Supporting Information

Synthesis and Structural Identification of Taxifolin Sulfate Conjugates

Yu Sasaki, Yuto Nishidono, Ken Tanaka and Tohru Kamitanaka*

Contents

General Information

NMR spectra were measured using a JNM-ECZ500R/S1 (Japan Electronic Co., Ltd., Tokyo, Japan) at 500 MHz (^{1}H) and 125 MHz (^{13}C). Deuterated dimethyl sulfoxide (DMSO-d_6) was obtained from Euriso-Top (Saint-Aubin, France). During analysis, DMSO-d_6 (^{1}H NMR: 2.49 ppm, ^{13}C NMR: 39.5 ppm) was used as the internal standard.

LC-QTOF-MS analysis was performed using a Xevo G2-XS QTOF mass spectrometer (Waters Corporation (Milford, Massachusetts, USA)) equipped with an electrospray ionization (ESI) interface in negative ion mode. The sample temperature was maintained at 4 °C using the ACQUITY UPLC H-CLASS Plus Sample Manager FTN-H (Waters Corporation, Milford, Massachusetts, USA). Additionally, LC-QTOF-MS analysis was performed after passing through a 0.20-μm PTFE membrane. ESI parameters were as follows: data acquisition mode, MS^{E}; collision energy, 20-50 eV; Acquisition mass range, 50–1500 Da; cone voltage, 35 eV; capillary voltage, 2.5 kV; source temperature, 150 °C; desolvation temperature, 400 °C; cone gas flow, 100.0 L/h; desolvation gas flow, 1000.0 L/h. Data acquisition mode, MSMS; collision energy, 20–50 eV; Acquisition mass range, 100–1000 Da; precursor ion, 383.0078 Da; target enhancement, 303.0510 Da; cone voltage, 40 eV; capillary voltage, 2.5 kV; source temperature, 150 °C; desolvation temperature, 400 °C; cone gas flow, 100.0 L/h; desolvation gas flow, 1000.0 L/h.

A TOSOH TSKgel ODS-100V column (2.0 × 150 mm, 3 μm) was used at a column temperature maintained at 30 °C. The mobile phase was a binary eluent of (A) water (containing 0.1% formic acid) and (B) acetonitrile under the following gradient conditions: A linear gradient from 5% to 100% over 0–60 minutes. Flow rate: 0.2 mL/min.

HPLC analysis and fractionation were performed using a Shimadzu SPD-M20A photodiode array UV-VIS detector (Shimadzu Corporation, Kyoto, Japan). An ODS column COSMOSIL 5C_{18}-AR-II (10 I.D. × 250 mm, 5 μm, 40 °C) was used to separate the components of the extract using a 5 mM potassium acetate aqueous solution/acetonitrile = 75:25 (v/v) mobile phase. Flow rate: 5.0 mL/min. Wavelength: 290 nm. Additionally, HPLC analysis and fractionation were performed after passing through a 0.45-μm PTFE membrane.

CD spectra were measured using a JASCO J-1500 (JASCO Corporation, Tokyo, Japan) at 600-200 nm and 20 °C. A 0.05 mg/mL methanol solution of the sample was prepared and analyzed.

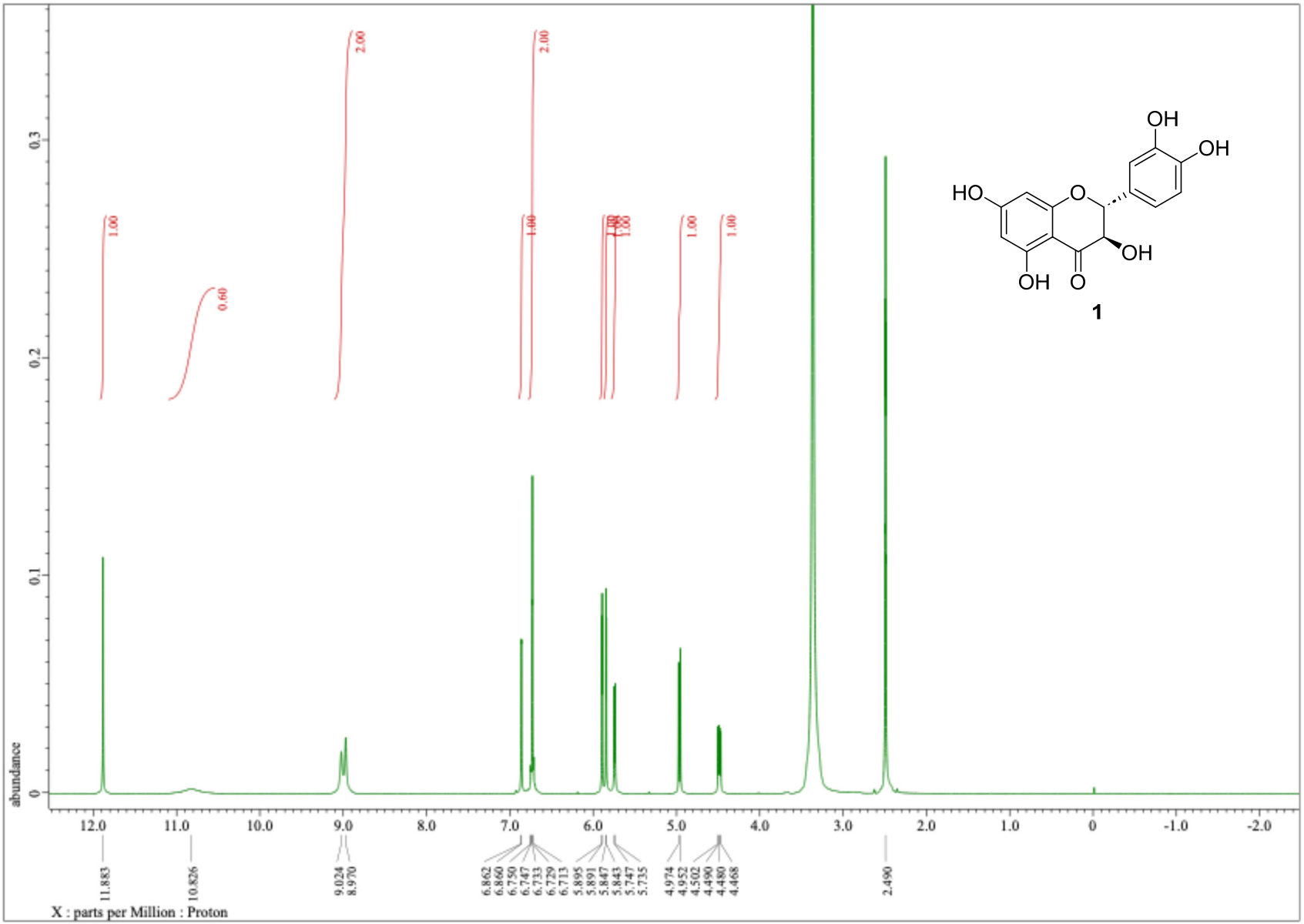

Figure S1. ^{1}H NMR spectrum of taxifolin (**1**) in DMSO-d_6.

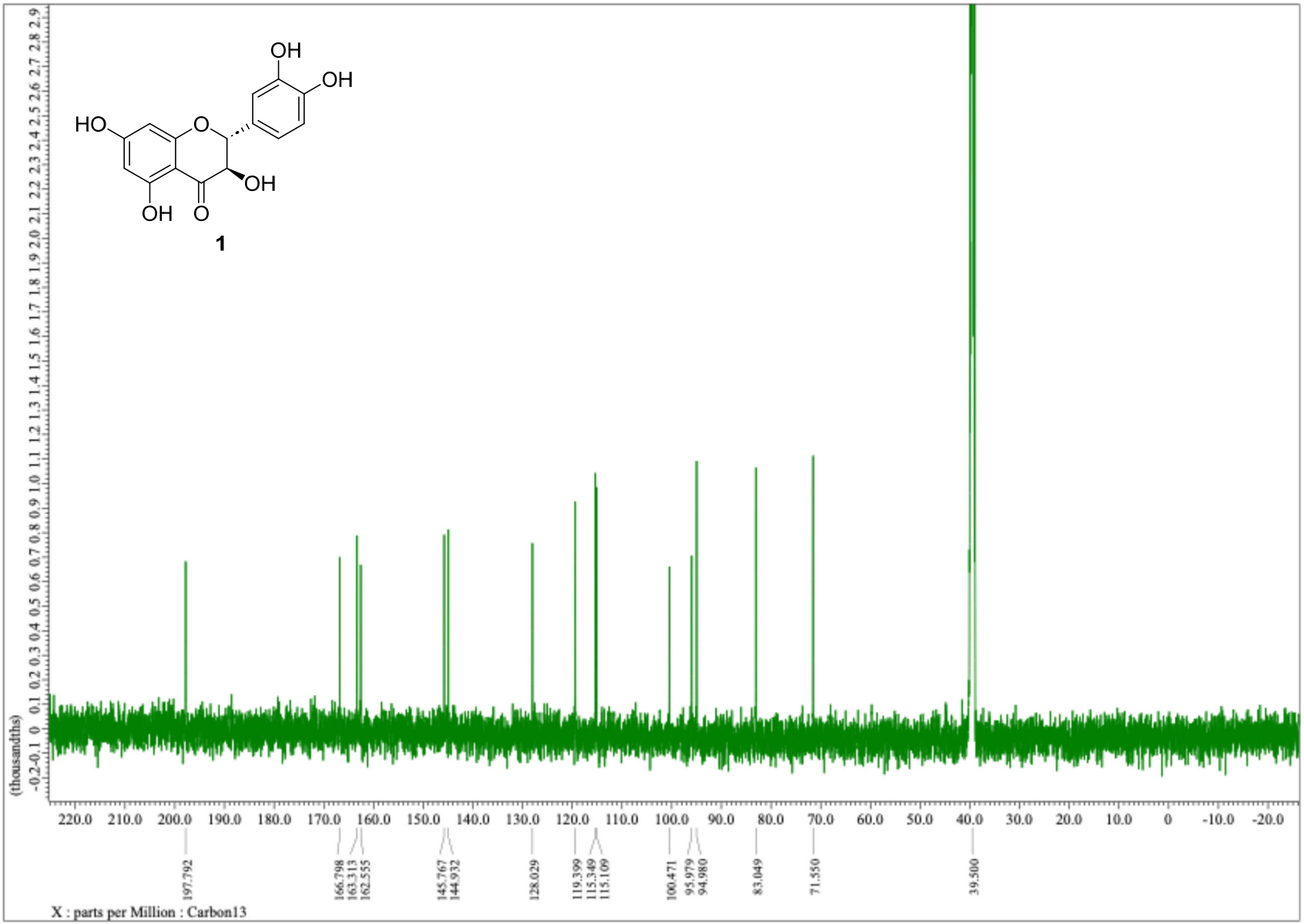

Figure S2. ^{13}C NMR spectrum of taxifolin (**1**) in DMSO-d_6.

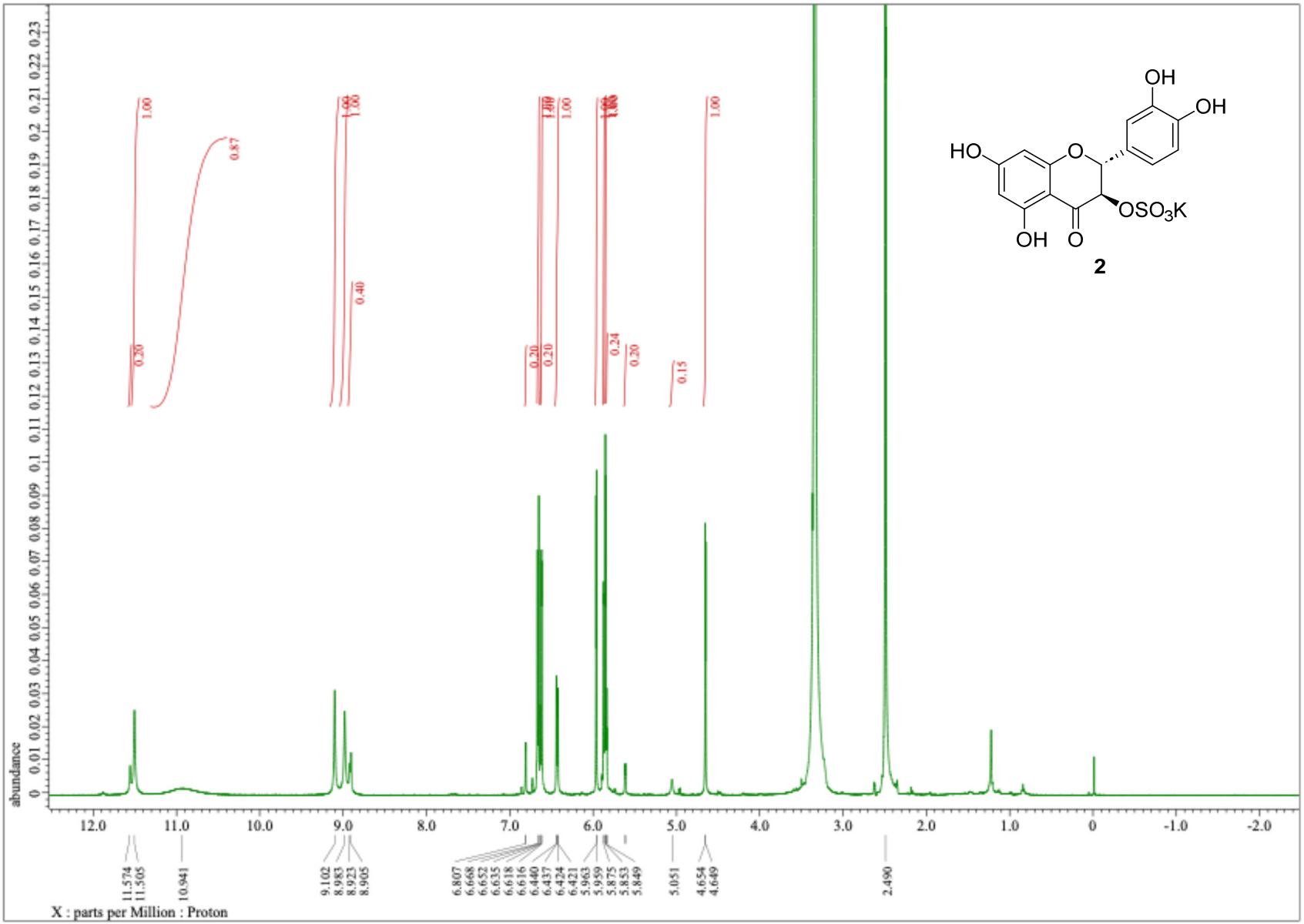

Figure S3. ^{1}H NMR spectrum of taxifolin 3-*O*-sulfate (**2**) in DMSO-d_6.

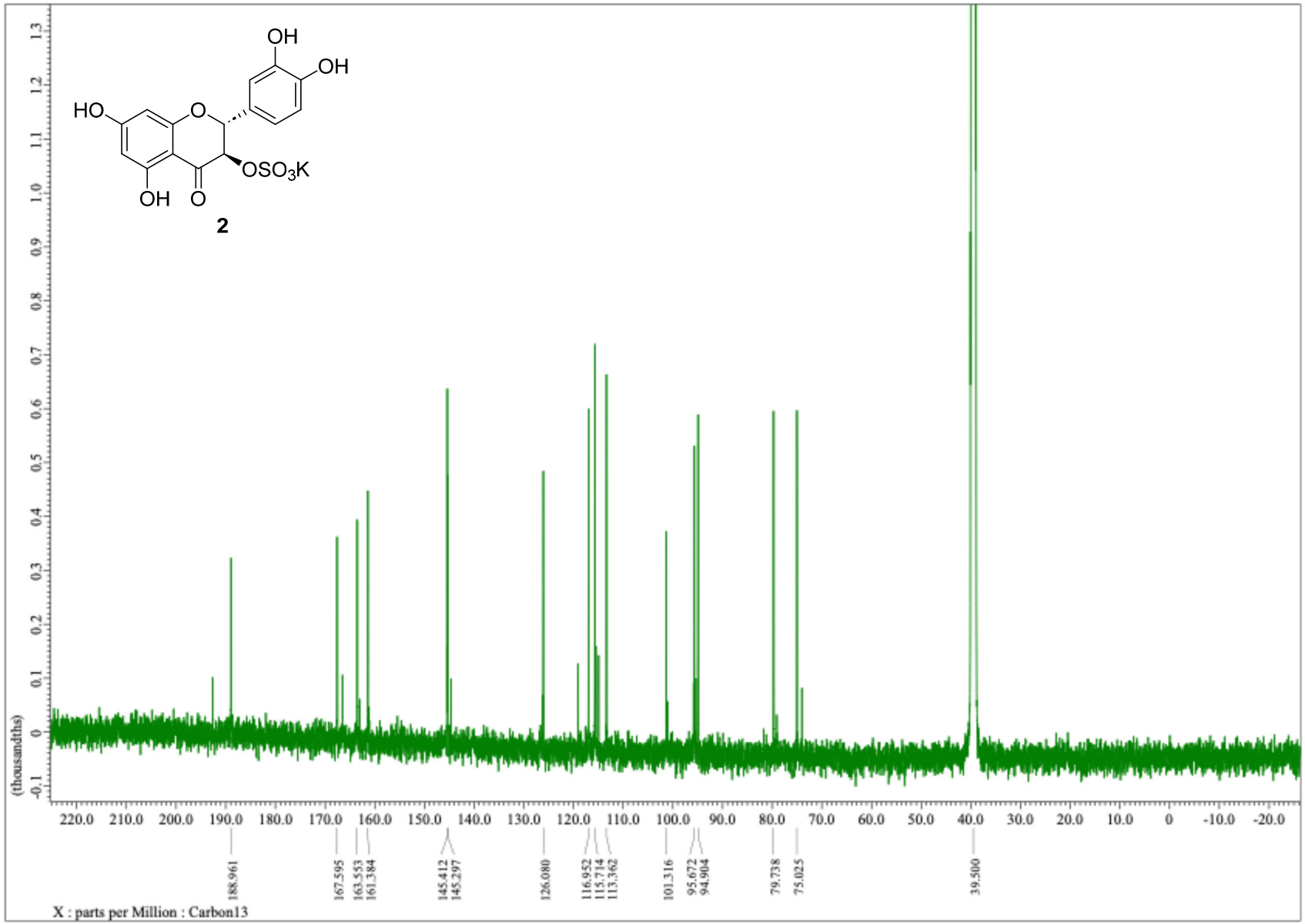

Figure S4. ^{13}C NMR spectrum of taxifolin 3-*O*-sulfate (**2**) in DMSO-d_6.

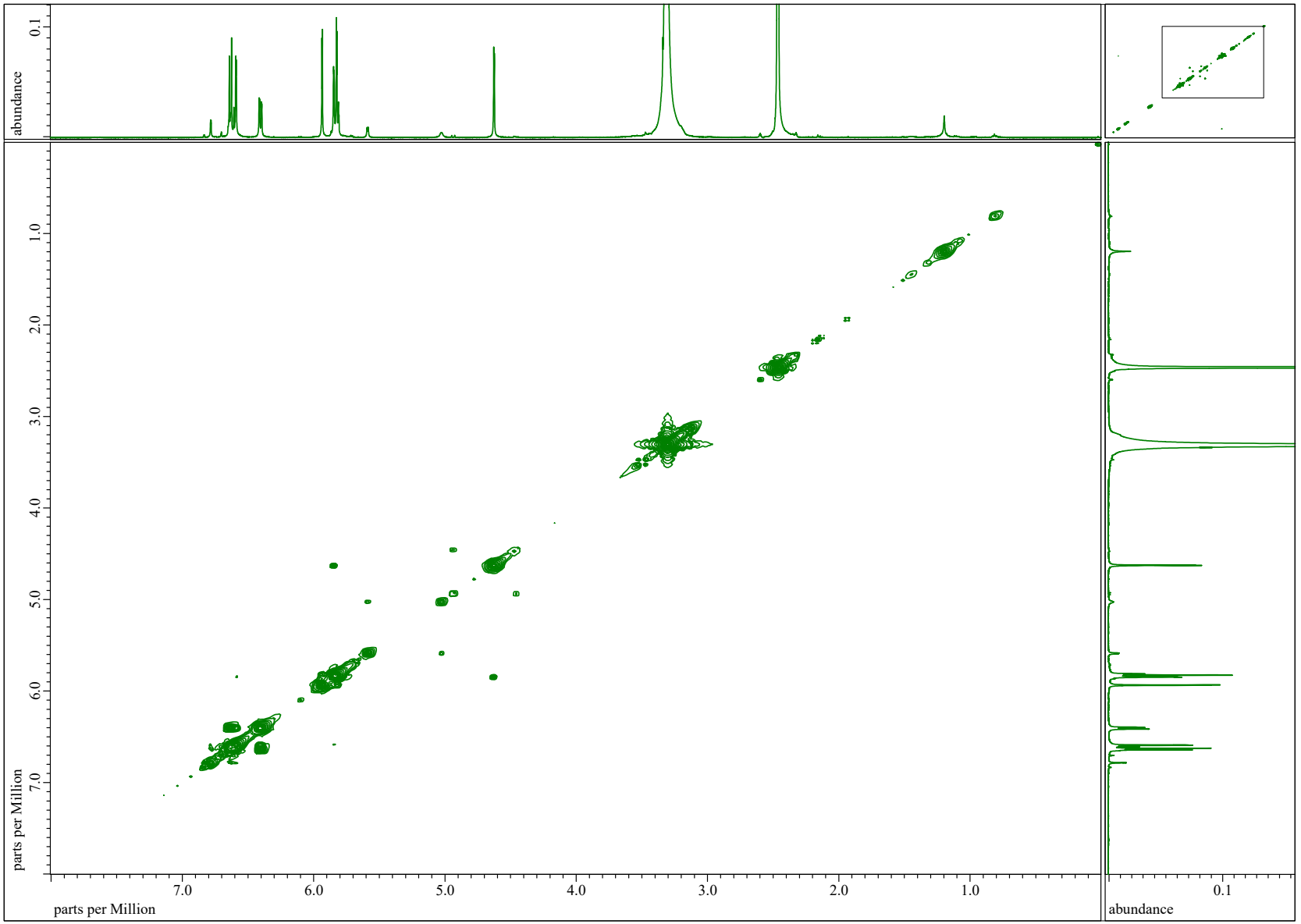

Figure S5. COSY spectrum of taxifolin 3-*O*-sulfate (**2**) in DMSO-d_6.

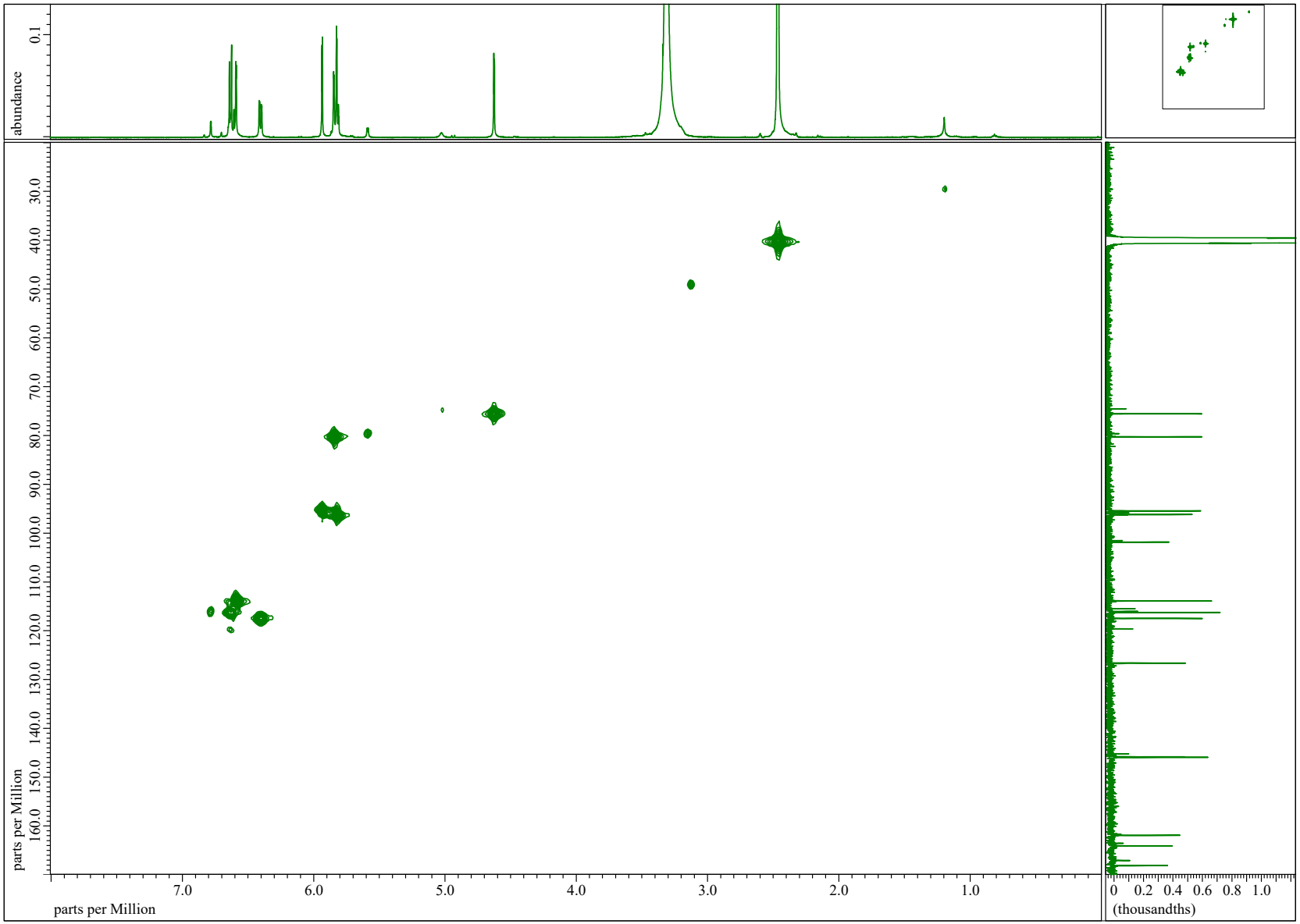

Figure S6. HMQC spectrum of taxifolin 3-*O*-sulfate (**2**) in DMSO-d_6.

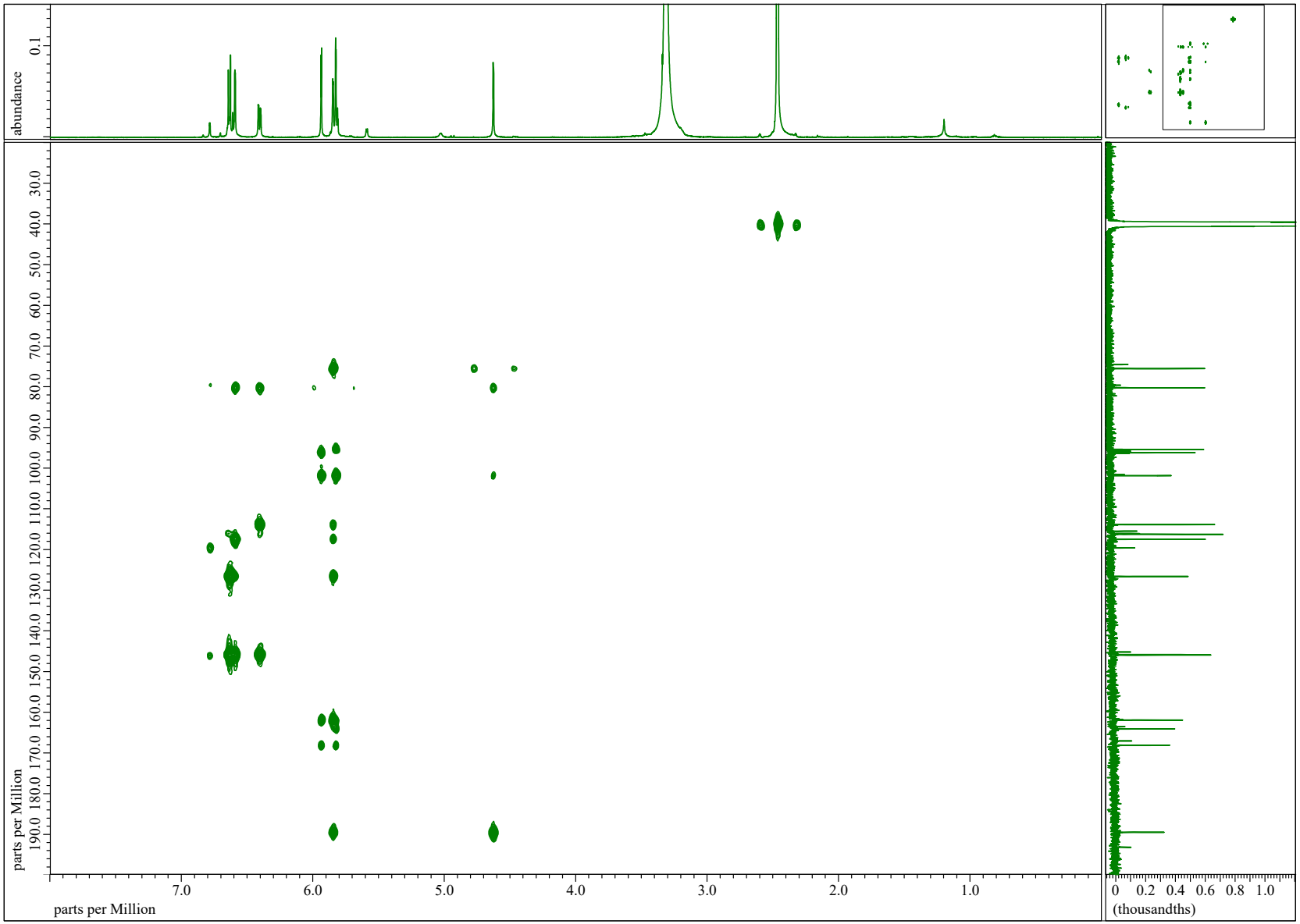

Figure S7. HMBC spectrum of taxifolin 3-*O*-sulfate (**2**) in DMSO-d_6.

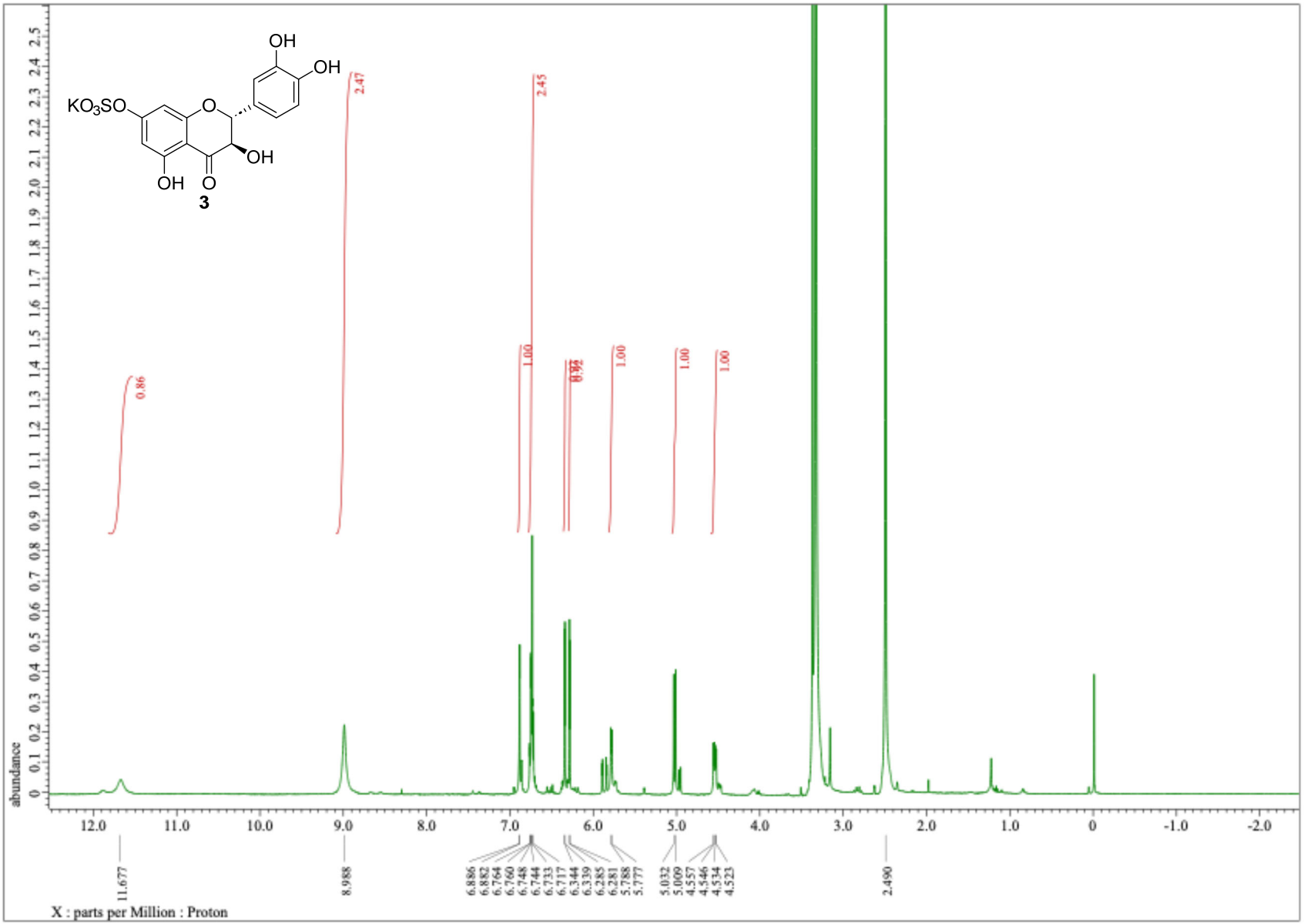

Figure S8. ^{1}H NMR spectrum of taxifolin 7-*O*-sulfate (**3**) in DMSO-d_6.

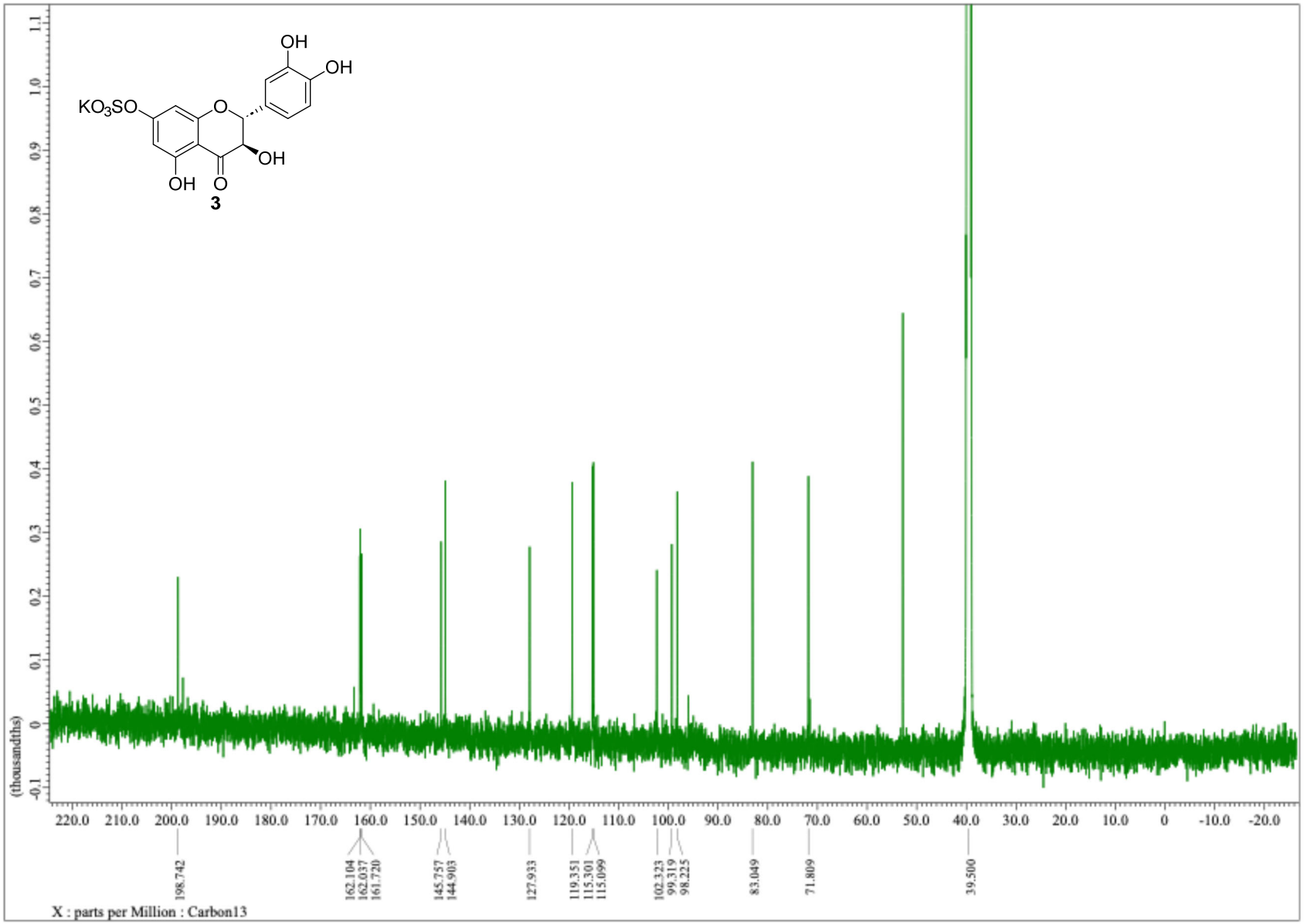

Figure S9. ^{13}C NMR spectrum of taxifolin 7-*O*-sulfate (**3**) in DMSO-d_6.

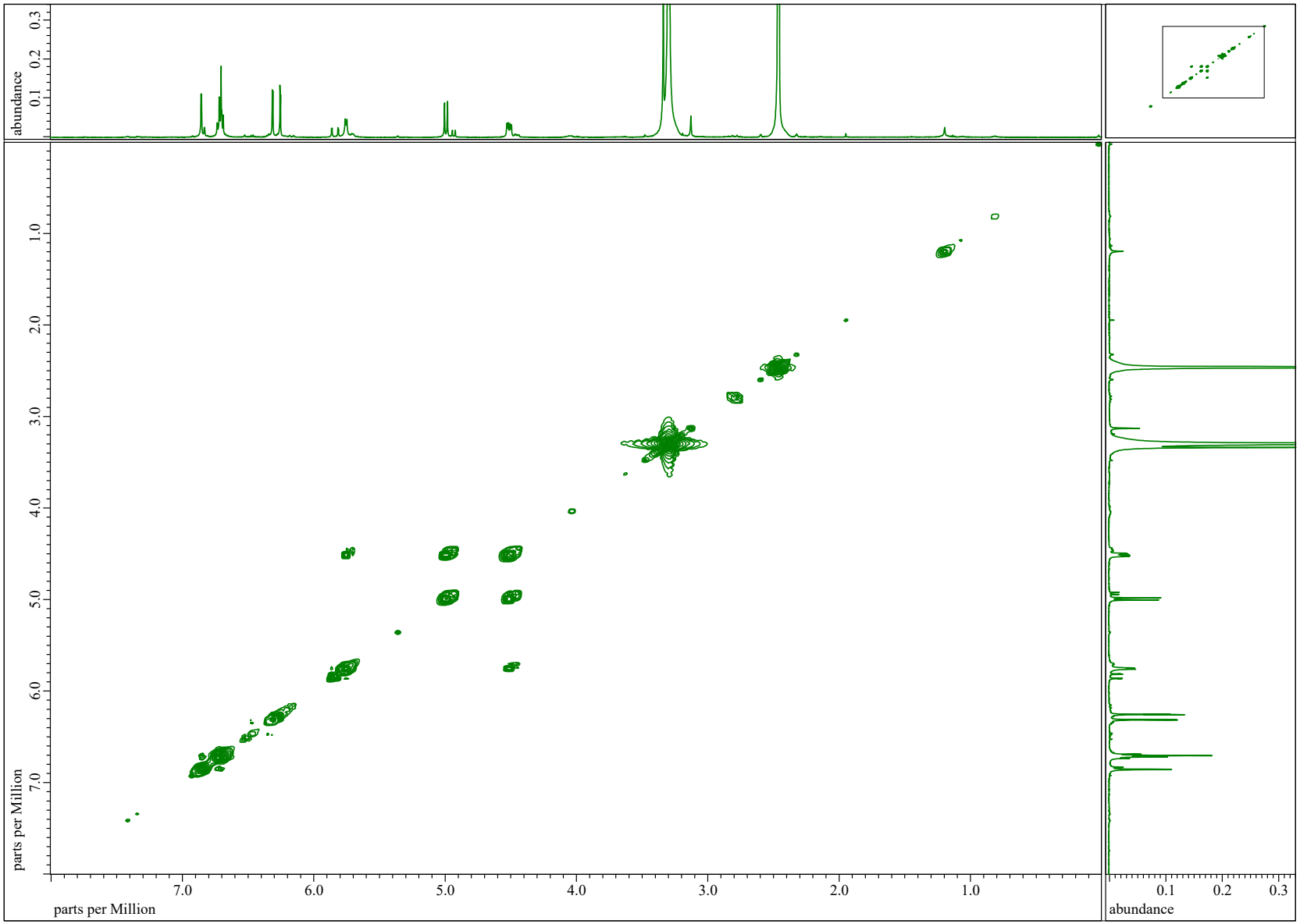

Figure S10. COSY spectrum of taxifolin 7-*O*-**sulf**ate (**3**) in DMSO-d_6.

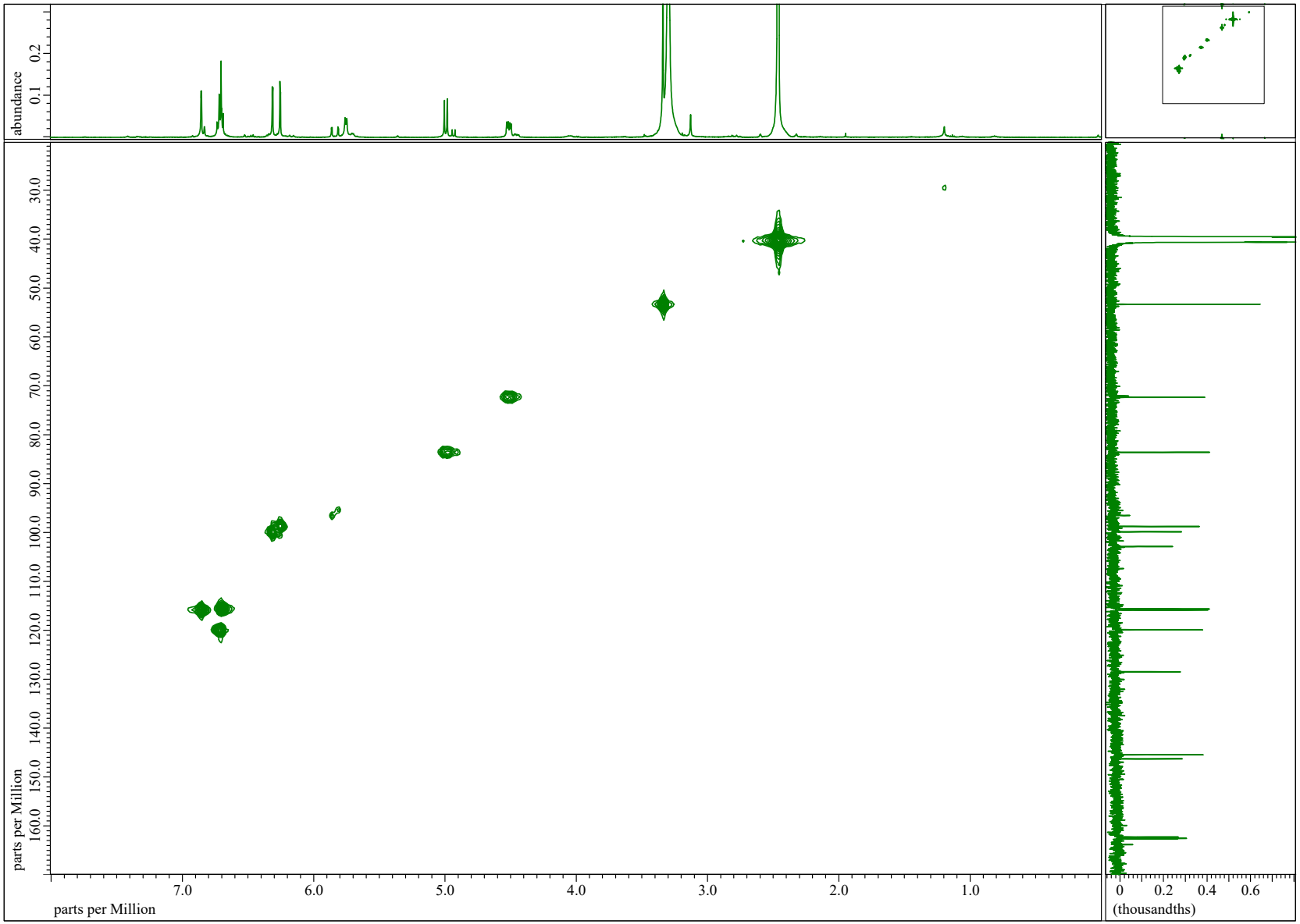

Figure S11. HMQC spectrum of taxifolin 7-*O*-**sulf**ate (**3**) in DMSO-d_6.

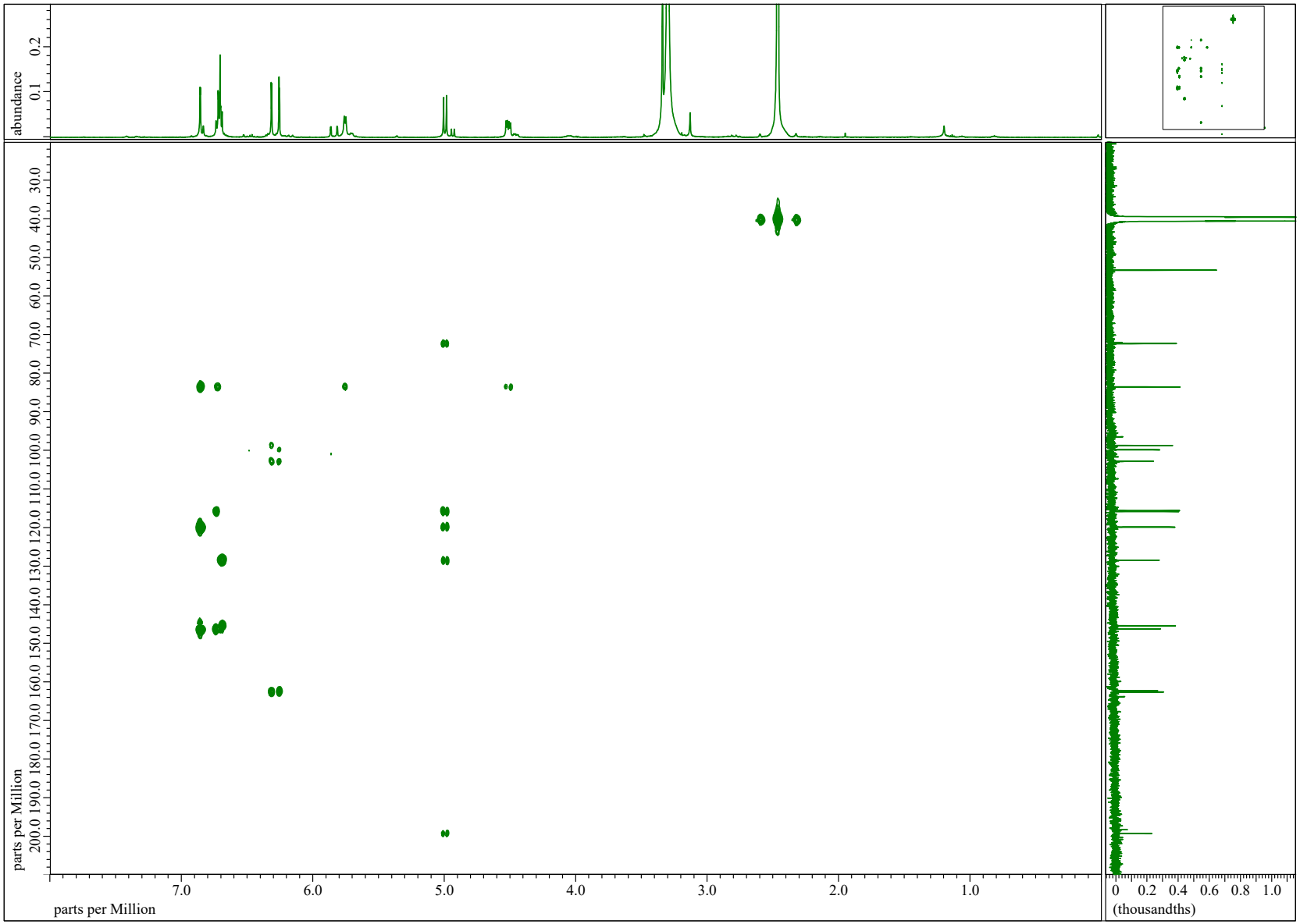

Figure S12. HMBC spectrum of taxifolin 7-*O*-**sulf**ate (**3**) in DMSO-d_6.

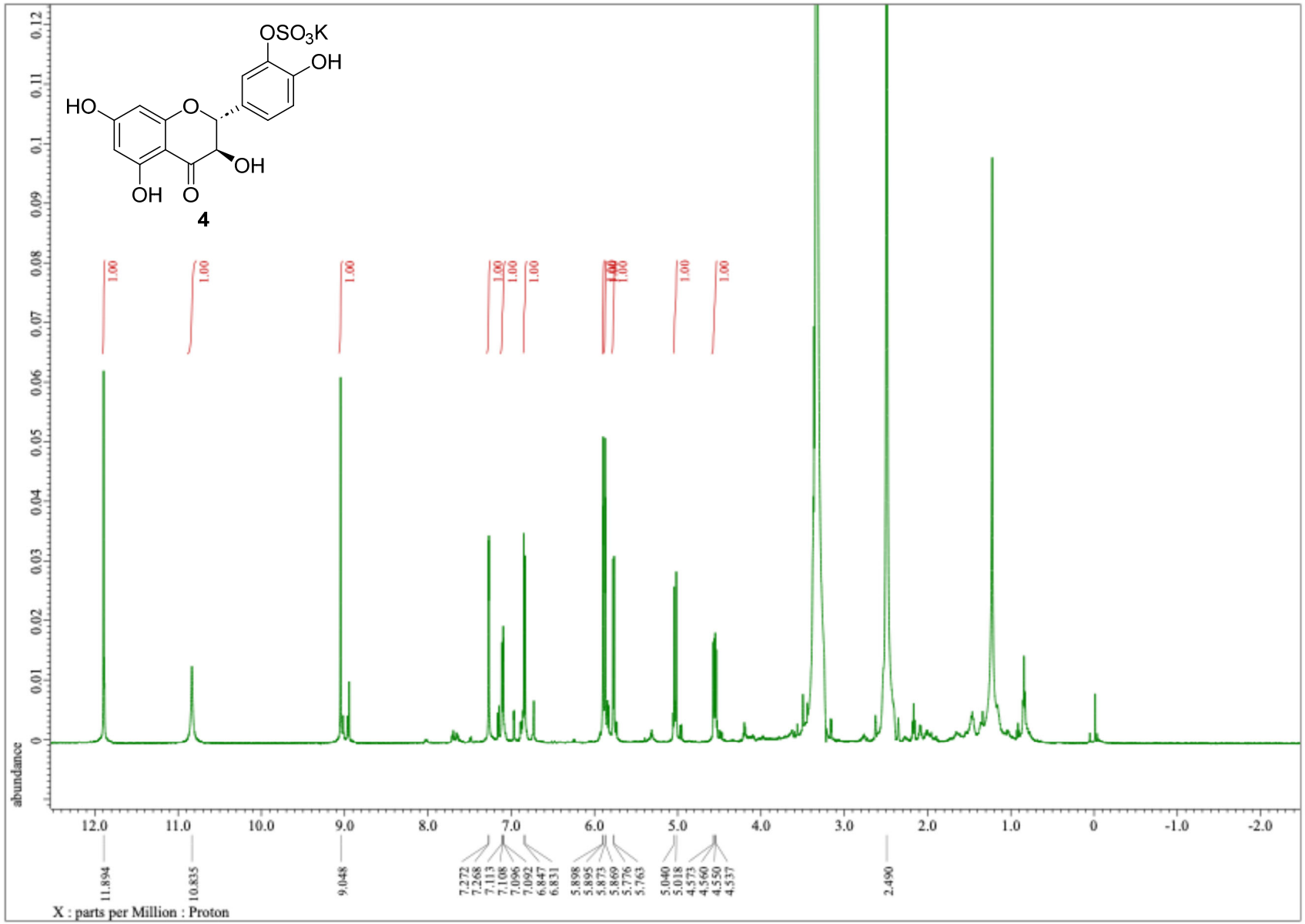

Figure S13. ^{1}H NMR spectrum of taxifolin 3′-*O*-sulfate (**4**) in DMSO-d_6.

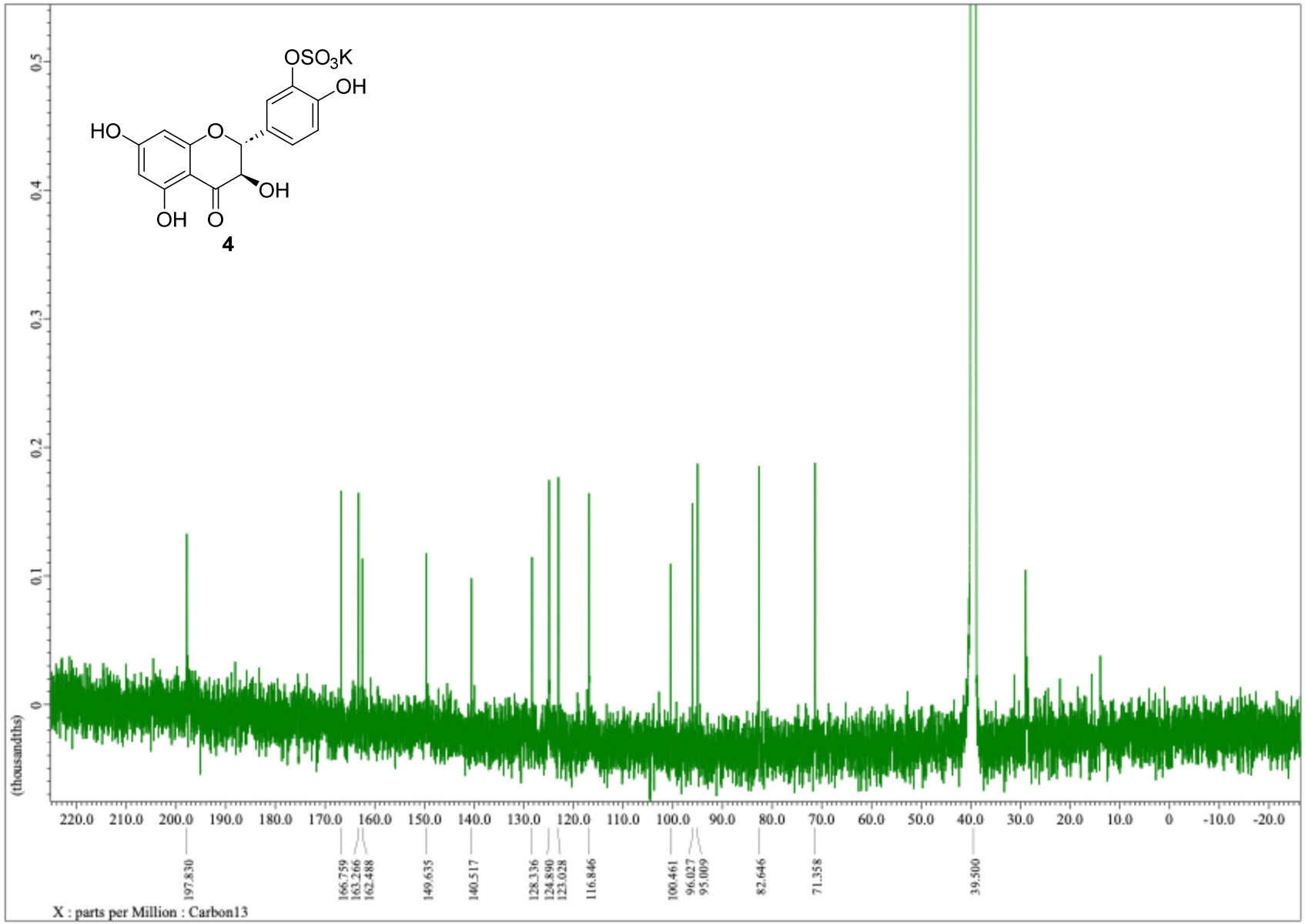

Figure S14. ^{13}C NMR spectrum of taxifolin 3′-*O*-sulfate (**4**) in DMSO-d_6.

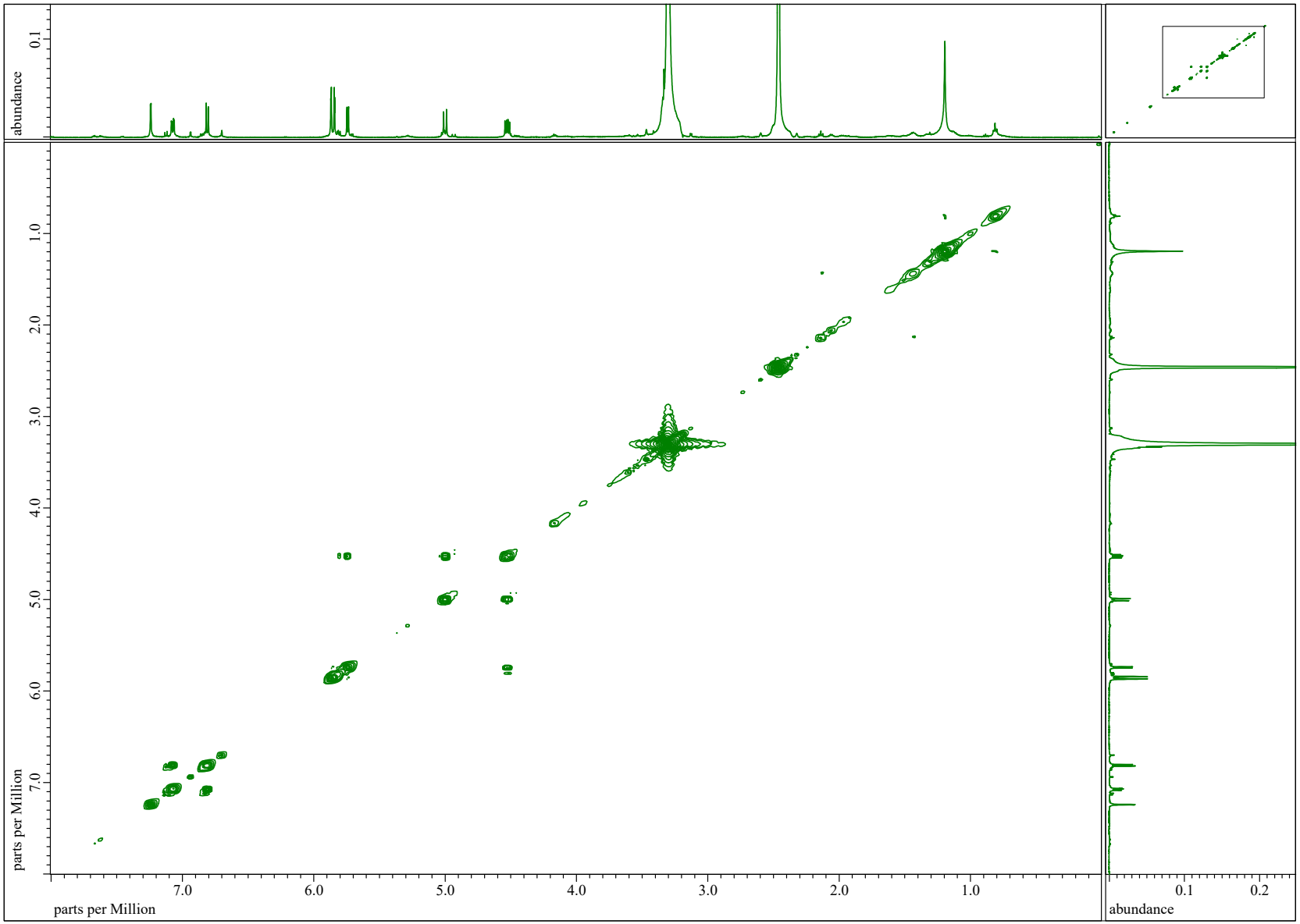

Figure S15. COSY spectrum of taxifolin 3′-*O*-sulfate (**4**) in DMSO-d_6.

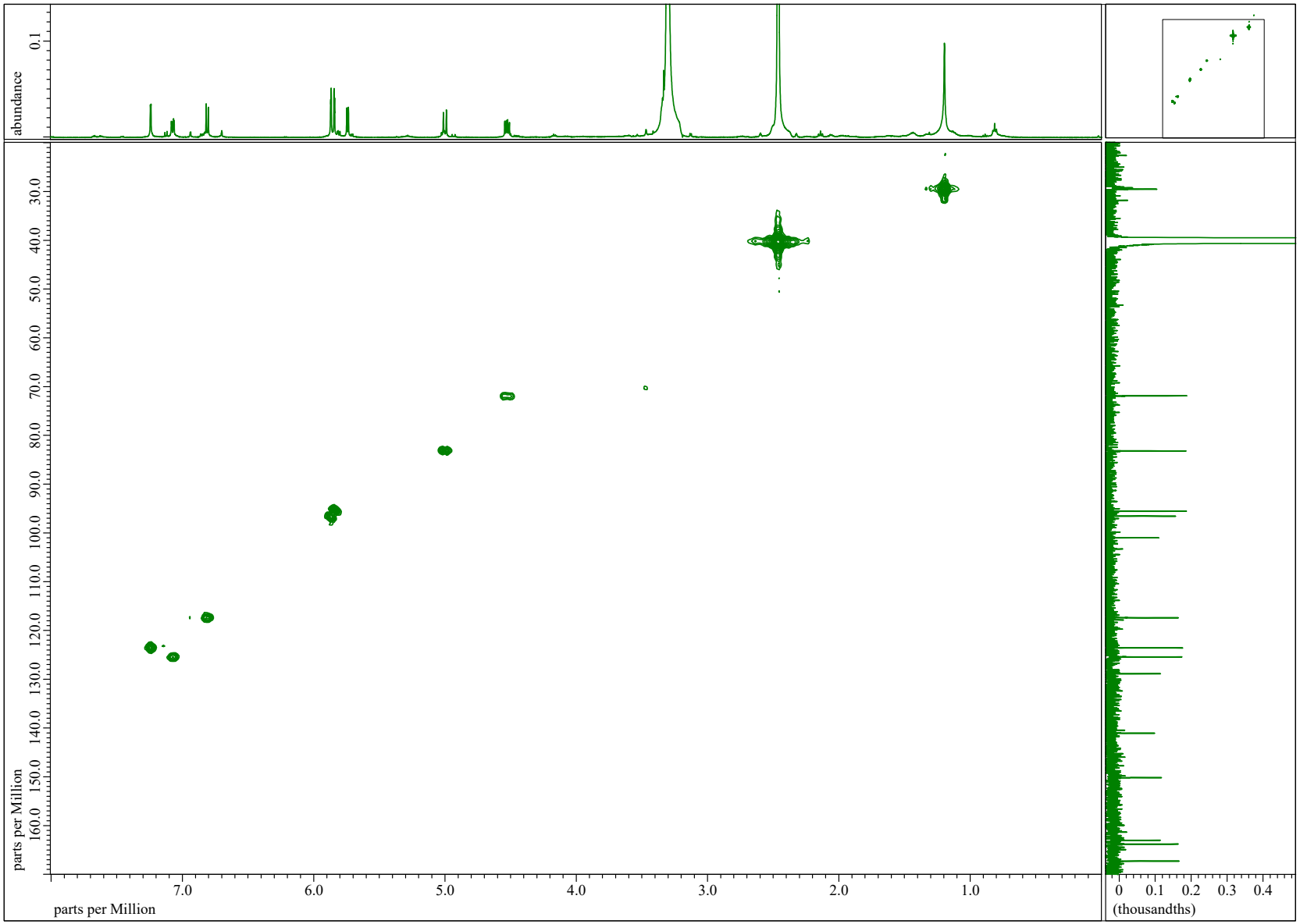

Figure S16. COSY spectrum of taxifolin 3′-*O*-sulfate (**4**) in DMSO-d_6.

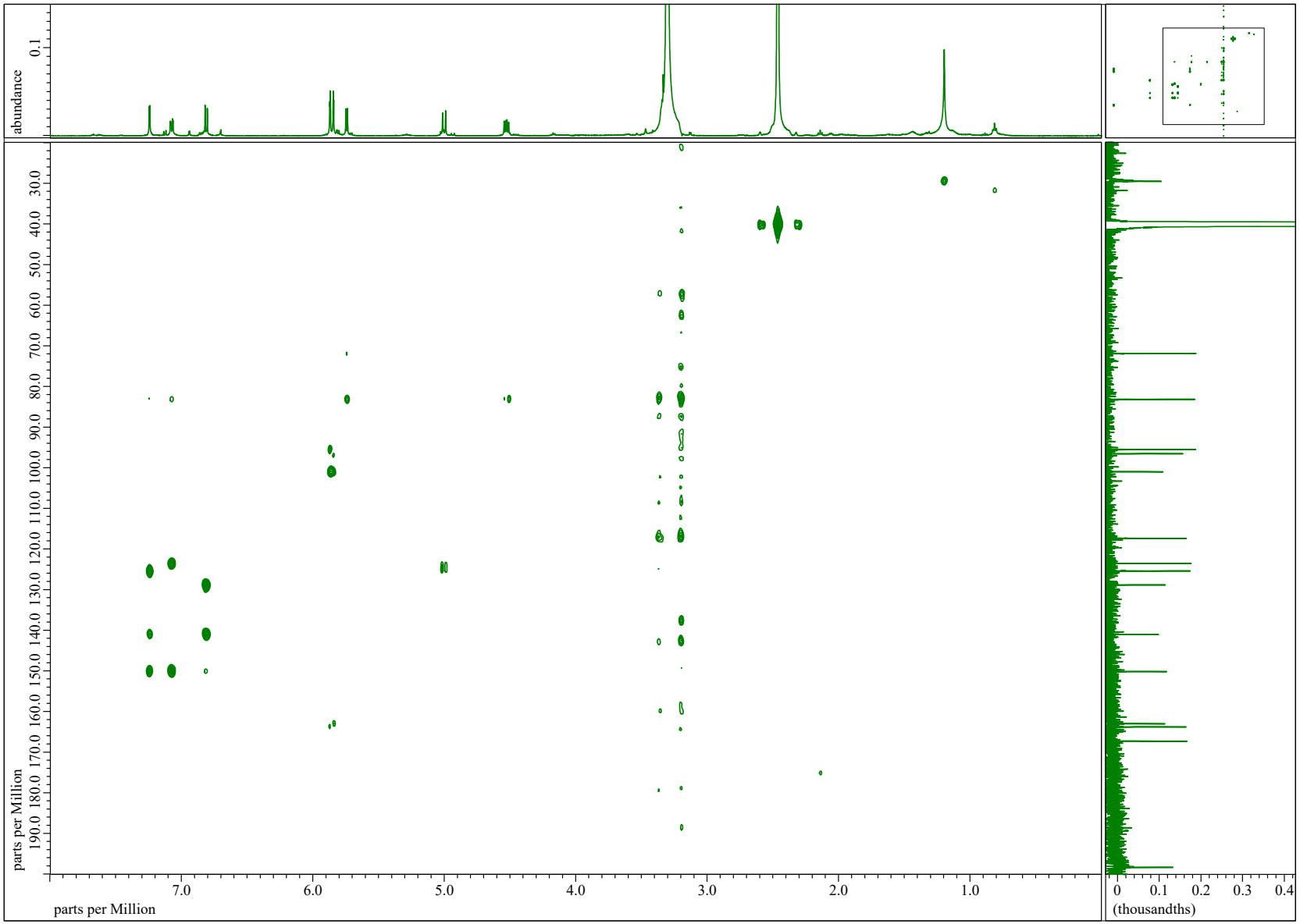

Figure S17. HMBC spectrum of taxifolin 3′-*O*-sulfate (**4**) in DMSO-d_6.

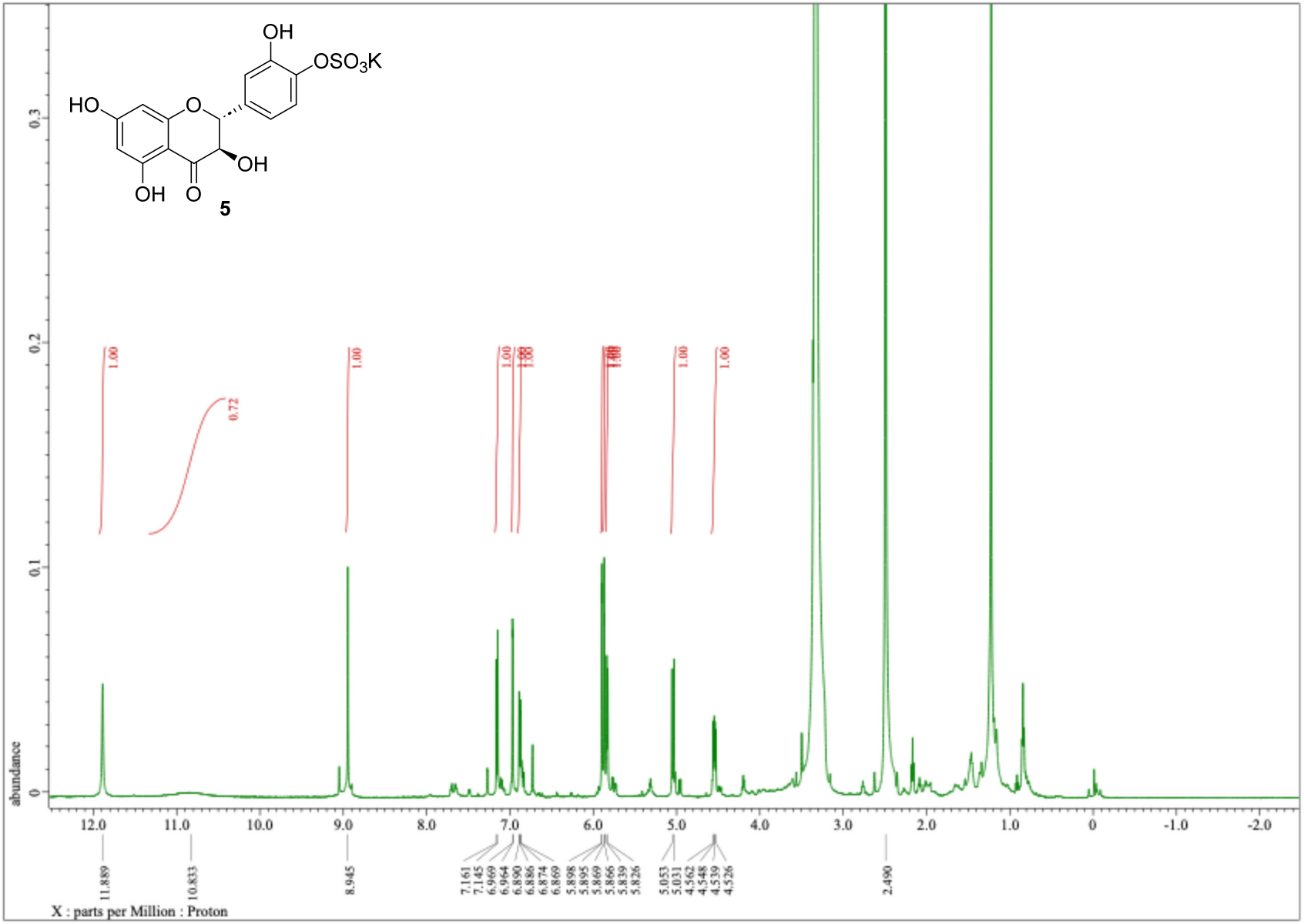

Figure S18. ^{1}H NMR spectrum of taxifolin 4′-*O*-sulfate (**5**) in DMSO-d_6.

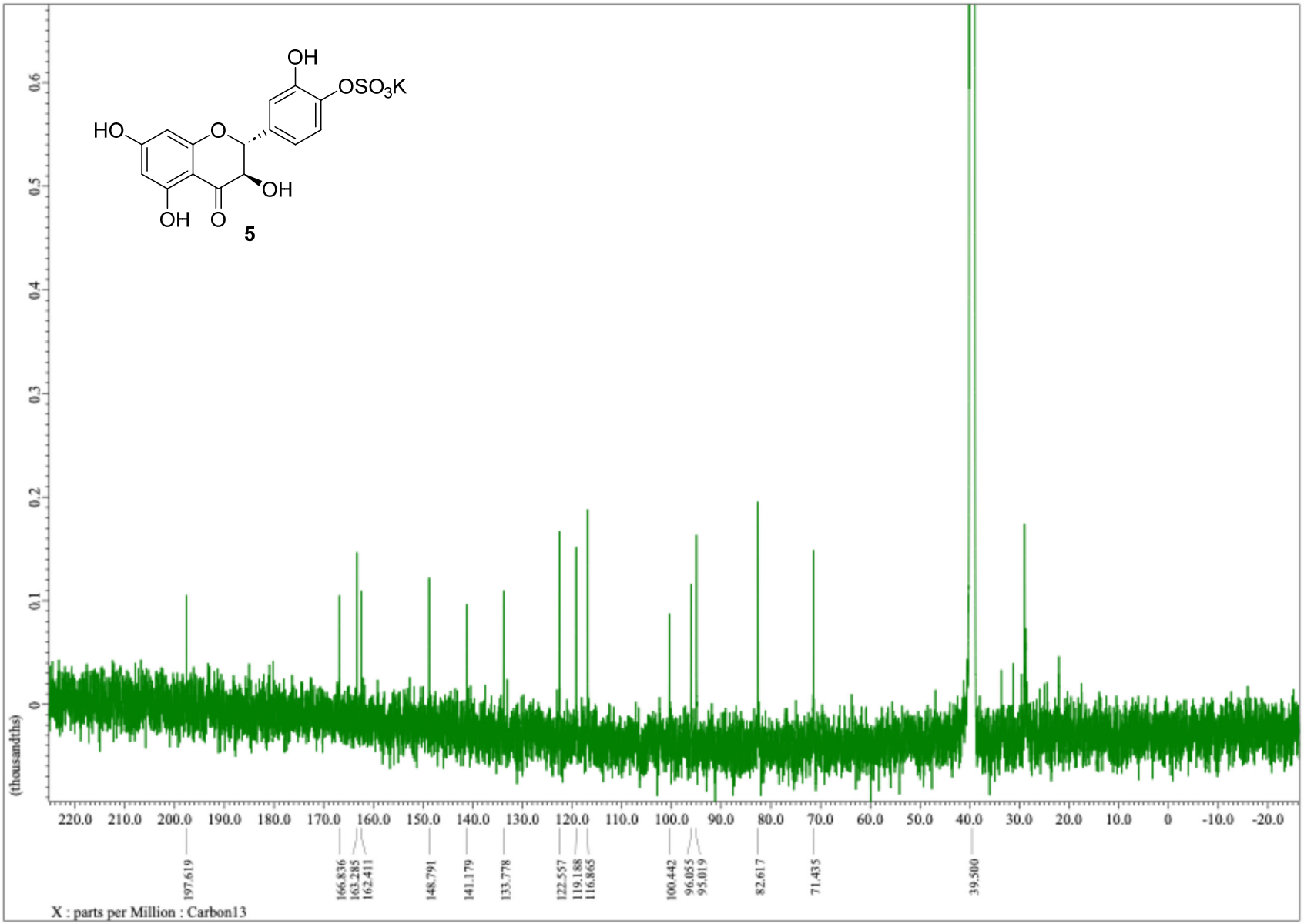

Figure S19. ^{13}C NMR spectrum of taxifolin 4′-*O*-sulfate (**5**) in DMSO-d_6.

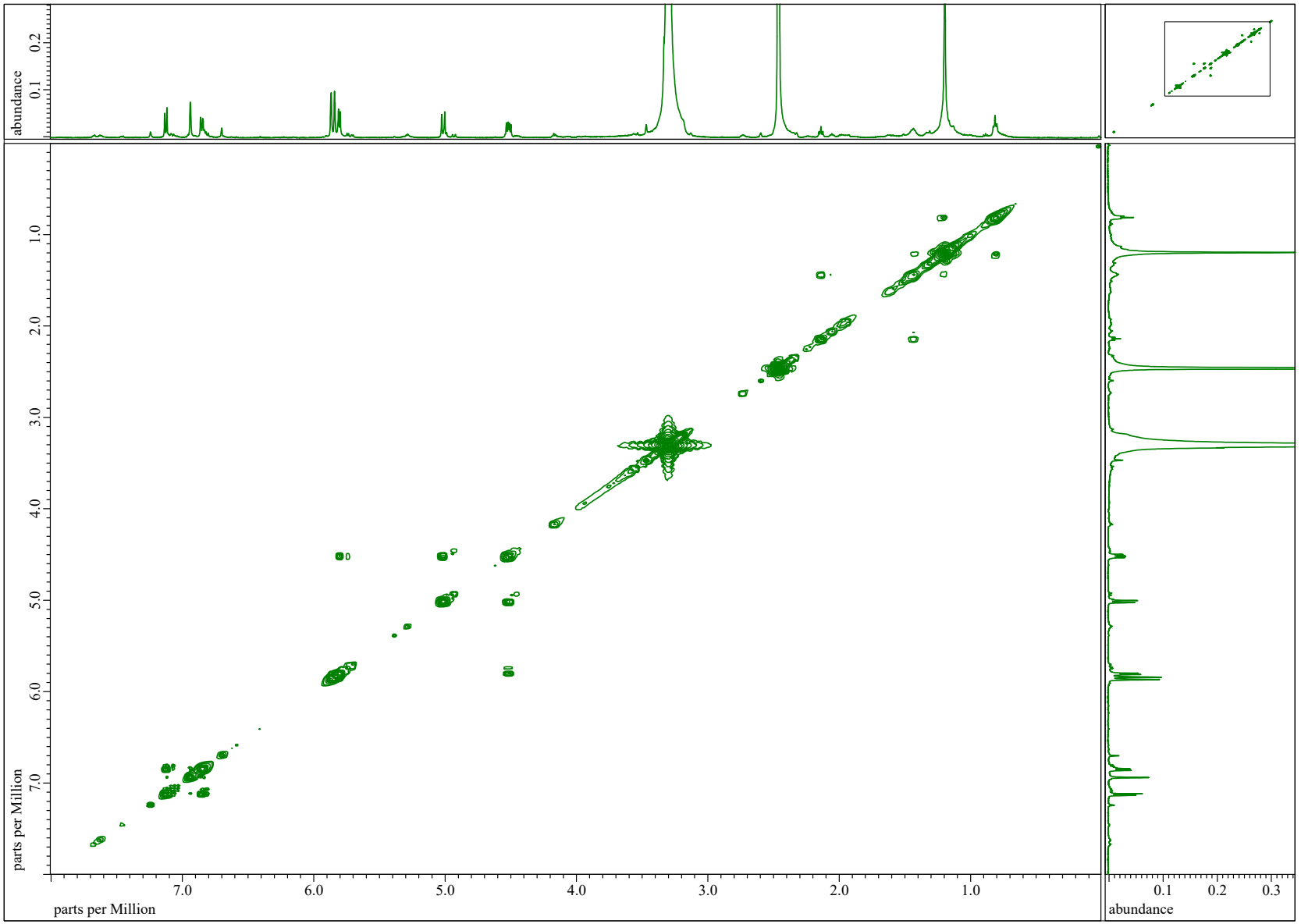

Figure S20. COSY spectrum of taxifolin 4′-*O*-sulfate (**5**) in DMSO-d_6.

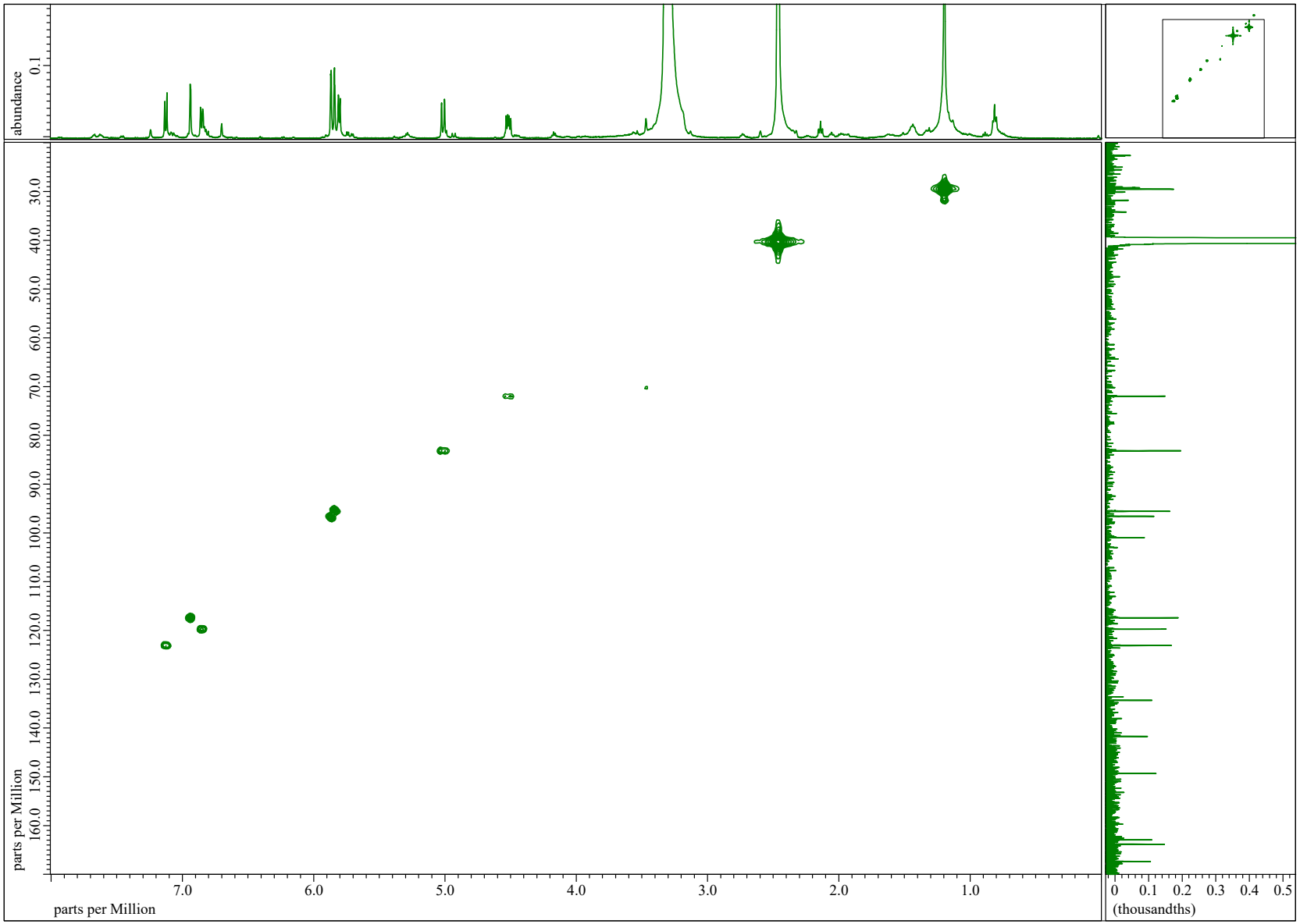

Figure S21. HMQC spectrum of taxifolin 4′-*O*-sulfate (**5**) in DMSO-d_6.

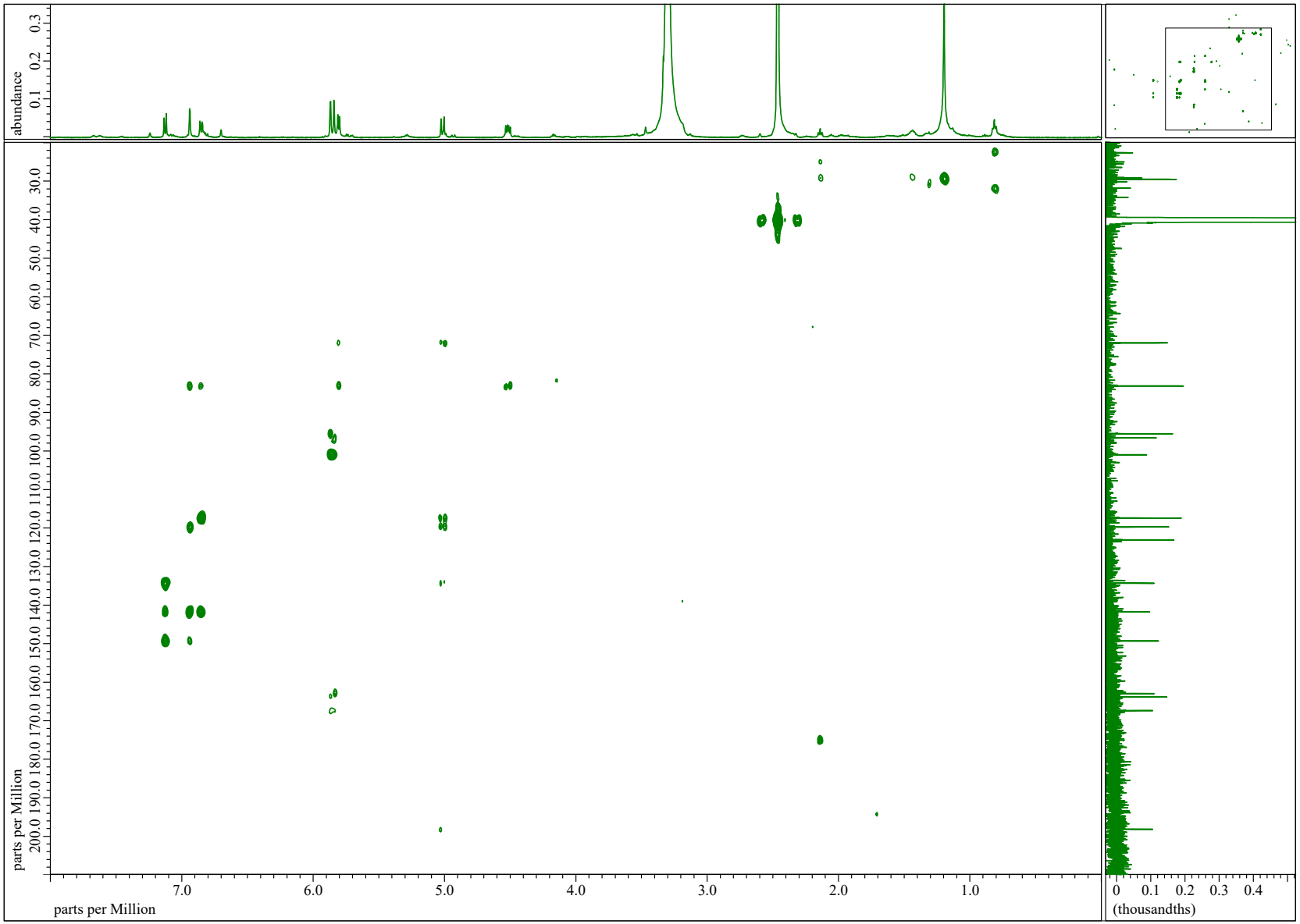

Figure S22. HMBC spectrum of taxifolin 4′-*O*-sulfate (**5**) in DMSO-d_6.

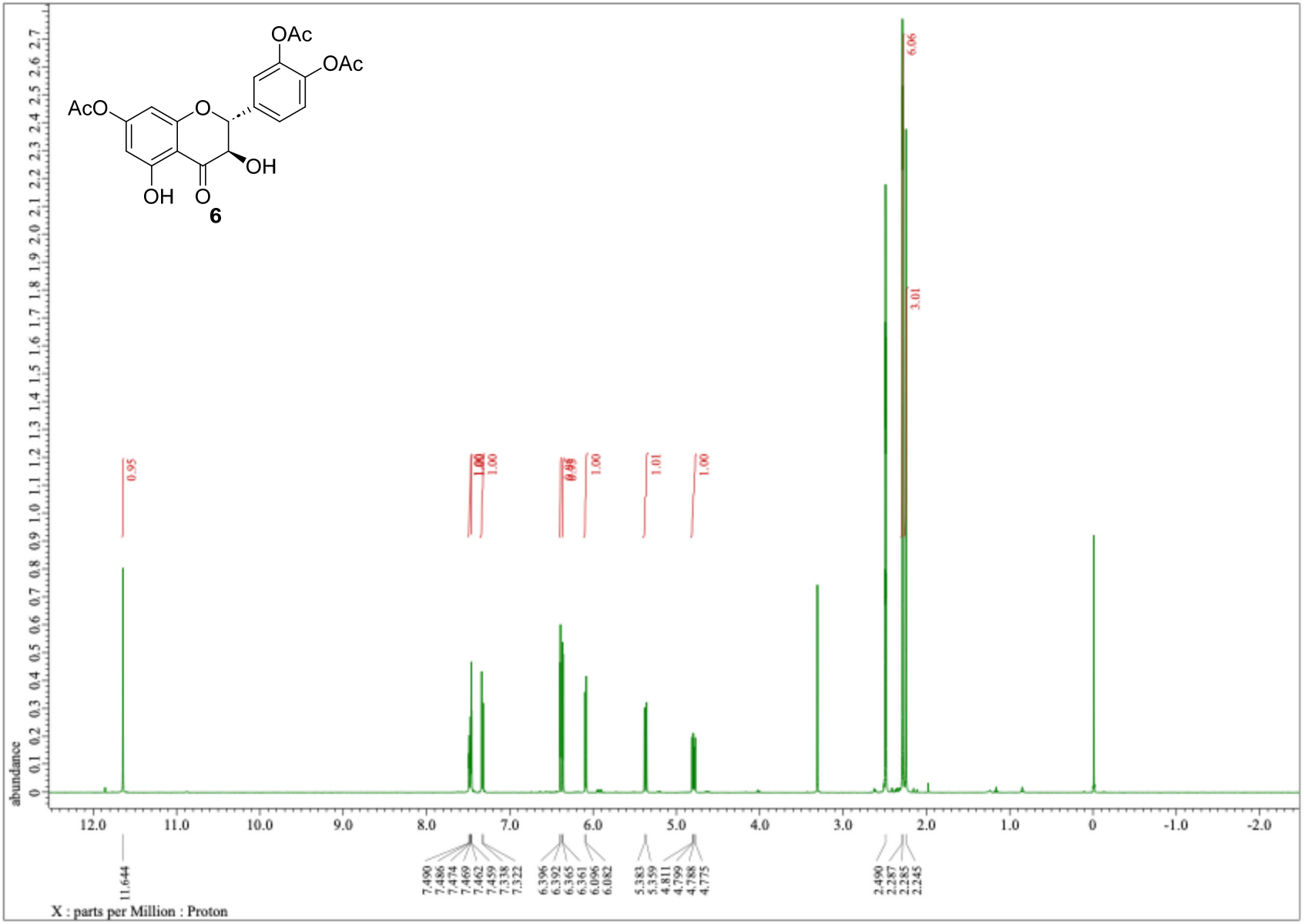

Figure S23. ^{1}H NMR spectrum of taxifolin 7,3′,4′-tri-*O*-acetate (**6**) in DMSO-d_6.

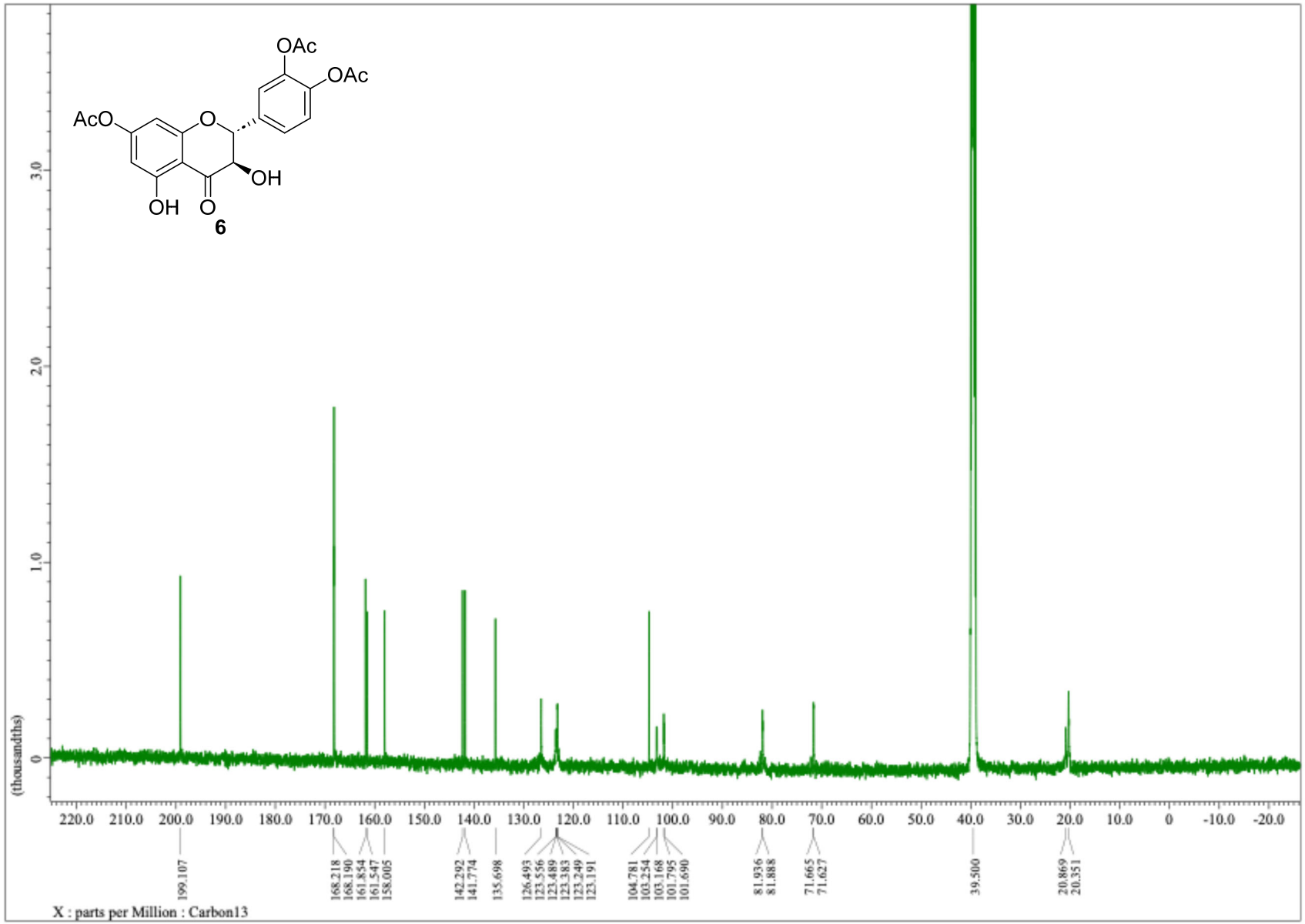

Figure S24. ^{13}C NMR spectrum of taxifolin 7,3′,4′-tri-*O*-acetate (**6**) in DMSO-d_6.

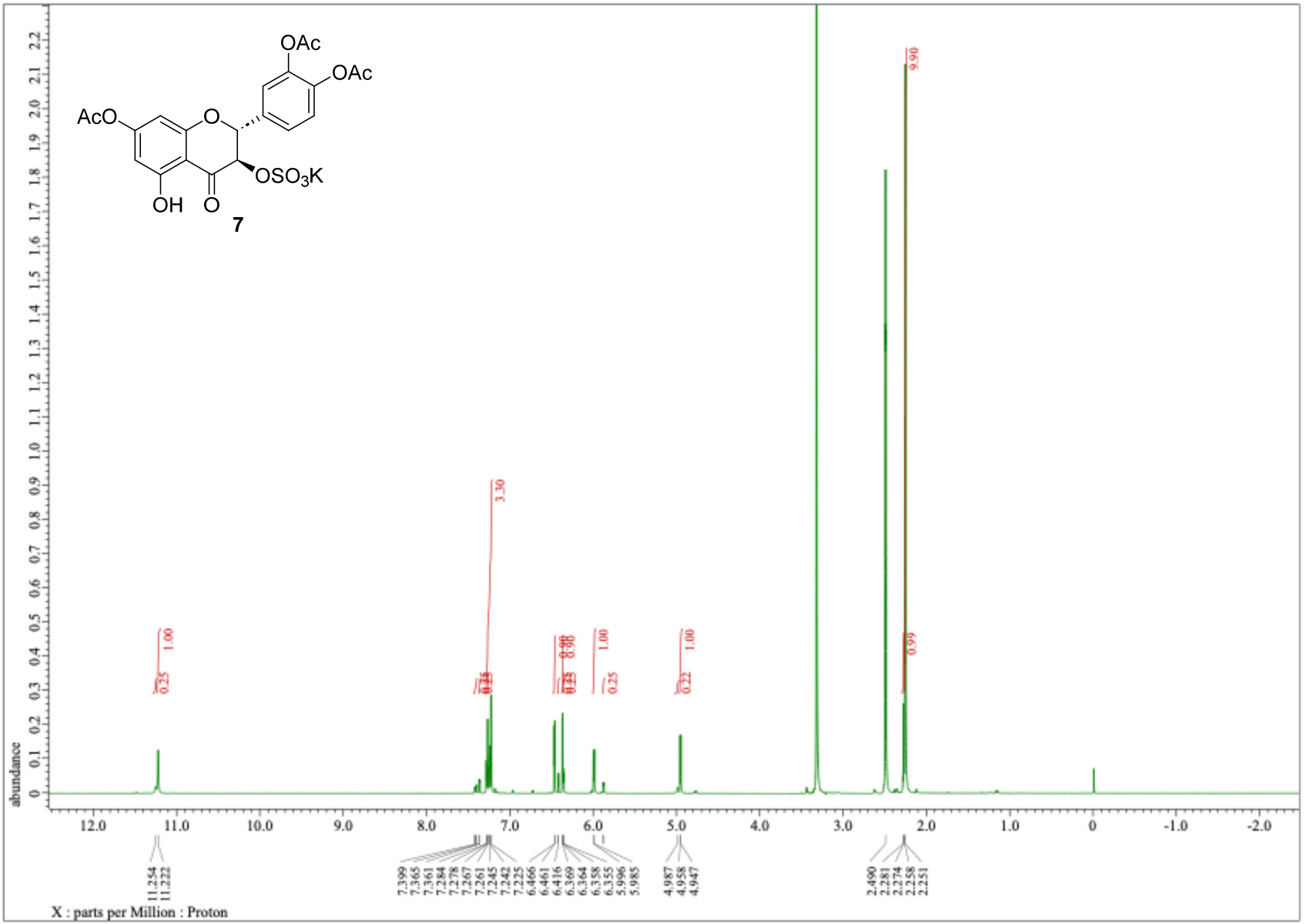

Figure S25. ^{1}H NMR spectrum of taxifolin 7,3′,4′-tri-*O*- acetyl-3-*O*-sulfate (**7**) in DMSO-d_6.

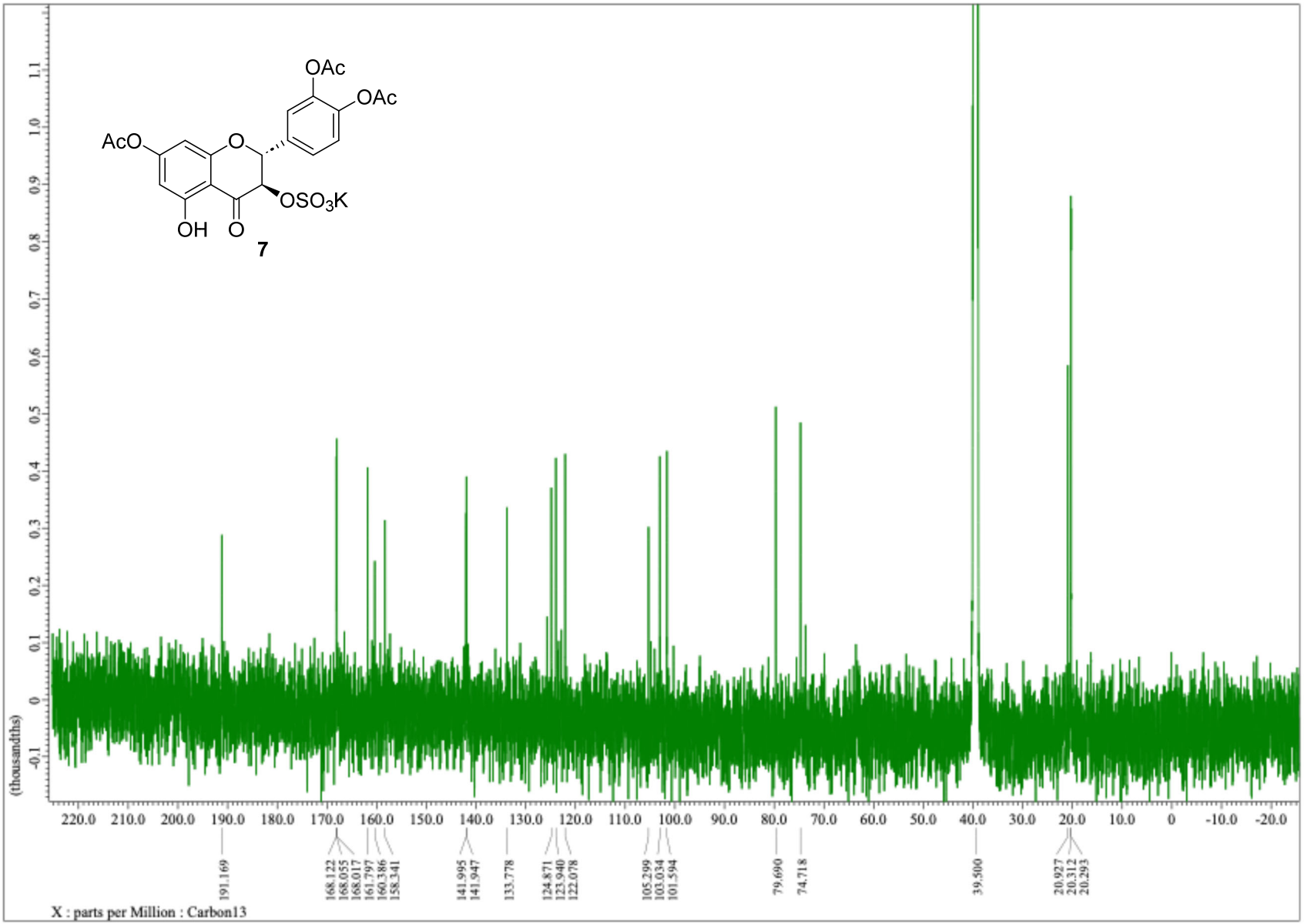

Figure S26. ^{13}C NMR spectrum of taxifolin 7,3′,4′-tri-*O*- acetyl-3-*O*-sulfate (**7**) in DMSO-d_6.

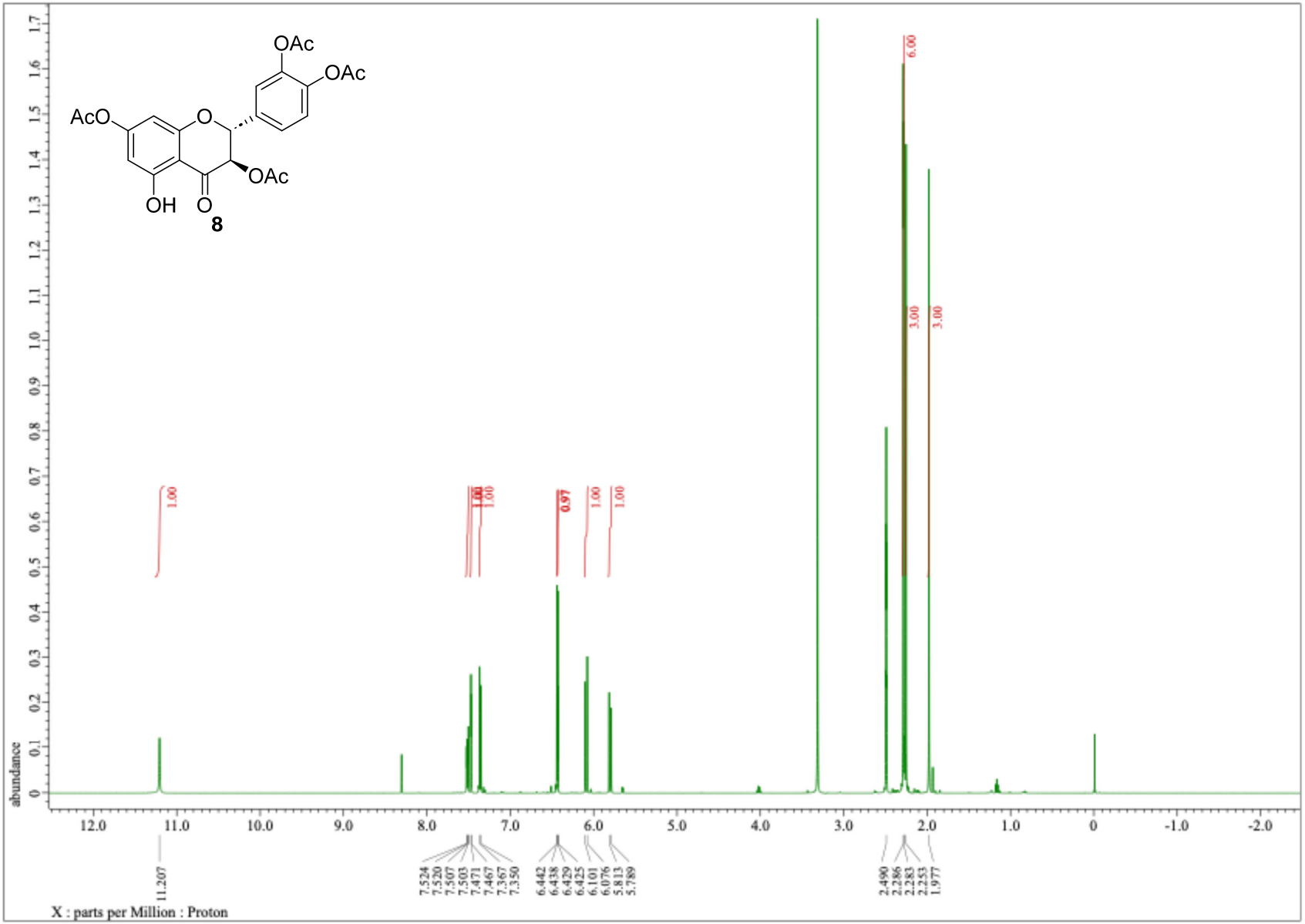

Figure S27. ^{1}H NMR spectrum of taxifolin 3,7,3′,4′-tetra-*O*-acetate (**8**) in DMSO-d_6.

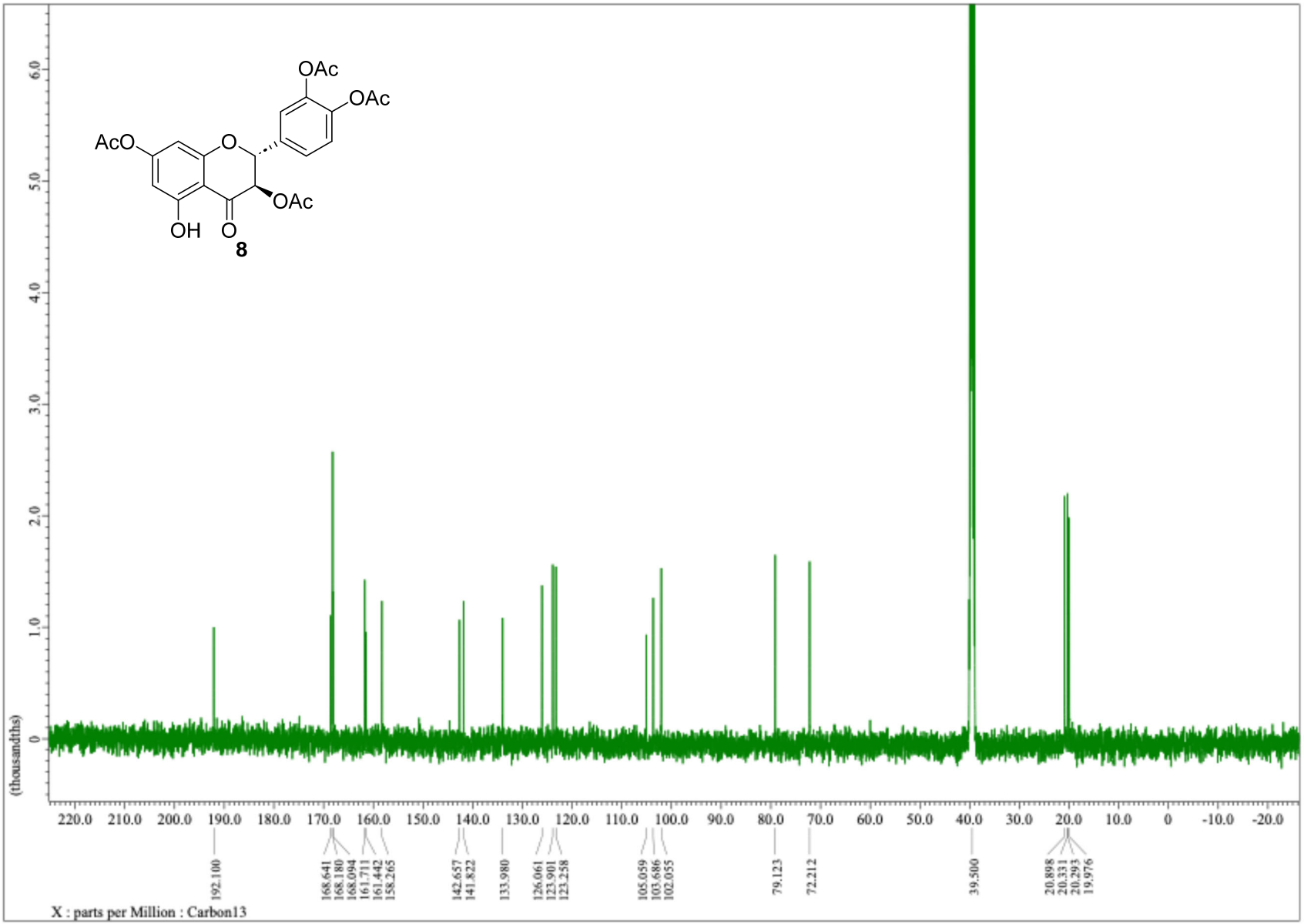

Figure S28. ^{13}C NMR spectrum of taxifolin 3,7,3′,4′-tetra-*O*-acetate (**8**) in DMSO-d_6.

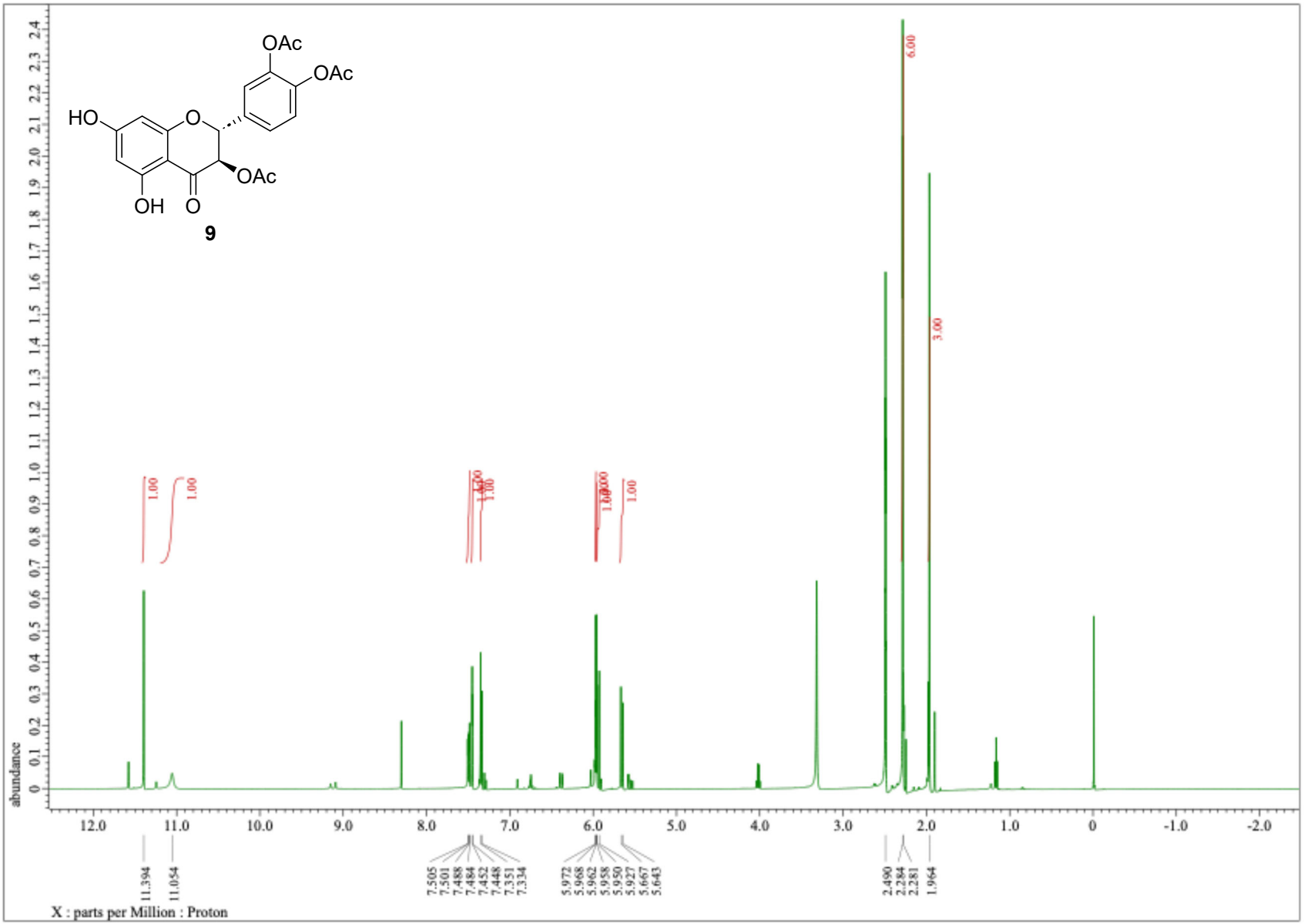

Figure S29. ^{1}H NMR spectrum of taxifolin 3,3′,4′-tri-*O*-acetate (**9**) in DMSO-d_6.

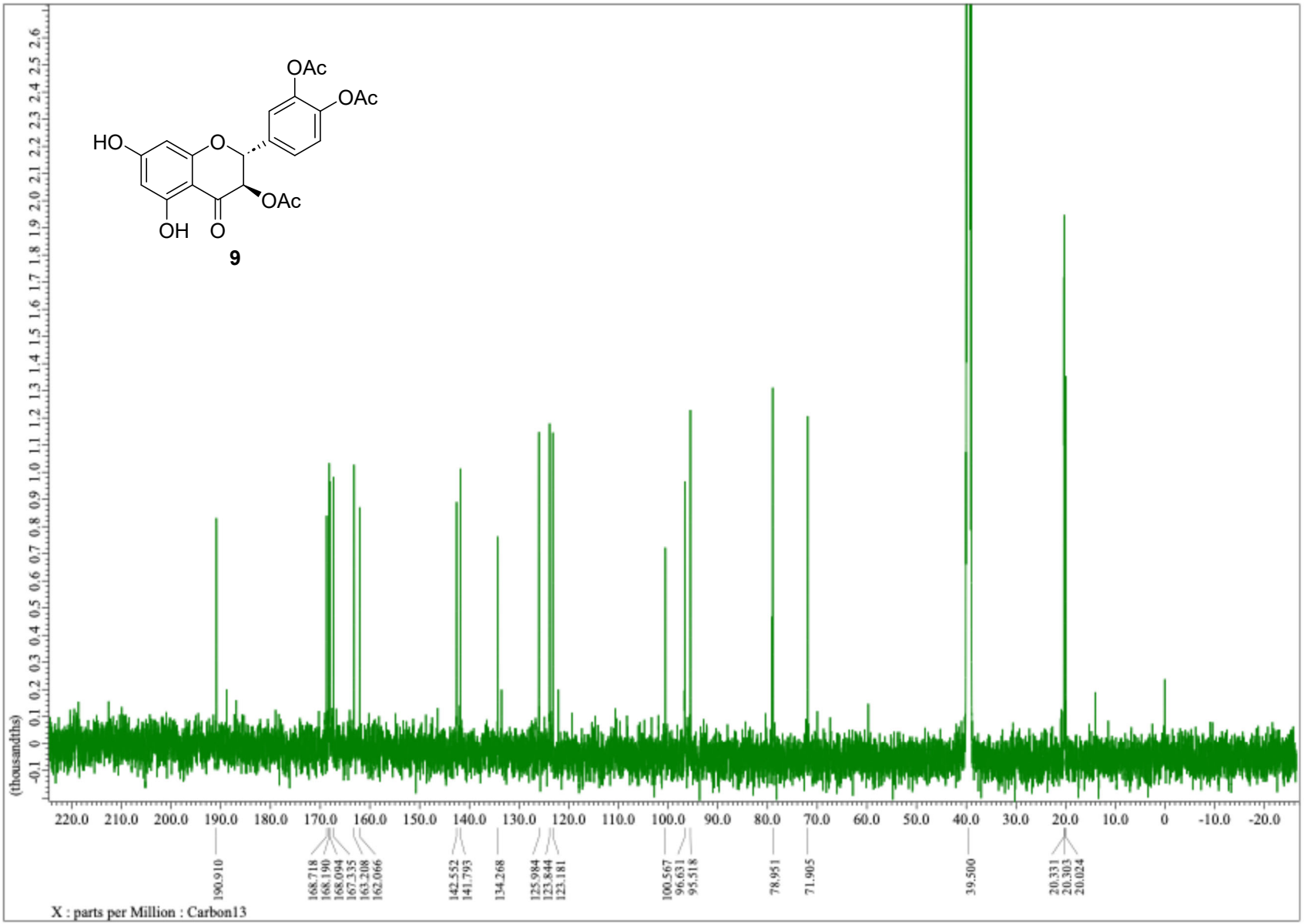

Figure S30. ^{13}C NMR spectrum of taxifolin 3,3′,4′-tri-*O*-acetate (**9**) in DMSO-d_6.

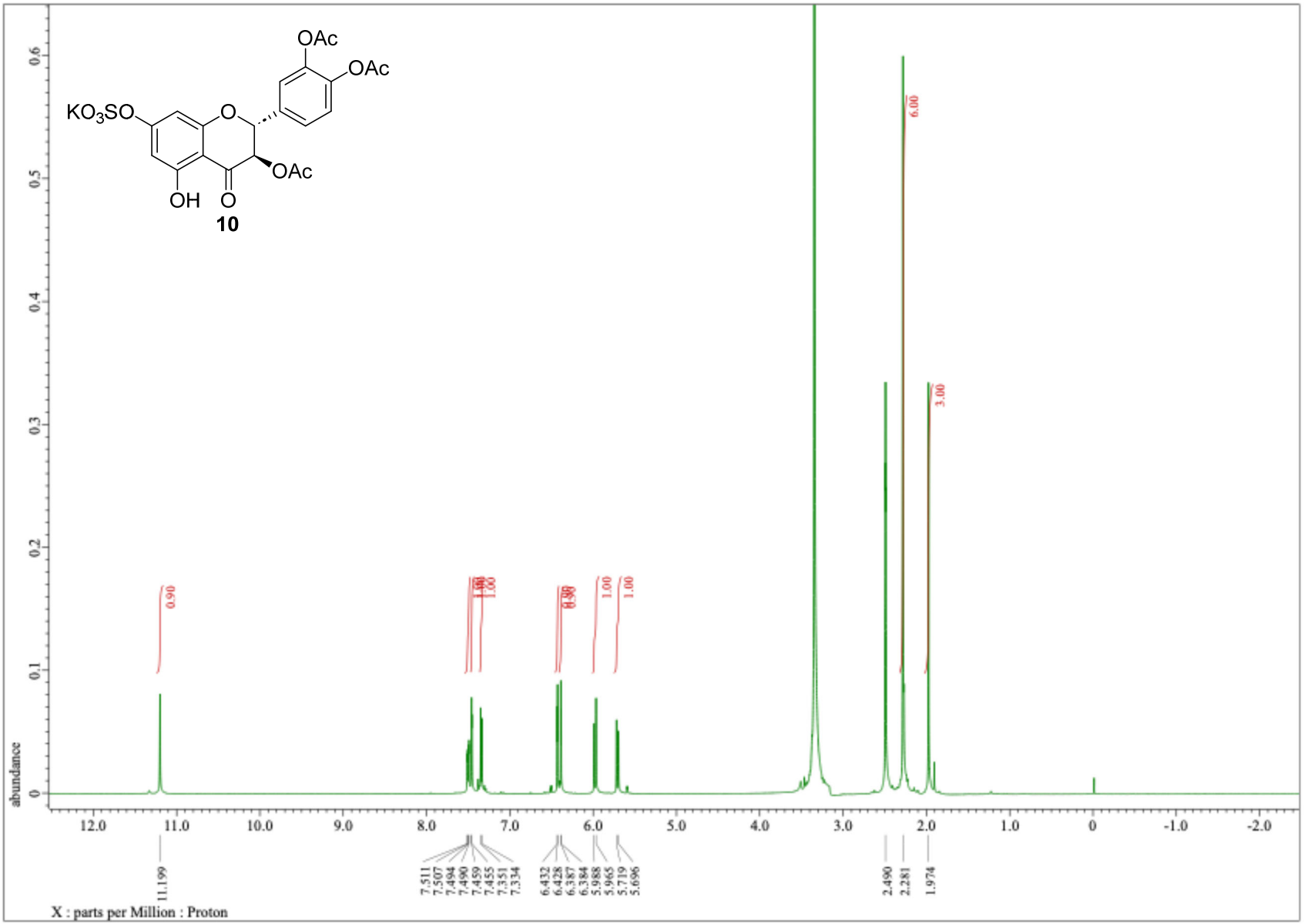

Figure S31. ^{1}H NMR spectrum of taxifolin 3,3′,4′-tri-*O*-acetyl-7-*O*-sulfate (**10**) in DMSO-d_6.

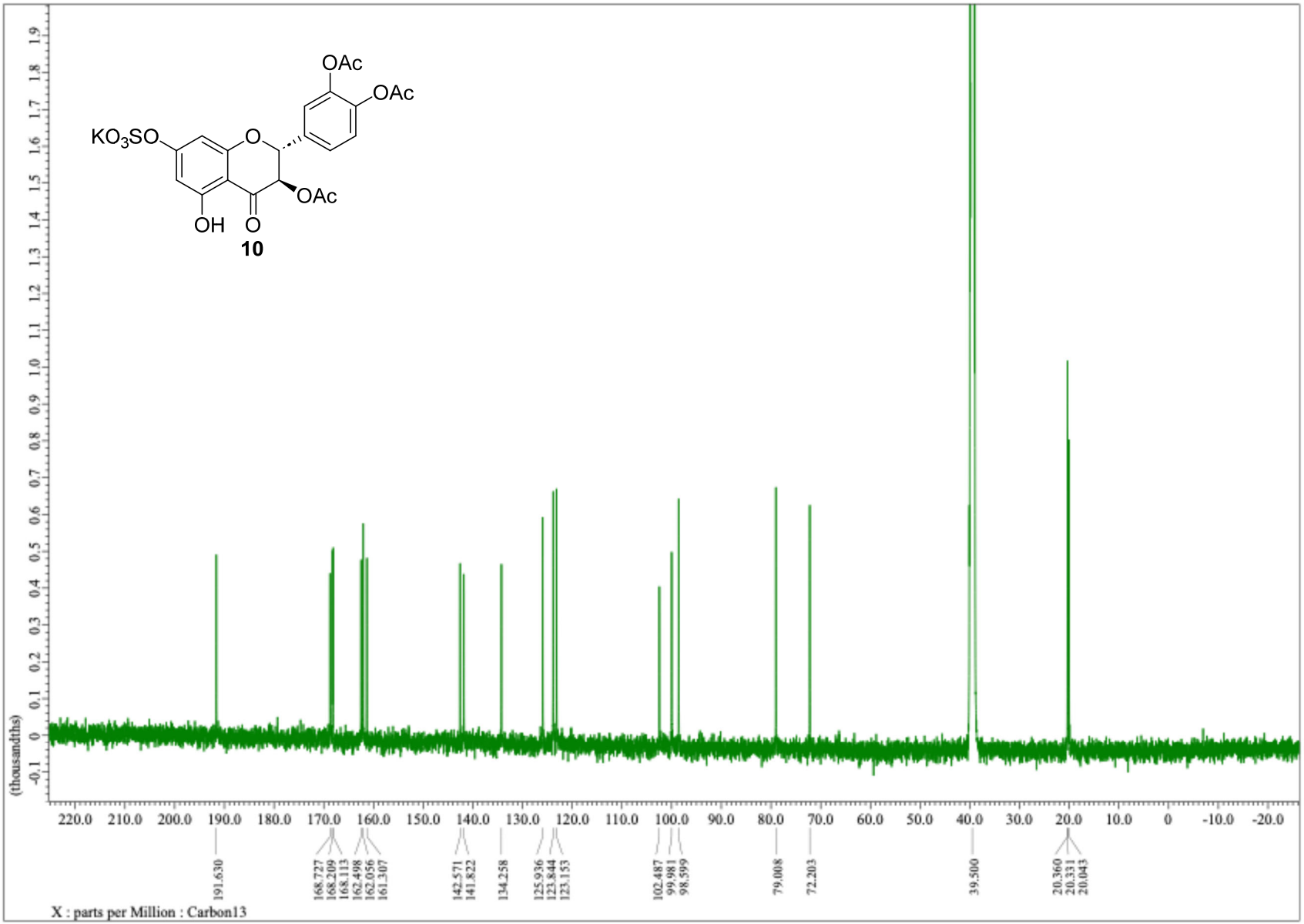

Figure S32. ^{13}C NMR spectrum of taxifolin 3,3′,4′-tri-*O*-acetyl-7-*O*-sulfate (**10**) in DMSO-d_6.

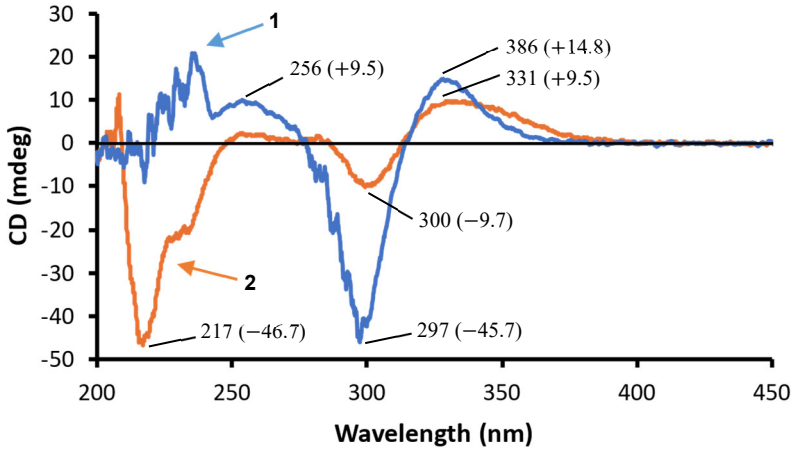

Figure S33. CD spectra of taxifolin (**1**) and taxifolin 3-*O*-sulfate (**2**).

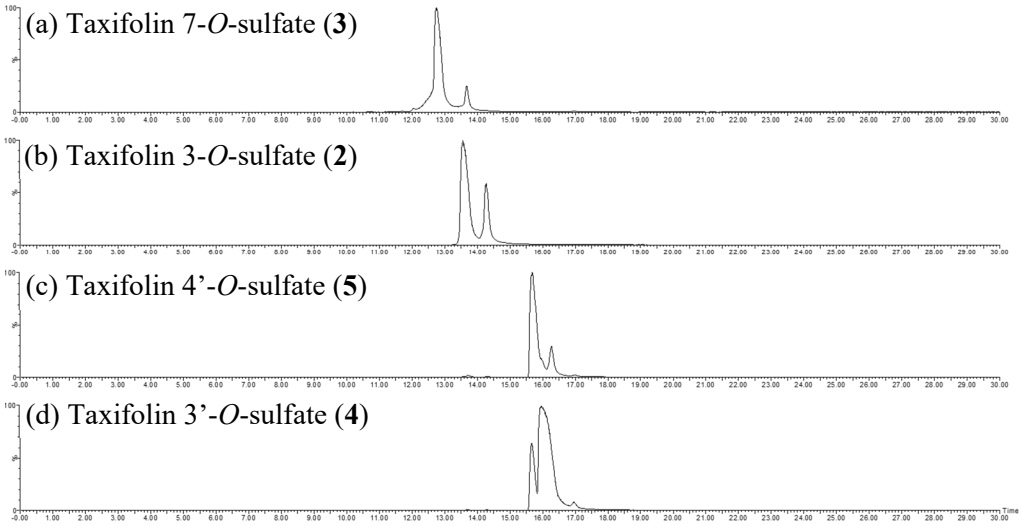

Figure S34. LC-MS chromatograms monitored by $[M-H]^-$ ions of taxifolin monosulfates (*m*/*z* 383.0078): (a) Taxifolin 7-*O*-sulfate (**3**); (b) Taxifolin 3-*O*-sulfate (**2**); (c) Taxifolin 4'-*O*-sulfate (**5**); (d) Taxifolin 3'-*O*-sulfate (**4**).

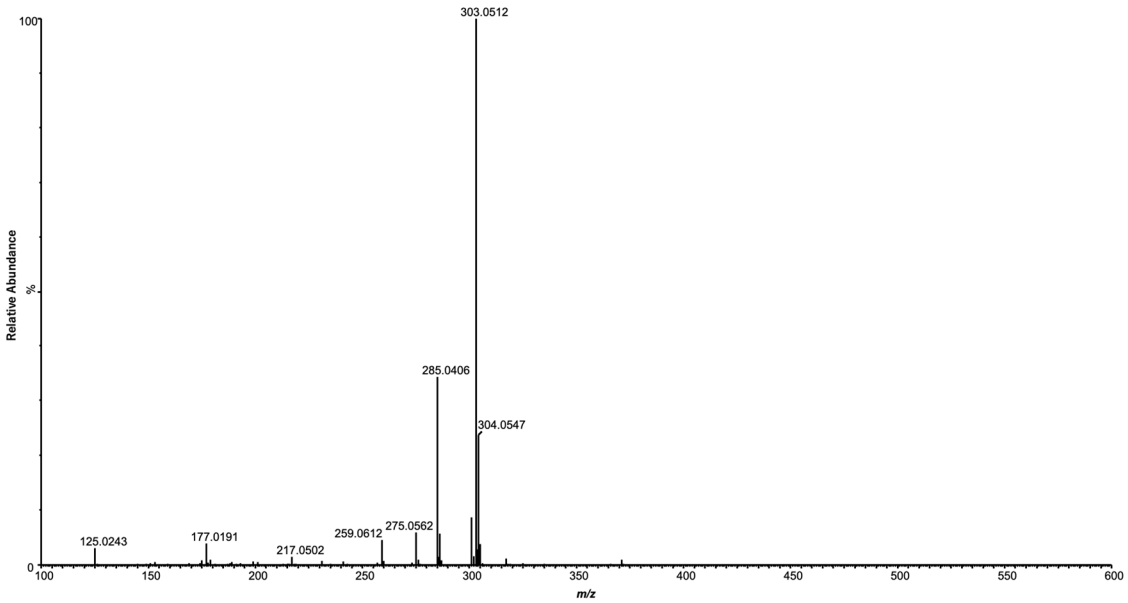

Figure S35. MS (ES-) data of taxifolin (**1**).

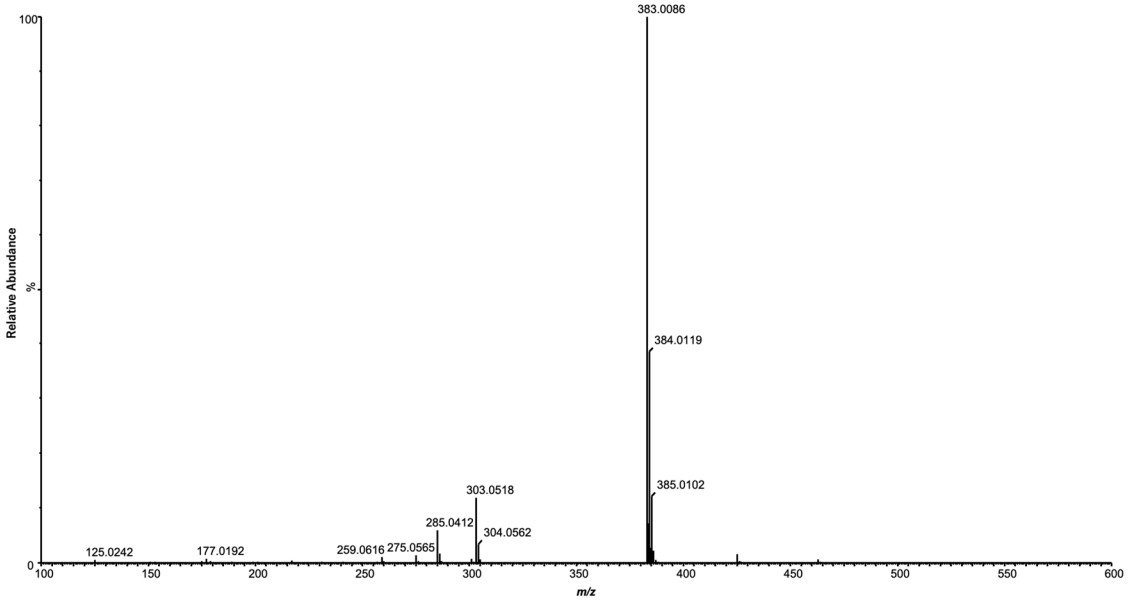

Figure S36. MS (ES-) data of taxifolin 3-*O*-sulfate (**2**) (at retention time 13.56 min).

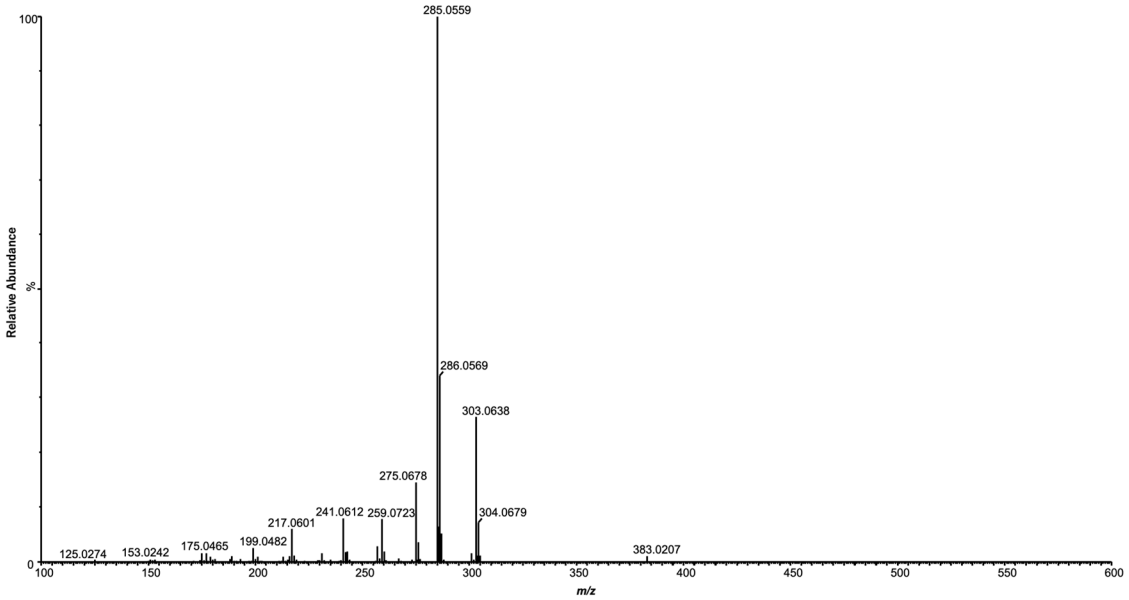

Figure S37. MS/MS (from the ion at *m/z* 383.0078) data of taxifolin 3-*O*-sulfate (**2**).

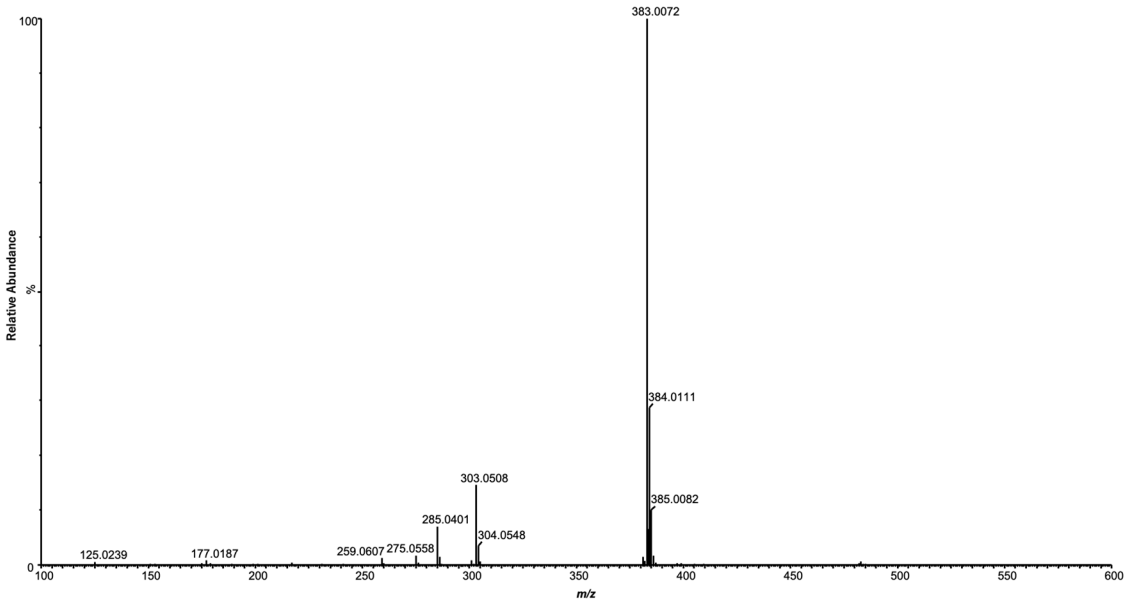

Figure S38. MS (ES-) data of taxifolin 7-*O*-sulfate (**3**) (at retention time 12.75 min).

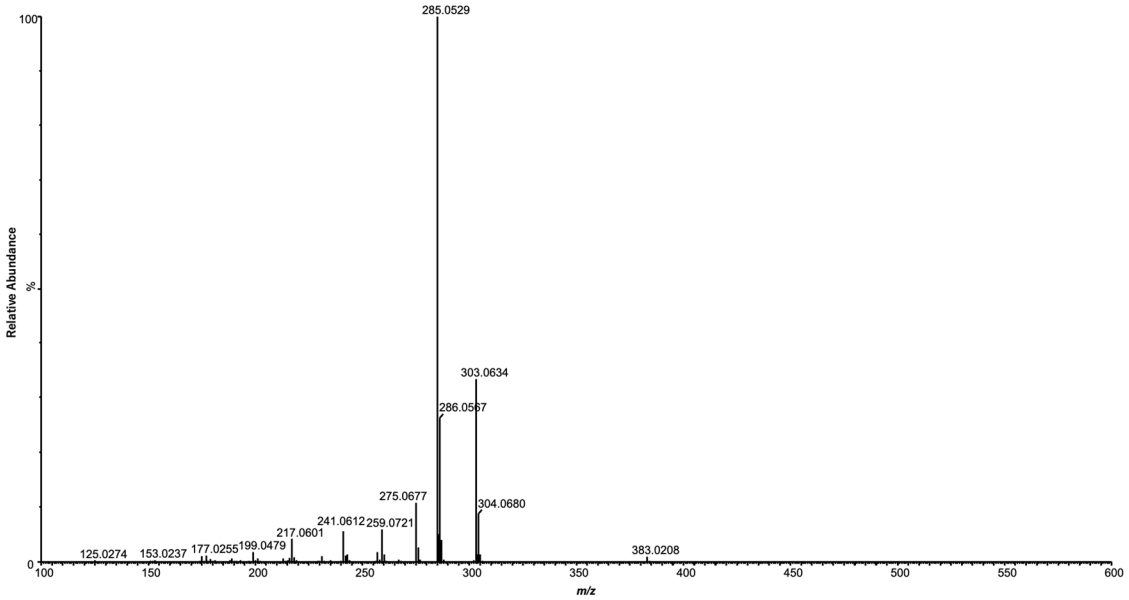

Figure S39. MS/MS (from the ion at *m/z* 383.0078) data of taxifolin 7-*O*-sulfate (**3**).

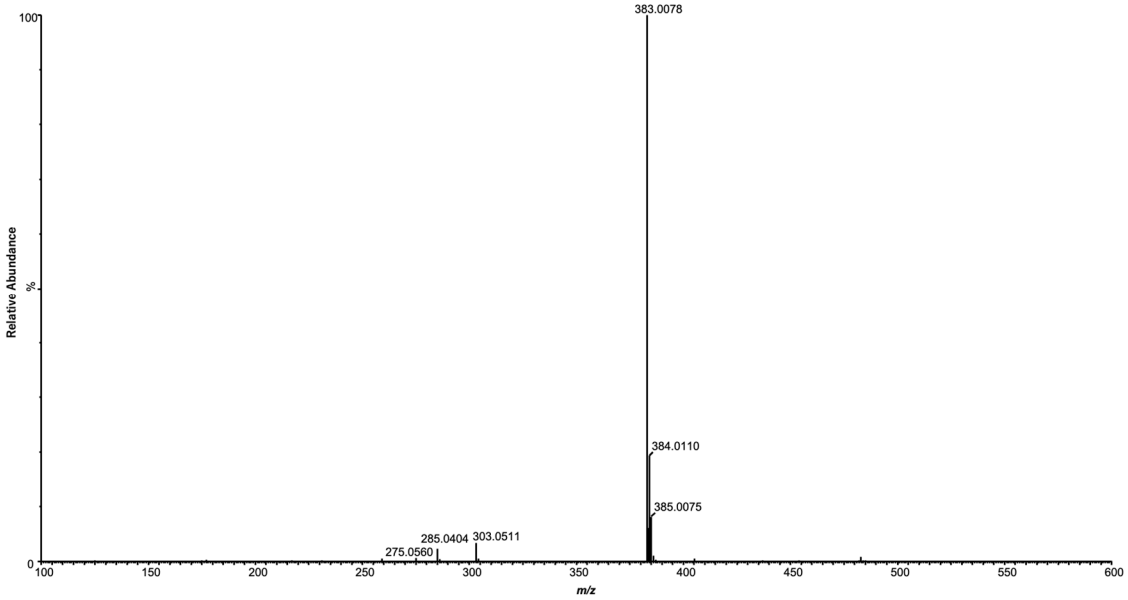

Figure S40. MS (ES-) data of taxifolin 3′-*O*-sulfate (**4**) (at retention time 15.96 min).

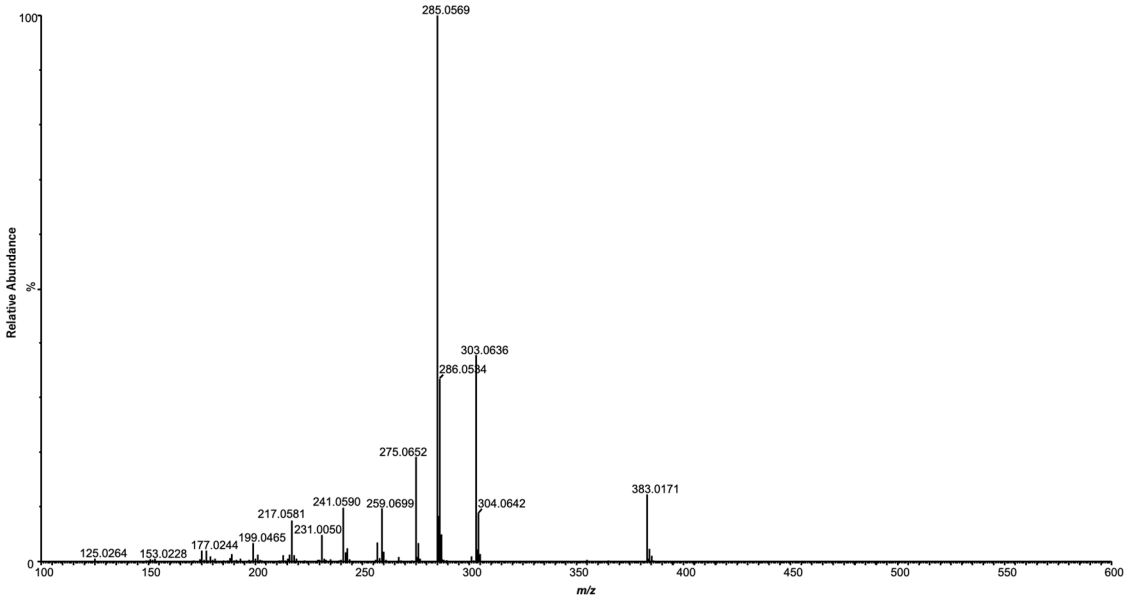

Figure S41. MS/MS (from the ion at *m/z* 383.0078) data of taxifolin 3′-*O*-sulfate (**4**).

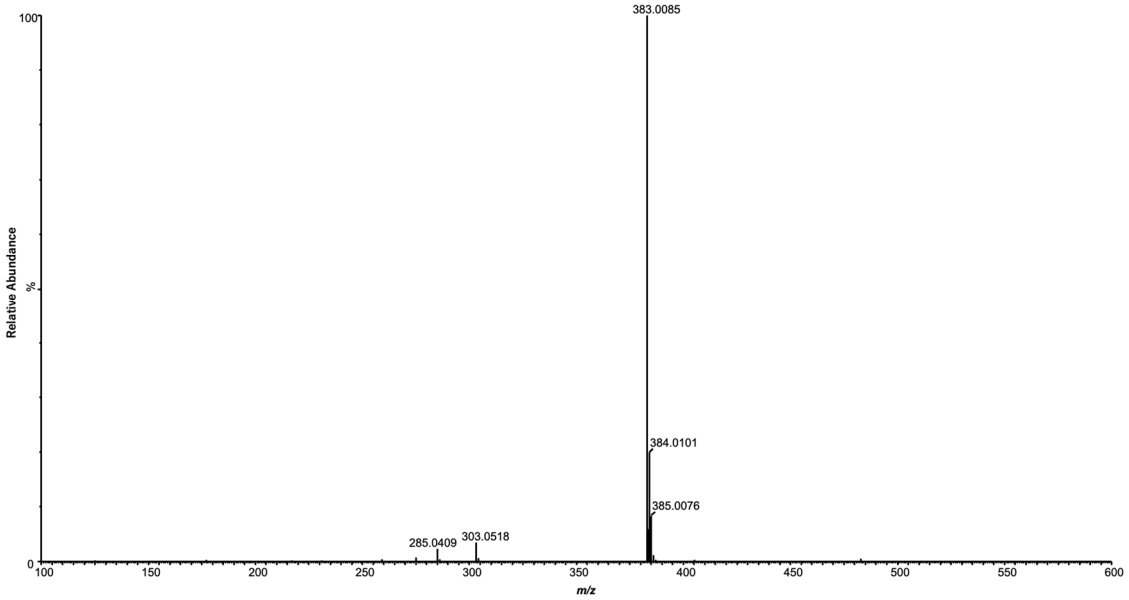

Figure S42. MS (ES-) data of taxifolin 4′-*O*-sulfate (**5**) (at retention time 15.67 min.).

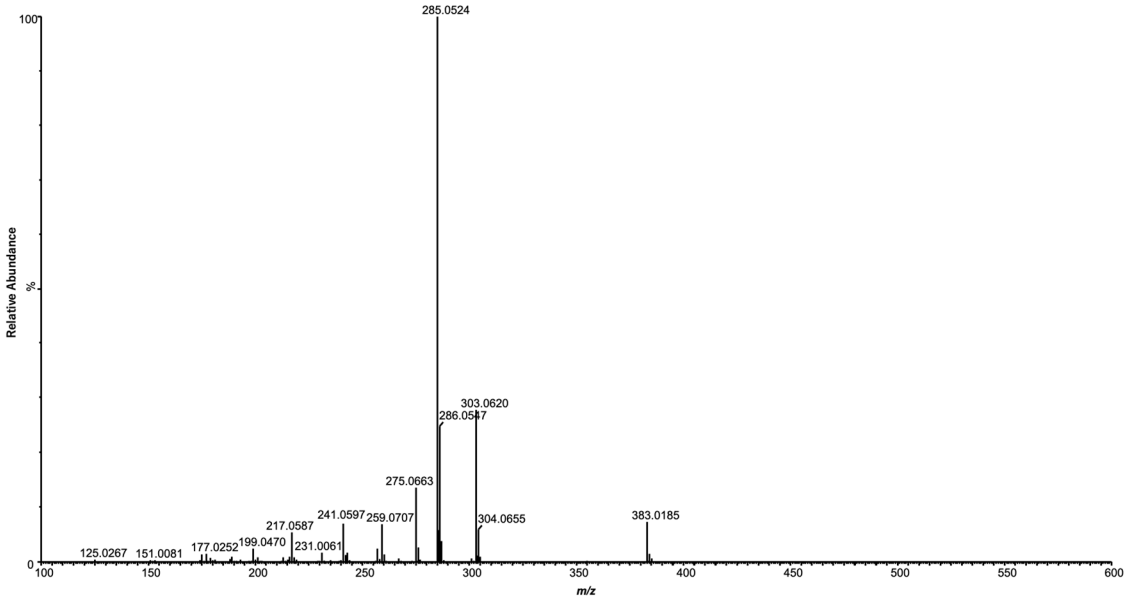

Figure S43. MS/MS (from the ion at *m/z* 383.0078) data of taxifolin 4′-*O*-sulfate (**5**).

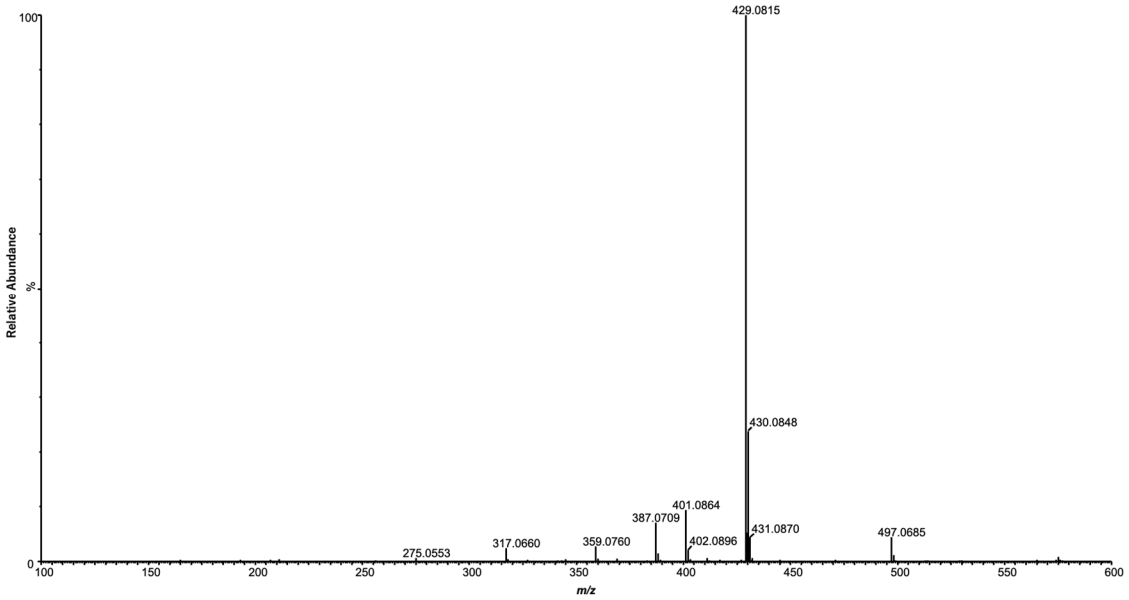

Figure S44. MS (ES-) data of taxifolin 7,3′,4′-tri-*O*-acetate (**6**).

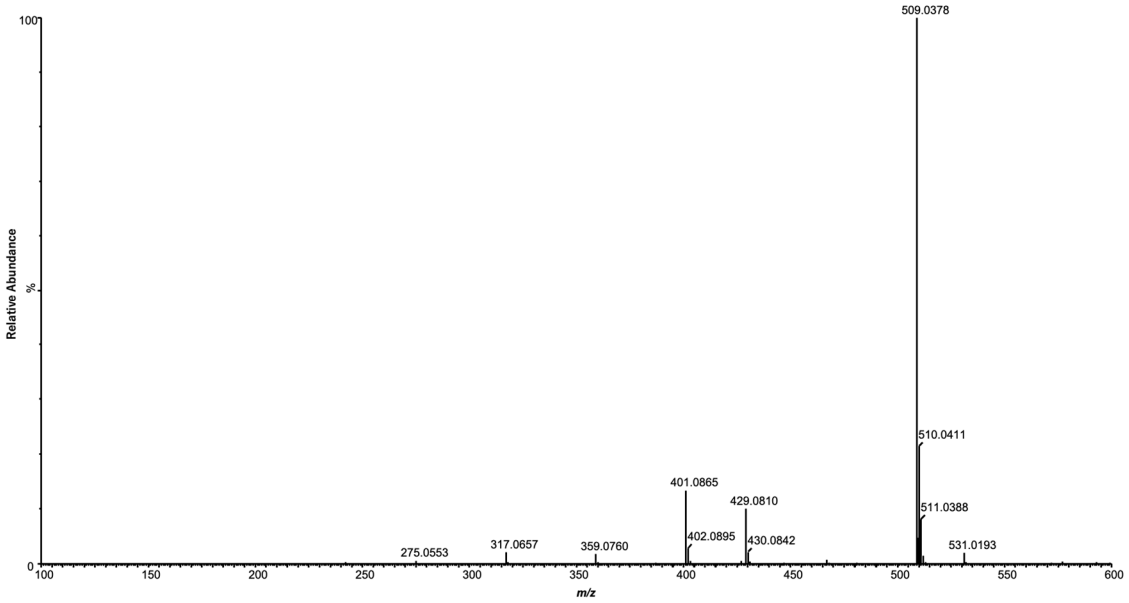

Figure S45. MS (ES-) data of taxifolin 7,3′,4′-tri-*O*- acetyl-3-*O*-sulfate (**7**).

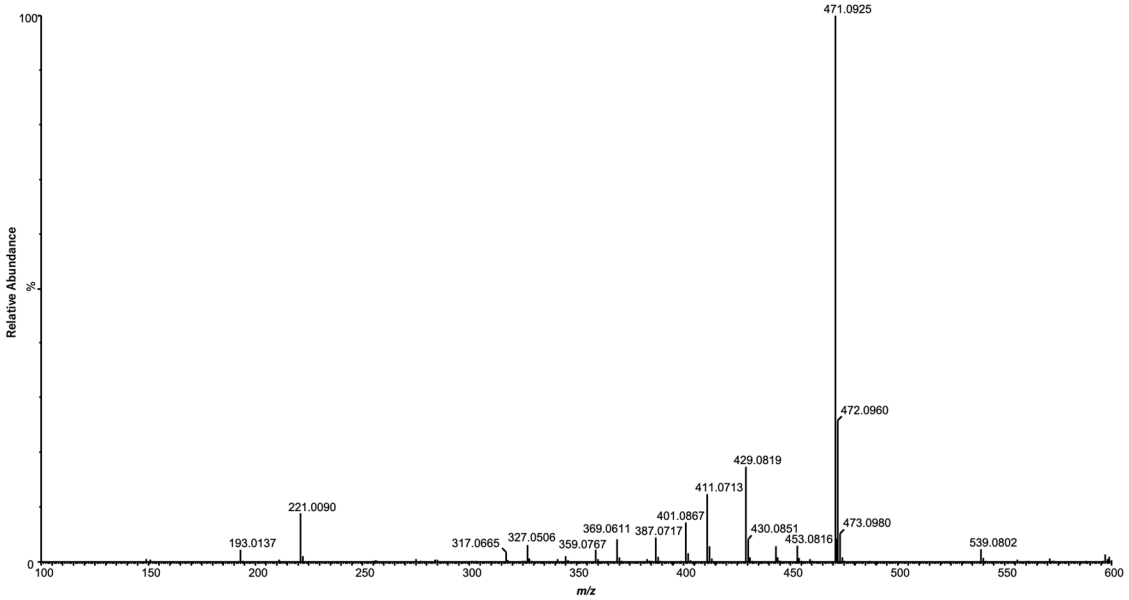

Figure S46. MS (ES-) data of taxifolin 3,7,3′,4′-tetra-*O*-acetate (**8**).

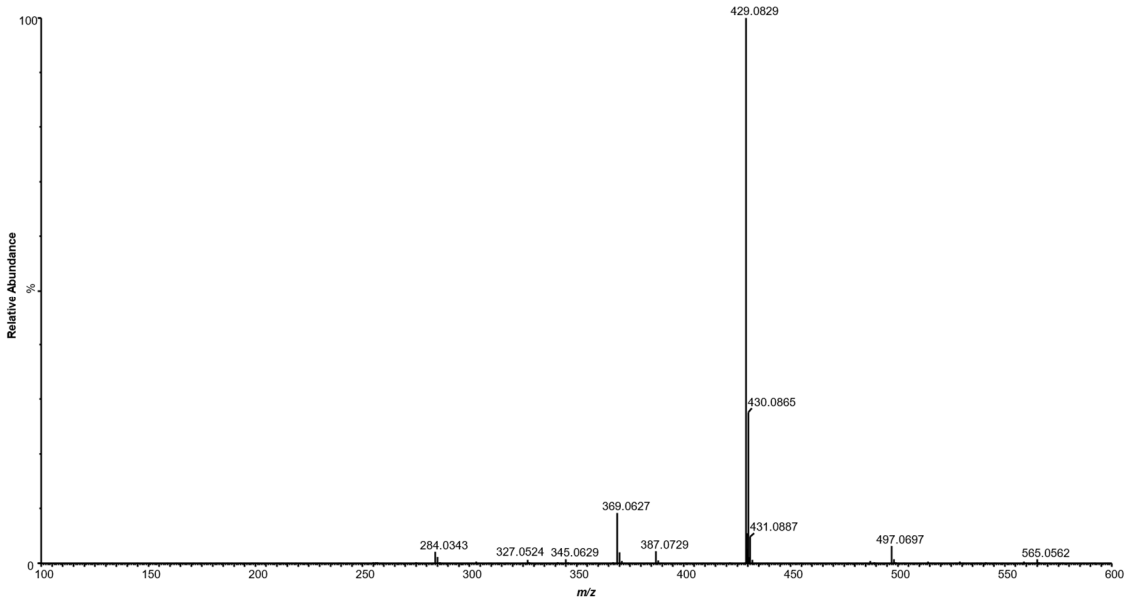

Figure S47. MS (ES-) data of taxifolin 3,3′,4′-tri-*O*-acetate (**9**).

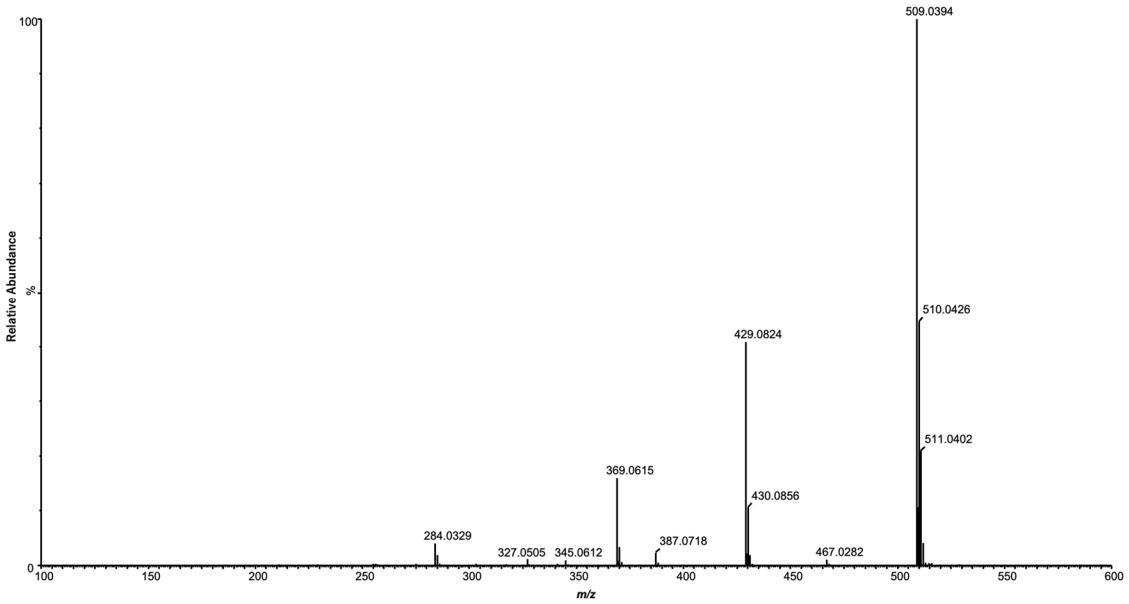

Figure S48. MS (ES-) data of taxifolin 3,3′,4′-tri-*O*-acetyl-7-*O*-sulfate (**10**).