1,2-Dihydroxymintlactone, a New Nematicidal Monoterpene Isolated from the Basidiomycete *Cheimonophyllum candidissimum* (Berk & Curt.) Sing.

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Isolated from the Basidiomycete *Cheimonophyllum candidissimum* (Berk & Curt.) Sing., a fungus that previously has yielded nematicidal sesquiterpenes. In this paper we wish to allow for the isolation, structure elucidation and bioactive metabolites in cultures of *C. candidissimum*, a fungus that previously has yielded nematicidal sesquiterpenes. The structure was determined by spectroscopic methods.

Introduction

*Cheimonophyllum candidissimum* is a small, wood-inhabiting Basidiomycete which belongs to the Tricholomataceae (Section Collybiae) (Singer, 1986). Mycelial cultures of the fungus were found to kill and consume nematodes on water agar (Stadler et al., 1994a). In a screening of fungal extracts for nematicidal activity, *C. candidissimum* showed strong effects. The major nematicidal principles were shown to be a series of new bisabolane sesquiterpenes (Stadler et al., 1994b), e.g. *cheimonophyllum A* (2) and *cheimonophyllal* (3). The latter also exhibited antimicrobial and cytotoxic properties (Stadler et al., 1994a). In a search for additional bioactive metabolites in cultures of *C. candidissimum*, small amounts of the new nematicidal monoterpene 1,2-dihydroxymintlactone (1) were isolated. In this paper we wish to report the isolation, structure elucidation and biological activities of 1,2-dihydroxymintlactone (1).

Results and Discussion

1,2-Dihydroxymintlactone (1) was isolated by bioassay-guided fractionation of an extract of *C. candidissimum* (prepared as previously reported by Stadler et al. (1994a)), using the free-living nematode *Caenorhabditis elegans* Maupas as test organism. High resolution mass spectroscopy suggested that its elemental composition is C_{10}H_{14}O_{4}. The structure was elucidated by the long-range ¹H–¹³C correlations observed in HMBC NMR experiments (summarized in Fig. 2). The relative configurations of C-1, C-2 and C-3 of dihydroxymintlactone (1) were found to be the same as in cheimonophyllal (3). The large ¹H–¹H coupling constant as well as the lack of a NOESY correlation between 2-H and 3-H show that the two are in a transdiaxial position, while the NOESY correlation between 7-H₃ and 2-H and the lack of correlation between 7-H₃ and 3-H places the 1-CH₃ group at the same side as 2-H.

1,2-Dihydroxymintlactone (1) belongs to the large p-menthane group of monoterpenses, only few of which have been reported from fungal sources (Chapman and Hall, 1994). The structurally related mintlactone, in which the 1-OH and 2-OH of 1 are replaced by hydrogens, is a constituent of peppermint (*Mentha piperita*) oil (Takahashi et al., 1980) and is reported to have an extremely sweet taste. The sweetness of compound (1), however, is not too pronounced. The biosynthesis of mintlactone in *M. piperita* as well as its chemical synthesis have been reported.

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et al., 1991; Chevan et al., 1993). p-Menthanes have been detected and isolated from ascomycetous and imperfect fungi, for example limonene from Gyromitra esculenta, Cronartium fusiforme and Fusicoccum amygdali (Turner and Aldridge, 1983). This is the first report of a p-menthane produced by a Basidiomycete.

1,2-Dihydroxymint-lactone (1) has the same ring system as cheimonophyllal (3), but it lacks the iso-pentenone side chain. TLC and analytical HPLC analyses of samples taken daily during the fermentation of C. candidissimum revealed that 1,2-dihydroxy-mint-lactone (1) is produced along with the sesquiterpenes, indicating a biosynthesis via the classical monoterpene pathway rather than being a degradation product of compound (2) or other bisabolanes.

The LD₈₀ of 1,2-dihydroxymint-lactone (1) towards C. elegans was 25 μg/ml. Up to 100 μg/paper disk 1 exhibited no antimicrobial activity in the plate diffusion assay towards fungi (Mucor miehei, Penicillium notatum, Paecilomyces variotii and Nematospora coryli) and bacteria (Bacillus brevis, B. subtilis and Micrococcus luteus). Its cytotoxic activity was very weak. L1210 cells were inhibited at 100 μg/ml. No phytotoxic effects in the plant germination assay towards Setaria italica and Lepidium sativum could be observed at 50 μg/paper disk.

The Experimental section details the methodology used in the study. For instance:

**Experimental**

_Cheimonophyllum candidissimum_ TA 8644 is maintained in the culture collection of Dr. T. Anke, University of Kaiserslautern, F.R.G., where also a voucher specimen has been deposited. The fungus was cultivated in YMG medium (yeast extract 0.4 %, malt extract 1%, glucose 0.4%, pH 5.5) in a Braun Biostat U 201 fermentor at 24 °C, with stirring (150 rpm) and an aeration rate of 4 l/min. The taxonomy of the producing organism, its fermentation and the preparation of crude extracts from the culture fluid have been described by Stadler et al. (1994a). 1,2-Dihydroxymint-lactone (1) was isolated by chromatography on silica gel with cyclohexane–ethyl acetate mixtures as eluents, followed by HPLC on reversed phase material (RP18) with water–methanol (1:1).

The assays for biological activities were carried out as described previously, nematocidal activity (Stadler et al., 1993), phytotoxic and cytotoxic activities (Anke et al., 1989). NMR spectra were recorded with a Bruker ARX500 spectrometer, mass spectra (direct inlet) with a Jeol SX102 spectrometer, IR spectra with a Bruker IFS 48 spectrophotograph and UV spectra with a Perkin Elmer Lambda 16 spectrophotometer. The optical rotation was determined with a Perkin Elmer 1541 polarimeter with a cell path of 10 cm.

1,2-Dihydroxymint-lactone (1) was obtained as a colourless oil. [α]D = +16° (c 0.5 in CDCl₃). UV (methanol) λ_max (ε): 221 nm (2260). IR (KBr): 3415, 2930, 1740, 1685, 1645, 1450, 1160, 1110, 1075, 1050 and 1005 cm⁻¹. ¹H NMR (500 MHz, CDCl₃, chemical shifts in ppm relative CHCl₃ [7.26 ppm]): 4.83, dm, J2-3 = 8.6, 3-H; 3.22, d, J2-3 = 8.6, 2-H; 2.60, m, 5-Ha; 2.58, m, 5-Hb; 2.09, ddd, J5a-6a = 3, J5b-6a = 4, J6a-6b = 14.1, 6-Ha; 1.82, brs, 10-H3; 1.42, ddd, J5a-6b = 8, J5b-6b = 10.
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