FURTHER CHEMICAL STUDY ON *AVICENNIA ALBA* Bl.

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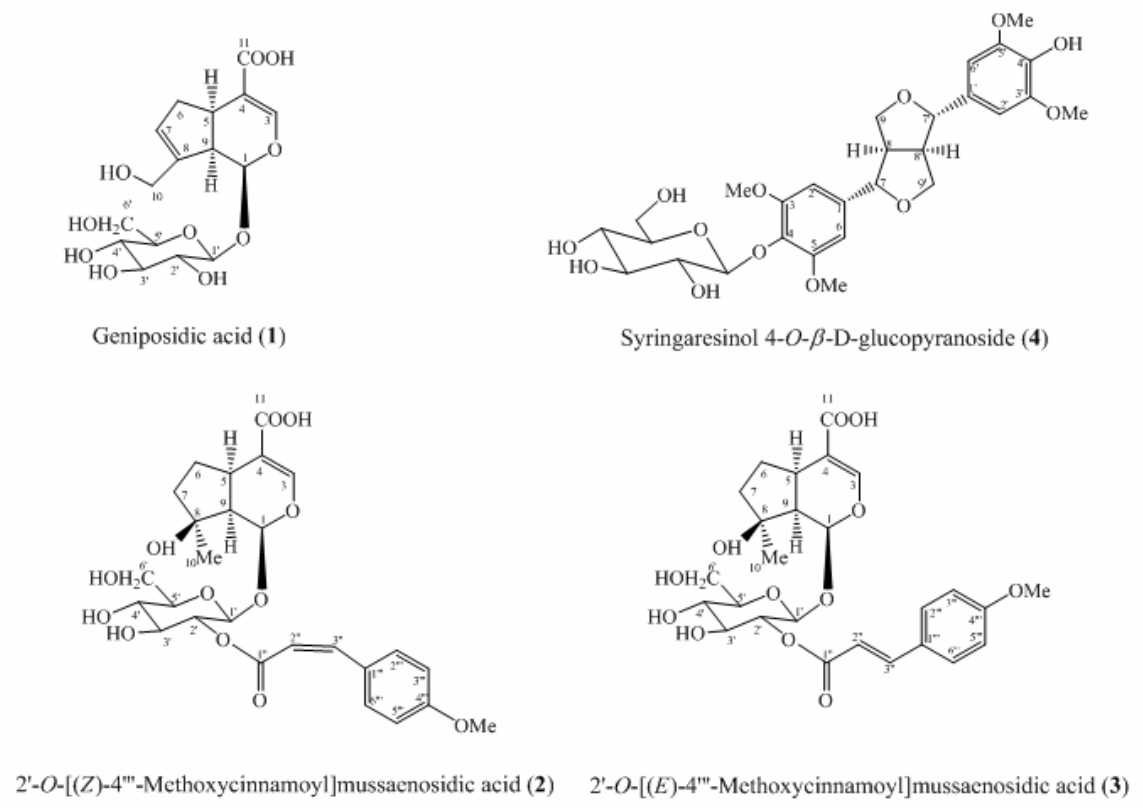
Abstract

Three iridoid glucosides and a lignan glucoside were isolated from the dried leaves of *Avicennia alba* Bl. (Avicenniaceae), including geniposidic acid **(1)**, a mixture of 2'-*O*-[(*Z*)-4'''-methoxycinnamoyl]mussaenosidic acid **(2)**, 2'-*O*-[(*E*)-4'''-methoxycinnamoyl]mussaenosidic acid **(3),** and syringaresinol 4-*O*-β-D-glucopyranoside **(4)**. Their chemical structures were elucidated by spectroscopic methods as well as compared with data in the literature. These substances were isolated for the first time from this genus.

Keywords: *Avicennia alba*, iridoid, lignan.



Geniposidic acid (**1**)

Syringaresinol 4-*O*-*β*-D-glucopyranoside (**4**)

2'-*O*-[(*Z*)-4'''-Methoxycinnamoyl]mussaenosidic acid (**2**)

2'-*O*-[(*E*)-4'''-Methoxycinnamoyl]mussaenosidic acid (**3**)

1. INTRODUCTION

Can Gio mangrove forest is an economically and ecologically important resource for Ho Chi Minh City. The mangrove plants are exploited for firewood, timber and used for erosional protection. The genus *Avicennia* (Avicenniaceae) comprises about 14 species which are mangrove woody trees or

shrubs and used as traditional medicine for treatment of leprosy, ringworms, snake bites and especially, tumors and ulcers [1]. These species have not much been studied in the phytochemistry as well as in the pharmacology. Therefore, it is necessary to chemically study on the mangrove trees to enhance the value of their uses.

Avicennia alba Bl., the mangrove woody tree, has been rarely chemically studied. The unique literature present the isolation of some naphthoquinones from *Avicennia alba* Bl. in Singapore [2]. Previously, we have isolated four triterpenes, such as β-amyrin acetate, ursolic acid, lupeol and betulin, along with four flavones, namely, luteolin, chrysoeriol, velutin and flavogadorinin from *Avicennia alba* Bl. growing in Can Gio mangrove forest. The chemical structure elucidation of these compounds was reported in an article to participate the Sixth National Conference of Science and Technology, Organic Chemistry section, going to be held in Hanoi in August 2012. In the present work, we will display the isolation and the elucidation of three iridoid glucosides and a lignan glucoside from this species.

2. EXPERIMENTAL

2.1. General experimental procedures

The NMR spectra were measured on a Bruker Avance spectrometer, at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. The above spectra were performed in the Center of Analysis, University of Science, National University – HCM City.

2.2. Plant material

The leaves of *Avicennia alba* Bl., collected in June 2010 in Can Gio mangrove forest, HCM City, were identified by Pharmacist Phan Duc Binh, Associate Editor-in-Chief of the Bimonthly Magazine of Drug and Health. A voucher specimen (US–B010) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, National University – HCM City.

2.3. Extraction and isolation

The dried leaves of *Avicennia alba* Bl. (856 g) was exhaustedly extracted with methanol by maceration to yield a residue of 260 g. The crude residue (260 g) was subjected to silica gel solid phase extraction and eluted with different solvents to give corresponding extracts, including petroleum ether (3.7 g), $CHCl_3$ (18 g) and EtOAc (102 g). The EtOAc extract (102 g) was separated into six fractions (EA.1 to EA.6) by silica gel column chromatography with the elution of petroleum ether – EtOAc from 9:1 to 8:2. Fraction EA.5 (22 g) was separated by silica gel chromatography with CH_3Cl-MeOH (7:3) to obtain **1** (17 mg), a mixture of **2** and **3** (12 mg) and **4** (10 mg).

∗ **Geniposidic acid (1):** Opalescent wax; ^{1}H and ^{13}C NMR ($CDCl_3$) shown in table 1.

Table 1: NMR spectral data of **1** (CD_3OD)

Pos.	δ_H, J (Hz)	δ_C
1	5.16 (1H, *d*, 7.5)	98.3
3	7.52 (1H, *s*)	153.3
4	-	112.8
5	3.17 (1H, *m*)	36.6
6	2.11 (1H, *dd*, 16.0, 7.5) 2.83 (1H, *dd*, 16.0, 7.5)	39.7
7	5.80 (1H, *s*)	128.5
8	-	144.7
9	2.72 (1H, *t*, 7.5)	47.1
10	4.32 (1H, *d*, 14.0) 4.18 (1H, *d*, 14.0)	61.4
11	-	170.9
1’	4.72 (1H, *d*, 8.0)	100.4
2’	3.22-3.41 (1H, *m*)	74.9
3’	3.22-3.41 (1H, *m*)	77.8
4’	3.22-3.41 (1H, *m*)	71.5
5’	3.22-3.41 (1H, *m*)	78.3
6’	3.64 (1H, *d,* 11.7) 3.86 (1H, *d*, 11.5)	62.6

Mixture of **2'-*O*-[(*Z*)-4”’-methoxy cinnamoyl]mussaenosidic acid (2)** and **2'-*O*-[(*E*)-4”’-methoxycinnamoyl]mussaenosidic acid (3)** in the ratio of 1:2: Opalescent wax; ^{1}H (DMSO-d_6), δ_H: 7.68 (1H, *d*, *J* = 8.5 Hz, H-2’’’$_n$, H-6’’’$_n$), 7.62 (1H, *d*, *J* = 16.0 Hz, H-3’’$_m$), 7.54 (2H, *d*, *J* = 9.0 Hz, H-2’’’$_m$, H-6’’’$_m$), 7.31 (0.5H, *s*, H-3$_n$), 7.27 (1H, *s*, H-3$_m$), 6.95 (2H, *d*, *J* = 9.0 Hz, H-3’’’$_m$, H-5’’’$_m$), 6.89 (1H, *d*, *J* = 9.0 Hz, H-3’’’$_n$, H-5’’’$_n$), 6.88 (0.5H, *d*, *J* = 12.5 Hz, H-3’’$_n$), 6.32 (1H, *d*, *J* = 16.0 Hz, H-2’’$_m$), 5.77 (0.5H, *d*, *J* = 12.5 Hz, H-2’’$_n$), 5.48 (1H, *d*, *J* = 3.0 Hz, H-1$_m$), 5.46 (0.5H, *d*, *J* = 3.0 Hz, H-1$_n$), 4.90 (1.5H, *d*, *J* = 8.0 Hz, H-1’$_m$, H-1’$_n$), 4.81 (1.5H, *dd*, *J* = 9.5, 8.0 Hz, H-2’$_m$, H-2’$_n$), 3.94 (1.5H, *d*, *J* = 12 Hz, H-6’a$_m$, H-6’a$_n$), 3.84 (3H, *s*, OCH_{3m}), 3.82 (1.5H, *s*, OCH_{3n}), 3.71 (1.5H, *dd*, *J* = 12.0, 5.5 Hz, H-6’b$_m$, H-6’b$_n$), 3.64 (1.5H, *m*, H-3’$_m$, H-3’$_n$),

3.41 (1.5H, *m*, H-5'$_m$, H-5'$_n$), 3.38 (1.5H, *m*, H-4'$_m$, H-4'$_n$), 3.01 (1.5H, *m*, H-5$_m$, H-5$_n$), 2.25 (1.5H, *dd*, *J* = 9.0, 3.0 Hz, H-9$_m$, H-9$_n$), 2.20 (1.5H, *m*, H-6a$_m$, H-6a$_n$), 1.70 (1.5H, *m*, H-7a$_m$, H-7a$_n$), 1.63 (1.5H, *m*, H-7b$_m$, H-7b$_n$), 1.46 (1.5H, *m*, H-6b$_m$, H-6b$_n$), 1.29 (1.5H, *s*, H-10$_n$), 1.28 (3H, *s*, H-10$_m$). ^{13}C NMR (DMSO-d_6), δ_C: 170.4 (C-11$_m$, C-11$_n$), 168.0 (C-1''$_m$), 167.0 (C-1''$_n$), 163.1 (C-4'''$_m$), 162.0 (C-4'''$_n$), 151.1 (C-3$_n$), 151.0 (C-3$_m$), 146.3 (C-3''$_m$), 145.1 (C-3''$_n$), 133.4 (C-2'''$_n$, C-6'''$_n$), 131.1 (C-2'''$_m$, C-6'''$_m$), 128.6 (C-1'''$_m$, C-1'''$_n$), 117.4 (C-2''$_n$), 116.1 (C-2''$_m$), 115.4 (C-3'''$_m$, C-5'''$_m$), 114.6 (C-4$_n$), 114.4 (C-4$_m$), 114.3 (C-3'''$_n$, C-5'''$_n$), 97.8 (C-1'$_m$), 97.6 (C-1'$_n$), 95.2 (C-1$_m$), 95.0 (C-1$_n$), 80.0 (C-8$_n$), 79.9 (C-8$_m$), 78.5 (C-5'$_m$, C-5'$_n$), 76.0 (C-3'$_m$), 75.9 (C-3'$_n$), 74.8 (C-2'$_m$), 74.4 (C-2'$_n$), 71.9 (C-4'$_n$), 71.8 (C-4'$_m$), 62.8 (C-6'$_m$, C-6'$_n$), 55.9 (OCH_{3m}), 55.8 (OCH_{3n}), 52.6 (C-9$_m$), 52.5 (C-9$_n$), 41.3 (C-7$_m$), 41.2 (C-7$_n$), 31.5 (C-5$_m$, C-5$_n$), 30.4 (C-6$_n$), 30.3 (C-6$_m$), 24.4 (C-10$_m$, C-10$_n$). *Note:* m for 2'-*O*-[(*E*)-4'''-methoxycinnamoyl]mussaenosidic acid (**3**) and n for 2'-*O*-[(*Z*)-4'''-methoxycinnamoyl] mussaenosidic acid (**2**).

Syringaresinol 4-*O*-β-D-glucopyranoside (4): Opalescent wax; ^{1}H and ^{13}C NMR ($CDCl_3$) shown in table 2.

Table 2: NMR spectral data of **4** (CD_3OD)

Pos.	δ_H, J (Hz)	δ_C
1	–	139.5
1'	–	133.1
2, 6	6.72 (2H, *s*)	104.7
2', 6'	6.65 (2H, *s*)	105.0
3, 5	–	154.4
3', 5'	–	149.4
4	–	135.7
4'	–	136.4
7	4.71 (1H, *d*, 4.5)	87.6
7'	4.76 (1H, *d*, 4.5)	87.2
8	3.13 (1H, *m*)	55.5
8'	3.13 (1H, *m*)	55.7
9, 9'	4.27 (2H, *m*) 3.91 (2H, *dd*, 9.5, 3.5)	72.9
CH_3O-3,5	3.85 (6H, *s*)	57.2
CH_3O-3',5'	3.86 (6H, s)	56.9
1''	4.85 (1H, d, 8.0)	105.3
2''	3.40–3.49 (1H, m)	75.7
3''	3.20 (1H, m)	78.3
4''	3.40–3.49 (1H, m)	71.4
5''	3.40–3.49 (1H, m)	77.8
6''	3.66 (1H, dd, 12.0, 5.0) 3.77 (1H, dd, 12.0, 2.0)	62.6

3. RESULTS AND DISCUSSION

The EtOAc extract (102 g), which was obtained from the dried leaves of *Avicennia alba* Bl. (856 g) was fractionated by silica gel solid phase extraction, followed by silica gel chromatography to yield four compounds, **1**–**4**.

Compound **1** was isolated as opalescent wax from the EtOAc extract. Analysis of the ^{1}H and ^{13}C NMR spectra (table 1) revealed **1** to be an iridoid glucoside with two acetal proton signals at δ_H 5.16 (1H, *d*, *J* = 7.5 Hz, H-1) and δ_H 4.72 (1H, *d*, *J* = 8.0 Hz, H-1') along with 16 carbon signals, of which 6 signals assigned to a β-glucopyranosyl moiety. The complete assignments of the ^{1}H and ^{13}C NMR data relied on the results of the COSY, HSQC and HMBC experiments. By COSY experiment, signals in the region of δ_H 3.22-4.72 were assigned for the β-glucopyranosyl moiety, which was suggested to attach at the C-1 position of the aglycon by the HMBC correlations between H-1'/C-1 and reversion. The other proton signals belonged to the aglycon with two olefinic protons at δ_H 7.52 (1H, *s*, H-3) and δ_H 5.80 (1H, *s*, H-7) and a pair of signals at δ_H 4.32 (1H, *d*, *J* = 14 Hz, H-10a) and 4.18 (1H, *d*, *J* = 14 Hz, H-10b) for a hydroxymethyl group. The ^{13}C NMR spectrum showed the presence of a carboxyl group with the signal at δ_C 170.9 (C-11), which linked to C-4 demonstrated by the HMBC correlation from δ_H 7.52 (1H, *s*, H-3) to 170.9 (C-11). The HMBC correlations between δ_H 4.32 (1H, *d*, *J* = 14 Hz, H-10a) and δ_C 144.7 (C-8) as well as δ_H 4.32 (1H, *d*, *J* = 14 Hz, H-10a) and δ_C 128.5 (C-7) confirmed that the hydroxymethyl group attached to C-8 of the aglycon. Based on these spectroscopic data and comparison with the published ones [3, 4], **1** was identified as geniposidic acid.

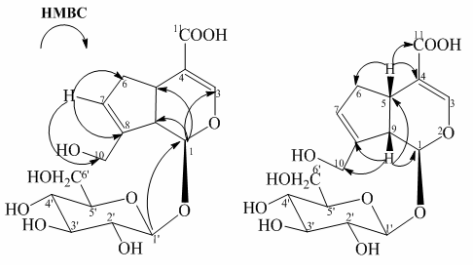


Figure 1: The HMBC correlations of **1**

Compounds **2** and **3** could not be separated by chromatography due to unstableness. Their NMR spectra showed characteristic signals of a mixture of two iridoid glucosides esterified with a cinnamoyl moiety. The ^{1}H NMR spectrum exhibited a doublet signal at δ_H 5.48 (1H, *d*, *J* = 3.0 Hz, H-1$_m$) for the

acetal proton at C-1 of an iridoid skeleton, an singlet olefinic proton signal at δ_H 7.27 (1H, *s*, H-3_m) for a double bond at C-3 and C-4 as usual in iridoid, a singlet signal at δ_H 1.28 (3H, *s*, H-10_m) for a methyl group attached to the oxygenated carbon and an anomeric proton at δ_H 4.90 (1H, *d*, *J* = 8.0 Hz, H-1'$_m$) for glucopyranosyl moiety. The *β*-configuration of C-1'$_m$ of the glucopyranose moiety was evidenced by the large coupling constant of the anomeric proton signal (*J* = 8.0 Hz). In addition, two *ortho*-coupled doublet proton signals at δ_H 7.54 (2H, *d*, *J* = 8.5 Hz, H-2'''$_m$, H-6'''$_m$) and 6.95 (2H, *d, J* = 9.0 Hz, H-3'''$_m$, H-5'''$_m$) indicated a 1,4-disubstituted aromatic ring. Furthermore, two coupling signals at δ_H 6.32 (1H, *d*, *J* = 16.0 Hz, H-2''$_m$) and 7.62 (1H, *d*, *J* = 16.0 Hz, H-3''$_m$) showed the presence of a *trans*-configuration double bond. Besides, the singlet signal at δ_H 3.84 (3H, *s*, OCH_{3m}) displayed a methoxy group. Similarly, the ^{1}H NMR spectrum also indicated the signals of another esterified iridoid glucoside in a lower quantity. The difference of this one from the above one was a *cis* double bond of the cinnamoyl moiety, demonstrated by a pair *cis*-coupled olefinic proton with the coupling constant of *J* = 12.5 Hz at δ_H 5.77 (0.5H, H-2''$_n$) and 6.88 (0.5H, H-3''$_n$). It was observed that was a mixture of two diastereoisomers: the *trans*-cinnamoyl moiety and a *cis*-cinnamoyl moiety in the ratio of 2:1, determined by the integration of some proton signals such as H-2''$_m$ (1H) and H-2''$_n$ (0.5H), H-1_m (1H) and H-1_n (0.5H). The ^{13}C NMR spectrum also supported the evidences for the above demonstration. The aromatic and olefinic carbon signals in the zone δ_C 114.3-163.1 indicated the presence of a cinnamoyl moiety in each molecule. The spectrum also indicated typical signals of iridoid *β*-D-glucopyranosyl skeleton of this mixture such as two pairs of olefinic carbon signals at δ_C 151.0 (C-3_m), 114.4 (C-4_m) and 151.1 (C-3_n), 114.6 (C-4_n), a pair of anomeric carbon signal at δ_C 97.8 (C-1'$_m$), 97.6 (C-1'$_n$) and five pairs of carbinol methine signals in the zone δ_C 62.8-78.5. Two pairs of signals at δ_C 168.0 (C-1''$_m$), 167.0 (C-1''$_n$) and 170.4 (C-11_m, C-11_n) showed carbonyl groups of an ester and a carboxylic acid in each molecular, respectively. Based on the above analyzed spectroscopic data and the comparison with the published ones,[5] the compounds **2** and **3** were suggested to be a mixture of two isomers: 2'-*O*-[(*Z*)-4'''-methoxycinnamoyl]mussaenosidic acid and 2'-*O*-[(*E*)-4'''-methoxycinnamoyl] mussaenosidic acid with the ratio of 1:2.

Compound **4** was isolated as opalescent wax from the EtOAc extract. The NMR spectra of **4** (table 2) indicated characteristic signals of a lignan glucoside. Its ^{1}H NMR spectrum exhibited two singlet aromatic proton signals at δ_H 6.72 (2H, *s*, H-2, H-6) and 6.65 (2H, *s*, H-2', H-6'), which were the characteristics of two 1, 3, 4, 6-tetrasubstituted benzene rings. Two singlet signals at δ_H 3.86 (6H, *s*) and 3.85 (6H, *s*) showed the presence of four methoxy groups. Additionally, the proton spectrum also showed eight saturated proton signals which were the feature of a diepoxytetrahydrofuran lignan skeleton at δ_H 4.71 (1H, *d*, *J* = 4.5 Hz, H–7), 3.13 (2H, *m*, H-8, H-8'), 4.27 (2H, *m*, H-9a, H-9'a), 3.91 (2H, *dd*, *J* = 9.5, 3.5 Hz, H-9b, H-9'b). The anomeric proton signal at δ_H 4.85 (1H, *d*, *J* = 8.0 Hz, H-1'') corresponding to δ_C 105.3 (C-1''), which had COSY correlation chains to the oxygenated protons in the zone δ_H 3.40-3.49 (3H, *m*, H-2'', H-4'', H-5''), 3.20 (1H, *m*, H-3''), 3.66 (1H, *dd*, *J* = 12.0, 5.0 Hz, H-6''a), 3.77 (1H, *dd*, *J* = 12.0, 2.0 Hz, H-6''b) suggested the presence of the glucopyranosyl moiety. Furthermore, the ^{13}C NMR spectrum showed the distinct signals of a *bis*-tetrahydrofuran ring with two oxymethylene signals at δ_C 72.9 (C-9, C-9'), two oxymethine signals at δ_C 87.6 (C-7) and 87.2 (C-7'), two aliphatic methine signals at δ_C 55.5 (C-8) and 55.7 (C-8'). The aromatic rings were demonstrated by resonance signals in the downfield from δ_C 104.7-154.4. Two signals at δ_C 57.2 (CH_3O-C3, C5) and 56.9 (CH_3O-C3', C5') were for four methoxy groups. The sugar moiety was proved by the acetal carbon signal at δ_C 105.3 (C-1'') as well as five other oxygenated methine signals at δ_C 75.7 (C-2''), 78.3 (C-3''), 71.4 (C-4''), 77.9 (C-5'') and 62.6 (C-6''). The ^{1}H and ^{13}C assignment were resumed by COSY, HSQC and HMBC experiments. The linking of molecular fractions was established by the HMBC experiment. Two aromatic rings directly attached to diepoxytetrahydrofuran skeleton at the position 7 and 7', which demonstrated by the HMBC correlations between δ_H 4.71 (1H, *d*, *J* = 4.5 Hz, H–7) and two carbon signals at δ_C 104.7 (C-2, C-6) as well as δ_H 4.75 (1H, *d*, *J* = 4,0 Hz, H–7') to two carbon signals at δ_C 105.0 (C-2', C-6'). The HMBC correlations between a couple of signals δ_H 3.85 (6H, *s*) and 3.86 (6H, *s*) for methoxy group to carbon signals at δ_C 154.4 (C-3, C-5) and 149.4 (C-3', C-5') indicated that four methoxy groups linked to two benzene rings at the positions 3, 5 and 3', 5', respectively. In addition, the HMBC correlation of the anomeric proton signal at δ_H 4.85 (H-1'') to carbon signal at δ_C 136.4 (C-4) showed that the *β*-D-glucopyranoside moiety attached to the benzene ring at the position 4. In the nature, the stereostructure of diepoxytetrahydrofuran lignans at H-8 and H-8'

always possessed the *cis*-configuration [6]. According to Kotaro Takahashi [7], the stereochemistry at C-7 and C-7' could be determined by the coupling constant of H-7/H-8 and H-7'/H-8'. If the coupling constant $J_{7,8} < 5.5$ Hz, two protons H-7 and H-8 *trans*-coupled; in contrast, if the coupling constant $J_{7,8} > 6.0$ Hz, these two protons *cis*-coupled. In the case of the compound **4**, two atoms H-7 and H-8 *trans*-coupled and two atoms H-7' and H-8' either because the two protons H-7 and H-7' possessed small coupling constants $J_{7,8} = J_{7',8'} = 4.5$ Hz. The HR-ESI-MS spectrum gave a pseudomolecular ion peak at *m*/*z* 603.2065 $[M+H]^+$ corresponding to the molecular formula of $C_{28}H_{36}O_{13}$ [($C_{28}H_{36}O_{13}$+H), M=603.2054, error 1.1 mili mass]. Based on the above spectroscopic data analysis and comparison with the published ones [8], **4** was identified as syringaresinol 4-*O*-*β*-D-glucopyranoside.

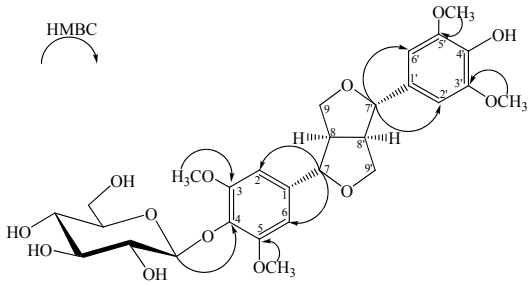


Figure 2: The HMBC correlations of **4**

4. CONCLUSION

From the leaves of *Avicennia alba Bl.* collected in June 2010 in Can Gio mangrove forest, HCM City, three iridoid glucosides and a lignan glucoside were isolated, including geniposidic acid **(1)**, a mixture of 2'-*O*-[(*Z*)-4"'-methoxycinnamoyl]-mussaenosidic acid **(2)** and 2'-*O*-[(*E*)-4"'-methoxycinnamoyl]mussaenosidic acid **(3)**, and syringaresinol 4-*O*-β-D-glucopyranoside **(4)**. These substances were isolated for the first time from this genus.

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