Fulicineroside, an Unusual Glycosidic Dibenzofuran Metabolite from the Slime Mold *Fuligo cinerea* (Schwein.) Morgan

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Fulicineroside, a glycosidic dibenzofuran metabolite from the slime mold *Fuligo cinerea* collected in the Czech Republic, has been isolated as a new natural product. Its structure was elucidated from UV, IR, MS, 1D and 2D NMR spectroscopic data and chemical degradation. The compound was highly active against Gram-positive bacteria and crown gall tumors. (© Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2005)

Introduction

The class Myxomycetes comprises approximately 500 species that live on moist soil, decaying wood, and dung.[1] *Fuligo cinerea*, which belongs to the order Physarales (Family Physaraceae, Phylum Gymnomycota, Class Myxomycetes) settles down to form a blob that sometimes is yellow or orange, crusting to a white blob with a black spore-mass inside.[1,2] The slime molds engulf their food, whereas fungi exude enzymes to digest their food and then reabsorb the products.[3] In this regard, a slime mold is on the animal branch of taxonomy, splitting off soon after the evolution of the nucleated cell that spawned animals, plants, and fungi.[4] The life history of a slime mold is complex, but usually ends up with a group of cells that join together and dissolve their cell walls to form a plasmodia. This amoeba-like creature oozes along, eating bits of plant and debris, and, when the time is ripe, forms a sporocarp.

Only a few papers have described novel compounds isolated from the *Fuligo* species. For instance, the plasmodial pigment fuligorubin A has been found in *Fuligo cinerea*,[5] and cycloanthranilylproline derivatives have been isolated from *Fuligo candida*.[6] During our search for new secondary metabolites from the myxomycetes, we have isolated polyunsaturated and methylene non-interrupted polyunsaturated fatty acids,[7] and a multibranched polyunsaturated fatty acid and its four glycosides[8] from field-collected fruit bodies of several myxomycete species.

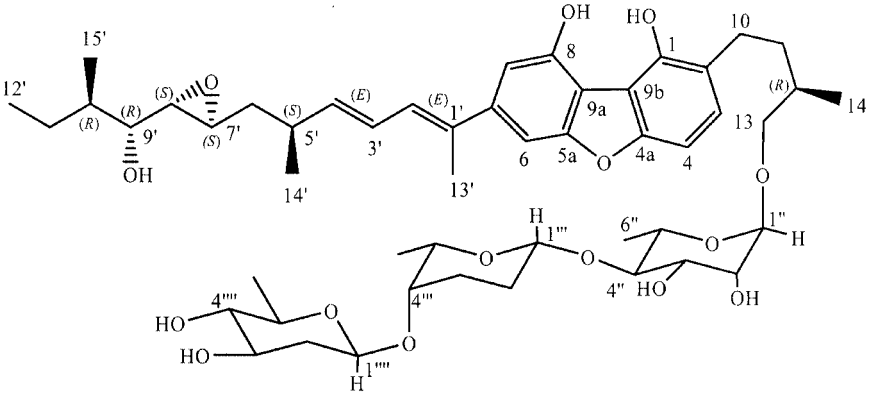

Figure 1. Fulicineroside (**1**), a dibenzofuran glycoside from the slime mold *Fuligo cinerea*.

Dibenzofuran-containing metabolites mostly occur in lichen species,[9] with the most well-known being usnic acid, which has become the most extensively studied lichen me-

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tabolite and one of the few that have been commercialized.[10] Usnic acid is only found in lichens, and is especially abundant in genera such as *Alectoria*, *Cladonia*, *Usnea*, *Lecanora*, *Ramalina*, and *Evernia*. Many lichens and extracts containing usnic acid have been utilized for medicinal, perfumery, cosmetic, and ecological applications.[11] Usnic acid as a pure substance has been utilized in creams, toothpaste, mouthwash, deodorants, and sunscreen products, in some cases as an active principle and in others as a preservative. In addition to antimicrobial activity against human and plant pathogens, usnic acid has been shown to exhibit antiviral, antiprotozoal, antiproliferative, anti-inflammatory, and analgesic activity.[10] Dictyomedin A and B, novel dibenzofuran metabolites that have been isolated from the slime mold *Dictyostelium medium*,[12] and other dibenzofuran phytoalexins have been isolated from the sapwood of *Cotoneaster acutifolius*, *Photinia*, *Pyracantha* and *Crataegus* species, and *Mespilus germanic*a.[13–15]

In the course of our continuing search for novel antimicrobial agents from myxomycetes,[16,17] we have isolated a novel alkyldibenzofuran metabolite, named fulicineroside (**1**; Figure 1) from *Fuligo cinerea*. In this paper, we describe the isolation and structure elucidation of **1** by extensive NMR spectroscopic analysis. The antimicrobial and antiviral activity of **1** are also reported.

Results and Discussion

A 19.65-g sample of *Fuligo cinerea* (Schwein.) Morgan was extracted with butanol and subsequently separated on a Sephadex LH-20 column. The fractions were further purified by RP-HPLC to give glycoside **1** (19.9 mg), which was identified by IR, UV, MS, and ^{1}H and ^{13}C NMR spectroscopic data and chemical degradation. Compound **1** was obtained as a white amorphous powder with $[\alpha]_D^{23}$ = +43, but without an exact melting point as the glycoside decomposed.

The positive HRFAB mass spectrum shows an $[M + Na]^+$ peak at *m/z* = 935.4774 (calcd. 935.4769) corresponding to the molecular formula $C_{50}H_{72}O_{15}$, as also deduced by ^{13}C NMR and DEPT analyses. The negative FAB mass spectrum of **1** shows a molecular anion peak $[M – H]^–$ at *m/z* = 911 with fragment ions at *m/z* = 781 $[M – H – 130]^–$ and *m/z* = 763 $[M – H – H_2O – 130]^–$, which are formed by the loss of the terminal dideoxyhexose unit, and an additional fragment ion at *m/z* = 667 $[M – H – 130 – 114]^–$ corresponding to the loss of a trideoxyhexosyl unit. Furthermore, the prominent fragment at *m/z* = 521 is formed by loss of the trisaccharide chain linked to the aglycon.

The IR spectrum displays absorptions at 1620 and 1590 cm^{-1} characteristic of aromatic ring(s), 825 cm^{-1} for tetrasubstituted benzene, and shows a broad absorption band at 3290 cm^{-1} consistent with the presence of OH functionalities.

The NMR spectroscopic data of fulicineroside (**1**; see Table 1) show the presence of three *O*-glycosidic hexopyranoses, i.e. three anomeric carbon signals at $\delta \approx$ 97.0–103.0 ppm and three anomeric protons ($\delta \approx$ 4.70–

Table 1. ^{1}H and ^{13}C NMR spectroscopic data (measured in $CDCl_3$) of fulicineroside (**1**).

Position	Aglycon	
	^{1}H	^{13}C
1	–	150.2
2	–	121.3
3	6.88 (d, *J* = 7.1 Hz, 1 H)	125.8
4	6.93 (d, *J* = 7.1 Hz, 1 H)	104.2
4a	–	154.1
5a	–	145.0
5	7.02 (d, *J* = 2.1 Hz, 1 H)	102.1
6	–	132.4
7	6.64 (d, *J* = 2.1 Hz, 1 H)	108.1
8	–	150.3
9a	–	106.9
9b	–	114.9
10	2.55 (m, 2 H)	23.4
11	1.58 (m, 2 H)	35.9
12	1.98 (m, 1 H)	32.6
13	4.33 (dd, *J* = 13.2, 2.4 Hz, 1 H) 4.52 (dd, *J* = 13.2, 7.1 Hz, 1 H)	71.5
14	1.06 (d, *J* = 6.9 Hz, 3 H)	16.4
1′	–	138.2
2′	6.57 (d, *J* = 10.2 Hz, 1 H)	128.5
3′	6.27 (dd, *J* = 10.2, 14.3 Hz, 1 H)	126.0
4′	5.72 (dd, *J* = 14.3, 8.0 Hz, 1 H)	138.6
5′	2.34 (dddq, *J* = 10.1, 8.0, 3.0, 6.8 Hz, 1 H)	35.0
6a′	1.52 (ddd, *J* = 4.0, 10.1, 14.2 Hz, 1 H)	39.6
6b′	1.72 (ddd, *J* = 9.6, 3.0, 14.2 Hz, 1 H)	–
7′	2.62 (ddd, *J* = 1.9, 9.6, 4.0 Hz, 1 H)	58.2
8′	2.80 (dd, *J* = 8.9, 1.9 Hz, 1 H)	64.0
9′	3.52 (dd, *J* = 8.9, 10.2 Hz, 1 H)	74.9
10′	1.78 (m, 1 H)	35.4
11′	1.29 (m, 2 H)	23.5
12′	0.96 (t, *J* = 6.8 Hz, 3 H)	11.8
13′	1.75 (s, 3 H)	12.3
14′	1.16 (d, *J* = 6.8 Hz, 3 H)	19.9
15′	1.06 (d, *J* = 6.7 Hz, 3 H)	10.4
	Rhamnose	
1′′	4.98 (d, *J* = 2.6 Hz, 1 H)	100.4
2′′	3.92 (dd, *J* = 2.6, 2.5 Hz, 1 H)	71.7
3′′	3.71 (dd, *J* = 2.5, 9.4 Hz, 1 H)	71.5
4′′	4.28 (t, *J* = 9.4 Hz, 1 H)	73.2
5′′	4.11 (dq, *J* = 9.4, 6.5 Hz, 1 H)	69.7
6′′	1.35 (d, *J* = 6.5 Hz, 3 H)	18.6
	Rhodinose	
1′′′	4.78 (dd, *J* = 8.8, 2.1 Hz, 1 H)	96.9
2a′′′	2.02 (m, 1 H)	32.1
2e′′′	1.46 (m, 1 H)	–
3a′′′	2.10 (m, 1 H)	27.3
3e′′′	1.43 (m, 1 H)	–
4′′′	3.05 (br. s, 1 H)	68.8
5′′′	3.28 (dq, *J* = 1.5, 6.3 Hz, 1 H)	69.0
6′′′	1.08 (d, *J* = 6.3 Hz, 3 H)	18.9
	Olivose	
1′′′′	4.77 (dd, *J* = 9.5, 2.0 Hz, 1 H)	102.9
2a′′′′	1.73 (ddd, *J* = 12.1, 11.8, 9.5 Hz, 1 H)	36.4
2e′′′′	2.44 (ddd, *J* = 12.1, 5.4, 2.0 Hz, 1 H)	–
3′′′′	3.87 (ddd, *J* = 11.8, 9.7, 5.4 Hz, 1 H)	74.6
4′′′′	4.56 (dd, *J* = 9.7, 9.2 Hz, 1 H)	73.8
5′′′′	3.39 (dq, *J* = 9.2, 6.6 Hz, 1 H)	70.7
6′′′′	1.23 (d, *J* = 6.6 Hz, 3 H)	16.5

5.00 ppm). The key resonance is a peak at δ = 18.6 ppm, which represents the C-6′′ signal of a 6-deoxy sugar. This signal was identified as a methyl carbon from the DEPT spectrum and the corresponding ^{1}H NMR chemical shift ($\delta \approx$ 1.35 ppm). This shift was used as a starting point in the homonuclear correlated spectra to determine all protons on the same sugar. The value of $J_{H\text{-}1''\text{–}H\text{-}2''}$ for compound **1** (2.6 Hz) and NOEs further confirmed that the deoxyhexose sugar was α-rhamnopyranose.[18] From enzymatic hydrolysis, we also deduced that the sugar must be L-rhamnose (6-deoxy-L-mannose; **2**).

The second monosaccharide moiety was determined to be rhodinose (**3**) from its ^{1}H and ^{13}C NMR chemical shifts and by analyzing the coupling constant between H-4′′′ and H-5′′′. This constant is very small ($J \approx$ 1.5 Hz), which confirms the H-4′′′$_{eq}$–H-5′′′$_{ax}$ configuration. The coupling constants for the anomeric proton ($J_{1,2ax}$ = 8.8 and $J_{1,2eq}$ = 2.1 Hz) and the chemical shift of the anomeric carbon at δ = 96.9 ppm suggested that the monosaccharide is attached to the rhamnose by a glycosidic bond. On the basis of the NMR spectroscopic data, the monosaccharide was determined to be β-rhodinose (**3**) (2,3,6-trideoxy-β-*threo*-hexopyranose).[19]

As to the third monosaccharide, strong NOEs were observed between H-l′′′′/H-3′′′′, H-l′′′′/H-5′′′′, and H-3′′′′/H-5′′′′, and strong *J* couplings between H-l′′′′/H-2′′′′$_{ax}$, H-2′′′′$_{ax}$/H-3′′′′, H-3′′′′/H-4′′′′, and H-4′′′′/H-5′′′′. These observations indicate that this monosaccharide is β-olivose (**4**; 2,6-dideoxy-β-*arabino*-hexopyranose).[20] The interglycosidic linkages were established by HMBC techniques (see Figure 2). The HMBC spectrum of **1** shows cross peaks between the signals at δ = 4.98 (rhamnose H-1′′) and 71.5 (C-13 of the aglycon), 4.78 (rhodinose H-1′′′) and 73.2 (rhamnose C-4′′), and 4.77 (olivose H-1′′′′) and 68.8 ppm (rhodinose C-4′′′).

The aglycon fulicinerine (**5**) was liberated from **1** by enzymatic hydrolysis with hesperidinase (EC, 3.2.1.40); it was extracted from water solution with ethyl acetate and further characterized. Mild acid hydrolysis of the water solution yielded three different sugars, which were purified by NH_2-HPLC. After evaporation of the eluent, the three saccharides were obtained as colorless syrups. The rhamnose isolated had an optical rotation of +9.0, which is essentially identical to the literature data ($[\alpha]_D$ = +9.1 and +8.9 for L-rhamnose).[21] The optical rotation of rhodinose (**3**) was found to be –10.8, which is also practically identical to the reported value for L-rhodinose ($[\alpha]_D^{20}$ = +14.2 for D-rhodinose and $[\alpha]_D^{27}$ = –11.8 for L-rhodinose).[22,23] The optical rotation of the olivose (**4**) in water ($[\alpha]_D$ = +21.7) was also in good agreement with the literature data ($[\alpha]_D^{23}$ = +22.0 for D-olivose).[24] These results indicate that two monosaccharides of **1** are in their L-forms and one in its D-form.

The molecular formula of **5** was determined as $C_{32}H_{42}O_6$ from the HRFAB-MS data (545.2880 for $[M + Na]^+$). This determination was further supported by the ^{1}H and ^{13}C NMR spectra.

The UV spectrum of the aglycon from the ethyl acetate extract has bands at λ = 206, 238, 260, and 338 nm, which suggest a dibenzofuran skeleton;[25] this was confirmed by the presence of 12 aromatic carbon signals in the ^{13}C NMR spectrum. From the presence of four aromatic methine groups, as indicated by the ^{1}H, ^{13}C, and DEPT NMR spectra, it was apparent that the dibenzofuran structure is tetrasubstituted. The ^{1}H NMR spectrum also features signals attributable to two hydroxy groups. Finally, a further D_2O-exchangeable proton (singlets at δ = 9.37 and 9.45 ppm, respectively) in the ^{1}H NMR spectrum indicates the presence of two phenolic groups.

The substitution pattern of the aromatic rings was elucidated by analysis of the ^{1}H-^{1}H coupling constants and 2D COSY, HMBC, and NOESY data. The *meta* relationship of H-5 (d, δ = 7.02 ppm) and H-7 (d, δ = 6.64 ppm) was established from their small mutual coupling constants ($J \approx$ 2 Hz). The large coupling constant ($J_{3,4}$ = 7.1 Hz) be-

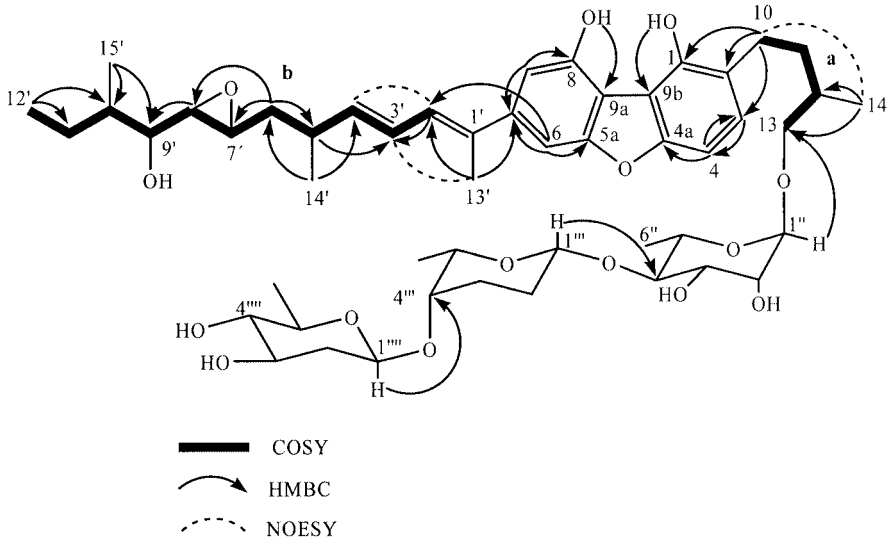


Figure 2. The HMBC, ^{1}H-^{1}H COSY, and NOE correlations of fulicineroside (**1**).

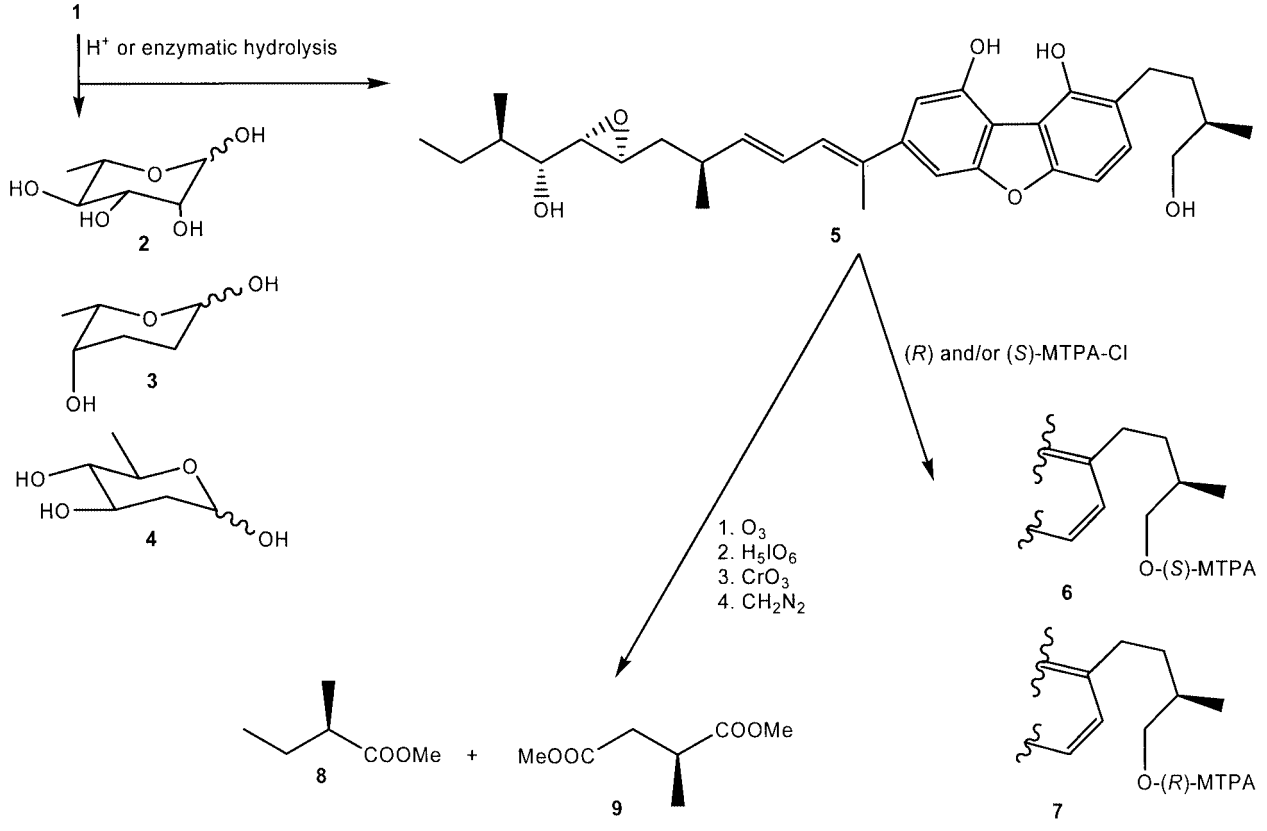
tween the aromatic protons resonating at δ = 6.88 and 6.93 ppm (H-3 and H-4, respectively) established their *ortho* relationship.

The observation of cross peaks between H-5 and C-5a (δ = 145.0 ppm) and H-4 and C-4a (δ = 154.1 ppm) located the two oxygen-bearing sp^2 carbons C-4a and C-5a, and completed the connectivity of both phenyl rings. All functional groups were assigned to the two phenyl rings, except for the substituents on quaternary carbons C-9a and C-9b. The only possibility is the direct connection of these two carbons since no other functional groups were available and therefore the biphenyl system has to be cyclized through an oxygen linkage to form a dibenzofuran system.

Detailed analyses of the ^{1}H and ^{13}C NMR spectra with the aid of the ^{1}H-^{1}H COSY experiment, coupled with the structural information from the UV and IR spectra, clearly revealed two partial structures **a** and **b** consisting of fragments C-10 to C-13 and C-1′ to C-12′. The connections of these two units and the remaining methyls (C-14 and C-13′ and C-14′ and C-15′, respectively) are suggested by the HMBC correlations (Figure 2). The methylene (H_2-10) in unit **a** is connected to C-2 (δ = 121.3 ppm), since a long-range correlation between H_2-10 and C-2 is unambiguously observed. The attachment of the methyl group (C-14; δ_C = 16.0, δ_H = 1.07 ppm) to the tertiary carbon (C-12) at δ = 32.6 ppm was suggested by the long-range correlations of H_3-14 to C-13 and C-12 and an NOE to C-10 in unit **a**.

The diene side-chain (unit **b**) is connected to the C-6 quaternary carbon, as evidenced by the long-range correlations of C-6 to the olefinic proton (H-2′) at δ = 6.57 ppm and the methyl proton (H_3-13′) at δ = 1.75 ppm. The geometry of the olefinic protons (H-3′, H-4′) was determined to be (*E*) from the vicinal coupling constant ($J_{3',4'}$ = 14.3 Hz). The high-field chemical shift of the methyl group (C-13′) at δ = 12.3 ppm indicates an (*E*) configuration of the double bond,[26] which was supported by NOEs between H-2′ and H-4′ and between the methyl proton (H_3-13′) and H-3′ (Figure 2). The oxirane ring on the side chain was unambiguously assigned as *threo* on the basis of the coupling constant ($J_{7',8'}$ = 1.9 Hz).[27] Thus, the structure of fulicinerine was found to be that of **5**. The absolute stereostructural elucidation of **5** is reported below.

To elucidate the absolute configuration at C-12, the esterification of **5** with (*R*)- and (*S*)-MTPACl was performed, and two compounds, the (*S*)-MTPA ester **6** and (*R*)-MTPA ester **7**, respectively, were obtained (see Scheme 1). A very similar structure has been found in sterols, where Mosher's method has been applied for the determination of the absolute stereochemistry of a methyl group at C-25 with a primary hydroxyl group at C-26. In the ^{1}H NMR spectra of the (*R*)-MTPA ester, two methylene-26 protons of the 25-(*S*) isomer are much closer ($\Delta\delta \approx 0.04$ ppm) to each other than those ($\Delta\delta \approx 0.14$ ppm) of the 25-(*R*) isomer, whereas in the (*S*)-MTPA esters this relationship is reversed.[28] The absolute configuration at C-12, where a methyl group is located, was elucidated on the basis of chemical-shift differences and signal patterns of the two geminal protons at C-13 of **6** and **7**. The methylene protons at C-13 of **6** appear



Scheme 1. The degradation of fulicineroside (**1**) and preparation of its derivatives.

as two separate doublet signals at δ_H = 4.12 and 4.17 ppm ($\Delta\delta$ = 0.05 ppm), while the $\Delta\delta$ value (0.16 ppm) of H_2-13 (δ_H = 4.03 and 4.19 ppm) for **7** is larger than that for **6**, indicating that the absolute configuration at C-12 is (*R*)[29,30] (see also Figure 3).

To determine the absolute configuration of the methyls at C-5′ and C-10′, compound **5** was oxidized with ozone, and the resulting oxirane was further exposed to periodate. The resulting triol was oxidized in a Jones oxidation to give two chiral compounds (Scheme 1), which were then esterified with diazomethane. The retention time (determined by chiral chromatography) of methyl 2-methylbutyrate (**8**) obtained from compound **1** had an identical retention time to the (*R*)-isomer obtained commercially, therefore the absolute configuration at C-10′ is (*R*). Analogously, the retention time of dimethyl methylsuccinate (**9**) from the natural specimen was identical with that of the commercial (*S*)-isomer (Table 2), thus indicating that the absolute configuration at C-5′ of compound **1** is (*S*). From the known relative configuration it is evident that both C-7′ and C-8′ have an (*S*) configurations and C-9′ has an (*R*) configuration. Therefore, the absolute configuration of all six chiral centers in compound **5** has been elucidated to be (5′*S*,7′*S*,8′*S*,9′*R*,10′*R*,12*R*). On the basis of these data, the structure of fulicineroside (**1**) is (1′*E*,3′*E*,5′*S*,7′*S*,8′*S*,9′*R*,10′*R*,12*R*)-13-β-D-olivosyl-(1′′′′→4′′′)-β-L-rhodinosyl-(1′′′→4′′)-α-L-rhamnosyloxy-6-(9′-hydroxy-7′,8′-epoxy-1′,5′,10′-trimethyl-dodeca-1′,3′-dienyl)-2-(13-hydroxy-12-methylbutyl)dibenzofuran-1,8-diol.

Table 2. The presence of degradation products (determined by chiral capillary GC) after oxidation of compound **5**.

Methyl ester of	t_R of products [min]	
	Standards	After degradation of **5**
(2*R*)-Methylbutyric acid	9.12	9.14
(2*S*)-Methylbutyric acid	9.23	–
(2*R*)-Methylsuccinic acid	14.22	–
(2*S*)-Methylsuccinic acid	14.71	14.73

The antimicrobial and antiviral activities of **1** are summarized in Table 3. Compound **1** inhibits the growth of the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. It also shows a modest growth inhibition of Gram-negative bacteria and some yeasts. These results indicate that **1** has a higher permeability into the cells and interacts more strongly with bacteria than with fungi.

The crown gall tumor inhibition test has been used to test the activity of antitumor agents produced in vivo by organisms and is also used to evaluate extracts for different pharmacological activities. The isolated compound was

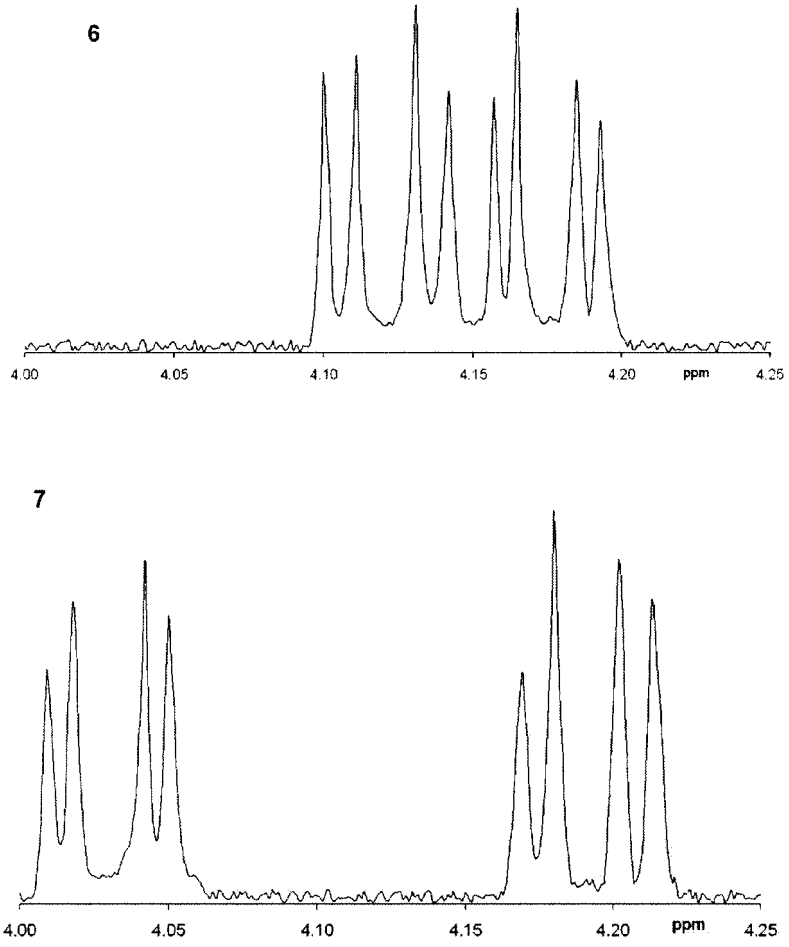


Figure 3. Proton signal patterns of 13-H_2 of the tetrakis-(*S*)- and (*R*)-MTPA esters (**6** and **7**, respectively).

Table 3. Bioactivities of fulicineroside (**1**).

Test organism[a]	**1**
Staphylococcus aureus	48
Bacillus subtilis	56
Escherichia coli	8
Saccharomyces cerevisiae	7
Candida albicans	2
Agrobacterium tumefaciens[b][c]	28 ± 3[d]

[a] Samples (10 μg) were applied on 6.35 mm paper disks, values are diameters [mm] of inhibitory zones. [b] See Experimental Section. [c] Presented values are means of three determinations. [d] Percentage of crown gall tumor inhibition (±S.D.).

evaluated by its ability to inhibit the growth of crown gall tumors on potato discs inoculated with *Agrobacterium tumefaciens* carrying a tumor-inducing plasmid. Compound **1** showed significant inhibition of the growth (about 83%) of crown gall tumors on potato disks, which suggests in vivo antitumor activity.

Experimental Section

General Experimental Procedures: UV/Vis spectra were measured in MeOH within the range of 220 to 550 nm with a Cary 118 (Varian) spectrometer. A Perkin–Elmer (Perkin–Elmer, Norwalk, CT, USA) model 1310 IR spectrophotometer was used for scanning IR spectra from KBr tablets. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. NMR spectra were recorded on a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^{1}H) or 125.7 MHz (^{13}C). High- and low-resolution mass spectra were recorded with a VG 7070E-HF spectrometer (70 eV). HRFABMS (negative ion mode) were obtained with a PEG-400 matrix. GC-MS of the methyl esters was done with a Finnigan 1020 B (Finnigan MAT, San Jose, CA, USA) single-state quadrupole GC-MS instrument in the EI mode. Gas chromatography analysis was made on a Hewlett Packard HP 5980 gas chromatograph (Hewlett Packard, Czech Republic). FS capillary column HYDRODEX β-3P ID 0.25 mm, length 25 m, with the stationary phase [heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin] from Macherey–Nagel GmbH & Co. KG, Düren, Germany. Oven temperature: 50 °C to 150 °C at 2 °C/min, then to 240 °C at 5 °C/min, carrier gas helium, 20 mL/s, detector FID, 300 °C, injection of 1 μL mixture in dichloromethane (for standards containing 0.5 mg/mL of each sample), split (100:1), 300 °C.

(2*R*)-Methylbutyric, (2*S*)-methylbutyric, (2*R*)-methylsuccinic, and (2*S*)-methylsuccinic acids, and hesperidinase (ECN, 3.2.1.40) from *Aspergillus niger* (contains both α-L-rhamnosidase and β-D-glucosidase) were purchased from Sigma–Aldrich (Prague, Czech Republic).

Plant Material: The slime mold was collected on 6th July 2003 at the Braitava, near Vranov nad Dyji, South Moravia (Czech Republic), on decayed wood of the European Beech.

Extraction and Isolation: A sample of slime mold (19.65 g dry weight) was extracted with 90% butanol. Chromatography of the extract on a Sephadex LH-20 column (100 × 5 cm) eluting with MeOH gave organic fractions (8 mL) that were checked by two-dimensional TLC [silica gel plates, *n*BuOH/AcOH/H_2O (12:3:5) and $CHCl_3$/MeOH/H_2O (40:9:1)]. Fraction E was further fractionated by RP-HPLC on a C18-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL/min) with MeOH/H_2O (4:1) to yield compound **1** (19.9 mg).

Acid Hydrolysis: A 6.0-mg portion of ester **1** was refluxed in 2 M HCOOH (0.5 mL) for 2 h. The hydrolysate was then extracted three times with EtOAc (5 mL). After separating the organic layer, the aqueous phase was neutralized with $NaHCO_3$ and lyophilized. The residue obtained after lyophilization was purified on a Sepharon SGX NH_2 column (7 μm, 3 × 150 mm) eluting with 90% MeCN (flow 0.7 mL/min) to yield 0.8 mg of L-rhamnose (t_R = 13.8 min) $[\alpha]_D^{22}$ = +9.0 (equilib.), 0.9 mg of L-olivose (t_R = 9.6 min) $[\alpha]_D^{23}$ = +21.7 (equilib.), and 0.8 mg of L-rhodinose (t_R = 5.2 min) $[\alpha]_D^{23}$ = –10.8 (equilib.).

Enzymatic Hydrolysis: A solution of glycoside (13.9 mg) in acetate buffer (pH 4.4, 10 mL) was treated with hesperidinase for 48 h at 37 °C. The reaction solution was evaporated to dryness, and the residue was chromatographed on a column of silica gel (10 g) with CH_2Cl_2/MeOH/H_2O (90:10:1) as eluent to give 5.5 mg of compound **5** for NMR analysis.

Mosher Esters. (*S*)-MTPA Esters 6: (–)-MTPA chloride (20 μL) was added to a stirred solution of 1.0 mg of compound **5** in 0.3 mL of dry pyridine. The mixture was stirred under N_2 at room temperature for 1 h and the solvent was then removed by blowing with N_2. The residue was redissolved in 2 mL of EtOAc/hexane (1:1; v/v) and filtered through a Sep-Pak silica column. After removing the solvent under vacuum, the residue was separated by RP-HPLC (ODS column, 100% acetonitrile) to yield 1.0 mg of the (*S*)-ester as a colorless gum. HRFABMS calcd. for $C_{72}H_{70}F_{12}NaO_{14}$ [M + Na]$^+$: 1409.4470; found 1409.4474. See Figure 3 for a partial ^{1}H NMR spectrum.

(*R*)-MTPA Esters 7: These were prepared as described for the (*S*)-ester from 1.0 mg of compound **5** and 20 μL of (+)-MTPA chloride to give 0.9 mg of the (*R*)-ester as a colorless gum. HRFABMS calcd. for $C_{72}H_{70}F_{12}O_{14}Na$ [M + Na]$^+$: 1409.4470; found 1409.4477.

Methyl Methylbutyrate and Dimethyl Methylsuccinate: A stream of 4% ozone was passed through a solution of fulicinerine (**5**, 3.5 mg) in dichloromethane (0.5 mL) at –78 °C for 5 min. The solution was then flushed with nitrogen and concentrated, and the residue was dissolved in 2 mL of dry diethyl ether. A solution of 2.6 mg of *para*-periodic acid in 1 mL of dry diethyl ether was added to this ethereal solution, and the reaction mixture was stirred at room temperature for 1 h. After removed of the solvent at reduced pressure, Jones' reagent (0.2 mL) in 1 mL of acetone was added dropwise to the residue in 1.0 mL of acetone. Two or three drops of isopropyl alcohol were then added after 10 min at 25 °C, followed by a large amount of water. This mixture was extracted with two 2-mL portions of diethyl ether/benzene (1:1), the solvents were removed under reduced pressure, and the residue treated with an excess of diazomethane in diethyl ether. The resultant oil was further separated by chiral GC. The mass spectrum was identical with commercially obtained methyl 2-methylbutyrate (**8**) and dimethyl methylsuccinate (**9**; see below). See Table 2 for retention times.

Fulicineroside (1): Amorphous pale-yellow powder (19.9 mg). $[\alpha]_D^{23}$ = +43 (*c* =0.01, MeOH). UV (MeOH): λ_{max} (log ε) = 221 nm (3.94), 240 (4.28), 267 (3.97), 303 (4.15), 313 (4.06), 358 (3.80). IR (KBr): $\tilde{\nu}_{max}$ = 3600 cm^{-1}, 3300, 1625. HRFABMS: *m*/*z* = 935.4774 [M + Na]$^+$, calcd. for [$C_{50}H_{72}O_{15}$+Na]$^+$ 935.4769; negative FABMS: *m*/*z* = 911 [M – H]$^-$, 781 [M – H – 130]$^-$, 763 [M – H/H_2O – 130]$^-$, 667 [M – H – 130 – 114]$^-$, 521 [aglycon]; see Table 1 for NMR spectroscopic data.

Fulicinerine (5): Pale-yellow microcrystals; m.p. 245–247 °C. Yield: 5.5 mg. $[\alpha]_D^{23}$ = +18 (*c* =0.008, MeOH). UV (MeOH): λ_{max} (log ε)

= 206 nm (4.38), 238 (4.26), 260 (4.07), 338 (4.16). IR (KBr): $\tilde{\nu}_{max}$ = 3290 cm^{-1}, 1620, 1590, 825. HRFABMS: *m/z* = 545.2880 [M + Na]$^+$, calcd. for [$C_{32}H_{42}O_6$+Na]$^+$ 545.2879. ^{1}H NMR ($CDCl_3$): δ = 1.58 (m, 2 H, H-11), 2.19 (m, 1 H, H-12), 4.58 (dd, *J* = 13.4, 2.5 Hz, 1 H, H-13) 4.74 (dd, *J* = 13.4, 7.0 Hz, 1 H, H-13), 1.07 (d, *J* = 7.0 Hz, 3 H, H-14) ppm. ^{13}C NMR ($CDCl_3$): δ = 37.1 (C-11), 32.6 (C-12), 75.8 (C-13), 16.0 (C-14) ppm. The other signals in the ^{1}H and ^{13}C spectra are identical with values measured for fulicineroside (**1**) given in Table 1.

Antibacterial Tests: The test organisms were *Bacillus subtilis, Staphyloccocus aureus*, *Escherichia coli*, and *Saccharomyces cerevisiae* (Czechoslovak Collection of Microorganisms, Brno). Antibacterial assays were carried out according to the literature.[30] The amounts used were 50 μg of compound per test disk (see Table 3).

Crown Gall Tumors on Potato Disks Test: The *Agrobacterium tumefaciens* potato disc assay for tumor/antitumor induction was performed according to the procedure described in the literature.[31] The potatoes were sterilized by immersion in 70% ethanol for 2 min and in 50% sodium hypochlorite solution (active chlorine 30 g/L) for 30 min. Then, the potatoes were rinsed several times with sterilized distilled water in a laminar flow hood. A core of tissue was extracted from each tuber with a sterilized 1.5 cm cork borer. Discs of 0.5 cm were cut with a scalpel. The potato discs were placed in 1.5% agar Petri dishes. To each potato disc was applied 0.05 mL of a solution containing 2 mL of a broth culture of *A. tumefaciens* (48 h culture of ca. 109 cells/mL), 1.5 mL of sterile H_2O, and 0.5 mL of the solution test extract (8 mg of extract in 2 mL of DMSO filtered through 0.22 mm filters). Control discs were prepared with sterile DMSO instead of test extract. A minimum of three Petri dishes (5 disks/dish, *n* = 15–25) was used for each test compound and the control. Following preparation, the Petri dishes were placed in an incubator at 27 °C for 12–21 days. To determine the number of tumors, the potato discs were stained with a solution of I_2 (1 g) and KI (2 g) in 300 mL of distilled H_2O. Significant activity was indicated when two independent assays gave 20% or greater inhibition.

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