ANTIBIOTICS FROM BASIDIOMYCETES

XXXVII†. NEW INHIBITORS OF CHOLESTEROL BIOSYNTHESIS FROM CULTURES OF *XERULA MELANOTRICHA* DÖRFELT

DOROTHÉE KUHNT, TIMM ANKE*

Lehrbereich Biotechnologie der Universität,
Paul-Ehrlich-Str. 23, D-6750 Kaiserslautern, FRG

HELMUT BESL

Institut für Botanik der Universität,
Postfach 397, D-8400 Regensburg, FRG

MONIKA BROSS, RUPERT HERRMANN, URSULA MOCEK,
BERT STEFFAN and WOLFGANG STEGLICH

Institut für Organische Chemie der Universität,
Gerhard-Domagk-Str. 1, D-5300 Bonn 1, FRG

(Received for publication May 7, 1990)

Three new inhibitors of cholesterol biosynthesis have been isolated from cultures of *Xerula melanotricha* and their structures elucidated by spectroscopic methods. Dihydroxerulin (**1**) in admixture with xerulin (**2**) strongly inhibits the incorporation of ^{14}C- acetate into cholesterol in HeLa cells while the incorporation of ^{14}C-mevalonate is not affected. Xerulinic acid (**3**) shows similar biological activities but a higher cytotoxicity.

In humans approximately 50% of the total cholesterol in the body is derived by de novo synthesis. Drugs interfering with this biosynthetic pathway are of potential value in treating hypercholesterolemia which is one of the primary causes of arteriosclerosis and coronary heart disease. Mevinolin (monacolin K)[2], a specific inhibitor of eucaryotic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase has been introduced into clinical practice. In a search for new inhibitors of cholesterol biosynthesis in human (HeLa) cells we detected that the yellow pigments produced by surface cultures of *Xerula melanotricha* Dörfelt[3,4] strongly interfered with the incorporation of ^{14}C-acetate into cholesterol[5]. From the same fungus the antifungal strobilurins A and B, and oudemansin B had been reported previously[6].

In the following we wish to describe the production, isolation, structural elucidation and biological characterization of the active compounds from *X. melanotricha*.

Materials and Methods

X. melanotricha Dörfelt, Strain 80241

Mycelial cultures were derived from tissue plugs of fruiting bodies collected in Deißlingen, Germany. The strain is deposited in the collection of the Lehrbereich Biotechnologie, University of Kaiserslautern.

Fermentation

For maintenance on agar slants *X. melanotricha* 80241 was grown in YMG-medium (yeast extract

† See ref 1.

0.4%, glucose 0.4%, malt extract 1%, pH 5.5). For the production of xerulins and xerulinic acid in surface culture *X. melanotricha* was grown in penicillin flasks containing 500 ml of YMGP-medium (yeast extract 1 g, maltose 20 g, glucose 10 g, peptone 2 g, KH_2PO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 1 g, $FeCl_3$ 10 mg, $ZnSO_4 \cdot 7H_2O$ 1.78 mg, $CaCl_2$ 73 mg, agar 30 g, water 1 liter). After 8~10 weeks at 22°C the mycelia had turned to an intense orange-brown color.

Isolation

The pigments were extracted from four agar cultures (2 liters of culture) with acetone (4 × 0.5 liters). The combined extracts were evaporated to dryness and the crude product (1.6 g) was applied to a column with silica gel (Merck 60, 7 × 12 cm). Elution with cyclohexane - EtOAc (8 : 2) afforded fractions containing dihydroxerulin (**1**) and xerulin (**2**) (296 mg) and subsequent elution with acetone yielded xerulinic acid (**3**) (227 mg). **1/2** and **3** were detected by agar plate diffusion assay using *Mucor miehei* as test organism. **1** was obtained by crystallization from acetone in admixture with **2**, yield 40 mg. As determined by NMR-spectroscopy **1** contained 10~35% **2**, depending on the cultivation and the work-up procedure. Separation of the two compounds could not be achieved by HPLC or other chromatographic methods. **3** was purified by chromatography on Sephadex LH-20 (2.5 × 52 cm, column) using MeOH as eluent, yield 14 mg.

Physical and Spectroscopic Data

The mp's were determined with a Reichert hot-plate microscope and are uncorrected. Spectral data were recorded on the following instruments: NMR, Bruker AM-400 and AM-500; IR, Pye-Unicam SP 1100; UV, Varian Cary-17; MS, A. I. E. MS-30 and with Silica gel 60 F_{254} Merck, Darmstadt, No. 5554.

Dihydroxerulin (**1**) and Xerulin (**2**) (2 : 1-mixture)

Yellow-orange Crystals: MP 143~153°C (dec); Rf 0.87 (CH_2Cl_2), 0.79 (toluene - acetone - AcOH, 70 : 30 : 1); UV λ_{max}^{MeOH} nm 400, 412; IR (KBr) cm^{-1} 2220 (w), 1772 (sst), 1745 (sst), 1630 (w), 1530 (m), 1335 (w), 1320 (w), 1103 (st), 1074 (w), 992 (st), 940 (m), 880 (st), 858 (w), 845 (m), 820 (w), 765 (m), 670 (m); ^1H NMR ($CDCl_3$, 500 MHz): **1**, δ 0.99 (t, J=7.5 Hz, 18-H), 1.57 (sextet, $J \approx 7$ Hz, 17-H), 2.32 (td, J=7 and 1.2 Hz, 16-H), 5.69 (br d, J=15.5 Hz, 11-H), 5.88 (br d, J=11.8 Hz, 5-H), 6.17 (dd, J=5.5 and $J \approx 0.5$ Hz, 2-H), 6.37~6.54 (m, 7-H, 8-H and 9-H), 6.74 (dd, J=15.5 and 10.5 Hz, 10-H), 6.80 (dd, J=14.5 and 11.8 Hz, 6-H), 7.35 (d, J=5.5 Hz, 3-H); **2**, δ 1.83 (dd, J=6.8 and 1.8 Hz, 18-H), 5.60 (dqd, J=15.8, 1.8 and 1 Hz, 16-H), 5.75 (br d, J=15.5 Hz, 11-H), 5.88 (br d, J=11.8 Hz, 5-H), 6.17 (dm, J=5.5 Hz, 2-H), 6.33 (dq, J=15.8 and 6.8 Hz, 17-H), 6.37~6.54 (m, 7-H, 8-H and 9-H), 6.76 (dd, J=15.5 and 10.5 Hz, 10-H), 6.81 (dd, J=14.5 and 11.8 Hz, 6-H), 7.35 (d, J=5.5 Hz, 3-H); EI-MS (direct inlet, 180°C) *m/z* (relative intensity %) 265 (20), 264.1144 (100, M^+ (**1**), calcd for $C_{18}H_{16}O_2$ 264.1150), 262.09987 (29, M^+ (**2**), calcd for $C_{18}H_{14}O_2$ 262.0994), 235 (18), 207 (23), 191 (29), 189 (27), 182 (22), 181 (23), 180 (30), 179 (60), 178 (75), 167 (31), 165 (83), 154 (66), 152 (69).

Xerulinic Acid (**3**)

Yellow-orange Crystals: MP > 250°C (dec), Rf 0.17 (CH_2Cl_2 - MeOH, 10 : 1), 0.35 (toluene - acetone - AcOH, 70 : 30 : 1); UV λ_{max}^{MeOH} nm (log ε) 416 (4.88), 432 (4.46); IR (KBr) cm^{-1} 3220~2500 (br), 2195 (w), 1790 (sst), 1715 (sst), 1610 (m), 1432 (w), 1305 (m), 1100 (m), 1065 (w), 985 (st), 935 (m), 875 (m), 835 (w), 810 (w), 760 (m), 665 (w), 540 (w); EI-MS (direct inlet, 180°C) *m/z* (relative intensity %) 292.0719 (22.3, M^+, calcd for $C_{18}H_{12}O_4$ 292.0735), 44 (100).

Biological Assays

The antimicrobial spectra and the cytotoxicity against cells of the ascitic form of Ehrlich carcinoma (ECA), L1210, BHK 21, and HeLa S3 cells were measured as described previously[7].

Macromolecular Syntheses in HeLa S3 Cells

HeLa S3 cells (ATCC CCL 2.2) were grown in suspension culture in F12 medium containing 10% of fetal calf serum. The cultures were incubated in a humidified atmosphere containing 5% of CO_2 at 37°C for 24 hours. The cells were washed twice with phosphate buffered saline (PBS) and resuspended in

PBS containing 1.1 mM $CaCl_2$, 0.6 mM $MgSO_4$, and 0.01% glucose to a cell density of 1×10^6 cells/ml. Then the cells were preincubated with the inhibitors at 37°C for 30 minutes. To test the effect on DNA-, RNA-, and protein synthesis, 1 ml aliquots of the cells suspension were incubated with 0.1 μCi [2-^{14}C]thymidine (55 mCi/mmol), 0.01 μCi [2-^{14}C]uridine (50 mCi/mmol), or 0.01 μCi [1-^{14}C]leucine for 30 minutes at 37°C. Then 1 ml of cold 10% TCA were added, the precipitate collected on cellulose nitrate filters and washed with 5 ml of 5% TCA. The radioactivity was measured in a liquid scintillation counter (LSC).

Incorporation of ^{14}C-Acetate and ^{14}C-Mevalonate into Cholesterol in HeLa S3 Cells

HeLa S3 cells were grown in F12 medium containing 10% of fetal calf serum in a humidified atmosphere containing 5% of CO_2 at 37°C until a cell density of 7.5×10^5 per plate (diameter 9 cm). The medium was removed and the cells were washed once with 2 ml of PBS, and were incubated in serum free medium[8]. After 24 hours the medium was replaced by serum free medium containing either 0.1 μCi of [2-^{14}C]acetate or 0.3 μCi [2-^{14}C]mevalonate. The inhibitors (dissolved in 20 μl of EtOH) were added and the cells were further incubated for 24 hours. After removing the medium the monolayers were washed once with PBS and suspended in 2 ml of 20% KOH in MeOH. Then 2 ml each of 0.5 N KOH were added and the suspensions incubated at 75°C for 1 hour. The protein content was determined in 100 μl samples using bicinchonic acid (BCA)-reagent (Pierce). After saponification, 1 nmol of cholesterol was added as an internal standard and the total cholesterol in the samples was extracted with petroleum ether. After the evaporation of the solvent the samples were applied to TLC plates (Macherey and Nagel, Alugram SIL G/UV_{254}) and developed in *n*-heptane - EtOAc (2 : 1). The radiolabeled cholesterol was localized with a TLC scanner and quantified by LSC.

HMG-CoA Synthase (EC 4.1.3.5.)

HeLa cells were grown in roller bottles in F12 medium with 10% fetal calf serum at 37°C in an atmosphere containing 5% CO_2 at 1 rpm to a cell density of 1.6×10^7 cells/ml. The cells were collected by centrifugation, washed once with PBS, suspended in 4 ml of 1 mM potassium phosphate buffer pH 7.3 containing 0.32 M sucrose, 1 mM $MgCl_2$, and sonicated three times for 10 seconds. The following steps were done according to the procedure of RAMACHANDRAN *et al.*[9]. The homogenate was centrifuged first for 10 minutes at $10,000 \times g$ and then for 1 hour at $100,000 \times g$. The resulting supernatant was dialyzed for 24 hours at 4°C against two changes of 2 liters of 20 mM potassium phosphate buffer pH 7.2, 0.1 mM EDTA, 0.5 mM dithiothreitol and was stored at −80°C. The protein content was determined according to BRADFORD[10].

The assay of HMG-CoA synthase was performed in a total volume of 200 μl: 0.1 M Tris-HCl, pH 8.0; 0.1 mM EDTA; 20 mM $MgCl_2$; 10 μM acetoacetyl-CoA; the $100,000 \times g$ supernatant containing 75 μg of protein. After 2 minutes of preincubation at 30°C 5 μM (0.05 μCi) ^{14}C-acetyl-CoA was added. After 4 minutes at 30°C the reaction mixture was cooled (4°C). A 50-μl aliquot was applied to a cellulose-polyethyleneimine plate (Macherey and Nagel, Polygram Cel PEI/UV) and developed in 0.4 M LiCl, 0.25 M $(NH_4)_2SO_4$ (pH 4.2). Radioactive HMG-CoA and acetyl-CoA were localized and quantified with a TLC scanner.

HMG-CoA Reductase (EC 1.1.1.34)

Five weeks old male Sprague-Dawley rats were fed with 10% of cholestyramine in their diet for 1 week. Six rats were sacrificed and their livers (36 g) removed. 3 g portions of liver were added each to 15 ml of buffer A (50 mM potassium phosphate pH 7.2, 100 mM sucrose, 50 mM KCl, 30 mM EDTA, 3 mM dithiothreitol) and homogenized in a glass-teflon homogenizor. The homogenate was centrifuged at 4°C for 10 minutes at $600 \times g$. The supernatant was centrifuged for 20 minutes at $13,000 \times g$, and the enzyme pelleted from the resulting supernatant (1 hour at $100,000 \times g$). Each $100,000 \times g$ pellet was suspended in 5 ml of buffer A and was centrifuged again for 1 hour at $100,000 \times g$. The resulting pellets containing the enzyme were resuspended in a total of 50 ml of buffer A. This enzyme preparation was stored in small aliquots in liquid nitrogen. HMG-CoA reductase assay: 60 μl of enzyme preparation (440 μg of protein) were preincubated for 30 minutes at 37°C in buffer B (0.28 M potassium phosphate buffer pH 6.8; 0.36 M KCl; 7 mM EDTA; 20 mM dithiothreitol (DTT); 0.1 mg/ml bovine serum albumine). Then the samples to be tested (dissolved in 20 μl of buffer B) were added and incubated for 15 minutes. The reaction

was started by adding 326 pmol (0.02 μCi) ^{14}C-HMG-CoA and 10 μl of a NADPH-regenerating system (0.2 M glucose-6-phosphate; 40 mM NADP; 20 U/ml glucose-6-phosphate dehydrogenase). After 30 minutes at 37°C the reaction was stopped by adding 20 μl of 5 N HCl. After standing at 37°C for 15 minutes for maximized lactonization of mevalonic acid the mixtures were applied to Extrelut columns (Merck 11738). Mevalonolactone was eluted from the column with twice times 6 ml of Et_2O - acetone (3 : 1). The solvent was evaporated and the radioactivity determined by LSC.

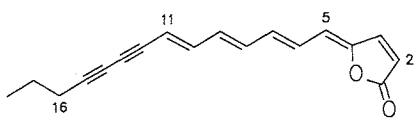
Results and Discussion

The inhibitors of cholesterol biosynthesis in HeLa cells were isolated as described in the Materials and Methods section. The best yields were obtained from surface cultures in YMGP medium. Although dihydroxerulin (**1**), xerulin (**2**) and xerulinic acid (**3**) were also produced in submerged cultures in the same medium, the yields were considerably lower. Since dihydroxerulin preparations containing different amounts of **2** (10~35%) exhibited identical biological activities and because the structures of the two compounds are very closely related it is assumed that **2** is equally active. In all the following biological tests a preparation of **1** containing 20% of **2** was used.

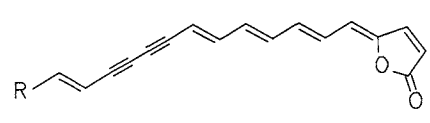
The dihydroxerulin - xerulin mixture exhibits intensive UV/Vis maxima at 412 and 400 nm and gives a dark-blue color reaction with conc H_2SO_4 which points to a polyene chromophore. In the IR spectrum (KBr) two bands at 1772 and 1745 cm^{-1} indicate an α,β-unsaturated γ-lactone moiety[11] and a weak absorption at 2220 cm^{-1} can be attributed to conjugated triple bonds. The EI-MS suggests the presence of two closely related compounds which give rise to molecular ions at m/z 264 ($C_{18}H_{16}O_2$) and 262 ($C_{18}H_{14}O_2$). This is confirmed by the ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) in which two separate sets of signals are deducible. Both compounds contain a 4-ylidenebutenolide system[12] which exhibits two doublets at δ 6.17 and 7.35 coupled with J=5.5 Hz. The small coupling constant ($J\approx$0.5 Hz) between the protons at C-2 and C-5 is consitent with the (*Z*)-configuration at the exocyclic double bond[13]. The 4-ylidenebutenolide unit is connected at C-5 with a polyunsaturated C_{13}-chain which in the case of dihydroxerulin (**1**) is terminated by a propyl group whereas xerulin (**2**) contains a (*E*)-1-propenyl group instead. According to the spectroscopic data the 4-ylidenebutenolide residue and the terminal groups must be connected by a C_8-unit which contains two double and two triple bonds. Since the protons at C-16 experience only small long range couplings ($^7J\approx$1 Hz) in both cases, the two triple bonds have to be placed next to them[14]. This leads to structures **1** and **2** for dihydroxerulin and xerulin, respectively. Since 6-H and 10-H exhibit coupling constants in the order of J=15 Hz, the corresponding double bonds must have the (*E*)-configuration. Due to severe signal overlap, the stereochemistry at the 8-double bond cannot be directly determined. It is assumed, however, to be *trans* because of the co-occurrence of **1** and **2** with xerulinic acid.

Xerulinic acid (**3**), $C_{18}H_{12}O_4$, is closely related to the less polar pigments **1** and **2**. Its structure was derived from a careful investigation of the ^{1}H and ^{13}C NMR spectra (Table 1).

The complete connectivities were obtained by ^{1}H, ^{1}H-COSY, ^{13}C, ^{1}H-COSY and correlation *via* long



1



2 R=CH_3
3 R=COOH

Table 1. ^{1}H and ^{13}C NMR data of xerulinic acid (**3**)[a].

Proton	δ (ppm) m	J (Hz)	C-Atom	δ (ppm) m	J (Hz)
			C-1	169.10 dd	14/8
2-H	6.43 d	5.4	C-2	118.77 dd	185/3
3-H	7.84 d	5.4	C-3	144.32 ddd	181/4/4
			C-4	149.63 m	
5-H	6.25 d	11.7	C-5	114.45 dd	162/7
6-H	6.85 dd	14.1/11.7	C-6	128.38 d (br)	159
7-H	6.73 dd	14.1/11.1	C-7	138.08 ddd	158/7/6
8-H	6.74 dd	14.9/11.1	C-8	137.47 ddd	158/6/4
9-H	6.63 dd	14.9/10.7	C-9	134.77 ddd	156/7/2
10-H	6.99 dd	15.6/10.7	C-10	146.41 dm	157
11-H	6.08 d	15.6	C-11	110.36 dd	168/5
			C-12	85.68 d	5
			C-13	77.45 t	2
			C-14	80.93 t	2
			C-15	81.11 d	5
16-H	6.87 d	16.1	C-16	121.97 d	169
17-H	6.48 d	16.1	C-17	135.73 ddd	166/4/2
			C-18	166.14 dd (br)	6/3

[a] 400 respective 100.6 MHz, DMSO-d_6 as solvent and internal standard (δ 2.49 ppm).
m: Multiplicity.

Fig. 1. Selected ^{1}H-^{13}C long range connectivities of **3** as determined by selective decouplings and COLOC experiments.

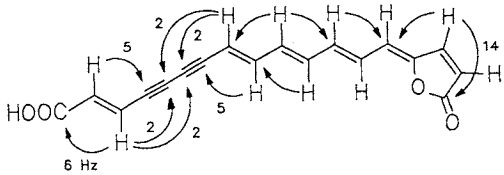


Table 2. Incorporation of [2-^{14}C]acetate and [2-^{14}C]-mevalonate into cholesterol in HeLa S3 cells.

	Dihydro-xerulin (μg/ml)	Protein (μg/assay)	Incorporation into cholesterol (cpm/assay)
^{14}C-Acetate	0	10.5	2,055
	0.1	10.1	1,349
	1.0	11.3	991
	10.0	10.5	705
^{14}C-Mevalonate	0	12.9	501
	0.1	11.3	490
	1.0	11.1	455
	10.0	13.4	396

range coupling (COLOC)[15] experiments as well as selective proton decouplings in the ^{1}H-coupled ^{13}C NMR spectrum and are depicted in Fig. 1. Signal separation in strongly overlapping regions was achieved by the addition of C_6D_6. These experiments led to a correction of the tentative formula given in literature[16].

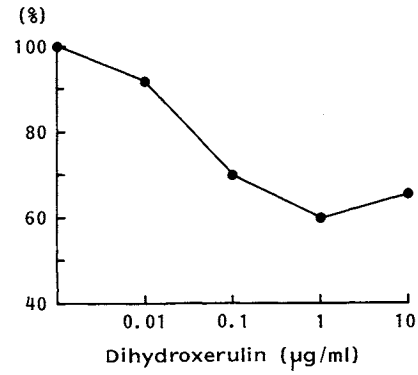
In serum free medium **1** strongly inhibited the incorporation of ^{14}C-acetate into cholesterol in HeLa S3 cells (Table 2). The incorporation of ^{14}C-mevalonic acid was hardly affected. No cytotoxic (lytic) effects were observed. The protein content of cells was identical in both the control and the **1** containing assays. In serum containing media growth of HeLa S3, L1210, BHK 21, and ECA cells was not inhibited by concentrations of up to 100 μg/ml. However, in serum-free, cholesterol-free media, HeLa S3 cells stopped growth and changed their morphology to spherical cells when dihydroxerulin was added starting from concentrations of 50 μg/ml. Since the biosynthesis of cholesterol starting from ^{14}C-acetate was strongly inhibited while the incorporation of ^{14}C-mevalonic acid was hardly affected, the inhibitory action of **1** on the two key enzymes of mevalonic acid synthesis was investigated. HMG-CoA reductase was prepared from cholestyramine-treated rats and assayed as described in the Materials and Methods section. The enzyme was inhibited 65% by 0.1 μg/ml of compactin but not at all by concentrations of up to 300 μg/ml

of **1**. For the preparation and assay of human HMG-CoA synthase from HeLa cells a procedure modified from that of RAMACHANDRAN *et al.*[9] was used. The synthesis of 3-hydroxy-3-methylglutaryl-coenzyme A from ^{14}C-acetyl-CoA and acetoacetyl-CoA by HMG-CoA synthase was strongly inhibited by concentrations of 1 μg/ml of **1** (Fig. 2). This inhibitory effect could not be increased by higher concentrations of **1**. This could be due to multiple forms of HMG-CoA synthases, as has been described for birds by CLINKENBEARD *et al.*[17]. The selective effect of **1** on HMG-CoA-synthase explains the inhibition of cholesterol biosynthesis in HeLa cells. The effects of **3** on the incorporation of acetate and mevalonate into cholesterol in HeLa cells and its effects on HMG-CoA synthase and HMG-CoA reductase were comparable with those obtained with **1**. However, the compound showed a higher cytotoxicity starting from 20 μg/ml (HeLa cells in serum-containg medium). This might be attributed to the carboxy group in **3**. While only small effects on macromolecular syntheses (incorporation of ^{14}C-thymidine, ^{14}C-uridine, and ^{14}C-leucine into acid-precipitable material) were observed for **1**, **3** strongly inhibited RNA-synthesis at concentrations starting from 10 μg/ml (Fig. 3).

No antibacterial activities were detected for **1** or **3** (MIC > 100 μg/ml for *Acinetobacter calcoaceticus*, *Bacillus brevis*, *Bacillus subtilis*, and *Micrococcus luteus*). The growth of fungi was inhibited by concentrations starting from 10 μg/disc of **1** (Table 3). The antifungal activities of **3** were very weak.

Similar biological activities are known from fungal β-lactones with long alkyl chains. 12-Hydroxy-13-hydroxymethyl-3,5,7-trimethyltetradeca-2,4-dienedioic acid-12,14-lactone from a *Cephalosporium* species[18] and analogous compounds

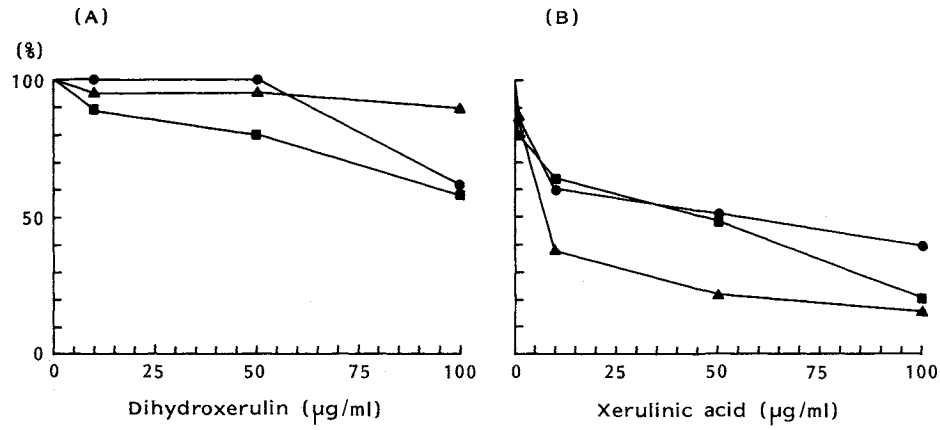
Fig. 2. Effect of dihydroxerulin on HMG-CoA synthase.



Incorporation of ^{14}C-acetyl-CoA into HMG-CoA without antibiotic (100%): 3,365 cpm.

Fig. 3. Effect of dihydroxerulin and xerulinic acid on the incorporation of ^{14}C-thymidine, ^{14}C-uridine, and ^{14}C-leucine in HeLa S3 cells.

(A) Dihydroxerulin, (B) xerulinic acid, ● [^{14}C]thymidine, ▲ [^{14}C]uridine, ■ [^{14}C]leucine.



Controls without antibiotics (100%): [^{14}C]Thymidine 3,400 cpm, [^{14}C]uridine 12,000 cpm, [^{14}C]leucine 17,000 cpm.

Table 3. Antifungal activities of dihydroxerulin (**1**) and xerulinic acid (**3**) in the agar diffusion assay.

Fungi	Diameter of inhibition zone (mm)					
	Dihydroxerulin			Xerulinic acid		
	10[a]	50[a]	100[a]	10[a]	50[a]	100[a]
Ascochyta pisi	10i	15	20	—	—	—
Aspergillus ochraceus	8	10	16	—	—	—
Fusarium oxysporum	—	7i	10i	—	—	—
Mucor miehei	10	15	15	—	10	12
Nematospora coryli	—	—	—	—	—	—
Neurospora crassa	—	15i	20i	—	—	—
Paecilomyces varioti	—	—	10i	—	—	—
Penicillium notatum	10i	15i	15i	—	—	—
Phytophthora infestans	—	23	30	—	—	—
Rhodotorula glutinis	—	20i	27i	—	—	—
Ustilago nuda	—	—	—	—	—	—
Venturia cerasi	—	20i	20i	—	—	—
Zygorhynchus moelleri	8i	15	15	—	—	—

[a] μg/disc (diameter 6 mm).
—: No inhibition zone.
i: Incomplete inhibition.

from *Scopulariopsis* sp. have been reported to inhibit the growth of fungi and to irreversibly inactivate HMG-CoA synthase from rat liver at very low concentrations[19].

Acknowledgments

We thank the Deutsche Forschungsgemeinschaft, the Bundesministerium für Forschung und Technologie, and Boehringer-Mannheim for financial support. Sprague-Dawley rats fed with cholestyramine were kindly provided by Dr. PILL of Boehringer-Mannheim. We thank Prof. H. DÖRFELT, Halle/Saale, for confirming our identification of *X. melanotricha*.

References

1) HARTTIG, U.; T. ANKE, A. SCHERER & W. STEGLICH: Antibiotics from basidiomycetes. XXXVI. Leaianafulvene, a sesquiterpenoid fulvene derivative from cultures of *Mycena leaiana* (Agaricales). Phytochemistry, in press
2) ENDO, A.: Monacolin K, a new hypocholesterolemic agent produced by a *Monascus* species. J. Antibiotics 32: 852~854, 1979
3) DÖRFELT, H.: Taxonomische Studien in der Gattung *Xerula* R. Mre. (IV). Feddes Repert 92: 255~291, 1981
4) HERRMANN, R.: Untersuchungen zur Konstitution, Synthese und Biosynthese von Pilzfarbstoffen. Ph. D. Thesis, Univ. Bonn, 1980
5) KUHNT, D.: Neue Inhibitoren der Cholesterinbiosynthese aus Basidiomyceten. Ph. D. Thesis, Univ. Kaiserslautern, 1989
6) ANKE, T.; H. BESL, U. MOCEK & W. STEGLICH: Antibiotics from basidiomycetes. XVIII. Strobilurin C and oudemansin B, two new antifungal metabolites from *Xerula* species (Agaricales). J. Antibiotics 36: 661~666, 1983
7) LEONHARDT, K.; T. ANKE, E. HILLEN-MASKE & W. STEGLICH: 6-Methylpurine, 6-methyl-9-β-D-ribofuranosylpurine and 6-hydroxymethyl-9-β-ribofuranosylpurine as antiviral metabolites of *Collybia maculata* (Basidiomycetes). Z. Naturforsch. 42C: 420~424, 1987
8) HUTCHINGS, S. E. & G. H. SATO: Growth and maintenance of HeLa cells in serum-free medium supplemented with hormones. Proc. Natl. Acad. Sci. U.S.A. 75: 901~904, 1978
9) RAMACHANDRAN, C. K.; S. L. GRAY & G. MELNYKOVYCH: Cytoplasmatic 3-hydroxy-3-methylglutaryl coenzyme A synthase and the regulation of sterol synthesis in tissue culture cells. Biochim. Biophys. Acta 618: 439~448, 1980
10) BRADFORD, M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248~254, 1976
11) JONES, R. N.; C. L. ANGELL, T. ITO & R. J. D. SMITH: The carbonyl stretching bands in the infrared spectra of

unsaturated lactones. Can. J. Chem. 37: 2007~2022, 1959

12) PATTENDEN, G.: Natural 4-ylidenebutenolides and 4-ylidenetetronic acids. Progr. Chem. Nat. Prod. 35: 133~198, 1978

13) BOHLMANN, F. & R. REINECKE: Polyacetylenverbindungen, CXV. Synthese des Thioenoletherbutenolids aus *Anthemis rigescens* Willd. Chem. Ber. 99: 3437~3440, 1966

14) BARFIELD, M. & B. CHAKRABARTI: Long range proton spin-spin coupling. Chem. Rev. 69: 757~778, 1969

15) KESSLER, H.; C. GRIESINGER, J. ZARBOCK & H. R. LOOSLI: Assignment of carbonyl carbons and sequence analysis of peptides by heteronuclear shift correlation *via* small coupling constants with broadband decoupling in t_1 (COLOC). J. Magn. Reson. 57: 331~336, 1984

16) GILL, M. & W. STEGLICH: Pigments of fungi (macromycetes). Progr. Chem. Nat. Prod. 51: 1~317, 1987

17) CLINKENBEARD, K. D.; T. SUGIYAMA, W. D. REED & M. D. LANE: Cytoplasmic 3-hydroxy-3-methylglutaryl coenzyme A synthase from liver. J. Biol. Chem. 250: 3124~3135, 1975

18) TOMODA, H.; H. KUMAGAI, H. TANAKA & S. ŌMURA: F-244 specifically inhibits 3-hydroxy-3-methylglutaryl coenzyme A synthase. Biochim. Biophys. Acta 922: 351~356, 1987

19) ALDRIDGE, D. C.; D. GILES & W. B. TURNER: Antibiotic 1233 A, a fungal β-lactone. J. Chem. Soc. Chem. Commun. 1971: 3888~3891, 1971