

Supporting Information for

**Uncovering the Unusual D-Ring Construction in Terretonin
Biosynthesis by Collaboration of a Multifunctional Cytochrome P450
and a Unique Isomerase**

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Supplementary Materials and Methods

General

Solvents and chemicals were purchased from Wako Chemicals Ltd. (Tokyo, Japan) or Kanto Chemical Co., Inc. (Tokyo, Japan), unless noted otherwise. Oligonucleotide primers were purchased from Eurofins Genetics (Tokyo, Japan), and are listed in Table S1. PCR was performed using a TaKaRa PCR Thermal Cycler Dice[®] Gradient (TaKaRa) with iProof DNA polymerase (BIO-RAD). Sequence analyses were performed by Eurofins Genetics (Tokyo). Analytical and preparative HPLC were performed on a Shimadzu Prominence system, using a TSK-gel ODS-80T_S column (4.6 i.d. x 150 mm, Tosoh Co. Ltd.) and an ODS-80T_M column (7.8 i.d. x 300 mm, Tosoh Co. Ltd.), respectively. Silica gel column chromatography was performed using Wakogel C-200. NMR spectra were obtained at 500 MHz (¹H) and 125 MHz (¹³C) with a JEOL ECX-500 or ECA-500 spectrometer, and chemical shifts were recorded with reference to solvent signals (¹H NMR: CDCl₃ 7.26 ppm, ¹³C NMR: CDCl₃ 77.0 ppm). Samples for LC-MS/MS analysis were injected into an Agilent 1100 series HPLC-Esquire 4000 mass spectrometer (Bruker Daltonics), using Electrospray Ionization with a COSMOSIL 2.5C₁₈-MS-II column (2.0 i.d. x 100 mm; Nacalai Tesque, Inc.). HR-FAB-MS analyses were performed with a JMS-700 MStation (JEOL). The glufosinate solution for the fungal transformation was extracted from Basta (Bayer) as previously described,¹ and used at a 50 µL/mL concentration.

Detailed purification procedure for each metabolite

Purification conditions for terretonin (**1**):

The extract from a 3 L culture of *A. oryzae* NSAR1 with *trt4*, *trt2*, *trt5*, *trt8*, *trt1*, *trt9*, *trt3*, *trt6*, *trt14*, and *trt7* was subjected to silica-gel column chromatography and eluted stepwise with a chloroform:methanol gradient (100:0 to 90:10). Fractions that contained **1** were further purified by reverse-phase preparative HPLC (47% aqueous acetonitrile, 3.0 mL/min) to yield 10.8 mg of a white solid.

Purification conditions for terretonin C (**2**):

The extract from a 2 L culture of *A. oryzae* NSAR1 with *trt4*, *trt2*, *trt5*, *trt8*, *trt1*, *trt9*, *trt3*, *trt6*, and *trt7* was subjected to silica-gel column chromatography, and eluted stepwise with a chloroform:methanol gradient (100:0 to 85:15). Fractions that contained **2** were further purified by reverse-phase preparative HPLC (50% aqueous acetonitrile, 3.0 mL/min) to yield 118.2 mg of a white solid.

Purification conditions for preterrenoid (**5**):

The extract from a 2 L culture of *A. oryzae* NSAR1 with *trt4*, *trt2*, *trt5*, *trt8*, *trt1*, and *trt9* was subjected to silica-gel column chromatography, and eluted stepwise with a chloroform:methanol gradient (100:0 to 95:5). Fractions that contained **5** were further purified by reverse-phase preparative HPLC (65% aqueous acetonitrile, 3.0 mL/min) to yield 26.4 mg of a white solid.

Purification conditions for terrenoid (**6**):

The extract of from a 4 L culture of *A. oryzae* NSAR1 with *trt4*, *trt2*, *trt5*, *trt8*, *trt1*, *trt9*, and *trt3* was subjected to silica-gel column chromatography, and eluted stepwise with a chloroform:methanol gradient (100:0 to 95:5). Fractions that contained **6** were further purified by reverse-phase preparative HPLC (60% aqueous acetonitrile, 3.0 mL/min) to yield 20.0 mg of a yellowish white solid.

Purification conditions for terrenoidol (**7**):

The extract from a 4 L culture of *A. oryzae* NSAR1 with *trt4*, *trt2*, *trt5*, *trt8*, *trt1*, *trt9*, and *trt3* was subjected to silica-gel column chromatography, and eluted stepwise using a chloroform:methanol gradient (100:0 to 95:5). Fractions that contained **7** were further purified by reverse-phase preparative HPLC (60% aqueous acetonitrile, 3.0 mL/min) to yield 10.0 mg of a white solid.

Purification conditions for terretonin H (**8**):

The extract from a 2 L culture of *A. oryzae* NSAR1 with *trt4*, *trt2*, *trt5*, *trt8*, *trt1*, *trt9*, *trt3*, and *trt6* was subjected to silica-gel column chromatography, and eluted stepwise with a chloroform:methanol gradient (100:0 to 95:5). Fractions that contained **8** were further purified by reverse-phase preparative HPLC (50% aqueous acetonitrile, 3.0 mL/min) to yield 32.0 mg of a white solid.

Purification conditions for terretonin I (**9**):

The extract from a 3 L culture of *A. oryzae* NSAR1 with *trt4*, *trt2*, *trt5*, *trt8*, *trt1*, *trt9*, *trt3*, and *trt6* was subjected to silica-gel column chromatography, and eluted stepwise with a chloroform:methanol gradient (100:0 to 90:10). Fractions that contained **9** were further purified by reverse-phase preparative HPLC (43% aqueous acetonitrile, 3.0 mL/min) to yield 5.95 mg of a colorless solid.

Purification conditions for terretonin J (**10**):

The extract from a 3 L culture of *A. oryzae* NSAR1 with *trt4*, *trt2*, *trt5*, *trt8*, *trt1*, *trt9*, *trt3*, *trt6*, *trt14*, and *trt7* was subjected to silica-gel column chromatography, and eluted stepwise with a chloroform:methanol gradient (100:0 to 95:5). Fractions that contained **10** were further purified by reverse-phase preparative HPLC (50% aqueous acetonitrile, 3.0 mL/min) to yield 1.02 mg of a

colorless solid.

Purification conditions for terretonin A (**11**):

The extract from a 3 L culture of *A. oryzae* NSAR1 with *trt4*, *trt2*, *trt5*, *trt8*, *trt1*, *trt9*, *trt3*, *trt6*, *trt14*, and *trt7* was subjected to silica-gel column chromatography, and eluted stepwise with a chloroform:methanol gradient (100:0 to 90:10). Fractions that contained **11** were further purified by reverse-phase preparative HPLC (47% aqueous acetonitrile, 3.0 mL/min) to yield 9.47 mg of a white solid.

Purification conditions for terretonin D (**12**):

The extract from a 3 L culture of *A. oryzae* NSAR1 with *trt4*, *trt2*, *trt5*, *trt8*, *trt1*, *trt9*, *trt3*, *trt6*, and *trt14* was subjected to silica-gel column chromatography, and eluted stepwise with a chloroform:methanol gradient (100:0 to 90:10). Fractions that contained **12** were further purified by reverse-phase preparative HPLC (47% aqueous acetonitrile, 3.0 mL/min) to yield 14.9 mg of a colorless solid.

Purification conditions for terretonin K (**13**):

The extract from a 3 L culture of *A. oryzae* NSAR1 with *trt4*, *trt2*, *trt5*, *trt8*, *trt1*, *trt9*, *trt3*, *trt6*, and *trt14* was subjected to silica-gel column chromatography, and eluted stepwise with a chloroform:methanol gradient (100:0 to 90:10). Fractions that contained **13** were further purified by reverse-phase preparative HPLC (50% aqueous acetonitrile, 3.0 mL/min) to yield 31.6 mg of a white amorphous solid.

Purification conditions for terretonin L (**14**):

Trt14 with an N-terminal His-tag was expressed from *E. coli* Rosetta pLys (DE3) harboring pET28a(+)-*trt14* and purified by Ni-affinity column chromatography. The purified Trt14 (50 μ M) was added to 10 mL of reaction buffer (50 mM HEPES pH 7.5, 1mM EDTA, 20% glycerol, and 1.5 mM 2-mercaptoethanol) and incubated with 1.2 mM terrenoid at 30 °C for 20 hrs. The extract that contained **14** was purified by reverse-phase preparative HPLC (60% aqueous acetonitrile, 3.0 mL/min) to yield 2.02 mg of white amorphous solid.

Purification conditions for labeled preterretonin A (**4**):

The extract from a 500 mL culture of *A. oryzae* NSAR1 with *trt4*, *trt2*, *trt5*, *trt8*, and *trt1* was subjected to silica-gel column chromatography, and eluted stepwise with a chloroform:methanol gradient (100:0 to 90:10). Fractions that contained the labeled preterretonin A were further purified by

reverse-phase preparative HPLC (54% aqueous acetonitrile, 3.0 mL/min) to yield 26.3 mg of a yellowish white solid.

Purification conditions for labeled terretonin (**1**):

The extract from a 360 mL culture of *A. oryzae* NSAR1 with *trt9*, *trt3*, *trt6*, *trt14*, and *trt7* was subjected to silica-gel column chromatography and eluted stepwise with a chloroform:methanol gradient (100:0 to 95:5). Fractions that contained the labeled terretonin were further purified by reverse-phase preparative HPLC (50% aqueous acetonitrile, 3.0 mL/min) to yield 2.3 mg of a white solid.

Supplementary Reference

(1) Chooi, Y.-H.; Cacho, R.; Tang, Y. *Chem. Biol.* **2010**, *17*, 483-494.

Table S1. Primers used in this study

Primer	Sequence (5' to 3')
EcoRI-trt9-F	GCCGCAGAATTCATGGCGCTGGACCAGGGC
EcoRI-trt9-R	GTAATAAAGAATTCTCAGATGGTTGACCAG
InF-pUNA-trt9-F	TCGAGCTCGGTACCCATGGCGAACCTCCAAGATAAAG
InF-pUNA-trt9-R	CTACTACAGATCCCCTCAGATGGTTGACCAGGCAT
InF-pUNA-trt3-R	CTACTACAGATCCCCTTATGTCCCCTTCCATGCAATTTT
KpnI-trt3-F	CAAGATGGTACCATGACTATCCCTTTTCAG
KpnI-trt3-R	CAGATAGGTACCTTATGTCCCCTTCCATG
EcoRI-trt6-F	CTCAAAGATGAATTCATGCTGGAACCGGTC
EcoRI-trt6-R	CAACCAGAATTCCTTAAAGAGCCACCTCTTC
InF-pAdeA-trt6-R	TAGTAGATCCTCTAGCAAAGGTATTTTGTCTGGGTGTG
KpnI-trt7-F	GAAAACCGGTACCATGACTGGCCAGGCCG
KpnI-trt7-R	GGATGGCGGTACCTCATGCCTCCCGCGATG
KpnI-trt14-F	GTATGGGTACCATGAGTCTACTCGC
KpnI-trt14-R	ATATGCCTGGTACCTCAAAAAGTGATCCC
NdeI-trt14-F	GTATGCATATGAGTCTACTCGCGAAGATCTGGTC
pET-28a(+)-XhoI-trt14-R	ATATGCCTCTCGAGTCAAAAAGTGATCCCATCGAG
InF-pUSA-AtCPR-F	CCGAATTTCGAGCTCGATGGCTCAACTCGACACTCT
InF-pUSA-AtCPR-R	ACTACAGATCCCCGGTCATGACCACACGTCCTCCT
InF-pTA-PamyB-F1	GCTCGCGAGCGCGTTGCATCATCAGTCTAG
InF-pTA-TamyB-R1	AACGCGCTCGCGAGCCCATACAGTACCGCG
InF-pPTRI-PamyB-F	TGATTACGCCAAGCTTCGACTCCAATCTTCAAGAGC
InF-pPTRI-TamyB-R3	GCAGGCATGCAAGCTTGTAAGATACATGAGCTTCG
InF-pTA-PamyB-F2	TCGCGTGCGCGTTTACCCATCATGGTGTTTTGATC
InF-pTA-TamyB-R2	TAAACGCGCACGCGACATTAATCCGGATCCTTTCC
InF-pAdeA-PamyB-F	CAGGTCGACTCTAGAGGATCCCAATCTTCAAGAGC
InF-pAdeA-TamyB-R	AGTAGATCCTCTAGAGTCGAATACATGAGCTTCGG

Table S2. Plasmids constructed in this study and PCR / ligation conditions

Plasmid	Insert	Primer 1	Primer 2	PCR Template	Ligation method
pTAex3-trt9	<i>trt9</i>	EcoRI-trt9-F	EcoRI-trt9-R	gDNA	DNA Ligation Kit Ver.2.1
pUNA-trt9	<i>trt9</i>	InF-pUNA-trt9-F	InF-pUNA-trt9-R	gDNA	In-Fusion® HD Cloning Kit
pTAex3-trt3	<i>trt3</i>	KpnI-trt3-F	KpnI-trt3-R	gDNA	DNA Ligation Kit Ver.2.1
pUNA-trt9+3	<i>trt9-TamyB</i>	InF-pUNA-trt9-F	InF-pTA-TamyB-R1	pTAex3-trt9	In-Fusion® HD Cloning Kit
	<i>PamyB-trt3</i>	InF-pTA-PamyB-F1	InF-pUNA-trt3-R	pTAex3-trt3	
pTAex3-trt6	<i>trt6</i>	EcoRI-trt6-F	EcoRI-trt6-R	gDNA	DNA Ligation Kit Ver.2.1
pTAex3-trt14	<i>trt14</i>	KpnI-trt14-F	KpnI-trt14-R	gDNA	DNA Ligation Kit Ver.2.1
pTAex3-trt7	<i>trt7</i>	KpnI-trt7-F	KpnI-trt7-R	gDNA	DNA Ligation Kit Ver.2.1
pBAR-trt6	<i>PamyB-trt6-TamyB</i>	InF-pPTRI-PamyB-F	InF-pPTRI-TamyB-R3	pTAex3-trt6	In-Fusion® HD Cloning Kit
pBAR-trt6+14	<i>PamyB-trt6-TamyB</i>	InF-pPTRI-PamyB-F	InF-pTA-PamyB-R1	pTAex3-trt6	In-Fusion® HD Cloning Kit
	<i>PamyB-trt14-TamyB</i>	InF-pTA-PamyB-F1	InF-pPTRI-TamyB-R3	pTAex3-trt14	
pBAR-trt6+7	<i>PamyB-trt6-TamyB</i>	InF-pPTRI-PamyB-F	InF-pTA-PamyB-R1	pTAex3-trt6	In-Fusion® HD Cloning Kit
	<i>PamyB-trt7-TamyB</i>	InF-pTA-PamyB-F1	InF-pPTRI-TamyB-R3	pTAex3-trt7	
pBAR-trt6+7+14	<i>PamyB-trt6-TamyB</i>	InF-pPTRI-PamyB-F	InF-pTA-PamyB-R1	pTAex3-trt6	In-Fusion® HD Cloning Kit
	<i>PamyB-trt7-TamyB</i>	InF-pTA-PamyB-F1	InF-pTA-PamyB-F2	pTAex3-trt7	
	<i>PamyB-trt14-TamyB</i>	InF-pTA-PamyB-F2	InF-pPTRI-TamyB-R3	pTAex3-trt14	
pAdeA-trt6	<i>PamyB-trt6-TamyB</i>	InF-pAdeA-PamyB-F	InF-pAdeA-PamyB-R	pTAex3-trt6	In-Fusion® HD Cloning Kit
pUSA-AtCPR	<i>AtCPR</i>	InF-pUSA-AtCPR-F	InF-pUSA-AtCPR-R	gDNA	In-Fusion® HD Cloning Kit
pET-28a(+)-trt14	<i>trt14</i>	NdeI-trt14-F	pET-28a(+)-XhoI-trt14-R	gDNA	DNA Ligation Kit Ver.2.1

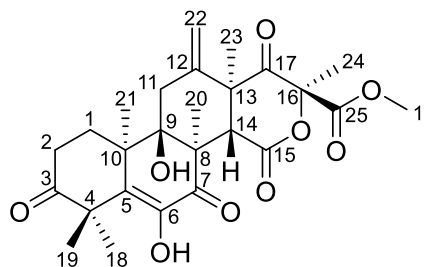


Table S3. NMR data for terretonin (1)

position	¹³ C		¹ H				
	δ (ppm)	δ (ppm)	intensity	multiplicity	HMBC correlation	COSY correlation	NOESY correlation
1	28.1	1.76 (α)	1H	dd (<i>J</i> = 14.0, 8.4 Hz)	3, 5, 9, 10, 21	H-1β, H-2α, H-2β	H-1β, H-2α, H-21
		2.36 (β)	1H	m	2, 10, 21	H-1α, H-2α, H-2β	H-1α, H-2β, H-11β
2	32.6	2.52 (α)	1H	m	1, 3	H-1α, H-1β, H-2β	H-1α, H-2β, H-18, H-21
		2.71 (β)	1H	dd (<i>J</i> = 19.1, 8.4 Hz)	1, 3, 10	H-1α, H-1β, H-2α	H-1β, H-2α, H-19
3	214.0						
4	47.8						
5	131.6						
6	138.7						
7	197.0						
8	52.3						
9	77.6						
10	43.1						
11	34.9	2.97 (α)	1H	brd (<i>J</i> = 14.0 Hz)	9, 12, 22	H-11β	H-11β, H-20, H-21, H-23
		2.26 (β)	1H	brd (<i>J</i> = 14.6 Hz)	8, 9, 10, 12, 13, 22	H-11α	H-1β, H-11α, H-22a
12	139.8						
13	49.4						
14	44.6	3.54	1H	s	7, 8, 13, 15, 17, 20, 23		9-OH
15	168.5						
16	85.5						
17	201.4						
18	21.2	1.47	3H	s	3, 4, 5, 19		H-2α
19	23.6	1.48	3H	s	3, 4, 5, 18		H-2β
20	19.8	1.93	3H	s	7, 8, 9, 14		H-11α, H-21, H-23
21	18.6	1.21	3H	s	1, 5, 9, 10		H-1α, H-2α, H-11α, H-20
22	117.1	5.09 (a)	1H	brs	11, 13	H-22b	H-11β, H-22b, 9-OH
		5.47 (b)	1H	brs	11, 12, 13	H-22a	H-22a, H-23
23	23.4	1.44	3H	s	12, 13, 14, 17		H-11α, H-20, H-22b
24	21.2	1.72	3H	s	16, 17, 25		
25	167.7						
1'	53.7	3.80	3H	s	25		
6-OH		6.14	1H	s	5, 6, 7		
9-OH		1.81	1H	s	8, 9, 10, 11		H-14, H-22a

¹H NMR: 500 MHz, ¹³C NMR: 125 MHz (in CDCl₃)

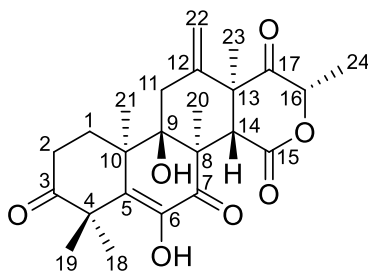


Table S4. NMR data for terretonin C (2)

position	¹³ C		¹ H				
	δ (ppm)	δ(ppm)	intensity	multiplicity	HMBC correlation	COSY correlation	NOESY correlation
1	28.2	1.79 (α)	1H	dd (<i>J</i> = 13.5, 8.4 Hz)	2, 3, 5, 9, 10, 21	H-1β, H-2α, H-2β	H-1β, H-2α, H-11β, H-21
		2.39 (β)	1H	m	2, 10	H-1α, H-2α, H-2β	H-1α, H-2β
2	32.6	2.54 (α)	1H	m	3	H-1α, H-1β, H-2β	H-1α, H-2β, H-21
		2.72 (β)	1H	dd (<i>J</i> = 19.1, 8.4 Hz)	1, 3, 10	H-1α, H-1β, H-2α	H-1β, H-2α
3	213.9						
4	47.8						
5	131.9						
6	138.7						
7	197.4						
8	52.1						
9	77.9						
10	43.2						
11	35.1	3.01 (α)	1H	brd (<i>J</i> = 14.0 Hz)	9, 12, 22	H-11β, H-22a, H-22b	H-11β, H-20, H-21, H-23
		2.29 (β)	1H	brd (<i>J</i> = 14.6 Hz)	8, 9, 10, 12, 13, 22	H-11α	H-1α, H-11α, H-22a
12	140.3						
13	49.3						
14	44.9	3.83	1H	s	7, 8, 13, 15, 17, 20, 23		H-16, 9-OH
15	169.2						
16	77.4	5.08	1H	q (<i>J</i> = 6.2 Hz)	17, 24	H-24	H-14, H-24
17	206.5						
18	21.2	1.48	3H	s	3, 4, 5, 19		
19	23.6	1.48	3H	s	3, 4, 5, 18		
20	19.9	1.97	3H	s	7, 8, 9, 14		H-11α, H-21, H-23
21	18.7	1.23	3H	s	1, 5, 9, 10		H-1α, H-2α, H-11α, H-20
22	116.5	5.06 (a)	1H	d (<i>J</i> = 1.1 Hz)	11, 13	H-11α, H-22b	H-11β, H-22b
		5.39 (b)	1H	brs	11, 12, 13	H-11α, H-22a	H-22a
23	22.8	1.42	3H	s	12, 13, 14, 17		H-11α, H-20
24	14.6	1.50	3H	d (<i>J</i> = 6.2 Hz)	16, 17	H-16	H-16
6-OH		6.15	1H	s	5, 6, 7		
9-OH		1.83	1H	s	8, 9, 10, 11		H-14

¹H NMR: 500 MHz, ¹³C NMR: 125 MHz (in CDCl₃)

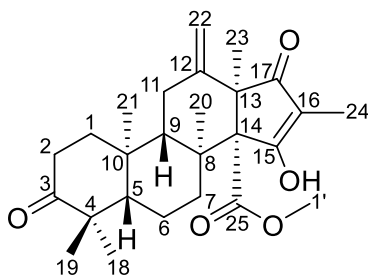


Table S5. NMR data for preterrenoid (5)

position	¹³ C		¹ H				
	δ (ppm)	δ (ppm)	intensity	multiplicity	HMBC correlation	COSY correlation	NOESY correlation
1	39.0	1.76 (α)	1H	dt (<i>J</i> = 11.2, 3.4 Hz)	2, 3, 5, 10, 21	H-1β, H-2	H-1β, H-21
		1.37 (β)	1H	m	2, 3, 9, 10, 21	H-1α, H-2	H-1α, H-5, H-11β
2	33.7	2.39	2H	m	1, 3	H-1α, H-1β	
3	216.9						
4	47.1						
5	54.9	1.22	1H	m	4, 9, 10, 18, 19, 21	H-6	H-1β, H-7β, H-9, H-19
6	19.7	1.49	2H	m	4, 5, 8, 10	H-5, H-7α, H-7β	
7	37.8	1.75 (α)	1H	m	5, 9, 14, 20	H-6, H-7β	H-7β, H-20
		1.53 (β)	1H	m	20	H-6, H-7α	H-5, H-7α
8	44.5						
9	44.5	1.35	1H	m	7, 8, 11, 14, 20	H-11α, H-11β	H-5, H-11β
10	37.8						
11	28.7	2.24 (α)	1H	dd (<i>J</i> = 16.8, 11.8 Hz)	9, 10, 12, 13, 22	H-9, H-11β, H-22a, H-22b	H-11β, H-20, H-21, H-23
		2.44 (β)	1H	m	9, 12	H-9, H-11α, H-22a, H-22b	H-1β, H-9, H-11α, H-22a
12	143.4						
13	57.6						
14	67.9						
15	177.4						
16	112.9						
17	205.2						
18	21.4	1.01	3H	s	3, 4, 5, 19		
19	26.9	1.06	3H	s	3, 4, 5, 18		H-5
20	19.3	0.97	3H	s	7, 8, 9, 14		H-7α, H-11α, H-1'
21	15.7	1.03	3H	s	1, 5, 9, 10		H-1α, H-11α
22	113.6	5.18 (a)	1H	brs		H-11α, H-11β	H-11β, H-22b
		5.12 (b)	1H	d (<i>J</i> = 2.8 Hz)	11, 12, 13	H-11α, H-11β	H-22a, H-23
23	23.4	1.31	3H	s	12, 13, 14, 17		H-11α, H-22b, H-1'
24	5.97	1.73	3H	s	15, 16, 17		
25	175.1						
1'	51.8	3.79	3H	s	25		H-20, H-23
OH		9.66	1H	brs			

¹H NMR: 500 MHz, ¹³C NMR: 125 MHz (in CDCl₃)

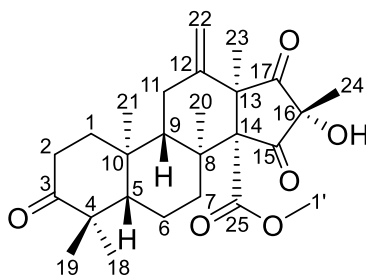


Table S6. NMR data for terrenoid (6)

position	¹³ C		¹ H				
	δ (ppm)	δ (ppm)	intensity	multiplicity	HMBC correlation	COSY correlation	NOESY correlation
1	39.1	1.94 (α) 1.41 (β)	1H 1H	m m	2, 3, 5, 10, 21 2, 3, 5, 9, 10, 21	H-1β, H-2 H-1α, H-2	H-1β, H-11β, H-21 H-1α, H-9
2	33.7	2.47	2H	m	1, 3, 4, 10	H-1α, H-1β	
3	216.9						
4	47.2						
5	53.5	1.52	1H	m	1, 4, 6, 7, 9, 10, 18, 19, 21	H-6	H-7β, H-9, H-19
6	18.9	1.57	2H	m	4, 5, 7, 8, 10	H-5, H-7α, H-7β	
7	32.8	2.14 (α) 2.94 (β)	1H 1H	dt (<i>J</i> = 14.0, 3.4 Hz) m	5, 6, 8, 9 8, 14, 20	H-6, H-7β H-6, H-7α	H-7β, H-20 H-5, H-7α
8	40.5						
9	50.3	1.30	1H	dd (<i>J</i> = 13.5, 3.9 Hz)	1, 5, 8, 10, 11, 12, 14, 20, 21	H-11α, H-11β	H-1β, H-5, H-11β
10	37.0						
11	28.1	2.62 (α) 2.37 (β)	1H 1H	ddt (<i>J</i> = 16.3, 14.0, 2.8 Hz) dd (<i>J</i> = 16.3, 3.9 Hz)	8, 9, 10, 12, 22 8, 9, 10, 12, 13, 22	H-9, H-11β, H-22a, H-22b H-9, H-11α, H-22a	H-11β, H-20, H-21, H-23 H-1α, H-9, H-11α, H-22a
12	143.7						
13	57.5						
14	73.6						
15	209.9						
16	75.2						
17	211.1						
18	21.2	1.06	3H	s	3, 4, 5, 19		H-5
19	26.8	1.13	3H	s	3, 4, 5, 18		
20	17.9	1.38	3H	s	7, 8, 9, 14		H-7α, H-11α, H-21, H-23, H-1'
21	15.5	1.01	3H	s	1, 5, 9, 10		H-1α, H-11α, H-20
22	116.3	5.06 (a) 4.90 (b)	1H 1H	d (<i>J</i> = 2.2 Hz) d (<i>J</i> = 2.8 Hz)	11, 13 11, 12, 13	H-11α, H-11β H-11α	H-11β, H-22b H-22a, H-23
23	23.6	1.53	3H	s	12, 13, 14, 17		H-11α, H-20, H-22b, H-1'
24	25.6	1.39	3H	s	15, 16, 17		
25	168.6						
1'	52.4	3.64	3H	s	25		H-20, H-23

¹H NMR: 500 MHz, ¹³C NMR: 125 MHz (in CDCl₃)

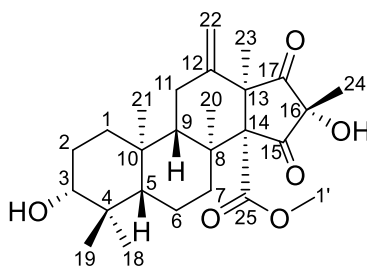


Table S7. NMR data for terrenoidol (7)

position	¹³ C		¹ H				
	δ (ppm)	δ (ppm)	intensity	multiplicity	HMBC correlation	COSY correlation	NOESY correlation
1	38.4	1.71 (α)	1H	dt (<i>J</i> = 12.5, 3.4 Hz)	2, 3, 5	H-1β, H-2α, H-2β	H-1β, H-11β, H-21
		0.91 (β)	1H	m	3	H-1α, H-2α, H-2β	H-1α, H-2β
2	27.0	1.63 (α)	1H	m	3, 4	H-1α, H-1β, H-2β, H-3	H-18, H-21
		1.58 (β)	1H	dd (<i>J</i> = 11.9, 3.4 Hz)	3, 4	H-1α, H-1β, H-2α, H-3	H-1β, H-3, H-19
3	78.4	3.25	1H	dd (<i>J</i> = 11.9, 5.1 Hz)	18, 19	H-2α, H-2β	H-2β, H-5, H-19
4	38.8						
5	53.8	0.89	1H	m	3, 6, 7, 18, 19	H-6α, H-6β	H-3, H-7β, H-9, H-19
6	17.7	1.46 (α)	1H	td (<i>J</i> = 12.5, 3.4 Hz)		H-5, H-7α, H-7β	H-6β, H-7α, H-18
		1.61 (β)	1H	m	4	H-5, H-7α, H-7β	H-6α, H-7α, H-7β, H-19
7	33.5	2.08 (α)	1H	dt (<i>J</i> = 13.0, 2.8 Hz)	5, 8, 9	H-6α, H-6β, H-7β	H-6α, H-6β, H-7β, H-20
		2.90 (β)	1H	td (<i>J</i> = 13.6, 4.5 Hz)	8, 20	H-6α, H-6β, H-7α	H-5, H-6β, H-7α
8	40.4						
9	50.9	1.21	1H	dd (<i>J</i> = 13.6, 4.0 Hz)	8, 10, 11, 14, 20, 21	H-11α, H-11β	H-5, H-11β
10	37.4						
11	27.6	2.56 (α)	1H	m	9, 12	H-9, H-11β, H-22a, H-22b	H-11β, H-20, H-21, H-23
		2.37 (β)	1H	dd (<i>J</i> = 16.4, 4.0 Hz)	8, 9, 12, 13, 22	H-9, H-11α, H-22a	H-1α, H-9, H-11α, H-22a
12	144.0						
13	57.4						
14	73.7						
15	210.1						
16	75.2						
17	211.4						
18	15.5	0.80	3H	s	3, 4, 5, 19		H-2α, H-6α, H-19
19	27.9	1.03	3H	s	3, 4, 5, 18		H-2β, H-3, H-5, H-6β, H-18
20	18.2	1.33	3H	s	7, 8, 9, 14		H-7α, H-11α, H-21, H-23
21	15.5	0.92	3H	s	1, 5, 9, 10		H-1α, H-2α, H-11α, H-20
22	116.2	5.04 (a)	1H	d (<i>J</i> = 1.7 Hz)	11, 13	H-11α, H-11β	H-11β, H-22b
		4.89 (b)	1H	d (<i>J</i> = 2.8 Hz)	11, 12, 13	H-11α	H-22a, H-23
23	23.6	1.52	3H	s	12, 13, 14, 17		H-11α, H-20, H-22b, H-1'
24	25.5	1.38	3H	s	15, 16, 17		
25	168.8						
1'	52.3	3.63	3H	s	25		H-23

¹H NMR: 500 MHz, ¹³C NMR: 125 MHz (in CDCl₃)

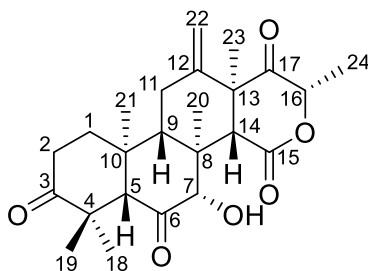


Table S8. NMR data for terretonin H (8)

position	¹³ C		¹ H				
	δ (ppm)	δ (ppm)	intensity	multiplicity	HMBC correlation	COSY correlation	NOESY correlation
1	40.0	2.19 (α)	1H	ddd (<i>J</i> = 12.9, 6.7, 2.8 Hz)	2, 3, 5, 10, 21	H-1β, H-2α, H-2β	H-1β, H-2α, H-2β, H-11β, H-21
2	33.4	1.59 (β)	1H	m	2, 3, 5, 9, 10, 21	H-1α, H-2α, H-2β	H-1α, H-2β, H-5, H-9
		2.78 (α)	1H	m	1, 3, 10	H-1α, H-1β, H-2β	H-1α, H-2β, H-18, H-21
3	213.0	2.30 (β)	1H	ddd (<i>J</i> = 15.2, 4.5, 2.8 Hz)	3, 10	H-1α, H-1β, H-2α	H-1α, H-1β, H-2α
4	46.6						
5	63.0	2.47	1H	s	1, 4, 6, 9, 10, 18, 19, 21		H-1β, H-7, H-9, H-19
6	206.0						
7	85.2	4.04	1H	d (<i>J</i> = 2.2 Hz)	6, 8, 9, 14, 20	OH	H-5, H-9, H-14, OH
8	49.2						
9	57.6	1.62	1H	dd (<i>J</i> = 15.7, 2.2 Hz)	1, 5, 7, 8, 10, 11, 12, 14, 20	H-11α, H-11β	H-1β, H-5, H-7, H-11β, H-14
10	43.0						
11	28.7	2.54 (α)	1H	t (<i>J</i> = 13.5 Hz)	8, 9, 12, 13, 22	H-9	H-11β, H-20, H-21, H-23
12	143.5	2.37 (β)	1H	dd (<i>J</i> = 14.0, 1.7 Hz)	8, 9, 10, 12, 13, 22	H-9	H-1α, H-9, H-11α, H-22a
13	51.5						
14	57.1	3.23	1H	s	7, 8, 9, 12, 13, 15, 17, 20, 23		H-7, H-9, H-16
15	171.0						
16	77.2	5.04	1H	q (<i>J</i> = 6.2 Hz)	17, 24	H-24	H-14, H-24
17	205.2						
18	21.8	1.51	3H	s	3, 4, 5, 19		H-2α, H-19, H-21
19	24.0	1.07	3H	s	3, 4, 5, 18		H-5, H-18
20	12.7	1.41	3H	s	7, 8, 9, 14		H-11α, H-21, OH
21	16.2	1.16	3H	s	1, 5, 9, 10		H-1α, H-2α, H-11α, H-18, H-20
22	111.6	4.94 (a)	1H	brs	11, 12, 13	H-22b	H-11β
23	22.9	4.99 (b)	1H	brs	11, 12, 13	H-22a	
24	14.3	1.36	3H	s	12, 13, 14, 17		H-11α
OH	14.3	1.46	3H	d (<i>J</i> = 6.2 Hz)	16, 17	H-16	H-16
		4.56	1H	d (<i>J</i> = 3.4 Hz)	6, 7, 8	H-7	H-7, H-20

¹H NMR: 500 MHz, ¹³C NMR: 125 MHz (in CDCl₃)

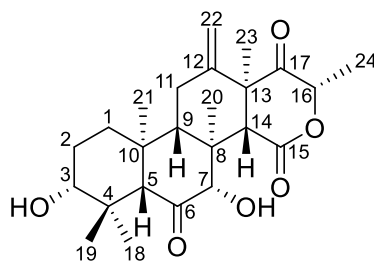


Table S9. NMR data for terretonin I (9)

position	¹³ C	¹ H					
	δ (ppm)	δ (ppm)	intensity	multiplicity	HMBC correlation	COSY correlation	NOESY correlation
1	39.0	1.91 (α)	1H	dt (<i>J</i> = 13.0, 3.4 Hz)	3, 5, 10	H-1β, H-2α, H-2β	H-1β, H-2α, H-2β, H-11β, H-21
		1.29 (β)	1H	m	21	H-1α, H-2α, H-2β	H-1α, H-2β, H-5, H-9
2	26.6	1.64 (α)	1H	m	4	H-1α, H-1β, H-2β, H-3	H-1α, H-2β, H-18, H-21
		1.73 (β)	1H	m	3, 10	H-1α, H-1β, H-2α, H-3	H-1α, H-1β, H-2α, H-3
3	78.1	3.14	1H	dd (<i>J</i> = 11.3, 3.0 Hz)		H-2α, H-2β	H-2β, H-5, H-19
4	37.5						
5	63.5	2.11	1H	s	1, 3, 6, 9, 10, 19, 21		H-1β, H-3, H-7, H-9, H-19
6	206.6						
7	85.4	4.01	1H	d (<i>J</i> = 2.3 Hz)	8, 20	7-OH	H-5, H-9, H-14, 7-OH
8	49.4						
9	58.3	1.55	1H	m	10, 11, 20, 21	H-11α, H-11β	H-1β, H-5, H-7, H-14
10	43.4						
11	28.5	2.46 (α)	1H	t (<i>J</i> = 13.0 Hz)	9, 12, 22	H-9, H-11β	H-11β, H-20, H-21, H-23
		2.38 (β)	1H	dd (<i>J</i> = 14.2, 2.8 Hz)	8, 9, 12, 22	H-9, H-11α	H-1α, H-11α, H-22a
12	144.2						
13	51.6						
14	57.3	3.18	1H	s	7, 8, 12, 13, 15, 17, 20, 23		H-7, H-9, H-16
15	170.4						
16	77.1	5.01	1H	q (<i>J</i> = 6.2 Hz)	24	H-24	H-14, H-24
17	205.4						
18	15.3	1.25	3H	s	3, 4, 5, 19		H-2α, H-19, H-21
19	27.3	1.03	3H	s	3, 4, 5, 18		H-3, H-5, H-18
20	12.6	1.38	3H	s	7, 8, 9, 14		H-11α, H-21
21	16.8	0.92	3H	s	1, 5, 9, 10		H-1α, H-2α, H-11α, H-18, H-20
22	111.2	4.94 (a)	1H	brs	11, 13	H-22b	H-11β, H-22b
		4.98 (b)	1H	brs	11, 12, 13	H-22a	H-22a
23	22.9	1.37	3H	s	12, 13, 14, 17		H-11α
24	14.4	1.48	3H	d (<i>J</i> = 6.2 Hz)	16, 17	H-16	H-16
7-OH		4.46	1H	d (<i>J</i> = 2.8 Hz)		H-7	H-7

¹H NMR: 500 MHz, ¹³C NMR: 125 MHz (in CDCl₃)

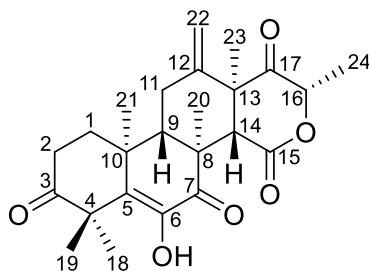


Table S10. NMR data for terretonin J (10)

position	¹³ C		¹ H				
	δ (ppm)	δ (ppm)	intensity	multiplicity	HMBC correlation	COSY correlation	NOESY correlation
1	34.3	2.22 (α) 1.73 (β)	1H 1H	dd (<i>J</i> = 13.6, 9.1 Hz) dd (<i>J</i> = 9.5, 3.7 Hz)	3, 5 21	H-1β, H-2α, H-2β H-1α, H-2α, H-2β	H-1β, H-2α, H-2β, H-11β, H-21 H-1α, H-2β
2	32.6	2.54 (α) 2.70 (β)	1H 1H	m dd (<i>J</i> = 19.3, 9.1 Hz)	1 1, 3, 10	H-1α, H-1β, H-2β H-1α, H-1β, H-2α	H-1α, H-2β, H-21 H-1α, H-1β, H-2α
3	213.8						
4	48.0						
5	137.7						
6	139.1						
7	198.1						
8	45.5						
9	52.4	1.69	1H	dd (<i>J</i> = 13.0, 2.8 Hz)	10, 21	H-11α, H-11β	H-11β, H-14
10	38.2						
11	29.2	2.54 (α) 2.36 (β)	1H 1H	m dd (<i>J</i> = 13.6, 2.3 Hz)	9, 12, 22 8, 12, 13, 22	H-9, H-11β H-9, H-11α	H-11β, H-20, H-21, H-23 H-1α, H-9, H-11α, H-22a
12	143.2						
13	50.3						
14	49.5	3.11	1H	s	8, 13, 15, 17, 20, 23		H-9, H-16
15	168.7						
16	77.3	5.02	1H	q (<i>J</i> = 6.8 Hz)	17, 24	H-24	H-14, H-24
17	206.2						
18	20.9	1.47	3H	s	3, 4, 5, 19		H-21, OH
19	23.8	1.47	3H	s	3, 4, 5, 18		
20	16.3	1.86	3H	s	7, 8, 9, 14		H-11α, H-21, H-23
21	16.8	1.13	3H	s	1, 5, 9, 10		H-1α, H-2α, H-11α, H-18, H-20
22	111.8	4.96 (a) 5.05 (b)	1H 1H	brs brs	11, 13 11, 12, 13		H-11β, H-22b H-22a, H-23
23	22.9	1.40	3H	s	12, 13, 14, 17		H-11α, H-20 H-22b
24	14.5	1.50	3H	d (<i>J</i> = 6.8 Hz)	16, 17	H-16	H-16
OH		6.20	1H	s	5, 7		H-18

¹H NMR: 500 MHz, ¹³C NMR: 125 MHz (in CDCl₃)

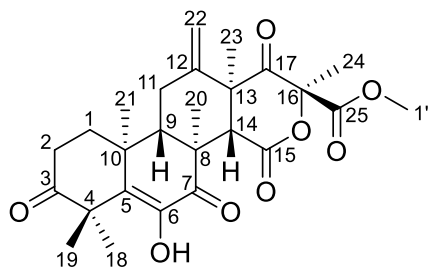


Table S11. NMR data for terretonin A (11)

position	¹³ C	¹ H					
	δ (ppm)	δ (ppm)	intensity	multiplicity	HMBC correlation	COSY correlation	NOESY correlation
1	34.3	2.18 (α)	1H	dd (<i>J</i> = 14.0, 9.0 Hz)	2, 3, 5, 10	H-1β, H-2α, H-2β	H-1β, H-2α, H-11β, H-18, H-21
		1.70 (β)	1H	dd (<i>J</i> = 10.1, 3.4 Hz)		H-1α, H-2α, H-2β	H-1α, H-2β, H-9
2	32.6	2.52 (α)	1H	m	1	H-1α, H-1β, H-2β	H-1α, H-2β
		2.69 (β)	1H	dd (<i>J</i> = 19.1, 9.0 Hz)	1, 3, 10	H-1α, H-1β, H-2α	H-1β, H-2α
3	213.8						
4	47.9						
5	137.2						
6	139.0						
7	197.9						
8	45.8						
9	52.9	1.54	1H	dd (<i>J</i> = 12.9, 2.8 Hz)	1, 8, 10, 11, 21	H-11α, H-11β	H-1β, H-11β, H-14
10	38.2						
11	29.1	2.51 (α)	1H	m	8, 9, 12, 22	H-9, H-11β	H-11β, H-20, H-21, H-23
		2.33 (β)	1H	dd (<i>J</i> = 13.5, 2.8 Hz)	8, 9, 12, 13, 22	H-9, H-11α	H-1α, H-9, H-11α, H-22a
12	143.0						
13	50.6						
14	49.1	2.84	1H	s	7, 8, 13, 15, 17, 20, 23		H-9
15	166.9						
16	85.9						
17	201.3						
18	20.9	1.46	3H	s	3, 4, 5, 19		H-1α, H-21, OH
19	23.7	1.47	3H	s	3, 4, 5, 18		
20	16.5	1.83	3H	s	7, 8, 9, 14		H-11α, H-21, H-23
21	16.9	1.11	3H	s	1, 5, 9, 10		H-1α, H-11α, H-18, H-20
22	112.1	4.98 (a)	1H	brs	11, 13		H-11β, H-22b
		5.17 (b)	1H	brs	11, 12, 13		H-22a
23	23.6	1.43	3H	s	12, 13, 17		H-11α, H-20
24	22.0	1.73	3H	s	16, 17, 25		H-1'
25	168.4						
1'	53.7	3.82	3H	s	25		H-24
OH		6.18	1H	s	6, 7		H-18

¹H NMR: 500 MHz, ¹³C NMR: 125 MHz (in CDCl₃)

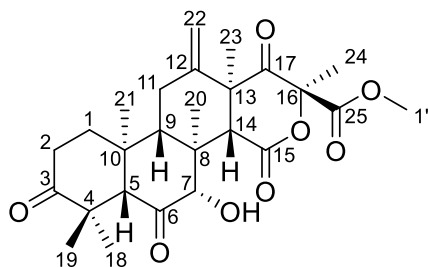


Table S12. NMR data for terretonin D (12)

position	¹³ C	¹ H					
	δ (ppm)	δ (ppm)	intensity	multiplicity	HMBC correlation	COSY correlation	NOESY correlation
1	39.9	2.17 (α)	1H	ddd (<i>J</i> = 13.0, 6.2, 2.8 Hz)	2, 3, 5, 10, 21	H-1β, H-2α, H-2β	H-1β, H-2α, H-2β, H-11β, H-21
		1.62 (β)	1H	td (<i>J</i> = 13.6, 4.5 Hz)	2, 3, 9, 10, 21	H-1α, H-2α, H-2β	H-1α, H-2β, H-5, H-9
2	33.3	2.78 (α)	1H	td (<i>J</i> = 14.2, 6.2 Hz)	1, 3, 10	H-1α, H-1β, H-2β	H-1α, H-2β, H-18, H-21
		2.30 (β)	1H	ddd (<i>J</i> = 15.3, 4.5, 2.8 Hz)	1, 3, 4, 10	H-1α, H-1β, H-2α	H-1α, H-1β, H-2α
3	212.9						
4	46.6						
5	62.9	2.44	1H	s	1, 3, 4, 6, 9, 10, 18, 19, 21		H-1β, H-7, H-9, H-19
6	205.7						
7	85.2	3.89	1H	d (<i>J</i> = 1.7 Hz)	6, 8, 9, 20	OH	H-5, H-9, H-14, OH
8	49.6						
9	58.2	1.44	1H	dd (<i>J</i> = 13.0, 2.3 Hz)	1, 5, 7, 8, 10, 11, 12, 14, 20, 21	H-11α, H-11β	H-1β, H-5, H-7, H-11β, H-14
10	42.9						
11	28.7	2.50 (α)	1H	t (<i>J</i> = 13.6 Hz)	8, 9, 10, 12, 13, 22	H-9, H-11β	H-11β, H-20, H-21, H-23
		2.35 (β)	1H	dd (<i>J</i> = 14.2, 2.3 Hz)	8, 9, 12, 13, 22	H-9, H-11α	H-1α, H-9, H-11α, H-22a
12	143.6						
13	52.0						
14	56.7	2.94	1H	s	7, 8, 9, 12, 13, 15, 17, 20, 23		H-7, H-9
15	169.0						
16	86.2						
17	200.3						
18	21.8	1.50	3H	s	3, 4, 5, 19		H-2α, H-19, H-21
19	24.0	1.08	3H	s	3, 4, 5, 18		H-5, H-18
20	13.0	1.39	3H	s	7, 8, 9, 14		H-11α, H-21, OH
21	16.3	1.15	3H	s	1, 5, 9, 10		H-1α, H-2α, H-11α, H-18, H-20
22	111.9	4.97 (a)	1H	brs	11, 12	H-22b	H-11β
		5.10 (b)	1H	brs	11, 12, 13	H-22a	H-23
23	23.6	1.40	3H	s	12, 13, 14, 17		H-11α, H-22b
24	22.1	1.71	3H	s	16, 17, 25		H-1'
25	168.2						
1'	53.9	3.84	3H	s	25		H-24
OH		4.59	1H	d (<i>J</i> = 2.8 Hz)	6, 7, 8	H-7	H-7, H-20

¹H NMR: 500 MHz, ¹³C NMR: 125 MHz (in CDCl₃)

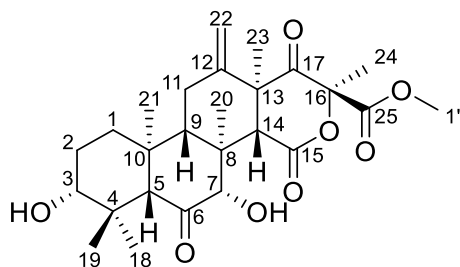


Table S13. NMR data for terretonin K (13)

position	¹³ C	¹ H					
	δ (ppm)	δ (ppm)	intensity	multiplicity	HMBC correlation	COSY correlation	NOESY correlation
1	38.9	1.88 (α)	1H	dt (<i>J</i> = 13.0, 3.4 Hz)	3, 5	H-1β, H-2α, H-2β	H-1β, H-2α, H-11β, H-21
		1.28 (β)	1H	m		H-1α, H-2α, H-2β	H-1α, H-3, H-5, H-9
2	26.6	1.64 (α)	1H	m	1, 3	H-1α, H-1β, H-2β, H-3	H-1α, H-2β, H-18, H-21
		1.73 (β)	1H	m	1	H-1α, H-1β, H-2α, H-3	H-2α, H-3
3	78.0	3.15	1H	dd (<i>J</i> = 11.9, 4.5 Hz)	18, 19	H-2α, H-2β	H-1β, H-2β, H-5, H-19
4	37.5						
5	63.3	2.06	1H	s	3, 4, 6, 9, 10, 19, 21		H-1β, H-3, H-7, H-9, H-19
6	206.4						
7	85.3	3.86	1H	brs	8, 14, 20	7-OH	H-5, H-9, H-14, 7-OH
8	49.7						
9	58.7	1.36	1H	dd (<i>J</i> = 12.5, 2.8 Hz)	10, 11, 20, 21	H-11α, H-11β	H-1β, H-5, H-7, H-11β, H-14
10	43.2						
11	28.5	2.42 (α)	1H	t (<i>J</i> = 12.5 Hz)	8, 10, 12, 13, 22	H-9, H-11β	H-11β, H-20, H-21, H-23
		2.34 (β)	1H	dd (<i>J</i> = 14.2, 2.8 Hz)	8, 9, 12, 13, 22	H-9, H-11α	H-1α, H-9, H-11α, H-22a
12	144.1						
13	52.0						
14	56.8	2.94	1H	s	7, 8, 9, 12, 13, 15, 20, 23		H-7, H-9
15	168.7						
16	86.1						
17	200.6						
18	15.3	1.23	3H	s	3, 4, 5, 19		H-2α, H-19, H-21, 7-OH
19	27.2	1.02	3H	s	3, 4, 5, 18		H-3, H-5, H-18
20	12.8	1.34	3H	s	7, 8, 9, 14		H-11α, H-21
21	16.9	0.89	3H	s	1, 5, 9, 10		H-1α, H-2α, H-11α, H-18, H-20
22	111.5	4.97 (a)	1H	brs	11, 13	H-22b	H-11β, H-22b
		5.09 (b)	1H	brs	11, 12, 13	H-22a	H-22a, H-23
23	23.6	1.39	3H	s	12, 13, 14, 17		H-11α, H-22b, H-24
24	22.2	1.71	3H	s	16, 17, 25		H-23, H-1'
25	168.4						
1'	53.8	3.87	3H	s	25		H-24
7-OH		4.51	1H	brs	8	H-7	H-7, H-18

¹H NMR: 500 MHz, ¹³C NMR: 125 MHz (in CDCl₃)

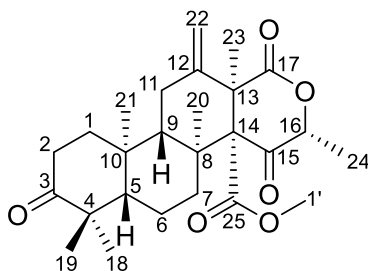


Table S14. NMR data for terretonin L (14)

position	¹³ C	¹ H					
	δ (ppm)	δ (ppm)	intensity	multiplicity	HMBC correlation	COSY correlation	NOESY correlation
1	39.1	1.98 (α)	1H	m	2, 3, 5, 10, 21	H-1β, H-2	H-1β, H-21
		1.41 (β)	1H	m	3	H-1α, H-2	H-1α
2	33.7	2.47	2H	m	1, 3	H-1α, H-1β	
3	216.7						
4	47.2						
5	53.9	1.35	1H	dd (<i>J</i> = 12.3, 3.4 Hz)	4, 6, 7, 10, 18, 19, 21	H-6α, H-6β	H-7β, H-19
6	19.1	1.60 (α)	1H	m	5, 8	H-5, H-6β, H-7α, H-7β	H-18, H-21
		1.55 (β)	1H	m		H-5, H-6α, H-7α, H-7β	H-7α, H-7β
7	33.6	2.08 (α)	1H	dt (<i>J</i> = 13.5, 3.4 Hz)	5	H-6α, H-6β, H-7β	H-6β, H-7β, H-20, H-1'
		2.22 (β)	1H	td (<i>J</i> = 12.9, 4.5 Hz)	20	H-6α, H-6β, H-7α	H-5, H-6β, H-7α, H-9
8	43.5						
9	51.5	1.21	1H	dd (<i>J</i> = 13.5, 3.4 Hz)	8, 10, 11, 20	H-11α, H-11β	H-7β, H-11β, H-16
10	37.4						
11	28.6	2.65 (α)	1H	m	9, 12	H-9, H-11β, H-22a, H-22b	H-11β, H-20, H-21, H-23
		2.45 (β)	1H	m	8, 9, 12, 13, 22	H-9, H-11α, H-22a	H-9, H-11α, H-22a
12	145.3						
13	54.3						
14	68.9						
15	203.0						
16	77.5	5.01	1H	q (<i>J</i> = 6.2 Hz)	24	H-24	H-9, H-24
17	171.9						
18	21.1	1.05	3H	s	3, 4, 5, 19		H-6α
19	26.7	1.09	3H	s	3, 4, 5, 18		H-5
20	19.7	1.47	3H	s	7, 8, 9, 14		H-7α, H-11α, H-21
21	15.7	1.00	3H	s	1, 5, 9, 10		H-1α, H-6α, H-11α, H-20
22	112.9	5.03 (a)	1H	d (<i>J</i> = 2.2 Hz)	11, 13	H-11α, H-11β	H-11β
		4.98 (b)	1H	d (<i>J</i> = 2.2 Hz)	11, 13	H-11α	H-23
23	25.3	1.68	3H	s	12, 13, 14, 17		H-11α, H-22b, H-1'
24	15.3	1.41	3H	d (<i>J</i> = 6.2 Hz)	15, 16	H-16	H-16
25	167.5						
1'	52.3	3.64	3H	s	25		H-7α, H-23

¹H NMR: 500 MHz, ¹³C NMR: 125 MHz (in CDCl₃)

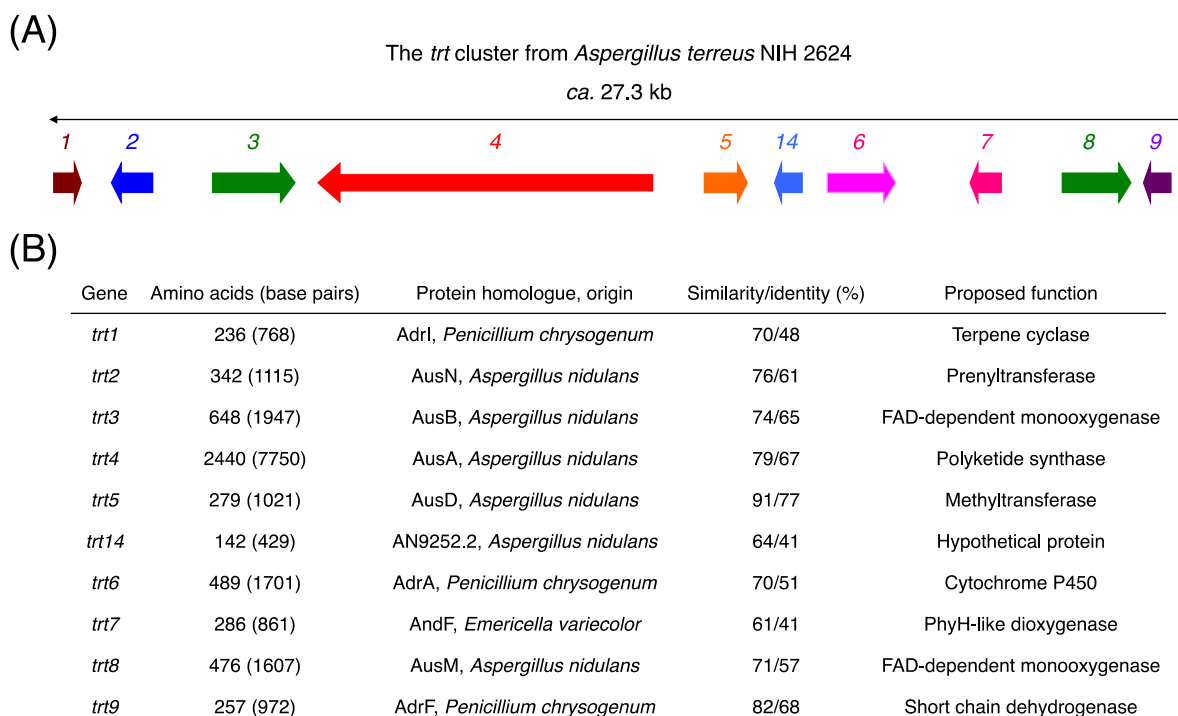


Figure S1. (A) Schematic representation of the *trt* cluster. The direction of the arrow indicates the direction from the start to the stop codon. (B) Annotation of each protein in the *trt* cluster. The deduced function of each open reading frame (ORF) and the amino acid sequence similarity/identity, as compared with the homologues found by a BLAST search at NCBI, are shown.

Database sequence	-----	
Revised sequence	A T G C G A A C C T C C A A G A T A A A G T A G C T G T T C A A T A A G A G T A C C C A T T G A A C A T T C A C T G A	60
Database sequence	-----	
Revised sequence	C A G C A A T A C A G G T C T T G T A T A T C A C T G G A A G T G C A T C G G G T A T C G G T C T T G C C G C A G C C T	120
Database sequence	- - A T G C G C T G G A C C A G G G C G C A A A A G T G T T C G G C G T T G A T A T C G C G G C A T C A C C C G C G C	58
Revised sequence	C G A T G G C G C T G G A C C A G G G C G C A A A A G T G T T C G G C G T T G A T A T C G C G G C A T C A C C C G C G C	180
Database sequence	C G C T G G C C A A C C A C C C A A A C T A C A A A T T C C T T A A G G C T G A T C T C T G T G A C A A G C A A A C A C	118
Revised sequence	C G C T G G C C A A C C A C C C A A A C T A C A A A T T C C T T A A G G C T G A T C T C T G T G A C A A G C A A A C A C	240
Database sequence	C T A A G G A T G T C G T G C A G G C T T G T G T C A A A A T G T T T G G C G G C C G C A T C G A C G G G C T G T T G A	178
Revised sequence	C T A A G G A T G T C G T G C A G G C T T G T G T C A A A A T G T T T G G C G G C C G C A T C G A C G G G C T G T T G A	300
Database sequence	A T A T C G C T G G G A T C A T G G A T A G T A A C C A G A G C G T T G A C A G T G T T T C C G A C G A A A T G T G G G	238
Revised sequence	A T A T C G C T G G G A T C A T G G A T A G T A A C C A G A G C G T T G A C A G T G T T T C C G A C G A A A T G T G G G	360
Database sequence	A C C G C T G T A T T G C G G T C A A T T T G A C G G C T C C G A T A C G A C T G A T G C G G G A G G T A A T T G C T A	298
Revised sequence	A C C G C T G T A T T G C G G T C A A T T T G A C G G C T C C G A T A C G A C T G A T G C G G G A G G T A A T T G C T A	420
Database sequence	T C A T G C G G G A G C A G A A G A G C G G T A A T A T C G T G A A T G T G G C C A G C A A A G C A G C A A C T A G T G	358
Revised sequence	T C A T G C G G G A G C A G A A G A G C G G T A A T A T C G T G A A T G T G G C C A G C A A A G C A G C A A C T A G T G	480
Database sequence	G T G C T G T T T C T G G C G T A G C G T A T A C A G C A A G T G A G T G G G A C G C C T T C C T C G C G T T C C C A T	418
Revised sequence	G T G C T G T T T C T G G C G T A G C G T A T A C A G C A A G T G A G T G G G A C G C C T T C C T C G C G T T C C C A T	540
Database sequence	A A G G T A T C G C G C T A C G G T G A T G T C G C G C T C G T A C T G A T T T G G T A C A A A T A G G C A A G C A T G	478
Revised sequence	A A G G T A T C G C G C T A C G G T G A T G T C G C G C T C G T A C T G A T T T G G T A C A A A T A G G C A A G C A T G	600
Database sequence	G A C T G G T G G G A G C G A C G A A G A A C G T T G C C T G G A G G T T C A A A C A C G A C A A T A T C C G T T G C A	538
Revised sequence	G A C T G G T G G G A G C G A C G A A G A A C G T T G C C T G G A G G T T C A A A C A C G A C A A T A T C C G T T G C A	660
Database sequence	A T G C G G T T T G T C C C G G A G G T A A G T A C G C T G C G A C T G T C C C G G C G T A G A T G G C T A T C C C A C	598
Revised sequence	A T G C G G T T T G T C C C G G A G G T A A G T A C G C T G C G A C T G T C C C G G C G T A G A T G G C T A T C C C A C	720
Database sequence	G G C A T G C G G T T A A C G G G G C G A T T A G G T G T G G T G G G A A C G G G A G T C C A C A A T G A A A T G G A T	658
Revised sequence	G G C A T G C G G T T A A C G G G G C G A T T A G G T G T G G T G G G A A C G G G A G T C C A C A A T G A A A T G G A T	780
Database sequence	A T T C A A A A A T G G G A C A A G G A G G C A A T G A A A A C A A T G T T T C T A T T C A T C A A T C G C A C T C A	718
Revised sequence	A T T C A A A A A T G G G A C A A G G A G G C A A T G A A A A C A A T G T T T C T A T T C A T C A A T C G C A C T C A	840
Database sequence	T G T G A C A A A G A C A A A G G G A T T G G G C T T C G C G C A G A A G A C A T C G C C C G C C C G C T T C T G T T T	778
Revised sequence	T G T G A C A A A G A C A A A G G G A T T G G G C T T C G C G C A G A A G A C A T C G C C C G C C C G C T T C T G T T T	900
Database sequence	C T T G T G T C G G A T A G A A G C A A A G G G A T C A A T G G A G C T A T C C T T C C T A T T G A C A A T G C C T G G	838
Revised sequence	C T T G T G T C G G A T A G A A G C A A A G G G A T C A A T G G A G C T A T C C T T C C T A T T G A C A A T G C C T G G	960
Database sequence	T C A A C C A T C T G A	850
Revised sequence	T C A A C C A T C T G A	972

Figure S2. Comparison of the revised DNA sequence of *trt9* with the original database sequence. Highlighted in magenta are the region predicted for an intron in our study.

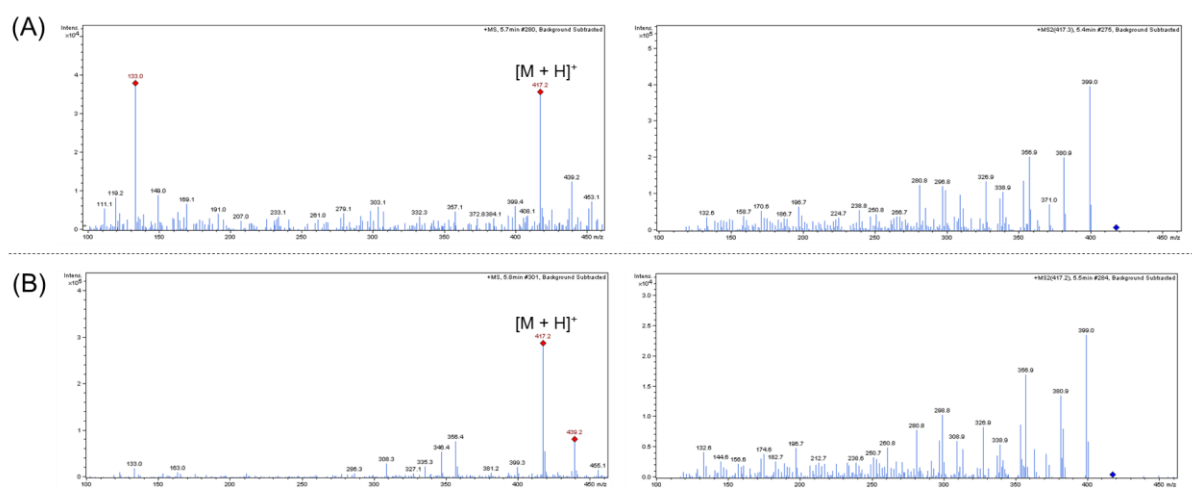


Figure S3. MS and MS² spectra of (A) terretonin H (12) and (B) enzymatic product from Trt6

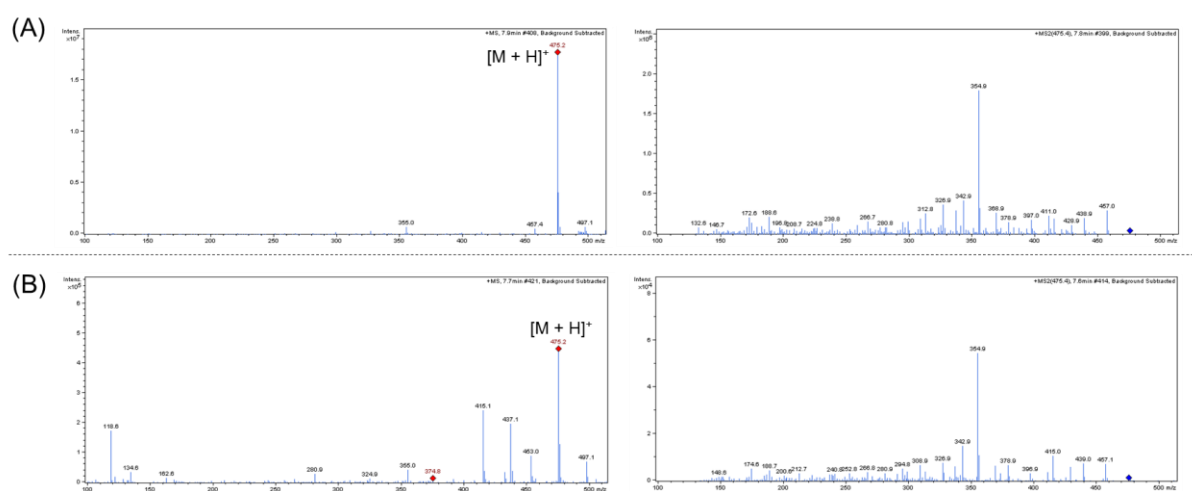


Figure S4. MS and MS² spectra of (A) terretonin D (3) and (B) enzymatic product from Trt6 and Trt14

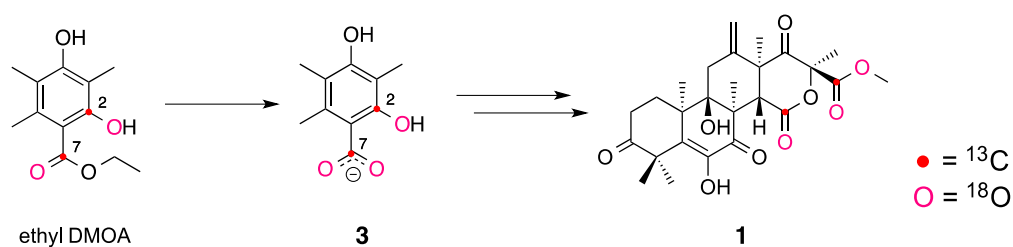


Figure S5. Labeling study of terretinin (1) with the doubly labeled DMOA (3)

	1	11	21	31	41	51
Trt14 (<i>Aspergillus terreus</i>)	MSPTR E DLVA	TAK L FI A KYN	EFT P ES I IS V	RTPNSV S H R L	FPT R NAT-RN	I- G ES M EACA
AusJ (<i>Aspergillus nidulans</i>)	MNT T RR H LLA	TAS R F V ETLE	SLD V D A M L AI	RSST C L H MC	CP S FRNY-S I	TNDQTREAFP
AusH (<i>Aspergillus nidulans</i>)	M P PT R DE L L C	TAL N F V GQFA	KLD V ES V LS F	MSP S CT L RS F	P S SLG K PALQ	TKE E SKAD F Q
AusF (<i>Aspergillus nidulans</i>)	M S T T R E K LL R	T T SR F V S T F G	S F DI E E I LS I	RTPT C LY H QC	CP S FN K N-V V	TNE E TRAN F P
	61	71	81	91	101	111
Trt14 (<i>Aspergillus terreus</i>)	NAKE V FKSLT	VS V IDNDNTI	VDE R TRK V VF	YLASRGDT I V	GEWKS E C I F I	FQMS E DGKLV
AusJ (<i>Aspergillus nidulans</i>)	QWKATITKYK	FGVLDD S QIL	VDE Q ARK V MI	HAETA A ETTV	GDYNN E Y V F I	LRMA E D C NTV
AusH (<i>Aspergillus nidulans</i>)	GLKD F FYNFQ	LR V KDGAEPV	TDE P ARK V VL	HIEGKGDS L V	GRFET E Y V Y I	LQIN E EGTMV
AusF (<i>Aspergillus nidulans</i>)	QFIAT F KRFD	FS I IEPDHTL	VDE A ARR V MI	RAKAS A ES I V	GAYEN E Y I F I	LKM T DD C RFI
	121	131	141	151	161	
Trt14 (<i>Aspergillus terreus</i>)	DRIWAG F DT A	YMDEFES R LD	G I T F - - - - -	- - - - -	- - -	
AusJ (<i>Aspergillus nidulans</i>)	DE I W E FYDT I	RLQDLR H R L E	ASH V PIGVDA	PAPFTTTASP	AAL	
AusH (<i>Aspergillus nidulans</i>)	ED F FQFAD S A	TRDAWG K K I E	AH F SARN- - -	- - - - -	- - -	
AusF (<i>Aspergillus nidulans</i>)	E E TI E YFYDT I	RLKDLQY R L E	AKHISYGDAA	PFKTRDTQL-	- - -	

Figure S6. Sequence alignment of Trt14 with its homologous proteins. Red asterisks indicate the conserved acidic amino acid residues. Sequences of *ausH* and *ausF* in the database were manually revised upon creating the alignment.

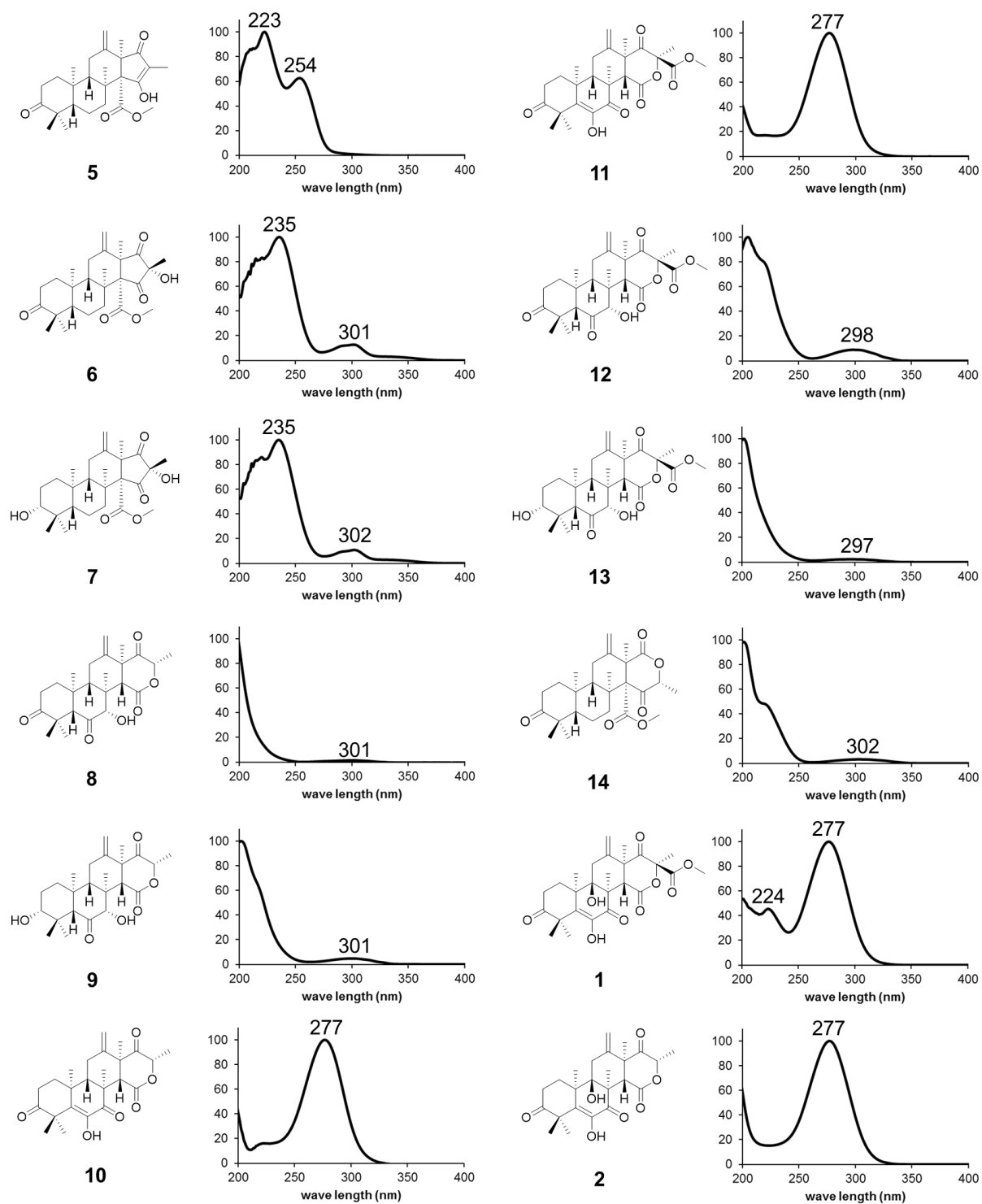


Figure S7. UV spectra of secondary metabolites isolated in this study

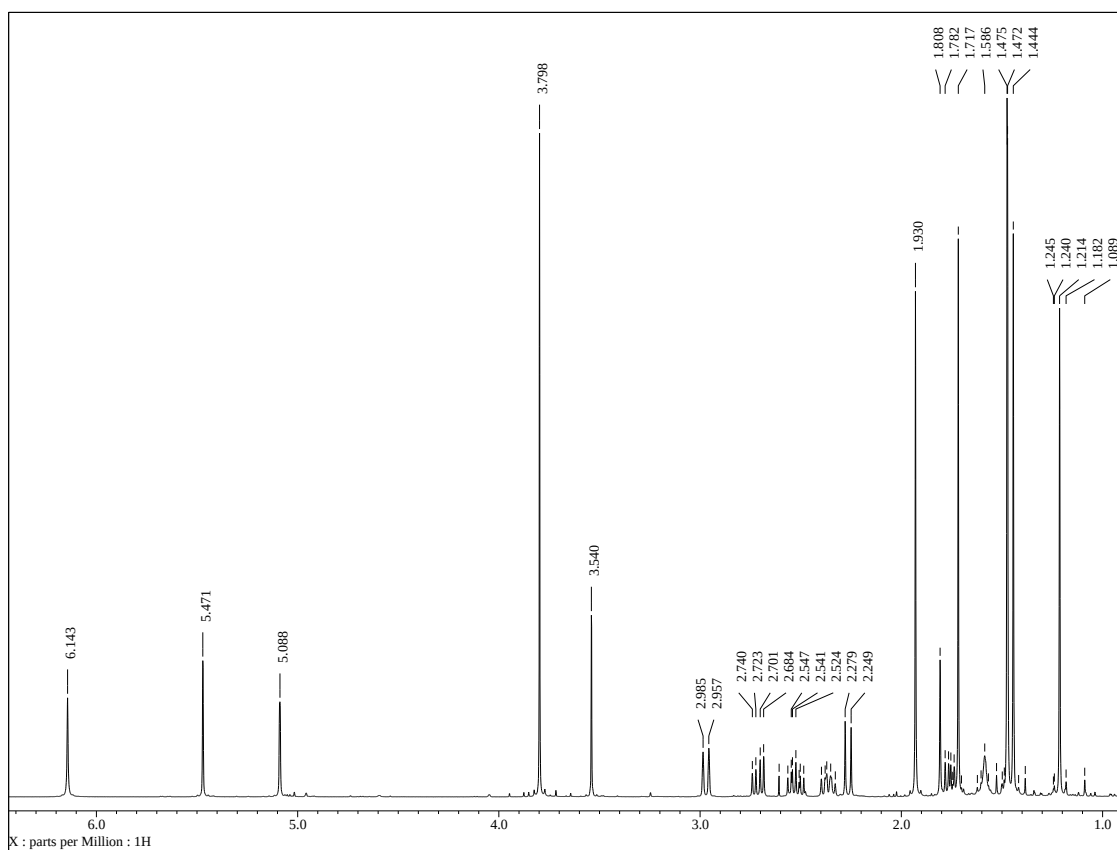


Figure S8. ¹H NMR spectrum of terretonin (1)

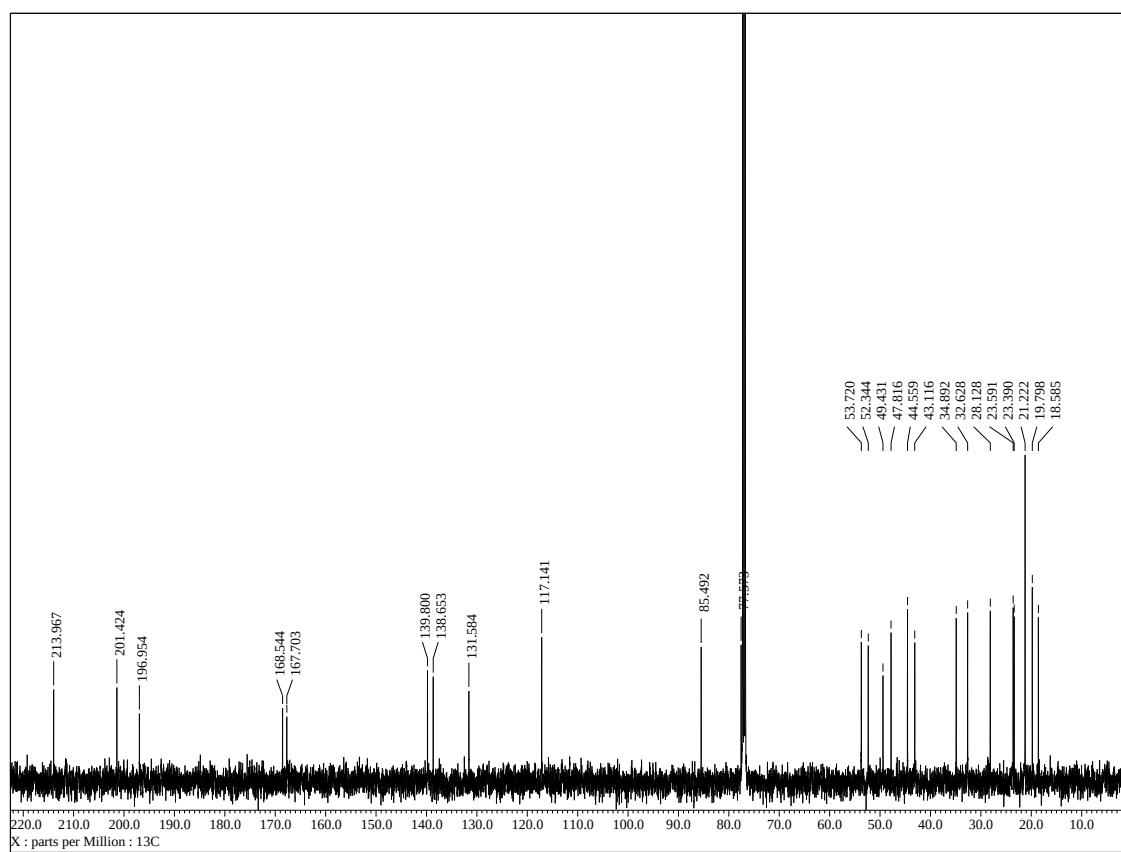


Figure S9. ¹³C NMR spectrum of terretonin (1)

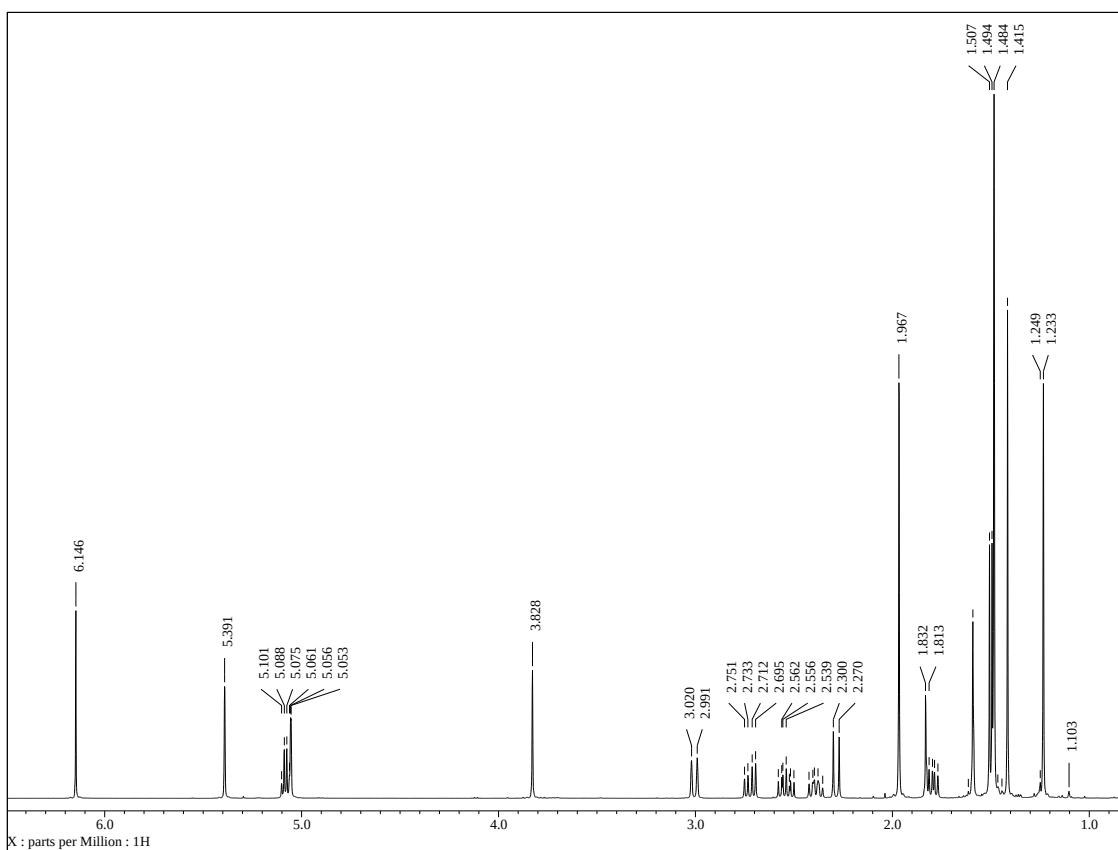


Figure S10. ¹H NMR spectrum of terretonin C (2)

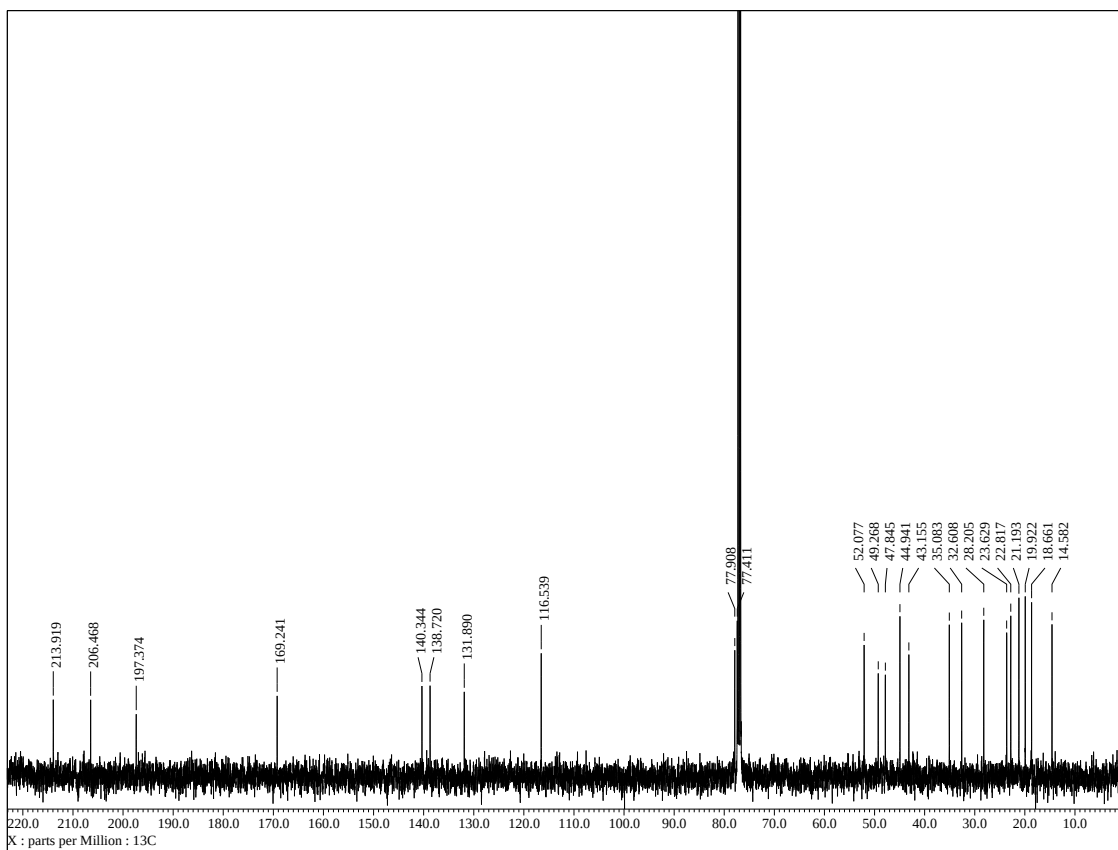


Figure S11. ¹³C NMR spectrum of terretonin C (2)

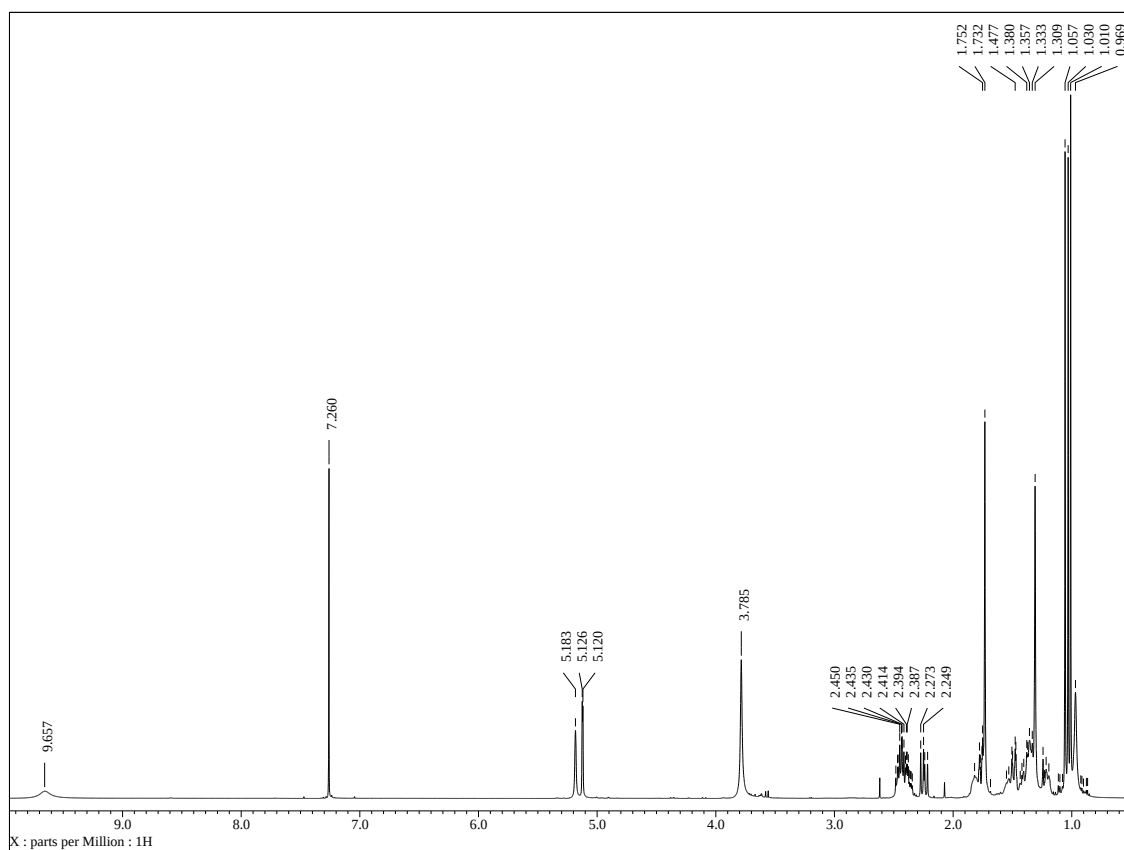


Figure S12. ¹H NMR spectrum of preterrenoid (5)

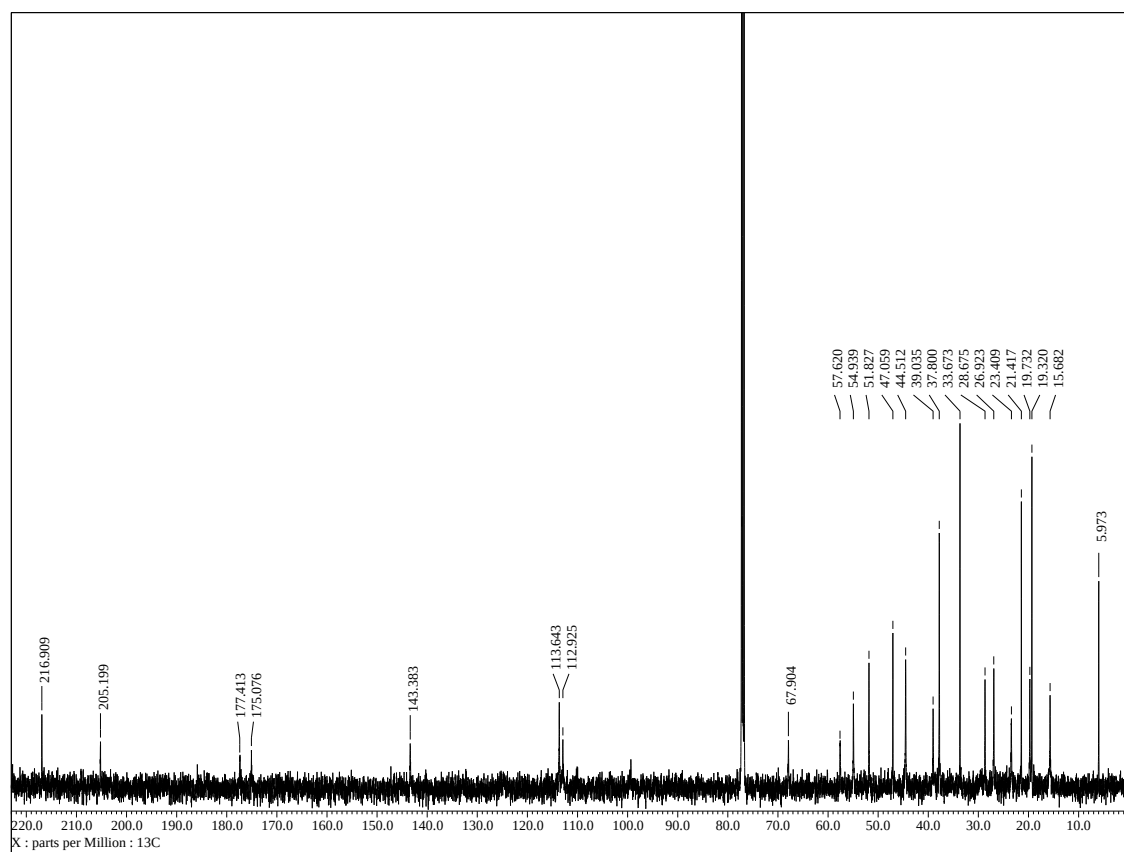


Figure S13. ¹³C NMR spectrum of preterrenoid (5)

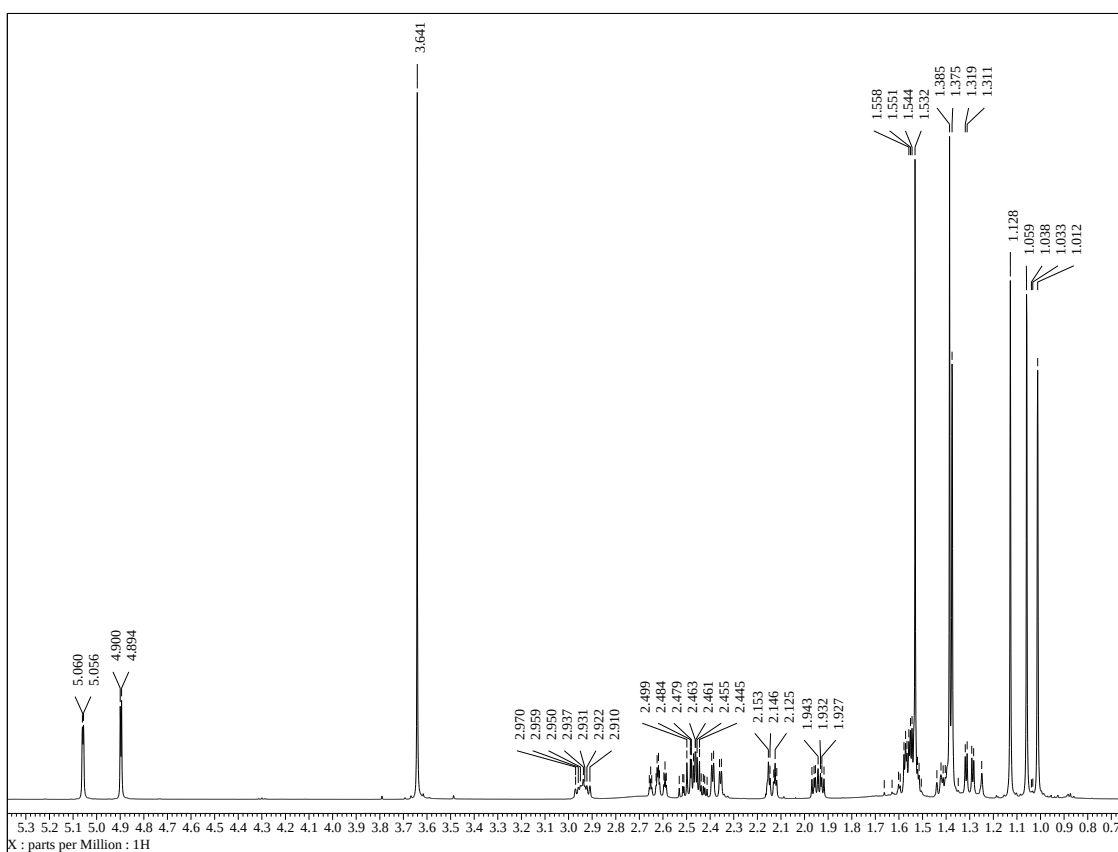


Figure S14. ¹H NMR spectrum of terrenoid (6)

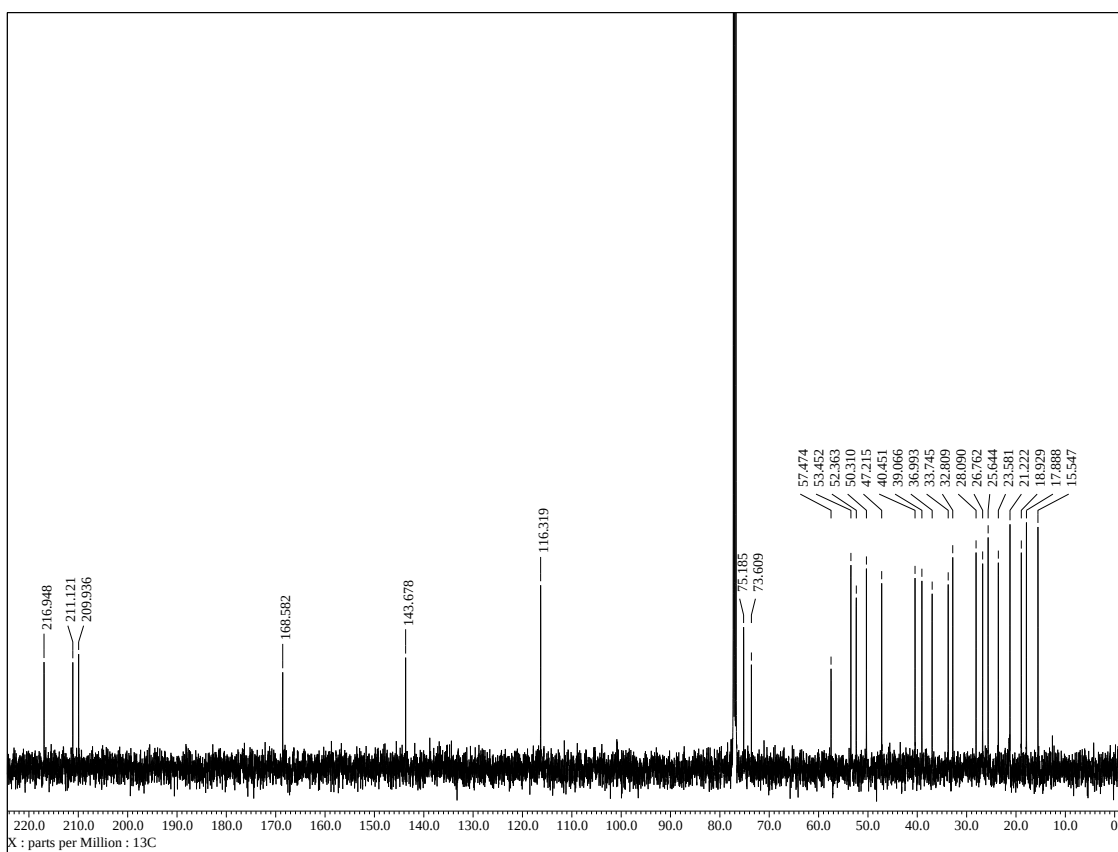


Figure S15. ¹³C NMR spectrum of terrenoid (6)

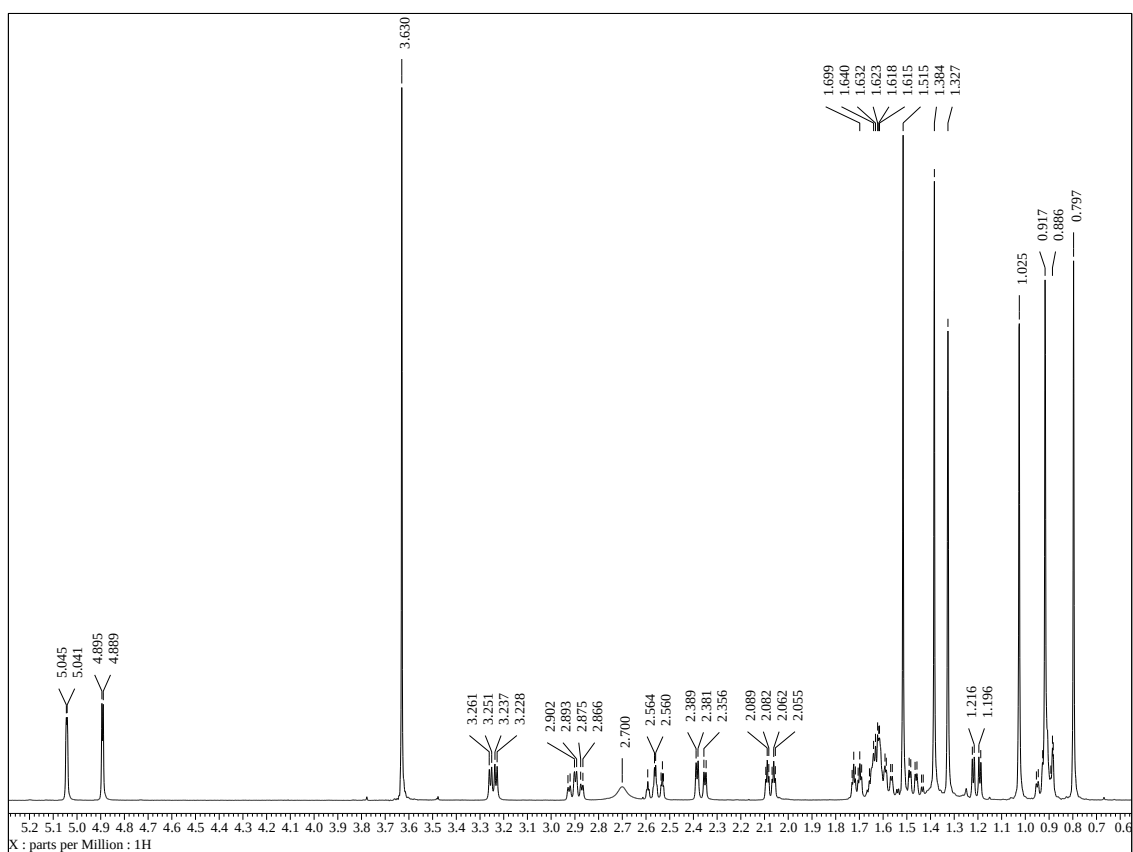


Figure S16. ¹H NMR spectrum of terrenoidol (7)

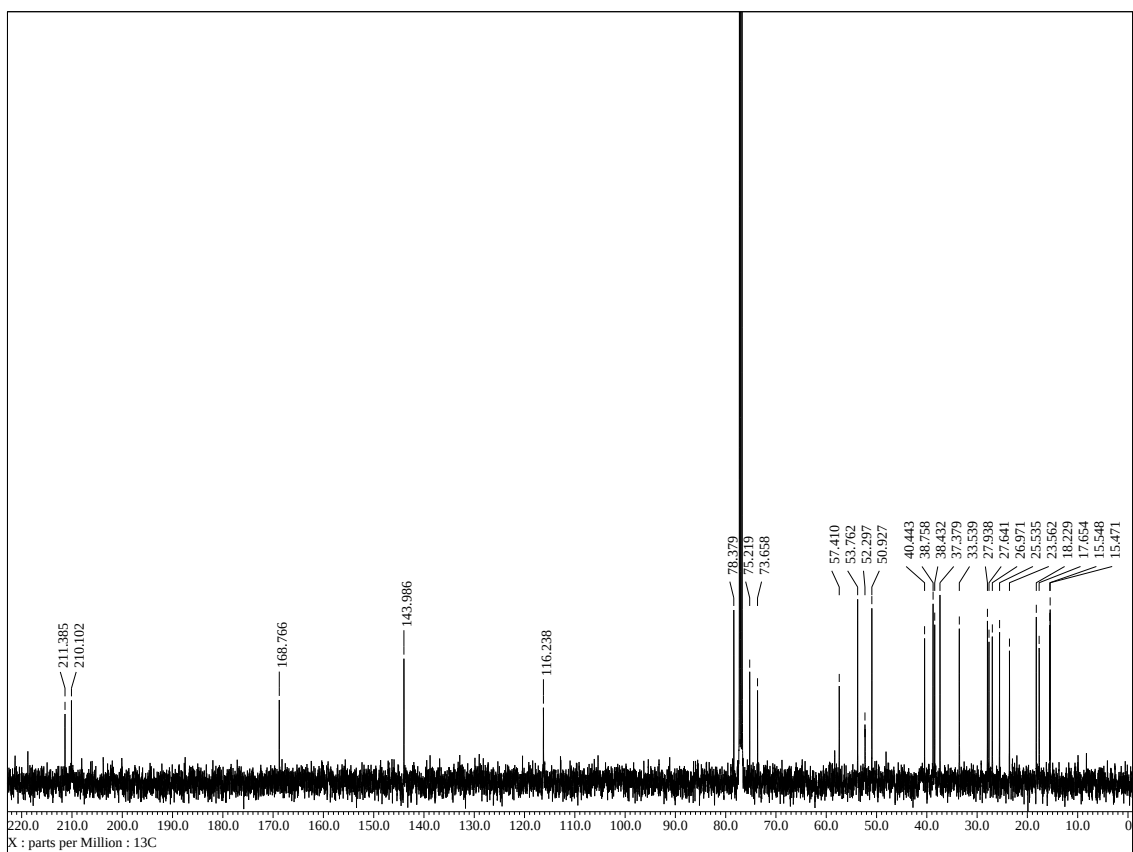


Figure S17. ¹³C NMR spectrum of terrenoidol (7)

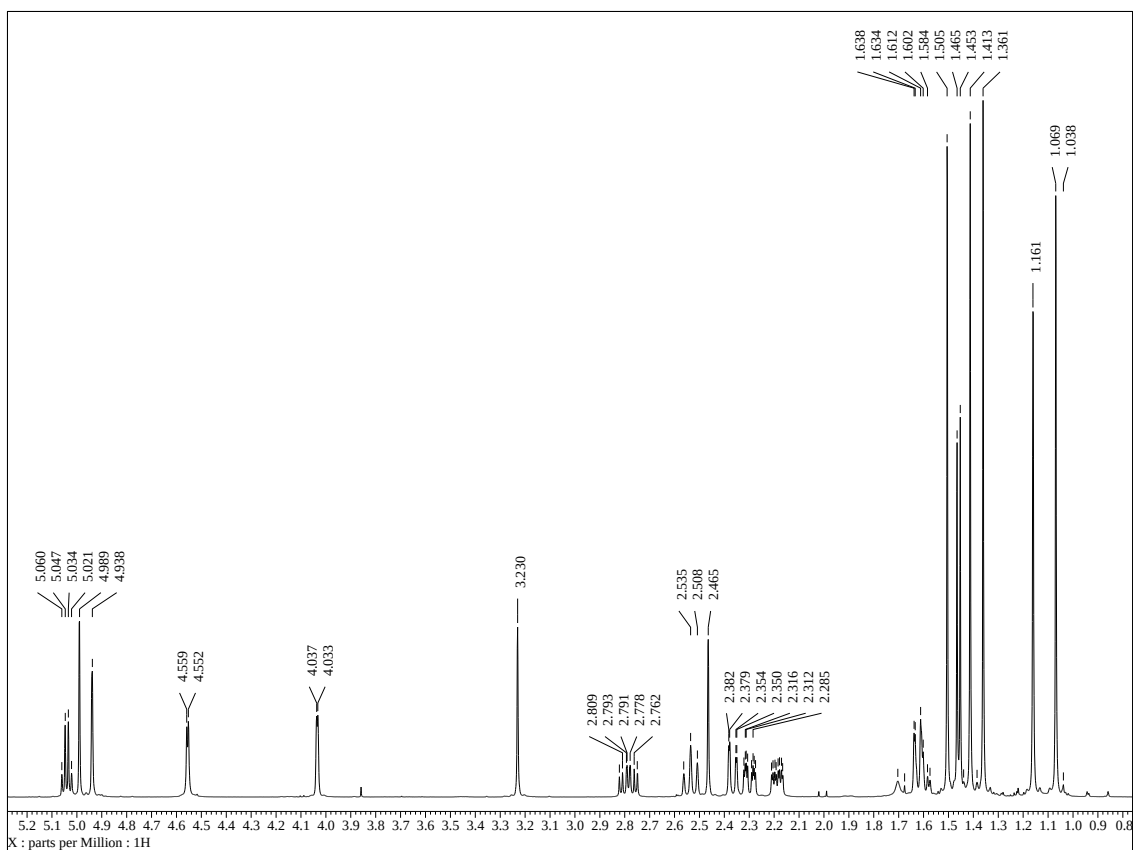


Figure S18. ¹H NMR spectrum of terretonin H (8)

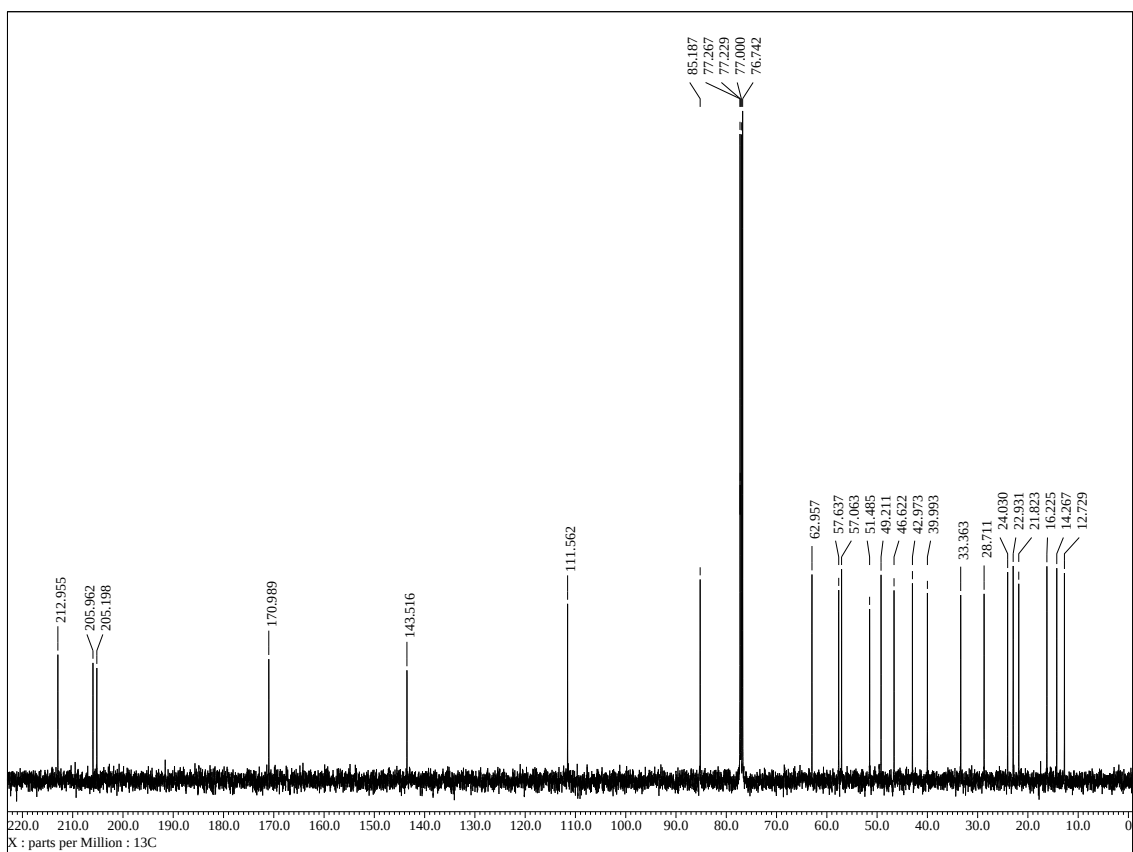


Figure S19. ¹³C NMR spectrum of terretonin H (8)

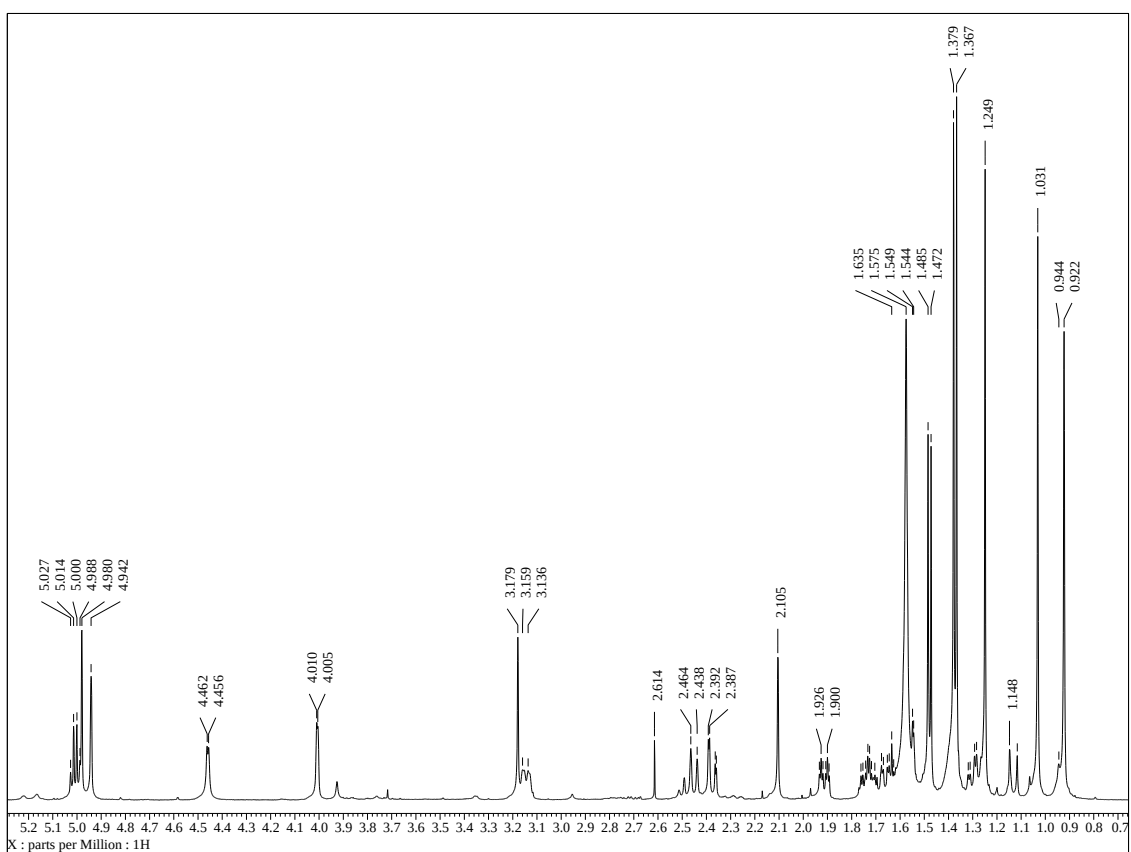


Figure S20. ¹H NMR spectrum of terretonin I (9)

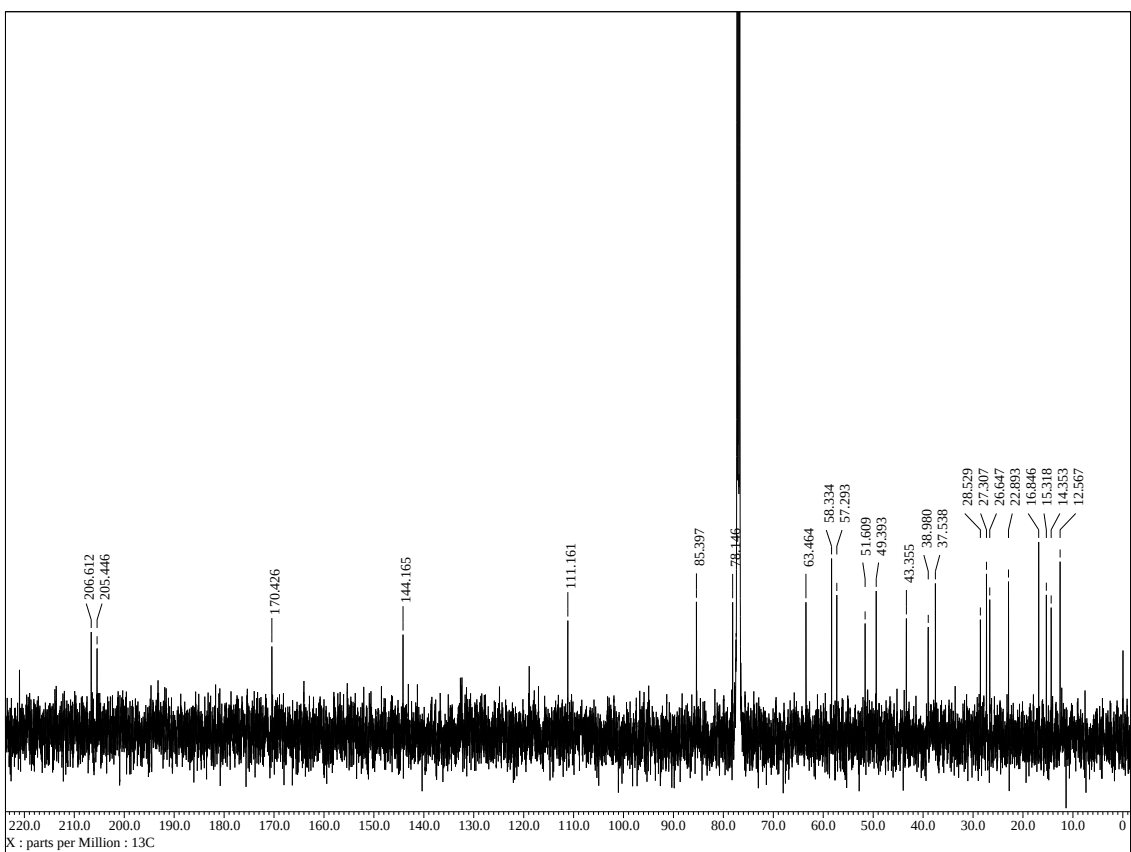
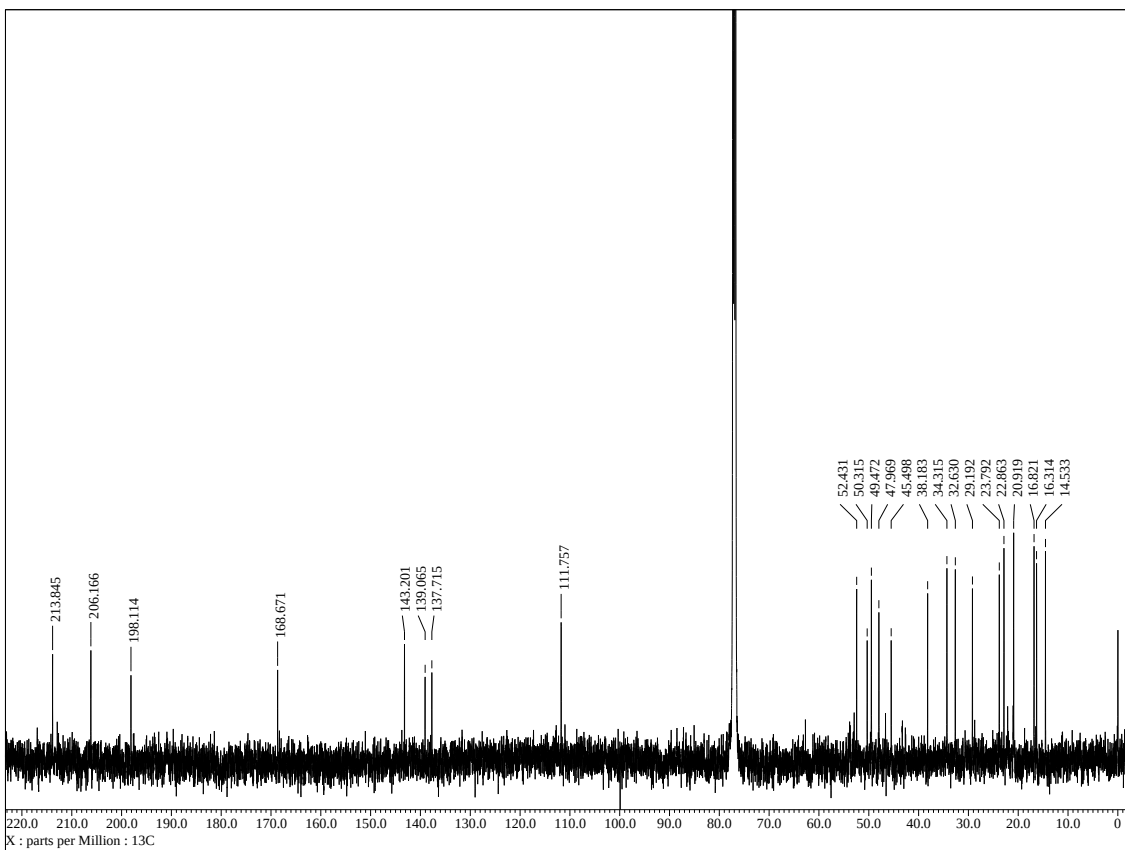
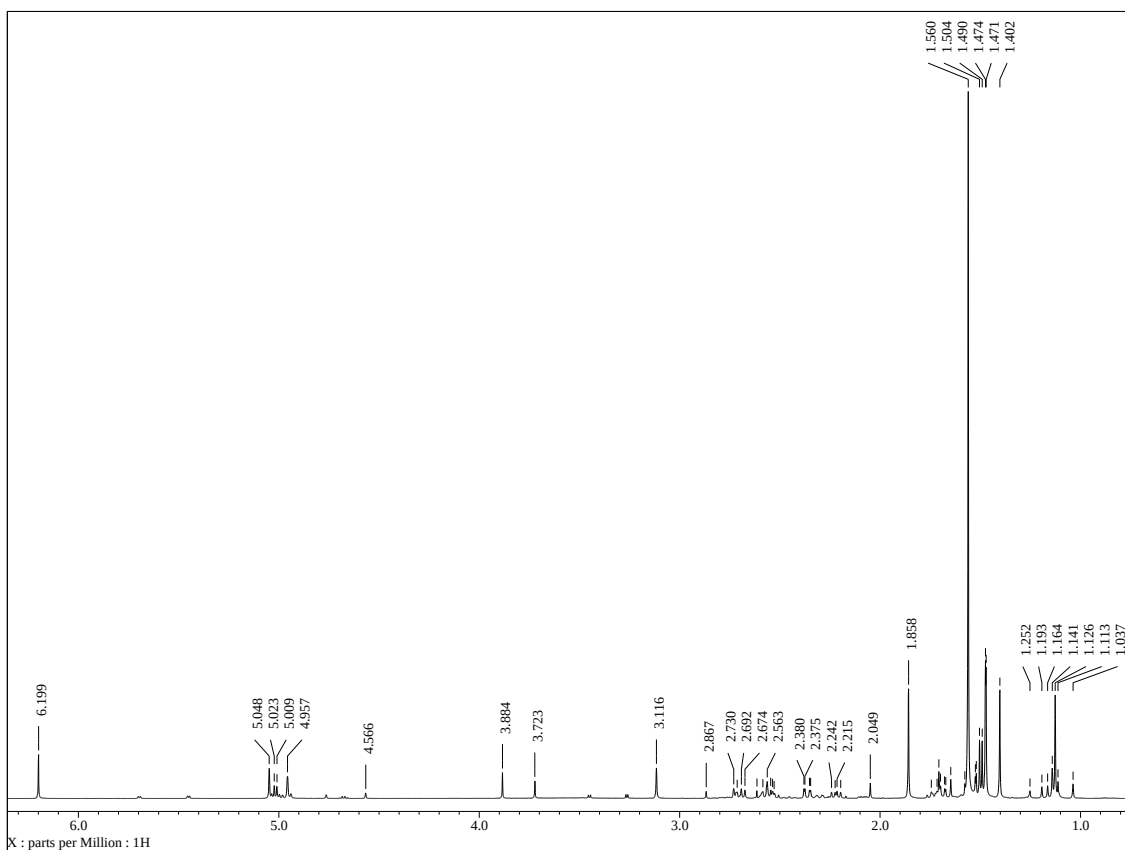


Figure S21. ¹³C NMR spectrum of terretonin I (9)



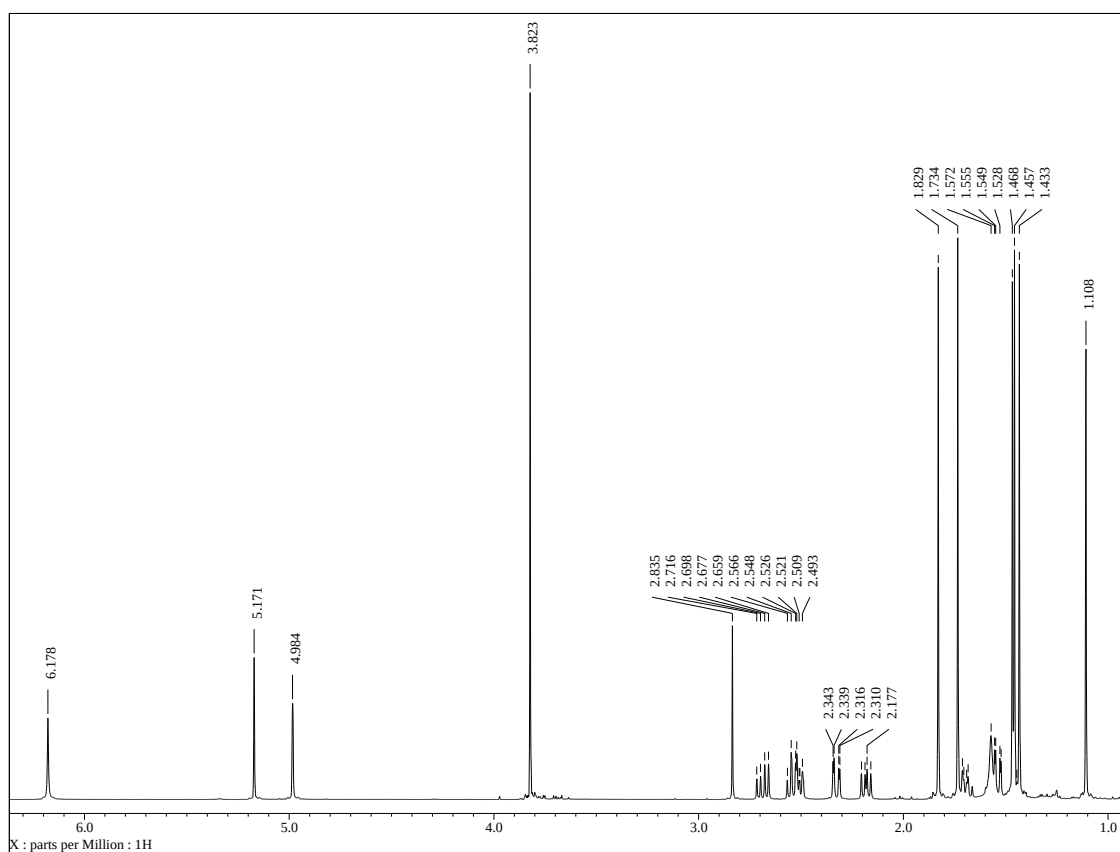


Figure S24. ¹H NMR spectrum of terretonin A (11)

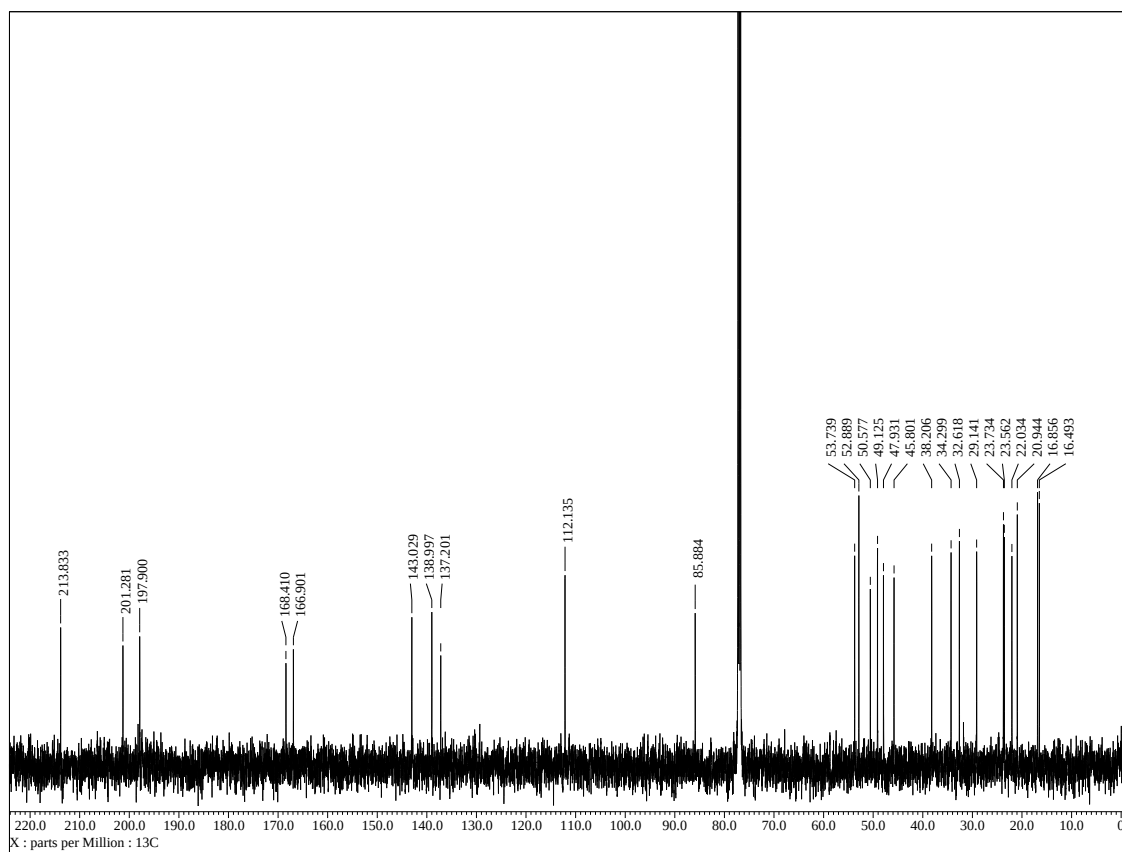


Figure S25. ¹³C NMR spectrum of terretonin A (11)

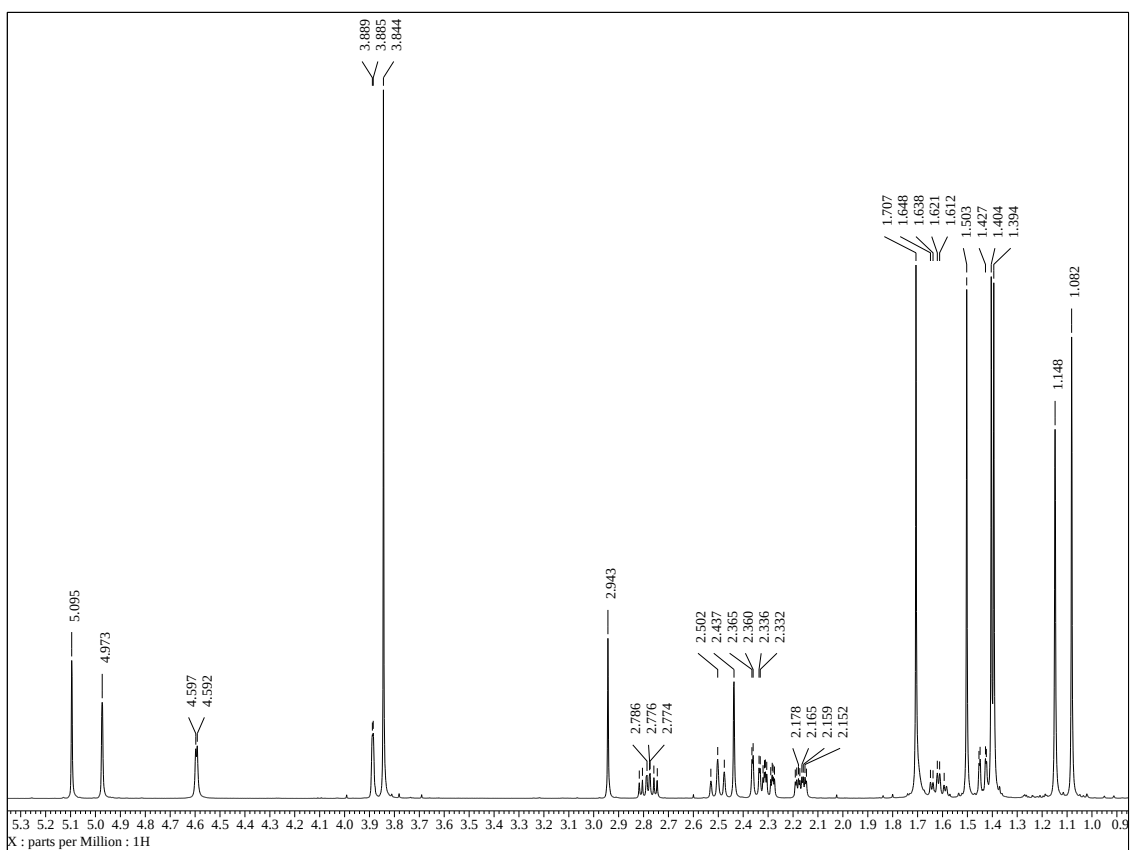


Figure S26. ¹H NMR spectrum of terretonin D (12)

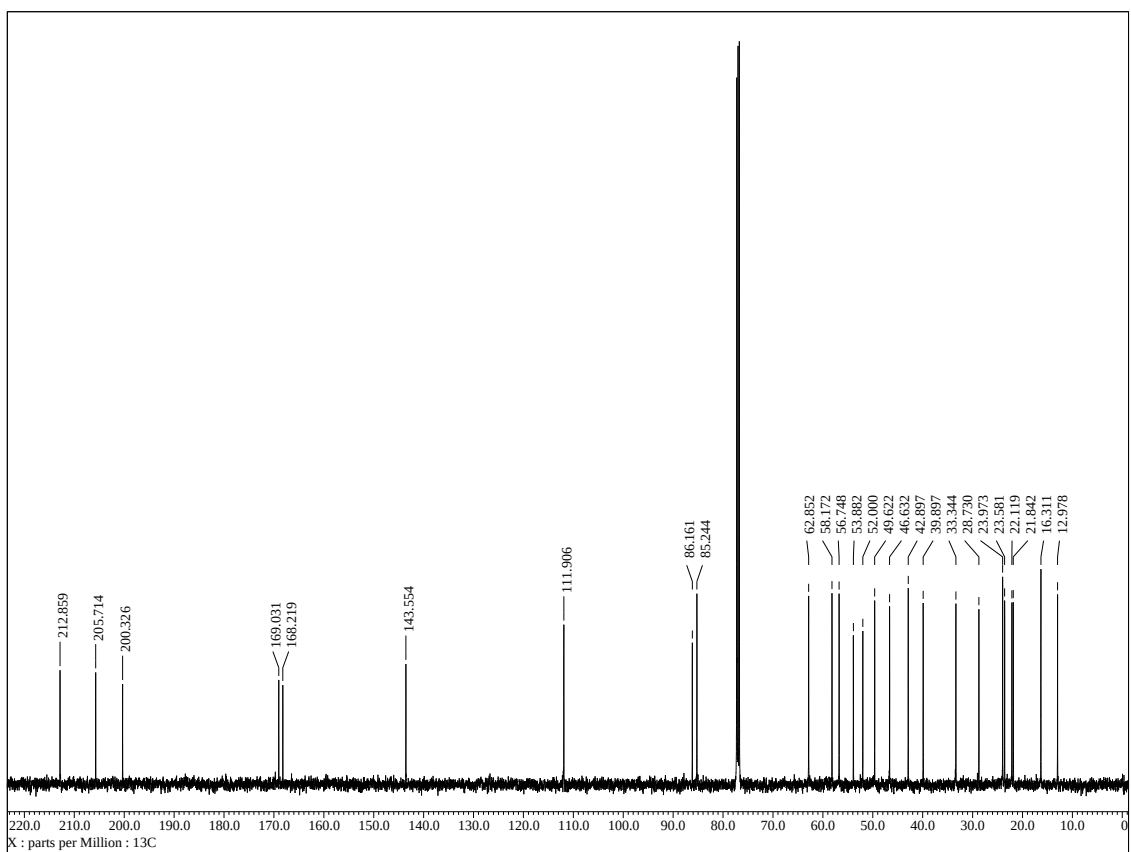


Figure S27. ¹³C NMR spectrum of terretonin D (12)

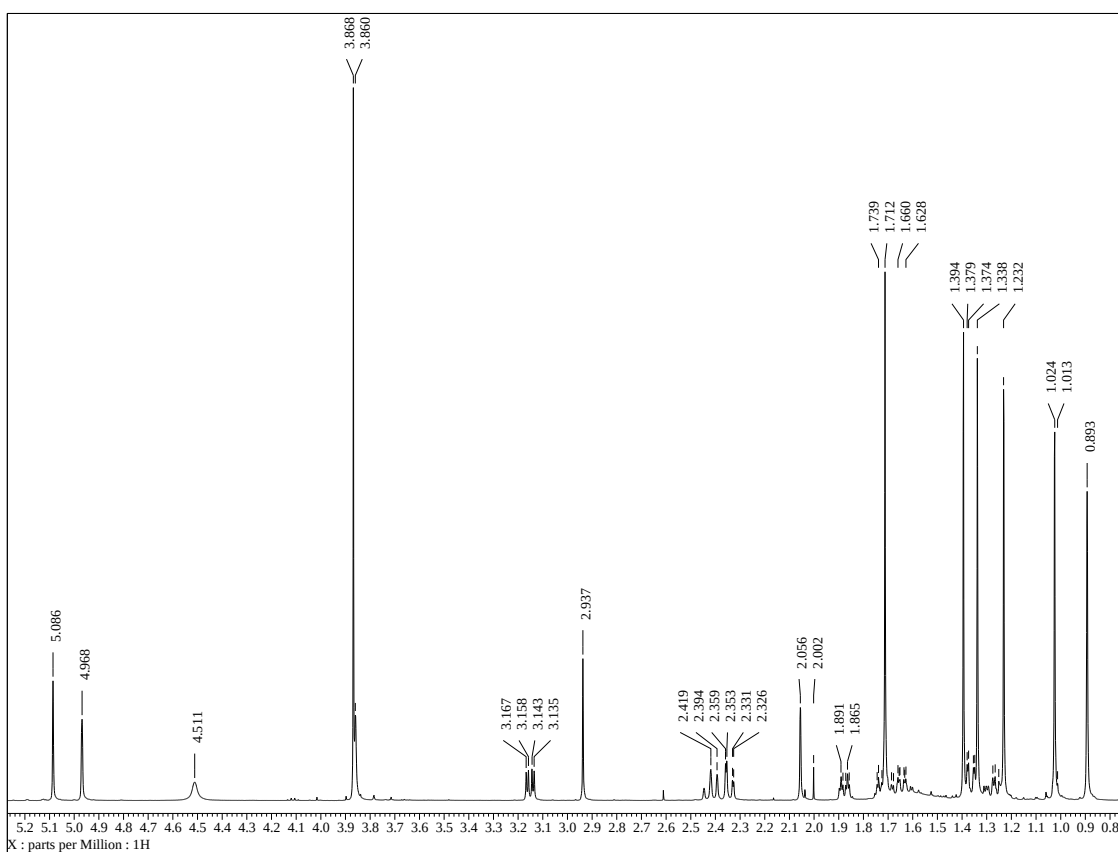


Figure S28. ¹H NMR spectrum of terretonin K (13)

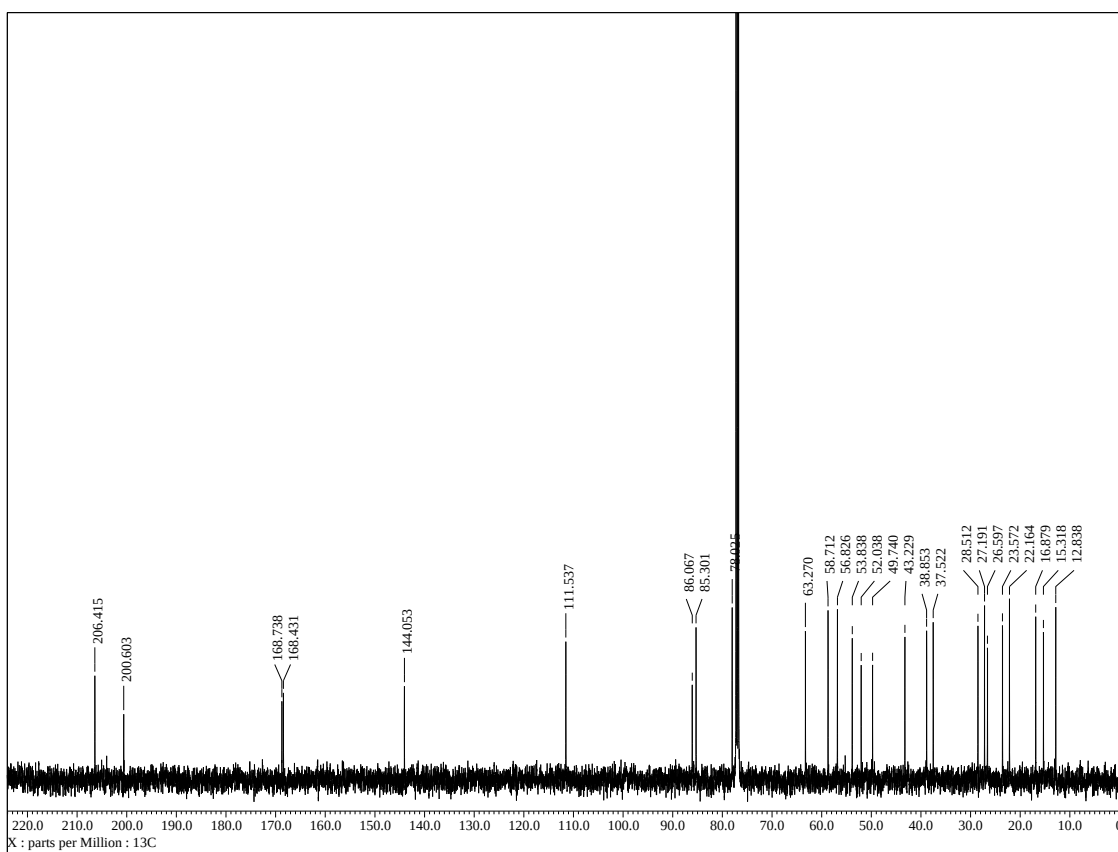


Figure S29. ¹³C NMR spectrum of terretonin K (13)

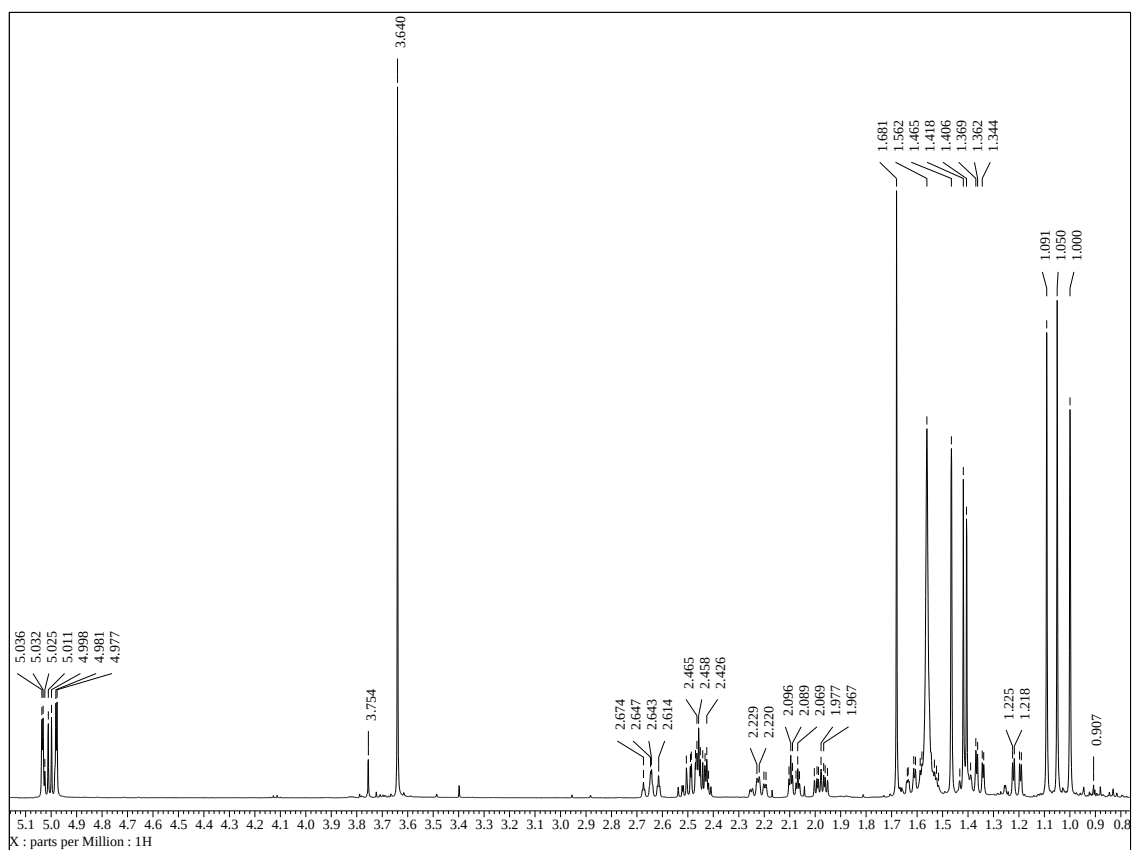


Figure S30. ¹H NMR spectrum of terretonin L (14)

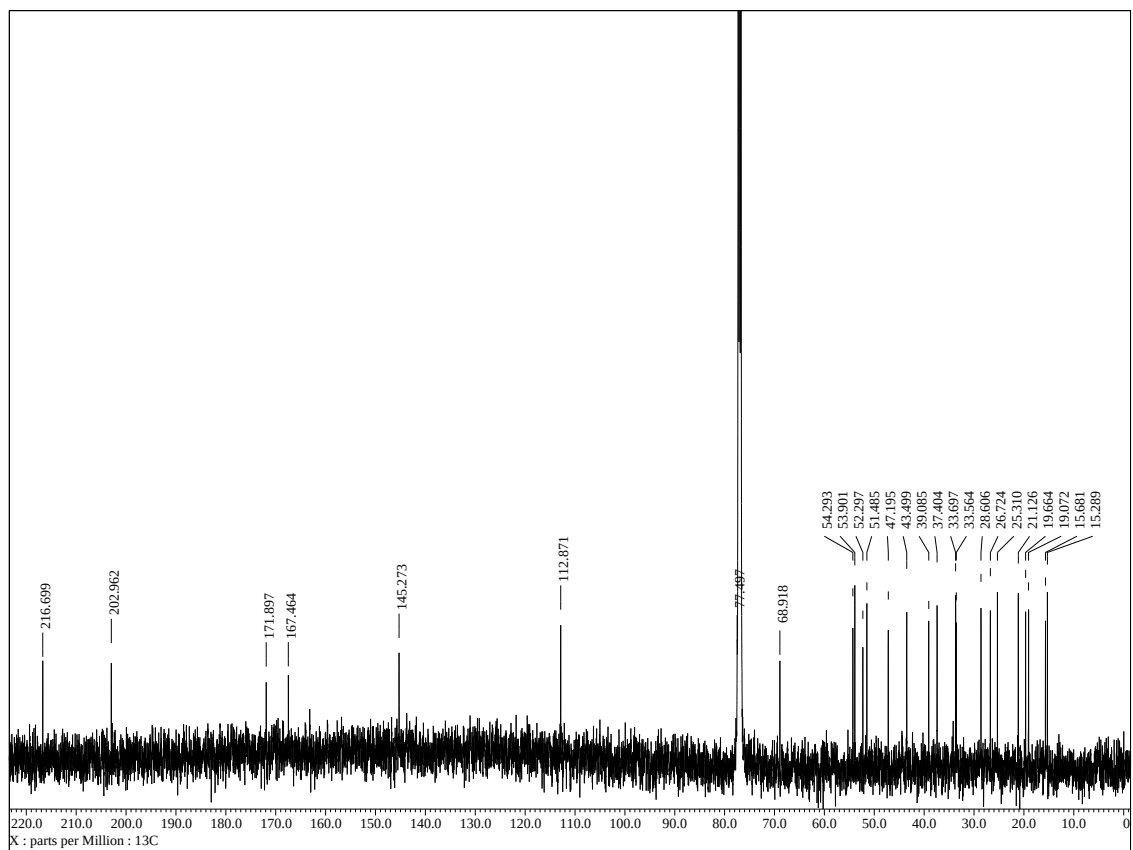


Figure S31. ¹³C NMR spectrum of terretonin L (14)

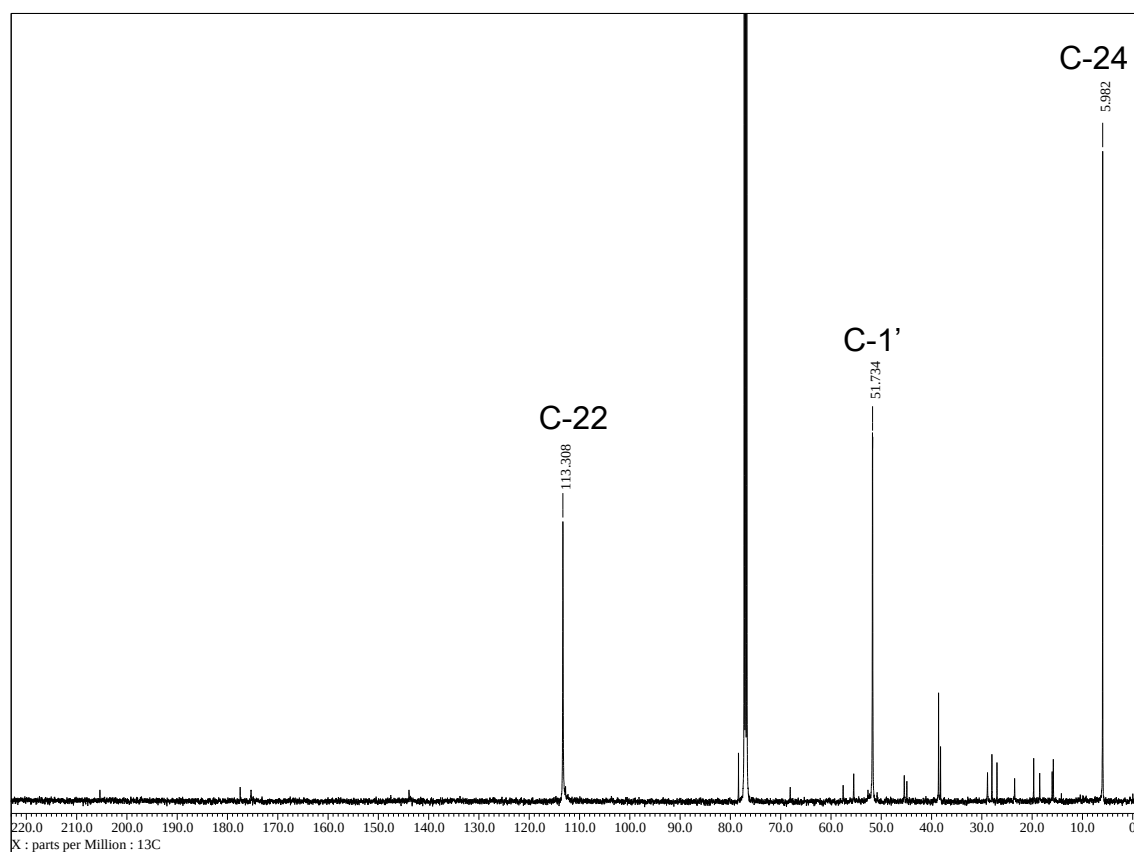


Figure S32. ¹³C NMR spectrum of the labeled preterretonin A

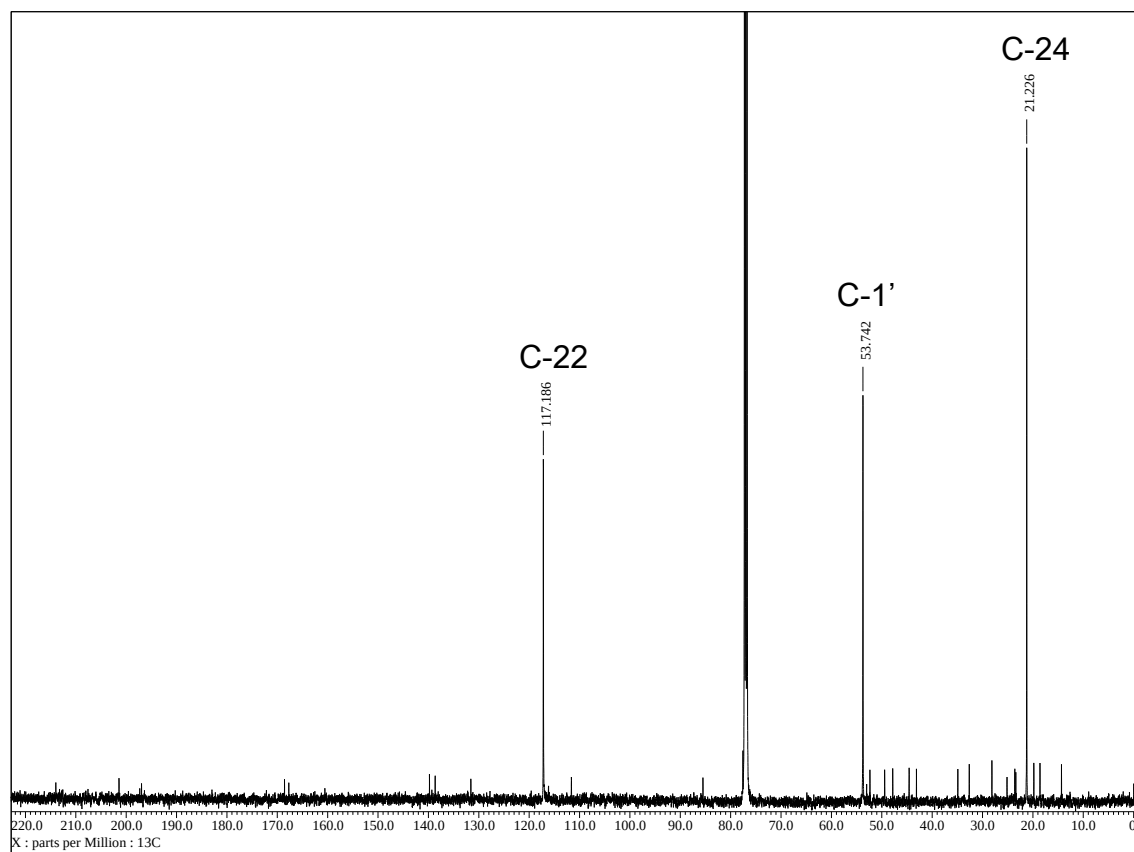


Figure S33. ¹³C NMR spectrum of the labeled terretonin