Terpenes from *Artemisia herba-alba*

Abou El-Hamd H. Mohamed*, Abeer M. Esmail, and Adila M. El-Saade

Department of Chemistry, Aswan-Faculty of Science, Aswan University, Aswan, Egypt. Fax: +020973480450. E-mail: abouhassan68@yahoo.com

* Author for correspondence and reprint requests

Z. Naturforsch. **68c**, 343–346 (2013); received December 22, 2012/May 5, 2013

Chromatographic investigation of the methylene chloride/methanol extract of the aerial parts of *Artemisia herba-alba* afforded a new monoterpene dimer, in addition to a known monoterpene and three known sesquiterpene lactones. The structures of the compounds were determined by comprehensive NMR analyses, including DEPT, COSY, HMQC, HMBC, and HRMS.

Key words: *Artemisia herba-alba*, Sesquiterpenes, Monoterpene

Introduction

The large genus *Artemisia*, family Asteraceae in the tribe Anthemideae, has been the subject of numerous chemical and biological studies (Saadali *et al.*, 2001). *Artemisia* species, widespread throughout the world, are important medicinal plants, which have been used for the treatment of diseases such as malaria, hepatitis, cancer, tracheitis, pharyngitis, pneumonia, inflammation, and infections by fungi, bacteria, and viruses (Kim *et al.*, 2002; Zheng *et al.*, 1996). Several species of *Artemisia* are important in folk medicine, thus, *A. herba-alba* has been used as an antihelminthic by local populations in Morocco (Boriky *et al.*, 1996), *A. mongolica* has been proven to cure inflammations and colds in Northwest China (Hu *et al.*, 1996), *A. pontica* is used as a sedative and appetizer in Bulgarian folk medicine (Todorova *et al.*, 1996). Of approximately 200 species growing in China, more than 50 have been used in traditional Chinese medicinal practice, for the treatment of gynaecopathy, amenorrhea, bruise, and rheumatic disease (Tan *et al.*, 1999; Kwak *et al.*, 1997). Furthermore, *A. vulgaris* is used to flavour tea and rice dishes in Asia and as a culinary herb for poultry and pork in western cultures. In oriental medicine, it has been employed as an analgesic agent, in conjunction with acupuncture therapy, and has been implemented in the treatment of painful menstruation and in the induction of labour or miscarriage (Lee *et al.*, 1998). From extensive studies, the genus *Artemisia* has been found to be a rich source of biologically active compounds such as monoterpenes, ses-

quiterpenes, triterpenes, and flavones (Kim *et al.*, 2002; Tan *et al.*, 1999; Tang *et al.*, 2000; Mohamed *et al.*, 2010). This paper describes the isolation, identification, and structure elucidation of a new monoterpene dimer, **1**, two germacranolides, **2** and **3** (Marco 1989; Marco *et al.*, 1994; Pathak and Khanna, 1987), an eudesmanolide, **4** (Ahmed *et al.*, 1990), and a monoterpene, **5** (Marco *et al.*, 1991), from the aerial parts of *A. herba-alba*.

Results and Discussion

Repetitive chromatographic steps in the fractionation of the methylene chloride/methanol (1:1) extract of the air-dried aerial parts of *A. herba-alba* afforded the new compound **1**, in addition to the known sesquiterpene lactones **2**, **3**, and **4** and the monoterpene **5**. Compound **1** was obtained as yellowish oil and showed a molecular ion peak $[M]^+$ at m/z 360, in accord with the molecular formula $C_{21}H_{28}O_5$, in the EI mass spectrum. Fragment ions at m/z 342 and 327 were due to the elimination of a water molecule and a methyl group, respectively. An interesting peak appearing at m/z 300 (12%) was due to the elimination of a CH_3COOH molecule from the $[M]^+$ ion. The strong peak at m/z 135 $[C_9H_{11}O]^+$ (33%) resulted from the elimination of $C_{10}H_{13}O_2$ from the $[M - CH_3COOH]^+$ ion. This ion could be formed by cleavage of the C9-C10 bond. The fragment ion at m/z 105 (40%) might be produced by elimination of the two methyl groups from the ion 135, while the fragment ion at m/z 91 could be produced by elimination of a CH_2 group from the ion 105. Exact mass determination of the ion at m/z 360

Fig. 1. Chemical structures of compounds **1**–**5** isolated from *Artemisia herba-alba*.

established the elemental composition $C_{21}H_{28}O_5$ (experimental 360.1937, calcd. 360.1902).

The 1H NMR spectrum showed seven singlet signals at δ_H 6.30 ppm, 1.10 ppm, 1.28 ppm, 5.08 ppm, 1.22 ppm, 1.12 ppm, and 3.70 ppm assigned to H-2', H-7', H-8', H-5, H-11, H-12, and the methyl ester, respectively. Furthermore, it revealed the presence of a doublet of doublets at δ_H 2.88 ppm ($J = 11.9, 4.3$ Hz, H-4'), correlated with a doublet of doublets at δ_H 2.52 ppm (dd, $J = 4.3, 17.0$ Hz, H-5'a) and 2.69 ppm (dd, $J = 11.9, 17.0$ Hz, H-5'b) in the 1H - 1H -COSY spectrum. It also showed a doublet signal at δ_H 4.56 ppm (d, $J = 4.55$ Hz, H-7), coupled with a carbon atom at δ_C 77.74 ppm (C-7) in the HMQC spectrum. The latter proton showed a correlation with a multiplet signal at δ_H 2.33 ppm (m, H-8) in the 1H - 1H -COSY spectrum. Moreover, it showed a multiplet signal at δ_H 2.14 ppm integrated for three protons (H-3a, H-9a, H-10a), and it showed a clear correlation in the 1H - 1H -COSY spectrum

with a multiplet signal at δ_H 2.38 ppm integrated for three protons (H-3b, H-9b, H-10b). The ^{13}C NMR and DEPT experiments of **1** displayed twenty one carbon signals: three carbonyl carbon atoms at δ_C 177.0 ppm (C-2), 197.27 ppm (C-6'), and 172.62 ppm (C-9'), five methyl carbon atoms at δ_C 28.27 ppm (C-11), 27.40 ppm (C-12), 22.91 ppm (C-7'), 28.93 ppm (C-8'), and 51.69 ppm (COOMe), four methylene carbon atoms at δ_C 34.08 ppm (C-3), 36.78 ppm (C-5'), 26.98 ppm (C-9), and 33.76 ppm (C-10), five methine carbon atoms at δ_C 154.37 ppm (C-2'), 49.40 ppm (C-4'), 133.49 ppm (C-5), 77.74 ppm (C-7), and 48.96 ppm (C-8), and four quaternary carbon atoms at δ_C 136.0 ppm (C-1'), 35.15 ppm (C-3'), 134.75 ppm (C-4), and 35.46 ppm (C-6). Confirmation of the structure of compound **1** was given by the HMBC analysis; the most important correlations were observed between: H-2' (δ_H 6.30 ppm, s) and C-4' (δ_C 49.40 ppm), C-6' (δ_C 197.27 ppm), C-10 (δ_C 33.76 ppm); H-4' (δ_H 2.88 ppm, dd)

and C-3' (δ_C 35.15 ppm), C-5' (δ_C 36.78 ppm), C-6' (δ_C 197.27 ppm), C-7' (δ_C 22.91 ppm), C-8' (δ_C 28.93 ppm), C-9' (δ_C 172.62 ppm); H-5' (δ_H 2.52 ppm, dd; 2.69 ppm, dd) and C-3' (δ_C 35.15 ppm), C-4' (δ_C 49.40 ppm), C-6' (δ_C 197.27 ppm); H-3 (δ_H 2.14 ppm, m; 2.38 ppm, m) and C-2 (δ_C 177.0 ppm); H-5 (δ_H 5.08 ppm, s) and C-6 (δ_C 35.46 ppm), C-7 (δ_C 77.74 ppm), C-8 (δ_C 48.96 ppm), C-9 (δ_C 26.98 ppm); H-7 (δ_H 4.56 ppm, d) and C-2 (δ_C 177.0 ppm), C-4 (δ_C 134.75 ppm), C-8 (δ_C 48.96 ppm); H-8 (δ_H 2.33 ppm, m) and C-6 (δ_C 35.46 ppm); H-9 (δ_H 2.14 ppm, m; 2.38 ppm, m), H-10 (δ_H 2.14 ppm, m; 2.38 ppm, m) and C-4 (δ_C 134.75 ppm), C-1' (δ_C 136.0 ppm); H-11 (δ_H 1.22 ppm), H-12 (δ_H 1.12 ppm) and C-5 (δ_C 133.49 ppm); H-7' (δ_H 1.10 ppm), H-8' (δ_H 1.28 ppm), and C-2' (δ_C 154.37 ppm), C-3' (δ_C 35.15 ppm), C-4' (δ_C 49.40 ppm). On the basis of these results, compound **1** was identified as a new monoterpene dimer, which was given the name herbalbin, a new natural product (Fig. 1).

Experimental

General

^1H NMR (600 MHz, CDCl_3), ^{13}C NMR (125 MHz, CDCl_3), and the 2D spectra were recorded on a JEOL (Hiroshima, Japan) 500-MHz Lambda spectrometer, with tetramethylsilane (TMS) as an internal standard. EI mass spectra were recorded on a JEOL SX102A mass spectrometer.

Plant material

The aerial parts of *A. herba-alba* Asso were collected during the flowering stage at Tebessa, Eastern Algeria. A voucher specimen has been deposited at the Department of Botany, El-Minia University, El-Minia, Egypt.

Extraction and isolation

Air-dried aerial plant material (400 g) was ground and extracted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1:1, v/v) at room temperature for 24–48 h. The extract was concentrated *in vacuo* to obtain a residue of 60 g. The residue was pre-fractionated by column

chromatography (CC) on a silica gel column (6 cm x 120 cm) eluted with *n*-hexane (2 L) followed by a gradient of *n*-hexane/ CH_2Cl_2 up to 100% CH_2Cl_2 and $\text{CH}_2\text{Cl}_2/\text{MeOH}$ up to 15% MeOH (2 L each of the solvent mixtures). The *n*-hexane/ CH_2Cl_2 (1:1, v/v, 6 g) fraction was subjected to silica gel CC (2 cm x 60 cm), eluted with *n*-hexane/ CH_2Cl_2 with increasing polarity up to 100% CH_2Cl_2 , to give pure compound **2** (10 mg). The CH_2Cl_2 (100%) fraction was chromatographed on a Sephadex LH-20 column eluted with *n*-hexane/ $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (7:4:0.5, v/v/v) to give compounds **3** (10 mg), **4** (15 mg), and **5** (11 mg). The $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (95:5, v/v) fraction was chromatographed on a Sephadex LH-20 column eluted with *n*-hexane/ $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (7:4:0.5) to give compound **1** (6 mg).

Herbalbin (1): Yellowish oil. – IR (KBr): ν = 1716 (broad) (C=O), 1623 cm^{-1} (C=C). – ^1H NMR [the ^1H assignments were achieved by ^1H - ^1H correlation spectroscopy (COSY)]: δ = 6.30 (s, H-2'), 2.88 (dd, J = 11.9, 4.3 Hz, H-4'), 2.52 (dd, J = 4.3, 17.0 Hz, H-5'a), 2.69 (dd, J = 11.9, 17.0 Hz, H-5'b), 1.10 (s, H-7'), 1.28 (s, H-8'), 2.14 (m, H-3a), 2.38 (m, H-3b), 5.08 (s, H-5), 4.56 (d, J = 4.55 Hz, H-7), 2.33 (m, H-8), 2.14 (m, H-9a), 2.38 (m, H-9b), 2.14 (m, H-10a), 2.38 (m, H-10b), 1.22 (s, H-11), 1.12 (s, H-12), 3.70 (s, methyl of acetate). – ^{13}C NMR (the ^{13}C assignments were achieved by HMQC and HMBC): δ = 136.0 (s, C-1'), 154.37 (d, C-2'), 35.15 (s, C-3'), 49.40 (d, C-4'), 36.78 (t, C-5'), 197.27 (s, C-6'), 22.91 (q, C-7'), 28.93 (q, C-8'), 172.62 (s, C-9'), 177.0 (s, C-2), 34.08 (t, C-3), 134.75 (s, C-4), 133.49 (d, C-5), 35.46 (s, C-6), 77.74 (d, C-7), 48.96 (d, C-8), 26.98 (t, C-9), 33.76 (t, C-10), 28.27 (q, C-11), 27.40 (q, C-12), 51.69 (q, methyl of acetate). – MS (EI, 70 eV): m/z (%) = 360 $[\text{M}]^+$ (100), 342 $[\text{M} - \text{H}_2\text{O}]^+$ (12), 327 $[\text{M} - \text{H}_2\text{O} - \text{CH}_3]^+$ (18), 300 $[\text{M} - \text{CH}_3\text{COOH}]^+$ (12), 284 (22), 237 (30), 135 $[\text{C}_9\text{H}_{11}\text{O}]^+$ (33), 105 $[\text{C}_9\text{H}_{11}\text{O} - 2 \text{CH}_3]^+$ (40), 91 $[105 - \text{CH}_2]^+$ (50). – $\text{C}_{21}\text{H}_{28}\text{O}_5$: calcd. 360.1902; found 360.1937.

Acknowledgement

The authors thank Prof. Ahmed A. Ahmed for his help, assistance, and his teaching.

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