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Cholinesterase Inhibitory Activity of Two Farnesylacetone Derivatives from the Brown Alga *Sargassum sagamianum*

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Two known farnesylacetone derivatives (**1** and **2**) were isolated from the Korean brown alga *Sargassum sagamianum* off Jeju Island, Korea. Compounds **1** and **2** were identified as (5*E*,10*Z*)-6,10,14-trimethylpentadeca-5,10-dien-2,12-dione and (5*E*,9*E*,13*E*)-6,10,4-trimethylpentadeca-5,9,13-trien-2,12-dione, respectively, by comparison with the literature data. Compounds **1** and **2** showed moderate acetylcholinesterase and butyrylcholinesterase inhibitory activities with IC_{50} values of 65.0~48.0 and 34.0~23.0 μM, respectively.

Key words: Marine brown algae, *Sragassum sagamianum*, Farnesylacetone derivatives, Cholinesterase inhibitory activity

INTRODUCTION

Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disease that is characterized symptomatically progressive deteriorations of activities of daily living, behavioral disturbances and cognitive loss. The neurodegerative features of AD include pathological changes in the brain, such as the formation of β-amyloid plaques and neurofibrillary tangles. Furthermore, AD is associated with substantial reductions in the activity of choline acetyltransferase and reduced levels of acetylcholine (ACh) in the brain as cholinergic neurons are lost and cholinergic neurotransmission declines (Greig *et al.*, 2001a). Cholinesterase (ChE) inhibitors retard the inactivation of ACh after synaptic release and represent the only approved treatment resulting in significant clinical benefit. Two types of ChE enzymes are found in the CNS, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Extraordinarily efficiently, they are both able to cleave >10,000 molecules of ACh per second (Bazelyansky *et al.*, 1986), at a rate that is limited more by the diffusion of ACh to the enzyme, rather than catalytic capacity. Until recently, the relative contribution of BuChE in the regulation of ACh level had been largely ignored. However, there is growing evidence that AChE and BuChE both play important roles in the regulation of ACh level and may also have an important role in the development and progression of AD (Greig *et al.*, 2001b).

The brown algae of the genus Sargassum are known to contain prenyl phenyl derivatives such as farnesylacetone derivatives and geranylgeranylphenyl derivatives (Ishitsuka *et al.*, 1979; Kusumi *et al.*, 1979a; Shizuri *et al.*, 1982; Kikuchi *et al.*, 1983; Segawa *et al.*, 1987). In the course of our investigations on the ChE inhibitor from Korean marine algae, we found moderate BuChE-inhibitory activity in the methanol extract of *S. sagamianum*. We herein report the isolation, structural characteristics and cholinesterase inhibitory activity of compounds **1** and **2**.

MATERIALS AND METHODS

General

UV spectrum was recorded with a HP8453 UV/VIS spectrophotometer. IR spectrum was performed on a Perkin-Elmer model 1750 FT-IR spectrophotometer. MS spectra were measured on a JEOL JMX-SX 102 mass spectrometer. High resolution mass measurement was done with a JEOL AX-505H mass spectrometer at high resolution. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ at 25°C on a Brucker ARX-400 NMR spectrometer. Chemical shifts (δ) are given relative to TMS, using the

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solvent peaks [$CDCl_3$ (δ_H 7.26, δ_C 77.1)] as an internal standard. TLC was performed on precoated Kiessel gel 60 F_{254} plates (Merck). The silica gel used for column chromatography was Kiessel gel 60 (70-230 mesh, Merck). Enzymes, substrates, and buffer agents used in the bioassay were purchased from SIGMA Co: AChE [EC3.1.1.7], Sigma C-5021; ATCh (acetylthiocholine chloride), A-5625; BuChE [EC3.1.1.8], C-1057; BuTCh (butyrylthiocholine chloride), B-3128; DTNB [5,5'-dithio-bis(2-nitrobenzoic acid)] D-8130.

Plant material

The brown alga, *Sargassum sagamianum* was collected off Jeju island, Korea in 2000. A voucher specimen (GD026) of the alga has been deposited at the Laboratory of Natural Products Chemistry, Hanbat National University.

Enzyme assay methods

Enzyme activities were determined at room temperature. UV absorbance was measured spectrophotometrically by a modification of a previously described method (Ellman *et al.*, 1961). To each cuvette was added DTNB (900 μL of 5.55 mM DTNB in 50 mM potassium phosphate buffer, pH 7.4) followed by the addition of ATCh (25 μL of a buffer of ATCh of varying concentration). The enzymatic reaction was initiated at 25°C by the addition of enzyme (75 μL of AChE, appropriately diluted in 50 mM, pH 7.4, potassium phosphate buffer to give 0.005 unit), and absorbance change was monitored at 412 nm for 60 sec as described (Berkman *et al.*, 1993). The slope of the absorbance change for this time is the initial rate of an enzyme reaction. Enzyme inhibition assay for butyrylcholinesterase (BuChE) was performed by the same method as that of AChE. Effect of inhibition for each sample was calculated as followed.

$$\text{Inhibition (\%)} = 100 - (ST/CT) \times 100$$

CT stands for the initial rate of control test and ST for the initial rate of sample test.

Extraction and isolation

The MeOH extract (6.4 g) of the dried algal sample (600 g) was suspended with 90% MeOH and extracted with *n*-hexane three times. The lower layer was concentrated *in vacuo* and further partitioned between 30% MeOH and $CHCl_3$. The $CHCl_3$ phase was subjected to ODS flash column chromatography [aqueous MeOH] as well as gel-filtration on Sephadex LH-20 [MeOH/$CHCl_3$(6:4)] to give a crude acetonic sesquiterpene (320 mg). This terpenoid fraction was finally purified by ODS HPLC with 75% MeCN to afford fanesylacetone derivatives **1** (7.2 mg) and **2** (36.6 mg).

Compound 1

Colorless oil; $[\alpha]_D^{23}$ +14.5° (*c* 2.4, $CHCl_3$); UV λ_{max} (ε) 239 nm (11,000); IR (film) ν_{max} cm^{-1} 1680 (aromatic C=C), 1715 (C=O); EIMS *m/z* 278 $[M]^+$, 221 $[M^+-CH_2COCH_3]$; HREIMS *m/z* 278.2238 $[M]^+$, calcd for $C_{18}H_{30}O_2$, 278.2245; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.00 (1H, s, H-11), 5.05 (1H, t, 7.0 Hz, H-5), 2.49 (2H, m, H-13), 2.43 (2H, m, H-7), 2.24-2.20 (1H, m, H-14), 2.21 (2H, m, H-4), 2.09 (2H, m, H-3), 2.10 (3H, s, H-1), 1.98 (2H, t, 7.6 Hz, H-9), 1.83 (3H, s, 10-Me), 1.59 (3H, s, 6-Me), 0.89 (3H, s, H-15), 0.88 (3H, s, H-15'); ^{13}C-NMR ($CDCl_3$, 100MHz) δ 209.1 (C-12), 200.8 (C-2), 159.1 (C-10), 136.4 (C-6), 124.4 (C-11), 122.9 (C-5), 53.7 (C-14), 43.9 (C-13), 39.9 (C-9), 33.6 (C-7), 30.1 (C-1), 26.7 (C-8), 25.7 (10-Me), 25.3 (C-3), 22.9 (C-15), 22.9 (C-15'), 22.6 (C-4), 16.0 (6-Me).

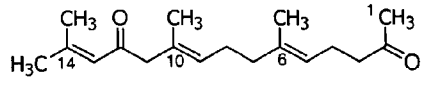
Compound 2

Colorless oil; UV λ_{max} (ε) 245 nm (21,000); IR (film) ν_{max} cm^{-1} 2950 (C_{sp3}-H), 1670 and 1625 (α,β-unsaturated carbonyl); EIMS *m/z* 276 $[M]^+$, 219 $[M^+-CH_2COCH_3]$; HREIMS *m/z* 276.2143 $[M]^+$, calcd for $C_{18}H_{28}O_2$, 278.2151; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.10 (1H, s, H-13), 5.22 (1H, t, 7.2 Hz, H-9), 5.08 (1H, t, 7.2 Hz, H-5), 3.02 (2H, s, H-11), 2.45 (2H, t, 7.2 Hz, H-3), 2.26 (2H, q, 7.2, 14.4 Hz, H-4), 2.13 (3H, s, H-15), 2.13 (3H, s, H-1), 2.13 (2H, m, H-8), 2.01 (2H, t, 7.6 Hz, H-7), 1.87 (3H, s, H-15'), 1.61 (3H, s, 6-Me), 1.61 (3H, s, 10-Me); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 209.1 (C-2), 199.7 (C-12), 155.8 (C-14), 136.3 (C-6), 129.9 (C-10), 129.2 (C-9), 123.1 (C-13), 123.0 (C-5), 55.6 (C-11), 44.0 (C-3), 39.5 (C-7), 30.2 (C-8), 27.9 (C-15'), 26.9 (C-1), 22.7 (C-4), 20.9 (C-15), 16.6 (10-Me), 16.2 (6-Me).

RESULTS AND DISCUSSION

The molecular formula of **1**, isolated as an oil $[\alpha]_D^{23}$ +14.5° (*c* 2.4, $CHCl_3$), was obtained by high resolution EIMS (*m/z* 278.2238 $[M]^+$, calcd for $C_{18}H_{30}O_2$, 278.2245) and ^{13}C-NMR data. The presence of a methyl ketone moiety and an acetyl group was deduced from the IR absorption bands at 1680 and 1715 cm^{-1}, the UV absorption maximum at 239 nm (ε 11,000). ^{1}H-NMR spectrum showed two

1



2

Table I. Cholinesterase inhibitory activities for compounds **1** and **2** and standard drugs

	IC_{50} (μM)	
	AChE	BuChE
compound **1**	65.0	34.0
compound **2**	48.0	23.0
tacrine	0.05	0.002
huperzine A	0.001	20.01
phenserine	0.008	0.53
cymserine	0.29	0.02
N-norcymserine	0.12	0.003
tolserine	0.004	0.68

olefinics [δ 6.00 (1H, s) and 5.05 (1H, t, 7.0 Hz)], a singlet ketonic methyl [2.10 (3H, s)], two singlet vinyl methyls [2.10 (3H, s) and 1.83 (3H, s)], and two doublet isopropyl methyl protons [0.89 (3H, d, 7.0 Hz) and 0.88 (3H, d, 7.0 Hz)]. The combination of ^{13}C-NMR spectrum with DEPT experimental data also revealed the presence of two carbonyls, two trisubstituted olefinics, two olefinic methines, six aliphatic methylenes, an aliphatic methine, and five methyl carbons. ^{1}H-^{1}H COSY spectrum suggested three partial structures [$(CH_3)_2CHCH_2$, $CH=C(CH_3)CH_2CH_2CH_2$, $(CH_3)C=CHCH_2CH_2$]. HMBC correlations of these subunits with two carbonyl carbons gave planar structure **1** as followed. The geometry of the double bonds at C-5 and C-10 was determined to be *E* and *Z*, respectively, from the chemical shifts of the methyl groups at 6-CH_3 (δ_H 1.59; δ_C 16.0) and 10-CH_3 (1.83; 25.7) (Bates *et al.*, 1963; Tanaka *et al.*, 1982; Ibata *et al.*, 1983). Therefore, this compound was identified as **1**.

Compound **2**, $C_{18}H_{28}O_2$; *m/e* 276.2143 (M^+) and 219 (M^+-CH_2COCH_3), was a dehydro derivative of **1**. Compound **2** showed a absorption maximum at 245 nm (ε 21,000) in its UV spectrum, and exhibited IR absorption bands due to α,β-unsaturated carbonyl system at 1670 and 1625 cm^{-1}. The presence of an isopropylidene group instead of isopropyl group in **1** was confirmed by its ^{1}H-NMR spectrum [δ 6.10 (1H, s, H-13), 5.08 (1H, t, 7.2 Hz, H-5), 3.02 (2H, s, H-11), 2.13 (6H, s, 15-Me and $COCH_3$), 1.87 (3H, s, 15-Me), 1.61 (6H, s, 6-Me and 10-Me)]. *E*-Configuration of the double bond at C-10 was obvious from the down field chemical shift (δ_H 1.61; δ_C 16.6) of the methyl group at C-10. Thus, compound **2** was identified.

Compounds **1** and **2** showed moderate AChE (IC_{50}, 65.0 μM and 48.0 μM, respectively) and BuChE (IC_{50}, 34.0 μM and 23.0 μM, respectively) inhibitory activities. Most of the potent cholinesterase inhibitors such as tacrine (Huegin, 1962), huperzine A (Liu *et al.*, 1986), phenserine (Brzostowska *et al.*, 1992), cymserine (Greig *et al.*, 1995c), *N*-norcymserine (Yu *et al.*, 1999), and tolserine (reig *et al.*, 1995c) are alkaloids or quaternary ammonium salts. Compounds **1** and **2** are, however, unexpectedly farnesylacetone skeleton. Potent BuChE-inhibitory plastoquinone derivatives such as sargaquinoic acid and sargachromenol (Kusumi *et al.*, 1979b), having similar skeleton with farnesylacetone, were also isolated from this Sargassum specimen (Ryu *et al.*, in preparation). Farnesylacetone derivatives have been described previously from the brown alga *Sargassum micracanthum* (Kusumi *et al.*, 1979c) and the authors suggested that these metabolites were derived from tocotrienols, present in other *Sargassum* species. The ubiquitous occurrence of tocotrienols in five *Cystophora* species previously described (Gregson *et al.*, 1977; Kazlauskas *et al.*, 1981) lends support to this suggestion and may be of taxonomic significance in that *C. moniliformis* has been placed as one of the most developed species of this genus.

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