# Inhibition of Acetylcholinesterase Activity by Dihydro-β-agarofuran Sesquiterpenes Isolated from Chilean Celastraceae

Julio Alarcón[a,*], Luís Astudillo[b], and Margarita Gutierrez[b]

[a] Departamento de Ciencias Básicas, Universidad del Bío-Bío, Chillán, Chile.
Fax: +56-42-25 31 56. E-mail: jualarcon@ubiobio.cl
[b] Instituto de Química, Universidad de Talca, Talca, Chile

* Author for correspondence and reprint requests

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A series of agarofuran compounds isolated from Chilean Celastraceae were evaluated *in vitro* for their cholinesterase inhibitory activities by UV spectroscopy.

*Key words:* Acetylcholinesterase Inhibition, Dihydro-β-agarofuran Sesquiterpenes, Alzheimer's Disease

## Introduction

Alzheimer's disease (AD) is characterized by upheavals of the mind abilities that are of gradual beginning, but of irreversible progress (Whitehouse *et al.*, 1982). The first clinical manifestation usually is the alteration of the memory of recent events, whereas the old memories are preserved relatively well during the progress of the disease. In conjunction with this progress of upheavals, other mind functions are impaired, among them the ability to carry out spatial calculations, exercises, skills and to manipulate common objects and instruments.

One criterion of main importance in the therapy of AD includes the effort for increasing the cholinergic brain function (Johnston, 1992). One of them is the use of precursors of acetylcholine (ACh), like choline chloride and phosphatidylcholine whose effectiveness has not been demostrated yet. Better results until now have been shown by certain inhibitors of acetylcholinesterase (AChE), which hydrolyzes acetylcholine. AChE may interact with the central cholinergic system to improve memory and cognitive deficits of the patients by diminishing the breakdown of ACh at the synaptics site of the brain. Galanthamine, which is an alkaloid and occurs in Amaryllidaceae, has been reported as the most effective medicine for AD (Thomsen and Kewitz, 1990; Thomsen *et al.*, 1991; Howes *et al.*, 2003; Howes and Hougton, 2003).

As part of our continuing program to search for bioactive natural compounds, we investigated the inhibition of the AChE activity by dihydro-β-agarofuran sesquiterpene polyol esters isolated from Chilean plants of the Celastraceae family (Alarcón *et al.*, 1998). We have previously reported the inhibition of AChE isolated from *Spodoptera frugiperda* by two agarofuran sesquiterpenes from Chilean Celastraceae (Cespedes *et al.*, 2001). This family is present in Chile in the endemic genus *Maytenus,* occurring with four species (*M. disticha, M. boaria, M. magellanica* and *M. chubutensis*). So, in the present paper, we report the inhibition of AChE from bovine erythrocytes by six kinds of sesquiterpenoids with the dihydroagarofuran skeleton.

## Experimental

### *General experimental procedures*

Analytical and preparative TLC were performed on silica gel 60 F254 E Merck plates, and the spots were visualized by spraying with a 10 % solution of $H_2SO_4$, followed by heating at 110 °C. HPLC was performed on a Merck-Hitachi La-Chrom model L-7100 instrument, equipped with a Kromasil KR100–5C18 column (250 × 4.6 mm) and a diode array detector (speed flux, 1.5 ml/min; mobile phase, MeOH/$H_2O$ 7:3 v/v). $^1H$ and $^{13}C$ NMR (300 and 50 MHz, respectively): Bruker AM-300; solvent: $CDCl_3$.

### *Plant material*

Aerial parts (steam and leaves) of *Maytenus disticha* and seeds of *Maytenus boaria* were collected in Chillán, VIII Región of Chile. A voucher speci-

men (R. Rodriguez and C. Marticorena) can be found at the ethnobotanical collection of the herbarium (CONC), Departamento de Botánica, Facultad de Ciencias Naturales y Oceanograficas, Universidad de Concepción, Concepción, Chile.

*Isolation, purification and identification of dihydro-β-agarofuran sesquiterpene polyol esters*

The aerial parts of *M. disticha* were extracted with methanol. This crude extract was dissolved in water and washed with *n*-hexane and acetyl acetate. The *n*-hexane fraction was chromatographed over silica gel, and eluted with *n*-hexane/AcOEt mixtures containing increasing portions of AcOEt to give four compounds.

Seeds of *M. boaria* were extracted with MeOH. The extract was partitioned using $CHCl_3$, AcOEt and water. The $CHCl_3$ extract was chromatographed on a silica gel column using *n*-hexane/AcOEt mixtures. Polar fractions were combined and separated by preparative TLC (*n*-hexane/AcOEt, 1:1 v/v) to give three bands, which were further separated by HPLC.

The structure of the compounds was established on the basis of spectroscopy data. The full assigment of $^1H$ and $^{13}C$ NMR signals (Tables I, II) for

Table II. $^1H$ NMR data of compounds isolated from seeds of *Maytenus boaria* (*J* in Hz).

| H | **5** | **6** |
|---|---|---|
| 1 | 5.27 (dd)<br>*J* = 4.0; 12 | 5.35 (dd)<br>*J* = 4.0; 12 |
| 2α | 1.20 (m) | 1.6 (m) |
| 2β | 1.50 (m) | 1.83 (m) |
| 3α | 1.90 (m) | 1.43 (m) |
| 3β | 1.70 (m) | 2.14 |
| 4 | | 2.25 (m) |
| 6 | 5.53 (s) | 5.87 (br s) |
| 7 | 2.32 (dd)<br>*J* = 3.0; 3.0 | 2.40 (br s)<br>*J* = 3.5 |
| 8α | 2.51 (ddd)<br>*J* = 3.0; 7.0; 16 | |
| 8β | 2.21 (dd)<br>*J* = 7.0; 16 | 4.27 (br d)<br>*J* = 3.5 |
| 9 | 4.98 (d)<br>*J* = 7.0 | 4.77 (br s) |
| 12 | 1.51 (s) | 1.34 (s) |
| 13 | 1.53 (s) | 1.41 (s) |
| 14 | 1.40 (s) | 1.45 (s) |
| 15 | 1.34 (s) | 1.01 (d)<br>*J* = 7.0 |

Table I. $^1H$ NMR data of compounds isolated from aerial parts of *Maytenus disticha* (*J* in Hz).

| H | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| 1 | 5.75 (d)<br>*J* = 3.3 | 5.65 (d)<br>*J* = 3.20 | 5.75 (d)<br>*J* = 3.30 | 5.71 (d)<br>*J* = 3.5 |
| 2 | 5.50 (dd)<br>*J* = 4.0; 6.8 | 5.60 (brd)<br>*J* = 6.6 | 5.59 (dd)<br>*J* = 4.0; 6.8 | 5.59 (dd)<br>*J* = 2.5; 3.5 |
| 3α | 1.83 (ddd)<br>*J* = 1.2; 2.5; 15 | 1.72 (ddd)<br>*J* = 1.2; 2.5; 15 | 1.83 (ddd)<br>*J* = 1.1; 2.5; 15 | 1.77 (ddd)<br>*J* = 1.2; 2.5; 15 |
| 3β | 2.49 (ddd)<br>*J* = 3.9; 6.5; 15 | 2.46 (m) | 2.61 (ddd)<br>*J* = 4.1; 6.5; 15 | 2.49 (ddd)<br>*J* = 4.0; 6.5; 15 |
| 4 | 2.39 (ddq)<br>*J* = 1.2; 6.4; 7.5 | 2.34 (m) | 2.39 (ddq)<br>*J* = 1.1; 6.4; 7.5 | 2.39 (ddq)<br>*J* = 1.2; 6.5; 7.5 |
| 6 | 5.58 (d)<br>*J* = 1.0 | 5.90 (d)<br>*J* = 1.0 | 5.89 (d)<br>*J* = 1.0 | 6.38 (d)<br>*J* = 1 |
| 7 | 2.38 (brd)<br>*J* = 3.0 | 2.36 (brd)<br>*J* = 3.0 | 2.38 (brd)<br>*J* = 3.0 | 2.38 (brd)<br>*J* = 3.0 |
| 8α | | 2.48 (m) | | |
| 8β | 5.32 (d)<br>*J* = 4.0 | 2.12 (m) | 5.31 (d)<br>*J* = 4.0 | 5.27 (d)<br>*J* = 3.0 |
| 9 | 5.90 (d)<br>*J* = 1.0 | 5.35 (d)<br>*J* = 1.0 | 5.89 (d)<br>*J* = 1.0 | 5.52 (s) |
| 12 | 1.44 (s) | 1.39 (s) | 1.54 (s) | 1.56 (s) |
| 13 | 1.40 (s) | 1.36 (s) | 1.53 (s) | 1.43 (s) |
| 14 | 1.23 (d)<br>*J* = 7.5 | 1.12 (d)<br>*J* = 7.5 | 1.18 (d)<br>*J* = 7.5 | 1.16 (d)<br>*J* = 7.5 |
| 15 | 4.32 (d)<br>*J* = 12.5<br>4.24 (d)<br>*J* = 12.5 | 5.10 (d)<br>*J* = 12.0<br>4.30 (d)<br>*J* = 12.5 | 4.79 (d)<br>*J* = 12.0<br>4.75 (d)<br>*J* = 12.5 | 5.10 (d)<br>*J* = 12.5<br>4.53 (d)<br>*J* = 12.5 |

all compounds was achieved using two-dimensional NMR techniques: 1$\alpha$,2$\alpha$,6$\beta$,8$\alpha$-tetraacetoxy-9$\beta$-benzoyloxy-15-hydroxy-$\beta$-agarofuran (**1**); 1$\alpha$,2$\alpha$,6$\beta$-triacetoxy-9$\beta$-benzoyloxy-15-hydroxy-$\beta$-agarofuran (**2**); 1$\alpha$,2$\alpha$,6$\beta$-triacetoxy-9$\beta$-benzoyloxy-8$\alpha$,15-dihydroxy-$\beta$-agarofuran (**3**); 1$\alpha$,2$\alpha$,6$\beta$,8$\alpha$,15-pentaacetoxy-9$\beta$-benzoyloxy-$\beta$-agarofuran (**4**); 1$\alpha$-acetoxy-6$\beta$,9$\beta$-difuroyloxy-4$\beta$-hydroxy-$\beta$-agarofuran (**5**); 6$\beta$,8$\alpha$-diacetoxy-9$\beta$-furoyloxy-1$\alpha$-hydroxy-$\beta$-agarofuran (**6**).

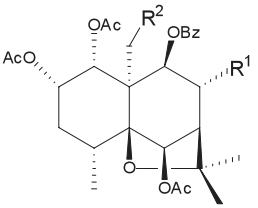
*Acetylcholinesterase inhibition assay*

The assay for measuring the acetylcholinesterase activity was carried out according to López *et al.* (2002). Briefly, 50 $\mu$l of AChE solution (0.25 U/ml) in phosphate buffer (8 mM $K_2HPO_4$, 2.3 mM $NaH_2PO_4$, 150 mM NaCl, 0.05 % Tween 20, pH 7.6) and 50 $\mu$l of the sample dissolved in the same buffer were added to the wells. The plates were incubated for 30 min at room temperature before the addition of 100 $\mu$l of the substrate solution [40 mM $Na_2HPO_4$, 0.2 mM 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), 0.24 mM acetylthiocholine iodide (ACTI) in distilled water, pH 7.5]. The absorbance was read in a Multiskan Ascent Instrument microplate reader (Optics Planet, Northbrook, USA) at 405 nm after 3 min. Enzyme activity was calculated as the percentage compared to a control using buffer and enzyme solution only. Compounds were tested at 0.5 mg/ml. When the enzyme inhibition was $>$ 50 % at this concentration, further dilutions of these samples were undertaken and the corresponding $IC_{50}$ values were determined. The $IC_{50}$ values were calculated from three individual determinations.

## Results and Discussion

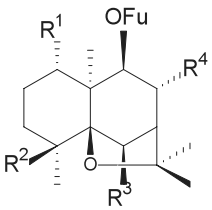
The inhibitory activity against AChE of six dihydro-$\beta$-agarofuran sesquiterpene polyol esters (Figs. 1 and 2) is shown in Table III and Fig. 3. According to the data in Table III, compound **1** shows a greater inhibitory activity than the other compounds assayed against AChE with an $IC_{50}$ value of 0.07 mg/ml, close to the reference compound used.

To demostrate that the biological activity can be related to the nucleus of the polyhydroxy-agarofurans, six compounds belonging to the polyhydroxy dihydroagarofuran sesquiterpenoid-type were used: alatol (**1**, **3** and **4**), 8-deoxy-alatol (**2**), 4$\beta$-hydroxy-celorbicol (**5**) and celapanol (**6**). Our




| | $R^1$ | $R^2$ |
|---|---|---|
| **1** | OAc | OH |
| **2** | H | OH |
| **3** | OH | OH |
| **4** | OAc | OAc |

Fig. 1. Compounds isolated from aerial parts of *Maytenus disticha.*




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| **5** | OAc | OH | OFu | H |
| **6** | OH | H | OAc | OAc |
| **7** | OAc | H | OAc | OAc |

Fig. 2. Compounds isolated from seeds of *Maytenus boaria.*

Table III. AChE inhibitory activity of the compounds isolated from Chilean Celastraceae.

| Compound | $IC_{50}$[a] ± SE[b] [mg/ml] | $IC_{50}$[a] ± SE[b] [mM] |
|---|---|---|
| **1** | 0.07 ± 0.002 | 0.12 ± 0.003 |
| **2** | 0.14 ± 0.002 | 0.26 ± 0.006 |
| **3** | 0.25 ± 0.002 | 0.40 ± 0.0 |
| **4** | 0.21 ± 0.003 | 0.33 ± 0.006 |
| **5** | 0.38 ± 0.004 | 0.74 ± 0.007 |
| **6** | 0.34 ± 0.016 | 0.74 ± 0.035 |
| Galanthamine | 0.001 | 0.10 |

[a] $IC_{50}$ is the concentration producing 50 % of AChE inhibition calculated by ANOVA ($P < 0.05$).
[b] Mean ± standard error.

results show that comparing the activities of the different compounds the agarofurans with a nucleus of the alatol-type are most active.

The presence of a free hydroxy group at C-15 and of the OAc group next to OBz could be responsible for the biological activity of these compounds.

Comparing the inhibitory activity of **4** ($IC_{50}$ 0.0057 mg/ml) and **7** ($IC_{50}$ 0.0029 mg/ml), previously reported by Cespedes *et al.* (2001), where enzyme obtained from *S. frugiperda* was used, with these new results obtained using enzyme from bo-

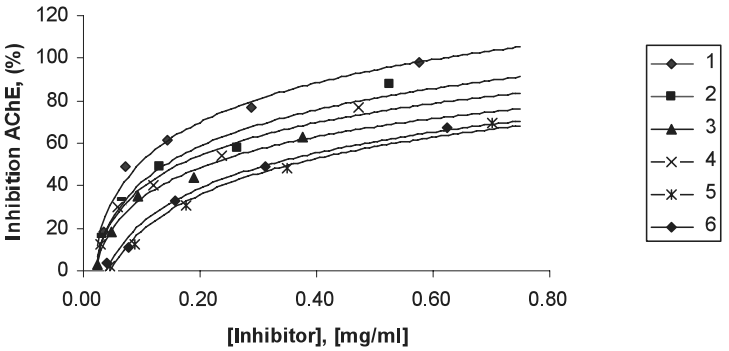



Fig. 3. Inhibitory effects of sesquiterpenes with agarofuran skeleton on AChE activity. The percentage of enzyme activity for the inhibitors was calculated as compared to the control activity (see Experimental).

vine erythrocytes, it is possible to observe that these $IC_{50}$ values possess lesser degree.

Probably, these results permit to verify a major affinity of these compounds to enzyme from insects. These facts explain the good insecticidal activity shown by these types of compounds (Spivey *et al.*, 2002).

On the other hand, from a structural point of view, many of the compounds used as AChE inhibitors reported in the literature present a skeleton of alkaloid-type (tacrine, donezepil, rivastigmine, galanthamine) (Wilkinson, 2007; Darreh-Shori *et al*., 2008). Nevertheless, our compounds with an epoxyeudesmane skeleton in a condensed structure have shown a very good activity. In summary, our study suggests that agarofuran compounds might serve as lead compounds to design new potential AChE inhibitors with high selectivty, low toxicity and additional pharmaceutical effects.

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