Omphalone, an Antibiotically Active Benzoquinone Derivative from Fermentations of *Lentinellus omphalodes* [1]

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Omphalone, a new antibiotic, cytotoxic, and phytotoxic pigment has been isolated from fermentations of a Canadian strain of *Lentinellus omphalodes*. Its structure has been established as 2-(4-methylfuran-2-yl)-1,4-benzoquinone (**1**) by spectroscopic investigations and conversion into leuco-acetate **2**.

Introduction

In the course of our investigations of secondary metabolites from basidiomycetes we found that strains of *Lentinellus*-species produced several antibiotic and cytotoxic compounds [2, 3, 4]. Of these, lentinellic acid, an antibiotically active protoilludane derivative has been described previously [5]. In the following we report the isolation, structural elucidation, and biological activities of a pigment from submerged cultures of *Lentinellus omphalodes* (Fr.) P. Karst. strain 80116 for which we propose the name omphalone.

Experimental

Lentinellus omphalodes

Mycelial cultures of *Lentinellus omphalodes* strain 80116 were obtained from the spore print of a specimen collected in Canada. For maintenance on agar slants the strain was kept on YMG medium composed of (g/liter): yeast extract 4, malt extract 10, glucose 4.

Fermentation

The medium used for fermentations contained (g/liter): yeast extract 5, peptone 2, glucose 50, K_2HPO_4 1, $MgSO_4 \times 7\ H_2O$ 1. For the production of omphalone *L. omphalodes* 80166 was grown in 20 liters of medium in a Biolafitte C-6 fermentation apparatus (1 l air/min, 200 rpm, 22 °C). Size of inoculum: 1%. During fermentation aliquots of the culture filtrate were extracted with ethyl acetate and the crude extracts separated by TLC [Alugram Sil G, Macherey-Nagel; toluene–Me_2CO–AcOH (70:30:1)]. Omphalone was quantified spectrophotometrically after elution of the red zone from the silica gel with methanol.

Isolation of omphalone and lentinellic acid from L. omphalodes 80166

After 16 days of fermentation, omphalone was extracted from the culture fluid (18 l) with 5 l of ethyl acetate. The crude product (5.1 g) was applied to a column with silica gel (Merck 60) and eluted with CH_2Cl_2. Pure omphalone was obtained from the first fractions after recrystallization from ethyl acetate.

Yield: 90 mg. Lentinellic acid was further purified by chromatography on Sephadex LH 20 (MeOH). Yield: 120 mg.

Physico-chemical properties of omphalone (1)

Dark-red crystals, m.p. 98–100 °C, R_f 0.81 [silica gel 60, cyclohexane–EtOAc–HCO_2H (120:40:5)]. – UV (MeOH) λ_{max} (log ε) = 213 (4.12, sh), 258 (4.08), 284 (3.87, sh), 320 (3.52, sh), 445 nm (3.48). – IR (KBr) cm^{-1} 3460–3440 (m, br), 3160 (m), 3130 (w), 3070 (w), 2940 (w), 1660 (sst), 1640 (sst), 1600 (sst), 1570 (sst), 1500 (m), 1460 (w), 1390 (m), 1350 (m), 1302 (sst), 1230 (w), 1165 (m), 1110 (m), 1050 (w), 1000 (w), 990 (m),

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980 (w), 925 (st), 905 (st), 875 (m), 840 (w), 815 (w), 670 (w), 605 (w). – ^{1}H and ^{13}C NMR see Table I; HR-MS (70 eV, DI 180 °C): *m/z* (relative intensity, %) 188.0479 (96, M^+, calcd for $C_{11}H_8O_3$ 188.0473), 160 (9, $C_{10}H_8O_2$), 132 (10, C_9H_8O), 106 (32, C_7H_6O), 78 (11, C_6H_6), 54 (9, C_3H_2O), 51 (11, C_4H_3), 44 (9).

Leuco-diacetate **2**

To a solution of **1** (10 mg) in pyridine (0.5 ml) zinc dust (15 mg) and acetic anhydride (0.5 ml) were added and the mixture was stirred for 1 h at room temperature. The mixture was poured in ice water, extracted with ether (3 × 5 ml) and evaporated *in vacuo*. The residue was purified by preparative TLC on silica gel 60 [C_6H_6–EtOAc (9:1), R_f 0.38]. Colorless oil. – ^{1}H NMR ($CDCl_3$) δ 2.21 (s br, 3H, 4′-CH_3), 2.43, 2.51 (each s, 6H, 2 $COCH_3$), 6.70 (s br, 1H, 3′-H), 7.12 (dd, J = 9 + 2.5 Hz, 1H, 5-H), 7.28 (dd, J = 9 + 0.5 Hz, 1H, 6-H), 7.40 (s, hidden, 1H, 5′-H), 7.66 (dd, J = 2.5 and 0.5 Hz, 1H, 3-H).

Biological assays

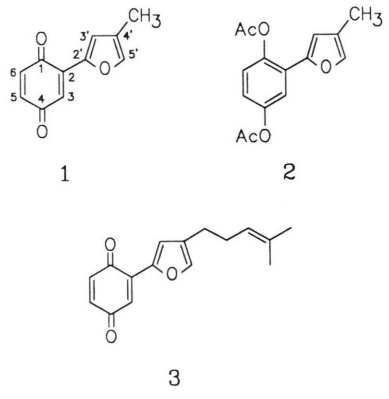
The antimicrobial spectra and the incorporation of radioactive precursors [1-^{14}C]leucine (52 Ci/mol), [2-^{14}C]uridine (52.4 Ci/mol), and [2-^{14}C]thymidine (52 Ci/mol) in Ehrlich carcinoma ascitic cells were evaluated as described previously [6, 7]. The phytotoxic activity of omphalone was assayed using seeds of *Setaria italica, Lepidium sativum* and *Lactuca sativa*. The compound and 150 µl of water were added to filter discs and incubated in a moist chamber for 2 days in the dark and then 2 days in daylight. Germination and growth of the seedlings was followed in 12 h intervals.

Results and Discussion

A fermentation of *Lentinellus omphalodes* 80116 is shown in Fig. 1. The production of omphalone starts when the growth has reached the stationary phase. The antibacterial activity of the culture broth is due to the formation of lentinellic acid, which starts much earlier and almost parallels growth.

Omphalone, $C_{11}H_8O_3$ ($M^+ = m/z$ 188), shows UV/Vis absorptions at λ_{max} (MeOH) 258 and 445 nm. The consecutive loss of two molecules of CO from the molecular ion and IR-bands (KBr) at 1660 and 1640 cm^{-1} are indicative for a quinone derivative. This is supported by the formation of a leuco-diacetate **2** on reductive acetylation with zinc dust-acetic anhydride.

From an inspection of the ^{1}H and ^{13}C NMR spectra (Table I) and selective ^{1}H-decouplings the structure of a 2-(4-methylfuran-2-yl)-1,4-benzoquinone (**1**) can be deduced for omphalone. The



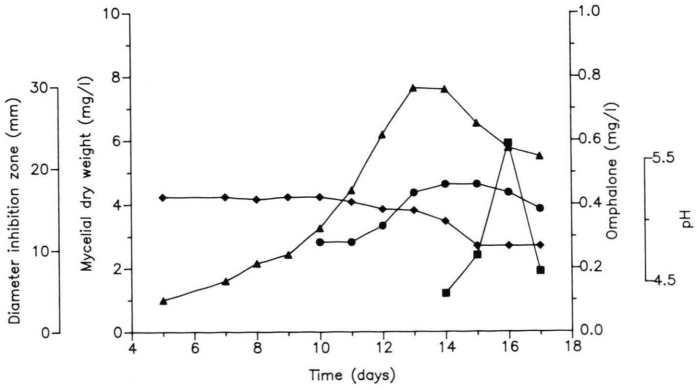


Fig. 1. Fermentation of *Lentinellus omphalodes*. ▲—▲ Dry weight of the mycelium; ●—● antibacterial activity (plate diffusion assay with *Bacillus brevis*); ■—■ omphalone content in the culture broth, ◆—◆ pH.

Table I. ^1H and ^{13}C NMR data of omphalone (**1**) and echinofuran B (**3**) ($CDCl_3$, 400 resp. 100.62 MHz, δ-values, TMS as int. standard).

H	**1** [^1H NMR] δ	C	**1** [^{13}C NMR] δ, *J* [Hz]	**3** [8]
		C-1	187.29 m	187.2
		C-2	133.58 s (br)	133.6
3-H	7.01 s (br)	C-3	125.08 D (br), 169 Hz	125.1
		C-4	185.46 m	185.4
5-H	6.74 "s"[1]	C-5	136.51 Dd, 170 + 5.2 Hz	136.4
6-H	6.75 "s"[1]	C-6	136.43 D (br)	136.5
		C-2′	146.02 dd, 7 + 7 Hz	146.0
3′-H	7.35 s (br)[2]	C-3′	121.11 Dpent, 178 + 5 Hz	120.2
		C-4′	123.91 m	129.0
5′-H	7.33 s (br)[2]	C-5′	142.50 Dpent, 201 + 6 Hz	142.1
4′-CH_3	2.10 dd[3]	CH_3-4′	9.62 Q, 128 Hz	

[1, 2] Signals may be interchanged.
[3] $J = 1 + 1$ Hz.

monosubstituted 1,4-benzoquinone ring follows from ^{13}C NMR signals at δ 185.5 and 187.3 as well as from three broadened singlets in the ^1H NMR spectrum at δ 7.01, 7.33, and 7.35. In the ^1H NMR spectrum of leuco-acetate **2** the corresponding signals indicate a 1,2,4-trisubstitution pattern for the benzene ring. The remaining ^1H NMR signals of **1** and **2** can be ascribed to a 2-substituted 4-methylfuran residue which leads to the structures given in the formulas. In the ^1H-coupled ^{13}C NMR spectrum of **1** the furan carbons C-3′ and C-5′ exhibit ^2J-couplings of 178 and 201 Hz, respectively, in accord with the literature [9].

The ^{13}C NMR data are in excellent agreement with those of echinofuron B (**3**) [8] which are included in Table I for comparison. The antibiotically active echinofurans have been isolated by Japanese authors [8, 10] from cell cultures of several *Boraginaceae*.

Omphalone exhibits both antibacterial and antifungal activities (Table II and III). The cytotoxic activities of **1** are quite high. Incorporation of the radioactive precursors leucine, uridine, and thymidine into trichloroacetic acid-precipitable material (protein, RNA, and DNA) was reduced 50% by concentrations of 2–3 μg/ml of omphalone (Fig. 2). The germination of *Lactuca sativa* seeds was completely inhibited at 100 μg/ml of omphalone. At this concentration the germination of *Setaria italica* seeds was partially delayed while *Lepidium sativum* was not affected. Omphalone readily reacts with cysteine yielding products which are completely devoid of antibacterial and cytotoxic activities.

Table II. Antimicrobial spectrum of omphalone (**1**) (serial dilution test).

Test organism	MIC* [μg/ml]
Acinetobacter calcoaceticus	50–100
Aerobacter aerogenes	20–50
Arthrobacter citreus	20–50
Bacillus brevis	10–20
Bacillus subtilis	20–50
Corynebacterium insidiosum	10–20
Eschericha coli K-12	>100
Leuconostoc mesenteroides	>100
Mycobacterium phlei	50–100
Proteus vulgaris	20–50
Pseudomonas fluorescens	>100
Sarcina lutea	20–50
Staphylococcus aureus	>100
Streptomyces spec. ATCC 23836	20–50
Candida albicans	50–100
Nadsonia fulvescens	10–20
Rhodotorula glutinis	20–50
Saccharomyces cerevisiae is 1	20–50
Saccharomyces cerevisiae αS 228 c	20–50

* Minimal inhibitory concentration.

Table III. Antifungal activity of omphalone (**1**) in the plate diffusion assay.

	Diameter inhibition zone [mm] µg/disk	
	50	100
Absidia glauca (+)	–*	13
Absidia glauca (–)	–	9
Alternaria porri	–	–
Ascochyta pisi	–	–
Aspergillus ochraceus	12	15
Curvularia lunata	–	11
Eurotium cristatum	–	–
Fusarium oxysporum	9	19
Mucor miehei	9	15
Nematospora coryli	–	10
Neurospora crassa	–	–
Paecilomyces varioti	–	10
Penicillium islandicum	–	10
Penicillium notatum	–	10
Penicillium steckii	–	11
Phytophthora infestans	–	10
Pleospora herbarum	–	–
Pythium debaryanum	–	12
Saprolegnia ferax	–	–
Ustilago nuda	–	–
Venturia cerasi	–	–
Zygorhynchus moelleri	–	–

* No inhibition.

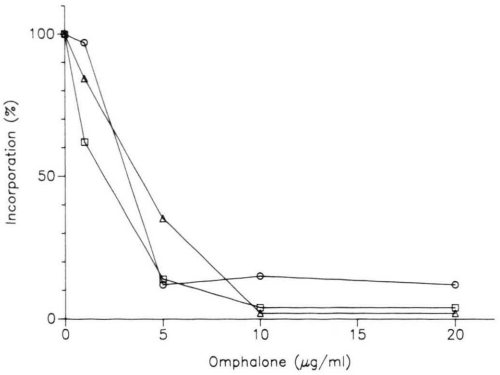


Fig. 2. Effect of omphalone on the syntheses of macromolecules in Ehrlich carcinoma ascites cells in percent of the controls without antibiotic. △—△ Protein synthesis; □—□ RNA synthesis; ○—○ DNA synthesis. Controls without antibiotic, incorporation into TCA-precipitable material per 10^6 cells: [^{14}C]leucine, 21446 cpm; [^{14}C]uridine, 9095 cpm; [^{14}C]thymidine, 1579 cpm.

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[1] Antibiotics from Basidiomycetes, XLII, XLI: G. Erkel, T. Anke, A. Gimenez, and W. Steglich, J. Antibiotics, submitted.
[2] A. Stärk, Dissertation, University of Kaiserslautern, 1985.
[3] U. Mocek, Dissertation, University of Bonn, 1985.
[4] E. Hillen-Maske, Dissertation, University of Bonn, 1987.
[5] A. Stärk, T. Anke, U. Mocek, W. Steglich, A. Kirfel, and G. Will, Z. Naturforsch. **43c,** 177 (1988).
[6] T. Anke, F. Oberwinkler, W. Steglich, and G. Schramm. J. Antibiotics **30,** 806 (1977).
[7] J. Kupka, T. Anke, F. Oberwinkler, G. Schramm, and W. Steglich, J. Antibiotics **32,** 130 (1979).
[8] H. Fukui, N. Yoshikawa, and M. Tabata, Phytochemistry **23,** 301 (1984).
[9] E. Breitmaier und W. Voelter, Carbon-13 NMR Spectroscopy, 3rd. edit., p. 146, VCH, Weinheim 1987.
[10] H. Inouye, H. Matsumura, M. Kawasaki, K. Inoue, M. Tsukada, and M. Tabata, Phytochemistry **20,** 1701 (1981).