Terpenes from *Artemisia herba-alba*

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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. mongolina* 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, sesquiterpenes, 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 monoterpen, **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 monoterpen **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$_{30}$O$_6$, 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$_3$COOH molecule from the [M]$^+$ ion. The strong peak at m/z 135 [C$_{10}$H$_{12}$O]$_3$ (33%) resulted from the elimination of C$_{10}$H$_{13}$O$_2$ from the [M – CH$_3$COOH]$^+$ 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$_3$ group from the ion 105. Exact mass determination of the ion at m/z 360...
The elemental composition of compound 1 was established as C_{21}H_{28}O_{5} (experimental 360.1937, calcd. 360.1902).

The $^1$H NMR spectrum showed seven singlet signals at $\delta_H$ 6.30 ppm, 1.10 ppm, 1.28 ppm, 5.08 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 $\delta_H$ 2.88 ppm ($J = 11.9, 4.3$ Hz, H-4'), correlated with a doublet of doublets at $\delta_H$ 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 $^1$H-$^1$H-COSY spectrum. It also showed a doublet signal at $\delta_H$ 4.56 ppm (d, $J = 4.55$ Hz, H-7), coupled with a carbon atom at $\delta_C$ 77.74 ppm (C-7) in the HMQC spectrum. The latter proton showed a correlation with a multiplet signal at $\delta_H$ 2.33 ppm (m, H-8) in the $^1$H-$^1$H-COSY spectrum. Moreover, it showed a multiplet signal at $\delta_H$ 2.14 ppm integrated for three protons (H-3a, H-9a, H-10a), and it showed a clear correlation in the $^1$H-$^1$H-COSY spectrum with a multiplet signal at $\delta_H$ 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 $\delta_C$ 177.0 ppm (C-2), 197.27 ppm (C-6'), and 172.62 ppm (C-9'), five methyl carbon atoms at $\delta_C$ 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 $\delta_C$ 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 $\delta_C$ 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 $\delta_C$ 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' ($\delta_H$ 6.30 ppm, s) and C-4' ($\delta_C$ 49.40 ppm), C-6' ($\delta_C$ 197.27 ppm), C-10 ($\delta_C$ 33.76 ppm); H-4' ($\delta_H$ 2.88 ppm, dd)
and C-3' (δC 35.15 ppm), C-5' (δC 35.46 ppm), C-4' (δC 49.40 ppm), C-6' (δC 28.93 ppm), C-9' (δC 77.74 ppm), C-8' (δC 197.27 ppm); H-3 (δH 2.14 ppm, m; 2.38 ppm, m) and C-2 (δC 134.75 ppm), C-7' (δC 136.00 ppm), C-9' (δC 133.49 ppm), C-6' (δC 154.37 ppm), C-3' (δC 125.00 ppm), C-4' (δC 35.15 ppm), C-4 (δC 177.00 ppm), C-2 (δC 22.91 ppm), 2.14 ppm, m; 2.38 ppm, m) and C-6 (δC 35.46 ppm); H-9 (δH 2.14 ppm, m; 2.38 ppm, m), H-10 (δH 2.14 ppm, m; 2.38 ppm, m) and C-4 (δC 134.75 ppm), C-1' (δC 136.00 ppm); H-11 (δH 1.22 ppm), H-12 (δH 1.12 ppm) and C-5 (δC 133.49 ppm); C-7' (δH 1.10 ppm), H-8' (δH 1.28 ppm), and C-2' (δC 154.37 ppm), C-3' (δC 35.15 ppm), C-4' (δC 49.40 ppm). On the basis of these results, compound 1 was identified as a new monoterpenic dimer, which was given the name herbalbin, a new natural product (Fig. 1).

**Experimental**

**General**

1H NMR (600 MHz, CDCl3), 13C NMR (125 MHz, CDCl3), 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 CH2Cl2/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/CH2Cl2 up to 100% CH2Cl2 and CH2Cl2/MeOH up to 15% MeOH (2 L each of the solvent mixtures). The n-hexane/CH2Cl2 (1:1, v/v, 6 g) fraction was subjected to silica gel CC (2 cm x 60 cm), eluted with n-hexane/CH2Cl2 with increasing polarity up to 100% CH2Cl2, to give pure compound 2 (10 mg). The CH2Cl2 (100%) fraction was chromatographed on a Sephadex LH-20 column eluted with n-hexane/CH2Cl2/MeOH (7:4:0.5, v/v/v) to give compounds 3 (10 mg), 4 (15 mg), and 5 (11 mg). The CH2Cl2/MeOH (95:5, v/v) fraction was chromatographed on a Sephadex LH-20 column eluted with n-hexane/CH2Cl2/MeOH (7:4:0.5) to give compound 1 (6 mg).

*Herbalbin* (1): Yellowish oil. – IR (KBr): δ = 1716 (broad) (C=O), 1623 cm–1. – 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). – 13C NMR (the 13C assignments were achieved by HMOC 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 [M]+ (100), 342 [M – H2O]++ (12), 327 [M – H2O – CH3]++ (18), 300 [M – CH3COOH]+ (12), 284 (22), 237 (30), 135 [C6H11O]++ (33), 105 [C4H9O – 2 CH3]++ (40), 91 [105 – CH3]++ (50). – C21H30O5: calcd. 360.1902; found 360.1937.

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