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ISOLATION AND CRYSTAL STRUCTURE OF (–)-BYSSOCHLAMIC ACID FROM MANGROVE FUNGUS (STRAIN NO. K38)

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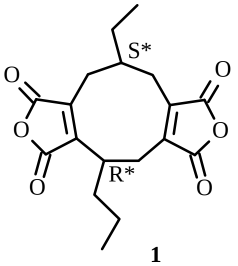
*(–)-Byssochlamic acid (**1**) was isolated from mangrove fungus (strain No. k38) from the South China Sea coast. This is the first time that **1** was found in nature. Its structure was confirmed by spectra data and X-ray diffraction analysis.*

Key words: marine, fungus, mangrove, Byssochlamic acid, x-ray structure analysis.

The marine mangrove fungus has attracted the attention of many researchers due to its importance in ecology and ability to produce various bioactive metabolites [1–8]. As part of our studies on the metabolites of mangrove fungi from the South China Sea coast, we examined the chemical constituents of mangrove fungus (strain No. k38). (–)-Byssochlamic acid (**1**), a structurally rare compound, was isolated from the fungus.



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(+)-Byssochlamic acid is one of the compounds known as nonadrides that comprises a small structure class, in which the core unit is a nine-membered carbocylic ring. It was first isolated from ascomycete byssochlamys fulva [9]. The structure of byssochlamic acid was solved using chemical transformations and spectral data, etc. [10–12]. However, its crystal structure has not yet been investigated. Erenow, (–)-byssochlamic acid (**1**), has not been isolated in nature and is a synthetic product [13]. We first isolated **1** in nature and elucidated its molecular and crystal structure by spectral data and x-ray diffraction analysis.

Culture of fungus k38 isolated from the South China Sea coast was filtered through cheesecloth; the filtrate was concentrated below 50°C and extracted with ethyl acetate. Repeated chromatography afforded (–)-byssochlamic acid as colorless blocks (20 mg).

^{1}H NMR ($CDCl_3$, TMS) and ^{13}C NMR ($CDCl_3$) data are shown in Table 1. Its spectral data and physical properties were consistent with that of (+)-byssochlamic acid reported in the literature except for the specific rotatory power [14]. The structure of **1** was finally confirmed by X-ray diffraction analysis, which showed the relative configuration of the compound to be (R*, S*).

Figure 1 shows the structure of **1** according to the XSA. It can be seen that the core unit is a nine-membered carbocyclic ring.

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TABLE 1. NMR Data of **1** ($CDCl_3$, δ, ppm, J/Hz)

δC (DEPT)	δH
11.9 (CH_3)	0.96 (3H, t, J = 7)
14.1 (CH_3)	1.11 (3H, t, J = 7)
21.0 (CH_2)	1.28-1.50 (2H, m)
28.4 (CH_2)	1.50-1.76 (4H, m)
29.6 (CH_2)	1.91 (1H, m)
29.7 (CH_2)	2.27-2.40 (2H, m)
30.4 (CH_2)	2.63 (1H, m)
35.1 (CH)	2.70 (1H, dd, J = 2.14)
36.5 (CH_2)	2.75-2.97 (2H, m)
40.1 (CH)	3.41 (1H, m)
143.3 (C)	
143.6 (C)	
144.3 (C)	
144.8 (C)	
165.0 (C)	
165.1 (C)	
165.4 (C)	
165.6 (C)	

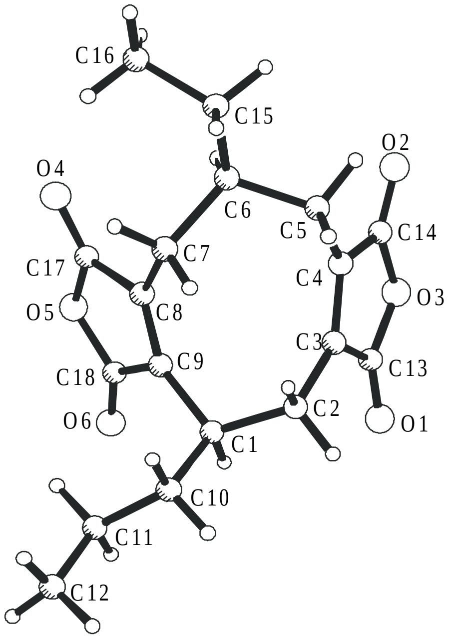

Fig. 1. Structure of **1**.

Two five-membered anhydrides are fused to the core, which also bears a pair of *n*-alkyl chains. The molecule adopts a U-shaped conformation similar to that of the bis-*p*-bromophenylhydrazide of byssochlamic acid [14]. It is interesting that they are quite different in shape from a chairlike conformation predicted by computing using the PM3 algorithm [13]. The atoms C (3), C (4), (13),C (14), and O (3) are planar within ±0.0082 Å, and the remaining O (2) and O (1) atoms are considered on the plane and have maximum deviations of 0.0399 and –0.0422 Å, respectively. Another five-membered ring has similar arrangements. The angle between the two rings is 109.6°C, and the nine-membered ring is not coplanar obviously. The bond lengths of C–C, C=C, C-O, C=O, =C–C, etc. are consistent with the normal values reported in the literature [15–16]. Since **1** has no free hydroxyls, the molecular packing in the crystal is determined by ordinary van der Waals interactions.

EXPERIMENTAL

M.p.: uncorrected. ^{1}H, ^{13}C, DEPT, NMR: Varian Inova300 NB spectrometer, in ppm, J/Hz. MS: VG-ZAB-HS mass spectrometer.

Fungus Material and Purification. A strain of the fungus k38 was isolated from the South China Sea coast. It is aposporous 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 10g/L, Peptone 2 g/L, yeast extract 1g/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. The cultures (200 L) were then 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 using gradient elution from petroleum to ethyl acetate to obtain (–)-byssochlamic acid from the ethyl acetate–petroleum ether (10:90) fraction. mp 164–165°C; $[\alpha]_D^{23}$ –104° (*c* 0.1, $CHCl_3$).

IR spectrum (KBr, ν, cm^{-1}): 2966, 2934, 1829 (C=O), 1766 (C=O), 1260, 927 cm^{-1}.

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 for the title compound: Triclinic system, space group: P1, a = 7.805 (3) Å, b = 10.705 (5) Å, c = 11.805 (5) Å, α = 95.846°, β = 97.179°, γ = 102.115°, V = 854.4 (6) Å^3, Z = 1, D_{calc} = 1.292 g/cm^3, F (000) = 352, μ = 0.097 cm^{-1}. 7301 reflections were collected at room temperature with $0.977° \le \theta \le 27.04°$. There were 3671 unique reflections (R(int) = 0.0159) and 2555 reflections were observed. The data were corrected for Lp effects, semi-empirical method, and the crystal habit. All the data were applied for the structure determination and refinement. The structure was solved by direct methods using the program SHELXS-97 and refined with anisotropic displacement parameters and full-matrix least-squares refinement with 438 refined parameters converging smoothly at R = 0.0457 and wR = 0.1240 for 2555 reflections with $I > 2\sigma (I)$, $w = 1/[\sigma^2(Fo^2) + (0.0536 * P)^2]$, $P = (Max (Fo^2, 0) + 2 * Fc^2)/3$; goodness of fit: 1.042. The highest peak and deepest hole in the final difference Fourier map were 0.310 and –0.154 e. Å^{-3}, respectively. H atoms were fixed geometrically as rider.

The coordinates of nonhydrogen atoms, cell constants, atomic coordinates, and bond lengths and angles were deposited in the Cambridge Crystallographic Database (reference No. CCDC 277564). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road Cambridge CB21 EZ, UK.

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