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A FUROSTANOL SAPONIN FROM FRUITS OF *BALANITES AEGYPTIACA*

M. S. KAMEL*

Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

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Key Word Index—*Balanites aegyptiaca*; Balanitaceae; balanitesin; furostanol saponin.

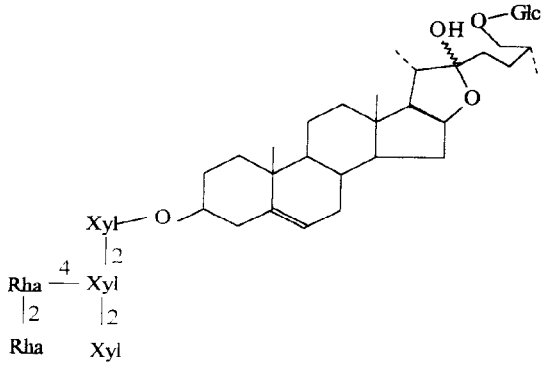
Abstract—A new furostanol saponin was isolated from the mesocarp of *Balanites aegyptiaca* fruits and identified as 26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-ene-3,22,26-triol 3-*O*-{[*α*-L-rhamnopyranosyl-(1 → 2)]-[*β*-D-xylopyranosyl-(1 → 2)]-*β*-D-xylopyranosyl-(1 → 2)}-*β*-D-xylopyranoside (balanitesin). © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Balanites aegyptiaca Del. is a widely distributed African plant of Medicinal interest [1–4]. In Egyptian folk-medicine, the fruits are used as an oral hypoglycemic drug. Our previous work on this plant resulted in isolation and characterization of new hypoglycemic furostanol saponins in addition to new steroidal glycosides [5–7]. This paper deals with isolation and elucidation of a new furostanol saponin from the mesocarp of the fruits.

RESULTS AND DISCUSSION

The methanolic extract of the mesocarp of *Balanites aegyptiaca* fruits was defatted with *n*-hexane and fractionated on a column of Diaion HP-20 to yield compound **1**. Compound **1** was assumed to be a furostanol saponin on the basis of the positive coloration with Ehrlich reagent [8]. The acid hydrolysis of **1** yielded D-glucose, L-rhamnose and D-xylose in addition to diosgenin as the aglycone. The sugars were identified using TLC by comparison with authentic samples and also by GLC of their trimethylsilyl derivatives. A peak at *m/z* 1283 $[M+H]^+$ in the positive ion FAB-mass was consistent with the molecular formula $C_{60}H_{98}O_{29}$. The following significant fragment peaks were also observed at *m/z* 1137 $[M+H-Rha]^+$, *m/z* 975 $[M+H-Rha-Glc]^+$, *m/z* 829 $[M+H-2Rha-Glc]^+$ and *m/z* 433 $[M+H-2Rha-Glc-3Xyl]^+$. The carbon signals due to the aglycone moiety in the ^{13}C NMR spectrum of **1** (Table 1) appeared at almost the same positions as those reported for the previously isolated 22-hydroxylated furostanol saponins from *Balanites aegyptiaca* [5, 6], indicating that compound **1** was also a 3,26-*O*-bisdesmosidic furostanol saponin with the same aglycone moiety. The carbon signals at *δ* 106.9, 104.6, 104.6, 104.4, 101.7 and 100.0 (Table 1) were clear for the anomeric carbons of six sugar units which were identified as one terminal *β*-glucopyranosyl, one terminal *α*-rhamnopyranosyl, one terminal *β*-xylopyranosyl, one substituted *α*-rhamnopyranosyl and two substituted *β*-xylopyranosyl units respectively [9]. The *β*-configuration of the D-glucopyranosyl and D-xylopyranosyl units was indicated from the coupling constants (6.5 Hz, 7.0 Hz, 7.2 Hz and 7.0 Hz) of the doublet signals at *δ* 4.81, 5.10, 5.13 and 5.22 respectively in the ^1H NMR spectrum [10], while the *α*-configuration of the L-rhamnopyranosyl units was deduced from the downfield shifts of C-5 (*δ* 69.4 each) in the ^{13}C NMR spectrum [9, 11]. The attachment sites of the substituted sugars have been determined by inspection of the other sugar signals which revealed the downfield shifts of C-2 of



* Author to whom correspondence should be addressed.

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

C	1	C	1	C	1
1	37.5*	22	110.7	*Subst. Rha*	
2	30.1	23	37.1	1	100.0
3	78.4	24	29.0	2	78.8
4	38.9	25	35.1	3	71.2
5	140.8	26	75.2	4	73.9
6	121.8	27	17.4	5	69.4
7	32.3	*26-Glc*		6	18.6
8	31.7	1	104.4	*Term. Xyl*	
9	50.1	2	75.3	1	106.9
10	37.1*	3	78.6	2	75.4
11	21.1	4	71.7	3	78.0
12	40.0†	5	78.2	4	70.9
13	40.3†	6	62.8	5	68.5
14	57.0	*Term. Rha*		*Subst. Xyl*	
15	32.8	1	101.7	1,1′	104.6, 104.6
16	81.2	2	72.4	2,2′	81.6, 81.6
17	62.1	3	72.7	3,3′	77.3, 76.1
18	16.4	4	74.9	4,4′	70.9, 78.0
19	19.3	5	69.4	5,5′	68.5, 67.3
20	40.7	6	18.6		
21	16.4				

*† Values may be interchangeable in each column.

the substituted rhamnopyranosyl unit and one of the substituted xylopyranosyl units (δ 78.8, 81.6 respectively) in addition to the downfield shifts of C-2 and C-4 (δ 81.6 and 78.0, respectively) of the second substituted xylopyranosyl unit [11]. The full assignment of the sugar moiety was established by ^1H detected Multiple Bond Connectivity (HMBC) based on the assignment of the sugar proton and carbon signals by ^{13}C–^1H Correlation Spectroscopy (2D COSY). The correlations (Fig. 1) were obvious between H-1 of the 2,4-disubstituted xylopyranosyl unit and C-2 of the 2-substituted xylopyranosyl unit, H-1 of the terminal xylopyranosyl and C-2 of the 2,4-disubstituted xylopyranosyl unit, H-1 of the 2-substituted rhamnopyranosyl unit and C-4 of the 2,4-disubstituted xylopyranosyl unit in addition to H-1 of the terminal rhamnopyranosyl unit and C-2 of the 2-substituted rhamnopyranosyl unit. Consequently, compound **1** was assigned the structure 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-ene-3,22,26-triol 3-*O*-{[α-L-rhamnopyranosyl-(1 → 2)-αL-rhamnopyranosyl-(1 → 4)]-[β-D-xylopyranosyl-(1 → 2)]-β-D-xylopy-ranosyl-(1 → 2)}-β-D-xylopyranoside (balanitesin).

EXPERIMENTAL

NMR spectra were recorded in pyridine-d_5 using a JEOL JNM-GX-400 spectrometer (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) with tetramethylsilane (TMS) as internal standard. The FAB-mass was 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}), Neutra Bond-1 column (0.25 mm i.d. × 30 m), column temp. 180°, injection temp. 225°. R_t L-rhamnose 6.0 min, D-xylose 7.9 min and D-glucose 21 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) $CHCl_3$–MeOH–H_2O (60:40:10); (III) 70% MeOH and (IV) 65% MeOH. Spray reagents: 10% H_2SO_4 and triphenyltetrazolium chloride (TTC). Plant material was collected in May 1993 from Al-Wady Al-Gadid area, western desert of Egypt. The identity of the plant was confirmed by Prof. A. Fayed, Faculty of Science, Assiut University. A voucher sample is kept in the Herbarium of the Faculty of Pharmacy, Assiut University, Egypt.

Extraction and isolation

The mesocarp (750 g) of *B. aegyptiaca* fruits was extracted 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 Me_2CO successively. The 40% MeOH eluate was chromatographed on silica gel (system II) affording three fractions. Fraction 2 was subjected to CC on RP-8 using system III followed by prep. HPLC

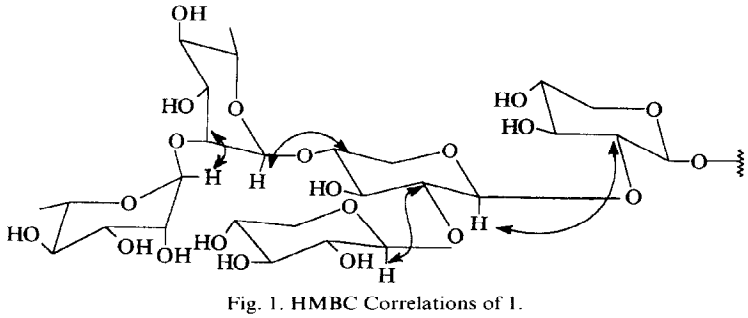


Fig. 1. HMBC Correlations of 1.

on an ODS column (system IV) to provide compound **1**.

Compound **1** (*balanitesin*). Powder (50 mg), $[\alpha]_D^{23}$ +25.2° (MeOH; *c* 0.31) and R_f 0.12 (system I). ^{1}H NMR (pyridine-d_5): δ 0.80 (3H, *s*, Me-18), 0.88 (3H, *d*, J = 7.0 Hz, Me-27), 1.02 (3H, *s*, Me-19), 1.4 (3H, *d*, J = 6.8 Hz, Me-21), 1.75 (6H, *d*, J = 6.1 Hz, 2Me-6 Rha), 4.81 (1H, *d*, J = 6.5 Hz, H-1 Glc), 5.10 (1H, *d*, J = 7.0 Hz, H-1 Xyl), 5.13 (1H, *d*, J = 7.2 Hz, H-1 Xyl), 5.22 (1H, *d*, J = 7.0 Hz, H-1 Xyl), 6.22 (1H, *bs*, H-1, Rha) and 6.24 (1H, *bs*, H-1 Rha). FAB-mass (positive) *m/z*: 1283 $[C_{60}H_{98}O_{29}+H]^+$, 1137, 975, 829 and 433.

Acid hydrolysis

A sample (30 mg) was heated with 2 M HCl in H_2O–dioxane (1:1) in a sealed tube at 80° for 3 h. 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 II (detection: TTC reagent). R_f values were 0.17, 0.32 and 0.25 for D-glucose, L-rhamnose and D-xylose, respectively. The aq. layer was concd (20 mg), trimethylsilylated and subjected to GLC analysis. The identification of the aglycone (diosgenin) isolated from the $CHCl_3$ layer was based on ^{13}C NMR analysis and comparison with reported data [9].

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