

Supplementary Information

Lithocarpins A-D, Four Tenellone–Macrolide Conjugated [4+2] Hetero-adducts from the Deep-sea Derived Fungus *Phomopsis lithocarpus* FS508

Jianlin Xu,^{a,b} Haibo Tan,^c Yuchan Chen,^a Saini Li,^a Zilei Huang,^a Heng Guo,^{a,b}
Haohua Li,^a Xiaoxia Gao,^b Hongxin Liu,^{*a} and Weimin Zhang^{*a}

^a State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Open Laboratory of Applied Microbiology, Guangdong Institute of Microbiology, Guangzhou 510070, China

^b College of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, China

^c Program for Natural Products Chemical Biology, Key Laboratory of Plant Resources Conservation and Sustainable Utilization, Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

Table of Contents

X-ray crystallographic data	4
¹H and ¹³C NMR data of compounds 1-4	4
Figure S1. HRESIMS spectrum of lithocarpin A (1).....	7
Figure S2. ¹ H NMR spectrum (500 MHz, CD ₃ OD) of lithocarpin A (1).....	7
Figure S3. ¹³ C NMR spectrum (125 MHz, CD ₃ OD) of lithocarpin A (1).....	8
Figure S4. HSQC spectrum of lithocarpin A (1).	8
Figure S5. ¹ H- ¹ H COSY spectrum (500 MHz, CD ₃ OD) of lithocarpin A (1).....	9
Figure S6. HMBC spectrum of lithocarpin A (1).	9
Figure S7. NOESY spectrum (500 MHz, CD ₃ OD) of lithocarpin A (1).....	10
Figure S8. CD spectrum of lithocarpin A (1).....	10
Figure S9. UV spectrum of lithocarpin A (1).....	11
Figure S10. IR spectrum of lithocarpin A (1)	11
Figure S11. HRESIMS spectrum of lithocarpin B (2).....	12
Figure S12. ¹ H NMR spectrum (500 MHz, CD ₃ OD) of lithocarpin B (2).....	12
Figure S13. ¹³ C NMR spectrum (125 MHz, CD ₃ OD) of Lithocarpin B (2).....	13
Figure S14. HSQC spectrum of lithocarpin B (2).	13
Figure S15. ¹ H- ¹ H COSY spectrum (500 MHz, CD ₃ OD) of lithocarpin B (2).....	14
Figure S16. HMBC spectrum of lithocarpin B (2).	14
Figure S17. NOESY spectrum (500 MHz, CD ₃ OD) of lithocarpin B (2).....	15
Figure S18. CD spectrum of lithocarpin B (2).	15
Figure S19. UV spectrum of lithocarpin B (2).	16
Figure S20. IR spectrum of lithocarpin B (2).....	16
Figure S21. HRESIMS spectrum of lithocarpin C (3).....	17
Figure S22. ¹ H NMR spectrum (500 MHz, CD ₃ OD) of lithocarpin C (3).....	17
Figure S23. ¹³ C NMR spectrum (125 MHz, CD ₃ OD) of lithocarpin C (3).....	18
Figure S24. HSQC spectrum of lithocarpin C (3).	18
Figure S25. ¹ H- ¹ H COSY spectrum (500 MHz, CD ₃ OD) of lithocarpin C (3).....	19
Figure S26. HMBC spectrum of lithocarpin C (3).	19

Figure S27. NOESY spectrum (500 MHz, CD ₃ OD) of lithocarpin C (3).....	20
Figure S28. CD spectrum of lithocarpin C (3).	20
Figure S29. UV spectrum of lithocarpin C (3).	21
Figure S30. IR spectrum of lithocarpin C (3).....	21
Figure S31. HRESIMS spectrum of lithocarpin D (4)	22
Figure S32. ¹ H NMR spectrum (500 MHz, CD ₃ OD) of lithocarpin D (4).	22
Figure S33. ¹³ C NMR spectrum (125 MHz, CD ₃ OD) of lithocarpin D (4).	23
Figure S34. HSQC spectrum of Lithocarpin D (4).....	23
Figure S35. ¹ H- ¹ H COSY spectrum (500 MHz, CD ₃ OD) of lithocarpin D (4).	24
Figure S36. HMBC spectrum of lithocarpin D (4).....	24
Figure S37. NOESY spectrum (500 MHz, CD ₃ OD) of lithocarpin D (4).....	25
Figure S38. CD spectrum of lithocarpin D (4).	25
Figure S39. UV spectrum of lithocarpin D (4).....	26
Figure S40. IR spectrum of lithocarpin D (4).....	26
Figure S41. ¹ H NMR spectrum (500 MHz, CDCl ₃) of tenellone B (5).	27
Figure S42. ¹³ C NMR spectrum (125 MHz, CDCl ₃) of tenellone B (5).	27
Figure S43. Cytotoxic activity of lithocarpins C and D against SF-268.....	28
Figure S44. Cytotoxic activity of lithocarpins C and D against MCF-7.....	28
Figure S45. Cytotoxic activity of lithocarpins C and D against HepG-2.....	29

X-ray crystallographic data

X-ray crystallographic data for lithocarpin A (1): $C_{38}H_{43}Cl_3O_{11}$, $M = 782.07$, monoclinic, size $0.15 \times 0.13 \times 0.11 \text{ mm}^3$, space group $P2_1$; $a = 12.0999 (3) \text{ \AA}$, $b = 8.9388 (2) \text{ \AA}$, $c = 17.0397 (4) \text{ \AA}$, $\alpha = \beta = 90.00^\circ$, $\gamma = 90.378^\circ$, $V = 1842.95 (8) \text{ \AA}^3$, $T = 100.00 \text{ K}$, $Z = 2$, $\rho_{\text{calcd}} = 1.409 \text{ g/cm}^3$, $F(000) = 820.0$, 17295 reflections in $-14 \leq h \leq 15$, $-10 \leq k \leq 11$, $-20 \leq l \leq 20$, measured in the range $7.31^\circ \leq \theta \leq 147.52^\circ$, GOOF = 1.036, Final R indices [$I > 2\sigma(I)$]: $R_1 = 0.0906$, $wR_2 = 0.2611$, Final R indices (all data): $R_1 = 0.0940$, $wR_2 = 0.2650$, Flack parameter $-0.087 (13)$, largest difference peak and hole = 1.20 and $-1.44 \text{ e. \AA}^{-3}$. Data were collected on Agilent Xcalibur Nova single-crystal diffractometer using $\text{CuK}\alpha$ radiation. The crystal structure was refined by full-matrix least-squares calculation. Crystallographic data for the structure of lithocarpin A (1) reported in this paper have been deposited in the Cambridge Crystallographic Data Centre. (Deposition number: CCDC 1586451). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html.)

X-ray crystallographic data for lithocarpin D (4): $C_{37}H_{42}O_{11}$, $M = 662.70$, orthorhombic, size $0.14 \times 0.13 \times 0.11 \text{ mm}^3$, space group $P2_12_12_1$; $a = 9.34542 (7) \text{ \AA}$, $b = 14.58845 (13) \text{ \AA}$, $c = 24.65138 (16) \text{ \AA}$, $\alpha = \beta = \gamma = 90.00^\circ$, $V = 3360.85 (4) \text{ \AA}^3$, $T = 100.01 \text{ K}$, $Z = 4$, $\rho_{\text{calcd}} = 1.310 \text{ g/cm}^3$, $F(000) = 1408.0$, 38401 reflections in $-11 \leq h \leq 11$, $-17 \leq k \leq 15$, $-30 \leq l \leq 30$, measured in the range $7.04^\circ \leq \theta \leq 147.20^\circ$, GOOF = 1.047, Final R indices [$I > 2\sigma(I)$]: $R_1 = 0.0340$, $wR_2 = 0.0889$, Final R indices (all data): $R_1 = 0.0349$, $wR_2 = 0.0897$, Flack parameter $-0.08 (5)$, largest difference peak and hole = 0.21 and $-0.23 \text{ e. \AA}^{-3}$. Data were collected on Agilent Xcalibur Nova single-crystal diffractometer using $\text{CuK}\alpha$ radiation. The crystal structure was refined by full-matrix least-squares calculation. Crystallographic data for the structure of lithocarpin D (4) reported in this paper have been deposited in the Cambridge Crystallographic Data Centre. (Deposition number: CCDC 1586450). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html.)

^1H and ^{13}C NMR data of compounds 1-4

Table S1 ¹H (500 MHz) and ¹³C (125 MHz) NMR data of **1** and **2** (δppm) in CD₃OD.

No.	1		2	
	δ _H (J in Hz)	δ _C	δ _H (J in Hz)	δ _C
1		144.5, C		144.5, C
2		131.7, C		131.7, C
3		148.3, C		148.3, C
4	6.70, d, (8.3)	116.4, CH	6.69, d, (8.3)	116.4, CH
5	6.86, d, (8.3)	130.8, CH	6.85, m	130.9, CH
6		127.7, C		127.6, C
7	2.84, s	29.8, CH ₂	2.85, t, (7.2)	29.8, CH ₂
8	5.02, t, (6.5)	125.3, CH	5.03, d, (7.2)	125.2, CH
9		132.6, C		132.4, C
10	1.29, s	17.7, CH ₃	1.30, s	17.7, CH ₃
11	1.59, s	25.9, CH ₃	1.59, s	25.7, CH ₃
1'		125.1, C		125.1, C
2'		143.0, C		143.0, C
3'		145.0, C		145.0, C
4'	6.84, d, (2.0)	120.0, CH	6.85, d, (1.9)	120.0, CH
5'		131.4, C		131.2, C
6'	7.01, d, (2.0)	121.4, CH	7.06, d, (1.9)	121.5, CH
7'	2.36, s	21.0, CH ₃	2.37, s	21.0, CH ₃
8a'	4.39, dd, (10.2, 2.1)	65.6, CH ₂	4.39, dd, (10.2, 2.1)	65.6, CH ₂
8b'	3.79, t (10.2)		3.79, t (10.2)	
9'	3.67, dd, (10.2, 2.1)	79.6, CH	3.66, dd, (10.2, 2.1)	79.7, CH
10'		71.3, C		71.2, C
11'	0.67, s	23.6, CH ₃	0.67, s	23.6, CH ₃
12'	0.88, s	27.2, CH ₃	0.86, s	27.2, CH ₃
2''		168.5, C		168.1, C
3''	3.39, overlapped	56.0, CH	3.41, d, (5.0)	55.1, CH
4''	3.39, overlapped	52.4, CH	3.37, d, (5.0)	52.3, CH
5''	4.01, d, (4.5)	42.6, CH	4.11, d, (4.5)	43.0, CH
6''	3.31, d, (4.5)	57.1, CH	3.34, d, (4.5)	55.5, CH
7''		204.6, C		209.5, C
8''	5.63, dd, (4.0, 2.1)	76.1, CH	4.82, m	74.1, CH
9a''	2.08, m	38.8, CH ₂	1.99, dd, (13.2, 2.4)	42.7, CH ₂
9b''	2.67, ddd, (16.2, 10.0, 4.0)		2.59, ddd, (13.2, 10.0, 2.4)	
10''	4.78, m	70.6, CH	4.82, m	70.0, CH
11''	1.29, s	20.2, CH ₃	1.23, d, (6.4)	20.2, CH ₃
12''		91.8, C		91.8, C
13''	5.75, s	79.2, CH	5.47, s	79.3, CH
14''		171.7, C		
15''	2.08, s	20.4, CH ₃		

Table S2 ¹H (500 MHz) and ¹³C (125 MHz) NMR data of **3** and **4** (δppm) in CD₃OD.

NO.	3		4	
	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}
1		144.9, C		144.4, C
2		131.6, C		131.3, C
3		148.8, C		148.8, C
4	6.60, d, (8.3)	115.9, CH	6.60, d, (8.3)	116.2, CH
5	6.82, d, (8.3)	130.4, CH	6.70, d, (8.3)	129.9, CH
6		127.7, C		126.9, C
7a	2.70, m	29.5, CH ₂	2.43, m	29.7, CH ₂
7b			2.72, dd, (16.3, 6.4)	
8	5.02, t, (6.3)	125.6, CH	4.99, tdd, (6.4, 2.9, 1.4)	124.4, CH
9		132.5, C		132.8, C
10	1.29, s	17.8, CH ₃	1.31, s	20.3, CH ₃
11	1.64, s	25.8, CH ₃	1.63, s	25.8, CH ₃
1'		125.1, C		125.5, C
2'		142.9, C		142.7, C
3'		145.3, C		144.8, C
4'	6.82, d, (2.0)	119.7, CH	6.84, d, (2.0)	120.0, CH
5'		130.5, C		130.1, C
6'	6.99, d, (2.0)	121.8, CH	7.49, d, (2.0)	121.9, CH
7'	2.35, s	21.0, CH ₃	2.43, s	21.0, CH ₃
8a'	4.35, m	65.7, CH ₂	4.37, dd, (9.0, 1.8)	65.6, CH ₂
8b'	3.85, m		3.78, t (9.0)	
9'	3.58, dd, (9.5, 2.1)	80.2, CH	3.61, m	79.9, CH
10'		71.3, C		71.2, C
11'	0.64, s	24.5, CH ₃	0.66, s	23.8, CH ₃
12'	0.77, s	26.2, CH ₃	0.85, s	27.0, CH ₃
2''		175.1, C		168.8, C
3a''	2.23, m	35.9, CH ₂	3.61, overlapped	60.2, CH
3b''	2.35, m			
4a''	2.13, m	30.8, CH ₂	3.61, overlapped	53.9, CH
4b''	2.23, m			
5''	3.38, m	46.8, CH	3.01, brs	45.5, CH
6''	3.07, d, (4.0)	56.8, CH	4.50, brs	56.5, CH
7''		215.9, C		204.6, C
8''	4.35, m	74.8, CH	5.54, brs	76.6, CH
9a''	2.03, m	44.2, CH ₂	2.15, ddd, (12.4, 6.2, 3.0)	40.3, CH ₂
9b''	2.23, m		2.56, t, (12.4)	
10''	5.30, m	67.7, CH	4.80, dqd, (9.4, 6.2, 3.0)	69.9, CH
11''	1.19, d, (5.3)	20.3, CH ₃	1.33, s	17.7, CH ₃
12''		92.4, C		93.1, C
13''	5.46, s	79.1, C	5.59, s	82.6, CH
14''				170.4, C
15''			1.88, s	20.8, CH ₃

Mass Spectrum SmartFormula Report

Analysis Info		Acquisition Date	10/26/2017 9:18:18 AM	
Analysis Name	D:\Data\MS\data\201710\xujianlin_w-36_neg_2_01_3761.d	Operator	SCSIO	
Method	LC_Direct Infusion_neg_100-1000mz.m	Instrument	maXis	255552.00029
Sample Name	xujianlin_w-36_neg			
Comment				

Acquisition Parameter					
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4000 V	Set Dry Heater	180 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Charging Voltage	0 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

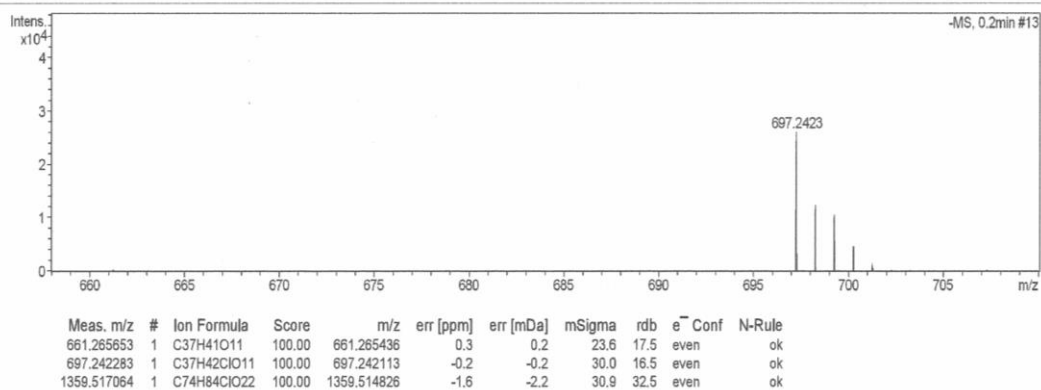


Figure S1. HRESIMS spectrum of lithocarpin A (**1**).

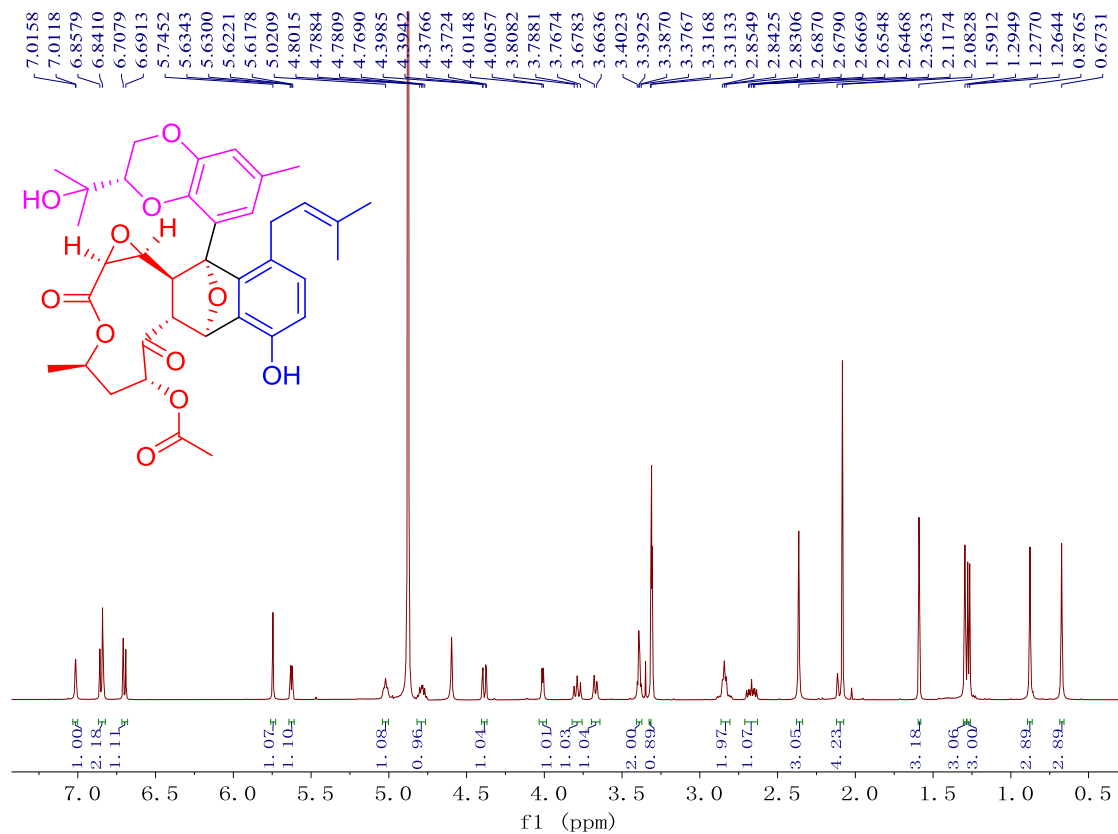


Figure S2. ¹H NMR spectrum (500 MHz, CD₃OD) of lithocarpin A (**1**).

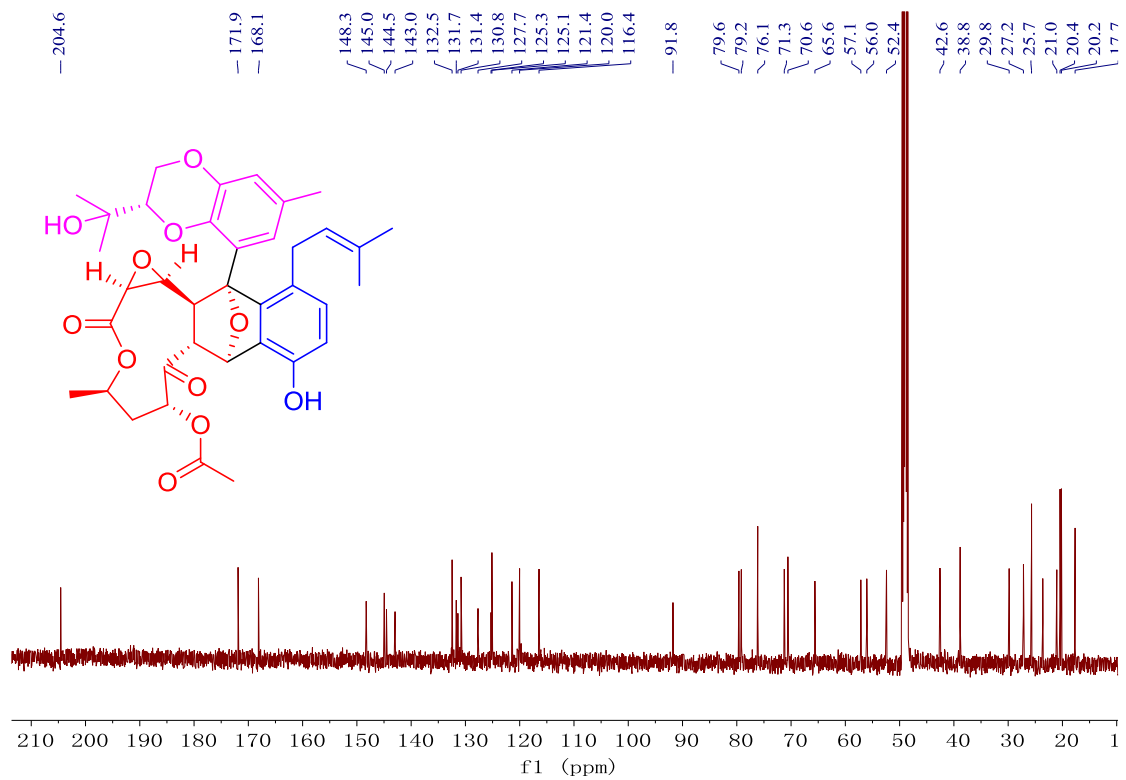


Figure S3. ^{13}C NMR spectrum (125 MHz, CD_3OD) of lithocarpin A (**1**).

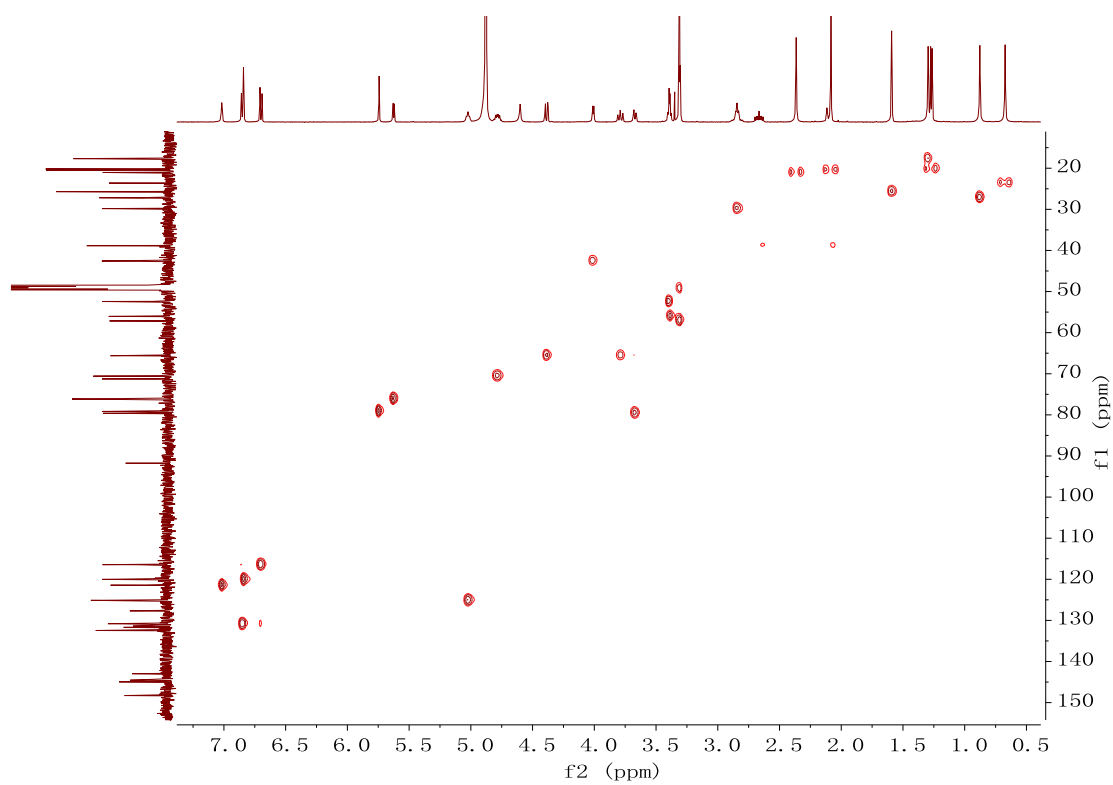


Figure S4. HSQC spectrum of lithocarpin A (**1**).

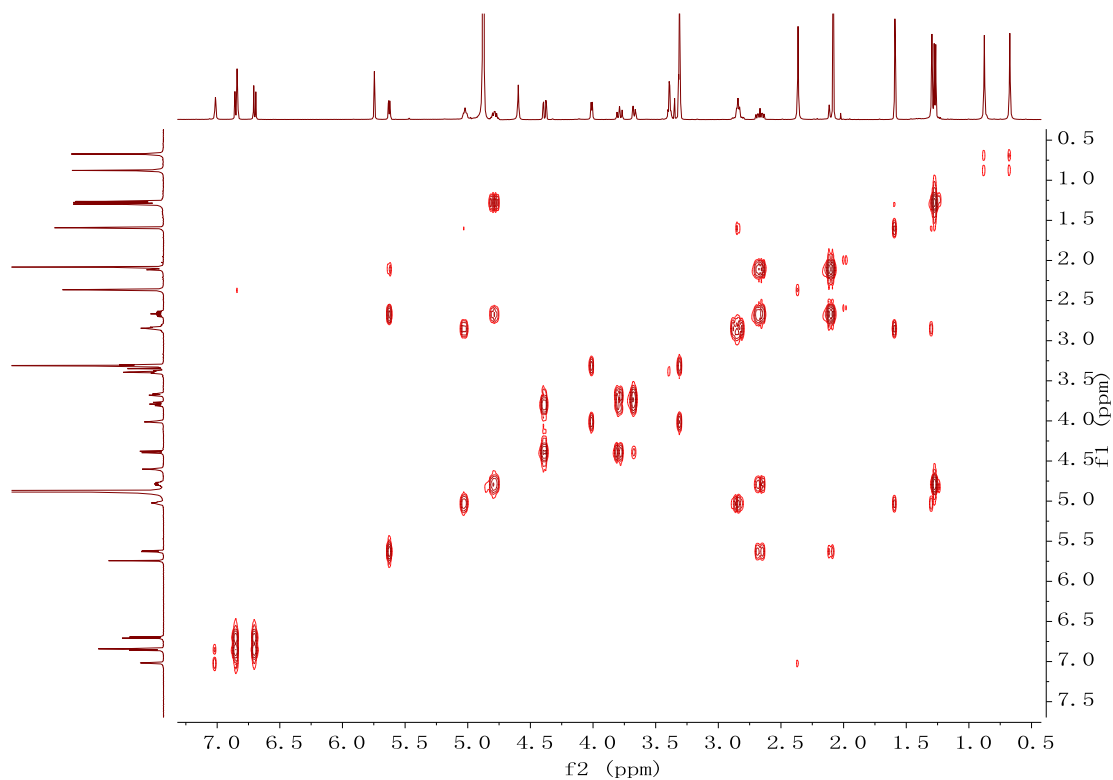


Figure S5. ¹H-¹H COSY spectrum (500 MHz, CD₃OD) of lithocarpin A (**1**).

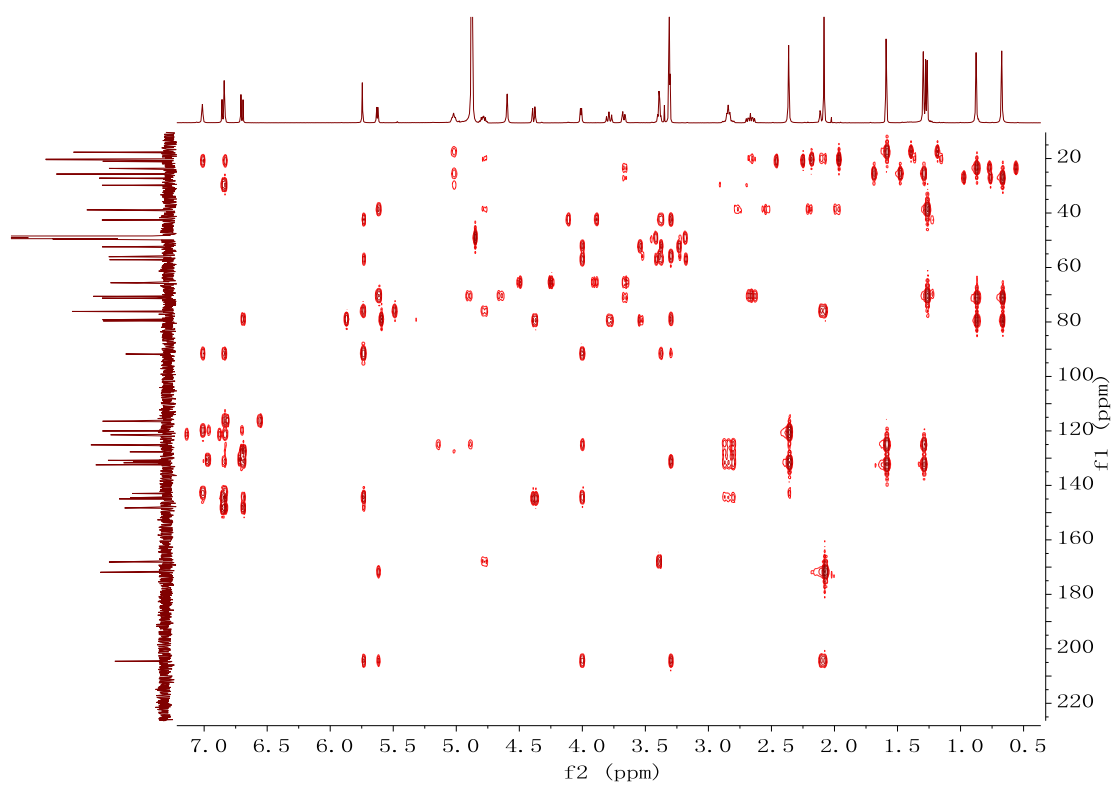


Figure S6. HMBC spectrum of lithocarpin A (**1**).

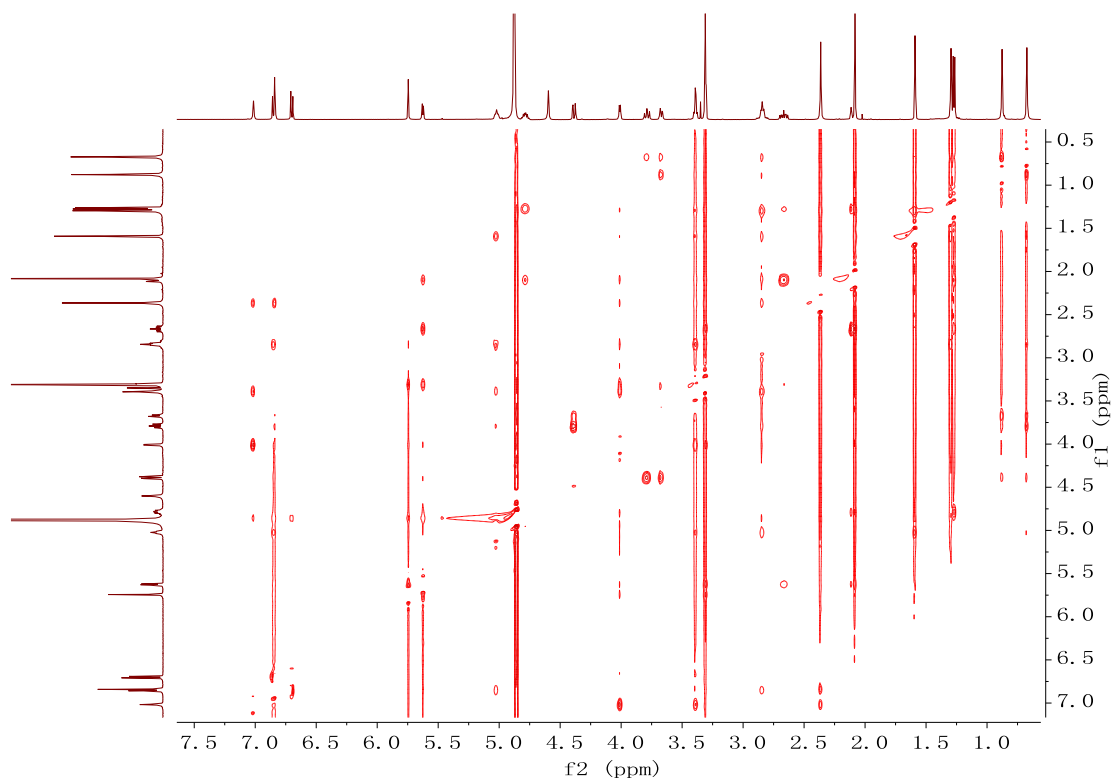


Figure S7. NOESY spectrum (500 MHz, CD₃OD) of lithocarpin A (**1**).

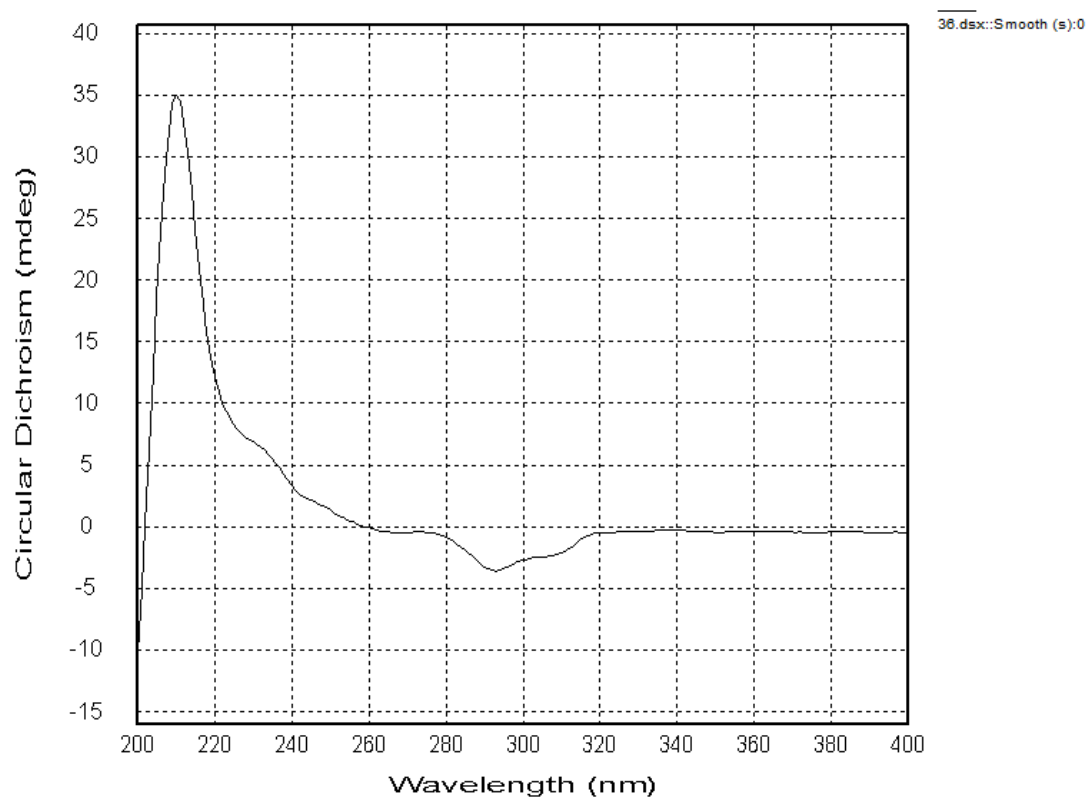


Figure S8. CD spectrum of lithocarpin A (**1**).

光谱峰值检测报告

数据集: w-36 - RawData

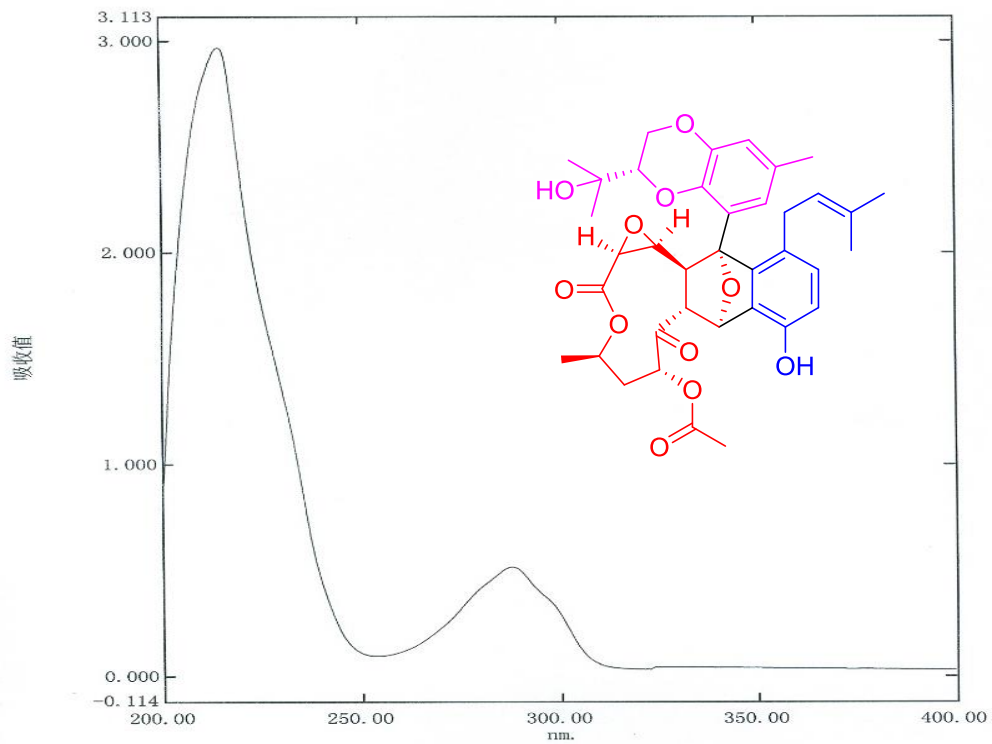


Figure S9. UV spectrum of lithocarpin A (1).

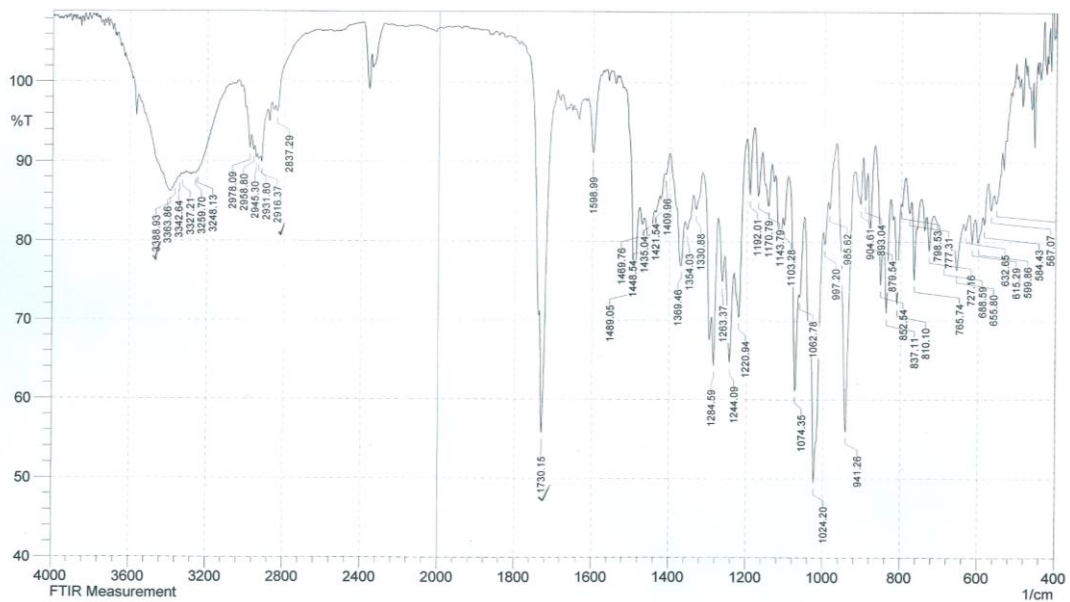


Figure S10. IR spectrum of lithocarpin A (1).

Mass Spectrum SmartFormula Report

Analysis Info		Acquisition Date	11/14/2017 9:09:04 AM	
Analysis Name	D:\Data\MS\data\201711\xujianlin_w-351_neg_63_01_3848.d	Operator	SCSIO	
Method	LC_Direct Infusion_neg_100-1000mz.m	Instrument	maxis	255552.00029
Sample Name	xujianlin_w-351_neg			
Comment				

Acquisition Parameter					
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4000 V	Set Dry Heater	180 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Charging Voltage	0 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

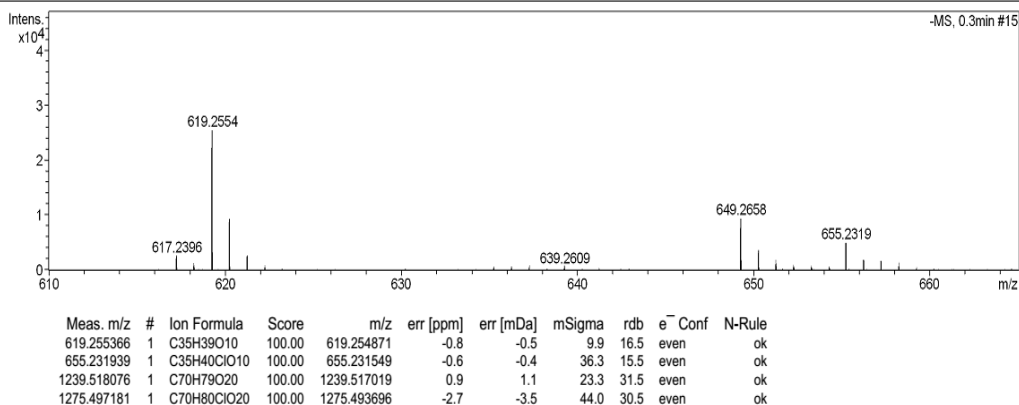


Figure S11. HRESIMS spectrum of lithocarpin B (2).

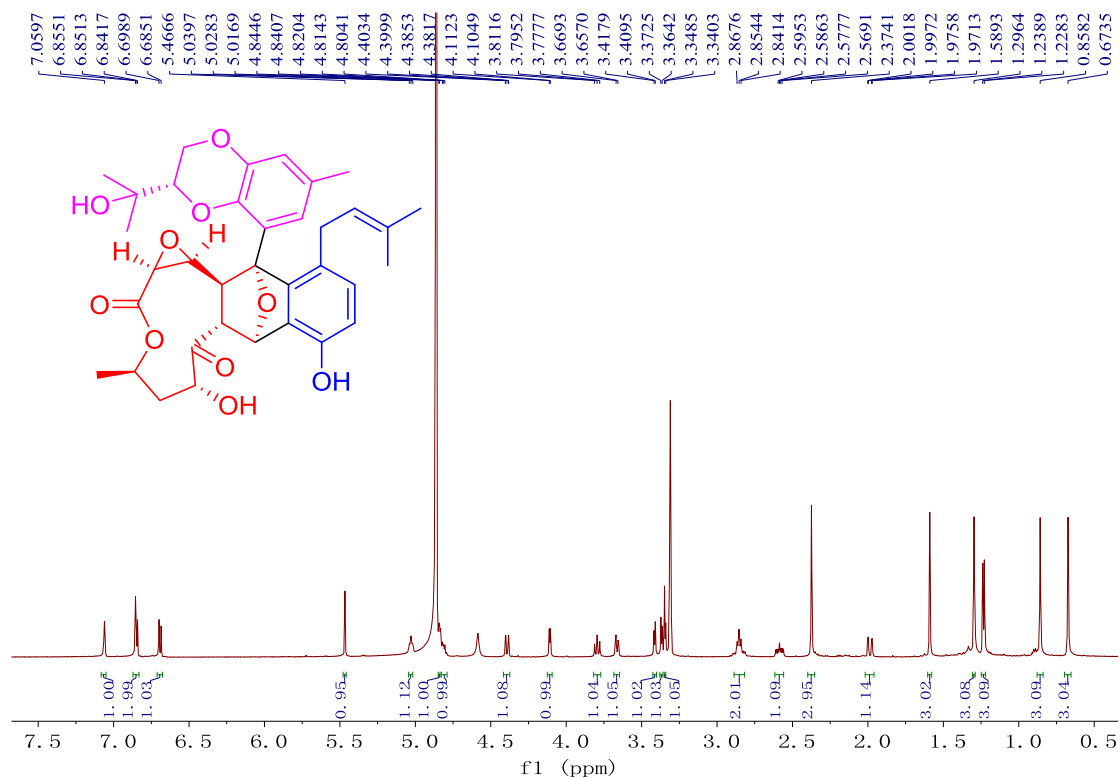


Figure S12. ¹H NMR spectrum (500 MHz, CD₃OD) of lithocarpin B (2).

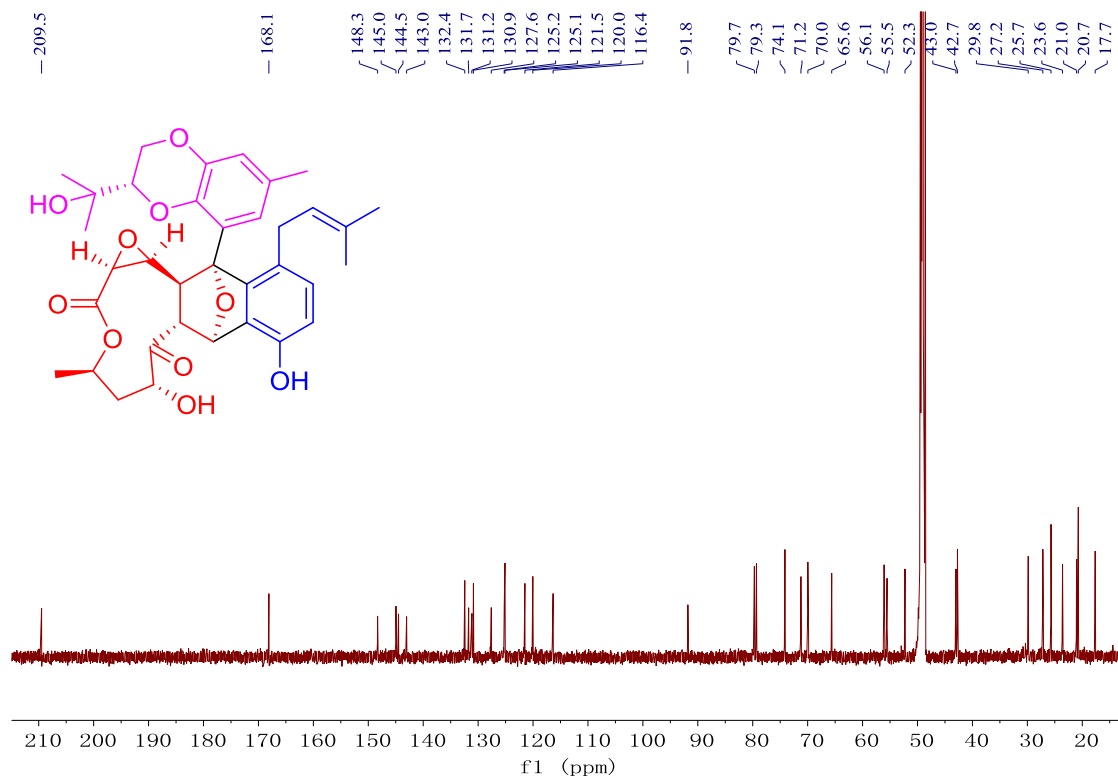


Figure S13. ^{13}C NMR spectrum (125 MHz, CD_3OD) of Lithocarpin B (**2**).

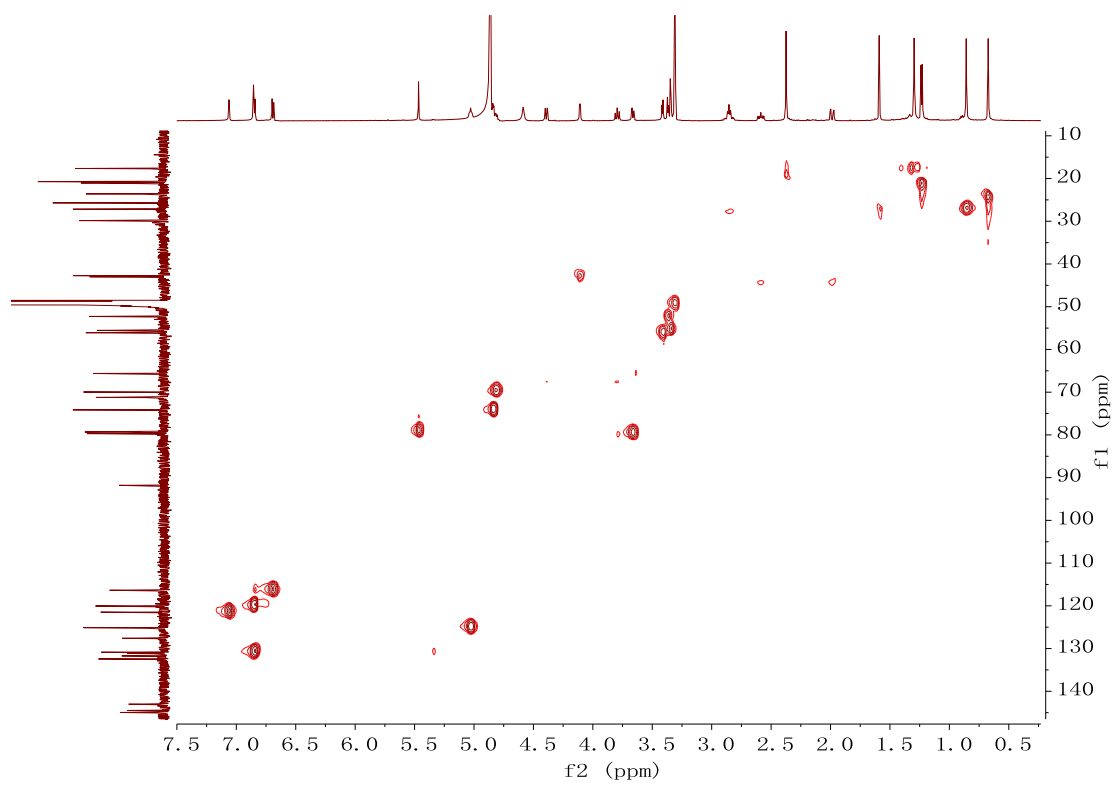


Figure S14. HSQC spectrum of lithocarpin B (**2**).

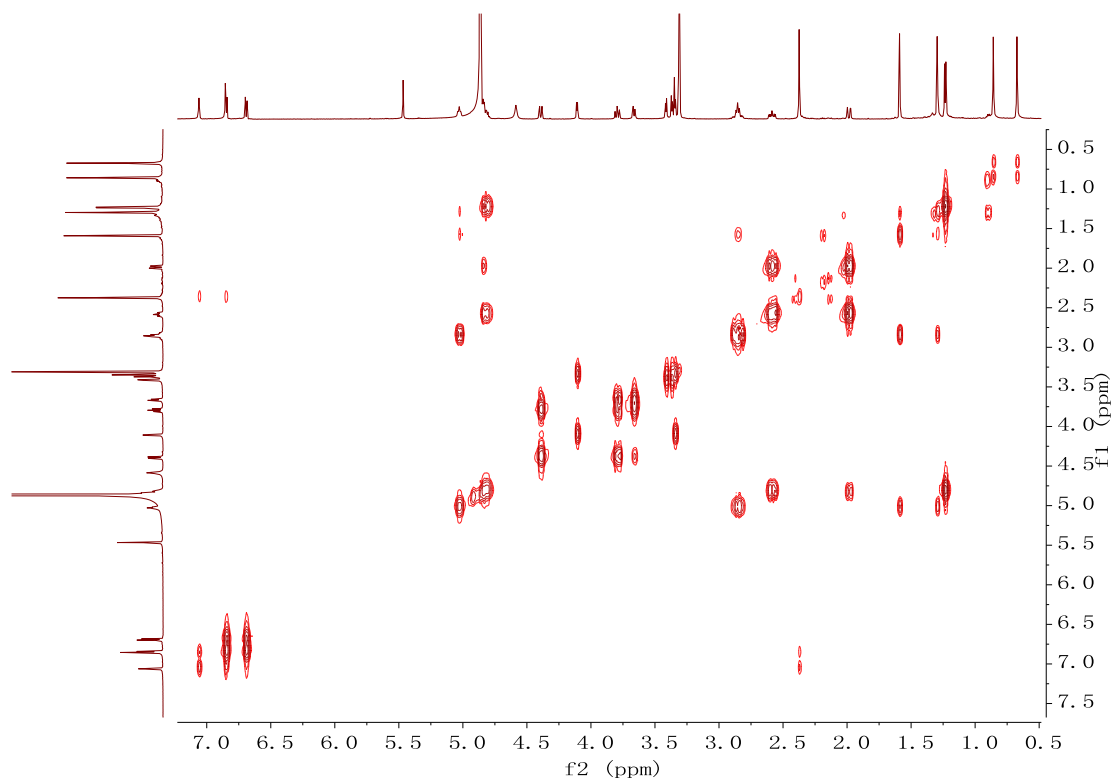


Figure S15. ^1H - ^1H COSY spectrum (500 MHz, CD_3OD) of lithocarpin B (**2**).

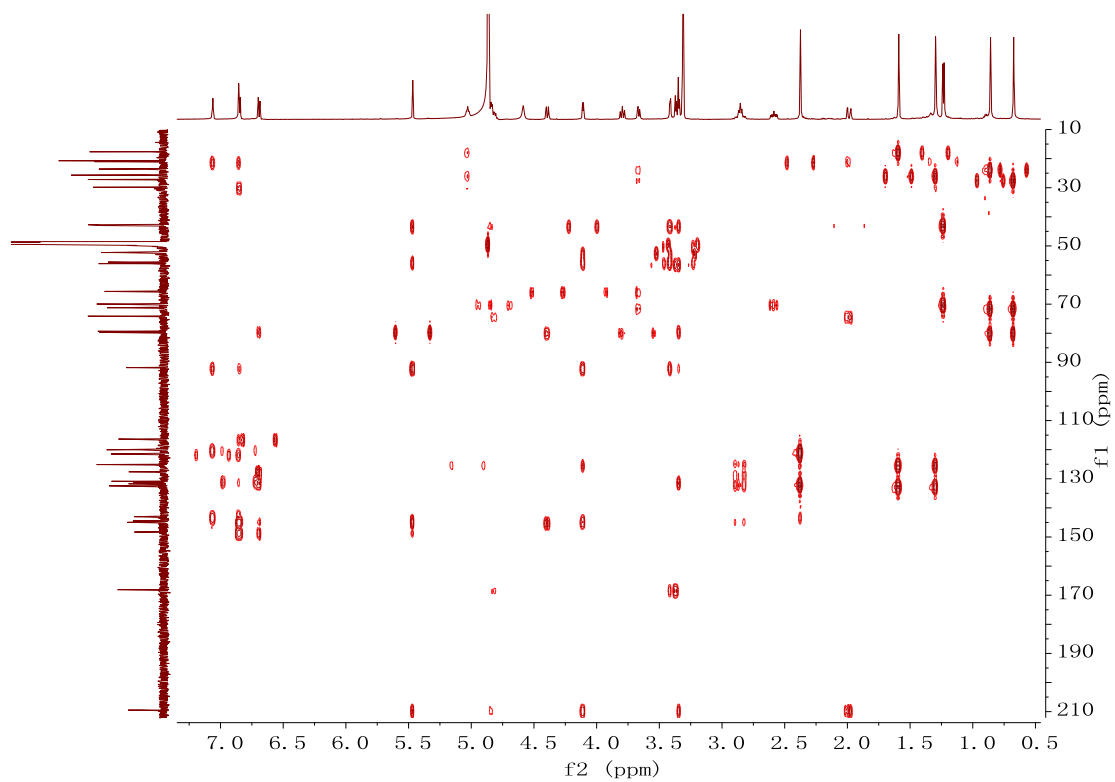


Figure S16. HMBC spectrum of lithocarpin B (**2**).

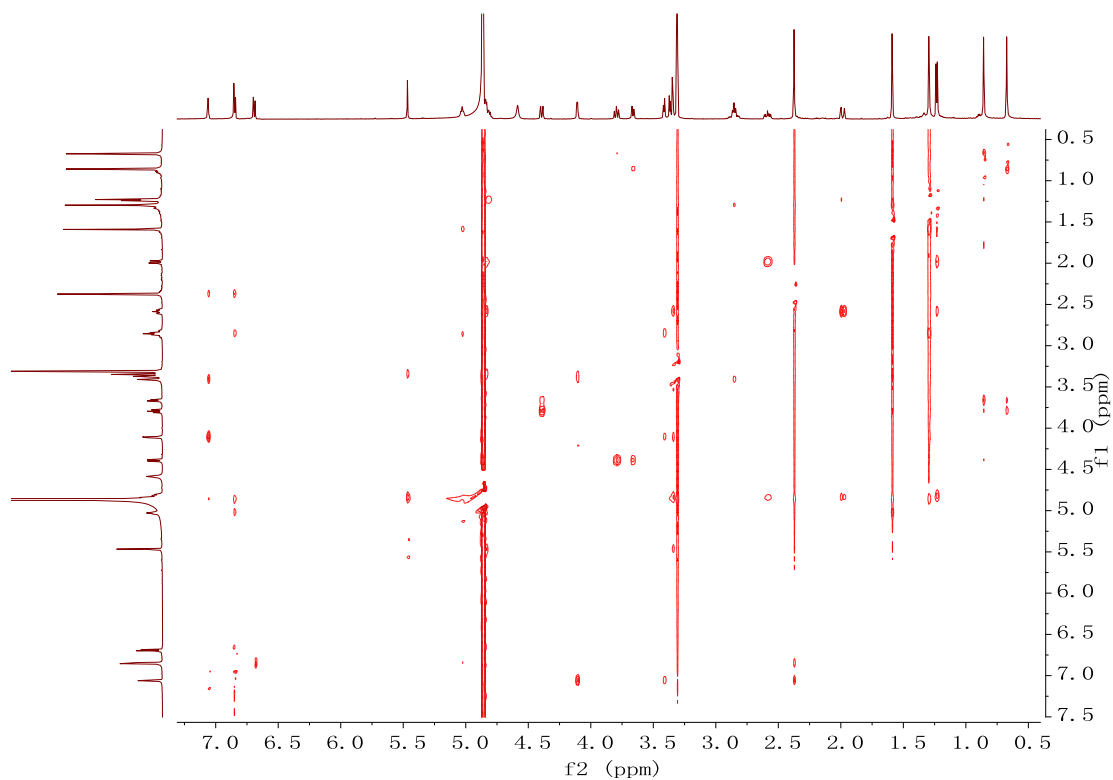


Figure S17. NOESY spectrum (500 MHz, CD₃OD) of lithocarpin B (**2**).

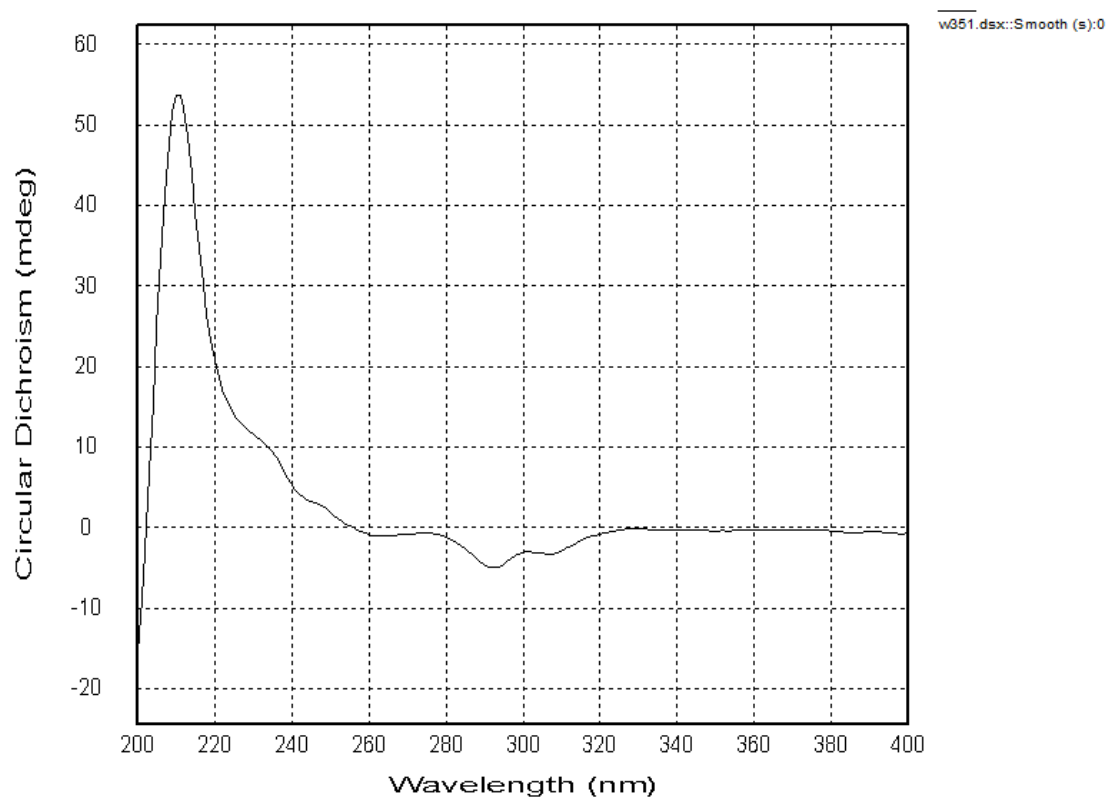


Figure S18. CD spectrum of lithocarpin B (**2**).

光谱峰值检测报告

数据集: W-351 - RawData

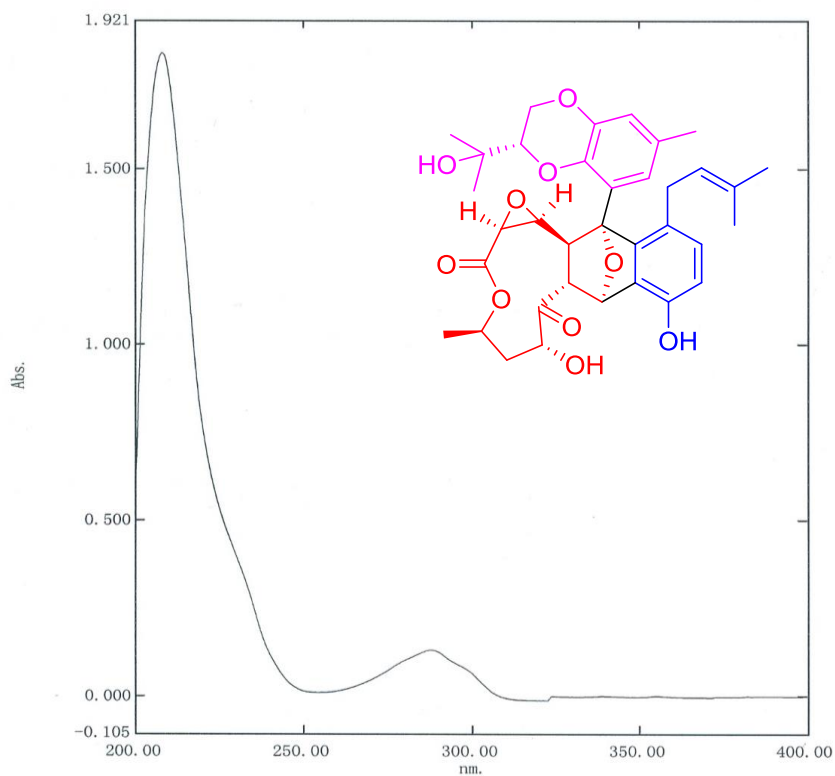


Figure S19. UV spectrum of lithocarpin B (2).

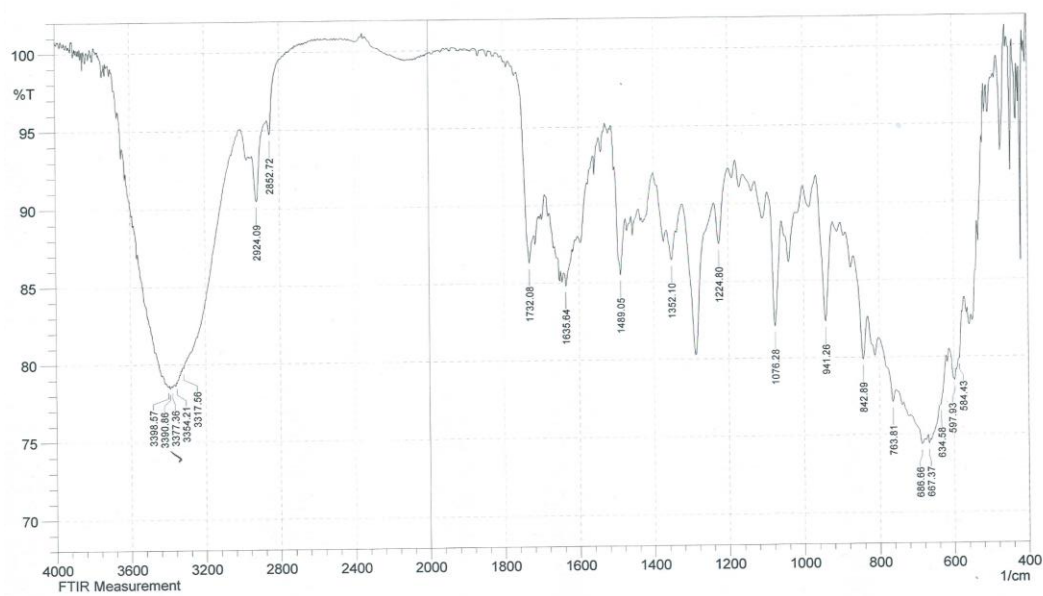


Figure S20. IR spectrum of lithocarpin B (2).

Mass Spectrum SmartFormula Report

Analysis Info		Acquisition Date	11/14/2017 9:02:07 AM	
Analysis Name	D:\Data\MS\data\201711\xujianlin_w-10_neg_61_01_3846.d	Operator	SCSIO	
Method	LC_Direct Infusion_neg_100-1000mz.m	Instrument	maXis	
Sample Name	xujianlin_w-10_neg		255552.00029	
Comment				

Acquisition Parameter					
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4000 V	Set Dry Heater	180 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Charging Voltage	0 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

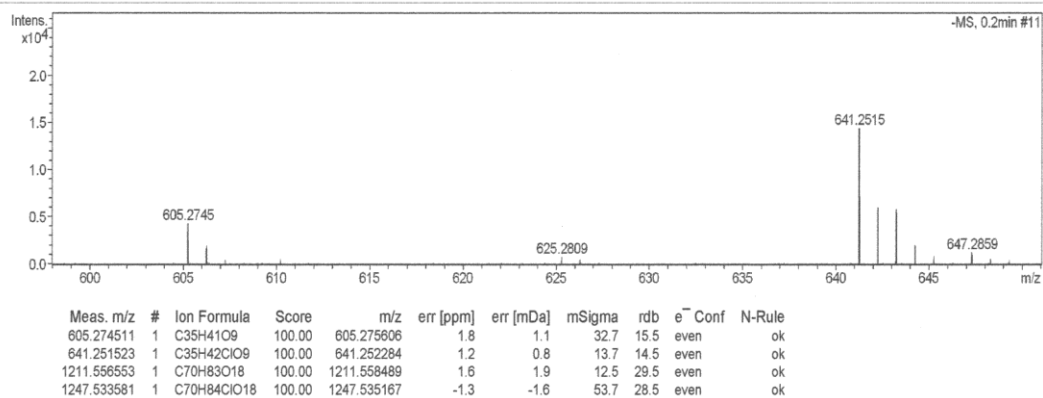


Figure S21. HRESIMS spectrum of lithocarpin C (**3**).

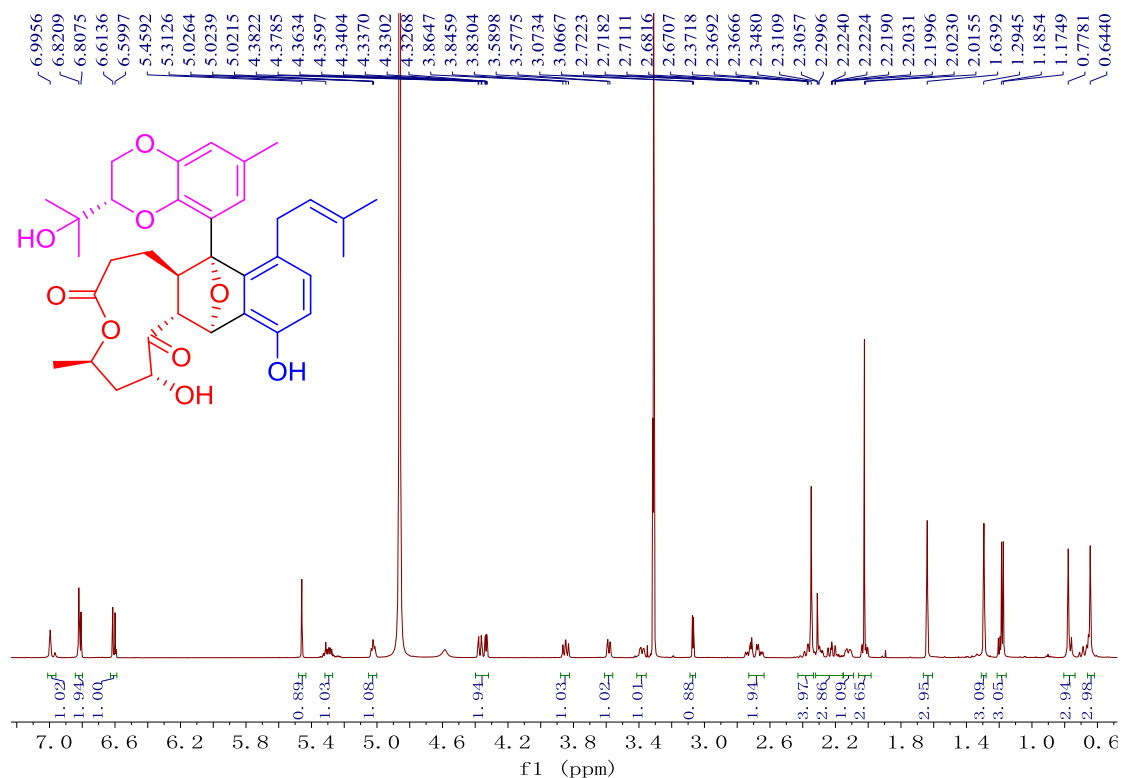


Figure S22. ¹H NMR spectrum (500 MHz, CD₃OD) of lithocarpin C (**3**).

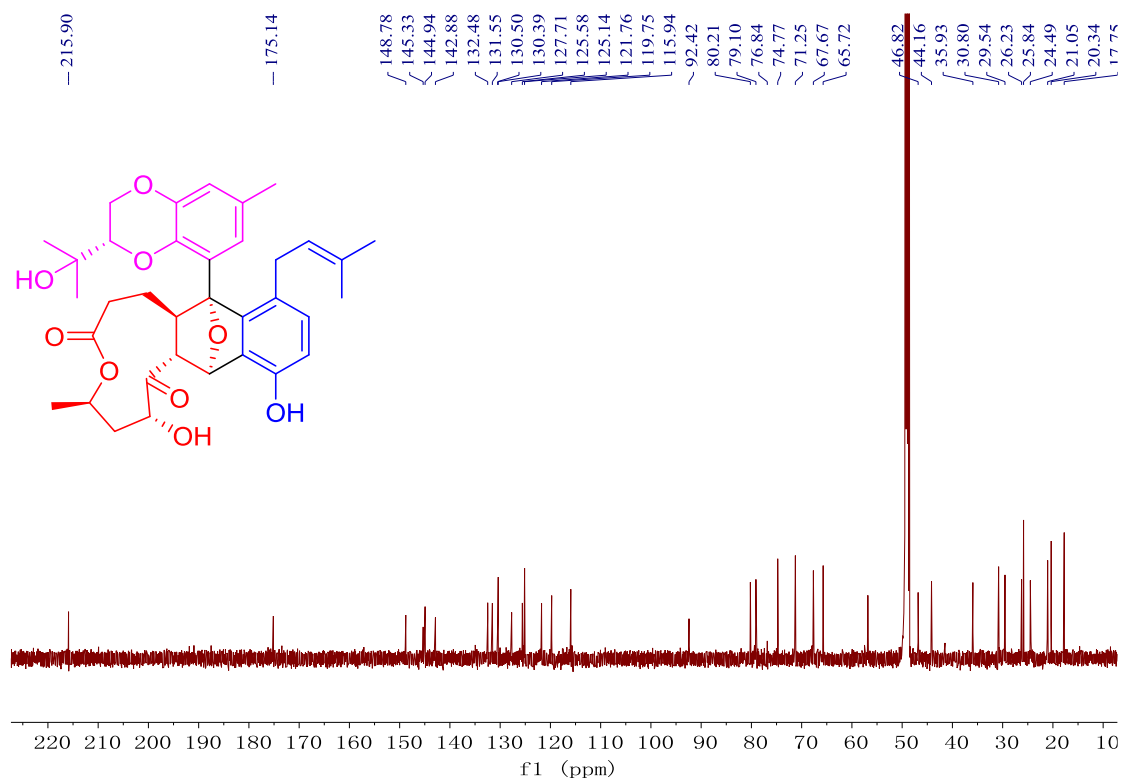


Figure S23. ^{13}C NMR spectrum (125 MHz, CD_3OD) of lithocarpin C (**3**).

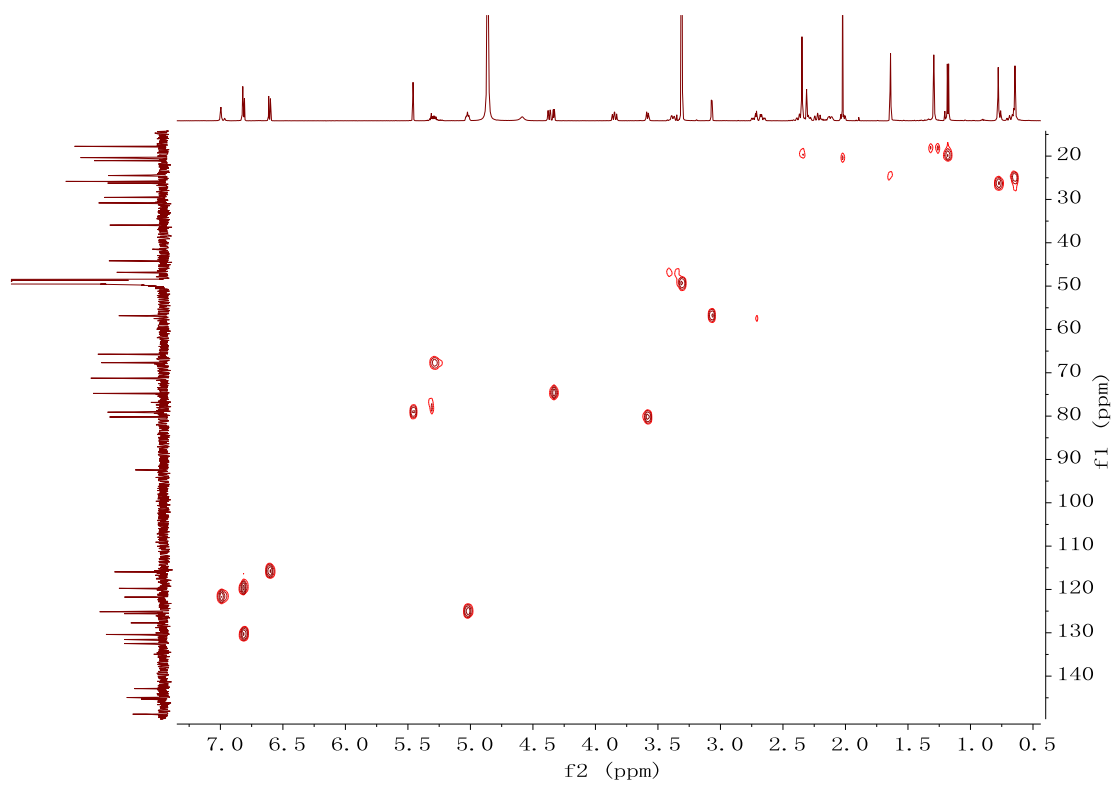


Figure S24. HSQC spectrum of lithocarpin C (**3**).

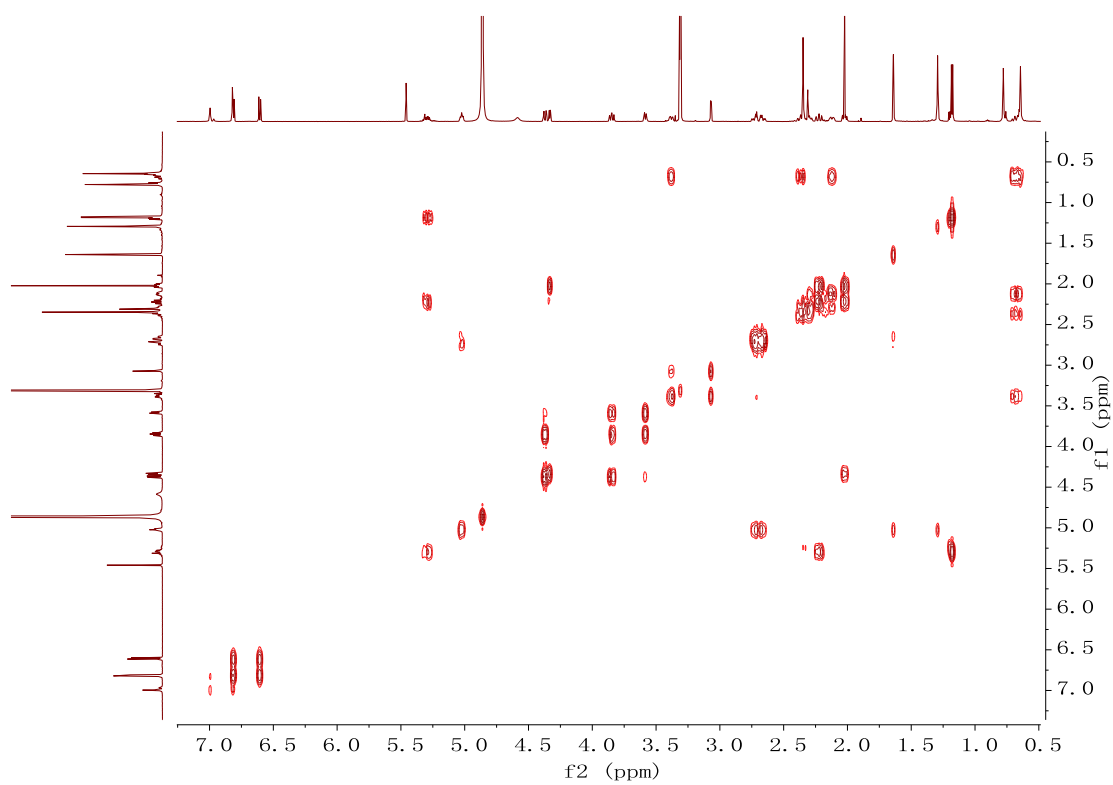


Figure S25. ^1H - ^1H COSY spectrum (500 MHz, CD_3OD) of lithocarpin C (**3**).

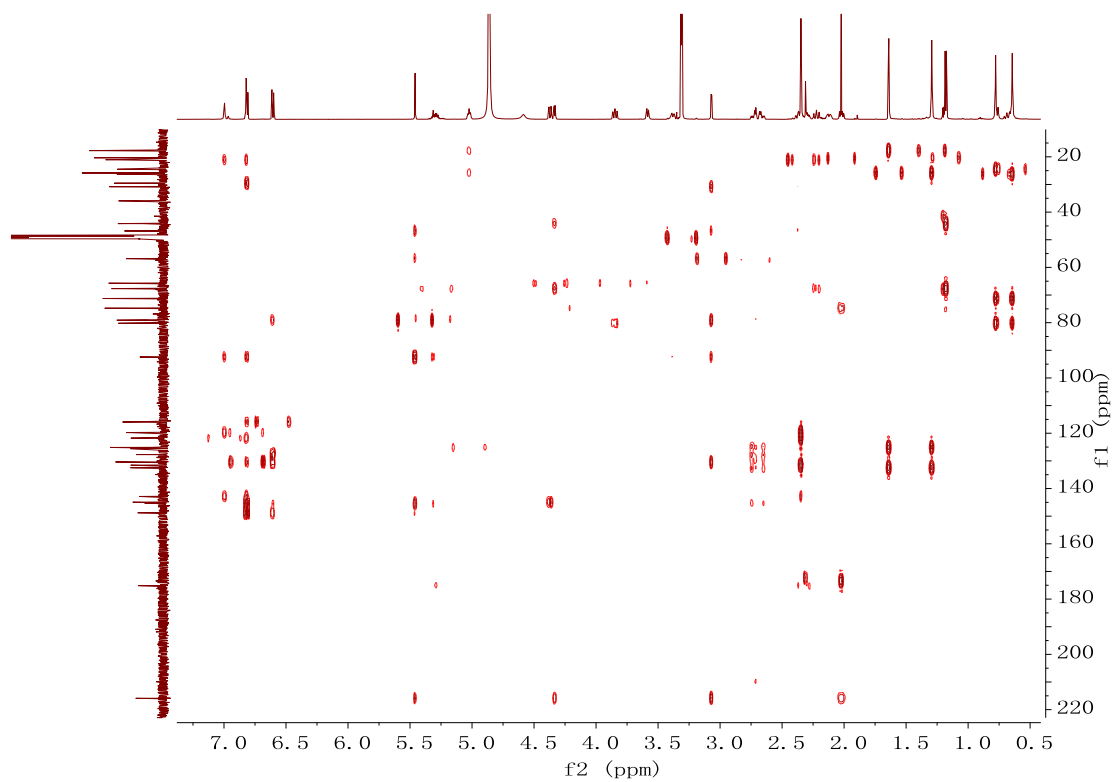


Figure S26. HMBC spectrum of lithocarpin C (**3**).

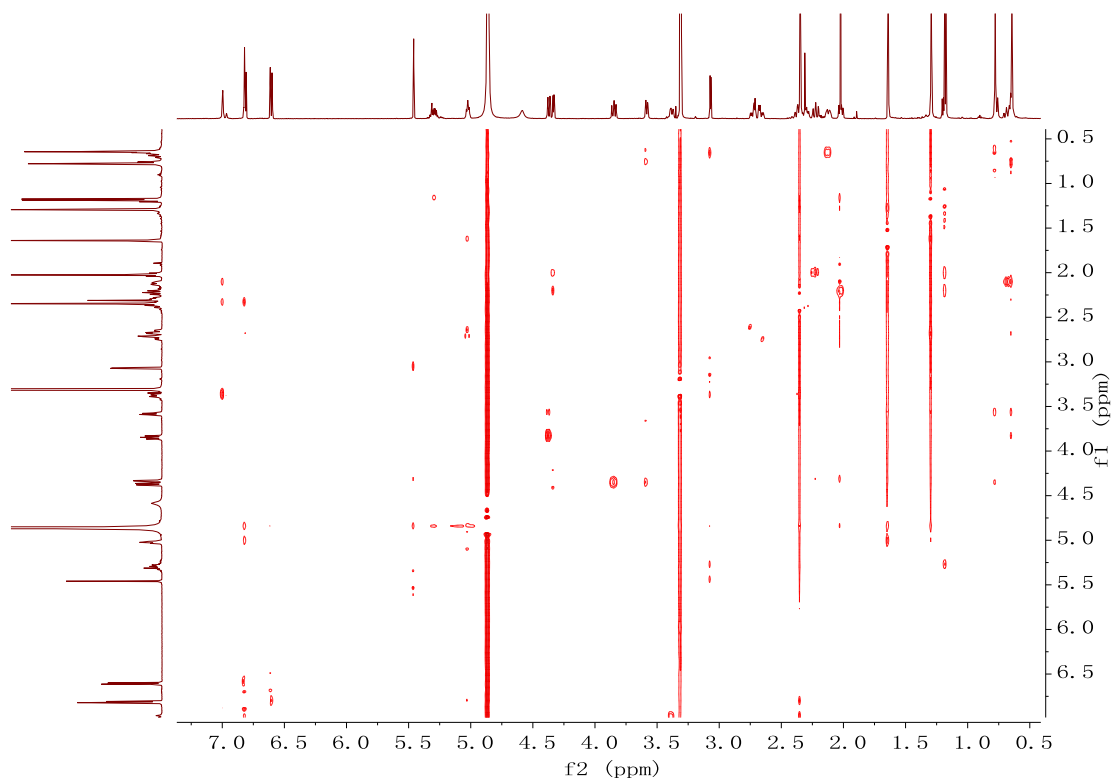


Figure S27. NOESY spectrum (500 MHz, CD₃OD) of lithocarpin C (**3**).

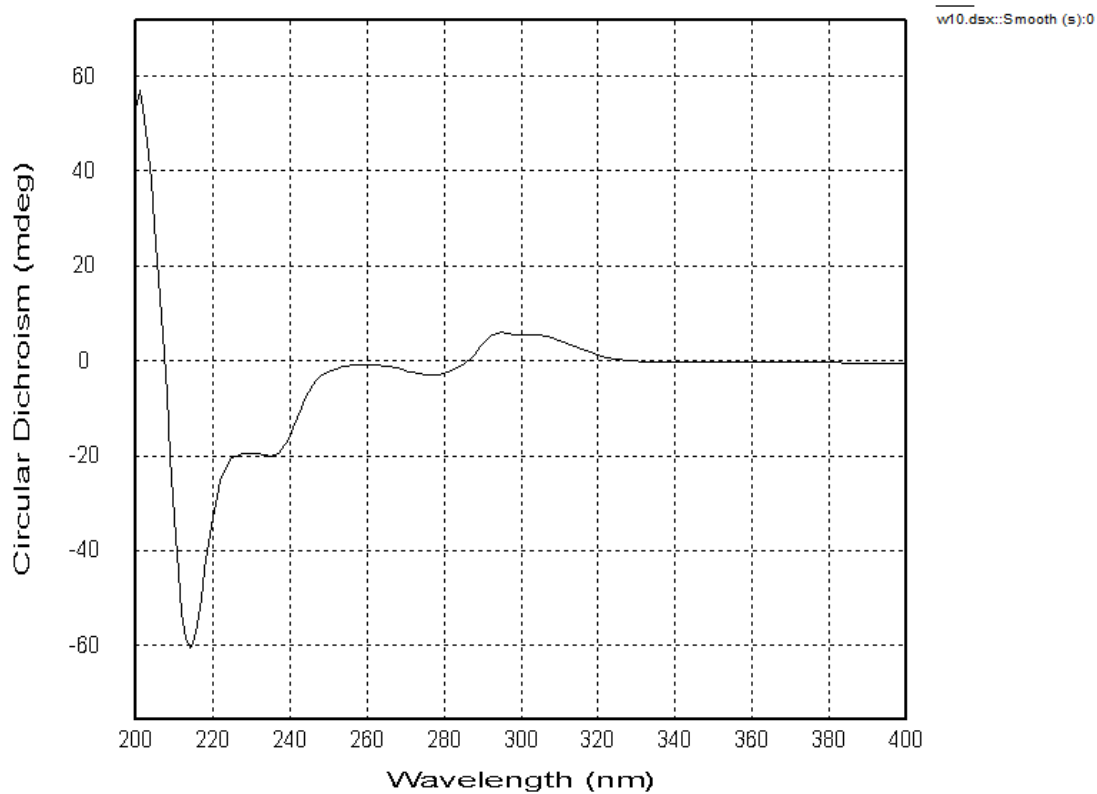


Figure S28. CD spectrum of lithocarpin C (**3**).

光谱峰值检测报告

数据集: W-101 - RawData

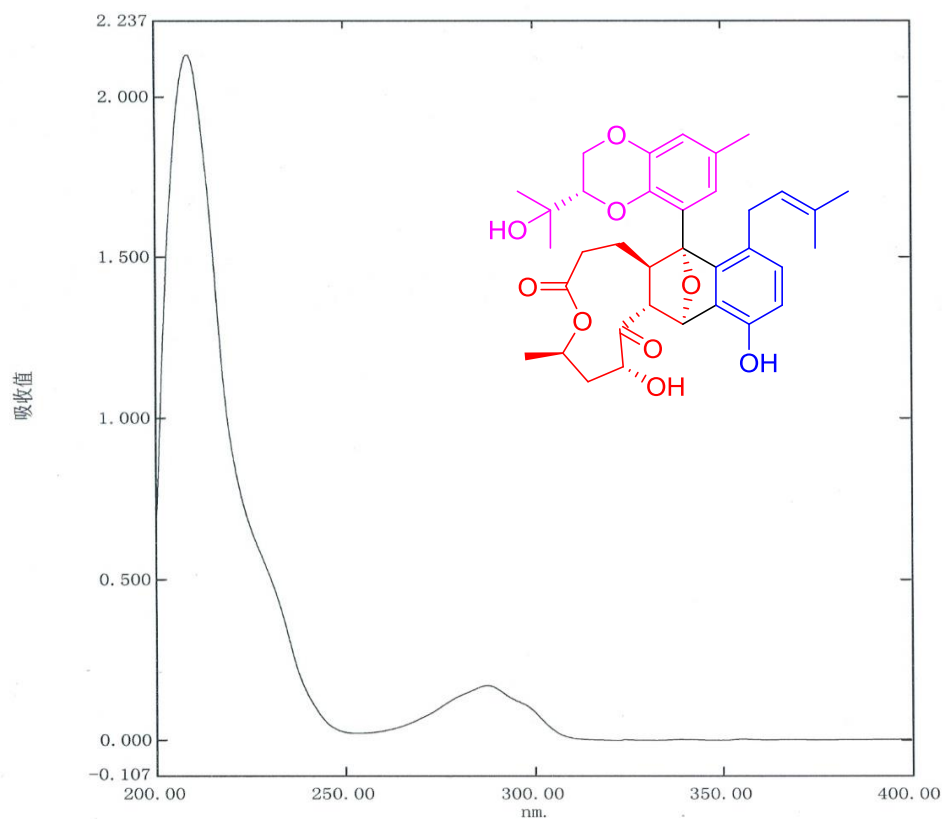


Figure S29. UV spectrum of lithocarpin C (3).

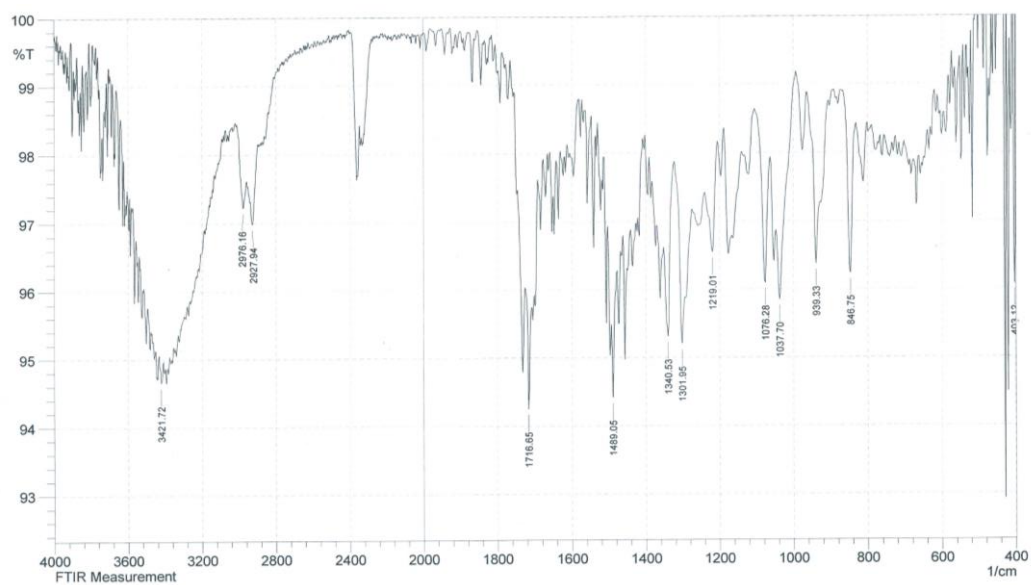


Figure S30. IR spectrum of lithocarpin C (3).

Mass Spectrum SmartFormula Report

Analysis Info		Acquisition Date	
Analysis Name	D:\Data\MS\data\201711\Xujianlin_w-55_neg_62_01_3847.d	11/14/2017 9:05:35 AM	
Method	LC_Direct Infusion_neg_100-1000mz.m	Operator	SCSIO
Sample Name	Xujianlin_w-55_neg	Instrument	maXis
Comment			255552.00029

Acquisition Parameter					
Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4000 V	Set Dry Heater	180 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Charging Voltage	0 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C

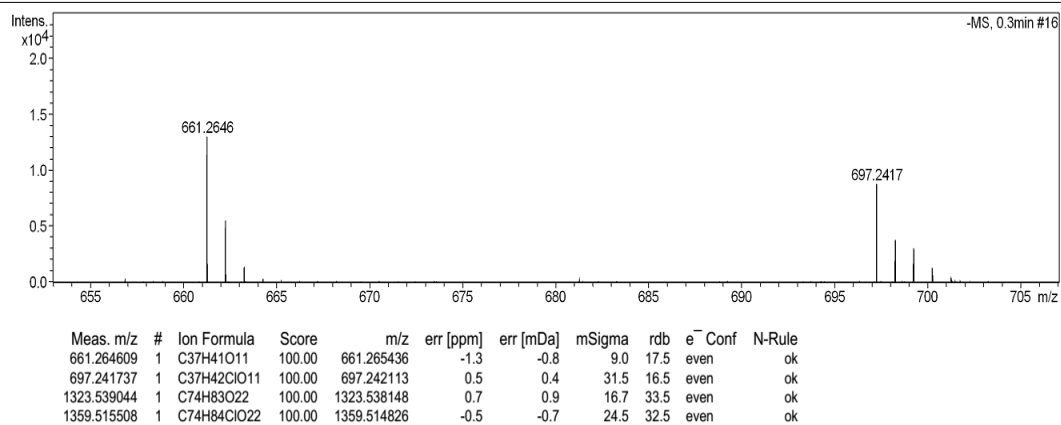


Figure S31. HRESIMS spectrum of lithocarpin D (4).

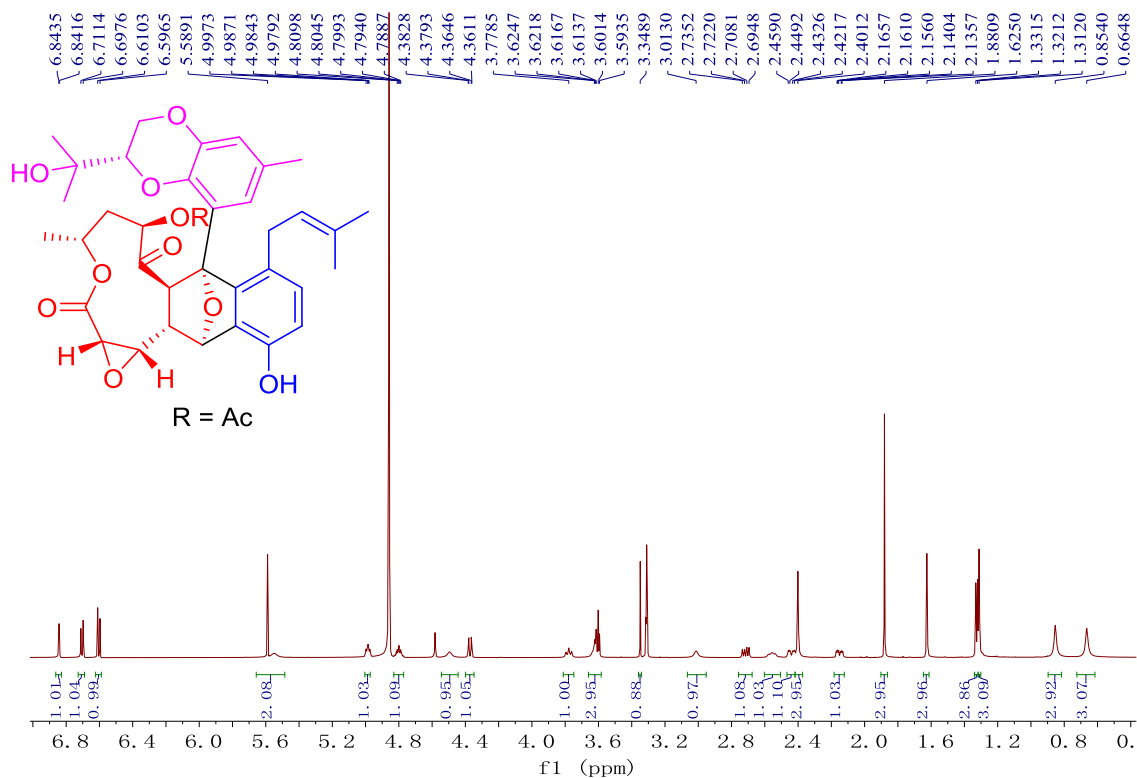


Figure S32. ¹H NMR spectrum (500 MHz, CD₃OD) of lithocarpin D (4).

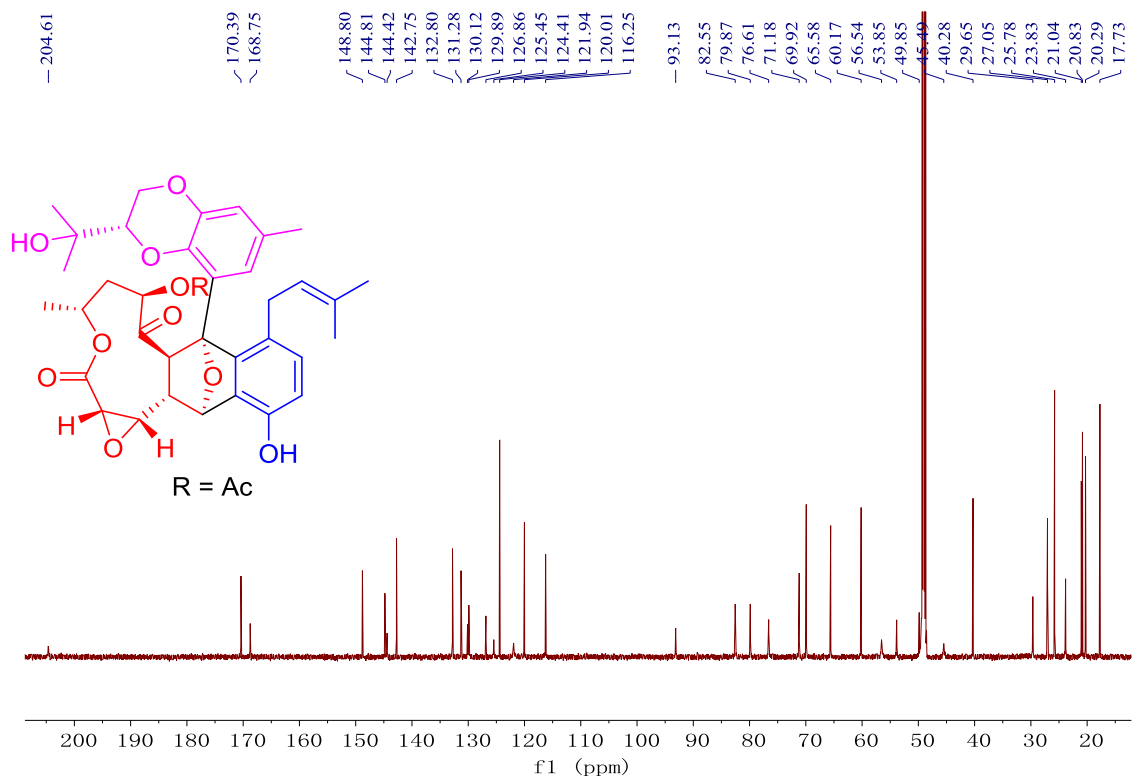


Figure S33. ¹³C NMR spectrum (125 MHz, CD₃OD) of lithocarpin D (4).

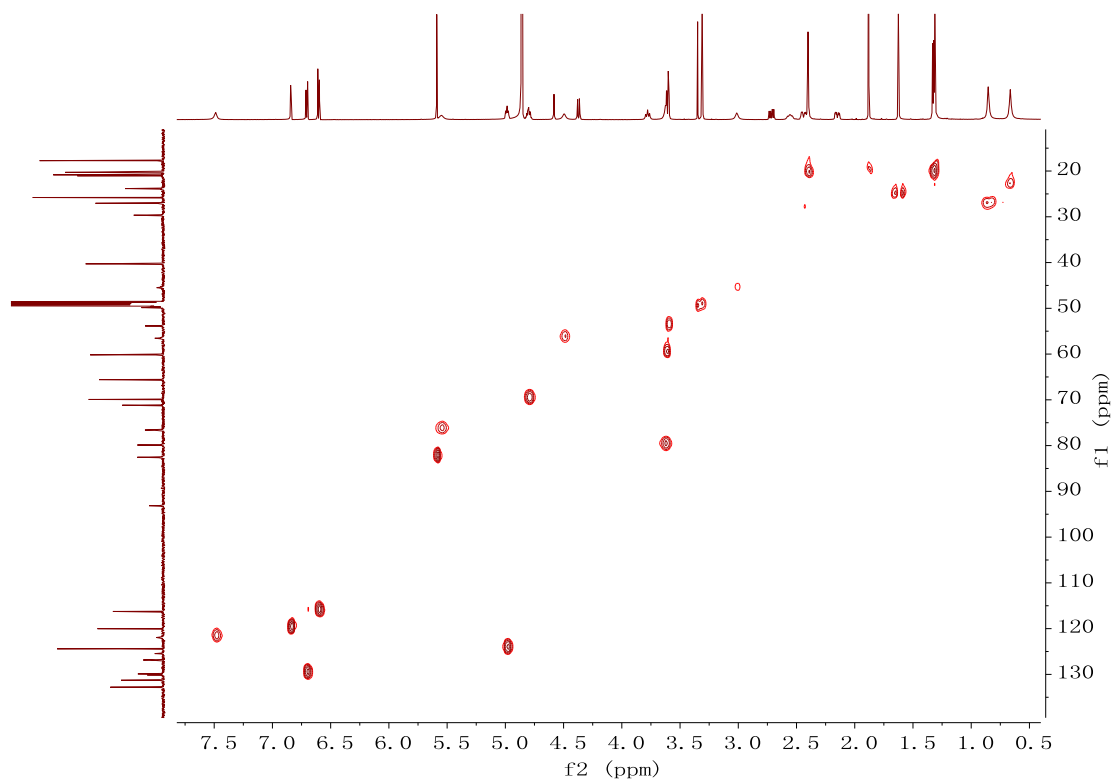


Figure S34. HSQC spectrum of Lithocarpin D (4).

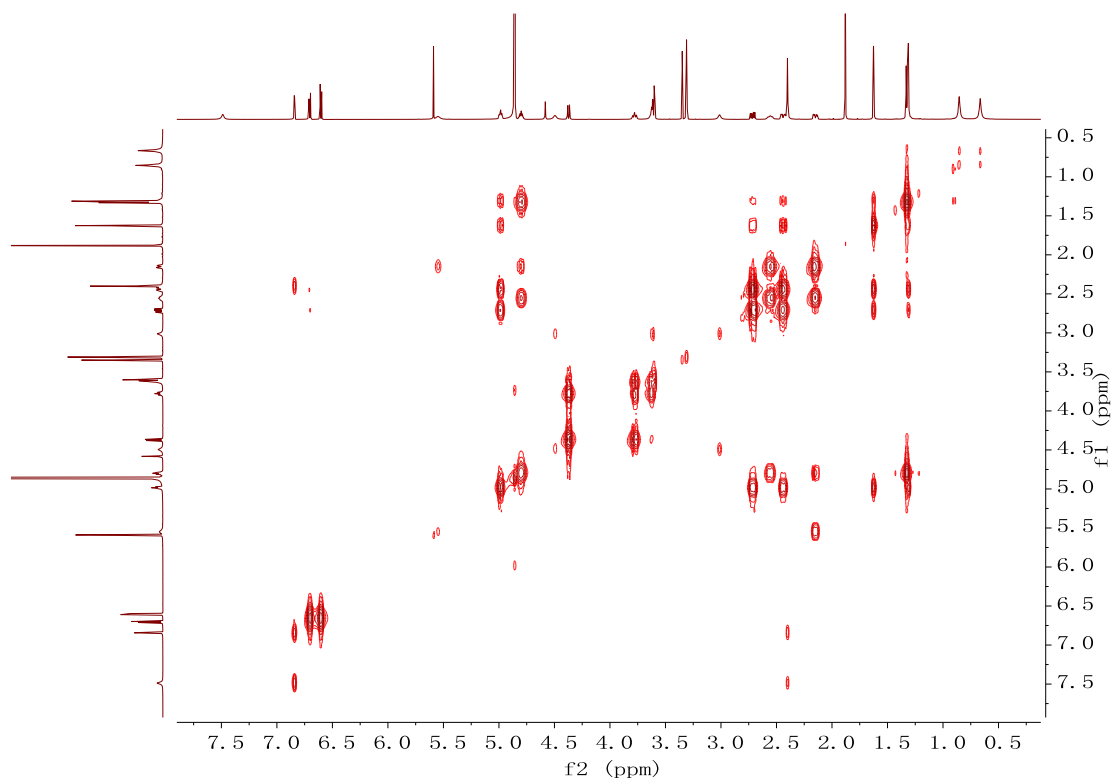


Figure S35. ^1H - ^1H COSY spectrum (500 MHz, CD_3OD) of lithocarpin D (**4**).

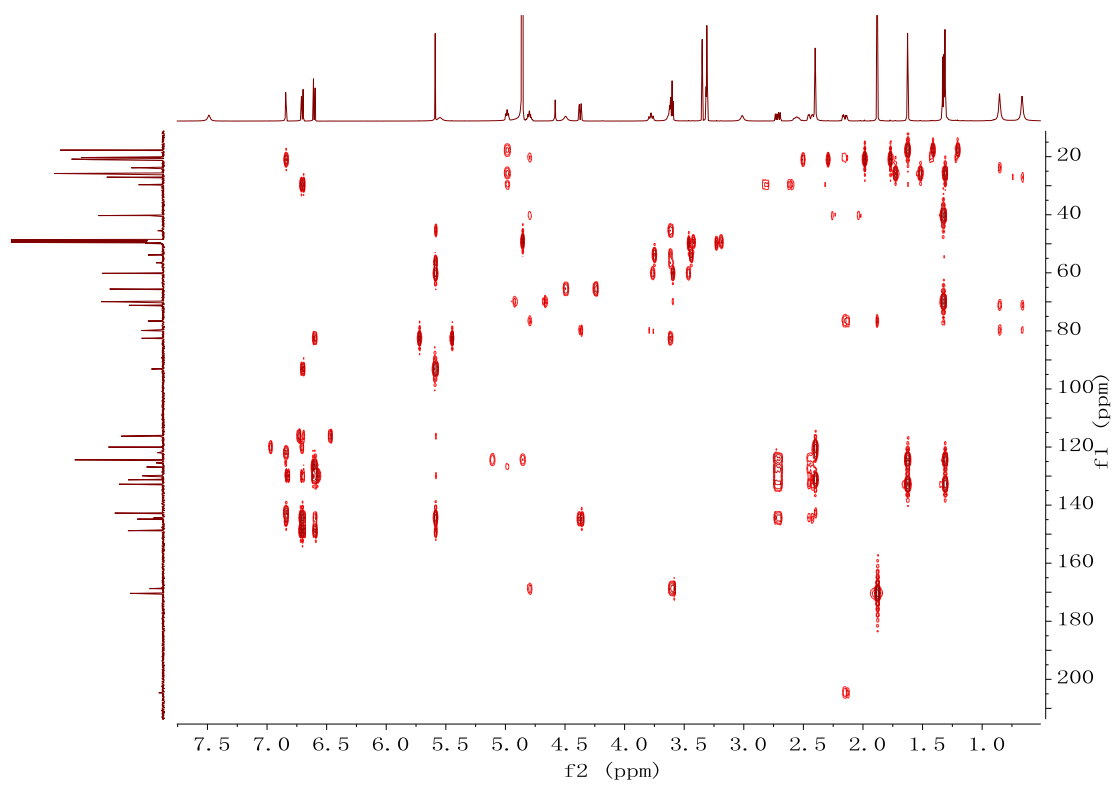


Figure S36. HMBC spectrum of lithocarpin D (**4**).

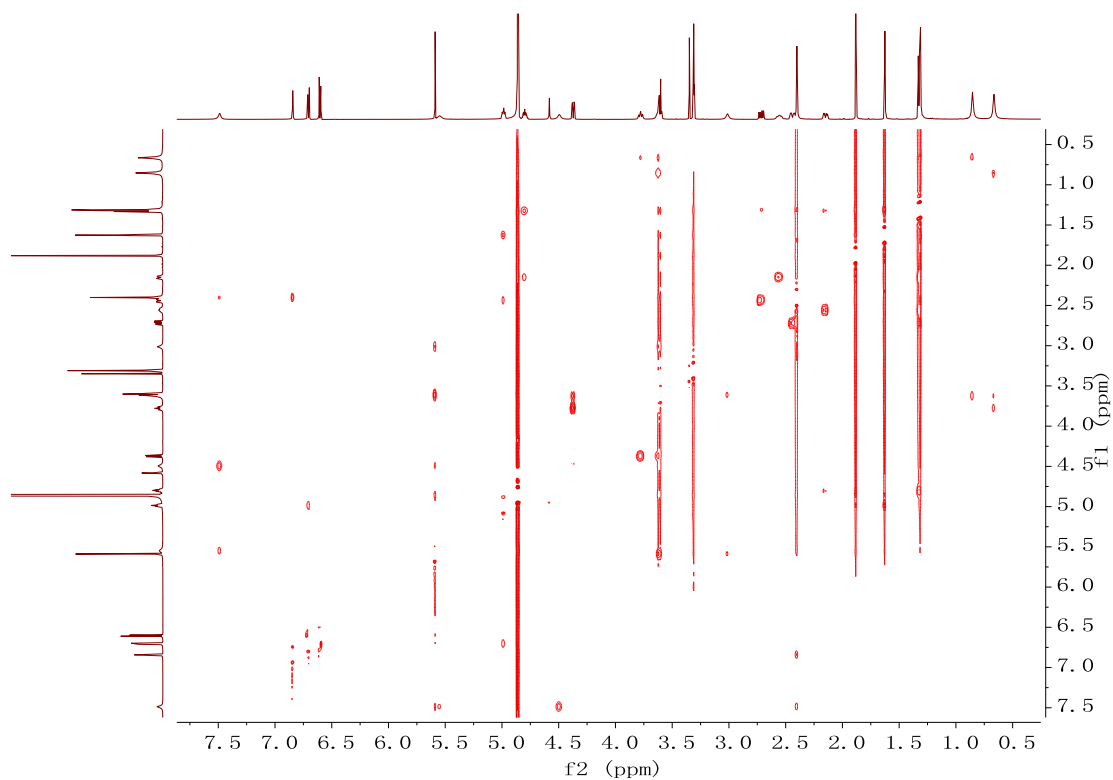


Figure S37. NOESY spectrum (500 MHz, CD₃OD) of lithocarpin D (**4**).

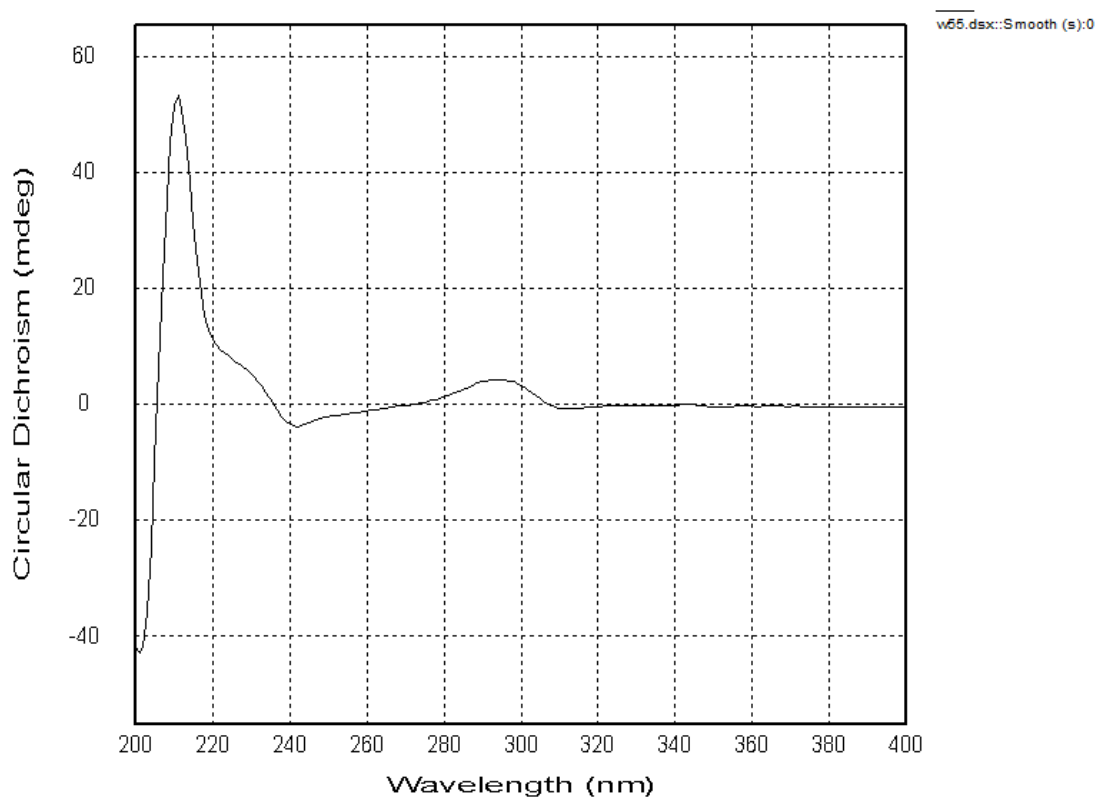


Figure S38. CD spectrum of lithocarpin D (**4**).

光谱峰值检测报告

数据集: W-55 - RawData

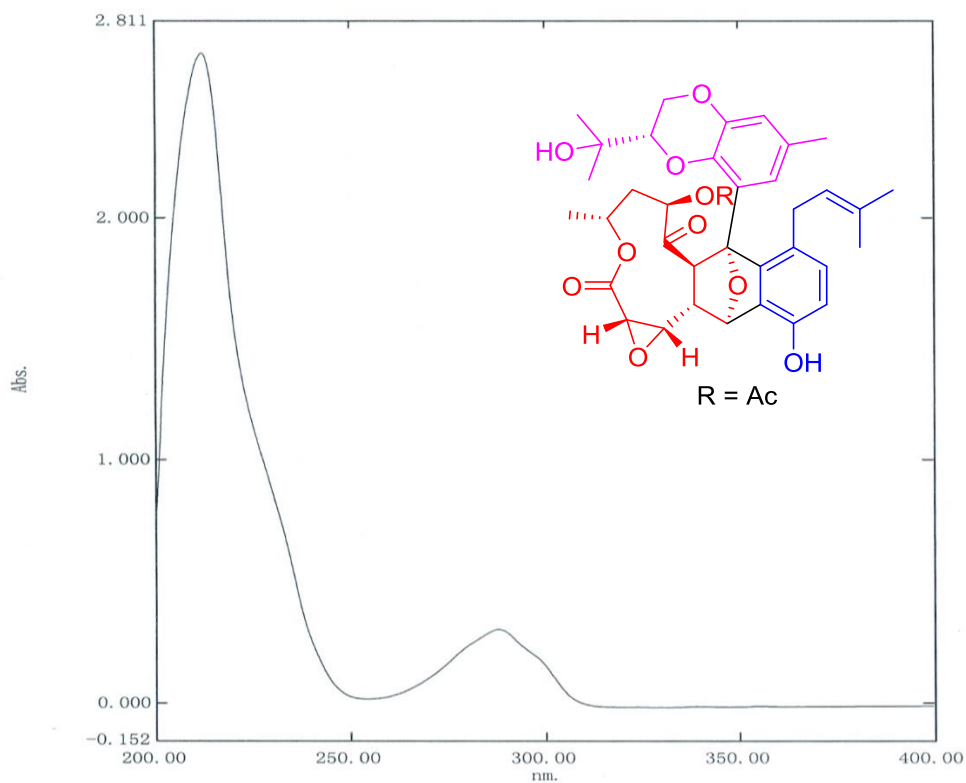


Figure S39. UV spectrum of lithocarpin D (4).

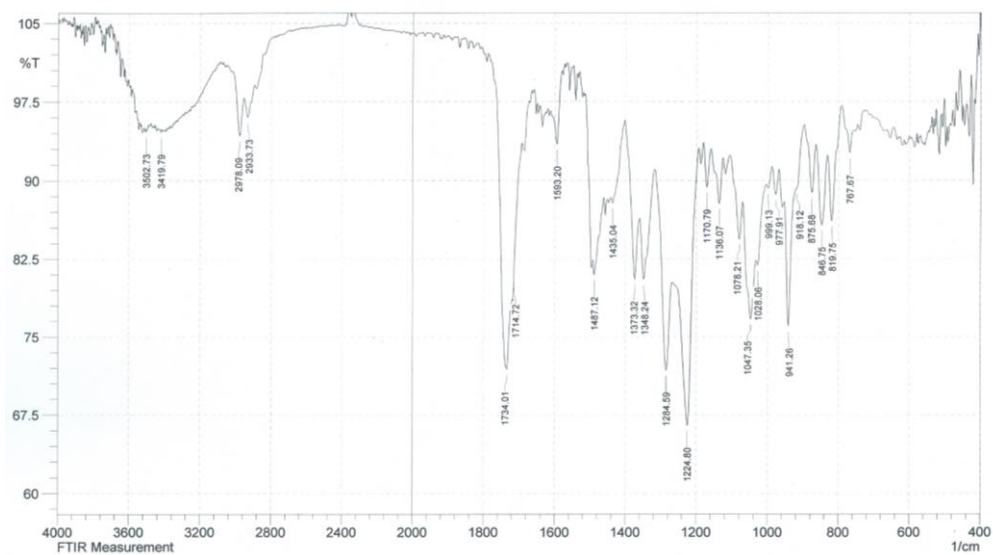


Figure S40. IR spectrum of lithocarpin D (4).

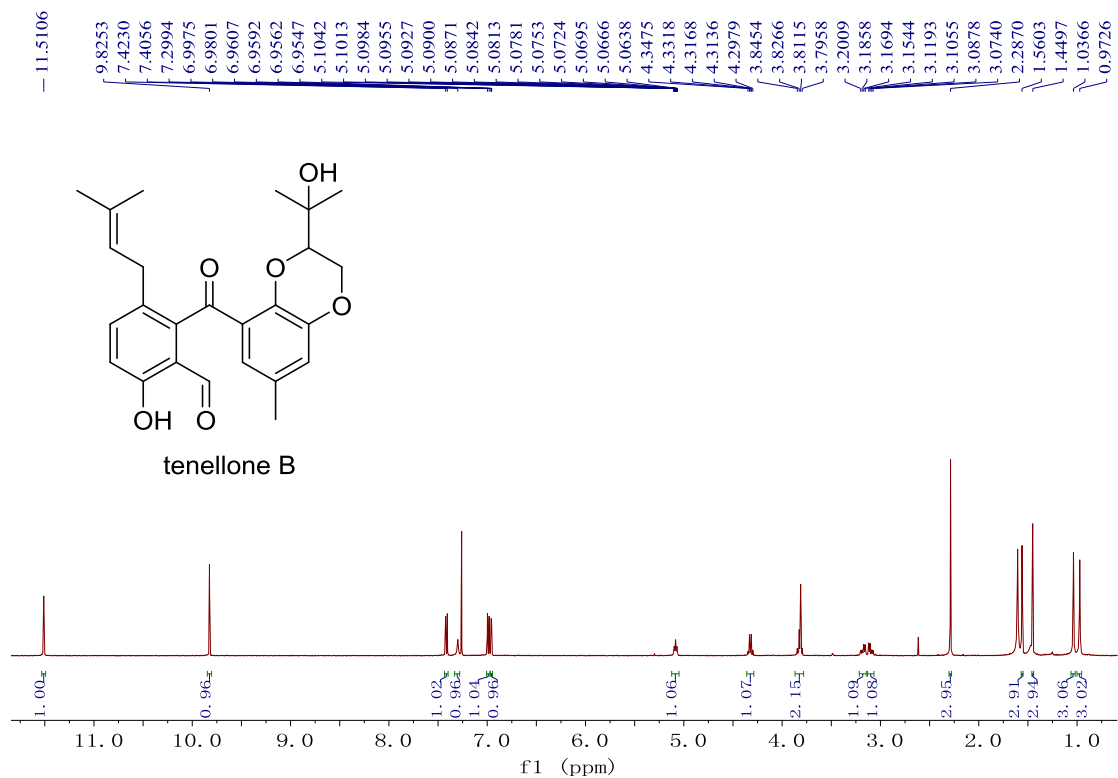


Figure S41. ¹H NMR spectrum (500 MHz, CDCl₃) of tenellone B (5).

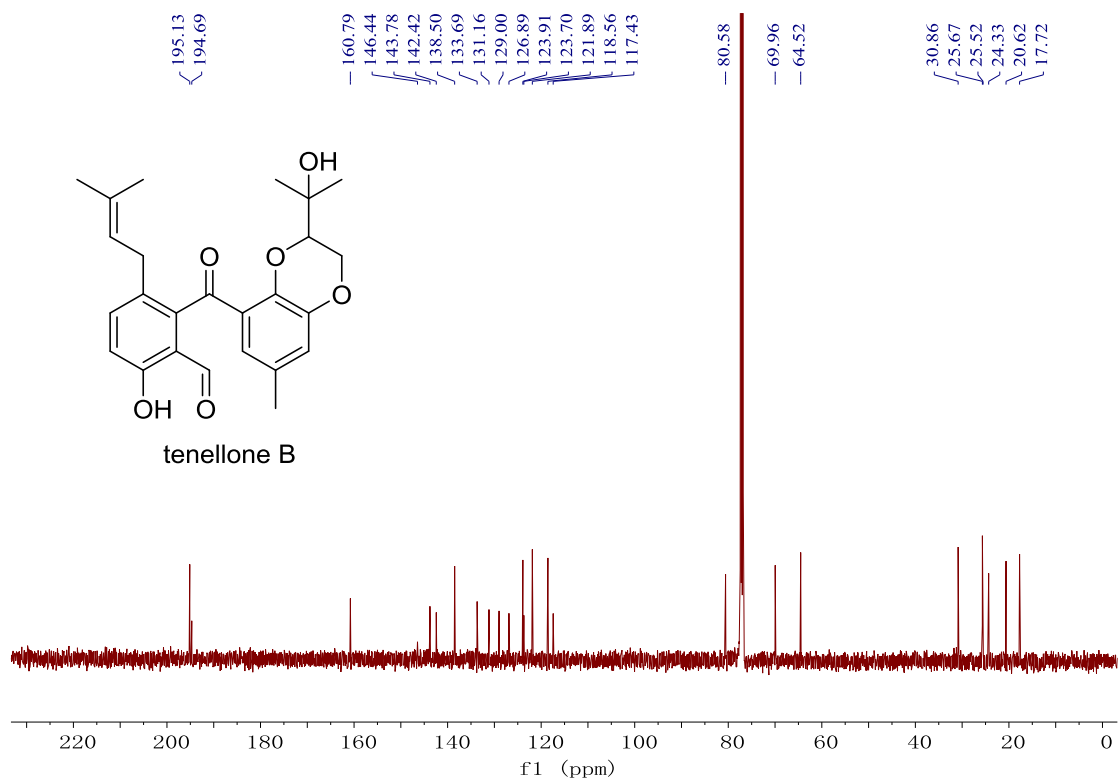


Figure S42. ¹³C NMR spectrum (125 MHz, CDCl₃) of tenellone B (5).

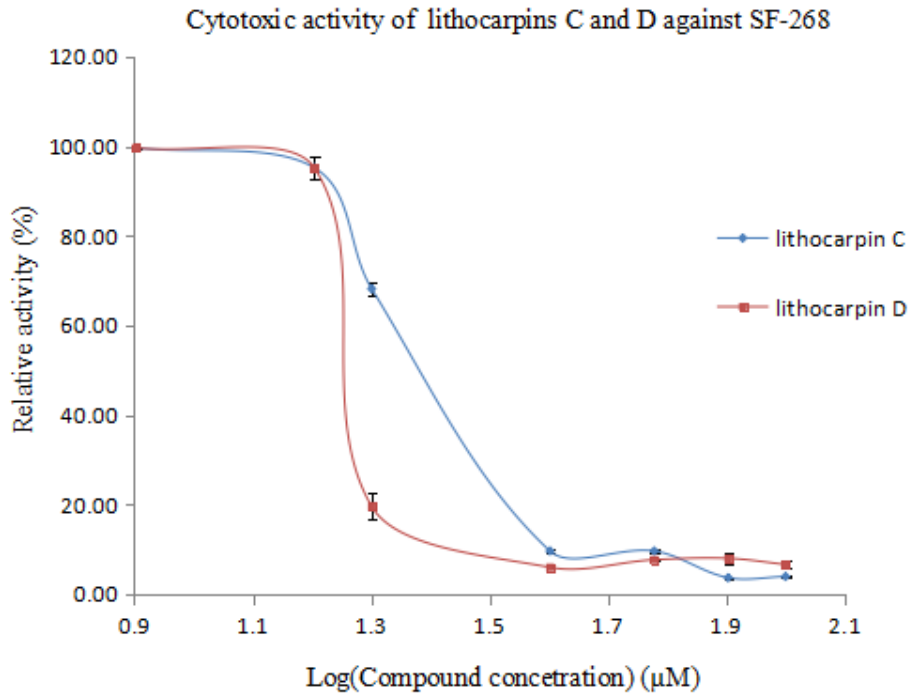


Figure S43. Cytotoxic activity of lithocarpins C and D against SF-268.

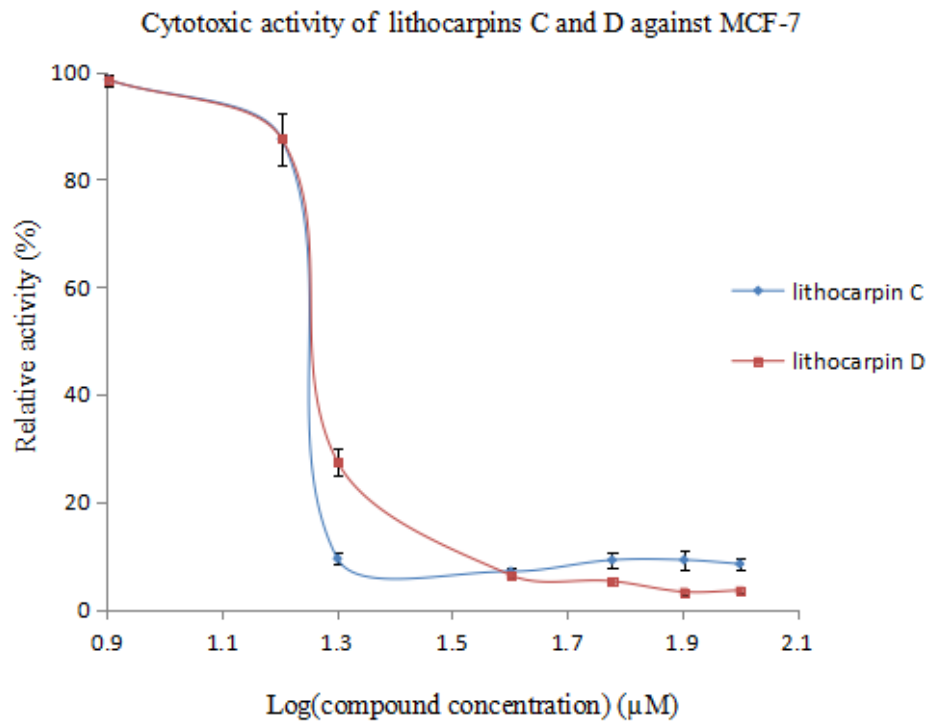


Figure S44. Cytotoxic activity of lithocarpins C and D against MCF-7.

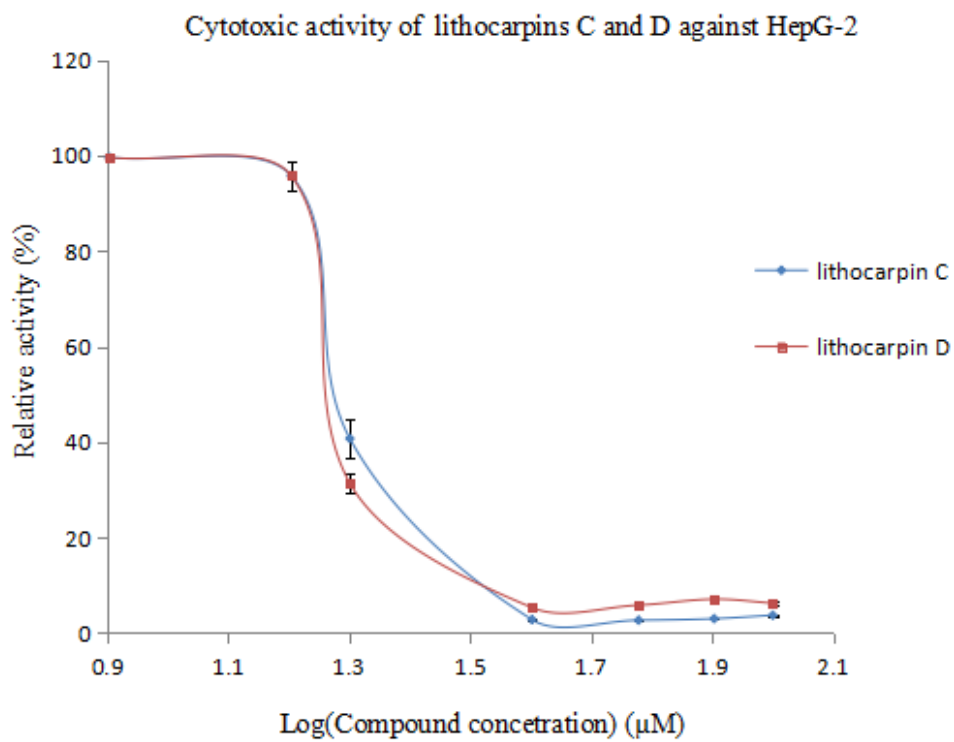


Figure S45. Cytotoxic activity of lithocarpins C and D against HepG-2.