Novel sesquiterpenoids from cultures of the basidiomycete *Irpex lacteus*

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Keywords:

*Irpex lacteus*

Sesquiterpenoids

Irlactins A–E

Sesquiterpenoids produced by higher fungi have been one of the most important secondary metabolites, which possess a rich variation of structures and significant biological activities, attracting great interest of many chemists.1 Most of them are formed via the humulene biosynthetic pathway.1 One pathway starting from humulene ends in the irregular sesquiterpenes of the tremulane type, which was first isolated from cultures of *Phellinus tremulae*,2 and recently from cultures of the basidiomycete *Conocybe siliginea*3 and *Phellinus igniarius*4.

*Irpex lacteus*, a pathogenic wood-decaying fungus, belongs to the family Polyporaceae.5 It has been used traditionally as drug for-the family Polyporacea.5 It has been used traditionally as drug for-the family Polyporacea.5 It has been used traditionally as drug for-

Irlactins A–D (1–4), four novel sesquiterpenoids with a rearranged 6/6 bicyclic system, together with their presumed biosynthetic precursor irlactin E (5), were isolated from cultures of the *Irpex lacteus*. Their structures were elucidated by means of spectroscopic methods, and the absolute configurations of 1–4 were established by single crystal X-ray diffraction analysis. A hypothetical biogenetic pathway for irlactins A–D (1–4) was proposed.

Compounds 2–4 are obtained as a mixture in solution, while a cocrystal of 3/4 mixture was obtained in methanol. The X-ray diffraction revealed the absolute configurations of 1–4. Irlactins A–D (1–4) possessed a new carbon skeleton in sesquiterpenoid family, which could be derived from irlactin E (5) via a ring rearrangement. In this Letter, we report the isolation, structure elucidation, and a hypothetical biogenetic pathway of irlactins A–E (1–5).

Irlactin A (1) was obtained as a colorless solid.6 The molecular formula was determined to be C16H26O4 with four degrees of unsaturation, as deduced by HREIMS at m/z 282.1822 [M]+ (calcd 282.1831). The IR absorption bands at 3432, 3441, and 1631 cm−1 revealed the existence of hydroxy groups and a double bond. The 13C and DEPT NMR spectra revealed four quaternary carbons (two olefinic carbons at δC 135.1 and 137.9, two sp3 quaternary carbons at δC 33.4 and 48.1), four sp3 methines (three oxygenated at δC 68.0, 81.1, and 110.2), four sp3 methylenes (one oxygenated at δC 61.5), and four methyis (one methoxyl at δC 55.1) (Table 1). These data suggested that 1 might be a sesquiterpenoid bearing a three-ring system.

The gross structure of 1 was initially deduced by comprehensive analysis on its 1D and 2D NMR spectra. Observations of HMBC correlations from the geminal methyls Me-14 (δH 0.78) and Me-15 (δH 1.00) to C-8, C-9, and C-10, from H-10a (δH 2.18) and H-10b (δH 1.74) to C-1, C-2, and C-6, and from H-13 (δH 0.92) to C-1, C-5, C-6, and C-7, together with the 1H–1H COSY cross peak between H-7 and H-8, gave a six-membered ring A (Fig. 1). The HMBC correlations from H-5 (δH 4.23) to C-1 and C-3, from H-11a (δH 4.17) and H-11b (δH 3.95) to C-1, C-2, and C-3, along with the 1H–1H COSY correlations of H-3/H-4/H-5, established a six-membered ring B (Fig. 1). The key HMBC correlations from H-12 (δH 4.68) to

![Diagram of sesquiterpenoids](image-url)
OH

Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>1 H</th>
<th>2 H</th>
<th>3 H</th>
<th>4 H</th>
<th>5 H</th>
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<td>137.9 s</td>
<td>131.9 s</td>
<td>131.9 s</td>
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<td>134.4 s</td>
<td>135.1 s</td>
<td>134.8 s</td>
<td>134.4 s</td>
<td>136.4 s</td>
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<tr>
<td>3</td>
<td>2.69 d (3.9)</td>
<td>44.7 d</td>
<td>2.64 m</td>
<td>45.7 d</td>
<td>2.60 m</td>
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<td>4a</td>
<td>2.09 m</td>
<td>28.9 t</td>
<td>2.17 m</td>
<td>28.2 t</td>
<td>2.16 m</td>
</tr>
<tr>
<td>4b</td>
<td>1.84 d (10.8)</td>
<td>1.86 m</td>
<td>1.83 m</td>
<td>1.54 m</td>
<td>1.68 d (12.7)</td>
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<tr>
<td>5</td>
<td>4.23 d (6.1)</td>
<td>81.1 d</td>
<td>4.25 m</td>
<td>80.7 d</td>
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<tr>
<td>6</td>
<td>4.12 dd (11.7, 5.6)</td>
<td>68.0 d</td>
<td>4.09 m</td>
<td>67.8 d</td>
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<tr>
<td>7</td>
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<td>1.50 m</td>
<td>44.2 t</td>
<td>1.50 m</td>
</tr>
<tr>
<td>8</td>
<td>33.4 s</td>
<td>38.4 t</td>
<td>38.2 t</td>
<td>38.2 t</td>
<td>38.2 t</td>
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<tr>
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<td>1.97 m</td>
</tr>
<tr>
<td>10a</td>
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<td>1.69 m</td>
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<td>11a</td>
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<td>4.26 m</td>
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<tr>
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<td>4.68 s</td>
<td>110.2 d</td>
<td>5.08 s</td>
<td>103.0 d</td>
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<tr>
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<td>0.92 s</td>
<td>16.2 q</td>
<td>0.92 s</td>
<td>16.0 q</td>
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<td>0.77 s</td>
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<td>0.92 s</td>
</tr>
<tr>
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<td>1.00 s</td>
<td>32.8 q</td>
<td>0.99 s</td>
<td>32.6 q</td>
<td>0.99 s</td>
</tr>
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</table>

a Data were assigned by the HSQC, HMBC, 1H–1H COSY and ROESY spectra.

b Spectra were measured in methanol-d4 (Bruker Drx-600).

c Spectra were measured in CDCl3 (Bruker Drx-600).

C-3 and C-5 indicated that a five-membered acetal ring formed between C-5 and C-12, while the HMBC correlation from 12-OC (δH 3.33) to C-12 suggested a methoxyl was connected to C-12.

In the ROESY spectrum (Fig. 1), the presence of correlations of H-3/H-12 indicated H-12 to be α oriented. Detailed analysis of these data suggested that the other parts of 1 were the same to those of 1, which suggested that the relative configuration of 2 was the same to that of 1. Therefore, compound 2 was elucidated as irilactin B, as shown.

The NMR data of irlactin C (Fig. 1) revealed four quaternary carbons (two olefinic carbons at δC 135.1, and 137.9), four sp3 methines (three oxygenated at δC 67.8, 80.7, and 103.0), four sp3 methylenes (one oxygenated at δC 61.4), and three methyls. These data were very similar to those of 1 except for a hydroxy at C-12 in 2 instead of the methoxyl in 1, which was confirmed by HMBC correlations from H-12 (δH 5.08) to C-2, C-3, C-4, and C-5. In the ROESY spectrum, the correlations of H-5/H-13, H-3/H-12, H-3/H-4x, and H-4/H-13 indicated that H-3, H-5, H-12, and Me-13 were in the same side. The correlations of H-7/H-13 and H-7/H-5 were not observed in the ROESY spectrum, implying that H-7 should be α oriented. In addition, the chemical shifts of C-3, C-5, C-6, and C-7 of 2 were very close to those of 1, which suggested that the relative configuration of 2 was the same to that of 1. Therefore, compound 2 was elucidated as irilactin B, as shown.

In the same 1D and 2D NMR spectra, irilactin C (3) was found to possess four quaternary carbons (two olefinic carbons), four methines (three oxygenated), four methylenes (one oxygenated), and three methyls (Table 1), which were very similar to those of 2. However, a hemiacetal ring was formed between C-11 and C-12 in 3 rather than between C-12 and C-5 in 2, as established by the HMBC correlation from H-12 (δH 5.37) to C-11. The ROESY correlation of H-3/H-12 indicated H-12 to be β oriented. Detailed analysis of other 2D NMR data suggested that the other parts of 3 were the same to those of 2.

The NMR data of irilactin D (4) showed features similar to those of 3. Detailed analysis of these data suggested that 4 was an epimer of 3, as indicated by a significant variation of 13C NMR signal at δC 104.5 (d) for C-12 in 4 (δC 99.0 for C-12 in 3). In addition, the absent ROESY correlation between H-3 and H-12 also suggested the relative configuration at C-12 in 4 was opposite to that in 3.

Irilactin E (5), a colorless oil, was assigned the molecular formula C20H26O11 by the positive HRESIMS (found at m/z 273.1463 [M+Na]+, calc. for 273.1466). The 13C and DEPT NMR spectra of 5 (Table 1) revealed 15 carbon resonances, including three sp3
quaternary carbons at \( \delta_c \) 171.4, 136.4, and 190.3, three methylene carbons at \( \delta_c \) 42.1, 46.6, and 28.7, three methyls at \( \delta_c \) 12.3, 26.4, and 28.7, five methine carbons at \( \delta_c \) 44.1, 41.3, 35.8, 84.7, and 102.7, and one quaternary carbon at \( \delta_c \) 37.5. These data exhibited similarities with those of conocenol D which suggested that compound 5 possessed the same tremulane type skeleton as that of conocenol D. The key differences between the two compounds were an aldehyde at C-2 and a hydroxy at C-12 in 5 instead of an oxymethylene and a methoxy respectively in conocenol D, which were confirmed by HMBC correlations from H-3 to C-11, from H-11 to C-2 and C-3, and from H-3, H-4, and H-5 to C-12. The ROESY correlations of H-7/H-12, H-7/H-6, H-3/H-4a, H-4a/H-5, H-4b/H-13, and H-7/H-14 indicated that H-3, H-5, Me-13, and Me-15 were in the same side, while H-6, H-7, Me-14, and
H-12 in the opposite side (Fig. 3). Therefore, compound 5 was established to be irlactin E.

Biogenetically, irlactin E (5) may be considered as a precursor of irlactins A–D (1–4). As shown in Scheme 1, irlactin E (5) underwent a ring arrangement, then formed a 6/6 ring system in irlactins A–D (1–4). In detail, the hemiacetal functionality in 5 might be opened to form the formyl group at C-12 and the hydroxy group at C-5. Then, the formation of a carbocation led to the ring rearrangement from a 5/7 ring system to a 6/6 ring system, producing a key intermediate 5a (Scheme 1). The latter interconverted into irlactins A–D (1–4) due to instability of the hemiacetal functionality in solution.

Fortunately, a cocrystal of the 3/4 mixture was obtained from methanol. In solution, compounds 3 and 4 were inseparable and underwent spontaneous α,β-anomerization similar to the process of mutarotation of the hemiacetal functionality in carbohydrates.11 The X-ray diffraction analysis (Fig. 4),12 in combination with the ROESY data analysis, as well as the biogenetical discussion, revealed the absolute configuration of 1–4 to be (3R,5S,6R,7S,12R)-irlactin A (1), (3R,5S,6R,7S,12R)-irlactin B (2), (3R,5S,6R,7S,12S)-irlactin C (3), and (3R,5S,6R,7S,12R)-irlactin D (4), respectively.

Due to the limited amount available of irlactins A–D (1–4), only irlactin E (5) was tested for its cytotoxicities against five human cancer cell lines using the MTT method13 with minor modification and for its inhibitory activity against isozymes of 11β-hydroxysteroid dehydrogenases (11β-HSD1) (see Supplementary data). Unfortunately, irlactin E (5) was inactive in these bioassays.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.03.038.

References and notes

8. Irlactin A (1): White solid; [α]D20 = −15.1 (c 0.03, MeOH); IR (KBr) νmax: 3432, 3452, 2024, 1630, 1464, 1384, 1286, 1252, 1044 cm−1; 1H (600 MHz) and 13C NMR (150 MHz) data (methanol-d4), see Table 1; HREIMS m/z 282.1822 [M]+ (calcd for C16H26O4, 282.1831).
9. Irlactins B–D (2–4): Colorless crystal, mp 156°C; [α]D20 = +3.5 (c 0.18, MeOH); IR (KBr) νmax: 3404, 2928, 1452, 1356, 1057, 995 cm−1; 1H (600 MHz) and 13C NMR (150 MHz) data (methanol-d4), see Table 1; HREIMS m/z 268.1654 [M]+ (calcd for C15H24O4, 268.1675).
10. Irlactin E (5): Colorless oil; [α]D20 = +7.9 (c 0.41, MeOH); IR (KBr) νmax: 3426, 2954, 1728, 1665, 1625, 1462, 1379, 1241, 1014, 1024 cm−1; 1H (400 MHz) and 13C NMR (100 MHz) data (CDCl3), see Table 1; positive ion HREIMS m/z 273.1463 [M+Na]+ (calcd for C16H22O3Na2, 273.1465).
12. Crystal data for irlactins C (3) and D (4): C15H24O4, MW = 268.34; orthorhombic, space group P21a21c; a = 6.2769(3) Å, b = 9.1477(4) Å, c = 24.4170(11) Å, α = γ = 90°, V = 1402.00(11) Å3, Z = 4, d = 1.271 g/cm3, crystal dimensions 0.52 × 0.24 × 0.13 mm3 was used for measurement on a Bruker AXS D800 with a graphite monochromator, Cu Kα radiation. There are 5321 reflections measured, including 2261 independent reflections (Rint = 0.0505). The final R1 values were 0.0755 (I > 2σ(I)). The final wR(F2) value was 0.1840 (I > 2σ(I)). The final R1 value was 0.0763 (all data). The final wR(F2) value was 0.1846 (all data). The goodness of fit on F2 was 1.176. The crystal structures of 3 and 4 were solved by a direct method (SHELXS-97 (Sheldrick, 1990) and expanded using the difference Fourier technique, refined by the program SHELXL-97 (Sheldrick, 1997) and the full-matrix least-square calculations. The overall population of the two epimers in the crystal is 69.4% and 30.6%, respectively. Crystallographic data for the structure of 3 and 4 have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 903730). Copies of these data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: +44 1223 316 033; or depository@ccdc.cam.ac.uk).