Illudin S, the Sole Antiviral Compound in Mature Fruiting Bodies of *Omphalotus illudens*

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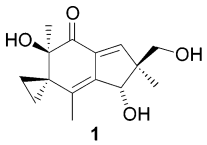
Received May 5, 2003

Crude extracts from the fruiting bodies of *Omphalotus illudens* displayed activity in the HSV-I/CV-1 antiviral assay. Bioactivity-guided isolation led to the known compound illudin S (**1**) as the sole antiviral component present in these extracts.

The biological activity of the fungus *Omphalotus illudens* (Schwein.) Singer (Paxillaceae) has been studied since the 1950s, when the first reports by Anchel and co-workers reported the isolation of two antibacterial crystalline compounds from the liquid cultures of *O. illudens*.[1] These two compounds were later identified as illudins S and M.[2,3] In addition to antibacterial activity, these compounds have been reported to have in vitro anticancer activity.[4−8] However, there have been no reports in the literature of the antiviral activity of either compound. In our continuing search for new antiviral compounds, we found that crude extracts of the fruiting bodies of the fungus *O. illudens* displayed antiviral activity. Investigation of mature fruiting bodies of *O. illudens* indicated the known compound illudin S (**1**) to be the sole antiviral component present.

The antiviral assay used to direct the isolation was a modified version of the HSV-I/CV-1 assay developed by Hughes and co-workers.[9] The assay was scored for cytotoxicity based on the activity of the compound toward the CV-1 cells on a scale from 0 (normal, viable cells) to 16 (no viable cells) and for antiviral activity toward HSV-I on a scale from − (no antiviral activity) to +++ (no visible viral plaques). The isolation scheme for purifying the antiviral compound is shown as Scheme S1 in the Supporting Information. One antiviral compound [0(+++) at 50 ng/disk and 0(+) at 25 ng/disk] was isolated from the organic extracts of mature fruiting bodies of *O. illudens* after liquid−liquid partitioning and chromatographic purification. HRFABMS established its pseudomolecular formula as $C_{15}H_{21}O_4$ (obsd *m/z* 265.1437, calcd 265.1434, ΔmDa = +0.3), which matched that of illudin S (**1**). The ^1H and ^{13}C NMR spectra (see Supporting Information) corresponded with published data for **1**.[4,10]

The only antiviral metabolite detected in the fruiting bodies of *O. illudens* was the known sesquiterpene **1**. Since no inactive compounds were isolated, no comment can be made about the potential antiviral activity of other compounds isolated from *O. illudens* and related species, as the majority of metabolites have been isolated from liquid cultures of the mycelium, not the fruiting bodies. The only other illudanes isolated from fruiting bodies have been dihydroilludin S from *Lampteromyces japonicus* and illudin M from *Omphalotus nidiformis*.[11,12] In vivo results with **1** as an anticancer drug have not been promising. In mouse models, the toxic effects of **1** appeared at or below therapeutic doses.[7] This raises doubt as to whether **1** could be used as an effective antiviral agent. However, analogues of **1** with reduced toxicity, such as Irofulvene (6-hydroxymethylacylfulvene, **2**) and dehydroilludin M (**3**) have been created and might also be safe, potent antiviral compounds.[13,14]



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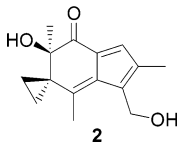

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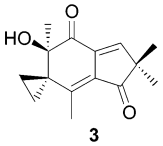

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Experimental Section

General Experimental Procedures. All ^1H NMR was performed on the Varian U500 (500 MHz ^1H) NMR spectrometer in the VOICE NMR Laboratory, School of Chemical Sciences, University of Illinois. Deuteriochloroform ($CDCl_3$) was the solvent, with residual chloroform ($CHCl_3$ δ 7.29 singlet ^1H) as the internal standard. Chemical shifts are in ppm. Second-order splitting patterns were solved using NUTS NMR Utility Transform Software from Acorn NMR.

All mass spectrometry was performed by the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois. Low-resolution FABMS were obtained on a ZAB-SE mass spectrometer. High-resolution and tandem FABMS were obtained on a VG 70-SE-4F. All FAB spectra were run in magic bullet (3:1 dithiothreitol/dithioerythritol).

HPLC was performed with either Beckman 114M pumps and a 421A controller with an Isco V^4 variable-wavelength detector (set at 225 nm), connected to a linear 1200 chart recorder, or Waters 510 pumps, an automated gradient controller, and a 990 photodiode array detector (set at 225 nm) connected to a NEC Powermate 2. ODS flash column chromatography was carried out on Whatman Partisil 40 ODS-3. Silica gel chromatography was carried out on 30−60 μm silica gel. All solvents used were reagent grade or spectrometric grade. Water used was filtered through a Millipore Milli Q Reagent Water System.

Biological Sample. Mature fruiting bodies of *Omphalotus illudens* (3095 wet wt) were collected in September 1997, in Mahomet, IL. The fresh sample was divided into six parts and stored frozen until used. A voucher specimen was deposited in the University of Illinois Herbarium (ILL).

Extraction and Isolation. Two samples (546 and 421 g wet wt) were homogenized in a Waring Blender with 1:1 CH_3-

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10.1021/np030205w CCC: $25.00
Published on Web 08/21/2003

OH/CH_2Cl_2 (2.2 L). The initial extraction was filtered, and the filtrate was washed (2 L). The filtrate was then further extracted with 1:1 CH_3OH/CH_2Cl_2 (3 × 2 L), followed by 3:1 CH_3OH/toluene (1 × 1.2 L and 2 × 0.8 L), and CH_3OH (12 L). All supernatants were combined and dried to obtain 46.15 g of crude extract. Two more samples (541 and 616 g wet wt) were extracted in a similar fashion to give a total crude extract of 94.16 g.

A portion of the extract (19.55 g) was partitioned between H_2O (500 mL) and hexane (5 × 300 mL), followed by CH_2Cl_2 (5 × 300 mL), EtOAc (1 × 500 mL and 3 × 400 mL), and 1-BuOH (4 × 400 mL). A saturated NaCl solution (200 mL) was added during the initial EtOAc extraction to disperse an emulsion layer that had formed. The CH_2Cl_2 (1.54 g) and EtOAc (3.33 g) extracts displayed antiviral activity in the HVS-CV-1 assay [both 0(+++) at 10 μg/disk and 0(+) at 2 μg/disk]. The CH_2Cl_2 and EtOAc extracts were combined. The remaining crude extract was partitioned in a similar manner to yield a total of 8.82 g of the active fraction. The active fraction was partitioned using 7:4:4:3 EtOAc/*n*-heptane/CH_3OH/H_2O. The lower layer (4.25 g) and upper layer (4.28 g) both displayed antiviral activity [0(+) at 1 μg/disk and 0(+) at 5 μg/disk, respectively].

The lower layer was subjected to repeated flash ODS column chromatography using a H_2O/MeOH step gradient. A portion of the active fraction (31.2 mg) was then subjected to ODS HPLC using a CH_3CN/H_2O gradient (10/90 to 100/0 over 41 min, 5 mL/min, Econosil C_{18} 10 μm, 22 × 250 mm). Only one compound, **1** (0.8 mg), was isolated with antiviral activity [0(+++) at 50 ng/disk and 0(+) at 25 ng/disk].

The upper layer (4.28 g) from the "magic solvent" partitioning was subjected to a silica gel flash chromatography column using a hexane to EtOAc step gradient (in 10% increments, 250 mL). The active fraction was then subjected to ODS HPLC using a CH_3CN/H_2O gradient. The purified active component was identical to **1** (<0.1 mg).

Illudin S (1): colorless, amorphous solid; ^{1}H and ^{13}C NMR data, comparable with literature values;[4,10] HRFABMS found *m*/*z* 265.1437; $C_{15}H_{21}O_4$ (calcd 265.1434, ΔmDa = +0.3).

Antiviral Assay. The procedure for the HSV-I/CV-1 antiviral assay was a modified version of the Hughes method, in which agar was substituted for methylcellulose for the overlay.[9] Monkey kidney cells (CV-1 cells) were placed into wells of a 24-well plate. Herpes simplex virus-I (HSV-I) was added to each well to infect the cells. The compound to be tested was absorbed onto a filter disk, which was then added to the well on top of the infected cells. After the incubation period, the cells were stained with Neutral Red and examined. The cytotoxicity level was expressed as a numerical score from 0 to 16. A score of 0 indicates the cells were normal and viable, with no indication of a dead zone of cells. When there was a cytotoxic effect, it was expressed as the diameter of the dead zone of cells in mm. When all the cells were dead, a score of 16 was recorded. The antiviral activity was scored using a qualitative system. A (+++) score meant no viral plaques were present in the well. A (++) score meant that plaques were present only at the extreme edge of the well. A (+) score meant that plaques that were present were reduced in size and/or number when compared with the control wells. A (−) score meant that plaques correspond in size and number with the controls. A (?) score meant that all CV-1 cells were dead, so no plaque reading could be made. Results for **1**: 16(?) at 10 μg/disk; 16(?) at 5 μg/disk; 16(?) at 2.5 μg/disk; 16(?) at 1.0 μg/disk; 16(?) at 0.5 μg/disk; 16(?) at 0.25 μg/disk; 0(+++) at 0.15 μg/disk; 0(+++) at 0.1 μg/disk; 0(+++) at 0.05 μg/disk; 0(+) at 0.025 μg/disk; 0(±) at 0.015 μg/disk; 0(−) at 0.010 μg/disk; 0(−) at 0.005 μg/disk.

Acknowledgment. The authors would like to thank Mr. Tryggvi Emilsson for providing the samples of *Omphalotus illudens*. NMR spectra were obtained in the Varian Oxford Instrument Center for Excellence in NMR Laboratory. Funding for this instrumentation was provided in part from the W.M. Keck Foundation, the National Institutes of Health (PHS 1 S10 RR104444-01), and the National Science Foundation (NSF CHE 96-10502). Mass spectra were obtained on instruments supported by grants from the National Institute of General Medical Sciences (GM 27029), the National Institutes of Health (RR 01575), and the National Science Foundation (PCM 8121494). Finally, the National Institute of General Medical Sciences (GM 54063) is thanked for their generous financial support of this research.

Supporting Information Available: The isolation scheme and ^{1}H and ^{13}C NMR spectra in $CDCl_3$ for illudin S (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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NP030205W