

A New Epimeric Sesquiterpene Lactone from *Achillea ligustica*

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Abstract: A new sesquiterpene guaianolide (**1**) and two known compounds matricarin (**2**) and 5-hydroxy-5,6-secocaryophyllen-6-one (**3**) were isolated from the methylene chloride/methanol (1:1) extract of the aerial parts of *Achillea ligustica* All. The structure was determined on the basis of comprehensive 1D and 2D (COSY, NOESY, HMQC, HMBC) NMR and MS analyses.

Keywords: *Achillea ligustica*; Asteraceae; guaianolide.

1. Introduction

The genus *Achillea* (family Asteraceae) comprises over 100 species, mainly distributed in the northern hemisphere (Europe and temperate areas of Asia) [1]. There is wide interest in research of these plants, especially their active components, because many of the plants are used in traditional medicine for the treatment of fever, cough, bronchitis, asthma, skin inflammation, and liver ailments [2-5], and as food flavoring and preservatives [6]. The species of this genus contain essential oils [7, 8], flavonoids [9, 10], sesquiterpene lactones [11, 12] and ionone glucosides [13].

Biological activities of plant-derived extracts are attributed to the presence of various secondary metabolites, such as sesquiterpene lactones that are abundant mainly in the Asteraceae family. Biological activities of sesquiterpene lactones are mediated by a Michael-type addition reaction of their α,β -unsaturated carbonyl electrophilic groups (α -methylene- γ -lactone or an α,β -unsaturated cyclopentenone) with thiol-containing enzymes and proteins [14, 15]. This interaction adversely affects cellular function and depends on the conformation, geometry, lipophilicity, chemical environment, and accessibility of the sesquiterpene molecule to the target proteins [16-18].

As part of our ongoing research on the genus *Achillea* [13, 19-21], we success to isolate a new stereoisomer sesquiterpene guaianolide (3 α -chloro-4 β ,10 β -Dihydroxy-1 β ,2 β -epoxy-5 α ,7 α H-guai-11(13)-en-12,6 α -olide (**1**) form the methylene chloride/methanol (1:1) extract of the aerial parts of *Achillea ligustica*.

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2. Experimental

2.1. General experimental procedures

Optical rotation was determined using a JASCO DIP-360 digital Polari meter. IR spectra were recorded with a Perkin–Elmer FT-IR 1725 IR spectrophotometer. ^1H and ^{13}C NMR spectra were acquired using a JEOL-LA 500 Lambda (500 and 125 MHz, respectively) spectrometer. Chemical shifts are given on δ (ppm) scale. EI and HR-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography (CC) was carried out on Kieselgel 60 (Merck; 230–400 mesh) and Sephadex LH-20 (Pharmacia Co., Tokyo, Japan), whereas TLC was performed on silica gel 60 F254 plated (0.25 mm, Merck Co.).

2.2. Plant material

The air-dried aerial parts of *A. ligustica* All were collected in 1996 from Mt. Parnitha (Greece), and identified by Dr. Th. Constandinidis (Institute of Systematic Botany, Agricultural University of Athens). A voucher specimen of the collection (OT-6) has been deposited in the Herbarium of the University of Athens (ATHU).

2.3. Extraction and isolation

Air-dried aerial plant tissue (1 kg) was crushed and extracted with CH_2Cl_2 –MeOH (1:1) (3L X 3) at room temperature for 24 hours. After solvent removal, the residue (35 g) was kept at -20°C . The CH_2Cl_2 –MeOH (1:1) extract (10g) was subjected to CC on silica gel and eluted with *n*-hexanes, CH_2Cl_2 and MeOH in increasing order of polarity up to 100% CH_2Cl_2 and then to 15% MeOH in CH_2Cl_2 (a total solvent volume of 3 L). The *n*-hexane– CH_2Cl_2 (1:3) and (1:5) fractions were combined (5 g) based on TLC similarities, concentrated to remove the solvent and subjected to Sephadex LH-20 except that the eluting solvent was *n*-hexanes: CH_2Cl_2 :MeOH (7:4:0.25) to afford compounds **1** (6 mg) and **2** (20 mg). Pooled fractions 30–35 (40 fractions in total) were re-purified by Sephadex LH-20 with the eluting solvent *n*-hexanes: CH_2Cl_2 :MeOH (7:4:0.5), followed by HPLC to afford compound **3** (8 mg).

3 α -Chloro-4 β ,10- β dihydroxy-1 β ,2 β -epoxy-5 α ,7 α H-guai-11(13)-en-12,6-olide (**1**). Pale yellow powder, $[\alpha]_{\text{D}}^{25}$ -98.0° (*c* 0.051, MeOH). IR (KBR) ν_{max} (KBr) = 3405, 1754, 1634, 1459, 1136, 1023 cm^{-1} .- HR-FABMS: 315.0897 $[\text{M}+\text{H}]^+$ (calcd. for $\text{C}_{15}\text{H}_{19}\text{O}_5\text{Cl}$ 315.0921); ^1H and ^{13}C NMR : see Table 1.

3. Results and Discussion

Chromatographic fractionation of the methylene chloride-methanol (1:1) extract of *Achillea ligustica* has led to the isolation of two guaianolide derivatives (**1** and **2**), in addition to one with a geminal dimethyl-substituted cyclo-butane ring (**3**); one of them is a new compound (**1**) (Fig. 1).

The positive HR-FABMS of **1** revealed a quasi-molecular ion peak at m/z 315.0897 $[\text{M}+\text{H}]^+$ consistent with the molecular formula $\text{C}_{15}\text{H}_{19}\text{O}_5\text{Cl}$ and two degrees of unsaturation (confirmed by ^{13}C NMR and DEPT analysis). Also, the presence of a chlorine atom was supported by an isotopic peak at m/z 317. The IR spectrum showed absorption bands characteristic of a hydroxyl (3405 cm^{-1}), lactone ring (1754 cm^{-1}), and double bond (1634 cm^{-1}). The ^1H NMR spectrum of **1** showed two methyl singlets at δ_{H} 1.20 (3H, s, H-14) and 1.54 (3H, s, H-15), and two exo-methylene proton signals at δ_{H} 6.16 (d, $J = 3.6\text{ Hz}$) and 5.43 (d, $J = 3.6\text{ Hz}$) which correlated with a signal integrating for one proton at δ_{H} 3.56 (1H, m, H-7) in the ^1H - ^1H COSY spectrum, suggesting the presence of an α -methylene- γ -

lactone ring [20-23]. The coupling constant $J_{5,6}$ (11.0 Hz) and $J_{6,7}$ (10.0 Hz) were in agreement with guaianolide skeleton with *trans*-diaxial disposition of H-6 (β) and H-7 (α) [20-23]. In addition, the ^1H - ^1H COSY spectrum showed correlations between H-5/H-6; H-7/H-6, H-8, H-13; and H-8/H-9. The ^{13}C NMR and DEPT experiments displayed 15 carbon signals. Among them, five methine carbon signals appeared at δ_{C} 63.3 (C-2), 64.1 (C-3), 49.8 (C-5), 78.4 (C-6), and 43.3 (C-7), three methylene carbon signals at δ_{C} 22.9 (C-8), 33.7 (C-9), and 119.2 (C-13), two methyl carbon signals at δ_{C} 27.9 (C-14), and 24.0 (C-15), and five quaternary carbon signals at δ_{C} 72.9 (C-1), 79.6 (C-4), 71.8 (C-10), 140.2 (C-11), 169.2 (C-12). Additionally, the three methine protons in the oxygenated carbon region were observed at δ 4.34 (dd, $J = 9.7, 10.7$, H-6), 4.09 (brd, $J = 0.9$, H-3) and 3.83 (brd, $J = 0.9$, H-2). along with the carbon signals at δ_{C} 78.4 (CH), 64.1 (CH), and 63.3 (CH) in the ^{13}C NMR spectrum, demonstrated the presence of an $1\beta,2\beta$ -epoxy- 3α -chlorid moiety on a five-membered ring supported by HMBC correlations as follows: H-2 with C-3 and C-4; H-3 with C-1, C-2, C-4 and C-5; and H-5 with C-1, C-3, and C-4 [20,23]. Furthermore, the HMBC spectrum exhibited correlations between H-6 with C-5, C-7 and C-8; H-9 with C-7, C-8 and C-10; and H-15 with C-3, C-4 and C-5.

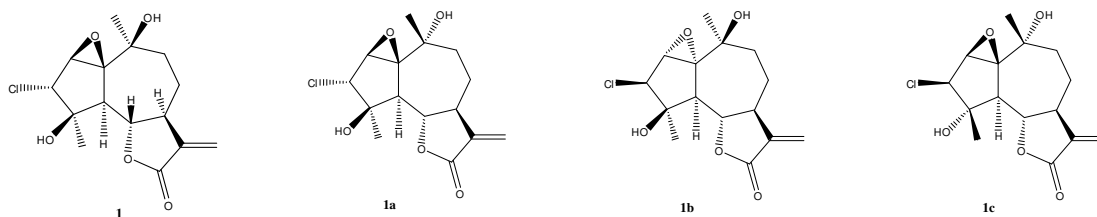
The relative stereochemistry of **1** was assigned on the basis of a study of the coupling constants and NOESY experiments relative to the biogenetically rationalized α -configuration for H-7 and a β -configuration for H-6 (Huneck *et al.*, 1991). The relative configuration and stereochemistry at C-5, C-6 and C-7 were derived from the coupling constants ($J_{5,6} = 10.9$ and $J_{6,7} = 10.7$ Hz), which were in agreement with the *trans*-diaxial disposition of the protons at C-5 (α), C-6 (β) and C-7 (α). The β -configuration of the epoxide was assigned by the presence of a NOE effect between H-2 and H-14. A proton of **1** vicinal to the epoxide (H-5) exhibited ($\delta_{\text{H}} = 2.78$) a downfield shift compared with the co-occurring $1\alpha,2\alpha$ -epoxy- 3α -chloro guaianolides, and the higher field shift of the C-5 resonance occurring at ($\delta_{\text{C}} = 49.8$), supported the β -configuration of the epoxide [23]. These results were confirmed by the coupling constant between H-2/H-3 ($J_{2,3} = 0.9$ Hz) [23]. These results were further supported by the NOESY spectrum, which showed NOE correlations between H-7 α and H-5 α ; and the absence of the correlations between H-6 β with the methyl groups CH₃-14 and CH₃-15, suggested the α -configuration of these methyl groups. Furthermore, the strong NOE which was observed between H-2 and H-14, supported the β -configuration of the hydroxyl group at C-14. From the above considerations, compound **1** showed different NOESY correlations (Fig. 2) compared with these published by Trifunovic *et al.*; 2006 [24, 25], which had showed a strong NOE between H₃-15 and H-5 and a much weaker one between H₃-15 and H-3 which revealed *cis*-H₃-15/H-5 and *trans*-H₃-15/H-3 relationships, corresponds to a 4α -Me (4β -OH) and 3α -Cl configuration and indicating that compound **1** is a new epimer [23-25] and was identified as 3α -chloro- $4\beta,10\beta$ -dihydroxy- $1\beta,2\beta$ -epoxy- $5\alpha,7\alpha$ H-guai-11(13)-en-12,6-olide.

Careful comparison between compounds **1**, **1a**, **1b**, and **1c** of the ^{13}C -NMR data (Table 2) showed most of carbon signals for compound **1** was quite similar to all of **1a** and **1b** for C-3, C-4 and C-15 and different in C-14. Additionally, for carbon signal for C-5 are completely different for all of **1**, **1a**, and **1b** than **1c** indicating the different stereochemistry of hydroxyl group at C-4. All of above mentioned data indicating that **1** is a new compound and identified as 3α -chloro- $4\beta,10\beta$ -dihydroxy- $1\beta,2\beta$ -epoxy- $5\alpha,7\alpha$ H-guai-11(13)-en-12,6-olide.

The known compounds matricarin (**2**) [22] and 5-hydroxy-5,6-secocaryophyllen-6-on (**3**) [22] were identified through direct comparison with published data.

Table 1. ^1H and ^{13}C NMR spectral data of **1** (CDCl_3 , 500 MHz, δ ppm).

No.	H	C	HMBC
1	--	72.9	H-3, 5, 9
2	3.83 brd (0.9)	63.3	H-4, 2, 15
3	4.09 brd (0.9)	64.1	H-5, 2, 4
4	--	79.6	H-5, 3, 8, 15
5	2.78 d (11.0)	49.8	3, 15
6	4.34 dd (10.0, 11.0)	78.4	5, ..
7	3.56 m	43.3	13, 5, 8, 9
8	2.30 m	22.9	----
9	1.88 ddd (9.5, 6.5)	33.7	15, 8
10	--	71.8	9, 15, 2
11	--	140.2	--
12	--	169.2	13
13a	6.16 d (3.6)	119.2	--
13b	5.43 d (3.6)	--	--
14	1.20 s	27.9	9,10
15	1.54 s	24.0	5, 3, 4

**Table 2.** ^{13}C NMR comparison of related compounds.

No.	1	1a [24]	1b [25]	1c [22]
C-1	72.9	73.0	75.1	73.4
C-2	63.3	63.5	63.2	64.7
C-3	64.1	64.0	63.6	64.4
C-4	79.5	80.0	80.3	83.4
C-5	49.8	50.0	50.9	57.9
C-6	78.4	78.5	77.2	79.9
C-10	71.8	72.0	69.8	72.2
C-14	27.9	28.0	21.9	26.5
C-15	24.0	24.0	24.0	22.1

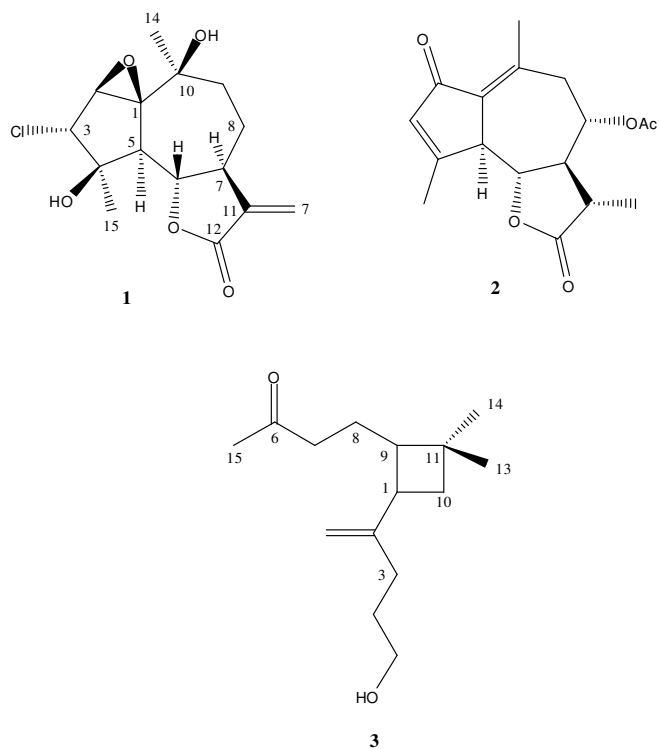


Figure 1. Compounds isolated from *Achillea ligustica*

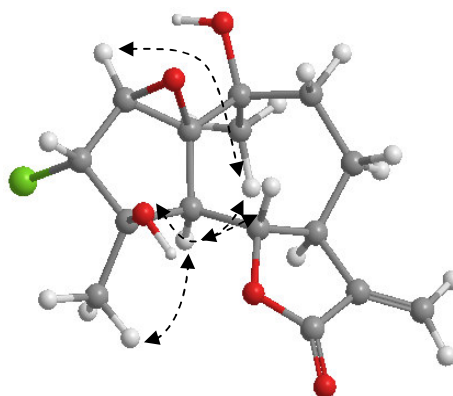


Figure 2. Selected NOE correlations of 1.

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Supporting Information:

Supporting information accompanies this paper on <http://www.acgpubs.org/RNP>

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