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ISOLATION AND STRUCTURE OF CYTOSTATIC STEROIDAL SAPONINS FROM THE AFRICAN MEDICINAL PLANT *BALANITES AEGYPTICA*[1]

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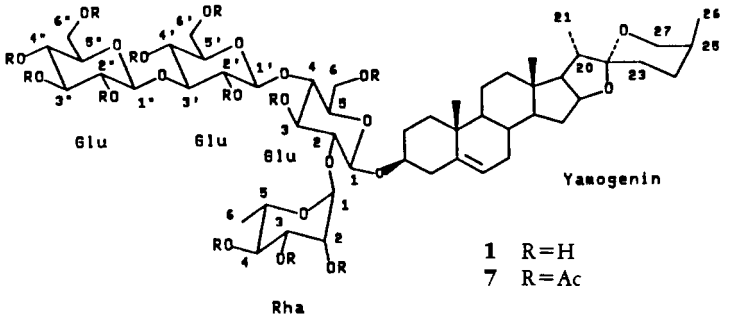
ABSTRACT.—Bioactivity-guided separation of a CH_2Cl_2/MeOH extract of *Balanites aegyptica* afforded four new cytostatic saponins, named balanitins 4 [**1**], 5 [**2**], 6 [**3**], and 7 [**4**]. On the basis of enzymatic hydrolyses and glycosidation nmr chemical shifts employing the peracetates, structures **1–4** were established as yamogenin 3β-*O*-β-D-glucopyranosyl-(1↦3)-β-D-glucopyranosyl-(1↦4)-[α-L-rhamnopyranosyl-(1↦2)]-β-D-glucopyranoside [**1**], yamogenin 3β-*O*-α-L-rhamnopyranosyl-(1↦3)-β-D-glucopyranosyl-(1↦4)-[α-L-rhamnopyranosyl-(1↦2)]-β-D-glucopyranoside [**2**], yamogenin 3β-*O*-β-D-glucopyranosyl-(1↦4)-[α-L-rhamnopyranosyl-(1↦2)]-β-D-glucopyranoside [**3**], and diosgenin 3β-*O*-β-D-xylopyranosyl-(1↦3)-β-D-glucopyranosyl-(1↦4)-[α-L-rhamnopyranosyl-(1↦2)]-β-D-glucopyranoside [**4**].

Balanites aegyptica Del. (Balanitaceae) is widely employed in Africa as a component of various primitive medicines used for abortifacient, antiseptic, antiviral (herpes zoster), antimalarial, molluscicidal, antisyphilitic, and vermifuge purposes (2,3). In 1982 the molluscicidal steroidal saponins balanitins 1–3, isolated from the root and bark of this plant, were reported (4). As part of our evaluation of higher plant antineoplastic and/or cytostatic constituents, we examined the seed components of this plant using the murine P-388 lymphocytic leukemia (PS system) in cell culture (5). A 1978 re-collection (107 kg) of seeds was crushed and extracted using CH_2Cl_2/MeOH (followed by dilution with H_2O) (6,7). The aqueous phase was successively partitioned (8) between MeOH-H_2O (9:1↦4:1↦1:1)↦H_2O and hexane↦CCl_4↦CH_2Cl_2↦*n*-BuOH. The most encouraging PS activity (ED_{50} 17 μg/ml) was located in the *n*-BuOH fraction. Bioassay-directed fractionation employing (8) Sephadex LH-20, Si gel, cc, and hplc afforded four new PS cytostatic constituents **1–4** designated balanitins 4–7 (4).

The mol wt of balanitin 6 [**3**] was deduced as 884 from the peaks at *m/z* 885 $[M + H]^+$ and 907 $[M + Na]^+$ in the fabms. In the ir spectrum, glycoside **3** exhibited absorption bands at 988, 920, and 900 cm^{-1} with the 920 band stronger than the 900 band, characteristic of a 25(*S*)-spirostan (9,10) along with hydroxyl group absorptions at 3430 and 3280 cm^{-1}. Comparison of the ^1H- and ^{13}C-nmr data of glycoside **3** with published values (4,11) suggested a glycoside of yamogenin (see Experimental and Table 1). A choice between yamogenin and diosgenin as the aglycone was based on the chemical shifts of H-27, C-23, and C-27 in the ^1H- and ^{13}C-nmr spectra, respectively, plus the ir bands near 988–900 cm^{-1}. Acetylation of glycoside **3** afforded nonaacetate **5**. The ^1H-nmr signals in the sugar moiety of this derivative were assigned on the basis of the ^1H-^1H homonuclear decoupling technique. That established the presence of two glucose and one rhamnose units (Table 2).

The anomeric configurations of glucose and xylose in the balanitins were deduced as β from the large J ^1H-^1H coupling and small J ^{13}C-^1H coupling values (12) of each anomeric position displayed by the peracetate derivatives. The rhamnose orientation

[1]Number 196 in the series Antineoplastic Agents. For Part 195 see Singh and Pettit (1).



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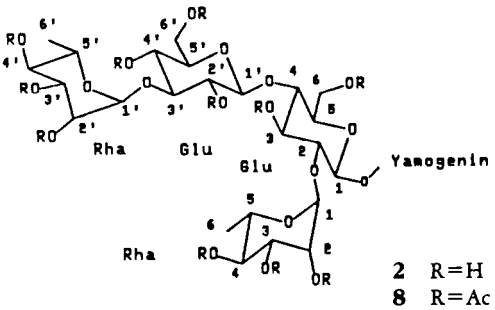
7 R=Ac



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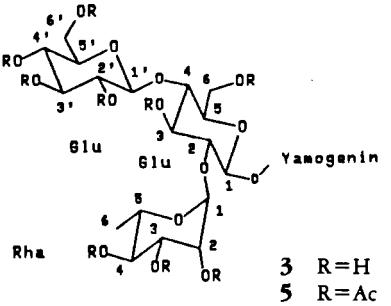
8 R=Ac



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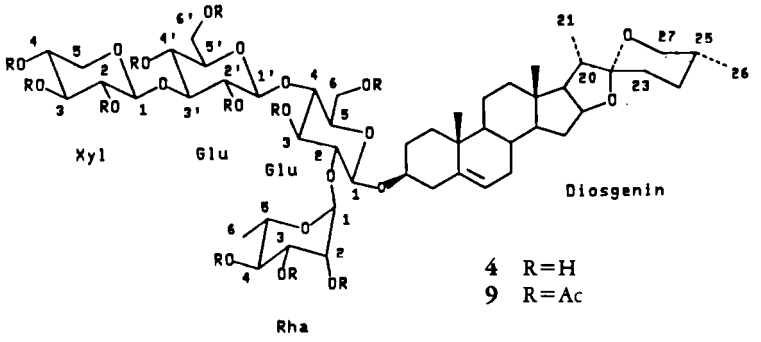
5 R=Ac



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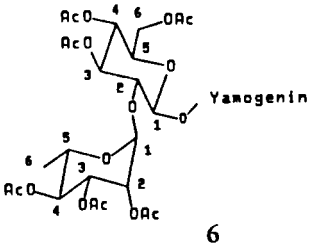
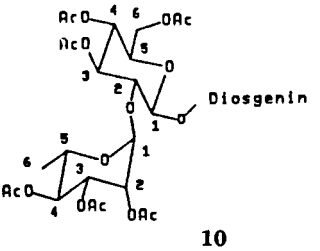
9 R=Ac



6



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was determined as α from the large J ^{13}C-^{1}H coupling value for the anomeric carbon in the corresponding peracetates. Also, good agreement was found between the ^{13}C-nmr chemical shifts (especially for C-3 and C-5 which are easily distinguishable from a β configuration) (13) in those peracetates with the values for methyl *O*-triacetyl-α-L-rhamnopyranoside (Table 3).

Once the glycoside configurations were established, the sequence was next under-

TABLE 1. ^{13}C-nmr Chemical Shifts (δ ppm) of Balanitins 4 [**1**], 5 [**2**], 6 [**3**] and 7 [**4**] in pyridine-d_5.

Carbon	Compound			
	1	**2**	**3**	**4**
C-1	37.48	37.52	37.50	37.51
C-2	29.98	30.12	30.14	30.15
C-3	78.31	78.29	78.29	78.27
C-4	38.92	38.92	38.92	38.95
C-5	140.78	140.81	140.80	140.81
C-6	121.78	121.78	121.78	121.81
C-7	32.32	32.20	32.32	32.24
C-8	31.70	31.70	31.70	31.71
C-9	50.31	50.29	50.30	50.31
C-10	37.16	37.16	37.13	37.15
C-11	21.11	21.10	21.10	21.13
C-12	39.84	39.87	39.86	39.87
C-13	40.43	40.47	40.45	40.48
C-14	56.62	56.65	56.64	56.65
C-15	32.20	32.31	32.21	32.24
C-16	81.18	81.18	81.18	81.12
C-17	62.71	62.75	62.74	62.93
C-18	16.34	16.37	16.32	16.35
C-19	19.38	19.43	19.40	19.42
C-20	42.46	42.46	42.46	42.00
C-21	14.90	14.90	14.89	15.06
C-22	109.73	109.73	109.72	109.27
C-23	26.38	26.42	26.40	31.86
C-24	26.18	26.22	26.21	29.30
C-25	27.54	27.53	27.54	30.63
C-26	65.08	65.07	65.08	66.88
C-27	16.34	16.37	16.32	17.34
G-1	101.75	101.78	101.79	101.80
G-6	61.74[a]	61.79[a]	62.09[a]	61.74[a]
G-1′	104.59	104.75	105.23	104.61
G-6′	61.47[a]	61.56[a]	61.96[a]	61.52[a]
G-1″	105.93			
G-6″	62.53			
R-1	99.95	99.98	100.00	99.98
R-6	18.66	18.63	18.66	18.68
R-1′		102.87		
R-6′		18.63		
X-1				106.36
X-5				62.75
Other sugar units	69.32	69.21	69.45	67.44
	69.46	69.47	71.21	69.01
	71.60	69.88	72.44	69.49
	72.47	72.41	72.77	70.95
	72.78	72.50	74.13	72.47
	73.70	72.64	74.95	72.79
	74.09	72.74	76.19	74.01
	75.56	74.10	77.28	74.16
	76.27	75.14	77.73	75.35
	77.21	76.22	78.12	76.29
	77.65	77.26	78.48	77.25
	78.01	77.65	82.07	77.66
	78.12	78.15		78.14
	78.71	81.33		81.51
	81.48	83.04		87.33
	88.33			

[a]Assignments may be interchanged.

TABLE 2. ^{1}H-nmr Chemical Shifts and Coupling Constants of Balanitins 6 Peracetate [**5**], 4 Peracetate [**7**], 5 Peracetate [**8**], and 7 Peracetate [**9**] in Pyridine-d_5.

Proton	Compound							
	5		**7**		**8**		**9**	
	δ ppm	J ^{1}H-^{1}H	δ ppm	J ^{1}H-^{1}H	δ ppm	J ^{1}H-^{1}H	δ ppm	J ^{1}H-^{1}H
H-3	3.92 m		3.93 m		3.95 m		3.93 m	
H-6	5.52 d	5.0	5.56 d	5.0	5.54		5.55 d	5
H-16	4.51 q	7.0	4.53[a]		4.57 q	7.0	4.55[a]	
H-18	0.87 s		0.88 s		0.88 s		0.89 s	
H-19	1.09 s		1.10 s		1.10 s		1.10 s	
H-21	1.17 d	7.0	1.17 d	6.9	1.17 d	6.9	1.16 d	6.8
H-27	1.09 d	6.5	1.09 d	6.9	1.09 d	6.7	0.71 d	5.3
G-1	4.88 (+0.05)[b] d	8.2	4.79 (−0.04) d	8	4.87 (+0.04) d	8	4.80 (−0.03) d	8
2	*4.03 (−1.43)*[d] dd	8.2, 10	*4.00 (−1.46)* dd	8, 10	*4.03 (−1.43)* dd	8, 10	*4.01 (−1.45)* dd	8.8, 9.9
3	5.74 (+0.01) t	10	5.68 (−0.05) t	10	5.74 (+0.01) t	10	5.68 (−0.05) t	9.9
4	*4.15 (−1.35)* t	10	*4.09 (−1.41)* t	10	*4.13 (−1.37)* t	10	*4.31 (−1.19)* t	9.9
5	3.91 (−0.19) m		3.85 (−0.25) m		3.93 (−0.17) m		3.86 (−0.24) m	
6	4.52 (+0.12) dd	6.4, 12	4.53 (+0.13) dd	6, 11	4.57 (+0.17) dd	6, 12	4.55 (+0.15) dd	5.2, 11
	4.84 (+0.27) br d	12	4.81 (+0.24) br d	11	4.84 (+0.27) br d	12	4.81 (+0.24) br d	11.6
G-1′	5.09 (+0.26) d	8.8	4.87 (+0.04) d	7.8	4.92 (+0.09) d	8.1	4.86 (+0.03) d	8.4
2′	5.42 (−0.04) dd	8.8, 10	5.43 (−0.12) dd	7.8, 10	5.45 (−0.01) dd	8.1, 9.8	5.40 (−0.06) dd	8.4, 10
3′	5.71 (−0.02) t	10	*4.48 (−1.25)* t	10	*4.24 (−1.49)* t	9.8	*4.31 (−1.42)* t	10
4′	5.50 (0) t	10	5.37 (−0.13) t	10	5.40 (−0.10) t	9.8	5.36 (−0.14) t	10
5′	4.16 (+0.06) m		4.04 (−0.06) m		3.99 (−0.11) m		4.02 (−0.08) m	
6′	4.33 (−0.13) dd	3, 12.4	4.32 (−0.08) dd	2, 12.4 }	4.32 (−0.08) br d	14	4.28 (−0.12) br d	12
	4.84 (+0.35) dd	5.2, 12.4	4.69 (+0.12) dd	4.2, 12.4 }[c]	4.65 (+0.08) dd	5, 14	4.65 (+0.08) dd	4, 12
G-1″			5.15 (+0.32) d	8.2				
2″			5.38 (−0.08) dd	8.2, 9.9				
3″			5.75 (+0.02) t	9.9				
4″			5.46 (−0.04) t	9.9				
5″			4.07 (−0.03) m					
6″			4.20 (−0.20) dd	2.2, 12.4 }				
			4.65 (+0.08) dd	4.2, 12.4 }[c]				

R-1	5.51 d	2	5.49 d	2.2	5.51 d	2	5.49 d	1.5
2	5.63 dd	2, 4	5.61 dd	2.2, 3.6	5.63 dd	2, 3.4	5.62 dd	1.5, 4
3	5.84 dd	4, 10	5.84 dd	3.6, 10.5	5.85 dd	3.4, 11	5.84 dd	4, 9.9
4	5.68 t	10	5.68 t	10.5	5.69 t	11	5.68 t	9.9
5	4.90 m		4.88 m		4.89 m		4.88 m	
6	1.50 d	6.3	1.45 d	6.3	1.51 d	6.3	1.50 d	6.3
R-1′					5.22 d	1.7		
2′					5.51 dd	1.7, 3.2		
3′					5.56 dd	3.2, 11.1		
4′					5.54 t	11.1		
5′					4.13 dq	6.3, 11.1		
6′					1.35 d	6.3		
X-1							5.01 d	6.9
2							5.29 dd	6.9, 8.7
3							5.64 t	8.7
4							5.22 dt	6, 8.7
5							4.29 dd	6, 12
6							3.63 dd	8.7, 12
CH_3CO	2.00 (6H)		2.00		1.96		1.98	
	2.02		2.017		2.01		2.017	
	2.04		2.02 (6H)		2.02		2.022	
	2.05 (6H)		2.036 (6H)		2.04		2.03	
	2.18		2.043		2.05		2.04	
	2.21		2.181 (6H)		2.08		2.05	
	2.42		2.184		2.10		2.08	
			2.32		2.12		2.16	
			2.41		2.18		2.18	
					2.45		2.32	
					2.48		2.40	

[a]Overlapping signals.
[b]Values in parentheses denote downfield (+) or upfield (−) shifts as compared to methyl tetraacetylglucoside.
[c]Assignments may be interchanged.
[d]Values in italics represent atoms involved in the glycoside linkages.

taken. The fabms of glycoside **3** gave ions at *m/z* 761 $[M + Na - 146]^+$ and 739 $[M + H - 146]^+$ due to loss of one rhamnose unit, and fragments at *m/z* 745 $[M + Na - 162]^+$ and 723 $[M + H - 162]^+$ arising from the loss of one glucose unit (Table 4) (4). Hence, both rhamnose and glucose were found linked to glucose in turn attached to yamogenin. The points of attachment of the sugar residues were based on glycosidation shifts (14, 15) observed in the peracetate **5**. The ^{13}C-nmr signals in the

TABLE 3. ^{13}C-nmr Chemical Shifts (δ ppm) of Balanitins 6 Peracetate [**5**], 4 Peracetate [**7**], 5 Peracetate [**8**], and 7 Peracetate [**9**] in Pyridine.

Carbon	Compound			
	5	**7**	**8**	**9**
C-1	37.42	37.44	37.42	37.45
C-2	29.97	29.96	29.95	29.93
C-3	78.61	78.51	78.54	78.48
C-4	38.63	38.59	38.59	38.56
C-5	140.39	140.39	140.37	140.36
C-6	122.42	122.49	122.46	122.45
C-7	32.19	32.32	32.31	32.20
C-8	31.67	31.68	31.67	31.65
C-9	50.30	50.33	50.30	50.30
C-10	37.14	37.17	37.15	37.15
C-11	21.18	21.21	21.19	21.19
C-12	39.90	39.93	39.90	39.90
C-13	40.48	40.50	40.48	40.49
C-14	56.66	56.68	56.66	56.66
C-15	32.30	32.21	32.21	32.29
C-16	81.18	81.18	81.18	81.09
C-17	62.74	62.78	62.75	62.92
C-18	16.41	16.42	16.41	16.40
C-19	19.36	19.39	19.36	19.36
C-20	42.48	42.49	42.48	41.99
C-21	14.89	14.92	14.89	15.04
C-22	109.76	109.78	109.73	109.28
C-23	26.39	26.41	26.40	31.81
C-24	26.22	26.23	26.21	29.28
C-25	27.56	27.57	27.56	30.61
C-26	16.32	16.33	16.31	17.34
C-27	65.40	65.13	65.10	66.88
G-1	99.22(−2.67)[a][163.0][b]	99.10(−2.79)[162.4]	99.13(−2.76)[162.0]	99.08(−2.81)[162.1]
2	*76.54(+4.55)*[c]	*76.48(+4.49)*	*76.53(+4.54)*	*76.46(+4.47)*
3	74.80(+1.22)	74.70(+1.12)	74.80(+1.22)	74.68(+1.10)
4	*77.58(+8.43)*	*77.24(+8.09)*	*77.33(+8.18)*	*77.19(+8.04)*
5	73.31(+1.09)	73.35(+1.13)	73.38(+1.16)	73.29(+1.07)
6	62.74[c](+0.35)	62.85[c](+0.46)	62.75[c](+0.36)	62.80[c](+0.41)
G-1′	101.47(−0.42)[162.0]	101.44(−0.45)[162.2]	101.44(−0.45)[162.5]	101.44(−0.45)[162.1]
2′	72.30(+0.31)	73.48(+1.49)	72.64(+0.65)	73.29(+1.30)
3′	73.65(+0.07)	*79.77(+6.19)*	*81.80(+8.22)*	*80.48(+6.90)*
4′	68.56(−0.59)	68.21(−0.94)	69.70(+0.55)	68.42(−0.73)
5′	72.44(+0.22)	72.01[d](−0.21)	72.42(+0.20)	72.42(+0.20)
6′	62.08[c](−0.31)	62.09[c](−0.30)	62.23[c](−0.16)	62.27[c](−0.12)
G-1″		101.52(−0.37)[162.0]		
2″		71.80(−0.19)		
3″		73.67(+0.09)		
4″		68.72(−0.43)		
5″		72.46[d](+0.24)		
6″		62.21[c](−0.18)		
R-1	97.87(−0.91)[174.5]	97.82(−0.96)[176.1]	97.83(−1.02)[174.0]	97.79(−1.06)[176.0]
2	71.01(+1.02)	71.04(+1.05)	71.01(+0.84)	71.01(+0.84)
3	69.40(−0.27)	69.40(−0.27)	69.38(−0.48)	69.41(−0.45)
4	71.64(+0.58)	71.65(+0.59)	71.64(+0.41)	71.62(+0.39)
5	67.11(+0.56)	67.13(+0.58)	67.11(+0.40)	67.10(+0.39)
6	17.69(+0.33)	17.70(+0.34)	17.69(+0.04)	17.67(+0.02)
R-1′			99.95(+1.10)[172.3]	
2′			71.01(+0.84)	
3′			70.29(+0.43)	
4′			69.63(−1.60)	
5′			67.92(+1.21)	
6′			17.48(−0.17)	

TABLE 3. (Continued).

Carbon	Compound			
	5	**7**	**8**	**9**
X-1				101.67 (−0.36) [161.5]
2				71.16 (−0.40)
3				72.03 (−0.30)
4				69.41 (−0.16)
5				62.18[c] (−0.17)
CH_3CO	20.43 (3C)	20.44 (3C)	20.51 (3C)	20.54 (6C)
	20.58 (3C)	20.57 (5C)	20.58 (4C)	20.78 (3C)
	20.67	20.80 (3C)	20.77	21.01
	20.77	21.05 (2C)	20.88	
	21.10		21.10 (2C)	
CH_3CO	169.47	169.39	169.41	169.39
	169.67	169.58	169.78	169.52
	170.12	169.68 (2C)	169.92	169.67
	170.28 (4C)	170.17	170.12 (2C)	169.98
	170.49	170.29 (2C)	170.26 (3C)	170.12
	170.57	170.37 (2C)	170.59 (2C)	170.26 (3C)
		170.48	170.65	170.51
		170.55		170.74
		170.78		

[a]Values in parentheses denote downfield (+) or upfield (−) shifts as compared to methyl tri- or tetra-acetylglycoside.
[b]J ^{13}C-^{1}H (Hz).
[c,d]Assignments with the same superscript for each compound may be interchanged.
[e]Values in italics denote glycoside linkage carbon atoms.

sugar moiety of peracetate **5** were assigned by correlation with the fully assigned proton signals in the ^{1}H-^{13}C heteronuclear shift-correlated (HETCOR) 2D nmr spectrum (Table 3). The C-2 and C-4 signals (δ 76.54 and 77.58 ppm) in one glucose unit appeared shifted downfield by 4.55 and 8.43 ppm, respectively, relative to methyl *O*-tetraacetylglucoside. This evidence indicated that the glycosidic linkages were at the glucose 2 and 4 positions.

Enzymatic hydrolysis of trisaccharide **3** with cellulase followed by acetylation gave hexaacetate **6**, where the sugar side chain consisted of one glucose and one rhamnose (deduced from the ^{1}H-nmr spectrum). The proton signal of the glucose 2-position in the hexaacetate **6** was found shifted upfield by 1.34 ppm, relative to methyl *O*-tet-

TABLE 4. Fabms for Balanitins 4 [**1**], 5 [**2**], 6 [**3**], and 7 [**4**].[a]

Compound			
1	**2**	**3**	**4**
1069 $[M+Na]^+$	1053 $[M+Na]^+$	907 $[M+Na]^+$	1039 $[M+Na]^+$
1047 $[M+H]^+$	1031 $[M+H]^+$	885 $[M+H]^+$	1017 $[M+H]^+$
923 $[M+Na-146]^+$	907 $[M+Na-146]^+$	761 $[M+Na-146]^+$	885 $(M+H-132]^+$
901 $[M+H-146]^+$	885 $(M+H-146]^+$	739 $[M+H-146]^+$	
			871 $[M+H-146]^+$
907 $[M+Na-162]^+$	739 $(M+H-292]^+$	745 $[M+Na-162]^+$	
885 $[M+H-162]^+$		723 $[M+H-162]^+$	761 $[M+Na-278]^+$
	723 $[M+H-308]^+$		739 $[M+H-278]^+$
723 $[M+H-324]^+$			
	578 $[M+H-452]^+$		745 $[M+Na-294]^+$
577 $[M+H-470]^+$			723 $[M+H-294]^+$

[a]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; 324, two glucose; 452, two rhamnose + glucose; 470, two glucose + rhamnose.

raacetylglucoside (Table 5). In turn this indicated that rhamnose was linked to the 2 position in glucose derivative **6**. Furthermore, the proton signals at the glycosidic linkage position were shifted upfield by ca. 1.2~1.5 ppm for glycosides **7–9** (Table 2). Based on this evidence, the structure of peracetate **5** was elucidated, and balanitin 6 was assigned structure **3**.

TABLE 5. ^{1}H-nmr Chemical Shifts and Coupling Constants of Balanitin 6 Hydrolyzate Peracetate [**6**] and Balanitin 7 Hydrolyzate Peracetate [**10**] in Pyridine-d_5.

Proton	**6**		**10**	
	δ ppm	J ^{1}H-^{1}H	δ ppm	J ^{1}H-^{1}H
H-3	3.93 m		3.93 m	
H-6	5.43[a]		5.43[a]	
H-16	4.55 m		4.55 m	
H-18	0.87 s		0.89 s	
H-19	1.10 s		1.10 s	
H-21	1.17 d	6.9	1.16 d	6.9
H-27	1.09 d	6.8	0.71 d	5.5
G-1	5.06 (+0.23)[b] d	7.8	5.06 (+0.23) d	7.8
2	4.12 (−1.34) dd	7.8, 9.6	4.12 (−1.34) dd	7.8, 9.6
3	5.83 (+0.10) t	9.6	5.83 (+0.10) t	9.6
4	5.44 (−0.06) t	9.6	5.44 (−0.06) t	9.6
5	4.12 (+0.02) ddd	3, 4.8, 9.6	4.12 (+0.02) ddd	3, 4.8, 9.6
6	4.42 (+0.02) dd	3, 12	4.42 (+0.02) dd	3, 12
	4.65 (+0.08) dd	4.8, 12	4.65 (+0.08) dd	4.8, 12
R-1	5.45 d	1.8	5.45 d	1.8
2	5.60 dd	1.8, 3.3	5.60 dd	1.8, 3.3
3	5.80 dd	3.3, 9.9	5.80 dd	3.3, 9.9
4	5.68 t	9.9	5.68 t	9.9
5	4.88 m	6.3, 9.9	4.88 dq	6.3, 9.9
6	1.50 d	6.3	1.50 d	6.3
MeCO	2.02		2.02	
	2.03		2.03	
	2.05 (6H)		2.05 (6H)	
	2.17		2.17	
	2.21		2.21	

[a]Overlapping signals.

[b]Values in parentheses denote downfield (+) or upfield (−) shifts as compared to methyl tetraacetylglucoside.

By application of the preceding structural approach, balanitin 4 [**1**] was shown to be a glycoside of yamogenin by analysis of the ir and ^{1}H- and ^{13}C-nmr spectra (Table 1). On acetylation steroidal saponin **1** gave dodecaacetate **7**. The peracetate ^{1}H-nmr spectrum indicated the presence of three glucose and one rhamnose units (Table 2). The fabms fragmentation of balanitin 4 suggested that rhamnose and glucose (bonded to a second glucose) were linked to glucose attached to yamogenin (Table 4). In the ^{13}C-nmr spectrum of the peracetate **7**, the C-2 and C-4 signals (δ 76.48 and 77.24 ppm) from one glucose unit and the C-3 signal (δ 79.77 ppm) of another glucose unit were shifted downfield by 4.49, 8.09, and 6.19 ppm, respectively (Table 3). Enzymatic hydrolysis of saponin **1** with naringinase followed by acetylation afforded balanitin 6 peracetate [**5**] and allowed assignment of structure **1** to balanitin-4.

That balanitin 5 [**2**] corresponded to a glycoside of yamogenin was deduced from the ir, ^{1}H- and ^{13}C-nmr spectra (Table 1). On acetylation saponin **2** afforded unde-

caacetate **8**, and the ^{1}H-nmr spectrum indicated the presence of two glucose and two rhamnose units (Table 2). Fragments in the fabms of glycoside **2** suggested that a rhamnose segment and a glucose unit attached to another rhamnose unit were in turn linked to glucose bonded to yamogenin (Table 4). The ^{13}C-nmr spectrum of peracetate **8** provided more useful data. The C-2 and C-4 signals (δ 76.53 and 77.33 ppm) of one glucose and the C-3 signals (δ 81.80 ppm) of another glucose appeared shifted downfield by 4.54, 8.18 and 8.22 ppm, respectively (Table 3). Enzymatic hydrolysis of steroidal saponin **2** with naringinase followed by acetylation gave peracetate **5**. The sum of this evidence led to structure **2** for balanitin 5.

In its ir spectrum, balanitin 7 [**4**] exhibited important bands at 982, 920, and 900 cm^{-1}. The 900 adsorption band was stronger than the 920 cm^{-1} band, typical of a 25(*R*)-spirostan (9,10). In addition, the ^{1}H- and ^{13}C-nmr spectral data suggested that saponin **4** was a glycoside of diosgenin (Table 1). Acetylation of glycoside **4** gave undecaacetate **9**. The ^{1}H-nmr spectrum of the peracetate indicated the presence of two glucose, one rhamnose, and one xylose unit (Table 2). Application of fabms to saponin **4** suggested that the rhamnose unit and a glucose segment attached to xylose were each linked to glucose which in turn was bonded to diosgenin (Table 4). Enzymatic takadiastase hydrolysis of **4** followed by acetylation provided hexaacetate **10**. General features of the ^{1}H-nmr spectrum of the peracetate were identical with those of peracetate **6** except that the proton signals of the aglycone corresponded to those of diosgenin (Table 5). Therefore, peracetate **10** was assumed to have a structure in which the yamogenin aglycone of saponin **6** was replaced by diosgenin. As expected in peracetate **9**, the C-2 and C-4 ^{13}C-nmr signals (δ 76.46 and 77.19) of one glucose unit and the C-3 signal (δ 80.48) of the second glucose unit were respectively shifted downfield by 4.47, 8.04, and 6.90 ppm (Table 3). The evidence summarized above allowed assignment of structure **4** to balanitin 7.

Balanitins 4–7 exhibited cytostatic activity against P-388 cultured cells as shown in Table 6. Further evaluation of these cell growth inhibitory plant constituents is in progress.

TABLE 6. Evaluation of Balanitins 4 [**1**], 5 [**2**], 6 [**3**], and 7 [**4**] Against the P-388 Lymphocytic Leukemia Cell Line.

Steroidal Saponin	P-388 (ED_{50} μg/ml)[a]
1	0.41
2	2.40
3	0.21
4	0.22
5-Fluorouracil (standard)	0.08

[a]DMSO was used as vehicle.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—General experimental procedures have been described previously (8). All mp's were determined employing a Yanagimoto micromelting point apparatus. Optical rotations were measured with a JASCO ORD/UV-5 spectropolarimeter. Hplc separations were performed using a Waters ALC-200 instrument equipped with a differential refractometer (R 401) and Shim-pack PREP-ODS (25 cm × 20 mm i.d.). Each of the balanitins was colorless. Spectroscopic measurements were conducted with the following instruments: ir, Hitachi EPI-G2; nmr (^{1}H and ^{13}C), Varian XL-300; fabms, Zab-se.

The three enzymes employed for cleavage of the glycoside bonds were obtained from commercial sources as noted at end of the acknowledgment section. These enzymes are known to hydrolyze the follow-

ing specific disaccharide linkages. The specificity of these hydrolytic cleavages has been confirmed by the following nmr studies, such as recorded here: for cellulase 1,6-glc-glc (16–19), 1,2-rha-glc (18), 1,3-glc-ara (19), 1,2-rha-ara (19), 1,6-xyl-glc (17), and 1,3-xyl-ara (19); for takadiastase 1,4-glc-rha (20), 1,4-glc-xyl (20,21), and 1,4-xyl-rha (22); for naringinase, 1,2-glc-xyl (21).

SEED EXTRACTIONS.—Dried, finely ground seeds (36 kg) of *B. aegyptica* (a voucher specimen of which is maintained by the ASU-CRI) were extracted with CH_2Cl_2-MeOH (1:1) (150 liters) at ambient temperature. The extract was separated into CH_2Cl_2 and aqueous phases on addition (25% by volume) of H_2O. The aqueous fraction was evaporated in vacuo and freeze-dried to give the PS active (ED_{50} 6.0 μg/ml) extract (1251.3 g).

SOLVENT PARTITION SEQUENCE.—The PS cell line active aqueous extract (542.5 g) was successively partitioned between MeOH-H_2O (9:1) and hexane, MeOH-H_2O (4:1) and CCl_4, and MeOH-H_2O (1:1) and CH_2Cl_2. Removal of solvents gave the hexane (350 mg), CCl_4 (30.2 g), CH_2Cl_2 (8 g), and H_2O (504.5 g) fractions. The remaining H_2O fraction was further partitioned between *n*-BuOH and H_2O (1:1) to afford 166.5 g and 337.1 g fractions, respectively, on evaporation.

ISOLATION OF BALANITINS 4 [**1**], 5 [**2**], 6 [**3**], AND 7 [**4**].—The *n*-BuOH extract (166.5 g, PS, ED_{50} 17 μg/ml) was subjected to steric exclusion chromatography on a Sephadex LH-20 column, using MeOH as eluent. The first fraction (96.5 g) was rechromatographed on a Sephadex LH-20 column, using CH_2Cl_2-MeOH (2:3) as eluent. The second fraction (79.4 g) from this step was chromatographed on a Si gel column previously washed (successively) with the lower layer of $CHCl_3$-MeOH-H_2O (65:35:10) and MeOH followed by gradient elution with $CHCl_3$→MeOH. Elution with $CHCl_3$-MeOH (9:1) gave two P-388 cytostatic fractions (0.743 g and 7.62 g). The first fraction (0.743 g) was subjected to hplc with MeOH-H_2O (9:1) to afford saponins **3** (76.7 mg) and **4** (61.7 mg). The second fraction (7.62 g) from the Si gel column was repeatedly chromatographed on a Si gel column treated as noted above. Elution with $CHCl_3$-MeOH (9:1) gave saponins **1** (0.196 g) and **2** (0.32 g).

BALANITIN 4 [**1**].—Needles: mp 271–272° (from MeOH); $[\alpha]^{20}D$ −61° (*c* = 0.90, pyridine); fabms *m/z* 1069 [M ($C_{51}H_{82}O_{22}$) + Na]$^+$; ir ν KBr max cm^{-1} 3380 (OH), 988, 920, 900 [intensity 920>900, (25*S*)-spiroketal]; and ^{1}H-nmr (C_5D_5N) ppm 0.83 (3H, s, H-18), 1.06 (3H, s, H-19), 1.09 (3H, d, *J* = 6.9 Hz, H-27), 1.16 (3H, d, *J* = 6.9 Hz, H-21), 1.78 (1H, d, *J* = 6.0 Hz, rha), 3.35, 5.34 (OH, CH-O-), 5.45 (1H, d, *J* = 4.0 Hz, H-6), 6.25 (1H, d, *J* = 2.0 Hz, R-1).

Acetylation of balanitin 4 [**1**] (22 mg) with Ac_2O/pyridine followed by chromatography on Si gel and elution with 0.5% MeOH in $CHCl_3$ afforded dodecaacetate **7** (15 mg) as colorless needles; mp 135–137° (from iPrOH), $[\alpha]^{20}D$ −59° (*c* = 1.1, $CHCl_3$); fabms *m/z* 1551 [M($C_{75}H_{106}O_{34}$) + H]$^+$; and ir ν max ($CHCl_3$) cm^{-1} 1730 (OAc).

BALANITIN 5 [**2**].—Needles: mp 203–207° (from MeOH); $[\alpha]^{20}D$ −78° (*c* = 1.47, pyridine); fabms *m/z* 1030 [M($C_{51}H_{82}O_{21}$) + Na]$^+$; ir ν max (KBr) cm^{-1} 3380 (OH), 990, 920, 900 [intensity 920>900, 25(*S*)-spiroketal]; ^{1}H-nmr (C_5D_5N) ppm 0.83 (3H, s, H-18), 1.04 (3H, s, H-19), 1.08 (3H, d, *J* = 7.2 Hz, H-27), 1.15 (3H, d, *J* = 6.9 Hz, H-21), 1.69 (3H, d, *J* = 6.3 Hz, R-6′), 1.75 (3H, d, *J* = 6.3 Hz, R- Acetylation of saponin **2** (62 mg) with Ac_2O/pyridine followed by chromatography on Si gel and elution with $CHCl_3$ afforded undecaacetate **8** (51 mg) as colorless needles: mp 145–149° (from iPrOH); $[\alpha]^{20}D$ −51° (*c* = 1.28, $CHCl_3$); fabms *m/z* 1493 [M($C_{73}H_{104}O_{32}$) + H]$^+$; ir ν max ($CHCl_3$) cm^{-1} 1740 (OAc).

BALANITIN 6 [**3**].—Needles: mp 278–280° (from MeOH); $[\alpha]^{20}D$ −89° (*c* = 0.67, pyridine); fabms *m/z* 907 [M($C_{45}H_{72}O_{17}$) + Na]$^+$; ir ν max (KBr) cm^{-1} 3430, 3280 (OH), 988, 920, 900 [intensity 920>900, 25(*S*)-spiroketal]; ^{1}H-nmr (C_5D_5N) ppm 0.84 (3H, s, H-18), 1.06 (3H, s, H-19), 1.09 (3H, d, *J* = 6.9 Hz, H-27), 1.16 (3H, d, *J* = 6.9 Hz, H-21), 1.78 (3H, d, *J* = 6.3 Hz, R-6), 3.35–5.35 (OH, CH-O-), 5.23 (1H, d, *J* = 4.3 Hz, H-6), 6.28 (1H, d, *J* = 2.0 Hz, R-1).

When glycoside **3** (30 mg) was acetylated with Ac_2O/pyridine and the product chromatographed on Si gel (elution with $CHCl_3$) nonaacetate **5** (25 mg) was obtained as colorless needles, mp 132–134° (iPrOH); $[\alpha]^{20}D$ −54° (*c* = 1.47, $CHCl_3$); fabms *m/z* 1263 [M($C_{63}H_{90}O_{26}$) + H]$^+$; ir ν max ($CHCl_3$) cm^{-1} 1730 (OAc).

BALANITIN 7 [**4**].—Needles, mp 273–280° (from MeOH), $[\alpha]^{20}D$ −83° (*c* = 0.83, pyridine); fabms *m/z* 1039 [M($C_{50}H_{80}O_{21}$) + Na]$^+$; ir ν max KBr cm^{-1} 3380 (OH), 982, 920, 900 [intensity 920<900, 25(*R*)-spiroketal]; and ^{1}H-nmr (C_5D_5N) ppm 0.70 (3H, d, *J* = 5.5 Hz, H-27), 0.84 (3H, s, H-18), 1.06 (3H, s, H-19), 1.15 (3H, d, *J* = 7.2 Hz, H-21), 1.79 (1H, d, *J* = 6.3 Hz, R-6), 3.35–5.30 (OH, CH-O-), 5.29 (1H, d, *J* = 4.0 Hz, H-6), 6.26 (1H, d, *J* = 2.0 Hz, R-1).

The peracetate derivative of saponin **4** (32 mg) was prepared and purified as summarized above for balanitin 6 peracetate [**5**] to afford undecaacetate **9** (26 mg) as colorless needles: mp 137–140° (iPrOH);

$[\alpha]^{21}D -58°$ ($c = 1.37$, $CHCl_3$); fabms *m/z* $[M(C_{72}H_{102}O_{32}) + H]^+$ 1479; ir ν max ($CHCl_3$) cm^{-1} 1740 (OAc).

ENZYMATIC HYDROLYSIS OF BALANITIN 4 [**1**] WITH NARINGINASE.—A suspension of glycoside **1** (28 mg) in EtOH (8 ml) and Na_2HPO_4/citric acid buffer (pH 4.0, 20 ml) was treated with naringinase (200 mg). The mixture was stirred at 37° for 8 days and extracted with *n*-BuOH. The *n*-BuOH solution was evaporated under reduced pressure, and the residue was acetylated with Ac_2O/pyridine. After purification by Si gel cc (elution with $CHCl_3$), balanitin 6 peracetate [**5**] was obtained (3.2 mg).

ENZYMATIC HYDROLYSIS OF BALANITIN 5 [**2**] WITH NARINGINASE.—A suspension of saponin **2** (21 mg) was treated with naringinase (200 mg) as described in the preceding experiment. Stirring at 37° was continued for 4 days. Following isolation and acetylation, 4 mg of balanitin 6 peracetate [**5**] was realized.

ENZYMATIC HYDROLYSIS OF BALANITIN 6 [**3**] WITH CELLULASE.—A suspension of saponin **3** (26 mg) in EtOH (8 ml) and Na_2HPO_4/citric acid buffer (pH 4.0, 20 ml) was treated with cellulase (200 mg). The mixture was stirred at 37° for 9 days and extracted with *n*-BuOH. The *n*-BuOH was evaporated in vacuo, and the residue was acetylated with Ac_2O/pyridine. Purification by Si gel cc (in $CHCl_3$) provided hexaacetate **6** (4.1 mg) as a colorless oil: fabms *m/z* $[M(C_{51}H_{74}O_{18}) + H]^+$ 975; ir ν max ($CHCl_3$) cm^{-1} 1730 (OAc).

ENZYMATIC HYDROLYSIS OF BALANITIN 7 [**4**] WITH TAKADIASTASE.—The preceding experiment was repeated with saponin **4** (17 mg) and crude takadiastase (100 mg), except for stirring at 37° for 8 days. Hexaacetate **10** (5 mg) was isolated as a colorless oil: fabms *m/z* 975 $[M(C_{51}H_{74}O_{18}) + H]^+$; and ir ν max ($CHCl_3$) cm^{-1} 1730 (OAc).

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