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Terreinol—a novel metabolite from *Aspergillus terreus*: structure and ^{13}C labeling ☆

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Abstract—A novel metabolite from *Aspergillus terreus*, named terreinol, was isolated and its biosynthetic origin was determined by NMR based on the incorporation of [1-^{13}C]-D-glucose. The labeling pattern indicated a predominant polyketide biosynthetic origin for this metabolite.

1. Introduction

Fungi are accomplished chemists that produce a wide range of complex organic molecules with important applications in the pharmaceutical industry. The biological properties of these compounds and the availability of ^{13}C labeling make fungal metabolites good candidates for studying biosynthetic pathways exploitations using NMR.[1]

The fungus *Aspergillus terreus* has a worldwide distribution in different soils. This microorganism produces lovastatin (mevinolin), a natural product used as a cholesterol lowering agent[2] and several other metabolites such as terrein[3] **2** and terreic[4] acid **3**, both of which have antibiotic activity (Fig. 2).[5]

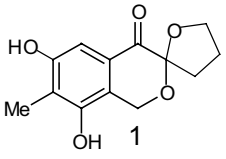

Figure 1. Terreinol: a novel metabolite from *A. terreus*.

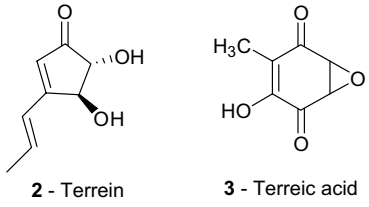

Figure 2. Structures of two other natural products of *A. terreus*.

We now report the isolation of a novel metabolite from *A. terreus*[6] (compound **1**, Fig. 1) and its biosynthetic origin, based on the pattern of ^{13}C incorporation.

2. Isolation and characterization of metabolite 1

While screening for enzymatic activity in malt extract cultures of Brazilian strains[6] of *A. terreus*, we observed that this fungus produced compounds **2** and **3**, and the new compound **1**, all of which were isolated from ethyl acetate extracts. Terreic acid was the major product in 2-day cultures whereas compound **1** was not present. On the other hand, in older cultures (>4 days), **1** was produced in higher amounts than compounds **2** and **3**, as confirmed by GC/MS analysis.

The production of these metabolites was time dependent and was also influenced by the culture medium. Thus, the formation of compound **1** was totally suppressed when culture media richer than malt extract were used.

Keywords: Terreinol; Novel metabolite; *Aspergillus terreus*; Isotopic labeling.

☆ Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2003.10.128

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Table 1. ^{1}H and ^{13}C NMR data for compound

Carbon	δ_C	δ_H (int, mult, J)	HMBC ($^{1}H \rightarrow {}^{13}C$)
1	59.7	4.94 (1H, d, 15.7 Hz); 4.73 (1H, d, 15.7 Hz)	106.6; 123.1; 128.0; 190.0
2	123.1	—	—
3	152.3	—	—
4	120.8	—	—
5	156.7	—	—
6	104.7	6.97 (1H, s)	120.8; 156.7; 190.7
7	128.0	—	—
8	190.7	—	—
9	106.6	—	—
10	33.8	2.64 (1H, dt, 12.6 and 8.8 Hz); 1.89 (1H, ddd, 12.6, 8.2, and 4.5 Hz)	26.0; 71.2; 106.6; 190.7
11	26.0	2.13 (1H, overlapping); 2.04 (1H, m)	26.0; 71.2; 106.6; 190.7
12	71.2	4.06 (1H, m); 3.97 (1H, dd, 14.6 and 7.7 Hz)	26.0; 33.8; 106.6
13	9.3	2.13 (3H, s)	9.3; 120.8; 152.3; 156.7

The spectra were acquired under the following conditions: 1–2 mg of **1**, CD_3OD, 25 °C, 499.885 MHz for ^{1}H and 125.696 MHz for ^{13}C.

Moreover, metabolite production was strongly influenced by the malt extract (ME) manufacturer.[7] To maximize the production of **1**, other experimental conditions were optimized and, to confirm its microbiological source, a blank assay using the appropriate medium without inoculation was run.

The structure of this new optically active[8] natural product was established by mass spectrometry and extensive spectroscopic analysis (UV,[9] IV, MS, COSY, HSQC, g-HMBC, and NOESY 1D).

The molecular ion at m/z 250.08485 (17%) in the HRMS agreed with the formula ($C_{13}H_{14}O_5$; calcd: 250.08413). The IR spectrum showed intense phenolic hydroxyl and carbonyl peaks at 3421 and 1735 cm^{-1}, respectively, while the sp^2 electron-rich aromatic ring carbon–carbon vibrations occurred at 1609 cm^{-1}.

The presence of a pentasubstituted aromatic ring was established based on the occurrence of only one aromatic methine group in the ^{1}H and ^{13}C NMR spectra (δ_H 6.97, δ_C 104.7), both of which depicted a correlation in the HSQC spectrum. Two doublets at δ_H 4.94 and δ_H 4.73 ($^{2}J = 15.7$ Hz) were assigned to the diastereotopic benzylic methylene H1a,b, an AX spin system clearly visualized in the H,H-COSY spectrum. The ^{1}H NMR signals of the diastereotopic methylene H12a,b and the methylene 11 were observed at δ 4.06, 3.97 and δ 2.13, 2.04, respectively, with the expected integrals and multiplicities.

The last methylenic hydrogen pair, H10a,b, which was strongly influenced by the proximity of a stereogenic center, was assigned to the NMR signals at δ_H 2.64 and δ_H 1.89. All of these four pairs of methylenic hydrogens showed homonuclear correlations in the COSY spectrum. Moreover, their respective attached carbon atoms were unequivocally assigned based on the correlations observed in the HSQC and HMBC 2D NMR spectra (Table 1).

Long range scalar couplings observed between the quaternary carbon C9 at δ 106.6 and the alicyclic hydrogens (H1, H10, H11, and H12) were taken as evidence for the presence of a dioxaspiro moiety. Similar chemical shift values have been reported[10a,b] for oxaspiro-type carbons.

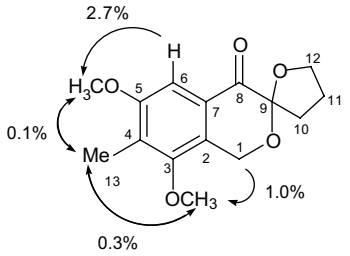

Figure 3. NOE increment data for derivative **1a**.

Finally, the signals at δ_C 9.3 and δ_H 2.13 were assigned to the aromatic methyl group. The presence of two phenolic groups was suggested by the deshielded aromatic carbons at δ_C 156.7 and δ_C 152.3, and this was confirmed by signals at 4.85 and 5.30 in NMR experiments using $CDCl_3$, in which **1** was only partially soluble.

The substitution pattern of the aromatic ring was assigned based on NOESY 1D experiments of the natural product and its dimethyl derivative **1a**.[11] The aromatic hydrogen of **1** displayed dipolar coupling only with the methyl group. The selective excitation of the methoxy group attached to the C5 of **1a** (δ_H 3.87) revealed its proximity to the CH and CH_3 groups by signal enhancement (Fig. 3) while the other methoxy group at δ_H 3.72 showed dipolar coupling with the benzylic and methyl hydrogens (Fig. 3). These data convincingly allowed the proposition of 6′,8′-dihydroxy-7′-methyl-4,5-dihydro-3*H*-spiro[furan-2,3′-isochromen]-4′(1′*H*)-one (compound **1**).

3. ^{13}C labeling experiments

One of the simplest and most widely used means of probing biosynthetic routes consists of incorporating labeled acetate into the natural product. However, the addition of the sodium acetate to the culture medium,

Table 2. NMR data for the [1-^{13}C]-D-glucose-labeled compound **1**

Carbon	δ_C	Integral
1	59.7	1.2
2	123.1	2.3
3	152.3	1.3
4	120.8	2.3
5	156.7	1.2
6	104.7	2.3
7	128.0	0.9
8	190.7	2.6
9	106.6	1.1
10	33.9	2.4
11	26.0	1.1
12	71.2	2.3
13	9.4	1.5

The spectra were acquired under the following conditions: <1 mg of **1**, 50 μL of CD_3OD, 125.696 MHz, 25 °C.

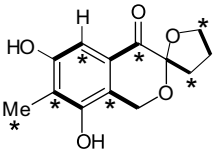

Figure 4. Pattern of [1-^{13}C]-D-glucose incorporation into compound **1**: * indicates the ^{13}C-labeled positions; bold bonds indicate the incorporation of intact acetyl units.

even in minimal amounts, completely suppressed the production of metabolite **1** under several different growth conditions. On the other hand, the use of [1-^{13}C]-D-glucose as a labeled precursor resulted in formation of this metabolite.

The positions of glucose incorporation into compound **1** were unequivocally observed with ^{13}C NMR spectroscopy using optimized[12] inverse gated decoupling.[13] Despite the small amounts of the compound (<1 mg) and the low levels of incorporation (~1.5%), the enrichment could still be accurately quantified (Table 2).

The pattern of incorporation shown in Figure 4 indicates that **1** had a predominantly polyketide origin[14] involving six acetyl/malonyl units added by CoA.

The labeling of the aromatic methyl, adjacent to another labeled position, may indicate alkylation of the polyketide intermediate by methionine.

Full delineation of the biosynthetic steps of compound **1** requires further studies involving putative labeled intermediates. These studies and determination of the configuration of the stereogenic center are currently underway.

The supplementary data (UV and NMR spectra) are available online with the paper in ScienceDirect.

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6. Strain CCT3320 isolated from the Brazilian Atlantic Rain Forest by Fundação Tropical André Tosello.
7. Difco malt extract (100 mL) (2%) in 500 mL Erlenmeyers was used. For the enrichment assays, 25 mg of [1-^{13}C]-D-glucose was added to 100 mL of this medium. In all experiments, the cultures were maintained at 28 °C with stirring at 170 rpm in a rotatory shaker.
8. **1a** was readily obtained treating **1** with an excess of freshly distilled diazomethane.
9. λ_{max} MeOH: 222, 282, and 336 nm.
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11. $[\alpha]_D^{20}$ −74° (*c* 1.15, CH_3OH).
12. Relaxation delay = 20 s; acquisition time = 1 s.
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