Supporting Information for

Schicagenins A–C: Three Cage-like Nortriterpenoids from Leaves and Stems of *Schisandra chinensis*

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Detailed experimental procedures

1. ***General Experimental procedures***

Optical rotations were measured with a JASCO DIP–370 digital polarimeter. UV data were obtained on a Shimadzu UV–2401A spectrophotometer. CD spectra were measured on a Chirascan instrument. A BioRad FtS–135 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM–400 and DRX–500 spectrometers. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. High–resolution electrospray–ionization (HRESIMS) were performed on a VG Autospec–3000 spectrometer under 70 eV. X-ray diffraction was realized on a Bruker APEX DUO instrument. Column chromatography was performed with silica gel (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), or MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Semipreparative HPLC was performed on an Agilent 1200 liquid chromatograph with a Zorbax SB–C_{18}, 9.4 mm × 25 cm column. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH. All solvents including petroleum ether (60–90 ^oC) were distilled prior to use.

2. ***Plant material***

The leaves and stems of *Schisandra chinensis* were collected from Yabuli mountain area of Heilongjiang province, People's Republic of China, in August 2010. Voucher specimens (KIB 2010-08-13) were deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, and were identified by Prof. Xi-Wen Li.

3. ***Extraction and isolation***

The air-dried and powdered leaves and stems (10 kg) were extracted with 70% aqueous Me_2CO (3 × 10 L, 3 days each) at room temperature, and concentrated under reduced pressure to give a crude extract, which was partitioned between H_2O and EtOAc. The EtOAc part (438 g) was chromatographed on a silica gel column with a gradien elution of $CHCl_3$/Me_2CO (1:0 to 0:1) to furnish seven fractions A–G. Fraction E (41 g) was subject to further separation over columns, first MCI eluted with 90%

MeOH, then on RP-18 with a gradien elution of MeOH/H_2O (3:7 to 1:0) to yield fractions E1–E6. Subsequently fraction E3 (2.26 g) was purified by a silica gel column (petrolum ether/Me_2CO_3 4:1 to 1:0) to give subfractions E3c (637mg) and E3d (700mg). Subfraction E3c was subjected to purified by smipreparative HPLC (3 mL/min, detector UV λ_{max} 275 nm, MeOH/MeCN/H_2O 40:15:45) to yield **1** (11 mg). Subfraction E3d was purified by smipreparative HPLC (3 mL/min, detector UV λ_{max} 275 nm, MeCN/H_2O 30:70) to yield **2** (6 mg) and **3** (3 mg).

Schicagenin A (**1**), colorless crystals; $[\alpha]_D^{21.7}$ +349.3 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε): 275 (3.44) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$): 307 (+15.95), 270 (–16.34); IR (KBr) ν_{max} 3441, 2973, 2932, 1769, 1698, 1626, 1462, 1387, 1374, 1310, 1295, 1226, 1178, 1113, 1073, 1046, 1020, 989, 937, 915, 843, 758 cm^{-1}; positive ESIMS $[M + Na]^+$ *m/z* 567; positive HRESIMS $[M + Na]^+$ *m/z* 567.2204 (calcd. $C_{29}H_{36}O_{10}Na$ $[M + Na]^+$, 567.2206).

Schicagenin B (**2**), white solid; $[\alpha]_D^{21.7}$ +276.0 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε): 275 (3.54) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$): 300 (+15.95), 270 (–16.59); IR (KBr) ν_{max} 3440, 2973, 2932, 1767, 1697, 1627, 1460, 1383, 1296, 1237, 1221, 1174, 1118, 1072, 1043, 1016, 991, 935, 914, 843, 758 cm^{-1}; positive ESIMS $[M + Na]^+$ *m/z* 583; positive HRESIMS $[M + Na]^+$ *m/z* 583.2151 (calcd. $C_{29}H_{36}O_{11}Na$ $[M + Na]^+$, 583.2155).

Schicagenin C (**3**), white solid; $[\alpha]_D^{21.7}$ –28.2 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε): 276 (3.31) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$): 300 (–14.72), 270 (+4.29); IR (KBr) ν_{max} 3440, 2968, 2931, 1766, 1701, 1626, 1461, 1382, 1296, 1252, 1237, 1221, 1174, 1117, 1073, 1018, 992, 935, 914, 842, 759 cm^{-1}; positive ESIMS $[M + Na]^+$ *m/z* 583; positive HRESIMS $[M + Na]^+$ *m/z* 583.2150 (calcd. $C_{29}H_{36}O_{11}Na$ $[M + Na]^+$, 583.2155).

X-ray Crystallographic Analysis of schicagenin A (**1**): $C_{29}H_{44}O_{14}$, M + $4H_2O$ = 616.64, colorless prism, size 0.18 × 0.18 × 0.60 mm^3, orthorhombic, space group $P2_12_12_1$; a = 11.1986(2) Å, b = 12.0920(2) Å, c = 23.6000(4) Å, $\alpha = \beta = \gamma$ = 90.00 °, V = 3195.76 (9) $Å^3$, T = 296(2) K, Z = 4, $\rho_{calcd.}$ = 1.282 g/cm^3, μ(Cu Kα) = 0.863 mm^{-1}, F(000) = 1320, 22863 reflections in h(–10/12), k(–14/13), l(–27/28), measured in the range 3.75° ≤ θ ≤ 68.04°, completeness θmax = 94.6%, 5453 independent reflections, Rint = 0.0307, 5277 reflections with $|F|^2 \geq 2\sigma|F|^2$, 396 parameters, 0 restraints, GOF =

1.061. Final *R* indices: R_1 = 0.0527, wR_2 = 0.1590. *R* indices (all data): R_1 = 0.0538, wR_2 = 0.1608. Flack parameter –0.1(2), largest difference peak and hole = 0.538 and –0.232 e Å^{-3}. The intensity data for **1** were collected on a Bruker APEX DUO diffractometer using graphitemonochromated Cu Kα radiation. The structure of **1** was solved by direct methods (SHELXS97), expanded using difference Founier techniques, and refined by the program and full-matrix least-squares calculations. The nonhydrogen atoms were refined anisotropically, and hydrogen atoms were fixed at calculated positions. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 825583). Copies of the data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk.

4. *Cellular Proliferation Assay*

Colorimetric assays were performed to evaluate compound activity. The following human tumor cell lines were used: the HL-60 human myeloid leukemia cell line, the SMMC-7721 human hepatocarcinoma cell line, the A549 lung cancer cell line, the MCF-7 breast cancer cell line, and the SW-480 human pancreatic carcinoma. All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (Hyclone) at 37 ℃ in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma,St. Louis, MO). Briefly, 100 μL adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with initial density of 1 × 105 cells/mL in 100 μL of medium. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with *cis*-Platin (Sigma) as positive control. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 h at 37 ℃. The cells were lysed with 100 μL of 20% SDS-50% DMF after removal of 100 μL of medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated by Reed and Muench's method.

Table S1. ^{1}H NMR assignments of (**1**–**3**) (pyridine-d_5, δ in ppm)[a]

position	**1**	**2**	**3**
	δ_H (mult., *J* in Hz)	δ_H (mult., *J* in Hz)	δ_H (mult., *J* in Hz)
1	4.19 (d, 5.5)	4.29 (d, 5.5)	4.32 (d, 5.6)
2α	2.59 (d, 18.1)	2.53 (d, 18.0)	2.56 (d, 18.1)
2β	2.82 (overlap)	2.81 (overlap)	2.93 (dd, 18.1, 5.7)
5	2.46 (s)	3.21 (s)	3.21 (s)
6	4.48 (d, 8.4)	4.76 (d, 8.6)	4.72 (d, 8.5)
7α	2.66 (m)	2.68 (m)	2.65 (m)
7β	2.20 (overlap)	2.30 (overlap)	2.29(overlap)
8	2.81 (overlap)	2.83 (overlap)	2.80 (d, 5.8)
11α	2.19 (overlap)	2.23 (overlap)	2.24 (overlap)
11β	1.39 (m)	1.40 (m)	1.36 (m)
12α	2.23 (overlap)	2.28 (overlap)	2.26 (overlap)
12β	1.54 (m)	1.56 (m)	1.55 (m)
16α	3.36 (d, 16.0)	3.33 (d, 15.8)	2.99 (d, 16.0)
16β	3.12 (d, 16.0)	3.10 (d, 15.8)	3.08 (d, 16.0)
18	1.08(s)	1.07 (s)	1.09 (s)
19α	1.97 (d, 16.0)	2.01 (d, 16.0)	2.03 (d, 15.8)
19β	2.22 (overlap)	2.30 (overlap)	2.32 (overlap)
20	5.43 (m)	5.39 (m)	4.97 (m)
21	1.81 (d, 4.8)	1.78 (d, 6.3)	1.51 (d, 7)
22	5.28 (d, 10.8)	5.26 (d, 10.8)	5.94 (d, 6.7)
24	7.01 (s)	6.99 (s)	7.72 (s)
27	1.82 (s)	1.81 (s)	1.72 (s)
29	1.10 (s)	3.73 (d, 11.7)	3.72 (d, 11.5)
		3.59 (d, 11.7)	3.57 (d, 11.7)
30	1.07 (s)	1.24 (s)	1.23 (s)

[a] Data for compounds **1-3** was recorded at 500 MHz, and the assignments were base on DEPT, HSQC, HMBC, COSY, and ROESY experiments.

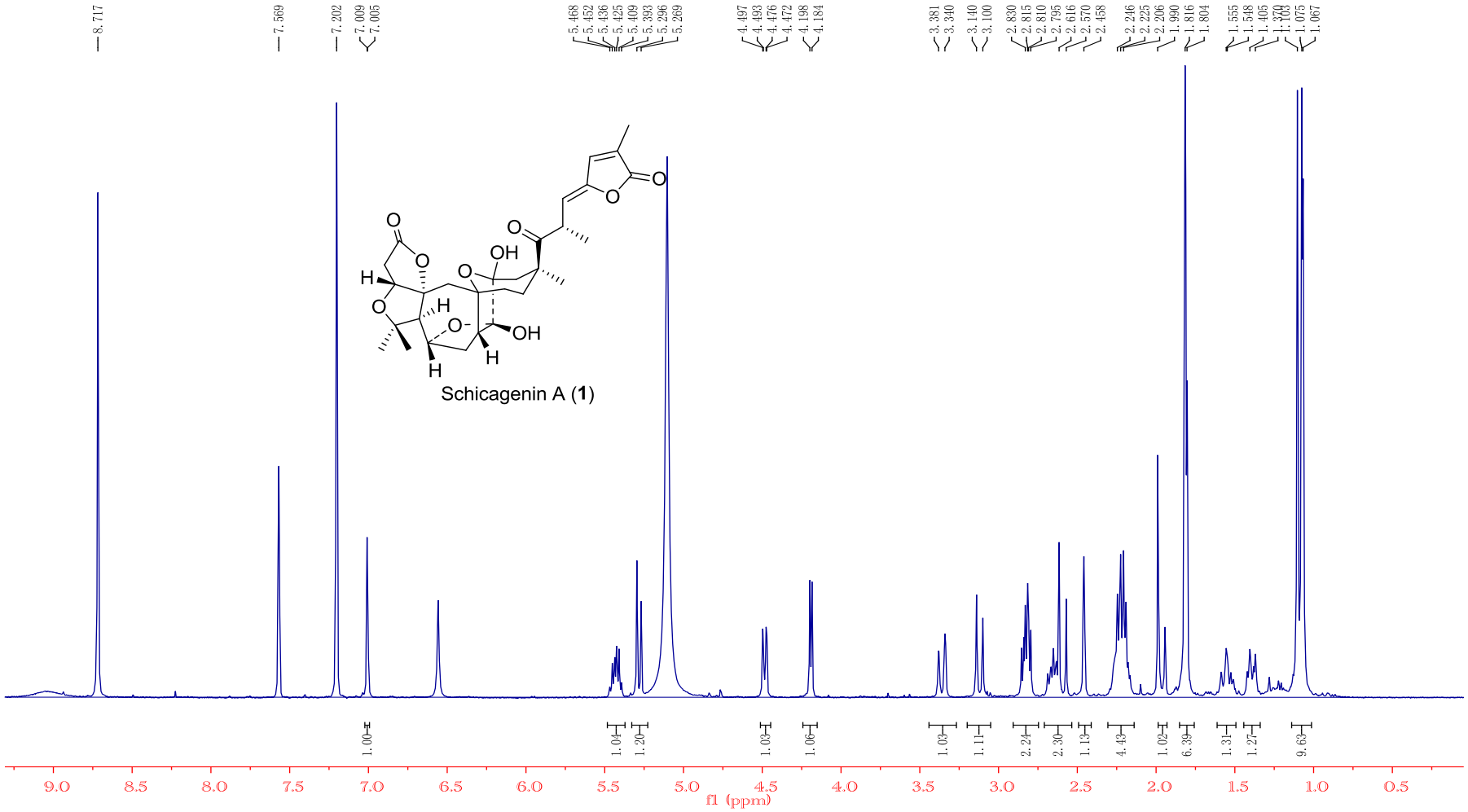

Figure S1. ^{1}H NMR spectrum of schicagenin A (**1**)

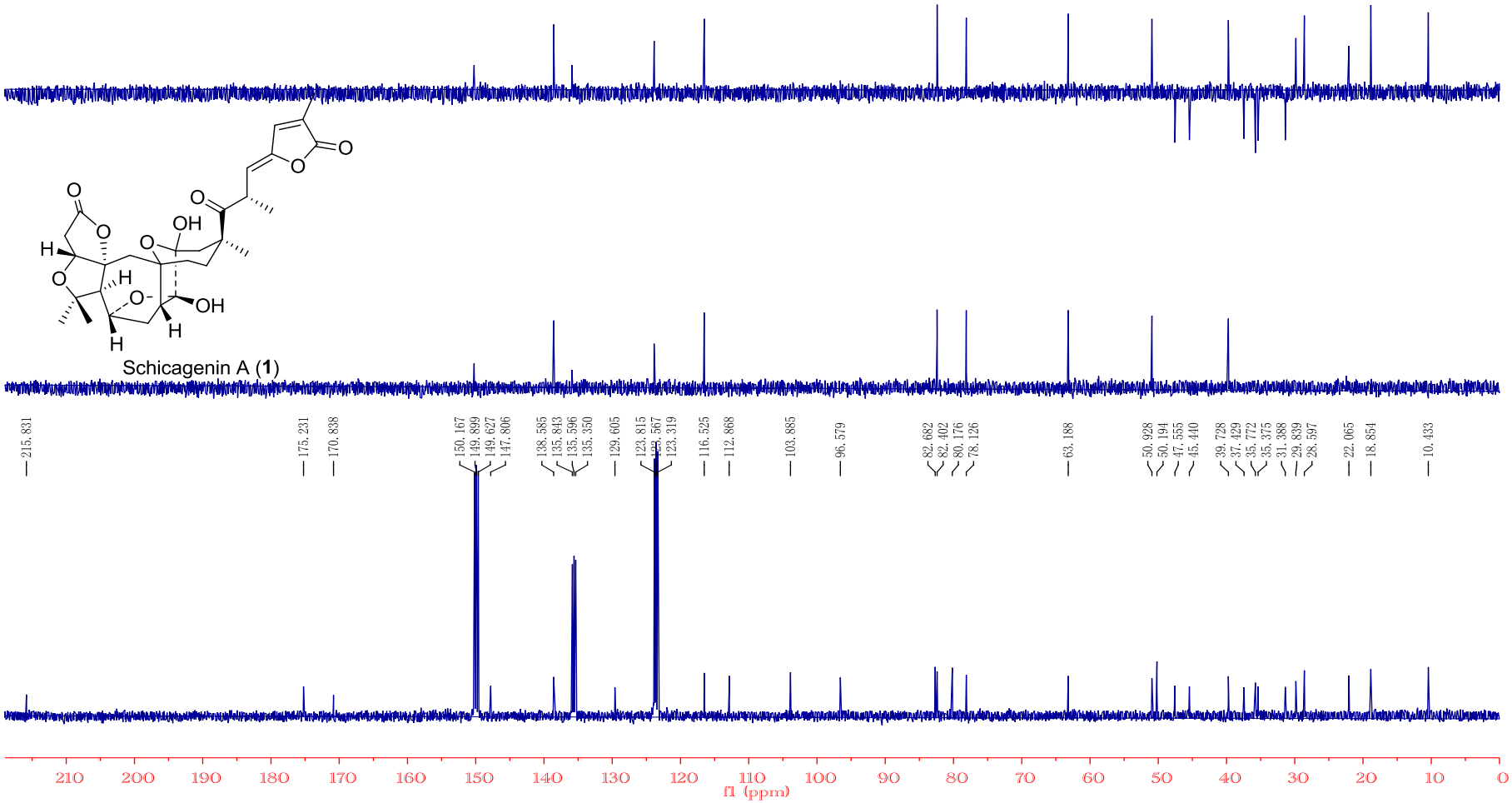

Figure S2. ^{13}C NMR spectrum of schicagenin A (**1**)

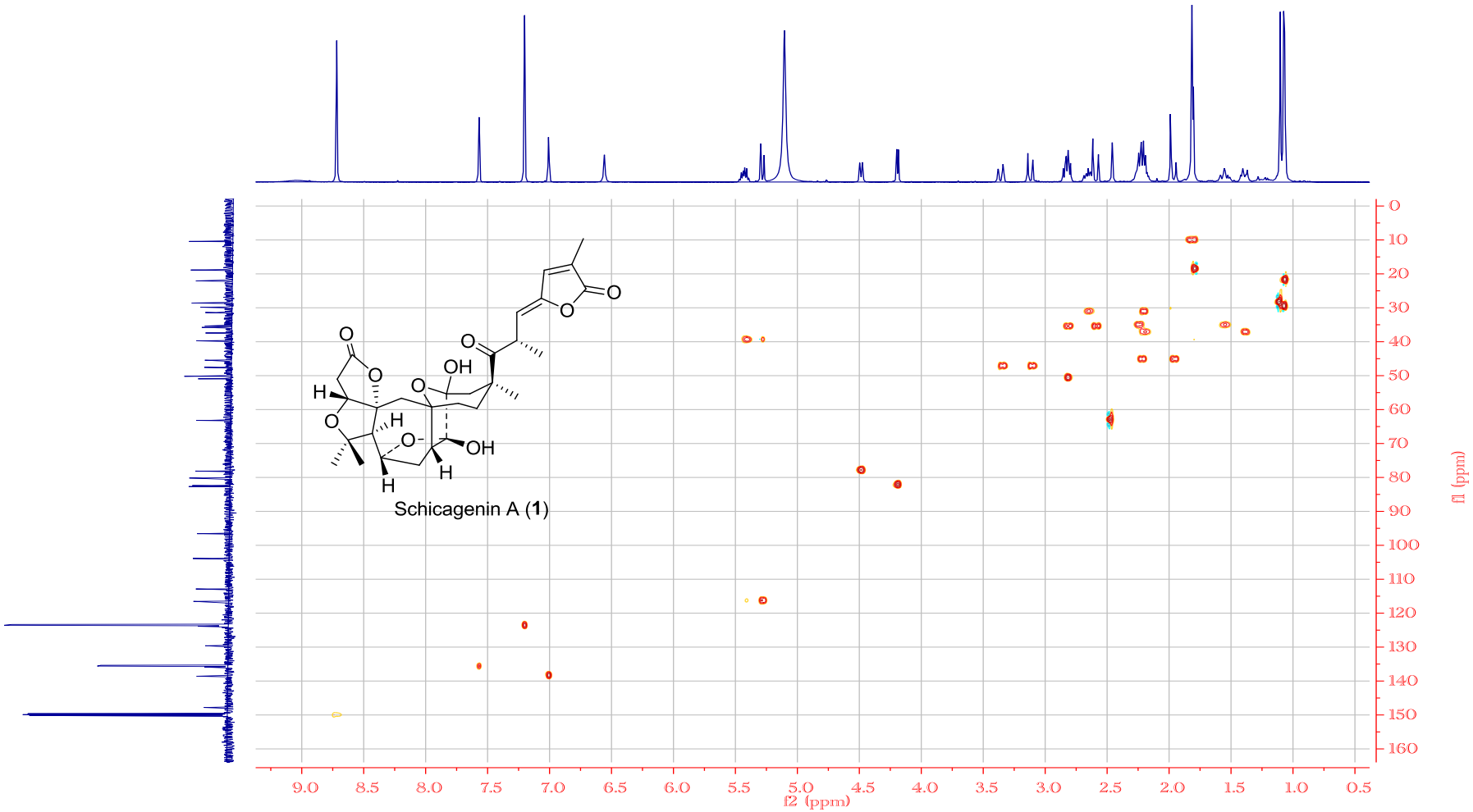

Figure S3. HSQC spectrum of schicagenin A (**1**)

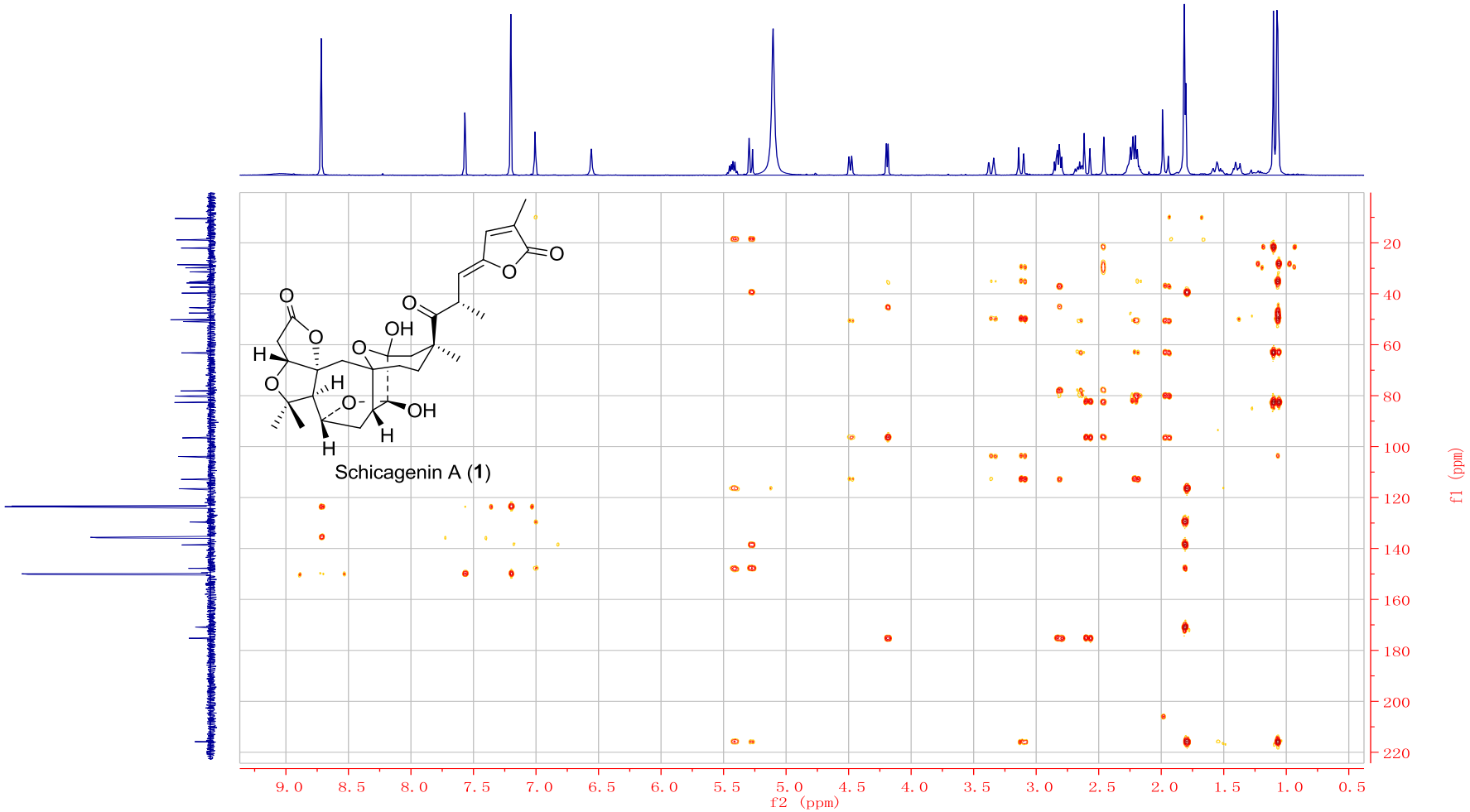

Figure S4. HMBC spectrum of schicagenin A (**1**)

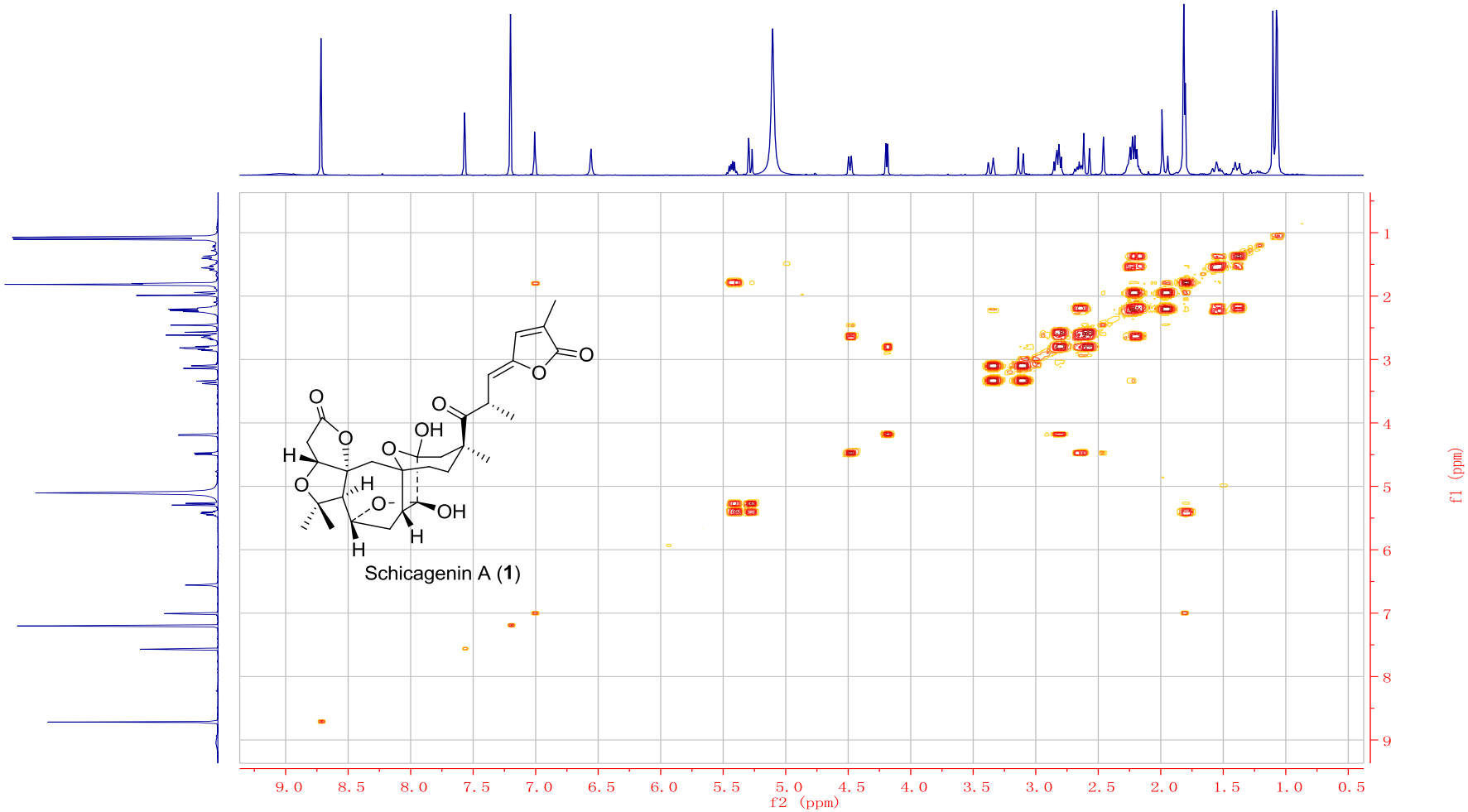

Figure S5. ^{1}H–^{1}H COSY spectrum of schicagenin A (**1**)

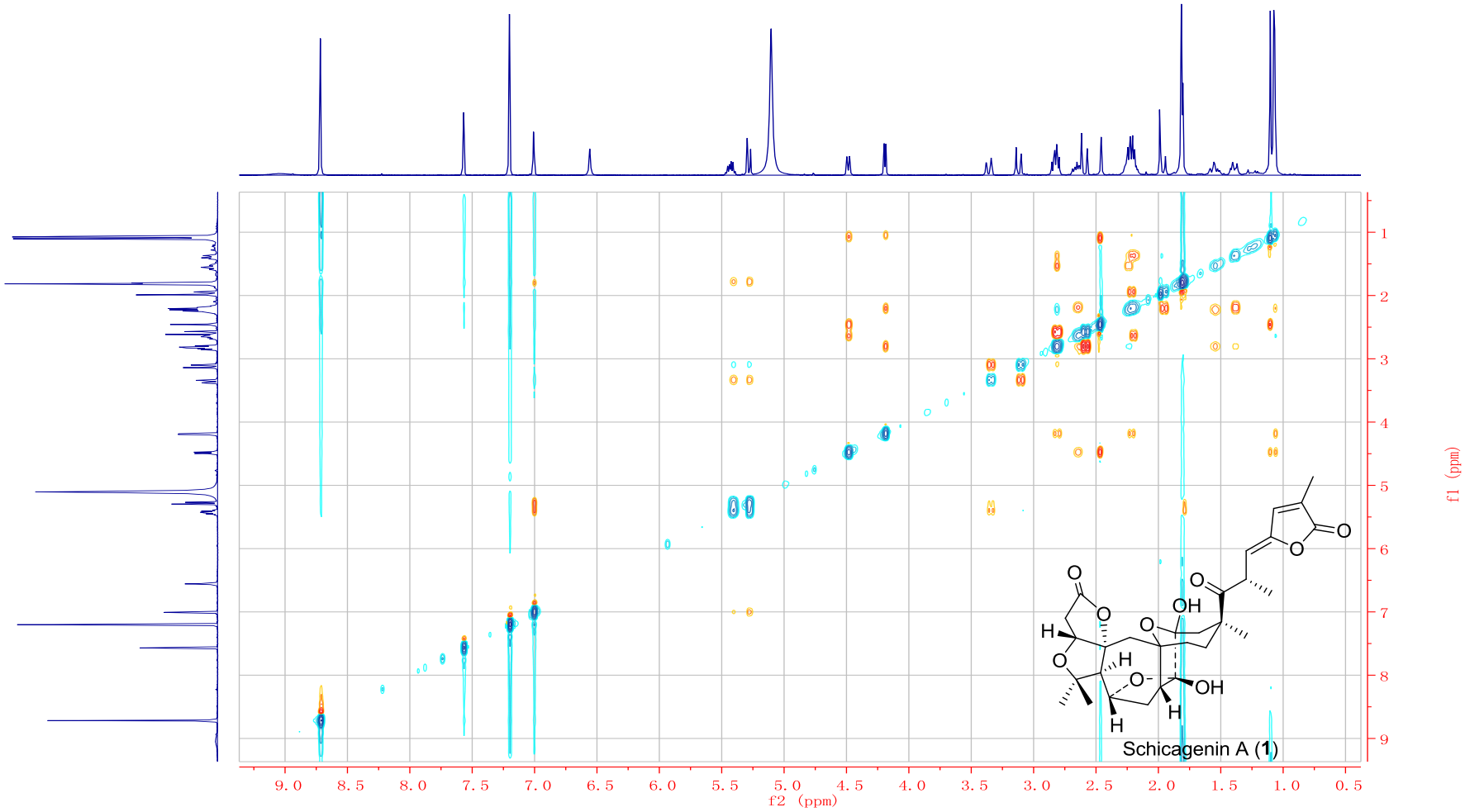

Figure S6. ROESY spectrum of schicagenin A (**1**)

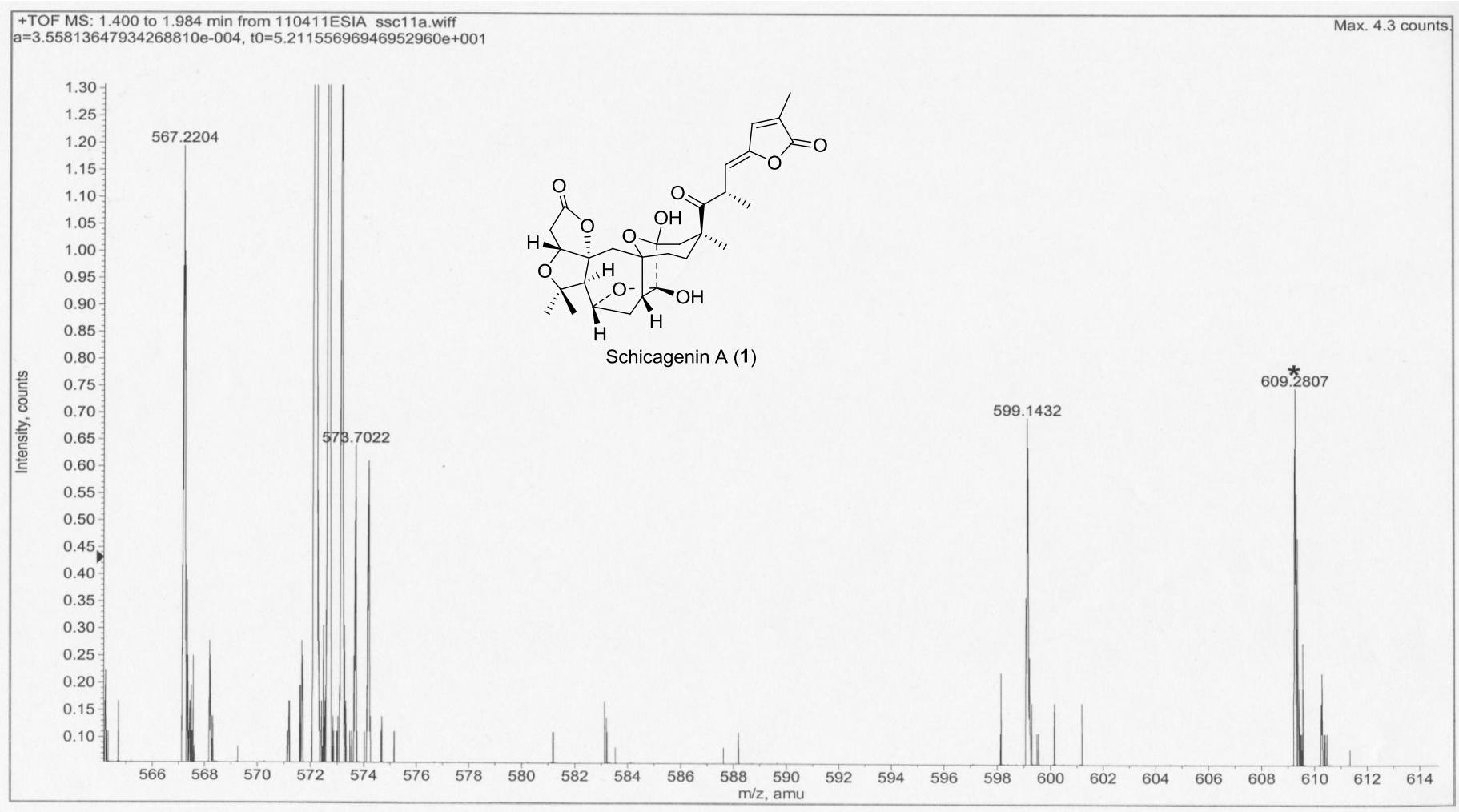

Figure S7. HRESIMS spectrum of schicagenin A (**1**)

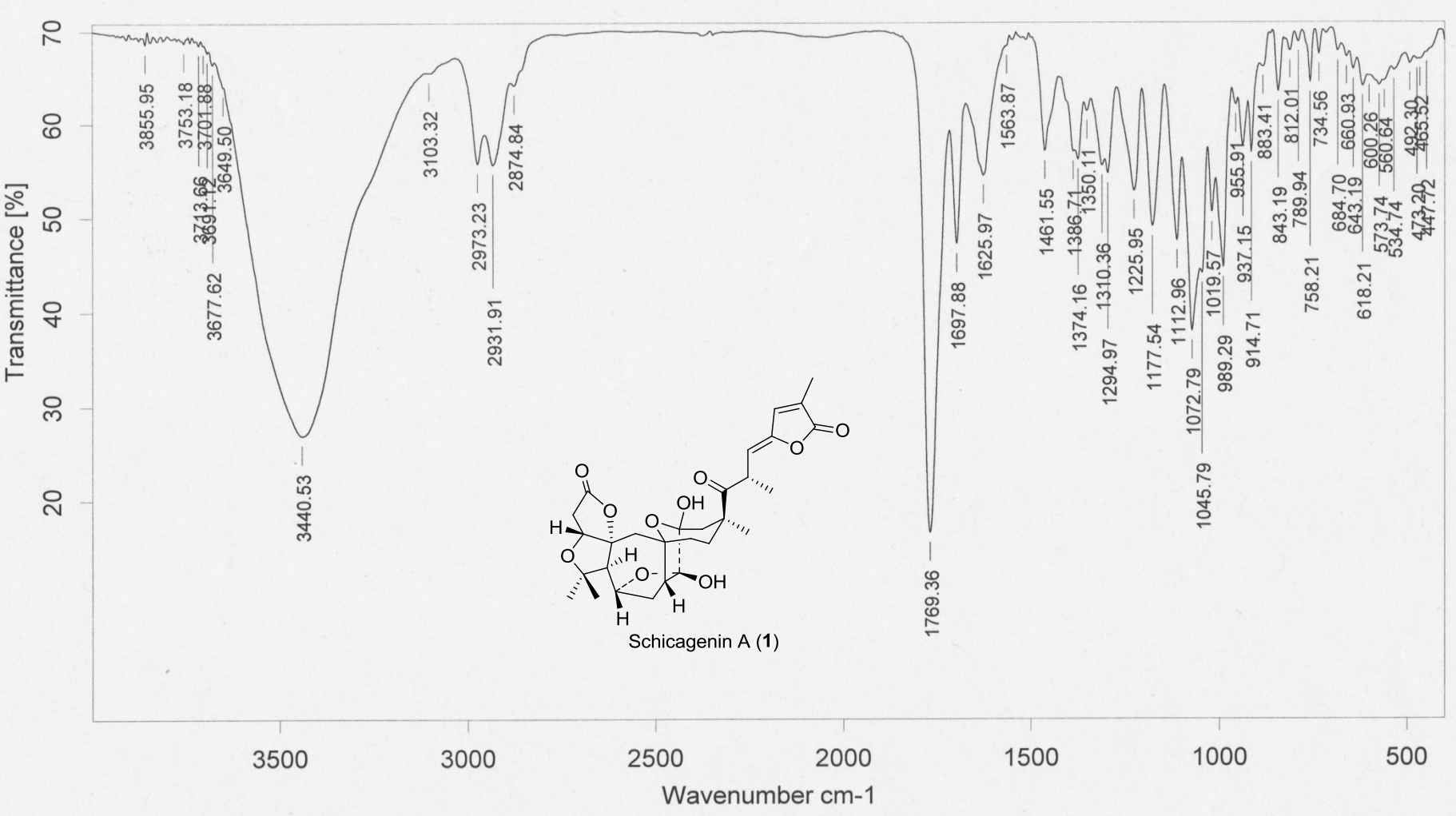

Figure S8. IR spectrum of schicagenin A (**1**)

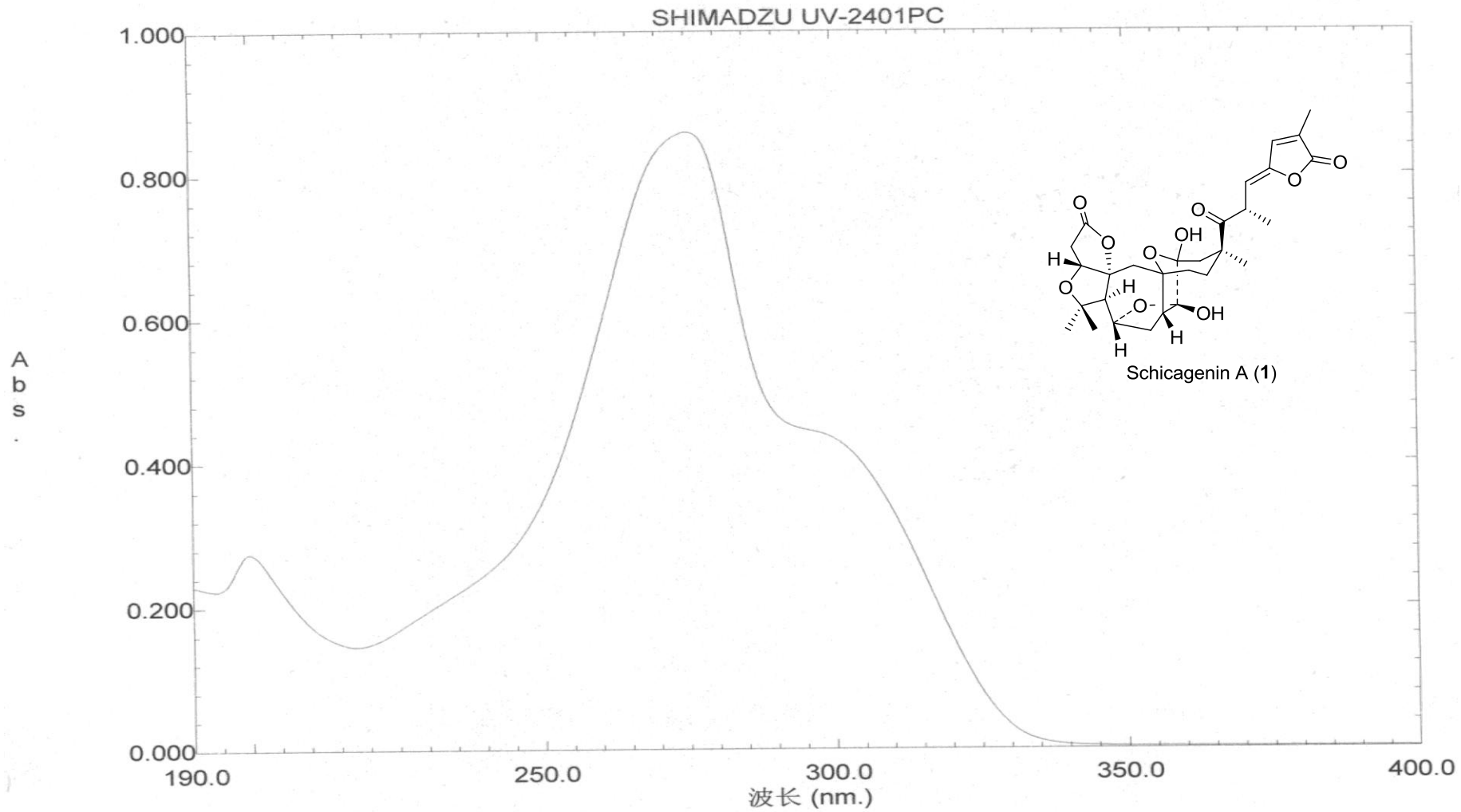

Figure S9. UV spectrum of schicagenin A (**1**)

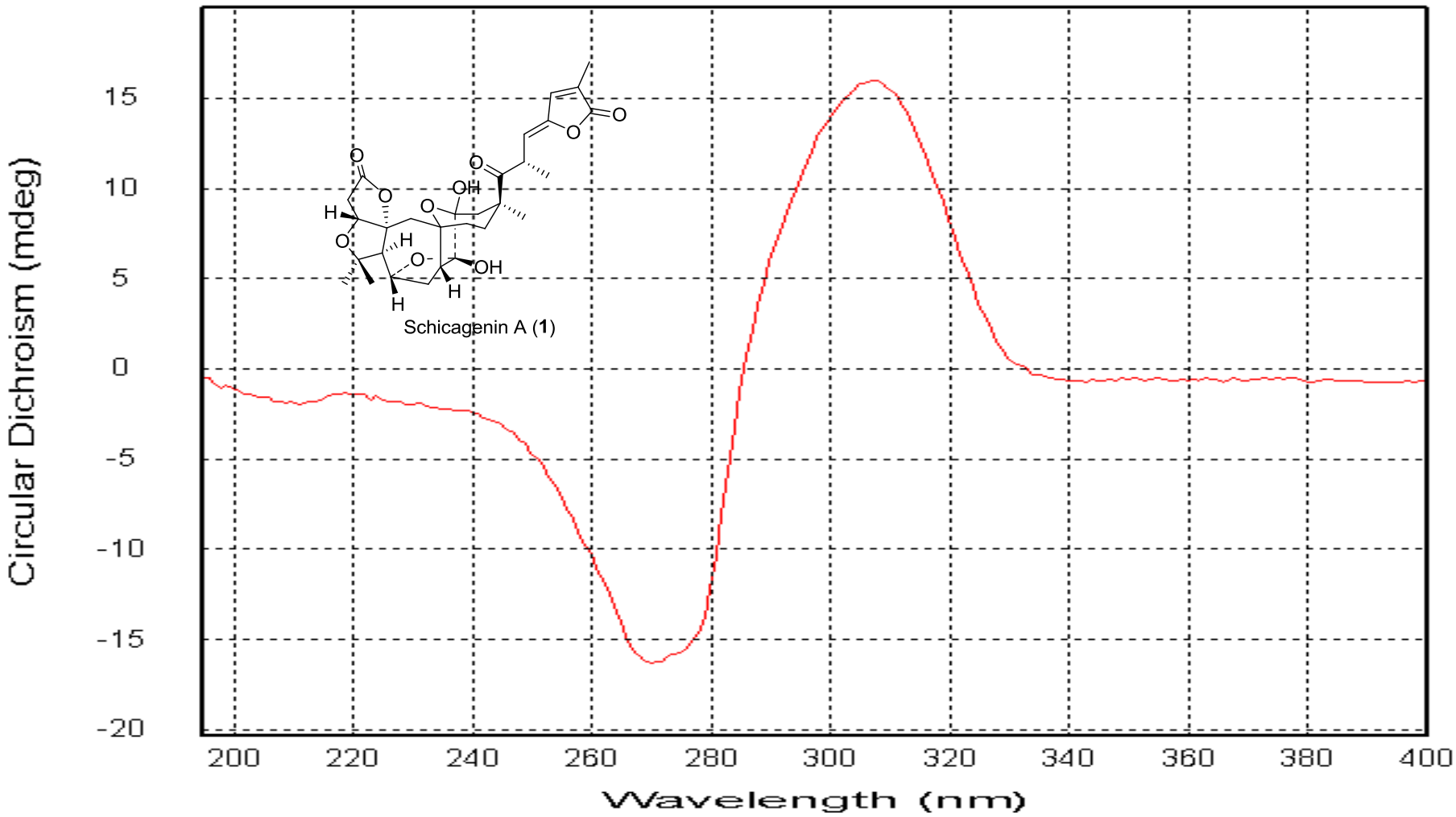

Figure S10. CD spectrum of schicagenin A (**1**)

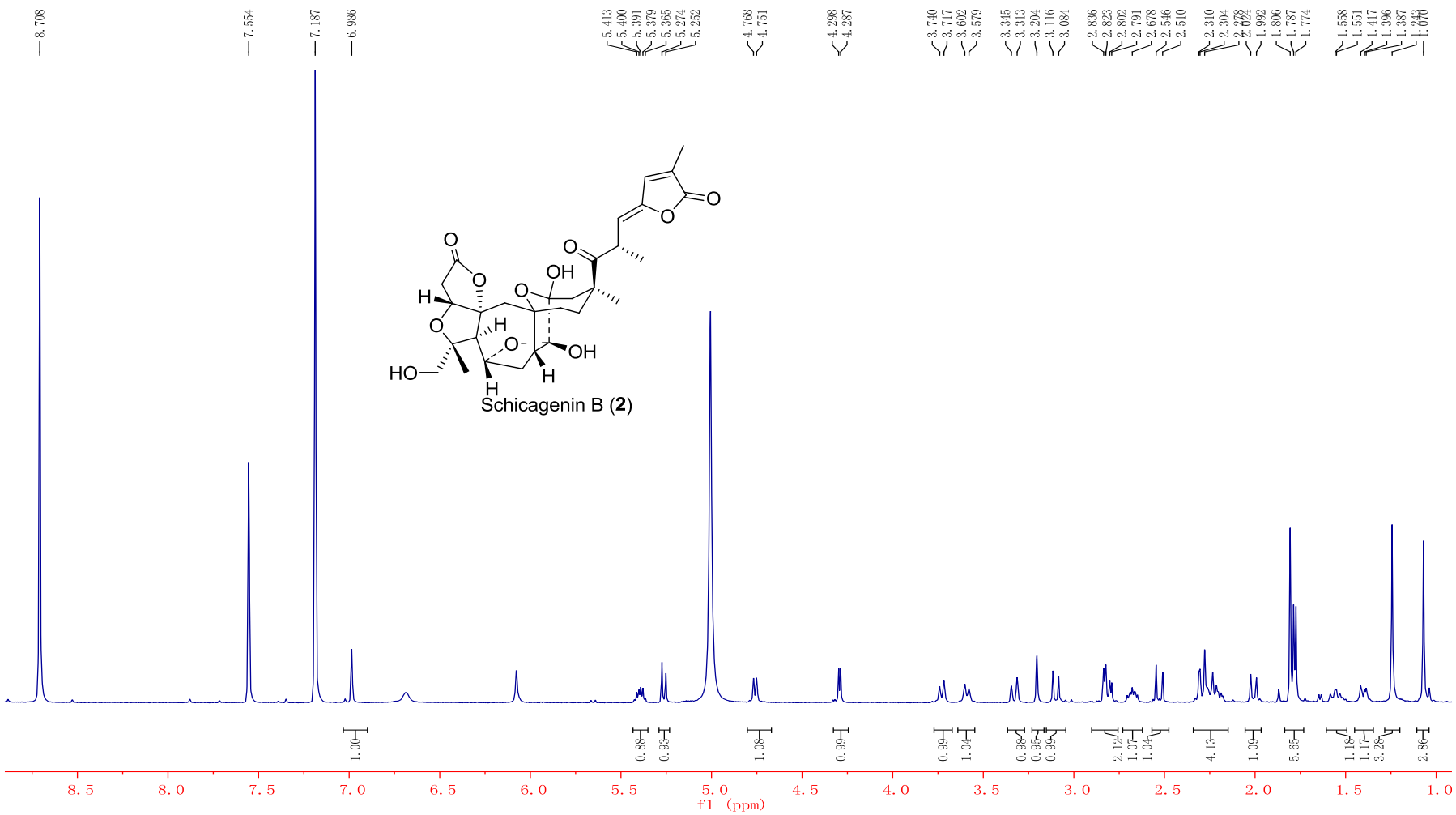

Figure S11. ^{1}H NMR spectrum of schicagenin B (**2**)

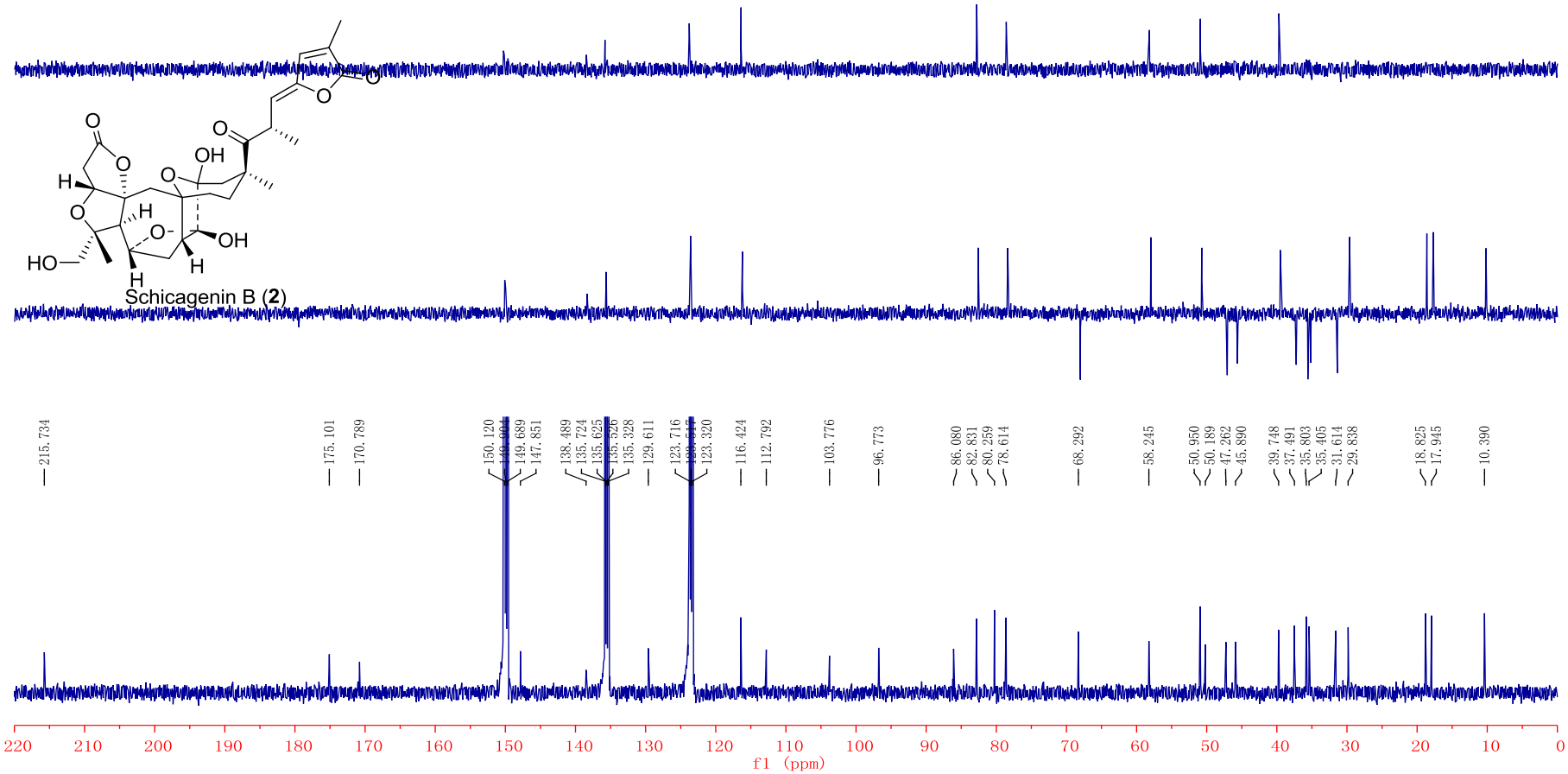

Figure S12. ^{13}C NMR spectrum of schicagenin B (**2**)

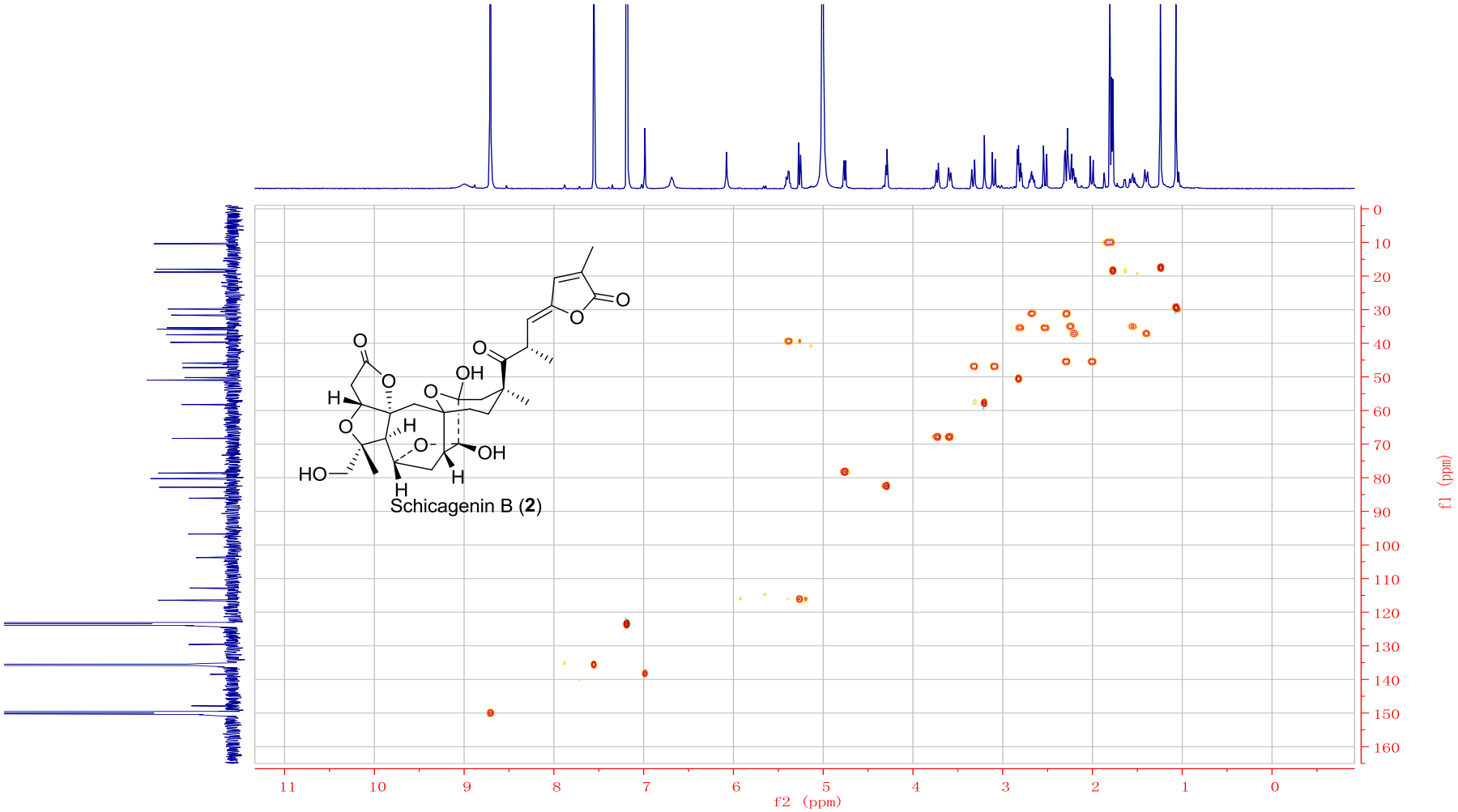

Figure S13. HSQC spectrum of schicagenin B (**2**)

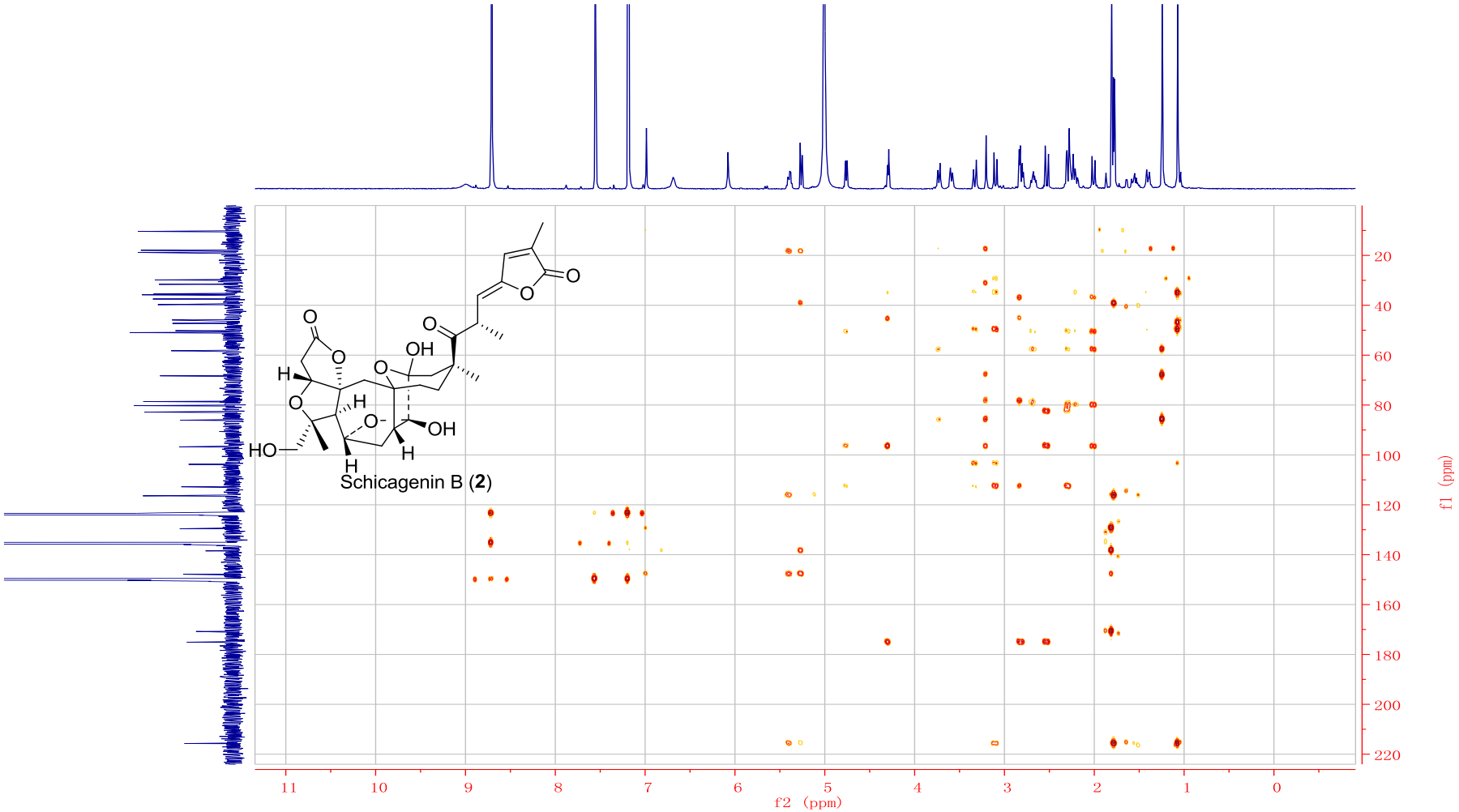

Figure S14. HMBC spectrum of schicagenin B (**2**)

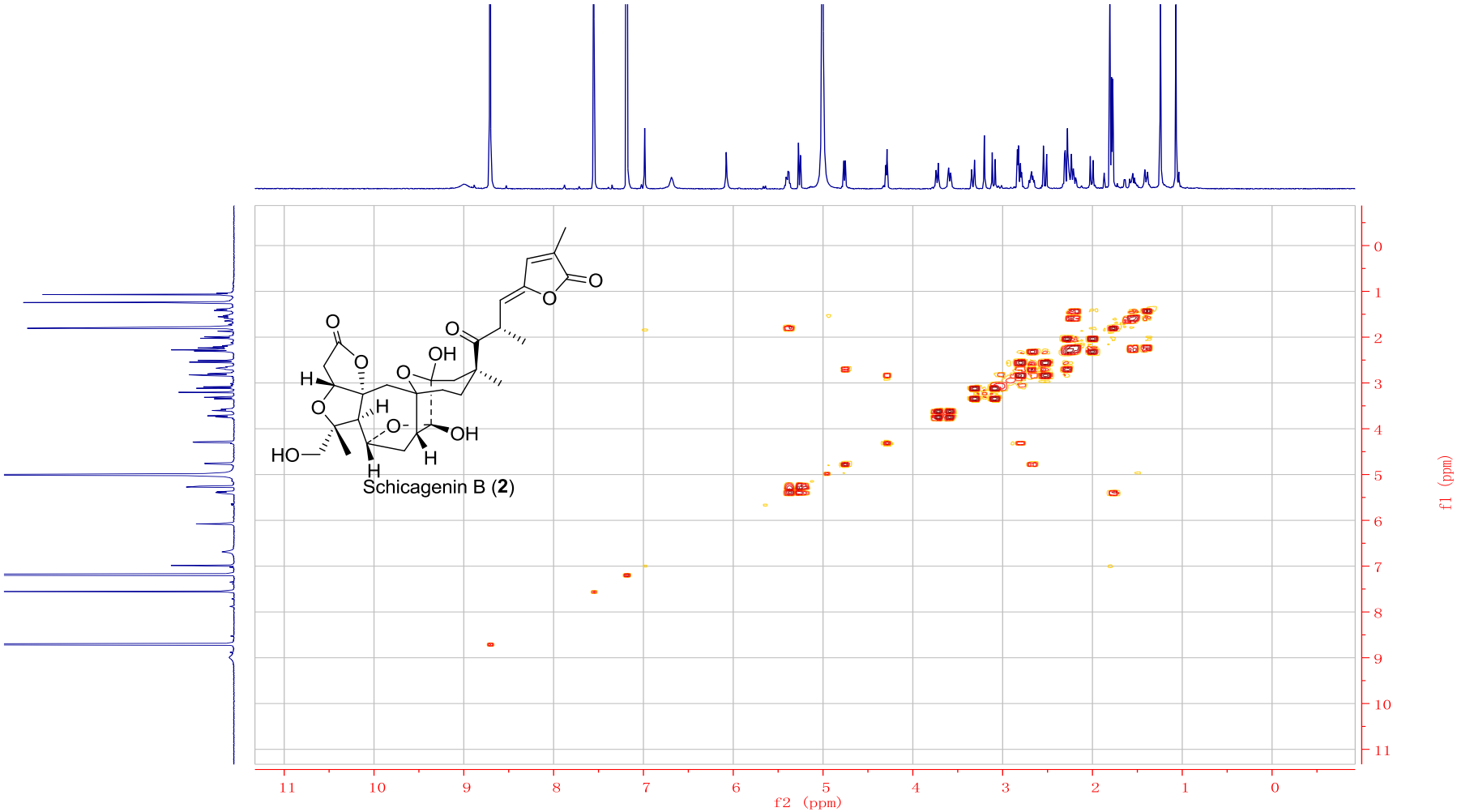

Figure S15. ^{1}H–^{1}H COSY spectrum of schicagenin B (**2**)

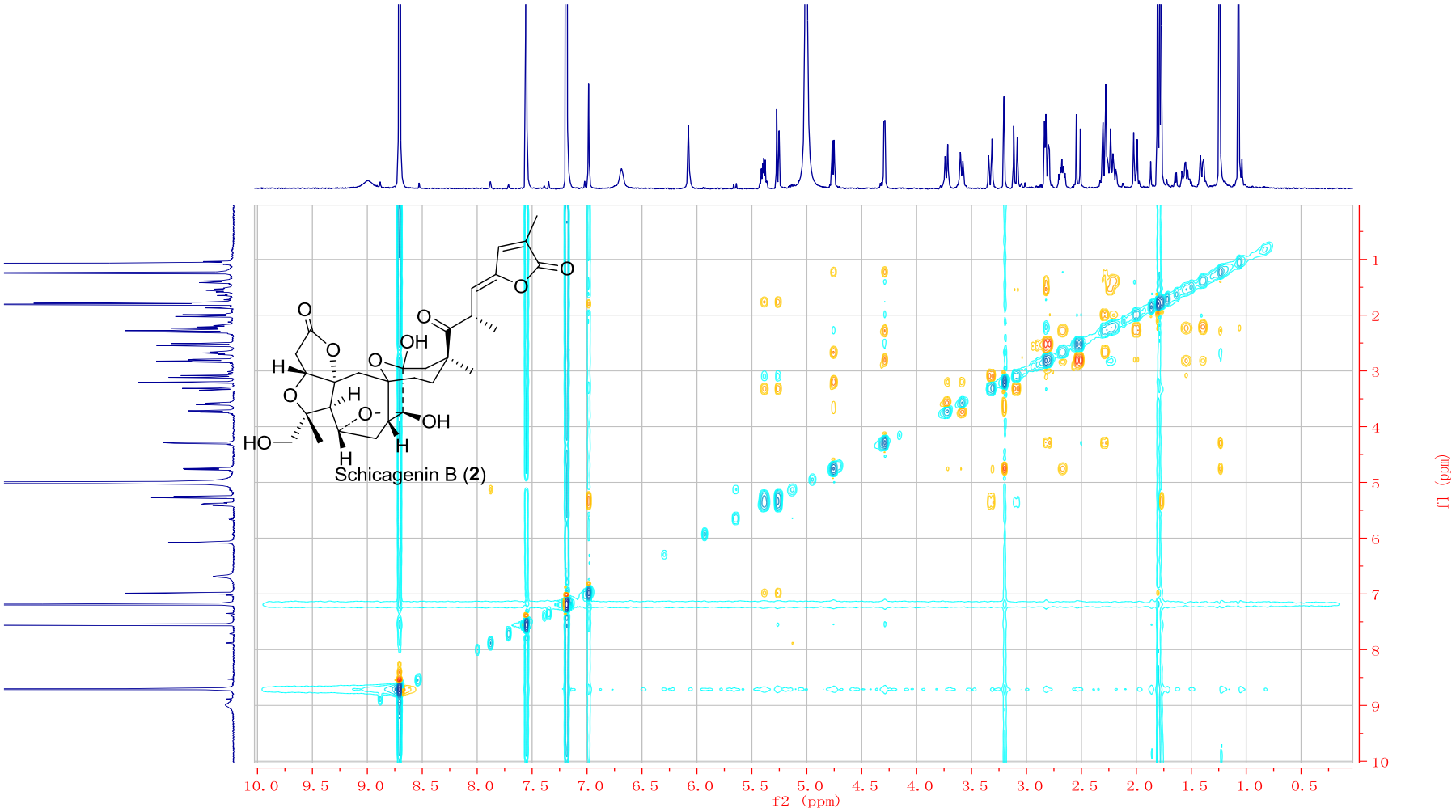

Figure S16. ROESY spectrum of schicagenin B (**2**)

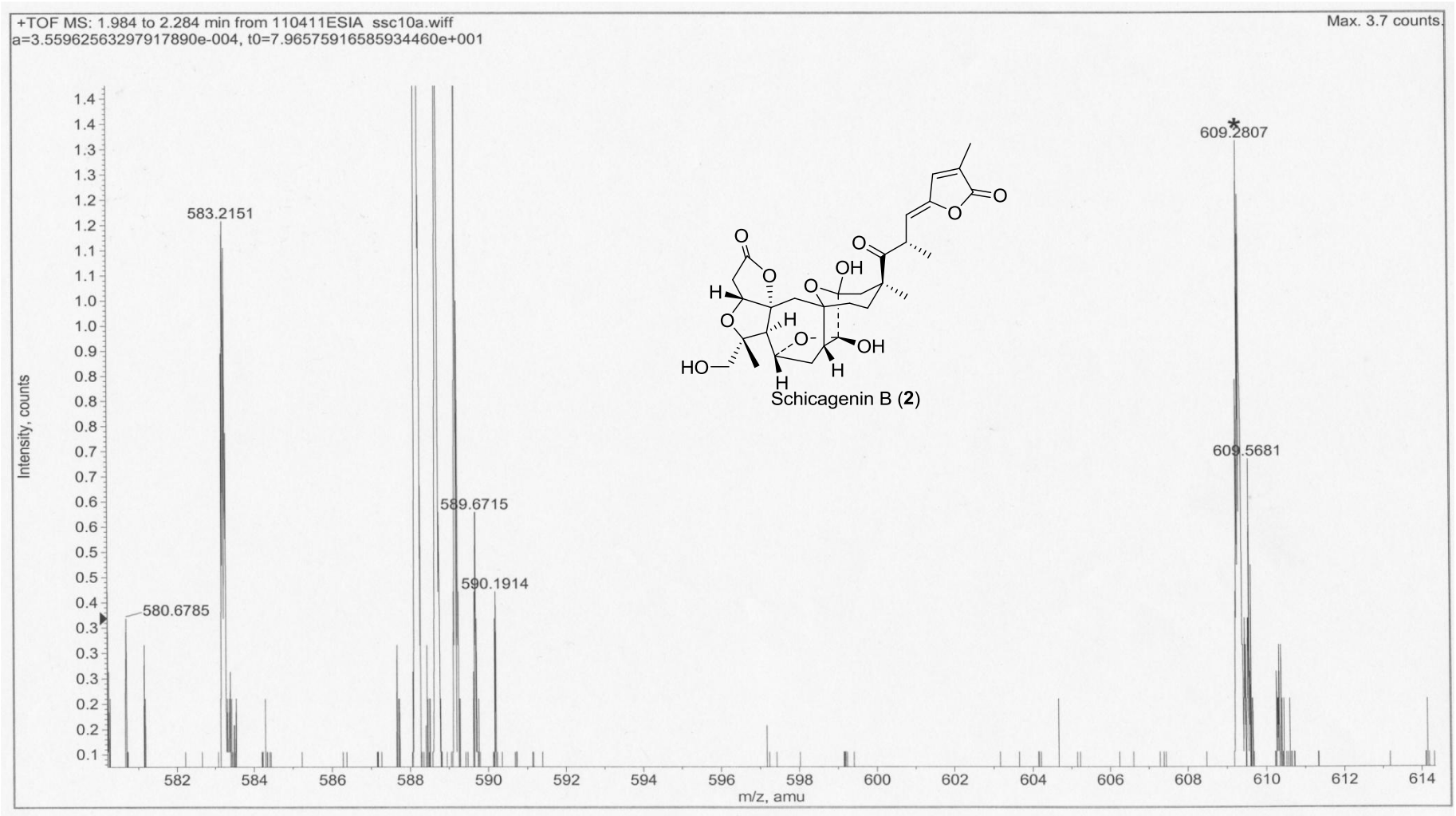

Figure S17. HRESIMS spectrum of schicagenin B (**2**)

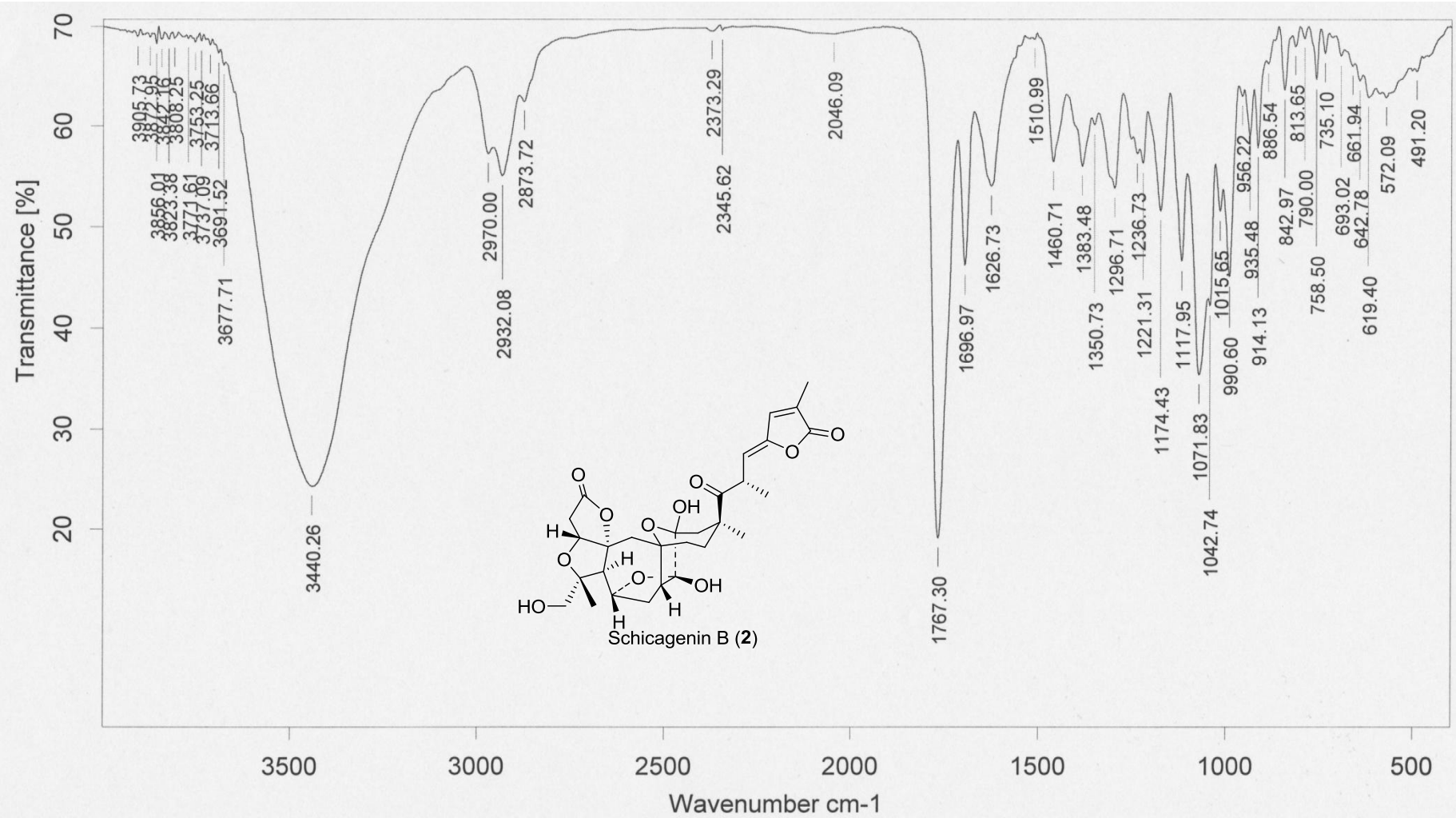

Figure S18. IR spectrum of schicagenin B (**2**)

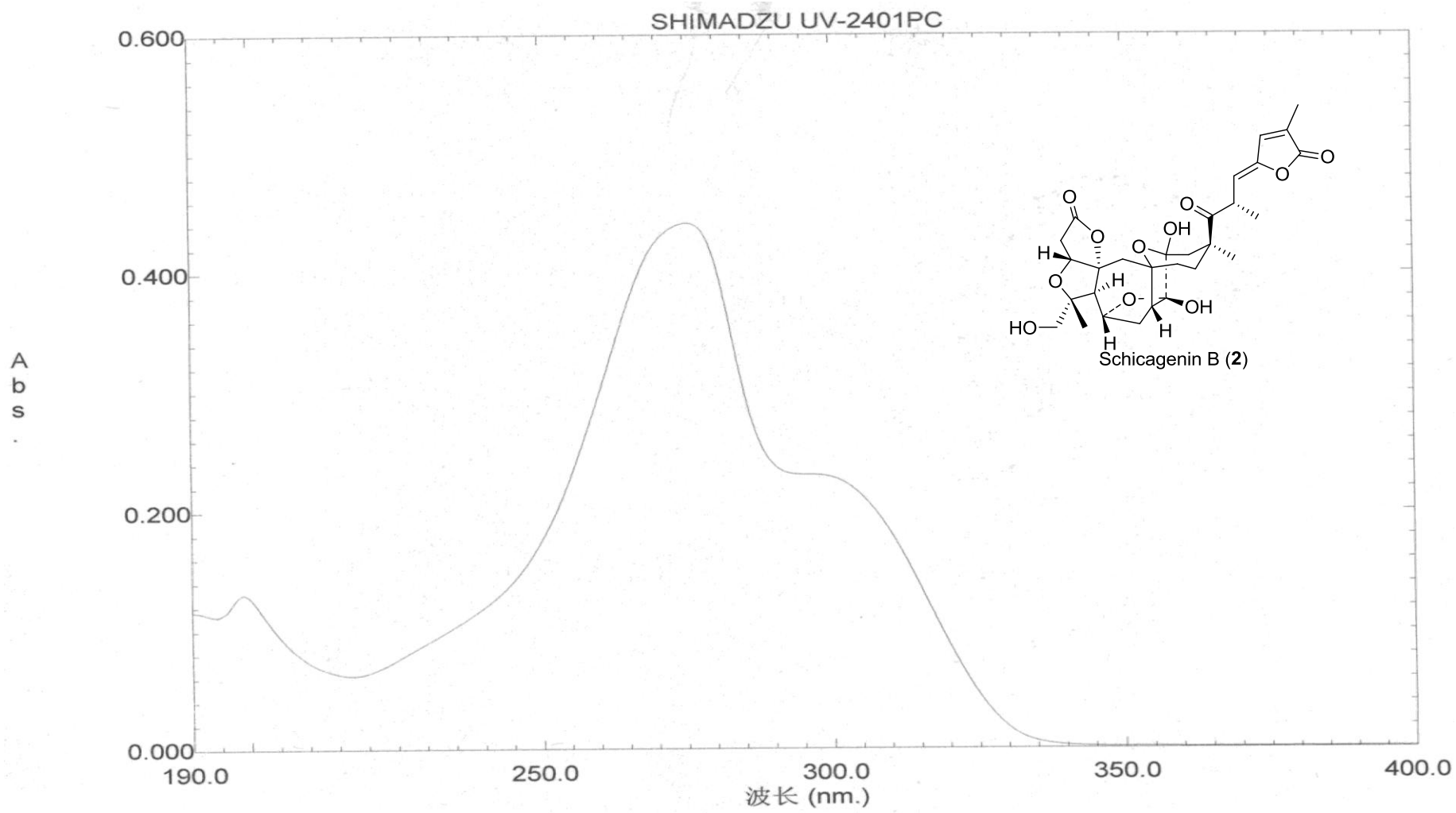

Figure S19. UV spectrum of schicagenin B (**2**)

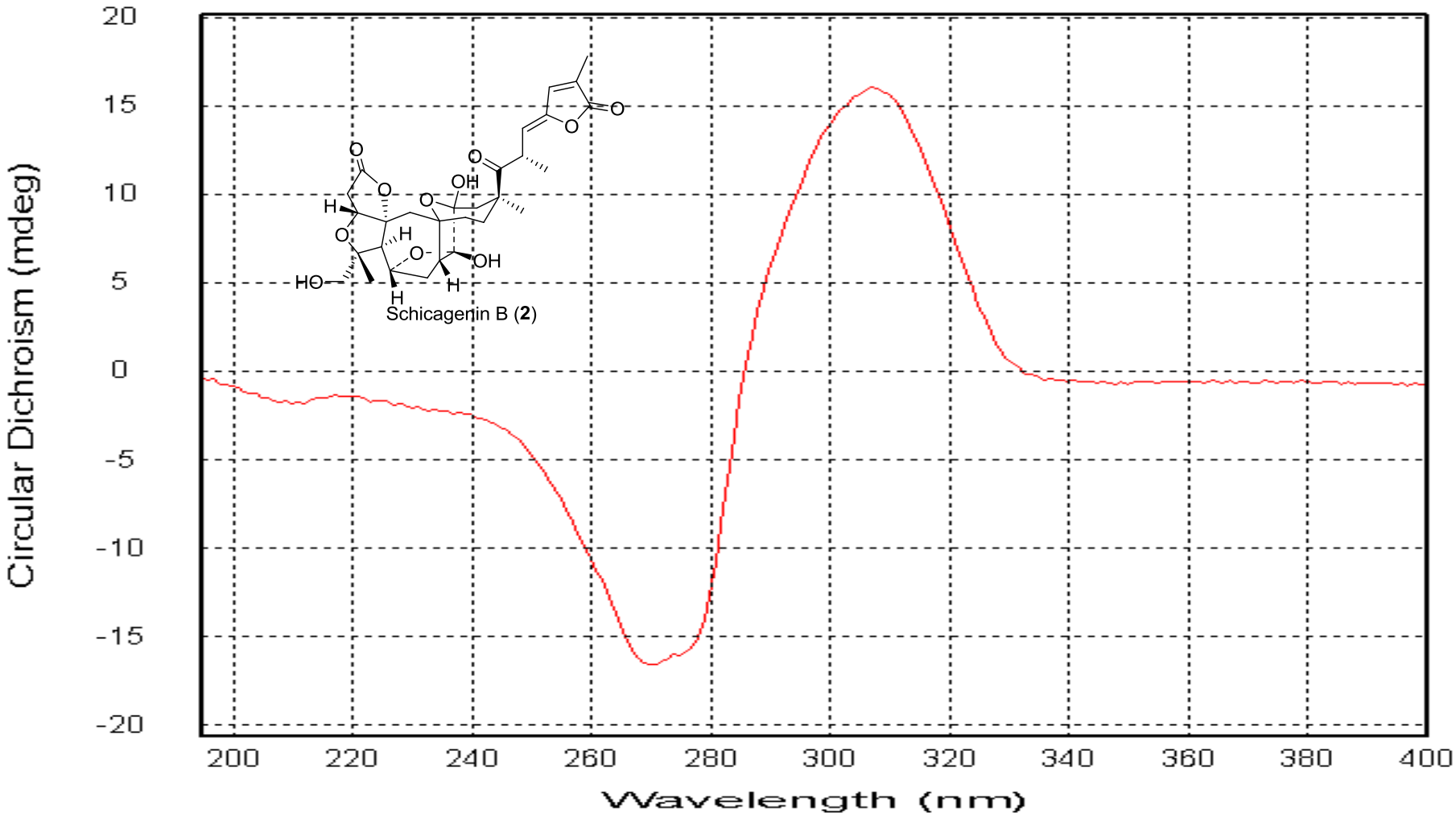

Figure S20. CD spectrum of schicagenin B (**2**)

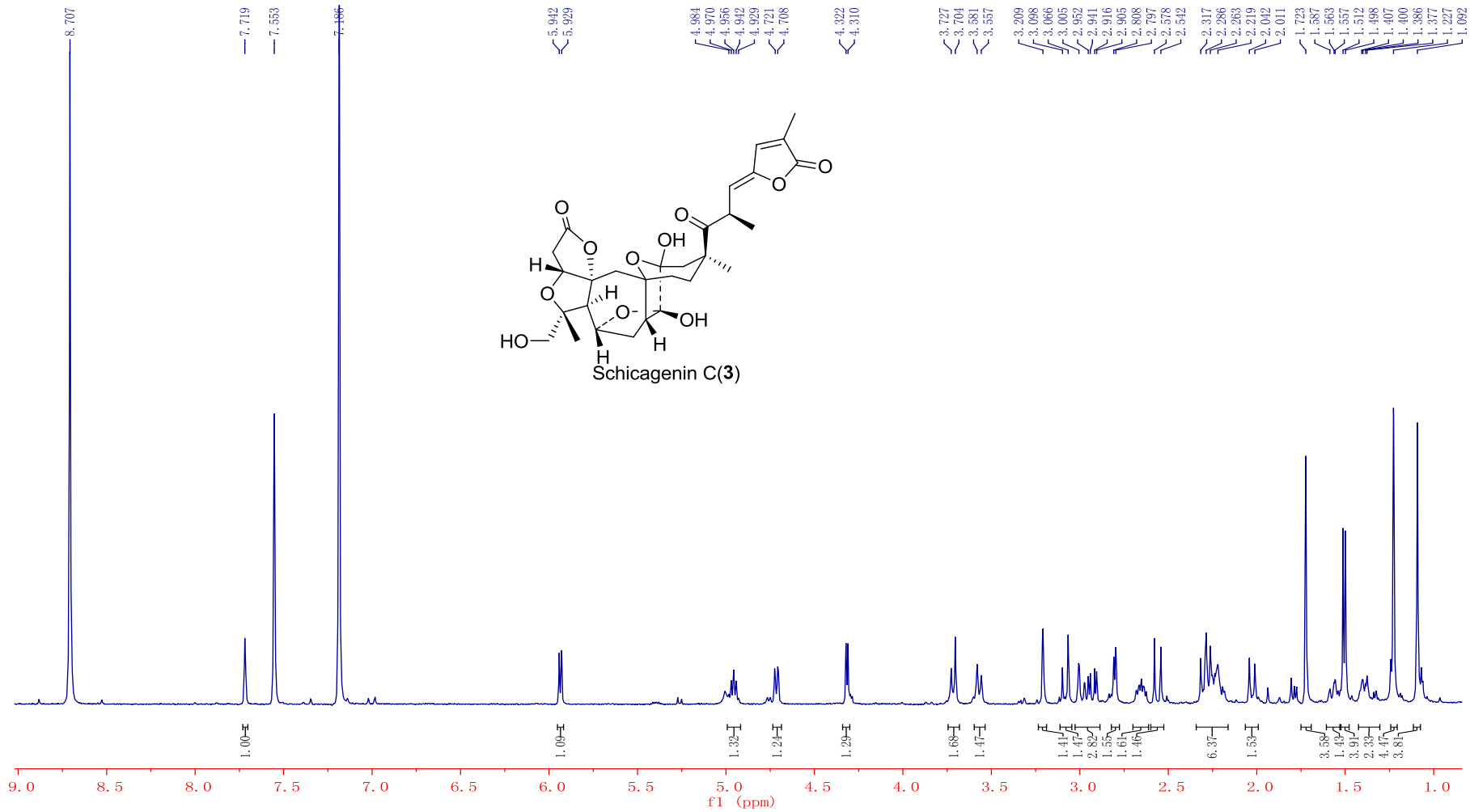

Figure S21. ^{1}H NMR spectrum of schicagenin C (**3**)

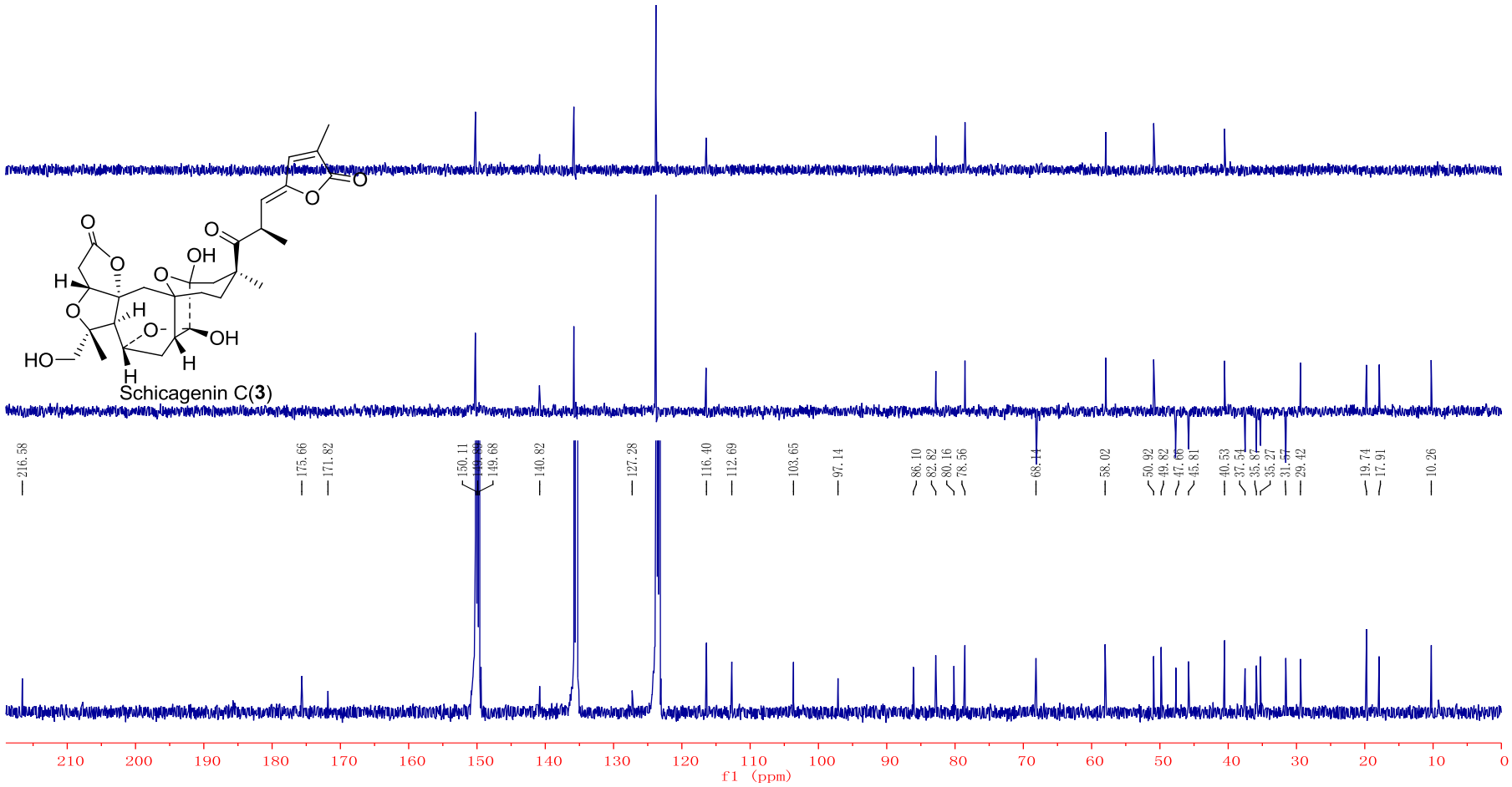

Figure S22. ^{13}C NMR spectrum of schicagenin C (**3**)

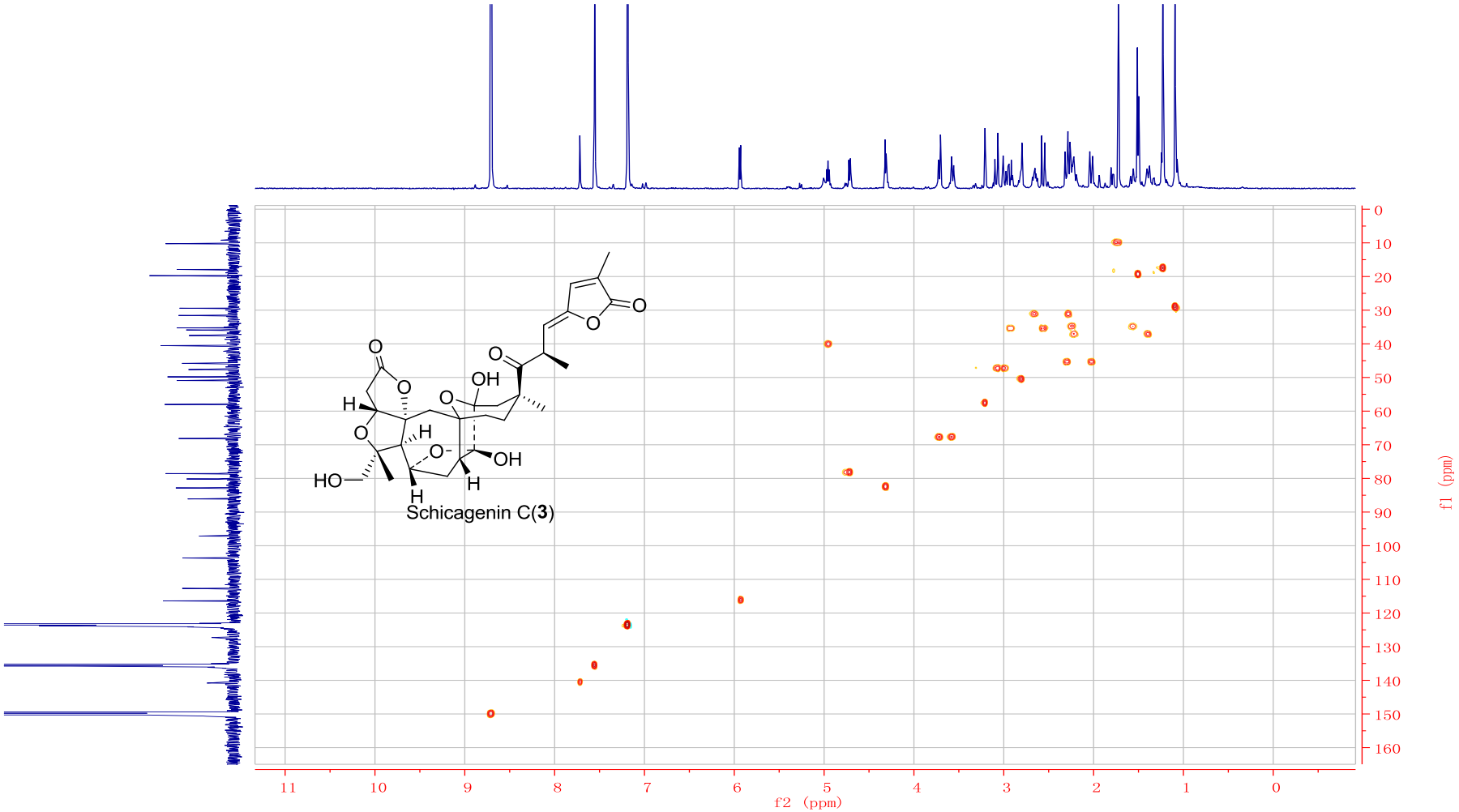

Figure S23. HSQC spectrum of schicagenin C (**3**)

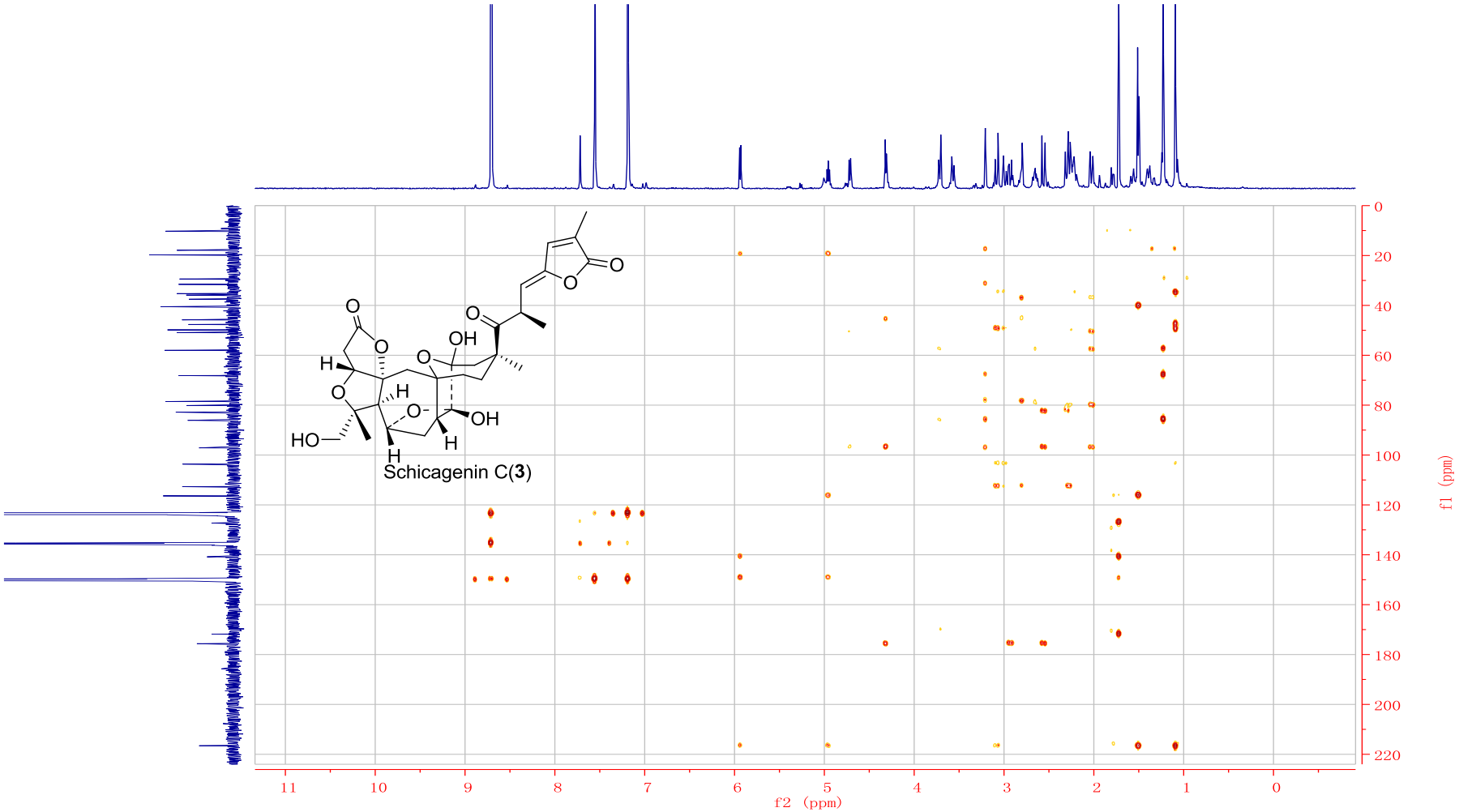

Figure S24. HMBC spectrum of schicagenin C (**3**)

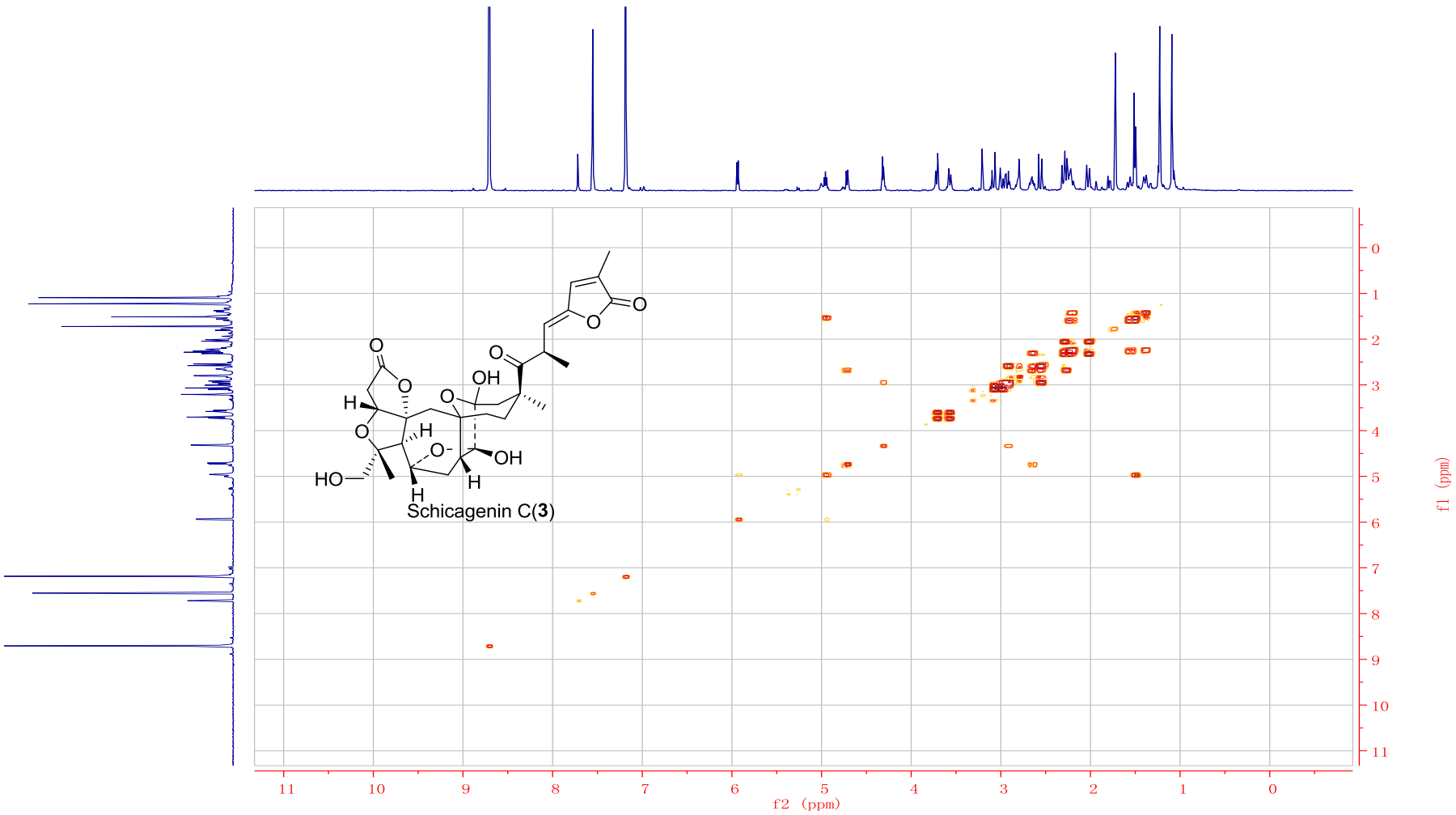

Figure S25. ^{1}H–^{1}H COSY spectrum of schicagenin C (**3**)

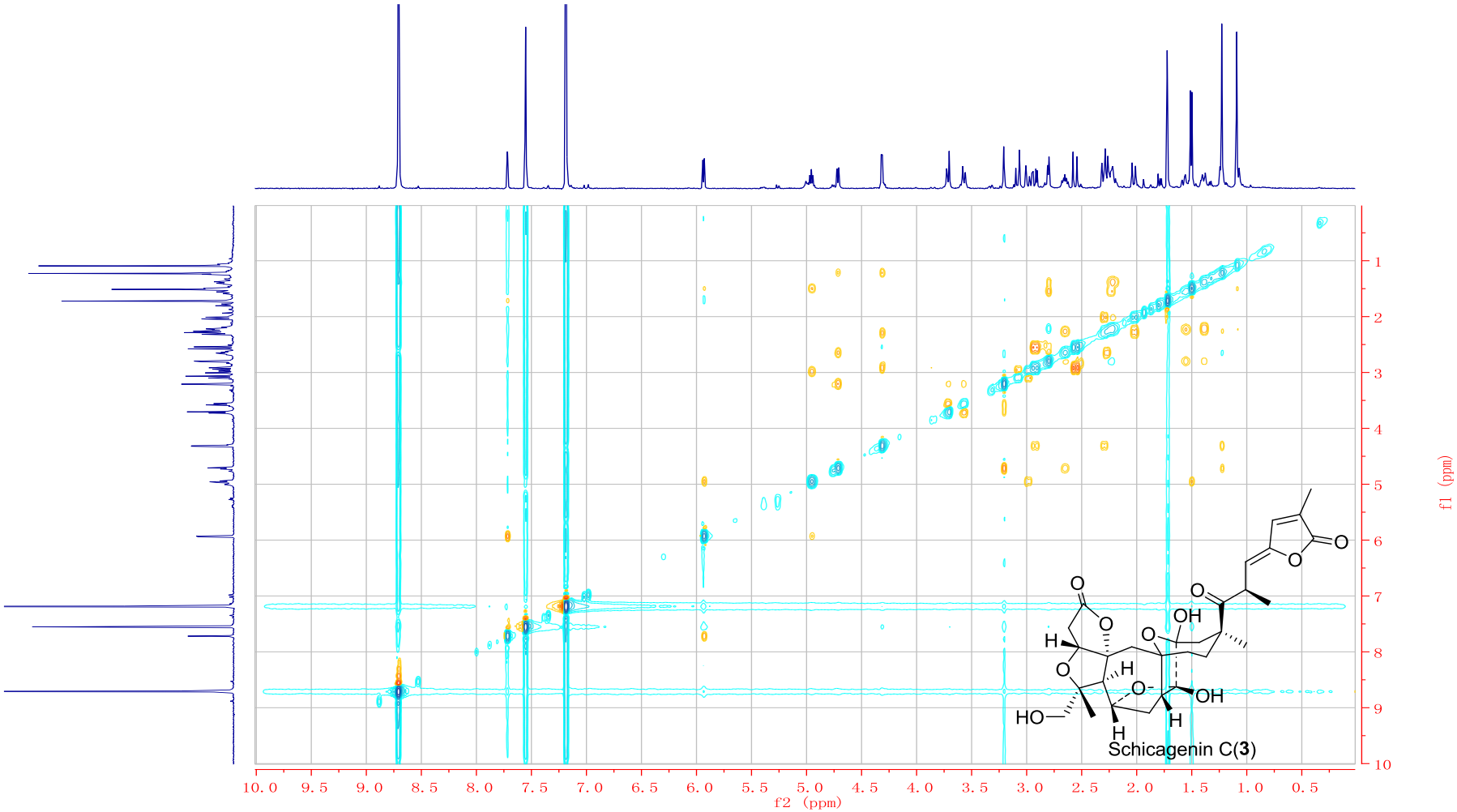

Figure S26. ROESY spectrum of schicagenin C (**3**)

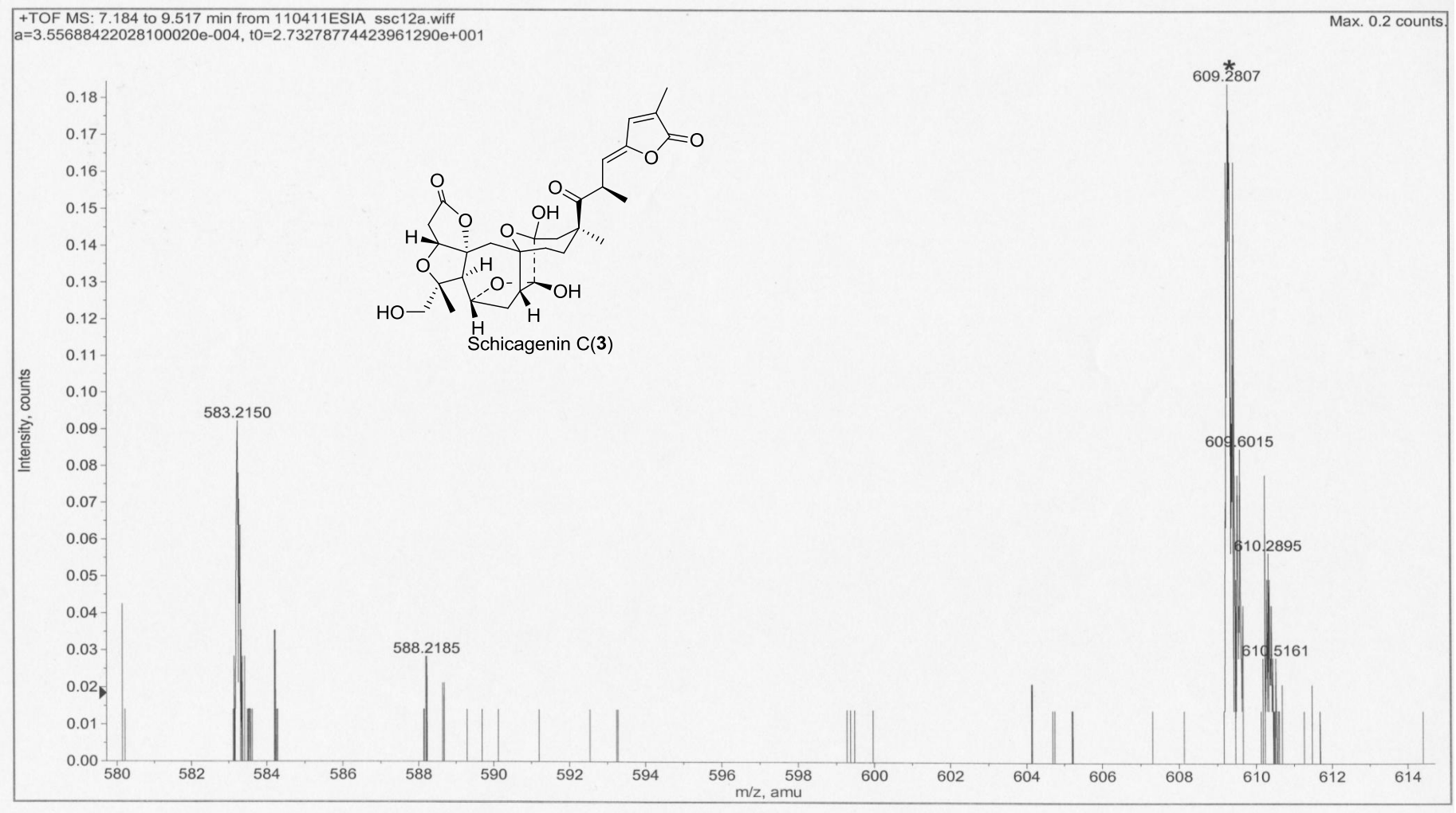

Figure S27. HRESIMS spectrum of schicagenin C (**3**)

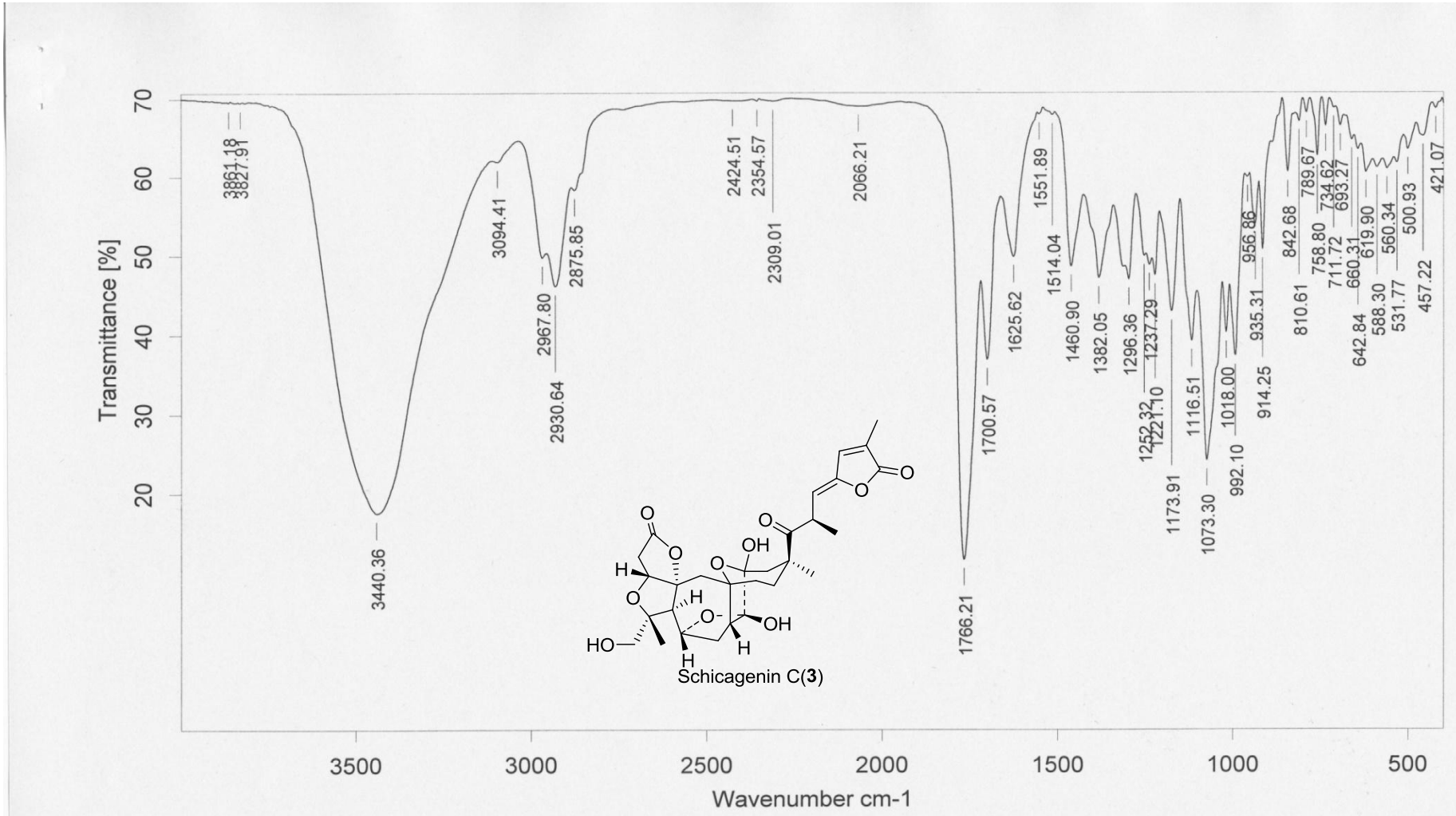

Figure S28. IR spectrum of schicagenin C (**3**)

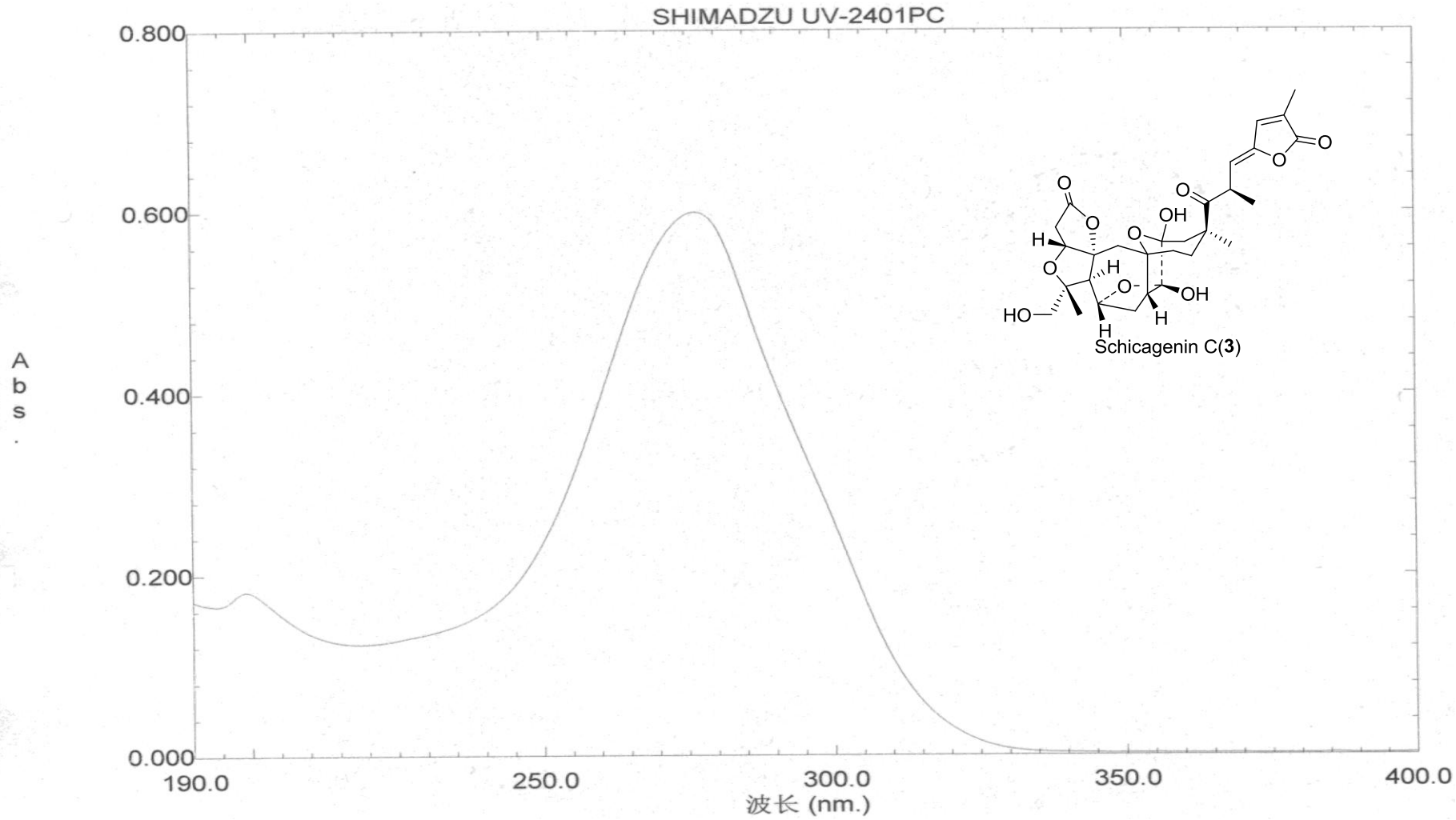

Figure S29. UV spectrum of schicagenin C (**3**)

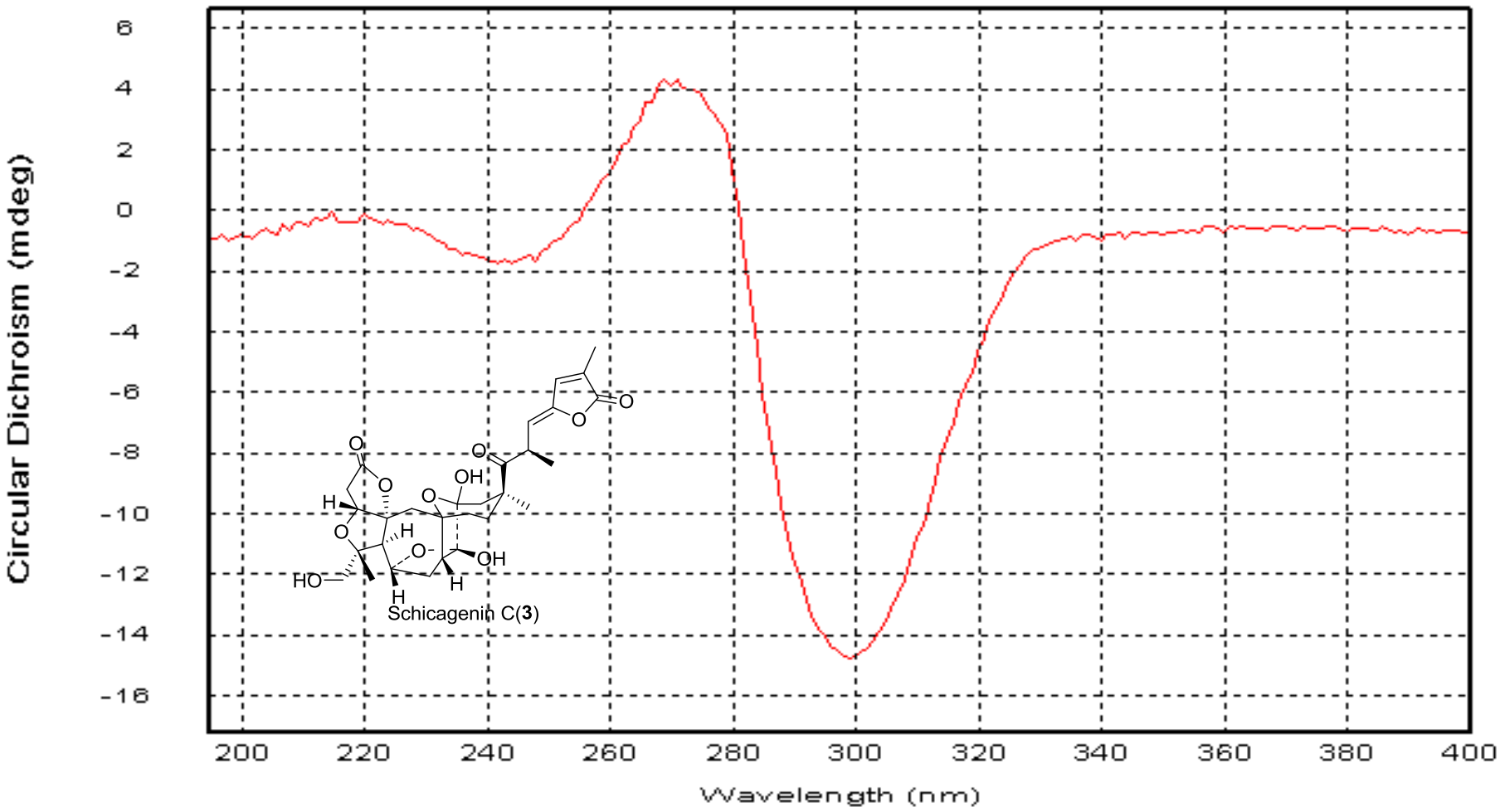

Figure S30. CD spectrum of schicagenin C (**3**)

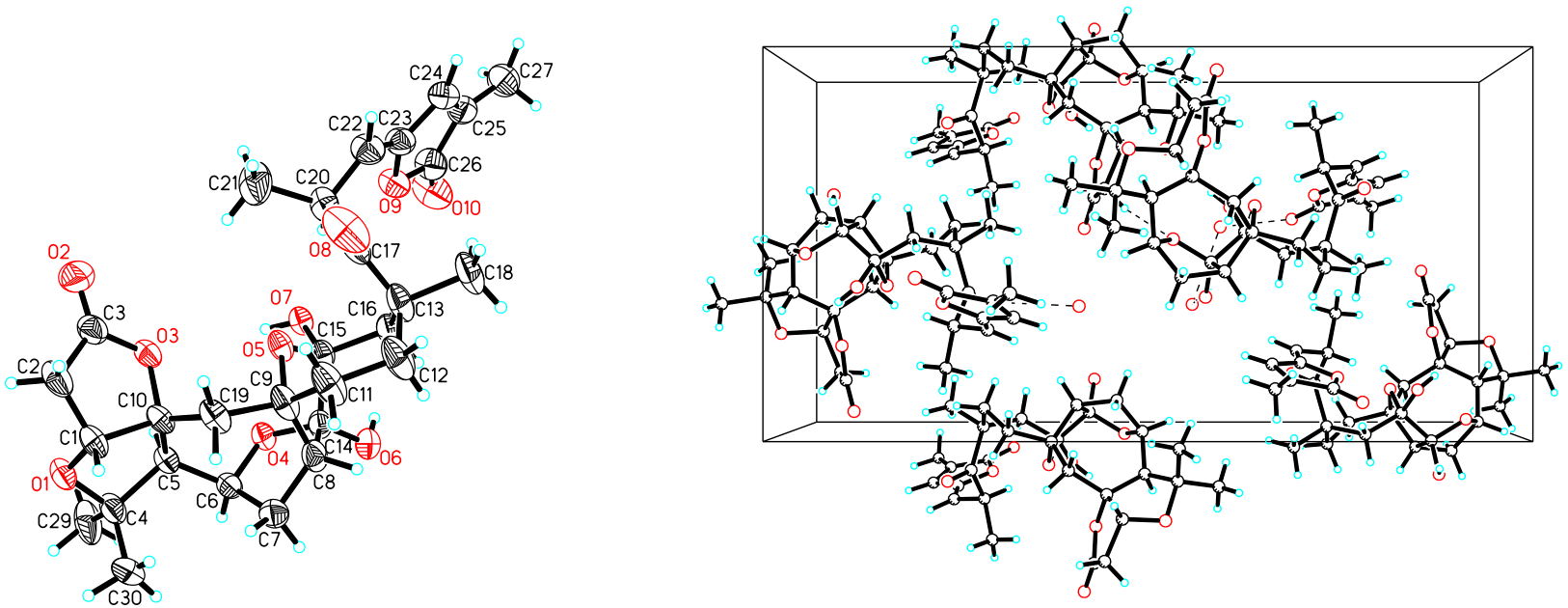

Figure S31. X-ray crystal structrue of schicagenin A (**1**)