



NOROLEANANE SAPONINS FROM *CELMISIA SPECTABILIS*

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(Received 5 August 1997)

Key Word Index—*Celmisia spectabilis*; Asteraceae; triterpenoid saponins; 2 β ,3 β ,17,23-tetrahydroxy-28-norolean-12-en-16-one.

Abstract—Two new saponins were isolated as the major components of the deacylated saponin extract from the underground parts of *Celmisia spectabilis*. Their structures were established by NMR and mass spectral data and derivative formation as 2 β ,3 β ,17,23-tetrahydroxy-28-norolean-12-en-16-one-3-*O*- α -L-arabinopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl(1 \rightarrow 6)- β -D-glucopyranoside and 2 β ,3 β ,17,23-tetrahydroxy-28-norolean-12-en-16-one-3-*O*- α -L-arabinopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl(1 \rightarrow 6)-[α -L-rhamnopyranosyl(1 \rightarrow 2)]- β -D-glucopyranoside. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genus *Celmisia* includes about 80 species, most of which are found in mountainous regions of New Zealand. The habit is often similar to that of the European *Bellis* and consequently the name mountain daisy has been given to plants of this genus. Some plants of the genus, *C. spectabilis* among them, are used for medicinal purposes as fine cotton lint for dressing wounds and for relieving asthma [1]. The presence of saponins in plants of this genus was first documented when noroleanane saponins were shown to be present in *C. petriei* [2].

Celmisia spectabilis Hook. f. (cotton daisy, cotton plant, tikumu) is a stout, rosulate herb found in alpine regions of New Zealand from Mt. Hikurangi southwards to North Otago. In a continuation of our systematic studies of the saponin containing medicinal plants of the tribe Astereae (Asteraceae), we have isolated and identified the two major saponins from the roots of this plant.

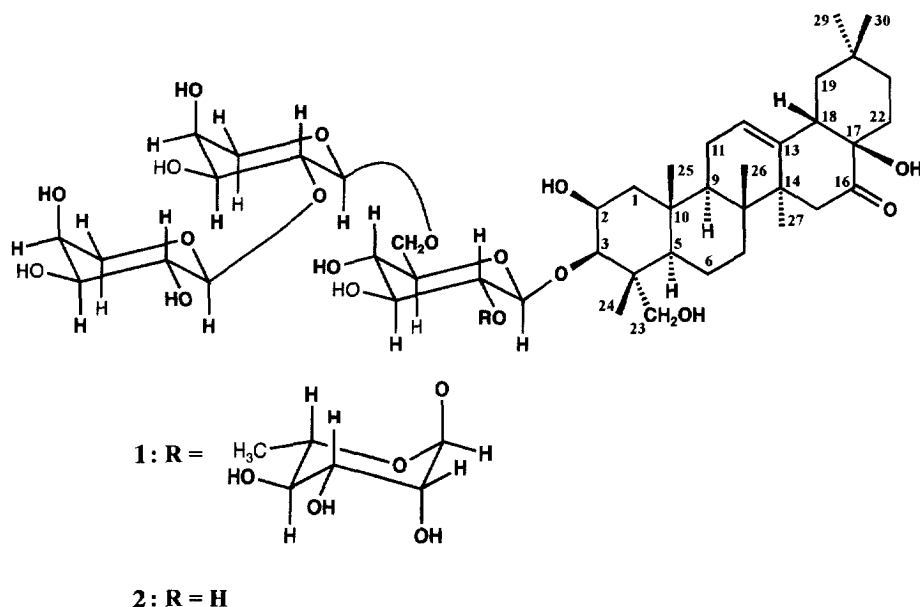
RESULTS AND DISCUSSION

Compounds **1** and **2** were isolated from alcoholic extracts after alkaline hydrolysis by column chromatography and HPLC as described in the Experimental section. Sugar component analysis [3] and the

determination of the absolute configuration of the sugars [4] revealed the presence of two molecules of L-arabinose and one molecule each of L-rhamnose and D-glucose in compound **1** and of two L-arabinoses and one D-glucose in **2**.

Electrospray ionisation mass spectrometry of compound **1** gave a molecular ion at m/z 1045 $[M-H]^-$ in the negative ion mode and m/z 1069 $[M+Na]^+$ in the positive ion mode. The fragmentation pattern obtained after collision induced dissociation (CID) of these molecular ions (see Experimental) indicated that **1** consisted of an aglycone with a molecular weight of 474 Dalton. From the 1H and ^{13}C NMR data (Table 2) the presence of four carbohydrate units and of an aglycone possessing six tertiary methyl, a hydroxymethylene and a keto function was evident. As 2 β ,3 β ,17,23-tetrahydroxy-28-norolean-12-en-16-one has been described as the aglycone of the major saponins obtained from *C. petriei* [1], a comparison of the ^{13}C NMR data was performed. Since slight differences were obvious, the structure of the aglycone was carefully established on the basis of 2D NMR experiments [1H - 1H correlation spectroscopy (COSY), 1H -detected heteronuclear multiple quantum coherence spectroscopy (HMQC), 1H -detected multiple bond ^{13}C multiple quantum coherence spectroscopy (HMBC)]. Most assignments were unambiguously established, as shown in Table 1 by starting from the 1H methyl signals which show intense long-range through-bond correlations to all carbons over two and three bonds in the HMBC spectrum. These, in conjunction with the direct correlations and subsequent through-bond 1H - 1H correlations, provide a network of correlations

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representative of this aglycone type. The exact nature of the aglycone was established by identification of the carbons of the carbonyl and tertiary alcohol carbons at C-16 and C-17, respectively. Thus cross peaks were observed between H-15 α (δ 3.35) and H-15 β (δ 1.68) with the carbonyl carbon at δ 217.1 together with cross peaks between H-15 β (δ 1.68), H-18 (δ 2.83) and H-19 α (δ 1.48) and a quaternary carbon at δ 77.5 to give a final proof of the identity of the aglycone.

Methylation analysis of the saponin afforded 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylarabitol, 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylarabitol, 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol and 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methylglucitol in approximately equal amounts indicating terminal arabinose (ara¹) and rhamnose, 1,2-linked arabinose (ara²) and 1,2,6-linked glucose compatible with a tetrasaccharide glycan moiety branched at the glucose residue. The MS-MS fragmentation pattern (see Experimental) confirmed these results and showed that glucose is the innermost carbohydrate residue linked to the aglycone. The NMR data of the sugar systems were assigned on the basis of the same 1D and 2D NMR experiments referred to above (see Table 2). The α -anomeric, ⁴C₁-configuration of arabinoses and the β -anomeric, ⁴C₁-configuration of glucose were evident from the H-1, H-2 coupling constants. The H-4-H-5 coupling constant of 9.6 Hz showed that rhamnose was present in a ¹C₄-configuration while the C-1-H-1 coupling constant of 169 Hz indicated an α -glycosidic linkage [5].

Cross peaks in the HMBC spectrum between C-1 of terminal arabinose (δ 105.5) and H-2 of the 1,2-linked arabinose (δ 3.82), C-1 of ara² (δ 103.3) and H-6A and H-6B of glucose (δ 4.04/3.80) and between H-1 of rhamnose (δ 5.41) and C-2 of glucose (δ 78.8) indicated the interglycosidic linkage positions. Finally, a cross peak between C-1 of glucose (δ 104.8)

and H-3 (δ 3.74) of the aglycone showed that the carbohydrate moiety was attached to the aglycone in position C-3. Hence, compound **1** was 2 β ,3 β ,17,23-tetrahydroxy-28-norolean-12-en-16-one-3-*O*- α -L-arabinopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl(1 \rightarrow 6)-[α -L-rhamnopyranosyl(1 \rightarrow 2)]- β -D-glucopyranoside.

Electrospray ionization mass spectrometry of compound **2** gave a molecular ion at m/z 899 [$M - H$]⁻ in the negative ion mode and m/z 923 [$M + Na$]⁺ in the positive mode. The fragmentation pattern obtained after CID (see Experimental) indicated that **2** also consisted of an aglycone of a molecular weight 474 Dalton linked to a trisaccharide with terminal pentose, a second pentose and a hexose bound to the aglycone. Sugar component analysis and determination of the absolute configuration gave L-arabinose and D-glucose in a 2:1 ratio. Methylation analysis afforded terminal arabinose, 1,2-linked arabinose and 1,6-linked glucose. The NMR data were assigned by comparison with compound **1** and on the basis of the COSY spectrum, as summarized in Table 2. From these data it is evident that compound **2** had the same basic structure as compound **1** except the rhamnose attached to the glucose moiety was absent. Hence, compound **2** was 2 β ,3 β ,17,23-tetrahydroxy-28-norolean-12-en-16-one-3-*O*- α -L-arabinopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl(1 \rightarrow 6)- β -D-glucopyranoside.

Both compounds are new triterpenoid saponins. However, compound **2** is an isomer of saponin (1a), previously obtained by Rowan and Newman from *Celmisia petriei* [2]. The only difference is the sequence of the carbohydrate chain. While compound **2** consists of an α -L-arabinopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl(1 \rightarrow 6)- β -D-glucopyranoside, in saponin (1a) arabinose is attached to the aglycone. The

Table I. Assignments of ^1H and ^{13}C NMR chemical shifts (δ) of the aglycone of **1** on the basis of COSY, HMQC and HMBC spectra (correlations are indicated by an arrow; direct correlations are illustrated in one line; long range and proton-proton correlations in different lines)

Methyl proton	\rightarrow HMQC (^{13}C)	\rightarrow HMBC (^{13}C)	\rightarrow HMQC (^1H)	\rightarrow COSY (^1H)	\rightarrow COSY (^1H)	\rightarrow HMQC (^{13}C)	Assigned C atom
1.35	\rightarrow 17.4						25
		\rightarrow 48.5	\rightarrow 1.59				9
				\rightarrow 2.18, 2.05		\rightarrow 24.8	11
		\rightarrow 48.5	\rightarrow 1.39		\rightarrow 5.46	\rightarrow 125.4	12
				\rightarrow 1.59		\rightarrow 18.4	5
					\rightarrow 1.67, 1.29	\rightarrow 33.5	6
		\rightarrow 44.4	\rightarrow 2.11, 1.26				7
				\rightarrow 4.41		\rightarrow 71.9	1
					\rightarrow 3.78	\rightarrow 82.8	2
		\rightarrow 37.4					3
							10
1.20	\rightarrow 27.4						27
		\rightarrow 142.9					13
		\rightarrow 49.8					14
		\rightarrow 43.9	\rightarrow 3.35, 1.68				15
		\rightarrow 41.1					8
1.16	\rightarrow 18.0						26
		\rightarrow 49.8					14
		\rightarrow 48.5	\rightarrow 1.59				9*
		\rightarrow 41.1					8
		\rightarrow 33.5	\rightarrow 1.67, 1.29				7*
1.01	\rightarrow 24.0						29
		\rightarrow 49.2	\rightarrow 1.48, 1.27				19
				\rightarrow 2.83		\rightarrow 53.5	18
		\rightarrow 37.8	\rightarrow 1.60, 1.30				22
				\rightarrow 2.05†, 1.45‡		\rightarrow 31.6	21
		\rightarrow 33.1	\rightarrow 0.90				30
		\rightarrow 31.6	\rightarrow 2.05, 1.45				21*
		\rightarrow 31.6					20
1.00	\rightarrow 14.9						24
		\rightarrow 82.8	\rightarrow 3.74				3*
		\rightarrow 65.4	\rightarrow 3.65, 3.33				23
		\rightarrow 48.5	\rightarrow 1.39				5*
		\rightarrow 43.2					4
0.90		\rightarrow 33.1					30
		\rightarrow 49.2	\rightarrow 1.48, 1.27				19*
		\rightarrow 37.8	\rightarrow 1.60, 1.30				22*
		\rightarrow 31.6	\rightarrow 2.05, 1.45				21*
		31.6					20
		\rightarrow 24.0	\rightarrow 1.01				29

* For correlations see upper part of Table.

† Cross peak only with proton at δ 1.60.

‡ Cross peak only with proton at δ 1.30.

sugar sequence of saponin (1a) was determined by ^{13}C NMR relaxation times and on the basis of inertness to hydrolysis with emulsin. Since these procedures do not give unambiguous results it can be assumed that compound **2** is identical with saponin (1a) from *C. petriei*.

EXPERIMENTAL

General experimental procedures

1D and 2D NMR spectra were recorded in CD_3OD at 300 K on Bruker ARX-400 NMR (^1H : 400.13 MHz,

Table 2. NMR data of compounds **1** and **2** in CD₃OD*

	Atom	1	2		1	2
Aglycone	C-1	44.4	44.1	H-1A	2.11 (<i>d</i> , <i>J</i> = 16.0 Hz)	2.11 (<i>dd</i> , <i>J</i> = 3.6/14.9 Hz)
				H-1B	1.26 (<i>d</i> , <i>J</i> = 16.0 Hz)	1.25 (<i>dd</i> , <i>J</i> = 4.8/14.9 Hz)
	C-2	71.9	71.2	H-2	4.41 (<i>m</i>)	4.39 (<i>m</i>)
	C-3	82.8	83.5	H-3	3.74 (<i>d</i> , <i>J</i> = 3.6 Hz)	3.70 (<i>d</i> , <i>J</i> = 3.5 Hz)
	C-4	43.2	43.2			
	C-5	48.5	48.5 [†]	H-5	1.39 (<i>d</i> , <i>J</i> = 11.3 Hz)	†
	C-6	18.4	18.5	H-6A/B	1.59 (<i>m</i>)	1.58 (<i>m</i>)
	C-7	33.5	33.5	H-7A	1.67 (<i>td</i> , 14.3/4.0 Hz)	1.67
				H-7B	1.29 (<i>d</i> , 14.3 Hz)	1.30
	C-8	41.1	41.1			
	C-9	48.5	48.7 [‡]	H-9	1.59 (<i>dd</i> , <i>J</i> = 11.8/7.1 Hz)	1.59 (<i>m</i>)
	C-10	37.4	37.5			
	C-11	24.8	24.7	H-11A	2.81 (<i>m</i>)	2.18 (<i>m</i>)
				H-11B	2.05 (<i>m</i>)	2.04 (<i>m</i>)
	C-12	125.4	125.4	H-12	5.46 (<i>t</i> , <i>J</i> = 3.6 Hz)	5.45 (<i>t</i> , <i>J</i> = 4.1 Hz)
	C-13	142.8	142.8			
	C-14	49.8	49.8			
	C-15	43.9	43.8	H-15A	3.35 (<i>d</i> , <i>J</i> = 14.9 Hz)	3.34 (<i>d</i> , <i>J</i> = 13.3 Hz)
				H-15B	1.68 (<i>d</i> , <i>J</i> = 14.9 Hz)	1.68 (<i>d</i> , <i>J</i> = 13.3 Hz)
	C-16	217.1	217.1			
	C-17	77.5	77.5			
	C-18	53.5	53.4	H-18	2.83 (<i>dd</i> , <i>J</i> = 15.0/4.2 Hz)	2.83 (<i>dd</i> , <i>J</i> = 14.2/3.9 Hz)
	C-19	49.2	49.2 [‡]	H-19A	1.48 (<i>t</i> , <i>J</i> = 15.0 Hz)	1.48 (<i>t</i> , <i>J</i> = 14.2 Hz)
				H-19B	1.27 (<i>dd</i> , <i>J</i> = 15.0/4.2 Hz)	1.27 (<i>d</i> , <i>J</i> = 14.2/3.9 Hz)
	C-20	31.6	31.6			
	C-21	31.6	31.6	H-21A	2.05 (<i>d</i> , 14.9 Hz)	2.06 (<i>d</i> , <i>J</i> = 13.0 Hz)
				H-21B	1.46 (<i>m</i>)	1.46 (<i>dt</i> , <i>J</i> = 4.8/13.0 Hz)
	C-22	37.8	37.8	H-22A	1.60 (<i>t</i> , 15.5 Hz)	1.58 (overlapped)
				H-22B	1.30 (<i>dd</i> , 15.5/3.2 Hz)	1.31 (overlapped)
	C-23	65.4	65.4	H-23A	3.65 (<i>d</i> , <i>J</i> = 12.2 Hz)	3.67 (<i>d</i> , <i>J</i> = 11.5 Hz)
				H-23B	3.33 (<i>d</i> , <i>J</i> = 12.2 Hz)	3.27 (<i>d</i> , <i>J</i> = 11.5 Hz)
	C-24	14.9	14.7	H ₃ -24	1.00 (<i>s</i>)	0.99 (<i>s</i>)
	C-25	17.4	17.4	H ₃ -25	1.35 (<i>s</i>)	1.36 (<i>s</i>)
	C-26	18.0	18.0	H ₃ -26	1.16 (<i>s</i>)	1.16 (<i>s</i>)
	C-27	27.4	27.4	H ₃ -27	1.20 (<i>s</i>)	1.20 (<i>s</i>)
	C-29	33.1	33.0	H ₃ -29	0.90 (<i>s</i>)	0.90 (<i>s</i>)
	C-30	24.0	24.0	H ₃ -30	1.01 (<i>s</i>)	1.01 (<i>s</i>)
Term. Ara	C-1	105.5	105.6	H-1	4.54 (<i>d</i> , <i>J</i> = 7.0 Hz)	4.53 (<i>d</i> , <i>J</i> = 7.1 Hz)
	C-2	72.6	72.7	H-2	3.69 (<i>dd</i> , <i>J</i> = 7.1/8.9 Hz)	3.68 (<i>t</i> , <i>J</i> = 7.1 Hz)
	C-3	74.2	74.2	H-3	3.60 (<i>dd</i> , <i>J</i> = 8.9/4.7 Hz)	3.60 (overlapped)
	C-4	69.5	69.5	H-4	3.86 (<i>d</i> , <i>J</i> = 4.7 Hz)	3.84 (<i>d</i> , <i>J</i> = 4.0 Hz)
	C-5	67.2	67.2	H-5A	4.01 (<i>bd</i> , <i>J</i> = 14.0 Hz)	4.00 (<i>dd</i> , <i>J</i> = 4.0/12.3 Hz)
				H-5B	3.63 (<i>bd</i> , <i>J</i> = 14.0 Hz)	3.62 (<i>bd</i> , <i>J</i> = 12.3 Hz)
Rha	C-1	102.1		H-1	5.41 (<i>d</i> , <i>J</i> = 1.8 Hz)	
	C-2	71.8		H-2	4.05 (<i>dd</i> , <i>J</i> = 1.8/4.0 Hz)	
	C-3	72.0		H-3	3.83 (<i>dd</i> , <i>J</i> = 4.0/9.6 Hz)	
	C-4	73.9		H-4	3.44 (<i>t</i> , <i>J</i> = 9.6 Hz)	
	C-5	70.3		H-5	3.99 (<i>dd</i> , <i>J</i> = 6.2/9.6 Hz)	
	C-6	18.0		H ₃ -6	1.28 (<i>d</i> , <i>J</i> = 6.2 Hz)	
1,2 Ara	C-1	103.3	103.0	H-1	4.53 (<i>d</i> , <i>J</i> = 6.8 Hz)	4.56 (<i>d</i> , <i>J</i> = 6.1 Hz)
	C-2	79.7	79.8	H-2	3.82 (overlapped)	3.81 (overlapped)
	C-3	73.2	73.3	H-3	3.79 (overlapped)	3.79 (overlapped)
	C-4	68.7	68.9	H-4	3.91 (<i>d</i> , <i>J</i> = 4.5 Hz)	3.87 (overlapped)
	C-5	65.6	64.4	H-5A	3.88 (<i>dd</i> , <i>J</i> = 4.5/13.3 Hz)	3.89 (overlapped)
				H-5B	3.58 (<i>dd</i> , <i>J</i> = 2.3/13.3 Hz)	3.57 (<i>d</i> , <i>J</i> = 10.0 Hz)

Table 2.—Continued.

Glc	C-1	104.8	105.6	H-1	4.62 (<i>d</i> , <i>J</i> = 7.2 Hz)	4.49 (<i>d</i> , <i>J</i> = 7.8 Hz)
	C-2	78.8§	73.8	H-2	3.53 (overlapped)	3.31 (overlapped)
	C-3	71.5	77.9	H-3	3.52 (overlapped)	3.39 (overlapped)
	C-4	79.4§	71.1	H-4	3.53 (overlapped)	3.40 (overlapped)
	C-5	76.2	76.4	H-5	3.48 (overlapped)	3.50 (overlapped)
	C-6	69.2	68.9	H-6A	4.04 (<i>dd</i> , <i>J</i> = 12.1/2.3 Hz)	4.05 (<i>bd</i> , <i>J</i> = 11.2 Hz)
				H-6B	3.80 (<i>dd</i> , <i>J</i> = 12.1/4.8 Hz)	3.80 (<i>dd</i> , <i>J</i> = 4.0/11.2 Hz)

* The atom numbering follows the accepted numbering of this system and consequently C-28 does not appear.

† Unambiguous assignment not possible.

‡ Obtained from DEPT spectrum.

§ Assignments interchangeable.

^{13}C : 100.61 MHz, compound **1**) or AM-300 NMR spectrometers (^1H : 300.13 MHz, ^{13}C : 75.48 MHz, compound **2**), MS were obtained on a Finnigan TSQ 700 equipped with a Finnigan electrospray source (ESI MS and MS-MS). Gas chromatography was performed on a Hewlett-Packard HP 5890 Series II with FID detection. GC/MS analyses were performed on a Finnigan GCQ ion trap mass spectrometer connected to a Finnigan gas chromatograph. $[\alpha]_D$ -values were measured on a Perkin-Elmer 241 C polarimeter. TLC was carried out on silica gel 60 plates or foils (Merck), column chromatography on Diaion HP-20 and silica gel 60.

Plant material

Plants were collected on 5 March 1996, in New Zealand, South Island, Canterbury at Mont Terako at an altitude of about 1700 m. The material was immediately air dried and later redried at 50–60°. A voucher specimen is deposited at the herbarium of the ZE Botanischer Garten und Botanisches Museum of the FU Berlin (B), herbal name *Celmisia spectabilis* Hook. f. (collection number Oberprieler 9666).

Extraction and isolation

The dried underground parts (65 g) were refluxed with 80% MeOH (1 h, 1 l). After removal of the MeOH extract the material was further refluxed with 96% EtOH (1 h, 1 l). After filtration, the solvents were removed under red. pres. to give 17.9 g of viscous MeOH extract and 1.0 g of solid EtOH extract. The combined extracts were hydrolyzed with 200 ml of 1% KOH for 2 h at room temp. After neutralization with HCl, the deacylated saponin mixture was chromatographed on a column of Diaion HP-20 using a H_2O , 50% MeOH, MeOH gradient. The MeOH eluate was dried to give 2.6 g of a saponin fraction. This was chromatographed on 270 g of silica gel 60 (Merck, particle size 0.063–0.200 mm, solvent EtOAc–MeOH– H_2O 68.6–17.1–14.3, upper layer), to give 95 mg of compound **1** and 540 mg of compound **2**.

Identification of the component monosaccharides

The determination was performed according to Kusumoto *et al.* [3] using 0.5 mg of each compound. GLC conditions: column J&W Scientific DB-17 (30 m \times 0.25 mm ID, film thickness 0.25 μm), oven temperature: 170° for 10 min, then increasing by 2° \times min $^{-1}$, 250° injection port temp., 280° detector temp., carrier gas He (14 cm s $^{-1}$), injection volume 1 μl , split ratio 1:50. *R*_f: arabinose 7.90, 8.27, 8.69 and 9.07 min (compounds **1** and **2**), glucose 18.65 and 19.04 (compounds **1** and **2**), rhamnose 8.65 and 8.99 min (compound **1**).

Determination of the absolute configuration of the sugars

The determination was performed according to Ref. [4] using about 3 mg of each compound. GLC conditions: column J&W Scientific DB-17 (30 m \times 0.25 mm I.D., film thickness 0.25 μm), oven temperature: 250°C; 260°C injection port temp. 280° detector temp.; carrier gas He (14 cm s $^{-1}$), injection volume 1 μl ; split ratio 1:50. *R*_f: L-arabinose 8.62 min (compounds **1** and **2**, D-arabinose 9.26 min), D-glucose 11.49 min (compounds **1** and **2**, L-glucose 12.05 min), L-rhamnose 9.39 min (compound **1**).

Preparation and analysis of partially methylated alditol acetates, [6, 7]

Each component (100 μg) was dissolved in 150 μl of DMSO and methylated according to Hakamori [8]. Hydrolysis was performed using trifluoroacetic acid, reduced using NaBH $_4$, and acetylated using acetic acid anhydride. GLC conditions: column J&W Scientific DB-5 (30 m \times 0.25 mm ID, film thickness 0.25 μm), temp. program: 1 min 80°, then 10° \times min $^{-1}$ to 300°. The respective partially methylated alditol acetates were identified by comparison with standard compounds, their characteristic EI-MS fragments and their retention times.

Compound 1. Yellow powder; mp 185–190°; $[\alpha]_D^{24}$

–4.6° ($c = 0.655$, MeOH–H₂O 75:25); TLC: R_f 0.24 (EtOAc–MeOH–H₂O 72:18:15); HPLC: R_t 6.0 min (LiChrosorb[®] RP-18, 7 μ m, 250 \times 4 mm I.D., MeOH–H₂O 65:35, 1 ml \times min^{–1}). ESI-MS (positive ion mode): m/z 1069 [M + Na]⁺; MS-MS of m/z 1069 (CID): 937 [M + Na – Pent]⁺, 923 [M + Na – dHex]⁺, 791 [M + Na – Pent – dHex]⁺, 659 [M + Na – 2Pent – dHex]⁺; ESI-MS (negative ion mode): m/z 1045 [M – H][–]; MS-MS of m/z 1045 (CID): 913 [M – H – Pent][–], 899 [M – H – dHex][–], 767 [M – H – Pent – dHex][–], 749 [M – H – Pent – dHex – H₂O][–], 635 [M – H – 2Pent – dHex][–], 473 [M – H – 2Pent – dHex – Hex][–]. ¹H and ¹³C NMR: see Table 2.

Compound 2. White powder; mp 194–196°; $[\alpha]_D^{24}$ –5.3° ($c = 0.624$, MeOH–H₂O 75:25); TLC: R_f 0.30 (EtOAc–MeOH–H₂O 72:18:15); HPLC: R_t 7.2 min (LiChrosorb[®] RP-18, 7 μ m, 250 \times 4 mm I.D., MeOH–H₂O 65:35, 1 ml \times min^{–1}). ESI-MS (positive ion mode): m/z 939 [M + K]⁺, 923 [M + Na]⁺; MS-MS of m/z 923 (CID): 791 [M + Na – Pent]⁺, 659 [M + Na – 2Pent]⁺; ESI-MS (negative ion mode): m/z 899 [M – H][–]; MS-MS of m/z 899 (CID): 767 [M – H – Pent][–], 749 [M – H – Pent – H₂O][–], 635 [M – H – 2Pent][–], 473 [M – H – 2Pent – Hex][–]. ¹H and ¹³C NMR: see Table 2.

Acknowledgement—We are grateful to H. Döhnert (HUB), B. Jaschok-Kentner, C. Kakoschke, C. Kamp and A. Waßmann (all GBF) for skilful technical assistance and I. Breitwieser, Ch. Oberprieler and R. Vogt for identification of plant material.

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