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Enteridinines A and B from slime mold *Enteridium lycoperdon*

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Abstract

Two novel deoxysugar esters, named enteridinines A and B, were isolated from the slime mold *Enteridium lycoperdon*. Their structures, including the absolute configurations of the hydroxyl and methyl groups, were determined by means of extensive spectroscopic data such as UV, IR, MS, 1D and 2D NMR spectra. Enteridinines A and B have unique structures containing 1,7-dioxaspiro[5.5]undecanes with an *O*-β-D-mycarosyl-(1→4)-β-D-olivosyl and an *O*-β-L-olivomycosyl-(1→4)-β-D-amicetosyl-(1→4)-β-L-digitoxosyl unit, respectively, and showed growth inhibitory activities against Gram positive bacteria.

Keywords: *Enteridium lycoperdon*; Myxomycetes; Deoxysugar esters; Spiro[5.5]undecane derivatives; Slime mold

1. Introduction

The slime molds are heterotrophic organisms once regarded as fungi but later classified with the Protista (Bonner, 1985). According to a recent system of classification based on analysis of nucleic acid sequences, slime molds have been classified in a major group called the eukaryota, which also includes plants and animals (Alexopoulos and Mims, 1979). There are two groups of slime molds, the plasmodial slime molds of the phylum Myxomycota (Myxomyceta) and the cellular slime molds of Acrasiomycota (Stephenson and Stempen, 1994). The slime molds are an unique group among living organisms, and produced many biological active compounds, including lipids (Murakami-Murofushi et al., 2002), alkaloids (Blumenthal et al., 2002), aromatic compounds (Takaya et al., 2001), and optical active pigments (Eisenbarth and Steffan, 2000). This report is to continue our investigation of slime mold active compounds (Řezanka and Dvořáková, 2003) and we report the isolation and structural determination of two novel deoxysugar esters, named enteridinine A (**1**) and B (**2**), from *Enteridium lycoperdon*.

2. Results and discussion

A 30.5 g of lyophilized slime mold *Enteridium lycoperdon* were extracted by *n*-BuOH and subsequently separated on a Sephadex LH-20 column. The fractions were further purified by RP-HPLC to give enteridinine A (**1**) and B (**2**) (Fig. 1).

Enteridinine A (**1**) was obtained as an amorphous powder with $[\alpha]_D^{23}$ −62° but without an exact melting point as the glycoside decomposed. The UV spectrum of **1** showed absorption maximum at 205 nm (log ϵ 2.41), suggesting that it had no conjugated system of double bonds. The IR spectrum of **1** showed absorption bands due to free hydroxyls (3600 cm^{-1}), carboxyl (1650 cm^{-1}) and ether (1170 and 1040 cm^{-1}) groups. The molecular weight determined by positive HRFABMS was 633.4216 $[M+H]^+$, which corresponds to a molecular formula of $C_{33}H_{60}O_{11}$, and negative FABMS gave two prominent ions at *m*/*z* 501 $[M-H-130]^-$ (cleavage of a dideoxyhexosyl unit) and *m*/*z* 357 $[M-H-130-144]^-$ (cleavage of a dideoxyhexosyl and a dideoxymethylhexosyl unit).

The ester was subjected to alkaline hydrolyzis and enteridic acid (**1a**) was isolated from the reaction mixture. The molecular formula of **1a** ($C_{21}H_{39}O_7$) was determined by HRFABMS ($[M+H]^+$ at *m*/*z* 403.2701).

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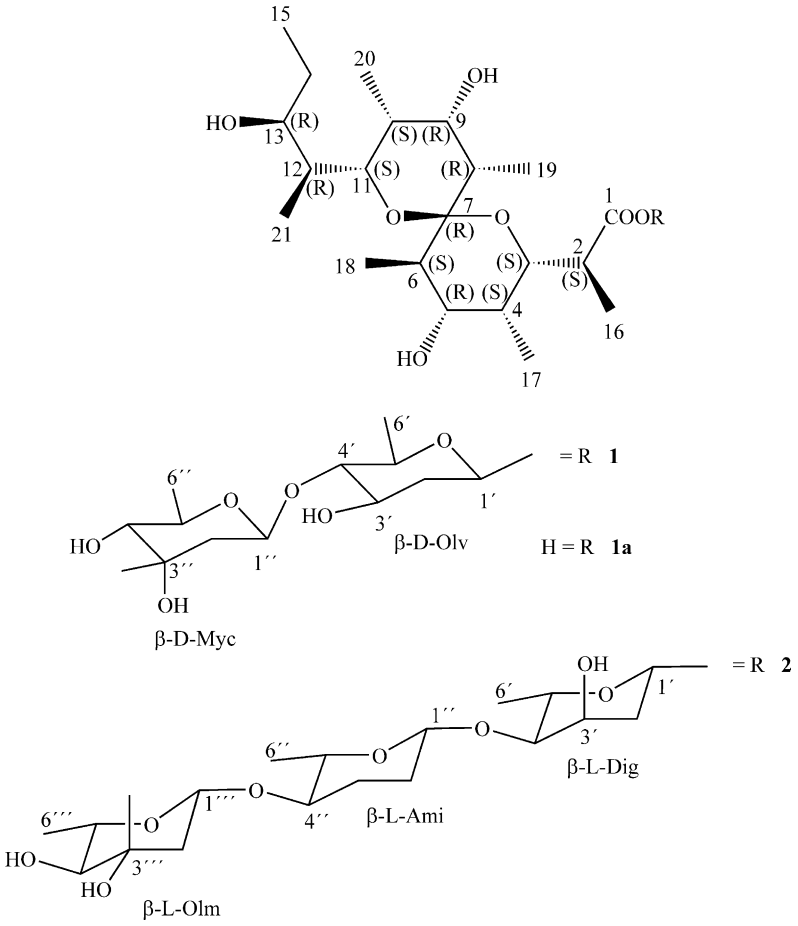


Fig. 1. Structures of **1** and **2**.

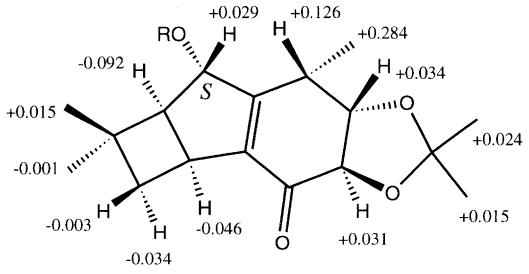
The ^{1}H and ^{13}C NMR data of the acid (**1a**) (Table 1) indicated the presence of six secondary methyls, one ethyl, and three oxymethine groups together with one carboxyl group. The planar structure of **1a** was determined by analysis of the COSY, HMBC and NOESY spectra (Figs. 2 and 3). Detailed analysis of the ^{1}H–^{1}H COSY spectrum of **1a** allowed construction of two partial structures (Fig. 2). Predominantly, the presence of an acetal group was deduced from a carbon signal at (δ 97.6). In the ^{13}C NMR spectrum the acetal carbon was shown to be connected to two ethereal oxygens at C-3 and C-11 to form a spiroacetal structure on the basis of the molecular formula. The HMBC data summarized in Fig. 2 allowed us to connect the both partial structures and the acetal group described above, establishing all of the carbon-carbon connectivities.

The vicinal spin–spin coupling constants ($J_{3,4}=3.6$ Hz, $J_{4,5}=4.5$ Hz, $J_{5,6}=10.5$ Hz) and the NOE correlations (H-2/H-6, H-6/H-17 and H-16/H-17) indicated that the tetrahydropyrane ring (C-3–C-7) had a chair conformation and that the four substituents at C-3, C-4, C-5, and C-6 of the tetrahydropyrane ring were equatorial, axial, equatorial, and again equatorial, respectively (Fig. 3). The conformation of the six-membered ring from C-7 to C-11 was deduced from the coupling

Table 1
^{1}H and ^{13}C NMR data of **1a**

	^{1}H	^{13}C
1	—	179.3 [−5.6]
2	2.56 (1H, *dq*, *J* = 6.7, 6.2) [−0.12]	42.1 [−2.8]
3	3.80 (1H, *ddd*, *J* = 6.2, 3.6, 1.2) [+0.09]	66.9 [+0.3]
4	1.75 (1H, *ddq*, *J* = 6.9, 4.5, 3.6)	31.7
5	4.07 (1H, *ddq*, *J* = 10.5, 4.5, 1.2)	74.5
6	1.65 (1H, *ddq*, *J* = 10.5, 7.0, 0.9)	37.4
7	—	97.6
8	1.58 (1H, *dddq*, *J* = 7.0, 3.8, 1.4, 0.9)	38.1
9	3.61 (1H, *ddd*, *J* = 4.5, 3.8, 1.2)	72.8
10	1.94 (1H, *ddddq*, *J* = 6.9, 4.5, 3.6, 2.1, 1.4)	33.8
11	3.86 (1H, *ddd*, *J* = 7.6, 3.6, 1.2)	69.0
12	2.04 (1H, *dddq*, *J* = 7.0, 7.6, 4.4, 2.1)	38.4
13	3.41 (1H, *ddd*, *J* = 8.8, 4.4, 3.6)	72.6
14	1.54 (1H, *ddq*, *J* = 14.6, 7.3, 3.6)	1.48
	(1H, *ddq*, *J* = 14.6, 8.8, 7.3)	29.1
15	0.87 (3H, *t*, *J* = 7.3)	10.1
16	1.19 (3H, *d*, *J* = 6.7) [−0.13]	10.3 [+1.3]
17	0.96 (3H, *d*, *J* = 6.9)	11.4
18	1.06 (3H, *d*, *J* = 7.0)	11.9
19	1.07 (3H, *d*, *J* = 7.0)	12.4
20	0.93 (3H, *d*, *J* = 6.9)	8.3
21	0.87 (3H, *d*, *J* = 7.0)	9.1

Values affected by ester's shifts are indicated in the square brackets.



3c : R= (*S*)-MTPA
3d : R= (*R*)-MTPA

Fig. 2. COSY, HMBC and NOE correlations of **1**.

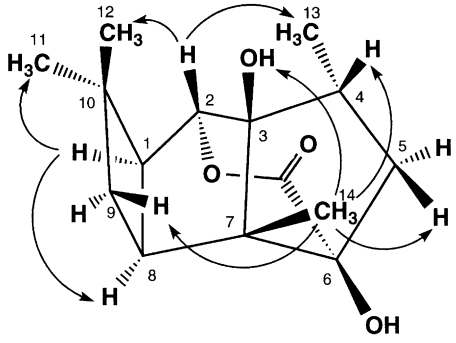


Fig. 3. NOE correlations of **1**.

constants between H-11 and H-12 ($J=7.6$ Hz), H-10 and H-11 ($J=3.6$ Hz), H-9 and H-10 ($J=4.5$ Hz), and H-8 and H-9 ($J=3.8$ Hz). The $^4J_{\text{H-9,H-11}}=1.2$ Hz was observed and furthermore, NOE correlation between H-8/H-10, established the stereochemistry at C-8, C-9, C-10 and C-11, respectively (Bextermöller et al., 1998). The stereochemistry at the spiroacetal C-7 was determined by NOE correlations between H-3/H-8, H-6/H-12, where both ketal oxygens are in axial positions. Thus, the relative stereochemistry (2*S**, 3*S**, 4*S**, 5*R**, 6*S**, 7*R**, 8*R**, 9*R**, 10*S**, 11*S**, 12*R**, 13*R**) of **1a** was established as depicted in Fig. 1.

The reaction MTPA-chloride (α-methoxy-α-trifluoromethylphenylacetyl chloride) with the acid (**1a**) having secondary hydroxyl groups on C-5, C-9 and C-13 proceed only slowly and the yield of required ester was very poor, therefore we used the method based on reaction of free acid with (*S*)- and (*R*)-PGME (2-phenylglycine methyl ester hydrochloride) for determination of absolute stereochemistry on C-2 (Nagai and Kusumi, 1995). The stereochemistry of the side chain was resolved by ^{1}H NMR analysis of (*S*)-PGME ester (**1b**) and (*R*)-PGME ester (**1c**) of **1a** (Table 2). The signal of methyl on C-2 (H-16) of **1b** appeared more downfield than that of **1c**, and the H-3, H-4 and H-17 protons of **1c** were shielded by the phenyl group and shifted more upfield than that of **1b**. These observations indicated that the configuration at C-2 was *S*. On basis of the all above mentioned information, the structure of enteridic acid (**1a**) was found to have the 2*S*, 3*S*, 4*S*, 5*R*, 6*S*, 7*R*, 8*R*, 9*R*, 10*S*, 11*S*, 12*R*, and 13*R* configurations.

The ^{1}H and ^{13}C NMR data of the saccharide units as well as decoupling experiments of **1** were used to assign the signals of the monosaccharide moieties (see Table 3). As to the first monosaccharide, strong NOESY were observed between H-l′/H-3′, H-l′/H-5′, and H-3′/H-5′, and strong *J* couplings appeared 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 (2,6-dideoxy-β-*arabino*-hexopyranose) (Roush and Brown, 1983). In the second sugar, the configuration of three ring substituents was established through COSY and selective 1D proton decoupling experiments. A large ^{1}H–^{1}H coupling ($J=9.5$ Hz) between H-4″ and H-5″ indicated a diaxial relationship; the hydroxyl and methyl groups are thus equatorial. The large and small ^{1}H–^{1}H coupling constants ($J=9.1$, 2.3 Hz) between the anomeric proton (H-1″) and the adjacent methylene protons (H-2″) indicated the β-anomeric configuration. The observed NMR data matched well with published data (Korte et al., 1964; Wohlert et al. 1999) for β-mycarose (2,6-dideoxy-3-C-methyl-β-*ribo*-hexopyranose). Hydrolysis of **1** with 5% HC1 in MeOH-H_2O afforded two saccharides. By comparison with the literature data, the first carbohydrate was identified as 2,6-dideoxy-D-*arabino*-hexopyranose ($[\alpha]_D^{23}$ +20°) (Miyamoto et al., 1964), whereas the second carbohydrate, as 2,6-dideoxy-3-C-methyl-D-*ribo*-hexopyranose ($[\alpha]_D^{23}$ +31°) (Regna et al., 1953; Flaherty et al., 1966).

In **1** the ester's shift at C-1 (cca-5.6 ppm) and the chemical shifts of H-1′ (δ 6.07) and C-1′ (δ 99.8) of olivose indicated that this monosaccharide was bonded to C-1 of **1a**. The signal due to the anomeric proton (H-1″) of mycarose (δ 5.01), correlating to the C-1″ resonance at δ 96.4 by the HETCOR spectrum indicated that the mycarose unit was linked to a secondary alcoholic carbon (C-4′ of the olivose) and was determined to be terminal by the absence of any glycosylation shift at H-3″, 4″ and/or C-3″, 4″. The structure of **1** was enteridic acid 1-*O*-β-D-mycarosyl-(1→4)-β- D-olivosyl ester.

Positive HRFABMS of **2** (enteridinine B) also gives a pseudomolecular ion at *m*/*z* 791.4800 $[M+H]^+$, corresponding to the formula $C_{40}H_{70}O_{15}$ and showed the negative FABMS with an $[M-H]^-$ ion at *m*/*z* 789 and with the prominent fragments at *m*/*z* 645 $[M-H-144]^-$ (cleavage of a dideoxymethylhexosyl), 531 $[M-H-144-114]^-$ (cleavage of dideoxymethylhexosyl and a trideoxyhexosyl), and 401 $[M-H-144-114-130]^-$ (cleavage of a dideoxymethylhexosyl, a trideoxyhexosyl and a dideoxyhexosyl).

The ^{1}H NMR spectrum of **2** was similar to those of **1**, except that there were different signals at δ 1.5–5.0, suggesting the presence of three different monosaccharide moieties in the molecule. The ^{13}C NMR spectrum of **2** was also similar to those of **1**. Comparison

Table 2
^{1}H NMR data of (*S*)-PGME amide (**1b**) and (*R*)-PGME amide (**1c**)

No.	**1b**	**1c**	Δδ
2	2.76 (1H, *dq*, *J* = 7.1, 6.1)	2.76 (1H, *dq*, *J* = 7.1, 6.1)	
3	3.44 (1H, *dd*, *J* = 6.1, 3.6)	3.38 (1H, *dd*, *J* = 6.1, 3.6)	[+0.06]
4	1.86 (1H, *ddq*, *J* = 6.9, 4.5, 3.6)	1.83 (1H, *ddq*, *J* = 6.9, 4.5, 3.6)	[+0.03]
5	4.21 (1H, *dd*, *J* = 10.5, 4.5)	4.21 (1H, *dd*, *J* = 10.5, 4.5)	
6	1.65 (1H, *dq*, *J* = 10.5, 7.0)	1.65 (1H, *dq*, *J* = 10.5, 7.0)	
8	1.58 (1H, *dq*, *J* = 7.0, 3.8)	1.58 (1H, *dq*, *J* = 7.0, 3.8)	
9	3.61 (1H, *dd*, *J* = 4.5, 3.8)	3.61 (1H, *dd*, *J* = 4.5, 3.8)	
10	1.94 (1H, *ddq*, *J* = 6.9, 4.5, 3.6)	1.94 (1H, *ddq*, *J* = 6.9, 4.5, 3.6)	
11	3.86 (1H, *dd*, *J* = 7.6, 3.6)	3.86 (1H, *dd*, *J* = 7.6, 3.6)	
12	2.04 (1H, *ddq*, *J* = 7.0, 7.6, 4.4)	2.04 (1H, *ddq*, *J* = 7.0, 7.6, 4.4)	
13	3.41 (1H, *ddd*, *J* = 8.8, 4.4, 3.6)	3.41 (1H, *ddd*, *J* = 8.8, 4.4, 3.6)	
14	1.54 (1H, *ddq*, *J* = 14.6, 7.3, 3.6)	1.54 (1H, *ddq*, *J* = 14.6, 7.3, 3.6)	
	1.48 (1H, *ddq*, *J* = 14.6, 8.8, 7.3)	1.48 (1H, *ddq*, *J* = 14.6, 8.8, 7.3)	
15	0.87 (3H, *t*, *J* = 7.3)	0.87 (3H, *t*, *J* = 7.3)	
16	1.25 (3H, *d*, *J* = 7.1)	1.34 (3H, *d*, *J* = 7.1)	[−0.09]
17	1.09 (3H, *d*, *J* = 6.9)	0.98 (3H, *d*, *J* = 6.9)	[+0.11]
18	1.06 (3H, *d*, *J* = 7.0)	1.06 (3H, *d*, *J* = 7.0)	
19	1.07 (3H, *d*, *J* = 7.0)	1.07 (3H, *d*, *J* = 7.0)	
20	0.93 (3H, *d*, *J* = 6.9)	0.93 (3H, *d*, *J* = 6.9)	
21	0.87 (3H, *d*, *J* = 7.0)	0.87 (3H, *d*, *J* = 7.0)	
C*H*N	4.71 (1H, *m*)	4.71 (1H, *m*)	
o-C*H*	7.36 (2H, *m*)	7.36 (2H, *m*)	
m-C*H*	7.34 (2H, *m*)	7.34 (2H, *m*)	
p-C*H*	7.27 (1H, *m*)	7.27 (1H, *m*)	
OCH_3	3.72 (3H, *s*)	3.72 (3H, *s*)	

Values affected by amide's shifts [$\Delta\delta = (\delta_S - \delta_R)$ values (ppm)] are indicated in the square brackets.

Table 3
^{1}H and ^{13}C NMR data for the sugar residues of **1** and B **2**

	^{1}H	^{13}C		^{1}H	^{13}C
	1			**2**	
1′	6.07 (1H, *dd*, *J* = 9.5, 2.0)	99.8	1′	5.98 (1H, *dd*, *J* = 9.6, 2.1)	98.6
$2_a'$	1.73 (1H, *ddd*, *J* = 12.1, 11.8, 9.5)	36.4	$2'_a$	1.66 (1H, *ddd*, *J* = 11,4, 9.6, 2.7)	38.9
$2_e'$	2.44 (1H, *ddd*, *J* = 12.1, 5.4, 2.0)		$2'_e$	1.98 (1H, *ddd*, *J* = 11.4, 3.5, 2.1)	
3′	3.87 (1H, *ddd*, *J* = 11.8, 9.7, 5.4)	74.6	3′	3.82 (1H, *ddd*, *J* = 3.5, 2.8, 2.7)	65.3
4′	4.56 (1H, *dd*, *J* = 9.7, 9.2)	73.8	4′	3.46 (1H, *dd*, *J* = 9.6, 2.8)	76.5
5′	3.39 (1H, *dq*, *J* = 9.2, 6.6)	70.7	5′	3.79 (1H, *dq*, *J* = 9.6, 6.2)	69.5
6″	1.23 (3H, *d*, *J* = 6.6)	16.5	6′	1.30 (3H, *d*, *J* = 6.2)	76.5
1″	5.01 (1H, *dd*, *J* = 9.1, 2.3)	96.4	1″	4.54 (1H, *d*, *J* = 8.0)	98.0
$2_a''$	1.64 (1H, *dd*, *J* = 13.5, 9.1)	41.8	$2''_a$	2.03 (1H, *m*)	32.1
$2_e''$	1.76 (1H, *dd*, *J* = 13.5, 2.3)		$2''_e$	1.75 (1H, *m*)	
3″	—	69.7	$3''_a$	1.92 (1H, *m*)	29.8
			$3''_e$	1.73 (1H, *m*)	
3″-CH_3	1.22 (3H, *s*)	26.8	4″	3.28 (1H, *ddd*, *J* = 9.9, 9.5, 5.1)	71.6
4″	4.63 (1H, *d*, *J* = 9.5)	76.9	5″	3.51 (1H, *dq*, *J* = 9.9, 6.4)	78.2
5″	3.87 (1H, *dq*, *J* = 9.5, 6.7)	66.8	6″	1.29 (3H, *d*, *J* = 6.4)	19.0
6″	1.24 (3H, *d*, *J* = 6.7)	16.4	1‴	4.77 (1H, *dd*, *J* = 9.5, 2.2)	98.5
			$2'''_a$	1.67 (1H, *dd*, *J* = 12.0, 9.5)	43.6
			$2'''_e$	2.06 (1H, *dd*, *J* = 12.0, 2.2)	
			3‴	–	71.5
			4‴	3.13 (1H, *d*, *J* = 9.3)	77.3
			5‴	3.28 (1H, *dd*, *J* = 9.3, 6.3)	68.4
			6‴	1.28 (3H, *d*, *J* = 6.3)	18.0
			C-3‴-Me	1.22 (3H, *s*)	22.1

of the ^{1}H and ^{13}C NMR data of **2** with those of **1**, revealed that **2** was a different ester.

The presence of the monosaccharides was evident by the signals at 4.5–6.0 ppm in the ^{1}H NMR and by the signal at ~98 ppm in the ^{13}C NMR spectrum, corresponding to the anomeric protons and carbons, respectively. For a dideoxyhexose, strong *J* coupling between H-1′/H-2_a′ and H-4′/H-5′ and weak coupling between H-1′/H-2_e′, and H-2_e′/H-3′, and H-3′/H-4′ were observed, while the NOE patterns were strong only between H-2′ and H-4′. The coupling constant of the anomeric proton of sugar was $J_{H\text{-}1'\text{–}H\text{-}2a'} = 9.6$ Hz and $J_{H\text{-}1'\text{–}H\text{-}2e'} = 2.1$ Hz. Thus the glycoside linkage of the sugar was determined to be β. These observations indicated that this monosaccharide is β-digitoxose (2,6-dideoxy-β-*ribo*-hexopyranose) (Roush and Brown, 1983). The coupling constants ($J = 9.9$ Hz) between H-4″ and H-5″ of the trideoxyhexose indicate diaxial orientations. The anomeric proton H-1″ appears as doublet ($J = 8.0$ Hz). On the basis of data from 2D NMR, i.e. COSY and NOE, the monosaccharide was determined to be β-amicetose (2,3,6-trideoxy-β-*erythro*-hexopyranose) (Igarashi et al., 2002). The anomeric proton in the third sugar was founded at δ 4.77 as double of doublets ($J = 9.5$, 2.2 Hz) which proved a methylene group at the adjacent carbon. These values indicated that anomeric proton is axial (i.e. β). The large $J_{H\text{-}4''',H\text{-}5'''} = 9.3$ Hz indicated that both hydrogens are axial and C-4‴-OH and C-5‴-Me are equatorial. The spectrum was very similar to mycarose, see above, but signals of H-1‴ and H-5‴ protons were observed at the higher field because of missing the anisotropy effect of C-3‴-OH. From the all above mentioned data unwound that the third sugar is β-olivomycose (2,6-dideoxy-3-C-methyl-β-*arabino*-hexopyranose) (Miyamoto et al., 1966). The monosaccharides from the hydrolysate of **2** were purified by NH_2-HPLC. After evaporation of eluent, the three saccharides were obtained as colorless syrups. The first peak (amicetose) showed the optical rotation of −38.5° (Catelani et al., 1989). Also the ^{1}H NMR spectrum was in good accordance with published data (Berti et al., 1983). Further, the optical rotation of the second peak (digitoxose) in water ($[\alpha]_D^{21}$ −37°) was in good agreement with literature data (Berlin et al., 1964; Tomita et al., 1980). The optical rotation of olivomycose was −21.5°, which was practically identical with the reported value for L-olivomycose ($[\alpha]_D$ −22°) (Berlin et al., 1964; Toshima et al., 1991). These results indicate that all monosaccharides of **2** are in L-forms.

Interglycosidic linkages were established by HMBC techniques. The HMBC spectrum of **2** showed cross peaks between the signals at 5.98 (digitoxose H-1′) and 179.3 (C-1 of the non-saccharidic part), 4.54 (amicetose H-1″) and 76.5 (digitoxose C-4′), and 4.77 (terminal olivomycose H-1‴) and 71.6 (amicetose C-4″). Thus, the structure of **2** was determined to be enteridic acid 1-O-β-L-olivomycosyl-(1→4)-β-L-amicetosyl-(1→4)-β-L-digitoxosyl ester.

The antimicrobial activities of **1** and **2** are summarized in Table 4. Compound **1** inhibited predominantly growth of Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. It also showed a modest growth inhibition of Gram-negative bacteria and some yeasts. The compound **2** showed 3-fold weaker activity than **1** against the bacterial strains tested. In contrast, it exhibited somewhat higher antifungal activity than **1**. These results indicate that **1** has a higher permeability into the cells and interacts more strongly with bacteria than with fungi.

Spiroacetal compounds are widespread as substructures of naturally occurring substances from many sources, including microorganisms, plants, fungi, and marine organisms (Perron and Albizati, 1989). The increasing pharmacological importance of compounds containing spiroacetal assemblies has triggered intense interest in their chemical synthesis (Kluge, 1986; Boivin, 1987) and they displays in vitro high activity against different tumors (Bai et al., 1991; Hamel, 1992; Luduena et al., 1993; Pettit, 1994). Polyether ionophores are also naturally occurring spiroacetals, which belonging to polyketide-derived polyether antibiotics produced by filamentous branching bacteria and microorganisms (Sun et al., 2003). Biosynthesis some natural compounds with spiroacetal units have recently been reported (Sun et al., 2003). Our finding showed that the slime mold produced similar structures with one spiroacetal unit.

Proposed biosynthesis of the compound **1a** is shown in Fig. 4. Garson et al. (1994) used [1-^{14}C]propionate for study of siphonarins biosynthesis by injection of [1-^{14}C]propionate into a tissue of pulmonate limpet *Siphonaria zelandica*. We concluded that the mechanism of biosynthesis **1a** could be the same as that of siphonarins.

It is possible that marine invertebrates use a C3-based *de novo* pathway rather than a C2 plus C1 route for synthesis of the compounds which contain this spiropyrone unit. Recently, the biosynthesis of a two toxins, DTX-5a and DTX-5b, produced by dinoflagellate *Prorocentrum maculosum* was studied, which also contain

Table 4
Antibacterial and antifungal activity of **1** and **2**

Test organism[a]	**1**	**2**
Staphylococcus aureus	28	10
Bacillus subtilis	34	11
Escherichia coli	6	5
Saccharomyces cerevisiae	9	41
Candida albicans	3	12

[a] Samples (10 μg) were applied on 6.35 mm paper disks, and values are diameters (mm) of inhibitory zones.

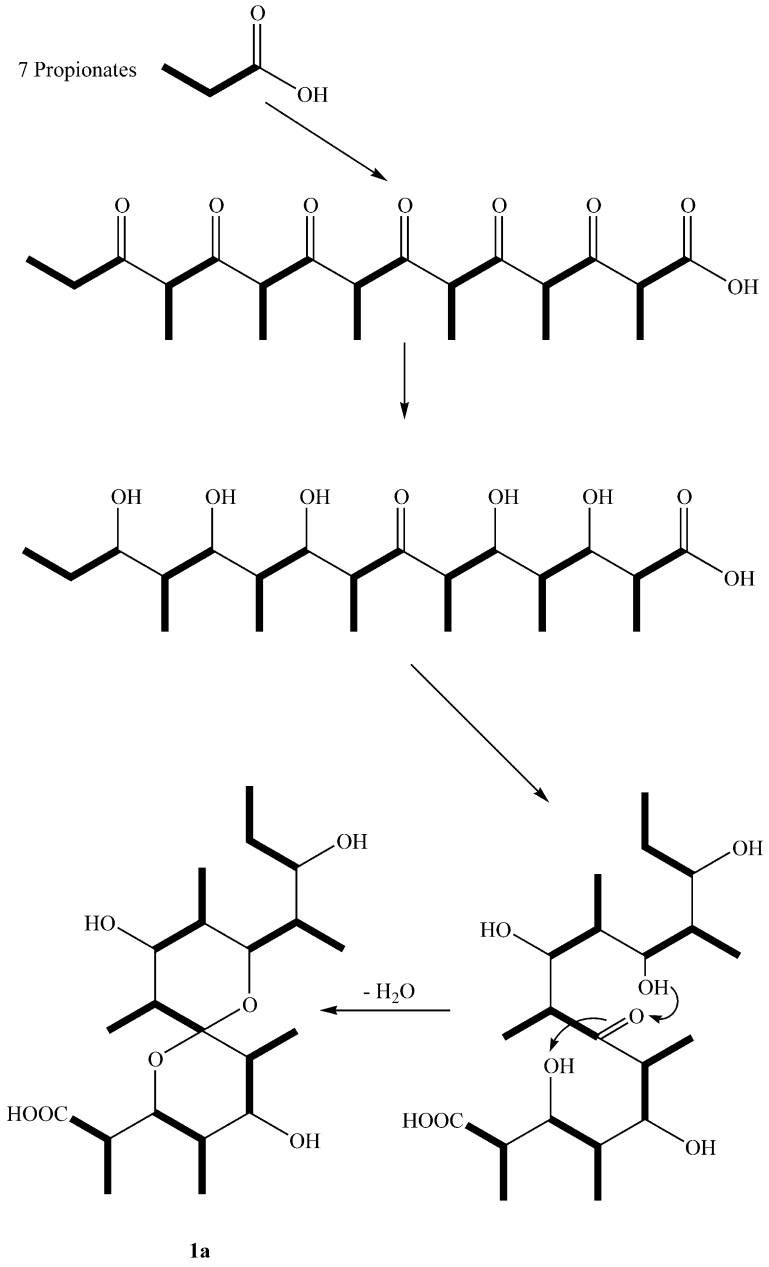


Fig. 4. Proposed biosynthetic pathways **1a**.

the same spiro-pyrone unit. MacPherson et al. (2003) showed that this fragment can be synthesized from acetate.

3. Experimental

3.1. General experimental procedures

UV spectra were measured in heptane within the range of 200-350 nm by a Cary 118 (Varian) apparatus. A Perkin-Elmer Model 1310 (Perkin-Elmer, Norwalk, CT, USA) IR spectrophotometer was used for scanning IR spectroscopy of glycosides as neat films. NMR spectra were recorded on a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^{1}H), 125.7 MHz (^{13}C) in the mixture of deuterated pyridine and CD_3OD (v/v 1:1). High- and also low-resolution MS were recorded using a VG 7070E- HF spectrometer (70 eV). HRFABMS (positive and/or negative ion mode) were obtained with a PEG-400 matrix. HPLC was carried out using Shimadzu gradient LC system (Shimadzu, Kyoto, Japan).

3.2. Plant material

The slime mold was collected near the Travni Dvur, 3.5 km southeastern of Hrusovany on Jevisovka (South Moravia), Czech Republic, on decayed stump-wood of

Salix alba. It was identified by the second author (R.D.) by its physical properties.

3.3. Extraction and isolation

Sample of slime-mold (30.5 g dry weight) was extracted by 90% *n*-BuOH. Chromatography of the extract on a Sephadex LH-20 column (100×5 cm) with elution of MeOH gave organic fractions (8 ml) checked by two-dimensional TLC [silicagel plates, *n*-BuOH–AcOH–H_2O (12:3:5) and $CHCl_3$–MeOH–H_2O (40:9:1)]. Fraction D 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 **1** (17.1 mg) and **2** (14.6 mg).

3.4. Acid hydrolysis

The ~12 mg of ester (**1**) was refluxed in 2 M HCOOH (0.5 ml) for 2 h. The hydrolysate was extracted three times with EtOAc (10 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 to yield 1.8 mg of D-mycarose $[\alpha]_D^{22}+31°$(equilib.) and 2.1 mg of D-olivose $[\alpha]_D^{21}+20°$ (equilib.). Analogically the glycoside **2** was work-up and water fraction give 1.6 mg of L-digitoxose $[\alpha]_D^{23}-37°$(equilib.), 1.1 mg of D-olivomycose $[\alpha]_D^{23}-21.5°$(equilib.) and 1.2 mg of L-amicetose $[\alpha]_D^{23}-38.5°$(equilib.).

3.5. Alkalic hydrolysis

A solution of **2** (14.2 mg) in 1 M NH_4OH in a sealed bottle was kept for 1 day at 60 °C. After acidifying to pH 7 with acetic acid, the mixture was extracted by CH_2Cl_2, the solution was then evaporated to dryness, and the residue was chromatographed on silica gel column (10 g), using CH_2Cl_2–MeOH–H_2O (90:10:1) to afford **1a** (8.1 mg).

3.6. (*S*)- and (*R*)-PGME amides of *1a*

To a stirred solution of **1a** (4 mg) and (*S*)-PGME (4 mg) (or (*R*)-PGME) in dry 1 ml DMF were successively added benzotriazole-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (8 mg), 1-hydroxybenzotriazole hydrate (5 mg), and *N*-methylmorpholine (30 μl) at 0 °C. After the mixture was stirred at room temperature for 3 h, ethyl acetate was added, and the resulting solution was successively washed with 5% HCl, saturated $NaHCO_3$ solution, and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to give a residue which was chromatographed on silica gel with hexane–ethyl acetate (3:1, v/v) as developing solvent to afford the amides **1b** (yield 2.9mg) and/or **1c** (yield 3.2 mg) as yellow oils (Yabuuchi and Kusumi, 2000). HRFABMS of **1b** (*m/z*) 550.3389 $[M+H]^+$, calcd for $[C_{30}H_{47}NO_8+H]^+$ 550.3380; HRFABMS of **1c** (*m/z*) 550.3386 $[M+H]^+$, calcd for $[C_{30}H_{47}NO_8+H]^+$ 550.3380; NMR data of both compounds, see Table 3.

Enteridinine A (**1**): white amorphous powder; $[\alpha]_D^{23}$ −62° (c 0.15, MeOH); UV (MeOH) λ_{max} 205 nm (log ϵ 2.41); IR (film) ν_{max} 3600 (OH), 2900, 1650 (COOH), C-O-C (1170 and 1040) cm^{-1}; HRFABMS (*m/z*) 633.4216 $[M+H]^+$, calcd for $[C_{33}H_{60}O_{11}+H]^+$ 633.4211; negative FABMS *m/z* 631 $[M-H]^-$, *m/z* 501 $[M-H-130]^-$ and *m/z* 357 $[M-H-130-144]^-$; NMR data see Tables 1 and 2.

Enteridinine B (***2***): white amorphous powder; $[\alpha]_D^{23}+102°$ (c 0.11, MeOH); UV (MeOH) λ_{max} 205 nm (log ϵ 2.40); IR (film) ν_{max} 3600 (OH), 2900, 1650 (COOH), C-O-C (1170 and 1040) cm^{-1}; HRFABMS (*m/z*) 791.4800 $[M+H]^+$, calcd for $[C_{40}H_{70}O_{15}+H]^+$ 791.4792; negative FABMS *m/z* 789 $[M-H]^-$, *m/z* 645 $[M-H-144]^-$, *m/z* 531 $[M-H-144-114]^-$ and 401 $[M-H-144-114-130]^-$; NMR data see Tables 1 and 2.

Enteridic acid (***1a***): white crystals, mp 131–133 °C; $[\alpha]_D^{23}+38°$ (c 0.08, CH_2Cl_2); UV (MeOH) λ_{max} 205 nm (log ϵ 2.25); IR (film) ν_{max} 3580 (OH), 2910, 1645 (COOH), C-O-C (1175 and 1040) cm^{-1}; HRFABMS (*m/z*) 403.2701 $[M+H]^+$, calcd for $[C_{21}H_{39}O_7+H]^+$ 403.2696; NMR data see Table 1.

References

Alexopoulos, C.J., Mims, C.W., 1979. Introductory Mycology, third ed. John Wiley & Sons, New York.

Bai, R., Paull, K.D., Herald, C.L., Malspeis, L., Pettit, G.R., Hamel, E., 1991. Halichondrin-B and homohalichondrin-B, marine natural-products binding in the vinca domain of tubulin-discovery of tubulin-based mechanism of action by analysis of differential cytotoxicity data. J. Biol. Chem. 266, 15882–15889.

Berlin, Yu.A., Esipov, S.E., Kolosov, M.N., Shemyakin, M.M., Brazhnikova, M.G., 1964. Olivomycin I., Methanolysis. Tetrahedron Lett. 5, 1323–1328.

Berti, G., Caroti, P., Catelani, G., Monti, L., 1983. Synthesis of D-amicetose and L-rhodinose from L-glutamic acid. Carbohydrate Research 124, 35–42.

Bextermöller, R., Redlich, H., Schnieders, K., Thormahlen, S., Fröhlich, R., 1998. Perhydroxylated 1,7-dioxaspiro[5.5]undecanes (“spiro sugars”): synthesis, stereochemistry, and structure. Angew. Chem., Int. Ed. 37, 2496–2500.

Blumenthal, F., Polborn, K., Steffan, B., 2002. The absolute configuration of polycephalin C from the slime mold *Physarum polycephalum* (Myxomycetes). Tetrahedron 58, 8433–8437.

Boivin, T.L., 1987. Synthetic routes to tetrahydrofuran, tetrahydropyran, and spiroketal units of polyether antibiotics and a survey of spiroketals of other natural products. Tetrahedron 43, 3309–3312.

Bonner, J.T., 1985. The Cellular Slime Molds, second ed. Princeton University Press, Princeton, NJ, USA.

Catelani, G., Colonna, F., Rollin, P., 1989. A new method for resolving racemic 2-deoxyhexoses- total synthesis of D-2,3,6-trideoxy-erythro-hexose (amicetose) and L-2,3,6-trideoxy-erythro-hexose (amicetose). Gazzetta Chimica Italiana 119, 389–393.

Eisenbarth, S., Steffan, B., 2000. Structure and biosynthesis of chrysophysarin A, a plasmodial pigment from the slime mould *Physarum polycephalum* (Myxomycetes). Tetrahedron 56, 363–365.

Flaherty, B., Overend, W.G., Williams, N.R., 1966. Branched-chain sugars. Part VII. The synthesis of D-mycarose D-cladinose. J. Chem. Soc. (C), 398–403.

Garson, M.J., Jones, D.J., Small, C.J., Liang, J., Clardy, J., 1994. Biosynthetic studies on polypropionates: a stereochemical model for siphonarins A and B from the pulmonate limpet *Siphonaria zelandica*. Tetrahedron Lett. 35, 6921–6924.

Hamel, E.H., 1992. Natural-products which interact with tubulin in the vinca domain—maytansine, rhizoxin, phomopsin-A, dolastatin-10 and dolastatin-15 and halichondrin-B. Pharm. Ther. 55, 31–51.

Igarashi, Y., Kajiura, T., Furumai, T., Hori, H., Higashi, K., Ishiyama, T., Uramoto, M., Uehara, Y., Oki, T., 2002. Biosynthesis of hibarimicins—III. Structures of new hibarimicin-related metabolites produced by blocked mutants. J. Antibiot. 55, 61–70.

Kluge, A.F., 1986. Synthesis of 1,7-dioxaspiro[5.5]undecanes. Heterocycles 24, 1699–1708.

Korte, F., Claussen, U., Snatzke, G., 1964. Zur Stereochemie der Mycarose und ihrer 3- und 4-Epimeren. Tetrahedron 20, 1477–1487.

Luduena, R.F., Roach, M.C., Prasad, V., Pettit, G.R., 1993. Interaction of halichondrin-B and homohalichondrin-B with bovine brain tubulin. Biochem. Pharmacol. 45, 421–427.

MacPherson, G.R., Burton, I.W., LeBlanc, P., Walter, J.A., Wright, J.L.C., 2003. Studies of the biosynthesis of DTX-5a and DTX-5b by the dinoflagellate *Prorocentrum maculosum*: regiospecificity of the putative Baeyer-Villigerase and insertion of a single amino acid in a polyketide chain. J. Org. Chem. 68, 1659–1664.

Miyamoto, M., Kawamatsu, Y., Shinohara, M., Nakadaira, Y., Nakanishi, K., 1966. Structures and properties of the sugars obtained from the chromomycins. Tetrahedron 22, 2785–2799.

Miyamoto, M., Kawamatsu, Y., Shinohara, M., Nakanishi, K., Nakadaira, Y., Bhacca, N.S., 1964. The four chromoses from chromomycin A3. Tetrahedron Lett. 5, 2371–2377.

Murakami-Murofushi, K., Uchiyama, A., Fujiwara, Y., Kobayashi, T., Kobayashi, S., Mukai, M., Murofushi, H., Tigyi, G., 2002. Biological functions of a novel lipid mediator, cyclic phosphatidic acid. Biochim. Biophys. Acta—Mol. Cell. Biol. Lipids 1582, 1–7.

Nagai, Y., Kusumi, T., 1995. New chiral anisotropic reagents for determining the absolute-configuration of carboxylic-acids. Tetrahedron Lett. 36, 1853–1856.

Perron, F., Albizati, K.F., 1989. Chemistry of spiroketals. Chem. Rev. 89, 1617–1661.

Pettit, G.R., 1994. Marine animal and terrestrial plant anticancer constituents. Pure Appl. Chem. 66, 2271–2281.

Regna, P.P., Hochstein, F.A., Wagner, R.L., Woodward, R.B., 1953. Magnamycin II. Mycarose, an unusual branched-chain desoxysugar from magnamycin. J. Am. Chem. Soc. 75, 4625–4626.

Rezanka, T., Dvorakova, R., 2003. Polypropionate lactones of deoxysugars glycosides from slime mold *Lycogala epidendrum*. Phytochemistry 63, 945–952.

Roush, W.R., Brown, R.J., 1983. Total synthesis of carbohydrates. 3. Efficient enantioselective syntheses of 2,6-dideoxyhexoses. J. Org. Chem. 48, 5093–5101.

Stephenson, S.L., Stempen, H., 1994. Myxomycetes: A Handbook of Slime Molds. Timber Press, Portland, Oregon.

Sun, Y.H., Zhou, X.F., Dong, H., Tu, G.Q., Wang, M., Wang, B.F., Deng, Z.X., 2003. Complete gene cluster from *Streptomyces nanchangensis* NS3226 encoding biosynthesis of the polyether ionophore nanchangmycin. Chemistry Biology 10, 431–441.

Takaya, Y., Kikuchi, H., Terui, Y., Komiya, J., Maeda, Y., Ito, A., Oshima, Y., 2001. Novel aromatic substances, dictyomedin A and B, from *Dictyostelium* cellular slime molds and their inhibitory effects on *Dictyostelium* development. Tetrahedron Lett. 42, 61–63.

Tomita, F., Tamaoki, T., Shirahata, K., Kasai, M., Morimoto, M., Ohkubo, S., Mineura, K., Ishii, S., 1980. Novel anti-tumor antibiotics tetrocarcins. J. Antibiot. 33, 668–670.

Toshima, K., Yoshida, T., Mukaiyama, S., Tatsuta, K., 1991. De novo highly stereocontrolled synthesis of 2,6-dideoxy sugars by use of 2,6-anhydro-2-thio sugars. Carbohydrate Res. 222, 173–188.

Wohlert, S.E., Kunzel, E., Machinek, R., Mendez, C., Salas, J.A., Rohr, J., 1999. The structure of mithramycin reinvestigated. J. Nat. Prod. 62, 119–121.

Yabuuchi, T., Kusumi, T., 2000. Phenylglycine methyl ester, a useful tool for absolute configuration determination of various chiral carboxylic acids. J. Org. Chem. 65, 397–404.