New Triterpenoid Saponins from *Achyrantes aspera* LINN.

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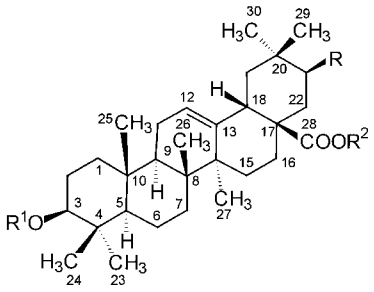
Two new bisdesmosidic triterpenoid saponins, *i.e.* **1** and **2**, were isolated, besides the three known saponins **3**–**5**, from the MeOH extract of the aerial parts of *Achyranthes aspera* LINN. (*Amaranthaceae*). Their structures were elucidated as β-D-glucopyranosyl 3β-[*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-glucopyranuronosyloxy]machaerinate (**1**) and β-D-glucopyranosyl 3β-[*O*-β-D-galactopyranosyl-(1 → 2)-*O*-α-D-glucopyranuronosyloxy]machaerinate (**2**) by NMR spectroscopy, including 2D-NMR experiments (machaerinic acid = 3β,21β-dihydroxyolean-12-en-28-oic acid). The other saponins were identified as β-D-glucopyranosyl 3β[*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-glucopyranuronosyloxy]oleanolate (**3**), β-D-glucopyranosyl 3-β-[*O*-β-D-galactopyranosyl-(1 → 2)-*O*-β-D-glucopyranuronosyloxy]oleanolate (**4**), and β-D-glucopyranosyl 3β-[*O*-β-D-glucopyranuronosyloxy]oleanolate (**5**) (oleanolic acid = 3β-hydroxyolean-12-en-28-oic acid).

Introduction. – *Achyranthes aspera* LINN. (*Amaranthaceae*) is a stiff erect herb, 0.3 to 0.9 m in height, distributed as a weed up to an altitude of 2500 m in many regions of Ethiopia, locally known as 'Mat'oya' or 'T'elenji' and used in the indigenous medical system for acute febrile illness, wound dressing, tonic, diuretic, expectorant, and various ailments [1]. Considerable phytochemical investigations of the roots and seeds of this plant growing in tropical regions of Asia have been undertaken and resulted in the isolation of saponins and long-chain fatty acids [2][3]. However, no detailed chemical investigation appears to have been done on the species growing in Africa, particularly in Ethiopia. Therefore, in the course of searching for the saponin constituent of this plant, we investigated the MeOH extract, and this paper describes the isolation and structural elucidation of the two new bisdesmosidic triterpene glycosides **1** and **2**.

Results and Discussion. – Saponins **1**–**5** were isolated from the BuOH-partitioned MeOH extract of the aerial parts of *A. aspera* by repeated chromatographic purification (normal-phase and reversed-phase silica gel and *Sephadex LH-20*). Structural determination of the saponins were mainly based on 1D-(^{1}H,^{13}C, ^{13}C-dept)

[1]) Part of the Ph. D. Thesis of *A.D.*

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 ^{1}H,^{1}H-ROESY). The ^{1}H- and ^{13}C-chemical shifts of compound **1** and of the more polar **2** are given in the *Table*.



1 R = OH, R^1 = α-L-Rha*p*-(1 ›3)-β-D-Glc*p*A-(1 ›, R^2 = β-D-Glc*p*-(1 ›

2 R = OH, R^1 = β-D-Gal*p*-(1→2)-α-D-Glc*p*A-(1→, R^2 = β-D-Glc*p*-(1→

3 R=H, $R^1 = \alpha$-L-Rhap-(1 → 3)-β-D-Glc*p*A-(1 → , $R^2 = \beta$-D-Glc*p*-(1 →

4 R=H, $R^1 = \beta$-D-Gal*p*-(1 → 2)-β-D-Glc*p*A-(1 → , $R^2 = \beta$-D-Glc*p*-(1- →

5 R=H, $R^1 = \beta$-D-Glc*p*A-(1 → , $R^2 = \beta$-D-Glc*p*-(1 →

Acid hydrolysis of **1** and **2** gave the aglycone machaerinic acid (= 3β,21β-dihydroxyolean-12-en-28-oic acid), which was identified by comparison of its ^{13}C-NMR spectrum with the reported one [4]. Alkaline hydrolysis of **1** furnished the corresponding prosapogenin and D-glucose (TLC, GC/MS), thus establishing the connection of D-glucose to C(28) of the aglycone. The sugars obtained from aqueous acid hydrolysis of **1** were identified as D-glucose, L-rhamnose, and D-glucuronic acid (GC/MS; TLC, and capillary electrophoresis) [5]. The monosaccharides obtained from acid hydrolysis of **2** were D-galactose and D-glucuronic acid, whereas D-glucose was observed in the alkaline hydrolysate (GC/MS, TLC, and capillary electrophoresis). The spectral data and the hydrolyses established the structure of **1** as β-D-glucopyranosyl 3β-[*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-glucopyranuronosyloxy]machaerinate and that of **2** as β-D-glucopyranosyl 3β-[*O*-β-D-galactopyranosyl-(1 → 2)-*O*-α-D-glucopyranuronosyloxy]machaerinate.

The ESI-MS of **1** and **2** showed the $[M-1]^+$ at *m*/*z* 955 and 971, respectively, suggesting a molecular formula $C_{48}H_{76}O_{19}$ and $C_{40}H_{75}O_{20}$, respectively. The ^{1}H-NMR of **1** and **2** indicated the presence of a olean-12-ene-type triterpene, exhibiting seven Me *s* and signals for an olefinic proton and two H-atoms geminal to OH groups (δ 3.18 (*dd*, *J* = 4.3, 11.6 Hz, H−C(3)) and 3.50 (*t*, *J* = 9.4 Hz, H−C(21)) for **1**, and δ 3.17 (br. *d*, *J* = 8.5 Hz, H−C(3)) and 3.50 (br. *s*, H−C(21)) for **2**). The position of the second OH group was determined to be C(21) by HMBC correlation of *H*−C(21) with *C*H$_3$(30) and C=O for **1** and **2**, respectively. The position of H−C(18) was confirmed to be β (axial) by the observed ROESY correlation between H−C(18), H−H$_3$(26) (δ 0.79 and 0.78). H$_{ax}$−C(15) (δ 1.75 and 1.74), and H$_{ax}$−C(19) (δ 1.80 and 1.81) for **1** and **2**, respectively. The α (axial) position of H−C(21) was determined for both compounds by ROESY correlation between H−C(21), CH$_3$(30), H$_{ax}$−C(16), and H$_{ax}$−C(19). The glycosylation shifts observed for C(3) (4–5 ppm downfield) and for C(28) (6–9 ppm upfield) in the ^{13}C-NMR indicated that the sugar moieties were attached at both positions.

The point of attachment of the sugar moieties and the interglycosidic linkage of **1** were determined by HMBC. Long-range correlations were observed between *H*−C(1) of the D-glucuronic acid moiety and C(3) of

Table. *^{1}H- and ^{13}C-NMR Chemical Shifts* δ [ppm] *of the Aglycone Parts of* **1** *and* **2** ($SiMe_4$ as internal standard; solvent CD_3OD, 30°)

	1		**2**			**1**		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)		δ(C)	δ(H) (J [Hz])	δ(C)	δ(H) (J [Hz])
CH_2(1)	40.2	0.99, 1.59	40.2	0.96, 1.60		Glc		Glc	
CH_2(2)	27.2	1.97, 1.67	27.4	1.69, 1.94	H–C(1)[a])	96.2	5.38 (J = 8.2)	96.4	5.38 (J = 7.5)
H–C(3)	91.2	3.18	91.8	3.17	H–C(2)[a])	74.3	3.33	74.4	3.32
C(4)	40.8	–	40.2	–	H–C(3)[a])	78.7	3.41	78.8	3.41
H–C(5)	57.4	0.78	57.5	0.76	H–C(4)[a])	71.6	3.35	71.6	3.35
CH_2(6)	19.6	1.38, 1.53	19.7	1.37, 1.53	H–C(5)[a])	79.1	3.35	79.1	3.35
CH_2(7)	34.1	1.47, 1.30	34.3	1.30, 1.46	CH_2(6)[a])	62.8	3.67, 3.81	63.0	3.66, 3.80
C(8)	41.0	–	41.1	–		Rha		Gal	
H–C(9)	49.4	1.57	49.5	1.56	H–C(1)[a])	102.9	5.17 (br. *s*)	105.4	4.67 (J = 7.4)
C(10)	38.3	–	38.2	–	H–C(2)[a])	72.9	3.93	76.8	3.23
CH_2(11)	24.9	1.88, 1.88	25.0	1.86, 1.88	H–C(3)[a])	72.9	3.72	72.4	3.27
H–C(12)	124.6	5.25	124.7	5.27	H–C(4)[a])	74.6	3.37	78.4	3.37
C(13)	144.1	–	144.3	–	H–C(5)[a])	70.2	4.09	78.7	3.26
C(14)	43.0	–	43.2	–	Me(6)[a]) or				
CH_2(15)	29.4	1.75, 1.08	29.5	1.08, 1.74	CH_2(6)[a])	18.2	1.24	63.5	3.81, 3.62
CH_2(16)	25.6	2.03, 1.85	25.6	1.84, 2.03		GlcA		GlcA	
C(17)	50.2	–	50.3	–	H–C(1)[a])	106.9	4.34 (J = 8.1)	105.9	4.47 (br. *s*)
H–C(18)	42.6	2.88	42.6	2.88	H–C(2)[a])	76.6	3.35	81.7	3.61
CH_2(19)	48.3	1.80, 1.23	48.4	1.81, 1.23	H–C(3)[a])	84.1	3.52	78.6	3.59
C(20)	37.6	–	37.3	–	H–C(4)[a])	72.9	3.50	74.3	3.48
H–C(21)	74.2	3.50	74.0	3.50	H–C(5)[a])	77.8	3.57	76.9	3.64
CH_2(22)	40.9	1.63, 1.76	41.0	1.62, 1.75	C(6)[a])	177.2	–	*	–
Me(23)	29.0	1.04	28.9	1.06					
Me(24)	16.8	0.84	17.3	0.85					
Me(25)	16.4	0.94	16.3	0.94					
Me(26)	18.2	0.79	18.2	0.78					
Me(27)	26.5	1.15	26.5	1.14					
C(28)	177.2	–	177.6	–					
Me(29)	29.9	0.96	29.9	0.95					
Me(30)	17.9	0.88	18.0	0.88					

[a]) Numbering of the carbohydrate moieties. [b]) The ^{13}C-NMR signal was not observed.

the aglycone and between H–C(1) of the L-rhamnose and C(3) of the D-glucuronic acid moieties. Further correlations appeared between H–C(1) (δ 5.38) of the D-glucose moiety and C(28) (δ 177.2) of the aglycone. Furthermore, fragment ions at m/z 791 ($[M-1-146)]^+$), 775 ($[M-1-162]^+$), 775 ($[M-1-180]^+$), and 632 ($[M-1-326]^+$) showed the successive elimination of deoxyhexosyl and two hexosyl moieties. The coupling constant value of the anomeric protons in the ^{1}H-NMR (*Table*) showed that D-glucose and D-glucuronic acid have β-D- whereas L-rhamnose has α-D-configuration.

The interglycosidic linkage as well as the nature of the sugar moieties linked to the aglycone of **2** were established by HMBC and ROESY experiments. Fragment ions at m/z 809 ($[M-1-162]^+$), 793 ($[M-1-178]^+$), and 471 ($[M-1-500]^+$) showed the presence of three hexosyl units, of which one could be glucuronic acid. The coupling constant value in the ^{1}H-NMR showed the α-D-configuration for the glucuronic acid moiety (equatorial position of the anomeric proton) and the β-D-configuration for the glucose and galactose moieties (axial position of the anomeric proton).

On acid hydrolysis, compounds **3**–**5** afforded oleanolic acid (= 3β-hydroxyolean-12-en-28-oic acid) as aglycone, which was identified by comparison with an authentic sample (TLC) and with reported ^{13}C-NMR data [6]. The sugars obtained from aqueous hydrolysates were determined (TLC, GC/MS, and capillary electrophoresis) as D-glucose, D-glucuronic acid, and L-rhamnose in the case of **3**, D-glucose, D-galactose, and D-glucuronic acid in the case of **4**, and D-glucose and D-glucuronic acid in the case of **5**. The glycosylation shift observed for C(3) of **3**–**5** (3–6 ppm downfield) and an upfield shift (4–5 ppm) for the carboxyl group as compared to the free oleanolic acid suggested 3-*O*- and 28-*O*-glycosidic linkages. This was further confirmed by alkaline hydrolysis that furnished the corresponding prosapogenins and D-glucose. The interglycosidic linkage as well as the linkage of the sugar moieties to the aglycone were established by HMBC. Thus, **3**–**5** were bisdesmosidic saponins with D-glucose attached at C(28) of the aglycone and with the structures β-D-glucopyranosyl 3β-[*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-glucopyranuronosyloxy]oleanolate (**3**), β-D-glucopyranosyl 3-β-[*O*-β-D-galactopyranosyl-(1 → 2)-*O*-β-D-glucopyranuronosyloxy]oleanolate (**4**), and β-D-glucopyranosyl 3β-[*O*-β-D-glucopyranuronosyloxy]oleanolate (**5**), respectively. These compounds were previously reported [7–9].

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Experimental Part

General. Capillary electrophoresis: *Prince Technologies* (Netherlands) instrument equipped with an on-column UV detector (*Bischoff*, Germany). Paper partition chromatography (PPC): *Whatman* No. *1* paper in descending mode; detection of the sugars by spraying with aniline/hydrogen phthalate. Column chromatography (CC): silica gel *60* (*Merck*, 230–240 μm), *RP18* (*Merck*, 40–63 μm), and *Sephadex LH-20* (*Biochemica*, 25–100 μm). TLC: silica gel (*Merck*) and *RP18* (*Merck*); detection by spraying with anisaldehyde/H_2SO_4 soln. followed by heating at 100° for 3 min. Solvents used for TLC and CC: *A:* $CHCl_3$/MeOH/H_2O 0:1:0.1 (→ *Fr. 1*), 5:1:0.1 (→ *Fr. 2*) and 1:1:0.1 and 1:2:0.1 (→ *Fr. 3*); *B*, $CHCl_3$/MeOH/H_2O 70:30:4; *C*, $CHCl_3$/MeOH/H_2O 60:40:7; *D*, MeOH/H_2O 8:2; *E*, MeOH/H_2O 7:3; *F*, AcOEt/MeOH/H_2O/AcOH 13:3:3:4. GLC/MS: *Hewlett-Packard 5890* (series 2 plus) instrument equipped with a *Hewlett-Packard-5989B* mass spectrometer. Melting points: open capillaries; electrothermal melting apparatus. Optical rotations: *Perkin-Elmer-241-MC* polarimeter. UV Spectra: *Shimadzu-UV-160A* UV/VIS-recording spectrophotometer. IR Spectra: *Perkin-Elmer-881-IR* spectrometer. ^{1}H- and 2D-NMR Spectra: see [10]. EI-MS: at 70 eV. LSI-MS: at 5 kV neg. ESI-MS: platform *LCZ* (*Micromass*); H_2O/MeCN 1:1 with 0.1% CF_3COOH as solvent for loop injection (cone voltage 40 eV).

Plant Material. The aerial parts of *Acyranthes aspera* LINN. were collected around Addis Ababa, Ethiopia, in August 1997, at an altitude of 2630 m, and identified by Dr. *Dawit Abebe* of EHNRI. A voucher specimen (Herbarium No. 1124) has been deposited at the Herbarium of the Department of Drug Research EHNRI, Ethiopia, and at the Institute of Pharmaceutical Chemistry, Karl-Franzens-Universität, Graz.

Extraction and Isolation of the Glycosides. Air-dried and finely powdered aerial parts (2.6 kg) were defatted with petrolum ether (40–60°) in a percolator at r.t. The solvent-free powder was exhaustively extracted with MeOH in a percolator to afford a greenish gum (218 g), which was taken up in H_2O and re-extracted with Et_2O until all the chlorophyll pigments were removed. The aq. phase was then partitioned with BuOH saturated with H_2O (3 × 300 ml). The BuOH extract, after evaporation, yielded a saponin mixture (54 g). This mixture was subjected to a first CC (silica gel, *A*) to afford *Fr. 1–3*, which were submitted to another CC (*Sephadex LH-20*, MeOH). Repeated further CC (silica gel *A–C*; *RP-18* silica gel, *D* and *E*) afforded **1** (27 mg), **2** (15 mg), **3** (71 mg), **4** (29 mg), and **5** (19 mg).

β-D-Glucopyranosyl 3β-[D-O-α-L-*Rhamnopyranosyl-(1 → 3)*-O-β-D-*glucopyranuronosyloxy]-21β-hydroxyolean-12-en-28-oate* (**1**). Partly solidified gum. $[\alpha]_{546}^{23} = -13$ ($c = 0.42$, MeOH). UV (MeOH): 213 (229). IR

(KBr): 3850, 3420, 2942, 1735, 1612, 1231, ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 955 (22, $[M-1]^+$), 791 (20, $[M-1-\text{hexose}]^+$), 775 (15, $[M-1-180]^+$), 632 (20, $[M-1-\text{Rha}-\text{GlcA}^+$), 470 (15, $[M-1-\text{Rha}-\text{GlcA}-\text{Glc}]^+$).

*β-*D*-Glucopyranosyl 3β-[*O*-β-*D*-Galactopyranosyl-(1→2)-*O*-α-*D*-glucopyranuronosyloxy]-21β-hydroxyolean-12-en-28-oate* (**2**). Partly solidified gum. $[\alpha]_{546}^{21} = -24$ ($c = 0.41$, MeOH). UV (MeOH): 211 (160). IR (KBr): 3855, 3409, 2945, 1732, 1616, 1260. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 971 (100, $[M-1]^+$), 809 (55, $[M-1-\text{hexose}]^+$), 793 (62, $[M-1-\text{GlcA}]^+$), 631 (40, $[M-1-\text{GlcA}-\text{Glc}]^+$), 471 (47, $[M-1-\text{Gal}-\text{GlcA}-\text{Glc}]^+$).

Acid Hydrolysis. The glycosides (10 mg each) were refluxed with 7% HCl soln. (20 ml) on a steam bath for 3 h. Extraction with $CHCl_3$ afforded the aglycone. The aglycone of **3**–**5** was identical with oleanolic acid (= 3β-hydroxyolean-12-en-28-oic acid; co-migration on TLC with authentic sample and comparison of ^{13}C-NMR data [6]). The neutralized (*Dowex* basic anion exchanger(Cl^-)) and lyophilized aq. hydrolysates contained D-glucose, L-rhamnose, and D-glucuronic acid for **3**, D-glucose, D-galactose, and D-glucuronic acid for **4**, and D-glucose and D-glucuronic acid for **5** (TLC, GC/MS, and capillary electrophoresis). The aglycone of **1** and **2** was identical with machaerinic acid (= 3β,21β-dihydroxyolean-12-en-28-oic acid; comparison of ^{13}C-NMR data [4]). The neutralized and lyophilized aq. hydrolysates contained D-glucose, L-rhamnose, and D-glucuronic acid for **1**, and D-glucose, D-galactose, and D-glucuronic acid for **2**. (*F*): R_f 0.28 (Glc), 0.26 (Gal), 0.35 (Rha), and 0.15 (GlcA). GC/MS (5% phenyl- and 95%-methylsilicone on *Ultra 2*, 0.2 × 46 m; column temp. 250°; carrier gas He, 0.8 ml/min; sample: trimethylsilyl derivatives): t_R(min) 15.76 and 17.49 for Glc from **1** a 15.79 and 17.43 Glc from **2**, 10.56 and 11.83 for Rha from **1**, 9.90 and 11.69 for Gal from **2**, 17.26 and 18.25 for GlcA from **1**, and 17.24 and 18.23 for GlcA from **2**.

Capillary Electrophoresis. To the residue (1 mg) of the lyophilized aq. phase of hydrolysis, 1M 5-amino naphthalene-2-sulfonic acid (10 µl) was added, and the mixture was heated at 90° for 10 min. Then, aq. $NaBH_4$ soln. (4 µl, 0.3 mg/ml) was added, and the mixture was heated for 60 min at 90°. The samples were then diluted with H_2O to concentrations of 1 mM. Similar reactions were carried out with authentic sugar samples. For analysis, 1 µl was used. To optimize resolution, 5 mM cyclodextrin and 10 mM borate were used. The D-configuration of glucose, galactose, and glucuronic acid, and the L-configuration of rhamnose was thus determined with authentic samples of D- and L-glucose, D- and L-galactose, L-rhamnose, and D-glucuronic acid for comparison [11].

Alkaline Hydrolysis. Each glycoside (5 mg) was refluxed in 5% NaOH soln. (5 ml) for 2 h. The mixture was neutralized to pH 6 with 1N HCl and then extracted with BuOH (sat. with H_2O; 2 × 3 ml). The aq. hydrolysates contained only D-glucose (co-migration on TLC with authentic D-glucose) for **1**–**5**.

REFERENCES

[1] D. Abebe, A. Ayehu, 'Medicinal Plants and Enigmatic Health Practices of Northern Ethiopia', Addis Ababa, B.S.P.E., 1983.
[2] V. Hariharan, S. Rangaswami, *Phytochemistry* **1970**, *9*, 409.
[3] V. Seshadri, A. K. Batta, S. Rangawami, *Indian J. Chem., Sect. B* **1981**, *20*, 773.
[4] K. Yoshikawa, Y. Satou, Y. Tokunaga, M. Tanaka, S. Arihara, S. K. Nigam, *J. Nat. Prod*. **1998**, *61*, 440.
[5] M. Stefansson, M. Novotny, *J. Am. Chem. Soc*. **1993**, *115*, 11573.
[6] K. Tori, S. Seo, A. Shimaoka, Y. Tomita, *Tetrahedron Lett*. **1974**, *48*, 4227.
[7] M. P. Gupta, K. Hostettmann, *Phytochemistry* **1987**, *26*, 2685.
[8] H. Kawaki, M. Nishida, Y. Tashiro, M. Kuroyanagi, A. Ueno, M. Satake, *Chem. Pharm. Bull*. **1989**, *37*, 2318.
[9] F. Mizui, R. Kasaki, K. Ohtani, O. Tanaka, *Chem. Pharm. Bull*. **1990**, *38*, 375.
[10] A. Debella, E. Haslinger, O. Kunert, G. Michl, D. Abebe, *Phytochemistry* **1999**, *51*, 1069.
[11] M. Stefansson, M. Novotny, *J. Am. Chem. Soc*. **1993**, *115*, 11573.

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