

# 1 SUPPLEMENTARY MATERIAL

## 2 Two new homoisoflavones from *Portulaca oleracea* L. and their activities

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### 21 Abstract

22 Two new compounds, identified as  
23 3-(2-hydroxybenzyl)-6,8-dimethoxy-4H-chromen-4-one (**1**), named oleracone J and  
24 3-(2-hydroxybenzyl)-6,8-dimethoxychroman-4-one (**2**), named oleracone K, were  
25 isolated from *Portulaca oleracea* L., and the structures of them were determined by

26 spectroscopy, including one- and two-dimensional nuclear magnetic resonance,  
27 high-resolution electrospray ionization time-of-flight mass spectrometry. The two  
28 compounds have scavenging activities in 1,1-diphenyl-2-picryl-hydrazyl (DPPH)  
29 radical quenching assay, with IC<sub>50</sub> values of 18.34, 23.92 μM, and anticholinesterase  
30 activities with IC<sub>50</sub> values of 59.08, 67.89 μM, respectively.

31 **Keywords:** *Portulaca oleracea* L.; homoisoflavone; antioxidant activity;  
32 anticholinesterase activity

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### 34 **Supporting information**

35 Supplementary material relating to this article is available online, alongside Tables  
36 S1-S3 and Figure S1-S23.

37 Table S1. Full NMR data for oleracone J (**1**) in CDCl<sub>3</sub>

38 Table S2. Full NMR data for oleracone K (**2**) in CDCl<sub>3</sub>

39 Table S3. IC<sub>50</sub> (μM) for antioxidant and acetylcholinesterase activities of compounds  
40 **1** and **2** (*n* = 5)

41 Figure S1. Key HMBC correlations of oleracone J (**1**)

42 Figure S2. Key <sup>1</sup>H-<sup>1</sup>H COSY and ROESY correlations of oleracone J (**1**)

43 Figure S3. Key HMBC correlations of oleracone K (**2**)

44 Figure S4. Key <sup>1</sup>H-<sup>1</sup>H COSY and ROESY correlations of oleracone K (**2**)

45 Figure S5. <sup>1</sup>H NMR (600 MHz) spectrum of oleracone J (**1**) in CDCl<sub>3</sub>

46 Figure S6. <sup>13</sup>C NMR (150 MHz) spectrum of oleracone J (**1**) in CDCl<sub>3</sub>

47 Figure S7. DEPT spectrum of oleracone J (**1**) in CDCl<sub>3</sub>

48 Figure S8. HSQC spectrum of oleracone J (**1**) in CDCl<sub>3</sub>

49 Figure S9. HMBC spectrum of oleracone J (**1**) in CDCl<sub>3</sub>

50 Figure S10. <sup>1</sup>H-<sup>1</sup>H COSY spectrum of oleracone J (**1**) in CDCl<sub>3</sub>

51 Figure S11. ROESY spectrum of oleracone J (**1**) in CDCl<sub>3</sub>

52 Figure S12. UHPLC-ESI-Q-TOF/MS of oleracone J (**1**)

53 Figure S13. <sup>1</sup>H NMR (600 MHz) spectrum of oleracone K (**2**) in CDCl<sub>3</sub>

54 Figure S14. <sup>13</sup>C NMR (150 MHz) spectrum of oleracone K (**2**) in CDCl<sub>3</sub>

55 Figure S15. DEPT spectrum of oleracone K (**2**) in CDCl<sub>3</sub>

56 Figure S16. HSQC spectrum of oleracone K (**2**) in CDCl<sub>3</sub>

57 Figure S17. HMBC spectrum of oleracone K (**2**) in CDCl<sub>3</sub>

58 Figure S18. <sup>1</sup>H-<sup>1</sup>H COSY spectrum of oleracone K (**2**) in CDCl<sub>3</sub>

59 Figure S19. ROESY spectrum of oleracone K (**2**) in CDCl<sub>3</sub>

60 Figure S20. UHPLC-ESI-Q-TOF/MS of oleracone K (**2**)

61 Figure S21. DPPH radical scavenging activities of compounds **1-2** and BHA (*n* = 5)

62 Figure S22. Anticholinesterase effect of compounds **1-2** and Eserine (*n* = 5)

63 Figure S23. Compounds **1-2** binding mode to AChE

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76 Table S1. Full NMR data for oleracone J (**1**) in CDCl<sub>3</sub>

Position	$\delta_C$	Type	$\delta_H$ , mult (J in Hz)	$^1H$ - $^1H$ COSY	HMBC	ROESY
1		O				
2	151.1	CH	7.87 s	9	3, 4, 8a, 9	9
3	125.5	C				
4	178.7	C				
4a	108.9	C				
5	92.6	CH	6.42 d (2.2)	7	4, 4a, 6, 7, 8a	6-OMe, 7
6	164.8	C				
6-OMe	56.3	CH <sub>3</sub>	3.88 s		6	5
7	96.6	CH	6.35 d (2.2)	5, 8-OMe	4a, 5, 6, 8	5, 8-OMe
8	161.3	C				
8-OMe	56.6	CH <sub>3</sub>	3.93 s	7	8	7
8a	160.7	C				
9	27.5	CH <sub>2</sub>	3.66 s	2, 6'	2, 3, 4, 1', 2', 6'	2, 6'
1'	126.2	C				
2'	155.4	C				
2'-OH			8.23 brs			
3'	118.7	CH	6.94 dd (8.1, 14.1)	4'	1', 2', 5'	4'
4'	128.6	CH	7.12 m	3', 5', 6'	2', 3', 5', 6'	3', 5', 6'
5'	120.3	CH	6.82 t (7.1)	4', 6'	1', 3', 4', 6'	4', 6'
6'	130.2	CH	7.10 m	9, 4', 5'	9, 1', 2', 4', 5'	9, 4', 5'

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88 Table S2. Full NMR data for oleracone K (**2**) in CDCl<sub>3</sub>

Position	$\delta_C$	Type	$\delta_H$ , mult (J in Hz)	<sup>1</sup> H- <sup>1</sup> H COSY	HMBC	ROESY
1		O				
2	70.5	CH <sub>2</sub>	a: 4.17 t (11.2) b: 4.53 dd (4.9, 11.2)	2b, 3, 9a, 9b 2a, 3, 9b	3, 4, 8a, 9 3, 4, 8a, 9	2b, 3, 9a, 9b 2a, 3, 9a, 9b
3	47.8	CH	3.04 m	2a, 2b, 9a, 9b	2, 4, 9, 1'	2a, 2b, 9a, 9b
4	194.1	C				
4a	105.5	C				
5	93.4	CH	6.05 q (2.3, 3.7)	7	4, 4a, 6, 7, 8a	6-OMe, 7
6	166.7	C				
6-OMe	55.8	CH <sub>3</sub>	3.83 s		6	5
7	93.1	CH	6.05 q (2.3, 3.7)	5, 8-OMe	5, 6, 8, 8a	5, 8-OMe
8	162.8	C				
8-OMe	56.0	CH <sub>3</sub>	3.88 s	7	8	7
8a	165.6	C				
9	26.9	CH <sub>2</sub>	a: 2.79 q (6.6, 16.7) b: 3.06 m	2a, 3, 9b 2a, 2b, 3, 9a	2, 3, 4, 1', 2', 6' 2, 3, 4, 1', 2', 6'	2a, 2b, 3, 9b, 6' 2a, 2b, 3, 9a
1'	125.2	C				
2'	155.1	C				
2'-OH			9.65 brs			
3'	117.8	CH	6.94 dd (8.1, 14.1)	4'	1', 2', 5'	4'
4'	128.6	CH	7.13 m	3', 5', 6'	2', 3', 5', 6'	3', 5', 6'
5'	120.4	CH	6.82 t (7.1)	4', 6'	1', 3', 4', 6'	4', 6'
6'	131.0	CH	7.04 dd (0.9, 7.1)	4', 5'	9, 1', 2', 4'	9a, 4', 5'

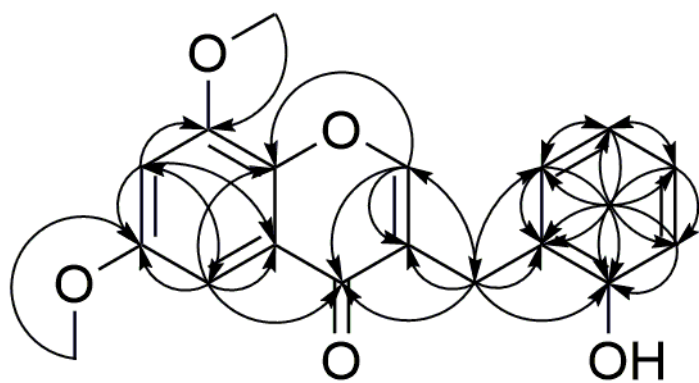
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90 Table S3. IC<sub>50</sub> (μM) for antioxidant and acetylcholinesterase activities of compounds

Compounds and standard Inhibitors	DPPH IC <sub>50</sub> (μM)	AChE IC <sub>50</sub> (μM)
1	18.34 ± 0.03	59.08 ± 0.05
2	23.92 ± 0.01	67.89 ± 0.09
Eserine	-	33.26 ± 0.03
BHA	57.41 ± 0.05	-

91 Eserine and BHA as positive control, and values are expressed as the means ± SD for

92 *n* = 5.



HMBC (H  $\longrightarrow$  C)

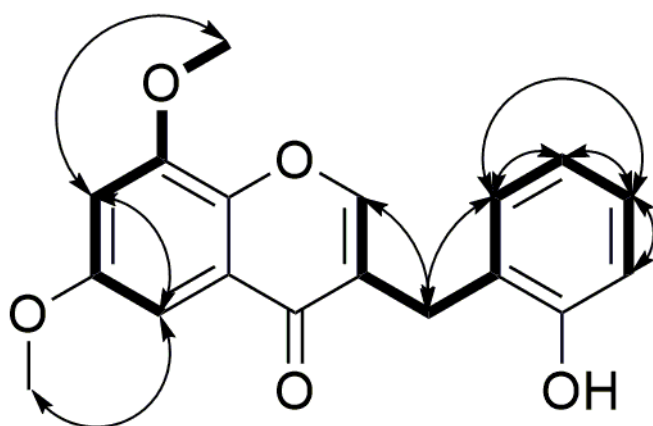
Oleracone J (1)

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95 Figure S1. Key HMBC correlations of oleracone J (1)

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ROESY  $\curvearrowright$

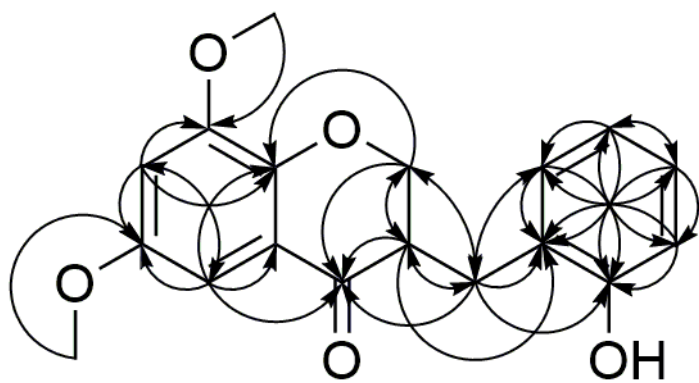
COSY  $\text{—}$

Oleracone J (1)

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99 Figure S2. Key  $^1\text{H}$ - $^1\text{H}$  COSY and ROESY correlations of oleracone J (1)



HMBC (H  $\longrightarrow$  C)

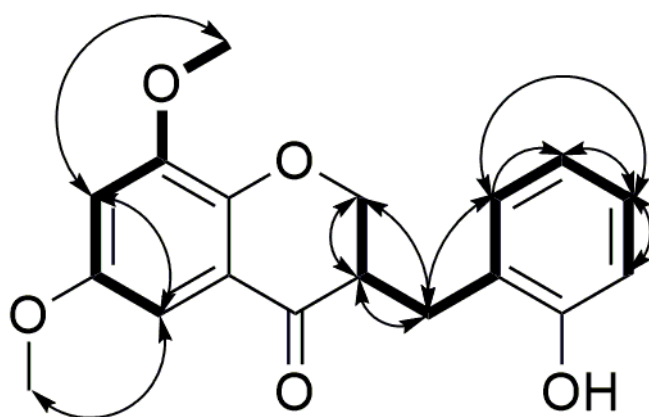
Oleracone K (**2**)

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102 Figure S3. Key HMBC correlations of oleracone K (**2**)

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ROESY  $\curvearrowright$

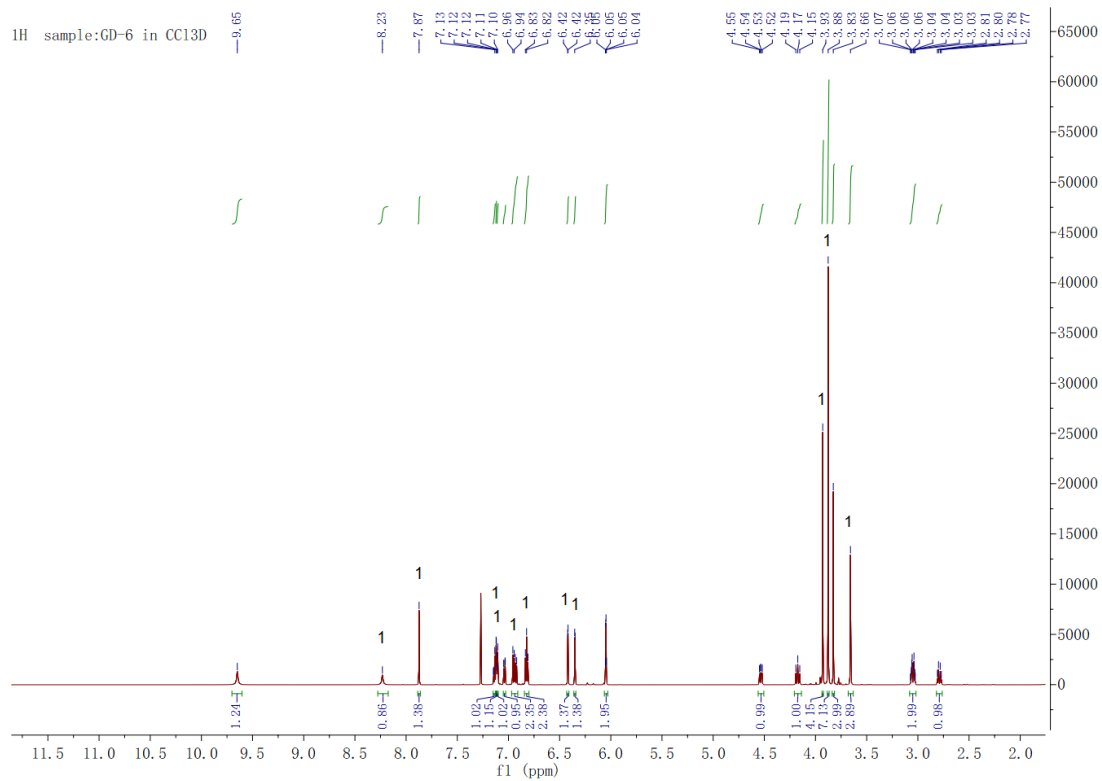
COSY —

Oleracone K (**2**)

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106 Figure S4. Key  $^1\text{H}$ - $^1\text{H}$  COSY and ROESY correlations of oleracone K (**2**)

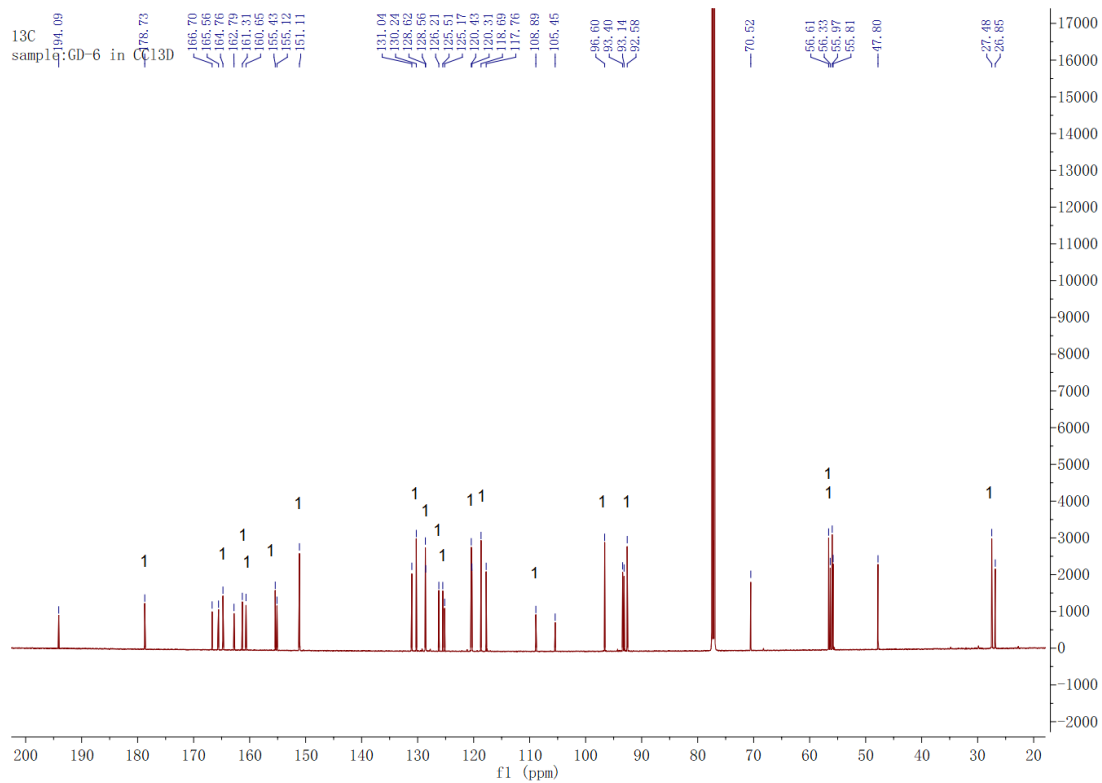


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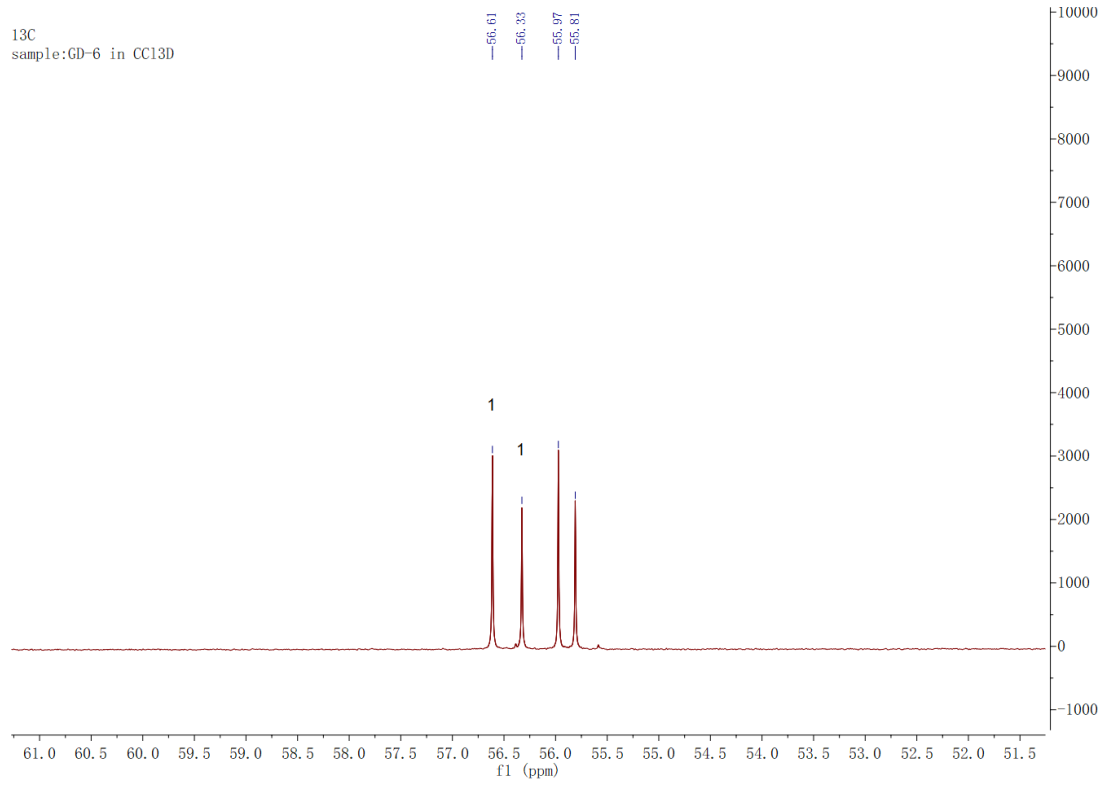
109 Figure S5. <sup>1</sup>H NMR (600 MHz) spectrum of oleracone J (**1**) in CDCl<sub>3</sub>

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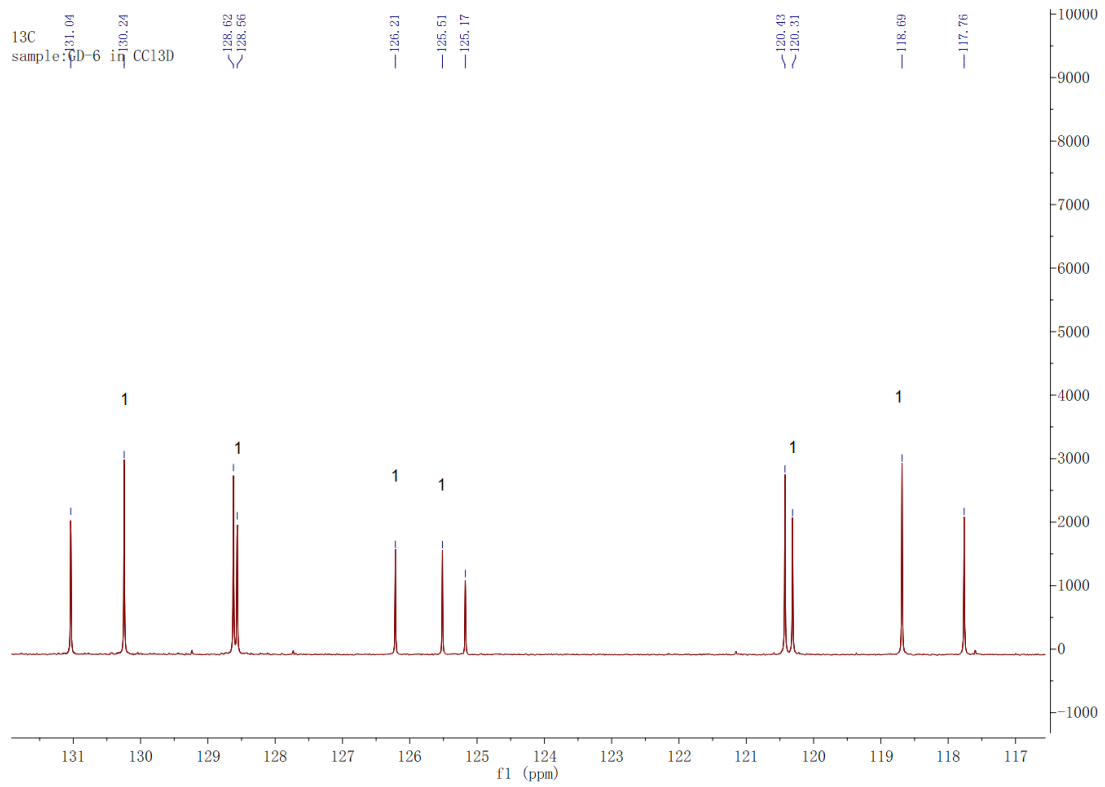
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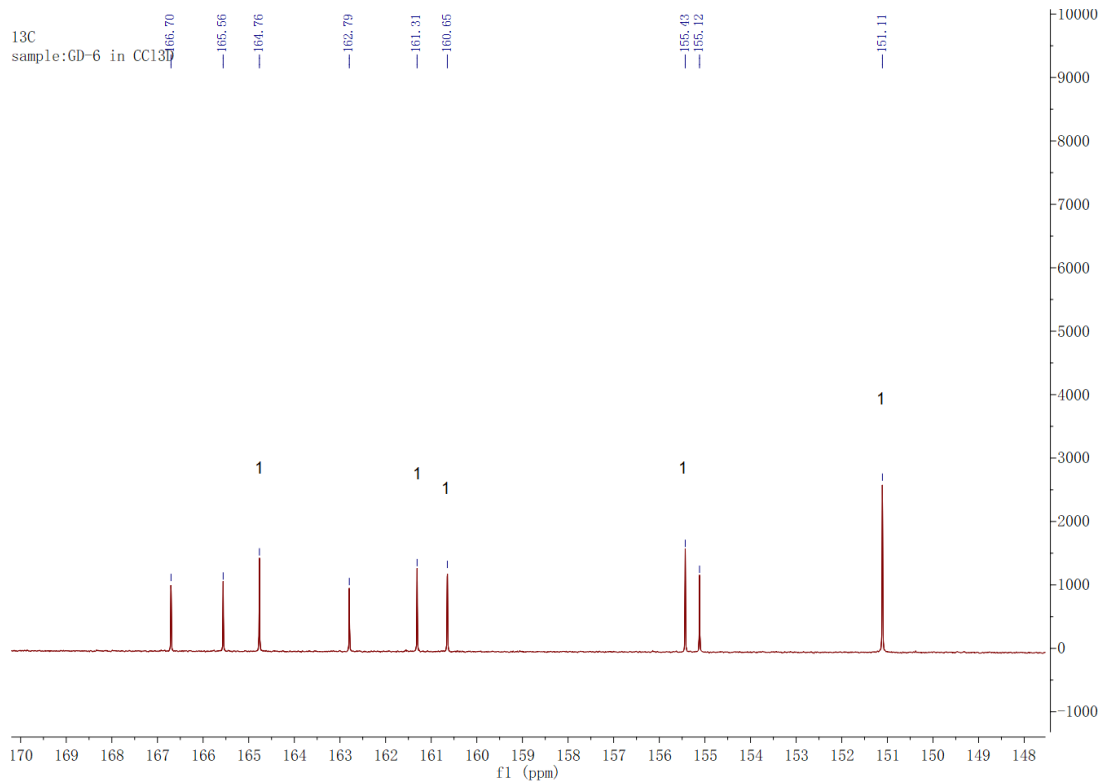


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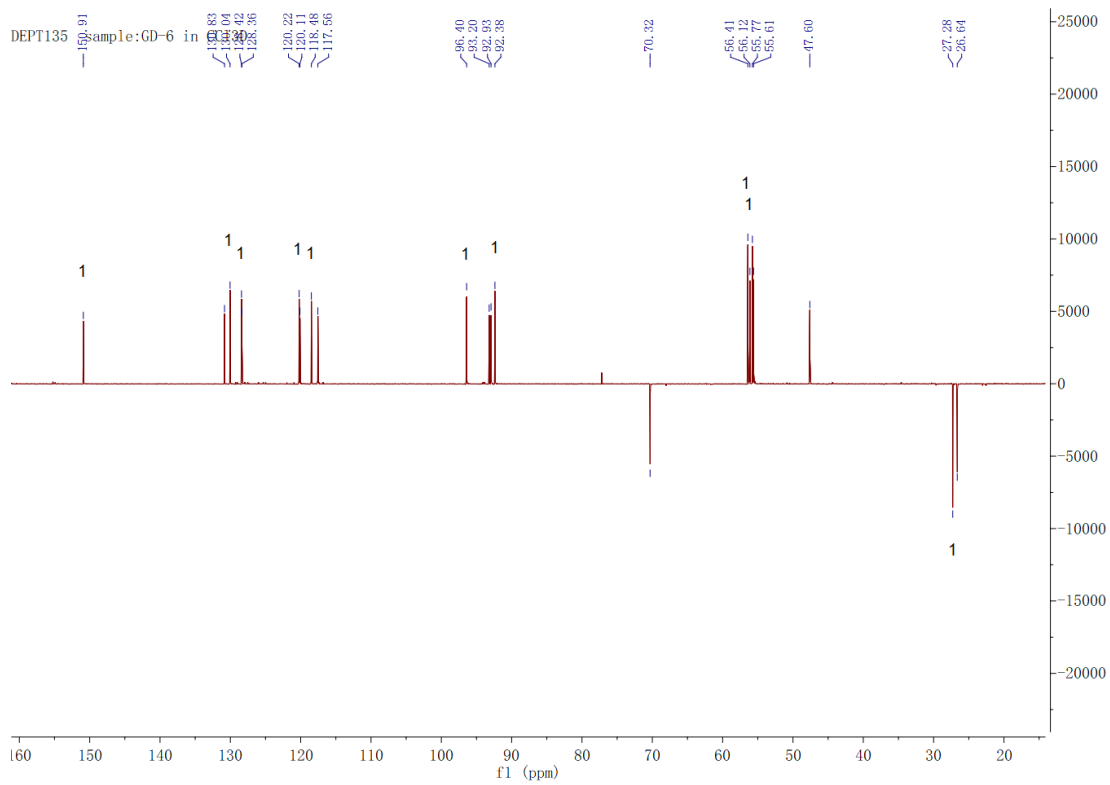


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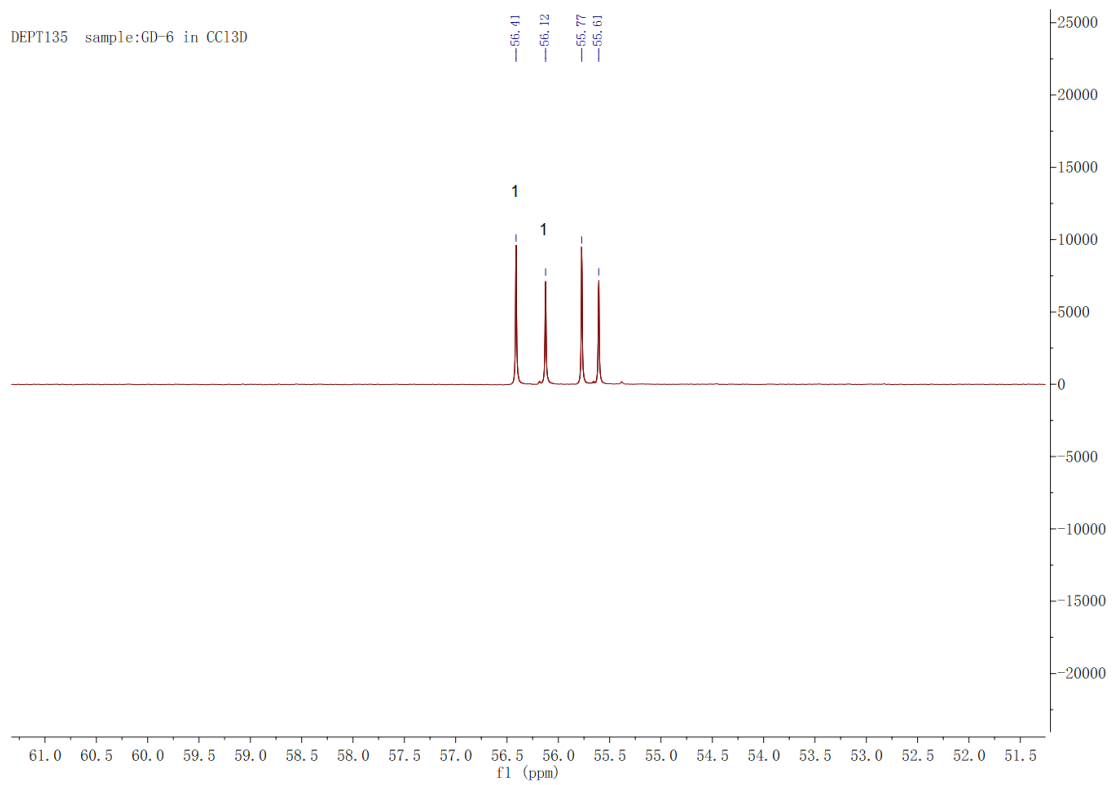
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117 Figure S6. <sup>13</sup>C NMR (150 MHz) spectrum of oleracone J (**1**) in CDCl<sub>3</sub>

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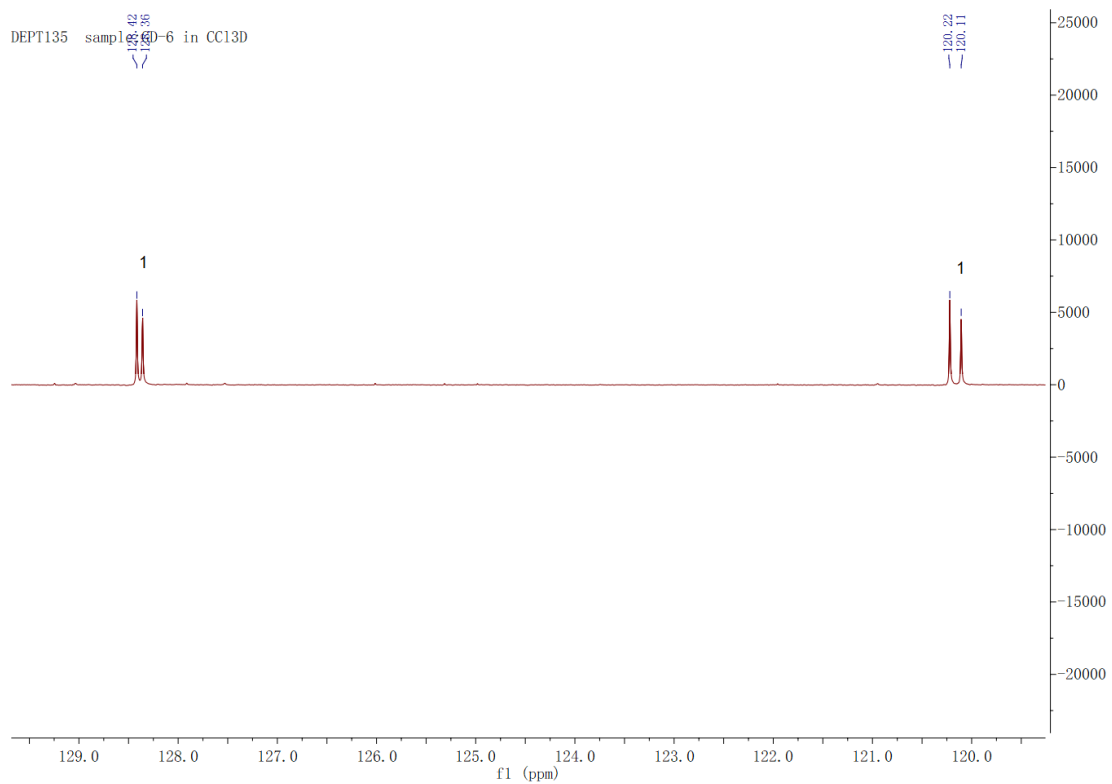


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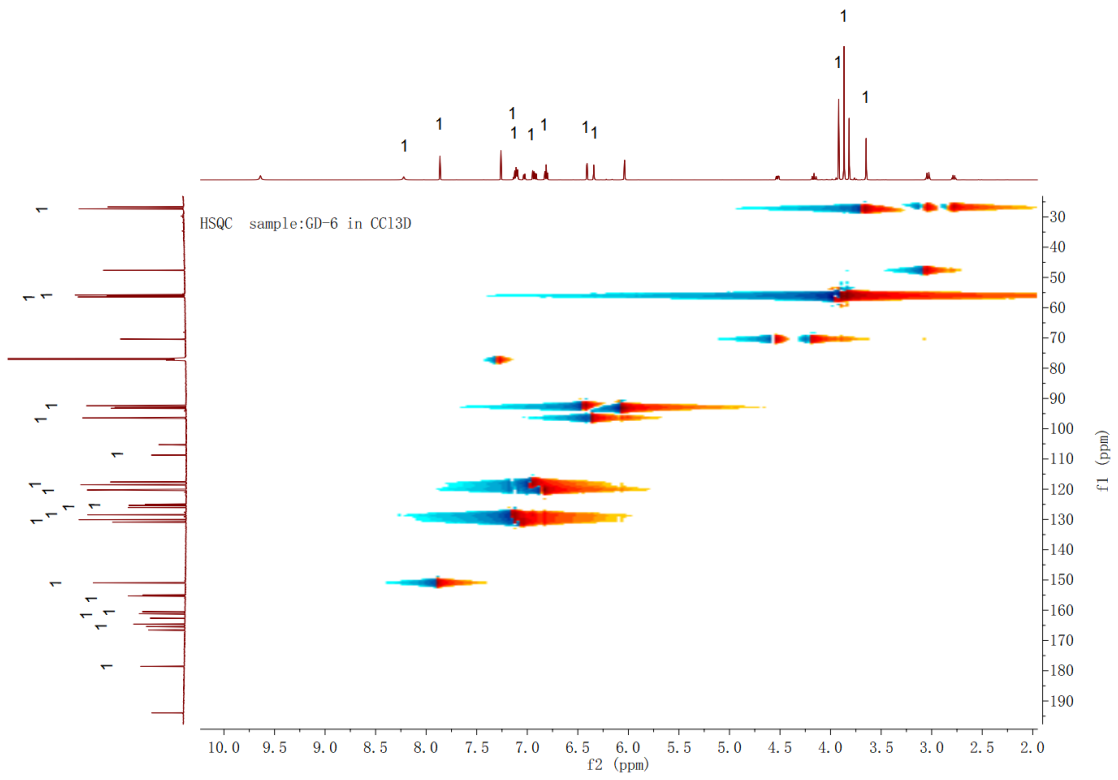
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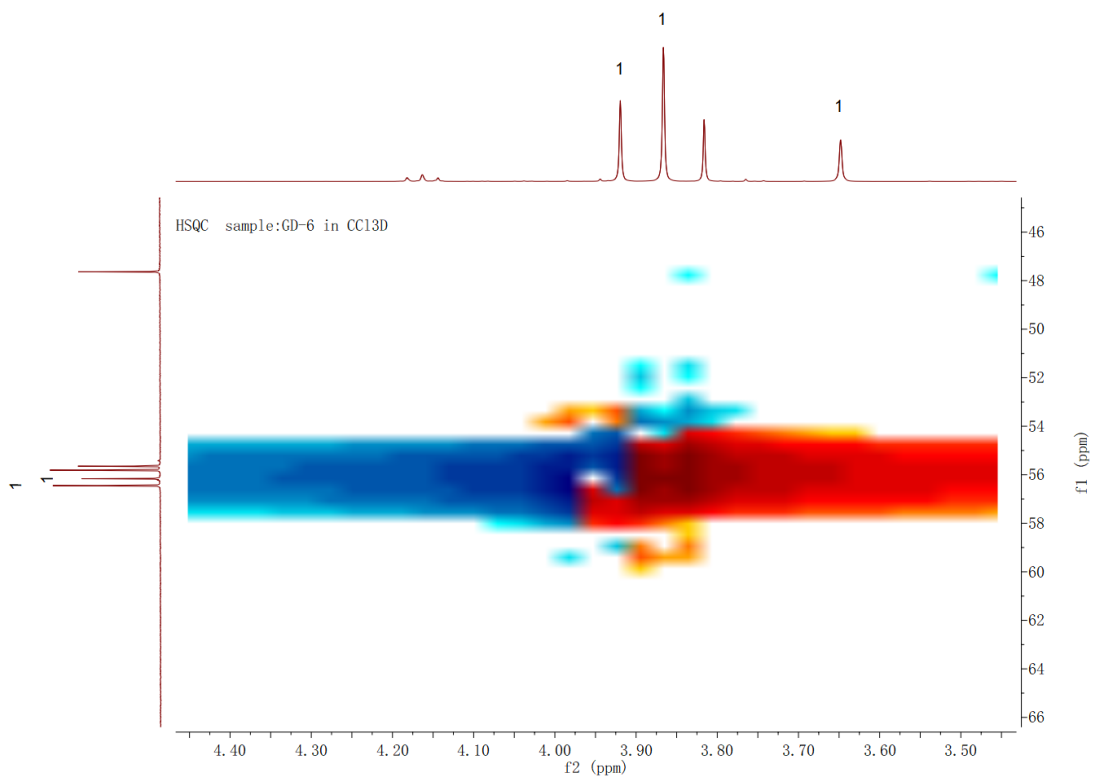
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124 Figure S7. DEPT spectrum of oleracone **J** (**1**) in CDCl<sub>3</sub>

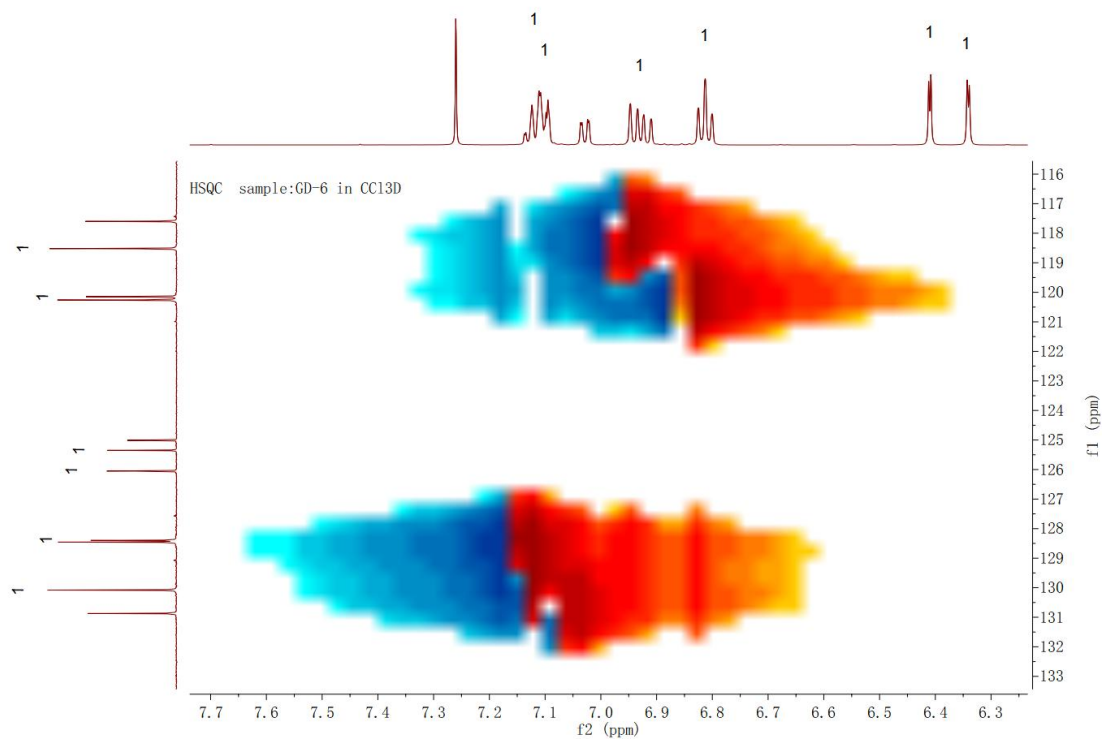


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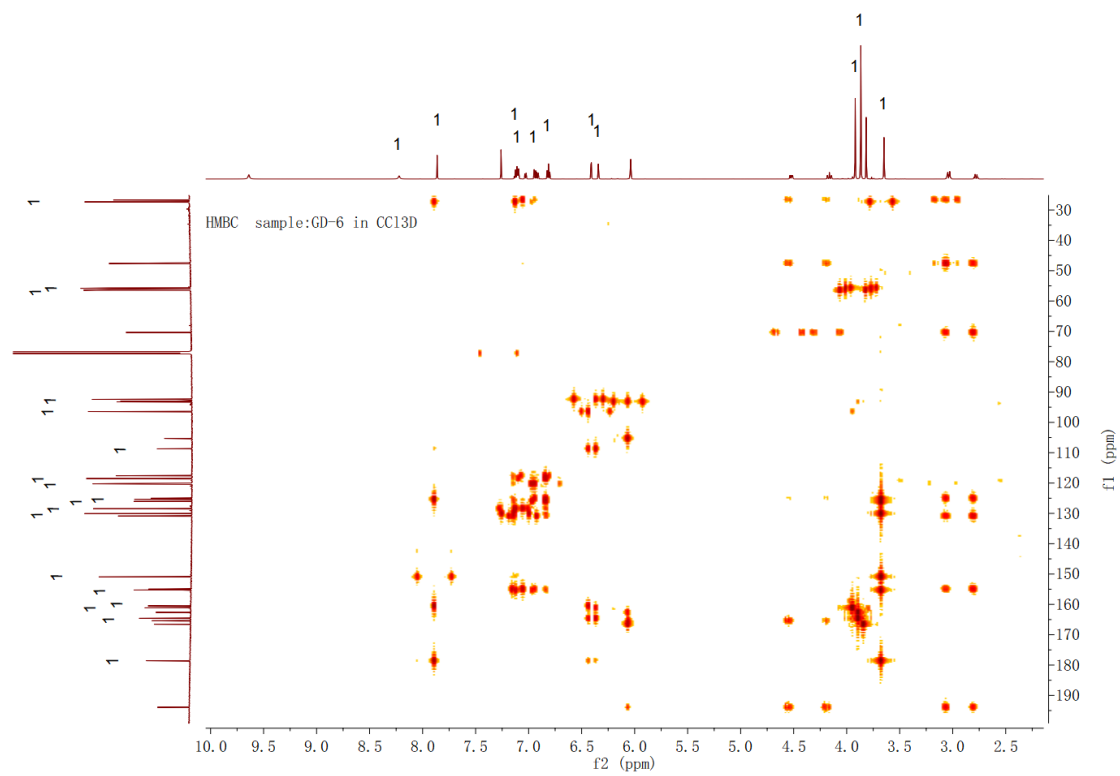


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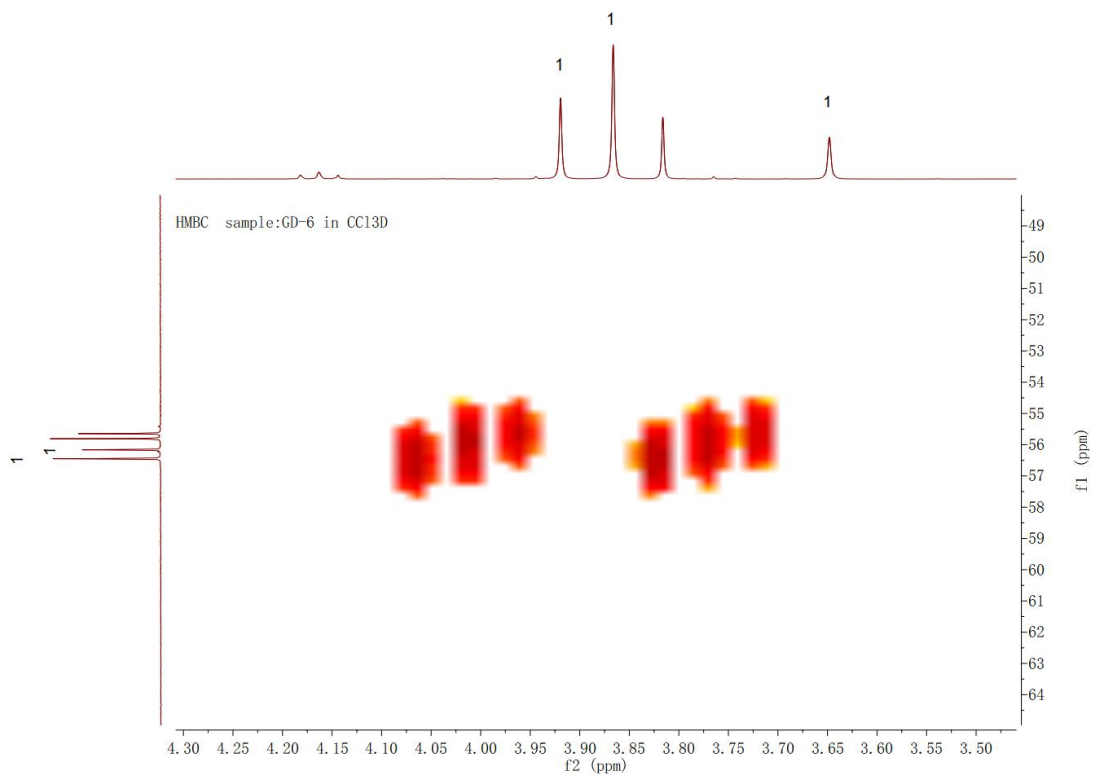
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130 Figure S8. HSQC spectrum of oleracone J (**1**) in CDCl<sub>3</sub>

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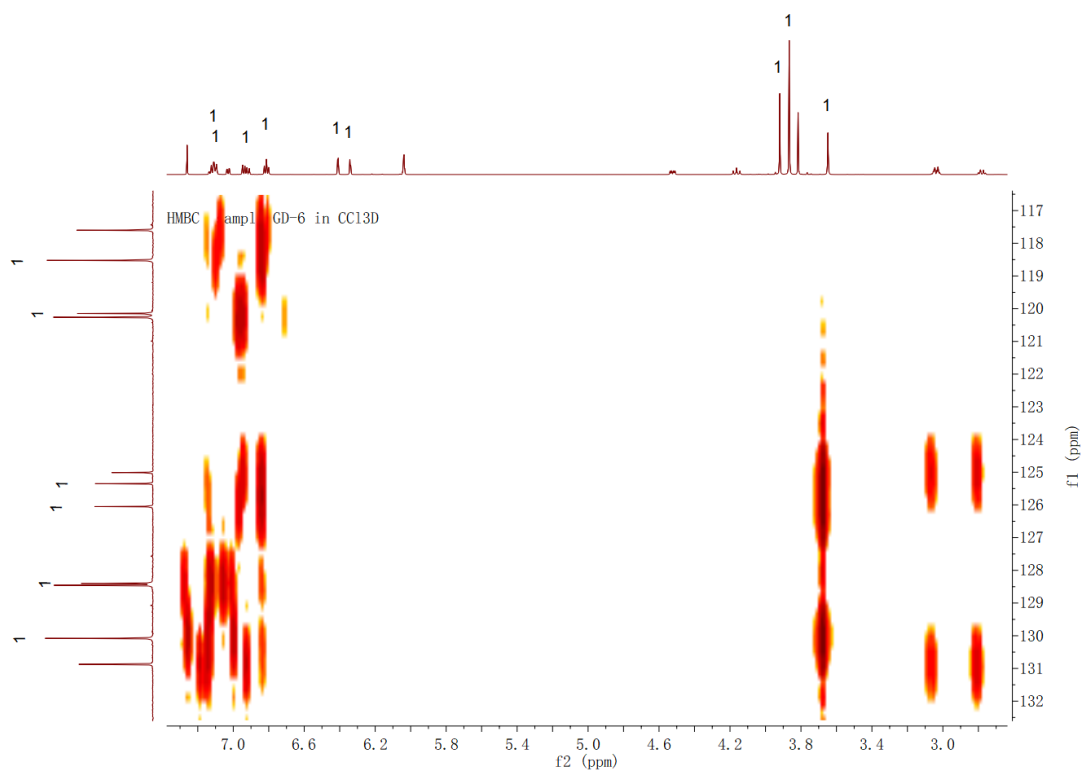


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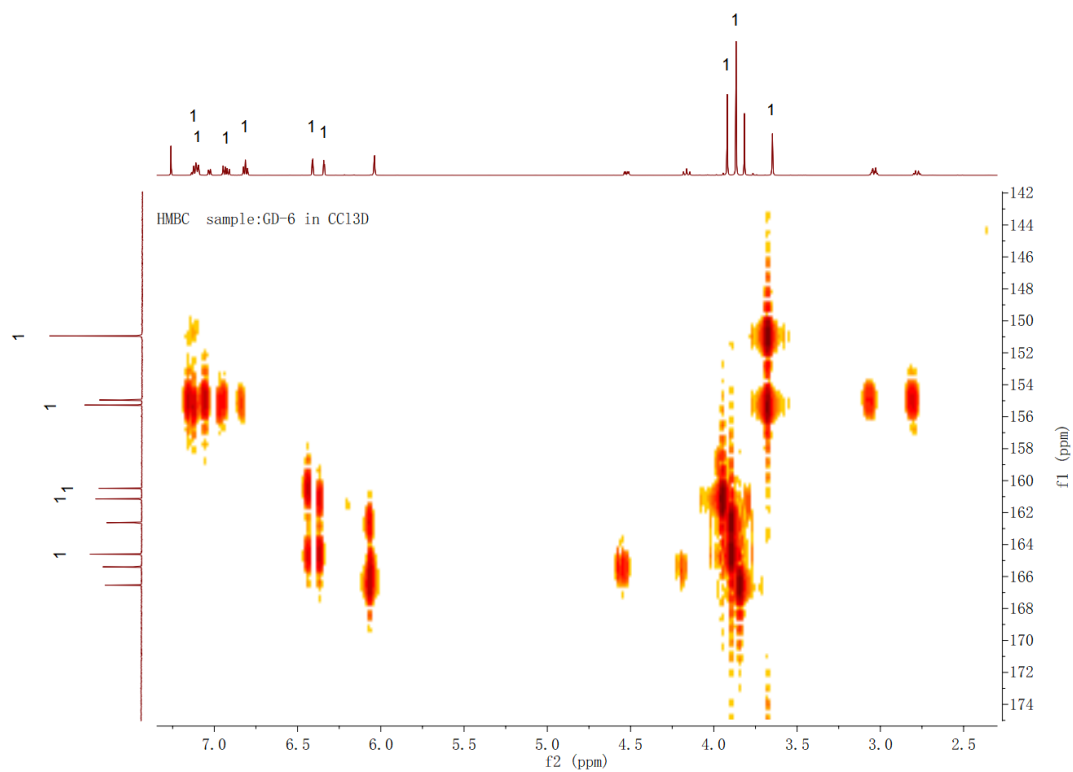


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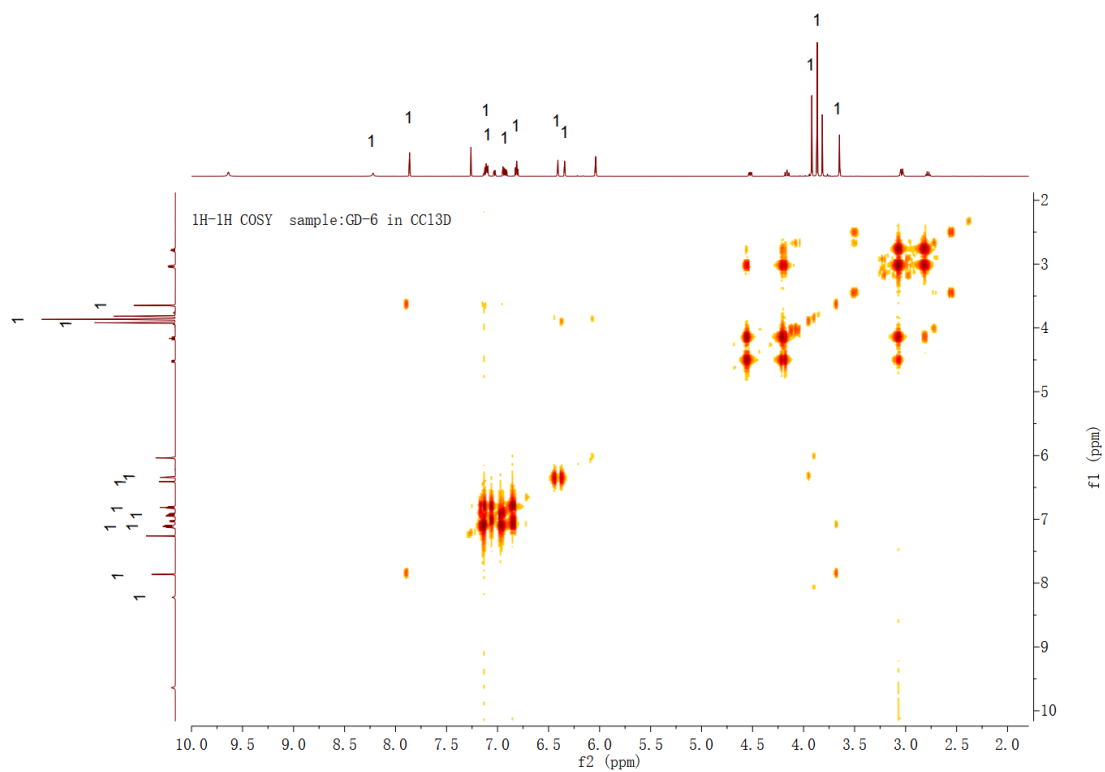


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138 Figure S9. HMBC spectrum of oleracone J (**1**) in CDCl<sub>3</sub>

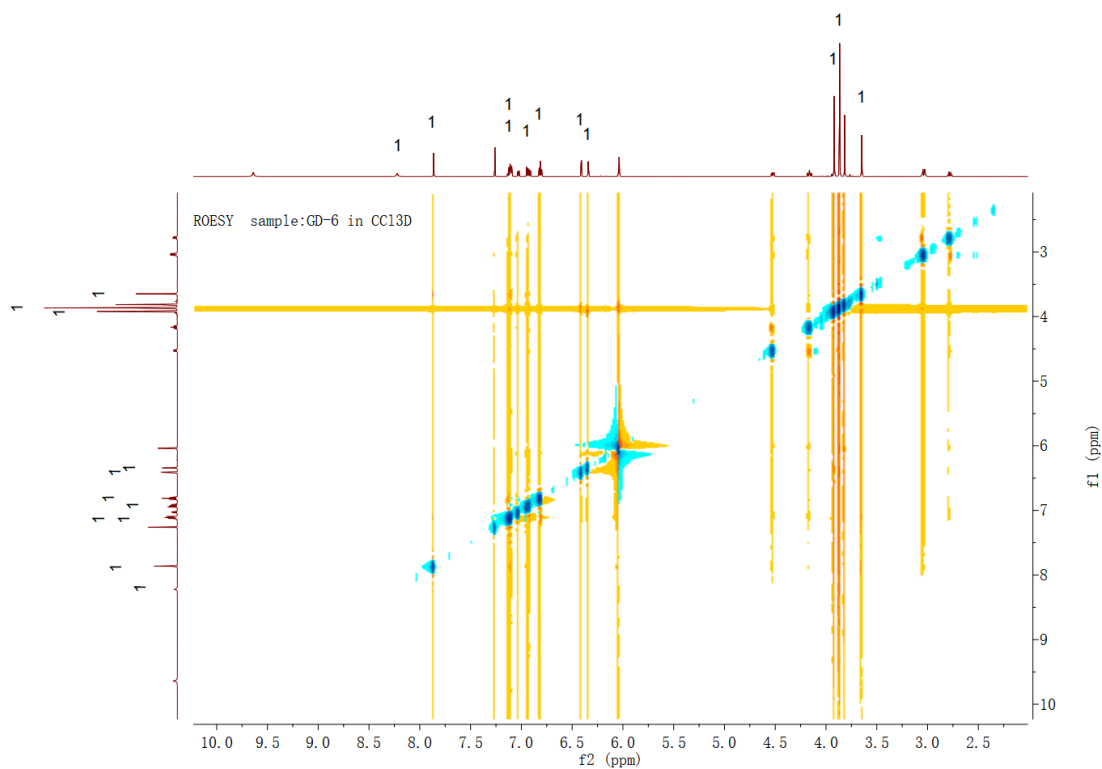
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141 Figure S10.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of oleracone J (**1**) in  $\text{CDCl}_3$

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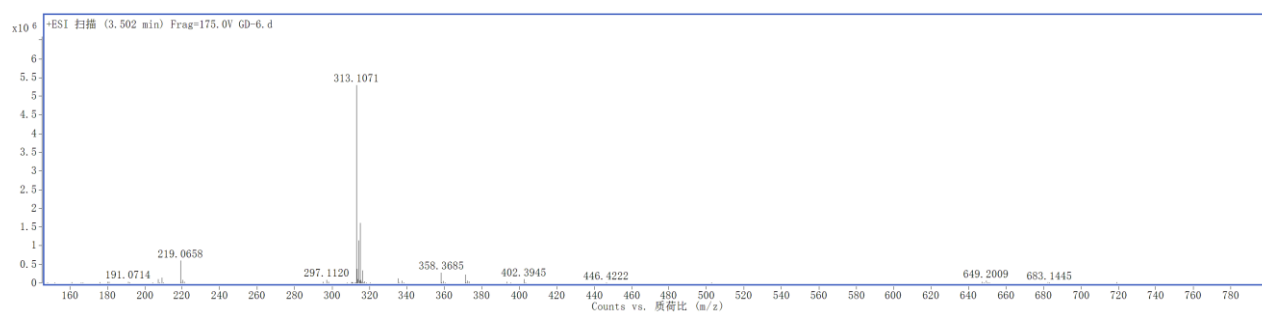


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145 Figure S11. ROESY spectrum of oleracone J (**1**) in  $\text{CDCl}_3$

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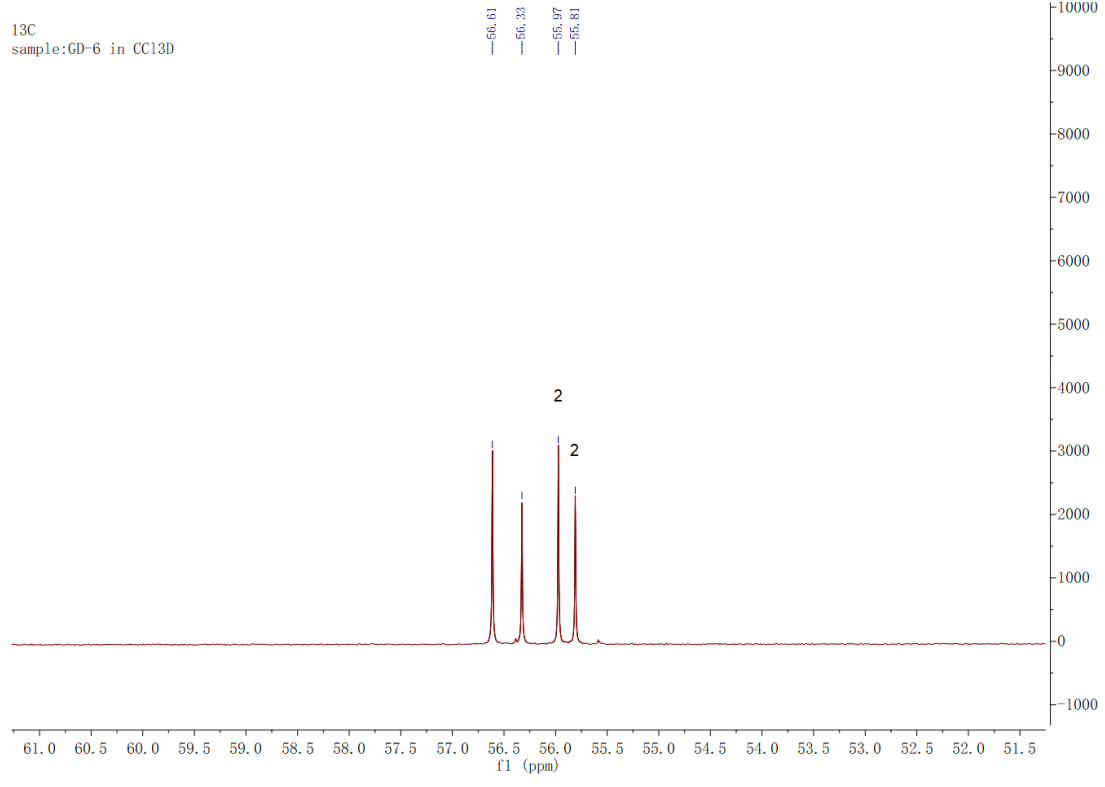
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149 Figure S12. UHPLC-ESI-Q-TOF/MS of oleracone J (**1**)

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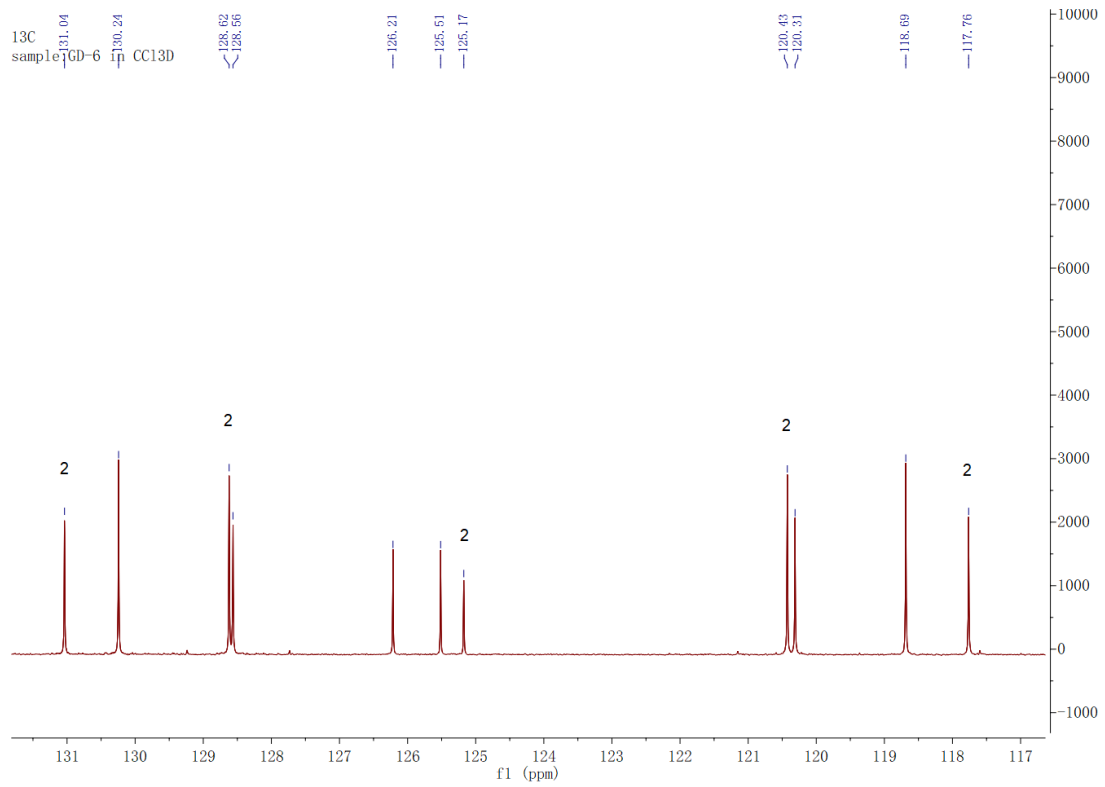




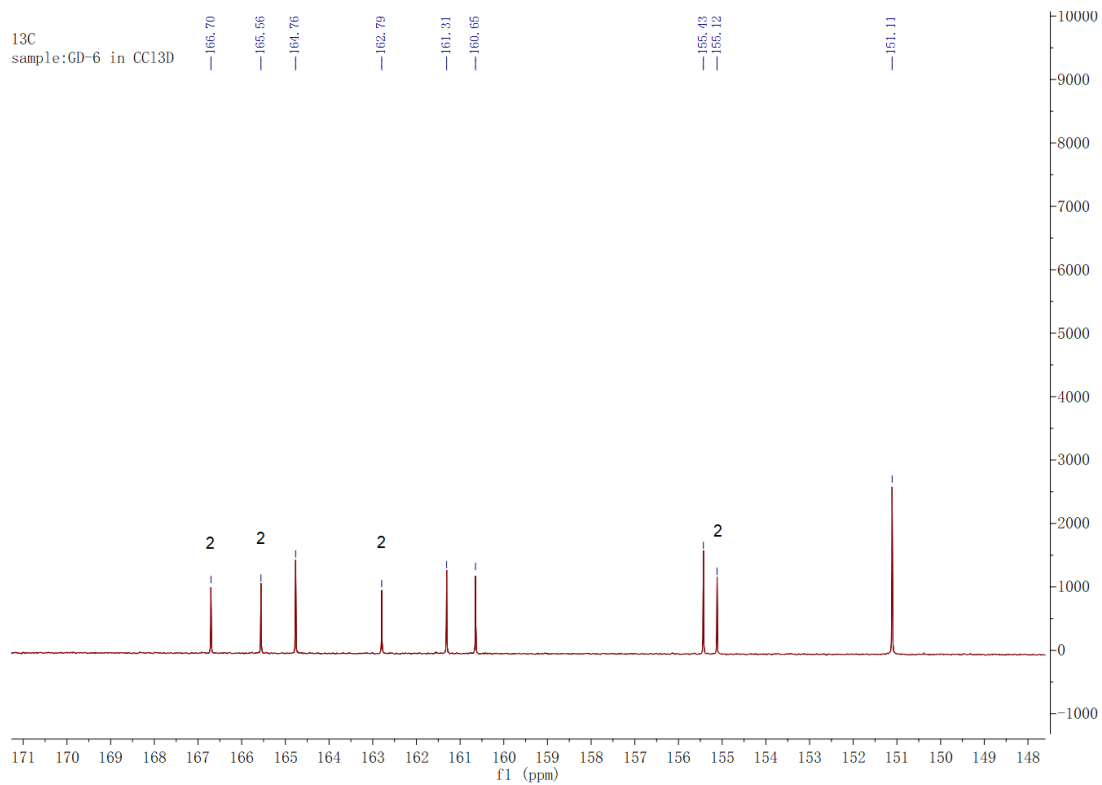


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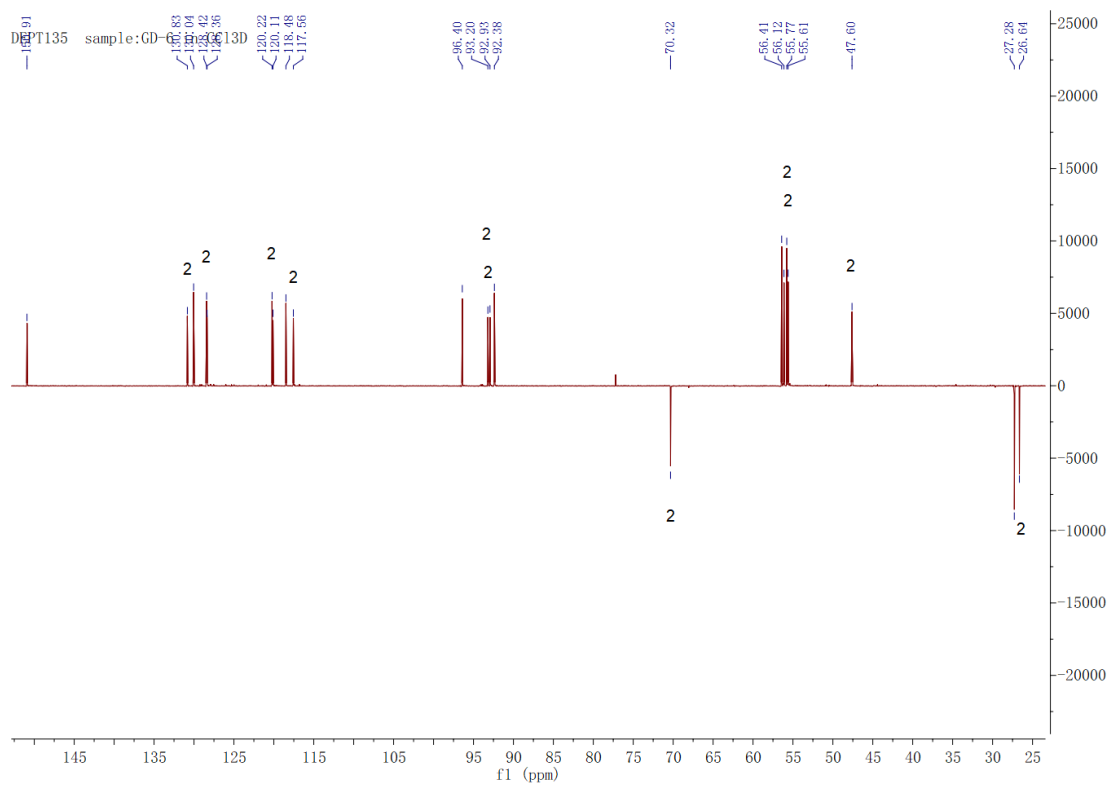


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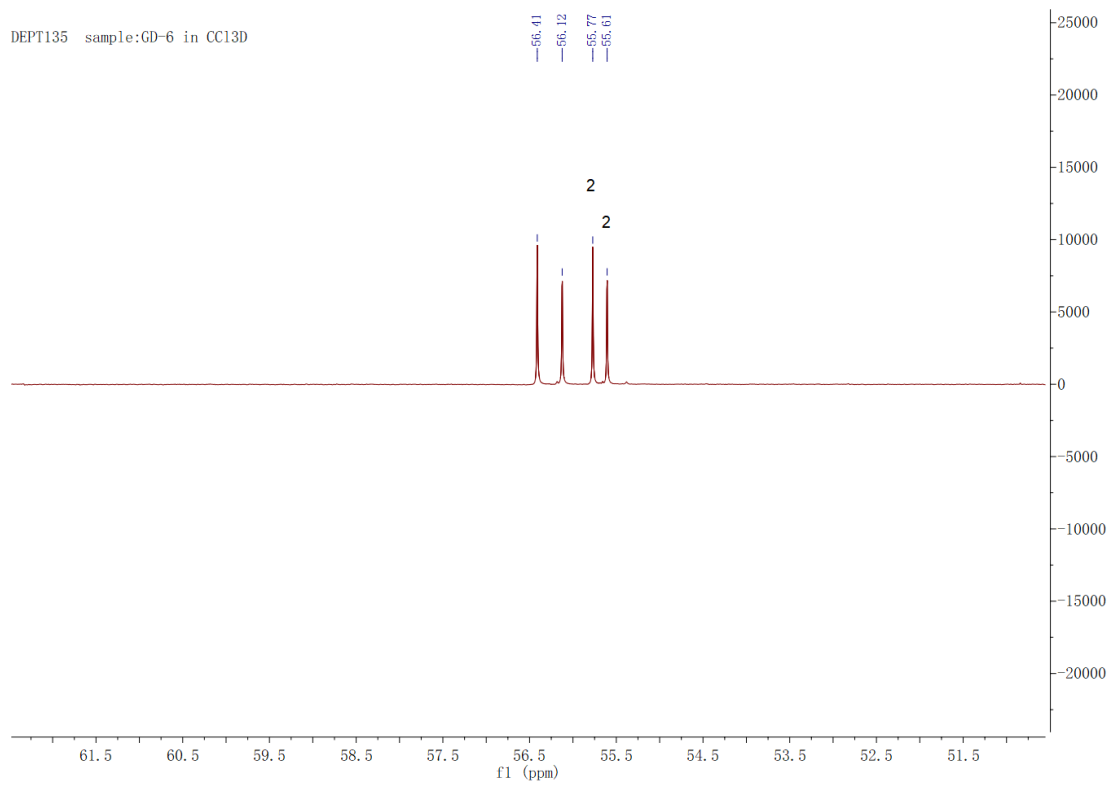
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161 Figure S14.  $^{13}\text{C}$  NMR (150 MHz) spectrum of oleracone K (**2**) in  $\text{CDCl}_3$

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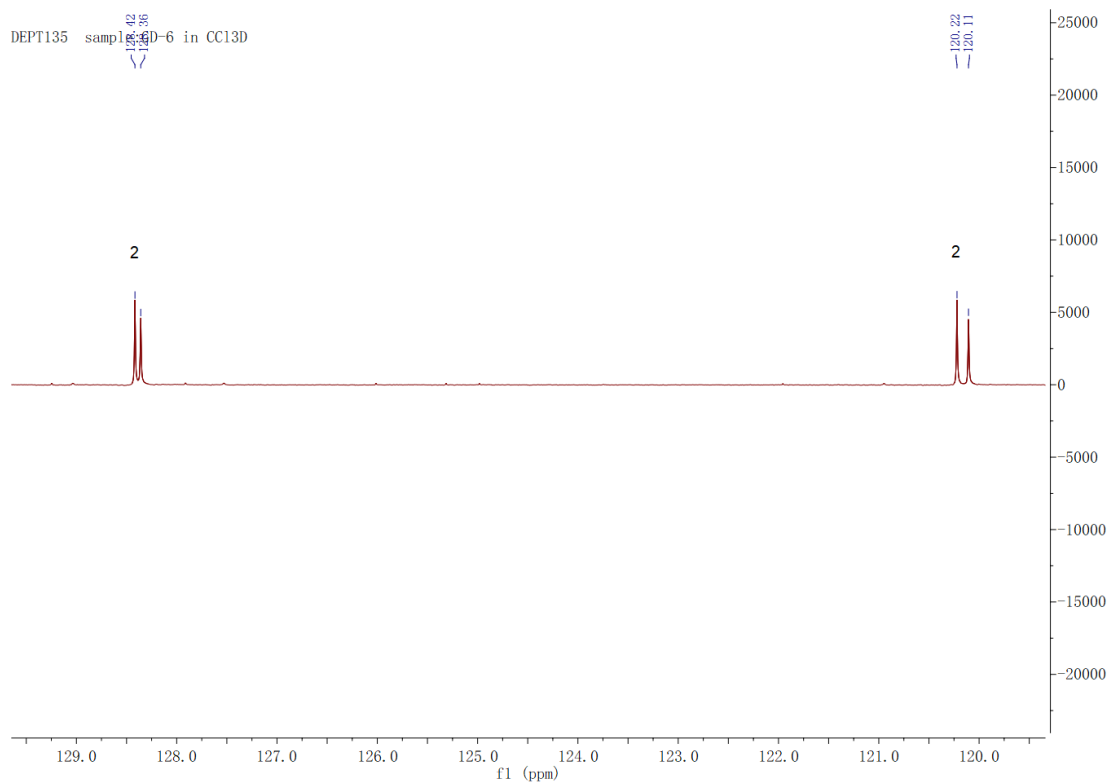


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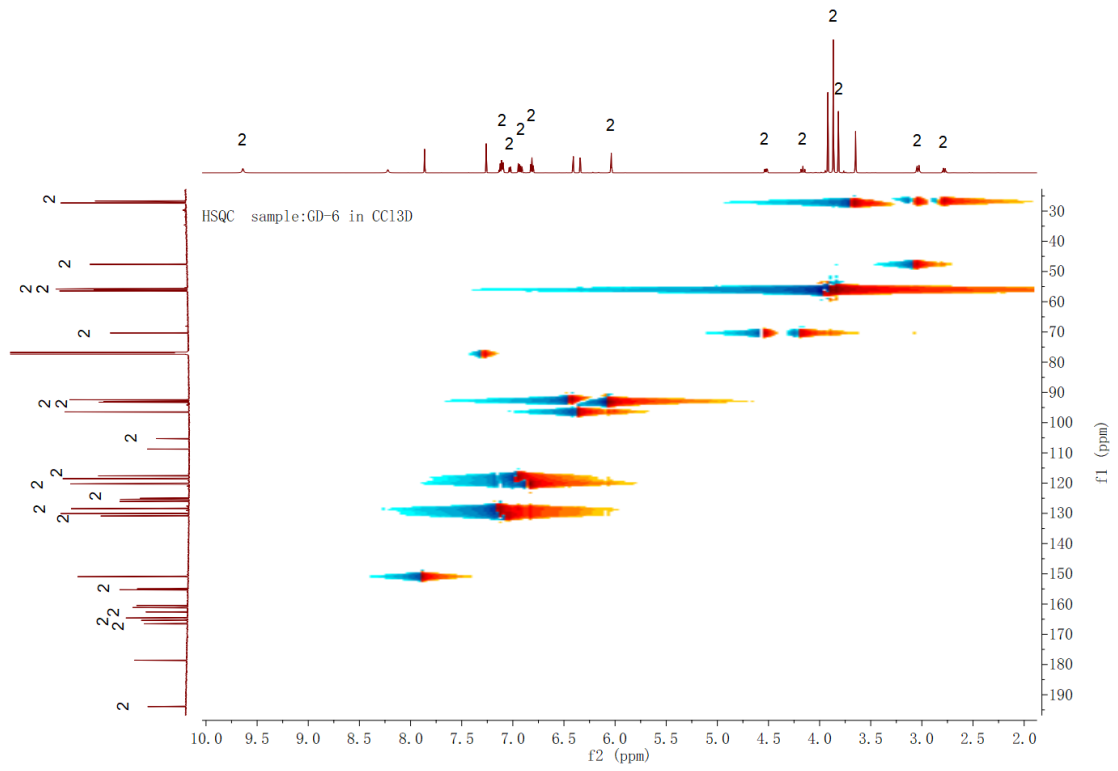
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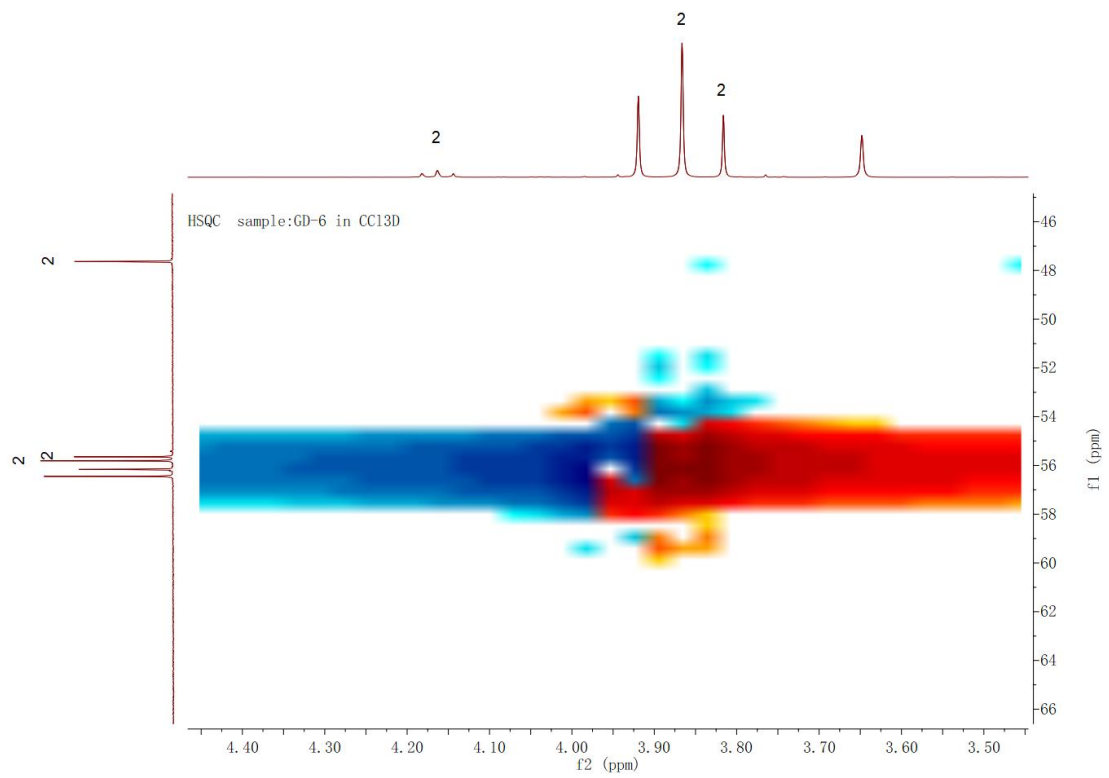
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168 Figure S15. DEPT spectrum of oleracone K (**2**) in CDCl<sub>3</sub>

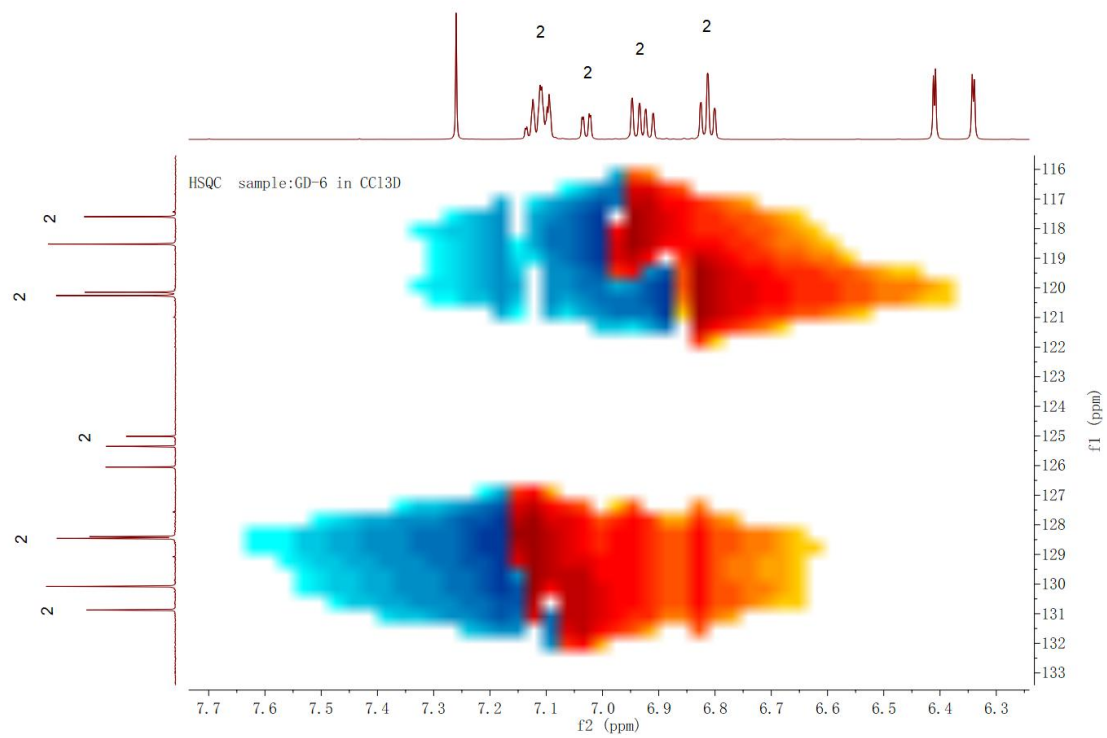


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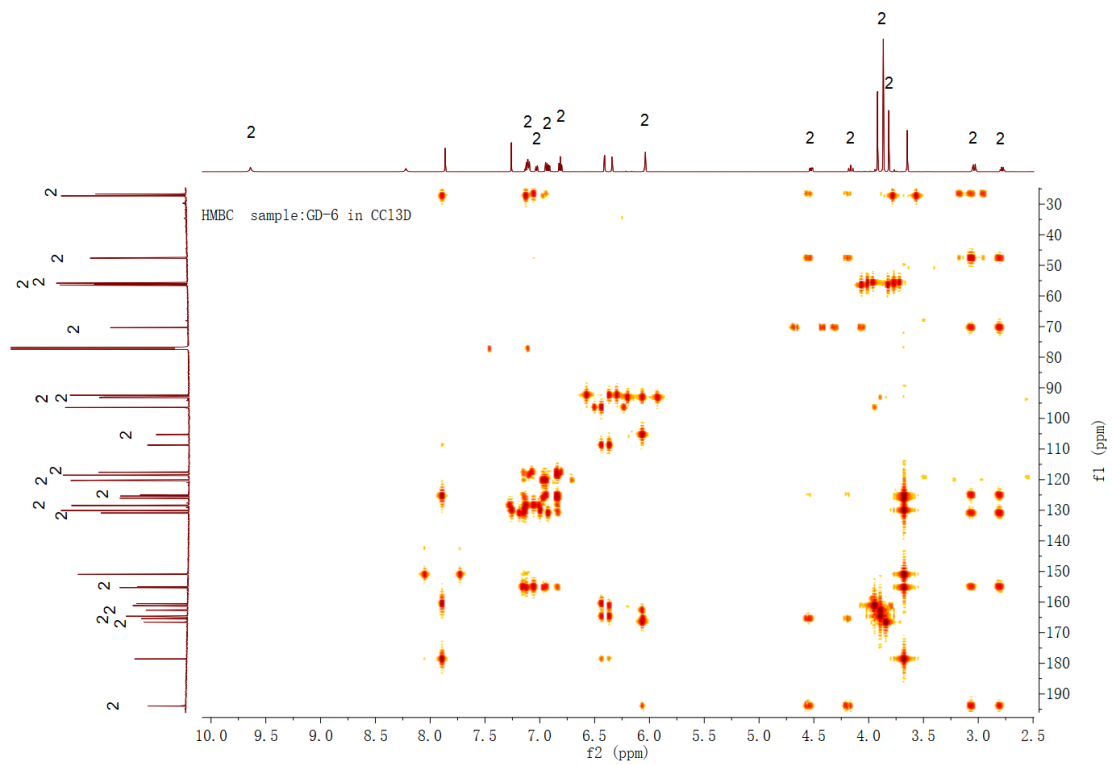


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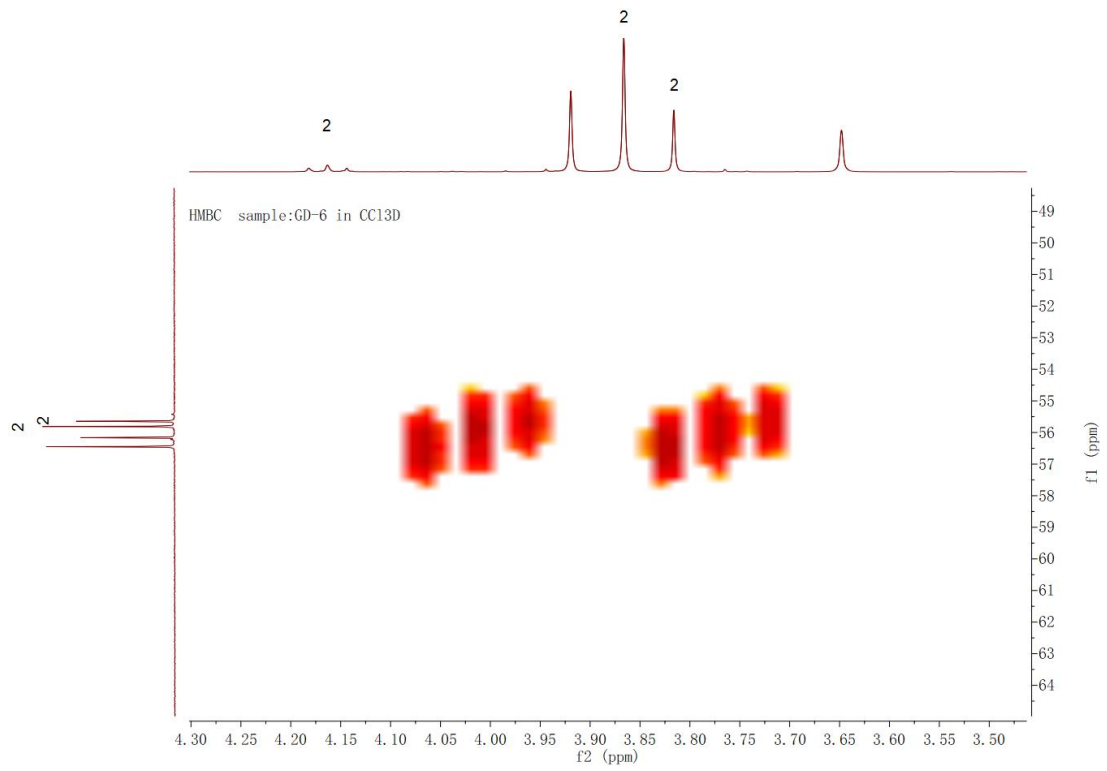
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174 Figure S16. HSQC spectrum of oleracone K (**2**) in CDCl<sub>3</sub>

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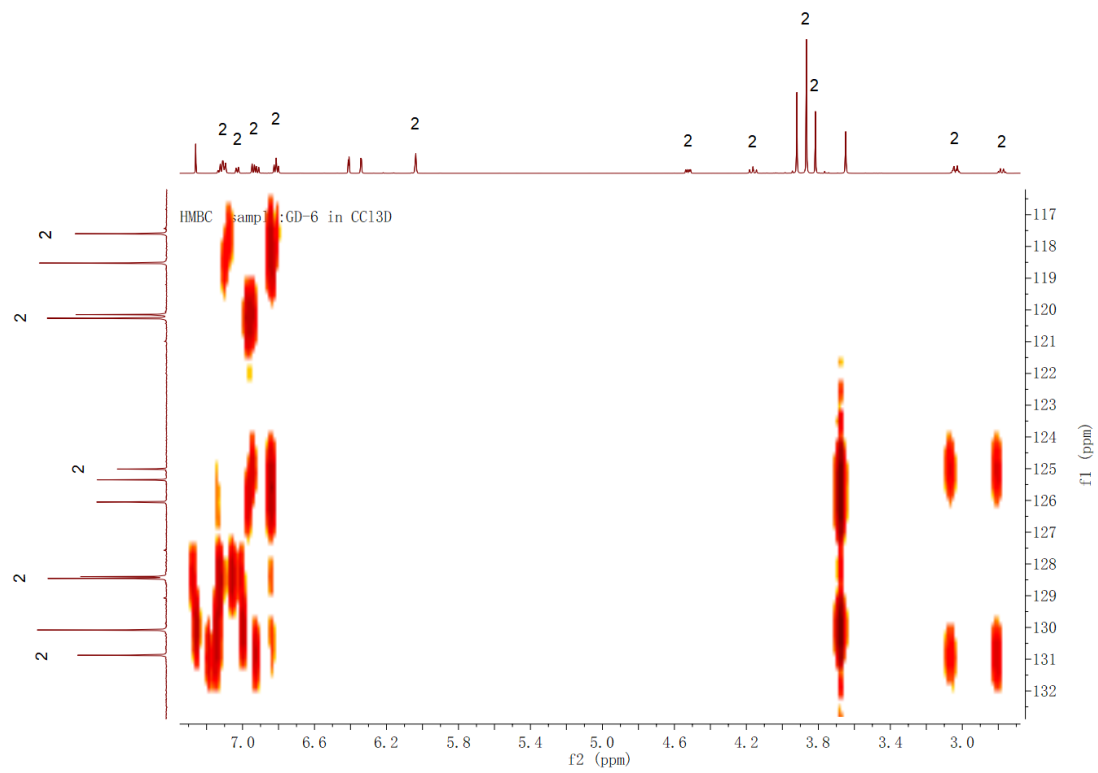


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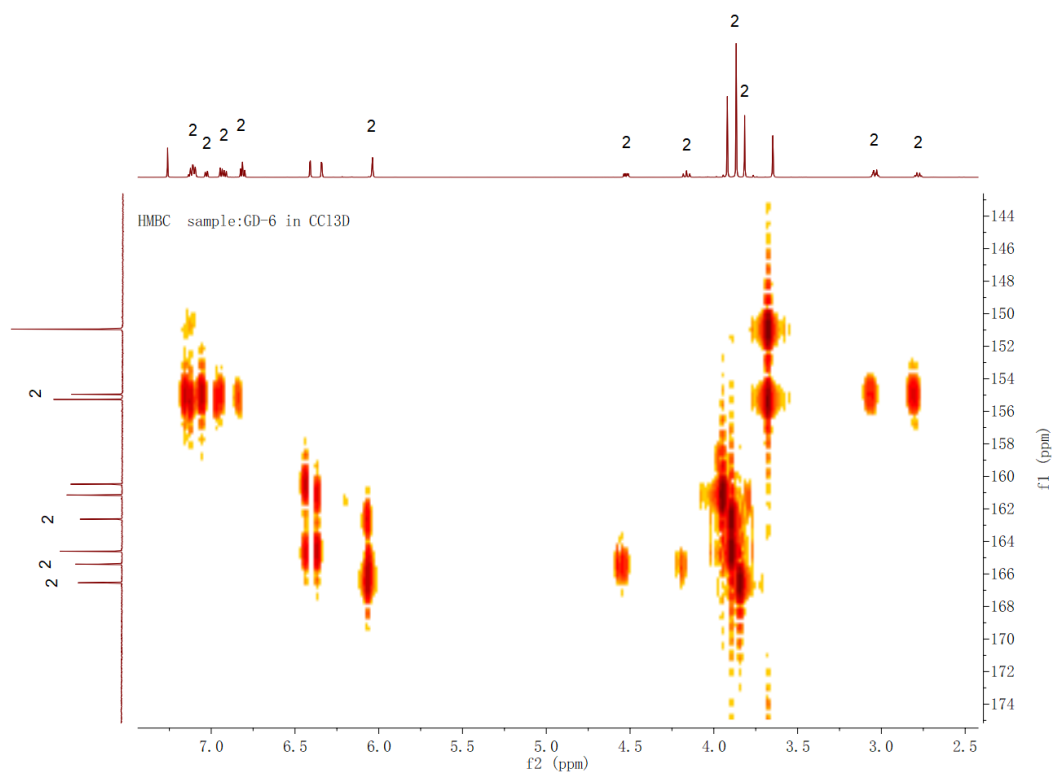


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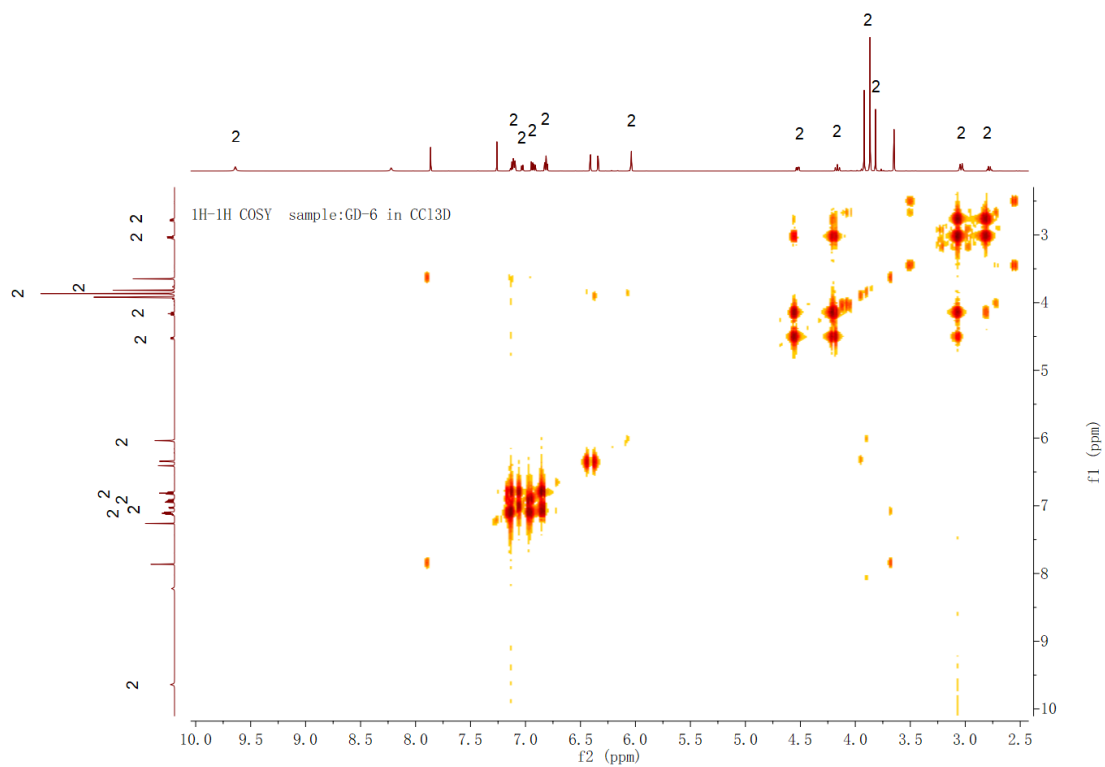


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182 Figure S17. HMBC spectrum of oleracone K (**2**) in CDCl<sub>3</sub>

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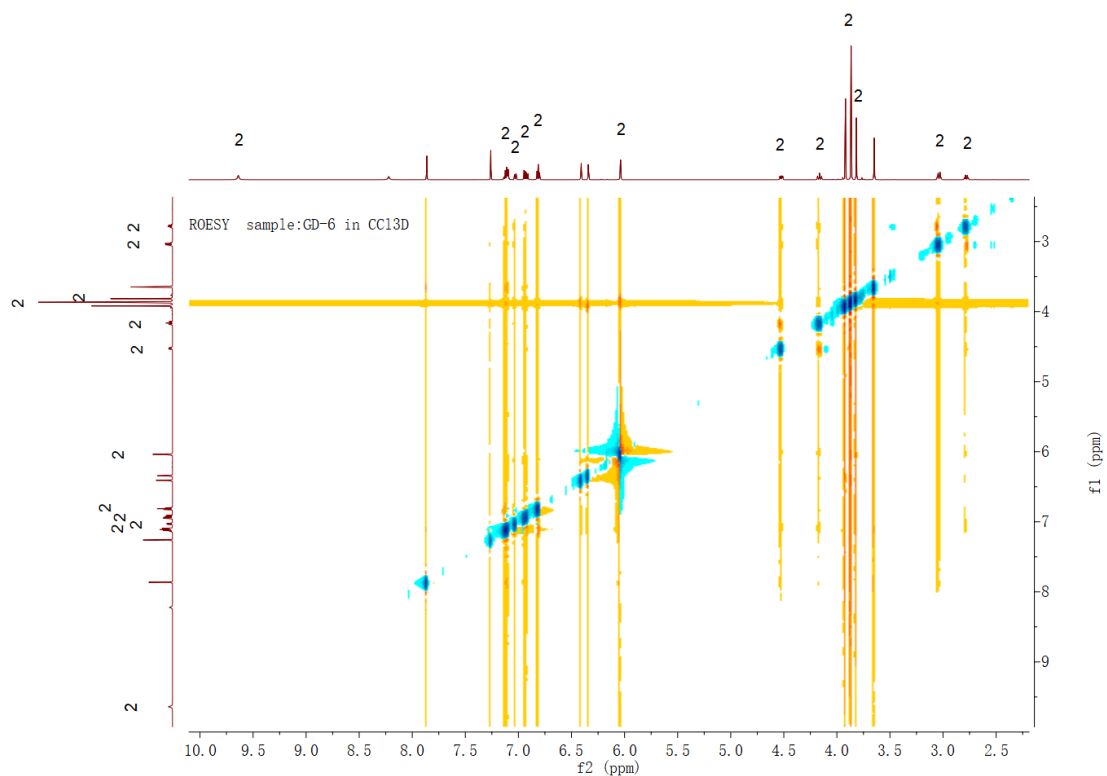


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185 Figure S18.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of oleracone K (**2**) in  $\text{CDCl}_3$

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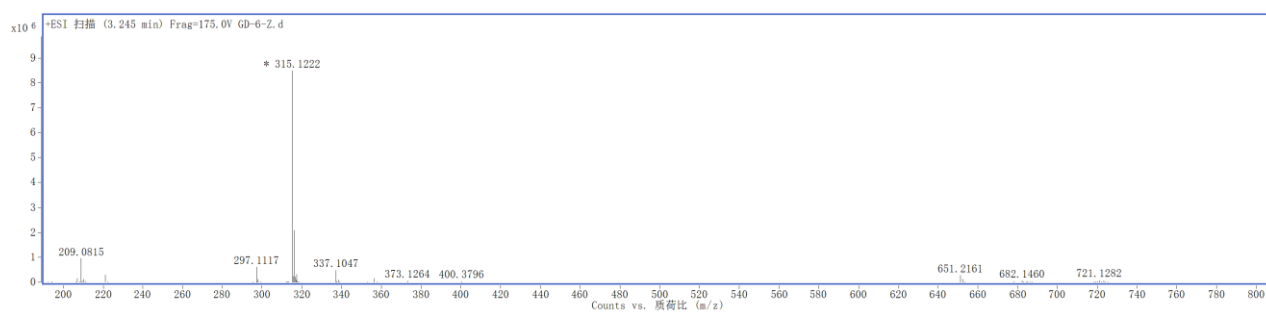


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189 Figure S19. ROESY spectrum of oleracone K (**2**) in  $\text{CDCl}_3$

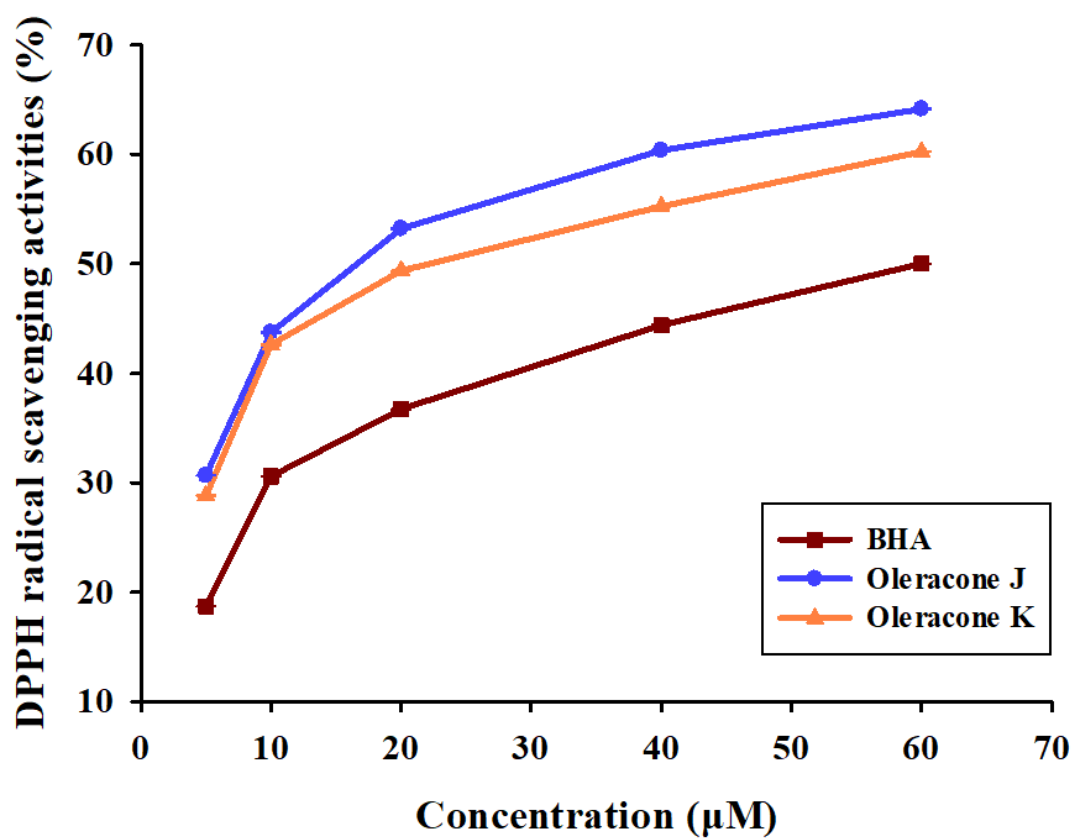
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193 Figure S20. UHPLC-ESI-Q-TOF/MS of oleracone K (**2**)

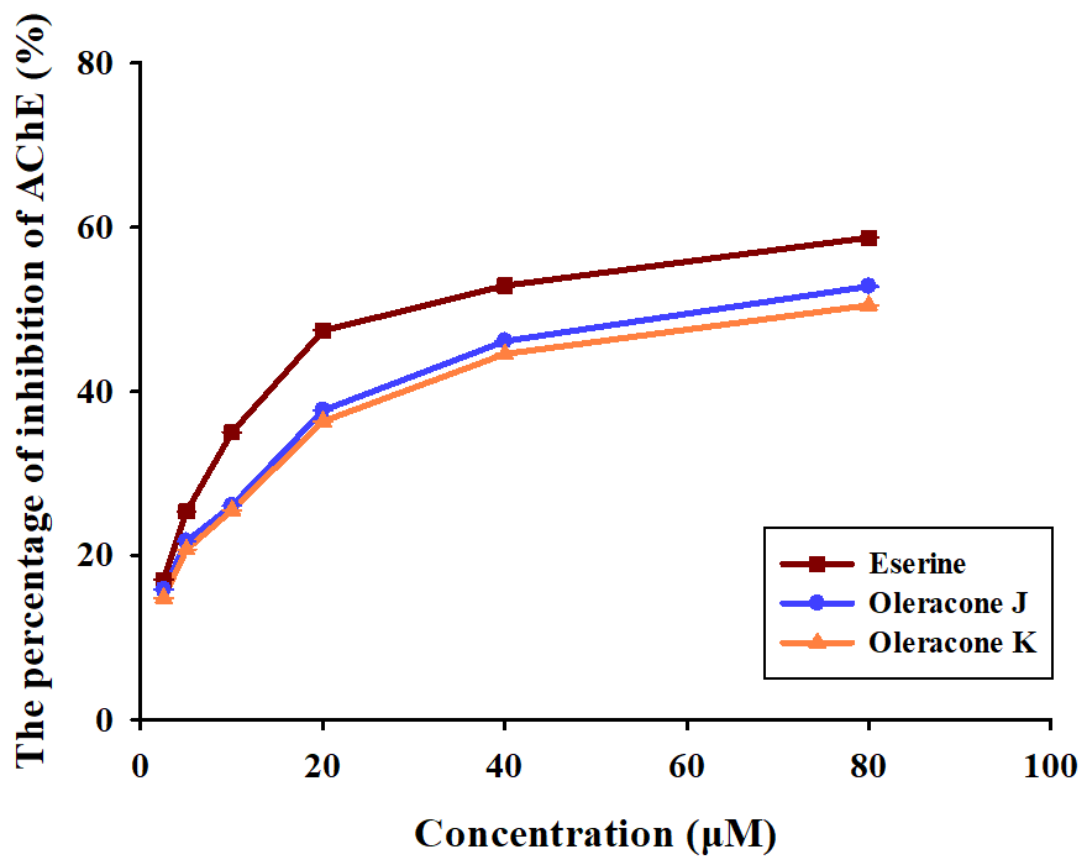


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196 Figure S21. DPPH radical scavenging activities of compounds **1-2** and BHA

197 The values are expressed as the means  $\pm$  SD for  $n = 5$ .

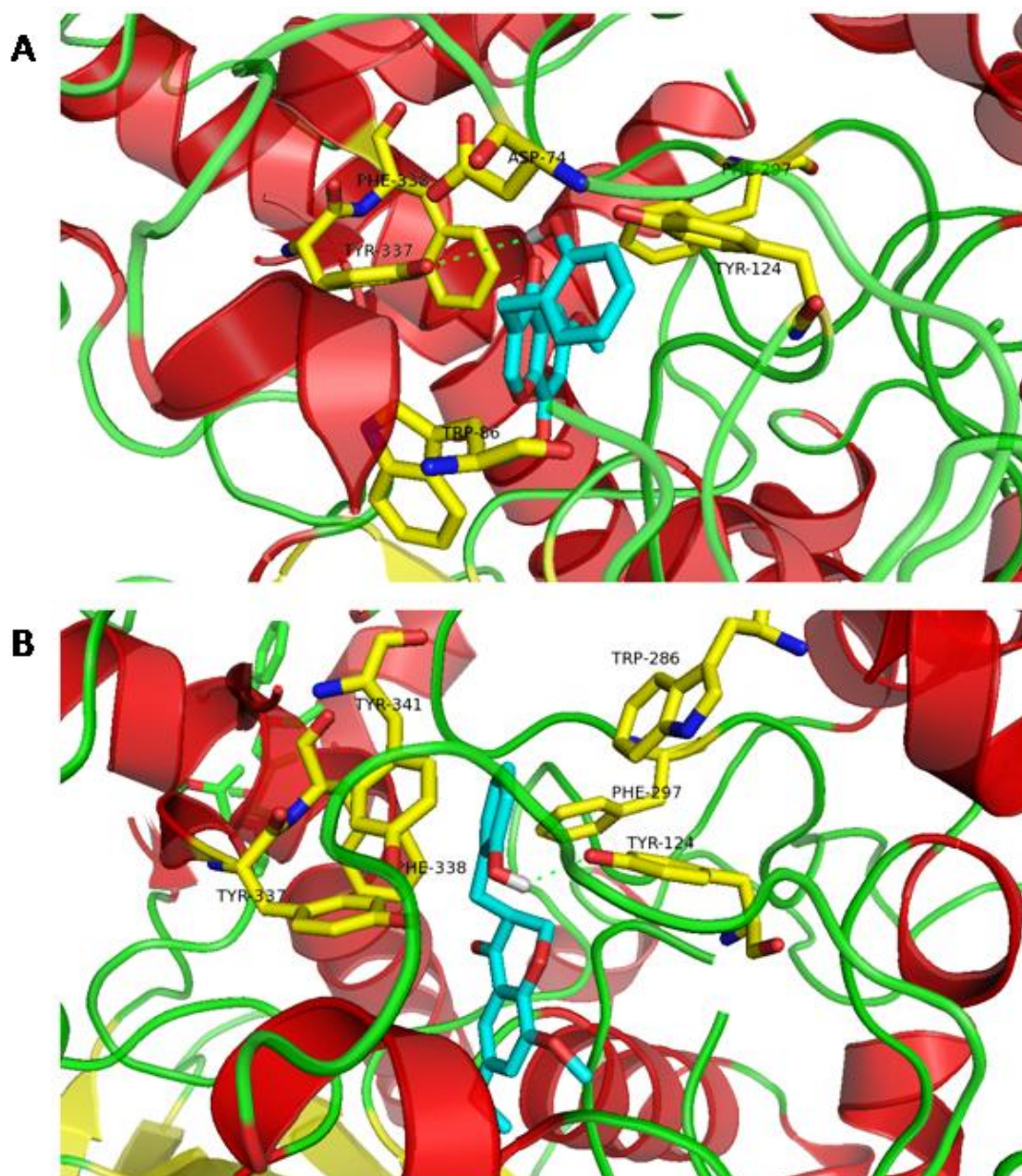


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200 Figure S22. Anticholinesterase effect of compounds 1-2 and Eserine

201 The values are expressed as the means  $\pm$  SD for  $n = 5$ .



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204 Figure S23. Compounds **1-2** binding mode to AChE

205 (**A**) oleracone J (**B**) oleracone K.

206 Carbons are colored in cyan in the compounds and yellow in the residues of protein.

207 Hydrogen bonds are drawn as green dashes.