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STRUCTURE OF TRICHODERMAOL, ANTIBACTERIAL SUBSTANCE PRODUCED IN COMBINED CULTURE OF *TRICHODERMA* SP. WITH *FUSARIUM OXYSPORUM* OR *FUSARIUM SOLANI*

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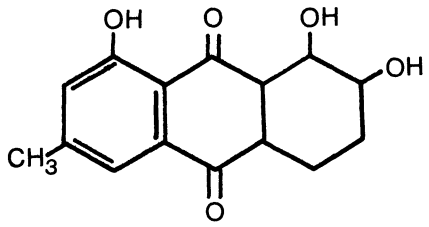
Trichodermaol, an antibacterial substance was isolated from the combined culture of *Trichoderma* sp. with *Fusarium oxysporum* or *Fusarium solani* and the structure was determined by spectroscopic and X-ray crystallographic analyses.

In the course of our study on biologically active metabolites of fungi, we have found that an appreciable amount of novel antibacterial substance, named trichodermaol, is produced in the combined culture of *Trichoderma* sp. with *Fusarium oxysporum* or *Fusarium solani*. Since only small traces of this substance was found in the cultured medium of *Trichoderma* sp. alone, and did not exist in those of *Fusarium oxysporum* or *Fusarium solani* alone, we concluded that it was produced practically by *Trichoderma* sp. in response to *Fusarium oxysporum* or *Fusarium solani*. Several metabolites of *Trichoderma* sp. have been reported,[1-7] but they all differ from trichodermaol. In this paper we wish to report the isolation and the structural elucidation of trichodermaol by spectroscopic and X-ray crystallographic analyses.

Trichoderma sp. was grown aerobically together with *Fusarium oxysporum* or *Fusarium solani* by shaking in the 500ml flask containing 2% sucrose-potato decoction pH ca. 5.5 for 5 days at 27°C. The cultured broth was adjusted to pH 2.2 and extracted with ethyl acetate. The extract was dried over anhydrous Na_2SO_4

and concentrated. The reddish brown residue gave a light yellow crystal, trichodermaol, on recrystallization from acetone, mp 204-206°C, $[\alpha]_D^{21}$+161.5(c, 0.5 EtOH). Yield was ca. 80 mg / 3 litre broth. UV spectrum of trichodermaol showed absorption maxima at 234.9 nm (ε=28,600), 277.9 nm (ε=6,480), 346.7 nm (ε=6,260) in MeOH, indicating the presence of acetophenone chromophore. Elemental analysis resulted in a composition of C;64.82 and H;5.78% (calcd for $C_{15}H_{16}O_5$: C;65.21, H;5.84%). Molecular ion in mass spectrum was observed at m/z 276.0980 ($C_{15}H_{16}O_5$) along with fragment ion peaks at m/z 258 ($M-H_2O$) and at m/z 203 ($M-C_3H_5O_2$). IR spectrum (KBr) indicated absorption bands at 3440 cm^{-1}(hydroxyl), 2940 cm^{-1} (methyl), 1687 cm^{-1}(non-chelated carbonyl) and 1633 cm^{-1}(chelated carbonyl). ^1H NMR spectrum [δ(ppm) in d_6-DMSO from int. TMS] exhibited the following signals: a 4H multiplet at 1.7 and a 2H broad singlet at 3.2 attributable to $-CH_2-CH_2-$ and $\rangle CH-CH\langle$, respectively, a 3H singlet at 2.4 ascribable to an aromatic methyl. A pair of doublets at 7.1 and 7.2 (1H each, J=1.2 Hz) due to aromatic protons meta to each other. Two doublets at 4.8 and 5.1 (1H each), and a singlet at 12.4 (1H) due to two secondary alcoholic hydroxyls and one chelated phenol, respectively. A multiplet at 3.7 and a broad triplet at 4.3, each coupling with each alcohol protons at 4.8 and 5.1 respectively. When D_2O was added, all hydroxyl protons disappeared and the methine proton at 4.3 changed to a doublet (J=3.9 Hz), and further to a singlet by irradiation of the methine at 3.7. Furthermore, the methine proton at 3.7 changed to a doublet (J=3.9 Hz) by adding D_2O and irradiation of the methylene at 1.7. Thus the partial structure $-CHOH-CHOH-CH_2-CH_2-$ was deduced. Fifteen carbons were detected in ^{13}C NMR spectrum (d_6-DMSO, TMS) at; 19.4 ($-CH_2-$), 21.6 ($-CH_3$), 25.4 ($-CH_2-$), 44.0 ($-CH\langle$), 49.0 ($-CH\langle$), 66.8 ($\rangle CH-O$), 67.4 ($\rangle CH-O$), 115.8 ($=C\langle$), 117.8 (=CH-), 122.5 (=CH-) 135.7 ($=C\langle$), 148.3 ($=C\langle$), 160.8 ($=C\langle$), 198.3 ($\rangle C=O$), 203.9 ($\rangle C=O$). From the foregoing spectroscopic observations, the structure of trichodermaol was concluded either 1 or 2.



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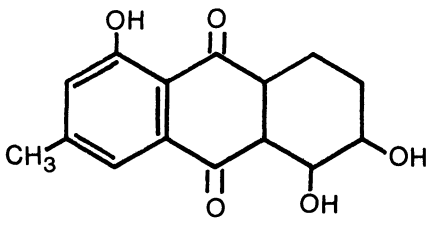

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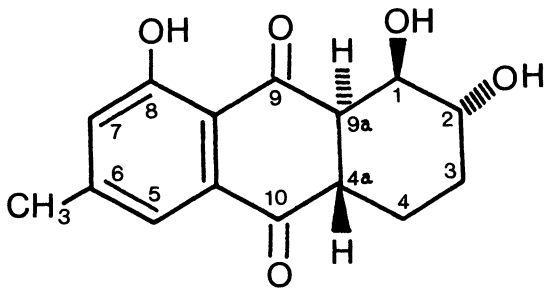
For further elucidation of the structure, we undertook an X-ray crystallographic analysis of trichodermaol. A single crystal (0.3 x 0.2 x 0.1 mm), recrystallized from acetone, belongs to the orthorhombic space group $p2_12_12_1$ with a=13.086(3), b=16.613(2), c=6.018(1) Å and z=4. A total of 1240 independent reflections were measured on a RIGAKU four-circle diffractometer using Cu-Kα radiation. The data was corrected for Lorentz and polarization factors, but not for absorption. The structure was solved by direct methods (MULTAN 78)[8] and refined by block-diagonal least squares method (HBLS VI).[9] From bond lengths and temperature factors, all carbons and oxygens were identified. After refinement using anisotropic temperature factors for carbon and oxygen, all the hydrogen atoms were located on a difference map and the R factor is 0.043 at the final stage. Though the absolute stereochemistry has not been established as yet, the molecular structure of trichodermaol was shown to be 3 or its mirror image.



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Interestingly, trichodermaol is stable with 9,10-diketo form of the structure. As the concept about factors attributing to the stability for the structure is not clear, this aspect is being investigated. Trichodermaol is the first naturally occurring 1,2,3,4,4a,9a-hexahydro-monoanthraquinone and exhibits antibacterial activity against Bacillus subtilis and Staphylococcus aureus at 10^{-4} x 1.8 M concentration and shows growth inhibition effect on mustard seedling at 10^{-4} x 3.6 M concentration. Further studies on the biological activity and production of trichodermaol are in progress, and will be reported later.

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