Supporting Information

Neuroprotective constituents from the aerial parts of *Cannabis sativa* L. subsp. *Sativa*

Jia Li[a], Guan Wang[a,b], Yu Qin[a], Xue Zhang[a], Hai-Feng Wang[a], Hong-Wei Liu[c], Ling-Juan Zhu*[,a] and Xin-Sheng Yao*[, a]

[a] College of Traditional Chinese Materia, Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China.
[b] State Key Laboratory of Biotherapy and Cancer Center and Department of Gastrointestinal Surgery, West China Hospital, Sichuan University, and Collaborative Innovation Center for Biotherapy, Chengdu 610041, China.
[c] State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

Contents:

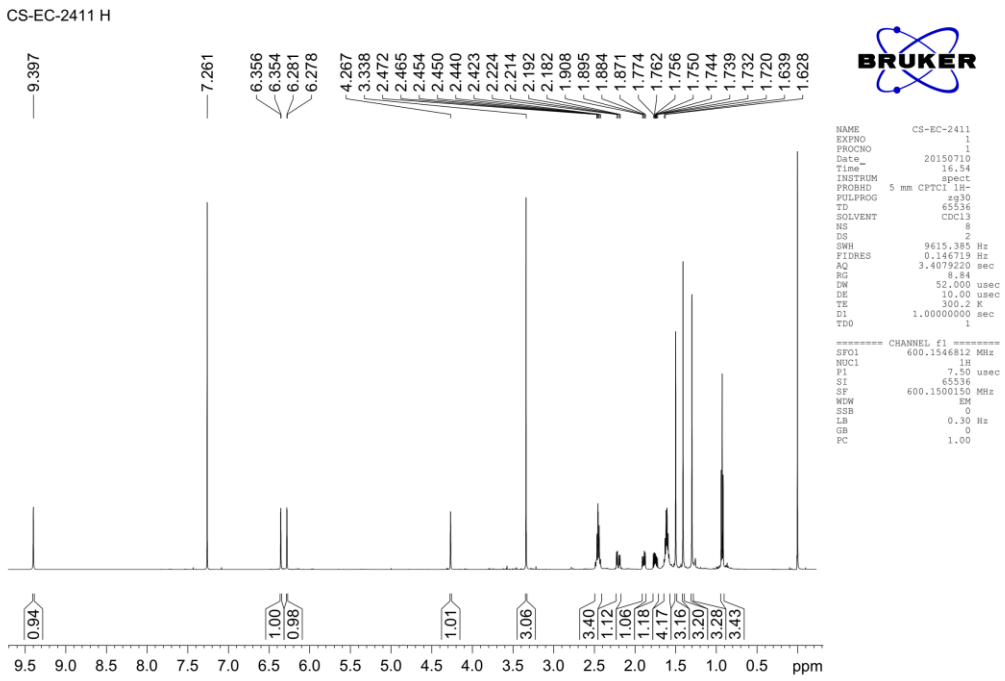


Figure S1. ^{1}H NMR spectrum of cannabisativas A (**1**) in $CDCl_3$.

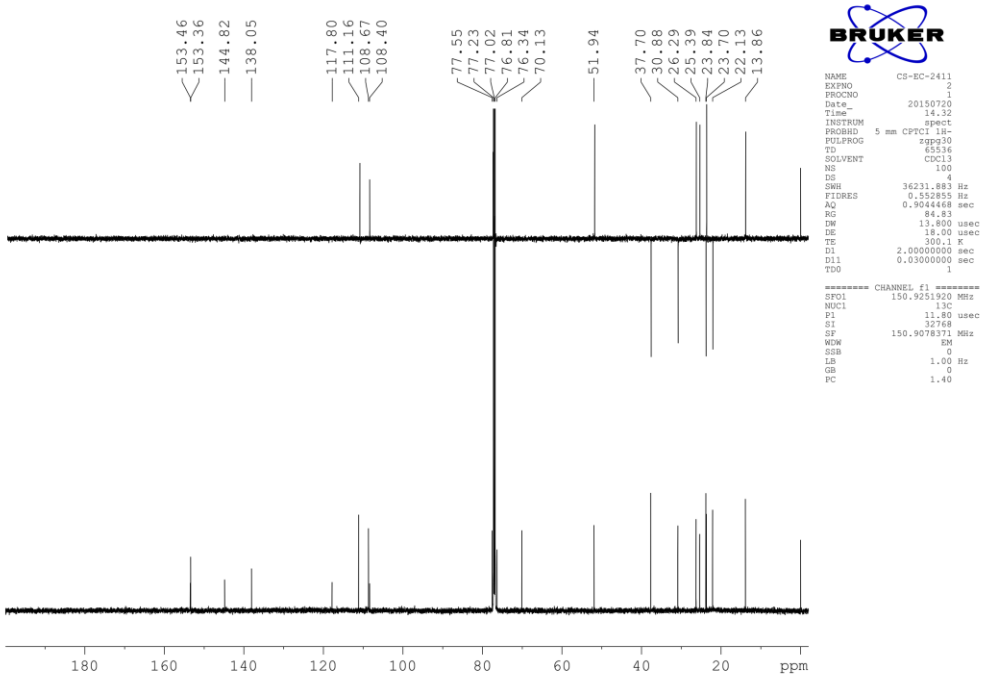


Figure S2. ^{13}C NMR and DEPT 135 spectra of cannabisativas A (**1**) in $CDCl_3$.

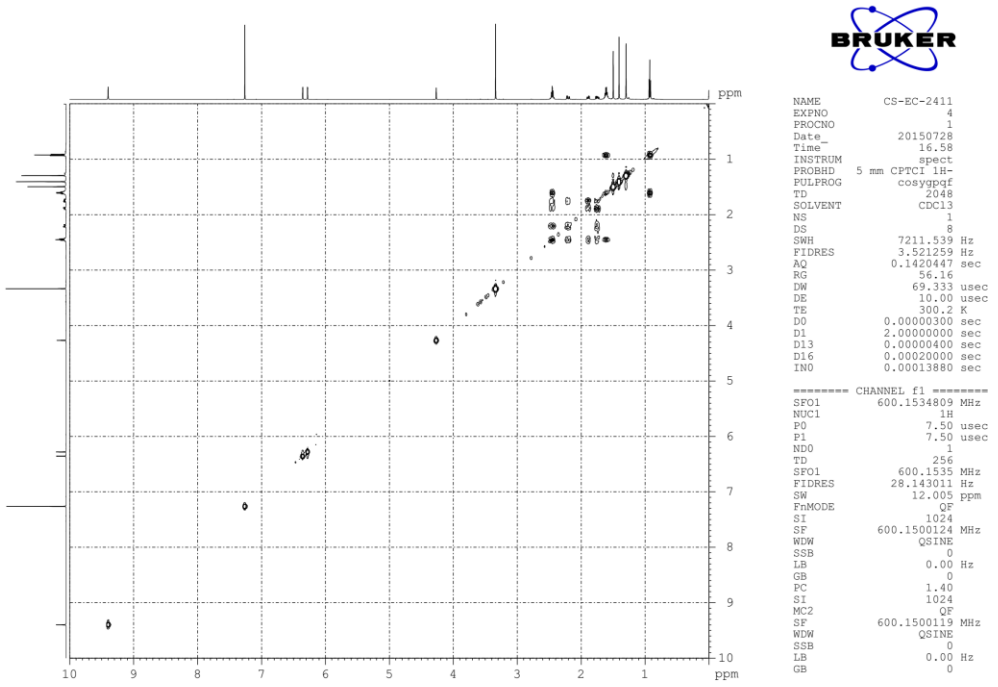


Figure S3. ^{1}H-^{1}H COSY spectrum of cannabisativas A (**1**) in $CDCl_3$.

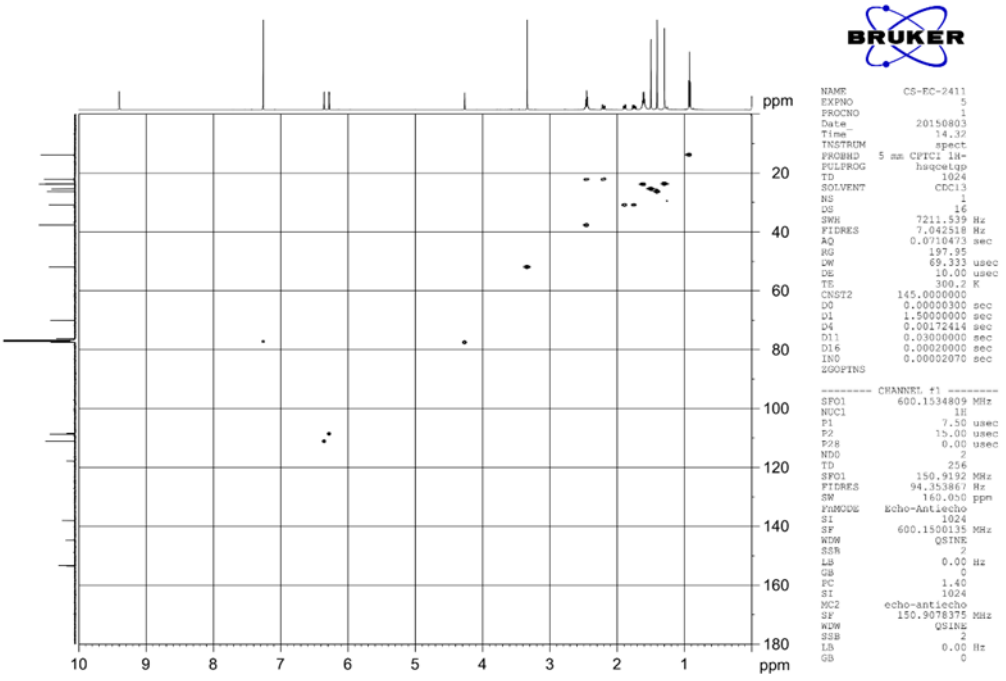


Figure S4. HSQC spectrum of cannabisativas A (**1**) in $CDCl_3$.

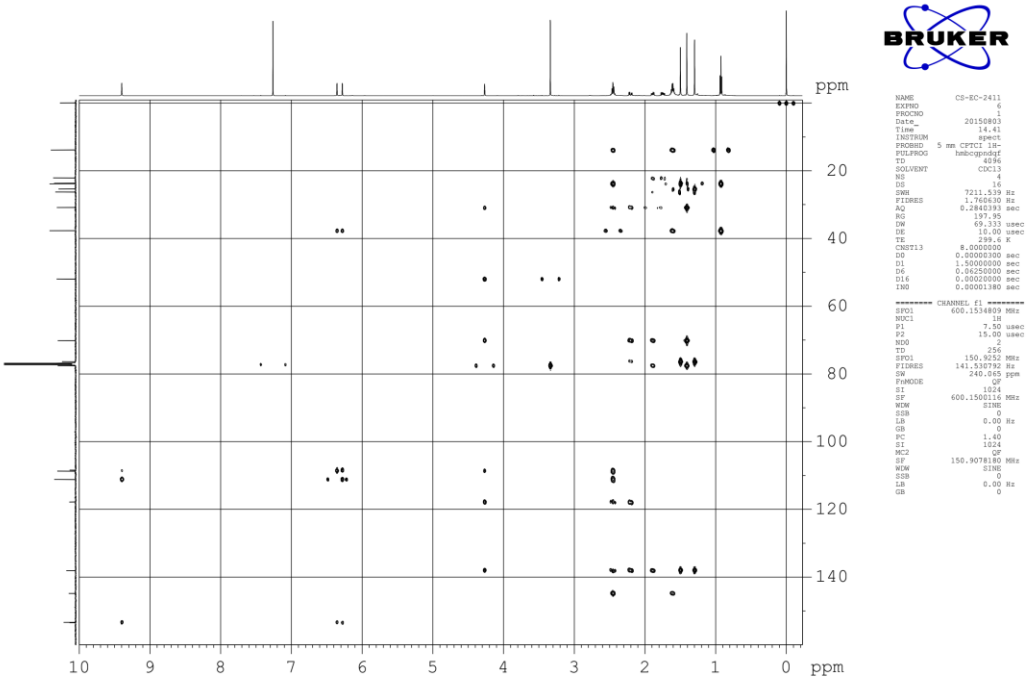


Figure S5. HMBC spectrum of cannabisativas A (**1**) in $CDCl_3$.

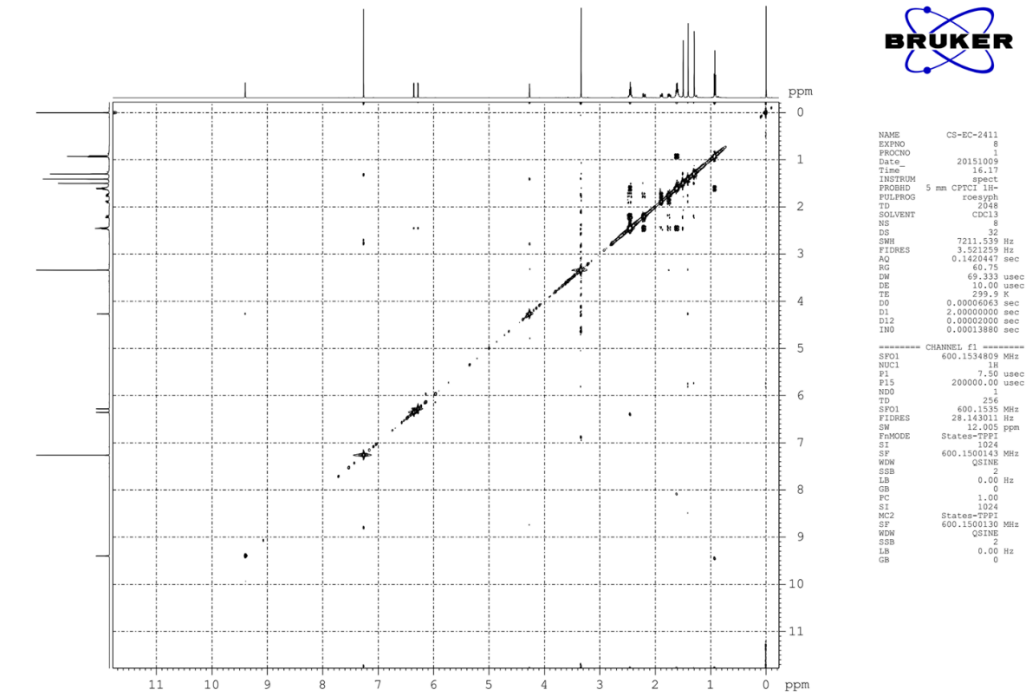
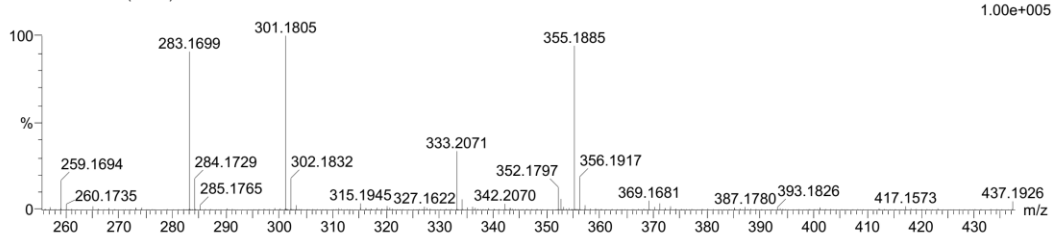


Figure S6. ROESY spectrum of cannabisativas A (**1**) in $CDCl_3$.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
40 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 20-20 H: 0-900 O: 0-80 23Na: 0-1
1
20170410-78 218 (1.765) 1: TOF MS ES+ 1.00e+005



Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
333.2071	333.2066	0.5	1.5	6.5	137.8	n/a	n/a	C20 H29 O4

Figure S7. HRESIMS spectrum of cannabisativas A (**1**).

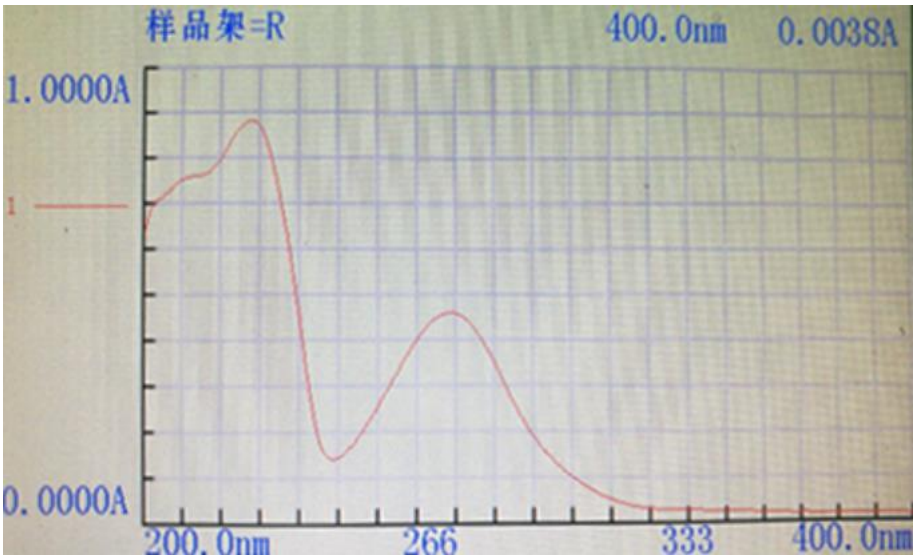


Figure S8. UV spectrum of cannabisativas A (**1**).

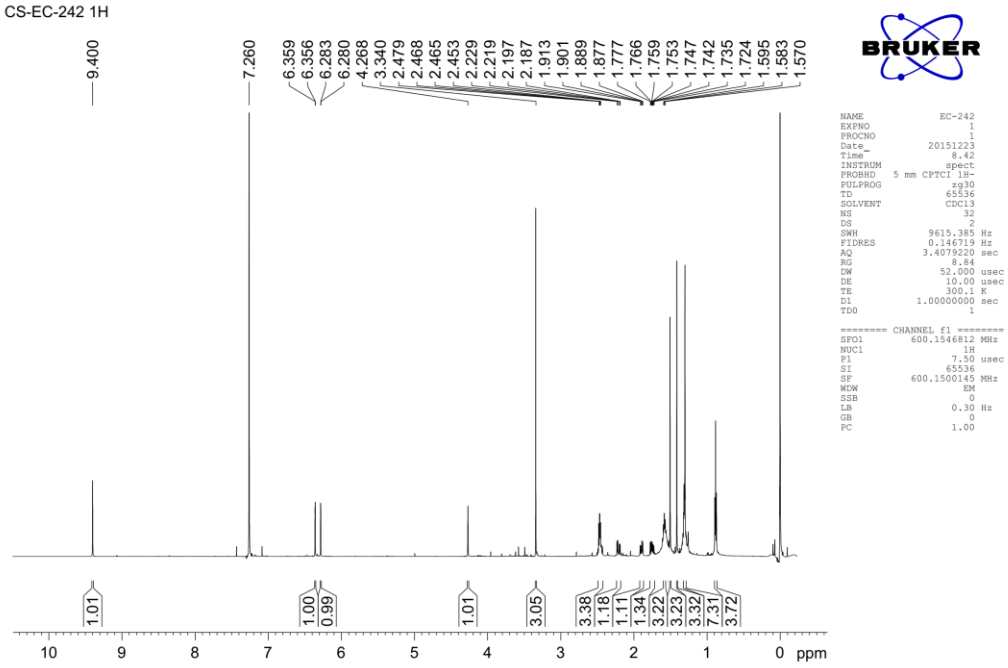


Figure S9. ^{1}H NMR spectrum of cannabisativas B (**2**) in $CDCl_3$.

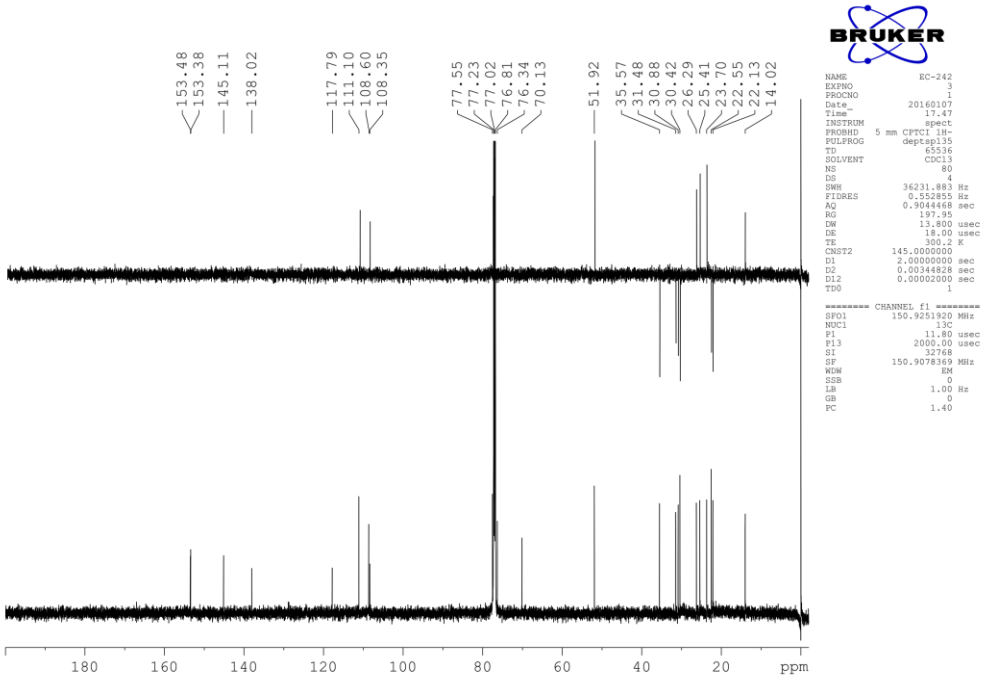


Figure S10. ^{13}C NMR and DEPT 135 spectra of cannabisativas B (**2**) in $CDCl_3$.

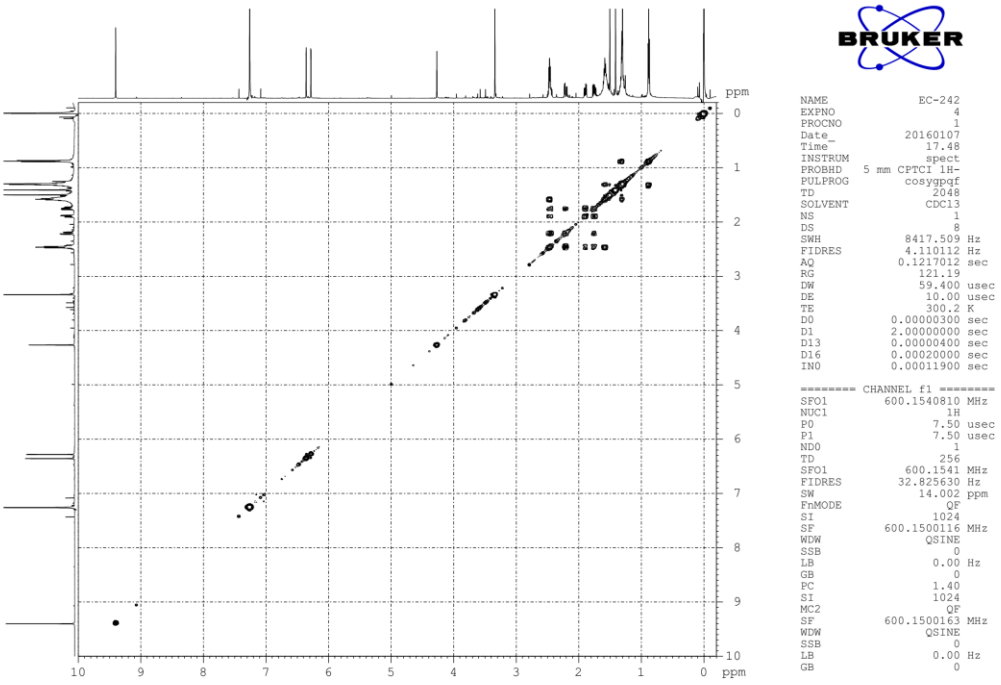


Figure S11. ^{1}H-^{1}H COSY spectrum of cannabisativas B (**2**) in $CDCl_3$.

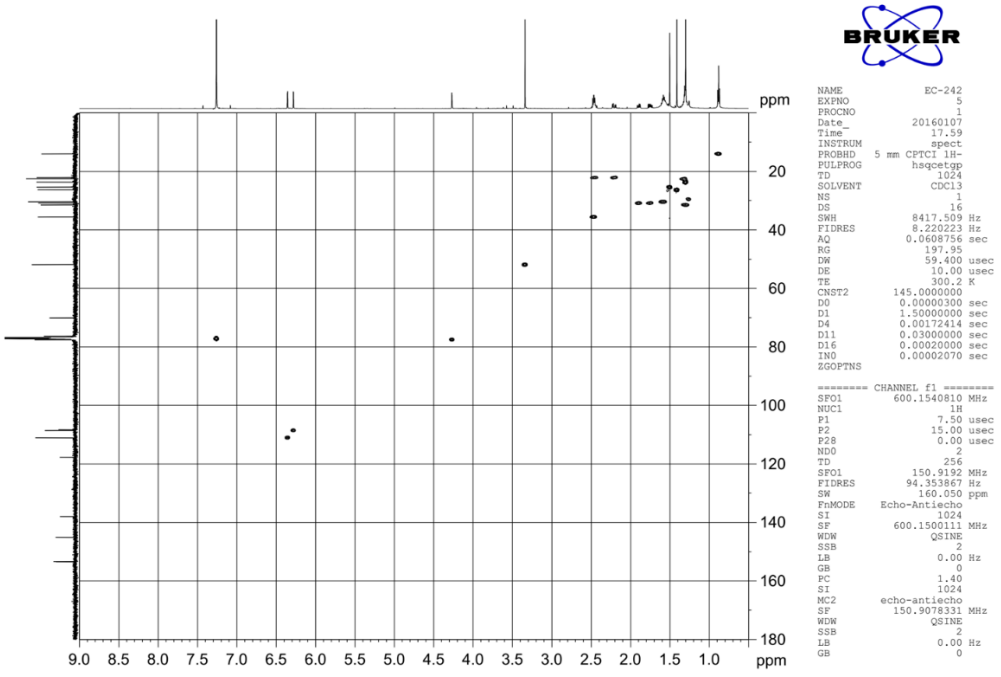


Figure S12. HSQC spectrum of cannabisativas B (**2**) in $CDCl_3$.

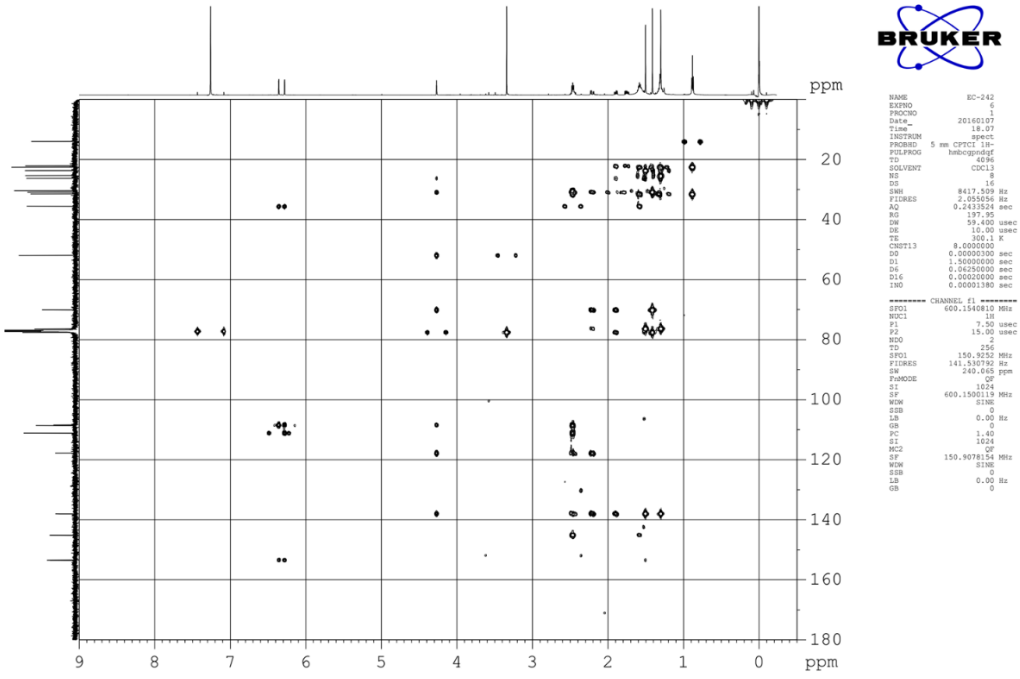


Figure S13. HMBC spectrum of cannabisativas B (**2**) in $CDCl_3$.

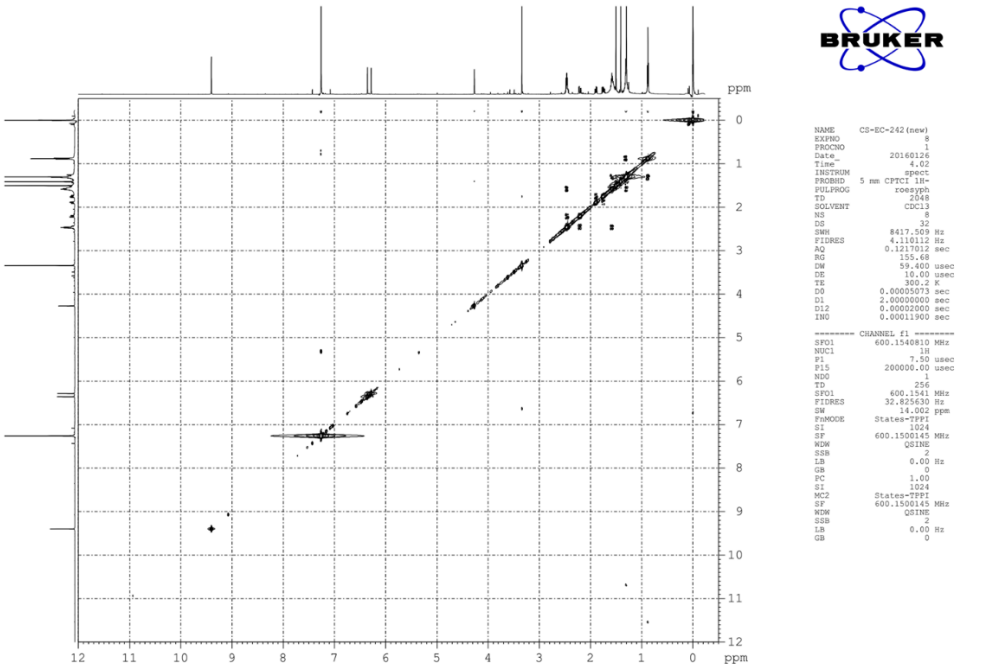
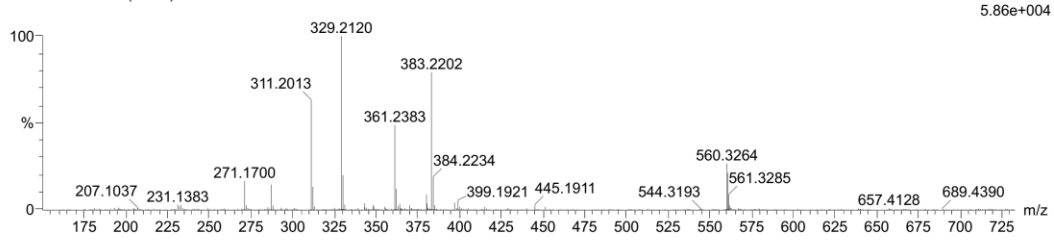


Figure S14. NOESY spectrum of cannabisativas B (**2**) in $CDCl_3$.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
76 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 22-30 H: 0-900 O: 0-80 23Na: 0-1
2
20170410-79 247 (1.993) 1: TOF MS ES+ 5.86e+004



Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
361.2383	361.2379	0.4	1.1	6.5	135.4	n/a	n/a	C22 H33 O4

Figure S15. HRESIMS spectrum of cannabisativas B (**2**).

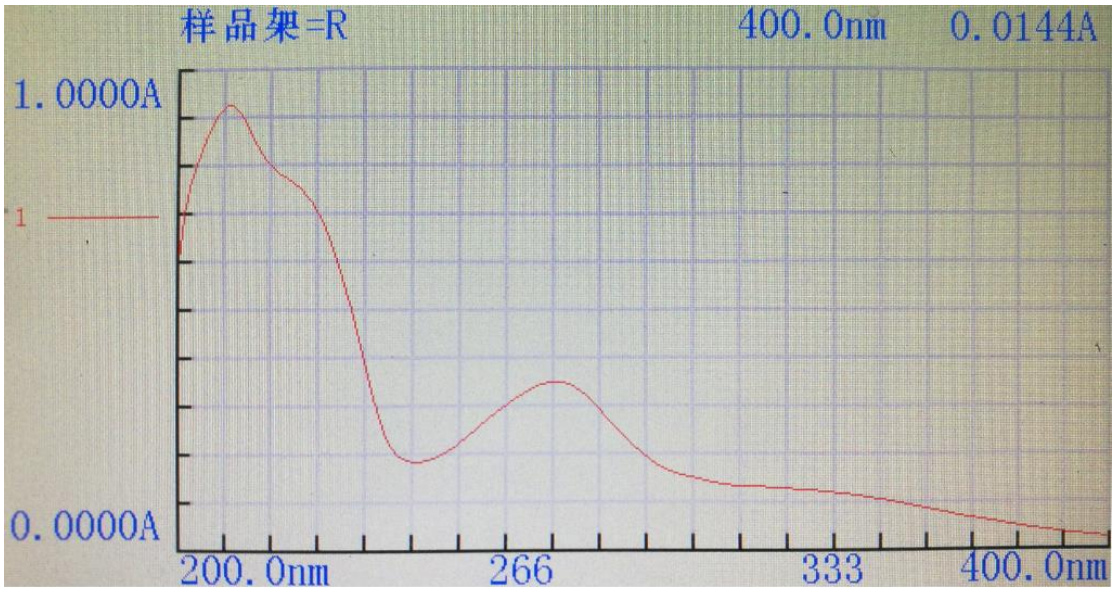


Figure S16. UV spectrum of cannabisativas B (**2**).

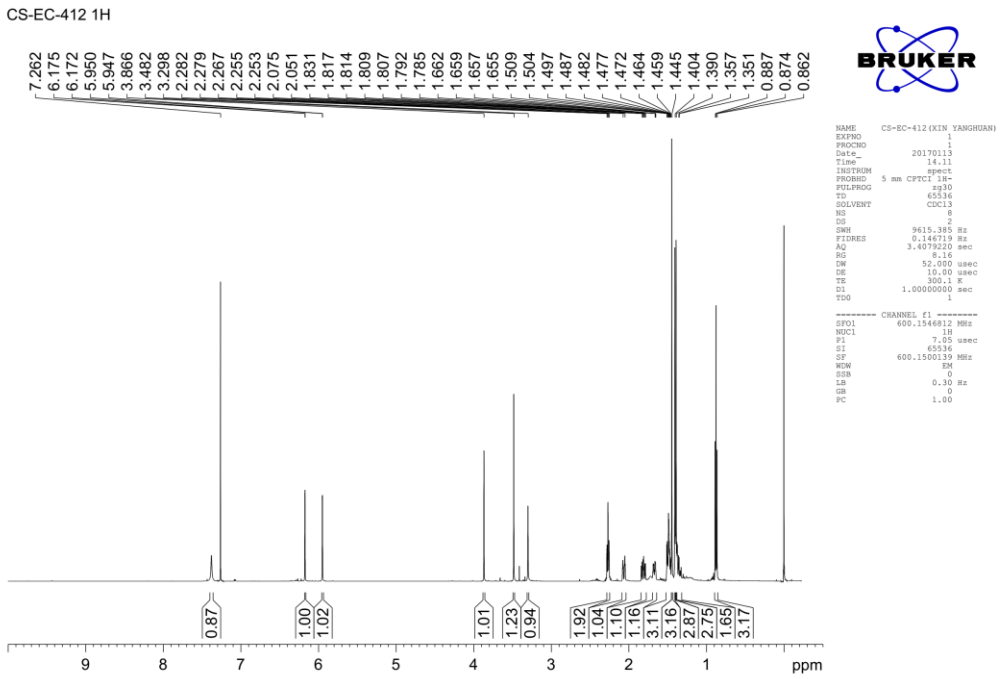


Figure S17. ^{1}H NMR spectrum of cannabisativas C (**3**) in $CDCl_3$.

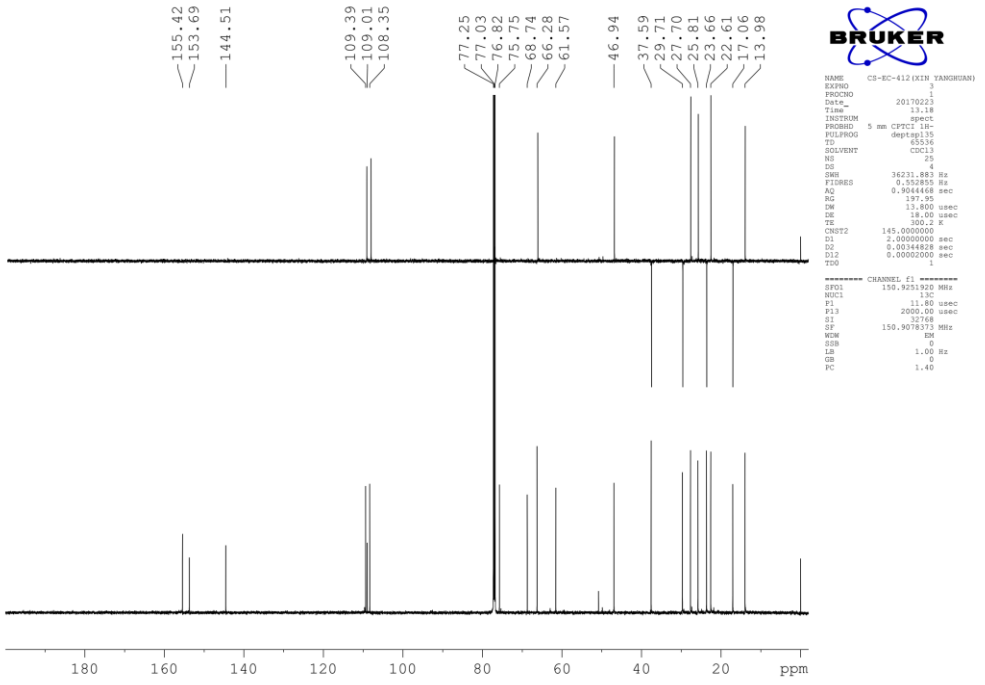


Figure S18. ^{13}C NMR and DEPT 135 spectra of cannabisativas C (**3**) in $CDCl_3$.

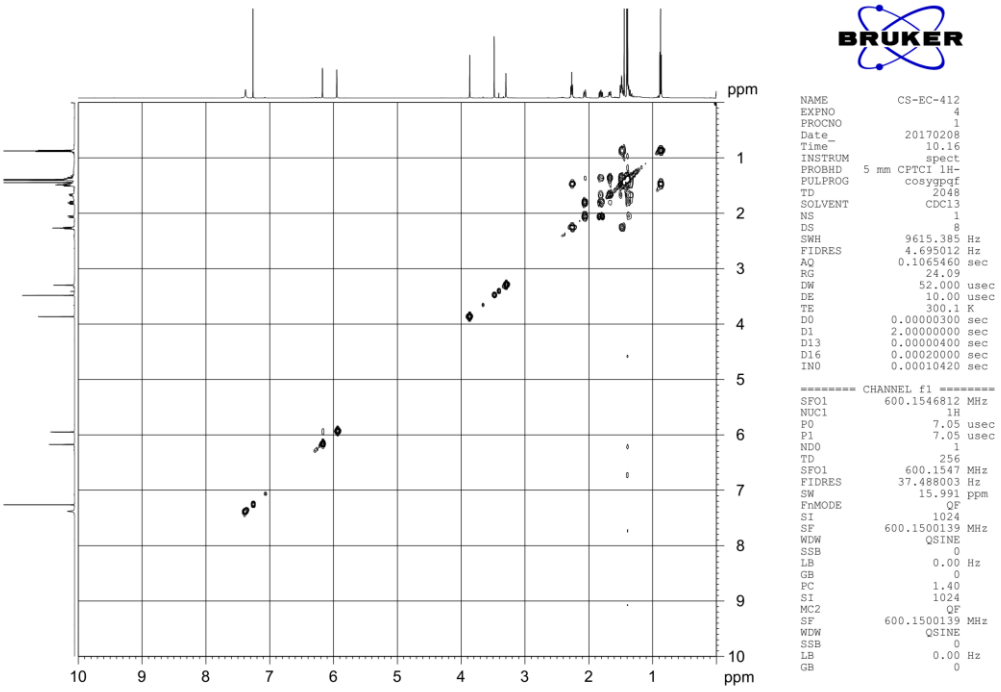


Figure S19. ^{1}H-^{1}H COSY spectrum of cannabisativas C (**3**) in $CDCl_3$.

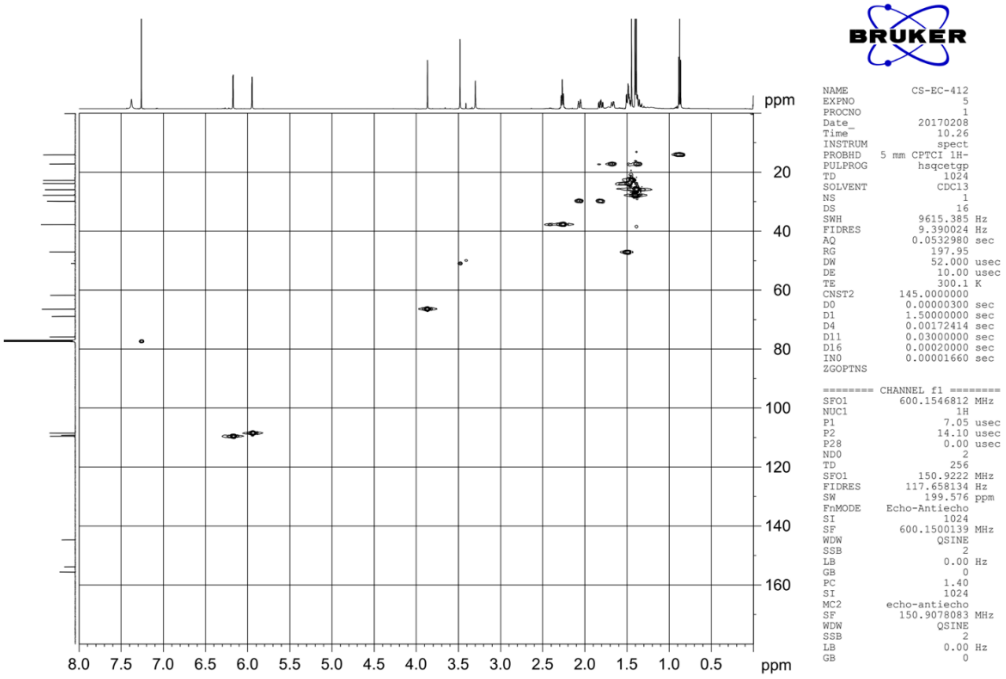


Figure S20. HSQC spectrum of cannabisativas C (**3**) in $CDCl_3$.

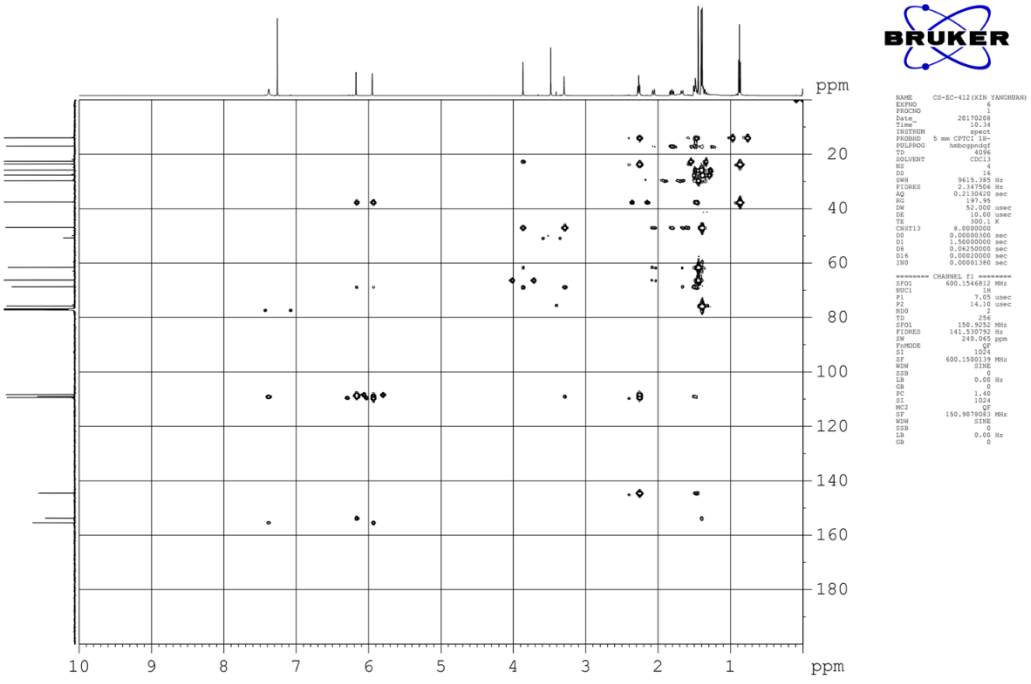


Figure S21. HMBC spectrum of cannabisativas C (**3**) in $CDCl_3$.

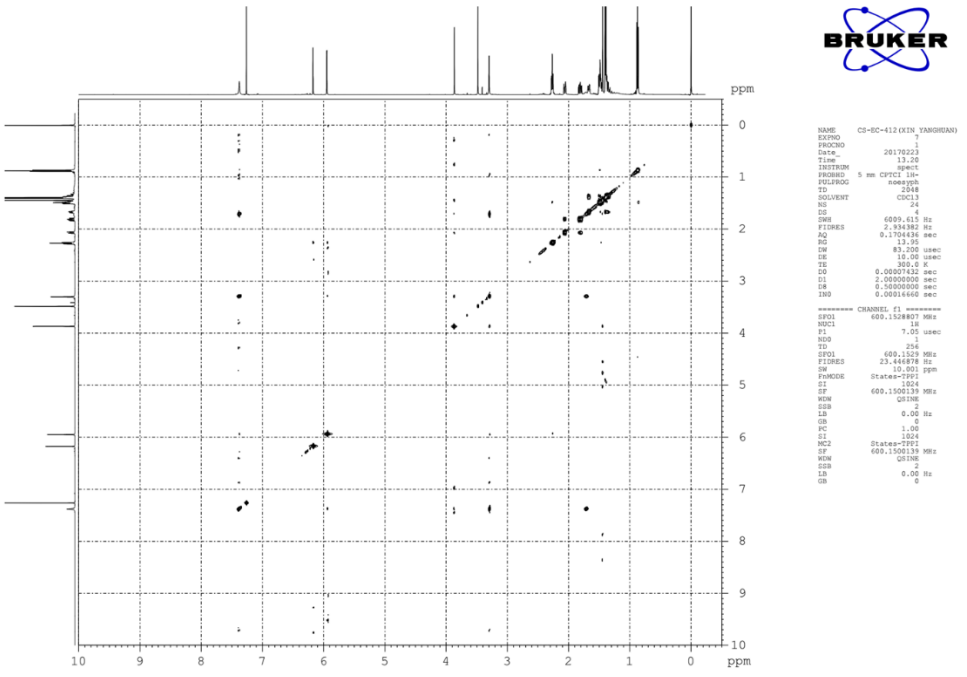


Figure S22. NOESY spectrum of cannabisativas C (**3**) in $CDCl_3$.

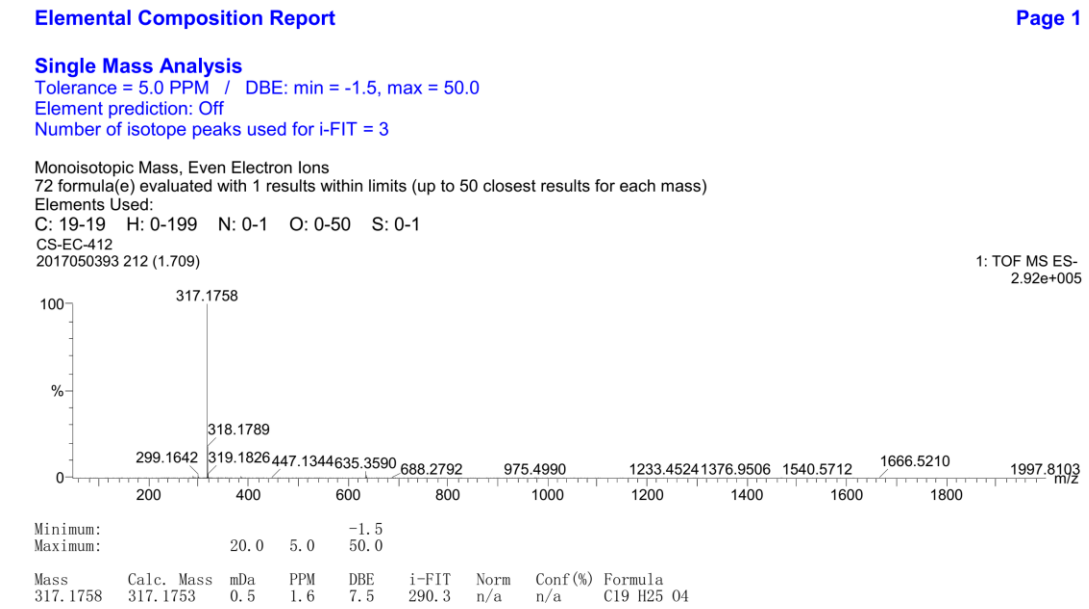


Figure S23. HRESIMS spectrum of cannabisativas C (**3**).

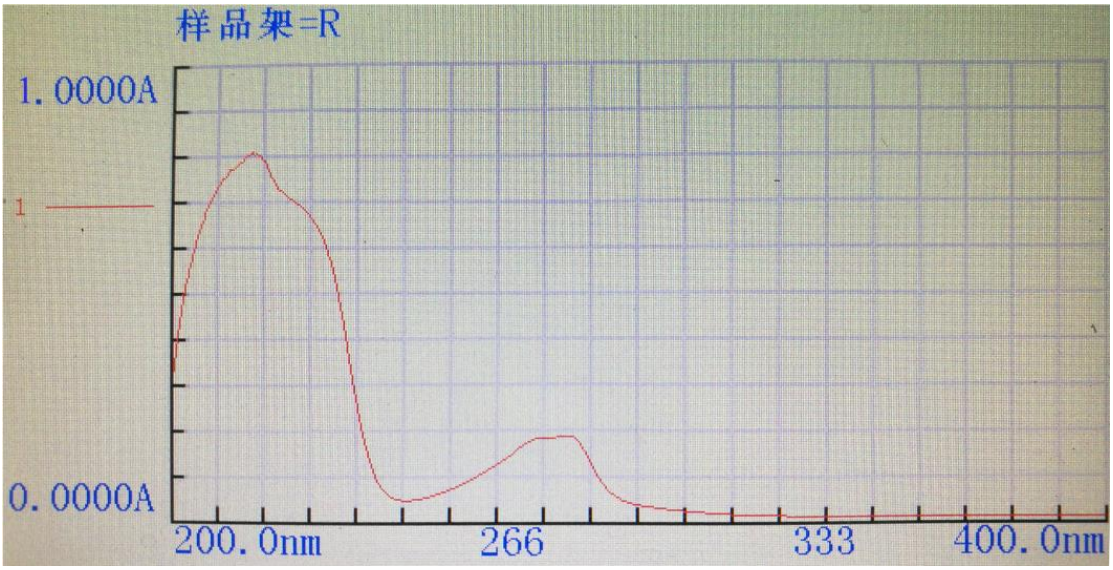


Figure S24. UV spectrum of cannabisativas C (**3**).

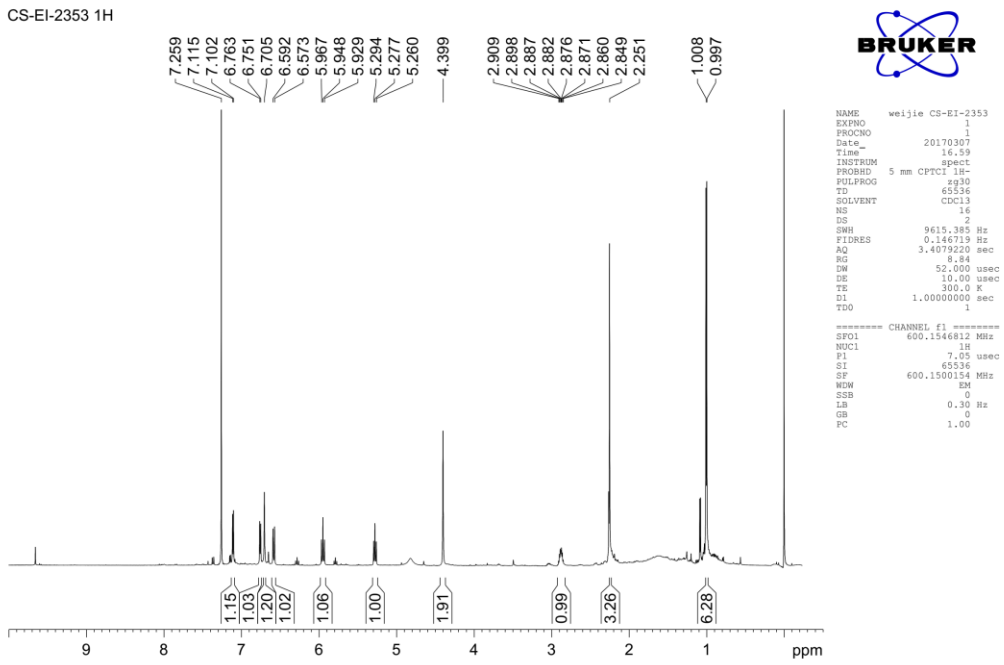


Figure S25. ^{1}H NMR spectrum of (7*Z*, 9*Z*)-cannabiphenolic acid A (**4**) in $CDCl_3$.

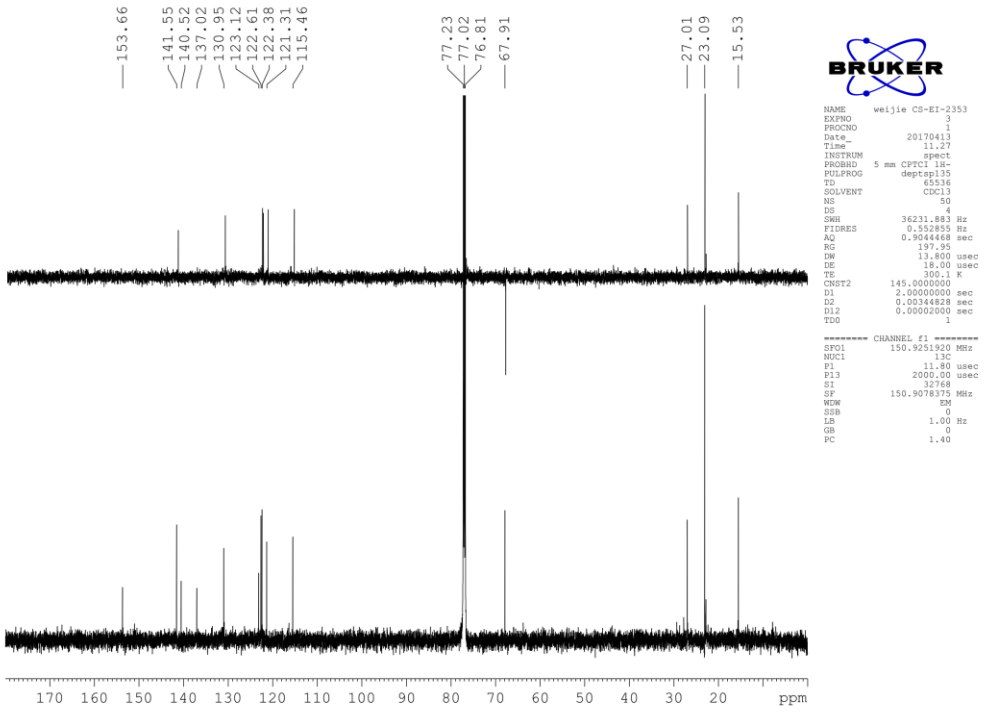


Figure S26. ^{13}C NMR and DEPT 135 spectra of (7*Z*, 9*Z*)-cannabiphenolic acid A (**4**) in $CDCl_3$.

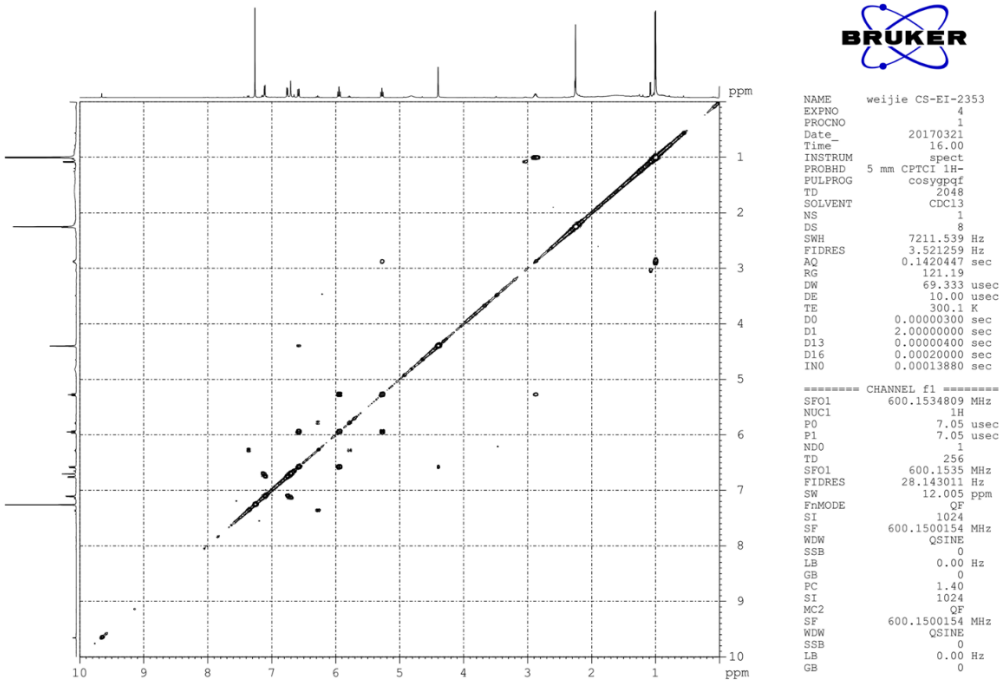


Figure S27. ^{1}H-^{1}H COSY spectrum of (7*Z*, 9*Z*)-cannabiphenolic acid A (**4**) in $CDCl_3$.

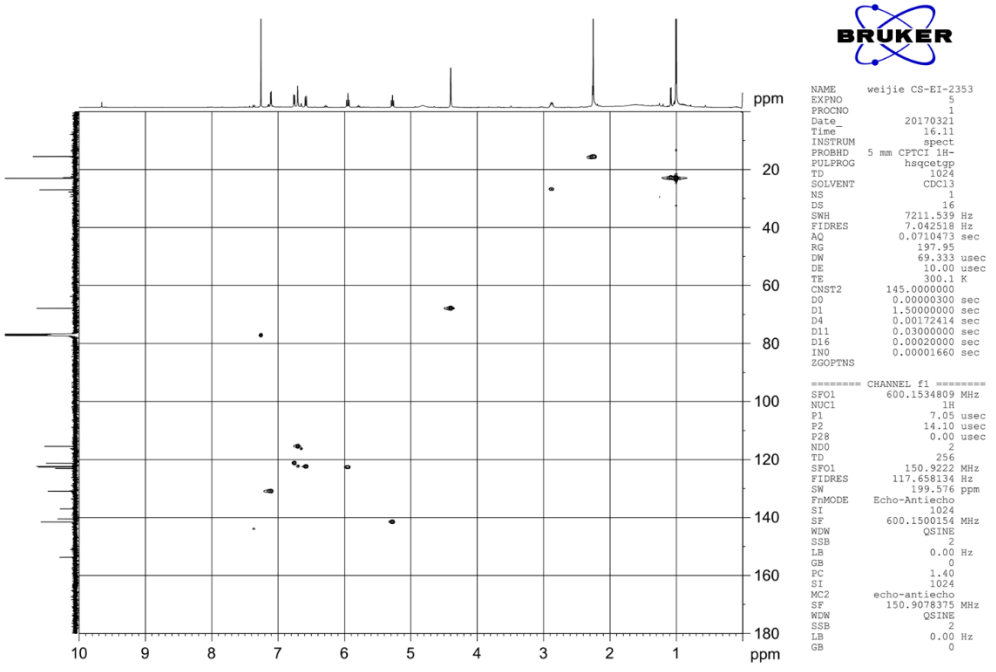


Figure S28. HSQC spectrum of (7*Z*, 9*Z*)-cannabiphenolic acid A (**4**) in $CDCl_3$.

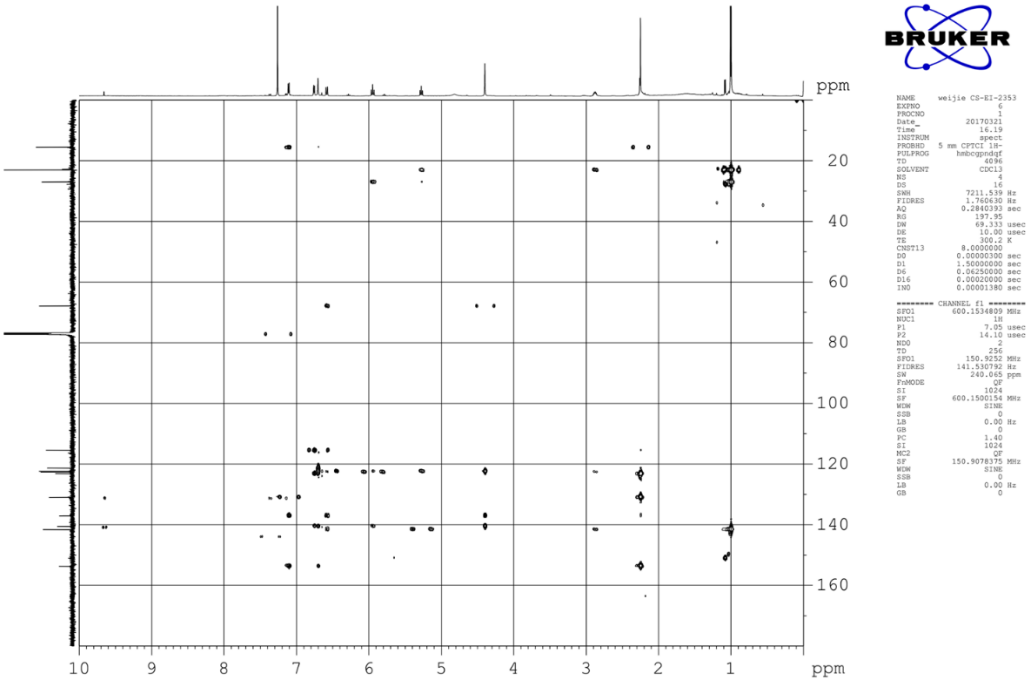


Figure S29. HMBC spectrum of (7*Z*, 9*Z*)-cannabiphenolic acid A (**4**) in $CDCl_3$.

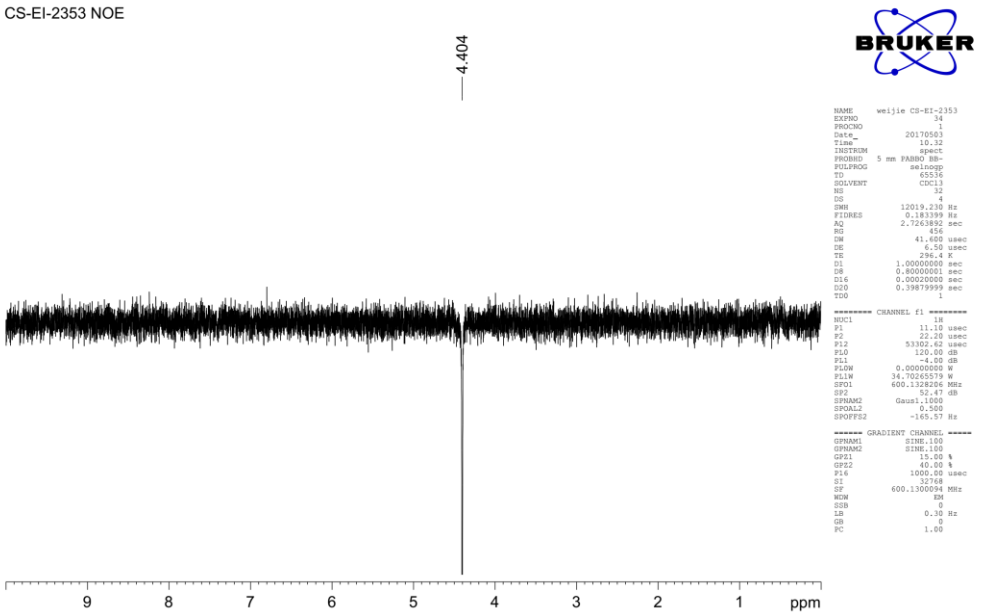


Figure S30. NOE difference spectrum (δ_H 4.40) of (7*Z*, 9*Z*)-cannabiphenolic acid A (**4**) in $CDCl_3$.

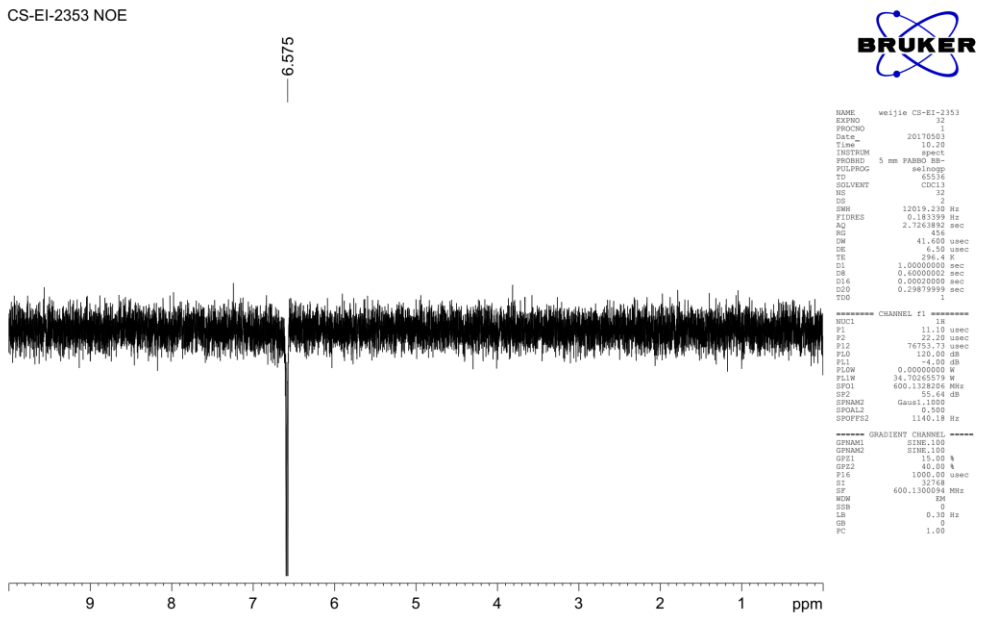


Figure S31. NOE difference spectrum (δ_H 6.58) of (7*Z*, 9*Z*)-cannabiphenolic acid A (**4**) in $CDCl_3$.

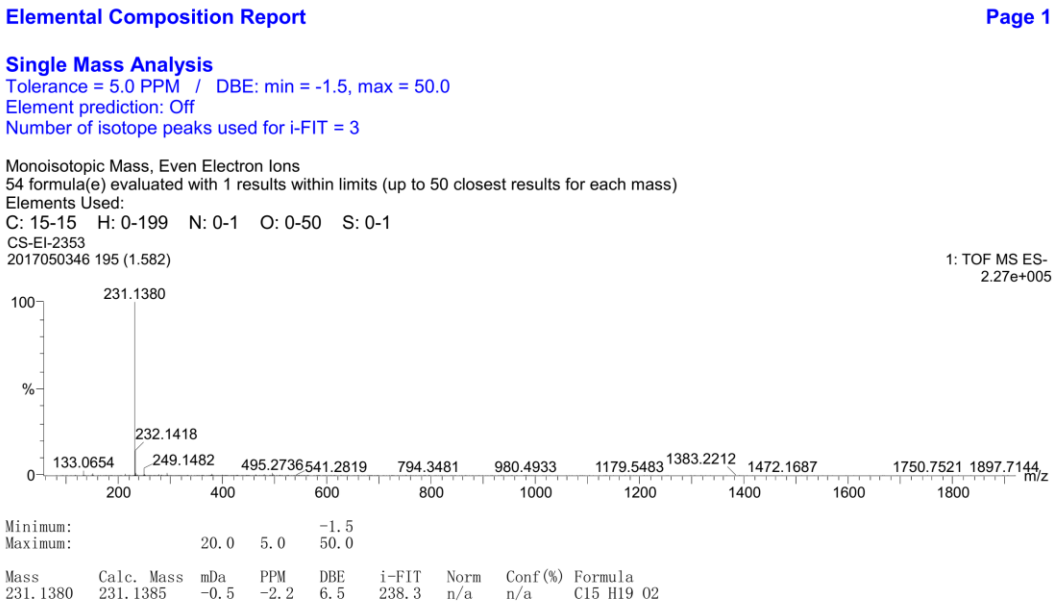


Figure S32. HRESIMS spectrum of (7*Z*, 9*Z*)-cannabiphenolic acid A (**4**).

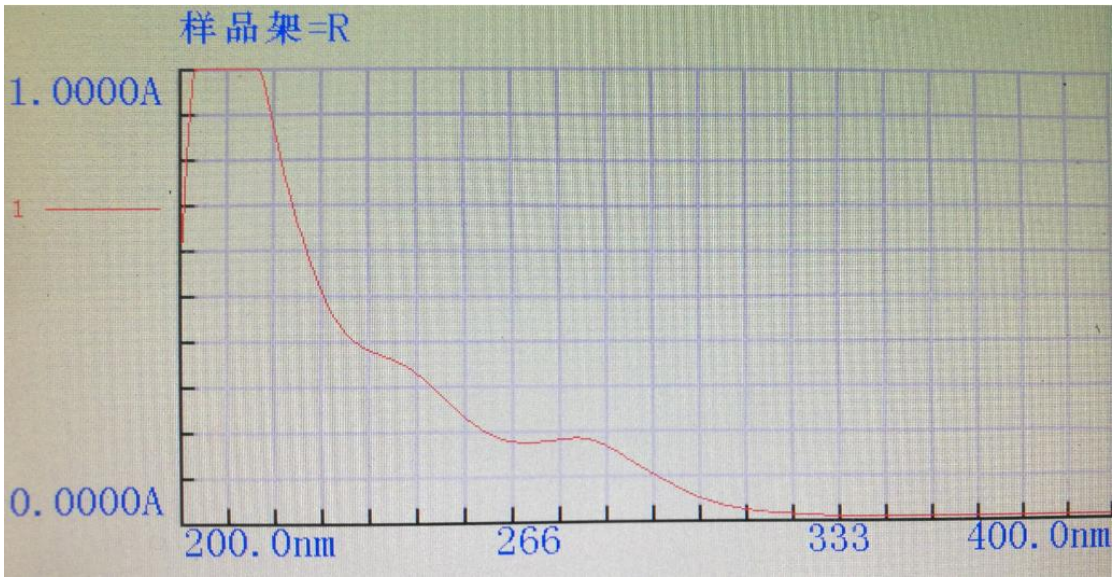


Figure S33. UV spectrum of (7*Z*, 9*Z*)-cannabiphenolic acid A (**4**).

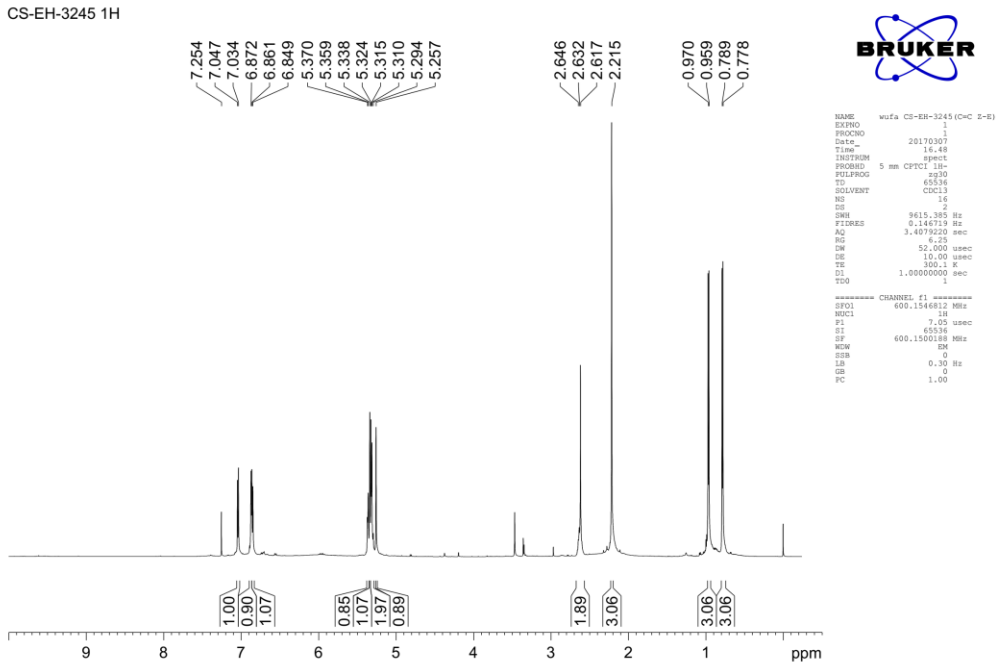


Figure S34. ^{1}H NMR spectrum of (8S, 9Z)-cannabiphenolic acid B (**5**) in $CDCl_3$.

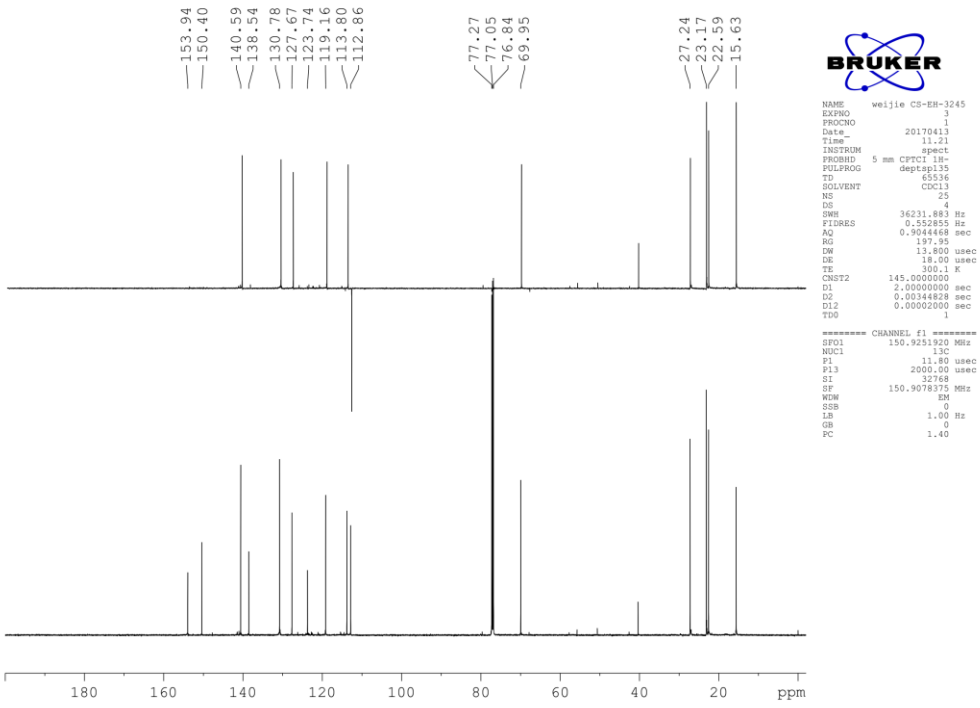


Figure S35. ^{13}C NMR and DEPT 135 spectra of (8*S*, 9*Z*)-cannabiphenolic acid B (**5**) in $CDCl_3$.

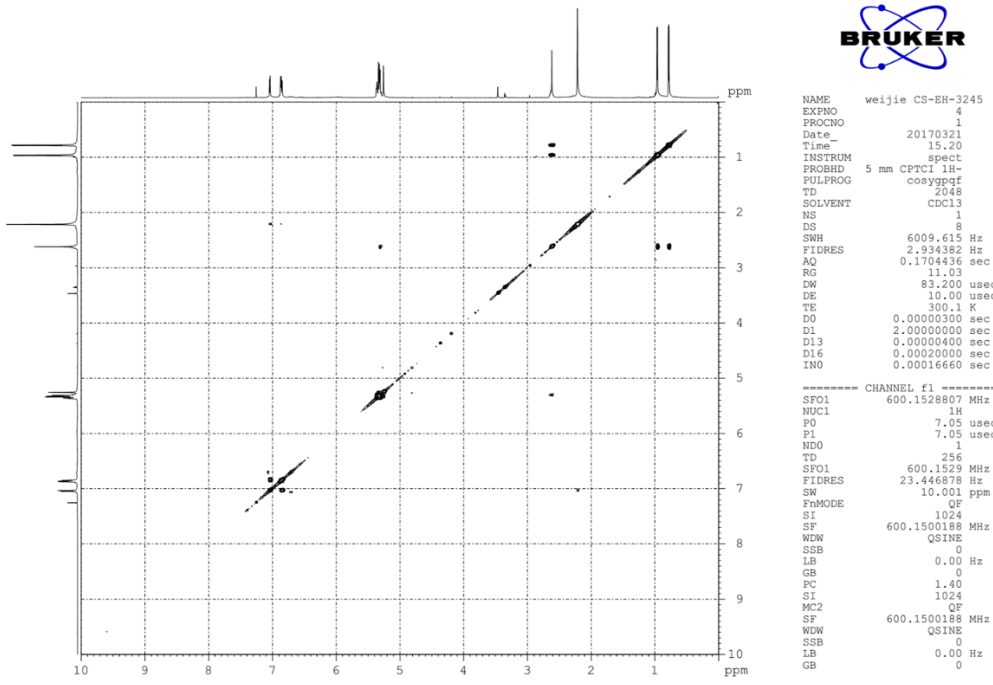


Figure S36. ^{1}H-^{1}H COSY spectrum of (8*S*, 9*Z*)-cannabiphenolic acid B (**5**) in $CDCl_3$.

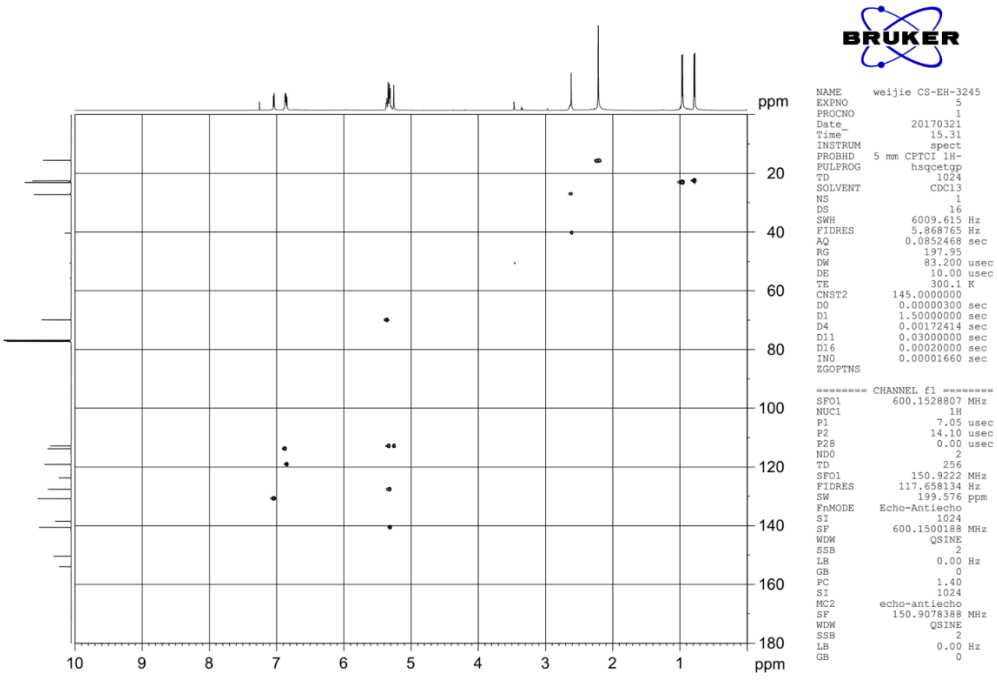


Figure S37. HSQC spectrum of (8*S*, 9*Z*)-cannabiphenolic acid B (**5**) in $CDCl_3$.

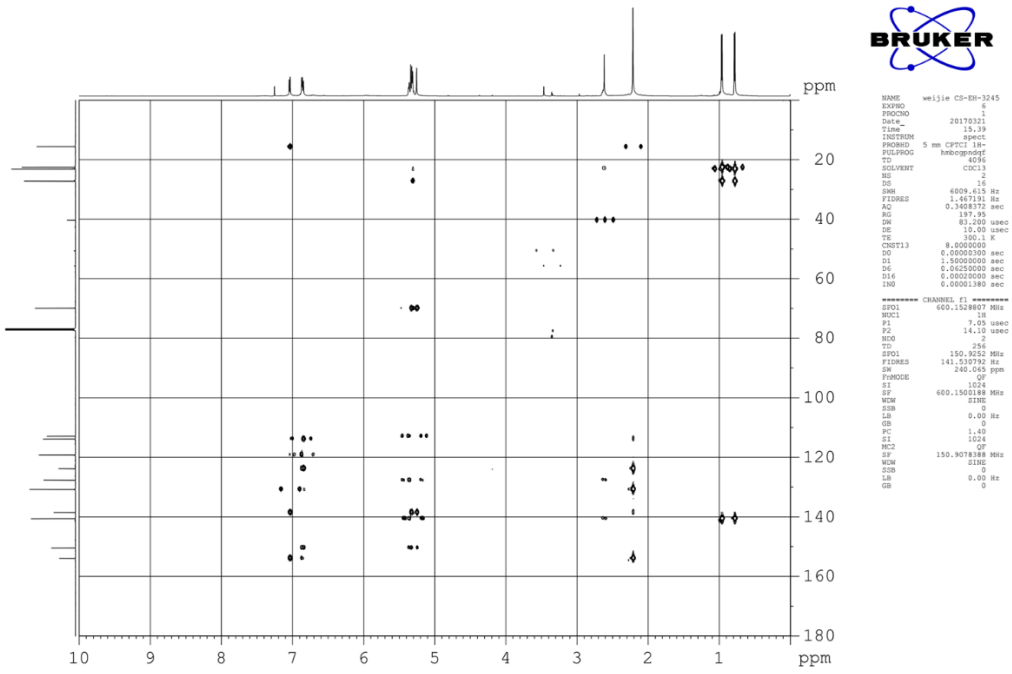


Figure S38. HMBC spectrum of (8*S*, 9*Z*)-cannabiphenolic acid B (**5**) in $CDCl_3$.

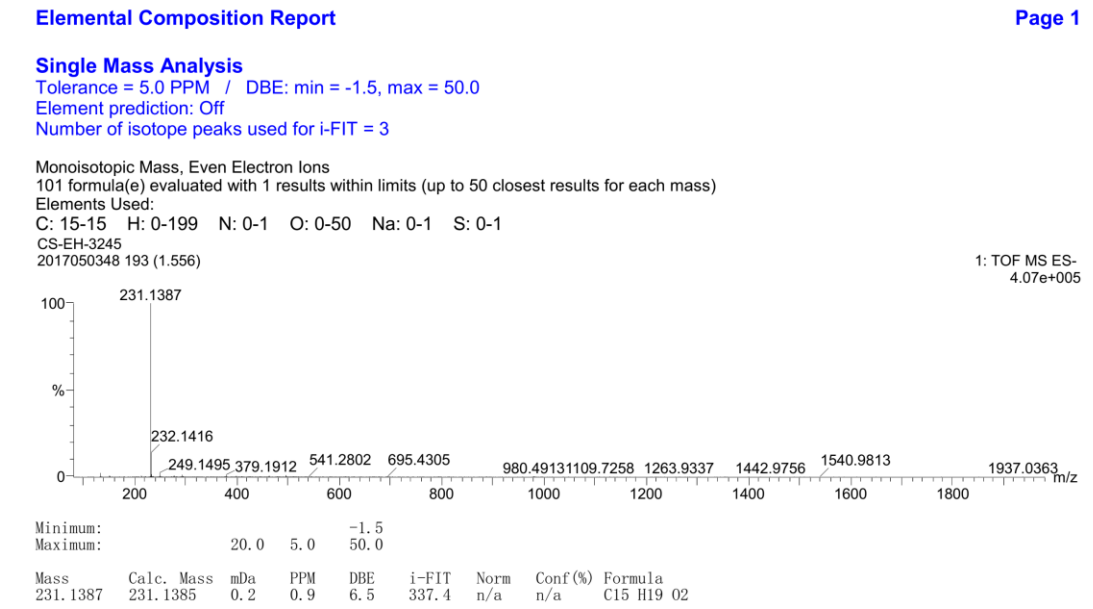


Figure S39. HRESIMS spectrum of (8*S*, 9*Z*)-cannabiphenolic acid B (**5**).

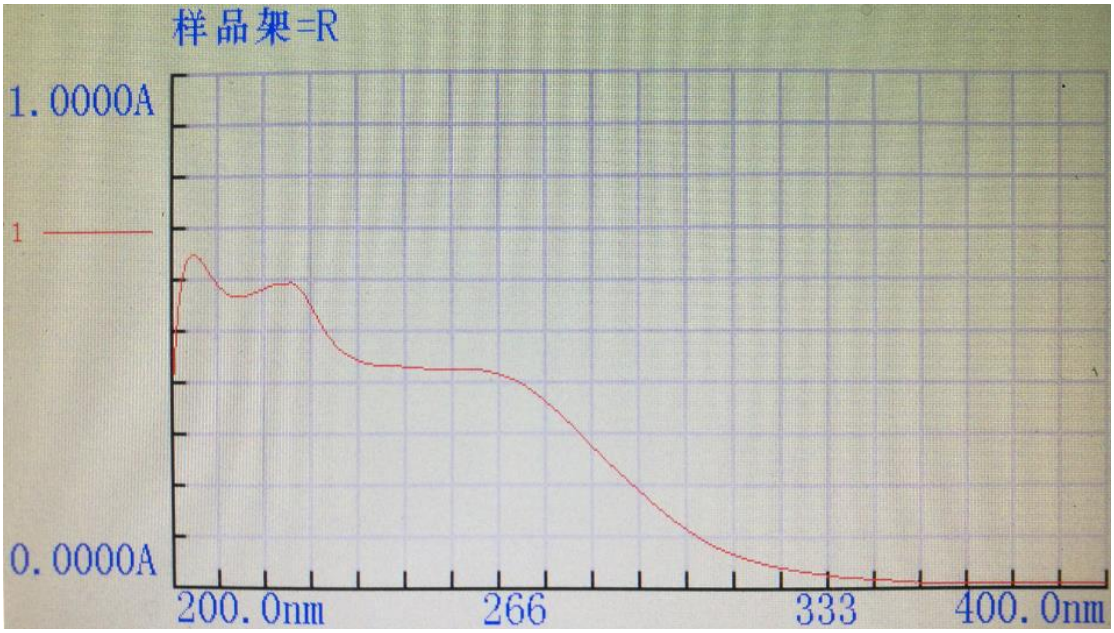


Figure S40. UV spectrum of (8*S*, 9*Z*)-cannabiphenolic acid B (**5**).

Functional	Solvent?	Basis Set	Type of Data
mPW1PW91	PCM	6-311+G(d,p)	Unscaled Shifts

		DP4+	0.00%	100.00%	-	-	-
Nuclei	sp2?	experiments	Isomer 1	Isomer 2	Isomer 3	Isomer 4	Isomer 5
C		15.5344	18.66921	18.64712			
C	x	123.1251	130.8866	131.57709			
C	x	153.6654	164.20831	163.5768			
C	x	115.4674	116.92795	120.40864			
C	x	137.0233	150.20307	146.2386			
C	x	140.5277	149.07616	152.03422			
C		67.9152	64.42602	74.71416			
C	x	122.3789	132.17665	130.53072			
C	x	122.6142	128.25533	130.47206			
C	x	141.5551	153.91382	152.83204			
C		27.0115	34.46574	34.66421			
C		23.0881	25.20402	25.20637			
C		23.0881	24.9941	25.17819			
C	x	121.3131	124.09433	127.20956			
C	x	130.9557	137.42589	137.22484			
H		2.2514	2.42538	2.44689			
H		2.2514	2.41829	2.44163			
H		2.2514	2.10521	2.13054			
H	x	6.705	7.29012	7.03457			
H		4.3997	4.47124	4.65955			
H		4.3997	5.08463	4.46226			
H	x	6.58275	7.16738	6.97964			
H	x	5.9486	6.73015	6.48497			
H	x	5.2775	5.78576	5.64706			
H		2.8773	3.1381	3.08682			
H		0.9977	1.06429	1.04998			
H		0.9977	1.25974	1.23863			
H		0.9977	0.93798	0.91876			
H		1.0087	1.22845	1.21345			
H		1.0087	1.00984	1.00926			
H		1.0087	0.90695	0.88608			
H	x	6.75745	7.293	7.07616			
H	x	7.1087	7.3652	7.38118			

Figure S41. Results of DP4+ analysis of (7*Z*, 9*Z*)-cannabiphenolic acid A (**4**).

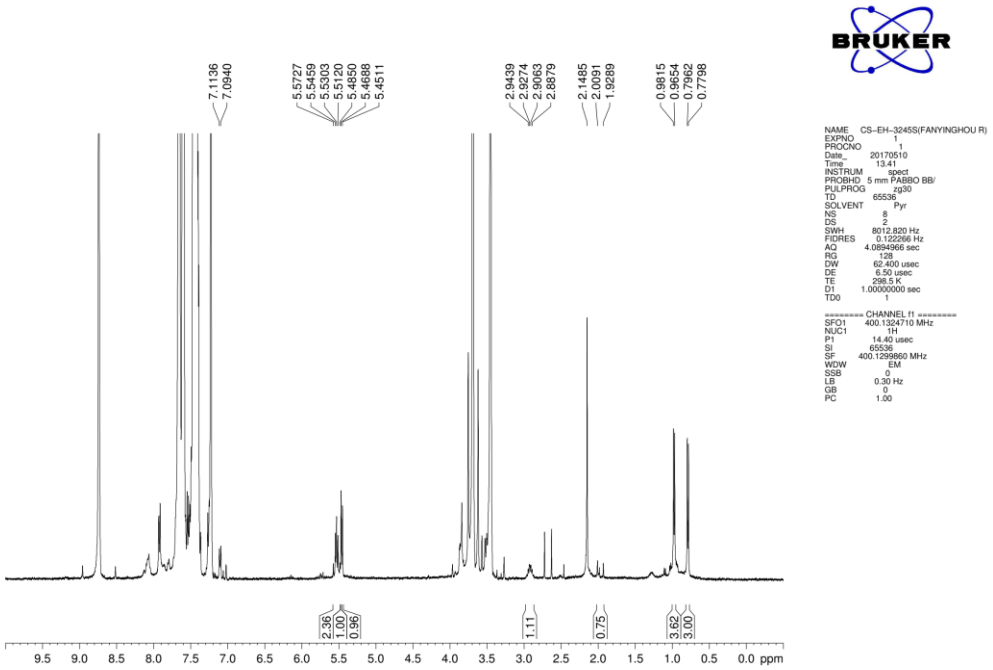


Figure S42. ^{1}H-NMR spectrum of (*R*)-AT Ester of (8*S*, 9*Z*)-cannabiphenolic acid B (**5**) in pyridine-d_5.

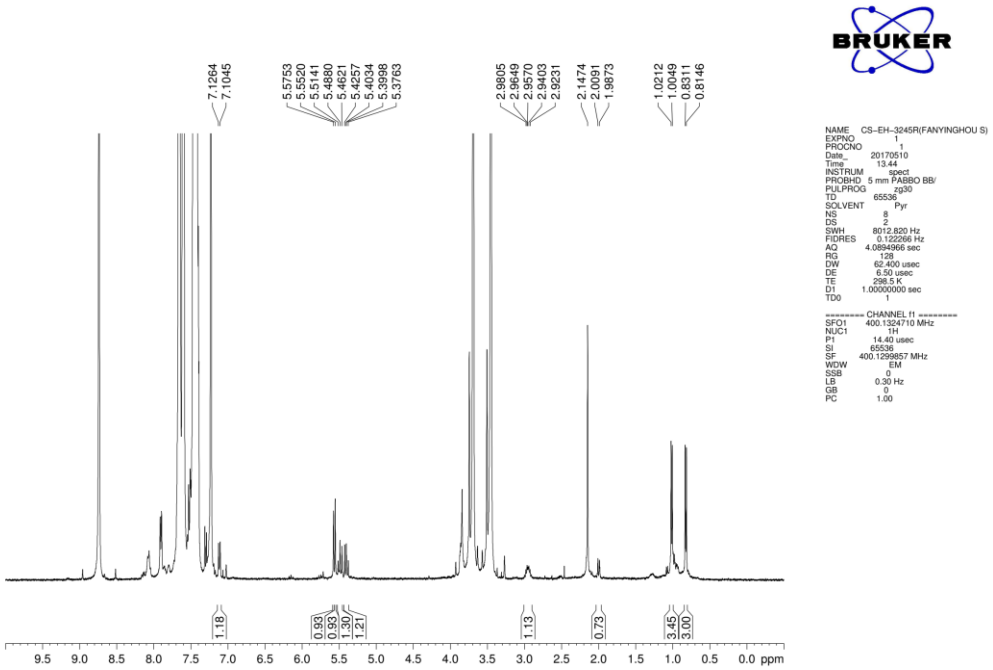


Figure S43. ^{1}H-NMR spectrum of (*S*)-AT Ester of (8*S*, 9*Z*)-cannabiphenolic acid B (**5**) in pyridine-d_5.

Table S1. Effects of compounds **1-5**, **7**, **8** and **12-16** against H_2O_2-induced cytotoxicity in PC 12 cells

Compound	Compound concentration (μM)	Cell viability of compound group (%)[a]	Cell viability of H_2O_2 + compound group (%)[a]	Rescue effect of compound (%)[b]
1	1	94.51 ± 1.26	77.26 ± 0.98	12.61
	10	118.36 ± 0.57	77.96 ± 1.05	13.63
	50	124.53 ± 0.95	72.71 ± 0.08	5.98
2	1	110.66 ± 0.09	74.72 ± 1.09	8.91
	10	107.10 ± 0.37	75.67 ± 0.83	10.29
	50	115.32 ± 0.85	75.09 ± 1.34	9.44
3	1	121.55 ± 0.48	71.99 ± 1.03	4.05
	10	118.46 ± 0.83	84.54 ± 1.30	22.19
	50	97.11 ± 0.28	75.44 ± 1.10	9.03
4	1	99.76 ± 1.31	84.19 ± 1.49	22.71
	10	103.04 ± 0.57	80.90 ± 1.49	17.91
	50	120.25 ± 1.02	66.43 ± 0.05	-3.31
5	1	120.82 ± 0.94	70.36 ± 0.75	1.69
	10	99.06 ± 0.16	68.08 ± 1.05	-1.60
	50	94.24 ± 0.59	54.80 ± 0.41	-20.80
7	0	100.00 ± 0.03	48.87 ± 0.03	
	3.125	96.44 ± 0.04	44.44 ± 0.02	-9.07
	6.25	96.71 ± 0.06	40.96 ± 0.04	-16.20
	12.5	93.87 ± 0.03	34.80 ± 0.01	-28.80
8	0	100.00 ± 0.02	48.93 ± 0.04	
	3.125	98.66 ± 0.02	44.46 ± 0.25	-9.13
	6.25	91.60 ± 0.03	27.95 ± 0.05	-42.87
	12.5	89.17 ± 0.01	42.48 ± 0.49	-13.17
12	0	100.00 ± 0.03	48.87 ± 0.03	
	6.25	98.11 ± 0.02	61.16 ± 0.04	25.15
	12.5	99.62 ± 0.02	63.94 ± 0.04	30.83
	25	101.85 ± 0.04	59.50 ± 0.03	21.74
	50	98.41 ± 0.03	53.78 ± 0.02	10.05
13	0	100.00 ± 0.04	48.87 ± 0.03	
	12.5	102.59 ± 0.07	57.63 ± 0.05	17.92
	25	97.39 ± 0.01	58.71 ± 0.02	20.13
	50	93.30 ± 0.03	58.09 ± 0.02	18.85
	100	92.04 ± 0.01	58.57 ± 0.01	19.83
14	0	100.00 ± 0.04	48.93 ± 0.04	
	12.5	96.54 ± 0.03	57.93 ± 0.03	18.39
	25	95.32 ± 0.03	60.22 ± 0.07	23.07
	50	94.54 ± 0.03	54.50 ± 0.05	11.38
	100	102.00 ± 0.01	51.90 ± 0.04	6.07
15	0	100.00 ± 0.02	48.93 ± 0.04	
	3.125	100.26 ± 0.01	51.32 ± 0.05	4.88
	6.25	92.86 ± 0.02	54.50 ± 0.04	11.39
	12.5	92.09 ± 0.01	57.78 ± 0.04	18.08
16	0	100.00 ± 0.02	51.50 ± 0.06	
	3.125	97.14 ± 0.02	45.45 ± 0.05	-11.74
	6.25	98.09 ± 0.02	48.12 ± 0.05	-6.57
	12.5	93.54 ± 0.02	46.50 ± 0.31	-9.70

[a] The cell viabilities are presented as mean ± SD, which are determined by at least three independent experiments.

[b] Rescue rate = $[(\text{cell viability})_{H2O2 + \text{compound}} - (\text{cell viability})_{H2O2}]/[(\text{cell viability}_{)H2O2}] \times 100\%$