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A GUAIANOLIDE FROM *TANACETUM ARGENTEUM* SUBSP. *FLABELLIFOLIUM*

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Key Word Index—*Tanacetum argenteum* subsp. *flabellifolium*; Compositae; sesquiterpene lactones; guaianolides; flabellin.

Abstract—A new guaianolide was isolated from the aerial parts of *Tanacetum argenteum* subsp. *flabellifolium*, which is endemic to Turkey. The structure of the new compound was elucidated by spectral methods, including NMR (^{1}H NMR, NOE, APT, DEPT and HETCOR) and X-ray techniques.

INTRODUCTION

In the flora of Turkey, *Tanacetum argenteum* is classified into three subspecies: *argenteum*, *flabellifolium* and *canum*, which are endemic [1]. *Tanacetum* species usually contain eudesmanolide, germacranolide and guaianolide types of sesquiterpene lactones. Our previous work on *T. argenteum* subsp. *argenteum* has mainly yielded germacranolides, two of them being new [2]. In this paper we report a new guaianolide and other constituents of *T. argenteum* subsp. *flabellifolium*.

RESULTS AND DISCUSSION

The aerial parts of *T. argenteum* (Lam.) Willd., subsp. *flabellifolium* (Boiss. et Heldr.) Grierson yielded α-amyrin (**2**), β-amyrin (**3**), sitosterol (**4**), parthenolide (**5**) [3], desacetyllaurenobiolide (**6**), spiciformin (**11**) [4], desacetyltulipinolide-1β,10α-epoxide (**7**) [5], tatridin A (**8**), 1-epi-tatridin B (**9**), tamirin (**13**) [6], desacetyl-β-cyclopyrethrosin (**10**), isospiciformin (**12**) [7], sivasinolide (**14**) [8], dentatin A (**15**) [9] and a new guaianolide (**1**).

The ^{1}H NMR (Table 1) spectrum of **1**, mp 175°, together with the IR and ^{13}C NMR data (Table 2) indicated an 8α,10α-dihydroxyguaian-4(15),11(13)-dien-6α,12-olide. The IR spectrum indicated hydroxyl groups, an α-methylene-γ-lactone and unsaturation absorptions at 3400, 1780 and 11680 cm^{-1}, respectively. The compound had a molecular formula of $C_{15}H_{20}O_4$, determined by HR mass spectrometry (*m/z* 264.14384). The ^{1}H NMR spectrum (Table 1) displayed a pair of doublet of doublets at δ 6.19 (*dd*, $J = 3.5$ and 1.5 Hz, H-13) and 6.09 (*dd*, $J = 3.0$ and 1.5 Hz, H-13′), which indicated the presence of an α-hydroxyl group attached to the carbon atom next to the lactone ring [10]. The ^{13}C NMR resonances were at δ 121.02 (*d*, C-13), 140.20 (*s*, C-11) and 170.21 (*s*, C-12). In the ^{1}H NMR spectrum, the typical ABC pattern for H-6, H-5, and H-7 was observed at δ 3.87 (*dd*, $J = 11$ and 11 Hz), 2.51 (*br dd*, $J = 11$ and 7 Hz) and 3.41 (*dddd*, $J = 3$, 3.5, 10 and 11 Hz), and the assignment was confirmed by spin decoupling experiments. Irradiation of the signal at δ 3.87 (H-6) collapsed the doublet of doublets at δ 2.51 to a doublet and the four-fold doublet at δ 3.41 into a three-fold doublet. The coupling constants among these protons indicated a *trans*-fused 6,12-lactone ring. Their ^{13}C NMR signals appeared at δ 76.60 (*d*, C-6), 52.95 (*d*,

Table 1. ^{1}H NMR spectral data for compounds **1** and **1a** (200 MHz, $CDCl_3$)

	1	**1a**
H-1	2.23 *m*	2.35 *m*
H-2	180 *m*	1.85 *m*
H-2′	1.53 *m*	1.55 *m*
H-3	2.44 *m*	2.36 *m*
H-3′	2.30 *m*	2.35 *m*
H-5	2.91 *br dd*	2.95 *br dd*
H-6	3.88 *dd*	3.93 *dd*
H-7	3.45 *dddd*	3.83 *dd*
H-8	3.88 *m*	5.15 *m*
H-9	2.16 *dd*	2.28 *dd*
H-9′	1.89 *dd*	1.85 *br d*
H-13	6.17 *dd*	6.18 *d*
H-13′	6.0 *dd*	5.49 *d*
H-14	1.33 *s*	1.27 *s*
H-15	5.16 *br s*	5.19 *br s*
H-15′	5.02 *br s*	5.02 *br s*
Oac	—	2.16 *s*

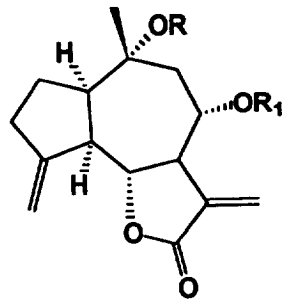
J (Hz): 5,6 = 6,7 = 7,8 = 11; 8,9 = 4.5; 8,9′ = 1.5; 9,9′ = 16; 13,13′ = 1.5; 7,13 = 3.5; 7,13′ = 3.0.

Table 2. ^{13}C NMR spectral data for compound **1** (50.37 MHz, $CDCl_3$)

	1	APT
C-1	53.99	(−)
C-2	26.73	(+)
C-3	29.46	(+)
C-4	148.95	(+)
C-5	52.95	(−)
C-6	76.60	(−)
C-7	52.22	(−)
C-8	71.36	(−)
C-9	40.55	(+)
C-10	75.42	(+)
C-11	140.20	(+)
C-12	170.21	(+)
C-13	121.04	(+)
C-14	33.36	(−)
C-15	111.47	(+)

C-5) and 52.22 (*d*, C-7). A quarternary methyl group [δ 1.33 (*s*)] and exocyclic methylene groups [δ 5.17 (*br s*) and 5.08 (*br s*)] exhibited ^{13}C NMR signals at δ 33.36 (*q*, C-14), 111.47 (*t*, C-15) and 148.95 (*s*, C-4). Spin decoupling experiments suggested that H-1 centred at δ 2.35 (*m*) was overlapped with H-9 (*dd*, $J = 4.5$ and 16 Hz) while H-8 (*m*) overlapped with H-6 at δ 3.87. In the ^1H NMR spectrum in benzene-d_6, the H-8 signal appeared at δ 3.43 (*m*), but H-6 now overlapped with H-7 at δ 2.96 (*m*). In the ^1H NMR of the acetyl derivative (**1a**) in $CDCl_3$, H-8 shifted downfield from δ 3.85 to 5.15 (*m*) and overlapped with the broadened singlet of H-15 at δ 5.19 while H-6 and H-7 overlapped at δ 3.93 and 3.85. The acetyl methyl resonance appeared at δ 2.16. The acetyl derivative gave better spectral dispersion in benzene-d_6 and displayed two broadened doublets at δ 6.19 (H-13, $J = 3.5$ Hz) and 5.46 (H-13′, $J = 3.0$ Hz), two broadened exocyclic methylene protons at δ 5.46 (H-15) and 5.02 (H-15′), a three-fold doublet at δ 4.85 (H-8, $J = 10$, 6 and 2.5 Hz), a four-fold doublet at δ 3.53 (H-7, 9.5, 9.5, 3.5 and 3.0 Hz), two double doublets at δ 3.08 (H-6, $J = 11$ and 9.5 Hz) and 2.61 (H-5, $J = 11$ and 7 Hz), and the other proton signals of the structure (Table 1). All the signals were assigned by spin decoupling experiments. The lower chemical shift of the H_2-15 as broadened singlets and the sharpening of the singlets with the irradiation of H-5 during spin decoupling experiments implied that the exocyclic methylene protons were located at C-4. 1D and 2D ^{13}C NMR (APT and HETCOR) spectra of **1** were in accordance with the structure giving one methyl, five methylene, five methine and four quarternary carbon signals (Table 2). NOE experiments confirmed the location of the exocyclic methylene group. Irradiation of H-5 caused enhancement of the signals of H-15′, H-7 and H-1, indicating that H-7, H-5 and H-1 are in the α-position, and H-15′ and H-5 are close to each other in space.

The relative configuration of the compound was determined by X-ray measurements (Table 3), and a thermal ellipsoid drawing is shown in Fig. 1. We named the compound flabellin.



1 : R=R$_1$=H

1a : R=H, R$_1$=Ac

EXPERIMENTAL

General. CC was carried out on Kieselgel 60 (0.063–0.200 mm, Merck) and Sephadex LH-20 (Pharmacia), TLC was performed on precoated silica gel 60 F_{254}, 0.2 mm plates (Merck), spots were detected under UV and spraying acidified ceric sulphate followed by

Table 3. Crystal data, data collection and refinement parameters for compound **1**

Compound	1
Empirical formula	$C_{15}H_{20}O_4$
Formula weight	264.32
Crystal colour, shape	Colourless, prismatic
Crystal dimensions (mm)	0.100 × 0.100 × 0.500
Crystal system	Orthorhombic
No. reflections used for unit cell determination	20 (31.0–57.0°)
Omega scan peak width at half-height	0.30
Lattice parameters	$a = 8.834$ (3) Å $b = 24.096$ (3) Å $c = 6.600$ (3) Å $V = 1405$ (1) Å^3
Space group	$P2_12_12_1$ (# 19)
Z value	4
D_{calc}	1.249 g cm^{-3}
F_{000}	568
$\mu_{(Cu\ K_\alpha)}$	6.97 cm^{-1}
Diffractometer	Rigaku AFC6S
Radiation	Cu K_α ($\lambda = 1.54178$ Å)
Temperature	23°
Scan type	$\omega = 2\theta$
Scan rate	8.0° min^{-1} (in omega) (3 rescans)
Scan width	$(1.26 + 0.30 \tan\theta)°$
$2\theta_{max}$	157.5°
No. of reflections measured	Total: 3409 Unique: 1706 ($R_{int} = 0.034$
No. observations ($I > 2.00\sigma(I)$)	1113
No. variables	252
Residuals: R; R_w	0.039; 0.032
GOF = $[\Sigma w(\|F_o\| - \|F_c\|)^2/(N_o - N_v)]^{1/2}$	1.71
Max. shift/error in final cycle	0.00
Max. peak in final diff. map	0.15 e$^-$ Å^{-3}
Min. peak in final diff. map	−0.17 e$^-$ Å^{-3}

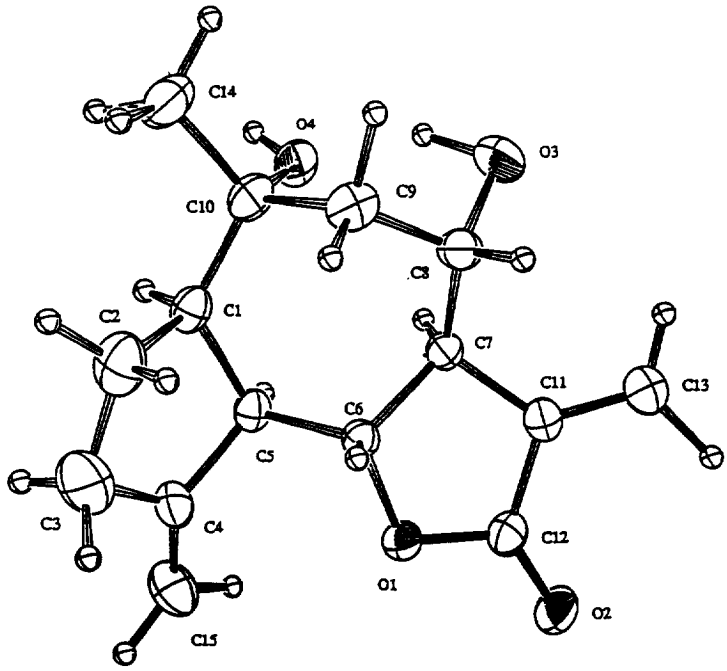


Fig. 1. Three-dimensional structure of compound **1**.

heating. IR spectra were run on a Pelkin-Elmer 983 instrument. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-200L spectrometer with $CDCl_3$, C_6D_6 as solvents and TMS as int. standard, operating at 200 and 50.32 MHz, respectively. GC-MS spectra were recorded on a VG Zabspec instrument. X-ray data were collected on a Rigaku AFC6S.

Plant material. Tanacetum argenteum subsp. *flabellifolium* was collected from southern Turkey (Ermenek). A voucher specimen (ISTE 65581) is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul, Turkey.

Extraction and isolation. Dried aerial parts (3 kg) were extracted with petrol, $CHCl_3$ and EtOH, respectively. The $CHCl_3$ and EtOH extracts were combined and treated with MeOH. The residue was applied to a silica gel column and eluted with petrol. A gradient of Et_2O was added up to 100% followed by MeOH. Frs obtained from the column were further sepd by prep. TLC and/or Sephadex LH-20. By these procedures, 180 mg **1**, 99 mg **2**, 35 mg **3**, 33 mg **4**, 17 mg **5**, 326 mg **6**, 20 mg **7**, 189 mg **8**, 4 mg **9**, 700 mg **10**, 48 mg **11**, 170 mg **12**, 86 mg **13**, 9 mg **14** and 27 mg **15** were obtained.

Flabellin (**1**). Isolated as prismatic crystals. Mp. 175°. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1780 (α,β-unsaturated-γ-lactone), 1680 (unsaturation), 1480, 1400, 1350, 1300, 1180, 1150, 1120, 1020, 970, 900, 800, 750, 650, 540. ^1H NMR: see Table 1. ^{13}C NMR (APT and HETCOR): see Table 2. HRMS *m/z* (rel. int.): calc. 264.13604, found 264.14384 $[M]^+$ ($C_{15}H_{20}O_4$) (0.2), 246 $[M-H_2O]$ (29), 228 $(246-H_2O)$ (38.5), 213 $(228-CH_3)$ (11), 203 (28 − C=CH) (55), 200 (228 − CO) (19), 188 $(203-CH_3)$ (67), 178 (30), 166 (13), 157 (26), 150 (30), 143 (20), 133 (228-lactone ring) (60), 121 (78), 107 (C_8H_{11}) (100), 97, (50), 91 (82), 84 (93), 79 (86), 69 (63), 63 (50), 58 (40).

Acetylation of **1**. Compound **1** (13 mg) was treated with pyridine (1.5 ml) and Ac_2O (1.5 ml) overnight. After vacuum evapn it was sepd by prep. TLC; thus, **1a** (9.5 mg) was obtained.

Acetyl derivative of **1** (**1a**). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1775 (α,β-unsaturated-γ-lactone), 1740, 1230 (ester), 1680 (unsaturation), 1440, 1380, 1030. ^1H NMR: see Table 1. EIMS *m/z* (rel. int.): 306 $[M]^+$ ($C_{17}H_{22}O_5$) (4), 246 $[M-CH_3COOH]^+$ (68), 228 $[246-H_2O]^+$ (100), 213 $[228-CH_3]^+$, 203 (70), 188 (85), 165 (70), 133 (65), 123 (70), 107 (71), 93 (64), 81 (73), 69 (44).

X-ray diffraction studies. Data were collected using the $\omega = 20$ mode with Cu K_α radiation. Lorentz polarization, absorption and extinction corrections were applied. The structure was solved by direct methods [11], and parameters were refined by a full-matrix least-squares technique. Compound **1** crystallized in orthorhombic space group $P2_12_12_1$ with $a = 8.834(3)$, $b = 24.096(3)$, $c = 6.600(3)$ Å, $V = 1405(1)$ Å^3, $d_c = 1.249$ gcm^{-3} and $\mu = 6.97$ cm^{-1}. All hydrogen atoms were located in a difference map and the model refined to $R_w = 0.032$ for 1113 reflections with $I \geq 2\sigma(I)$. The atomic coordinates as well as distances and angles are deposited at the Cambridge Crystallographic Data Centre.

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