Electronic Supplementary Material (ESI) for RSC Advances.

Streptospirodienoic Acids A and B, 6,6-Spiroketal Polyketides from *Streptomyces* sp.

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List of Supporting Information

1 General Experimental Procedure.

Optical rotations were measured on a JASCO P-1020 digital polarimeter at room temperature (JASCO Inc., Japan). UV spectra were recorded in MeOH using a JASCO V-550 UV/VIS spectrophotometer (JASCO Inc., Japan). IR spectra were recorded on a JASCO FT/IR-480 plus Fourier transform infrared spectrometer (JASCO Inc., Japan). NMR spectra were recorded on a Bruker AV 400 spectrometer at 300 K (Bruker, Switzerland). The chemical shifts were given in δ with the solvent signals (CD_3OD: δ_H 3.31/δ_C 49.2) as an internal standard. HR-ESI-TOF-MS was recorded on a Micromass Q-TOF mass spectrometer (Waters, America). TLC analysis was carried out on pre-coated silica gel GF254 plates (Qingdao Haiyang Chemical Group Corp, China). HPLC was performed on a SHIMADZU LC-6AD LC-6AD Liquid Chromatography with SPD-20A Detector, using an ODS column [YMC-Pack ODS-A (ϕ 5 μm, 10.0 × 250 mm)] at 220 nm. Column chromatography was carried out on ODS (60-80 mesh; YMC Ltd., Japan). All solvents used in column chromatography were of analytical grade (Tianjin Damao Chemical Plant, Tianjin, China).

2 Strain and Fermentation

The strain of *Streptomyces* sp. (No. 0950134) was isolated from rhizosphere soil of *Hibiscus tilisaceus* in year 2004 from the mangrove soil (Wenchang, Hainan, China).

The strain was grown on YE plates (yeast extract, 4 g L^{-1}; glucose, 4 g L^{-1}; maltose, 10 g L^{-1}; sea salt, 18 g L^{-1}; agar, 20 g L^{-1} pH 7.4 with 0.5 M NaOH) for 7 days at 28 °C. For metabolites production, the strain was scraped from the t agar plate and inoculated into a 500 mL Erlenmeyer flask containing 100 mL of seed medium FM2 (yeast extract, 2 g L^{-1}; glucose, 10 g L^{-1}; soluble starch, 30 g L^{-1}; casein hydrolysate, 4 g L^{-1}; sea salt, 18 g L^{-1}; MOPS, 50 g L^{-1}; K_2SO_4, 8 g L^{-1}; NaCl, 1 g L^{-1}; pH 7.0 with 0.5 M NaOH), which was incubated at 28 °C in an orbital shaker set at 220 r.p.m. After 3 days, 50 mL of the culture was transferred into 1L Erlenmeyer flask each containing 200 mL of the FM2 media and incubated at 28 °C for 7 days on a rotary shaker at 180 r.p.m.

3 Extraction and Isolation.

Total of 40 liters of fermentation broth was centrifuged at 3600 rpm for 20 min. The supernatant was adsorbed with macroporous resin Diaion HP 20 and was eluted with acetone. The eluent was extracted repeatedly with EtOAc, and the organic solvent was evaporated under reduced pressure to afford the crude extract (3.2 g). The extract was subjected to ODS medium pressure liquid chromatography (MPLC) eluted with a gradient of MeOH in H_2O (35%, 55%, 75%, 100%) to afford seven subfractions (F1~F7). F4 (0.20 g) was purified by reversed-phase HPLC on an YMC-Pack ODS-A column (5 μm, 10.0 × 250 mm, 4.0 mL min^{-1}) with MeOH and H_2O (70: 30, v/v, 0.1% HCOOH) as the eluent to yield compounds **1** (15.0 mg, t_R = 8.4 min), **2** (11.6 mg, t_R = 13.2 min), and **3** (2.6 mg, t_R = 17.9 min).

4 Spectroscopic data of 1-3.

***Streptospirodienoic acid A* (1):** colorless crystals (MeOH); $[\alpha]_D^{25}$ + 40.5 (*c* 0.6, MeOH); UV (MeOH) λ_{max} nm (logε): 260 (3.2); IR (KBr) ν_{max}: 3459 (-OH), 2945 (-CH_2-), 1686 (-CO-), 1457, 861 cm^{-1}; ^1H (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; HR-ESI-MS *m/z* 449.2514 $[M + Na]^+$ (calcd for $C_{23}H_{38}O_7$ Na, 449.2515).

***Streptospirodienoic acid B* (2)**: white amorphous powder; $[\alpha]_D^{25}$ + 21.8 (*c* 0.6, MeOH); UV (MeOH) λ_{max} nm (logε): 260 (3.5); IR (KBr) ν_{max}: 3423 (-OH), 2968 (-CH_2-), 1678 (-CO-), 1457, 985 cm^{-1}; ^1H (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 2; HR-ESI-MS *m/z* 449.2511 $[M + Na]^+$ (calcd for $C_{23}H_{38}O_7$ Na, 449.2515).

***Streptospirodienoate A* (3)**: white amorphous powder; $[\alpha]_D^{25}$ + 37.1 (*c* 0.6, MeOH); UV (MeOH) λ_{max} nm (logε): 260 (3.4); IR (KBr) ν_{max}: 3423 (-OH), 2968 (-CH_2-), 1689 (-CO-) , 1457, 983 cm^{-1}; ^1H (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; HR-ESI-MS (ESI) *m/z* 463.2664 $[M + Na]^+$ (calcd for $C_{24}H_{40}O_7$ Na，463.2672).

5 X-ray Crystallographic Analysis of 1.

Upon crystallization from MeOH using the vapor diffusion method, needles of **1** were obtained. Colorless blocks, $C_{23}H_{38}O_{7.50}$, monoclinic, $P2_1$, a = 9.9993(2), b = 15.1269(3), c = 9.5353(2) Å, β = 96.722(2), V = 1243.06(5) Å^3, Z = 2, d_x = 1.161 Mg/m^3, F (000) = 472. Data collection was performed on a SMART CCD using graphite monochromated radiation (λ = 1.54184 Å) under low temperature (nitrogen gas); 2869 unique reflections were collected to θ_{max} = 62.65, in which 3870 reflections were observed [$F^2 > 4\sigma(F^2)$]. The structures were solved by direct methods (SHELXTL version 5.1) and refined by full-matrix least-squares on F^2. In the structure refinements, non-hydrogen atoms were refined anisotropically. Hydrogen atoms bonded to carbons were placed on the geometrically ideal positions by the 'ride on' method. Hydrogen atoms bonded to oxygen were located by the difference Fourier method and were included in the calculation of structure factors with isotropic temperature factors. The final R = 0.0341, Rw = 0.0905 and S = 1.031. Crystallographic data of **1**: CCDC 1007092 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallo-graphic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

6 MTT Assay

Cells were counted by a hemocytometer and equally distributed in 96-well plates (2.0 × 10^3 cells per well) and treated with compounds 1-3 for 72 h, To determine cell viability, the medium was removed and cells were incubated with 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) at a final concentration of 0.5 mg/mL in RPMI 1640 medium containing 10% FBS for 3 h in the dark at 37 °C. Then 100 μL DMSO was added to the wells. Cultures were incubated at room temperature (RT) for 15 min, read at 550 nm.

7 The 1D and 2D NMR spectra of *Streptospirodienoic acid A* (1)

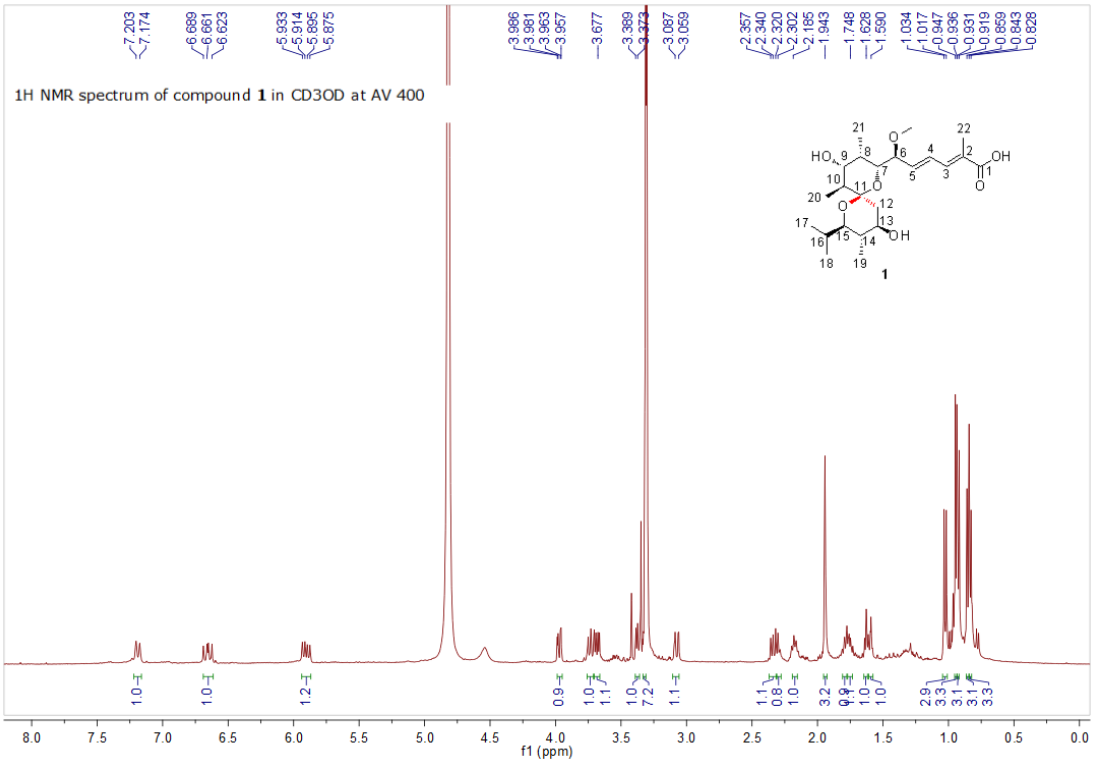

Figure 1. The ^{1}H NMR spectrum of **1** in CD_3OD

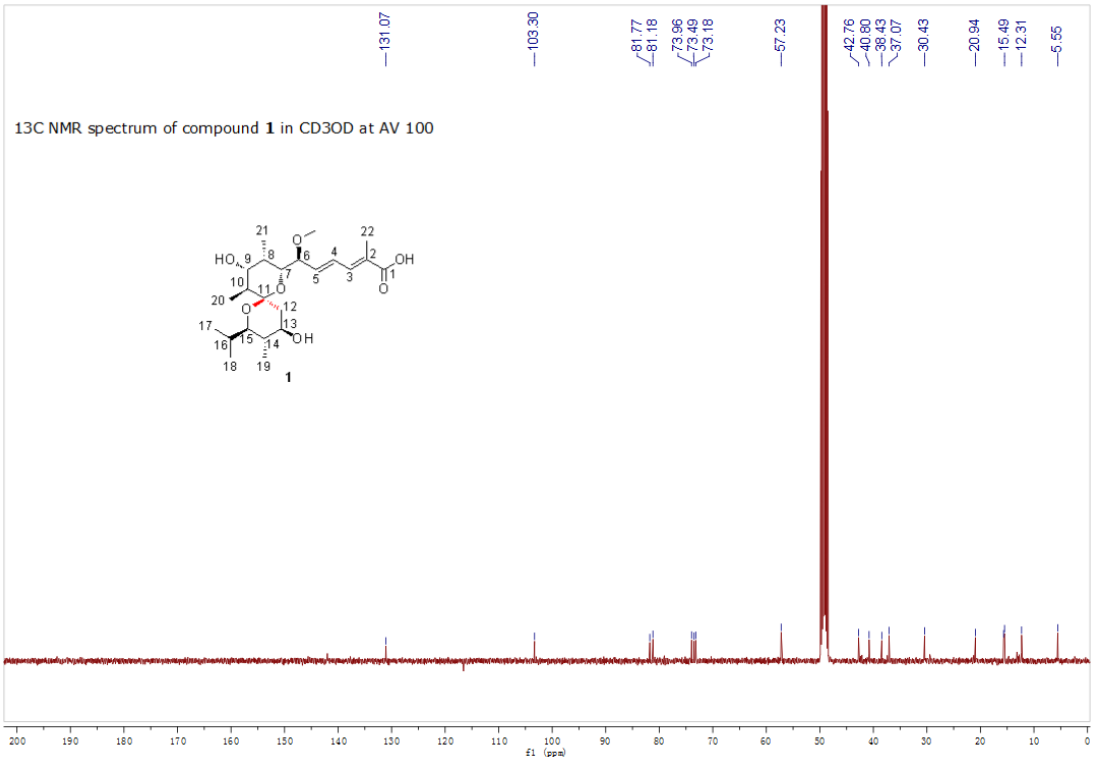

Figure 2. The ^{13}C NMR spectrum of **1** in CD_3OD

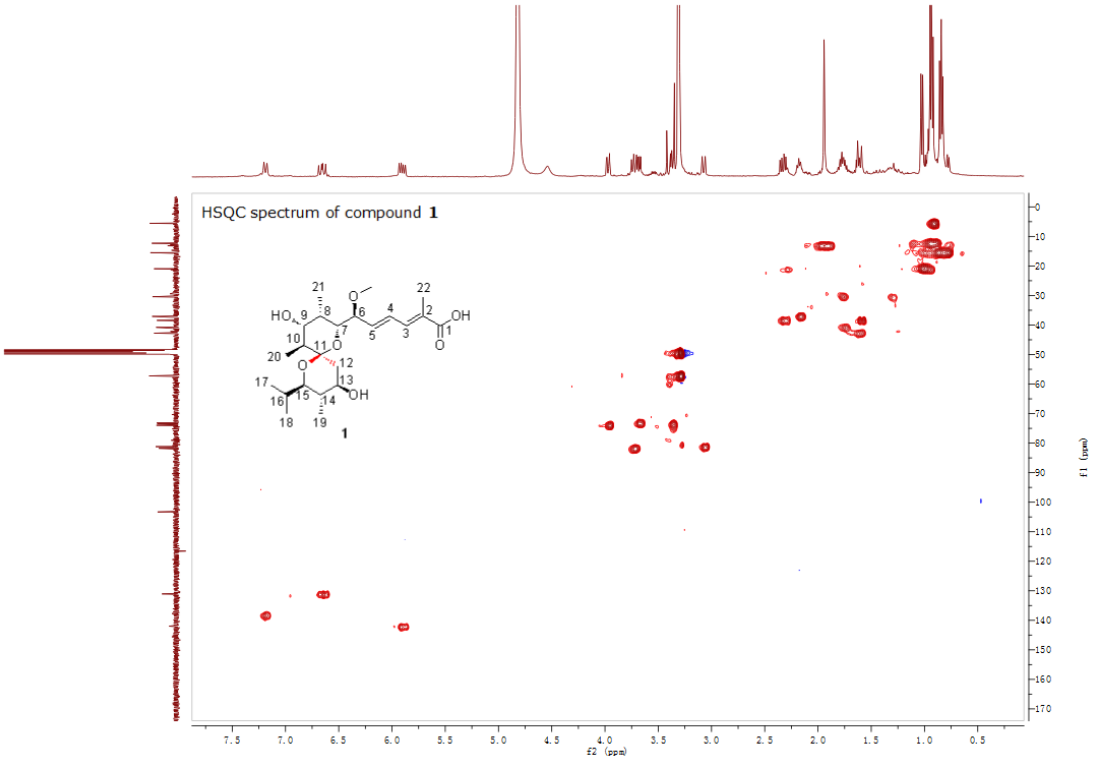

Figure 3. The HSQC spectrum of **1** in CD_3OD

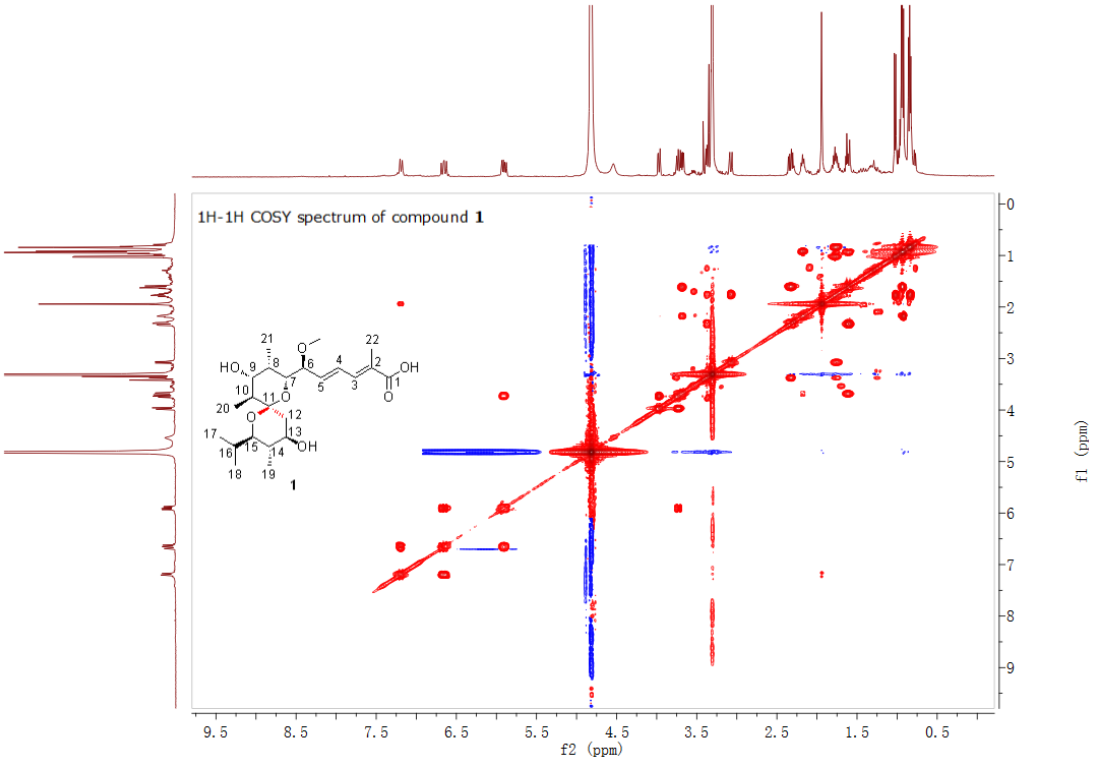

Figure 4. The ^{1}H-^{1}H COSY spectrum of **1** in CD_3OD

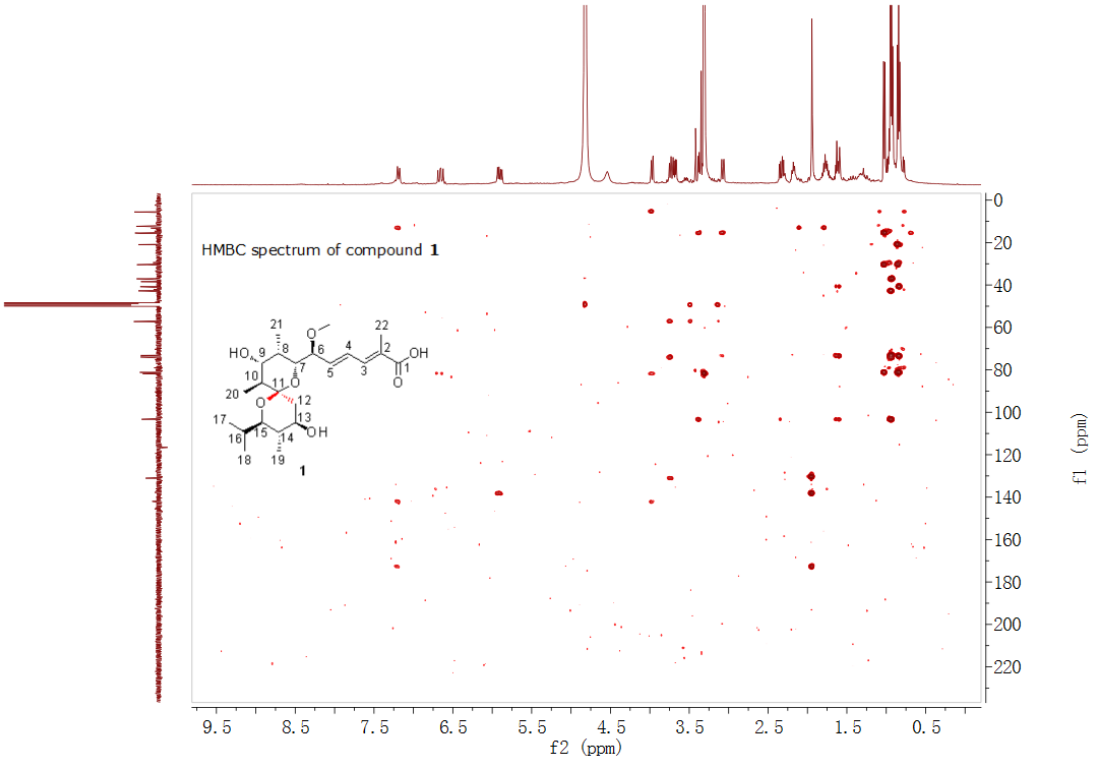

Figure 5. The HMBC spectrum of **1** in CD_3OD

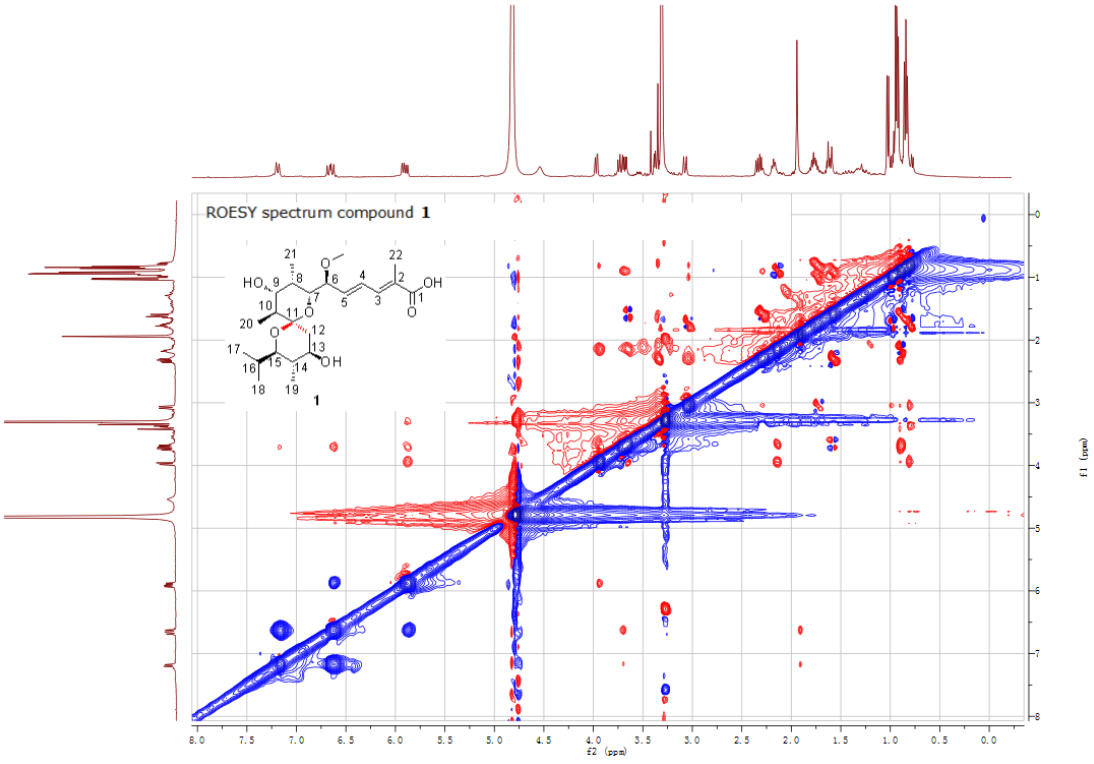

Figure 6. The ROESY spectrum of **1** in CD_3OD

8 The 1D and 2D NMR spectra of *Streptospirodienoic acid B* (2)

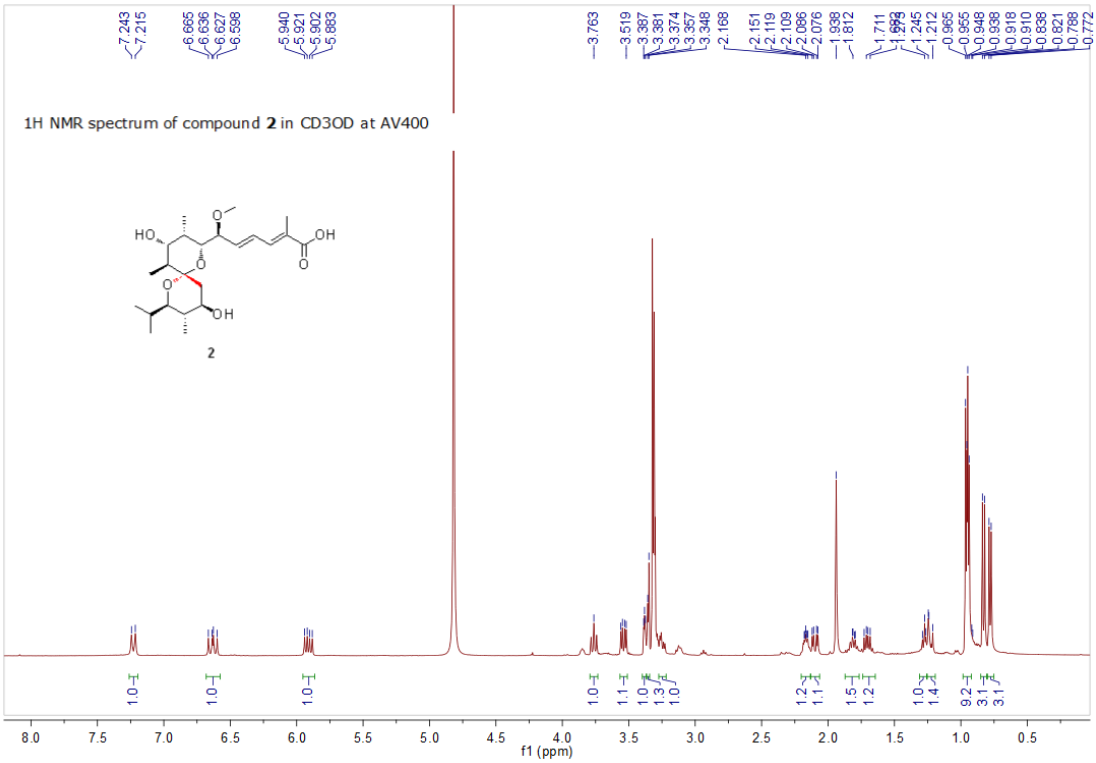

Figure 1. The ^{1}H NMR spectrum of **2** in CD_3OD

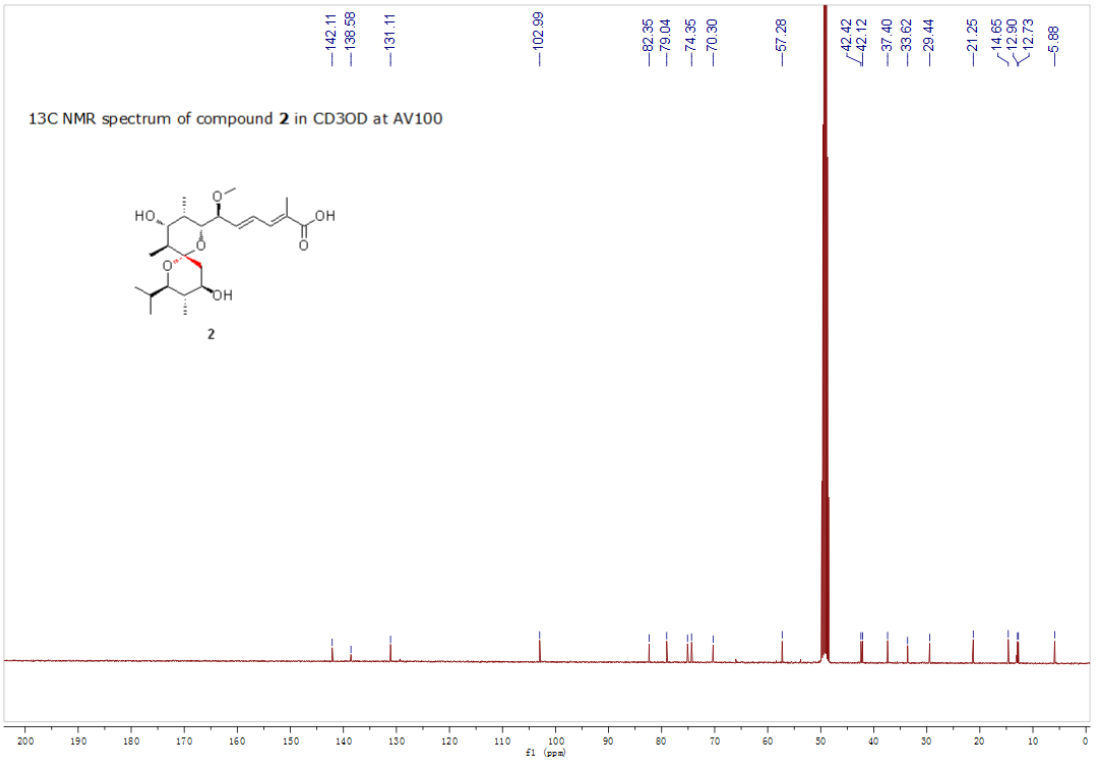

Figure 2. The ^{13}C NMR spectrum of **2** in CD_3OD

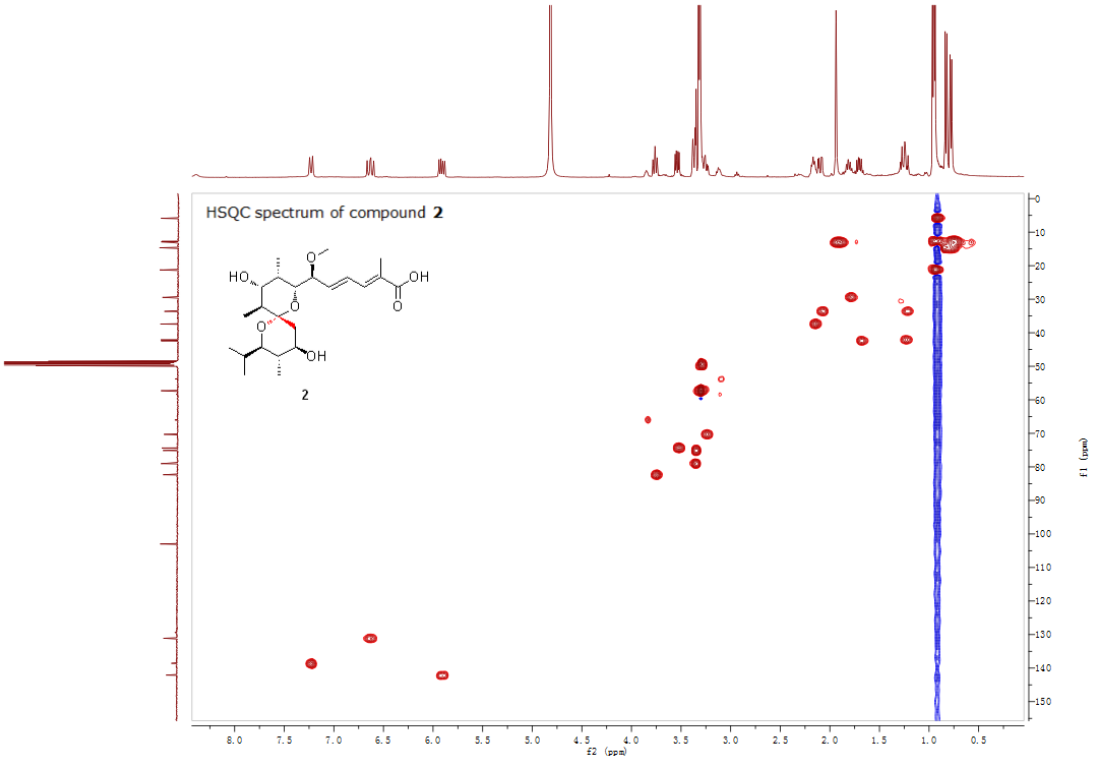

Figure 3. The HSQC spectrum of **2** in CD_3OD

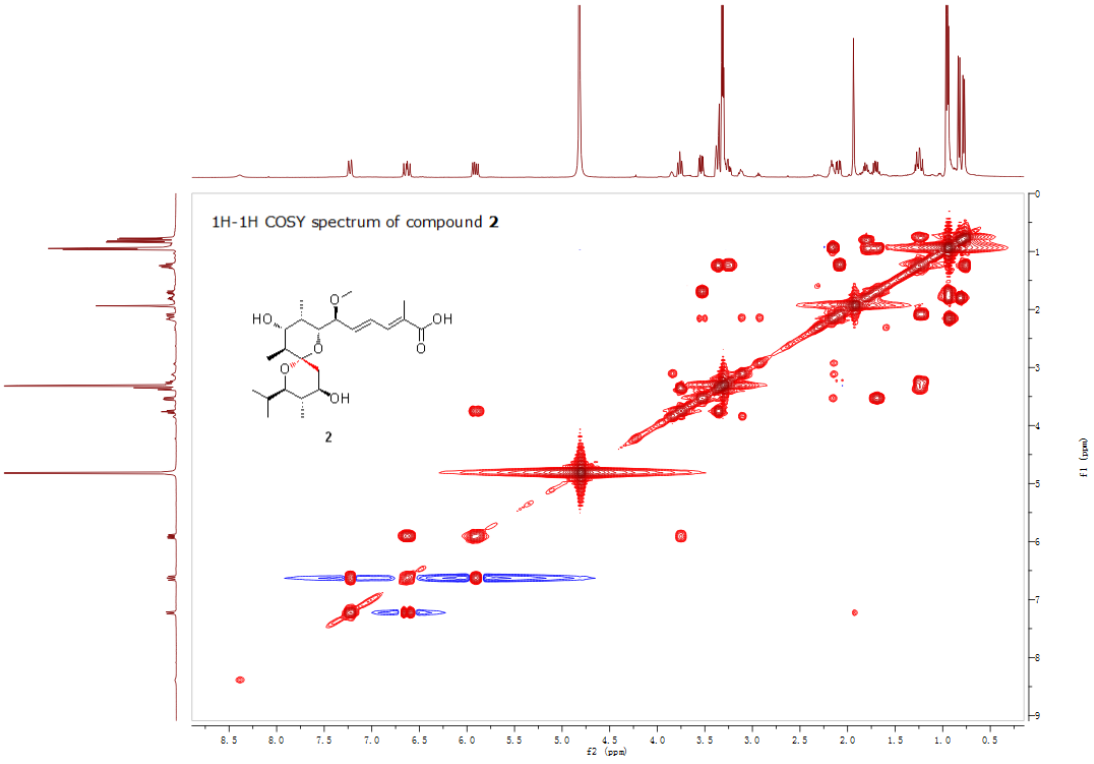

Figure 4. The ^1H-^1H COSY spectrum of **2** in CD_3OD

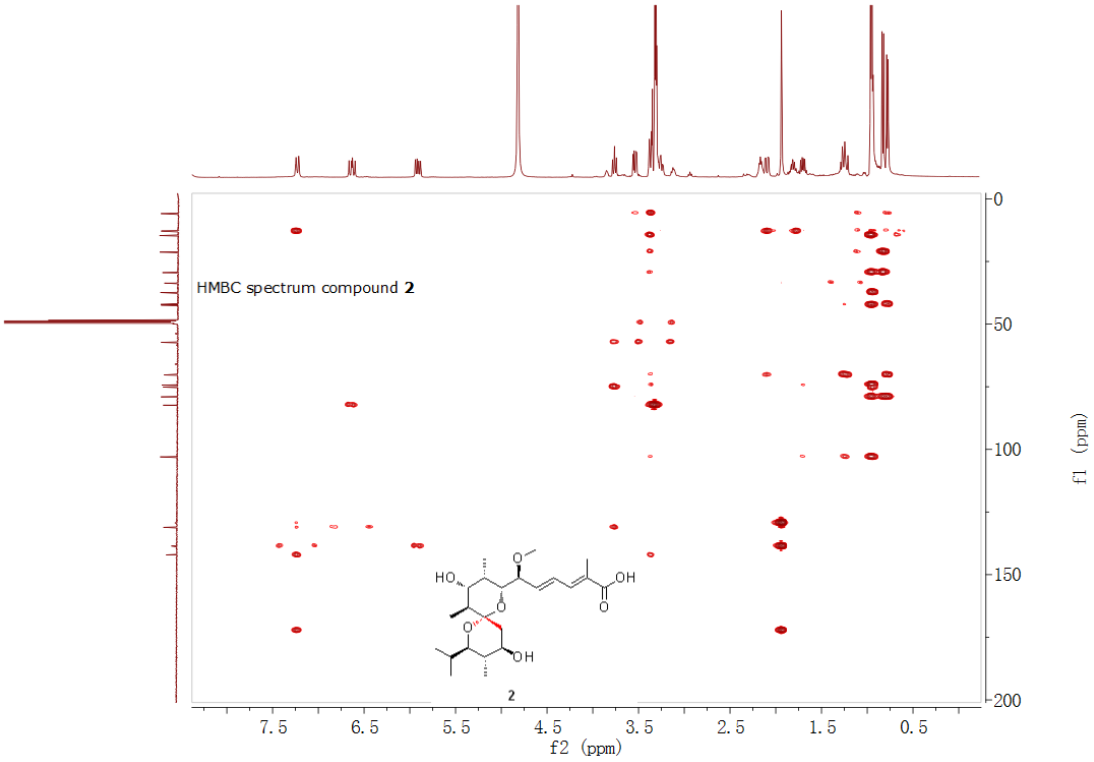

Figure 5. The HMBC spectrum of **2** in CD_3OD

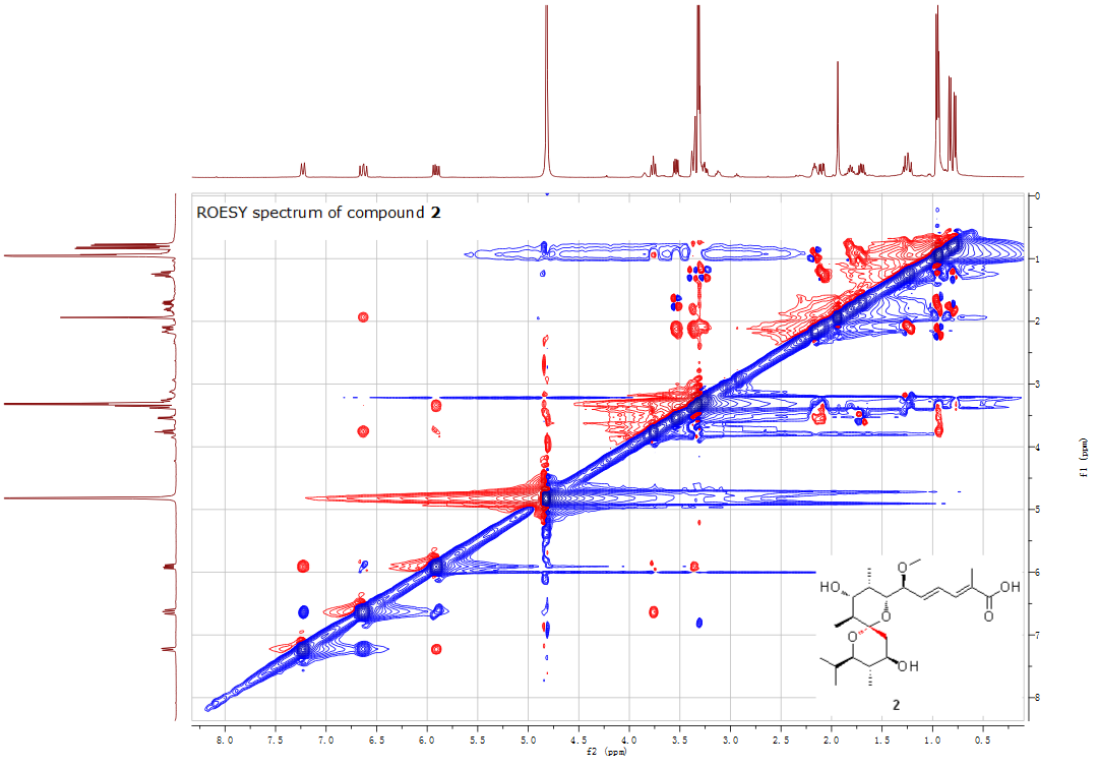

Figure 6. The ROESY spectrum of **2** in CD_3OD

9 The 1D and 2D NMR spectra of *Streptospirodienoate A* (3)

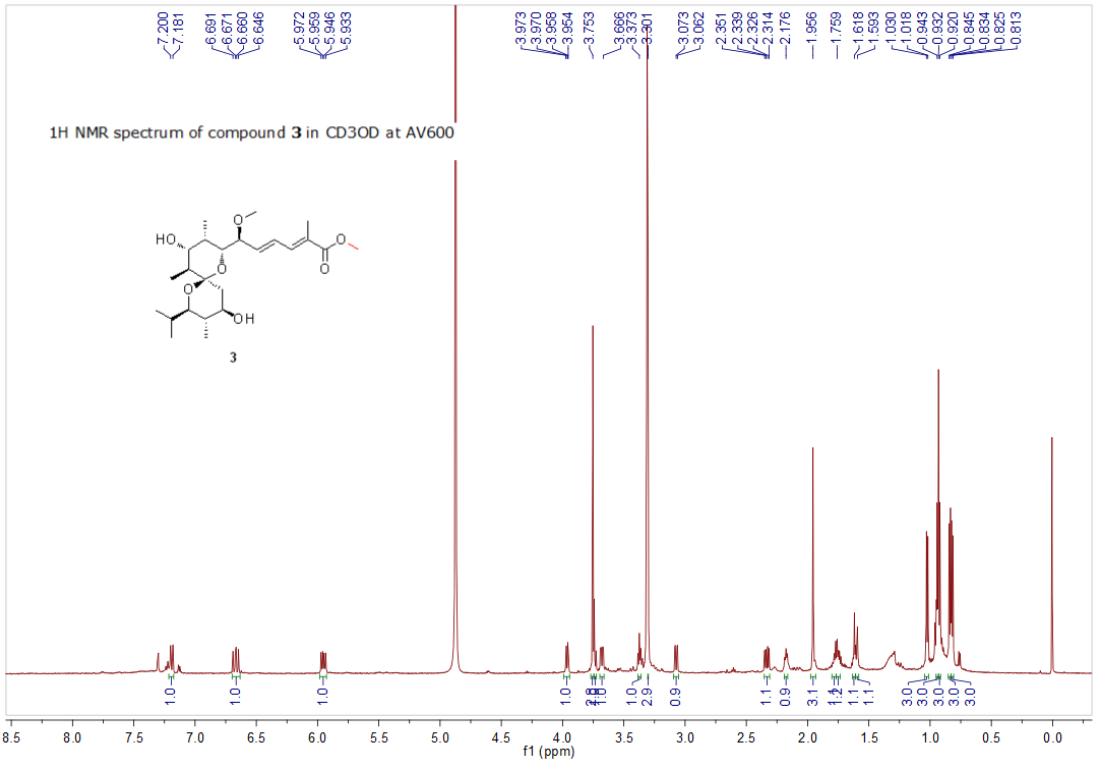

Figure 1. The ^{1}H NMR spectrum of **3** in CD_3OD

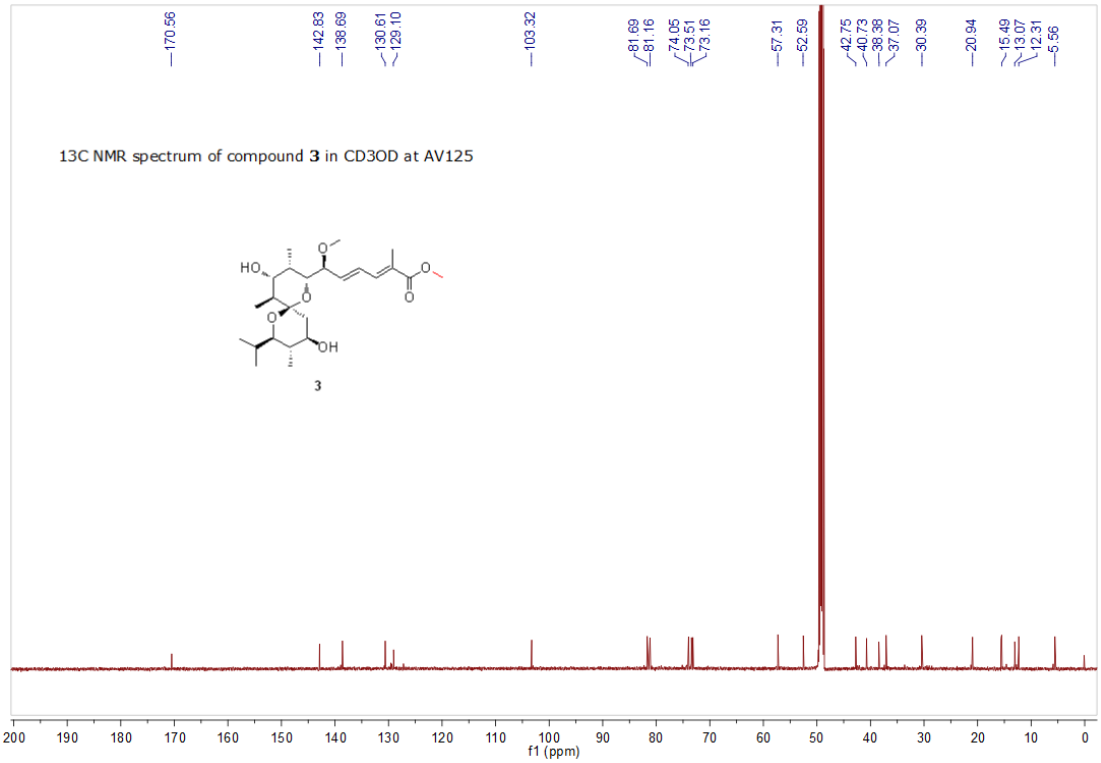

Figure 2. The ^{13}C NMR spectrum of **3** in CD_3OD

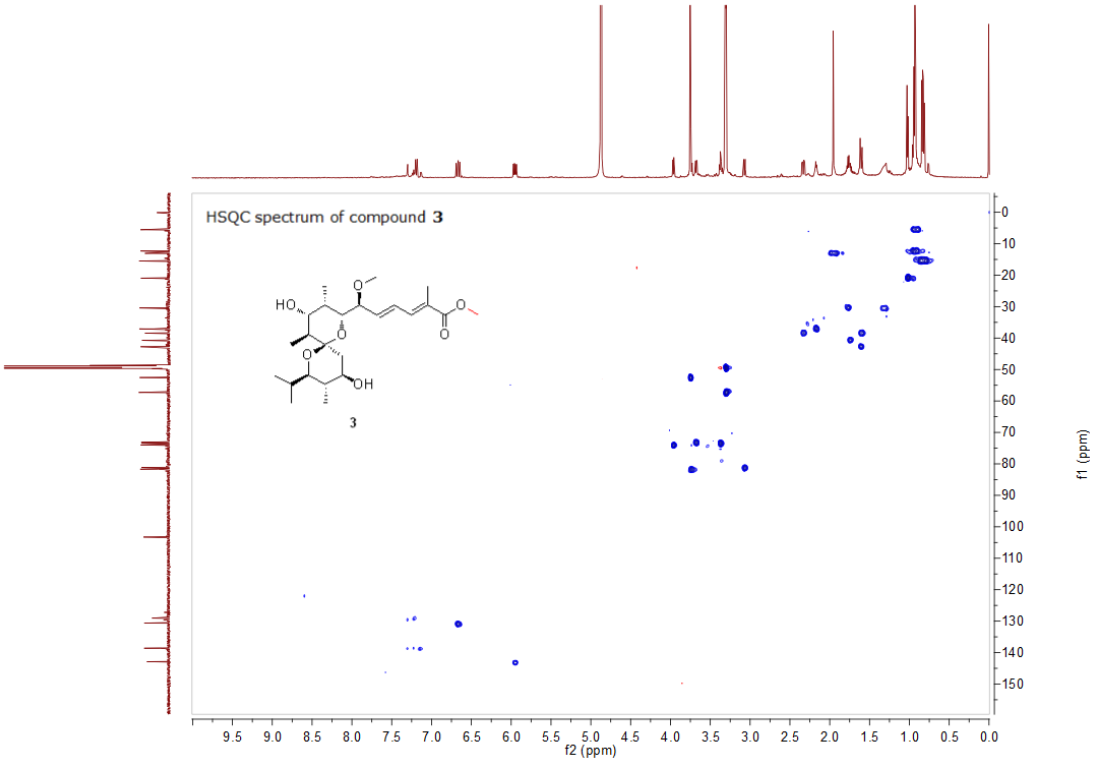

Figure 3. The HSQC spectrum of **3** in CD_3OD

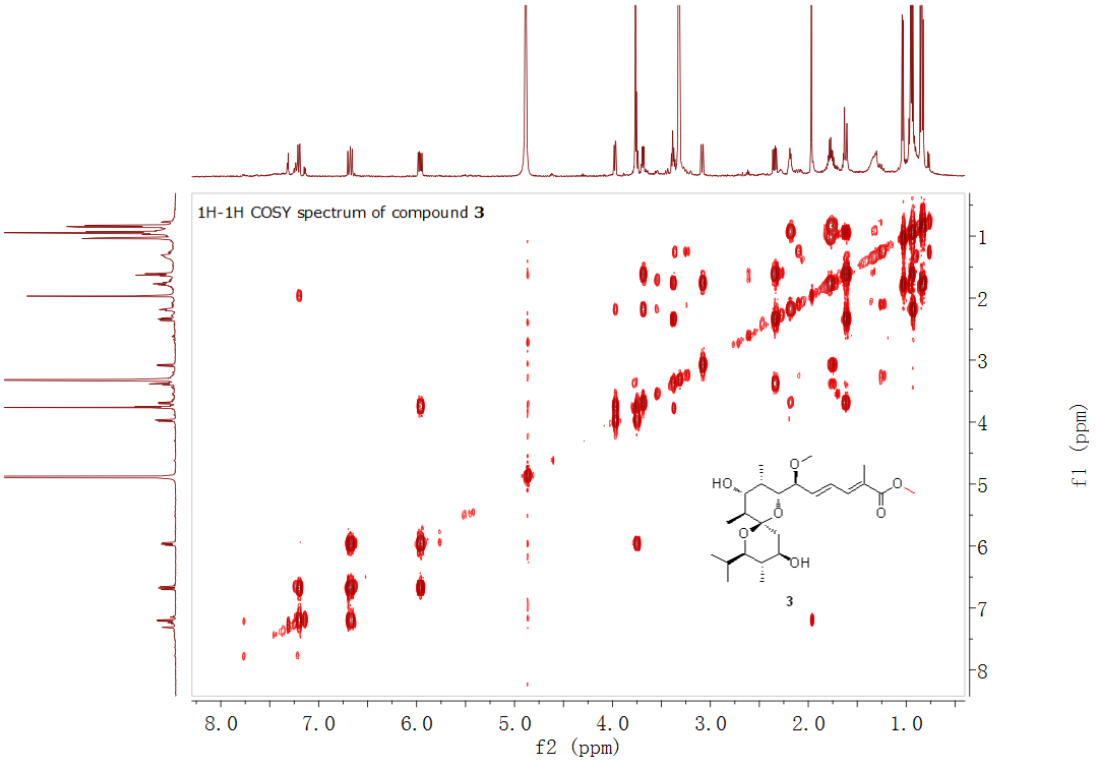

Figure 4. The ^1H-^1H COSY spectrum of **3** in CD_3OD

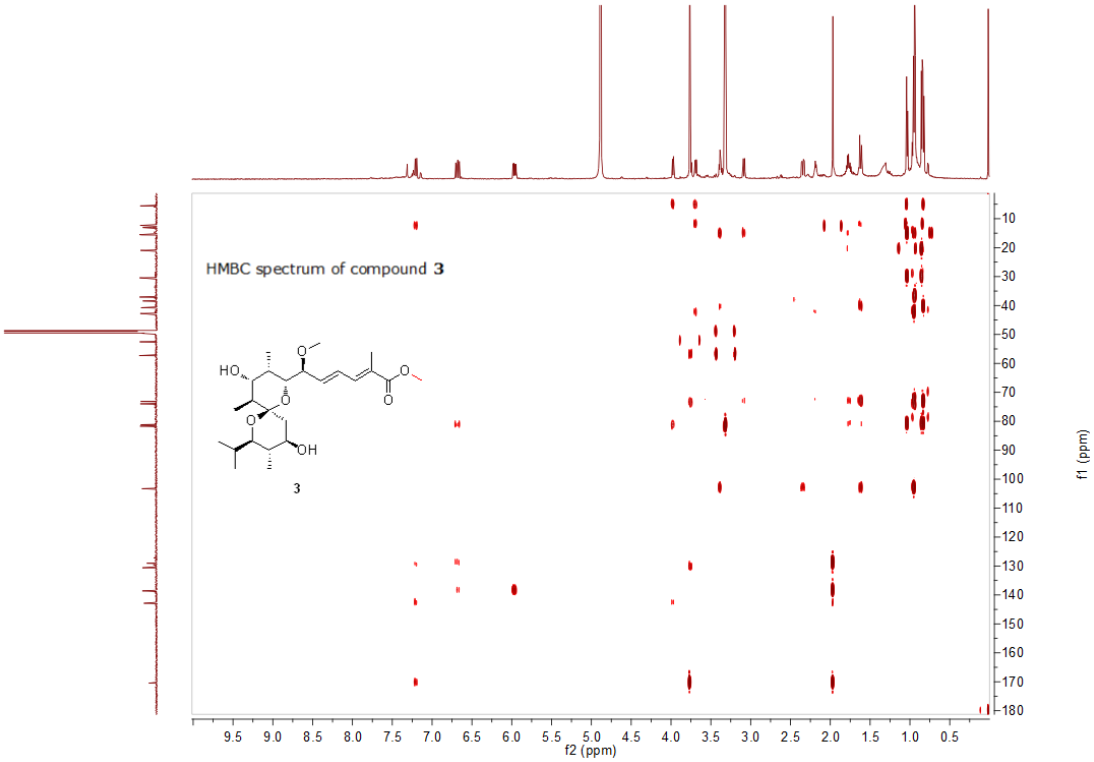

Figure 5. The HMBC spectrum of **3** in CD_3OD