Endophyte inspired chemical diversity from beta-caryophyllene

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**General Procedures**

UV spectra were obtained using a Evolution 300 UV-vis Spectrophotometer (Thermo Fisher Scientific Inc., USA). IR were recorded on a Bruker Tensor 27 spectrophotometer with KBr disks (Bruker Corp., German). Optical rotations were measured on a Autopol III automatic polarimeter (Rudolph Research Analytical, USA). CD spectrum were obtained on a Chirascan CD Spectrometer (Applied Photophysics Ltd., United Kingdom). ESI-MS was performed on a LTQ Fleet instrument (Thermo Fisher Scientific Inc., USA). HR ESI-MS was performed on an Agilent 6520 Accurate-Mass Q-TOF LC/MS spectrometer (Agilent Technologies Ltd., USA). 1D and 2D NMR spectra were recorded on a AVANCE III (500 MHz) instrument (Bruker Corp., German). Chemical shifts were reported using solvent residual as the internal standard. High performance liquid chromatography (HPLC) analysis and semi-preparation was performed on a Waters 1525 instrument (Waters Corp.). Column chromatography was performed on silica gel (90–150 μm; Qingdao Marine Chemical Inc., China), MCI gel (75–150 μm; Mitsubishi Chemical Corp., Japan), Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Chromatorex C$_{18}$ gel (40–75 μm; Fuji Silysia Chemical LTD., JPN). GF$_{254}$ plates (Qingdao Marine Chemical Inc., China) were used for thin-layer chromatography (TLC).

**Fermentatiun Biotransformation of β-Caryophyllene**

The microorganism *Aspergillus tubingensis* KJ-9 was incubated in potato dextrose agar (PDA) at 25°C for 3 days. One piece (approximately 7 mm$^2$) of mycelium and liquid state β-Caryophyllene with no solvent (333 μL) was fermented aseptically to a
500 ml Erlenmeyer flask containing 150 mL of potato dextrose (PD) liquid medium. 47 flasks were shook at 130 rpm, 27±0.5 °C for 10 days. Another 3 parallel controls were conducted at the same time. Ethyl acetate (EtOAc) extraction of the fermentation and the controls were compared on HPLC with an Agilent TC-C18 column (250×4.6 mm, 10–100% methanol in 30 min, 1 ml/min, detected at 254 and 210 nm).

The fermentation broth was extracted by EtOAc to yield 12 g of residue A. The residue was then chromatographed on a silica flash column (eluted with gradient Chloroform-Methanol) to give four fractions (Fr.1-Fr.4). Fr.2 (3.4 g) was subjected on a Sephadex LH-20 (Methanol) and silica flash chromatography (eluted with gradient Petroleum ether-Acetone) to give three subfractions (Fr.21-Fr.23). Fr.21 (760 mg) was purified on a semi-preparative HPLC C18 column (Thermo BDS Hypersil 250×10 mm, eluted with 64% Methanol) to afford compound 3 (14 mg), compound 5 (24 mg) and compound 7 (21 mg). Fr.23 (380 mg) was separated on the semi-preparative HPLC C18 column (eluted with 55% Methanol) to yield compound 1 (16 mg), compound 2 (2.8 g), compound 4 (24 mg). Fr.3 (3.9 g) was chromatographed with Sephadex LH-20 (methanol) and flash ODS column to give seven subfractions (Fr.31-Fr.37). Fr.36 (197 mg) was separated on the semi-preparative HPLC C18 column (eluted with 63% methanol) to yield compound 6 (18 mg).

**Compound 1**: white amorphous solid; [α]D^29 −129.0 (c 0.14, CHCl3); UV (MeCN) λ_max(logε) 248 nm (3.91); CD (MeCN) λ (Δε in cm^−1 M^−1) 205 (−9.3), 224 (−3.1), 248 (−7.8), 329 (+1.1) nm; IR (KBr) ν_max 3402, 2956, 2873, 2801, 1653, 1602, 1459, 1380, 1283, 1205, 1048, 1031 cm^−1; 1H NMR and 13C NMR data, see Figure S2; ESIMS m/z 235.10 [M+H]^+; HR ESIMS m/z 257.1508 [M+Na]^+ (calcd. for C_{15}H_{22}O_{2} [M+Na]^+ 257.1512).

**Compound 2**: white amorphous solid; [α]D^26 −115.6 (c 0.09, CHCl3); UV (MeCN) λ_max(logε) 244 nm (3.98); CD (MeCN) λ (Δε in cm^−1 M^−1) 210 (+10.8), 246 (−7.4), 335 (+1.7) nm; IR (KBr) ν_max 3423, 2925, 2873, 1645, 1613, 1461, 1379, 1272, 1234, 1032 cm^−1; 1H NMR and 13C NMR data, see Figure S2; ESIMS m/z 235.21 [M+H]^+; HR ESIMS m/z 257.1504 [M+Na]^+ (calcd. for C_{15}H_{22}O_{2} [M+Na]^+ 257.1512).

**Compound 3**: colorless oil; [α]D^26 −11.8 (c 0.12, CHCl3); UV (MeCN) λ_max(logε) 193 nm (3.85); CD (MeCN) λ (Δε in cm^−1 M^−1) 207 (−4.7), 239 (+8.1) nm; IR (KBr) ν_max 3464, 2952, 2928, 1669, 1459, 1388, 1373, 1308, 1265, 1198, 1089, 1024 cm^−1; 1H NMR and 13C NMR data, see Figure S2; ESIMS m/z 253.23 [M+H]^+; HR ESIMS m/z 257.1505 [M+Na]^+ (calcd. for C_{15}H_{22}O_{2} [M+Na]^+ 257.1512).

**Compound 4**: colorless oil; [α]D^26 −89.3 (c 0.08, CHCl3); UV (MeCN) λ_max(logε) 237 nm (4.13); CD (MeCN) λ (Δε in cm^−1 M^−1) 201 (+8.4), 234 (−4.4) nm; IR (KBr) ν_max 3440, 2949, 2870, 2852, 1660, 1442, 1379, 1685, 1319, 1296, 1252, 1048 cm^−1; 1H NMR and 13C NMR data, see Figure S2; ESIMS m/z 257.12 [M+Na]^+; HR ESIMS m/z 257.1506 [M+Na]^+ (calcd. for C_{15}H_{22}O_{2} [M+Na]^+ 257.1512).

**Compound 5**: colorless oil; [α]D^26 −93.1 (c 0.11, CHCl3); UV (MeCN) λ_max(logε) 193 nm (4.28); CD (MeCN) λ (Δε in cm^−1 M^−1) 200 (+11.7), 226 (−3.0) nm; IR (KBr) ν_max 3437, 2952, 2931, 2874, 1726, 1665, 1631, 1437, 1378, 1316, 1250, 1134 cm^−1; 1H NMR and 13C NMR data, see Figure S2; ESIMS m/z 263.20 [M+H]^+; HR ESIMS m/z 285.1452 [M+Na]^+ (calcd. for
Compound 6: crystal; [α]D\textsuperscript{21} +60.5 (c 0.20, CHCl\textsubscript{3}); IR (KBr) ν\textsubscript{max} 3277, 2959, 2924, 2868, 1448, 1386, 1033, 993 cm\textsuperscript{-1}; \textsuperscript{1}H NMR and \textsuperscript{13}C NMR data, see Figure S2; EIMS m/z 236 [M]\textsuperscript{+}; HR EIMS m/z 236.1775 [M]\textsuperscript{+} (calcd. for C\textsubscript{16}H\textsubscript{20}O\textsubscript{2} [M]\textsuperscript{+} 236.1776).

Compound 7: colorless oil; [α]D\textsuperscript{29} +46.1 (c 0.12, CHCl\textsubscript{3}); IR (KBr) ν\textsubscript{max} 3441, 2927, 2868, 1459, 1732, 1379, 1068, 1016 cm\textsuperscript{-1}; \textsuperscript{1}H NMR and \textsuperscript{13}C NMR data, see Figure S2; EIMS m/z 250 [M]\textsuperscript{+}; HR EIMS m/z 250.1935 [M]\textsuperscript{+} (calcd. for C\textsubscript{16}H\textsubscript{20}O\textsubscript{2} [M]\textsuperscript{+} 250.1933).

Calculation Procedures

A preliminary conformational search was performed in Conflex6.7 using MMFF94s forcefield. Conformers were saved and further optimized using the density functional theory (DFT) method and CPCM solvent model at B3LYP/6-311++G(d,p) level in Gaussian 09 software package. Frequency was calculated at the same level of theory to check optimized results. The stable conformers with populations greater than 1% and without imaginary frequencies were submitted to ECD calculation by the TDDFT [B3LYP/aug-cc-pVDZ or cam-B3LYP/TZVP] method associated with CPCM solvent model in MeCN. The excitation energies (E), oscillator strength (f), rotatory strength in velocity form (R\textsubscript{vel}), and rotatory strength in length form (R\textsubscript{len}) of the lowest 32 excited states were calculated. ECD spectra of different conformers were summated in SpecDis 1.62 according to their Boltzmann-calculated distributions. The half bandwidths and UV-shifts of CD summation were recorded as Table S1.

Table S1 Parameters in CD curves summation

<table>
<thead>
<tr>
<th>Compd.</th>
<th>NC*</th>
<th>CD calculation method</th>
<th>half bandwidth (σ, eV)</th>
<th>UV-shifts (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>Cam-B3LYP/TZVP</td>
<td>0.30</td>
<td>+6</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>B3LYP/aug-cc-pVDZ</td>
<td>0.30</td>
<td>–11</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Cam-B3LYP/TZVP</td>
<td>0.30</td>
<td>+5</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>B3LYP/aug-cc-pVDZ</td>
<td>0.29</td>
<td>–9</td>
</tr>
</tbody>
</table>

*NC, number of stable conformers

Figure S1. HPLC profiles of *A. tubingensis* KJ-9 fermentation

Chromatographi ccondition: Agilent TC-C18 column, gradient 10-100% MeOH in 30 min, detected by 230 nm
Figure S2. Assignments of $^1$H (500M Hz) and $^{13}$C (125M Hz) NMR Data
δ in ppm, J in Hz; *overlapped
Figure S3. $^1$H (500M Hz) and $^{13}$C (125M Hz) NMR and DEPT spectra of 1
Figure S4. HSQC of 1
Figure S5. $^1$H-$^1$H COSY
Figure S6. HMBC of 1
Figure S7. NOESY of 1
Figure S8. $^1$H (500M Hz) and $^{13}$C (125M Hz) NMR and DEPT spectra of 2
Figure S9. HSQC spectrum of 2
Figure S10. COSY spectrum of 2
Figure S11. HMBC spectrum of 2
Figure S12. NOESY spectrum of 2
Figure S13. $^1$H (500M Hz) and $^{13}$C (125M Hz) NMR and DEPT spectra of 3
Figure S14. HSQC of 3
Figure S15. COSY of 3
Figure S16. HMBC of 3
Figure S17. NOESY of 3
Figure S18. $^1$H (125M Hz) and $^{13}$C NMR of 4
Figure S19. HSQC of 4
Figure S20. $^1$H-$^1$H COSY of 4
Figure S21. HMBC of 4
Figure S22. NOESY of 4
Figure S23. $^1$H (500M Hz) and $^{13}$C (125M Hz) NMR and DEPT spectra of 5
Figure S24. HSQC of 5
Figure S25. $^1$H-$^1$H COSY of 5
Figure S26. HMBC of 5
Figure S27. NOESY of 5
Figure S28. $^1$H (500M Hz) and $^{13}$C (125M Hz) NMR spectra of 6
Figure S29. HSQC of 6
Figure S30. $^1$H-$^1$H COSY of 6
Figure S31. HMBC of 6
Figure S32. NOESY of 6
Figure S33. $^1$H (500M Hz) and $^{13}$C (125M Hz) NMR and DEPT spectra of 7
Figure S34. HSQC of 6
Figure S35. $^1$H-$^1$H COSY of 6
Figure S36.  HMBC of 6
Figure S37. NOESY of 6