Pergamon

0031-9422(94)00866-3

Phytochemistry, Vol. 39, No. 1, pp. 63-68, 1995

0031-9422/95 $9.50 + 0.00

MOLLUSCICIDAL SAPONINS FROM *CATUNAREGAM NILOTICA*

ELSE LEMMICH,* CLAUS CORNETT, PETER FURU,† CAMILLA L. JØRSTIAN, ANNE D. KNUDSEN, CARL E. OLSEN,‡ ASIA SALIH§ and SUSANNE T. THILBORG

Department of Medicinal Chemistry, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark; †Danish Bilharziasis Laboratory, Jægersborg Allé 1D, DK-2920 Charlottenlund, Denmark; ‡Department of Chemistry, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark; §Bilharzia Research Unit, National Health Laboratory, Khartoum, Sudan

(*Received in revised form* 19 *September* 1994)

Key Word Index—*Catunaregam nilotica*; *Lachnosiphonium nilotica*; *Randia nilotica*; *Xeromphis nilotica*; Rubiaceae; triterpene saponins; oleanolic acid; molluscicidal activity; haemolytic activity.

Abstract—Two new saponins were isolated from the fruits of *Catunaregam nilotica* Stapf, syn. *Lachnosiphonium nilotica*; *Randia nilotica*; *Xeromphis nilotica*. Their structures were determined mainly by spectroscopic methods as 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-[*O*-β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranosyl}oleanolic acid and 28-*O*-β-D-glucopyranosyl-3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-[*O*-β-D-glucopyranosyl]-β-D-glucopyranosyl}oleanolate. The monodesmosidic saponin is a potent molluscicide against the schistosomiasis transmitting snail *Biomphalaria glabrata* with a LC_{50} value of 3 ppm. In addition two known saponins, 3-*O*-[2′,3′-di-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl]oleanolic acid and 3-*O*-[*O*-β-D-glucopyranosyl(1 → 3)-β-D-glucopyranosyl]oleanolic acid, were identified and their molluscicidal activity determined, the LC_{50} values being 26 and 3 ppm, respectively. Initial molluscicidal screening of the crude water and ethanol extracts revealed 100% snail mortality at concentrations of 100 and 50 ppm, respectively. The haemolytic activity of the molluscicidal saponins was determined as well and the HC_{50} values towards bovine erythrocytes found to be 3 ppm for the new saponin, and 16 and 2 ppm, respectively, for the two known saponins.

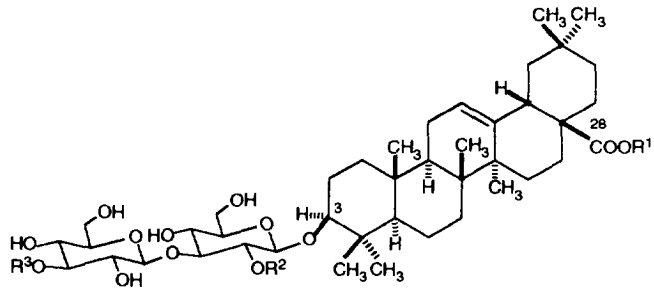
INTRODUCTION

Schistosomiasis is a major health problem in many tropical countries, especially in Africa. The infection is transmitted by freshwater snails acting as intermediate hosts. Despite the success of some control programmes, the prevalence of schistosomiasis remains constant, largely because population growth and development of man-made water resources is continuing [1]. Treatment of water bodies with molluscicidal compounds is considered an important element in an integrated strategy for morbidity control, but as the use of synthetic molluscicides is impeded by the high costs, there is a demand for inexpensive alternatives [2].

The interest in studying plant material containing molluscicidal compounds is based on the idea of a local supply of molluscicides, which can be produced at low costs by simple technologies. The plant *Phytolacca dodecandra* is presently a very promising candidate for a plant molluscicide and the development of a safe and affordable molluscicide from the berries of this plant is in progress [3–6]. Nevertheless, alternative molluscicides with different qualities are interesting and screening of plant material for molluscicidal activity is still justifiable.

In a screening performed on a number of indigenous plants, commonly used in folk medicine in the Sudan, several plants showed molluscicidal activity, among these *Catunaregam nilotica*, a lowland shrub or tree, widespread in the Sudan and reported from lowland habitats in Central and East Africa as well as Cameroun and Nigeria [7]. The fruits from *C. nilotica* were among the most potent material and a content of saponins in the extracts were indicated by the ability to produce foam and by haemolysing erythrocytes [8]. In this paper we report the isolation of two new saponins **1** and **3**, a bidesmosidic and a monodesmosidic saponin, respectively, of which **3** is a very potent molluscicide with an LC_{50} value of 3 ppm against the schistosomiasis transmitting snail *Biomphalaria glabrata*. Furthermore, two previously described saponins **2** and **4** were isolated and shown to be molluscicidal with LC_{50} of 26 and 3 ppm, respectively. The haemolytic activity was determined for the saponins, which showed molluscicidal activity, in order to investigate whether these two biological activities occurred in the same concentration range as previously seen [6, 9]. The concentration causing 50% haemolysis of bovine erythrocytes, HC_{50}, for **2**–**4** were found to be 16, 3 and 2 ppm, respectively.

*Author to whom correspondence should be addressed.



1:	R^1=β-D-Glu*p*	R^2=H	R^3=α-L-Rha*p*
2:	R^1=H	R^2=β-D-Glu*p*	R^3=H
3:	R^1=H	R^2=H	R^3=α-L-Rha*p*
4:	R^1=H	R^2=H	R^3=H

RESULTS AND DISCUSSION

Column chromatography of the ethanolic extract of the fruits afforded three fractions, which showed molluscicidal activity against *Biomphalaria glabrata*. Compounds **1** and **2** were isolated from the most polar fraction, **3** from the fraction of medium polarity and **4** was isolated from the least polar fraction. Purification of **2**–**4** was performed by repeated chromatography on silica gel and on RP8 material, whereas **1**, despite several attempts, was not obtained in a completely pure state.

A TLC investigation of the reaction mixture obtained by hydrolysis of each of the compounds, using H_2SO_4 as a catalyst, indicated the presence of oleanolic acid and glucose in all of them, and in the reaction mixture from **1** and **3** rhamnose was detected in addition. The structure elucidation of **1** and **3** and the identification of **2** and **4** were performed by a combination of different spectroscopic methods [10]. The M_r of the compounds was obtained from the *m/z* value of the quasi-molecular ion $[M - H]^-$ present in the FAB-mass spectra (negative mode). In addition, fragments formed on cleavage of the glycosidic bonds indicated the size of the aglycone and the size and number of sugars. In cases with sugar residues of different size the fragments in addition indicated the sequence of the sugars. Peracetylation of compounds **2**–**4** afforded 5–7, respectively. The FAB-mass spectrum (negative mode) of the derivative **5** showed a $[M - H]^-$ ion at *m/z* 1361, proving that 10 hydroxy groups in **2** had been acetylated. The FAB-mass spectrum of **6** showed a quasi-molecular ion at *m/z* 1303, corresponding to a nonaacetyl derivative of **3** and the FAB-mass spectrum of **7** showed a quasi-molecular ion at *m/z* 1073, corresponding to a heptaacetyl derivative of **4**.

The ^{13}C NMR data of the aglycone moiety of the saponins **1**–**4** and of the acetates **5**–**7** confirmed the presence of oleanolic acid with a glycosidic chain at C-3 [11, 12]. The signal arising from the carboxylic carbon in the saponins **2**–**4** and in the acetates **5**–**7** appeared at δ 180.2, indicating a free carboxylic acid group, whereas in **1** C-28 gave rise to a signal at δ 177.0, corresponding to that of a bidesmosidic saponin with an ester bonded sugar in this position [13].

In the ^{13}C NMR spectrum of saponin **3**, 18 signals appeared from the sugar part of the molecule (Table 1). The anomeric carbons gave rise to signals at δ 102.8, 105.6, and 106.4. A signal at δ 18.7, corresponding to a methyl group, was in accordance with the presence of a rhamnose residue, which was identified after acid hydrolysis, and a signal at δ 88.4 indicated a C-3 substituted glucose residue [10]. The rest of the signals appeared in the range δ 62–83. The FAB-mass spectrum of **3** showed an intense peak *m/z* 779 $[M - H - 146]^-$, indicating a terminal position of a deoxyhexose.

In the ^{1}H NMR spectrum of **3** the signals from the three anomeric protons were found at δ 4.89, 5.25, and 6.25 (Table 2). Analogously, signals from the anomeric protons in **6** were observed at δ 4.77, 5.03, and 5.26. TOCSY spectra of **3** and **6** enabled us to group the proton signals as arising from three separated spin systems, each corresponding to one monosaccharide residue. A combined interpretation of the COSY, NOESY and HETCOR spectra allowed the signals from the sugar moiety to be assigned to the carbons and protons of the two glucose residues and of the terminal rhamnose. From the *J*-resolved ^{1}H NMR spectra of **3** and **6** most coupling constants could be read. The coupling constants of the anomeric protons in the two glucose residues indicated a β-configuration, and the coupling constant of the anomeric proton in rhamnose indicated that the relative configuration was α. The interglycosidic linkages were established from the NOESY spectra and were in accordance with the ^{1}H and ^{13}C NMR spectra of saponin **3** and the acetyl derivative **6**. The structure was concluded to be **3**, a compound which has not previously been isolated. The absolute configuration of the monosaccharides has not been determined for any of the saponins, therefore the configurations most frequently found in nature have been assumed.

Table 1. ^{13}C NMR spectral data of sugar moieties of saponins **1–4**, and the peracetylated saponins **5–7** from *Catunaregam nilotica*

C	1*	2	3	4	5	6	7
1′	106.4	105.1	106.4	106.4	103.9	103.3	103.3
2′	74.4	79.4	74.4	74.4	78.5	73.9	73.8
3′	88.4	88.8	88.4	88.9	80.8	79.6	79.8
4′	69.9	70.1	69.9[a]	69.9	69.4	69.1[a]	69.1
5′	78.0	77.9	78.0	78.0[a]	72.1	72.2	72.1
6′	62.2[a]	63.4	62.6[b]	62.6[b]	63.0	62.7[b]	62.7
1″	105.6	103.9	105.6	106.0	100.2	101.6	101.5
2″	76.0	75.5	76.0	75.4	72.9	72.2	72.0
3″	83.4	78.7	83.3	78.7	73.8	82.1	73.8
4″	69.9	72.7[a]	69.6[a]	71.6	68.9[a]	70.1[a]	68.8
5″	78.6	77.9	78.6	78.3[a]	72.6	72.2	72.0
6″	62.2[a]	62.4[b]	62.2[b]	62.5[b]	62.4[b]	62.5[b]	62.3
1‴	102.8	104.8	102.8		100.5	99.9	
2‴	72.6[b]	76.5	72.6[c]		72.9	70.3	
3‴	72.7[b]	78.7	72.7[c]		73.8	71.0	
4‴	74.4	71.6[a]	74.4		69.2[a]	69.6	
5‴	69.9	78.7	69.9		72.6	67.9	
6‴	18.7	62.7[b]	18.7		62.5[b]	18.5	
1⁗	95.8						
2⁗	74.4						
3⁗	78.9						
4⁗	71.1						
5⁗	78.9						
6⁗	62.6[a]						

*Assignment by comparison with data of **3** and literature [12].
[a–c]The assignment of the signals may be interchanged in each vertical column.

The ^{13}C NMR spectrum of **1** contained 24 signals from the sugar moiety of the molecule (Table 1), confirming that the molecule contains four hexoses. The signals from the anomeric carbons appeared at δ95.8, 102.8, 105.6, and 106.4, and the first of these had a chemical shift value as expected for an anomeric carbon in an ester bonded sugar [13]. The rest of the anomeric carbons have chemical shifts identical to those of saponin **3**. Comparison of the ^{13}C and ^{1}H NMR data (Table 2) of **1** and **3** reveals that the data corresponding to the presence in **1** of an additional ester bonded glucose residue is the only difference. On TLC examination of the reaction mixture from a basic hydrolysis of **1** [13] a compound cochromatographing with **3** appeared, and thus it was concluded that the compound was the bidesmosidic saponin **1**.

The ^{13}C NMR spectrum of saponin **2** contained, besides the signals arising from the oleanolic acid, 18 signals from the sugar part confirming the presence of three hexoses in the molecule (Table 1). The signals from the three anomeric carbons were found at δ103.9, 104.8, and 105.1 and the remaining signals from the sugar moiety appeared in the range δ62–80 except for one signal at δ88.8, which indicated a substitution at C-3 of one of the glucoses [10].

In the ^{1}H NMR spectrum of saponin **2** the signals from the three anomeric protons were found at δ4.84, 5.37, and 5.72 (Table 2). Analogously, signals from the anomeric protons in **5** were observed at δ4.81, 5.37, and 5.55. TOCSY spectra of **2** and **5** indicated the three separated spin systems, from which the proton signals arise and which correspond to three glucose residues. A combination of the information from COSY, NOESY and HETCOR spectra of **2** and **5** allowed most of the signals from the sugar moiety to be assigned to the carbons and protons in the three glucoses, but a severe overlap of the signals from **2** impeded the assignment of all carbon signals. From the *J*-resolved spectra of **2** and **5** almost all coupling constants could be read. The coupling constants for the anomeric protons indicated β-configuration of all three sugars and the coupling constants for the remaining protons were as expected for glucose units. The interglycosidic linkages were established from the NOESY spectra and were in accordance with the ^{1}H and ^{13}C NMR data of saponin **2** and the acetyl derivative **5**, and the structure was thus concluded to be **2**. A saponin, anchusosid 2, with this structure has previously been isolated from *Anchusa officinalis* [14]. The structure elucidation was performed by chemical methods, and in addition the chemical shift and the coupling constant of one of the anomeric protons was published, but a closer verification of the identity of **2** with anchusosid 2 is not possible. The optical rotations of the two compounds are slightly different.

In the ^{13}C NMR spectrum of **4** the sugar moiety of the molecule gave rise to 12 signals (Table 1), of which signals at δ106.0 and 106.4 corresponding to the anomeric

Table 2. ^{1}H NMR spectral data of sugar moieties of the saponins **1–4** and the peracetylated saponins **5–7** from *Catunaregam nilotica*

H	1*	2	3	4	5	6	7
1′	4.88 *d*	4.84 *d*	4.89 *d*	4.91 *d*	4.81 *d*	4.77 *d*	4.80 *d*
2′	4.04 *dd*	4.39 *dd*	4.01 *dd*	4.08 *dd*	4.25 *dd*	5.45 *dd*	5.44 *dd*
3′	4.19 *t*	4.26 *t*[a]	4.20 *t*	4.23 *t*	4.56 *t*	4.41 *t*	4.46 *t*
4′	4.40 *t*	4.04 *dd*	4.08 *t*[a]	4.14 *t*	5.40 *t*	5.40 *dd*	5.37 *t*
5′	3.92 *ddd*	3.83 *ddd*	3.93 *ddd*	3.96 *ddd*	4.06 *ddd*	4.10[a]†‡	4.13 *ddd*
6a′	4.31 *dd*	4.33 *dd*	4.51 *dd*[b]	4.35 *dd*	4.61 *dd*	4.62 *dd*	4.62 *dd*
6b′	4.50 *dd*	4.48 *dd*	4.31 *dd*	4.55 *dd*	4.40 *dd*	4.35[b]†‡	4.44 *dd*
1″	5.25 *d*	5.72 *d*	5.25 *d*	5.32 *d*	5.55 *d*	5.03 *d*	5.18 *d*
2″	4.07 *dd*	4.09 *dd*	4.05 *dd*	4.10 *t*	5.60 *dd*[a]	5.43 *dd*	5.36 *dd*
3″	4.34 *t*	4.28 *t*[a]	4.41 *t*	4.25 *t*	5.79 *t*	4.35 *t*	5.72 *t*
4″	4.40 *t*	4.17 *dd*[b]	4.20*t*[a]†‡	4.27 *t*	5.55[b]†‡	5.37 *dd*	5.46 *dd*
5″	3.96 *ddd*	3.89 *ddd*	3.96 *ddd*	4.05 *ddd*	4.25 †‡	4.03 *ddd*[a]	4.18 *ddd*
6a″	4.31 *dd*	4.45†‡	4.47 *dd*[b]	4.35 *dd*	4.64 *dd*	4.67 *dd*	4.70 *dd*
6b″	4.48 *dd*	4.30†‡	4.31†‡	4.57 *dd*	4.46 *dd*	4.44[b]†‡	4.34 *dd*
1‴	6.25 *d*	5.37 *d*	6.25 *d*		5.37 *d*	5.26 *d*	
2‴	4.76 *dd*	4.20†‡	4.75†‡		5.55[a]†‡	5.60 *dd*	
3‴	4.57 *dd*	4.22 *t*	4.58 *dd*		5.69 *t*	5.50†‡	
4‴	4.37 *t*	4.16 *t*[b]	4.34 *t*		5.61 *dd*[b]	5.53†‡	
5‴	5.03 *dq*	4.05†‡	5.04 *dq*		4.14 *ddd*	4.13 *dq*	
6a‴	1.70 *d*	4.54 *dd*	1.70 *d*		4.76 *dd*	1.35 *d*	
6b‴		4.30†‡			4.30†‡		
1⁗	6.34 *d*						
2⁗	4.22 *dd*						
3⁗	4.34 *t*						
4⁗	4.04 *dd*						
5⁗	†						
6a⁗	†						
6b⁗	†						
J							
1′,2′	8.0	7.7	7.8	7.8	7.6	8.0	8.1
2′,3′	9.4	9.0	9.5	9.3	9.4	9.6	9.7
3′,4′	9.1	9.2	9.1	9.8	9.3	9.2	9.6
4′,5′	10.1	9.5	9.5	8.8	9.6	10.3	10.1
5′,6a′	5.3	5.7	5.5	5.7	5.5	5.0	5.0
5′,6b′	2.4	3.1	2.7	2.7	2.9	†	2.9
6a′,6b′	12.0	11.6	12.3	12.3	12.3	12.5	12.5
1″,2″	8.1	8.9	7.9	7.9	7.7	8.2	8.1
2″,3″	9.5	9.3	9.6	8.0	9.6	9.5	9.6
3″,4″	9.5	9.0	9.1	9.6	9.5	9.1	9.3
4″,5″	10.1	9.9	10.1	8.8	†	10.2	10.2
5″,6a″	5.7	5.6	5.2	5.7	5.5	4.6	4.4
5″,6b″	2.9	3.5	2.8	2.7	2.9	2.3	2.8
6a″,6b″	12.4	†	12.0	12.0	12.4	12.6	12.7
1‴,2‴	2.2	7.8	2.0		7.8	2.7	
2‴,3‴	3.7	†	3.6		†	3.4	
3‴,4‴	9.4	8.9	9.3		9.3	†	
4‴,5‴	9.5	9.1	9.3		10.1	10.4	
5‴,6a‴	6.4	†	6.3		4.7	6.0	
5‴,6b‴		2.7			2.5		
6a‴,6b‴		12.0			12.2		
1⁗,2⁗	8.1						
2⁗,3⁗	9.4						
3⁗,4⁗	9.6						
4⁗,5⁗	†						
5⁗,6a⁗	†						
5⁗,6b⁗	†						
6a⁗,6b⁗	†						

*Assignment by comparison with **3** and the literature [12].

†Signal and/or multiplicity not found owing to severe overlapping.

‡Tentative assignment owing to severe overlapping.

[a,b]The assignment of the signals may be interchanged in each vertical column.

carbons confirmed the presence of two hexoses, and a signal at δ88.9, collapsing with the signal from C-3 in oleanolic acid, indicated that the terminal glucose was attached to C-3 of the internal glucose. From the ^{1}H NMR spectrum (Table 2) of saponin **4** and of the acetyl derivative **7** combined with the TOCSY, COSY, NOESY and *J*-resolved spectra the structure **4** could be established. Combination of these spectra and the HETCOR spectrum of **7** allowed the assignment of all hydrogens and carbons in **4** and **7**. Furthermore, from the ^{1}H *J*-resolved spectra all proton coupling constants could be read. A saponin, randianin, has previously been isolated from *Randia dumetorum* [15], and the ^{13}C NMR data of the methyl ester of randianin are in accordance with those of **4**, except that the chemical shifts for C-19 and C-21 in oleanolic acid ought to be interchanged [10, 11]. The ^{1}H NMR chemical shifts reported for the acetate of randianin are generally 0.5 ppm lower than those of **7** (Table 2) owing to the solvents used, as randianin peracetate was dissolved in chloroform-*d*, whereas **7** was dissolved in pyridine-d_5. The optical rotations of the two compounds show a difference probably owing to the difficulty of purifying saponins.

The crude water and ethanol extracts of the fruit material were initially tested for molluscicidal activity, giving 100% mortality at 100 and 50 ppm, respectively. The saponins **2**–**4** were subsequently tested for molluscicidal activity. Saponin **1** was not tested, because bidesmosidic saponins usually have no or a very low molluscicidal activity [16] and the results would be unreliable owing to the minor impurities of other saponins with unknown activity. The LC_{50} value of saponin **2** was 26 ppm, which, owing to shortage of the chemical compound, was determined with five snails for each concentration. For saponin **3** and **4** the LC_{50} value of both were 3 ppm, determined with 20 and 10 snails, respectively, for each concentration. The concentration causing 50% haemolysis of bovine erythrocytes, HC_{50}, was determined for the saponins **2**–**4** and found to be 16, 3 and 2 ppm, respectively.

The fruits of *C. nilotica* thus contain some very potent molluscicidal saponins, which are closely related to the saponins from *P. dodecandra*, in moderate amounts. On examination of the saponin content in the different parts of the fruit, saponins were found only in the mesocarp, which in the dried fruit is a thin layer covering the seed and making up a minor part, in this case around 2%, of the whole fruit. The content of molluscicidal saponins thus explains only part of the activity, to which the reported content of tannins [8] most likely also contributes.

EXPERIMENTAL

The plant material was provided and identified by the Medicinal and Aromatic Plants Research Institute, National Council for Research, Khartoum, the Sudan in 1990, in collaboration with the Department of Botany, University of Khartoum, where the herbarium specimens are deposited.

The NMR spectra were measured in pyridine-d_5 and were recorded at 400 MHz (Bruker) for the ^{1}H NMR, COSY, TOCSY, NOESY and *J*-resolved ^{1}H NMR experiments and 100.6 MHz for the ^{13}C NMR. Chemical shifts are given in δ values relative to TMS. FAB-MS were obtained in negative mode on a Jeol JMS-AX505W instrument. Hydroxyethyldisulphide was used as a matrix. Analytical HPLC was performed isocratically on a Spherisorb S5 C8 column (4.6 × 250 mm) with MeCN–0.05 M $NH_4H_2PO_4$, pH 4.4 (21:29) or (7:13) as eluent, flow rate 1.2 ml min^{-1} and peak monitoring at 203 nm. LPLC was performed on LiChroprep RP8, 40–63 μm, with eluents consisting of MeOH–0.05 M NH_4OAc, pH 8.0, (3:2) → (4:1) or (3:1) → (17:3). Before mass and NMR spectra and biological testing were performed the remaining salt was removed by absorption/desorption using small prepacked RP18 columns (1 g). TLC examinations during fractionation and purification were performed on silica gel with $CHCl_3$–MeOH–H_2O (35:14:1) or (29:19:2) as eluent, or on RP8 with MeOH–H_2O (18:7), and detection with naphthoresorcinol–H_2SO_4.

Heparinized bovine blood was obtained from the National Veterinary Laboratory, Copenhagen. The erythrocytes were separated by centrifugation, washed with saline soln three times and diluted with buffer to a suspension containing 2.0×10^8 cells ml^{-1}. Isotonic buffer solution: $Na_2HPO_4 \cdot 2H_2O$ 3.95 g; KH_2PO_4 0.76 g; NaCl 7.2 g, H_2O to 1000 ml.

Isolation of saponins. Fruits from *C. nilotica* (105 g) were crushed and extracted with EtOH. Evapn of the EtOH left 15.5 g. CC of this extract on silica gel using mixtures of $CHCl_3$–MeOH–H_2O (40:10:1 → 5:5:1) as eluents provided 3 fractions. The most polar fraction contained saponins **1** and **2**, the medium polar contained saponin **3**, and the least polar contained saponin **4**. Repeated chromatography on silica gel and on RP8 afforded saponins **2** (190 mg), **3** (166 mg), and **4** (210 mg) in a pure state, while saponin **1** was not obtained in a pure state.

28-O-β-D-*Glucopyranosyl*-3-O{O-α-L-*rhamnopyranosyl*-(1 → 3)-O-[O-β-D-*glucopyranosyl*-(1 → 3)]-β-D-*glucopyranosyl*}*oleanolate* (**1**). Obtained as an amorphous powder, not quite pure. FAB-MS *m/z*: 1087 [M − H]$^-$, 925 [M − H − 162]$^-$, 779 [M − H − 308]$^-$, 617 [M − H − 470]$^-$, and 455 [M − H − 632]$^-$. ^{13}C and ^{1}H NMR data of the sugar moieties: see Tables 1 and 2.

3-O-[2′,3′-*di*-O-(β-D-*glucopyranosyl*)-β-D-*glucopyranosyl*]*oleanolic acid* (**2**). Obtained as an amorphous powder. $[\alpha]_D^{25}$ + 6.8° (MeOH; *c* 0.10) (lit. [12] $[\alpha]_D^{25}$ + 28.0° (MeOH; *c* 0.64)). Data obtained from FAB-MS and ^{13}C NMR were in agreement with previously published data for oleanolic acid glycosides [9, 12]. ^{13}C and ^{1}H NMR data of the sugar moietiy: see Tables 1 and 2.

3-O-{O-α-L-*rhamnopyranosyl*-(1 → 3)-O-[O-β-D-*glucopyranosyl*-(1 → 3)]-β-D-*glucopyranosyl*}*oleanolic acid* (**3**). Obtained as an amorphous powder. $[\alpha]_D^{25}$ = + 52.5° (MeOH; *c* 0.12). FAB-MS *m/z*: 941 [M − H]$^-$, 795 [M − H − 146]$^-$, 633 [M − H − 308]$^-$, 471 [M − H − 470]$^-$. ^{13}C and ^{1}H NMR data of the sugar moietiy: see Tables 1 and 2.

3-O-[O-β-D-*glucopyranosyl*-(1 → 3)-β-D-*glucopyranosyl*}*oleanolic acid* (**4**). Obtained as an amorphous powder. $[\alpha]_D^{25} = +19.6°$ (MeOH; *c* 0.074). FAB-MS *m/z*: 779 $[M-H]^-$, 617 $[M-H-162]^-$, and 455 $[M-H-324]^-$. ^{13}C and ^1H NMR data of the sugar moiety: see Tables 1 and 2. Literature data [15] for randianin in accordance with the ^{13}C NMR data, but the ^1H NMR chemical shifts of the peracetate were generally 0.5 ppm lower, because the NMR spectra were registered in $CDCl_3$, while we, as a standard procedure, dissolved both the saponins and the corresponding peracetates in pyridine-d_5.

Peracetylation of saponins **2**–**4**. Compounds **2**–**4** (28, 25 and 27 mg, respectively) were dissolved in pyridine (1 ml) and acetylated with Ac_2O (0.5 ml) at room temp. for 16 hr. The reaction products were evapd to dryness and the residues chromatographed on silica gel using toluene–EtOAc–MeOH–HOAc (78:20:1:1) → (73:25:1:1) as eluents to give **5**–**7** (22, 21 and 27 mg, respectively) as amorphous powders. FAB-MS of compound **5** *m/z*: 1361 $[M-H]^-$ (decaacetate). FAB-MS of **6** *m/z*: 1302 $[M-H]^-$ (nonaacetate). FAB-MS of **7** *m/z*: 1072 $[M-H]^-$ (heptaacetate). ^{13}C and ^1H NMR data of the sugar moieties: see Tables 1 and 2.

Acid hydrolysis of **1**–**4**. Compounds **1**–**4** (3 mg of each) were hydrolysed with H_2SO_4 0.5 M (500 μl) at 100° for 24 hr. The ppt. was extracted with EtOAc and identified as oleanolic acid by comparison with an authentic sample on TLC (silica gel, toluene–EtOAc, 1:1, visualization with vanillin–H_2SO_4). The aq. layer on TLC (silica gel, CH_2Cl_2–MeOH–H_2O, 8:7:1, visualization with naphthoresorcinol–H_2SO_4) showed the presence of glucose in the reaction mixture from all the compounds and in addition rhamnose in the reaction mixture from **1** and **3**.

Basic hydrolysis of compound **1**. Compound **1** (2 mg) in MeOH was heated in 2 ml 0.5 M KOH for 2 hr. The mixture was adjusted to pH 6 with Dowex 50wx8 (acid form) and submitted to a TLC examination (silica gel, $CHCl_3$–MeOH–H_2O, 35:14:1, visualization with naphthoresorcinol), which showed that saponin **1** had disappeared and that saponin **3** had formed.

Determination of molluscicidal activity. Initially the molluscicidal activity of the crude water and ethanol extracts of the whole fruits of *C. nilotica* was assessed against groups of 20 *Biomphalaria glabrata* snails according to the WHO provisional technique [17], modified according to Duncan and Sturrock [18]. Using the same test the molluscicidal activity of the monodesmosidic saponins **2**–**4** was assessed, but with varying snail numbers (between 5 and 20) owing to shortage of saponin. Stock solns of the saponins were prepared using DMSO as solvent. The resulting concentration of DMSO was 0.5% v/v in all tests. Controls with the same DMSO concentration were included.

Determination of haemolytic activity. All saponins were dissolved in EtOH and diluted with buffer to the desired concns. The concn of EtOH was adjusted to 10% at each saponin concn. Saponin solns (2.50 ml) were incubated with buffer (1.50 ml) at 37° for 5 min. An erythrocyte suspension (1.00 ml) was added and the mixtures were incubated for another 30 min at 37° and then centrifuged at 1500 *g* for 5 min. The supernatants were sepd and their optical density determined at 540 nm. Percentage of haemolysis was determined by comparison with a sample for which 100% haemolysis was obtained.

Acknowledgements—The Alfred Benzon Foundation, The Danish Technology Council and The Danish Research Council are acknowledged for the provision of the Bruker AMX 400 spectrometer.

REFERENCES

1. WHO (1993) *Wld Hlth Org. Tech. Rep. Ser.* 830.
2. Mott, K. E. (ed.) (1987) *Plant Molluscicides.* Wiley, Chichester.
3. Lambert, J. D. H., Temmink, J. H. M., Marquis, J., Parkhurst, R. M., Lugt, C. B., Lemmich, E., Wolde-Yohannes, L. and DeSavigny, D. (1991) *Regul. Toxicol. Pharmacol.* **14**, 189.
4. Parkhurst, R. M., Mthupha, B. M., Liang, Y.-S., Bruce, J. I., Lambert, J. D. H., Collier, T. L., ApSimon, J. W., Wolde-Yohannes, L., Heath, G. E., Jones, W. O., Stobaeus, J. K. and Makhubu, L. P. (1989) *Biochem. Biophys. Res. Commun.* **158**, 436.
5. Ndamba, J. (1993) Ph.D. Thesis. Royal Danish School of Pharmacy, Danish Bilharziasis Laboratory and Blair Research Laboratory.
6. Thiilborg, S. T. (1993) Ph.D. Thesis. Royal Danish School of Pharmacy and Danish Bilharziasis Laboratory.
7. *Flora of Tropical East Africa* (1988). Royal Botanical Gardens Kew and East African Herbarium.
8. Ahmed, E. H. M., Bashir, A. K. and El Kheir, Y. M. (1984) *Planta Med.* **50**, 74.
9. Thiilborg, S. T., Brøgger Christensen, S., Cornett, C., Olsen, C. E. and Lemmich, E. (1994) *Phytochemistry* **36**, 753.
10. Thiilborg, S. T., Brøgger Christensen, S., Cornett, C., Olsen, C. E. and Lemmich, E. (1993) *Phytochemistry* **32**, 1167.
11. Tori, K., Seo, S., Shimaoka, A. and Tomita, Y. (1974) *Tetrahedron Letters* 4227.
12. Tori, K., Seo, S., Yoshimura, Y., Arita, H. and Tomita, Y. (1977) *Tetrahedron Letters* **2**, 179.
13. Domon, B. and Hostettmann, K. (1984) *Helv. Chem. Acta* **67**, 1310.
14. Romussi, G., Ciarello, G., Falsone, G. and Schneider, C. (1979) *Liebigs Ann. Chem.* 2038.
15. Sotheeswaran, S., Bokel, M. and Kraus, W. (1989) *Phytochemistry* **28**, 1544.
16. Hostettmann, K., Kizu, H. and Tomimori, T. (1982) *Planta Med.* **44**, 34.
17. WHO (1961) *Wld Hlth Org. Techn. Rep. Ser.* **214**, 30.
18. Duncan, J. and Sturrock, R. F. (1987) in *Plant Molluscicides* (Mott, K. E., ed.), p. 257. John Wiley, New York.