

**Acutifoliside, a novel benzoic acid glycoside from *Salix acutifolia***

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UHPLC-MS profiling of a polar solvent extract of juvenile stem tissue of *Salix acutifolia* Willd. identified a range of phenolic metabolites. Salicortin, 1, a well-known salicinoid, was the major compound present and the study identified young stem tissue of this species as a potential source of this compound for future studies. Three further known metabolites (salicin 2, catechin 3 and tremuloidin 4) were also present. The UHPLC-MS analysis also revealed the presence of a further, less polar, unknown compound, which was isolated via HPLC peak collection. The structure was elucidated by high resolution mass spectroscopic and 1- and 2-dimensional NMR analysis, chemical derivatisation and comparison with literature values and was shown to be a novel benzoic acid glycoside 5, which we have named as acutifoliside.

**Keywords:** *Salix acutifolia*; phenolic glycoside; benzoic acid glycoside; acutifolisde; UHPLC-MS; NMR.

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| Number | $^1$H (ppm) | $^1$J (Hz) | $^{13}$C (ppm) | $^1$H-$^1$H correlation to: | $^1$H-$^{13}$C HMBC correlation to |
|--------|-------------|------------|----------------|----------------------------|-----------------------------------|
| 1      | -           | -          | 121.9          |                           |                                   |
| 2      | -           | -          | 153.3          |                           |                                   |
| 3      | -           | -          | 147.3          |                           |                                   |
| 4      | 7.16 (1H, dd) | 8.1, 1.4   | 122.3          | H-5                       | 127.2 (C-6), 147.3 (C-3), 153.3 (C-2) |
| 5      | 6.55 (1H, t)  | 8.0        | 120.7          | H-4, H-6                  | 121.9 (C-1), 147.3 (C-3), 153.3 (C-2) |
| 6      | 7.43 (1H, dd) | 8.0, 1.4   | 127.2          | H-5                       | 122.3 (C-4), 153.3 (C-2), 177.8 (C-7) |
| 7      | -           | -          | 177.8          |                           |                                   |
| 1"     |             |            | 131.9          |                           |                                   |
| 2"/6"  | 7.99 (2H, dd) | 8.3, 1.2   | 132.4          | H-3" & H-5"               | 131.9 (C-1"), 132.0 (C-2")/(C-5"), 136.7 (C-4"), 170.9 (C-7") |
| 3"/5"  | 7.54 (2H, dd) | 8.3, 7.5   | 132.0          | H-4" & H-2"/H-6"          | 131.9 (C-1"), 136.7 (C-4") |
| 4"     | 7.70 (1H, dd)| 7.5, 1.2   | 136.7          | H-3" & 5"                 | 132.4 (C-2")& (C-6") |
| 7"     |             |            | 170.9          |                           |                                   |
| 1'      | 5.08 (1H, d)  | 7.5        | 103.3          | H-2'                      | 147.3 (C-3)                      |
| 2'      | 3.68 (1H, dd)| 9.1, 7.5   | 75.8           | H-1' & H-3'               | 78.5 (C-3')                      |
| 3'      | 3.64 (1H, t)  | 8.9        | 78.5           | H-4'                      | 75.8 (C-2'), 73.3 (C-4')         |
| 4'      | 3.59 (1H, dd)| 9.9, 8.9   | 73.3           | H-5'                      | 78.5 (C-3'), 76.6 (C-5')         |
| 5'      | 3.95 (1H, ddd)| 9.9, 7.9, 2.4 | 76.6   | H-6' & H-6'               |                                   |
| 6'α     | 4.71 (1H, dd)| 12.0, 2.4  | 67.0           | H-5'                      | 170.9 (C-7")                     |
| 6'β     | 4.52 (1H, dd)| 12.0, 8.1  | 67.0           | H-5'                      | 170.9 (C-7"), 76.6 (C-5")       |

Table S1. 1 and 2-D-NMR data of acutifoliside 5 in D$_2$O:CD$_3$OD (80:20 containing 0.01% w/v d$_4$-TSP)
Figure S1. Total ion chromatogram from UHPLC-MS analysis (negative mode) of the polar plant extract (H₂O:CH₃OH, 8:2) from juvenile stem tissue of *Salix acutifolia*. The LC-retention times are indicated. 1: salicortin; 2: salicin; 3: catechin; 4: tremuloidin; 5: acutifoliside.
Figure S2. UHPLC-MS data from juvenile *Salix acutifolia*. A: Extract of total ion chromatogram indicating the peak at 26.60 minutes; B: mass spectrum; C: MS² fragmentation of m/z 419 ion.
Figure S3. UHPLC-MS analysis (negative mode) of purified acutifoliside, 5. A: total ion chromatogram; B: electrospray mass spectrum; C: MS-MS fragmentation spectrum of m/z 419.
$^1$H-NMR spectrum of acutifoliside, 5

600 MHz, 80:20 D$_2$O:CD$_3$OD containing 0.01 % w/v d$_4$-TSP

Number of integrated protons; Assignments

Figure S4. $^1$H-NMR spectrum of isolated acutifoliside 5, collected at 600 MHz.
$^1$H-$^1$H COSY spectrum of acutifoliside, 5

600 MHz, 80:20 D$_2$O:CD$_3$OD

Figure S5. $^1$H-$^1$H COSY spectrum of polar extract of juvenile *Salix Acutifolia.*
$^1$H- $^{13}$C HSQC spectrum of acutifoliside, 5

600 MHz, 80:20 D$_2$O:CD$_3$OD

Figure S6. $^1$H- $^{13}$C HSQC spectrum of polar extract of juvenile Salix Acutifolia.
**1H-13C** HMBC spectrum of acutifoliside, 5

600 MHz, 80:20 D2O:CD3OD

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Figure S7. **1H-13C** HMBC spectrum of polar extract of juvenile *Salix acutifolia*. 
Figure S8. Key HMBC correlations (from H to C) of 5.
$^1$H-NMR spectrum of acutifoliside methyl ester

600 MHz, 80:20 D$_2$O:CD$_3$OD containing 0.01 % w/v d$_4$-TSP

Number of integrated protons; Assignments

Figure S9. $^1$H-NMR spectrum of acutifoliside methyl ester, collected at 600 MHz.
Figure S10. UHPLC-MS data from acutifolside methyl ester. A: Extract of total ion chromatogram indicating the peak at 31.89 minutes; B: mass spectrum; C: MS$^2$ fragmentation of m/z 433 ion.
Figure S11. Replicated total ion chromatograms of *S. acutifolia* stem extracts obtained from 3 separate solvent extractions. Table indicates peak areas for compounds 1-5 and their relative standard deviations.