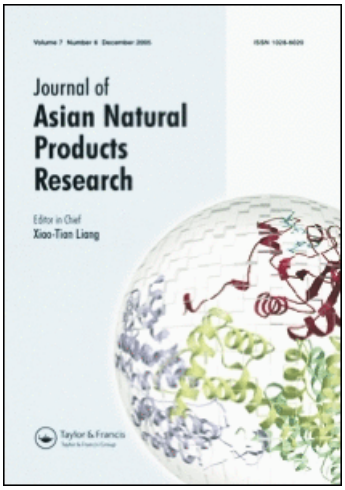

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A new sulphated nor-sesquiterpene from mangrove *Laguncularia racemosa* (L.) Gaertn. F.

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A new sulphated nor-sesquiterpene, 3α-hydroxysulphonyloxy-5α,6α-epoxy-megastigmen-9-one (**1**), and a known sulphated lipid, 5′-(hydroxysulphonyloxy) jasmonic acid (**2**), have been isolated from the twigs and leaves of mangrove *Laguncularia racemosa* (L.) Gaertn. F. The structure of the new compound was elucidated on the basis of detailed analysis of spectroscopic data and chemical reaction.

Keywords: Mangrove; *Laguncularia racemosa*; Nor-sesquiterpene; Half esters of sulphuric acid

1. Introduction

Laguncularia racemosa (L.) Gaertn. F., a mangrove plant in the family Combretaceae, is native to the coast of western Africa, the Atlantic and Pacific coast of America. *L. racemosa* is reported to be an astringent and tonic used as a folk remedy for dysentery. The bark of the title plant is used in traditional medicine for the treatment of aphthae, fever, and scurvy[1]. However, to the best of our knowledge, there were only a few reports[2,3] regarding the gum polysaccharides of this plant and no other chemical studies about its secondary metabolites were performed. In the course of our research on biologically active substances from Chinese mangrove plants[4–6], a sample of *L. racemosa* was recently collected from Haikou, Hainan Province, China, and chemically investigated. Separation of the *n*-BuOH-soluble fraction of the methanolic extract led to the isolation of a new uncommon sulphated nor-sesquiterpene, 3α-hydroxysulphonyloxy-5α,6α-epoxy-megastigmen-9-one (**1**), and a known sulphated lipid, 5′-(hydroxysulphonyloxy) jasmonic acid (**2**). In the present paper, we report the isolation and structural elucidation of the new compound.

2. Results and discussion

The chipped twigs and leaves of *L. racemosa* were extracted exhaustively with MeOH. The MeOH extract was partitioned consecutively between H_2O and EtOAc, H_2O and *n*-BuOH. The *n*-BuOH fraction was subjected to repeated column chromatography (Diaion HP-20, Silica gel, RP-C18 silica gel and Sephadex LH-20) to give compounds **1** and **2**, respectively.

Compound **1** was obtained as an optically active colourless powder, $[\alpha]_D^{24}$ −67 (*c* 0.28, MeOH). Its ESI–MS displayed a pseudo-molecular ion at *m*/*z* 303.1 ($[M - H]^-$). The HRESI–MS experiment established its molecular formula as $C_{13}H_{20}O_6S$ (*m*/*z* 303.0930 $[M - H]^-$, calcd. 303.0902), indicating four degrees of unsaturation. The IR spectrum of **1** showed absorption bonds for carbonyl (1673 cm^{-1}), double bond (1627 cm^{-1}) and sulphate (1217 and 1080 cm^{-1}) groups[7]. Acid hydrolysis of **1**, followed by treatment with $BaCl_2$, gave a white precipitate confirming the presence of a sulphate function in the molecule[8]. The ^1H NMR spectrum (Table 1) of **1** displayed the presence of four methyls [δ 0.97 (3H, s, H-11), 1.18 (3H, s, H-12), 1.22 (3H, s, H-13), and 2.29 (3H, s, H-10)], two methylenes [δ 1.46 (1H, dd, $J = 13.3$, 9.9 Hz, H-2α); 1.79 (1H, ddd, $J = 13.3$, 3.1, 1.4 Hz, H-2β); 1.94 (1H, dd, $J = 14.5$, 8.0 Hz, H-4α); 2.50 (1H, ddd, $J = 14.5$, 5.4, 1.3 Hz, H-4β)], one oxygenated methine [δ 4.51 (1H, m, H-3)], and two olefinic protons [δ 6.18 (1H, d, $J = 15.8$ Hz, H-8); 7.16 (1H, d, $J = 15.8$ Hz, H-7). The corresponding carbons were assigned through HMQC correlations. The ^{13}C NMR and DEPT spectroscopic data (Table 1) were in good agreement with the above analysis, and showed 13 carbon signals consisting of four methyls, two methylenes, three methines and four quaternary carbons. The ^1H NMR and ^{13}C NMR spectral data, in combination with the molecular composition, clearly showed compound **1** to be a sulphated nor-sesquiterpene. The final structure of **1** was mainly determined by extensive study of 2D NMR (^1H–^1H COSY, HMQC, HMBC, ROESY) spectra.

Analysis of ^1H–^1H COSY (Figure 2) and HMQC spectra, in combination with ^{13}C NMR spectral data,

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Table 1. ^{1}H NMR and ^{13}C NMR spectral data of compound **1**[a] and ^{13}C NMR spectral data of **3**[b] (δ in ppm, J in Hz).

	1		**3** [9]
Position	δ_H, J (Hz)	δ_C	δ_C
1		36.5 (s)	35.2 (s)
2α	1.46 (dd, 13.3, 9.9)	44.4 (t)	46.7 (t)
2β	1.79 (ddd, 13.3, 3.1, 1.4)		
3	4.51 (m)	73.8 (d)	64.1 (d)
4α	1.94 (dd, 14.5, 8.0)	39.1 (t)	40.7 (t)
4β	2.50 (ddd, 14.5, 5.4, 1.3)		
5		68.6 (s)	67.3 (s)
6		71.3 (s)	69.6 (s)
7	7.16 (d, 15.8)	145.4 (d)	142.4 (d)
8	6.18 (d, 15.8)	134.5 (d)	132.7 (d)
9		200.7 (s)	197.5 (s)
10	2.29 (s)	27.9 (q)	28.4 (q)
11	0.97 (s)	26.0 (q)	25.1 (q)
12	1.18 (s)	29.7 (q)	29.4 (q)
13	1.22 (s)	20.6 (q)	19.9 (q)

[a] ^{1}H NMR (CD_3OD, 400 MHz); ^{13}C NMR (CD_3OD, 100 MHz); chemical shifts (ppm) are referenced to CH_3OH ($\delta_H = 3.33$, $\delta_C = 49.0$); assignments were deduced by analysis of 1D and 2D NMR spectra.
[b] Measured in $CDCl_3$.

readily identified two spin–spin systems [**a** (C-2 to C-4), **b** (C-7 to C-8)] and a tetrasubstituted epoxy moiety (C-5 to C-6). Furthermore, a series of significant HMBC (Figure 2) correlations between H-12 (δ 1.18)/C-1 (δ 36.5), C-2 (δ 44.4) and C-6 (δ 71.3), H-7 (δ 7.16)/C-1 and C-6, and H-13 (δ 1.22)/C-4 (δ 39.1), C-5 (δ 68.6) and C-6 suggested that two partial structures **a** and **b** were connected to each other through the quaternary carbons C-1, C-5 and C-6. The connectivity of C-8 (δ 134.5) to C-10 (δ 27.9) was revealed by the cross-peaks of H-10 (δ 2.29)/C-8 and C-9 (δ 200.7), and H-7 and H-8 (δ 6.18)/C-9. Thus, the gross structure of **1** was established as shown in Figure 1.

The relative stereochemistry of **1** was deduced from analysing its ROESY spectrum. The ROESY (Figure 2) correlations of H-12/H-3 (δ 4.51), H-7 and H-13/H-4β (δ 2.50), H-7 indicated the α orientation of the half ester of sulphuric acid at C-3 and the epoxide group at C-5, C-6. The splitting pattern of H-4α (J = 14.5, 8.0 Hz) and H-4β (J = 14.5, 5.4 Hz) further confirmed that H-3 was axially (β) oriented. Thus, the structure of **1** was elucidated as depicted in Figure 1. Literature survey revealed that the structure of **1** was very similar to the model compound **3**, 3α-hydroxy-5α,6α-epoxy-7-megastigmen-9-one, which was isolated previously from *Saussurea medusa*[9]. In fact, the ^{13}C NMR spectral data of **1** and **3** (Table 1) were almost the same except for C-3 in agreement with the 80 mass units difference ($-SO_3-$) between them. Thus, the structure of **1** was unambiguously elucidated as 3α-hydroxysulphonyloxy-5α,6α-epoxy-megastigmen-9-one.

The known compound **2** was identified as 5′-(hydroxysulphonyloxy) jasmonic acid[10] by analysis of its NMR spectral data and comparison with the reported data in the literature.

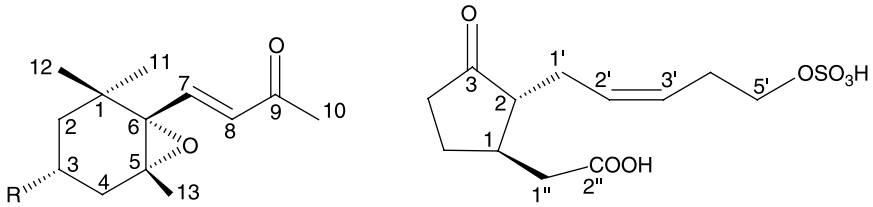
It may be important to point out that the natural products containing sodium sulphovinate ($-OSO_3Na$) group are frequently encountered in marine invertebrates while half esters of sulphuric acid as in **1** and **2** represent a rare but characteristic structural group in constituents of higher plants, and are typically present in plant hormones of the turgorin-type[11]. Both compounds **1** and **2** were isolated for the first time from the title plant.

Further study should be conducted to understand the real biological/ecological role of these metabolites in the life cycle of the mangrove as well as to test their biological activities such as cytotoxic, anti-inflammatory and anti-fouling activities.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin–Elmer 341 polarimeter. IR spectrum was recorded on a Nicolet Magna FT-IR 750 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer.



1 R = OSO_3H

3 R = OH

2

Figure 1. Structures of compounds **1**–**3**.

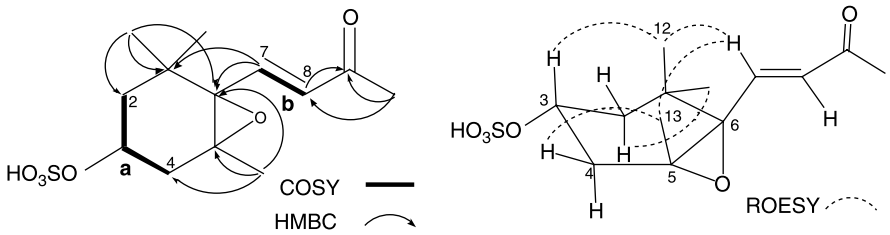


Figure 2. Selected ^{1}H–^{1}H COSY, HMBC and ROESY combinations for compound **1**.

Chemical shifts (δ) are reported in ppm relative to an internal TMS standard, coupling constant (J) in Hz. ^{1}H NMR and ^{13}C NMR assignments were supported by ^{1}H–^{1}H COSY, HMQC, HMBC and ROESY experiments. The HRESI–MS was recorded on a Q-TOF-Micro-LC–MS–MS spectrometer. Commercial silica gel plates (Qing Dao Hai Yang Chemical Group Co.) were used for TLC. The chromatograms were detected by a UV lamp at 254 nm, and successively sprayed with 0.1% $Ce(SO_4)_2$ in 2N H_2SO_4 and heating at 80°C for 5 min.

3.2 Plant material

The twigs and leaves of *L. racemosa* was collected from Haikou, Hainan Province, China in May 2002 and identified by Associate Professor J.-G. Shen of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher sample (No. 0603P-30) is available for inspection at the Herbarium of SIMM-CAS.

3.3 Extraction and isolation

The chipped twigs and leaves of *L. racemosa* (3.5 kg) were extracted exhaustively with MeOH. The MeOH extracts were concentrated *in vacuo* to give a residue (330 g), which was partitioned consecutively between H_2O and EtOAc, H_2O and *n*-BuOH. The *n*-BuOH fraction was applied to Diaion HP-20 polymeric resin and subsequently fractionated with H_2O and increasing concentrations of MeOH [H_2O/MeOH (10:0, 6:4, 4:6, 2:8, 0:10)] to give five fractions (fractions 1–5). Fraction 1 (4.2 g) was further chromatographed on MCI gel column eluting with H_2O and increasing concentrations of MeOH [H_2O/MeOH (10:0, 6:4, 4:6, 2:8, 0:10)] to give five subfractions (fractions 5.1–5.5). Fraction 5.2 (242 mg) was subjected to silica gel column and eluted with EtOAc/MeOH (9:1, 8:2) to give fraction 5.2.1 and fraction 5.2.2. Fraction 5.2.1 (60 mg) was subjected to RP-C18 silica gel column eluting with H_2O/MeOH (90:10) to afford compound **1** (25 mg). Fraction 5.2.2 (43 mg) was applied to a Sephadex LH-20 column eluting with MeOH to yield compound **2** (18 mg).

3.3.1 3α-Hydroxysulphonyloxy-5α,6α-epoxy-megastigmen-9-one (*1*)

Amorphous powder; $[\alpha]_D^{24}$ −67 (*c* 0.28, MeOH); IR ν_{max} (KBr) cm^{-1}: 3448, 2966, 2929, 1673, 1627, 1216, 1080, 970, 619; ^{1}H NMR and ^{13}C NMR spectral data: see Table 1; ESI–MS: 303.1[M − H]$^-$; HRESI–MS: *m/z* 303.0930 [M − H]$^-$ (calcd for $C_{13}H_{19}O_6S$, 303.0902).

3.3.2 Detection of the sulphate group of *1*

A 5 mg aliquot of the sample was refluxed with 10% HCl 10 ml for 4 h and then extracted with $CHCl_3$. An aliquot of the aqueous layer was treated with 70% $BaCl_2$ to give a white precipitate ($BaSO_4$).

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