Monatshefte für Chemie 131, 195–204 (2000)

Monatshefte für Chemie
Chemical Monthly

Three Saponins, a Steroid, and a Flavanol Glycoside from *Achyrantes aspera*

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Summary. Three bisdesmosidic saponins, 20-hydroxyecdysone, and quercetin-3-O-β-*D*-galactoside were isolated from the methanol extract of the aerial parts of *Achyranthes aspera* L. (*Amaranthaceae*). Their structures were established on the basis of NMR spectroscopic analysis; the complete ^{1}H and ^{13}C assignments of the compounds were achieved by means of 2D NMR studies.

Keywords. Saponins; NMR spectroscopy; Natural products; Structure elucidation.

Introduction

Achyranthes aspera L. (*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* it is 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 also in the 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 performed on the species growing in Africa, particularly in Ethiopia. This paper describes the isolation and structure elucidation of the bisdesmosidic triterpene glycosides **1**–**3** as well as that of 20-hydroxyecdysone (**4**) and quercetin 3-O-β-*D*-galactopyranoside (**5**) from the aerial parts of this plant.

Results and Discussion

Compounds **1**–**5** were isolated from the *n*-BuOH-partitioned MeOH extract of the aerial parts of *A. aspera* by repeated chromatographic purification over normal phase, reversed phase silica, and Sephadex LH-20. Structural analysis was mainly based on 1D (^{1}H, ^{13}C, ^{13}C-DEPT) and 2D NMR experiments (^{1}H, ^{1}H-COSY, ^{1}H,

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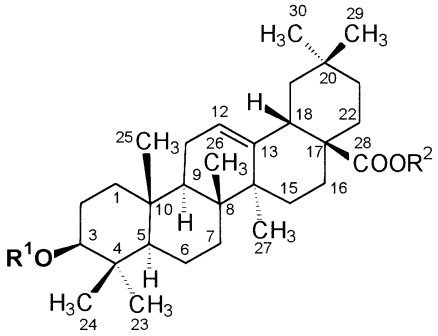
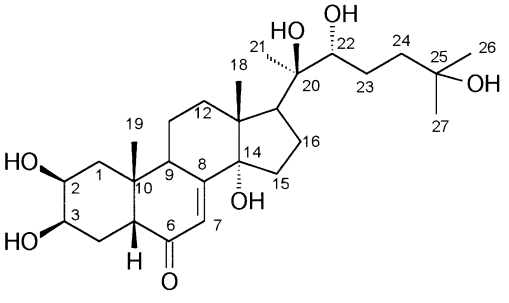


1 R^1 = α-L-Rhap-(1→3)–β–D-glpA- R^2 = β-D-glcp-

2 R^1 = β-D-Galp-(1→3)–β–D-glpA- R^2 = β-D-glcp-

3 R^1 = β–D-GlpA- R^2 = β-D-glcp-



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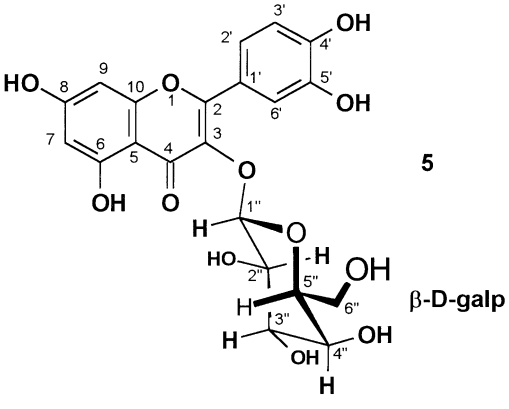

5

β-D-galp

^{1}H-TOCSY, ^{1}H, ^{13}C-HMBC, ^{1}H, ^{13}C-HSQC, ^{1}H, ^{13}C-HSQC-TOCSY, ROESY). The ^{1}H and ^{13}C chemical shifts of **1–5** are given in Tables 1–4.

Upon acidic hydrolysis, **1–3** afforded oleanolic acid as the aglycone. It was identified by comparison with an authentic sample (TLC) and the reported ^{13}C

Table 1. ^{1}H and ^{13}C NMR shifts of the aglycones of **1–3** (*TMS*, CD_3OD, 30°C)

Compound	**1**		**2**		**3**	
Position	δ_C/ppm	δ_H/ppm	δ_C/ppm	δ_H/ppm	δ_C/ppm	δ_H/ppm
1	39.7	1.61, 0.98	39.80	1.61, 1.02	40.3	1.60, 0.98
2	26.3	1.69, 1.95	26.7	2.02, 1.70	27.1	1.97, 1.69
3	90.8	3.17	91.2	3.22	91.3	3.19
4	40.0	–	40.4	–	40.7	–
5	56.7	0.78	56.9	0.78	57.4	0.77
6	18.9	1.50, 1.40	19.1	1.54, 1.39	19.6	1.54, 1.39
7	33.6	1.49, 1.33	33.8	1.49, 1.32	34.2	1.48, 1.31
8	40.4	–	40.7	–	41.0	–
9	48.7	1.58	49.0	1.58	49.4	1.57
10	37.5	–	37.9	–	38.4	–
11	24.3	1.90, 1.90	24.5	1.89, 1.89	24.9	1.90, 1.90
12	123.7	5.25	123.8	5.26	124.1	5.27
13	144.4	–	144.7	–	145.3	–
14	42.7	–	42.9	–	43.3	–
15	26.6	1.81, 1.10	28.7	1.80, 1.09	29.2	1.80, 1.08
16	23.6	1.72, 2.05	23.8	2.05, 1.72	24.3	2.04, 1.71
17	47.8	–	48.1	–	48.3	–
18	42.4	2.86	42.6	2.86	43.1	2.85
19	46.7	1.17, 1.71	46.9	1.71 1.16	47.5	1.70, 1.15
20	31.2	–	31.4	–	31.7	–
21	34.6	1.22, 1.40	34.7	1.4 1.22	35.2	1.39, 1.21
22	32.7	1.60, 1.75	33.1	1.75 1.60	33.5	1.73, 1.61
23	28.6	1.06	28.3	1.08	28.9	1.06
24	16.8	0.86	16.7	0.86	17.3	0.85
25	15.7	0.96	15.8	0.96	16.3	0.95
26	17.6	0.81	17.7	0.81	18.1	0.80
27	26.0	1.16	26.1	1.16	26.7	1.15
28	177.9	–	178.2	–	178.4	–
29	33.2	0.92	33.4	0.92	33.9	0.91
30	23.7	0.95	23.8	0.94	24.4	0.93

chemical shifts [4, 5]. The glycosylation shift observed at C-3 (3–6 ppm downfield) and an upfield shift (4–5 ppm) of the carboxyl group as compared to the free oleanolic acid for **1–3** suggested 3- and 28-O-glycosidic linkages. This was further confirmed by alkaline hydrolysis that furnished the corresponding prosapogenins and glucose. Thus, **1–3** were identified as bisdesmosidic saponins with glucose esterified at position C-28 of the aglycone.

The El-MS of **1** showed the [M-1]peak at $m/z = 939$ which suggested a molecular formula of $C_{48}H_{76}O_{18}$. Signals observed at $m/z = 791$ $[(M-1)-146]^+$ and 631 $[(M-1)-308]^+$ corresponded to the successive elimination of one rhamnosyl and one glucuronic acid moieties; thus, rhamnose is the terminal sugar. A signal at $m/z = 777$ $[(M-1)-162]^+$ indicated the elimination of a glucosyl moiety, possibly

Table 2. ^{1}H and ^{13}C NMR data of the carbohydrate moieties of **1–3** (*TMS* (CD_3OD, 30°C)

Compound	**1**		**2**		**3**	
Position	δ_C/ppm	δ_H/ppm (*J*, Hz)	δ_C/ppm	δ_H/ppm (*J*, Hz)	δ_C/ppm	δ_H/ppm (*J*, Hz)
	Glucose		Glucose		Glucose	
1	95.4	5.39 (7.8)	95.8	5.38 (8.5)	96.1	5.40 (7.7)
2	73.7	3.55	73.7	3.33	74.1	3.35
3	78.0	3.44	78.2	3.41	78.2	3.43
4	71.0	3.37	71.0	3.36	71.2	3.38
5	78.4	3.37	78.5	3.35	78.8	3.38
6	62.3	3.82, 3.69	62.3	3.82/3.68	62.7	3.84, 3.71
	Rhamnose		Galactose			
1	102.2	5.18 (br s)	104.7	4.68 (7.1)		
2	72.0	3.97	76.1	3.23		
3	72.0	3.75	71.6	3.24		
4	73.8	3.39	77.7	3.37		
5	69.6	4.00	78.0	3.25		
6	17.7	1.25	62.9	3.82/3.64		
	GlucA		GlucA		GlucA	
1	106.1	4.35 (8.2)	105.2	4.45 (7.5)	106.7	4.38 (7.8)
2	75.9	3.37	81.3	3.60	75.5	3.28
3	83.6	3.54	78.1	3.60	73.7	3.48
4	72.2	3.52	72.2	3.48	78.6	3.45
5	76.9	3.62	76.2	3.55	76.7	3.64
6	177	–	176.7	–	172.4	–

from the C-28 position of the aglycone. The sugars obtained from acid aqueous hydrolysates were identified as glucose, glucuronic acid, and rhamnose (GC-MS and TLC). Their configurations were determined by capillary electrophoresis [6]. Furthermore, the ^{1}H and ^{13}C NMR spectra (Table 2) showed the presence of three sugars, two of which were attached to oleanolic acid at position C-3 and the remaining one at C-28. The coupling constant value showed that glucose and glucuronic acid have β-configuration, whereas rhamnose has α-configuration. The interglycosidic linkage as well as the nature of the sugar chains linked to the aglycone were established by HMBC experiments. Long-range correlations were observed between H-1 (4.35 ppm) of glucuronic acid and C-3 (90.8 ppm) of the aglycone as well as H-1 (5.18 ppm) of rhamnose and C-3 (83.6 ppm) of glucuronic acid. Further correlations appeared between H-1 (5.39 ppm) of glucose and C-28 (177.9 ppm) of the aglycone. Based on the above data, the structure of **1** was established as β-*D*-glucopyranosyl 3-(α-*L*-rhamnopyranosyl-(1$\rightarrow$3)-(β-*D*-glucopyranosyloxy)-oleanate. This compound has been already reported to be contained in *Swartzia simplex* [7].

Compound **2** was more polar than **1** and isolated only in small quantity. The El-MS revealed an ion peak at $m/z = 955$ $[M\text{-}1]^+$ that suggested a molecular formula of $C_{48}H_{76}O_{19}$. The sugars obtained from acidic hydrolysis were identified as

Table 3. ^{1}H and ^{13}C chemical shifts of **4** in CD_3OD and of the peracetate of **4** in pyridine-d_6 at 30°C *vs.* internal *TMS*

Position	**4** δ_C/ppm	δ_H/ppm	**4** Peracetylated δ_C/ppm	δ_H/ppm
1	33.5	1.46, 1.83	37.4	1.67, 2.10
2	68.1	3.88	68.6	5.33
3	66.8	3.9	68.3	5.47
4	28.4	1.74, 1.78	32.7	1.72, 1.87
5	50.6	2.42	51.7	2.63
6	201.9	–	206	–
7	121.6	5.8	122	6.19
8	165	–	168	–
9	33.3	3.2	35.39	3.55
10	37.5	–	39.5	–
11	20.1	1.78, 2.02	21.0	1.71, 1.96
12	30.9	1.98, 2.18	32.4	1.94, 2.48
13	47.1	–	48.7	–
14	83.0	–	85	–
15	30.6	1.64, 2.02	31.7	1.85, 2.13
16	20.5	1.76, 1.86	21.4	2.15, 2.51
17	49.4	2.43	50.6	2.94
18	16.8	0.94	17.2	1.15
19	22.9	1.02	24.7	1.07
20	75.4	–	78.1	–
21	19.4	1.24	21	1.60
22	79.7	3.36	78.5	5.44
23	25.2	1.71, 1.32	27.3	1.98, 2.28
24	40.7	1.46, 1.84	42.3	1.82, 1.90
25	68.1	–	71.6	–
26	28.9	1.24	28.8	1.36
27	28.7	1.28	29.4	1.36

D-glucose, *D*-galactose, and *D*-glucuronic acid (GC-MS, TLC, and capillary electrophoresis). The El-MS also showed fragment ion peaks at $m/z = 793$ [(M-1)-162]$^+$ and $m/z = 631$ [(M-1)-324]$^+$ due to loss of one resp. two hexosyl moieties. The peak at $m/z = 455$ [(M-1)-500]$^+$ showed the elimination of two hexosyl moities and one glucuronic acid. The coupling constant values in the ^{1}H NMR spectrum showed an axial orientation of the anomeric protons. The point of attachment of the saccharide part and the interglycosidic linkage were established by means of ^{13}C NMR and HMBC experiments. Thus, the structure of **2** was determined as β-*D*-glucopyranosyl 3-(β-*D*-glactopyranosyl (1$\rightarrow$2) (β-*D*-glucopyranosyloxy))-oleanate which has been previously reported in the literature [8].

Compound **3** was found to contain one sugar less as compared to the other compounds. The El-MS showed an [M-1]$^+$ peak at $m/z = 793$, suggesting a molecular formula of $C_{42}H_{66}O_{14.}$ The ^{1}H and ^{13}C NMR data (Table 2) revealed two

Table 4. ^{1}H and ^{13}C chemical shifts of **5** in pyridine-d_6 at 30°C *vs.* internal *TMS*

Position	δ_H/ppm	δ_C/ppm	Position	δ_H/ppm	δ_C/ppm
2	–	157.7	1′	–	122.5
3	–	134.0	2′	8.4, d ($J = 1.8$ Hz)	117
4	–	178.5	3′	–	146.4
5	–	162.3	4′	–	150.4
6	6.67, d ($J = 1.8$ Hz)	99.5	5′	7.2, d ($J = 8.9$ Hz)	115
7	–	165.6	6′	8.1, dd ($J = 1.8$; 8.9 Hz)	121
8	6.62, d ($J = 1.8$ Hz)	94.2			
9	–	157.3		Galactose	
10	–	104.8			
			1″	6.0, d ($J = 7.8$ Hz)	105.2
			2″	4.8	73.0
			3″	4.28	75.0
			4″	4.30	75.1
			5″	4.16	77.2
			6″	4.32, 4.58	61.6

anomeric protons and carbons ($\delta = 5.40$ ppm (d, $J = 7.7$ Hz) and $\delta = 96.1$ ppm; $\delta = 4.38$ ppm (d, $J = 7.8$ Hz) and $\delta = 106.7$ ppm for glucose and glucuronic acid, respectively. Furthermore, significant fragment peaks at $m/z = 630$ $[(M-1)\text{-}163]^+$ and 592 $[(M-1]\text{-}201]^+$ also indicated the presence of hexosyl and glucuronic acid residues. The monosaccharides obtained from the acid aqueous hydrolysate showed the presence of *D*-glucose and *D*-glucuronic acid, whereas *D*-glucose was identified from the alkaline aqueous hydrolysate (GC-MS, TLC, and capillary electrophoresis). The interglycosidic linkage was also established by an HMBC experiment. Accordingly, the structure of **3** was determined as β-*D*-glucopyranosyl 3-(β-*D*-glucopyranosyloxy)-oleanate, which has already been isolated from the bran of *Chenopodium quinoa* [9].

Compound **4** showed its $[M-1]^+$ peak at $m/z = 479$ and $[(M-1)\text{-}H_2O]^+$ at $m/z = 461$ in the El-MS, suggesting a molecular formula of $C_{27}H_{44}O_7$. The presence of an α, β-unsaturated ketone in **4** was revealed from ist spectroscopic data (IR: $\nu = 1653\ cm^{-1}$; UV:$\lambda_{max} = 242$ nm; ^{1}H NMR: $\delta = 5.8$ (1H, br s) ppm; ^{13}C NMR: $\delta = 122$, 168, 206 ppm. The ^{13}C NMR spectrum (Table 3) showed the presence of five tertiary methyls, eight methylenes, and seven methines three of which were attached to an oxygen function. Olefinic carbons and carbonyl carbons were also detected. The ^{1}H and ^{13}C NMR chemical shifts of three methyls indicated that they were attached to carbons bearing hydroxyl groups. Accordingly, the remaining two methyl groups could be placed at C-10 and C-13. Acetylation gave a triacetate suggesting that three hydroxyl groups were sterically unhindered. The remaining three hydroxyl functions were considered as tertiary hydroxyl groups, possibly sterically hindered for acetylation, since three quaternary carbons attached to oxygen were observed in the ^{13}C NMR spectrum.

The proton signals at $\delta = 6.19$ ppm (H-7) in acetylated **4** showed HMBC correlations with the carbon signals at $\delta = 83.0$ (C-14), 50.6 (C-5), and 33.3 (C-9) ppm. Long-range correlations were also observed from the proton at $\delta = 1.07$ ppm

Table 5. UV spectral data of **5** in MeOH and with shift reagents added

Medium	λ_{max}/nm (log ε)
MeOH	259 (0.777), 359 (0.716)
$AlCl_3$	274 (0.939), 438 (0.920)
$AlCl_3$/HCl	268 (0.878), 406 (0.637)
NaOAc	273 (0.977), 328 (sh) (0.483), 383 (0.655)
NaOAc/H_3BO_3	263 (0.966), 378 (0.836)
NaOMe	272 (1.003), 333 (sh), (0.377), 410 (0.919)

(CH_3-19) with carbons at $\delta = 68.1$ (C-2), 50.6 (C-5), 37.5 (C-10), and 33.3 (C-9) ppm and from the proton at $\delta = 1.60$ ppm (H-21) with carbons at $\delta = 79.7$ (C-22), 75.4 (C-20), and 49.4 (C-17) ppm. The above data identified **4** as 20-hydroxyecdysone. It has recently been reported to occur in the mushroom *Paxillus atrotomentosus* [10].

Compound **5** was isolated as a pale yellow amorphous solid of m.p. = 222 – 224°C (dec). The IR spectrum displayed absorption bands at $\nu = 3383$ (OH) and 1655 (chelated carbonyl) cm^{-1}. The El-MS showed a peak at $m/z = 463$ $[M\text{-}1]^+$, suggesting a molecular formula of $C_{21}H_{20}O_{12}$. The UV spectrum showed band-I and band-II absorption maxima (MeOH) at 359 and 259 nm (Table 5). A bathochromic shift with NaOAc (+14 nm), $AlCl_3$ (+15 nm), and $AlCl_3$/HCl (+11 nm) [11] revealed a phenolic 5,7-dihydroxy ring structure (ring A). The presence of two *meta*-coupled doublets ($J = 1.8$ Hz) at $\delta = 6.67$ (H-6) and 6.62 (H-8) ppm in the ^1H NMR spectrum (Table 4) further supported the UV results. A bathochromic shift with NaOAc/H_3BO_3 (+19) of the band-I absorption maximum in MeOH (359 nm) showed *ortho*-dihydroxylation of ring B. This was also evident from the proton resonances at 8.4 (d, $J = 1.8$ Hz, H-2′), 8.1 (d, $J = 8.9$ Hz and 2.2 Hz, H-6′), and 7.2 (d, $J = 8.9$ Hz, H-5′) ppm that indicated 3′,4′-dihydroxylation. Fragment ions at $m/z = 301$ $[M\text{-hexose}]^+$ and 163 (hexose) showed that a hexose unit was linked to the aglycone. Furthermore, the ^1H and ^{13}C NMR spectra of **5** displayed the presence of an anomeric proton and carbon (Table 4). The coupling constant indicated an axial orientation. The point of attachment of the monosaccharide was determined by the HMBC correlation between H-1 ($\delta = 6.0$ ppm) of the sugar and C-3 ($\delta = 134.2$ ppm) of the aglycone. Acidic hydrolysis of **5** gave quercetin as the aglycone which was identified by comparison with reported ^{13}C chemical shifts [12]. From the aqueous hydrolysates, *D*-galactose was obtained and identified by TLC, GC-MS, and capillary electrophoresis. From these experimental evidence, the structure of **5** was established as quercetin-3-O-β-*D*-galactoside.

Experimental

General

Melting points were determined in open capillaries in an electrothermal melting apparatus. El-MS were run at 70 eV and LSIMS at 5 kV neg. NMR spectra were recorded as given in Ref. [13]. 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 Hewlett Packard 5989B mass spectrometer. UV spectra were obtained from a Shimadizu UV 160A UV-visible recording spectrophotometer. Capillary electrophoresis was performed with a Prince Technologies apparatus (the Emmen, 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; detection was carried out with aniline/hydrogen phthalate as spraying reagent. Column chromatography (CC) was carried out on Silicagel-60 (Merck, 230–240 μm), RP18 (Merck, 40–63 μm), and Sephadex LH-20 (Biochemica, 25–100 μm).

The homogeneity of fractions was tested on TLC (Silicagel and RP18, Merck). The spots were visualized 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 the following: A: $CHCl_3$/MeOH/H_2O (10:1:0.1 yielding fraction 1, 5:1:0.1 yielding fraction 2, 1:1:0.1 and 1:2:0.1 yielding fraction 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: EtOAC/MeOH/H_2O/HOAc (13:3:3:4).

Plant material

The aerial parts of *Acyranthes aspera* L. 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 University, Graz, Austria).

Extraction and isolation of the glycosides

Air dried and finely powdered aerial parts (2.6 kg) were defatted with petroleum ether (40–60°C) in a percolator at room temperature. The solvent free powder was exhaustively extracted with methanol in a percolator to afford a greenish gum (218 g) which was taken up in water and re-extracted with diethyl ether until all the chlorophyll pigments were removed. The aqueous phase was then partitioned with *n*-butanol saturated with water ($3\times300\,cm^3$). After concentration under reduced pressure, the *n*-butanol extract yielded a saponin mixture (54 g). This mixture was subjected to CC on silicagel (solvent A) to afford fractions 1 to 5 which were rechromatographed over Sephadex LH-20 with methanol as eluent followed by repeated chromatography on silicagel (solvent A–C) and RP-18 silicagel with solvents D and E to afford compounds: **1** (71 mg), **2** (29 mg), **3** (19 mg), **4** (120 mg), and **5** (53 mg).

β-D-Glucopyranosyl 3-(O-α-L-rhamnopyranosyl (1→3) (O-β-D-glucopyranosyloxy)-oleanolate (**1**; $C_{48}H_{76}O_{18}$)

White amorphous powder (acetone); m.p.: 227–229°C (dec.); $[\alpha]_D^{23} = -14°$ ($c = 0.41$, MeOH); UV (MeOH):$\lambda_{max}(\log\,\varepsilon) = 215$ (2.30) nm; IR (KBr): $\nu = 3854$, 3421, 2944, 1733, 1610, 1229 cm^{-1}; El-MS: *m/z* (%) = 939 (100) $[M\text{-}1]^+$, 791 (42) $[(M\text{-}1)\text{-Rha}]^+$, 777 (40) $[(M\text{-}1)\text{-Glc}]^+$ 631 (20) [(M-1)-Rha-Glc], 455 (30) [(M-1)-Rha-GlcA-Glc]; ^{13}C and ^1H NMR: Tables 1 and 2.

β-D-Glucopyranosyl 3-(O-β-D-glactopyranosyl (1→2)(O-β-D-glucopyranosyloxy))-oleanolate (**2**; $C_{48}H_{76}O_{19}$)

Partly solidified gum; $[\alpha]_{546}^{21} = -10°$ ($c = 0.26$, MeOH); UV (MeOH): λ_{max} ($\log\,\varepsilon$) = 213 (2.274) nm; IR (KBr): $\nu = 3854$, 3418, 2944, 1731, 1616, 1416, 1260 cm^{-1}; El-MS: *m/z* (%) = 955 (60) $[M\text{-}1]^+$,

783 (42) [(M-1)-hexose]$^+$, 631 (30) [(M-1)-2 ×hexose], 455 (25) [(M-1)-Gal-GlcA-Glc]:^{13}C and ^{1}H NMR: Tables 1 and 2.

β-D-Glucopyranosyl 3-(O-β-D-glucopyranosyloxy)-oleanolate (**3**; $C_{42}H_{66}O_{14}$)

Partly solidified gum; $[\alpha]_{546}^{21} = -10.2°$ ($c = 0.25$, MeOH); UV (MeOH): λ_{max} (logε) = 212 (2.174) nm; IR (KBr): $\nu = 3854$, 3422, 2954, 1734, 1615, 14120, 1364, 1260 cm^{-1}, El-MS: *m/z* (%) = 794 [M]$^+$ (54), 793 (40) [M-1]$^+$, 630 (30) [(M-1)-hexose], 455 (20) [(M-1)-GlcA-Glc]; ^{13}C and ^{1}H NMR: Tables 1 and 2.

20-Hydroxyecdysone (**4**; $C_{27}H_{44}O_7$)

Oil; $[\alpha]_D^{23} = +53°$ ($c = 0.26$ MeOH); UV (MeOH): λ_{max} (logε) = 210 (0.879); 242 (1.181) nm; IR (KBr): $\nu = 3417$, 2961, 1653, 1604, 1382, 1056 cm^{-1}; El-MS: *m/z* (%) = 476 [M-1]$^+$ (100), 461 [(M-1)-H_2O]$^+$ (10), 319 [(M-1)-side chain-H_2O]$^+$ (5); ^{1}H and ^{13}C NMR: Table 3.

Quercetin-3-O-galactoside (**5**; $C_{21}H_{21}O_{12}$)

Pale yellow solid (EtOAc); m.p.: 222–224°C (dec.); $[\alpha]_{546}^{21} = -10.5°$ ($c = 0.11$, MeOH); UV (MeOH and with shift reagents): see Table 5; IR (KBr): $\nu = 3383$, 1789, 1655, 1364, 1171, 997 cm^{-1}; El-MS: *m/z* (%) = 463 [M-1]$^+$ (20), 301 [M-hexose]$^+$ (20), 154 [RDA ring A]$^+$ (85), 147 [RDA ring B] (25); ^{1}H and ^{13}C NMR: Table 4.

Acidic hydrolysis

The glycosides (10 mg each) were refluxed with 20 cm^3 7% HCl on a steam bath for 3 h. Extraction with $CHCl_3$ afforded the aglycone. The aglycones of **1**, **2**, and **3** were found to be identical with oleanolic acid, whereas the aglycone of **5** was identified as quercetin. The neutralized (Dowex basic anionic ion exchanger (Cl^-)) and lyophilized aqueous hydrolysates contained glucose, rhamnose, and arabinose for **1**, glucose, galactose, and glucuronic acid for **2**, glucose and glucuronic acid for **3**, and galactose for **5** (PPC, solvent system F, $R_f = 0.28$ (glucose), 0.26 (galactose), and 0.15 (glucuronic acid)). GC-MS (Column: 5% phenyl and 95% methyl silicone on ultra 2, 0.2×46 m, column temperature: 250°C, carrier gas: He (0.8 cm^3/min), sample: trimethylsilyl derivatives; t_R (min): glucose: 15.78 and 17.48 for **1**, 15.79 and 17.45 for **2**, 15.73 and 17.45 for **3,** rhamnose: 10.58 and 11.85 for **1**; galactose: 9.93 and 11.71 for **2**, 9.91 and 11.74 for **5**, glucuronic acid: 17.28 and 18.27 for **2**, 9.91, 17.26, and 18.25 for **3**.

The *D*-configuration of glucose, galactose, and glucuronic acid and the *L*-configuration of rhamnose was determined by capillary electrophoresis as complexes with borate and β-cyclodextrin as chiral selector using authentic samples of *D*- and *L*-glucose, *D*- and *L*-rhamnose, *D*- and *L*-galactose, and *D*-glucuronic acid.

Acknowledgements

A. D. and *E. M.* gratefully acknowledge ÖAD for an Austrian scholarship award and EHNRI, Addis Ababa, for facilitating the collection of plant material.

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Received July 21, 1999. Accepted August 26, 1999