PERGAMON

Phytochemistry 58 (2001) 509–516

PHYTOCHEMISTRY

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Glycoside esters from lichens of central Asia

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Received 5 December 2000; received in revised form 17 May 2001

Abstract

Ten compounds isolated from the extract of the central Asian lichens comprised new glycosides and glycoside esters having 18*R*-hydroxy-dihydroalloprotolichesterinic, 18*S*-hydroxy-dihydroprotolichesterinic and 18*S*-hydroxy-neodihydroprotolichesterinic acids, as the aglycones and a saccharide moiety linked at C-18 and also at C-21 made by glucose, xylose or rhamnose. The structures were elucidated using extensive spectroscopic analysis (1D and 2D NMR, MS, IR, UV and ORD) and by biochemical methods.

Keywords: Glycoside esters; Lichens of central Asia; NMR, MS, IR, ORD and UV spectra

1. Introduction

The lichens of the genera *Acarospora gobiensis, Cladonia furcata, Lecanora fructulosa, Leptogium saturninum, Peltigera canina, Rhizoplaca peltata, Xanthoparmelia camtschadalis, X. tinctina* and *Xanthoria elegans* are a central Asia species found in Tian-Shan mountains. The present study is the general phytochemical investigation carried out on these species and we report herein on the isolation and structure elucidation of seven new glycosides and glycoside esters.

Although many tens of thousands of plant glycosides and glycoside esters have been discovered in nature (Ikan, 1999), only a few of these have as yet been identified from lichens. These include, e.g. 1-(*O*-β-D-glucopyranosyl)-3*S*,25*R*-hexacosanediol from *Solorina crocea* (Huneck and Yoshimura, 1996), galapagin, lobodirin, mollin and roccellin (Huneck et al. 1992).

In our continuing search for unusual bioactive compounds from central Asian lichens (Řezanka and Dembitsky, 1998, 1999; Řezanka and Guschina, 1999, 2000a,b), we have examined the nine above-mentioned lichens. The ten compounds isolated from the extract of the thallus comprised new glycosides (**4**–**10**) having 18*R*-hydroxy-dihydroalloprotolichesterinic, 18*S*-hydroxy-dihydroprotolichesterinic and 18*S*-hydroxy-neodihydroprotolichesterinic acids as the aglycones and an oligosaccharide moiety linked at C-18 and C-21 made by glucose, xylose or rhamnose.

2. Results and discussion

In recent papers (Řezanka and Gushina, 2000a,b), we reported the isolation of lichen's acids, i.e. hydroxy-murolic, hydroxy-protoconstipatic, hydroxy-allo-murolic acids and their glycosides from the water–methanolic extract of the central Asian lichens. All nine lichen species were extracted independently and the extracts were also chromatographed separately by means of column chromatography over Sephadex LH-20 eluted with EtOH-i-PrOH (fraction 1), EtOH (fraction 2) and then MeOH (fraction 3). Repeated RP–HPLC of fraction 1 afforded new compounds (**1**), together with (**2**) and (**3**) (Table 1). Fraction 2 accounted for the largest amount of the EtOH extract by weight. Repeated column chromatography of this fraction afforded compounds (**4**–**6**) (Table 1). Fraction 3 was also purified by a further RP–HPLC method to give compounds (**7**–**10**) (Table 1). Comparing their spectral data, i.e. predominantly 1D and 2D NMR, MS, IR, UV and ORD with previously reported data performed the identification of the above-mentioned compounds. All compounds (**1**–**10**) are new natural products.

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0031-9422/01/$ - see front matter
PII: S0031-9422(01)00261-8

Table 1
Occurrence of acids and their derivatives from lichens of Tian Shan mountains

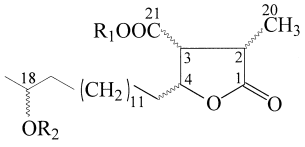
Name of lichen	Name of compounds; mg/100 g of dry weight									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Acarospora gobiensis	22.5	–[a]	–	8.7	–	–	–	–	–	–
Cladonia furcata	–	24.3	–	–	0.5	–	–	2.9	0.8	–
Lecanora fructulosa	–	13.5	–	–	3.8	–	–	3.4	1.9	–
Leptogium saturninum	–	–	19.8	–	–	6.8	7.1	–	–	3.9
Peltigera canina	–	–	25.4	–	–	0.9	0.9	–	–	1.4
Rhizoplaca peltata	–	19.1	–	–	4.2	–	–	6.3	1.4	–
Xanthoparmelia camtschadalis	–	22.7	–	–	0.2	–	–	5.7	3.1	–
X. tinctina	13.8	–	–	1.8	–	–	–	–	–	–
Xanthoria elegans	10.3	–	–	2.9	–	–	–	–	1.7	–

[a] –, Less than 0.1 mg/100 g of dry weight.

The unknown compound **1** isolated as white solid crystals was 18*R*-hydroxy-dihydroalloprotolichesterinic acid and was identified by comparison of its ^{1}H, ^{13}C NMR, MS, and ORD data with our and previously published values for dihydroalloprotolichesterinic acid (Huneck and Takeda, 1992). The HREIMS of **1** showed a molecular ion at *m*/*z* 370.5293. These data are consistent with the molecular formula of $C_{21}H_{38}O_5$. The ^{13}C NMR spectrum of **1** showed 21 signals corresponding to 21 carbons of acid, and DEPT measurements revealed the presence of two methyl groups, 13 methylenes, four methines (two bearing oxygen), and two oxygenated quaternary carbons at δ 170.1 and 177.5 assigned to carboxylic acid and lactone, respectively. The ^{1}H NMR spectrum of **1** showed signals at δ 2.93 (1H, *dq*, $J=7.0$, 7.5 Hz, H-2), 3.30 (1H, *dd*, $J=7.0$, 8.0 Hz, H-3), and 4.64 (1H, *ddd*, $J=8.0$, 8.0, 5.0 Hz, H-4), typical for a trisubstituted γ-butyrolactone acid. Examination of NMR, MS, and physical data for compound **1** showed that they are very similar to the data of (−)-dihydropertusaric and dihydroalloprotolichesterinic acids. The relative stereochemistry of **1** was assigned on the basis of the 2D NOESY spectrum, which exhibited the presence of NOEs, indicating that the Me on C-2 (C-20), H-3 and H-4 are oriented on the same side of the molecule, while H-2 has the same orientation as the lipophilic side chain (Fig. 1), suggesting that **1** have the following relative stereo configuration, i.e. 2*R*,3*S*,4*S*. The presence of signals at δ 1.17 (3H, *d*, $J=6$ Hz, H-19) and 3.74 (1H, *m*, H-18) were of the terminal hydroxyethyl group. The absolute configuration at C-18 of all three acids, i.e. **1**, **2** and **3** was determined by the modified Mosher's method (Ohtani et al., 1991a,b; Rieser et al., 1992).

Treatment of each hydroxy acid 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 7.

Compound **2**, i.e. 18*S*-hydroxy-dihydroprotolichesterinic acid, had according to the high-resolution mass spectrum the formula $C_{21}H_{38}O_5$. Its UV spectrum showed a maximum at 207 nm (log ε 2.74), indicative of a saturated γ-lactone. The ^{1}H NMR spectrum (Table 2) and ORD data (Fig. 2) of 18*S*-hydroxy-dihydroprotolichesterinic acid gave the structure and absolute configuration shown in formula 2 (Fig. 1). The ORD curve of compounds **2** is very similar to the ORD curve of (−)-dihydroprotolichesterinate (Huneck and Takeda, 1992), thus proving the same relative and absolute configuration of both compounds. Contrary to this the ORD spectra of 18*R*-hydroxy-dihydroalloprotolichesterinic acid (**1**) and 18*S*-hydroxy-neodihydroprotolichesterinic acid (**3**)



Compound	R_1	R_2	Carbon 2	Carbon 3	Carbon 4	Carbon 18
1	H	H	*R*	*S*	*S*	*R*
2	H	H	*S*	*R*	*S*	*S*
3	H	H	*R*	*R*	*S*	*S*
4	Rha	H	*R*	*S*	*S*	*R*
5	Glc	H	*S*	*R*	*S*	*S*
6	Glc	H	*R*	*R*	*S*	*S*
7	Rha	Glc	*R*	*S*	*S*	*R*
8	Glc	Xyl	*S*	*R*	*S*	*S*
9	Glc	Rha	*R*	*R*	*S*	*S*
10	Glc	Glc	*R*	*R*	*S*	*S*

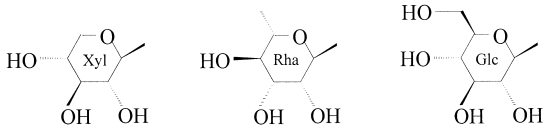


Fig. 1. The structures of hydroxy-lichesterinic acids (**1**, **2** and **3**) and their glycosides and glycoside esters (**4**–**10**) from lichens.

Table 2
^{1}H NMR spectral data of 18*R*-hydroxydihydroalloprotolichesterinic acid (**1**), 18*S*-hydroxydihydroprotolichesterinic acid (**2**) and 18*S*-hydroxyneodihydroprotolichesterinic acid (**3**), solvent see Experimental

H No.	**1**	**2**	**3**
2	2.93 (1H, *dq*, $J_{2,3}=7.0$, $J_{2,20}=7.5$)	2.97 (1H, *dq*, $J_{2,3}=9.0$, $J_{2,20}=7.5$)	2.99 (1H, *dq*, $J_{2,3}=11.1$, $J_{2,20}=7.2$)
3	3.30 (1H, *dd*, $J_{3,2}=7.0$, $J_{3,4}=8.0$)	3.12 (1H, *dd*, $J_{3,2}=9.0$, $J_{3,4}=6.5$)	3.30 (1H, *dd*, $J_{3,2}=11.1$, $J_{3,4}=9.5$)
4	4.64 (1H, *ddd*, $J_{4,3}=8.0$, $J_{4,5}=8.0$ and 5.0)	4.70 (1H, *ddd*, $J_{4,3}=6.5$, $J_{4,5}=8.0$ and 4.5)	4.44 (1H, *ddd*, $J_{4,3}=9.5$, $J_{4,5}=8.0$ and 4.0)
5	1.66 (2H, *m*)	1.69 (2H, *m*)	1.71 (2H, *m*)
6–16	1.26 (22H, *m*)	1.28 (22H, *m*)	1.27 (22H, *m*)
17	1.53 (2H, *m*)	1.58 (2H, *m*)	1.56 (2H, *m*)
18	3.74 (1H, *m*)	3.81 (1H, *m*)	3.78 (1H, *m*)
19	1.17 (3H, *d*, $J=6$)	1.16 (3H, *d*, $J=6$)	1.18 (3H, *d*, $J=6$)
20	1.31 (3H, *d*, $J_{20,2}=7.5$)	1.29 (3H, *d*, $J_{20,2}=7.5$)	1.33 (3H, *d*, $J_{20,2}=7.2$)

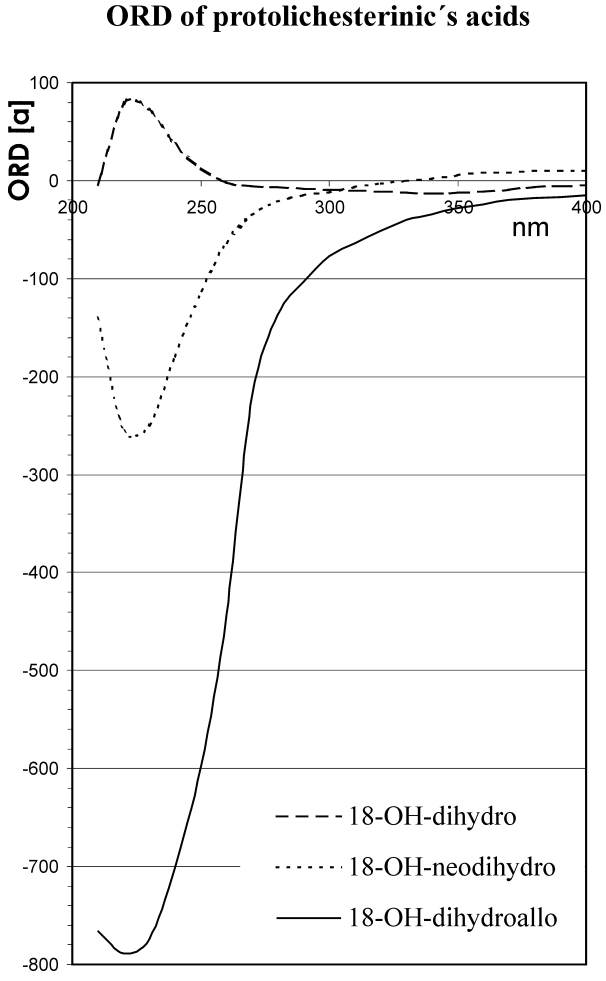


Fig. 2. The ORD spectra of lichen's acids [18*R*-hydroxy-dihydroalloprotolichesterinic (**1**), 18*S*-hydroxy-dihydroprotolichesterinic (**2**) and 18*S*-hydroxy-neodihydroprotolichesterinic (**3**)].

were quite different (Fig. 2). As Table 2 shows, the signal of H-18 in **2** (δ 3.81) is shifted downfield in relation to the chemical shift of H-18 in dihydroprotolichesterinic acid (δ 1.26) (Huneck and Takeda, 1992). The deshielding of the H-18 through the OH group in 2 causes this shift. Hence dihydro acid has relative stereochemistry 2*S*,3*R*,4*S*,18*S*.

HREIMS of compounds **3** showed the molecular ion at 370.5295 corresponding to the formula $C_{21}H_{38}O_5$, which implies three unsaturated sites. Its IR spectrum displayed two, intense carbonyl absorption, the stronger at 1740 cm^{-1} (lactone) and the second at 1705 cm^{-1} (acid). The remaining unsaturation was assigned to the lactone ring.

The spectrum of **3** showed double quartet (1H) at δ 2.99 ppm as well as a double doublet (1H) at δ 3.30 ppm and also signal at δ 4.44 (1H, *ddd*). The high relative values of coupling constants ($J_{2,3}=11.1$ Hz and $J_{3,4}=9.5$ Hz) indicate trans orientations of hydrogen on C-2, the hydrogen on C-3 and also the hydrogen on C-4. The absolute configuration on C-18 determined by Mosher's method is *S*. For data of chemical shifts, see Table 7. Thus, the structure of compound **3** is 18*S*-hydroxy- neodihydroprotolichesterinic acid.

Treatment of esters (**4**–**10**) with esterase (E.C. 3.1.1.1, from porcine liver) gave compounds (**4a**–**10a**) and free sugars. Their analysis by GC–MS according to Gerwig et al. (1978) indicated that the ester bound sugars were rhamnose (**4** and **7**) and glucose (**5**, **6**, **8**–**10**). According to ^{1}H NMR and ORD spectra, free acids **4a**–**6a** were identical with compounds **1**–**3**. Enzymatic hydrolysis of the compounds **7a**–**10a** by β-glucosidase (E.C. 3.2.1.21) liberated free sugars, i.e. glucose from **7** and **10**, xylose from **8** and rhamnose from **9**, and aglycones **7b**–**10b**. The latter were again identical with free acids **1** and **3**, according of their ^{1}H NMR and ORD spectra.

The combined positive ion HRFABMS (see Experimental) and NMR data (Tables 3 and 4) confirmed the molecular formula and identity of the acids and sugars present. The positive HRFABMS of al three esters (**4**–**6**) showed pseudomolecular ions $(M+H)^+$ and the negative ion FABMS spectra $(M-H)^-$ and $(M-H-146)^-$ for a deoxyhexosyl unit (**4**) and $(M-H-162)^-$ for a hexosyl unit (**5** and **6**). Substitution of the sugar at C-21 of the acid was unambiguously established from correlations of this carbon with the appropriate anomeric protons in the 2D HMBC NMR spectra.

Analysis of the ^{13}C NMR chemical shifts of sugar moieties (**7**–**10**), conducted by a combination of homo-

Table 3
^{13}C NMR spectral data of compounds **1**–**6**, solvent see Experimental

C No.	1	2	3	4	5	6
1	177.5, *s*	178.9, *s*	177.8, *s*	177.7, *s*	178.0, *s*	177.9, *s*
2	36.4, *d*	36.0, *d*	36.8, *d*	36.1, *d*	35.4, *d*	36.4, *d*
3	51.8, *d*	52.0, *d*	51.5, *d*	50.9, *d*	50.1, *d*	50.0, *d*
4	77.5, *d*	79.3, *d*	78.6, *d*	77.1, *d*	78.2, *d*	78.2, *d*
5	31.2, *t*	32.1, *t*	32.3, *t*	31.9, *t*	32.2, *t*	31.7, *t*
6	25.6, *t*	25.8, *t*	24.6, *t*	25.9, *t*	25.4, *t*	25.6, *t*
7–15			28.1–29.8			
16	25.1, *t*	25.5, *t*	24.9, *t*	25.3, *t*	25.9, *t*	25.3, *t*
17	38.1, *t*	38.5, *t*	38.6, *t*	38.7, *t*	38.5, *t*	38.4, *t*
18	68.5, *d*	68.8, *d*	68.6, *d*	68.0, *d*	68.1, *d*	68.8, *d*
19	22.8, *q*	23.4, *q*	22.9, *q*	22.4, *q*	23.4, *q*	23.0, *q*
20	12.8, *q*	13.9, *q*	14.9, *q*	12.9, *q*	13.4, *q*	13.9, *q*
21	170.1, *s*	168.7, *s*	169.5, *s*	163.7, *s*	162.8, *s*	163.0, *s*
1′	–	–	–	95.7, *d*	95.7, *d*	95.2, *d*
2′	–	–	–	73.7, *d*	73.9, *d*	75.0, *d*
3′	–	–	–	70.8, *d*	78.7, *d*	77.7, *d*
4′	–	–	–	75.2, *d*	71.2, *d*	71.1, *d*
5′	–	–	–	68.0, *d*	78.3, *d*	78.1, *d*
6′	–	–	–	18.6, *q*	62.4, *t*	62.4, *t*

and heteronuclear COSY spectra, indicated their unsubstituted nature (Huneck and Takeda, 1992).

These data indicated that one monosaccharide was linked to the C-18 position of the aglycone, the other monosaccharide being linked to the C-21 of the aglycone through an ester bond.

Comparison of the ^{13}C NMR chemical shifts of glycoside **7** with those of **1** (Tables 3 and 6) revealed that a glycosidation shift at C-19 (3.6 ppm) larger than that at C-17 (3.1 ppm) was observed in pyridine-(d_5) as solvent. Application of the glycosidation shift rule (Seo et al., 1978; Tori et al., 1977; Kasai et al., 1977) to these shifts indicated the configuration at C-18 of glycoside **7** to be *R*; the glycoside **7** was thus confirmed to be 18*R*-glycoside. In the spectrum of **8**, **9** and **10**, the glycosidation shift is the opposite (Δ at C_{19} = 3.5–3.7 ppm and Δ at C_{17} = 3.8–4.1 ppm), and the configuration in **8**, **9** and **10** is thus *S*.

A correlation in the HMQC spectrum of **7** showed that the rhamnose residue was attached to the carboxylic group of the aglycone by an ester linkage. HMBC experiments showed that the second sugar (glucose) was connected by a glycosidic bond to hydroxyl group on C-18.

The chemical shifts of C-18 δ 76.6 and C-21 δ 163.8 indicated that **8** was at the same time a glycoside and glycoside ester, with xylose linked to C-18 and glucose to C-21, as indicated from their ^{13}C NMR data (Table 4).

Compound **9** again contained two sugar residues in a HMQC NMR experiment. The anomeric ^{13}C-NMR signals gave cross-peaks with anomeric protons and long-range ^{1}H–^{13}C correlations were observed between the signals at δ_C 163.4 and δ_H 6.25, δ_C 77.1 and δ_H 5.82. Accordingly, the glucose unit was again linked to C-21 of the aglycone, while the rhamnose units were linked to the C-18 position of the aglycone. These linkages were confirmed from the NOESY spectrum of **9**.

The HMBC spectrum of **10** showed that one glucose unit was at the C-21 position of the aglycone and another glucose exhibited correlation peaks between H-1 δ 5.14 of glucose and C-18 δ 77.2 of the aglycone. The names of compounds **4**–**10** are given in Experimental.

To summarize the present study, the chemical structures and structural relationships among the new 10 component of lichen acids produced by lichens from Central Asia are shown in Fig. 1.

We have isolated three hydroxy substituted γ-lactonic carboxylic acids of which only the optical antipode, i.e. neodihydromurolic acid has previously been described (Huneck et al., 1979). The absolute configuration of the hydroxyl group of all three acids (**1**–**3**) were determined and also the all three were described for the first time in

Table 4
^{1}H NMR spectral data of compounds **4**–**6**, solvent see Experimental

H No.	4	5	6
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)
1′	6.18 (1H, *d*, *J* = 1.7)	6.31 (1H, *d*, *J* = 8.0)	6.30 (1H, *d*, *J* = 7.8)
2′	4.20 (1H, *dd*, *J* = 1.7, 3.4)	4.22, (1H, *dd*, *J* = 8.0, 9.0)	4.23 (1H, *dd*, *J* = 7.8, 9.1)
3′	4.41 (1H, *dd*, *J* = 3.4, 9.0)	4.28, (1H, *t*, *J* = 9.0)	4.24 (1H, *t*, *J* = 9.1)
4′	4.08 (1H, *t*, *J* = 9.0)	4.37 (1H, *dd*, *J* = 9.0, 10.0)	4.31 (1H, *dd*, *J* = 9.1, 10.2)
5′	4.77 (1H, *dq*, *J* = 9.0, 6.5)	4.03 (1H, *ddd*, *J* = 9.6, 6.1, 2.3)	4.04 (1H, *m*)
6′	1.31 (3H, *d*, *J* = 6.5)	4.40 (1H, *dd*, *J* = 12.0, 5.0)	4.38 (1H, *dd*, *J* = 11.8, 4.8)
		4.43 (1H, *dd*, *J* = 12.0, 2.5)	4.44 (1H, *dd*, *J* = 11.8, 2.7)

Table 5
^{1}H NMR spectral data of compounds **7**–**10**, solvent see Experimental

H No.	**7**	**8**	**9**	**10**
2	2.89 (1H, *dq*, $J_{2,3}=7.0$, $J_{2,20}=7.5$)	2.92 (1H, *dq*, $J_{2,3}=9.0$, $J_{2,20}=7.5$)	2.94 (1H, *dq*, $J_{2,3}=11.1$, $J_{2,20}=7.3$)	2.95 (1H, *dq*, $J_{2,3}=11.1$, $J_{2,20}=7.3$)
3	3.28 (1H, *dd*, $J_{3,2}=7.0$, $J_{3,4}=8.0$)	3.10 (1H, *dd*, $J_{3,2}=9.0$, $J_{3,4}=6.5$)	3.27 (1H, *dd*, $J_{3,2}=11.1$, $J_{3,4}=9.5$)	3.26 (1H, *dd*, $J_{3,2}=11.1$, $J_{3,4}=9.5$)
4	4.58 (1H, *ddd*, $J_{4,3}=8.0$, $J_{4,5}=8.0$; 5.0)	4.64 (1H, *ddd*, $J_{4,3}=6.5$, $J_{4,5}=8.0$; 4.5)	4.39 (1H, *ddd*, $J_{4,3}=9.5$, $J_{4,5}=8.0$; 4.0)	4.40 (1H, *ddd*, $J_{4,3}=9.5$, $J_{4,5}=8.0$; 4.0)
5	1.67 (2H, *m*)	1.69 (2H, *m*)	1.71 (2H, *m*)	1.71 (2H, *m*)
6–16	1.26 (22H, *m*)	1.26 (22H, *m*)	1.26 (22H, *m*)	1.26 (22H, *m*)
17	1.73 (2H, *m*)	1.78 (2H, *m*)	1.76 (2H, *m*)	1.77 (2H, *m*)
18	3.98 (1H, *m*)	3.94 (1H, *m*)	3.96 (1H, *m*)	3.99 (1H, *m*)
19	1.24 (3H, *d*, $J=6$)	1.26 (3H, *d*, $J=6$)	1.25 (3H, *d*, $J=6$)	1.26 (3H, *d*, $J=6$)
20	1.31 (3H, *d*, $J_{20,2}=7.5$)	1.29 (3H, *d*, $J_{20,2}=7.5$)	1.33 (3H, *d*, $J_{20,2}=7.3$)	1.33 (3H, *d*, $J_{20,2}=7.3$)
1′	6.25 (1H, *d*, $J=1.6$)	6.28 (1H, *d*, $J=7.9$)	6.25 (1H, *d*, $J=8.0$)	6.29 (1H, *d*, $J=7.8$)
2′	4.26 (1H, *dd*, $J=3.5$, 1.6)	4.19 (1H, *dd*, $J=7.9$, 9.1)	4.20 (1H, *dd*, $J=8.0$, 9.0)	4.18 (1H, *dd*, $J=7.8$, 9.1)
3′	4.39 (1H, *dd*, $J=8.9$, 3.5)	4.26 (1H, *t*, $J=9.1$)	4.28 (1H, *t*, $J=9.0$)	4.29 (1H, *t*, $J=9.2$)
4′	4.12 (1H, *t*, $J=8.9$)	4.34 (1H, *t*, $J=9.1$)	4.31 (1H, *t*, $J=9.0$)	4.33 (1H, *t*, $J=9.2$)
5′	4.74 (1H, *dd*, $J=8.9$, 6.0)	4.03 (1H, *m*)	4.01 (1H, *m*)	4.01 (1H, *m*)
6′	1.36 (1H, *d*, $J=6.0$)	4.42 (1H, *dd*, $J=11.0$, 4.7);	4.44 (1H, *dd*, $J=10.8$, 5.5);	4.41 (1H, *dd*, $J=10.8$, 4.9);
		4.48 (1H, *dd*, $J=11.8$, 2.9)	4.47 (1H, *dd*, $J=11.3$, 3.7)	4.46 (1H, *dd*, $J=11.6$, 3.5)
1″	5.15 (1H, *d*, $J=7.9$)	4.92 (1H, *d*, $J=7.4$)	5.82 (1H, *d*, $J=1.5$)	5.14 (1H, *d*, $J=7.9$)
2″	4.04 (1H, *dd*, $J=9.0$, 7.9)	3.96 (1H, *dd*, $J=7.4$, 8.8)	4.06 (1H, *dd*, $J=3.0$, 1.5)	4.03 (1H, *dd*, $J=9.0$, 7.9)
3″	4.14 (1H, *dd*, $J=9.0$, 8.7)	4.04 (1H, *dd*, $J=8.8$, 8.2)	3.94 (1H, *dd*, $J=9.0$, 3.0)	4.13 (1H, *dd*, $J=9.2$, 9.0)
4″	4.21 (1H, *t*, $J=8.9$)	4.19 (1H, *ddd*, $J=10.5$, 8.2, 5.2)	4.02 (1H, *t*, $J=9.0$)	4.24 (1H, *dd*, $J=9.2$, 9.1)
5″	3.94 (1H, *m*)	3.68 (1H, *dd*, $J=10.7$, 10.5)	3.93 (1H, *dd*, $J=9.0$, 5,6)	3.98 (1H, *m*)
		4.35 (1H, *dd*, $J=10.7$, 5.2)		
6″	4.28 (1H, *dd*, $J=11.2$, 4.0);	–	1.33 (1H, *d*, $J=5.6$)	4.25 (1H, *dd*, $J=11.4$, 3.9);
	4.45 (1H, *dd*, $J=11.2$, 3.8)			4.50 (1H, *dd*, $J=11.4$, 4.1)

Table 6
^{13}C NMR spectral data of compounds **7**–**10**, solvent see Experimental

C No.	7	8	9	10
1	177.6, *s*	177.4, *s*	177.3, *s*	177.7, *s*
2	36.1, *d*	36.3, *d*	36.2, *d*	36.5, *d*
3	50.6, *d*	50.4, *d*	50.1, *d*	50.0, *d*
4	77.0, *d*	77.6, *d*	77.5, *d*	77.6, *d*
5	31.9, *t*	32.2, *t*	31.7, *t*	31.7, *t*
6	25.9, *t*	25.4, *t*	25.6, *t*	25.6, *t*
7–15		28.1–29.4		
16	26.6, *t*	26.5, *t*	25.3, *t*	25.9, *t*
17	35.0, *t*	34.4, *t*	34.5, *t*	34.8, *t*
18	76.5, *d*	76.6, *d*	77.1, *d*	77.2, *d*
19	19.2, *q*	19.7, *q*	19.4, *q*	19.3, *q*
20	12.6, *q*	12.8, *q*	12.9, *q*	13.4, *q*
21	163.7, *s*	163.8, *s*	163.4, *s*	164.0, *s*
1′	95.0, *d*	94.8, *d*	94.8, *d*	95.6, *d*
2′	72.8, *d*	75.7, *d*	75.7, *d*	74.1, *d*
3′	71.8, *d*	78.0, *d*	78.0, *d*	78.8, *d*
4′	73.5, *d*	71.3, *d*	71.6, *d*	71.0, *d*
5′	67.5, *d*	78.5, *d*	78.2, *d*	79.2, *d*
6′	18.5, *q*	62.3, *t*	62.1, *t*	62.0, *t*
1″	105.4, *d*	106.3, *d*	104.4, *d*	105.7, *d*
2″	75.4, *d*	75.3, *d*	72.7, *d*	75.5, *d*
3″	77.9, *d*	78.1, *d*	72.6, *d*	78.6, *d*
4″	71.5, *d*	71.1, *d*	73.8, *d*	71.6, *d*
5″	78.2, *d*	67.1, *t*	69.0, *d*	78.3, *d*
6″	62.7, *t*	–	18.5, *q*	62.6, *t*

Table 7
Stereochemical analysis of compounds **1**, **2** and **3** 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
1	+12.8	−30.1	*R*
2	−16.9	+26.7	*S*
3	−15.8	+34.2	*S*

[a] The aglycones from glycosides **4**–**10** are identical from **1** or **2** or **3**.

nature. Absolute new is the discovery of glycoside esters containing rhamnose, xylose and glucose.

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. Optical rotatory dispersion (ORD) measurement was carried out under a 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 (^1H), 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. RP–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 sro, Czech Republic).

3.2. Plant material

The specimens of *Acarospora gobiensis* H. Magn., *Cladonia furcata* (Huds.) Schrad, *Lecanora fructulosa* (Dicks.) Ach., *Leptogium saturninum* (Dicks.) Nyl., *Peltigera canina* (Neck.) Hoffm., *Rhizoplaca peltata* Ram., *Xanthoparmelia camtschadalis* Ach., *X. tinctina* Mah. et Gill., and *Xanthoria elegans* (Link.) Th. Fr. were collected in July 1996 along the shore of Lake Issyk Kul, Tian-Shan Mountains.

3.3. Extraction and isolation

All nine lichen species (100 g of air-dried lichens, see Řezanka and Guschina, 2000a) were extracted independently by the Blight and Dyer (1959) method and the extracts were also chromatographed separately be means of the column chromatography over Sephadex LH-20 eluted with EtOH-i-PrOH (fraction 1), EtOH (fraction 2) and then MeOH (fraction 3). Fraction 1 was further fractionated by RP–HPLC on a C18-Bondapak (Waters sro, Czech Republic) column (30 cm×7.8 mm, flow rate 2.0 ml/min) with ACN–H_2O (1:2) to yield compounds **1**–**3**. Fraction 2 was separated by RP-HPLC with ACN–H_2O (2:5) to yield the compounds **4**–**6**. Fraction 3 using ACN–H_2O (1:3), yielded compounds **7**–**10**.

The identification and the D or L configuration of sugars (i.e. D-xylose, L-rhamnose and D-glucose) was determined using GC–MS (according to the method of Gerwig et al., 1978), with some modifications, as previously described (Řezanka and Guschina, 2000a) with SPB-1 (Supelco) column (30 m×0.25 mm I.D.). The column temperature was programmed to rise 5 °C/min from 170 to 260 °C. The flow rate of the helium carrier gas through the column was 1.5 ml/min. The temperature of the injector was 270 °C.

3.4. Acid hydrolysis of *4*

The glycoside (~1 mg) was refluxed in 2 N HCl (0.5 ml) for 2 h. The aglycone was 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 (1 mg) in acetate buffer (pH 4.4, 10 ml) was treated with β-glucosidase (20 mg) 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's acids for ^{1}H NMR and ORD analysis.

Glycosides (~0.5 mg) were suspended in $(NH_4)_2SO_4$ solution of enzyme (E.C. 3.1.1.1, from porcine liver) at pH ~8 and incubated 15 min at 25 °C. 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

(*S*)-MTPA esters of free acids (**1**–**3**) and aglycones from glucosides **4**–**10** (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 evaporation of solvent, the residue was purified by Silica gel TLC (hexane–AcOEt, 2:1) to provide the (*S*)-MTPA ester as a colorless oil.

(*R*)-MTPA Esters of free acids (**1**–**3**) and of aglycones from glucosides **4**–**10**. 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.

18*R*-Hydroxydihydroalloprotolichesterinic acid **1**, white crystals, m.p. 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), see also Fig. 2; IR (KBr) (cm^{-1}): 3450 (OH), 2950, 2920, 1740 (lactone), 1705 (acid); HREIMS *m/z*: 370.5293 (M^+, calc. for $[C_{21}H_{38}O_5]^+$ 370.5297; LREIMS *m/z* (%), 370 $[M]^+$ (2), 355 $[M–Me]^+$ (21), 352 $[M–H_2O]^+$ (56), 334 $[M–2xH_2O]^+$ (67), 326 $[M–CO_2]^+$ (14), 308 $[M–CO_2–H_2O]^+$ (58); ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3.

18*S*-Hydroxydihydroprotolichesterinic acid **2**, 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), see also Fig. 2); IR (KBr) (cm^{-1}): 3450 (OH), 2950, 2920, 1740 (lactone), 1705 (acid); HREIMS *m/z*: 370.5301 (M^+, calc. for $[C_{21}H_{38}O_5]^+$ 370.5297; LREIMS *m/z* (%), 370 $[M]^+$ (3), 355 $[M–Me]^+$ (19), 352 $[M–H_2O]^+$ (67), 334 $[M–2xH_2O]^+$ (81), 326 $[M–CO_2]^+$ (10), 308 $[M–CO_2–H_2O]^+$ (51); ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3.

18*S*-Hydroxyneodihydroprotolichesterinic acid **3**, 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), see also Fig. 2; IR (KBr) (cm^{-1}): 3450 (OH), 2950, 2920, 1740 (lactone), 1705 (acid); HREIMS *m/z*: 370.5295 (M^+, calc. for $[C_{21}H_{38}O_5]^+$ 370.5297; LREIMS *m/z* (%), 370 $[M]^+$ (4), 355 $[M–Me]^+$ (17), 352 $[M–H_2O]^+$ (63), 334 $[M–2xH_2O]^+$ (73), 326 $[M–CO_2]^+$ (9), 308 $[M–CO_2–H_2O]^+$ (42); ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3.

21-*O*-α-L-Rhamnopyranosyl-18*R*-hydroxydihydroalloprotolichesterinate **4**, colorless powder, mp 146.5 °C, $[\alpha]_D^{24}$ −188° (*c* 0.15, MeOH); UV 219 nm (log ε 3.88); IR (KBr) (cm^{-1}): 3400 (OH), 2950, 2920, 1750 (lactone), 1710 (ester); HRFABMS *m/z* 517.6801 $[M+H]^+$, calc. for $[C_{27}H_{49}O_9]^+$ 517.6807; negative LRFABMS *m/z* 515 $(M–H)^-$, 369 $(M–H-146)^-$ and ^{1}H and ^{13}C NMR spectra see Table 3 and 4.

21-*O*-β-D-Glucopyranosyl-18*S*-hydroxydihydroprotolichesterinate **5**, colorless powder, mp 157 °C, $[\alpha]_D^{24}$ −179° (*c* 0.14, MeOH); UV 218 nm (log ε 3.82); HRFABMS *m/z* 533.6806 $(M+H)^+$ calc. for $[C_{27}H_{49}O_{10}]^+$ 533.6800; negative LRFABMS *m/z* 531 $(M–H)^-$, 369 $(M–H–162)^-$, and ^{1}H and ^{13}C NMR spectra, see Table s3 and 4.

21-*O*-β-D-Glucopyranosyl-18*S*-hydroxyneodihydrolichesterinate (**6**), white amorphous powder, $[\alpha]_D^{23}$ −14°; HRFABMS *m/z* 533.6797 $(M+H)^+$, calculated for $[C_{27}H_{49}O_{10}]^+$ 533.6800; negative LRFABMS *m/z* 531 $(M–H)^-$, 369 $(M–H–162)^-$ and 353 $(M–H–178)^-$; and ^{1}H NMR and ^{13}C NMR spectra see Tables 3 and 4.

18-*O*-β-D-Glucopyranosyl-18*R*-hydroxydihydroalloprotolichesterinate 21-*O*-α-L-rhamnopyranoside (**7**), white amorphous powder, $[\alpha]_D^{23}$ −35°; HRFABMS *m/z* 679.8234 $(M+H)^+$, calc. for $[C_{33}H_{59}O_{14}]^+$ 679.8231; negative LRFABMS *m/z* 677 $(M–H)^-$, 531 $(M–H–146)^-$, 515 $(M–H–162)^-$, 499 $(M–H–178)^-$, 369 $(M–H–162–146)^-$; ^{1}H and ^{13}C NMR spectra, see Tables 5 and 6.

18-*O*-β-D-Xylopyranosyl-18*S*-hydroxydihydroprotolichesterinate 21-*O*-β-D-glucopyranoside (**8**), white powder, $[\alpha]_D^{23}$ −18°; HRFABMS *m/z* 665.7961 $(M+H)^+$, calc. for $[C_{32}H_{57}O_{14}]^+$ 665.7960; negative FABMS *m/z* 663 $(M–H)^-$, 501 $(M–H–162)^-$, 369 $(M–H–162–132)^-$; ^{1}H and ^{13}C NMR spectra, see Tables 5 and 6.

18-*O*-α-L-Rhamnopyranosyl-18*S*-hydroxyneodihydroprotolichesterinate 21-*O*-β-D-glucopyranoside (**9**), white powder, $[\alpha]_D^{23}$ −52.7°; HRFABMS *m/z* 679.8233 $(M+H)^+$, calc. for $[C_{33}H_{59}O_{14}]^+$ 679.8231; negative LRFABMS *m/z* 677 $(M–H)^-$, 515 $(M–H–162)^-$, 369 $(M–H–162–146)^-$; ^{1}H and ^{13}C NMR spectra, see Tables 5 and 6.

18-*O*-β-D-Glucopyranosyl-18*S*-hydroxyneodihydroprotolichesterinate 21-*O*-β-D-glucopyranoside (**10**), colorless powder, $[\alpha]_D^{23}$ −42.0°; HRFABMS *m/z* 695.8224 $(M+H)^+$, calc. for $[C_{33}H_{59}O_{15}]^+$ 695.8221; negative LRFABMS *m/z* 693 $(M–H)^-$, 531 $(M–H–162)^-$, 369 $(M–H–162–162)^-$; ^{1}H and ^{13}C NMR spectra, see Tables 5 and 6.

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