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First isolation and Characterization of Chemical Constituents from *Achillea biebersteinii.*

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ABSTRACT

Investigation of the aerial part of *Achillea biebersteinii* (family Asteraceae) resulted in the isolation of five compounds for the first time, including germacranolide derivatives (**1** and **2)**, one coumarin (**3**), one monoterpene (**4**), and one flavonoid (**5**). Structures were determined by interpretation of their spectroscopic data (1D, 2D-NMR and MS).

Keywords: *Achillea biebersteinii*, Asteraceae, Flavonoid, Monoterpene, Germacranolide.

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INTRODUCTION

Achillea comprises more than 100 species distributed worldwide [1]. The *Achillea* species represent a good source of secondary metabolites of different classes, including flavonoids, sesquiterpene lactones and polyacetylenes [2-4]. Yarrow (*Achillea*) has been used as an ayurvedic medicinal herb. The American folk use the leaves as a remedy for colds, diarrhea, fevers, flu, headaches, indigestion, obesity, tuberculosis and varicose veins [5]. In 1997, the yarrow was approved by the German Commission E as a remedy for indigestion, gall bladder problems, liver problems and a loss of appetite. The modern medicine confirmed the traditional uses that the aerial parts from different species of the genus *Achillea* proved to be effective as bitter aromatics, astringents, chemostyptics, choleretics and antiphlogistics [6-7]. The fractionation of the $CHCl_3$ extract of the aerial parts of *Achillea biebersteinii* Afan, collected in Saudi Arabia, by Normal phase column chromatography (CC) using different solvent polarities followed by reversed-phase (RP-C18) HPLC yielded five known compounds **1**-**5**. These results represent a new source of the isolated compounds (**1**-**5**) which indicate that the genus of *Achillea* is a promising source for further investigation.

MATERIALS AND METHODS

General experimental

NMR study employed a JEOL JNM EX-400 spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C, Including COSY, NOESY, HMQC, and HMBC. Mass spectra (EIMS and HREIMS) were recorded on a JEOL JMS D-300 mass spectrometer using a direct inlet and electron impact ionization. The IR spectra ($CHCl_3$) were recorded on a Perkin-Elmer FT-IR-spectrometer.

Plant material

The aerial parts of *A. biebersteinii* were collected in El-Baha, Saudi Arabia, April, 2014. A voucher specimen was deposited in Pharmacognosy Department, College of Pharmacy, King Abdulaziz University.

Extraction and isolation

The air-dried and powdered aerial parts of *A. biebersteinii* (300 g) were extracted by maceration with CH_2Cl_2-MeOH (1:1). The extract was fractionated by silica gel column chromatography (CC) eluted successively with *n*-hexane-EtOAc and EtOAc-MeOH in a step gradient by using different ratios. The fractions *n*-hexane-EtOAc (1:3) was purified on a Sephadex LH-20 column (*n*-hexane-CH_2Cl_2-MeOH, 5:4:1) to **1** (12 mg), **2** (6 mg), **3** (9 mg), **4** (20 mg) and **5** (4 mg).

Sintenin (1): was isolated as gummy oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.20 (1H, dd, *J* = 14, 5 Hz, H-1), 5.12 (1H, dd, *J* =11,6 Hz, H-3), 5.09 (1H, dd, *J* = 7, 5 Hz, H-9), 4.77 (1H, dd, *J* = 15, 11 Hz, H-6), 4.75 (1H, d 8.5 Hz,, H-5), 2.50. (2H, m, H_2-2), 1.80 (1H, m, H-7), 2.30 (2H, m, H_2-8), 2.70 (1H, m, H-11), 1.20 (3H, d, *J* = 7.5 Hz, H_3-13), 1.67 (3H, s, H_3-14), 1.43 (3H, s, H_3-15), 2.10 (3H, s, H_3-17), 2.09 (3H, s, H_3-19); ^{13}C NMR [125 MHz, ($CDCl_3$)] δ 178.7 (s, C-12), 169.9 (s, C-18), 169.8 (s, C-16), 137.4 (s, C-10), 136.4 (s, C-4), 127.9 (d, C-5), 125.6 (d, C-1), 80.6 (d, C-9), 78.5 (d, C-3), 79.4 (d, C-6), 45.7 (s, C-7), 40.4 (d, C-11), 21.2 (s, C-17), 21.0 (q, C-19), 12.3 (q, C-13).

Micranthin (2): was isolated as yellowish oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (1H, dd, *J* =11.0, 6.0, H-3), 5.31 (1H, d, *J* = 11.0, H-5), 4.79 (1H, dd, *J* = 11.0, 11.0 Hz, H-6), 4.33 (1H, dd, *J* = 2.5, 11.0 Hz, H-9), 2.9 (dd, J = 2.5, 11.0, H-1), 2.70 (1H, m, H-11), 2.50 (2H, m, H_2-2), 2.25 (1H, m, H-7), 2.10 (3H, s, H_3-17), 2.09 (3H, s, H_3-19)1.95 (2H, m, H_2-8), 1.76 (3H, s, H_3-15), 1.30 (3H, s, H_3-14), 1.20 (3H, d, *J* = 7.5 Hz, H_3-13),; ^{13}C NMR [125 MHz, ($CDCl_3$)] δ 178.5 (s, C-12), 169.9 (s, C-18), 169.8 (s, C-16), 141.0 (s, C-4), 123.5 (d, C-5), 80.1 (d, C-9), 79.0 (d, C-6), 75.6 (d, C-3), 62.5 (d, C-1), 61.2 (s, C-10), 45.5 (s, C-7), 40.4 (d, C-11), 21.2 (s, C-17), 20.9 (q, C-19), 11.0 (q, C-13).

7-Hydroxy-6,8-dimethoxy-2*H*-chromen-2-one (3): was isolated as a whit powder (20.0 mg); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (1H, d, 9.3, H-4), 6.68 (1H, s, H-5), 6.30 (1H, d, 9.3, H-3), 4.10 (3H, s, H_3-12), 3.95 (3H, s, H_3-11), ^{13}C NMR [75 MHz, ($CDCl_3$)] δ 160.7 (s, C-2), 144.6 (s, C-9), 143.9 (s, C-4), 143.1 (d, C-6), 142.5 (d, C-8), 134.5 (d, C-7), 134.5 (s, C-7), 113.5 (s, C-3), 111.2 (s, C-10), 61.6 (s, C-12), 56.5 (s, C-11).CIMS *m/z* 223 $[M+H]^+$ calcd for $C_{11}H_{10}O_5$ *m/z* 222.0528.

(-)-(*1R,2S,3S,4S*)-1,2,3,4-Tetrahydroxy-p-menthane (4): was isolated as a greenish oil; ^{1}H NMR (400 MHz, $CDCl_3$); δ 3.75 (1H, br d, *J* = 9bHz, H-2), 3.53 (1H, br d, J = 9 Hz, H-3), 2.00 (1H, qq, J= 7,7, Hz, H-8), 1.98 (3H, m, H-6, H-5a), 1.36 (3H, s, H-7), 1.26 (1H, dddd, J = 14, 3.5, 3, 1 Hz, H-5b), 0.97 (3H, d, J= 7, H-9), 0.96 (3H, d, J= 7, H-10); ^{13}C NMR ($CDCl_3$, 100 MHZ) δ 91.2 (s, C-1), 70.3 (d, C-2), 73.4 (d, C-3), 84.54 (s, C-4), 29.1 (t, C-5), 25.0 (t, C-6), 20.0 (d, C-7), 32.7 (d, C-8), 17.6 (q, C-9), 17.5 (q, C-10), 45.7 (s, C-7), 40.4 (d, C-11), 21.2 (s, C-17), 21.0 (q, C-19), 12.3 (q, C-13); CIMS *m/z* 187 $[M\text{-}H_2O]^+$ and HREIMS *m/z* 187.1330 $[M\text{-}H_2O]^+$ calcd for $C_{10}H_{19}O_4$.

5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,6-dimethoxy-4*H*-chromen-4-one (quercetagetin 3,6,4'-trimethyl ether) (5): was isolated as a yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (1H, s, H-12), 7.55 (1H, d, *J* = 7.5, H-15); 6.90 (1H, s, H-14), 6.45 (3H, s, H-8), 3.87 (3H, s, OCH_3-3) 3.85 (3H, s, OCH_3-6) 3.60 (3H, s, OCH_3-14); CIMS *m/z* 361 $[M+H]^+$ calcd for $C_{18}H_{16}O_8$ *m/z* 360.3148.

RESULT AND DISCUSSION

Compound **1** was isolated as yellowish oil, and its IR spectrum indicated the presence of γ-lactone (1768 cm^{-1}), carbonyl group (1730 $cm^{-}1$), and a double bond (1665 cm^{-1}). The molecular formula of **1** was deduced by accurate mass measurement (*m/z*: 350) to be $C_{19}H_{26}O_6$ with seven elements of unsaturation. In the ^{13}C NMR and DEPT spectrum of **1**, nineteen resonances, attributable to 5 x CH_3, 2 x CH_2, 7 x CH, and 5 x C groups (Table 1); which indicated that we deal with a bicyclic molecule. ^{1}H NMR spectrum showed two olefinic methyl singlet signals at δ_H1.67 (H_3-14) and δ_H 1.43 (H_3-15), and two acetyl singlet at δ_H 2.09 and 2.10. From the ^{1}H-^{1}H COSY NMR spectrum of **1**, correlation between H_2-2 and H-1 and H-3, between H-6 and H-5 and H-7 were observed, and between H-8 and H-7 and H-9, as well as between H-7 and H-11. Diagnostic long-range ^{1}H-^{13}C correlations between H-1 to those of C-2, C-3, C-9 and C-10, between H-7 to C-5, C-6, C-8 and C-9, and between H-3 and C-2, C-4 and C-5 were evident. These couplings established the C-C bonds from C-1 to C-10, resulted in ring closure of the cyclodecadiene. Additionally, correlations observed between the resonances of H-7 to those of C-11, C-12, and C-6 and correlation between H-6 and C-11 and C-12, led to cyclization of the dihydrofurane ring, As well as correlations between H-9 and C-18 and between H-3 and C-16 led to deposition of two acetate groups on C-3 and C-9. ^{1}H-^{13}C long-range correlations observed between the resonances of H_3-15 and C-4 and correlation between H_3-14 and C-10 resulted in the two methyl CH_3-15 and CH_3-14 directly bonded to C-4 and C-10 respectively. This deduction was supported by the ^{13}C chemical shift of C-10 (δ 137.4, s) and C-4 (δ 136.4, s). The stereochemistry at C-3 and C-9 were tentatively deduced from the coupling ($J_{9,8}$ = 7.5 Hz and $J_{2,3}$ = 10 Hz) and by comparison with haageanolide acetate (**1a**) [8]. Finally, compound **1** is sesquiterpenes lactone of the *trans* germacranolide type. Thus **1** was identified as Sintenin [9], a new source from *Achillea biebersteinii*.

Compound **2** was isolated as yellowish oil, and its IR spectrum indicated the presence of γ-lactone (1768 cm^{-1}), carbonyl group (1730 cm^{-1}), a double bond (1665 cm^{-1}) and an epoxide (1255 cm^{-1}). The low resolution mass spectrum showed the molecular ion peak $[M]^+$ at *m/z* 366. The HREIMS exhibited the molecular ion peak $[M]^+$ at *m/z* 366.1668 (calcd for $C_{19}H_{26}O_7$: 366.1664). The molecular formula of $C_{19}H_{26}O_7$ was also confirmed by ^{13}C NMR and DEPT analyses. Careful comparison of ^{1}H and ^{13}C NMR spectrum analyses between compound **1** and **2**, indicated that compound **2** was quite similar to those of **1**, except for differences in proton signal chemical shift at C-1 and C-10 [8-10].

The ^{1}H NMR spectrum revealed the presence of methyl singlet signals at δ_H 1.76 (H_3-15), 1.30 (H_3-14), a doublet signal at δ_H 1.20 (H_3-13, J = 7), and two acetyl singlet signals at δ_H 2.09 and 2.10. The ^{13}C NMR spectrum exhibited nineteen carbon signals were classified by a DEPT as follows: five quaternary carbon signals, seven methines, two methylenes, and five methyl carbon signals. All ^{1}H and ^{13}C NMR resonances were assigned using HMQC, and HMBC measurements of **2**. The oxygenated proton H-1 appeared as a doublet of doublet signal at δ_H 2.9 (dd, J = 2.5, 11.0), showed a clear correlation with the oxygenated proton at δ_H 4.33 (dd, J = 2.5, 11.0. H-9) in ^{1}H-^{1}H COSY spectrum analysis. The oxygenated protons appear doublet of doublets at δ_H 5.40 (dd, J = 6.0, 11.0, H-3) and a doublet of doublet at δ_H 4.33 (dd, J = 2.5, 11.0. H-9), which showed clear correlations with oxygenated carbon signals at δ_C 81.3 (C-3) and 80.6 (C-9), respectively. The connectivity of the partial moieties and the position of the acetyl groups and epoxy group were established by the HMBC spectrum of **1** (Fig. 2). The correlation in the HMBC experiments, between H-1 to those of C-2, C-3, C-9 and C-10, between the resonance of H-7 to C-5, C-6, C-8 and C-9, and also correlations between H-3 and C-2, C-4 and C-5. These couplings established the C-C bonds from C-1 to C-10, resulted in ring closure of the cyclodecadiene. Additionally, long-range correlations observed between the resonances of H-7 to those of C-11, C-12, and C-6 and correlation between H-6 and C-11 and C-12, led to cyclization of the dihydrofurane ring; correlations between H-9 and C-18, and

between H-3 and C-16 led to deposition of the two acetate on C-3 and C-9. HMBC long-range correlations observed between the resonances of H_3-14 and C-10 and, correlation between H_3-15 and C-4 resulted in the two methyl CH_3-14 and CH_3-15 directly located on C-10 and C-4 respectively, in addition to, the clear correlation between H-1 and C-3, C-9, C-10 and C-1, indicated the position of epoxy group to be at C-1/C-10.

The relative stereochemistry of **2** was assigned on the basis of the study of the coupling constants and NOESY experiments, which indicated the *α*-configuration for H-7 and β-configuration for H-6 [8, 10]. The stereochemistry at C-3 and C-9 were tentatively deduced from the coupling ($J_{9,8}$ = 11.0 Hz and $J_{2,3}$ = 11.0) [8]. The high value coupling constant of H-1 (J = 11.0) indicated the *α*-orientation of the H-1 and *β* orientation of epoxy group. The above results were confirmed by NOESY experiment. The clear correlation between H-1 (2.9, dd) with H-7 (2.5, m) together with H-8β (1.70, m), and H-13 (1.22, d) were observed. Therefore, compound **2** was assigned to Micranthin [9].

The molecular formula of **3** was deduced by accurate mass measurement to be $C_{11}H_{10}O_5$. In the ^{13}C NMR spectrum of **3**, eleven resonances, attributable to 2 x OCH_3, 3 x CH, and 6 x C groups (Table 2), were evident. It was also clear from these data that since five of the seven degrees of unsaturation indicating the presence of bicyclic molecule. The ^1H and ^{13}C NMR data consistent with the presence of a trisubstituted coumarin ring [δ 7.62 (d, J = 9.3, H-4); 6.30 (d, J = 9.3, H-3); 6.68, (s)]. All ^1H and ^{13}C NMR resonances were assigned using HMQC, and HMBC measurements of **3**. Therefore, compound **3** was assigned to 6,8-dimethoxy-7-hydroxycoumarin

The ^1H NMR and ^{13}C of compound **4** were identical with a monoterpene which reported by one of the authors from *Chenopodium ambrosioides* [11]. The structure of compound **5** could be easily determined from its ^1H NMR spectrum [11].

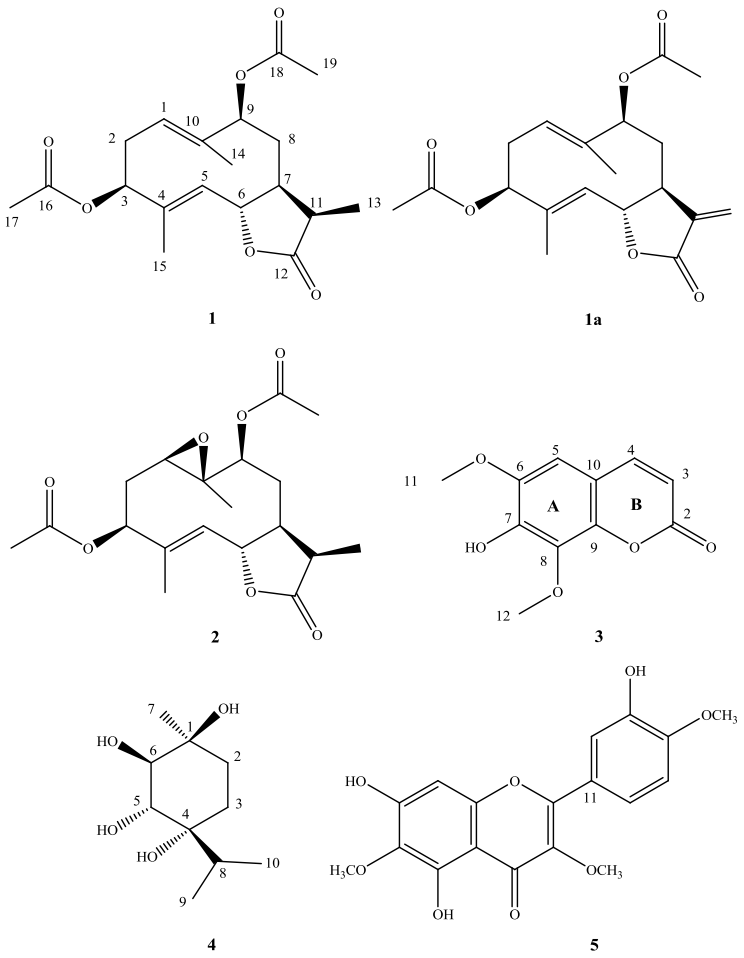

Figure 1: Isolated compounds from *Achillea biebersteinii.*

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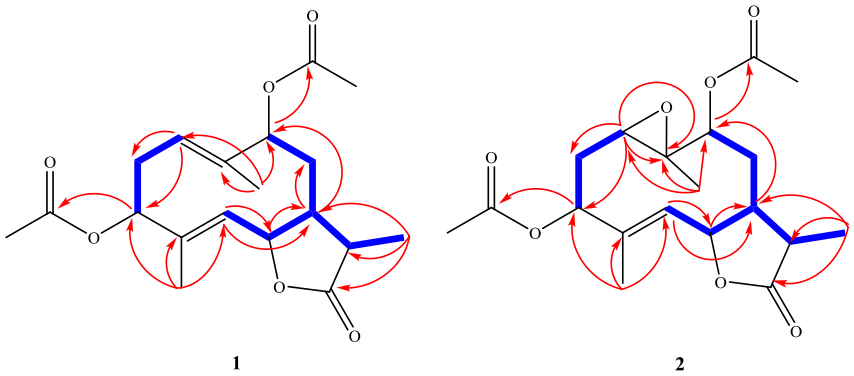

1 2

Figure 2: Selected ^{1}H-^{1}H COSY (—) and HMBC (→) correlations of 1, 2.

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