Supplementary data

Acylated quinic acids are the main salicortin metabolites in the specialist herbivore *Cerura vinula*

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A) Spectroscopic data for structure elucidation

A.1) 3-*O*-salicyloyl quinic acid (1):

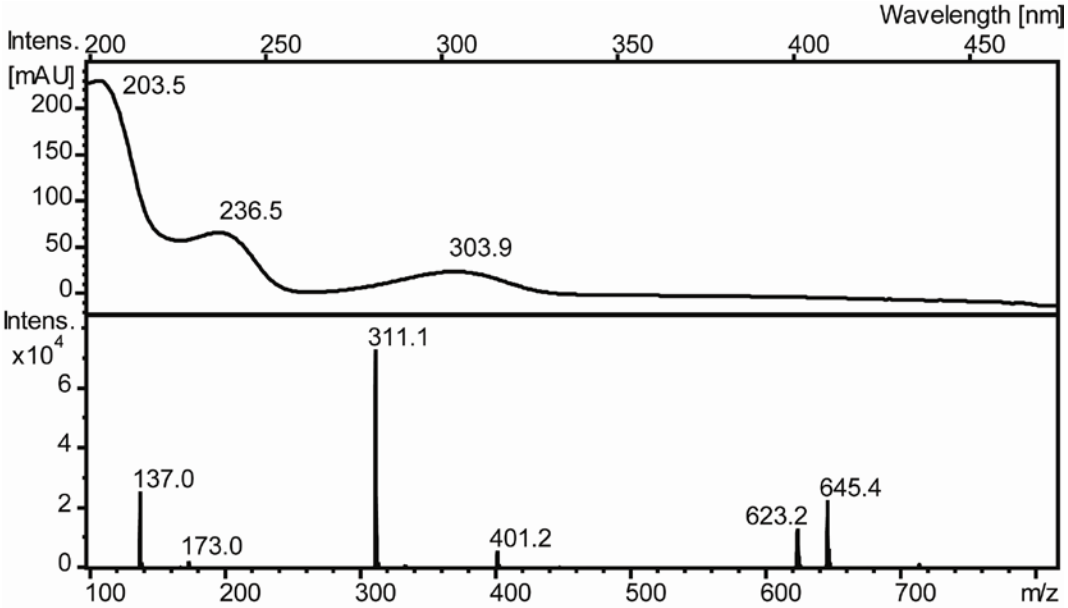


Figure A.1-1 Compound **1**, UV spectrum obtained from HPLC-DAD (top) and mass spectrum (*m/z* 311.1 $[M-H]^-$, bottom).

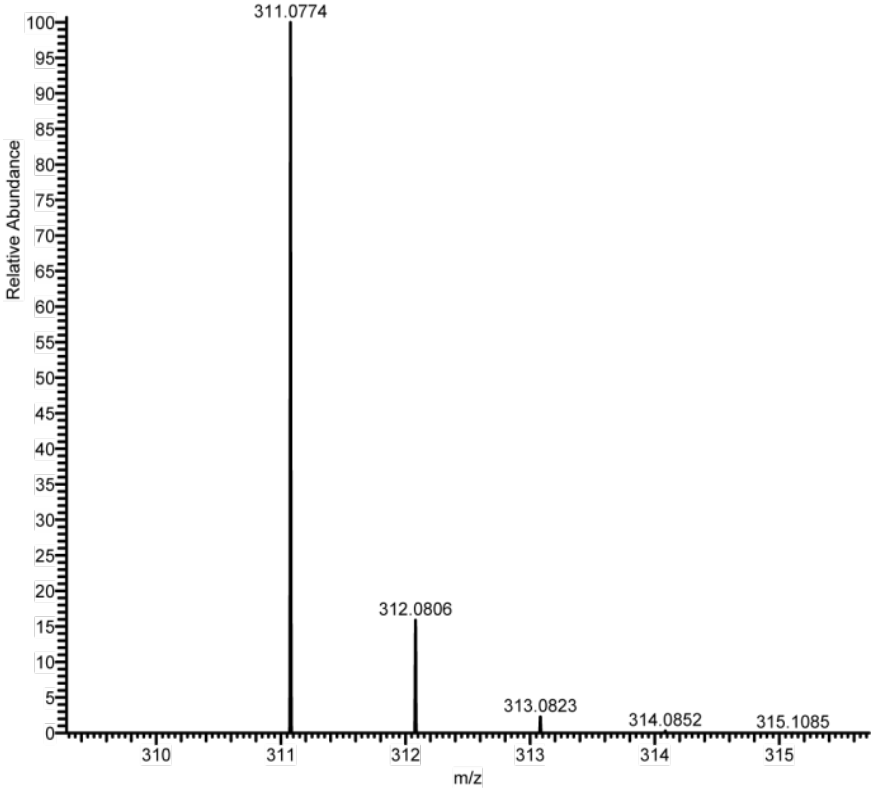


Figure A.1-2 Compound **1**, HRESIMS spectrum, *m/z* 311.0774 $[M-H]^-$.

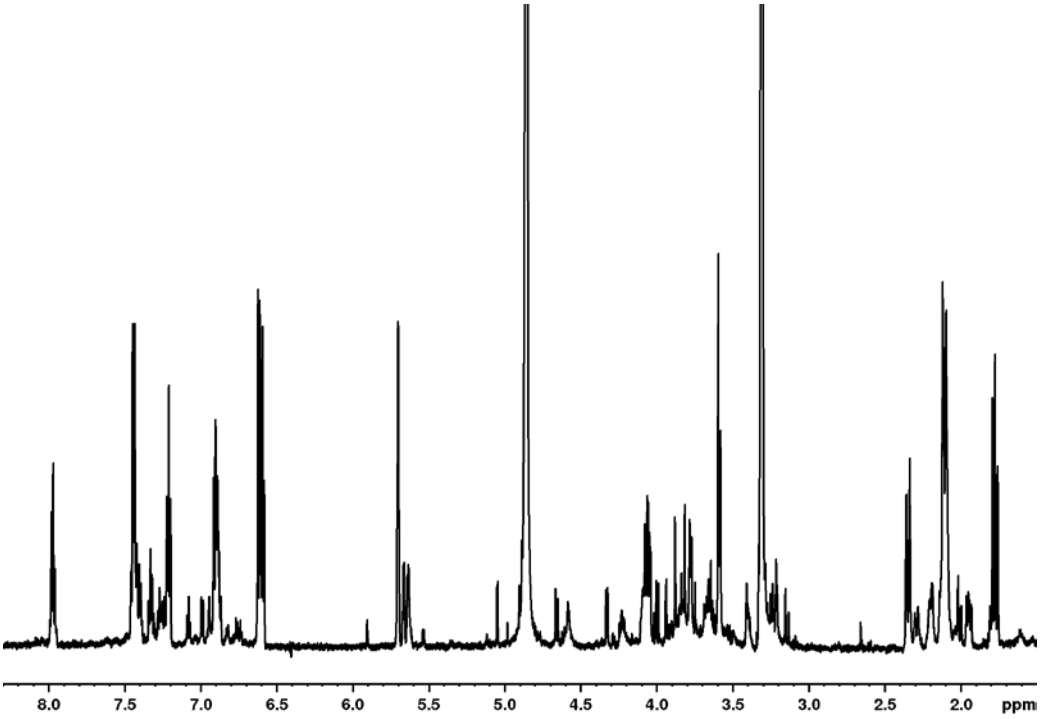


Figure A.1-3 Compound **1**, ^{1}H NMR spectrum (700 MHz, MeOH-d_4).

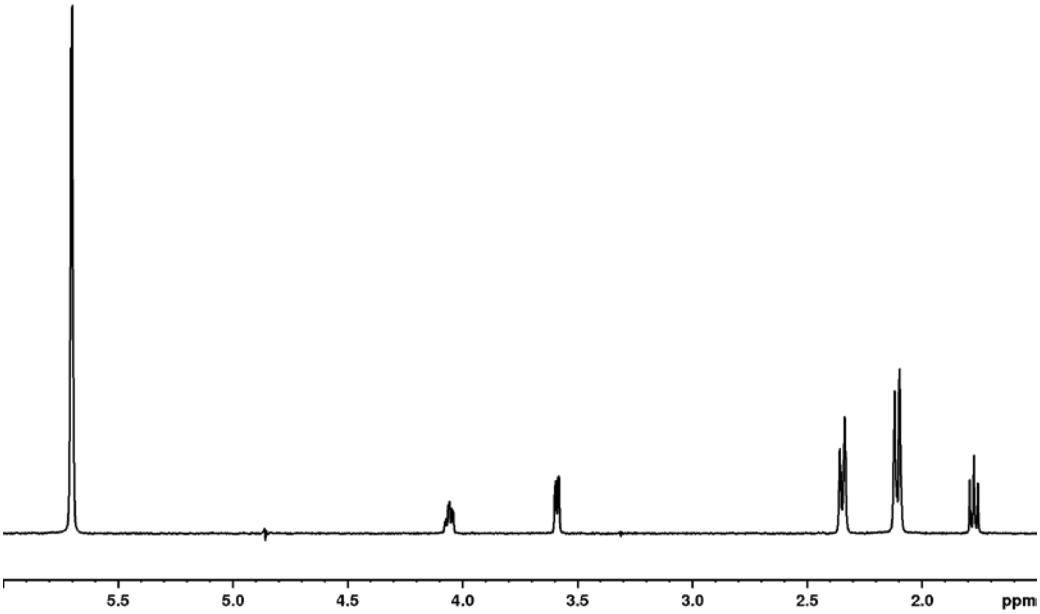


Figure A.1-4 Compound **1**, selective 1D TOCSY spectrum (700 MHz, MeOH-d_4, o1p = 5.70 ppm).

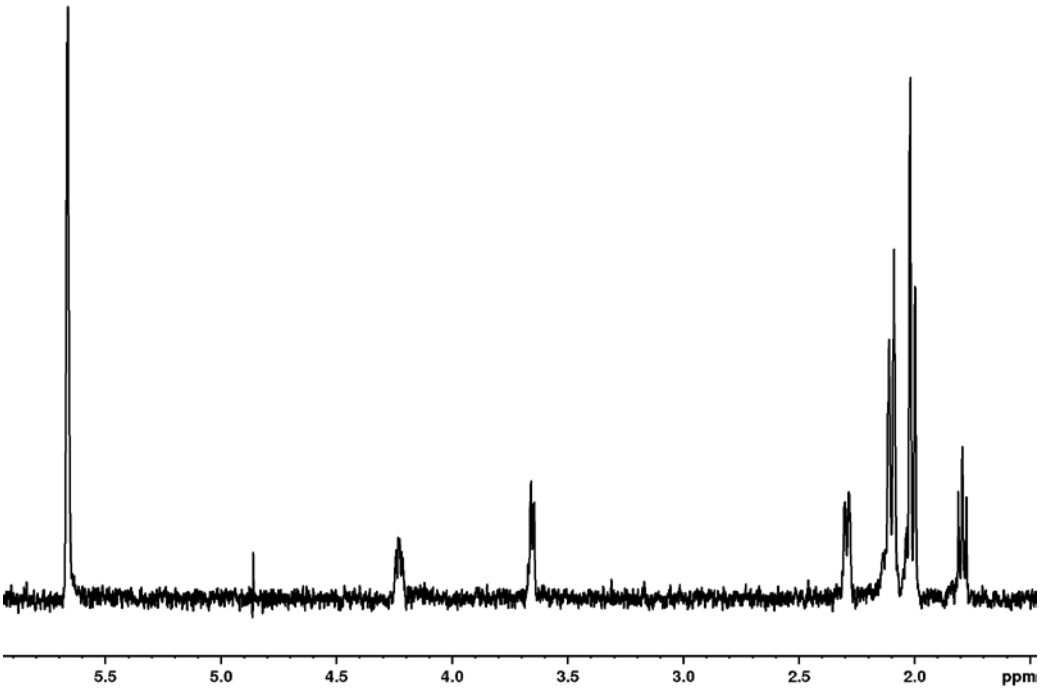


Figure A.1-5 Compound **1**, selective 1D TOCSY spectrum (700 MHz, MeOH-d_4, o1p = 5.66 ppm).

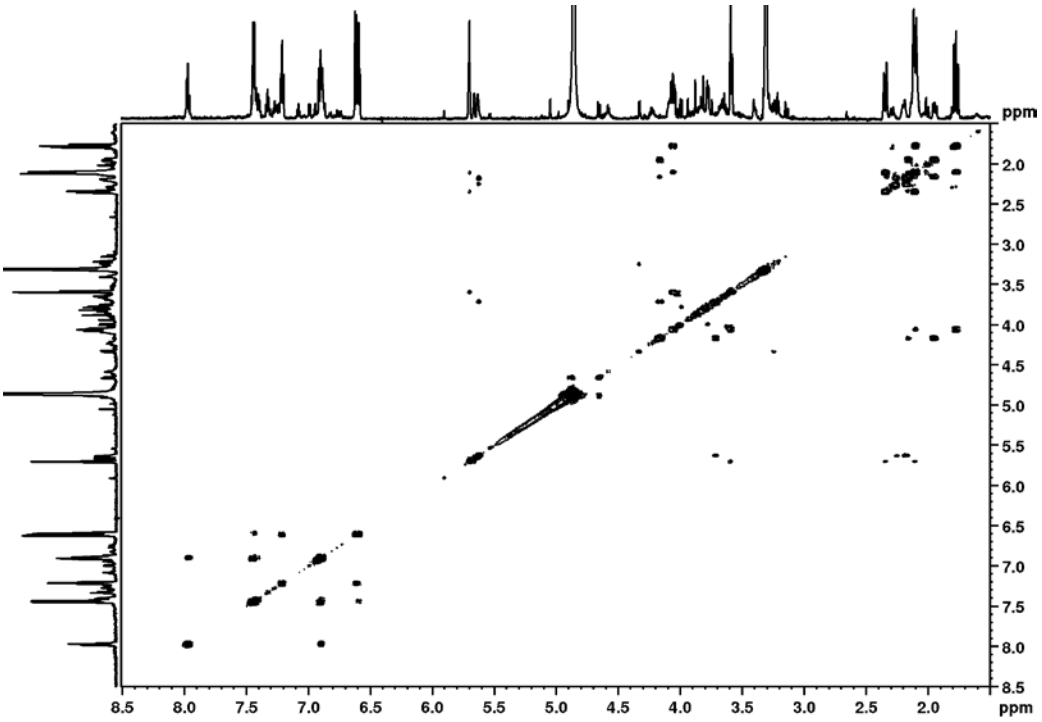


Figure A.1-6 Compound **1**, ^{1}H-^{1}H COSY spectrum (700 MHz, MeOH-d_4).

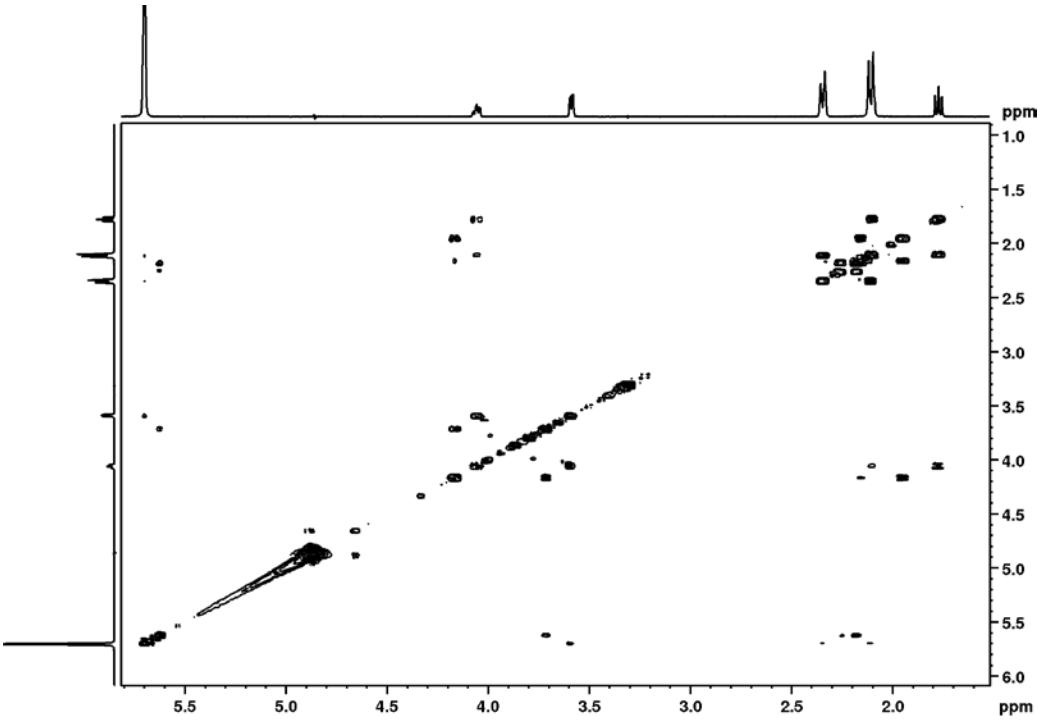


Figure A.1-7 Compound **1**, ^{1}H-^{1}H COSY spectrum (700 MHz, MeOH-d_4) with selective 1D TOCSY projections (**Fig. A.1-5**).

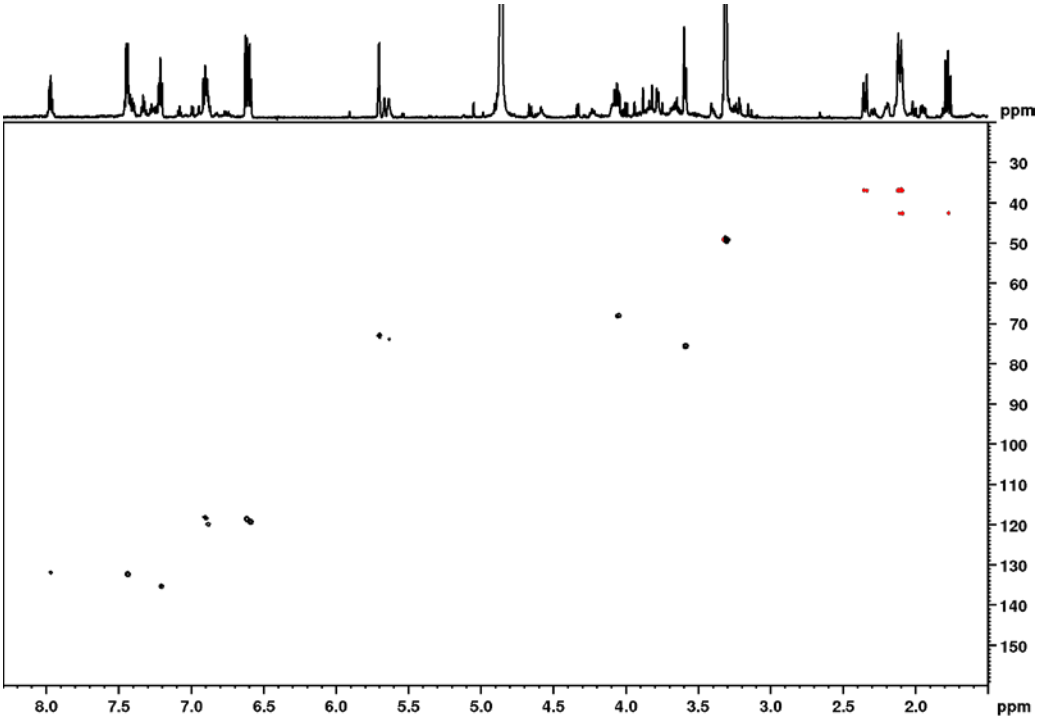


Figure A.1-8 Compound **1**, ^{1}H-^{13}C HSQC spectrum (700 MHz, MeOH-d_4).

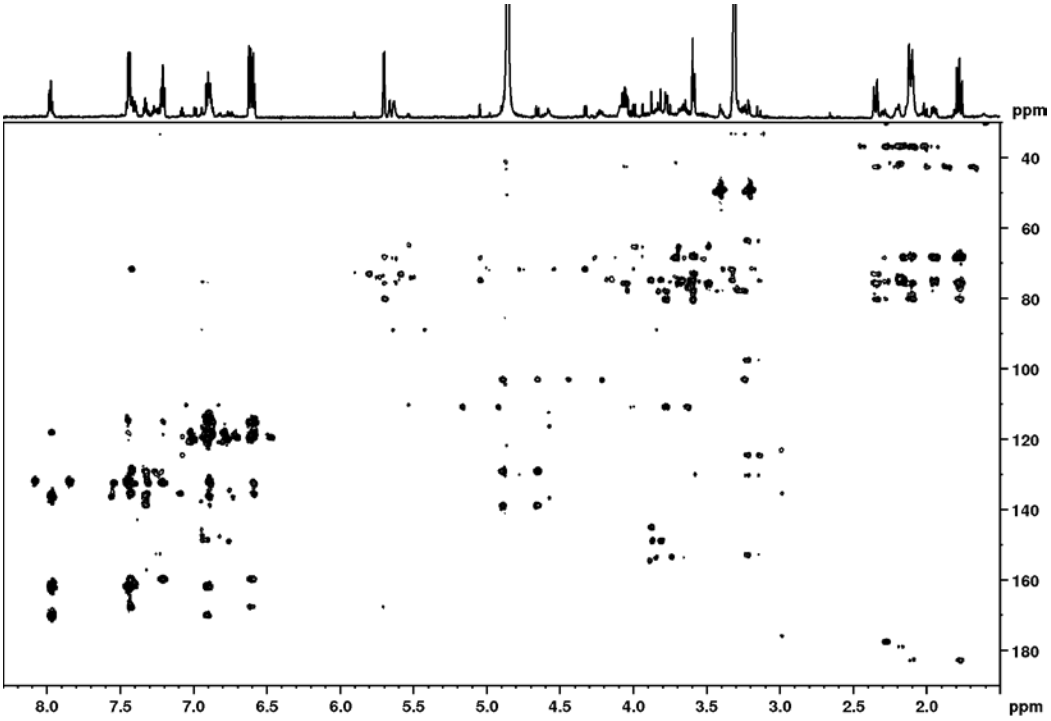


Figure A.1-9 Compound **1**, ^{1}H-^{13}C HMBC spectrum (700 MHz, MeOH-d_4).

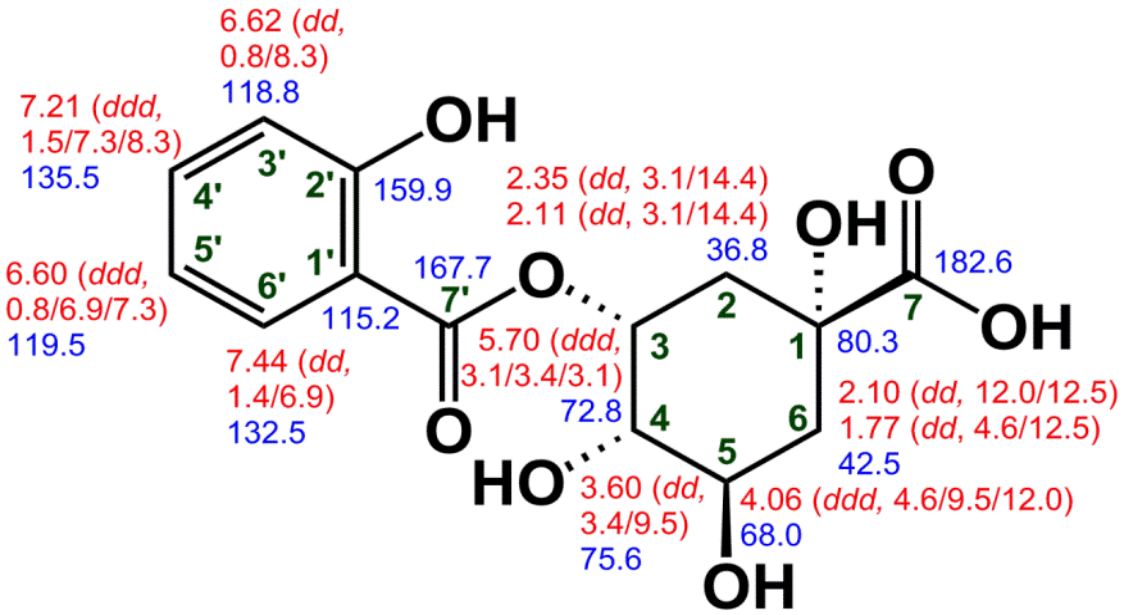


Figure A.1-10 Compound **1**, chemical structure with chemical shifts (MeOH-d_4).

A.2) 4-*O*-Salicyloyl quinic acid (2):

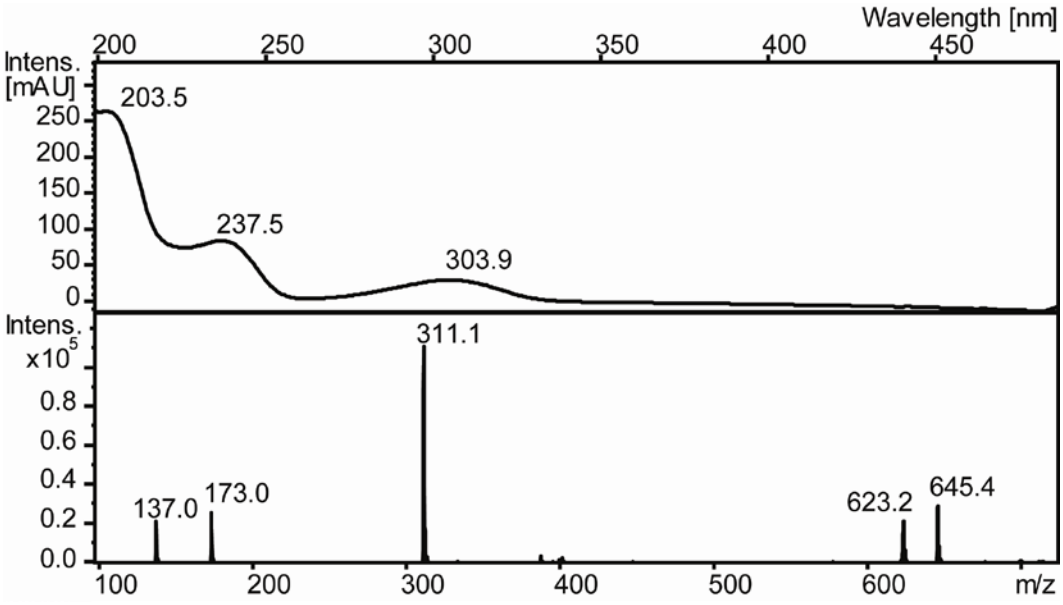


Figure A.2-1 Compound **2**, UV spectrum obtained from HPLC-DAD (top) and mass spectrum (*m/z* 311.1 $[M-H]^-$, bottom).

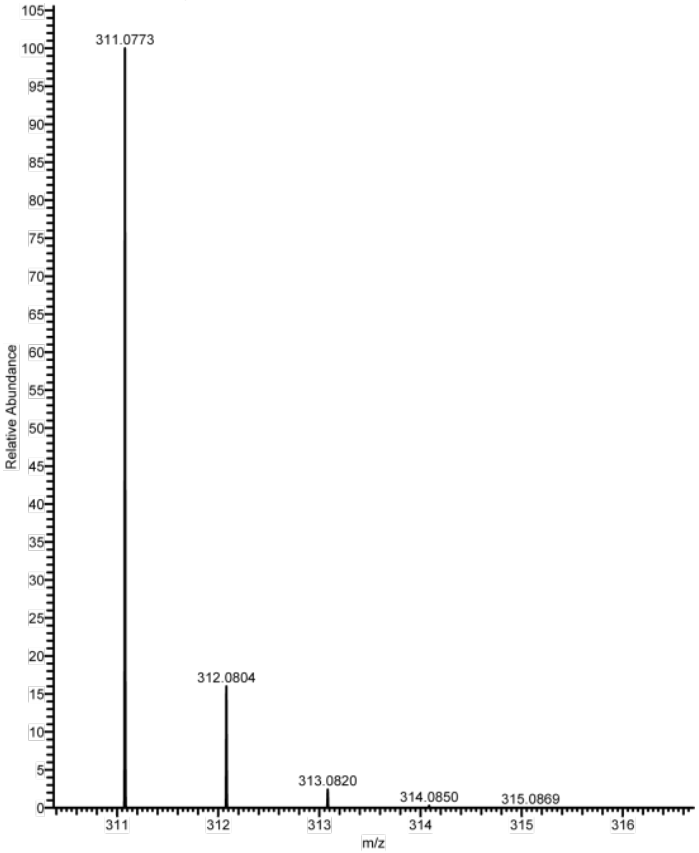


Figure A.2-2 Compound **2**, HRESIMS spectrum, *m/z* 311.0773 $[M-H]^-$.

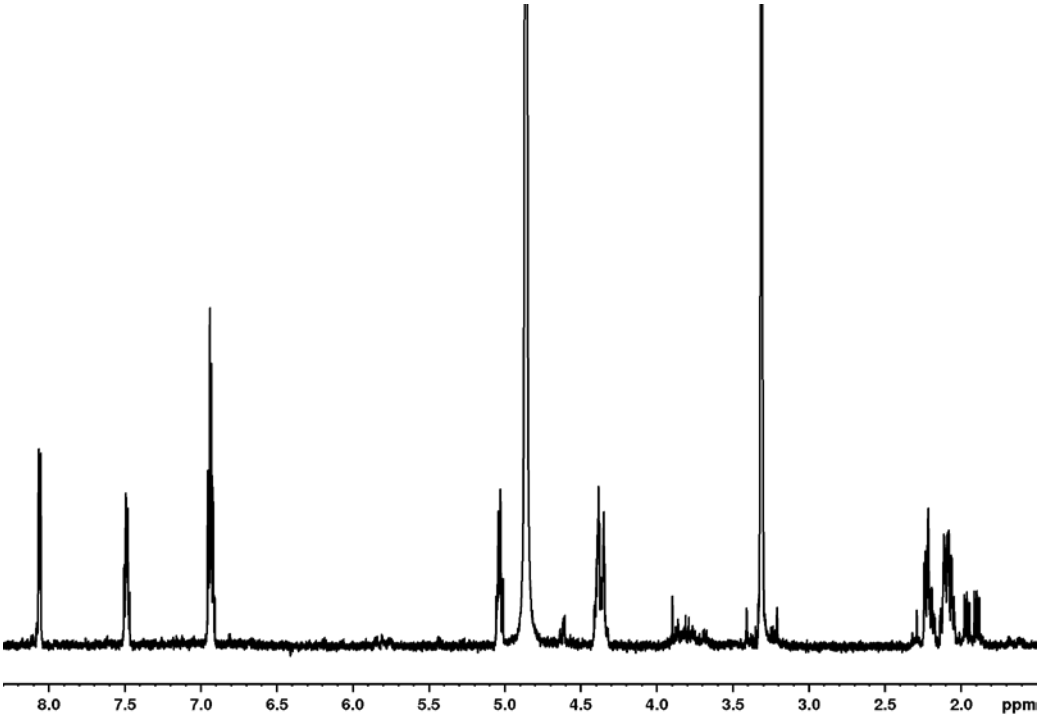


Figure A.2-3 Compound **2**, ^{1}H NMR spectrum (700 MHz, MeOH-d_4)

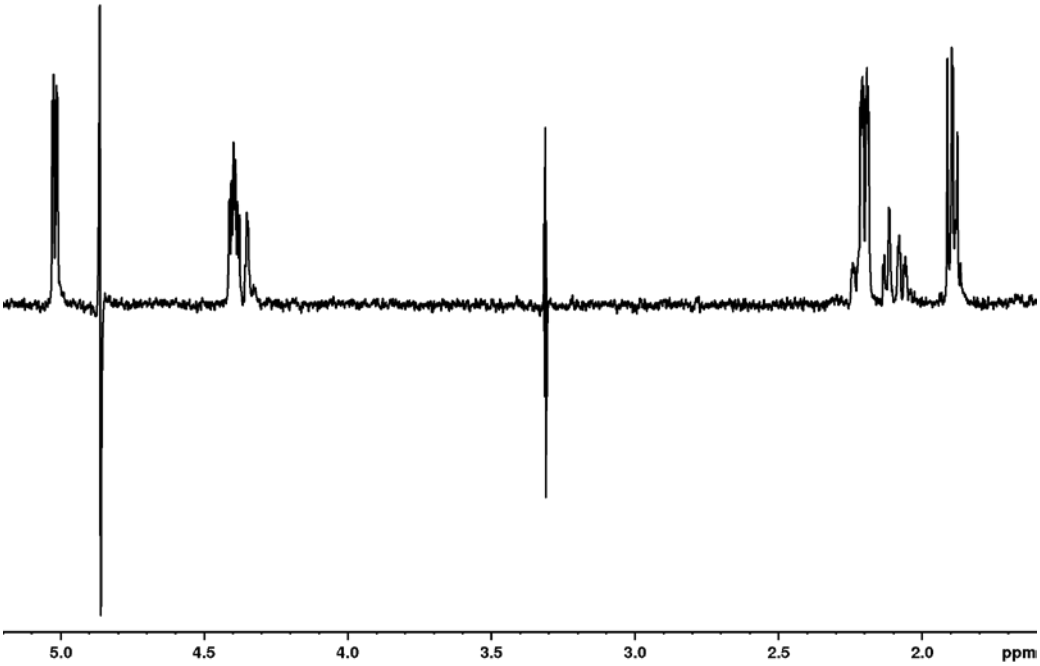


Figure A.2-4 Compound **2**, selective 1D TOCSY spectrum (700 MHz, MeOH-d_4, o1p = 1.89 ppm).

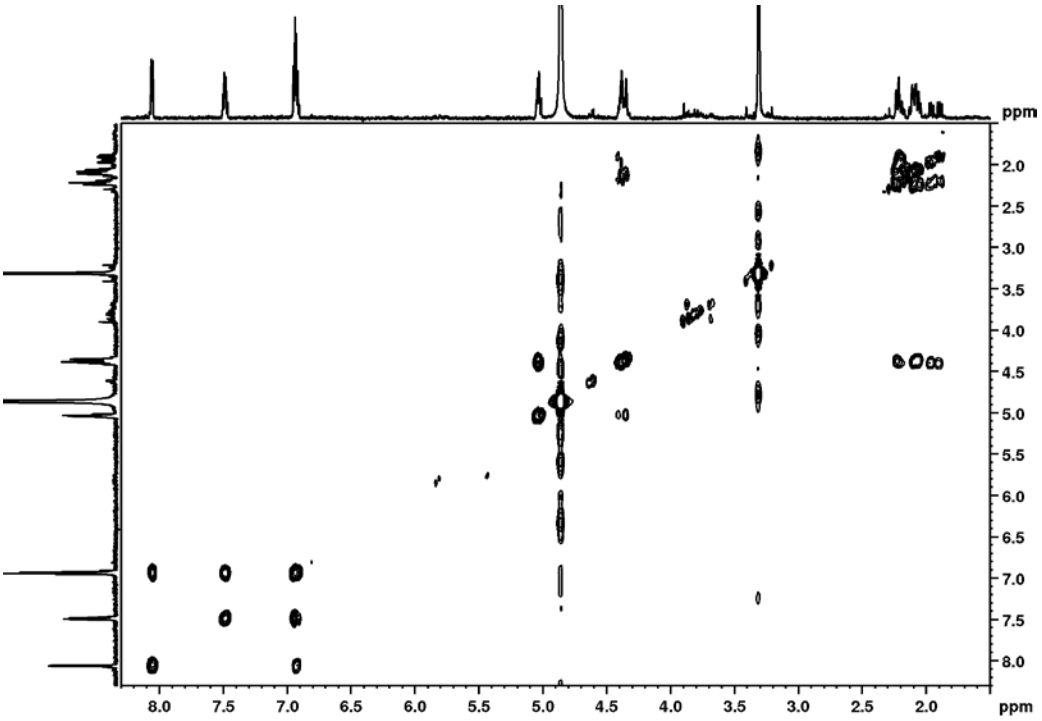


Figure A.2-5 Compound **2**, ^{1}H-^{1}H COSY spectrum (700 MHz, MeOH-d_4).

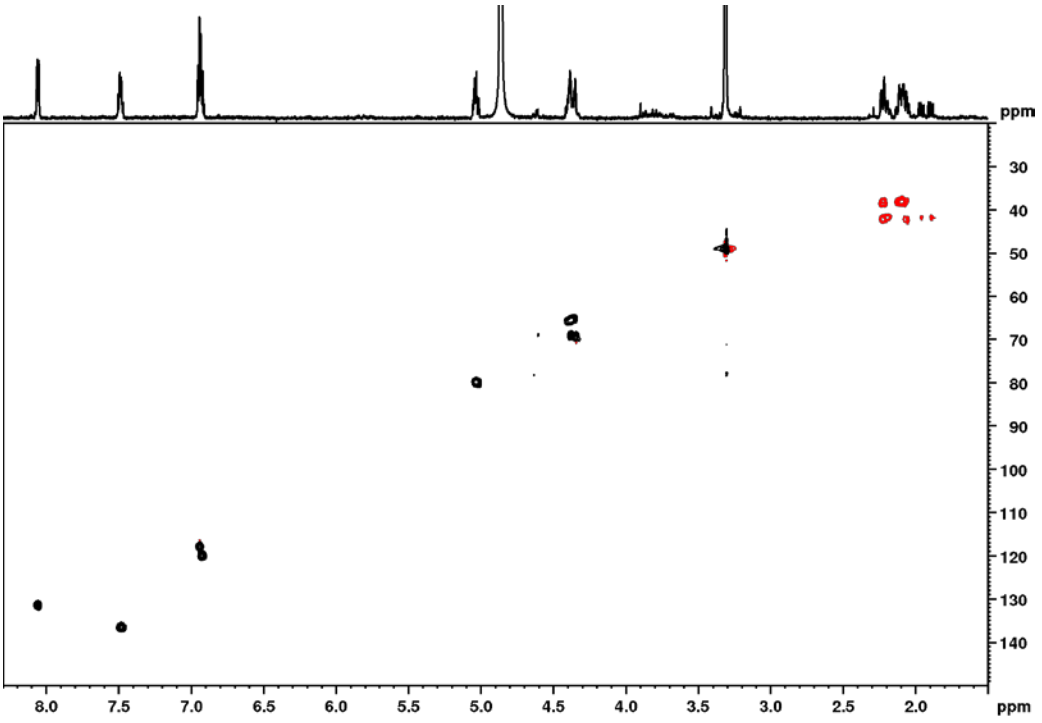


Figure A.2-6 Compound **2**, ^{1}H-^{13}C HSQC spectrum (700 MHz, MeOH-d_4).

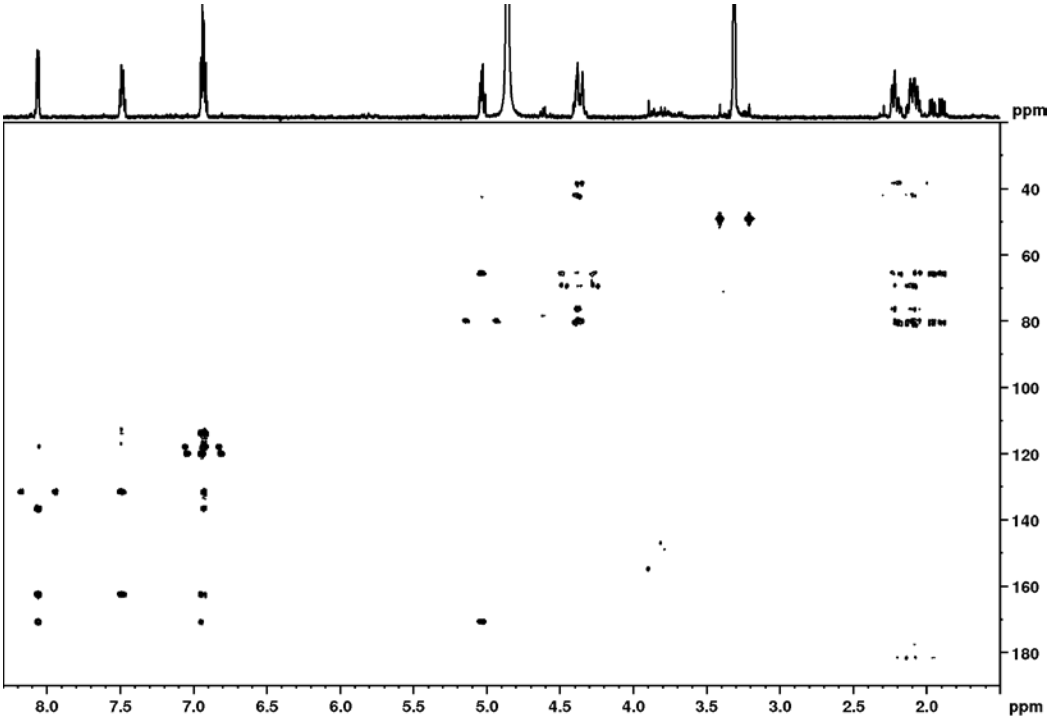


Figure A.2-7 Compound **2**, ^{1}H-^{13}C HMBC spectrum (700 MHz, MeOH-d_4).

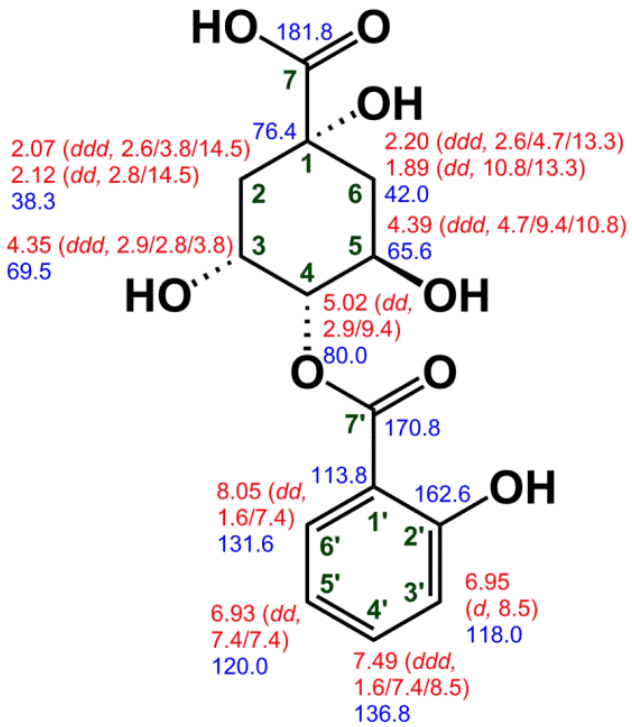


Figure A.2-8 Compound **2**, chemical structure with chemical shifts (MeOH-d_4).

A.3) 5-*O*-Salicyloyl quinic acid (3):

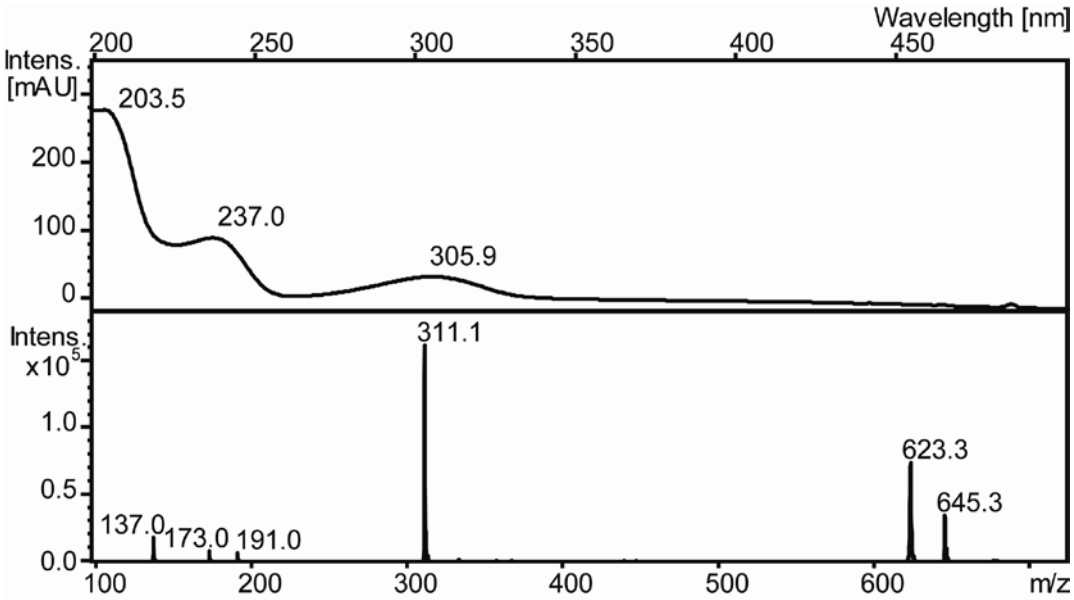


Figure A.3-1 Compound **3**, UV spectrum obtained from HPLC-DAD (top) and mass spectrum (*m/z* 311.1 $[M-H]^-$, bottom).

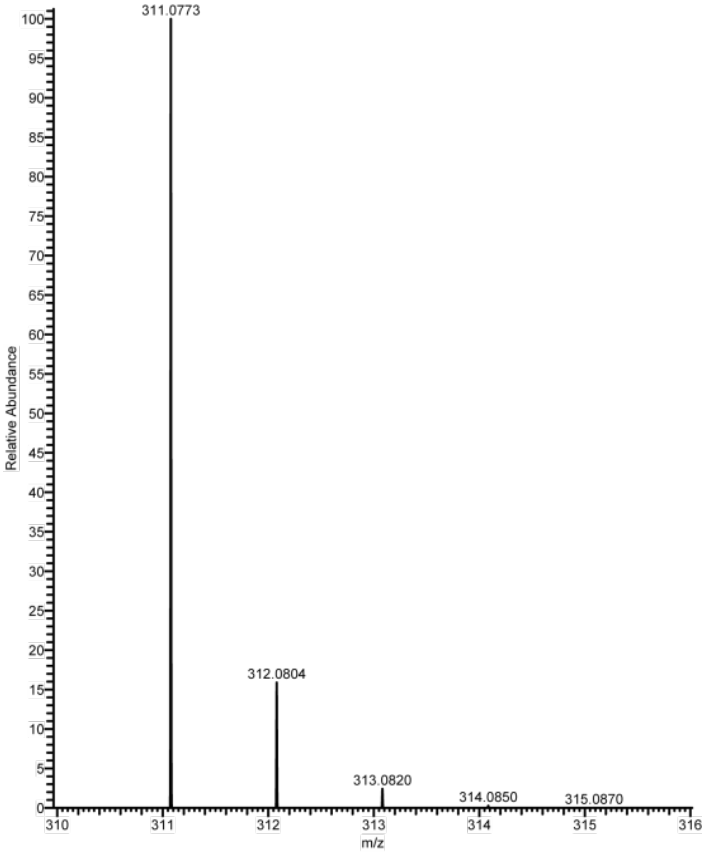


Figure A.3-2 Compound **3**, HRESIMS spectrum, *m/z* 311.0773 $[M-H]^-$.

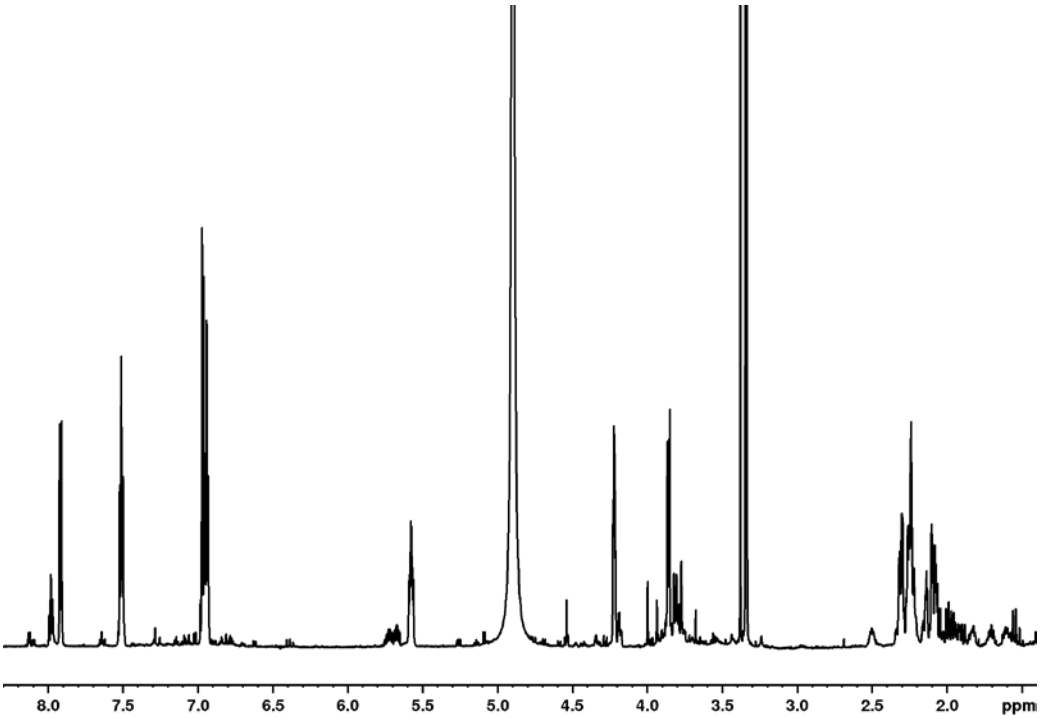


Figure A.3-3 Compound **3**, ^{1}H NMR spectrum (700 MHz, MeOH-d_4).

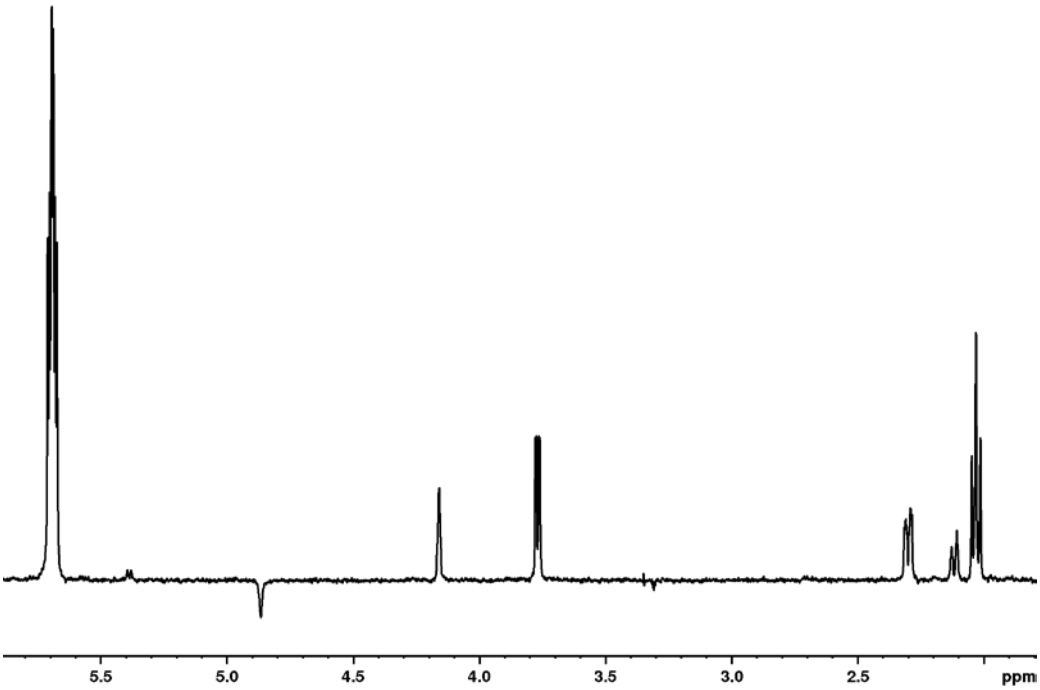


Figure A.3-4 Compound **3**, selective 1D TOCSY spectrum (700 MHz, MeOH-d_4, o1p = 5.55 ppm).

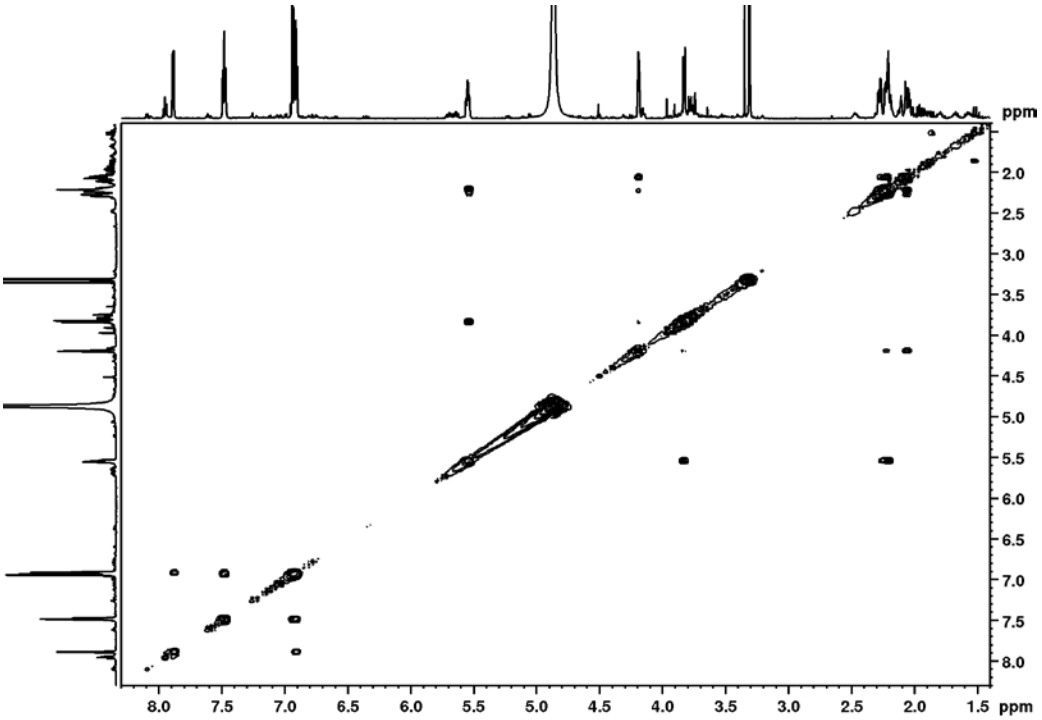


Figure A.3-5 Compound **3**, ^{1}H-^{1}H COSY spectrum (700 MHz, MeOH-d_4).

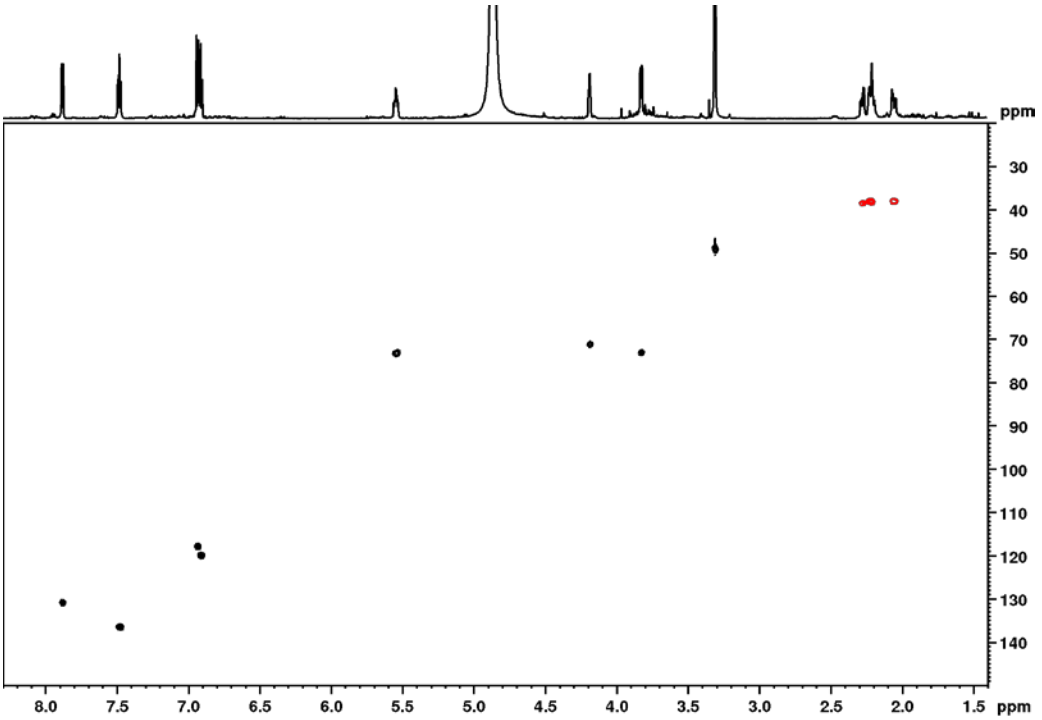


Figure A.3-6 Compound **3**, ^{1}H-^{13}C HSQC spectrum (700 MHz, MeOH-d_4).

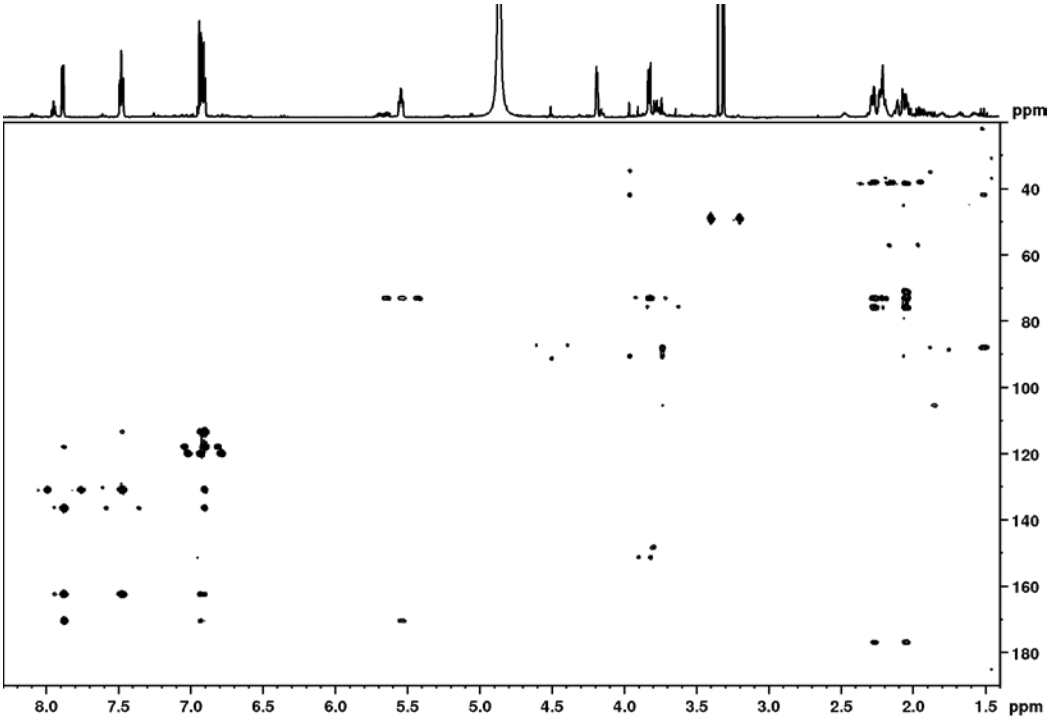


Figure A.3-7 Compound **3**, ^{1}H-^{13}C HMBC spectrum (700 MHz, MeOH-d_4).

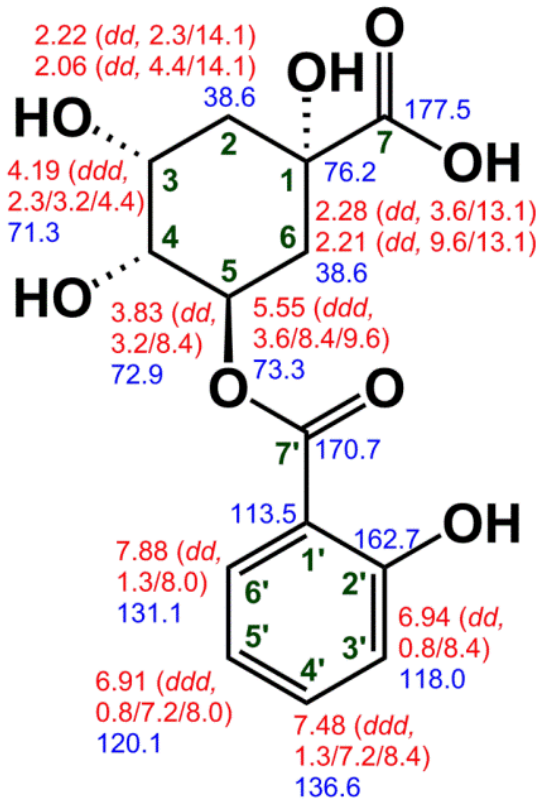


Figure A.3-8 Compound 3, chemical structure with chemical shifts (MeOH-d_4).

A.4) 4-*O*-Benzoyl quinic acid (4):

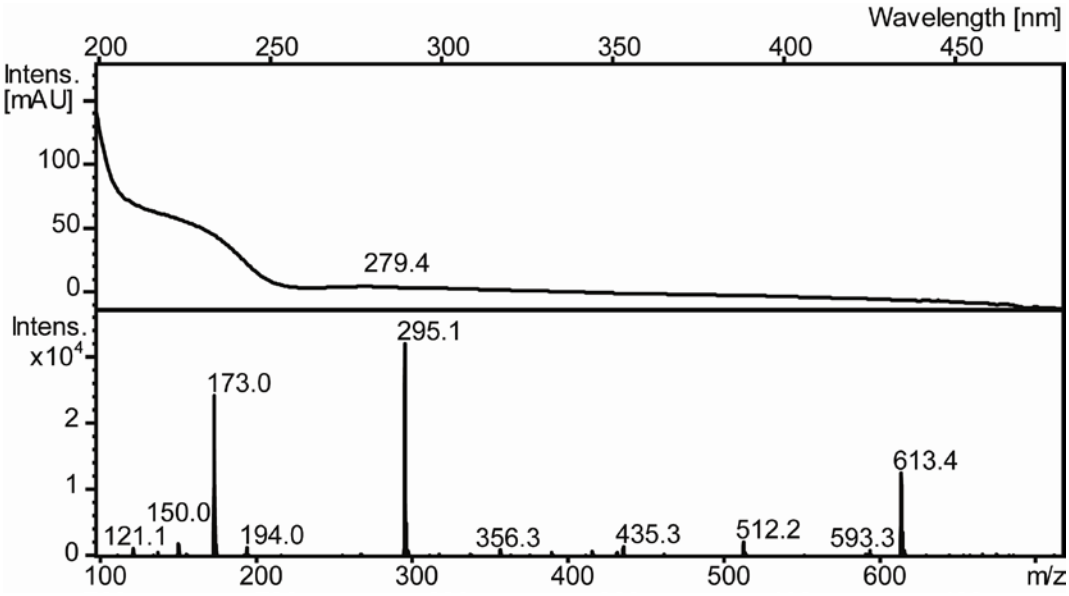


Figure A.4-1 Compound **4**, UV spectrum obtained from HPLC-DAD (top) and mass spectrum (*m/z* 295.1 $[M-H]^-$, bottom).

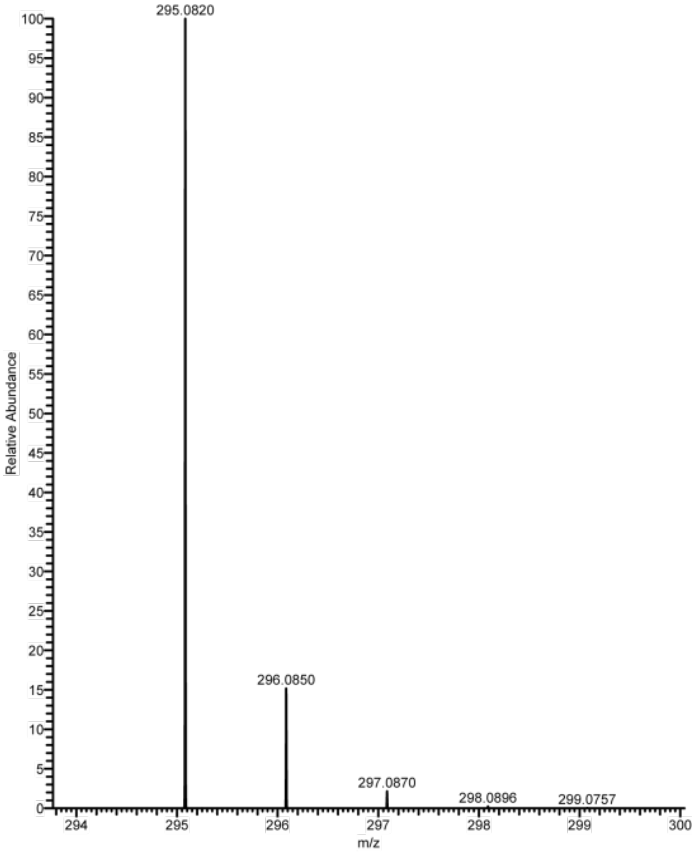


Figure A.4-2 Compound **4**, HRESIMS spectrum, *m/z* 295.0820 $[M-H]^-$.

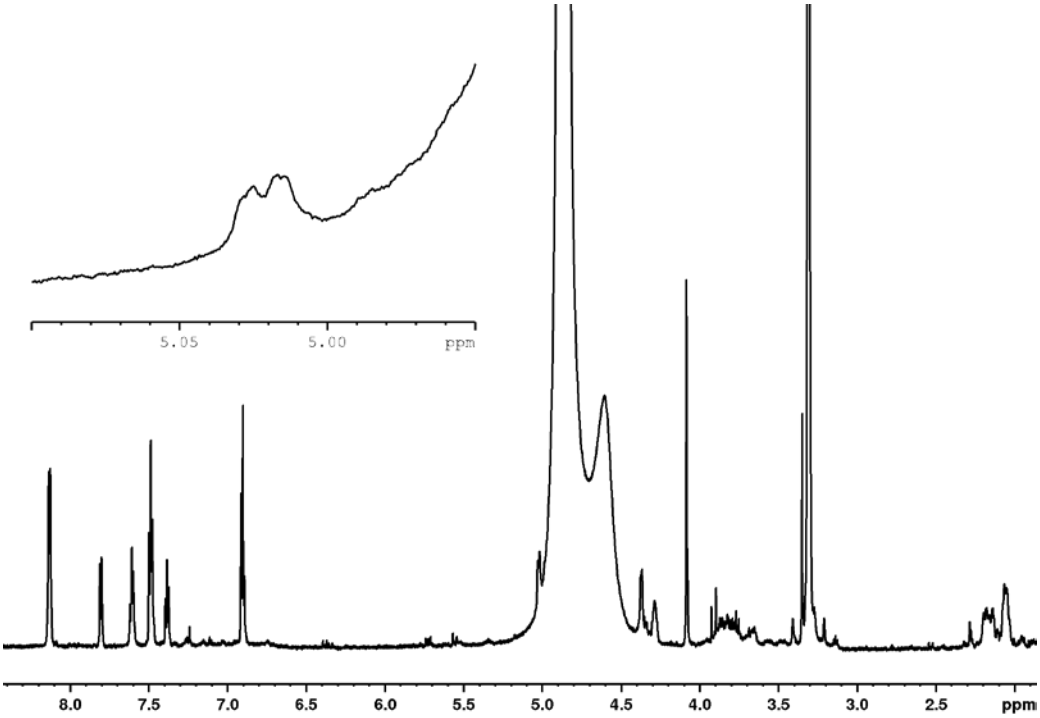


Figure A.4-3 Compound **4**, ^{1}H NMR spectrum (700 MHz, MeOH-d_4); insert: magnified signal of H-4 (δ_H 5.02).

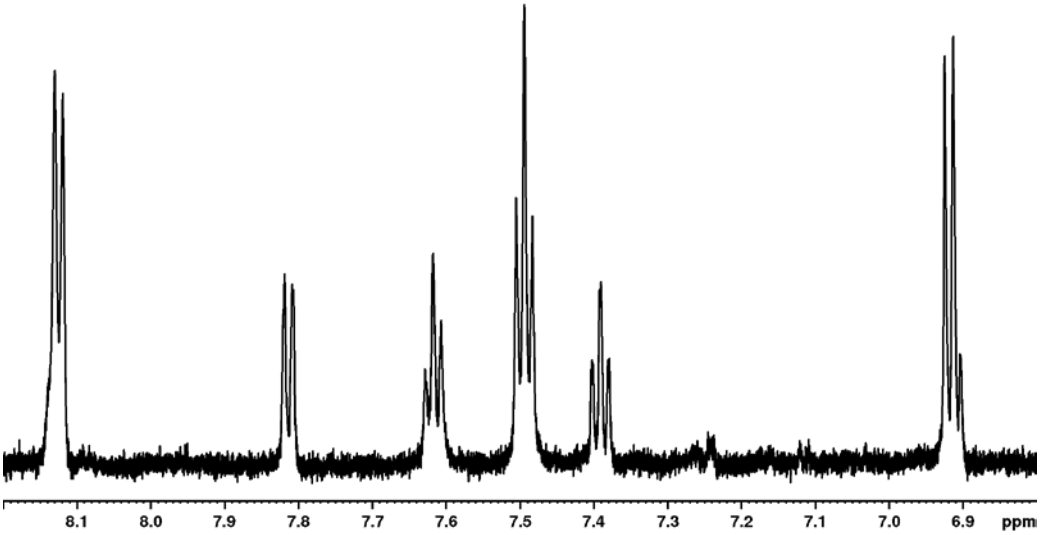


Figure A.4-4 Compound **4**, ^{1}H NMR spectrum (700 MHz, MeOH-d_4); 6.8 to 8.2 ppm.

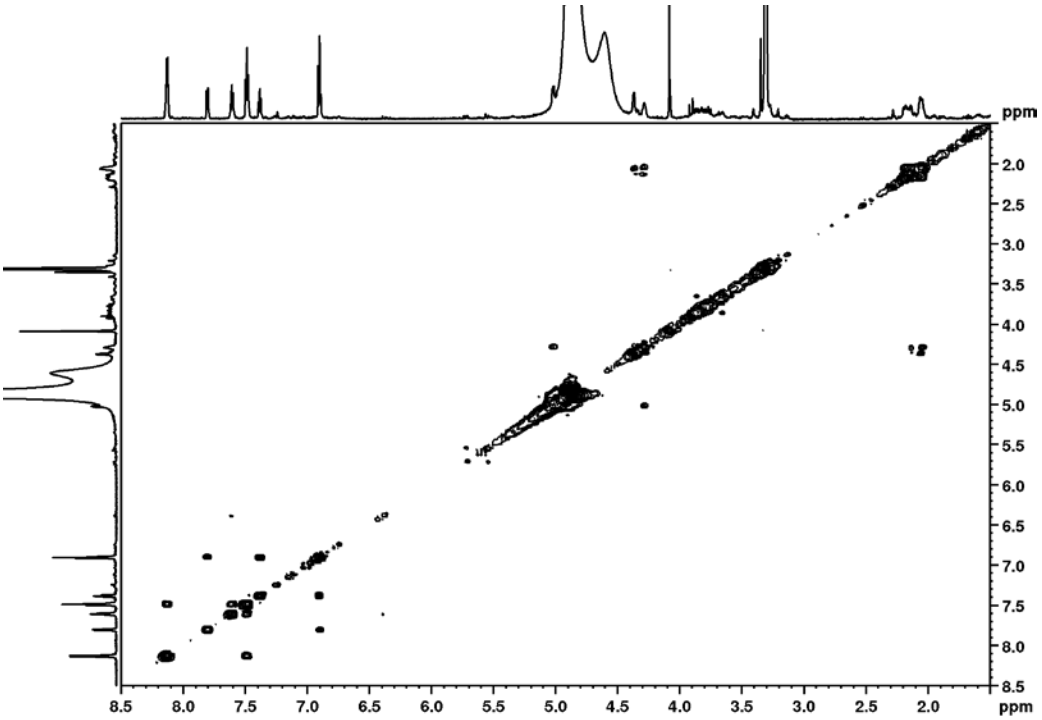


Figure A.4-5 Compound **4**, ^{1}H-^{1}H COSY spectrum (700 MHz, MeOH-d_4).

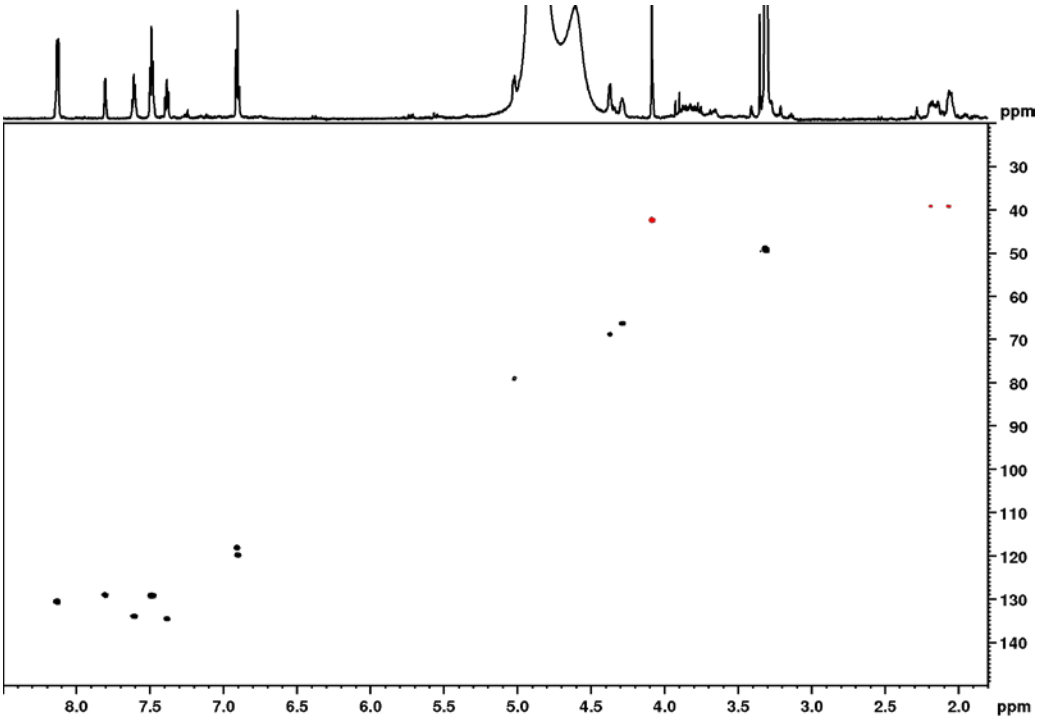


Figure A.4-6 Compound **4**, ^{1}H-^{13}C HSQC spectrum (700 MHz, MeOH-d_4).

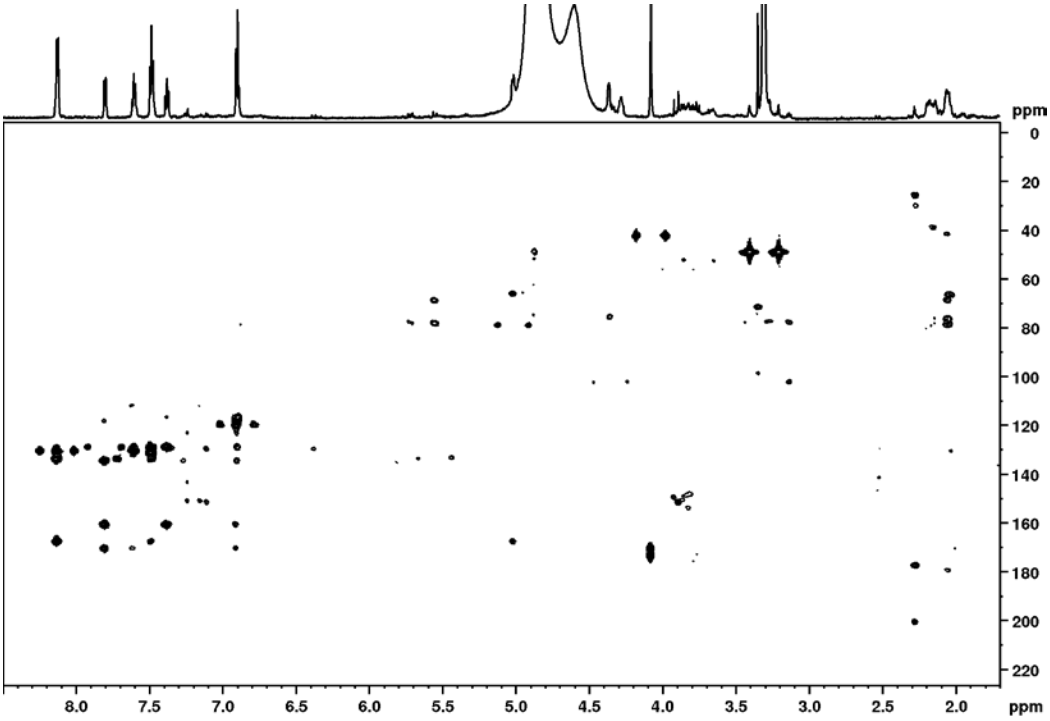


Figure A.4-7 Compound **4**, ^{1}H-^{13}C HMBC spectrum (700 MHz, MeOH-d_4).

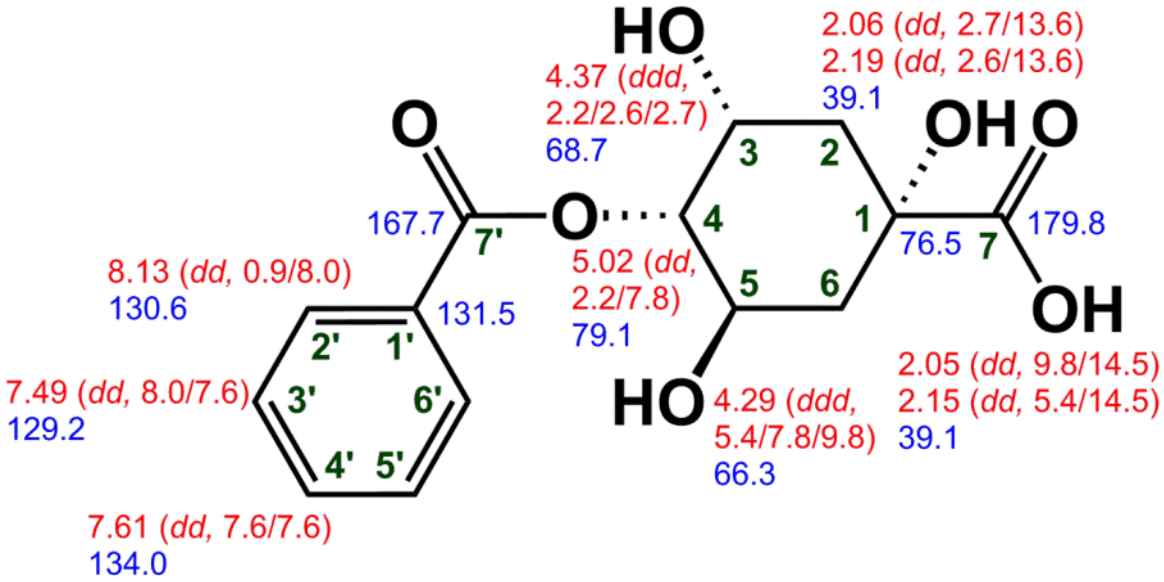


Figure A.4-8 Compound **4**, chemical structure with chemical shifts (MeOH-d_4).

A.5) 5-*O*-Benzoyl quinic acid (5):

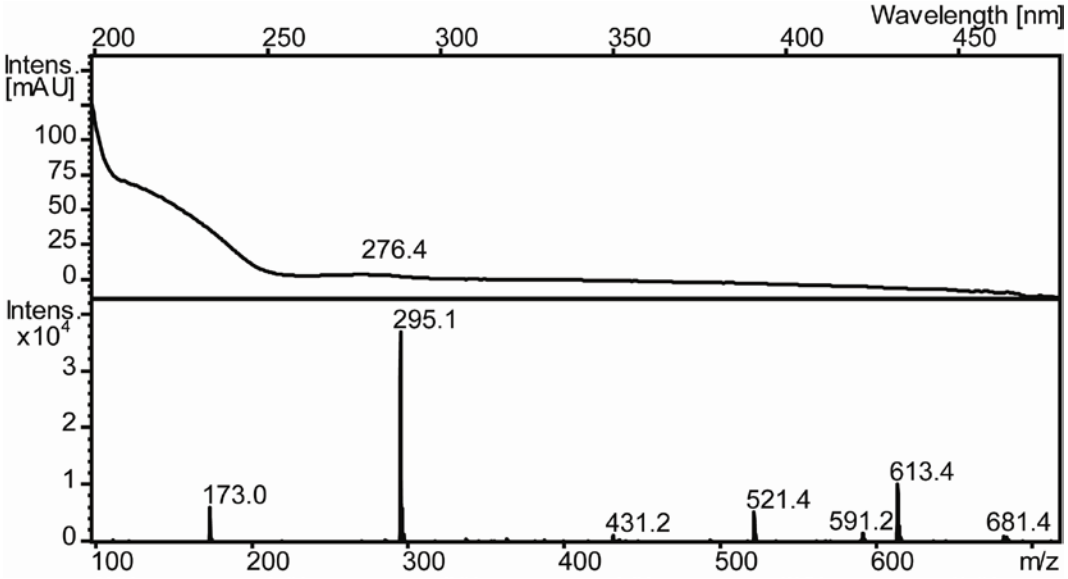


Figure A.5-1 Compound **5**, UV spectrum obtained from HPLC-DAD (top) and mass spectrum (*m/z* 295.1 $[M-H]^-$, bottom).

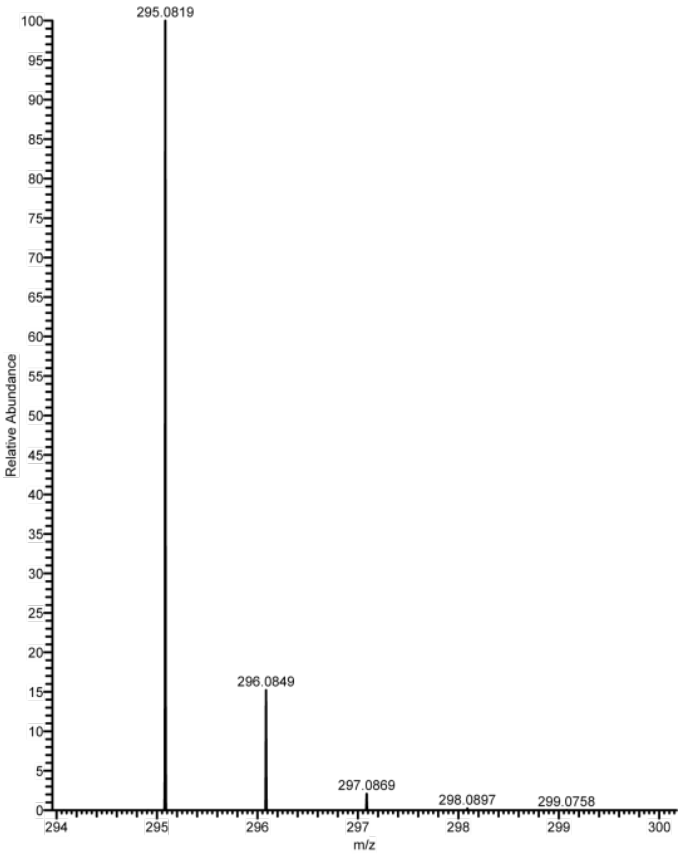


Figure A.5-2 Compound **5**, HRESIMS spectrum, *m/z* 295.0819 $[M-H]^-$.

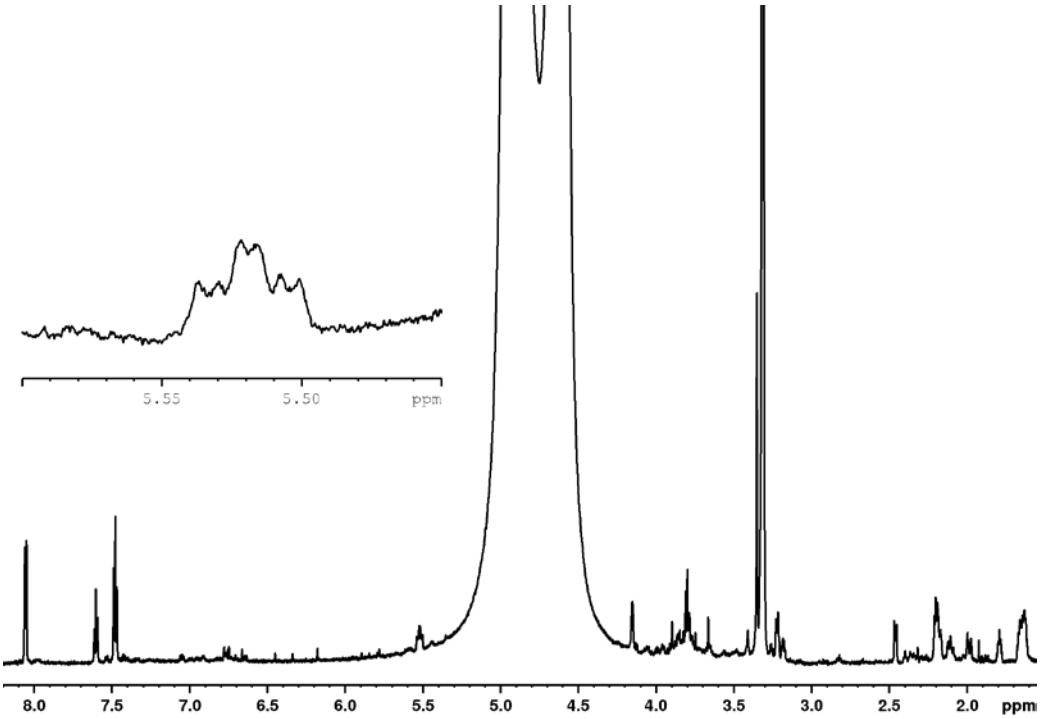


Figure A.5-3 Compound **5**, ^{1}H NMR spectrum (700 MHz, MeOH-d_4); Insert: magnified signal of H-5 (δ_H 5.52).

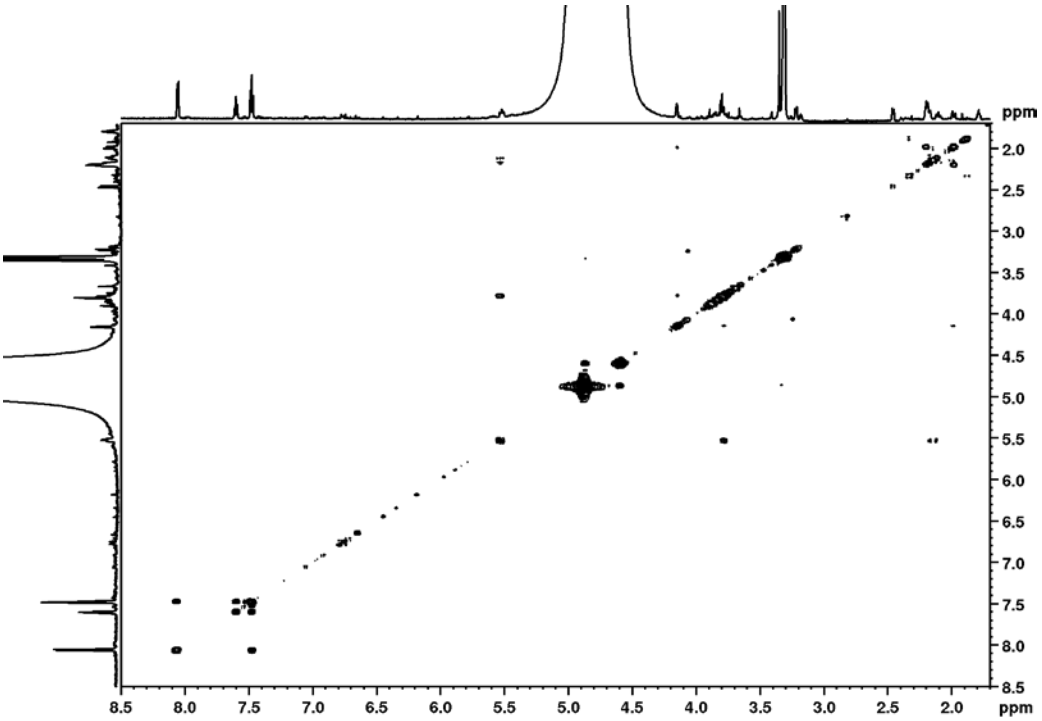


Figure A.5-4 Compound **5**, ^{1}H-^{1}H COSY spectrum (700 MHz, MeOH-d_4).

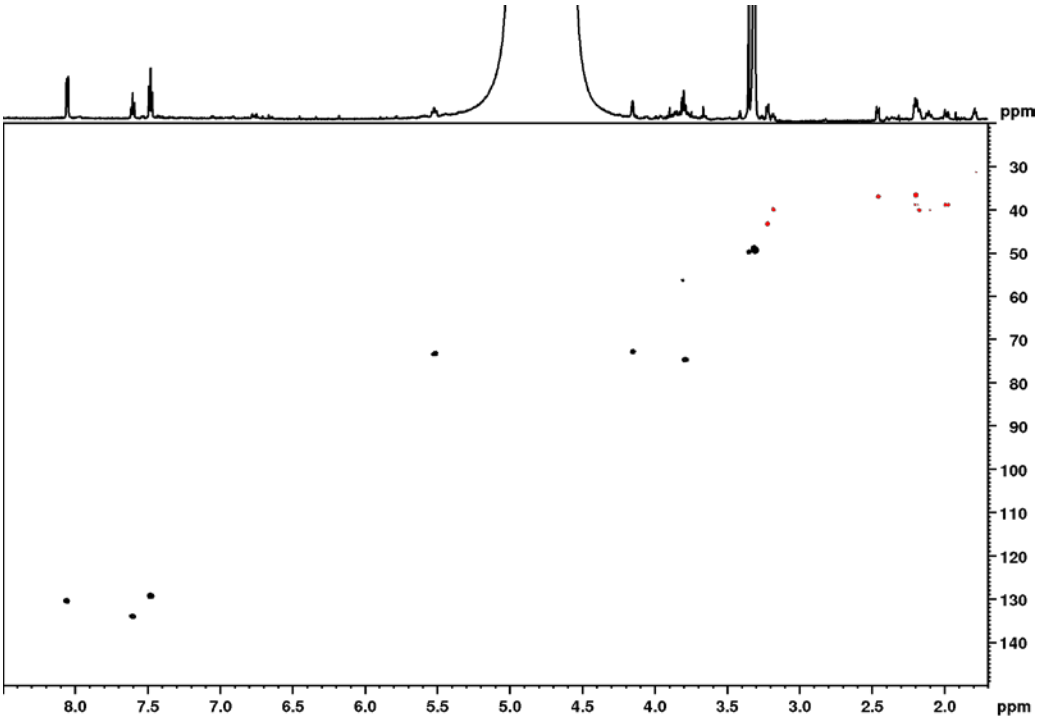


Figure A.5-5 Compound **5**, ^{1}H-^{13}C HSQC spectrum (700 MHz, MeOH-d_4).

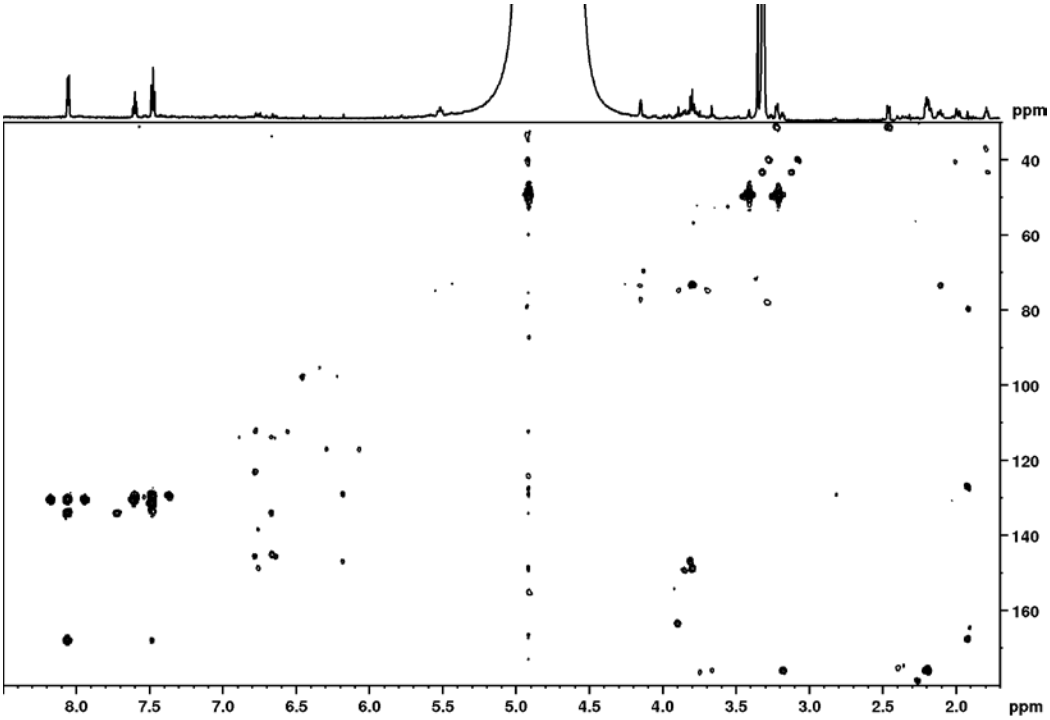


Figure A.5-6 Compound **5**, ^{1}H-^{13}C HMBC spectrum (700 MHz, MeOH-d_4).

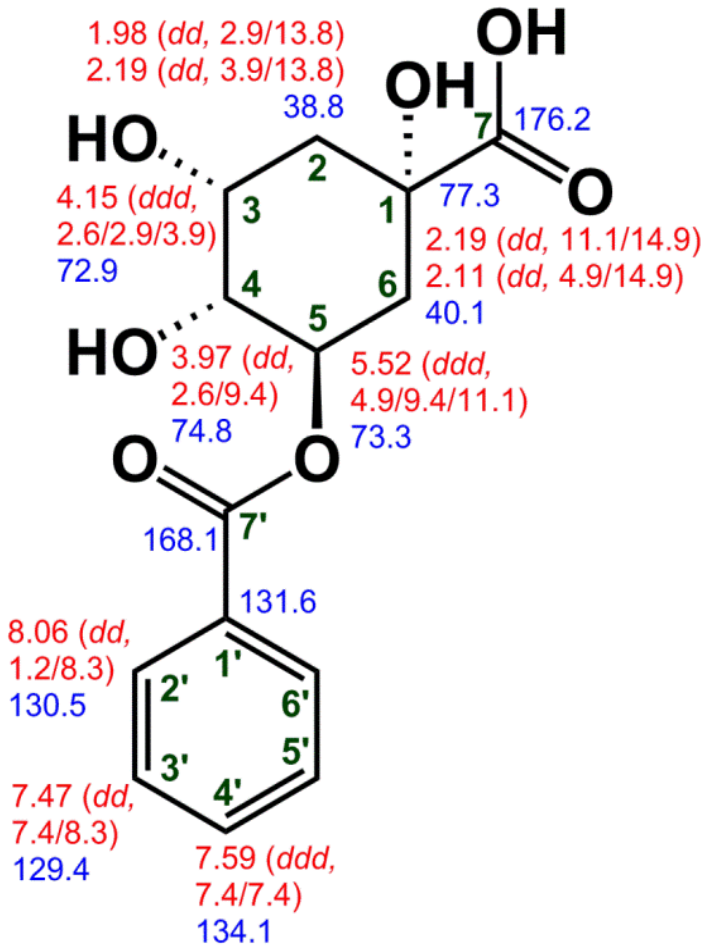


Figure A.5-7 Compound **5**, chemical structure with chemical shifts (MeOH-d_4).

A.6) 3-*O*,4-*O*-Disalicyloyl quinic acid (6):

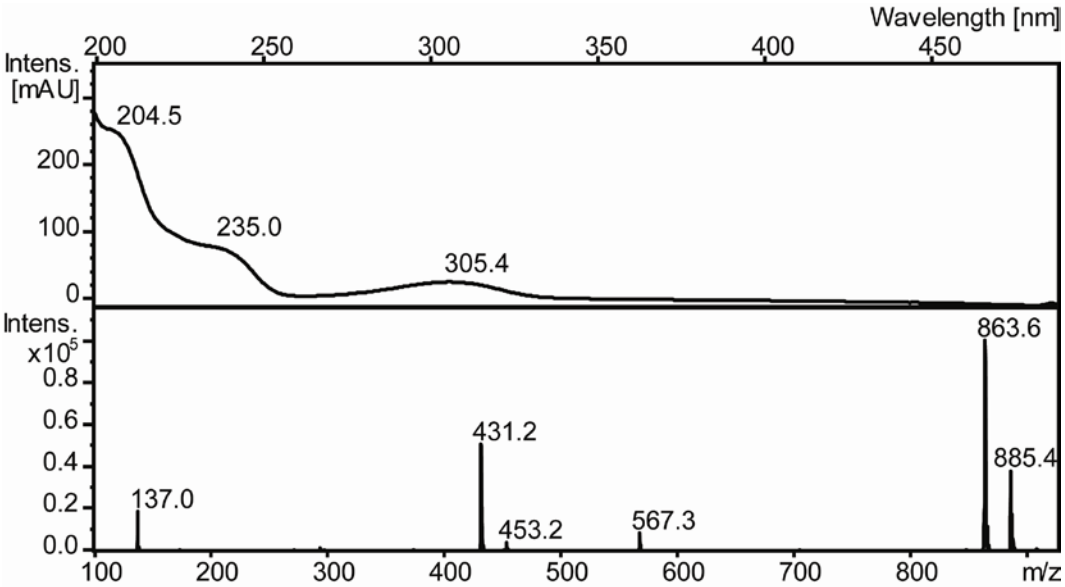


Figure A.6-1 Compound **6**, UV spectrum obtained from HPLC-DAD (top) and mass spectrum (*m/z* 431.2 $[M-H]^-$, bottom).

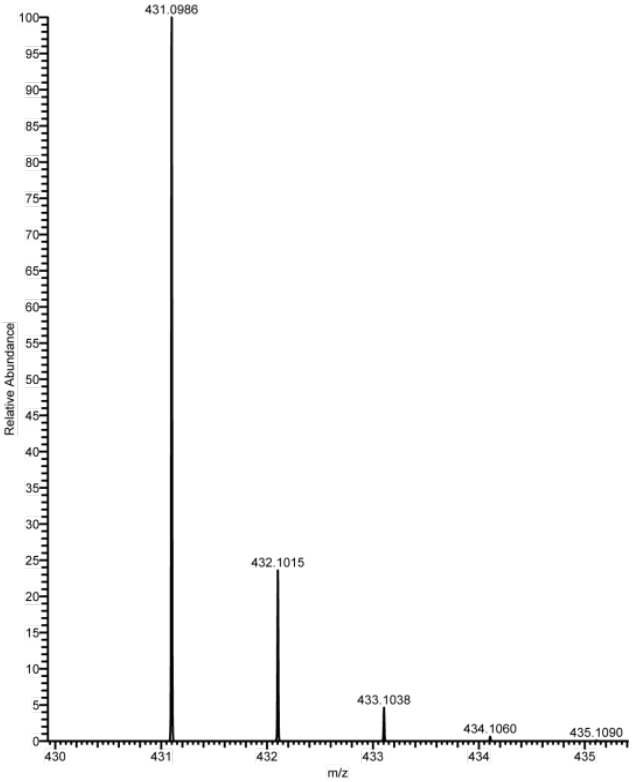


Figure A.6-2 Compound **6**, HRESIMS spectrum, *m/z* 431.0986 $[M-H]^-$.

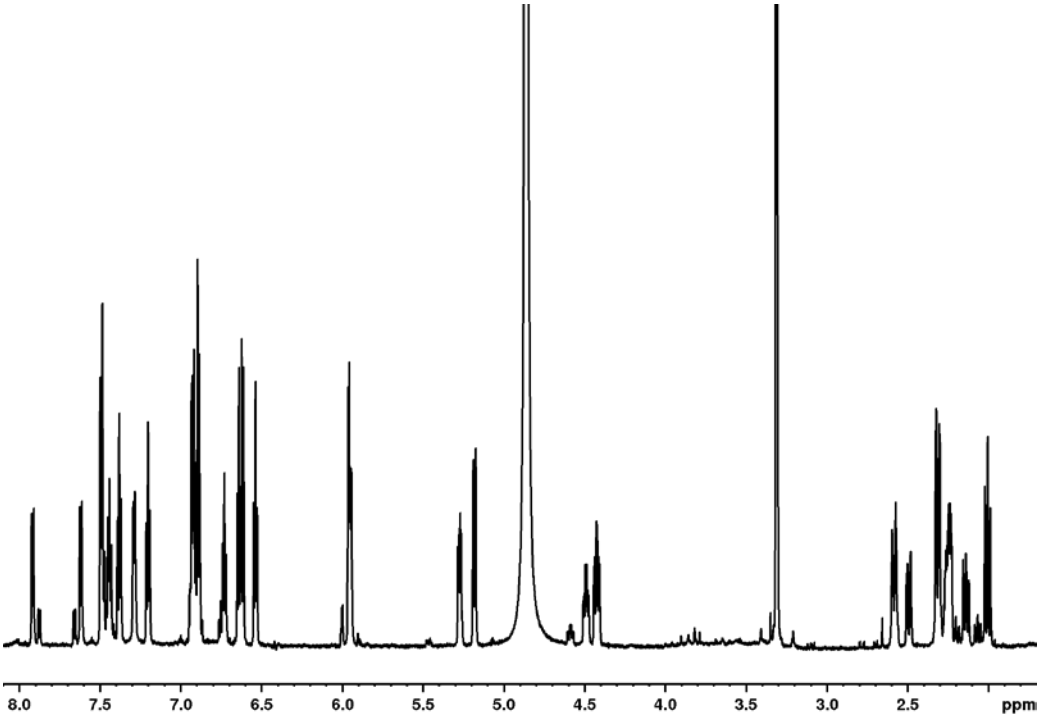


Figure A.6-3 Compound **6**, ^{1}H NMR spectrum (700 MHz, MeOH-d_4).

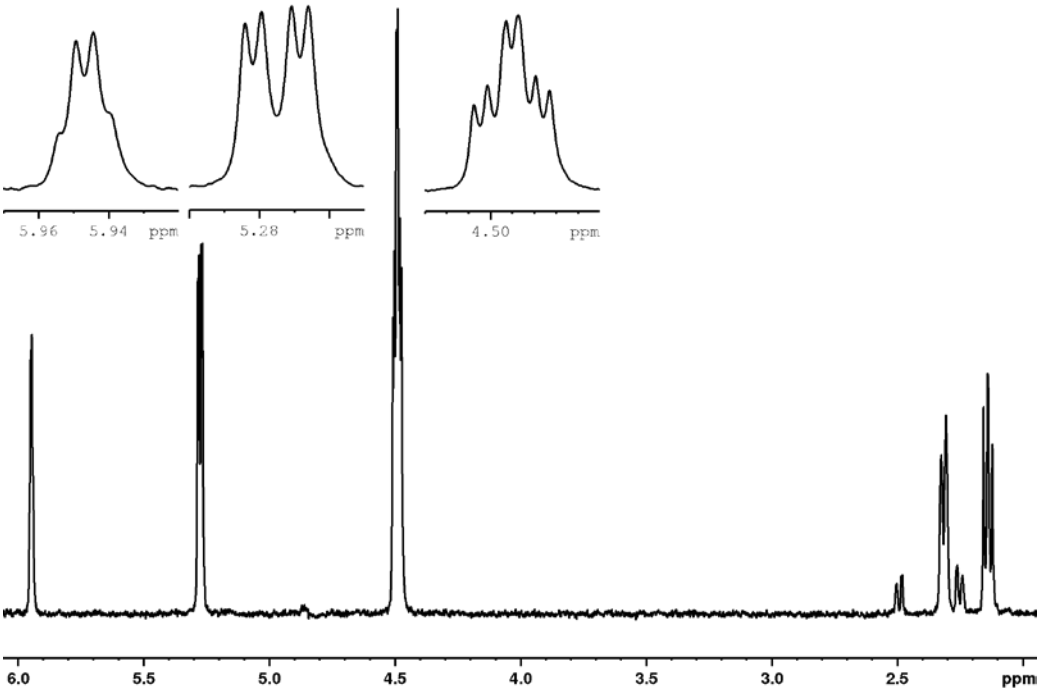


Figure A.6-4 Compound **6**, selective 1D TOCSY spectrum (700 MHz, MeOH-d_4, o1p = 4.48 ppm); Inserts: magnified signals of H-3 (δ_H 5.95), H-4 (δ_H 5.28) and H-5 (δ_H 4.48).

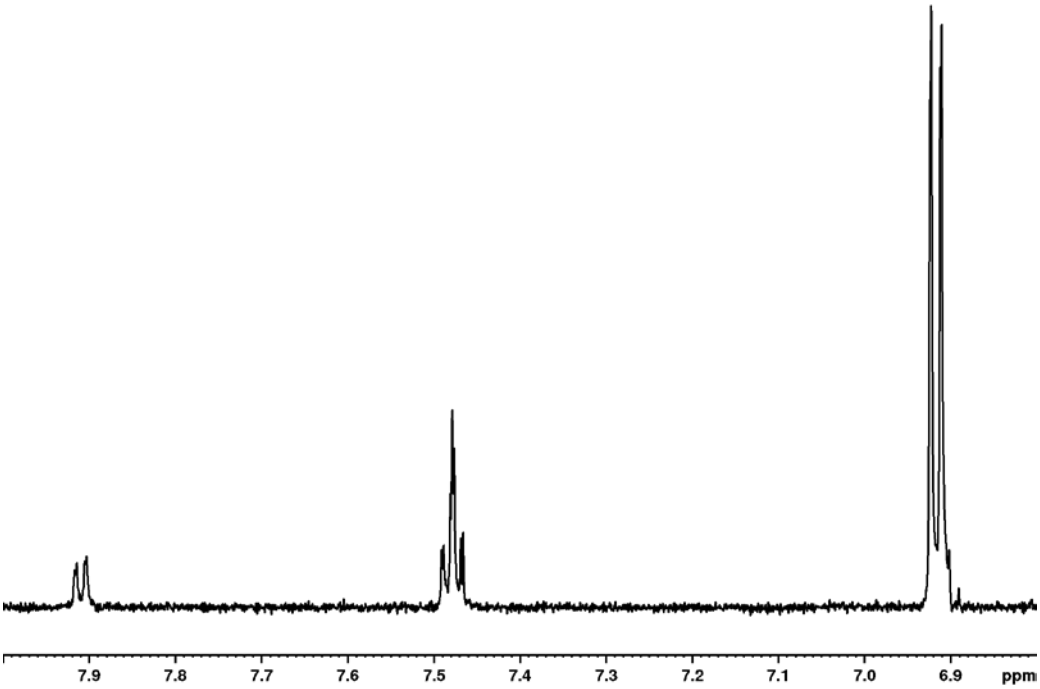


Figure A.6-5 Compound **6**, selective 1D TOCSY spectrum (700 MHz, MeOH-d_4, o1p = 7.91 ppm); signals displayed for H-6' (δ_H 7.91), H-4' (δ_H 6.92), H-3' (δ_H 7.48) and H-5' (δ_H 6.90).

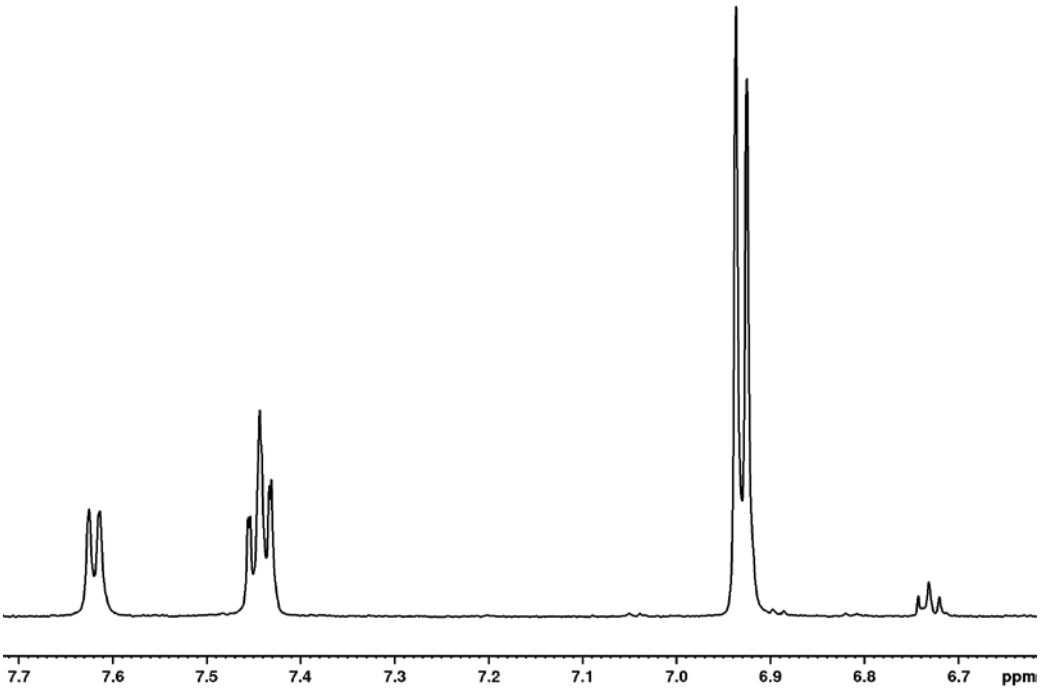


Figure A.6-6 Compound **6**, selective 1D TOCSY spectrum (700 MHz, MeOH-d_4, o1p = 7.62 ppm); signals displayed for H-6'' (δ_H 7.62), H-4'' (δ_H 7.44), H-3'' (δ_H 6.93) and H-5'' (δ_H 6.74).

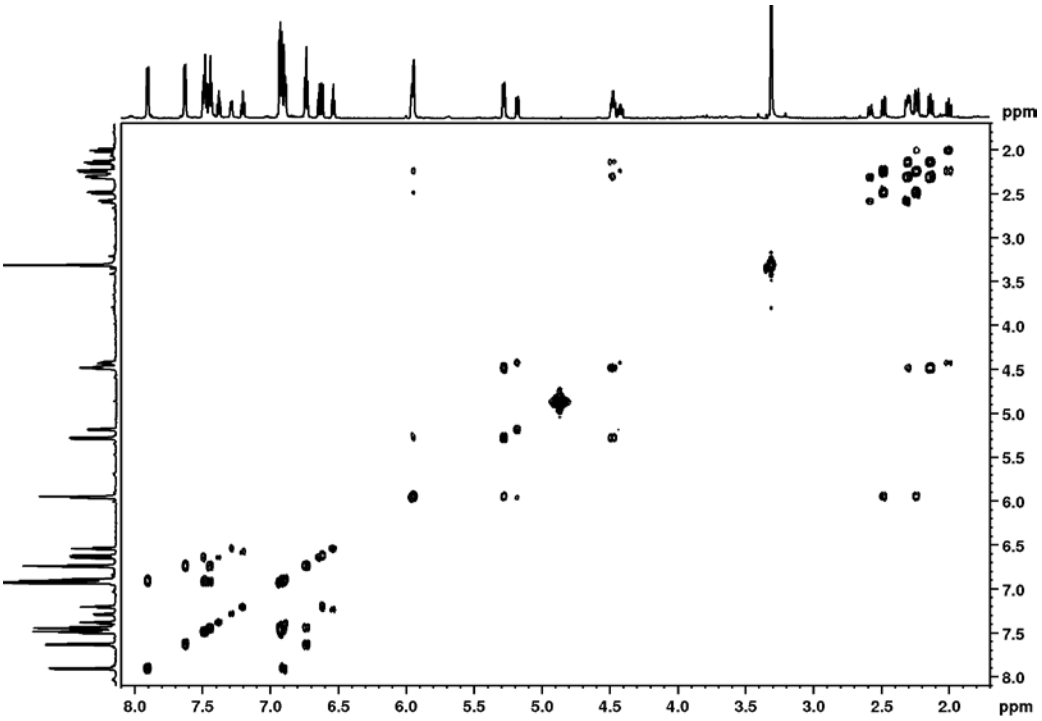


Figure A.6-7 Compound **6**, ^{1}H-^{1}H COSY spectrum (700 MHz, MeOH-d_4).

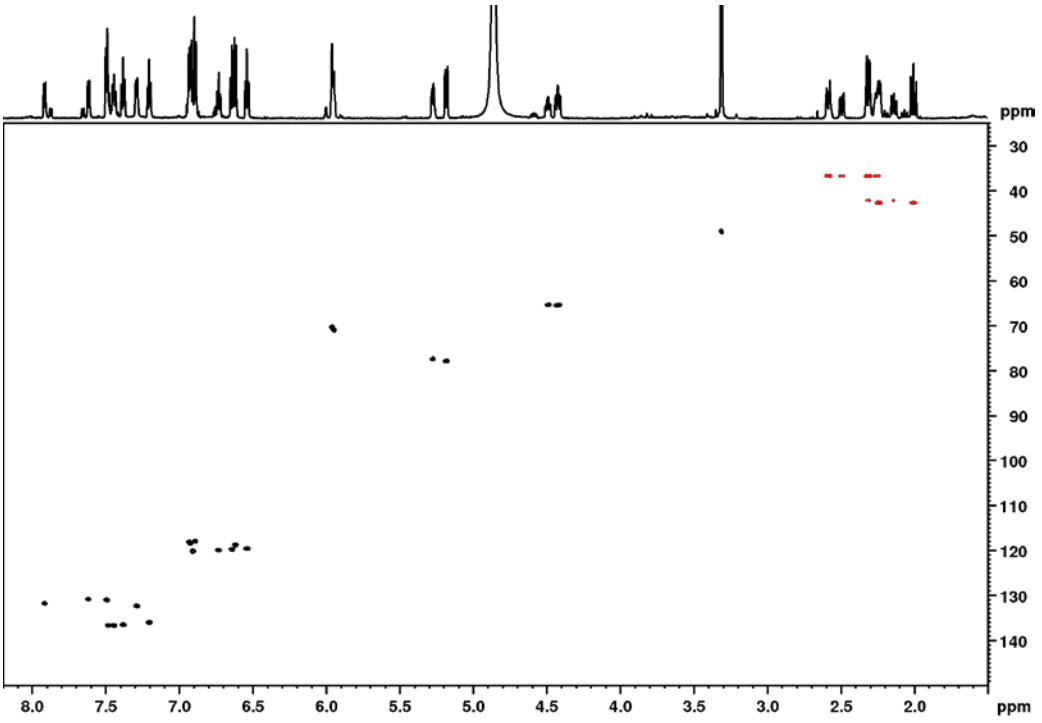


Figure A.6-8 Compound **6**, ^{1}H-^{13}C HSQC spectrum (700 MHz, MeOH-d_4).

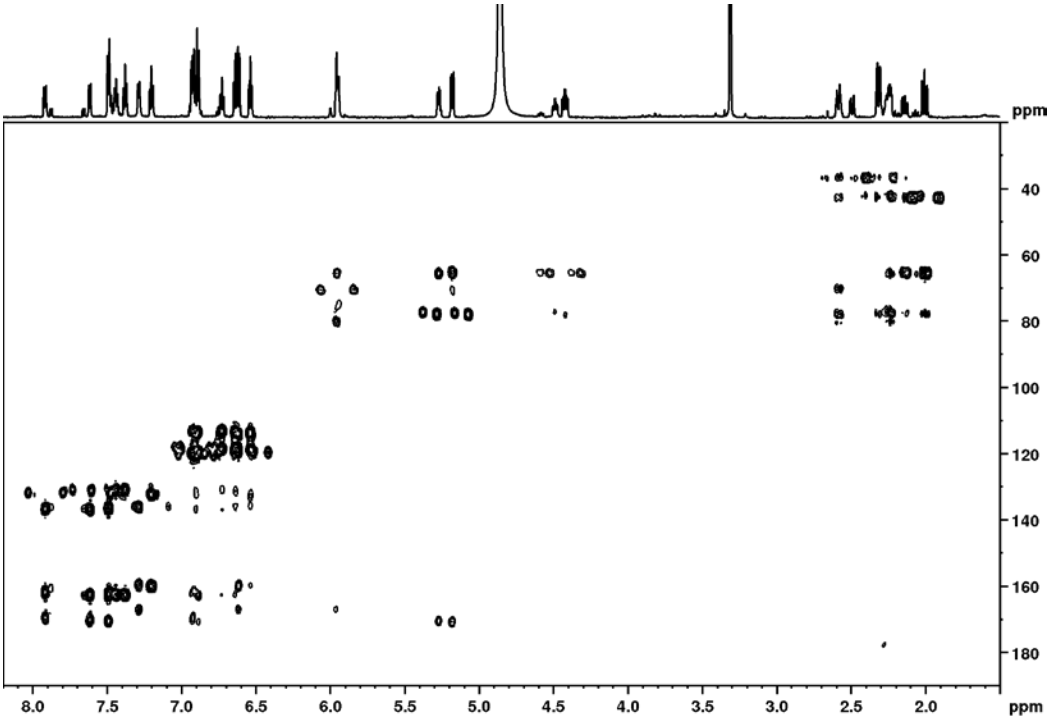


Figure A.6-9 Compound **6**, ^{1}H-^{13}C HMBC spectrum (700 MHz, MeOH-d_4).

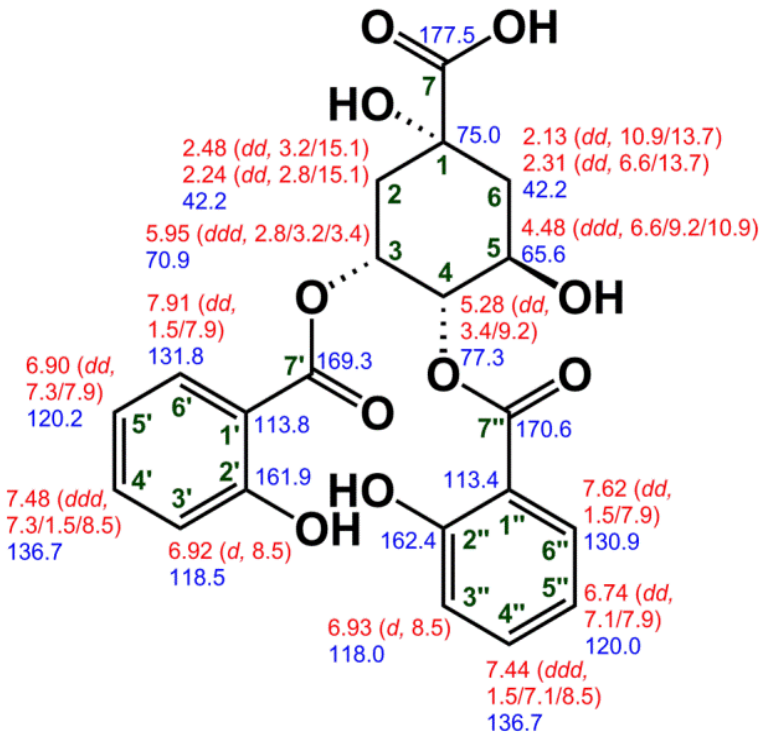


Figure A.6-10 Compound **6**, chemical structure with chemical shifts (MeOH-d_4).

A.7) 3-*O*,5-*O*-Disalicyloyl quinic acid (7):

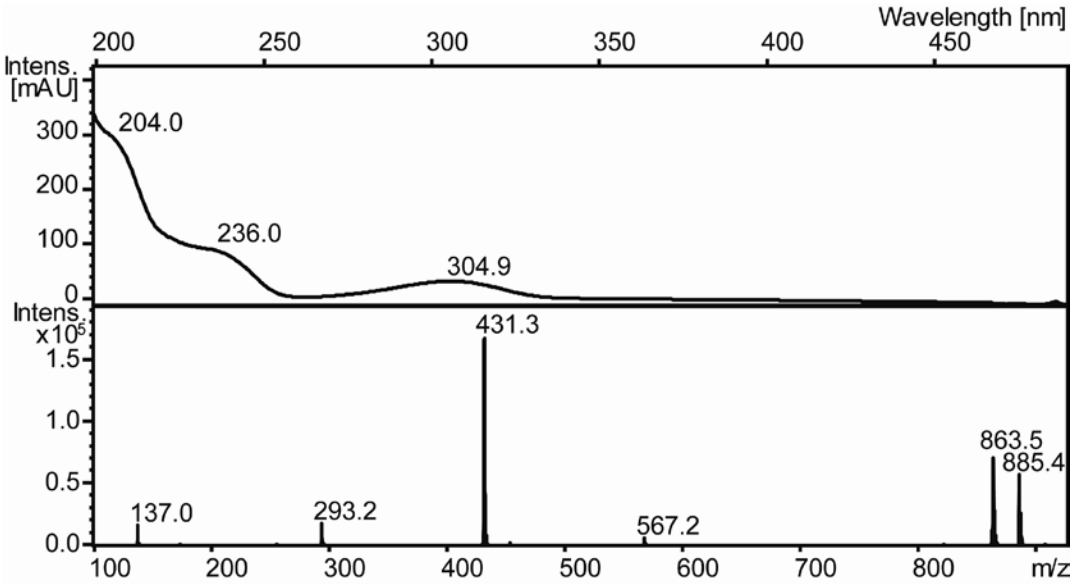


Figure A.7-1 Compound **7**, UV spectrum from HPLC-DAD (top) and mass spectrum (m/z 431.3 $[M-H]^-$, bottom).

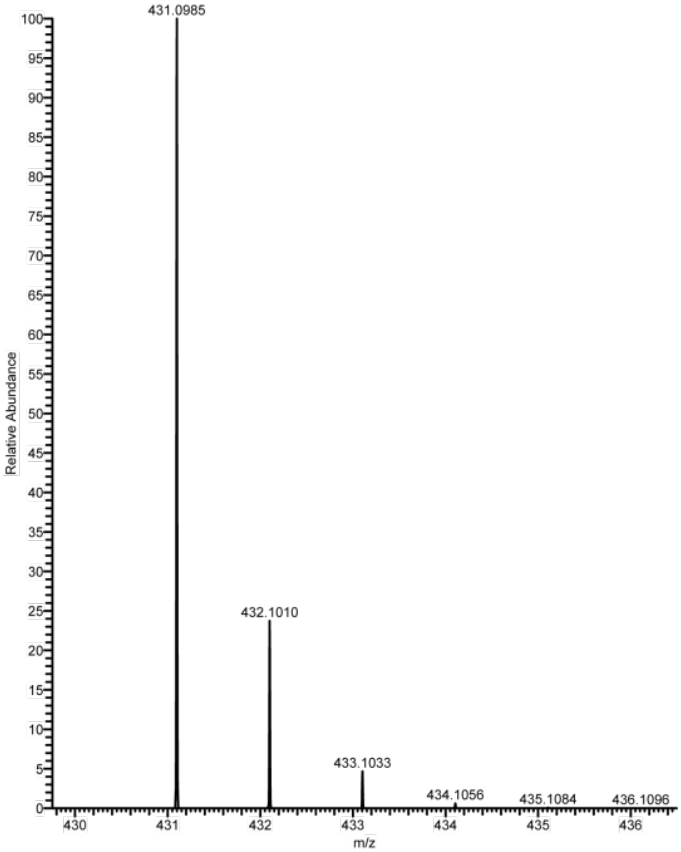


Figure A.7-2 Compound **7**, HRESIMS spectrum, *m/z* 431.0985 $[M-H]^-$.

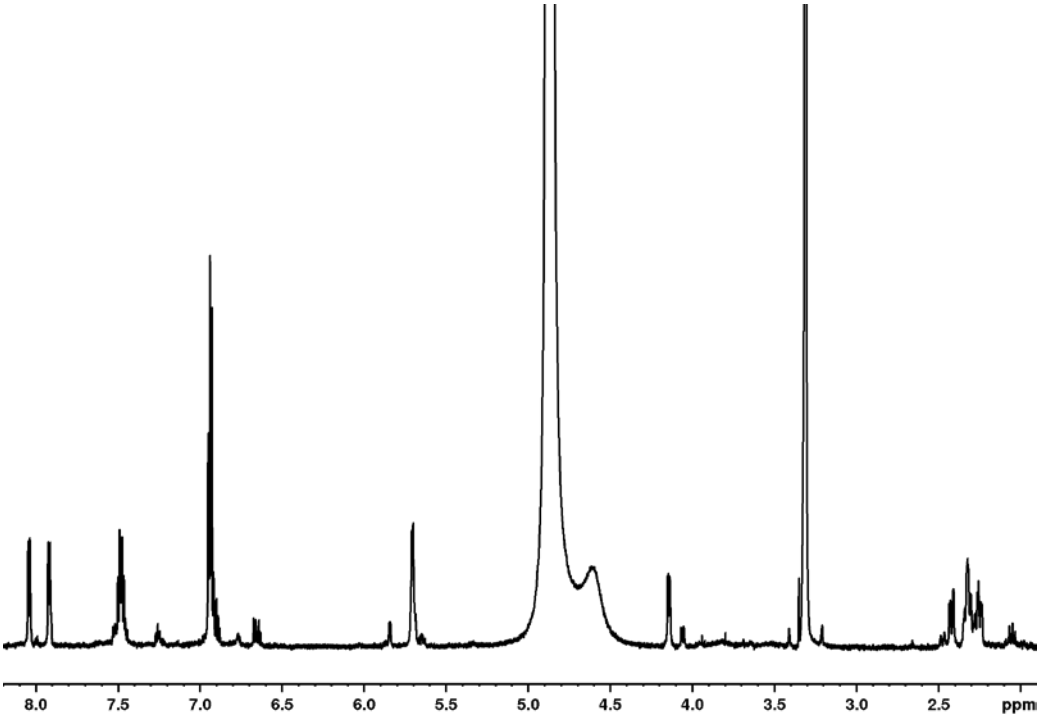


Figure A.7-3 Compound **7**, ^{1}H NMR spectrum (700 MHz, MeOH-d_4).

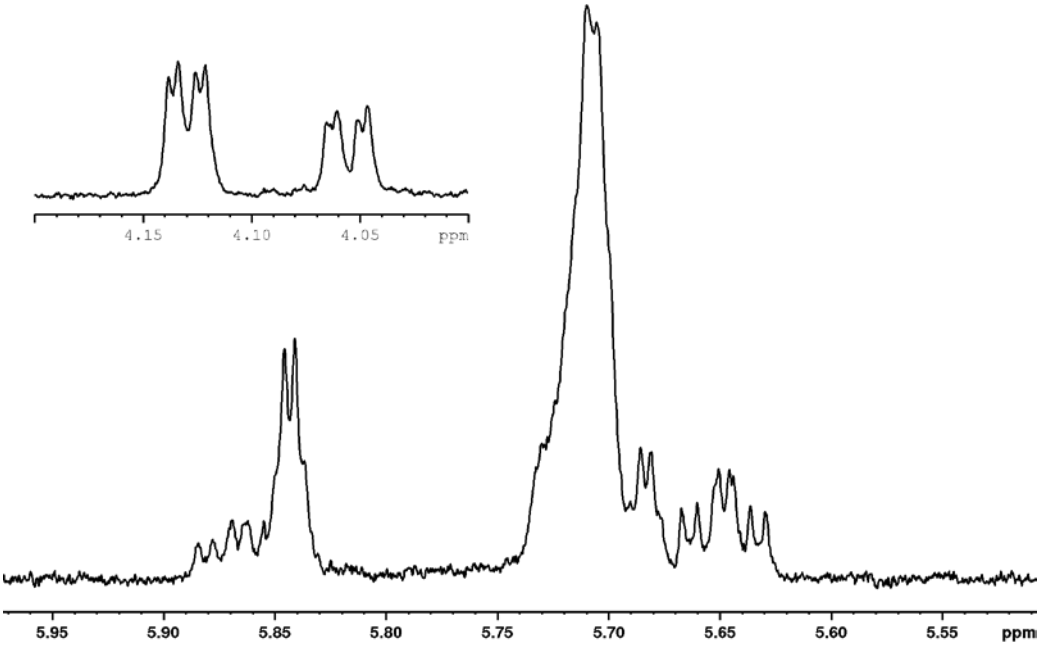


Figure A.7-4 Compound **7**, ^{1}H NMR spectrum (700 MHz, MeOH-d_4); region from 5.55 to 5.95 ppm, signal H-3 and H-5 are overlapping (δ_H 5.71); insert: magnified signal of H-4 (δ_H 4.13).

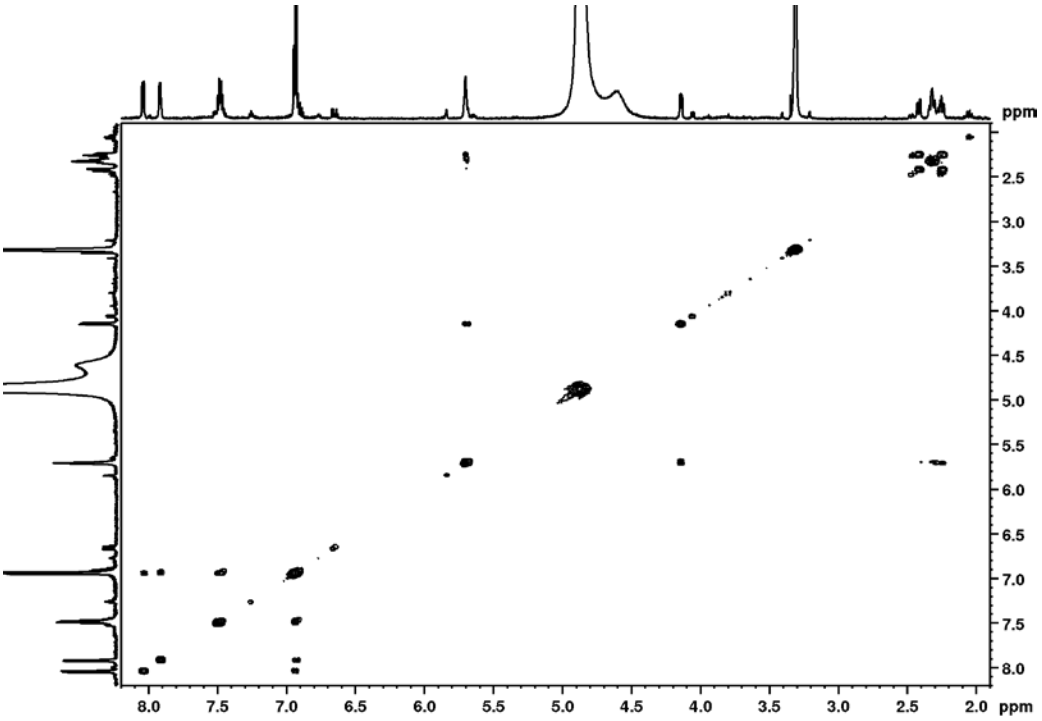


Figure A.7-5 Compound **7**, ^{1}H-^{1}H COSY spectrum (700 MHz, MeOH-d_4).

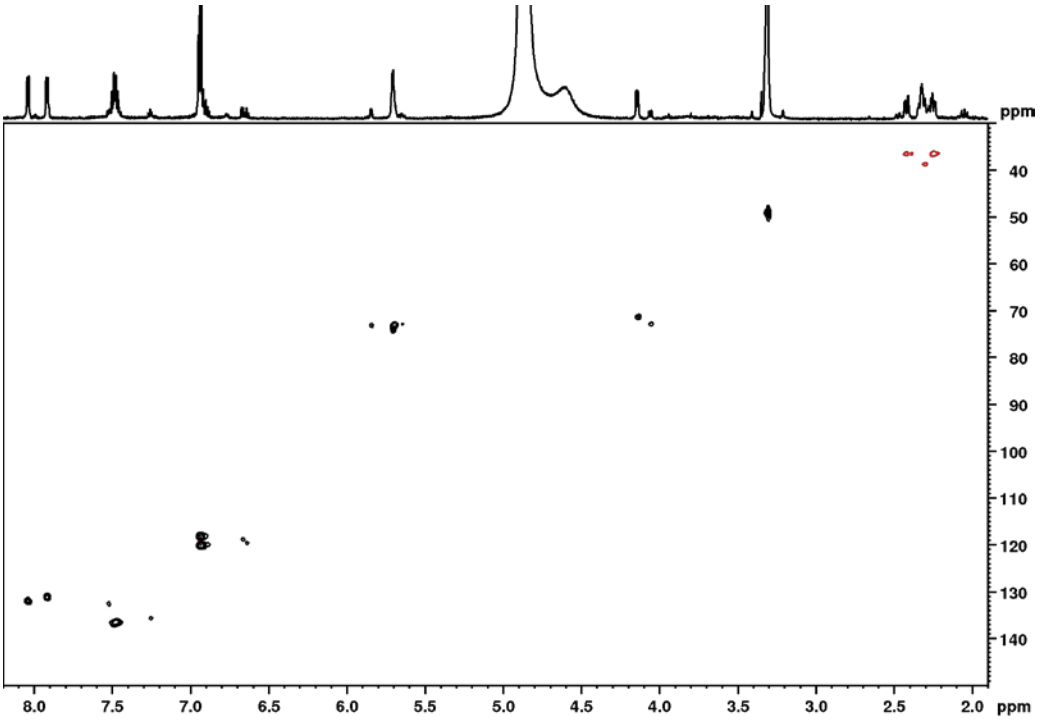


Figure A.7-6 Compound **7**, ^{1}H-^{13}C HSQC spectrum (700 MHz, MeOH-d_4).

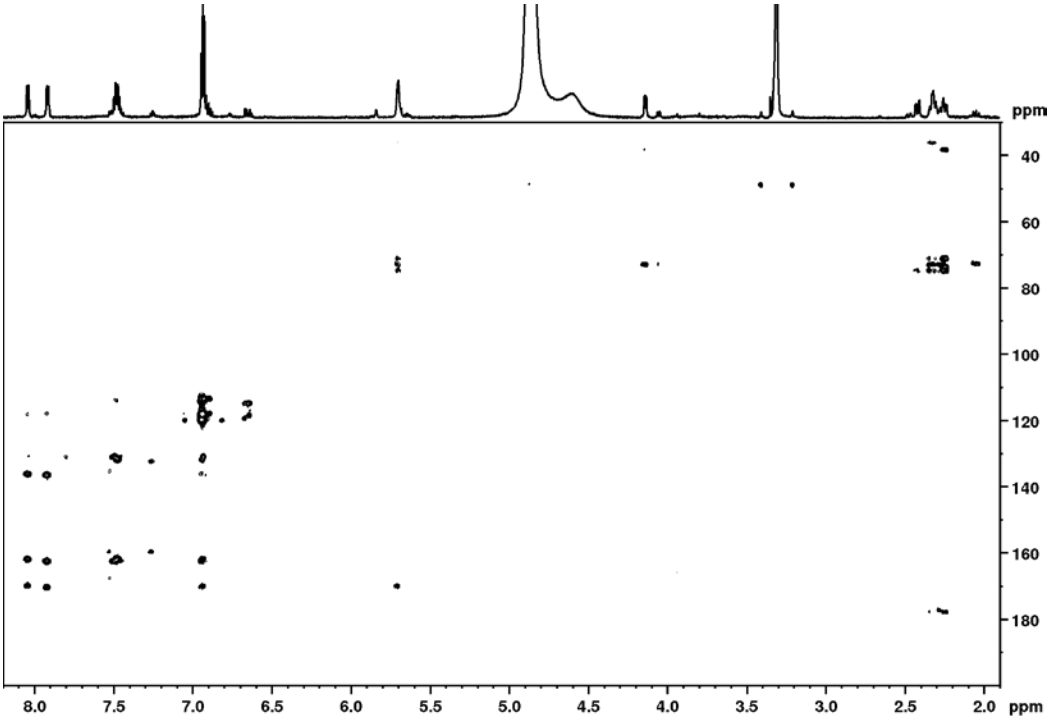


Figure A.7-7 Compound **7**, ^{1}H-^{13}C HMBC spectrum (700 MHz, MeOH-d_4).

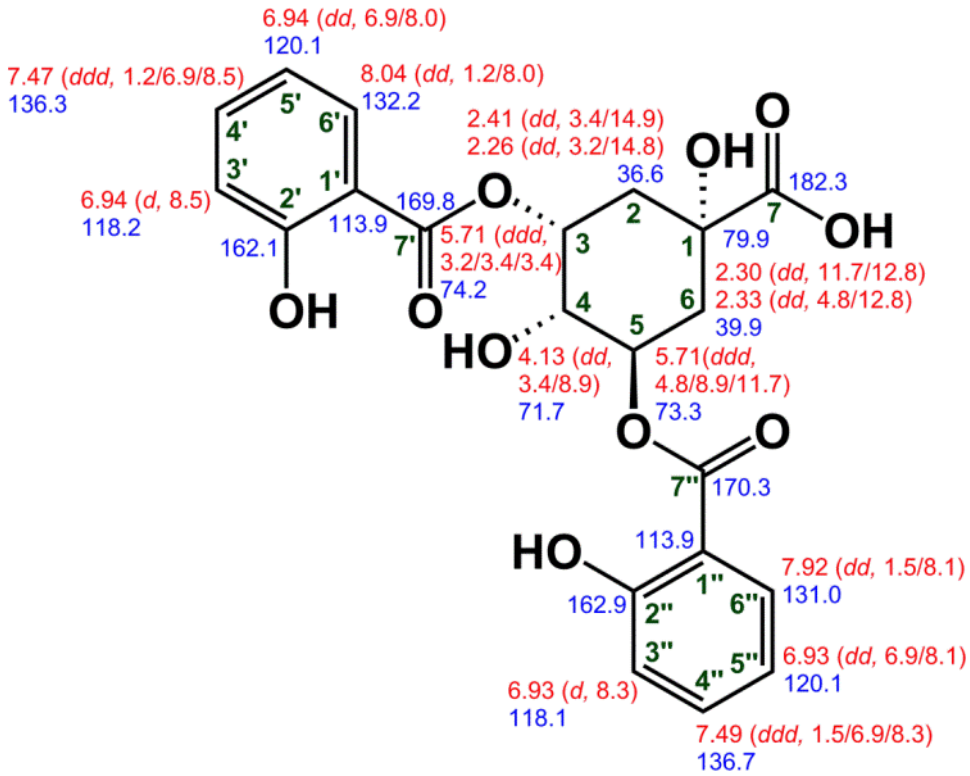


Figure A.7-8 Compound **7**, chemical structure with chemical shifts (MeOH-d_4).

A.8) 4-*O*,5-*O*-Disalicyloyl quinic acid (8):

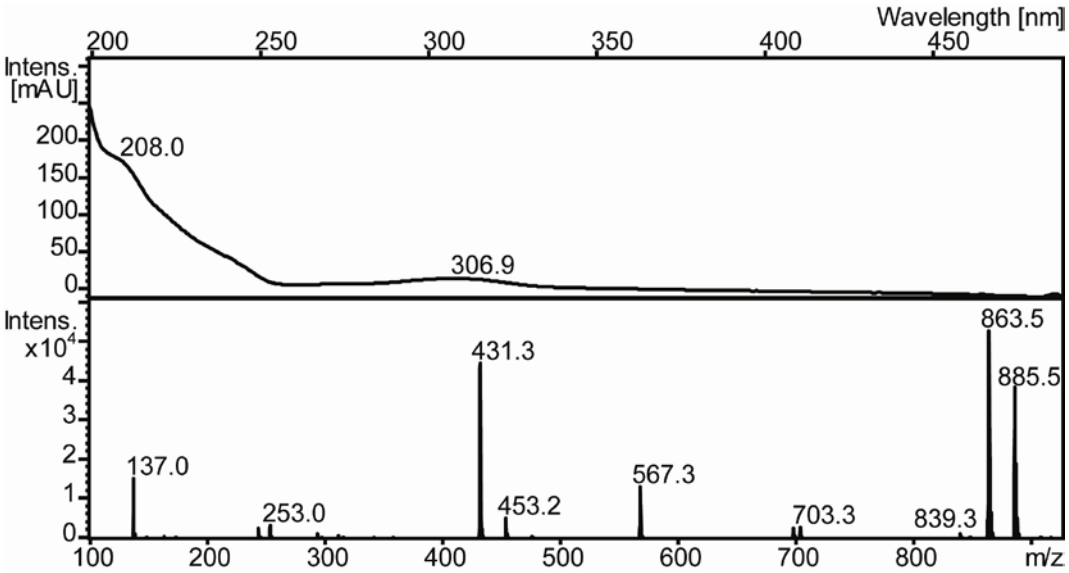


Figure A.8-1 Compound **8**, UV spectrum obtained from HPLC-DAD (top) and mass spectrum (*m/z* 431.3 $[M-H]^-$, bottom).

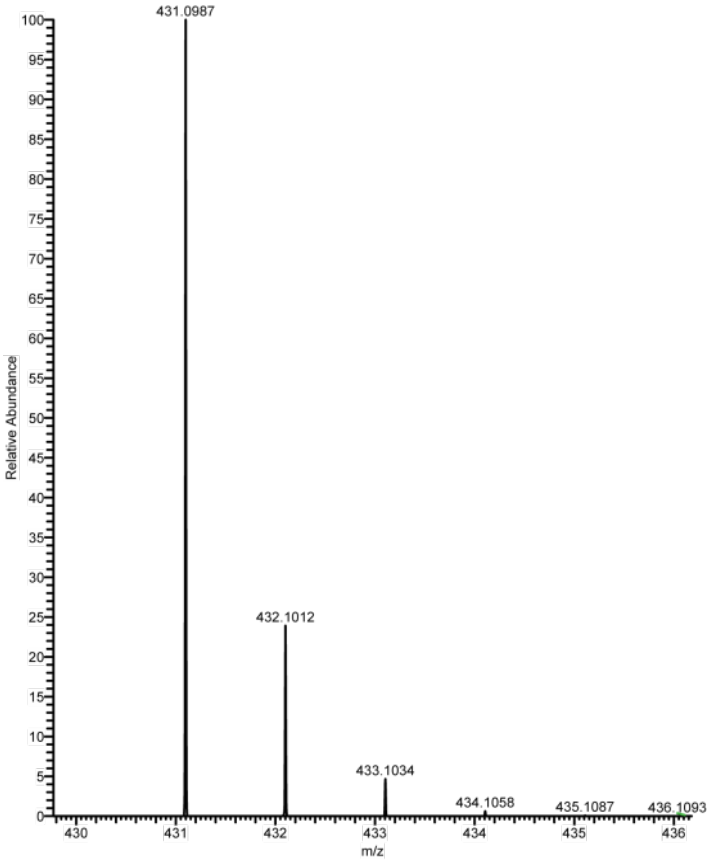


Figure A.8-2 Compound **8**, HRESIMS spectrum, *m/z* 431.0987 $[M-H]^-$.

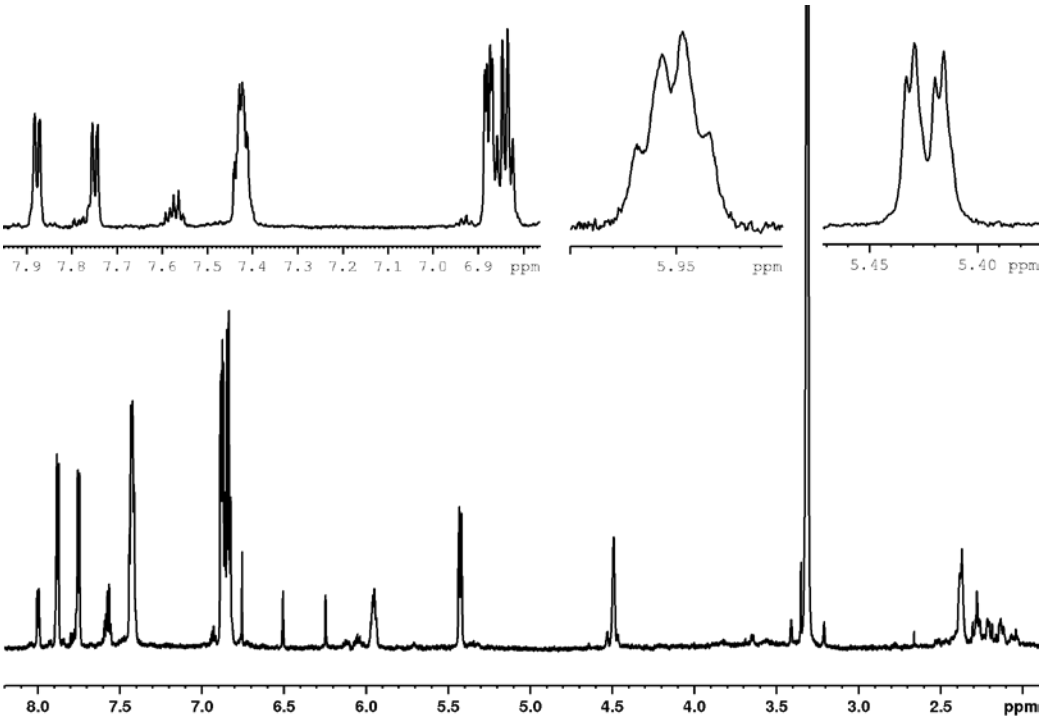


Figure A.8-3 Compound **8**, 1D NOESY spectrum (700 MHz, MeOH-d_4, o1p = 4.92 ppm), region 7.9 to 6.8 ppm; inserts: magnified signals of H-5 (δ_H 5.95) and H-4 (δ_H 5.42).

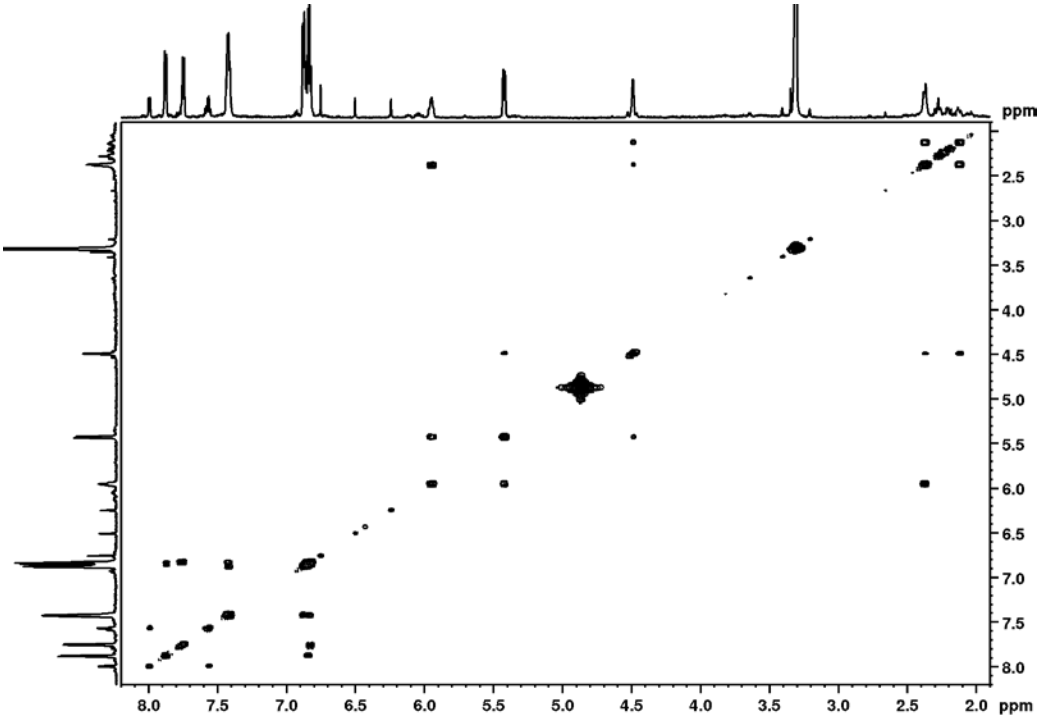


Figure A.8-4 Compound **8**, ^{1}H-^{1}H COSY spectrum (700 MHz, MeOH-d_4).

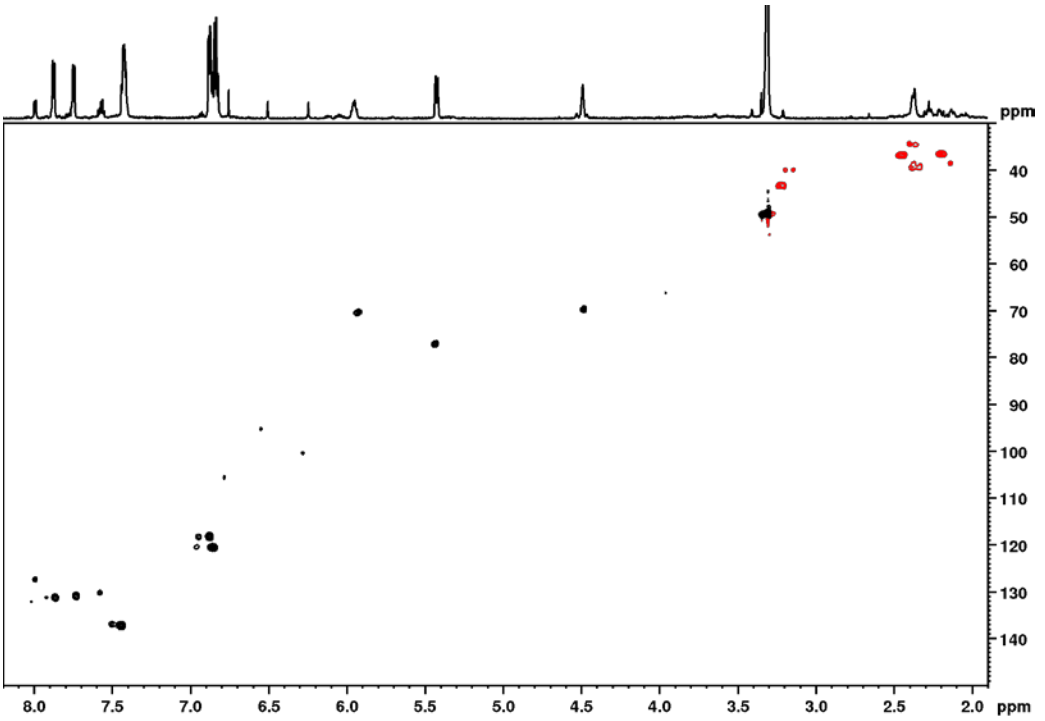


Figure A.8-5 Compound **8**, ^{1}H-^{13}C HSQC spectrum (700 MHz, MeOH-d_4).

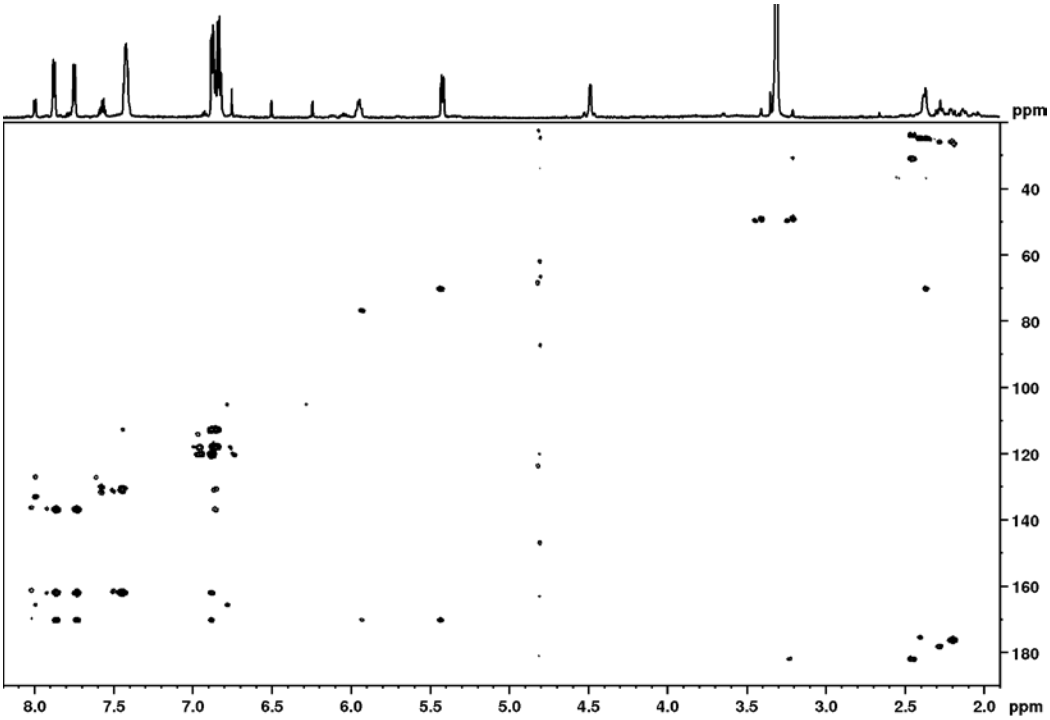


Figure A.8-6 Compound **8**, ^{1}H-^{13}C HMBC spectrum (700 MHz, MeOH-d_4).

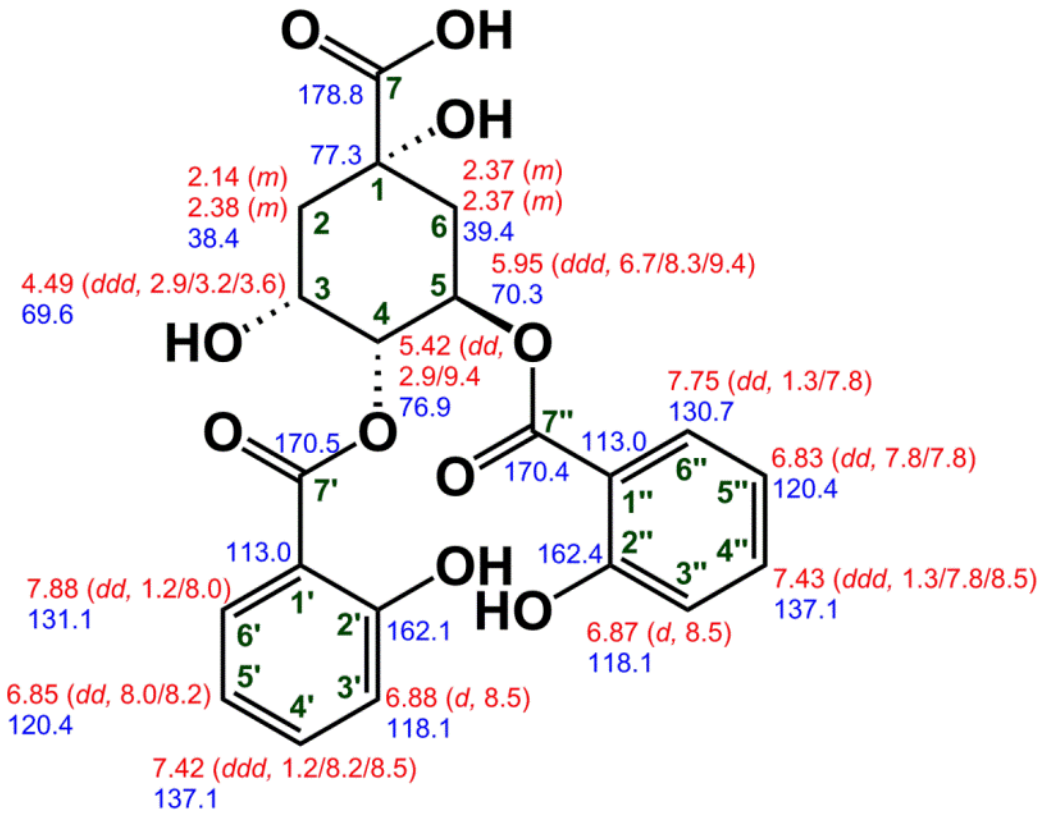


Figure A.8-7 Compound **8**, chemical structure with chemical shifts (MeOH-d_4).

A.9) 3-*O*-Salicyloyl-4-*O*-benzoyl quinic acid (9):

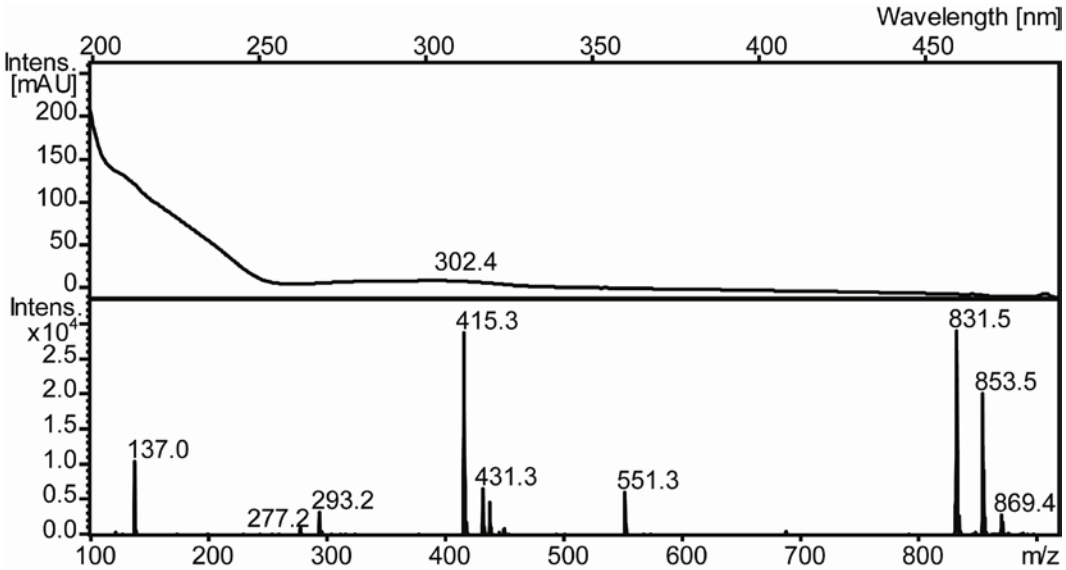


Figure A.9-1 Compound **9**, UV spectrum obtained from HPLC-DAD (top) and mass spectrum (m/z 415.3 $[M-H]^-$, bottom).

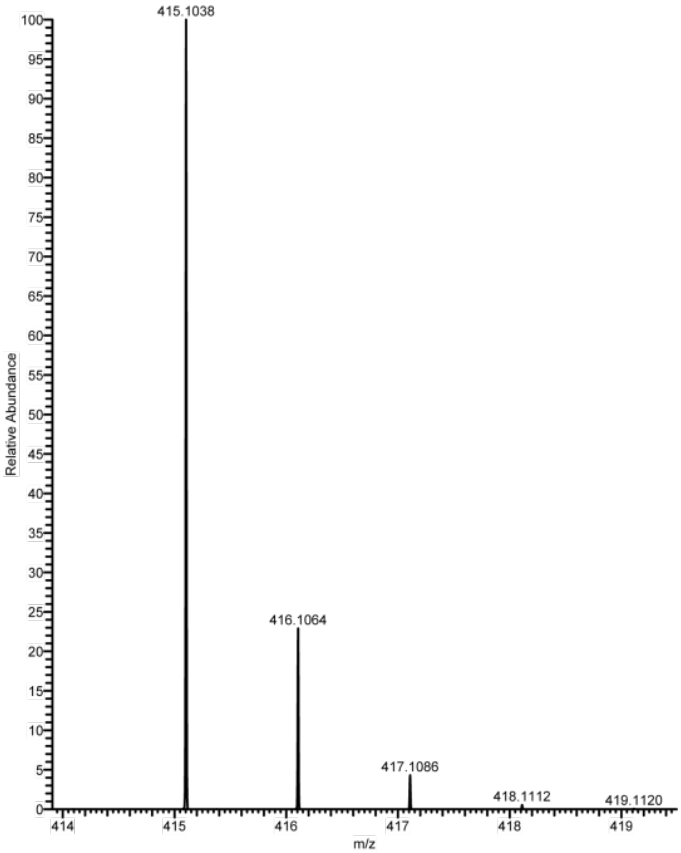


Figure A.9-2 Compound **9**, HRESIMS spectrum, *m/z* 415.1038 $[M-H]^-$.

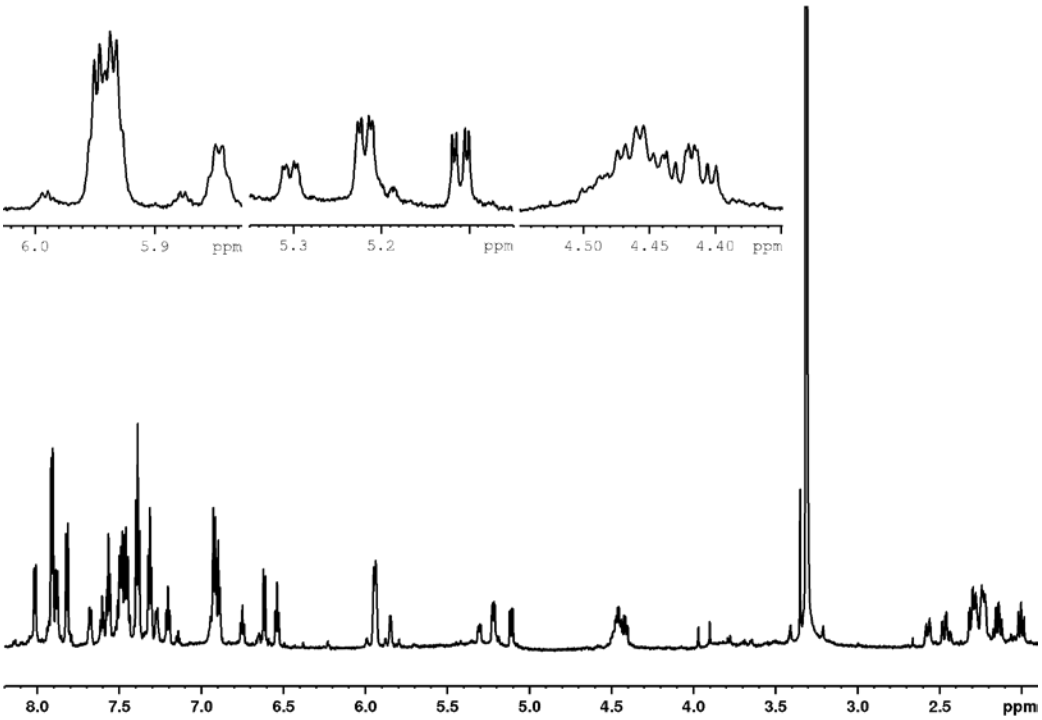


Figure A.9-3 Compound **9**, 1D NOESY spectrum (700 MHz, MeOH-d_4, o1P = 4.92 ppm); inserts: magnified signals of H-3 (δ_H 5.93), H-4 (δ_H 5.22) and H-5 (δ_H 4.46).

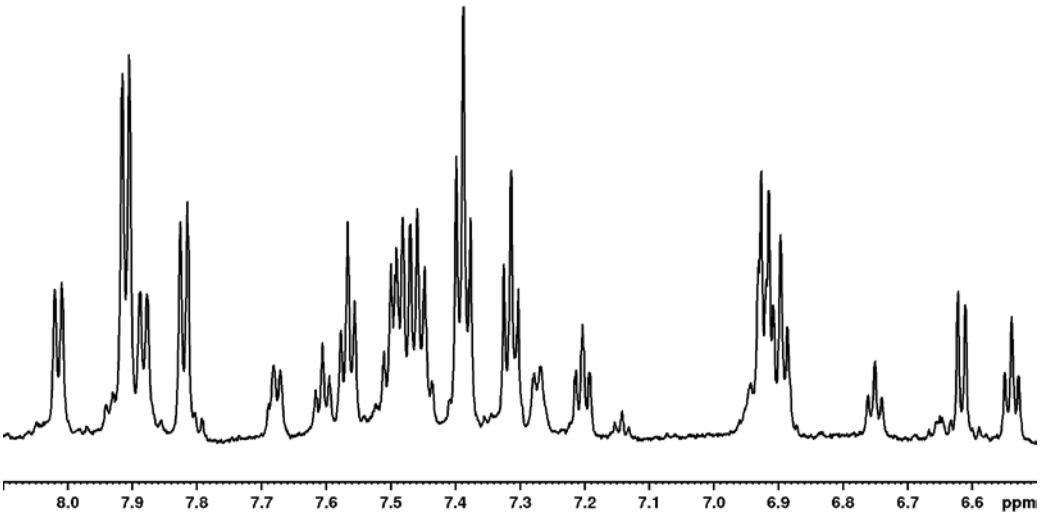


Figure A.9-4 Compound **9**, 1D NOESY spectrum (700 MHz, MeOH-d_4, o1p = 4.92 ppm); region 8.1 to 6.5 ppm.

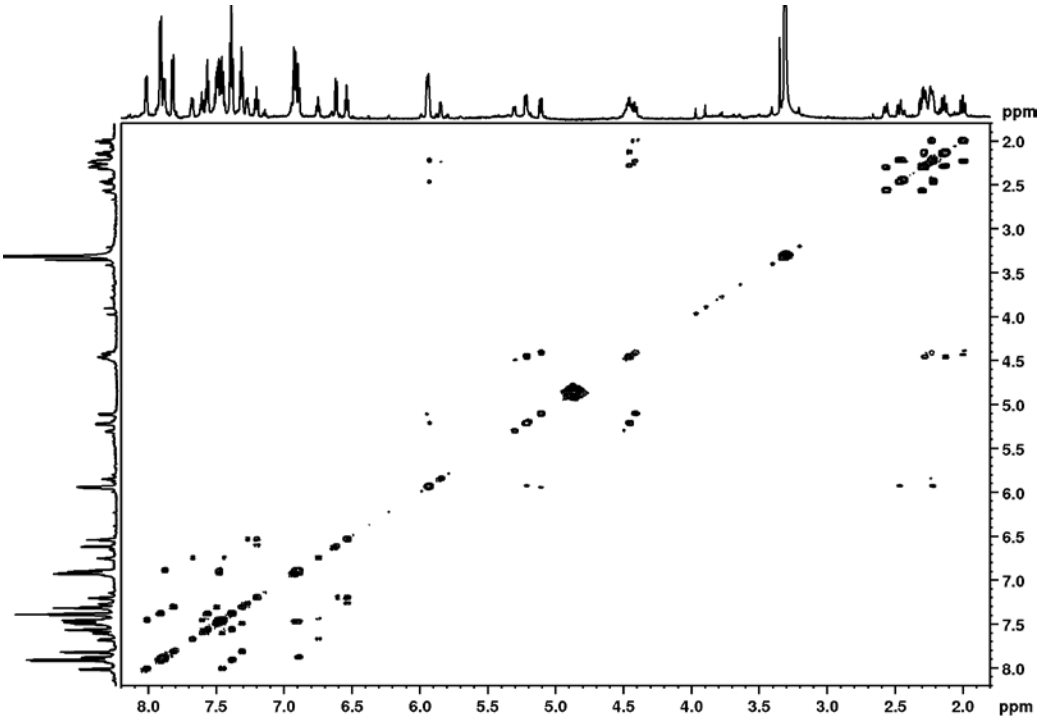


Figure A.9-5 Compound **9**, ^{1}H-^{1}H COSY spectrum (700 MHz, MeOH-d_4).

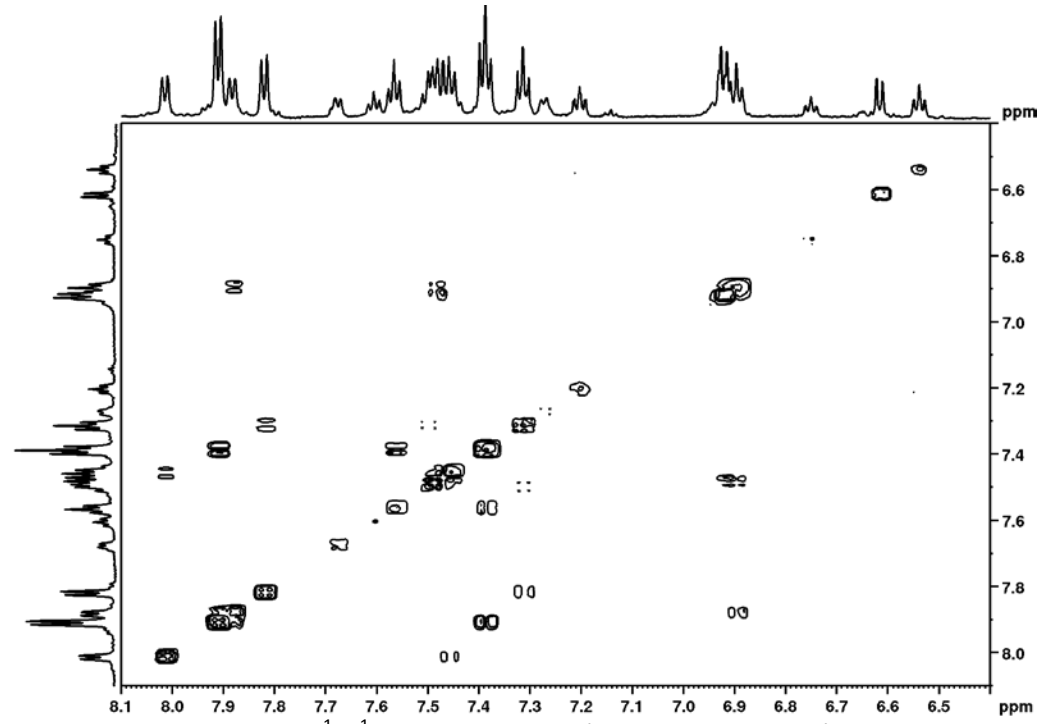


Figure A.9-6 Compound **9**, ^{1}H-^{1}H COSY spectrum (700 MHz, MeOH-d_4); region 8.1 to 6.5 ppm.

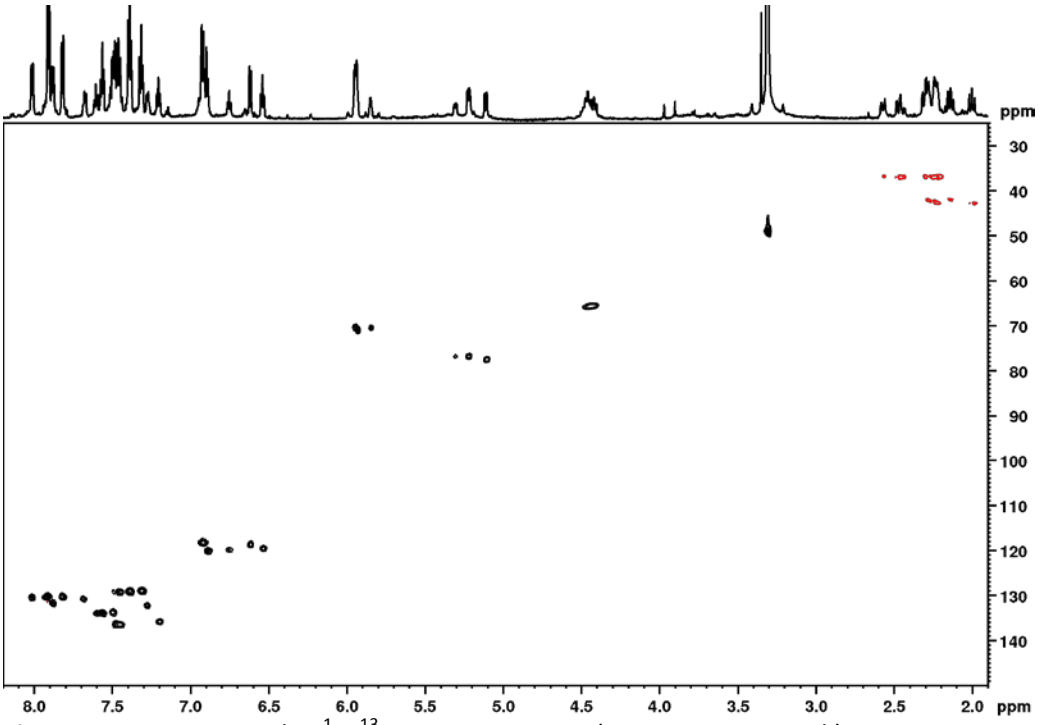


Figure A.9-7 Compound **9**, ^{1}H-^{13}C HSQC spectrum (700 MHz, MeOH-d_4).

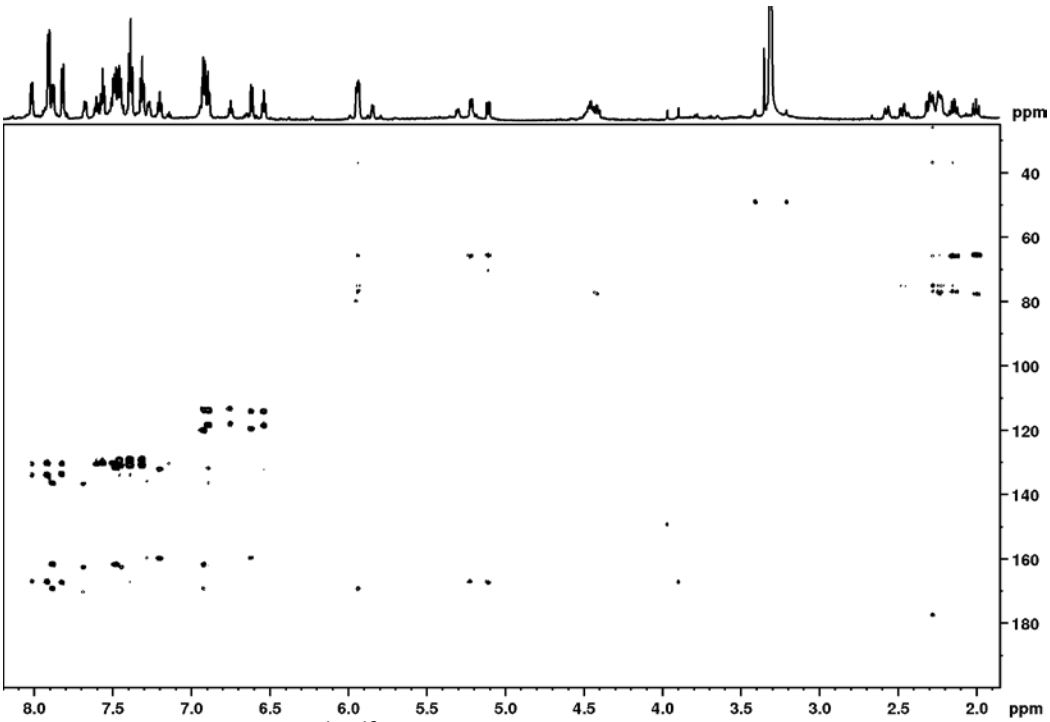


Figure A.9-8 Compound **9**, ^{1}H-^{13}C HMBC spectrum (700 MHz, MeOH-d_4).

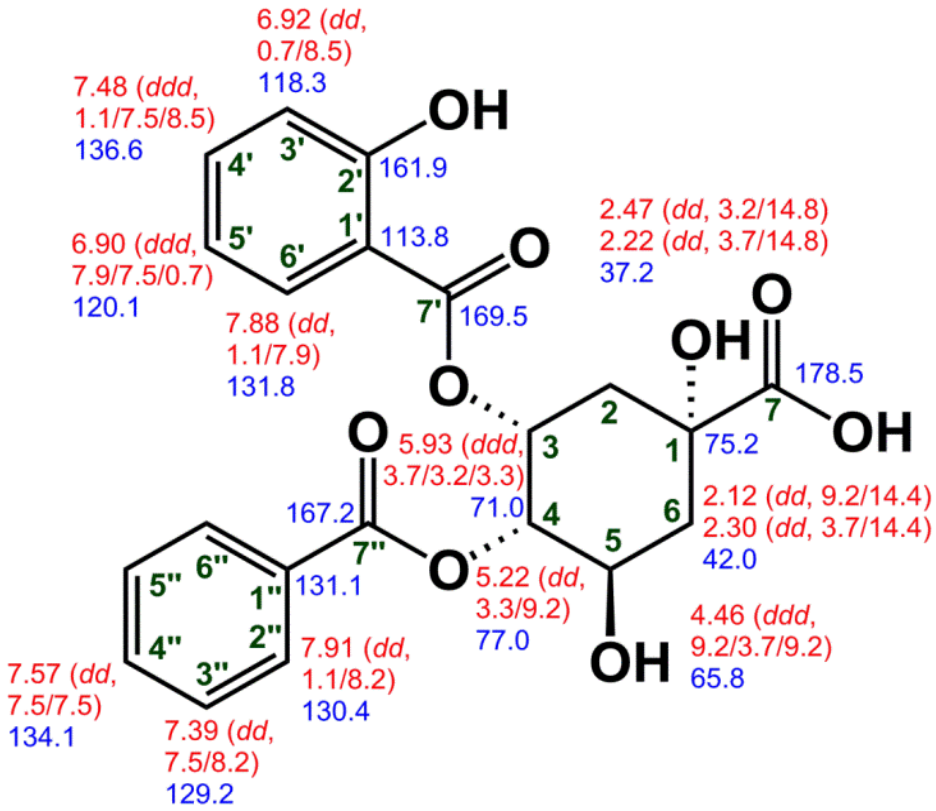


Figure A.9-9 Compound **9**, structure with chemical shifts (MeOH-d_4).

A.10) 3-*O*-Salicyloyl-5-*O*-benzoyl quinic acid (10):

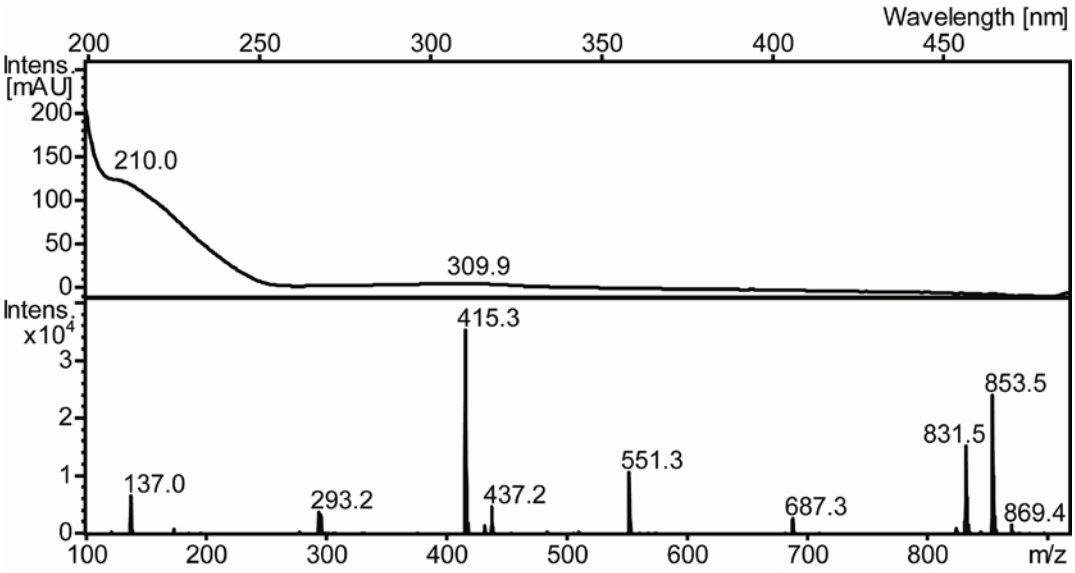


Figure A.10-1 Compound **10**, UV spectrum obtained from HPLC-DAD (top) and mass spectrum (*m/z* 415.3 $[M-H]^-$, bottom).

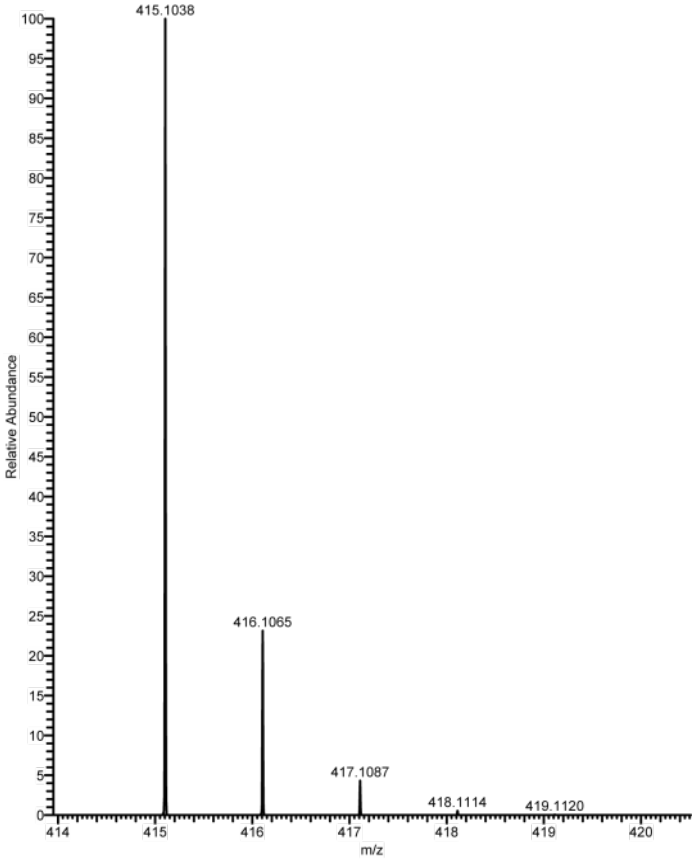


Figure A.10-2 Compound **10**, HRESIMS spectrum, *m/z* 415.1038 $[M-H]^-$.

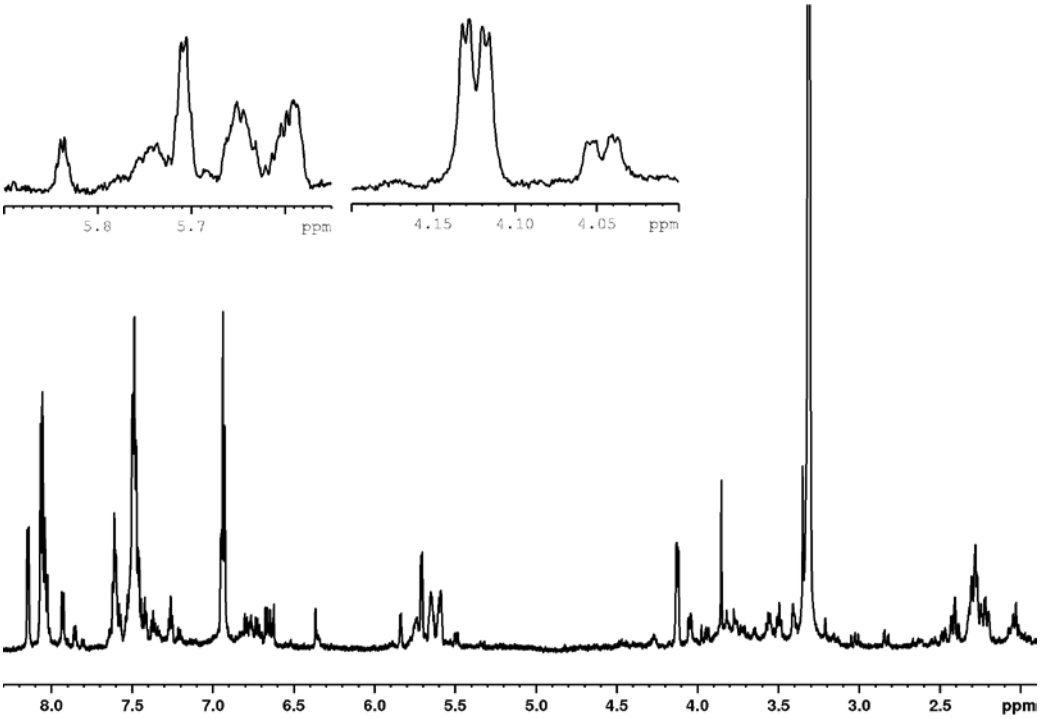


Figure A.10-3 Compound **10**, 1D NOESY spectrum (700 MHz, MeOH-d_4, o1P = 4.92 ppm); inserts: magnified signals of H-3 (δ_H 5.71), H-5 (δ_H 5.56) and H-3 (δ_H 4.12).

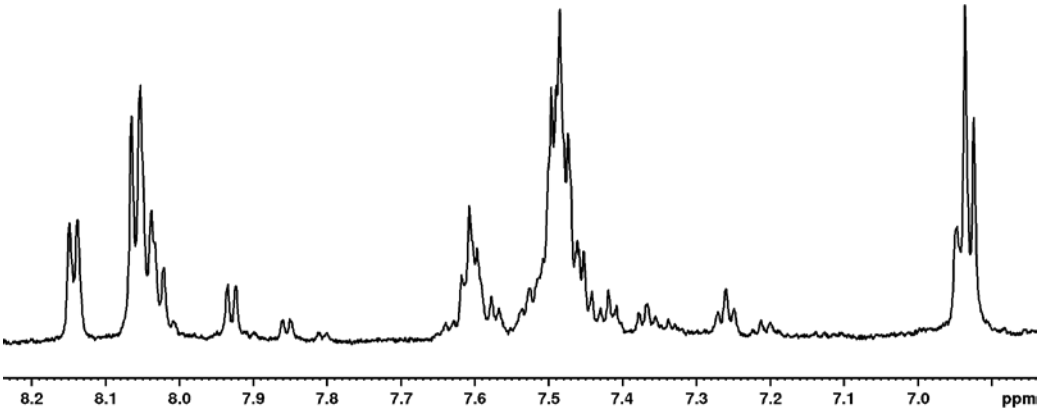


Figure A.10-4 Compound **10**, 1D NOESY spectrum (700 MHz, MeOH-d_4, o1P = 4.92 ppm); region 8.2 to 6.8 ppm.

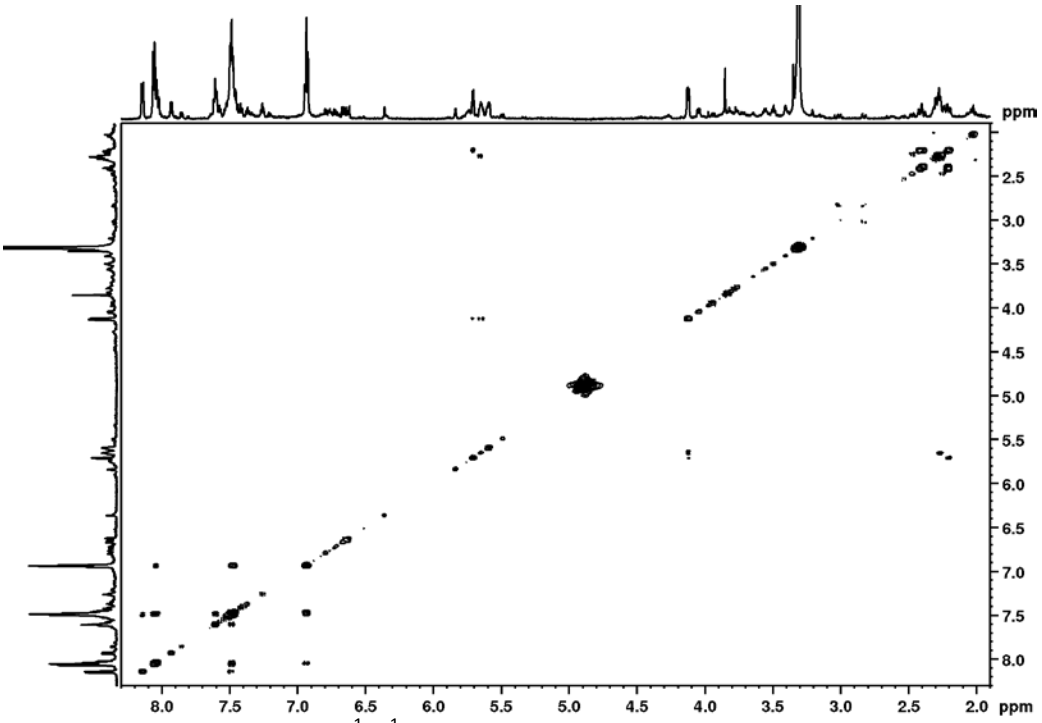


Figure A.10-5 Compound **10**, ^{1}H-^{1}H COSY spectrum (700 MHz, MeOH-d_4).

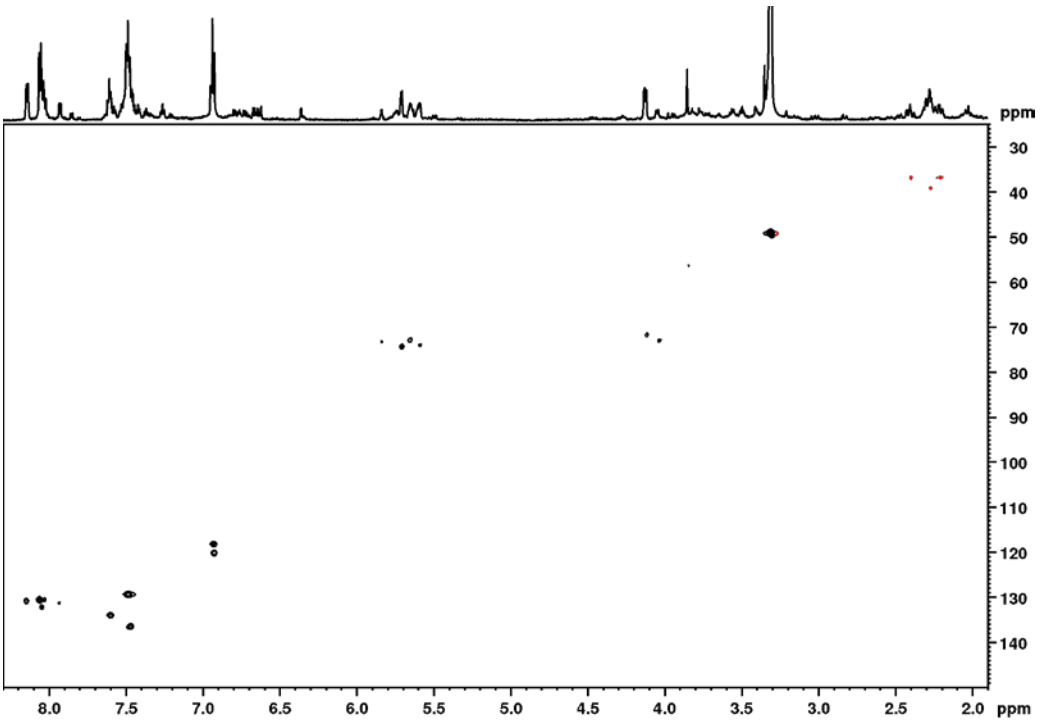


Figure A.10-6 Compound **10**, ^{1}H-^{13}C HSQC spectrum (700 MHz, MeOH-d_4).

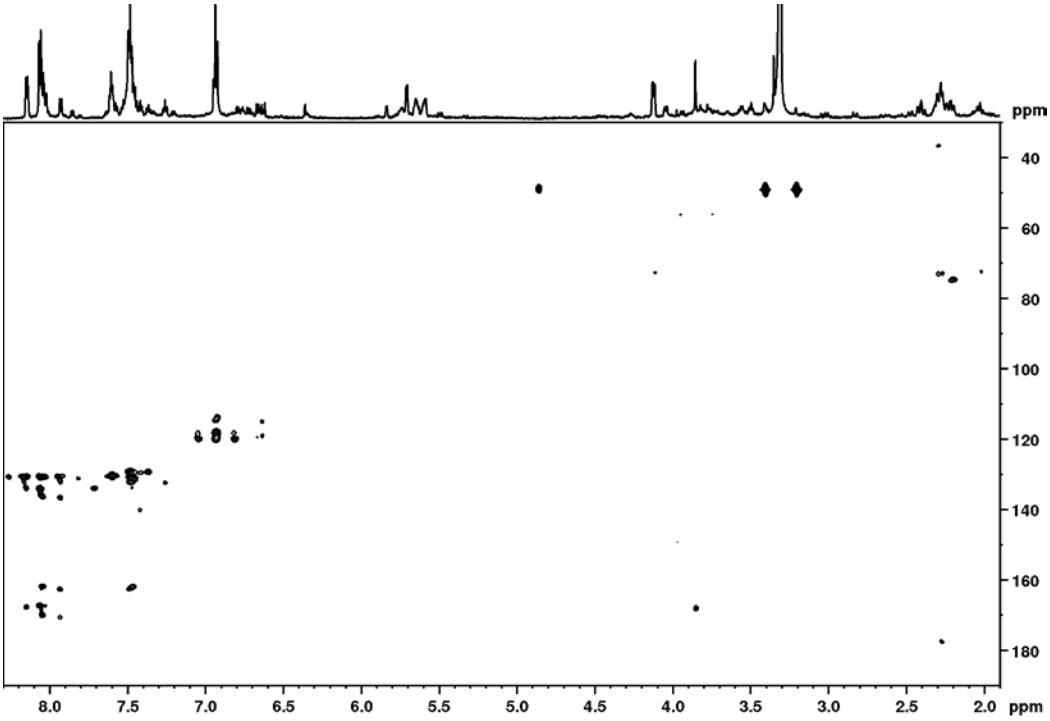


Figure A.10-7 Compound **10**, ^{1}H-^{13}C HMBC spectrum (700 MHz, MeOH-d_4).

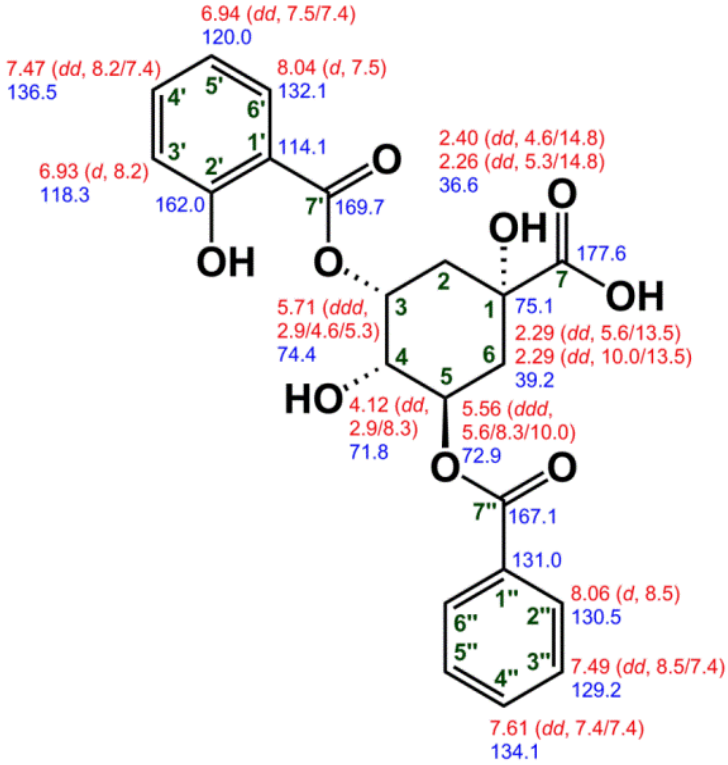


Figure A.10-8 Compound **10**, structure with chemical shifts (MeOH-d_4).

A.11) 4-*O*-Salicyloyl-5-*O*-benzoyl quinic acid (11):

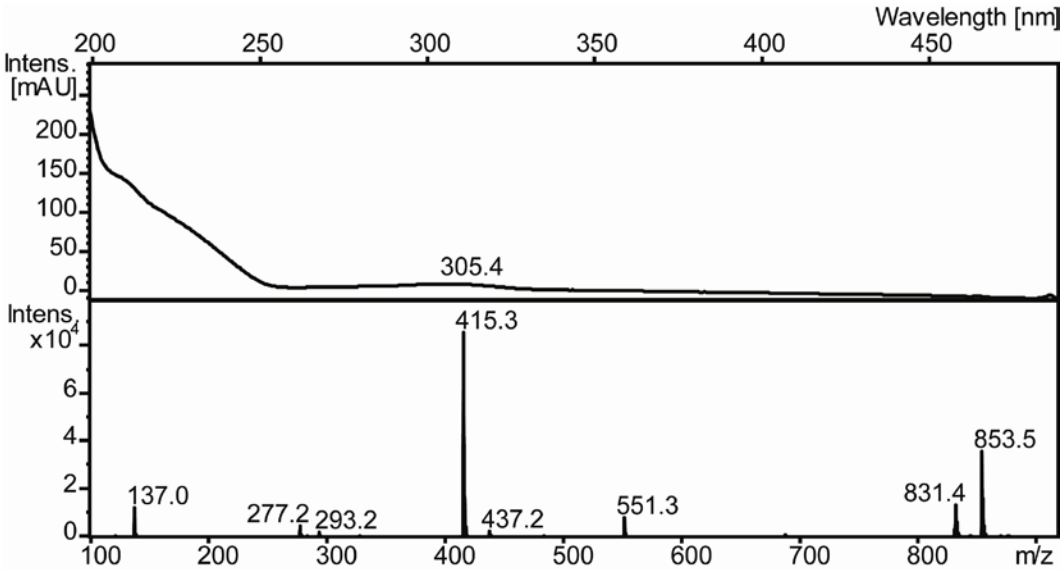


Figure A.11-1 Compound **11**, UV-spectra from HPLC-DAD (top) and mass spectrum (*m/z* 415.3 $[M-H]^-$, bottom).

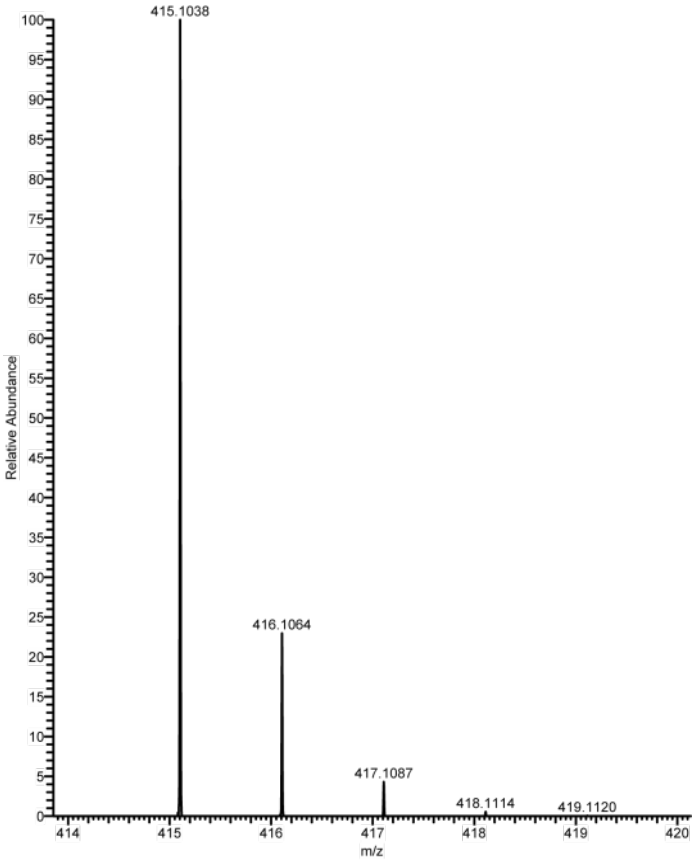


Figure A.11-2 Compound **11**, HRESIMS spectrum, *m/z* 415.1038 $[M-H]^-$.

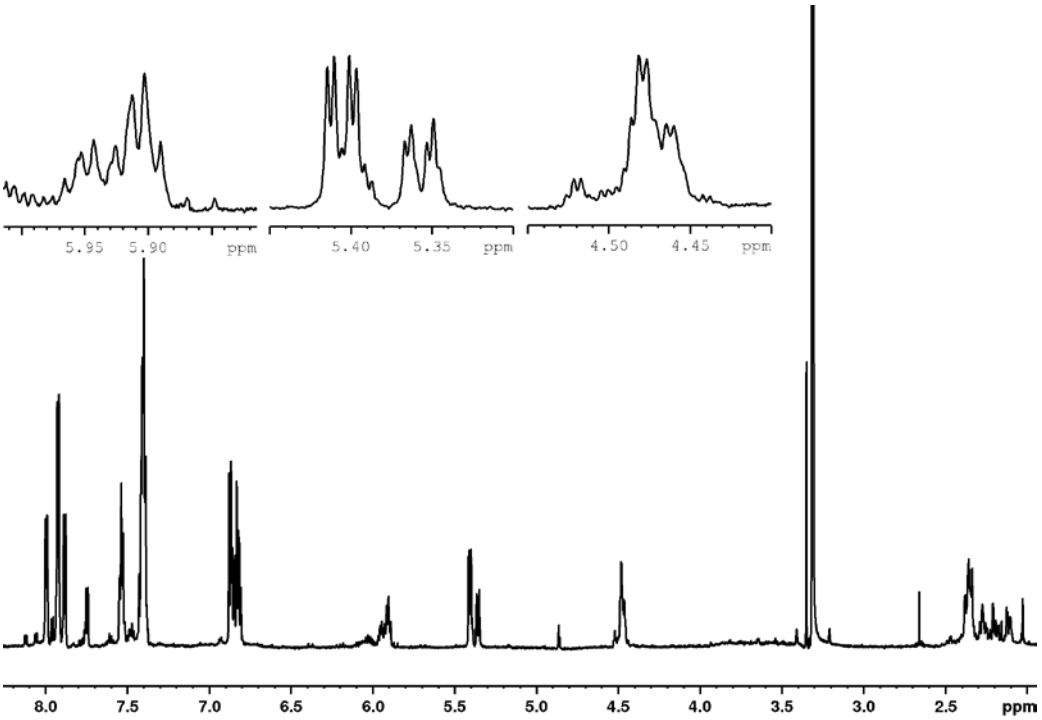


Figure A.11-3 Compound **11**, 1D NOESY spectrum (700 MHz, MeOH-d_4, o1P = 4.92 ppm); signal H-5 (δ_H 5.91), H-4 (δ_H 5.41) and H-3 (δ_H 4.47) are magnified.

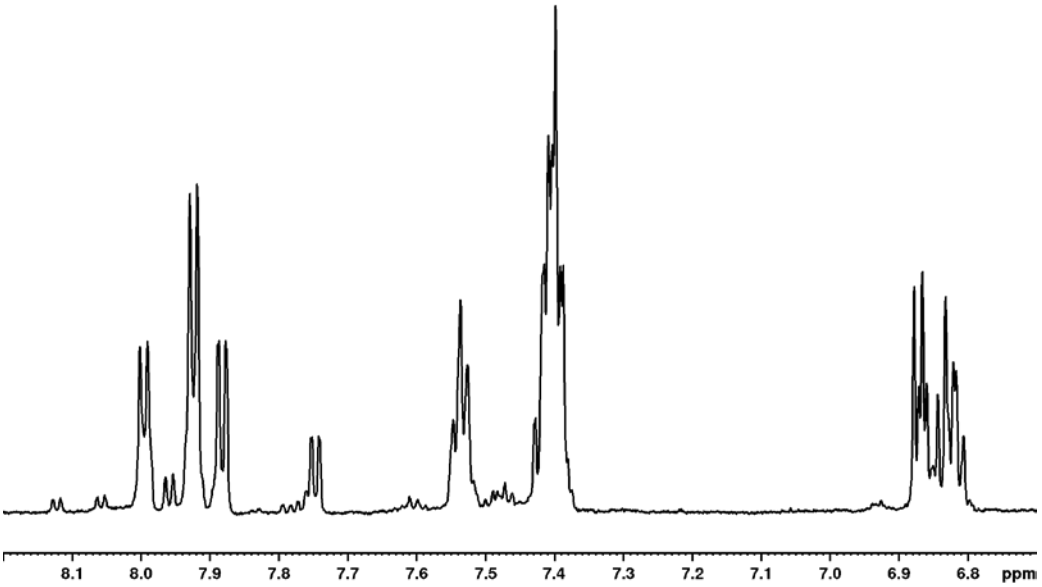


Figure A.11-4 Compound **11**, 1D NOESY spectrum (700 MHz, MeOH-d_4, o1P = 4.92 ppm); region 8.2 to 6.7 ppm.

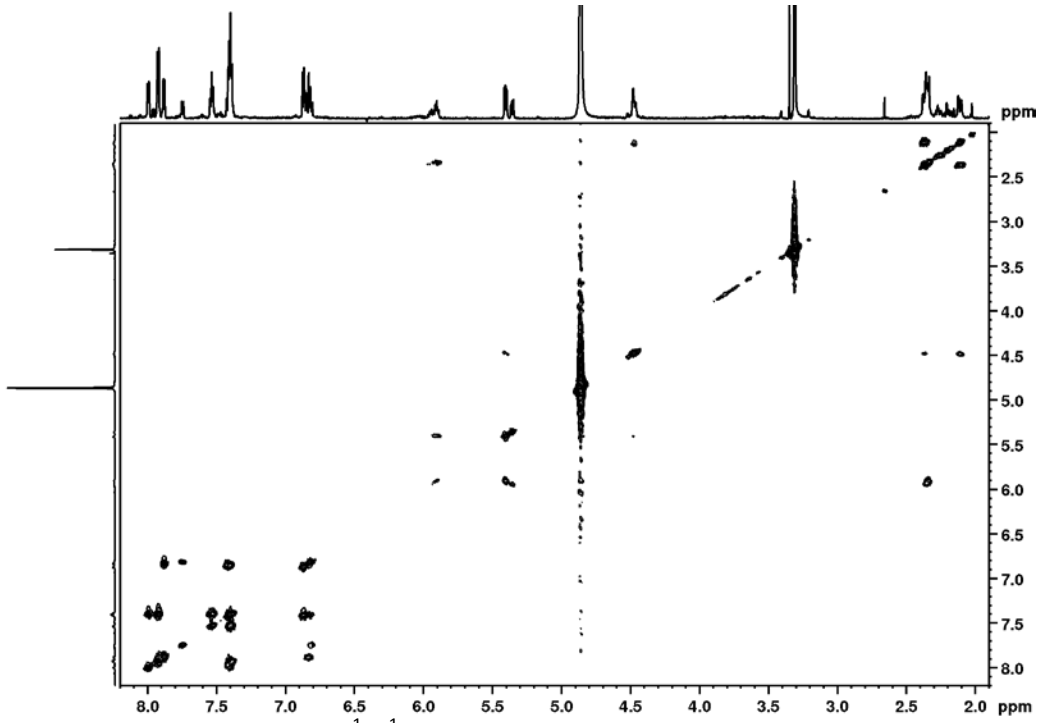


Figure A.11-5 Compound **11**, ^{1}H-^{1}H COSY spectrum (700 MHz, MeOH-d_4).

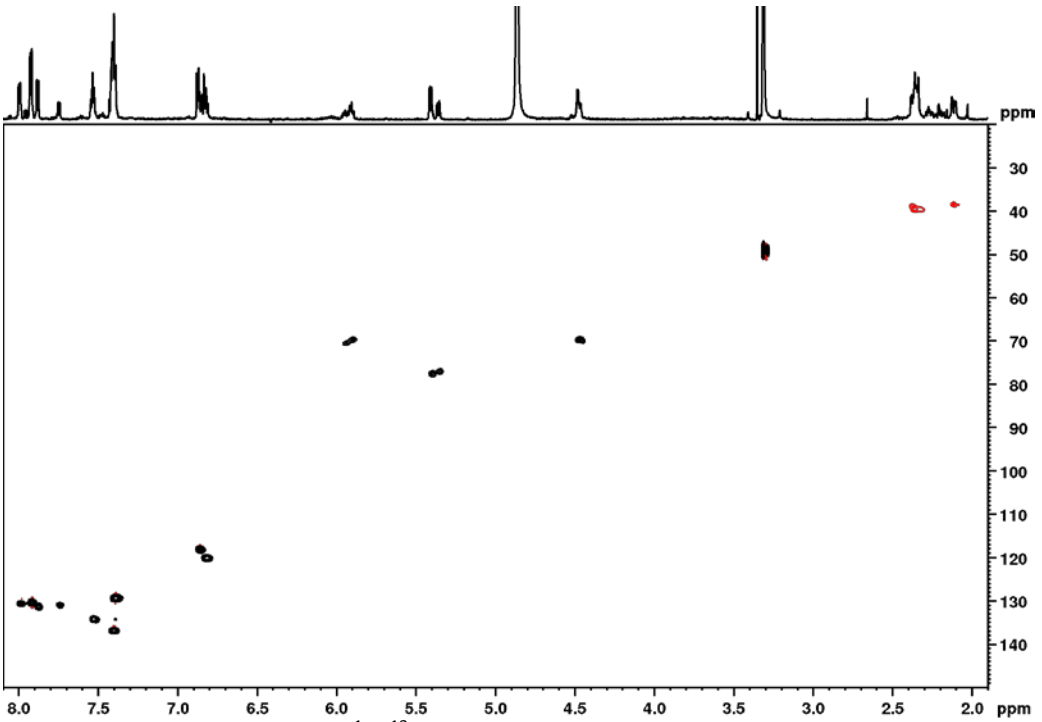


Figure A.11-6 Compound **11**, ^{1}H-^{13}C HSQC spectrum (700 MHz, MeOH-d_4).

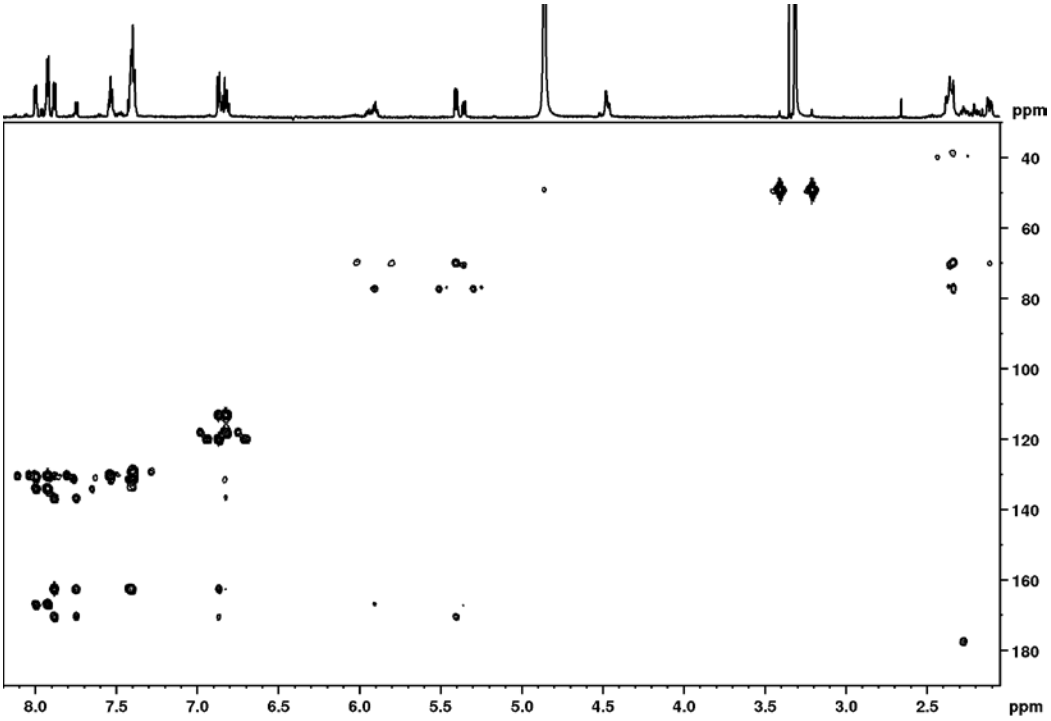


Figure A.11-7 Compound **11**, ^{1}H-^{13}C HMBC spectrum (700 MHz, MeOH-d_4).

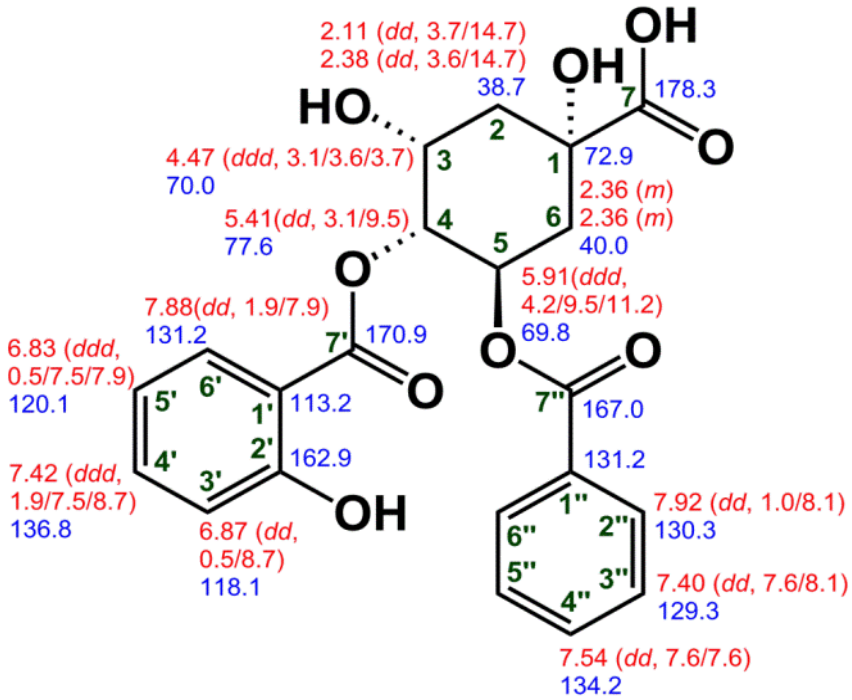


Figure A.11-8 Compound **11**, chemical structure with chemical shifts (MeOH-d_4).

A.12) Salicortin (12):

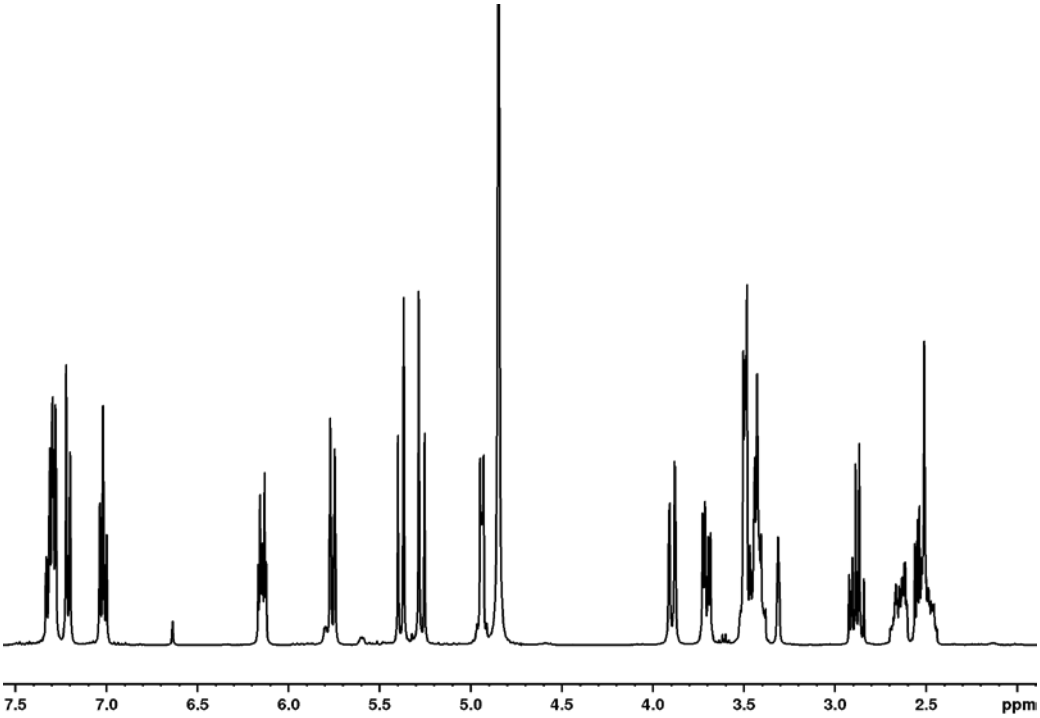


Figure A.12-1 Salicortin (**12**), ^{1}H NMR spectrum (400 MHz, MeOD-d_4).

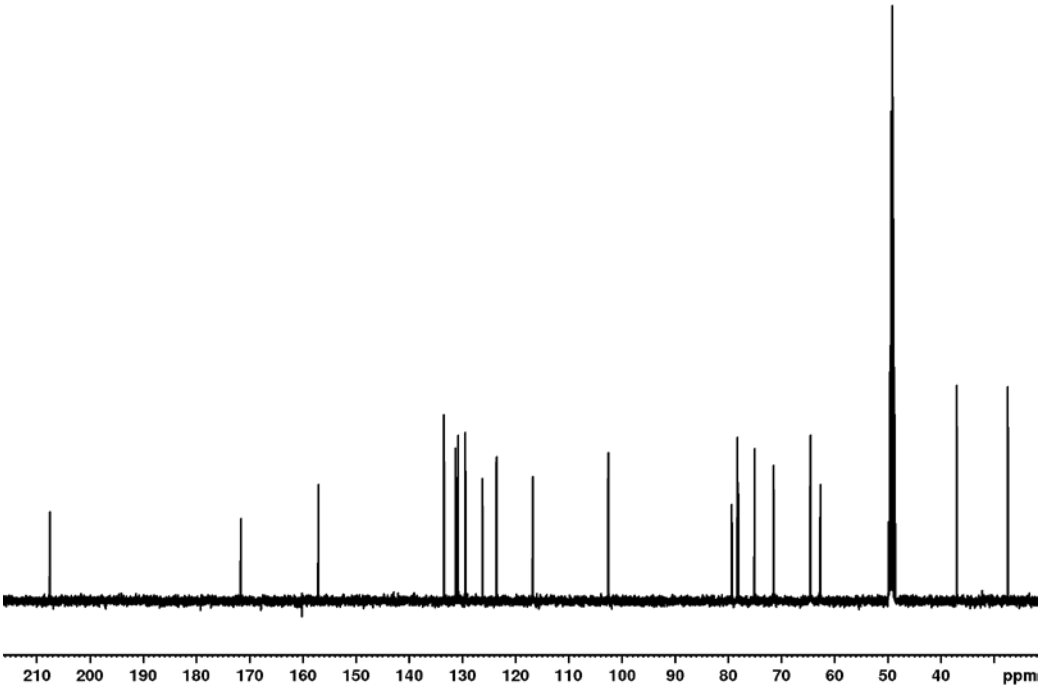


Figure A.12-2 Salicortin (**12**), ^{13}C NMR spectrum (100 MHz, MeOD-d_4).

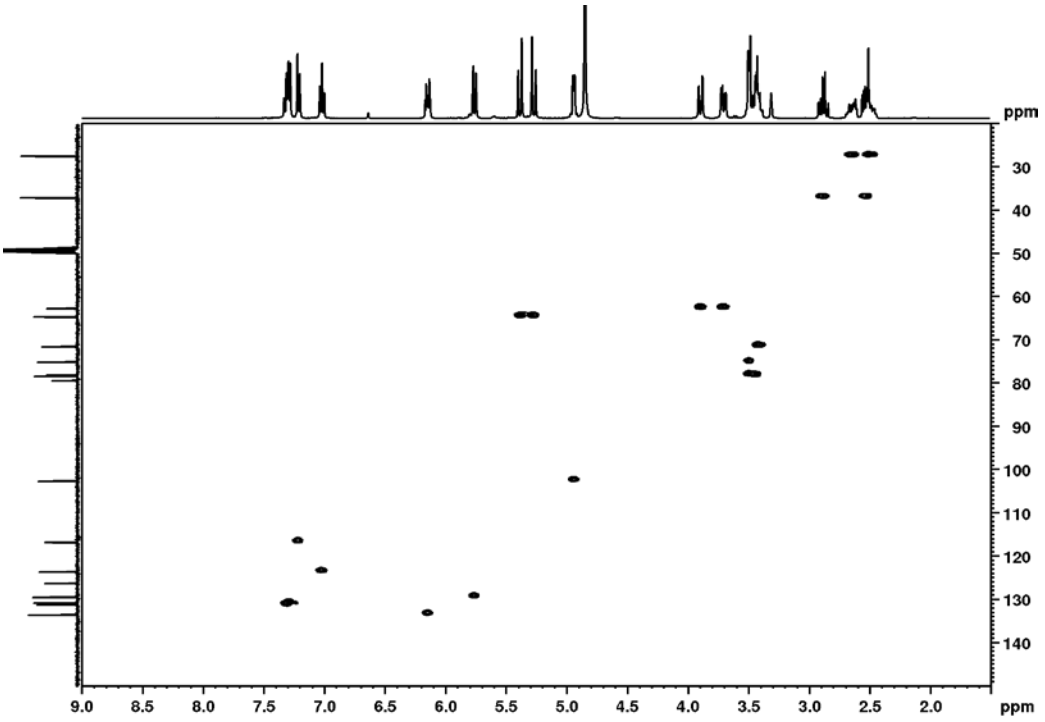


Figure A.12-3 Salicortin (**12**), ^{1}H-^{13}C HSQC spectrum (400 MHz, MeOD-d_4).

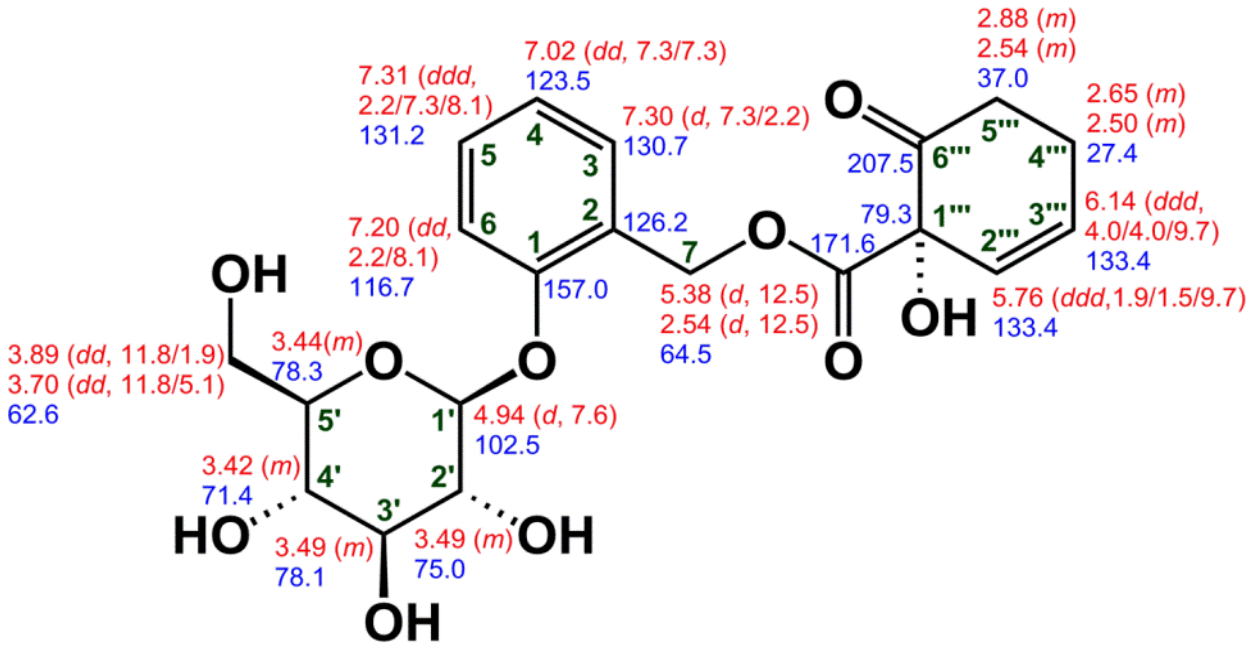


Figure A.12-4 Salicortin (**12**), chemical structure with chemical shifts (MeOH-d_4).

A.13) Quinic acid (13):

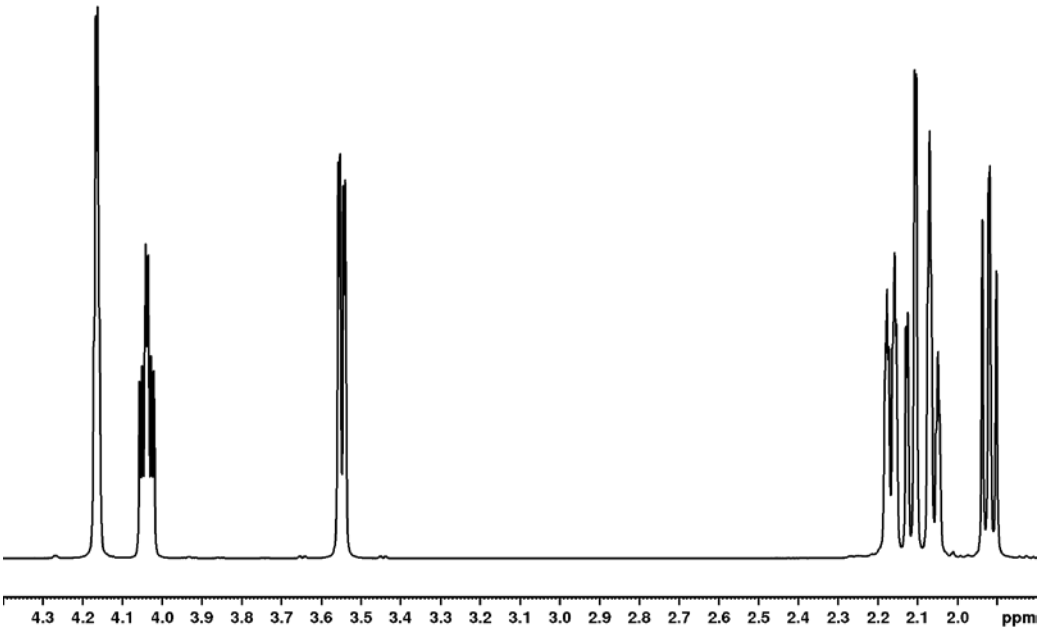


Figure A.13-1 Quinic acid (**13**), ^{1}H NMR spectrum (700 MHz, D_2O).

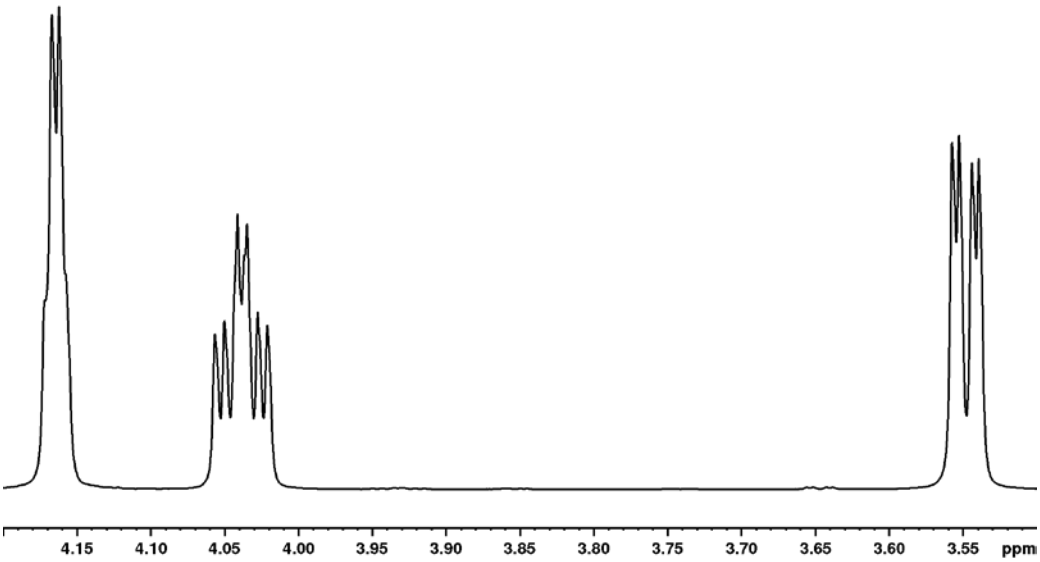


Figure A.13-2 Quinic acid (**13**), Partial ^{1}H NMR spectrum (700 MHz, D_2O); region 3.5 to 4.2 ppm.

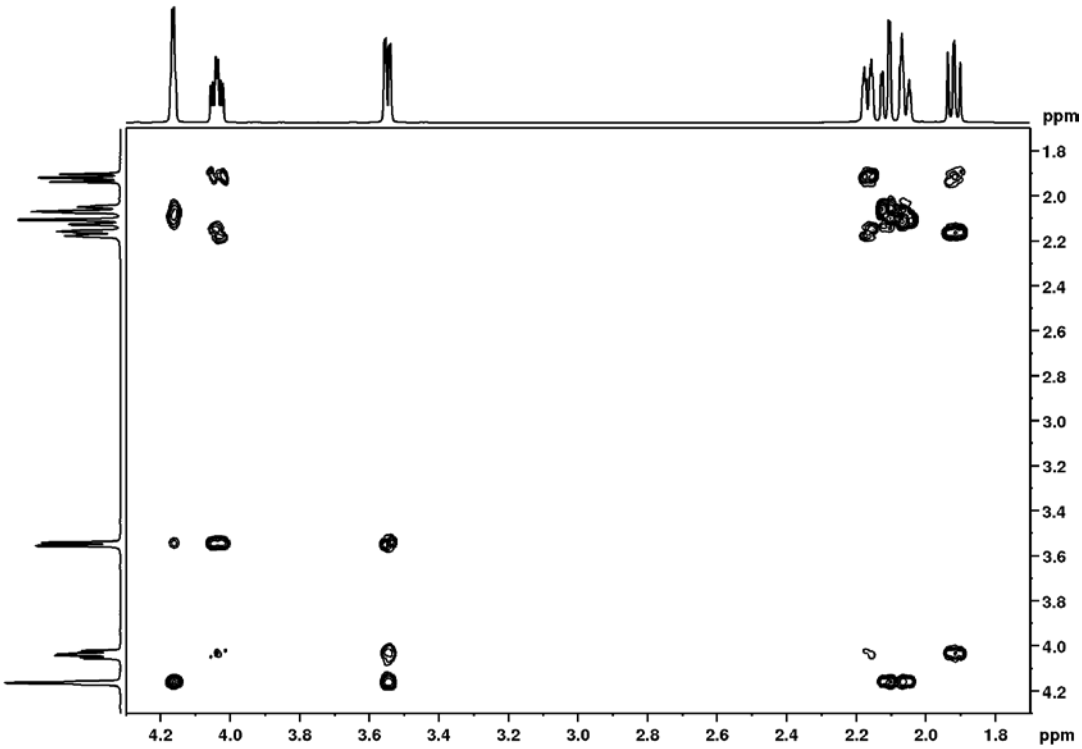


Figure A.13-3 Quinic acid (**13**), ^{1}H-^{1}H COSY spectrum (700 MHz, D_2O).

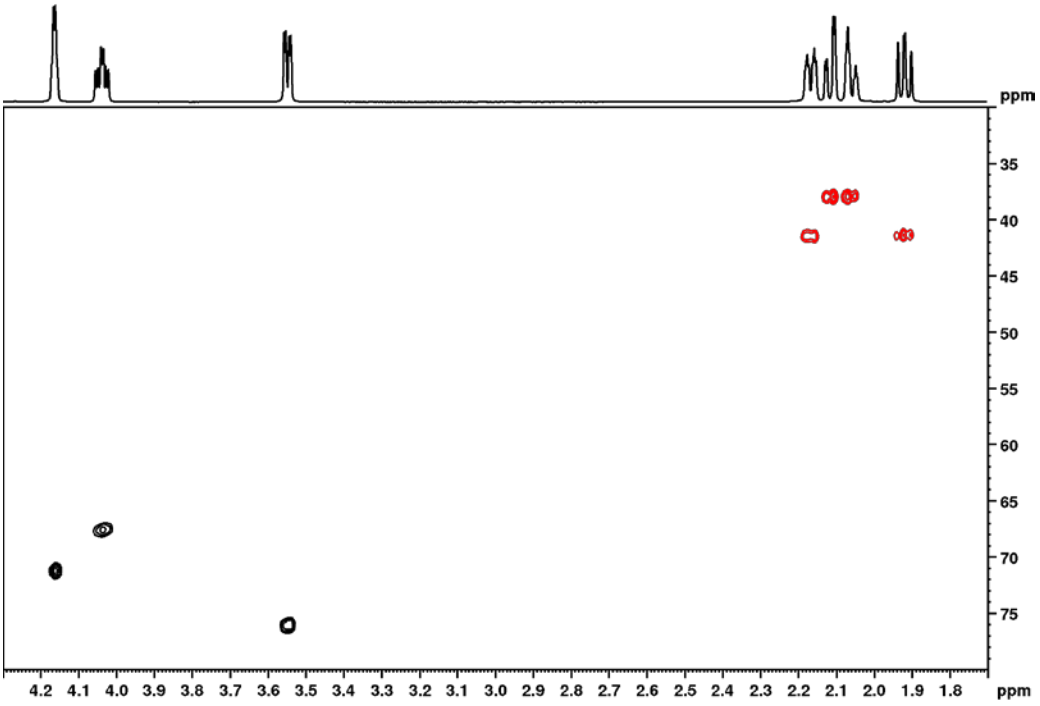


Figure A.13-4 Quinic acid (**13**), ^{1}H-^{13}C HSQC spectrum (700 MHz, D_2O).

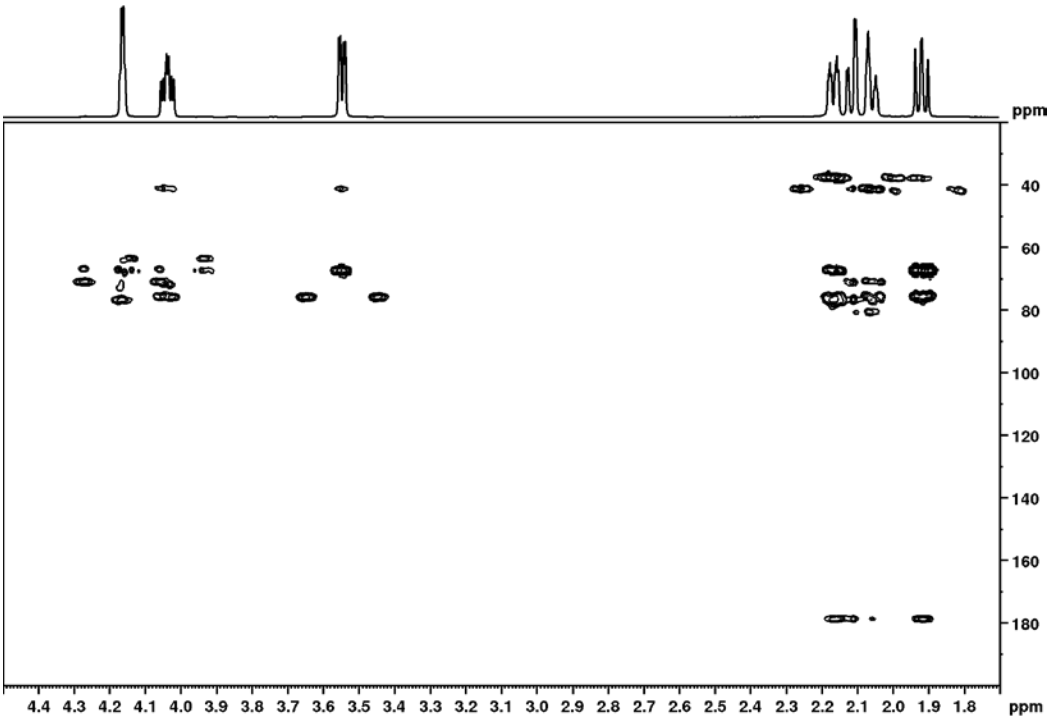


Figure A.13-5 Quinic acid (**13**), ^{1}H-^{13}C HMBC spectrum (700 MHz, D_2O).

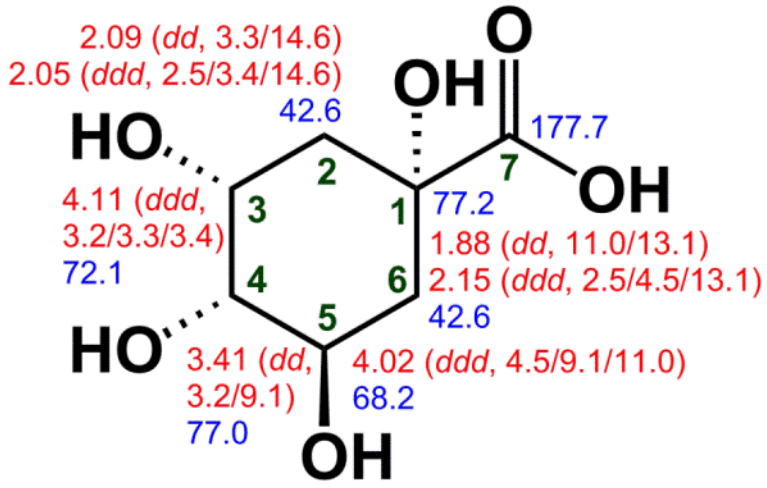


Figure A.13-6 Quinic acid (**13**), chemical structure with chemical shifts (D_2O).

B) *In vivo* ^{13}C-labeling of salicortin (12)

B.1) Growth enclosure – Labeling process

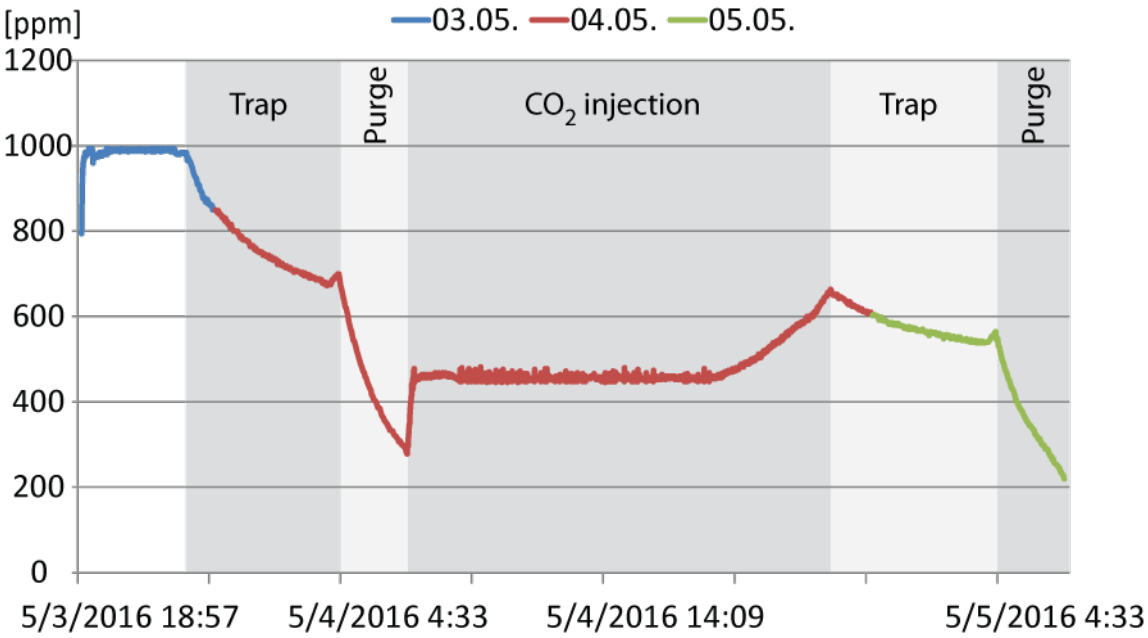


Figure B.1-1 CO_2-curve [in ppm] of the 1st (blue), 2nd (red) and 3rd (green) experimental day.

- The highlighted areas represent different daily modes of action of the labeling setup:

  Purge (4:30-7:00) – strong CO_2 scrubbing with soda lime

  Trap (22:30-4:15) – weak CO_2 scrubbing and trapping with saturated $Ba(OH)_2$ solution

  CO_2 injection (7:00-22:00) –automatized pulse-labeling with $^{13}CO_2$ gas from lecture bottle

- The CO_2 level was set to 450 ppm. The CO_2-IR sensor probe did not discriminate between $^{12}CO_2$ and $^{13}CO_2$. Therefore the CO_2-value was corrected by use of the isotope ratio factor for $^{13}CO_2$:$^{12}CO_2$:

  Gas bottle with a $^{13}CO_2$ conc. of 380 ppm and constant volume flow gave a mean signal of 57.09 ppm, yielding an isotope factor of 6.7:1 ($^{12}CO_2$:$^{13}CO_2$).

- $^{13}CO_2$ bottles were empty at day 12 and 19 and therefore exchanged for full bottles (see Fig. B.1-2).
- During the night between day 21 and 22, leaf samples were taken in order to distinguish the ^{13}C-enrichment (see B.2).
- Last bottle was emptied at early evening of day 25. Afterwards plants were kept in the chamber for further 3 days in order to consume the remaining $^{13}CO_2$.

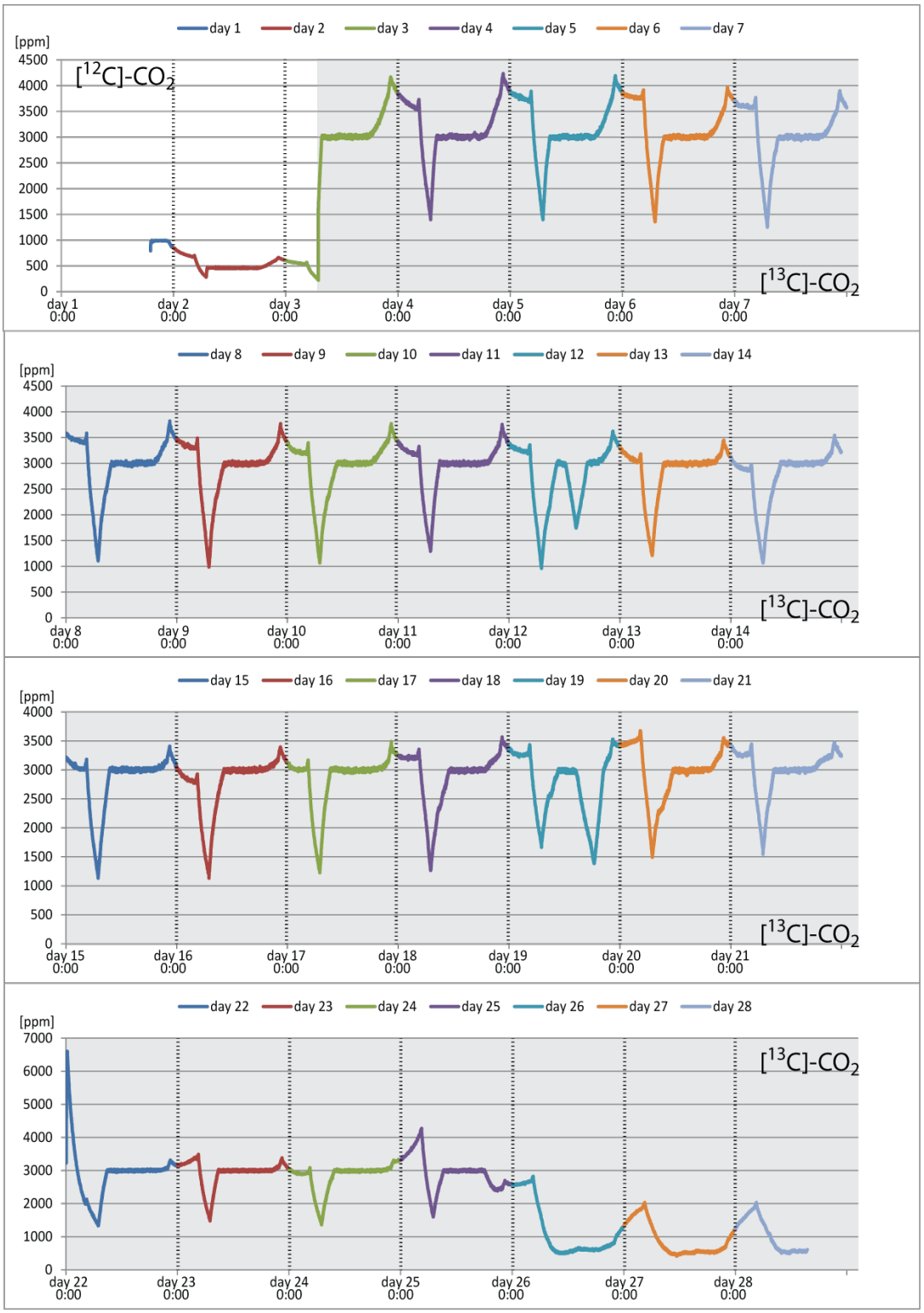


Figure B.1-2 Overall CO_2-curve [in ppm] of the $^{13}CO_2$ labeling experiment.

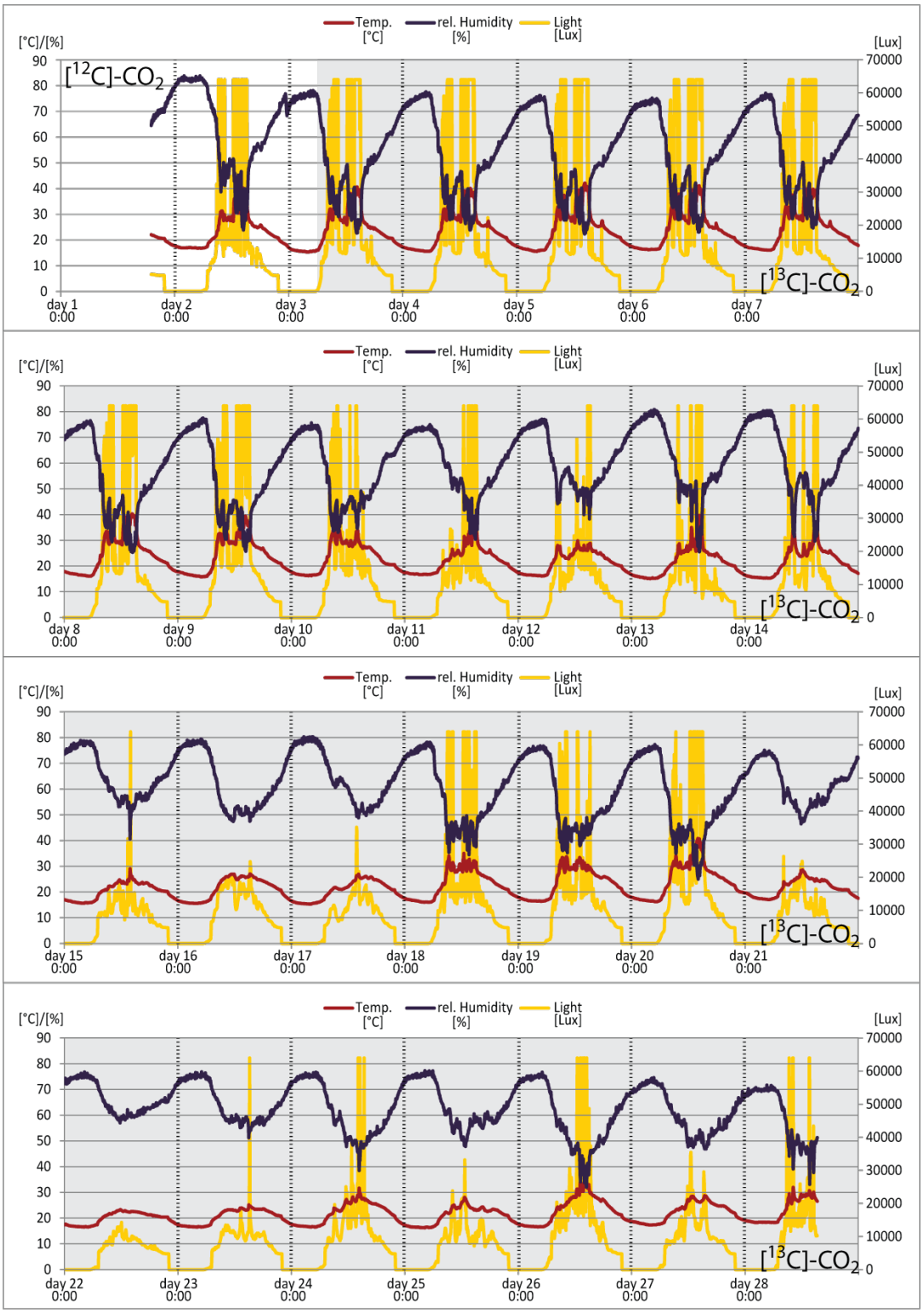


Figure B.1-3 Overall temperature [°C], rel. humidity [%] and light-curve [Lux] of the $^{13}CO_2$ feeding experiment.

Figure B.1-4 Pictures of the automated growth enclosure for stable isotope $^{13}CO_2$ labeling experiments.

Left – Enclosure located in the greenhouse of the MPI-CE during $^{13}CO_2$ labeling of *P. beaupré.*
Right – The chamber is equipped with six specimen of *P. beaupré* during the labeling experiment.

B.2) Characterization of in vivo generated [U-^{13}C]salicortin

In order to investigate the ^{13}C enrichment, leaf samples of old (source) and young, newly grown (sink) leaf tissue were collected during the night between days 21 and 22, lyophilized and balanced, yielding 42.58 mg (source) and 45.02 mg (sink) dried material. Afterwards, the samples were transferred into homogenizer vials (2 mL) with ZrO_2 beats (1.4 mm, 700 mg), homogenized 3 times (60 sec; 5000 rpm; 15 sec breaks) with 70% MeOH (1 mL) and subsequently centrifuged (10 min, 13200 rcf). The supernatant was collected and transferred to LC-MS (10 µL injection) and NMR.

HPLC-ESI-MS was performed on an Agilent 1100 HPLC system, consisting of a degasser, quaternary solvent delivery pump G1311A, an autosampler G1313A (Agilent Technologies, Waldbronn, Germany), a photodiode array detector (detection 200-700 nm; J&M Analytik, Aalen, Germany) and an Esquire 3000 ion trap mass spectrometer (Bruker Daltonik, Bremen, Germany). An Isis RP-18e column (250 x 4.6 mm, 5 µm particle size) (Macherey-Nagel, Düren, Germany) was used for separation. Column temperature was set to 35 °C, and the solvent flow rate was 0.8 mL min^{-1} using 0.1% formic acid in water and 0.1% formic acid in MeOH as binary solvent system. An HPLC gradient was used starting with a 5 min isocratic flow of 100% H_2O, and then decreasing linearly for 5 min to 85%, 25 min to 70% and finally 50 min to

50% H_2O. Afterwards, the column was washed for 10 min with 100% MeOH and equilibrated for 10 min with 100% H_2O.

NMR spectra were recorded on a Bruker Avance III HD 400 MHz spectrometer equipped with a 5 mm BBFO probe (Bruker Biospin, Rheinstetten, Germany). NMR tubes of 5 mm outer diameter were used for measurements.

Identification and characterization was done by means of ^{1}H, ^{13}C and ^{1}H-^{13}C NMR (Fig. B.2-6 to B.2-8) and ESI-MS in comparison to spectra of an unlabeled reference (B.2-1 to B.2-5). The ^{1}H-^{13}C HSQC was in accordance with the spectrum of the reference, confirming its identity as salicortin (**12**, spectra see A.12). Furthermore, as a result of spin-spin coupling between adjacent ^{13}C atoms, the ^{13}C NMR spectrum showed multiplet signal structures for every carbon resonance in the molecule (Schneider, 2007; Schneider et al., 2003) (Fig. B.2-9). These characteristic multiplets indicated uniform ^{13}C-incorporation into the molecule. That observation was confirmed by the ^{1}H NMR spectra, which also showed numerous satellites for all signals resulting from ^{1}H-^{13}C spin-spin couplings.

The mass spectra obtained from extracts of young leaf tissues displayed peaks corresponding to the salicortin isotopologues of *m/z* 423 to 444 (Table B.2-1). An average ^{13}C enrichment of 82% (R^2 = 0.994) was calculated based on the comparison of theoretical and experimental MS data, according to a method previously described (Taubert et al., 2011). Accordingly, the isotopologue patterns of characteristic salicortin fragments, generated by in-source fragmentation, were extracted from the mass spectra (Table B.2-2) and used to determine the ^{13}C enrichment of the different parts of the molecule. The calculation yielded an average ^{13}C enrichment of 82% for the glucose (*m/z* 161 $[M\text{-}262\text{-}H]^-$; R^2 = 0.987) as well as the 1-hydroxy-6-oxocyclohex-2-en-1-oyl (HCH) fragment (*m/z* 111 $[M\text{-}312\text{-}H]^-$; R^2 = 0.952) and 81% for the salicin fragment (*m/z* 285 $[M\text{-}138\text{-}H]^-$; R^2 = 0.947).

The salicortin mass spectrum from the extract of old leaves, however, also showed an isotopologue pattern which reached from *m/z* 423 to 443. Unlike the molecular ion peaks in the spectra of young plant tissue, the molecular ion peak *m/z* 423 $[M\text{-}H]^-$ is the by far most intense signal in the mass spectrum of old leaves (Table B.2-1). Furthermore, the signal intensity of the ^{13}C-enriched isotopologues appeared to be notably weak, which assumed that only a minor amount of $^{13}CO_2$ had been incorporated. The calculation of ^{13}C-enrichment yielded 1.1% (R^2 = 0.989), confirming that assumption.

References:

Schneider, B., 2007. Nuclear magnetic resonance spectroscopy in biosynthetic studies. Prog. Nucl. Magn. Reson. Spectrosc. 51, 155-198.

Schneider, B., Gershenzon, J., Graser, G., Hölscher, D., Schmitt, B., 2003. One-dimensional ^{13}C NMR and HPLC-^{1}H NMR techniques for observing carbon-13 and deuterium labelling in biosynthetic studies. Phytochem. Rev. 2, 31-43.

Taubert, M., Jehmlich, N., Vogt, C., Richnow, H.H., Schmidt, F., von Bergen, M., Seifert, J., 2011. Time resolved protein-based stable isotope probing (Protein-SIP) analysis allows quantification of induced proteins in substrate shift experiments. Proteomics 11, 2265-2274.

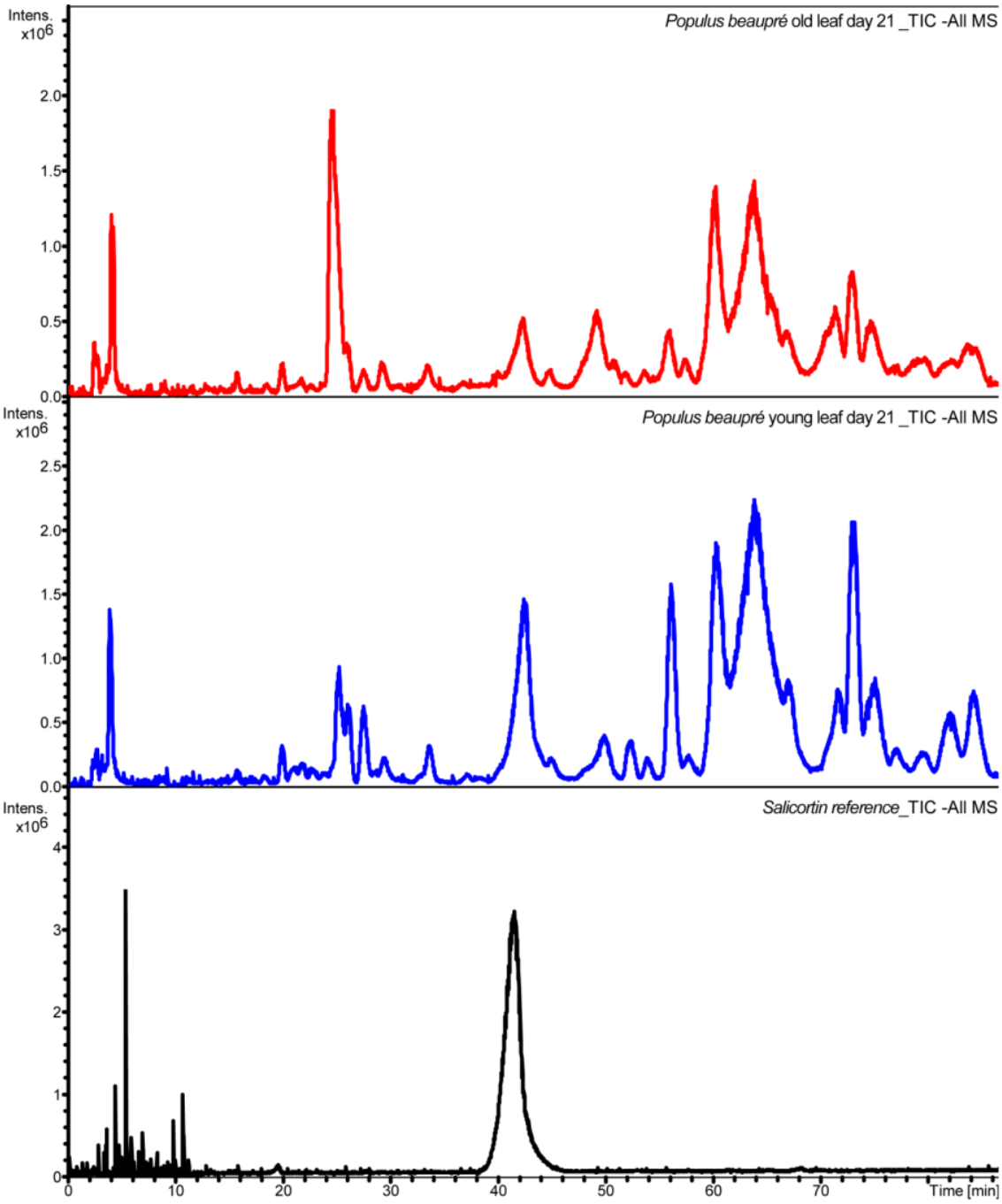


Figure B.2-1 Stacked TIC spectra of young (blue) and old (red) leaf sample extracts of *P. beaupré* in comparison with a salicortin (**12**) reference (black).

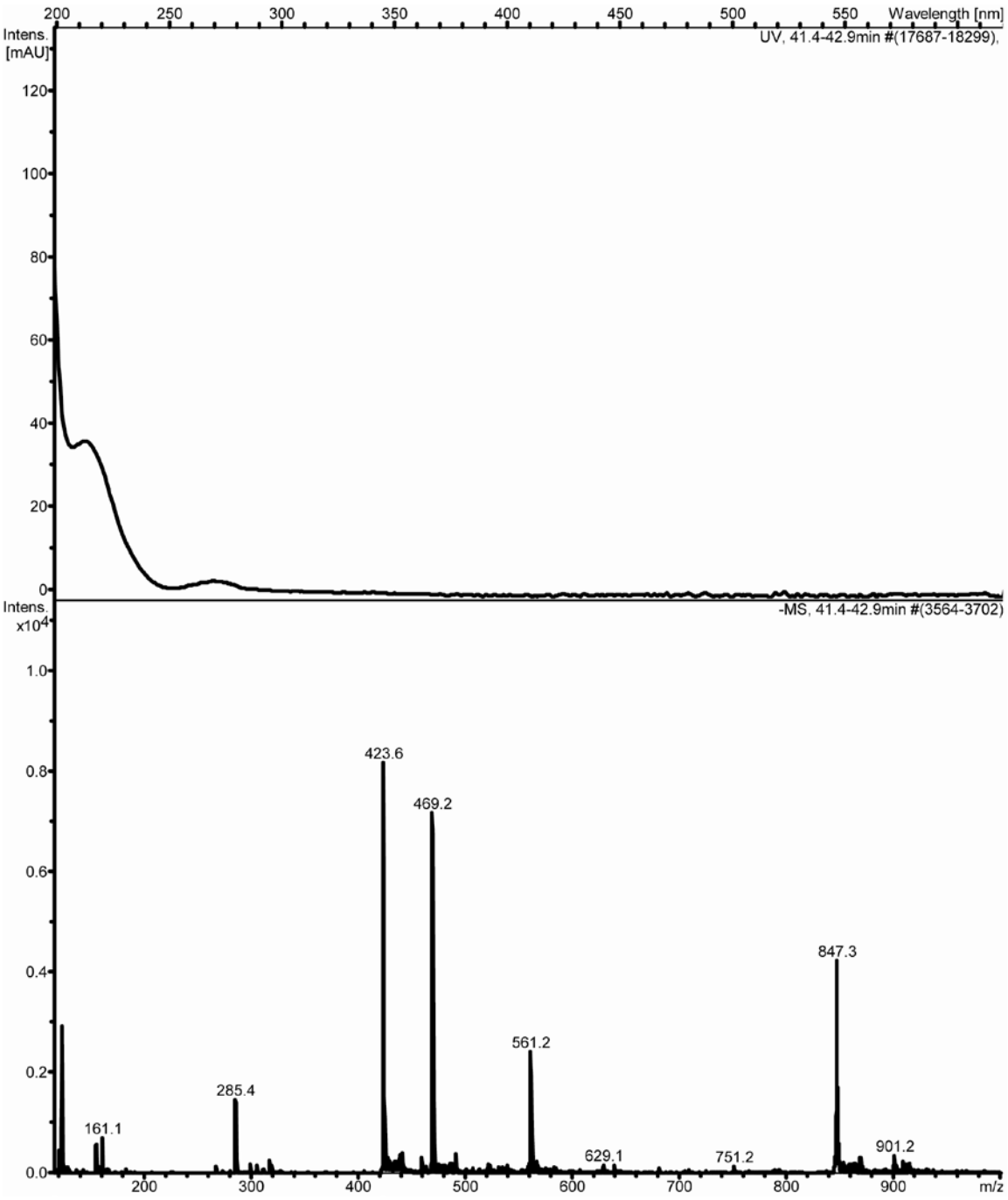


Figure B.2-2 UV spectrum (top) and ESI ion trap mass spectrum (bottom) of salicortin (**12**) (*m/z* 423.6 $[M-H]^-$, $^{12}C_{20}H_{23}O_{10}$) in the old leaf sample.

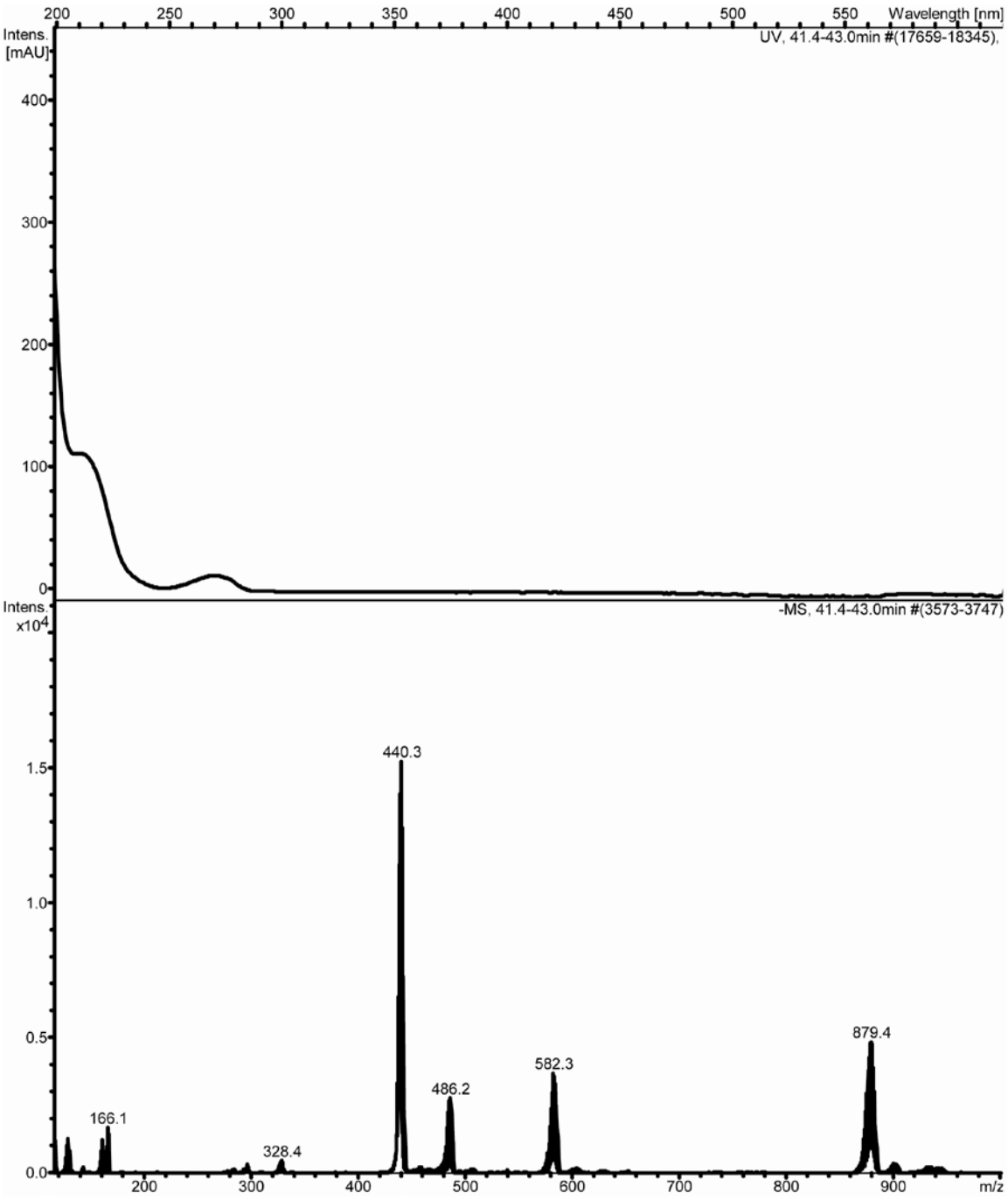


Figure B.2-3 UV spectrum (top) and ESI ion trap mass spectrum (bottom) of salicortin (**12**) (*m/z* 440.3 $[M-H]^-$, $^{13}C_{17}{}^{12}C_3H_{23}O_{10}$) in the young leaf sample.

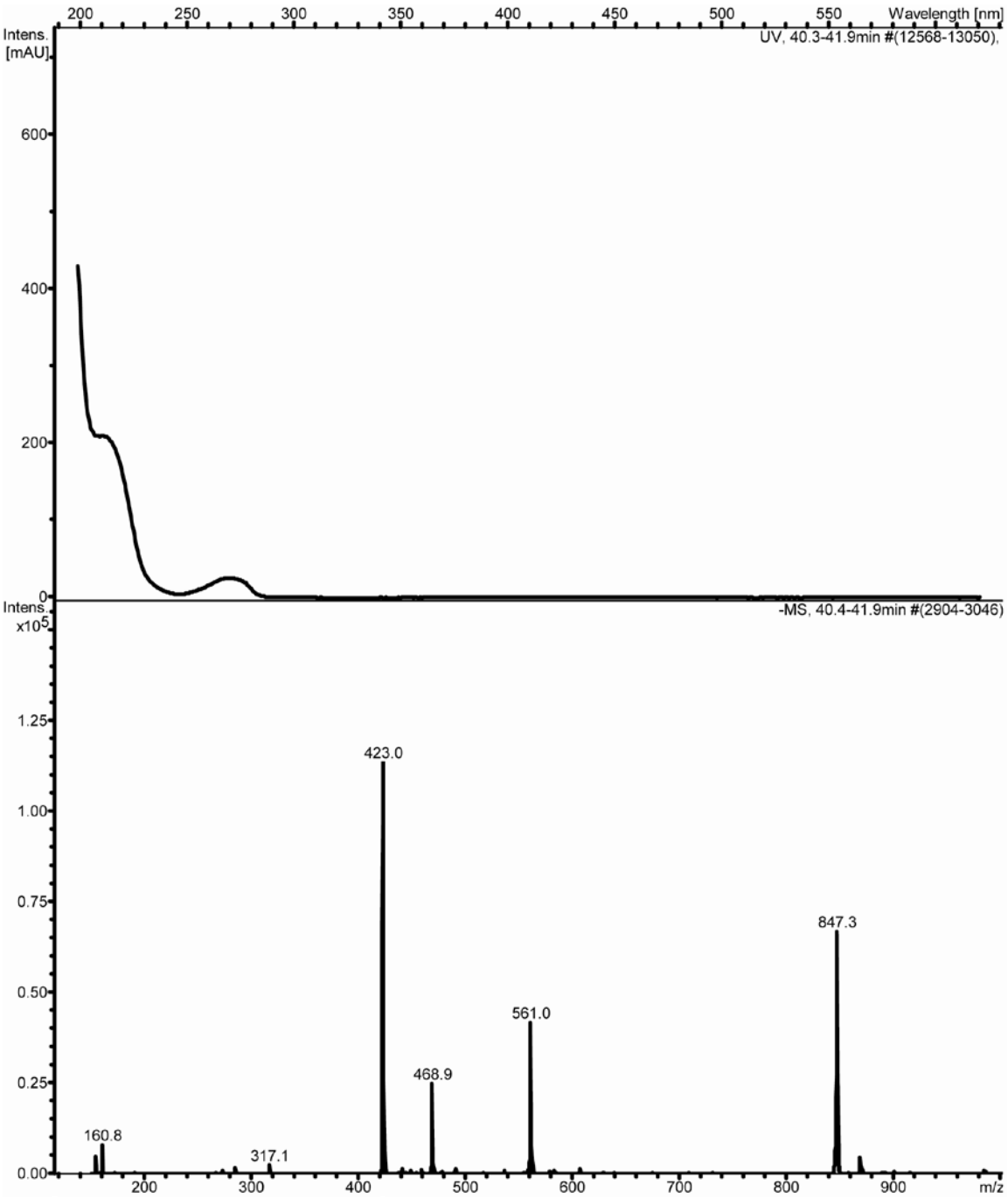


Figure B.2-4 UV spectrum (top) and ESI ion trap mass spectrum (bottom) of the salicortin (**12**) reference (*m/z* 423.6 $[M-H]^-$, $^{12}C_{20}H_{23}O_{10}$).

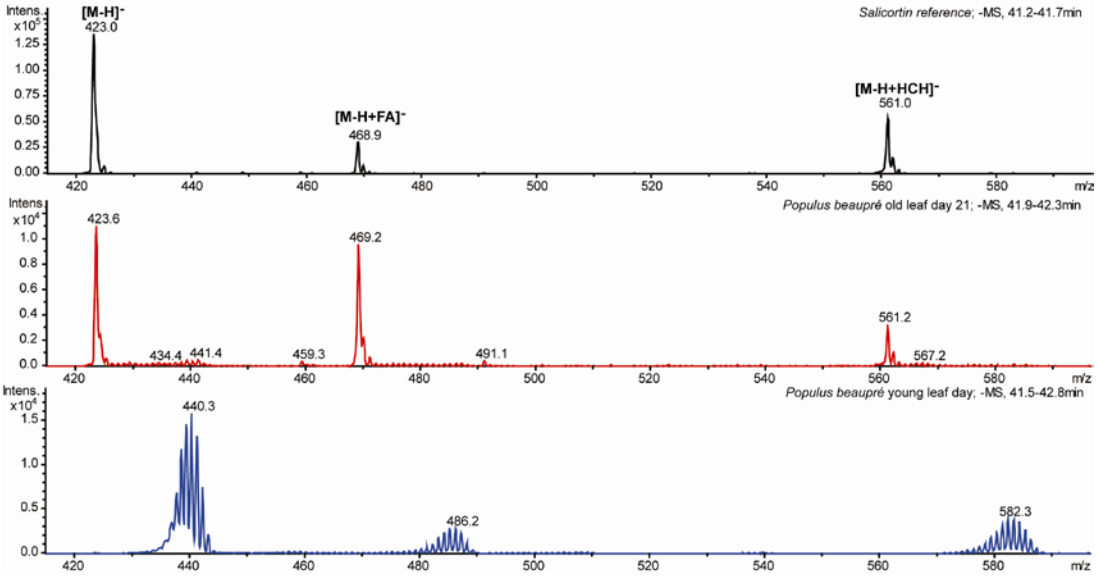
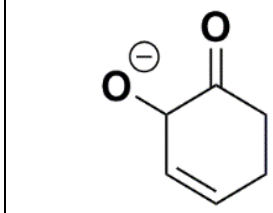
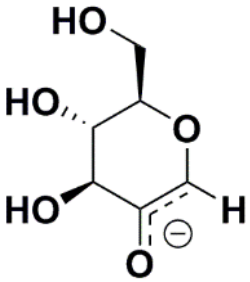
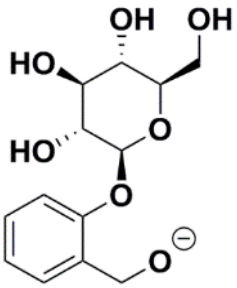
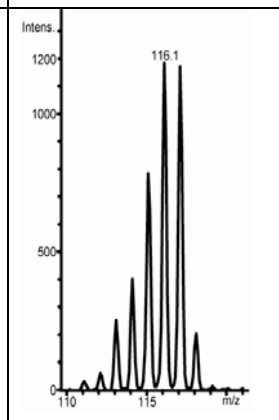
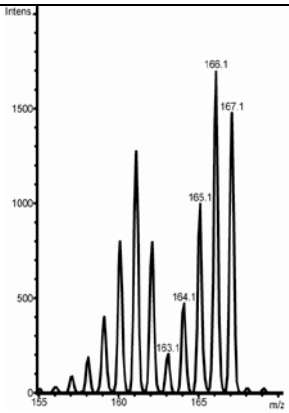
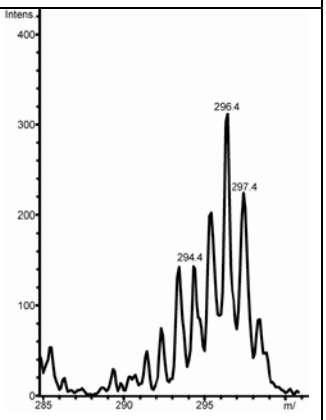


Figure B.2-5 Stacked mass spectra of salicortin (**12**) from young (blue) and old (red) leaf sample extract in comparison with a salicortin (**12**) reference (black). The mass range from *m/z* 420 to 600 is displayed.

Table B.2-1 Extracted MS data which were used for the calculation of the ^{13}C-enrichment of the salicortin isotopologues (*m/z*) together with their signal intensity and their signal-to-noise ratio (S/N). Only signals of S/N > 2 were extracted. *Italic* values were artificially added for the calculation.

C-isotope		Salicortin in old leaves				Salicortin in young leaves		
^{12}C	^{13}C	*m/z*	intensity	S/N		*m/z*	intensity	S/N
20	0	423.6	10223	468.9		*423.0*	*0*	-
19	1	424.4	2321	106.5		*424.0*	*0*	-
18	2	425.4	611	28.0		*425.0*	*0*	-
17	3	426.3	191	8.8		*426.0*	*0*	-
16	4	427.3	227	10.4		*427.0*	*0*	-
15	5	428.4	237	10.9		*428.0*	*0*	-
14	6	429.4	243	11.2		*429.0*	*0*	-
13	7	430.4	202	9.3		430.6	109	2.1
12	8	431.4	137	6.3		431.7	139	2.7
11	9	432.4	152	7.0		432.5	194	3.7
10	10	433.4	208	9.5		433.7	335	6.4
9	11	434.4	270	12.4		435.0	654	12.5
8	12	435.4	233	10.7		435.9	1420	27.2
7	13	436.4	259	11.9		436.9	3370	64.5
6	14	437.4	244	11.2		437.8	6567	125.7
5	15	438.4	341	15.7		438.6	11446	219.0
4	16	439.4	433	19.8		439.4	14074	269.3
3	17	440.4	381	17.5		440.3	15363	294.0
2	18	441.4	447	20.5		441.3	12920	247.2
1	19	442.4	196	9.0		442.3	7304	139.8
0	20	443.4	106	4.9		443.3	2079	39.8

Table B.2-2 Isotopologue patterns (*m/z*) observed for the fragment ions of labeled salicortin (**12**). MS data were used to calculate the ^{13}C-enrichment. Only signals of S/N > 1 were extracted from the mass spectra. *Italic* values were artificially added for the calculation. Values marked with (*) resulted from overlapping signals and were assumed as zero for calculation.

	HCH fragment ion			glucose fragment ion			salicin fragment ion		
									
									
^{13}C	*m/z*	intensity	S/N	*m/z*	intensity	S/N	*m/z*	intensity	S/N
0	111	*0*	-	161.1	(1274)*	(24.7)*	285	*0*	-
1	112.1	63	1.2	162.1	(797)*	(15.5)*	286	*0*	-
2	113.1	254	4.9	163.1	206	4.0	287	*0*	-
3	114.1	404	7.8	164.1	473	9.2	288	*0*	-
4	115.1	785	15.2	165.1	1000	19.4	289	*0*	-
5	116.1	1184	23.0	166.1	1701	33.0	290	*0*	-
6	117.1	1175	22.8	167.1	1479	28.7	291	*0*	-
7							292.3	75	1.5
8							293.4	143	2.8
9							294.4	144	2.8
10							295.4	202	3.9
11							296.4	313	6.1
12							297.4	224	4.3
13							298.3	85	1.7

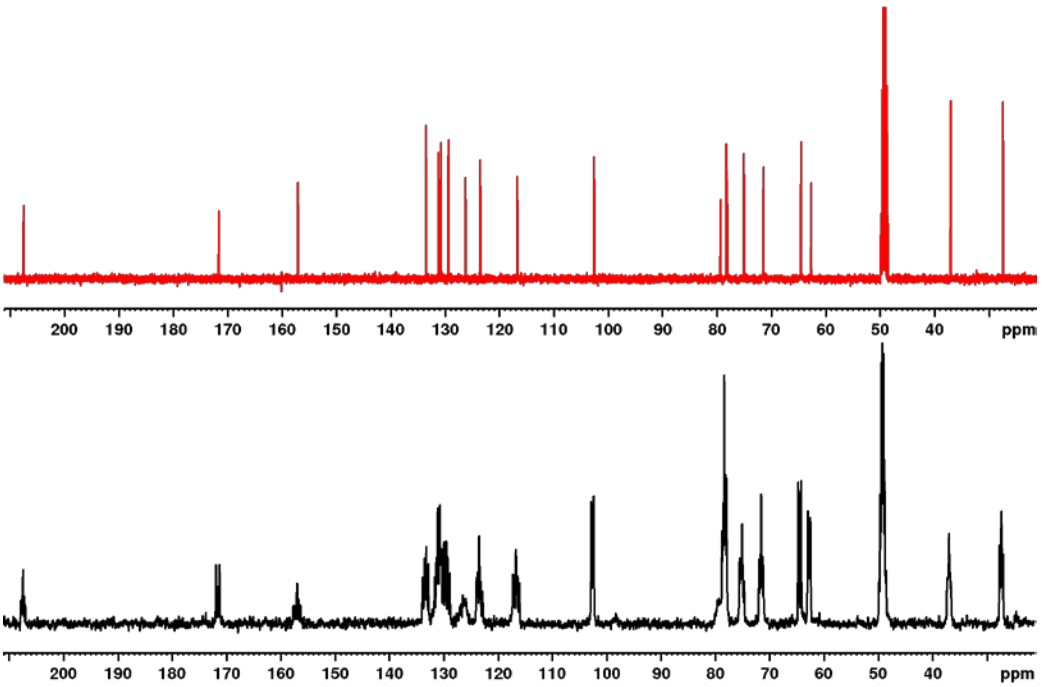


Figure B.2-6 ^{13}C NMR spectra (100 MHz, MeOH-d_4) of salicortin (**12**) isolated from young leaf tissue of the ^{13}C- enriched *P. beaupré* plants (black) and the reference (red).

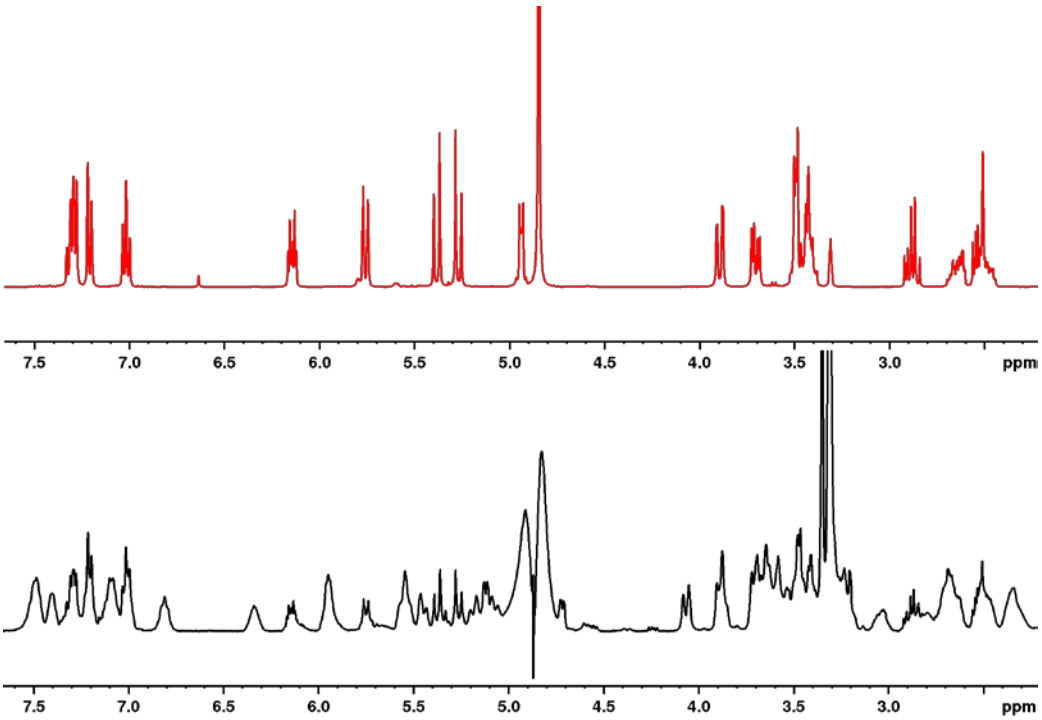


Figure B.2-7 ^{1}H NMR spectra (400 MHz, MeOH-d_4) of salicortin (**12**) isolated from young leaf tissue of the ^{13}C- enriched *P. beaupré* plants (black; with water suppression o1p = 4.887 ppm) and the reference (red).

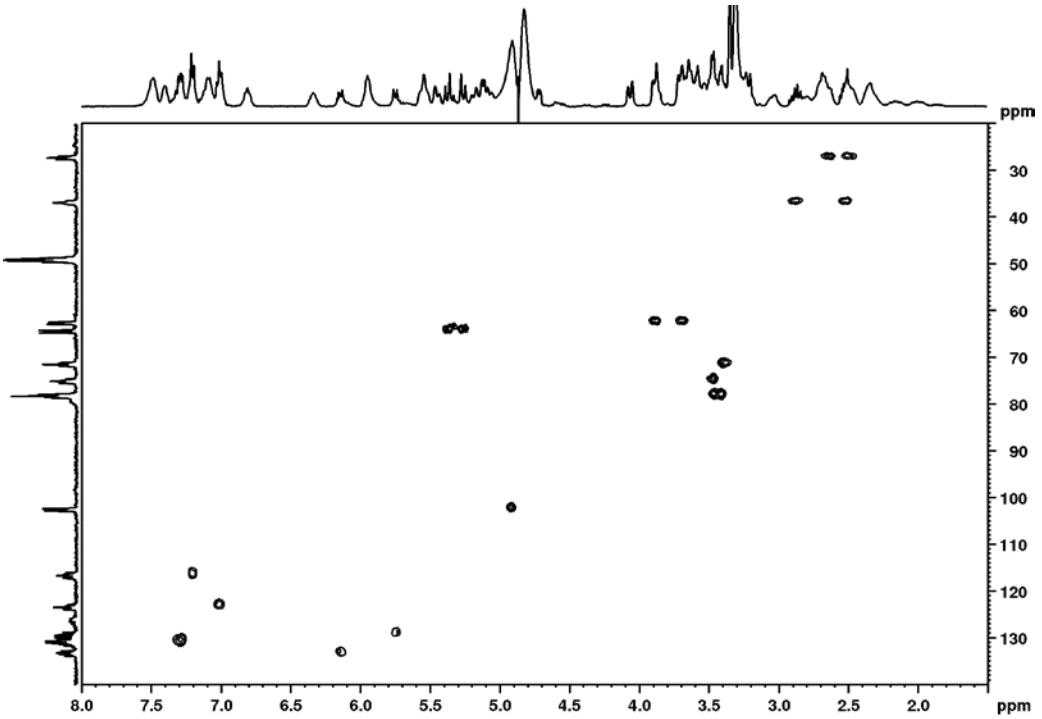


Figure B.2-8 ^{1}H-^{13}C HSQC spectrum of salicortin (**12**) isolated from young leaf tissue of the ^{13}C-enriched *P. beaupré* plants.

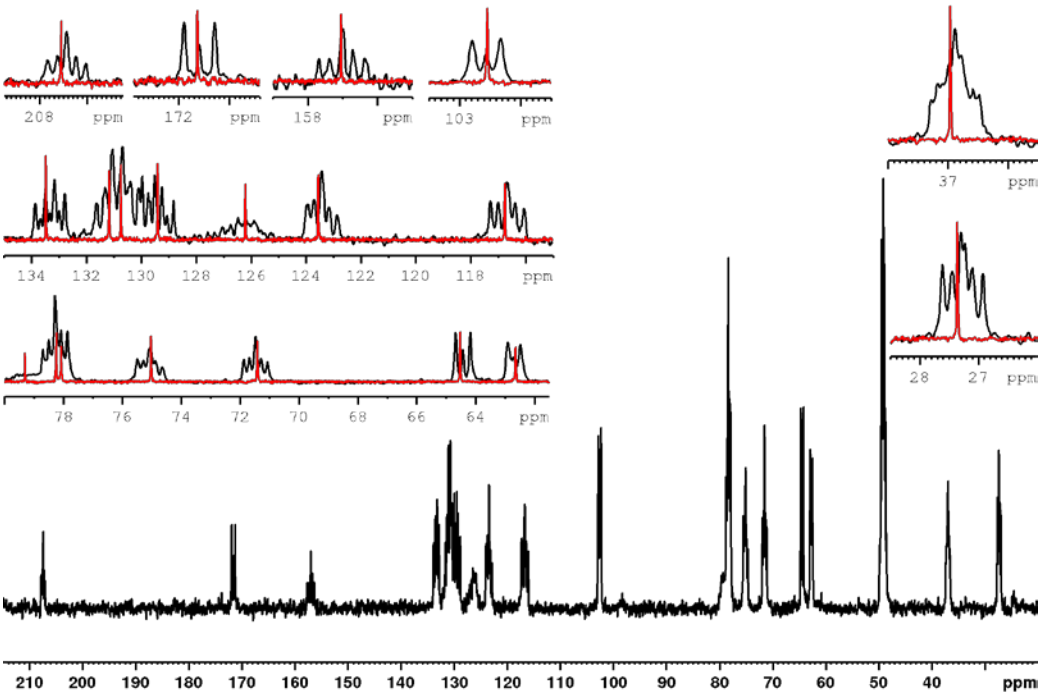


Figure B.2-9 ^{13}C NMR spectrum (100 MHz, MeOH-d_4) of salicortin (**12**) isolated from young leaf tissue of the ^{13}C-enriched *Populus beaupré* plants (black). The inserts show multiplets, which result from ^{13}C-^{13}C spin-spin coupling (black), in comparison to the singlet signals of the unlabeled reference (red).

C) [U-^{13}C]Salicortin *C. vinula* larvae feeding

C.1) Experimental setup

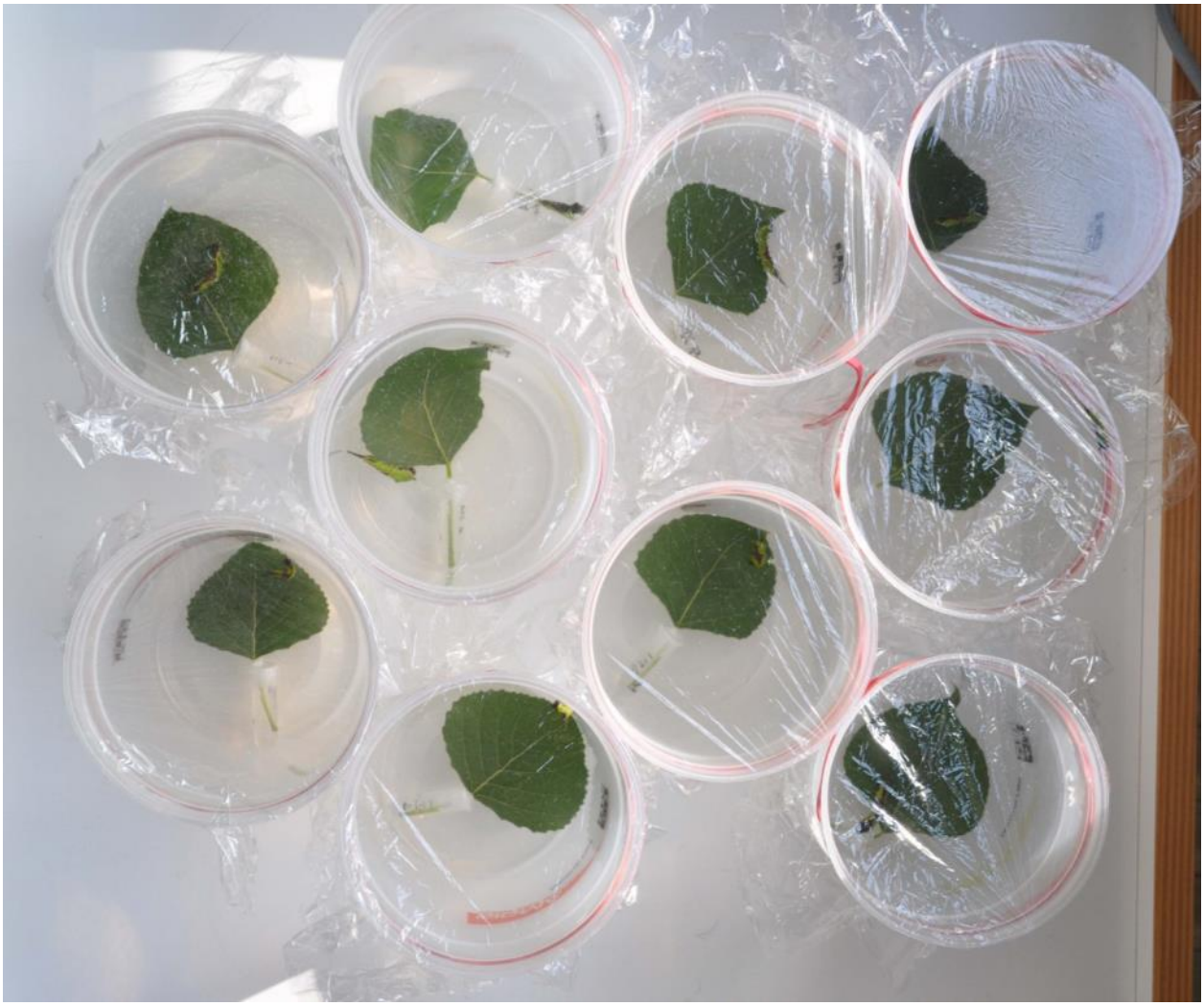


Figure C.1-1 Arena setups including coated leaves and *C. vinula* larvae

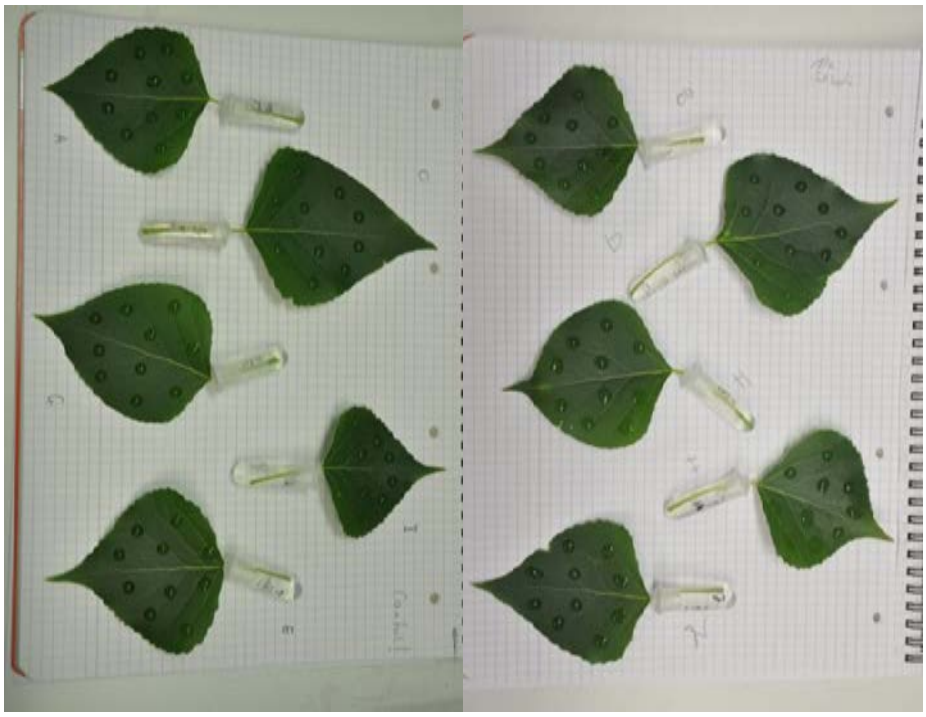


Figure C.1-2 Leaves of *P. beaupré* coated with H_2O (left) and [U-^{13}C]salicortin solution (right).

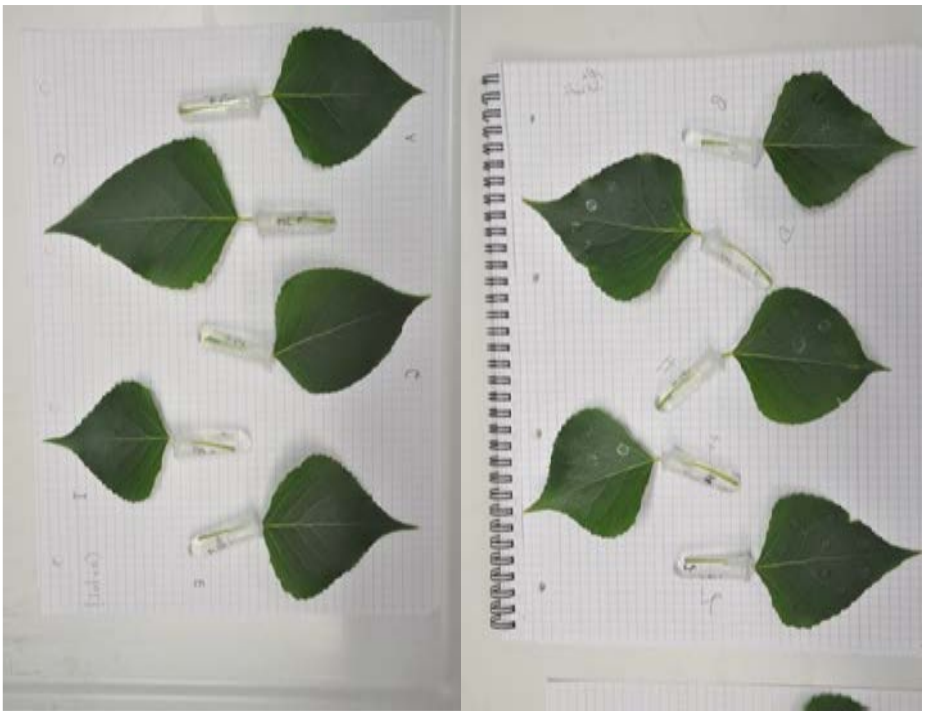


Figure C.1-3 Leaves of *P. beaupré* coated with H_2O (left) and [U-^{13}C]salicortin solution (right) after drying.

C.2) NMR spectra

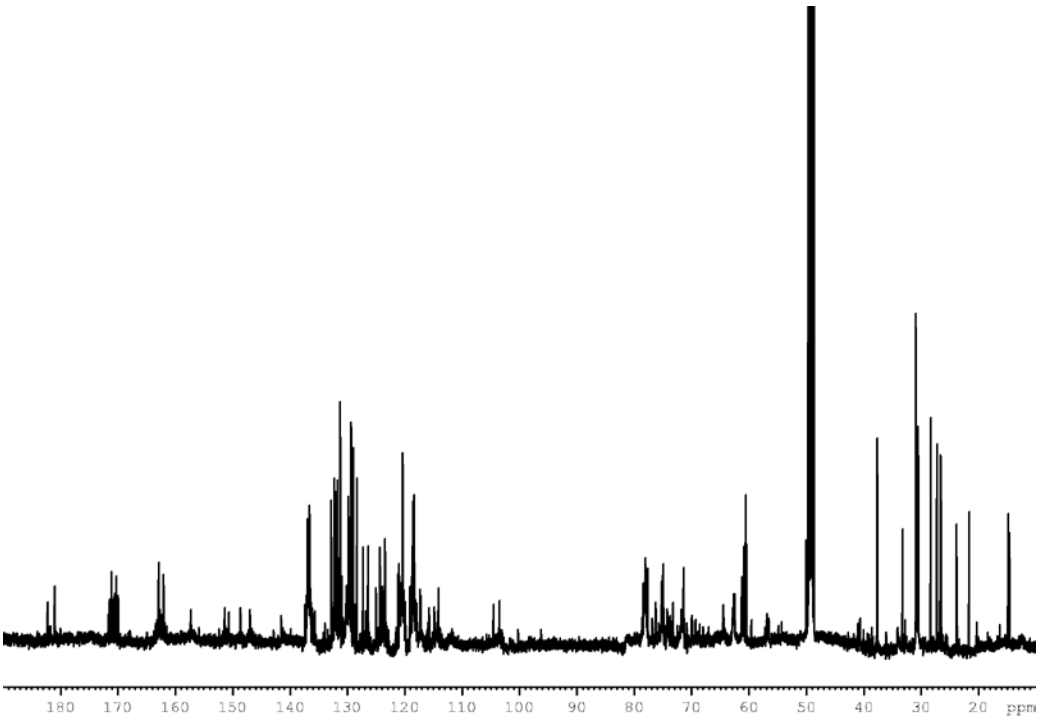


Figure C.2-1 ^{13}C NMR spectrum (125 MHz; MeOH-d_4; 20k scans) of feces from *C. vinula* which fed on *P. nigra* diet supplemented with [U-^{13}C]salicortin.

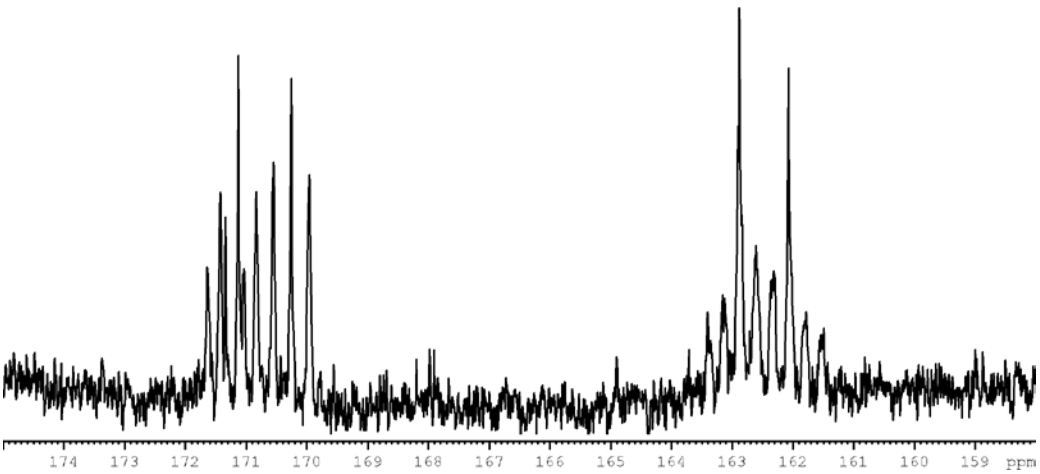


Figure C.2-2 Partial ^{13}C NMR spectrum (125 MHz; MeOH-d_4; 20k scans; 155-175 ppm) of feces from *C. vinula* which fed on *P. nigra* diet supplemented with [U-^{13}C]salicortin.

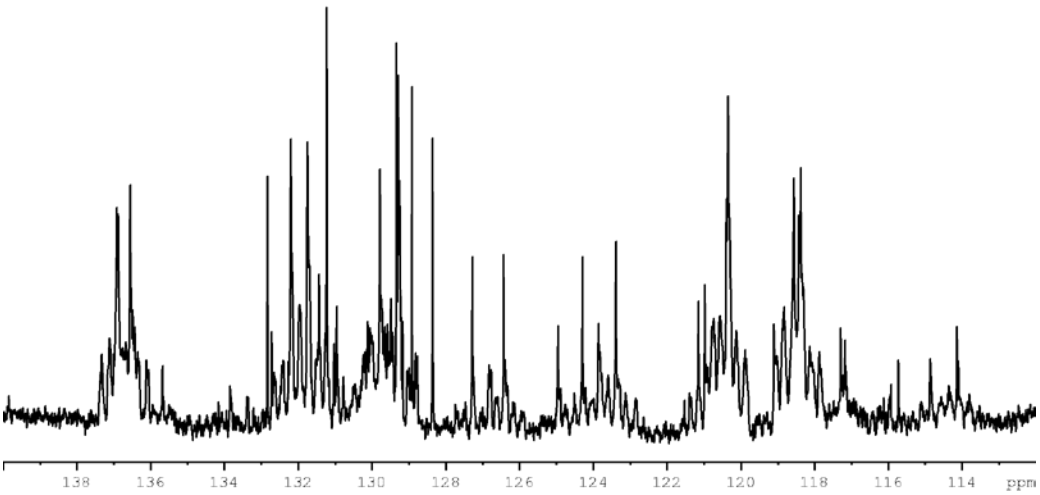


Figure C.2-3 Partial ^{13}C NMR spectrum (125 MHz; MeOH-d_4; 20k scans; 110-140 ppm) of feces from *C. vinula* which fed on *P. nigra* diet supplemented with [U-^{13}C]salicortin.

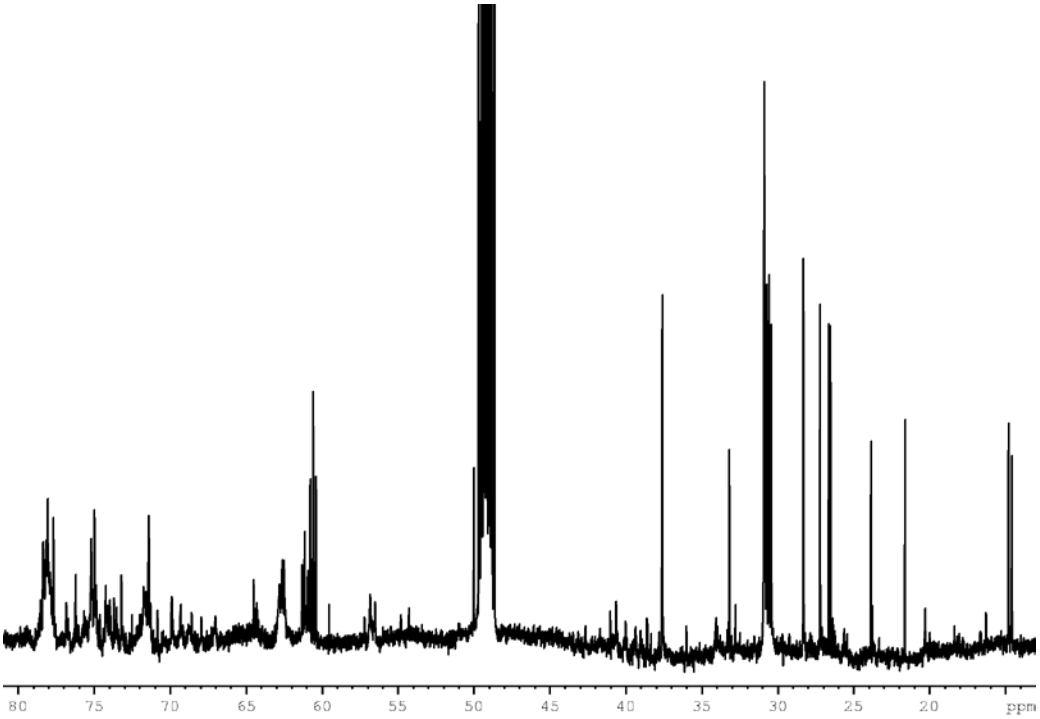


Figure C.2-4 Partial ^{13}C NMR spectrum (125 MHz; MeOH-d_4; 20k scans; 1-80 ppm) of feces from *C. vinula* which fed on *P. nigra* diet supplemented with [U-^{13}C]salicortin.

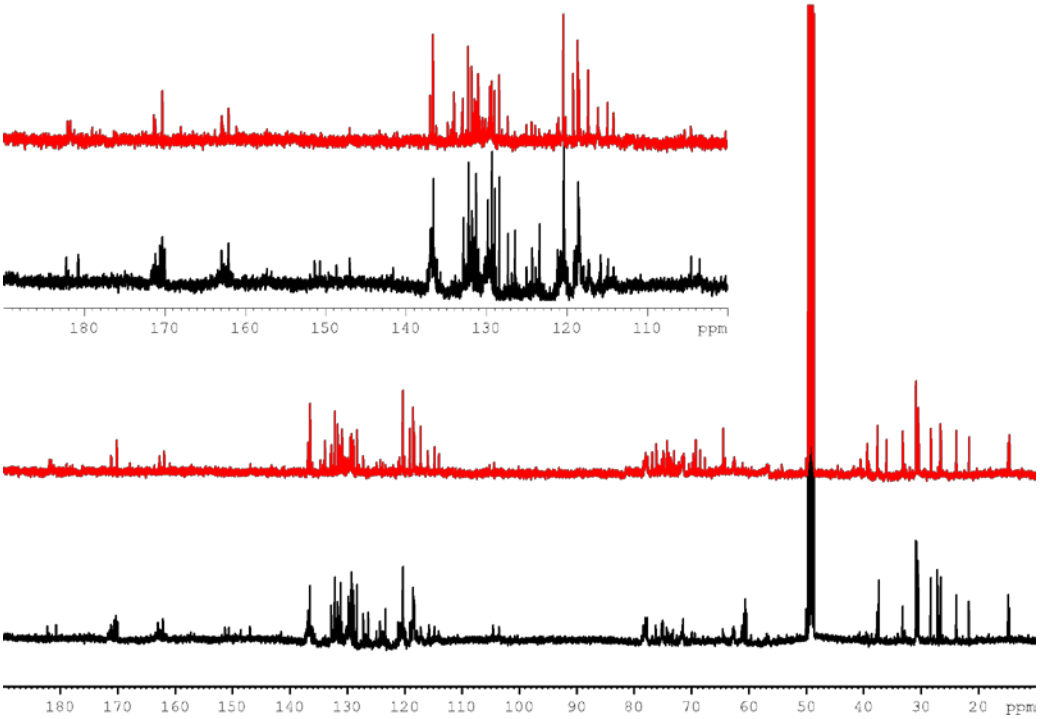


Figure C.2-5 ^{13}C NMR spectra (125 MHz; MeOH-d_4; 6k scans; 10-190 ppm) of feces from *C. vinula* which fed on *P. nigra* diet supplemented with [U-^{13}C]salicortin (black) and the control leaves (red). The insert shows the partial spectra between 100 and 190 ppm.

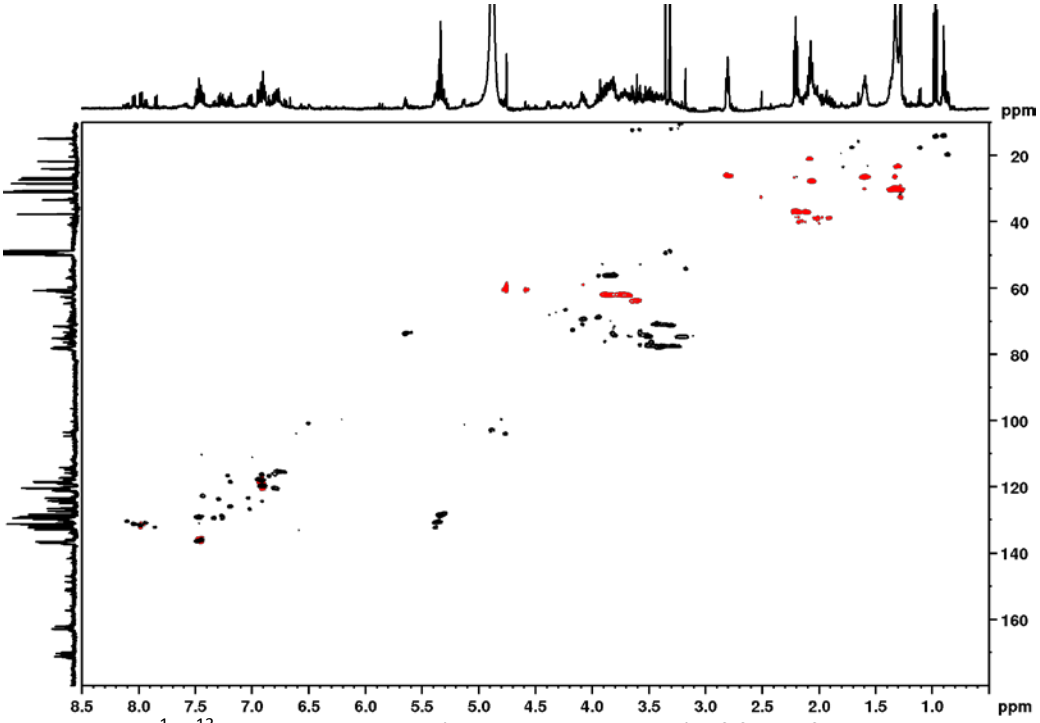


Figure C.2-6 ^{1}H-^{13}C HSQC spectrum (500 MHz, MeOH-d_4) of feces from *C. vinula* which fed on *P. nigra* diet supplemented with [U-^{13}C]salicortin.

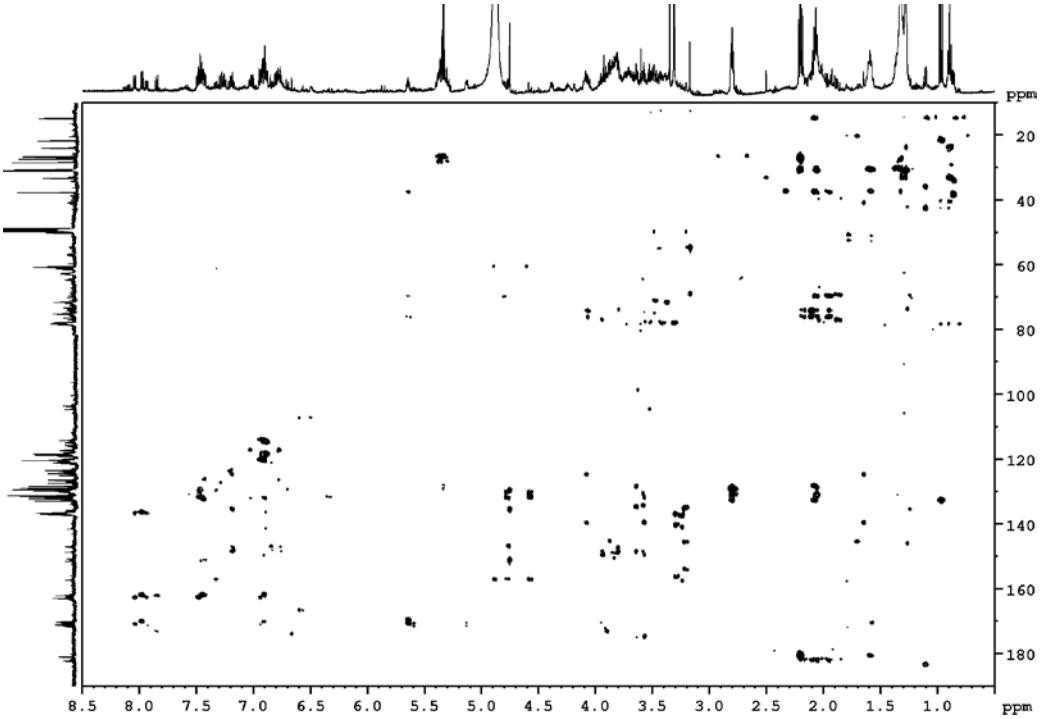


Figure C.2-7 ^{1}H-^{13}C HMBC spectrum (500 MHz, MeOH-d_4) of feces from *C. vinula* which fed on *P. nigra* diet supplemented with [U-^{13}C]salicortin.

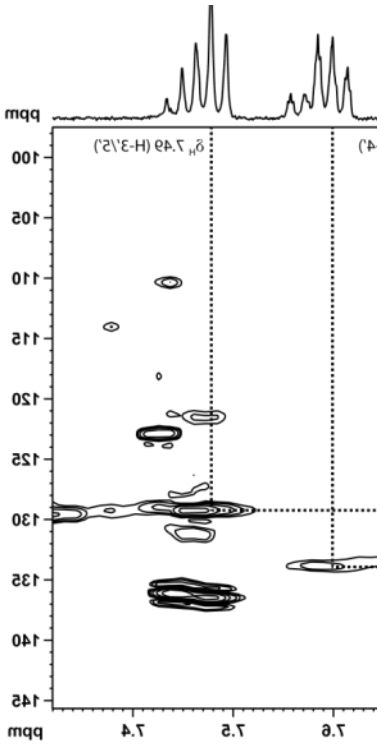


Figure C.2-8 ^{1}H-^{13}C HSQC spectrum (500 MHz, MeOH-d_4) with selective 1D TOCSY spectrum (top) (500 MHz, MeOH-d_4; o1p= 8.13 ppm with 16 Hz) and ^{13}C NMR spectrum (left) (125 MHz).

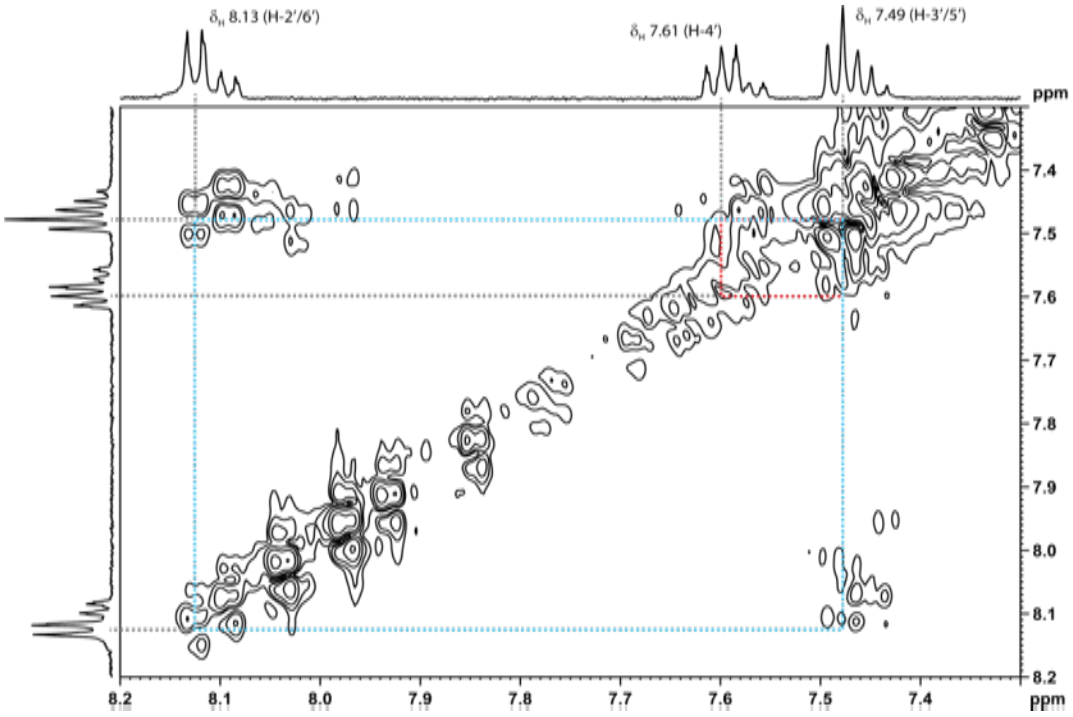


Figure C.2-9 ^{1}H-^{1}H COSY (500 MHz, MeOH-d_4) with selective 1D TOCSY spectrum (top and left) (500 MHz, MeOH-d_4; o1p= 8.13 ppm with 16 Hz).

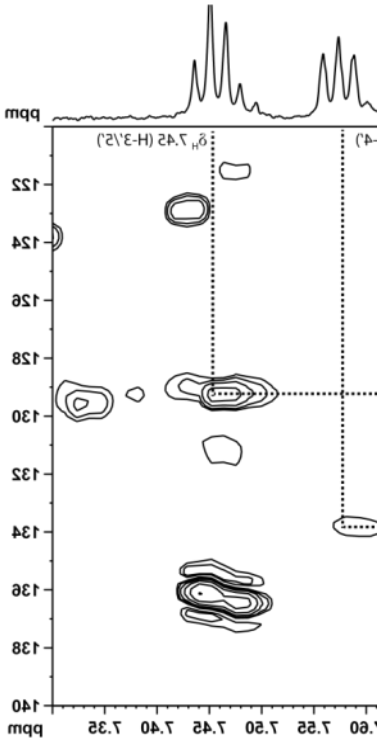


Figure C.2-10 ^{1}H-^{13}C HSQC spectrum (500 MHz, MeOH-d_4) with selective 1D TOCSY spectrum (top) (500 MHz, MeOH-d_4; o1p= 8.09 ppm with 16 Hz) and ^{13}C NMR spectrum (left) (125 MHz).

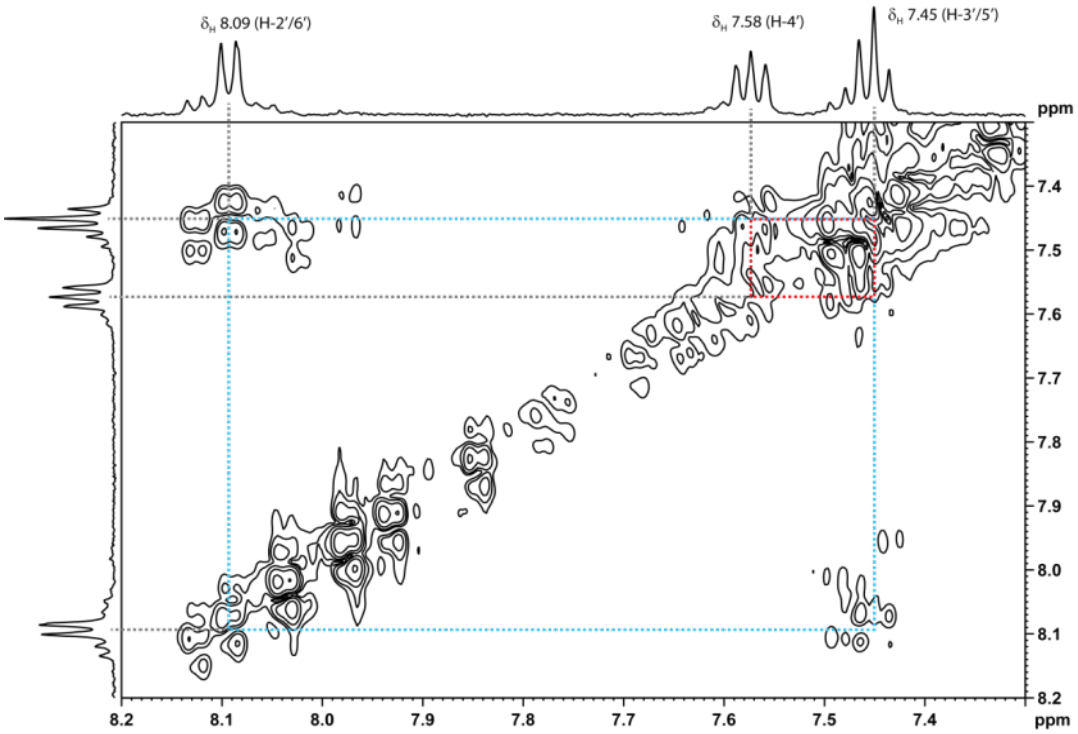


Figure C.2-11 ^{1}H-^{1}H COSY spectrum (500 MHz, MeOH-d_4) with selective 1D TOCSY spectrum (top and left) (500 MHz, MeOH-d_4; o1p= 8.09 ppm with 16 Hz).

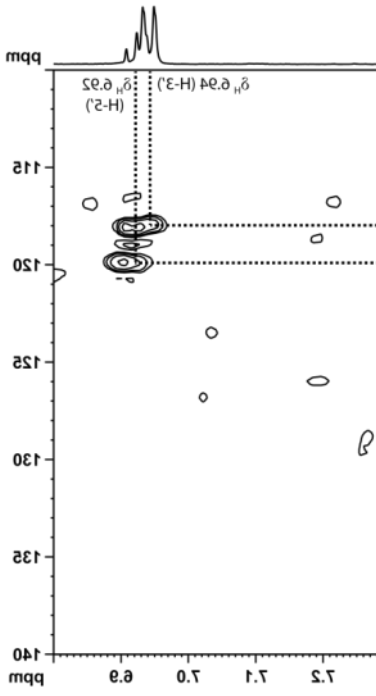


Figure C.2-12 ^{1}H-^{13}C HSQC spectrum (500 MHz, MeOH-d_4) with selective 1D TOCSY spectrum (top) (500 MHz, MeOH-d_4; o1p= 8.04 ppm with 16 Hz) and ^{13}C NMR spectrum (left) (125 MHz).

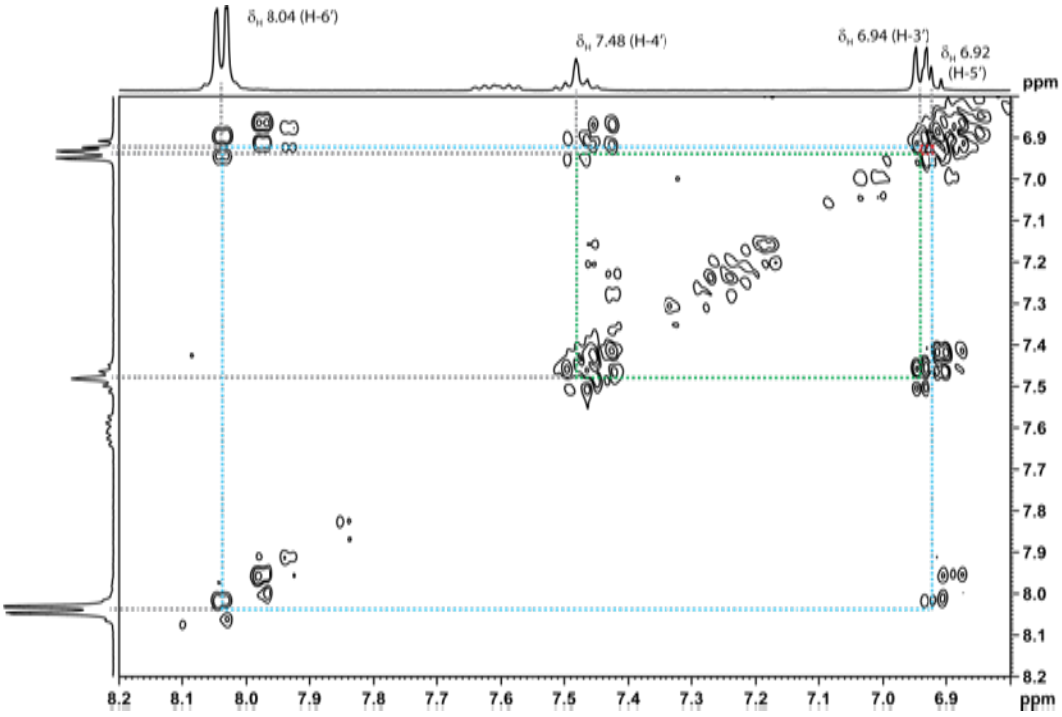


Figure C.2-13 ^{1}H-^{1}H COSY spectrum (500 MHz, MeOH-d_4) with selective 1D TOCSY spectrum (top and left) (500 MHz, MeOH-d_4; o1p= 8.04 ppm with 16 Hz).

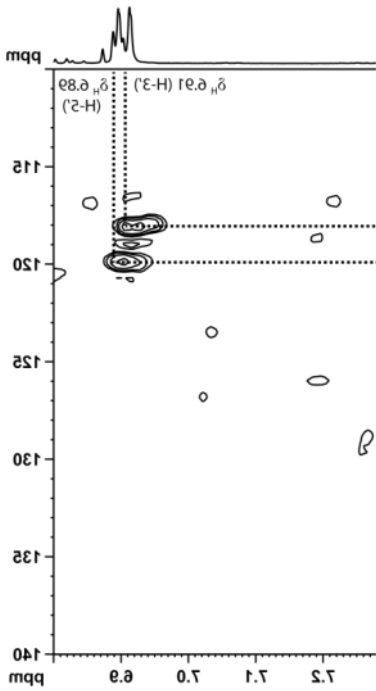


Figure C.2-14 ^{1}H-^{13}C HSQC spectrum (500 MHz, MeOH-d_4) with selective 1D TOCSY spectrum (top) (500 MHz, MeOH-d_4; o1p= 7.98 ppm with 16 Hz) and ^{13}C NMR spectrum (left) (125 MHz).

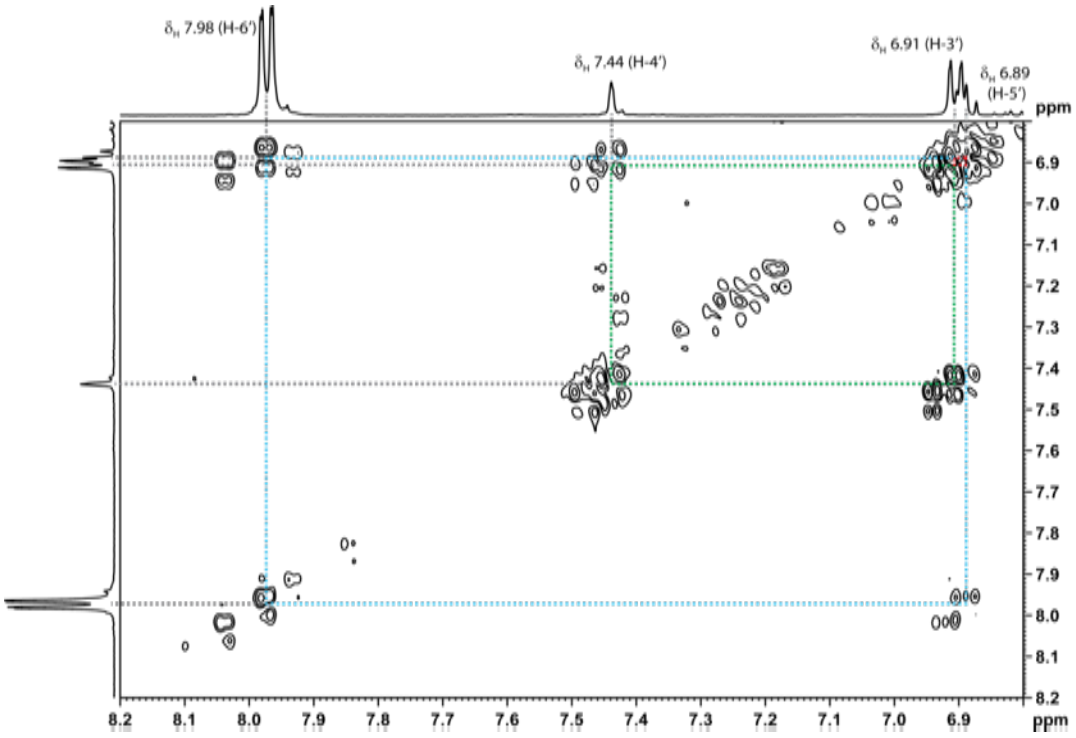


Figure C.2-15 ^{1}H-^{1}H COSY spectrum (500 MHz, MeOH-d_4) with selective 1D TOCSY spectrum (top and left) (500 MHz, MeOH-d_4; o1p= 7.98 ppm with 16 Hz).

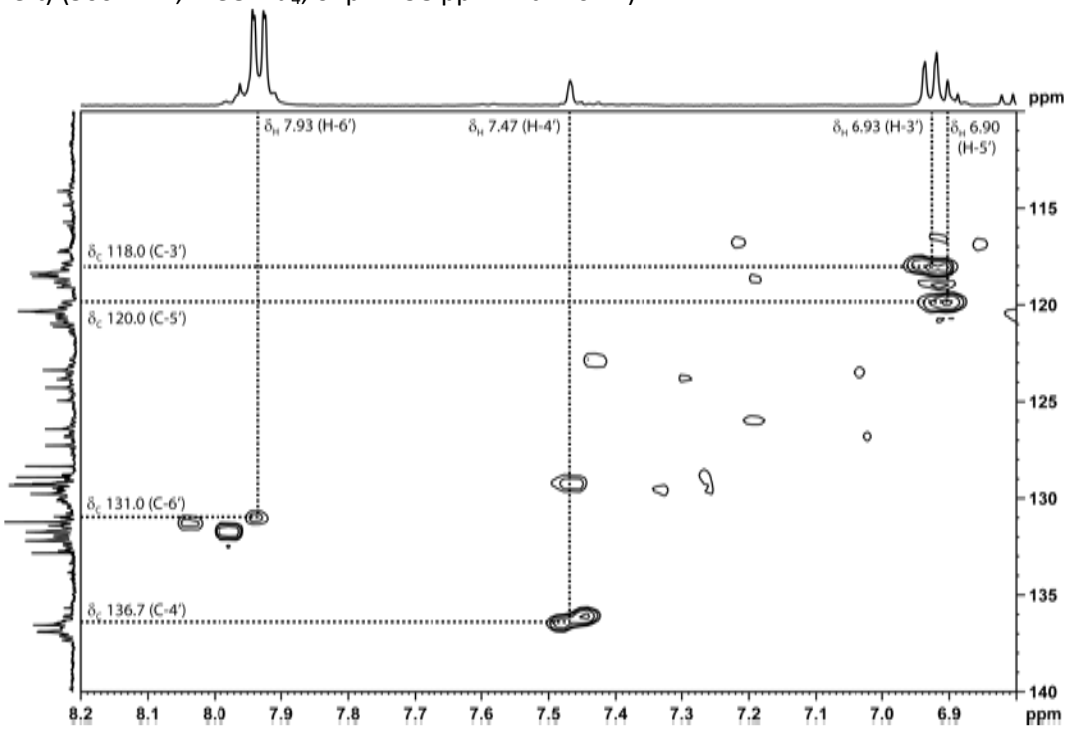


Figure C.2-16 ^{1}H-^{13}C HSQC spectrum (500 MHz, MeOH-d_4) with selective 1D TOCSY spectrum (top) (500 MHz, MeOH-d_4; o1p= 7.93 ppm with 16 Hz) and ^{13}C NMR spectrum (left) (125 MHz).

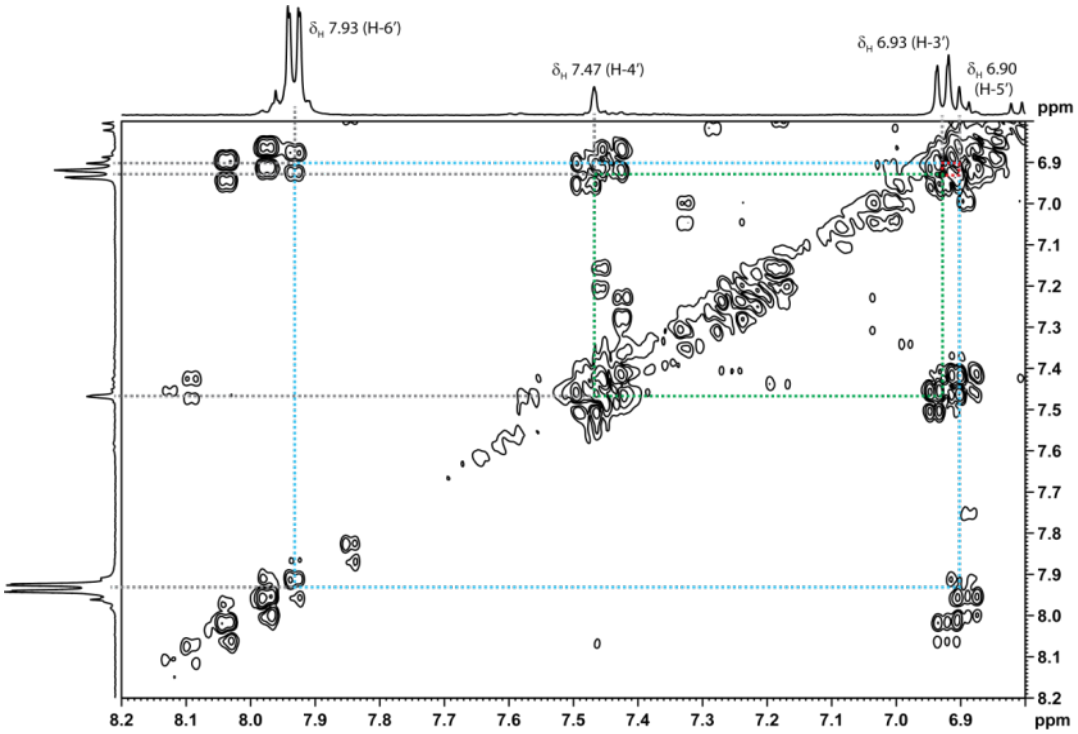


Figure C.2-17 ^{1}H-^{1}H COSY spectrum (500 MHz, MeOD-d_4) with selective 1D TOCSY spectrum (top and left) (500 MHz, MeOH-d_4; o1p= 7.93 ppm with 16 Hz).

C.3) HRESIMS spectra

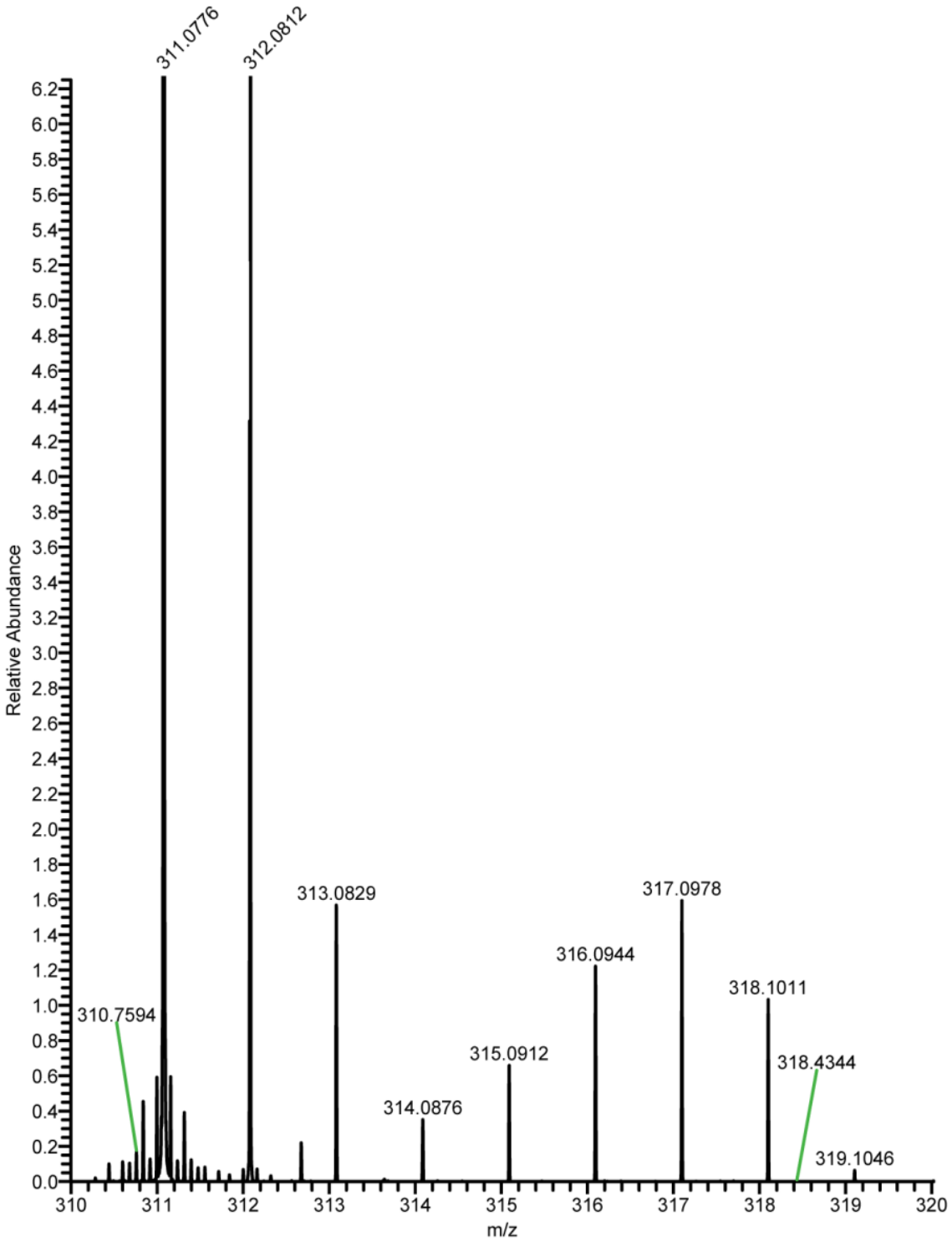


Figure C.3-1 Compound **1**; HRESIMS spectrum (*m/z* 311.0776 $[M-H]^-$) of *C. vinula* feces after consumption of leaves labeled with [U-^{13}C]salicortin.

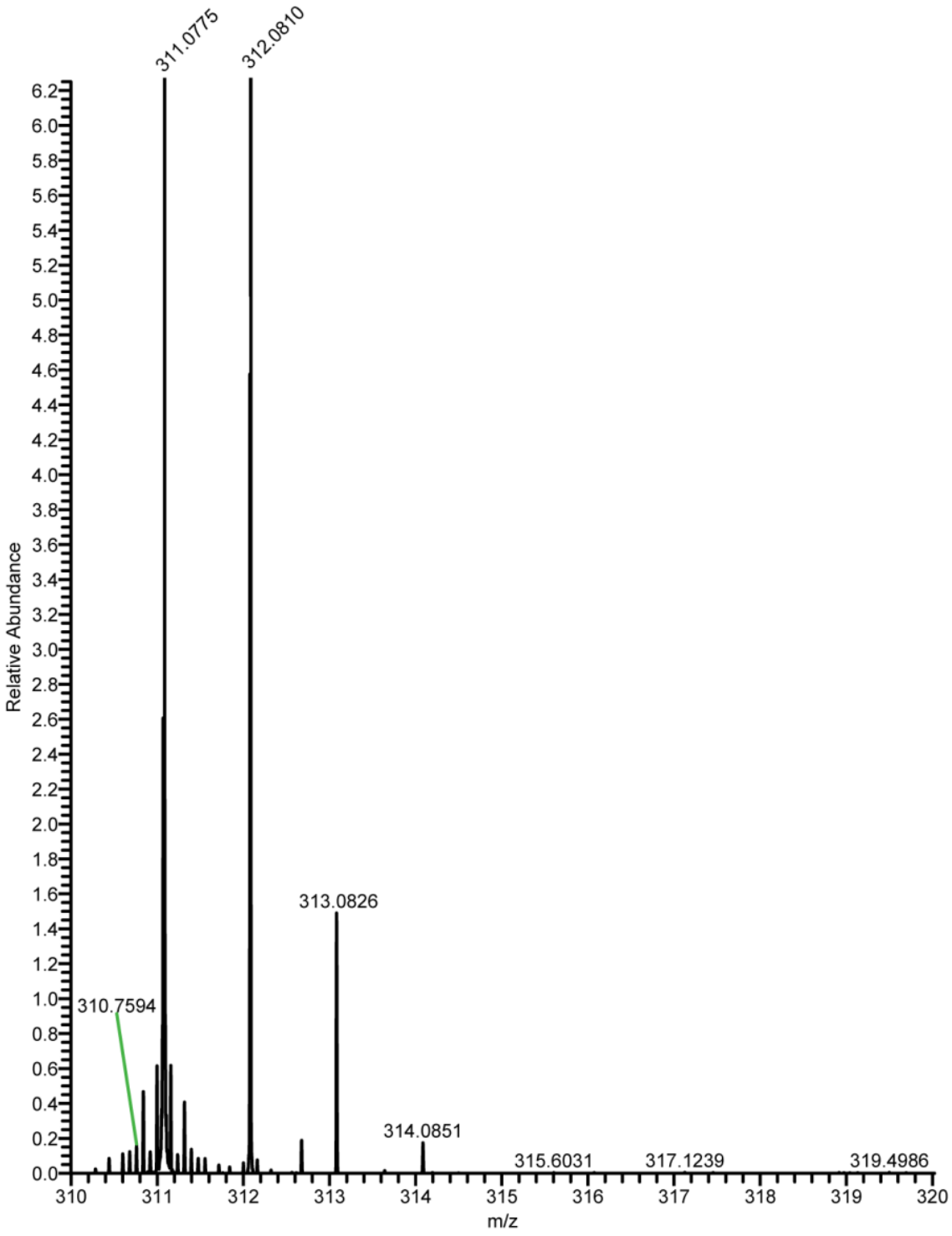


Figure C.3-2 Compound **1**; HRESIMS spectrum (*m/z* 311.0775 $[M-H]^-$) of *C. vinula* feces after consumption of control tissue.

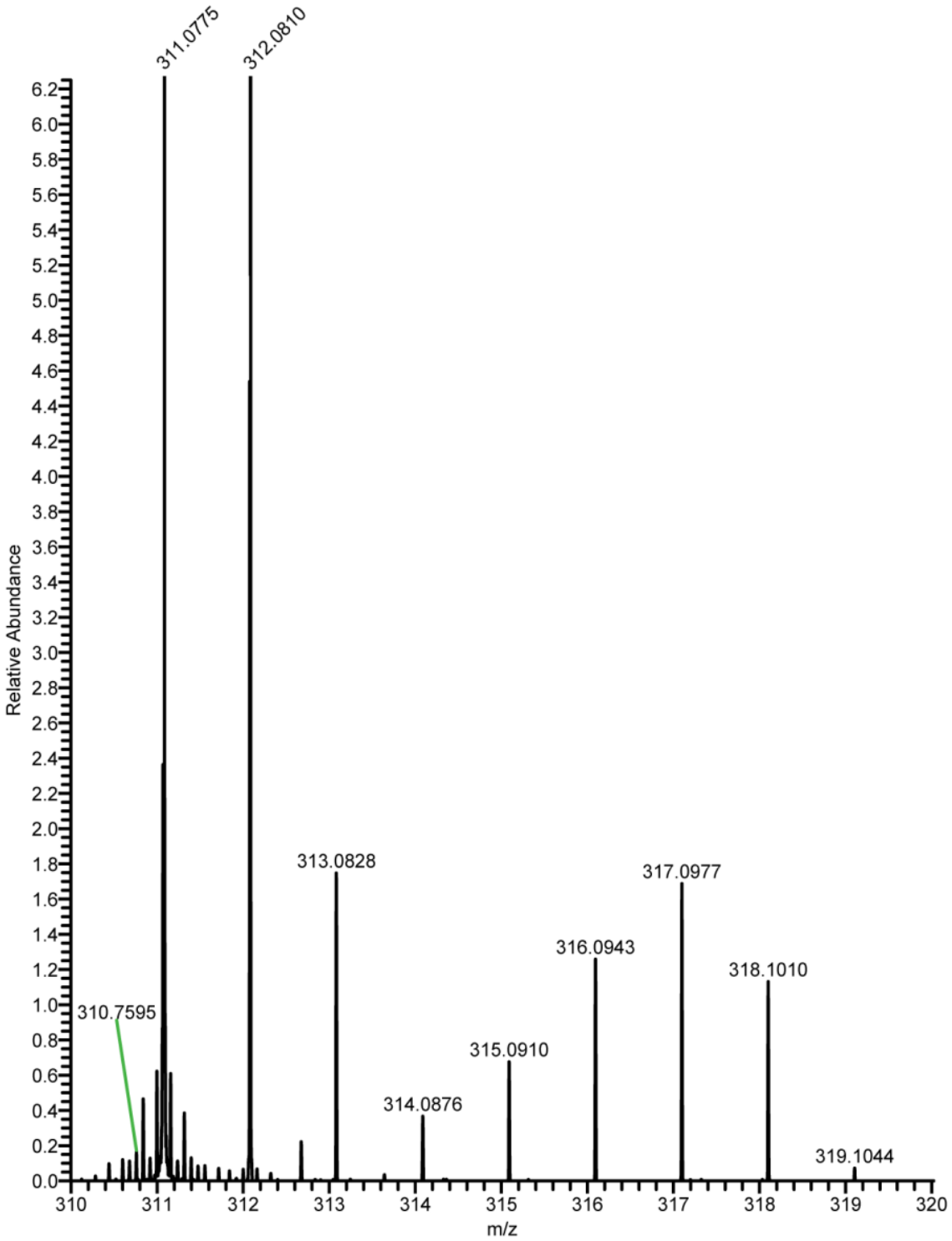


Figure C.3-3 Compound **2**; HRESIMS spectrum (*m/z* 311.0775 $[M-H]^-$) of *C. vinula* feces after consumption of leaves labeled with [U-^{13}C]salicortin.

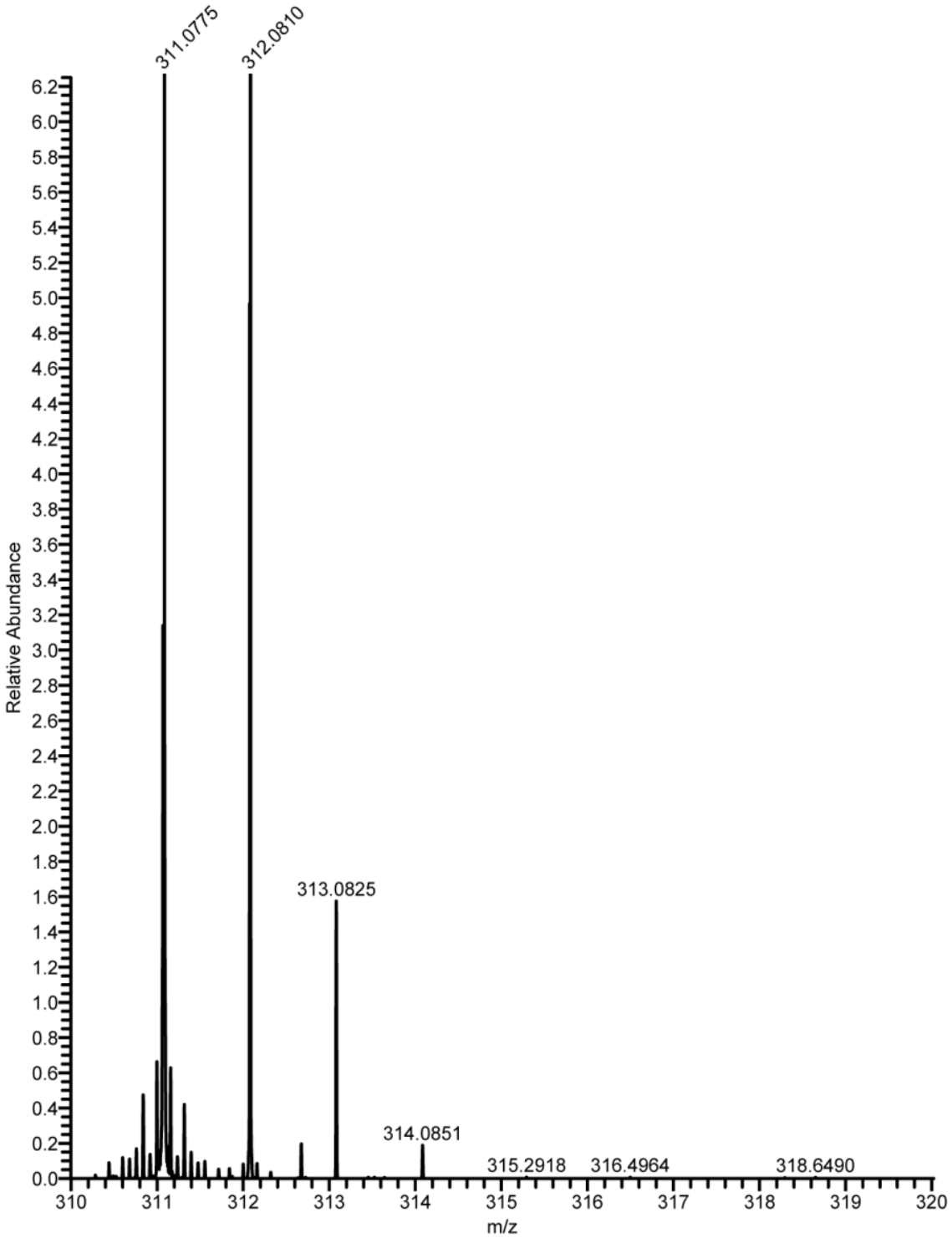


Figure C.3-4 Compound **2**; HRESIMS spectrum (*m/z* 311.0775 $[M-H]^-$) of *C. vinula* feces after consumption of control tissue.

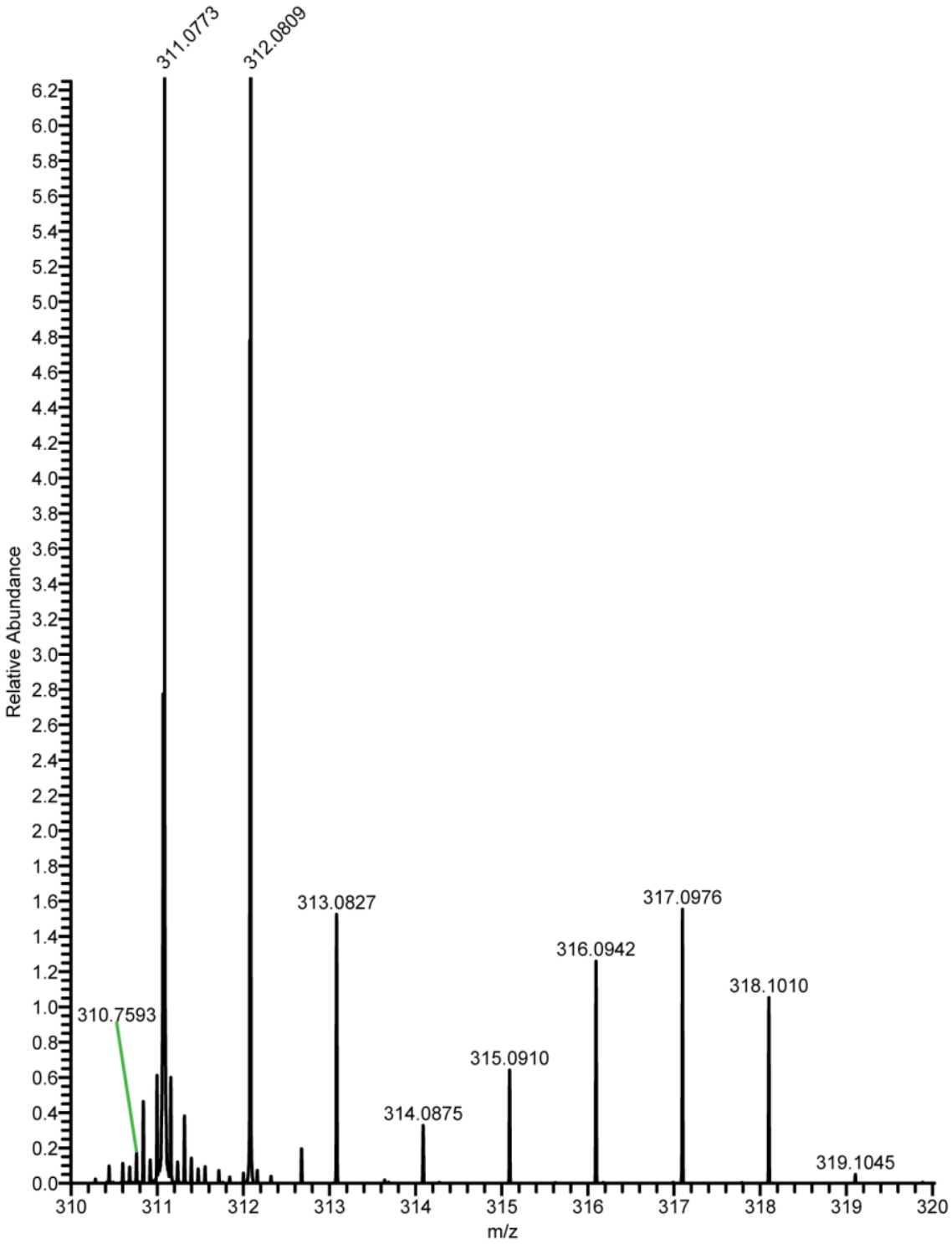


Figure C.3-5 Compound **3**; HRESIMS spectrum (*m/z* 311.0773 $[M-H]^-$) of *C. vinula* feces after consumption of leaves labeled with [U-^{13}C]salicortin.

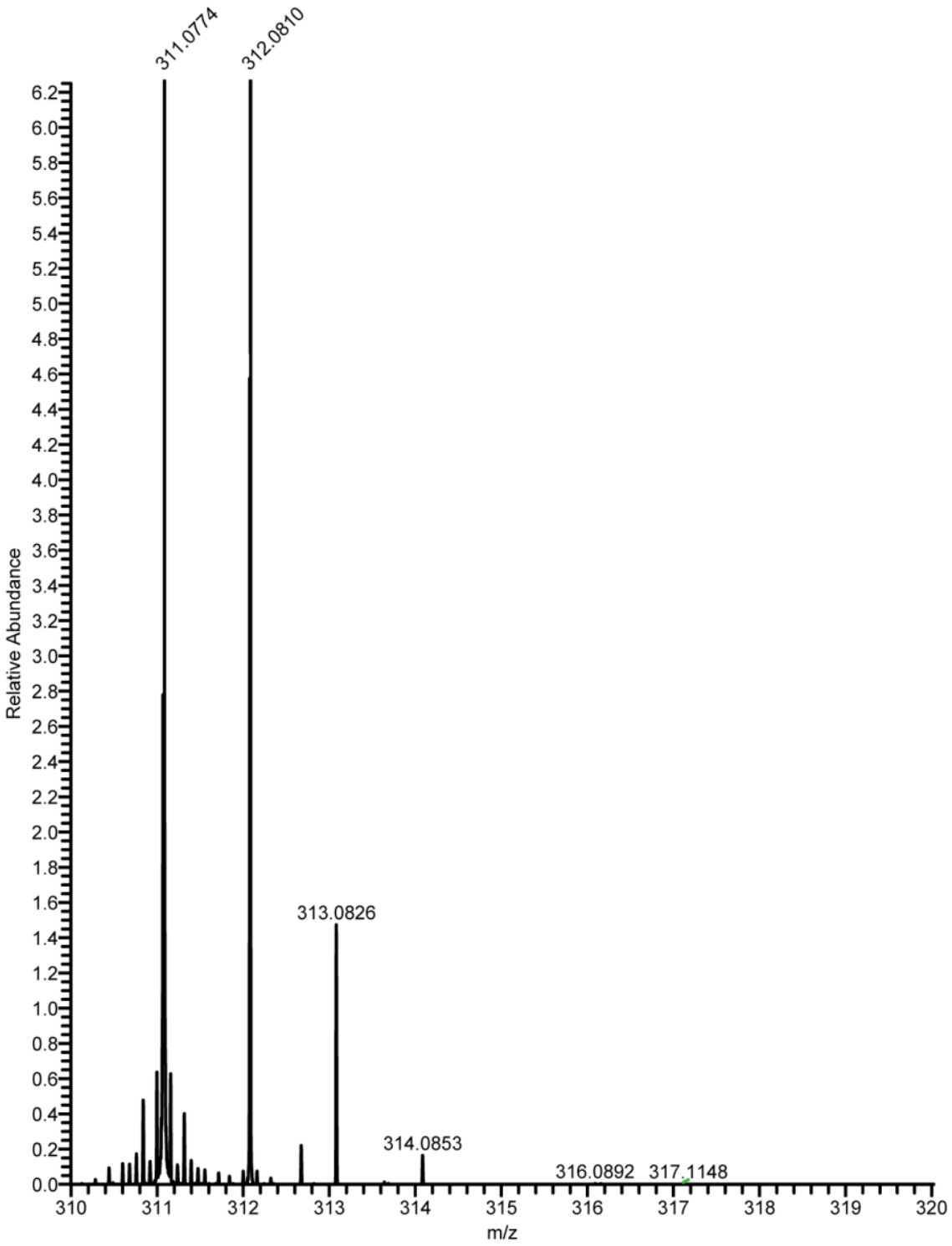


Figure C.3-6 Compound **3**; HRESIMS spectrum (*m/z* 311.0774 $[M-H]^-$) of *C. vinula* feces after consumption of control tissue.

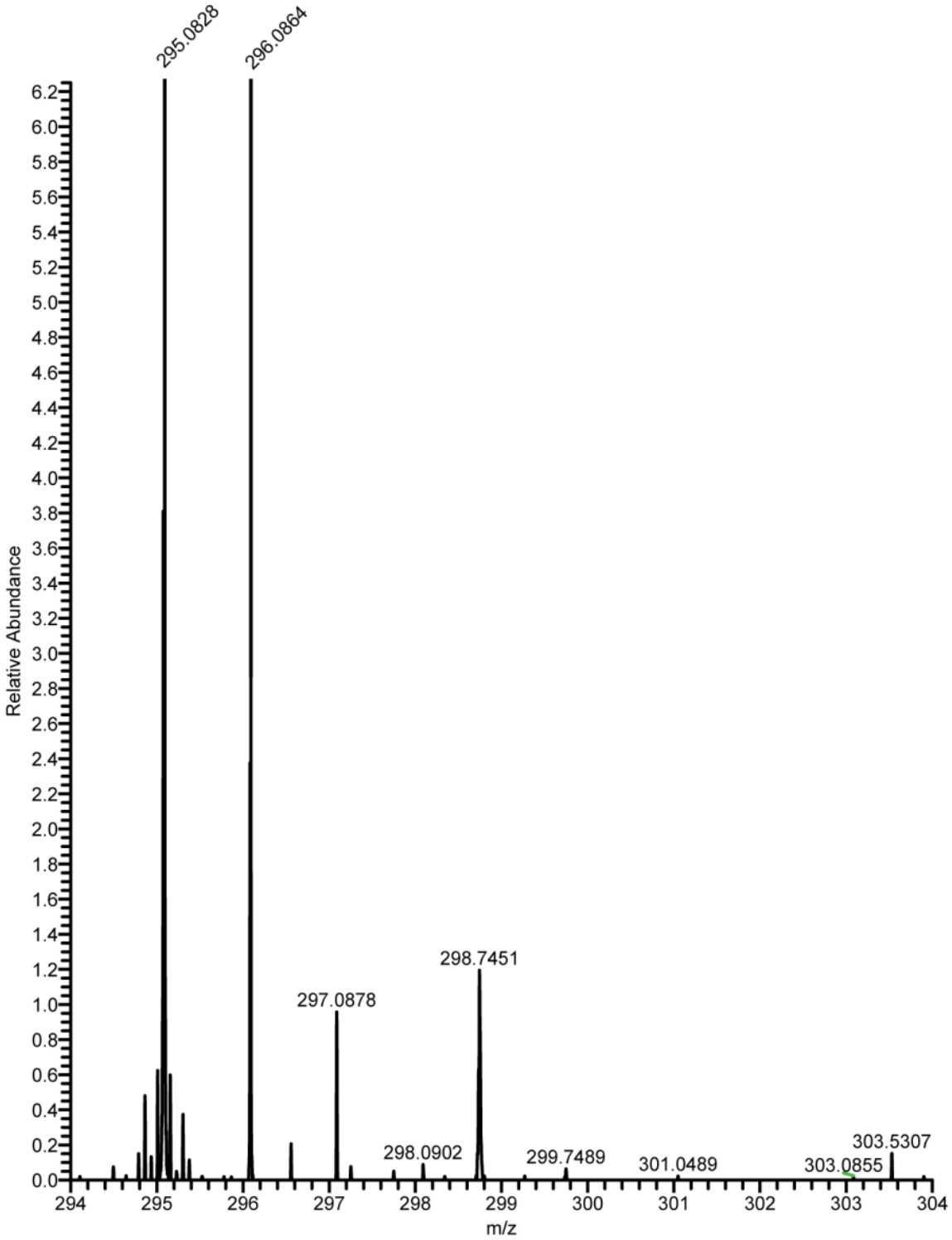


Figure C.3-7 Compound **4**; HRESIMS spectrum (*m/z* 295.0828 $[M-H]^-$) of *C. vinula* feces after consumption of leaves labeled with [U-^{13}C]salicortin.

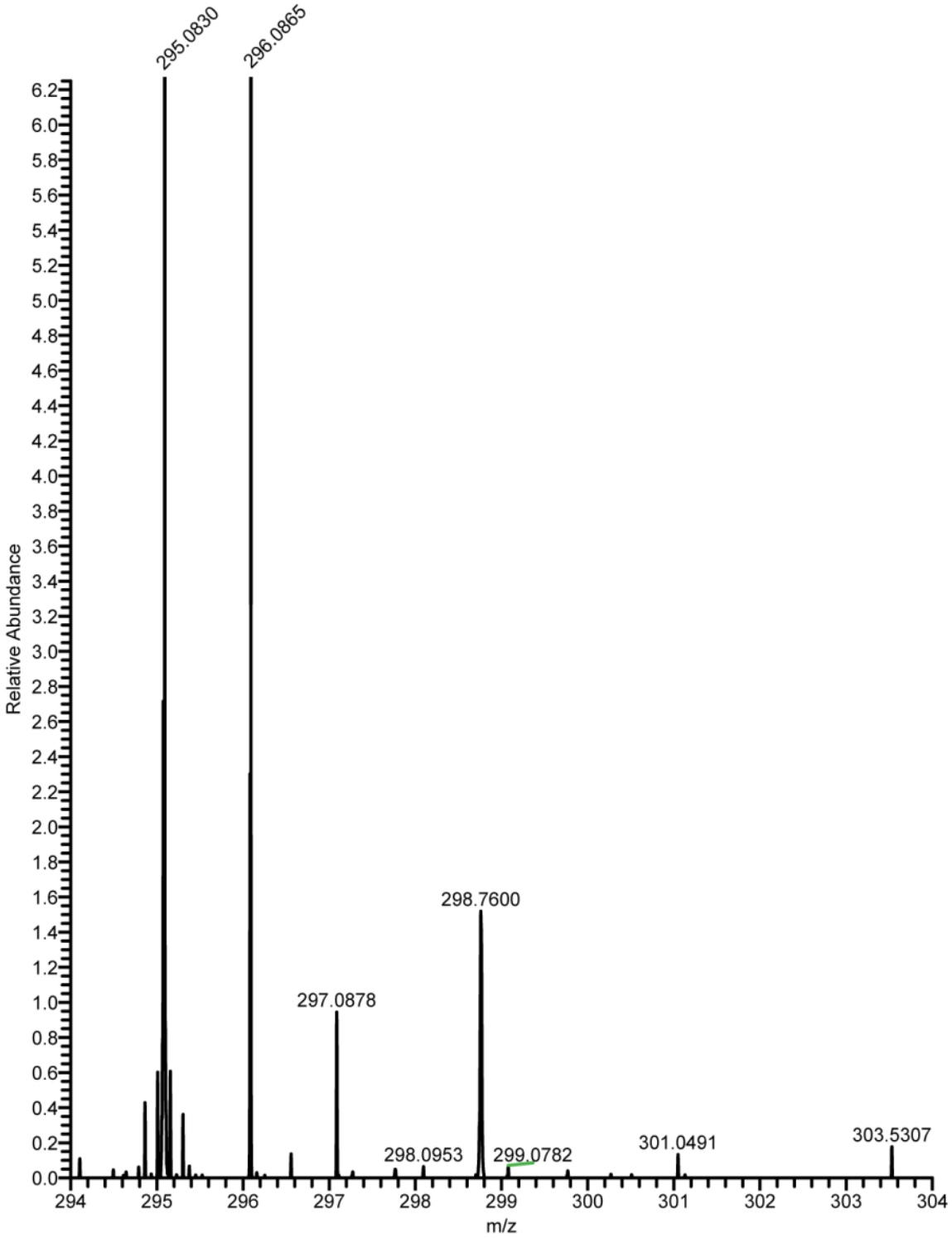


Figure C.3-8 Compound **4**; HRESIMS spectrum (*m/z* 295.0830 $[M-H]^-$) of *C. vinula* feces after consumption of control tissue.

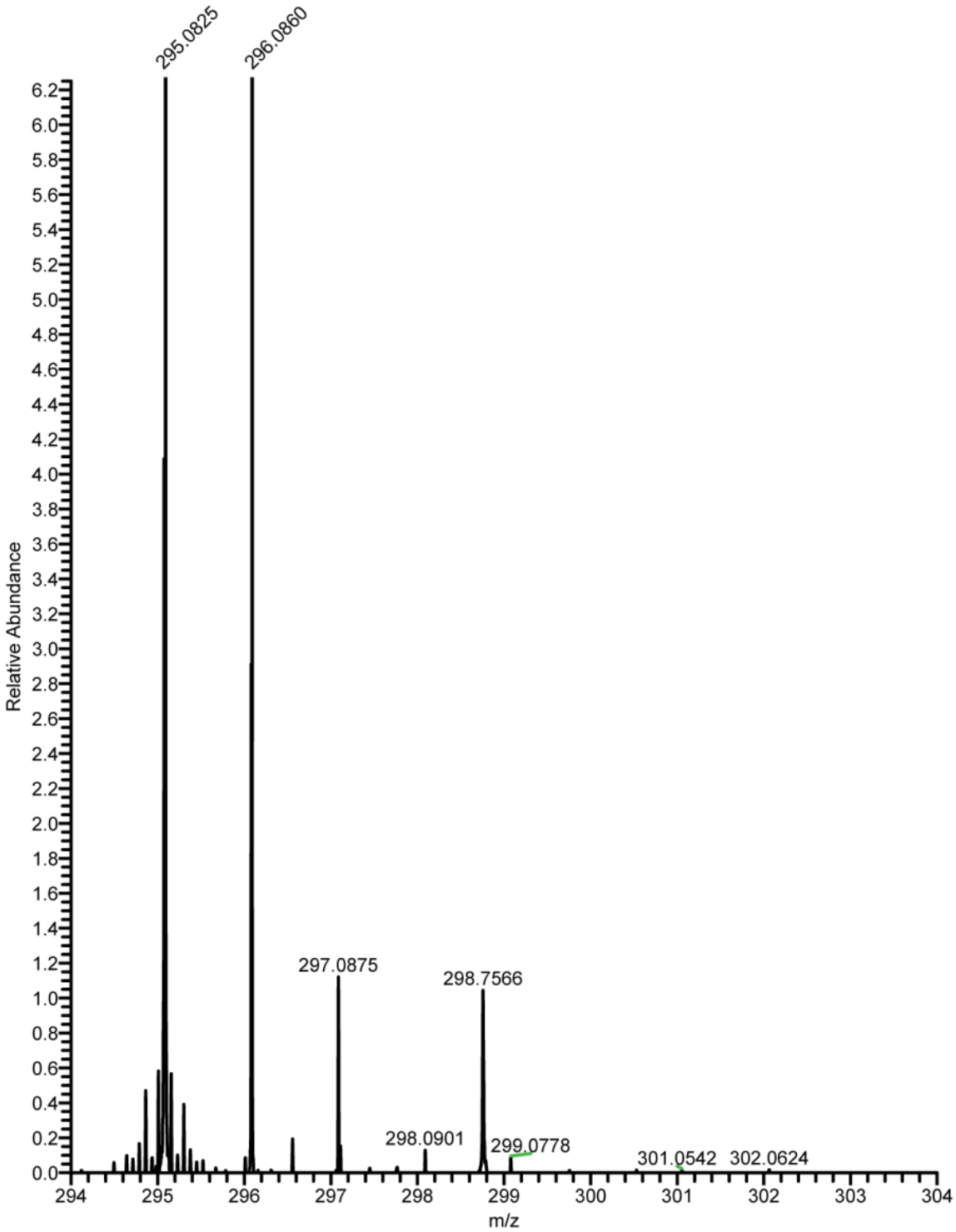


Figure C.3-9 Compound **5**; HRESIMS spectrum (*m/z* 295.0825 $[M-H]^-$) of *C. vinula* feces after consumption of leaves labeled with [U-^{13}C]salicortin.

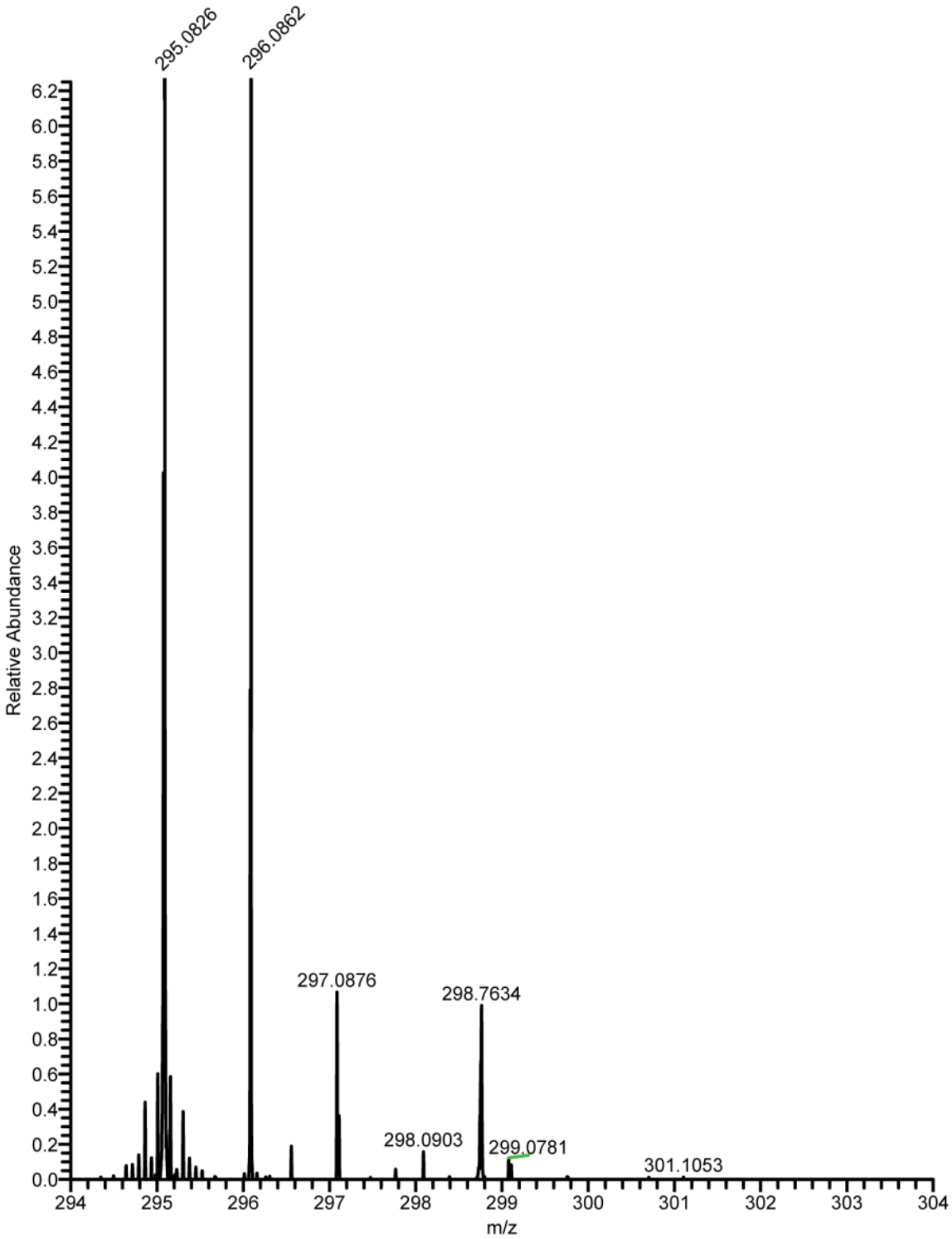


Figure C.3-10 Compound **5**; HRESIMS spectrum (*m/z* 295.0826 $[M-H]^-$) of *C. vinula* feces after consumption of control tissue.

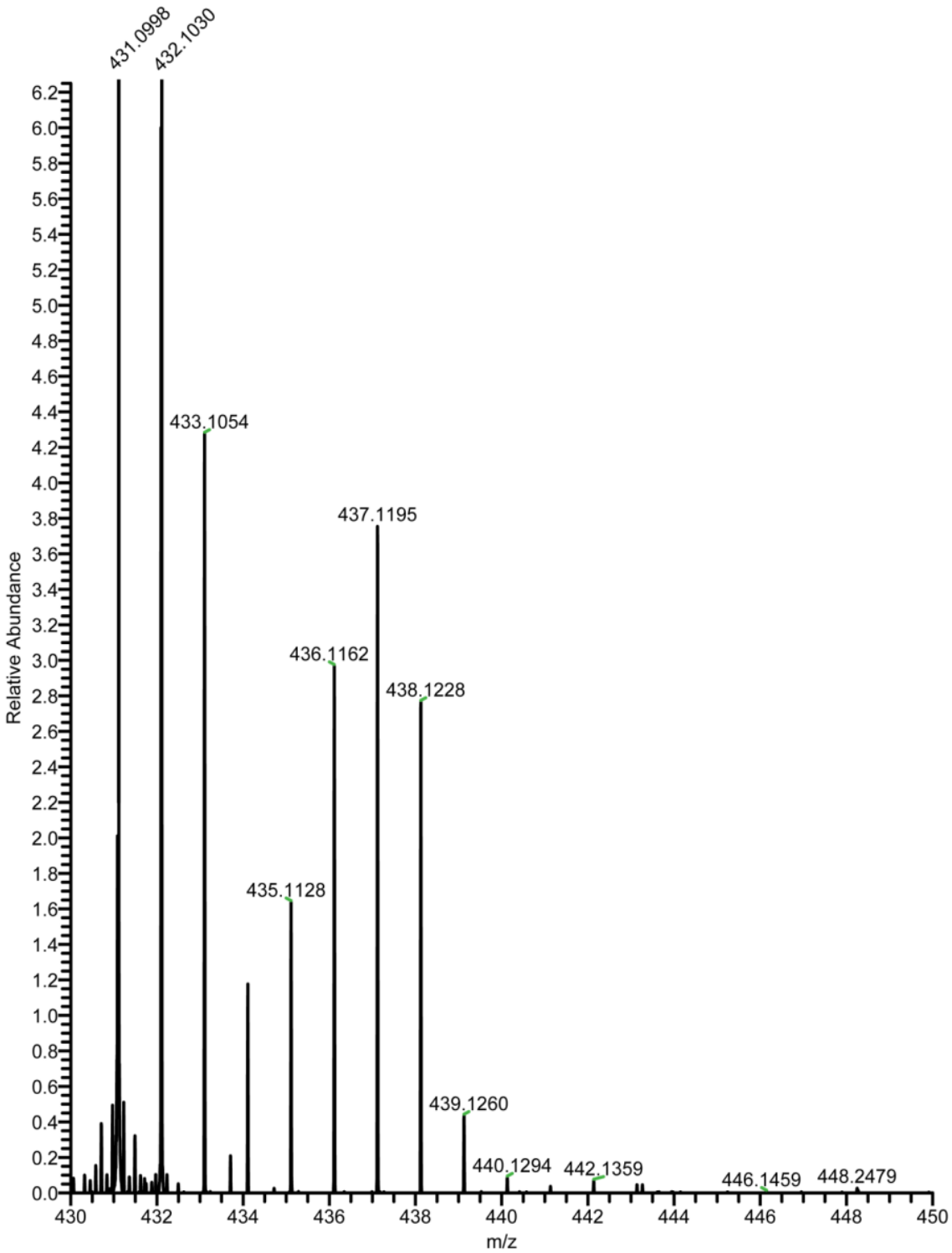


Figure C.3-11 Compound **6**; HRESIMS spectrum (*m/z* 431.0998 $[M-H]^-$) of *C. vinula* feces after consumption of leaves labeled with [U-^{13}C]salicortin.

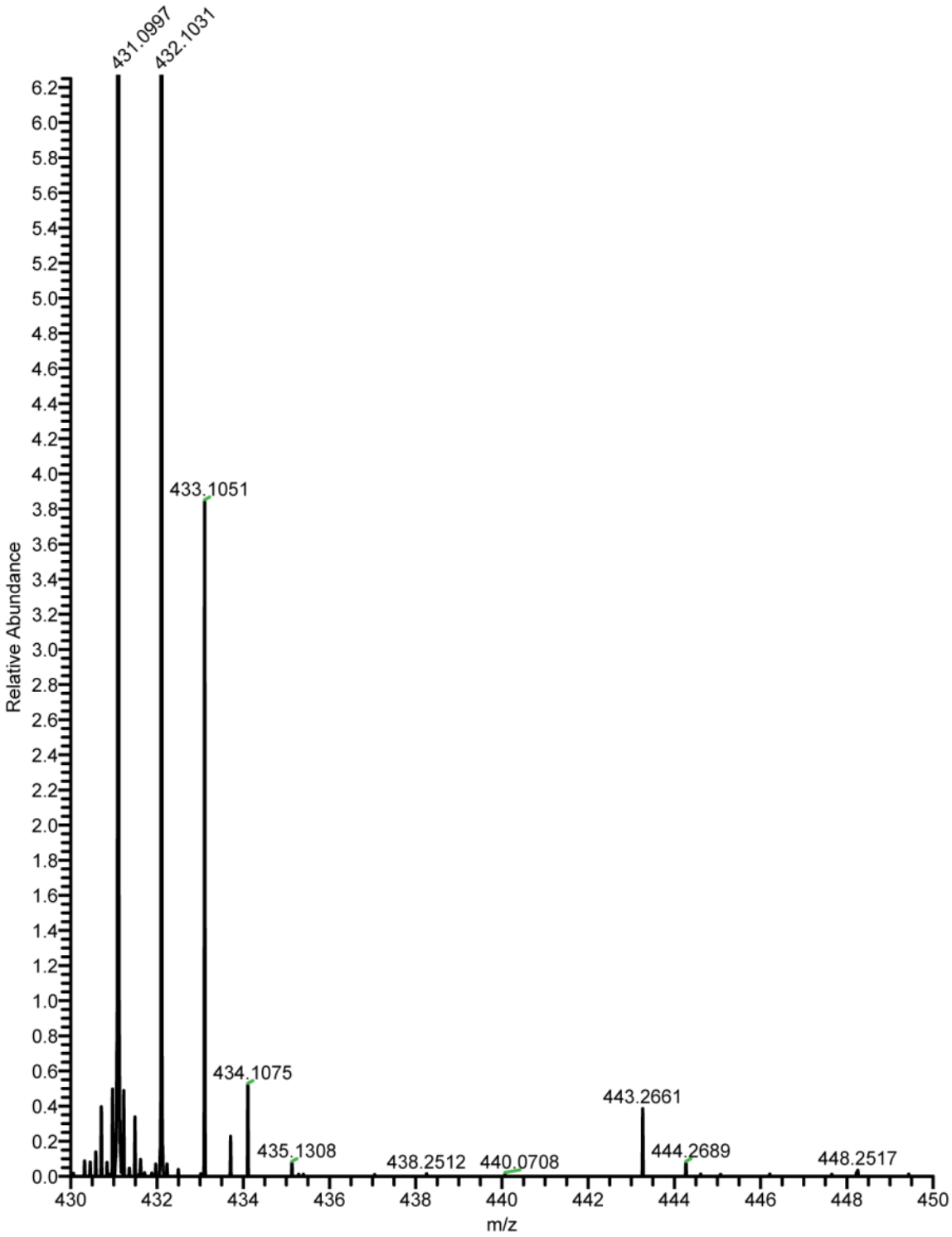


Figure C.3-12 Compound **6**; HRESIMS spectrum (*m/z* 431.0997 $[M-H]^-$) of *C. vinula* feces after consumption of control tissue.

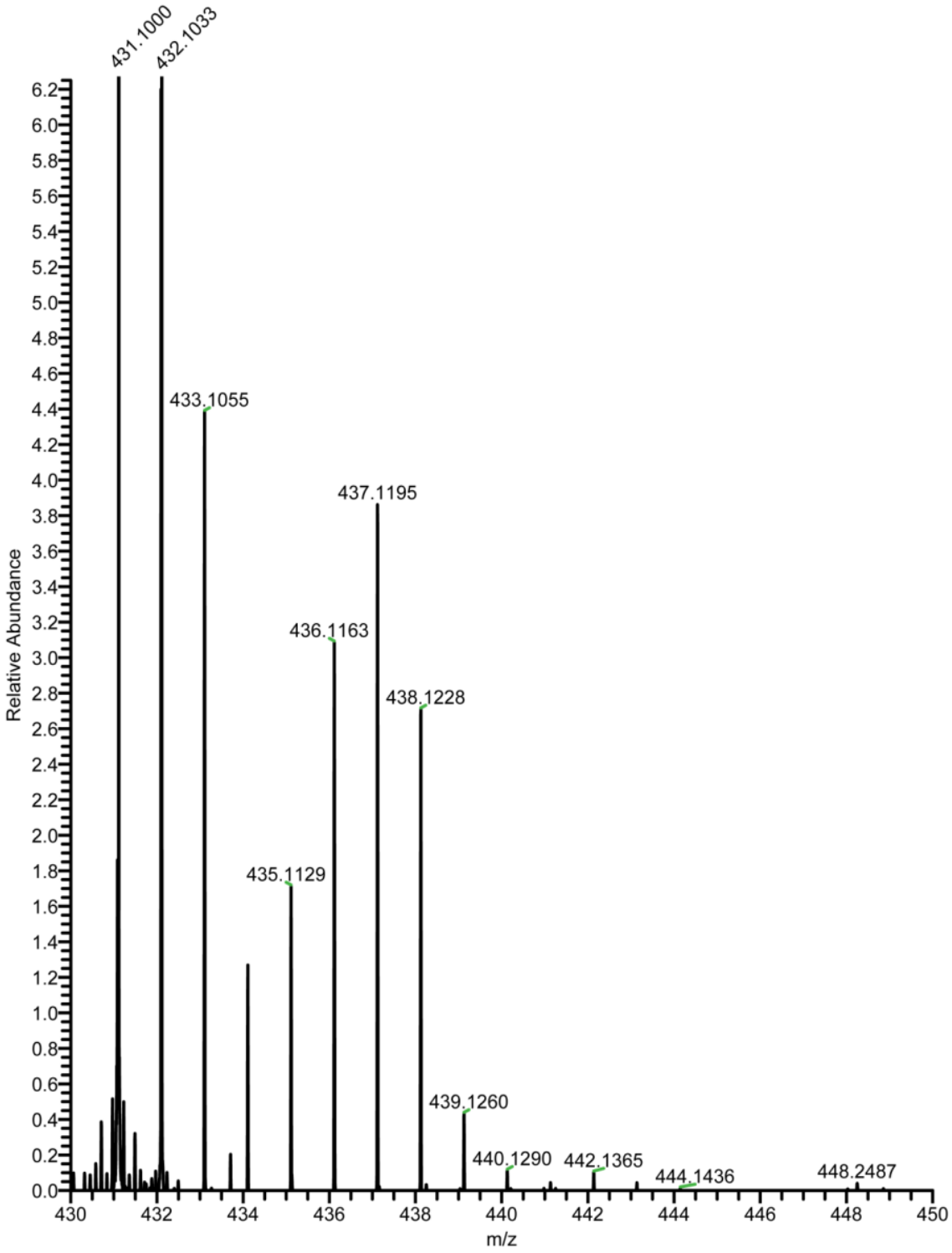


Figure C.3-13 Compound **7**; HRESIMS spectrum (*m/z* 431.1000 $[M-H]^-$) of *C. vinula* feces after consumption of leaves labeled with [U-^{13}C]salicortin.

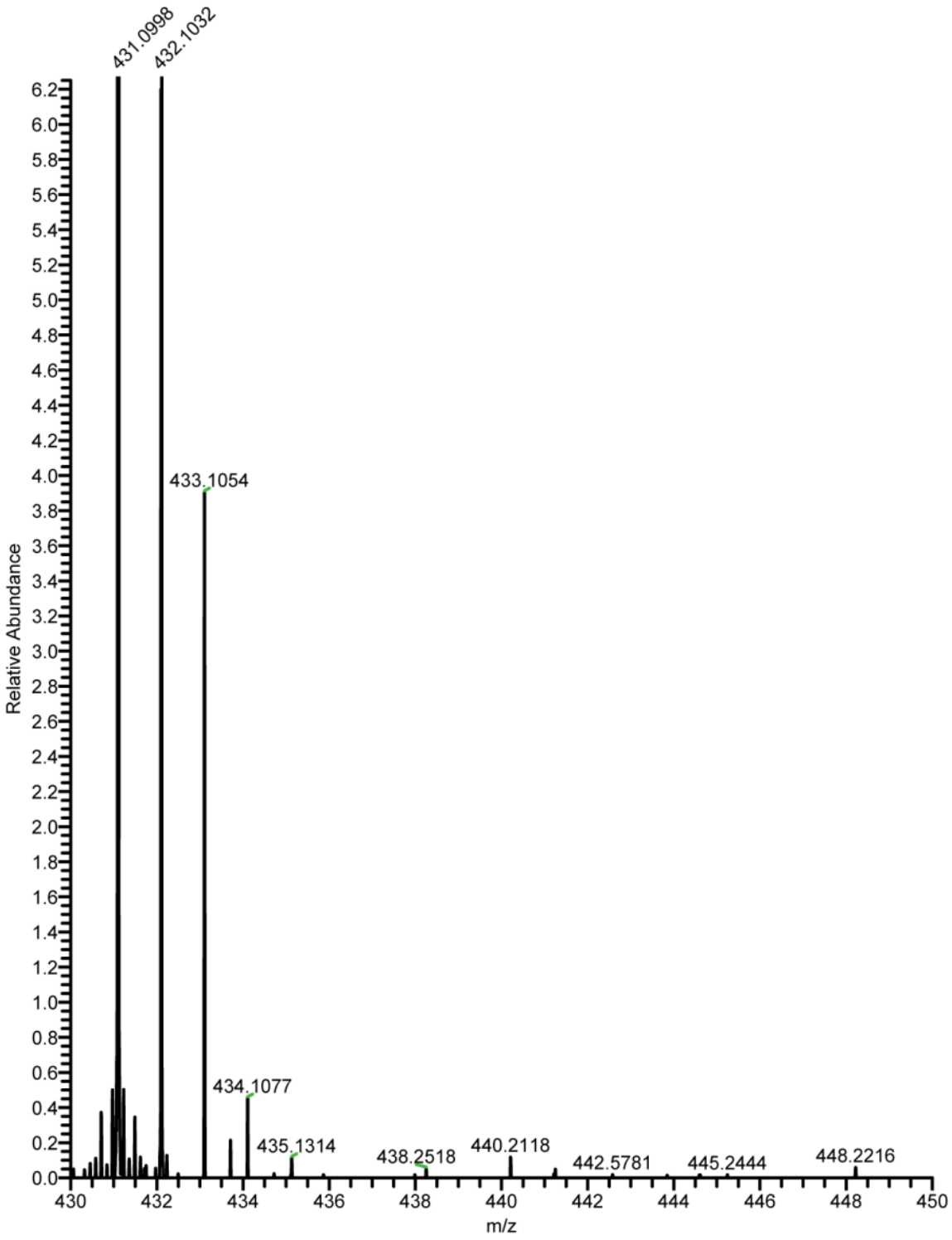


Figure C.3-14 Compound **7**; HRESIMS spectrum (*m/z* 431.0998 $[M-H]^-$) of *C. vinula* feces after consumption of control tissue.

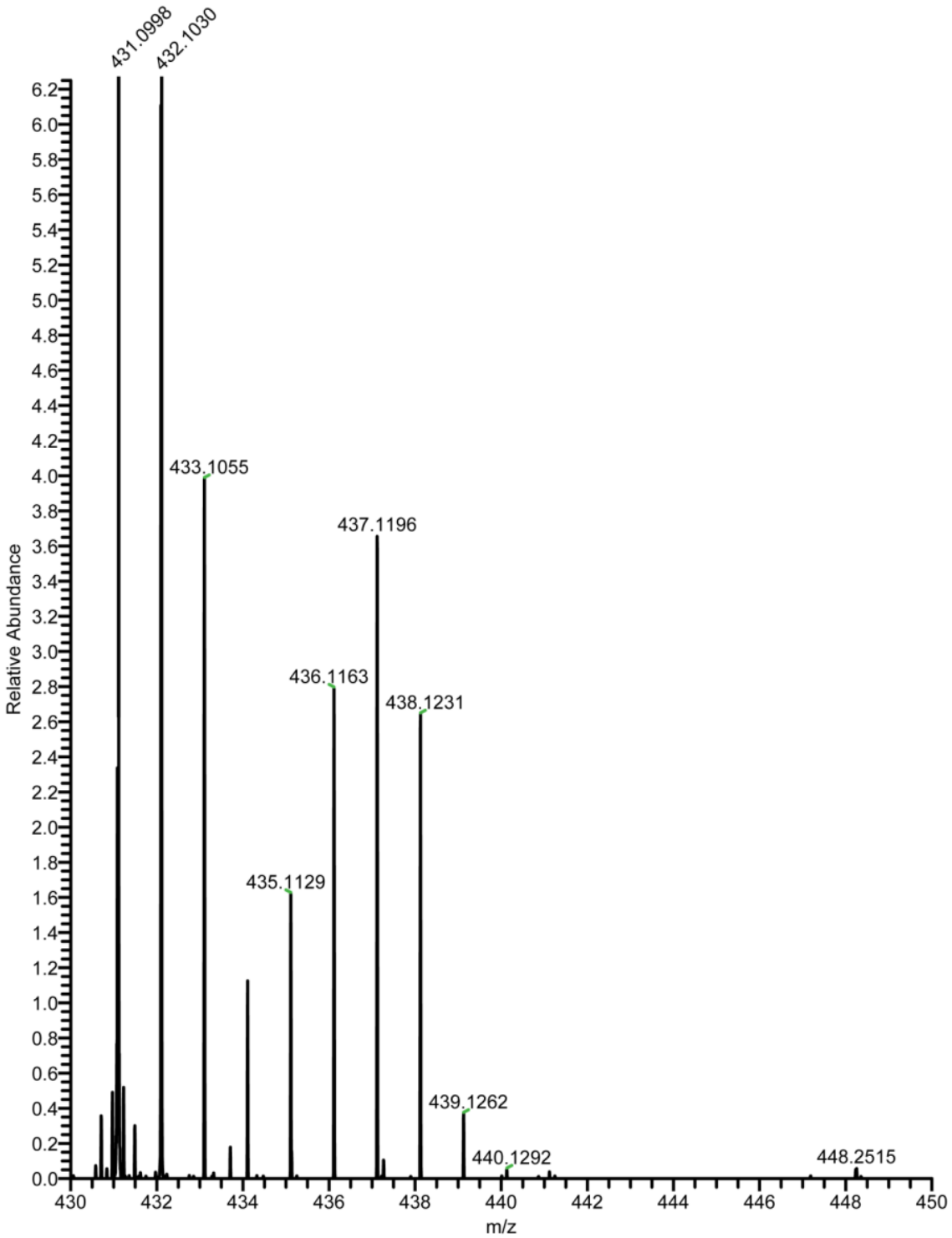


Figure C.3-15 Compound **8**; HRESIMS spectrum (*m/z* 431.0998 $[M-H]^-$) of *C. vinula* feces after consumption of leaves labeled with [U-^{13}C]salicortin.

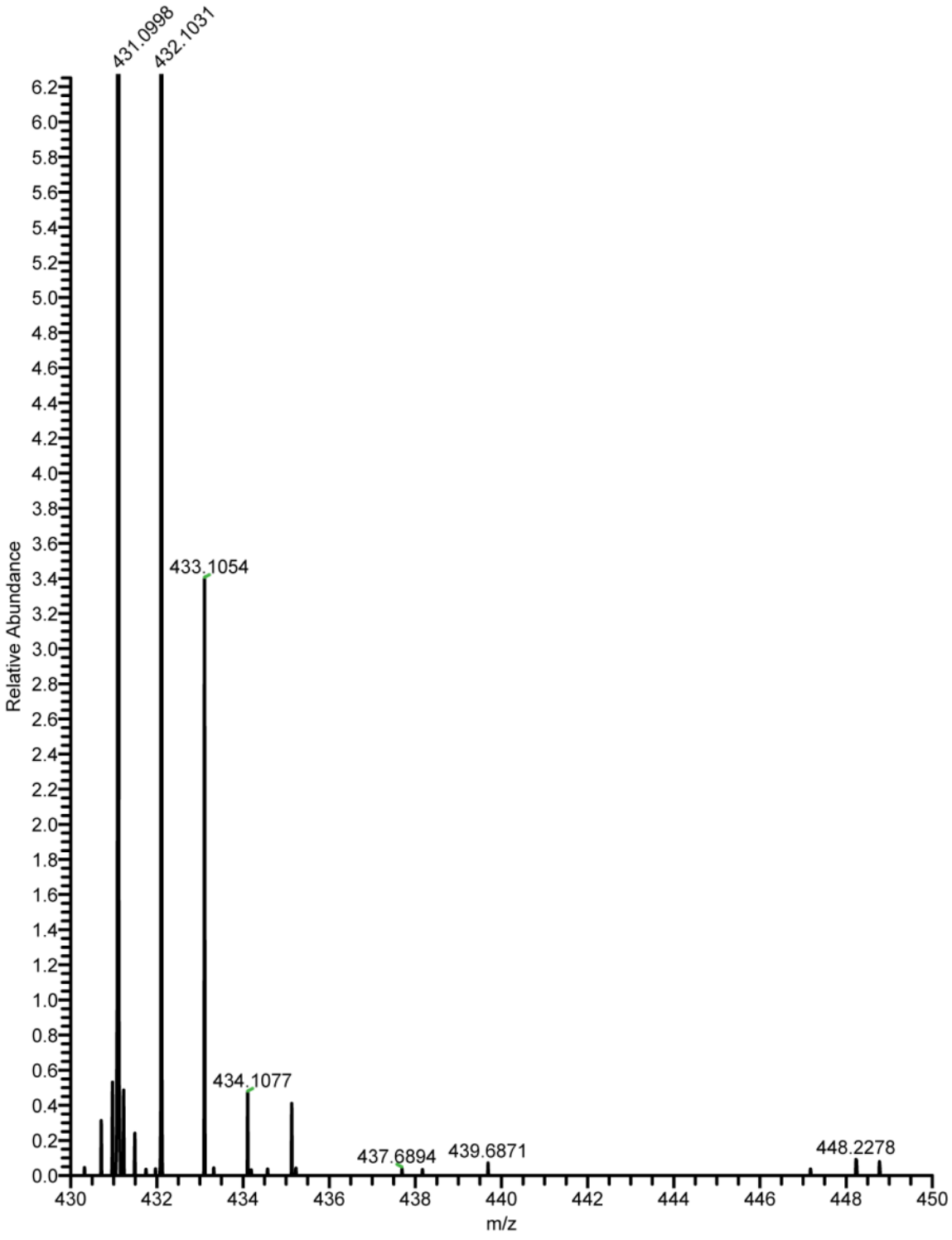


Figure C.3-16 Compound **8**; HRESIMS spectrum (*m/z* 431.0998 $[M-H]^-$) of *C. vinula* feces after consumption of control tissue.

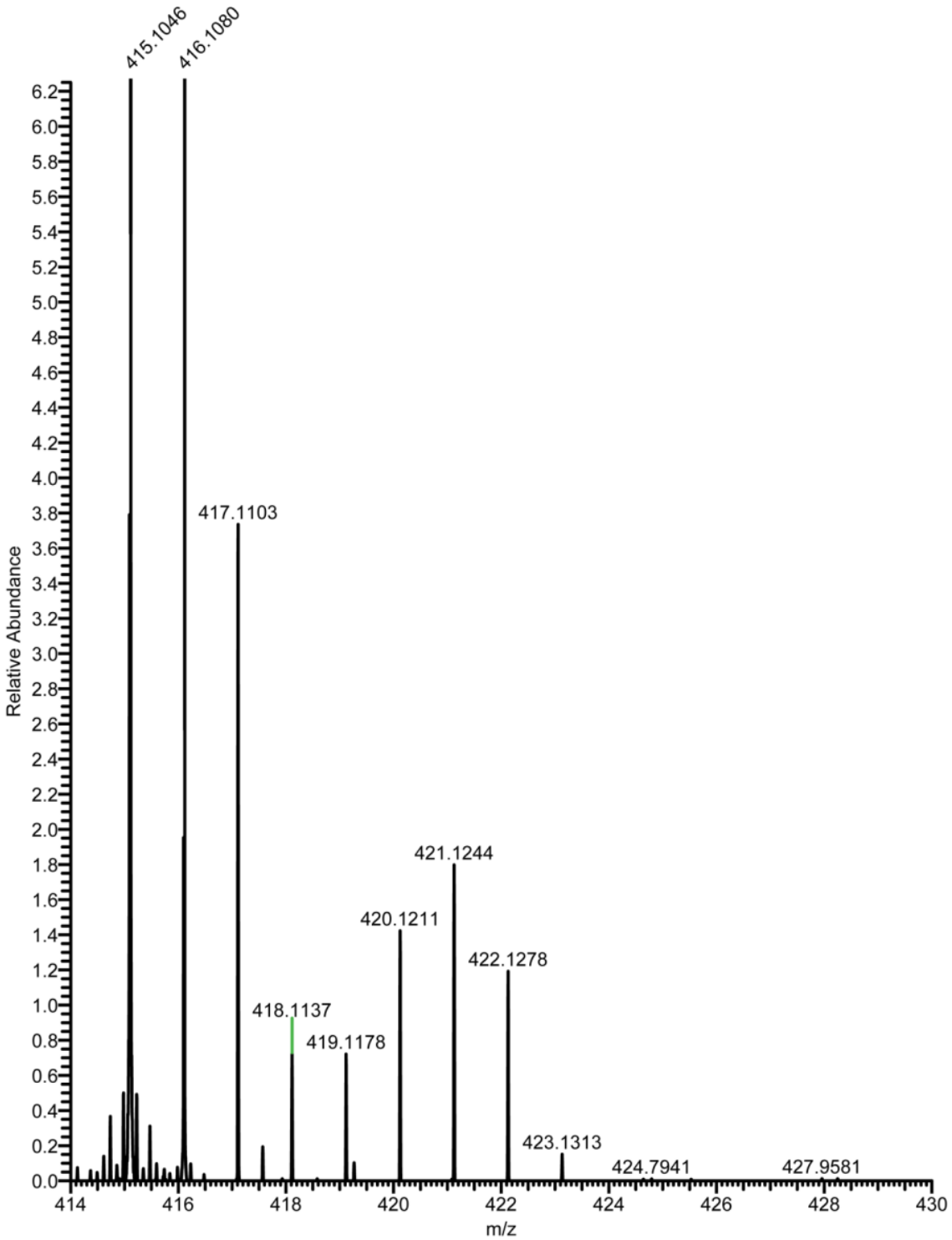


Figure C.3-17 Compound **9**; HRESIMS spectrum (*m/z* 415.1046 $[M-H]^-$) of *C. vinula* feces after consumption of leaves labeled with [U-^{13}C]salicortin.

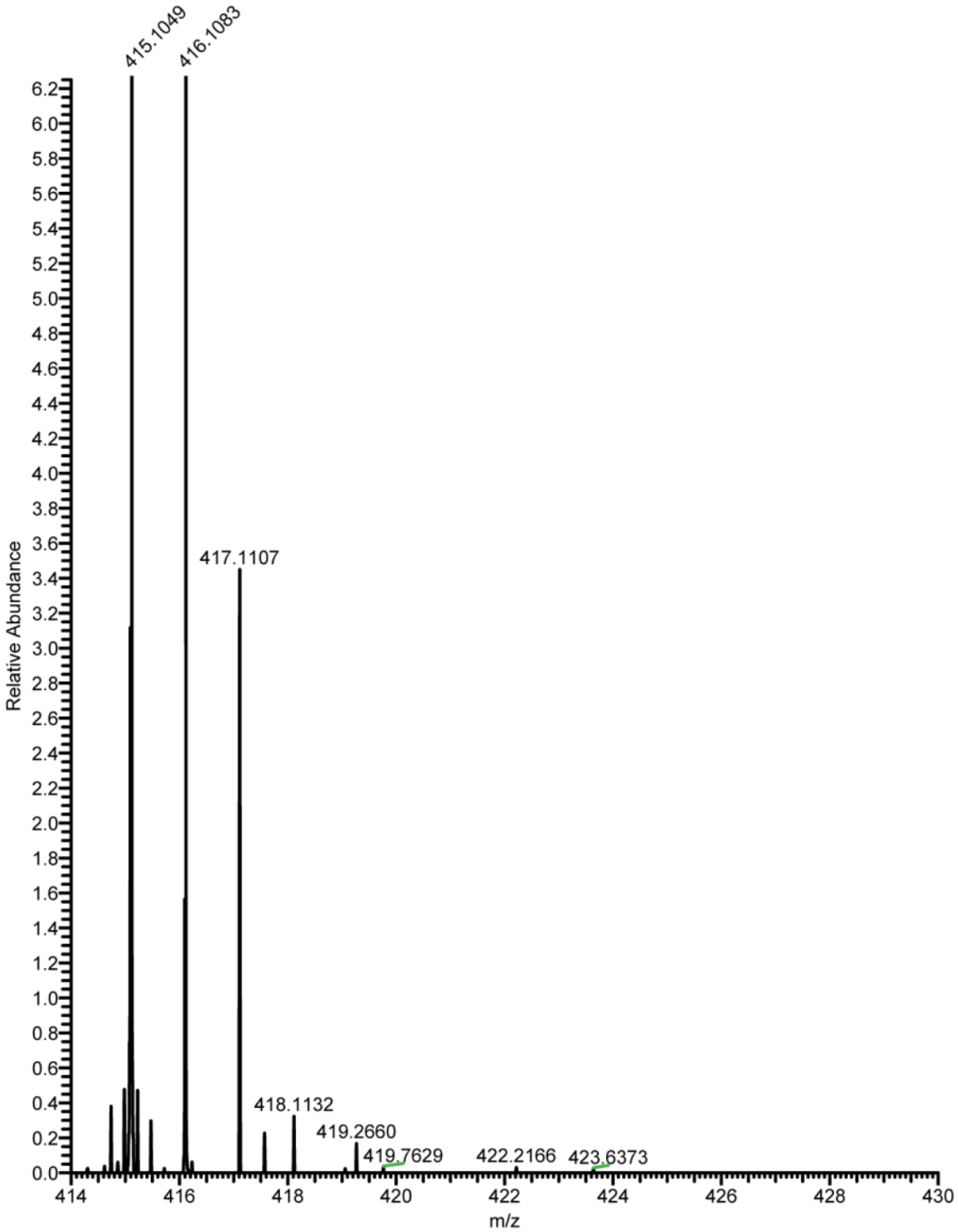


Figure C.3-18 Compound **9**; HRESIMS spectrum (*m/z* 415.1049 [M-H]$^-$) of *C. vinula* feces after consumption of control tissue.

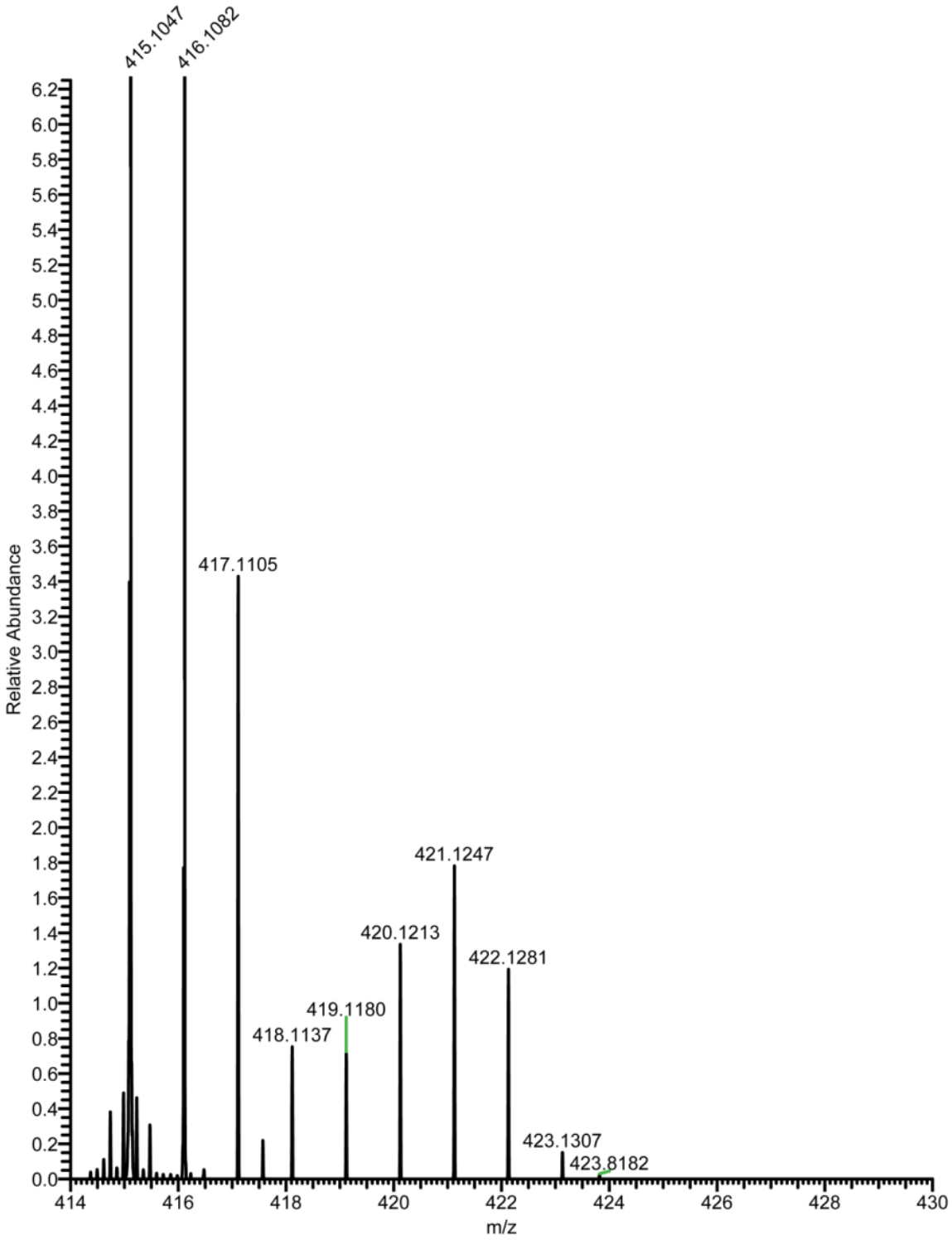


Figure C.3-19 Compound **10**; HRESIMS spectrum (*m/z* 415.1047 $[M-H]^-$) of *C. vinula* feces after consumption of leaves labeled with [U-^{13}C]salicortin.

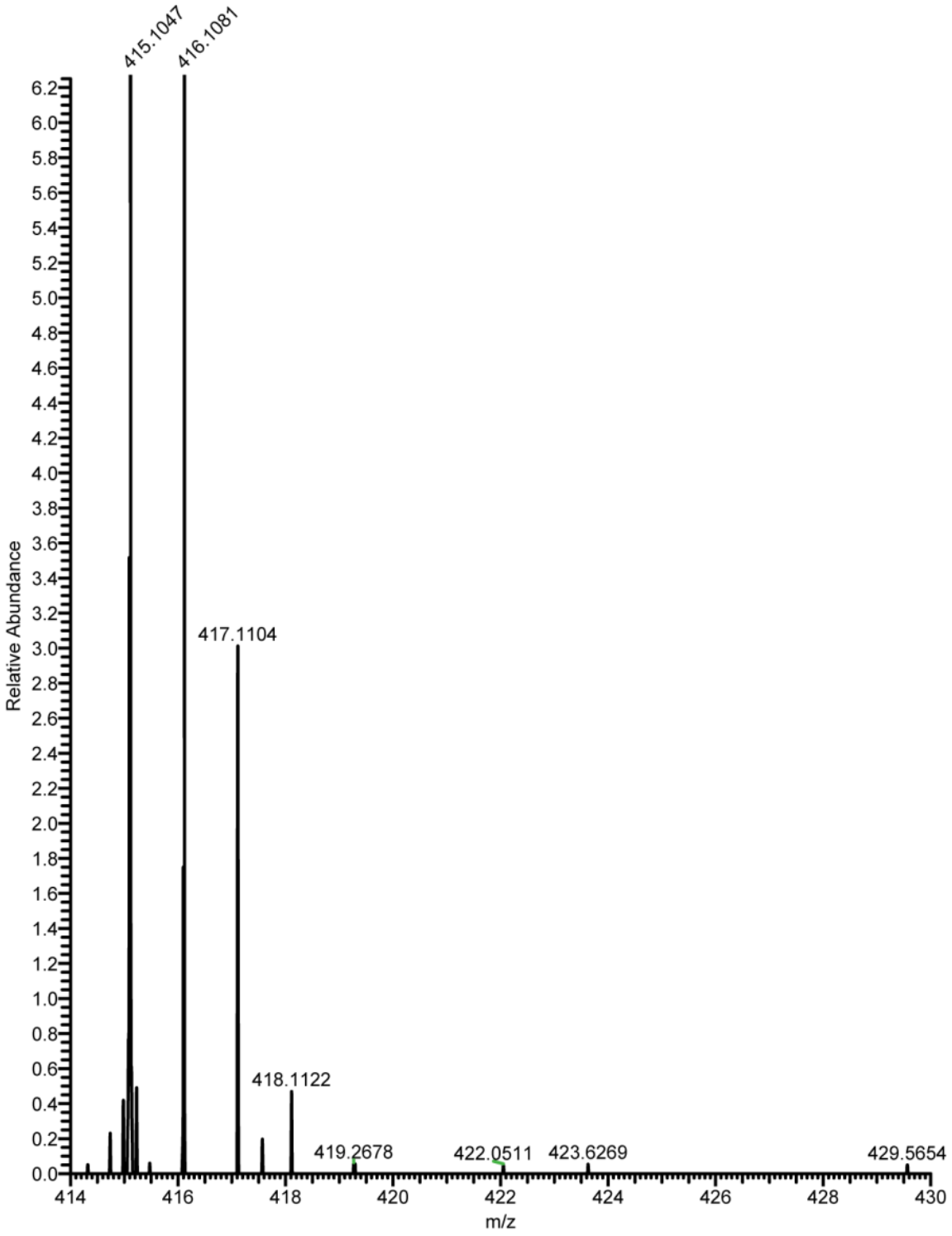


Figure C.3-20 Compound **10**; HRESIMS spectrum (*m/z* 415.1047 $[M-H]^-$) of *C. vinula* feces after consumption of control tissue.

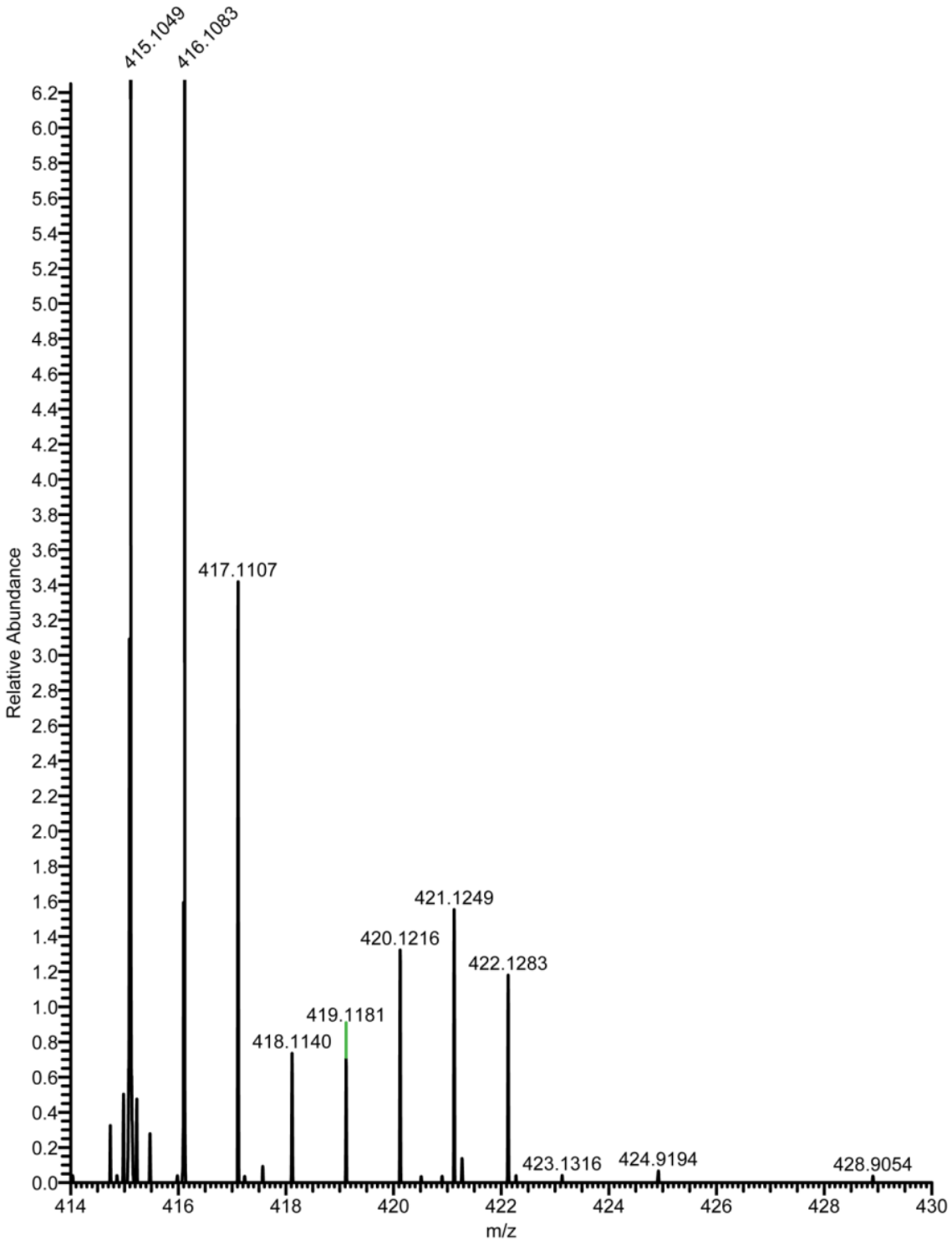


Figure C.3-21 Compound **11**; HRESIMS spectrum (*m/z* 415.1049 $[M-H]^-$) of *C. vinula* feces after consumption of leaves labeled with [U-^{13}C]salicortin.

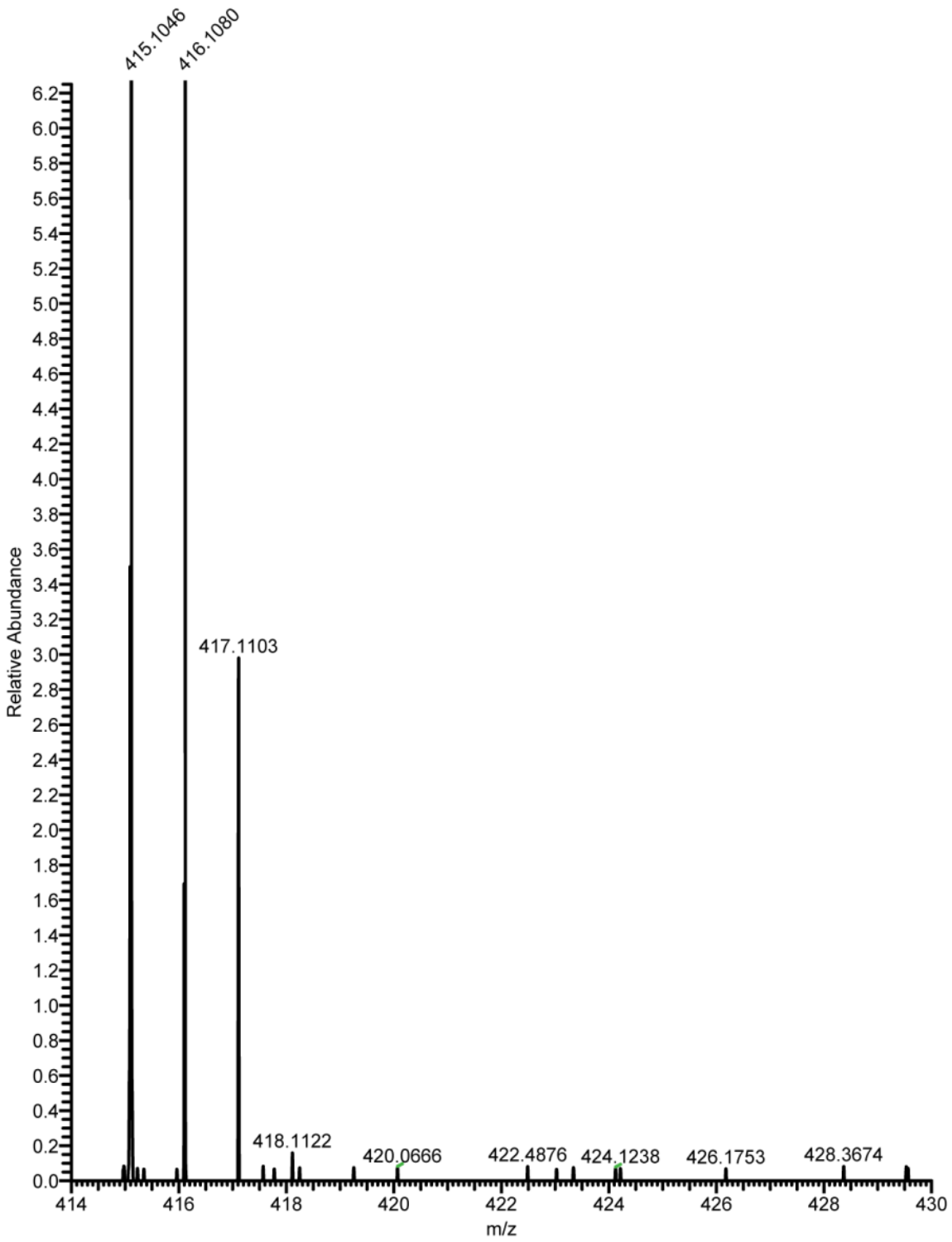


Figure C.3-22 Compound **11**; HRESIMS spectrum (*m/z* 415.1046 $[M-H]^-$) of *C. vinula* feces after consumption of control tissue.

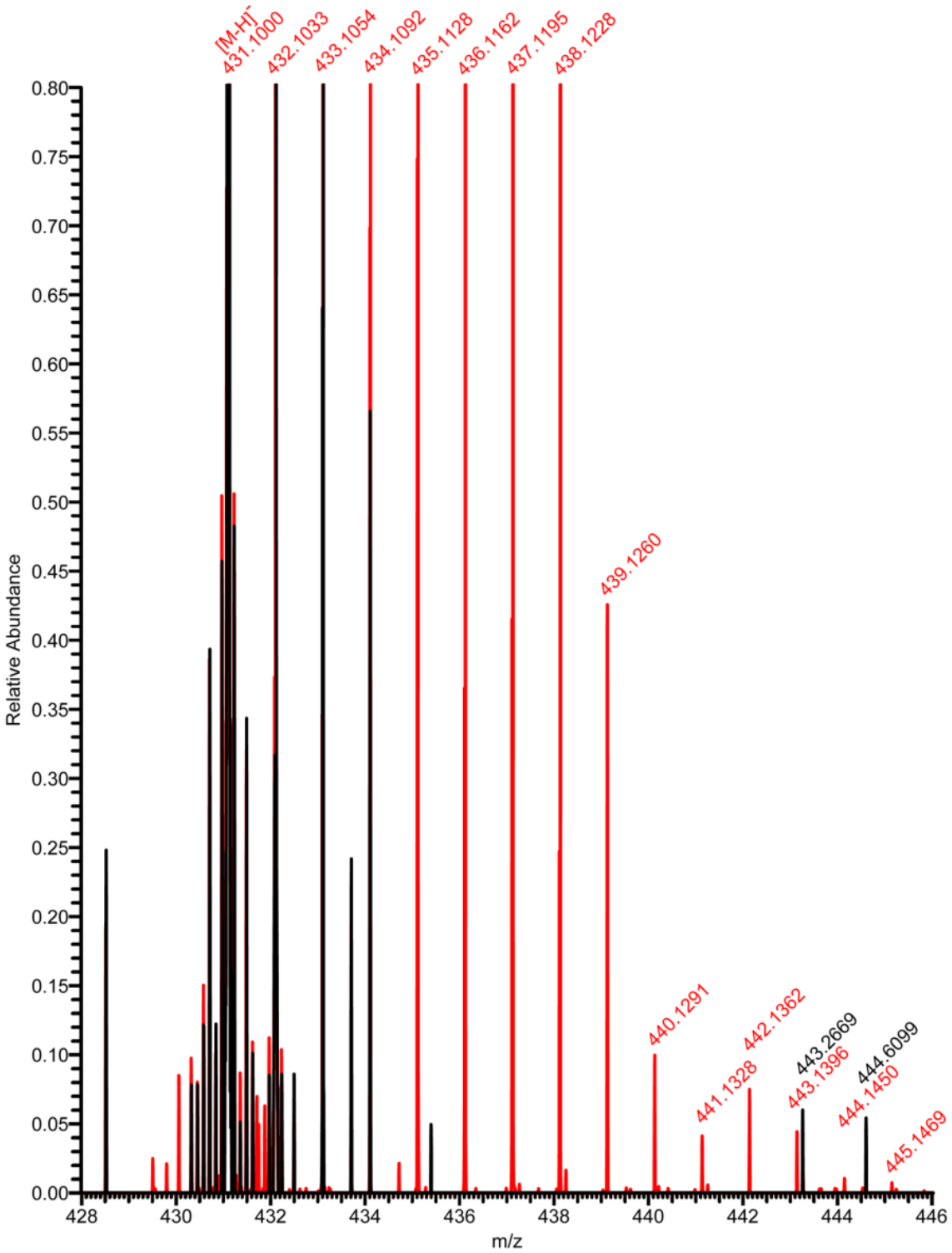


Figure C.3-23 Strongly amplified, superimposed HRESIMS spectrum of compound **7** (*m/z* 431) from *C. vinula* feces after consumption of control tissue (black) and leaves labeled with [U-^{13}C]salicortin (red). The spectrum is strongly amplified to show occurrence of isotopologues up to $[M-H+14]^-$.

D) Qualitative analysis of *C. vinula* hemolymphs:

D.1) HRESIMS of six caterpillars testes (I to VI)

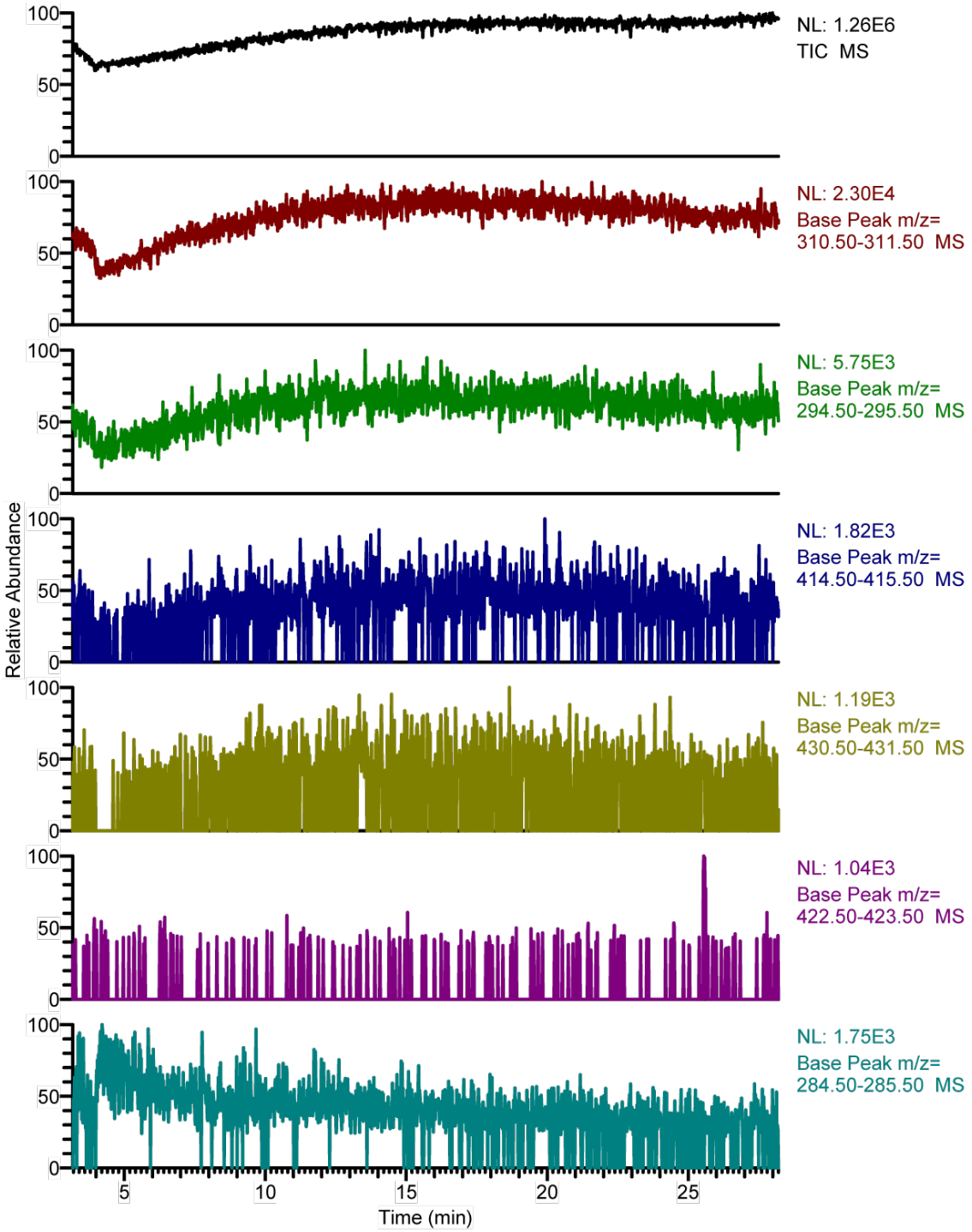


Figure D.1-1 HRESIMS; TIC and base peak chromatograms of hemolymphs from *C. vinula* larva I.

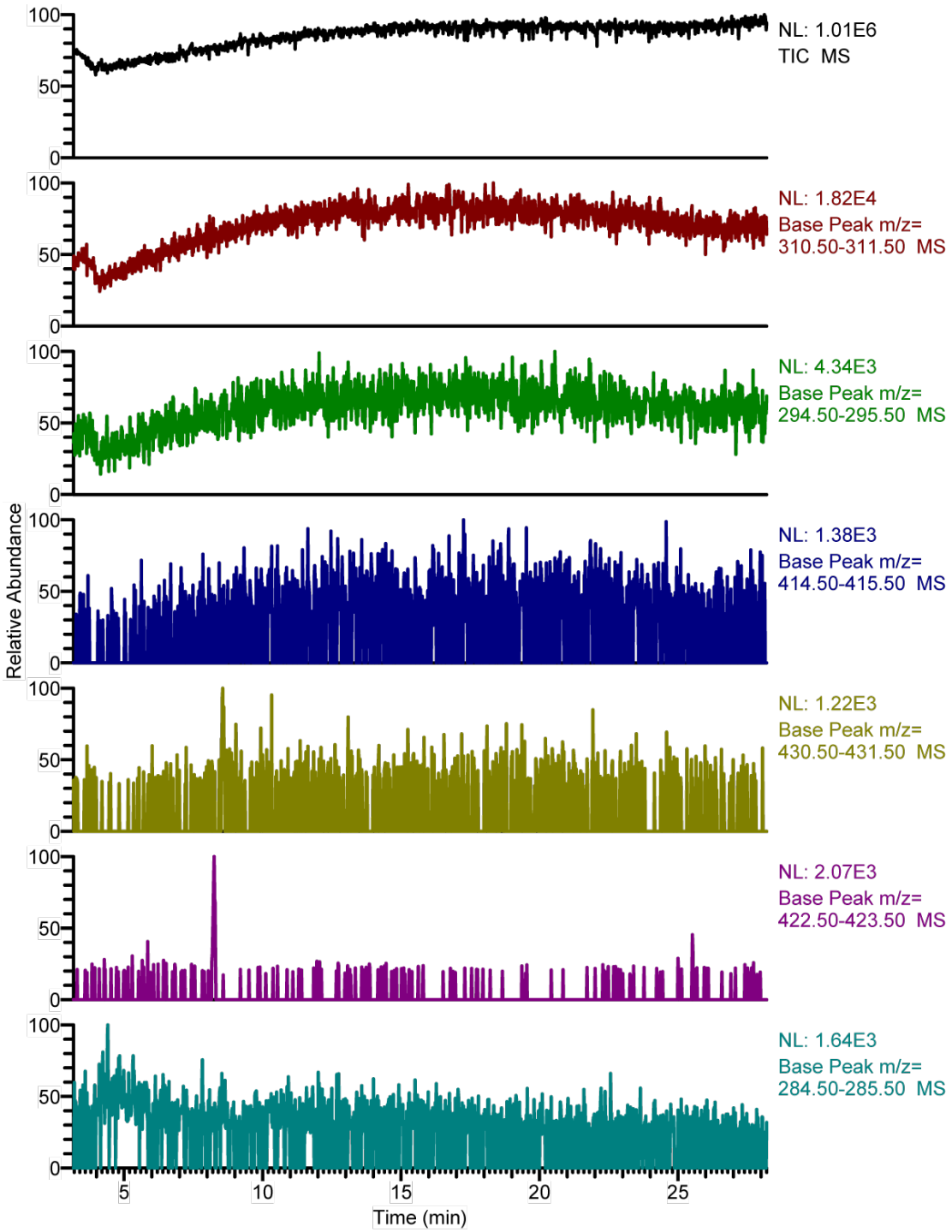


Figure D.1-2 HRESIMS; TIC and base peak chromatograms of hemolymphs from *C. vinula* larva II.

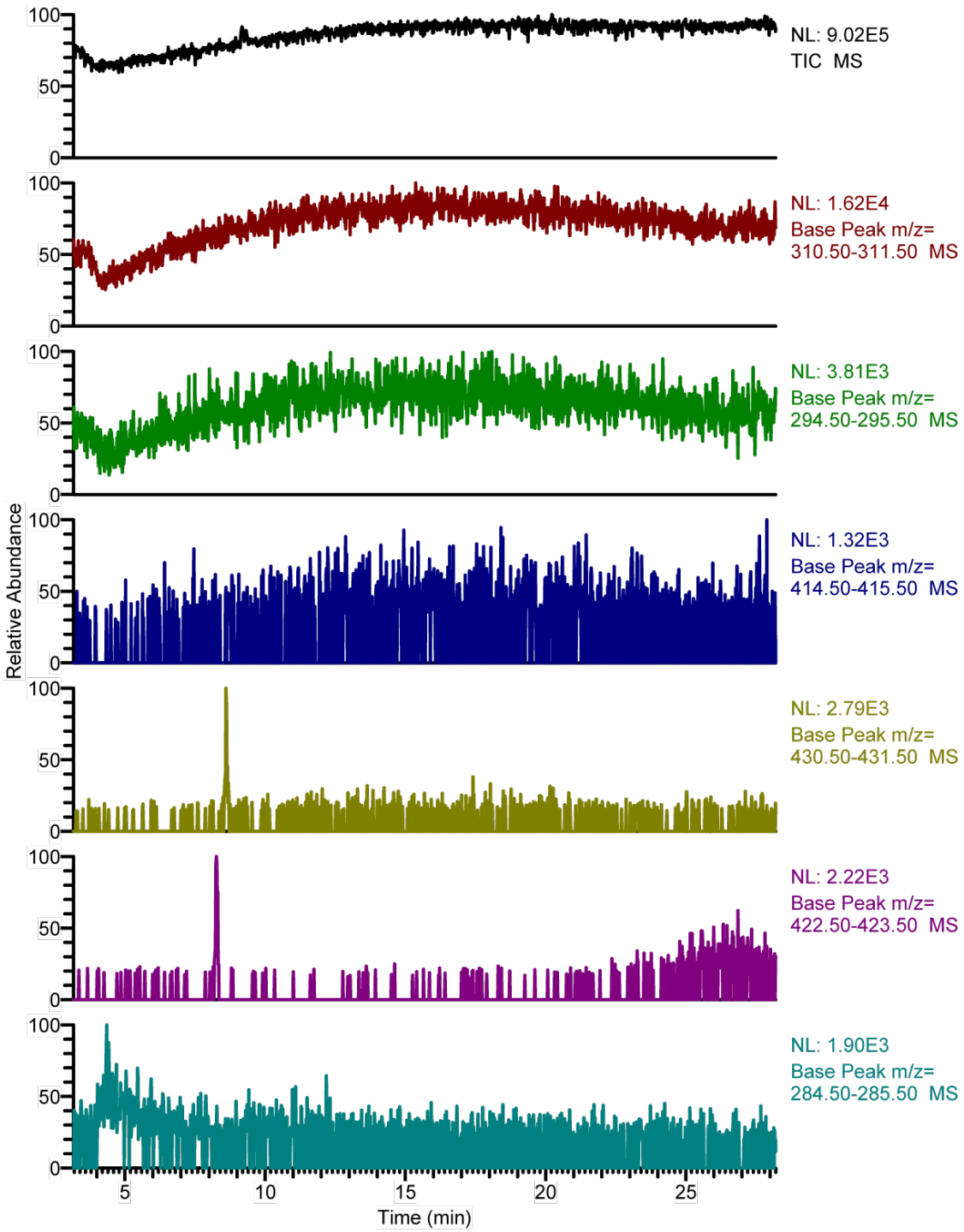


Figure D.1-3 HRESIM; TIC and base peak chromatograms of hemolymphs from *C. vinula* larva III.

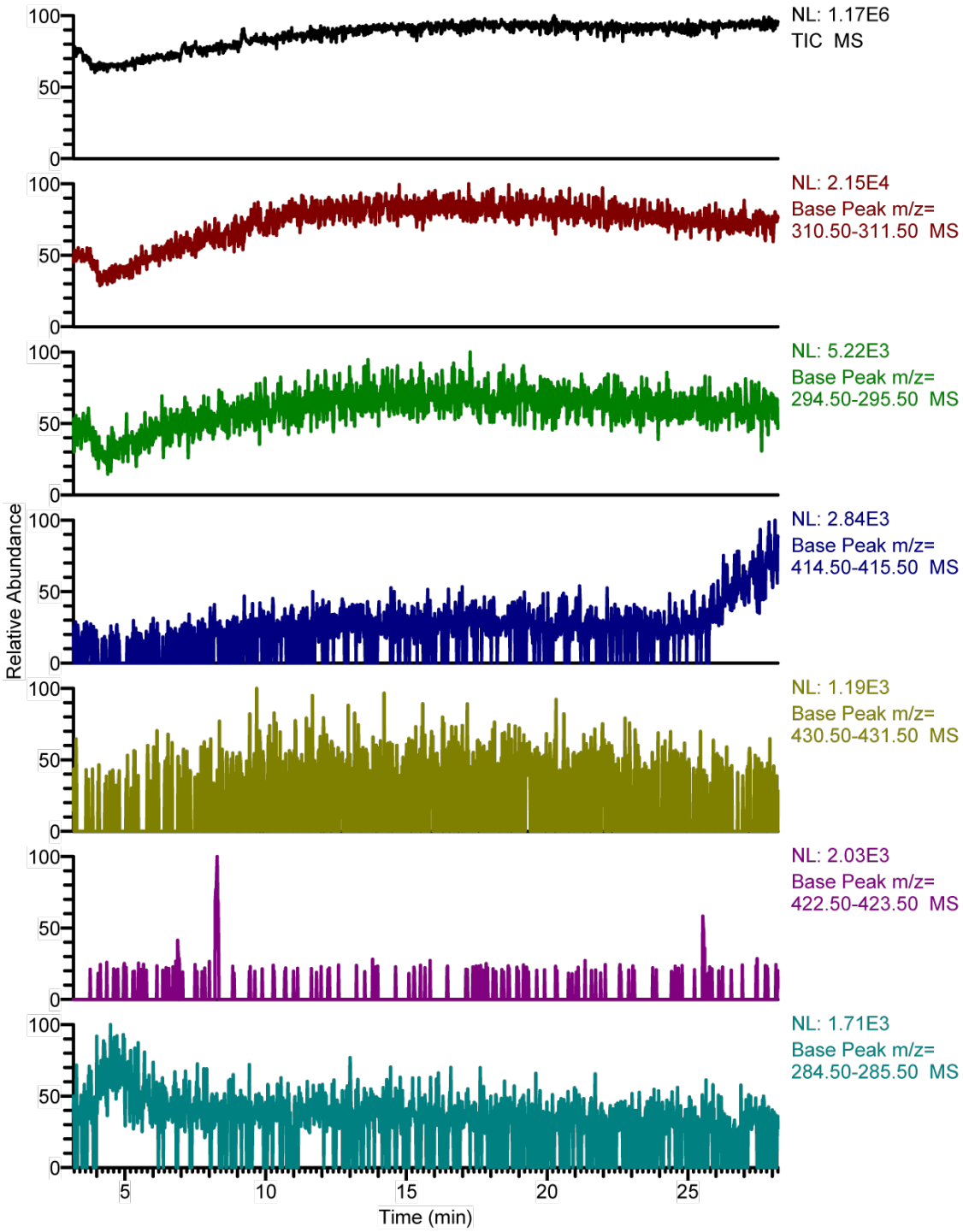


Figure D.1-4 HRESIMS; TIC and base peak chromatograms of hemolymphs from *C. vinula* larva IV.

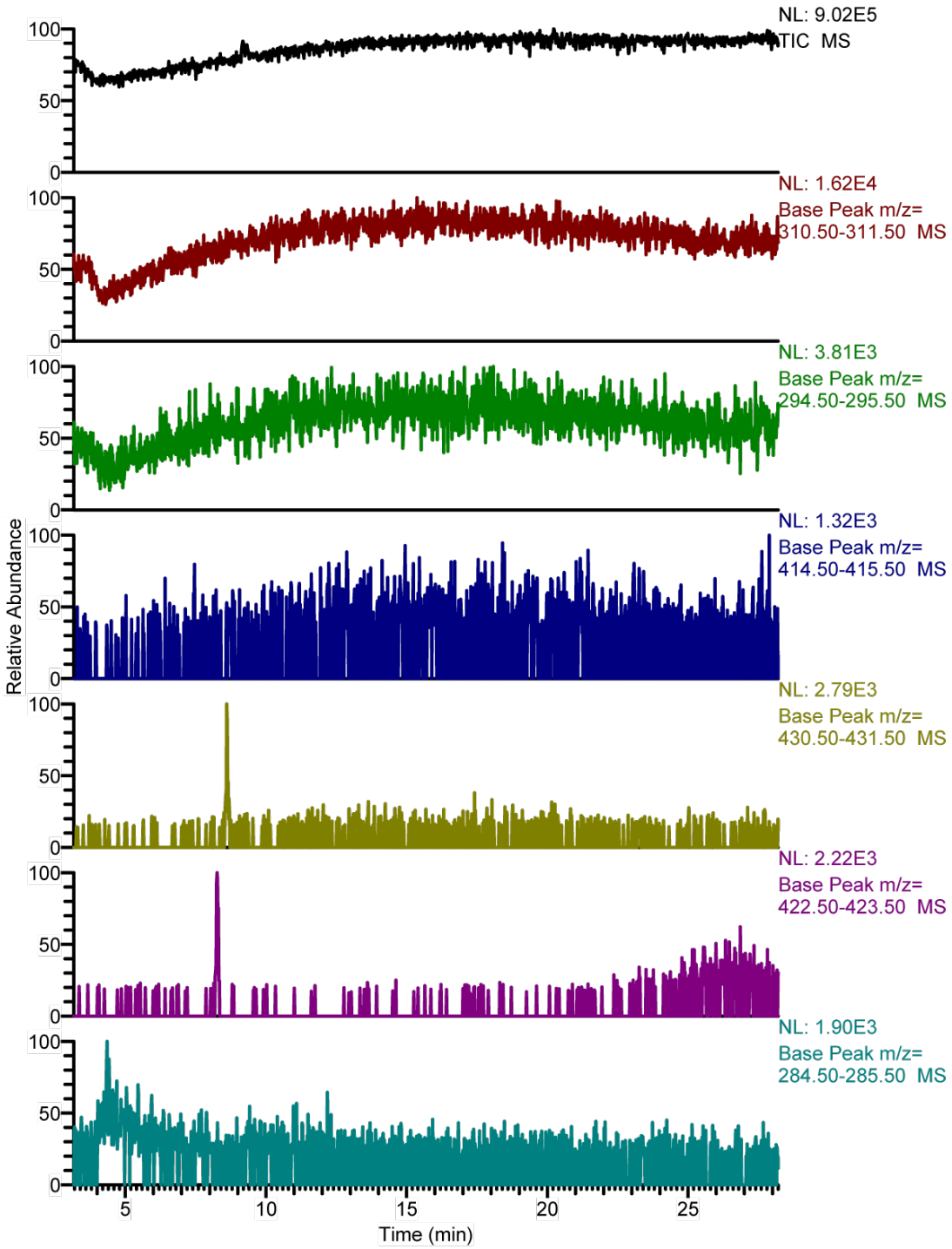


Figure D.1-5 HRESIMS; TIC and base peak chromatograms of hemolymphs from *C. vinula* larva V.

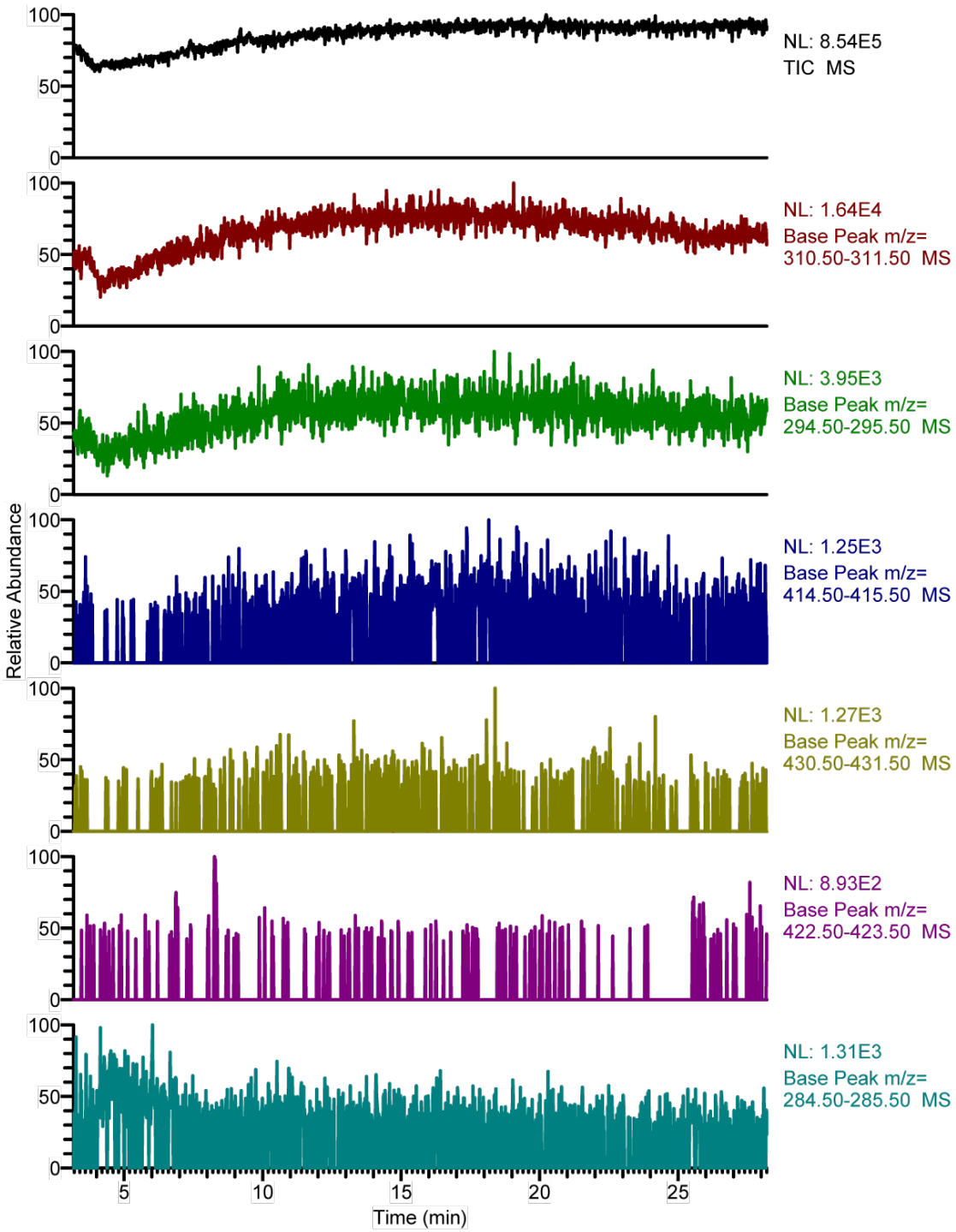


Figure D.1-6 HRESIMS; TIC and base peak chromatograms of hemolymphs from *C. vinula* larva VI.

D.2) HRESIMS of references

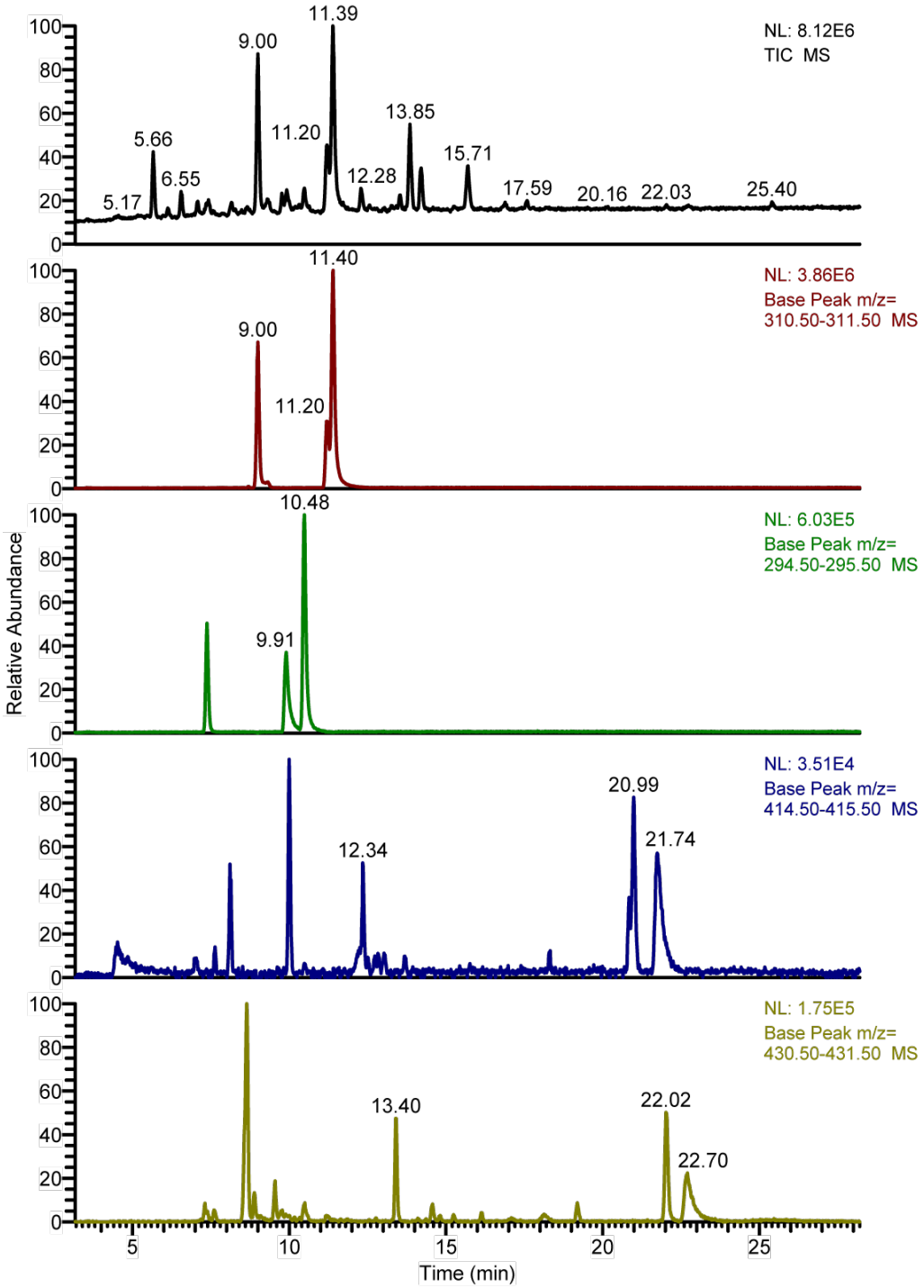


Figure D.2-1 HRESIMS; TIC and base peak chromatograms of *C. vinula* feces. The peaks of quinic acid esters are labeled with retention times. R_t (compound): 9.00 (**1**), 9.91 (**4**), 10.48 (**5**), 11.20 (**2**), 11.40 (**3**), 12.34 (**10**), 13.40 (**7**), 20.99 (**9**), 21.74 (**11**), 22.02 (**6**), 22.70 (**8**).

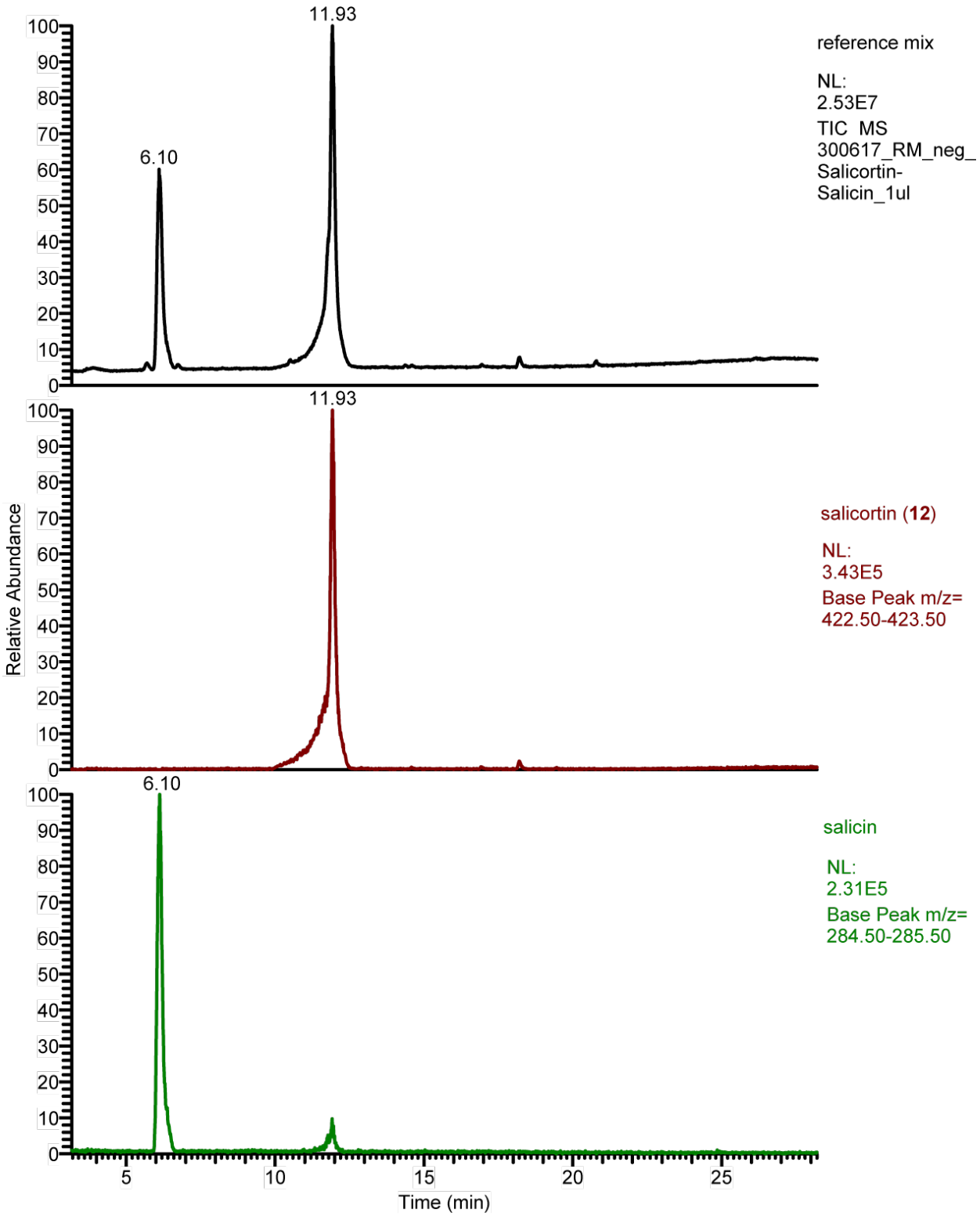


Figure D.2-2 HRESIMS; TIC and base peak chromatograms from a reference mix of salicortin (**12**) (red; 11.93 min) and salicin (green; 6.10 min).