Isolation and Structure of Isoculmorin from the Marine Fungus *Kallichroma tethys*

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A new tricyclic sesquiterpene—isoculmorin—was isolated from the marine fungus *Kallichroma tethys* and its structure determined by X-ray crystallography.

During the past decade interest in the secondary metabolites of marine fungi has been increasing at a slow but definite pace. The initial results from a limited number of publications clearly demonstrate the presence of chemically diversified secondary metabolites in marine fungi (e.g., nectriapyrones,[1] leptosins,[2,3] trichoharzin,[4] phomactins,[5] and chlorilins,[6] to mentione a few). However, their potential as producers of novel secondary metabolites has not been conclusively established. About 70−80% of the secondary metabolites that have been isolated from marine fungi are biologically active.

In our search for secondary metabolites of marine fungi with cytotoxicity and/or novel chemical structures, we have investigated the marine fungus *Kallichroma tethys* (Kohlm. and Volkm. Kohlm.; family Hypocreaceae, Hypocreales) and report the isolation and structure of a new tricyclic sesquiterpene, isoculmorin, from the culture broth of this fungus.

The fungus (isolate no. 6175, Portsmouth Culture Collection) was cultured for 21 days in an enriched seawater medium. The culture broth, after the mycelium was separated by filtration through cheese cloth, was extracted with EtOAc. Evaporation of the organic extract gave a yellowish residue that was chromatographed twice on Si gel columns to afford a colorless crystalline solid. LRMS showed the M^+ at m/z 238.2 [HRMS 220.18210 (M^+-H_2O, Δ −2.81 mmu), $C_{15}H_{24}O$], which, along with the ^{13}C- and an APT-NMR spectra established the molecular formula as $C_{15}H_{26}O_2$. The ^1H NMR spectrum showed the presence of three methyls (δ 0.830, 0.876, 0.958, 3H each, s), a methine (δ 3.870, 1H, J = 4.2 Hz), and an oxygen-bearing methylene (δ 3.424, 2H, d, J = 16 Hz), the remaining proton resonances were centered between δ 1 and 2.1. The ^{13}C-NMR spectrum had 15 resonances including two oxygen-bearing carbons (78.4, 69.4 ppm). The APT spectrum showed the presence of three methyls, six methylenes (one oxygen bearing), three methines (one oxygen bearing), and three quaternary carbons. Because of the crystalline nature of **1**, a sample was subjected to a single crystal X-ray crystallographic analysis, while other NMR data were recorded for structure elucidation.

The final atomic parameters of the non-hydrogen atoms are listed in Table 1. A perspective ORTEP drawing of a single molecule of **1** is shown in Figure 1, which shows the relative configuration of the isoculmorin molecule and the atom numbering scheme. The

Table 1. Positional and Equivalent Thermal Parameters and Their ESDs

atom	*x*	*y*	*z*	U_{eq}[a]
O-1	0.1826(2)	0.9463(1)	1.0670(2)	0.0391(6)
O-2	0.2431(2)	0.9193(1)	0.4159(3)	0.0490(7)
C-1	−0.0161(3)	0.9092(2)	0.6848(3)	0.0309(7)
C-2	−0.0979(3)	0.8671(2)	0.8274(4)	0.0334(8)
C-3	−0.0696(3)	0.7797(2)	0.8450(4)	0.0382(9)
C-4	0.0176(3)	0.7424(2)	0.7057(4)	0.042(1)
C-5	0.1605(3)	0.7583(2)	0.7258(4)	0.0400(9)
C-6	0.2071(3)	0.8396(1)	0.6786(4)	0.0321(8)
C-7	0.1220(2)	0.9032(1)	0.7564(3)	0.0279(7)
C-8	0.1000(3)	0.8996(1)	0.9619(3)	0.0296(7)
C-9	−0.0428(3)	0.9164(2)	0.9833(3)	0.0329(8)
C-10	−0.0671(3)	0.9990(2)	0.9176(4)	0.0392(9)
C-11	−0.0549(3)	0.9929(2)	0.7106(4)	0.040(1)
C-12	−0.0920(3)	0.9013(2)	1.1722(4)	0.043(1)
C-13	−0.2421(3)	0.8767(2)	0.8000(5)	0.050(1)
C-14	0.2112(3)	0.8442(2)	0.4736(4)	0.0355(8)
C-15	0.3434(3)	0.8499(2)	0.7512(4)	0.0417(9)

[a] $U_{eq} = (1/3)\sum_i\sum_j U_i U_j a^* i^a * j a_i \cdot a_j$.

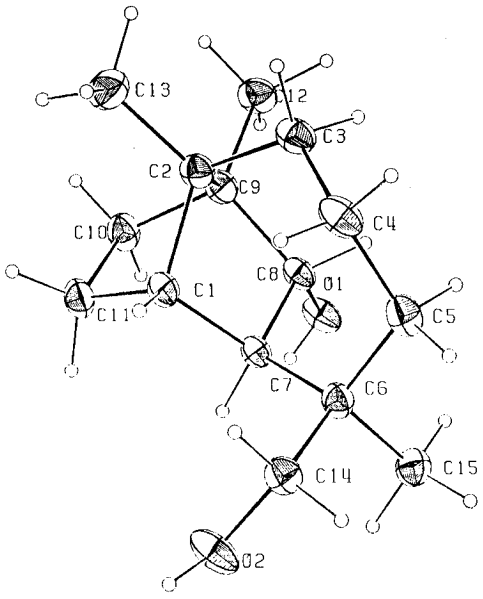

Figure 1. Perspective ORTEP drawing of **1** showing the atom numbering. Thermal ellipsoids are at the 30% level.

isoculmorin differs from culmorin (**2**),[7] and all other known culmorin derivative,[8−10] in that it lacks a hydroxyl or keto group at C-11. It also differs in its C-6 stereochemistry from those culmorin derivatives where C-6 is chiral, such as 15-hydroxyculmorin (**3**) and 15-hydroxyculmorone.[8] The bond distances and bond angles are comparable to those observed in 5-hydroxyculmorin[9] and indicate a strained polycyclic system. The

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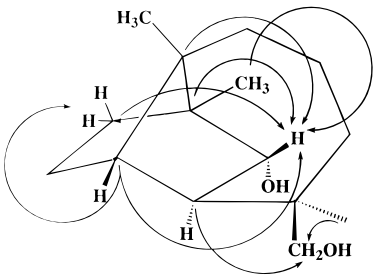
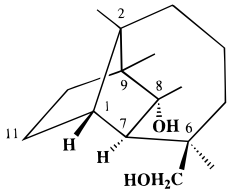


Figure 2. $^1H/^{13}C$ long-range correlations observed in the HMBC spectrum of **1**.

two five-membered rings are in envelope conformation, with C-2 as the common flap. The eight-membered ring is folded. Both hydroxyl groups form two hydrogen bonds and act as both a donor and an acceptor.



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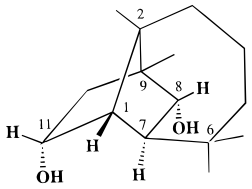

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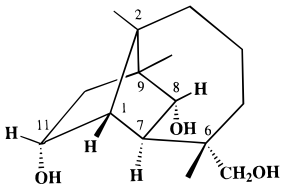

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The ^1H-^1H connectivities were established by utilizing a ^1H,^1H COSY spectrum, while the assignment of protons to individual carbons was accomplished by the use of HMQC spectrum. A HMBC spectrum was used to assign correlations between protons and carbons that are three to four bonds apart (Figure 2).

Although the first isolation of culmorin was reported in 1937,[10] its structure was established only in 1967.[7] A re-examination of *Fusarium culmorin* metabolites[8] has resulted in the identification of a number of hydroxy derivatives of culmorin, which were produced during the first 60 hours of fermetation. In the later stages of culturing (85 h) the fungus produced a number of trithecosenes such as 3 ADON.[7] In our 21-day cultures culmorin was detected only in trace amounts when fractions after the separation of **1** were combined and analyzed by ^1H-NMR spectrometry. A detailed study of the relationship between the secondary metabolites of *Kallichroma tethys* and the age of culture is currently in progress.

Experimental Section

General Experimental Procedures. ^1H- and ^{13}C-NMR spectra were recorded in both $CDCl_3$ and pyridine on a GE-300 spectrometer operating at 300 and 75 MHz frequencies. ^1H,^1H COSY; APT; HMQC; and HMBC spectra were recorded, in pyridine-d_5, on a Bruker 600 spectrometer operating at 600 and 150 MHz frequencies. Chemical shifts were referenced to solvent peaks: δ_H 7.26 (residual $CHCl_3$) and δ_H 7.19 (residual C_5H_5N). MS was recorded on a Finnigan Model 1020 spectrometer equipped with an Incos data system. HRMS was recorded at the Nebraska Mass Spectrometry Center, Department of Chemistry, University of Nebraska, Lincoln, Nebraska. Rotation was recorded on a Perkin-Elmer Model 241 M polarimeter, and the mp was recorded on a Fisher-Johns hot stage mp apparatus and is uncorrected.

Table 2. ^1H and ^{13}C Assignments of Isoculmorin

	^1H [J]	^{13}C	
carbon no.	pyridine-d_5	pyridine-d_5	$CDCl_3$
1	1.953 (d, 4.2)	58.66	43.14
2		50.11	49.70
3	α, 1.570 (m); β, 1.30 (dd)	35.33	35.42
4	1.57 (m)	22.72	21.90
5	1.552 (m); 1.436 (m)	30.35	34.70
6		36.33	37.78
7	1.835 (d, 4.6)	43.75	48.11
8	4.155 (d, 3.5)	78.20	78.72
9		51.26	50.47
10	1.815 (m); 1.31 (m)	38.79	43.15
11	1.153 (m)	26.42	29.38
12	0.819 (s)	13.38	12.20
13	1.040 (s)	22.59	22.18
14	1.389 (s)	23.87	25.87
15	3.751 (d, 16)	69.36	69.14

Culture Conditions. A plug of starter culture was aseptically transferred into a 125 mL flask containing 50 mL of enriched sea-water medium,[11] and the flask was incubated for 21 days on an orbital shaker (120 rpm) at 22 °C. At the end of the incubation period, the mycelium was homogenized in a sterilized blender and the homogenate used to inoculate two 5-gal solution bottles, each with 8 L of medium. The cultures were incubated for 21 days at 22 °C and were aerated by bubbling 0.45 μ of filtered air and were exposed to a cycle of 8 hours of light and 16 hours of dark.

Extraction and Isolation. At the end of the incubation period the mycelium was were separated by filtration through cheese cloth, and the culture broth was extracted with EtOAc (2 × 1L/8L), which, upon evaporation, gave a pale yellow residue (50.4 mg from 32 L of culture broth). The organic residue was layered on a Si gel (230−400 mesh, E. Merck) column (0.6 × 60 cm), which was eluted with $CHCl_3$ (50 mL) followed by a linear gradient of 0−10% MeOH in $CHCl_3$ (total volume 100 mL). Fractions of 4-mL volume were collected and combined on the basis of TLC [Si gel, $CHCl_3$-MeOH (9:1 v/v)]. Fractions containing the desired compound were combined and rechromatographed, as above, to give a residue, which, upon crystallization with MeOH, gave colorless needles, mp 165−167 °C, $[a]^{25}_D$ +156 (*c* 0.2, MeOH). ^1H- and ^{13}C-NMR data are presented in Table 2.

X-ray Crystallography. Compound **1** crystallized from MeOH solution as colorless rods. A crystal of size 0.48 × 0.12 × 0.10 mm was used for all X-ray measurements. Cell dimensions were determined by a least-squares fit to $\pm 2\theta$ of 25 reflections ($12° < \theta < 23°$) measured at −45 °C using CuKα_1 radiation.

Crystal Data: $C_{15}H_{26}O_2$, fw = 238.4; orthorhombic, $P2_12_12_1$; a = 10.461(2) Å, b = 17.470(3) Å, c = 7.470(2) Å, V = 1365.2 Å^3, Z = 4, D_x = 1.160 gm/cm^3, μ(Cu Kα) = 5.1 cm^{-1}, λ = 1.541 78 Å.

Intensities of 1655 unique reflections within $0 < 2\theta < 150°$ were collected on an Enraf-Nonius CAD-4 diffractometer fitted with a N_2 low-temperature device using CuKα radiation. The ω-2θ scan technique was

employed with a variable scan angle, (0.90 + 0.2 tan θ)° and a variable horizontal aperture, (3.5 + 0.86 tan θ) mm. Three intensity control monitors were measured every 7200 seconds of X-ray exposure time, and they showed a maximum variation of < 1%. Intensities were corrected for Lorentz and polarization factors, but no absorption correction was made. In all, 1382 reflections with intensities greater than 2 σ (I) were considered "observed," and these were used in the structure refinement.

The structure was solved by the direct methods using the program MITHRIL.[12] The structure was refined by a full-matrix least-squares routine using the program SHELX76.[13] The quantity $\sum w(Fo - Fc)^2$ was minimized, where w is the weighting function, $1/\sigma^2$ (Fo). All the hydrogen atoms were located from the difference Fourier map, and hydrogen atom positions were refined isotropically. The refinement converged to R = 0.042, Rw = 0.045 for 1382 observations and 258 variables; S = 1.6, (Δ/σ)max = 0.02, $\Delta\rho$max = 0.22/Å^3.

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