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Glycosidic compounds of murolic, protoconstipatic and allo-murolic acids from lichens of Central Asia

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Abstract

Eleven compounds isolated from the extract of the Central Asian lichens comprised eight new glycosides having murolic, protoconstipatic and allo-murolic acids, as the aglycones and a saccharide moiety linked at C-18 made up of one or two sugars (glucose and apiose or rhamnose or xylose or arabinose). The structures were elucidated by using extensive spectroscopic analysis (1D and 2D NMR, MS, IR, UV and CD) and chemical methods.

Keywords: Lichens; Murolic acid; Glycosides; Glucore; Central Asia; Aglycones

1. Introduction

Protolichesterinic acid was first isolated at the beginning of this century by Zopf from various species of the lichen *Cetraria* (Zopf, 1907) and was structurally elucidated some 30 years later by Asahina and Asano (1932). Interestingly, both the dextro- and levorotatory forms of protolichesterinic acid have been isolated from different sources of *Cetraria* (Asahina and Yanagita, 1937).

Dextrorotatory protolichesterinic acid has, additionally, been found by several groups in *Parmelia* species (Shah, 1954) indigenous to India. Today's protolichesterinic acid is probably the best-known member of this class of lactone fatty acids, which includes murolic, alloprotolichesterinic and protoconstipatic acids (Huneck and Yoshimura, 1986).

Although tens of thousands of plant glycosides were discovered in nature (Ikan, 1999), only a few of them have been identified from lichens. These include, 1-(*O*-α-D-glucopyranosyl)-3*S*,25*R*-hexacosanediol from *Solorina crocea* (Huneck and Yoshimura, 1986), 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), we have examined *Acarospora gobiensis*, *Cladonia furcata*, *Lecanora fructulosa*, *Leptogium saturninum*, *Peltigera canina*, *Xanthoparmelia camtschadalis*, *X. tinctina* and *Xanthoria elegans*. The eleven compounds isolated from the extract of the thallus comprised eight new glycosides (**4**–**11**) having murolic **1**, protoconstipatic **2** and *allo*-murolic **3** acids as the aglycones and an oligosaccharide moiety linked at C-18 made up of one or two sugars (glucose and apiose or rhamnose or xylose or arabinose).

2. Results and discussion

The aqueous-MeOH (upper) layer after extraction (Blight and Dyer, 1959) of lipids from the thallus of lichens (Table 1) was chromatographed on a Sephadex

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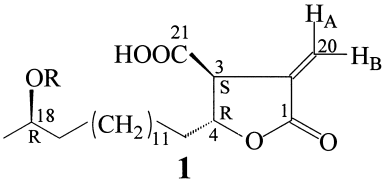
Table 1
Occurrence of acids and their glycosides from lichens of Tian–Shan mountains

	Name of compounds; mg/100 g of dry weight											
Name of lichen	**1**	**2**	**3**	**4a**	**4b**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Acarospora gobiensis	22.5	–[a]	–	8.7	–	–	–	–	–	–	–	3.2
Cladonia furcata	–	24.3	–	–	0.5	–	–	2.9	0.8	–	7.8	–
Lecanora fructulosa	–	13.5	–	–	3.8	–	–	3.4	1.9	–	1.5	–
Leptogium saturninum	–	–	19.8	–	–	6.8	7.1	–	–	3.9	–	–
Peltigera canina	–	–	25.4	–	–	0.9	0.9	–	–	1.4	–	–
Xanthoparmelia camtschadalis	–	19.1	–	–	4.2	–	–	6.3	1.4	–	4.3	–
X. tinctina	–	22.7	–	–	0.2	–	–	5.7	3.1	–	0.7	–
Xanthoria elegans	13.8	–	–	1.8	–	–	–	–	–	–	–	6.1

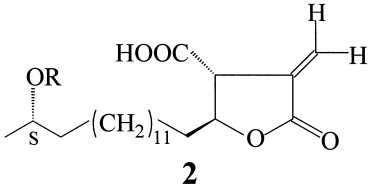
[a] Less than 0.1 mg/100 g of dry weight.

LH-20 column and reverse-phase HPLC to give compounds **1**–**11** (Figs. 1 and 2).

The compounds were identified as murolic acid **1**, protoconstipatic acid **2** and allo-murolic or allo-protoconstipatic acid **3**, (the term allo-murolic acid is better because it is shorter; moreover, this acid has an identical configuration at C-18, i.e. on the secondary alcoholic group), respectively, in agreement with spectral data, i.e. UV, IR, ^{13}C and ^{1}H NMR and CD (Chester and Elix, 1979; David et al., 1990; Ghogomu and



1



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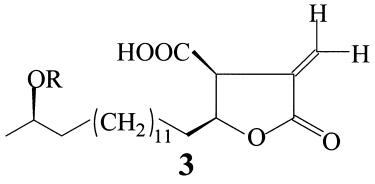
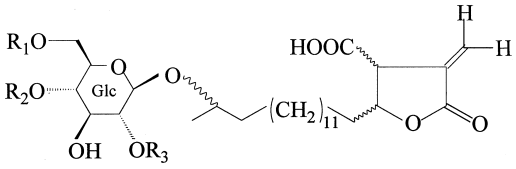


3

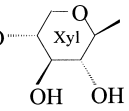
R = H or R = MTPA

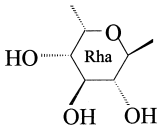
Fig. 1. The structures of murolic, protoconstipatic and allo-murolic acids from lichens.



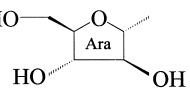
4a $R_1 = R_2 = R_3 = H$ 3S, 4R, 18R

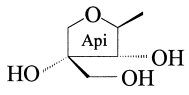
4b $R_1 = R_2 = R_3 = H$ 3S, 4S, 18S

5 R_1 =  $R_2 = R_3 = H$ 3S, 4S, 18R

6 R_1 =  $R_2 = R_3 = H$ 3S, 4S, 18R

7 R_2 = Rha $R_1 = R_3 = H$ 3R, 4S, 18S

8 R_1 =  $R_2 = R_3 = H$ 3S, 4S, 18R

9 R_1 =  $R_2 = R_3 = H$ 3S, 4S, 18R

10 R_2 = Api $R_1 = R_3 = H$ 3R, 4S, 18S

11 R_3 = Api $R_1 = R_2 = H$ 3S, 4R, 18R

Fig. 2. The structures of glycosides (**4**–**11**) from lichens.

Table 2
^{1}H and ^{13}C NMR of murolic **1**, protoconstipatic **2** and allo–murolic **3** acids

No. of atom	**1** (^{13}C)	**2** (^{13}C)	**3** (^{13}C)	**1** (^{1}H)	**2** (^{1}H)	**3** (^{1}H)
1	173.5	174.2	173.8	–	–	–
2	133.1	133.7	132.9	–	–	–
3	49.7	49.6	50.1	3.61 (1H, *ddd*, $J_{3,4}=6$; $J_{3,2}0=3$)	3.59 (1H, *dt*, $J_{3,4}=5.7$; $J_{3,20}=3$)	3.61 (1H, *dt*, $J_{3,4}=7.5$; $J_{3,20}=2.3$)
4	79.3	81.0	80.5	4.83 (1H, *dt*, $J_{4,3}=6$; $J_{4,5}=8$ and 11.5)	4.80 (1H, *dt*, $J_{4,3}=5.7$; $J_{4,5}=7$)	4.63 (1H, *dt*, $J_{4,3}=7.5$; $J_{4,5}=4$ and 8.2)
5	35.8	34.1	34.8	1.74 (2H, *m*)	1.74 (2H, *m*)	1.70 (2H, *m*)
6	24.7	24.0	24.5	1.28 (18H, *br.s*)	1.27 (18H, *br s*)	1.26 (18H, *br s*)
7–15	28.1–29.4	28.4–28.9	28.1–28.8	1.28 (18H, *br.s*)	1.27 (18H, *br s*)	1.26 (18H, *br s*)
16	25.6	25.9	25.1	1.28 (18H, *br.s*)	1.27 (18H, *br s*)	1.26 (18H, *br s*)
17	39.0	38.9	38.5	1.46 (2H, *m*)	1.45 (2H, *m*)	1.46 (2H, *m*)
18	68.2	68.4	68.8	3.74 (1H, sextet)	3.88 (1H, *m*)	3.74 (1H, *m*)
19	23.3	23.8	23.0	1.15 (1H, *d*, $J_{18,19}=6$)	1.18 (1H, *d*, $J_{18,19}=6$)	1.15 (1H, *d*, $J_{18,19}=6$)
20	125.6	126.1	125.4	6.02 ($1H_a$, *d*, $J_{20,3}=3$) 6.37 (1Hb, *d*, $J_{20,3}=3$)	5.91 ($1H_a$, *d*, $J_{20,3}=2.7$) and 6.40 (1Hb, *d*, $J_{20,3}=3$)	5.83 ($1H_a$, *d*, $J_{20,3}=2$) and 6.41 ($1H_b$, *d*, $J_{20,3}=2$)
21	168.5	169.0	168.9	–	–	–

Bodo, 1982; Huneck and Takeda, 1992; Huneck et al., 1979, 1986).

The spectroscopic properties of **1** showed similarities to both murolic acid and (+)-protolichesterinic acid (Huneck et al., 1979; Huneck and Takeda, 1992). The ^{1}H NMR spectrum of **1** exhibited a three-proton doublet at δ_H 1.15 ($J=6$ Hz) and one-proton sextet at δ_H 3.74 indicative of the terminal 1-hydroxyethyl group. Two further one-proton multiplets at δ_H 6.02 and 6.37 were assigned to the 20-methylene protons, while the one-proton signals at δ_H 3.61 and 4.83 were assigned to H-3 and H-4, respectively (Table 2).

In a similar manner, the spectroscopic properties of **2** showed remarkable similarities to protolichesterinic acid and protoconstipatic acid (Chester and Elix, 1979). The ^{1}H NMR spectrum of **2** exhibited two one-proton doublets at δ_H 5.91 and 6.40 assigned to the 20-methylene protons, while one-proton signals at δ_H 3.59 and 4.80 were assigned to H-3 and H-4, respectively. A comparison of the ^{1}H NMR spectrum of **2** with those of (−)-protolichesterinic acid and protoconstipatic acid confirmed the latter assignments. The ^{1}H NMR spectrum of **2** also established the relative stereochemistry shown in formula **2**. The coupling constant $J_{3,4}$ of 5.7 H*z* corresponds exactly with that observed in (−)-protolichesterinic acid and protoconstipatic acid (Chester and Elix, 1979; Huneck and Takeda, 1992) thus establishing the *trans* stereochemistry for the hydrogen atoms bonded to C-3 and C-4.

The absolute configurations of **1** and **2** as shown in Fig. 3 followed from a comparison of the CD spectral data with those reported for murolic and protolichesterinic acids (Huneck et al., 1979, 1986).

Compound **3** had the molecular formula $C_{21}H_{36}O_5$ according to its high-resolution mass spectrometry and showed a maximum at 209 nm (log ε 3.92) in the UV spectrum, indicative of an α-methylene-γ-lactone. The ^{1}H NMR spectrum (Table 2) of **3** gave the structure and relative stereochemistry shown in formula **3** (Fig. 1). Inspection of a model shows that in the most stable conformation of the molecule, the protons at C-3 and C-4 include an angle of ca. 15° which, according to the Karplus relation, corresponds to a coupling constant of ca. 8 Hz, in good agreement with the observed value of 7.5 Hz. Furthermore, the NMR data are in excellent agreement with the corresponding data of (−)-allo-protolichesterinic acid (Huneck and Takeda,

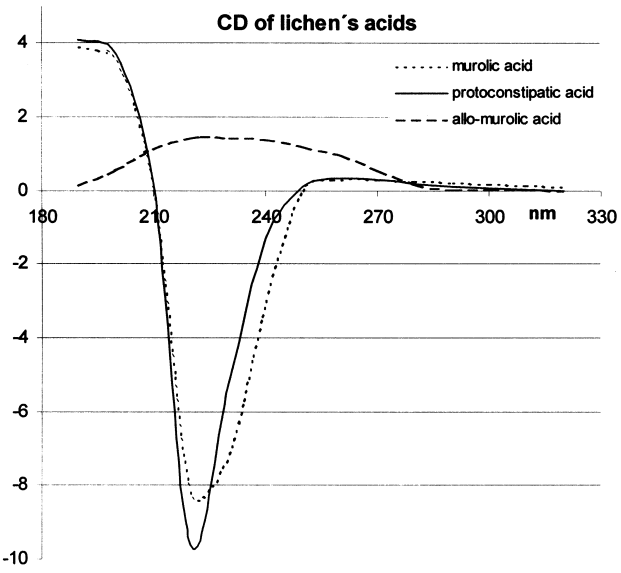


Fig. 3. The CD spectra of lichen's acids (murolic, protoconstipatic and allo-murolic).

1992). The absolute configuration of allo-murolic acid as shown in formula 3 follows from the nearly identical CD spectra (Fig. 3) of **3** and (−)-allo-protolichesterinic acid.

The absolute configuration at C-18 of all three acids, i.e. **1**, **2** and **3** was determined by the modified Mosher method (Ohtani et al., 1991a, 1991b; 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 **1** and **3** was (*R*) while that of **2** was (*S*) (Ohtani et al., 1991b). The data are showed in Table 6.

Compounds **4a** and **4b** were obtained as colorless powders, $[\alpha]_D^{23} -187^\circ$ (c 0.14, MeOH), $[\alpha]_D^{23} +180^\circ$ (c 0.15, MeOH), respectively. The molecular formulae of **4a** and **4b** were determined to be $C_{27}H_{46}O_{10}$ on the basis of HRFABMS. The UV spectra of both **4** derivatives had a maximum band at 218 nm (log ε 3.84). Their IR spectra showed absorption bands due to a hydroxy (3500 cm^{-1}), a γ-lactone (1740 cm^{-1}) and a carboxy (1708 cm^{-1}) groups. An absorption band at 1665 cm^{-1} was attributed to an olefinic group.

In the ^{1}H NMR spectrum of **4a** the signals of five oxymethine protons in diaxial conformation ($J = 7.0 - 9.0$ Hz) and one oxymethylene group indicated the presence of a β-glucopyranosyl group. The glucosyl residue was located at the 18-*O*-position of the aglycone skeleton according to long-range HMBC correlations between C-18 at δ_C 60.5 and the anomeric H-1′ at δ_H 4.80, as well as H-19 and H-17 protons at δ_H 1.24, and at δ_H 1.69, respectively. Subsequently, glucoside **4a** was hydrolyzed by β-glucosidase, furnishing an aglycone and D-glucose.

To determine the absolute configuration of carbohydrates in the glycosides, the acetylated-2-butyl derivatives prepared from glycosides were analyzed by gas chromatography using a glass-capillary column (Supelco SPB-1) (Gerwig et al., 1978). The (acetylated (+)-2-butyl) derivatives were eluted as a peak with retention time of 12.55 min, which was identical with that of tetraacetyl (+)-2-butyl-D-glucose. These results revealed that **4** contain D-glucose. The carbohydrates from glycosides **5**–**11** were transformed to butyl glycosides and analyzed by GC. The appropriate carbohydrates were identified as shown below.

In the ^{1}H NMR spectrum of **4a**, the unusual 18-*O*-glycosylation was indicated by downfield shifts of H-19 (+0.09 ppm) and H-17 (+0.23 ppm) with respect to murolic acid as a model compound. Similarly, in the ^{13}C NMR spectra of **4a** (Table 3) 18-*O*-glycosylation was confirmed by the diagnostic downfield shift of C-18 (+7.7 ppm) and by upfield shift of the related C-19 (−4.1 ppm) and C-17 (−3.2 ppm) carbons with respect to murolic acid.

The absolute configuration of **4a** was established by ^{13}C NMR spectroscopy. Comparison of the ^{13}C NMR chemical shifts of glycoside **4a** with those of **1** (Tables

Table 3
^{1}H and ^{13}C NMR of 18-*O*-β-glycopyranoside of murolic (**4a**) and protoconstipatic acids (**4b**)

No. of atom	**4a** (^{13}C)	**4b** (^{13}C)	**4a** (^{1}H)	**4b** (^{1}H)
1	173.5	174.2	–	–
2	133.1	133.7	–	–
3	49.7	49.6	3.59 (1H, *dt*, $J_{3,4} = 5.7$; $J_{3,20} = 3$)	3.61 (1H, *ddd*, $J_{3,4} = 6$; $J_{3,20} = 3$)
4	79.3	81.0	4.80 (1H, *dt*, $J_{4,3} = 5.7$; $J_{4,5} = 7$)	4.83 (1H, *dt*, $J_{4,3} = 6$; $J_{4,5} = 8$ and 11.5)
5	35.8	34.1	1.74 *m* (2H)	1.74 *m* (2H)
6	24.7	24.0	~1.27 (2H, *br s*)	~1.28 (2H, *br s*)
7–15	28.1–29.4	28.4–28.9	~1.27 (18H, *br s*)	~1.28 (18H, *br s*)
16	26.6	26.5	~1.27 (2H, *br s*)	~1.28 (2H, *br s*)
17	35.8	41.3	1.69 (2H, *m*)	1.66 (2H, *m*)
18	75.9	75.8	3.98 (1H, *m*)	3.87 (1H, sextet)
19	19.2	20.7	1.24 (1H, *d*, $J_{18,19} = 6$)	1.22 (1H, *d*, $J_{18,19} = 6$)
20	125.6	126.1	5.91 (1Ha, *d*, $J_{20,3} = 2.7$) and 6.40 (1H$_b$, *d*, $J_{20,3} = 3$)	6.02 (1Ha, *d*, $J_{20,3} = 3$) and 6.37 (1H$_b$, *d*, $J_{20,3} = 3$)
21	168.5	169.0	–	–
1′	105.2	105.0	4.80 (1H, *d*, $J = 7.1$)	4.80 (1H, *d*, $J = 7.3$)
2′	74.2	74.7	3.52 (1H, *dd*, $J = 8.9$, 7.1)	3.54 (1H, *dd*, $J = 9.0$, 7.3)
3′	76.9	77.2	3.58 (1H, *t*, $J = 8.9$)	3.56 (1H, *t*, $J = 9.0$)
4′	71.0	71.4	3.41 (1H, *t*, $J = 8.9$)	3.42 (1H, *t*, $J = 9.0$)
5′	78.9	78.6	3.45 (1H, *m*)	3.47 (1H, *m*)
6′	62.3	62.6	3.72 (1H, *dd*, $J = 12.1$, 5.2) and 3.93 (1H, *dd*, $J = 11.8$, 2.3)	3.74 (1H, *dd* $J = 12.0$, 5.0) and 3.93 (1H, *dd*, $J = 12.0$, 2.0)

2 and 3) revealed that a larger glycosidation shift at C-19 (4.1 ppm) than that at C-17 (3.2 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 the glycoside **4a** to be R and thus the glycoside **4a** was confirmed to be (18*R*)-18-*O*-β-D-glucopyranoside of murolic acid. In the spectrum of **4b**, the glycosidation shift is the opposite (3.1 and 2.4 ppm), and the configuration in **4b** thus must be *S* and this glycoside is derived from (−)-protoconstipatic acid **2**. These results were confirmed on isolating **1** and/or **2** after enzymatic hydrolysis.

The FABMS (negative mode) spectrum of **5** showed a molecular anion peak at *m/z* 662 and fragment ions at *m/z* 529 $(M-H-132)^+$ and 367 $(M-H-132-162)^+$, corresponding to sequential losses of pentosyl and hexosyl units, respectively.

The ^1H and ^{13}C NMR spectra of compound **5** displayed the signals of two sugar residues in δ_H 3.5–5.5 and δ_C 65–105 regions, in addition to aglycone resonances. The hexose and the pentose, as revealed by ^1H–^1H and ^1H–^{13}C COSY, were glucose and xylose, respectively, in view of the diaxial ^1H–^1H coupling constants (7–10 Hz). HMBC experiments provided evidence for a connection of the 1″-β-xylosyl residue to C-6′ of glucose, while 1′-β-glucosyl group was bonded to C-18 of the aglycone as shown by cross-peaks between H-1″ and C-6′; H-1′ and C-18, respectively. The glycosides could be hydrolyzed by HCl and gave rise to glucose, xylose and allo-murolic acid. The structure of 5 was identified as (18*R*)-18-*O*- 1-β-xylopyranosyl-(1-6)-β-D-glucopyranoside of allo-murolic acid.

The negative ion FABMS spectrum of **6** showed a molecular anion peak at *m/z* 675 and fragment ions at *m/z* $(M-H-146)^-$ and $(M-H-146-162)^-$ corresponding to the loss of a deoxyhexosyl or a hexosyl unit.

Table 4
^{13}C NMR spectra of glycosides (**5**–**11**)

No. of atom	**5**	**6**	**7**	**8**	**9**	**10**	11
1′	105.4	101.3	100.6	104.4	104.6	105.3	97.9
2′	74.8	73.8	73.7	75.1	75.3	75.1	75.7
3′	77.5	77.6	75.2	78.0	78.2	76.3	76.6
4′	71.1	71.1	76.5	72.0	71.7	79.3	70.1
5′	77.4	77.2	75.4	76.7	77.6	76.5	73.8
6′	70.0	67.3	60.1	68.1	68.9	61.4	63.4
1″	105.8	101.9	100.4	109.9	111.3	111.1	108.9
2″	74.8	72.2	70.7	85.9	78.1	77.7	76.2
3″	78.0	71.8	70.5	78.9	80.7	80.2	79.4
4″	70.9	74.5	71.8	83.2	75.1	74.9	74.1
5″	66.9	69.6	68.6	63.1	65.8	64.6	64.3
6″		17.7	17.6				

The exact disposition of the two monosaccharide units and the position of the interglycosidic linkages in compound **6** were determined using 2D NMR spectroscopy. HOHAHA experiments allowed the resolution of the overlapping spectral region of the disaccharide moiety of **6** into a subset of individual monosaccharide spectra. 2D-COSY experiments established the proton sequence within each sugar fragment starting from the well-resolved anomeric proton signals of a β-D-glucopyranose (δ_H 5.10, *d*, $J = 7.5$ Hz), and one α-L-rhamnopyranose unit (δ_H 5.00, *d*, $J = 1.5$ Hz) in compound **6**. HETCOR correlated all proton signals with those of each corresponding carbon leading to the assignments (Tables 4 and 5). Chemical shifts, multiplicity of the proton signals, values of coupling constants, and chemical shifts of carbons indicated that the sugars must be in the β-D-glucopyranosyl and in the α-L-rhamnopyranosyl forms.

In compound **6**, a glycosidation shift at C-6′ (i.e. 5.0 ppm) and the chemical shifts of H-1′ (δ_H 5.10) and C-1′ (δ_H 101.3) of glucose indicates these monosaccharides to be glycosidated at C-6 and linked at the aglycon. The one signal due to anomeric proton of rhamnose (δ_H 5.00 *d*, $J = 1.5$ Hz), correlating to the C-l″ resonance at δ_C 101.9 by HETCOR, indicated that the rhamnose unit was linked to a primary alcoholic group (C-6′ of the glucose). Rhamnose 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 glucose (δ_H 5.10) and C-18 (δ_C 60.5) of the aglycon, between H-1″(δ_H 5.00) of the rhamnose unit and C-6′ (δ_C 67.3) of glucose, between H-5″(δ_H 4.12) and C-3″ (δ_C71.8). The sugar moieties were shown to be rutinose, and the glycoside would be (18*R*)-18-*O*-α-L-rhamnopyranosyl-(1-6)-β-D-glucopyranoside of murolic acid.

The FABMS of compound **7** displayed a $[M+H]^+$ peak at *m/z* 677.8075, consistent with a molecular formula of $C_{33}H_{56}O_{14}$.

The majority of resonances for this glycoside (**7**) were found in the 60–105 ppm range in the ^{13}C NMR and 3–5 ppm in the ^1H NMR. In compound **7**, the key resonance is peak at δ_C 17.6, which represents the C-6″ signal of 6-deoxy sugar. This signal was identified as a methyl carbon from the DEPT spectrum and the corresponding ^1H NMR chemical shift was determined for this signal from an HMQC spectrum, which was at δ_H 1.41. This was used as a starting point in the homonuclear correlated spectra (COSY, TOCSY, and DQF-COSY) to determine all the protons on the same sugar ring. Thus, the methyl protons were correlated to H-5″ at δ_H 3.82, which was further coupled to H-4″ and so on. With the help of the HMQC data the corresponding carbon chemical shifts were assigned for the 6-deoxy-hexose unit. The DEPT spectrum showed

Table 5
^{1}H NMR spectra of glycosides (**5**–**11**)

No. of atom	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1′	5.42 (1H, *d*, *J* = 7.3)	5.10 (1H, *d*, *J* = 7.5)	5.25 (1H, *d*, *J* = 7.8)	5.24 (1H, *t*, *J* = 7.9)	5.56 (1H, *d*, *J* = 8.0)	5.26 (1H, *d*, *J* = 7.3)	5.22 (1H, *d*, *J* = 7.1)
2′	4.39 (1H, *dd*, *J* = 7.3, 9.0)	4.35 (1H, *dd*, *J* = 7.5, 9.0)	4.24 (1H, *dd*, *J* = 7.8, 8.8)	4.15 (1H, *dd*, *J* = 8.9, 7.8)	4.33 (1H, *t*, *J* = 8.0)	4.28 (1H, *t*, *J* = 7.6)	4.35 (1H, *m*)
3′	4.30 (1H, *t*, *J* = 9.0)	4.28 (1H, *t*, *J* = 9.0)	4.32 (1H, *t J* = 8.0)	4.32 (1H, *m*)	4.37 (1H, *t*, *J* = 8.0)	4.31 (1H, *t*, *J* = 7.6)	4.33 (1H, *m*)
4′	4.22 (1H, *t*, *J* = 9.0)	4.24 (1H, *t*, *J* = 9.0)	4.30 (1H, *t*, *J* = 7.4)	4.28 (1H, *m*)	3.96 (1H, *dd*, *J* = 9.6, 8.0)	4.03 (1H, *dd*, *H* = 9.5, 8.0)	4.01 (1H, *dd*, *J* = 9.0, 9.0)
5′	4.35 (1H, *m*)	4.33 (1H, *m*)	4.15 (1H, *m*)	4.42 (1H, *ddd*, *J* = 9.5, 6.0, 2.4)	4.28 (1H, *td*, J = 9.6, 2.4)	4.19 (1H, *td*, *J* = 9.5, 2.1)	4.17 (1H, *td*, *J* = 9.8, 8.1)
6′	4.31 (1H, *dd*, *J* = 11.0, 5.0) and 4.81 (1H, *dd*, *J* = 11.0, 2.0)	4.36 (1H, *dd*, *J* = 12.0, 3.0) and 4.80 (1H, *dd*, *J* = 12.0, 5.0)	4.58 (1H, *dd*, *J* = 11.0, 8.0) and 4.42 (1H, *dd*, *J* = 12.0, 5.0)	5.00 (1H, *dd*, *J* = 11.1, 2.4) and 4.60 (1H, *dd*, *J* = 11.0, 5.9)	4.09 (1H, *dd*, *J* = 10.8, 9.6) and 4.68 (1H, *dd*, *J* = 10.8, 2.4)	4.07 (1H, *dd*, *J* = 11.0, 7.6) and 4.38 (1H, *dd*, *J* = 11.0, 1.2)	4.06 (1H, *dd*, *J* = 11.3, 6.9) and 4.36 (1H, *dd*, *J* = 11.3, 1.4)
1″	4.95 (1H, *d*, *J* = 7.8)	5.00 (1H, *d*, *J* = 1.5)	4.68 (1H, *d*, *J* = 1.2)	4.95 (1H, *d*, *J* = 1.3)	5.61 (1H, *d*, *J* = 3.7)	5.28 (1H, *d*, *J* = 3.6)	5.36 (1H, *br s*)
2″	4.02 (1H, *dd*, *J* = 8.5, 7.8)	3.90 (1H, *dd*, *J* = 1.5, 2.5)	3.95 (1H, *dd*, *J* = 1.2, 2.4)	3.98 (1H, *dd*, *J* = 3.3, 1.5)	4.79 (1H, *d*, J = 3.7)	4.70 (1H, *d*, *J* = 3.6)	4.76 (1H, *br s*)
3″	4.13 (1H, *t*, *J* = 8.5)	3.70 (1H, *dd*, *J* = 2.5, 9.5)	3.71 (1H, *dd*, *J* = 2.4, 10.0)	3.81 (1H, *dd*, *J* = 5.8, 3.3)	–	–	–
4″	4.21 (1H, *m*)	4.30 (1H, *t*, *J* = 9.5)	3.91 (1H, *t*, *J* = 9.4)	3.96 (1H, *ddd*, *J* = 5.8, 5.8, 3.4)	4.37 (1H, *d*, *J* = 10.8) and 4.74 (1H, *d*, *J* = 10.8)	4.33 (1H, *d*, *J* = 9.6) and 4.58 (1H, *d*, *J* = 9.6)	4.39 (1H, *d*, *J* = 9.3) and 4.35 (1H, *d*, *J* = 9.3)
5″	3.65 (1H, *dd*, *J* = 11.2, 10.0) and 4.33 (1H, *dd*, *J* = 11.0, 6.5)	4.12 (1H, *dq*, *J* = 9.5, 6.5)	3.82 (1H, *dq*, *J* = 9.4, 6.2)	3.73 (1H, *dd*, *J* = 11.7, 3.3) and 3.63 (1H, *dd*, *J* = 11.7, 5.5)	4.28 (1H, *d*, *J* = 9.6) and 4.71 (1H, *d*, *J* = 9.6)	4.45 (1H, *d*, *J* = 8.7) and 4.83 (1H, *d*, *J* = 8.7)	4.35 (1H, *d*, *J* = 9.0) and 4.67 (1H, *d*, *J* = 9.0)
6″	–	1.30 (3H, *d*, *J* = 6.5)	1.41 (3H, *d*, *J* = 6.2)	–	–	–	–

the presence of a methylene carbon at δ_C 60.1 (C-6′) not associated with the aglycon portion of the compound. Then, using the ^{13}C–^{1}H correlations from the HMQC data, the corresponding proton chemical shifts were determined to be δ_H 4.58 and δ_H 4.42 (H-6′), respectively. By using these methylene proton signals and their counterparts as the starting points, the sugar intra-residue connectivity was deciphered for the remaining sugar in the same manner as for the deoxyhexose. Chemical shifts for the glycosidic moieties are listed in Tables 4 and 5.

The glycosidic linkages between H-1″ (δ_H 4.68) and H-4′ (δ_H 4.30) were clearly observed in the NOESY spectrum. In addition, cross-peaks between H-1′ (δ_H 5.25) and H-18 of the aglycone were observed, revealing the point of attachment of the glycoside to the aglycone. The absolute stereochemistry at C-18 carbon of aglycone was determined by means of the Mosher's method after enzymatic hydrolysis and also by glycosidation shifts, which are in accordance with the above mentioned data for **4b**. Thus, **7** was found to have the linkage (18*S*)-18-*O*-α-L-rhamnopyranosyl-(1-4)-β-D-glucopyranoside to protoconstipatic acid.

Compound **8** was obtained as a colorless wax-like semisolid, $[a]_D^{23}-75.0^\circ$. Its positive ion HRFABMS exhibited a peak at m/z 685.7623 $[M+H]^+$, compatible with the molecular formula $C_{32}H_{54}O_{14}$. Its ^1H NMR spectrum (Table 5) revealed the presence of two anomeric protons at δ_H 5.24 and δ_H 4.95. Acid hydrolysis of **8** yielded D-glucose and D-arabinose (identified by $[\alpha]_D$ and GLC).

The sequence of the sugar part could be determined by the MS fragmentation peak at m/z 529 $(M-H-132)^-$, which indicated that the D-arabinose moiety was the terminal sugar. The ^{13}C NMR chemical shift of C-6′ in glucose at δ_C 68.1 indicated glycosylation at this position, and the long-range correlation between δ_H 4.95 (1H, *d*, $J=1.3$ Hz, anomeric proton of D-arabinose) and δ_C 68.1 (C-6′ of glucose) in the HMBC spectrum of **8** confirmed the interglycosidic linkage to be arabinose-(1-6)-glucose. The coupling constants of the anomeric protons in arabinose ($J=1.3$ Hz) and glucose ($J=7.9$ Hz) showed these sugars to have the α and β configurations, respectively, and the ^{13}C NMR shifts of the arabinose carbons indicated that arabinose was in the furanose ring form. The absolute stereochemistry at C-18 carbon was S, as determined by hydrolysis and converting of aglycone to Mosher's method and also by glycosidation shifts (see compound **4b** and **7**, respectively). Based on these data, **8** is (18*S*)-18-*O*-α-D-arabinofuranosyl-(1-6)-β-D-glucopyranoside of protoconstipatic acid.

Glycoside **9**, an amorphous powder, was obtained from the MeOH extract by chromatography. The UV spectrum showed an absorbtion maximum at 209 nm (log ε 3.87). The IR spectrum showed absorptions for a hydroxy group at 3426 cm^{-1}.

In the negative-ion FABMS spectrum of **9**, the expected molecular ion peak ($C_{32}H_{54}O_{14}$) was observed at m/z 661 $(M-H)^+$. In addition, the peaks at m/z 499 $(M-H-162)^-$ and 367 $(M-H-162-132)^-$ indicated that the sugar chain, which consisted of hexose and pentose, was linked to the C-18 hydroxyl group of the aglycone and that the sequence is aglycone–hexose–pentose.

The subtraction of the carbon signals corresponding to this aglycone from the total number of signals observed in the ^{13}C NMR spectra of **9** allowed the assignment of the resonances due to two saccharide moieties [(C–O); $(CH–O)_7$; $(CH_2--O)_3=C_{11}H_{13}O_9$ (two ether functions)], corresponding to one pentose and one hexose moiety. A β-D-apiofuranoside moiety was recognized by a chemical shift at δ_C 111.3 attributed to an anomeric dioxymethine carbon –O–CH–O– (C-1″) in a furanoside ring observed in the ^{13}C NMR spectrum of **9**, along with the alcoholic signals of one quaternary at δ_C 80.7 (C-3″), two oxymethylenes at δ_C 75.1 (C-4″) and δ_C 65.8 (C-5″), and one oxymethine at δ_C 78.1 (C-2″) (Table 4). This deduction was confirmed by ^{13}C–^{1}H COSY spectrum of **9** through cross-peaks revealing heteronuclear coupling of quaternary C-3″ (δ_C 80.7) and methine C-1″ (δ_C 111.3), and hydrogens H-4″ δ_H 4.37 and δ_H 4.74, as shown in Table 5. Additional confirmation was obtained from the HMBC spectrum of **9**, which showed coupling of the carbons C-4″ (δ_C 75.1) and C-3″ (δ_C 80.7) with hydrogens H-1″ (δ_H 5.61) and H_a-4″ (δ_H 4.37), respectively.

The remaining ^{13}C signals correlated to alcoholic carbon atoms and were used to establish a β-D-glucopyranoside moiety: one anomeric dioxymethine [δ_C 104.6 (C-1′)], four monooxymethine [δ_C 75.3 (C-2′), 78.2 (C-3′), 71.7 (C-4′), and 77.6 (C-5′)], and one oxymethylene [δ_C 68.9 (C-6′)] carbons (Table 4). The chemical shift of the oxymethylene carbon C-6′ (δ_C 68.9) was used to define the disaccharide linkage as apiofuranosyl (1″-6′)–glucopyranoside, because the chemical shift of a monosaccharide oxymethylene group appears at about δ_C 62 (Table 3). The HMBC spectrum of **9** was used to confirm this deduction, which revealed cross-peaks corresponding to long-range coupling of carbon C-6′ (δ_C 68.9) and hydrogen H-1″ (δ_H 5.61, $^3J_{CH}$), along with the coupling of the C-1″ (111.3) and 2H-6″ [δ_H 4.28 (H_a-5″) and δ_H 4.71 (H_b-5″)].

The location of the 6-*O*-D-β-apiofuranosyl-β-D-glucopyranosyl moiety attached to the oxygenated carbon 18 of the aglycon was suggested by the slight modification observed in the ^{13}C signals of the tertiary C-18 and methylene CH_2-17 and methyl CH_3-19 carbon atoms revealed by comparative analysis of the ^{13}C

NMR spectra of **9** δ_C 60.5 (C-18) and δ_C 27.4 and δ_C 42.2 (C-19 and C-17), respectively. On the above evidences the structure of **9** was established to be (18*R*)-18-*O*-β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside of allo-murolic acid.

The ^{13}C and ^{1}H NMR spectra of **10** displayed signals for two sugar residues (Tables 4 and 5). One of these was easily identified from its quaternary carbon at δ_C 80.2 as a β-D-apiose moiety ($J_{H1-H2} = 3.6$ Hz). The other sugar residue was identified as a β-D-glucose unit. The anomeric proton of this glucose residue appeared as a doublet at δ_H 5.26 ($J = 7.3$ Hz), indicating that this glucose was substituted at the C-4 position δ_C 79.3. Analysis of the ^{13}C NMR chemical shifts of this glucose moiety, conducted by a combination of homo- and heteronuclear COSY spectra, indicated its substituted nature. Thus, **10** was found to have the (18*S*)-18-*O*-β-D-apiofuranosyl-(1-4)-β-D-glucopyranoside linkage to protoconstipatic acid.

The presence of three methylenes at δ_C 63.4, 64.3, and 74.1 among the remaining 8 carbons indicated that the aglycon was linked to a hexose and a pentose, and that the pentose was most probably apiose. The apiosyl anomeric carbon C-1″ showed a signal at δ_C 108.9, along with the signals of one quaternary carbon at δ_C 79.4 (C-3″), an oxymethine at δ_C 76.2 (C-2″), and two oxymethylenes at δ_C 74.1 (C-4″) and δ_C 64.3 (C-5″). For the inner sugar, ^{13}C chemical shifts were in accord with those reported for glucoside, mentioned earlies. 3-Bond correlations between the apiosyl anomeric proton H-l″ δ_H 5.36 and the glucosyl carbon C-2′ δ_C 75.7, as well as correlations between the glucosyl proton H-2′ (δ_H 4.35) and the apiosyl anomeric carbon C-1″ (δ_C 108.9), established the linked position between the two sugar moieties. The large coupling constant between H-l′ and H-2′ ($J = 7.1$ Hz) was typical of a β-glucosidic linkage to the aglycone. The glycosidation position was unambiguously determined by a 3-bond correlation between the glucosyl anomeric proton H-1′ (δ_H 5.22) and secondary alcohol of murolic acid, using gradient-selected HMBC. Finally, acid hydrolysis of the glycoside followed by GLC analysis showed that D-glucose was one of the two sugars, in addition to D-apiose. The structure of **11** was thus established as (18*R*)-18-*O*-β-D-apiofuranosyl-(1-2)-β-D-glucopyranoside of murolic acid.

To summarize the present study, the chemical structures and structural relationships among the 11 component lichen acids produced by lichens from Central Asia are shown in Figs. 1 and 2.

We have isolated three hydroxy substituted γ-lactonic carboxylic acids of which only murolic acid has previously been described. The absolute configuration of the hydroxyl group of protoconstipatic and allo-murolic acids was determined and allo-murolic acid was described for the first time in nature. The discovery of glycosides containing apiose, rhamnose, xylose and arabinose is most recent. The above-mentioned saccharides were identified in lichens for the first time.

In contrast to glycosidic compounds from higher (flower) plants, the first glycosides identified from lichens with different carbohydrates exhibit both a higher variability of aglycone and disaccharides. This may be caused by the adverse climate of the environment in which lichens grow, namely temperatures as high as 80°C, exposure to wind (lack of moisture follows from this) and salinated soil.

3. Experimental

3.1. General experimental procedures

UV spectra were recorded on a Cary 118 (Varian) apparatus in heptane within the range of 200–350 nm. Circular dichroism (CD) measurement was carried out on a Jasco-500A spectropolarimeter at 24°C, under dry N_2. 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). HRFABMS (positive and/or negative ion mode) were obtained with a PEG-400 as a matrix.

3.2. Plant material

The specimens of *Acarospora gobiensis*, *Cladonia furcata*, *Lecanora fructulosa*, *Leptogium saturninum*, *Peltigera canina*, *Xanthoparmelia camtschadalis*, *X. tinctina* and *Xanthoria elegans* were collected in July 1996 along the shore of lake Issyk Kul, Tian–Shan mountain, Uzbekistan.

3.3. Extraction and isolation

Samples of 100 g of air-dried lichen were extracted by the method of the Blight and Dyer (1959). The aqueous-MeOH (upper) layer after extraction of lipids from lichens was used for further work. Concentration of the H_2O–MeOH layer gave an organic fraction (8 g). Chromatography of this fraction on a Sephadex LH-20 column, with elution of MeOH (100 × 5 cm) gave organic fractions (8 ml) checked by TLC [silica-gel plates, *n*-BuOH–AcOH–H_2O (12:3:5) and $CHCl_3$–MeOH–H_2O (40:9:1)] and combined in three main fractions. Fraction A 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 (1:1) to yield

compounds **1**–**3**. Fraction B was separated by RP-HPLC with MeOH–H_2O (9:11) to yield only the compounds **4a** and **4b**. Fraction C separated by using MeOH–H_2O (2:3) yielded compounds **5-11**.

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. Identification of glycoside's component

A solution of **5**–**11** (each ~1 mg) was carried out in the same way as described for **4**. The aglycone parts of glycosides were transformed by Mosher's method to appropriate esters according to the process described later. The data of esters are described in Table 6. The identification was done and the D or L configuration of the sugar was determined using gas chromatography according to the method of Gerwig (Gerwig et al., 1978) with some modifications. Nitrogen was bubbled through a solution of the lyophilized sample (0.5 mg) after methanolysis of **4** in (+)-2-butanol (300 μl) and acetyl chloride (50 μl), and the ampoule was then sealed. After butanolysis at 80°C for 8 h, the solution was neutralized with Ag_2CO_3. After centrifugation at 2000 rpm for 10 min the supernatant solution was concentrated under reduced pressure at 45°C. The residue was treated with acetic anhydride (100 μl) in pyridine (200 μl). Heating at 70°C for 30 min brought about acetylation. The resultant compounds were subsequently analyzed by gas chromatography using a fused silica column (30 m × 0.25 mm ID, Supelco SPB-1). 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 and the detector was 300°C. Authentic 1-((+)-2-butyl)-2,3,4,6-tetraacetyl-D- and L-glucose, and 1-methyl-2,3,4,6-tetraacetyl-D-glucose were eluted as peaks with retention times of 12.55, 13.06 and 8.36 min, respectively.

3.6. Enzymatic hydrolysis and determination of glycosides

A solution of **4a** and/or **4b** (2 mg) in acetate buffer (pH 4.4, 10 ml) was treated with β-glucosidase (20 mg), and the solution was left at 37°C for 48 h. 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 afford **1** or **2** (1 mg), ^{1}H NMR data (Table 2).

3.7. (S)-MTPA esters

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 the solvent, the residue was purified by Si gel TLC (hexane–AcOEt, 2:1) to afford the (*S*)-MTPA ester (**4s**–**11s**) as colorless oil. The esters of the aglycones from above mentioned glycosides (**4**–**11**) are identical with one of **1** or **2** or **3**. The data of esters (see above) are described in Table 6.

3.8. (R)-MTPA ester

Aglycone (0.3 mg) was treated with (*S*)-(+)-MTPACl (2.0 mg) using the same procedure as described above to afford the (*R*)-MTPA ester (**4r**–**11r**) as colorless oil. The esters of the aglycones from above mentioned glycosides (**4**–**11**) are identical with one of **1** or **2** or **3**. The data of esters (see above) are described in Table 6.

Murolic acid (**1**) colorless plates, m.p. 112.5°C, $[\alpha]_D^{24}$ +11.2 (*c* 0.14, $CHCl_3$), UV λ_{max} (MeOH, nm) 217 (log ε 3.80); CD $\Delta\varepsilon_{200} = +3.50$ sh, $\Delta\varepsilon 220 = -8.21$, $\Delta\varepsilon 260 = +0.29$ (Fig. 3); IR (KBr) (cm^{-1}): 3500 (OH), 2950, 2900, 1740 (lactone), 1702 (acid), 1650 (C=CH_2); HRFABMS *m/z*: 369.5213 (MH^+, calcd for $[C_{21}H_{36}O_5+H]^+$ 369.5218; LREIMS *m/z* (%), 368 $[M]^+$ (3), 353 $[M-Me]^+$ (45), 350 $[M-H_2O]^+$ (37), 332 $[M-2\times H_2O]^+$ (61), 324 $[M-CO_2]^+$ (100), 306 $[M-CO_2-H_2O]^+$ (80); ^{1}H and ^{13}C-NMR spectra (Table 2).

Table 6
Stereochemical analysis of compounds **1**, **2** and **3** with (*R*)- and (*S*)-MTPA (Mosher) derivatives[a]

$\Delta\delta = \delta_{S-ester} - \delta_{R-ester}$ (Hz)			
MTPA esters of compound no.	H-19	H-17	Carbinol configuration
1	+14.5	−38.0	*R*
2	−12.5	+22.5	*S*
3	+15.5	−42.0	*R*

[a] The aglycones from glycosides **4**–**11** are identical with one of **1** or **2** or **3**.

Protoconstipatic acid (**2**) white flakes, m.p. 103.5°C, $[\alpha]_D^{24}$ −12.3 (*c* 0.15, $CHCl_3$), UV λ_{max} (MeOH, nm) 218 (log ε 3.91); CD Δε200 = +3.69 sh, Δε220 = −9.62, Δε260 = +0.32 (Fig. 3); IR (KBr) 3500 (OH), 2945, 2905, 1743 (lactone), 1710 (acid), 1470, 1436, 1404, 828 (C=CH_2) cm^{-1}; HRFABMS *m/z* 369.5221 $(MH^+$, calcd for $[C_{21}H_{36}O_5+H]^+$ 369.5218; LREIMS *m/z* 368 $[M]^+$ (4), 353 $[M-Me]^+$ (51), 350 $[M-H_2O]^+$ (23), 332 $[M-2\times H_2O]^+$ (54), 324 $[M-CO_2]^+$ (100), 306 $[M-CO_2-H_2O]^+$ (65). ^1H and ^{13}C-NMR spectra (Table 2).

Allo-murolic acid (**3**) white plates, m.p. 89°C, $[\alpha]_D^{24}$ −15.7 (c 0.10, $CHCl_3$), UV λ_{max} (MeOH, nm) 209 (log ε 3.92); CD Δε200 = +0.57 sh, Δε220 = +1.44, Δε260 = +0.97 (Fig. 3); IR (KBr) 3510 (OH), 2955, 2900, 1738 (lactone), 1705 (acid), 1655 (C=CH_2), 1475, 830 (C=CH_2) cm^{-1}; HRFABMS *m/z* 369.5217 $(MH^+$, calcd for $[C_{21}H_{36}O_5+H]^+$ 369.5218; LREIMS *m/z* 368 $[M]^+$ (5), 353 $[M-Me]^+$ (47), 350 $[M-H_2O]^+$ (51), 332 $[M-2\times H_2O]^+$ (49), 324 $[M-CO_2]^+$ (100), 306 $[M-CO_2-H_2O]^+$ (66); ^1H and ^{13}C-NMR spectra (Table 2).

(18*R*)-18-*O*-β-D-Glucopyranoside of murolic acid (**4a**) colorless powder, m.p. 147°C, $[\alpha]_D^{24}$ −187° (c 0.14, MeOH); UV 218 nm (log ε 3.84); IR 3500 (OH), 1740 (γ-lactone), 1708 (carboxyl) 1665 (C=C) cm^{-1}; HRFABMS *m/z* 531.6645 $[MH]^+$, calcd for $[C_{27}H_{46}O_{10}+H]^+$ 531.6642; negative LRFABMS *m/z* 529 $(M-H)^-$, 367 $(M-H-162)^-$; ^1H and ^{13}C-NMR spectra, (Table 3).

(18*S*)-18-*O*-β-D-Glucopyranoside of protoconstipatic acid (**4b**) colorless powder, m.p. 157°C, $[\alpha]_D^{24}$−180° (*c* 0.15, MeOH); UV 217 nm (log ε 3.80); IR 3500 (OH), 1740 (γ-lactone), 1708 (carboxyl) 1665 (C=C) cm^{-1}; HRFABMS *m/z* 531.6648 $(MH)^+$, calcd for $[C_{27}H_{46}O_{10}+H]^+$ 531.6642; negative LRFABMS *m/z* 529 $(M-H)^-$, 367 $(M-H-162)^-$; ^1H and ^{13}C-NMR spectra, (Table 3).

(18*R*)-18-*O*-1-β-Xylopyranosyl-(1-6)-β-D-glucopyranoside of allo-murolic acid (**5**) colorless powder, m.p. 211°C; $[\alpha]_D^{24}$ −63°; HRFABMS *m/z* 663.7800 $(MH)^+$, calcd for $[C_{32}H_{54}O_{14}+H]^+$ 663.7803; negative FABMS *m/z* 662 $(M-H)^-$, 529 $(M-H-132)^-$ and 367 $(M-H-132-162)^-$; ^1H and ^{13}C-NMR spectra (Tables 4 and 5).

(18*R*)-18-*O*-α-L-Rhamnopyranosyl-(1-6)-β-D-glucopyranoside of murolic acid (**6**) colorless powder, m.p. 205°C; $[\alpha]_D^{24}$ −51°; HRFABMS *m/z* 677.8075 $(MH)^+$, calcd for $[C_{33}H_{56}O_{14}+H]^+$ 677.8072; negative FABMS *m/z* 675 $(M-H)^-$, 529 $(M-H-146)^-$, 367 $M-H-146-162)^-$; ^1H and ^{13}C-NMR spectra (Tables 4 and 5).

(18*S*)-18-*O*-α-L-Rhamnopyranosyl-(1-4)-β-D-glucopyranoside of protoconstipatic acid (**7**) colorless powder, m.p. 234°C; $[\alpha]_D^{24}$ −28.0°; HRFABMS *m/z* 677.8079 $(MH)^+$, calcd for $[C_{33}H_{56}O_{14}+H]^+$ 677.8072; negative FABMS *m/z* 675 $(M-H)^-$, 529 $(M-H-146)^-$, 367 $M-H-146-162)^-$; ^1H and ^{13}C-NMR spectra (Tables 4 and 5).

(18*S*)-18-*O*-α-D-Arabinofuranosyl-(1-6)-β-D-glucopyranoside of protoconstipatic acid (**8**) colorless wax-like semisolid, m.p. 134°C; $[\alpha]_D^{24}$ −75.0°; HRFABMS *m/z* 663.7812 $(MH)^+$, calcd for $[C_{32}H_{54}O_{14}+H]^+$ 663.7803; negative FABMS *m/z* 661 (M–H)$^-$, 529 $(M-H-132)^-$ and 367 $(M-H-132-162)^-$; ^1H and ^{13}C-NMR spectra (Tables 4 and 5).

(18*R*)-18-*O*-β-D-Apiofuranosyl-(1-6)-β-D-glucopyranoside of allo-murolic acid (**9**) colorless powder, m.p. 278°C; $[\alpha]_D^{24}$ −63.0°; HRFABMS *m/z* 663.7804 $(MH)^+$, calcd for $[C_{32}H_{54}O_{14}+H]^+$ 663.7803; negative FABMS *m/z* 661 (M-H)$^-$, 529 $(M-H-132)^-$ and 367 $(M-H-132-162)^-$; ^1H and ^{13}C-NMR spectra (Tables 4 and 5).

(18*S*)-18-*O*-β-D-Apiofuranosyl-(1-4)-β-D-glucopyranoside of protoconstipatic acid (**10**) colorless powder, m.p. 257°C; $[\alpha]_D^{24}$ −41.0°; HRFABMS *m/z* 663.7806 $(MH)^+$, calcd for $[C_{32}H_{54}O_{14}+H]^+$ 663.7803; negative FABMS *m/z* 661 (M-H)$^-$, 529 $(M-H-132)^-$ and 367 $(M-H-132-162)^-$; ^1H and ^{13}C-NMR spectra (Tables 4 and 5).

(18*R*)-18-*O*-β-D-Apiofuranosyl-(1-2)-β-D-glucopyranoside of murolic acid (**11**) colorless powder, m.p. 212°C; $[\alpha]_D^{24}$ −56.0°; HRFABMS *m/z* 663.7814 $(MH)^+$, calcd for $[C_{32}H_{54}O_{14}+H]^+$ 663.7803; negative FABMS *m/z* 661 (M-H)$^-$, 529 $(M-H-132)^-$ and 367 $(M-H-132-162)^-$; ^1H and ^{13}C-NMR spectra (Tables 4 and 5).

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