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Macrolactone glycosides of three lichen acids from *Acarospora gobiensis*, a lichen of Central Asia

Tomáš Řezanka[a,*], Irina A. Guschina[b]

[a]*Institute of Microbiology, Vídeňská 1083, 14220 Prague, Czech Republic*
[b]*Institute of Ecology of the Volga River Basin of the Russian Academy of Sciences, Togliatti 445003, Russia*

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Abstract

The compounds isolated from the extract of Central Asian lichen (*Acarospora gobiensis* H. Magn.) comprised three new glycosides having 18-hydroxy-dihydroalloprotolichesterinic, 18-hydroxy-neodihydroprotolichesterinic and 18-hydroxy-dihydroprotolichesterinic acids as aglycones and a di- or trisaccharide moiety linked at C-18 and at the carboxylic group. These compounds, called gobienines A–C, were found to be di- or trisacharides forming a macrolactone with the aglycone. The structures were elucidated by using extensive spectroscopic analysis (1D and 2D NMR, MS, IR and ORD) and chemical and enzymatic methods. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Macrolactone glycoside esters; Lichens of Central Asia; *Acarospora gobiensis*; Acarosporaceae; NMR; MS; IR spectra

1. Introduction

Macrolactone glycosides containing an aglycone and di- or trisaccharides are found very rarely in nature (Ikan, 1999). At present only a few examples of these unusual compounds are known to come from living organisms. The best known are compounds isolated from tropical and subtropical plants, mainly of the genus *Ipomaea* and/or family Convolvulaceae (Noda et al., 1988; Hernandez-Carlos et al., 1999; Lehmann et al., 2000). Further examples of macrocyclic lactones were isolated from a Japanese mountain shrub (*Polygala*) or roots of the genera *Sonchus*; all are glycosides that have a terpenic aglycone and two sugars (Iwagawa and Hase, 1983; Helal et al., 2000; Kobayashi et al., 2000).

Similar compounds have also been reported in *Candida bogoriensis* and *Torulopsis* (*Candida*) *bombicola* (Spencer et al., 1970). The glycolipids produced by these yeast strains contain the disaccharide sophorose (β-D-Glc-(1-2)-D-Glc) linked to a lipid moiety containing a 17-hydroxyoctadecanoic acid.

As a continuation of our search for unusual bioactive compounds from Central Asian lichens (Řezanka and Dembitsky, 1998, 1999; Řezanka and Guschina, 1999, 2000, 2001a,b), we examined the lichen *Acarospora gobiensis* H. Magn.

2. Results and discussion

Detailed investigation of fractions of oligosaccharide mixtures from *A. gobiensis* led to the isolation of three additional glycosides. These compounds were purified to homogeneity by HPLC using an amino bonded silica gel column. Structural investigation of these three new macrolactone di- and trisaccharides of lichen acids, gobienine A (**1**), gobienine B (**2**) and gobienine C (**3**) was performed by NMR and FAB–MS. Their aglycones are the following lichen acids: 18-hydroxy-dihydroalloprotolichesterinic, 18 - hydroxy - neodihydroprotolichesterinic and 18-hydroxy-dihydroprotolichesterinic acid. All were present in the relatively small amounts ($<0.01\%$ of dry mass).

Compound **1** gave either positive or negative-ion FAB–MS. The positive ion in the mass spectrum of gobienine A (**1**) was a pseudo-molecular ion at m/z 677.3751 $(M+H)^+$ which indicates the molecular formula $C_{33}H_{56}O_{14}$.

* Corresponding author. Tel.: +42-2-4752300; fax: +42-2-4752347.
E-mail address: rezanka@biomed.cas.cz (T. Řezanka).

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In addition to the common fragment peaks produced by a glycosidic cleavage of the sugar moieties in each of the oligosaccharide cores (Fig. 1), i.e. m/z 513 $(M–H-162)^-$, m/z 351 (513–162 (hexose); (aglycone))$^-$, the negative ions of compound **1** determined by FAB–MS were observed at m/z 675 $(M–H)^-$.

Treatment of **1** with esterase (EC 3.1.1.1 from porcine liver) gave compound **1a** (37.1%) together with unreacted **1**. The positive ion FAB mass spectrum of **1a** exhibited a $[M+H]^+$ ion peak at m/z 695. The negative FAB–MS of **1a** showed an $(M–H)^-$ peak at m/z 693 along with the fragment peak at m/z 369 (M–H-324 (2× hexose units))$^-$ and a peak at m/z 531 (M–H-162 (hexose unit))$^-$ in place of that detected at m/z 513 in the spectrum of **1**. The difference of 18 mass units indicated (see above) that the ester linkage of the lichen acid is placed at C-2 of second glucose (Glu″).

The use of various one- and two-dimensional NMR spectroscopic methods provided information about the interglycosidic linkage, the sequence of monosaccharide residues and the site of esterification in all these complex oligosaccharides. The ^{1}H NMR spectrum of **1a**, when compared with that of **1**, showed an upfield shift (δ 1.52 ppm) of H-2″ of the second glucose (Glc″) and a disappearance of the signal at δ 5.73 indicating a loss of lactonization. The ester linkage of the aglycone is therefore located at 2-OH of Glc″.

The acid-catalyzed hydrolysis of **1a** gave a mixture of degradation products from the aglycone and the monosaccharide. The two saccharide units were identified as D-glucose by using the acetylated-2-butyl derivatives prepared from glycosides and analyzed by gas chromatography using a capillary column (Supelco SPB-1) (Gerwig et al., 1978).

The ^{1}H NMR spectrum of **1a** showed the signal of H-18 at δ 3.76. The proton and carbon signals arising from the sugar moiety were assigned with the aid of ^{1}H–^{1}H and ^{1}H–^{13}C two-dimensional shift-correlated NMR (COSY) analyses (Tables 2 and 4).

The ^{13}C NMR spectrum of **1a** showed glycosylation shifts only at C-2′ (Δ 5.7 ppm) of the first glucose relative to those of corresponding methyl pyranoside (δ_{C-2} = 74.8) (Seo et al., 1978).

The enzymatic hydrolysis of **1a** by β-glucosidase (EC 3.2.1.21) gave **1b** (EI–MS, m/z 370 $(M)^+$). The ^{1}H NMR, ^{13}C NMR and also ORD spectra of **1b** revealed it to the 18-hydroxydihydroallo-protolichesterinic acid, previously described by Řezanka and Guschina (2001b). The coupling constants of both anomeric proton signals of the sugar moiety indicated that the mode of glycosidic linkage of the two glucopyranose units is β (Kasai et al., 1977; Tori et al., 1977). The above data indicated that **1a** is (18*R*)-18-hydroxydihydroalloprotolichesterinic acid 18-*O*-β-D-glucopyranosyl-(1″-2′)-*O*-β-D-glucopyranoside.

To complete the determination of the primary structure of compound **1**, ^{1}H and ^{13}C NMR spectra of this compound were recorded. Gobienine A (**1**) showed a well resolved ^{1}H NMR spectrum (no-overlap in the proton region between δ 3.8 and 4.4). No derivatization was needed to achieve the total structural assignment of gobienine A (**1**) which was readily made through an extensive investigation using NMR spectroscopy. A combination of ^{1}H NMR spectra with 2-D homonuclear techniques including DQF-COSY and TOCSY allowed all C-bonded protons to be sequentially assigned within

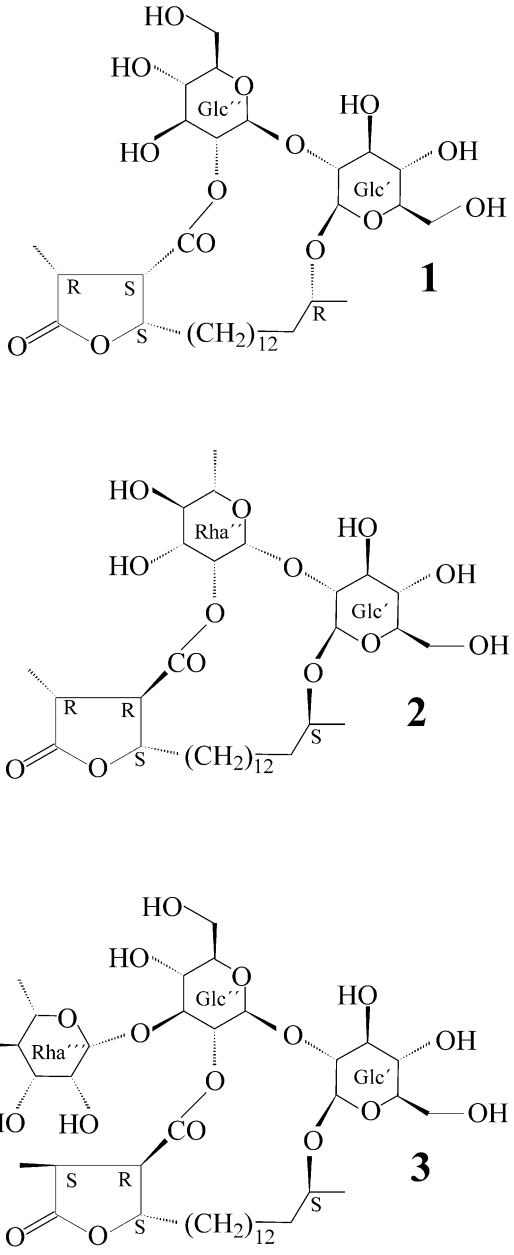

Fig. 1. The structures of gobienines A–C: (**1**) (18*R*)-18-hydroxydihydroalloprotolichesterinic acid 18-*O*-β-D-glucopyranosyl-(1″-2′)-*O*-β-D-glucopyranoside-(21,2″-lactone), (**2**) (18*S*)-hydroxyneodihydroprotolichesterinic acid 18-*O*-α-L-rhamnopyranosyl-(1″-2′)-*O*-β-D-glucopyranoside-(21,2″-lactone), (**3**) (18*S*)-hydroxydihydroprotolichesterinic acid 18-*O*-α-L-rhamnopyranosyl-(1‴-3″)-*O*-β-D-glucopyranosyl-(1″-2′)-*O*-β-D-glucopyranoside-(21,2″-lactone).

Table 1
^{1}H NMR data of **1b**–**3b**

H No.	**1b**	**2b**	**3b**
2	2.88 (1H, *dq*, $J_{2,3}=7.0$, $J_{2,20}=7.5$)	2.91 (1H, *dq*, $J_{2,3}=9.0$, $J_{2,20}=7.5$)	2.92 (1H, *dq*, $J_{2,3}=11.1$, $J_{2,20}=7.2$)
3	3.24 (1H, *dd*, $J_{3,2}=7.0$, $J_{3,4}=8.0$)	3.09 (1H, *dd*, $J_{3,2}=9.0$, $J_{3,4}=6.5$)	3.26 (1H, *dd*, $J_{3,2}=11.1$, $J_{3,4}=9.5$)
4	4.57 (1H, *ddd*, $J_{4,3}=8.0$, $J_{4,5}=8.0$ and 5.0)	4.65 (1H, *ddd*, $J_{4,3}=6.5$, $J_{4,5}=8.0$ and 4.5)	4.40 (1H, *ddd*, $J_{4,3}=9.5$, $J_{4,5}=8.0$ and 4.0)
5	1.68 (2H, *m*)	1.67 (2H, *m*)	1.70 (2H, *m*)
6–16	1.26 (22H, *m*)	1.26 (22H, *m*)	1.26 (22H, *m*)
17	1.54 (2H, *m*)	1.57 (2H, *m*)	1.56 (2H, *m*)
18	3.76 (1H, *m*)	3.80 (1H, *m*)	3.79 (1H, *m*)
19	1.16 (3H, *d*, $J=6$)	1.17 (3H, *d*, $J=6$)	1.17 (3H, *d*, $J=6$)
20	1.30 (3H, *d*, $J_{20,2}=7.5$)	1.31 (3H, *d*, $J_{20,2}=7.5$)	1.32 (3H, *d*, $J_{20,2}=7.2$)

Table 2
^{1}H NMR data of glycosides **1**–**3**

	1	**1a**	**2**	**3**
Glc 1′	5.78 (*d*, $J=7.1$)	5.64 (*d*, $J=7.6$)	5.81 (*d*, $J=7.8$)	5.65 (*d*, $J=7.7$)
2′	4.18 (*dd*, $J=7.1$, 8.1)	4.12 (*dd*, $J=7.6$, 8.1)	4.07 (*dd*, $J=7.8$, 8.0)	4.20 (*dd*, $J=8.5$, 7.7)
3′	4.26 (*dd*, $J=8.1$, 8.2)	4.18 (*dd*, $J=8.1$, 8.0)	4.12 (*dd*, $J=8.0$, 8.1)	4.10 (*dd*, $J=8.5$, 8.4)
4′	4.10 (*dd*, $J=8.2$, 8.1)	4.05 (*dd*, $J=8.0$, 8.2)	4.05 (*dd*, $J=8.1$, 8.1)	4.04 (*dd*, $J=8.4$, 8.8)
5′	3.91 (*ddd*, $J=8.1$, 6.5, 2.9)	3.90 (*ddd*, $J=8.2$, 6.1, 2.8)	3.91 (*ddd*, $J=8.1$, 6.2, 2.9)	3.94 (*ddd*, $J=8.8$, 6.2, 3.0)
6′	4.21 (*dd*, $J=11.0$, 6.5)	4.23 (*dd*, $J=10.9$, 6.1)	4.20 (*dd*, $J=10.5$, 6.2)	4.22 (*dd*, $J=10.9$, 3.0)
	4.34 (*dd*, $J=11.0$, 2.9)	4.31 (*dd*, $J=10.9$, 2.8)	4.27 (*dd*, $J=10.5$, 2.9)	4.34 (*dd*, $J=10.9$, 6.2)
Glc1″	5.61 (*d*, $J=7.5$)	5.60 (*d*, $J=7.7$)		5.68 (*d*, $J=7.3$)
2″	5.73 (*dd*, $J=7.5$, 8.0)	4.21 (*dd*, $J=7.7$, 8.0)		5.81 (*dd*, $J=7.3$, 8.0)
3″	4.23 (*dd*, $J=8.0$, 8.0)	4.25 (*dd*, $J=8.0$, 8.0)		4.16 (*dd*, $J=8.0$, 8.0)
4″	4.07 (*dd*, $J=8.0$, 8.1)	4.08 (*dd*, $J=8.0$, 8.1)		4.06 (*dd*, $J=8.0$, 8.1)
5″	3.88 (*ddd*, $J=8.1$, 6.1, 3.2)	3.92 (*dd*, $J=8.1$, 6.8, 3.2)		3.96 (*ddd*, $J=8.1$, 6.5, 3.1)
6″	4.19 (*dd*, $J=10.8$, 6.1)	4.16 (*dd*, $J=10.9$, 6.8)		4.18 (*dd*, $J=11.2$, 6.5)
6″	4.30 (*dd*, $J=10.8$, 3.2)	4.27 (*dd*, $J=10.9$, 3.2)		4.32 (*dd*, $J=11.2$, 3.1)
Rha1″			6.19 (*d*, $J=1.2$)	
2″			6.14 (*dd*, $J=1.2$, 3.7)	
3″			4.62 (*dd*, $J=9.5$, 3.7)	
4″			4.29 (*dd*, $J=9.5$, 9.7)	
5″			4.87 (*dq*, $J=9.7$, 6.6)	
6″			1.59 (*d*, $J=6.6$)	
Rha1‴				6.08 (*d*, $J=1.3$)
2‴				4.78 (*dd*, $J=1.3$, 3.9)
3‴				4.65 (*dd*, $J=9.9$, 3.9)
4‴				4.29 (*dd*, $J=9.9$, 9.5)
5‴				4.74 (*dq*, $J=9.5$, 6.8)
6‴				1.55 (*d*, $J=6.8$)

each ring system (Table 2) and all the resonances in the ^{13}C NMR spectra were assigned by using a ^{1}H-detected (^{1}H–^{13}C) one-bond correlation experiment (HMQC) (Table 4). The interglycosidic connectivities, as well as the site of lactonization, were established entirely on the basis of the observed ^{13}C–^{1}H long-range cross peaks ($^2J_{CH}$ and $^3J_{CH}$) in HMBC experiments. For example, ^{13}C–^{1}H long-range cross peaks (^3J) were observed in the spectrum of gobienine A (**1**) between C-1′ (δ 103.4) of the first glucose and H-18 of the aglycone and C-1″ of the second glucose (δ_C 103.8) and H-2′ of the first glucose (δ_H 4.18). A series of gated decoupling experiments was performed for further determination of the anomeric configuration of the glycosyl linkages, from the completely resolved multiplets for the two anomeric ^{13}C atoms of compounds **1** (Table 4), all $^1J_{CH}$ values of the glucoses were found to be <165 Hz. Such coupling constants established the mode of glycosidic linkage to be β (Collins and Ferrier, 1995) type for each of the monosaccharide units.

From the ^{1}H NMR spectra (Table 2), the esterified positions on the disaccharide cores were easily identified through strong deshielding effects relative to the non-esterified equivalents on **1a**. In this way, the primary structure of gobienine A (**1**) was established as (18*R*)-18-hydroxydihydroalloprotolichesterinic acid 18-*O*-β-D-

glucopyranosyl-(1″-2′)-*O*-β-D-glucopyranoside-(21,2″-lactone).

In **2**, both positive and negative FAB–MS were obtained (*m/z* 661.3801, $(M+H)^+$, i.e. $C_{33}H_{56}O_{13}$,). For compound **3**, the positive ion mode revealed a quasi-molecular ion peak $(M+H)^+$ at *m/z* 823.4331 indicating a molecular formula of $C_{39}H_{66}O_{18}$. The negative ion FAB–MS of compounds **2** and **3** showed the same fragmentation pathways that resulted from the cleavage of glucoside-ester bonds that yields high-mass fragment ions representing each monosaccharide and aglycone. Compounds **2** and **3** showed also the pseudomolecular peaks at *m/z* 659 $(M–H)^-$ and *m/z* 821 $(M–H)^-$, respectively. In **2** the appropriate cleavages resulted in the presence of the ions at *m/z* 497 $(M–H\text{-}162)^-$, 351 [$(M–H\text{-}162–146)^-$; (aglycone)] while **3** yielded ions at *m/z* 821 $(M–H)^-$, 675 $(M–H\text{-}146)^-$, 513 $(M–H\text{-}146–162)^-$, 351 [$(M–H\text{-}146\text{-}2\times162)^-$; (aglycone)].

The site of lactonization of **2** was placed at C-2″ of rhamnose based on the observed ^3J-coupling between the carbonyl carbon of the aglycone (δ 162.7) and its geminal proton (δ ca. 6.4) in the pyranose unit. For **3**, the position of lactonization was verified by the observed connectivities between the carbonyl group of the aglycone (δ 163.1) and H-2″ of the second glucose.

The position of lactonization was also verified by the $^3J_{CH}$ connectivities between the carbonyl group of the aglycone and proton H-2 of the monosaccharide unit in both natural disaccharides as in compound **1**.

A series of gated decoupling experiments was performed for further determination of the anomeric configuration of the glycosyl linkages and the completely resolved multiplets for the two anomeric ^{13}C atoms of compounds **2** (Table 4) showed that all $^1J_{CH}$ values of the glucoses were <165 Hz. Such coupling constants established the mode of glycosidic linkage as β of the pyranose (Collins and Ferrier, 1995) type for each of the monosaccharide units. A different diglycoside was identified as the oligosaccharide core of gobienine B (**2**) and the structure of this natural product was determined to be (18*S*)-hydroxyneodihydroprotolichesterinic acid 18-*O*-α-L-rhamnopyranosyl-(1″-2′)-*O*-β-D-glucopyranoside-(21,2″-lactone).

The ^1H and ^{13}C signals arising from the sugar moieties in the spectra of compound **3** were assigned by ^1H–^1H DQ-COSY and ^1H–^{13}C HETCOR analyses (Tables 2 and 4). The ^{13}C NMR spectrum of **3** showed the glycosylation shifts at C-3″ (Δδ 6.3) of the second glucose relative to those of corresponding methyl pyranosides (Seo et al., 1978). The selective INEPT technique (Cordell and Kinghorn, 1991), applied to gobienine C (**3**), confirmed the nature of the saccharide substitution. Thus, irradiation of the anomeric proton H-1′ of the first glucose at δ 5.65 ($^3J_{CH}=6$ Hz) selectively enhanced C-18 of the aglycone. When H-2′ of the first glucose (δ 4.20) was irradiated ($^3J_{CH}=6$ Hz), a clear enhancement of the anomeric carbon C-1″ of the second glucose resulted (δ 103.1). Irradiation of the inner glucose H-3″ at δ 4.16 ($^3J_{CH}=6$ Hz) resulted in the polarization transfer to the anomeric carbon C-1‴ (δ 102.1) of the terminal rhamnose. The application of a COLOC NMR experiment in **3** further confirmed the sequence of the sugar moiety. ^{13}C-^1H long-range cross peaks were observed and unambiguously assigned as those between C-1′ of glucose and H-18 of the aglycone and C-1‴ of the terminal rhamnose and H-3″ of inner glucose.

Table 3
^{13}C NMR data of **1b**–**3b**

C No.	1b	2b	3b
1	177.5	178.9	177.8
2	36.4	36.0	36.8
3	51.8	52.0	51.5
4	77.5	79.3	78.6
5	31.2	32.1	32.3
6	25.6	25.8	24.6
7–15		28.1–29.8	
16	25.1	25.5	24.9
17	38.1	38.5	38.6
18	68.5	68.8	68.6
19	22.8	23.4	22.9
20	12.8	13.9	14.9
21	170.1	168.7	169.5

Table 4
^{13}C NMR data of glycosides **1**–**3**

	1	1a	2	3
Glc1′	103.4	101.8	104.5	103.7
2′	80.3	80.5	80.9	80.8
3′	77.4	78.9	78.1	77.1
4′	72.1	71.2	72.0	73.0
5′	76.1	74.9	75.1	75.0
6′	63.4	64.2	64.5	64.6
6′				
Glc1″	103.8	102.8		103.1
2″	80.2	74.5		80.4
3″	78.1	78.4		86.7
4″	72.6	72.7		72.0
5″	77.9	78.1		78.3
6″	64.1	63.3		64.4
6″				
Rha1″			101.9	
2″			79.8	
3″			72.2	
4″			74.1	
5″			69.1	
6″			18.3	
Rha1‴				102.1
2‴				71.6
3‴				72.4
4‴				73.9
5‴				69.6
6‴				18.7

In compound **3**, the $^1J_{CH}$ showed the following coupling value: 163.1 Hz for the first glucose, 164.7 Hz for the second glucose and 172.5 Hz for the rhamnose. The latter value is typical for the equatorial configuration of the anomeric proton, confirming the α-L-rhamnose residue (Etse et al., 1991).

Long-range heteronuclear coupling correlations were used to determine the linkages within the trisaccharide core of gobienine C (**3**). For instance a connectivity was observed between H-2″ of the second glucose at δ 5.81 and the anomeric carbon of the first glucose at δ 103.7, and between H-3‴ of rhamnose (δ 4.65) and C-1″ of the second glucose (δ 103.1).

The signals for the sugar moiety in the ^{1}H NMR spectra of **3** and **1a** were compared in order to establish the site of lactone linkage at the oligosaccharide core. Significant downfield shifts owing to acylation were observed for the inner glucose proton H-2″ (Δ 1.60 ppm).

The above mentioned spectral evidence indicated that compound **3** is a glycoside similar to those isolated from this source, having the carbonyl group of the aglycone linked to an oligosaccharide core to form a macrocyclic lactone. Its correct structure is (18*S*)-hydroxydihydroprotolichesterinic acid 18-*O*-α-L-rhamnopyranosyl-(1‴-3″)-*O*-β-D-glucopyranosyl-(1″-2′)-*O*-β-D-glucopyranoside-(21,2″-lactone).

3. Experimental

3.1. General experimental procedures

UV spectra were measured in heptane in the range of 200–350 nm by a Cary 118 (Varian) apparatus. Optical rotatory dispersion (ORD) measurement was carried out under dry N_2 on a Jasco-500A spectropolarimeter at 24 °C. A Perkin-Elmer Model 1310 (Perkin-Elmer, Norwalk, CT, USA) IR spectrophotometer was used for scanning IR spectroscopy of acids and 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). High- and also low-resolution MS were recorded using a VG 7070E-HF spectrometer (70 eV). HR–FAB–MS (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 chromatographic analysis was done on a Hewlett Packard HP 5980 gas chromatograph (Hewlett Packard Ltd., Czech Republic).

3.2. Plant material

The specimen of *Acarospora gobiensis* H. Magn., (Acarosporaceae, Lecanorales, Ascolichenes) was collected in July 1996 along the shore of Lake Issyk Kul, Tian-Shan mountains. The voucher specimens are deposited in the collection of the second author (I.A. Guschina).

3.3. Extraction and isolation

The lichen specimen [100 g of air-dried lichen, see Řezanka and Guschina, (2000)] was extracted by the Blight and Dyer (1959) method and the extract was chromatographed on a column of Sephadex LH-20 eluted with EtOH–i–PrOH (fraction 1), EtOH (fraction 2) and then MeOH (fraction 3). Fraction 3 was further fractionated by HPLC on a NH_2-Bondapak (Waters Ltd., Czech Republic) column (30 cm×7.8 mm, flow rate 1.8 ml/min) with ACN–H_2O (2:5) to yield compounds **1** (2.1 mg), **2** (1.4 mg) and **3** (0.6 mg).

The identification and the D or L configuration of sugars was determined using gas chromatography according to the method of Gerwig et al. (1978), with some modifications as previously described (Řezanka and Guschina, 2000).

3.4. Acid hydrolysis of *1a*

The glycoside (~0.1 mg) was refluxed in 2 N HCl (0.5 ml) for 2 h. The degradation products of the aglycone were extracted three times with EtOAc (10 ml). After separating the organic layer, the aqueous phase was neutralized with $NaHCO_3$ and lyophilized.

3.5. Enzymatic hydrolysis

A solution of glycoside (~0.1 mg) in acetate buffer (pH 4.4, 10 ml) was treated with β-glucosidase (EC 3.2.1.21) for 48 h at 37 °C. The reaction solution was evaporated to dryness, and the residue was chromatographed on a column of silica gel (10 g), using CH_2Cl_2–MeOH–H_2O (90:10:1) to provide lichen acids for ^{1}H NMR and ORD analysis.

Glycosides (~ 0.1 mg) were suspended in $(NH_4)_2SO_4$ solution of esterase (EC 3.1.1.1, from porcine liver) at pH ~8 and incubated 15 min at 25 °C. The aglycone was extracted three times with EtOAc (10 ml). After separating the organic layer, the aqueous phase was neutralized with 0.5 N HCl and lyophilized.

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

Treatment of each hydroxy acid (**1b**, **2b** and **3b**) with (*R*)-(−)-MTPACl or (*S*)-(+)-MTPACl converted them into the (*S*)- and (*R*)-2-methoxy-2-trifluoromethylphenylacetic acid (MTPA) esters (**1s**, **2s**, and **3s** and **1r**, **2r** and **3r**, respectively). The values of $\Delta\delta = [\delta(S\text{-MTPA ester}) - \delta(R\text{-MTPA ester})]$ in the ^{1}H NMR spectra suggested that the absolute configuration at C-18 of compound **1** was (*R*) while that of **2** and **3** were (*S*) (Ohtani et al., 1991b). The data are shown in Table 5.

Table 5
Stereochemical analysis of compounds **1b**, **2b** and **3b** with (*R*)- and (*S*)-MTPA (Mosher) derivatives[a]

MTPA esters of compound No.	$\Delta\delta = \delta_{S\text{-ester}} - \delta_{R\text{-ester}}$ (Hz)		
	H-19	H-17	Carbinol configuration
1b	+12.8	−30.1	*R*
2b	−16.9	+26.7	*S*
3b	−15.8	+34.2	*S*

[a] The aglycones from glycosides **1**–**3** are identical from one from **1b** or **2b** or **3b**.

(*S*)-MTPA esters of free acids (**1**–**3**) (Ohtani et al., 1991a, b). To a CH_2Cl_2 solution (100 μl) of aglycone (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 the evaporation of the solvent, the residue was purified by Silica gel TLC (hexane–AcOEt, 2:1) to provide the (*S*)-MTPA ester as colorless oil.

(*R*)-MTPA Esters of free acids (**1**–**3**). Each aglycone (0.3 mg) was treated with (*S*)-(+)-MTPACl (2.0 mg) by the same procedure as described above to provide the (*R*)-MTPA ester as colorless oil. The data are given in Table 5.

18*R*-Hydroxydihydroalloprotolichesterinic acid **1b**, white crystals, mp 143 °C, $[\alpha]_D^{24}$ −48° (*c* 0.19, $CHCl_3$), UV λ_{max} (EtOH, nm) 218 (log ε 2.28); ORD: $[\alpha]_{222}^{24}$ nm (−790), ^{1}H NMR, ^{13}C NMR and MS spectra, see (Řezanka and Guschina, 2001b).

18*S*-Hydroxydihydroprotolichesterinic acid **2b**, white small needles, mp 105–107 °C, $[\alpha]_D^{24}$ +7.9° (*c* 0.10, $CHCl_3$), UV λ_{max} (EtOH, nm) 207 (log ε 2.74); ORD: $[\alpha]_{220}^{24}$ nm (+90), ^{1}H NMR, ^{13}C NMR and MS spectra, see (Řezanka and Guschina, 2001b).

18*S*-Hydroxyneodihydroprotolichesterinic acid **3b**, white plates, mp 127.5 °C, $[\alpha]_D^{24}$ −28.5° (*c* 0.21, $CHCl_3$), UV λ_{max} (EtOH, nm) 217 (log ε 2.42); ORD: $[\alpha]_{220}^{24}$ nm (−260), ^{1}H NMR, ^{13}C NMR and MS spectra, see (Řezanka and Guschina, 2001b).

Gobienine A, (18*R*) - 18 - hydroxydihydroalloprotolichesterinic acid 18-*O*-β-D-glucopyranosyl-(1″-2′)-*O*-β-D-glucopyranoside-(21,2″-lactone) **1**, colorless powder, mp 187 °C, $[\alpha]_D^{24}$ −61° (*c* 0.07, MeOH); UV 219 nm (log ε 3.88); IR (KBr) (cm^{-1}): 3400 (OH), 2950, 2920, 1765 (lactone), 1710 (ester); HR–FAB–MS *m*/*z* 677.3751 $(M+H)^+$, calc. for $[C_{33}H_{56}O_{14}]^+$ 677.3748; negative FAB–MS *m*/*z* 675 $(M-H)^-$, 513 (M–H-162)$^-$, 351 (M–H-162-162)$^-$; ^{1}H NMR and ^{13}C NMR spectra see Tables 1–4.

(18*R*) - 18 - Hydroxydihydroalloprotolichesterinic acid 18-*O*-β-D-glucopyranosyl-(1″-2′)-*O*-β-D-glucopyranoside **1a**, colorless powder, mp 142 °C, $[\alpha]_D^{24}$ −82° (*c* 0.12, MeOH); UV 219 nm (log ε 3.88); IR (KBr) (cm^{-1}): 3400 (OH), 2950, 2920, 1770 (lactone), 1710 (ester); positive FAB *m*/*z* 695 $(M+H)^+$, negative FAB–MS *m*/*z* 693 (M–H)$^-$, 531 (M–H-162)$^-$, 369 (M–H-162-162)$^-$.

Gobienine B, (18*S*)-hydroxyneodihydroprotolichesterinic acid 18-*O*-α-L-rhamnopyranosyl-(1″-2′)-*O*-β-D-glucopyranoside-(21,2″-lactone) **2**, colorless powder, mp 105 °C, $[\alpha]_D^{24}$ −59° (*c* 0.08, MeOH); UV 219 nm (log ε 3.88); IR (KBr) (cm^{-1}): 3400 (OH), 2950, 2920, 1765 (lactone), 1710 (ester); HR–FAB–MS *m*/*z* 661.3801 $(M+H)^+$, calc. for $[C_{33}H_{56}O_{13}]^+$ 661.3798; negative FAB–MS *m*/*z* 659 (M–H)$^-$, 497 (M–H-162)$^-$, 351 (M–H-162-146)$^-$; ^{1}H NMR and ^{13}C NMR spectra see Tables 1–4.

Gobienine C, (18*S*)-hydroxydihydroprotolichesterinic acid 18-*O*-α-L-rhamnopyranosyl-(1‴-3″)-*O*-β-D-glucopyranosyl-(1″-2′)-*O*-β-D-glucopyranoside-(21,2″-lactone) **3**, colorless powder, mp 115 °C, $[\alpha]_D^{24}$ −152° (*c* 0.05, MeOH); UV 219 nm (log ε 3.88); IR (KBr) (cm^{-1}): 3400 (OH), 2950, 2920, 1765 (lactone), 1710 (ester); HR–FAB–MS *m*/*z* 823.4331 $(M+H)^+$, calc. for $[C_{39}H_{66}O_{18}]^+$ 823.4327; negative FAB–MS *m*/*z* 821 (M–H)$^-$, 675 (M–H-146)$^-$, 513 (M–H-146-162)$^-$, 351 (M–H-146-2×162)$^-$; ^{1}H NMR and ^{13}C NMR spectra see Tables 1–4.

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