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SAPONINS FROM *ALBIZZIA ANTHELMINTICA*

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Key Word Index—*Albizzia anthelmintica*; Leguminosae; Mimosaceae; root bark; echinocystic acid glycosides; structure determination; 2-acetamido-2-deoxy-β-D-glucopyranosyl moiety; molluscicidal activity.

Abstract—The structures of three new saponins from the bark of *Albizzia anthelmintica* were established to be 3-*O*-[β-D-glucopyranosyl(1→3)] [α-L-arabinopyranosyl(1→2)] [α-L-arabinopyranosyl(1→6)]2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid; 3-*O*-[α-L-arabinopyranosyl(1→2)][α-L-arabinopyranosyl(1→6)] 2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid and, present in small amounts, 3-*O*-[α-L-arabinopyranosyl(1→6)] 2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid.

INTRODUCTION

Albizzia anthelmintica (Brongn.), Leguminosae, is a bush, commonly occurring from Abyssinia and Somaliland to South West Africa. Root bark preparations have long been used as an anthelmintic, particularly against tapeworm. Minor uses are in childbirth, malaria, stomach and intestinal disorders, rheumatism and venereal diseases [1]. The constituents of the bark so far described are musennin, histamine and unknown saponins [2, 3]. We now report the isolation and the structure of three new saponins from *A. anthelmintica* root bark. One of these compounds has been found active as a molluscicidal against *Biomphalaria glabrata.*

RESULTS AND DISCUSSION

The methanol extract of root bark of *A. anthelmintica* was partitioned between *n*-butanol and water. After digestion with diethyl ether, the butanol extract (3% of plant material) showed on TLC a sequence of several plum coloured spots. Three main compounds (**1**–**3**) were separated by means of gel filtration (Sephadex LH-20 and Fractogel TSK-HW 40) and droplet counter-current chromatography [4].

The most polar compound **1**, after hydrolysis with 1 M CF_3CO_2H, extraction and subsequent treatment of the sapogenin with ethereal diazomethane, gave echinocystic acid methyl ester (comparison of its physical data: mp, $[\alpha]_D^{25}$, ^{1}H NMR with literature [5, 6]). The hydrolysate in the aqueous layer was examined by TLC and GC (as peracetylated derivatives) and showed the presence of glucose, arabinose and *N*-acetyl-D-glucosamine (1:2:1). Treatment of **1** with ethereal diazomethane gave a methyl ester as confirmed by its ^{1}H NMR spectrum (δ3.79, *s*). The fast atom bombardment mass spectrum, obtained in the negative ion mode, showed a quasi-molecular ion peak at *m/z* 1100 $[M-H]^-$; the fragments at *m/z* 939, 807 and 675 corresponded to the successive elimination of one glucosyl and two arabinosyl moieties. Thus, glucose is a terminal sugar. Treatment of **1** with β-glucosidase from *Helix pomatia* digestive juice [7] showed the rapid formation of glucose (TLC and GC of the acetylated derivative) and of a compound which showed the same behaviour on TLC and the same ^{1}H and ^{13}C NMR spectra as compound **2**. Enzymatic hydrolysis under the same conditions, of **2** was prolonged by up to 40 days; the main product (**4**) was isolated and ^{13}C NMR resonances of its glycosidic part were consistent with those reported for *O*(3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-oleanolic acid [8].

The signal at δ63.8 (*t*) in the ^{13}C NMR spectrum of **4** attributable to C-6 of *N*-acetylglucosamine was shifted to δ70.2 in the ^{13}C NMR spectrum of **2**, whilst the C-5 resonance was shifted upfield by *ca* 2 ppm. These glycosylation shifts indicated that an arabinosyl moiety in compound **2** was linked to the hydroxymethylene group of *N*-acetyl-D-glucosamine. It remained to locate the interglycosidic linkage between the two arabinosyl units. First of all, the anomeric configuration of the glycosyl moiety in **2** was confirmed as β- for glucosamine and α- for arabinose based on the ^{13}C NMR signals as observed for alkyl glycopyranosides [9]. In a previous paper [10], various 2-*O*-glycosylated α- and β-L-arabinopyranosides were synthesized and the ^{13}C NMR signals assigned by the application of selective deuteration. Our data were compared with those in the literature [10] and were in good agreement with those reported for a 2-*O*-α-L-arabinopyranosyl-α-L-arabinopyranosyl moiety. From these considerations compound **2** is 3-*O*-[α-L-arabinopyranosyl(1→2)] [α-L-arabinopyranosyl(1→6)]2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid. Methylation analysis of **2** yielded methyl 3,4 di-*O*-methyl-arabinopyranoside and methyl 2,3,4-tri-*O*-methyl-arabinopyranoside.

The linkage of the terminal glucose to the α-L-arabinopyranosyl moiety in compound **1**, was deduced by a comparison of the ^{13}C NMR spectra of **1** and **2**, **2** being the desgluco derivative of **1**. It revealed that only the

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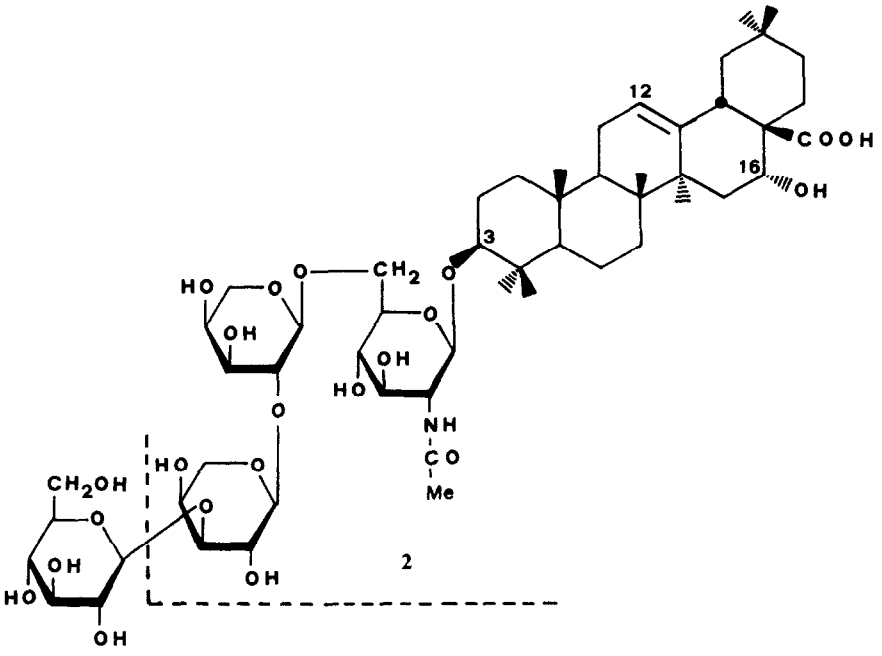
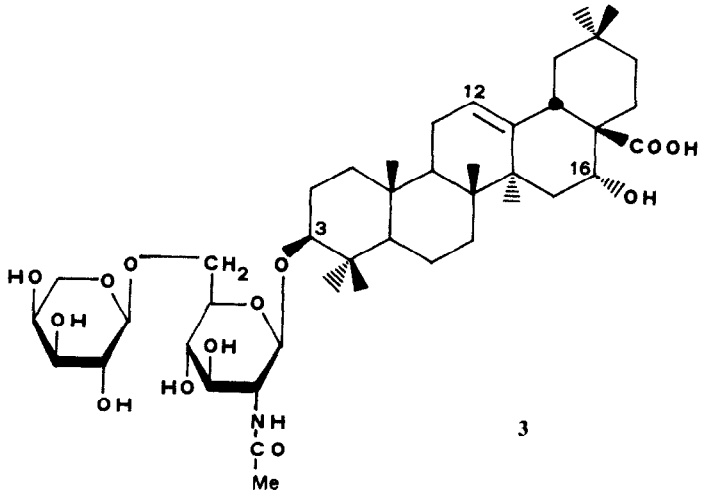


1



signals at δ74.8 (C-3‴), 73.3 (C-2‴) and 69.4 (C-4‴) in the ^{13}C NMR spectrum of **2** were significantly shifted. They can be found at δ83.3 (+8.5 ppm), at 71.7 (−1.6 ppm) and 68.6 (−0.8 ppm) in the ^{13}C NMR spectrum of **1**. From these considerations, **1** is 3-*O*-[β-D-glucopyranosyl(1→3)] [α-L-arabinopyranosyl(1→2)] [α-L-arabinopyranosyl (1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid.

The sequence of sugar components of **1** was also confirmed by methylation analysis. Compound **1** was permethylated by the method described for monosaccharides [11] then hydrolysed with 0.5 M HCl/MeOH. Methyl 2,4-di-*O*-methylarabinopyranoside, methyl 3,4-di-*O*-methylarabinopyranoside and methyl 2,3,4,6-tetra-*O*-methylglucopyranoside were detected by TLC and capillary GC analysis.

A third saponin, **3**, less polar than **1** and **2**, was isolated in small quantity. The FAB mass spectrum showed a quasi-molecular ion peak at *m/z* 806 $[M-H]^-$ and loss of 132 mass units from it. The ^{13}C NMR spectrum of **3** (Table 1) is consistent with the proposed structure 3-*O*-[α-L-arabinopyranosyl(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid.

Compounds **1** and **2** were tested for their molluscicidal activity on the snail *Biomphalaria glabrata* [12]. Only **2** exhibited activity at a concentration of 25 ppm, while **1** was inactive. All three compounds did not show any anti-inflammatory activity using the carrageenin-induced pleurisy test in rats [13].

EXPERIMENTAL

Plant material was collected in Tanzania in June 1986. Mps: uncorr. Precoated Kieselgel 60 F_{254} and RP8 (Merck) were used for TLC. Spots were detected by spraying with H_2SO_4–MeOH (1:9)

Table 1. ^{13}C NMR chemical shift values (δ) of the aglycone moiety of compounds **1–4** (52.4 MHz, C_5D_5N)

C	1	2	3	4
1	39.1	39.5	39.1	39.5
2	26.6	27.2	26.7	27.0
3	89.6	89.9	89.9	89.9
4	39.4	39.9	39.5	39.9
5	56.2	56.7	56.7	56.7
6	18.8	19.3	18.9	19.3
7	33.7	34.2	33.7	33.8
8	40.2	40.8	40.4	40.7
9	47.5	48.1	47.6	47.9
10	37.3	37.8	37.4	37.7
11	24.0	24.5	24.1	24.5
12	122.5	122.7	122.6	123.0
13	145.2	145.9	145.2	145.7
14	42.3	42.9	42.5	42.8
15	36.3	36.8	36.4	36.8
16	74.8	75.4	74.9	75.4
17	49.4	50.2	*	49.8
18	41.8	41.8	42.0	42.2
19	47.5	48.1	47.6	47.9
20	31.0	31.5	31.0	31.6
21	36.3	36.8	36.4	36.8
22	32.3	32.3	*	33.0
23	28.4	28.9	28.4	28.9
24	17.1	17.6	17.1	17.6
25	15.7	16.3	15.8	16.2
26	17.7	18.3	17.1	18.2
27	27.4	28.0	*	27.9
28	180.6	181.3	*	180.4
29	33.3	34.0	33.4	34.2
30	25.2	26.2	*	25.6

*These signals were not unambiguously assigned.

followed by heating. Gel filtration was carried out on Sephadex LH-20 (Pharmacia) and Fractogel TSK-HW 40 (Merck), eluting with MeOH. Kieselgel 60 (70–230 mesh, Merck) was employed for CC. Reverse phase CC was carried out on a Lobar RP-18 column (Merck, 1 × 20 cm) eluting with MeOH–H_2O (7:3). Droplet counter-current chromatography (DCCC) was performed with $CHCl_3$–MeOH–*n*-PrOH–H_2O (5:6:1:4) in the ascending mode (flow rate 0.2 ml/min, 300 tubes 400 × 2 mm). Samples for NMR were dissolved in $CDCl_3$ or C_5D_5N. FAB-MS were obtained from samples dissolved in a glycerol matrix and placed on a steel target prior to bombardment with Ar atoms of energy 7–8 kV. Acetylated sugars were analysed by GC on a 3% SE 30 column, 6 m × 3 mm i.d., carrier N_2, 1.4 kg/cm^2, inj. and det. temp 310°, prog. at 180–300° at 5°/min; 3% SP-2340 column, 6 m × 3 mm i.d., carrier N_2, 1.8 kg/cm^2, inj. and det. temp 270°, prog. from 210–270° at 5°/min. Methylated sugars were analysed on a WCOT-CP-Sil 5-CB fused silica capillary column (Chrompack), 25 m × 0.32 mm i.d., film thickness 0.11 μm, carrier H_2, 0.5 kg/cm^2, split flow 80 ml/min, inj. and det. temp 310°, prog. at 80–300° at 4°/min.

Extraction and isolation. Thinly minced bark (1720 g) was exhaustively extd by percolation with MeOH (18 l) at room temp. The concd ext was taken up in H_2O and extracted with *n*-BuOH; the organic phase was then concd *in vacuo* to yield a crude residue (66.4 g) which was washed with Et_2O in order to obtain an extract free from less polar compounds (59.6 g). This extract (5 g) was dissolved in MeOH and applied to a Fractogel TSK-HW 40 (70 × 2 cm i.d.) column eluting with MeOH (700 ml) at a flow rate of 0.5 ml/min; fractions (2 ml) were collected according to their composition, taken to dryness (2.3 g) and applied to a column of Sephadex LH-20 (70 × 2 cm i.d.), eluted with MeOH (300 ml); fractions (2 ml) 68–99, enriched in the three main compounds, were collected and taken to dryness (1.8 g). This mixt. (580 mg) was submitted to DCCC; fractions were collected according to their composition and afforded 154 mg of **1**, 195 mg of **2** and 17 mg of **3**.

Compound **1** was obtained from EtOAc–EtOH–MeOH (14:6:1) as an amorphous powder, mp 196–197° (dec), $[\alpha]_D^{25}$ +8.64 (MeOH; *c* 0.5); FAB MS *m/z* ($C_{54}H_{87}NO_{22}$): 1100 $[M-H]^-$, 939 $[M-162]^-$, 807 $[M-162-132]^-$, 675 $[M-162-2\times132]^-$; ^1H NMR (200 MHz, C_5D_5N): δ8.55 (*br d*, –NH), 5.65 (*dd*, $J_{12-11\alpha}=J_{12-11\beta}=3$ Hz, H-12), 2.20 (*s*, MeCONH), 1.80, 1.22, 1.20, 1.08, 1.05, 1.00, 0.95 (*s*, –Me). ^{13}C NMR (52.4 MHz, C_5D_5N): see Tables 1 and 2.

Acid hydrolysis of **1**. A soln of **1** (15 mg) in 2 ml of 1 M CF_3COOH was refluxed for 3 hr. The soln was extracted with $CHCl_3$ to afford 5 mg of the aglycone (**5**) that was treated with CH_2N_2–Et_2O, evapd and purified by CC on silica gel eluting with $CHCl_3$–MeOH (99:1) to afford 3 mg of echinocystic acid Me ester, which was crystallized from *iso*-PrOH, mp 209–210° (lit [5] 210–212°), $[\alpha]_D^{25}$ +37° (EtOH; *c* 0.3) (lit [6] +37.08, EtOH); ^1H NMR (200 MHz, $CDCl_3$): δ5.38 (*dd*, $J_{12-11\alpha}=J_{12-11\beta}=3$ Hz, H-12), 4.50 (*dd*, $J_{16-15\alpha}=J_{16-15\beta}=4$ Hz, H-16), 3.60 (*s*, –COOMe), 3.20 (*dd*, 10, 6.5 Hz, H-3), 1.34, 0.98, 0.96, 0.90, 0.89, 0.78, 0.72 (*s*, –Me); the aq. phase from acid hydrolysis (9 mg) was acetylated with Ac_2O–pyridine (1:1) and the sugars obtained identified comparing their $[\alpha]_D^{25}$ and GC R_t values with pure samples of acetyl derivatives of D-glucose, L-arabinose and *N*-acetyl-D-glucosamine.

Enzymatic hydrolysis of **1**. Compound **1** (54 mg) was hydrolysed with the hepatopancreatic juice of 10 snails (*Helix pomatia*) dil. with H_2O (10 ml) and filtered. The soln was stirred at 35° for 18 hr; the mixt. was then extracted with *n*-BuOH (2 × 15 ml) to afford

Table 2. ^{13}C NMR chemical shift values (δ) of the sugar moieties of compounds **1–4** (52.4 MHz, C_5D_5N)

C		1	2	3	4
GlcNAc	1	104.5	105.2	104.6	105.2
	2	58.1	58.7	58.5	58.9
	3	75.8	76.5	76.5	76.7
	4	72.7	73.3	73.2	73.6
	5	75.5	76.2	76.0	78.6
	6	69.6	70.2	69.9	63.8
Me	(CO–NH–)	23.5	24.2	24.2	24.2
Me	(CO–NH–)	171.2	170.9	171.0	170.8
Ara	1	102.4	103.2	104.9	
	2	79.9	80.0	72.3	
	3	72.4	73.5	74.2	
	4	67.7	68.4	68.6	
	5	64.6	65.3	65.8	
Ara	1	105.4	105.9		
	2	71.7	73.3		
	3	83.3	74.8		
	4	68.6	69.4		
	5	66.7	67.1		
Glc	1	105.4			
	2	75.8			
	3	78.3			
	4	71.7			
	5	78.1			
	6	62.8			

48 mg, which were purified by DCCC. The main compound was identified as **2** by TLC and ^{13}C NMR (see Tables 1 and 2).

Permethylation of compound **1**. **1** (50 mg) was dissolved in 1 ml of dry DMSO, 157 mg of *tert*-BuONa (Merck), 30 mg of dry powdered NaOH and then 0.5 ml of MeI were added with stirring at room temp. After 3 hr the mixt. was poured into ice and extracted with $CHCl_3$ (3 × 2.5 ml). The organic phase was washed with H_2O and evapd to dryness; the product of permethylation was treated with 2 ml of 0.5 M HCl–MeOH (Merck) with stirring at 50° under N_2. After 2 hr the mixt. was cooled to room temp. and compared by TLC and C-GC with samples of α- and β-Me 2,3,4,6-tetraMe glucoside (R_t 10.20 [α], 11.43 [β]), α- and β-Me 2,4-diMe arabinoside (R_t 7.36 [β], 7.40 [α]) and α- and β-Me 3,4-diMe arabinoside (R_t 7.60 [β], 8.42 [α]).

Compound **2** was crystallized from EtOAc–EtOH (3:2), mp 211–212° (dec), $[\alpha]_D^{25}$ +7.03° (MeOH; *c* 0.5); FAB MS *m/z* ($C_{48}H_{77}NO_{18}$): 938 $[M-H]^-$, 807 $[M-132]^-$, 675 $[M-2\times132]^-$, 453 $[M-2\times132-222]^-$; ^1H NMR (200 MHz, C_5D_5N): δ8.40 (*br d*, –NH), 5.65 (*dd*, $J_{12-11\alpha}=J_{12-11\beta}=3$ Hz, H-12), 2.15 (*s*, MeCONH), 1.80, 1.22, 1.20, 1.08, 1.05, 1.02, 0.95 (*s*, –Me).

Acid hydrolysis of **2**. A soln of **2** (53 mg) was treated under the same conditions as described for **1**. After extraction (3 × 2.5 ml $CHCl_3$), the organic phase was evapd to give 4 mg of the aglycone (**5**). The aq. phase was acetylated in the usual manner and the sugars obtained were identified by comparing their $[\alpha]_D^{25}$ and GC R_t values with those of pure samples of acetyl derivatives of L-arabinose and *N*-acetyl-D-glucosamine.

Enzymatic hydrolysis of **2**. Compound **2** (61 mg) was hydrolysed under the same conditions as those used for **1** for 40 days at 35° with stirring; the mixt. was extracted with EtOAc (3 × 10 ml) and *n*-BuOH (3 × 15 ml) to afford 7 mg of aglycone and 50 mg of BuOH phase, which was purified by RPCC to afford 14 mg of compound **4** (^{13}C NMR: see Tables 1 and 2).

Permethylation of **2**. Permethylation was carried out using the same conditions as those used for **1**; after 3 hr the mixt. was poured into ice and extd with $CHCl_3$ (3 × 3 ml). The product of permethylation was dissolved in 0.5 M HCl–MeOH (1.6 ml) and stirred at 50° under N_2. After 2 hr the mixt. was cooled to room temp and compared by TLC and GC with pure samples of α- and β-Me 2,3,4-triMe arabinoside (R_t 7.60 [α, β] and α- and β-Me 3,4-diMe arabinoside (R_t 7.60 [β], 8.42 [α]).

Compound **3** was crystallized from EtOAc–EtOH–MeOH (2:2:1), mp 237–240° (dec), $[\alpha]_D^{25}$ +2.7° (MeOH; *c* 0.5); FAB MS *m/z* ($C_{43}H_{69}NO_{13}$): 806 $[M-H]^-$, 675 $[M-132]^-$, 453 $[M-132-222]^-$; ^1H NMR (200 MHz, C_5D_5N): δ8.85 (*br d*, –NH), 5.65 (*dd*, $J_{12-11\alpha}=J_{12-11\beta}=3$ Hz, H-12), 2.22 (*s*, MeCONH), 1.83, 1.22, 1.20, 1.10, 1.0, 1.0, 0.83 (*s*, –Me).

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