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THE STRUCTURES OF BALANITINS, POTENT MOLLUSCICIDES ISOLATED FROM *BALANITES AEGYPTIACA*

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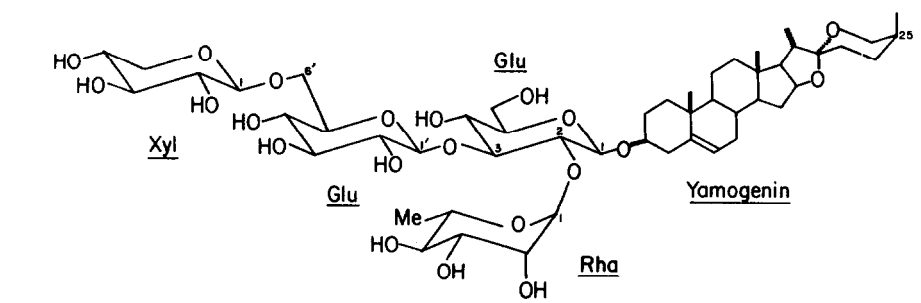
Abstract—The structures of three potent molluscicides balanitin-1, -2 and -3 which were isolated from the East African tree *Balanites aegyptiaca* have been determined to be **1**, **2** and **3**, respectively.

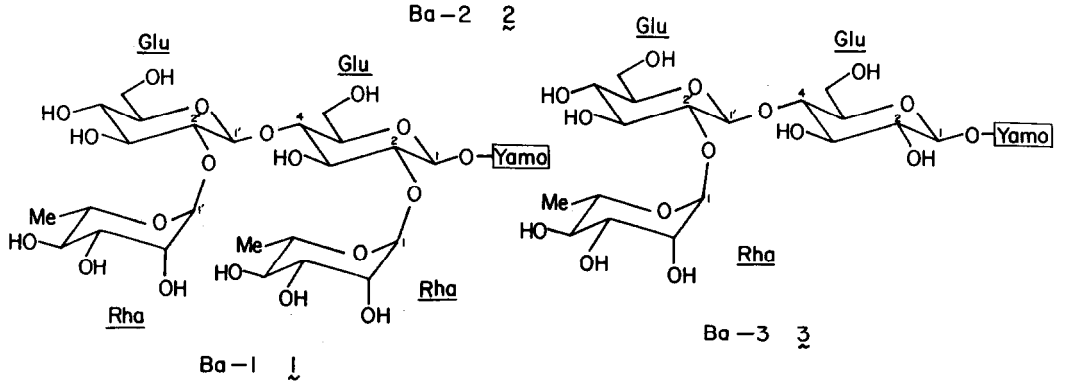
Balanites aegyptiaca Del. (Balanitaceae) is widely used in East Africa in various folk medicines.[1,2] The root is used for treatment of abdominal pains, as a purgative, and as an anthelmintic, while the bark is employed as a detergent, fish poison and also as a remedy for malaria and syphilis. The leaf of this plant is edible and has been once regarded as an effective medicine for sleeping sickness. The seeds, fruits and even the flowers are sold in African food markets. The root, bark, kernel, fruit and branch have been shown to be lethal to molluscs, and a concentrated emulsion of the berry has been recommended for treating ponds and canal dead-ends. The planting of the trees alongside infested waters was once suggested[3] so that the fruit could drop into the water spontaneously.

In continuation of our studies on biologically active substances from medicinal plants, we have investigated the molluscicidal fraction of *Balanites aegyptiaca* (collected in Kenya). Aqueous methanol extracts of the root and bark of this plant exhibited insect antifeedant activity against the Mexican bean beetle *Epilachna varivestis*, antimicrobial activity against *Bacillus subtilis*, *Penicillium crustosum*, *Saccharomyces serevisiae*,[4] and molluscicidal activity against African snails *Lymnaca natalenses*, *Biomphalaria pfeifferi*, and the South American snail *B. glabratus*.[5]

The 60% aqueous methanol extract of the root and bark was evaporated to dryness, suspended in water, and fractionated into the hexane extract, ether extract, methanol extract and aqueous residue. Bioassay of the various fractions showed that the molluscicidal activity resided in the methanol fraction. Further fractionation of the methanol extract, as monitored by molluscicidal bioassay, was carried out as outlined in Fig. 1. Final separation of the active constituents was accomplished efficiently by droplet counter-current chromatography[6] which yielded 31 mg of balanitin-1 (Ba-1) **1**, 10 mg of Ba-2 **2** and 15 mg of Ba-3 **3**.

The results of field-desorption (FD) MS carried out on the underivatised saponins Ba-1, -2, and -3 are tabulated in Table 1 (see also fig. 2). That the molecular weight of balanitin-1 is 1030 is clear from the doublet of peaks at





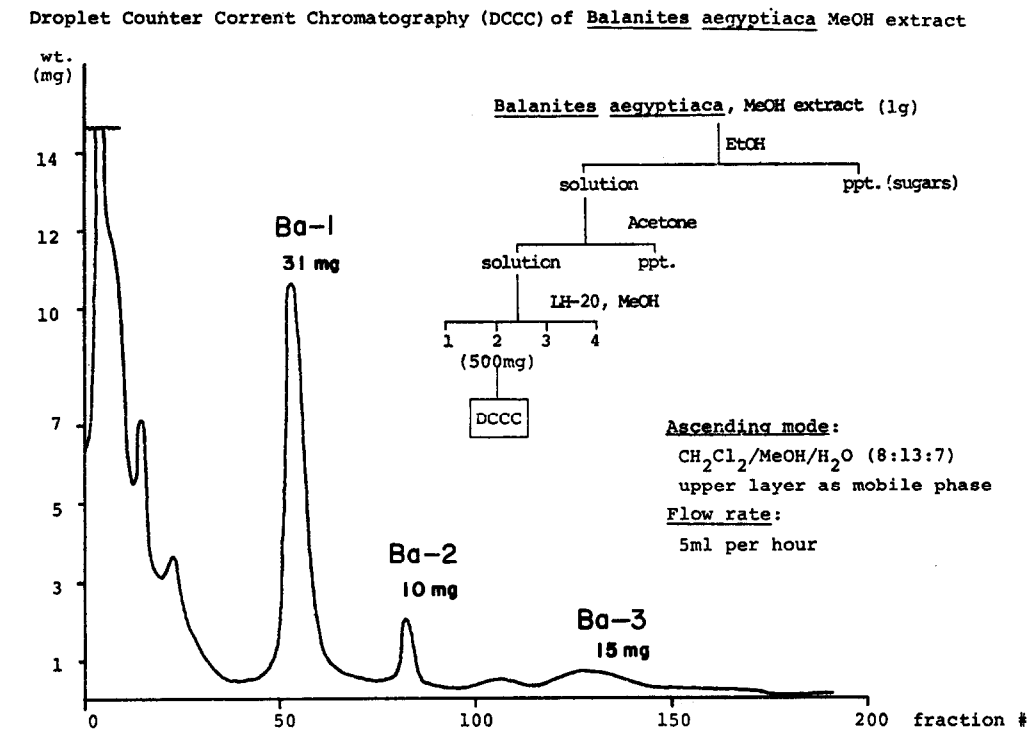


Fig. 1. Isolation scheme and droplet counter-current chromatography (DCCC) trace of *Balanites aegyptiaca* methanol extract. The eluate from DCCC was fractionated into 3 ml aliquots of 200 fractions. Each fraction tube was evaporated *in vacuo* to dryness, and the weight of the residue was plotted against fraction number to give the chromatogram.

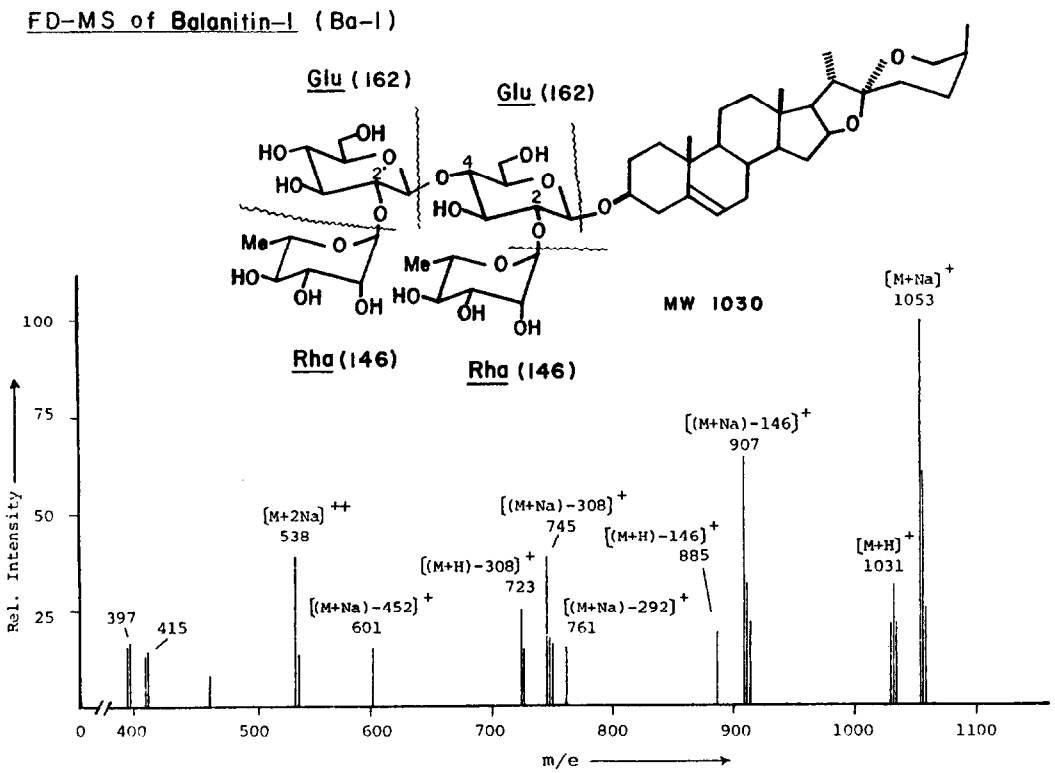


Fig. 2. FD-MS of Balanitin-1 (Ba-1) 1.

Table 1. FD-MS data of Ba-1 (1), Ba-2 (2) and Ba-3 (3)

Ba-1	Ba-2	Ba-3
1053 (M+Na)		
1031 (M+H)	1039 (M+Na)	
907 ({M+Na}-146)	907 ({M+Na}-132)	907 (M+Na)
885 ({M+H}-146)	893 ({M+Na}-146)	885 (M+H)
761 ({M+Na}-292)	761 ({M+Na}-278)	761 ({M+Na}-146)
745 ({M+Na}-308)	745 ({M+Na}-294)	745 ({M+Na}-162)
723 ({M+H}-308)		
601 ({M+Na}-452)	599 ({M+Na}-440)	599 ({M+Na}-308)
538 $(M+2Na)^{++}$	531 $(M+2Na)^{++}$	
465 $(\{M+2Na\}-146)^{++}$	465 $(\{M+2Na\}-132)^{++}$	465 $(M+2Na)^{++}$
415 ({M+H}-616)	414 (M-602)	414 (M-470)

Assignments of peaks are shown in brackets.
The mass units which are lost correspond to the following fragments:
132: xylose, 146: rhamnose, 162: glucose,
278: xylose+rhamnose, 292: two rhamnose, 294: xylose+glucose,
308: rhamnose+glucose, 440: xylose+rhamnose+glucose,
452: two rhamnose+glucose, 470: rhamnose+two glucose,
602: xylose+rhamnose+two glucose, 616: two rhamnose+two glucose.

1053 $(M+Na)^+$ and 1031 $(M+H)^+$. In addition, a doubly charged ion generated by a second cationization of the sodium cation complex of Ba-1 is observed at 538 $(M+2Na)^{2+}$. The 907 $(M+Na-146)^+$/885 $(M+H-146)^+$ peaks correspond to a loss of a deoxyhexose unit (rhamnose), whereas the peaks at 761 $(M+Na-146-146)^+$ and at 745 $(M+Na-146-162)^+$/723 $(M+H-146-162)^+$ correspond to further losses of a deoxyhexose (rhamnose) and a hexose (glucose), respectively. The ion at *m/e* 601 $(M+Na-452)^+$ arises from the loss of two deoxyhexose (two rhamnose) and one hexose (glucose); the 415 peak is ascribable to the mass of the aglycone (aglycone + H)$^+$. The facile cleavage of the interglycosidic linkage is presumably triggered by addition of alkali metals (Na^+) to the glycosidic oxygen atoms.[7] The sugar sequence in Ba-1 is hence deoxyhexose$_1$ (rha)-hexose$_2$ (glu)-hexose$_1$ (glu)-aglycone with another deoxyhexose$_2$(rha) attached to hexose$_1$. Similarly, the molecular weights of Ba-2 1016 and Ba-3 884 can be deduced directly from their cation complexes. The straightforward fissions of the glycosidic linkages clarify the sequence of the sugar residues in Ba-2 to be pentose(xyl)-hexose$_2$(glu)-hexose$_1$(gly)-aglycone with a deoxyhexose (rha) attached to hexose$_1$; in Ba-3 this is deoxyhexose (rha)-hexose$_2$(glu)-hexose$_1$(glu)-aglycone (Table 1).

Acid hydrolysis with HCl-dioxane-H_2O of Ba-1, Ba-2, and Ba-3 afforded the same aglycone, m.p. 201°, with molecular formula $C_{27}H_{42}O_3$ [CI-MS 415 $(M^+ + 1)$]. Of decisive importance in establishing the aglycone structure was the product obtained upon treatment of the aglycone from Ba saponins with HCl in boiling ethanol, i.e. the usual condition employed for the isomerization of the sapogenin side chain.[8] The ^{1}H-NMR spectrum of this product is almost identical to that of the original aglycone except that the doublet 25-Me ^{1}H-NMR signal originally at 1.089 ppm (J = 6.99 Hz) is now shifted to 0.798 ppm (J = 6.33 Hz). The acid-isomerised product was identified as diosgenin[9] (25-eq-methyl in aglycone moiety shown in **1**) by comparison with an authentic specimen. The original aglycone with the lower 25-Me signal thus should be yamogenin[10] (as shown in **1**).

The sugars obtained from the saponin hydrolysates were examined by TLC. The presence of rhamnose in all three saponins and xylose in Ba-2 was indicated by their R_f values and characteristic colors upon spraying the TLC plate with 10% sulfuric acid and heating (rhamnose, yellow; xylose, black). The co-existing hexose, as demonstrated by FD-MS, in Ba-1, -2, -3 was found to be either glucose, galactose or mannose by TLC.

Although these three sugars are frequently encountered in natural products, they cannot be characterized in a straightforward manner due to overlapping retention times. The following microanalytical method was therefore developed during the course of this study. Namely, it was found that when the common sugars shown in Fig. 3 were perbenzoylated with benzoyl chloride and pyridine, each sugar usually yielded only one major anomeric perbenzoate (α-anomer, by ^{1}H-NMR), which gave baseline separated HPLC peaks on a μ-Porasil column with 10% ether in hexane as solvent (Fig. 3). The amount of sugar required is in the range of 1–2 μg. In the CD of these compounds, the benzoates are all coupled and are subject to the recently discovered additivity relation in the CD amplitudes;[11] CD data of the separated HPLC peaks would therefore offer further criteria for sugar identification, if required.[12]

The aqueous acid hydrolysate of 1 mg of Ba-3 **3** was perbenzoylated and the product mixture was separated by HPLC for sugar identification. Calibration of the integrated peak area against known concentrations unambiguously showed that two glucose and one rhamnose units were present in Ba-3. It was shown similarly that Ba-1 contains two units each of glucose and rhamnose, whereas Ba-2 contains two units of glucose and one unit each of rhammnose and xylose.

The sugar linkages of Ba-1, -2 and -3 were determined from NMR data (Table 2) as described in the following; they have also been corroborated by an entirely new CD dependent micromethod for oligosaccharide structure determination currently under development.[13] The anomeric configurations were generally deduced from the δ and J values of the anomeric protons. The points of attachment of sugar residues, on the other hand, were based on glycosidation shifts clarified by Tanaka *et al.*[14] and Tori *et al.*[15] Namely, when the hydroxyl groups are derivatised, i.e. glycosylated, methylated (or acetylated), the α- and β- carbons of both the sugar and aglycone moieties undergo characteristic shifts: i.e. the α-CH sig-

nals are shifted downfield (denoted by plus signs) by 6–9 ppm (or 1.3–2 ppm), while the β-C's are shifted upfield (denoted by minus signs) (shift is due to the general γ-upfield shift).

Partial hydrolysis of Ba-1 **1** and Ba-3 **3** yielded Ba-X **4**, while hydrolysis of Ba-2 **2** gave Ba-Y **5** (see Fig. 6 for structures). The ^{13}C-NMR shifts listed in Table 2 were assigned by comparison between the natural products and partial hydrolysis products.

Balanitin-3 (*Ba*-3) **3** (*Fig.* 4)

Ba-3 contains one rhamnose and two glucose units. The rhamnose unit is α-linked in view of the good agreement in the R-1-H (anomeric proton of rhamnose) peak at 4.917 ppm (br s), R-3 (C-3 carbon of rhamnose) peak at 72.5 ppm and R-5 peak at 69.5 ppm with the following values given for methyl α-L-rhamnopyranoside:[16] 5.04, 72.1 and 69.5 ppm, respectively (the values for the β-anomer are 4.55, 75.3 and 73.4 ppm). The two glucose units are β-linked from the large *J* values (7.74 and 7.35 Hz). The downfield glycosidation shifts at G-4 and G-2′, the upfield glycosidation shifts at G-3, G-5, and G-3′, and the obtention of Ba-X **4** upon partial hydrolysis lead to the yamogenin O - α - L - rhamnopyranosyl - (1→2) - β - D - glucopyranosyl - (1→4) - β - D - glycopyranoside structure shown.

Balanitin-1 (*Ba*-1) **1** (*Fig.* 5)

Ba-1 contains two units each of rhamnose and glucose. The FD-MS data (Table 1, Fig. 2) show a peak at *m/e* 761 due to the loss of two rhamnose units, and peaks at *m/e* 745/723 arising from the loss of one rhamnose unit and one glucose unit. It follows that the rhamnose residues cannot be attached to the same glucose residue. The R-1′-H and R-1-H peaks at 5.011 and 4.942 ppm, and the R-3, 3′, 5, 5′ peaks indicate that the L-rhamnose units are α-linked. As in Ba-3 **3**, the downfield glycosidation shifts at G-2, G-4 and G-2′, the upfield glycosidation shifts at G-3, G-5 and G-3′, and the obtention of Ba-X **4** allows one to determine the structure of Ba-1 as yamogenin O-α-L-(1→2) - β - D - glucopyranosyl - (1→4) - [α - L - rhamnopyranosyl - (1→2) - β - glucopyranoside **1**.

Balanitin-2 (*Ba*-2) **2** (*Fig.* 6)

Ba-2 contains one rhamnose, one xylose and two glucose units. Partial hydrolysis of Ba-2

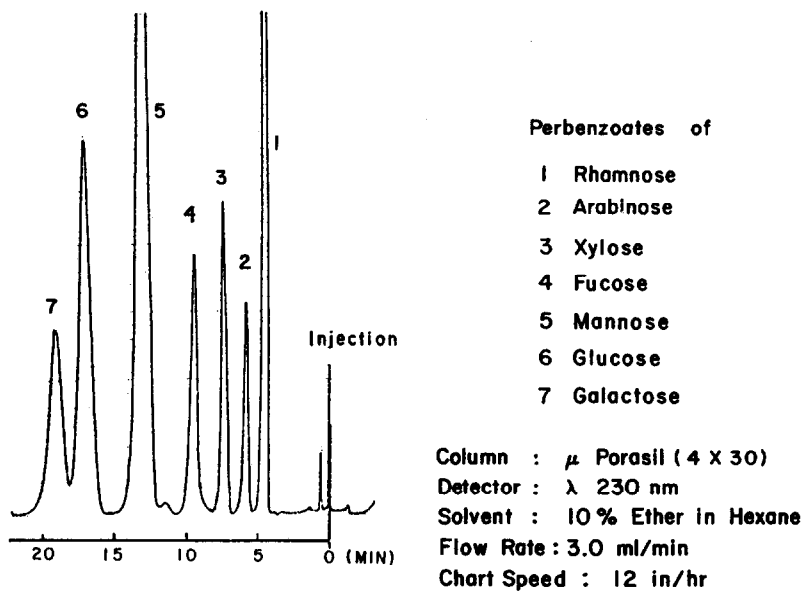


Fig. 3. HPLC separation of perbenzoates (α-anomers) of some common sugars.

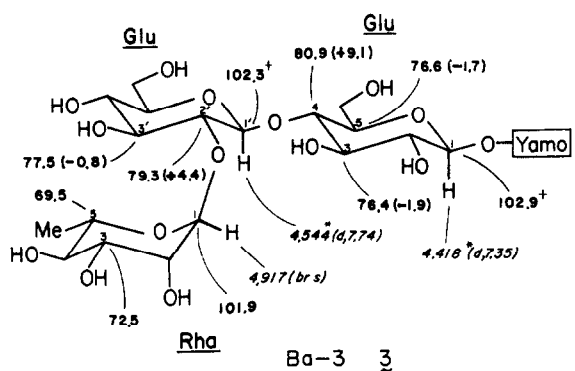


Fig. 4. Pertinent ^{13}C-NMR peaks are shown; +(−) values in parentheses denote downfield (upfield) shifts as compared to methyl β-D-glucopyranoside. Italicised values are ^{1}H-NMR shifts of anomeric protons of B-3 peracetate. Assignments are interchangable between the asterisked protons.

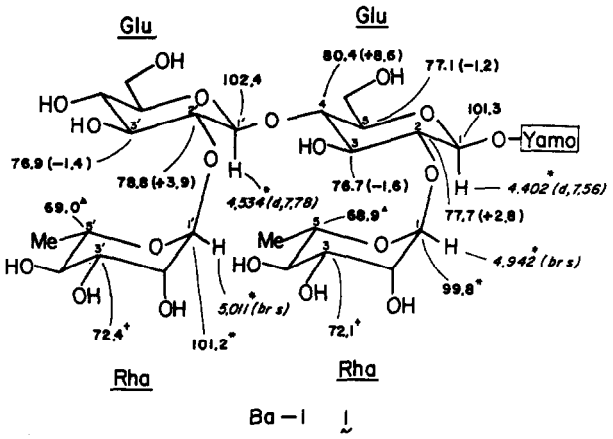


Fig. 5. Pertinent ^{13}C-NMR peaks are shown; +(−) values in parentheses denote downfield (upfield) shifts as compared to methyl β-D-glucopyranoside. Italicised values are ^{1}H-NMR shifts of anomeric protons of Ba-1 peracetate. Assignments are interchangeable between chemical shifts marked with the same sign, e.g. *, †, etc.

Table 2. ^{13}C chemical shifts (δ in ppm) of Ba-1 (1), -2(2), -3(3), -X(4) and -Y(5)

^{13}C	Ba-1[a,b]	Ba-2[a]	Ba-3[a]	Ba-X[a]	Ba-Y[a]
1	37.2	37.4	37.4	37.5	37.4
2	29.8	29.9	30.2	30.0	30.1
3	78.1	78.2	78.2	78.5	78.5
4	38.7	39.6	39.4	39.3	39.4
5	140.6	141.2	141.2	140.9	140.8
6	121.2	121.5	121.6	121.7	121.6
7	32.0	32.1	32.3	32.3	32.3
8	31.5	31.6	31.7	31.7	31.7
9	50.2	50.3	50.3	50.3	50.3
10	36.8	37.1	37.0	37.1	37.1
11	20.8	21.0	21.1	21.2	21.2
12	39.2	39.8	40.0	39.9	39.9
13	40.2	40.4	40.5	40.5	40.4
14	56.5	56.6	56.7	56.7	56.7
15	31.9	32.3	32.2	32.2	32.2
16	80.9	81.1	81.2	81.2	81.2
17	62.6	61.5	61.8	62.2	62.0
18	15.9	16.2	16.3	16.3	16.2
19	19.0	19.3	19.3	19.4	19.3
20	42.3	42.4	42.5	42.5	42.5
21	14.3	14.8	14.8	14.8	14.8
22	109.3	109.6	109.8	109.7	109.8
23	27.2	27.5	27.5	27.6	27.5
24	25.9	26.1	26.2	26.3	26.2
25	26.2	26.4	26.4	26.4	26.4
26	64.9	65.0	65.2	65.1	65.2
27	15.9	16.2	16.3	16.4	16.2
G-1	101.3	101.7	102.9*	102.3	102.8
G-2	77.7	81.4	75.0	74.8*	73.8
G-3	76.7	87.3	76.4	76.5	88.7
G-4	80.4	69.0	80.9	81.2	69.9
G-5	77.1	77.5†	76.6	76.9	77.7
G-6	61.3†	62.7	62.3†	62.5†	62.5†
G-1'	102.4	104.4	102.3*	105.0	104.9
G-2'	78.8	73.8	79.3	74.9*	75.2
G-3'	76.9	77.3†	77.5	78.3	78.3
G-4'	71.9	70.8	71.8	71.6	71.9
G-5'	77.5	76.8	78.5	78.3	78.3
G-6'	61.8†	68.2	62.8†	62.8†	62.8†
R-1	99.8[b]	100.0	101.9		
R-2	71.5	72.3	72.1		
R-3	72.1	72.6	72.5		
R-4	73.3	74.0	74.4		
R-5	68.9	69.4	69.5		
R-6	18.0	18.5	18.4		
R-1'	101.2[b]				
R-2'	71.6				
R-3'	72.4				
R-4'	74.0				
R-5'	69.0				
R-6'	18.1				
X-1		106.1			
X-2		75.1			
X-3		78.0			
X-4		70.7			
X-5		67.3			

[a] Measured in pyridine-d_5.

[b] Assignments are interchangable between the corresponding carbons in the two rhamnose residues.

†* Assignments may be interchanged between the two carbons in the same column.

5 which contains two-glucose residues linked 1→3 as derived from the glycosidation shifts at G-3 (downfield shift), and G-2/G-4 (upfield shift). The MS peak at Ba-2 at *m/e* 745 corresponding to the loss of xylose *and* glucose units leads to two important conclusions: (i) the xylose must be linked to the terminal (or second) glucose unit of Ba-Y 5; and hence (ii) the rhamnose must be linked to the central (or first) glucose unit of Ba-Y 5.

The glycosidation shifts of Ba-2 clearly indicate that *one glucose unit is substituted at position* 6 (68.2 ppm, or downfield shift of +5.4 ppm), whereas the *other glucose unit is substituted at position* 2 (81.4 ppm, or downfield shift of +6.5 ppm). The two glucose anomeric carbon signals appear at 104.4 and 101.7 ppm; when compared to the G-1 shifts in Ba-3 3 (102.9), BaX 4 (102.3) and Ba-Y 5 (102.8), the 104.4 ppm signal has to be assigned to G-1′ (as in Fig. 6). From the low chemical shift of 104.4 ppm, the second glucose unit cannot be substituted at G-2′. Namely, xylose is attached to G-6′ and rhamnose is attached to G-2, i.e., Ba-2 2 is yamogenin O-β-D-xylopyranoxyl - (1→6) - β -D-glucopyranosyl- (1→3) - [α - L - rhamnopyranosyl-(1→2)]-glucopyranoside; this deduction has been established by an independent CD micromethod.[13]

Dilute (5–10 ppm) solutions of balanitin-1, -2 or -3 kill *Biomphalaria glabratus* snails within 24 h, and hence they are potent molluscicides.

EXPERIMENTAL

The spectroscopic measurements were carried out with the following instruments: MS (EI and CI), Finnigan 3300; FD-MS, Varian MAT 731; UV, JASCO UVIDEC-505; CD, JASCO J-40; NMR (^{1}H and ^{13}C), Bruker WM-250. The analytical HPLC was performed on a Waters Model 6000A pump equipped with a U6K injector, a Schoeffel Model SF770 variable wavelength detector. DCCC separation was made on a DCC-A apparatus manufactured by Tokyo Rikakikai, Nishikawa Bldg., Toyama-cho, Kanda, Chiyoda, Tokoyo; 300 glass tubes (length 400 mm, i.d. 2 mm) were used in these studies.

Isolation of balanitin-1 (*Ba*-1), -2 (*Ba*-2) *and* -3 (*Ba*-3). The air-dried root and bark of *Balanites aegyptiaca* was extracted and fractionated as indicated in Results and Discussions (see text). The biologically active methanol extract (2g) was first treated with ethanol, the ethanol extract was evaporated to dryness, and the residue was then treated with acetone in order to precipitate various polysaccharides and concomitant nonpolar inpurities. This partially purified material was chromatographed on a Sephadex LH-20 column (MeOH) to yield 1.5 g of a dark-brown active fraction (fr. 2). TLC ($CHCl_3$/MeOH/H_2O = 6:3:1, lower phase) of this fraction revealed the presence of three major components (Ba-1, R_f 0.32; Ba-2, R_f 0.36; Ba-3, R_f 0.47) accompanied by numerous minor products. The isolation of Ba-1, -2 and -3 could be achieved by conventional open column and preparative thin layer chromatography employing various solvent systems, e.g. EtOAC/EtOH/H_2O (15:5:4), $CHCl_3$/n-PrOH/MeOH/H_2O (45:5:60:40), EtOAC/n-PrOH/H_2O (4:2:7); however, droplet countercurrent chromatography (DCCC) offered a much easier and quicker alternative for this separation. As shown in Fig. 1, the second fraction eluted from the LH-20 column which exhibited the molluscicidal activity was submitted directly to DCCC. Although the main fraction which eluted with the solvent front (CH_2Cl/MeOH/H_2O = 8:13:7, ascending mode) was still a mixture of more polar compounds, Ba-1 (31 mg), Ba-2 (10 mg) and Ba-3 (15 mg) were obtained in nearly pure form. Further purification by DCCC ($CHCl_3$/MeOH/H_2O = 65:35:10, ascending mode) afforded pure balanitine-1, -2 and -3.

Hydrolysis of Ba-1 1, *Ba*-2 2 *and Ba*-3 3. Ba-1, Ba-2 and Ba-3 (3 mg each) were hydrolyzed with 2N HCl (4N HCl:50% dioxane/1:1 (v/v)) on a boiling water bath for 5 h. The reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, 5% $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated to dryness. The aqueous layer was neutralized with Amberlite IR-45 (or Ag_2CO_3) and evaporated *in vacuo* to dryness.

The $CHCl_3$ soluble fraction (sapogenin) was purified by Prep TLC on silica gel using 15% EtOAc/benzene, R_f = 0.30, to afford yamogenin, m.p. 201° (from ethanol), which gave the following physical data: CI-MS(CH_4): 415 $(M+1)^+$ for $C_{27}H_{42}O_3$; EI-MS: 414 (M^+), 355, 345, 342, 139 (base peak), 115; IR ($CHCl_3$): 3400 (OH), 3030 (C=C), 981, 920, 899, 864 cm^{-1} (spirostane); ^{1}H-

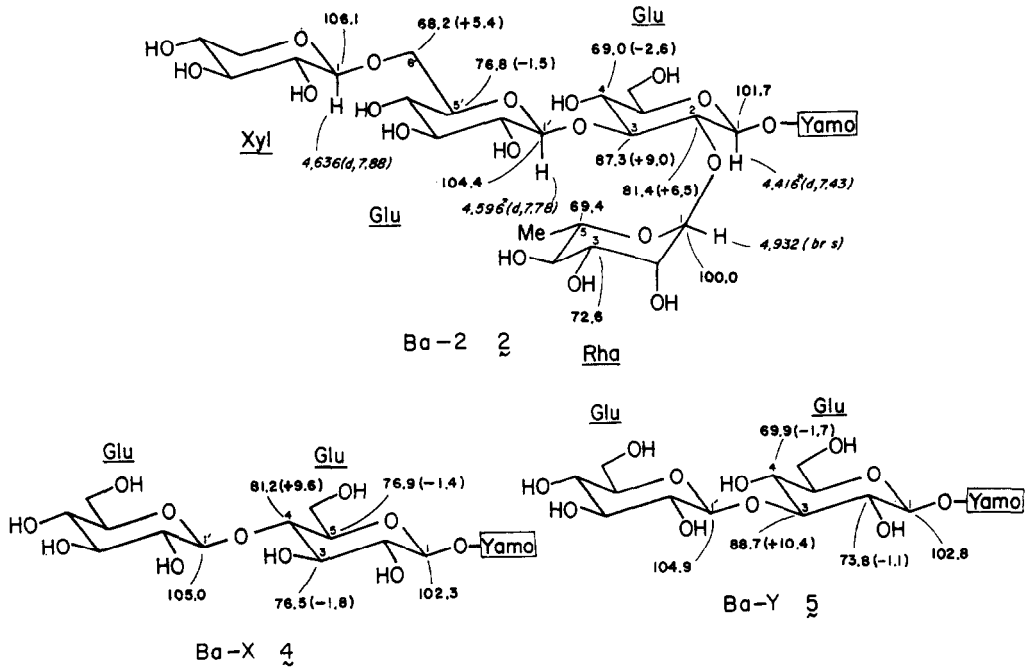


Fig. 6. Pertinent ^{13}C-NMR peaks are shown; +(−) values in parentheses denote downfield (upfield) shifts as compared to methyl β-D-glucopyranoside. Italicised values are ^{1}H-NMR shifts of anomeric protons of Ba-2 peracetate. Assignments are interchangable between the asterisked protons.

NMR ($CDCl_3$): 5.359 (6-H, brd, J = 5.2), 4.429 (16-H, dd, J = 14.4, 7.4), 3.514 (3-H, m), 1.089 (25-Me, d, J = 6.99), 1.034 (10-Me, s), 1.009 (20-Me, d, J = 6.62), 0.797 (13-Me, s); ^{13}C-NMR ($CDCl_3$): 140.8 (C-5), 121.4 (C-6), 109.5 (C-22), 80.9 (C-16), 71.7 (C-3), 65.1 (C-26), 62.0 (C-17), 56.5 (C-14), 50.1 (C-9), 42.3 (C-20), 42.1 (C-4), 40.2 (C-13), 39.8 (C-12), 37.2 (C-1), 36.6 (C-10), 32.0 (C-7), 31.8 (C-15), 31.6 (C-2), 31.5 (C-8) (C-8), 27.1 (C-23), 26.0 (C-25), 25.8 (C-24), 20.9 (C-11), 19.4 (C-19), 16.2 (C-18), 16.0 (C-27), 14.3 (C-21).

The sugars in the water-soluble fractions were identified by TLC by comparison with authentic samples. TLC ($BuOH/AcOH/H_2O$=4:1:5, upper phase): for Ba-1: R_f = 0.16 (glucose), 0.49 (rhamnose); for Ba-2: R_f = 0.16 (glucose), 0.49 (rhamnose), and 0.35 (xylose); for Ba-3: R_f = 0.16 (glucose), 0.49 (rhamnose). TLC ($CHCl_3/MeOH/H_2O$ = 65:35:10, lower phase): for Ba-1: R_f = 0.08 (glucose), 0.30 (rhamnose); for Ba-2: R_f = 0.08 (glucose), 0.30 (rhamnose), and 0.23 (xylose); for Ba-3: R_f = 0.08 (glucose), 0.30 (rhamnose).

Partial Hydrolysis of Ba-1 **1**, *Ba-2* **2** *and Ba-3* **3**. Ba-1 (20 mg), Ba-2 (10 mg) and Ba-3 (10 mg) were partially hydrolysed by boiling in 4% HCl in MeOH for 2 h, respectively. The reaction mixture was lyophilised overnight to dryness and separated on DCCC ($CHCl_3/MeOH/H_2O$ = 65:35:10, ascending mode). Both Ba-1 and Ba-3 gave 13 mg and 6 mg of the same prosapogenin Ba-X **4**, R_f = 0.56 on TLC ($CHCl_3/MeOH/H_2O$ = 6:3:1, lower phase); under the same conditions, Ba-1 has R_f = 0.20 and Ba-3 has R_f = 0.33. On the other hand, partial hydrolysis of Ba-2 yielded 6 mg of a white powder, Ba-Y **5**, R_f = 0.50 ($CHCl_3/MeOH/H_2O$ = 6:3:1, lower phase).

Conversion of yamogenin to diosgenin. To a solution of 1.5 mg of yamogenin, isolated from hydrolysis of Ba-1, -2 and -3, in 0.15 ml of 95% EtOH, was added a mixture of 0.1 ml of 95% EtOH and 0.045 ml of concentrated HCl. After heating at reflux for 12 h, the solution was poured into water and the mixture was extracted with $CHCl_3$. The organic extract was washed with water, 5% $NaHCO_3$ solution and dried over Na_2SO_4. Excess solvent was evaporated *in vacuo* and the residue was purified by PrepTLC (25% EtOAc/benzene) to afford pure diosgenin in 45% yield. TLC (15% EtOAc/benzene): R_f (diosgenin) = 0.262, R_f (yamogenin) = 0.258. Diosgenin was recrystallised from acetone (m.p. 205°), and was identified by comparison with authentic material.

Perbenzoylation of the Hydrolysates Obtained from Hydrolysis of Ba-1 **1**, *-2* **2** and *-3* **3**. The water-soluble fractions (sugars) obtained from the hydrolysis of Ba-1, -2 and -3 were perbenzoylated with benzoyl chloride in pyridine at room temperature for 12 h. The reaction mixture was diluted with MeOH and toluene (or benzene), and the excess solvent was evaporated *in vacuo*. The residue was filtered through a silica gel column with $CHCl_3$ and then injected into HPLC for sugar identification (see Fig. 3).

Acetylation of Ba-1 **1**, *Ba-2* **2** *and Ba-3* **3**. Ba-1, Ba-2 and Ba-3 (5 mg each) were acetylated with acetic anhydride and pyridine for 12 h at room temperature. The reaction mixture was quenched with MeOH and the excess solvent was evaporated *in vacuo* after the addition of toluene (or benzene). The residue was purified by PrepTLC on silica gel (3% $MeOH/CHCl_3$) or flash column chromatography ($CHCl_3$) to give the peracetylated product. TLC (3% $MeOH/CHCl_3$): R_f (Ba-1 peracetate) = 0.44, R_f (Ba-2 peracetate) = 0.40, and R_f (Ba-3 peracetate) = 0.33. Yield: Ba-1 peracetate 87%, Ba-2 peracetate 80% and Ba-3 peracetate 85%.

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