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SECONDARY MOLD METABOLITES, PART 49. ISOLATION, STRUCTURAL ELUCIDATION, AND BIOMIMETIC SYNTHESIS OF TRAMETOL, A NEW 1-ARYLPROPANE-1,2-DIOL PRODUCED BY THE FUNGUS *TRAMETES* SP.

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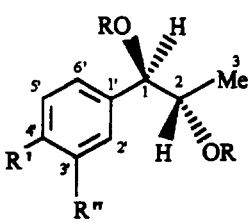
ABSTRACT.—Investigations of the culture extracts of *Trametes* sp. led to the isolation of the 1-arylpropane-1,2-diols **1** and **2**. Their structures and stereochemistry were elucidated by chemical and spectroscopic methods and confirmed by biomimetic synthesis.

In our search for new active compounds produced by Basidiomycetae (fungi), grown on artificial media, we have previously reported the isolation of a series of benzofuran derivatives from *Laurilia taxodii* and also described a 1,2-propane-diol derivative, biogenetically related to the phytopathogenic aldehyde, fomannoxin (1,2). This diol derivative was previously isolated along with other related compounds from the wood-rot fungus *Fomes annosus* (3).

In our present study, two new 1,2-arylpropane diols [**1** and **2**] were isolated from a strain of *Trametes* sp., isolated in 1993, during a test to evaluate the microbial pollution of the air of a flour mill. The structures and absolute configurations of two new natural compounds, **1** and **2**, were confirmed by biomimetic synthesis from the corresponding aldehydes by reductive C_2-homologation with fermenting Baker's yeast. The genus *Trametes* has been previously reported to produce a series of triterpenes, as well as substituted hydroxybenzoic acids, and phenylacetic acids (4).



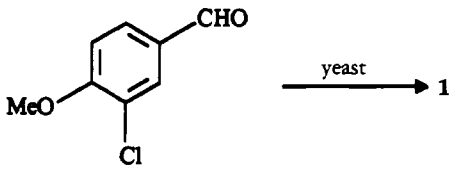
1 R=H, R′=OMe, R″=Cl
2 R=R′=R″=H
3 R=$OCOC_6H_5$, R′=OMe, R″=Cl

The strain of *Trametes* sp. IPV-F640 was grown on MPG (malt extract-peptone-glucose) for three weeks. EtOAc extracts of cultures were evaporated and subjected to repeated prep. Si gel chromatography to give the novel trametol [**1**] and 1-phenylpropane-1,2-diol [**2**], described previously (5). Compound **1** was isolated as a solid, mp 74°, $[\alpha]_D$ −29.2° (c=1, $CHCl_3$). The ir spectrum exhibited absorptions bands at 3450 and 1610, 1510 cm^{-1} due to hydroxy groups and an aromatic ring. The molecular formula $C_{10}H_{13}O_3Cl$ is in agreement with the mass spectral data obtained.

The ^{1}H-nmr spectrum of **1** showed the presence of a methyl doublet (H_3-3) and two methine protons adjacent to hydroxyl groups. Benzoylation of **1** afforded a dibenzoate [**3**] in which H-1 and H-2 were shifted downfield (see Experimental). Comparison of the coupling constants of H-1 and H-2 in compound **1** with those of known propane-diols (2,3) was in agreement with an erythro configuration ($J_{1\text{-}2}\cong$4 Hz). The remaining signals were attributable to a trisubstituted aromatic ring. NOe nmr experiments (see Experimental) were in agreement with the proposed aromatic substitution.

Definitive evidence for structure **1** was obtained by synthesis starting with a 3-chloro-4-hydroxybenzoic acid reaction with CH_2N_2 in dry Et_2O. Direct reduc-

tion of the resulting ester with diisobutylaluminum hydride in Et_2O at $-70°$ gave rise to the corresponding alcohol only, and not the aldehyde [**4**] as expected. Therefore, 3-chloro-4-methoxybenzylic alcohol was oxidized to the corresponding aldehyde [**4**] with pyridinium chlorochromate (see Experimental). Aldehyde **4** was treated with fermenting baker's yeast to give trametol [**1**] in a 10% yield (Scheme 1). Compound **2** was synthesized from benzaldehyde and fermenting baker's yeast as described previously (5).



4

SCHEME 1

The diastereo- and enantioselectivity of this reaction effected by *Saccharomyces cerevisiae* has been demonstrated to be very high for a series of substituted aromatic aldehydes (5). C_2-homologation of aldehydes gives rise only to an erythro isomer having the 1*R*,2*S* absolute configuration. The optical rotation, nmr coupling constants, cd spectra, and hplc analysis (with a chiral column) of the corresponding dibenzoates [**3**] obtained from natural and synthetic trametol [**1**], respectively, were identical. Therefore, the absolute configuration of the asymmetric carbons in compound **1** is 1*R*,2*S*.

The natural methyl-diols **1** and **2** probably arise from reduction of α-ketols formed from aldehydes and a C_2 unit. In fact, 3-chloro-4-methoxybenzaldehyde and other isomers have been isolated from Basidiomycetae species (4).

Metabolite **1** was inactive against *Bacillus cereus, B. subtilis, Escherichia coli, Saccharomyces cerevisae,* and *Cladosporium cladosporioides* at dosages of 150 μg per plate.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler apparatus and are uncorrected. Ir spectra were recorded with a Perkin-Elmer 177 instrument; optical rotations on a Jasco DIP-181polarimeter; cd spectra on a Jasco-500A Dichrograph; and ms on a Finnigan-MAT TSQ70 spectrometer. Nmr spectra were acquired on a Bruker AC 250L spectrometer operating at 250.1 MHz for ^1H; chemical shifts are in ppm (δ) from $SiMe_4$ as internal standard. Flash cc was performed with Merck Si gel (0.04–0.06 mm) and tlc with Merck HF_{254} or RP-18 $F_{254}Si$ gel. The purity of the compounds was determined by hplc analysis using a Merck-Hitachi L6000A apparatus, using a Daicel Chiralcel OD 0.45×25 cm column with hexane-*i*-PrOH (95:5) as eluent at a nominal flow rate of 0.5 $cm^3\ min^{-1}$.

FUNGAL MATERIAL.—To identify the strain IPV-F640, deposited by Istituto di Patologia Vegetale, Facoltà di Agraria, Università di Milano, a taxonomic system proposed by J.A. Staplers was used (6). From morphological-cultural characteristics, the strain is similar to *Trametes meyenii*. The fungus shows positive peroxidase, growth rate >70 mm in 7 days, and clamp connections, possesses an aerial white mycelium with a granulose, woolly, pellicular, felty aspect; produces crystals in the aerial mycelium; and shows skeletal hyphae rare branched and thick-walled generative hyphae.

EXTRACTION AND ISOLATION.—The fungal material was inoculated in 20 Roux flasks containing 50 ml of MPG (malt extract-peptone-glucose, 20:3:20 g/liter) with a mycelium suspension at 24° and in still culture (or shaken at 140 rpm). After three weeks for still cultures (or 10 days for shaken) flasks were extracted with EtOAc containing MeOH (1%) and the extracts were evaporated to give 150 mg of a mixture of crude metabolites (or 100 mg from shaken cultures). Purification of the above mixtures of compounds **1** and **2** by prep. tlc on Si gel 60 plates, using hexane-EtOAc (2:1) gave compounds **2** (70 mg) and **1** (30 mg) from still cultures, and compound **1** (60 mg) alone from shaken cultures.

Trametol [**1**].—Mp 74°; $[\alpha]_D$ −29.2° (*c*=1.4 $CHCl_3$); *anal.*, found C, 55.4, H, 5.9, Cl, 16.3%, $C_{10}H_{13}O_3Cl$ requires C, 55.44, H, 6.05, Cl, 16.36; eims *m/z* 216, 170, 142, 107, 43; ir (nujol) ν max 3450, 1610, 1510 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.39 (1H, d, *J*=2.0 Hz, H-2′), 7.22 (1H, dd, *J*=8.5 and 2.0 Hz, H-6′), 6.92 (1H, d, *J*=8.5 Hz, H-5′), 4.61 (1H, d, *J*=4.2 Hz, H-1), 3.98 (1H, dq, *J*=4.2 and 6.0 Hz, H-2), 3.90 (3H, s, H_3-7′), 2.20 (2H, br, OH-1 and -2), 1.07 (3H, d, *J*=6.0 Hz, H_3-3); nOe experiments ($CDCl_3$) {H-1} enhanced H-2′ (4.0%), H-6′ (3.5%) and H-2 (4.5%); {H-2}

enhanced H-1 (3%), H_3-3 (1.5%), H-2′ (1.5%), and H-6′ (1.5%); {H_3-3} enhanced H-2′ (1.5%), H-6′ (1.5%), H-1 (2.5%), and H-2 (6.5%); {H-2′} enhanced H-2 (1.5%) and H-1 (2.5%); {H-5′} enhanced H-6′ (6%) and H_3-7′ (2.5%); {H-6′} enhanced H-5′ (5.5%), H-1 (2.5%), and H-2 (1.0%); {H_3-7} enhanced H-5′ (11%); {OH} enhanced H-1 (3.5%), H-2 (2%), H_3-3 (1%), H-2′ (1%), and H-6′ (1.5%).

1-Phenyl-1,2-propanediol [**2**].—Mp 92°; $[\alpha]^{25}D$ −35° (*c*=1, $CHCl_3$); eims *m/z* 152.0817 ($C_9H_{12}O_2$ requires 152.0837).

TRAMETOL DIBENZOATE [**3**].—Compound **1** (20 mg) was dissolved in dry pyridine (1.0 ml) and treated with benzoyl chloride (0.1 ml) at 0°. After 20 minutes at 0°, H_2O (3 ml) was added and the solution extracted with EtOAc. Evaporation of solvent and prep. tlc gave **3** as an oil (15 mg), cd (c 2.6 10^{-2} g dm^{-3}, MeOH) 233 nm ($\Delta\epsilon$ +13.8). ^1H nmr ($CDCl_3$) δ 8.1–7.2 (12H, m, ArH), 6.92 (1H, d, *J*=8.5 Hz, H-5′), 6.14 (1H, d, *J*=4.2 Hz, H-1), 5.59 (1H, dq, *J*=4.2 and 6.0 Hz, H-2), 3.89 (3H, s, H_3-7′), 1.42 (3H, d, *J*=6.0 Hz, H_3-3).

REDUCTIVE C_2-HOMOLOGATION OF 3-CHLORO-4-METHOXYBENZALDEHYDE [**4**] BY FERMENTING BAKER'S YEAST.—Aldehyde **4** was obtained from the corresponding ester by reduction of $LiAlH_4$ and subsequent oxidation with pyridine chlorochromate in dry CH_2Cl_2. Direct reduction of the ester with diisobutyl-aluminum hydride gave rise only to the corresponding benzylic alcohol. Dry yeast (12.5 g) and 10 g of glucose were added to 50 ml of H_2O, under stirring. Then a solution of aldehyde **4** (600 mg) in 2 ml of EtOH was added with stirring. After 1 h, yeast (6.2 g) and glucose (5 g) were added to the reaction mixture, followed by stirring for an additional 3 h. The reaction mixture was extracted twice with 100 ml of EtOAc and the combined organic layers were dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give an oil. The diol **1** was purified by cc on Si gel, hexane-EtOAc (4:1), and yielded 60 mg.

BIOLOGICAL TESTS.—Antibacterial and antifungal activity were tested with paper disks (6-mm diameter), soaked with **1** (150 and 50 μg) dissolved in EtOH, which were placed in suitable culture medium, cooled at 45°, and poured into petri dishes with *Bacillus cereus, B. subtilis, Escherichia coli, Saccharomyces cerevisiae,* and *Cladosporium cladosporioides* as test microorganisms.

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