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Sesquiterpenes from *Omphalotus illudens*

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Abstract

Three sesquiterpenes, illudosone hemiacetal (**1a**), isoomphadione (**2**) and illudiolone (**3**) were isolated from the liquid culture extract of *Omphalotus illudens*. Their structures were elucidated by spectroscopic techniques as well as by X-ray crystallographic analysis.

Keywords: Sesquiterpenes; *Omphalotus illudens*; Basidiomycetes; X-ray crystal structure

1. Introduction

Many sesquiterpenes have been isolated from the basidiomycete *Omphalotus illudens* (*Omphalotus olearius*, formerly *Clitocybe illudens*). Among them are illudin S and illudin M as well as illudol, which were the first in this family to be identified (McMorris and Anchel, 1963). Illudin S and M are highly cytotoxic and exhibit antitumor activity, though the therapeutic index is low. Certain derivatives of these compounds show greatly increased selectivity in toxicity toward malignant cells compared to normal cells. In particular, a derivative of illudin S, irofulven (hydroxymethylacylfulvene), has been extensively investigated and is currently in phase II clinical trials (McMorris et al., 2001).

Illudin S is produced by fermentation of *O. illudens*. After extraction of the culture liquid with ethyl acetate, illudin S can be obtained by crystallization. The mother liquor contains several compounds as reported earlier (McMorris et al., 2000). We now report identification of new metabolites which we have designated illudosone hemiacetal (**1a**), illudosone (**1b**), isoomphadione (**2**) and illudiolone (**3**).

2. Results and discussion

The carbon skeletons of **1**, **2** and **3** are reminiscent of those of illudosin (Arnone et al., 1991), omphadiol (McMorris et al., 2000) and illudol (McMorris et al., 1967) previously reported metabolites of *Omphalotus*. Illudosone hemiacetal (**1a**) was obtained crystalline [mp 87–89 °C, $[\alpha]_D^{25}$ −44.3° (0.1, EtOH)] after repeated chromatography. The compound had a molecular formula of $C_{15}H_{22}O_3$, derived from HRMS (MALDI-FTMS) (MNa^+, 273.1439, calc. 273.1429). The structure (**1a**) was established by X-ray crystallographic analysis (ORTEP diagram, Fig. 1). While the ^1H NMR spectrum determined in DMSO was consistent with the hemiacetal structure the spectrum in $CDCl_3$ solution showed a strong aldehyde proton signal δ 9.98 (structure **1b**) and a weak signal at δ 5.34 about 37% of the signal at δ 9.98, which could be assigned to the hemiacetal proton. Thus it appears that **1a** and **1b** exist as a mixture of hemiacetal and free aldehyde,

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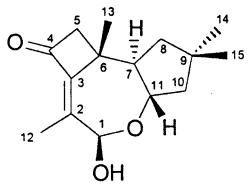
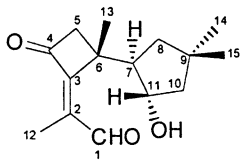


1a



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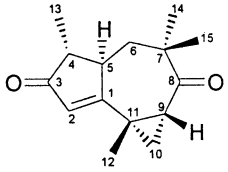

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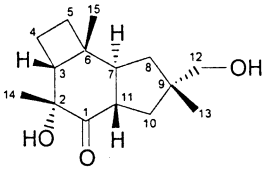

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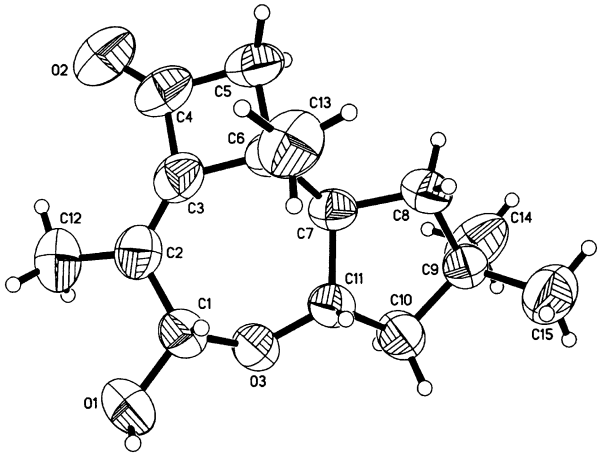

Fig. 1. ORTEP view of X-ray molecular structure of compound **1a**.

the former being favored in the solid state and in the polar solvent DMSO. The IR absorptions ($CHCl_3$) occur at 3452, 2952, 2864, 1744, 1684, 1644 cm^{-1} consistent with a conjugated ketone and free aldehyde and also the UV absorption at λ_{max} 250 (ε 3745) nm. ^1H and ^{13}C NMR spectral data are given in Table 1.

Isoomphadione (**2**) was also obtained crystalline from CH_2Cl_2–hexane [mp 68–70 °C, $[\alpha]_D^{25}$ −112.2° (0.05, EtOH)]. The compound had a molecular formula of $C_{15}H_{22}O_2$; HRMS (MALDI-FTMS) (M^+, 233.1541, calc. 233.1536). It showed IR absorptions at 1700 and 1669 cm^{-1} for two carbonyl groups and NMR spectral data were consistent with the structure (see Table 1). Structure **2** was established by X-ray crystallographic analysis (ORTEP diagram, Fig. 2).

The most polar metabolite, illudiolone (**3**), could not be isolated pure despite repeated chromatography. It was the major component of a mixture of four compounds which was separated by conversion to the 3,5-dinitrobenzoate derivatives. These were separated by chromatography and **3** was regenerated by basic hydrolysis. Pure illudiolone [mp 80–82 °C, $[\alpha]_D^{25}$ +33.1° (0.1, EtOH)] had molecular formula $C_{15}H_{24}O_3$, HRMS (MALDI-FTMS) (MNa^+ 275.1617; calc. 275.1614). It showed IR absorptions at 3484, 3463, and 1707 cm^{-1} and NMR data consistent with structure **3** (see Table 1). The structure was established by X-ray crystallographic analysis (ORTEP diagram Fig. 3).

3. Experimental

3.1. General

Melting points were determined on a Kofler apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were obtained at 300 or 400 and 100 MHz respectively. Spectra were recorded in $CDCl_3$ or DMSO-d_6 with Me_4Si as internal standard (Varian Mercury) HRMS were determined at the Scripps Research Institute, La Jolla, CA (IonSpec Ultima FTMS). Column chromatography was carried out with Si gel (Davisil 230–425 mesh, Fisher scientific). Analytical TLC was carried out on Whatman 4420 222 Si gel plates. Reactions were routinely monitored by TLC. Single crystal X-ray diffraction measurements were made with a Siemens P3/V diffractometer using Wyckoff scans, λ = 0.71073 Å from MoK_α graphite monochromator, SHELXTL PLUS for structure solution and refinement.

3.2. Production of metabolites

O. illudens 4499 was grown in large fermentors (ca. 100 l) to produce Illudin S, at PANLABS, Inc., Bothell, WA (as previously reported, McMorris et al., 2000).

For isolation of illudosone hemiacetal (**1a**), omphadione (**2**) and illudiolone (**3**), a sample (ca. 10 g) of the residue from the mother liquor was repeatedly subjected to Si gel chromatography with EtOAc–hexane (1:10 increasing gradually to 1:1) yielding, in order of elution, acylfulvene (100 mg), illudosone hemiacetal (**1a**, 25 mg), illudin M (500 mg) omphadione (**2**, 15 mg), illudosin (2 g), illudiolone (**3**, 30 mg) and illudin S (4.5 g). Spectral data for **1a**, **1b**, **2** and **3** are given above and in Table 1.

3.3. X-ray crystal structure analysis of *1a*

$C_{15}H_{22}O_3$ at 296 K; $P2_12_12_1$, a = 6.015 (6) Å, b = 10.971 (12) Å; c = 22.24 (3) Å, Z = 4, calc. density = 1.133 mg/m^3, crystal size 2.30×0.40×0.30 mm, 2 to 5°/min 0.6°

Table 1
^{1}H and ^{13}C NMR spectroscopic data for compounds **1**, **2** and **3**

Proton	^{1}H			Carbon	^{13}C		
	1b	**2**	**3**		**1a**[a]	**2**	**3**
1	9.98 (*s*)	–	–	1	95.2	183.1	214.7
2	–	6.11 (*s*)	–	2	145.6	128.7	77.1
3	–	–	2.56 (*t*, 9.4)	3	134.8	214.2	58.5
4α	–		1.85 (*m*)	4	191.2	50.4	19.7
4β		2.32 (*m*)	1.66 (*m*)				
5α	3.06 (*d*, 17.6)	2.22 (*m*)	1.56 (*m*)	5	46.8	45.6	29.7
5β	2.81 (*d*, 17.6)	1.48 (*m*)					
6α	–	1.78 (*t*, 12.6)	–	6	28.0	46.3	40.4
6β	–	1.84 (*m*)	–				
7	1.85 (*dd*, 8.0, 5.6)	–	2.30 (*m*)	7	43.1	40.3	54.0
8α	1.60 (*m*)	–	1.76 (*dd*, 2.6,7.0)	8	36.6	209.3	37.2
8β	1.17 (*t*, 12.0)		1.25 (*t*, 12.4)				
9	–	2.02 (*m*)	–	9	24.8	47.3	42.6
10α	1.55 (*m*)	1.40 (*dd*, 7.8, 4.8)	1.39 (*m*)	10	47.2	23.5	33.6
10β	1.66 (*m*)	1.46 (*t*, 4.8)	1.91 (*m*)				
11	4.06 (*m*)	–	2.86 (*m*)	11	57.0	25.6	46.3
12	2.03 (*s*)	1.46 (*s*)	3.39 (2*d*, J_{AB} 10.2, δ_{AB} 1.6ppm)	12	11.5	24.4	71.3
13	1.73 (*s*)	1.16 (*d*, 4.8)	1.09 (*s*)	13	20.8	16.3	26.8
14	1.11 (*s*)	1.18 (*s*)	1.32 (*s*)	14	30.6	27.1	28.5
15	1.00 (*s*)	1.23 (*s*)	1.16 (*s*)	15	29.8	27.9	22.2

[a] Spectral data taken in DMSO-d_6.

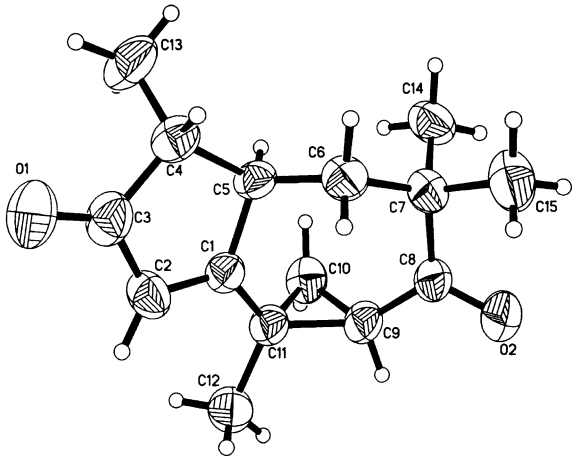

Fig. 2. ORTEP view of X-ray molecular structure of compound **2**.

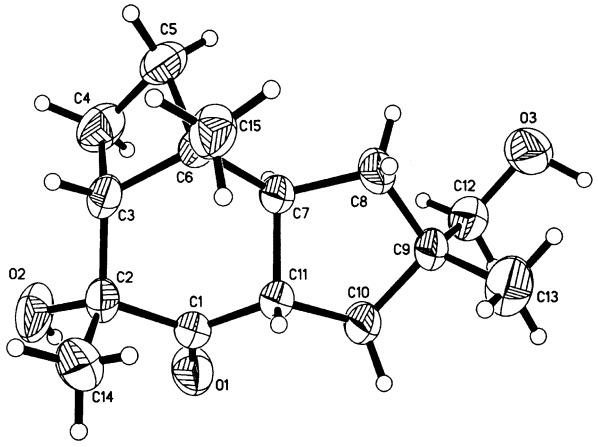

Fig. 3. ORTEP view of X-ray molecular structure of compound **3**.

scan range, 2130, 1475 observed [I > 2 > (I)] from 1.83 to 27.50°, $R = 5.13\%$, residual electron density 0.116 and −0.151 e/ Å^3.

3.4. X-ray crystal structure analysis of *2*

$C_{15}H_{20}O_2$ at 296 K; $P2_12_12_1$, a = 8.902 (8) Å, b = 10.619 (8) Å; c = 13.605 (10) Å, Z = 4, calc. density = 1.200 mg/m^3, crystal size 1.00×0.30×0.20 mm, 2°/min 0.6° scan range, 1429, 1093 observed [I > 2 > (I)] from 2.43 to 25.00°, $R = 5.07\%$, residual electron density 0.173 and −0.129 e/Å^3.

3.5. X-ray crystal structure analysis of *3*

$C_{15}H_{24}O_3$ at 296 K; C_2, a = 19.37 (2) Å, b = 6.141 (4) Å; c = 15.046 (9) Å, Z = 4, calc. density = 1.207 mg/m^3, crystal size 0.80×0.60×0.20 mm, 2 to 10°/min 0.6° scan range, 1790, 1559 observed [I > 2 > (I)] from 1.74 to 27.49°, $R = 4.05\%$, residual electron density 0.207 and −0.127 e/ Å^3.

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