

A NEW GUAIANOLIDE FROM *TANACETUM FRUTICULOSUM* LEDEB.

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ABSTRACT

A new guaianolide, 3β , 4β -epoxy- 8α -isobutyryloxyguaia-1(10), 11(13)-dien-12,6 α -olide (Carlaolide) (1) from *Tanacetum fruticosum* was isolated, and its structure was elucidated on the basis of spectroscopic data.

Key Words: *Tanacetum fruticosum*, Compositae, Sesquiterpene lactone, Guaianolide.

INTRODUCTION

The genus *Tanacetum* (syn. *Chrysanthemum*), with Ca.200 species, is distributed over Europe and west Asia. *Tanacetum parthenium* has been used since ancient times for a variety of medicinal purposes, and recently has gained considerable prominence due to its ability to alleviate the symptoms of migraine (1), arthritis and psoriasis (2), and to inhibit blood platelet aggregation (3). Parthenolide and a number of related sesquiterpene lactones are considered to be responsible for these activities.

Pervious chemical investigations on different species of *Tanacetum* have shown the presence of acetylenes (4), flavonoides (5), sesquiterpene lactones (6) and essential oils (7-9). According to reports twenty six different species from the genus of *Tanacetum* grow in various region of Iran (10) and the essential oil of *Tanacetum fruticosum* from Iran have been investigated by GC, GC/MS and NMR spectroscopy and some new farnesyl derivatives have been identified by their NMR spectra (11).

The guaianolides represent one of the largest groups of sesquiterpene lactones covering over 650 known naturally occurring compounds. Much attention has been paid to the antitumor properties.

Sesquiterpene lactones have been reported to have multiple biological effects including cytotoxic, antibacterial, anti-inflammatory, hypotensive and many others.

Guaianolides are common constituents of the most Iranian genera of Compositae family (12-

16). In view of the fact that these compounds show cytotoxic and in vivo anticancer activity (17-18) and as a continuation of our work on sesquiterpene lactones of the Iranian species of Compositae (19-22), we have carried out an examination of the constituents of *Tanacetum* (syn. *Chrysanthemum fruticosum* Ledeb. B. Fedtsch) a plant collected from Shahmirzad, Province of Semnan, Iran.

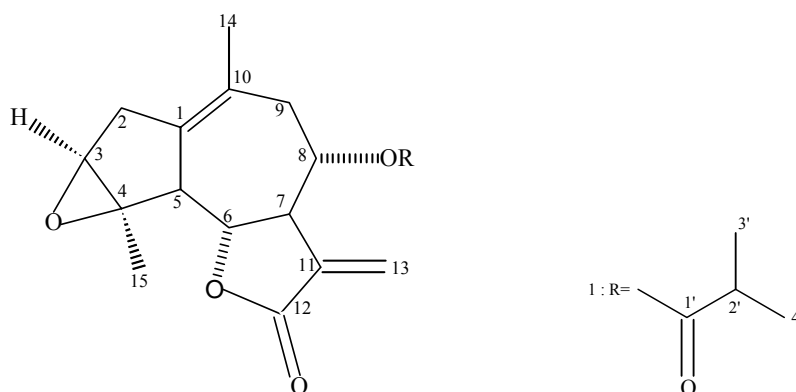
MATERIALS AND METHODS

Plant materials

Tanacetum fruticosum was collected in August 2000 from Shahmirzad, near Semnan, in northwest Iran at an altitude of 2200m. Voucher specimens with Herbarium number 59523 (TARI) are deposited at the Herbarium of the Research Instituted of Forests and Rangelands, Tehran, Iran.

Methods

The optical rotations were measured on a polarimeter using a sodium lamp (589 nm). IR spectra were recorded on a Shimadzu IR-470 spectrometer. ¹H and ¹³C-NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker AM 500 instrument and under Aspect X32 control and TMS was used as internal standard. The 2-D NMR spectra were obtained using Brukers microprograms. Low-resolution and high-resolution mass spectra were recorded on an AEI MS-50 spectrometer. Silica gel 60 (70-230 and 230-400 mesh) and TLC plate (silica gel 60 F₂₅₄) were purchased from Merck (Germany). For separation of mixtures, a



Compound 1

Table 1. NMR Data of Compound 1^a

Position	δ_C	δ_H	HMBC
1	128.5 s	-	
2	33.2 t	2.72 brd (14) 2.46 brd (14)	
3	63.5 d	3.41 brs	C-1, C-5
4	66.8 s	-	
5	51.3 d	3.09 d (10.4)	C-10, C-3
6	77.9 d	3.71 t (10.4)	C-1, C-11, C-4
7	56.9 d	3.15 ddt (3,10.4,3)	C-12, C-5
8	69.9 d	4.8 dt (2,10.4)	C-10, C-11
9	41.2 t	2.46 dd (15,10.4 α) 2.15 dd (2 β ,15)	
10	136.4 s	-	
11	137.0 s	-	
12	168.8 s	-	
13	120.5 t	6.15 d (3) 5.56 d (3)	
14	22.0 q	1.73 s	
15	18.7 q	1.68 s	
1'	176.0 s	-	
2'	34.1 d	2.58 sep (7.0,7.0)	
3'	18.9 q	1.21 d (7.0)	C-1'
4'	18.9 q	1.19 d (7.0)	C-1'

^aChemical shifts were determined at 500 (¹H) and 125 (¹³C) MHz in CDCl₃. ¹H and ¹³C NMR chemical shifts refer to CHCl₃ at 7.26 ppm and CDCl₃ at 77.0 ppm, respectively. J values in Hertz are in parentheses. Multiplicities established by DEPT experiments.

Knauer HPLC instrument with a 1001 pump and a 2500-differential refractometer detector and column a C₁₈ (120 × 8mm ID) were used.

Experimental

The whole air dried plant material (500g) was finely ground and extracted twice with CHCl₃ at room temperature for 48h. The CHCl₃ solution was evaporated to dryness, weighed, and suspended in hot MeOH (15ml) and then cooled to -15°C. After standing for a night at -15°C, the

waxy precipitate was removed by filtration and the filtrate evaporated in vacuo to afford a syrupy residue (22g). The residue was subjected to column chromatography on Silica gel and eluted with a stepwise gradient of light petroleum (b.p. 40-60 °C) and increasing amounts of diethyl ether(5:1→1:1, v/v) and then by CHCl₃-MeOH (50:1→5:1, v/v) and 50 ml fractions were collected to give eight subfractions, (fr.1-fr.8). Fraction 4 (1.3g) was

chromatographed on a Silica gel column with a solvent mixture of n-Hexan-EtOAc (1:1 v/v) and then CHCl_3 -MeOH (99:1→20:1, v/v), which among these fractions, semipreparative HPLC (RP-8 MeOH : H_2O , 7.5: 2.5, v/v) of the fraction of 36 afforded 8mg of 1. 3β , 4β -epoxy- 8α -isobutyryloxyguaia-1(10), 11(13) -dien-12,6 α -olide (Carlaolide) (1): colorless oil $[\alpha]_D^{25}$ - 16.5° (C 1.0, CHCl_3); IR (CHCl_3) λ max 1760, 1735, 1635 cm^{-1} ; ^1H and ^{13}C NMR, see Table 1; EIMS (direct inlet) m/z 332 $[\text{M}]^+$ (5), 314 $[\text{M}-\text{H}_2\text{O}]^+$ (2), 244 $[\text{M}-(\text{CH}_3)_3\text{COOH}]^+$ (26), 229 $[\text{244-Me}]^+$ (5) 71 (100); HREIMS m/z 332.1819 (calcd for $\text{C}_{19}\text{H}_{24}\text{O}_5$, 332.1821).

RESULT AND DISCUSSION

In this research a new sesquiterpene lactone with guaianolide structure was isolated from the air-dried *Tanacetum fruticosum*. The presence of guaianolide structure is an interesting chemotaxonomic marker in various species of *Tanacetum*. The sesquiterpene lactone with guaianolide structure have been reported in many other species of *Tanacetum*; *T. annum* (23), *T. argenteum* (24), *T. densum* (25) and *T. parthenium* (26). The structure elucidation of compound **1** is as follow; compound **1** (Carlaolide) showed IR bands for γ -lactone and ester groups at 1760, and 1735 cm^{-1} , respectively. Its molecular formula was $\text{C}_{19}\text{H}_{24}\text{O}_5$ as followed from its mass spectrum, which showed $[\text{M}]^+$ at m/z 332. Mass spectral peaks at m/z 244 $[\text{M}-(\text{CH}_3)_3\text{COOH}]^+$ and 229 $[\text{244-Me}]^+$ indicated the presence of an isobutyrate and a methyl group. The ^1H -NMR spectrum showed the typical signals of isobutyrate residue at 2.58 (s, $J = 7.0$, 7.0 Hz), 1.21 (d, $J = 7.0$ Hz) and 1.19 (d, $J = 7.0$ Hz).

An α -methylene- γ -lactone moiety was evident by the two doublets at δ 6.15 ($J = 3.0$ Hz) and 5.56 ($J = 3.0$ Hz) in the ^1H -NMR spectrum. The ^{13}C -NMR spectrum showed 19 signals corresponding to four $-\text{CH}_3$, three $-\text{CH}_2-$, six $-\text{CH}-$ and six quaternary carbons, as deduced from a DEPT experiment, which was in agreement with the molecular formula obtained from the mass spectrum. ^{13}C -NMR spectrum also indicated the presence of a lactone moiety, which showed signals at δ 168.8 (C-12), 137.0 (C-11) and 120.5 (C-13). The two methyl singlets in the ^1H -NMR (δ 1.73 and 1.68 ppm) correlated with the carbon signals at δ 22.0 (C-14) and 18.7 (C-15), respectively and the two methyl doublets (δ 1.21 and 1.19) correlated with carbon signals at δ 19.1 (C-3') and 18.9 (C-4') respectively in the HMQC spectrum. The ^1H , ^1H COSY experiment showed that the signal at δ 6.15 was allylic coupled with the signal at δ 3.15 and very small geminal coupling with the signal at δ 5.56 ppm. The coupling constant between H-7 and H-8 (10.4 Hz) indicated that ester residue at C-8 is α -oriented. To confirm the β -orientation of the epoxide at C-3 and C-4, an NOE experiment irradiating the H-3 signal showed enhancement of the signal at 1.68 (6%) and 3.15 (4%) corresponding to H-15 and H-7 respectively. Furthermore the NOESY spectrum of **1** showed a cross-peak between H-3 and H-15, H-5 and H-7 but none between H-6 and H-7 which indicated 6,7 trans configuration. Compound **1** was named Carlaolide.

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