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Trichoharzin, a New Polyketide Produced by the Imperfect Fungus *Trichoderma harzianum* Separated from the Marine Sponge *Micale cecilia*

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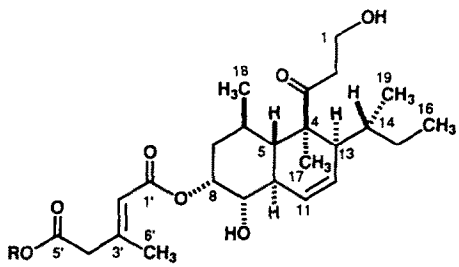
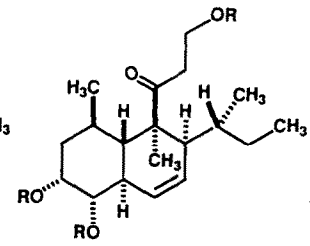
Abstract: Trichoharzin (1) has been isolated from a culture of the imperfect fungus *Trichoderma harzianum*, which was separated from the marine sponge *Micale cecilia*, and the absolute stereostructure elucidated. Trichoharzin (1) is a new polyketide constructed with an alkylated decalin skeleton and esterified with 3-methylglutaconic acid, a rare acyl moiety.

The worldwide effort has been made to find new leads with the view of "drugs from the sea", and an increasing number of marine natural products have been discovered from various marine invertebrates and marine algae as well. In recent years, the chemical metabolites of marine microorganisms have also been paid attention, and as a part of our search for new bioactive marine natural products,[1] we have been investigating the metabolites of marine microorganisms, which are separated from marine sponges. This paper communicates the absolute stereostructure elucidation of a new polyketide named trichoharzin (1), which was isolated from a culture of the imperfect fungus *Trichoderma harzianum* Rifai separated from the fresh marine sponge *Micale cecilia*.

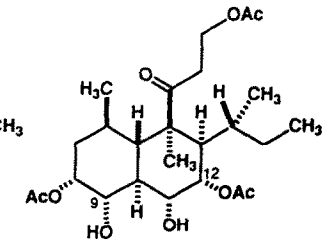
The fungus, separated from the sponge *Micale cecilia* collected at Amami Island, Kagoshima Prefecture in July 1990, was grown in the Wickerham medium prepared with sea water. The cultivation was carried out in 5 *l* round flasks with vigorous shaking at 25°C for 10 days. The combined culture (30 *l*) was filtered with satin and the filtrate was partitioned with AcOEt. The AcOEt soluble portion was evaporated under reduced pressure to give 1.5 g of the extractive. Since *Trichoderma harzianum* Rifai is a widespread soil fungus and is known to produce antibiotics active against other microscopic fungi,[2] we have compared by TLC the extractive from the salty medium with that obtained from the culture in fresh water medium.



trichoharzin (1) : R= H
2 : R= CH_3



3 : R= H
4 : R= Bz



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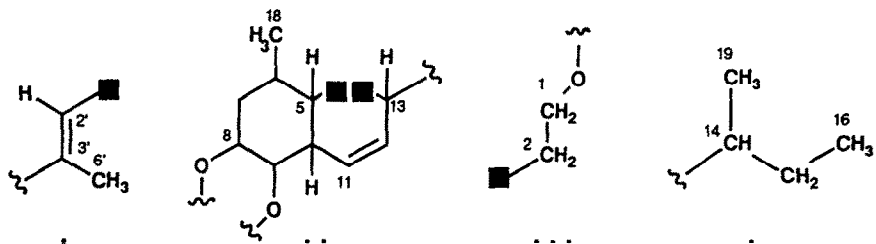
The chemical constituents of both extractives differed significantly, and silica gel column chromatographic and reversed phase HPLC separations of the extractive from the salty medium provided a new polyketide named trichoharzin (1)(15 mg) as a characteristic major metabolite.

Trichoharzin (**1**) was obtained as a colorless glassy solid: $[\alpha]_D$ +38° (MeOH); UV (MeOH): 222 nm (ε= 26000); IR (KBr): 3380, 1700, 1651 cm^{-1}. The FAB-MS of **1** showed a quasi-molecular ion peak at *m/z* 451 $(M+H)^+$ and the molecular formula was determined as $C_{25}H_{38}O_7$ by HR-FABMS in conjunction with NMR analysis (Table I). Treatment of **1** with trimethylsilyl diazomethane furnished the methyl ester **2**.[3] The detailed analyses of ^1H-^1H COSY and C-H COSY spectra of **1** have led to the following four partial structures i∼iv (Fig. 1).



i ii iii iv

Fig. 1 ■ denotes a quaternary carbon.

The connectivities of respective partial structures (i∼iv) have been shown from the following HMBC correlations. 1) The acyl moiety: the cross peaks observed between H-6' (methyl protons) and C-1', C-2', C-3', and C-4'; between H-4' (methylene protons) and C-2', C-3', and C-5'. 2) The adjacency of the partial structures **ii** and **iii**: the cross peaks observed between H-17 (methyl protons) and C-3, C-4, C-5, and C-13; between H-1 (methylene protons) and C-2 and C-3. 3) The adjacency of the partial structures **ii** and **iv**: a cross peak observed between H-19 (methyl

protons) and C-13. Furthermore, the following COLOC correlations have shown the connectivities of those partial structures. 1) The adjacency of the acyl moiety **i** and the partial structure **ii**: a cross peak observed between C-1' and H-8. 2) The adjacency of the partial structures **ii** and **iv**: a cross peak observed between C-15 and H-13.

As shown in Fig. 2, the NOESY correlations in **1** were observed, which further have demonstrated the connectivities of the partial structures (**i**∼**iv**) thus elaborating the relative stereostructure of **1**, including the R configuration at C-14. Based on the above accumulated evidence, the relative stereostructure of trichoharzin (**1**) has been elucidated as shown.

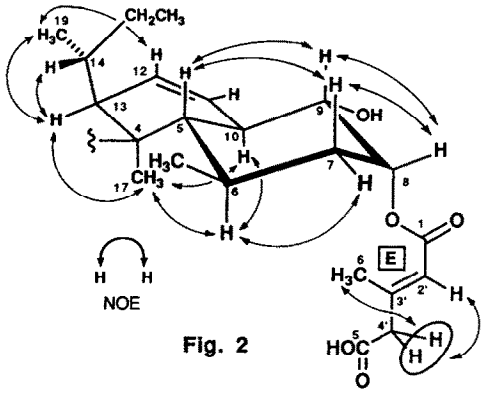


Fig. 2

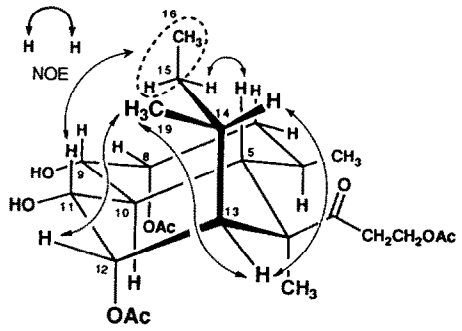


Fig. 3

The absolute stereostructure of **1** has been determined in the following manner. Treatment of **1** with aqueous KOH-methanol furnished the triol **3**,[4] which was then treated with benzoyl chloride and pyridine to provide the tribenzoate **4**.[5] The CD spectrum of **4** showed a negative maximum ($\Delta\varepsilon$ -51.3) at 236 nm and a positive maximum ($\Delta\varepsilon$ +16.9) at 219 nm, which may arise from the exciton coupling between two benzoate chromophores at C-8 and C-9, thus indicating the 8R, 9S configurations.

In order to confirm the stereochemistry at the C-14 methyl of trichoharzin (**1**), the following conversion was undertaken. Acetylation of **3** gave the 1,8,9-triacetate, which was subjected to osmium tetroxide oxidation in benzene-pyridine (10.1) to provide the pentaol triacetate **5**.[6] The NOESY correlations of **5** have supported the stereochemistry of the C-14 methyl as shown in Fig. 3. Consequently, the absolute stereostructure of trichoharzin has been confirmed to be shown as **1**.

Trichoharzin (**1**) is an octaketide with a decalin framework which is alike to *e.g.*

Table I. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data for Trichoharzin (1) in $CDCl_3$

carbon	^{13}C (mult.)	^{1}H (mult., J in Hz)	carbon	^{13}C (mult.)	^{1}H (mult., J in Hz)
1	57.9 (t)	3.84 (td-like, ca 5, 12)	13	52.3 (d)	1.94 (brs, wh/2=10)
		3.91 (ddd, 3.5, 7, 12)	14	37.1 (d)	1.12 (m)
2	41.1 (t)	2.69 (ddd, 3.5, 6, 18.5)	15	24.4 (t)	0.7-0.8 (m)
		2.84 (ddd, 4, 7, 18.5)			1.47 (m)
3	215.6 (s)		16	12.5 (q)	0.76 (d-like, ca 4)*
4	52.5 (s)		17	19.3 (q)	1.26 (s)
5	43.1 (d)	1.98 (dd-like, ca. 10, 10)	18	22.2 (q)	0.58 (d, 6.5)
6	31.4 (d)	1.62 (m)	19	19.4 (q)	0.93 (d, 6.5)
7	39.0 (t)	1.55 (dd-like, ca 14, 14)	1'	166.2 (s)	
		1.86 (d-like, ca. 14)	2'	119.8 (d)	5.87 (brs)
8	72.7 (d)	5.26 (brs, wh/2=10)	3'	151.7 (s)	
9	74.4 (d)	3.55 (brd, ca. 10)	4'	45.5 (t)	3.18 (2H, brs)
10	40.2 (d)	2.12 (dd-like, ca. 10, 10)	5'	174.0 (s)	
11	123.8 (d)	6.04 (brd, ca 10)	6'	19.2 (q)	2.26 (brs)
12	125.8 (d)	5.70 (brd, ca. 10)			

* Due to partial overlapping with one of the 15-H_2 signals, the 16-H_3 signals were observed as d-like

betaenone B previously isolated from the culture filtrate of *Phoma betae* Fr., a causal fungus of leaf spot disease of sugar beat.[7] It is noteworthy to mention that the acyl moiety, 3-methylglutaconic acid, is hitherto known to occur only in a lichen chromone[8] and is presumed to be derived from mevalonic acid. The biological activity of trichoharzin (1) is under investigation.

ACKNOWLEDGEMENT The authors are grateful to Prof K Tsubaki, Faculty of Pharmaceutical Sciences, Nippon University for identification of the fungus. Thanks are due to the Ministry of Education, Science, and Culture of Japan for financial support

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3) **2**: FAB-MS: *m/z* 465 $(M+H)^+$, IR (KBr): 3430, 1738, 1701, 1655 cm^{-1}, ^{1}H NMR ($CDCl_3$, δ). 3.72 (3H, s); ^{13}C NMR ($CDCl_3$, δc)· 52.2 (q), 170.3 (s, 5'-C).

4) **3**: FAB-MS. *m/z* 325 $(M+H)^+$; IR (KBr) 3385, 1698 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ) 4.06 (brs, H-8)

5) **4**: FAB-MS: *m/z* 637 $(M+H)^+$, IR (KBr) 1721 (br), 1279 cm^{-1}, ^{1}H NMR ($CDCl_3$, δ) 4.62∼4.72 (2H, m, H-1), 5.11 (dd, *J*=3.5, 11.5 Hz, H-9), 5.72∼5.76 (m, H-8)

6) The acetyl moiety initially attached to the 9-hydroxyl in the 1,8,9-triacetate [δ 2.02, 2.03, 2.09 (each 3H, s) in $CDCl_3$] was presumed to be migrated *via* the 11-hydroxyl to the 12-hydroxyl both introduced by osmium tetroxide oxidation **5**. FAB-MS m/z 483 $(M+H)^+$, IR (KBr): 3400, 1738 (br), 1244 cm^{-1}, ^{1}H NMR ($CDCl_3$, δ) 2.01, 2.14, 2.16 (each 3H, s), 3.85 (dd, *J*= 3.5, 10, H-9), 3.98 (dd, *J*=3.5, 10, H-11), 5.14(q-like, *J*=*ca* 3, H-8), 5.25 (dd-like, *J*=*ca*.3.5, 3.5, H-12).

7) A. Ichihara, H. Oikawa, K Hayashi, S Sakamura, A Furusaki, and T Matsumoto, *J Am Chem. Soc.*, **105**, 2907 (1983).

8) D. J. Aberhart, K. H. Overton, and S Huneck, *J Chem Soc* (C), **1969**, 704 The geometry of the 2'-olefin moiety was not determined in that paper

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