

## CRYSTAL STRUCTURE OF TRICHODIMEROL

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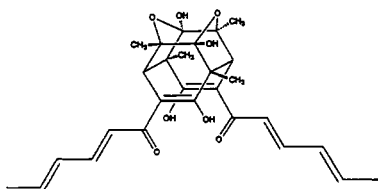
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**ABSTRACT.**—The proposed structure of trichodimerol (**1**), isolated from a culture broth of *Penicillium chrysogenum*, has been confirmed by an X-ray crystallographic study. The relative stereochemistry of the molecule was established and its solid state conformation was determined. The results show a caged dimeric molecule with a pseudo twofold symmetry and two sorbyl side-chains of almost fully extended conformations. The crystal structure consists of a double-layered network by solvent-mediated hydrogen bonding.

Inhibitors of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) production may have potential for the treatment of bacterial-induced septic shock. In a program to screen for such inhibitors, the fungal metabolite trichodimerol (**1**) was identified as a lead molecule (1–3). Using a promoter-based screen (2), bioassay-guided fractionation of a culture broth of *Penicillium chrysogenum* (3) led to the isolation of this previously reported symmetrical dimer (1). Structure **1** was initially proposed based on spectroscopic studies. Recrystallization from EtOH/H<sub>2</sub>O at room temperature afforded pale-yellow hexagonal plates suitable for X-ray single-crystal analysis, which confirmed the proposed structure. It was later found that the spectral data for our isolate were in full agreement with published data (1). We report herein the crystal structure and solid state conformation of **1**.

### RESULTS AND DISCUSSION

The crystallographic study showed that **1** has a dimeric structure as proposed by spectroscopic methods. The relative stereochemistry of the molecule was established in this study. The absolute configuration shown in Figure 1 was assigned based on the cd exciton chirality method (4), which is also consistent with the absolute structure reported (1). Compound **1** contains three six-membered rings and two five-membered rings fused to form an open-ended cage structure. The two six-membered rings consisting only of carbon atoms C-1 to C-6 and C-1' to C-6', respectively, are in boat conformations and have their concaves facing each other. The two boats are bonded at the ends with C-4–C-1' 1.583(7) Å and C-1'–C-4' 1.586(7) Å. The third six-membered ring formed by O-1, C-2, C-3, O-1', C-2', and C-3', also adopts a boat conformation with its convex facing to the cage. The two five-membered rings, one involving O-1, C-3, C-4, C-1', C-2' and the other involving O-1', C-3', C-4', C-1, C-2, are back-to-back and exist in envelope conformations. In the cage, C-C bonds range from 1.546(7) to 1.597(9) Å, with an average value of 1.577(8) Å, and are generally longer than normal C-C bonds. Lengthened C-C bonds have been observed in cage structures (5,6) caused by constraints



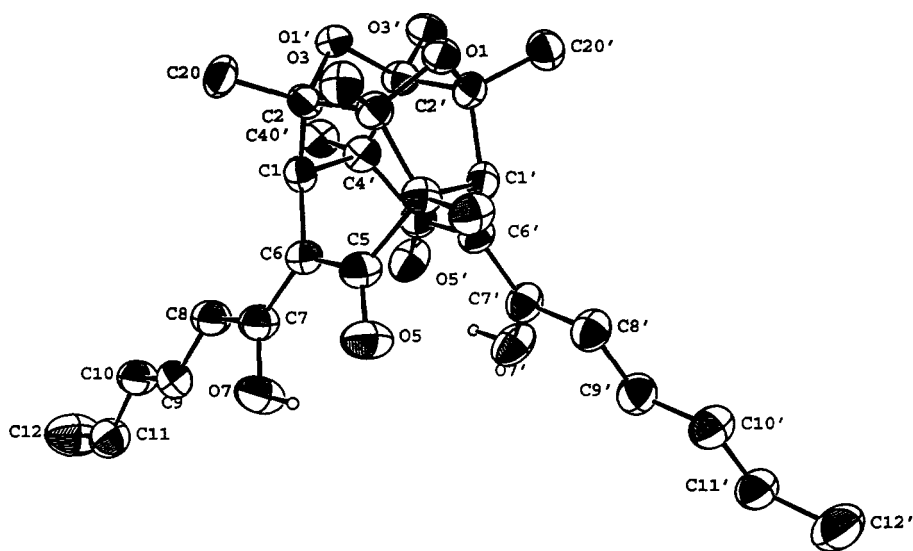


FIGURE 1. ORTEP drawing of **1** showing the atomic labeling scheme. The thermal ellipsoids were drawn at 50% probability level. For clarity, H-atoms are not shown.

of smaller C-C-C bond angles. In our case, significantly smaller angles are found in the cage, e.g., C-3'-C-4'-C-1 = 100.1(4)° and C-3-C-4-C-1' = 101.5(3)°. At the open end of the cage, e.g., C-5-C-6, 1.419(9) Å, and C-5'-C-6', 1.414(9) Å, are much shorter than normal C-C single bonds. These apparent double-bond features are due to their coplanarity with adjacent carbonyl and C=C groups. On the other hand, C-5'=O-5' 1.272(6) Å, C-5=O-5 1.243(5) Å, C-6'=C-7' 1.412(7) Å, and C-6=C-7 1.382(9) Å are longer than normal C=O and C=C bonds. The non-bonded intramolecular distances at the open end of the cage are 2.803 Å (C-5'...C-6) and 2.854 Å (C-5...C-6').

At the open end of the cage, the conjugated system including the sorbyl side-chain C-5' to C-11' and O-5' and its neighboring atoms C-1', C-4', C-12', and O-7' are planar while the system including the second sorbyl side-chain C-5 to C-11 and O-5 and corresponding neighbors are in a common plane with significant deviations. The two planes form a dihedral angle of 132.9(2)°. Intramolecular hydrogen bonds from O-5 to O-7 and O-5' to O-7' are found in both side-chains with the following geometry: O-5...O-7 = 2.462 Å, O-5...H(O-7) = 1.707 Å and O-5-H...O-7 = 132.4° and O-5'...O-7' = 2.452 Å, O-5'...H'(O-7') = 1.687 Å and O-5'-H'...O-7' = 134.3°. All C=C bonds in the side-chains have trans configurations and are almost fully extended. Only one small kink exists in the middle of the chains as indicated by the torsion angles C-8-C-9-C-10-C-11 = 174.3(8)° and C-8'-C-9'-C-10'-C-11' = 174.6(9)°. The C=C bonds in the chains range from 1.32(1) Å to 1.41(1) Å and the C-C bonds range from 1.43(1) Å to 1.51(1) Å. The thermal motions of the C atoms increase gradually towards the terminal methyls. Although the chemical structure of **1** has C<sub>2</sub> symmetry, the molecules occupy general positions in the crystal instead of crystallographic twofold symmetry and have pseudo two-fold axes. In the cage portion, C<sub>2</sub> symmetry is very well kept. The two sorbyl chains break down the symmetry, especially at the ends of the chains because of their slightly different orientations with respect to the cage.

In the crystal, **1** exists as a complex with methanol and water molecules of a formula C<sub>28</sub>H<sub>32</sub>O<sub>8</sub>·CH<sub>3</sub>OH·2H<sub>2</sub>O. A stereoscopic drawing of the crystal structure is shown in Figure 2. The crystal packing is constructed from a double-layered network formed by

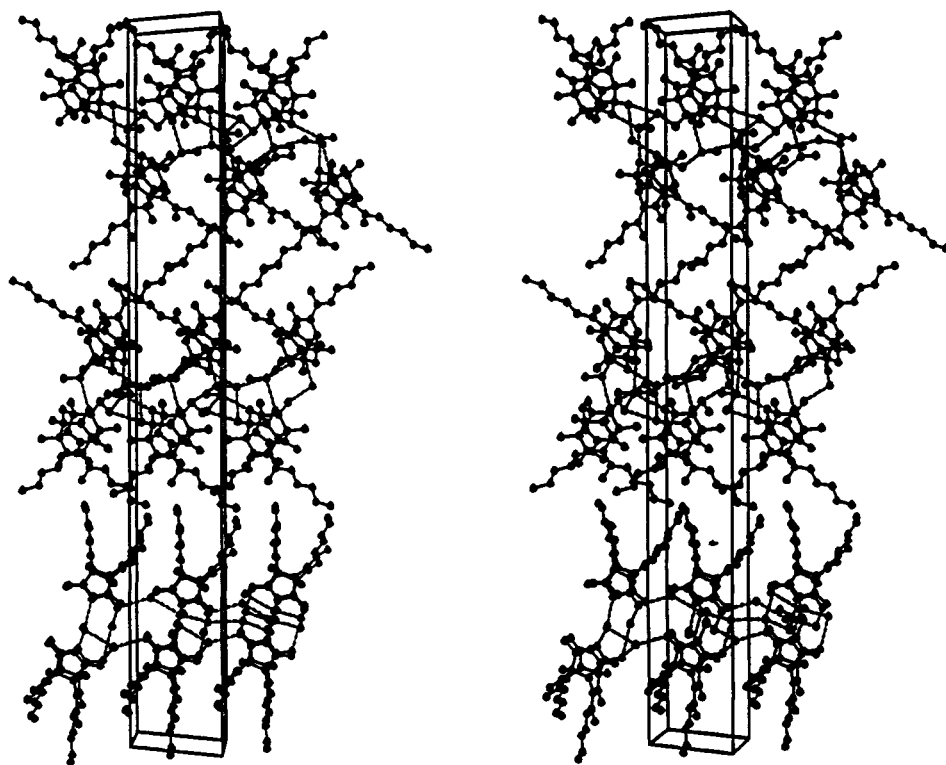


FIGURE 2. Molecular packing in the crystal structure of trichodimerol [1] showing hydrogen-bonding scheme. Dotted lines indicate hydrogen bonds.

intermolecular hydrogen bonding. Parallel to the crystallographic *ab* plane, molecules of **1** first form single layers through hydrogen bonds between the more polar close end of the cage and solvents and then each two single layers form a double layer, in a head-to-head fashion, with the solvent molecules staying in the middle. All interactions within the layer are solvent-mediated. Nine independent hydrogen bonds are involved, six of them between molecules of **1** and the solvents and the rest between solvent molecules. The O...O distances of these hydrogen bonds are in the range 2.772 to 2.973 Å. The double layers are stacked along the *c* axis and the interactions between double layers are of van der Waals-type involving hydrophobic sorbyl chains.

### EXPERIMENTAL

**EXTRACTION AND ISOLATION.**—The fermentation broth of *Penicillium chrysogenum* was prepared as described elsewhere (3). Whole broth (10 liters) was mixed with diatomaceous earth and vacuum filtered. The filtrate was extracted with *n*-BuOH. The *n*-BuOH extract (3 g) was taken up in MeOH-H<sub>2</sub>O (9:1) and extracted with hexane (3 ×). The aqueous MeOH layer was diluted to 25% H<sub>2</sub>O in MeOH and extracted with CCl<sub>4</sub> (3 ×). The CCl<sub>4</sub> extract was further chromatographed by Si gel vlc (Merck LiChroprep Si 60, 25–40 μm). Elution was begun with CHCl<sub>3</sub>, followed by increasing percentages of MeOH in CHCl<sub>3</sub>. Activity was present in the 1% and 2.5% MeOH in CHCl<sub>3</sub> fractions; these were pooled and further developed by Si gel prep. tlc (CHCl<sub>3</sub>-MeOH 95:5), affording a yellow residue (35 mg). Final purification was achieved by C<sub>18</sub> prep. hplc (Rainin Instruments, Dynamax semi-prep. C<sub>18</sub> column, 10.0 mm i.d. × 25 cm length, 8 μm particle size, 60 Å pore size). The mobile phase was MeOH-H<sub>2</sub>O (1:1) with a linear gradient to MeOH-H<sub>2</sub>O (85:15) over a 10-min period, followed by a linear gradient to MeOH-H<sub>2</sub>O (95:5) over a 60-min period at a flow rate of 4 ml/min, with uv detection at 254 nm. A typical sample size/injection was 5 mg/0.5 ml MeOH. The major peak (17 min) was collected and the solvent evaporated to give **1** (total yield 17 mg) as a yellow-white crystalline solid, mp 166–167°. The spectroscopic data were in full agreement with published values (1).

X-RAY DATA FOR TRICHODIMEROL [1].<sup>1</sup>—Crystals suitable for X-ray diffraction experiments were obtained after recrystallization from a mixture of EtOH and H<sub>2</sub>O by slow evaporation at room temperature. A fragment of approximate size 0.10×0.40×0.60 mm cut from a large crystal was mounted on a quartz fiber with epoxy adhesive and was used for preliminary examination and data collection. Diffraction experiments were carried out at room temperature on a Rigaku AFC5R diffractometer using graphite monochromated CuK $\alpha$  radiation ( $\lambda=1.5418$  Å). Crystals are trigonal and cell constants were obtained from a least-squares fit to data for 25 well-centered reflections in the range  $8.85^\circ \leq \theta \leq 17.76^\circ$ . Unit cell constants are  $a=b=9.002(3)$ ,  $c=59.855(9)$  Å and  $V=4200(2)$  Å<sup>3</sup>. From the systematic absences, the space group was determined to be  $P3_121$  and there are six molecules in the unit cell. Intensity data were collected with  $0 \leq h \leq 8$ ,  $0 \leq k \leq 8$ ,  $0 \leq l \leq 67$  up to  $\theta=60^\circ$ . As a check of crystal stability, three reflections were remeasured every 2 h during data collection and showed no significant crystal decay. A total of 2632 unique reflections was measured using an  $\omega/2\theta$  scan mode. The Lorentz and polarization effects were corrected. Absorption corrections were based on  $\psi$ -scans of a selected reflection. After data reduction, the unique data set contains 2571 reflections, 2136 of them are observed with  $I \geq 3\sigma(I)$ .

The structure was solved by direct methods using program SHELXS-86 (7) and was refined by full-matrix least-squares techniques using computer software MolEN (8). The final refinements included 326 parameters, a scale factor and atomic coordinates and anisotropic temperature factors for non-hydrogen atoms. All hydroxyl hydrogens, including those of some solvent molecules, were located in difference Fourier maps and the positions of all the other hydrogen atoms, except the methyl hydrogens of the methanol, were calculated from an idealized geometry with standard bond lengths and angles. All hydrogen atoms were assigned isotropic temperature factors and were included in structure factor calculations with fixed parameters. The refinement converged at  $R(F)=0.067$  and  $wR(F)=0.105$ , where  $w=1/\sigma^2(F)$  and  $S=3.029$  for 2136 reflections. The final difference Fourier map showed no recognizable residual features ( $-0.23 \geq \Delta\rho \geq 0.394$  eÅ<sup>-3</sup>). The final atomic coordinates are listed in Table 1.

TABLE 1. Fractional Atomic Coordinates and Equivalent Isotropic Temperature Factors (Å<sup>2</sup>).

Atom	$x/a$	$y/b$	$z/c$	$B_{eq}^a$
O-1	0.5675 (4)	0.5465 (4)	0.13362 (5)	3.56 (8)
O-1'	0.5600 (4)	0.2767 (4)	0.13415 (5)	3.47 (8)
O-3	0.8454 (4)	0.6846 (5)	0.14534 (6)	4.04 (9)
O-3'	0.2912 (5)	0.1435 (5)	0.11832 (6)	4.1 (1)
O-5	1.0061 (5)	0.7784 (5)	0.08067 (6)	4.9 (1)
O-5'	0.5220 (5)	0.2294 (5)	0.05609 (6)	5.0 (1)
O-7	1.1025 (5)	0.5916 (5)	0.06378 (7)	5.3 (1)
O-7'	0.5388 (6)	0.4669 (5)	0.03404 (6)	5.3 (1)
C-1	0.7442 (6)	0.3443 (6)	0.10415 (8)	3.2 (1)
C-1'	0.5806 (7)	0.5726 (7)	0.09510 (8)	3.6 (1)
C-2	0.7310 (6)	0.4039 (6)	0.12791 (8)	3.3 (1)
C-2'	0.4692 (6)	0.4549 (6)	0.11478 (7)	3.5 (1)
C-3	0.7363 (6)	0.5821 (7)	0.12842 (8)	3.4 (1)
C-3'	0.4586 (6)	0.2725 (7)	0.11534 (7)	3.5 (1)
C-4	0.7662 (7)	0.6727 (7)	0.10577 (8)	3.6 (1)
C-4'	0.5519 (6)	0.2405 (6)	0.09557 (8)	3.5 (1)
C-5	0.8951 (7)	0.6518 (7)	0.09107 (8)	3.7 (1)
C-5'	0.5396 (6)	0.3155 (7)	0.07373 (8)	3.7 (1)
C-6	0.8780 (6)	0.4866 (7)	0.08956 (8)	3.6 (1)
C-6'	0.5551 (7)	0.4798 (6)	0.07339 (8)	3.6 (1)
C-7	0.9810 (7)	0.4615 (7)	0.07471 (8)	4.0 (1)
C-7'	0.5546 (7)	0.5510 (7)	0.05240 (9)	4.1 (1)
C-8	0.9652 (7)	0.2965 (7)	0.07008 (9)	4.3 (2)

<sup>1</sup>Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

TABLE 1. Continued.

Atom	<i>x/a</i>	<i>y/b</i>	<i>z/c</i>	$B_{eq}^a$
C-8'	0.5669 (7)	0.7152 (7)	0.04898 (8)	4.4 (1)
C-9	1.0609 (7)	0.2740 (7)	0.05470 (9)	4.4 (1)
C-9'	0.5630 (7)	0.7758 (8)	0.02911 (9)	4.6 (2)
C-10	1.0393 (7)	0.1109 (8)	0.0481 (1)	4.7 (2)
C-10'	0.5693 (8)	0.9359 (7)	0.02431 (9)	4.8 (2)
C-11	1.1274 (8)	0.0934 (8)	0.0315 (1)	5.3 (2)
C-11'	0.5516 (8)	0.9778 (7)	0.00362 (8)	4.6 (2)
C-12	1.109 (1)	-0.074 (1)	0.0248 (1)	8.2 (3)
C-12'	0.552 (1)	1.1336 (9)	-0.0026 (1)	7.2 (2)
C-20	0.8537 (7)	0.3928 (7)	0.14403 (9)	4.1 (1)
C-20'	0.2970 (7)	0.4423 (8)	0.11607 (9)	4.6 (1)
C-40'	0.4946 (7)	0.0506 (7)	0.09337 (9)	4.1 (1)
C-40	0.8214 (7)	0.8586 (7)	0.10849 (9)	4.2 (1)
Ow1	0.3862 (8)	0.1853 (7)	0.53474 (9)	8.5 (2)
Ow2	0.1292 (8)	0.1893 (9)	0.83414 (9)	9.8 (2)
OMe	0.4507 (8)	0.4816 (8)	0.82201 (9)	9.6 (2)
CMe	0.277 (2)	0.451 (2)	0.8230 (2)	19.7 (6)

$$^a B_{eq} = (8\pi^2/3) \sum_i \sum_j U_{ij} a_i^* a_j$$

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