods of Bruker.

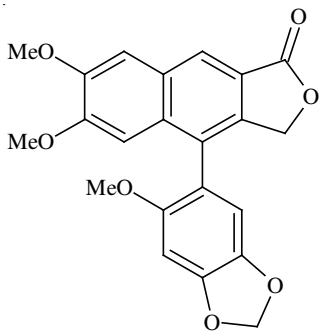
The accuracy of the ^{1}H and ^{13}C chemical shifts were 0.01 ppm; ^{1}H/^{1}H spin-spin coupling constants 0.2 Hz. IR spectra were recorded on a Shimadzu FTIR 8400S in KBr pellets. UV spectra were recorded in Beckman DU 700 UV spectrophotometer. Column chromatography (CC) was carried out on silica gel (kieselgel 60-120 and 70-230 mesh, Merck). TLC were conducted on Si-gel (E-Merck and BDH) coated on a thin glass plate (0.25 mm thickness containing 13 % $CaSO_4$ as binder). PC was carried out on Whatman filter paper No. 1 (descending) and spots were detected by spraying with aniline hydrogen phthalate (AHP) followed by heating. Melting points were recorded in Boetius microscopic apparatus. Optical rotations were recorded in methanol on Jasco DIP-140 digital polarimeter.

The aerial parts of *J. prostrata* Gamble were collected from Botanical garden, Forest Research Institute, New Forest, Dehradun, Uttarakhand, India, during January 2008. The plant was identified by Dr. Sumer Chand, Systematic Botany Division, FRI, Dehradun, Uttarakhand, India. A voucher specimen (HR No. 156) was deposited in the Botany Department, Govt. P.G. College, Uttarkashi, Uttarakhand, India.

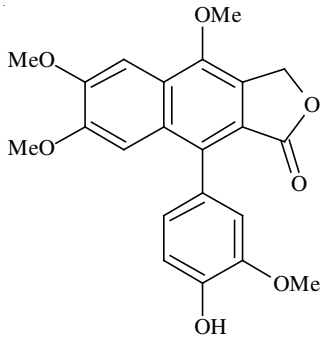
Extraction and isolation: The Air dried and powdered whole plants of *J. prostrata* Gamble (2.5 kg) were exhaustively extracted with light petroleum ether (60-80 ºC) and the petroleum free mass was further extracted with MeOH. After evaporation of solvent the MeOH extract (150 g) was subjected to column chromatography over Si-gel eluted with $CHCl_3$:MeOH with increasing content of MeOH afforded various fractions. Fraction 1, on repeated CC over Si-gel eluted with *n*-hexane:EtOAc (3:1), CH_2Cl_2:EtOAc (15:1) afforded procumphthalide A (**2**) (25 mg) and cilinaphrhalide A (**3**) (31 mg). Fraction 3 was subjected to repeated CC over Si-gel eluted with *n*-hexane:EtOAc (3:1), CH_2Cl_2:EtOAc (15:1 and 1:1) yielded compound 3 (7 mg) and justicidinoside B (**4**) (21 mg).



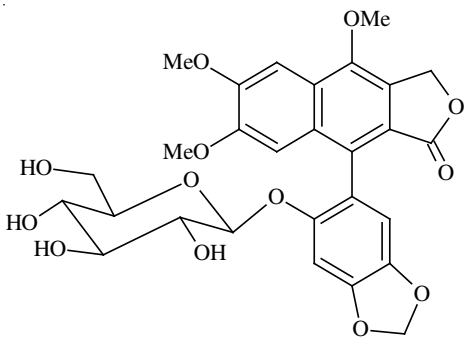
Structure of compound **1**



Structure of compound **2**



Structure of compound **3**



Structure of compound **4**

Fraction 4 on CC over Si-gel eluted with *n*-hexane: EtOAc: MeOH (2:4:1) gave three fractions, **a-c**. Fraction **a** on repeated column chromatography over Si-gel eluted with CH_2Cl_2: MeOH (5:1; 4:2 and 1:1) afforded 4-O-β-D-apiofuranosyl-(1'''→6")-β-D-glucopyranosyldiphyllin (**1**) (24 mg). The known compounds were identified by spectroscopic methods and comparison with reported data.

4-O-β-D-apiofuranosyl-(1'''→6")-β-D-glucopyranosyldiphyllin (1): Colourless amorphous solid (MeOH); $[\alpha]_D^{25}$ -33° (c, 0.10, MeOH); UV (λ_{max}, MeOH): 215 (log ε 3.80), 260 (3.29), 315 (2.71), 355 (3.75) nm; IR (KBr, ν_{max}, cm^{-1}): 3390, 2939, 1756, 1627, 1508, 1490, 1260, 1215, 1165, 1072, 1035, 1007 *etc*. Positive MS-FAB: m/z 673 $[M-H]^-$ (5), 359 (100), 365 (11), 319 (9), 183 (5), 97 (10); HRFAB-MS: m/z 673.1780 $[M-H]^-$, calcd. for $C_{32}H_{33}O_{16}$, 673.1769; ^1H and ^{13}C NMR data: given in Table-1.

TABLE-1
NMR SPECTRAL DATA OF COMPOUND **1**

C/H	δ_C*	δ_H	HMBC correlations
1	136.5[s]	–	–
2	128.8[s]	–	–
3	120.2[s]	–	–
4	146.0[s]	–	–
5	105.2[d]	7.70, 1H, s	4, 6, 7, 10
6	153.3[s]	–	–
7	151.8[s]	–	–
8	107.1[d]	7.02, 1H, (s)	6, 7, 9, 10
9	128.0[s]	–	–
10	132.1[s]	–	–
11	172.1[s]	–	–
12	68.9[t]	5.51 1H, d (*J* = 14.6 Hz)	2, 3, 4, 11
		5.63 1H, d (*J* = 14.6 Hz)	2, 4, 11
1′	130.2[s]	–	–
2′	111.7[d]	6.72 1H, d (*J* = 1.5 Hz)	1, 1′, 3′, 4′, 6′
3′	149.0[s]	–	–
4′	149.0[s]	–	–
5′	109.0[d]	6.94 1H, d (*J* = 7.6Hz)	1′, 4′, 6′,
6′	124.7[d]	6.69 1H, dd (*J* = 7.6, 1.5 Hz)	1, 1′, 2′, 4′, 5′
7′	102.9[t]	6.01 2H, *s*	3′, 4′
1″	99.7[d]	4.66 1H, d (*J* = 8.0 Hz)	4, 2″, 3″
2″	74.6[d]	3.20 1H, dd (*J* = 8.8, 8.0 Hz)	1″, 3″
3″	77.9[d]	3.71 1H, t (*J* = 8.8 Hz)	1″, 2″, 4″
4″	71.4[d]	3.30 1H, *m*	–
5”	77.7[d]	3.46 1H, *m*	–
6″	68.5[t]	3.62 1H, dd (*J* = 6.0, 12.0 Hz)	1‴, 4″
		4.01 1H, dd (*J* = 4.0, 12.0 Hz)	1‴, 4″, 5″
1‴	110.9[d]	4.99 1H, d (*J* = 4.0 Hz)	6″, 2‴
2‴	77.1[d]	3.91 1H, d (*J* = 4.0 Hz)	1‴, 3‴
3‴	80.4[s]	–	2‴, 5‴
4‴	74.9[t]	3.96 1H, d (*J* = 9.6 Hz)	–
		3.75 1H, d (*J* = 9.6 Hz)	2‴, 3‴
5‴	65.5[t]	3.56 1H, brs	3‴, 4‴
6-OCH_3	56.9[q]	3.86 3H, s	6
7-OCH_3	56.0[q]	4.04 3H, s	7

*Multiplicity of carbon signals were determined by DEPT spectrum.

Acid hydrolysis of 1: Compound **1** (7 mg) were dissolved in 5 mL of 7 % H_2SO_4 and refluxed for 3.5 h on water bath.

The reaction mixture was cooled and diluted with H_2O and extracted with $CHCl_3$. The aqueous layer was neutralized with saturated solution of $BaCO_3$ and filtered. The sugar was identified by PC (*n*-BuOH-C_5H_5N-H_2O; 6:4:3; ascending) and spraying with aniline hydrogen phthalate.

RESULTS AND DISCUSSION

The methanol extract of whole plant of *J. prostrata* Gamble on repeated column chromatography over Si-gel successively eluted with various solvents yielded, 4-O-β-D-apiofuranosyl-(1"'→6")-β-D-glucopyranosyldiphyllin (**1**). It was obtained from methanol as colourless amorphous powder. The negative-ion HRFABMS of **1** showed molecular ion peak at m/z 673.1780, which corresponded to molecular formula of $C_{32}H_{34}O_{16}$. Its IR spectrum displayed characteristic absorption band for hydroxyl groups (3390 cm^{-1}), an aromatic γ-lactone ring (1756 cm^{-1}), an aromatic ring (1627 cm^{-1}) and methylenedioxy group (1007 cm^{-1}) and the UV spectrum exhibited characteristic absorption bands at 210, 258, 315, 355 nm, which were similar to those of diphyllin[7] and procumbenoside B[8] indicating presence of an arylnaphthalene nucleus[9].

The ^1H and ^{13}C NMR spectrum of **1** was strongly reminiscent of that reported for diphyllin[7] in addition, to signals assignable for protons of hexose and pentose sugars (Table-1). The ^1H and ^{13}C resonances were confirmed by ^1H-^1H-COSY and HMQC experiments. The ^1H NMR spectrum displayed presence of three ABX type protons at 6.72 (d, *J* = 1.5 Hz, H-2'), 6.94 (d, *J* = 7.6 Hz, H-5') and 6.69 (dd, *J* = 7.6, 1.5 Hz, H-6') and two singlets, each for one proton, at 7.70 and 7.02 in the aromatic region. In aliphatic region it displayed two non-equivalent proton signals at 5.51 and 5.63 coupling with each other (*J* = 14.8 Hz) ascribable to γ-lactone methylene group, a two protons singlet at 6.01 for dioxygenated methylene group and two singlet, each for three protons, at 3.86 and 4.04 for two methoxy groups. The anomeric proton signals at δ 4.46 (d, *J* = 8.0 Hz) and 4.99 (d, *J* = 4.0 Hz) indicated presence of two sugar units. The negative ion FABMS confirmed the presence of sugar units, which revealed a significant fragment ion peak at m/z 379 due to cleavage of one pentose and one hexose unit. Compound **1** on acid hydrolysis yields agylcone and sugars. The sugars were identified as apiose and glucose by PC. The β-orientation of sugars were determined from the value of coupling constant.

The ^{13}C NMR spectrum of **1** showed presence of 31 carbon atoms (two equivalent carbons), the DEPT spectrum revealed the presence of 12 methine, 5 methylene, 2 methyl and 12 quaternary carbon atoms. The presence of 2 methoxyl groups displayed by ^1H NMR spectrum at 3.86 and 4.04 were confirmed by its ^{13}C resonances at 56.0 and 56.9. The presence of γ-lactone methylene group was confirmed by ^{13}C-chemical shifts of carbonyl carbon at 172.1 and methylene carbon at 68.9. The dioxygenated methylene carbon signal resonated downfield at 102.9. The ^{13}C-chemical shift of anomeric proton at 99.7 and 110.9 confirmed the presence of two sugar moieties, namely β-D-apiofuranosyl and β-D-glucopyranosyl in the molecule. The present spectral data confirmed that compound **1** contains a diyphyllin nucleus with 2 sugar moieties attached to it.

The location of sugar moieties at 4-position of diphyllin moiety was established by the glycosylation shifts (-3.10 ppm) at C-4 signals[7]. The connectivity of sugar residues with the diphyllin nucleus was confirmed from the HMBC spectrum, which showed a correlation between anomeric proton of glucose (δ 4.46, H-1") and C-4 (δ 146.0) of diphyllin nucleus. The chemical shift of anomeric proton of apiofuranosyl at δ 4.99 (H-1"'), which appeared upfield when compared with anomeric proton of procumbenoside A[8] (δ 5.45) and B[2] (δ 5.85) suggest that this sugar is terminal. The downfield chemical shift of C-6" (δ 68.5) of glucose when compared with C-6" of procunbenoside B (δ 62.1) suggested that the terminal apiofuranosyl was attached to the C-6" of glucose. The 1→6 linkage between apiose and glucose was confirmed by HMBC experiment which displayed correlation between anomeric proton of apiose (δ 4.99 H-1"') and C-6" of glucose (Table-1).

The above discussed spectral evidences led to the identification of a new compound characterized as 4-O-β-D-apiofuranosyl-(1"'→6")-β-D-glucopyranosyldiphyllin (**1**). Compounds **2-4** were identified as procumphthalide A[8], cilinaphthalide A[10] and justicidinoside B[11], respectively by comparison of their physical constant and spectral data with the literature values.

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