Molecular Structure of Terrecyclodiol: A Derivative of the Antifungal Metabolite Terrecyclic Acid A from *Aspergillus terreus*

Faramak Almassi, Emilio L. Ghisalberti,* Brian W. Skelton, and Allan H. White

Department of Chemistry, University of Western Australia, Nedlands, Western Australia 6907

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A strain of *Aspergillus terreus,* which was isolated from organic mulch and inhibited the growth of the plant pathogen *Phytophthora cinnamomi*, produces an antifungal metabolite when grown in liquid culture. This metabolite was isolated by bioassay-guided fractionation and identified as terrecyclic acid A (**1**). X-ray diffraction studies and spectroscopic details of the derived terrecyclodiol (**2**) are described.

Phytophthora cinnamomi (Pythiaceae) Rands is a cosmopolitan, primarily soil-borne, plant pathogen that causes many destructive diseases mainly by root- and crown-rot.[1] It has been noted that organic mulch applied around avocado trees suppresses root-rot caused by *P. cinnamomi*.[2] In a study of this phenomenon, it was found that the most frequently isolated fungi from the mulch (oaten straw–chicken manure), before and after infestation with the pathogen, were *Aspergillus* species (~11%) (3). Of these, an isolate of *Aspergillus terreus* Thom (Deuteromycotina) (Hyphomycetes) was subsequently shown to inhibit significantly (83%) the growth of the pathogen in dual culture assays. Furthermore, this fungus exhibited a high level of pathogen and disease suppression in pot trials with snapdragon seedlings grown in soil infested with the pathogen.[3] We were interested in determining whether these interactions were mediated by antifungal metabolites produced by *A. terreus*. We now report on the bioassay-guided isolation, identification, and characterization of the antifungal metabolite that accumulates in liquid cultures of this fungus.

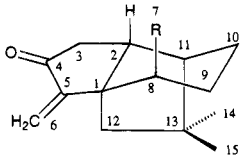
The *A. terreus* isolate (IMI 356242) was cultured in a liquid medium consisting of 1/5 potato dextrose broth for 6 weeks. After removal of the mycelium, the liquid medium was extracted with EtOAc to give a fraction that was shown to inhibit the growth of *P. cinnamomi*. The organic extract was separated by CC to yield three main fractions of which only the first, and major, fraction retained antifungal activity. Chromatographic and spectroscopic analyses indicated that this fraction was essentially homogenous and consisted of a keto acid, M^+ 236, $C_{15}H_{24}O_3$.

The ^{1}H-NMR, ^{13}C-NMR, and MS parameters of this compound were consistent with those reported for terrecyclic acid A (**1**) previously isolated from a strain of *A. terreus* Thom No. 14.[4–7] Although the sample was predominantly one compound (>95%), the optical rotation ($[\alpha]_D$ +11°) did not increase to that reported for **1** ($[\alpha]_D$ +33.9°),[4] despite repeated purification attempts. It was unclear whether this difference reflected the presence of a contaminant in our sample or an isomeric relationship between the two compounds. Further characterization and purification was achieved by reduction with $LiAlH_4$ in Et_2O to the corresponding diol.

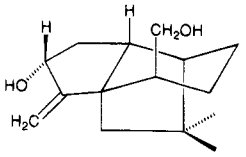
Table 1. Ring Torsion Angles (deg) (Atoms are Designated by Numbers Only)

atoms	angle	atoms	angle
5-1-2-3	−27(2)	8-1-2-11	78(1)
1-2-3-4	41(2)	1-2-11-10	−76(1)
2-3-4-5	−39(2)	2-11-10-9	60(2)
3-4-5-1	23(2)	11-10-9-8	−42(3)
4-5-1-2	3(2)	10-9-8-1	39(3)
12-1-2-11	−40(1)	9-8-1-2	−58(2)
1-2-11-13	47(1)	12-1-8-9	55(2)
2-11-13-12	−32(1)	9-10-11-13	−58(2)
11-13-12-1	6(1)	10-11-13-12	84(1)
13-12-1-2	22(1)	13-12-1-8	−95(1)

Recrystallization of this diol from hexane-ethyl acetate gave crystals suitable for X-ray diffraction studies.



1 R = COOH
3 R = CH_2OH



2

The results presented below indicate that the structure of the diol is as shown in **2** and confirm that the antifungal metabolite is terrecyclic acid A (**1**). This also establishes that hydride reduction occurs by delivery of the hydride ion from the *si*-face of the carbonyl group. This outcome is the same as that observed for the sodium borohydride reduction of terrecyclic acid A, evidence for which was obtained from chiroptical studies.[8,9] Interestingly, reduction with diisobutyl aluminium hydride provides mainly the epimeric alcohol.[9] The diol obtained from sodium borohydride reduction of terrecyclol (**3**) has been shown to be identical to that obtained from $LiAlH_4$ reduction of **1**, but the stereochemistry at C-4 had not been assigned.[8] This diol can now be said to have the stereochemistry shown in **2**. It is worthwhile noting that, from the ring torsion angle data (Table 1) and Figure 1, the five-membered ring containing carbon atoms 1 to 5 adopts a classical envelope conformation. Thus, C-2, C-1, C-5, and C-4 are almost coplanar and C-3 is out of the plane. Furthermore, this conformation forces the hydrogens at C-2 and C-4 to approach a *syn*-parallel disposition. That this conformation is maintained in solution is suggested by the significant allylic coupling observed between H-4–H-6a (J = 2.8 Hz) and H-4–H-6b (J = 2.4 Hz),

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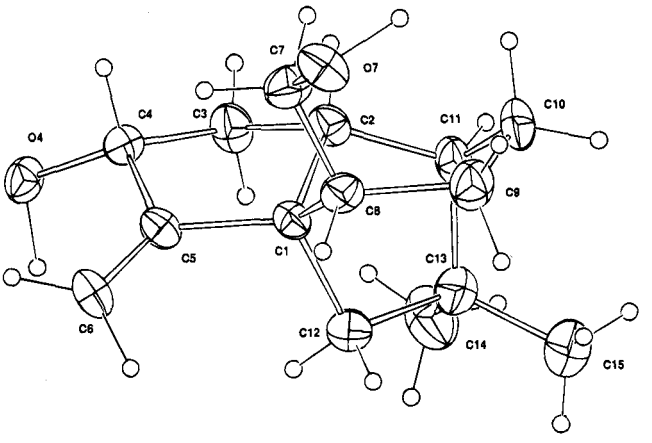

Figure 1. Molecular projection of **2**; 20% thermal ellipsoids are shown for the non-hydrogen atoms; hydrogen atoms having arbitrary radii of 0.1Å.

indicative of an orthogonal relationship between these pairs of hydrogens.[10]

Terrecyclic acid A has been shown to exhibit antitumor activity (<0.5 μg mL^{-1}) towards lymphocytic leukemia P-388 in mice, antibiotic activity towards bacteria, and, to a lesser extent, fungicidal activity towards fungi such as *Penicillium chrysogenum* and *Fusarium oxysporum*.[4] It is worthwhile noting that terrecyclic acid A inhibits the growth of *P. cinnamomi* and appears, to a large extent, to be responsible for the antagonistic activity shown by the *A. terreus* isolate towards this pathogen (You and Almassi, unpublished results).

The structure determination of **2** was carried out as follows. A unique room temperature diffractometer data set (ENRAF-Nonius CAD-4 instrument; $2\theta/\theta$ scan mode; monochromatic Mo $K\alpha$ radiation, λ 0.7107$_3$ Å, T ~295 K) was measured within the limit $2\theta_{max}$ = 50 °C, yielding 1,234 independent reflections; 992 of these with $I > 3\sigma(I)$ were considered "observed" and used in the full matrix least-squares refinement after solution of the structure by direct methods and without absorption correction. Anisotropic thermal parameters were refined for C, O; $(x, y, z, U_{iso})_H$ were included constrained at estimated values after location in difference maps. Conventional residuals R, R_w on $|F|$ were 0.079, 0.093 [statistical weights, derivative of $\sigma^2(I) = \sigma^2(I_{diff}) + 0.0004\sigma^4(I_{diff})$]; neutral atom complex scattering factors were employed, computation used the XTAL 3.2 program system implemented by S. R. Hall[11] and chirality being assigned from the chemistry. Ring torsion angles are given in Table 1 and the atomic coordinates for non-hydrogen atoms are given in Table 2. Lists of other parameters are deposited with the Cambridge Crystallographic Data Centre.

Crystal Data: $C_{15}H_{24}O_2$, M_r 236.4; trigonal, space group $P3_2$ (C_3^3, No. 145), a = 14.081(5) Å, c = 6.157(5) Å, V = 1057 Å^3, D_c (Z = 3) 1.11 g·cm^{-3}, $F(000)$ 520, μ_{Mo} 0.7 cm^{-1}, specimen 0.75 × 0.38 × 0.10 mm.

An undesirably high residual was not reduced substantially by repetition of the experiment with a different specimen and, in the absence of other substantial abnormalities, seemingly in the context of a correct cell and space group and a satisfactorily meagre residual electron density maximum of 0.24 e Å^{-3}, provides cause for a caveat in the context of a seemingly otherwise normal and sensible result. No evidence from the chemistry or the determination suggested any appreciable admixture of related compounds or isomers. O(7)···H,O(7) (1 − x + y, 2 − x, z − 1/3) are 1.6, 2.66(3) Å, and O(4)···H, O(4) (1 − y, 1 + x − y, 1/3 + z) are 1.7, 2.77(3) Å.[12]

Table 2. Non-hydrogen Positional and Isotropic Displacement Parameter

atom	x/a	y/b	z/c	U_{eq}Å^2
C(1)	0.3278(7)	0.2485(6)	0.0(−)[a]	0.055(4)
C(2)	0.3750(6)	0.3214(7)	−0.201(2)	0.057(4)
C(3)	0.4972(7)	0.3526(7)	−0.195(2)	0.070(5)
C(4)	0.4894(6)	0.2438(6)	−0.127(2)	0.053(4)
O(4)	0.5918(4)	0.2560(5)	−0.053(2)	0.062(3)
C(5)	0.3990(6)	0.1972(6)	0.040(2)	0.053(4)
C(6)	0.3836(7)	0.1228(8)	0.189(2)	0.072(5)
C(7)	0.1843(7)	0.0855(8)	−0.212(2)	0.070(5)
O(7)	0.0702(4)	0.0019(5)	−0.231(2)	0.072(3)
C(8)	0.2036(6)	0.1675(7)	−0.036(2)	0.057(4)
C(9)	0.1480(8)	0.2335(8)	−0.079(2)	0.080(5)
C(10)	0.2127(9)	0.3371(9)	−0.223(2)	0.086(6)
C(11)	0.3344(7)	0.4017(7)	−0.164(2)	0.067(5)
C(12)	0.3453(7)	0.3322(7)	0.184(2)	0.067(5)
C(13)	0.3582(8)	0.4413(8)	0.073(2)	0.079(6)
C(14)	0.473(1)	0.5371(9)	0.102(2)	0.110(7)
C(15)	0.280(1)	0.475(1)	0.163(2)	0.122(9)

[a] Defines origin.

Experimental Section

General Experimental Procedures. Experimental details have been recorded previously.[13] The *A. terreus* isolate was identified at the International Mycological Institute (IMI 356242). Antifungal activity testing of fractions was carried out as for the dual culture assay but replacing the inoculum plugs of the antagonist with wells containing an MeOH solution (10 μL) of the fraction (10 mg/mL).

Fungal Material. The organism (*Aspergillus terreus*) was cultured in potato dextrose broth (PDB; 3 L) at 1/5 (5 g L^{-1}) of the normal concentration. The cultures were allowed to grow without shaking for 6 weeks at 25 °C.

Extraction and Isolation. The culture broth (3 L) was extracted with EtOAc (3 × 300 mL), the organic layer dried over $MgSO_4$, and the solvent was removed under vacuum. TLC and ^{1}H-NMR spectroscopy of the oily extract (806 mg) obtained showed this material to contain mostly a single compound (~90%). The extract was separated by absorption on a Si gel column. Gradient elution (petroleum ether-EtOAc; 4:6, to EtOAc) gave three fractions (a) 674 mg; R_f 0.6 (EtOAc), (b) 56 mg; R_f 0.5, and (c) 15 mg; R_f 0.4. Of these, only the first showed significant antibiotic activity. The ^{1}H-NMR, ^{13}C-NMR, and MS parameters of this compound were in good agreement with those reported[4-7] for terrecyclic acid A (**1**), but the optical rotation, $[\alpha]_D$ +11° (*c* 6; $CHCl_3$) did not match that reported[4] for terrecyclic acid A, $[\alpha]_D$ +33.9°; (*c* 0.177; $CHCl_3$). Because recrystallization of the compound was difficult, it was subjected to repeated chromatography but with no increase of the value for the optical rotation.

Hydride Reduction of Terrecyclic Acid A (1). A solution of **1** (0.15 g) in Et_2O (10 mL) was treated with $LiAlH_4$ (0.26 g), and the mixture was left standing for 18 h. The product (91 mg), recovered in the usual way, was adsorbed on a column of Si gel (5 g). Gradient elution from 20% petroleum ether-EtOAc to EtOAc gave

three major fractions, the most polar of which (20 mg) was crystalline and was recrystallized from hexane-EtOAc.

Terrecyclodiol (2). Needles; mp 195–196 °C; R_f 0.33 (Si gel; petroleum ether-EtOAc; 1:1). ^{1}H NMR (500 MHz; pyridine-d_5) δ 1.03 and 1.06 (each 3H, s, H_3-14 and H_3-15), 1.73 (1H, d, J = 13.8 Hz, H-12a), 1.85 (1H, d, J = 13.8 Hz, H-12b), 2.05 (1H, dt, J = 12.9, 10.6 Hz; H-3a), 2.15 (1H, dt, J = 6.9, 10.6 Hz; H-3b), 2.20 (1H, m, H-8), 3.75 (1H, t, J = 9.9 Hz, H-7a), 4.02 (1H, ddd, J = 9.9, 2.9, 1.9 Hz; H-7b), 4.62 (1H, dddd, J = 10.6, 6.9, 2.8, 2.4 Hz; H-4), 5.01 (1H, dd, J = 2.8, 1.4 Hz; H-6a), 5.54 (1H, dd, J = 2.4, 1.4 Hz; H-6b); ^{13}C NMR (125 MHz; pyridine-d_5) δ 21.4 (t, C-9), 26.9 and 35.0 (each a q, C-14 and C-15), 28.6 (t, C-10), 38.8 (s, C-13), 39.2 (t, C-3), 47.97 and 48.0 (each a d, C-2 and C-8), 49.3 (d, C-11), 55.8 (t, C-12), 56.6 (s; C-1), 62.9 (t, C-7), 77.3 (d, C-4), 104.0 (t, C-6), 163.5 (s, C-5).

References and Notes

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