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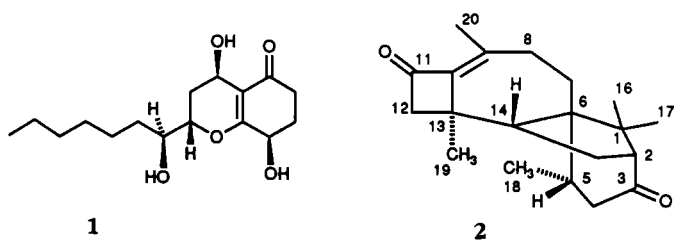
HARZIANDIONE, A NEW CLASS OF DITERPENE FROM
TRICHODERMA HARZIANUM

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ABSTRACT.—The isolation and structural elucidation of harziandione [**2**] from the biological control agent *Trichoderma harzianum* is described. Harziandione represents a new class of diterpenes and is the first diterpene isolated from *Trichoderma* species. The structure and relative stereochemistry were determined from an X-ray diffraction study.

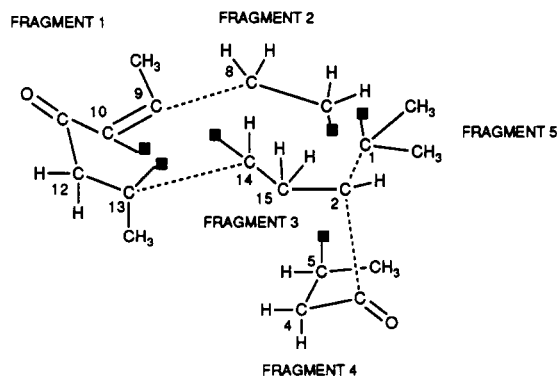
Trichoderma harzianum Rifai (Deuteromycotina) has attracted considerable attention for use in the biological control of plant pathogens. Recent studies by several groups have implicated various metabolites as being involved in suppression of disease (1). Compound **1** and a group of lipophilic peptides are some of the metabolites which contribute to the antifungal activity of different isolates of this species (1–5). In continuation of our interest in characterizing the metabolite produced in growing liquid cultures of this fungus (2), we report the isolation and structural determination of a novel diterpene dione. The diterpene, trivially named harziandione [**2**], contains a new skeleton and represents the first such metabolite to be isolated from *Trichoderma* species. The structure was determined by single-crystal X-ray diffraction methods, the results of which are reported here together with spectroscopic data and assignments.



The EtOAc extract obtained from a liquid culture of *T. harzianum* was fractionated by radial chromatography on Si gel. Elution with CH_2Cl_2 yielded a fraction which appeared homogeneous on tlc and crystallized from diisopropyl ether. The compound, mp 74–75°, from ms and ^{13}C -nmr spectroscopy had a molecular formula of $\text{C}_{20}\text{H}_{28}\text{O}_2$ and contained two carbonyl groups (δ_{C} 214.4, 197.8; ν_{max} 1730, 1700 cm^{-1}). Since the ^{13}C -nmr spectrum showed signals for only two other sp^2 -hybridized carbons, the dione is carbotetracyclic. The ^1H -nmr spectrum contained signals assignable to three tertiary, one secondary, and one vinylic methyl groups. Extensive ^1H - ^1H decoupling experiments, ^1H - ^{13}C HETCOR, and DQF-COSY measurements, summarized in Table 1, provided support for the presence of fragments 1–5 in the molecule (Figure 1). Fragment 1 could be assembled from the observation that one of the methylene protons α to a carbonyl showed long range coupling to the vinylic methyl, whereas the other showed similar coupling to a tertiary methyl. Fragment 2 was a 1,2-disubstituted ethane fragment in which one of the protons was long-range-coupled to the vinylic methyl group. Fragment 3 was a 1,3-disubstituted propane, a methine proton of which showed long range interaction with a proton of a methylene group α to the second carbonyl function. This proton belonged to fragment 4 with the connectivity as shown. The final fragment contained a 2,2-disubstituted propane as evidenced from the long range

TABLE 1. Nmr Assignments for Harziandione [2].^a

Position	$\delta^{13}\text{C}$ (CDCl_3)	$\delta^1\text{H}$ (CDCl_3)	$\delta^1\text{H}$ (C_6D_6)	Multiplicity (J in Hz)	Long-range correlations ^b
1	39.9				C-1-H-15
2	59.3	2.30	2.20	ddd (8,1,1)	
3	214.4				
4 β		2.90	2.60	dd (18,11)	
4	42.6				
4 α		2.05	1.95	ddd (18,1,1)	H-2
5	29.9	2.95	2.40	ddq (1,11,7.2)	
6	51.6				
7 β		1.44	0.87	m	
7	29.8				
7 α		1.90	1.30	ddd (14,6.5,1)	
8 β		2.00	1.35	m	
8	29.5				
8 α		2.45	1.87	ddd (14,12,1)	H-20
9	149.4 ^c				
10	146.4 ^c				
11	197.8				
12 β		2.58	2.20	dq (16,1)	C-11; H-19
12	59.9				
12 α		2.45	2.15	dq (16,1)	H-20
13	49.5				
14	53.0	2.5	2.0	dd (13,10)	
15 β		2.03	1.45	ddd (13,13,8)	
15	26.6				
15 α		1.55	1.1	ddd (13,10,1)	
16	25.0	1.00	0.80	br s	C-16-H-2
17	23.2	0.99	0.56	br s	
18	20.9	1.12	0.69	d (7.2)	C-18-H-4
19	20.7	1.52	1.00	br s	C-19-H-2, H-14
20	22.5	2.12	2.00	br s	C-20-H-8 α

^a ¹H nmr, 300 MHz; ¹³C nmr, 75.1 MHz.^b ¹H-¹H from DQF-COSY; ¹H-¹³C from COLOC ($J=4$ Hz) experiments.^c Values may be interchanged.FIGURE 1. Connectivity established for **2** by nmr spectroscopy. Dashed bonds are inferred from long-range interactions.

interactions between the methyl protons in the DQF-COSY spectrum. This left a tetrasubstituted carbon, which must link two or more of the fragments identified. A COLOC ($J=4$ Hz) measurement gave limited information but established a three-bond coupling between the methylene proton in fragment 3 with the central carbon in fragment 5 and H-14 and C-19. At this stage the isoprene nature of the skeleton was apparent, particularly in the sequences involving C-8–C-14, C-1–C-5, and associated pendant groups, and consideration of the structures derivable on biogenetic grounds suggested that the compound contained a new diterpene nucleus. To determine the structure and its relative configuration, we undertook an X-ray diffraction study of the

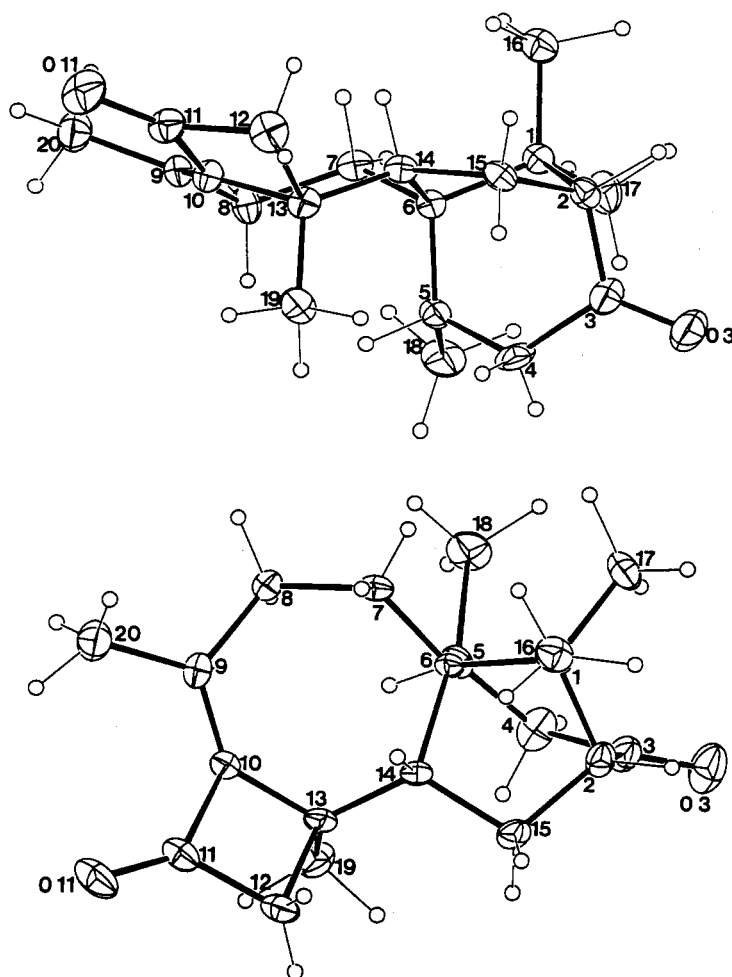
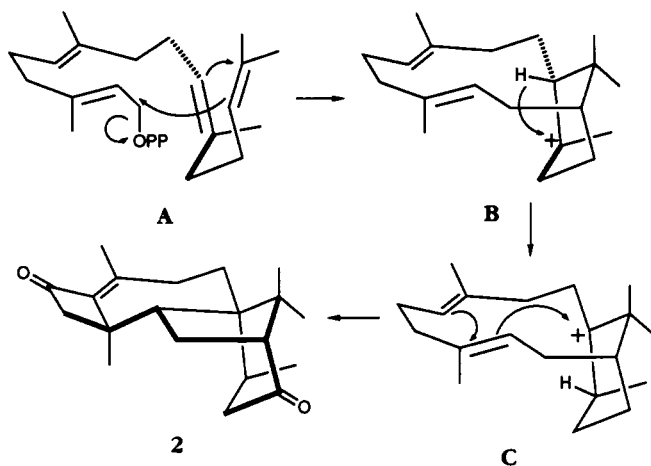


FIGURE 2. Projections of the molecule normal to the planes of the six and seven-membered rings. Atom numbering and 20% thermal ellipsoids are shown for the non-hydrogen atoms; hydrogen atoms have arbitrary radii of 0.1 Å.

dione (Figure 2). The results disclosed that the compound has the structure shown in **2**. From a biosynthetic point of view, it is a regular diterpene whose carbotetracyclic nucleus may be assembled along a pathway depicted in **A–C** (Scheme 1). Apart from the



SCHEME 1.

novelty of the structure, it is significant that this represents the first diterpene to be identified in any *Trichoderma* species. Although sesquiterpenoid metabolites are known to be produced by these species, polyketide-derived metabolites are more common (1).

*Crystal/refinement data.*¹—C₂₀H₂₈O₂, *M*_r 303, orthorhombic, space group *P*2₁2₁2₁ (No. 19), *a* = 17.008(7), *b* = 11.308(5), *c* = 8.765(3) Å, *V* = 1685 Å³, *D*_c 1.18 g·cm⁻³; 1712 unique diffractometer data measured at ca. 295 K [2θ max 50°; 2θ/θ scan mode, monochromatic Mo Kα radiation (λ 0.7107₃ Å)], 990 with *I* > 3σ(*I*) considered observed and used in the full matrix least-squares refinement without absorption correction (μ_{Mo} = 0.4 cm⁻¹; specimen 0.4 × 0.22 × 0.05 mm). Anisotropic thermal parameter refinement for C, O; (x, y, z U_{iso})_H also refined. *R* = 0.050; *R*' = 0.046 (statistical weights derivative of σ²(*I*) = σ²(*I*_{diff}) + 0.0004σ⁴(*I*_{diff})).

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—The preparation of liquid cultures and general methods are as described previously (2).

ISOLATION OF HARZIANDIONE FROM A LIQUID CULTURE OF *T. HARZIANUM*.—The liquid medium (10 liters) of 28-day-old cultures of *T. harzianum* WU 71 (culture deposited with the Commonwealth Mycological Institute IMI 311090) was extracted repeatedly with EtOAc, and the combined organic layer was dried over Na₂SO₄ and evaporated under reduced pressure. The extract (1.26 g) was fractionated by radial plate chromatography using gradient elution (petroleum ether to EtOAc). Elution with petroleum ether-EtOAc (3:1) gave a fraction (84 mg) of a compound (*R*_f 0.2, CH₂Cl₂) which solidified. Crystallization from diisopropyl ether gave harziandione [2], mp 74–75°, [α]_D + 116.5° (*c* = 0.9; CHCl₃); ir ν max (CHCl₃) 1720, 1700, 1660 cm⁻¹; ¹H and ¹³C nmr see Table 1; eims *m/z* [M]⁺ 300 (9) 285 (28), 272 (3), 257 (6), 215 (9), 201 (17), 189 (7), 187 (23), 177 (12), 175 (17), 173 (15), 163 (14), 161 (24), 159 (32), 151 (28), 150 (29), 149 (47), 148 (39), 147 (30), 137 (46), 136 (31), 135 (34), 133 (44), 131 (20), 121 (40), 119 (51), 107 (42), 105 (62), 93 (51), 91 (60), 83 (96), 55 (81), 41 (100).

ACKNOWLEDGMENTS

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¹ Atomic coordinates for this compound have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

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