Versicones E–H and arugosin K produced by the mangrove-derived fungus Aspergillus versicolor HDN11-84

Feng Li, Wenqiang Guo, Qian Che, Tianjiao Zhu, Qianqun Gu and Dehai Li

Four new xanthonoids, versicones E–H (1–4), and a biogenetically related new derivative arugosin K (5), were isolated from a culture extract of the mangrove-derived fungus Aspergillus versicolor HDN11-84. Their structures were elucidated on the basis of extensive NMR spectroscopic data analysis. Versicone E (1) represents the first example of naturally occurring xanthonoids containing a 2-butenamide moiety. Among them, 5 showed the best cytotoxicity against Hela cell line with an IC50 value of 9.2 µM. The Journal of Antibiotics advance online publication, 27 July 2016; doi:10.1038/ja.2016.95

INTRODUCTION

Xanthonoids, a family of polyketides with a polyphenolic dibenzo-γ-pyrone core, are widely distributed in plants, fungi and lichens,1–6 and show various biological activities partly depending on the variation of substituents.7 Prenylation is an important post-modification process during the biosynthesis of xanthonoids,1,8 through which, diverse prenylated xanthones are provided with a range of enhanced bioactivities including antimicrobial, antioxidant, cytotoxic, antiviral, antiallergic, anti-inflammatory, antimalarial and antihyperglycemic effects.7,9–10

Xanthones from lichens and fungi are mainly derived from acetate units.7,11,12 Among naturally occurring xanthones, prenylated derivatives make up an important subgroup.11,13 Typically, the five-carbon dimethylallyl moiety is directly attached to the aromatic rings of xanthonoids via the C–C bond. Some rare instances are the structures where the prenyl units are linked via oxygen bridges, for which only a few cases have been reported, such as variecoxanthone A14 and versicone A–D.15 The O-prenylation of xanthones was reported to be catalyzed by XptB under the presence of dimethylallyl diphosphate.1

During our searching for novel bioactive secondary metabolites from microorganisms derived from various habitats,16–18 a mangrove rhizosphere-derived Aspergillus versicolor strain HDN11-84, was selected based on the characteristic HPLC–UV profile of the ethyl acetate (EtOAc) extract. Guided by the UV characteristics, five new polyketides, including four xanthonoids versicones E–H (1–4) and a biogenetically related derivative arugosin K (5) (Figure 1) were isolated from the culture extract, together with four known ones versicone A, B, D (6–8) and variecoxanthone A (9). The cytotoxicity of new compounds was tested against K562, HL-60, Hela, HCT-116 and NB4 cell lines. Compound 5 showed the best activity against Hela cell line with an IC50 (half maximal inhibitory concentration) value of 9.2 µM.

EXPERIMENTAL PROCEDURE

General

UV spectra were recorded on a Beckman DU 640 spectrophotometer (Beckman, Shanghai, China). IR spectra were recorded on a Nicolet NEXUS 470 spectrophotometer (Thermo Scientific, Beijing, China) in Kbr disks. NMR spectra were recorded on JEOL JUM-ECP 600 (JEOL, Beijing, China) and Agilent 500 MHz DD2 spectrometers (Agilent, Beijing, China) using tetramethylsilane as an internal standard, and the chemical shifts were recorded as δ values. HRESIMS and ESIMS data were obtained on a Thermo Scientific LTQ Orbitrap XL mass spectrometer. TLC and column chromatography were performed either on plates precoated with silica gel GF254 (10×20 cm) or over silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China). Size-exclusion chromatography was performed using the Sephadex LH-20 (GE Healthcare, Bio-Sinences, Piscataway, NJ, USA). Semi-preparative HPLC was performed using an octadecylsilyl (ODS) column (YMC-Pack ODS-A, 10 × 250 mm, 5 µm).

Fungal resource

The fungus A. versicolor HDN11-84 was isolated from rhizosphere soil of mangrove plant Thespesia populnea, collected in Guangxi Province, China. The isolate was identified by ITS-rDNA sequence analysis. The ITS1-5.8S-ITS2 rDNA sequence of the strain HDN11-84 was submitted to GenBank with the accession number KU950433. The voucher specimen has been deposited in our laboratory at ~ 80°C. The working strain was prepared on potato dextrose agar in slants and stored at 4°C.

Fermentation and extraction

A small spoon of spores growing on potato dextrose agar slant was inoculated into a 500 ml Erlenmeyer flask containing 150 ml culture medium comprising of 20.0% potato broth, 1% maltose, 0.5% peptone, 2.0% glucose, and 2.0%...
mannitol, 0.3% yeast extract (in sea water) cultured at 28 °C for 2 days on a rotary shaker at 160 r.p.m. Then 3 ml seed culture was inoculated into 1000 ml medium containing 300 ml of the above culture medium (pH 5.7). The fungus was grown under the static condition in 200 Erlenmeyer flasks (1 L). After 3 weeks of fermentation (pH 7.3), the mycelium and broth were separated by filtration and the broth extracted with equal volumes of EtOAc twice. The mycelium was macerated and extracted with acetone, which was evaporated in vacuum and then extracted with EtOAc. All of the EtOAc extracts were combined to give a crude gum (25 g).

### Purification

The EtOAc extract (25 g) was subjected to ODS over a silica gel column using a gradient elution with MeOH/H2O to give five fractions (fraction 5.1–5.5). Fraction 5.5 was subjected to Sephadex LH-20 chromatography (MeOH: CH2Cl2 = 1:1) to afford five fractions (fraction 5.5.1–5.5.5). The fraction 5.5.5 was further purified by HPLC (90% MeOH/H2O, 3 ml min⁻¹) to obtain compounds 3 (tR 9.3 min; 13.5 mg) and 2 (tR 14.6 min; 10.5 mg). Fraction 5.5.4 was subjected to Sephadex LH-20 chromatography (MeOH: CH2Cl2 = 1:1) to yield four fractions (fraction 5.5.4.1–5.5.4.4). Fraction 5.5.4.1 was subjected to semi-preparative HPLC (65% MeOH/H2O, 3 ml min⁻¹) and further purified by HPLC (80% MeOH/H2O, 3 ml min⁻¹) to afford compounds 1 (tR 12.3 min; 13.4 mg) and 4 (tR 14.6 min; 4.6 mg). The MS and NMR spectra of 1–5 are in Supplementary Information.

**Versicone E (1).** Yellow oil; UV (MeOH) λmax (log ε) 220 (2.0), 342 (0.6) nm; IR: 2973, 2929, 1743, 1610, 1475, 1457, 1247, 1072, 1029 cm⁻¹; for 1H and 13C NMR data, see Tables 1 and 2; HRESIMS m/z 384.1451 [M+H]+ (calcd for C21H22O6N, 384.1442).

**Versicone F (2).** Yellow amorphous powder; UV (MeOH) λmax (log ε) 220 (2.0), 342 (0.6) nm; IR: 2973, 2929, 1743, 1643, 1425, 1276, 1070 cm⁻¹; for 1H and 13C NMR data, see Tables 1 and 2; HRESIMS m/z 385.1646 (calcd for C21H22O6Na, 385.1646).

**Versicone G (3).** Brownish yellow oil; UV (MeOH) λmax (log ε) 221 (2.0), 343 (0.3) nm; IR: 2931, 2858, 1735, 1548, 1483, 1459, 1429, 1276, 1247, 1072, 1029 cm⁻¹; for 1H and 13C NMR data, see Tables 1 and 2; HRESIMS m/z 427.1754 [M+H]+ (calcd for C23H22O7, 427.1751).

**Versicone H (4).** Yellow oil; UV (MeOH) λmax (log ε) 218 (1.3), 349 (0.3) nm; IR: 2973, 2942, 2902, 1643, 1612, 1486, 1465, 1417, 1280, 1068, 1018 cm⁻¹; for 1H and 13C NMR data, see Tables 1 and 2; HRESIMS m/z 353.0998 [M+Na]+ (calcd for C15H24O7Na, 353.0996).

**Arugosin K (5).** Yellow amorphous powder; UV (MeOH) λmax (log ε) 258 (1.2) nm; IR: 2973, 2929, 1743, 1610, 1475, 1457, 1228, 1120 cm⁻¹; for 1H and 13C NMR data, see Table 3; HRESIMS m/z 481.2227 [M+H]+ (calcd for C24H32O14, 481.2221).

### Cytotoxicity assay

Cytotoxic activities of 1–5 were evaluated using K562, Hela, NB4, HL-60 (using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method) and HCT-116 (using the sulforhodamine B (SRB) method). Doxorubicin hydrochloride was used as positive control (IC50 values 0.30, 0.60, 0.40, 0.02 and 0.20 μM, respectively). Compounds 3 and 5 exhibited cytotoxicity against Hela, HL-60 and NB4 cell lines with IC50 values ranging from 9.2 to 21.7 μM.

### RESULTS AND DISCUSSION

Versicone E (1) was obtained as yellow oil. Its formula C21H22O6N was determined by the protonated peak at HRESIMS peak at m/z...
Table 2 $^1$H NMR data (500 MHz) of compounds 1–4

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<thead>
<tr>
<th>Position</th>
<th>$^{1}$ $\delta$ (J in Hz)</th>
<th>$^{2}$ $\delta$ (J in Hz)</th>
<th>$^{3}$ $\delta$ (J in Hz)</th>
<th>$^{4}$ $\delta$ (J in Hz)</th>
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<tr>
<td>2</td>
<td>6.97 (1H, d, 8.4)</td>
<td>7.28 (1H, d, 9.2)</td>
<td>7.29 (1H, d, 9.2)</td>
<td>7.58 (1H, d, 9.3)</td>
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<td>3</td>
<td>7.72 (1H, t, 8.4)</td>
<td>7.13 (1H, d, 9.2)</td>
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<td>7.32 (1H, d, 9.3)</td>
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<td>4</td>
<td>7.44 (1H, s)</td>
<td>7.12 (1H, s)</td>
<td>7.27 (1H, s)</td>
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<tr>
<td>11</td>
<td>4.89 (2H, d, 6.0)</td>
<td>5.56 (2H, s)</td>
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<td>4.68 (1H, t, 6.9)</td>
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<td>2.40 (3H, s)</td>
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<td>3.91 (3H, s)</td>
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<td>3.87 (3H, s)</td>
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<td>14</td>
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<td>1′</td>
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<td>4′</td>
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<td>1.46 (3H, s)</td>
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<td>5′</td>
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<td>1.46 (3H, s)</td>
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<td>4′-NH$_2$</td>
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<td>2.06 (3H, s)</td>
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*a* in dimethylsulfoxide (DMSO).

*b* in CDCl$_3$.

Figure 2 The key 2D NMR correlations of 1–5.
The molecular formula was determined as C_{28}H_{32}O_{7} evidenced by the differences belonging to the signals of the substituent groups at C-1 and C-7 (\( \delta_{C} 170.9 \)) and H3-21 (H 3.69) to C-3 indicated the substituent of C-3 was a methoxy group, instead of a hydroxyl group in arugosin E.

Versicone F (2) was obtained as yellow amorphous powder with a 1D NMR data (Tables 1 and 2) showed 21 carbon signals classified by DEPT and HSQC spectra as three methyls, five aromatic/olefinic methines and 11 non-protonated carbons including two carbonyls (\( \delta_{C} 177.9 \) and 170.0). The \(^1\)H and \(^{13}\)C NMR data of 1 suggested a xanthone core skeleton, which was further indicated by the COSY (H-2/H-3) and HMBC correlations (Figure 2). Comparing with the reported 7-dihydroxy-6-methyl-8-hydroxymethylxanthone,1 the major differences of two compounds were the substituents at C-3 (\( \delta_{C} 151.6 \)) and C-7 (\( \delta_{C} 130.0 \)) to C-3′ (\( \delta_{C} 134.2 \)) in 2-butenamide. Compound 1 was named as versicone E.

Versicone G (2) and H (3 and 4) were obtained with molecular formulae C_{22}H_{24}O_{6} and C_{22}H_{24}O_{6}, respectively. Compound 3 showed similar NMR data to versicone B (7),13 and their difference was the replacement of the OH group in 7 by an acetate-substituted hydroxymethyl as evidenced from the HMBC correlations of H3-19 (\( \delta_{H} 5.11 \)) to C-7 and C-1″ (\( \delta_{C} 170.4 \)), H3-2″ (\( \delta_{H} 1.75 \)) to C-1″. The HMBC correlation of H3-1 (\( \delta_{H} 3.69 \)) to C-3 indicated the substituent of C-3 was a methoxy group, instead of a hydroxyl group in arugosin E.

In conclusion, nine polyketides including five new compounds (1–5) was tested on five human cancer cell lines, K562 (myelogenous leukemia), Hela (human cervical cancer), NB4 (acute promyelocytic leukemia), HL-60 (human leukemia) and HCT-116 (human colon cancer). Compounds 3 and 5 showed cytotoxicity against Hela, HL-60 and NB4 cell lines with IC\textsubscript{50} values ranging from 9.2 to 21.7 \( \mu \)M (Table 4).

In conclusion, nine polyketides including five new compounds (1–5) and four known ones (6–9) were discovered from the mangrove-derived fungus A. versicolor HDN11-84. Among them, versicone E (1) is the first example of natural xanthanonoids containing 2-butenamide moiety.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.
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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)