

Notes

A New Isodrimeninol from *Pestalotiopsis* sp.

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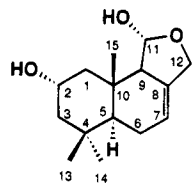
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A new sesquiterpene 2 α -hydroxydrimeninol **1** was isolated as the most polar metabolite produced by a cultured *Pestalotiopsis* sp., a fungus associated with *Taxus* sp. Its structure was established by spectroscopic and X-ray diffraction analyses.

The recent report that taxol and taxanes are produced by an endophytic fungus of the Pacific yew tree¹ has generated a systematic exploration of the metabolites produced by fungal endophytes associated with members of the genus *Taxus*. We recently found that two strains of *Pestalotiopsis* sp. isolated from the bark and leaves of *Taxus brevifolia* produce several compounds when grown on a modified S7 medium. The compounds were isolated by applying the crude culture extract to silica gel column and developing with the solvent system CHCl₃–MeOH (95:5). Some of these metabolites have been identified by both spectroscopic and X-ray diffractometric analyses as new sesquiterpenes of the caryophyllene type.² However, the most polar compound among them, which is the subject of this note, is a new derivative of the drimen type (**1**).



Compound **1** (2 α -hydroxydrimeninol) was detected on Si gel TLC as a red-purple spot when the plate was sprayed with anisaldehyde-H₂SO₄ reagent. It was originally isolated as a yellow oil and recrystallized from H₂O–EtOH to give colorless needles (mp 74 °C) suitable for X-ray analysis (12.5 mg/L). The loss of 18 mass units (H₂O) from m/z 235 is the only significant peak in the FABMS. The presence of hydroxy groups is also indicated by the IR (KBr) bands at 3496, 3406, and 3243 cm⁻¹.

Analysis of the carbon and proton NMR spectra identified three methyl, four methylene, five methine, and three quaternary carbons. One quaternary carbon and one methine carbon belong to an olefinic system. One moiety each of CH₂O, CHO, and CHO₂ was also readily apparent from the NMR spectra.

The HRMS had a prominent peak at 234.1619 amu that fitted very well with a formula C₁₅H₂₂O₂ (calcd 234.1620), and the FABMS showed a loss of 18 mass

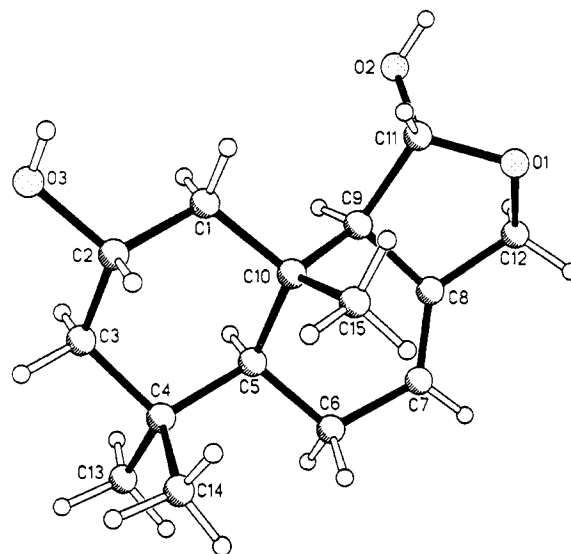


Figure 1. A computer-generated perspective view of 2 α -hydroxydrimeninol (**1**) showing one (of three) independent molecules in the crystal. No absolute configuration is implied. units (H₂O) from m/z 235 as the only significant peak. Because only one double bond was evident from the NMR spectra, the molecule must be tetracyclic to satisfy the molecular formula. Reconstruction of the skeleton by means of the PFG-HMQC,³ PFG-HMBC,⁴ and PFG-DQFCOSY⁵ NMR spectra, however, showed that the two-oxygen formula could not be correct because it required an impossible oxygen bridge between C-2 and C-11. Moreover, the two-oxygen formula meant that no OH group could be present, and a hydroxy group was indicated by the IR (KBr) bands at 3496, 3406, and 3243 cm⁻¹. Thus, the real molecular formula had to be C₁₅H₂₄O₃, and the peak at m/z 235 arose from the loss of H₂O.⁶

The three-oxygen formula, as well as the structure, was clearly indicated by single-crystal X-ray analysis (Figure 1). The initial analysis was complicated because the asymmetric unit contained three molecules of **1** and two waters of crystallization, but the final structure converged nicely. The structure and conformation of all three molecules in the asymmetric unit are the same, and the conformation found in the solid state is in good

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accordance with the one in solution as deduced by NOE difference spectroscopy experiments.

We suggest the trivial name 2 α -hydroxyisodrimeninol for compound **1**. Drimane sesquiterpenes of this type have been isolated from plants⁶ as well as from fungi⁷ and even sponges.⁸ Although there are literature reports of isodrimeninols that are unsubstituted or C-2, C-3 disubstituted, or C-3 only substituted, this is the first report of a substituent exclusively at C-2. A compound with a 2-hydroxy group, 2-hydroxypolygodial, with potential antitumor activity has been prepared by microbial oxidation of the unsubstituted compound.⁹ Drimanes such as polygodial and waburganal are known also for their feeding-deterrent properties, and, although this activity seems to point to dialdehyde equivalent substituents at positions 8 and 9, the masked monoaldehyde alcohol of **1** could play a similar role or serve as a precursor.⁶

Experimental Section

General Experimental Procedures. The ¹H NMR and all the 2D NMR spectra were recorded on a JEOL JNM- α 600 at 600 MHz, while ¹³C-NMR spectra were recorded on a Bruker AC-300 at 75.5 MHz. Both FABMS and HRMS measurements were taken on a JEOL JMS-AX505WA mass spectrometer, while the IR spectrum was measured using a Nicolet Impact 400 instrument. For the mp a Yanaco Micro melting point apparatus was used, and the specific rotation was recorded on JASCO DIP-370 digital polarimeter. The TLC R_f value was determined by using a Merck TLC plate Si gel 60F₂₅₄, of 20-cm length and layer thickness of 0.25 mm, using CHCl₃-MeOH (96:4) as solvent system; after development, the compound was visualized with a solution of anisaldehyde and H₂SO₄ (5% w/v) in MeOH.

Fungal Material. Leaves of *Taxus brevifolia* were collected in Bozeman, Montana. The strain was selected by culturing several small leaf pieces onto agar and subsequently transferring the mycelial tips several times. The strain was identified as *Pestalotiopsis* sp. Small agar plugs were then transferred into a culture broth consisting of: 1 mg biotin, 1 mg thiamine, 1 mg calcium pantothenate, 1 mg pyridoxin, 3.6 mg MgSO₄, 6.5 mg Ca(NO₃)₂, 1 mg Cu(NO₃)₂, 2.5 mg ZnSO₄, 5 mg MnCl₂, 2 mg FeCl₃, 5 mg phenylalanine, 100 mg sodium benzoate, 1 g glucose, 3 g fructose, 6 g sucrose, 1 g sodium acetate, 1 g (Bacto) Soytone, and 1 mL of 1 M KH₂PO₄ per liter. The fungus was grown in still culture at 25 °C for 46 days.

Extraction and Isolation. The culture was filtered through cheesecloth, and the fluid (4 L) was extracted with CH₂Cl₂ (2 L). This extract was concentrated under vacuum (628 mg) and applied to Si gel (2 \times 13 cm). The column was developed with CHCl₃-CH₃OH (95:5), and the eluent was collected in 2.5-mL fractions. Tubes 58–

137 yielded a yellow oil (56 mg) on evaporation, which was crystallized from H₂O-EtOH, giving 50.5 mg of pure compound **1**.

2 α -Hydroxyisodrimeninol (1). R_f 0.14; solid crystalline, mp 74 °C; [α]_D²² -18.5° (c 0.88, MeOH); IR ν_{\max} (KBr) 3550–3150, 2927, 1020 cm⁻¹; ¹H-NMR (CDCl₃, TMS, 600 MHz) δ 0.86 (3H, s, H-15), 0.94 (3H, s, H-14), 0.96 (3H, s, H-13), 1.21 (1H, dd, J = 12.2, 11.8 Hz; H-3 α), 1.23 (1H, dd, J = 12.4, 11.8 Hz; H-1 α), 1.28 (1H, dd, J = 11.6, 5.1 Hz, H-5), 1.81 (1H, ddd, J = 12.2, 3.9, 2.5 Hz; H-3 β), 1.89 (1H, dd, J = 16.5, 11.6 Hz; H-6 β), 2.13 (1H, ddd, J = 12.4, 3.9, 2.5 Hz; H-1 β), 2.14 (1H, br. d, J = 16.5 Hz, H-6 α), 2.29 (1H, br. s, H-9), 3.90 (1H, dddd, J = 11.8, 11.8, 3.9, 3.9 Hz; H-2), 4.17 (1H, d, J = 5.7 Hz, H-12a), 4.48 (1H, d, J = 5.7 Hz, H-12b), 5.28 (1H, d, J = 2.4 Hz, H-11), 5.51 (1H, br. s, H-7); ¹³C-NMR (CDCl₃, TMS, 300 MHz) δ 14.9 (q, C-15), 22.4 (q, C-14), 23.5 (t, C-6), 33.1 (q, C-13), 34.5 (s, C-4), 35.0 (s, C-10), 48.6 (t, C-1), 49.1 (d, C-5), 51.1 (t, C-3), 61.1 (d, C-9), 64.5 (d, C-2), 68.6 (t, C-12), 99.0 (d, C-11), 117.1 (s, C-8), 136.2 (d, C-7); HREIMS m/z 234.1619 (calcd for C₁₅H₂₂O₂: 234.1620); FABMS (glycerol matrix) m/z 235 ([M+H]⁺ - 18), 217, 93.

X-Ray Crystallographic Analysis of 1.¹⁰ Single crystal X-ray diffraction was used to characterize compound **1**. Crystals of **1** belong to the orthorhombic space group $P2_12_12_1$ ($Z = 4$) with $a = 10.275(2)$ Å, $b = 16.021(3)$ Å, and $c = 26.656(5)$ Å, $V = 4388(2)$ Å³. The structure was solved by direct methods and refined by full-matrix least-squares with 2,534 observed ($I > 2\sigma(I)$) reflections, anisotropic nonhydrogen atoms and isotropic hydrogens in calculating positions. The final R -factor is 4.9%, and the weight for a reflection was calculated using standard SHELEX 93 scheme.

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Supporting Information Available: Crystal data, atomic coordinates, and bond lengths and angles for **1** (7 pages). See any current masthead page for ordering information.

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- (10) Atomic coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

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