

Isolation and Structure Determination of Three New Sesquiterpenoids from *Achillea millefolium*

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Phytochemical investigation of the whole plant of *Achillea millefolium* led to the isolation of three new sesquiterpenes which were trivially named as sesquiterpene lactone-esters A and B (**1** and **2**), and sesquiterpene lactone-diol (**3**). The structures of these compounds were determined with the help of one- and two-dimensional (1D and 2D) NMR techniques including ¹H NMR, ¹³C NMR, HMQC, HMBC, COSY, and NOESY experiments.

Key words: *Achillea millefolium*, Sesquiterpenes, Lactone-ester, Lactone-diol, Compositae

Introduction

The Asteraceae (Compositae), also referred to as the sunflower family, is the largest family of vascular plants. The family has more than 22,750 currently accepted species spread across 1,620 genera, and 12 subfamilies. The genus *Achillea* is composed of 115 species of perennial herbs, all native to temperate regions of the Northern hemisphere. A large number of them are cultivated in gardens for their ornamental foliage [1]. *Achillea millefolium* is a perennial herb distributed in different parts of Pakistan. It was collected from Abbottabad, Pakistan, in July 2010. The plant is an erect, up to 1 m tall, basally woody shrublet with obtuse-angled, punctuate-glandulose, woolly pilose twigs [2]. Medicinally it is very important. The use of *A. millefolium* seems to be originated in European folk medicine and perhaps was carried from there to the East. The flower clusters are slightly antispasmodic, and the leaves are astringent. An infusion or a decoction of the flowers and leaves is useful in colds, to quiet the nerves, to treat colic, to suppress hemorrhages, and as an emmenagogue [3, 4]. The essential

oils of *A. millefolium* have shown *in vitro* antioxidant and antimicrobial properties [5]. The plant also has a long history as a powerful ‘healing herb’ used topically for wounds, cuts and abrasions. The phytochemical investigation of this plant has been initiated in view of its medicinal importance and the fact that no phytochemical investigation has been done on this plant before. Herein, we report the isolation and structure elucidation of three new sesquiterpenes (**1–3**) (Fig. 1) from this plant.

Results and Discussion

The ethyl acetate-soluble fraction of the air-dried whole plant of *Achillea millefolium* was subjected to column chromatography which resulted in the isolation of three new sesquiterpenes (**1–3**). The structures of these compounds were established by 1D and 2D NMR, IR, UV spectroscopy and mass spectrometry.

Compound **1** was isolated as a gummy solid. The molecular formula was deduced from the HR-EIMS at $m/z = 362.1371$ corresponding to the molecular formula C₁₉H₂₂O₇. The IR spectrum contained absorp-

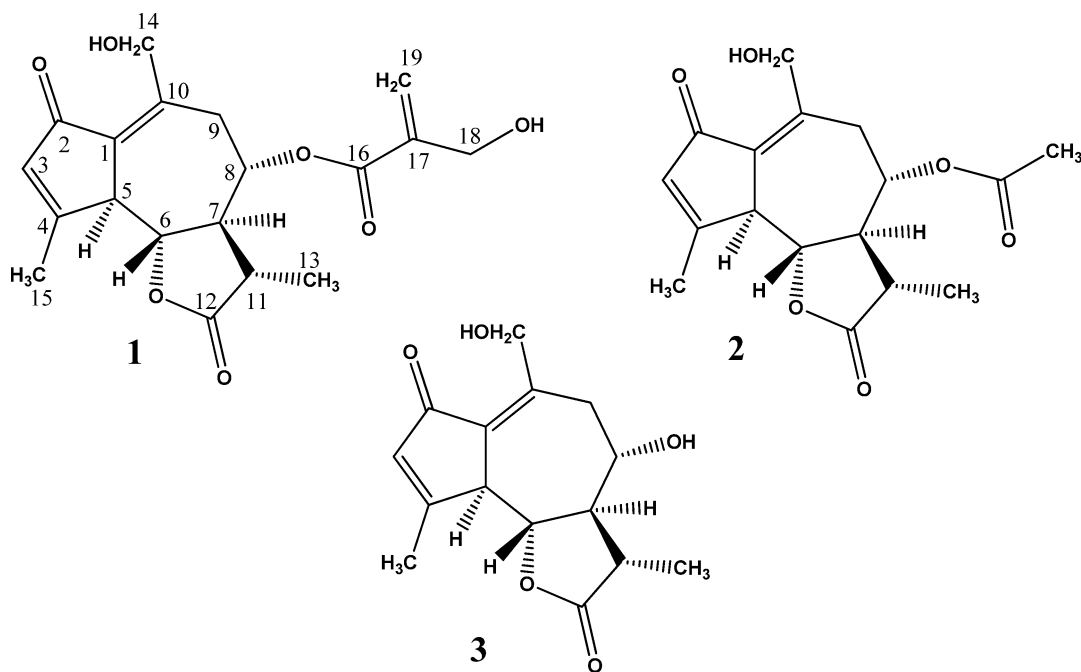


Fig. 1. Structures of compounds **1–3**.

tion bands at 3470–3120 (OH), 1760 (γ -lactone), 1710 (ester carbonyl), 1680 (α,β -unsaturated cyclopentenone), and 1640 cm^{-1} (C=C) [6, 7]. The UV spectrum showed absorptions at 225 and 243 nm. The ^1H NMR spectrum of compound **1** showed the typical signals of a guaianolide sesquiterpene skeleton which was supported by the ^{13}C NMR spectra (BB and DEPT) that revealed the presence of 19 carbon signals comprising four methylene, six methine, two methyl and seven quaternary carbons. The ^1H NMR spectrum showed signals of a proton geminal to the lactone oxygen atom (lactone proton) as a doublet of doublets at $\delta = 3.85$ ($J = 12.0, 10.1$ Hz, H-6). ^1H - ^1H correlations revealed its coupling to two vicinal protons which are characteristic for guaianolides with a 6,7-fused lactone. The spectrum further showed a signal due to *exo*-methylene protons at $\delta = 4.10$ (s, H-14) [8]. The signal at $\delta = 3.85$ showed ^1H - ^1H correlations to vicinal protons at $\delta = 3.38$ (d, $J = 12.0$ Hz) and $\delta = 2.15$ (ddd, $J = 11.5; 10.1; 8.1$ Hz) which could subsequently be assigned to H-5 and H-7, respectively. The resonances in the up-field region of the ^1H NMR spectrum at $\delta = 1.40$ (d, $J = 8.4$ Hz), and $\delta = 2.30$ (s), each integrating for 3H, were assigned to the methyl hydrogens at C-13 and C-15, respectively. A singlet at $\delta = 6.10$ (s) corre-

sponds to the olefinic proton at C-3, and the doublet of doublets at $\delta = 2.15$ ($J = 11.5; 8.4$ Hz) could subsequently be assigned to C-11. The coupling constants, $J_{5,6} = 12.0$ Hz and $J_{6,7} = 10.1$ Hz, suggested a *trans*-axial arrangement for the protons at C-5, C-6, and C-7; consequently we assigned the β -configuration to H-6, and the α -configuration to H-5 and H-7. A multiplet at $\delta = 5.10$ was assigned to the proton at C-8; comparison with literature data revealed H-8 to be β -oriented [9, 10]. A doublet at $\delta = 4.38$ ($J = 9.0$ Hz) and another doublet at $\delta = 6.40$ ($J = 1.3$ Hz) were assigned to H-18 and H-19, respectively [11].

The basic skeleton of compound **1** was similar to sesquiterpene lactone-esters isolated from *Achillea vermicularis*, *Scorzonera austriaca* and *Eupatorium perfoliatum* [12–14]. The resonances at $\delta = 163.3, 143.1, 60.4$, and 124.6 were due to the presence of a side chain ester and could be assigned to C-16, C-17, C-18, and C-19, respectively. The point of attachment of the ester moiety at C-8 was supported by the downfield shift of H-8 ($\delta = 5.10$ Hz) as well as by the ^{13}C NMR signal for C-8 at $\delta = 73.4$. This attachment was supported by the HMBC spectrum in which H-8 showed correlations with C-16, C-9 and C-7. Two signals in the ^{13}C NMR spectrum at $\delta = 62.4$ and $\delta =$

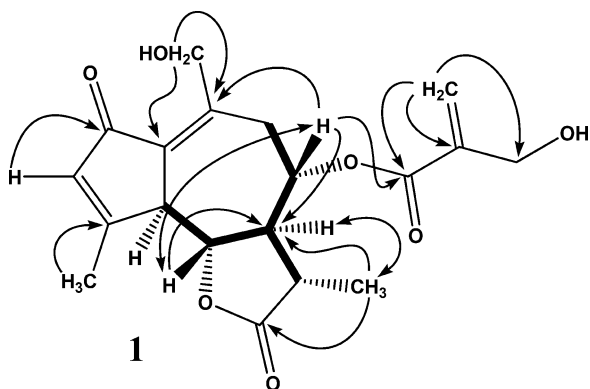


Fig. 2. Selective COSY (—), HMBC (---) and NOESY (↔) correlations of **1**.

60.4 were respectively assigned to the two methylene groups C-14 and C-18 having hydroxyl groups. The positions of both methylenes were also supported by HMBC correlations. The methyl group attached to C-11 appeared at $\delta = 14.6$ in the ^{13}C NMR spectrum and is α -oriented, while the methyl group at C-11, which is β -oriented, should have a chemical shift of less than 10 ppm [6, 9]. The stereochemistry at all the stereogenic centers was confirmed by a NOESY experiment and by comparison with literature data (Fig. 2) [9].

Compound **2** was isolated as a gummy solid. The HR-EIMS showed the molecular ion peak at $m/z = 320.1263$ corresponding to the molecular formula $\text{C}_{17}\text{H}_{20}\text{O}_6$. The IR spectrum contained absorption bands at 3490, 1752, 1740, 1680, and 1630 cm^{-1} , characteristic of an OH, γ -lactone, OAc, α,β -unsaturated cyclopentenone, and a C=C double bond, respectively. Compound **2** exhibited absorption maxima at 230 and 254 nm in the UV spectrum. The NMR spectra (^1H and ^{13}C) of compound **2** were identical to those of **1** except that the side chain at C-8 in **1** is replaced by an OAc in compound **2**. In the ^1H NMR spectrum the signal at $\delta = 2.19$ (s) was assigned to H₃-17, and a signal centered at $\delta = 4.20$ was assigned to H₂-14 (*exo*-methylene having a hydroxyl group). Two methyl signals resonating at $\delta = 1.18$ (d, $J = 8$ Hz) and $\delta = 2.45$ (s) were assigned to CH₃-13 and CH₃-15, respectively. An olefinic proton appeared in the down-field region at $\delta = 6.15$ and was assigned to C-3, while five methine protons were centered at $\delta = 3.28$ (d, $J = 9.8$ Hz, H-5), 3.70 (dd, $J = 9.8$; 10.9 Hz, H-6), 2.08 (m, H-7), 4.95 (m, H-8), and 2.38 (dd, $J = 8$; 11.4 Hz, H-11). The ^{13}C NMR spectrum disclosed the presence of the

three methyls at $\delta = 16.6$, 22.1 and 23.2 for C-13, C-15 and C-17, respectively. Three carbons resonating at $\delta = 196.4$, 176.3 and 171.4 could be assigned to C-2, C-12 and C-17, respectively. The attachment of a side group at C-8 and the position of different substituents were confirmed by HMBC analysis, while the relative stereochemistry at various chiral centers was confirmed by a NOESY experiment and by comparison with literature data.

Compound **3** was also isolated as a gummy solid. The HR-EIMS showed the $[\text{M}]^+$ peak at $m/z = 278.1162$ corresponding to the molecular formula $\text{C}_{15}\text{H}_{18}\text{O}_5$. The UV spectrum showed absorption maxima at 223 and 250 nm. The IR spectrum exhibited absorption bands at 3396–3150, 1760, 1688, and 1625 cm^{-1} which indicated the presence of a hydroxyl group, a γ -lactone, an α,β -unsaturated cyclopentenone, and a C=C double bond, respectively. The ^1H NMR, ^{13}C NMR and 2D NMR data indicated that compound **3** is structurally similar to compounds **1** and **2**. In compound **3** there is a lack of the side chain on C-8. On comparison with compound **2** there was a loss of the $\text{C}_2\text{H}_2\text{O}$ fragment ($m/z = 42$) in the EIMS. This was further supported by a DEPT experiment which disclosed the presence of 15 carbon atoms. In the ^1H NMR spectrum, the proton at C-8 ($\delta = 3.90$ m) correlated (HMBC) with C-6 ($\delta = 83.2$), C-7 ($\delta = 65.7$), C-9 ($\delta = 38.5$), and C-10 ($\delta = 141.3$). Similarly, in the ^{13}C NMR spectrum the *exo*-methylene with the hydroxyl group was attached to C-14 ($\delta = 63.8$). In the ^{13}C NMR spectrum two signals appeared at $\delta = 191.8$ and $\delta = 173.5$ which were assigned to two carbonyls at C-2 and C-13, respectively. The positions of all groups in the compound were confirmed by HMBC and COSY experiments, while the stereochemistry of all chiral centers was confirmed by a NOESY experiment.

Experimental Section

General

Column chromatography (CC): silica gel (E. Merck, 230–400 mesh); TLC: pre-coated silica gel G-25-UV₂₅₄ plates, and detection was done at 254 nm and by spraying with ceric sulfate in 10% H_2SO_4 solution. Optical rotations: measured on a JASCO-DIP-360 digital polarimeter by using a 10 cm cell tube. UV and IR spectra: Hitachi-UV-3200 and JASCO-320-A spectrometers, respectively. ^1H NMR and ^{13}C NMR: Bruker AM-400 spectrometer with tetramethylsilane (TMS) as an internal standard, 2D NMR spectra: Bruker

Table 1. ^1H (400 MHz) and ^{13}C (125 MHz) NMR data of compounds **1**–**3** in CDCl_3 .

Position	1		2		3	
	δ_{C}	δ_{H} (mult., J in Hz)	δ_{C}	δ_{H} (mult., J in Hz)	δ_{C}	δ_{H} (mult., J in Hz)
1	136.4	–	133.9	–	139.1	–
2	192.9	–	196.4	–	191.8	–
3	133.4	6.10 (s)	136.8	6.15 (s)	135.8	6.13 (s)
4	167.4	–	168.6	–	172.1	–
5	50.4	3.38 (d, 12)	50.9	3.28 (d, 9.8)	49.3	3.40 (d, 10.6)
6	80.8	3.85 (dd, 10.1, 12)	83.4	3.70 (dd, 9.8, 10.9)	83.2	3.60 (dd, 9.5, 10.6)
7	61.3	2.15 (ddd, 8.4, 10.1, 11.5)	63.1	2.08 (m)	65.7	1.95 (ddd, 9.5, 10.4, 11.6)
8	73.4	5.10 (m)	70.8	4.95 (m)	73.6	3.90 (m)
9	39.1	2.70 (m), 2.42 (m)	41.3	2.30 (m), 2.60 (m)	38.5	2.50–2.58 (m)
10	141.9	–	140.6	–	141.3	–
11	41.3	2.50 (dd, 8.4, 11.5)	38.6	2.38 (dd, 8, 11.4)	40.4	2.20 (dd, 8.9, 11.6)
12	177.8	–	176.3	–	173.5	–
13	14.6	1.40 (d, 8.4)	16.6	1.18 (d, 8)	15.9	1.28 (d, 8.9)
14	62.4	4.10 (s)	66.8	4.20 (s)	63.8	4.32 (s)
15	20.4	2.30 (s)	22.1	2.45 (s)	21.5	2.15 (s)
16	163.3	–	171.4	–	–	–
17	143.1	–	23.2	2.19 (s)	–	–
18	60.4	4.38 (d, 9)	–	–	–	–
19	124.6	6.40 (d, 1.3)	–	–	–	–

AMX 500 MHz NMR spectrometer. Chemical shift (δ) values were reported in ppm and coupling constants J in Hz. Mass spectra (EI and HR-EIMS) were measured in an electron impact mode on Finnigan MAT 312 spectrometers, and ions are given in m/z .

Plant material

The air-dried whole plant of *Achillea millefolium* was collected from Abbottabad, Pakistan, in July 2010. Taxonomic identification was done by Dr. Manzoor Ahmad, Department of Botany, Postgraduate College No. 1, Abbottabad, Pakistan. A voucher specimen (# ACH-899) has been deposited in the herbarium of the above department.

Extraction and purification

The air-dried whole plant (10 kg) was grinded and extracted with methanol (3×15 L) at room temperature. The methanol extract was evaporated under reduced pressure to yield a residue (550 g) which was partitioned between *n*-hexane (60 g), ethyl acetate (45 g) and *n*-butanol (80 g), and the remainder was the water-soluble fraction. The ethyl acetate fraction was subjected to CC over a silica gel column using *n*-hexane with a gradient of ethyl acetate up to 100% followed by methanol. Sixteen fractions were collected. Fractions 11–15 showed similar TLC spots and were recombined and further subjected to column chromatography using flash silica 230–400 mesh, and eluted with *n*-hexane-ethyl acetate 3 : 2. Gradually increasing the percentage of

ethyl acetate, at 1 : 1 ethyl acetate-*n*-hexane gave the pure compounds **1** (8.9 mg) and **2** (13 mg). Similarly, fractions 16–18 were combined and subjected to column chromatography using *n*-hexane-ethyl acetate 2 : 3 to purify compound **3** (9.1 mg). The purity of the compounds was checked on TLC and HPTLC plates.

Compound 1

Gummy solid. – UV/Vis (MeOH): λ_{max} ($\log \epsilon_{\text{max}}$) = 225 (6.13), 243 nm (4.61). – $[\alpha]_{\text{D}}^{25} = +33^{\circ}$ (0.92, CHCl_3). – IR (KBr): $\nu = 3470$ – 3120 (OH), 1760 (γ -lactone), 1710 (ester carbonyl), 1680 (α, β -unsaturated cyclopentenone), 1640 ($\text{C}=\text{C}$) cm^{-1} . – ^1H NMR (400 MHz, CDCl_3) and ^{13}C NMR (125 MHz, CDCl_3): see Table 1. – MS ((+)-EI): m/z (%) = 362 (70.6), 344 (50.5), 326 (40.2), 278 (100), 262 (80.0), 178 (34.6). – HRMS ((+)-EI): m/z = 362.1371 (calcd. 362.1366 for $\text{C}_{19}\text{H}_{22}\text{O}_7$, $[\text{M}]^+$).

Compound 2

Gummy solid. – UV/Vis (MeOH): λ_{max} ($\log \epsilon_{\text{max}}$) = 230 (3.34), 254 (3.82) nm. – $[\alpha]_{\text{D}}^{25} = +33^{\circ}$ (0.92, CHCl_3). – IR (KBr): $\nu = 3490$ (OH), 1752 (γ -lactone), 1740 (OAc), 1680 (α, β -unsaturated cyclopentenone), 1630 ($\text{C}=\text{C}$) cm^{-1} . – ^1H NMR (400 MHz, CDCl_3) and ^{13}C NMR (125 MHz, CDCl_3): see Table 1. – MS ((+)-EI): m/z (%) = 320 (100), 302 (60.4), 284 (30.8), 278 (80), 276 (56.1), 262 (87.1), 152 (23), 91 (93). – HRMS ((+)-EI): m/z = 320.1263 (calcd. 320.1259 for $\text{C}_{17}\text{H}_{20}\text{O}_6$, $[\text{M}]^+$).

Compound 3

Gummy solid. – UV/Vis (MeOH): λ_{\max} ($\log \epsilon_{\max}$) = 223 (4.28), 250 (4.82) nm. – $[\alpha]_{\text{D}}^{25} = +62^{\circ}$ (1.06, CHCl_3). – IR (KBr): $\nu = 3396\text{--}3150$ (OH), 1760 (γ -lactone), 1688 (α, β -unsaturated cyclopentenone), 1625 (C = C) cm^{-1} . – ^1H NMR (400 MHz, CDCl_3) and ^{13}C NMR (125 MHz, CDCl_3): see Table 1. – MS ((+)-EI): m/z (%) = 278 (100), 262 (73.1),

250 (48.7), 254 (60.3), 234 (85.6), 222 (20.1), 152 (90). – HRMS ((+)-EI): m/z = 278.1162 (calcd. 278.1154 for $\text{C}_{15}\text{H}_{18}\text{O}_5$, $[\text{M}^+]$).

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