64. Molluscicidal Saponins from *Swartzia madagascariensis* DESVAUX

by **Christian Borel** and **Kurt Hostettmann***

Institut de Pharmacognosie et Phytochimie, Ecole de Pharmacie, Université de Lausanne, 2, rue Vuillermet, CH–1005 Lausanne

(3.II.87)

The phytochemical investigation of the dried fruits of *Swartzia madagascariensis* (Leguminosae) afforded the five triterpenoid saponins **1**–**5**. They were shown to be glucuronides of oleanolic acid and of gypsogenin by chemical and spectral means (FAB-MS, ^{13}C-NMR, GC/MS). One of the isolated compounds, identified as *3-O-[O-α-L-rhamnopyranosyl-(1→3)-(β-D-glucopyranosyluronic acid)]oleanolic acid*, was responsible for the high molluscicidal activity of *Swartzia madagascariensis* fruits against the schistosomiasis-transmitting snails *Biomphalaria glabrata* and *Bulinus globosus*.

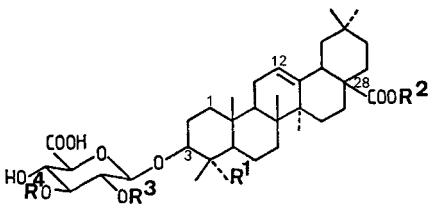
Introduction. – Schistosomiasis (or bilharzia) affects millions of people living in African, Asian, and South-American countries. This disease is linked with certain species of aquatic snails because they serve the parasite as intermediate hosts. Molluscicidal or snail-killing activities of plants are of special importance for the control of schistosomiasis as they seem to be less expensive than synthetic compounds. Among the different promising natural molluscicides, *Phytolacca dodecandra* L'HERIT (Phytolaccaceae) has been intensively studied [1]. Nevertheless, the geographical distribution of *Phytolacca dodecandra* is restricted to Ethiopia, and thus it has to be cultivated if required for use in other countries. Therefore, other plant molluscicides have to be taken into account.

Swartzia madagascariensis DESVAUX (Leguminosae), is a common tree in many regions of Africa. Since 1939, it has been reported that fruits of this plant have been used effectively in controlling the populations of schistosomiasis-transmitting snails in natural ponds [2]. Previous phytochemical investigations of this plant have afforded flavonoids [3], pterocarpans [4], and mixtures of saponins [5]. The present work reports the isolation and the structure elucidation of saponins responsible for the molluscicidal activity of an aqueous extract of *Swartzia madagascariensis* fruits. It is part of a more extensive programme in collaboration with the *Swiss Tropical Institute* (Basel) and its Field Laboratory at Ifakara in South-Eastern Tanzania [6].

Results. – Dried ground pods (40 g) of *Swartzia madagascariensis* were extracted with distilled H_2O. The aqueous extract was partitioned between BuOH and H_2O. Separation of the BuOH extract by different chromatographic techniques afforded saponins **1**–**5** (70, 2, 150, 8, and 15 mg, resp.).

On acid hydrolysis, saponins **1**, **3**, and **5** afforded oleanolic acid as aglycone, identified by comparison with an authentic sample (TLC, DCI-MS). The sugars obtained from the saponin hydrolysates were identified as rhamnose and glucuronic acid for **1**, rhamnose, glucuronic acid, and glucose for **3** and **5**. Acid hydrolysis of saponins **2** and **4** afforded gypsogenin as aglycone identified by comparison with an authentic sample (TLC, DCI-

Molluscicidal Saponins from Swartzia madagascariensis



Saponin[a])	R^1	R^2	R^3	R^4	Moll. activity[b])
1	CH_3	H	H	Rha	3
2	CHO	H	H	Rha	25
3	CH_3	H	Glc	Rha	25
4	CHO	H	Glc	Rha	> 50
5	CH_3	Glc	Glc	Rha	no activity
6	CH_3	H	Glc	H	6.25
7	CHO	H	Glc	H	not tested
8	CH_3	H	H	H	3

[a]) Glc = β-D-Glucopyranosyl, Rha = α-L-rhamnopyranosyl.

[b]) Molluscicidal activity against *Biomphalaria glabrata* [mg/l].

MS). The sugars rhamnose and glucuronic acid were obtained from **2**, whereas rhamnose, glucuronic acid, and glucose were obtained from **4**. Only saponin **5** was affected by alkaline treatment and furnished glucose as sugar. Thus, **5** is a bidesmosidic saponin with a sugar esterified at position C(28) of oleanolic acid.

The spectroscopic data of **1–5** and of some of their derivatives established their structures.

Saponin **1** was submitted to fast-atom-bombardment MS (FAB-MS; negative-ion mode) in order to establish the sugar sequence. A quasimolecular ion was observed at *m/z* 777 ($[M - H]^-$), indicating a mol. wt. of 778. Signals at *m/z* 631 ($[(M - H) - 146]^-$) and 455 ($[(M - H) - 322]^-$) corresponded to the successive elimination of 1 rhamnosyl and of 1 glucuronic-acid moiety; thus, rhamnose is the terminal sugar. ^{13}C-NMR spectroscopy was used for clarification of the location of the disaccharide moiety on the aglycone of **1** and for the determination of the interglycosidic linkage. Signals for oleanolic acid corresponded to those published [7], except for the C(3) signal (82.9 ppm) which was significantly shifted downfield (C(3): 78.8 ppm for non-substituted oleanolic acid); thus, the disaccharide was linked at C(3) of oleanolic acid. The signals of the anomeric C-atoms appeared at 107.0 and 102.9 ppm indicating a pyranose form for the sugars [8]. Although the interglycosidic linkage was postulated to be rhamnosyl-(1→3)-glucuronic acid by ^{13}C-NMR spectroscopy, the glycosylation shift observed for C(3') of glucuronic acid (4 ppm) was smaller than that usually observed for this position (8 ppm) [9]. Furthermore, the upfield shift for the C(2') signal deduced from the *Konishi* rule [10] was absent. Consequently, it was necessary to confirm the substitution position by GC/MS of the methylated alditol acetates obtained from **1**; the procedure was carried out as described by *Gunzinger et al.* [11]. GC/MS analysis showed a 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-6-deoxyhexitol, corresponding to a terminal rhamnose. Substitution at position C(3') on glucuronic acid was clearly indicated by the presence in the EI-MS of peaks at *m/z* 117 and 189, obtained from the fragmentation of a 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methylhexitol [12]. The configurations of the sugars were deduced from the ^{1}H-NMR data of the permethylated derivative of **1**. Among other signals, a *s* appeared at 5.32 ppm, ascribed to the anomeric proton of the α-L-rhamnose moiety, and a *d* (J = 7 Hz) was observed at 4.3 ppm corresponding to the anomeric proton of a glucuronic-acid unit in the β-configuration [13] [14]. Consequently, the structure of saponin **1** is *3-O-[O-α-L-rhamnopyranosyl-(1→3)-(β-D-glucopyranosyluronic acid)]oleanolic acid.*

FAB-MS (negative-ion mode) of saponin **3** showed a quasimolecular ion at *m/z* 939 ($[M - H]^-$), indicating a mol. wt. of 940. The signals at *m/z* 793 ($[(M - H) - 146]^-$) and 777 ($[(M - H) - 162]^-$) corresponded to the simultaneous elimination from the quasimolecular ion of 1 rhamnosyl moiety and 1 glucosyl moiety. Additional signals for the glucuronic-acid-oleanolic-acid moiety and the oleanolic-acid moiety were observed at *m/z* 631 ($[(M - H) - 308]^-$) and 455 ($[(M - H) - 484]^-$), respectively. From this fragmentation pattern, it could be deduced that rhamnose and glucose were both terminal sugars. Signals for the oleanolic-acid moiety in the ^{13}C-NMR spectrum corresponded to those already described [7], except for C(3) which appeared at 83.3 ppm, thus indicating substitution of the sugar chain in this position. Therefore, the terminal rhamnosyl and glucosyl moieties are linked to the glucuronic acid. Substitution of glucuronic acid was found to be at positions C(2') and C(3') since C(2') and C(3') of **3** are shifted downfield by 3.7 and 5.2 ppm, respectively, and C(1') and C(4') are shifted upfield by

Table. *^{13}C-NMR Chemical Shifts of the Glycosidic Moieties in (D_5)Pyridine*

Sugar moiety[a])	C-Atom	1	2	3	4	5	6	8
GlcA	1	107.0	105.3	103.8	103.3[b])	103.9[b])	105.9	107.1
	2	76.0	75.5	79.3	78.9	79.1[c])	82.9	75.6
	3	82.2	82.3	83.4	84.6	85.0	76.9[b])	78.2
	4	72.8[b])	72.8[b])	72.8[b])	72.7[c])	72.7[d])	73.3	73.5
	5	77.3	77.5	76.4[c])	77.5[d])	77.8[e])	77.9[c])	77.6
	6	173.8	172.7	175.6	172.2	176.2	173.8	172.9
Rha	1	102.9	103.0	102.9	103.3	103.3[b])		
	2	71.9	71.5	72.4[b])	71.4	71.6		
	3	72.6[b])	72.6[b])	72.6[b])	72.0[e])	72.4[d])		
	4	74.2	74.2	74.2	73.9	74.0[f])		
	5	69.8	70.0	69.9	70.4	70.4		
	6	18.8	18.9	18.5	18.4	18.6		
Glc	1			104.9	103.9[b])	105.0	105.1	
	2			76.0[c])	76.0[d])	75.9	76.6[b])	
	3			78.2[d])	78.4[e])	78.3	77.9[c])	
	4			72.6[b])	72.5[c])	72.6[d])	71.9	
	5			78.1[d])	78.0[e])	77.8[e])	78.0	
	6			63.5	63.2	63.5	62.9	
Glc-C(28)	1					95.8		
	2					74.2[f])		
	3					78.9[c])		
	4					71.5		
	5					78.9[c])		
	6					62.5		

[a]) GlcA = β-D-Glucopyranosyluronic acid, Rha = α-L-rhamnopyranosyl, Glc = β-D-glucopyranosyl.
[b])[c])[d])[e])[f]) Assignments in the vertical column with the same sign may be alternated although those given here are preferred.

3.3 and 0.7 ppm, respectively, as compared to **8** (see *Table*). The anomeric C-atoms of **3** appeared at 103.8, 102.9, and 104.9 ppm, indicating a pyranose form for all sugars [8]. The substitution scheme was confirmed by GC/MS analysis of the methylated alditol acetates obtained from **3**. In the case of a 2',3'- or 2',4'-di-*O*-substituted glucuronic acid, the two major MS fragments obtained are the same (*m/z* 261, 189) for both types of substitution; thus $LiAlD_4$ was used instead of $LiAlH_4$ for the reduction of the carboxylic group on glucuronic acid. The characteristic fragments of a 2',3'-di-*O*-substituted glucuronic acid at *m/z* 191 and 261 can be clearly distinguished from those at *m/z* 189 and 263 arising from a 2',4'-di-*O*-substituted glucuronic acid.

Mild acid hydrolysis of saponin **3** afforded saponins **6** and **8**. FAB-MS of **6** showed a quasimolecular ion at *m/z* 793 ($[M - H]^-$). Signals at *m/z* 631 ($[(M - H) - 162]^-$) and 455 ($[(M - H) - 338]^-$) corresponded to the successive elimination of 1 glucosyl moiety and 1 glucuronic-acid moiety. ^{13}C-NMR values of **6** showed, by comparison with data from [9], that the interglycosidic linkage was glucosyl-(1→2)-glucuronic acid. FAB-MS of **8** showed a quasimolecular ion at *m/z* 631 ($[M - H]^-$). Signals at *m/z* 455 ($[(M - H) - 176]^-$) corresponded to the elimination of 1 glucuronic-acid moiety. ^{13}C-NMR data of **8** corresponded to those previously described for this compound [9].

Sugar configurations of **3** were deduced from ^{1}H-NMR data of the permethylated saponin. A *s* at 5.17 ppm indicated that the rhamnose moiety had an α configuration, whereas the 2 *d* (J = 7 Hz) at 4.55 ppm and 4.47 ppm indicated that the glucose and glucuronic-acid unit had the β configuration [13] [9]. Thus, the structure of saponin **3** is *3-O-{O-β-D-glucopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→3)]-(β-D-glucopyranosyluronic acid)}oleanolic acid.*

FAB-MS (negative-ion mode) of saponin **5** showed a quasimolecular ion at *m/z* 1101 ($[M - H]^-$) indicating a mol. wt. of 1102. Additional signals were observed at *m/z* 955 ($[(M - H) - 146]^-$), 939 ($[(M - H) - 162]^-$), 793 ($[(M - H) - 308]^-$), 777 ($[(M - H) - 324]^-$), 631 ($[(M - H) - 470]^-$), and 455 ($[(M - H) - 646]^-$). FAB-MS of the prosapogenin obtained after alkaline treatment of saponin **5**, showed a quasimolecular ion at *m/z* 939 ($[M - H]^-$) and a similar fragmentation pattern to that of saponin **3**.

The ^{13}C-NMR spectrum of saponin **5** gave a signal at 95.8 ppm, attributed to the anomeric C-atom of a β-glucosyl ester of oleanolic acid [9]. Signals in the ^{13}C-NMR spectrum of the prosapogenin obtained from **5** (see above) corresponded to those of saponin **3**. Substitution of glucuronic acid at positions C(2′) and C(3′) was confirmed by GC of the methylated alditol acetates obtained from saponin **5**.

The ^{1}H-NMR anomeric signals of the permethylated derivative of **5** (4.47 ppm (*d*, J = 7 Hz, 1 H); 4.52 (*d*, J = 6 Hz, 1 H); 5.17 (*s*, 1 H); 5.37 (*d*, J = 8 Hz, 1 H)) enabled the assignments of the anomeric configurations of the glucuronic acid and glucosyl units as β and the rhamnosyl unit as α [13][9]. Consequently, saponin **5** is *β-D-glucopyranosyl3-O-{O-β-D-glucopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→3)]-(β-D-glucopyranosyluronic acid)}oleanolate*.

FAB-MS (negative-ion mode) of saponin **2** showed a quasimolecular ion at m/z 791 ([M – H]$^-$) indicating a mol. wt. of 792. Signals observed at m/z 645 ([(M – H) – 146]$^-$), and 469 ([(M – H) – 322]$^-$) corresponded to the successive elimination of 1 rhamnosyl moiety and 1 glucuronic-acid moiety. As observed in the ^{13}C-NMR spectrum of **2**, the sugar chain is linked at position C(3) on gypsogenin; all signals of the aglycone corresponded to those of literature values [7], except the signal for C(3) which was significantly shifted downfield (82.6 ppm) when compared with non substituted gypsogenin (71.8 ppm) [7]. Two signals of anomeric C-atoms appeared at 105.3 and 103.0 ppm, indicating that both sugars were in the pyranose form. The downfield shift for C(3′) (3.9 ppm) and the upfield shift for the adjacent C(2′) and C(4′) (0.3 and 1.2 ppm, resp.) as compared to 3-*O*-(β-D-glucuronic acid)gypsogenin [15] showed that the interglycosidic linkage was rhamnosyl(1→3)glucuronic acid. These results are in agreement with the glycosylation rule previously established by *Konishi et al.* [10]. Sugar configurations were deduced from ^{1}H-NMR data of the permethylated derivative of **2**. Thus, the structure of saponin **2** is *3-O-[O-α-L-rhamnopyranosyl-(1→3)-(β-D-glucopyranosyluronic acid)]gypsogenin*.

FAB-MS (negative-ion mode) of saponin **4** showed a quasimolecular ion at m/z 953 ([M – H]$^-$) indicating a mol. wt. of 954. Additional signals were observed at m/z 807 ([(M – H) – 146]$^-$), 791 ([(M – H) – 162]$^-$), 645 ([(M – H) – 308]$^-$), and 469 ([(M – H) – 484]$^-$). This fragmentation pattern indicated a simultaneous elimination of 1 rhamnosyl and 1 glucosyl unit from the quasimolecular ion and thus both were terminal sugars. Glucuronic acid was found to be substituted at C(2′) and C(3′) by ^{13}C-NMR spectroscopy since C(2′) and C(3′) were shifted downfield (3.7 and 6.2 ppm, resp.) and C(1′) and C(4′) were shifted upfield (1.4 and 1.3 ppm, resp.) as compared to 3-*O*-(β-D-glucuronic acid)gypsogenin [15]. The substitution pattern was confirmed by GC/MS analysis of the methylated alditol acetates of **4**. Mild acid hydrolysis of **4** afforded saponin **7**. Investigation of the latter by FAB-MS afforded a quasimolecular ion at m/z 807 ([M – H]$^-$). Additional signals were observed at m/z 645 ([(M – H) – 162]$^-$) and 469 ([(M – H) – 338]$^-$), corresponding to the successive elimination of 1 glycosyl moiety and 1 glucuronic-acid moiety, respectively. GC/MS analysis of the methylated alditol acetates of **7** showed peaks corresponding to a terminal glucose and a 2′-substituted glucuronic acid. Therefore, the structure of saponin **4** is *3-O-{O-β-D-glucopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→3)]-(β-D-glucopyranosyluronic acid)}gypsogenin*.

Discussion. – Five triterpenoid saponins were isolated from the aqueous extract of *Swartzia madagascariensis* fruits by a combination of silica-gel and reversed-phase chromatography. Structures were established by ^{13}C-NMR and ^{1}H-NMR spectroscopy, FAB-MS, GC/MS of methylated alditol acetates, and on the basis of acid and basic hydrolyses. They were found to be glucuronides of oleanolic acid and gypsogenin. Saponins **2**–**5** have not been reported previously, whereas saponin **1** is a known compound already isolated from *Zexmenia buphthalmiflora* (Compositae) [14] and *Putranjiva roxburghii* (Euphorbiaceae) [16].

The results of biological testing have shown that saponin **1** presented the highest molluscicidal activity (3 mg/l) of the isolated compounds against schistosomiasis-transmitting snails *Biomphalaria glabrata*. Saponins with disubstituted glucuronic acid as well as those with gypsogenin as aglycone had a lower activity ($\geq$ 25 ppm). In accordance with general structure-activity relationships of other molluscicidal saponins [17], bidesmosidic saponin **5** had no snail-killing activity.

Recently, field trials performed at Ifakara, Tanzania, have shown that *Swartzia madagascariensis* is a viable candidate as a natural molluscicide [18] [19]. Thus, toxicological studies have been undertaken in order to determine whether large-scale applications

of *Swartzia madagascariensis* extracts can be realised. Experimental tests are also underway in order to standardise the extraction procedure.

Financial support has been provided by the *Swiss National Science Foundation* and the *UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases*. Grants were received from the *Directorate of Development Cooperation and Humanitarian Aid of the Swiss Governement* and from the *Stiftung für Experimentelle Zoologie*, Basel, for field work in Tanzania. The authors wish to thank Dr. *R. Suter*, Dr. *M. Tanner*, Dr. *A. A. Degrémont* and Prof. *T. A. Freyvogel, Swiss Tropical Institute*, Basel, for their very useful cooperation. Thanks are also due to Dr. *K. E. Mott, WHO*, Geneva, for helpful advice and to Prof. *W. J. Richter* (*Ciba-Geigy AG*, Basel) and Prof. *G. Bodenhausen* (University of Lausanne) for spectral measurements. The research programme was supported by the *National Institute for Medical Research (NIMR)*, Tanzania (Director General, Prof. *W. L. Kilama*), and Research Clearance was granted by the *Tanzanian National Scientific Research Council* (UTAFITI, Director General Prof. *A. S. Msangi*) as per Ref. No. NSR/CONF R. C. 29th July 1982 and 19th April 1983 and NSR/RA. 47 of the 18th June 1984.

Experimental Part

General. Normal-phase column chromatography: silica gel *60* (40–63 μm; *Merck*, Darmstadt). Reversed-phase chromatography: *Lobar Lichroprep RP-8* column (40–63 μm; i.d. 2.5 × 27 cm; *Merck*, Darmstadt), equipped with a *Duramat-80* pump (*Chemie und Filter*, Regensdorf), and *Büchi B-681 MPLC* system with a 2.6 × 46 cm column packed with *RP-8* material (*Merck* No. *9324*). TLC: silica-gel-precoated Al sheets (*Merck*, Darmstadt) with $CHCl_3$/MeOH/H_2O 65:35:5 afforded the following R_f values for saponins **1**–**5**: 0.25, 0.25, 0.1, 0.1, 0.05; detection with *Godin* reagent [20] showed a violet colour for oleanolic-acid derivatives and a blue colour for gypsogenin derivatives. GC/MS: *Dani 6500* apparatus using a 0.22 mm × 25 m fused-silica column packed with *SE 54* (injection temp. 210°, column temp. 130°/3 min and 5°/min up to 250°), coupled with a *Nermag R 3010* mass spectrometer. GC: *Hewlett Packard-5790* chromatograph using a 0.25 mm × 10 m capillary column *SP-2330* (injection temp. 240°, column temp. 170°/6 min and 5°/min up to 240°/6 min). M.p.: *Mettler F. P. 80/82* hot-stage apparatus; uncorrected. Before recording NMR spectra of glucuronides, the samples were desalted with *Amberlite 200* (*Fluka* No. *06437*) in MeOH. ^{1}H-NMR: in (D_5)pyridine and $CDCl_3$; *Bruker WP-360* at 360 MHz; *Varian VXR-200* at 200 MHz. ^{13}C-NMR: in (D_5)pyridine; *Bruker WP-360* at 90.5 MHz; *Bruker WP-200* at 50.29 MHz; *Varian X-L 400* apparatus at 101 MHz; δ of the aglycones for **1**–**8** corresponded to those previously described for oleanolic acid and gypsogenin [7], except for C(3) and C(28) when these C-atoms were substituted by sugars. Fast-atom-bombardment (FAB) MS: negative-ion mode, *ZAB-1S* spectrometer; the target was bombarded with 5-keV Xe-atoms; samples were suspended in thioglycerol.

Extraction and Isolation. The dried ground pods of *Swartzia madagascariensis* (40 g) collected near Ifakara (Tanzania) were extracted with dist. H_2O (200 ml) during 24 h. The aq. extract (20 g) was partitioned between BuOH and H_2O (500:900 ml). Part of the org. phase (3 g) was then separated on a silica-gel column with $CHCl_3$/MeOH/H_2O 58:35:7→65:35:5. Separation was monitored by TLC, and fractions *I*–*VI* were obtained. Fraction *II* (230 mg) was chromatographed in 2 portions on a *Lobar RP-8* column with MeOH/H_2O 75:25 to afford **1** (70 mg) and **2** (2 mg). From fraction *V*, **3** (150 mg) and a mixture **4**/**5** were obtained by medium-pressure reversed-phase chromatography (MPLC) [21] with MeOH/H_2O 65:35. Saponins **4** and **5** were further separated on a *Lobar RP-8* column with MeOH/H_2O 55:45 to give pure **4** (8 mg) and **5** (15 mg).

Acid Hydrolysis. The saponin (2 mg) in 1 ml MeOH was refluxed in 10 ml of 4N HCl for 4 h. The aglycone was extracted with AcOEt and identified by comparison with an authentic sample by TLC on silica gel with (i-Pr)$_2$O/acetone 7:3 and DCI-MS. The aq. layer was adjusted to pH 6 with $NaHCO_3$. After evaporation to dryness, the sugars were extracted with pyridine from the residue and analyzed by TLC on silica gel with AcOEt/MeOH/H_2O/AcOH 65:15:15:20; detection with *p*-anisidine phthalate.

Partial Acid Hydrolysis. The saponin (60 mg) was refluxed in 0.1N HCl (50 ml) for 5 h. The mixture was extracted with BuOH (3 × 50 ml). Then, the org. layer was washed with H_2O (3 × 50 ml). The products were purified on a silica-gel column (1 × 30 cm) with $CHCl_3$/MeOH/H_2O 65:35:5.

Basic Hydrolysis. The saponin (30 mg) was refluxed in 0.5N aq. KOH (20 ml) for 2 h. The mixture was adjusted to pH 6 with 1N aq. HCl and then extracted with 2 × 30 ml of BuOH; the org. phase was washed with 3 × 30 ml of H_2O.

Methylated Alditol Acetates. They were obtained as described previously [11].

3-O-[O-α-L-Rhamnopyranosyl-(1→3)-(β-D-glucopyranosyluronic acid)]oleanolic Acid (= *3β-{[O-α-L-Rhamnopyranosyl-(1→3)-(β-D-glucopyranosyluronic acid)]oxy}olean-12-en-28-oic Acid;* **1**): white powder, m.p. 264–277° (dec.). ^{13}C-NMR (101 MHz, (D_5)pyridine): Signals of the sugar moieties, see *Table*. FAB-MS (thioglycerol, negative ions): 777 ($[M-H]^-$), 631 ($[(M-H)-146]^-$), 455 ($[(M-H)-322]^-$).

Acid hydrolysis of **1** afforded rhamnose, glucuronic acid, and oleanolic acid.

3-O-[O-α-L-Rhamnopyranosyl-(1→3)-(β-D-glucopyranosyluronic acid)]gypsogenin (= *23-Oxo-3β-{[O-α-L-rhamnopyranosyl-(1→3)-(β-D-glucopyranosyluronic acid)]oxy}olean-12-en-28-oic Acid;* **2**): white powder, m.p. 278–286° (dec.). ^{13}C-NMR (101 MHz, (D_5)pyridine): Signals of the sugar moieties, see *Table*. FAB-MS (thioglycerol, negative ions): 791 ($[M-H]^-$), 645 ($[(M-H)-146]^-$), 469 ($[(M-H)-322]^-$).

Acid hydrolysis of **2** afforded rhamnose, glucuronic acid, and gypsogenin.

3-O-{O-β-D-Glucopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→3)]-(β-D-glucopyranosyluronic acid)}-oleanolic Acid (= *3β-{[O-β-D-Glucopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→3)]-(β-D-glucopyranosyluronic acid)]oxy}olean-12-en-28-oic Acid;* **3**): white powder, m.p. 207–213° (dec.). ^{13}C-NMR (101 MHz, (D_5)pyridine): Signals of the sugar moieties, see *Table*. FAB-MS (thioglycerol, negative ions): 939 ($[M-H]^-$), 793 ($[(M-H)-146]^-$), 777 ($[(M-H)-162]^-$), 631 ($[(M-H)-308]^-$), 455 ($[(M-H)-484]^-$).

Acid hydrolysis of **3** afforded rhamnose, glucose, glucuronic acid, and oleanolic acid.

3-O-{O-β-D-Glucopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→3)]-(β-D-glucopyranosyluronic acid)}-gypsogenin (= *3β-{[O-β-D-Glucopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→3)]-(β-D-glucopyranosyluronic acid)]oxy}-23-oxoolean-12-en-28-oic Acid;* **4**): white powder, m.p. 210–219° (dec.). ^{13}C-NMR (90.5 MHz, (D_5)pyridine): Signals of the sugar moieties, see *Table*. FAB-MS (thioglycerol, negative ions): 953 ($[M-H]^-$), 807 ($[(M-H)-146]^-$), 791 ($[(M-H)-162]^-$), 645 ($[(M-H)-308]^-$), 469 ($[(M-H)-484]^-$).

Acid hydrolysis of **4** afforded rhamnose, glucose, glucuronic acid, and gypsogenin.

β-D-Glucopyranosyl 3-O-{O-β-D-Glucopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→3)]-(β-D-glucopyranosyluronic acid)}oleanolate (= *β-D-Glucopyranosyl 3β-{[O-β-D-Glucopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→3)]-(β-D-glucopyranosyluronic acid)]oxy}olean-12-en-28-oate;* **5**): white powder, m.p. 215–223° (dec.). ^{13}C-NMR (50.29 MHz, (D_5)pyridine): Signals of the sugar moieties, see *Table*. FAB-MS (thioglycerol, negative ions): 1101 ($[M-H]^-$), 955 ($[(M-H)-146]^-$), 939 ($[(M-H)-162]^-$), 793 ($[(M-H)-308]^-$), 777 ($[(M-H)-324]^-$), 631 ($[(M-H)-470]^-$), 455 ($[(M-H)-646]^-$).

Acid hydrolysis afforded rhamnose, glucose, glucuronic acid, and oleanolic acid. Basic hydrolysis afforded glucose and **3**.

3-O-[O-β-D-Glucopyranosyl-(1→2)-(β-D-glucopyranosyluronic acid)]oleanolic Acid (= *3β-{[O-β-D-glucopyranosyl-(1→2)-(β-D-glucopyranosyluronic acid)]oxy}olean-12-en-28-oic Acid;* **6**): white powder, m.p. 255–263° (dec.). ^{13}C-NMR (101 MHz, (D_5)pyridine): Signals of the sugar moieties, see *Table*. FAB-MS (thioglycerol, negative ions): 793 ($[M-H]^-$), 631 ($[(M-H)-162]^-$), 455 ($[(M-H)-338]^-$).

Acid hydrolysis of **6** afforded glucose, glucuronic acid, and oleanolic acid.

3-O-[O-β-D-Glucopyranosyl-(1→2)-(β-D-glucopyranosyluronic acid)]gypsogenin (= *3β-{[O-β-D-glucopyranosyl-(1→2)-(β-D-glucopyranosyluronic acid)]oxy}-23-oxoolean-12-en-28-oic Acid;* **7**): white powder, m.p. 268–275° (dec.). FAB-MS (thioglycerol, negative ions): 807 ($[M-H]^-$), 645 ($[(M-H)-162]^-$), 469 ($[(M-H)-338]^-$).

3-O-[O-β-D-Glucopyranosyluronic acid]oleanolic Acid (= *3β-[(O-β-D-Glucopyranosyluronic acid)oxy]olean-12-en-28-oic Acid;* **8**): white powder, m.p. 255–263° (dec.). ^{13}C-NMR (101 MHz, (D_5)pyridine): Signals for glucuronic acid, see *Table*. FAB-MS (thioglycerol, negative ions): 631 ($[M-H]^-$), 455 ($[(M-H)-176]^-$).

Acid hydrolysis of **8** afforded glucuronic acid, and oleanolic acid.

REFERENCES

[1] A. Marston, K. Hostettmann, *Phytochemistry* **1985,** *24,* 639.

[2] A. Mozley, 'The Control of Bilharzia in Southern Rhodesia', Rhodesian Printing and Publishing Co. Ltd., Salisbury, 1944.

[3] L. Bézanger-Bauquesne, M. Pinkas, *C. R. Séances Acad. Sci.* **1967,** 401.

[4] S.H. Harper, A.D. Kemp, W.G.E. Underwood, *J. Chem. Soc., Chem. Commun.* **1965,** 309.

[5] K. Jewers, J.K. Coker, R.D. Dougan, J.M. Sandberg, *Phytochemistry* **1971,** *10,* 2763.

[6] M. Tanner, A.A. Degrémont, D. De Savigny, T.A. Freyvogel, Ch. Mayombana, S. Tayari, *Acta Trop.* **1987,** in press.

[7] K. Tori, S. Seo, A. Shimaoka, J. Tomika, *Tetrahedron Lett.* **1974,** 4227.
[8] P. A. J. Gorin, M. Mazurek, *Can. J. Chem.* **1975,** *53,* 1212.
[9] R. L. Nie, T. Morita, R. Kasai, J. Zhou, C.-J. Wu, O. Tanaka, *Planta Med.* **1984,** *48,* 322.
[10] T. Konishi, A. Tada, J. Shoji, R. Kasai, O. Tanaka, *Chem. Pharm. Bull.* **1978,** *26,* 668.
[11] J. Gunzinger, J. D. Msonthi, K. Hostettmann, *Phytochemistry* **1986,** *25,* 2501.
[12] P. E. Jansson, L. Kenne, H. Liedgren, B. Lindberg, J. Lönngren, *Chem. Commun.* **1976,** 8.1 (Stockholm Univ.).
[13] L. W. Jaques, J. B. Macaskill, W. Waltner, *J. Phys. Chem.* **1979,** *83,* 1412.
[14] C. D. Schteingart, A. B. Pomilio, *Phytochemistry* **1984,** *23,* 2907.
[15] C. Borel, M. P. Gupta, K. Hostettmann, *Phytochemistry,* in press.
[16] V. Hariharan, *Indian J. Chem.* **1974,** *12,* 447.
[17] K. Hostettmmann, H. Kizu, T. Tomimori, *Planta Med.* **1982,** *44,* 34.
[18] R. Suter, M. Tanner, C. Borel, K. Hostettmann, T. A. Freyvogel, *Acta Trop.* **1986,** *43,* 69.
[19] R. Suter, Ph. D. Thesis, University of Basel, 1986.
[20] P. Godin, *Nature* **1954,** *174,* 134.
[21] D. Schaufelberger, K. Hostettmann, *J. Chromatogr.* **1985,** *346,* 396.