Sumalactones A–D, four new curvularin-type macrolides from a marine deep sea fungus *Penicillium Sumatrense*†

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Sumalactones A–D (1–4), four new curvularin-type macrolides, together with two known analogues, curvularin (5) and dehydrocurvularin (6), were isolated from *Penicillium Sumatrense*, a marine fungus isolated from deep-sea sediments. Sumalactones C (3) and D (4) are unprecedented curvularin-type macrolides bearing a rare 11-membered macrolide skeleton. Their structures were elucidated on the basis of intensive spectroscopic analysis. The absolute configurations of compounds 1–4 were determined by CD spectra and modified Mosher’s method. Compound 6 showed significant inhibition activity towards LPS-induced nitric oxide production in RAW 264.7 macrophages with IC₅₀ value of 0.91 μM.

**Introduction**

Among fungal macrolides, resorcinylid acid lactones (RALs) and dihydroxyphenylactic acid lactones (DHALs) belong to a unique family of naturally occurring homologous macrolides, which are characterized by possessing a macrocyclic core structure fused to a resorcinol aromatic ring.¹ Curvulins, featuring a substituted resorcinol fragment fused to the β, γ-positions of the macrocyclic lactone ring, are produced by a number of fungal species mainly from the genera *Aspergillus*,² *Alternaria*,³ *Astragalus*,⁴ *Curvularia*,⁵ *Cochliobolus*,⁶ and *Penicillium*⁷ with diverse biological activities. They are biogenetically derived from the polyketide synthase pathways in bacterial and fungi, and have brought great interest and challenges for total synthesis and biosynthesis studies.⁸

In the course of our ongoing research on new bioactive secondary metabolites from marine fungi,⁹ *Penicillium sumatrense* was isolated from a deep-sea sediment sample (−2500 m depth) of the Indian Ocean, which resulted in the isolation of four new curvularin derivatives, sumalactones A–D (1–4) with 10- or 11- or 12-membered macrolide skeletons, as well as two known compounds curvularin (5)¹⁰ and dehydrocurvularin (6).¹¹ Compounds 3 and 4 with 11-membered macrolides skeleton are considered rare in nature and haven’t been reported before. All the compounds were evaluated for their inhibitory effect on the production of nitric oxide (NO) induced by lipopolysaccharide (LPS) in RAW 264.7 macrophages. Herein we report the isolation, structure elucidation, and biological activities of these compounds (Fig. 1).

**Results and discussion**

Sumalactone A (1) gave an HRESIMS ion peak at *m/z* 307.1197 [M − H]⁺, corresponding to the molecular formula C₁₆H₂₀O₆, which required seven degrees of unsaturation. The UV spectrum showed absorption maxima at 204, 218, 269, and 297 nm. The ¹H signals (Table 1) suggested a pair of meta-coupled aromatic protons at δH 6.12 (d, *J* = 1.8 Hz, H-4), and 6.24 (d, *J* = 2.0 Hz, H-6) and one methyl group at δH 1.12 (3H, d, *J* = 6.2 Hz, CH₃-16).

Fig. 1 Chemical structures of 1–6.

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†† Electronic supplementary information (ESI) available: Spectroscopic data of 1–4, preparation of [S]- and [R]-MTPA esters of 1 and 3, and the 1D and 2D NMR spectra of 1–4. See DOI: 10.1039/c7ra06933b

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Analysis of $^{13}$C NMR and DEPT spectra data (Table 1) together with the HSQC data indicated the presence of six $sp^2$ quaternary carbons including one ketone carbonyl carbon ($\delta_{C} 171.8$) and one ester carbonyl carbon ($\delta_{C} 171.8$), two $sp^2$ methine carbons ($\delta_{C} 110.6$ and 102.4), two $sp^3$ oxygenated methine carbons ($\delta_{C} 76.3$ and 65.5), five $sp^3$ methylene carbons, and one methyl carbon ($\delta_{C} 23.9$). The $^1$H and $^{13}$C NMR data were nearly identical when reacted with R- and S-MTPA chloride, 1 gave the corresponding (S)- and (R)-MTPA esters, respectively. The observed chemical shift differences $\Delta\delta_{H(S-R)}$ (Fig. 3) clearly defined the S configuration at C-15. The absolute configuration of C-13 was determined by CD spectrum (Fig. 4). We suggest that 2 is epimeric at C-13 relative to compound 1. Consequently, the absolute configuration of 2 was assigned as 13S, 15S.

Cotton effect at 316 nm of 1 suggested the $R$ configuration at C-13, comparing to that of xestodecalactone A with opposite sign. Therefore, the absolute configuration of 1 was established as 13R, 15S.

Sumalactone B (2) was isolated as a yellow oil and it was determined to be C$_{16}$H$_{20}$O$_{6}$ on the basis of negative HRESIMS, indicating that 2 is isomeric to 1. Indeed, analysis of the NMR data indicated that compound 2 had the same planar structure as 1. Considering that the only difference observed between 2 and 1 was the completely opposite CD curves (Fig. 4), we suggest that 2 is epimeric at C-13 relative to compound 1. Consequently, the absolute configuration of 2 was assigned as 13S, 15S.

### Table 1 $^1$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) spectroscopic data of 1–4 in CD$_3$OD ($\delta$ in ppm, $J$ in Hz)

| No. | $\delta_{H}$ | $\delta_{C}$ | $\delta_{H}$ | $\delta_{C}$ | $\delta_{H}$ | $\delta_{C}$ | $\delta_{H}$ | $\delta_{C}$ |
|-----|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 1   | 171.8       | 172.7       | 172.4       | 172.5       | 172.4       | 172.5       | 172.4       | 172.5       |
| 2   | 41.3 $^a$   | 41.1 $^a$   | 41.1 $^a$   | 41.1 $^a$   | 41.1 $^a$   | 41.1 $^a$   | 41.1 $^a$   | 41.1 $^a$   |
| 3   | 136.3 $^a$  | 136.3 $^a$  | 136.3 $^a$  | 136.3 $^a$  | 136.3 $^a$  | 136.3 $^a$  | 136.3 $^a$  | 136.3 $^a$  |
| 4   | 110.6 $^a$  | 110.6 $^a$  | 110.6 $^a$  | 110.6 $^a$  | 110.6 $^a$  | 110.6 $^a$  | 110.6 $^a$  | 110.6 $^a$  |
| 5   | 161.0       | 161.0       | 161.0       | 161.0       | 161.0       | 161.0       | 161.0       | 161.0       |
| 6   | 102.4 $^a$  | 102.4 $^a$  | 102.4 $^a$  | 102.4 $^a$  | 102.4 $^a$  | 102.4 $^a$  | 102.4 $^a$  | 102.4 $^a$  |
| 7   | 158.8       | 158.8       | 158.8       | 158.8       | 158.8       | 158.8       | 158.8       | 158.8       |
| 8   | 122.6       | 122.6       | 122.6       | 122.6       | 122.6       | 122.6       | 122.6       | 122.6       |
| 9   | 211.6       | 211.6       | 211.6       | 211.6       | 211.6       | 211.6       | 211.6       | 211.6       |
| 10  | 46.7 $^a$   | 46.7 $^a$   | 46.7 $^a$   | 46.7 $^a$   | 46.7 $^a$   | 46.7 $^a$   | 46.7 $^a$   | 46.7 $^a$   |
| 11  | 23.4 $^a$   | 23.4 $^a$   | 23.4 $^a$   | 23.4 $^a$   | 23.4 $^a$   | 23.4 $^a$   | 23.4 $^a$   | 23.4 $^a$   |
| 12  | 35.4 $^a$   | 35.4 $^a$   | 35.4 $^a$   | 35.4 $^a$   | 35.4 $^a$   | 35.4 $^a$   | 35.4 $^a$   | 35.4 $^a$   |
| 13  | 76.3 $^a$   | 76.3 $^a$   | 76.3 $^a$   | 76.3 $^a$   | 76.3 $^a$   | 76.3 $^a$   | 76.3 $^a$   | 76.3 $^a$   |
| 14  | 45.3 $^a$   | 45.3 $^a$   | 45.3 $^a$   | 45.3 $^a$   | 45.3 $^a$   | 45.3 $^a$   | 45.3 $^a$   | 45.3 $^a$   |
| 15  | 65.5 $^a$   | 65.5 $^a$   | 65.5 $^a$   | 65.5 $^a$   | 65.5 $^a$   | 65.5 $^a$   | 65.5 $^a$   | 65.5 $^a$   |
| 16  | 23.9 $^a$   | 23.9 $^a$   | 23.9 $^a$   | 23.9 $^a$   | 23.9 $^a$   | 23.9 $^a$   | 23.9 $^a$   | 23.9 $^a$   |

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**Fig. 2** Key $^1$H–$^1$H COSY and HMBC correlations of 1 and 3.

**Fig. 3** $\Delta(\delta_{S} - \delta_{R})$ values (in ppm) for the MTPA esters of 1 and 3.

**Fig. 4** CD spectra of 1–4.
Sumalactone C (3) was obtained as a yellow oil, for which the molecular formula was assigned as C_{16}H_{20}O_{6} by HRESIMS, from a [M − H]− ion at 307.1191 (calc. 307.1182). The UV absorptions together with 1H and 13C NMR data indicated the curvularin-type macrolide skeleton similar to that of compound 1. The 1H-1H COSY correlations between H-13/H-14/H-15/H-16, and HMBC correlation from H-14 (δH 4.75) to C-1 (δC 172.4), confirmed the 11-membered macrolide in compound 3, which was rare in nature and was first example of oxygenation at C-14 in curvularin skeleton. The absolute configuration at C-14 was assigned to be R because of the almost identical CD spectrum to that of 1 (Fig. 4). The OH group at C-15 was determined as S by the modified Mosher’s method (Fig. 3). Therefore, the absolute configuration of 3 was established as 14R, 15S.

Sumalactone D (4) was attributed the molecular formula C_{16}H_{20}O_{6} from its HRESIMS at m/z 307.1014 ([M − H]− 307.1014, calc. 307.1182). On analysis of its 1H and 13C NMR spectra, similar features to that of 3 were evident, but with an opposite CD curve (Fig. 4). Therefore, the absolute configuration at C-14 was assigned as S. Thus, the absolute configuration of 4 was assigned as 14S, 15S.

In addition to compounds 1–4, the known curvularin (5)\(^\text{\textsuperscript{a}}\) and dehydrocurvularin (6)\(^\text{\textsuperscript{a}}\) were also isolated and identified from the fungal strain P. sumatrense MCCC 3A00612. All isolated compounds were tested for inhibitory activities against LPS-induced NO production in RAW 264.7 macrophages. As the results, only compound 6 showed significant NO production inhibitory activity with IC_{50} of 0.91 ± 0.03 μM, which was comparable to that of the positive control L-NMMA (IC_{50} of 41.91 ± 1.27 μM). The cell viability measured by the MTS assay showed that compound 6 had no significant cytotoxicity to the RAW 264.7 cells at the effective concentration for the inhibition of NO production.

**Experimental section**

**General experimental procedures**

Optical rotations were measured on an MCP 300 polarimeter, and UV spectra were measured on a U-2910 spectrometer. IR spectra were measured on an Affinity-1 FT-IR spectrometer. The CD spectra were recorded in MeOH using a Chirascan spectropolarimeter at room temperature. HRESIMS spectra were obtained on Waters Synapt G2 TOF mass spectrometer. The NMR data were acquired with a Bruker AV 500 NMR spectrometer using solvent signals (CD_{2}OD: δ_{t} 3.30/δ_{c} 49.0) as standards. Column chromatography (CC) was carried out on Sephadex LH-20 (Pharmacia, USA), and ODS (60–80 μm, YMC). TLC was performed on silica gel plate (SGF254, 0.2 mm, Merck, Germany). Analytical and semi-preparative HPLC were performed on an Agilent HPLC system equipped with a G1311B pump, a G1329B automated sample injector, a G1316A column compartment, and a G1315D diode array detector using a Phenomenex Kinetex C18 column (4.6 × 250 mm, 5 μm), a Waters T3 C18 column (4.6 × 250 mm, 5 μm) and a Phenomenex Kinetex C18 column (10.0 × 250 mm, 5 μm).

**Fungus material**

The fungus P. sumatrense MCCC 3A00612 was isolated from deep-sea sediments collected from the Indian Ocean. The strain was identified by Dr Zongze Shao, and a voucher specimen (P. sumatrense MCCC 3A00612) has been deposited in the Marine Culture Collection of China.

**Extraction and isolation**

The fresh mycelia of P. sumatrense were grown on PDA medium at 28 °C for 4 days. Agar plugs were cut into small pieces and were selected to inoculate 10 Erленмeyer flasks (500 mL) each containing 200 mL of PDB. The seed cultures were incubated at 28 °C on a rotary shaker (150 rpm) for 5 days and were then inoculated into 60 × 500 mL conical flasks on rice solid medium (80 g rice, 0.36 g sea salt, and 120 mL filtered water) for 30 days at 28 °C. The fermented cultures were extracted with 70% acetone/water, and evaporated under reduced pressure to afford an aqueous solution, which was then extracted three times with EtOAc and afforded the EtOAc extract (10.4 g). The EtOAc extract (10.4 g) was fractionated by silica gel column chromatography (CC) eluting with CHCl_{3}-MeOH (100 : 0, 95 : 5, 98 : 2, 9 : 1, 8 : 2, 1 : 1, and 0 : 100, v/v) to afford seven fractions (J1–J7). Fraction J5 (877.8 mg) was further subjected to Sephadex LH-20 CC using CHCl_{3}-MeOH (1 : 1, v/v) to afford four subfractions (J5-1 to J5-4). Subfraction J5-3 (374.0 mg) was isolated as a yellow oil, for which the molecular formula was assigned as C_{16}H_{20}O_{6} by HRESIMS, from a [M − H]− ion at 307.1197 (calc. 307.1182). On analysis of its 1H and 13C NMR spectra, similar features to that of compound 3 were evident, but with an opposite CD curve (Fig. 4). Therefore, the absolute configuration at C-14 was assigned as S. Thus, the absolute configuration of 4 was established as 14S, 15S.

In addition to compounds 1–4, the known curvularin (5)\(^\text{\textsuperscript{a}}\) and dehydrocurvularin (6)\(^\text{\textsuperscript{a}}\) were also isolated and identified from the fungal strain P. sumatrense MCCC 3A00612. All isolated compounds were tested for inhibitory activities against LPS-induced NO production in RAW 264.7 macrophages. As the results, only compound 6 showed significant NO production inhibitory activity with IC_{50} of 0.91 ± 0.03 μM, which was comparable to that of the positive control L-NMMA (IC_{50} of 41.91 ± 1.27 μM). The cell viability measured by the MTS assay showed that compound 6 had no significant cytotoxicity to the RAW 264.7 cells at the effective concentration for the inhibition of NO production.

**Summary**

**Sumalactone A (1).** Yellow oil (MeOH); [\alpha]_{D}^{20} = −124.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.13), 218 (4.08), 269 (3.76), 297 (3.68) nm; CD (0.81 mM, MeOH) λ_{max} (Δε) 211 (1.61), 226 (−0.22), 266 (6.52), 317 (−7.72) nm; IR (MeOH) ν_{max} 3194, 2968, 2938, 1715, 1663, 1607, 1589, 1472, 1339, 1267, 1161, 1136, 1024, 1007, 845, 669 cm⁻¹; HRESIMS m/z 307.1197 (M − H)− (calcd for C_{16}H_{20}O_{6}: 307.1182); the 1H and 13C NMR data, see Table 1.

**Sumalactone B (2).** Yellow oil (MeOH); [\alpha]_{D}^{25} = +86.1 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.02), 218 (3.94), 268 (3.62),
Although the con...1H and 13C NMR data, see Table 1. Sumalactone C (3). Yellow oil [MeOH]; [α]25D −41.6 (c 0.1, MeOH); UV (MeOH) λmax (log e) 203 (4.04), 219 (3.95), 268 (3.61), 297 (3.54) nm; CD (0.81 mM, MeOH) λmax (Δε) 214 (1.30), 231 (−1.90), 265 (4.53), 318 (−6.30) nm; IR (MeOH) νmax 3256, 2934, 1703, 1651, 1607, 1591, 1462, 1335, 1261, 1161, 1088, 1042, 993, 847 cm−1; HRESIMS m/z 307.1193 [M − H]− (calcd for C16H19O6, 307.1182); the 1H and 13C NMR data, see Table 1. Sumalactone D (4). Yellow oil [MeOH]; [α]25D +35.8 (c 0.1, MeOH); UV (MeOH) λmax (log e) 203 (4.04), 219 (3.96), 268 (3.63), 298 (3.54) nm; CD (0.81 mM, MeOH) λmax (Δε) 213 (−1.45), 231 (1.76), 265 (−4.75), 317 (5.87) nm; IR (MeOH) νmax 3304, 2943, 1703, 1651, 1607, 1462, 1337, 1265, 1161, 1040, 993, 847 cm−1; HRESIMS m/z 307.1014 [M − H]− (calcd for C16H19O6, 307.1182); the 1H and 13C NMR data, see Table 1. Curvularin (5). [α]25D −22.5 (c 0.1, MeOH); literature value [α]25D −28.6 (c 0.4, EtOH). Dehydrocurvularin (6). [α]25D −45.6 (c 0.1, MeOH); literature value [α]25D −65.9 (c 1.8, EtOH).

NO production bioassay

The murine macrophage cell line RAW 264.7 was obtained from Cell Bank of Chinese Academy of Sciences. RAW 264.7 cells were seeded in 96-well cell culture plates (1.5 × 105 cells per well) and treated with serial dilutions of the compounds with a maximum concentration of 25 μM in triplicate, followed by stimulation with 1 μg mL−1 LPS (Sigma, St. Louis, MO, USA) for 18 h. NO production in the supernatant was assessed by Griess reagents (Reagent A & Reagent B, respectively, Sigma). The absorbance at 540 nm was measured with a microplate reader (Thermo, Waltham, MA, USA). N6-methyl-L-arginine acetate salt (L-NMMA, Sigma), a well-known nitric oxide synthase (NOS) inhibitor, was used as a positive control.14 The viability of RAW 264.7 cells was evaluated by the MTS assay simultaneously to the NO production bioassay.

Conflicts of interest

There are no conflicts to declare.

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