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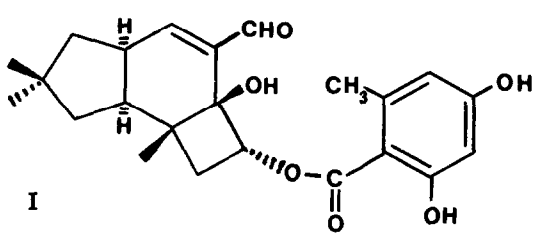
MELLEOLIDE, A NEW ANTIBIOTIC FROM ARMILLARIA MELLEA

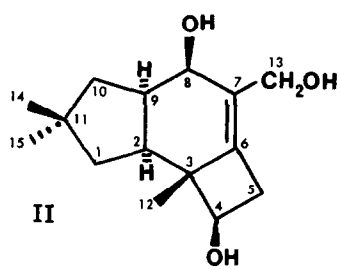
Sharon L. Midland, Richard R. Izac, Richard M. Wing,
A.I. Zaki, D.E. Munnecke, and James J. Sims*
Departments of Plant Pathology* and Chemistry, University of California, Riverside, CA. 92521

Summary: Melleolide, a new sesquiterpenoid orsellinate, has been isolated from cultures of Armillaria mellea. Its structure was elucidated by X-ray crystallography.

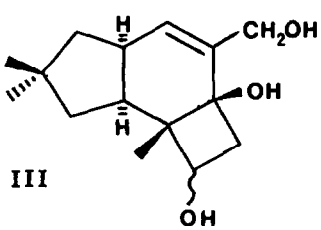
Armillaria mellea the prevalent "oak root fungus" is known to produce chemical defense substances to eliminate its bacterial predators.[1] By X-ray crystallography we have now identified the first such compound isolated from laboratory cultures of this fungus. Melleolide (I) is the orsellinate of a sesquiterpenediol of the protoilludane skeleton. It exhibits antifungal activity against Cladosporium cucumerinum[2] and is one of a family of related esters which have antibiotic activity against a number of gram positive bacteria.[3] The only previously isolated protoilludane derivatives are illudol (II)[4] and neoilludol (III)[5] from Clitocybe illudens, and Δ^6-protoilludene and Δ^7-protoilluden-6-ol from Fomitopsis insularis.[6] Other naturally occurring esters of orsellinic acid are the lichen depsides, lecanoric and gyrophoric acid, and a fungal pigment, mitorubin (IV)[7] from Penicillium rubrum. Orsellinic acid and several of its simple aliphatic esters have been shown to be effective antifungal and antibacterial agents.[8] No terpenoid orsellinates have been previously reported.[9]



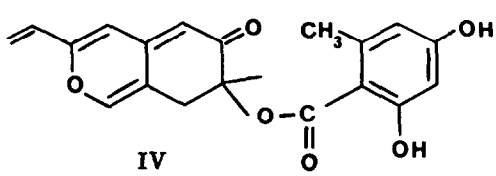
I



II



III



IV

Armillaria mellea was grown in still cultures of potato dextrose broth containing a trace of ethanol to stimulate growth. After three weeks, extraction of the culture filtrate with methylene chloride gave small amounts of an oily mixture with antifungal activity. This material was chromatographed on a Florisil column and active fractions were purified by repeated hplc. Final purification of melleolide was achieved using a Diol column (Brownlee Labs) with 6% isopropanol in hexanes as the mobile phase. The extracts of the fungal rhizomorphs and mycelia contained relatively less antifungal material. A number of other related antifungal compounds which were concurrently isolated are still under investigation.

Chemical ionization mass spectra (methane) of melleolide indicated a probable molecular weight of 400. Major fragments were observed at m/e 233 and 151, corresponding to cleavage on either side of an ester oxygen. The ultraviolet spectrum showed bands at λmax. nm(ϵ) 218(31000), 267(16500), and 305(6700) which shifted to 218(37000), 240(22000), 313(24000) in the presence of sodium hydroxide. The position of these bands and the observation of a 46nm bathochromic shift is explained by a p-hydroxy aromatic carbonyl compound. Infrared absorbance at 3380 cm^{-1} and a D_2O-exchangeable pmr signal at 11.6 δ indicate a hydrogen-bonded phenol. Assuming proton doublets (J=2.4 Hz) at 6.16 and 6.22 δ to be aromatic meta hydrogens, substructure A was deduced as a starting point for crystallography. Carbon-13 nmr data[10] confirmed the presence of such an oxygenated aromatic system and one olefinic double bond, a conjugated aldehyde, and an ester.

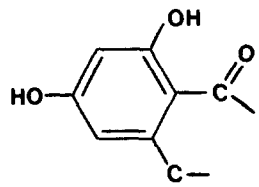


Figure 1. Fragment A

The remaining pmr data indicated quaternary methyl groups at 0.98, 1.0, and 1.3δ, oxygenated methines at 3.03 δ (br t) and 5.64 δ (t, J=8.8 Hz), an olefinic proton at 6.82 δ (d, J=1.8 Hz), and an aldehyde singlet at 9.45 δ. The methylene envelope, unresolved at 90 MHz, could be clarified at 500 MHz with judicious choice of solvent and eventually assigned by double irradiation techniques[11] and by correlation with double off-resonance ^{13}C spectra. Fortuitous positionings of the upfield protons however precluded structure elucidation by spectroscopy alone.

Crystals suitable for X-ray diffraction were obtained by slow evaporation of a water-methanol solution of I.* Crystallographic cell constants were $\underset{\sim}{a}$=17.251(2), $\underset{\sim}{b}$=14.072(2), $\underset{\sim}{c}$=14.614(4), space group $P2_12_12_1$. The observed density, 1.246 g/ml, indicated 8 molecules per unit cell or 2 independent molecules per asymmetric unit; $M_{calc.}$=400.8. Data collection on a Syntex $P\bar{1}$ diffractometer using Cu K_α radiation to 2θ=100.1° gave 2494 observed reflections including 350 E's above 1.4. A $\emptyset$ scan showed that no absorption correction was needed. The structure was solved with a single run of MULTAN 80+ [12], supplying fragment A as a random group. The remaining atoms were defined by Karle recycling and weighted Fourier synthesis. Preliminary refinement of these atomic coordinates by block diagonal least squares followed by full matrix refinement of isotropic carbons and anisotropic oxygens gave R=10.9%. Final full matrix

refinement of all atoms, including hydrogens, brought R to 6.0% (weighted), 7.9% (unweighted).

Comparison of the two independent molecules of the asymmetric unit showed conformational similarity of the sesquiterpene portions, but some rotation about the ester and the aldehyde linkage. Major torsion angle differences between the two molecules at the ester bonds were 26° about C5-O1 and -6° about C16-O1; the aldehyde substituents showed a torsion angle difference of 16° about C7-C13. The relative orientations of the sesquiterpene and aromatic portions of the two independent molecules differed by 20°.

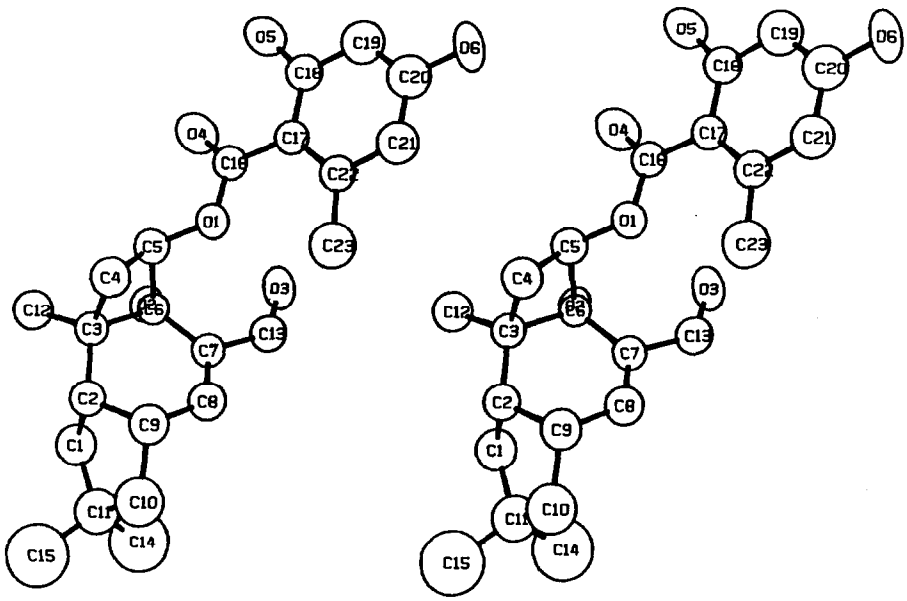


Figure 2. Stereo representation of melleolide.

We wish to acknowledge the use of the Southern California Regional NMR Facility and NSF Grant No. CHE-7916324 for acquisition of our 500MHz pmr data. Our CMR data was obtained on the UCR Bruker WH90 instrument, supported by NSF Grant No. MPS 75-06138 and NIH Biomedial Science Grant No. 5-S05-RR07010.

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9. An orsellinate of an illudol derivative has recently been isolated from A. mellea.; D. Donnelly, personal communication.*

10. cmr (CD_2Cl_2): δ 196.6(C13), 171.2(C16), 165.8(C18), 160.9(C20), 159.1(C8), 144.1(C22), 137.7(C7), 111.5(C21), 105.3(C18), 101.5(C19), 77.6(C5), 75.6(C6), 46.8(C4), 44.5(C2), 42.0(C1), 40.8(C9), 38.2 and 37.9(C3 or C11), 33.1(C10), 31.6 and 31.2 (C14 or C12), 24.6(C23), 21.3(C15).

11. 500 MHz pmr (CD_2Cl_2): δ 11.60s(OH), 9.45s (H13), 6.82d J=1.84(H8), 6.22d J=2.41(H19), 6.16d J=2.41 (H21), 5.63t J=8.83 (H5), 4.35br (OH), 3.03dddd J=9.63, 7.23,, 2.41, 1.84 (H9), 2.30s (Me23), 2.26ddd J=12.82, 7.26, 7.23 (H2), 2.04dd J=11.25, 8.83 (H4A), 2.02dd J=13.65, 9.63 (H10A), 1.61dd J=11.25, 8.83, (H4B), 1.59dd J=13.65, 2.41 (H10B), 1.50dd J=13.57, 7.26 (H1A), 1.30s (Me12), 1.26dd J=13.57, 12.82 (H1B), 1.03s (Me14), 0.99s (Me15).

12. P. Main, S.J. Fiske, S.E. Hull, L. Lessinger, G. Germain, J-P. DeClercq, and M.M. Woolfson, Multan 80, A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data, Dept. of Physics, University of York, York,England.

13. Crystallographic data is available upon request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratories, Lensfield Road, Cambridge CB2 1EW.

* Note added in press.

a. Reference 9 has now appeared. D. Donnelly, S. Sanada, J. O'Reilly, J. Polonsky, T. Prangé, C. Pascard, J. C. S. Chem. Comm. 135 (1982).

b. The melting point of melleolide was 198-200°C; its rotation, $[\alpha]_D$ + 183.3° (c 0.93, $CHCl_3$).

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