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AN ANTIFUNGAL POLYACETYLENIC COMPOUND FROM THE CULTURE FILTRATE OF THE FUNGUS *TRAMETES PUBESCENS*

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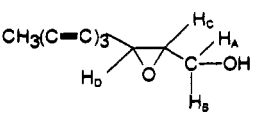
ABSTRACT.—A new antifungal compound has been isolated from the culture filtrate of the fungus *Trametes pubescens* and was characterized by spectral data and chemical transformations as *trans*-2,3-epoxydeca-4,6,8-triyn-1-ol [**1**].

The polypore *Trametes pubescens* (Fr.) Pil. (Polyporaceae) is a cosmopolitan, wood-rotting fungus with a wide distribution in East Africa, particularly in Ethiopia, Kenya and Tanzania (1). In the genus *Trametes,* 20 species in Africa (1) and 18 in North America (2) have been described. In previous work, triterpenes have been reported from the fruit bodies (3,4) and monoterpenes from cultures of *Trametes odorata* (5). To our knowledge there is no report in the literature on the chemical constituents of *T. pubescens*. We describe here the isolation, characterization and biological activities of the new antifungal compound *trans*-2,3-epoxydeca-4,6,8-triyn-1-ol [**1**], obtained from the culture filtrate of *T. pubescens*.

Bioautography was used to identify the active component in the culture filtrate of *T. pubescens*. Cultivation on a larger scale and extraction and isolation of the antifungal compound as described in the Experimental section yielded 25 mg of **1**.

The molecular formula of **1** was determined to be $C_{10}H_8O_2$ by hrms. The presence in **1** of an acetylenic functional group was deduced from the ir spectrum that showed the characteristic conjugated C-C triple bond absorption band at 2250 cm^{-1}, and from the uv spectrum which displayed finger-like absorption maxima typical of polyacetylenes (6).

The high-field ^{1}H-nmr spectrum showed resonances at δ 1.85 (3H, s), 3.19 (1H, m), 3.36 (1H, d, J=2.5 Hz), 3.52 (1H, dd, J=13 and 4 Hz), and 3.69 (1H, dd, J=13 and 3 Hz). The sharp singlet at δ 1.85 was assignable to a terminal methyl group on an acetylenic chain. The two sets of doublet of doublets at δ 3.52 and 3.69 (J=13 and 4 Hz) were due to two geminal protons on an oxygenated carbon and further coupled to a methine proton. The presence of two methine protons was evident from the multiplet at δ 3.19 and the doublet at δ 3.36. These observations together with ir absorption bands at 3400 (OH), 1070, and 860 cm^{-1} (epoxide) (7,8) suggested the presence of an epoxide flanked by a triyne and a CH_2OH group. The sharpness of the methyl singlet in the ^{1}H-nmr spectrum indicated that it was separated from the methine proton by more than two C-C triple bonds (9). The ^{13}C-nmr spectrum (DEPT experiment) of **1** displayed six quaternary, two tertiary, one secondary, and one primary carbon atoms. The primary carbon resonance which appeared at δ 4.1 is in accord with a terminal methyl group attached to an sp-hybridized carbon atom. The chemical shifts of the tertiary (42.9 and 60.5 ppm) and the secondary (60.4 ppm) carbon atoms also are consistent with the presence of an epoxide and CH_2OH groups, respectively. The trans-configuration of the epoxide



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ring was deduced from the coupling constant (J=2.5 Hz) for H_C and H_D (10). The acetylenic carbon resonances appeared at 64.5, 64.8, 66.8, 69.6, 73.0, and 77.2 ppm. Thus, structure **1** was assigned to this new fungal metabolite.

The structure of **1** was confirmed through conversion to known derivatives. Catalytic hydrogenation of **1** yielded 1,2-decanediol and acetylation of the diol with Ac_2O/pyridine gave the diacetate. Oxidation of 1,2-decanediol afforded nonaldehyde, which was spontaneously oxidized in air to nonanoic acid. The nonanoic acid thus obtained was then esterified with CH_2N_2 and the ester was found to be identical to an authentic sample (gc, ms and ^{1}H nmr).

Polyacetylenic C_{10} compounds have been isolated from diverse plant types and cultures of higher fungi. Compound **1** can be considered as the epoxide of *trans*-dec-2-ene-4,6,8-triyn-1-ol, which has been isolated from various Basidiomycetes (11).

Serial dilution assays were carried out to determine the antibiotic spectrum of compound **1**. It demonstrated antifungal properties as shown by its MIC values for *Candida albicans*, *C. tropicalis*, and *Rhodotorula glutinis*, and inhibition of the spore germination of two *Aspergillus* species tested (Table 1). Compound **1** did not show antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, or *Agrobacterium tumefaciens*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Boetius hot-stage melting point apparatus and are uncorrected. ^{1}H-Nmr spectra were measured on Varian (300 MHz) and Bruker WM (400 MHz) instruments and ^{13}C-nmr spectra on a JEOL FX90Q (22.5 MHz) spectrometer, hrms on an AEI MS-50 (70 eV) mass spectrometer, ir spectra on a Perkin-Elmer 727B spectrophotometer, and the optical rotation was obtained with a Perkin-Elmer 241 polarimeter. A Paar instrument was used for hydrogenation. Gc was carried out on a Varian GC 3700 using a SE-54 capillary column.

FUNGAL MATERIAL.—The polypore *Trametes pubescens* was collected from the Munesa forest, southern Ethiopia, in July 1990. A voucher specimen is deposited at the National Herbarium, Addis Ababa University, under the cipher ADA-202. Culture was made from the fresh specimen and is maintained on 3% malt extract (Oxoid) as ADA-202 in D.A.'s laboratory.

CULTURE CULTIVATION.—The fungus was cultivated in submerged culture on a rotatory shaker (120 rpm) in broth medium containing: (g/liter distilled H_2O) maltose 20, glucose 10, peptone 2, yeast extract 1, $MgSO_4 \cdot H_2O$ 1, KH_2PO_4 0.5, $CaCl_2$ 0.05, $FeCl_3$ 0.01, and $ZnSO_4$ 0.012. The culture was filtered after 10 days of growth to yield 5.8 liters of filtrate (pH 4.2).

ISOLATION OF *TRANS*-2,3-EPOXYDECA-4,6,8-TRIYN-1-OL [**1**].—The culture filtrate was extracted with an equal volume of EtOAc twice and solvent removed *in vacuo* to give a crude extract (390 mg). The crude extract was active against

TABLE 1. Antimicrobial Evaluation of Compound **1** (Using Serial Dilution Assays).

Test organism	MIC[a] (μg/ml)	Incubation temperature
Bacteria		
Bacillus cereus DSM 318	>100	37
Staphylococcus aureus ATCC 13709	>100	37
Agrobacterium tumefaciens ATCC 23308	>100	27
Fungi		
Candida albicans ATCC 10231	50–100	37
C. tropicalis DSM 1346	50–100	37
Rhodotorula glutinis DSM 70398	10–20	27
Aspergillus niger DSM 737[b]	1–5	27
A. ochraceus DSM 63304[b]	1–5	27

[a]Minimum inhibitory concentration.
[b]Spores.

Aspergillus niger and *Candida albicans*. Cc of the extract on Si gel, eluting with increasing concentrations of MeOH in CH_2Cl_2, yielded fractions containing the active compound. Final purification with prep. tlc [Si gel 0.25 mm, toluene-Me_2CO-HOAc (70:30:1)] gave 25 mg of **1**.

trans-*2,3-Epoxydeca-4,6,8-triyn-1-ol* [**1**].—Yellow crystals (unstable when exposed to air and light); mp 98–102° (dec); $[\alpha]_D$ −3° (c=0.04, $CHCl_3$); R_f=0.64 [toluene-Me_2CO-HOAc (70:30:1)], brown color upon reaction with vanillin-H_2SO_4 spray reagent; uv λ max (MeOH) 242, 262, 270, 293, 312 nm; ir ν max (KBr) 3400, 2250, 1385, 1070, 860 cm^{-1}; hrms *m/z* 160.0517 $[M]^+$ (11.8) (calcd for $C_{10}H_8O_2$, 160.0522), 130 $[M-CH_2O]^+$ (10.5), 100 $[M-C_2H_4O_2]^+$ (100); ^1H nmr (400 MHz, $CDCl_3$/MeOH δ 1.85 (3H, s, CH_3), 3.19 (1H, m, H_C), 3.36 (1H, d, J=2.5 Hz, H_D), 3.52 (1H, dd, J=13, 4 Hz, H_B), 3.69 (1H, dd, J=13, 3 Hz, H_A); ^{13}C nmr (22.5 MHz, $CDCl_3$) δ 4.1 (C-10), 42.9 (C-3), 60.5 (C-2), 60.4 (C-1), 64.5, 64.8, 66.8, 69.6, 73.0 and 77.2 (3×C≡C).

1,2-Decanediol and 1,2-diacetoxydecane.—Compound **1** (15 mg) was dissolved in MeOH-Et_2O (1:1) and hydrogenated using PtO_2 as catalyst. A light yellow oil was obtained, which after recrystallization from MeOH yielded 10 mg of 1,2-decanediol, colorless crystals: mp 47–49° [lit. (12) mp 48–49°]; R_f=0.93 [$CHCl_3$-MeOH (99:1)], orange color with vanillin-H_2SO_4 spray reagent. 1,2-Decanediol (7 mg) was acetylated using Ac_2O/pyridine to yield 1,2-diacetoxydecane (5 mg) after the usual workup: oil. R_f=0.32 [toluene-Me_2CO-HOAc (70:30:1)].

Nonaldehyde, nonanoic acid, and methyl nonanoate.—1,2-Decanediol (8 mg) was oxidized using $Pb(OAc)_4$ in C_6H_6 to yield nonaldehyde, which was spontaneously oxidized to nonanoic acid (4.5 mg); ^1H nmr (90 MHz, $CDCl_3$) δ 0.88 (3H, t, CH_3), 1.28 (12H, br s, [$(CH_2)_{3-8}$]) 2.38 [2H, t, $(CH_2)_2$]. The nonanoic acid thus obtained was esterified using CH_2N_2 in Et_2O solution to yield methyl nonanoate (5 mg), which was found to be identical with an authentic sample (co-tlc, eims, ^1H nmr and gc).

BIOLOGICAL ASSAYS.—The biological activity of compound **1** against eight test organisms (Table 1) was measured using serial dilution assays. The test organisms were grown for 24 h, with the bacteria in nutrient and yeasts in Sabouraud's broth. Spores of *Aspergillus* spp. were obtained from cultures grown on agar plates for seven days. Bacteria, yeasts and mold spores about 10^6 cells/ml were added to various amounts (1, 5, 20, 50 and 100 μg/ml) of compound **1** and incubated at optimal temperatures for 24 h. The minimum inhibitory concentrations (MIC) were then determined.

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