TRITERPENOIDS FROM THE FRUIT BARKS OF *PERIPLOCA LAEVIGATA* GROWING IN TUNISIA

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ABSTRACT: Three compounds, previously identified in other natural sources, have been isolated for the first time from the fruit barks of *Periploca laevigata* (Asclepiadaceae): oleanolic acid **1**, maslinic acid **2** and β-sitosterol-3-β-D-glucoside **4**. β-amyrine **3** has been also re-isolated. Their structures have been established by spectroscopic procedures.

Key words: *Periploca laevigata*, Asclepiadaceae, Triterpenoids, Sterol, NMR.

RESUME: Trois composés connus, identifiés dans d'autres sources naturelles, ont été isolés pour la première fois des écorces de fruits de la plante *Periploca laevigata* (Asclépiadacées) : acide oléanolique **1**, acide maslinique **2** et 3β-O-(β-D-glucoside)sitostérol **4**. Le β-amyrine **3** a été aussi re-isolé. Les structures de ces produits naturels ont été établies à l'aide de techniques spectroscopiques.

Mots clés: *Periploca laevigata*, Asclépiadacées, Triterpènes, Stérol, RMN.

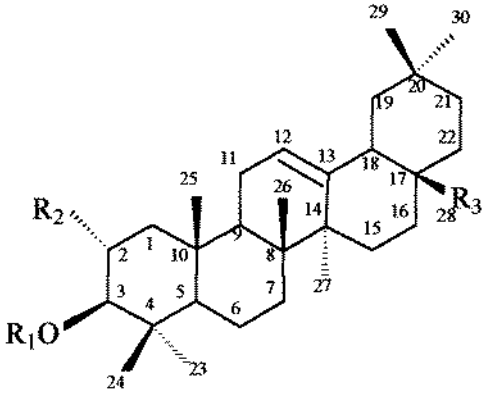
INTRODUCTION

Periploca laevigata (Asclepiadaceae) is widely distributed through North Africa and found abundantly in Tunisia [1]. It is used in traditional herbal medicine for several treatments such as the diabetic property of the roots [2]. Following our study of the chemical composition of this plant we report in this paper the results of an investigation of the acetone extract of the dried fruit barks of *P. laevigata* and the identification of three triterpenoids, two are isolated for the first time: oleanolic acid **1** and maslinic acid **2**, together with β-amyrine **3** previously isolated from the same source [3]. We also report, for the first time, the identification of an heteroside, β-sitosterol-3-β-D-glucoside **4**.The acetylated derivatives **1a**, **2a**, **3a** and **4a** have been also prepared. The structure elucidation was established by spectroscopic methods and chemical correlations.

RESULTS AND DISCUSSION

Compound **1** was isolated as a white powder. The elemental analysis and the EIMS showing the molecular ion peak at m/z 456 indicated a molecular formula of $C_{30}H_{48}O_3$ and seven degrees of insaturation. The significant fragment ion at m/z 248 is due to a typical retro-Diels Alder rearrangement, diagnostic to $\Delta^{12\text{-}13}$ double band in pentacyclic structure. Its IR spectrum revealed absorptions attributable to free hydroxyl (3500 cm^{-1}) and carboxylic acid (1715 cm^{-1}) groups. The ^{1}H-NMR spectrum of **1** showed a large singlet at 5.19 ppm and a multiplet at 3.50 ppm assigned as being H_{12} and H_3, respectively. The ^{13}C and the DEPT NMR spectra indicated the presence of ethylenic tertiary and quaternary carbons at 122.9 and 143.1 ppm attributable to C_{12} an C_{13}, respectively. A signal at 178.6 ppm has been also observed and assigned as C_{28} (COOH). These

spectral data, as well as those of the acetylated derivative **1a** (Table 1 and experimental) are in good agreement with reported values for oleanolic acid [4] and oleanolic acid acetate [5].

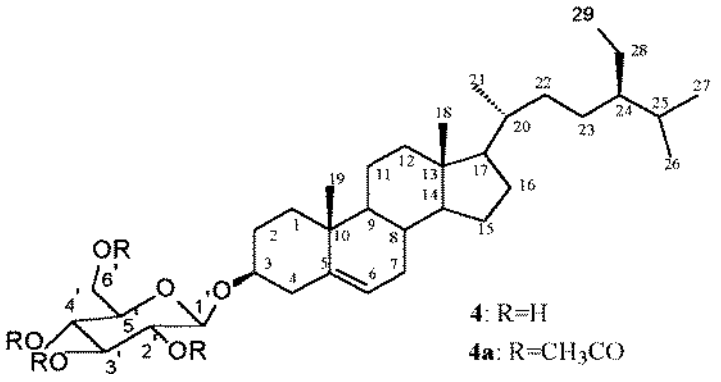


1. R_1=H, R_2=H, R_3=COOH
1a. R_1=$COCH_3$, R_2=H, R_3=COOH
2. R_1=H, R_2=OH, R_3=COOH
2a. R_1=$COCH_3$, R_2=$OCOCH_3$, R_3=COOH
3. R_1=H, R_2=H, R_3=CH_3
3a. R_1=$COCH_3$, R_2=H, R_3=CH_3

Compound **2** was obtained as a white powder. The EIMS of its acetylated derivative **2a** showed fragment ion peaks at m/z 511 $[M\text{-}COOH]^+$, m/z 436 $[M\text{-}2\ CH_3COO]^+$ and m/z 248 analogous to that observed with compound **1**. The analysis of the ^{1}H-NMR spectrum of **2a** permitted to observe two singlets at 2.00 and 2.04 ppm attributable to two CH_3COO groups. The spectrum showed a doublet at 4.74 ppm (J = 9.6 Hz) assigned to H_3 and a ddd at 5.08 ppm ($J_1 = J_2 = 9.6$ Hz, $J_3 = 4.8$ Hz) attributable to H_2. These assignments were confirmed by the HMQC experiment showing the ^1J correlation of H_2 and H_3 with carbons at 70.0 (C_2) and 80.6 (C_3) ppm, respectively. Furthermore, the presence of the two ester groups at C_2 and C_3 was confirmed by the COSY spectrum of **2a**, witch displays the vicinal correlation H_2-H_3. The comparison of the spectral data deduced from the latter experiments with those of the literature [6] for maslinic acid and the corresponding diacetate, confirmed the structure of **2** and **2a**.

Compound **3** was obtained as a white powder. The EIMS of its acetylated derivative **3a** showed ion peaks at m/z 468 $[M^{+\cdot}]$, 250 and 218 relative to a retro-Diels Alder fragmentation confirming the presence of the Δ^{12-13} insaturation in ring C. The structure of **3a** and consequently of **3** were supported by the comparison of the ^{1}H- and ^{13}C-NMR spectral data of **3a** with those of **1a** and literature [7].Thus, compound **3** is β-amyrine.

Compound **4** was isolated as a white solid. Its FABMS showed ion peaks at m/z 599 $[M+Na]^+$, m/z 414 relative to the aglycone (β-sitosterol), m/z 396 $[\beta\text{-sitosterol} - H_2O]^+$, m/z 255 relative to the loss of the aliphatic chain fixed at C_{17} of the β-sitosterol and m/z 163 attributable to the β-D-glucoside moiety. The comparison of the spectral data (see experimental) deduced from the extension of some regions of the ^{1}H-NMR spectrum of its acetylated derivative **4a** with those of β-sitosterol [8] and β-sitosterol-β–D-glucoside, β-sitosterol-β–D-glucoside tetraacetate [9] permitted us to confirm the structures of **4** and **4a**. The hydrolysis of the glycoside **4** was carried out and the resulting apolar fraction (aglycone) of the molecule was identified by GC/MS as β-sitosterol.



4: R=H
4a: R=CH_3CO

Table 1 ^{13}C-NMR spectral data of **1** ($DMSOd_6$), **2a, 3a** ($CDCl_3$) and **4** (CD_3OD)

Position	1	1a	2a	3a	4
1	38.1	38.0	45.8	37.7	37.0
2	27.2	27.7	70.0	23.6	31.5
3	76.9	80.9	80.6	80.9	73.7
4	38.4	39.0	39.3	37.7	38.5
5	54.9	55.3	54.8	55.3	140.8
6	18.0	18.1	18.0	18.2	121.5
7	33.4	32.8	32.3	32.7	31.5
8	45.5	39.5	39.3	39.5	31.5
9	47.1	48.0	47.5	47.6	50.8
10	36.6	37.7	38.2	36.9	36.3
11	22.9	24.0	23.4	23.0	21.0
12	121.5	122.5	122.2	122.9	39.7
13	143.7	143.6	143.6	143.1	42.0
14	41.3	41.5	41.5	41.3	56.4
15	26.9	28.0	27.6	29.4	24.0
16	22.6	23.4	22.8	25.9	28.8
17	45.5	46.6	46.1	33.0	55.6
18	40.8	40.9	40.8	47.6	12.4
19	45.7	45.8	46.5	46.0	19.0
20	30.4	30.7	30.4	31.5	35.6
21	32.4	33.8	33.7	36.9	18.7
22	32.1	32.5	32.4	37.7	33.5
23	28.2	29.7	28.4	28.1	25.6
24	15.9	16.7	16.4	17.2	45.3
25	15.1	15.5	17.1	15.4	29.4
26	16.8	17.2	17.6	16.7	19.8
27	25.6	25.9	25.8	25.9	19.2
28	178.6	184.1	184.2	27.5	22.7
29	32.9	33.1	33.0	33.7	11.9
30	23.4	23.6	23.5	23.5	
$CH_3\underline{C}OO$		171.0	170.7	171.2	
$\underline{CH_3}COO$		22.8	20.9	23.1	
$CH_3\underline{C}OO$			171.0		
$\underline{CH_3}COO$			21.1		
1'					101.1
2'					77.0
3'					77.2
4'					70.3
5'					77.0
6'					61.3

3. EXPERIMENTAL SECTION

3.1 General experimental procedures. Optical rotation was run on a Perkin-Elmer 241-MC polarimeter. FTIR spectra was measured on a Perkin-Elmer 157G infrared spectrophotometer. Shimadzu QP-1000EX and MS-80RF spectrometers were used in the EI and FAB experiments, respectively. GC/MS HP 5972 equipped with the mass spectral library Chem-station HP NBS75 K.L was also used. ^{1}H (250 and 400 MHz) and ^{13}C (62.5 and 100 MHz) one- and two-dimensional NMR spectra were recorded on a Bruker ARX-400 and WM-400 spectrometers, using $CDCl_3$, CD_3OD and $DMSOd_6$ as solvents and TMS as internal standard. Melting points were determined on a Büchi 510 apparatus using capillary tubes.

3.2 Plant Material. Plant material was harvested in june 1999 in Jemmal, Tunisia. A voucher specimen is deposited at the herbarium of the Ecole Supérieure d'Horticulture de Chott mériem, Université du Centre, Sousse, Tunisia.

3.3 Extraction and isolation. Dried and finely fruit barks of *Periploca laevigata* (2 Kg) were successively extracted in a Soxhlet apparatus with petroleum ether (40°C-60°C), acetone, methanol and water. The crude acetone extract obtained after filtration and evaporation of the solvent (200 g) was dissolved in methanol. After two weeks of storage at room temperature this solution afforded a white precipitate which was recuperated by filtration, then purified by recristallisation from methanol, yielding compound **1** (21 g). The remaining liquid phase was concentrated in vacuo and separated by Si gel cc eluted with petroleum ether/EtOAc/MeOH mixtures, providing several fractions from which compounds **2** and **3** were isolated in their acetylated form **2a** (4 mg) and **3a** (16 mg) after additional purification by successive Si gel cc and acetylation. The more polar fraction obtained from the same column was subjected to Si gel cc and eluted with EtOAc/MeOH 9:1 and 8:2 to give compound **4** (10 mg).

Oleanolic acid 1: white powder; mp 310°C; $[\alpha]^{20}_D$ +60.0 (c = 0.01, $CHCl_3$); IR ν_{max} cm^{-1} 3500, 2950, 2850, 1715, 1450, 1370; EIMS m/z (rel. intensity) 456 (M^+) (5), 412 (3), 248 (100), 203 (50), 167 (25), 44 (51). ^{1}H-NMR ($DMSOd_6$, 250 MHz) δ 0.75 (3H, s, CH_3-26), 0.87 (3H, s, CH_3-24), 0.89 (3H, s, CH_3-23), 0.91 (3H, s, CH_3-25), 0.96 (3H, s, CH_3-30), 1.12 (3H, s, CH_3-27), 2.87 (1H, m, H-18), 3.23 (1H, m, H-3), 5.28 (1H, m, H-12); ^{13}C-NMR (see Table 1).

Oleanolic acid acetate 1a: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.67 (3H, s, Me-26), 0.79 (3H, s, Me-29), 0.87(3H, s, Me-24), 0.88 (3H, s, Me-23), 1.00 (3H, s, Me-30), 1.05 (3H, s, Me-25), 1.18 (3H, s, Me-27), 2.06 (3H, s,OAc-31), 2.78 (1H, m, H-18), 4.42 (1H, m, H-3), 5.19 (1H, m, H-12); ^{13}C-NMR (see Table 1).

Maslinic acid diacetate 2a: white powder; mp 156-158°C; EIMS m/z (rel. intensity) 556 (M^+) (1), 511 (2), 436 (3), 307 (1), 248 (100), 203 (82), 190 (13), 189 (24); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.80 (3H, s, CH_3-27), 0.86 (3H, s, CH_3-30), 0.87 (3H, s, CH_3-25), 0.92 (3H, s, CH_3-29), 0.93 (3H, s, CH_3-26), 0.94 (3H, s, CH_3-24), 1.05 (1H, m, H-1b), 1.14 (1H, m, H-19b), 1.59 (1H, m, H-19a), 1.88 (1H, m, H-11), 1.99 (1H, m , H-la), 2.06 (3H, s,$C\underline{H}_3CO$); 2.80 (1H, dd, J_1 = 3.6, J_2 =13.6 Hz, H-18), 4.74 (1H, d, J=9.6 Hz, H-3), 5.08 (1H, ddd, J_1=J_2=9.6, J_3 =4.8 Hz, H-2), 5.25 (1H, m, H-12); ^{13}C-NMR (see Table 1).

β-amyrine acetate 3a: white powder; mp 264-266 °C; IR ν_{max} cm^{-1} 2925, 2851, 1734, 1462, 1246; EIMS m/z (rel. intensity) 393 (5), 250 (13), 248 (62), 203 (87), 190 (25), 189 (25), 43 (100); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 4.50 (1H, m, H-3), 5.31 (1H, m, H-12), 2.83 (1H, m, H-18), 0.94 (3H, s, CH_3-24), 0.87 (3H, s, CH_3-25), 0.93 (3H, s, CH_3-26), 0.80 (3H, s, CH_3-27), (3H, s, CH_3-28), 0.92 (3H, s, CH_3-29), 0.86 (3H, s, CH_3-30), 2.06 (3H, s,$C\underline{H}_3CO$); ^{13}C-NMR (see Table 1).

β-sitosterol-3-β-D-glucoside 4: white solid; ^{1}H-NMR (CD_3OD, 400 MHz) δ 0.62 (3H, s, CH_3-18), 0.77 (3H, s, CH_3-26), 0.79 (3H, s, H-27), 0.88 (3H, s, CH_3-29), 0.93 (3H, s, CH_3-21), 0.95 (1H, m, H-17), 0.96 (3H, s, CH_3-19), 1.12 (1H, m, H-1α), 1.21(1H, m, H-12), 1.21(1H, m, H-22b), 1.28 (1H, m, H-23b), 1.35 (1H, m, H-8), 1.47 (1H, m, H-7), 1.47 (1H, m, H-11), 1.59 (1H, m, H-2α), 1.60 (1H, m, H-15β), 1.78 (1H, m, H-2β), 1.91 (1H, m, H-1β), 2.32 (1H,m, H-4), 3.59 (1H, m, H-3), 5.29 (1H, d, J=4.8 Hz, H-6), glycopyranosyl part: δ 4.19 (1H, ddd, J_1=2.3, J_2=4.7, J_3=9.6 Hz, H-5'), 4.43 (1H, dd, J_1=8.1, J_2=9.6 Hz, H-2'), 4.85 (1H, t, J=9.6 Hz, H-4'), 4.89 (1H, t, J=9.6 Hz, H-3'), 4.94 (1H, dd, J_1=2.3, J_2=12.2 Hz, H-6'a), 4.99 (1H, dd, J_1= 4.8, J_2=12.2 Hz, H-6'b), 5.09 (1H, d, J=8.1Hz, H-1'); ^{13}C-NMR (see Table 1).

β-sitosterol-3-β-D-glucopyranoside tetracetate 4a: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.67 (3H, s, CH_3-18), 0.80 (3H, s, H-26), 0.82 (3H, s, H-27), 0.84 (3H, s, CH_3-29), 0.91 (1H, m, H-9), 0.93 (3H, s, CH_3-21), 0.99 (1H, m, H-17), 1.01 (3H, s, CH_3-19), 1.01 (1H, m, H-1α), 1.02 (1H, m, H-15α), 1.04 (1H, m, H-14), 1.15 (1H, m, H-12), 1.25 (1H, m, H-22b), 1.28 (1H, m, H-23), 1.46 (1H, m, H-

8), 1.49 (1H, m, H-11), 1.55 (1H, m, H-2α), 1.55 (1H, m, H-7), 1.56 (1H, m, H-15β), 1.80 (1H, m, H-16), 1.84 (1H, m, H-1β), 1.87 (1H, m, H-2β), 2.24 (1H, m, H-4), 3.49 (1H, m, H-3), 5.35 (1H, d, J=4.8 Hz, H-6); glycosyl tetracetate unit: δ 3.69 (1H, ddd, J_1=2.3, J_2=4.7, J_3=9.6 Hz, H-5'), 4.11 (1H, dd, J_1=2.3, J_2=12.2 Hz, H-6'a), 4.25 (1H, dd, J_1=4.8, J_2=12.2 Hz, H-6'b), 4.59 (1H, d, J=8.1Hz, H-1'), 4.95 (1H, dd, J_1=8.1, J_2=9.6 Hz, H-2'), 5.07 (1H, t, J=9.6 Hz, H-4'), 5.20 (1H, t, J=9.6 Hz, H-3'), 2.00, 2.02, 2.05, 2.08 (3H, s, $C\underline{H}_3CO$).

Acetylation of compounds **1**, **2**, **3** and **4**: each compound was dissolved in 1 ml of a dried C_5H_5N adding immediately 1 ml Ac_2O and the mixture was stirred at room temperature for 4 hr. The mixture was diluted with water to decompose the excess of Ac_2O and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated to yield **1a** (5 mg); **2a** (4.5 mg), **3a** (5 mg) and **4a** (5 mg).

Acid hydrolysis of compound **4**: compound **4** (10 mg) was refluxed with 2N HCl-EtOH (1:1). After 2 hr the EtOH was evaporated under red. pres., diluted with H_2O and the hydrolysate was then extracted with $CHCl_3$. The organic layer was washed with an excess of 10 % $NaHCO_3$ then with water, dried over Na_2SO_4 and evaporated to yield a residue identified as β-sitosterol by GC/MS.

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