Deoxynortrichoharzin, a New Polyketide from the Saltwater Culture of a Sponge-Derived *Paecilomyces* Fungus

Lisa Rahbæk,[1] Sam Sperry, Janet E. Piper, and Phillip Crews*

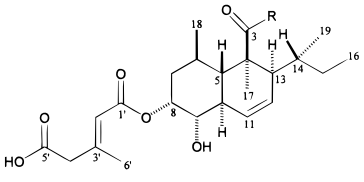
Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95064

Received June 5, 1998

The saltwater culture of a *Paecilomyces* cf. *javanica* isolated from the marine sponge *Jaspis* cf. *coriacea* has yielded a new polyketide, deoxynortrichoharzin (**1**), and two known diketopiperazines (**2** and **3**).

Recent investigations of marine-derived fungi indicate that their extracts exhibit a high rate of antibacterial activity[2] and might be better as a source of bioactive metabolites[3] than fungi derived from terrestrial origins. Some of our recent discoveries of new chemotypes from sponge-derived fungi are also consistent with this promising outlook. One strategy guiding this program involves choosing chemically prolific coral reef sponges as sources of fungal cultures. Once obtained, the fungi are grown in saltwater cultures, and their extracts are monitored by a combination of ESIMS data and bioassay results to highlight extracts that might contain new bioactive constituents.[4] It was this pathway that uncovered two different series of unique halogenated compounds, including the chloriolins as fermentation products of an unidentified fungus separated from *Jaspis* aff. *johnstoni*[4a] and the chlorocarolides obtained from the culture of *Aspergillus* cf. *ochraceus*, isolated from *Jaspis* cf. *coriacea*.[4b] Herein we report the isolation of a new polyketide, deoxynortrichoharzin (**1**), and a mixture of known diketopiperazines (**2** and **3**) derived from a *Paecilomyces* cf. *javanica* also obtained from *J.* cf. *coriacea*.

The molecular formula of **1**, $C_{24}H_{36}O_6$, was established based on an ESIMS *m*/*z* at 433 $[M + Na]^+$. Additional confirmation came from NMR analysis and the MH^+ peak observed by HRFABMS. The HMBC and $^1H-^1H$ COSY data supported the presence of an 11,12-dehydrodecalin framework substituted with groups consisting of acetyl, *sec*-butyl, an OH, an OR, and two methyls. This Decalin core and its array of substituents were analogous to those present in trichoharzin (**4**).[5] In fact, comparison of the ^1H and ^{13}C NMR data (see Table 1) to that of **4** justified the assignment of OR as a 3-methylglutaconic acid moiety, thereby completing the gross structure of **1**.



1 R=CH_3
4 R=CH_2CH_2OH

There were minor complications in the structure elucidation of **1** that deserve comment. At first it was not obvious that the 3-methylglutaconic acid moiety was present because C4′ appeared as a methine group in the ^1H NMR and DEPT spectra, presumably due to H−D exchange of our sample stored for a prolonged period in CD_3OD. Furthermore, no signal was observed for the C5′ carbonyl carbon. Also, the C16 methyl appeared as an apparent doublet with a spacing of 4 Hz in the ^1H NMR. This was due to the accidental isochrony of H_316 and one of the diasterotopic protons of H_215. This isochrony effectively eliminates the splitting expected due to *J*-coupling for nonequivalent protons.[6] Stereochemical relationships within the Decalin core were established from characteristic *J* values, which indicated that H5, H6, H9, and H10 were axial, while H7α and H8 were equatorial. Important NOESY correlations were observed, including those from H_317 to H6 and to H13 indicating that all three groups were on the same face. Several NOESY correlations were observed to groups attached to C14, but these are not diagnostic of relative configuration at that site.[7] Alterna-

Table 1. NMR Data for **1** and **4**

atom no.	**1** (CD_3OD)		**4** ($CDCl_3$)[a]	
	^{13}C	^1H	^{13}C	^1H
1			57.9	3.84 (td-like, ca. 5, 12) 3.91 (ddd, 3.5, 7, 12)
2	28.7	2.22 (s)	41.1	2.69 (ddd, 3.5, 6, 18.5) 2.84 (ddd, 4, 7, 18.5)
3	215.4		215.6	
4	54.2		52.5	
5	44.9	1.92 (t, 10.0)	43.1	1.98 (dd-like, ca. 10, 10)
6	32.6	1.65 (m)	31.4	1.62 (m)
7	40.4	1.52 (m) ax	39.0	1.55 (dd-like, ca. 14, 14)
		1.80 (dt, 14.5, 3) eq		1.86 (d-like, ca. 14)
8	73.6	5.19(q, 3.0)	72.7	5.26 (br s, wh/2=10)
9	75.1	3.42 (dd, 11, 3)	74.4	3.55 (br d, ca. 10)
10	41.4	2.21(tq, 11, 2.2)[b]	40.2	2.12 (dd-like, ca. 10, 10)
11	127.9	6.04 (dt, 10.5, 2)	123.8	6.04 (br d, ca. 10)
12	124.7	5.71 (ddd, 10.5, 4.5, 2.5)	125.8	5.70 (br. d, ca. 10)
13	53.1	2.01 (dt, 2.5 4.5)	52.3	1.94 (br s, wh/2=10)
14	38.3	1.15 (m)	37.1	1.12 (m)
15	25.8	0.76 (below H16)	24.4	0.7−0.8 (m)
		1.48 (m)		1.47 (m)
16	12.8	0.76 (d, 4.0)	12.5	0.76 (d-like, ca. 4)
17	20.1	1.29 (s)	19.3	1.27 (s)
18	22.6	0.59 (d, 7.0)	22.2	0.58 (d, 6.5)
19	19.5[c]	0.96 (d, 6.5)	19.4	0.93 (d, 6.5)
1′	167.7		166.2	
2′	119.6	5.84 (br s)	119.8	5.87 (br s)
3′	155.6		151.7	
4′	49.0[d]	3.11 (br s)	45.5	3.18 (2H, br s)
5′	not obs.		174.0	
6′	19.4[c]	2.22 (br s)	19.2	2.26 (br s)

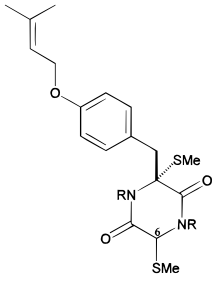
[a] NMR data from Kitagawa et al.[5] [b] $J_{5,10} = J_{9,10} = 11$ Hz and $J_{10,11} = J_{10,12} = J_{10,13} = 2.2$ Hz. [c] Signals are interchangeable. [d] Signal under solvent.

* To whom correspondence should be addressed. Tel.: (831) 459-2603. Fax: (831) 459-2935. E-mail: phil@chemistry.ucsc.edu.

10.1021/np980230f CCC: $15.00 © 1998 American Chemical Society and American Society of Pharmacognosy
Published on Web 10/31/1998

tively, comparison of the very similar NMR (see Table 1) and optical rotation data obtained for **1** ($[\alpha]_D$ +26°) to those previously reported for **4** ($[\alpha]_D$ +38°) strongly supports that they have the same overall relative configuration at both C14 and the other stereocenters as shown. An unexpected intense HMBC correlation across four bonds was observed between H14 and C17, which could be rationalized if these atoms lie trans-axial to each other, thus adopting a *W* arrangement.

The dereplication process used to establish the structures of an HPLC fraction containing a mixture of **2** and **3** was concise and employed a combination of ESIMS and ^{1}H NMR data. An intense ESIMS *m/z* peak at 431.2 $[M + Na]^+$ plus sets of SMe and NMe proton signals implied that a mixture of diketopiperazines **2** and **3** were present. That compound **3** was actually the major isomer (ratio of 60:40%) could be established by the relative areas of the Me signals along with those of the H6 singlets (**2** δ 4.45 and **3** δ 4.93). These compounds have also been isolated from a terrestrial fungus, namely in our study of a saltwater fermentation of *Coriolus consors*.[8] Additionally, compound **2** (H6 δ 4.18) was reported from *Gliocladium deliquescenes*,[9] and both **5** (H6 δ 4.25) and **6** (H6 δ 4.93) were isolated from a *Tolypocladium* sp.[10]



2 α-6-SMe, R=CH_3
3 β-6-SMe, R=CH_3
5 α-6-SMe, R=H
6 β-6-SMe, R=H

A fascinating aspect of this work is that the related trichoharzins **1** and **4** have been isolated in a somewhat parallel approach, but under rather different circumstances. In this study **1** was obtained from a *Paecilomyces* cf. *javanica* isolated from a Fiji collection of *Jaspis* cf. *coriacea*. In contrast, Kitagawa obtained **4** from a *Trichoderma harzianum* (Rifai) derived from a marine sponge *Micale cecilia* collected in Japan.[5] The soil fungus *T. harzianum* is widespread and known to produce antibiotics active against other fungi.[5] Similarly, the *Paecilomyces* spp. are known as common soil fungi in addition to being insect parasites.[11] The *Paecilomyces* are chemically prolific but have not previously been reported to be a source of the compounds discovered in this study. It is reasonable to assume that (+)-deoxynortrichoharzin (**1**), which differs from (+)-trichoharzin (**4**)[5] only by the absence of the C1 hydroxy-
methylene, is its close biogenetic relative. Significantly, both **1** and **4** were isolated from sponge-derived fungi fermented in saltwater media. It is known that metabolite profiles expressed by fungi and their activity are sometimes dependent on media salinity.[12] In this regard, a preliminary study of the interdependence of trichoharzin (**4**) production as a function of saltwater was carried out by Kitagawa, and it appears that salt is essential for its biosynthesis,[5] but this question deserves further study. Compound **1** was tested for cytotoxicity in solid-tumor cells in culture but did not show any activity. Finally, it is noteworthy that an analogous Decalin skeleton is found in the phytotoxic betaenones isolated from *Phoma betae* Fr., which causes leaf-spot disease of sugar beet.[13]

Experimental Section

General Experimental Procedures. NMR spectra were recorded in CD_3OD solution at 500 or 125.7 MHz for ^{1}H and ^{13}C, respectively. Optical rotation was measured on a JASCO DIP-370 digital polarimeter. The IR spectrum was determined on a Varian 1600 series FTIR spectrometer. UV data were obtained on a Hewlett−Packard 8452A Diode Array Spectrophotometer, ESIMS spectra on a VG Quattro II, and HR-FABMS on a VG 70-SE−4F mass spectrometer.

Collection and Fermentation. The fungus (coll. no. 961331) was isolated from *Jaspis* cf. *coriacea* collected in the Fiji Islands. The sponge was aseptically removed and placed in a sterile plastic bag using scuba. On land, pieces of the sponge were surface sterilized and placed on solid agar, and the pure *Paecilomyces* cf. *javanica* fungus strain was isolated.

The following fungal description is based on comparison of our sample with that in the literature (see, for example, Domsch et al.[11]). Macroscopic: Moderately slow growing; diameter of colony 5 cm at 2 weeks on Malt extract agar (MEA) and PDA at room temperature. Surface raised, woolly, and white, gradually becoming pale smoky-violet after 10 days. Reverse white. No diffusable pigment. Microscopic: Hyaline hyphae, erect conidiophores bearing hyaline bottle-shaped phialides with bent tapering axes; some singly placed but predominately in whorls. Conidia are smooth, ovoid, becoming fusiform with age; produced basipetally in long chains.

The fungus was grown on 8 L of MEA prepared with filtered (0.2 μm) Monterey Bay seawater. The fermentation was carried out in 500 mL shake flasks rotated at 120 rpm for 4 weeks at room temperature for initial cultures.

Extraction and Separation. The broths were extracted with EtOAc and partitioned between 10% aqueous MeOH and hexanes and then between 50% aqueous MeOH and CH_2Cl_2. The CH_2Cl_2-soluble fraction (314 mg) was chromatographed on Sephadex LH20 with MeOH−CH_2Cl_2 70:30. One of the fractions obtained was further separated by repeated reversed-phase HPLC using MeOH and H_2O, and pure **1** (2.7 mg) and a mixture of **2** and **3** (2 mg) were obtained. All separations were guided by the $[M + Na]^+$ peaks using ESIMS.

Deoxynortrichoharzin (1): glassy solid; $[\alpha]_D$ +26° (*c* 0.16, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 217 (4.29); $C_{24}H_{36}O_6$, IR (neat) 3378, 3032, 2901, 2929, 2875, 1703, 1652, 1582, 1455, 1381, 1354, 1255, 1148, 1080, 1042 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRFABMS, *m/z* 421.2585 (Δ 0.5 mmu calcd for $C_{24}H_{37}O_6$).

Acknowledgment. We are grateful for the support by NIH grant CA52955 and a grant from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, under grant no. NA36RG0537, project no. R/MP-78 through the California Sea Grant College, and in part by the California State Resources agency. NMR equipment grants from NSF BIR-94-19409 and the Elsa U. Pardee Foundation, and ESIMS equipment grant from the Keck Foundation. We also thank Deanna Sutton from the Fungus Testing Laboratory at the University of Texas Health Science Center, Dr. Keith A. Seifert, ECORC, Ottawa, Canada, and Dr. Gary J. Samuels at U.S. Department of Agriculture for assistance in identifying the fungus. We also acknowledge Bethel M. Borgeson for help in the initial culturing work.

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(7) Kitagawa suggests a 14*R* configuration for **4** based on NOE correlations from H12 and H13 to H19.[5] Our measurements of distances using PC model (6.0) for structures in which the dihedral angle H13−C13−C14−H14 is defined by $J_{H13,\ H14} = 4.5$ Hz does not justify this assignment as both possible diastereomers (14*R* or 14S) have distances as short as 2.3 to 2.5 Å, thereby making the observed NOE correlations possible for both configurations. However, the configuration of **4** was secured by Kitagawa via the pentaol triacetate of **4**.[5]

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NP980230F