Phenylpropanoids from *Umbilicus pendulinus*

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Phytochemical investigation of the leaves of *Umbilicus pendulinus* afforded in addition to 2-O-cafeoyl malate, isoquercitrin and Z-venusol, the new isomer E-venusol. Special NMR experiments were carried out to elucidate the configuration of the two latter compounds.

**Key words** *Umbilicus*; Crassulaceae; phenylpropanoid; venusol; 2-O-cafeoyl malate; isoquercitrin

*Umbilicus pendulinus* DC. (Crassulaceae) is a robust perennial herb widespread in western and southern Europe. Species from this calcifuge genus grow generally on rocks, walls and more rarely on trees. Only four studies concerning isolation or characterization of secondary metabolites have been carried out on the genus. Some authors mentioned the presence of betaines\(^1\) and leucodelphinidin.\(^6\) Others studies led to the isolation of Z-venusol, a phenylpropanoid glycoside.\(^3,4\) Our preliminary investigations of HPLC chromatograms obtained from crude extract of *Umbilicus* leaves showed the presence of compounds with UV spectra close to Z-venusol. In the present paper, we wish to describe the isolation and the structural elucidation from the lyophilised leaves of *Umbilicus pendulinus* of a O-glucosylflavonol and 3 phenylpropanoids among which E-venusol is described for the first time.

**Results and Discussion**

Lyophilised leaves of *Umbilicus pendulinus* were directly extracted by a mixture of CH\(_2\)Cl\(_2\)--MeOH (1:1). The crude extract was partitioned against solvents of increasing polarity. The EtOAc layer was fractionated by a combination of gel filtration and chromatographic techniques including C\(_{18}\), Diol bonded silica and polyamide CC. This led to the isolation of Z- and E-venusol (1, 2), 2-O-cafeoyl malate 3 and isoquercitrin 4. Compound 3 was first isolated from *Phaseolus vulgaris.*\(^3\) Compound 4 is of common occurrence in the plant kingdom.\(^9\) Compounds 3 and 4 have been identified by comparison of their spectral data with the literature.\(^2,8\)

In the course of the isolation of 1, we noticed that prolonged exposure to MeOH led to a major compound 5 in mixture with 1 as an impurity. Analysis of the \(^1\)H-NMR spectrum (Table 1) of 5 indicates the presence of a p-disubstituted aromatic ring (\(\delta\) 6.75, H-6,8, d, \(J=8.7\) Hz; \(\delta\) 7.72, H-5,9, d, \(J=8.7\) Hz), an anomic sugar signal (\(\delta\) 5.04, H-1', d, \(J=7.5\) Hz), an OMe group (\(\delta\) 3.73, 3H, s) and an olefinic proton (H-3) as a singlet at 6.77 ppm. The \(^13\)C-NMR spectrum of 5 (Table 1) displays characteristic signals for an O-glucose moiety. On the HMBC spectrum of 5, the OMe at 3.73 ppm is found to be strongly correlated with the carbon signal at 164.9 ppm (C-1), suggesting the presence of a COOMe function. In addition, a \(^{3}\)J correlation between H-3 and the aromatic carbons C-5 and C-9 demonstrate that the latter proton is localized near the aromatic ring. Furthermore, H-3 is correlated \(^{2}\)J to the quaternary C at 139.5 ppm (C-2). The chemical shift of C-2, together with the presence of a \(^{3}\)J correlation.

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**Table 1. NMR Spectral Data for 5 in DMSO-d_6 (\(^1\)H: 125 MHz, \(^1\)C: 500 MHz, \(\delta\) ppm, \(J\) Hz)**

<table>
<thead>
<tr>
<th>Position</th>
<th>(^1)H</th>
<th>(^1)C</th>
<th>HMBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>164.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>139.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>123.70</td>
<td>6.77 s</td>
<td>C-1; C-2, C-5/9</td>
</tr>
<tr>
<td>4</td>
<td>125.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/9</td>
<td>133.04</td>
<td>7.72 d (8.7)</td>
<td>C-3; C-7, C-5/9</td>
</tr>
<tr>
<td>6/8</td>
<td>116.11</td>
<td>6.75 d (8.7)</td>
<td>C-4; C-7, C-6/8</td>
</tr>
<tr>
<td>7</td>
<td>159.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OMe</td>
<td>52.75</td>
<td>3.73 s</td>
<td>C-1</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1'</td>
<td>101.42</td>
<td>5.04 d (7.5)</td>
<td>C-2</td>
</tr>
<tr>
<td>2'</td>
<td>74.94</td>
<td>3.17---3.24 m</td>
<td></td>
</tr>
<tr>
<td>3'</td>
<td>77.42</td>
<td>3.17---3.24 m</td>
<td>C-4'; C-2'</td>
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<tr>
<td>4'</td>
<td>70.73</td>
<td>3.10 m</td>
<td></td>
</tr>
<tr>
<td>5'</td>
<td>78.44</td>
<td>3.06 m</td>
<td></td>
</tr>
<tr>
<td>6'a</td>
<td>61.55</td>
<td>3.58 m</td>
<td></td>
</tr>
<tr>
<td>6'b</td>
<td></td>
<td>usu(^{a})</td>
<td></td>
</tr>
</tbody>
</table>

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\(^{a}\) Under solvent.

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with H-1' indicates that this carbon is linked to the glucosyl moiety through an osidic bond. This data suggest a modified cinnamoyl unit in which C-2 of the olefinic bond bears an oxygen atom. Thus compound 5 is identified as methyl lespedeza.

Both Z- and E-methyl lespedeza have been synthesized by Shigemori et al. The 1H-NMR data for the two isomers (CD3OD) and a few related compounds indicates that H-3 is more deshielded in the case of the Z-isomer than that of the E-isomer. Similar examination of the proton spectra (DMSO-d6) identifies 5 as Z-methyl lespedeza (H-3: δ=6.77 ppm).

FAB-MS analyses of 1 and 2 demonstrate that they have the same molecular ion [M+H]⁺ at m/z 325. Therefore 1 and 2 are isomers each other, differing by 5 from 32 mu. 1H-NMR spectra of 1 and 2 are close to those of 5, except for the absence of the OMe signal and a significant deshielding of the glucose H-2' (approximately +0.9 ppm). This suggests that position 2' of glucose is esterified. This is confirmed i) by the 4.70 ppm deshielding of C-2' in 1 (acetone-d6) as compared to 5 (DMSO-d6), and ii) by the 5.55 ppm shielding of C-1' in 1 as compared to 5. A similar pattern is observed between 5 and 2. Therefore, compounds 1 and 2 are two isomers of venusol, probably differing by the configuration of the double bond. Visualization of Z- and E-venusol by molecular modeling indicates that the main difference between the two compounds is the distance between H-1' of the sugar moiety and H-5/9 of the aromatic ring (Z-venusol: 0.341 nm and E-venusol: 0.453 nm). Thus to assess the configuration of 1 and 2, Nodified experiments were carried out. Irradiation of H-5/9 in compound 1 leads to a 1.2% increase of the H-1' signal. A similar experiment on 2 does not show any effect, suggesting that 1 and 2 are Z- and E-venusols, respectively. Further confirmation is obtained after measurement of the 1H-13C heteronuclear coupling constant between H-3 and C-1 with 1D long range 1H-13C correlation experiments. The Z-isomer 1 displays a constant of 10 Hz while a 4 Hz constant is obtained in the case of the E-isomer 2.

Z-venusol has been already characterised by Prolliac et al. On the contrary, E-venusol is reported here for the first time in the plant Kingdom. Natural coexistence of E and Z isomers of cinnamoyl derivatives has already been reported in the past. However light-induced trans/cis isomerization of Z-venusol cannot be excluded as the source of E isomer. Furthermore, we noticed that Z-methyl lespedeza can easily originate from methanolic solution of Z-venusol. Therefore the natural origin of the above mentioned compounds was checked by performing the complete extraction-purification processes in the dark and in the absence of MeOH. HPLC analyses of the resulting fractions showed the natural occurrence of E-venusol only.

Experimental

General Methods
FAB-MS were obtained in the positive ion mode using thiglycol acid as matrix. Molecular modeling of Z- and E-venusol used Chemsite software. 1H- and 13C-NMR spectra were measured at 500 and 125 MHz respectively on a BRUKER DRX500 spectrometer. Nodified experiments used the standard BRUKER normal pulse sequence. The 1D long range 1H-13C correlation experiments used a home made sequence based on a classical 2D gradient HMQC and incorporating a Dano-Z pulse for the selection of the 13C signal. Column chromatography was performed over Sephadex LH-20 (Pharmacia). MPLC was carried out on silica 40-63 μm (100 g) or silica gel 60 (100-230 μm). The subsequent solvent combinations were used for chromato graphic separation: solvent 1 (CHCl3, to iso-PROH gradient); solvent 2 (H2O/MeOH; initial gradient from 90/10 to 80/20); solvent 3 (H2O/MeOH; gradient from 90/10 to 50/50); solvent 4 (gradient of MeOH in CHCl3); solvent 5 (H2O-MeOH-TFA 2:80:0.1); solvent 6 (gradient of MeOH in toluene); solvent 7 (H2O-MeOH-TFA 75:25:0.1).

Plant material
Umbilicus pentalinus leaves were collected during winter at St Michel and Courrlieu (Loire and Rhône, France). A voucher specimen has been deposited at Tinctifine Laboratories.

Extraction and Isolation of 1, 2, and 4
Dried lyophilized and powdered leaves (5 kg) were extracted with a mixture of CH2Cl2-MeOH (1:1). After filtration and removal of the solvent, the residue (700 g) was partitioned according to the previous method modified as follows: 80 g of the total crude extract were dissolved in MeOH (800 ml) and hexane (800 ml) plus H2O (88 ml) were added. The two phases were separated and the hypophase was extracted twice with hexane (2×800 ml). After addition of 248 ml H2O the hypophase was extracted again with hexane (800 ml). The last hypophase was concentrated to one third of its original volume, dissolved with H2O (400 ml) and extracted with CHCl3 (2×800 ml). The aqueous phase was finally partitioned against 2×800 ml of EtOAc. The total EtOAc extract (2×44 g) was fractionated into six fractions (1–6) by MPLC on Diol (solvent 1).

Fraction 5 (5 g) was submitted to MPLC on C18 (solvent 2) to give fraction 5a (pure 1, 900 mg) and 5b (223 mg). MPLC fractionation of fraction 5b on Diol using solvent 3 gave 2 (12 mg). 4 (4 mg) was isolated from fraction 6 (2.4 g) by two successive MPLC on C18 (solvent 3) and Polyamide (MeOH).
Extraction and Isolation of 3. Following the same process as described above, another EtOAc extract (4 g) from 1 kg of dried hypophylosed leaves was produced and subjected to successive gel filtration on Sephadex LH-20 using EtOAc and MeOH. Fractions containing 3 were submitted successively to two successive MPLC on Diaion (solvent 4) and C₁₈ (solvent 5). Final purification was achieved by HPLC (250 × 10 mm, Lichrospher® ODS 5 μm, solvent 5, 5 mL/min) to give pure 3 (3.8 mg).

HPLC Demonstration of the Natural Occurrence of 1—2. All steps were performed in the dark and in the absence of MeOH. Dried material (50 g) was extracted (2 l) by a mixture of CH₃Cl₂-EtOH (1:1) for 48 h. After filtration and evaporation, the resulting crude extract was dissolved in toluene and chromatographed by MPLC on Polyamide using solvent 6. From the twelve fractions obtained, aliquots were analysed by HPLC (250 × 4.6 mm, Lichrospher® ODS 5 μm, solvent 7, 1 mL/min) for their content in compounds 1—2.

Compound 1: UV λ_max (nm): 323; ¹H- and ¹³C-NMR: Table 2; FAB-MS (rel. int.): m/z: 325 [M+H]+ (41).

 Compound 2: UV λ_max (nm): 326; ¹H- and ¹³C-NMR: Table 2; FAB-MS (rel. int.): m/z: 325 [M+H]+ (16).

Compound 5: UV λ_max (nm): 300; ¹H- and ¹³C-NMR: Table 1; FAB-MS (rel. int.): m/z: 379 [M+Na]+ (23), 357 [MH]+ (14), 195 [M-Glc+H₂O+H]+ (55).

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