Supporting Information

Pestaloporonins: Caryophyllene-Derived Sesquiterpenoids from a Fungicolous Isolate of *Pestalotiopsis* sp.

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General Experimental Procedures. Optical rotations were measured on an AUTOPOL® III automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ). UV data were obtained using a Varian Cary III UV/vis spectrophotometer, and ECD data were recorded with an Olis Cary-17 spectrophotometer (1-cm cell). HPLC separations were carried out using a Beckman System Gold instrument with a model 166P variable-wavelength UV detector connected to a 128 solvent module, equipped with either a preparative Dynamax C18 column (Rainin, 2.0 × 30 cm, 8 μm) or semi-preparative Apollo C18 column (Alltech, 1.5 × 25 cm, 5 μm) under UV detection at 210 nm. ^{1}H NMR spectra were recorded using Bruker DRX-400 or AVANCE-600 spectrometers. ^{13}C (150 MHz) and 2D (COSY, HSQC, HMBC, NOESY) NMR spectra were recorded on the Bruker AVANCE-600 spectrometer. ^{13}C NMR multiplicities were not directly measured, but all assignments were fully consistent with HSQC data. All NMR experiments were performed at 294 K, using methanol-d_4 or acetone-d_6 as the solvent. Chemical shifts were referenced to residual solvent signals for methanol-d_4 (δ_H/δ_C, 3.31/49.0) or acetone-d_6 (δ_H/δ_C, 2.05/29.8). NMR data processing was carried out with MestReNova v6.0.2 software. HRESITOFMS data were obtained on a Waters Q-ToF Premier mass spectrometer.

Fungal Material. *Pestalotiopsis* sp. MYC-709 was isolated from a basidioma of *Stereum complicatum* found on a dead hardwood branch in FDR State Park, Warm Springs, Georgia, USA. Fermentation was carried out in six 500-mL Erlenmeyer flasks, each containing 50 g of rice. Distilled H_2O (50 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in.^{2} for 30 min. The flasks were cooled to room temperature, inoculated with 3 mL of spore suspension, and incubated for 30 days at 25°C. After incubation, the fermented rice substrate was mechanically fragmented and then extracted repeatedly with EtOAc (3 × 100 mL) per flask. The combined EtOAc extracts were filtered and evaporated to give 687 mg of an oily residue that was employed for the isolation

processes described below. Efforts to deposit this organism in the NRRL culture collection at the USDA National Center for Agricultural Utilization Research led to the realization that the culture was no longer viable. However, NMR analysis of extract fractions from another, already-deposited *Pestalotiopsis* culture in our laboratory from which biogenetically related punctaporonin analogues had been isolated (*P. disseminata* NRRL 36915, Genbank deposition number GU183121.1; another fungicolous isolate also collected from Georgia) revealed the presence of the same compounds. This result indicated that *Pestalotiopsis* sp. MYC-709 was likely *P. disseminata*, although this could not be definitively established. The procedure described below was that used for the MYC-709 extract, but the same compounds could be produced by cultivation of the NRRL 36915 culture deposited at the USDA NCAUR.

Extraction and Isolation. The EtOAc extract (687 mg) was partitioned between hexane (3 × 8 mL) and MeCN (8 mL). The MeCN–soluble portion (388 mg) was subjected to silica gel column chromatography (3.2 × 16 cm, 63–200 μm particles) eluting with a stepwise gradient of hexane/EtOAc (200 mL each of 1:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:1), followed by pure MeOH (300 mL) to afford nine fractions after combination based on TLC data (Fr. 1 to 9). Fr. 9 (157 mg) was fractionated over C_{18} HPLC (2.0 × 30 cm, 8 mL/min) with 15% MeCN/H_2O over 30 min to obtain six subfractions (Fr. 9-1 to 9-6). Fr. 9-4 (2.2 mg) was further divided into two fractions (Fr. 9-4-1 and 9-4-2) using C_{18} HPLC (2.0 × 30 cm, 8 mL/min) with 15% MeCN/H_2O over 30 min. Fr. 9-4-2 (1.5 mg) was purified by C_{18} HPLC (1.0 × 25 cm, 2 mL/min) using 13% MeCN/H_2O for 30 min to give **1** (0.9 mg, t_R = 26.7 min). Fr. 9-6 (1.7 mg) was subjected to C_{18} HPLC (2.0 × 30 cm, 8 mL/min) with 15% MeCN/H_2O for 30 min to yield **2** (0.9 mg, t_R = 29.6 min). Compound **3** (2.8 mg, t_R = 26.8 min) was isolated from Fr. 5 (28 mg) by C_{18} HPLC (1.0 × 25 cm, 2 mL/min) eluting with 30% MeCN/H_2O for 15 min, followed by a linear increase to 60% MeCN over 45 min.

X-ray Crystallographic Analysis of Pestaloporonin A (1). A colorless plate (0.04 x 0.03 x 0.01 mm) was isolated from the sample crystallized from dichloromethane. Intensity data were collected at 150K on a D8 goniostat equipped with a Bruker APEXII CCD detector at Beamline 11.3.1 at the Advanced Light Source (Lawrence Berkeley National Laboratory) using synchrotron radiation tuned to λ=1.2399Å. Data collection frames were measured for a duration of 1-s at 0.3° intervals of ω with a maximum 2θ value of ~60°. The data frames were collected using the program APEX2 and processed using the SAINT routine within APEX2.[1] The data were corrected for absorption and beam corrections were based on the multi-scan technique as implemented in SADABS.[2] The structure contains a water molecule involved in the hydrogen bonding pattern. All non-hydrogen atoms were refined anisotropically with all hydrogen atoms (except the water H-atoms) included in calculated positions with the riding model. The water molecule hydrogen atoms were refined isotropically with the O-H distance restrained to be 0.85(5)Å. The absolute configuration was assigned based on the refined Flack parameter, x = 0.1(4).

Notes

(1) APEX2 v2013.6.2 and SAINT v8.32B data collection and data processing programs, respectively. Bruker Analytical X-ray Instruments, Inc., Madison, WI.

(2) SADABS v2012/1 semi-empirical absorption and beam correction program. G.M. Sheldrick, University of Göttingen, Germany.

Table S1. Crystal Data and Structure Refinement for Pestaloporonin A (**1**)

Identification code	Glo13_6
Empirical formula	C16 H26 O6
Formula weight	314.37
Temperature	150(2) K
Wavelength	1.23990 A
Crystal system, space group	Monoclinic, P 21
Unit cell dimensions	a = 6.5502(13) A alpha = 90 deg. b = 13.639(3) A beta = 91.21(3) deg. c = 9.1291(18) A gamma = 90 deg.
Volume	815.4(3) A^3
Z, Calculated density	2, 1.280 Mg/m^3
Absorption coefficient	0.408 mm^-1
F(000)	340
Crystal size	0.04 x 0.03 x 0.01 mm
Theta range for data collection	4.7 to 50.6 deg.
Limiting indices	-8<=h<=8, -16<=k<=16, -10<=l<=11
Reflections collected / unique	5979 / 2855 [R(int) = 0.0565]
Completeness to theta = 50.60	96.4 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9959 and 0.9839
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2855 / 3 / 211
Goodness-of-fit on F^2	1.051
Final R indices [I>2sigma(I)]	R1 = 0.0499, wR2 = 0.1342
R indices (all data)	R1 = 0.0529, wR2 = 0.1375
Absolute structure parameter	0.1(4)
Largest diff. peak and hole	0.310 and -0.229 e.A^-3

Table S2. ^{1}H and ^{13}C NMR Data for Pestaloporonin C (**3**) in Acetone-d_6

Position	δ_C[a]	δ_H, mult. (J in Hz)[b]
1	137.1	–
2	51.8	3.63, dd (9.7, 5.4)
3a	35.4	2.26, dd (15.6, 5.4)
3b		2.23, dd (15.6, 9.7)
4	143.5	–
5	217.1	–
6	76.7	4.69, dd (11.2, 6.0)
7a	48.8	2.66, dd (11.2, 6.0)
7b		2.39, t (11.2)
8	136.7	–
9	129.8	4.85, br d (10.3)
10	76.7	4.67, br d (10.3)
11	133.1	5.80, br s
12a	64.3	4.23, d (13.5)
12b		3.94, d (13.5)
13	22.6	1.76, s
14a	112.1	4.80, br s
14b		4.71, br s
15	18.4	2.11, d (1.3)
OCH_3	55.5	3.23, s

[a]Data were collected at 600 MHz. [b]Data were collected at 150 MHz.

Figure S1. ^{1}H NMR Spectrum of Pestaloporonin A (**1**; 600 MHz, methanol-d_4)

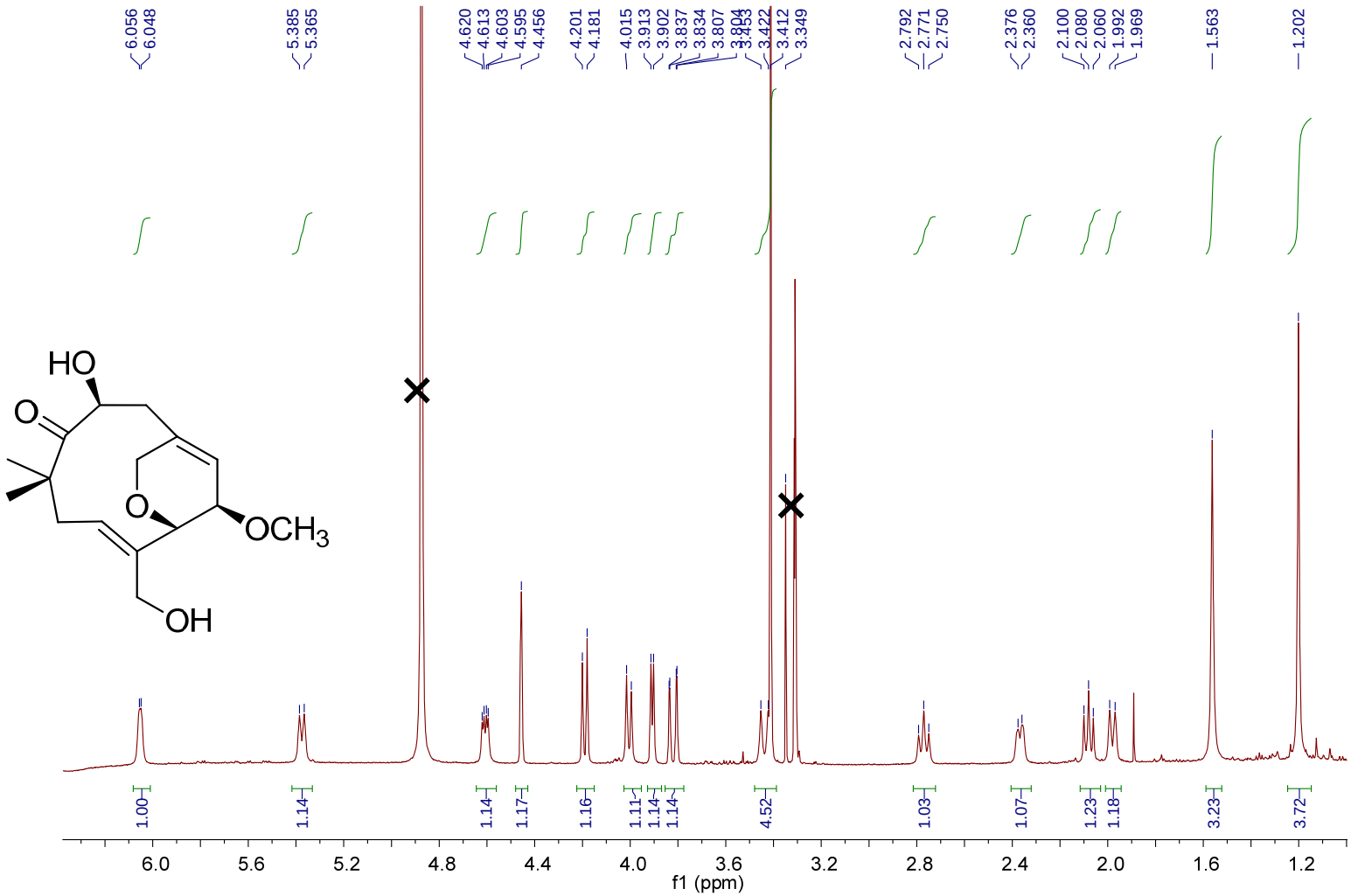
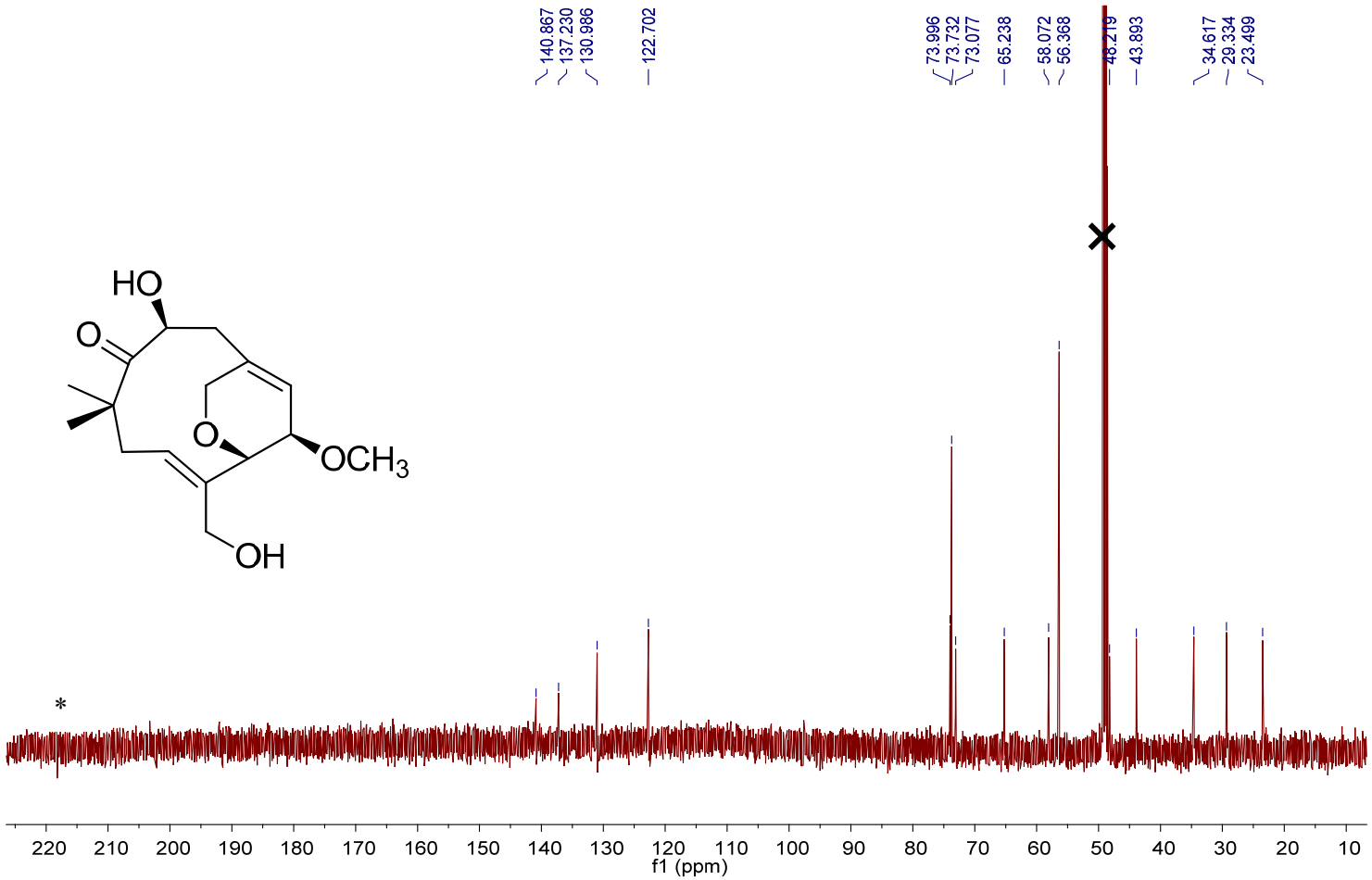


Figure S2. ^{13}C NMR Spectrum of Pestaloporonin A (**1**; 150 MHz, methanol-*d*4)



*Ketone signal too weak to be observed here, but was assigned on the basis of strong HMBC correlations (see Figure S3)

Figure S3. HMBC Spectrum of Pestaloporonin A (**1**; 600 MHz, methanol-*d*4)

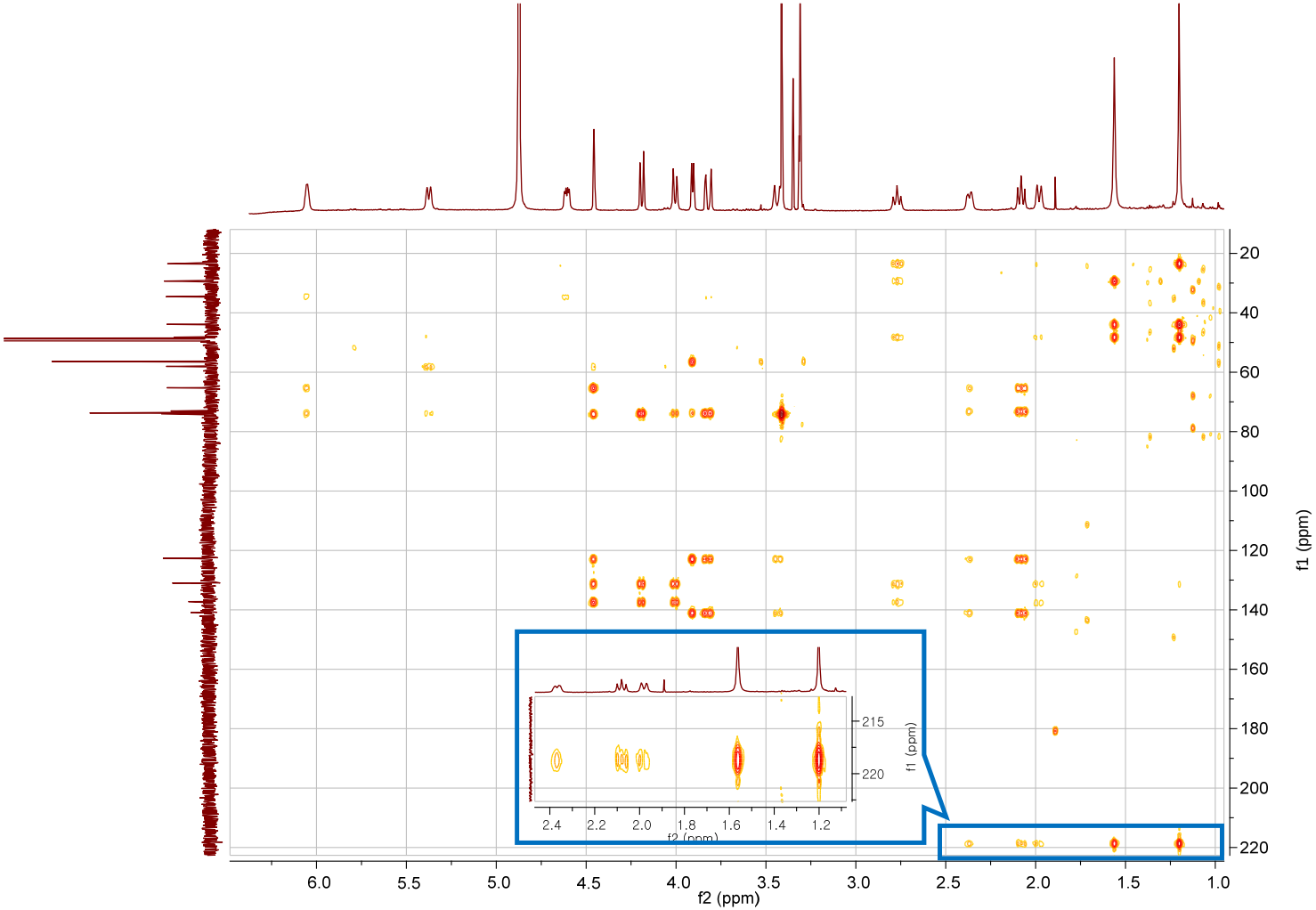


Figure S4. ECD Spectrum of Pestaloporonin A (**1**) in Methanol

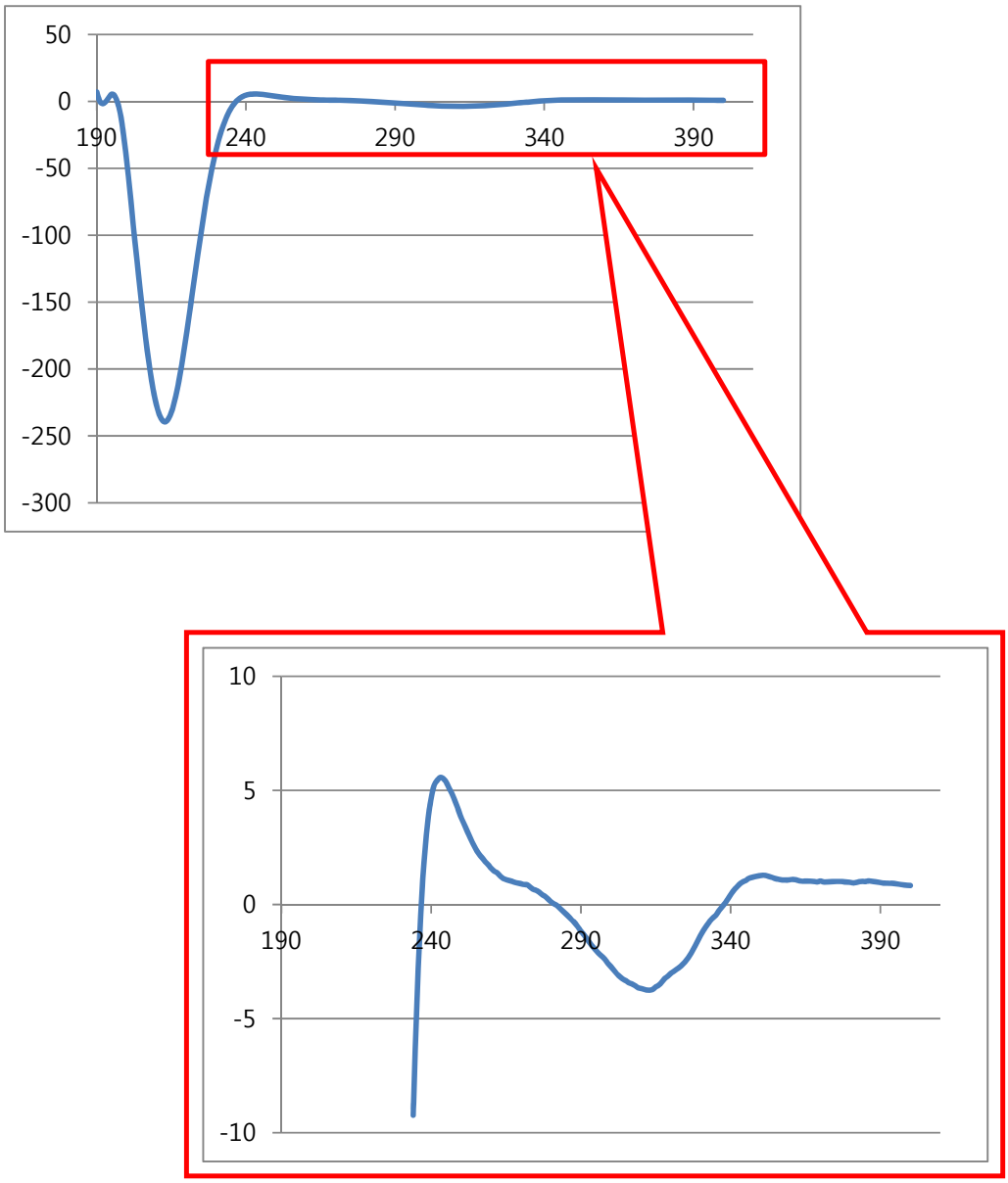


Figure S5. ^{1}H NMR Spectrum of Pestaloporonin B (**2**; 600 MHz, methanol-d_4)

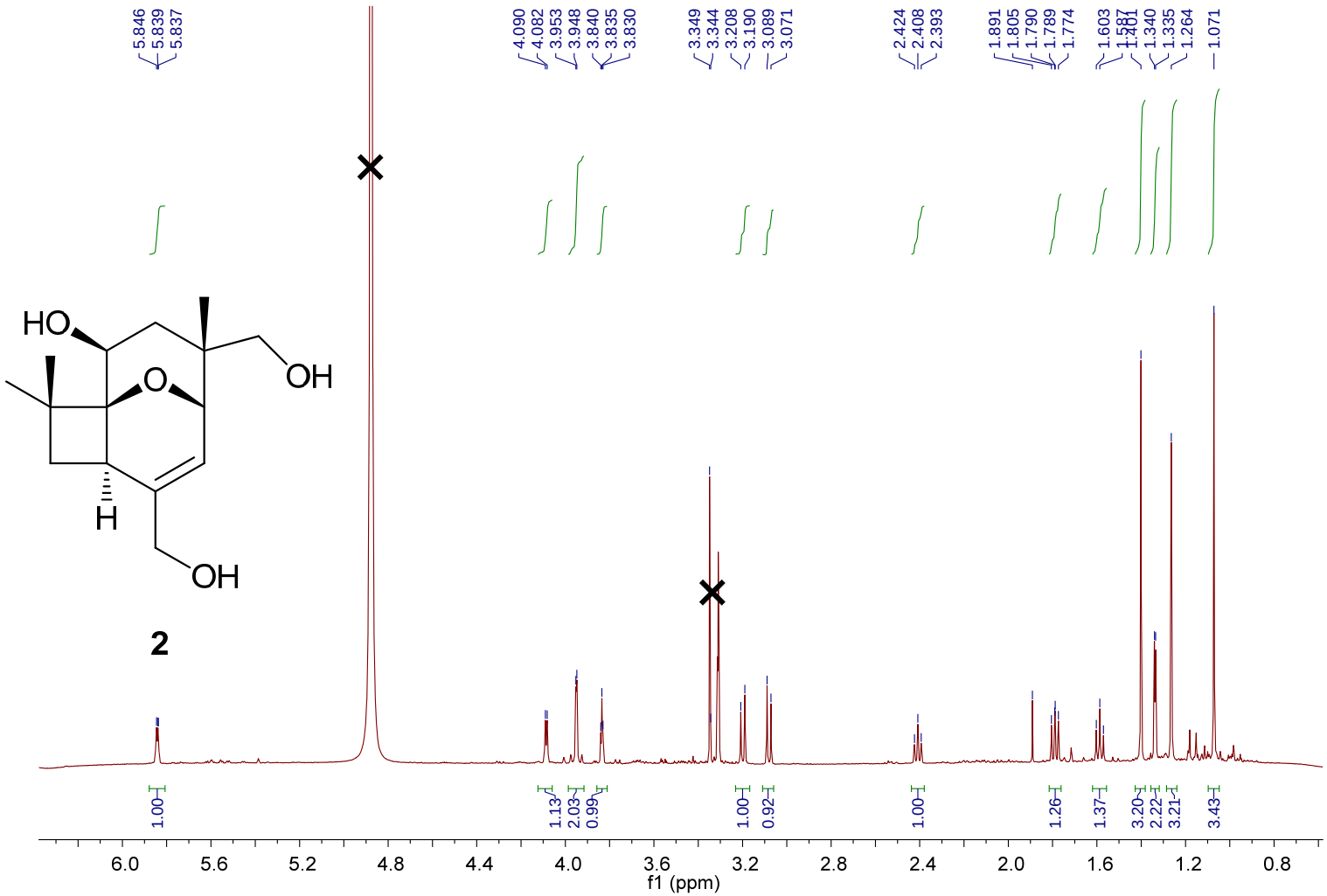


Figure S6. ^{13}C NMR Spectrum of Pestaloporonin B (**2**; 150 MHz, methanol-*d*4)

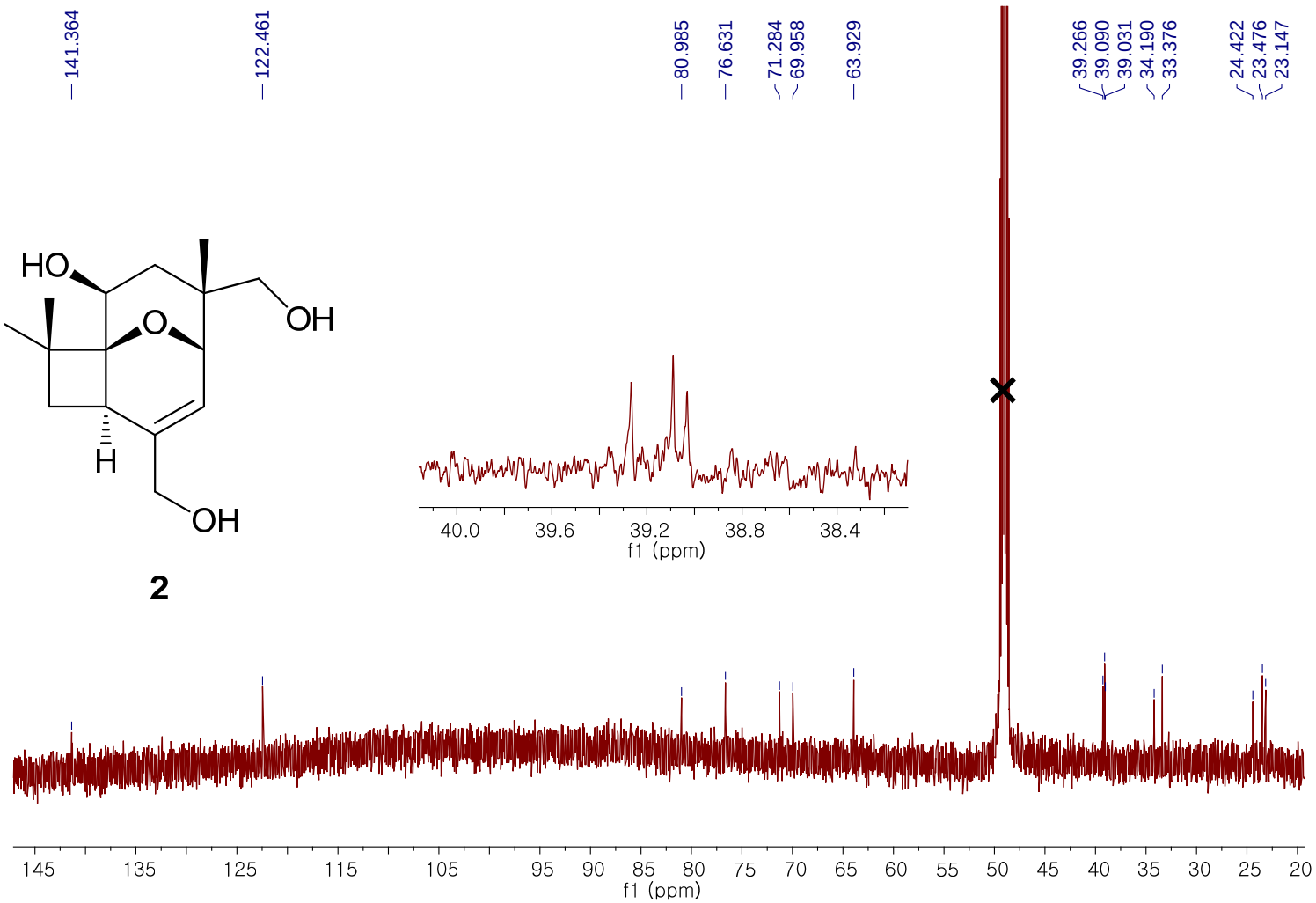


Figure S7. HMBC Spectrum of Pestaloporonin B (**2**; 600 MHz, methanol-*d*4)

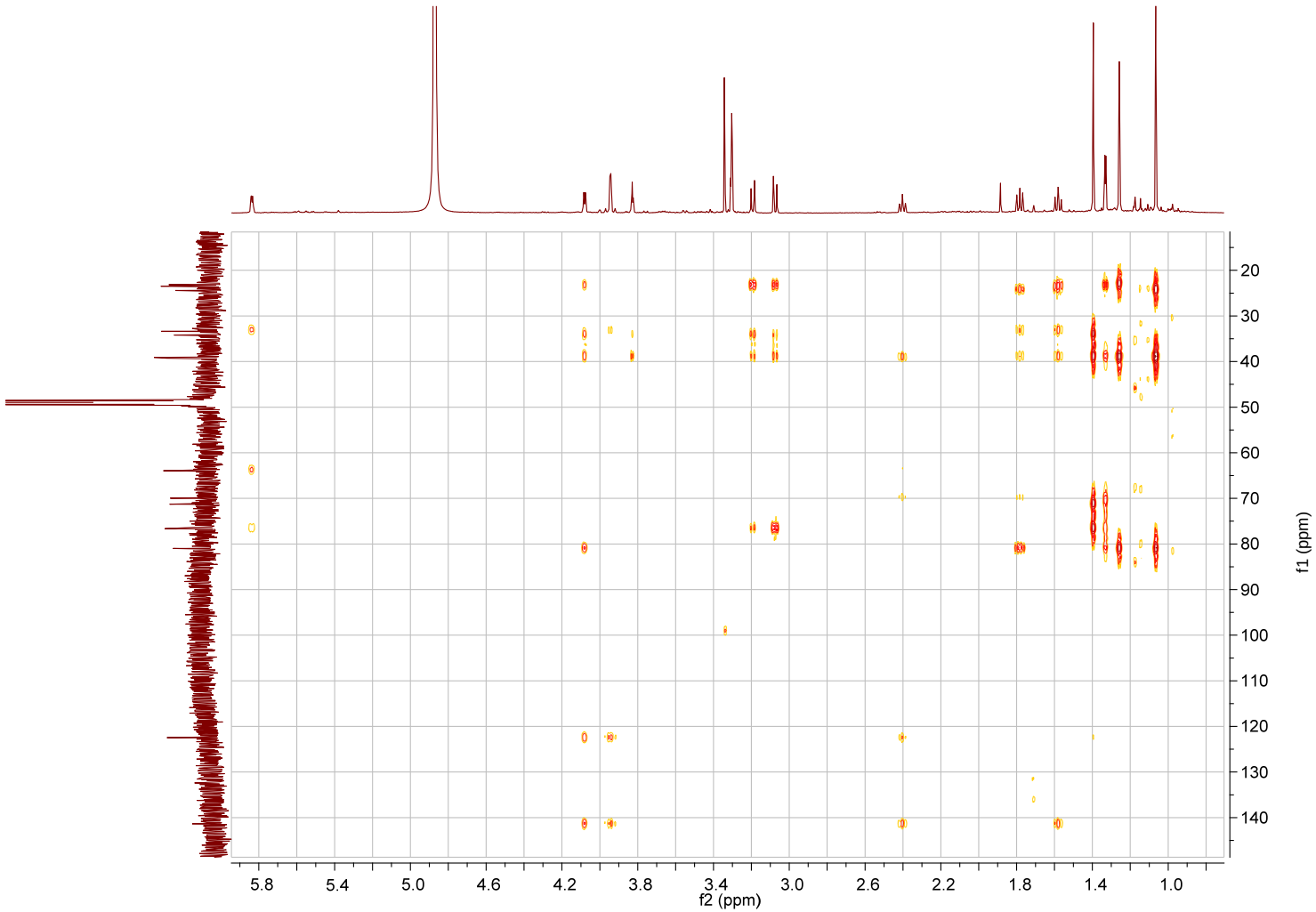


Figure S8. ^{1}H NMR Spectrum of Pestaloporonin C (**3**; 600 MHz, acetone-d_6)

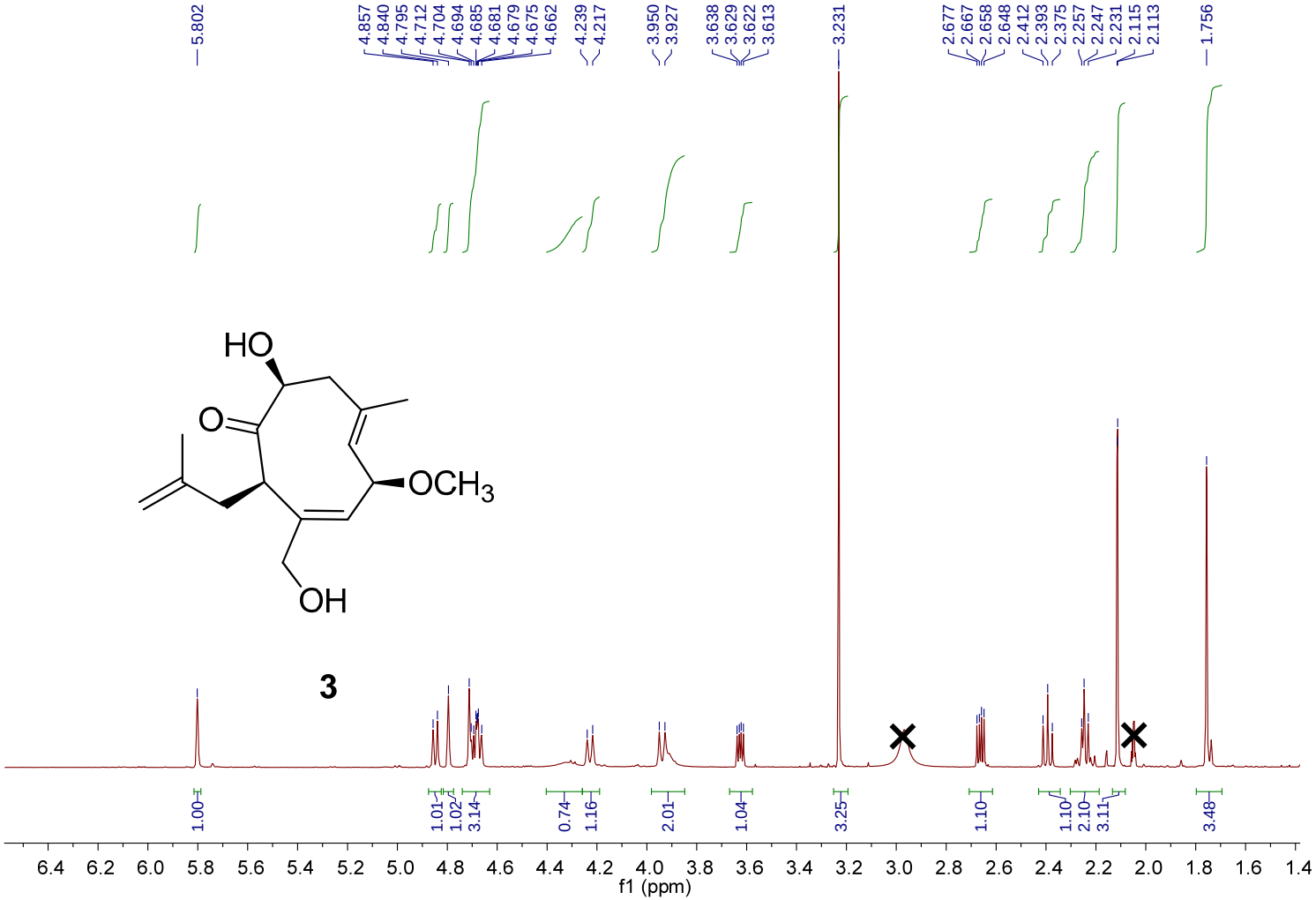


Figure S9. ^{13}C NMR Spectrum of Pestaloporonin C (**3**; 150 MHz, acetone-d_6)

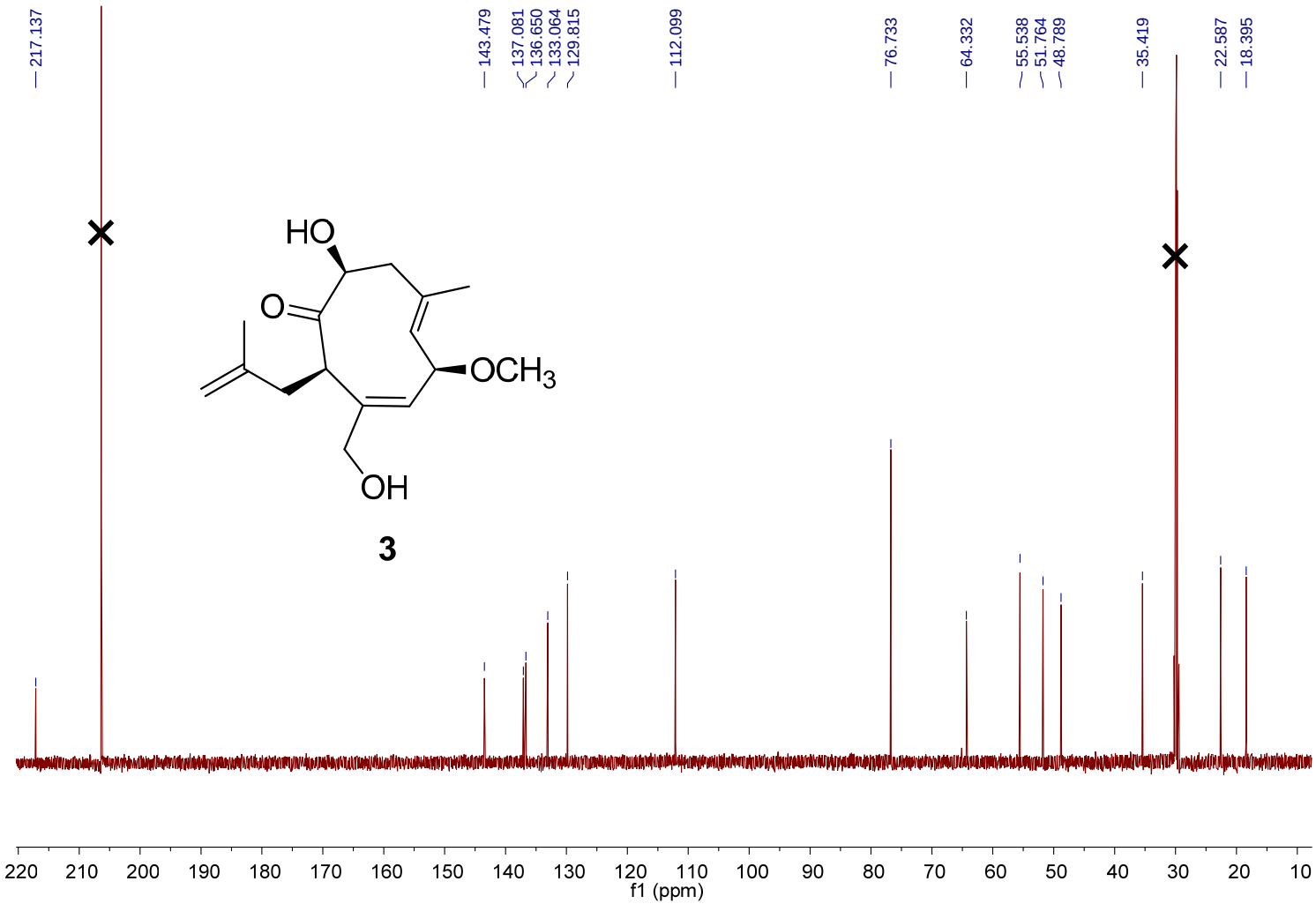


Figure S10. HMBC Spectrum of Pestaloporonin C (**3**; 600 MHz, acetone-d_6)

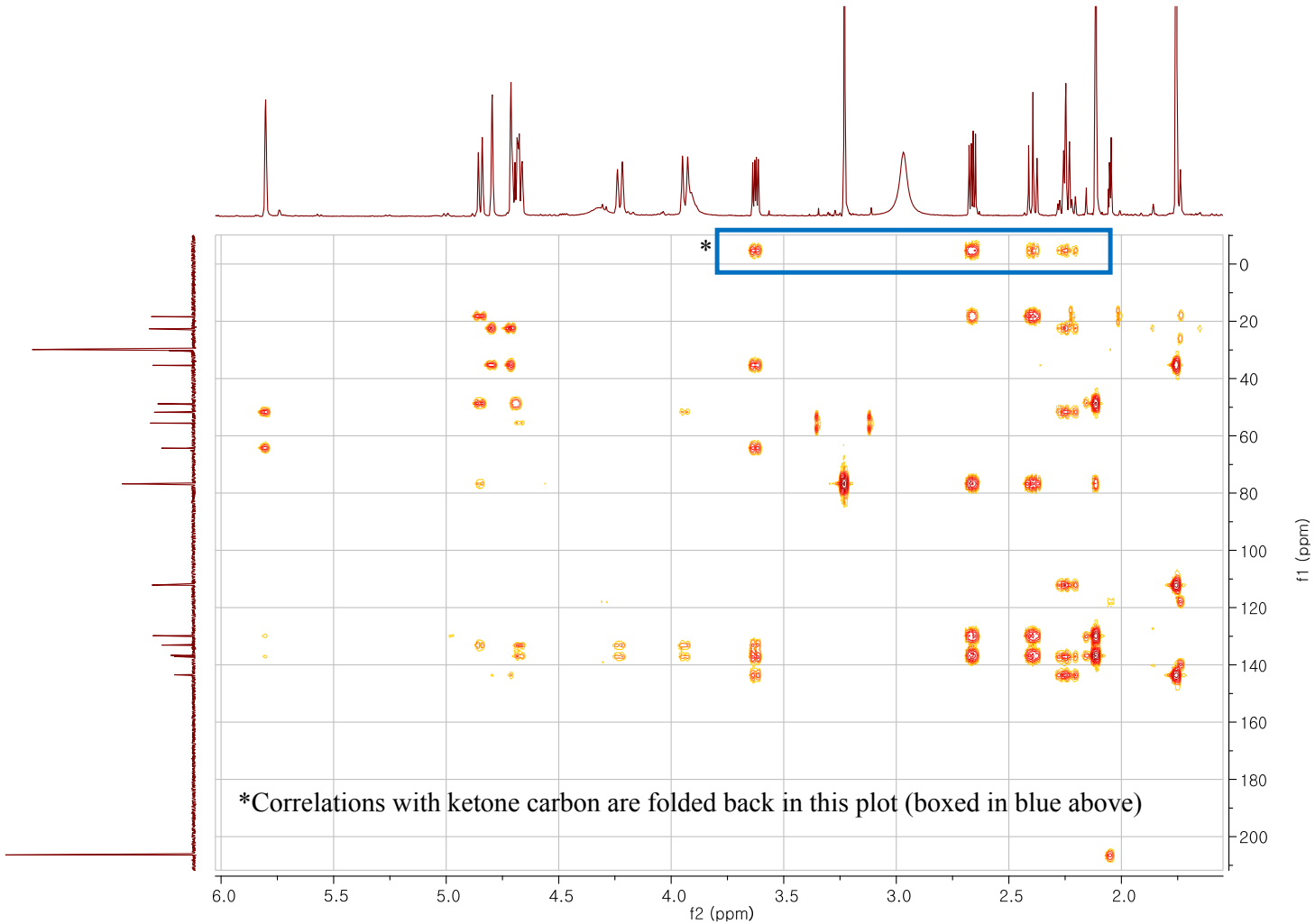


Figure S11. ECD Spectrum of Pestaloporonin C (**3**) in Methanol