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Triterpenoid saponins and sapogenin lactones from *Albizia gummifera*

Asfaw Debella[a], Ernst Haslinger[a,*], Martin G. Schmid[a], Franz Bucar[d], Günter Michl[b], Dawit Abebe[c], Olaf Kunert[a]

[a]*Institut für Pharmazeutische Chemie, Universität Graz, Universitätsplatz 1 A-8010, Graz, Austria*
[b]*Schering A.G. Berlin Müllerstr., 170-178 D-13342, Berlin, Germany*
[c]*Ethiopian Health and Nutrition Research Institute, P.O.Box 1242 Addis, Abeba, Ethiopia*
[d]*Institut für Pharmakognosie, Universität Graz, Universitätsplatz 4 A-8010, Graz, Austria*

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Abstract

The structures of two new monodesmosidic and bisdesmosidic triterpenoid saponins (**1** and **2**) and the known compound Δ^5-stigmasterol-3-*O*-β-D-glucopyranoside (**3**) as well as two new oleanane type triterpene lactone glycosides **4**, **5** and a new sapogenin lactone **6** isolated from the stem bark of *Albizia gummifera* C.A. Smith (Mimosaceae) have been elucidated as 3-*O*-{β-D-glucopyranosyl(1 → 2)-[α-L-arabinopyranosyl(1 → 6)]-β-D-glucopyranosyl}-oleanolic acid (**1**), β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl 3-*O*-{β-D-glucopyra-nosyl(1 → 2)-[α-L-arabinopyranosyl(1 → 6)]-β-D-glucopyranosyl}-oleanolate (**2**), 3β-{*O*-D-glucopyranosyl-(1 → 2)-[*O*-α-L-arabinopyranosyl(1 → 6)]β-D-glucopyranosyloxy}-machaerinic acid γ-lactone (**4**), 3β-*O*-β-D-glucopyranosiduronic acid (1 → 2)-β-D-glucopyranosyloxy]-machaerinic acid γ-lactone (**5**), and A-homo-3a-oxa-5β-olean-12-en-3-one-28-oic acid (**6**), respectively. The complete assignment of the ^{1}H and ^{13}C resonances of **1**, **2**, **4** and **6** and of the peracetate of **5** were achieved by means of 2D-NMR studies.

Keywords: *Albizia gummifera* (C.A. Smith); Mimosaceae; Machaerinic acid lactone glycosides; A-homo-3a-oxa-olean-12-en-28-oic acid; Oleanolic acid glycosides; Stigmasterol glycoside; Stem bark

1. Introduction

Albizia gummifera C.A. Smith (Mimosaceae), local name '*Ambabesa*', is an umbrella-like crown tree about 50 m high widely distributed in the southwest highland of Ethiopia. It is used in the indigenous medical system for various ailments (Abate, 1989; Abebe & Ayehu, 1983). There is no report on the chemical constituents of this plant to date. Related species have been recorded as yielding a wide range of triterpene saponins (Carpani, Orsini, Sisti & Verottta, 1989; Pal, Achari, Yoshikawa & Arihara, 1995; Yoshikawa, Satou, Tokunaga, Tanaka, Arihara & Nigam, 1998). In our search for the presence of saponins we examined the methanol extract, and this paper describes the isolation and structural elucidation of new monodesmosidic and bisdesmosidic triterpene glycosides (**1** and **2**),Δ^5-stigmasterol-3-*O*-β-D-glucopyranoside (**3**) as well as two new machaerinic acid γ-lactone glycosides (**4** and **5**) and a new lactone-sapogenine (**6**).

2. Results and discussion

Solvent partition and repeated chromatographic purification over silica, RP-18 and LH-20 Sephadex of the saponin mixture obtained from the methanol

* Corresponding author. Tel.: +43-316380-5366; fax: +43-316380-9846.
E-mail address: haslinger@kfunigraaz.ac.at (E. Haslinger).

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Table 1
^{1}H and ^{13}C chemical shifts of the aglycone part of **1** and **2** (pyridine-d_5, 40°C, TMS internal standard)

Compound	**1**			**2**		
Position	δ_C	δ_H (J [Hz])		δ_C	δ_H (J [Hz])	
1	38.7	1.57/1.03		38.8	1.57/1.00	
2	26.7	2.32/1.88		26.7	2.30/1.88	
3	89.2	3.24	*dd* (11.5, 4.6)	89.2	3.24	
4	39.6			39.5		*d* (11.4)
5	55.9	0.74	*d* (11.9)	55.9	0.70	
6	18.5	1.50/1.31		18.6	1.46/1.32	
7	33.3	1.45/1.30		33.1	1.42/1.32	
8	39.8			40.0		
9	48.0	1.64		48.1	1.61	
10	37.0			37.0		
11	23.8	1.91/1.91		23.8	1.90/1.90	
12	122.6	5.45	*s*	123.0	5.25	*s*
13	143.9			144.1		
14	42.0			42.2		
15	28.4	2.18/1.20		28.5	2.28/1.15	
16	24.1	2.12/1.97		23.4	2.07/1.94	
17	46.6			47.0		
18	42.2	3.30	*dd* (14.3, 4.6)	41.7	3.19	
19	46.7	1.84/1.33		46.4	1.77/1.26	
20	31.0			30.8		
21	34.3	1.48/1.24		34.1	1.35/1.14	
22	33.3	2.06/1.83		32.6	1.92/1.78	
23	28.2	1.25		28.2	1.24	
24	16.8	1.10		16.8	1.10	
25	15.5	0.87		15.6	0.91	
26	17.7	1.00		17.6	1.08	
27	26.2	1.28		26.1	1.22	
28	180.3			176.5		
29	33.3	0.98		33.2	0.91	
30	23.8	1.03		23.7	0.91	

Table 2
^{1}H and ^{13}C chemical shifts of carbohydrate moieties of **1** and **2** (pyridine-d_5, 40°C, TMS internal standard)

Compound	**1**		**2**	
	δ_C	δ_H (J [Hz])	δ_C	δ_H (J [Hz])
glcp I				
1	105.0	4.82 (7.8)	105.0	4.80 (7.6)
2	83.2	4.11	83.2	4.10
3	78.2	4.22	78.2	4.21
4	71.7	4.02	71.7	4.02
5	76.3	3.99	76.2	3.98
6	69.9	4.74 *dd* (11.6, 2.2) /4.20	69.7	4.73 *d* (10.4)/4.19
glcp II				
1	106.0	5.28 (7.7)	105.9	5.27 (8.6)
2	77.0	4.06	77.0	4.05
3	78.0	4.19	77.9	4.19
4	71.9	4.26	71.8	4.25
5	78.1	3.88	78.1	3.87
6	62.9	4.42/4.42	62.7	4.41/4.41
arap				
1	105.1	4.92 (6.5)	105.1	4.90 (6.6)
2	72.2	4.42	72.2	4.41
3	74.2	4.15	74.2	4.15
4	68.8	4.31	68.8	4.30
5	66.1	4.29/3.77	66.1	4.28/3.75 *d*(12.3)
glcp III				
1			95.7	6.20 (8.3)
2			73.9	4.09
3			78.4	4.18
4			71.1	3.25
5			77.9	4.08
6			69.5	4.65 *d* (10.9)/4.32
glcp IV				
1			105.2	4.99 (7.3)
2			75.1	3.95
3			78.2	4.15
4			71.6	4.15
5			78.3	3.85
6			62.7	4.30/4.42

extract of the stem bark of *A. gummifera* afforded two new triterpenoid saponins **1**, **2** and Δ^5-stigmasterol-3-*O*-β-D-glucopyranoside (**3**), as well as two new triterpenoid saponin lactones **4**, **5** and a sapogenin lactone **6**. Structural determination of these compounds was mainly based on 1D- and 2D- NMR experiments (^{1}H-^{1}H-COSY, ^{1}H-^{1}H-TOCSY, ^{1}H-^{13}C-HMBC, ^{1}H-^{13}C-HSQC, ^{1}H-^{13}C-HSQC-TOCSY and ROESY) and on NOE experiments. For structural determination of **5**, the corresponding peracetyl derivative was prepared. This offers better solubility, enhanced dispersion and allowed unambiguous assignment. The ^{1}H and ^{13}C chemical shifts of compounds **1**, **2**, **4**, **6** and of peracetate **5** are given in Tables 1–4 respectively.

Acid hydrolysis of **1** gave the aglycone oleanolic acid, identified by co-TLC with authentic sample and comparison of ^{13}C-NMR chemical shift from the literature (Tori, Seo, Shimaoka & Tomita, 1974). The monosaccharides obtained after hydrolysis of **1** were analysed as trimethylsilyl derivatives by GC–MS using authentic samples as references. Glucose and arabinose in the relative proportion of 2:1 were detected. **1** ($C_{47}H_{76}O_{17}$) exhibited in the ES–MS a pseudomolecular ion at m/z 935 $[M+Na]^+$ and 913 $[M+H]^+$. Fragment ions at m/z 802 $[(M+Na)$-arabinose$]^+$, 611 $[(M+Na)–2 \times$ glucose$]^+$ and 442 $[(M+H)$-arabinose–$2 \times$ glucose$]^+$ confirmed the presence of a branched chain trisaccharide, hexosyl-(pentosyl)-hexosyl, linked to the oxygen at C-3 of the aglycone. The ^{13}C-NMR data together with information from ^{1}H-NMR (seven methyl singlets and a broad triplet vinyl proton at δ 5.45) suggested that the aglycone is an olean-12-ene skeleton (Doddrell, Khong & Lewis, 1974; Miana & Al-Hazimi, 1987; Seo, Tomita & Tori, 1975). Furthermore, in the IR spectrum a broad band at 3378 cm^{-1} (OH), a peak at 1653 cm^{-1} (C=C stretching) and 786 cm^{-1} (C–H bending) for **1** characteristic of a double bond at position 12 (13) in a pentacyclic triterpenoid were visible (Cole & Thornton, 1957).

Table 3
^{1}H and ^{13}C shifts of the aglycone parts, TMS as internal standard. **4**: solvent pyridine-d_5 40°C; **5**: shifts for the peracetate, solvent benzene-d_6, 40°C; **6**: solvent $CDCl_3$, 27°C

Compound	**4**		**5** (peracetate)		**6**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
Position						
1	38.6	1.03/1.56	38.9	1.50/0.87	34.2	2.31/1.67
2	26.8	2.35/1.92	26.5	1.91/1.84	29.1	2.53/2.20
3	89.3	3.27	90.3	3.03	180.4	
4	39.8		39.9		76.1	
5	56.4	0.74	56.8	0.71	51.5	1.35
6	19.1	1.54/1.34	19.1	1.54/1.34	22.5	1.45/1.45
7	33.3	1.28/1.28	33.1	1.27/1.27	32.0	1.26/1.38
8	40.0		40.2		39.1	
9	48.2	1.64	48.3	1.60	39.1	1.75
10	37.6		37.8		41.2	
11	24.1	2.00/1.80	24.1	1.93/1.74	23.0	1.89/1.89
12	122.9	5.48	122.4	5.33	122.7	5.28
13	141.1		141.5		143.1	
14	42.4		42.2		42.1	
15	24.7	1.76/1.05	24.4	1.71/0.98	27.7	1.68/1.10
16	27.1	2.44/1.48	26.9	2.56/1.33	22.9	1.97/1.61
17	43.7		43.3		46. 6	
18	40.9	2.49	40.6	2.41	40.9	2.83
19	35.3	1.95/1.46	34.6	1.73/1.42	45.7	1.61/1.16
20	34.2		33.7		30.6	
21	84.3	4.11	83.7	3.72	33.9	1.34/1.22
22	37.3	2.39/1.79	37.2	2.13/1.41	32.4	1.77/1.56
23	28.7	1.27	28.3	1.12	27.5	1.24
24	17.1	1.13	17.1	1.02	33.9	1.30
25	16.0	0.87	15.8	0.84	19.8	1.08
26	16.8	0.70	16.7	0.68	17.0	0.78
27	24.2	1.09	23.7	0.99	25.5	1.13
28	181.9		181.1		184.35	
29	27.3	0.95	27.0	0.74	33.0	0.91
30	27.8	1.01	28.2	0.94	23.5	0.93

The ^{1}H-NMR spectrum of **1** displayed three doublets at δ 4.82 ($J = 7.8$ Hz), δ 5.28 ($J = 7.7$ Hz) and δ 4.92 ($J = 6.5$ Hz) for the anomeric protons. The coupling constants indicated an axial position for each of them. The ^{13}C-NMR showed a C-3 signal (δ 89.2) shifted down field by 10 ppm as compared to oleanolic acid, due to the glycosylation shift, suggesting that the sugar moieties are linked to the oxygen at C-3 of the aglycone.

The point of attachment of the saccharide part to the genin and the interglycosidic linkages were established by a HMBC experiment. Long range correlation's were observed between H-1 of glucose-I and C-3 of the aglycone, H-1 of arabinose and C-6 of glucose-I and H-1 of the terminal glucose-II and C-2 of glucose-I. The D-configuration of glucose and the L-configuration of arabinose were determined by capillary electrophoresis as complexes with borate and β-cyclodextrin as chiral selector, using authentic samples as references (Stefansson & Novotny, 1993). All the above data identified **1** as 3-*O*-{β-D-glucopyranosyl(1 → 2)-[α-L-arabinopyranosyl(1 → 6)-]-β-D- glucopyranosyl}-oleanolic acid.

Structure determination of **2** was performed in the same way as described above. Alkaline hydrolysis of **2** furnished a prosapogenin identical with **1** as judged by its physical data (co-TLC). The aqueous hydrolysate contained only D-glucose, the identity of which was confirmed by GC–MS. By acid hydrolysis of **2**, oleanolic acid was obtained and identified by co-TLC with authentic sample and by comparing the ^{13}C-NMR chemical shifts with values from the literature (Tori et al., 1974). From the aqueous hydrolysate, D-glucose and L-arabinose were obtained in a 4:1 ratio (GC–MS). Their configuration (D or L) was determined by capillary electrophoresis as described before. ES–MS of **2** gave peaks at 1259 $[M + Na]^+$ and 1275 $[M + K]^+$ in agreement with a molecular formula of $C_{59}H_{96}O_{27}$. The ^{13}C chemical shifts of C-3 (δ 89.2) and C-28 (δ 176.5) showed that **2** was a bisdesmosidic glycoside. The ^{1}H- and ^{13}C-NMR of **2** displayed five anomeric sugar resonances. The proton coupling constants are characteristic for pyranoses with axial oriented anomeric protons (see Tables 1 and 2).

The structure of **2** was further confirmed by ES–MS peaks at *m/z* 911 $[M - 325]^+$, and 778 $[M - 132–325]^+$, which arose by the loss of two hexose units

Table 4
^{1}H and ^{13}C spectral data for sugar moieties in benzene-d_6 for peracetate of **5** and in pyridine-d_5 for **4** at 40°C (TMS as internal standard)

Compound	**4**		**5** (peracetate)	
	δ_C	δ_H (J [Hz])	δ_C	δ_H (J [Hz])
Glcp-I			Glcp	
1	105.4	4.84 (9.0)	103.6	4.25 (9.7)
2	83.6	4.10	77.9	3.67
3	78.5	4.21	75.4	5.34
4	72.1	4.03	69.4	5.16
5	76.7	3.99	72.2	3.31
6	70.1	4.74/4.22	62.5	4.43/4.10
Glcp-II			GluAp	
1	106.4	5.29 (7.0)	101.1	4.67 (9.0)
2	77.4	4.06	72.2	5.28
3	78.5	4.20	73.3	5.43
4	72.3	4.26	69.7	5.55
5	78.5	3.89	78.5	3.89
6	62.9	4.42/4.42	167.2	
Arap				
1	105.5	4.92 (7.0)		
2	72.6	4.42		
3	74.7	4.16		
4	68.8	4.33		
5	66.4	4.29/3.77		

probably from C-28 and one pentose unit together with two hexose moieties from C-3, respectively. Similar HMBC correlations were observed as for **1** for sugars linked to C-3 of the aglycone. Further correlations appeared between H-1 of glucose-III and C-28 of the aglycone and H-1 of glucose-IV and C-6 of glucose-III. Thus, the structure of **2** was determined as β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl{3-*O*-β- D-glucopyranosyl(1 → 2)-[α-L-arabinopyranosyl(1 → 6)]-β-D-glucopyranosyl}-oleanolate.

3 was characterised as Δ^5-stigmasterol-3-*O*-β-D-glucopyranoside. The aglycone, obtained after acid hydrolysis of **3** was identified by comparison with reported data (IR, ^{13}C-NMR & MS) (Tandon, Shukta & Thakur, 1990). Glucose was found in the aqueous hydrolysate and the D-configuration was determined by capillary electrophoresis, as described above.

The seven methyl singlets and a broad triplet vinyl proton in the ^{1}H-NMR together with information from ^{13}C-NMR: seven sp^3 and two sp^2 hybrid carbons (Table 3) indicated that the aglycone of **4** and **5** belongs to the Δ^{12}-oleanene type (Tori et al., 1974; Doddrell et al., 1974; Miana & Al-Hazimi, 1987). For structure determination of **5**, the corresponding peracetyl derivative was prepared to obtain better solubility.

4 ($C_{47}H_{74}O_{17}$) exhibited in the ESI–MS a molecular ion at *m/z* 910 $[M]^+$. The IR absorption band at 1745 cm^{-1} and the peak at δ 181.9 in the ^{13}C-NMR spectrum showed the presence of a γ-lactone ring (Pal et al., 1995; Tursch et al., 1963). The position of OH at C-21 and its formation of a lactone ring with the carboxyl group at C-17 was determined by HMBC correlation of H-21 (δ 4.11) with CH_3-30 (δ 0.95) and carboxyl carbon (C-28, δ 181.9). The configuration of the proton at C-18 was confirmed to be axial (β) by the observed NOE and ROESY correlations between H-18 (δ 2.49), methyl protons at C-26 (δ 0.70), H-15ax (δ 1.76) and H-19ax (δ 1.47).

The ^{1}H- and ^{13}C-NMR spectra of **4** displayed three anomeric sugar protons and carbons. The coupling constants are characteristic for pyranoses with axial oriented anomeric protons. Acid hydrolysis yielded a machaerinic acid γ-lactone, that was identified by comparison of the ^{13}C-chemical shift with the published data (Delagado, Silva & Fo, 1984). The ^{13}C-NMR signal of C-3 (δ 89.3) in **4** exhibited a glycosylation shift of +10.8 ppm in comparison with machaerinic acid γ-lactone and suggested that the sugar moieties are linked to the oxygen at C-3. The sugar components obtained after hydrolysis of **4** were identified as trimethylsilyl derivatives by GC–MS using authentic samples as references. Glucose and arabinose in the relative proportion of 2:1 were detected. The D-configuration of glucose and the L-configuration of arabinose were determined by capillary electrophoresis as described before.

The point of attachment of the saccharide part and interglycosidic linkage was established by an HMBC experiment. Long range correlations were observed between H-1 (δ 4.84) of glucose-I and C-3 (δ 89.3) of the aglycone, H-1 (δ 4.92) of arabinose and C-6 (δ 70.1) of glucose-I, and H-1 (δ 5.29) of the terminal glucose-II and C-2 (δ 83.6) of glucose-I. Thus, the structure of **4** was established to be 3β-{*O*-β-D-glucopyranosyl-(1 → 2)-[*O*-α-L- arabinopyranosyl(1 → 6)]β-D-glucopyranosyloxy}-machaerinic acid γ-lactone.

5 had a molecular formula of $C_{42}H_{64}O_{14}$ determined from a pseudomolecular ion peak at *m/z* 815 $[M+Na]^+$ in the ESI–MS. The mass spectra also showed significant peaks at *m/z* 791 $[M\text{-}H]^-$ and 792 $[M]^-$. All the ^{1}H- and ^{13}C-NMR signals of its aglycone moiety were consistent with the corresponding resonances of **4**. Furthermore, two anomeric protons and carbons were displayed (Table 4). The coupling constants are characteristic for pyranoses with axial oriented anomeric protons. The ^{13}C-NMR showed a C-3 signal (δ 90.3) shifted down-field by 11.8 ppm, due to the glycosylation shift, which suggested that the sugar moieties are linked to the oxygen at C-3 of the aglycone. Acid hydrolysis of **5** gave the aglycone machaerinic acid lactone, identified by comparison of ^{13}C chemical shifts in the literature (Delagado et al., 1984) (see Tables 3 and 4).

From the aqueous hydrolysate glucose and glucuronic acid were identified in a ratio of 1:1 (GC–MS). Their D-configuration was determined by capillary electrophoresis using authentic samples as references (Stefansson & Novotny, 1993). The interglycosidic linkage was established by long range correlation obtained from HMBC experiment. Correlations were observed between H-1 (δ 4.25) of glucose and C-3 (δ 90.3) of the aglycone, and H-1 (δ 4.67) of glucuronic acid and C-2 (δ 77.9) of glucose-I. The structure of **5** was thus determined to be 3β-[*O*-β-D-glucopyra nosiduronic acid (1 → 2)-β-D-glucopyranosyloxy]-machaerinic acid γ-lactone.

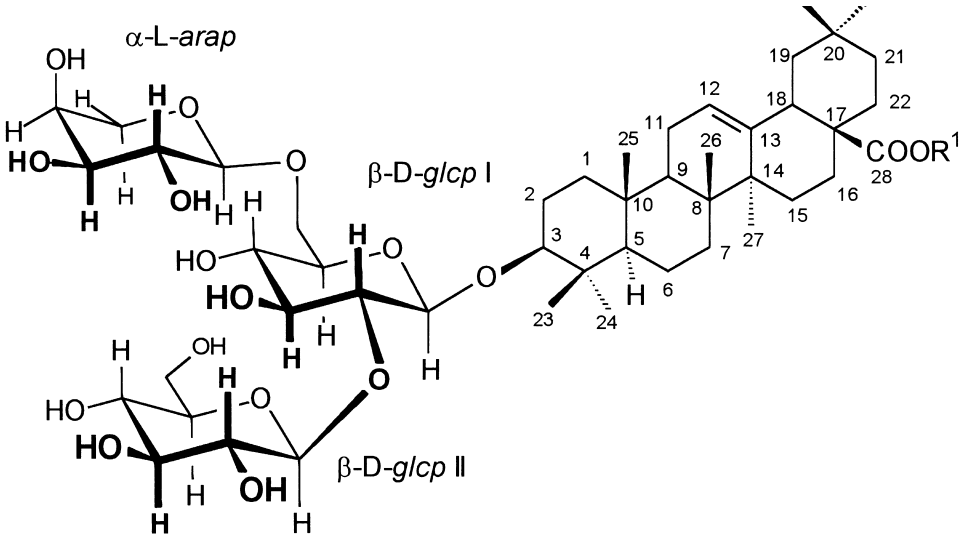
6 ($C_{30}H_{46}O_4$) exhibited in ESI–MS a pseudomolecular ion at *m/z* 471 $[M+H]^+$ and 488 $[M+H_2O]^-$. The ^{1}H- and ^{13}C-NMR contained signals for olefinic proton and carbons, and seven methyl groups attached to quaternary carbon atoms. The presence of two carboxyl carbons (δ 180.4 and 184.2) in **6** was also inferred from the ^{13}C-NMR (Table 3). Comparison of the ^{1}H- and ^{13}C-chemical shift data of **6** (with the exception of ring A and the lactone ring between ring D and E) with **4** and **5** indicated that they were almost similar. The signals of H-2ax and H-2eq (δ 2.53 and 2.20) in the ^{1}H-NMR spectra of **6** appeared down-field compared to the signal of **4** (δ 2.35 and 1.92) and **5** (δ 1.91 and 1.84). The main differences between the ^{13}C-NMR spectra of ring A for **6**, **4** and **5** were the chemical shifts of C-3 and C-4. HMBC correlation was

observed between H-2 (δ 2.53 and 2.20) and the carbonyl carbon C-3 (δ 180.4). The *cis* fusion of ring A was confirmed by the observed NOE between the β oriented methyl protons at C-10 (δ 41.22) and H-5 (δ 1.35). From these observations, we conclude that **3** has a Δ^{12}-oleanene skeleton with oxygen between C-3 and C-4 to form a seven membered lactone ring A. Therefore, **6** is A-homo-3a-oxa-5β-olean-12-en-3-one-28-oic acid. This is the first isolation and report of such a coprostanol type compound from a plant source. A similar lactone derived from oleanolic acid, however belonging to the cholestanol type, has been synthesised by enzymatic oxidation (Shirane, Hashimoto, Ueda, Takenaka & Katoh, 1996).

3. Experimental

3.1. General

Melting points were determined in open capillaries in an electrothermal melting apparatus. ESI–MS was recorded on a platform-LCZ (Micromass). ^{1}H and two dimensional NMR spectra were recorded as given in Ref. (Debella, Kunert, Haslinger, Michl & Abebe, 1999). IR spectra were obtained with a PERKIN ELMER 881 IR spectrometer. Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter. Gas–liquid chromatography was run on a Hewlett-Packard 5890 (series 2 plus) equipped with a FID and



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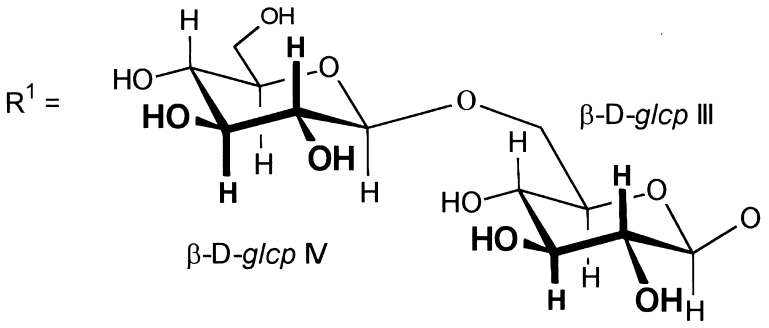

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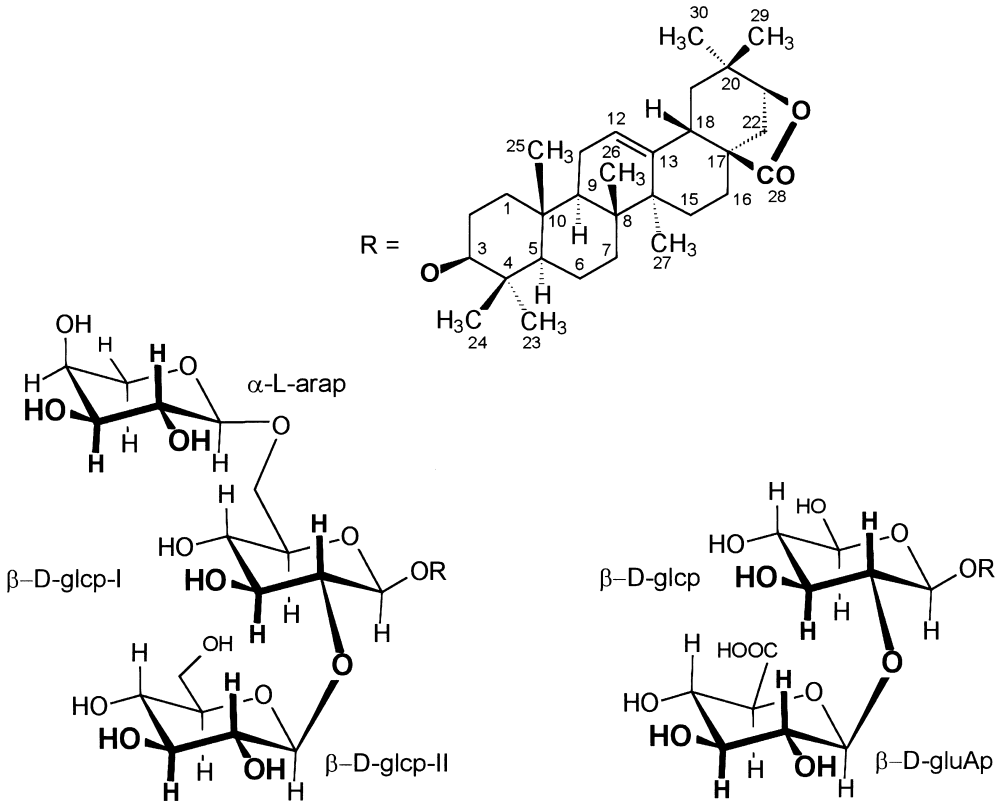

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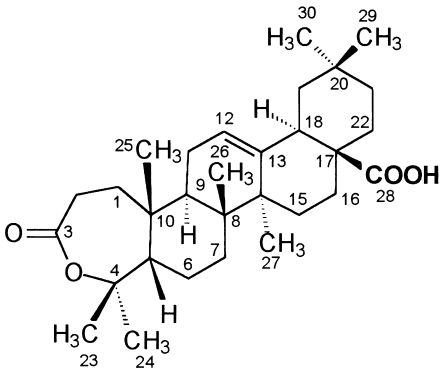

6

Hewlett Packard 5989B mass spectrometer. UV spectra were obtained with a Shimadzu UV 160A UV-visible recording. Capillary electrophoresis was recorded with Prince Technologies (the Emmen, The Netherlands) equipped with an on column UV detector (Bischoff, Germany). Paper partition chromatography (PPC) of sugars was conducted on Whatman No. 1 paper using a descending mode and detection was carried out with aniline/hydrogen phthalate as a spraying reagent. Column chromatography (CC) was carried out over Silica-60 (Merck, 230–240 μm), RP18 (Merck, 40–63 μm) and Sephadex LH-20 (Biochemica, 25–100 μm). Homogeneity of fractions was tested on TLC (Silica-gel, Merck) and (RP18, Merck). The spots were visualised by spraying with anisaldehyde-sulfuric acid reagent followed by heating at 100°C for 3 min. Unless otherwise noted, solvents used for TLC and CC were as follows: A: $CHCl_3/MeOH/H_2O$ A: (10:1:0.1, yielding fraction 1; 5:1:0.1, yielding fraction 2; 1:1:0.1, yielding fraction 3; 1:4:0.1, yielding fraction 4); B: $CHCl_3/MeOH/H_2O$ (60:40:7); C: $MeOH/H_2O$ (8:1) and D: *n*-BuOH/AcOH/H_2O (5:1:4).

3.2. Plant material

The stem bark of *Albizia gummifera* was collected

near Jimma a town 324 km southwest of Addis Ababa, Ethiopia in August 1997 at an altitude of 1700 m and identified by Dr. Dawit Abebe of EHNRI. A voucher specimen (Herbarium No. 1119) has been deposited at the Herbarium of the Department of Drug Research (EHNRI, Ethiopia) and at the Institute of Pharmaceutical Chemistry (Karl-Franzens-University, Graz).

3.3. Extraction and isolation of the glycosides

The stem bark was air dried, coarsely powdered (1.7 kg) and defatted with petroleum ether in a percolator at room temperature. The solvent free powder was exhaustively re-extracted with MeOH in a percolator to afford a brown gum (40 g), which was taken up in H_2O and extracted with *n*-BuOH (3 × 300 ml). The *n*-BuOH extract after concentration under reduced pressure yielded a saponin mixture (19.2 g). This mixture was subjected to CC on silica (solvent A) to afford fraction 1–4 which were rechromatographed over Sephadex LH-20 with MeOH as eluting solvent followed by repeated chromatography on silica (solvent B) and RP-18 silica with solvent C to afford compounds: **1** (22 mg), **2** (24 mg), **3** (85 mg), **4** (10 mg), **5** (52 mg) and **6** (32 mg).

3.4. Glycosides

3.4.1. 3-O-{β-D-glucopyranosyl(1 → 2)-[α-L-arabinopyranosyl(1 → 6)]-β-D-glucopyranosyl}-oleanolic acid (*1*)

White flakes (acetone) mp: 198–201°C. $[\alpha]_{Hg}^{20} = -32$ (MeOH, *c* 0.12); UV: λ_{max}^{MeOH} nm 216; IR ν_{max}^{KBr} cm^{-1}: 3378, 2920, 2851, 1653, 1467, 1258, 786; ES–MS: *m/z* (rel. int.) 935 (90) $[M+Na]^+$, 913 (20) $[M+H]^+$, 912 (60) $[M]^+$, 802 (5) $[(M+Na)-133]^+$, 611 (**5**) $[(M+Na)-2 \times glc]^+$, 455 (50) aglycone, 442 (100) $[(M+H)\text{-ara}-2 \times glc]^+$; ^{1}H- and ^{13}C-NMR (see Tables 1 and 2).

3.4.2. β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl {3-O-β-D-glucopyranosyl(1 → 2)-[α-L-arabinopyranosyl(1 → 6)]-β-D-glucopyranosyl}-oleanolate (*2*)

White flakes (acetone); mp: 204–206.7°C; $[\alpha]_{Hg}^{20} = -24$ (MeOH, *c* 0.08); UV: λ_{max}^{MeOH} nm 213; IR ν_{max}^{KBr} cm^{-1} 3386, 2927, 1743, 1636, 1458, 1387, 1261, 1161, 1074, 782; ES–MS: *m/z* (rel. int.) 1259 (5) $[M+Na]^+$, 1275 (30) $[M+K]^+$, 1236 (20) $[M]^+$, 911 (10) $[M-2 \times glc]^+$, 778 (5) $[M-2 \times glc\text{-ara}]^+$, 456 (3) aglycone; ^{1}H- and ^{13}C-NMR see Tables 1 and 2.

3.4.3. 3β-{O-β-D-glucopyranosyl-(1 → 2)-[O-α-L-arabinopyranosyl(1 → 6)]β-D-glucopyranosyloxy}-machaerinic acid γ-lactone (*4*)

White flakes (MeOH) mp: 289–292°C. $[\alpha]_{546}^{20} = -15$ (MeOH, *c* 0.1); UV: λ_{max} (MeOH) 211 nm, $\varepsilon = 1.956$; IR (KBr) λ_{max}: 3407, 2926, 2362, 1745, 1364, 1075, 470 cm^{-1}; ESI–MS: *m/z* (rel. int.) 910 (60) $[M]^+$; ^{1}H- and ^{13}C-NMR: (see Tables 3 and 4).

3.4.4. 3β-[O-β-D-glucopyranosiduronic acid (1 → 2)-β-D-glucopyranosyloxy]-machaerinic acid γ-lactone (*5*)

White amorphous (MeOH). mp: 295–297°C (dec.); $[\alpha]_{546}^{20} = -21$ (MeOH, *c* 0.09); UV: λ_{max} (MeOH) 207 nm, $\varepsilon = 1.203$; IR (KBr) ν_{max}: 3396, 2930, 1775, 1618, 1390, 1076, 594 cm^{-1}; ESI–MS: *m/z* (rel. int.) 815 (10) $[M+Na]^+$, 791 (100) $[M\text{-}H]^-$, 792 (40) $[M]^-$, 599 (4) $[M\text{-}H\text{–}glc]^-$, 455 (100) aglycone; ^{1}H- and ^{13}C-NMR: (see Tables 3 and 4).

3.4.5. A-homo-3a-oxa-olean-12-en-3-one-28-oic acid (*6*)

White flakes (MeOH) mp: 185–187°C; $[\alpha]_{546}^{20} = +25.8$ (methanol, *c* = 0.12); UV (MeOH) λ_{max} 214 nm, $\varepsilon = 1.499$; IR (KBr) ν_{max}: 3421, 2933, 2361, 1695, 1459, 1385, 1269, 1202, 737 cm^{-1}; ESI–MS: *m/z* (rel. int.) 471 (20) $[M+H]^+$, 488 (100) $[M\text{-}H_2O]^-$, 471 (12) $[M\text{-}H]^-$; ^{1}H- and ^{13}C-NMR: (see Tables 3 and 4).

3.5. Acid hydrolysis

The glycosides (10 mg each) were refluxed with 20 ml 7% HCl on a steam bath for 3 h. Extraction with $CHCl_3$ afforded the aglycone. The aglycone of **1** and **2** found to be identical with oleanolic acid (co-TLC with authentic sample and comparison with reported ^{13}C-NMR chemical shifts [6]) while the aglycone of **3** was stigmasterol (co-TLC and identical ^{13}C shifts with authentic sample and published data [12]). The neutralised [Dowex basic anionic ion exchanger (Cl^-)] and lyophilised aqueous hydrolysates of compounds **1** and **2** contained glucose and arabinose while compound **3** gave only glucose [PCC: solvent D, R_f = 0.19 (glucose), 0.21 (arabinose)].

The aglycone of **4** and **5** was found to be identical with machaerinic acid lactone (comparing with the reported ^{13}C-NMR chemical shifts [14]). The neutralised (Dowex basic anionic ion exchanger(Cl^-)) and lyophilised aqueous hydrolysates of **4** contained glucose and arabinose while **5** gave glucose and glucuronic acid [PC, solvent D, R_f = 0.18 (glucose), 0.21 (arabinose) and 0.11 (glucuronic acid)]. GC–MS (Column: 5% phenyl and 95% methyl silicone on ultra 2, 0.2 × 46 m, column temp.: 250°C, carrier gas: He 0.8 ml/min, sample: trimethylsilyl derivatives: t_R (min) glucose (15.78 and 17.48 for **4** and 15.79 and 17.45 for **5**) arabinose (10.37 and 10.97 for **4**) and glucuronic acid (17.28 and 18.27 for **5**).

3.6. Capillary electrophoresis

To 1 mg of the lyophilised aqueous phase of hy-

drolysis, 10 μl of 5-amino-2-naphthalene sulfonic acid (1 M) was added and the mixture was heated at 90°C for 10 min. Then 4 μl aqueous sodium borohydride (0.3 mg/ml) was added and the mixture was heated for 60 min at 90°C. The samples were then diluted with water to concentrations of 1 mM. Similar reactions were carried out with authentic sugar samples. 1 μl was used for analysis. 5 mM cyclodextrin and 10 mM borate were used to optimise resolution. The D-configuration of glucose and glucuronic acid and the L-configuration of arabinose was determined using authentic samples of D- and L-glucose and D- and L-arabinose, and D-glucuronic acid.

3.7. Alkaline hydrolysis

5 mg **2** were refluxed in 5% aqueous NaOH (5 ml) for 2 h. The mixture was neutralised to pH 6 with 1 N HCl and then extracted two times with 3 ml *n*-BuOH (saturated with H_2O). The organic phase yielded a prosapogenine identical with **1** (co-TLC). The aqueous hydrolysate contained only D-glucose (GC–MS, t_R 15.75, 17.45).

3.8. Acetylation

0.1 ml pyridine and 3 ml of Ac_2O were added to 20 mg of **5** and allowed to stand overnight. Work up as usual gave a peracetate of **5**.

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