Isolation of Isodrimenediol, a Possible Intermediate of Drimane Biosynthesis from *Polyporus arcularius*

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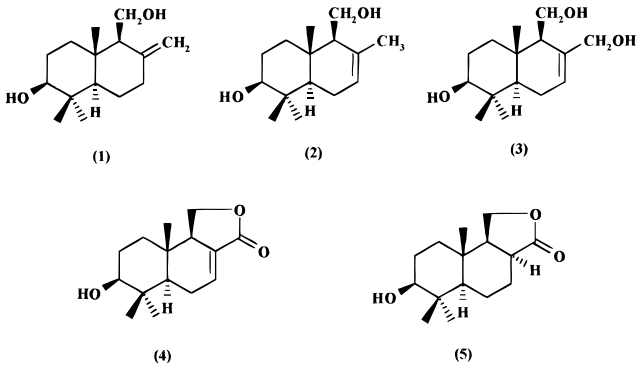
Isodrimenediol, 5-(hydroxymethyl)-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol (**1**), was isolated as a new microbial metabolite from *Polyporus arcularius* DSM 1021. The strain coproduces 7-drimene-3,11,12-triol (**3**) and two related drimane metabolites (**4** and **5**), suggesting that **1** is the first intermediate of biosynthesis of drimane-type structures.

Norsesquiterpene structures such as drimanes are frequently occurring metabolites of fungi such as *Marasmius oreades, Alternaria brassicae,* and *Lactarius noidus*.[1−4] The biosynthesis of these sesquiterpenes has previously been suggested to involve, in the fungi, the oxidative cyclization of farnesyl pyrophosphate.[4] This feature was demonstrated by chemical methods such as, e.g., the oxidative cyclization of farnesyl acetate in the presence of benzoyloxy radicals.[4] The resulting synthetic product, 5-(hydroxymethyl)-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol (**1**), was obtained as a single stereoisomer. This was shown by isomerization of the exo to the endo double bond to yield the known 7-drimene-3,11-diol (**2**).[4] But so far **1** has never been described as a microbial metabolite and intermediate of sesquiterpene biosynthesis. Here, we report on the isolation of **1** from the culture broth of the fungal strain, *Polyporus arcularius* DSM 1021, which coproduces the structurally similar but already known sesquiterpene antibiotic, 7-drimene-3,11,12-triol (**3**)[1−3] and the related drimane metabolites 3β-hydroxycinnamolide (**4**) and 3β-hydroxydihydroconfertifolin (**5**).[5]



The new 5-(hydroxymethyl)-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol (isodrimenediol (**1**)) and the related drimane-type metabolites **3**−**5** were isolated from the culture extract of *P. arcularius* DSM 1021 by several subsequent chromatographic steps. Two weakly antifungal components and two inactive compounds were isolated as pure samples that stained reddish on TLC by use of 1% vanillin/H_2SO_4 concd as reagent.

Due to the EI mass spectra, both **1** and **3** contain three double bond equivalents but differ in one oxygen atom (*m*/*z* 238.2 and 254.2). Moreover, the presence of two hydroxyl groups in **1** was confirmed by the occurrence of $M^+ - H_2O$ and $M^+ - 2H_2O$ (*m*/*z* 220.2 and 202.1). From the ^{1}H NMR and ^{1}H,^{1}H COSY NMR spectra of **1** and **3** it easily could be determined that the former compound is distinguishable from the latter by an exocyclic double bond. The ^{13}C, DEPT, HSQC, and HMBC spectra showed that **3** was constitutionally identical with the known 7-drimene-3,11,12-triol. NMR data confirmed the structure of **1** as the new natural product 5-(hydroxymethyl)-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol.[4] Full support to this contention was derived from the HMBC ^{1}H-^{13}C long-range correlations (Figure 1a).

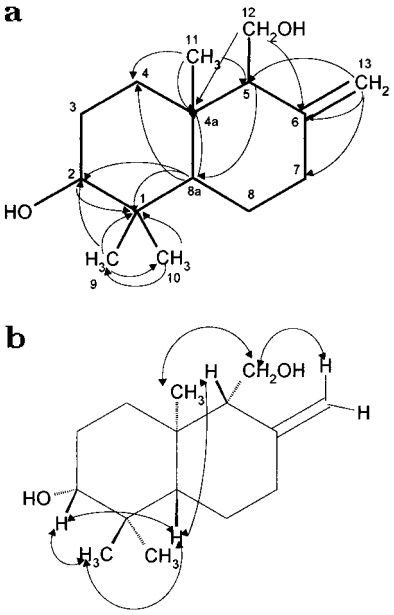


Figure 1. Structural assignation of **1** according to the NMR data (a) Important long range ^{1}H-^{13}C couplings as detected in the HMBC spectrum. (b) Relative stereochemistry according to NOESY experiments.

The relative stereochemistry of **1**, as inferred from the NOESY data (Figure 1b), was shown to be the same as that reported for the other members of the drimane family of antifungal antibiotics.[1] Similar to **3**, compound **1** displays moderate antimicrobial activity against some Gram-positive bacteria such as *Staphylococcus aureus* SG511 (MIC: 10 μg/mL) and yeasts such as *Sporobolomyces salmonicolor* 549 (MIC: 5 μg/mL) during the agar diffusion assay.[6] But under the same conditions it displayed approximately one-fifth of the

† DSMZ.

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activity of **3**. The structures of the two other coproduced metabolites, **4** ($C_{15}H_{22}O_3$) and **5** ($C_{15}H_{24}O_3$), were assigned in a similar manner by MS and NMR methods. Both compounds were isolated recently from the fungus *Peniophora polygonia*.[5] The occurrence of 5-(hydroxymethyl)-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol (**1**) provides one more example of a natural product that was synthesized first but later discovered in natural sources. The isolation of **1** from a fungal strain producing the related 7-drimene-3,11,12-triol (**3**) and the drimane sesquiterpenes **4** and **5** supports the view that these structures could be biosynthesized via **1** as an intermediate.[5]

Experimental Section

General Experimental Procedures. HPLC was carried out using a Gilson binary gradient HPLC system equipped with a UV detector (210 nm). High-resolution electron-impact mass spectra (EI-MS) were recorded on a AMD 402 double-focusing mass-spectrometer with BE geometry (AMD, Intectra, Harpstedt, Germany). NMR spectra were recorded at 300 K in $CDCl_3$ on an Avance DPX-300 spectrometer (Bruker, Karlsruhe, Germany) with sample concentrations of 10 mM. Chemical shifts are given in ppm relative to internal TMS. HSQC and NOESY (mixing time 0.7 s) data were obtained in the phase-sensitive mode using TPPI. The HMBC spectra were optimized on a long-range coupling constant of 7 Hz.

Microorganism and Cultivation. *P. arcularius* DSM 1021 was obtained from the DSMZ culture collection, Braunschweig, Germany. Fifteen day agar-plate cultures (25 °C) were prepared as inoculum using a medium composed as follows (g/L): malt extract 40, yeast extract 4, agar 15, deionized water, pH 6.0. Areals (4−5 cm^2) of the agar-plate cultures were used to inoculate a liquid medium composed of the following: (g/L) malt extract 20, glucose 10, yeast extract 1, $(NH_4)_2HPO_4$ 5, pH 6.0. The surface cultivation was carried out under sterile conditions in 5 L polypropylene boxes containing 500 mL of the above medium for 2 weeks at 25 °C.

Extraction and Isolation. The whole culture broth was extracted twice by two volumes of ethyl acetate. The residue of the evaporated ethyl acetate extract from 40 L of culture (7 g) was applied to a silica gel column (silica gel 60, Merck; 0.063−0.2 μm, in *n*-hexane). Elution was performed with 500 mL portions of *n*-hexane, *n*-hexane/ethyl acetate (9:1), and *n*-hexane/ethyl acetate (7:3). The product from the latter elution (350 mg) was separated by preparative HPLC on a reversed-phase column (RP_{18} Spherisorb, 25 mm × 250 mm) using a binary gradient (water/acetonitrile 95:5 to 5:95; 30 min). The individual peaks were collected and purified by the same preparative HPLC procedure.

Isodrimenediol (**1**) was obtained as waxy mass: yield 12 mg; $[\alpha]^{20}_D$ +9.7 (*c* 0.5, MeOH); IR (KBr) ν max 3340 (OH), 2935 (CH, aliphatic), 1452, 1408, 1379, 1352, 1332, 1281, 1250, 1182, 1131, 1101, 1032, 1005, 964, 930, 885 cm^{-1}; ^{1}H-NMR (CDCl3, 300 MHz) δ 4.96 (1H, d, J = 1.4 Hz, H-13β), 4.66 (1H, d, J = 1.4 Hz, H-13α), 3.80 (2H, m, CH_2-12), 3.28 (1H, dd, J = 11.4, 4.2 Hz, H-2), 2.44 (1H, ddd, J = 13.0, 4.4, 2.5 Hz, H-7β), 2.03 (1H, dt, J = 13.0, 5.1 Hz, H-7α), 1.94 (1H, dd, J = 9.2, 3.3 Hz, H-5), 1.77 (1H, m, H-8β), 1.73 (1H, m, H-4β), 1.72 (1H, m, H-3β), 1.59 (1H, m, H-3α), 1.42 (1H, m, H-8α), 1.37 (1H, m, H-4α), 1.13 (1H, dd, J = 12.1, 2.8 Hz, H-8α), 1.01 (3H, s, Me-9), 0.78 (3H, s, Me-10), 0.73 (3H, s, Me-11); ^{13}C-NMR (75.4 MHz, CDCl3) δ 147.2 (s, C-6), 106.9 (t, C-13), 78.6 (d, C-2), 58.8 (d, C-5), 58.7 (t, C-12), 54.4 (d, C-8a), 39.11 (s, C-1), 38.7 (s, C-4a), 37.7 (t, C-7), 36.9 (t, C-4), 28.3 (q, C-9), 27.7 (t, C-3), 23.7 (t, C-8), 15.4 (q, C-10), 15.3 (q, C-11); EIMS (70 eV) *m/z* $[M]^+$ 238.1940 (calcd 238.1937 for $C_{15}H_{26}O_2$), 220.18 ($M^+ - H_2O$); *m/z* 202.17 ($M^+ - 2H_2O$).

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