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DERMATOLACTONE, A CYTOTOXIC FUNGAL SESQUITERPENE WITH A NOVEL SKELETON

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Abstract—The nematicidal 5-pentyl-2-furaldehyde and the cytotoxic sesquiterpene dermatolactone were isolated from the extracts of an Ascomycete belonging to the Dermateaceae. The furan has previously been reported from the Basidiomycete *Irpex lacteus*, while dermatolactone is a new compound the structure of which was determined by spectroscopic methods. Copyright © 1996 Elsevier Science Ltd

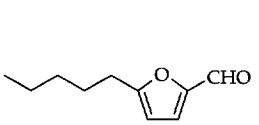
INTRODUCTION

In an ongoing screening of fungal extracts for nematicidal, antimicrobial and cytotoxic metabolites, the extracts of an ascomycete (strain A4990) belonging to the Dermateaceae showed both nematicidal and cytotoxic activity. Two bioactive metabolites were isolated, the nematicidal 5-pentyl-2-furaldehyde (**1**) which previously has been reported from the Basidiomycete *Irpex lacteus* [1], and the novel sesquiterpene **2** possessing cytotoxic activity. The structure of the latter, for which we suggest the name dermatolactone, was determined by spectroscopic methods, and in this paper the isolation, structure determination and biological activities of compounds **1** and **2** are described.

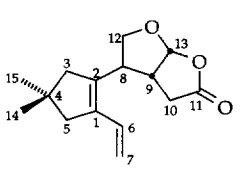
RESULTS AND DISCUSSION

The biological activities of extracts of the culture broth of strain A4990 grown in YMG medium peaked after four days of fermentation, and the two active metabolites **1** and **2** were isolated as described in Experimental. The compounds were not present in the mycelia which were discarded. The structures of compounds **1** and **2** were determined by NMR spectroscopy and mass spectrometry. The elemental composition of dermatolactone (**2**) was indicated by high resolution EI-MS measurements, and the structure and relative stereochemistry was determined by HMQC, HMBC and NOESY experiments. Pertinent HMBC and NOESY correlations are summarized in Fig. 1. It is reasonable to assume that dermatolactone (**2**) is a sesquiterpene, although its carbon skeleton to our knowledge has never been reported.

5-Pentyl-2-furaldehyde (**1**) exhibits nematicidal activity towards *Caenorhabditis elegans* Maupas (LD_{50} = 75 $\mu g\,ml^{-1}$; LD_{90} = 100 $\mu g\,ml^{-1}$) and *Meloidogyne incognita* (Kofoid & White) Chitwood (LD_{50} = 50 $\mu g\,ml^{-1}$; LD_{90} = 75 $\mu g\,ml^{-1}$), while dermatolactone (**2**) is inactive at concentrations up to 100 $\mu g\,ml^{-1}$. The cytotoxic activities of **1** and **2** towards four different mammalian cell lines are summarized in Table 1. While compound **1** was weakly cytotoxic, dermatolactone (**2**) inhibited the growth of HL 60-cells at 5 $\mu g\,ml^{-1}$. Dermatolactone (**2**) possesses weak antimicrobial activity, inhibiting *Nematospora coryli* at 100 $\mu g\,ml^{-1}$ and several Gram-positive bacteria (*Bacillus brevis*, *B. subtilis*, *Corynebacterium insidiosum* and *Micrococcus luteus*) at 50 $\mu g\,ml^{-1}$. Compound **1** only inhibits *N. coryli* at 100 $\mu g\,ml^{-1}$. *Arthrobacter citreus*, *Mycobac-*



1



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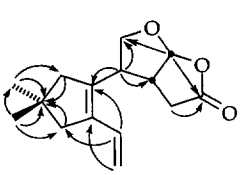

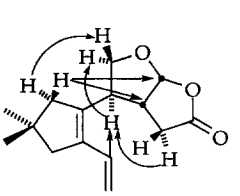

Fig. 1. Pertinent HMBC (left) and NOESY (right) correlations observed with dermatolactone (**2**).

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terium phlei, *Streptomyces* spec. ATCC 23836, *Acinetobacter calcoaceticus*, *Esherichia coli* K12, *Salmonella typhimurium* TA 98, *Nadsonia fulvescens*, *Rhodotorula glutinis*, *Saccharomyces cerevisiae* S 288c, *Fusarium oxysporium*, *Mucor miehei*, *Paecilomyces variotii*, *Penicillium notatum* and *Ustilago nuda* were not sensitive to either compound at concentrations up to 100 μg ml^{-1}. In the plant germination assay with *Lactuca sativa*, *Lepidium sativum* and *Setaria italica*, only compound **1** inhibited the germination of the seeds. At 50–100 μg ml^{-1} 50% of the seeds did not germinate and the seedlings were shorter as compared to the control. None of the compounds exhibited haemolytic activity towards bovine erythrocytes at 100 μg ml^{-1}.

Table 1. Cytotoxic activities of 5-pentyl-2-furaldehyde (**1**) and dermatolactone (**2**) towards four different mammalian cell lines

	1		2				
Cell line	50	100	1	5	15	50	100 (μg ml^{-1})
L1210	i	+	–	–	i	++	++
HL 60	i	+	–	i	+	++	++
HeLa-S3	–	–	–	–	i	+	++
BHK 21	–	–	–	–	i	+	++

–, no effect.

i, 50% inhibition of growth.

+, concentration causing 50% lysis of the cells after 48 hr incubation.

++, concentration causing 90% lysis of the cells after 48 hr incubation.

EXPERIMENTAL

Fermentation. Fruiting bodies of the ascomycete A4990 growing on a dead herbaceous stem, were collected in Queensland, Australia in 1990. The mycelial culture is deposited in the culture collection of the LB Biotechnology, University of Kaiserslautern. The fungus was maintained on solid YMG medium, composed of (g l^{-1}): yeast extract 4, malt extract 10, glucose 4. A4990 was fermented in a Biostat U 20 apparatus at 24°, with stirring (150 rpm) and an aeration rate of 3 l min^{-1}. For inoculum 200 ml of a well grown culture in YMG medium was used. The nematicidal activity against *Meloidogyne incognita* was measured in a microtitre plate assay [2]. After four days the cultures were harvested. The mycelium was separated from the fluid by filtration and discarded.

Isolation. The culture filtrate was applied onto HP 21 resin (Mitsubishi), and the resin was washed with water. Elution with MeOH followed by elution with Me_2CO yielded two crude extracts (3.8 g and 1.5 g). The crude extracts were fractionated on silica gel (Merck 60, 60–200 μm) with cyclohexane–EtOAc gradients. 5-Pentyl-2-furaldehyde (**1**) (90 mg) was isolated from the fr. of the Me_2CO crude extract obtained by elution with 100% cyclohexane, by HPLC (LiChroPrep Diol, 7 μm, column size 250 × 25 mm, flow rate 5 ml min^{-1}) with cyclohexan-*tert* butyl methyl ether (4:1). Dermatolactone (**2**) (5 mg) was isolated from the fraction of the MeOH crude extract obtained by elution with cyclohexane–EtOAc (7:3), by HPLC (same column as above) with *tert* butyl methyl ether.

Spectroscopy. EI-MS: direct inlet, 70 eV; NMR: ARX 500 spectrometer, $CDCl_3$, chemical shifts reported in ppm with the solvent signals (δ_H = 7.26 and δ_C = 77.0) as reference.

Dermatolactone (**2**) was obtained as an oil $[\alpha]_D$ +52° (*c* 0.3 in chloroform). UV λ_{max}^{MeOH} nm (ε): 243 (6,100); IR_{max}^{KBr} cm^{-1}: 2952, 2864, 1801, 1639, 1465, 1421, 1362, 1176, 1096, 990, and 907; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.57 (*dd*, $J_{6\text{-}7a}$ = 10.7, $J_{6\text{-}7b}$ = 16.2 Hz, 6-H), 6.08 (*d*, $J_{9\text{-}13}$ = 5.2 Hz, 13-H), 5.12 (*d*, $J_{6\text{-}7a}$ = 10.7 Hz, 7-Ha), 5.11 (*d*, $J_{6\text{-}7b}$ = 16.2 Hz, 7-Hb), 4.17 (*dd*, $J_{8\text{-}12a}$ = 6.9, $J_{12a\text{-}12b}$ = 9.3 Hz, 12-Ha), 3.86 (*dd*, $J_{8\text{-}12b}$ = 4.7, $J_{12a\text{-}12b}$ = 9.3 Hz, 12-Hb), 3.23 (*ddd*, $J_{8\text{-}9}$ = 4.7, $J_{8\text{-}12a}$ = 6.9, $J_{8\text{-}12b}$ = 4.7 Hz, 8-H), 2.90 (*dddd*, $J_{8\text{-}9}$ = 4.7, $J_{9\text{-}10a}$ = 9.5, $J_{9\text{-}10b}$ = 2.0, $J_{9\text{-}13}$ = 5.2 Hz, 9-H), 2.84 (*dd*, $J_{9\text{-}10a}$ = 9.5, $J_{10a\text{-}10b}$ = 17.7, 10-Ha; 2.53, *dd*, $J_{9\text{-}10b}$ = 2.0, $J_{10a\text{-}10b}$ = 17.7 Hz, 10-Hb), 2.32 (*m*, 5-H_2), 2.27 (*d*, $J_{3a\text{-}3b}$ = 16.7 Hz, 3-Ha), 2.17 (*d*, $J_{3a\text{-}3b}$ = 16.7 Hz, 3-Hb), 1.09 (*s*, 14-H_3), 1.09 (*s*, 15-H_3); ^{13}C NMR ($CDCl_3$, 125 MHz), δ: 174.2 C-11, 137.0 C-2, 136.3 C-1, 129.9 C-6, 115.3 C-5, 108.6 C-13, 71.8 C-12, 48.3 C-3, 47.4 C-5, 44.4 C-9, 44.1 C-8, 36.3 C-4, 34.9 C-10, 29.5 C-14, 29.5 C-15; EI-MS, *m/z* (rel. int.): 248.1423 ([M]$^+$ (84), $C_{15}H_{20}O_3$ requires 248.1412), 230 (41), 215 (45), 176 (77), 161 (89), 159 (81), 149 (80), 107 (85), 105 (93), 86 (100).

Biological tests. The assays for antimicrobial [3], phytotoxic [3], cytotoxic [4], and haemolytic [5], activities were carried out as described previously.

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