ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 11:1 (2017) 43-50

records of natural products

Melilotoside Derivatives from *Artemisia splendens* (Asteraceae)

Fariba H. Afshar[1], Abbas Delazar[1], Hossein Nazemiyeh[2], Lutfun Nahar[3], Sedigheh B. Moghaddam[1], Blessing O. Mbaebie[4], Simon Gibbons[4] and Satyajit D. Sarker[3*]

[1]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Research Center for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Medicinal Chemistry and Natural Products Research Group, School of Pharmacy and Biomolecular Sciences, Liverpool Jhon Moores University, James Parsons Building, Byron Street, Liverpool L3 3AF, UK
[4]UCL School of Pharmacy, 29-39 Brunswick Square WC1N 1 AX, London, UK

(Received February 26, 2016; Revised July 12, 2016; Accepted August 03, 2016)

Abstract: A combination of solid-phase-extraction (SPE) and reversed-phase preparative high-performance liquid chromatography (prep-HPLC) of the methanolic extract of the aerial parts of *Artemisia splendens* (common name: "Asia Minor Wormwood"), an endemic Iranian species, afforded *Z*- and *E*-melilotosides (**1** and **2**), *Z*- and *E*-4-methoxy-melilotosides (**3** and **4**), and a new dimer, *bis-ortho-Z*-melilotoside (**5**, named: splendenoside). Whilst the structures of these compounds (**1**-**5**) were elucidated unequivocally by spectroscopic means, the *in vitro* free-radical-scavenging property of **1**-**5** was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. This is the first report on the occurrence of any melilotoside derivatives in the genus *Artemisia*.

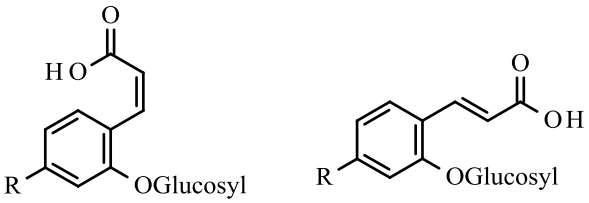
Keywords: *Artemisia splendens;* Asteraceae; 2,2-diphenyl-1-picrylhydrazyl (DPPH); free-radical scavenger; melilotoside; splendenoside.

1. Introduction

Artemisia splendens Willd., commonly known as "Asia Minor wormwood", is one of the 34 endemic Iranian species of the genus *Artemisia* L. (family: Asteraceae, tribe: Anthemidae) [1-3]. Like *Artemisia annua*, the best-known species of this genus for its antimalarial properties and its artemisinin content, other species of this genus have also been used by many cultures for the treatment of various ailments [4-7]. With the exception of the reports on the composition of the essential oil and the occurrence of terpenoids in the aerial parts of *A. splendens* [8,9], and our recent work on the essential oils [10], to the best of our knowledge, there is no report available to date on any other phytochemical or bioactivity studies on this species. As part of our continuing studies on Iranian *Artemisia* species [10-12], we now report on the isolation and structure determination of *Z*- and *E*-melilotosides (**1** and **2**), and their derivatives, *Z*- and *E*-4-methoxy-melilotoside (**3** and **4**), and a new dimer, *bis-ortho-Z*-melilotoside (**5**, named: splendenoside) (Figure 1), and their free-radical-scavenging properties evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

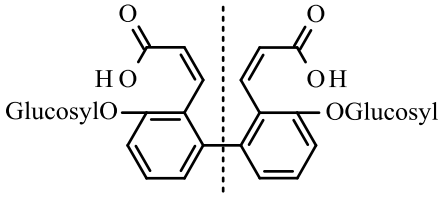
*Corresponding author: E-Mail: S.Sarker@ljmu.ac.uk;

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 09/21/2016 EISSN:1307-6167



1 R = H
3 R = OMe

2 R = H
4 R = OMe



5

Figure 1. Melilotosides (**1** and **2**) and their derivatives (**3**-**5**) from *Artemisia splendens.*

2. Materials and Methods

2.1. General Experimental Procedure

Preparative reversed-phase HPLC (prep-HPLC) analyses were performed on a Knauer preparative HPLC system, coupled with a Knauer PDA Detector 2800 (detection at 220, 254, 280 and 360 nm), Germany, using a reversed-phase Reprosil 100 C_{18} column (10 µm, 250 × 20 mm i.d.), Dr. Maisch, Germany. The NMR spectroscopic analyses were performed on a Bruker 300 NMR spectrometer (300 MHz for ^{1}H, and 75 MHz for ^{13}C) or on a Bruker DRX500 NMR spectrometer (500 MHz for ^{1}H, and 125 MHz for ^{13}C). Chemical shifts are given on δ (ppm) scale with TMS as the initial standard. UV-visible spectra were recorded using a Shimadzu-1600 spectrophotometer. MS analyses were performed on a Finnigan MAT95 spectrometer.

2.2. Plant Material

The aerial parts of *Artemisia splendens* Willd. were collected from Kaleibar (Gharedagh) at E: 46° 48', N: 38° 49' (altitude of 2300) from the Eastern Azarbaijan province during June 2010. The identity of the plant was confirmed by anatomical examination in comparison with the herbarium specimens and a voucher specimen (Tbz-FPh 717) has been maintained in the Herbarium of the Faculty of Pharmacy, Tabriz University of Medical Sciences, Iran.

2.3. Extraction and Isolation

Air-dried and ground aerial parts of *A. splendens* (100 g) were Soxhlet-extracted, successively, with *n*-hexane, dichloromethane (DCM) and MeOH (1.1 L each). All of these extracts were separately concentrated using a rotary evaporator at a maximum temperature of 45°C. A portion of the MeOH extract (2 g) was subjected to solid-phase extraction (SPE) using a C_{18} SEP-PAK cartridge (10 g, Waters, Ireland), eluting with a step gradient of MeOH-water mixtures (10:90, 20:80, 40:60, 60:40, 80:20 and 100:0). The SPE fraction eluted with 10% MeOH was analyzed by prep-HPLC using the mobile phase: 0-40 min, linear gradient of 0-55% MeOH in water; 40-45 min, maintained at 55% MeOH in water, to isolate compound **5** (t_R = 16.3 min). Similar prep-HPLC analyses of the 20% methanolic SPE fraction (mobile phase: 0-50 min, linear gradient of 20-40% MeOH in water; 50-60 min, maintained in 40% MeOH in water) afforded compounds **1**, **2** and **3** (t_R =

9.7, 9.9 and 12.5 min, respectively), whilst 40% methanoilc SPE fraction (mobile phase: 0-30 min, linear gradient of 40-60% MeOH in water; 30-35 min, maintained in 60% MeOH in water) produced compound **4** (t_R = 15.2 min). In all of the above prep-HPLC analyses, the flow rate of the mobile phase was 8.0 mL/min. The structures of all compounds (**1**-**5**) (Figure 1) were elucidated conclusively by spectroscopic means.

*2.3.1. Z-Melilotoside (**1**)*

Light brown amorphous powder (5.6 mg); UV λ_{max} (MeOH) 253 and 290 (sh) nm; HRESIMS *m/z* 349.0890 $[M+Na]^+$ (calc. for $C_{15}H_{18}O_8Na$, 349.0899) in positive mode, 325.0920 $[M- H]^-$ (calc. for $C_{15}H_{17}O_8$, 325.0923) in negative ion mode; see Tables 1 and 2 for ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data.

*2.3.2. E-Melilotoside (**2**)*

Brown amorphous powder (12.5 mg); UV λ_{max} (MeOH) 275 and 320 (sh) nm; HRESIMS *m/z* 349.0891 $[M+Na]^+$ (calc. for $C_{15}H_{18}O_8Na$, 349.0899) in positive ion mode, 325.0921 $[M - H]^-$ (calc. for $C_{15}H_{17}O_8$, 325.0923) in negative ion mode; see Tables 1 and 2 for ^1H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD) data.

*2.3.3. Z-4-Methoxy-melilotoside (**3**)*

Light brown amorphous powder (6.0 mg); UV λ_{max} (MeOH) 268 and 300 (sh) nm; HRESIMS *m/z* 379.1003$[M+Na]^+$ (calc. for $C_{16}H_{20}O_9Na$, 379.1004) in positive mode, 355.1028 $[M- H]^-$ (calc. for $C_{16}H_{19}O_9$, 355.1029) in negative ion mode; see Tables 1 and 2 for ^1H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD) data.

*2.3.4. E-4-Methoxy-melilotoside (**4**)*

Brown amorphous powder (5.0 mg); UV λ_{max} (MeOH) 286 and 320 (sh) nm; HRESIMS *m/z* 379.1003$[M+Na]^+$ (calc. for $C_{16}H_{20}O_9Na$, 379.1004) in positive mode, 355.1027 $[M-H]^-$ (calc. for $C_{16}H_{19}O_9$, 355.1029) in negative ion mode; see Tables 1 and 2 for ^1H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD) data.

2.3.5.bis-ortho-Z-Melilotoside (*Splendenoside, **5**)*

Light brown amorphous powder (3.0 mg); UV λ_{max} (MeOH) 253 and 290 (sh) nm; HRESIMS *m/z* 651.1928 $[M+H]^+$ (calc. for $C_{30}H_{35}O_{16}$, 651.1925) in positive mode, 649.1766 $[M-H]^-$ (calc. for $C_{30}H_{33}O_{16}$, 649.1768) in negative ion mode; see Tables 1 and 2 for ^1H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD) data.

2.4. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH), molecular formula $C_{18}H_{12}N_5O_6$, was obtained from Fluka Chemie AG, Bucks. Quercetin was obtained from Avocado Research Chemicals Ltd, Shore road, Heysham, Lancs. The method used by Takao *et al.* [13] was adopted with suitable modifications as outlined by Kumarasamy *et al.* [14,15] and Chima *et al.* [16]. DPPH (8 mg) was dissolved in MeOH (100 mL) to obtain a concentration of 80 μg/mL.

2.4.1. Qualitative DPPH assay

Test sample solutions (10 mg/mL) were applied on a TLC plate and sprayed with DPPH solution using an atomiser. It was allowed to develop for 30 min. The colour changes (purple on white) were noted.

2.4.2. Quantitative assay

All test compounds (**1**-**5**) as well as the positive control, quercetin, were dissolved individually in MeOH to obtain the stock concentration of 1 mg/mL Dilutions were made to obtain concentrations of 0.1, 0.01, 0.001, 0.0001 and 0.00001 mg/mL. Diluted solutions (1.00 mL each) were mixed with DPPH (1.00 mL) and allowed to stand for 30 min for any reaction to take place. The UV absorbance was recorded at 517 nm. The experiment was performed in triplicate and the average absorption was noted for each concentration. The DPPH inhibitory activity (% inhibition) at each concentration was calculated by using the following formula: inhibition (%) = ($A_{control}$-A_{sample}) x 100 / $A_{control}$, where $A_{control}$ was the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} was the absorbance of the test/reference. A concentration *vs* % inhibition curve was constructed, and from the slope of this curve, the RC_{50} value, which is the concentration at which 50% inhibition of DPPH absorbance at 517 nm occurs, was calculated for each test sample.

3. Results and Discussion

3.1. Structure elucidation

A combination of solid-phase-extraction (SPE) and reversed-phase prep-HPLC purification of the methanol (MeOH) extract of the aerial parts of *A. splendens* afforded four known compounds, *Z*- and *E*-melilotosides (**1** and **2**), *Z*- and *E*-4-methoxy-melilotosides (**3** and **4**), and a new dimer, *bis-ortho-Z*-melilotoside (**5**, named: splendenoside) (Figure 1). The structures of these compounds (**1**-**5**) were elucidated unequivocally by spectroscopic means. This is the first report on the occurrence of melilotosides (**1** and **2**) and their derivatives (**3**-**5**) in the genus *Artemisia*.

HRESIMS analyses (positive and negative ion modes) of compound **1** revealed the *pseudo*-molecular ions at *m/z* 349.0890 $[M+Na]^+$ and 325.0920 $[M-H]^-$, which corresponded to the *pseudo*-molecular formula $C_{15}H_{18}O_8Na$ and $C_{15}H_{17}O_8$, respectively. The ^1H NMR spectrum (Table 1) displayed signals for four aromatic methine protons at δ 7.14 (1H, dd, *J* = 1.0, 8.5 Hz), 7.20 (1H, ddd, *J* = 1.5, 8.0, 8.5 Hz), 6.94 (1H, ddd, *J* = 1.0, 8.0, 8.5 Hz) and 7.60 (1H, dd, *J* = 1.5, 8.0 Hz) suggesting the presence of a 1,2-disubstituted benzene ring system, two *cis*-olefinic protons at δ 6.83 (1H, d, *J* = 12.5 Hz) and 6.05 (1H, d, *J* = 12.5 Hz) indicating the presence of an α,β-unsaturated carbonyl functionality, and signals for five oxymethine (δ 4.90, 3.52, 3.48, 3.41 and 3.40) and an oxymethylene (δ 3.87 and 3.70) groups corresponding to a glucosyl moiety. The ^1H-^1H COSY spectrum confirmed all possible ^1H-^1H scalar couplings. The ^{13}C NMR spectrum together with a DEPT 135 experiment (Table 2) showed signals for a total of 14 carbons, including signals for a carbonyl of a carboxylic acid (δ 176.3), two aromatic quaternary (δ 128.1 and 156.1), one of which was oxygenated (δ 156.1), four aromatic methines (δ 116.5, 130.9, 123.0 and 131.6), five oxymethine (δ 102.5, 74.9, 78.2, 71.3 and 78.1) and an oxymethylene (δ 62.5) carbon atoms, and the remaining carbon signal (δ 128.3) could be assigned to two olefinic methine carbon atoms. The ^1H-^{13}C HSQC experiment showed that both *cis*-olefinic proton signals δ 6.83 and δ 6.05 were directly linked to the carbon resonating at δ 128.3, and confirmed that compound **1** actually has 15 carbon atoms, not 14. All ^1H-^{13}C direct correlations obtained from the ^1H-^{13}C HSQC spectrum established assignment of all proton signals to corresponding carbon signals. At this point, it was obvious that the compound was in fact 2-glucosyloxy-*cis*-cinnamic acid. The attachment of the glucosyl moiety at C-2 was confirmed from the ^1H-^{13}C long-range ^3J correlation from the glucose anomeric proton signal (δ 4.90) to the aromatic oxygenated quaternary carbon C-2 (δ 156.1) observed in the ^1H-^{13}C HMBC spectrum (Table 3). Thus, compound **1** was identified unequivocally as *Z*- melilotoside.

HRESIMS analyses (positive and negative ion modes) of compound **2** revealed *pseudo*-molecular ions at *m/z* 349.0891 $[M+Na]^+$ and 325.0921 $[M-H]^-$, which corresponded to the *pseudo*-molecular formulae $C_{15}H_{18}O_8Na$ and $C_{15}H_{17}O_8$, respectively. The MS data indicated that this compound was an isomer of compound **1**. The ^1H (Table 1) and ^{13}C (Table 2) NMR data were comparable to those of *Z*- melilotoside (**1**) with the main exception being the coupling constant of the olefinic protons, which was 16.1 Hz in **2** as opposed to 12.5 Hz in **1**, confirming the presence of a

trans-double bond instead of a *cis*. Thus, compound **2** was identified conclusively as *E*- melilotoside (**2**).

Table 1. ^{1}H NMR data of melilotosides and their derivatives (**1**-**5**) (CD_3OD, chemical shift δ in ppm, coupling constant *J* in Hz in parentheses).

Positions	**1**	**2**	**3**	**4**	**5**[c]
Cinnamoyl moiety					
3	7.14 (1H, *dd*, 1.0, 8.5)	7.30 (1H, *dd*, 1.0, 8.5)	6.75 (1H, *d*, 2.2)	6.86 (1H, *d*, 2.2)	7.15 (1H, *dd*, 1.0, 8.5)
4	7.20 (1H, *ddd*, 1.5, 8.0, 8.5)	7.40 (1H, *ddd*, 1.5, 8.0, 8.5)	-	-	7.27 (1H, *dd*, 8.0, 8.5)
4-OMe	-	-	3.77 (3H, *s*)	3.82 (3H, *s*)	-
5	6.94 (1H, *ddd*, 1.0, 8.0, 8.5)	7.08 (1H, *ddd*, 1.0, 8.0, 8.5)	6.55 (1H, *dd*, 2.2, 8.5)	6.55 (1H, *dd*, 2.2, 8.6)	6.90 (1H, *dd*, 1.0, 8.0)
6	7.60 (1H, *dd*, 1.5, 8.0)	7.66 (1H, *dd*, 1.5, 8.0)	7.60 (1H, *d*, 8.5)	7.56 (1H, *d*, 8.6)	-
7	6.83 (1H, *d*, 12.5)	8.10 (1H, *d*, 16.1)	6.76 (1H, *d*, 12.5)	7.97 (1H, d, 16.0)	6.67 (1H, d, 12.2)
8	6.05 (1H, d, 12.5)	6.54 (1H, *d*, 16.1)	6.00 (1H, *d*, 12.5)	6.45 (1H, *d*, 16.0)	6.00 (1H, *d*, 12.2)
Glucosyl moiety					
1'	4.90[a] (1H)	5.00 (1H, *d*, 7.6)	4.90[a] (1H)	5.02 (1H, *d*, 7.7)	5.00 (1H, *d*, 7.7)
2'	3.52[b](1H)	3.58[b] (1H)	3.51[b] (1H)	3.58[b] (1H)	3.49[b] (1H)
3'	3.41[b](1H)	3.48[b] (1H)	3.42[b] (1H)	3.48[b] (1H)	3.41[b] (1H)
4'	3.40[b](1H)	3.47[b] (1H)	3.40[b] (1H)	3.47[b] (1H)	3.39[b] (1H)
5'	3.48[b](1H)	3.48[b] (1H)	3.47[b] (1H)	3.48[bv](1H)	3.48[b] (1H)
6'	3.87 (1H, *dd*, 1.0, 12.0) 3.70 (1H, *ddd*, 1.0, 6.8, 12.0)	3.90 (1H, *dd*, 1.0, 12.2) 3.72 (1H, *ddd*, 1.0, 6.8, 12.2)	3.88 (1H, *bd*, 12.0) 3.71 (1H, *dd*, 6.8, 12.0)	3.90 (1H, *bd*, 12.2) 3.72 (1H, *dd*, 6.8, 12.2)	3.84 (1H, *bd*, 12.0) 3.74 (1H, *dd*, 6.8, 12.0)

Except for compound **1** (500 MHz), all ^{1}H NMR data were obtained at 300 MHz

[a]Masked by the water peak, but detected from COSY, HSQC and HMBC experiments; [b]Overlapped peaks, identified from COSY. HSQC and HMBC experiments; [c]Because of symmetry (Figure 1) in the molecule, only one set of ^{1}H NMR data was observed for one half of the dimer.

The ^{1}H and ^{13} C NMR spectra (Tables 1 and 2) of compound **3** were similar to those of *Z*-melilotoside (**1**), with the exception that instead of four aromatic methine signals as in **1**, there were only three such methine signals in **3**, and in addition, there was also a methoxy signal (δ_H 3.77 s; δ_C 54.3), suggesting that compound **3** was in fact a methoxy derivative of **1**. Close examination of the splitting patterns of the aromatic methine signals confirmed the presence of an *ortho* doublet (δ 7.60, J = 8.5 Hz), a *meta* doublet (δ 6.75, J = 2.2 Hz) and an *ortho-meta* doublet of doublets (δ 6.55, J = 2.2, 8.5 Hz) indicating the attachment of the methoxy signal at either C-4 or C-5 of the aromatic ring. The relatively shielded nature of the ^{13}C NMR signal for C-3 carbon (δ101.3) suggested that this carbon was in between two oxygenated quaternary carbon atoms, and that could only be possible, if the methoxy group was placed at C-4 (as C-2 had the glucosyloxy group). A combination of ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC confirmed all of the ^{1}H and ^{13}C NMR assignments (Table 3). The HRESIMS analyses revealed the *pseudo*-molecular ions at *m/z* 379.1003 $[M+Na]^+$ (calc. for $C_{16}H_{20}O_9Na$, 379.1004) in positive ion mode, and 355.1028 $[M-H]^-$ (calc. for $C_{16}H_{19}O_9$, 355.1029) in negative ion mode, which further confirmed the molecular formula of **3**. Therefore, this compound was identified as *Z*-4-methoxy-melilotoside (**3**), the presence of which was previously reported from *Lavendula officinalis* [17].

HRESIMS analyses (in both positive and negative ion modes) of compound **4** revealed the *pseudo*-molecular ions at *m/z* 379.1003 $[M+Na]^+$ and 355.1027 $[M-H]^-$, which corresponded to the *pseudo*-molecular formulae $C_{16}H_{20}O_9Na$ and $C_{16}H_{19}O_9$, respectively. The MS data indicated that this compound was an isomer of compound **3**. The ^{1}H (Table 1) and ^{13}C NMR (Table 2) data were comparable to those of *Z*-4-methoxy-melilotoside (**3**) with the main exception being the coupling constant of the olefinic protons, which was 16.0 Hz in **4** as opposed to 12.5 Hz in **3**, confirming the presence of a *trans*-double bond instead of a *cis*. Thus, compound **4** was identified conclusively as *E*-

4-methoxy-melilotoside (**4**), the presence of which was also previously reported from *Lavendula officinalis* [17].

Table 2. ^{13}C NMR data of melilotosides and their derivatives (**1**-**5**) (chemical shift δ in ppm)

Positions	**1**	**2**	**3**	**4**	**5**[a]
Cinnamoyl moiety					
1	128.1	125.6	122.1	121.1	126.0
2	156.1	157.5	159.1	159.2	153.4
3	116.5	116.8	101.3	101.0	115.1
4	130.9	132.5	160.2	160.1	124.1
5	123.0	123.6	107.2	107.6	107.2
6	131.6	128.6	130.1	129.1	128.0
7	128.3	140.1	138.1	141.1	129.4
8	128.3	121.1	123.8	122.1	122.8
9	176.3	172.1	178.0	172.9	175.5
4-OMe	-	-	54.3	55.0	-
Glucosyl moiety					
1'	102.5	102.5	101.4	102.4	100.1
2'	74.9	74.8	73.1	74.1	72.8
3'	78.2	78.3	76.8	77.3	75.4
4'	71.3	71.3	70.4	71.3	69.3
5'	78.1	78.0	76.7	77.2	76.0
6'	62.5	62.5	61.1	62.0	60.4

Except for compound **1** (125 MHz), all ^{13}C NMR data were obtained at 75.0 M
[a]Because of symmetry in the molecule, only one set of ^{13}C NMR data was observed for one half of the dimer.

Table 3. ^{1}H-^{13}C long-range (^{2}J and ^{2}J) correlations observed in the ^{1}H-^{13}C HMBC spectra of melilotosides and their derivatives (**1**-**5**).

	^{1}H-^{13}C long-range correlations	
	^{2}J	^{3}J
Cinnamoyl moiety		
H-3	C-2, C-4	C-1, C-5
H-4[a]	C-3, C-5	C-2, C-6
H-5	C-4, C-6	C-1, C-3
H-6[b]	C-1, C-5	C-2, C-7
H-7	C-1, C-8	C-2, C-6, C-9
H-8	C-7, C-9	C-1
4-OMe[c]	-	C-4
Glucosyl moiety		
H-1'		C-2, C-3', C-5'
H-2'		C-4'
H-3'	C-2', C-4'	C-1', C-5'
H-4'	C-3'	C-2', C-6'
H-5'	C-4'	C-1', C-3'
H-6'		C-4'

[a]This correlation was absent in compounds **3** and **4**, as there was no H-4 methine proton due to methoxylation at C-4;
[b]This correlation was absent in compound **5**, as there was no H-6 methine proton due to C-6/C-6' dimerization;
[c]This correlation was only present in compounds **3** and **4**, as there was no methoxylation in any other compounds

HRESIMS spectra of compound **5** displayed the molecular ions at *m/z* 651.1928 $[M+H]^+$ (calc. for $C_{30}H_{35}O_{16}$, 651.1925) in positive ion mode, and 649.1766 $[M-H]^-$ (calc. for $C_{30}H_{33}O_{16}$, 649.1768) in negative ion mode, suggesting an elemental composition of $C_{30}H_{34}O_{16}$ for this compound. This also suggested that this compound could be a dimer of compound **1** (mol wt 326, $C_{15}H_{18}O$). The ^{1}H and ^{13}

C NMR spectra (Tables 1 and 2) of compound **5** were similar to those of *Z*-melilotoside (**1**), with the exception that instead of four aromatic methine signals as in **1**, there were only three such methine signals in **3**, and there was no additional signal. Considering the molecular formula obtained from the HRESIMS spectra, together with the NMR data, it was obvious that compound **5** was a symmetrical dimer originating from two units of *Z*-melilotoside (**1**) linked (C-C) through the benzene ring carbons. A detailed examination of the splitting patterns of the aromatic methine signals confirmed the presence of an *ortho-meta* doublet of doublets (δ 7.15, *J* = 1.0, 8.5 Hz), *ortho-ortho* doublet of doublets (δ 7.27, *J* = 8.0, 8.5 Hz) and an *ortho-meta* doublet of doublets (δ 6.90, *J* = 1.0, 8.0 Hz) indicating dimerization through C-3/C-3' or C-6/C-6' of the aromatic rings. In the ^{1}H-^{13}C HMBC spectrum, a ^{3}J ^{1}H-^{13}C long-range correlation from H-7/7' (δ 6.67) to the aromatic quaternary carbon C-6/6' (δ 128.0), confirmed that dimerization was indeed between C-6 and C-6' of the aromatic rings of two *Z*-melilotoside (**1**) units. Therefore, compound **5** was identified as bis-*ortho*-*Z*-melilotoside (splendenoside, **5**), which is also a new natural product.

3.2. Free-radical-scavenging activity

The *in vitro* free-radical-scavenging activity of all isolated compounds (**1**-**5**) was assessed by the DPPH assay and compared with that of the positive control, quercetin (Table 4), a well-known natural antioxidant [14]. The free-radical-scavenging potency of **1**-**5** was quite similar, only with *Z*-4-methoxy-melilotoside (**3**) being slightly more potent (RC_{50} = 9.55 x 10^{-2} mg/mL) than others (RC_{50} = 156 x 10^{-1} – 3.22 x 10^{-1} mg/mL). All of these compounds (**1**-**5**) exhibited much less potency than that of quercetin. It is interesting to note that dimerization of **1** leading to the formation of **5** slightly decreased the free-radical-scavenging potency.

Table 4. Free-radical-scavenging activity of melilotosides and their derivatives (**1**-**5**) observed in the DPPH assay.

Test samples	RC_{50} value in mg/mL
Z-Melilotoside (**1**)	2.66 x 10^{-1}
E-Melilotoside (**2**)	1.56 x 10^{-1}
Z-4-Methoxy-melilotoside (**3**)	9.55 x 10^{-2}
E-4-Methoxy-melilotoside (**4**)	2.93 x 10^{-1}
bis-ortho-Z-Melilotoside (Splendenoside,**5**)	3.22 x 10^{-1}
Positive control quercetin	2.55 x 10^{-3}

4. Conclusions

The cinnamic acid-based compounds, melilotosides and their derivatives (**1**-**5**), have been reported here for the first time from the genus *Artemisia*. Among the compounds **1**-**4** are known compounds, to the best of our knowledge, the dimer, *bis-ortho-Z*-melilotoside (**5**, named: splendenoside) is a new natural products. All compounds (**1**-**5**) showed a low level of *in vitro* free-radical-scavenging property in the DPPH assay.

Acknowledgments

Mass spectrometry data were acquired at the EPSRC UK National Mass Spectrometry Facility at Swansea University.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP.

References

[1] V. A. Mozaffarian (1996). Dictionary of Iranian Plant Names. Tehran, Iran: Farhange Moaser, Tehran, pp. 56-58.

[2] K. M. Valant-Vetschera, R. Fischer and E. Wollenweber (2003). Exudate flavonoids in species of *Artemisia* (Asteraceae-Anthemideae): new results and chemosystematic interpretation, *Biochem. Syst. Ecol.* **31**, 487-498.

[3] S. Salido, L. R. Valenzuela, J. Altarejos, M. Nogueras, A. Sánchez and E. Cano (2004). Composition and intraspecific variability of *Artemisia herba-alba* from southern Spain, *Biochem. Syst. Ecol.* **32**, 265-277.

[4] J. H. Kim, H-K. Kim, S. B. Jeon, K-H. Son, E. H. Kim, S. K. Kang, N. D. Sung and B. M. Kwon (2002). New sesquiterpene–monoterpene lactone, artemisolide, isolated from *Artemisia argyi*, *Tetrahedron Letts*. **43**, 6205-6208.

[5] M. Laid, M-E. F. Hegazy, A. A. Ahmed, K. Ali, D. Belkacemi and S. Ohta (2008). Sesquiterpene lactones from Algerian *Artemisia herba-alba*, *Phytochem. Letts.* **1**, 85-88.

[6] Mamun-Ur-Rashid, M. Alamzeb, S. Ali, A. A. Khan, J. O. Igoli, V. A. Ferro, A. I. Gray and M. R. Khan (2015). A new ceramide along with eight known compounds from the roots of *Artemisia incisa* Pamp, *Rec. Nat. Prod.* **9**, 294-304.

[7] N. Manika, C. S. Chanotiya, M. Darokar, S. Singh and G. D. Bagchi (2010). Compositional characters and antimicrobial potential of *Artemisia stricta* Edgew. F. *stricta* Pamp. essential oil, *Rec. Nat. Prod.* **10**, 40-46.

[8] S. V. Serkirov and A. N. Aleskerova (1991). *Artemisia splendens* terpenoids, *Khim. Prirod. Soed*. issue **2**, 203-206.

[9] M. Kazemi, M. R. Zand, K. Roshanaei, M. Mehrzad and A. Rustaiyan (2010). Composition of the volatile oils of *Artemisia armenica* Lan. and *Artemisia splendens* Willd. from Iran, *J. Vol. Oil. Res.* **22**, 126-128.

[10] F. H. Afshar, A. Delazar, O. Janneh, H. Nazemiyeh, L. Nahar and S. D. Sarker (2015). Chemical composition, free-radical-scavenging and insecticidal properties, and general toxicity of volatile oils of two Artemisia species growing wild in Iran, *J. Essen. Oil Bearing Plants* **19**, 1406-1416.

[11] F. H. Afshar, A. Delazar, O. Janneh, H. Nazemiyeh, S. Asnaashari, L. Nahar and S. D. Sarker (2011). Evaluation of antimalarial, free-radical-scavenging and insecticidal activities of *Artemisia scoparia* and *A. spicigera* (Asteraceae), *Braz. J. Pharmacog.* **21**, 986-990.

[12] M. Mojarrab, A. Delazar, S. B. Moghadam, H. Nazemiyeh, L. Nahar, Y. Kumarasamy, S. Asnaashari, A. Hadjiakhoondi and S. D. Sarker (2011). Armenin and isoarmenin – two prenylated coumarins from the aerial parts of *Artemisia armeniaca*, *Chem. Biodiversity*. **8**, 2097-2103.

[13] T. Takao, N. Watanabe, I. Yagi and K. Sakata (1994). A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish, *Biosci. Biotechnol. Biochem.* **58**, 1780-1783.

[14] Y. Kumarasamy, M. Fergusson, L. Nahar and S. D. Sarker (2002). Biological activity of moschamindole from *Centaurea moschata*, *Pharm. Biol.* **40**, 307-310.

[15] Y. Kumarasamy, M. Byres, P. J. Cox, M. Jaspars, L. Nahar and S. D. Sarker (2007). Screening seeds of some Scottish plants for free-radical scavenging activity, *Phytother. Res.* **21**, 615-621.

[16] N. K. Chima, L. Nahar, R. R. T. Majinda, S. Celik and S. D. Sarker (2014). Assessment of free-radical scavenging activity of the extracts and fractions of *Gypsophila pilulifera*: Assay-guided isolation of the active component, *Braz. J. Pharmacog*. **24**, 38-43.

[17] S. A. Brown (1963). Biosynthesis of the coumarins IV. The formation of coumarin and herniarin in lavender, *Phytochemistry* **2**, 137-144.