Supporting Information

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A New Ingenane Diterpenoid from Euphorbia jolkinii

Wenxing Liu^{1,2}, Ying-Jun Zhang³, Min Zhou^{1*} and Ping Zhao^{2*}

¹ School of Chemistry and Environment, Yunnan Minzu University, Kunming, 650500, China

²Key Laboratory of State Forestry and Grassland Administration on Highly-Efficient Utilization of Forestry Biomass Resources in Southwest China, Southwest Forestry University, Kunming 650224, China

³ State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

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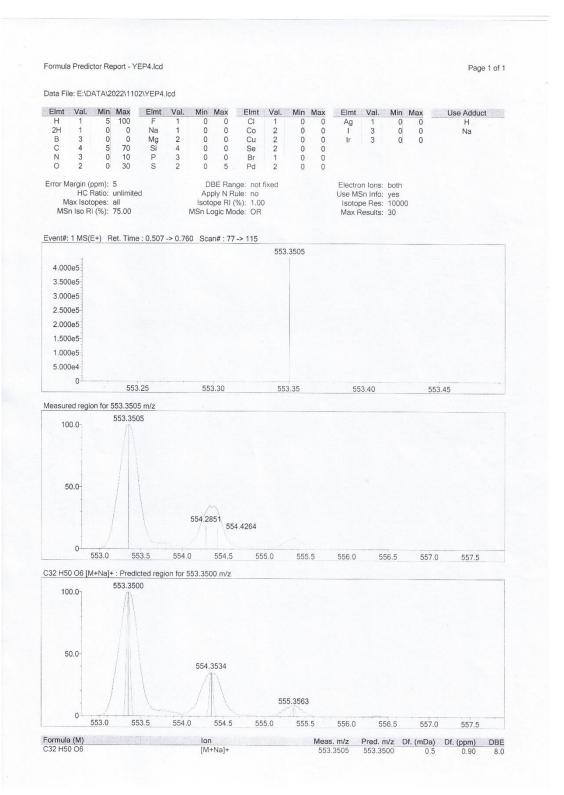


Figure S1: The HR-ESI-MS spectrum of 1

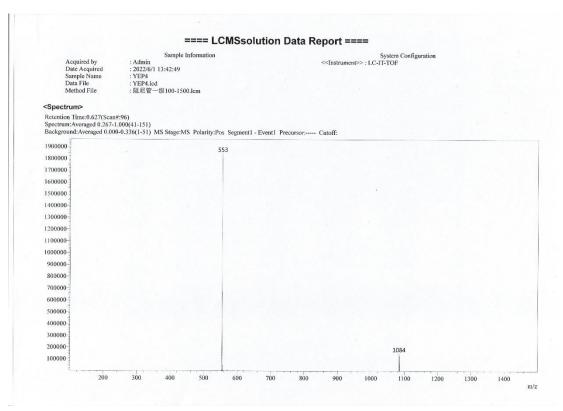


Figure S2: The ESI-MS spectrum of 1

Rudolph Research Analytical

This sample was measured on an Autopol VI, Serial #91058 Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : Friday, 24-FEB-2023

Set Temperature : 20.0 Time Delay : Disabled

Delay between Measurement : Disabled

<u>n</u> 5	<u>Average</u> 7.27	Std.Dev. % RS 0.28 3.85	<u>Maxin</u> 7.67	<u>num</u> <u>Mini</u> 7.00	<u>imum</u>				
S.No	Sample ID	<u>Time</u>	<u>Result</u>	<u>Scale</u>	OR °Arc	WLG.nm	<u>Lg.mm</u>	Conc.g/100m	<u>l Temp.</u>
1	YEP4	12:17:29 PM	7.67	SR	0.023	589	100.00	0.300	20.2
2	YEP4	12:17:35 PM	7.33	SR	0.022	589	100.00	0.300	20.1
3	YEP4	12:17:42 PM	7.00	SR	0.021	589	100.00	0.300	20.1
4	YEP4	12:17:53 PM	7.33	SR	0.022	589	100.00	0.300	20.0
5	YEP4	12:17:59 PM	7.00	SR	0.021	589	100.00	0.300	20.0

Figure S3: The optical rotation of 1

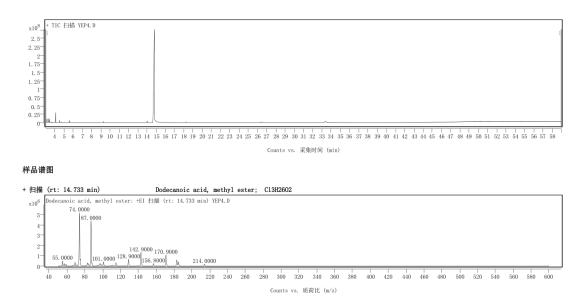


Figure S4: The GC-MS spectrum of the hydrolysis of dodecanoate unit of 1

分析报告									Agilent Trusted Answers		
谱图	识别表										
最佳	ID 来源	名称	分子式	种类	m/z	差 (ppm) CAS	分数	分数 分 (Lib)	数 (DB)	分数 谱库/数据库 (MFG)	
Yes	Lib 检索	Dodecanoic acid, methyl ester	C13H2602			111-82-0	82. 19	82. 19		NIST20. L	
No	Lib 检索	Undecanoic acid, 10- methyl-, methyl ester	C13H2602			5129-56-6	83. 92	83. 92		NIST20. L	
No	Lib 检索	Dodecanoic acid, methyl ester	C13H2602			111-82-0	80. 01	80. 01		NIST20. L	
No	Lib 检索	Dodecanoic acid, methyl ester	C13H2602			111-82-0	79. 27	79. 27		NIST20. L	
No	Lib 检索	Dodecanoic acid, methyl ester	C13H2602			111-82-0	78. 38	78.38		NIST20. L	
No	Lib 检索	Dodecanoic acid, 2- methyl-	C13H2602			2874-74-0	76. 21	76. 21		NIST20. L	
No	Lib 检索	Dodecanoic acid, 2- methyl-	C13H2602			2874-74-0	71. 29	71. 29		NIST20. L	
No	Lib 检索	Methyl 11-methyl- dodecanoate	C14H2802			1000336- 45-1	69. 93	69. 93		NIST20. L	
No	Lib 检索	Undecanoic acid, 10- methyl-, methyl ester	C13H2602			5129-56-6	69. 88	69. 88		NIST20. L	
No	Lib 检索	Methyl 13- methyltetradecanoate	C16H32O2			1000424- 50-7	68. 10	68. 10		NIST20. L	
最佳	名称	分子式	m/z (prec.) CAS	RT (DB)	RT 差 分数	分数 (Lib)	分数 (Fwd)	分数(Rev	v) 谱库/数据库	
Yes	Dodecanoic ester	acid, methyl C13H2602		111-82-0	24. 683	82. 19	82. 19			NIST20. L	

Figure S5: The library search data of hydrolysis of dodecanoate unit of **1** GC-MS analysis detail

Column: DB-5MS capillary column (30 m \times 0.25 mm, 0.25 μ m); injector temperature: 250 °C; initial temperature: 40 °C, increased at 40 °C/min to 120 °C, held for 5 min; increased at 5 °C/min to 240 °C, held for 5 min; increased at 5 °C/min to 300 °C, held for 10 min; carrier gas: helium; flow rate: 1mL/min; injection volume: 1 μ L. Mass: El voltage, 70 eV; Mass scanned-mass range: m/z 50-600.

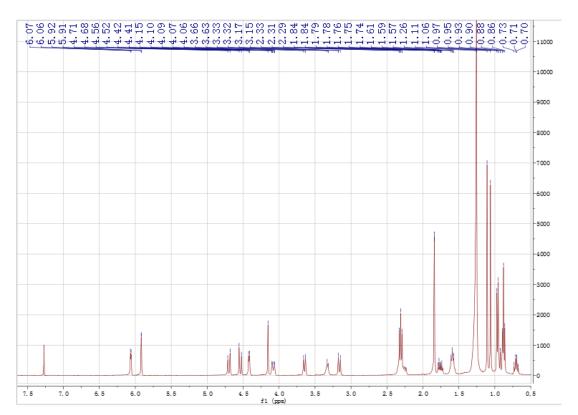


Figure S6: The ¹H-NMR (400 MHz, CDCl₃) spectrum of 1

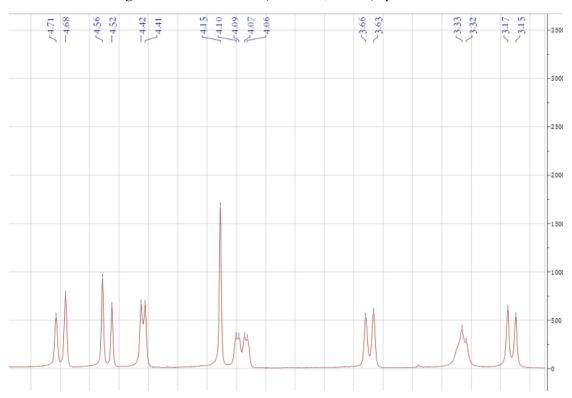


Figure S7: The ¹H-NMR (400 MHz, CDCl₃) spectrum of **1** (From $\delta_{\rm H}$ 3.0 ppm to $\delta_{\rm H}$ 4.8 ppm)

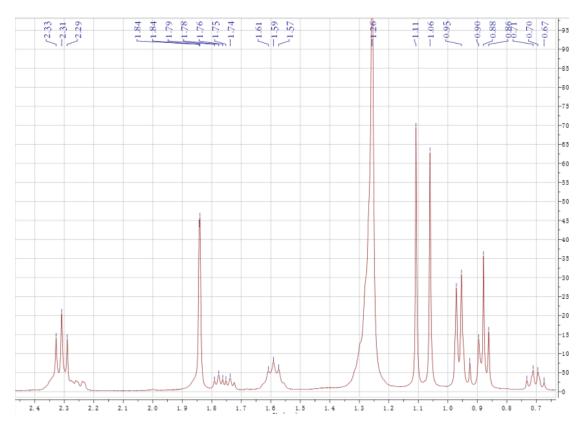


Figure S8: The ¹H-NMR (400 MHz, CDCl₃) spectrum of **1** (From $\delta_{\rm H}$ 0 ppm to $\delta_{\rm H}$ 2.4 ppm)

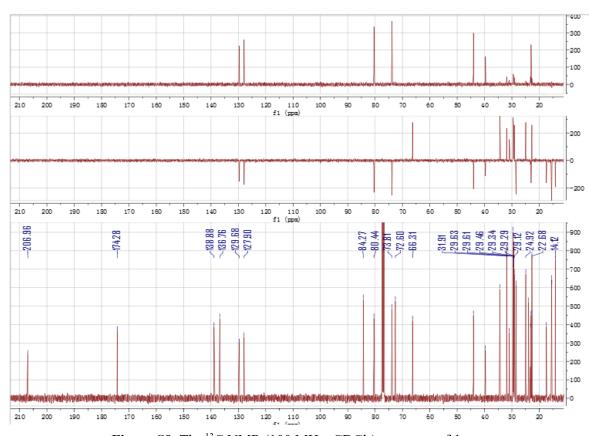


Figure S9: The ¹³C NMR (100 MHz, CDCl₃) spectrum of 1

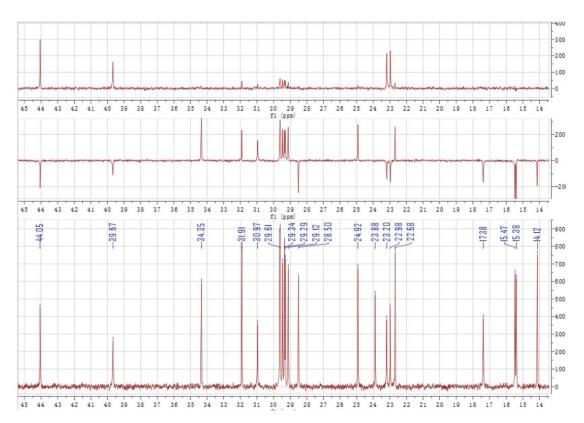


Figure S10: The ¹³C NMR (100 MHz, CDCl₃) spectrum of **1** (From δ_C 14 ppm to δ_C 45 ppm)

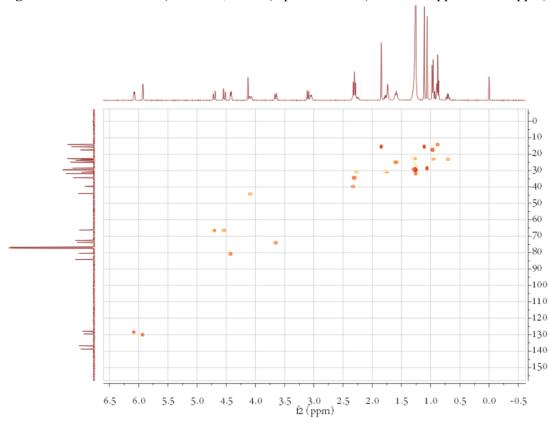


Figure S11: The HSQC spectrum of 1

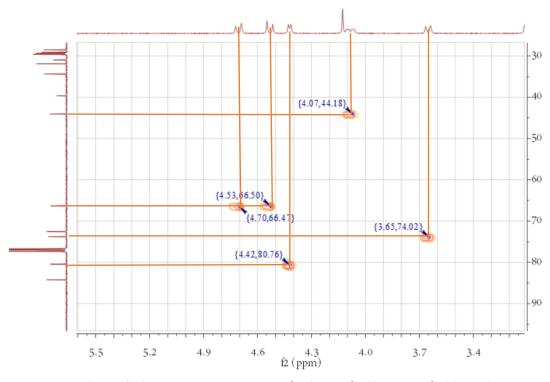


Figure S12: The HSQC spectrum of **1** (From δ_C 40 ppm to δ_C 90 ppm)

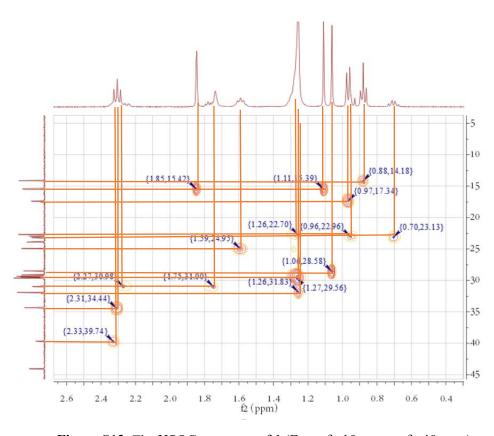


Figure S13: The HSQC spectrum of **1** (From δ_C 10 ppm to δ_C 40 ppm)

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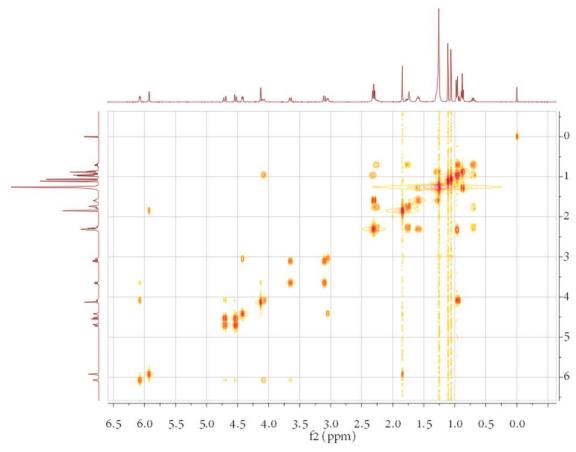


Figure S14: The ¹H-¹H COSY spectrum of 1

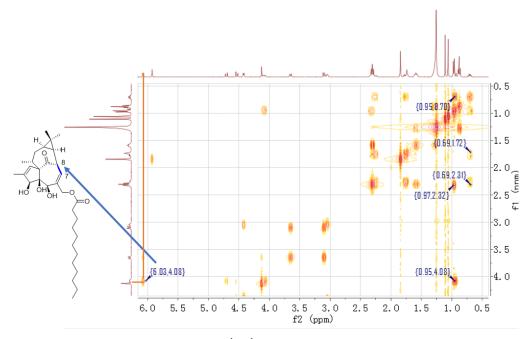


Figure S15: The ¹H-¹H COSY of H-7/H-8 of 1

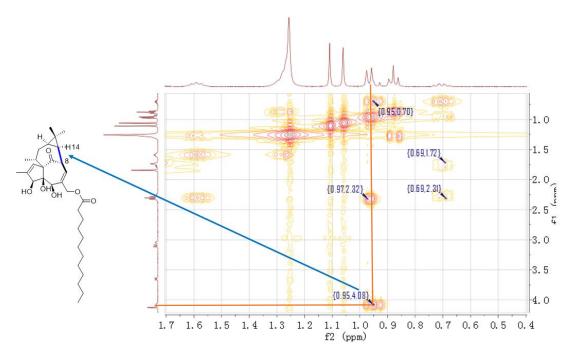


Figure S16: The ¹H-¹H COSY of H-8/H-14 of 1

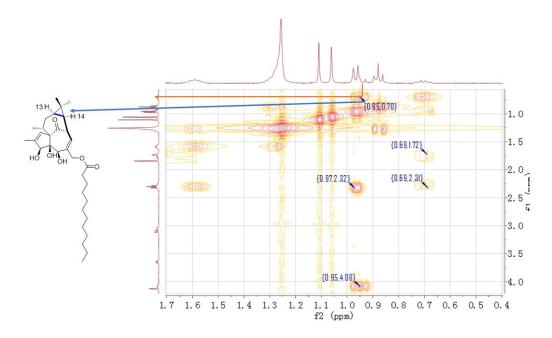


Figure S17: The ${}^{1}\text{H}$ - ${}^{1}\text{H}$ COSY of H-13/H-14 of 1

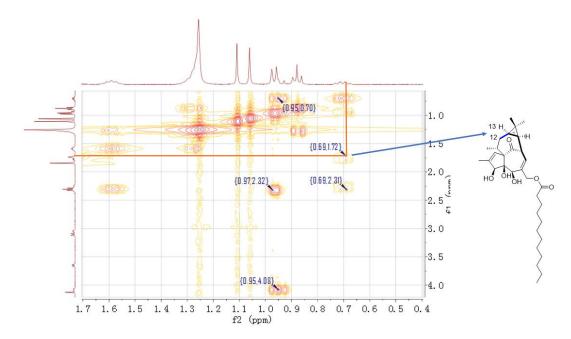


Figure S18: The ¹H-¹H COSY of H-12/H-13 of **1**

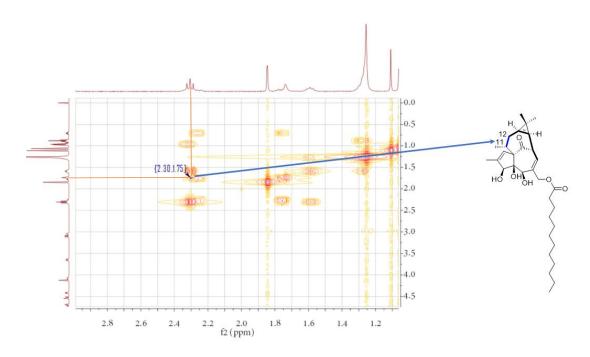


Figure S19: The ¹H-¹H COSY of H-11/H-12 of **1**

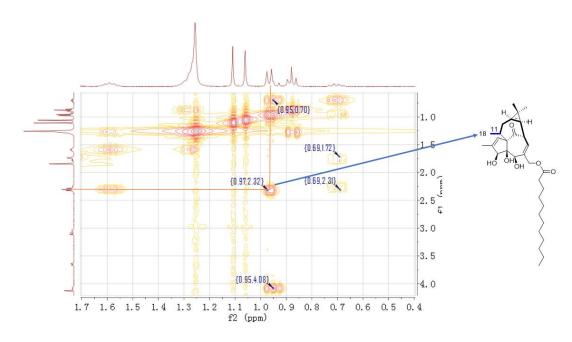


Figure S20: The ${}^{1}H$ - ${}^{1}H$ COSY of H-11/C \underline{H}_{3} -18 of 1

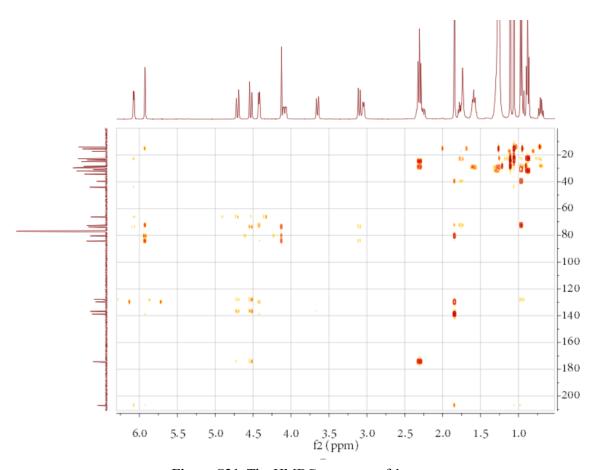


Figure S21: The HMBC spectrum of 1

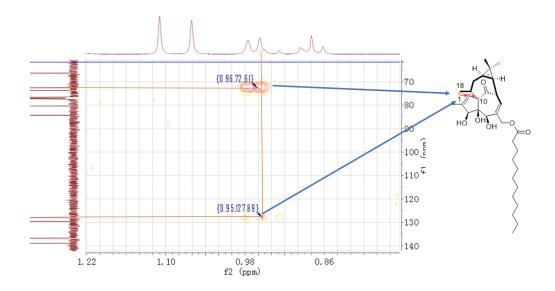


Figure S22: The HMBC correlations of $C\underline{H_3}$ -18 to C-1 and C-10 of 1

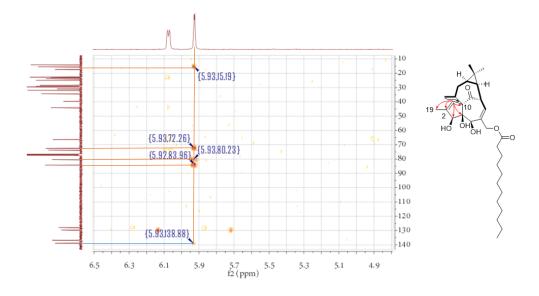


Figure S23: The HMBC correlations of H-1 to C-2, C-3, C-4, C-10 and C-19 of 1

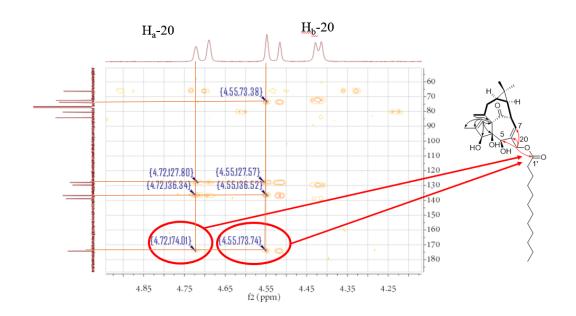


Figure S24: The HMBC correlations of H₂-20 to C-5, C-6, C-7, and C-1' of 1

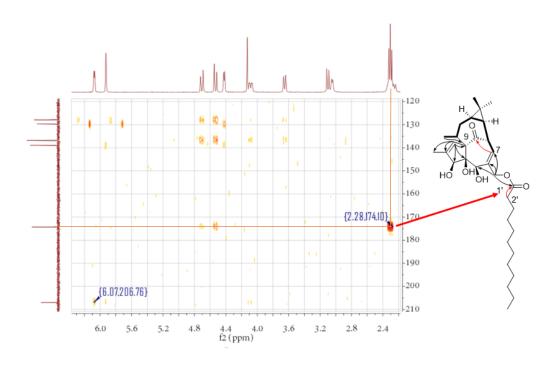


Figure S25: The HMBC correlations of H₂-2' to C-1' of 1

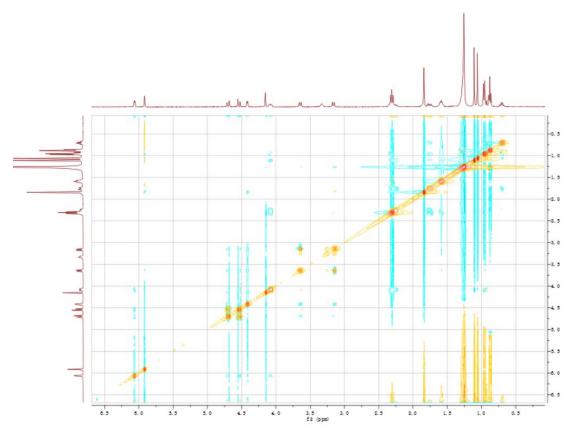


Figure S26: The ROESY spectrum of 1

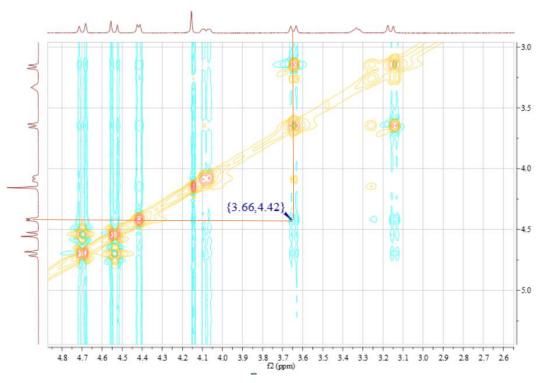


Figure S27: The ROESY correlation of H-5/H-3 of 1

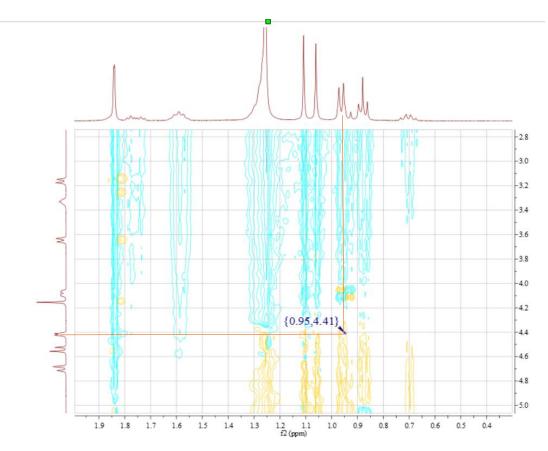


Figure S28: The ROESY correlation of H-3/C $\underline{\text{H}}_3$ -18 of 1

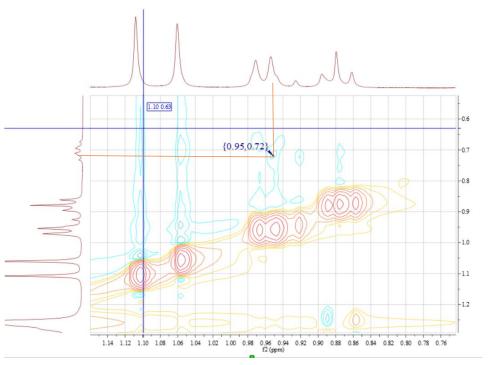


Figure S29: The ROESY correlation of $C\underline{H}_3$ -18/H-13 of 1

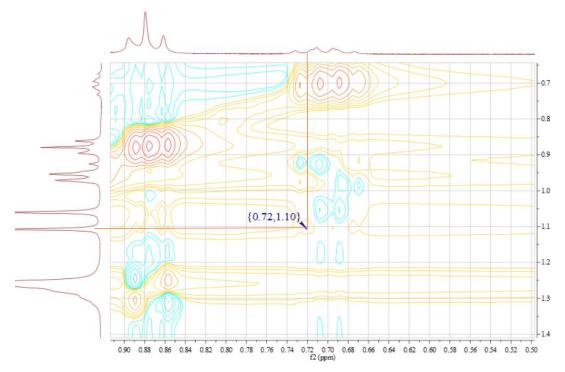


Figure S30: The ROESY correlation of CH₃-17/H-13 of 1

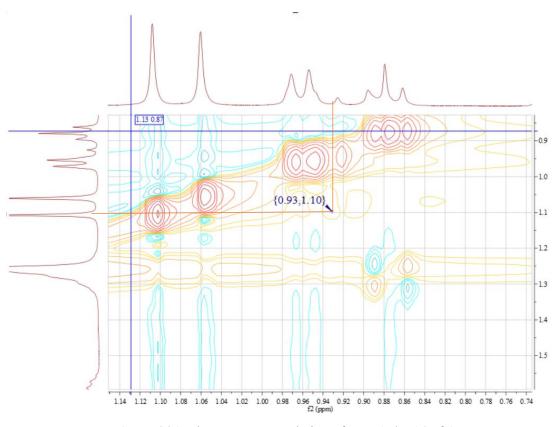
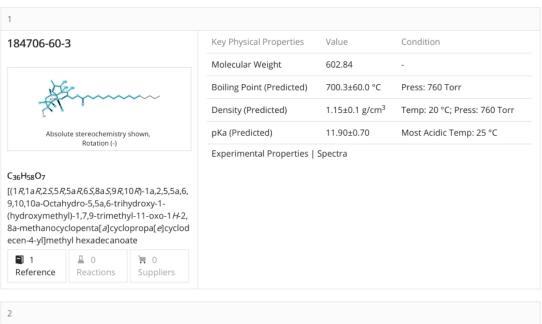


Figure S31: The ROESY correlation of $C\underline{H_3}$ -17/H-14 of 1



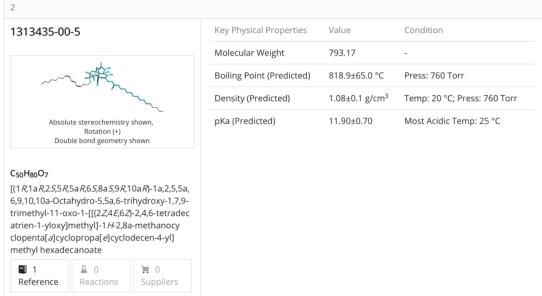


Figure S32: The exact search report from scifinder of 1

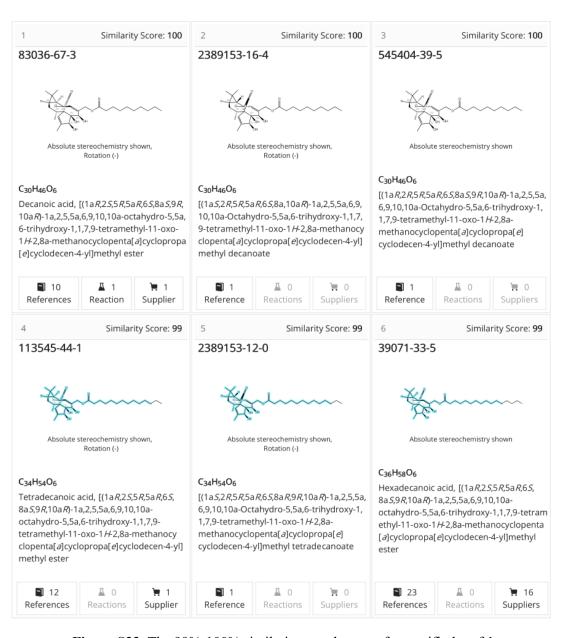


Figure S33: The 99%-100% similarity search report from scifinder of 1

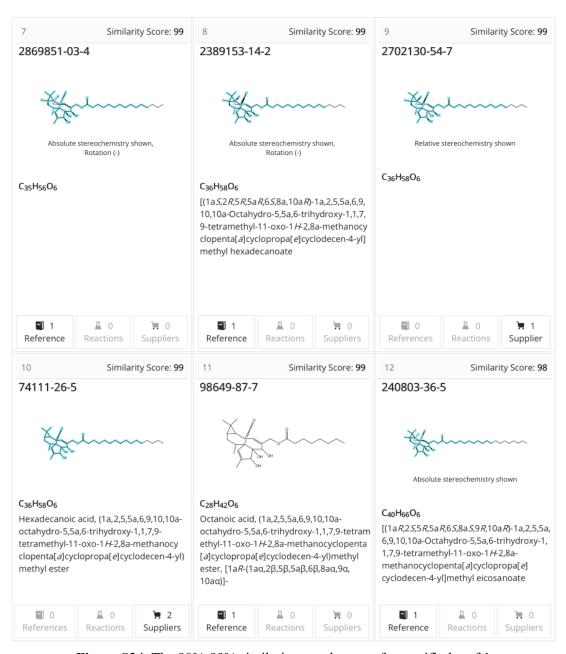


Figure S34: The 98%-99% similarity search report from scifinder of 1

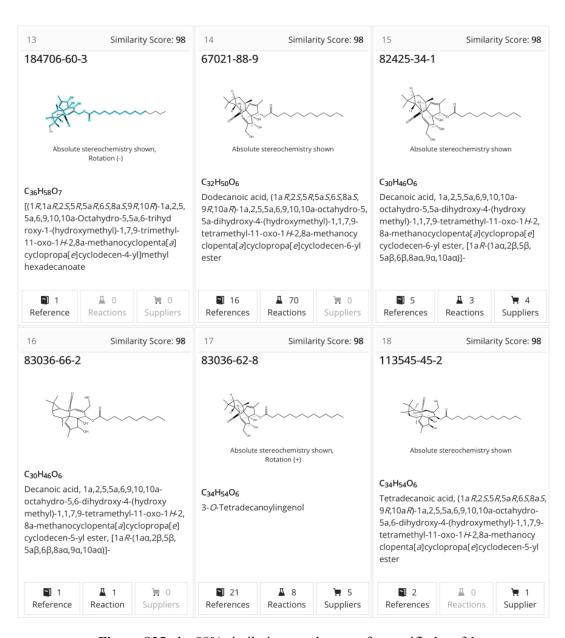


Figure S35: the 98% similarity search report from scifinder of 1

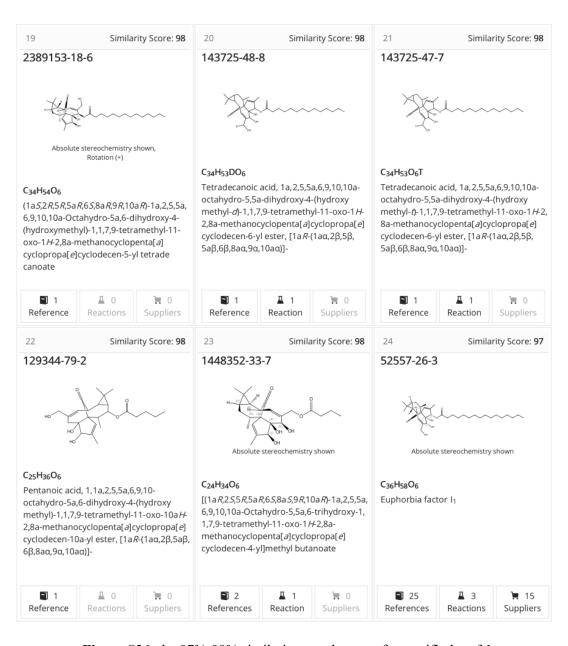


Figure S36: the 97%-98% similarity search report from scifinder of 1

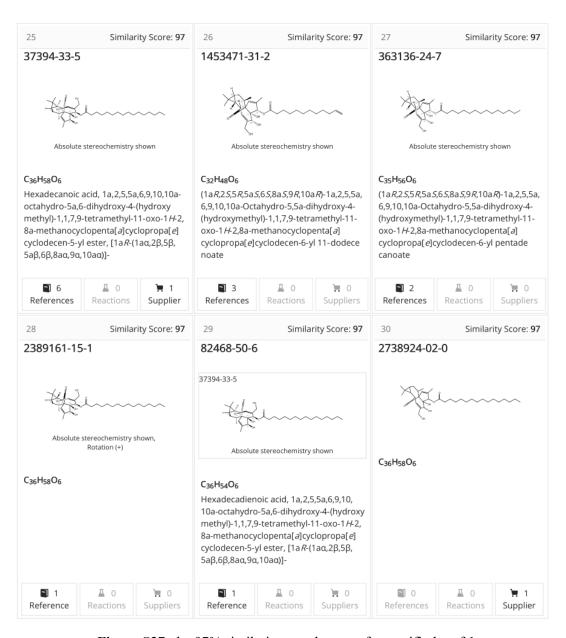


Figure S37: the 97% similarity search report from scifinder of 1

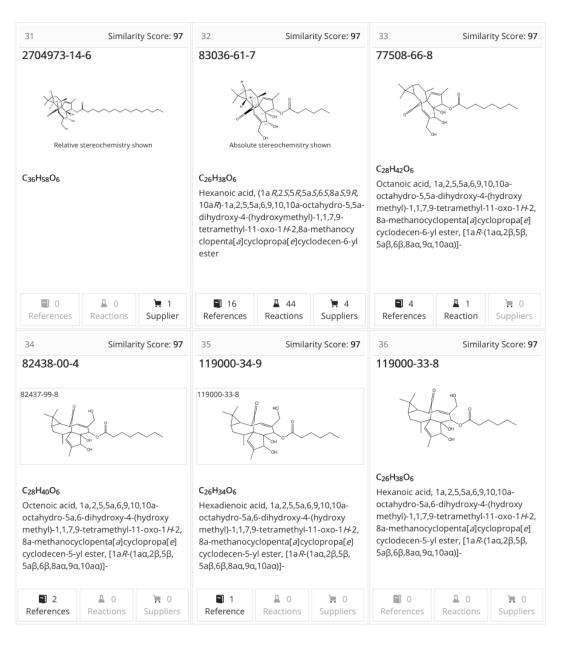


Figure S38: the 97% similarity search report from scifinder of 1

Juli made o

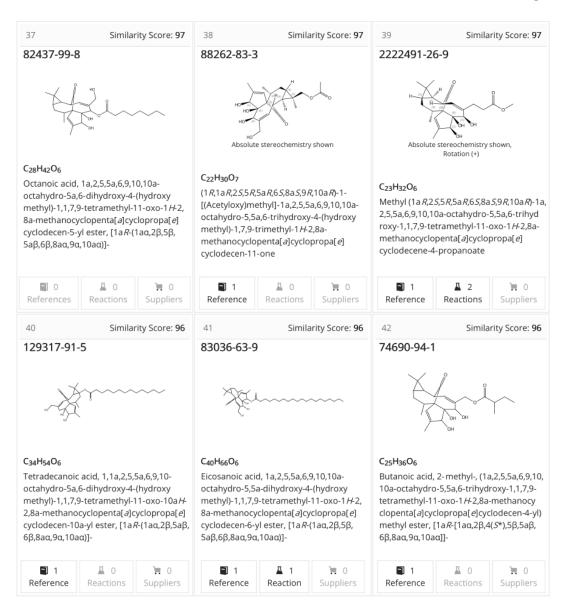


Figure S39: the 96%-97% similarity search report from scifinder of 1

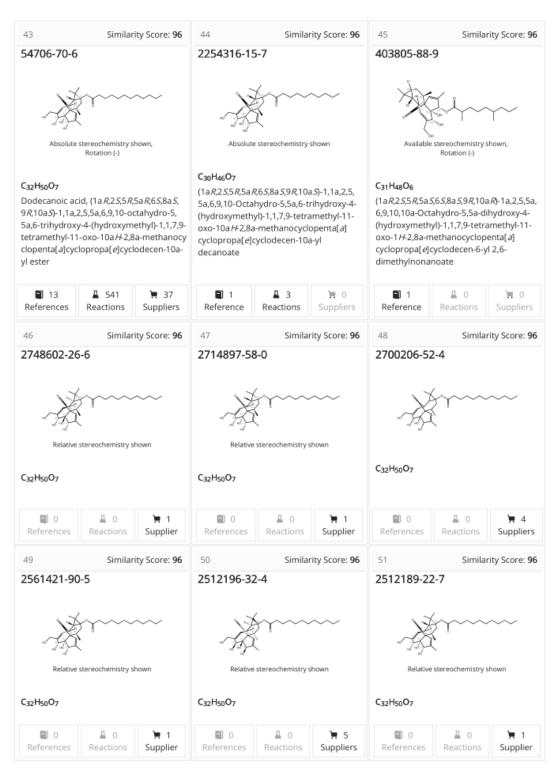


Figure S40: the 96% similarity search report from scifinder of 1

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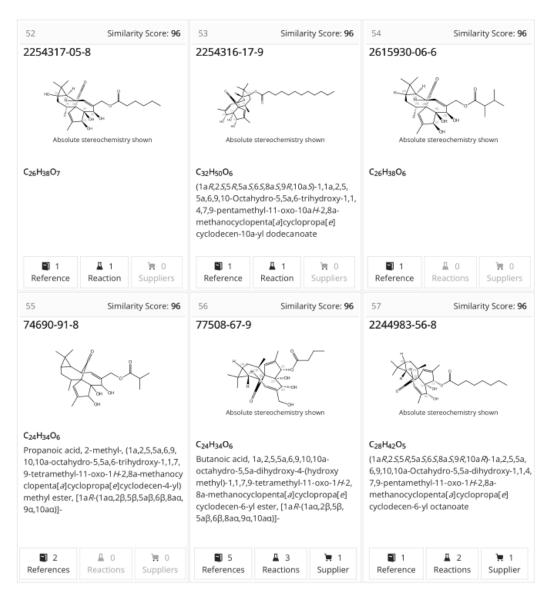
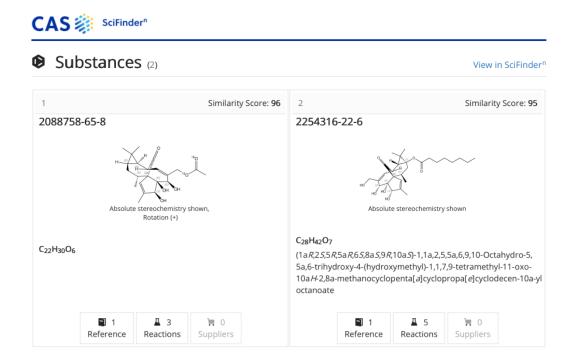


Figure S41: the 96% similarity search report from scifinder of 1

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Figure S42: the 95% similarity search report from scifinder of 1



Figure S43: The image of the *Euphorbia jolkinii*The herbarium number of *Euphorbia jolkinii* registered at https://sweetgum.nybg.org/science/ih/ was 4048695.

Table S1: The NMR data of the compound 1-3 (1 H NMR 400 MHz, 13 C NMR100 MHz, in CDCl₃)*

	1	2	3	1 2		3	
	$\delta_{ m C}$	$\delta_{\!\scriptscriptstyle m C}$	$\delta_{\!\scriptscriptstyle m C}$	$\delta_{\! ext{H}}$	$\delta_{\! ext{H}}$	$\delta_{\! ext{H}}$	
1	129.7	129.1	129.3	5.92, d (1.5)	5.90, br.s	5.90, br.s	
2	138.9	139.0	139.0				
3	80.4	80.1	80.2	4.42, d (5.5)	4.42, d (5.5)	4.42, d (5.5)	
4	84.3	84.2	84.2				
5	73.8	73.8	73.8	3.65, d (10.6)	3.64, d (11.0)	3.64, d (10.9)	
6	136.8	136.8	136.7				
7	127.9	127.4	127.6	6.07, d (3.7)	6.05, d (3.9)	6.05, d (3.7)	
8	44.0	44.0	44.0	4.07, dd (3.8, 11.7)	4.08, dd (3.8, 11.5)	4.08, dd (3.8, 11.7)	
9	207.0	207.5	207.3				
10	72.6	72.6	72.6				
11	39.7	39.5	39.6	2.32, m	2.32, m	2.32, m	
12	31.0	30.9	30.9	2.25, m	2.25, m	2.25, m	
				1.74, brs	1.74, brs	1.74, brs	
13	23.2	23.2	23.1	0.70, dd (8.5, 14.9)	0.70, dd (8.2, 15.0)	0.70, dd (8.5, 14.9)	
14	23.0	22.9	22.9	0.94, m	0.94, m	0.94, m	
15	23.9	23.8	23.9				
16	28.5	28.5	28.5	1.06, s	1.06, s	1.06, s	
17	15.5	15.5	15.5	1.11, s	1.11, s	1.11, s	
18	17.4	17.4	17.4	0.97, d (6.9)	0.95, d (6.9)	0.97, d (6.9)	
19	15.4	15.4	15.4	1.84, d (1.1)	1.83, br.s	1.83, br.s	
20	66.3	66.3	66.3	4.70, d (12.9)	4.69, d (12.9)	4.69, d (12.9)	
				4.53, d (12.9)	4.54, d (12.9)	4.54, d (12.9)	
1′	174.3	174.5	174.4				
2'	34.4	34.3	34.3	2.30, t (6.5)	2.30, t (6.5)	2.30, t (6.5)	
3′	24.9	24.9	24.9	1.59, m	1.59, m	1.59, m	
4′	29.1	29.3	29.3	1.26, m	1.25, m	1.26, m	
5′	29.3	29.5	29.5	1.26, m	1.25, m	1.26, m	
6′	29.6	29.7	29.7	1.26, m	1.25, m	1.26, m	
7′	29.6	29.7	29.7	1.26, m	1.25, m	1.26, m	

8′	29.5	29.7	29.7	1.26, m	1.25, m	1.26, m
9′	29.3	29.7	29.6	1.26, m	1.25, m	1.26, m
10'	31.9	29.7	29.6	1.26, m	1.25, m	1.26, m
11'	22.7	29.7	29.6	1.26, m	1.25, m	1.26, m
12'	14.1	29.7	29.5	0.88, t (6.8)	1.25, m	1.26, m
13′		29.7	22.7		1.25, m	1.26, m
14′		29.5	14.1		1.25, m	0.88, t (6.8)
15'		22.7			1.25, m	
16′		14.1			0.88, t (6.7)	

As the ¹H NMR and ¹³C NMR data shown in the Table S1, these data were very similar, while the differences were the molecular formula of compound **2** was $C_{36}H_{58}O_6$ by ESI-MS m/z 585 ([M-H]⁻), and compound **3** was $C_{34}H_{54}O_6$ by ESI-MS m/z 1139 ([2M+Na]⁺).

S1:Cytotoxic Activity Assay

Firstly, prepared the single cell suspension with the 10% fetal bovine serum (DMEM or RMPI1640), inoculated 5000 cells each well onto the 96-well plate, and the volume of each well was $100 \,\mu\text{L}$. Cells were inoculated and cultured 24 hours in advance. Secondly, the testing compound was dissolved with DMSO in $40 \,\mu\text{M}$, $8 \,\mu\text{M}$, $1.6 \,\mu\text{M}$, $0.32 \,\mu\text{M}$ and $0.064 \,\mu\text{M}$, the final volume of each hole was $200 \,\mu\text{L}$. After 48 hours of culturing at 37 °C, removed the culture solution in the hole, and added $20 \,\mu\text{L}$ MTS solution to each hole and culture solution $100 \,\mu\text{L}$, set 3 blank holes. Then hatched for 4 hours to measure the light absorption value after the reaction was fully carried out. Finally, set the wavelength of the multifunctional enzyme marker at 492 nm to read the light absorption value, and then calculated IC50 value of the tested compound by the Reed and Muench method, the Positive control were cisplatin (DDP) and paclitaxel (Taxol).