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11-HYDROXY-4-METHYL-2,4,6-DODECATRIENOIC ACID FROM FERMENTATIONS OF A *MUCOR* SPECIES

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Key Word Index—*Mucor* species; Zygomycetes; cytotoxic activity; 11-hydroxy-4-methyl-2,4,6-dodecatrienoic acid.

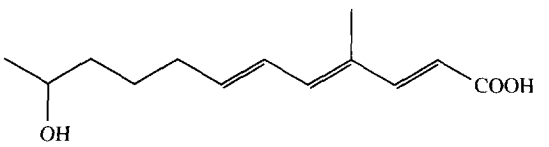
Abstract—11-Hydroxy-4-methyl-2,4,6-dodecatrienoic acid was isolated from fermentations of the *Mucor* species, strain KL 94-42 aq. The compound exhibits cytotoxic activity and the structural elucidation, as well as the biological properties of the new compound, are described. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The genus *Mucor* contains within the zygomycetes the largest number of species (300). Since only a few bioactive secondary metabolites have been reported from Zygomycetes (examples are the indole alkaloid, agroclavine [1], and the steroid antibiotic, fusidic acid [2]) and little is known about their secondary metabolism, we have screened submerged cultures of Zygomycetes for metabolites possessing antimicrobial activity, cytotoxicity and other bioactivities. In the present paper, we describe the isolation, structural elucidation and biological activities of a cytotoxic secondary metabolite detected in extracts of a *Mucor* species, strain KL 94-42 aq.

RESULTS AND DISCUSSION

11-Hydroxy-4-methyl-2,4,6-dodecatrienoic acid (**1**) was produced and isolated as described in the Experimental section; its structure was determined by NMR and mass spectrometry. High resolution EI mass spectral measurements suggested that its elemental composition is $C_{13}H_{20}O_3$ and this was supported by the presence of 13 signals in the ^{13}C NMR spectrum. COSY correlations show that the terminal methyl group (C-12) is connected to an oxygenated secondary carbon



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(δ_C 67.2 and δ_H 3.61), which in turn is connected to a chain of three methylenes (C-10, C-9 and C-8) and three protonated olefinic carbons (C-7, C-6 and C-5). 5-H gives a HMBC correlation to C-3 and both 3-H and 5-H give HMBC correlations to a methyl carbon. The protons of this methyl give HMBC correlations to C-3, C-4 and C-5; consequently, the methyl group must be positioned on C-4. 3-H couples with 2-H, and both 2-H and 3-H give HMBC correlations to C-1. In order to account for the elemental composition (see above), the C-11 substituent must be a hydroxyl group and C-1 must be a free carboxylic acid group; this is also in accordance with the NMR data. The configuration of all three carbon–carbon double bonds are *E*, as shown by the magnitude of the coupling constants between 2-H and 3-H (15.6 Hz), as well as between 6-H and 7-H (14.4 Hz) and the correlations observed in the NOESY spectrum. Both 2-H and 6-H, but not 3-H or 5-H, give strong NOESY correlations to the C-4 methyl group, while 3-H correlates to 5-H, which in turn correlates to 7-H.

Compound **1** does not exhibit antibacterial (*Bacillus brevis*, *B. subtilis*, *Enterobacter dissolvens* or *Micrococcus luteus*) or antifungal (*Mucor miehei*, *Penicillium notatum*, *Paecilomyces variotii* or *Naematospora coryli*) activities in the agar diffusion assay with up to 100 μg added to the filter discs. Its cytotoxic activities are weak; the IC_{50} for L1210 cells (mouse lymphocytic leukemia) was determined to be 25 μg ml^{-1}, while COS 7 (African green monkey) cells and BHK (baby hamster kidney) cells were less sensitive (IC_{50} 100 μg ml^{-1}). No effects on phospholipase A_2 (human synovial), on platelet aggregation or on the nematodes, *Meloidogyne incognita* and *Chaenorrhabditis elegans*, (100 μg ml^{-1}), which have been reported to be sensitive to several fatty acids [3, 4], were detected.

EXPERIMENTAL

Organism. Mucor sp., strain KL 94-42 aq, was isolated from detritus collected at a lake shore in the vicinity of Kaiserslautern. Samples were placed on Petri dishes containing a YMG medium [g l^{-1}: malt extract–glucose–yeast extract–streptomycin–penicillin (10:4:4:1:0.5), pH 5.5]. For maintenance on agar slants, the fungus was grown on YMG medium without antibiotics and stored at 4°. The areal velvety mycelium of the fungus has a pale magenta colour; the substrate mycelium remained colourless. Sporangiophores are branched and the sporangia contain a smooth, spherical columella. Although the strain showed all morphological characteristics of the genus *Mucor*, the species could not be identified since no zygospores were formed.

Fermentation. Fermentations were carried out in 5 l flasks containing 2.5 l of DMPG medium (g l^{-1}: 40 malt extract, 10 glucose, 2 peptone from soya, 1 yeast extract, 0.5 KH_2PO_4, 1 $MgSO_4 \cdot 7H_2O$, 0.0736 $CaCl_2$, 0.01 $FeCl_3$, 0.00178 $ZnSO_4 \cdot 7H_2O$, pH 5.5). After 10 days, cultures were harvested when the glucose was consumed and the pH value had increased to 6.1.

Isolation. Mycelia were sepd from culture fluid by filtration. Fermention broth was extracted with EtOAc and the organic phase was evapd. The crude extract (850 mg) was applied to a column (15 × 15 cm) containing silica gel (0.063 × 0.2 mesh, Merck 60) and eluted with 1 l cyclohexane–EtOAc (1:1). Further bioassay-guided purification of the active fr. (63.5 mg) was achieved by prep. HPLC (column 25 × 25 cm, LiChrosorb DIOL, 7 μm, 250 × 25 mm, Merck, elution with cyclohexane–*tert*-butylmethylether, 3:7). Yield: 19.9 mg pure **1**.

Spectroscopy. EIMS were recorded via direct inlet at 70 eV. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temp. using an inverse 5 mm probe equipped with a shielded gradient coil. COSY, HMQC and HMBC experiments were performed with gradient enhancements using sine-shaped gradient pulses. For the 2D-heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH}$ = 145 Hz and $^2J_{CH}$ = 10 Hz. Chemical shifts are reported in δ with the solvent signals (δ_H = 7.26 and δ_C = 77.0) as ref.

(2E,4E,6E)-11-*Hydroxy*-4-*methyl*-2,4,6-*dodecatrienoic acid* (**1**). Oil. $[\alpha]_D$ +2° (*c* 0.3 in MeOH). UV (MeOH) λ_{max} (ε): 307 nm (16 000). IR (KBr): 3400, 2930, 2680, 1685, 1605, 1515, 1400, 1270, 1205, 1020, 985, 855 cm^{-1}. ^{1}H NMR ($CDCl_3$–CD_3OD, 9:1, 500 MHz): δ 7.17 (*d*, J_{2-3} = 15.6 Hz, 3-H), 6.26 (*ddt*, J_{5-6} = 11.4, J_{6-7} = 14.4, J_{6-8} = 1 Hz, 6-H), 6.20 (*d*, J_{5-6} = 11.4 Hz, 5-H), 5.80 (*dt*, J_{6-7} = 14.4, J_{7-8} = 7.1 Hz, 7-H), 5.67 (*d*, J_{2-3} = 15.6 Hz, 2-H), 3.61 (*tq*, J_{10-11} = 6, J_{11-12} = 6 Hz, 11-H), 2.05 (*dd*, J_{7-8} = 7, J_{8-9} = 7 Hz, 8-H_2), 1.72 (*s*, 4-Me), 1.35 (*m*, 9-H_2), 1.29 (*m*, 10-H_2), 1.02 (*d*, J_{11-12} = 6.2 Hz, 12-H_3). ^{13}C NMR ($CDCl_3$–CD_3OD, 9:1, 125 MHz): δ 169.7 (C-1), 149.8 (C-3), 140.2 (C-7), 138.8 (C-5), 131.1 (C-4), 126.4 (C-6), 115.8 (C-2), 67.2 (C-11), 38.2 (C-10), 32.9 (C-8), 24.9 (C-9), 22.6 (C-12), 11.9 ((C-4)-CH_3). EIMS *m/z* (rel. int.): 224.1412 ($[M]^+$, 4, $C_{13}H_{20}O_3$ requires 224.1427), 206.1316 ($[M - H_2O]^+$ 11, $C_{13}H_{18}O_2$ requires 206.1307), 188 (6), 173 (9), 152 (63), 107 (100).

Biological tests. Assays for antimicrobial [5], cytotoxic [6] and nematicidal [3, 4] activities, and of human synovial phospholipase A_2 [7] and platelet aggregation activities [8], were carried out as described previously.

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