PERGAMON

Phytochemistry 59 (2002) 79–84

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum*

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Received 29 May 2001; received in revised form 23 August 2001

Abstract

Solanum aculeastrum Dunal was investigated for the presence of molluscicidal compounds. This led to the isolation of solaculine A, from the root bark in addition to known steroidal alkaloids; solamargine and β-solamarine from the berries. The structures were elucidated by spectroscopic techniques. Molluscicidal activity of the aqueous extracts of the berries and root bark, and the isolated compounds were investigated.

Keywords: *Solanum aculeastrum*; Solanaceae; Berries; Root bark; Steroidal alkaloid glycosides; Solamargine; Solamarine; Solaculine A; Molluscicidal activity

1. Introduction

Over 250 million people in tropical countries are infected by schistosomiasis and many more are at risk of getting infected (Marston and Hostettmann, 1985). Evidence indicates there is increasing transmission of the disease due to increased water exploitation (Rollinson and Johnston, 1996; Young et al., 1996).

The most efficient method of preventing the spread of the disease despite many limiting factors, is destruction of the host snails by use of synthetic molluscicides (Brackenbury, 1997). This concern has prompted a worldwide drive to develop alternative methods for controlling the disease. One of these alternatives is to search for the plant molluscicides (Hostettmann, 1980). The discovery of active saponins in the berries of *Phytolacca dodecandra* L'Hérit (endod), led to the evaluation of various plant species for molluscicides (Parkhust, 1975). In the light of the variety of biological activities reported in the *Solanum* species (Hostettmann et al., 1982; Marston and Hostettmann, 1985) and the observation that the aq. extract of the berries of *S. aculeastrum* (Solanaceae) was potent to snails (Mkoji et al., 1989) led to the choice of this plant for molluscicidal and phytochemical investigation. It is a thorny perennial plant widely distributed in Kenya and grows up to 2–3 m high with white flowers and lemon shaped berries that become yellow-green when ripe. It is not invaded by harmful insects and locally used as hedges. The fresh and boiled froth from the ripe berries are used as a cure for jigger wounds and gonorrhoea respectively (Agnew and Agnew, 1994). Solaculine A (**1**) from methanol extracts of the root bark is reported here for the first time. β-Solamarine (**2**) and solamargine (**3**) isolated from the berries showed high molluscicidal activity.

2. Results and discussion

Purification of the crude methanol extracts of the root bark extract of *S. aculeastrum* was achieved by repeated droplet counter current chromatography and column chromatography. This led to isolation of solaculine (**1**). The berries extracts were similarly purified and β-solamarine (**2**) and solarmargine (**3**) obtained.

Compound **1** was identified as a tomatidenol tetraose by comparison of its ^{1}H NMR and ^{13}C NMR data with those of **2** and **3**. The structures of **2** and **3** were established by their spectroscopic and other physicochemical data (Mahato et al., 1980; Lin et al., 1987) in addition to

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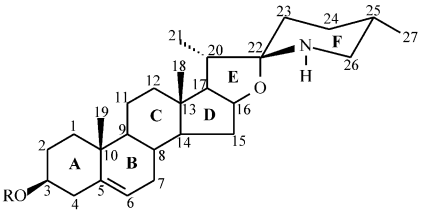
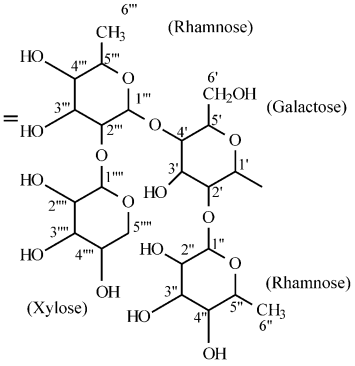
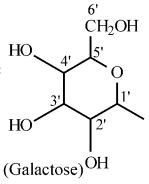
0031-9422/02/$ - see front matter
PII: S0031-9422(01)00424-1



1 R = 

1a R = 

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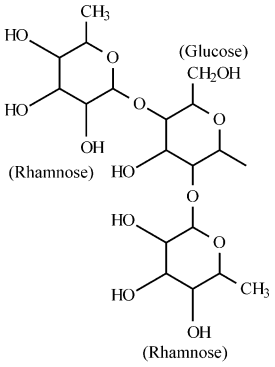
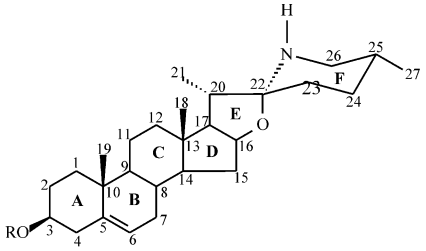
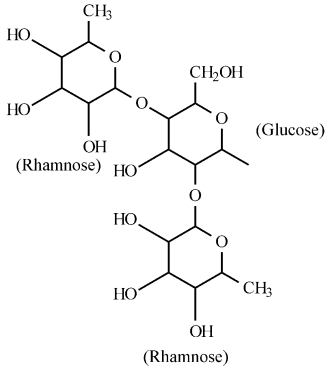
2 R = 



3 R = 

comparison with authentic samples obtained from *S. dulcamara* (Ehmke and Eilert, 1993). FABMS showed $[M+H]^+$ peak for **1** at *m/z* 1000. Other peaks observed were at *m/z*: 868, 722 and were due to loss of one mole each of pentose and deoxyhexose in terminal positions (Ripperger and Porzel, 1997). A peak at *m/z* 414 due to loss of a deoxyhexose and hexose from *m/z* 722. This fragmentation pattern was characteristic of tetraglycoside steroidal alkaloids that have been isolated from *Solanum* species (Ripperger and Schreiber, 1981; Harbone and Dey, 1993).

The ^{1}H NMR indicated four characteristic steroidal aglycone methyls at δ 0.93 (3H, *s*), 1.05 (3H, *s*), 1.10 (3H *d*, $J=6.4$ Hz) and 0.97 (3H, *d* $J=6.0$ Hz). Two doublets ($J=6.6$ Hz) at δ 1.27 was due to 6-deoxyhexose methyls while a doublet ($J=4.7$ Hz) at δ 5.39 was due to an olefinic proton at C-6. Four anomeric proton signals were observed at δ 4.47 (*d*, $J=7.7$ Hz), 5.22 (*s*), 5.09 (*s*) and 4.39 (*d*, $J=7.1$ Hz). ^{13}C NMR spectrum showed 50 signals with six methyls (Table 1). The DEPT experiment of **1** indicated extra anomeric and methylene carbon signals at δ 106.6 and 66.4 which were assigned to C-1 and C-5 respectively of a pentose sugar (Bock and Thogersen, 1982; Agrawal et al., 1985). However, carbon signal of C-23 was shifted upfield relative to that of **3** suggesting difference in stereochemistry in the vicinity of C-22 and C-25. The observed shift is attributed to the strong γ interaction of C-23 and C-21. Hence the presence of the

Table 1
^{13}C NMR spectral data for compounds **1** to **1b** and ^{1}H NMR for **1**

	Carbon	1	1a	1b	DEPT	^{1}HNMR (1)	
						α	β
	1	37.7	37.9	36.9	CH_2	1.41	1.33
	2	30.2	30.2	31.6	CH_2	1.95	1.63
	3	79.4	79.5	71.7	CH	3.68	2.30
	4	38.9	39.2	42.3	CH_2	2.45	
	5	141.0	141.1	141.0	C	–	
	6	122.0	122.2	121.0	CH	5.37	
	7	32.8	33.0	32.1	CH_2	1.85–2.0[a]	
	8	32.0	32.0	31.4	CH	1.55–1.65[a]	
	9	50.8	50.8	50.1	CH	1.00	
	10	37.5	37.4	37.3	C	–	
	11	21.4	21.4	20.9	CH_2	1.55–1.78[a]	
	12	40.4	40.4	40.0	CH_2	1.55–1.78[a]	
	13	41.3	41.3	40.6	C	–	
	14	56.6	56.6	56.0	CH	1.15	
	15	32.6	32.7	32.8	CH_2	1.35–2.21[a]	
	16	78.9	79.5	78.5	CH	4.24	
	17	62.6	62.7	61.9	CH	1.79	
	18	17.0	17.1	16.8	CH_3	0.93	
	19	19.6	19.7	19.4	CH_3	1.05	
	20	42.6	43.3	43.0	CH	2.80	
	21	15.6	15.9	15.9	CH_3	1.10	
	22	99.4	99.4	99.1	C	–	
	23	26.6	27.1	26.7	CH_2	1.48–1.90[a]	
	24	28.0	28.8	28.6	CH_2	1.34–1.48[a]	
	25	30.1	30.1	31.1	CH_2	1.60	
	26	49.9	50.1	50.2	CH_2	2.40	2.70
	27	19.2	19.6	19.4	CH_3	0.97	
Gal	1′	99.9	101.8			4.47	
	2′	78.4	70.5			4.20	
	3′	77.3	77.2			4.14	
	4′	80.4	76.8			3.84	
	5′	77.1	74.2			3.70	
	6′	61.5	62.2			3.92	
Rha	1″	101.5				5.22	
	2″	71.8				4.20	
	3″	70.3				3.94	
	4″	73.4				3.67	
	5″	69.9				4.20	
	6″	17.7				1.27	
Rha	1‴	101.2				5.09	
	2‴	81.6				3.86	
	3‴	71.8				3.66	
	4‴	73.4				3.38	
	5‴	69.1				4.21	
	6‴	17.6				1.27	
Xyl	1⁗	106.6				4.39	
	2⁗	74.4				3.41	
	3⁗	75.9				3.65	
	4⁗	71.3				3.56	
	5⁗	66.4				3.35–4.30[a]	

3-*O*-β-solaculinetetranoside of tomatidenol (25*S*)-22β*N*-spirosol-5-en-3β-ol. These considerations were in agreement with HMBC experiment as well as comparison of the ^{13}C NMR data of **1** and those of tomatine (Radeglia et al., 1977, Wenkert et al., 1977; Willker and Leibfritz, 1992). Establishment of the type of sugars was achieved by complete and mild hydrolysis of **1** where galactose, xylose and rhamnose were detected by co-spotting with authentic sugars. A monoglycoside (**1a**), after hydrolysis of **1** had $[M+H]^+$ at m/z 576 and 33 carbon signals in the ^{13}C NMR experiment. The aglycone, **1b**, obtained after complete acid hydrolysis of **1a** indicated $[M+H]^+$ at m/z 414 and 27 carbon signals. The pyranoside on **1a** was established to be galactose. Interglycosidic linkages were ascertained from FABMS fragmentation patterns and NMR studies using ^{1}H–^{1}H COSY and HMBC spectra (Table 2) as well as similar compounds reported in literature (Wenkert et al., 1977; Bock and Thogersen, 1982; Ripperger and Porzel, 1997; Usubillaga et al., 1997).

Table 2
HMBC spectral data for compound **1**

Proton	^{1}HNMR (J in Hz)	Correlated C-atom
		HMBC
H-18	0.93 *s*	C-14, C-13, C-12
H-19	1.05 *s*	C-10, C-9, C-5, C-1
H-21	1.10 *d*(6.4)	C-20, C-22, C-17
H-27	0.97 *d*(6.0)	C-26, C-25, C-24
H-1′	4.47 *d*(7.7)	C-3
H-2′	4.20 *d*(7.6)	C-1″
H-4′	3.84 *s*	C-1‴
H-1″	5.22 *s*	C-2′
H-1‴	5.09 *s*	C-4′
H-2‴	3.86 *d*(1.9)	C-1⁗
H-1⁗	4.39 *d*(7.1)	C-2‴

The assignment of proton and carbon chemical shifts of **1** and the corresponding sugar moieties were established by analysis of NMR (Table 1). The coupling constants for non anomeric protons on sugars could not easily be ascertained from ^{1}H NMR due to superimposition. Nevertheless, the sugar moieties were recognized. The β-linkages for galactose and xylose were established from the coupling constant of the anomeric protons. The D, D, L and L configuration for galactosyl, xylosyl, and rhamnosyl residues, respectively were assumed as these usually occur in glycosides of higher plants (Ripperger and Porzel, 1997).

Molluscicidal activity of the freeze dried aqueous and methanol extracts of the berries and root bark as well as the isolated compounds were evaluated according to WHO guidelines (WHO, 1965) The results (Table 3) show that the aqueous berries extract was more potent to the test snails than the root bark with 50 ppm killing 100% of the snails tested. Compound **1** did not show any activity up to 30 ppm. Compound **2** and **3** obtained from the berries extract showed higher activity than the crude extract. Compound **2** and **3** killed over 85% of the test snail at 10 ppm while the mixture of **2** and **3** (1:1) killed 100% at 8 ppm. Low molluscicidal activity of the root bark extract may be attributed to the concentration of steroidal saponins and alkaloids in the root bark. Comparing the aglycones and sugar moieties of **1**, **2** and **3**, it is probable that the molluscicidal activity is affected

Table 3
Molluscicidal activities of the crude extracts and isolated compounds against *Biomphalaria feifferi*

Dose (ppm)	Berries	Root bark	% Mortality[a]		
			2	**3**	**2+3**
75	100	43	nt	nt	nt
50	100	33	nt	nt	nt
25	57	7	nt	nt	nt
12.5	nt	nt	100	100	100
10	nt	nt	87	94	100
8	nt	nt	67	73	100
5	nt	nt	23	43	67
2.5	nt	nt	nt	nt	33
0	3	3	3	0	3

[a] nt = Not tested.

by the number and type of sugars as well as interglycosidic linkages but not the type of aglycone.

3. Experimental

3.1. General experimental procedures

Mps uncorr. TLC precoated Kieselgel 60 F_{254} (0.25 mm, Merck). CC silica gel (mesh 230–400) eluted with MeOH–$CHCl_3$ (3:2) or MeOH–$CHCl_3$–cyclohexane (3:2:1) unless otherwise stated. Dragendorff's reagent and anisaldehyde were used for detection of alkaloids and glycosides, respectively. IR: KBr. $[\alpha]_D$ in MeOH–$CHCl_3$, 1:1, *c* 0.2) at 24° unless stated. Positive FABMS with Xe at 8 keV from glycerol matrix. ^{13}C and ^{1}H NMR (Bruker DPX 75.5 MHz and 300 MHz, respectively) using solvent CD_3OD:$CDCl_3$ (1:1, TMS). DCCC (Tokyo, Rikakikai Co. Ltd.) fitted with 9 racks each with 25 columns of 4 ml (i.d. 2 mm). Pump pressure of 5–10 mbar and a temp. of 30 °C (ascending mode) were used.

3.2. Plant material

The berries (6 kg, fr. wt) and root bark (5 kg, fr. wt) of *S. aculeastrum* were obtained from Mount Elgon, Western Province, Kenya. The plants were verified by Mr. Simon Mathenge, Botany department, Nairobi University, Nairobi and a voucher specimen SAC/0198/2000 was deposited at the Nairobi University Herbarium.

3.3. Extraction and isolation of alkaloids

The root bark was extracted with cold MeOH. The MeOH extract was conc. under red. pres. and the dark brown residue freeze dried. Part of this (3 g) extract was dissolved in a mixture of mobile and stationary phases (1:1) and injected in the DCCC. Elution was done with MeOH–$CHCl_3$–H_2O (7:13:8) and satd. $CHCl_3$ as stationary phase. Alkaloid rich fractions were combined and conc. under red. pres. giving CR1 (833 mg). This was injected again in the DCCC and eluted with MeOH–$CHCl_3$–H_2O–NH_3 (8:13:6:1) and after alkaloidal fractions being pooled gave CRR2 (640 mg). This was further injected in the DCCC and eluted with MeOH–$CHCl_3$–H_2O–NH_3-iso-PrOH (65:35:40:1:5) resulting in fractions CR40 (240 mg), CR41 (220 mg) and CR42 (154 mg) based on TLC and FABMS analysis. Fraction CR41 was subjected to CC on silica gel repeatedly eluting with MeOH–$CHCl_3$ (3:2) and MeOH–$CHCl_3$–NH_3 (14:11:1) to give **1** (69 mg) of R_f 0.76. The above DCCC procedure was followed for the purification of the berries extract. Fraction CB41 (404 mg) was subjected to CC eluting with MeOH–$CHCl_3$ (3:2) which gave a large spot on TLC. This was rechromatographed and eluted with MeOH–$CHCl_3$–cyclohexane (3:2:1) and yielded **2** (120 mg) and **3** (130 mg) of R_f 0.3 and 0.5 respectively.

3.4. Solaculine A, (25S)-3β-{O-α-L-rhamnopyranosyl-(1→2)-[O-β-d-xylopyranosyl-(1→2)-O-α-L-rhamnopyranosyl-(1→4)]-β-D-galactopyranosyloxy}-22βN-spirosol-5-ene (*1*)

White pellets, mp 220–224 °C, $[\alpha]_D^{24}$ −52°. IR ν_{max}^{KBr} cm^{-1}: 3404 (OH or NH), 1625 (C=C), 1025 (C–O or C–N). FABMS *m/z* (rel. int.): 1000 (100), 722 (11), 414 (38), 396 (100). ^{1}H NMR spectral data (300 MHz, CD_3OD:$CDCl_3$): cf. Table 1. ^{13} C NMR spectral data (75.5 MHz, CD_3OD:$CDCl_3$): cf. Table 1.

3.5. Solamarine, (25S)-3β-{O-α-L-rhamnopyranosyl-(1→2)-[O-α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyloxy}-22βN-spirosol-5-ene (*2*)

White crystals, mp 268–270 °C, $[\alpha]_D^{24}$ −81.5°. IR ν_{max}^{KBr} cm^{-1}: 3398 (OH or NH, sharp), 1638 (C=C). FABMS *m/z* (rel. int.): 868 (53), 722 (5), 706 (10), 414 (7), 396 (21). ^{1}H NMR spectral data (300 MHz, CD_3OD:$CDCl_3$): δ 0.89 (3H, *s*, H-18), 1.05 (3H, *s*, H-19), 0.91 (3H, *d*, *J* = 6.3, H-27), 1.02 (3H, *d*, J = 6.9, H-21), 1.29 (6H, *m*, rhamnosyl CH_3), 1.4–2.35 (many overlapped peaks), 2.7 (2H, *m*, *br*, H–26), 3.2–4.1 (overlapped sugar protons), 4.22 (1H, *m*, H-16), 4.48 (1H, *d*, *J* = 8.8, H-1′), 4.85 (1H, *d*, *J* = 1.5, H-1‴), 5.23 (1H, *d*, *J* = 1.5, H-1″), 5.37 (1H, *d*, *J* = 5.0, 6-H). ^{13}C NMR spectral data (75.5 MHz, CD_3OD:$CDCl_3$): δ 37.9 (C-1), 31.0 (C-2), 79.7 C-3), 38.9 (C-4), 141.0 (C-5), 122.0 (C-6), 32.7 (C-7), 32.0 (C-8), 50.9 (C-9), 37.5 (C-10), 21.4 (C-11), 40.4 (C-12) 41.2 (C-13), 56.6 (C-14) 33.0 (C-15) 79.0 (C-16) 62.6 (C-17) 17.1 (C-18) 19.6 (C-19) 43.2 (C-20) 15.7 (C-21) 99.3 (C-22) 27.0 (C-23) 28.6 (C-24) 30.1 (C-25) 50.2 (C-26) 19.5 (C-27). Sugar moiety: δ 99.9 (C-1′), 79.0 (C-2′), 75.6 (C-3′), 77.9 (C-4′), 78.4 (C-5′), 61.4 (C-6′), 101.5 (C-1″), 71.6 (C-2″), 71.3 (C-3″), 73.4 (C-4″), 70.2 (C-5″) 17.6 (C-6″), 102.4 (C-1‴) 71.8 (C-2‴) 71.3 (C-3‴), 73.4 (C-4‴), 69.0 (C-5‴), 17.7 (C-6‴).

3.6. Solamargine, (25R)-3β-{O-α-L-rhamnopyranosyl-(1→2)-[O-α-L-rhamnopyranosyl-(1→4)]- β-D-glucopyranosyloxy}-22αN-spirosol-5-ene (3)

White crystals, mp 284–286 °C, $[\alpha]_D^{24}$ −91°. IR ν_{max}^{KBr} cm^{-1}: 3393 (OH or NH), 1639 (C=C). FABMS *m/z* (rel. int.): 868 (100), 722 (12), 706 (10), 414 (27), 396 (71). ^{1}H NMR spectral data (300 MHz, CD_3OD:$CDCl_3$): δ 0.84 (3H, *s*, H-18), 1.05 (3H, *s*, H-19), 0.87 (3H, *d*, *J* = 6.0, H-27), 0.97 (3H, *d*, *J* = 7.0, H-21), 1.29 (6H, *m*, rhamnosyl CH_3), 1.4–2.35 (many overlapped peaks), 2.6 (2H, *m*, br, H-26′), 3.2–4.1 (overlapped sugar protons), 4.33 (1H, *m*, H-16), 4.48 (1H, *d*, *J* = 7.8, H-1′), 4.87 (1H, *s*, H-1″), 5.23 (1H, *s*, H-1″), 5.37 (1H, *d*, *J* = 4.2, 6-H). ^{13}C NMR spectral data (75.5 MHz, CD_3OD:$CDCl_3$): δ 37.9 (C-1), 30.5 (C-2), 79.0 C-3), 40.4 (C-4), 141.1 (C-5), 122.2 (C-6), 32.5 (C-7), 32.1 (C-8), 50.7 (C-9), 37.5 (C-10), 21.4 (C-11), 38.9 (C-12) 41.1 (C-13), 57.1 (C-14) 30.1 (C-15) 79.7 (C-16) 63.2 (C-17) 16.8 (C-18) 19.6 (C-19) 42.0 (C-20) 15.4 (C-21) 98.9 (C-22) 34.4 (C-23) 32.7 (C-24) 31.3 (C-25) 47.8 (C-26) 19.6 (C-27). Sugar moiety: δ 99.9 (C-1′), 79.5 (C-2′), 77.3 (C-3′), 75.7 (C-4′), 78.6 (C-5′), 61.4 (C-6′), 102.4 (C-1″), 71.8 (C-2″), 71.5 (C-3″), 73.3 (C-4″), 69.1 (C-5″) 17.7 (C-6″), 101.5 (C-1‴) 71.6 (C-2‴) 71.3 (C-3‴), 73.0 (C-4‴), 70.1 (C-5‴), 17.6 (C-6‴) .

3.7. Sugar analysis of 1

Compound **1** (5 mg) was refluxed in 5 ml of 5% HCl–MeOH soln. for 2 h. The soln. was then diluted with distilled H_2O and extracted with $CHCl_3$ and the aglycones separated (org. layer). The aq. filtrate was neutralized with $BaCO_3$ and evaporated. The resulting conc. soln. was chromatographed on TLC ($CHCl_3$–MeOH–Me_2CO–H_2O, 3:3:3:1) against reference sugars (Saerragiotto et al., 1998). The sugar components were identified as galactose, rhamnose and xylose.

3.8. Partial and complete acid hydrolysis of 1

Compound **1** (25 mg) was partially hydrolysed by refluxing in 10 ml of 0.5 M HCl for 30 min. The resulting soln. was diluted and extracted with $CHCl_3$. This was put on CC and eluted with MeOH–$CHCl_3$(4:1). Frs. 25–35 of R_f 0.5 and showing green colouration on being sprayed with anisaldehyde and heating were combined to give **1a** (10 mg). Compound **1a** (5 mg) was further refluxed with 5 ml of 5% HCl–MeOH soln. for 2 h and extracted with $CHCl_3$. CC of the extract afforded **1b** (3 mg). The sugar appended to the aglycone was established to be galactose.

3.9. (25S)-3β-{O-β-D-Galactopyranosyloxy}-22βN-spirosol-5-ene (1a)

White powder, mp 263–266 °C, $[\alpha]_D^{24}$ –41.5°. IR ν_{max}^{KBr} cm^{-1}: 3393 (OH or NH), 1655 (C=C) and 1025 (C–O or C–N). FABMS *m/z* (rel. int.): 576 (100), 576 (49), 414 (5). ^{1}H NMR spectral data (300 MHz, $CDCl_3$): δ 0.85 (3H, *s*, H-18), 1.06 (3H, *s*, H-19), 0.89 (3H, *d*, *J* = 6.1, H-27), 1.01 (3H, *d*, *J* = 6.6, H-21), (1.15–2.32 (many overlapped peaks), 2.3 (1H, *d*, *J* = 3.8), 2.4 (1H, *d*, *J* = 4.2), 2.7 (2H, *m*, H-26), 3.3 (*m*, *br*), 3.4 (*m*), 3.60 (1H, *m*, H-3), 3.72 (1H, *d*, *J* = 5.0), 3.75 (1H, *d*, *J* = 5.1), 3.8 (1H, *d*, *J* = 2.3), 3.9 (1H, *d*, *J* = 2.0), 4.20 (1H, *m*, H-16), 4.40 (1H, *d*, *J* = 7.8, H-1′), 5.37 (1H, *d*, *J* = 5.1, H-6). ^{13}C NMR spectral data (75.5 MHz, $CDCl_3$): cf. Table 1.

3.10. Tomatidenol (25S)-3β-hydroxy-22βN-spirosol-5-ene (1b)

White powder, mp 230–232 °C, $[\alpha]_D^{24}$ −23.5°. FABMS *m/z* (rel. int.): 414 (69). ^{1}H NMR spectral data (300 MHz, $CDCl_3$): δ 0.82 (3H, *s*, H-18), 1.01 (3H, *s*, H-19), 0.83 (3H, *d*, *J* = 6.2, H-27), 0.95 (3H, *d*, *J* = 6.7, H-21), 1.15–2.65 (many overlapped peaks), 2.7 (2H, *m*, H-26), 3.47 (1H, *m*, H-3), 4.12 (1H, *m*, H-16), 5.32 (1H, *d*, *J* = 5.8, H-6). ^{13}C NMR spectral data (75.5 MHz, $CDCl_3$): cf. Table 1.

3.11. Molluscicide extraction and bioassay

Fr. berries (1.5 kg) and root bark (1.2 kg) of *S. aculeastrum* were macerated and extracted separately with cold distilled H_2O (5 l) by soaking and shaking for 10 h. The H_2O extracts were then freeze dried and stored under cool dry conditions until used. Immediately before use, the freeze dried material was reconstituted in distilled H_2O. The snails used to test for molluscicidal activity of the extracts and isolated compounds were laboratory reared, or were field-collected and laboratory acclimatized.

3.12. Biomphalaria pfeifferi

B. pfeifferi were collected from shallow rivers in Machakos District, Kenya and maintained in the laboratory aquaria at Kenya Medical Research Institute (KEMRI), Kenya. Molluscicidal activity was evaluated according to the established procedures (WHO, 1965). Ten adult snails (8–14 mm in diameter) were placed in a plastic dish, containing 400 ml molluscicide suspension at final concentrations ranging from 2 to 100 ppm. Each test concentration was set in triplicate. Snails were exposed to potential molluscicide for 24 h at room temp. and were kept under normal diurnal lighting. After 24 h, the suspension was decanted, the snails rinsed twice with aerated tap water and offered lettuce leaves as food. The test snails were then left in water for another 24 h, and at the end of this period were examined to assess mortality. Snails were considered dead if they either remained motionless, did not respond to the presence of food or if the shell looked discolored. In

control experiments, snails were not exposed to the potential molluscicide suspension and these remained in H_2O during the experiment. The number of snails killed was expressed as % mortality. The results are in Table 3.

Acknowledgements

This work was supported by German Academic Exchange Service (DAAD). We are very grateful to Ms. Doring, Chemistry Department, Technical University of Braunschweig and Dr. Ray of GBF, Germany for running FABMS and NMR respectively.

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