Phenyl Polypropanoids from *Lindelofia stylosa*

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A phytochemical investigation on the aerial parts of *Lindelofia stylosa* has resulted in the isolation of seven phenyl propanoids 1—7. This includes three analogs of lithospermic acid 1—3, along with rosmarinic acid (4) and its ester derivatives 5—7. Compound 1 was identified as a new natural product. These compounds were studied for their antioxidant properties.

**Key words** *Lindelofia stylosa*; rosmarinic acid; rosmarinate derivative; lithospermic acid; antioxidant activity

About ten species of genus *Lindelofia* (Boraginaceae) are found in Central and West Asia and in Himalayan regions of Pakistan. *Lindelofia stylosa* (Kar. & Kir.) is a perennial herb reported to contain pyrrolizidine alkaloids. 1—8 In continuation of our studies on the biologically active phytochemicals, we investigated the whole herb of *L. stylosa* of Pakistani origin. The present work resulted in the isolation of compounds 1—7. The structures of the isolated compounds were elucidated mainly with the help of NMR spectroscopic techniques.

Three members of the phenyl propanoid class (compounds 1—3) were isolated from the EtOAc fraction of the plant which were identified to be the derivatives of lithospermic acid.

New compound 1 was isolated as a brown yellow gum. The compound showed an M⁺ at *m/z* 579.1493 in HR-FAB-MS (-ve), in agreement with the formula C₃₀H₂₈O₁₂ (Calcd 565.1346). Its IR spectrum showed the presence of an α,β-unsaturated carbonyl system at 1722 cm⁻¹. The overall spectral data of compound 1 closely resembled the known compound dimethyl lithospermate (2),9 the only difference between the two compounds being the appearance of signals for O–CH₂CH₃ moiety in the NMR spectra of 1. The O–CH₂CH₃ protons appeared as an AB quartet at δ 4.14 (J=14.2 Hz, J=7.0 Hz), and a triplet at δ 1.19 (J=7.0 Hz), which corresponded to the carbons resonated at δ 62.4 and 14.3, respectively. This indicated that compound 1 is an ethyl ester of lithospermic acid.

Compound 2 exhibited an M⁺ at *m/z* 565.1343 in the HR-FAB-MS (-ve), which corresponded to the formula C₂₉H₂₆O₁₂ (Calcd 565.1346). The spectral data of compound 2 was found to be identical with the reported dimethyl lithospermate, which was first isolated from *Salvia mitiorrhiza*.9

Compound 3 showed an M⁺ at *m/z* 745.1766 in the HR-FAB-MS (-ve), suggestive of the formula C₃₂H₃₀O₁₆ (Calcd 745.1768). The overall spectral data of compound 3 was identified as a known methyl ester derivative of lithospermic acid B, which was isolated from *Salvia przewalskii*.10

The comparison of ¹H- and ¹³C-NMR data and physical data with those reported in the literature, indicated compounds 4—7 were esters of rosmarinic acid (4),11,12 O-methyl rosmarinate (5),13 ethyl rosmarinate (6)14 and butyl derivative (7).15 Their isolation from *L. stylosa* is reported here for the first time.

The isolated compounds 1—7 were found to possess radical scavenging properties in DPPH radical assay, among which, compounds 3 and 4 have exhibited the strongest activities. The rosmarinic acid (4) was found to be as active as the standard (3-tert-buty1-4-hydroxanisole).

Compound 4 has been reported in previous studies to be potent antioxidant.16,17 It increases the prostaglandin E₂ production and reduces the production of leukotriene B₄ in human polymorphonuclear leukocytes.18 It is also an inhibitor of complement C3-convertase⁹,²⁰ as well as an inhibitor of lipid peroxidation.2¹

Our results have indicated that the derivatives of lithospermic acid, possessing a C-19 lactate moiety, are more active than those without such substituents, such as compounds 1 and 2. The study of antioxidant potentials of compounds 1—7 was carried out by using Fe²⁺-chelating and superoxide scavenging assays. The results demonstrated the selectivity of compounds 3—5 in these two assays, while the others were inactive. Interestingly, compound 3 has shown activity which is comparable to the standard propyl gallate used in the two assays. Our results demonstrated that the rosmarinate ester derivatives 4—5 and compound 3 possess significant antioxidant activities in various assays (see Table 1).

**Experimental**

**General Experimental Procedures** Melting points were determined on a Yanaco apparatus. UV spectra were measured on a Shimadzu UV240 ma-

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Table 1. Activities of Compounds 1—7 (IC$_{50}$, nm) in Various Antioxidant Assays

<table>
<thead>
<tr>
<th>Compound</th>
<th>DPPH radical scavenging activity</th>
<th>Superoxide anion scavenging activity</th>
<th>Iron chelating assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl lithospermate (1)</td>
<td>0.1028</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dimethyl lithospermate (2)</td>
<td>0.1197</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Methyl lithospermate B (3)</td>
<td>0.0506</td>
<td>0.113</td>
<td>0.053</td>
</tr>
<tr>
<td>Rosmarinic acid (4)</td>
<td>0.0801</td>
<td>0.282</td>
<td>0.034</td>
</tr>
<tr>
<td>Methyl rosmarinic acid (5)</td>
<td>0.1456</td>
<td>0.443</td>
<td>0.092</td>
</tr>
<tr>
<td>Ethyl rosmarinic acid (6)</td>
<td>0.0412</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Butyl rosmarinic acid (7)</td>
<td>0.2706</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3-butyl-4-hydroxyanisole$^a$</td>
<td>0.0442</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Propyl gallate$^a$</td>
<td>0.03</td>
<td>0.106</td>
<td>0.064</td>
</tr>
</tbody>
</table>

$^a$ Standard reference compound. $^b$ Inactive.

chine in MeOH solutions. IR spectra were recorded as KBr discs on a JASCO A-302 spectrometer. $^1$H- (500 MHz) and $^{13}$C-NMR (125 MHz) spectra were recorded in CD$_2$OD solutions on a Bruker AV-500 machine with tetramethylsilane (TMS) as an internal standard and the data is given in $^d$ (ppm). 2D NMR spectra were taken on a Bruker AMX 500 NMR spectrometer. Electron impact mass spectra (EI-MS) were taken at 70 $eV$ on a Finnigan MAT-112 or MAT-312 instrument and major ions are presented as m/z (%). Fast bombardment (FAB)-MS were measured as glycerol matrix on a JEOL HX110 Mass spectrometer. TLC purification was carried out on pre-coated silica gel cards (E. Merck) and the spots were observed first under UV (254 nm) and then sprayed with cerium(IV)sulfate reagent and heated until coloration developed. Recycling preparative HPLC (RP-HPLC) was used for final purification (JAI LC-908W, Japan Analytical Industry Co. Ltd.) as a mobile phase, H-80 column and a flow rate of 4 ml/min. The fraction eluted at MeOH–EtOAc: 1 : 1 (5.0 g) resulted into two unresolved fractions A and B. These fractions were subjected again to RPHPLC with modified separation conditions using a H-80 column and a gradient of 10—25% MeOH–CHCl$_3$ were combined and subjected to RPHPLC conditions as those of compound 1.

For the reaction mixture was prepared by mixing 200 µl of 0.1 M ferrous chloride and 1 ml of 0.1 M ferrous sulfate buffer (pH 7.5). The mixture was shaken and left at room temperature for 10 mins. The absorbance of the resulting mixture was measured at 562 nm by microplate reader. A lower absorbance of the reaction mixture indicated a higher Fe$^{2+}$-chelating ability.

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**References**


**Superoxide Anion Scavenging Assay** The reaction mixture was prepared by mixing 280 µM β-nicotinamide adenine dinucleotide reduced form (NADH), 80 µM nitrate (NADPH), 8 µM phosphate buffer (pH 7.5) and various concentrations of test samples in 200 µl of 0.1 M phosphate buffer (pH 7.5). The mixture was incubated at 37 °C for 30 min. The absorbance at 515 nm was measured by microplate reader (Spectramax plus 384 Molecular Device, USA.), and percent radical scavenging activity was determined in comparison with the DMSO-treated sample. The mixture was incubated at 37 °C for 30 min. The absorbance at 515 nm was measured by microplate reader (Spectramax plus 384 Molecular Device, USA.), and percent radical scavenging activity was determined in comparison with the DMSO-treated sample.