Extraction and identification of triterpenoids from *Pergularia Tomentosa L*

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ABSTRACT: Two new triterpenoids pergularine A and pergularine B **[1]**, along with five known triterpenoids were isolated from the ethanolic extract of the aerial parts of *P. tomentosa* L. (Asclepiadaceae). The structures of the isolated known compounds were identified as 3-epi-Micromeric acid **(1)**, taraxasterol acetate **(2),** taraxasterol **(3),** 16α-hydroxytaraxasterol acetate **(4)** and α- amyrine **(5)** and were reported for the first time in the genus *Pergularia*. Structures were elucidated by chemical and spectroscopic methods (2D-NMR, IR, and MS) and by comparison with literature data.

KEYWORDS: *P. tomentosa* L., Asclepiadaceae, pergularine, 3-epi-Micromeric acid, 16α-hydroxytaraxasterol acetate, taraxasterol acetate, α- amyrine

1. Introduction

The milkweed family (Asclepiadaceae) comprises about 200 genera and 2500 species of perennial shrubs and herbs distributed through the tropics and temperate areas of the world. The family is reputed for cardenolides-containing plants, notably in the genera *Asclepias*, *Pergularia*, *Gomphocarpus* and *Calotropis* [2].

Pergularia tomentosa L., commonly known as Ghalqa in Algeria, is a perennial shrub about 50-60 cm high, reaching 1 m in good conditions [3-5]. It is a poisonous plant that is known to be distributed in the Saharian and Sub-Saharian countries of North Africa [1] including Algeria, Niger, and Egypt [6]. This plant is common also in the Middle-East region including Saudi Arabia [7] and Jordan [8]. The plant is known to produce corrosive white latex that may severely harm the skin.

In spite of being poisonous, *P. tomentosa* is used extensively in traditional medicine by North Saharian and Sub-Saharian populations. A decoction of the leaves and stems is used for the treatment of bronchitis and tuberculosis, a medication that should be taken with great care and is forbidden for pregnant women. The plant was reported to have molluscidal activity [9] and persistent hypoglyceaemic effects [6]. The roots are used for the treatment of bronchitis, constipation and skin diseases [10]. Several reports mentioned this plant which used for the treatment of asthma and as antirheumatic agent [10, 11].

Previous phytochemical investigations of this plant resulted in isolation and characterization of several cardenolide glycosides [12] and flavonoid glycosides [13] in addition to β-sitosteryl glucoside [2]. Careful literature survey on this plant revealed that triterpenoids were never isolated.

In our on-going investigation of new compounds from Mediterranean medicinal plants, we have investigated the ethanolic extract of *P. tomentosa* from Algerian. Here we report the isolation and characterization of two new triterpenes along with five other known compounds reported for the first time from *P. tomentosa*.

2. Results and discussion

The crude ethanol extract of the air dried (7.0 kg), ground and defatted plant material (254 g) was partitioned according to the procedure described in the experimental section into chloroform soluble and water soluble extracts. The chloroform extract (PC, 175 g) was further subjected to column chromatography on silica gel (650 g, 65 cm × 7 cm) column packed in chloroform and eluted with a gradient of $CHCl_3$/MeOH. The fractions collected were grouped according to their TLC behaviour into seven major fractions (CPI-CPVII). Each fraction was further purified by a combination of column chromatography (CC) and TLC. Ten compounds were obtained from the chloroform extract, two of which are reported for the first time from a natural source. The new compound is named as pergularine. The known compounds were identified as 3-epi-Micromeric acid (1), taraxasterol acetate (2) (Figure 1), taraxasterol (3), 16 α-hydroxytaraxasterol acetate (4) and α- amyrine (5) [14].

The IR spectrum of compound taraxasterol acetate (2) showed an absorption band at 1733 cm^{-1} corresponding to a carboxyl functionality and 1454 cm^{-1} indicating the presence of carbon- carbon double bond. The molecular formula $C_{32}H_{52}O_2$ was confirmed by mass spectroscopy, which showed a stable fragment (M-H) at m/z 413.26 attributed to the loss of acetate group.

The ^1H – NMR ($CDCl_3$) of compound (2) indicated the presence of 7 methyl groups in the region 0.82-0.99 ppm. The spectrum also contained: two broad singlets at 4.64 and 4.57 ppm for two olefinic protons and one doublet of doublets at 4.45 ppm corresponding to the proton on C-3 of ring A. The spectrum also shows a triple at 2.34 ppm (J= 5.61 Hz) integrating to one proton for H-18 and a double at 0.79 ppm corresponding to the protons of C29-CH_3. All the other methyl signals appear as singlets. The ^{13}C–NMR spectrum showed the presence of 32 carbon atoms. The multiplicity of these carbons was determined by the DEPT experiment which confirmed the presence of 8CH_3, 11 CH_2, 6 CH and 7 quaternary carbons. The spectrum showed signals for two olefinic carbons at δ150.81 and 109.43 belonging to C-30 and C-20 respectively, a signal at δ 170.90 for the acetate group, and a signal at δ 80.89 correspond to the methine adjacent to the oxygen of the OH group at C3, the structure of (2) was confirmed as shown in Figure 1.

The experimental results, unambiguously characterize the ursane skeleton of taraxasterol with five cyclohexane rings, seven methyls, one *exo*-methylene group on C-20, and an acetate on C-3. The COSY H-H experimental showed correlations between H-5 (δ 0.79 m), 0.93 ppm (6H singulet), and H-2, H-2' (1.87dd), and the correlations between H-3 (δ 4.5 dd) and H-5 (δ 0.78 m) and H-24(23) 0.91 ppm (6H singlet), and H-2, H-2′, and H-3 characterize C-3 a correlation with the C atom of the carboxylic group at 170.9 ppm is also observed

HMBC and HMQC significant the correlations between H-3 and C-1 (δc 38.42), C-2 (δc 23.69), C-4 (δc 38,03), C-23 (δc 27,98), C-24 (δc 16,51) and a quaternary carbon ethylenic attributable to the C-5 (δc 55.33). Figure 2 illustrates the key HMBC correlations for taraxasterol acetate (2).

3. Experimental

3.1. General experimental procedures

The optical rotations were recorded on a Perkin-Elmer polarimeter 141 (Shelton, USA). The IR spectra were recorded on Thermo-Nicolet Nexus 870 FT-IR spectrophotometer (Thermo Scientific, Wisconsin, USA). ^1H-NMR spectra were recorded on a Bruker DPX-300 MHz spectrometer with TMS as an internal standard. ^{13}C-NMR spectra were recorded at 75.5 MHz (Strasbourg, France). HRMS were measured in positive ion mode using electrospray ionization (ESI) technique on a Bruker APEX-2 instrument (Bremen, Germany). Column chromatography (CC) was performed on silica gel 60 (0.063-0.200 mm, Fluka, Steinheim, Germany) or silica gel S (Ridel de-haën, Seelze-Hannover, Germany). Purification of the compounds was achieved by routine Thin Layer Chromatography (TLC) on silica gel G-UV_{254} glass plates (0.25 mm, Macherey-Nagel, Easton, PA, USA). Compounds were visualized by spraying with sulfuric acid – anisaldehyde spray reagent followed by heating at 120 °C.

3.2. Plant material

Pergularia tomentosa.L was collected from the surroundings of Ghardaia (South Algeria) during the flowering period (April 2008). The plant was identified by Prof. Dawud AL-Eisawi (Department of Biological Sciences, Faculty of Science, University of Jordan). A voucher specimen of the plant (BAU/08/AP-Alg) is deposited at the Herbarium of the Department of Biological Sciences, University of Jordan.

3.3. Extraction and isolation

The ground whole plant material (7.0 kg) was defatted by soaking in petroleum ether at room temperature and then repeatedly soaked in ethanol at room temperature (50 L, 4 times, 5 days each). The residue obtained upon removal of EtOH under reduced pressure (255 g) was partitioned between water and chloroform. The chloroform soluble fraction (PC, 175 g) was chromatographed on silica gel S column eluted with a gradient of $CHCl_3$/MeOH of increasing polarity to give seven major fractions (PCI-PCVII). Each fraction was purified by a combination of CC, TLC and recrystallization.

Fraction PCI (23 g) afforded (1) (360 mg) and (2) (4640 mg). PC II (61.0 g) afforded compounds (3) (4950 mg), (4) (300 mg) and (5) (500 mg).

Taraxasterol acetate (**2**) was isolated from the fraction PCI (23 g) about 9.1 g of this solid was adsorbed on 15 g of silica gel DF_{254} and loaded on a column (200 g, 50 cm length and 4.5 cm diameter) packed in hexane and the polarity was then gradually increased by adding of benzene. The 12 fractions collected (100 ml each) were grouped into 6 groups according to their TLC behaviour to give compound (2).

3.3.1. taraxasterol acetate (**2**):

white solid (4.640 g), m.p 250°C (literature value 256-257 °C). Rf value 0.46 (Benzene), IR (KBr)(cm^{-1}) 1733 ($COOCH_3$) , 1454 (C=C). EIMS [M +H^+] m\z (%): 413.23 (100), 429.32 (26.9), 301(13.4).

Table 1: **Spectral data of ^1H (300 MHz) and ^{13}C NMR (75 MHz) for compound (2).**

	($CDCl_3$)	
N°	δ_H (*J* in Hz)	
1	1.64(1H,m),1.54 (1H, m)	38.42
2	1.87(2H, m)	23.69
3	4.45(dd; J =10.6,5.6)	80.89
4	-	38.03
5	0.78(H,br s)	55.33
6	1.37 (1H, m), 1.42 (1H, m)	18.20
7	1.35(2H, m)	34.21
8	-	39.98
9	1.32(1H, m)	50.32
10	-	50.03
11	1.24(2H, m)	20.94
12	1.54 (1H, m), 1.64 (1H, m)	25.07
13	1.581 (1H, m)	45.20
14	-	47.98
15	1.64 (1H, m), 0.86 (1H, m)	25.07
16	1.06 (1H, m), 1.24(1H, m)	35.56
17	-	27.93
18	2.34 (1H, m)	48.27
19	2.00 q	38.38
20	-	150.81

21	2.00 (1H, m), 1.23 (1H, m)	29.92
22	1.35 (2H, m)	39.98
23	0.91(3H, s)	27.98
24	0.90(3H, s)	16.51
25	0.82(3H, s)	16.49
26	0.93(3H, s)	16.17
27	0.97(3H, s)	15.97
28	0.93(3H, br s)	18.20
29	0.79(3H, s)	20.94
30	4.64 , 4.57	109.43
1’	2.10	170.90

The structure of the taraxasterol acetate (**2**) and the cosy (H-H); HMBC and HMBC (H-C) correlation are shown in the figure 1 and figure 2.

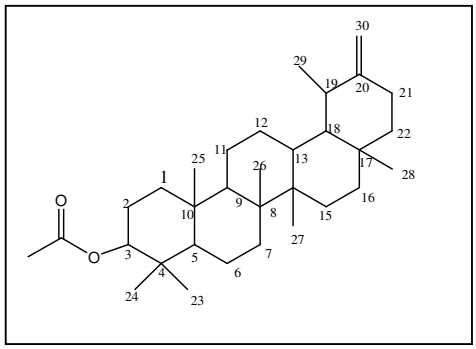

Figure 1: Structure of the taraxasterol

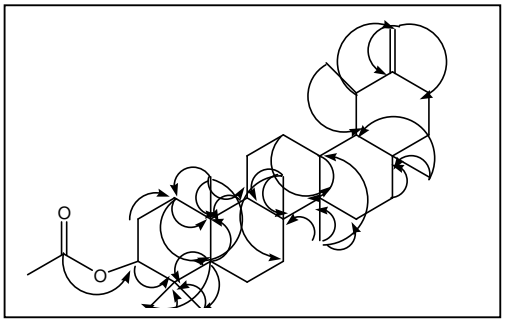

Figure 2: COSY (H-H) and HMBC and HMBC (H→C) correlation

4. Conclusion

The chemical investigation of the secondary metabolism of the plant, the endemic Algerian plant *Pergularia tomentosa* L., has resulted in the isolation of a plethora of natural products including seven compounds which are isolated for first time from this plant.

The structures of this new compound isolated from the plant was determined by means of spectroscopic methods, mainly 1D and 2D NMR techniques (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, HMBC experiments). Known molecules were identified by comparison the spectroscopic data with those of authentic samples. Additional 2D NMR experiments were conducted in some cases with the aim of fully characterising compounds for which a partial NMR assignment had been reported in the literature.

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