SUPPLEMENTARY MATERIAL

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; acutifoliside; UHPLC-MS; NMR.

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Number	^{1}H(ppm)	J_{H-H}(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'$_{\beta}$ & H-6'$_{\alpha}$	
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_2O:CD_3OD (80:20 containing 0.01% w/v d_4-TSP)

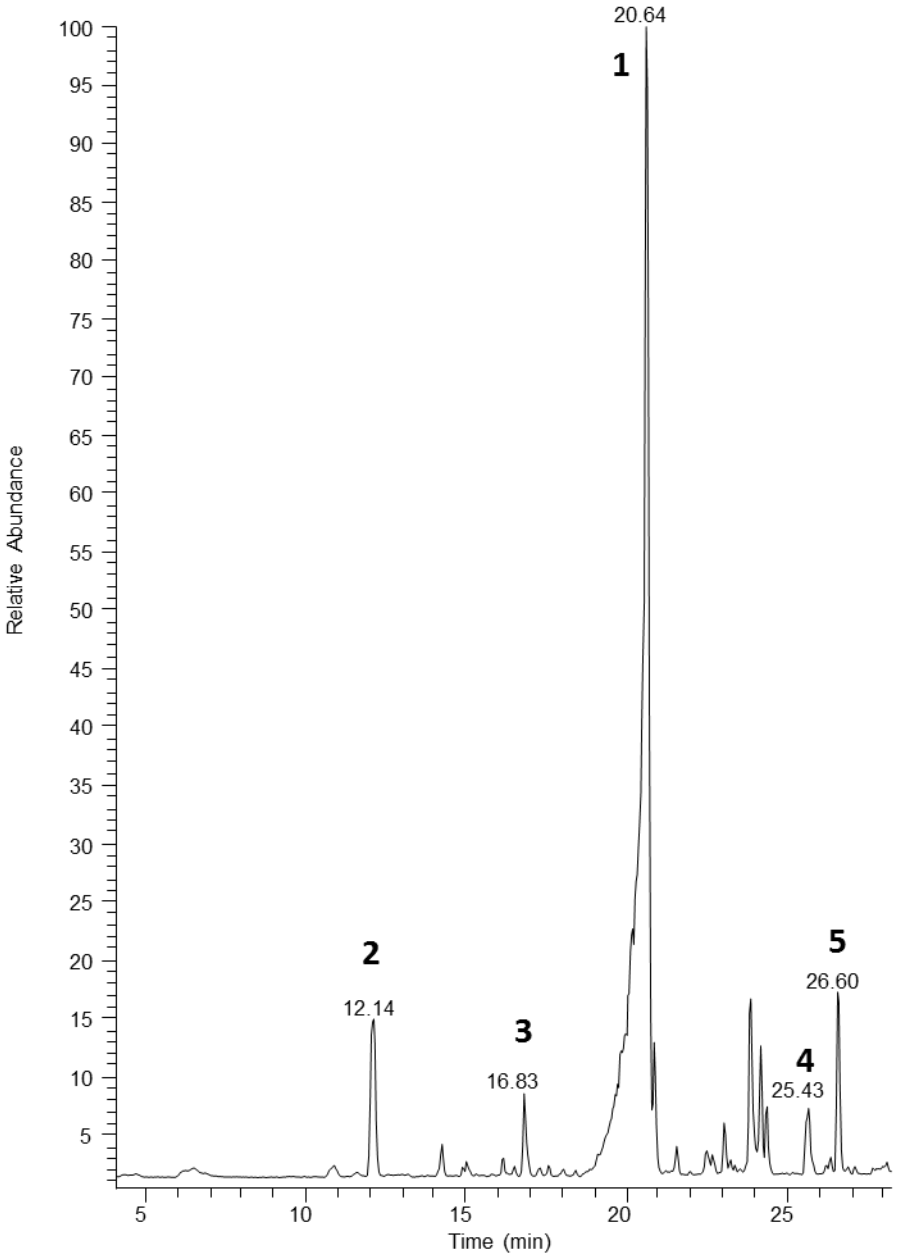


Figure S1. Total ion chromatogram from UHPLC-MS analysis (negative mode) of the polar plant extract (H_2O:CH_3OH, 8:2) from juvenile stem tissue of *Salix acutifolia*. The LC-retention times are indicated. 1: salicortin; 2: salicin; 3: catechin; 4: tremuloidin; 5: acutifoliside.

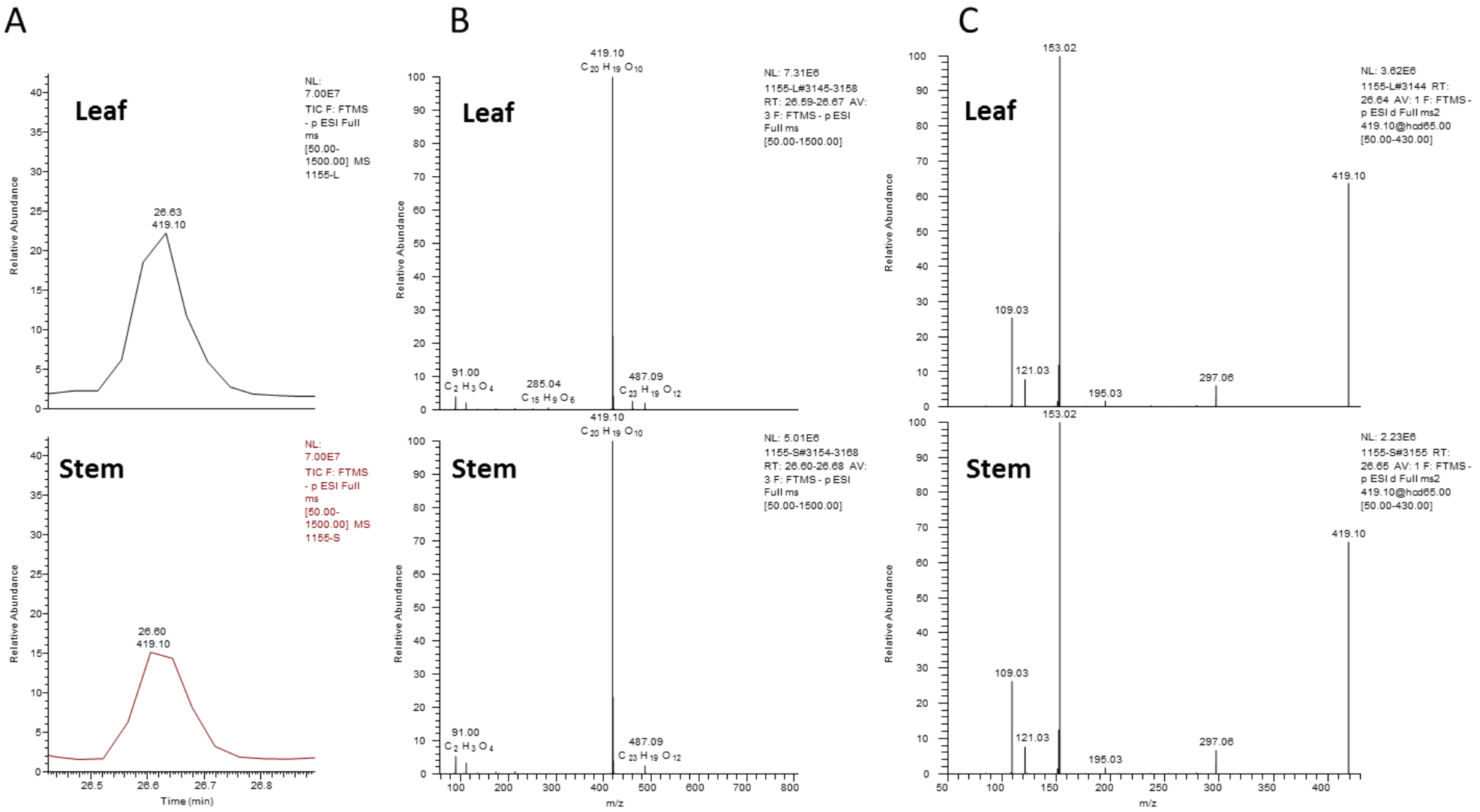


Figure S2. UHPLC-MS data from juvenile *Salix acutifolia.* A: Extract of total ion chromatogram indicating the peak at 26.60 minutes; B: mass spectrum; C: MS^2 fragmentation of m/z 419 ion.

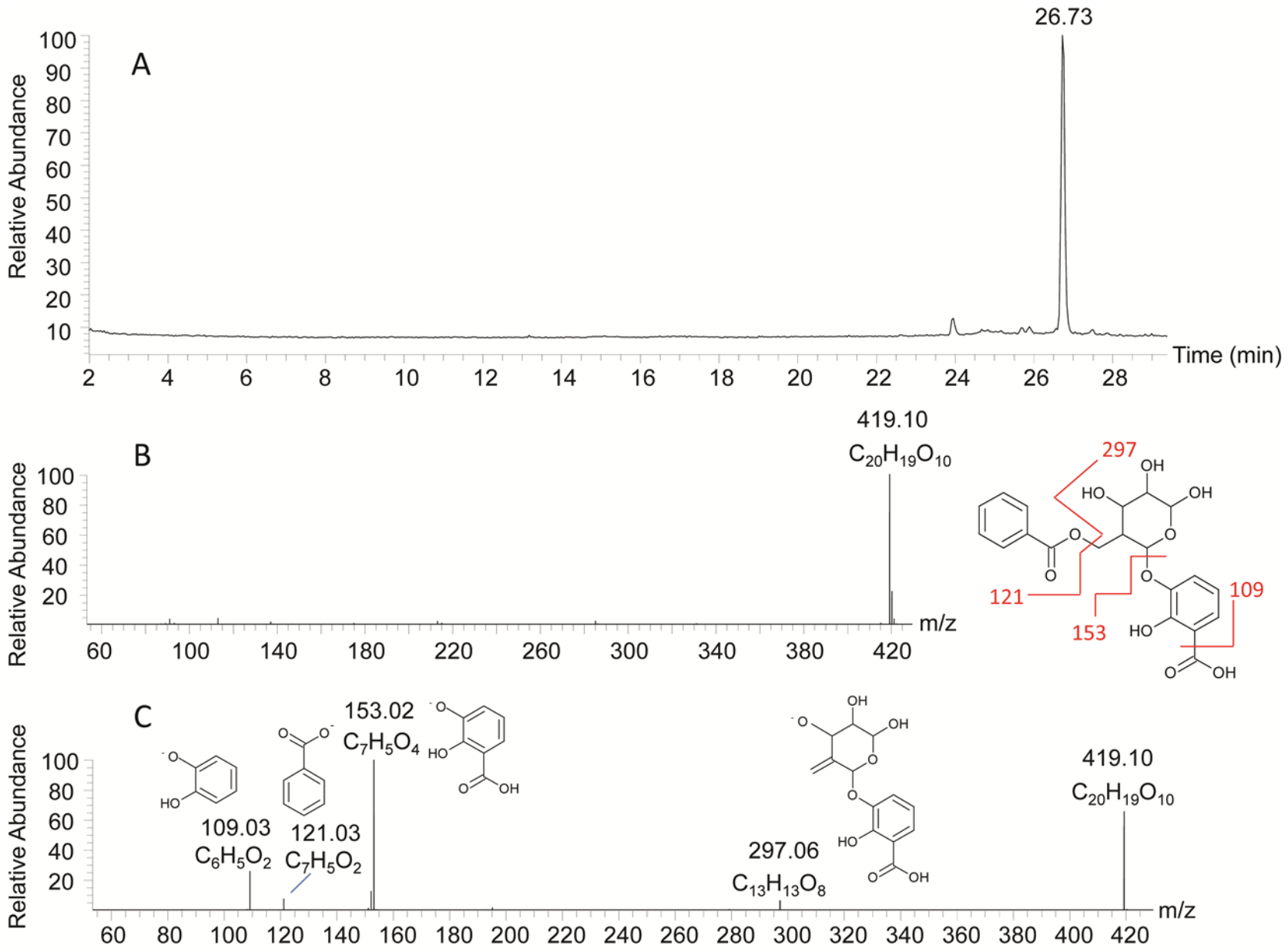


Figure S3. UHPLC-MS analysis (negative mode) of purified acutifoliside, 5. A: total ion chromatogram; B: electrospray mass spectrum; C: MS-MS fragmentation spectrum of m/z 419.

^{1}H-NMR spectrum of acutifoliside, **5**

600 MHz, 80:20 D_2O:CD_3OD containing 0.01 % w/v d_4-TSP

Number of integrated protons ; Assignments

Frequency (MHz)	600.05
Nucleus	1H
Number of Transients	128
Original Points Count	32768
Points Count	65536
Pulse Sequence	zgpr
Receiver Gain	1820.00
SW(cyclical) (Hz)	7183.91
Spectrum Offset (Hz)	2882.5244
Sweep Width (Hz)	7183.80

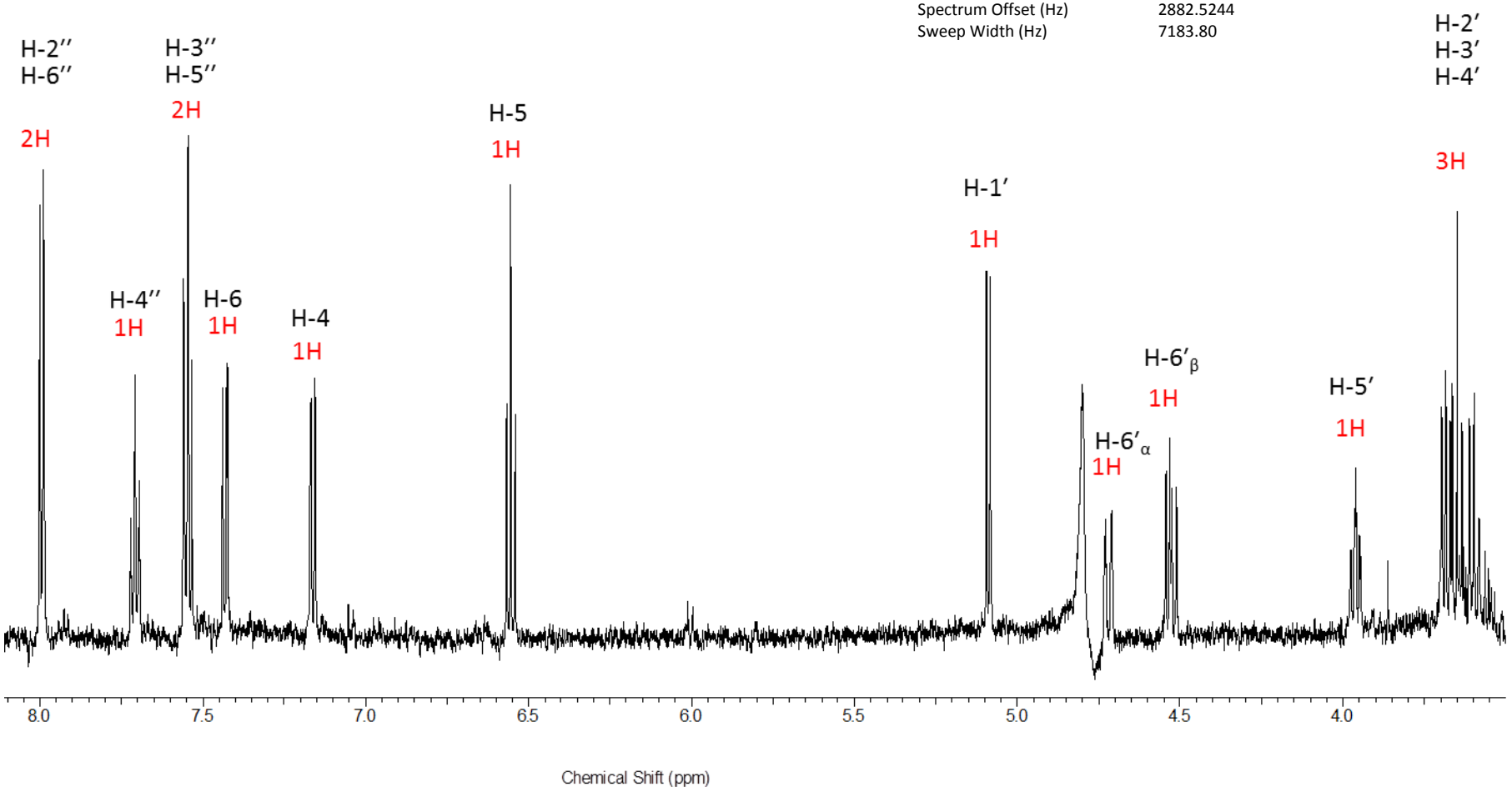
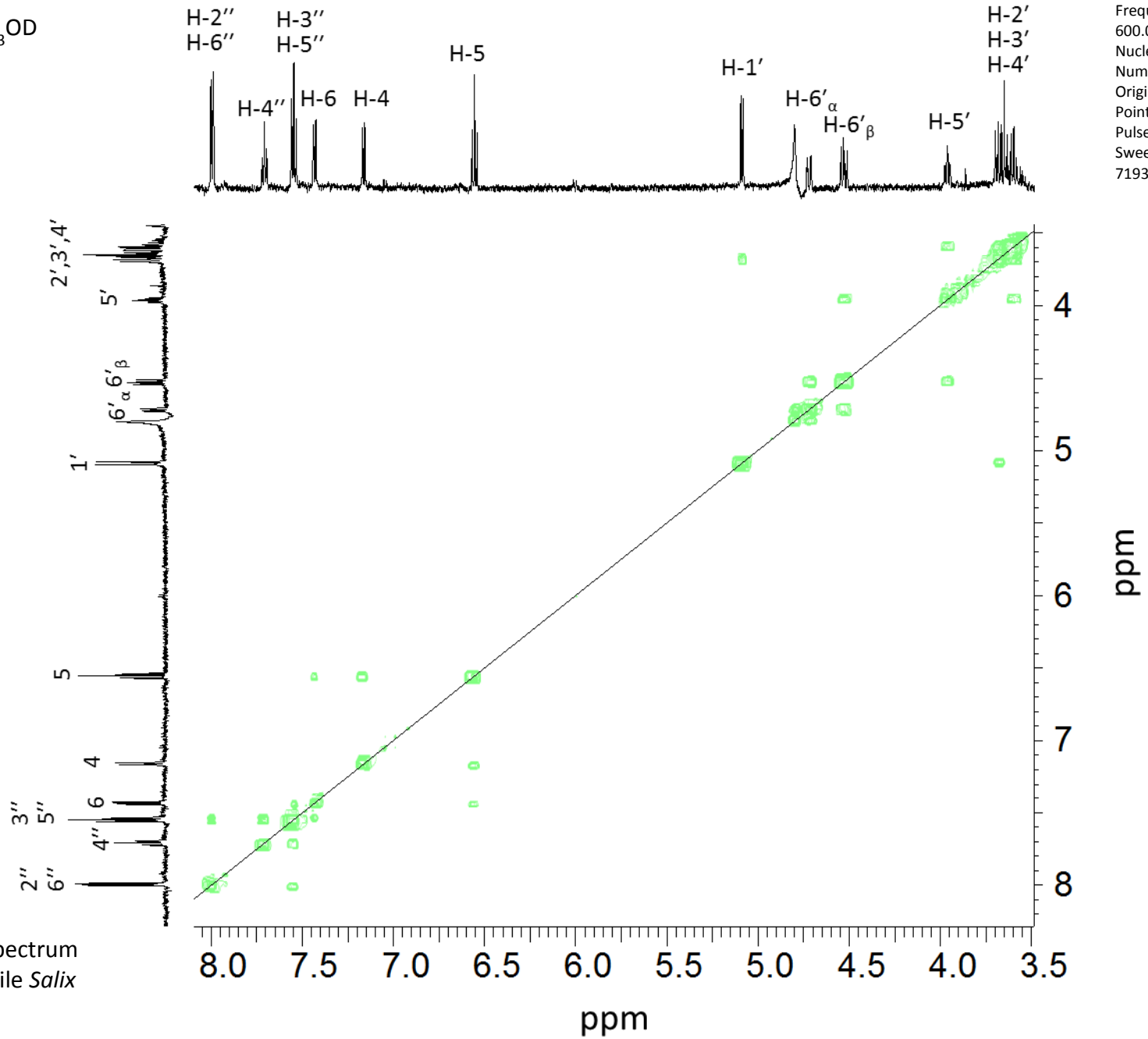


Figure S4. ^{1}H-NMR spectrum of isolated acutifoliside **5**, collected at 600 MHz.

^{1}H-^{1}H COSY spectrum of acutifoliside, **5**

600 MHz, 80:20 D_2O:CD_3OD



Frequency (MHz) (600.05, 600.05)
Nucleus (1H, 1H)
Number of Transients 32
Original Points Count (1024, 128)
Points Count (1024, 1024)
Pulse Sequence cosyprqf
Sweep Width (Hz) (7176.89, 7193.60)

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_2O:CD_3OD

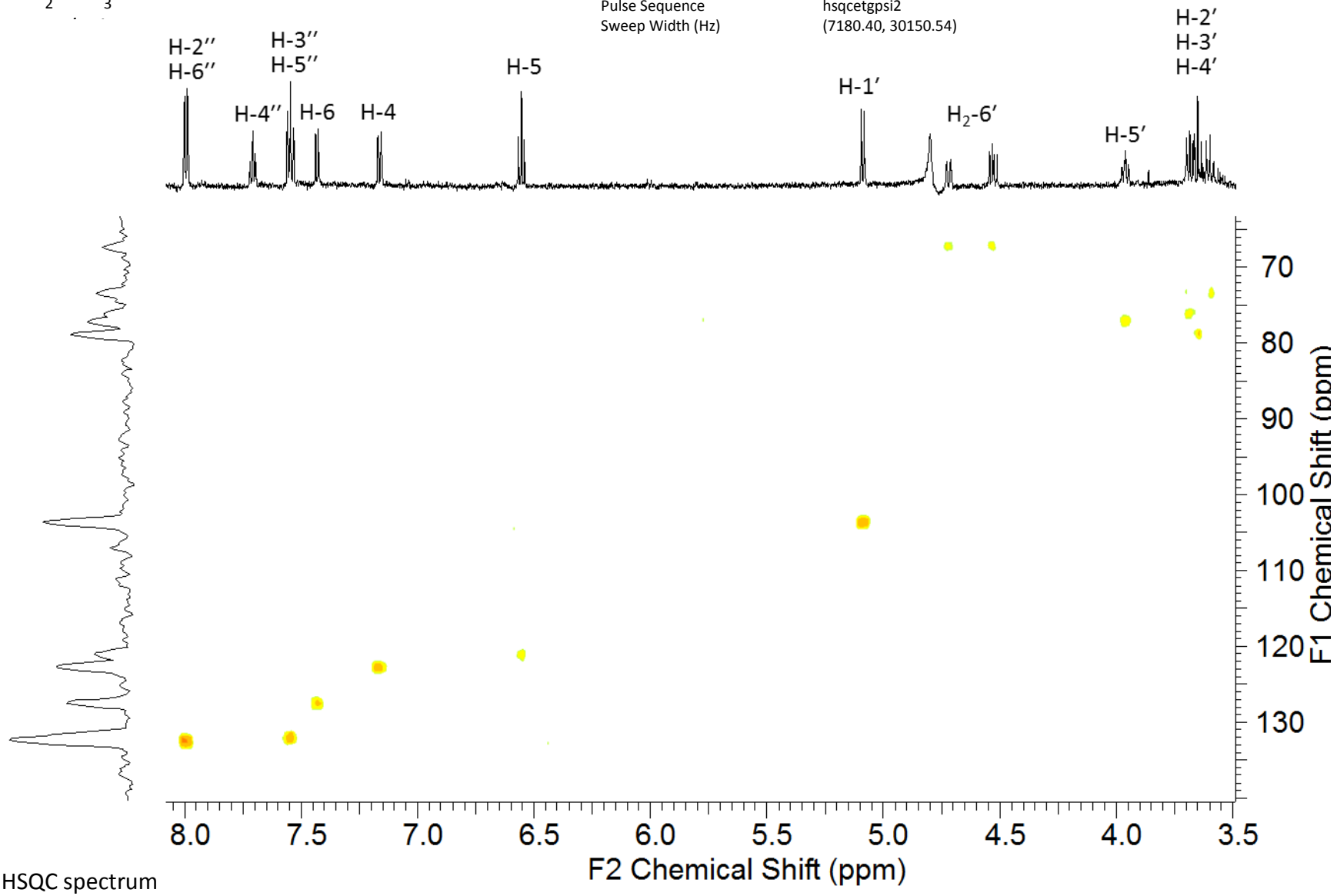


Figure S6. ^{1}H-^{13}C HSQC spectrum of polar extract of juvenile *Salix Acutifolia.*

^{1}H-^{13}C HMBC spectrum of acutifoliside, **5**

600 MHz, 80:20 D_2O:CD_3OD

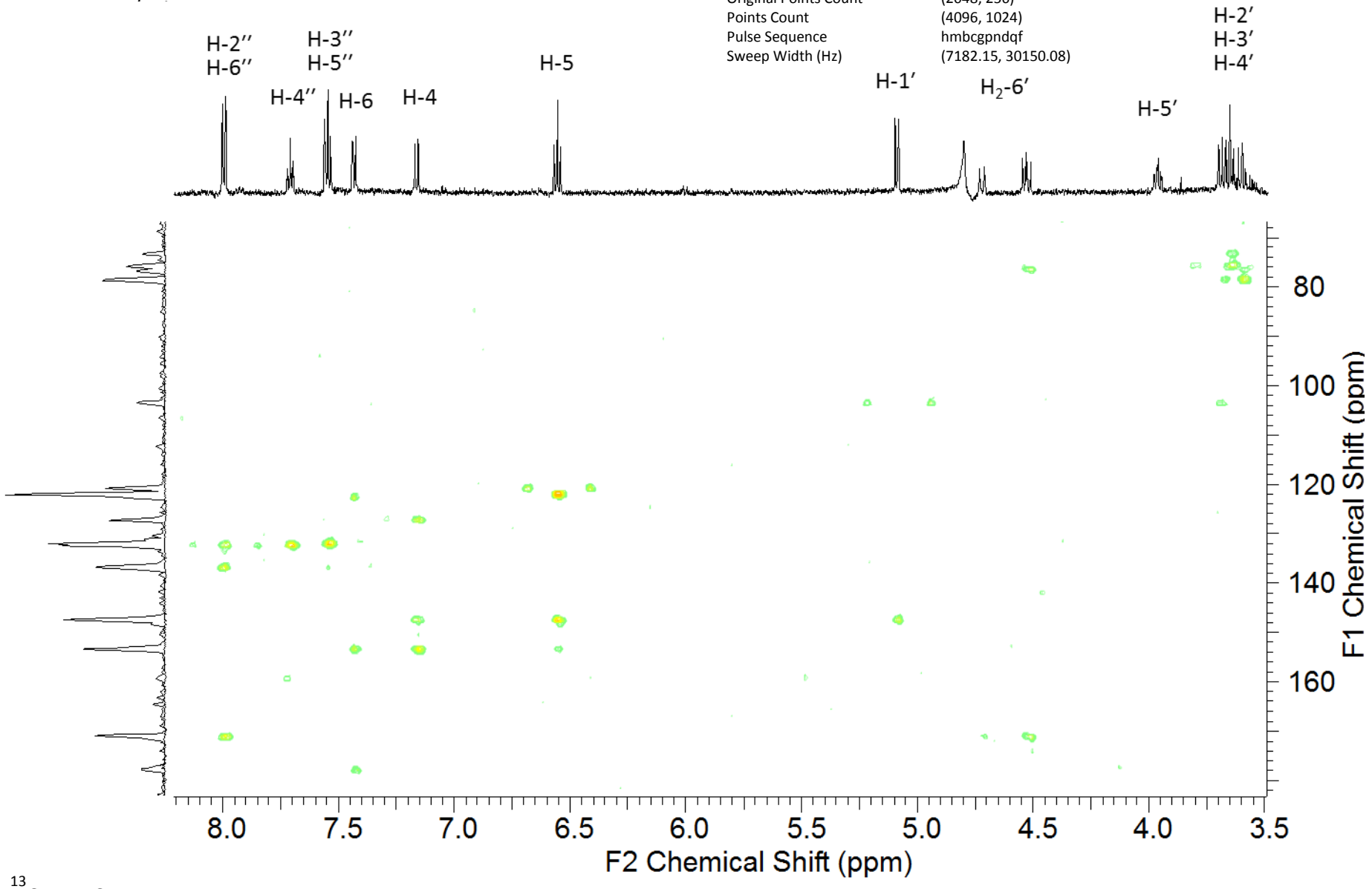
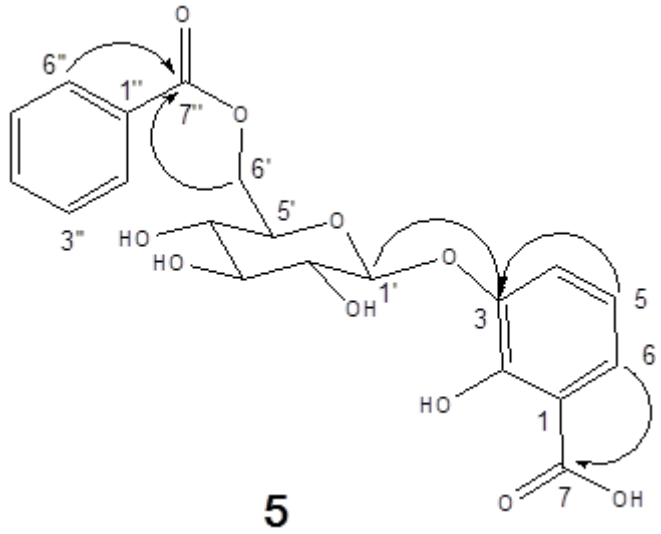


Figure S7. ^{1}H-^{13}C HMBC spectrum of polar extract of juvenile *Salix acutifolia.*



5

Figure S8. Key HMBC correlations (from H to C) of **5**.

^{1}H-NMR spectrum of acutifoliside methyl ester

600 MHz, 80:20 D_2O:CD_3OD containing 0.01 % w/v d_4-TSP

Number of integrated protons ; Assignments

Frequency (MHz)	600.05
Nucleus	1H
Number of Transients	128
Original Points Count	32768
Points Count	65536
Pulse Sequence	zgpr
Receiver Gain	1820.00
SW(cyclical) (Hz)	7183.91
Spectrum Offset (Hz)	2882.5244
Sweep Width (Hz)	7183.80

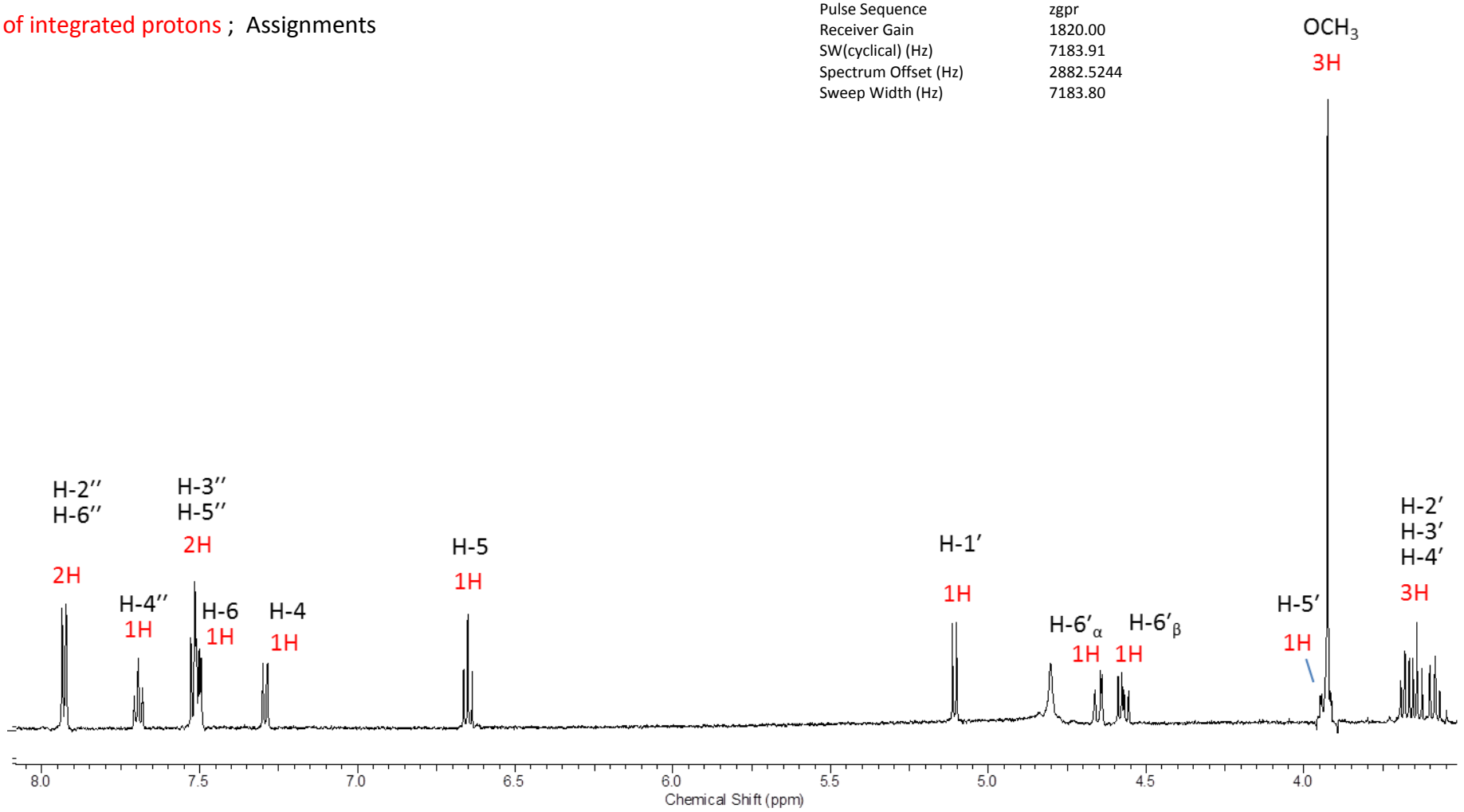


Figure S9. ^{1}H-NMR spectrum of acutifoliside methyl ester, collected at 600 MHz.

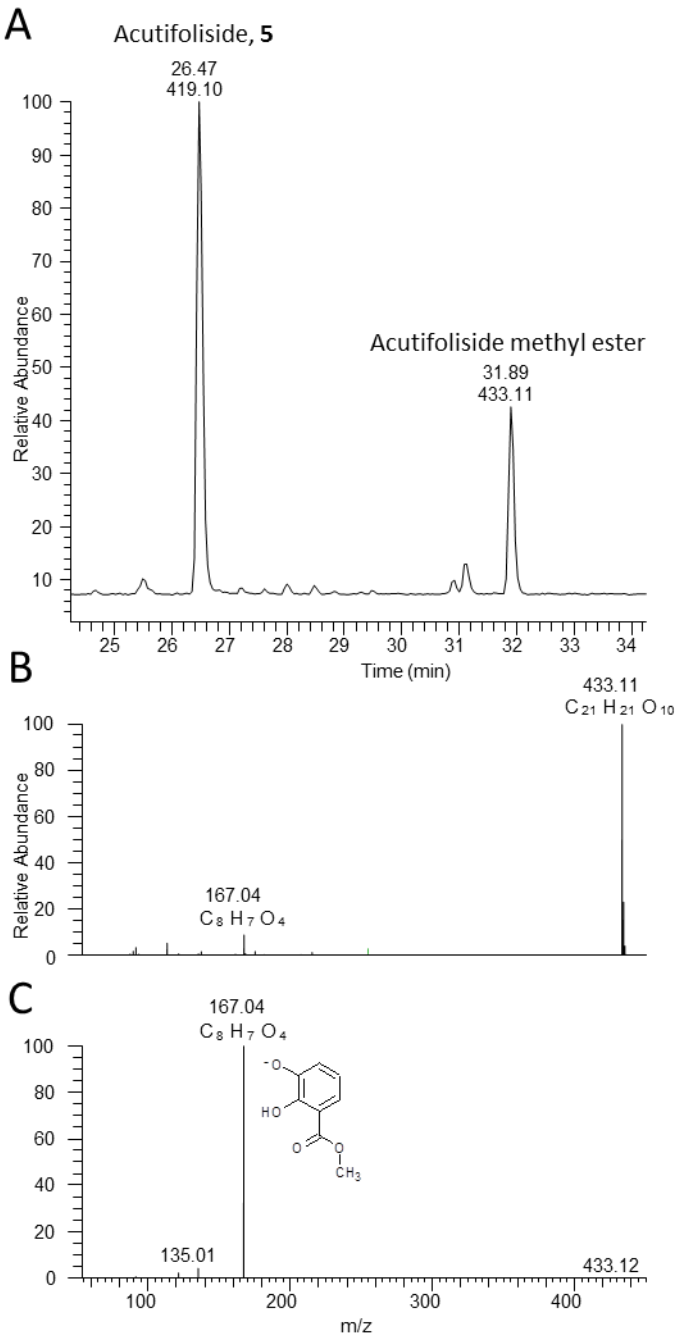
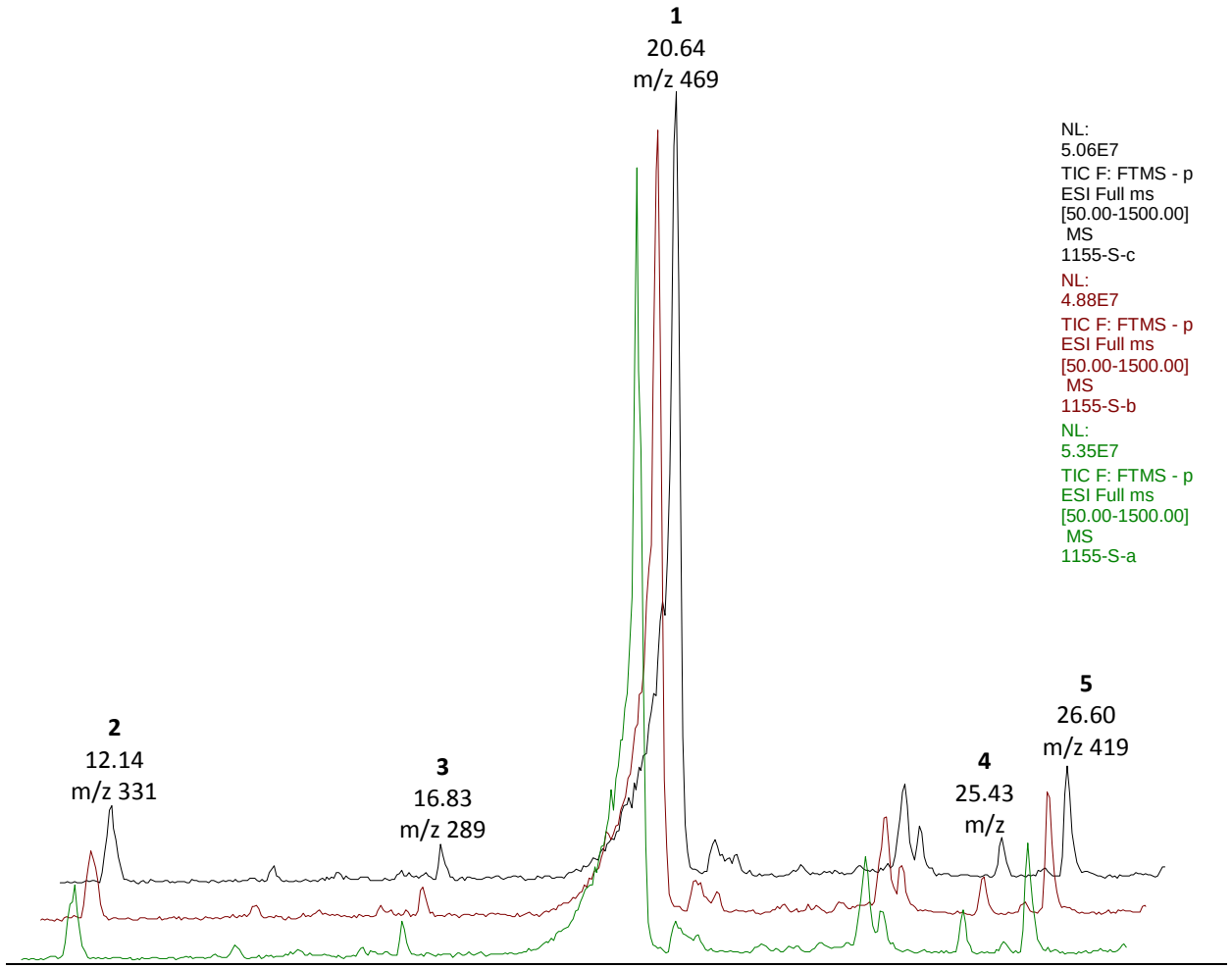


Figure S10. UHPLC-MS data from acutifoliside methyl ester. A: Extract of total ion chromatogram indicating the peak at 31.89 minutes; B: mass spectrum; C: MS^2 fragmentation of m/z 433 ion.



				Peak Areas					
Peak No	RT	m/z	Metabolite	S-1	S-2	S-3	Mean	Standard Deviation	% RSD
1	20.64	469	Salicortin [M-H+formate]-	9.00E+08	8.59E+08	9.08E+08	8.89E+08	2.61E+07	2.94
2	12.14	331	Salicin [M-H+formate]-	5.67E+07	5.42E+07	5.66E+07	5.58E+07	1.46E+06	2.62
3	16.83	289	Catechin	1.45E+07	1.34E+07	1.54E+07	1.44E+07	1.01E+06	6.99
4	23.9	435	Tremuloidin [M-H+formate]-	2.24E+07	2.29E+07	1.94E+07	2.15E+07	1.89E+06	8.77
5	26.6	419	Acutifolisode [M-H]-	6.30E+07	7.39E+07	6.45E+07	6.71E+07	5.89E+06	8.78

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