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Sesquiterpenes from the Basidiomycete *Omphalotus illudens*

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A new sesquiterpene, omphadiol (**4**), has been isolated from cultures of *Omphalotus illudens*. Several known compounds, including illudosin (**1**), were also obtained. Structures were determined using MS, NMR, and X-ray techniques.

The basidiomycete *Omphalotus illudens* (*Omphalotus olearius, Clitocybe illudens)* is the source of a large number of sesquiterpenes, several of which possess antibacterial properties.[1] Among these are illudin S and illudin M, which were the first in this family to be identified.[2] Illudins S and M are extremely 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, hydroxymethylacylfulvene, has been extensively investigated and has progressed to phase II human clinical trials.[3]

Hydroxymethylacylfulvene (also designated MGI 114) can be obtained by treating illudin S with paraformaldehyde in dilute H_2SO_4, and this method is used to prepare the compound for clinical trials. Certain strains of *O. illudens* on fermentation yield substantial quantities of illudin S (>4 g/L culture medium). After extraction of the culture liquids with ethyl acetate, illudin S can be obtained by crystallization. The mother liquors contain several compounds, including illudin M, acylfulvene,[4] and dehydroilludin M.[5] The latter two compounds have improved antitumor properties compared to the illudins (S and M) and may now be regarded as bona fide natural products.

Two more compounds from the mother liquors have been identified. The first (**1**), obtained as a colorless oil, had molecular formula $C_{15}H_{24}O_3$, derived from HRMS (MH^+, 253.1805). There were peaks at *m/z* 235 and 217 consistent with loss of one and two molecules of water from the molecular ion. The compound also showed IR absorptions at 3390 and 1670 cm^{-1} and UV absorption, λ_{max} 251 nm (ϵ 7100). The ^1H NMR spectrum contained a signal for one aldehydic proton, which, together with IR and UV evidence, suggested the presence of an α,β-unsaturated aldehyde. The broad-band ^1H decoupled ^{13}C NMR spectrum of **1** showed 15 carbon resonances, the multiplicities of which were obtained by DEPT experiments and analysis of the ^1H-coupled ^{13}C NMR spectrum. Three of them (δ 133.0, 173.3 and 191.4) were assigned to the fully substituted α,β-unsaturated moiety, while the remaining resonances were assigned to four methyl, three methylene, and three methine carbons, two of which were oxygen bearing and two quaternary sp^3-hybridized carbon atoms.

In seeking evidence for the size of each ring in **1**, the compound was oxidized with pyridinium dichromate in CH_2Cl_2. The ^{13}C NMR spectrum of the product (**2**) showed signals for two new carbonyl carbons (δ 216.8 and 198.7), while the IR spectrum had two new carbonyl absorption peaks, at 1735 and 1753 cm^{-1}, consistent with cyclopentanone and cyclobutanone rings. Moreover, this diketo aldehyde was nicely crystalline and suitable for X-ray crystallographic analysis. Thus, the structure of the diketo aldehyde was established as **2** (ORTEP diagram, Figure 1). NMR data were in complete agreement with this structure.

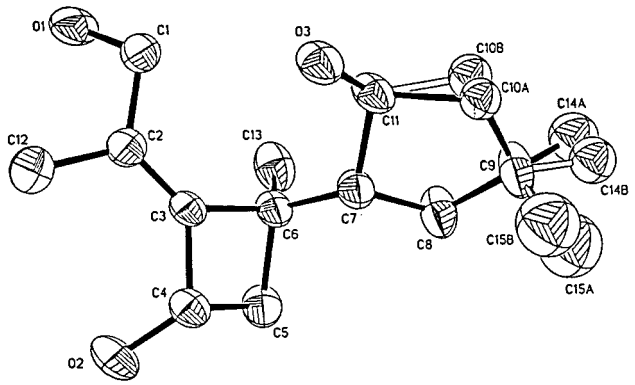

Figure 1. ORTEP view of X-ray molecular structure of compound **2**.

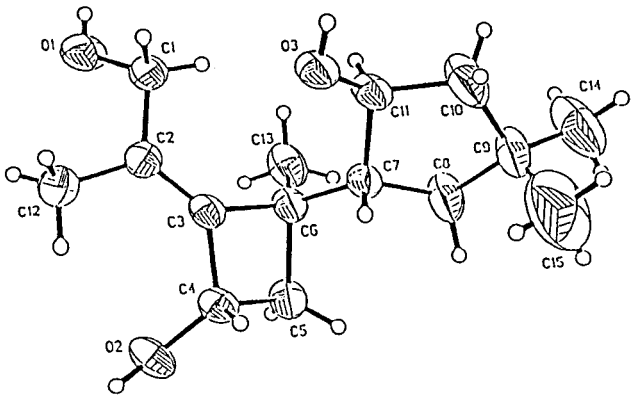

Figure 2. ORTEP view of X-ray molecular structure of compound **3**.

A structure could also be written for the parent compound **1**, though the assignment of stereochemistry at the oxygen-bearing carbons was tentative. This problem was resolved by reduction of **1** with $NaBH_4$ in 2-propanol, which afforded a crystalline triol (**3**). X-ray crystallographic analysis established the structure of the triol (ORTEP diagram, Figure 2) and hence of the parent compound.

A check of the literature revealed that isolation of compound **1** from *O. olearius* had been reported previously by Arnone et al.,[6] who established the structure using 1D

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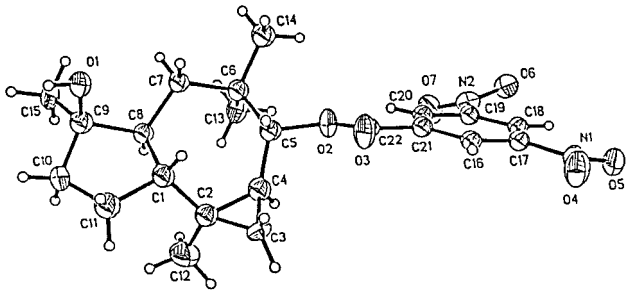
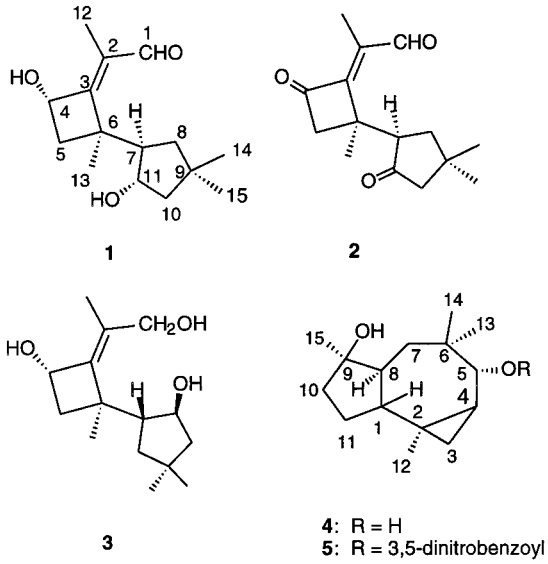


Figure 3. ORTEP view of X-ray molecular structure of compound **5**.

and 2D NMR techniques. The absolute configuration of the compound (designated illudosin) was determined, by applying the exciton chirality method to the corresponding dibenzoate. (The absolute configurations depicted in structures **1**, **2**, and **3** follow from this work.) The possibility that illudosin might be a biosynthetic intermediate to the sesquiterpene fomannosin was also noted.[6]

The second compound (**4**), from the mother liquors mentioned above, did not crystallize after repeated chromatography. However, a 3,5-dinitrobenzoate derivative (**5**) was obtained in crystalline form. The ^{1}H NMR spectrum of **5** showed the presence of four methyl groups and a methine proton on carbon bearing oxygen. The ^{13}C NMR spectrum showed three oxygen-bearing carbons but no olefinic carbons (other than the six benzene carbons). X-ray crystallographic analysis revealed a structure (**5**) (relative stereochemistry; ORTEP diagram, Figure 3) having a seven-membered ring fused to three-membered and five-membered rings. The sesquiterpenoid diol **4** (designated omphadiol), to the best of our knowledge, is a new natural product. However, a number of closely related sesquiterpenes have been isolated from soft corals (*Lemnalia africana*,[7] *Sinularia erecta*,[8] *Sinularia polydactyla*,[9] *Sinularia dissecta*,[10]) and the ascomycete *Leptographium lundbergii*.[11] These include africanol, which has the same carbon skeleton as (**4**), and leptographiol,[11] which lacks the C-5 hydroxyl of **4**.



Illudosin (**1**) was found to have antibacterial activity against *Bacillus subtilis* (ATCC 6633 at 10 μg per disk) and genotoxic activity (at 50 μg per disk) against *Escherichia coli* (PQ-37). No activity was found against *Staphyloccocus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), or *Candida albicans* (ATCC 32354). The diketo aldehyde (**2**) showed slight activity against only *B. subtilis*, while the triol (**3**) was inactive against all organisms tested. Likewise, the dinitrobenzoate (**5**) was inactive in all tests. Omphadiol itself was not tested because of insufficient material.

Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1**−**3**

	^{1}H				^{13}C		
proton	**1**	**2**	**3**	carbon	**1**	**2**	**3**
1	9.88	9.92	4.02	1	191.4	191.0	76.2
4	4.74		4.49	2	133.0	161.9	132.4
5a	1.79		1.72	3	173.3	134.3	147.4
5b	2.47		2.29	4	66.4	216.8	67.7
7	2.26	2.97	2.19	5	39.1	55.2	44.9
8a	1.54	1.85	1.47	6	45.1	33.2	49.8
8b	1.05	1.55	1.06	7	55.5	55.5	56.0
10a	1.51	2.02	1.53	8	43.1	43.1	40.2
10b	1.79	2.20	1.54	9	35.9	41.7	37.3
11	3.96		3.86	10	50.9	54.3	52.2
12	1.79	2.07	1.74	11	74.9	198.7	63.1
13	1.59	1.83	1.47	12	9.9	11.8	15.2
14	1.00	1.08	0.99	13	29.1	27.7	28.0
15	1.09	1.19	1.09	14	29.6	27.6	30.1
				15	30.5	33.2	31.1

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained at 400 or 600 and 100 MHz, respectively. Spectra were recorded in $CDCl_3$ or CD_3OD with Me_4Si as internal standard. HRMS were determined at the University of Minnesota Mass Spectrometry Service Laboratory. 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 Mo Kα graphite monochromator, SHELXTL PLUS for structure solution and refinement.

Production of Metabolites. *Omphalotus illudens* 4499 was grown in large fermentors (ca. 100 L) to produce illudin S, at PANLABS, Inc., Bothell, WA. In a typical experiment, extraction of culture liquid (108 L) with EtOAc (116 L) yielded 860 g of crude solid from which 412 g of pure illudin S were obtained by crystallization. For isolation of compounds **1** and **2**, a sample (ca. 750 mg) of the residue from the mother liquor was chromatographed on Si gel with EtOAc−hexanes (1:10 increasing gradually to 1:1) yielding, in order of elution, acylfulvene (5 mg), dehydroilludin M (3 mg), illudin M (143 mg), omphadiol (**4**, 10 mg), illudosin (**1**, 58 mg), and illudin S (73 mg).

Illudosin (1): obtained as an oil; IR ν_{max} 3400, 2952, 2865, 1669 cm^{-1}; HRFABMS *m/z* 253.1805 [MH$^+$, 20], 235 (50), 217 (100); UV λ_{max} (EtOH) 251 nm (ϵ 7100); ^{1}H and ^{13}C NMR spectroscopic data are reported in Table 1.

Oxidation of Illudosin (1). Compound **1** (52.7 mg, 0.2 mmol) was dissolved in CH_2Cl_2 (6 mL), and pyridinium dichromate (256 mg, 0.68 mmol) was added with stirring. After 6 h at room temperature, the mixture was filtered and the filtrate washed several times with water, then dried over $MgSO_4$. The diketone (**2**, 21.3 mg) was obtained in crystalline form, after chromatography with EtOAc−hexanes, as needles, mp 81−83 °C; IR ν_{max} 2957, 2925, 2867, 1753, 1735, 1681 cm^{-1}.

X-ray crystal structure analysis of 2: $C_{15}H_{20}O_3$, at 295 K; $P2_1$ a = 9.520(3) Å, b = 6.283(3) Å, c = 12.955(5) Å, β = 111.42(3)°, Z = 2, calcd density = 1.143 Mg/m^3, crystal size 1.00 × 0.40 × 0.20 mm, 3 and 0.5°/min 0.6° Ω scan range, 1495 observed [$I > 2\sigma(I)$] from 1.69 to 27.50°, R = 4.75%, residual electron density 0.16 and −0.12 e/Å^3.

Reduction of Illudosin. To a solution of **1** (21.1 mg, 0.08 mmol) in 2-propanol (1 mL) was added sodium borohydride

Table 2. ^{1}H and ^{13}C NMR Data for Compounds **4** and **5**

	^{1}H			^{13}C	
proton	**4**	**5**	carbon	**4**	**5**
1	1.44 m	1.61 m	1	49.1	49.2
			2	22.64	19.3
3a[a]	0.73 dd (7.8, 4.8)	0.77 m	3	22.62	23.2
3b[a]	0.44 t (4.8)	0.71 m			
4	0.54 m	0.84 m	4	29.8	26.7
5	3.14 d (8.4)	4.86 d (9)	5	80.9	85.7
			6	38.0	37.9
7a[a]	1.19 t (12.6)	1.53 dd (2.4, 13.5)	7	42.1	41.8
7b[a]	1.44 m	1.41 t (13.5)			
8	1.60 m	1.67 m	8	48.2	48.0
			9	81.1	80.9
10a[a], 10b[a]	1.60 m, 1.68 m	1.65 m, 1.71 m	10	41.4	41.5
11a[a], 11b[a]	1.82 m, 1.68 m	1.89 m, 1.74 m	11	23.1	23.1
12	0.97 s	1.03 s	12	19.1	19.9
13	1.02 s	1.18 s	13	28.7	28.6
14	0.99 s	0.96 s	14	19.4	20.4
15	1.26 s	1.28 s	15	25.6	25.6
16[b]		9.14 s	16		129.4
17			17		148.6
18		9.2 s	18		122.1
21			21		134.6
22			22		161.7

[a] Order of assignments may be reversed. [b] For numbering, see Figure 3.

(10 mg, 0.3 mmol) with stirring. The mixture was kept for 40 min at room temperature, then quenched with a drop of acetic acid. The mixture was concentrated under reduced presssure and the residue chromatographed to give the triol **3** (13 mg). It was crystallized from EtOAc−MeOH; mp 168−170 °C; IR ν_{max} 3415, 2964, 2930, 1647 cm^{-1}.

X-ray crystal structure analysis of 3: $C_{15}H_{26}O_3$, at 295 K; $P2_12_12_1$, a = 6.5260(13) Å, b = 10.028(2) Å, c = 22.817(5) Å, Z = 4, calcd density = 1.131 Mg/m^3, crystal size 1.00 × 0.15 × 0.12 mm, 3 to 15°/min 0.6° Ω scan range, 1082 observed [$I > 2\sigma(I)$] from 1.79 to 25.08°, R = 6.17%, residual electron density 0.22 and −0.21 e/Å^3.

Omphadiol 4: obtained as a noncrystalline solid, which showed a single spot on TLC (R_f = 0.4, EtOAc−hexane 2:3); IR ν_{max} 3362, 2951, 2925 cm^{-1}; $[\alpha]^{25}_D$ +22°(c 0.02, EtOH); HREIMS m/z 238.1930 (calcd for $C_{15}H_{26}O_2$, 238.1934); ^{1}H and ^{13}C NMR data, see Table 2.

On treatment of the compound (**4**, 10 mg) with 3,5-dinitrobenzoyl chloride (30.8 mg, 0.1 mmol) and pyridine (a few drops) in CH_2Cl_2 (3 mL), one product was obtained crystalline after chromatography, and X-ray analysis showed it to be the 3,5-dinitrobenzoate derivative **5**. Crystals (from EtOAc) had mp 50−52 °C; IR ν_{max} 3422, 3108, 2964, 1723, 1630 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2.

X-ray crystal structure analysis of 5: $C_{22}H_{28}N_2O_7$, at 187 K; $P2_1$, a = 9.083(5) Å, b = 7.698(4) Å, c = 16.315(9) Å, β = 103.82(4)°, Z = 2, calcd density = 1.297 Mg/m^3, crystal size 0.50 × 0.20 × 0.05 mm, 2.5 to 5°/min 0.6° Ω scan range, 1538 observed [$I > 2\sigma(I)$] from 2.31 to 25.00°, R = 5.63%, residual electron density 0.26 and −0.24 e/Å^3.

Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-(0)1223−336033 or e-mail: deposit@ccdc.cam.ac.uk].

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