Phenylpropanoids from *Umbilicus pendulinus*

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Phytochemical investigation of the leaves of *Umbilicus pendulinus* afforded in addition to 2-*O*-caffeoyl 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*-caffeoyl 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 betaïnes\textsuperscript{1} and leucodelphinidin.\textsuperscript{2} Others studies led to the isolation of *Z*- and *E*-venusol, a phenylpropanoid glycoside.\textsuperscript{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 that of *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\textsubscript{2}Cl\textsubscript{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\textsubscript{18}, Diol bonded silica and polyamide CC. This led to the isolation of *Z*- and *E*-venusol (\textbf{1}, \textbf{2}), 2-*O*-caffeoyl malate \textbf{3} and isoquercitrin \textbf{4}. Compound \textbf{3} was first isolated from *Phaseolus vulgaris*.\textsuperscript{5} Compound \textbf{4} is of common occurrence in the plant kingdom.\textsuperscript{6} Compounds \textbf{3} and \textbf{4} have been identified by comparison of their spectral data with the literature.\textsuperscript{7–8}

In the course of the isolation of \textbf{1}, we noticed that prolonged exposure to MeOH led to a major compound \textbf{5} in mixture with \textbf{1} as an impurity. Analysis of the \textsuperscript{1}H-NMR spectrum (Table 1) of \textbf{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 anomeric sugar signal (\(\delta\) 5.04, H-1\textsuperscript{′}, \(d, J=7.5\) Hz), an OMe group (\(\delta\) 3.73, 3H, s) and an olefinic proton (H-3) as a singlet at 6.77 ppm. The \textsuperscript{13}C-NMR spectrum of \textbf{5} (Table 1) displays characteristic signals for an *O*-glucose moiety. On the HMBC spectrum of \textbf{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 \(^3J\) 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 (\(^2J\)) to the quaternary C at 139.5 ppm (C-2). The chemical shift of C-2, together with the presence of a \(^3J\) correlation

<table>
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<th>Position</th>
<th>(^1H)</th>
<th>(^13C)</th>
<th>HMBC</th>
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<tr>
<td>1</td>
<td>164.94</td>
<td></td>
<td>C-1; C-2; C-5/9</td>
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<tr>
<td>2</td>
<td>139.52</td>
<td></td>
<td></td>
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<tr>
<td>3</td>
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<td>123.70</td>
<td>C-1; C-2; C-5/9</td>
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<td>4</td>
<td></td>
<td>125.01</td>
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</tr>
<tr>
<td>5/9</td>
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<td>133.04</td>
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<td>6/8</td>
<td>6.75 d (8.7)</td>
<td>116.11</td>
<td>C-4; C-7; C-6/8</td>
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<td>7</td>
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<tr>
<td>OMe</td>
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<td>52.75</td>
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<tr>
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\(\textsuperscript{a}\) 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 5H-3 is more deshielded in the case of the Z-isomer than that of the E-isomer. Similar examination of the proton spectra of the resulting fractions showed that the natural occurrence of compound 5 is 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 from 5 by 32 amu. 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 by the 4.70 ppm deshielding of C-2’ in 1 (acetone-d6) as compared to 5 (DMSO-d6), and 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 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, Noediff 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 1J 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.
Extraction and Isolation of 3  Following the same process as described above, another EtOAc extract (4 g) from 1 kg of dried lyophilized 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 Diol (solvent 4) and C_{18} (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 CH₂Cl₂–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).

References
2) Combier H., Thèse de docteur-ingénieur, Lyon (1968).