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

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http://dx.doi.org/10.13005/ojc/330432

(Received: May 18, 2017; Accepted: July 12, 2017)

**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 mg/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\(^1,2\). 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\(^6\), 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. 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. Furthermore, antioxidant triterpenes were isolated from S. baryosma and S. somalensis and new antioxidant bibenzyl derivative and isoflavonoid were isolated from S. tetrandra. Our previous work on the genus led to salsolanol and biphenylsalsinol isolation from S. villosa and cleomiscosin D, norisoprenoid, long-chain hydroxyl fatty acids, taxiphyllin, trans-N-feruloyltymamine S-(-)-trans-N-feruloyloctopamine and coumarinolignan from S. tetrandra. Recent research on Salsola imbricata showed the presence of triterpene saponins from the methanolic extract of roots and new isorhamnetin derivatives from the leaves. Thus, in order to continue our research on the genus 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 isolate new bioactive compounds from the roots of S. imbricata 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.

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-1800 spectrophotometer. Infrared spectra were measured on a Perkin-Elmer 157G. 'H, 13C and 2D NMR spectra of isolated 1 and 2 were obtained in CD3OD 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, 50:50, 500 mL each ). After TLC monitoring the column chromatographic fractions were combined into eight fractions (F1-F8). Fraction F1 (426 mg) was separated on a silica gel column (mesh 70-230, 70 × 2 cm, i.d.) that was eluted with CHCl3/MeOH (100:0 to 60:40) to obtained four subfractions (A to D). The purification of the fraction of the subfraction A (62mg) by using preparative TLC 85:15 (CHCl3/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)\textsuperscript{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 was fixed for the lowest concentration that showed the totally absence of the growth.

Antioxidant activities

Free radical scavenging ability using DPPH\textsuperscript{•} radical

The protocol was used as described, previously, by Tepe, B. et al. 2005\textsuperscript{18}. Briefly, 2 mL of DPPH solution (100 µg/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 (100 µg/mL, EtOH) was added to 0.5 mL of methanol. The IC\(_{50}\) was determined by the formula mentioned before in DPPH assays.

\textbf{Compound 1} (Fig. 1) has a molecular formula of C\(_{12}\)H\(_{12}\)O\(_6\), as deducted from the ESI-HRMS (m/z = m/z 397.1260 [M+Na])\textsuperscript{+}. 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 \(\delta_h\) 6.98 (H-2’, d, \(J = 1.9\)Hz) and 6.84 (H-6’, d, \(J = 1.9\)Hz), attributable to the meta-coupled protons, of the terasubstituted aromatic ring A and two aromatic proton signals at \(\delta_h\) 7.01 (H-5, d, \(J = 8.1\)Hz) and 6.78 (H-6, d, \(J = 8.1\)Hz) attributable to the two ortho-coupled protons of the terasubstituted aromatic ring B. The same spectrum displayed the presence of two trans-olefinic protons resonating at \(\delta_h\) 5.55 (1H, d, \(J = 15.9\)Hz) and at \(\delta_h\) 6.25 (1H, dt, \(J = 15.9\)Hz, \(J = 6.0\)Hz), attributable to H-7 and H-8, respectively, as well as two methoxy groups at \(\delta_h\) 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 hydroxymethylene protons (H-9) provided evidence for the propenol moiety (-CH=CH-CH\(_2\)OH). The \(^2\)J and \(^3\)J correlation of the olefinic proton H-7 (\(\delta_h\) 6.25) with C-3 (\(\delta_c\) 151.2), C-4 (\(\delta_c\) 126.4), and C-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 \(\text{noe} H-7'/H-10'(\text{OMe})\). The detection of signals at \(\delta_H 6.58 (2H, s)\) 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 \(\delta_H 6.87 (d, J = 1.8Hz)\) and at \(\delta_H 7.01 (d, J = 1.8Hz)\) attributed to H-2 and H-6. In addition, the spectrum showed a singlet at \(\delta_H 3.78 (6H, s)\) attributed to the two equivalent methoxyl groups H-10'and H-11' (-OCH\(_3\)) attached to the aromatic ring A and another singlet at \(\delta_H 3.76 (3H, s)\) corresponding to the methoxyl group H-10 (-OCH\(_3\)) attached to the second aromatic ring B. The \(^1{\text{H}}\)-NMR, \(^{13}{\text{C}}\)-NMR and HMQC spectra exhibited characteristic resonances of two disubstituted epoxides\(^{12,21,22}\).

The \(^{13}{\text{C}}\) 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 methoxyl carbons at \(\delta_C 56.8\) and six
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sp\(^3\) oxygenated carbons in the region \(\delta_c\) 56.0-87.3 (Table 1).

The \(^1^H-^1^H\) COSY experiment (Fig. 3) showed correlations H-8 with H-7 and H-9 on 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 \(^2^J\) and \(^3^J\) 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 (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 (\(\delta_H\) 5.51) with C-4 (\(\delta_C\) 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 \(^3^J\) C-H correlations of the protons resonating at \(\delta_H\) 3.78 (H-10, 11) and the aromatic quaternary carbons C-3', C-5' (\(\delta_C\) 152.2) (Fig. 3). This result was reinforced by the noe cross peak between the proton H-7' (\(\delta_H\) 5.46) and the equivalent protons of the two methoxy groups H-10' and H-11' (\(\delta_H\) 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 performed: Table 1: NMR spectral data of compounds 1 and 2 (CD\(_3\)OD, 300 MHz, \(J\) in Hz)

<table>
<thead>
<tr>
<th>Position</th>
<th>(^{13})C((\delta))</th>
<th>(^1^H(\delta))</th>
<th>(^{13})C((\delta))</th>
<th>(^1^H(\delta))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>139.5</td>
<td>-</td>
<td>138.7</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>149.0</td>
<td>-</td>
<td>112.4</td>
<td>7.01(1H, d, J=1.8)</td>
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<tr>
<td>3</td>
<td>151.2</td>
<td>-</td>
<td>150.1</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>126.4</td>
<td>-</td>
<td>126.8</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>112.5</td>
<td>7.01 (1H, d, J=8.1)</td>
<td>152.4</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>116.7</td>
<td>6.78 (1H, d, J=8.1)</td>
<td>110.2</td>
<td>6.67 (1H, d, J=1.8)</td>
</tr>
<tr>
<td>7</td>
<td>132.5</td>
<td>6.25 (1H, d, J=15.9)</td>
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<tr>
<td>8</td>
<td>127.9</td>
<td>5.55 (1H, dt, J=15.9 J= 6.0)</td>
<td>56.2</td>
<td>3.42 (1H, m)</td>
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<tr>
<td>9</td>
<td>64.5</td>
<td>4.21 (2H, d, J = 5.7)</td>
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<td>10(OMe)</td>
<td>56.4</td>
<td>3.84 (3H, s)</td>
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<tr>
<td>1'</td>
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<td>-</td>
<td>138.4</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>113.9</td>
<td>6.98 (1H, d, J = 1.9)</td>
<td>108.6</td>
<td>6.58 (1H, s)</td>
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<tr>
<td>3'</td>
<td>149.5</td>
<td>-</td>
<td>152.2</td>
<td>-</td>
</tr>
<tr>
<td>4'</td>
<td>126.3</td>
<td>-</td>
<td>126.2</td>
<td>-</td>
</tr>
<tr>
<td>5'</td>
<td>150.1</td>
<td>-</td>
<td>152.2</td>
<td>-</td>
</tr>
<tr>
<td>6'</td>
<td>110.4</td>
<td>6.84 (1H, J = 1.8)</td>
<td>108.6</td>
<td>6.58 (1H, s)</td>
</tr>
<tr>
<td>7'</td>
<td>87.4</td>
<td>5.52 (1H, d, J = 6.3)</td>
<td>87.1</td>
<td>5.46 (1H, m)</td>
</tr>
<tr>
<td>8'</td>
<td>56.3</td>
<td>3.42 (1H, m)</td>
<td>56.0</td>
<td>3.40 (1H, m)</td>
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<tr>
<td>9'</td>
<td>64.3</td>
<td>4.31 (2H, m)</td>
<td>64.4</td>
<td>4.32 (2H, m)</td>
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<td>10'(OMe)</td>
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<td>3.78 (3H, s)</td>
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</table>
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 ± 1.4 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*.
shown the same activity towards the tested bacteria, except *M. luteus* which exhibited more sensitivity against compound 2.

**ACKNOWLEDGMENTS**

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at Northern Borders University for its funding of this research through the research project No. SCI-2016-F-5705.

**REFERENCES**


17. Tripathi, V.D.; Agarwal, S.K.; Rastogi, R.P. An antibacterial biphenyl derivative and


