




Three New Phenolic Sulfates from *Acrostichum aureum* Collected from Coastal Area of Thai Binh Province, Vietnam and Their Cytotoxic Activity

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Abstract: The analysis of a methanol extract of mangrove plant *Acrostichum aureum* collected from Thai Binh coastal area led to isolation of three new compounds, 4-(3'-*O*-sulfate-4'-hydroxyphenyl)-2-butanone (1), 4-(3'-*O*-sulfate-4'-hydroxyphenyl)-2(*R*)-butanol (2), and dihydrodehydrodiconiferyl alcohol 9-*O*-sulfate (3) beside five known phenolic compounds (4-8). The structures of isolated compounds were determined by extensive analysis of their spectroscopic evidence (IR, 1D and 2D NMR, HR ESI-MS) as well as comparison with the data reported in the literature. The cytotoxic activity of these compounds was evaluated on SK-LU-1, HepG2, and MCF7 cell lines using SRB assay. The results showed that compound 4 exhibited weak cytotoxicity against three tested cell lines. The other compounds were considered as inactive in this test.

Keywords: *Acrostichum aureum*; Pteridaceae; 4-arylbutanoid sulfate; neolignan sulfate; cytotoxicity. © 2021 ACG Publications. All rights reserved.

1. Introduction

Swamp fern species *Acrostichum aureum* L. is a big terrestrial plant belonging to the family Pteridaceae and occurs in tropical and subtropical areas worldwide. This plant grows widely in mangrove forests and other wetlands from the north to the south of Vietnam. In Vietnamese traditional medicine, the rhizomes and leaves of this species have long been used for treatment of asthma, fever, ulcers, and boils [1]. The ethanol and methanol extracts from *A. aureum* have been reported to possess antioxidant, tyrosinase inhibition, anti-inflammatory, analgesic, antifertility in rats, antibacterial, and anticancer properties [2]. Phytochemical investigation of the aerial parts of *A. aureum* has demonstrated the presence of flavonoids, phthalates, sterols, terpenoids, and other compounds as patriscabratine, 2-butanone, tetracosane [3-5]. Previous pharmacological studies have shown that phytosterols, (2*S*,3*S*)-sulfated pterosin C and patriscabratine exhibited cytotoxic activity against

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different human cancer cell lines [5-7]. Nevertheless, there are few reports about lignans and other phenolics from *A. aureum*. As part of our program in study the traditional medicine plants from mangrove areas of Vietnam for anticancer activity, the extracts of *A. aureum* have been investigated. This paper deals with the isolation and structural elucidation of three new compounds as 4-arylbutanoid sulfates **1-2** and a neolignan sulfate **3** together with five known phenolic compounds **4-8** from the MeOH extract of the aerial parts of *A. aureum*. To our best knowledge, this is the first isolation of compounds **4-8** from *A. aureum*. The cytotoxicity of compounds **1-8** effect against three human cancer cell lines SK-LU-1, HepG2 and MCF7 was also reported.

2. Materials and Methods

2.1. General Experimental Procedures

Optical rotations were measured on a JASCO P-2000 polarimeter (Hachioji, Tokyo, Japan). Circular dichroism (CD) spectra were recorded with a Chirascan spectrometer (Applied Photophysics, UK). HR-QTOF-MS were measured on an Agilent 6530 Accurate-Mass spectrometer (CA, USA). NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer with tetramethylsilane (TMS) as internal standard. FT-IR spectra were recorded with a FTIR Affinity-1S spectrophotometer (Shimadzu, Japan). For column chromatography (CC), Diaion HP 20 (Mitsubishi chemical Co.), silica gel 60 (0.04-0.063mm, Merck), RP-18 resins (150 μ m, YMC) and Sephadex LH-20 (25-100 μ m, Sigma-Aldrich) were used. The TLC was performed on Merck precoated TLC DC-Alufolien silica gel 60F₂₅₄ and RP-18F₂₅₄S, and compounds were detected by UV fluorescence and spraying 1% vanillin-H₂SO₄ in MeOH, followed by heating at 100°C for 1-2 min.

2.2. Plant Material

The aerial parts of *A. aureum* were collected in 2019 from coastal area of Thai Binh province in Northern Vietnam. The plant material was identified by Prof. Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen (HUST.N03) is kept in the laboratory of the Organic Department, Hanoi University of Science and Technology (HUST), Vietnam.

2.3. Extraction and Isolation

The dry powdered aerial parts (3.2 kg) of *A. aureum* were extracted three times with MeOH (10 L each) at 45°C for 1 h under sonication and then concentrated to 1.5 L *in vacuo*. The extract was partitioned with *n*-hexane (3 \times 1 L) and then the MeOH layer was concentrated under reduced pressure. The obtained residue (214.5 g) was suspended in H₂O (1.5 L) and further extracted with EtOAc (3 \times 1 L) to give EtOAc and water residues 61.0 g and 135.8 g, respectively after removal of the solvents. The aqueous layer (135.8 g) was subjected to column chromatography on Diaion HP20 and eluted with water and increasing concentration of MeOH in water (20, 40, 80, 100, v/v). The fraction eluted with 20% MeOH (21.6 g) was rechromatographed over silica gel eluted with a gradient solvent system of CH₂Cl₂/MeOH (10 : 1, 5 : 1, 2.5 : 1, 1 : 1, v/v) to afford five fractions *Fr.1-Fr.5*. *Fr.1* (1.2 g) was further separated by sephadex LH-20 column eluted with MeOH and then on an RP 18 column eluted with MeOH/H₂O (2 : 3, v/v) to yield compounds **5** (4.8mg) and **7** (7.5mg). *Fr.2* (4.3 g) was separated by sephadex LH-20 column eluted with methanol to afford 3 subfractions (*Fr.2.1-Fr.2.3*). Purification of the *Fr.2.1* and *Fr.2.2* by RP 18 CC eluted with acetone/H₂O (1 : 3, v/v) to yield compound **8** (102.6 mg) and **6** (2.1 mg), respectively. *Fr.2.3* was further separated by RP18 CC eluted with MeOH/H₂O (1 : 2.5, v/v) to yield compounds **1** (33.2 mg), **2** (41.1 mg), and **4** (67.6 mg). Compound **3** (26.5 mg) was obtained from the fraction *Fr.3* (1.2 g) by RP18 CC eluted with MeOH/H₂O (1 : 3, v/v).

4-(3'-O-sulfate-4'-hydroxyphenyl)-2-butanone (1): White amorphous powder. IR (KBr) ν_{\max} 3388, 2914, 2848, 1728, 1605, 1467, 1178, 1024, 993, 825, 761 cm^{-1} ; HR ESI-MS: m/z 259.0291 [$M - H$] $^-$ (calcd. for $\text{C}_{10}\text{H}_{11}\text{O}_6\text{S}$, 259.0282); ^1H NMR (500 MHz, $\text{DMSO}-d_6$) and ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) data are given in Table 1.

4-(3'-O-sulfate-4'-hydroxyphenyl)-2(R)-butanol (2): White amorphous powder. $[\alpha]_D^{25} = -28.6$ (c 0.1, MeOH). IR (KBr) ν_{\max} 3383, 2914, 2848, 1602, 1512, 1269, 1024, 993, 825, 761 (cm^{-1}); HR ESI-MS: m/z 261.0446 [$M - H$] $^-$ (calcd. for $\text{C}_{10}\text{H}_{13}\text{O}_6\text{S}$, 261.0448); ^1H NMR (500 MHz, $\text{DMSO}-d_6$) and ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) data are given in Table 1.

Dihydrodehydrodiconiferyl alcohol 9-O-sulfate (3): White amorphous powder. $[\alpha]_D^{25} = 0$ (c 0.1, MeOH). IR (KBr) ν_{\max} 3358, 2939, 2839, 1604, 1531, 1492, 1384, 1249, 1195, 1128, 1062, 985, 815 cm^{-1} ; CD (MeOH, c 3.8×10^{-4} M) mdeg (λ_{\max}): +0.92 (204 nm), +0.55 (224 nm), +0.23 (242 nm), +0.26 (290 nm); HR ESI-MS: m/z 439.1074 [$M - H$] $^-$ (calcd. for $\text{C}_{20}\text{H}_{23}\text{O}_9\text{S}$, 439.1068); ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data are given in Table 2.

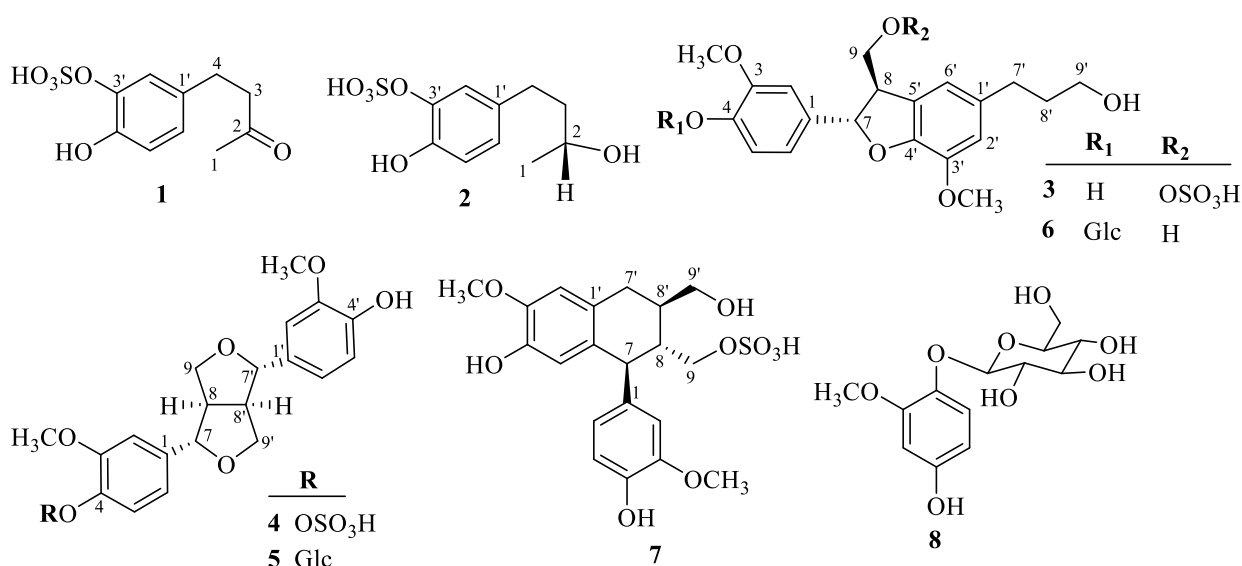


Figure 1. Chemical structures of compounds **1-8**

2.4. Cytotoxic Assay

The cytotoxicity of isolated compounds (**1-8**) were evaluated against three human cancer cell lines, SK-LU-1 (lung carcinoma), MCF7 (breast carcinoma), and HepG2 (hepatocellular carcinoma) using the sulforhodamine B assay [8]. These cells were supplied by Professor J. M. Pezzuto (Long-Island University, US) and Professor Jeanette Maier (Milan University, Italia) and cultured in Dulbeccos Modified Eagle Medium (DMEM) with 10% of Fetal Bovine Serum (FBS) at 37°C in a humidified atmosphere (5% CO_2 and 95% air). A volume of 180 μL of the cell suspension (3×10^4 cells/mL) was transferred to each well of a 96-well, followed by 20 μL of testing samples at four concentrations (100, 20, 4 and 0.8 μM). After incubation for 72 h, the cell layers were fixed with 20% (w/v) trichloroacetic acid (TCA), washed three times with acetic acid, and stained for 30 min with sulforhodamine B reagent (SRB) at 37°C. The cell density after sample treatment was then measured optical density (OD) at wavelength of 540 nm. The control sample without sample treatment, was incubated for 1 h, and fixed with TCA 20%. The negative reference sample was treated with 10% DMSO. Meanwhile, ellipticine with four different concentrations (50, 10, 2 and 0.4 μM) was used as a positive control. Cell viability was measured and IC_{50} values calculated using Table Curve 2Dv4 (System Software, USA).

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was obtained as a white amorphous powder. The molecular formula $C_{10}H_{12}O_6S$ was determined by HR ESI-MS at m/z 259.0291 [$M - H$]⁻ (calcd. 259.0282) and NMR data (Table 1). The IR spectrum showed absorption bands due to hydroxyl (3388 cm^{-1}), carbonyl (1728 cm^{-1}), aromatic (1605 and 1467 cm^{-1}), and sulfate (1178 , 1024 and 993 cm^{-1}) groups [9]. The 1H NMR spectrum of **1** indicated the presence of a phenolic hydroxyl group at δ_H 8.67 (1H, *s*, 4' -OH), a tri-substituted benzene ring with an *ABX* system at δ_H 6.92 (1H, *d*, $J = 2.0$ Hz, H-2'), 6.77 (1H, *dd*, $J = 2.0$, 8.5 Hz, H-6'), and 6.70 (1H, *d*, $J = 8.5$ Hz, H-5'). Also, the proton signals of two methylenes at δ_H 2.70 (2H, *m*, H₂-3) and 2.64 (2H, *m*, H₂-4), and a methyl signal at δ_H 2.07 (3H, *s*, H₃-1) were observed. The ^{13}C NMR and DEPT spectra of **1** exhibited 10 carbon signals corresponding to a methyl at δ_C 29.1 (C-1), two methylenes at δ_C 44.3 (C-3) and 28.1 (C-4), a ketone carbonyl at δ_C 207.7 (C-2), three aromatic methines at δ_C 124.5 (C-6'), 122.8 (C-2'), 117.0 (C-5'), and three aromatic quaternary carbons at δ_C 147.2 (C-4'), 140.5 (C-3'), 132.1 (C-1'). This spectroscopic evidence suggested that **1** is an arylbutanone derivative. The exact proton and carbon signals were assigned according to HSQC, 1H - 1H COSY and HMBC experiments (Figure 2). The cross peaks between H₂-3 and H₂-4 in the 1H - 1H COSY spectrum and the correlations from H₃-1 to C-2 and C-3 in the HMBC spectrum indicated the presence of a $-CH_2CH_2COCH_3$ moiety, which was determined to be attached at C-1' of the aromatic ring based on the HMBC correlations from H₂-3 to C-1', and from H₂-4 to C-2', C-6'. The HMBC correlations from the proton of the hydroxyl group (4' -OH) to C-4', C-3' and C-5' inferred that a hydroxyl group was linked to the aromatic ring at C-4'. Detailed analysis of the NMR data indicated that compound **1** was similar to 4-(3',4' -dihydroxyphenyl)-2-butanone, a known arylbutanone which was previously isolated from *A. aureum* [10], except that an upfield shielding for the substituted C-3' was observed (Δ - 4.3 ppm), while both the ortho and para carbons of C-3' a significant downfield shielding (Δ + 3.6 to 6.9 ppm) [10-11] (Table S1). Furthermore, the HR ESI-MS indicated that the molecular weight of **1** was 260 amu which was 80 mass units higher than that calculated for 4-(3',4' -dihydroxyphenyl)-2-butanone. The mass difference between these two compounds suggested that a sulfate group was substituted at the C-3' position of the benzene ring. The effect of *O*-sulfate as an electron-withdrawing group was indicated in the ^{13}C NMR spectrum [12-13]. Therefore, compound **1** was determined to be 4-(3' -*O*-sulfate-4' -hydroxyphenyl)-2-butanone (Figure 1).

Table 1. 1H (at 500 MHz) and ^{13}C NMR (at 125 MHz) data for compounds **1-2** in DMSO- d_6

Position	1		2	
	δ_C in ppm	δ_H in ppm, <i>J</i> in Hz	δ_C in ppm	δ_H in ppm, <i>J</i> in Hz
1	29.1 (CH ₃)	2.07 (3H, <i>s</i>)	23.5 (CH ₃)	1.06 (3H, <i>d</i> , $J = 6.5$)
2	207.7 (C)		65.2 (CH)	3.57 (1H, <i>m</i>)
3	44.3 (CH ₂)	2.70 (2H, <i>m</i>)	41.0 (CH ₂)	1.54 (2H, <i>m</i>)
4	28.1 (CH ₂)	2.64 (2H, <i>m</i>)	30.6 (CH ₂)	2.50 (1H, <i>m</i>), 2.43 (1H, <i>m</i>)
1'	132.1 (C)	-	133.4 (C)	-
2'	122.8 (CH)	6.92 (1H, <i>d</i> , $J = 2.0$)	122.8	6.91 (1H, <i>d</i> , $J = 2.0$)
3'	140.5 (C)	-	140.5 (C)	-
4'	147.2 (C)	-	146.9 (C)	-
5'	117.0 (CH)	6.70 (1H, <i>d</i> , $J = 8.5$)	116.9 (CH)	6.70 <i>d</i> (1H, <i>d</i> , $J = 8.5$)
6'	124.5 (CH)	6.77 (1H, <i>dd</i> , $J = 2.0$, 8.5)	124.5 (CH)	6.76 <i>dd</i> (1H, <i>dd</i> , $J = 2.0$, 8.5)
4' -OH		8.67 (1H, <i>s</i>)		8.61 (1H, <i>s</i>)
2-OH				4.42 (<i>d</i> , $J = 4.5$)

Compound **2** was isolated as a white amorphous powder. The molecular formula of $C_{10}H_{14}O_6S$ was derived from its quasi molecular ion peak at m/z 261.0446 [$M - H$]⁻ (calcd. 261.0438) in the HR ESI-MS. The IR spectrum of **2** indicated the presence of hydroxyl (3383 cm^{-1}), aromatic (1602 and 1512 cm^{-1}), and sulfate (1269 , 1024 and 993 cm^{-1}) groups. The 1H and ^{13}C NMR spectroscopic data (Table 1) of **2** was very similar to that of **1**, except the presence of a hydroxyl group instead of a carbonyl group at C-2 position in **2**. Moreover, the HMBC correlations from the proton signal of

hydroxyl group 2-OH (δ_H 4.42) to C-2 (δ_C 65.2), from H₃-1 (δ_H 1.06) to C-2 (δ_C 65.2) and C-3 (δ_C 41.0), from H₂-3 (δ_H 1.54) to C-1' (δ_C 133.4), and from H₂-4 (δ_H 2.50 and 2.43) to C-2' (δ_C 122.8) and C-6' (δ_C 124.5) indicated the presence of a -CH₂CH₂CHOHCH₃ moiety, which was attached to C-1' of benzene ring. Thus, compound **2** was identified to be 4-(3' -O-sulfate-4' -hydroxyphenyl)-2-butanol. Compound **2** has a chiral center at C-2 position. The configuration at C-2 was determined by comparison its optical rotation with those of the closely related compounds, 4-aryl-2(*R*)-butanol with negative optical rotation and 4-aryl-2(*S*)-butanol with positive optical rotation [14]. Compound **2** showed a negative optical rotation [α]_D²⁵ - 28.6 (*c* 0.1, MeOH) suggesting the absolute configuration at C-2 is 2*R*. Based on this evidence, compound **2** was elucidated as 4-(3' -O-sulfate-4' -hydroxyphenyl)- 2(*R*)-butanol.

Table 2. ¹H (at 500 MHz) and ¹³C NMR (at 125 MHz) data for compound **3** in CD₃OD

Position	δ_C in ppm	δ_H in ppm, <i>J</i> in Hz	Position	δ_C in ppm	δ_H in ppm, <i>J</i> in Hz
1	134.4 (C)	-	1'	137.1 (C)	-
2	110.6 (CH)	7.03 (1H, <i>d</i> , <i>J</i> = 2.0)	2'	114.4 (CH)	6.75 (1H, <i>s</i>)
3	149.0 (C)	-	3'	145.2 (C)	-
4	147.3 (C)	-	4'	147.6 (C)	-
5	116.1 (CH)	6.77 (1H, <i>d</i> , <i>J</i> = 8.0)	5'	128.8 (C)	-
6	119.4 (CH)	6.89 (1H, <i>dd</i> , <i>J</i> = 2.0, 8.0)	6'	118.1 (CH)	6.79 (1H, <i>s</i>)
7	88.8 (CH)	5.60 (1H, <i>m</i>)	7'	32.8 (CH ₂)	2.64 (2H, <i>t</i> , <i>J</i> = 7.5)
8	52.8 (CH)	3.70 (1H, <i>d</i> , <i>J</i> = 6.0)	8'	35.7 (CH ₂)	1.84 (2H, <i>m</i>)
9	70.0 (CH ₂)	4.30 (1H, <i>dd</i> , <i>J</i> = 6.0, 10.5)	9'	62.2 (CH ₂)	3.58 (2H, <i>t</i> , <i>J</i> = 6.5)
		4.19 (1H, <i>dd</i> , <i>J</i> = 8.0, 10.5)			
3-OCH ₃	56.4 (CH ₃)	3.85 (3H, <i>s</i>)	3'-OCH ₃	56.8 (CH ₃)	3.88 (3H, <i>s</i>)

Compound **3** was isolated as a white amorphous powder. It had a molecular formula of C₂₀H₂₄O₉S, determined by HR ESI-MS at *m/z* 439.1074 [*M* - H]⁻ (calcd. 439.1068) and NMR data (Table 2). The IR spectrum indicated the presence of hydroxyl (3358 cm⁻¹), aromatic (1604, 1531 and 1492 cm⁻¹), and sulfate (1249, 1062 and 985cm⁻¹) groups. The ¹³C NMR spectrum of **3** showed 20 carbon signals including 12 aromatic carbons (δ_C 110.6-149.0), one oxygenated methine carbon (δ_C 88.8), two oxygenated methylene carbons (δ_C 70.0 and 62.2), two methoxy carbons (δ_C 56.8 and 56.4), one methine carbon (δ_C 52.8) and two methylene carbons (δ_C 35.7 and 32.8). The observation of three aromatic protons of a typical *ABX* system [δ_H 7.03 (1H, *d*, *J* = 2.0 Hz, H-2), 6.89 (1H, *dd*, *J* = 2.0, 8.0 Hz, H-6), and 6.77 (1H, *d*, *J* = 8.0 Hz, H-5)] in ¹H NMR spectrum suggested the presence of a tri-substituted phenolic ring. Besides, the aromatic proton signals at δ_H 6.79 (1H, *s*, H-6') and 6.75 (1H, *s*, H-2') indicated the presence of another tetra-substituted phenolic ring. Also, one oxygenated methine signal at δ_H 5.60 (1H, *d*, *J* = 6.0 Hz, H-7), two oxygenated methylene signals at δ_H 4.30 (1H, *dd*, *J* = 5.5, 10.0 Hz, H_a-9), 4.19 (1H, *dd*, *J* = 8.0, 10.5 Hz, H_b-9) and 3.58 (2H, *t*, *J* = 6.5 Hz, H₂-9'), two methoxy signals at δ_H 3.88, 3.85 (each, *s*), one methine proton at δ_H 3.70 (1H, *m*, H-8), and two methylene signals at δ_H 2.64 (2H, *t*, *J* = 7.5 Hz, H₂-7'), 1.84 (2H, *m*, H₂-8') were observed. All information mentioned above suggested that compound **3** is a dihydrobenzofuran type neolignan derivative. The ¹H and ¹³C NMR spectroscopic data of **3** were similar with those of dihydrodehydrodiconiferyl alcohol-4-*O*-glucoside (**6**), except the loss of signals due to the glucosyl moiety. The results obtained from IR and HR ESI-MS spectra of **3** indicated the presence a sulfate group (-OSO₃H) in the molecule. The ¹³C NMR chemical shift at C-9 (δ_C 70.0) in **3** is shifted downfield by + 4.9 ppm compared with that of compound **6** (δ_C 65.1), suggesting the hydroxyl group at C-9 to be replaced with a sulfate substituent (Table S2). Furthermore, by comparing the NMR data of **3** with those published for dihydrodehydrodiconiferyl alcohol 9' -*O*-sulfate [15], it was found that the carbon signal of C-9 shifted downfield by + 5.0 ppm, while the carbon signal of C-9' shifted upfield by - 6.0 ppm (Table S3), which further confirmed that the sulfate group was located at C-9. The HSQC, ¹H-¹H COSY, and HMBC experiments permitted assignments of all ¹H and ¹³C signals of **3**. The ¹H-¹H COSY spectrum indicated correlations of H-7 (δ_H 5.60)/H-8 (δ_H 3.70)/H₂-9 (δ_H 4.30 and 4.19) and H₂-7' (δ_H 2.64)/H₂-8' (δ_H 1.84)/H₂-9' (δ_H 3.58). In the HMBC spectrum (Figure 2), the protons H-2 and H-6 showed strong correlations with carbons C-7 and C-4, as well as, the correlations

from H-7 to C-2, C-6, C-1, C-4', C-8, and C-9. Also, the correlations from H_a-9 and H_b-9 to C-5', from H-7' to C-6' and C-2', from H-8' to C-1' were observed. The position of two methoxy groups assigned through the correlations of the signals at δ_H 3.85 and δ_H 3.88 with the carbon C-3 and C-3', respectively. From the analysis of NMR spectroscopic data and the results of HR ESI-MS compound **3** was determined to be a new neolignan sulfate, namely dihydrodehydrodiconiferyl alcohol-9-*O*-sulfate. The relative configuration of **3** was indicated based on NOESY spectroscopic analysis (Figure 2). The NOESY spectrum of **3** displayed strong correlations between proton H-7 and H_a-9, as well as between H-8 and H-2 but only weak correlation between H-7 and H-8, indicating the threo configuration of H-7 and H-8 [16]. The large coupling constant ($J = 6.0$ Hz) between H-7 and H-8 also supported this conformation [17]. The absence of optical rotation ($[\alpha]_D^{25} = 0$, c 0.1, MeOH) along with the CD experiment (Figure S37) suggested **3** to be a racemate. By comparison with the CD data for 7*S*, 8*R*- $[\Delta\epsilon + 5.49$ (290 nm)] and 7*R*, 8*S*- $[\Delta\epsilon - 9.70$ (283 nm)] isomers of dihydrodehydrodiconiferyl alcohol-4-*O*-glucoside [15], the CD spectrum of **3** exhibited a weak positive Cotton effect at 290 nm (+ 0.26) suggested that the 7*S*, 8*R* isomer was slightly predominant.

By using the same spectroscopic methods and comparing with the reported data, the known compounds were identified as (+)-pinoresinol-4-*O*-sulfate (**4**) [18], (+)-pinoresinol-4-*O*-glucoside (**5**) [19], dihydrodehydrodiconiferyl alcohol-4-*O*-glucoside (**6**) [15], (+)-isolarisiresinol-9-*O*-sulfate (**7**) [20], and isotachioside (**8**) [21].

Sulfated compounds belonging to the classes of terpenoids, steroids and phenolics are widely distributed in marine sponges and mangrove plants [22-23]. Previously, two new sesquiterpenes containing sulfate group have been isolated from the mangrove fern *A. aureum* [6]. This is the first report of sulfated phenolic compounds from this mangrove plant.

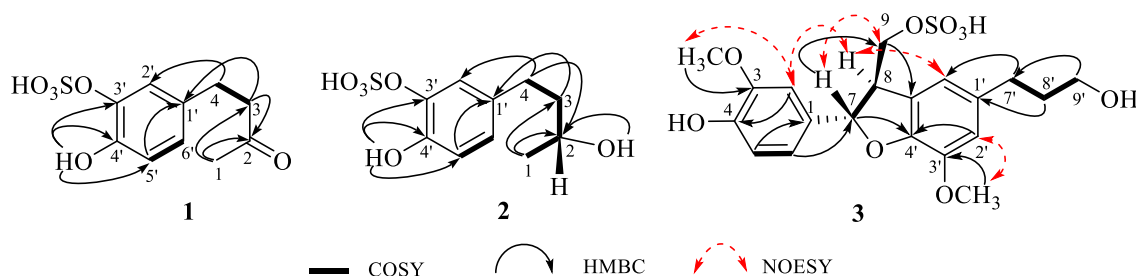


Figure 2. ^1H - ^1H COSY and the selected HMBC, NOESY correlations of compounds **1-3**

3.2. Cytotoxicity Activity

The *in vitro* cytotoxicity of compounds **1-8** toward three human cancer cell lines (LU-1, MCF7, and Hep-G2) was investigated by means of sulforhodamine B (SRB) assay using ellipticine as the positive control [8]. As the result, among the compounds tested, (+)-pinoresinol-4-*O*-sulfate (**4**) exhibited weak activity against three tested cell lines with the IC_{50} values of 65.54 ± 6.47 , 73.78 ± 5.86 and 64.73 ± 5.33 μM , respectively. The other compounds were inactive in this test ($\text{IC}_{50} > 100$ μM). Our result showed that compound **4** with a sulfate group at C-4 position is the most active compound, while compound **5** with a glucosyl moiety at C-4 as inactive recognized in this test.

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Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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