

Butyrylcholinesterase Inhibitory Guaianolides from *Amberboa ramosa*

Sher Bahadar Khan, Azhar-ul-Haq, Shagufta Perveen, Nighat Afza¹, Abdul Malik, Sarfraz Ahmad Nawaz, Muhammad Raza Shah, and Muhammad Iqbal Choudhary

International Centre for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi; Karachi-75270, Pakistan and ¹Pharmaceutical Research Centre, PCSIR Labs Complex Karachi, Karachi-75280, Pakistan

(Received November 11, 2004)

Phytochemical investigation of the whole plant of *Amberboa ramosa* led to the isolation of six sesquiterpene lactones which could be identified as 8 α -hydroxy-11 β -methyl-1 α H, 5 α H, 6 β H, 7 α H, 11 α H-guai-10(14), 4(15)-dien-6, 12-olide(1), 3 β , 8 α -dihydroxy-11 α -methyl-1 α H, 5 α H, 6 β H, 7 α H, 11 β H-guai-10(14), 4(15)-dien-6, 12-olide (2), 3 β , 4 α , 8 α -trihydroxy-4 β -(hydroxymethyl)-1 α H, 5 α H, 6 β H, 7 α H-guai-10(14), 11(13)-dien-6, 12-olide (3), 3 β , 4 α , 8 α -trihydroxy-4 β -(chloromethyl)-1 α H, 5 α H, 6 β H, 7 α H-guai-10(14), 11(13)-dien-6, 12-olide(4), 3 β , 4 α , dihydroxy-4 β -(hydroxymethyl)-1 α H, 5 α H, 6 β H, 7 α H-guai-10(14), 11(13)-dien-6, 12-olide(5), 3 β , 4 α -dihydroxy-4 β -(chloromethyl)-8 α -(4-hydroxymethacrylate)-1 α H, 5 α H, 6 β H, 7 α H-guai-10(14), 11(13)-dien-6, 12-olide (6) by spectroscopic methods. All of them showed inhibitory potential against butyrylcholinesterase.

Key words: *Amberboa ramosa*, Compositae, Guaianolides, Butyrylcholinesterase inhibition

INTRODUCTION

Cholinesterases are enzymes that share extensive sequence homology and distinct substrate specificity and inhibitor sensitivity. Cholinesterases are potential target for the symptomatic treatment of Alzheimers disease and related dementias. It has been found that butyrylcholinesterase (BChE) inhibition is an effective tool for the treatment of AD and related dementias (Yu *et al.*, 1999). It has been found that BChE is found in significantly higher quantities in Alzheimers plaques than in plaques of normal age-related non-demented brains. BChE is produced in the liver and enriched in the circulation. In addition, it is also present in adipose tissue, intestine, smooth muscle cells, white matter of the brain and many other tissues (Silver, 1974). It is generally viewed as a back up for the homologous AChE and to act as a scavenger for anti-cholinesterase compounds (Schwarz *et al.*, 1995).

Amberboa ramosa (Compositae) is an annual herbaceous plant which is grown in India and Pakistan. The plant has tonic, aperient, febrifuge, deobstruent, cytotoxic and anti-

bacterial activities (Akhtar *et al.*, 1993). Previously triterpenoids, flavanoids, steroids, and sesquiterpene lactones have been reported from *Amberboa* species (Akhtar *et al.*, 1993; Harrison and Kulshrestha, 1984). In the present investigation, a methanolic extract of the *Amberboa ramosa* showed positive cytotoxicity in the brine shrimp lethality test (Meyer *et al.*, 1982). Further biological screening of the chloroform soluble fraction revealed significant inhibitory activity against the *butyrylcholinesterase*. This prompted us to carry out bioassay-guided isolation studies on this plant. Herein we report the isolation and structure elucidation of six guaianolides (1-6). All of them showed inhibitory activity against butyrylcholinesterase; compounds 3-6 are more potent than 1 and 2.

MATERIALS AND METHODS

General experimental procedure

Optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were recorded on a 460 Shimadzu spectrometer. EIMS and HRFABMS were recorded on JMS-HX-110 with a data system and on JMS-DA 500 mass spectrometers. The ¹H- and ¹³C-NMR, HMQC, and HMBC spectra were recorded on Bruker spectrometers operating at 400 MHz for ¹H- and 100.6 MHz for ¹³C-NMR,

Correspondence to: Abdul Malik, International Centre for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi; Karachi-75270, Pakistan
E-mail: abdul.malik@iccs.edu

respectively. The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are in Hz. Aluminum sheets precoated with silica gel 60 F₂₅₄ (20 × 20 cm, 0.2 mm thick; E-Merck) were used for TLC and flash silica (230-400 mesh) was used for column chromatography.

Materials

The whole plant of *Amberboa ramosa* Jafri (Compositae) was collected in June 2002, from Karachi (Pakistan) and identified by Dr. Surraiya Khatoon, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen (no.KU 312 b) has been deposited. Horse-serum BChE (E.C 3.1.1.8), acetylthiocholine iodide, butyrylthiocholine chloride, 5,5'-dithiobis [2-nitrobenzoic acid] (DTNB) and galanthamine were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

Extraction and isolation

The shade dried plant material (20 kg) was extracted three times with methanol at room temperature. The residue from the methanolic extract was partitioned between *n*-hexane and water. The water soluble fraction was further extracted with chloroform, ethyl acetate and *n*-butanol. The chloroform soluble fraction (55 g) was subjected to silica gel column chromatography eluting with *n*-hexane: ethyl acetate, ethyl acetate, ethyl acetate:methanol and methanol in increasing order of polarity. The fractions which were obtained from *n*-hexane:ethyl acetate (7.5:2.5), were combined and further subjected to column chromatography using *n*-hexane:ethylacetate (8:2) as eluent to afford the pure compounds **1** (15 mg) and **2** (26 mg) from the head and tail fractions, respectively. The fractions which eluted with *n*-hexane:ethyl acetate (6.5:3.5) showed two major and two minor spots on TLC. It was further subjected to column chromatography using *n*-hexane:ethyl acetate (7:3) as eluent to afford pure compounds **3** (35 mg) and **5** (20 mg), respectively. The fractions which were obtained from *n*-hexane:ethyl acetate (5.5:4.5), were combined and further subjected to column chromatography using *n*-hexane:ethylacetate (6:4) as eluent to afford compounds **4** (15 mg) and **6** (17 mg), respectively.

8 α -Hydroxy-11 β -methyl-1 α H,5 α H,6 β H,7 α H,11 α H-guai-10(14),4(15)-dien-6,12-olide (1)

Colourless crystals. M.p. 129-131 °C; $[\alpha]_D^{20} +20$ ($c = 0.2$, CHCl₃). IR (KBr) ν_{\max} cm⁻¹ 3447 (OH), 1755 (γ -lactone), 1656 (double bond). HREIMS: m/z 248.1419 (calcd. for C₁₅H₂₀O₃ 248.1414). EIMS m/z (rel. int.): 248 [M]⁺ (52.7), 233 [M-Me]⁺ (9.4), 230 [M-H₂O]⁺ (47.3), 215 [M-Me-H₂O]⁺ (4.9), 105 (62.4), 55 (100). ¹H-NMR (CDCl₃, 400 MHz) δ : 2.79 (ddd, $J = 10.6, 9.3, 8.1$ Hz, H-1), 2.04 (ddd, $J = 13.5, 8.1, 5.9$ Hz, H-2 α), 2.21 (ddd, $J = 13.5, 9.3, 7.4$ Hz, H-2 β)

2.71 (m, H-3), 3.03 (dd, $J = 10.6, 9.8$ Hz, H-5), 4.27 (dd, $J = 11, 9.8$ Hz, H-6), 2.63 (ddd, $J = 11, 10.3, 9.8$ Hz, H-7), 4.05 (ddd, $J = 9.8, 6.6, 4.9$ Hz, H-8), 2.02 (dd, $J = 12.9, 4.9$ Hz, H-9 α), 2.37 (dd, $J = 12.9, 6.6$ Hz, H-9 β), 2.84 (dq, $J = 10.3, 6.1$ Hz, H-11), 1.33 (d, $J = 7.1$ Hz, H-13), 4.94 (br s, H-14), 5.13 (brs, H-14'), 5.21 (brs, H-15), 5.30 (brs, H-15'). ¹³C-NMR (CDCl₃, 100 MHz) δ : 48.4 (C-1), 29.7 (C-2), 33.6 (C-3), 150.3 (C-4), 52.9 (C-5), 79.4 (C-6), 50.7 (C-7), 70.0 (C-8), 36.2 (C-9), 144.4 (C-10), 42.6 (C-11), 178.2 (C-12), 16.1 (C-13), 116.0 (C-14), 110.7 (C-15).

3 β ,8 α -Dihydroxy-11 α -methyl-1 α H,5 α H,6 β H,7 α H,11 β H-guai-10(14),4(15)-dien-6,12-olide (2)

Colorless crystals. M.p. 136-137 °C; $[\alpha]_D^{20} +12.8^\circ$ ($c = 0.01$, CHCl₃). IR ν_{\max} cm⁻¹: 3500 (OH), 1750 (γ -lactone), and 1645 (double bond); HREIMS: m/z 264.1367 (calcd for C₁₅H₂₀O₄ 264.1362); EIMS m/z 264 [M]⁺, 246 [M-H₂O]⁺ (5.1), 228 [M-2H₂O]⁺ (7.5). ¹H-NMR (CDCl₃, 400 MHz) δ : 2.80 (ddd, $J = 10.5, 9.2, 8.0$ Hz, H-1), 2.09 (ddd, $J = 13.2, 8.0, 5.8$ Hz, H-2 α), 2.25 (ddd, $J = 13.2, 9.2, 7.3$ Hz, H-2 β) 4.0 (dd, $J = 7.3, 5.8$ Hz, H-3), 2.85 (dd, $J = 10.5, 9.9$ Hz, H-5), 4.29 (dd, $J = 11, 9.9$ Hz, H-6), 2.56 (ddd, $J = 11, 10.3, 9.8$ Hz, H-7), 4.10 (ddd, $J = 9.8, 6.5, 4.7$ Hz, H-8), 2.10 (dd, $J = 12.9, 4.7$ Hz, H-9 α), 2.43 (dd, $J = 12.9, 6.5$ Hz, H-9 β), 2.80 (dq, $J = 10.3, 6.1$ Hz, H-11), 1.34 (d, $J = 7.0$ Hz, H-13), 4.96 (br s, H-14), 5.10 (br s, H-14'), 5.21 (br s, H-15), 5.32 (br s, H-15'). ¹³C-NMR: (CDCl₃, 100 MHz) δ : 46.3 (C-1), 32.0 (C-2), 74.0 (C-3), 150.4 (C-4), 51.8 (C-5), 79.1 (C-6), 50.0 (C-7), 70.9 (C-8), 36.9 (C-9), 144.2 (C-10), 42.4 (C-11), 179.0 (C-12), 16.0 (C-13), 116.2 (C-14), 110.9 (C-15).

3 β ,4 α ,8 α -Trihydroxy-4 β -(hydroxymethyl)-1 α H,5 α H,6 β H,7 α H-guai-10(14),11(13)-dien-6,12-olide (3)

Colourless crystals. M.p. 195-196 °C; $[\alpha]_D^{20} +15^\circ$ ($c = 0.02$, CH₃OH). IR ν_{\max} cm⁻¹: 3483 (OH), 1752 (γ -lactone), 1645 (double bond); HREIMS: m/z 296.1267 (calcd. 296.1260 for C₁₅H₂₀O₆); EIMS m/z (rel. int.): 278 [M-H₂O]⁺ (1.8), 265 [M-CH₂OH]⁺ (2.1), 260 [M-2H₂O]⁺ (1.5). ¹H-NMR (CD₃OD, 500 MHz) δ : 3.15 (ddd, $J = 10.6, 9.3, 8.1$ Hz, H-1), 2.0 (ddd, $J = 13.5, 8.1, 5.9$ Hz, H-2 α), 2.20 (ddd, $J = 13.5, 9.3, 7.4$ Hz, H-2 β), 4.04 (dd, $J = 7.4, 5.9$ Hz, H-3), 2.21 (dd, $J = 10.6, 9.8$ Hz, H-5), 4.07 (dd, $J = 11, 9.8$ Hz, H-6), 2.16 (ddd, $J = 11, 10.3, 9.8$ Hz, H-7), 4.0 (ddd, $J = 9.8, 6.1, 4.9$ Hz, H-8), 2.75 (dd, $J = 13.0, 4.9$ Hz, H-9 α), 2.05 (dd, $J = 13.0, 6.1$ Hz, H-9 β), 6.15 (d, $J = 3.2$ Hz, H-13), 5.91 (d, $J = 3.2$ Hz, H-13'), 5.06 (br s, H-14), 5.0 (br s, H-14'), 3.92 (d, $J = 11.7$ Hz, H-15a), 3.87 (d, $J = 11.7$ Hz, H-15b). ¹³C-NMR (CD₃OD, 125 MHz) δ : 48.3 (C-1), 37.8 (C-2), 77.9 (C-3), 85.8 (C-4), 55.5 (C-5), 79.4 (C-6), 51.6 (C-7), 70.7 (C-8), 36.8 (C-9), 144.7 (C-10), 139.5 (C-11), 173.2 (C-12), 122.7 (C-13), 116.3 (C-14), 64.5 (C-15).

3 β ,4 α ,8 α -Trihydroxy-4 β -(chloromethyl)-1 α H,5 α H,6 β H,7 α H-guai-10(14),11(13)-dien-6,12-olide (4)

Colourless amorphous powder. M.p. 180-182 °C; $[\alpha]_D^{20} +21^\circ$ ($c = 0.02$, CH₃OH). IR ν_{\max} cm⁻¹: 3483 (OH), 1752 (γ -lactone), 1645 (double bond); HREIMS: m/z 314.5766 (calcd. 314.5762 for C₁₅H₁₉O₅Cl); EIMS m/z (rel. int.): 296 [M-H₂O]⁺ (1.8), 278 [M-2HO₂]⁺ (1.5), 260 [M-H₃ClO]⁺ (2.1), 41 (100). ¹H-NMR: (CD₃OD, 500 MHz) δ : 3.09 (ddd, $J = 10, 9.6, 7.9$ Hz, H-1), 2.20 (ddd, $J = 13, 7.9, 6.0$ Hz, H-2 α), 2.25 (ddd, $J = 13, 9.6, 7.4$ Hz, H-2 β), 4.01 (dd, $J = 7.4, 6.0$ Hz, H-3), 2.25 (dd, $J = 10.0, 9.7$ Hz, H-5), 4.10 (dd, $J = 10.5, 9.7$ Hz, H-6), 2.17 (ddd, $J = 10.5, 10.0, 9.5$ Hz, H-7), 4.12 (ddd, $J = 9.5, 6.5, 4.9$ Hz, H-8), 2.35 (dd, $J = 12.8, 4.9$ Hz, H-9 α), 2.10 (dd, $J = 12.8, 6.5$ Hz, H-9 β), 6.0 (d, $J = 2.2$ Hz, H-13), 5.90 (d, $J = 2.2$ Hz, H-13'), 5.10 (br s, H-14), 4.98 (br s, H-14'), 3.91 (d, $J = 11.5$ Hz, H-15a), 3.80 (d, $J = 11.7$ Hz, H-15b). ¹³C-NMR: (CD₃OD, 125 MHz) δ : 48.0 (C-1), 37.1 (C-2), 78.0 (C-3), 85.5 (C-4), 52.5 (C-5), 79.5 (C-6), 49.9 (C-7), 70.5 (C-8), 36.4 (C-9), 144.2 (C-10), 139.7 (C-11), 173.5 (C-12), 122.4 (C-13), 116.5 (C-14), 50.6 (C-15).

3 β ,4 α -Dihydroxy-4 β -(hydroxymethyl)-8 α -(4-hydroxymethacrylate)-1 α H,5 α H,6 β H,7 α H-guai-10(14),11(13)-dien-6,12-olide (5)

Colourless crystals. M.p. 72-74 °C; $[\alpha]_D^{20} +27^\circ$ ($c = 2.01$, CH₃OH). IR: ν_{\max} (KBr) cm⁻¹: 3495 (OH), 1751 (γ -lactone), 1705 (ester carbonyl group) and 1650 (double bond). HREIMS: m/z : [M]⁺ 380.1633 (calcd. 380.1630 for C₁₉H₂₄O₈); EIMS: m/z (rel. int.): 362() [M-H₂O]⁺, 344 () [M-2H₂O], 278 (9) [M-C₄H₆O₃]⁺, 85 (100) [C₄H₅O₂]⁺, 57(32) [C₃H₅O]⁺. ¹H-NMR: (CD₃OD, 500 MHz) δ : 3.20 (ddd, $J = 10.5, 9.5, 8.0$ Hz, H-1), 1.85 (ddd, $J = 13.0, 8.0, 5.7$ Hz, H-2 α), 2.20 (ddd, $J = 13.0, 9.5, 7.5$ Hz, H-2 β), 4.12 (dd, $J = 7.5, 5.7$ Hz, H-3), 2.27 (dd, $J = 10.5, 9.9$ Hz, H-5), 4.15 (dd, $J = 11, 9.9$ Hz, H-6), 2.17 (ddd, $J = 11, 10, 9.8$ Hz, H-7), 5.07 (ddd, $J = 9.8, 6.4, 5.2$ Hz, H-8), 2.65 (dd, $J = 13.5, 5.2$ Hz, H-9 α), 2.11 (dd, $J = 13.5, 6.4$ Hz, H-9 β), 6.05 (d, $J = 3.0$ Hz, H-13), 5.92 (d, $J = 3.0$ Hz, H-13'), 5.12 (br s, H-14), 4.98 (br s, H-14'), 4.0 (d, $J = 11.5$ Hz, H-15a), 3.80 (d, $J = 11.5$ Hz, H-15b), 4.28 (d, $J = 9.8$ Hz, H-18), 6.20 (d, $J = 1.2$ Hz, H-19), 5.90 (d, $J = 1.2$ Hz, H-19'); ¹³C-NMR: (CD₃OD, 125 MHz) δ : 48.1 (C-1), 38.0 (C-2), 77.2 (C-3), 85.5 (C-4), 56.5 (C-5), 79.0 (C-6), 50.6 (C-7), 75.7 (C-8), 36.4 (C-9), 144.5 (C-10), 139.1 (C-11), 172.7 (C-12), 122.4 (C-13), 116.2 (C-14), 64.4 (C-15), 165.7 (C-16), 141.0 (C-17), 61.8 (C-18), 125.1 (C-19).

3 β ,4 α -Dihydroxy-4 β -(chloromethyl)-8 α -(4-hydroxymethacrylate)-1 α H,5 α H,6 β H,7 α H-guai-10(14),11(13)-dien-6,12-olide (6)

Colourless crystals. M.p. 177-178 °C; $[\alpha]_D^{20} +71^\circ$ ($c = 2.01$, CH₃OH). IR: ν_{\max} (KBr) cm⁻¹: 3490 (OH), 1750 (γ -lactone),

1710 (ester carbonyl group) and 1651 (double bond). HREIMS: m/z 398.5977 (calcd. 398.5974 for C₁₉H₂₃O₇Cl); EIMS: m/z (rel. int.): 296 [M-C₄H₆O₃]⁺ (6), 278 [M-C₄H₆O₄]⁺ (9), 242 [M-C₄H₉O₄Cl]⁺ (7), 85 [C₄H₅O₂]⁺ (100), 57 [C₃H₅O]⁺ (32). ¹H-NMR: (CD₃OD, 500 MHz) δ : 3.59 (ddd, $J = 11.0, 5.4, 10.5$ Hz, H-1), 2.49 (ddd, $J = 14.7, 10.5, 6.0$ Hz, H-2 α), 1.54 (dd, $J = 14.7, 7.1$ Hz, H-2 β), 4.10 (d, $J = 6.0$ Hz, H-3), 2.25 (dd, $J = 11.0, 10.0$ Hz, H-5), 4.91 (dd, $J = 10.0, 9.3$ Hz, H-6), 3.18 (dd, $J = 9.3, 9.0$ Hz, H-7), 5.16 (ddd, $J = 9.0, 5.0, 1.5$ Hz, H-8), 2.43 (dd, $J = 14.5, 1.5$ Hz, H-9 α), 2.71 (dd, $J = 14.5, 5.0$ Hz, H-9 β), 6.08 (d, $J = 3.1$ Hz, H-13), 5.61 (d, $J = 3.1$ Hz, H-13'), 5.09 (br s, H-14), 4.78 (br s, H-14'), 4.18 (d, $J = 11.5$ Hz, H-15a), 3.82 (d, $J = 11.5$ Hz, H-15b), 4.28 (d, $J = 9.8$ Hz, H-18), 6.26 (d, $J = 1.2$ Hz, H-19), 5.96 (d, $J = 1.2$ Hz, H-19'). ¹³C-NMR: (CD₃OD, 125 MHz) δ : 48.9 (C-1), 39.6 (C-2), 77.0 (C-3), 85.5 (C-4), 59.1 (C-5), 78.7 (C-6), 47.9 (C-7), 75.6 (C-8), 36.1 (C-9), 144.4 (C-10), 139.2 (C-11), 171.0 (C-12), 122.3 (C-13), 117.4 (C-14), 50.2 (C-15), 166.5 (C-16), 141.5 (C-17), 61.6 (C-18), 126.2 (C-19).

In vitro butyrylcholinesterase inhibition assay

Butyrylcholinesterase inhibiting activity was measured

Table I: *In vitro* quantitative inhibition of BChE by compounds 1-6

Compounds	IC ₅₀ ± SEM ¹⁾ [μM]
1	78.5 ± 0.06
2	51.2 ± 0.01
3	39.2 ± 0.02
4	21.1 ± 0.01
5	34.0 ± 0.01
6	15.0 ± 0.05
Gаланthamine ²⁾	8.6 ± 0.05

¹⁾ Standard mean error of five assays

²⁾ Positive control used in the assay

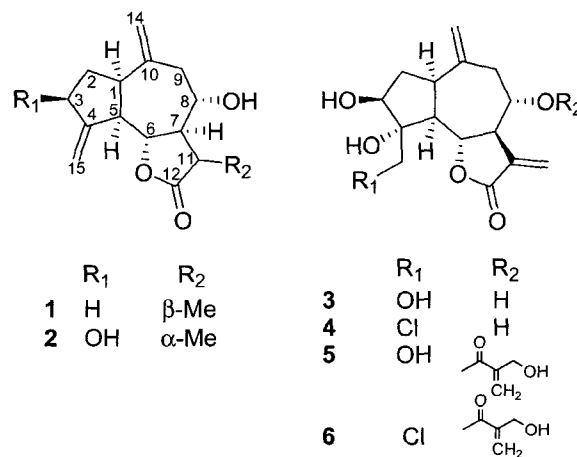


Fig.1. Structures of compounds 1-6

by the spectrophotometric method previously reported in literature (Ellman *et al.*, 1961). Butyrylthiocholine chloride was used as substrate to assay butyrylcholinesterase. The reaction mixture contained 150 μL of (100 mM) sodium phosphate buffer (pH 8), 10 μL of DTNB, 10 μL of test-compound solution and 20 μL of butyrylcholinesterase solution, mixed and incubated for 15 minutes (25 °C). The reaction was then initiated by the addition of 10 μL butyrylthiocholine. The hydrolysis of butyrylthiocholine was monitored by the formation of yellow 5-thio-2-nitrobenzoate anions as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of butyrylthiocholine at a wavelength of 412 nm (15 min.). Test compounds and the positive control (Galanthamine) were dissolved in EtOH. All the reactions were performed in triplicate in 96-well micro-titre plates in *SpectraMax 340* (Molecular Devices, U.S.A.). The percentage (%) inhibition was calculated as follows $(E - S)/E \times 100$, where E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound.

Determination of IC_{50} values

The concentrations of test compounds that inhibited the hydrolysis of substrate (butyrylthiocholine) by 50% (IC_{50}) were determined by monitoring the effect of various concentrations of these compounds in the assays on the inhibition values. The IC_{50} values were then calculated using the EZ-Fit Enzyme Kinetics program (*Perrella Scientific Inc.*, Amherst, U.S.A.).

RESULTS AND DISCUSSION

Compound **1** was isolated as colourless crystals and showed the molecular ion peak in HREIMS at m/z 248.1414 corresponding to the molecular formula $\text{C}_{15}\text{H}_{20}\text{O}_3$. The IR spectrum contained absorption bands at 3447, 1755, 1656 cm^{-1} characteristic of a hydroxyl group, the carbonyl of γ -lactone and the double bond, respectively. The $^1\text{H-NMR}$ spectrum showed the signals of proton geminal to the lactone oxygen atom (lactone proton) as doublet of doublet at δ_{H} 4.27 ($J = 11.0, 9.8$ Hz, H-6). The $^1\text{H-}^1\text{H}$ correlations revealed its coupling to two vicinal protons which is characteristic of a guaianolide with a 6,7-fused lactone. The spectrum further showed signals due to exomethylene groups at δ_{H} 5.13 (H-14) and 4.94 (1H each, br s, H-14) and at δ_{H} 5.30 and 5.21 (1H each, br s, H-15). The presence of secondary hydroxyl group was deduced from the downfield signal of methine proton at δ_{H} 4.05 (ddd, $J = 9.8, 6.6, 4.9$ Hz) The signal at δ_{H} 4.27 showed $^1\text{H-}^1\text{H}$ correlations to vicinal proton at δ_{H} 3.03 (dd, $J = 10.6, 9.8$ Hz) and 2.63 (ddd, $J = 11, 10.3, 9.8$ Hz) which could subsequently be assigned to H-5 and H-7, respectively. The signals at δ_{H} 1.33 (d, $J = 7.1$ Hz, H-13),

2.04 (ddd, $J = 13.5, 8.1, 5.9$ Hz, H-2a), 2.21 (ddd, $J = 13.5, 9.3, 7.4$ Hz, H-2b), 2.02 (dd, $J = 12.9, 4.9$ Hz, H-9a), 2.37 (dd, $J = 12.9, 6.6$ Hz, H-9b), 2.79 (ddd, $J = 10.6, 9.3, 8.1$ Hz, H-1) and 2.84 (dq, $J = 10.3, 6.1$ Hz, H-11) were assigned to methyl, methylene and methine protons, respectively. The $^{13}\text{C-NMR}$ spectrum (BB and DEPT) showed 15 carbon signals comprising of five methylene, six methine, one methyl and three quaternary carbons (see experimental part). The position of substituents were confirmed by the HMBC and COSY experiments, while the relative stereochemistry at various chiral centers of **1** were assigned through the 2D NOESY spectrum. The data identified **1** as 8 α -hydroxy-11 β -methyl-1 α H,5 α H,6 β H,7 α H,11 α H-guai-10(14),4(15)-dien-6,12-olide, reported earlier from *Saussurea involucreata* (Huikang *et al.*, 1986).

Compound **2** was isolated as colourless crystals and showed the molecular ion peak in HREIMS at m/z 264.1367 corresponding to the molecular formula $\text{C}_{15}\text{H}_{20}\text{O}_4$. The IR, $^1\text{H-}$ and $^{13}\text{C-NMR}$ spectra of **2** were identical to those of **1** except an additional signal which appeared at δ 4.10 (ddd, $J = 9.8, 6.5, 4.7$ Hz) in $^1\text{H-NMR}$ and at δ 70.9 in $^{13}\text{C-NMR}$ spectra. The structure was also confirmed through HMBC, COSY and NOESY experiments. Comparison of data with literature identified **2** as 3 $\alpha,8\alpha$ -dihydroxy-11 α -methyl-1 α H,5 α H,6 β H,7 α H,11 β H-guai-10(14),4(15)-dien-6,12-olide reported earlier from *Tricholepis glaberrima* (Fernandez *et al.*, 1989; Singhal *et al.*, 1982).

Compound **3** was isolated as colorless amorphous crystals. HREIMS showed the molecular ion peak at m/z 296.1267 corresponding to the molecular formula $\text{C}_{15}\text{H}_{20}\text{O}_6$. The IR spectrum contained absorption bands at 3490, 1749, 1645 cm^{-1} characteristic of a hydroxyl group, the carbonyl of γ -lactone and the double bond, respectively. The $^1\text{H-NMR}$ spectrum showed the signals for a proton geminal to the lactone oxygen atom (lactone proton) as a doublet of doublet at δ_{H} 4.07 ($J = 11.0, 9.8$ Hz). The spectrum further showed signals at δ_{H} 5.06, 5.0 (1H each, br s) and at δ_{H} 6.15 (d, $J = 3.2$ Hz, H-13) and 5.91 (d, $J = 3.2$ Hz, H-13') for the olefinic protons. The signal at δ_{H} 4.07 (dd, $J = 11.0, 9.8$ Hz) showed $^1\text{H-}^1\text{H}$ correlations to vicinal proton at δ_{H} 2.21 (dd, $J = 10.6, 9.8$ Hz) and 2.16 (ddd, $J = 11.0, 10.3, 9.8$ Hz) which could subsequently be assigned to H-5 and H-7, respectively. The signals for the oxymethine protons appeared at δ_{H} 4.0 (ddd, $J = 9.8, 6.1, 4.9$ Hz, H-8) and 4.04 (dd, $J = 7.4, 5.9$ Hz, H-3) while the signals at δ 3.92 (d, $J = 11.7$ Hz, H-15a) and 3.87 (d, $J = 11.7$ Hz, H-15b) could be assigned to hydroxymethylene protons. The $^{13}\text{C-NMR}$ spectrum (BB and DEPT) showed 15 carbon signals, five methylene, six methine and four quaternary carbons (see experimental). The position of substituents were confirmed by the HMBC and COSY experiments while the relative stereochemistry at various chiral centers were assigned through the 2D NOESY

spectrum. On the basis of these evidences the compound **3** could be assigned the structure as 3 β ,4 α ,8 α -trihydroxy-4 β -(hydroxymethyl)-1 α H,5 α H,6 β H,7 α H-guai-10(14),11(13)-dien-6,12-olide, reported earlier from *Centaurea scoparia* (Oksuz *et al.*, 1994; Youssef and Frahm, 1994).

Compound **4** was isolated as colourless amorphous powder. HREIMS showed the molecular ion peak in at m/z 314.6236 corresponding to the molecular formula C₁₅H₁₉O₅Cl. The IR, ¹H- and ¹³C-NMR spectra of **4** were identical to those of **3** with additional signals at δ 3.91 (d, J = 11.5 Hz) and 3.80 (d, J = 11.5 Hz) in ¹H-NMR and at δ 51.0 in ¹³C-NMR spectra for the chloromethylene group. The data suggested the structure as 3 β ,4 α ,8 α -trihydroxy-4 β -(chloromethyl)-1 α H,5 α H,6 β H,7 α H-guai-10(14),11(13)-dien-6,12-olide, reported earlier from *Centaurea scoparia* (Youssef and Frahm, 1994).

Compound **5** was isolated as colourless crystals and showed the molecular ion peak in HREIMS at m/z 382.1633 corresponding to the molecular formula C₁₉H₂₄O₈. The IR, ¹H- and ¹³C-NMR spectra of **5** were identical to those of **3** with additional signals at δ 4.28 (d, J = 9.8 Hz, H-18) and two doublets at δ 6.20 (J = 1.2 Hz, H-19) and 5.90 (J = 1.2 Hz, H-19') in ¹H-NMR and at δ 61.9, 125.1, 141.0 and 165.5 in ¹³C-NMR spectra due to the presence of a side chain ester. The point of attachment of ester moiety was shown to be C-8 based on downfield shifts of H-8 (δ 5.02) as well as C-8 (δ 75.7) which was also confirmed by HMBC experiment. The basic skeleton was also confirmed through HMBC, COSY and NOESY experiments. The foregoing data suggested that the structure of (**5**) is 3 β ,4 α ,-dihydroxy-4 β -(hydroxymethyl)-8 α -(4-hydroxymethacrylate)-1 α H,5 α H,6 β H,7 α H-guai-10(14),11(13)-dien-6,12-olide, reported earlier from *Saussurea candicans* (Singh and Bhala, 1988).

Compound **6** was isolated as colourless crystal and showed the molecular ion peak in HREIMS at m/z 382.1633 corresponding to the molecular formula C₁₉H₂₃O₇Cl. The IR, ¹H- and ¹³C-NMR spectra of **6** were identical to those of **5** with different signals for chloromethylene group which appeared at δ 3.20 (d, J = 11.5 Hz), 3.18 (d, J = 11.5 Hz) in ¹H-NMR and at δ 51.2 in ¹³C-NMR spectra. The data suggested the structure as 3 β ,4 α ,-dihydroxy-4 β -(chloromethyl)-8 α -(4-hydroxymethacrylate)-1 α H,5 α H,6 β H,7 α H-guai-10(14),11(13)-dien-6,12-olide, reported earlier from *Centaurea scoparia* (Youssef and Frahm, 1994).

Compounds **1** and **6** displayed weak (78.5 \pm 0.06) and strong (15.0 \pm 0.05) inhibitory potential, respectively, against BChE. From the results (Table I) it is clear that the chlorine containing compounds **4** and **6** are the most active

inhibitor of BChE; the latter is more potent than former due to the presence of hydroxyl and carbonyl functionalities in the side chain. The gradual increase in inhibitory activity of compounds **1**, **2**, **3**, and **5** may be due to the increasing number of the hydroxyl groups. These hydroxyl moieties can form H-bonding within the active site of BChE and thus can enhance the inhibitory potential of the compounds.

REFERENCES

- Akhtar, N., Malik, A., Afza, N., and Badar, Y., Cycloartane type triterpenes from *Amberboa ramosa*. *J. Nat. Prod.*, 56, 295-299 (1993).
- Ellman, G. L., Courtney K. D., Andres, V., and Featherstone, R. M., A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, 7, 88-95 (1961).
- Fernandez, I., Garcia, B., Grancha, F. J., and Pedro, J. R., Sesquiterpenes lactones, flavonoids and coumarins from *Centaurea collina*. *Phytochemistry*, 28, 2405-2407 (1989).
- Harrison, D. A. and Kulshrestha, D. K., Chemical constituents of *Amberboa ramosa*. *Fitoterapia*, LV, 189-192 (1984). Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., and McLaughlin, J. L., Brine shrimp: A conventional general bioassay for active plant constituents. *Planta Med.*, 45, 31-34 (1982).
- Huikang, W., Zhangdia, I., Kan, H., and Shuwen, W., Studies on the chemical constituents of *Saussurea involucrate*. *Yaoxue Xuebao*, 21, 680-682 (1986).
- Oksuz, S., Serin, S., and Topcu, G., Sesquiterpene lactones from *Centaurea hermanii*. *Phytochemistry*, 35, 435-438 (1994).
- Schwarz, M., Glick, D., Loewensten, Y., and Soreq, H., Engineering of human cholinesterases explains and predicts diverse consequences of administration of various drugs and poisons. *Pharmacol. Ther.*, 67, 283-322 (1995).
- Silver A., The Biology of Cholinesterases. North Holland Publishing Comp., *Amsterdam Elsevier* (1974).
- Singh, P. and Bhala, M., Guaianolides from *Saussurea candicans*. *Phytochemistry*, 27, 1203-1205 (1988).
- Singhal, A. K., Chowdhury, P. K., Sharma, R. P., Baruah, J. N., and Herz, W., Guainolides from *Tricholepis glaberrima*. *Phytochemistry*, 21, 462-463 (1982).
- Youssef, D. and Frahm, A. W., Constituents of the Egyptian *Centaurea scoparia*; chlorinated guaianolides of the aerial part. *Planta Med.*, 60, 267-270 (1994).
- Yu, S. Q., Holloway, H. W., Utsuki, T., Brossi, A., and Greig, N. H., Synthesis of novel phenserinebasedselective inhibitors of butyrylcholinesterase for Alzheimers disease. *J. Med. Chem.*, 42, 1855-1861 (1999).