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Novel sesquiterpenoids from cultures of the basidiomycete *Irpex lacteus*

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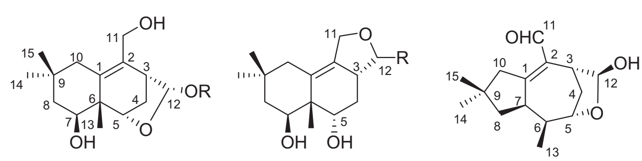
ABSTRACT

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.

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 *Phelinus 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 Polyporacea.[5] It has been used traditionally as drug formulations in Traditional Chinese Medicine for the treatment of chronic glomerulonephritis in clinic.[6] Previously, only three new nematicidal metabolities were isolated from a culture filtrate of *I. lacteus*.[7] In the current study, an EtOAc layer (16 g) of a 25 L culture broth of *I. lacteus* was separated by silica gel, RP-18, and Sephadex LH-20 to give four sesquiterpenoids, irlactin A (**1**, 0.8 mg), a mixture of irlactins B–D (**2–4**, 3.6 mg), and irlactin E (**5**, 20.3 mg).



1 R = CH_3
2 R = H

3 R= alpha OH
4 R= beta OH

5

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 biosynthetic pathway of irlactins A–E (**1–5**).

Irlactin A (**1**) was obtained as a colorless solid.[8] The molecular formula was determined to be $C_{16}H_{26}O_4$ 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 ^{13}C and DEPT NMR spectra revealed four quaternary carbons (two olefinic carbons at δ_C 135.1 and 137.9, two sp^3 quaternary carbons at δ_C 33.4 and 48.1), four sp^3 methines (three oxygenated at δ_C 68.0, 81.1, and 110.2), four sp^3 methylenes (one oxygenated at δ_C 61.5), and four methyls (one methoxyl at δ_C 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 (δ_H 0.78) and Me-15 (δ_H 1.00) to C-8, C-9, and C-10, from H-10a (δ_H 2.18) and H-10b (δ_H 1.74) to C-1, C-2, and C-6, and from H-13 (δ_H 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 (δ_H 4.23) to C-1 and C-3, from H-11a (δ_H 4.17) and H-11b (δ_H 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 (δ_H 4.68) to

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Table 1
^{1}H and ^{13}C NMR data of **1–5** (δ in ppm, *J* in Hz)[a]

No.	1[b]		2[b]		3[b]		4[b]		5[c]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		137.9 s		137.9 s		131.9 s		131.9 s		171.4 s
2		134.4 s		135.1 s		134.8 s		134.4 s		136.4 s
3	2.69 d (3.9)	44.7 d	2.64 m	45.7 d	2.60 m	46.0 d	2.36 m	47.4 d	3.51 d (8.0)	41.3 d
4a	2.09 m	28.9 t	2.17 m	28.2 t	2.16 m	28.8 t	2.01 m	31.0 t	2.13 m	28.7 t
4b	1.84 d (10.8)		1.86 m		1.83 m		1.54 m		1.68 d (12.7)	
5	4.23 d (6.1)	81.1 d	4.25 m	80.7 d	3.80 m	80.0 d	3.80 m	80.0 d	4.64 dd (6.9, 7.3)	84.7 d
6		48.1 s		47.7 s		44.2 s		44.4 s	2.13 m	35.8 d
7	4.12 dd (11.7, 5.6)	68.0 d	4.09 m	67.8 d	4.13 m	71.4 d	4.08 m	71.3 d	3.47 m	44.1 d
8	1.49 m	44.3 t	1.50 m	44.2 t	1.50 m	44.4 t	1.50 m	44.1 t	1.48 m	42.1 t
9		33.4 s		33.9 s		33.2 s		33 s		37.5 s
10a	2.18 m	38.4 t	2.18 m	38.2 t	1.97 m	40.2 t	1.94 m	39.1 t	2.97 d (16.6)	46.6 t
10b	1.74 d (13.8)		1.74 m		1.69 m		1.72 m		2.23 d (16.7)	
11a	4.17 d (12.1)	61.5 t	4.19 d (12.1)	61.4 t	4.39 m	68.3 t	4.39 m	67.7 t	9.82 s	190.3 d
11b	3.95 d (12.1)		3.92 d (12.1)		4.26 m		4.26 m			
12	4.68 s	110.2 d	5.08 s	103.0 d	5.37 d (4.7)	99.0 d	4.84 d (6.9)	104.5 d	5.35 s	102.7 s
13	0.92 s	16.2 q	0.92 s	16.0 q	1.22 s	17.7 q	1.21 s	17.9 q	0.74 d (7.0)	12.3 q
14	0.78 s	25.7 q	0.77 s	25.5q	0.92 s	25.6 q	0.88 s	25.6 q	1.16 s	28.7 q
15	1.00 s	32.8 q	0.99 s	32.6 q	0.99 s	32.4 q	0.99 s	32.3 q	0.92 s	26.4 q
OMe	3.33 s	55.1 q								

[a] Data were assigned by the HSQC, HMBC, ^{1}H–^{1}H COSY and ROESY spectra.
[b] Spectra were measured in methanol-d_4 (Bruker Drx-600).
[c] Spectra were measured in $CDCl_3$ (Bruker Drx-600).

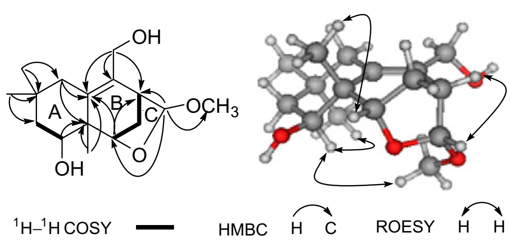
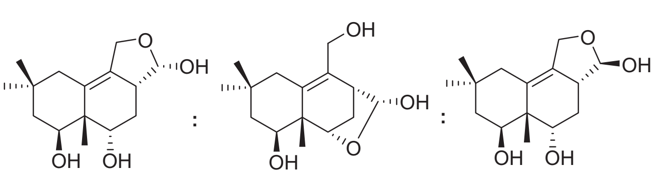


Figure 1. Key 2D NMR correlations of **1**.

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-OCH_3 (δ_H 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-5/H-13, H-3/H-12, H-7/H-14, and H-7/12-OCH_3 suggested that H-3, H-5, H-12, and Me-13 were in the same side, while H-7 and Me-14 in the opposite side.

Irlactins B–D (**2**–**4**) were isolated in the form of an inseparable mixture.[9] In the HREIMS, the molecular ion peak was observed at *m*/*z* 268.1654 [M]$^+$ (calcd 268.1675), corresponding to the molecular formula $C_{15}H_{24}O_4$. In the ^{1}H and ^{13}C NMR spectra of this mixture, the resonances appeared as pairs or were overlapped. However, careful analysis of the 1D and 2D NMR spectra allowed for the unambiguous assignments of the signals for each compound, which revealed that compounds **2**–**4** existed in solution as a solvent-dependent mixture of three stereoisomers in a ratio



	3		**2**		**4**
a	21	:	9	:	8
b	21	:	21	:	8

Figure 2. The ratio of **2**/**3**/**4** in ^{13}C NMR (a: in pyridine-d_5; b: in methanol-d_4).

of 9:21:8 in pyridine-d_5 and 21:21:8 in methanol-d_4, respectively (Fig. 2).

The ^{13}C and DEPT NMR spectra (Table 1) of irlactin B (**2**) revealed four quaternary carbons (two olefinic carbons at δ_C 135.1, and 137.9), four sp^3 methines (three oxygenated at δ_C 67.8, 80.7, and 103.0), four sp^3 methylenes (one oxygenated at δ_C 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 (δ_H 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-4α, and H-4β/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 irlactin B, as shown.

In the same 1D and 2D NMR spectra, irlactin 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 (δ_H 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 irlactin D (**4**) showed features similar to those of **3**. Detailed analysis of these data suggested that **4** was an *epi*-isomer of **3**, as indicated by a significant variation of ^{13}C NMR signal at δ_C 104.5 (d) for C-12 in **4** (δ_C 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**.

Irlactin E (**5**),[10] a colorless oil, was assigned the molecular formula $C_{15}H_{22}O_3$ by the positive HRESIMS (found at *m*/*z* 273.1463 [M+Na]$^+$, calcd for 273.1466). The ^{13}C and DEPT NMR spectra of **5** (Table 1) revealed 15 carbon resonances, including three sp^2

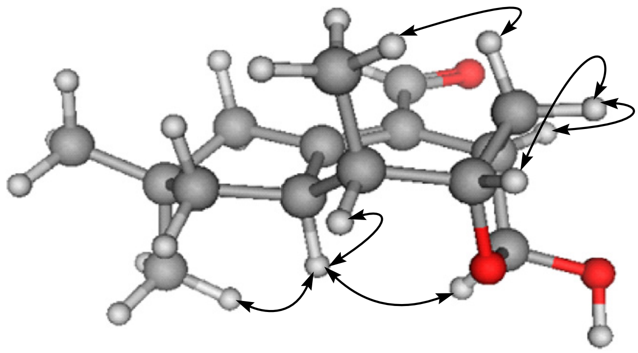
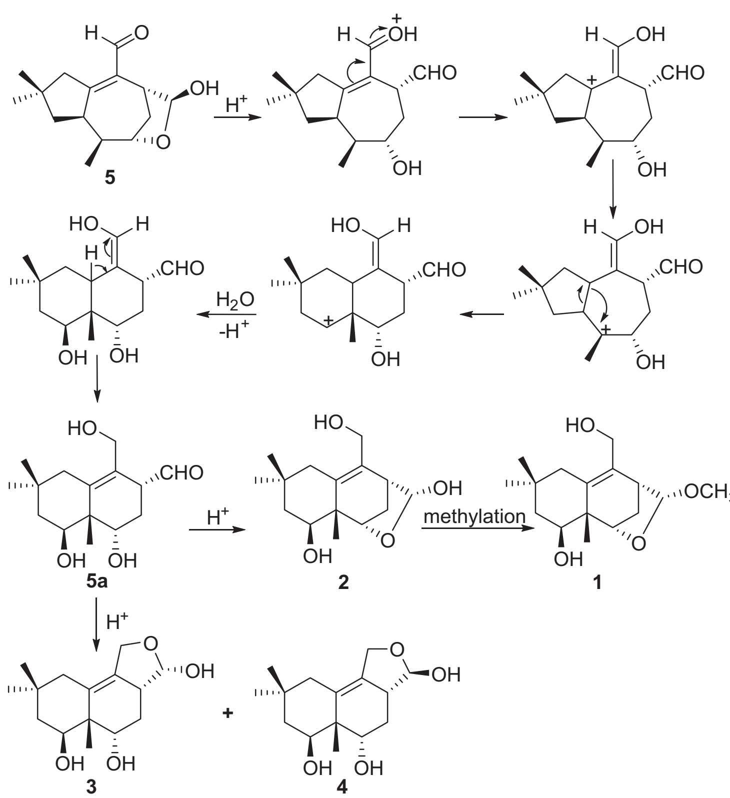


Figure 3. Key ROESY correlations of **5**.

quaternary carbons at δ_C 171.4, 136.4, and 190.3, three methylene carbons at δ_C 42.1, 46.6, and 28.7, three methyls at δ_C 12.3, 26.4, and 28.7, five methine carbons at δ_C 44.1, 41.3, 35.8, 84.7, and 102.7, and one quaternary carbon at δ_C 37.5. These data exhibited similarities with those of conocenol D[3] 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-4α, H-4α/H-5, H-4β/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



Scheme 1. Plausible biogenetic pathway of **1**–**4**.

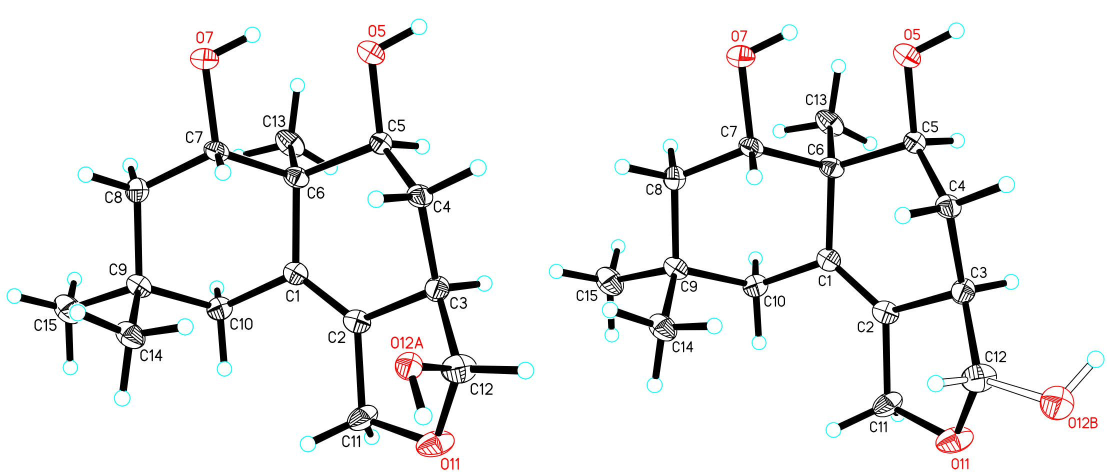


Figure 4. ORTEP drawing of cocrystals of **3** (left) and **4** (right) showing the absolute configuration. The atoms represented as outlines are the minor (30.6%) components **4**.

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 carbenium ion 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 (3*R*,5*S*,6*R*,7*S*,12*R*)-irlactin A (**1**), (3*R*,5*S*,6*R*,7*S*,12*R*)-irlactin B (**2**), (3*R*,5*S*,6*R*,7*S*,12*S*)-irlactin C (**3**), and (3*R*,5*S*,6*R*,7*S*,12*R*)-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 method[13] 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.

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8. Irlactin A (**1**): White solid; $[\alpha]_D^{24}$ −15.1 (*c* 0.03, MeOH); IR (KBr) ν_{max}:3440, 3432, 2924, 1630, 1464, 1384, 1286, 1252, 1044 cm^{-1}; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data (methanol-d_4), see Table 1; HREIMS *m*/*z* 282.1822 [M]$^+$ (calcd for $C_{16}H_{26}O_4$, 282.1831).
9. Irlactins B–D (**2**–**4**): Colorless crystal, mp 156 °C; $[\alpha]_D^{19}$ +3.5 (*c* 0.18, MeOH); IR (KBr) ν_{max}: 3404, 2928, 1452, 1356, 1057, 1036, 995 cm^{-1}; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data (methanol-d_4), see Table 1; HREIMS *m*/*z* 268.1654 [M]$^+$ (calcd for $C_{15}H_{24}O_4$, 268.1675).
10. Irlactin E (**5**): Colorless oil; $[\alpha]_D^{19}$ +87.9 (*c* 0.41, MeOH); IR (KBr) ν_{max} 3426, 2954, 1728, 1665, 1625, 1462, 1367.9, 1241.7, 1041, 1024 cm^{-1}; ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data ($CDCl_3$), see Table 1; positive ion HRESIMS *m*/*z* 273.1463 [M+Na]$^+$ (calcd for $C_{15}H_{22}O_3Na$, 273.1466).
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12. Crystal data for irlactins C (**3**) and D (**4**): $C_{15}H_{24}O_4$, MW = 268.34; orthorhombic, space group $P2_12_12_1$; a = 6.2769(3) Å, b = 9.1477(4) Å, c = 24.4170(11) Å, $\alpha = \beta = \gamma$ = 90.00, V = 1402.00(11) Å^3, Z = 4, d = 1.271 g/cm^3, crystal dimensions 0.52 × 0.24 × 0.13 mm^3 was used for measurement on a Bruker APEX DUO with a graphite monochromater, Cu Kα radiation. There are 5321 reflections measured, including 2261 independent reflections (R_{int} = 0.0505). The final R_1 values were 0.0755 ($I > 2\sigma(I)$). The final $wR(F^2)$ value was 0.1840 ($I > 2\sigma(I)$). The final R_1 value was 0.0763 (all data). The final $wR(F^2)$ value was 0.1846 (all data). The goodness of fit on F^2 was 1.176. The crystal structures of **3** and **4** were solved by a direct method SHLXS-97 (Sheldrick, 1990) and expanded using the difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997) and the full-matrix least–square calculations. The overall population of the two epimers in the crystal is 69.4% **3** and 30.6% **4**. 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 336 033; or desposit@ ccdc.cam.ac.uk).
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