Antifungal New Oxepine-Containing Alkaloids and Xanthones from the Deep-Sea-Derived Fungus Aspergillus versicolor SCSIO 05879

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Supporting Information

ABSTRACT: Phytopathogenic fungi remain a continuous and huge threat in the agricultural fields. The agrochemical industry has made great development of the use of microbial natural products, which has been regarded as an effective strategy against phytopathogenic fungi. Antifungal bioassay-directed fractionation was used to isolate two new oxepine-containing alkaloids (1 and 2), two new 4-aryl-quinolin-2-one alkaloids (3 and 4), and four new prenylated xanthones (5–8) from the deep-sea-derived fungus Aspergillus versicolor SCSIO 05879. Extensive NMR spectroscopic analysis, quantum mechanical calculations, and X-ray single-crystal diffraction were used to elucidate their structures, including their absolute configurations. Versicoloids A and B, versicorne A, and cottoquinazoline A showed antifungal activities against three phytopathogenic fungi. The antifungal activities of these bioactive compounds strongly depend on the fungal species. Especially versicoloids A and B showed strong fungicidal effect (MIC of 1.6 μg/mL) against Colletotrichum acutatum, compared with that of the positive control cycloheximide (MIC of 6.4 μg/mL). The results of antifungal experiments indicated that versicoloids A and B may be regarded as candidate agents of antifungal agrochemicals.

KEYWORDS: deep-sea-derived, Aspergillus versicolor, ECD calculations, oxepine, phytopathogenic fungi, antifungal activities

INTRODUCTION

Effective and sustained control of fungal pathogens is an important project in the agricultural fields. The global losses caused by phytopathogenic fungi are estimated to be ~20% reductions in the major food and cash crops in spite of the continued release of new resistant cultivars and antifungal agrochemicals.1,2 Agrochemicals from microbial natural products have been regarded as an effective strategy against phytopathogenic fungi. Many natural antifungal agents such as kasugamycin, polyoxins, validamycin, and blasticidin-S and antibacterial agents such as oxytetracycline and streptomycin have been isolated from microbial resources.3,4 Over the past 50 years, approximately 20 000 natural products have been reported from marine flora and fauna, but it was rarely reported from deep-water marine organisms, about <2%.5 Methods for sample collection, identification, and culturing technologies of microorganisms have developed rapidly in recent years. At the same time, chemical investigations on deep-sea microorganisms have shown a sharp increase.6 As part of our ongoing efforts to discover structurally novel bioactive natural compounds from marine-derived microorganism-inhabiting unique environments,7–10 a fungal strain SCSIO 05879, identified as Aspergillus versicolor, was isolated from a deep-sea sediment (depth = ~3927 m), collected from the Indian Ocean (6°00’00” N, 87°30’50” E). It was found that A. versicolor could produce a number of secondary metabolites with various chemical structures, such as anthraquinones, chromones, lactones, and alkaloids, and some of them exhibited intriguing biological activities.11–16 The ethyl acetate extract of the fermentation broth of A. versicolor SCSIO 05879 displayed significant in vitro antifungal activity against phytopathogenic fungi Colletotrichum acutatum and contained a variety of secondary metabolites with similar UV absorptions as shown by high-performance liquid chromatography (HPLC) analysis with photodiode array. Two new oxepine-containing diketopiperazine-type alkaloids, versicoloids A and B (1 and 2), two new 4-aryl-quinolin-2-one alkaloids, 3,6-O-dimethylviridicatin (3) and 3-O-methylviridicatol (4), and four new prenylated xanthones, versicones A–D (5–8), together with four known compounds cottoquinazoline A (9),19 (–)–cyclopropenol (10),16,17 variecoxanthone A (11),18 and dioncrinol (12),9 were isolated from the culture extract of A. versicolor SCSIO 05879 (Figure 1). Their structures, including absolute configurations, were elucidated on the basis of extensive NMR spectroscopic data analysis, time-dependent density functional theory ECD calculations, and X-ray single-crystal diffraction. All the isolated compounds (1–12) were tested for their antifungal activities against three phytopathogenic fungi (Colletotrichum acutatum, Magnaporthe oryzae, and Fusarium oxysporum).
photophysics, LTD.). 1H, 13C NMR, distortionless enhancement by
recorded with a Chirascan circular dichroism spectrometer (Applied
spectrophotometer (Shimadzu). Circular dichroism spectra were
performed on plates precoated with silica gel GF254 (100–200 μm) and
over silica gel (200–300 mesh) (Qingdao Marine Chemical Factory)
and Sephadex LH-20 (Amersham Biosciences, Sweden), respectively.
Vacuum-liquid chromatography (VLC) used silica gel H (Qingdao
Marine Chemical Factory). All solvents used were of analytical grade
(Tianjin Fuyu Chemical and Industry Factory). Semipreparative
HPLC was performed using an ODS column (YMC-pack ODS-A,
10 × 250 mm, 5 μm, 4 mL/min).

Fungal Material. The fungus A. versicolor SCSIO 05879 was isolated
from a marine sediment sample collected in May, 2014, from
the Indian Ocean (6°00′00″ N, 87°30′50″ E) at a depth of 3927 m.
The fungus was identified using a molecular biological protocol by
daNkul and sequencing of the ITS region. A BLAST search
result showed that the sequence was most similar (100%) to the
sequence of Aspergillus versicolor (compared to accession no.
AY373883). This strain has been deposited in China General
Microbiological Culture Collection Center with a deposition number
CGMCC 11123.

Extraction and Isolation. A. versicolor SCSIO 05879 was grown
under static conditions at 25 °C for 40 days in one hundred 1000 mL
conical flasks containing liquid medium (300 mL/flask) composed of
starch soluble (10 g/L) and polypeptone (1 g/L), and tap water after
adjusting its pH to 7.5. The fermented whole broth (30 L) was
stirred for 40 days in one hundred 1000 mL

| 1H (500 MHz) and 13C (125 MHz) NMR Data for 1–4 in Dimethyl Sulfoxide (DMSO-d6) (TMS, δ ppm) |
|---|---|---|---|
| position | δC | δH (J in Hz) | δC | δH (J in Hz) | δC | δH (J in Hz) | δC | δH (J in Hz) |
| 1 | 166.5, C | 166.9, C | 158.0, C | 158.0, C | 12.00, s | 12.05, s |
| 2 | 57.7, CH | 6.46, s | 58.4, C | 58.4, C | 6.50, s | 6.50, s |
| 3 | 154.0, C | 153.6, C | 136.8, C | 136.8, C | 12.00, s | 12.05, s |
| 4 | 159.0, C | 159.7, C | 158.4, C | 158.4, C | 6.40, d (2.8) | 6.40, d (2.8) |
| 5 | 145.1, CH | 6.36, d (5.6) | 144.5, CH | 144.5, CH | 6.18, d (6.0) | 6.18, d (6.0) |
| 6 | 115.3, CH | 5.65, d (5.6, 2.0) | 115.9, CH | 115.9, CH | 5.53, dd (6.0, 1.8) | 5.53, dd (6.0, 1.8) |
| 7 | 156.3, C | 157.4, C | 154.2, C | 154.2, C | 7.01, d (7.9) | 7.01, d (7.9) |
| 8 | 95.1, CH | 5.72, s | 94.9, CH | 94.9, CH | 7.07, t (7.5) | 7.07, t (7.5) |
| 9 | 108.0, C | 110.5, C | 110.5, C | 110.5, C | 7.39, t (7.5) | 7.39, t (7.5) |
| 10 | 106.9, C | 161.4, C | 108.5, C | 108.5, C | 7.33, d (7.9) | 7.33, d (7.9) |
| 11 | 60.5, CH | 4.76, d (6.9) | 61.2, CH | 61.2, CH | 5.08, d (5.4) | 5.08, d (5.4) |
| 12 | 35.8, CH | 2.40, m | 43.9, CH | 43.9, CH | 2.56, m | 2.56, m |
| 13 | 22.9, CH2 | 1.25, m | 25.2, CH2 | 25.2, CH2 | 0.97, m | 0.97, m |
| 14 | 12.3, CH3 | 0.81, t (6.9) | 11.9, CH3 | 11.9, CH3 | 0.86, t (7.2) | 0.86, t (7.2) |
| 15 | 15.0, CH3 | 1.07, d (6.9) | 11.0, CH3 | 11.0, CH3 | 1.12, d (7.2) | 1.12, d (7.2) |
| 16 | 55.0, CH2 | 3.64, s | 55.4, CH2 | 55.4, CH2 | 3.72, s | 3.72, s |
| 17 | 30.6, CH | 2.19, m | 33.1, CH | 33.1, CH | 2.39, m | 2.39, m |
| 18 | 19.9, CH3 | 0.91, d (6.4) | 20.2, CH3 | 20.2, CH3 | 1.14, d (6.9) | 1.14, d (6.9) |
| 19 | 18.4, CH3 | 0.96, d (6.4) | 18.1, CH3 | 18.1, CH3 | 1.04, d (6.9) | 1.04, d (6.9) |

3-OCH3 | 59.4, CH3 | 59.5, CH3 | 3.67, s | 3.67, s |
6-OCH3 | 55.2, CH3 | 55.2, CH3 | 3.56, s | 3.56, s |
13-OH | 9.60, s | 9.60, s |
nine subfractions by reverse-phase silica gel (ODS) using step-gradient elution with MeOH–H$_2$O (20%–100%). Subfraction 1-6 was directly separated by HPLC (55% MeOH/H$_2$O) to yield 1 (4.1 mg, $t_{R} = 21.6$ min). Fraction 1-7 was directly separated by HPLC (65% MeOH/H$_2$O) to yield 2 (3.6 mg, $t_{R} = 163$ min) and 1 (2.6 mg, $t_{R} = 22.6$ min). Fraction 1-8 was further purified by HPLC (57% acetonitrile/H$_2$O) to yield 7 (9.7 mg, $t_{R} = 163$ min), 5 (28.6 mg, $t_{R} = 23.1$ min), and 6 (42.8 mg, $t_{R} = 28.2$ min). Fraction 1-9 was further purified by HPLC (77% MeOH/H$_2$O) to yield 11 (44.5 mg, $t_{R} = 28.5$ min) and 8 (64.4 mg, $t_{R} = 32.0$ min). Fraction 3 was divided into five parts (fractions 3-1–3-5) by Sephadex LH-20 (MeOH). Fraction 3-3 was further purified by HPLC (57% MeOH/H$_2$O) to yield 3 (5.1 mg, $t_{R} = 18.8$ min). Fraction 4 was divided into three parts (fractions 4-1–4-3) by Sephadex LH-20 (MeOH). Fraction 4-3 was further purified by HPLC (41% MeOH/H$_2$O) to yield 10 (131.7 mg, $t_{R} = 8.0$ min) and 4 (3.5 mg, $t_{R} = 22.2$ min). Fraction 5 was divided into three parts (fractions 5-1–5-3) by Sephadex LH-20 (MeOH). Fraction 5-3 was further purified by HPLC (50% MeOH/H$_2$O) to yield 9 (15.9 mg, $t_{R} = 33.0$ min).

_X-ray Crystallographic Data for 4, 6, and 8._ Yellow crystals of 4, 6, and 8 were obtained in the solvent of methanol. Crystal data of 4 (Cu K$_\alpha$ radiation) and 6 (Cu K$_\alpha$ radiation) were obtained on a CrysAlis PRO CCD area detector diffractometer with graphite-monochromated Cu K$_\alpha$ radiation ($\lambda = 1.54178$ Å). Crystal data of 8 (Mo K$_\alpha$ radiation) were obtained on a Bruker Smart CCD area detector diffractometer with graphite-monochromated Mo K$_\alpha$ radiation ($\lambda = 0.70737$ Å). Crystallographic data for 4, 6, and 8 have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 1416778, 1416778, and 1417166, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB21 1EZ, U.K. [Fax: +44 (0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

_X-ray Crystallographic Data for 4, 6, and 8._ Monoclinic, space group P2(1)/ c with $a = 12.0134(10)$ Å, $b = 7.8855(8)$ Å, $c = 13.9221(11)$ Å, $V = 1304.1(2)$ Å$^3$, $Z = 4$, $D_{calc} = 1.361$ g/cm$^3$, $R = 0.0999$, $wR_{c} = 0.1045$, $T = 293(2)$ K. Crystal size, $0.20 \times 0.08 \times 0.06$ mm$^3$.

_X-ray Crystallographic Data of 6._ Monoclinic, space group P2(1)/ c with $a = 4.1217(2)$ Å, $b = 39.0750(2)$ Å, $c = 14.1681(8)$ Å, $V = 2275.62(2)$ Å$^3$, $Z = 4$, $D_{calc} = 1.122$ g/cm$^3$, $R = 0.1004$, $wR_{c} = 0.2142$, $T = 293(2)$ K. Crystal size, 0.30 $\times$ 0.14 $\times$ 0.10 mm$^3$.

_X-ray Crystallographic Data of 8._ Triclinic, space group P-1 with $a = 7.2746(7)$ Å, $b = 12.0963(12)$ Å, $c = 12.4076(14)$ Å, $V = 979.61(17)$ Å$^3$, $Z = 2$, $D_{calc} = 1.310$ g/cm$^3$, $R = 0.0888$, $wR_{c} = 0.1737$, $T = 298(2)$ K. Crystal size, 0.43 $\times$ 0.38 $\times$ 0.21 mm$^3$.

**Versicoloid A (1).** Yellow oil; $[^1]H/NMR$: $\delta$ = 230.4 (0.3, MeOH); UV (MeOH) $\lambda_{max}$ (log e) = 344 (3.65) nm; IR (KBr) $\nu_{max}$ = 1661, 1653, 1582, 1537, 1404, 1377, 1290, 1219, 1192, 1173, 1103, 1015 cm$^{-1}$; H NMR and $^{13}$C NMR data, see Table 1; HRESIMS $m/z$ 382.1726 [M + Na]$^+$ (calcld for C$_{17}$H$_{23}$N$_2$O$_4$Na $^{398.168}$).

**Versicoloid B (2).** Yellow oil; $[^1]H/NMR$: $\delta$ = -114.9 (0.4, MeOH); UV (MeOH) $\lambda_{max}$ (log e) = 253 (3.78), 350 (3.66) nm; IR (KBr) $\nu_{max}$ = 3246, 1657, 1616, 1584, 1537, 1406, 1379, 1290, 1217, 1127, 1152, 1020 cm$^{-1}$; H NMR and $^{13}$C NMR data, see Table 1; HRESIMS $m/z$ 398.1689 [M + Na]$^+$ (calcld for C$_{17}$H$_{23}$N$_2$O$_4$Na $^{398.1686}$).

**3,6-Dimethylviridicatrin (3).** Yellow amorphous solid; UV (MeOH) $\lambda_{max}$ (log e) = 205 (4.14), 238 (4.13), 347 (3.42) nm; IR (KBr) $\nu_{max}$ = 1653, 1647, 1558, 1541, 1506, 1489, 1456, 1423, 1339, 1277, 1217, 1152, 1020 cm$^{-1}$; H NMR and $^{13}$C NMR data, see Table 1; HRESIMS $m/z$ 304.0938 [M + Na]$^+$ (calcld for C$_{14}$H$_{20}$N$_2$O$_4$Na $^{304.0944}$).

**3-O-Methylviridicatol (4).** Faint yellow crystal; UV (MeOH) $\lambda_{max}$ (log e) = 203 (4.02), 267.3 (3.47), 322 (3.15) nm; IR (KBr) $\nu_{max}$ = 1684, 1647, 1636, 1558, 1541, 1506, 1489, 1456, 1423, 1339, 1278, 1209 cm$^{-1}$; H NMR and $^{13}$C NMR data, see Table 1; HRESIMS $m/z$ 268.0960 [M + H]$^+$ (calcld for C$_{14}$H$_{20}$NO$_2$ $^{268.0968}$).

**Versicone A (5).** Yellow amorphous solid; UV (MeOH) $\lambda_{max}$ (log e) = 204 (4.20), 237 (4.21), 243 (4.21), 251 (4.20), 286 (3.87), 357 (3.68) nm; IR (KBr) $\nu_{max}$ = 1616, 1601, 1474, 1420, 1379, 1265, 1211, 1190, 1098, 1084, 975 cm$^{-1}$; H NMR and $^{13}$C NMR data, see Table 2; HRESIMS $m/z$ 377.1355 [M + Na]$^+$ (calcld for C$_{20}$H$_{27}$N$_3$O$_4$ 377.1359).
The ethyl acetate extract of the fermentation broth of A. versicolor SCSIO 05879 was subjected to silica gel column chromatography and further purified by HPLC to obtain eight new (1–8) and four known (9–12) compounds (Figure 1). The known compounds were identified by comparison of spectroscopic data with those reported in the literature as cottoquinazoline A (9),16 (--)-cyclopenol (10),16,17 varieco-anthene A (11),18 and diorcinol (12).19

**Compound 1** was initially obtained as a yellow oil. Its molecular formula, C19H25N3O4, was established by HRESIMS at m/z 382.1726 [M + Na]+ (calcd 382.1737), implying the presence of 9 degrees of unsaturation. The 1H NMR and heteronuclear multiple quantum correlation (HMQC) spectra (measured in DMSO-d6) indicated the presence of 25 protons, including one exchangeable proton signal at δH 8.46 (2-NH), three unsaturated proton signals [δH 6.36 (d, J = 5.6 Hz, H-8), 5.72 (s, H-11), and 5.65 (dd, J = 5.6, 2.0 Hz, H-9)], two proton signals attached heteroatoms at δH 4.76 (d, J = 6.9 Hz, H-15) and δH 4.66 (brs, H-3), one methoxyl group at δH 3.64 (H-17), two methine signals at δH 2.40 (H-16) and δH 2.19 (H-21), one methylene at δH 1.25 (H-13), and four methyl groups [δH 0.96 (H-9), 1.07 (H-15), 1.29 (H-19), and 0.81 (H-22)]. The 13C NMR (Table 1) and DEPT spectra revealed the presence of 19 carbons, namely, 5 methyls (one oxygenated), 1 methylene, 4 saturated methines, 3 olefinic methines, and 6 quaternary carbons (including 4 olefinic ones and 2 carbonyls), which accounted for the 9 degrees of unsaturation. Four olefinic signals (δC 145.1, 151.1, 157.4, and 161.6), two quaternary carbons (δC 156.3) were typical for an oxepine skeleton. The signals for valine were observed in the corresponding unit of alanine in oxepinamides B;20 indicating that they shared the same skeleton. The signals for valine were observed in the corresponding unit of alanine in oxepinamides B;20 indicating that they shared the same skeleton. The signals for valine were observed in the corresponding unit of alanine in oxepinamides B;20 indicating that they shared the same skeleton. The signals for valine were observed in the corresponding unit of alanine in oxepinamides B;20 indicating that they shared the same skeleton. The signals for valine were observed in the corresponding unit of alanine in oxepinamides B;20 indicating that they shared the same skeleton. The signals for valine were observed in the corresponding unit of alanine in oxepinamides B;20 indicating that they shared the same skeleton. The signals for valine were observed in the corresponding unit of alanine in oxepinamides B;20 indicating that they shared the same skeleton. The signals for valine were observed in the corresponding unit of alanine in oxepinamides B;20 indicating that they shared the same skeleton. The signals for valine were observed in the corresponding unit of alanine in oxepinamides B;20 indicating that they shared the same skeleton.

Figure 2. Key 1H–1H COSY (bold) and HMBC (arrows) correlations of 1–8.
and H-21 in the NOESY spectrum indicated the noncofacial orientation of H-3 and H-15 (Figure 3). Because the chiral center C-16 in 1 was far from the chromophore, the simplified structure of 1 (Model-I) was used for conformational analysis.22,23 Therefore, the absolute configurations of C-3 and C-15 in 1 were determined as 3R and 15S, respectively, by comparing the calculated ECD spectrum with its experimental values (Figure 4).

Figure 4. CD and calculated ECD spectra of 1 and 2.

Compound 2, yellow oil, was assigned the molecular formula C10H8N4O3 on the basis of HRESIMS data, with one oxygen atom more than 1, and showed similar spectral features to those of 1 (Table 1). However, significant differences in chemical shift between 1 and 2 were observed, particularly for H-3 and C-3. The chemical shifts of the methine (CH-3, δC 57.7) in 1 were changed dramatically to the oxygenated quaternary carbon (C-3, δC 84.8) in 2, which was supported by HMBC correlations from H2-17 (δH 0.97, m) and H3-19 (δH 1.12, d, J = 7.2 Hz) to C-3 (Figure 2). In addition, the similarity of the CD curve [CD (ε 0.02, MeOH) (Δεmax) 217 (+13.5), 252 (−16.4) and specific rotation ([α]23D; −114.9°) of 2 with those of 1 [CD (ε 0.02, MeOH) (Δεmax) 218 (+21.1), 247 (−15.0) and specific rotation ([α]23D; −230.4°)] indicated the same absolute configuration between 2 and 1 (Figure 4). Similarly, this deduction was further supported by the ECD calculations of the simplified analogue (Model-II) with that determined experimentally. Because compounds 1 and 2 exhibited almost identical ECD spectra, the absolute configuration of 2 was also established as 3R and 15S (Figure 4).

Compound 3 was obtained as a yellow amorphous powder with the molecular formula C16H13NO3 as determined by HRESIMS at m/z 291.0526 [M + Na]+ (calcd 291.0523), indicating 11 degrees of unsaturation. In the 1H NMR spectrum, one exchangeable proton signal was observed at δH 12.00 ppm, which indicated that the NH or OH chelated with the carbonyl group. The 1H, 13C, and DEPT spectra showed the presence of 14 aromatic carbons, including 8 methine ones and 6 aromatic quaternary ones, and 1 amide carbonyl group (δC 162.0), which corresponded to 10 degrees of 11 unsaturation equivalents required by the molecular formula and indicated that compound 3 had three rings. The presence of a monosubstituted benzene system was indicated by the 1H NMR signals at δH 7.30 (2H, t, J = 7.6 Hz), 7.47 (1H, d, J = 7.3 Hz), and 7.32 (2H, overlap). Another three aromatic proton signals at δH 7.30 (1H, d, J = 8.5 Hz), 7.10 (1H, dd, J = 8.5, 2.8 Hz), and 6.40 (1H, d, J = 2.8 Hz) were assigned to be a typical ABX system. Detailed analysis of the 1D- and 2D-NMR spectra data of 3 revealed that they were very similar to those of 3-methylviridicatin,17 indicating that they shared the same skeleton. The main difference in the 1H NMR spectrum was the presence of a methoxy group at δH 3.56 for 6-OCH3 in 3. This deduction was further supported by the HMBC correlation of 6-OCH3 with C-6 (Figure 2). Therefore, compound 3 was the methylolation derivative of 3-O-methylviridicatin and was named 3,6-O-dimethylviridicatin.

Compound 4, a faint yellow crystal, had the molecular formula C17H15NO3 as determined by HRESIMS at m/z 290.0780 [M + Na]+ (calcd 290.0788), indicating 11 degrees of unsaturation. The similarity of 1H and 13C NMR spectra of 4 with 3 (Table 1) suggested that they were analogues, and the key difference was at the benzene ring. In the 1H NMR spectrum, the aromatic proton signals and the coupling constants at δH 7.57 (1H, t, J = 7.5 Hz), 7.53 (1H, d, J = 7.9 Hz), 7.07 (1H, t, J = 7.5 Hz), and 7.01 (1H, t, J = 7.9 Hz) indicated the presence of an ortho-disubstituted benzene system. Another four aromatic proton signals at δH 7.29 (1H,
t, J = 7.8 Hz), 6.83 (1H, d, J = 7.6 Hz), 6.69 (1H, d, J = 7.6 Hz), and 6.67 (1H, s) were assigned to the 1,3-disubstituted benzene ring. These deductions were further supported by the COSY correlations of H-5/H-6/H-7/H-8 and H-14/H-15/H-16, as well as by the HMBC correlation of H-12 with C-14 and C-16. The exchangeable proton signal at δH 9.60 (13-0H) was located at C-13, supported by the HMBC correlation of 13-0H with C-13 and C-14 (Figure 2). To verify the proposed structure, compound 4 was subjected to single-crystal X-ray diffraction analysis (Figure 5). Thus, compound 4 was the methylated derivative of viridicaticol and was named 3-O-methylviridicaticol.

Compound 5 was isolated as a yellow amorphous solid. Its molecular formula was determined to be C21H22O5 by HRESIMS at m/z 407.1469 [M + Na]+ (calcd 407.1465) with 10 degrees of unsaturation. Detailed analysis of the 1D- and 2D-NMR spectra of 5 revealed that they were very similar to those of 11, indicating that they shared the same skeleton. The main difference in the 1H NMR spectrum was the presence of a methoxy group at δH 4.01 for C-21 in 5, and in the 13C NMR spectrum, a methoxy group at δC 65.6 for 5-OCH3 was observed in 5, instead of a hydroxy group located at C-5 in 11. This deduction was further supported by the HMBC correlation of H-5 with C-5 (Figure 2). Compound 5 was thus elucidated as the methylated derivative of 11 and was named versicone A.

The molecular formula of compound 6 was determined to be C22H24O6 by HRESIMS at m/z 407.1469 [M + Na]+ (calcd 407.1465) with 10 degrees of unsaturation. The resemblance of the 1H and 13C NMR data (Table 2) between 6 and 5 indicated that they had the same skeleton. Interpretation of the 2D NMR spectra of 6 revealed that the aromatic proton signal H-6 (δH 9.51 (6-OH) was located at C-6, supported by the HMBC correlation of H-6 with C-5 in 11. This deduction was further supported by the HMBC correlation of H-1,21 with C-5 (Figure 2). Ultimately, the structure of 6 was confirmed by single-crystal X-ray diffraction (Figure 5).

Compound 7, a yellow amorphous solid, was assigned the molecular formula C21H22O6 on the basis of HRESIMS data, with one CH2 less than 6, and showed similar spectral features to those of 6 (Table 2). The distinct differences between them were the disappearance of the methoxy group 6-OCH3 and the signal at δH 8.3 Hz), 6.83 (1H, d, J = 8.3 Hz) in 5 was replaced by the corresponding methoxy group (δC 61.7) in 6. This deduction was further supported by the HMBC correlation of 6-OCH3 with C-6 (Figure 2).

The molecular formula of compound 8 was determined to be C21H22O5 by HRESIMS at m/z 377.1364 [M + Na]+ (calcd 377.1359), possessing the same molecular formula with 5. The resemblance of the 1H and 13C NMR data (Table 2) between 8 and 5 indicated that they had the same backbone, and the key difference between them was at the isopentene group. In the 1H NMR spectrum, the olefinic proton signals and the coupling constants at δH 6.60 (1H, dd, J = 16.8, 10.6 Hz), 5.27 (1H, d, J = 16.8 Hz), and 5.20 (1H, d, J = 10.6 Hz) indicated the presence of a terminal double bond. The HMBC correlations from H-18/H-19 (δH 1.35) to C-15 (δC 76.7) and C-16 (δC 143.1), and H-20 (δH 5.26) to C-15, revealed the presence of an oxygenated isopentene unit at C-20. To verify the proposed structure, compound 8 was subjected to single-crystal X-ray diffraction analysis (Figure 5). Consequently, the structure of 8 was deduced with the trivial name versicone D.

To discover the structurally novel bioactive natural compounds from the deep-sea-derived microorganism to control fungal pathogens, these compounds (1–12) produced by the A. versicolor SCSIO 05879 isolated from the Indian Ocean deep-sea sediment (depth = −3927 m) were preliminarily investigated for their antifungal activities against three phytopathogenic fungi (C. acutatum, M. oryzae, and F. oxysporum), which commonly infect major food and cash crops. Cycloheximide was used as the corresponding positive control. Among these compounds, versicoloids A and B (1 and 2), versicone A (5), and cottoquinazoline A (9) exhibited antifungal activities against three phytopathogenic fungi (Table 3). The antifungal activities of these bioactive compounds strongly depend on the fungal species. Especially versicoloids A and B (1 and 2) showed strong antifungal activities (MIC of 1.6 µg/mL) against C. acutatum, compared with that of the positive control cycloheximide (MIC of 6.4 µg/mL). In contrast, other compounds displayed weak antifungal activities with MIC values of >30 µg/mL against the pathogens tested.

In conclusion, two new oxepine-containing diketopiperazine-type alkaloids (1 and 2), two new 4-arylquinolin-2-one alkaloids (3 and 4), and four new prenylated xanthones (5–8) were isolated from the deep-sea-derived fungus A. versicolor SCSIO 05879. Their structures were elucidated on the basis of extensive NMR data analysis, time-dependent density functional theory (TDDFT) ECD calculations, and X-ray single-crystal diffraction. To the best of our knowledge, the structures of oxepine-containing diketopiperazine alkaloids are very rarely discovered in nature, featuring both oxepine and diketopiperazine ring systems. The antifungal experiments exhibited that four isolated compounds (1, 2, 5, and 9) showed antifungal activities against three phytopathogenic fungi. Moreover, versicoloids A and B (1 and 2) were identified as the most active components against C. acutatum, and they may be regarded as candidate agents of antifungal agrochemicals in the agricultural field.

Table 3. Inhibitory Effects of Tested Compounds on Phytopathogenic Fungi

<table>
<thead>
<tr>
<th>compound</th>
<th>phytopathogenic fungi (MIC, µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colletotrichum acutatum</td>
</tr>
<tr>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
</tr>
<tr>
<td>3</td>
<td>&gt;200</td>
</tr>
<tr>
<td>4</td>
<td>&gt;200</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td>6</td>
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<td>7</td>
<td>&gt;200</td>
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<tr>
<td>8</td>
<td>&gt;200</td>
</tr>
<tr>
<td>9</td>
<td>128</td>
</tr>
<tr>
<td>10</td>
<td>&gt;200</td>
</tr>
<tr>
<td>11</td>
<td>&gt;200</td>
</tr>
<tr>
<td>12</td>
<td>&gt;200</td>
</tr>
<tr>
<td>cycloheximide</td>
<td>6.4</td>
</tr>
</tbody>
</table>

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b00527.
ITS gene sequence data of SCSIO 05879 and NMR and HRESIMS spectra of 1−8 (PDF)

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Notes
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