Supporting Information

Rubesanolides A and B: Diterpenoids from *Isodon rubescens*

Juan Zou,[†,‡] Lutai Pan,[*,†] Qiji Li,[†] Junhua Zhao,[†] Jianxin Pu,[‡] Ping Yao,[§] Ningbo Gong,[ll] Yang Lu,[ll] Tamara P. Kondratyuk,[⊥] John M. Pezzuto,[⊥] Harry H. S. Fong,[§] Hongjie Zhang,[*,§] and Handong Sun[‡]

[†] Guiyang College of Traditional Chinese Medicine, Guiyang 550002, Guizhou, People's Republic of China

[‡] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Kunming 650204, People's Republic of China

[§] Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Street, Chicago, Illinois 60612, United States

[ll] Institute of Materia Medica Chinese Academy of Medical Sciences, Beijing 100050, People's Republic of China

[⊥] University of Hawaii at Hilo, College of Pharmacy, 34 Rainbow Dr., Hilo, HI 96720, United States

Table of Contents

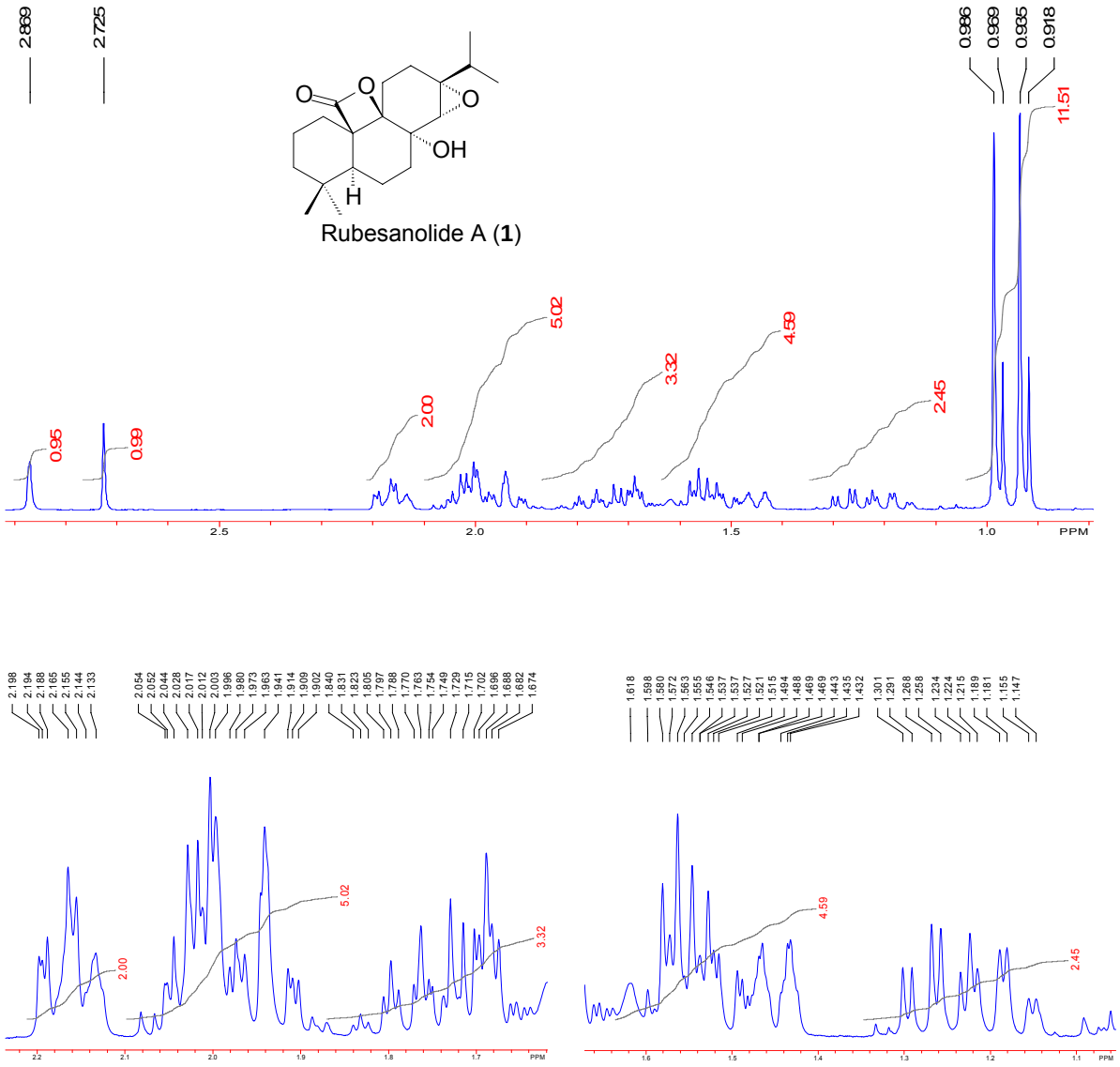
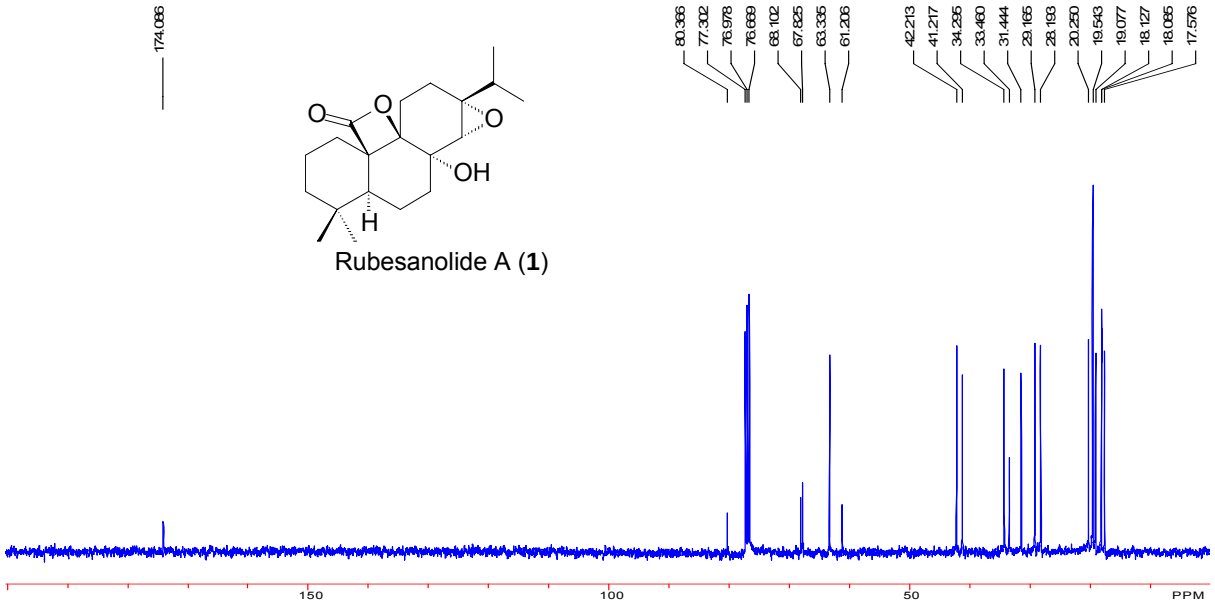


Figure S1. ^{1}H NMR spectrum of rubesanolide A (**1**)

A)



B)

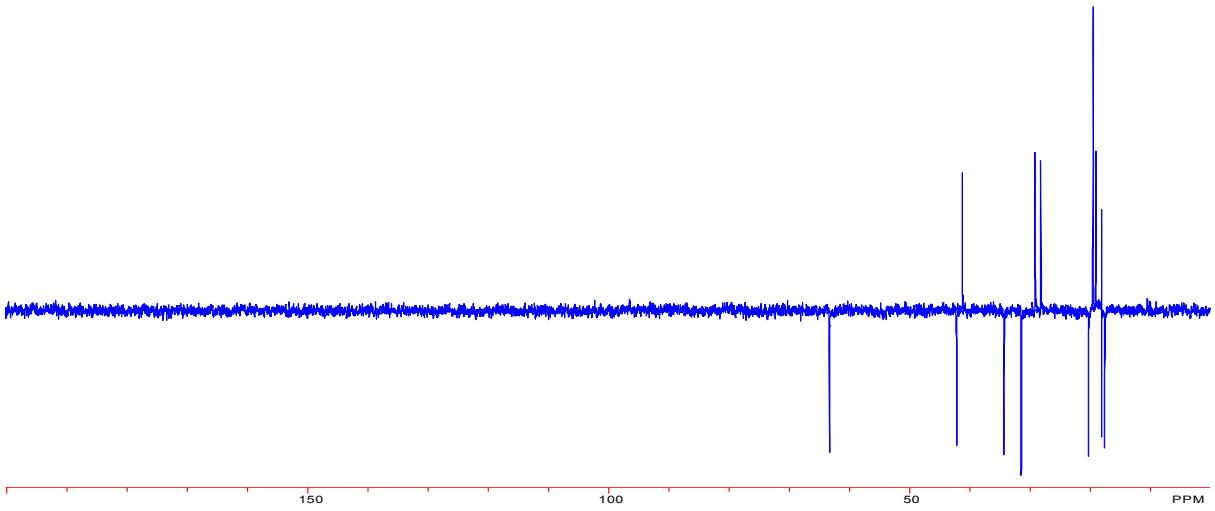


Figure S2. A) ^{13}C NMR, and B) DEPT-135 spectra of rubesanolide A (**1**)

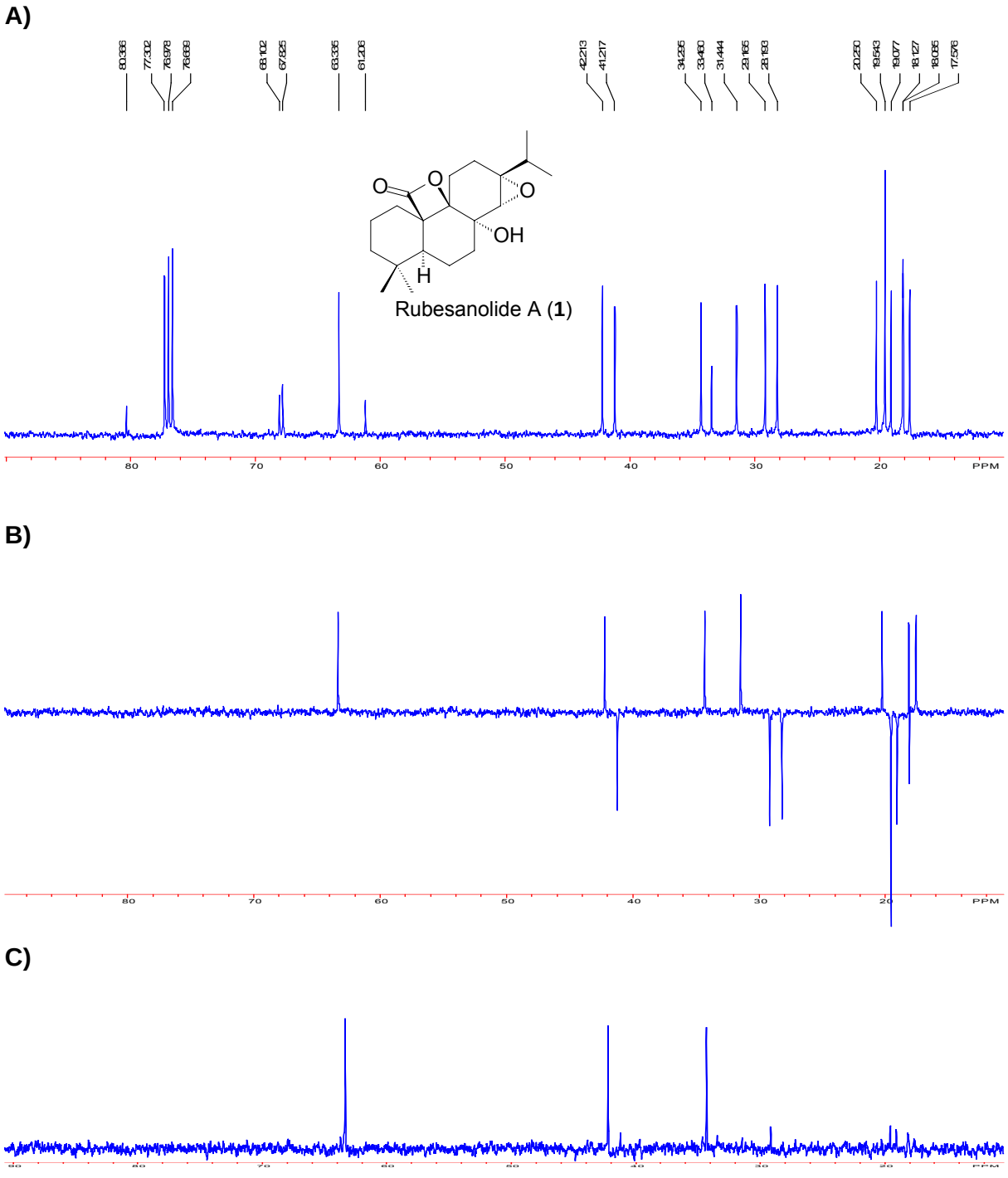


Figure S3. A) ^{13}C NMR, B) DEPT-135, and C) DEPT-90 spectra of rubesanolide A (**1**)

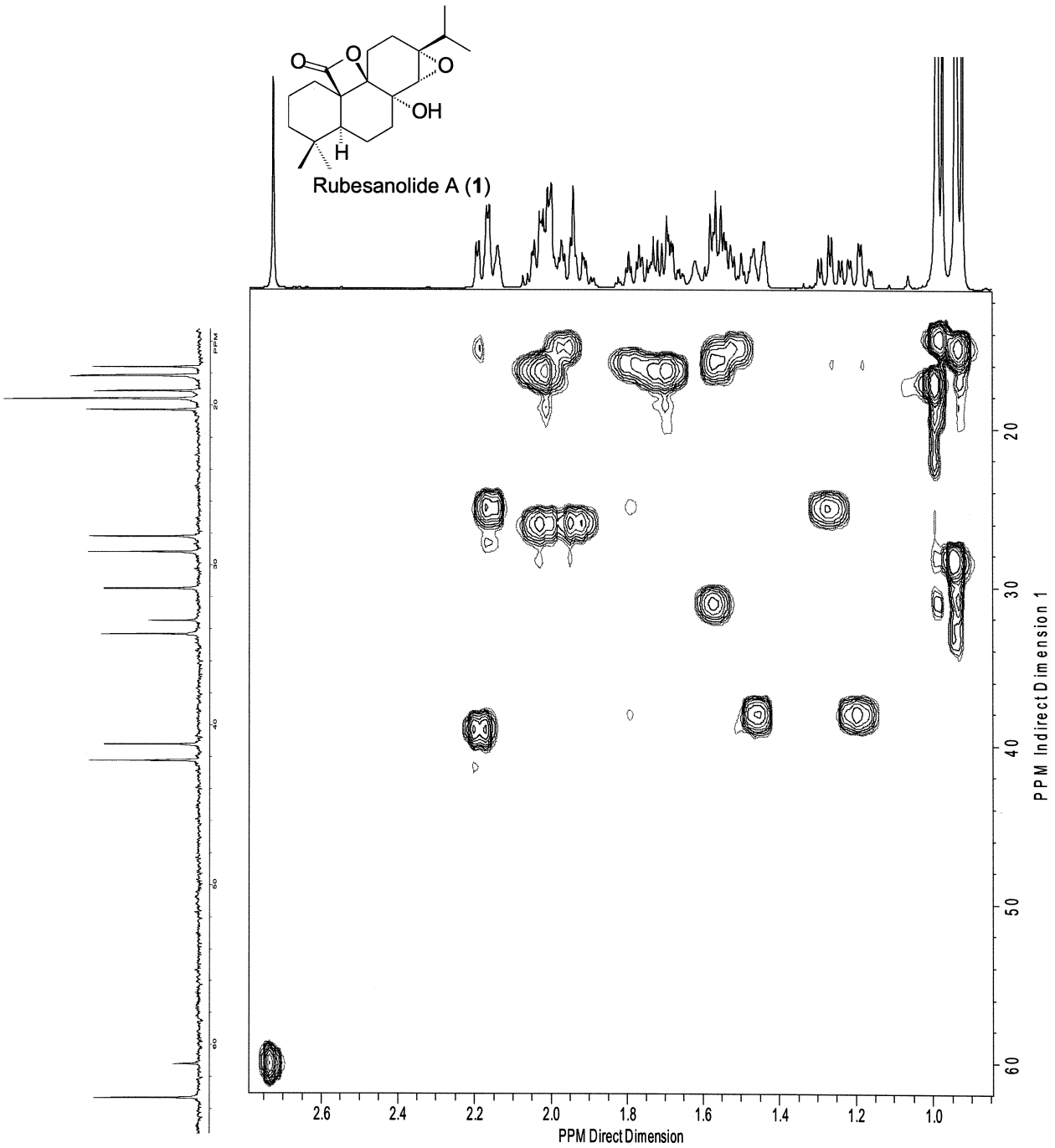


Figure S4. HSQC spectrum of rubesanolide A (**1**)

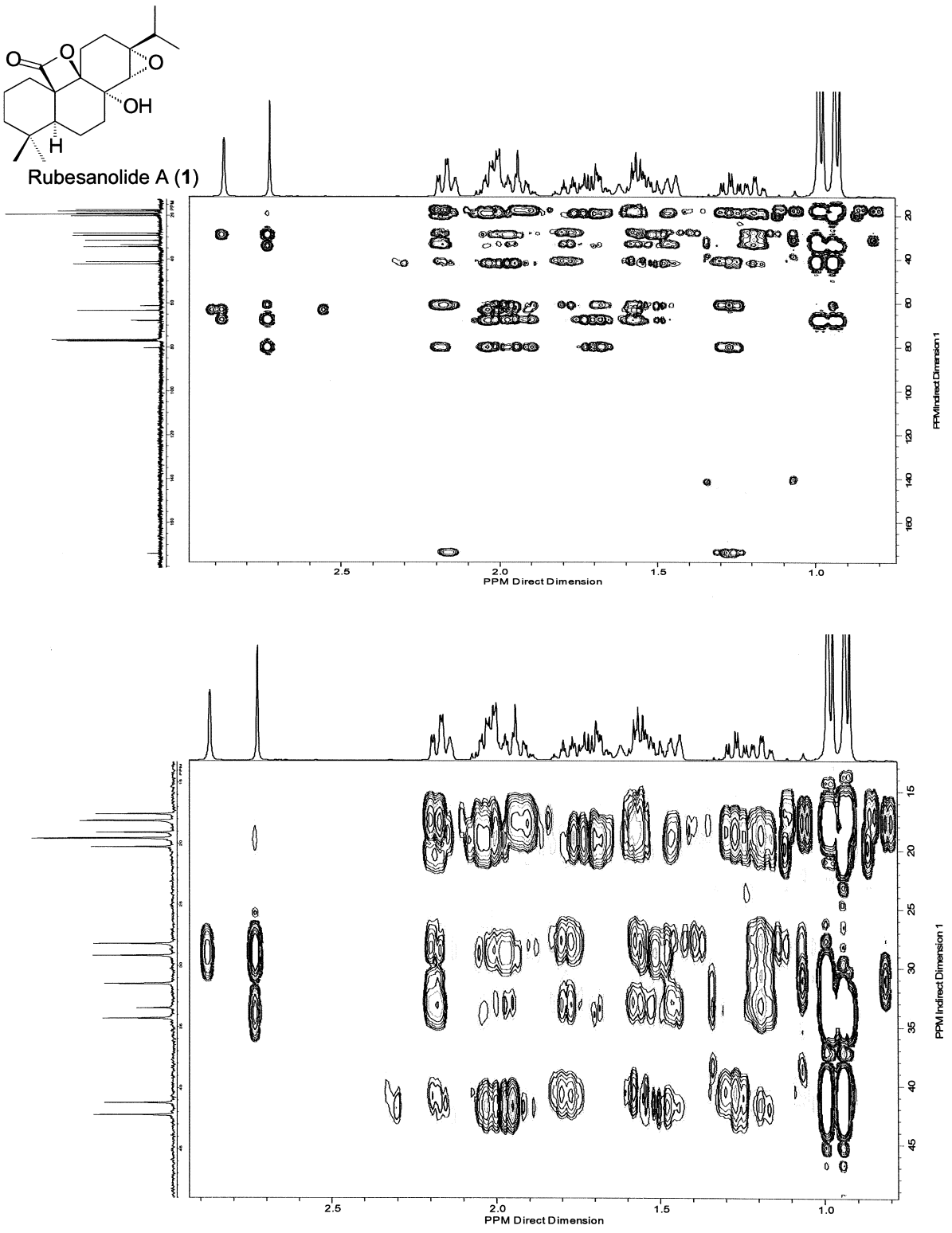


Figure S5. HMBC spectrum of rubesanolide A (**1**)

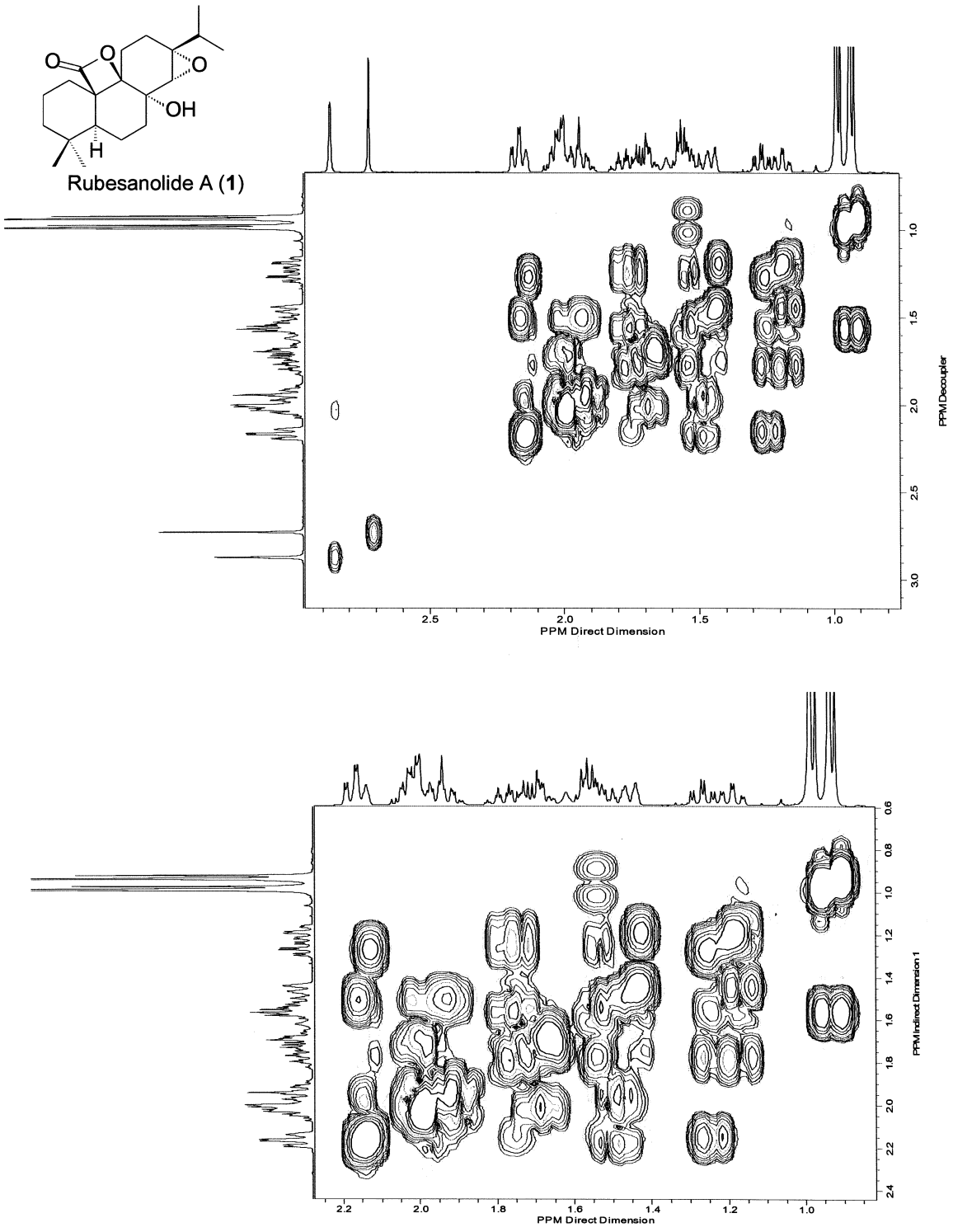


Figure S6. ^{1}H-^{1}H COSY spectrum of rubesanolide A (**1**)

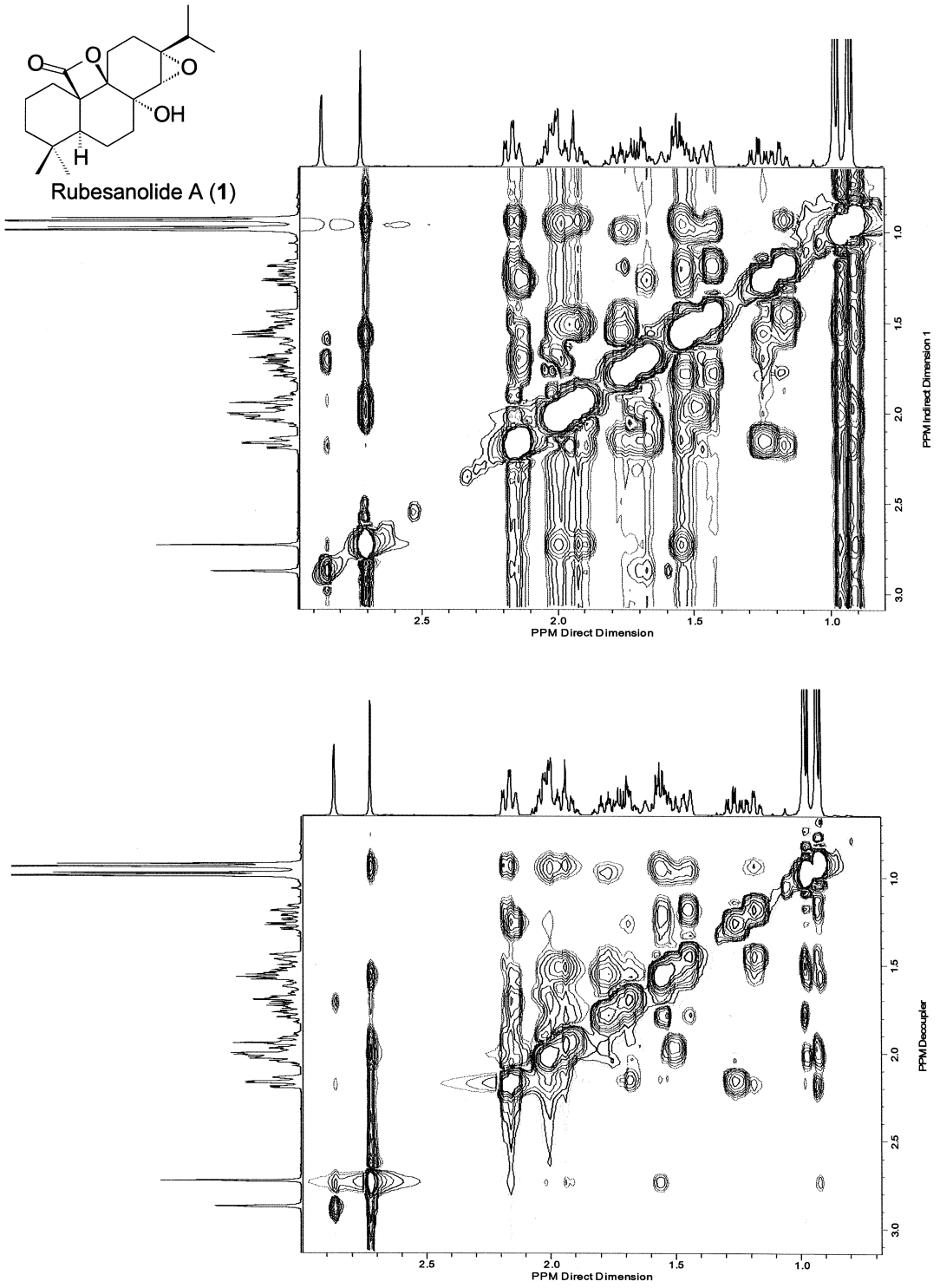


Figure S7. ROESY spectrum of rubesanolide A (**1**)

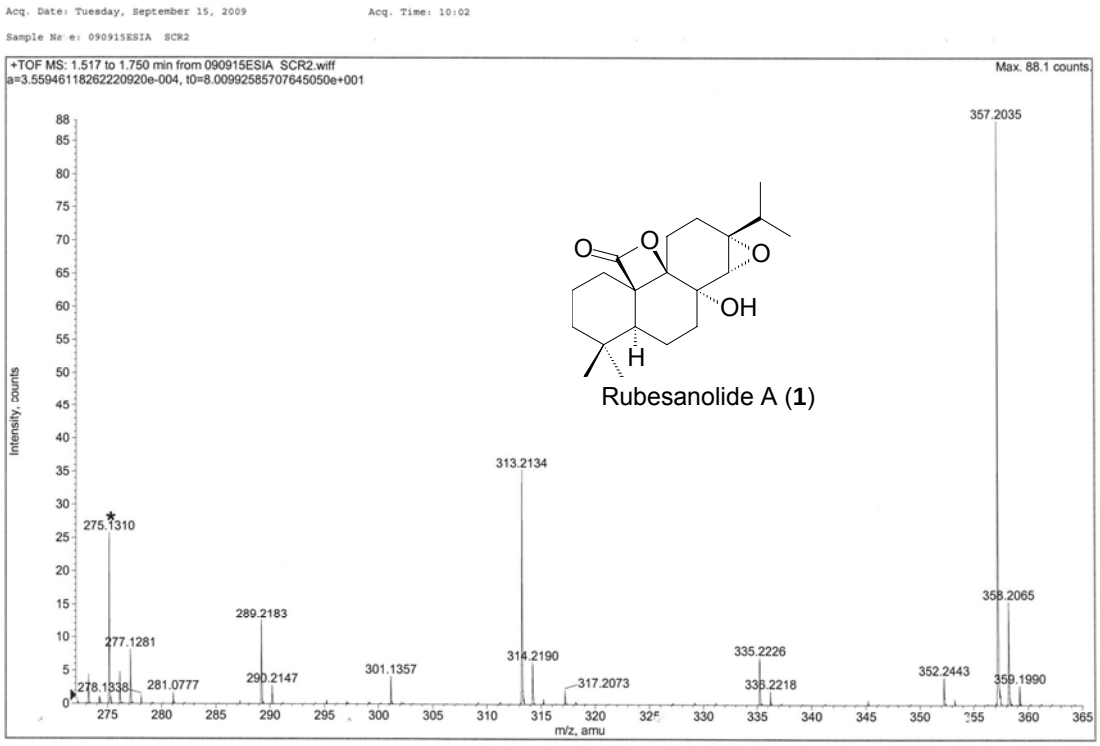
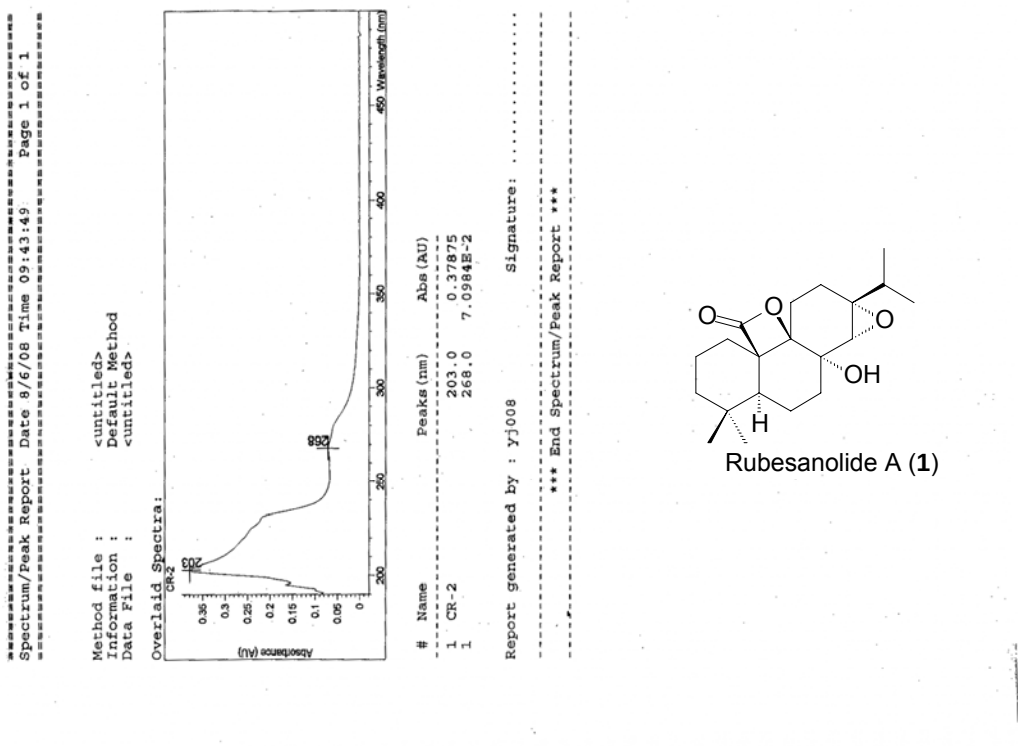
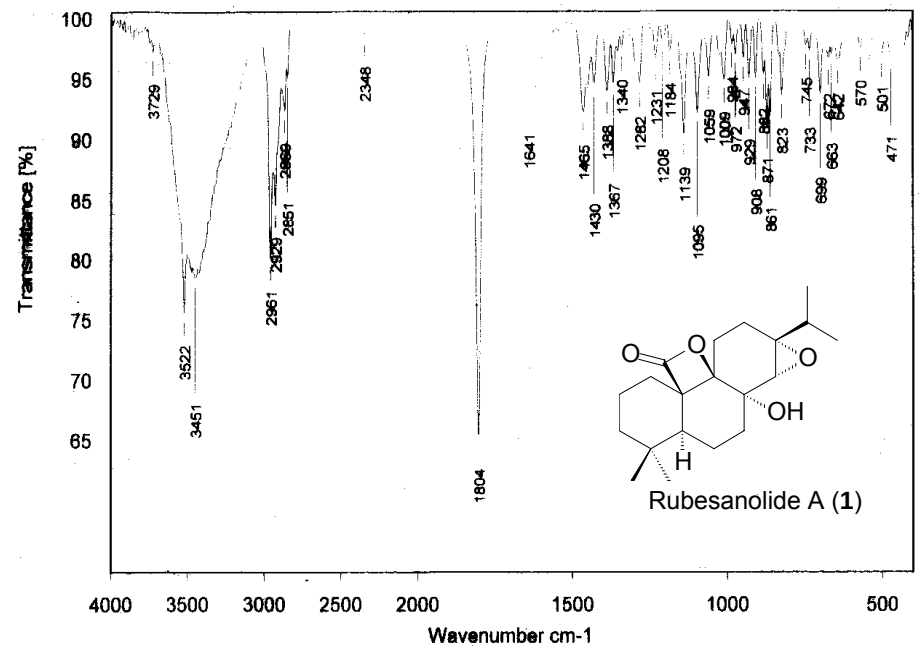


Figure S8. HRESIMS spectrum of rubesanolide A (**1**)



Rubesanolide A (**1**)

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



Path of File D:\OPUS\MEAS\CPX
Sample Name CR-2
Absolute Peak Pos in Laser*2 60693
Resolution 4
Number of Sample Scans 1

Filename 080806.1
Sample Form GYZYXY
Backward Peak Amplitude
Signal Gain, Sample Automatic
Scan time (sec) 0.712328

Figure S10. IR spectrum of rubesanolide A (**1**)

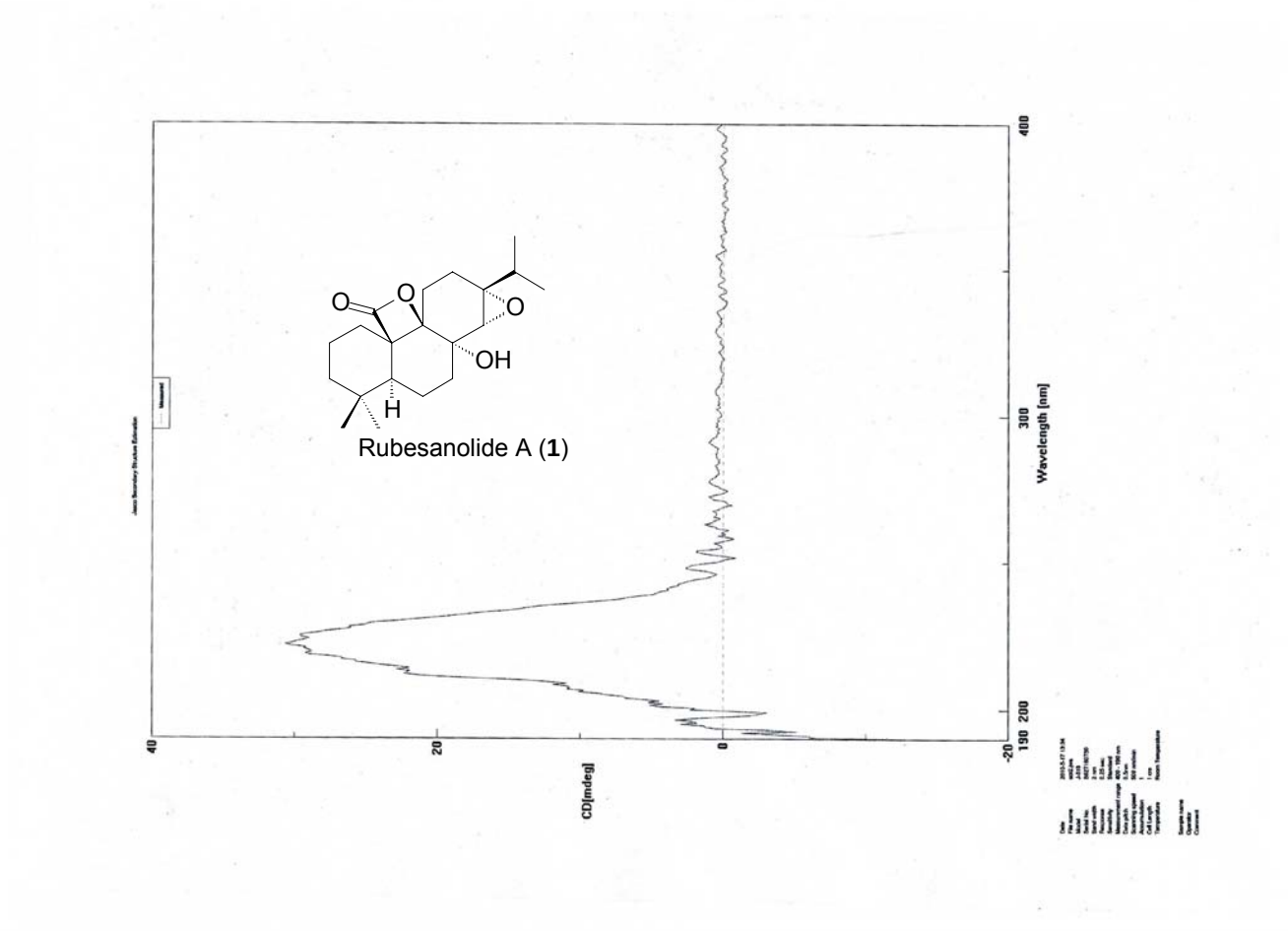


Figure S11. CD spectrum of rubesanolide A (**1**)

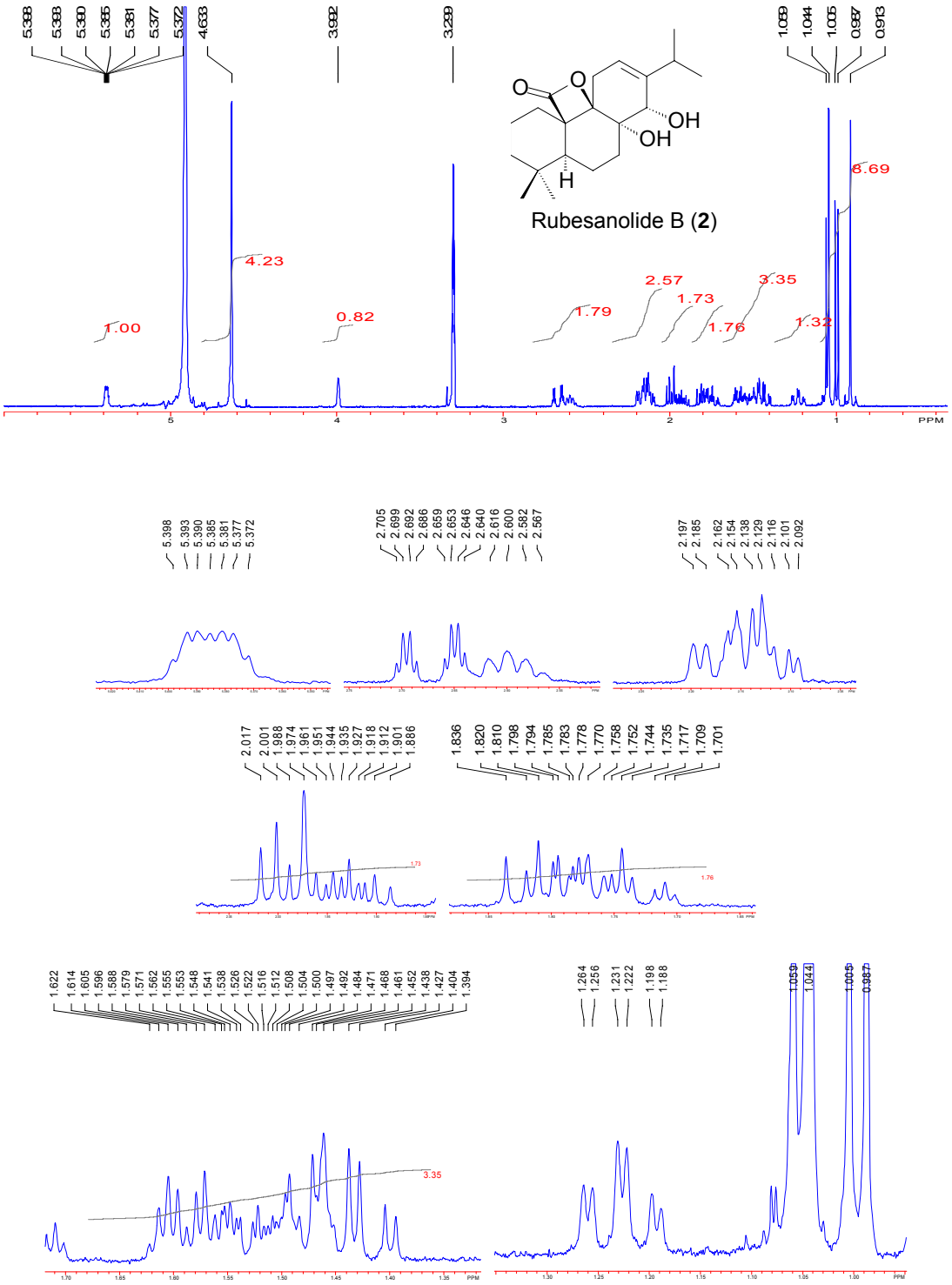


Figure S12. ^{1}H NMR spectrum of rubesanolide B (**2**)

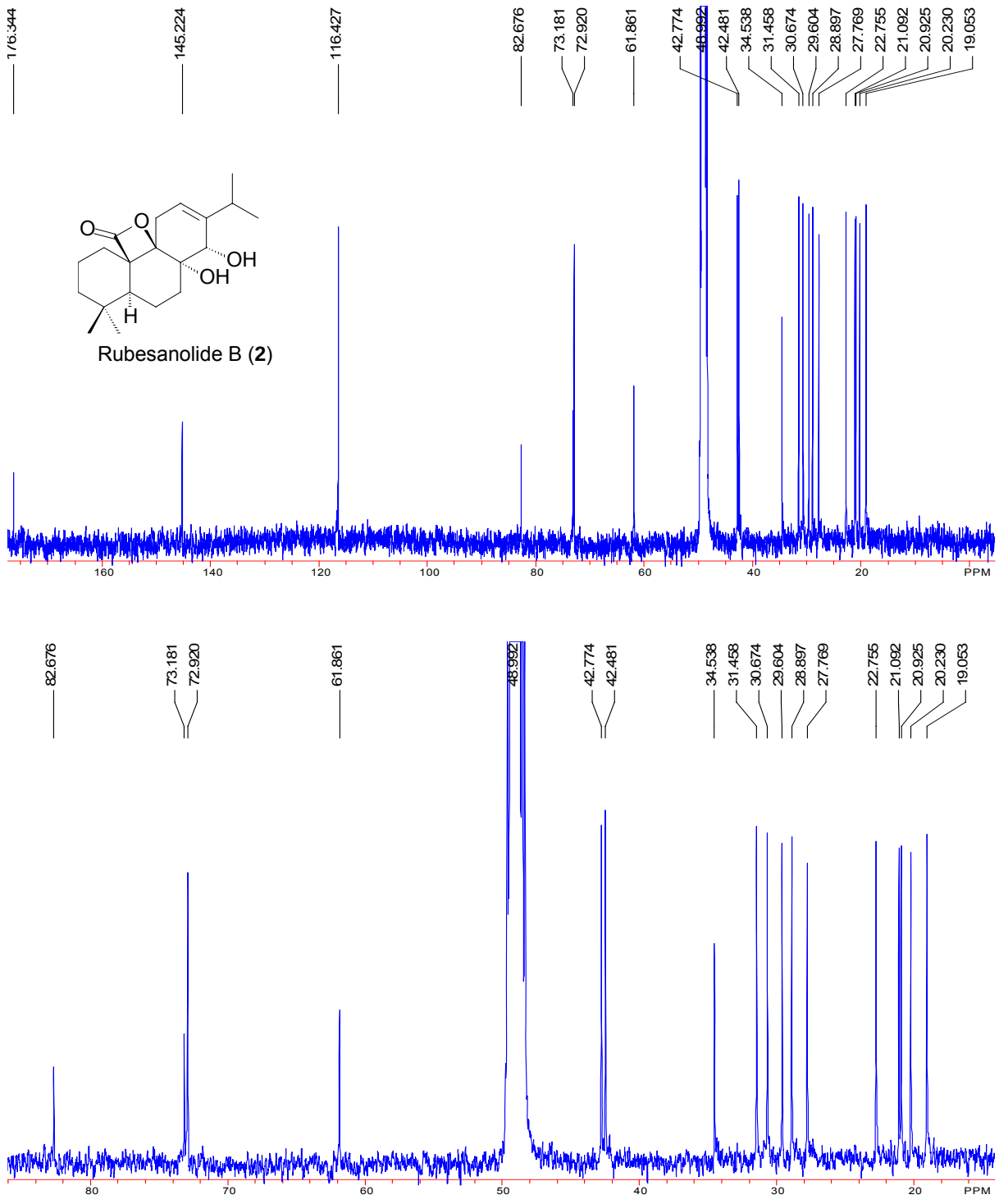
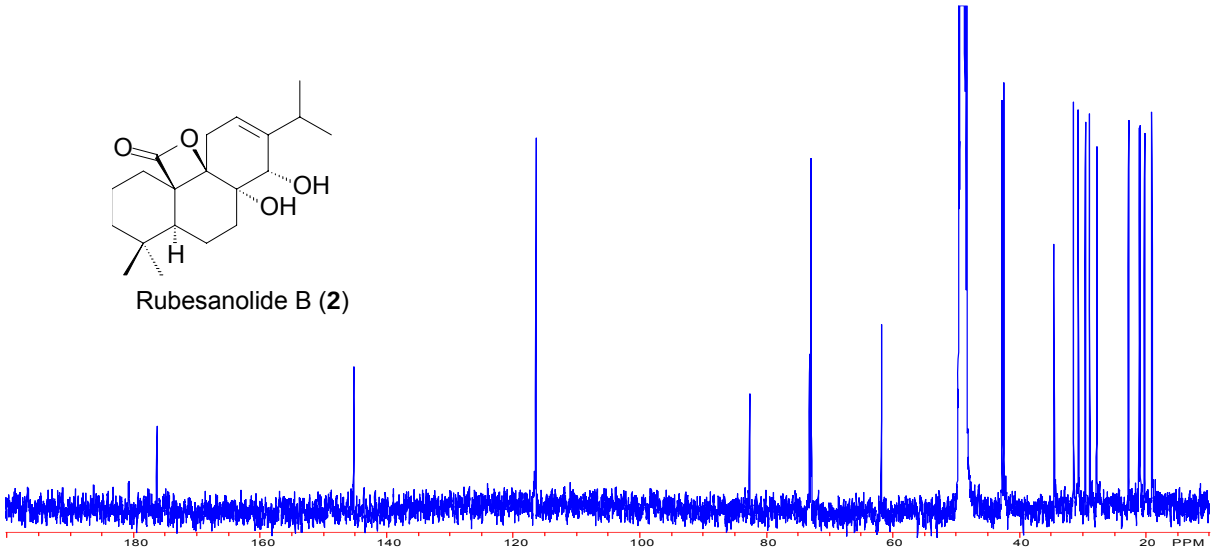


Figure S13. ^{13}C NMR spectrum of rubesanolide B (**2**)

A)



Rubesanolide B (**2**)

B)

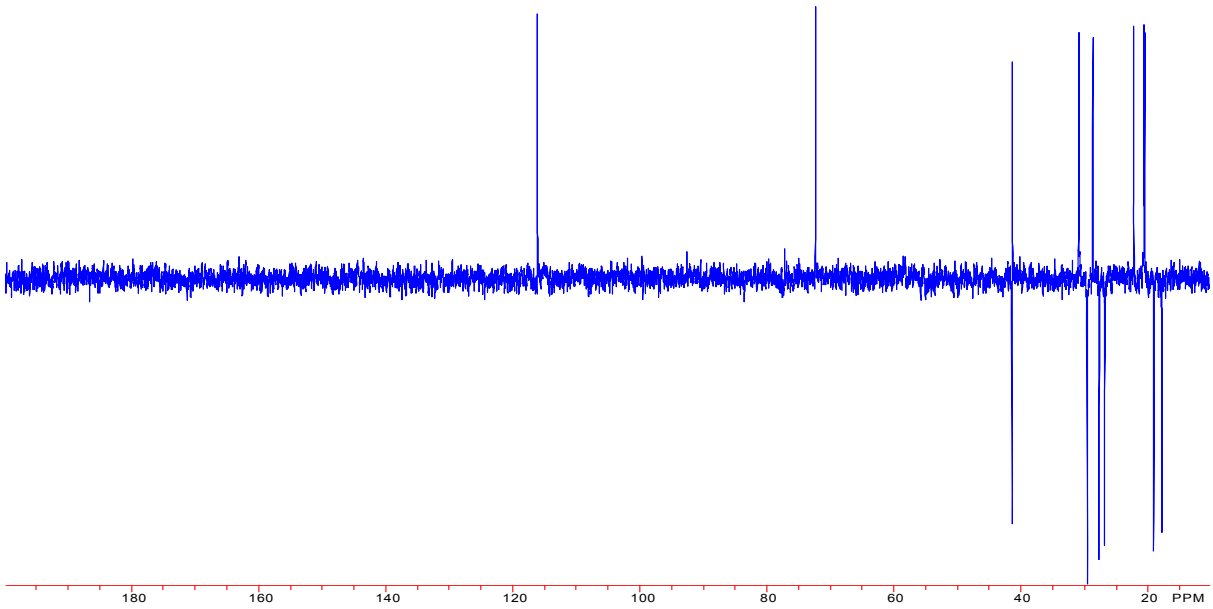


Figure S14. A) ^{13}C NMR, and B) DEPT-135 spectra of rubesanolide B (**2**)

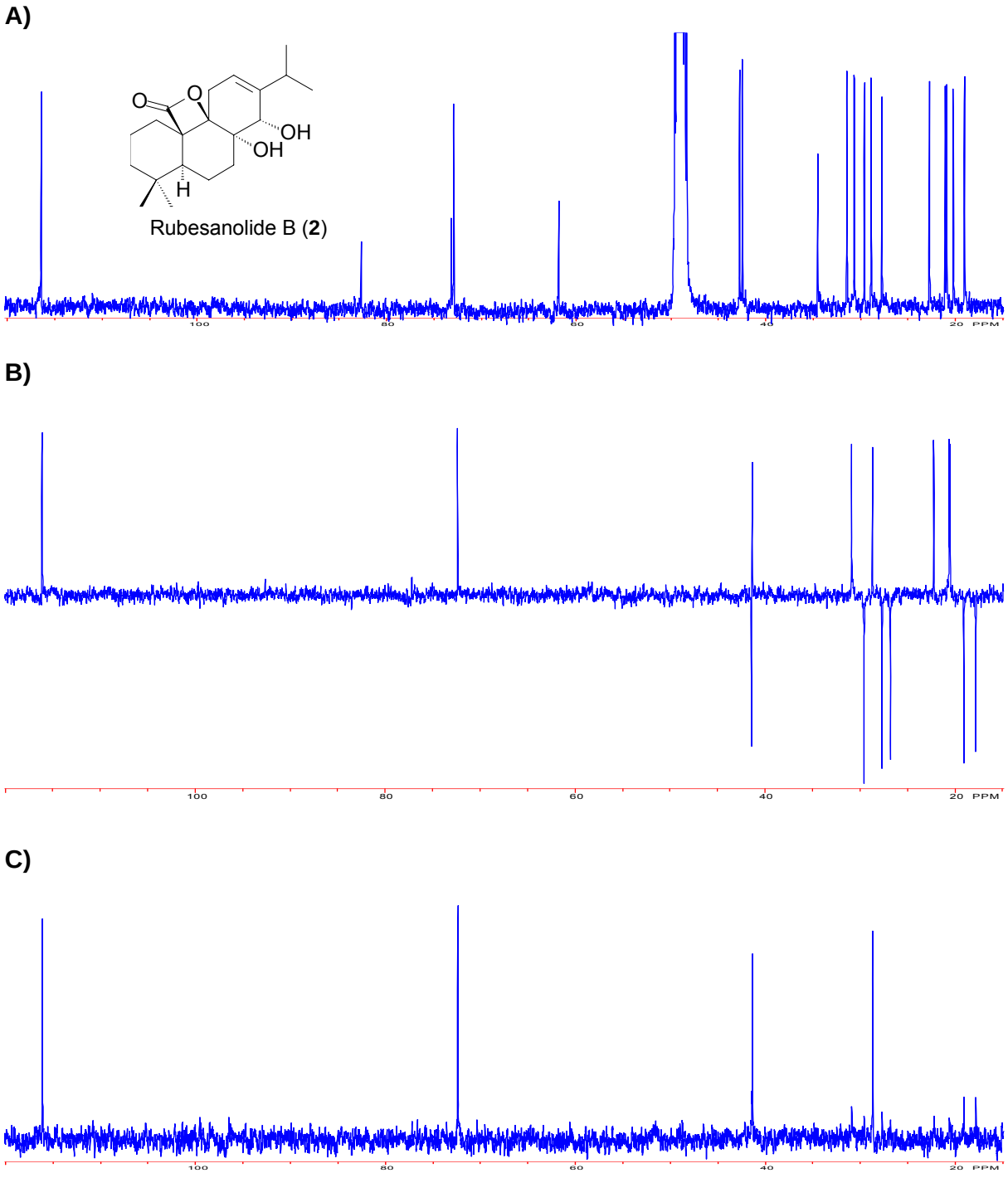


Figure S15. A) ^{13}C NMR, B) DEPT-135, and C) DEPT-90 spectra of rubesanolide B (**2**)

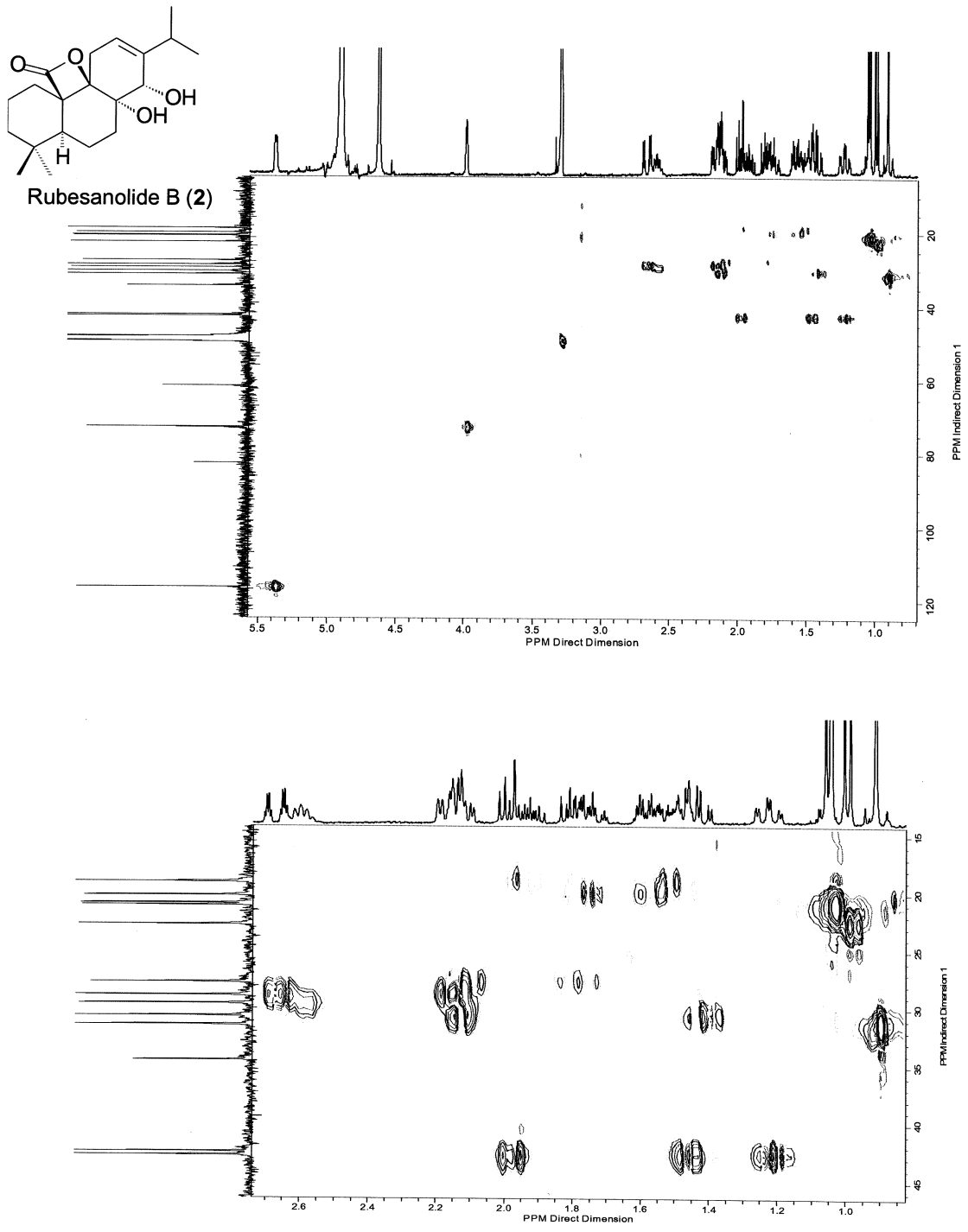


Figure S16. HMQC spectrum of rubesanolide B (**2**)

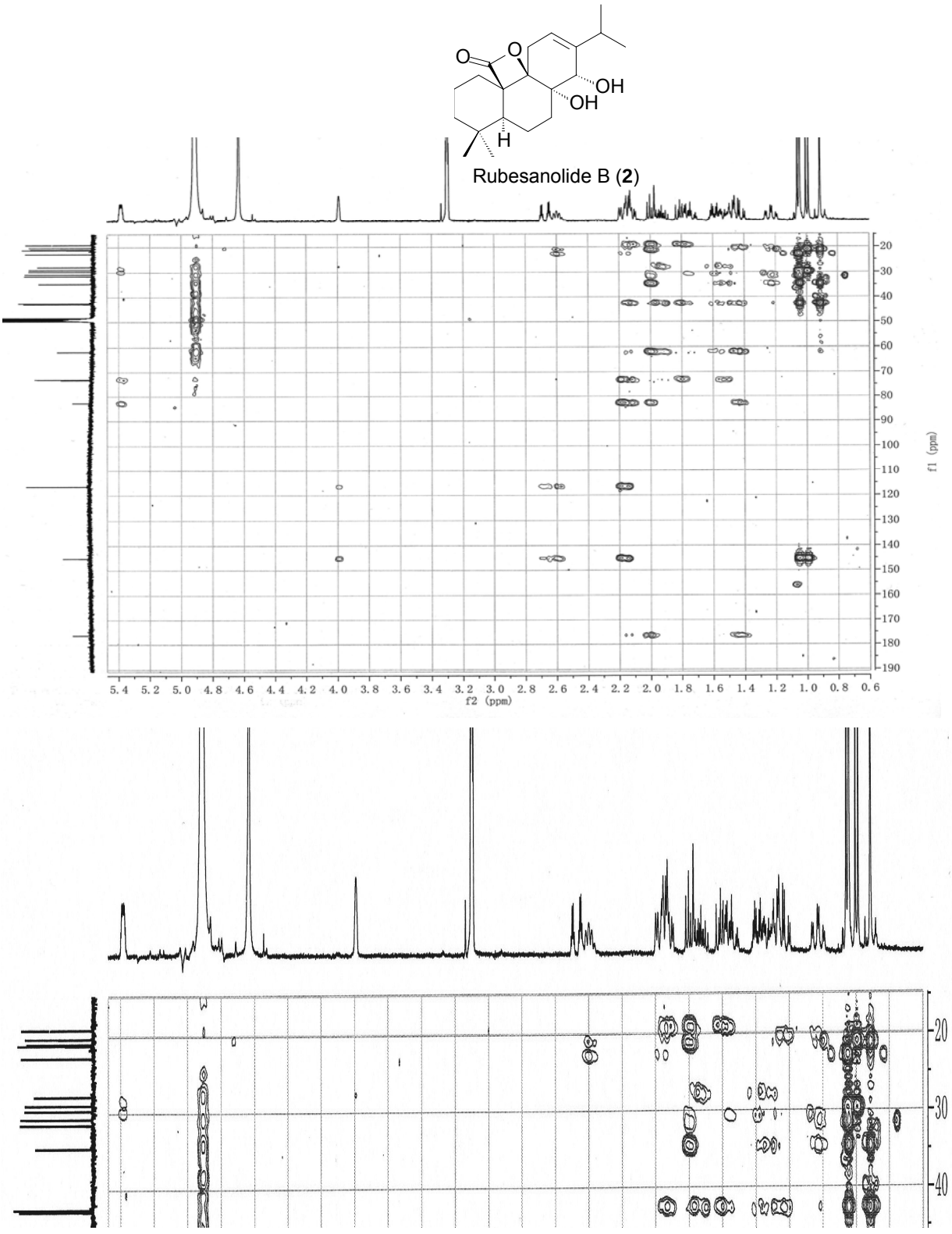


Figure S17. HMBC spectrum of rubesanolide B (**2**)

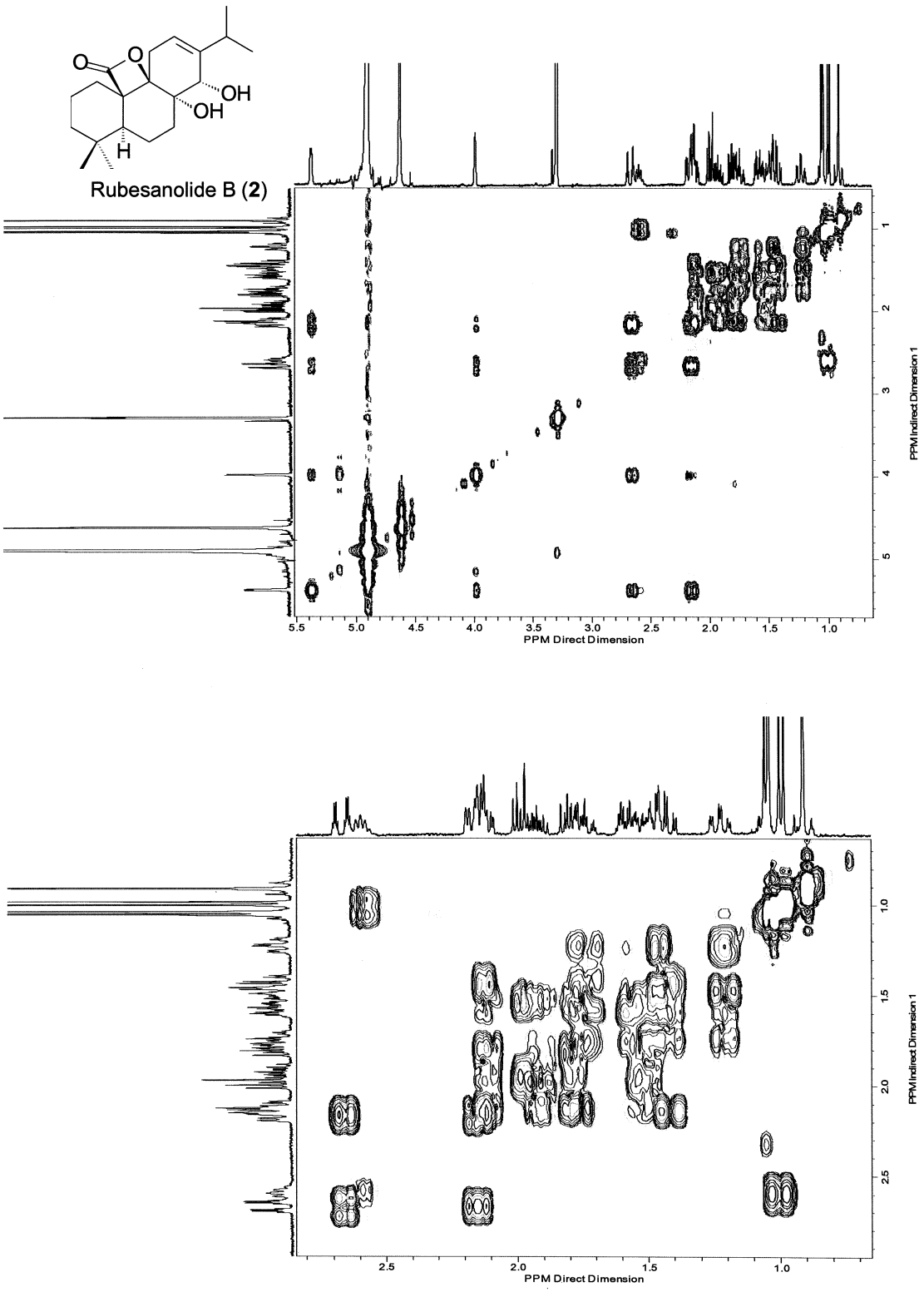


Figure S18. ^{1}H-^{1}H COSY spectrum of rubesanolide B (**2**)

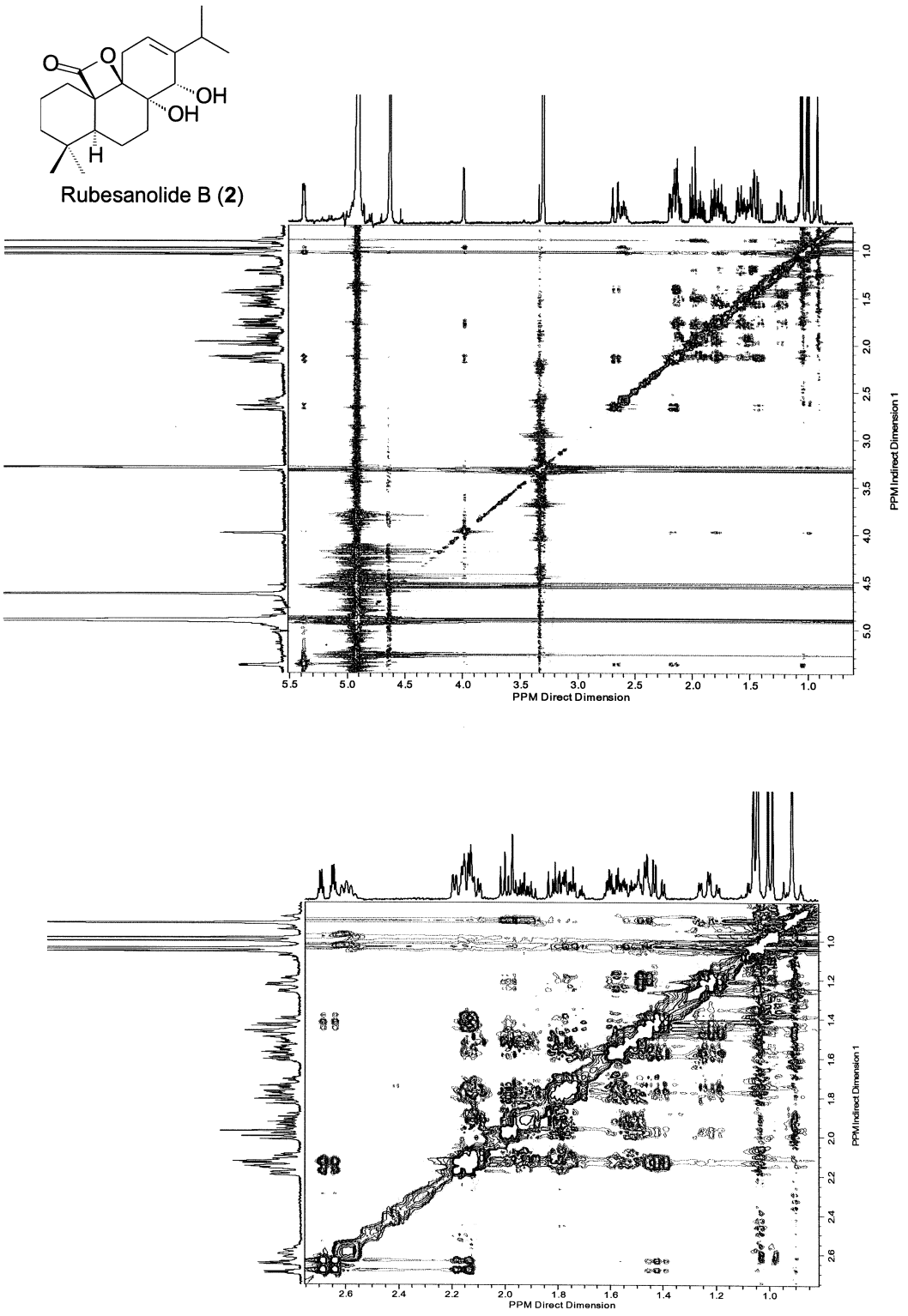


Figure S19. NOESY spectrum of rubesanolide B (**2**)

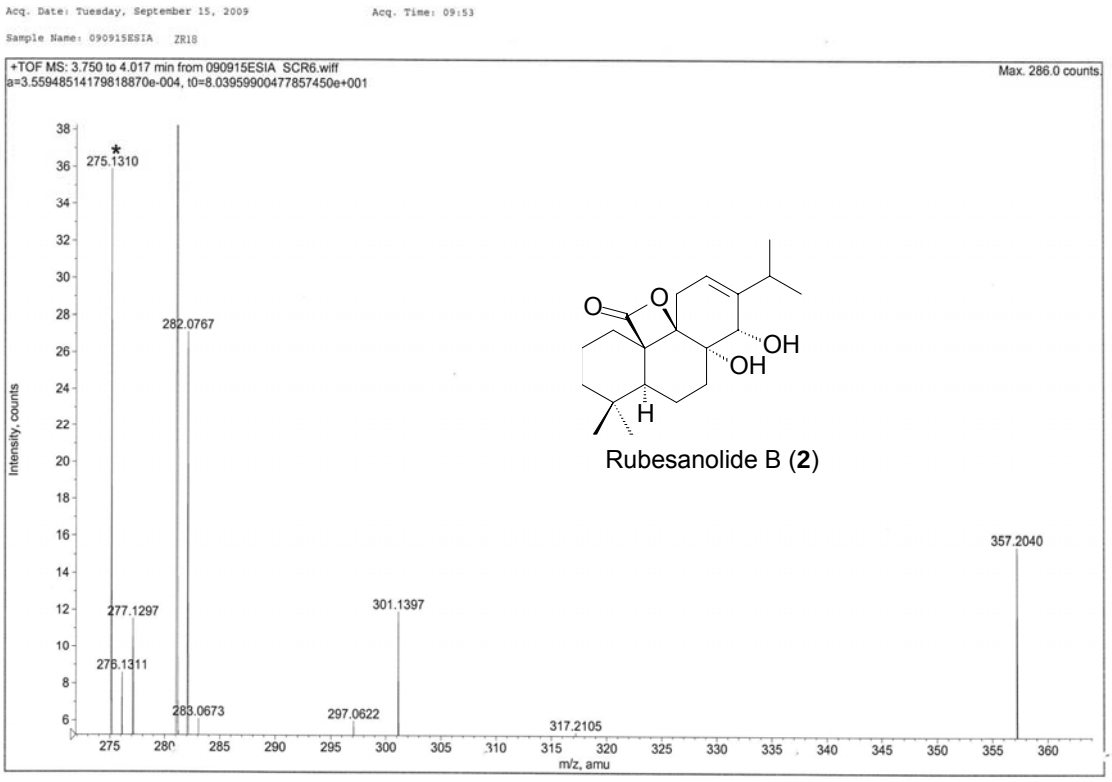
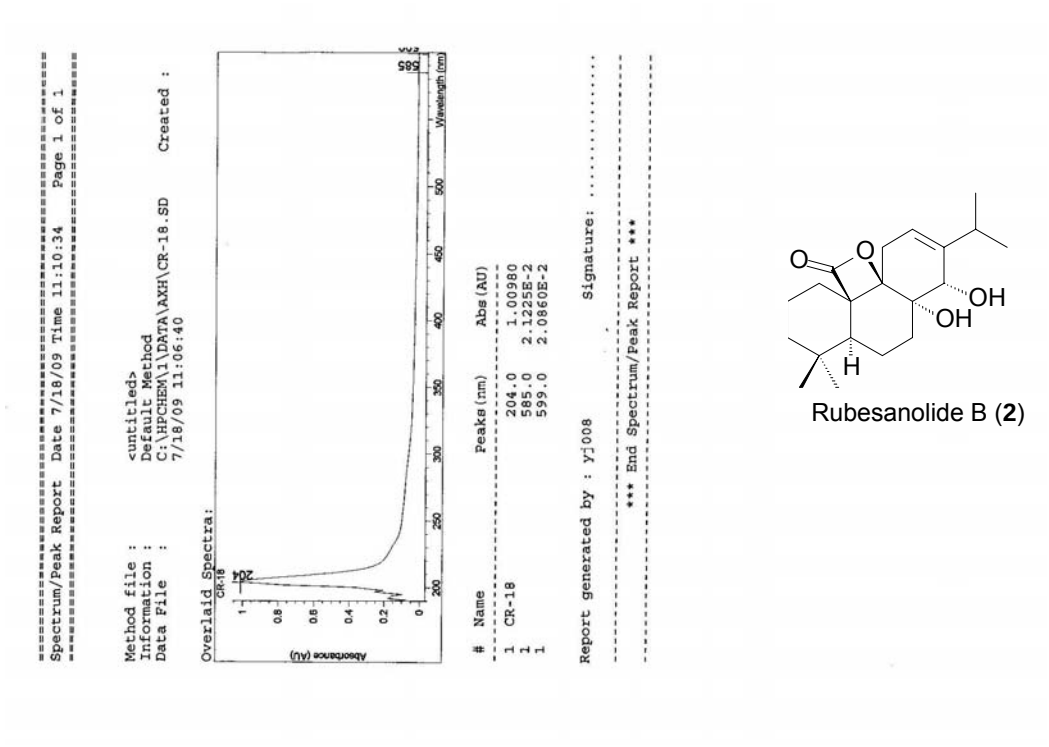


Figure S20. HRESIMS spectrum of rubesanolide B (**2**)



Rubesanolide B (**2**)

Figure S21. UV spectrum of rubesanolide B (**2**)

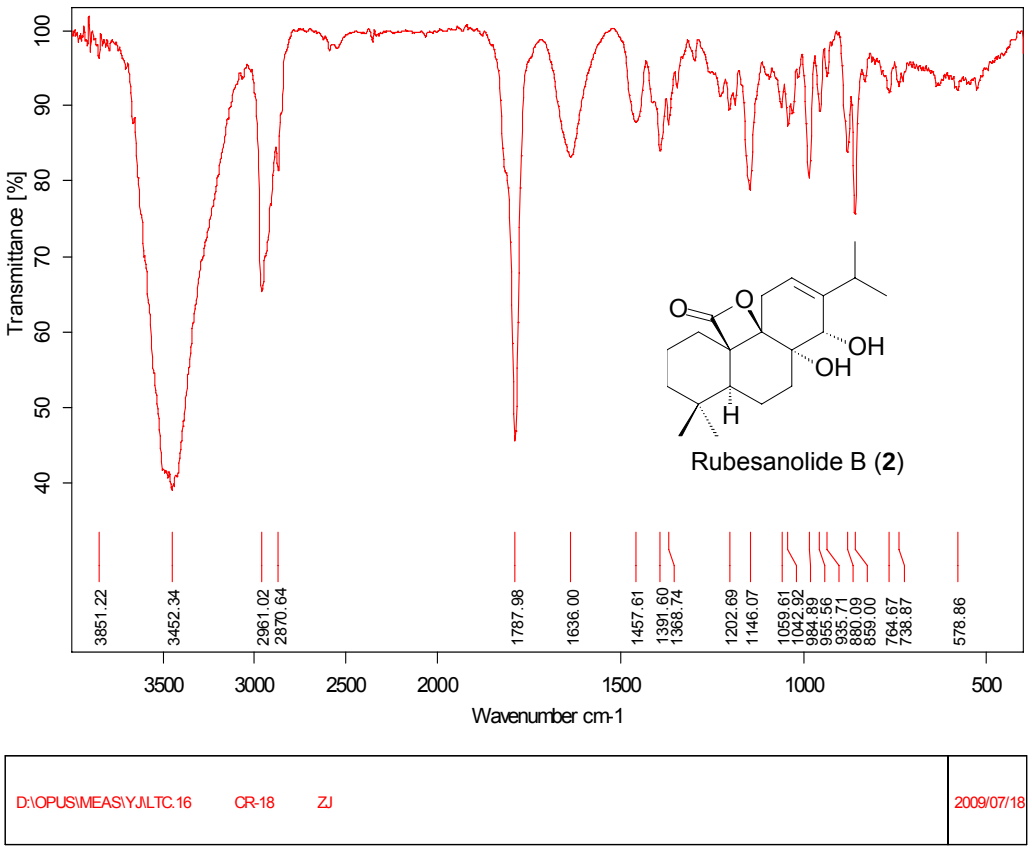
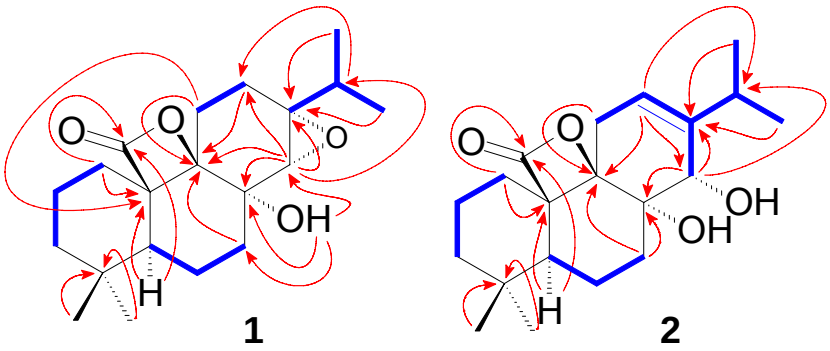
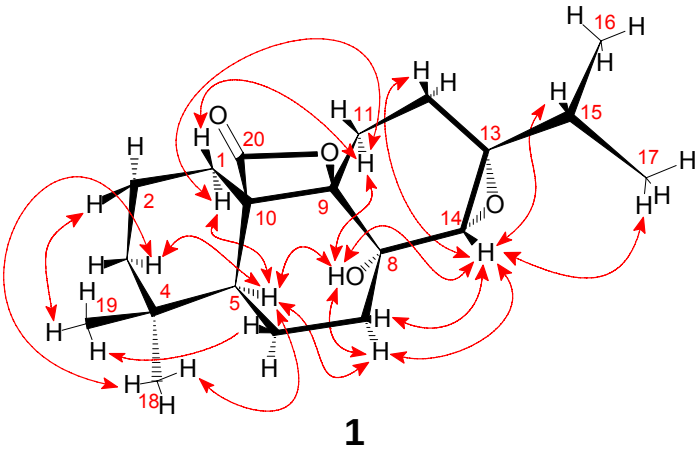


Figure S22. IR spectrum of rubesanolide B (**2**)

A)



B)



C)

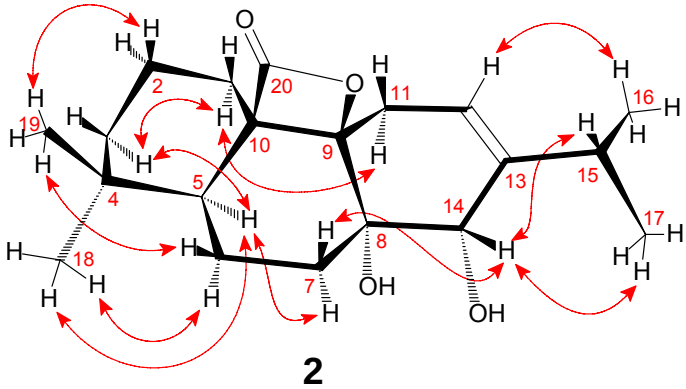


Figure S23. Key 2D correlations A) COSY (— in blue) and HMBC (→ in red) rubesanolides A (**1**) and B (**2**), B) ROESY of rubesanolide A (**1**), C) NOESY of rubesanolide B (**2**)

Detailed Experimental Procedures

1. General Experimental Procedures

Optical rotations were measured with a Rudolph digital polarimeter. UV data were obtained on a HP8453 spectrophotometer. VECTOR22 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400, DRX-500 and INOVA-400 spectrometers. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. High–resolution electrospray–ionization mass spectra (HRESIMS) were performed on a VG Autospec-3000 spectrometer under 70 eV. Column chromatography was performed with silica gel (200-300 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China). 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^o) were distilled prior to use.

2. Plant Material

The leaves of *Isodon rubescens* (Hemsl.) Hara were collected in Longli Prefecture, Guizhou Province, China, in October, 2006. The voucher specimen is deposited at Guiyang College of Traditional Chinese Medicine, Guiyang, Guizhou, China and was identified by Prof. Deyuan Chen.

3. Extraction and Isolation

The dried and milled plant material (8.5 kg) was extracted with 100% MeOH, and then concentrated in vacuo to give a crude extract (1450 g), which was treated with activated charcoal in MeOH to remove most of the chlorophylls. The filtered solution was concentrated and absorbed in silica gel, which was subjected to a silica gel column eluted with a gradient solvent system of petroleum ether/ethyl acetate and Et_2OAc/MeOH to yield five fractions. The petroleum ether/Et_2OAc 9:1 fraction was further separated by repeated silica gel column chromatography to afford rubesanolides A (1) (15mg) and B (2) (5mg).

Rubesanolide A (1): colorless laminate crystals (MeOH); mp 138~140^o; $[\alpha]_D^{12}$ +145.8^o (*c* 0.165, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.88) nm; CD (MeOH) (225 nm, $\Delta\varepsilon$+3.10); IR (KBr) λ_{max} 3451, 2961, 1804, 1641, 1430, 1367, 1208, 1139, 1096 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; positive EIMS showed a $[M]^+$ at *m/z* 334; HR-ESIMS ($[M+Na]^+$ *m/z* 357.2035, calcd 357.2041 for $C_{20}H_{30}O_4Na$).

Rubesanolide B (2): a white amorphous powder; $[\alpha]_D^{31}$ +15.1^o (*c* 0.531，MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.80) nm, IR (KBr) λ_{max} 3452, 2961, 1787, 1638, 1457, 1391, 1208, 1146, 1042 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; positive EIMS showed a $[M]^+$ at *m/z* 334; HR-ESIMS ($[M+Na]^+$ *m/z* 357.2040, calcd 357.2041 for $C_{20}H_{30}O_4Na$).

4. Evaluation of Biological Activity

NF-κB luciferase, aromatase, quinone reductase 1 (QR1), nitric oxide (NO) and cytotoxicity assays were conducted as previously described.

NF-κB Luciferase Assay: Yang, J. H.; Kondratyuk, T. P.; Marler, L. E.; Qiu, X.; Choi, Y. S.; Cao, H. M.; Sturdy, M.; Pegan, S.; Liu, Y,; Wang, L. Q.; Mesecar, A. D.; van Breemen, R. B.; Pezzuto, J, M.; Fong, H. H. S.; Chen, Y. G.; Zhang, H. J. *Phytochemistry* **2010**, *71*, 641-647.

Aromatase Assay: Yang, J. H.; Kondratyuk, T. P.; Marler, L. E.; Qiu, X.; Choi, Y. S.; Cao, H. M.; Sturdy, M.; Pegan, S.; Liu, Y,; Wang, L. Q.; Mesecar, A. D.; van Breemen, R. B.; Pezzuto, J, M.; Fong, H. H. S.; Chen, Y. G.; Zhang, H. J. *Phytochemistry* **2010**, *71*, 641-647.

Nitric Oxide (NO) Assay: Yang, J. H.; Kondratyuk, T. P.; Marler, L. E.; Qiu, X.; Choi, Y. S.; Cao, H. M.; Sturdy, M.; Pegan, S.; Liu, Y,; Wang, L. Q.; Mesecar, A. D.; van Breemen, R. B.; Pezzuto, J, M.; Fong, H. H. S.; Chen, Y. G.; Zhang, H. J. *Phytochemistry* **2010**, *71*, 641-647.

Quinone Reductase 1 (**QR1) Assay:** Homhual, S.; Zhang, H. J.; Bunyapraphatsara, N.; Kondratyuk, T.; Santarsiero, B. D.; Mesecar, A. D.; Herunsalee, A.; Chaukul, W.; Pezzuto, J. M.; Fong, H. H. S. *Planta Med.* **2006**, *72*, 255-260.

Cytotoxicity Assay of A549 and K562 Cell Lines: Niu, X. M.; Li, M. L.; Zhao, Q. S.; Mei, S. X.; Na, Z.; Wang, S. J.; Lin, Z. W.; Sun, H. D. *Planta Med*. **2002**, *68*, 528-533.

Cytotoxicity Assay of Hela and MCF7 Cell Lines: Jutiviboonsuk, A.; Zhang, H. J.; Tan, G. T.; Ma, C. Y.; Hung, N. V.; Cuong, N. M.; Bunyapraphatsara, N.; Soejarto, D. D.; Fong, H. H. S. *Phytochemistry* **2005**, *66*, 2745-2751.