Glucosides from Mangrove Plant Avicennia Marina

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Abstract

*Avicennia marina (Forsk.) Vierh. (Verbenaceae) is a species of mangrove plant, distributed in the coastal mangrove forests of Northern and Southern Vietnam. Using combined chromatographic separations, three iridoid glucosides 2'-O-(4-methoxycinnamoyl)mussaenosidic acid (**1**), 2'-cinnamoyl-mussaenosidic acid (**2**), marinoid D (**3**), and a lignan glucoside syringaresinol-β-D-glucopyranoside (**4**) were isolated from the methanol extract of leaves of mangrove plant Avicennia marina. Structures of the isolated compounds were elucidated by spectroscopic methods including one dimesional (1D)- and (2D)- NMR, electrospray ionization mass spectrometry (ESI - MS), and also by comparison with the literature data. Compound **4** is reported for the first time from this plant.*

Keywords: Avicennia marina, iridoid glucoside, lignan glucoside.

1. Introduction

Avicennia marina (Forsk.) Vierh. (Verbenaceae) is a species of mangrove plant, distributed in the coastal mangrove forests of Northern and Southern Vietnam. The bark and root of this plant have been used in traditional medicine to treat leprosy and contraception. Besides that, the leaves are used by coastal residents to drive mosquitoes away [1, 2]. Previous phytochemical studies on *A. marina* have demonstrated the presence of iridoid glucosides [3] [4], phenylpropanoid glycosides, abietane diterpenoid glucosides, lignan glycosides [5], and flavonoids [6]. In this article, we report the isolation and structural elucidation of three iridoid glucosides (**1**-**3**) and one lignan glucoside (**4**).

2. Experimental

2.1. General experimental procedures

The ESI-MS was measured on Agilent 1260 series single quadrupole LC/MS systems. NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (Bruker, Billerica, MA, U.S.A.) using TMS as an internal standard. Medium pressure liquid chromatography (MPLC) was carried out on a Biotage - Isolera One system. Column chromatography (CC) was performed using a silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany) or YMC RP-18 resins (30 - 50 μm, Fuji Silysia Chemical Ltd, Aichi, Japan). Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck, Darmstadt, Germany) and RP-18 F_{254S} plates (1.15685.0001, Merck, Darmstadt, Germany) and compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 3–5 minutes.

2.2. Plant material

The leaves of *Avicennia marina* (Forsk.) Vierh. were collected at Bai Tu Long bay, Quang Ninh province, Vietnam in July 2016 and identified by Dr. Nguyen The Cuong (Institute of Ecology and Biological resources, VAST). A voucher specimen (No. ĐTCB-HSB 17) was deposited at the Institute of Marine Biochemistry, VAST.

2.3. Extraction and isolation

The air dried and powdered leaves of *A. marina* (2.5 kg) were extracted three times with methanol at 40°C. Methanolic extracts were combined and evaporated under vacuum. This extract (650 g) was suspended in water and partitioned in turn with *n*-hexane and CH_2Cl_2. The water layer (590 g) was chromatographed on a Diaion HP-20 column and eluted with increasing concentration of MeOH in water (0, 25, 75, and 100%) to obtain four fractions, W1-W4. Fraction W3 was further subjected to RP-18

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MPLC with increasing concentration of MeOH in H_2O (33-100%) to give ten subfractions, W3A-W3K. Subfraction W3C was separated by silica gel CC eluted with a solvent system of CH_2Cl_2/MeOH/H_2O 8/1/0.05 to afford compound **1** (6.7 mg). Compound **4** (7.2 mg) was purified from subfraction W3E after subjecting it to silica gel CC eluted with CH_2Cl_2/MeOH/H_2O 4/1/0.1 and followed by silica gel CC eluting with CH_2Cl_2/MeOH/H_2O 10/1/0.1. Fraction W3G was separated by silica gel CC using CH_2Cl_2/MeOH/H_2O 5/1/0.1 and purified by Sephadex LH-20 CC to give compound **3** (22 mg). Fraction W3H was separated by silica gel CC eluting with CH_2Cl_2/MeOH 7/1 and followed by silica gel CC using CH_2Cl_2/MeOH/H_2O 4/1/0.1 as eluent to obtain compound **2** (8 mg).

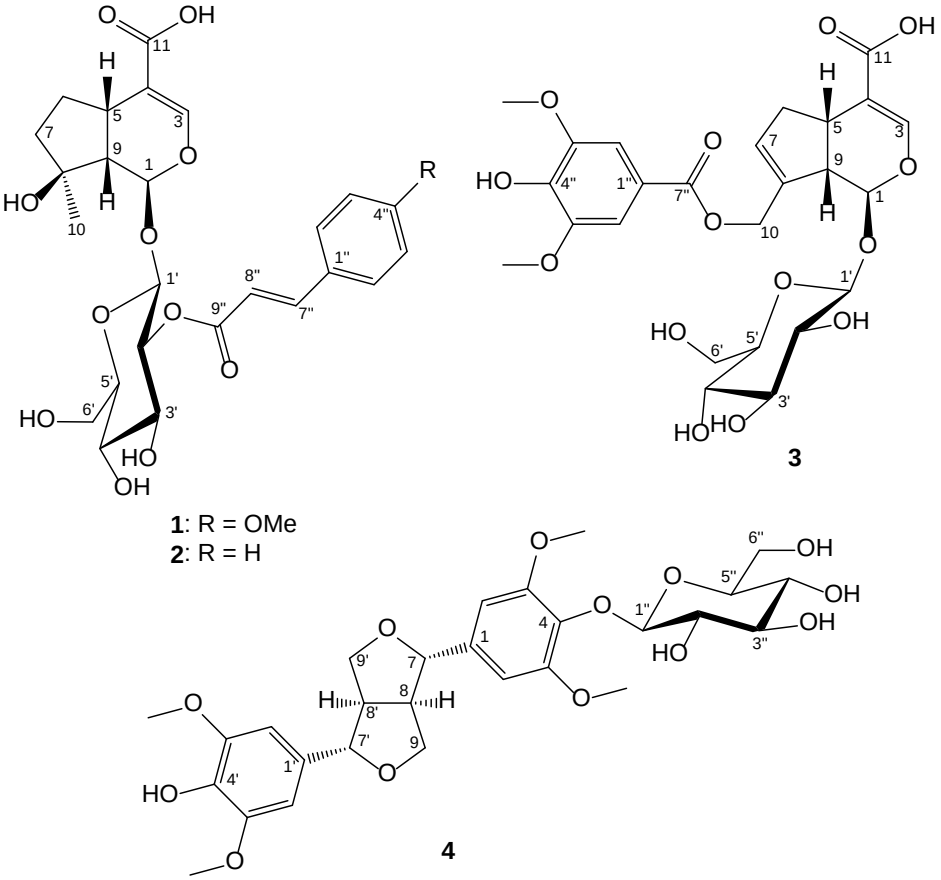

Fig. 1. Structure of compounds **1** - **4**

2'-*O*-(4-methoxycinnamoyl)mussaenosidic acid (**1**): White powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 1. Positive ESI-MS *m/z* 559 $[M+Na]^+$.

2'-cinnamoyl-mussaenosidic acid (**2**): White powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 1. Positive ESI-MS *m/z* 529 $[M+Na]^+$.

Marinoid D (**3**): White powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 2. Positive ESI-MS *m/z* 555 $[M+H]^+$.

Syringaresinol-β-D-glucopyranoside (**4**): White powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 2. Positive ESI-MS *m/z* 581 $[M+H]^+$.

3. Result and discussion

Compound **1** was obtained as a white powder. The ESI-MS spectrum of **1** exhibited a ion peak $[M+Na]^+$ at *m/z* 559, which is in agreement with the molecular formula $C_{26}H_{32}O_{12}$. The ^{13}C NMR spectrum along with the HSQC experiment showed the presence of 26 carbon corresponding to six quaternary, fifteen methane, three methylene, and two methyl carbon atoms. Among them, one ester carbonyl (δ 167.99), one carboxylic carbon (δ 170.40), one methoxy group (δ 55.86), and ten olefinic carbons were evident. Moreover, the presence of a glucose moiety was suggested by six carbons resonating at δ 97.82, 74.80, 76.02, 71.73, 78.53, and 62.76. The ^{1}H NMR spectrum of **1** exhibited characteristic signals of the common iridoid glycoside. The signals of an acetal proton at δ 5.49 (1H, d, *J* = 2.5 Hz), an olefinic proton at δ 7.28

(1H, s), a singlet methyl at δ 1.29 (3H, s), and an anomeric proton at δ 4.91 (1H, d, *J* = 8.0 Hz) were assigned to H-1, H-3, H-10 of iridoid skeleton and H-1' of β-glucose moiety, respectively. Furthermore, the signals of a set of AA'BB' aromatic ring at δ 7.55 and 6.97 (each 2H, d, *J* = 8.5 Hz), a *trans*-double bond at δ 7.64 and 6.33 (each 1H, d, *J* = 16.0 Hz), and a methoxy group at δ 3.85 (3H, s) were attributed to a *E-p*-methoxycinnamoyl moiety. In the HMBC spectrum, the cross-peaks from H-1 to C-3, C-5, from H-3 to C-4, C-5, C-11, from H-7 to C-5, C-6, C-8, C-9, and from H-10 to C-7, C-8, C-9 confirmed the structure of iridoid skeleton (fig. 2). The location of the glucose moiety was etablished by HMBC correlations from H-1' to C-1 and H-1 to C-1', whereas the *E-p*-methoxycinnamoyl fragment was attached to C-2' of sugar unit due to the cross-peak from H-2' to C-9''. On the basis of the above evidences as well as the good agreement of the NMR data of **1** with those reported in literature (see table 1), the structure of **1** was determined to be 2'-*O*-(4-methoxycinnamoyl)mussaenosidic acid [7].

Compound **2** was isolated as a white powder. Its molecular formula was determined as $C_{25}H_{30}O_{11}$ on the basis of an ion peak $[M+Na]^+$ at *m/z* 529 in ESI-MS. The ^1H and ^{13}C NMR spectral data of **2** were very similar to those of **1** (see table 1), except for the absence of the methoxy group at C-4''. In the ^1H NMR spectrum, the signals of a mono-substituted aromatic ring were observed at δ 7.61 (2H, m, H-2'', H-6''), 7.42 (2H, m, H-3'', H-5'') and 7.41 (1H, m, H-4''). The connection of each fragment in the molecule was established by HMBC correlations. Thus, compound **2** was identified as 2'-cinnamoyl-mussaenosidic acid.

Compounds **3** and **4** were elucidated as marinoid D [8] and syringaresinol-β-D-glucopyranoside [9] by detailed analysis of their 1D, 2D NMR data, and comparison of the ^{13}C-NMR data (Table 2) with the values reported in the literatures. In addition, compound **4** have not been previously isolated from *A. marina.*

Table 1. The NMR data of compounds **1** and **2**

C	$^{\#}\delta_C$ [a]	1		2	
		δ_C [a,b]	δ_H [a,c]	δ_C [a,b]	δ_H [a,c]
1	95.1	95.14	5.49 (1H, d, 2.5)	95.16	5.49 (1H, d, 3.0)
3	151.2	151.10	7.28 (1H, s)	150.97	7.28 (1H, s)
4	114.2	114.28	-	114.30	-
5	31.4	31.45	3.02 (1H, m)	31.51	3.03 (1H, m)
6	30.3	30.30	1.47 (1H, m) 2.22 (1H, m)	30.31	1.48 (1H, m) 2.22 (1H, m)
7	41.4	41.33	1.63 (1H, m) 1.71 (1H, m)	41.33	1.63 (1H, m) 1.71 (1H, m)
8	79.9	79.89	-	79.90	-
9	52.6	52.55	2.25 (1H, br d, 9.5)	52.57	2.26 (1H, dd, 2.5, 10.0)
10	24.4	24.39	1.29 (3H, s)	24.40	1.29 (3H, s)
11	170.2	170.40	-	170.60	-
1'	97.8	97.82	4.91 (1H, d, 8.0)	97.80	4.92 (1H, d, 8.0)
2'	74.8	74.80	4.83 (1H, m)	74.96	4.82 (1H, m)
3'	76.0	76.02	3.65 (1H, t, 8.5)	75.99	3.66 (1H, dd, 8.5, 9.0)
4'	71.7	71.73	3.41 (1H, m)	71.71	3.41 (1H, m)
5'	78.6	78.53	3.42 (1H, m)	78.53	3.42 (1H, m)
6'	62.8	62.76	3.72 (1H, dd, 5.0, 12.0) 3.95 (1H, br d, 12.0)	62.75	3.72 (1H, dd, 5.5, 12.0) 3.95 (1H, dd, 1.5, 12.0)
1''	128.6	128.56	-	135.93	-
2'', 6''	131.1	131.08	7.55 (2H, d, 8.5)	129.35	7.61 (2H, m)
3'', 5''	115.4	115.37	6.97 (2H, d, 8.5)	129.95	7.42 (2H, m)
4''	163.1	163.08	-	131.36	7.41 (1H, m)
7''	146.4	146.35	7.64 (1H, d, 16.0)	146.50	7.68 (1H, d, 16.0)
8''	116.0	116.04	6.33 (1H, d, 16.0)	118.75	6.48 (1H, d, 16.0)
9''	168.0	167.99	-	167.54	-
OMe	55.9	55.86	3.85 (3H, s)	-	-

[a] recorded in CD_3OD, [b]125 MHz, [c] 500 MHz, [#]δ_C of 2'-O-(4-methoxycinnamoyl)mussaenosidic acid [7]

Table 2. The NMR data of compounds **3** and **4**

3				4			
C	**$^{\#}\delta_C$**	**$\delta_C^{a,b}$**	**$\delta_H^{a,c}$**	**C**	**$^{\#\#}\delta_C$**	**$\delta_C^{a,b}$**	**$\delta_H^{a,c}$**
1	96.2	98.11	5.30 (1H, d, 7.5)	1	139.62	139.54	-
3	151.1	153.18	7.54 (1H, s)	2, 6	104.96	104.86	6.74 (2H, s)
4	112.5	113.85	-	3, 5	154.49	154.42	-
5	35.1	36.29	3.26 (1H, m)	4	135.72	135.61	-
6	38.9	39.92	2.19 (1H, m) 2.91 (1H, m)	7	87.26	87.19	4.79 (1H, d, 3.5)
7	130.2	131.47	5.93 (1H, br s)	8	55.57	55.49	3.15 (1H, m)
8	138.5	139.62	-	9	72.93	72.85	3.93 (1H, m) 4.30 (1H, dd, 8.5, 15.0)
9	46.7	47.83	2.88 (1H, m)	1’	133.17	133.08	-
10	63.0	64.14	5.01 (1H, d, 14.0) 5.06 (1H, d, 14.0)	2’, 6’	104.66	104.56	6.67 (2H, s)
11	169.1	170.95	-	3’, 5’	149.44	149.37	-
1’	99.2	100.55	4.75 (1H, d, 8.0)	4’	136.35	136.26	-
2’	73.7	74.85	3.27 (1H, t, 8.5)	7’	87.65	87.59	4.74 (1H, d, 4.0)
3’	77.0	77.94	3.41 (1H, t, 8.5)	8’	55.78	55.70	3.15 (1H, m)
4’	70.4	71.46	3.33 (1H, m)	9’	72.99	72.92	3.93 (1H, m) 4.30 (1H, dd, 8.5, 15.0)
5’	77.7	78.34	3.30 (1H, m)	1’’	105.43	105.34	4.88 (1H, d, 7.5)
6’	61.5	62.77	3.66 (1H, dd, 4.5, 12.0) 3.86 (1H, d, 12.0)	2’’	75.78	75.70	3.50 (1H, m)
1’’	119.7	121.35	-	3’’	77.90	77.82	3.44 (1H, m)
2’’, 6’’	107.3	108.25	7.37 (1H, s)	4’’	71.43	71.33	3.43 (1H, m)
3’’, 5’’	148.0	148.95	-	5’’	78.40	78.33	3.22 (1H, m)
4’’	141.1	142.09	-	6’’	62.67	62.57	3.68 (1H, dd, 5.0, 12.0) 3.79 (1H, dd, 2.0, 12.0)
7’’	166.8	167.92	-	3, 5-OMe	57.16	57.10	3.88 (3H, s)
OMe	56.5	56.89	3.91 (3H, s)	3’, 5’-OMe	56.90	56.84	3.86 (3H, s)

[a] recorded in CD_3OD, [b] 125 MHz, [c] 500 MHz, $^{\#}\delta_C$ of marinoid D [8]; $^{\#\#}\delta_C$ of syringaresinol-β-D-glucopyranoside [9]

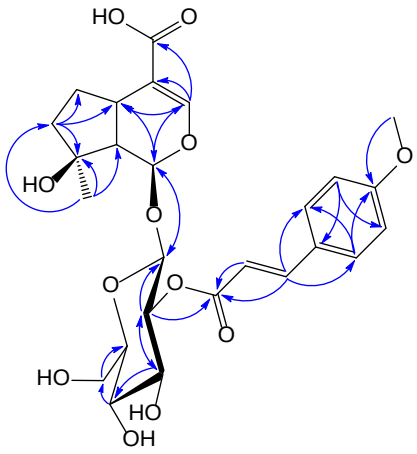

Fig. 2. Key HMBC correlations of compound 1

4. Conclusions

From the MeOH extract of the leaves of mangrove plant *Avicennia marina*, using various chromatography methods, four known compounds 2’-*O*-(4-methoxycinnamoyl)mussaenosidic acid (**1**), 2’-cinnamoyl-mussaenosidic acid (**2**), marinoid D (**3**), and syringaresinol-β-D-glucopyranoside (**4**) were isolated. Their structures were identified by comparison of the spectroscopic data with those reported in the literature. This is the first report for the isolation of compound **4** from this species.

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