SUPPLEMENTARY MATERIAL

Jatrophacine, a 4,5-*seco*-rhamnofolane diterpenoid with potent anti-inflammatory activity from *Jatropha curcas*

Yang Hu[a,#], Huimin Zhao[a,#], Aiping Yang[a], Qi Lv[a], Ning Ding[a], Tu-lin Lu[a,b], Lihong Hu[a,*] and Xiachang Wang[a,b,*]

[a]*Jiangsu Key Laboratory for Functional Substances of Chinese Medicine, Nanjing University of Chinese Medicine, Nanjing 210023, People's Republic of China*
[b]*Jiangsu Hongdian Research Institute of Traditional Chinese Medicine Industry Co.,LTD, Nanjing 210046, People's Republic of China*

[#]Yang Hu and Huimin Zhao contributed equally to this work.

*Corresponding author. Phone: +86-25-85811293. Fax: +86-25-85811293. E-mail: xiachangwang@njucm.edu.cn, lhhu@njucm.edu.cn.

Abstract:

A new diterpenoid named jatrophacine (**1**), with an unusual 4,5-*seco*-rhamnofolane skeleton, was isolated from the roots of *Jatropha curcas*, together with eleven known diterpenoids. The structure of the new compound was elucidated through a detailed analysis of its 1D- and 2D-NMR spectra. The X-ray structure of jatrophol (**2**) is also presented. Anti-inflammatory activity with LPS-induced RAW 264.7 macrophages revealed that compound **1** strongly inhibited the production of nitric oxide (IC_{50} = 0.53 μM).

Keywords: *Jatropha curcas*; Euphorbiaceae; Rhamnofolane diterpenoid; Jatrophacine; Anti-inflammatory

EXPERIMENTAL

General experimental procedure

Optical rotations were measured on a Perkin-Elmer 341 polarimeter (Waltham, MA, USA). IR Spectra were recorded on a Nicolet FTIR 750 spectrophotometer (in cm^{-1}) (Thermo Scientific, West Palm Beach, FL, USA). All NMR spectra were determined on Bruker AMX-300 and AV-500 NMR spectrometers (Kontich, Belgium). HRESIMS was measured on a Thermo Q-Extractive Plus mass spectrometer (West Palm Beach, FL, USA). ESIMS was measured on an Agilent 1290-6120 Quadrupole MSD mass spectrometer (Santa Clara, CA, USA). Single-crystal X-ray diffraction was measured on a Bruker APEX SMART-CCD diffractometer (Kontich, Belgium). Microplate reader was from (Epoch, Bio-Tek, USA). HPLC analyses were performed on a Waters e2690 system equipped with a 2998 photodiode array (PDA) detector (Milford, MA, USA) and a Phenomenex C18 column (250 × 4.6 mm, 5 μm; Torrance, CA). Semi-preparative HPLC separation was performed on a Waters 1525e preparative system equipped with 2998 PDA detector using a Waters Sunfire C18 column (250 × 19 mm, 10 μm, Milford, MA, USA). Column chromatography (CC): silica gel GF_{254} (200–300 mesh) was purchased from Qingdao Marine Chemical Ltd. (Qingdao, Shandong, China); MCI gel CHP 20P (75–150 μm) was purchased from Mitsubishi Chemical Ind. (Tokyo, Japan); RP-18 (20-45 μm) was purchased from Fuji Silysia Chemical Ltd. (Kasugai Aichi, Japan). Silica gel GF_{254} for TLC was purchased from Yantai Huiyou Inc. (Yantai, Shandong, China). McCoy's 5A was purchased from SIGMA (Beijing, China). Newborn Calf Serum was purchased from Hanzhou Sijiqing Biological Engineering Material Co., Ltd. (Hangzhou, China). MTT and DMSO were purchased from Amresco (Beijing, China). RAW 264.7 cells were obtained from Cell Bank of Chinese Academic of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium was from Gibco (NY, USA). L-glutamine and 1 mM nonessential amino acids were from Biosharp (Hefei, Anhui, China). Griess reagent was from Beyotime (Shanghai, China). All solvents used were of Anal. grade (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China).

Plant Material

The roots of *J. curcas* were collected from Panzhihua City, Sichuan Province, China, in

June 2017 and identified by Xian-Feng He (Panzhihua Institute of Forest Research, Sichuan, P. R. China). A voucher sample (20170606) was deposited at the Jiangsu Key Laboratory for Functional Substances of Chinese Medicine, Nanjing, China.

Extraction and Isolation

The dried and pulverized roots of *J. curcas* (5.0 kg) were extracted with 95% ethanol at room temperature for three times. The solvent was subsequently evaporated *in vacuo* and combined to afford 298 g of brown crude extract, which was suspended in H_2O and then partitioned with petroleum ether (PE) and ethyl acetate (EA) successively. The PE extract (86 g) was subjected to a silica gel column chromatography eluted with PE-EtOAc (50:1 to 1:5) to give six fractions P1−P6. Fraction P2 (4.4 g) was chromatographed over a silica gel column eluted with PE-EtOAc (20:1) to yield **6** (1.5 g) and **7** (945 mg). Fraction P4 (4.8 g) was chromatographed over a silica gel column eluted with PE-EtOAc (9:1) to yield **4** (25 mg) and **5** (13 mg). Fraction P5 (8.2 g) was chromatographed over a silica gel column eluted with PE-EtOAc (10:1-1:4) to yield **8** (146 mg) and **9** (42 mg). Fraction P6 (5.6 g) was firstly chromatographed over a silica gel column eluted with $CHCl_3$-Acetone (30:1-1:2) to get five sub-fractions, P6A-P6E. Fraction P6E (1.1 g) was then purified by a preparative HPLC eluted with 60% MeOH to yield **10** (95 mg). The EA extract (60 g) was subjected to a MCI gel column chromatography eluted with MeOH−H_2O (20:80-100:0) to give five fractions E1−E5. Fraction E2 (9.0 g) was firstly chromatographed over a silica gel eluted with $CHCl_3$-MeOH (25:1-1:1) to get six sub-fractions, E2A-E2F. Fraction E2B (1.2 g) was then purified by a preparative HPLC eluted with 25% MeCN to yield **2** (18 mg) and **3** (17 mg). Fraction E4 (11.8 g) was firstly chromatographed over a silica gel eluted with $CHCl_3$-MeOH (20:1-1:1) to give four fractions E4A−E4D. Fraction E4A (3.9 g) was then purified by a silica gel column eluted with $CHCl_3$-Acetone (25:1-1:2) to yield **11** (31 mg) and **12** (35 mg). Fraction E5 (9.2 g) was chromatographed over a silica gel column and then an RP-18 column to yield **1** (35 mg).

Jatrophacine (**1**): $C_{20}H_{26}O_4$, colorless powder; $[\alpha]_D^{20}$ + 51.7 (*c* 0.3, MeOH); IR (KBr) ν_{max} 3423, 2929, 2856, 1697, 1579, 1380, 1296, 887 cm^{-1}; ^1H and ^{13}C NMR see Table S1; ESI-MS *m/z* 331.2 $[M + H]^+$, 329.4 $[M - H]^-$; HREIMS *m/z* 331.1903 $[M + H]^+$ (calcd for $C_{20}H_{27}O_4$, 331.1909), 329.1756 $[M - H]^-$ (calcd for $C_{20}H_{25}O_4$, 329.1753).

Methylation of 1. To a stirred solution of compound **1** (0.015 mmol, 5 mg) in approximately MeOH (200 uL), 0.08 mmol $TMSCHN_2$ (40 uL) was added dropwise. Two further amounts of $TMSCHN_2$ (40 uL/80 uL) were added dropwise respectively at 6 hr and 9 hr after LC-MS trace analysis (Figure S24). The dimethylation products were yielded and concentrated to give **1a** and **1b** as mixture (one peak in HPLC, 5 mg) after 36 h reaction.

Crystallographic data of 2. colorless prism crystal (MeOH), $C_{20}H_{26}O_4$, MW = 312.39, monoclinic, space group P2(1); a = 6.742 (3), b = 16.268(6), c = 7.738(3) Å, α = 90.00, β = 91.183(5), γ = 90.00, V = 848.4(6) $Å^3$, Z = 2, ρ_{calc} = 1.223 g/cm^3, crystal dimensions 0.15×0.12×0.10 mm was used for measurement on a Bruker APEX SMART- CCD diffractometer with a graphite monochromater. The total number of reflections measured was 2125, of which 1305, were observed, $I > 2\sigma(I)$. Final indices: R = 0.0899, wR2 = 0.1254. The crystal structure of **2** was solved by direct method SHLXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for compound **2** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 1865355). Copies of the data can be obtained free of charge from CCDC, 12 Union Road, Cambridge CB21EZ, UK (Fax: +44-1223-336-033, or email: deposit@ccdc.cam.ac.uk).

Measurement of Cell Viability and Nitrite

RAW 264.7 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, and 1 mM nonessential amino acids. Cells were plated on 96-well plates at a concentration of 4×10^5 cells/well. After incubation of cells at 37℃ with 5% CO_2 for 4 h, test compound and indomethacin dissolved in 0.1% DMSO, with the final concentrations ranging from 0.1 to 50 μM, were added to the plates at 20 μL/well. Following incubation for 1 h, the LPS solution (10 μL/well, 5 μg/mL) was added, and the cells were incubated for a further 20 h. Cell viability was determined by a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Nitrite was measured by adding 50 μL of the Griess reagent to 50 μL of cell suspension for 5 min. The absorbance was measured at 540 nm with a microplate reader. All samples were tested in triplicate.

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Table S1. ^{13}C and ^{1}H Spectroscopic Data for **1**

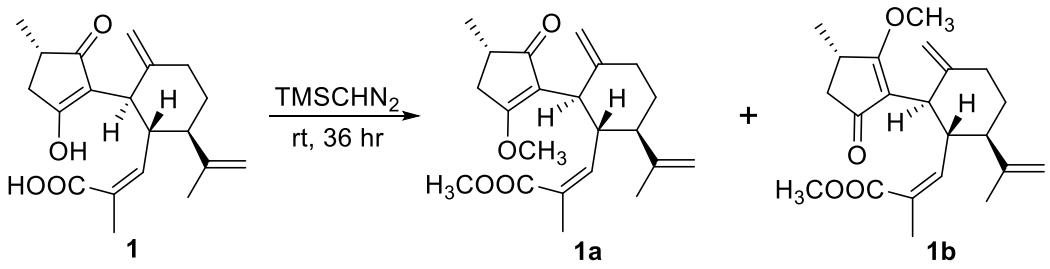
No.	δ_C,[a,d] type	δ_H[a,e] (*J* in Hz)	δ_C,[b,f] type	δ_H[b,g] (*J* in Hz)	δ_C,[c,f] type	δ_H[c,g] (*J* in Hz)
1	-		-		199.6, C	
2	-	-	37.7, CH	2.69, m	36.8, CH	2.35, m
3	-	-	38.5, CH_2	2.25, m	38.4, CH_2	1.90, dd (2.5, 17.7)
				2.92, dd (7.1, 16.9)		2.58, dd (7.1, 17.2)
4	-		-		190.0, C	
5	168.7, C		170.6, C		169.0, C	
6	126.8, C		128.3, C		127.1, C	
7	143.7, CH	5.25, d (10.5)	144.0, CH	5.71, dd (1.1, 10.7)	144.2, CH	5.27, dd (1.65, 10.7)
8	42.7, CH	3.41, s	42.8, CH	4.24, q (10.8)	43.1, CH	3.48, q (10.8)
9	42.1, CH	2.80, 2.84, d (11.1)	43.1, CH	3.55, d (11.0)	42.5, CH	2.83, d (10.9)
10	114.8, C		115.4, C		114.7, C	
11	144.5, C		145.5, C		144.2, C	
12	35.0, CH_2	2.06, 2.37, m	35.7, CH_2	2.23, 2.48, m	35.4, CH_2	2.09, 2.39, m
13	31.2, CH_2	1.62, 1.53, m	31.6, CH_2	1.51, 1.63, m	31.6, CH_2	1.47, 1.62, m
14	52.0, CH	2.05, m	53.0, CH	2.23, m	52.4, CH	2.04, m
15	148.2, C		148.9, C		148.5, C	
16	110.8, CH_2	4.64, 4.54, s	110.9, CH_2	4.75, 4.90, s	111.1, CH_2	4.54, 4.65, s
17	18.9, CH_3	1.62, s	19.2, CH_3	1.84, s	19.3, CH_3	1.62, s
18	107.9, CH_2	4.61, 4.32, s	108.2, CH_2	4.99, m	108.2, CH_2	4.33, 4.62, m
19	18.2, CH_3	1.02, d (6.9)	17.7, CH_3	1.20, d (7.3)	17.8, CH_3	1.02, d (7.3)
20	21.2, CH_3	1.62, s	21.6, CH_3	2.03, s	21.6, CH_3	1.62, s

[a]Measured in DMSO-d_6. [b]Measured in pyridine-d_6. [c]Measured in DMSO with 1% TFA-*d*. [d]Measured in 75 MHz. [e]Measured in 300 MHz. [f]Measured in 125 MHz. [g]Measured in 500 MHz. '-' undetected.

Table S2. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data for **1a** and **1b** in CD_3OD.

No.	**1a**		**1b**	
	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1	210.2, C		193.0, C	
2	40.6, CH	2.40, m	32.4, CH	3.17, m
3	34.1, CH_2	2.36, 2.96, m	44.3, CH_2	1.96, 2.70, m
4	188.5, C		206.8, C	
5	170.2, C		-	
6	128.4, C		-	
7	146.2, CH	5.42, t (10.5, 11)	-	-
8	44.9, CH	3.46, m	-	-
9	43.8, CH	2.96, m	-	-
10	119.0, C		119.0, C	
11	145.7, C		-	
12	36.6, CH_2	2.17, 2.46, m	-	-
13	33.0, CH_2	1.56, 1.69, m	-	-
14	53.9, CH	2.10, m	-	-
15	149.6, C		-	
16	110.6, CH_2	4.60, 4.66, s	-	-
17	19.6, CH_3	1.64, s	-	-
18	108.6, CH_2	4.35, 4.67, s	-	-
19	17.3, CH_3	1.13, d (7.5)	20.7, CH_3	1.21, m
20	21.3, CH_3	1.74, s	-	-
1-OCH_3			51.7	3.66, d (3.5)
4-OCH_3	51.7	3.66, d (3.5)		
14-OCH_3	57.6	3.95, s	57.8	4.00, s

"-": overlapped with **1a**.



Scheme S1. Di-methylation of **1**.

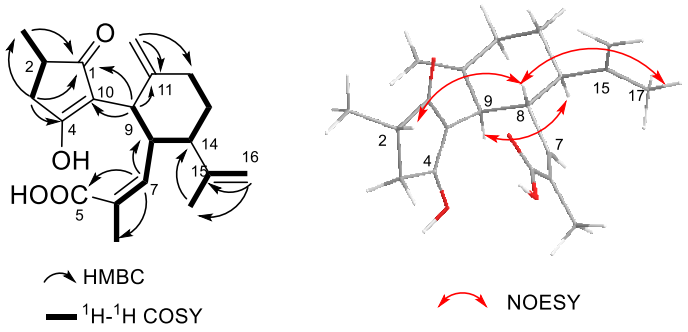


Figure S1. Key ^{1}H-^{1}H COSY, HMBC and ROESY correlations of **1**

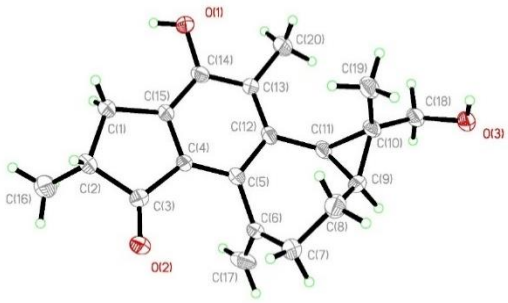


Figure S2. X-ray crystal structure analysis of jatrophol (**2**)

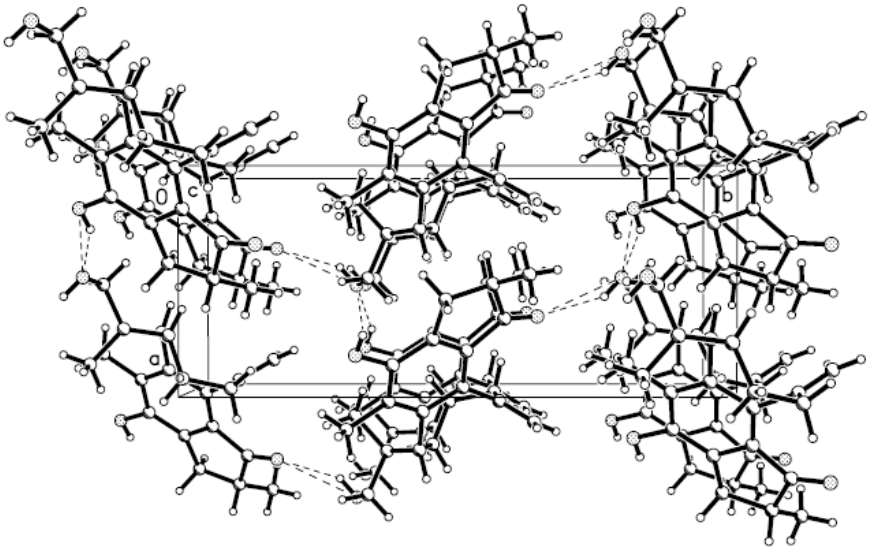


Figure S3. The crystal packing of jatrophol (**2**)

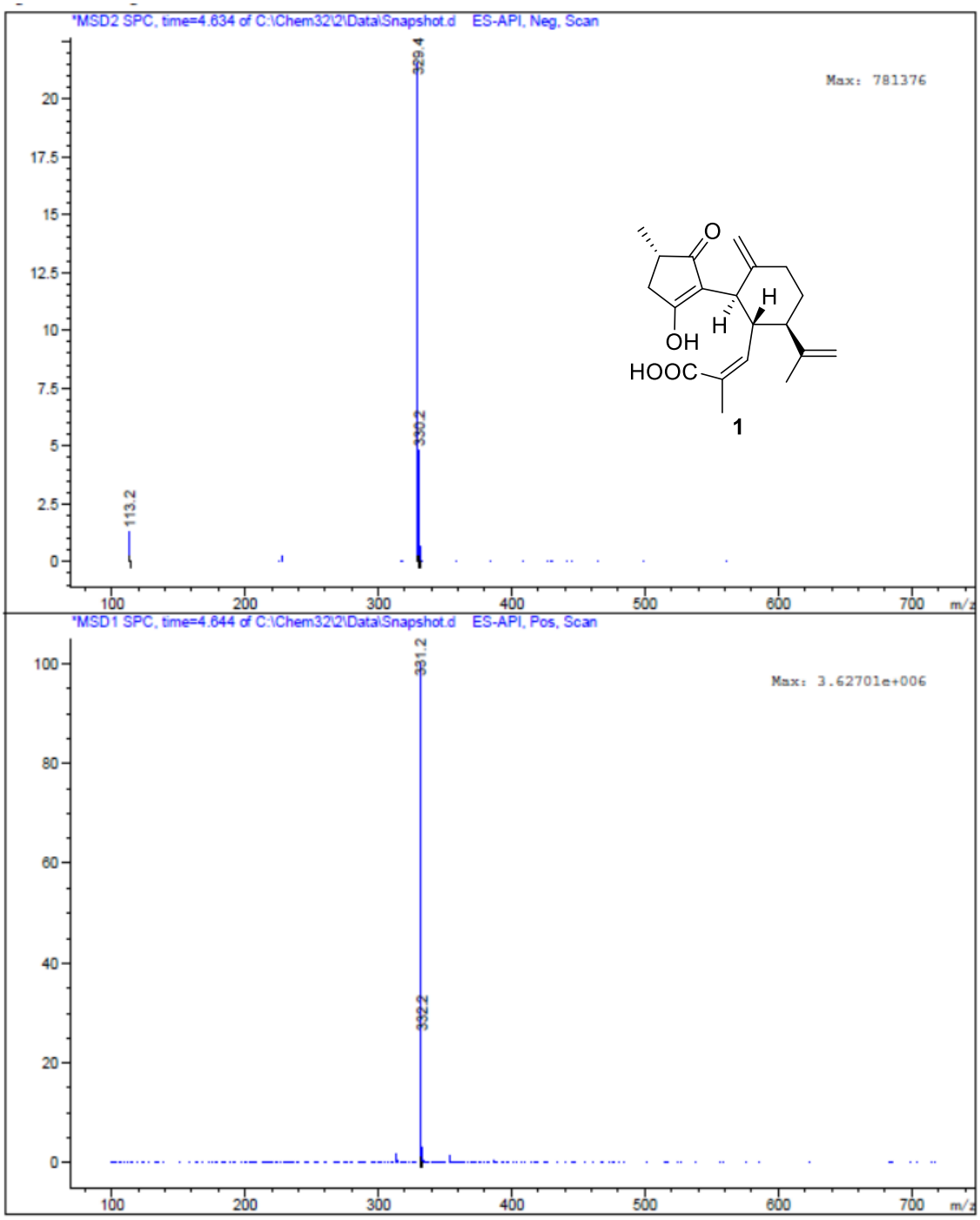


Figure S4. (±)-ESIMS spectrum of jatrophacine (**1**).

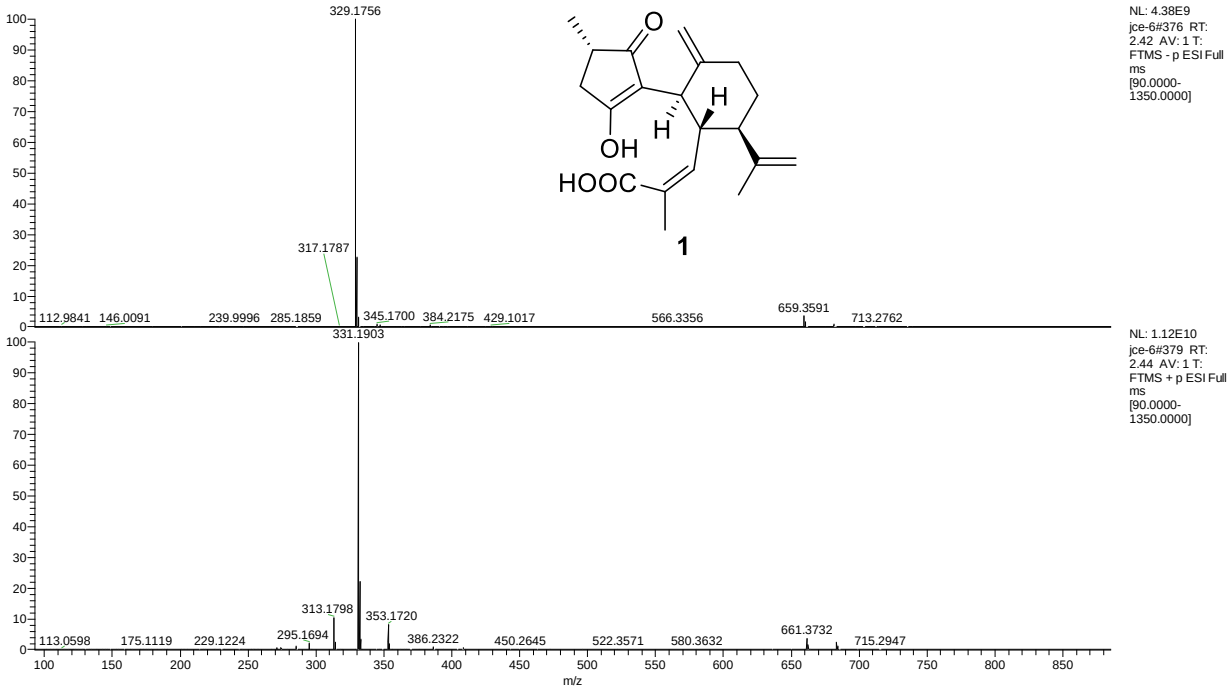


Figure S5. (±)-HRESIMS spectrum of jatrophacine (**1**).

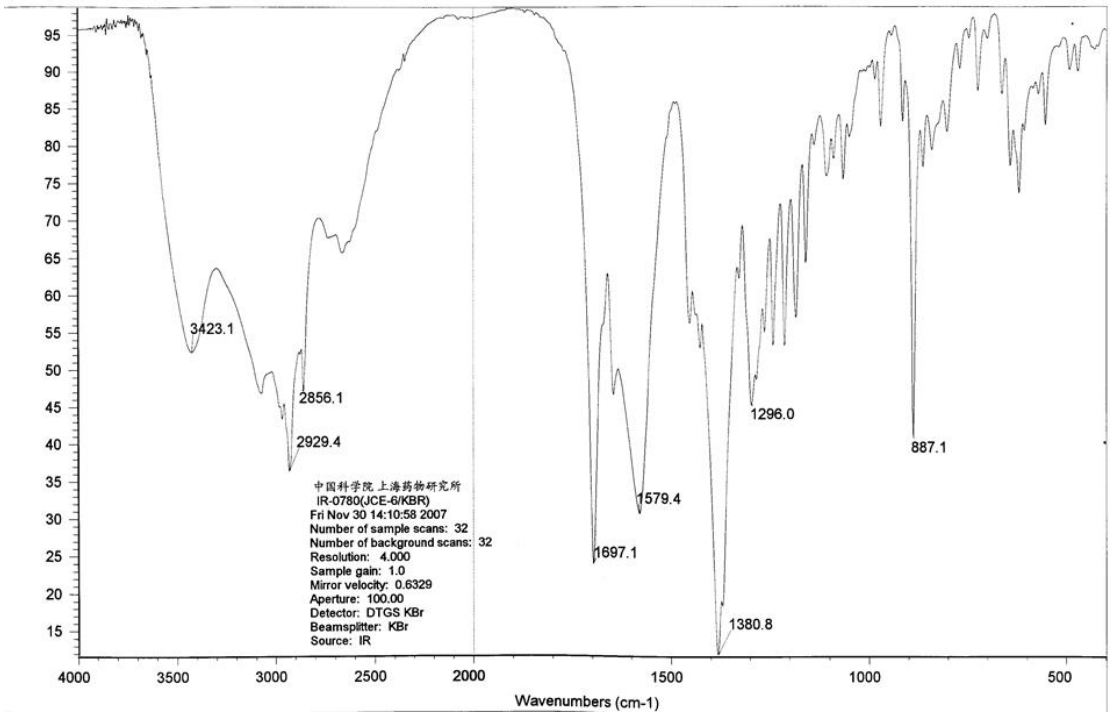


Figure S6. IR spectrum (KBr) of jatrophacine (**1**).

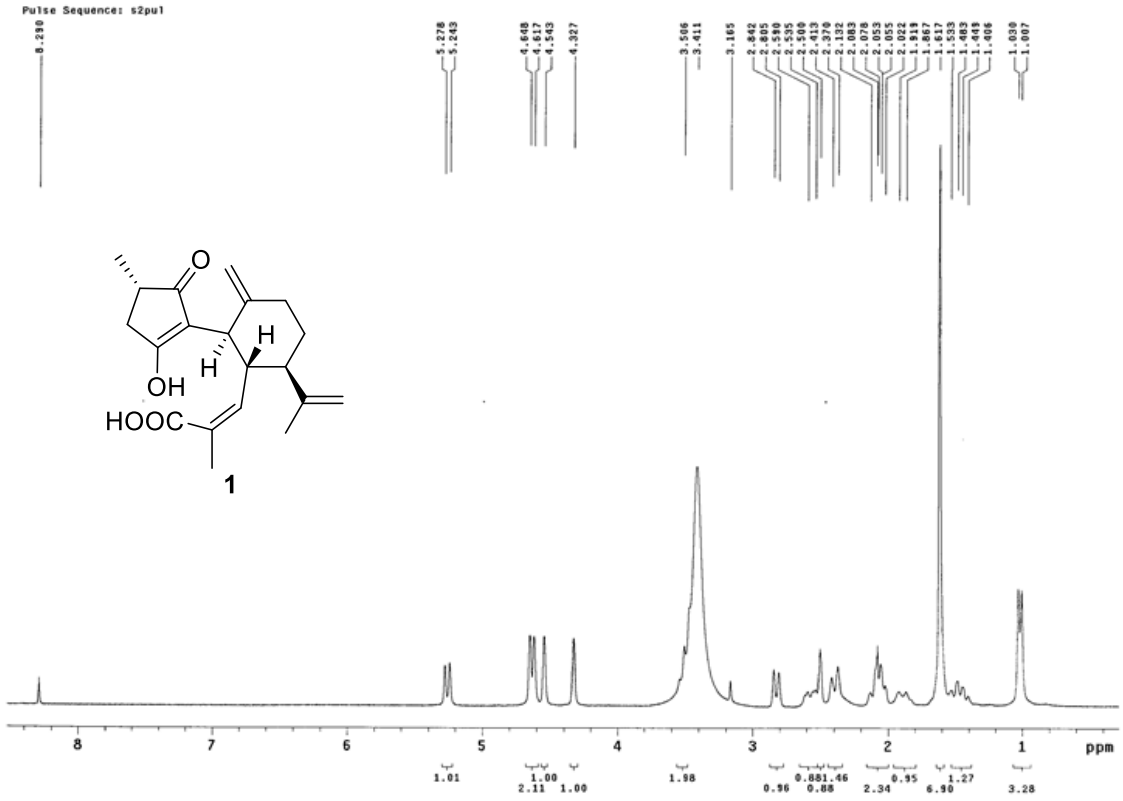


Figure S7. ^{1}H NMR spectrum (300 MHz) of jatrophacine (**1**) in DMSO-d_6.

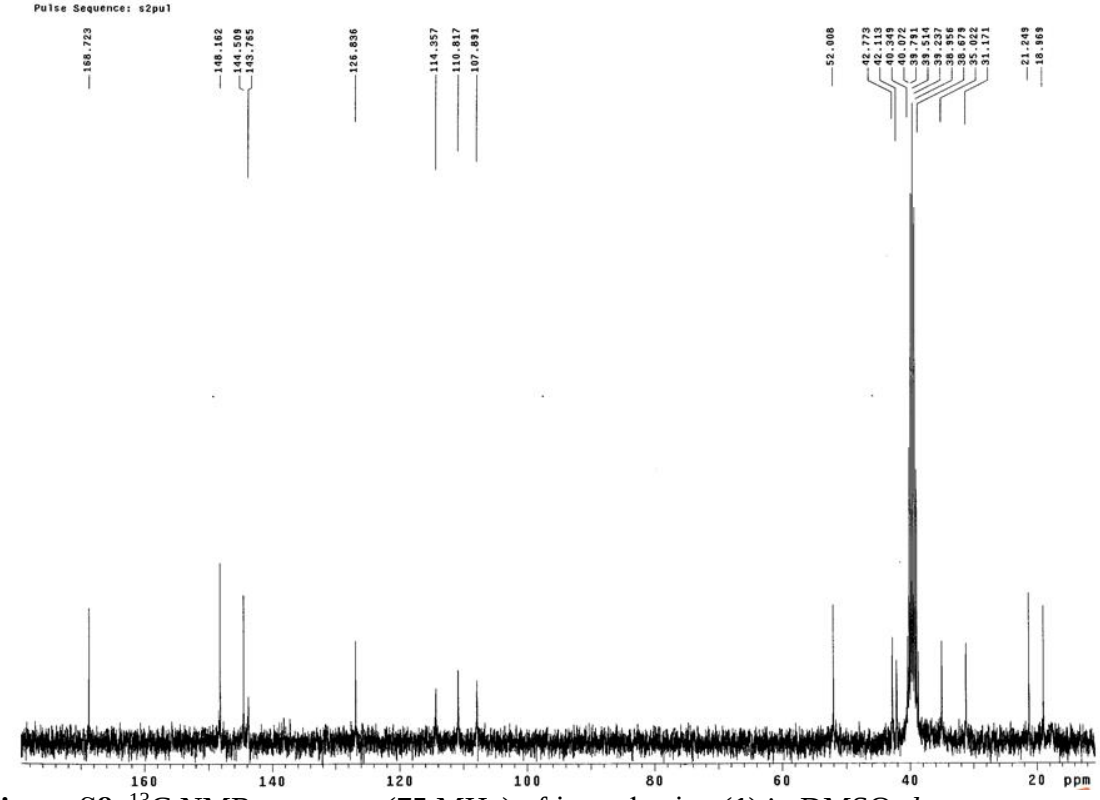


Figure S8. ^{13}C NMR spectrum (75 MHz) of jatrophacine (**1**) in DMSO-d_6.

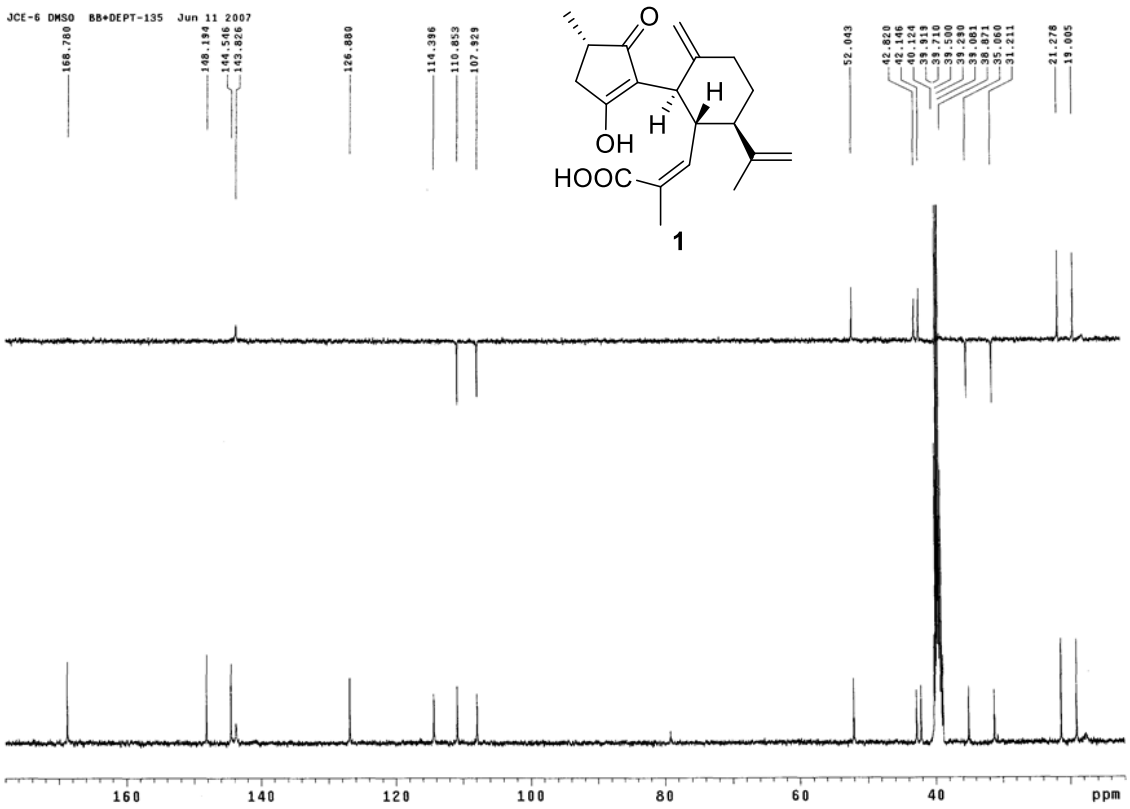


Figure S9. DEPT spectrum (75 MHz) of jatrophacine (**1**) in DMSO-d_6.

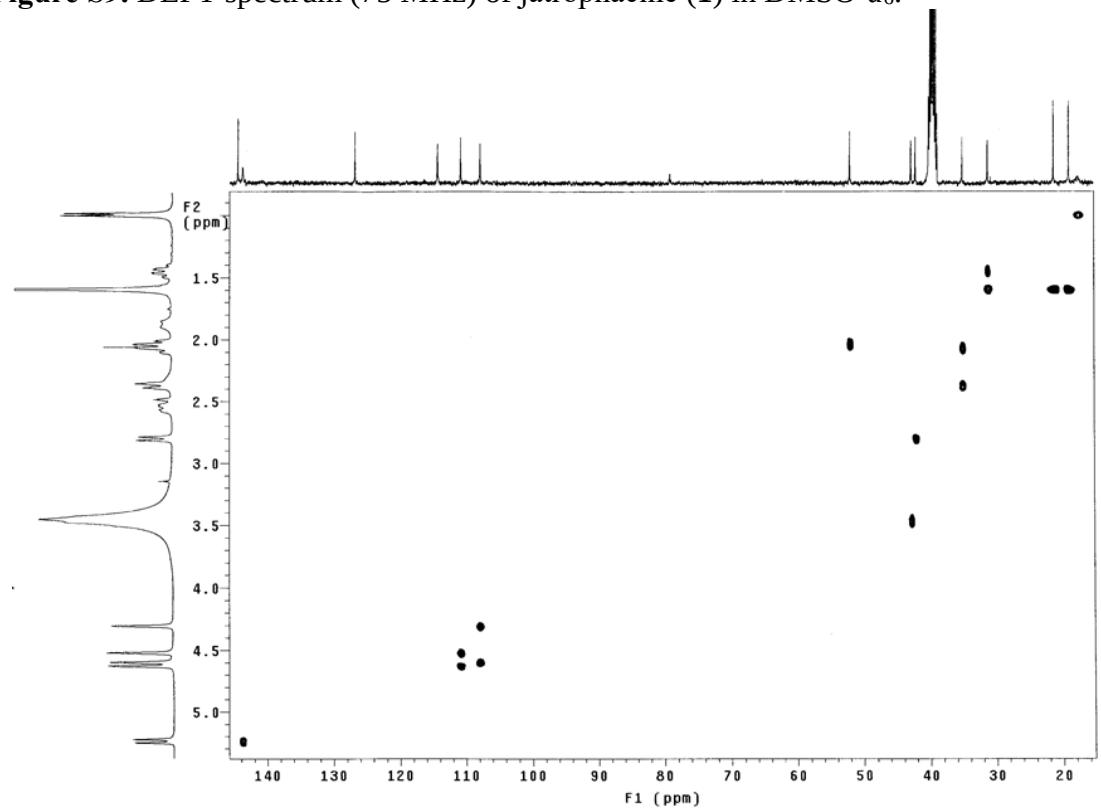


Figure S10. HSQC spectrum (300 MHz) of jatrophacine (**1**) in DMSO-d_6.

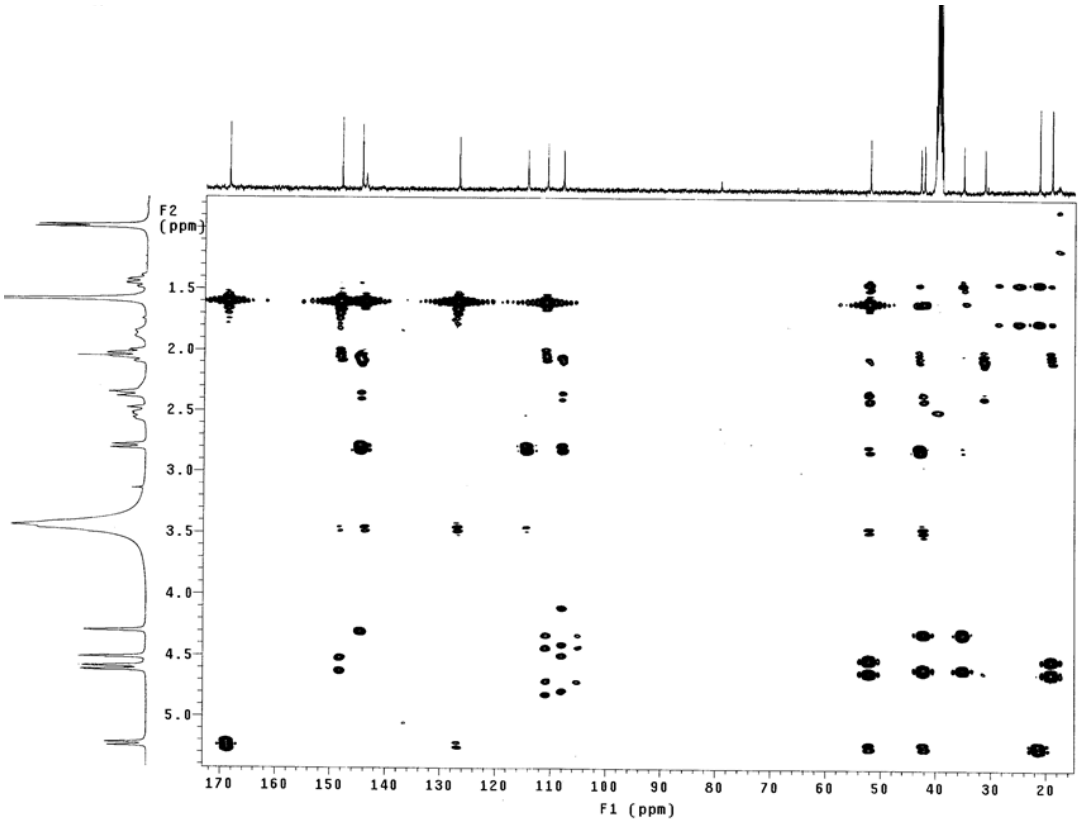


Figure S11. HMBC spectrum (300 MHz) of jatrophacine (**1**) in DMSO-d_6.

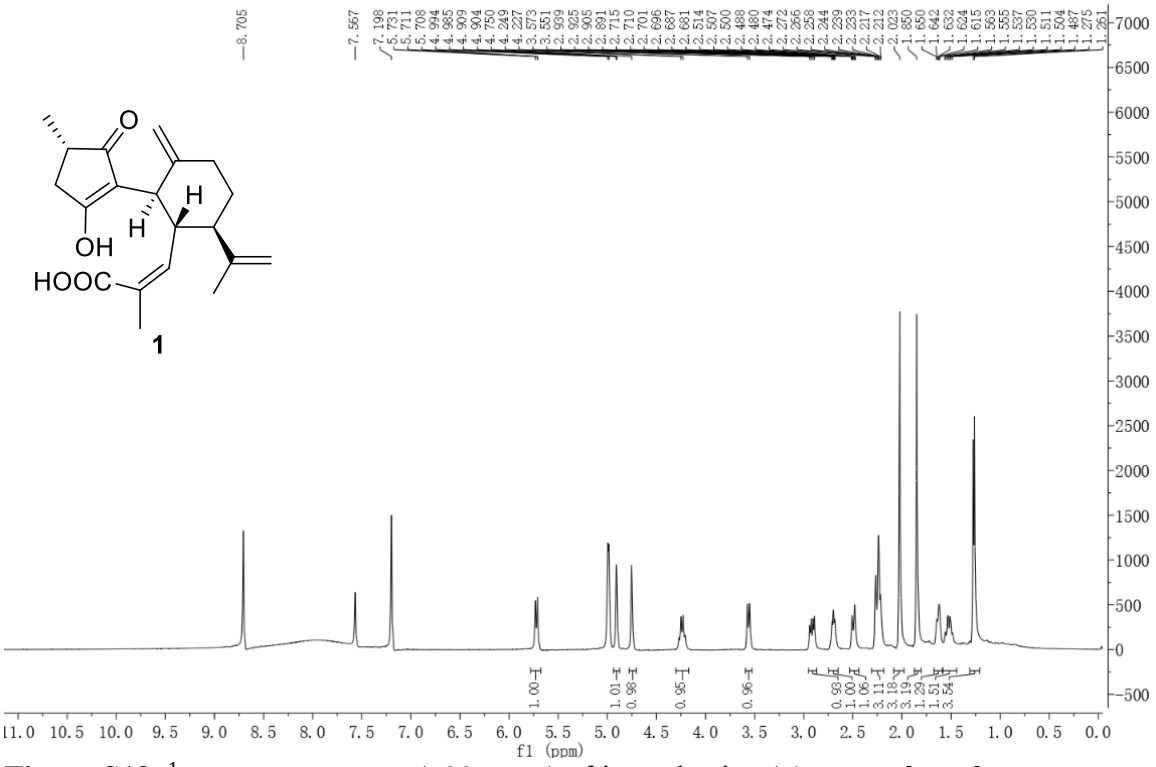


Figure S12. ^{1}H NMR spectrum (500 MHz) of jatrophacine (**1**) in pyridine-d_6.

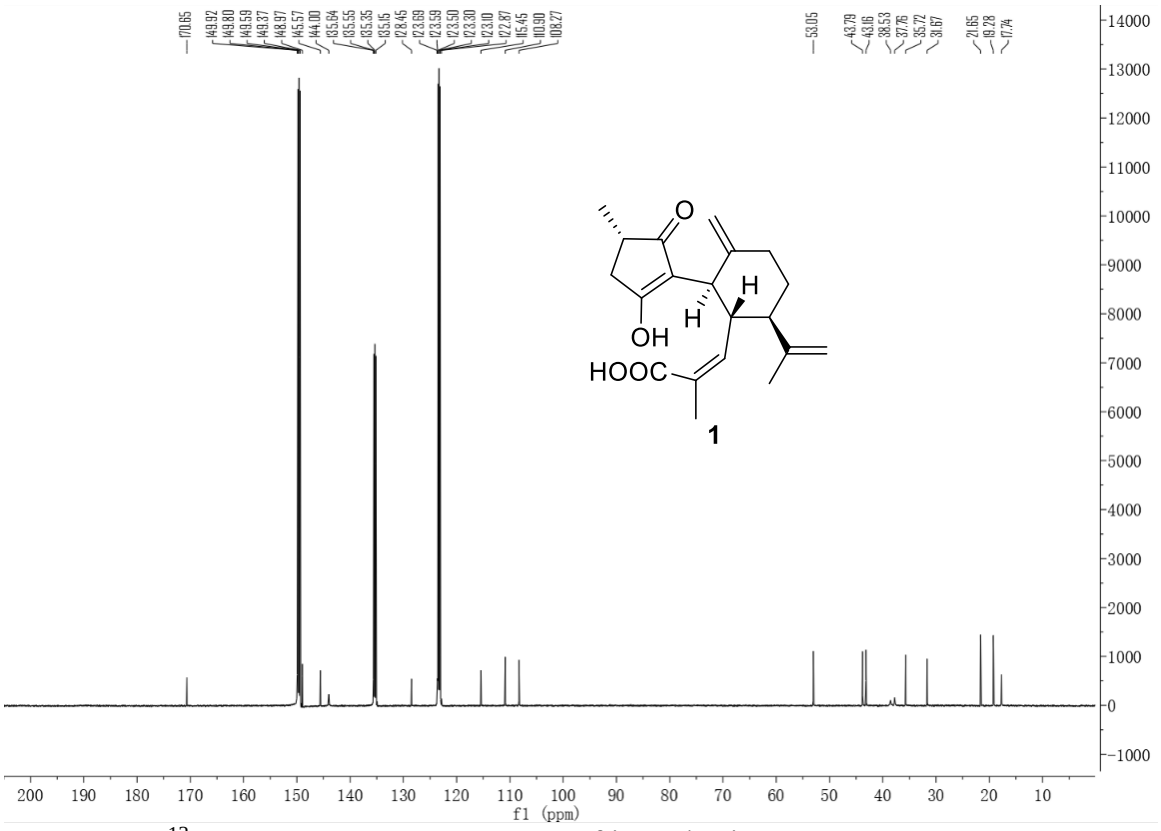


Figure S13. ^{13}C NMR spectrum (125 MHz) of jatrophacine (**1**) in pyridine-d_6.

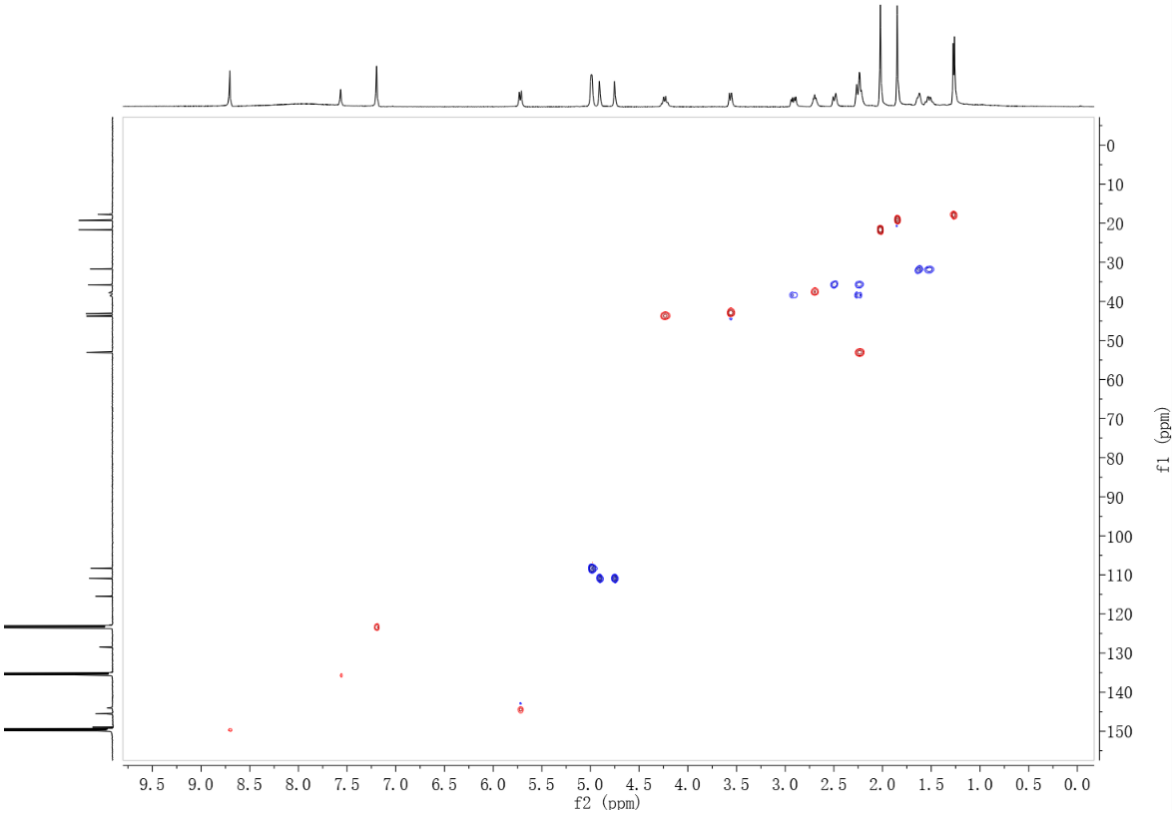


Figure S14. HSQC spectrum (500 MHz) of jatrophacine (**1**) in pyridine-d_6.

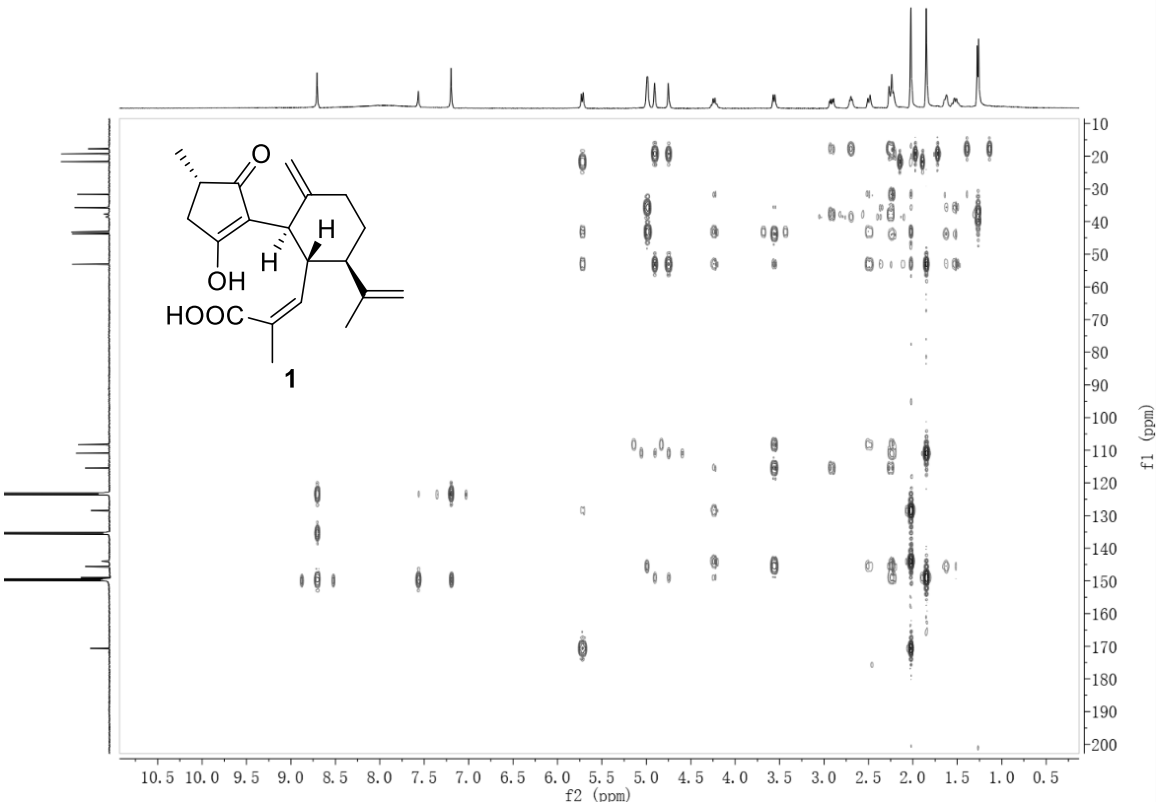


Figure S15. HMBC spectrum (500 MHz) of jatrophacine (**1**) in pyridine-d_6.

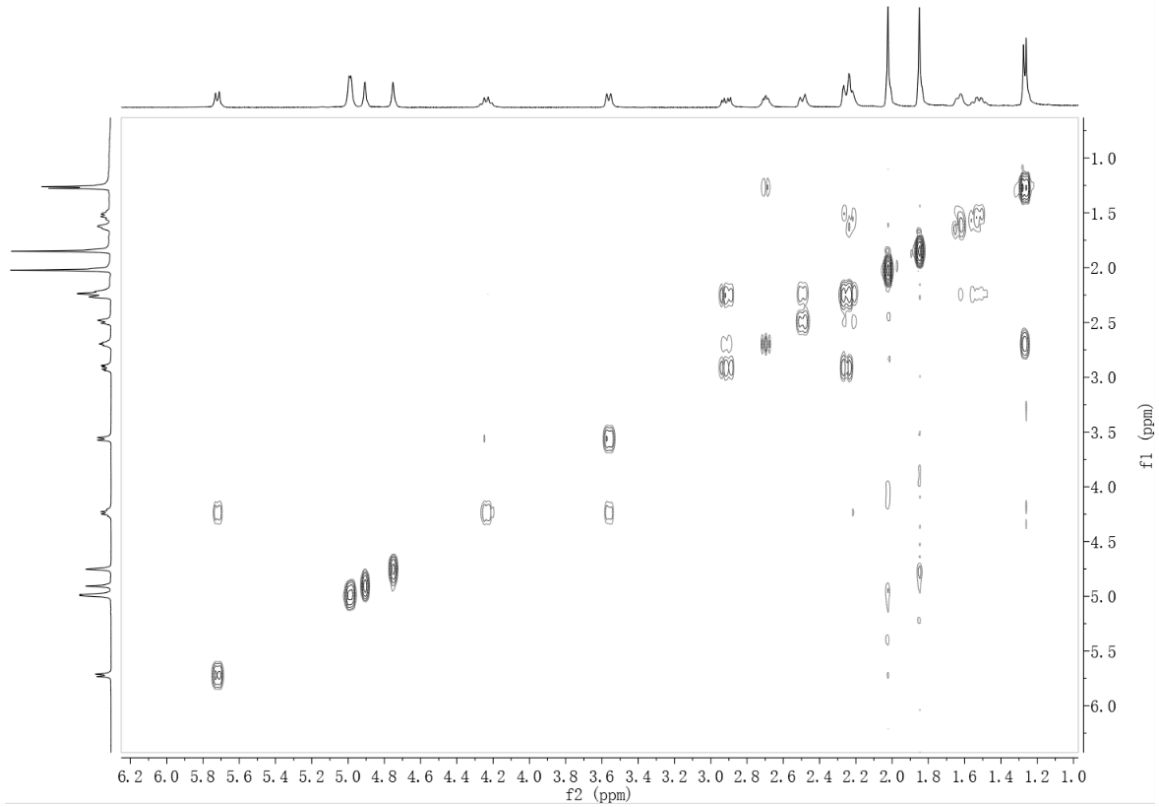


Figure S16. $^{1}H-^{1}H$ COSY spectrum (500 MHz) of jatrophacine (**1**) in pyridine-d_6.

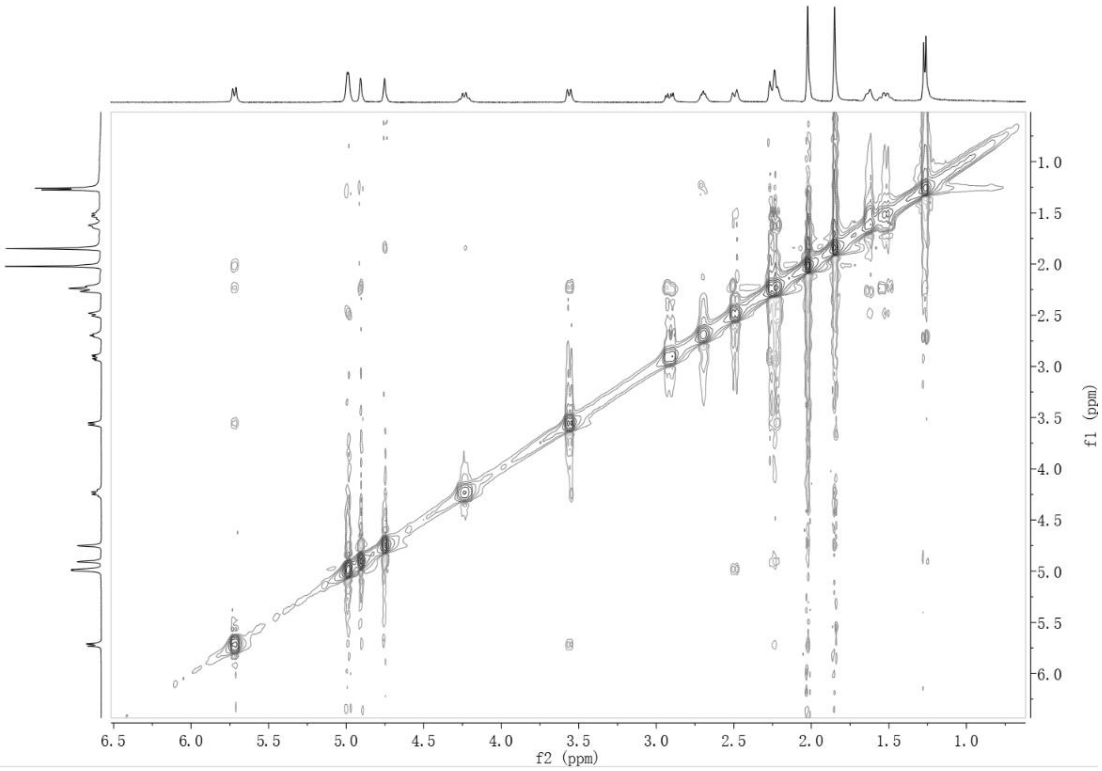


Figure S17. ROESY spectrum (500 MHz) of jatrophacine (**1**) in pyridine-d_6.

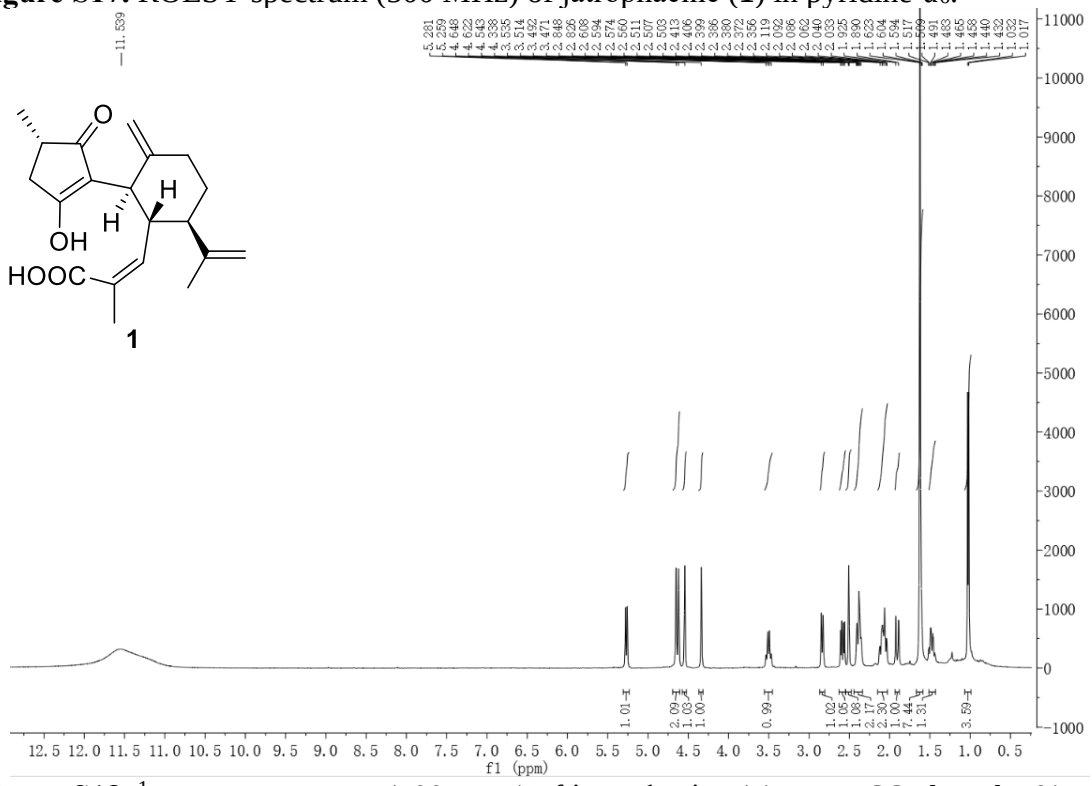


Figure S18. ^{1}H NMR spectrum (500 MHz) of jatrophacine (**1**) in DMSO-d_6 with 1% TFA-*d*.

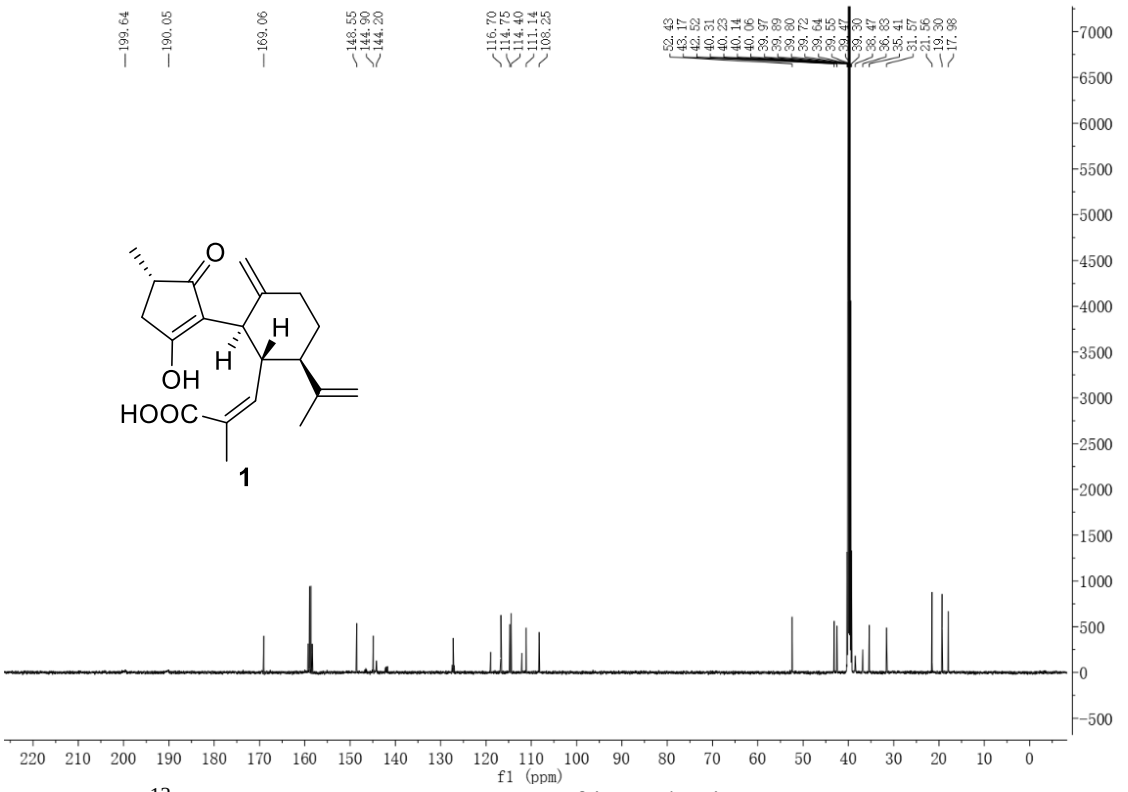


Figure S19. ^{13}C NMR spectrum (125 MHz) of jatrophacine (**1**) in DMSO-d_6 with 1% TFA-*d*.

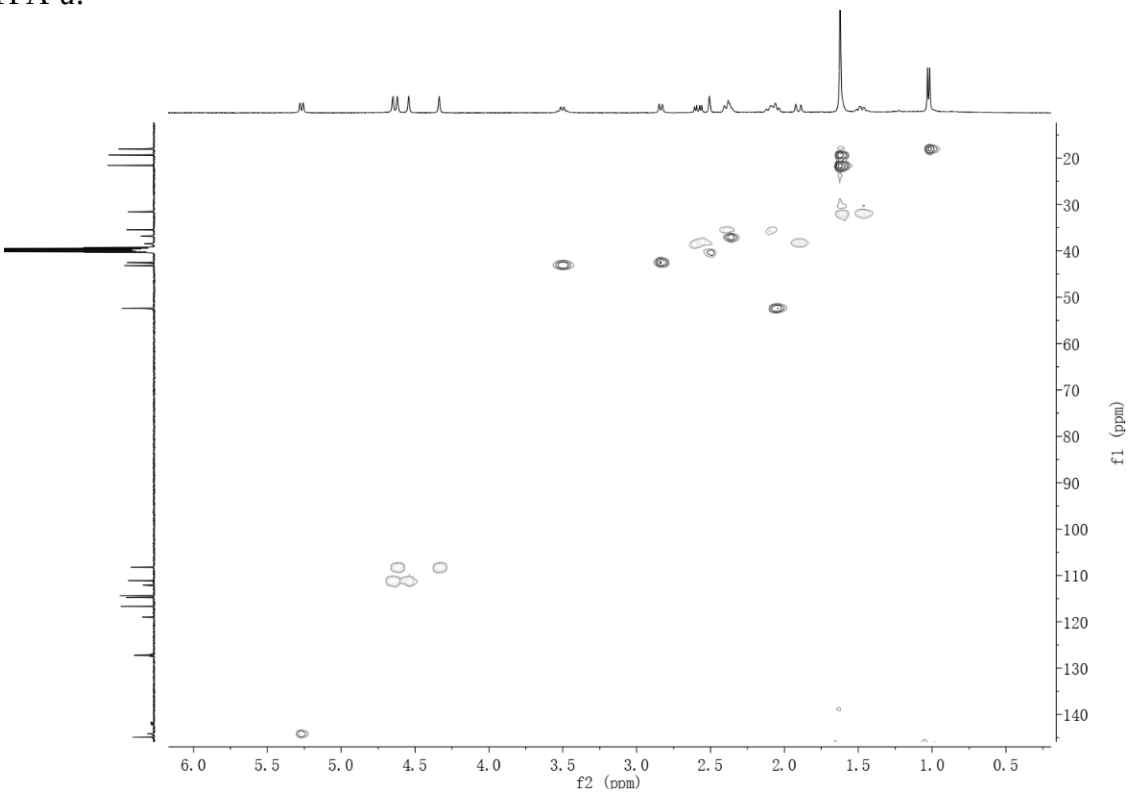


Figure S20. HSQC spectrum (500 MHz) of jatrophacine (**1**) in DMSO-d_6 with 1% TFA-*d*.

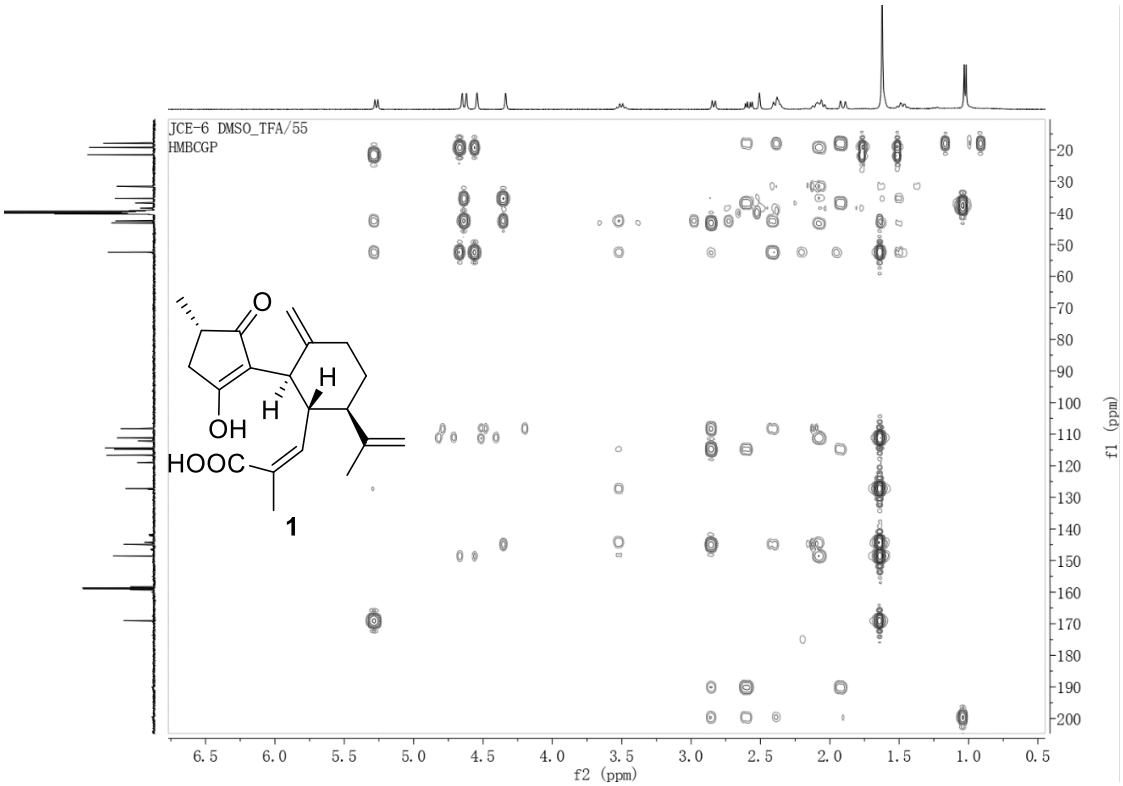


Figure S21. HMBC spectrum (500 MHz) of jatrophacine (**1**) in DMSO-d_6 with 1% TFA-*d*.

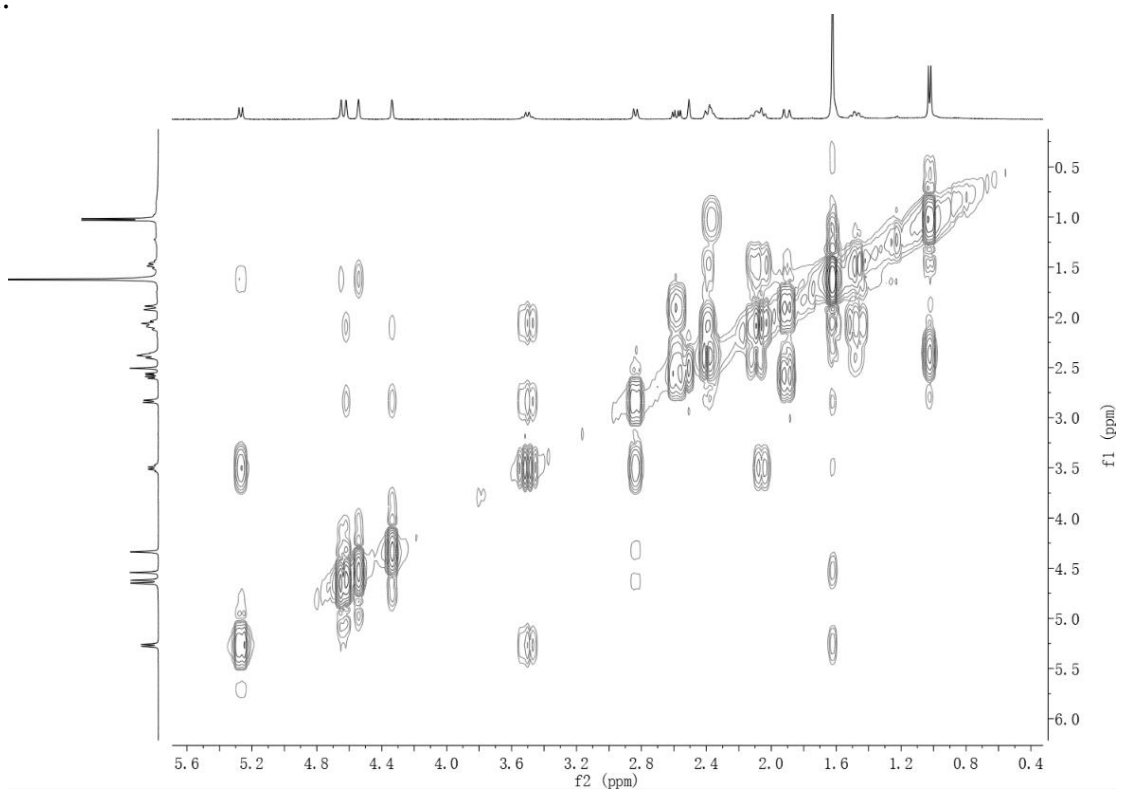


Figure S22. ^{1}H−^{1}H COSY spectrum (500 MHz) of jatrophacine (**1**) in DMSO-d_6 with 1% TFA-*d*.

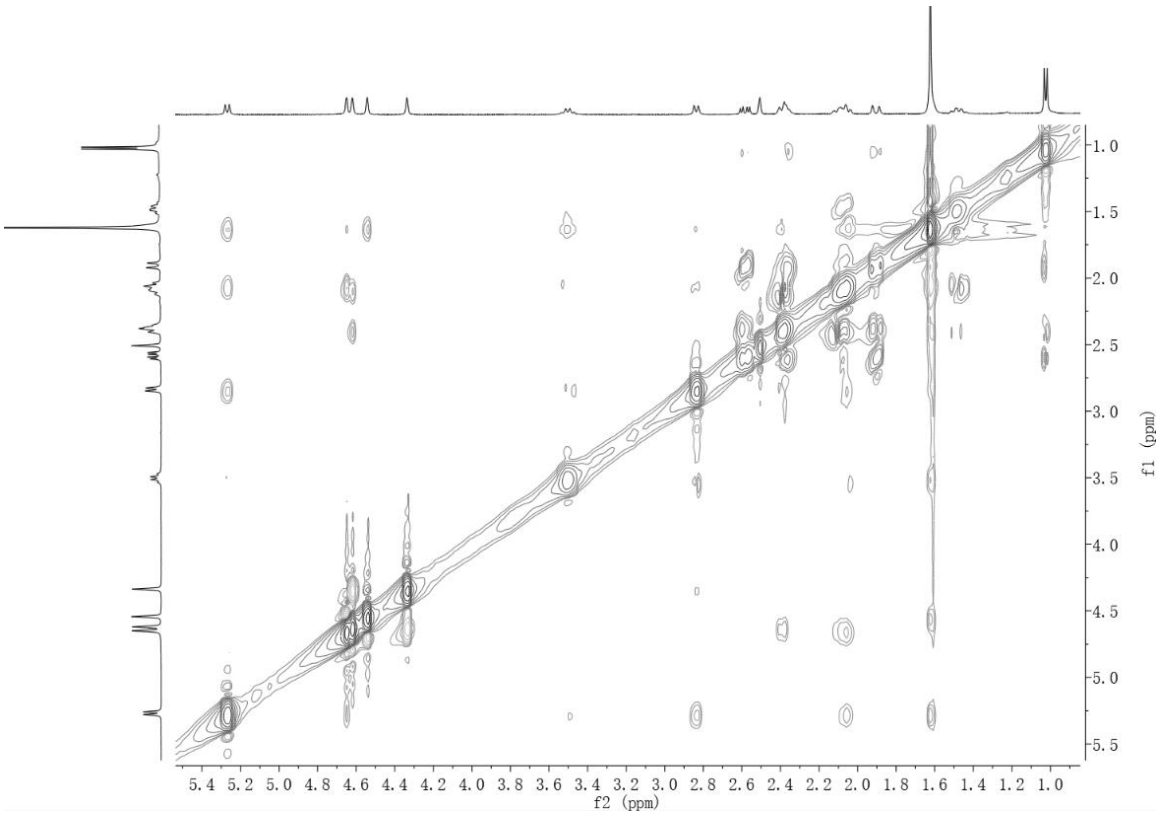


Figure S23. ROESY spectrum (500 MHz) of jatrophacine (**1**) in DMSO-d_6 with 1% TFA-*d*.

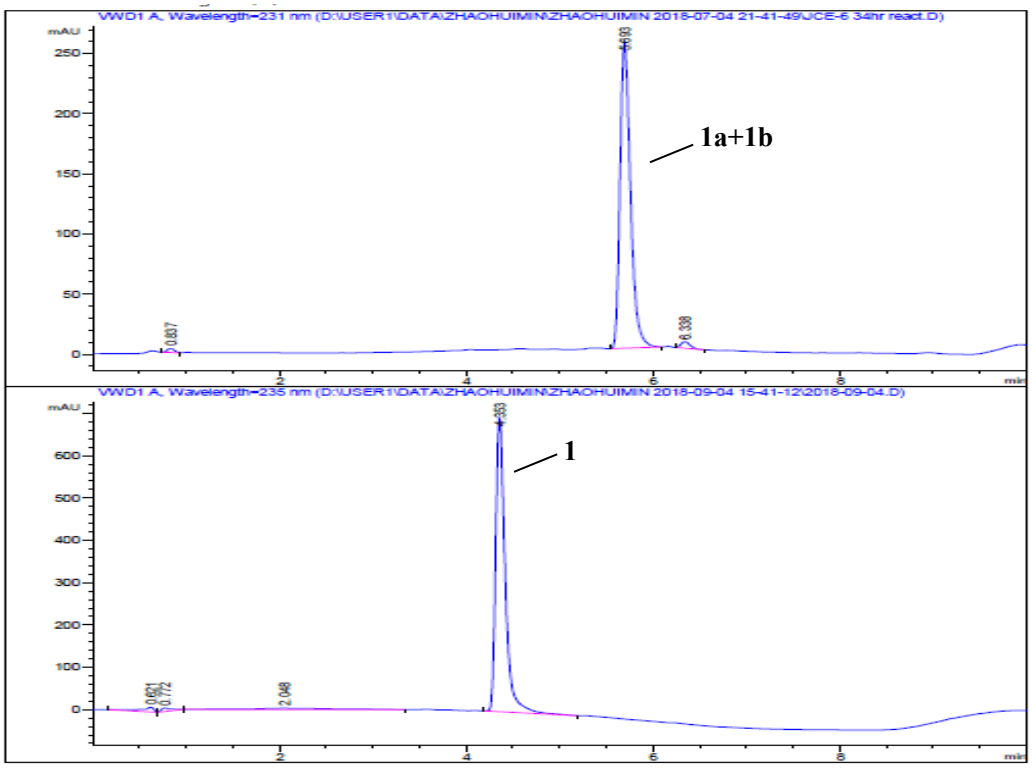


Figure S24. HPLC traces of dimethylation of **1**.

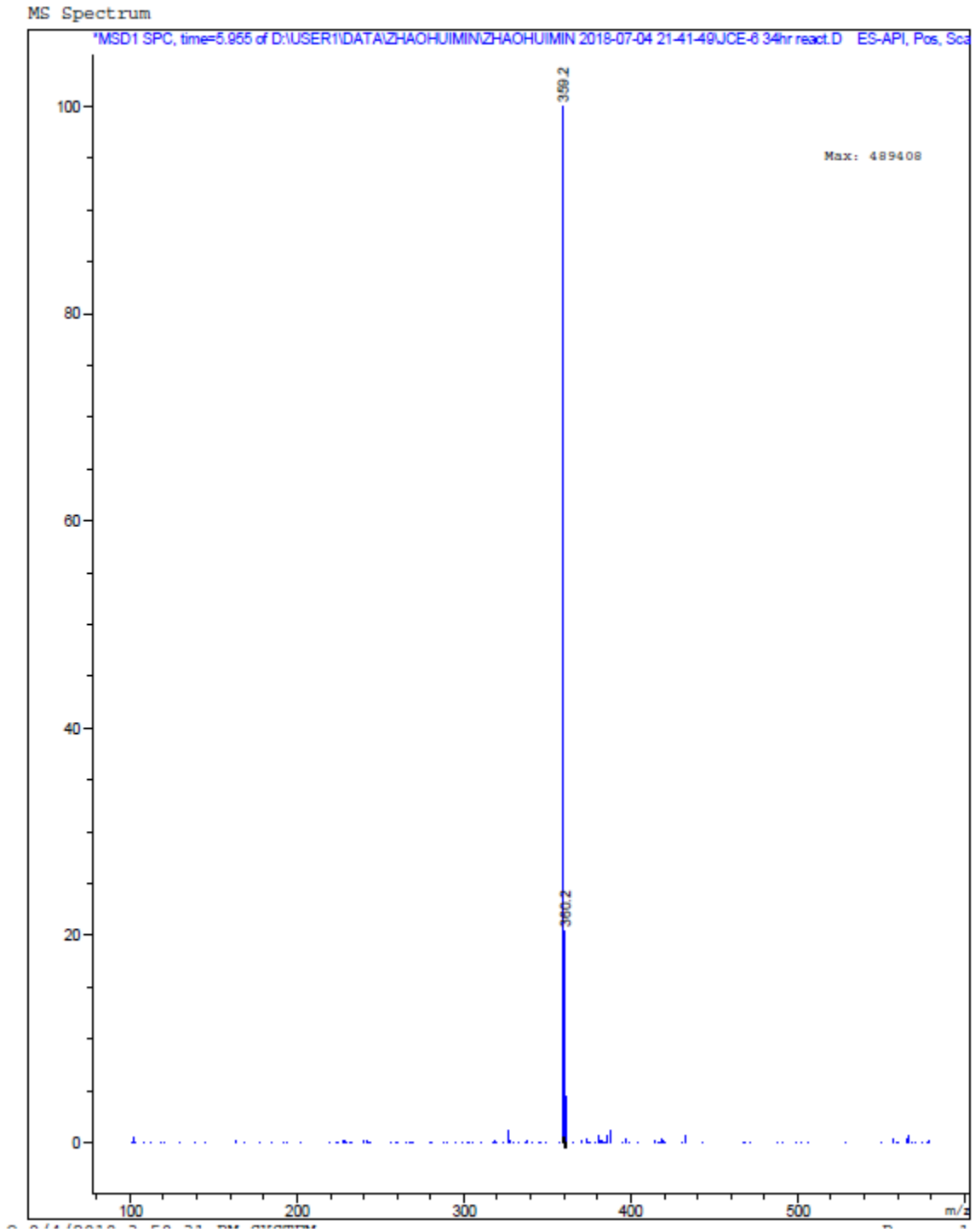


Figure S25. (+)-ESIMS spectrum (positive mode) of dimethyl-jatrophacine (**1a+1b**).

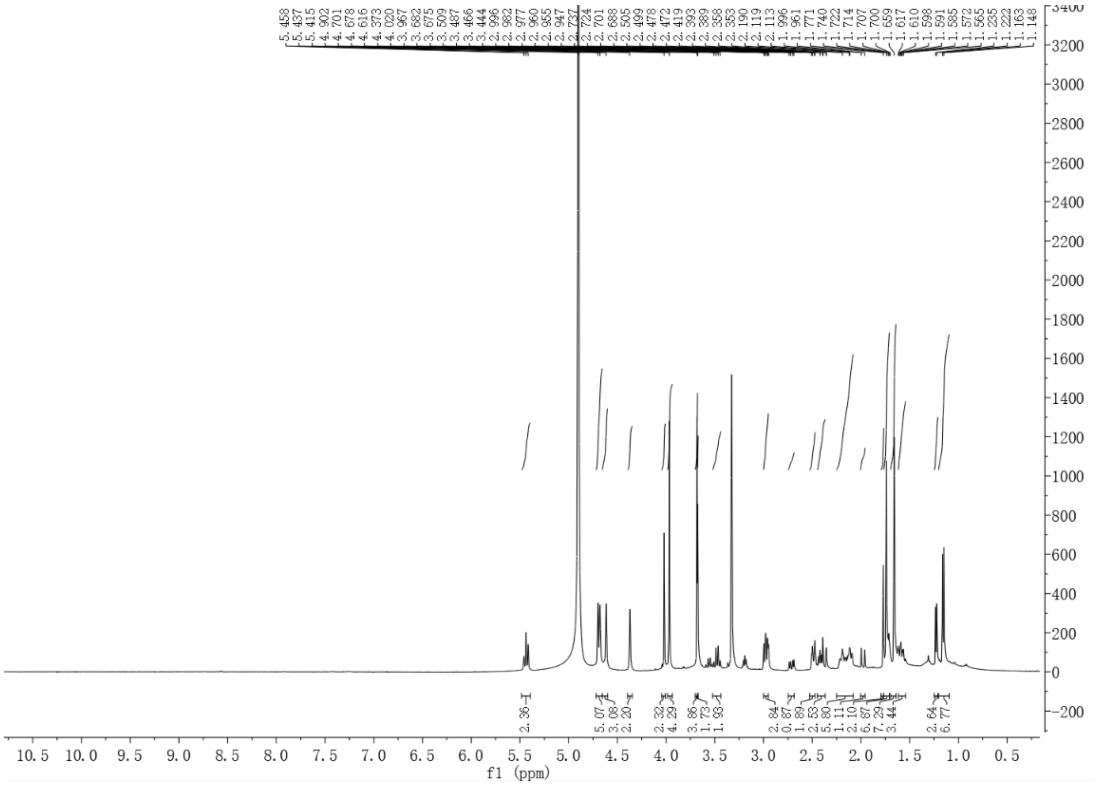


Figure S26. ^{1}H NMR spectrum (500 MHz) of dimethyl-jatrophacine (**1a+1b**) in CD_3OD.

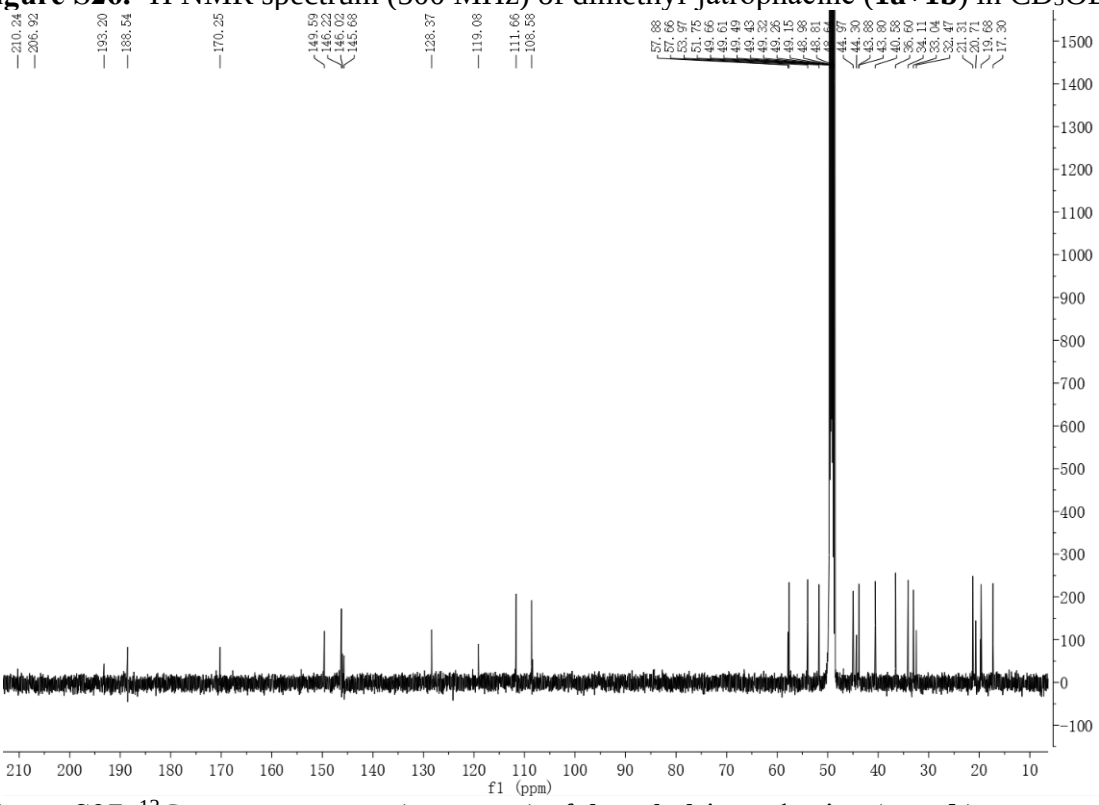


Figure S27. ^{13}C NMR spectrum (125 MHz) of dimethyl-jatrophacine (**1a+1b**) in CD_3OD.

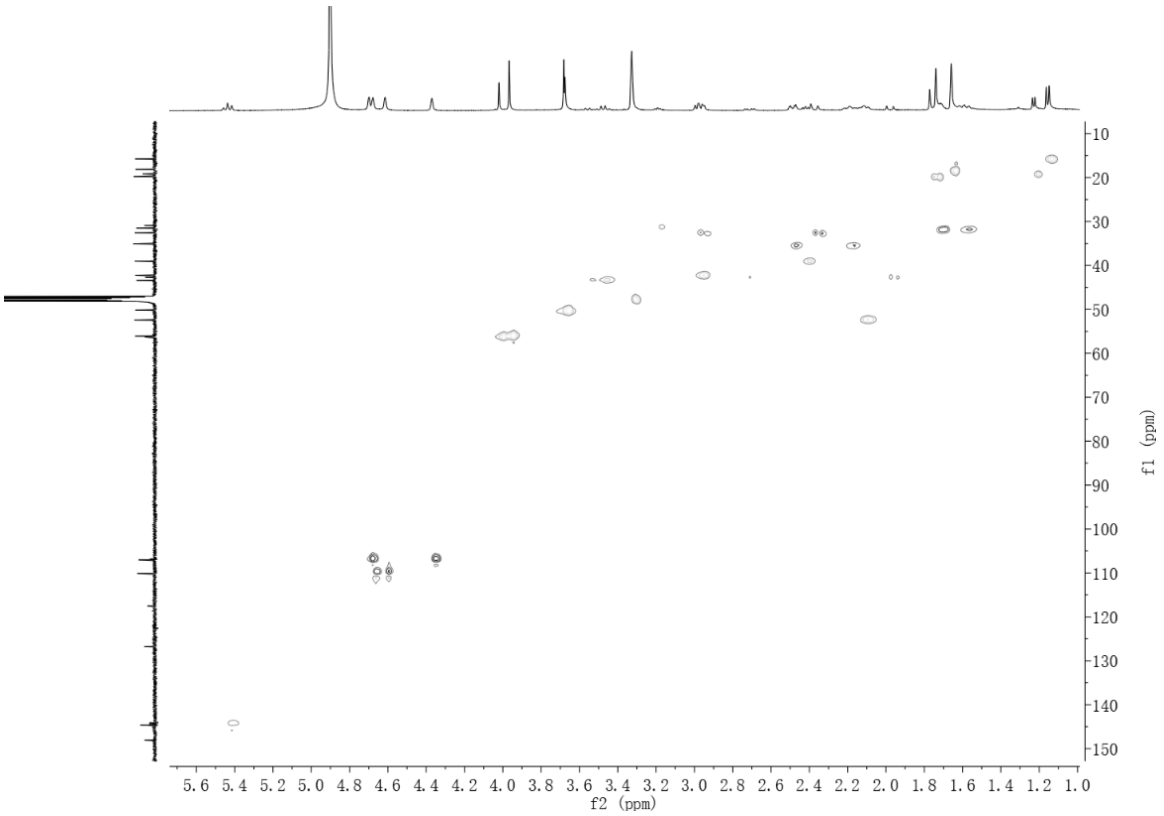


Figure S28. HSQC spectrum (500 MHz) of dimethyl-jatrophacine (**1a+1b**) in CD_3OD.

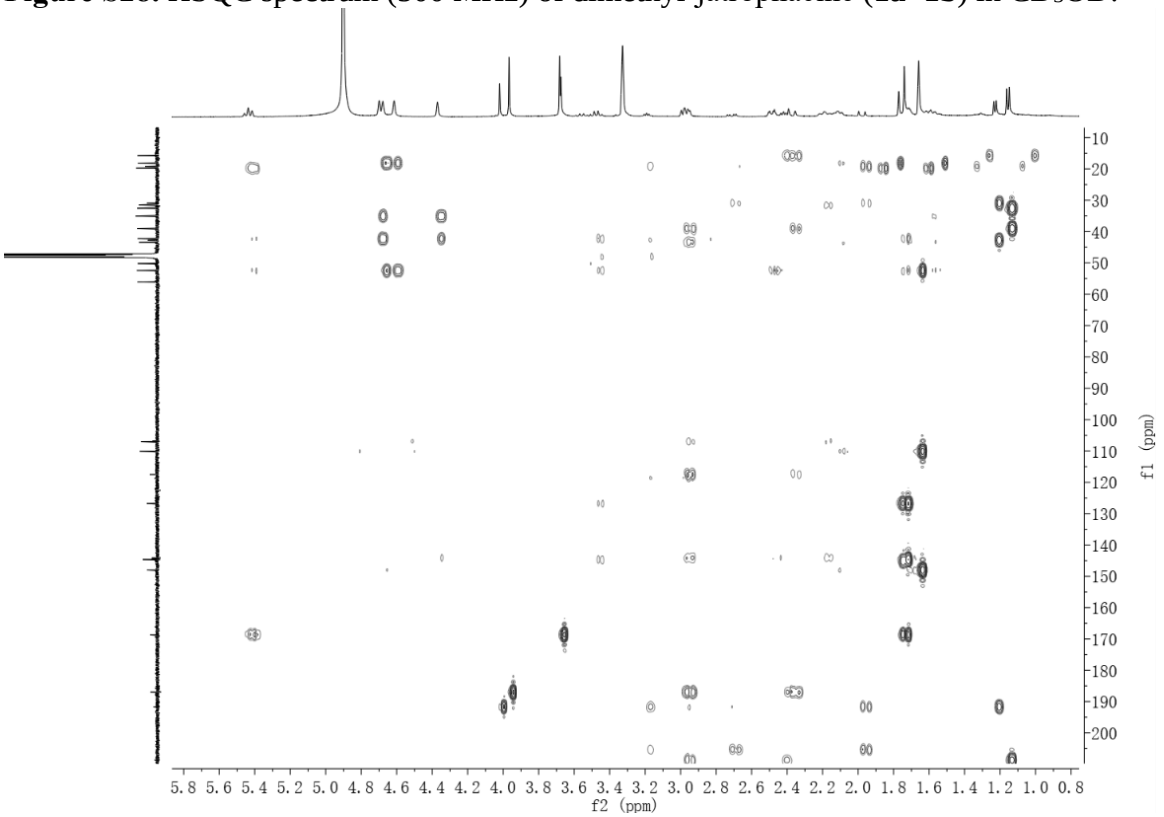


Figure S29. HMBC spectrum (500 MHz) of dimethyl-jatrophacine (**1a+1b**) in CD_3OD.

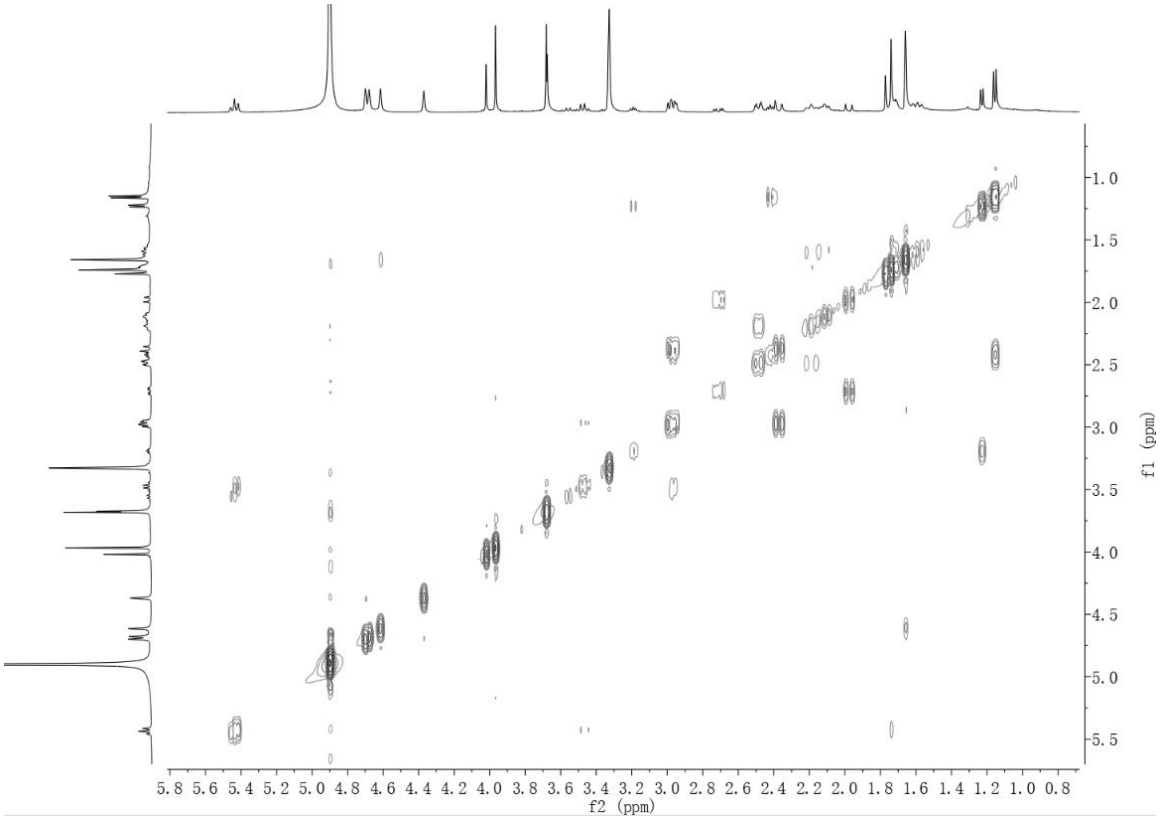


Figure S30. $^{1}H-^{1}H$ COSY spectrum (500 MHz) of dimethyl-jatrophacine (**1a+1b**) in CD_3OD.

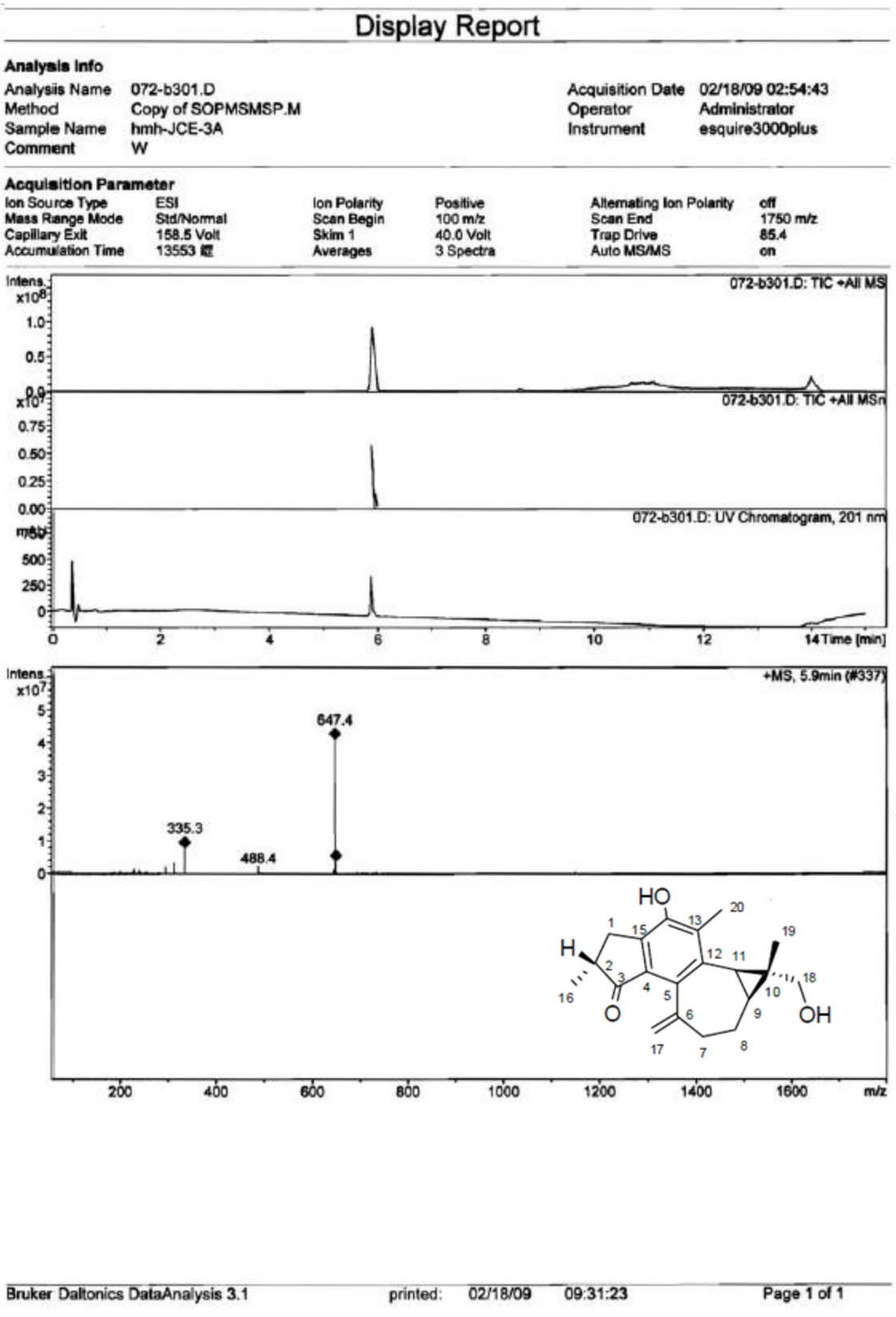


Figure S31. (+)-ESIMS spectrum of jatrophol (**2**).

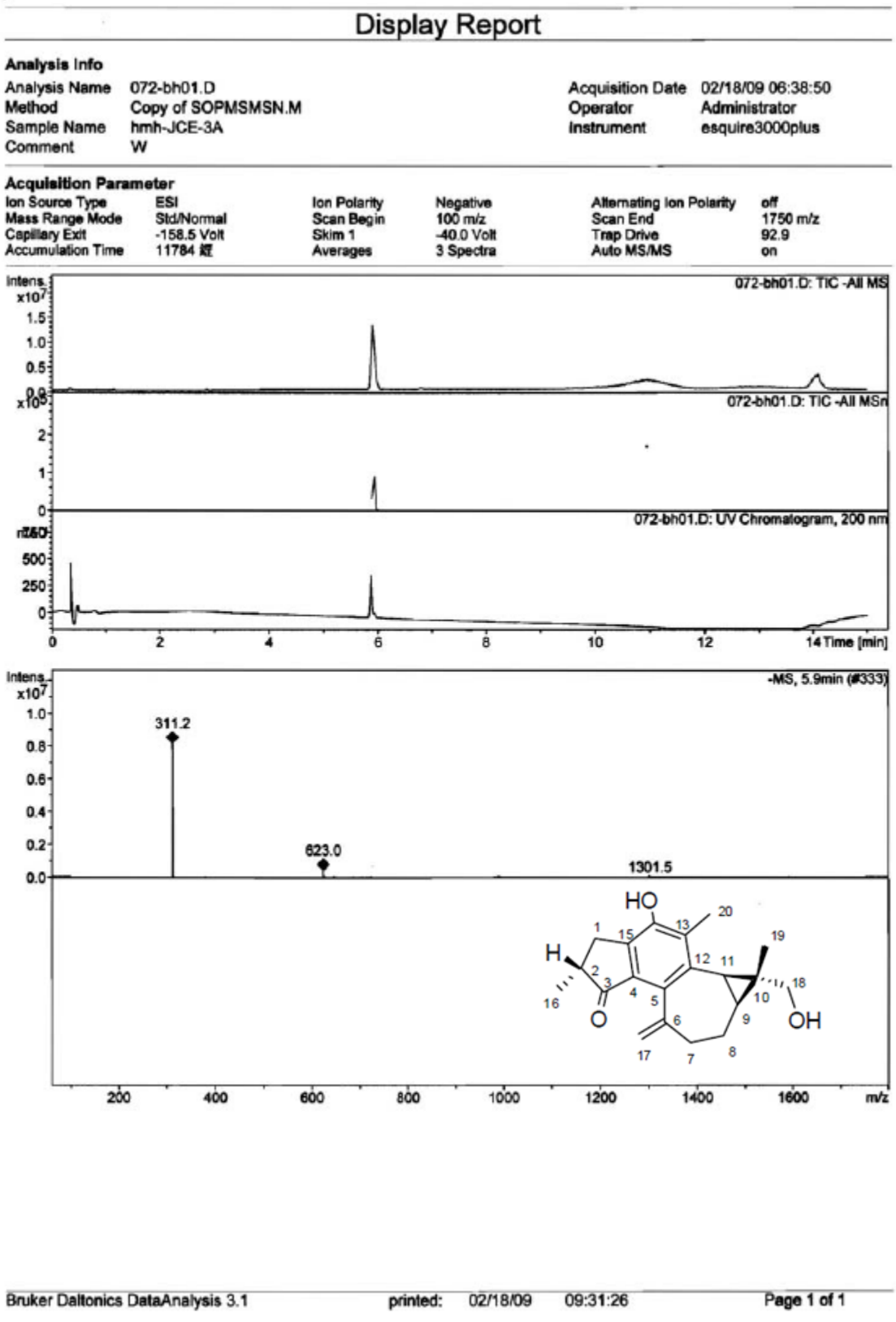


Figure S32. (–)-ESIMS spectrum of jatrophol (**2**).

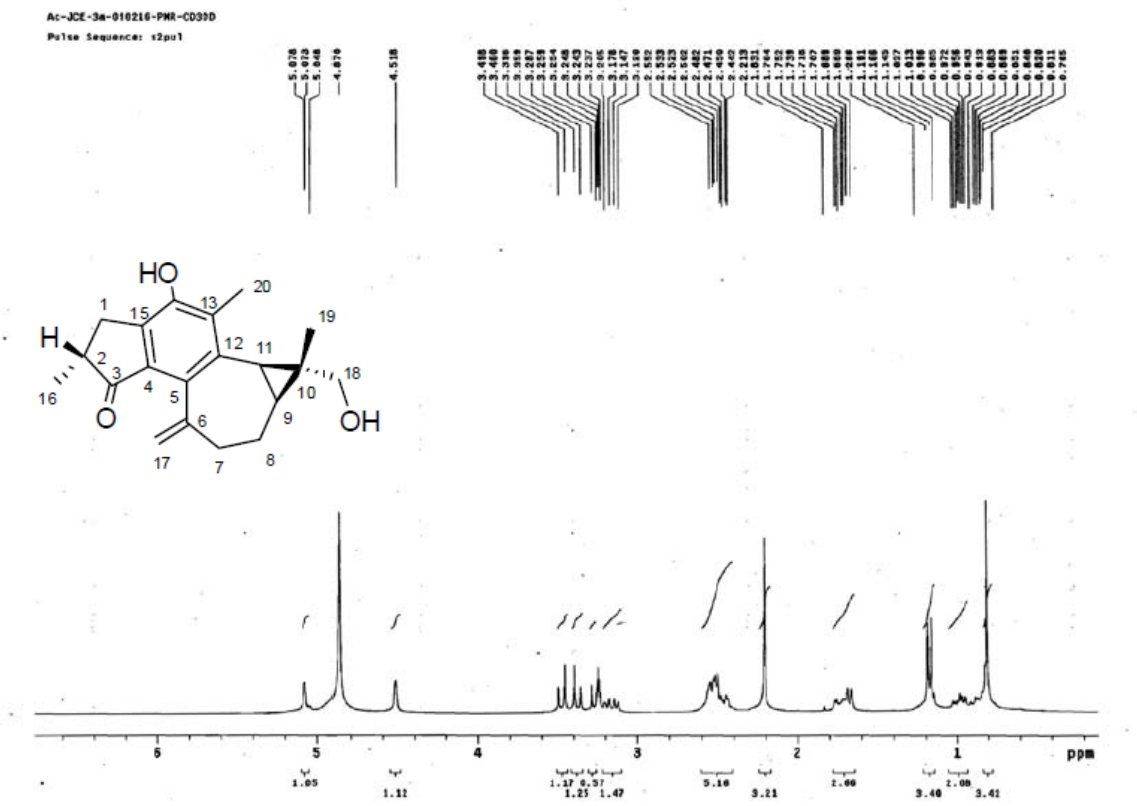


Figure S33. ^{1}H NMR spectrum (300 MHz) of jatrophol (**2**) in CD_3OD.

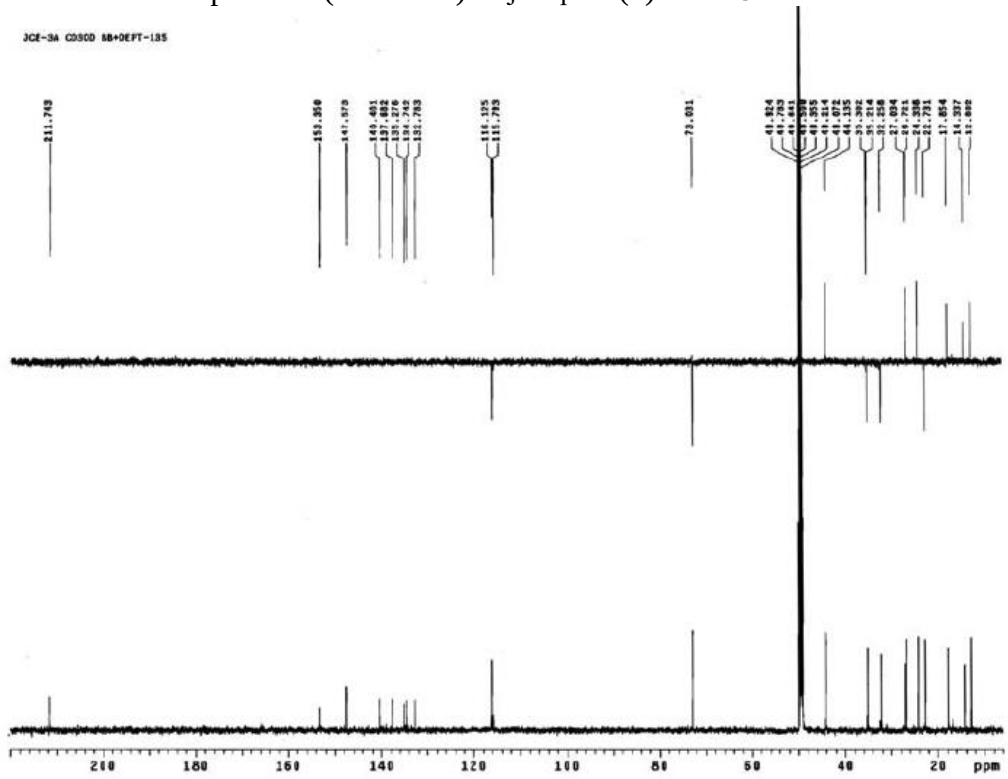


Figure S34. ^{13}C NMR spectrum (75 MHz) of jatrophol (**2**) in CD_3OD.

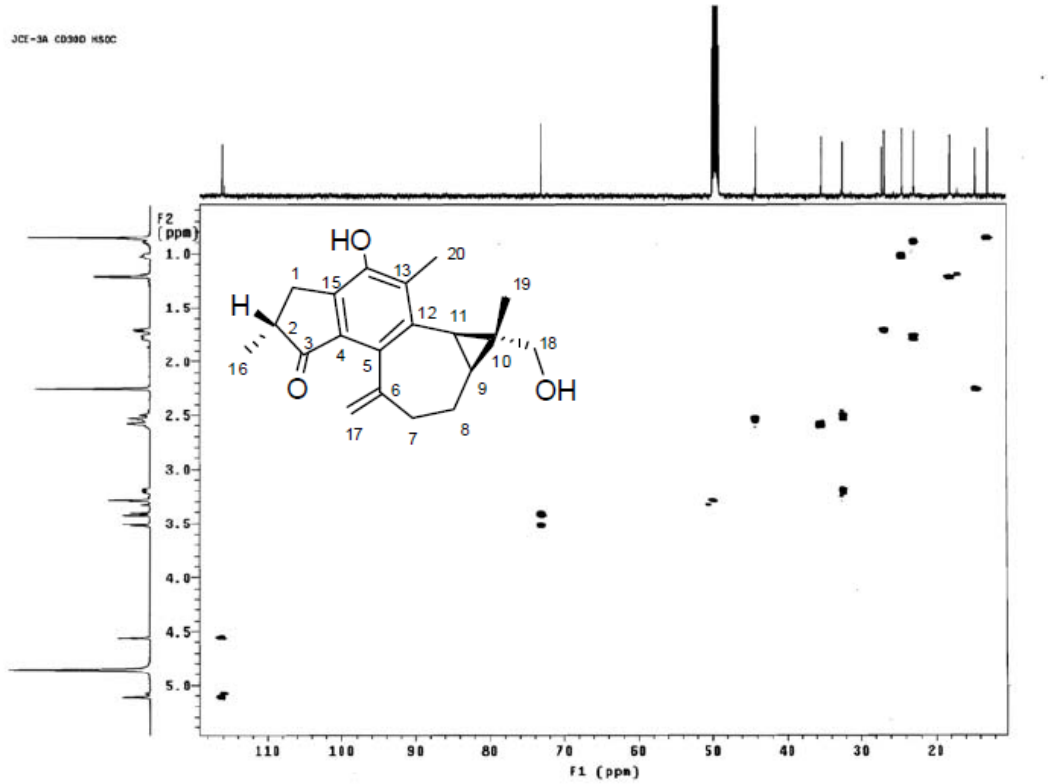


Figure S35. HSQC spectrum (300 MHz) of jatrophol (**2**) in CD_3OD.

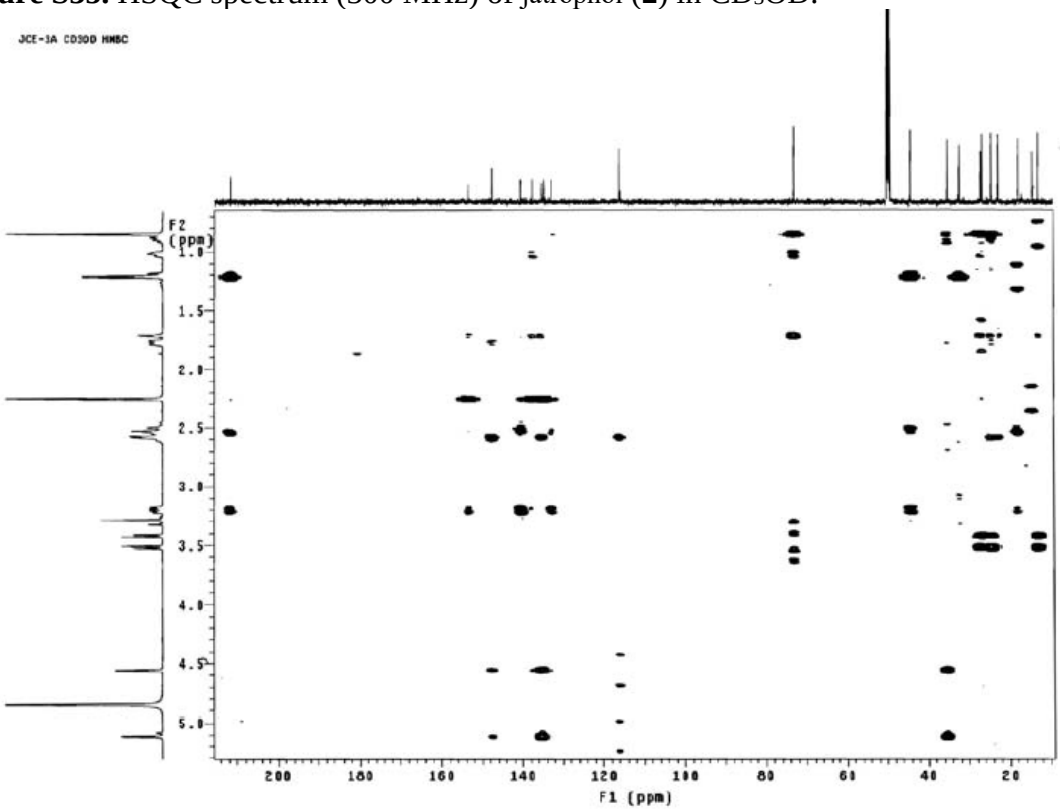


Figure S36. HMBC spectrum (300 MHz) of jatrophol (**2**) in CD_3OD.