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Pestalotriols A and B, new spiro[2.5]octane derivatives from the endophytic fungus *Pestalotiopsis fici*

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EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Rudolph Research Analytical automatic polarimeter, and UV data were obtained on a Shimadzu Biospec-1601 spectrophotometer. CD spectra were recorded on a JASCO J-815 spectropolarimeter. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. ^{1}H and ^{13}C NMR data were acquired with Varian Mercury-500 and NMR system-600 spectrometers using solvent signals (acetone-d_6: δ_H 2.05/δ_C 29.8, 206.1) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS and HRESIMS data were obtained using an Agilent Accurate-Mass-Q-TOF LC/MS 6520 instrument equipped with an electrospray ionization (ESI) source. The fragmentor and capillary voltages were kept at 125 and 3500 V, respectively. Nitrogen was supplied as the nebulizing and drying gas. The temperature of the drying gas was set at 300 °C. The flow rate of the drying gas and the pressure of the nebulizer were 10 L/min and 10 psi, respectively. All MS experiments were performed in positive ion mode. Full-scan spectra were acquired over a scan range of *m/z* 100–1000 at 1.03 spectra/s. HPLC separations were performed on an Agilent 1260 instrument (Agilent, USA) equipped with a variable-wavelength UV detector.

Fungal Material. The culture of *P. fici* was isolated from the branches of *Camellia sinensis* (Theaceae) in a suburb of Hangzhou, People's Republic of China, in April, 2005. The isolate was identified as *P. fici* by one of the authors (L.G.) based on sequence (GenBank Accession number DQ812914) analysis of the ITS region of the ribosomal DNA and assigned the accession number AS 3.9138 (= W106-1) in the China General Microbial Culture Collection (CGMCC) at the Institute of Microbiology,

Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 10 days. Agar plugs were cut into small pieces (about 0.5 × 0.5 × 0.5 cm^3) under aseptic conditions and 15 pieces were used to inoculate three Erlenmeyer flasks (250 mL), each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract); the final pH of the media was adjusted to 6.5 and sterilized by autoclave. Three flasks of the inoculated media were incubated at 25 °C on a rotary shaker at 170 rpm for five days to prepare the seed culture. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1 × 10^6/mL. Fermentation was carried out in 36 Fernbach flasks (500 mL), each containing 80 g of rice. Distilled H_2O (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 psi for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented material was extracted repeatedly with EtOAc (4 × 4.0 L), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (33 g), which was fractionated by silica gel vacuum liquid chromatography (VLC) using petroleum ether/EtOAc gradient elution. The fraction (400 mg) eluted with 60% EtOAc was separated by Sephadex LH-20 column chromatography (CC) using 1:1 CH_2Cl_2–MeOH as eluents. The resulting subfractions were combined and further purified by semipreparative RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 40–80% CH_3CN in H_2O for 30 min; 2 mL/min) to afford **1** (3.1 mg, t_R 18.5 min) and **2** (2.2 mg, t_R 19.8 min).

Computational Details. Systematic conformational analyses for **3a** and **3b** were

performed via the Molecular Operating Environment (MOE) ver. 2009.10. (Chemical Computing Group, Canada) software package using the MMFF94 molecular mechanics force field calculation. The MMFF94 conformational analyses were further optimized using TDDFT at the B3LYP/6-31G(d) basis set level. The stationary points have been checked as the true minima of the potential energy surface by verifying they do not exhibit vibrational imaginary frequencies. The 30 lowest electronic transitions were calculated, and the rotational strengths of each electronic excitation were given using both dipole length and dipole velocity representations. ECD spectra were stimulated using a Gaussian function with a half-bandwidth of 0.45 eV. Equilibrium populations of conformers at 298.15 K were calculated from their relative free energies (ΔG) using Boltzmann statistics. The overall ECD spectra were then generated according to Boltzmann weighting of each conformer. The systematic errors in the prediction of the wavelength and excited-state energies are compensated for by employing UV correlation. All quantum computations were performed using the Gaussian03 package[1] on an IBM cluster machine located at the High Performance Computing Center of Peking Union Medical College.

MTS Assay.[2] In a 96-well plate, each well was plated with (2–5) × 10^3 cells (depending on the cell multiplication rate). After cell attachment overnight, the medium was removed, and each well was treated with 100 μL of medium containing 0.1% DMSO, or appropriate concentrations of the test compounds and the positive control cisplatin (100 mM as stock solution of a compound in DMSO and serial dilutions; the test compounds showed good solubility in DMSO and did not precipitate when added to the cells). The plate was incubated for 48 h at 37 °C in a humidified, 5% CO_2 atmosphere.

Proliferation assessed by adding 20 μL of MTS (Promega) to each well in the dark, followed by a 90 min incubation at 37 °C. The assay plate was read at 490 nm using a microplate reader. The assay was run in triplicate.

Reference

1. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03,* Revision E. 01; Gaussian, Inc.: Wallingford, CT, 2004.

2. Zhang, N.; Chen, Y.; Jiang, R.; Li, E.; Chen, X.; Xi, Z.; Guo, Y.; Liu, X.; Zhou, Y.; Che, Y.; Jiang, X. *Autophagy* **2011**, *7*, 598–612.

Figure S1. ^{1}H NMR Spectrum of Pestalotriol A (**1**; 600 MHz, Acetone-d_6)

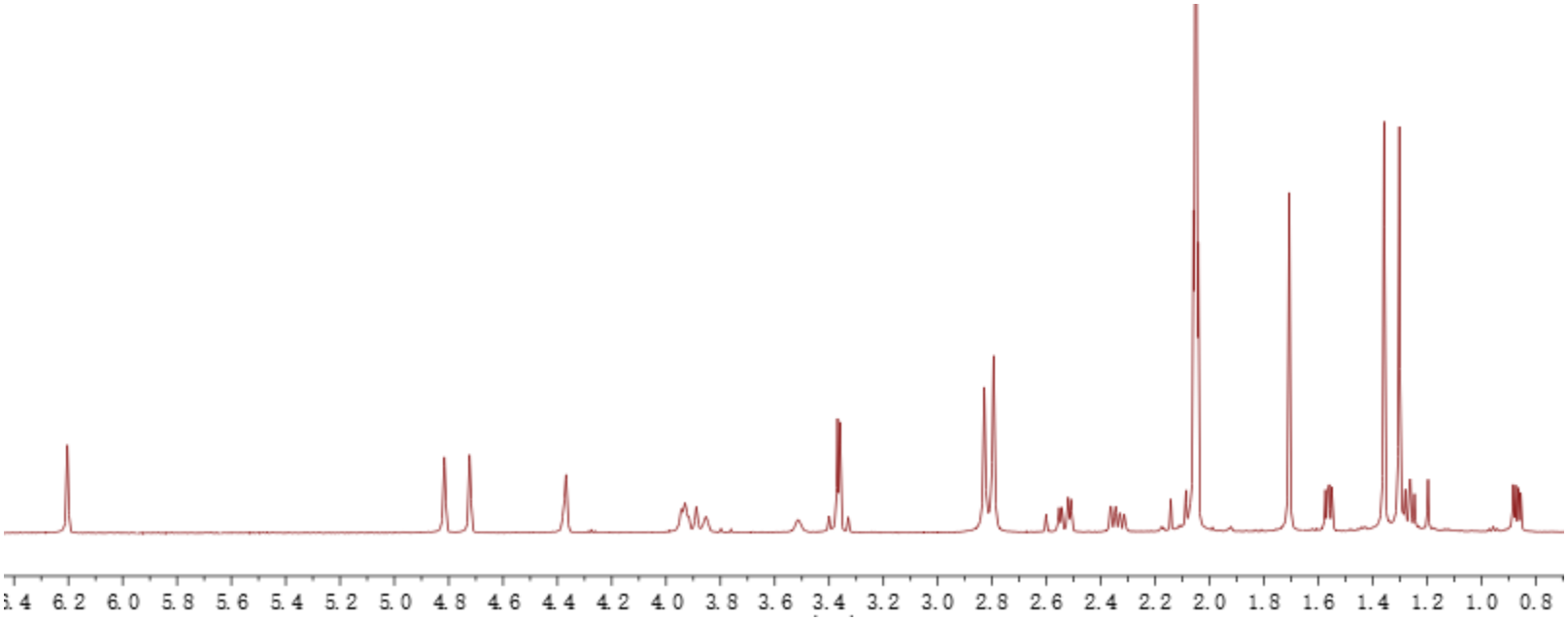


Figure S2. ^{13}C NMR Spectrum of Pestalotriol A (**1**; 150 MHz, Acetone-d_6)

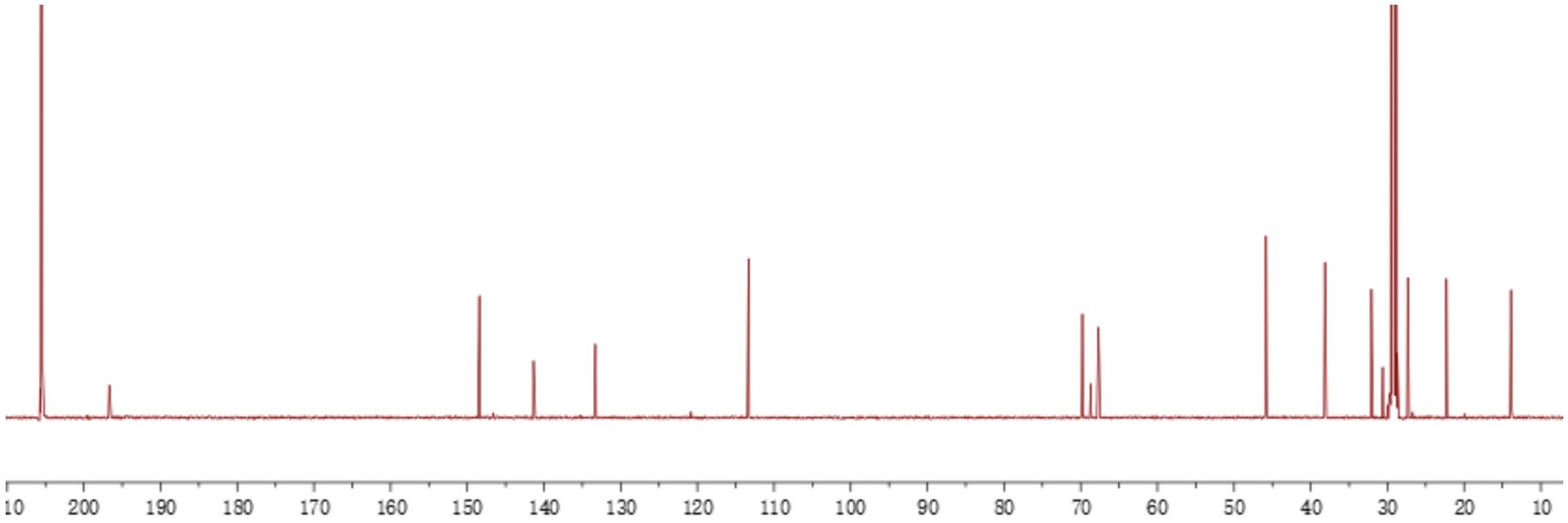


Figure S3. HMQC Spectrum of Pestalotriol A (**1**; 600 MHz, Acetone-d_6)

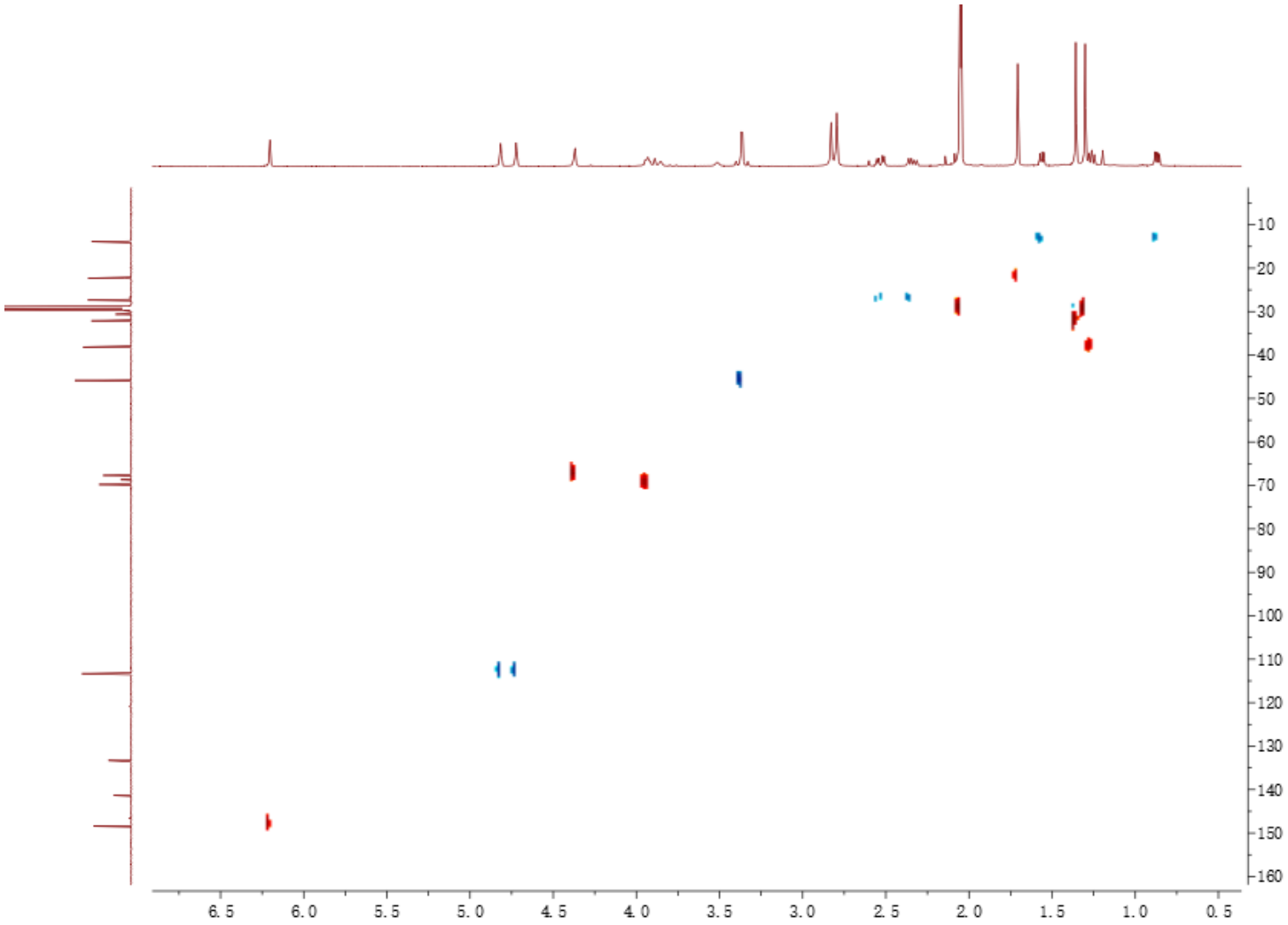


Figure S4. ^{1}H–^{1}H COSY Spectrum of Pestalotriol A (**1**; 600 MHz, Acetone-d_6)

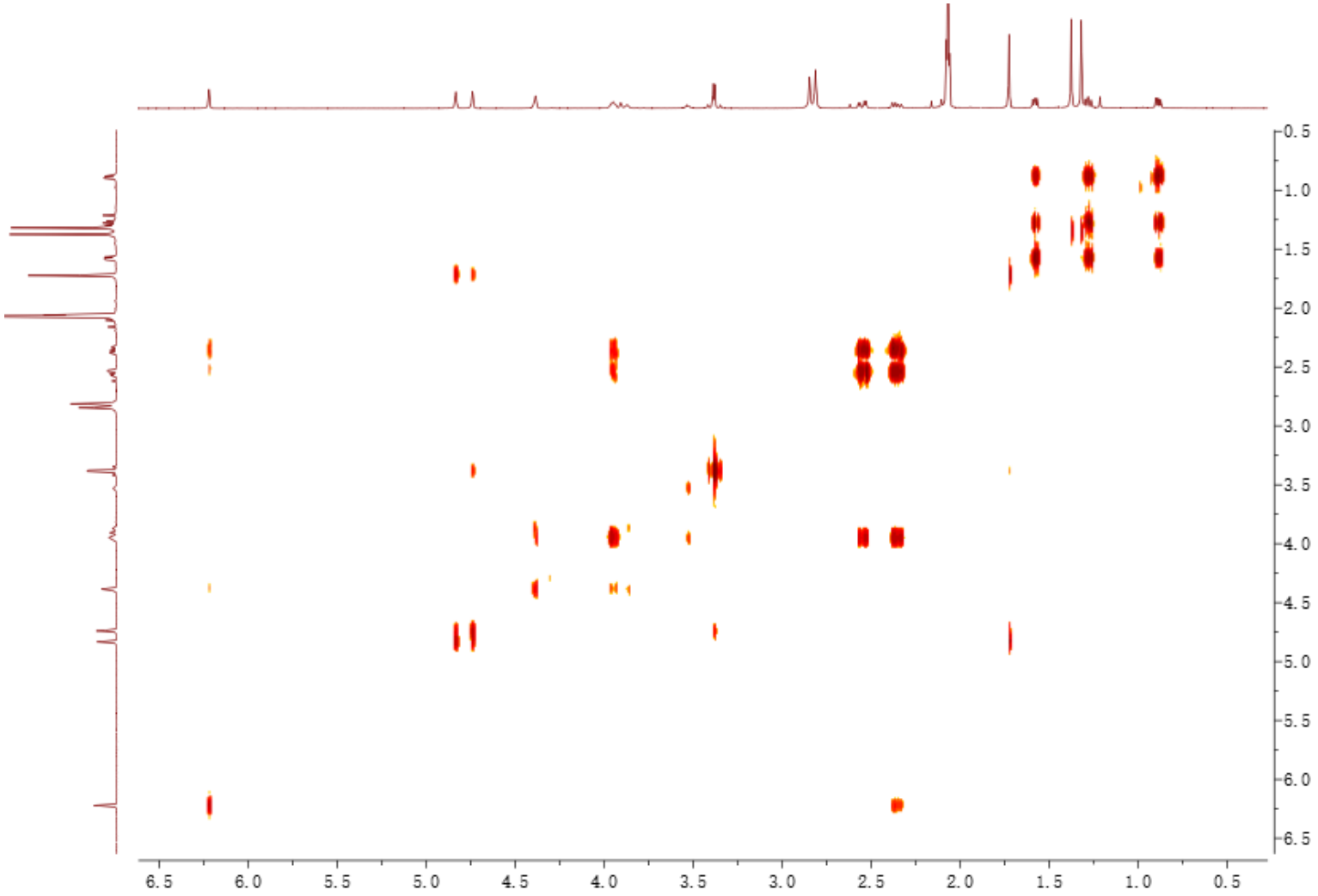


Figure S5. HMBC Spectrum of Pestalotriol A (**1**; 600 MHz, Acetone-d_6)

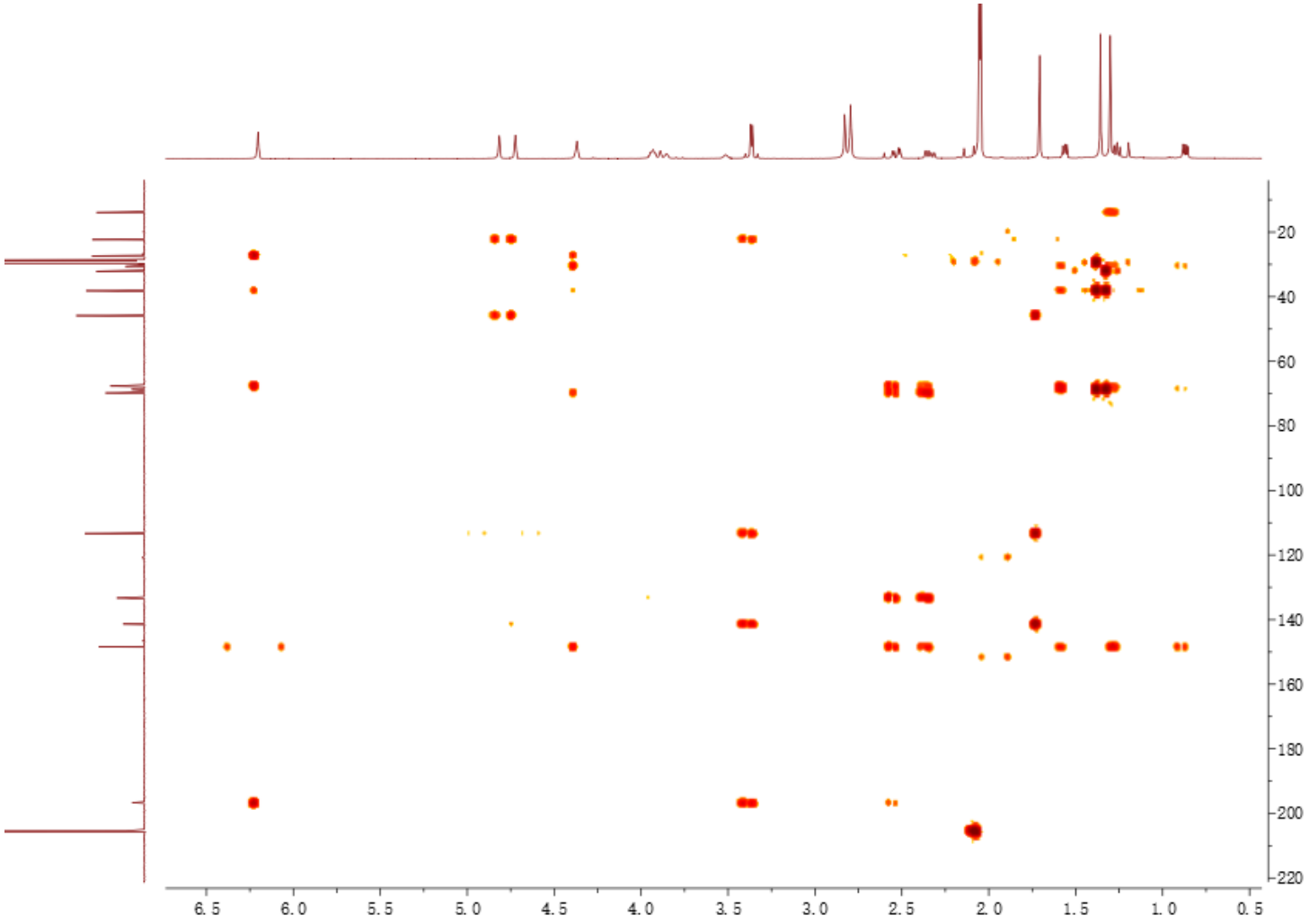


Figure S6. NOESY Spectrum of Pestalotriol A (**1**; 600 MHz, Acetone-d_6)

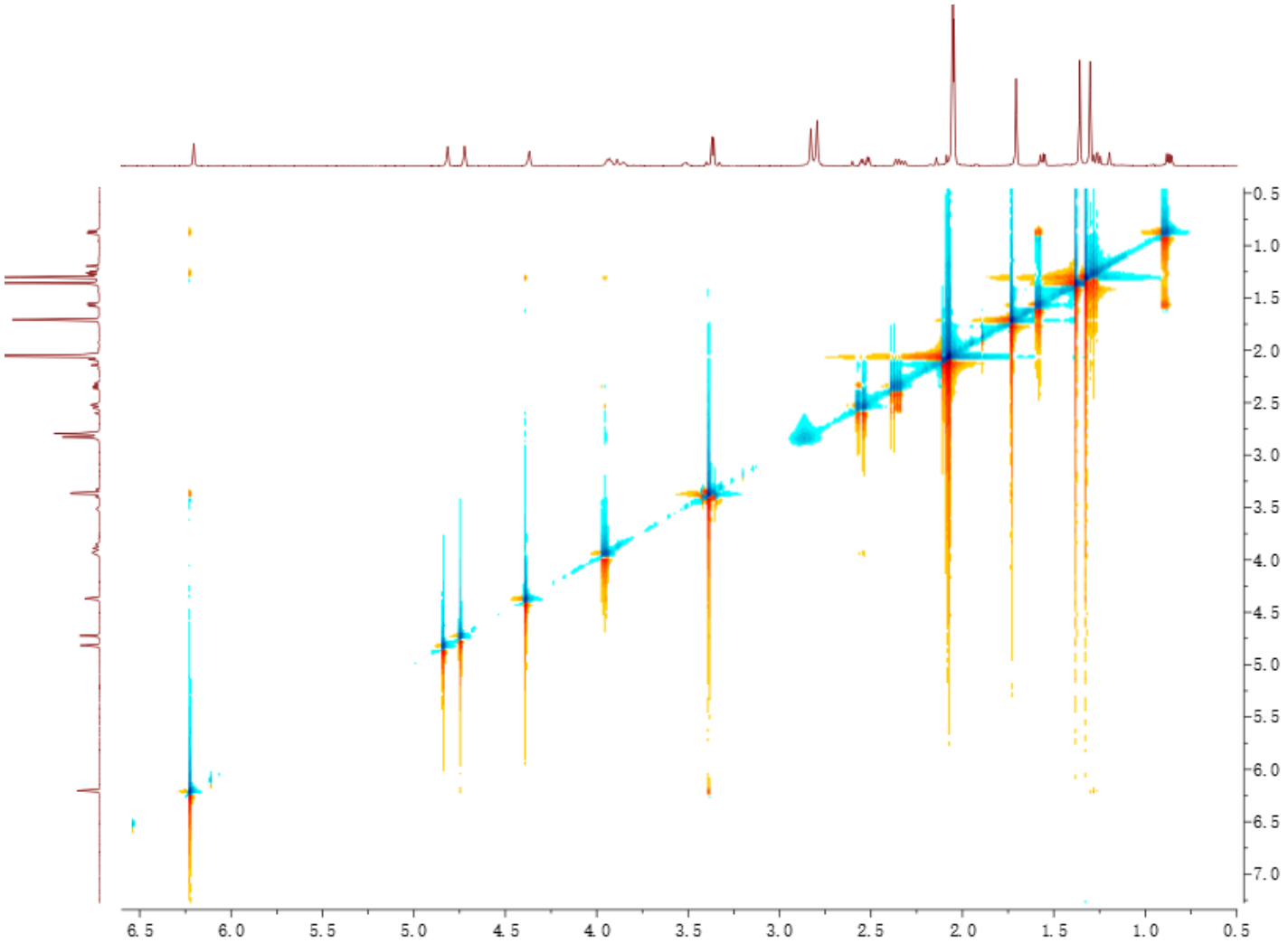


Figure S7. ^{1}H NMR Spectrum of Pestalotriol B (**2**; 500 MHz, Acetone-d_6)

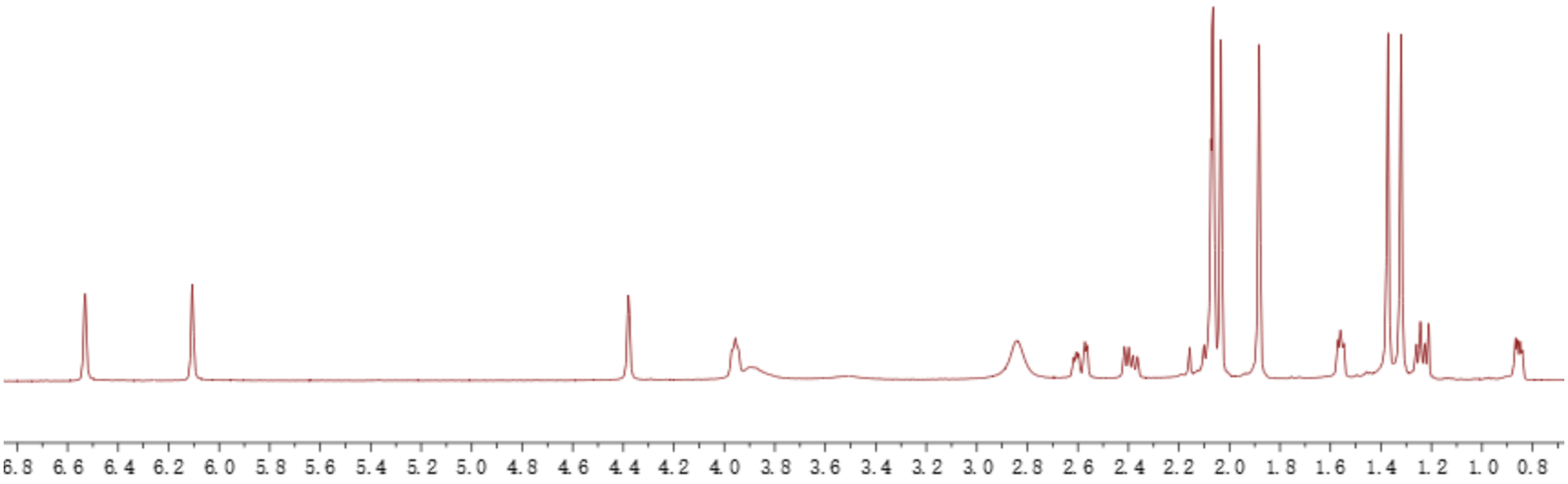


Figure S8. ^{13}C NMR Spectrum of Pestalotriol B (**2**; 150 MHz, Acetone-d_6)

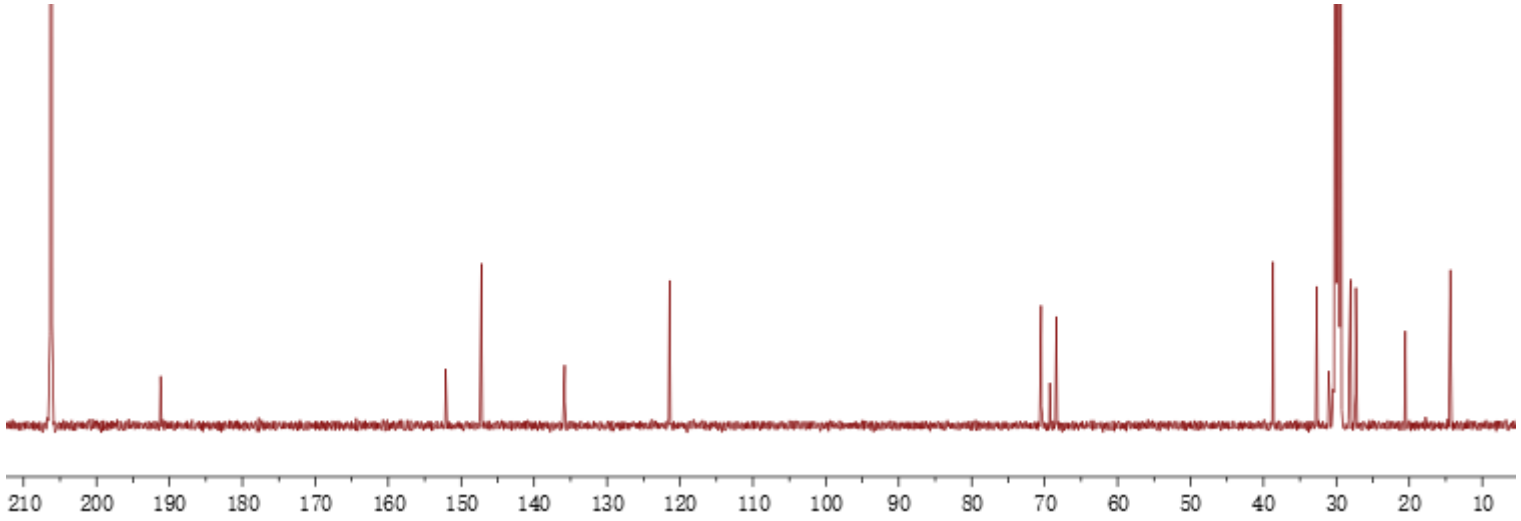


Figure S9. HMQC Spectrum of Pestalotriol B (**2**; 500 MHz, Acetone-d_6)

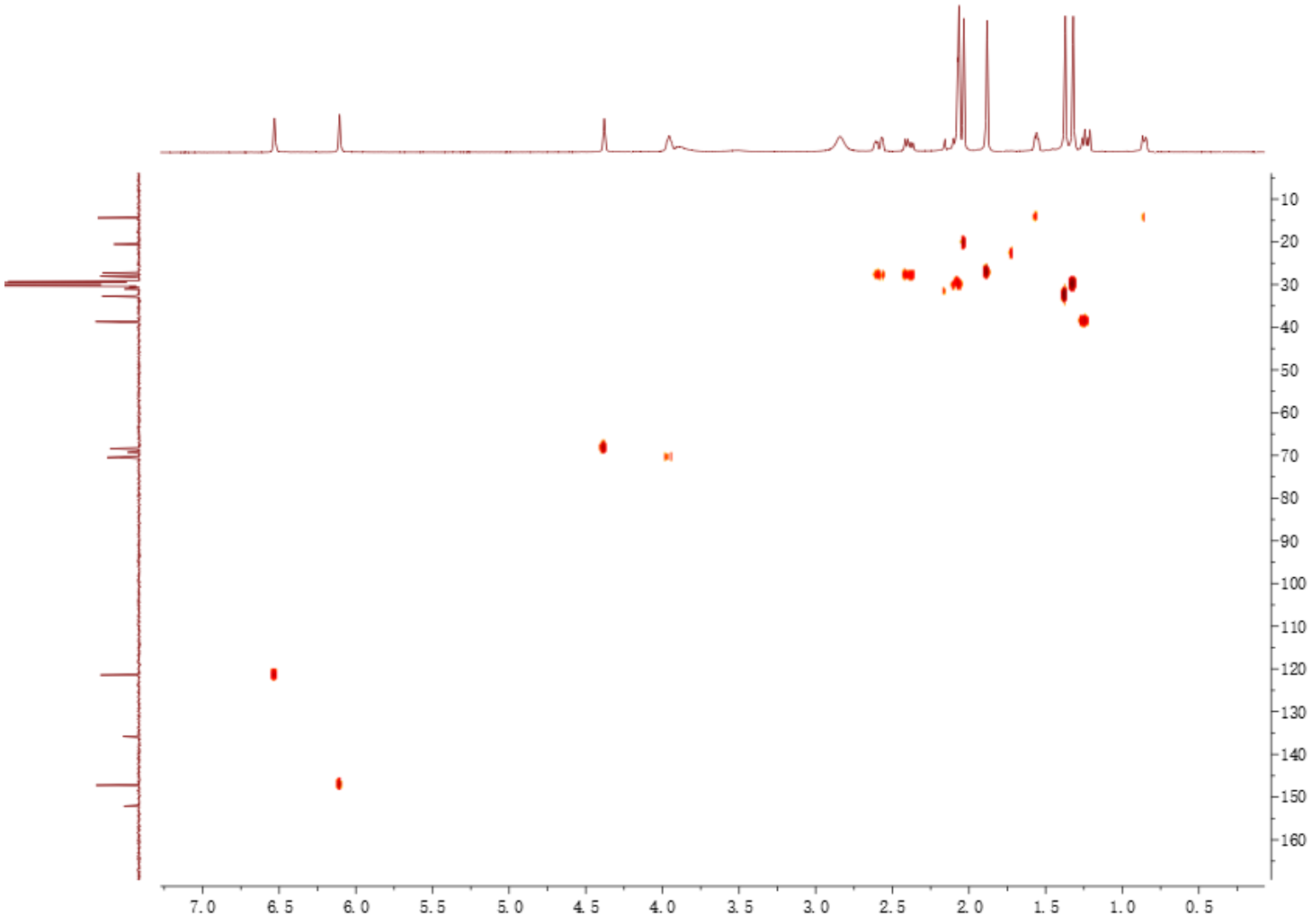


Figure S10. ^{1}H–^{1}H COSY Spectrum of Pestalotriol B (**2**; 500 MHz, Acetone-d_6)

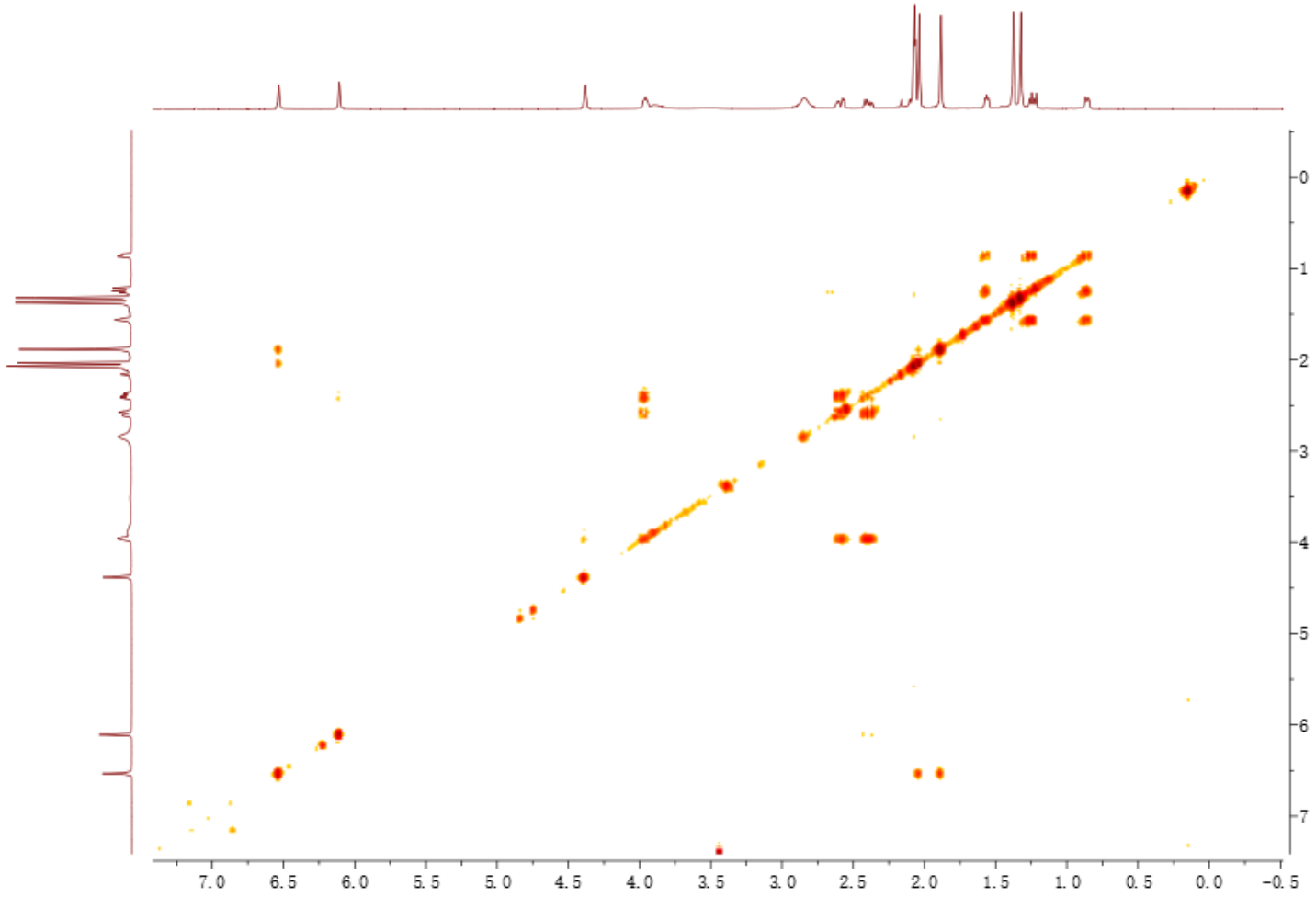


Figure S11. HMBC Spectrum of Pestalotriol B (**2**; 500 MHz, Acetone-d_6)

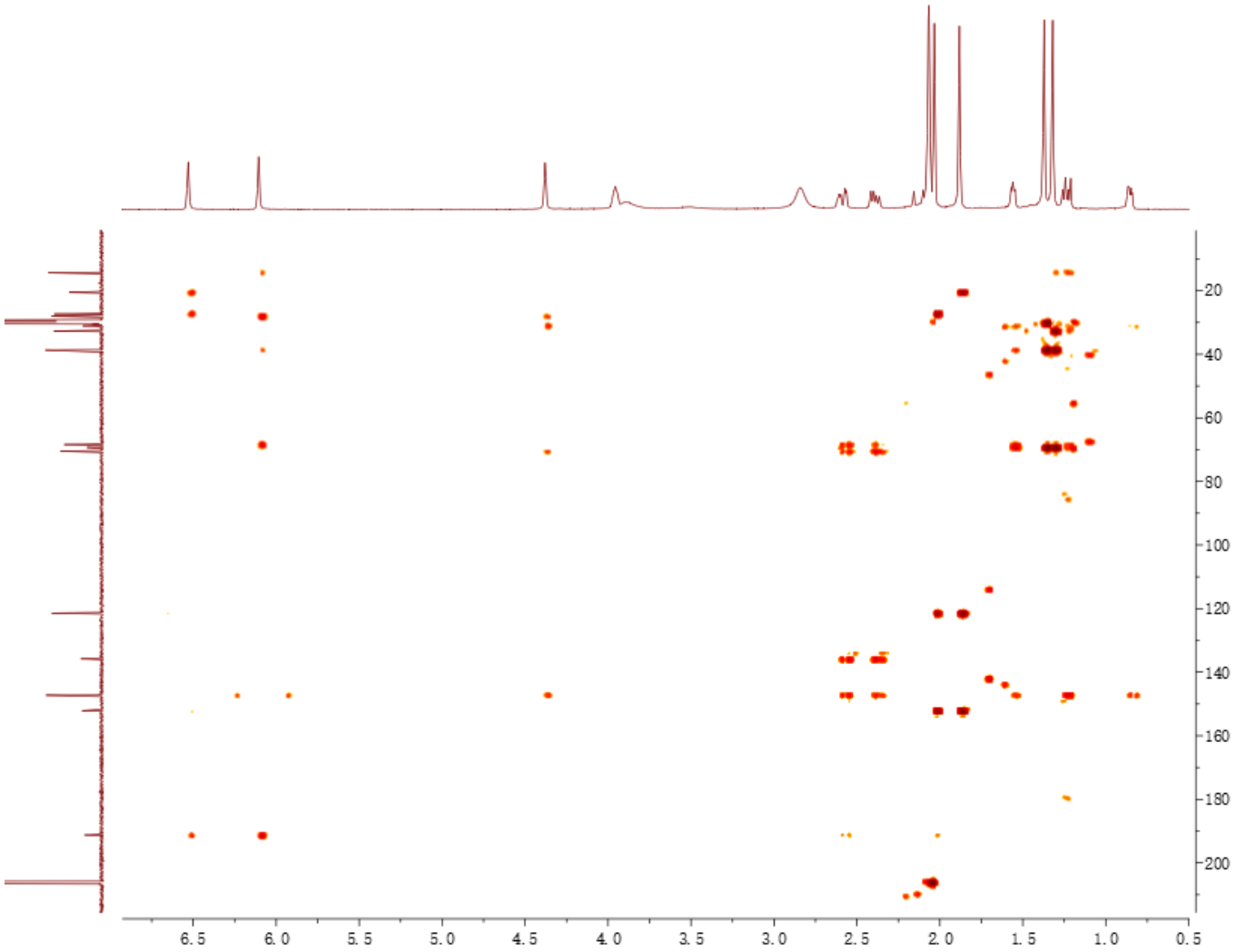


Figure S12. NOESY Spectrum of Pestalotriol B (**2**; 500 MHz, Acetone-d_6)

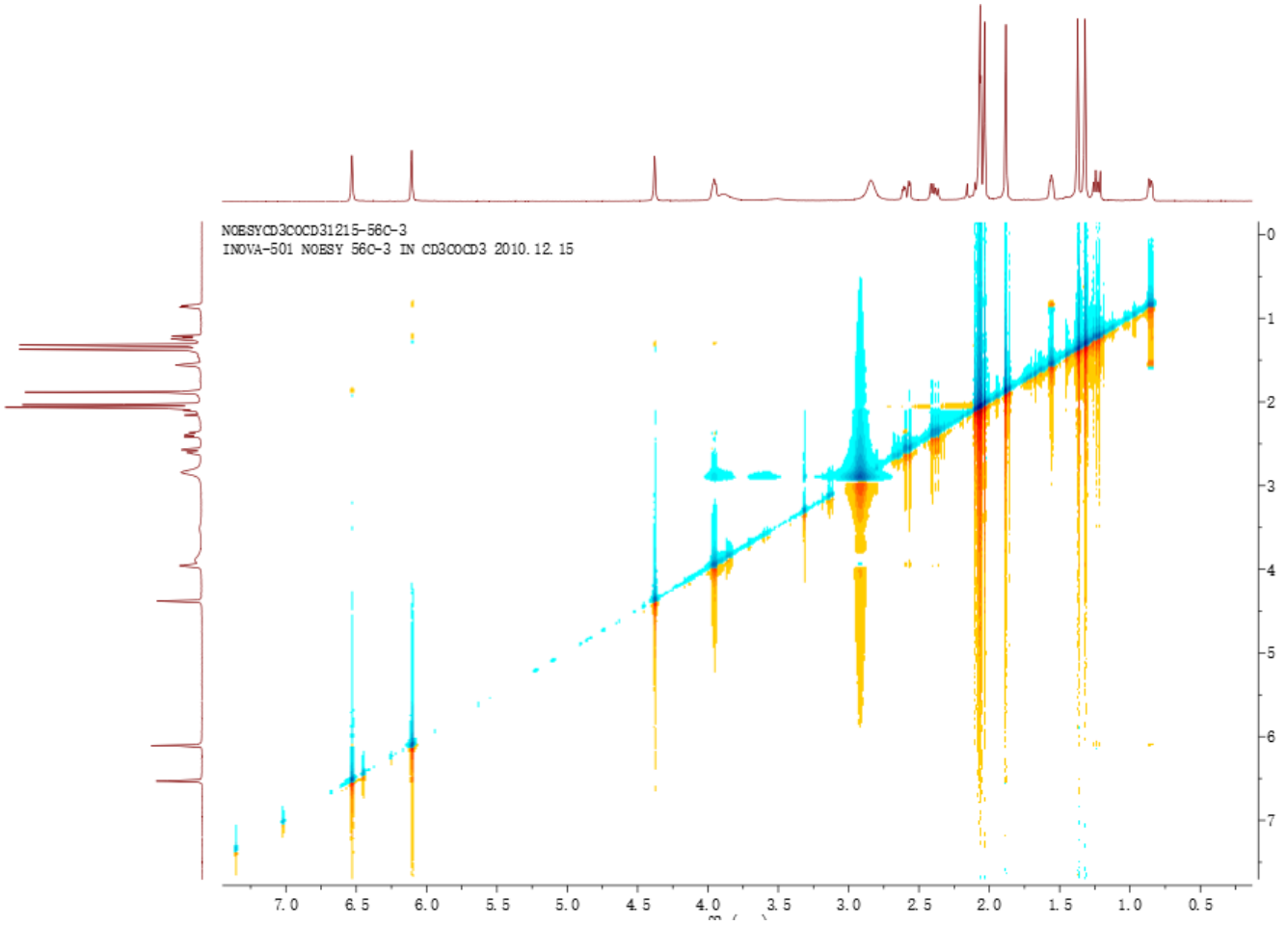


Figure S13. The Optimized Conformers for **3a**

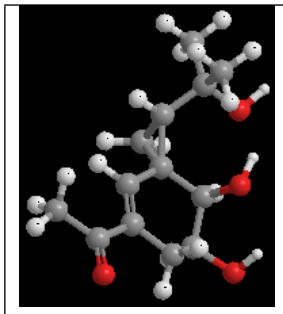
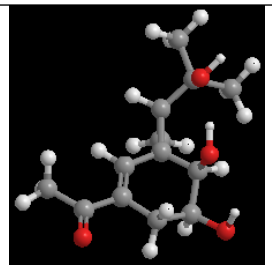
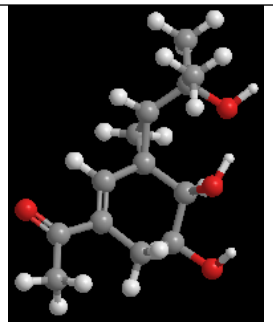
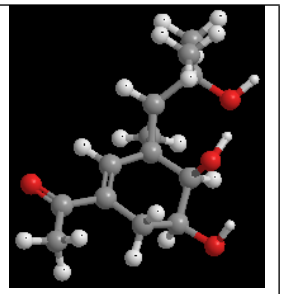
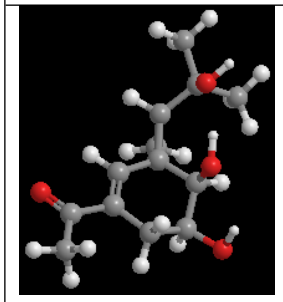
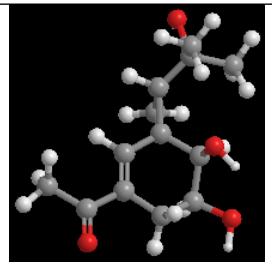
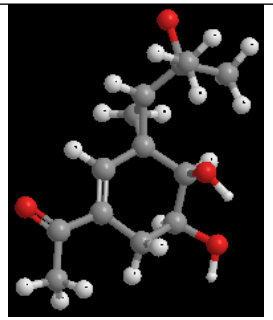
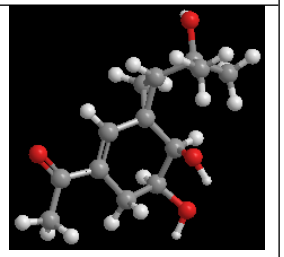
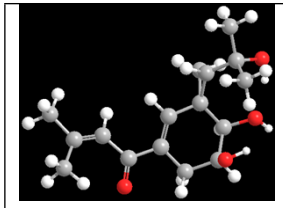
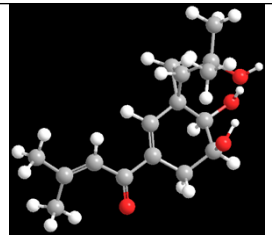
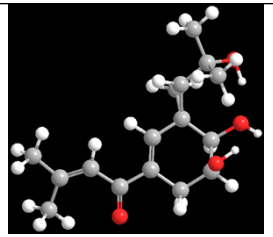
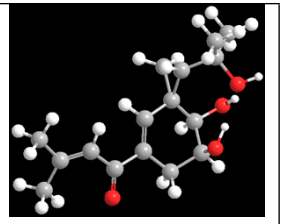
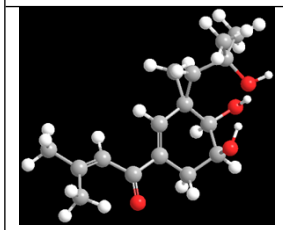
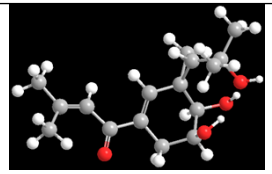
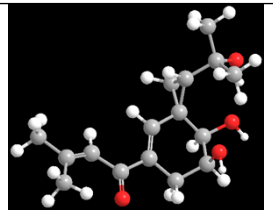
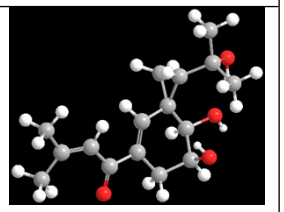
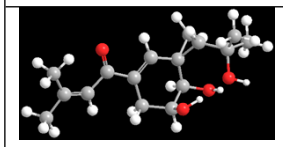
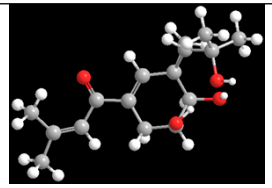
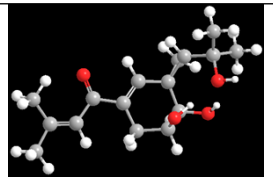
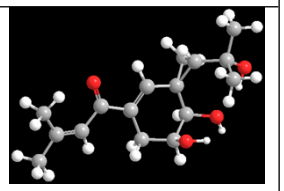
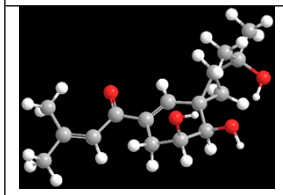
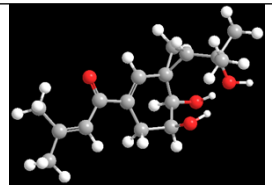
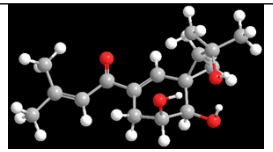
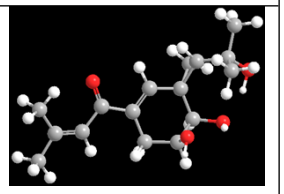
			
3aC1 (65.086%)	**3a** C2 (14.042%)	**3a** C3 (9.349%)	**3a** C4 (9.329%)
			
3a C5 (2.131%)	**3a** C6 (0.054%)	**3a** C7 (0.005%)	**3a** C8 (0.005%)

Figure S14. The Optimized Conformers for **2a**

			
2a C1 (16.961%)	**2a** C2 (10.929%)	**2a** C3 (9.862%)	**2a** C4 (9.820%)
			
2a C5 (8.422%)	**2a** C6 (8.038%)	**2a** C7 (3.640%)	**2a** C8 (3.489%)
			
2a C9 (3.427%)	**2a** C10 (3.387%)	**2a** C11 (3.369%)	**2a** C12 (2.999%)
			

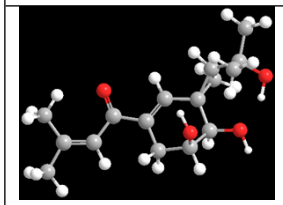
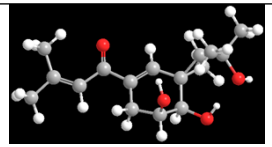
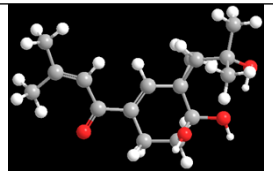
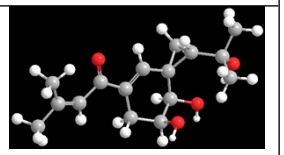
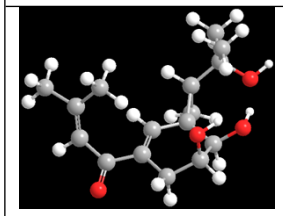
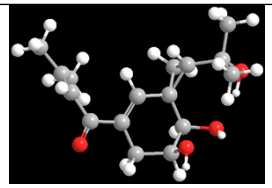
2a C13 (2.929%)	**2a** C14 (2.877%)	**2a** C15 (2.714%)	**2a** C16 (2.194%)
			
2a C17 (1.922%)	**2a** C18 (1.240%)	**2a** C19 (1.065%)	**2a** C20 (0.434%)
			
2a C20 (0.182%)	**2a** C22 (0.101%)		

Figure S15. UV spectrum of pestalotriol A (**1**)

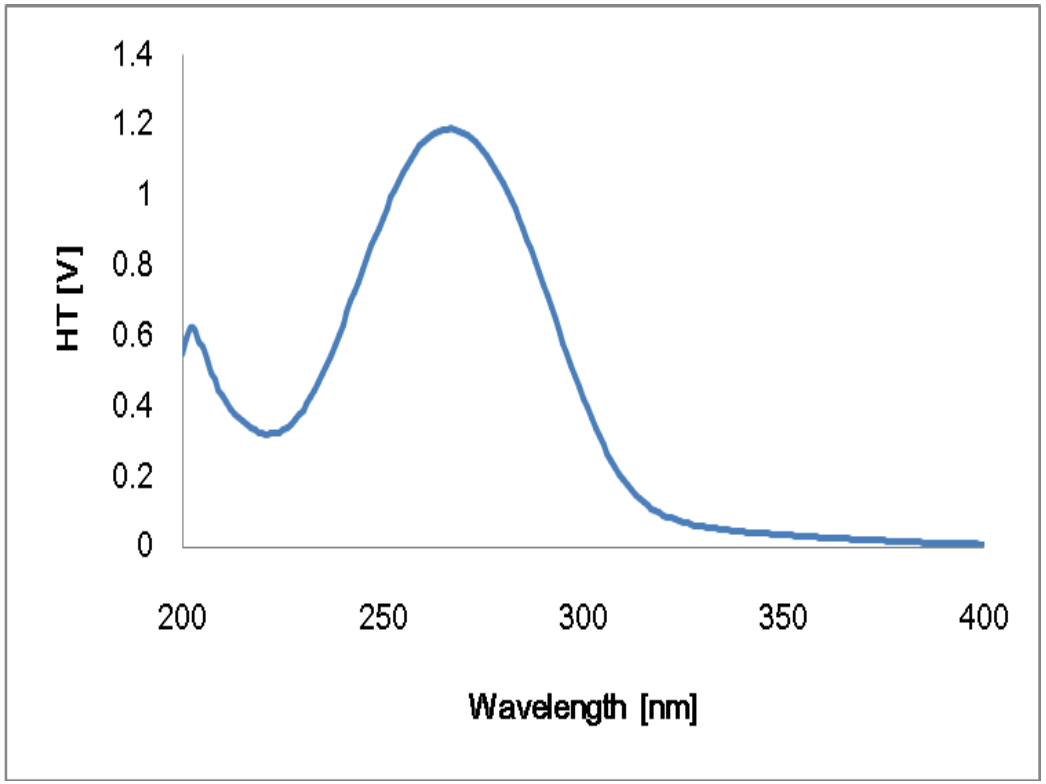


Figure S16. IR spectrum of pestalotriol A (**1**)

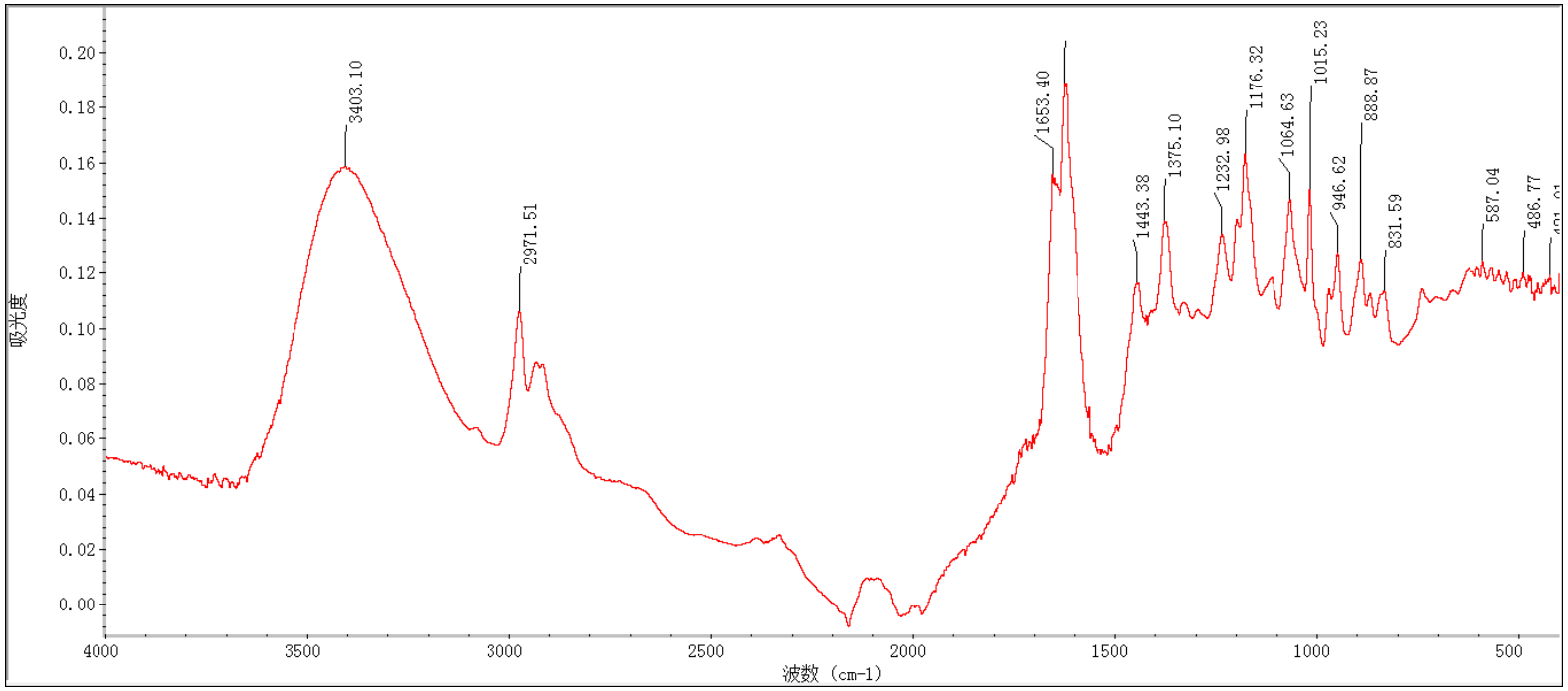


Figure S17. HRESIMS spectrum of pestalotriol A (**1**)

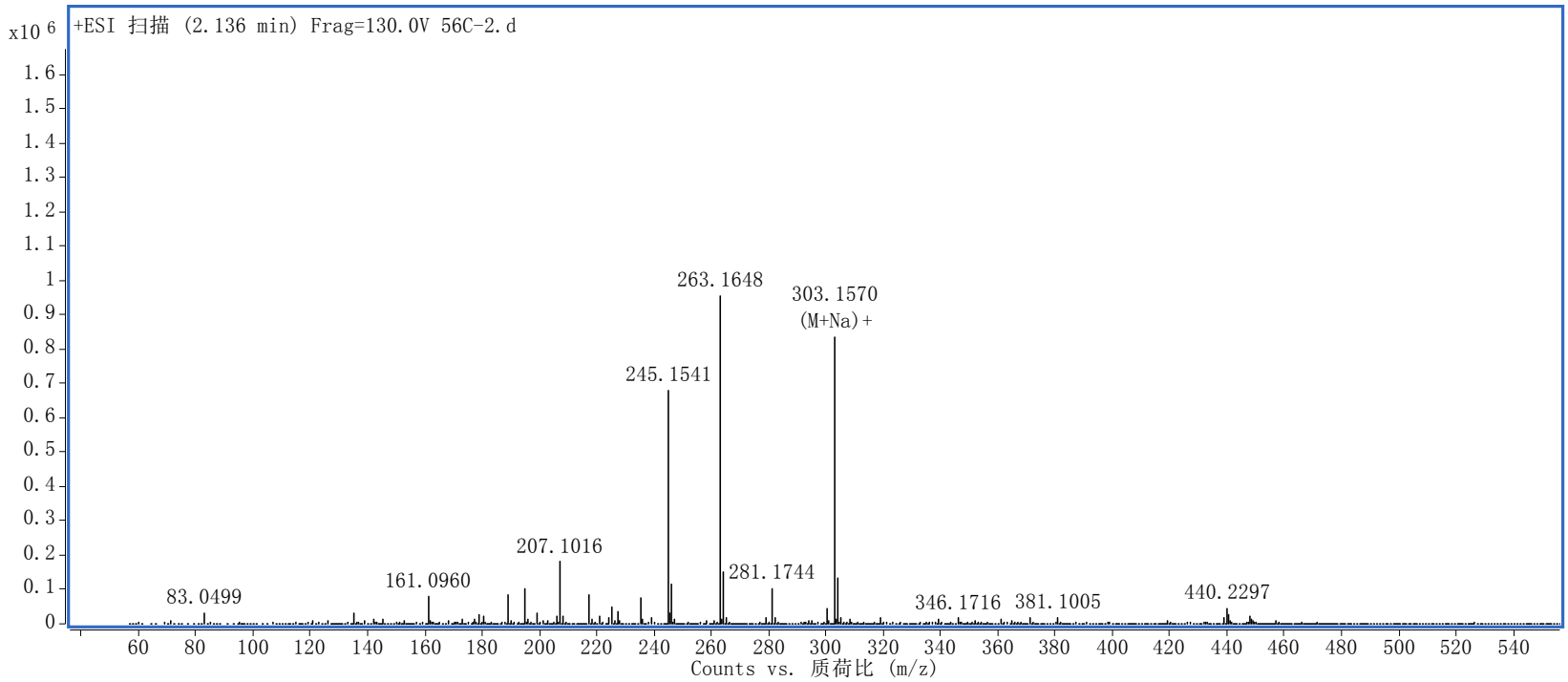


Figure S18. UV spectrum of pestalotriol B (**2**)

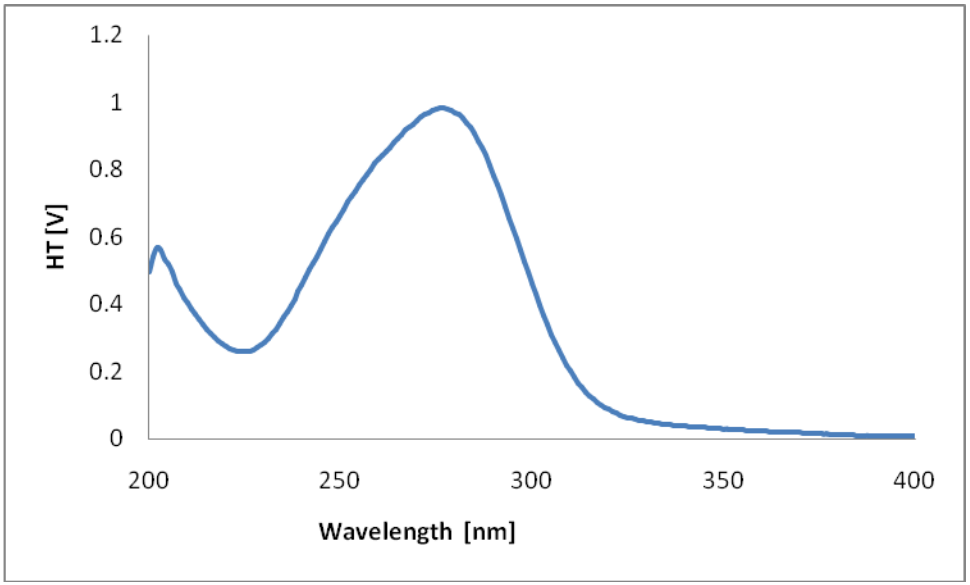


Figure S19. IR spectrum of pestalotriol B (**2**)

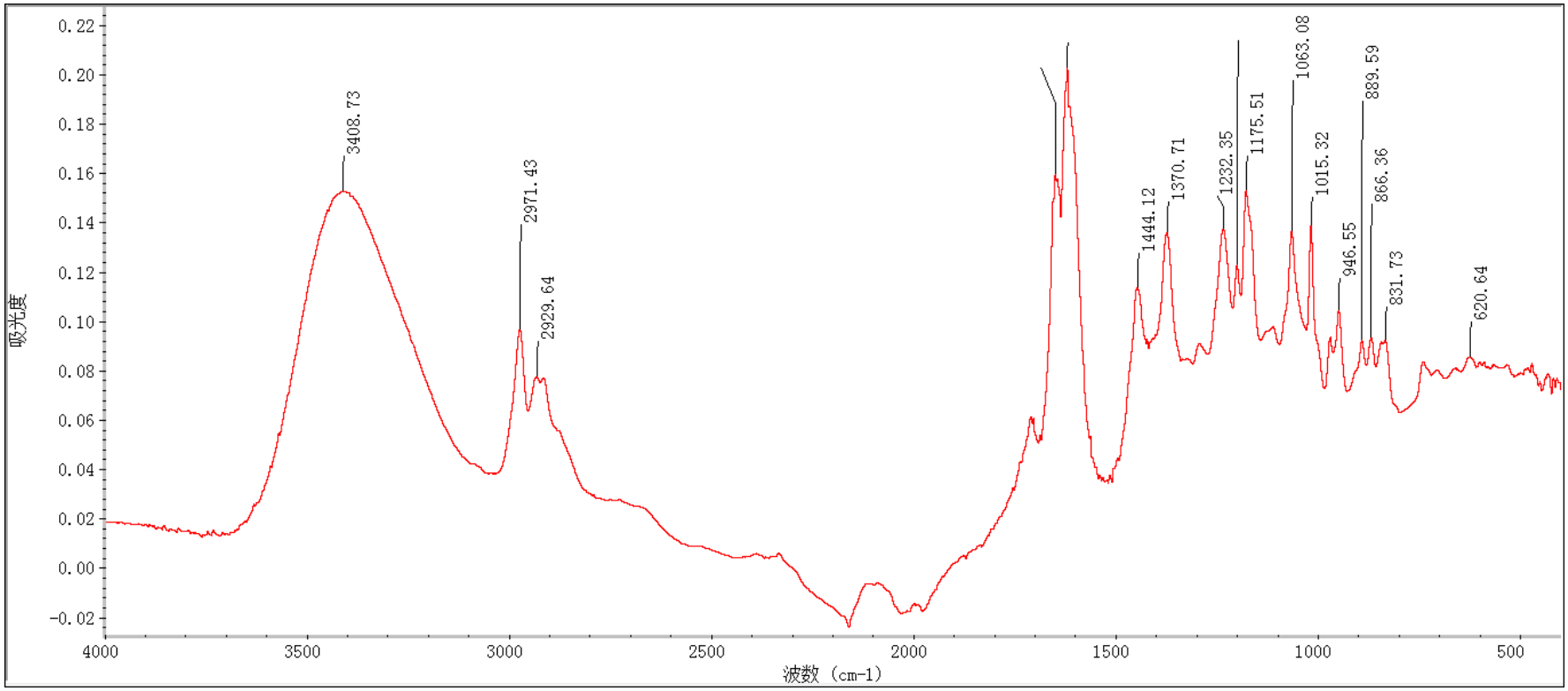


Figure S20. HRESIMS spectrum of pestalotriol B (**2**)