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SAIKOSAPONINS FROM *TAVERNIERA AEGYPTIACA*

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Key Word Index—*Taverniera aegyptiaca*; Leguminosae; root bark; saikosaponins.

Abstract—By tracing the UV triplet of heteroannular dienes, three novel saikosaponins were isolated from the root bark of *Taverniera aegyptiaca*. They were identified as 22β-hydroxyolean-11,13(18)-dien-3β-yl-β-D-glucopyranoside, 1β,22β-dihydroxyolean-11,13(18)-dien-3β-yl-β-D-glucopyranoside and 1β,22β-dihydroxy-olean-11,13(18)-dien-3β-yl-β-D-xylopyranosyl(1 → 2)β-D-glucopyranoside on the basis of chemical and spectral evidences.

INTRODUCTION

Taverniera aegyptiaca Boiss is a plant common to the Red Sea coastal region of Egypt. The polar fraction of the methanol extract of the root bark of this plant contains a complicated mixture of hemolytic saponins. In a recent report [1] we described the isolation of novel oleanene saponins from the aforementioned fraction. Further chromatographic investigation of the saponins of this fraction revealed the presence of saponins related in their basic skeleton to the medicinally reputed saikosaponins of *Bupleurum* species (Umbelliferae) [2–6]. By tracing the UV absorption spectra of the column eluates, three novel saponins (**1**–**3**) were isolated, two of which have a new triterpene aglycone of the rare 1β-hydroxylation pattern.

RESULTS AND DISCUSSION

Acid hydrolysis of saponins **2** and **3** afforded the same aglycone (**4**) besides D-glucose from **2** and D-glucose and D-xylose from **3** (TLC and GLC). The HRMS and DEPT ^{13}C NMR spectra of **4** established the molecular formula as $C_{30}H_{48}O_3$. The ^{1}H NMR spectrum of **4** showed eight sharp three proton singlets at δ 0.75–1.02 and two olefinic protons at δ 6.29 (1H, *dd*, J = 10.2, 3 Hz; H_{11}) and 6.70 (1H, *br d*, J = 10.2 Hz; H_{12}). The UV spectrum of **4** revealed a heteroannular diene triplet absorption at λ_{max}^{MeOH} 242, 251, 261 nm (log ε 4.42, 4.49, 4.27, respectively). These data together with the ^{13}C NMR signals at δ 125.0, 131.2, 135.7 and 135.9 (Table 1) are indicative of olean-$\Delta^{11,13(18)}$-diene system in **4** [7, 8].

The ^{1}H NMR spectrum of **4** also showed three axial carbinolic methine protons at δ 3.29 (1H, *dd*, J = 11.8, 4.6 Hz; H_3), 3.45 (1H, *dd*, J = 10, 7.4 Hz; H_{22}) and 3.56 (1H, *dd*, J = 11.2, 4.6 Hz; H_1) indicating the equatorial nature of the three hydroxyl groups in **4**. This was confirmed by the DEPT ^{13}C NMR CH signals at δ 75.6, 76.7 and 79.9 and also by the easy acetylation of **4** (Ac_2O–pyridine at 0°C) to give triacetate **4a** having three ^{1}H NMR signals for acetoxyl methyl groups at δ 2.03, 2.04 and 2.05 (each 3H, *s*) and the shift of [M$^+$] from m/z 456 in **4** to m/z 582 in **4a**. One of these hydroxyl functions could be unambiguously located at C-22 following observation of downfield shifts of C-21 and C-17 and of an upfield shift of C-28 due to a γ gauche effect. Resonances of C-6, C-7, C-15 and C-16 were comparable with those of the corresponding carbon of a series of saikogenins and other related sapogenins [8]. This conclusion was further confirmed by ^{1}H-^{1}H COSY, HMQC and HMBC of **4a** where significant correlations were observed between H-22 and C-17, C-20, C-21 and C-28 (Fig. 1).

The HRMS of **4** showed an intense fragment (100%) at m/z 235.1694 calculated for $C_{15}H_{23}O_2$ and pertaining to rings A, B and C. A vicinal glycol in **4** was excluded since Jones oxidation of **4** gave triketone derivative **4b** which exhibited β-diketone IR absorption bands at ν_{max}^{KBr} 1700 and 1725 cm^{-1} and an extra UV maximum at λ_{max}^{MeOH} 257 nm undergoing a 32 nm bathochromic shift in the presence of NaOH and indicating a 1,3 dione system [9, 10]. This is possible only in ring A of normal pentacyclic triterpenes. This conclusion was confirmed by the comparison of the ^{13}C NMR spectra of **4** and related compounds [8] where downfield shifts of C-2 and C-10 (10 and 4.3 ppm, respectively) and upfield shifts of C-25 and C-5 (1.7 and 3.4 ppm, respectively) were observed. The presence of the 1,3-dihydroxy system of ring A was finally confirmed from ^{1}H-^{1}H COSY of **4a** where cross peaks occurred between H-1 and H-3 and two proton multiplets centered at δ 1.8 and 1.98 (each 1H, *m*, $2H_2$) and

Table 1. DEPT ^{13}C-NMR analysis of compounds **1–5**

Carbon no.	**1**	**5**	**2**	**3**	**4**	**4a**
1	38.2	37.8	76.7	76.8	76.7	80.7
2	25.6	27.9	35.3	35.1	37.5	30.0
3	89.0	78.3	89.0	88.9	79.6	77.2
4	39.1	38.9	39.0	38.9	39.3	37.9
5	55.1	55.2	51.9	51.8	51.8	52.2
6	18.7	18.6	18.4	18.3	18.3	17.2
7	32.4	32.5	32.2	32.1	32.2	32.2
8	40.7	40.5	40.6	40.7	40.6	40.0
9	54.9	54.7	54.3	54.3	54.6	54.0
10	36.9	36.8	41.0	41.2	41.0	41.0
11	126.2	126.2	126.2	126.2	125.0	126.2
12	125.2	125.2	131.0	131.1	131.2	128.0
13	135.9	135.8	135.8	135.8	135.9	135.5
14	42.5	42.4	42.4	42.3	42.3	42.2
15	34.4	34.2	32.3	32.2	32.2	32.3
16	24.8	24.7	24.8	24.6	24.6	24.0
17	43.1	43.0	43.0	42.9	42.9	41.3
18	135.8	135.8	135.8	135.7	135.7	135.5
19	38.7	38.7	38.9	38.9	38.9	37.3
20	33.2	33.4	33.4	33.2	33.5	32.1
21	44.5	44.3	44.3	44.4	44.4	39.0
22	75.5	75.5	75.6	75.6	75.6	79.5
23	27.4	27.8	27.9	27.8	28.3	27.9
24	15.0	15.1	15.7	15.6	15.5	16.0
25	18.5	18.4	14.9	14.8	14.9	14.9
26	17.4	17.2	17.1	17.0	17.0	16.7
27	25.2	25.2	18.3	18.3	18.3	19.3
28	20.6	20.5	20.4	20.3	20.3	19.9
29	25.0	25.1	24.9	24.9	25.0	25.0
30	32.6	32.4	32.4	32.5	32.4	32.4
Inner sugar moiety						
1′	105.6		105.4	102.9	AcCO at C_1	169.9
2′	75.5		75.2	82.1	AcCO at C_1	21.0
3′	78.1		77.9	76.3	AcCO at C_3	170.6
4′	71.7		71.7	71.5	AcCO at C_3	21.3
5′	78.3		78.2	78.2	AcCO at C_{22}	170.3
6′	62.8		62.7	62.7	AcCO at C_{22}	21.7
Terminal sugar moiety						
1′				106.7		
2′				76.5		
3′				78.0		
4′				71.2		
5′				67.2		

from HMBC of **4a** where significant correlations were found between H-1 and H-3 and C-2. Other confirmatory correlations are shown in Fig. 2. The structure of **4** was then deduced to be 1β,3β,22β-trihydroxyolean-11,13(18)-diene which is a new aglycone.

It is interesting to note that H-12 and C-12 are downfield shifted in **4** in comparison with spectra of **5** and other saikogenins (ca 1.1, 6 ppm, respectively). These effects may be caused by 1β-hydroxylation and are lost from the ^{1}H NMR spectrum after acetylation (see Experimental and Table 1).

Acid hydrolysis of saponin **1** afforded D-glucose and aglycone **5**. The molecular formula of **5** was established as $C_{30}H_{48}O_2$ (HRMS and ^{13}C NMR). Its spectral analysis (MS, UV, ^{1}H and ^{13}C NMR) showed that it is closely related to **4** but with one less hydroxy function. Comparison of the ^{1}H NMR spectrum of **5** with that of **4** showed an upfield shift of H-3 (0.04 ppm) and H-12 (1.13 ppm) and an identical position of H-22 indicating the absence of the 1β-hydroxy function in **5**. The ^{13}C NMR spectrum of **5** (Table 1) further confirmed this conclusion by revealing normal ring A hydroxylation at C-3 only. Thus the structure of **5** was established at 1β,22β dihydroxyolean-11,13(18)-diene which is also a new aglycone.

The negative FAB-mass spectrum of **1** afforded a

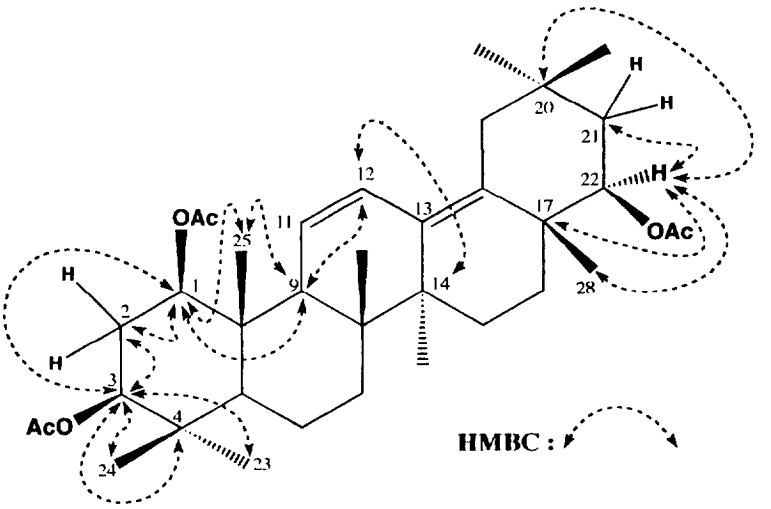
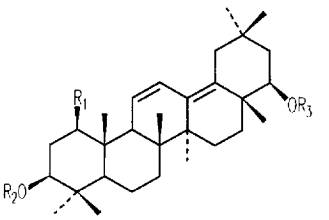


Fig. 1.



Compound	R_1	R_2	R_3
1	H	β-D-glucose	H
2	β-OH	β-D-glucose	H
3	β-OH	β-D-xylose (1→2) β-D-glucose	H
4	β-OH	H	H
4a	OAC	AC	Ac
5	H	H	H
5a	H	AC	Ac

Fig. 2.

quasi molecular anion peak at *m/z* 601$[M-H^+]^-$ and a further peak at *m/z* 439 assigned for loss of the glucose moiety. The ^1H NMR and ^{13}C NMR spectra of **1** showed the anomeric proton and carbon of a β-D-glucopyranoside structure at δ 4.51; (1H, *d*, *J* = 7.5 Hz) and δ 105.4 respectively. Glycosylation was assigned to C-3 of **5** due to the downfield shift of C-3 and upfield shift of C-2 in comparison with its aglycone **5** (see Table 1). As such its structure was deduced as 3β-O-β-D-glucopyranosylolean-11,13(18)-diene-3β,22β-diol.

Similarly the structure of saponin **2** was deduced as 3β-O-β-D-glucopyranosylolean-11,13(18)-diene-1β,3β,22β-triol (see Table 1 and Experimental).

Saponin **3** showed a molecular formula $C_{41}H_{66}O_{12}$ (FAB-mass spectrum and DEPT ^{13}C NMR). The negative FAB-mass spectrum of **3** revealed a quasi molecular anion at *m/z* 749 $[(M-H^+]^-$ and further peaks at *m/z* 617 and *m/z* 455 assigned for successive losses of one xylose and one glucose and one xylose units respectively from the $[M-H^+]^-$ peak. The ^1H NMR spectrum of **3** displayed signals for two β-anomeric protons at δ 4.53 (1H, *d*, *J* = 7.5 Hz) and at δ 4.60 (1H, *d*, *J* = 8 Hz). Comparison of the ^{13}C NMR spectrum of **3** with that of **2** (Table 1) showed the downfield shift of C-2 of glucose (6.9 ppm), upfield shift of both C-1 and C-3 (2.5 and 1.6 ppm, respectively) indicating that the terminal β-D-xylosyl moiety was linked to C-2 of β-D-glucose [11, 12]. As in **1** and **2**, glycosylation was also assigned to C-3 of **4** [see Table 1]. The structure of **3** was established therefore as 3β-*O*-β-D-xylo-

pyranosyl(1 → 2)-β-D-glucopyranosylolean-11,13(18)-diene-1β,3β,22β-triol.

It is pertinent to mention that saponins with a related basic skeleton have been reported to exhibit antiviral, cytotoxic, anti-inflammatory, anti-hepatotoxic and plasma cholesterol lowering activities [2–6]. They have been previously found in *Bupleurum* species [*Umbelliferae*] [3, 7, 13, 14], *Corchorous acutangulus* (Tiliaceae) [15], *Polycarpone loeffingiae* (Caryophyllaceae) [16], *Verbascum songaricum* (Scrophulariaceae) [17], *Glycyrrhiza yunnanensis* and *G. uralensis* (Leguminosae) [18–20], *Phyllanthus flexuosus* (Euphorbiaceae) [21], *Clinopodium polycephalum* (Labiatae) [22] and *Prunella vulgaris* (Labiatae) [23]. Finally the occurrence of diene saponins without a function on C-28 is rare and has been previously isolated only once from *G. uralensis* [20]. Also the rare 1β-hydroxylation in triterpenes has been encountered so far only in anagadiol [24] although the 1α-pattern was encountered in imberbic acid [10].

EXPERIMENTAL

Plant material, method of extraction and instruments have been described in previous work [1]. Mps: uncorr.; ^{1}NMR of aglycones in $CDCl_3$ and for glycosides in pyridine-d_5. TLC was carried out on precoated silica gel sheets "E-Merck" with the following systems (A) $CHCl_3$–MeOH (99:1), (B) $CHCl_3$–MeOH (92:8), (C) $CHCl_3$–MeOH (80:20), (D) $CHCl_3$–MeOH–H_2O (65:30:6.5), (E) acetonitrile–H_2O (85:15).

The saponin fraction (50 g) was fractionated on silica gel CC using a $CHCl_3$–MeOH gradient. Fraction collection was monitored by UV and TLC using systems (C) and (D) and only fractions showing UV absorption characteristics of heretoannular dienes were subjected to further investigation. Elution with $CHCl_3$–MeOH (90:10) afforded a mixture of three saponins (380 mg) HR_F 80, 75, 65 on system C. Further investigation of this mixture on silica gel CC afforded **1** (75 mg) HR_F 75 on system C and **2** (120 mg) HR_F 65 on the same system. Elution with $CHCl_3$–MeOH (65:35) afforded a mixture of two saponins (800 mg) HR_F 63, 60 on system D. Final separation of **3** was achieved on RP_{18} CC using MeOH–H_2O (3:7) to give **3** as an amorphous powder (250 mg) HR_F 60 on system D. The other component of this fraction is still under investigation.

Acid hydrolysis of saponins **1–2**

Each glycoside (30 mg) was refluxed with 10 ml 1 M aq. TFA for 2 h. The solns were then evaporated separately under vacuum, sugars were extracted with 2 ml H_2O and identified by TLC (using system E) and GLC through their alditol acetates using the procedure of Ref. [25]. Saponin **1** and **2** yielded D-glucose while **3** afforded D-xylose and D-glucose. Moreover, compound **1** afforded the aglycone **5** while **2** and **3** afforded the aglycone **4**.

Jones oxidation of **4**

Compound **4** (20 mg) was dissolved in 1 ml pyridine and oxidized with CrO_3 in pyridine (250 mg in 6 ml). The soln was agitated for 3 h at 0°C and filtered, diluted with H_2O and extracted with Et_2O. The residue obtained was purified by silica gel CC to give **4b** mp 136–139°C, IR ν_{max}^{KBr} cm^{-1}: 1725, 1700 (β-diketo), 1695 (C_{22} keto). UV λ_{max}^{MeOH} nm: 245, 251, 257, 263, (log ε 4.42, 4.47, 4.51, 4.27), $\lambda_{max}^{MeOH/NaOH}$ nm: 242, 251, 261, 289.

Acetylation of **4** *and* **5**

Compounds **4** (10 mg) and **5** (5 mg) were acetylated with Ac_2O–pyridine. The acetates **4a** and **5a** were precipitated by addition of H_2O, and crystallized from MeOH to give **4a** as needles mp 258–260°C and **5a** as amorphous powder mp 156–58°C.

Preparation of alditol acetates [25]

The aq. soln of each hydrolysed glycoside (2 ml) was reduced by 1 ml of 2% $NaBH_4$ in 0.1 ml 1N NH_3 at 60°C for 90 min. It was then acetylated with 2 ml Ac_2O using 0.2 ml of 1-methyl imidazole as catalyst. The alditol acetates were extracted from the reaction mixture with CH_2Cl_2 and subjected to GLC.

Compound **1**. Amorphous powder (MeOH), mp 175–179°C, HR_F 75 on system C. Positive FABMS *m/z*: 603 $[M+H^+]^+$; negative FABMS *m/z*: 601 $[M-H^+]^-$, 439 $[M-H^+-\text{glucose}]^-$. ^{1}H NMR: δ 6.40 (1H, *dd*, *J* = 10.7, 3.1 Hz, H-11), 5.59 (1H, *br d*, *J* = 10.7 Hz, H-12), 4.51 (1H, *d*, *J* = 7.5 Hz, H-1 glucose). ^{13}C NMR: see Table 1.

Compound **2**. Needles from MeOH, mp 207–209°C, HR_F65 on system C. Positive FABMS *m/z*: 619 $(M+H^+)^+$, 439 $[M-H^+-\text{glucose}-H_2O]^+$. Negative FABMS *m/z*: 617 $[M-H^+]^-$, 455 $[M-H^+-\text{glucose}]^-$, 437 $[M-H^+-\text{glucose}-H_2O]^-$. ^{1}H NMR: δ 6.76 (1H, *dd*, *J* = 10.2, 1 Hz, H-12), 6.35 (1H, *dd*, *J* = 10.2, 3 Hz, H-11), 4.56 (1H, *d*, *J* = 7.5 Hz, anomeric H-1′ of glucose. ^{13}C NMR: see Table 1.

Compound **3**. Amorphous powder mp 196–198°C (MeOH). Positive FABMS *m/z*: 751 $(M+H^+)^+$, 439 $[M+H^+-\text{xylose}-\text{glucose}-H_2O]^+$. Negative FABMS *m/z*: 749 $[M-H^+]^-$, 617 $[M-H^+-\text{xylose}]^-$, 455 $[M-H^+-\text{xylose}-\text{glucose}]^-$, 437 $[M-H^+-\text{xylose}-\text{glucose}-H_2O]^-$. ^{1}H NMR: δ 6.76 (1H, *br d*, *J* = 10.1 Hz, H-12), 6.36 (1H, *dd*, *J* = 10.1, 3 Hz, H-11), 4.60 (1H, *d*, *J* = 8 Hz), 4.53 (1H, *d*, *J* = 7.5 Hz). ^{13}C NMR see Table 1.

Identification of compound **4**. Mp 268–270°C (MeOH). HR_F 75 on system B. IR ν_{max}^{KBr} cm^{-1}: 3550–3150 br, 3020, 2980, 2960, 2880, 2870, 1660, 1640, 1475, 1400, 1370, 1320, 1300, 1090, 1040, 1030, 1000, 985, 940, 650. HRMS *m/z* (rel. int): 456.35965 (4) calcd for $C_{30}H_{48}O_3$ (required 456.36035), 438 (25), 423 (8), 420 (6), 405 (5), 235.16949 (100) calcd for $C_{15}H_{23}O_2$ (required 235.16980), 221 (2), 219 (15), 218 (20), 217

(9), 215 (28). ^{1}H NMR ($CDCl_3$): δ 0.75, 0.77, 0.80, 0.98 (3 Me), 0.99, 1.02 (8 Me groups), 3.29 (1H, *dd*, J = 11.8, 4.6 Hz; H-3), 3.45 (1H, *dd*, J = 10, 7.4 Hz, H-22), 3.56 (1H, *dd*, J = 11.2, 4.6 Hz, H-1), 6.29 (1H, *dd*, J = 10.2, 3 Hz, H-11), 6.70 (1H, *br d*, J = 10.2 Hz, H-12). ^{13}C NMR: see Table 1.

Triacetate **4a**. Mp 258–260°C. HRMS *m/z* (rel. in.): 582 (19.3), 522 (100), 462 (42), 402 (3.2), 327 (11.3), 201 (40). ^{1}H NMR: δ 0.73, 0.86 (3 Me), 0.98, 1.08, 1.09 (8 Me groups), 1.8, 1.98 (each 1H, *m*, $2H_2$) 2.03, 2.04, 2.05 (each 3H, *s*, 3 acetoxyl groups), 2.18 (1H, *d*, J = 2.9 Hz, H-9) 4.61 (1H, *dd*, J = 12.2, 4.6 Hz, H-3α), 4.72 (1H, *dd*, J = 9.8, 7.5 Hz, H-22α), 4.77 (1H, *dd*, J = 11.4, 4.6 Hz, H-1α), 5.83 (1H, *dd*, J = 10.9, 1 Hz, H-12), 6.27 (1H, *dd*, J = 10.9, 2.9 Hz, H-11); ^{13}C NMR see Table 1.

Compound **5**. Mp 220–222°C, HR_F 66 (system A), HRMS *m/z* (rel. int.): 440.3664 (100) for $C_{30}H_{28}O_2$ required 440.3654, 425 (8.9), 422 (3.8), 270 (6.4), 245 (12.8), 231 (17.9), 220 (10.2). ^{1}H NMR: δ 0.72, 0.78, 0.80, 0.89, 0.95, 0.99 (2 Me), 1.02 (8 Me groups), 3.24 (1H, *dd*, J = 10.7, 5.6 Hz, H-3), 3.45 (1H, *dd*, J = 9.8, 7.6 Hz, H-22), 5.57 (1H, *br d*, J = 10.6, 1 Hz, H-12), 6.34 (1H, *dd*, J = 10.7, 2.7 Hz, H-11), ^{13}C NMR: see Table 1.

Compound **5a**. Mp 156–158°C, HRMS *m/z* (rel. int): 524 (100%), 464 (15), 404 (2), 368 (6), 273 (12.5), 227 (16.2), 213 (12.5), 201 (25).

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