

Hypatulins A and B, Meroterpenes from *Hypericum patulum*

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Experimental Section

1. General Experimental Procedures. Optical rotations and IR spectra were recorded on a JASCO P-2200 digital polarimeter and a JASCO FT/IR-6200 spectrophotometer, respectively. UV spectra were recorded using a Hitachi UV-3900H spectrophotometer. CD spectrum was recorded on a JASCO J-1500 spectrophotometer. NMR spectra were measured by a Bruker AVANCE-500 instrument using tetramethylsilane as an internal standard. HRESIMS spectra were recorded on a Waters LCT PREMIER 2695.

2. Plant Material. *Hypericum patulum* was cultivated at Mori Botanical Garden in Tokushima, Japan, and collected in July 2013. The voucher specimen (HYP201307) was deposited at the herbarium of Graduate School Pharmaceutical Sciences, Tokushima University.

3. Extraction and Isolation. The dried leaves of *Hypericum patulum* (1.48 kg) was extracted with MeOH to afford the extract (629 g), which was partitioned with *n*-hexane and water. The *n*-hexane soluble fraction was subjected to silica gel column chromatography (*n*-hexane/EtOAc, 9:1 to 8:2) to afford five fractions (frs. 1–5). Fr. 4 was loaded on a silica gel column (*n*-hexane/CHCl₃, 1:1 to 0:1) to give six fractions (frs. 4.1–6). Fr.4.5 was separated by an ODS column (MeOH/H₂O, 8:2 to 10:0) and a silica gel column (CHCl₃) chromatographies, and purified using ODS HPLC (YMC-Triart C18, YMC CO., LTD, 10 x 250 mm, MeOH/H₂O, 9:1) to give hypatulins A (**1**, 36.9 mg) and B (**2**, 3.6 mg).

3.1. Hypatulin A (**1**): colorless amorphous solid; $[\alpha]_D^{25} +40.4$ (*c* 0.046, MeOH); IR (KBr) ν_{\max} 3455, 1786, 1738, and 1672 cm⁻¹; UV (MeOH) λ_{\max} 245 (ϵ 9200) nm; ECD (MeOH) $\Delta\epsilon$ (nm) – 1.1 (334), –1.0 (281), and +5.1 (243); ¹H and ¹³C NMR (Table 1); HRESIMS: *m/z* 511.2807 [M+Na]⁺ (calcd for C₃₂H₄₀O₄Na, 511.2824).

3.2. Hypatulin B (**2**): colorless amorphous solid; $[\alpha]_D^{25} +27.0$ (*c* 0.17, MeOH); IR (KBr) ν_{\max} 3507, 1738, and 1672 cm⁻¹; UV (MeOH) λ_{\max} 243 (ϵ 10500); ¹H and ¹³C NMR (Table 1); HRESIMS: *m/z* 543.3064 [M+Na]⁺ (calcd for C₃₃H₄₄O₅Na, 543.3086).

4. Calculations. Conformational searches and DFT calculations were carried out on Spartan

14 program^{S1} and Gaussian 09 program,^{S2} respectively. Possible enantiomers **1a** (1*S*,3*R*,4*R*,6*R*,7*R*,8*S*) and **1b** (1*R*,3*S*,4*S*,6*S*,7*S*,8*R*) were submitted to conformational searches at the Molecular Mechanics (MMFF94s). The initial stable conformers for **1a** and **1b** with Boltzmann distributions over 1% (13 and 12 conformers, respectively) were further optimized by DFT calculations at the B3LYP/6-31G(d) level in the presence of MeOH with a polarizable continuum model (PCM). The stable conformers for **1a** and **1b** with Boltzmann distributions over 1% (12 and 11 conformers, respectively) were subjected to TDDFT calculations at the B3LYP/6-31G(d) level in the presence of MeOH with a PCM. The resultant rotatory strengths of the lowest 30 excited states for each conformer were converted into Gaussian-type curves with half-bands (0.3 eV) using SpecDis v1.61.^{S3} The calculated CD spectra for **1a** and **1b** were composed after correction based on the Boltzmann distribution of the stable conformers and blue-shifted by 15 nm, respectively.

5. Chemical conversion of hypatulin A (**1**) into hypatulin B (**1c**).

A mixture of hypatulin A (**1**, 5 mg) and 4-dimethylaminopyridine (12 mg) in MeOH (5 mL) was stirred at rt for 12 h. The reaction mixture was diluted by water (30 mL) and extracted with EtOAc (20 mL x 3). The EtOAc layer was washed with 1M HCl and brine, and concentrated to give a residue, which was purified by ODS HPLC (YMC-Triart C18, 10x250 mm, MeOH:H₂O, 9:1) to give hypatulin B (**1c**, 2.3 mg).

5.1. Hypatulin B (**1c**) derived from hypatulin A (**1**): colorless amorphous solid; $[\alpha]_D^{25} +25.4$ (*c* 0.11, MeOH); ¹H NMR spectrum of **1c** (Fig. S15) was identical to that of natural hypatulin B (**2**) (Fig. S8).

6. Antimicrobial Assay.

Test microorganisms. Seven clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) strains, three clinical isolates of methicillin-sensitive *S. aureus* (MSSA) strains, *S. aureus* 209P and Smith, one *Bacillus subtilis* strain, and four *Escherichia coli* strains were used in this study. All the strains of microorganisms used in this work were kindly supplied by Dr. C. Sano, the School of Medicine, Shimane University (Shimane, Japan).

Susceptibility testing. The MICs were determined by broth microdilution method in 96-well microtiter plates with cation-supplemented Mueller-Hinton broth (CAMHB;

Ca²⁺, 25 µg/mL; Mg²⁺, 12.5 µg/mL; Becton Dickinson, Sparks, MD) according to the current guidelines of the Clinical and Laboratory Standards Institute (CLSI). All the strains of microorganisms were inoculated at a final bacterial density of about 5×10^5 CFU/ml. Then, *S. aureus* strains were incubated at 35 °C for 20 h, and *B. subtilis* and *E. coli* strains were incubated at 37 °C for 20 h before the MICs were determined.

7. References.

(S1) Spartan 14, Wavefunction Inc., Irvine, CA.

(S2) Gaussian 09, Revision C.01, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V. Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; 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.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; and Fox, D. J. Gaussian, Inc., Wallingford, CT, 2010.

(S3) Bruhn, T.; Schaumlöffel, A.; Hemberger, Y.; Bringmann, G. SpecDis, Version 1.61, University of Wuerzburg, Germany, 2013.

Figure S1. ^1H NMR spectrum of hypatulin A (1) in CD_3OD (500 MHz).

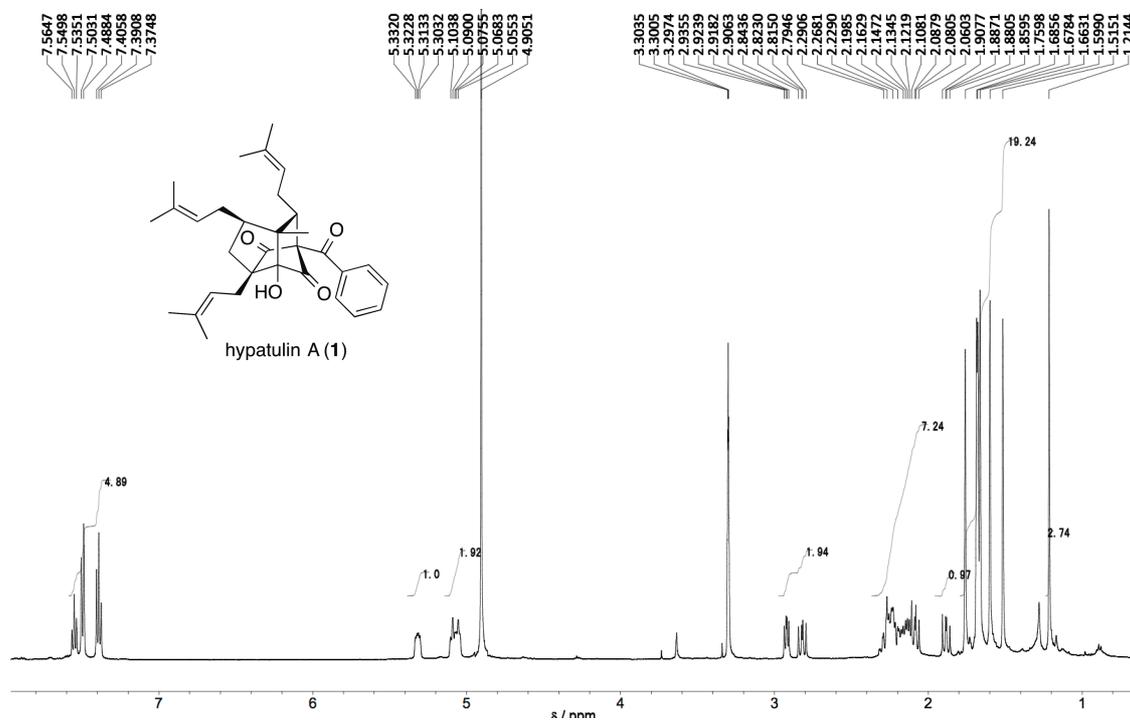


Figure S2. ^{13}C NMR spectrum of hypatulin A (1) in CD_3OD (125 MHz).

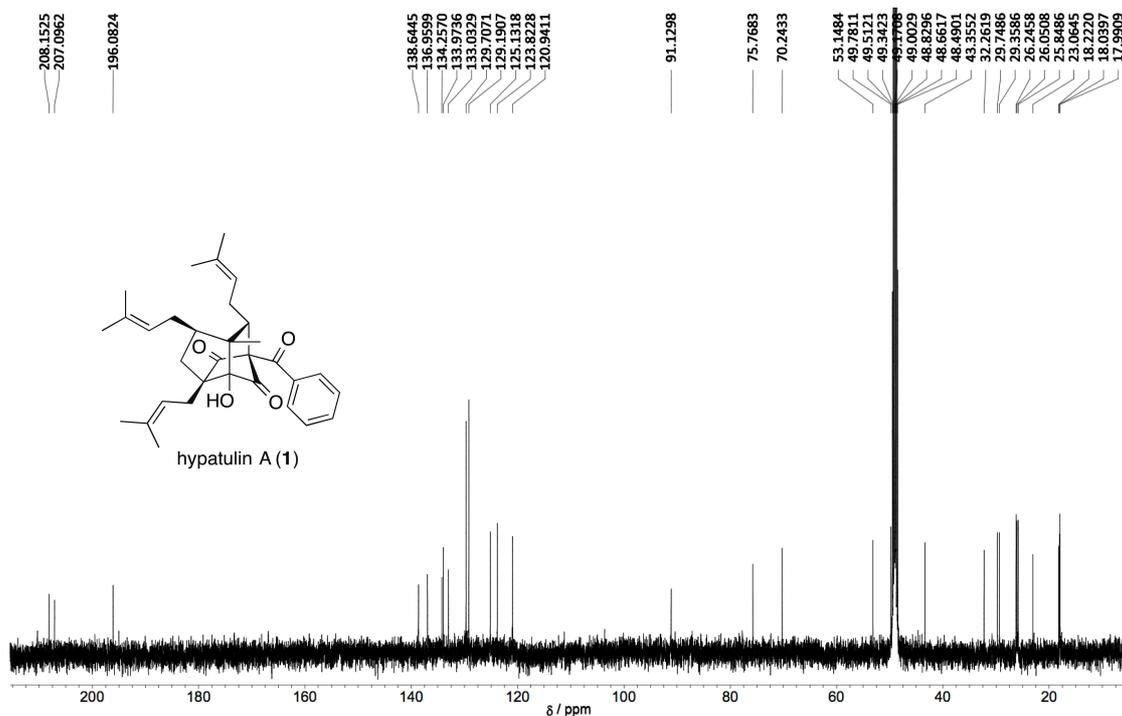


Figure S3. ^{13}C NMR spectrum of hypatulin A (**1**) in CD_3OH (125 MHz).

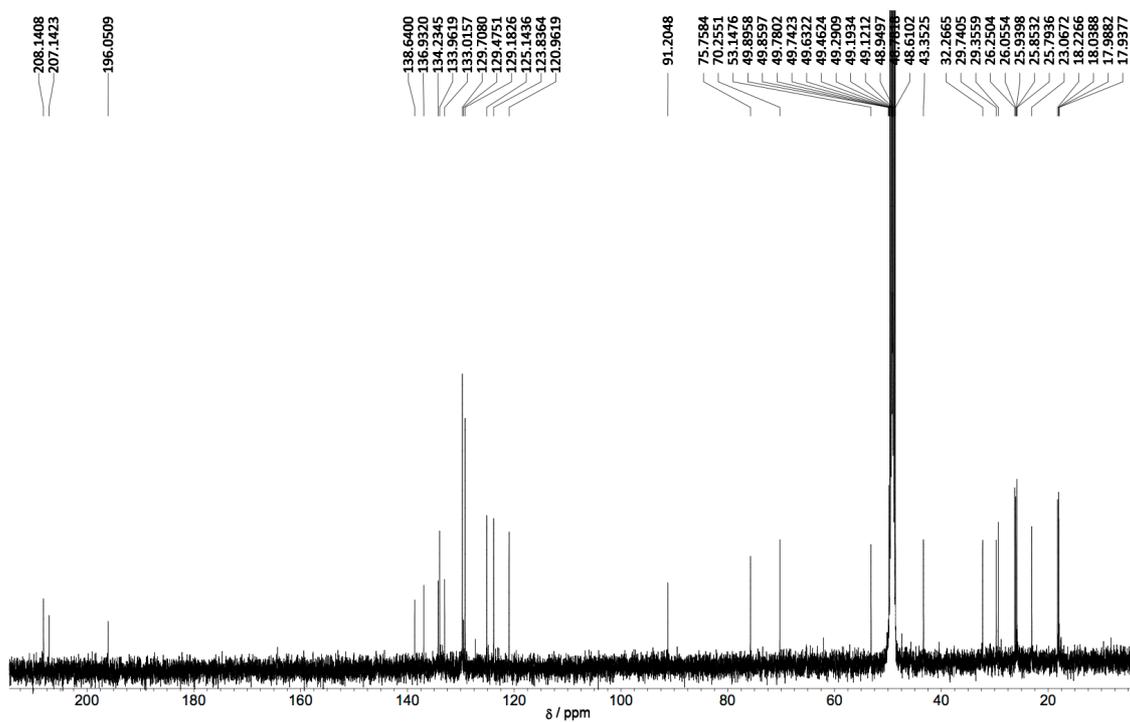


Figure S4. ^1H - ^1H COSY spectrum of hypatulin A (**1**) in CD_3OD (500 MHz).

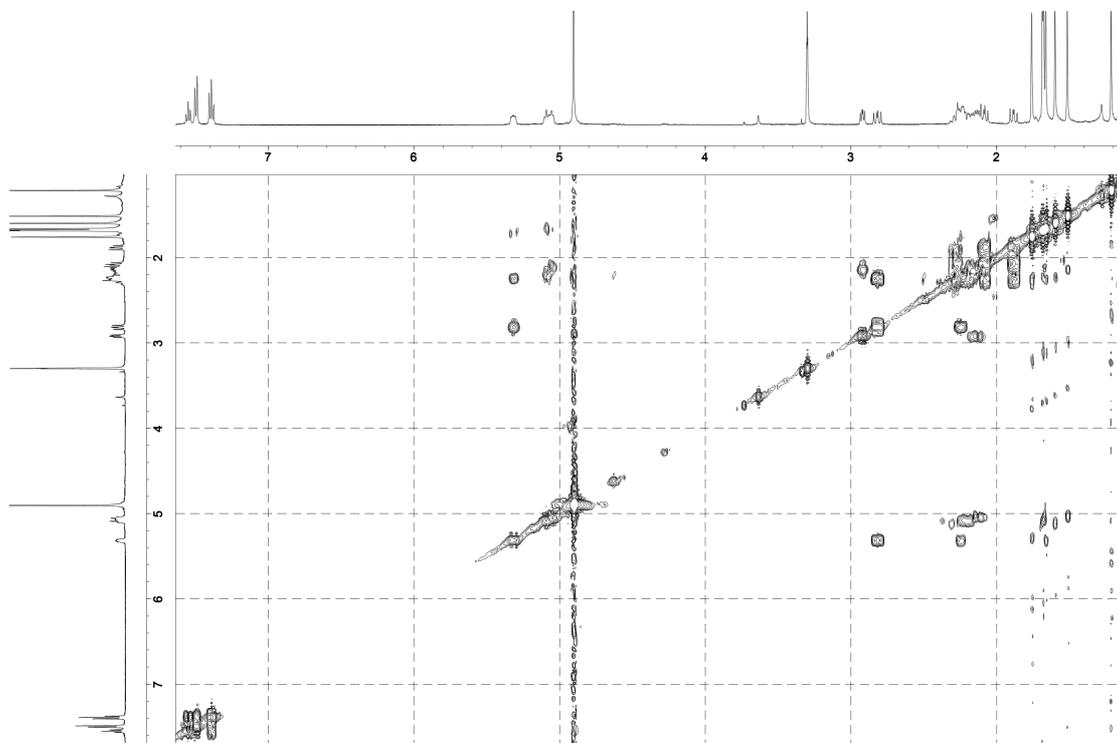


Figure S5. HSQC spectrum of hypatulin A (**1**) in CD₃OD (500 MHz).

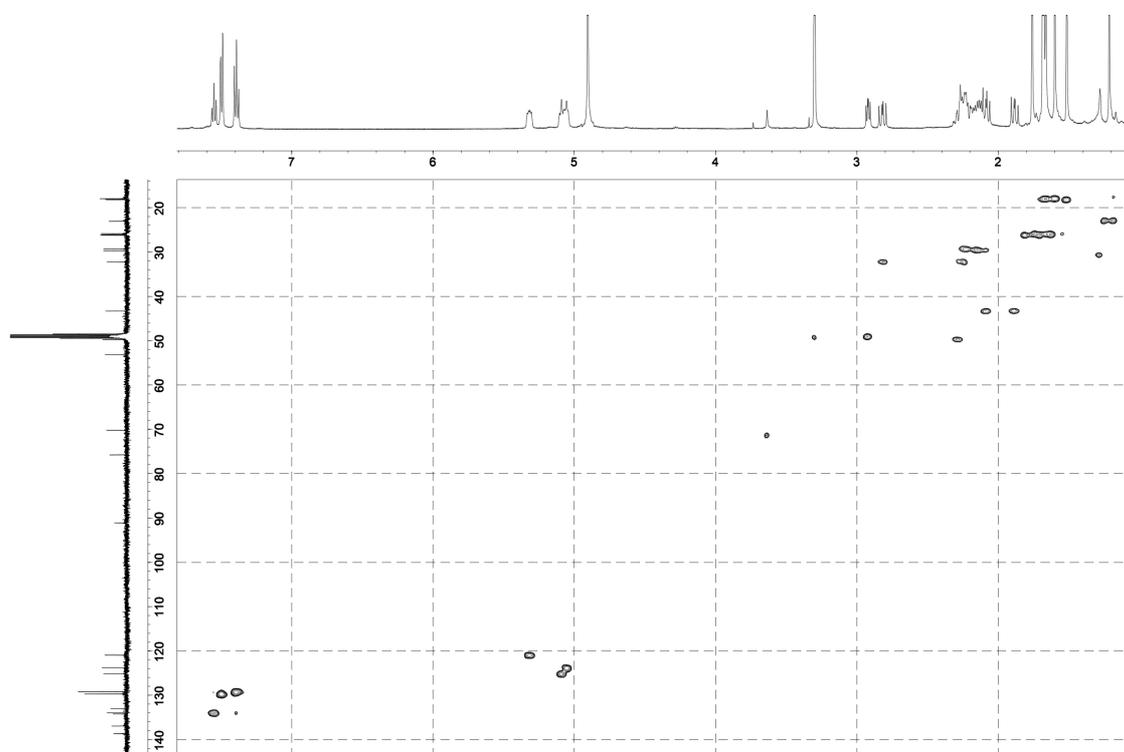


Figure S6. HMBC spectrum of hypatulin A (**1**) in CD₃OD (500 MHz).

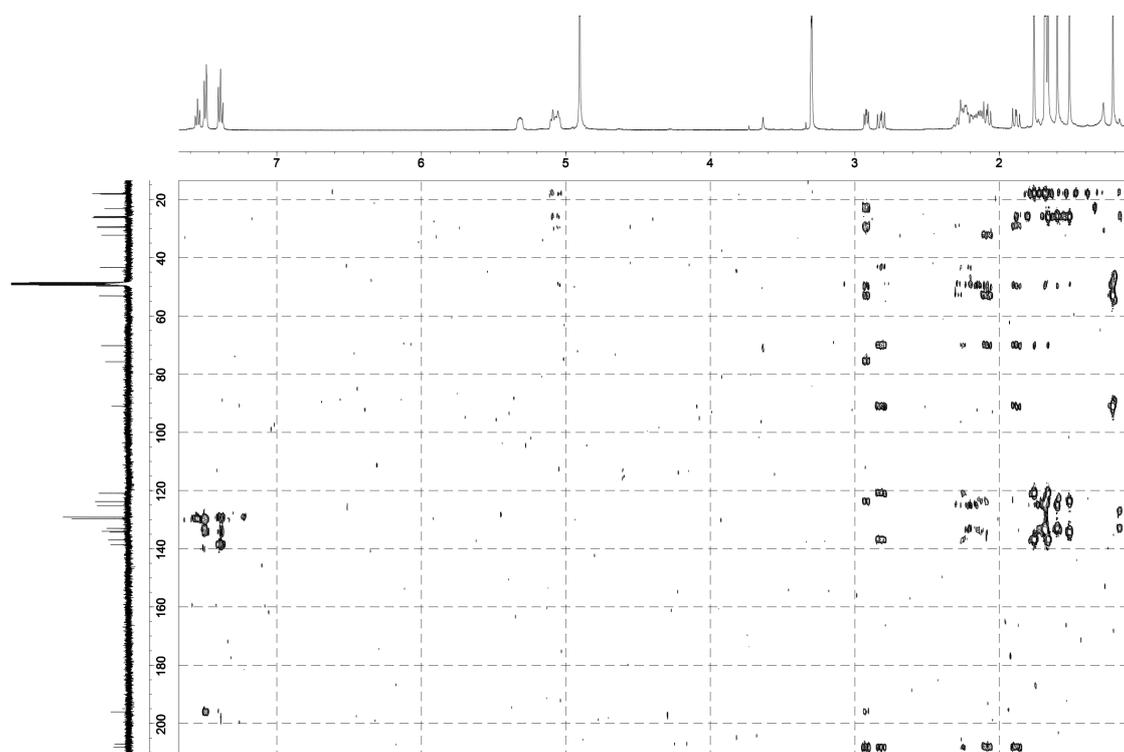


Figure S7. NOESY spectrum of hypatulin A (1) in CD₃OD (500 MHz).

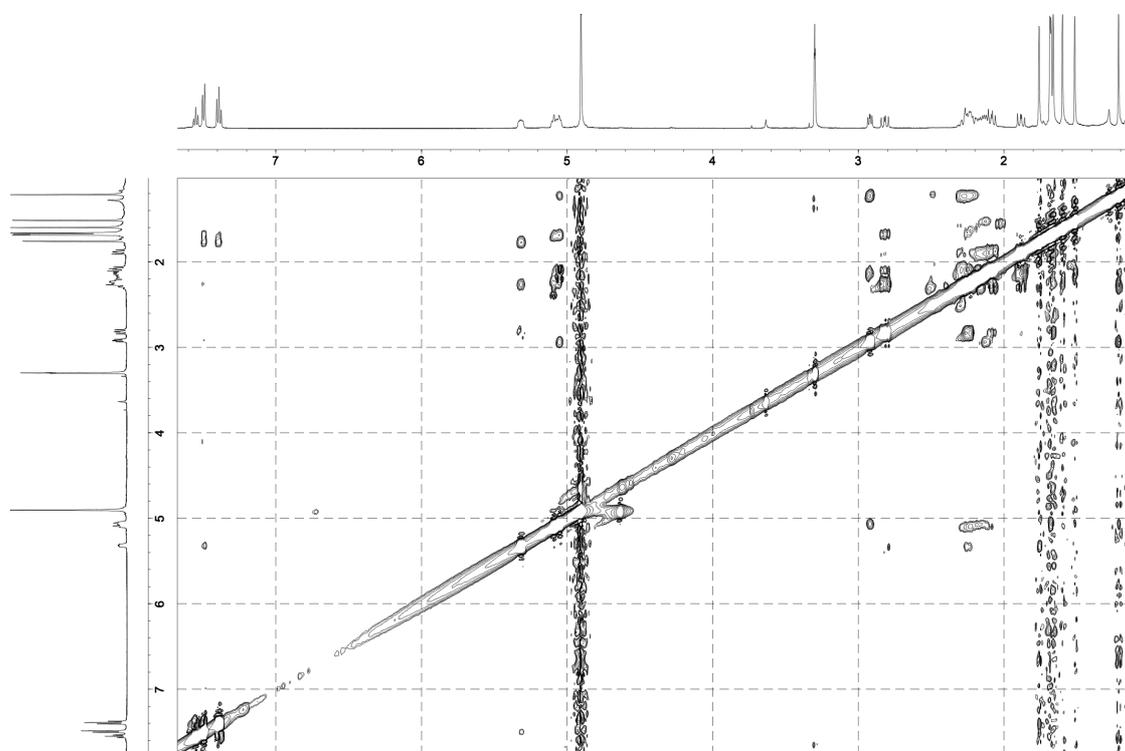


Figure S8. ¹H NMR spectrum of hypatulin B (2) in CD₃OD (500 MHz).

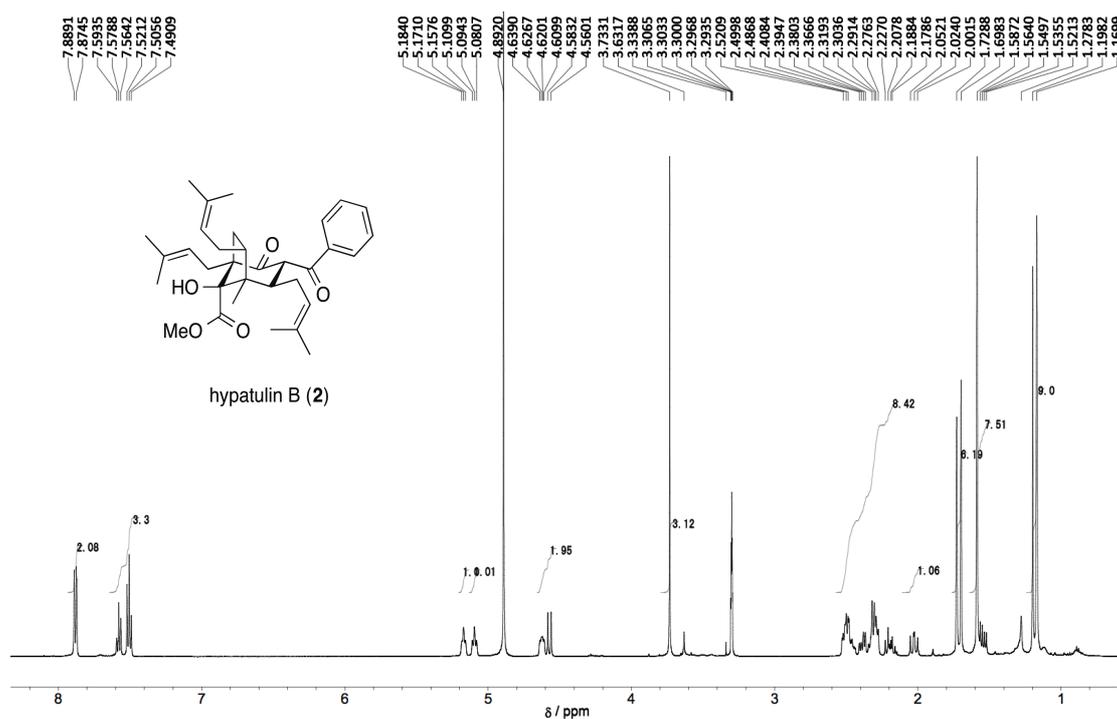


Figure S9. ^{13}C NMR spectrum of hypatulin B (**2**) in CD_3OD (125 MHz).

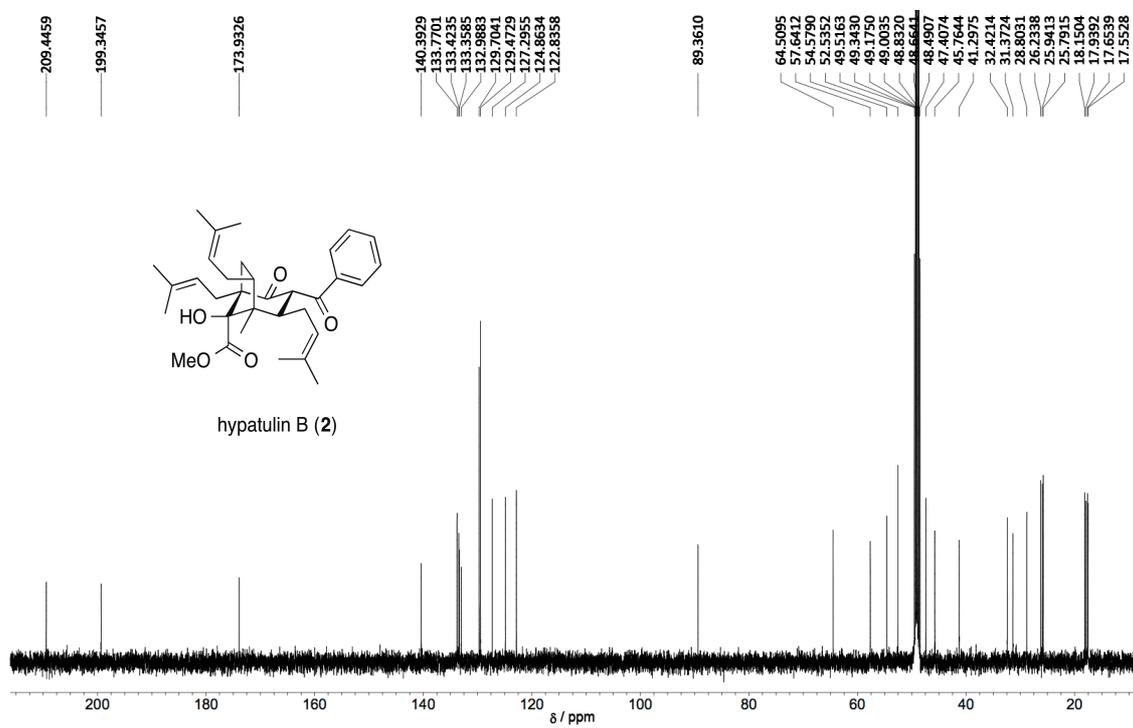


Figure S10. ^{13}C NMR spectrum of hypatulin B (**2**) in CD_3OH (125 MHz).

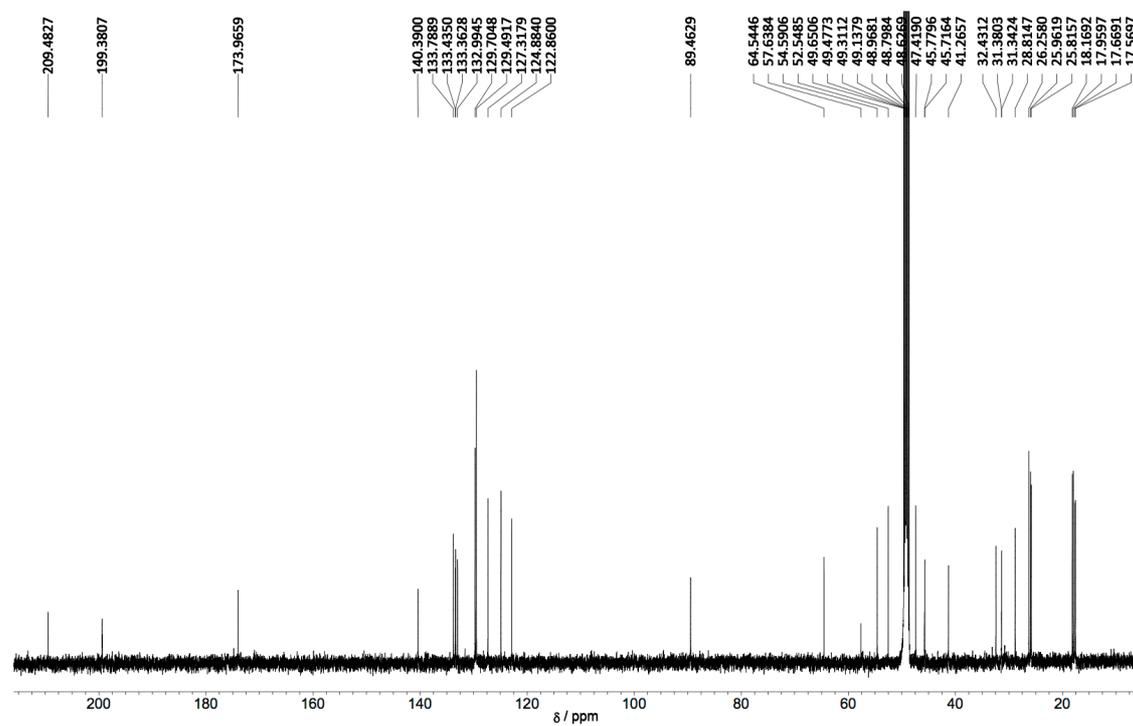


Figure S11. ^1H - ^1H COSY spectrum of hypatulin B (**2**) in CD_3OD (500 MHz).

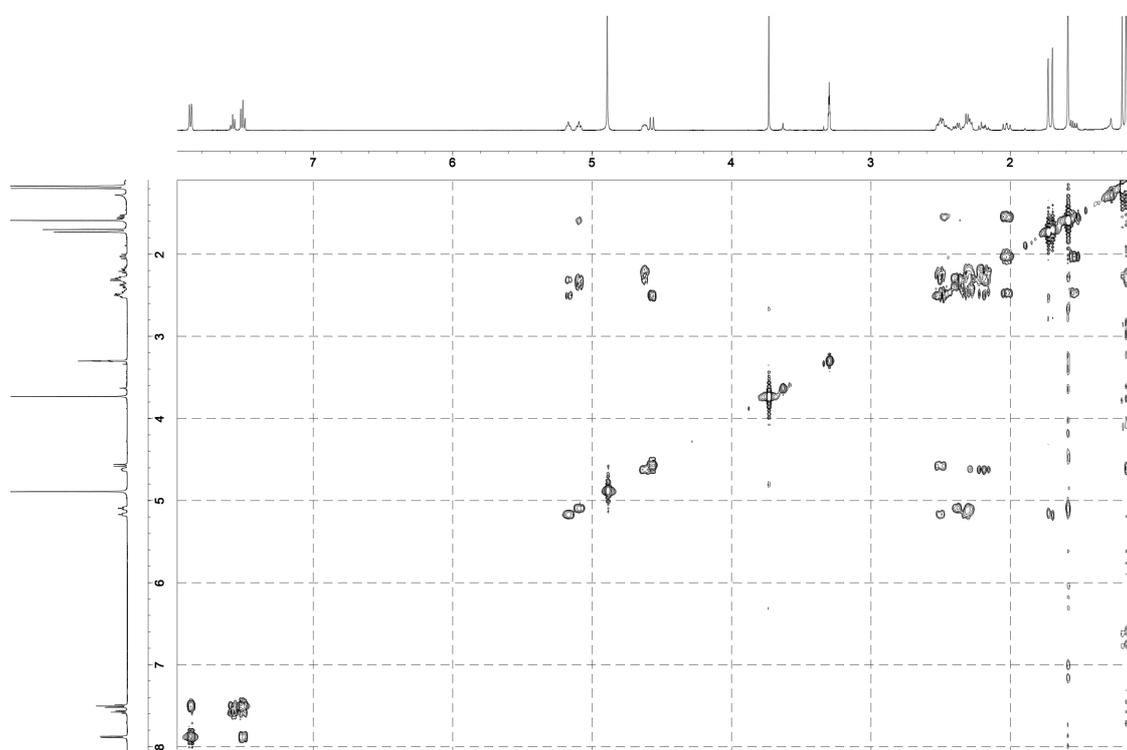


Figure S12. HSQC spectrum of hypatulin B (**2**) in CD_3OD (500 MHz).

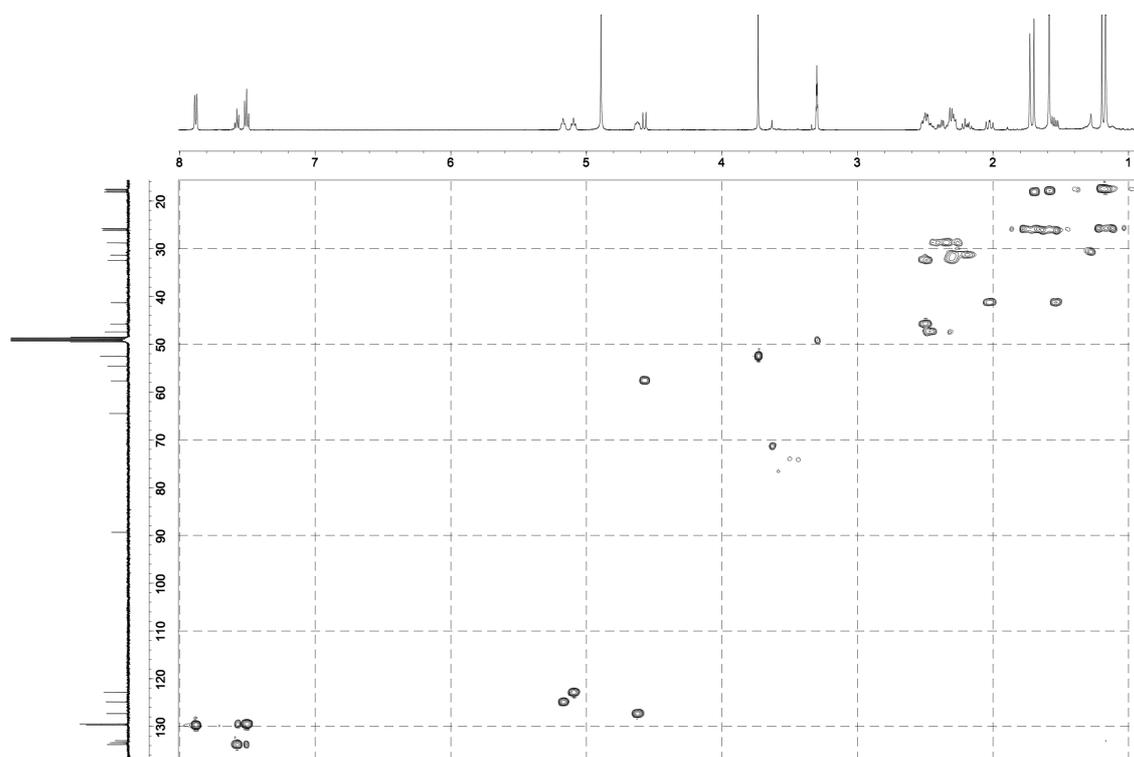


Figure S13. HMBC spectrum of hypatulin B (2) in CD₃OD (500 MHz).

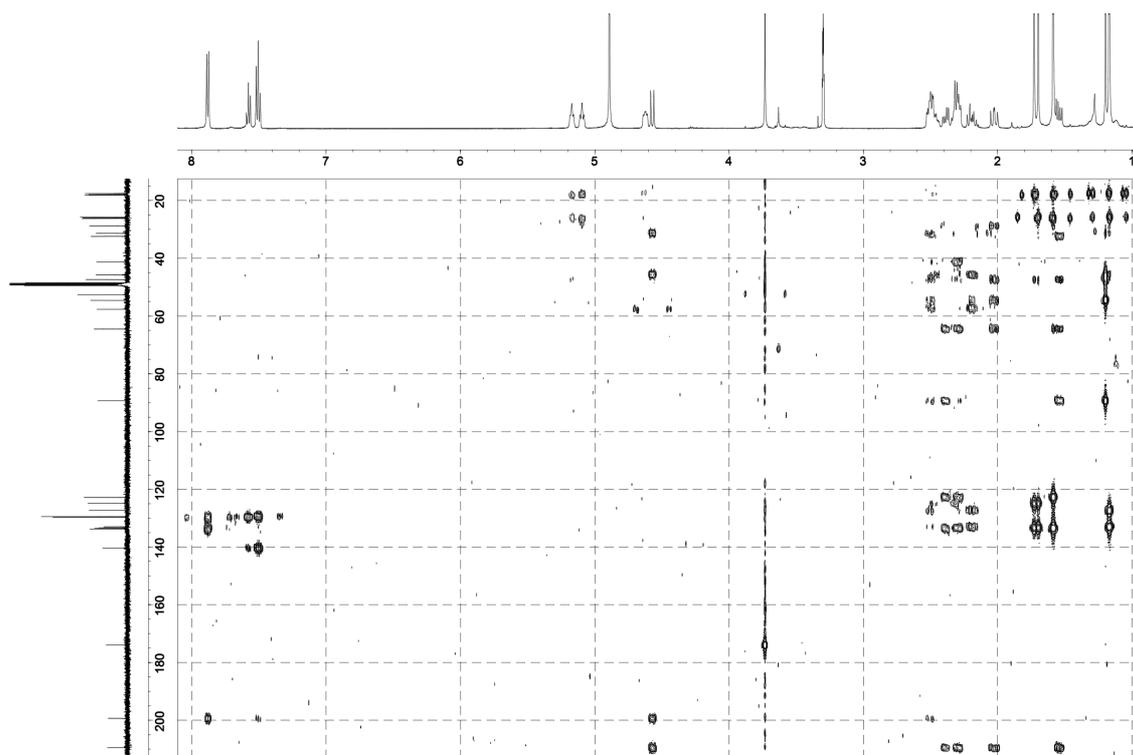


Figure S14. NOESY spectrum of hypatulin B (2) in CD₃OD (500 MHz).

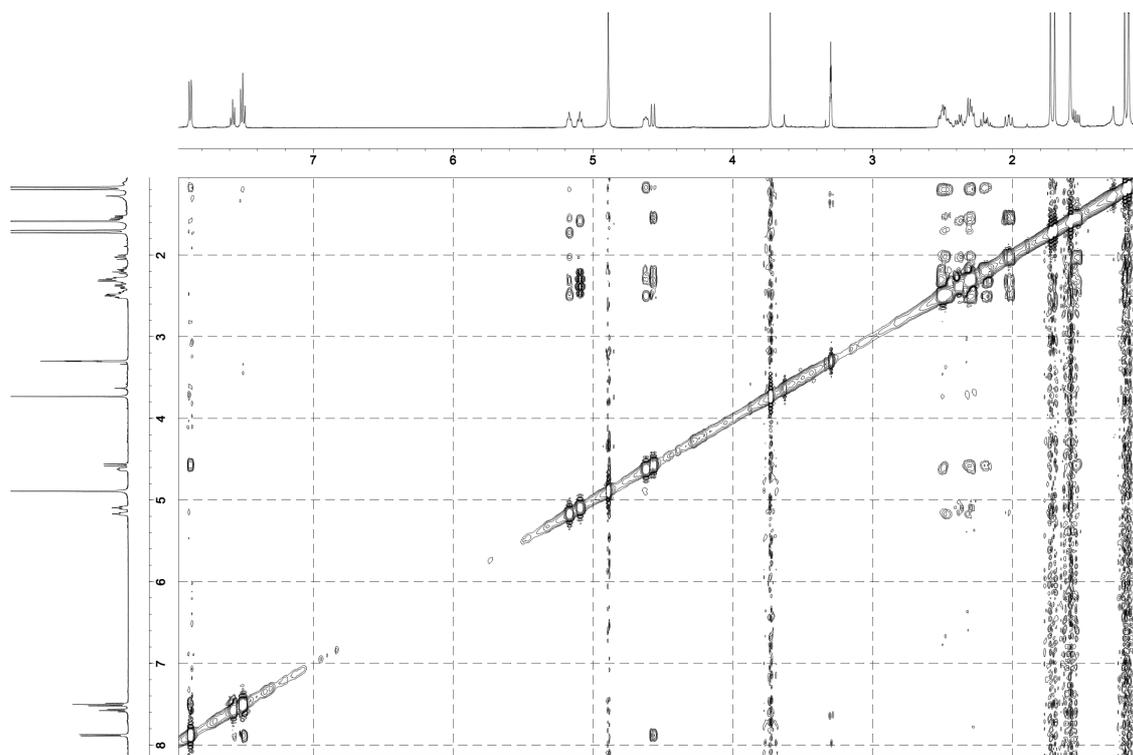


Figure S15. ^1H NMR spectrum of hypatulin B (**1c**) derived from hypatulin A (**1**) in CD_3OD (500 MHz).

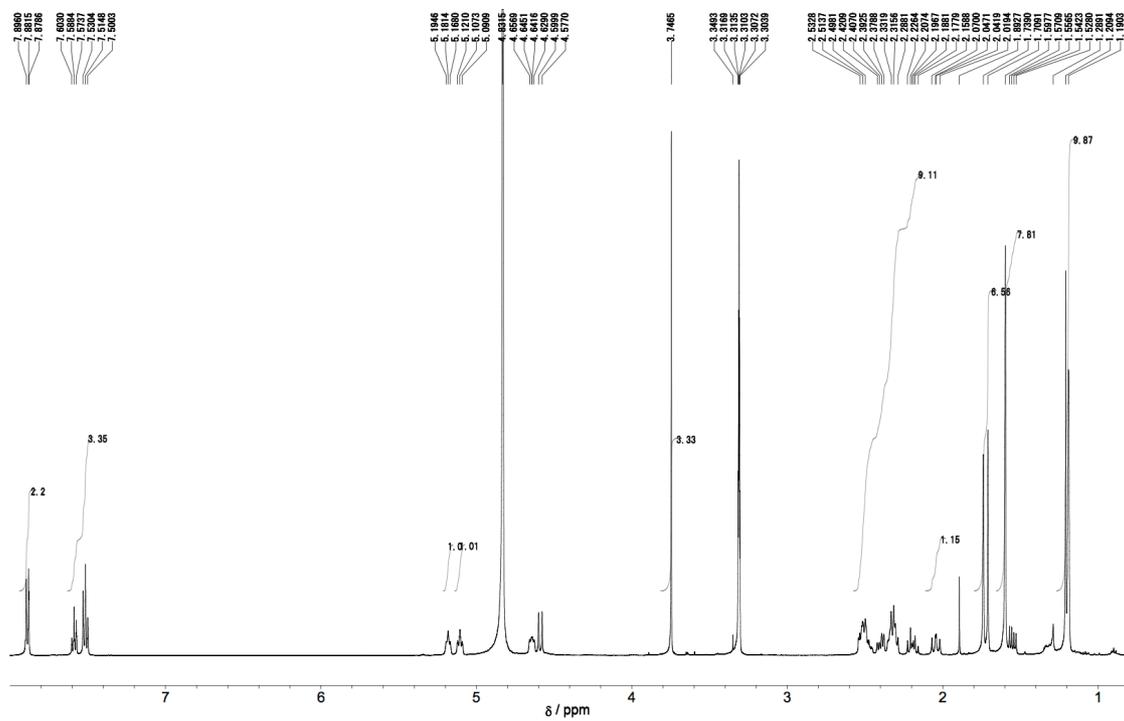


Table S1. ^{13}C NMR Data in CD_3OH and CD_3OD and Deuterium-induced Isotope Shifts for Hypatulins A (**1**) and B (**2**).

position	1			2		
	^{13}C	^{13}C	$\Delta\delta$	^{13}C	^{13}C	$\Delta\delta$
	(CD_3OH)	(CD_3OD)	($\text{CD}_3\text{OH}-\text{CD}_3\text{OD}$)	(CD_3OH)	(CD_3OD)	($\text{CD}_3\text{OH}-\text{CD}_3\text{OD}$)
1	75.76	75.77	-0.01	57.63	57.64	-0.01
2	207.14	207.10	0.04	173.96	173.93	0.03
3	91.20	91.13	0.07	89.45	89.36	0.09
4	70.26	70.24	0.02	64.53	64.51	0.02
5	43.35	43.36	-0.01	41.26	41.30	-0.04
6	49.9 ^a	49.8 ^a	-	47.41	47.41	0.00
7	53.15	53.15	0.00	54.58	54.58	0.00
8	49.2 ^a	49.2 ^a	-	45.77	45.76	0.01
9	208.14	208.15	-0.01	209.47	209.45	0.02
10	23.07	23.06	0.01	17.56	17.55	0.01
11	32.27	32.26	0.01	28.8	28.80	0.00
12	120.96	120.94	0.02	122.85	122.84	0.01
13	136.93	136.96	-0.03	133.42 ^b	133.42 ^c	0.00
14	17.99	17.99	0.00	17.95	17.94	0.01
15	26.25	26.25	0.00	26.25	26.23	0.02
16	29.36	29.36	0.00	32.42	32.42	0.00
17	125.14	125.13	0.01	124.87	124.86	0.01
18	133.02	133.03	-0.01	133.35 ^b	133.36 ^c	-0.01
19	18.04	18.04	0.00	18.16	18.15	0.01
20	25.85	25.85	0.00	25.95	25.94	0.01
21	29.74	29.75	-0.01	31.37	31.37	0.00
22	123.84	123.82	0.02	127.31	127.30	0.01
23	134.23	134.26	-0.03	132.98	132.99	-0.01
24	18.23	18.22	0.01	17.66	17.65	0.01
25	26.06	26.05	0.01	25.80	25.79	0.01
26	196.05	196.08	-0.03	199.37	199.35	0.02
27	138.64	138.64	0.00	140.38	140.39	-0.01
28,32	129.71	129.71	0.00	129.69	129.70	-0.01
29,31	129.18	129.19	-0.01	129.48	129.47	0.01
30	133.96	133.97	-0.01	133.78	133.77	0.01
2-Ome				52.54	52.54	0.00

^a Overlapped with signal of CD_3OH or CD_3OD .

^{b,c} Interchangeable.