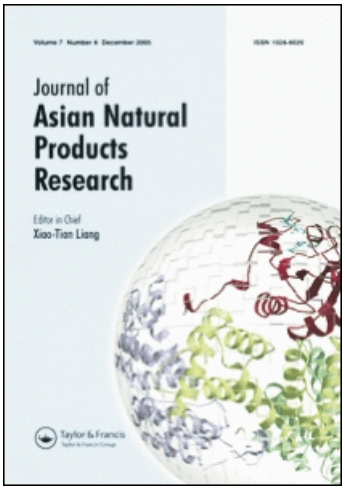

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A new nonadride derivative from mangrove fungus (strain No. k38)

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A new nonadride derivative, (−)-1-hydroxybyssochlamic acid (**1**) and the known (−)-byssochlamic acid (**2**) were isolated from mangrove fungus (strain No. k38) collected from the South China Sea coast. The structure and relative configuration of **1** were elucidated by spectral data and X-ray diffraction analysis. Primary bioassays showed that **2** had medium cytotoxic activity against HEp-2 and HepG2 Cells, and **1** exhibited weak activity.

Keywords: Fungus; (−)-1-Hydroxybyssochlamic acid; X-ray diffraction; Cytotoxic

1. Introduction

The natural products known collectively as nonadrides comprise a small structural class in which the core unit is a nine-membered carbocyclic ring. Two five-membered anhydrides or an anhydride and a lactol are fused to the core, which also bears a pair of n-alkyl chains [1]. Various pharmacological activities have been reported for compounds belonging to the class [2–6]. Because of the specific structure and bioactivities, they have attracted many research groups to their synthesis [7–11].

As a part of our studies on the metabolites of mangrove fungi from the South China Sea coast [12–17], we examined the chemical constituents of mangrove fungus (strain No. k38). A new nonadride derivative, named (−)-1-hydroxybyssochlamic acid (**1**), was isolated from the fungus. Compound **2**, which is the same as (−)-byssochlamic acid known as a synthetic product, whose specific rotation is contrary to the natural (+)-byssochlamic acid [19], were also found. Their cytotoxic effects against HEp-2 cells and HepG2 cells were measured.

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2. Results and discussion

(−)-1-Hydroxybyssochlamic acid (**1**) was isolated as a colourless block (10 mg) (figure 1). The molecular formula $C_{18}H_{20}O_7$ was established by elemental analysis and FAB-MS, which was larger than **2** by one oxygen atom. The ^{13}C NMR spectrum (table 1) showed four carbonyl carbon signals at δ 164.59, 165.20, 165.31, and 165.50, four olefinic carbon signals at δ 144.04, 144.22, 144.35, and 144.56. The nine unsaturation equivalents required by the molecular formula suggested that the compound has three rings. Compound **1** showed similar anhydride absorption (1854 and 1775 cm^{-1}) in the IR spectrum. However, the hydroxyl absorption (3517 cm^{-1}) of **1** is absent in **2**. The UV spectrum of **1** had a maximum absorption at 249 nm ($\log \Sigma = 3.89$). The NMR spectra of **1** and **2** are very similar. But the ^{13}C NMR spectrum of **1** has one methine more and one methylene less than **2**; the ^1H NMR spectrum of **1** has one signal at δ 4.97 that was missing in **2**, which is the signal of the proton at bearing-oxygen carbon. This presumed **1** to be a hydroxyl substituted byssochlamic acid.

The assignments of protons attached to their corresponding carbons were readily accomplished by the HMQC technique. The construction of the molecular framework was based on the analysis of $^1H-^1H$ COSY and HMBC experiments (table 1). In the ^1H NMR spectrum of **1**, two signals of a three-proton triplet at δ 0.97 and 1.14 suggested the presence of two alkyl groups. An ethyl side chain was revealed by the contiguous correlations from H-1′ to H-2′ in $^1H-^1H$ COSY and correlations from H-2′ to C-1 and C-2 in HMBC. The correlations from H-3′ to H-4′ and H-4′ to H-5′ in $^1H-^1H$ COSY, and correlations from H-6 to C-7′ and C-5 in HMBC showed the presence of a propyl side chain. While the positions of two five-membered anhydrides were determined by the HMBC correlations from H-3 to C-4 and C-10, from H-6 to C-5 and C-11, and from H-1 to C-9 and C-13, and between H-2 and C-9, respectively (figure 2). Additionally the position of hydroxyl was determined by the HMBC correlations from H-3a and H-2′ to C-1, and between H-1 and C-9. Thus the overall structure of **1** was unambiguously established (table 1 and figure 1).

This structure was also confirmed by X-ray diffraction analysis (figure 3). The relative configuration is 1S*, 2S*, 7R*. The molecule adopts a U-shaped conformation similar to that of the bis-*p*-bromophenylhydrazide of byssochlamic acid. It is interesting that the U-shaped

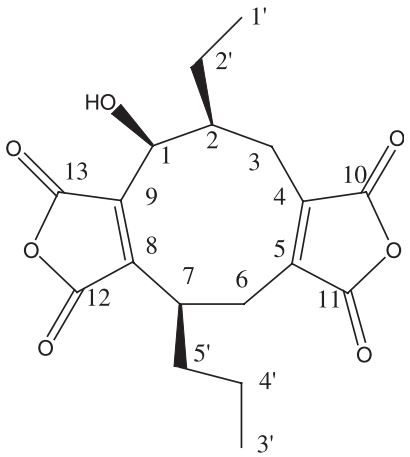

Figure 1. The structure of **1**.

Table 1. ^{1}H NMR and ^{13}C NMR data of **1** (δ in ppm, J in Hz).

	^{1}H NMR	*^{13}C NMR (DEPT)*	*^{1}H–^{1}H COSY*	*HMBC*
1′	1.14 (t), $J = 7$ Hz	11.8 (CH_3)	H-2′	C-2′, 2
2′	1.64, 1.70 (overlap)	28.4 (CH_2)	H-1′, 2	C-1′, 1, 2
3′	0.97 (t), $J = 7$ Hz	13.9 (CH_3)	H-4′a, 4′b	C-4′, 5′
4′	a 1.53 (qd, $J = 7{,}14$ Hz)	21.0 (CH_2)	4′aH-4′b, 3′,5′	C-3′, 5′, 7
	b 1.35 (qd, $J = 7{,}14$ Hz)		4′bH-4′a, 3′,5′	
5′	1.78 (m)	37.7 (CH_2)	H-4′a, 4′b, 7	C-3′, 4′, 6, 7
1	4.97 (d), $J = 5$ Hz	67.9 (CH)	H-OH	C-2′, 3, 9, 13
2	1.79 (overlap)	43.7 (CH)	H-3a, 3b, 2′	C-3, 9
3	a,2.50 (qd, $J = 13{,}1.5$ Hz)	22.3 (CH_2)	3aH-3b, 2	C-1, 2, 2′, 4, 10
	b,2.82 (bd, $J = 13$ Hz)		3bH-3a, 2	
4		144.2 (C)		
5		144.0 (C)		
6	a, 2.85 (dd, $J = 11$, 4 Hz)	28.0 (CH_2)	6aH-6b, 7	C-5, 7, 11
	b, 3.32 (bd, $J = 11$ Hz)		6bH-6a, 7	
7	3.36 (m)	35.3 (CH)	H-5′,6a, 6b	
8		144.4 (C)		
9		144.6 (C)		
10		165.5 (C)		
11		164.6 (C)		
12		165.2 (C)		
13		165.3 (C)		
—OH	2.50 (d, $J = 5$ Hz)		H-1	

conformation is quite different in shape from a chairlike conformation predicted by computing on a PM3 algorithmc for byssochlamic acid [9]. The atoms of the nine-membered ring are not co-planar obviously and the five-membered anhydrides are planar. The bond lengths of C—C, C═C, C—O, C═O, and ═C—C etc. are consistent with the normal values. The molecular packing in the crystal is determined by ordinary van-der-Waals interactions. CCDC 284556 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif or the author.

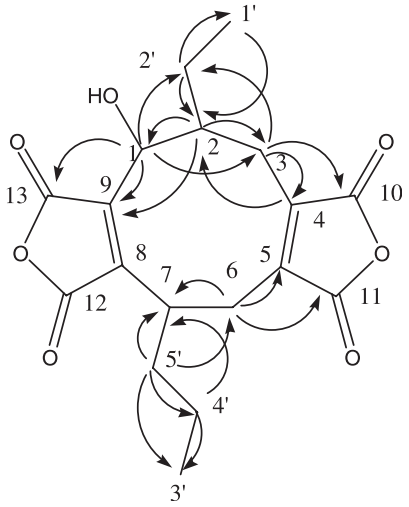

Figure 2. The Key HMBC correlations of **1**.

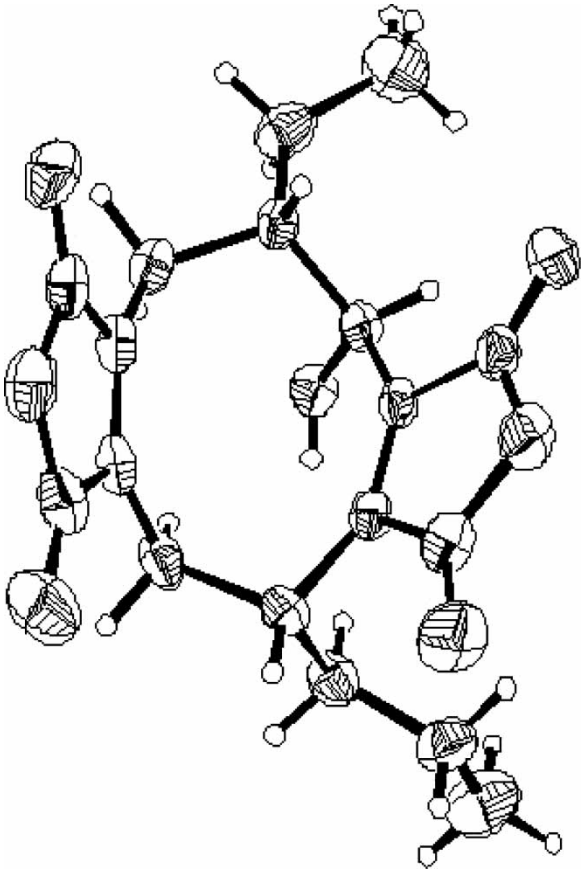

Figure 3. The X-ray crystallgraphic structure of **1**.

Primary bioassays showed that **2** has medium cytotoxic effect against HEp-2 and HepG2 cells, and **1** exhibited weak activity (see table 2).

Compound **2** is the enantio isomer of natural (+)-byssochlamic acid. It was a product synthesised by James D. White [9], but was first isolated from nature by us.

3. Experimental

3.1 General experimental procedures

Melting points were detected on a Fisher–Johns hot-stage apparatus and are uncorrected. NMR data were recorded on a Varian Inova-500 NB spectrometer, using $CDCl_3$ as solvent and TMS as internal standard. Mass spectra were acquired on a VG-ZAB mass spectrometer,

Table 2. 50% Growth inhibition (IC_{50}) values of **1** and **2** (μg/ml).

Compound	*IC_{50} of HEp-2 cells*	*IC_{50} of HepG2 cells*
1	>50	>50
2	37	35

IR spectra were obtained on a Nicolet 5DX-FTIR spectrophotometer, and UV spectra were measured on a Shimadzu UV-240 spectrophotometer. Elemental analysis was carried out on an Elementar Vario EL CHNS-O spectrometer. X-ray structure analysis was performed on a Bruker SMART 1000 CCD diffractometer (Mo Kα-radiation, graphite monochromator). Chromatography was carried out on silica gel column (200–300 mesh; Qingdao haiyang chemicals).

3.2 Fungus material

A strain of the fungus k38 was isolated from the South China Sea coast. It is apospory and its general species has not been identified. Starter cultures were maintained on cornmeal seawater agar. Plugs of agar supporting mycelial growth were cut and transferred aseptically to a 250-ml Erlenmeyer flask containing 100 ml of liquid medium (glucose 10 g/L, Peptone 2 g/L, yeast extract 1 g/L, NaCl 30 g/L). The flask was incubated at 30°C on a rotary shaker for 5–7 days. The mycelium was aseptically transferred to 500-ml Erlenmeyer flasks containing culture liquid (200 ml). The flasks were then incubated at 30°C for 25 days.

3.3 Extraction and isolation

The cultures (100 L) were filtered through cheesecloth. The filtrate was concentrated to 5 L *in vacuo* below 50°C and extracted five times by shaking with an equal volume of ethyl acetate. The combined extracts were chromatographed repeatedly on silica gel column using gradient elution from petroleum to ethyl acetate to obtain (−)-1-hydroxybyssochlamic acid (**1**) from the ethyl acetate/petroleum ether (15:85) fraction and (−)-byssochlamic acid (**2**) from the ethyl acetate/petroleum ether (10:90).

3.3.1 (−)-1-Hydroxybyssochlamic acid (1). Colourless blocks (10 mg), mp ($CHCl_3$) 162–163°C, $[\alpha]_D^{20}$ −53, UV (EtOH) λ_{max} 249 nm, IR bands (KBr) λ_{max} (cm^{-1}): 3517(OH), 2965, 2933, 2875, 1854 (O—C═O), 1775 (O—C═O), 1261, 924, FAB-MS *m/z*, 349 $[M + 1]^+$, 331. Elemental anal. C 62.12%, H 5.68%, N 0. calcd for $C_{18}H_{20}O_7$, C 62.07%, H 5.75%, N 0. ^1H NMR and ^{13}C NMR (500 MHz $CDCl_3$) data: see table 1.

3.3.2 (−)-Byssochlamic acid (2). Colourless blocks (20 mg). mp ($CHCl_3$) 164–165°C, $[\alpha]_D^{20}$ −104, IR bands (neat) 2966, 2934, 1829 (O—C═O), 1766 (O—C═O), 1260, 927 cm^{-1}; Elemental anal. C, 65.09%, H 6.03%, N, 0. calcd. for $C_{18}H_{20}O_6$: C 65.06%, H 6.02%, N 0.^1H NMR (300 MHz, $CDCl_3$) δ 0.96 (3H, t, *J* 7 Hz), 1.12 (3H, t, *J* 7 Hz), 1.28–1.50 (2H, m), 1.50–1.75 (4H, m),1.90 (1H, m), 2.25–2.43 (2H, m), 2.65 (1H, m), 2.72 (1H, dd, *J* 2,14 Hz), 2.77–2.98 (2H, m), 3.41 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$) 11.6 (—CH_3), 13.7 (—CH_3), 20.6 (—CH_2), 28.1 (—CH_2), 29.2 (—CH_2), 29.7 (—CH_2), 30.0 (—CH_2), 34.7 (—CH_2), 36.0 (—CH), 40.4 (—CH), 143.2 (—C═C), 143.4 (—C═C),144.1 (—C═C), 144.7 (—C═C), 164.6 (O—C═O), 165.4 (O—C═O), 164.9 (O—C═O),165.7 (O—C═O).

3.4 X-ray structure analysis

A transparent single crystal obtained from ethanol solution was measured on a Bruker SMART 1000 CCD diffractometer (Mo Kα-radiation, graphite monochromator). Crystal data of 1: $C_{18}H_{20}O_7$ monoclinic system, space group P2 1, $a = 10.373(4)$ Å, $b = 14.732(5)$ Å, $c = 22.872(9)$ Å, $\alpha = 90°$, $\beta = 97.310(6)°$, $\gamma = 90°$. $V = 3467(2)$ Å3, $Z = 8$, Dcalc = 1.335 g/cm^3, $M_r = 348.34$, F(000) = 1472, $\mu = 0.103\,mm^{-1}$, $-13 \le h \le 13$; $-15 \le k \le 18$; $-24 \le l \le 29$, 21067 data collected, 13608 independent data [R(int) = 0.0270]. Reflections observed (>2sigma) were 7403, Final R indices [I > 2σ(I)], R1 = 0.0470, wR2 = 0.1028.

3.5 Bioassays for cytotoxic activity

The cytotoxic assays were performed using the MTT assay method [18]. The HEp-2 cells and HepG2 cells were cultured in RPMI 1640 medium (Nissui) supplemented with 5% heat-inactivated fetal bovine serum (FBS) and kanamycin (5.3 ml/L) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. The cell suspension (100 ml) was added to each well of a 96-microwell plate and incubated for 24 h. Test compounds were dissolved in DMSO in various concentrations (100, 50, 25, 12.5, 6.25 mg/ml) and 10 μl of the test solution or DMSO (control) was added to each well. The plate was kept in an incubator for 48 h. After terminating cell culture by adding 20 ml MTT (5% in PBS) to each well, the plate was further incubated for 4 h. To each well, 100 ml of 10% SDS/0.01 N HCl was added. The plate was read on a microplate reader (MPR A4i, Tosoh) at 492 nm. A dose–response curve was plotted for each compound and the concentrations giving 50% inhibition of cell growth (IC_{50}) were recorded. Primary bioassays showed that **2** has medium cytotoxic effect, and **1** exhibited weak cytotoxicity against HEp-2 and HepG2 cells (see table 2).

Acknowledgements

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