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PREGNANE GLYCOSIDES FROM FRUITS OF *BALANITES AEGYPTIACA*

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Key Word Index—*Balanites aegyptiaca*; Balanitaceae; balagyptin; pregnane glycosides.

Abstract—From the mesocarp of *Balanites aegyptiaca* fruits, two pregnane glycosides were isolated. One is new and identified as pregn-5-ene-3β,16β,20(*R*)-triol 3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside (balagyptin), while the other is known and assigned as pregn-5-ene-3β,16β,20(*R*)-triol 3-*O*-β-D-glucopyranoside.

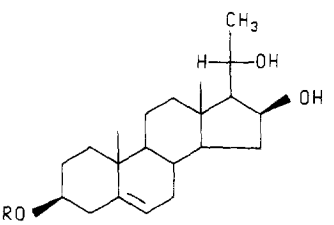
INTRODUCTION

Balanites aegyptiaca Del. is a widely distributed African plant of medicinal interest [1–4]. Our previous work on this plant revealed isolation and identification of new hypoglycaemic steroidal glycosides in addition to known furostanol saponins [5, 6]. This paper deals with isolation and characterization of two pregnane glycosides from the mesocarp of the fruits.

RESULTS AND DISCUSSION

The methanolic extract of the mesocarp of *B. aegyptiaca* fruits was defatted with *n*-hexane and fractionated on a column of Diaion HP-20. From the 80% methanol eluate, two compounds designated **1** and **2** were isolated.

The acid hydrolysis of compound **1** with 2 M HCl [5] produced D-glucose and L-rhamnose in addition to the aglycone **1a**. The sugars were identified by TLC alongside with authentic samples and GLC of their trimethylsilyl derivatives. ^{13}C NMR spectral data of **1** (Table 1) were almost similar to those reported for pregn-5-ene-3β,16β,20(*R*)-triol previously isolated form *Periploca sepium* [7]. However, the downfield shift of C-3 (δ78.3) as well as the upfield shifts of C-2 and C-4 (δ30.0 and 37.0, respectively) indicated that it is a monodesmosidic glycoside with one sugar moiety attached to C-3 [8]. This was confirmed by comparison of the ^{13}C NMR spectrum of **1** with that of **1a** (Table 1). The upfield shift of C-3 (δ71.5) was obvious in the latter. At the same time, inspection of the ^{1}H NMR spectrum of **1** revealed the presence of three methyl groups at positions C-18, C-19 and C-21 from the signals at δ0.93 (*s*), 1.02 (*s*) and 1.2 (*d*), respectively, in addition to two methyl groups at δ1.7 (*d*) for two rhamnosyl units. Conversely, the signals at δ104.2, 101.0 and 100.0 in the ^{13}C NMR spectrum were clear for the anomeric carbons of one β-glucosyl and 2α-rhamnosyl



	R
1	Glc(2→Rha, 6→Rha)
1a	H
2	Glc

Glc: β-D-glucopyranose Rha: α-L-rhamnopyranose

units. The α-configuration of the L-rhanmosyl units was also indicated from the upfield shifts of C-5 (69.5, 69.2) [9], while the β-configuration of D-glucose was confirmed from the coupling constant (7.1 Hz) of the doublet signal at δ5.1 for the anomeric proton in the ^{1}H NMR spectrum [10]. Inspection of other sugar signals in the ^{13}C NMR spectrum revealed the presence of two terminal rhamnopyranosyl units and one 2,6-disubstituted glucopyranosyl unit [8]. The substitution at C-2 and C-6 of glucose was obvious from their downfield shifts (δ82.1 and 66.7, respectively) and confirmed by methylation analysis which revealed the presence of a 2,6-linked glucosyl unit, together with the terminal rhamnosyl units. Furthermore, the negative FAB-mass spectrum of **1** exhibited a peak at *m/z* 787 consistent with the molecular formula $C_{39}H_{64}O_{16}$ $[M-H]^-$ and other significant peaks were at *m/z* 641 $[M-H-Rha]^-$ and 333 $[M-H-2Rha-Glc]^-$. Consequently, the structure of compound **1** was assigned as pregn-5-ene-3β,16β,20(*R*)-triol 3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside and termed balagyptin.

In the ^{13}C NMR spectrum of compound **2** (Table 1), the chemical shifts of the aglycone moiety could be

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Table 1. ^{13}C NMR spectral data of compounds **1**, **1a** and **2** (100 MHz, pyridine-d_5)

C	1	1a*	2
1	37.4[a]	37.2	37.1
2	30.0	31.4	30.0
3	78.3	71.5	78.0
4	37.0[a]	42.0	37.1
5	141.3	140.4	140.9
6	121.5	121.6	121.8
7	31.8[c]	31.4[c]	31.6[c]
8	31.4[c]	31.1[c]	31.3[c]
9	50.8	50.3	50.8
10	37.0[a]	36.8	36.9
11	20.9	20.8	21.0
12	39.0	39.2	38.9
13	41.5	41.3	41.6
14	54.8	54.5	54.8
15	35.6	35.4	35.3
16	73.7	73.5	73.8
17	62.8	63.0	62.9
18	15.9	14.1	15.6
19	19.3	19.4	19.0
20	67.2	66.9	67.0
21	23.9	23.7	23.9
Glc			
1	104.2		105.2
2	82.1		75.4
3	76.2		78.4
4	71.6[b]		71.8
5	77.3		78.3
6	66.7		61.8
Rha			
1	101, 100		
2	72.4, 71.2[b]		
3	73.7, 72.8		
4	75.0, 74.2		
5	69.5, 69.2		
6	18.6, 18.6		

*In $CDCl_3$.

[a, b, c]Values may be interchangeable in each column.

superimposed on those of **1** while the signals of the sugar moiety were consistent with one β-glucopyranosyl unit. The identity of the sugar was confirmed by acid hydrolysis which provided D-glucose. The β-configuration was also clear from the doublet ($J = 7.0$ Hz) at $\delta 5.2$ for the anomeric proton in the ^1H NMR spectrum. The FAB-mass spectrum of **2** revealed a peak at m/z 495 $[M - H]^-$ consistent with the molecular formula $C_{27}H_{44}O_8$ and m/z 333 $[M - H - Glc]^-$. Therefore, the structure of compound **2** was identified as pregn-5-ene-3β,16β,20(*R*)-triol 3-*O*-β-D-glucopyranoside. This is the first report of the isolation of pregnane glycosides from the family Balanitaceae.

EXPERIMENTAL

NMR spectra were recorded in C_5D_5N using a JEOL JNM-GX-400 spectrometer (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) with tetramethylsilane (TMS) as int. standard. FAB-MS and GC-MS were recorded by a Kratos MS 80RF mass spectrometer. GLC conditions: Perkin-Elmer GC apparatus equipped with flame ionization detector, carrier gas He (3 ml min^{-1}), OV-101 column (0.25 mm i.d. × 30 m), column temp. 200°, injection temp. 225°. R_t D-glucose 6.2 min and L-rhamnose 2.3 min. TLC was carried out on precoated silica gel plates (Kieselgel 60 F_{254}, Merck). For CC, silica gel G (E. Merck), Lichroprep RP-8 (40–63 μm, Merck) and Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd) were used. The solvent systems were: (I) $CHCl_3$–MeOH–H_2O (65:35:10, lower phase); (II) 65% MeOH; (III) 55% MeOH and (IV) $CHCl_3$–MeOH–H_2O (60:40:10). Spray reagents: 10% H_2SO_4 and triphenyltetrazolium chloride (TTC). Plant material was collected in May, 1993 from the western desert of Egypt. The identity of the plant was confirmed by Prof. A. Fayed, Fac. of Science, Assiut Univ. A voucher sample is kept in the Herbarium of the Fac. of Pharmacy, Assiut Univ., Egypt.

Extraction and isolation. The mesocarp (750 g) of *B. aegyptiaca* fruits was extraced with MeOH. After removal of the solvent by evapn the residue was defatted with *n*-hexane. The 'mother' liquor was chromatographed on Diaion HP-20 and eluted with H_2O, 40% MeOH, 80% MeOH, MeOH and H_2O successively. 80% MeOH eluate was chromatographed on silica gel (system I) affording four fractions. Fraction 4 was subjected to CC on RP-8 using system II followed by prep. HPLC on ODS column (system III) to provide two compounds designated **1** and **2**.

Compound **1** (balagyptin). Powder (65 mg), 231–233°, $[\alpha]_D^{23}$ + 11.3° (MeOH; *c* 0.2) and R_f 0.15 (system I). ^1H NMR (C_5D_5N): δ0.93 (3H, *s*, Me-18), 1.02 (3H, *s*, Me-19), 1.2 (3H, *d*, $J = 6.2$ Hz, Me-21), 1.7 (6H, *d*, $J = 6.2$ Hz, 2Me-6Rha), 3.8 (1H, *m*, H-20), 4.7 (1H, *bs*, H-1 Rha) and 5.1 (1H, *d*, $J = 7.1$ Hz, H-1 Glc).

Compound **1a**. Powder (5 mg), 212–215°, $[\alpha]_D^{23}$ − 12.3° (MeOH; *c* 0.15). ^1H NMR ($CDCl_3$): δ5.4 (1H, *m*, H-6), 3.59 (1H, *m*, H-3), 4.3 (1H, *m*, H-20), 0.90 (3H, *s*, Me-18), 1.01 (3H, *s*, Me-19), and 1.2 (3H, *d*, $J = 6.1$ Hz, Me-21).

Compound **2**. Powder (40 mg), 220–222°, $[\alpha]_D^{23}$ + 17.5° (MeOH; *c* 0.15) and R_f 0.30 (system I). ^1H NMR (C_5D_5N): δ0.90 (3H, *s*, Me-18), 1.01 (3H, *s*, Me-19), 1.2 (3H, *d*, $J = 6.4$ Hz, Me-21) and 5.2 (1H, *d*, $J = 7.0$ Hz, H-1 Glc).

Acid hydrolysis of the isolated glycosides. A sample (20 mg) was heated with 2 M HCl in H_2O–dioxane (1:1) in a sealed tube at 80° for 3 hr. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The aq. layer was neutralized with Ag_2CO_3 and subjected to TLC on silica gel using system IV (detection: TTC reagent). R_f values were 0.17 and 0.32 for D-glucose and L-rhamnose, respectively. The aq. layer was concd (12 mg), trimethylsilylated and subjected to GLC analysis. The identification of the aglycone (5 mg) was based on comparison of physical characters and NMR spectral analysis with reported data [7, 11].

Methylation analysis of compound **1**. A soln of compound **1** in DMSO (5 mg 100 μl^{-1}) was subjected to GC-MS analysis according to the procedure reported in

ref. [5]. MS showed the following significant peaks at (*m/z*) 43, 131, 101, 117, 89, 115, 161 for terminal rhamnose (1,5-di-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methylhexitol) and *m/z*: 129, 43, 87, 189, 99 for 2,6-disubstituted glucose (1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methylhexitol).

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REFERENCES

1. Kokwano, J. (1976) in *Medicinal Plants of East Africa*, p. 34. East Africa Literature Bureau, Kampala.
2. Duke, J. (1983) in *Medicinal Plants of the Bible*, p. 28. Trado Med. Books, New York.
3. Liu, H. and Nakanishi, K. (1982) *Tetrahedron* **38**, 513.
4. Pettit, G., Doubek, D. and Herald, D. (1991) *J. Nat. Prod.* **54**, 1491.
5. Kamel, M., Ohtani, K., Kurokawa, T., Assaf, M., El-Shanawany, M., Ali, A., Kasai, R., Ishibashi, S. and Tanaka, O. (1991) *Chem. Pharm. Bull.* **39**, 1229.
6. Kamel, M., Mohamed, M., El-Moghazy, S. and Ali, A. (1992) *Proc. First Natl Symp. Herb. Med., Assiut, Egypt*, p. 22.
7. Itokawa, H., Xu, J. and Takeya, K. (1988) *Phytochemistry* **27**, 1173.
8. Bradbury, H. and Jenkins, J. (1984) *J. Carbohydr. Res.* **126**, 125.
9. Agrawal, P., Jain, D., Gupta, R. and Thakur, R. (1985) *Phytochemistry* **24**, 2479.
10. Agrawal, P. (1992) *Phytochemistry* **31**, 3307.
11. Zechmeister, L. (1979) *Progress in the Chem. of Org. Nat. Products* **36**, p. 108. Springer-Verlag, Wien.