Lycoperdinoside A and B, New Glycosides from the Slime Mold *Enteridium lycoperdon*

Tomáš Řezanka,*[a] Radmila Dvořáková,[b] Lumír O. Hanuš,[c] and Valery M. Dembitsky[c]

Keywords: Lycoperdinoside A and B / Glycosides / *Enteridium lycoperdon* / Structure elucidation

Two, novel, six-membered lactone glycosides (lycoperdinoside A and B) were isolated from the slime mold *Enteridium lycoperdon*. Their structures, including the absolute configurations of the hydroxy and methyl groups, were determined by means of extensive spectroscopic data such as MS, IR, UV, and 1D and 2D NMR spectra and chemical degradation. The compounds have structures containing a β-L-amicetosyl-(1→4)-α-I-amicetosyl unit and a β-L-amicetosyl-(1→4)-α-L-amicetosyl-(1→4)-β-L-rhodinosyl unit, respectively.

Introduction

The myxomycetes have long been the subject of intensive research in cell biology, physiology, genetics, and also in chemistry.[1,2] The polyenes physarochrom A, physarorubinic acid A and B, the polycephalins B and C, and a yellow, optically-active pigment, chrysophysarin A have been isolated from the yellow plasmodium of the slime mold *Physarum polycephalum*.[3−7]

Two novel amino sugar analogs, furanodictine A and B, were isolated from a methanol extract of the multicellular fruit body of *Dictyostelium discoideum*. They are the first 3,6-anhydrosugars to be isolated from a natural source. These furanodictines show the potential to induce neuronal differentiation of rat pheochromocytoma (PC-12) cells.[8]

A novel, chloro-containing, antibacterial substance, AB0022, was isolated from the cellular slime mold *Dictyostelium purpureum* K1001. It inhibited the growth of Gram-positive bacteria, and its MICs ranged from 0.39 to 50 μg/ml.[9]

The phospholipids and phospholipase D of the true slime mold *Physarum polycephalum*, as well as the fatty acid compositions of some slime molds have been studied.[10−12]

Three novel triacylglycerols, lycogarides A−C, were isolated from *Lycogala epidendrum* while in two other studies, which were independent of each other, the same three 3,4-bis(indol-3-yl)pyrrole-2,5-dicarboxylic acid derivatives, lycogalic acid dimethyl esters A−C, were obtained. Four new acylglycerols, lycogarides D−G were also isolated from the slime mold *Lycogala epidendrum*.[13−16]

In the course of our screening program for new compounds from unusual sources, we isolated two new glycosides from the slime mold *Lycogala epidendrum*.[12] In this paper, we report further isolation, physico-chemical properties and structure determination of two new lactone glycosides, i.e. lycoperdinosides A and B, from *Enteridium lycoperdon*.

Results and Discussion

A sample 22.1 g of *Enteridium lycoperdon* (Bull.) Farr (syn. *Reticularia lycoperdon* Bull.) slime mold was extracted by butanol and subsequently separated on a Sephadex LH-20 column. The fractions were further purified by RP-HPLC to give two glycosides (**1,** 11.3 mg and **2,** 7.2 mg; see Figure 1), which were 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 $[\alpha]_D^{23} = +63$, but without an exact melting point as the glycoside decomposed. The HRFABMS established the molecular formula **1** as $C_{39}H_{60}O_9$, i.e. m/z = 673.4320 [M + H]$^+$. Negative FABMS gave three prominent ions at m/z = 671 [M − H]$^-$, m/z = 557 [M − H − 114]$^-$ and m/z = 443 [M − H − 114 − 114]$^-$ corresponding to the loss of one and two trideoxyhexosyl units, respectively.

The compound exhibited IR absorption peaks corresponding to hydroxy groups (3460 cm^{-1}) and carbonyl groups (1725 cm^{-1}). The UV spectrum of **1** showed absorption maxima at 243 nm (log ε 4.45) ascribable to diene and

[a] Institute of Microbiology,
Vídeňská 1083, Prague, 14220 Czech Republic
Fax: (internat.) + 420-241-062347
E-mail: rezanka@biomed.cas.cz
[b] South-Moravian Museum,
Přemyslovců 8, 66902 Znojmo, Czech Republic
[c] Department of Pharmaceutical Chemistry and Natural Products, School of Pharmacy, Hebrew University of Jerusalem,
P. O. Box 12065, Jerusalem 91190, Israel

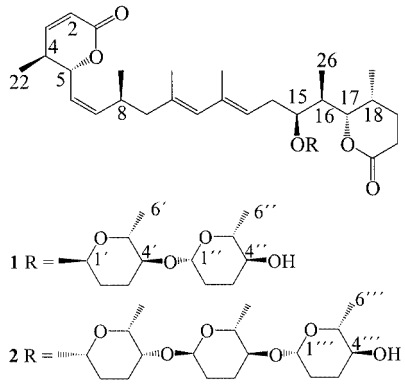

Figure 1. Structure of new glycosides, i.e. lycoperdinoside A and B (**1** and **2**) from slime mold *Enteridium lycoperdon*

212 nm (log 3.48) and 283 nm (log 2.64) ascribable to α,β-unsaturated carbonyl.

The ^{13}C NMR spectrum of **1** confirmed the presence of 39 carbon atoms. An HMQC experiment established all one-bond ^{1}H-^{13}C connectivities. A COSY experiment revealed two spin networks to generate partial structures as shown in Figure 2. The HMBC spectrum displayed ^{1}H-^{13}C long-range couplings from CH_3-10 (i.e. C-24) to C-9, C-10 and C-11, and from H-8, H-9 and H-11 to C-10, and from C-25 (CH_3–12) to H-11 and H-13, indicating the connection between the partial structures via C-10 and C-12 as shown in Figure 2. In addition, δ-lactone rings were constructed from ^{1}H-^{13}C long-range correlations from H-2 (H-20), H-3 (H-19) and H-5 (H-17) to an ester carbonyl carbon (C-1; C-21) to establish the structure of **1**, except for the stereochemistry. The relative configuration of the unsaturated δ-lactone shown in Figure 1 was assumed on the basis of the following considerations. The carbon framework was practically identical to model compounds. The *syn* 4,5-substituents have $\delta_{H\text{-}3}$ at ≈ 7.0 ppm with $J_{3,4} > 6$ Hz, whereas the *anti* 4,5-substituents showed $\delta_{H\text{-}3}$ at ≈ 6.65 ppm and $J_{3,4} \approx 3$ Hz, which is in good agreement with the measured data ($\delta_{H\text{-}3} = 6.61$, $J_{3,4} = 3.1$ Hz).[17,18]

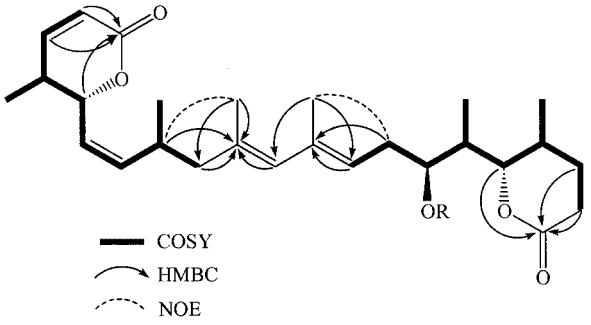

Figure 2. The H-H COSY, HMBC and NOE correlations of aglycone part of lycoperdinoside A (**1**)

The geometrical configurations of Δ^2 and Δ^6 were determined to be (2*Z*) and (6*Z*) by $J_{2,3} = 9.9$ Hz and $J_{6,7} = 9.1$ Hz. The high-field shifts for CH_3-10 (δ = 16.7 ppm) and CH_3-12 (δ = 15.9 ppm) indicated (10*E*) and (12*E*) configurations.

The absolute configuration of compound **1** was determined in two steps. The first step was the elucidation of the saccharide components after hydrolysis. In the second step, the structure of aglycon (after hydrolysis, ozonolysis, and further reactions) was clarified.

The ^{1}H NMR and ^{13}C NMR spectroscopic data of the monosaccharide units of **1** are also shown in Table 1. ^{1}H and ^{13}C NMR spectroscopic data, as well as decoupling experiments on **1**, were used to assign the signals of the monosaccharide moieties. The coupling constants ($J = 9.7$ and/or $J = 9.8$ Hz) between H-4′ and H-5′ (and H-4′′, H-

Table 1. ^{1}H and ^{13}C NMR of compound **1**

No.	^{1}H	^{13}C
1	–	166.1
2	5.98 (dd, J = 9.9, 1.0, 1 H)	121.8
3	6.61 (dd, J = 9.9, 6.1, 1 H)	144.9
4	2.36 (qdd, J = 7.2, 6.1, 0.8, 1 H)	33.1
5	4.70 (dd , J = 8.4, 0.8, 1 H)	81.4
6	5.76 (dd, J = 9.1, 8.4, 1 H)	123.9
7	6.12 (dd, J = 9.1, 9.7, 1 H)	134.2
8	2.61 (dqd, J = 9.7, 7.4, 0.8, 1 H)	33.6
9	2.45 (ddd, J = 0.7, 6.6, 13.1, 1 H); 1.98 (m, 1 H)	44.6
10	–	136.1
11	5.68 (br. s, 1 H)	132.8
12	–	135.2
13	5.32 (dd, J = 8.0, 6.2, 1 H)	124.7
14	2.40 (ddd, J = 14.6, 6.2, 1.9, 1 H) 2.54 (ddd, J = 14.6, 8.0, 9.9, 1 H)	34.1
15	3.71 (ddd, J = 9.9, 1.9, 1.5, 1 H)	75.2
16	1.77 (dqd, J = 10.3, 7.3, 1.5, 1 H)	39.3
17	3.80 (dd, J = 10.3, 3.6, 1 H)	79.3
18	1.90 (qbrd, J = 6.8, ~3, 1 H)	33.8
19	1.79 (m, 1 H) 1.65 (m, 1 H)	31.7
20	2.38 (ddd, J = 17.7, 8.5, 6.8, 1 H) 2.56 (ddd, J = 17.7, 6.9, 6.7, 1 H)	34.9
21	–	172.7
22	1.04 (d, J = 7.2, 3 H)	12.3
23	1.06 (d, J = 7.4, 3 H)	20.1
24	1.83 (s, 3 H)	16.7
25	1.82 (d, J = 0.8, 3 H)	15.9
26	1.00 (d, J = 7.3, 3 H)	12.7
27	0.98 (d, J = 6.8, 3 H)	17.4
1′	5.00 (brd, J = 2.4, 1 H)	96.9
2a′	2.02 (m, 1 H)	24.7
2e′	1.46 (m, 1 H)	
3a′	2.10 (m, 1 H)	30.2
3e′	1.43 (m, 1 H)	
4′	3.05 (ddd, J = 9.7, 9.2, 4.2, 1 H)	71.0
5′	3.28 (dq, J = 9.7, 6.3, 1 H)	73.5
6′	1.08 (d, J = 6.3, 3 H)	18.9
1′′	4.46 (dd, J = 8.2, 1.8, 1 H)	104.4
2a′′	1.87 (m, 1 H)	28.1
2e′′	1.42 (m, 1 H)	
3a′′	1.95 (m, 1 H)	27.5
3e′′	1.40 (m, 1 H)	
4′′	3.08 (ddd, J = 9.8, 8.8, 4.4, 1 H)	69.0
5′′	3.85 (dq, J = 9.8, 6.6, 1 H)	72.1
6′′	1.19 (d, J = 6.6, 3 H)	18.6

5′′) of the monosaccharide part of **1** indicate their diaxial orientations. The anomeric proton (H-1′) of the first monosaccharide from **1** appears as a doublet (J = 2.4 Hz) and the anomeric proton (H-1′′) of the second monosaccharide as double doublets (J = 8.2, 1.8 Hz). On the basis of the data from 2D NMR, i.e. COSY and NOE (Figure 3), the monosaccharides were determined to be α-amicetose and β-amicetose (α- and β-2,3,6-trideoxy-*erythro*-hexopyranoses), respectively.

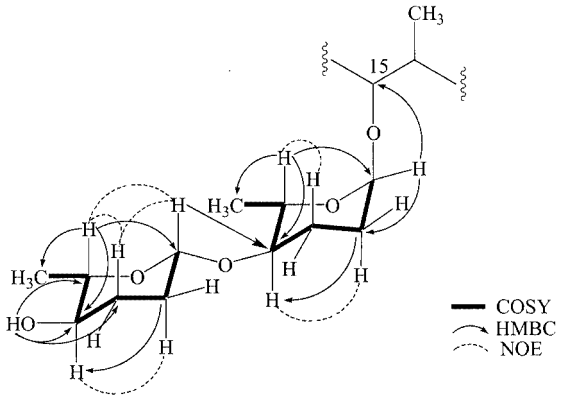

Figure 3. The H-H COSY, HMBC and NOE correlations of saccharidic part of lycoperdinoside A (**1**)

The monosaccharides obtained after hydrolysis of **1** were evaporated and an α,β-anomeric mixture of L-amicetose ($[\alpha]_D^{21}$ = −39.4) was obtained as a colorless syrup, while Catelani reported $[\alpha]_D$ = −39.0. The ^{1}H NMR spectrum of our L-amicetose is in good agreement with those of the published data.[19,20]

Connectivities between the monosaccharide moieties and between the monosaccharide moiety and the aglycon were determined by HMBC spectroscopy (Figure 3). This data show that the disaccharide has 1→4 linkage and that β-L-amicetosyl-(1→4)-α-L-amicetose is glycosidically connected to C-15 carbon of the aglycon (Figure 1).

We also report the determination of the absolute configurations of all the chiral centers of **1**, as shown in Figure 1. The studies were mainly performed using the products obtained by the degradation sequence presented in Figure 4. These degradation products facilitated the determination of the stereochemistry of **1**.

The configurations of the chiral carbon atoms (C-4, C-5 and C-8) were determined by comparison of the retention times of the isolated and synthesized compound **3** and both (*R*) and (*S*) methylsuccinic acid (purchased from Sigma) to compound **8**, on a chiral capillary column (Figure 4).

After chromatographic analysis, it was determined that compound **3** is (2*S*,3*S*)-3-methylbutane-1,2,4-triol and that **8** is (2*S*)-2-methylsuccinic acid. The absolute configurations

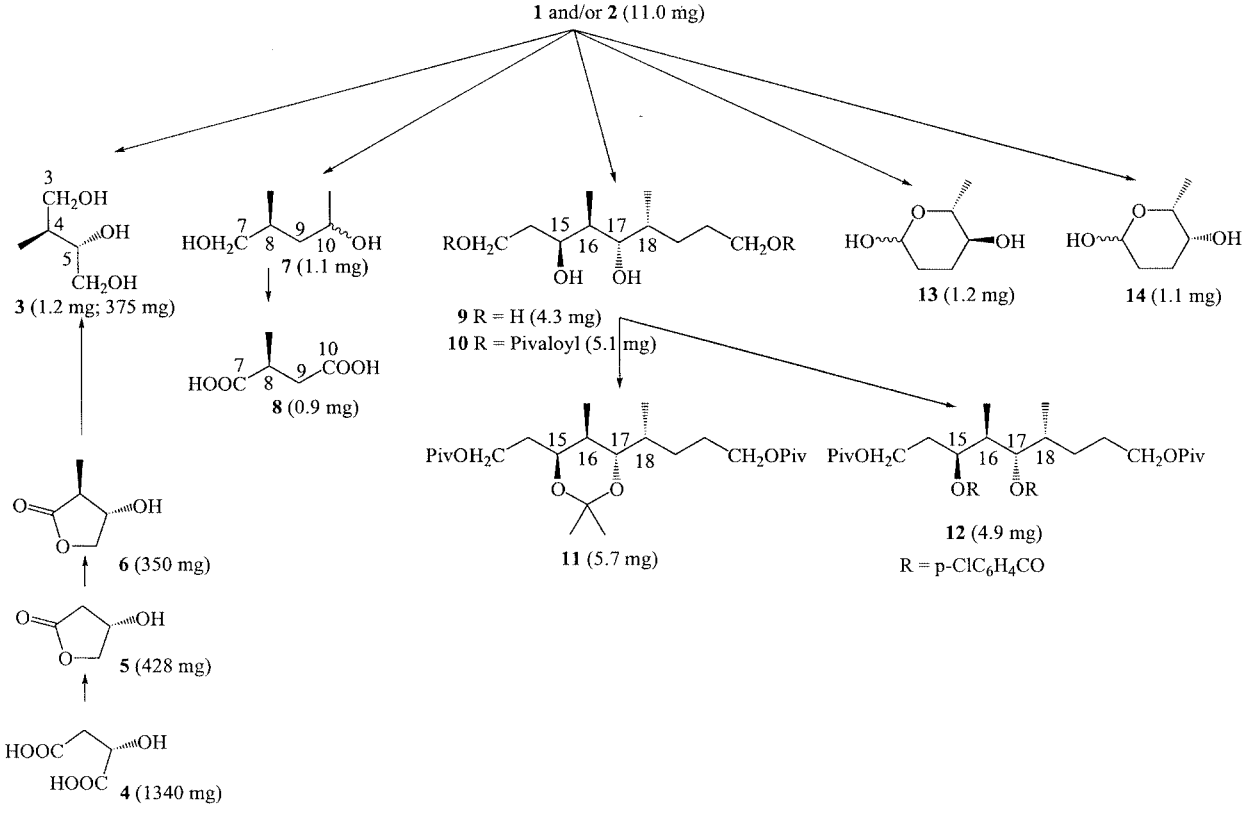

Figure 4. Reaction schema of degradation compounds from glycoside **1** (**2**) and synthesis of standard (2*S*,3*S*)-3-methyl-butane-1,2,4-triol (**3**)

of all others carbon atoms (i.e. C15−C19) were assigned by a combination of the chemical and physical methods, see below.

The relative configuration of the 1,3-diol units and the relative configuration of the methyl groups at C-2 (i.e. C-16 in **1**) in the degradation products was determined using the NOE between methyl protons at C-2 and H-1 (or H-3) in the case of the *anti*-1,3-diol-acetonide.[21]

To determine the absolute configuration of **10**, we synthesized a *p*-chlorobenzoate derivative and applied the exciton chirality method.[22]

Degradation product **10** possesses four asymmetric centers at C-15, C-16, C-17, and C-18. In order to determine the relative configurations of **9** having two primary alcohol functions, they were protected with acetonide.

The relative configuration at C-15/C-17 was established as 15,17-*anti* by Rychnovsky's method, because the two acetonide methyl carbon atoms and one ketal carbon appeared at δ = 23.6, 24.6, and 101.1 ppm, respectively, in the ^{13}C NMR spectrum of **11**. The *anti*-acetonide exists in the twisted boat conformation owing to the 1,3-diaxial interaction. The vicinal coupling constants $J_{15,16}$ = 3.4 Hz and $J_{16,17}$ = 1.4 Hz in the ^{1}H NMR spectrum of **11** indicate that the three substituents at C-15, C-16, and C-17 are *syn* and *anti* disposed, respectively. The relative configuration at C-17/C-18 was determined by the vicinal coupling constant $J_{17,18}$ = 1.7 Hz. This value showed that the substituents were 17,18-*syn*. From these results, the relative configurations of **10** were determined to be 15,16-*syn*, 16,17-*anti* and again 17,18-*syn* (i.e. 15*S**, 16*R**, 17*S**, and 18*R**). Consequently, the CD exciton chirality method was applied and the CD spectrum of **12** showed a large positive first Cotton effect at 247 nm (Δ = +27.5) and a second negative Cotton effect at 230.8 nm (Δ = −12.7), indicating that the absolute configurations of **1** were 15*S*, 16*R*, 17*S*, and 18*R*. On the basis of this information, it was concluded that the structure of the glycoside lycoperdinoside A (**1**) is (4*S*,5*R*,8*S*,15*S*,16*R*,17*S*,18*R*)-15-β-L-amicetosyl-(1→4)-α-L-amicetosyloxy-5,17-dihydroxy-4,8,10,12,16,18-hexamethylheneicosa-2,6,10,12-tetraene-di-1,5:21,17-olide.

Positive HRFABMS of **2** also give the pseudomolecular ion at *m*/*z* = 787.5001 $[M + H]^+$, corresponding to the formula $C_{45}H_{71}O_{11}$ and showed the negative FABMS with $[M - H]^-$ ion at *m*/*z* = 785 and with prominent fragments at *m*/*z* = 671 $[M - H - 114]^-$, 557 $[M - H - 2 \times 114]^-$ and 443 $[M - H - 3 \times 114]^-$ (cleavage of one, two and three trideoxyhexose units, respectively).

The ^{1}H NMR spectrum of **2** was similar to that of **1**, except that there were different signals in the region δ = 3.1−5.1 ppm, suggesting the presence of three 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 spectroscopic data of **2** with those of **1** revealed that **2** was a different glycoside.

The presence of the monosaccharides was evidenced by the signals at δ = 4−5 ppm in the ^{1}H NMR and by the signal at ≈ 100 ppm in the ^{13}C NMR spectrum, corresponding to the anomeric protons and carbon atoms, respectively. The first monosaccharide moiety was determined to be rhodinose, as a result of the ^{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* = 2.5 Hz) which confirms the equatorial (H-4′) − axial (H-5′) configuration. The coupling constant values (*J* = 7.7, 1.5 Hz) for the anomeric proton $J_{1,2ax}$ and $J_{1,2eq}$ and the chemical shift of the anomeric carbon at δ = 104.4 ppm suggested that the monosaccharide is attached to the aglycon by a glycosidic bond. On the basis of the NMR spectroscopic data, the monosaccharide was determined to be β-rhodinose; the chemical shifts and coupling constants were similar to the reported values of L-rhodinose-containing antibiotic.[23] The long-range coupling of H-1′ (anomeric proton) to C-15 (d, 83.5) in the HMBC experiment showed the linkage of this monosaccharide to the aglycon part at C-15. The absolute configuration of rhodinose was determined after hydrolysis and separation by NH_2-HPLC by measurement of $[\alpha]_D$ = −10.1. The literature data described $[\alpha]_D^{20}$ = +14.2 for D-rhodinose and $[\alpha]_D^{27}$ = −11.8 for L-rhodinose.[24−26]

The second and third monosaccharides have nearly identical spectra, as described above and shown in Table 2. On the basis of this data, the structure of lycoperdinoside B (**2**) is (4*S*,5*R*,8*S*,15*S*,16*R*,17*S*,18*R*)-15-β-L-amicetosyl-(1→4)-α-L-amicetosyl-(1→4)-β-L-rhodinosyloxy-5,17-dihydroxy-4,8,10,12,16,18-hexamethylheneicosa-2,6,10,12-tetraene-di-1,5:21,17-olide.

Table 2. ^{1}H- and ^{13}C-NMR of saccharidic part of compound **2**

No.[a]	^{1}H	^{13}C
1′	4.74 (dd, *J* = 8.8, 2.1, 1 H)	96.7
2a′	2.21 (m, 1 H)	32.8
2e′	1.50 (m, 1 H)	
3a′	2.11 (m, 1 H)	26.9
3e′	1.73 (m, 1 H)	
4′	3.58 (br. s, 1 H)	68.7
5′	4.11 (dq, *J* = 6.3, 1.5, 1 H)	69.0
6′	1.27 (d, *J* = 6.2, 3 H)	17.9
1′′	5.02 (d, *J* = 2.4, 1 H)	97.1
2a′′	2.03 (m, 1 H)	25.2
2e′′	1.51 (m, 1 H)	
3a′′	2.12 (m, 1 H)	32.0
3e′′	1.53 (m, 1 H)	
4′′	3.08 (ddd, *J* = 9.9, 9.5, 4.3, 1 H)	71.4
5′′	3.31 (dq, *J* = 9.9, 6.4, 1 H)	76.2
6′′	1.11 (d, *J* = 6.4, 3 H)	18.5
1′′′	4.48 (dd, *J* = 8.1, 1.6, 1 H)	104.8
2a′′′	1.81 (m, 1 H)	33.4
2e′′′	1.41 (m, 1 H)	
3a′′′	1.96 (m, 1 H)	32.4
3e′′′	1.43 (m, 1 H)	
4′′′	3.10 (ddd, *J* = 10.0, 9.0, 4.5, 1 H)	72.5
5′′′	3.87 (dq, *J* = 10.0, 6.8, 1 H)	78.4
6′′′	1.21 (d, *J* = 6.8, 3 H)	19.3

[a] The differences of chemical shifts in ^{1}H and ^{13}C NMR spectra of aglycone parts from compound **1** and **2** are less than ± 0.15 ppm or ± 0.5 ppm, respectively.

The obtained compounds suggested that slime molds may exhibit diversity in their secondary metabolite biosyn-

Table 3. ^1H and ^{13}C NMR data of both pivaloyl esters **11** and **12**

No	**11**		**12**	
^1H	^{13}C	^1H	^{13}C	
1	4.31	61.6	4.15	61.6
4.24	4.06			
2	1.90	33.7	2.12	31.7
1.84				
3	3.48 (ddd, J = 9.6, 8.4, 4.1, 1 H)	67.0	5.29	74.3
4	1.63 (qdd, J = 6.8, 10.4, 4.1, 1 H)	38.2	2.33	37.2
5	3.77 (dd, J = 10.4, 1.8, 1 H)	71.4	5.62 (dt, J = 3.4, 6.1, 1 H)	70.4
6	1.97	39.2	2.24	31.2
7	1.25	27.7	1.25	27.7
8	1.57	27.4	1.57	27.4
9	4.03	67.6	4.31 (m)	69.1
10	0.85 (d, J = 6.8, 3 H)	11.4	1.19 (d, J = 7.3)	11.9
11	0.92 (d, J = 6.7, 3 H)	11.9	1.06	12.5
Me_3-C	1.16	27.5	1.17	27.5
Me_3-C	-	38.4	–	38.2
Me_2-C	1.46	24.9	–	–
Me_2-C	1.37	23.4	–	–
Me_2-C	–	101.7	–	–
Aryl-CO	–	–	–	164.0
Aryl	–	–	7.84 (d, J = 8.4, 2 H)	138.1
Aryl	–	–	7.83 (d, J = 8.4, 2 H)	139.7
Aryl	–	–	7.37 (d, J = 8.4, 2 H)	128.6
Aryl	–	–	7.29 (d, J = 8.4, 2 H)	131.1

thetic pathways, and prompted us to find a variety of chemical constituents. The structure elucidation and syntheses of two novel acyl derivatives of *N*-acetylglucosamines have recently been described. Deoxy sugars have not been previously isolated from slime mold, but both glucoamines and deoxy sugars could probably have a common biosynthesis scheme, but different starting precursors.[27–29]

Lycogalinosides A and B, which are present in *Lycogala epidendrum*, could structurally belong to the multimethyl-branched fatty acids family, which have been previously isolated from widespread microorganisms belonging to strains of *Actinomycetales*.[30–32]

In our previous paper we showed that lycogalinosides A and B are probably not the products of actinomycetes, but rather metabolites produced by engulfed bacterial cells. In this paper, we suggest that even these metabolites are produced by the engulfed bacteria. This assumption is supported by the structural similarity between the multi-branched fatty acids contained in *Actinomycetales* and lycoperdinosides. In addition, the presence of deoxy sugars, typical components of antibiotics produced by streptomycetes, also favors this hypothesis.[10–12]

Experimental Section

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 with a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^1H), 125.7 MHz (^{13}C) in mixture of deuterated pyridine and CD_3OD (v/v 1:1). High- and 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. Circular dichroism (CD) measurements were carried out with a Jasco-500A spectropolarimeter at 24 °C, under dry N_2. HPLC was carried out using a Shimadzu gradient LC system (Shimadzu, Kyoto, Japan). GC analysis was performed with a Hewlett–Packard HP 5980 gas chromatograph (Hewlett–Packard, Czech Republic). FS capillary column HYDRODEX ß-3P ID 0.25 mm, length 25 m, with the stationary phase [heptakis(2,6-di-*O*-metyl-3-*O*-pentyl)-β-cyclodextrin] from Macherey–Nagel GmbH & Co. KG, Dren, Germany was used. Oven temperature: 50 °C to 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 dichloromethane (for standards: containing 0.5 mg/ml of each sample), split (100:1), 300 °C.

Acetic acid, acetone, malonic, oxalic, pyruvic, (2*R*)- and (2*S*)-methylsuccinic acids were purchased from Sigma–Aldrich (Prague, Czech Republic).

The slime mold was collected in the Karlov pack reservation, near Znojmo, Czech Republic. It was identified by the second author (R.D.) by its physical properties.

A sample of slime mold (22.1 g dry weight) was extracted by 90% butanol. Chromatography of the extract on a Sephadex LH-20 column (100 × 5 cm) with elution with MeOH gave eight fractions (8 mL) 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 C 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** (11.3 mg) and **2** (7.2 mg).

Compound **1** (8.8 mg) was dissolved in 5% hydrogen chloride (8 mL) and heated under reflux for 4 h. The reaction mixture was neutralized with silver carbonate, filtered and the filtrate extracted

with diethyl ether. In addition, the water layer was evaporated to dryness and an α,β-anomeric mixture of L-amicetose $[\alpha]_D^{21} = -39.4$ ($c = 0.32$, acetone) was obtained as a colorless syrup.

The water-soluble material after hydrolysis of **2** was chromatographed on an NH_2-HPLC column with water/acetonitrile (8:2) to yield α,β-anomeric mixtures of L-amicetose (see above) and L-rhodinose $[\alpha]_D^{21} = -10.1$ ($c = 0.12$, MeOH) which was also obtained as a colorless syrup.

The evaporated diethyl ether extract (after hydrolysis of **1** and **2**) was treated with 5% sodium hydroxide-water for 1 h under reflux. The solution was then acidified with dilute HCl, evaporated to dryness, and dissolved in methanol (10.0 mL) that was cooled in a dry ice-acetone bath (−78 °C). Ozone was bubbled through the solution for 5−10 min. Excess ozone was then removed with a stream of nitrogen for 2 min. Sodium cyanoborohydride (23.4 mg) was added at −78 °C and the mixture was stirred for 1 h, then acetic acid (29 μL) was added. After an additional 30 min stirring at room temperature, the solution was evaporated to dryness. The residue was dissolved in water and applied to an ODS cartridge. The cartridge was washed with distilled water to remove inorganic salts and the crude ozonolysis products were eluted with methanol and further chromatographed on a silica-gel column with methanol (flow rate 1 mL/ min) to yield products **3**, **7** and **9** (checked by TLC on silica gel *n*BuOH/AcOH/H_2O, 60:15:25).

Compound **3** was dissolved in methanol (0.5 mL) and 10% *N,O*-bis(trimethylsilyl)trifluoracetamide (BSTFA) in hexane (0.5 mL) was added. After 3 min, 2 μL of the reaction solution was analyzed by chiral GC (see above for conditions).

Compound **7** was further oxidized as described previously.[33] Briefly, **7** was added gradually to a mixture containing 1.5 parts of nitric acid, 1 part of water and 0.0125 parts of ammonium metavanadate. The mixture was then heated to 110−114 °C for 60 min and treated with ethereal diazomethane solution. The resulting dimethyl ester of **8** was chromatographed by chiral GC.

Compound **9** was dissolved in pyridine (0.5 mL), and pivaloyl chloride (12 μL) was added. After 15 min the reaction solvents were evaporated to dryness. The residue was subjected to TLC (benzene/ethyl acetate, 1:1) to yield 1.5 mg of dipivaloyl ester **10**. This was dissolved in 2,2-dimethoxypropane (1 mL), and a catalytic amount of *p*-toluenesulfonate was added. After 2 h the reaction mixture was diluted with diethyl ether (4.0 mL) and passed through a cartridge with basic alumina; the eluent was evaporated to yield **11**. HRMS calcd. for $C_{24}H_{44}O_6$ $[M]^+$: 428.3138, found 428.3140.

Dipivaloyl ester **10** was dissolved in pyridine (1.0 mL), and 4-chlorobenzoyl chloride (20 μL) and a catalytic amount of 4-dimethylaminopyridine were added. After 18 h methanol (2.0 mL) and hexane (1 mL) were added, and the reaction solvents were evaporated to dryness. The residue was subjected to TLC (benzene/EtOAc, 9:1) to yield **12**. HRMS calcd. for $C_{35}H_{46}{}^{35}Cl_2O_8$ $[M]^+$: 664.3192, found 664.3201.

(3*S*)-3-Hydroxy-4-butanolide (5): Borane−dimethyl sulfide complex (11 mmol) was added to a solution of **4** [(*S*)-malic acid; 10 mmol] in THF (10 mL) and the mixture was stirred at room temperature for 30 min. Then, sodium borohydride (1.2 mmol) was added to the mixture and stirred for an additional 30 min. Methanol (2 mL) was added and the mixture was concentrated. The residue was treated with trifluoroacetic acid (100 μL) in CH_2Cl_2 (2 mL) at room temperature for one day. After concentration, the residue was treated again with trifluoroacetic acid (150 μL) in CH_2Cl_2 (2 mL) at room temperature for two days. The mixture was concentrated and purified by TLC (hexane/EtOAc, 2:3) to afford the lactone **5** as a colorless oil, $[\alpha]_D^{22} = -82.0$ ($c = 0.4$, EtOH). $[\alpha]_D^{25} = -85.9$ ($c = 2.2$, EtOH).[34] ^{1}H NMR (CD_3OD): δ = 2.47 (dd, $J = 18.1$, 0.9 Hz, 1 H, H-2a), 2.82 (dd, $J = 18.1$, 5.9 Hz, 1 H, H-2b), 4.22 (dd, $J = 10.9$, 1.1 Hz, 1 H, H-4b), 4.30 (dd, $J = 10.9$, 4.7 Hz, 1 H, H-4a), 4.47 (dddd, $J = 5.9$, 4.7, 1.1, 0.9 Hz, 1 H, H-3) ppm. HRMS calcd. for $C_4H_6O_3$ $[M]^+$: 102.0317, found 102.0321.

(2*S*,3*S*)-3-Hydroxy-2-methyl-4-butanolide (6): A solution of lactone **5** (4.2 mmol) in THF (8 mL) was added to a dry flask containing lithium diisopropylamide [17 mmol; prepared from diisopropylamine (18 mmol) and *n*-butyllithium (17 mmol, 1.60 M in hexane)] in THF (15 mL) at −78 °C. After 1 h at −78 °C, the resulting solution was transferred to a stirred, cooled (−78 °C) solution of methyl iodide (0.11 mmol) in THF (30 mL). After 6 h the reaction was quenched with glacial acetic acid (1 mL). The reaction mixture was warmed to room temperature and stirred overnight. The resulting insoluble material was removed by filtration and the filtrate was concentrated. TLC (hexane/EtOAc, 1:1) afforded **6** as a pale oil, $[\alpha]_D^{22} = -63.5$ ($c = 0.3$, $CHCl_3$). $[\alpha]_D^{25} = -64.2$ ($c = 1.0$, $CHCl_3$).[34] ^{1}H NMR (CD_3OD): δ = 1.52 (d, $J = 7.5$ Hz, 3 H, H-5), 2.58 (dd, $J = 7.5$, 6.5 Hz, 1 H, H-2), 4.13 (dd, $J = 10.1$, 5.1 Hz, 1 H, H-4b), 4.32 (ddd, $J = 6.5$, 5.6, 5.1 Hz, 1 H, H-3), 4.51 (dd, $J = 10.1$, 5.9 Hz, 1 H, H-4a) ppm. HRMS calcd. for $C_5H_8O_3$ $[M]^+$: 116.0473; found 116.0476.

(2*S*,3*S*)-3-Methylbutane-1,2,4-triol (3): $LiAlH_4$ (75 μL, 1.0 M in diethyl ether) was added to a solution of **6** (3 mmol) in diethyl ether (4 mL) at −78 °C under an argon atmosphere. The resulting solution was stirred for 30 min and then quenched by the addition of 1 M $KHSO_4$ (1.0 mL). The mixture was extracted with EtOAc (10 mL). The organic layer was washed with brine (3 mL), dried (Na_2SO_4), filtered, and then concentrated. The residue gave **3** as a colorless oil, which was used for the next step without further purification. $[\alpha]_D^{22} = -5.5$ ($c = 0.14$, $CHCl_3$), $[\alpha]_D^{22} = -5.7$.[34] ^{1}H NMR (CD_3OD): δ = 0.84 (d, $J = 7.0$ Hz, 3 H, H-5), 1.73 (dddd, $J = 8.6$, 7.0, 3.0, 0.7 Hz, 1 H, H-3), 3.18 (ddd, $J = 7.3$, 3.0, 2.6 Hz, 1 H, H-2), 3.23 (dd, $J = 11.7$, 2.6 Hz, 1 H, H-1a), 3.52 (dd, $J = 11.7$, 7.3 Hz, 1 H, H-1b), 3.62 (dd, $J = 11.2$, 8.6 Hz, 1 H, H-4a), 3.82 (dd, $J = 11.2$, 0.7 Hz, 1 H, H-4b) ppm.

Lycoperdinoside A (1): Colorless powder (11.3 mg). $[\alpha]_D^{23} = +63$ ($c = 0.015$, CH_2Cl_2). UV (MeOH) : $\lambda_{max.}$ (log ε) = 243 (4.45) nm. IR (film): $\tilde{\nu}_{max.}$ = 3460 (OH), 2900, 1725 (C=O) cm^{-1}. HRFABMS: m/z = 673.4320 $[M + H]^+$, calcd. for $[C_{39}H_{60}O_9 + H]^+$ 673.4315; negative FABMS: m/z = 671 $[M - H]^-$, 557 $[M - H - 114]^-$, 443 $[M - H - 2 \times 114]^-$. NMR spectroscopic data see Table 1 and 2.

Lycoperdinoside B (2): Colorless powder (7.2 mg). $[\alpha]_D^{23} = +102$ ($c = 0.01$, CH_2Cl_2). UV (MeOH): $\lambda_{max.}$ (log ε) = 243 (4.45) nm. IR (film): $\tilde{\nu}_{max.}$ = 3460 (OH), 2900, 1725 (C=O) cm^{-1}. HRFABMS: m/z = 787.5001 $[M + H]^+$, calcd. for $[C_{45}H_{71}O_{11} + H]^+$ 787.4996; negative FABMS: m/z = 785 $[M - H]^-$, 671 $[M - H - 114]^-$, 557 $[M - H - 2 \times 114]^-$, 443 $[M - H - 3 \times 114]^-$. NMR spectroscopic data see Table 1 and 2.

[1] L. Rakoczy, in *The Blue Light Syndrome* (Ed.: H. Senger), Berlin: Springer, **1980**; pp. 570−583.
[2] T. Nakagaki, S. Umemura, Y. Kakiuchi, T. Ueda, *Photochem. Photobiol.* **1996**, *64*, 859−862.
[3] B. Steffan, M. Praemassing, W. Steglich, *Tetrahedron Lett.* **1987**, *28*, 3667−3670.
[4] A. Nowak, B. Steffan, *Liebigs Ann. Recl.* **1997**, 1817−1821.

[5] A. Nowak, B. Steffan, *Angew. Chem. Int. Ed.* **1998**, *37*, 3139−3141.
[6] S. Eisenbarth, B. Steffan, *Tetrahedron* **2000**, *56*, 363−365.
[7] F. Blumenthal, K. Polborn, B. Steffan, *Tetrahedron* **2002**, *58*, 8433−8437.
[8] H. Kikuchi, Y. Saito, J. Komiya, Y. Takaya, S. Honma, N. Nakahata, A. Ito, Y. Oshima, *J. Org. Chem.* **2001**, *66*, 6982−6987.
[9] T. Sawada, M. Aono, S. Asakawa, A. It, K. Awano, *J. Antibiotics* **2000**, *53*, 959−966.
[10] P. Comes, H. Kleinig, *BBA − Lipid. Lipid Met.* **1973**, *316*, 13−18.
[11] T. Rezanka, *Phytochemistry* **2002**, *60*, 639−646.
[12] T. Rezanka, R. Dvorakova, *Phytochemistry* **2003**, *63*, 945−952.
[13] T. Hashimoto, K. Akazawa, M. Toil, Y. Kan, T. Kusumi, H. Takahashi, A. Asakawa, *Chem. Pharm. Bull.* **1994**, *42*, 1531−1533.
[14] T. Hashimoto, A. Yasuda, K. Akazawa, S. Takaoka, M. Toil, A. Asakawa, *Tetrahedron Lett.* **1994**, *35*, 2559−2560.
[15] R. Frode, C. Hinze, I. Josten, N. Schmidt, B. Steffan, W. Steglich, *Tetrahedron Lett.* **1994**, *35*, 1689−1690.
[16] M. S. Buchanan, T. Hashimoto, Y. Asakawa, *Phytochemistry* **1996**, *41*, 791−794.
[17] M. Kobayashi, W. Q. Wang, Y. Tsutsui, M. Sugimoto, N. Murakami, *Tetrahedron Lett.* **1998**, *39*, 8291−8294.
[18] F. Yokokawa, H. Fujiwara, T. Shioiri, *Tetrahedron* **2000**, *56*, 1759−1775.
[19] G. Catelani, F. Colonna, P. Rollin, *Gazz. Chim. Ital.* **1989**, *119*, 389−393.
[20] G. Berti, P. Caroti, G. Catelani, L. Monti, *Carbohyd. Res.* **1983**, *124*, 35−42.
[21] S. D. Rychnovsky, B. N. Rogers, T. I. Richardson, *Accounts Chem. Res.* **1998**, *31*, 9−17.
[22] N. Harada, A. Saito, H. Ono, J. Gawronski, K. Gawronska, T. Sugioka, H. Uda, T. Kuriki, *J. Am. Chem. Soc.* **1991**, *113*, 3842−3850.
[23] N. Matsumoto, T. Tsuchida, H. Nakanuta, R. Sawa, Y. Takahashi, H. Naganawa, H. Jinyma, T. Sawa, T. Takeuchi, M. Shiro, *J. Antibiot.* **1999**, *52*, 276−280.
[24] L. M. Canedo, J. L. F. Puentes, J. P. Baz, X. H. Huang, K. L. Rinehart, *J. Antibiot.* **2000**, *53*, 479−483.
[25] S. Hatakeyama, K. Sakurai, S. Takano, *Heterocycles* **1986**, *24*, 633−636.
[26] S. Servi, *J. Org. Chem.* **1985**, *50*, 5865−5867.
[27] Y. Takaya, H. Kikuchi, Y. Terui, J. Komiya, K. Furukawa, K. Seya, S. Motomura, A. Ito, Y. Oshima, *J. Org. Chem.* **2000**, *65*, 985−989.
[28] Y. Takaya, H. Kikuchi, Y. Terui, J. Komiya, Y. Maeda, A. Ito, Y. Oshima, *Tetrahedron Lett.* **2001**, *42*, 61−63.
[29] H. Kikuchi, J. Komiya, Y. Saito, J. Sekiya, S. Honma, N. Nakahata, Y. Oshima, *Tetrahedron Lett.* **2002**, *43*, 1477−1480.
[30] S. Omura, H. Tomoda, N. Tabata, Y. Ohyama, T. Abe, M. Namikoshi, *J. Antibiot.* **1999**, *52*, 586−589.
[31] H. Tomoda, Y. Ohyama, T. Abe, N. Tabata, M. Namikoshi, Y. Yamaguchi, R. Masuma, S. Omura, *J. Antibiot.* **1999**, *52*, 689−694.
[32] N. Tabata, Y. Ohyama, H. Tomoda, T. Abe, M. Namikoshi, S. Omura, *J. Antibiot.* **1999**, *52*, 815−826.
[33] O. Buddenberg, in *Houben-Weyl Methoden der Organischen Chemie*, Georg Thieme Verlag: Stuttgart, **1981**; Band 4, Teil 1a, Oxidation, Anorganische Stickstoff-Verbindungen als Oxidationsmittel, pp. 720−726.
[34] A. Bernardi, S. Cardani, C. Scolastico, R. Villa, *Tetrahedron* **1990**, *46*, 1987−1998.

Received September 14, 2003

