

Two new metabolites of a marine endophytic fungus (No. 1893) from an estuarine mangrove on the South China Sea coast

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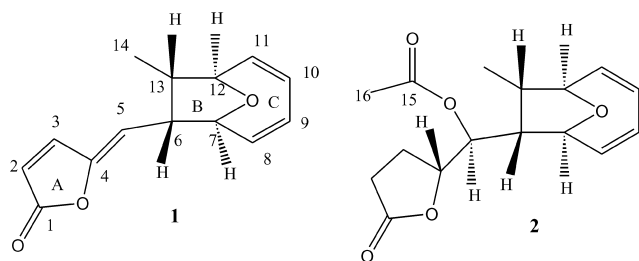
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Abstract—The endophytic fungus No. 1893 was isolated from the dropper of *Kandelia candel* from an estuarine mangrove on the South China Sea coast. The ethyl acetate extract of the fermentation broth of this fungus strain exhibited cytotoxicity toward NCI4460 and Bel-7402, and high activities against *Heliothis armigera* (Hühner) and *Sinergasilus spp.* Two new lactones, 1893A (**1**) and B (**2**), together with 5-(*p*-hydroxybenzyl)hydantoin and two cyclodipeptides, cyclo-(Ser-Leu) and cyclo-(Phe-Gly), were isolated from the extract. Their structures were determined by spectroscopic experiments, including X-ray diffraction. © 2003 Elsevier Science Ltd. All rights reserved.

1. Introduction

In the course of our ongoing search for potent antitumor natural products from marine mangrove fungi,^{1–3} the extract of the endophytic fungus No. 1893 was discovered to exhibit cytotoxicity toward NCI4460 and Bel-7402, and high activities against *Heliothis armigera* (Hühner) and *Sinergasilus spp.*⁴ This endophytic fungus strain was collected from the dropper of *Kandelia candel* from an estuarine mangrove on the South China Sea coast. This species is unidentified. We have isolated two new lactones, 1893A (**1**) and 1893B (**2**), together with 5-(*p*-hydroxybenzyl)hydantoin and two cyclodipeptides, cyclo-(Ser-Leu) and cyclo-(Phe-Gly), from the fermentation broth of this fungus.



A 200 L fermentation broth was concentrated and extracted with ethyl acetate. The extract was repeatedly chromatographed on silica gel. Compound **1** was obtained as

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colorless needle-shaped crystals, mp 95–96°C, $[\alpha]_D^{20} = +4.0$ (*c* 0.07, acetone) and determined to have the molecular formula $C_{14}H_{14}O_3$ by HREI-MS data (*m/z* 230.0941, calcd. 230.0943). The number of hydrogen and carbon atoms observed in the ¹H and ¹³C NMR spectra are

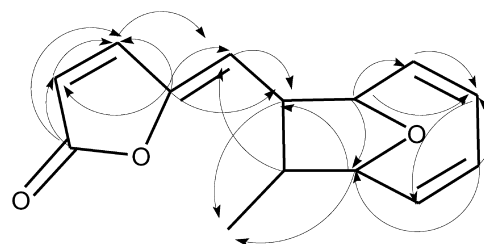


Figure 1. The selective correlations of the HMBC of 1893A.

Table 1. NMR data for 1893A (**1**) (CD₃COCD₃, TMS)

No.	¹³ C	¹ H	COSY	HMBC
1	170.1 (C)			H-2,3
2	120.1 (CH)	6.28 (d, 5.5 Hz)	H-3	H-3
3	145.2 (CH)	7.74 (d, 5.5 Hz)	H-2	H-2,5
4	150.9 (C)			H-2,3,5,6
5	115.4 (CH)	5.54 (d, 11 Hz)	H-6	H-6,7
6	54.9 (CH)	3.71 (ddd, 3.5, 7, 11 Hz)	H-5,7,13	H-14
7	83.7 (CH)	4.43 (dd, 3.5, 5.5 Hz)	H-6,8,9	H-5,8,9,12
8	138.1 (CH)	6.18 (dd, 5.5, 8.5 Hz)	H-7,9	H-9
9	126.1 (CH)	5.88 (dd, 7, 8.5 Hz)	H-8	H-7,8,11
10	125.7 (CH)	5.89 (dd, 7, 8.5 Hz)	H-11	H-9,12
11	138.9 (CH)	6.23 (dd, 5, 8.5 Hz)	H-10,12	H-10
12	85.4 (CH)	4.25 (dd, 3.5, 5 Hz)	H-11,13	H-6,7,10,11,14
13	52.5 (CH)	2.83 (ddq, 3.5, 7, 7 Hz)	H-6,12,14	H-5,14
14	15.5 (CH ₃)	1.02 (d, 7 Hz)	H-13	H-12

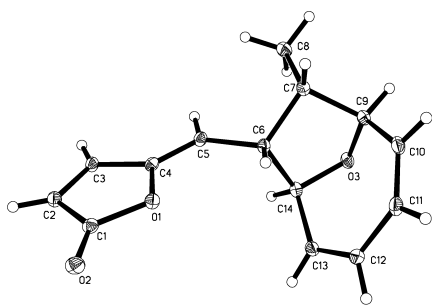


Figure 2. Molecular structure of 1893A (1).

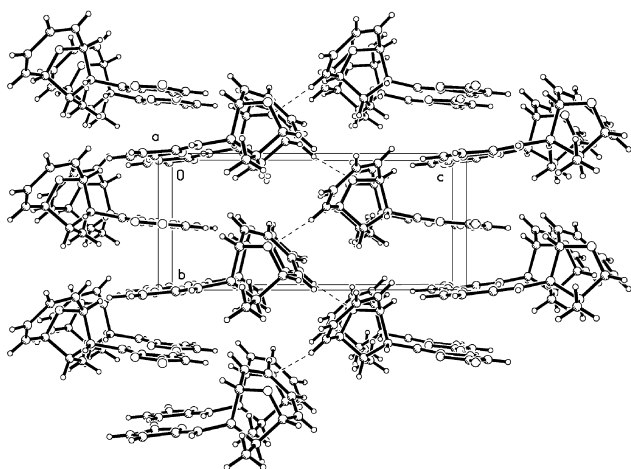


Figure 3. The Molecular stack of 1893A (1).

in agreement with this molecular formula. The IR spectrum of **1** showed the presence of one carbonyl group with band at 1776 cm^{-1} . In the ^{13}C NMR spectrum, one carbonyl carbon signal (δ 170.1), eight olefinic carbon signals (δ 150.9, 145.2, 138.9, 138.1, 126.1, 125.7, 120.1, 115.4), and two methine bearing oxygen signals (85.4, 83.7), two methines signals (δ 54.9, 52.5) and one methyl group (δ 15.5) were observed. The eight unsaturation equivalents required by the molecular formula indicated that the compound has three rings. The partial structure of rings A and B in **1** was

revealed by the COSY spectrum that showed a contiguous sequence of coupled signals from H-5 to H-14. The HMBC data established the overall structure of **1** (Fig. 1 and Table 1), especially the multiple correlations between H-2, H-3, H-5, H-6 and C-4 defined that the connection points between rings A and B are C-4 and C-6. Similarly the correlation between H-7 and C-12 defined that the connection points between rings B and C are C-7 and C-12. The geometries of C-2, C-8, C-10 were proved to be all *Z* by the coupling constants of $J_{2,3}=5.5\text{ Hz}$, $J_{8,9}=8.5\text{ Hz}$ and $J_{10,11}=8.5\text{ Hz}$, respectively. The structure of **1** was finally confirmed by X-ray diffraction analysis that showed the relative configuration of the compound to be (6*S**, 7*R**, 12*S**, 13*S**) (Fig. 2). It was found from the X-ray diffraction analysis that a weak hydrogen bond was formed between the oxygen of the B-ring and the hydrogen at C-11 of another molecule (Fig. 3).⁵

Compound 1893B (**2**) was obtained as optically active ($[\alpha]_{\text{D}}^{20}=\pm 10.8$) colorless crystals and its molecular formula of $\text{C}_{16}\text{H}_{20}\text{O}_5$ was determined by HREI-MS (m/z 292.1305, $\text{C}_{16}\text{H}_{20}\text{O}_5$ calcd. 292.1311). The ^1H and ^{13}C NMR data obtained for **2** were very similar to those of **1** (Tables 1 and 2), suggesting that the two molecules were closely related. Differences in the ^1H NMR spectrum of **2** compared to that of **1** included the absence of three olefinic hydrogen ($=\text{CH}$) resonances at 7.74, 6.28 and 5.54 ppm, and the appearance of a series of correlated saturated resonances (δ 2.50, 2.43, 2.28, 2.06, 4.57 and 5.15) and one acyl methyl signal (δ 2.11). The differences in the ^{13}C NMR spectra of **1** and **2** are similar to those observed in the ^1H NMR spectra. Taken together, the NMR and MS evidence suggested that double bonds (C₂₋₃, C₄₋₅) in **1** are replaced by saturated bonds (C₂₋₃, C₄₋₅) in **2**, and one additional acetyl group is connected to C-5 in **2**. According to the coupling constants of H-4 and H-5 ($J_{4,5}=2.5\text{ Hz}$) and H-5 and H-6 ($J_{5,6}=10.5\text{ Hz}$), the relative configuration of **2** can be tentatively determined as 4*R**, 5*S**, 6*S**, 7*R**, 12*S**, 13*S**. However, this must be confirmed through further experiments.

The ROESY spectrum of **2** provided little information. Only the correlations between H-4 and H-3b, H-4 and H-5 were observed.

Table 2. NMR Data of 1893B (2) (CDCl_3 , TMS)

No.	^{13}C	^1H	COSY	HMBC
1	176.2 (C)			H-2,3,4
2	28.1 (CH_2)	(a) 2.43 (ddd, 6, 10, 18 Hz) (b) 2.50 (ddd, 8, 10, 18 Hz)	H-3a,3b	H-3a
3	23.9 (CH_2)	(a) 2.24~2.32 (m) (b) 2.02~2.08 (m) H-2,3a	H-2,3b	H-2
4	80.1 (CH)	4.57 (ddd, 2.5, 7, 8 Hz)	H-3,5	H-2,3b,6
5	72.6 (CH)	5.15 (dd, 2.5, 10.5 Hz)	H-4,6	H-3,4,6,7
6	51.5 (CH)	2.98 (ddd, 6, 7, 10.5 Hz)	H-5,7,13	H-5,7,8,12,13,14
7	76.7 (CH)	4.42 (t, 6, 6 Hz)	H-8,6	H-6,8,9,12,13
8	137.5 (CH)	6.24 (dd, 6, 11 Hz)	H-7,9	H-6,7,9
9	126.5 (CH)	5.94 (dd, 5.5, 11 Hz)	H-8	H-7,10,11
10	125.0 (CH)	6.05 (dd, 5.5, 10.5 Hz)	H-11, 12	H-12
11	136.9 (CH)	5.90 (dd, 4.5, 10.5 Hz)	H-10	H-9,12,13
12	86.2 (CH)	4.23 (d, 4.5 Hz)	H-11,13	H-7,10,11,14
13	52.3 (CH)	2.72 (ddq, 4.5, 7, 7 Hz)	H-6,14,12	H-5,6,7,12,14
14	14.4 (CH_3)	0.98 (d, 7 Hz)	H-13	H-6,12,13
15	170.5 (C)			H-5,16
16	20.9 (CH_3)	2.11 (s)		

Through comparing our data with that for mycoepoxydiene, which was isolated from a land fungi by Cai,⁶ we conclude that 1893A and B are probably rearranged metabolites.

2. Experimental

2.1. General

NMR data were recorded on a Varian Inova-500 NMR spectrometer, mass spectra on a VG-ZAB mass spectrometer and a VG Autospec-500 mass spectrometer, IR spectra on a Nicolet 5DX-FTIR spectrophotometer, UV spectra on a Shimadzu UV-240 spectrophotometer, optical rotations on a Horiba High Sensitivity Polarimeter SEPA-300, and X-ray data on a Bruker Smart 1000 CCD system diffractometer.

2.2. Fungal strain

A strain of fungus (No. 1893) was isolated from the dropper of *Kandelia candel* from an estuarine mangrove on the South China Sea, and has been deposited in the Department of Applied Chemistry, Zhongshan University, Guangzhou, P.R. China.

2.3. Culture conditions

Starter cultures (from Professor E. B. Gareth Jones and Dr L. L. P. Vrijmoed) 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 GYT (glucose 5 g/L, peptone 1 g/L, yeast extract 0.5 g/L, beef extract 0.5 g/L, NaCl 3 g/L). The flask was incubated at 30°C on a rotary shaker for 5–7 days, and the mycelium was aseptically transferred to a 500 mL Erlenmeyer flask containing culture liquid (200 mL). The flask was then incubated at 30°C for 40 days.

2.4. Extraction and separation of metabolites

The cultures (200 L) were filtered through cheesecloth. The filtrate was concentrated to 5 L in vacuo below 50°C and extracted three times by shaking with an equal volume of ethyl acetate. The combined organic extracts were applied to a silica gel column, eluting with a gradient of petroleum ether to ethyl acetate to offer compounds **1** (25 mg), **2** (20 mg), 5-(*p*-hydroxybenzyl)-hydantoin (25 mg), cyclo-(Ser-Leu) (50 mg) and cyclo-(Phe-Gly) (30 mg).

2.4.1. 1893A (1). Colorless needle-shaped crystals, mp 95–96°C, $[\alpha]_D^{20}=+3.97$ (*c* 0.07, acetone), IR (KBr) 3028, 2966, 1766, 1753, 1673, 1557, 1456, 1372, 1132, 917, 878, 691 cm^{-1} ; UV: λ_{max} (MeOH) 278 nm ($\log \epsilon$ 4.7); ¹H NMR (CD₃COCD₃, TMS), ¹³C NMR and 2D NMR, see Table 1; HREIMS *m/z* [M]⁺ 230.0941, C₁₄H₁₄O₃, requires 230.0943. EIMS *m/z* 230 [M]⁺, 215, 201, 185, 135.

2.4.2. 1893B (2). Colorless crystals, mp 140–142°C, $[\alpha]_D^{20}=+10.8$ (*c* 0.02, acetone); IR (KBr) 2925, 1774, 1731, 1459, 1344, 1241, 1194, 1020, 961, 695 cm^{-1} ; UV: λ_{max} (MeOH) 274 nm ($\log \epsilon$ 4.3); for ¹H NMR (CDCl₃,

TMS), ¹³C NMR and 2D NMR, see Table 2; HREIMS *m/z* [M]⁺ 292.1305, C₁₆H₂₀O₅, requires 292.1311. EIMS *m/z* 292 [M]⁺, 232[M–CH₃COOH]⁺, 217, 203, 176, 157, 135.

2.5. X-Ray crystallographic data of 1893A

Crystal system, space group monoclinic, *P*2(1); Unit cell dimensions *a*=6.5948(13) Å, α =90°; *b*=6.1502(12) Å, β =91.47(3)°; *c*=13.855(3) Å, γ =90°; Volume=561.78(19) Å³, *Z*=2, *D*_{calcd}=1.361 mg/m³, *m*=0.095 mm⁻¹, *F*(000)=244. All single-crystal data was collected using the hemisphere technique on a Bruker SMART 1000 CCD system diffractometer with graphite-monochromated Mo K α radiation λ =0.71073 at 293(2) K. The structures were solved by direct methods using SHELXTLV5.0 (Siemens Industrial Automation Inc. Madison, WI) and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were applied with the Siemens Area Detector Absorption Program (SADABS). The final value of *R* was 0.0342, *wR*₂=0.0945 [*I*>2 σ (*I*)].

Acknowledgements

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