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Polypropionate lactones of deoxysugars glycosides from slime mold *Lycogala epidendrum*

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Abstract

Two novel polypropionate lactone glycosides (**1** and **2**, i.e. lycogalinosides A and B) were isolated from the slime mold *Lycogala epidendrum*. Their structures, including the absolute configurations of the hydroxyl and methyls groups, were determined by means of extensive spectroscopic data such as mass, IR, UV, and 1D and 2D NMR spectra and chemical degradation followed by spectroscopic and chromatographic analysis. Compounds **1** and **2** are unique in structure containing a 2-deoxy-α-L-fucopyranosyl-(1–4)-6-deoxy-β-D-gulopyranosyl unit and a β-D-olivopyranosyl-(1–4)-β-D-fucopyranosyl unit, respectively, and showed growth inhibitory activities against Gram-positive bacteria.

Keywords: Myxomycetes; Glycosides of deoxysugars; Polypropionate lactones; True slime molds; *Lycogala epidendrum*

1. Introduction

True slime molds are a phylogenetically unique group of organisms including about 1000 legitimately described subgeneric taxa (Stephenson and Stempen, 2000). The life cycle starts with haploid, unicellular myxoflagellates and myxamoebae hatching from spores. The diploid plasmodium is formed as the second trophic stage usually by undergoing somatogamy. This stage consists of multinuclear protoplasm mass, surrounded only by a simple membrane and a slime sheath, and moves by protoplasmatic streaming. During fructification, almost the entire biomass of the plasmodium turns into fruiting bodies, which in many species achieve macroscopical dimensions (Gray and Alexopoulos, 1968). Because of these unique characteristics, true slime molds are frequently used to study differentiation in various fields of biology.

Although differentiations inducing factors (Masenko et al., 1988), discadenine (Abe et al., 1976) and several other compounds (Nowak and Steffan, 1998) including pigments (Steglich, 1989) have been reported as the metabolites produced by slime molds, no antibacterial compound has been isolated. Because slime molds have not previously been considered as a source for new compounds, we investigated their use as a possible source of antibacterial compounds. Using a screening program (Řezanka, 2002), we isolated new antibacterial substances **1** and **2** from the pulvinate aethalia of *Lycogala epidendrum* (L.) Fries. The novel triacylglycerols, lycogarides A–C (Hashimoto et al., 1994a) and lycogarides D–G, were isolated from the slime mold *Lycogala epidendrum* (Buchanan et al., 1996). Three novel dimethyl pyrroledicaboxylates, lycogarubins A–C, were also obtained (Hashimoto et al., 1994b) from the same slime mold.

In this paper, we determined the structure of compound **1** and **2**, see Fig. 1. Because both **1** and **2** were highly methyl-substituted compounds, it was impossible to determine their structures using spectral data alone. We therefore deduced the structures from their degradation products, as shown in Fig. 2.

2. Results and discussion

The slime molds were collected near the village Hluboke Masuvky, 13 km north of Znojmo, Czech

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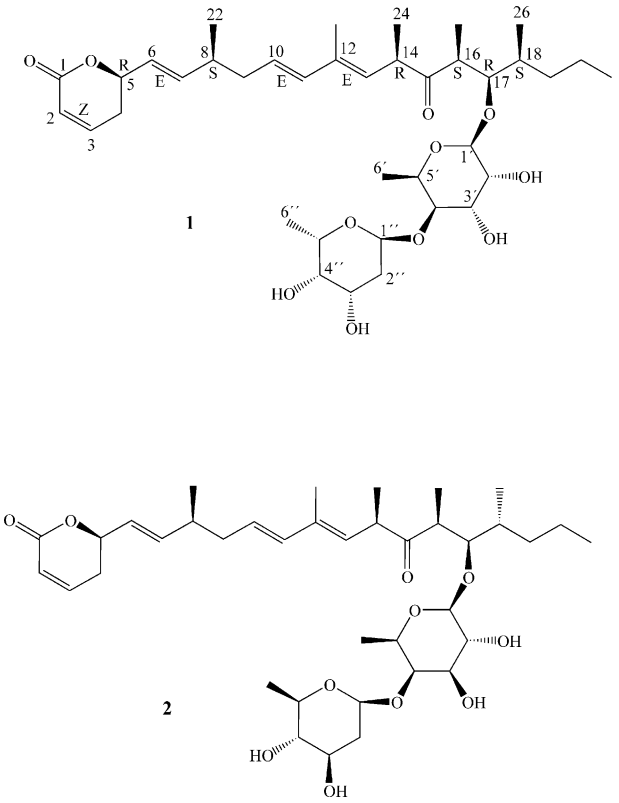

Fig. 1. The structures of hydroxy-polyenoic glycosides (**1** and **2**) from slime-mold.

Republic. They were identified by the second author (R.D.) by their physical properties.

The beige to dark-gray pulvinate aethalia of the slime mold were extracted by butanol and the extract was separated on Sephadex LH-20. The fractions were further purified by RP-HPLC to give two glycosides **1** and **2** (Fig. 1).

Compound **1** was obtained as a white amorphous powder with $[\alpha]_D^{23} = -19^\circ$ but without an exact melting point as the glycoside decomposed. The UV spectrum of **1** showed absorption maxima at 243 nm (log ε 4.72) and 300 nm (log ε 3.38) which can be ascribed to α,β-unsaturated carbonyl and diene chromophores. The IR spectrum of **1** showed absorption bands due to free hydroxyls (3400 cm^{-1}), α,β-unsaturated δ-lactone (1730 cm^{-1}) and ketone (1710 cm^{-1}) group. The molecular weight determined by positive HRFABMS was 693.4218 $[M+H]^+$, which corresponds to a molecular formula of $C_{37}H_{58}O_{11}$, and negative FABMS gave two prominent ions at m/z 561 $[M-H\text{-}130]^-$ (cleavage of a dideoxyhexosyl unit) and m/z 415 $[M-H\text{-}130\text{-}146]^-$ (cleavage of a dideoxyhexosyl and a deoxyhexosyl unit).

The ^1H and ^{13}C NMR data of the aglycone part of compound **1** indicated the presence of four secondary methyls, one propyl, one olefinic methyl, and two oxymethine groups together with eight olefinic carbons and two carbonyl carbons. The COSY spectrum of **1**

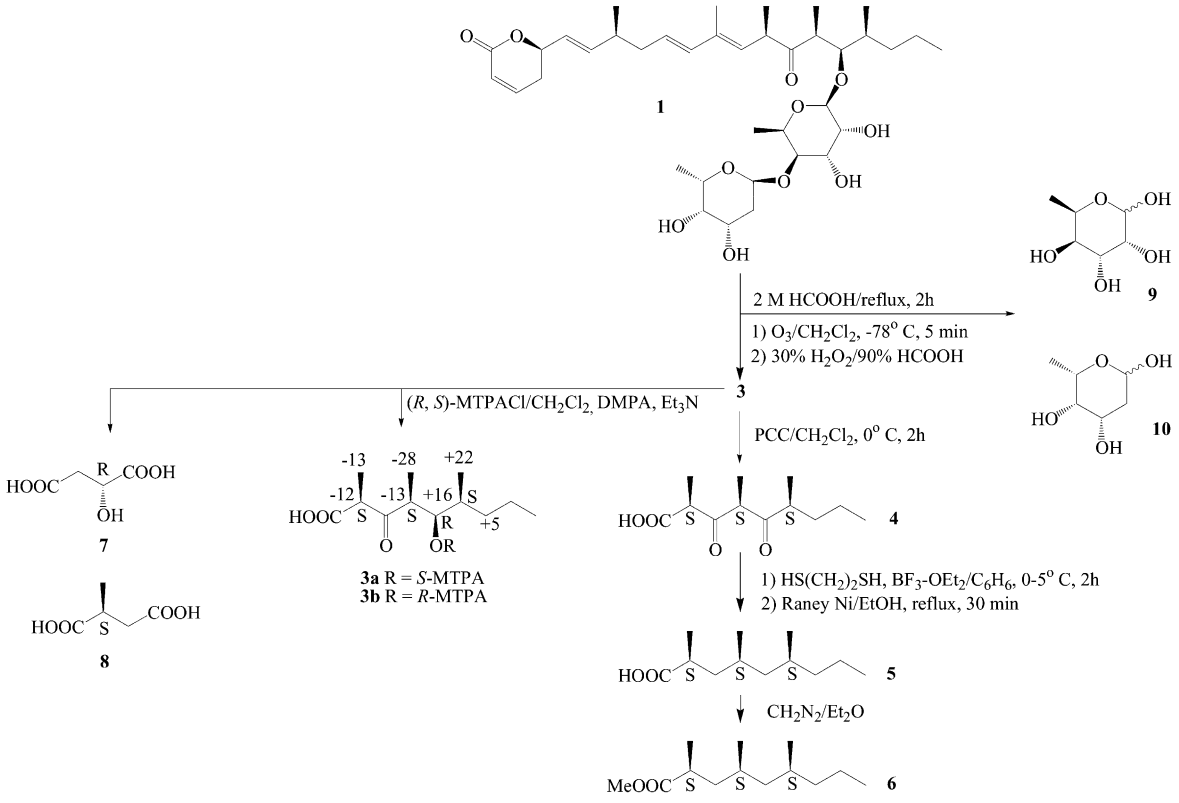

Fig. 2. Reaction schema of degradation compounds from glycoside **1**.

revealed the presence of three partial structures (fragment A: C-1–C-11, fragment B: C-13–C-14 together with a C-12 methyl group and fragment C: C-16–C-21). The connectivity between the C-23 methyl group and C-12 was clarified by allylic coupling between H-13 and H_3-23. The presence of these partial structures in **1** has also been substantiated by a HOHAHA experiment. The connectivities of these three partial structures have been figured out on the basis of the following HMBC correlations: (1) adjacency of fragments A and B: cross peaks between H-11 and C-12, 13, 23; H-9 and C-23; (2) adjacency of fragments B and C through the ketone carbonyl, i.e. cross peaks between H-14, H_3-25 and C-15. The presence of terminal δ-lactone was supported by the chemical shift of H-5 (δ 4.77 ppm). The geometries of Δ^2, Δ^6 and Δ^{10} double bonds were determined as 2*Z*, 6*E* and 10*E* by the coupling constants $J_{2,3}=9.8$ Hz, $J_{6,7}=16.1$ Hz and $J_{10,11}=15.8$ Hz, respectively. The geometry of the Δ^{12} double bond was established as 12*E* by the NOEs between H-10 and H-14. Based on the accumulated evidence, we elucidated the plane structure aglycone part of **1**.

The absolute configuration of **1** was determined in two steps. In the first step, the structures of degradation products (after hydrolysis and ozonolysis) were explored while the structures of monosaccharide components were elucidated in the second step. Acid hydrolysis of **1** with 2 M HCOOH yielded an aglycone and a sugar fraction. Ozonolysis of the aglycone gave a mixture of several degradation products.

To elucidate the absolute configuration at C-17 in compound **1**, we used modified Mosher's method (Ohtani et al., 1991a,b). Thus, **3** was treated with *R*-(+)- or *S*-(−)-MTPA, to furnish the 5-*O*-*S*-(+)-MTPA ester **3a** and 5-*O*-*R*-(−)-MTPA ester **3b**, respectively. All proton signals of both **3a** and **3b** were assigned and the absolute configuration at C-5 was determined as *R* by the analysis of Δδ values (Δδ = [δ(*S*-MTPA ester)-δ(*R*-MTPA ester)]). The data are shown in Fig. 2.

To identify the configuration of C-14, C-16, and C-18 methyls in **1**, we analyzed the NMR data by comparing them with those of related polyketides having similar partial structures (Davies-Colman and Garson, 1998). The proton and carbon chemical shifts and coupling constants (i.e. $J_{16,17}=4.4$ Hz, $J_{17,18}=6.6$ Hz) of the structure of **1** from C-14 to C-20 including 23, 24 and 25-methyls were very similar to those in previously described compounds (Uotani et al., 1982; Paterson and Hulme, 1995). These findings led us to presume that the respective relative configurations from C-14 to C-18 were 14*R**, 16*S**, 17*R**, and 18*S**.

The full confirmation of the proposed structure, see above, was obtained by hydrolysis and ozonolysis and measurement of optical activity of chiral acid (**5**), or by comparing the retention times of ozonolysis products (**7**, **8**) and commercial standards.

In order to establish the nature of this branched chain, **3** was subjected to oxidative degradation by ozonolysis (Fig. 2). First, 90% of the reaction mixture was oxidized with PCC to **4**. The dioxo acid **4** was treated with 10 equivalents of 1,2-ethanedithiol and 2.5 equivalents of BF_3-OEt_2 to give a bis(dithioketal). Next, Raney-nickel desulfurization of the bis(dithioketal) in ethanol gave 2,4,6-trimethyl nonanoic acid (**5**), which was isolated and further methylated with an ethereal solution of diazomethane to its methyl ester. The optical rotation of the methyl ester was +22.1°, which agrees with the value reported by Odham (1967) $[\alpha]_D=+21.9°$, or Beukes and Davies-Coleman (1999) $[\alpha]_D=+19°$ for the 2*S*, 4*S*, 6*S* isomer. Further, two substituted succinic acids (**7** and **8**) were isolated from the remaining 10% of the reaction mixture. After methylation by diazomethane, the reaction mixture was analyzed on a chiral capillary column. 2*R*-hydroxy succinic and 2*S*-methyl succinic acid methyl esters were identified among other non-chiral derivatives by chiral gas chromatography (Table 2). The above results indicate that the absolute stereochemistry of the aglycone part is 2*Z*, 5*R*, 6*E*, 8*S*, 10*E*, 12*E*, 14*R*, 16*S*, 17*R* and 18*S*.

The ^1H NMR and ^{13}C NMR data of the monosaccharides of **1** are also shown in Table 1. ^1H and ^{13}C NMR data as well as decoupling experiments were used to assign the signals of the monosaccharide moieties of **1**.

The small vicinal coupling constants, $^3J_{3'-4'}=3.4$ Hz and $^3J_{4'-5'}=1.3$ Hz, and the NOE between H-1′ and H-5′ indicated that H-1′ and H-5′ protons were located in an axial position. The missing observation of NOE between H-2′ and H-3′ protons suggested that H-2′ and H-3′ were oriented in an axial and equatorial position, respectively. β-Configuration was determined by a NOE correlation from H-1′ to H-5″, and by a small coupling constant $^1J_{C\text{-}1'-H\text{-}1'}=158.1$ Hz (<166 Hz) (Kasai et al., 1977). Detailed inspection of the ^1H–^1H COSY spectrum in conjunction with the HOHAHA data resulted in the identification of the spin-coupling system of the monosaccharide, suggesting that it is 6-deoxy-β-gulopyranose (**9**).

As to the other monosaccharide, strong *J* coupling between H-2_{ax}″ and H-3″ and weak coupling between H-1″/H-2_{ax}″, and H-1″/H-2_{eq}″, and H-2_{eq}″/H-3″, and H-3″/H-4″ were observed, while the NOE patterns were strong only between H-3″ and H-5″ and weak between H-1″/H-3″ and H-2″/H-4″. The coupling constant of the anomeric proton of the sugar was $J_{1''-2_{ax}''}=3.1$ Hz and $J_{1''-2_{eq}''}=2.2$ Hz. Thus the glycoside linkage of sugar was determined to be α. These observations indicated that this monosaccharide is 2-deoxy-α-fucose (2,6-dideoxy-α-*lyxo*-hexose) (**10**).

The absolute configuration of both monosaccharides was determined by optical rotation. The $[\alpha]_D^{22}$ value obtained for 2-deoxy-fucopyranose was −59°, while the literature (Butterworth and Hanessian, 1971; Kawai et

Table 1
^{1}H NMR and ^{13}C NMR data of **1** and **2,** solvent see Experimental

No.	**1**		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	–	165.0	–	164.8
2	5.96 (1H, *dd*, *J* = 9.8, 1.1)	121.4	5.98 (1H, *dd*, *J* = 9.9, 1.1)	121.0
3	6.65 (1H, *ddd*, *J* = 9.8, 11.8, 2.7)	144.3	6.63 (1H, *ddd*, *J* = 9.9, 11.6, 2.7)	145.4
4	2.36 (2H, *m*)	33.1	2.37 (2H, *m*)	33.7
5	4.77 (1H, br*dt* , *J* = 14.1, 7.1)	80.8	4.75 (1H, *brdt*, *J* = 14.2, 7.3)	81.0
6	5.70 (1H, *dd*, 16.1, 7.1)	124.5	5.71 (1H, *dd*, 16.0, 7.3)	124.7
7	6.17 (1H, *dd*, 16.1, 9.2)	133.8	6.18 (1H, *dd*, 16.0, 9.3)	133.5
8	2.58 (1H, *ddd*, *J* = 9.2, 7.4, 0.8)	34.1	2.60 (1H, *ddd*, *J* = 9.3, 7.5, 0.8)	34.4
9	2.04 (2H, br*t*, *J* = 7.4)	40.6	2.08 (2H, *brt*, *J* = 7.5)	40.3
10	5.59 (1H, *dt*, *J* = 15.8, 7.4)	128.1	5.61 (1H, *dt*, *J* = 15.9, 7.5)	128.0
11	6.02 (1H, *d*, *J* = 15.8)	135.0	6.08 (1H, *d*, *J* = 15.9)	135.2
12	–	137.2	–	137.1
13	5.45 (1H, *dq*, *J* = 9.9, 1.1)	126.9	5.48 (1H, *dq*, *J* = 10.0, 1.0)	127.0
14	3.46 (1H, *dq*, 9.9, 6.4)	44.9	3.52 (1H, *dq*, 10.0, 6.4)	45.9
15	–	218.8	–	217.4
16	2.95 (1H, *dq*, *J* = 7.2, 4.4)	44.3	2.95 (1H, *dq*, *J* = 7.3, 4.5)	45.0
17	3.78 (1H, *dd*, *J* = 6.6, 4.4)	80.4	3.78 (1H, *dd*, *J* = 6.4, 4.5)	80.9
18	1.43 (1H, *m*)	33.1	1.34 (1H, *m*)	33.4
19	1.26 (1H, *m*); 1.31 (1H, *m*)	38.7	1.28 (1H, *m*). 1.32 (1H, *m*)	39.3
20	1.23 (2H, *m*)	19.8	1.25 (2H, *m*)	19.9
21	0.94 (3H, *t*, *J* = 6.4)	14.4	0.94 (3H, *t*, *J* = 6.4)	14.3
22	1.06 (3H, *d*, *J* = 7.5)	20.3	1.06 (3H, *d*, *J* = 7.5)	19.9
23	1.81 (3H, *d*, *J* = 1.1)	16.6	1.81 (3H, *d*, *J* = 1.1)	16.8
24	1.13 (3H, *d*, *J* = 6.6)	16.2	1.13 (3H, *d*, *J* = 6.6)	16.1
25	1.09 (3H, *d*, *J* = 7.0)	9.8	1.09 (3H, *d*, *J* = 7.0)	9.9
26	0.98 (3H, *d*, *J* = 6.8)	14.3	0.98 (3H, *d*, *J* = 6.8)	14.2
1′	4.70 (1H, *d*, *J* = 8.2)	103.7	5.07 (1H, *d*, *J* = 9.4)	103.1
2′	3.60 (1H, *dd*, *J* = 8.2, 3.2)	70.8	4.26 (1H, *dd*, *J* = 9.4, 3.3)	69.5
3′	4.19 (1H, *dd*, *J* = 3.4, 3.2)	73.6	4.39 (1H, *dd*, *J* = 3.4, 3.3)	72.9
4′	3.93 (1H, *dd*, *J* = 3.4, 1.3)	81.4	4.55 (1H, *dd*, *J* = 3.4, 1.4)	81.1
5′	4.03 (1H, *dq*, *J* = 6.6, 1.3)	69.8	4.42 (1H, *dd*, *J* = 6.7, 1.4)	68.3
6′	1.30 (1H, *d*, *J* = 6.6)	16.8	1.52 (1H, *d*, *J* = 6.7)	17.1
1″	4.77 (1H, *dd*, 2.2, 3.1)	99.5	4.70 (1H, *dd*, 2.1, 10.3)	102.8
2 $_{ax}$″	1.75 (1H, *ddd*, *J* = 12.0, 11.6, 3.1)	33.6	1.72 (1H, *ddd*, *J* = 12.1, 11.3, 10.3)	37.2
2 $_{eq}$″	1.99 (1H, *dddd**, *J* = 12.0, 4.4, 2.2, 1.0)		2.08 (1H, *ddd*, *J* = 12.1, 5.5, 2.1,)	
3″	3.95 (1H, *ddd*, *J* = 11.6, 4.4, 2.8)	65.6	3.74 (1H, *ddd*, *J* = 11.3, 8.6, 5.5)	72.3
4″	3.69 (1H, br*dd*, *J* = 2.8, 1.1)	72.4	2.89 (1H, *dd*, *J* = 8.6, 8.5)	75.8
5″	3.51 (1H, *dq*, *J* = 6.4, 1.1)	68.5	3.42 (1H, *dq*, *J* = 6.3, 8.5)	69.4
6″	1.30 (1H, *d*, *J* = 6.4)	17.7	1.28 (1H, *d*, *J* = 6.3)	17.8

*Apart from vicinal and geminal couplings, H-2$_{eq}$″ showed long-range coupling behavior with *J* = 1 Hz, i.e. H-4″.

Table 2
The presence of degradation products (determined by chiral capillary GC) after oxidation of compounds **1** and **2**

Methyl ester of acid	Room temp. of products after degradation (min^{-1})		
	Standards	**1**[a]	**2**[a]
Acetic	2.15	2.17	2.17
Pyruvic	6.58	6.60	6.59
Oxalic	8.12	8.11	8.12
Malonic	11.76	11.77	11.75
2*R*-Methyl-succinic	14.22	–	–
2*S*-Methyl-succinic	14.71	14.74	14.72
2*R*-Hydroxy-succinic	38.11	38.14	38.15
2*S*-Hydroxy-succinic	38.56	–	–

al., 1987) gives the value $[\alpha]_D^{14}$ $-61.6\pm2^\circ$ or -53°, respectively, for 2-deoxy-L-fucopyranose. Also the optical rotation of our 6-deoxy-gulose in water ($[\alpha]_D^{21} = -39^\circ$) is in good agreement with the literature data (Butterworth and Hanessian, 1971) ($[\alpha]_D = +40.8^\circ$ for L and $[\alpha]_D = -38^\circ$ for D configuration).

A glycosidation shift at C-4′ (ca. +8.5 ppm) and the chemical shifts of H-1′ (δ 4.70) and C-1′ (δ 103.7) of the 6-deoxy-gulose of **1** indicated that this monosaccharide was glycosidated at C-4′ and linked to the aglycone. The one signal due to the anomeric proton of 2-deoxy-fucose (δ 4.77), correlating to the C-1″ resonance at δ 99.5 by HETCOR, indicated that the 2-deoxy-fucose unit was linked to a secondary alcoholic carbon (C-4′ of the

6-deoxy-gulose). The 2-deoxy-fucose was determined to be terminal by the absence of any glycosylation shift. These deductions were confirmed by a COLOC spectrum, which showed some diagnostic long-range correlations between H-1′ of 6-deoxy-gulose (δ 4.70) and C-17 (δ 81.4) of the aglycone, and between H-1″ (δ 4.77) of the 2-deoxy-fucose unit and C-4′ (δ 80.4) of 6-deoxy-gulose (see Table 1). The structure of glycoside **1** (lycogalinoside A) was determined as the 2-deoxy-α-L-fucopyranosyl-(1–4)-6-deoxy-β-D-gulopyranoside of lycogaline.

Positive HRFABMS of **2** gave also a pseudomolecular ion at m/z 693.4221 $[M+H]^+$, corresponding to the formula $C_{37}H_{58}O_{11}$, and showed the same negative FABMS with $[M-H]^-$ ion at m/z 691 and with prominent fragments at m/z 561 $[M-H\text{-}130]^-$ (cleavage of a dideoxyhexosyl unit) and 415 $[M-H\text{-}130\text{-}146]^-$ (cleavage of a dideoxyhexose and a deoxyhexosyl unit).

The ^{1}H NMR spectrum of **2** was similar to that of **1**, except for the different signals at δ 1.7–5.1, suggesting the presence of two different monosaccharide moieties in the molecule. The ^{13}C NMR spectrum of **2** was also similar to that of **1**. Comparison of the ^{1}H and ^{13}C NMR data of **2** with those of **1** revealed that **2** has different monosaccharides.

The ^{1}H-^{1}H COSY of **2** revealed a contiguous coupling between H-1′ and H-2′; H-2′ and H-3′; H-3′ and H-4′; and H-5′ and 5′-CH_3. The coupling constants of the anomeric proton (δ 5.07, $J_{1'-2'}=9.4$ Hz) and H-2′ (δ 4.26, $J_{2'-3'}=3.3$ Hz) of **2** suggest an axial orientation for the anomeric proton, thus confirming the β (equatorial) hemiacetal linkage to the aglycone and an axial orientation for both H-1′. The double–doublet signal of H-4′ suggested that H-4′ was equatorial. Selected NOE difference experiments were used to confirm the configuration of the monosaccharide residues in **2**; irradiation at H-5′ resulted in NOE enhancement at H-3′, confirming that both hydrogens were axial and that 5-CH_3 was equatorial. Finally, on the basis of these spectral data, it was concluded that the monosaccharide unit of **2** was β-fucopyranose (6-deoxy-galactose). The presence of a β-fucopyranose moiety in **2** was confirmed by comparing the ^{13}C NMR chemical shifts of the monosaccharide unit with those of known monosaccharides (Bock and Pedersen, 1983).

For a dideoxy-hexose, strong NOEs were observed between H-l″ and H-3″, H-l″ and H-5″, and H-3″ and H-5″, and strong J couplings were shown to occur between H-l″ and H-2_{ax}″, H-2″a and H-3″, H-3″ and H-4″, and H-4″ and H-5″. These observations indicate that this monosaccharide is 2,6-dideoxy-β-*arabino*-hexopyranose (β-olivose).

After total acid hydrolysis of **2** with 1 M HCl, the $[\alpha]_D$ of our fucopyranose was +74°; literature (Butterworth and Hanessian, 1971) reported $[\alpha]_D^{23}=-76°$ for L or +75° for D form, respectively. The optical rotation value of our olivose was +24°, which is practically identical with the value reported (Miyamoto et al., 1964) for D-olivose $[\alpha]_D^{23}=+22°$. This result indicates that both monosaccharides of compound **2** are in the D-forms.

The first saccharide moiety is connected to C-17 of the aglycone, as judged from the HMBC correlations between H-l′ of the fucose (δ 5.07) and C-17 of the aglycone (δ 80.9), while H-l″ of the 2-deoxyfucose (δ 4.70) is connected to C-4′ of the fucose (δ 81.1).

Compounds **7** and **8** were obtained after hydrolysis and ozonolysis and **11** after further reactions of **2**. Their absolute sterical structures were determined in the same way as for the degradation products of compound **1**. As shown in Table 2, the absolute configuration of C-5 and C-8 (5*R* and 8*S*) was identified by chiral chromatography of ozonolysis products. Because MTPA esters (**12a** and **12b**) show same chemical shift in ^{1}H NMR as was determined for compounds **3a** and **3b**, the configuration of the hydroxyl group on C-17 has to be equal to that one in substance **1**, i.e. *R*.

The 2,4,6-trimethyl nononanoic acid (**11**) derived from **2** possesses different $[\alpha]_D^{22}=-1.8°$. This value differs from the data published for other four synthetic acids (Odham, 1967). Its absolute value is analogous to $[\alpha]_D=+1.7°$, which was earlier determined for an *R*,*R*,*S* isomer. Therefore, our compound is its enantiomer, which has the chirality *S*,*S*,*R*. The ^{1}H and ^{13}C NMR spectra looked like those of natural ebalacton, which also has an α-methyl configuration on last chiral carbon (Uotani et al., 1982; Paterson and Hulme, 1995).

On the basis of the above observations, the structure of **2** (lycogalinoside B) was determined to be β-D-olivopyranosyl-(1–4)-β-D-fucopyranoside of lycogaline, where the aglycone part has the configuration (2*Z*, 5*R*, 6*E*, 8*S*, 10*E*, 12*E*, 14*R*, 16*S*, 17*R* and 18*R*) (Fig. 1).

The antimicrobial activities of **1** and **2** are summarized in Table 3. Compound **1** inhibited the growth of the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, with a MIC of 52 μg/ml and 12 μg/ml, respectively. It also showed a modest growth inhibition of Gram-negative bacteria and some yeasts. The stereoisomer **2** showed 10-fold weaker activity than **1** against the tested bacterial strains. 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.

Table 3
Bioactivities of compounds (**1**–**2**)

Test organism[a]	**1**	**2**
Staphylococcus aureus	52	6.4
Bacillus subtilis	12	1.6
Escherichia coli	8	2
Saccharomyces cerevisiae	7	32
Candida albicans	2	9

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

The chemical structure of either of the above compounds or rather their aglycone parts resembles the structure of some compounds isolated from *Streptomyces*. Examples of such structural similarity include e.g., NK10958P (Tsuchiya et al., 1997), pironetin (Kobayashi et al., 1994), leptomycin B (Hamamoto et al., 1983), leprofuranins (Hayakawa et al., 1996), anguinomycins C and D (Hayakawa et al., 1995) or ebelactones (Uotani et al., 1982). In addition, YM-47522 (Sugawara et al., 1996) from *Bacillus* and lagunapyrones (Lindel et al., 1996) that were isolated from an unidentified marine actinomycete have very similar structures. The presence of deoxy- and a dideoxyhexoses in the two glycosides **1** and **2** which are usually produced by *Streptomyces* (Johnson and Liu, 1999) lends support to our assumption that the two compounds are not inherent by original metabolites of the slime-mold, but are a relict from the amoeba or flagellate stage where *Streptomyces* and other soil microorganisms were an important part of the feeding chain of the slime-mold.

These facts may indicate the participation of symbiotic microorganisms used as nutrition in the biosynthesis of glycosides **1** and **2** in the slime-mold *Lycogala epidendrum*.

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 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). Gas chromatography analysis was made on a Hewlett Packard HP 5980 gas chromatograph (Hewlett Packard, Czech Republic).

The following compounds: acetic acid, acetone, malonic, oxalic, pyruvic, 2*R*- and 2*S*-methyl-succinic, 2*R*- and 2*S*-hydroxy-succinic acids were purchased from Sigma-Aldrich (Prague, Czech Republic).

3.2. Plant material

The slime mold was collected near the village Hluboke Masuvky, 13 km north of Znojmo, Czech Republic, on decayed wood. It was identified by the second author (R.D.) by its physical properties.

3.3. Extraction and isolation

Sample of slime mold (3.27 g dry weight) was extracted by 90% butanol. Chromatography of extract on a Sephadex LH-20 column (100×5 cm) with elution with 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 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 two compounds **1** (9.8 mg) and **2** (6.4 mg).

3.4. Acid hydrolysis

The ~7 mg of glycoside (**1**) was refluxed in 2 M HCOOH (0.5 ml) for 2 h. The hydrolysate was extracted three times with EtOAc (10 ml) and combined extracts were evaporated to give an aglycone. After separating the organic layer, the aqueous phase was neutralized with $NaHCO_3$ and lyophilized. The residue obtained after lyophilization was purified on a Separon SGX NH_2 column (7 μm, 3×150 mm) eluting with 90% MeCN to yield 0.8 mg of 2-deoxy-L-fucopyranose $[\alpha]_D^{22} = -59^\circ$ and 0.7 mg of 6-deoxy-D-gulose $[\alpha]_D^{21} = -39^\circ$.

Analogically the glycoside **2** was work-up and the non-polar fraction was ozonolysed, after which in water fraction the 0.5 mg D-olivopyranose $[\alpha]_D^{23} = +24^\circ$ and 0.6 mg D-fucopyranose $[\alpha]_D^{23} = +74^\circ$ were identified.

3.5. Oxidative cleavage

A stream of 4% ozone was passed through a solution of the aglycone (~4 mg) in dichloromethane (2 ml) at −78 °C for 5 min. The solution was flushed with nitrogen and concentrated. The residue was dissolved in 90% HCOOH (0.7 ml) and 30% hydrogen peroxide (0.3 ml) was added. After gentle heating the mixture was heated under reflux for 70 min. The mixture was concentrated and the 1/10 residue was dissolved in methanol (0.5 ml) and treated with etheral diazomethane, vice-versa 9/10 were further treated chemical reactions.

To 9/10 solution of **3** in 1 ml of dry CH_2Cl_2 at 0 °C, was added an excess of PCC (3 mg) and the mixture was stirred for 2 h. After work-up, the crude **4** was reduced, see bellow. A solution of the diketone **4** (1.2 mg), ethanedithiol (0.2 ml), and $BF_3{\cdot}OEt_2$ (0.05 ml) in dry benzene (0.5 ml) was magnetically stirred at 0–5 °C for 2 h. The reaction was quenched with aqueous $NaHCO_3$ solution and extracted with ether. The ether extract was washed with 5% aqueous NaOH solution. The solvent was evaporated and thioketal was dissolved in ethanol. To a magnetically stirred solution of the thioketal in dry ethanol was added an excess of Raney Ni, and the resulting mixture was refluxed for 30 min. The reaction mixture was filtered through a short silica gel column to

remove the catalyst, the solvent was evaporated and residue was purified by TLC (EtOAc/hexane = 2/7) to afford 1.3 mg of **5** (2*S*, 4*S*, 6*S*-trimethyl-nonanoic acid), $[\alpha]_D^{22} = 15.7°$ ($c = 0.12$, CH_2Cl_2). The free acid was treated with etheral diazomethane and resulting methyl 2*S*, 4*S*, 6*S*-trimethyl-nonanoate (**6**) had $[\alpha]_D^{22} = 22.1°$ ($c = 0.18$, CH_2Cl_2). The non-polar part after hydrolysis and ozonolysis of compound **2** was work-up analogically and two substituted succinic acids (**7** and **8**) and methyl 2*S*,4*S*,6*R*-trimethyl-nonanoate (**11**), $[\alpha]_D^{22} = -1.8°$ ($c = 0.18$, CH_2Cl_2) were identified.

3.6. Chiral chromatography

FS capillary column HYDRODEX β-3P ID 0.25 mm, length 25 m, with the stationary phase [heptakis-(2,6-di-*O*-metyl-3-*O*-pentyl)-β-cyclodextrine] from Macherey-Nagel GmbH & Co. KG, Düren, Germany was used. Oven temperature: 50 – 150 °C at 2 °C/min, then to 240 °C at 5 °C/min, carrier gas helium, 20 cm/s, detector FID, 300 °C, injection of 1 μl mixture in methylene chloride (for standards: containing 0.5 mg/ml of each sample), split (100:1), 300 °C.

3.7. (S)-MTPA and (R)-MTPA esters

(*S*)-MTPA ester of free acid (**3a**) (Ohtani et al., 1991a, b). To a CH_2Cl_2 solution (100 μl) of acid (**3**) (0.3 mg), DMAP (1.0 mg), and Et_3N (2 μl) was added (*R*)-(−)-MTPACl (2.0 mg) at room temperature, and stirring was continued for 3 h. After evaporation of solvent, the residue was purified by silica gel TLC (hexane/AcOEt, 2/1) to provide the (*S*)-MTPA ester (**3a**) as colorless oil.

(*R*)-MTPA esters of free acid (**3b**). Acid (**3**) (0.3 mg) was treated with (*S*)-(+)-MTPACl (2.0 mg) by the same procedure as described above to provide the (*R*)-MTPA ester (**3b**) as colorless oil. The esters **12a** and **12b** were prepared by analogical procedures.

1 (lycogalinoside A), white powder (9.8 mg), $[\alpha]_D^{23} = -19°$ ($c = 0.09$, MeOH), UV λ_{max} (EtOH, nm) 243 (log ε 4.72) and 300 (log ε 3.38); IR (KBr) (cm^{-1}): 3400 (OH), 2950, 2920, 1730 (C=C-C=O), 1710 (C=O); HRFABMS *m*/*z* 693.4218 $(M+H)^+$, calculated for $[C_{37}H_{58}O_{11}]^+$ 693.4213; negative FABMS *m*/*z* 691 $[M-H]^-$, *m*/z 561 $[M-H\text{-}130]^-$ and *m*/*z* 415 $[M-H\text{-}130\text{-}146]^-$; ^1H and ^{13}C NMR spectra, see Table 1.

2 (lycogalinoside B), white powder (6.4 mg), $[\alpha]_D^{23} = -41°$ ($c = 0.08$, MeOH), UV λ_{max} (EtOH, nm) 243 (log ε 4.72) and 300 (log ε 3.38); IR (KBr) (cm^{-1}): 3400 (OH), 2950, 2920, 1730 (C=C-C=O), 1710 (C=O); HRFABMS *m*/*z* 693.4221 $[M+H]^+$, calculated for $[C_{37}H_{58}O_{11}]^+$ 693.4213; negative FABMS *m*/*z* 691 $[M-H]^-$, *m*/z 561 $[M-H\text{-}130]^-$ and *m*/*z* 415 $[M-H\text{-}130\text{-}146]^-$; ^1H and ^{13}C NMR spectra, see Table 1.

Methyl 2*S*,4*S*,6*S*-trimethyl-nonanoate (**6**), $[\alpha]_D^{22}$ +22.1° ($c = 0.18$, CH_2Cl_2), ^1H NMR ($CDCl_3$) 0.84 (3H, *t*, *J* = 6.9 Hz, H-9), 0.88 (6H, *m*, Me on C-4 and Me on C-6), 1.18 (3H, *d*, *J* = 7.0 Hz, Me on C-2), 1.21 (2H, *m*, H-5), 1.25 (2H, *m*, H-7), 1.33 (2H, *m*, H-8), (1.53 (2H, *m*, H-3), 1.65 (1H, *m*, H-4), 1.74 (1H, *m*, H-6), 2.58 (1H, *m*, H-2), 3.64 (3H, *s*, COO*Me*); EI-MS 214 (M^+), 183, 171, 101, 88 (base peak).

Methyl 2*S*,4*S*,6*R*-trimethyl-nonanoate (**11**), $[\alpha]_D^{22}$ −1.8° ($c = 0.18$, CH_2Cl_2); ^1H NMR and MS spectra are identical with methyl 2*S*,4*S*,6*S*-trimethyl-nonanoate (**6**).

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