A New Chlorine-containing Sesquiterpene Lactone from *Achillea ligustica*

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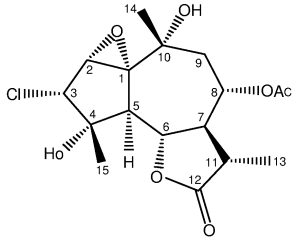
Chromatographic investigation of the methylene chloride/methanol extract of the aerial parts of *Achillea ligustica* afforded a new chlorine-containing sesquiterpene lactone as well as two known compounds. The structures of the compounds were determined by comprehensive NMR studies, including DEPT, COSY, NOE, HMQC, HMBC and HRMS. The neurotrophic activity of the new compound has been measured.

Key words: Achillea ligustica, Chlorine-containing Sesquiterpene Lactone, Neurotrophic Activity

Introduction

Achillea (Asteraceae) comprises 115 species, which are mainly distributed in Europe, Asia and North Africa and also is an introduced plant in the New World [1]. *Achillea* species have been used as anti-inflammatory, anti-spasmodic, diaphoretic, diuretic, and emmenagogic agents and for treatment of hemorrhage, pneumonia, rheumatic pain and wounds since antiquity [2]. In particular, an essential oil obtained from the leaves is used medicinally [3 – 5]. In continuation of our chemical investigation of the genus *Achillea* [6 – 12], herein we report on the isolation and identification of a new chlorine-containing sesquiterpene lactone in *A. ligustica* together with two known compounds.



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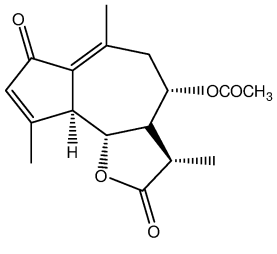

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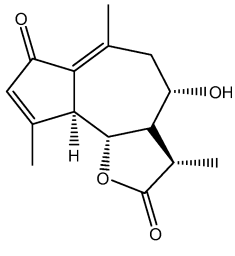

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Results and Discussion

Repetitive chromatographic steps of the methylene chloride/methanol (1 : 1) extract of the air-dried aerial parts afforded a new chlorine-containing sesquiterpene lactone **1** together with two known compounds (**2**, **3**). Compound **1** was isolated as a white material, $[\alpha]_D^{25} = +6.5^\circ$ ($c = 1.0$, $CHCl_3$) and its IR spectrum showed absorption bands at 3475 cm^{-1} (OH groups) and at 1765 and 1720 cm^{-1} (C=O groups). The CI-MS of **1** gave a molecular ion peak $[M+H]^+$ at m/z = 375, and exact mass determination at m/z = 375.1198 established the elemental composition as $C_{17}H_{24}O_7Cl$ (confirmed by ^{13}C NMR and DEPT analysis). Also, the presence of a chlorine atom was supported by an isotopic peak at $m/z = 377$. Three fragments at $m/z = 357$, 339 and 279, resulting from loss of two molecules of water and acetic acid, respectively, were indicating the presence of two hydroxyls and one acetoxy group. The ^1H and ^{13}C NMR spectral data of **1** established the presence of a guaianolide-type sesquiterpene with an acetoxy

Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **1**.

H atom	δ_H	C atom	δ_C
		C-1	72.8
H-2	3.68 br s	C-2	64.3
H-3	4.60 br s	C-3	64.2
		C-4	83.1
H-5	2.66 d (10.5)	C-5	57.2
H-6	4.28 dd (10.5, 10.0)	C-6	76.4
H-7	3.10 ddd (10.0, 10.0, 10.0)	C-7	51.3
H-8	5.18 ddd (11.0, 10.0, 3.0)	C-8	72.9
H-9α	2.15 dd (13.0, 3.0)	C-9	43.1
H-9β	2.18 dd (13.0, 11.0)		
		C-10	71.7
H-11	2.44 dq (10.0, 7.0)	C-11	41.4
		C-12	177.1
H-13	1.30 d (7.0)	C-13	15.1
H-14	1.46 s	C-14	26.9
H-15	1.54 s	C-15	22.2
OAc	2.17 s	OAc	21.3, 170.2

group at δ_H = 2.17 (s, 3H) in the ^{1}H NMR and δ_C = 21.3 (q) and 170.2 (s) in the ^{13}C NMR spectrum. Additionally, the ^{1}H NMR spectrum showed a clear doublet at δ_H = 1.30 (3H, d, J = 7.0) correlated with a methyl carbon at δ_C = 15.1 (C-13) in the HMQC spectrum. The latter proton correlated with a signal integrated for one proton at δ = 2.44 (1H, dq, J = 10.0, 7.0, H-11) in the ^{1}H-^{1}H COSY spectrum, indicating the presence of an α-methyl-γ-lactone. Moreover, it showed H-6 as a doublet of doublets at δ_H = 4.28 (J = 10.5, 10.0) correlated with two signals at δ_H = 2.66 (1H, d, J = 10.5) and 3.10 (1H, ddd, J = 10.0, 10.0, 10.0) for H-5 and H-7, respectively.

The ^{13}C NMR and DEPT experiments exhibited 17 carbon signals. Among them, seven methine carbon signals appeared at δ_C = 64.3 (C-2), 64.2 (C-3), 57.2 (C-5), 76.4 (C-6), 51.3 (C-7), 72.9 (C-8), and 41.4 (C-11), one methylene carbon signal at δ_C = 43.1 (C-9), four methyl carbon signals at δ_C = 15.1 (C-13), 26.9 (C-14), 22.2 (C-15), and 21.3 (OAc), and five quaternary carbon signals at δ_C = 72.8 (C-1), 83.1 (C-4), 71.7 (C-10), 177.1 (C-12), and 170.2 (OAc). The HMBC experiment indicated the following correlations: H-2 with C-3 and C-4; H-3 with C-1, C-2, C-4 and C-5; H-5 with C-1, C-3, C-4 and C-6; H-6 with C-5, C-7 and C-8; H-9 with C-7, C-8 and C-10; H-15 with C-3, C-4 and C-5, suggesting an epoxy moiety at the 1,2 position. All proton and carbon signals were identified by ^{1}H-^{1}H COSY, HMQC and HMBC (Table 1).

The relative stereochemistry of **1** was assigned on the basis of the study of the coupling constants and

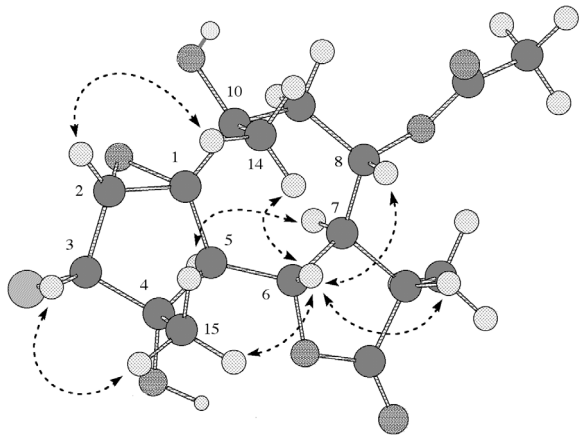

Fig. 1. Selected NOE correlations of **1**.

NOESY experiments (Fig. 1). The relative configuration and stereochemistry at C-5, C-6 and C-7 were derived from the coupling constants ($J_{5,6}$ = 10.5 and $J_{6,7}$ = 10 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 α-configuration of the epoxide was assigned by the presence of the NOE between H-2 and H-14. A proton of **1** vicinal to the epoxide (H-5) exhibited (δ_H = 2.65) a different chemical shift as compared with the published 1β,2β-epoxy-3α-chloro guaianolides, and the downfield shift of the C-5 resonance (δ_C = 57.2) supported the α-configuration of the epoxide [13]. These results were further supported by the NOESY spectrum, which showed NOE correlations between H-6β and the methyl groups CH_3-14 and CH_3-15, and with H-8β and H-11β. Furthermore, strong NOEs were observed between H-7α and H-5α, and between H-15β and H-3β.

Neuronal death contributes greatly to the brain deficits in neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease [14]. Ideal treatment and prevention of these diseases would interrupt the degenerative procedure specific to each disease. Offering protective agents to rescue damaged neurons from death is regarded as a practicable strategy for the control of neurodegenerative diseases [15]. Several compounds acting with different protective mechanisms are currently in development and few are in clinical trials, such as antioxidants and neurotrophins [16, 17].

Compound **1** was evaluated by PC12 cells [18]. It had no neurotrophic activity but showed toxicity against PC12 cells at a concentration of 10 μM. For more information on the neuroprotective ef-

fects please visit http://p.bunri-u.ac.jp/~fukuyama/fukuyama/index.htm.

Experimental Section

General

In the ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) experiments TMS was used as an internal standard. CI-MS spectra were recorded on a JEOL SX102A mass spectrometer. IR spectra were recorded on a JASCO FT/IR-5300 spectrometer.

Plant material

The air-dried aerial parts of *A. ligustica* 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 has been deposited in the Herbarium of the University of Athens (ATHU).

Extraction and isolation

The aerial parts (500 g) of *A. ligustica* were powdered and extracted with CH_2Cl_2-MeOH (1 : 1) at r. t. The extract was concentrated *in vacuo* to obtain a residue of 15 g. The residue was fractionated on a silica gel column (6 × 120 cm) eluting with *n*-hexane (2 L) followed by a gradient of *n*-hexane-CH_2Cl_2 up to 100 % CH_2Cl_2 and CH_2Cl_2-MeOH up to 15 % MeOH (2 L each of the solvent mixture). The CH_2Cl_2 (100 %) fraction was chromatographed on a Sephadex LH-20 column eluted with *n*-hexane-methylene chloride-methanol (7 : 4 : 0.5) to give compound **1** (10 mg), while the *n*-hexane-CH_2Cl_2 (1 : 3) fraction was purified on a Sephadex LH-20 column eluted with *n*-hexane-methylene chloride-methanol (7 : 4 : 0.25) to give matricarin **2** (13 mg) and desacetyl-matricarin **3** (15 mg).

*4α,10α-Dihydroxy-1α,2α-epoxy-5α,7αH-guaia-11(13)-en-12,6α-olide (**1**)*

Colorless material. – $[\alpha]_D^{25}$ = +6.5 (*c* = 1.0, $CHCl_3$). – IR (KBr) ν = 3475, 2920, 1765, 1720 cm^{-1}. – ^{1}H NMR ($CDCl_3$): The ^{1}H assignment was achieved by ^{1}H-^{1}H correlation spectroscopy (COSY), see Table 1. – ^{13}C NMR ($CDCl_3$): The ^{13}C attributions were achieved by HMQC and HMBC, see Table 1. – CI-MS: m/z = 375 $[M+H]^+$, 357 $[M+H–H_2O]^+$, 339 $[M+H–2(H_2O)]^+$, 279 $[M+H–(2H_2O+CH_3COOH)]^+$. – HRMS: m/z = 375.1198 (calcd. 375.1211 for $C_{17}H_{24}O_{17}Cl$, $[M+H]^+$).

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