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Two New Bioactive Biphenylpropanoids from the Roots of *Salsola imbricata* (Chenopodiaceae) Growing in Saudi Arabia

MOHAMED HABIB OUESLATI[1,2]*, JALLOUL BOUAJILA[3] and HICHEM BEN JANNET[4]

[1]Northern Border University, College of Science, Department of Chemistry , P.O. Box 1231, Arar 91431, Kingdom of Saudi Arabia.
[2]Carthage University, Department of Chemistry, Preparatory Institute for Scientific and Technical Studies, P.O. Box 51, La Marsa 2070, Tunisia.
[3]Laboratoire des IMRCP UMR CNRS 5623, Faculté de Pharmacie de Toulouse, Université de Toulouse, Université Paul-Sabatier, 118 Route de Narbonne, Toulouse F-31062, France.
[4]Laboratoire de Chimie hétérocyclique, Produits Naturels et Réactivité Faculté des Sciences de Monastir, Université de Monastir, Monastir 5019, Tunisia.
*Corresponding author E-mail address: oueshabib@yahoo.fr (M. H. Oueslati)

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ABSTRACT

Phytochemical investigation of the roots of *Salsola imbricata* allowed to two new bioactive biphenylpropanoids (1) and (2) named, biphenylsalsonoid A and B, respectively. Their structures were established through spectroscopic methods (1D and 2D NMR, (ES)-HRMS, and IR). The isolates were tested for their antioxidant activity using DPPH• and ABTS•+ assays. 1 and 2 showed a moderate activity towards DPPH (IC_{50} = 86.5 ± 1.3 and 122.3 ± 0.63 µg/mL, respectively) and ABTS (IC_{50} = 95 ± 1.5, 137.7 ± 1.2 µg/mL, respectively). The antibacterial effect of the ethyl acetate extract and the isolates were assessed. Results obtained revealed that compounds showed important antibacterial activities against S. *aureus*, *S. epidermidis*, *M. luteus*, and *E. coli* with MIC values ranging from 16 to 32 µg/mL.

Keywords: *Salsola imbricata*, biphenylsalsonoids, NMR, antioxidant activities, antibacterial activities.

INTRODUCTION

The genus *Salsola* includes halophyte species and belongs to the family of Chenopodiaceae[1-3]. The genus is widespread in the dry regions of Middle East, Africa, and Europe. Many species among the genus are used in traditional medicine. In the Middle East, *Salsola baryosma* is used as a diuretic agent and against some inflammations [4]. This plant also exhibits antioxidant activities [5], alkaloids (salsolin

and salsolidin) have been isolated from *Salsola tragus* (synonym: *Salsola kali*) used in the treatment of hypertension by stimulating the activity of sleep[6,7]. At present, only few species of the genus have been studied chemically and biologically and were found to be a good source of phenolic compounds identified in *S. kali*, *S. soda*, *S. oppositifolia* and *S. collina* [8,9] . Furthermore, antioxidant triterpenes were isolated from *S. baryosma* and *S. somalensis* [10] and new antioxidant bibenzyl derivative and isoflavonoid were isolated from *S. tetrandra*[11]. Our previous work on the genus led to salsolanol and biphenylsalsinol isolation from *S. villosa* [12] and cleomiscosin D, norisoprenoid, long-chain hydroxyl fatty acids, taxiphyllin, trans-*N*-feruloyltyramine *S*-(-)-trans-*N*-feruloyloctopamine and coumarinolignan from *S. tetrandra* [13,14]. Recent research on *Salsola imbricata* showed the presence of triterpene saponins from the methanolic extract of roots [14] and new isorhamnetin derivatives from the leaves[15]. Thus, in order to continue our research on the genus of *salsola* growing in Saudi Arabia. We focused our study on the ethyl acetate extract from the roots of *S. imbricata* because it has not been studied previously. Indeed, the present study suggests isolating new compounds with important biological activities. We try to the isolation of new bioactive compounds from the roots of *S. imbricate* and the evaluation of its antibacterial activity against Gram-positive and Gram-negative bacteria. Furthermore, the *in vitro* antioxidant activity was tested by using DPPH• and ABTS•$^{+}$ assays of isolated compounds.

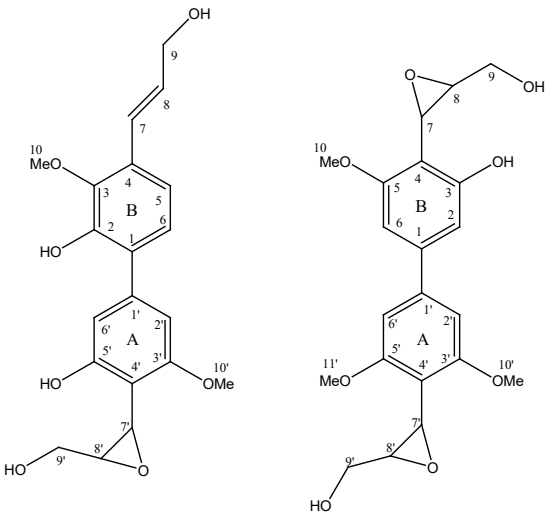

Fig. 1: Structure of compounds 1 and 2

MATERIALS AND METHODS

General Procedures

The optical rotations were recorded on a Perkin-Elmer 241-MC polarimeter. UV spectra were measured by using a Shimadzu UV-2401A spectrophotometer. Infra-red spectra were measured on a Perkin-Elmer 157G. ^{1}H, ^{13}C and 2D NMR spectra of isolated 1 and 2 were obtained in CD_3OD on Bruker 300 MHz, 75 MHz(spectrometer using internal reference the residual solvent resonance. Coupling constants were measured in Hertz and chemical shifts were reported in ppm. ESI-HRMS was measured on a Shimadzu LC-MS Spectrometer.

Plant material

Roots of *Salsola imbricata* Delile. ex Schul. Were collected from Arar, Saudi Arabia, on November 2015. The plant was identified by Dr. Ahmed K. Osman, College of Sciences, Department of Biology, Kingdom of Saudi Arabia and deposited in the herbarium (Sv-26) of the above department.

Extraction and Isolation

The roots from *S. imbricata* were dried and then ground into powder. 1Kg of powder was extracted with methanol (5L). After that the crude extract was evaporated in vacuo yielding a residue of 65.4g (%). The residue was dissolved in water (2 L) and then extracted successively with petroleum ether, ethyl acetate and *n*-butanol yielding 12.4, 16.2 and 24.6 g sub-extracts, respectively. The ethyl acetate extract was fractionated on a column chromatography (silica gel- mesh 70-230, 70 × 5 cm, i.d.) eluted with mobile phase of n-hexane/ EtOAc (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100, 1L each) and EtOAc/MeOH (90/10 80:20, 70:30, 60:40, 50:50, 500 mL each). After TLC monitoring the column chromatographic fractions were combined into eight fractions (F_1- F_8). Fraction F_4 (426 mg) was separated on a silica gel column (mesh 70-230, 70 × 2 cm, i.d) that was eluted with $CHCl_3$/MeOH (100:0 to 60:40) to obtained four subfractions (A_1 to A_4**).** The purification of the fraction of the subfraction A_2 (62mg) by using preparative TLC 85:15 ($CHCl_3$/MeOH) to yield 16 mg of **1** and 12 mg of **2**.

Antibacterial Activities

The antibacterial activities were tested against Gram-positive (*Staphylococcus aureus*, *S. epidermidis* and *Micrococcus luteus*) and Gram-negative strains (*Pseudomonas aeruginosa,*

Escherichia coli, and *Salmonella typhimurium*). The tested extracts were respectively: Methanol crude of the roots, the isolated compound **1** and the isolated compound **2.** Mueller-Hinton agar (5ml) was used for the culture of bacteria (stored at -70 °C stock) and the media were incubated for 24 h at 37 °C.

The antibacterial activity was evaluated by minimum inhibitory concentration (MIC)[16,17]. Serial tube dilution was used to determine the values of MIC for the methanol crude extract and for the two isolated compounds. To obtain stock solution, 0.5 mg of plant extracts (methanol crude, compound 1 and 2) was suspended in 2 mL of distilled water and 2 drops of between-80 for the homogenization. The suspensions of micro-organisms consist of a medium with the concentration fixed at 10^7 organisms/mL and one drop of suspension (0.02 mL) was added to the broth dilution. The temperature of the incubation was fixed at 37 °C for 18 h and the tubes were examined for the growth. The MIC of the tested extract/products was fixed for the lowest concentration that showed the totally absence of the growth for the micro-organisms. The negative and the positive control consist, respectively, of distilled water with 2 drops of tween-80 and kanamycin.

Antioxidant activities

Free radical scavenging ability using DPPH• radical

The protocol was used as described, previously, by Tepe, B. et al. 2005[19]. Briefly, 2 mL of DPPH solution (100 ìg/mL, EtOH) was added to 0.5 mL of compounds (0.01–1 mg/mL). After 30 min, the absorbance was read at 517 nm. The blank consists of 2 mL of DPPH solution and 0.5 mL of methanol. The IC_{50} was determined by the following equation:

$$\%\text{Inhibition} = [(A_{blank} - A_{sample})/A_{blank})] \times 100$$

A_{blank} and A_{sample} were, respectively, the absorbance values of the control and the test sample. Vitamin C was used as reference and tests were measured in triplicate.

Free radical scavenging ability using ABTS•+ radical cation

ABTS was dissolved in distilled water and the concentration was fixed at 7 mmol/L[20]. For the completion of radical generation, ABTS•+ radical cation was generated by adding the potassium persulfate (2.45 mmol/L) to the ABTS solution. The mixture was conserved in the darkness for 12–16 h at room temperature. After a dilution of the mixture with ethanol, the wavelength was fixed at 734 nm until to obtain an absorbance value of 0.70 ± 0.02. ABTS solution (50 mL) was added to 950 mL of compounds (0.01–1 mg/mL) and after 6 min. the absorbance was measured at 734 nm. The blank consists of 50 mL of ABTS solution and 950 mL of ethanol. The IC_{50} was determined by the formula mentioned before in DPPH assays.

Compound 1 (Fig. 1) has a molecular formula of $C_{20}H_{22}O_7$ as deducted from the ESI-HRMS (*m/z* = *m/z* 397.1260 $[M+Na]^+$). The IR spectrum revealed the presence of hydroxyl group (3446 cm^{-1}) and aromatic ring (1625 cm^{-1}).

In the aromatic region of the spectrum ^{1}H NMR of **1** (Table 1) displayed proton signals at δ_H 6.98 (H-2', d, *J* = 1.9Hz) and 6.84 (H-6', d, *J* = 1.9Hz), attributable to the meta-coupled protons, of the terasubstituted aromatic ring A and two aromatic proton signals at δ_H 7.01 (H-5, d, *J* =8.1Hz) and 6.78 (H-6, d, *J* = 8.1Hz) attributable to the two ortho-coupled protons of the tetrasubstituted aromatic ring B. The same spectrum displayed the presence of two *trans*-olefinic protons resonating at δ_H 5.55 (1H, d, *J* =15.9Hz) and at δ_H 6.25 (1H, dt, *J* =15.9Hz, *J* = 6.0Hz), attributable to H-7 and H-8, respectively, as well as two methoxy groups at δ_H 3.76 (3H, s) and 3.84 (3H, s) assignable to H-10 and H-10', respectively.

The ^{13}C-NMR and DEPT spectra of **1** showed signals for 14 sp^2 carbons (seven methines and seven quaternary carbons) and 6 sp^3 carbons (two methyl, two methylene and two methine groups) (Table 1).

The full analysis of the ^{1}H and ^{13}C NMR spectra were obtained by using 2D NMR. The correlations observed in the ^{1}H-^{1}H COSY spectrum between the olefinic protons (H-7 and H-8) and the hydroxymethylenic protons (H-9) provided evidence for the propenol moiety (-CH=CH-CH_2OH). The ^2J *and* ^3J correlation of the olefinic proton H-7 (δ_H 6.25) with C-3 (δ_C 151.2), C-4 (δ_C 126.4), and C-5

(δ_C 112.5) revealed in the HMBC spectrum (Fig. 2) showed that the propenol moiety is connected to the ring B at C-4 (Fig. 2). This position was consolidated by the appearance of the *noe* H-7/H-10(OMe). The detection of signals at δ_H/ δ_C 5.52/ 87.7 and 3.42 / 56.3 suggested the presence of a 1,2-disubstituted epoxide in the molecule[12,21,22]. The correlations H-7'/H-8' and H-8'/H-9' observed in ^{1}H-^{1}H COSY spectrum (Fig. 2) indicated an epoxy substituted propanoid moiety. HMBC cross-peaks of C-4'(δ_C 126.3), C-3'(δ_C 149.5), and C-5'(δ_C 150.1) with H-7' (δ_H 5.52) confirmed the attachment of this epoxy propanoid moiety to the trisubstituted aromatic ring A at C-4' (Fig. 2). This result was consolidated by the *neo* between H-7' and methoxy H-10' revealed in the NOESY spectrum (Fig. 2). The biphenylic structure of compound[23-24] and the connection of two ring A and B at C-1 and C-1' were evidenced by the ^3J correlations H-6/C-1' and H-2'/C-1 observed in HMBC spectrum (Fig. 2). The position of the two methoxy groups was confirmed by the ^3J correlations H-10 (OMe)/C-3 and H-10'(OMe)/C-3' in the HMBC spectrum (Fig. 2) and by the appearance of the *noe* cross peaks H-7/ H-10(OMe) and H-7'/H-10'(OMe) in the NOESY spectrum. The above data, were found to be consistent with a new biphenylpropanoid structure identified to be 4'-(9'- (hydroxymethyl) oxiran-7'-yl)-4-((*E*)-3-hydroxyprop-7-en-7-yl)-3,3'-dimethoxy-[1,1'-biphenyl]-2,5'-diol named biphenylsalsonoid **A**

Compound 2 Fig.1 has a molecular formula of $C_{21}H_{24}O_8$ as deducted from the ESI-HRMS (*m/z* = *m/z* 427.1365 $[M+Na]^+$). The IR spectrum revealed the presence of hydroxyl group (3448 cm^{-1}) and aromatic ring (1622 cm^{-1}).

The aromatic region of the ^{1}H-NMR **2** (table 1) spectrum showed characteristic singlets at δ_H 6.58 (2H, s) which were attributed to the equivalent protons H-2' and H-6' of the tetrasubstituted aromatic ring A. The same region exhibited two aromatic signals at δ_H 6.87 (d, *J* = 1.8Hz) and at δ_H 7.01 (d, *J* = 1.8Hz) attributed to H-2 and H-6. In addition, the spectrum showed a singletat δ_H 3.78 (6H, s) attributed to the two equivalent methoxy goups H-10'and H-11' (-OCH_3) attached to the aromatic ring A and another singlet at δ_H 3.76 (3H, s) corresponding to the methoxy group H-10 (-OCH_3) attached to the second aromatic ring B. The ^{1}H-NMR,^{13}C-NMR and HMQC spectra exhibited characteristic resonances of two disubstituted epoxides[12,21,22]. at δ_H/δ_C 5.51 (H-7, m)/ 87.3 (C-7), 3.42 (H-8, m)/ 56.2 (C-8), 5.46 (H-7', m)/ 87.1(C-7') and 3.40 (H-8', m)/ 56.0 (C-8').

The ^{13}C spectrum of **2** showed resonance of 12 sp^2 carbons attributable to eight quaternary carbons which four are oxygenated and four tertiary carbons (Table 1). The same spectrum also showed three methoxy carbons at δ_C 56.8 and six

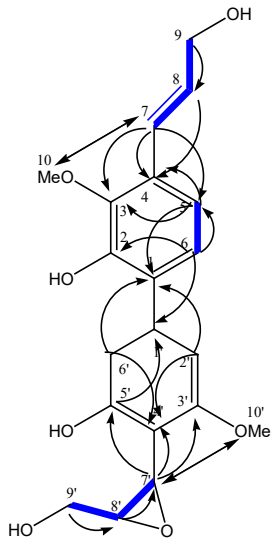

Fig. 2: Relevant HMBC (⌒), COSY (▬) and NOEs (↔) correlations for compound 1

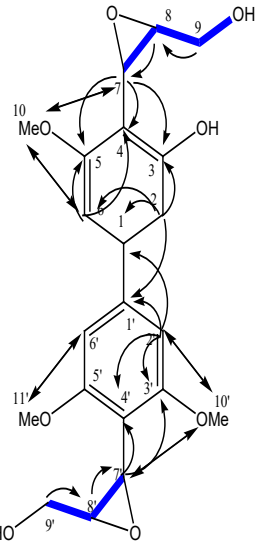

Fig. 3: Relevant HMBC (⌒), COSY (▬) and NOEs (↔) correlations for compound 2

sp^3 oxygenated carbons in the region δ_C 56.0-87.3 (Table 1).

The ^{1}H-^{1}H COSY experiment (Fig. 3) showed correlations H-8 with H-7and H-9on the one hand and /H-8' with H-7'and H-9'on the other hand provided evidence for the two epoxy propanoid moieties. The above spectral data indicate that **2** and **1** are two analogous compounds.

The HMBC long-range ^2J and ^3J correlations H-2 with C-1, C-3 and C-6; H-6 with C-4; and H-2' with C-1, C-3' and C-4' indicated **2** should be processed a biphenyl skeleton [23,24](Fig. 3). The location of the hydroxyl function, the three methoxy groups and the two epoxy propanoids one the biphenyl skeleton were evidenced by the HMBC and the NOESY experiments. The presence of the first epoxy propanoid at C-4 was established by the correlations of the proton H-7 (δ_H 5.51) with C-4 (δ_C 126.8), C-3 (150.1) and C-5 (152.4) observed in HMBC spectrum. The location of the methoxy group at C-5 was confirmed by the ^3J correlations H-10/C-5 deduced from the HMBC spectrum (Fig. 3) and by the appearance of the *noe* between H-7 and H-10(-OMe) observed in the NOESY spectrum. The second epoxy propanoid at C-4' was clearly indicated by the correlations of the proton H-7' (δ_H 5.46) with (C-3'and C-5', δ_C 152.2) and C-4' (δ_C 126.2) observed in HMBC spectrum (fig 3). The position of the two equivalent methoxy groups at C-3' and C-5' was established by the $^3J_{C\text{-}H}$ correlations of the protons resonating at δ_H 3.78 (H-10',11') and the aromatic quaternary carbons C-3', C-5' (δ_C 152.2) (Fig. 3). This result was reinforced by the *noe* cross peak between the proton H-7' (δ_H 5.46) and the equivalent protons of the two methoxy groups H-10' and H-11' (δ_H 3.78) (Fig. 3). The above data, were found to be consistent with a new biphenylpropanoid structure identified to be 4,4'-bis-(9-hydroxymethyl) oxiran-7-yl)-5,3',5'-trimethoxy [1,1'biphenyl]-3-ol named biphenylsalsonoid **B**.

Antioxidant activities

For the antioxidant activities of the isolated compounds 1 and 2, two assays have been

Table 1: NMR spectral data of compounds 1 and 2 (CD_3OD, 300 MHz, *J* in Hz)

	Compound 1		Compound 2	
Position	**^{13}C(δ)**	**^{1}H(δ)**	**^{13}C(δ)**	**^{1}H(δ)**
1	139.5	-	138.7	-
2	149.0	-	112.4	7.01(1H, d, *J* =1.8)
3	151.2	-	150.1	-
4	126.4	-	126.8	-
5	112.5	7.01 (1H, d, *J* =8.1)	152.4	-
6	116.7	6.78 (1H, d, *J* = 8.1)	110.2	6.87 (1H, d , *J* =1.8)
7	132.5	6.25 (1H, d, *J* =15.9)	87.3	5.51 (1H, m)
8	127.9	5.55 (1H, dt, *J* =15.9 *J*= 6.0)	56.2	3.42 (1H, m)
9	64.5	4.21 (2H, d, *J* = 5.7)	64.6	4.32 (1H, m)
10(OMe)	56.4	3.84 (3H, s)	57.2	3.76 (3H, s)
1'	136.0	-	138.4	-
2'	113.9	6.98 (1H, d, *J* = 1.9)	108.6	6.58 (1H, s)
3'	149.5	-	152.2	-
4'	126.3	-	126.2	-
5'	150.1	-	152.2	-
6'	110.4	6.84 (1H, *J* = 1.8)	108.6	6.58 (1H, s)
7'	87.4	5.52 (1H, d, *J* = 6.3)	87.1	5.46 (1H, m)
8'	56.3	3.42 (1H, m)	56.0	3.40 (1H, m)
9'	64.3	4.31 (2H, m)	64.4	4.32 (2H, m)
10' (OMe)	57.2	3.76 (3H, s)	56.8	3.78 (3H, s)
11'(OMe)	-	-	56.8	3.78 (3H, s)

Table 2. Antioxidant (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ Assays) activities of compounds 1 and 2

	IC_{50} values (μg/mL)		
	1	**2**	**Vitamin C**
DPPH$^{\bullet}$	86.5 ± 1.3	122.3 ± 1.4	26.0 ± 1.2
ABTS$^{\bullet+}$	95.1 ± 1.5	137.7 ± 1.2	22.4 ± 0.5

According to the results given in Table 3. The compounds 1 and 2 display the same activity against the three Gram-negative used and the Gram-positive strains *S. aureus* and *S. epidermidis*. This finding could be due to the common biphenyl skeleton bearing in the same position (C-4') present in both 1 and 2. On the other hand, Compound **2** (MIC = 16 μg/mL) was found to be two times more active than compound 1 against *M. luteus*.

Table 3. Antibacterial activities of EtOAc extract and compounds 1 and 2

Strains	**MIC values (μg/mL)**			
	EtOAc Extract	**1**	**2**	**Kanamycin**
Gram-positive				
S. epidermidis	32	32	32	2
S. aureus	16	16	16	2
M.s luteus	16	32	16	4
Gram-negative				
E. coli	16	16	16	4
P. aeruginosa	64	64	64	8
S. typhimurium	16	16	16	4

MIC: Minimum inhibitory concentration
Kanamycin: antibiotic

assessed: DPPH free radical scavenging and ABTS system (Table 2).

The isolate biphenylpropanoids **1** and **2** showed a moderate antioxidant activity towards DPPH with IC_{50} values of 86.5 ± 1.3 and 122.3 ± 0.63 μg/mL, respectively, but less potent when compared to Vitamin C. On the other hand, the isolated biphenylpropanoids showed antioxidant activity against ABTS in the similarly order as against DPPH (IC_{50} = 95 ± 1.5, 137.7 ± 1.2 μg/mL, respectively). Compound 1 has a relatively high activity due to the presence of two phenol groups by comparison with **2** bearing one phenol group.

Antibacterial Activities

The *in vitro* antibacterial activity of the EtOAc extract of the roots of *S. villosa***,** compounds 1 and compound 2 was assessed using the MIC method against three Gram-positive bacteria; *S. aureus*, *S. epidermidis* and *Micrococcus luteus* and three Gram-negative bacteria; *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*.

This dual sensitivity against the compound **2** might be explained by the difference in structure between the two compounds, mainly the apparition of a new epoxy moiety at C-4 and another methoxy group at C-5'. In addition to the modification in the position of the hydroxyl group by comparison to compound **1**.

CONCLUSION

Two new natural biphenylpropanoid analogous 1 and 2 (named biphenylsalsonoids A and B) were isolated from the roots of *Salsola villosa*. Their structures were elucidated by spectroscopic methods including 1D, 2D-NMR experiments. Compounds 1 and 2 showed a moderate activity of radical-scavenging towards DPPH and ABTS. The ABTS scavenging activity of compounds was similar to the DPPH free radical scavenging. Their antibacterial activity was evaluated by the MIC method against *Staphylococcus aureus*, *S. epidermidis*, *Micrococcus luteus*, *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*. The two compounds have

shown the same activity towards the tested bacteria, except *M. luteus* which exhibited more sensitivity against compound **2**.

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