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

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1,2-Dihydroxymintlactone (**1**), a new monoterpene possessing nematicidal activity was isolated as a minor metabolite from the culture filtrate of the Basidiomycete *Cheimonophyllum 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. cheimonophyllon 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 $^1H-^{13}C$ 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-H_3 and 2-H and the lack of correlation between 7-H_3 and 3-H places the 1-CH_3 group at the same side as 2-H.

1,2-Dihydroxymintlactone (**1**) belongs to the large *p*-menthane group of monoterpenes, 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. (Akhila

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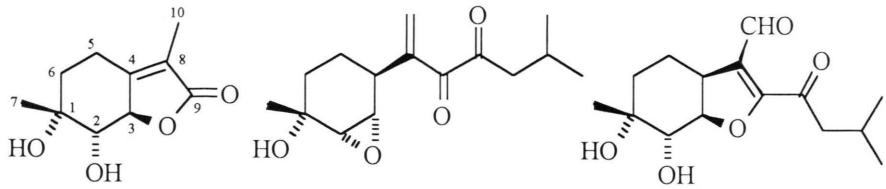

1 2 3

Fig. 1. Structures of 1,2-dihydroxymintlactone (**1**), cheimonophyllon A (**2**) and cheimonophyllal (**3**).

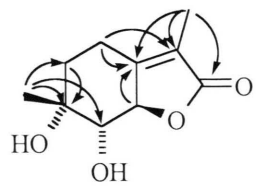

Fig. 2. Significant long-range heteronuclear correlations for 1,2-dihydroxymintlactone (**1**).

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-Dihydroxymintlactone (**1**) has the same ring system as cheimonophyllal (**3**), but it lacks the isopentenone side chain. TLC and analytical HPLC analyses of samples taken daily during the fermentation of *C. candidissimum* revealed that 1,2-dihydroxymint-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 LD_{50} of 1,2-dihydroxymintlactone (**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.

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 20 l 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-Dihydroxymintlactone (**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, nematicidal 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-Dihydroxymintlactone (**1**) was obtained as a colourless oil. $[\alpha]_D$ +16° (c 0.5 in $CDCl_3$). 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, $CDCl_3$, chemical shifts in ppm relative $CHCl_3$ [7.26 ppm]): 4.83, dm, J_{2-3} = 8.6, 3-H; 3.22, d, J_{2-3} = 8.6, 2-H; 2.60, m, 5-Ha; 2.58, m, 5-Hb; 2.09, ddd, J_{5a-6a} = 3, J_{5b-6a} = 4, J_{6a-6b} = 14.1, 6-Ha; 1.82, brs, 10-H_3; 1.42, ddd, J_{5a-6b} = 8, J_{5b-6b} = 10,

J_{6a-6b} = 14.1, 6-Hb; 1.33, s, 7-H_3. ^{13}C NMR (125 MHz, $CDCl_3$, chemical shifts in ppm relative $CDCl_3$ [77.0 ppm]): 174.8 C-9; 159.3 C-4; 120.8 C-8; 83.8 C-3; 79.9 C-2; 72.5 C-1; 36.4 C-6; 26.1 C-7; 21.3 C-5; 8.4 C-10. MS (EI, 70 eV), *m/z*: 198.0876 (M^+, 9%, $C_{10}H_{14}O_4$ requires 198.0892), 180 (62%), 165 (63%), 151 (63%), 137 (20%), 125 (27%), 110 (100%), 82 (43%), 43 (47%).

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