CALOPOROSIDE, A NEW INHIBITOR OF PHOSPHOLIPASES C FROM *Caloporus dichrous* (Fr.) Ryv.†

WOLFGANG WEBER, PETER SCHU, TIMM ANKE,
ROBERT VELTEN†† and WOLFGANG STEGLICH††

Department of Biotechnology, University of Kaiserslautern,
Paul-Ehrlich-Str. 23, D-67663 Kaiserslautern, Germany
†† Department of Organic Chemistry, University of Munich,
Karlstr. 23, D-80333 München, Germany

(Received for publication June 29, 1994)

A new salicylic acid derivative, caloporoside, was isolated from fermentations of *Caloporus dichrous*. Its structure was elucidated by a combination of chemical and spectroscopic methods. Caloporoside exhibits weak antibacterial and antifungal activities and is a quite selective inhibitor of phospholipase C isolated from pig brain (*Ki* 12, 3 μM).

Caloporus dichrous (*Polyporaceae*, *Aphyllophorales*) is a medium sized saprophytic polypore growing on wood or decaying fruiting bodies of other basidiomycetes, *e.g. Inonotus obliquus*[2]. It can be found in the northern parts of America, Europe or Asia. From mycelial cultures of strains derived from collections in Germany, the USA, and South Korea, a compound exhibiting weak antimicrobial activities was isolated. During a later screening, this compound was found to inhibit strongly phospholipase C isolated from pig brain. In the following we wish to describe the fermentation, isolation, structure elucidation and the biological activities of caloporoside, the first inhibitor of a phospholipase from a basidiomycete.

Materials and Methods

General

Spectral data were recorded on the following instruments: ^{1}H and ^{13}C NMR, Bruker AM-400 and AMX-600; EI-MS, A.E.I. MS-50; FAB-MS, Kratos Concept H-System; IR, Perkin-Elmer 1420; UV, Varian Cary 17. Optical rotations were recorded with a Perkin-Elmer 241 polarimeter. Aluminium foil coated with silica gel Merck 60 F_{254} was used for TLC. PTLC was carried out on glass plates precoated with silica gel (Merck 60 F_{254}, 2 mm). Pharmacia Sephadex LH-20 was used for column chromatography. All solvents were distilled prior to use.

Caloporus dichrous Strains 8365, 8366

Mycelial cultures were obtained from spore prints of fruiting bodies growing on wood in the Great Smoky Mountains, U.S.A. The specimen show the characteristics of the genus and species as described by RYVARDEN[2]. Voucher specimen and cultures are deposited in the collection of the Lehrbereich Biotechnologie, University of Kaiserslautern.

Fermentation

For maintenance, the fungi were cultivated in YMG medium composed of: Yeast extract 0.4%, malt extract 1%, glucose 0.4%, and agar 1.5%. For the production of caloporoside, a well grown seed culture of *Caloporus dichrous* 8365 (150 ml) in YMG was used to inoculate 20 liters of BAF medium (g/liter:

† Antibiotics from basidiomycetes XLV, see ref 1.

maltose 20, glucose 10, peptone 2, yeast extract 0.2, KH_2PO_4 0.5, $MgSO_4 \cdot 7\ H_2O$ 1, $FeCl_3$ 0.01, $ZnSO_4$ 0.001, $CaCl_2$ 0.055, and μg/liter: thiaminium dichloride 50, folic acid 100) in a Biolafitte C6 fermentation apparatus. The fermenter was incubated at 23°C with an aeration of 1.5 liters/minute and agitation (200 rpm). The production of caloporoside was followed by estimating the inhibitory effect of 10 μl of a crude EtOAc extract (concentrated 50 times as compared to the culture fluid) in the plate diffusion assay using *Bacillus subtilis* as test organism.

Isolation of Caloporoside (1)

During purification, caloporoside was detected by its antibacterial activity towards *B. subtilis*. After removal of the mycelia by filtration, caloporoside was extracted from the culture filtrate (19 liters) with EtOAc (two times 5 liters). Evaporation of the organic phase yielded a crude extract (8 g) that was further purified by chromatography on silica gel (Merck 60; elution with CH_2Cl_2 - MeOH, 10 : 1), resulting in 2.71 g of an enriched product. This was further purified by preparative HPLC (LiChrosorb RP8, column 2.5 × 25 cm, elution with MeOH - H_2O, 8 : 2) to yield 0.82 g of caloporoside (**1**).

Caloporoside (1)

Colourless oil; Rf 0.37 (*n*-butanol - H_2O - EtOH, 4 : 2 : 1); $[\alpha]_D^{20}$ −32° (*c* 1.15, MeOH); UV λ_{max}^{MeOH} nm (log ε) 208 (4.44), 238 (sh, 3.89), 300 (3.69); IR (KBr) cm^{-1} 3440, 2925, 2860, 1735, 1660, 1605, 1450, 1378, 1240, 1170, 1072, 1033; ^1H NMR, Table 1; ^{13}C NMR, Table 1; (+)-FAB-MS (thioglycerol) *m/z* 861 $(M-H+2Na)^+$, 839 $(M+Na)^+$, 465, 377, 357, 221, 205; (−)-FAB-MS (thioglycerol) *m/z* 815 $(M-H)^-$, 391, 373, 347, 329.

Alkaline Hydrolysis of Caloporoside (1)

Caloporoside (**1**) (25 mg) was treated at 20°C for 1 hour with 1 N NaOH. After acidification with 1 N HCl to pH 3 the mixture was extracted with EtOAc (3 × 10 ml). The combined organic phase was washed successively with saturated aqueous $NaHCO_3$ (2 × 20 ml) and brine (20 ml), and the dried organic phase (Na_2SO_4) was evaporated to dryness. Purification of the residue by PTLC on silica gel using toluene - acetone - AcOH (70 : 30 : 1) afforded **3** (8.5 mg) as colourless oil; Rf 0.41 (toluene - acetone - AcOH, 70 : 30 : 1); $[\alpha]_D^{20}$ 0° (*c* 0.53, $CHCl_3$); UV λ_{max}^{MeCN} nm (log ε) 210 (4.43), 240 (sh, 3.73), 308 (3.51); IR (KBr) cm^{-1} 3470, 3200 ~ 2600, 2920, 2855, 1730, 1675, 1599, 1573, 1464, 1448, 1395, 1330, 1312, 1249, 1219, 1169, 1120, 1105, 828, 817, 775; ^1H NMR (400 MHz, $CDCl_3$) δ 1.21 (3H, d, *J* = 6.1 Hz, 24-H), 1.25 ~ 1.50 (26H, m, 9 ~ 21-H), 1.55 ~ 1.65 (4H, m, 8-H, 22-H), 2.94 (2H, t, *J* = 7.8 Hz, 7-H), 3.84 (1H, sext, *J* = 6.1 Hz, 23-H), 4.02 (1H, s, br, 23-OH), 6.73 (1H, dd, *J* = 7.5 and 1.1 Hz, 4-H), 6.83 (6H, dd, *J* = 8.1 and 1.1 Hz, 6-H), 7.31 (1H, dd, *J* = 8.1 and 7.5 Hz, 5-H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 23.24 (C-24), 25.65 (C-21), 29.40 ~ 29.46, 29.85 (C-10 ~ 20), 32.10 (C-9), 36.49 (C-8), 39.06 (C-22), 68.68 (C-23), 110.85 (C-2), 115.67 (C-4), 122.55 (C-6), 134.92 (C-5), 147.51 (C-7), 163.45 (C-3), 175.01 (C-1); EI-MS (180°C) *m/z* (%) 392.2923 (8, M^+, calcd for $C_{24}H_{40}O_4$ 392.2926), 374 (19, $C_{24}H_{38}O_3$), 359 (9, $C_{23}H_{35}O_3$), 356 (34, $C_{24}H_{36}O_2$), 348 (11, $C_{23}H_{40}O_2$), 338 (19 ($C_{24}H_{34}O$), 330 (28, $C_{23}H_{38}O$), 161 (21), 152 (35), 147 (21), 120 (28), 108 (100), 107 (50).

Acetylation of Caloporoside (1)

To a solution of caloporoside (**1**) (20 mg) in THF (25 ml) was added dropwise a solution of triethylamine (0.35 ml), acetic anhydride (0.24 ml) and 4-(dimethylamino)pyridine (15 mg) in THF (10 ml). After stirring for 6 hours at 20°C the mixture was diluted with chloroform (100 ml) and saturated aqueous NH_4Cl (100 ml). The aqueous layer was extracted with chloroform (5 × 30 ml). The combined organic phase was dried over Na_2SO_4 and evaporated *in vacuo* to give an oil that was chromatographed on Sephadex LH-20. Elution with MeOH - CH_2Cl_2 (5 : 1) afforded peracetate **2** (26.3 mg) as colourless oil; Rf 0.42 (CH_2Cl_2 - MeOH, 10 : 1); $[\alpha]_D^{20}$ −12° (*c* 0.64, $CHCl_3$), [O. STERNER, University of Lund, personal communication: $[\alpha]_D$ −16° (*c* 1.5, $CHCl_3$)]; UV λ_{max}^{MeCN} nm (log ε) 300 (sh, 3.48); IR (KBr) cm^{-1} 2920, 2845, 1740, 1450, 1365, 1215, 1069, 1040; ^1H NMR (CD_3OD), Table 1; ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 19.30, 20.44, 20.50, 20.58 (2C), 20.63, 20.68, 20.70, 20.74, 20.90 (C-24, 9 CH_3CO_2), 25.13 (C-21), 29.37, 29.44, 29.52, 29.60 (7C), 29.67 (C-10 ~ 20), 31.39 (C-9), 33.74, 35.65 (C-8, C-22), 60.68, 62.66 (C-6′, C-6″), 66.09, 68.18, 68.51, 68.71, 69.42, 70.93, 71.83, 72.62, 73.30 (C-23, C-2′ ~ 5′, C-2″ ~ 5″), 95.43 (C-1″), 120.23

(C-4), 126.25 (C-2), 127.28 (C-6), 130.49 (C-5), 142.92 (C-7), 148.07 (C-3), 166.85, 168.96, 169.24, 169.57, 169.67, 169.70, 170.09, 170.67, 170.75 (2C) (C-1', 9 CH_3CO_2), C-1 signal not clearly observed; (+)-FAB-MS (mNBA + NaOAc) *m/z* 1155 $(M-H+2Na)^+$, 1133 $(M+Na)^+$, 1113, 1091, 1073, 717, 511, 331, 329, 311; (−)-FAB-MS (thioglycerol) *m/z* 1109 $(M-H)^-$, 1067, 1025, 391, 389, 373, 329.

Biological Assays

The assays for antimicrobial and cytotoxic activities were performed as described previously[3]. Cells of the ascitic form of Ehrlich carcinoma (ECA) were grown in suspension culture in F-12 medium containing 15% of horse serum at 37°C for two days. The cells were washed twice with sterile phosphate buffered saline (PBS) and resuspended in PBS to a cells density of $0.5 \sim 1 \times 10^6$ cells/ml. The incorporation of radiolabelled thymidine, uridine, and leucine into DNA, RNA, and proteins were determined as described previously[4].

Phosphoinositol-specific Phospholipase C

Fresh pig brain (200 ~ 300 g) was chopped and homogenized in a Potter homogenizer in 200 ~ 300 ml 0.1 M Tris-HCl buffer, pH 8.0. The homogenate was centrifugated at $2,700 \times g$ for 30 minutes and the supernatant decanted and centrifugated at $100,000 \times g$ for 90 minutes at 4°C. From the resulting supernatant phospholipase C was precipitated with 40% of ammonium sulfate. The precipitate was dissolved in 20 mM Tris-HCl, pH 8.5, and dialysed overnight against 2×2 liters of the same buffer. The dialysate was then applied to a DEAE ion-exchange column (Whatman DE-52; 20 mM Tris-HCl, pH 8.5) and eluted with a linear gradient of KCl (0 ~ 300 mM). The fractions containing the enzyme were concentrated by ultrafiltration (Amicon, PM 10) and the solution (18 mg of protein in 25 ml 20 mM Tris-HCl, 100 mM KCl) stored frozen at −20°C.

Assay of phospholipase C

Phosphatidyl-[*U*-^{14}C]inositol (Amersham-Buchler) was added to unlabeled compound to yield a specific activity of 30 dpm/nmol, and 100 nmol portions of this substrate in toluene - EtOH were transferred to assay tubes, dried and resuspended in 450 μl 50 mM Tris-maleate, 1 mM $CaCl_2$, pH 5.5. Then 10 ~ 15 μg of phospholipase C was added and the reaction mixture incubated for 10 minutes at 37°C. The reaction was stopped by the addition of 1 ml of ethyl acetate - acetone - acetic acid (66 : 33 : 1). The tubes were shaken vigorously and the two phases separated by centrifugation. The radioactive inositolphosphate released by the enzyme was measured in the aqueous phase in liquid scintillation counter. In addition, the products of the enzymic reaction were identified by TLC[5].

Assays of other Phospholipases

The following enzymes were purchased from Sigma, St. Louis, USA: Phospholipase A_2 (porcine pancreas) was assayed titrimetrically[6] using phosphatidylcholine as substrate and deoxycholate as detergent. Phospholipases A_2 (from *Naja Naja* and *Apis mellifica*) were assayed according to DEEMS and DENNIS[7] using phosphatidylcholine as substrate and triton X-100 as detergent at pH 8.9. Phospholipase A_2 from *Vipera russelli* was assayed at pH 6.5. For the assay of phospholipases C from *Bacillus cereus* and *Clostridium perfringens p*-nitrophenylphosphorylcholine was used as substrate[8]. Phospholipase D was assayed according to KATES and SASTRY[9] with subsequent determination of the liberated choline by the method of APPLETON[10]. Lipases (porcine pankreas, *Chromobacterium viscosum* and *Rhizopus arrhizus*) were assayed turbidimetrically[11] with triolein as substrate. Triacetin was used as substrate for wheat germ lipase in a titrimetric assay[6]. Acetylcholine esterase was assayed according to WHITTAKER[12] using acetylthiocholine as substrate.

Results and Discussion

Structure Elucidation

Caloporoside (**1**) was obtained as a colourless oil, $[\alpha]_D^{20}$ −32° (*c* 1.15, MeOH). Its molecular weight was determined as 816 by observation of a pseudomolecular ion $[M-H]^-$ at *m/z* 815 in the negative ion

FAB-MS, and cluster ion peaks $[M+Na]^+$ and $[M-H+2Na]^+$ at *m/z* 839 and 861, respectively, in the positive ion FAB-MS. The NMR spectra (Table 1) indicated that caloporoside (**1**) contains a 1,2,3-trisubstituted benzene ring, an alkyl chain with a terminal $-CH(OR)CH_3$ group, a complex sugar moiety, and two acetyl residues. On hydrolysis with 1 N aqueous NaOH, **1** yielded aglycone **3**, $C_{24}H_{40}O_4$, which according to its ^1H and ^{13}C NMR data proved to be identical with the dihydro deriva-

Fig. 1. Structures of caloporoside (**1**) and its peracetate (**2**).

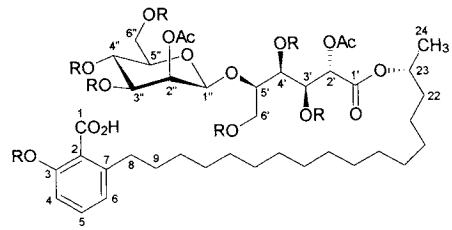


Table 1. NMR data for caloporoside (**1**) and its peracetate (**2**).

Carbon	Caloporoside (**1**, in CD_3OD)		Caloporoside peracetate (**2**, in CD_3OD)[a]
	δ_C (100.6 MHz)	δ_H (*J* in Hz, 400 MHz)	δ_H (*J* in Hz, 600 MHz)
Aglycone moiety			
1	[b] (s)	—	—
2	118.0 (s)	—	—
3	162.17 (s)	—	—
4	115.27 (d)	6.68 (dd, 8.0, 1.0)	7.17[c] (dd, 7.7, 0.9)
5	132.72 (d)	7.15 (dd, 8.0, 7.8)	7.34 (dd, 8.1, 7.7)
6	122.52 (d)	6.66 (dd, 7.8, 1.0)	6.99[c] (dd, 8.1, 0.9)
7	146.98 (s)	—	—
8	36.51 (t)	3.06 (t, br, 7.7)	2.76 (t, br, 7.9)
9	33.29 (t)	1.50~1.75 (m)	~1.6 (m)
10~20	30.5~31.0 (t)	1.30~1.45 (m)	1.3~1.4 (m)
21	26.33 (t)	1.30~1.45 (m)	1.3~1.4 (m)
22	36.94 (t)	1.50~1.75 (m)	~1.6 (m)
23	73.48[d] (d)	5.01 (sext, 6.5)	4.97 (sext, 6.3)
24	20.21 (t)	1.27 (d, 6.5)	1.26 (d, 6.3)
2-*O*-Acetyl-mannonic acid moiety			
1′	171.86 (s)	—	—
2′	73.91 (d)	4.83 (d, 9.8)	5.02 (d, 7.0)
3′	70.02 (d)	4.32 (dd, 9.8, 1.0)	5.54 (dd, 7.0, 3.2)
4′	69.47 (d)	3.60 (dd, 9.5, 1.0)	5.48 (dd, 8.7, 3.2)
5′	77.91 (d)	3.91~4.00 (m)	4.22 (ddd, 8.7, 3.6, 2.7)
6′	62.99[e] (t)	3.62~3.71 (m),	4.06 (dd, 12.8, 3.6),
		3.91~4.00 (m)	4.51 (dd, 12.8, 2.7)
2′-OAc	20.39 (q)	2.12 (s)	[a]
	171.57 (s)	—	—
2-*O*-Acetyl-mannopyranosyl moiety			
1″	99.12 (d)	4.95 (s, br)	5.11 (d, 1.0)
2″	73.50[d] (d)	5.42 (d, br, 3.3)	5.42 (dd, 2.9, 1.0)
3″	73.40 (d)	3.69 (dd, 9.5, 3.3)	5.19 (m)
4″	69.30 (d)	3.50 (dd, 9.5, 9.5)	5.19 (m)
5″	78.07 (d)	3.35 (m)	3.86 (ddd, 9.7, 5.7, 2.4)
6″	63.13[e] (t)	3.62~3.71 (m),	4.15 (dd, 12.2, 2.4),
		3.91~4.00 (m)	4.26 (dd, 12.2, 5.7)
2″-OAc	21.17 (q)	2.19 (s),	[a]
	172.88 (s)	—	—

[a] Nine OAc signals at δ 2.00, 2.07, 2.09, 2.11, 2.12, 2.13, 2.14, 2.24, 2.28.
[b] Not clearly observed.
[c~e] Assignments may be interchanged.

Fig. 2. Structures of aglycone **3** and merulinic acid B (**4**).

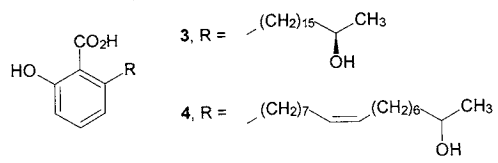


Fig. 3. ^{1}H-^{13}C long range couplings from COLOC experiments in partial structure A of caloporoside (**1**); arrows are directed from H to C.

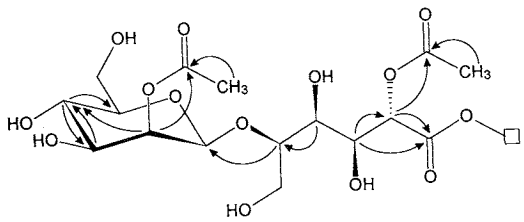
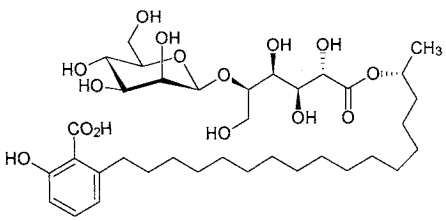


Fig. 4. Structure of bis-deacetyl caloporoside (**5**).



5

tive of merulinic acid B (**4**), an antibiotic from fruiting bodies of *Merulius tremellosus* and *Phlebia radiata*[13].

Table 2. Antibacterial activity of caloporoside by the serial dilution assay.

Organism	MIC (μg/ml)
Acinetobacter calcoaceticus	>100
Arthrobacter citreus	25~50
Bacillus brevis	>100
Bacillus subtilis	10~25
Corynebacterium insidiosum	25~50
Escherichia coli K12	>100
Micrococcus luteus	25~50
Streptomyces sp.	>100

Inoculum 10^6 cells/ml.

Table 3. Antifungal activity of caloporoside by the agar diffusion assay.

Organism	Diameter inhibition zone (mm) 100 μg/disc
Absidia glauca (+)	13
Absidia glauca (−)	15
Ascochyta pisi	15
Aspergillus ochraceus	—
Curvularia lunata	13
Fusarium fujikuroi	9
Fusarium oxysporum f. sp. *cubense*	10
Mucor miehei	12
Nematospora coryli	9
Paecilomyces varioti	—
Penicillium islandicum	12
Penicillium notatum	—
Saccharomyces cerevisiae is 1	—
Ustilago nuda	—
Venturia cerasi	—
Zygorhynchus moelleri	9

—: No inhibition zone.

The high field NMR spectra of **1** in CD_3OD showed the presence of a 2-*O*-acetyl-mannopyranosyl and an open chain 2-*O*-acetyl-mannonic acid moiety, which were readily identified by their characteristic signal patterns and by two-dimensional NMR experiments (Table 1, Fig. 3). The location of the acetyl residues was confirmed by cross peaks between its carbonyl signals and H-2′ and H-2″, respectively. A long range correlation between 5′-H of the mannonic acid part and C-1″ of the mannopyranosyl unit was observed, which proves the (1→5)-linkage between both sugars and leads to partial structure A (Fig. 3). The anomeric proton of the mannopyranosyl unit exhibits a CH-coupling $^{1}J_{CH}=156$ Hz which is in accord with a β-glycosidic linkage[14].

As indicated by the chemical shift of the side chain carbinol proton H-23 (δ 5.01), the mannonic acid forms an ester linkage to the corresponding hydroxy group which leads to structure **1** for caloporoside.

On acetylation, caloporoside yields a peracetate **2** which shows the expected acetylation shifts in its ^{1}H NMR spectrum (Table 1).

Recently, a biologically active glycoside corresponding to bis-deacetyl caloporoside (**5**) (Fig. 4) has

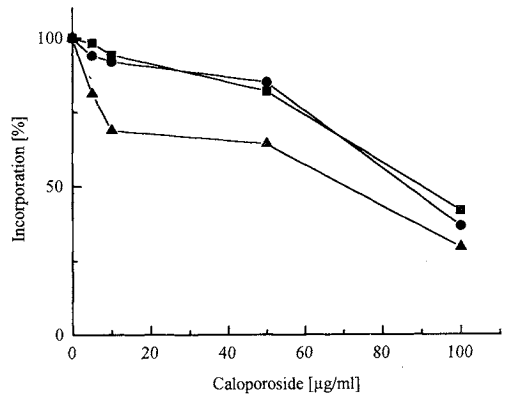
been obtained from cultures of an imperfect fungus isolated from fruiting bodies of a Mycena species[15]. On acetylation **5** afforded a peracetate that proved to be identical with **2** by comparison of its $[\alpha]_D$ value and spectroscopic data (O. STERNER, personal communication). Since the configuration at C-23 in **5** has been suggested to be *R* based on NOESY experiments with a co-metabolite[15], the same stereochemistry can be applied to caloporoside (**1**).

Table 4. Comparison of the inhibitory activities of caloporoside and mepacrine on phospholipases, triacylglycerol lipase, and acetylcholine esterase.

Enzyme	Inhibition (%) Compound*	
	Caloporoside	Mepacrine
Phospholipase A_2 (EC 3.1.1.4)		
Pig pancreas	—+	80
Naja naja venom	—	15
Vipera russelli venom	—	10
Apis mellifera venom	—	20
Phospholipase C (EC 3.1.4.10)		
Pig brain	100[a]	100[b]
Phospholipase C (EC 3.1.4.3)		
Clostridium welchii	20	20
Bacillus cereus	440	—
Phospholipase D (EC 3.1.4.4)		
Cabbage	—	—
Triacylglyceride lipase (EC 3.1.1.3)		
Pig pancreas	—	10
Wheat germ	—	—
Rhizopus arrhizus	—	20
Chromobacterium viscosum	—	20
Acetylcholine esterase (EC 3.1.1.7)		
Human erythrocytes	—	100[c]

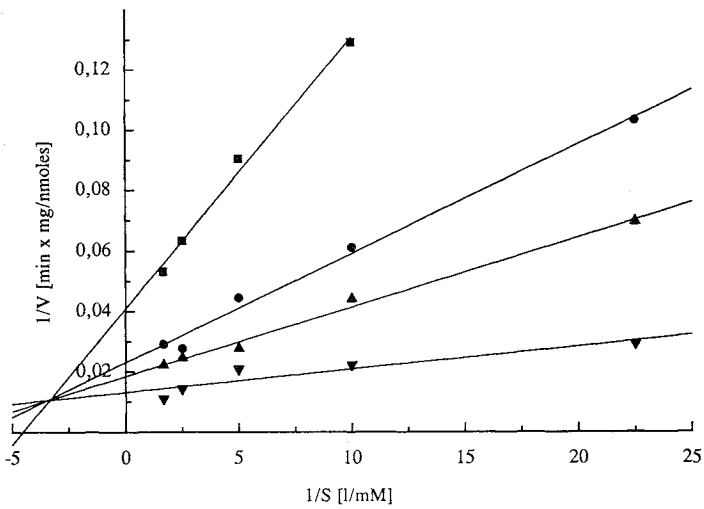
* 100 μg/ml (123 μM caloporoside, 250 μM mepacrine).
\+ —: no inhibition.
[a] $IC_{50} = 18 \sim 31$ μM; [b] $IC_{50} = 70 \sim 80$ μM; [c] $IC_{50} = 1$ μM.

Fig. 5. Effect of caloporoside on the incorporation of [^{14}C]thymidine (▲), [^{14}C]uridine (●), and [^{14}C]-leucine (■), into DNA, RNA, and proteins of Ehrlich carcinoma ascitic cells.



Controls without antibiotic (100%): [^{14}C]thymidine 4.719 cpm, [^{14}C]uridine 12.783 cpm, [^{14}C]leucine 27.651 cpm.

Fig. 6. Inhibition of phospholipase C by caloporoside, Lineweaver-Burk plot.
No inhibitor (▼), 61.5 μM (■), 24.6 μM (●), 12.3 μM (▲) caloporoside.



Biological Properties

The antibacterial and antifungal activities of caloporoside are quite weak (Tables 2 and 3). No cytotoxic (lytic) effects on L 1210, HeLa S3, and Ehrlich ascitic tumor cells could be observed at concentrations up to 100 μg/ml. The incorporation of radioactive precursors into DNA, RNA, and proteins of Ehrlich ascitic tumor cells in the presence of varying concentrations of caloporoside is shown in Fig. 5. The incorporation of uridine, the most sensitive macromolecular synthesis, is inhibited 50% at 90 μg/ml. Caloporoside strongly inhibits phospholipase C from pig brain (Fig. 6). The *Ki* value was determined to 10 μg/ml (12.3 μM). Table 4 shows that caloporoside exhibits—as compared to mepacrine—a marked selectivity towards phospholipase C of pig brain. Phospholipases C from *Clostridium welchii* and *Bacillus* cereus, which act on other substrates, are inhibited to a much lesser extent. Phospholipases A_2, D, triglyceride lipases, and acetylcholinesterase are not inhibited.

Acknowledgments

The financial support of the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged. We thank Dr. OLOV STERNER, University of Lund, for kindly providing us with spectra for comparison.

References

1) ERKEL, G.; T. ANKE, R. VELTEN, A. GIMENEZ & W. STEGLICH: Antibiotics from basidiomycetes. XLIV. Hyphodontal, a new antifungal inhibitor of reverse transcriptases from *Hyphodontia* sp. (*Corticiaceae*, *Basidiomycetes*), Z. Naturforsch. 49c: 1994, in press.
2) RYVARDEN, L.: The Polyporaceae of North Europe. Fungiflora, Oslo, Norway, 1976
3) LEONHARDT, K.; T. ANKE, E. HILLEN-MASKE & W. STEGLICH: 6-Methylpurine, 6-methyl-9-β-D-ribofuranosilpurine, and 6-hydroxymethyl-9-β-D-ribofuranosylpurine as antiviral metabolites of *Collybia maculata* (Basidiomycetes). Z. Naturforsch. 42c: 420~424, 1987
4) KUPKA, J.; T. ANKE, F. OBERWINKLER, G. SCHRAMM & W. STEGLICH: Antibiotics from Basidiomycetes. VII. Crinipellin, a new antibiotic from the basidiomycetous fungus *Crinipellis stipitaria* (Fr.) Pat. J. Antibiotics 32: 130~135, 1979
5) HANES, C. S. & F. A. ISHERWOOD: Separation of the phosphoric esters on the filter paper chromatogram. Nature 164: 1107~1112, 1949
6) SCHU, P.: Neue Inhibitoren der Phospholipase A_2 und der Phospholipase C aus Basidiomyceten und Imperfekten Pilzen. Ph. D. Thesis, Univ. Kaiserslautern, Germany, 1988
7) DEEMS, R. A. & E. A. DENNIS: Phospholipase A_2 from cobra venom (*Naja naja*). *In* Methods in Enzymology. Vol 71. *Ed.*, J. M. LOWENSTEIN, pp. 703~710, Acad. Press, London, 1981
8) KURIOKA, S. & M. MATSUDA: Phospholipase C assay using p-nitrophenylphosphorylcholine together with sorbitol and its application to studying the metal and detergent requirement of the enzyme. Anal. Biochem. 75: 281~289, 1976
9) KATES, M. & P. S. SASTRY: Phospholipase D. *In* Methods in Enzymology. Vol. 1. *Ed.*, J. M. LOWENSTEIN, pp. 197~204, Acad. Press, London, 1969
10) APPLETON, H. D.; B. N. LA DU, B. B. LEVY, J. M. STEELE & B. B. BRODIE: A chemical method for the determination of free choline in plasma. J. Biol. Chem. 205: 803~813, 1953
11) ZIEGENHORN, J.; U. NEUMANN, K. W. KNITSCH & W. ZWEZ: Determination of serum lipase. Clin. Chem. 25: 1067, 1979
12) WHITTAKER, M.: Cholinesterases. *In* Methods of Enzymatic Analysis. Enzymes 2: Esterases. Vol 4. *Ed.*, H. BERGMEYER, pp. 52~63, Verlag Chemie, Weinheim, 1984
13) GIANNETTI, B. M.; W. STEGLICH, W. QUACK, T. ANKE & F. OBERWINKLER: Merulinsäuren A, B und C, neue Antibiotika aus *Merulius tremellosus* Fr. und *Phlebia radiada* Fr. Z. Na turforsch. 33c: 807~816, 1978
14) Bock, K. & C. PEDERSEN: A study of ^{13}CH coupling constants in hexopyranoses. J. Chem. Soc. Perkin Trans. II, 1974: 293~297
15) SHAN, R.; H. ANKE, M. NIELSEN, O. STERNER & M. R. WITT: The isolation of two new fungal inhibitors of ^{35}S-TBPS binding to the brain $GABA_A$/benzodiazepine chloride channel receptor complex. Nat. Prod. Lett. 4: 171~178, 1994