

## Supporting Information

### Systematic characterization of metabolic profiles of Ingenol in rat by UPLC-Q/TOF-MS

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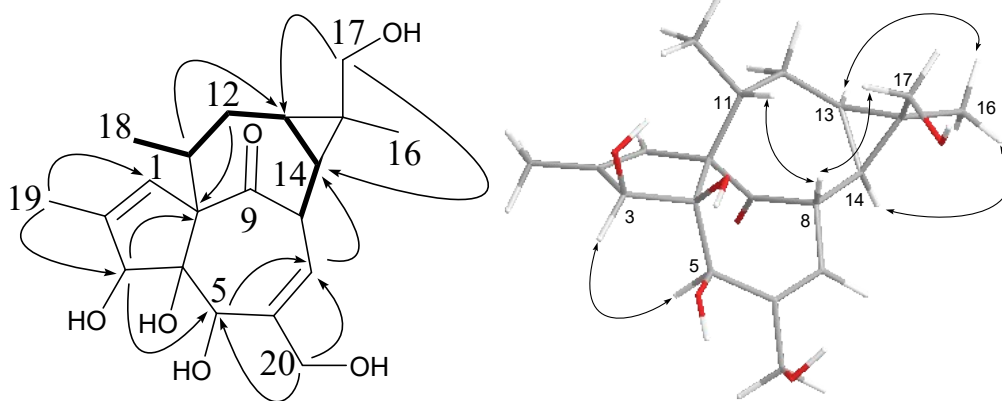


Figure S1. Key  $^1\text{H}$ - $^1\text{H}$  COSY (—), HMBC (---) and ROESY (····) correlations of **M3**

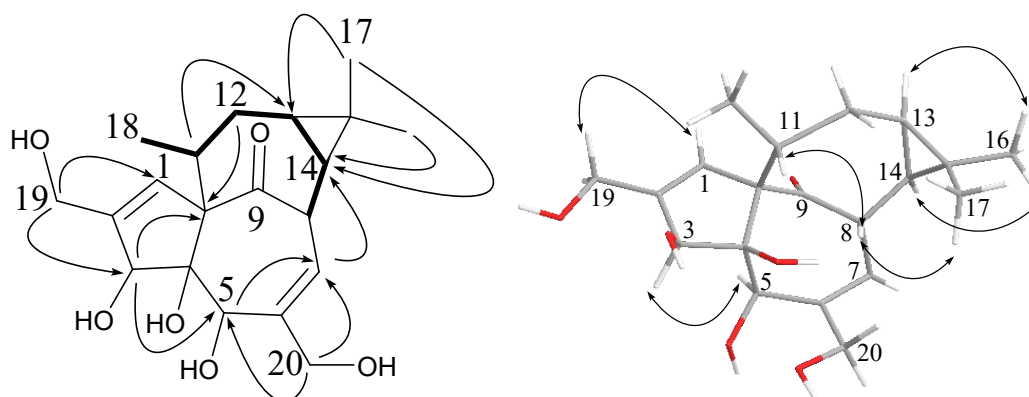
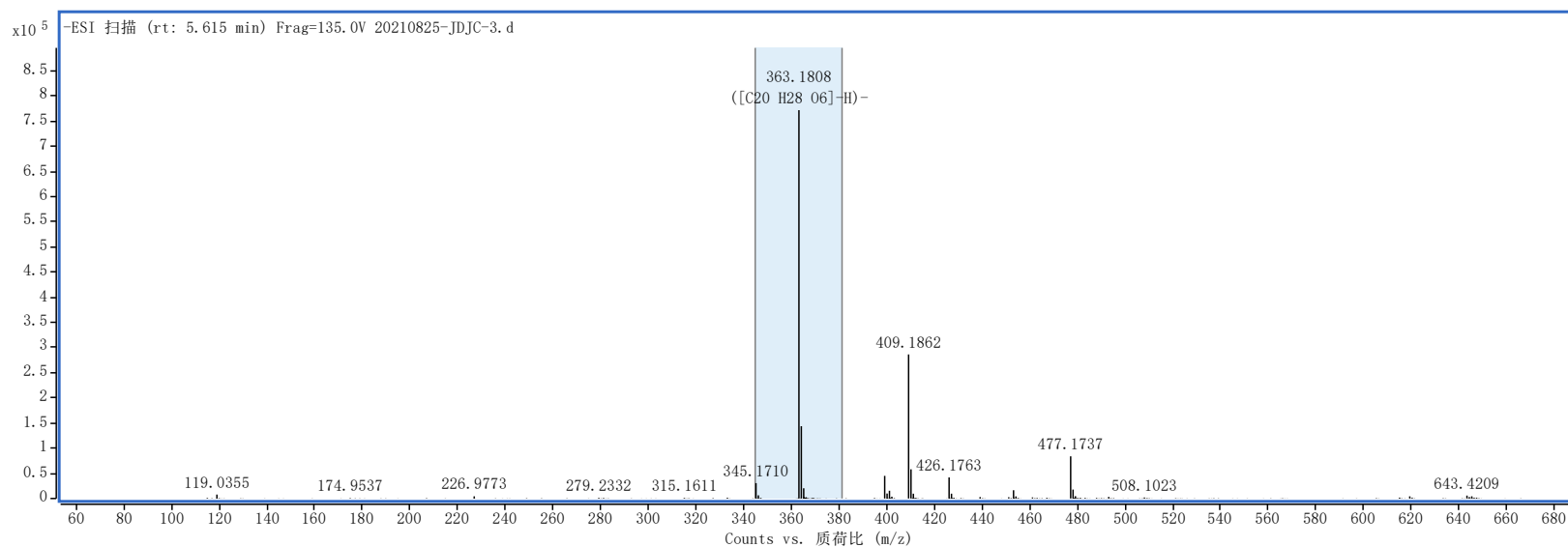


Figure S2. Key  $^1\text{H}$ - $^1\text{H}$  COSY (—), HMBC (---) and ROESY (····) correlations of **M4**



Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z
743524	79.3	100	363.1813	0.5	771702.1	100	82.3	363.1808

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M-H)-	363.1808	90.64	98.6	96.65	96.65	99.95	771702.1	C20 H27 O6

Figure S3. HRESIMS spectrum of 16 $\alpha$ -hydroxy ingenol (**M2**)

XSJ-JDJC-3 <sup>1</sup>H NMR (500MHz, CD<sub>3</sub>OD)

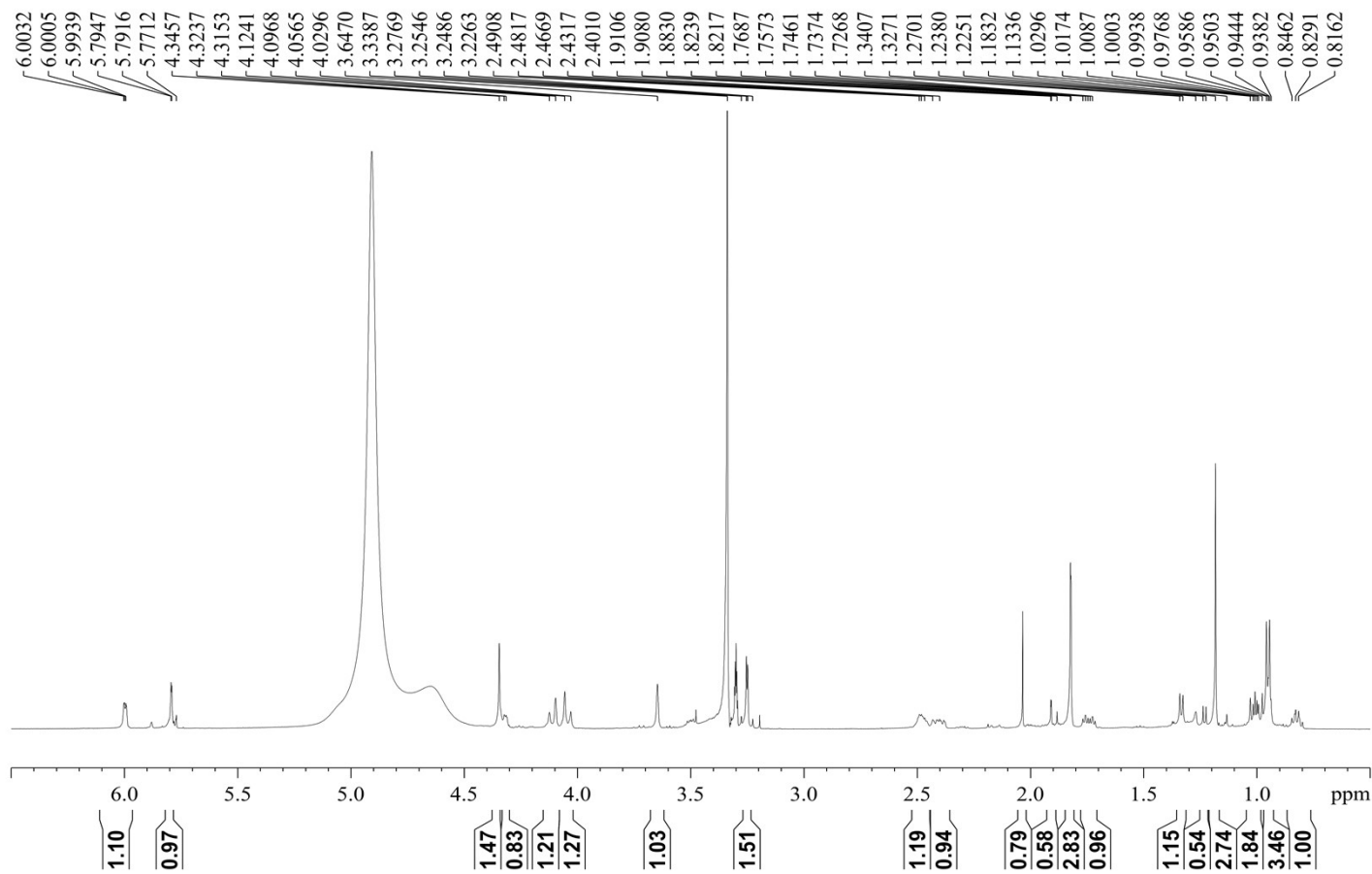


Figure S4. <sup>1</sup>H-NMR spectrum of 16 $\alpha$ -hydroxy ingenol (M2) in methanol-d<sub>4</sub>

XSJ-JDJC-3 C13 and DEPT NMR (125 MHz, CD3OD)

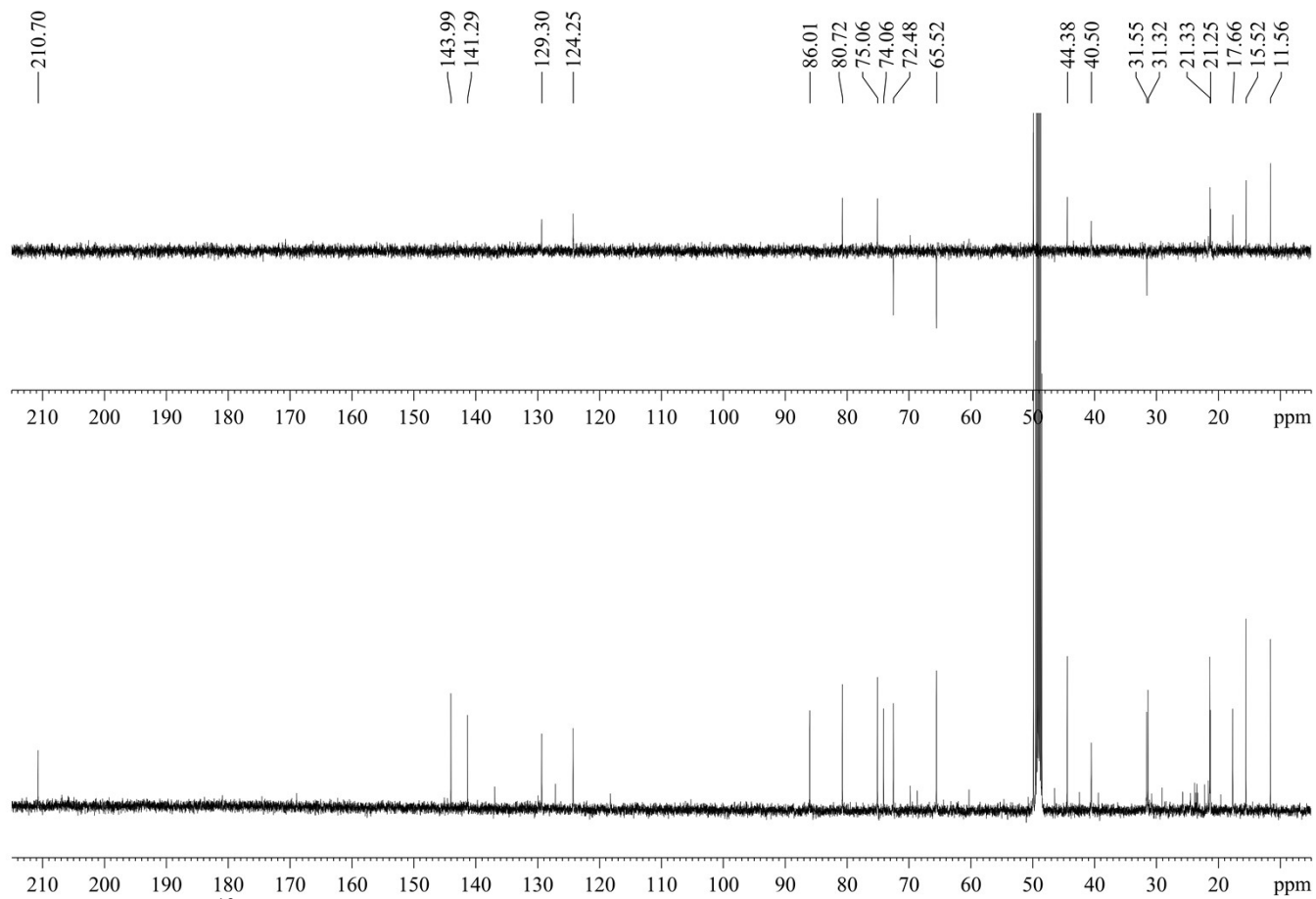
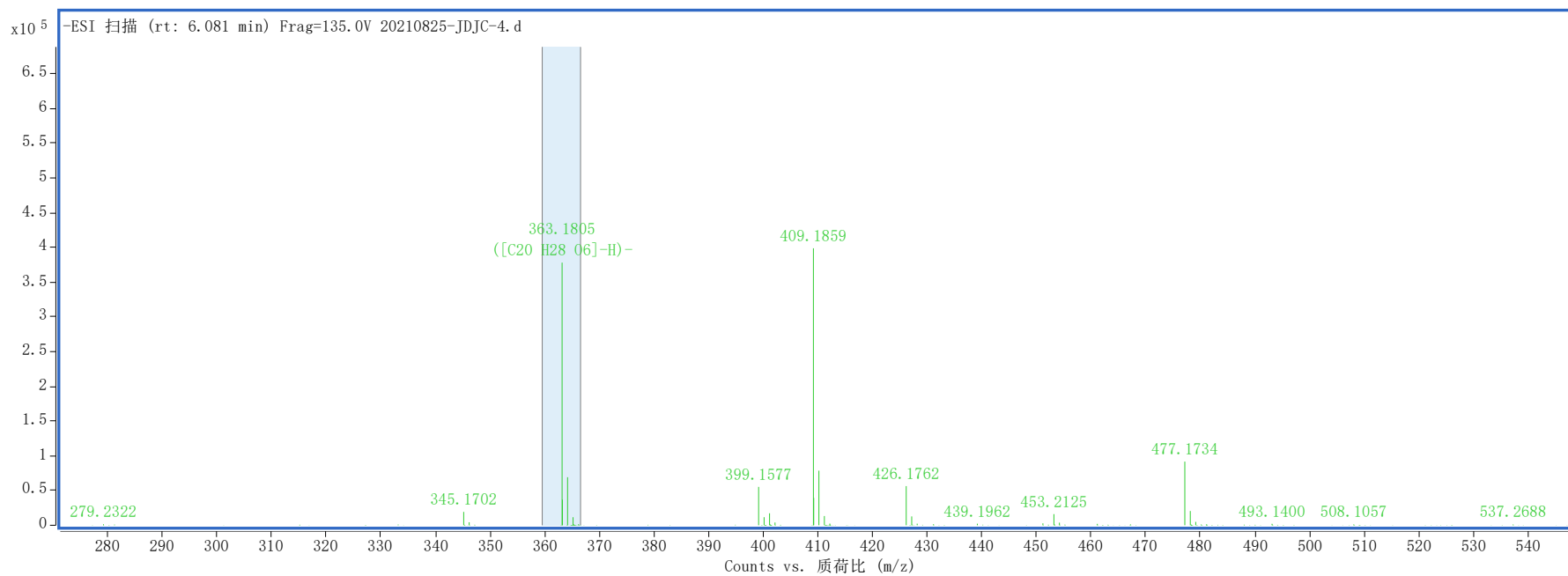


Figure S5.  $^{13}\text{C}$  and DEPT-135 NMR spectrum of  $16\alpha$ -hydroxy ingenol (**M2**) in methanol- $d_4$



Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z
364762	79.5	100	363.1813	0.8	377952.3	100	82.4	363.1805

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M-H)-	363.1805	89.15	96.87	95.17	95.17	99	377952.3	C <sub>20</sub> H <sub>27</sub> O <sub>6</sub>

Figure S6. HRESIMS spectrum of 17 $\beta$ -hydroxy ingenol (**M3**)

## Rudolph Research Analytical

Monday, 07/05/2021

This sample was measured on an Autopol VI, serial number 90079,  
manufactured by Rudolph Research Analytical, Hackettstown, NJ.

LotID : JDJC-4  
Set Temperature : 20.0  
Temp Corr : OFF

<b>n</b>	<b>Average</b>	<b>Std.Dev.</b>	<b>Maximum</b>	<b>Minimum</b>
6	-8.933	0.1886	-8.800	-9.200

<b>S.No</b>	<b>Sample ID</b>	<b>Time</b>	<b>Result</b>	<b>Scale</b>	<b>OR ° Arc</b>	<b>WLG</b>	<b>Lg.mm</b>	<b>Conc.</b>	<b>Temp.</b>	<b>Comment</b>
1	JDJC-4	02:59:01 PM	-8.800	SR	-0.022	589	100.00	0.250	19.7	
2	JDJC-4	02:59:08 PM	-9.200	SR	-0.023	589	100.00	0.250	19.7	
3	JDJC-4	02:59:15 PM	-9.200	SR	-0.023	589	100.00	0.250	19.7	
4	JDJC-4	02:59:22 PM	-8.800	SR	-0.022	589	100.00	0.250	19.7	
5	JDJC-4	02:59:30 PM	-8.800	SR	-0.022	589	100.00	0.250	19.7	
6	JDJC-4	02:59:37 PM	-8.800	SR	-0.022	589	100.00	0.250	19.8	

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Signature

Figure S7. OR Value of 17 $\beta$ -hydroxy ingenol (**M3**) in CH<sub>3</sub>OH



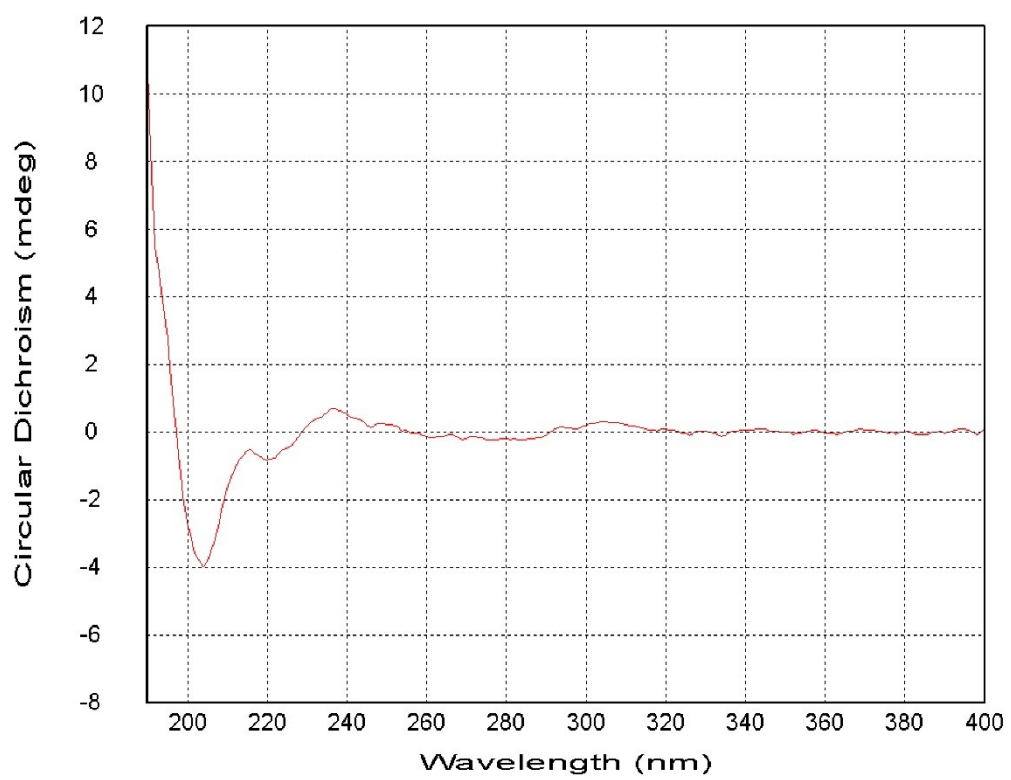


Figure S8. CD Value of 17β-hydroxy ingenol (**M3**) in CH<sub>3</sub>OH

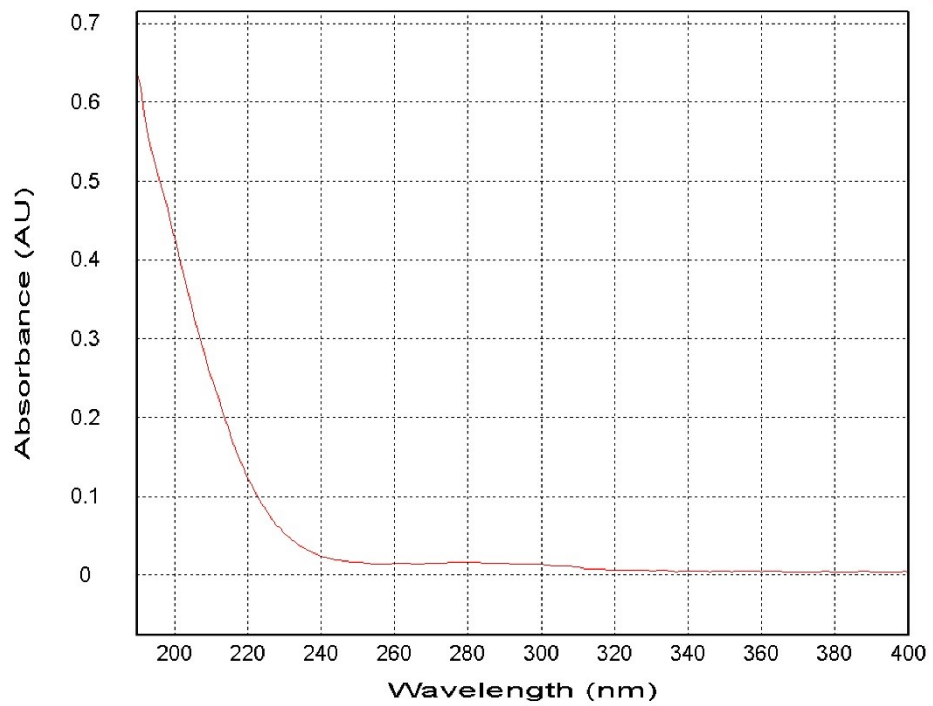


Figure S9. UV spectrum of 17β-hydroxy ingenol (**M3**) in CH<sub>3</sub>OH

XSJ-JDJC-4 <sup>1</sup>H NMR (500 MHz, CD3OD)

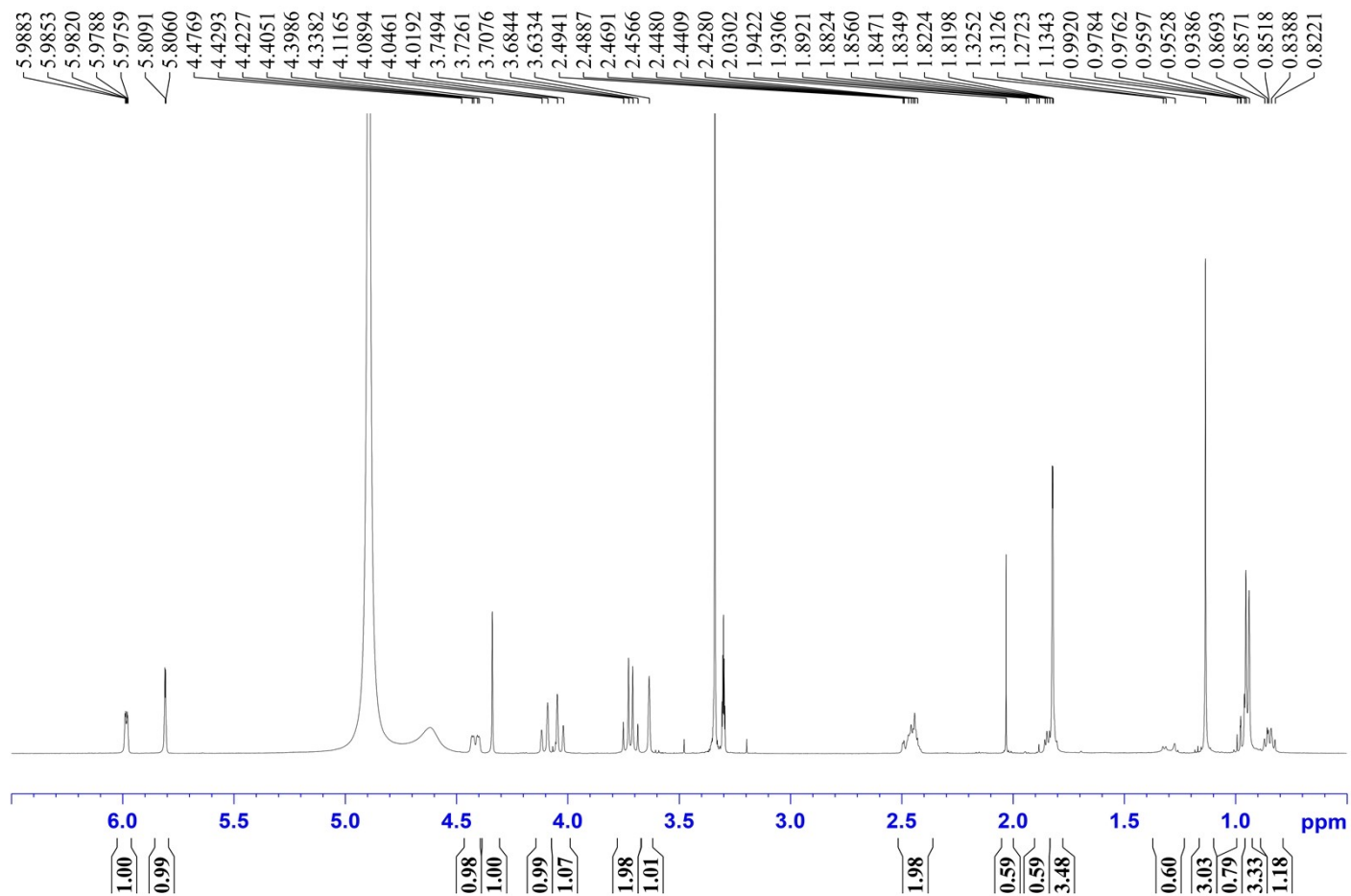


Figure S10. <sup>1</sup>H-NMR spectrum of 17 $\beta$ -hydroxy ingenol (**M3**) in methanol-d<sub>4</sub>

XSJ-JDJC-4 C13 NMR (125 MHz, CD3OD)

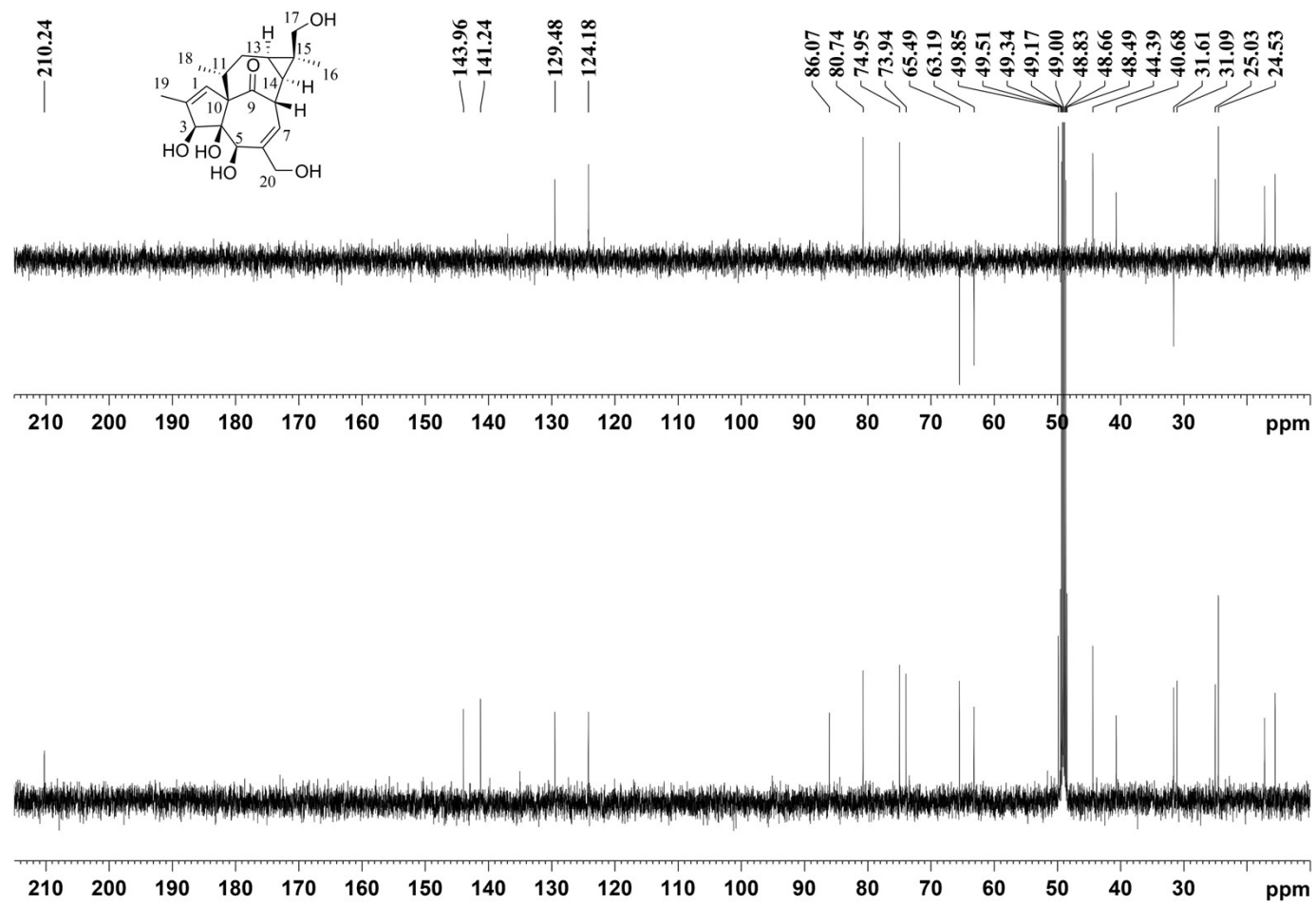


Figure S11. <sup>13</sup>C and DEPT-135 NMR spectrum of 17β-hydroxy ingenol (M3) in Methanol-d4

XSJ-JDJC-4 1H 1H COSY

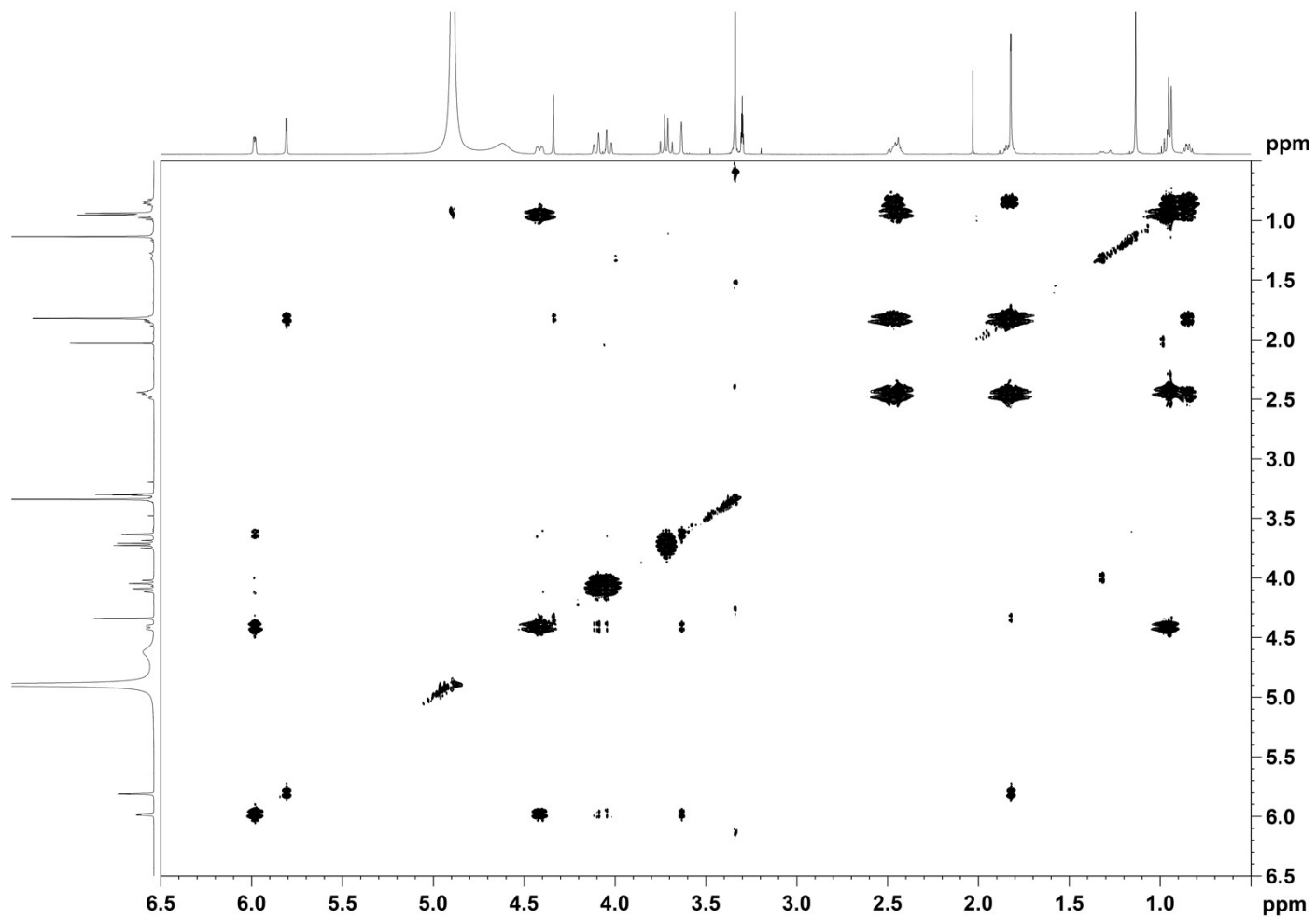


Figure S12.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of 17 $\beta$ -hydroxy ingenol (**M3**) in methanol- $d_4$

XSJ-JDJC-4 HSQC

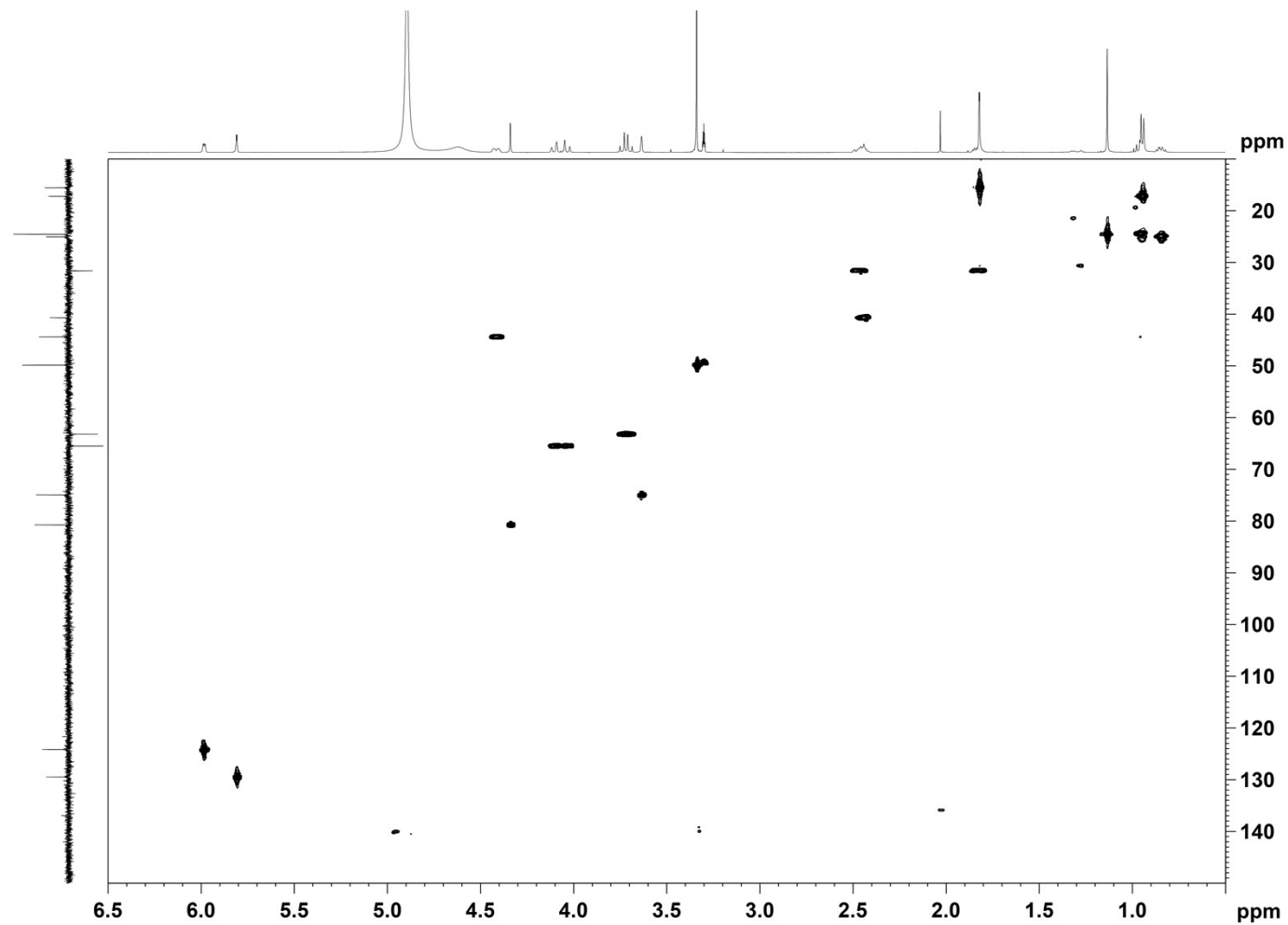


Figure S13. HSQC spectrum of 17 $\beta$ -hydroxy ingenol (**M3**) in methanol-d<sub>4</sub>



XSJ-JDJC-4 HMBC

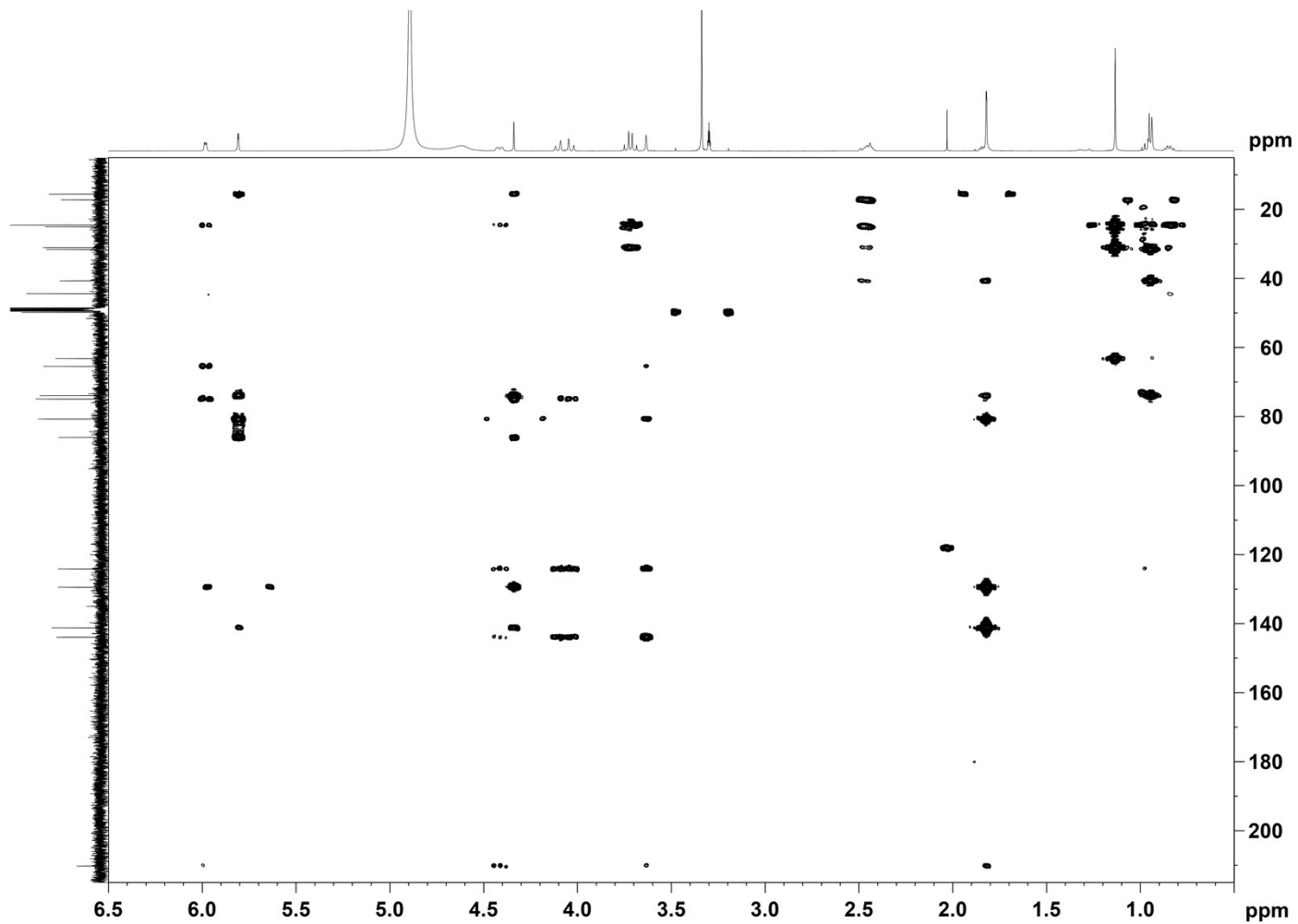


Figure S14. HMBC spectrum of 17 $\beta$ -hydroxy ingenol (**M3**) in methanol-d<sub>4</sub>

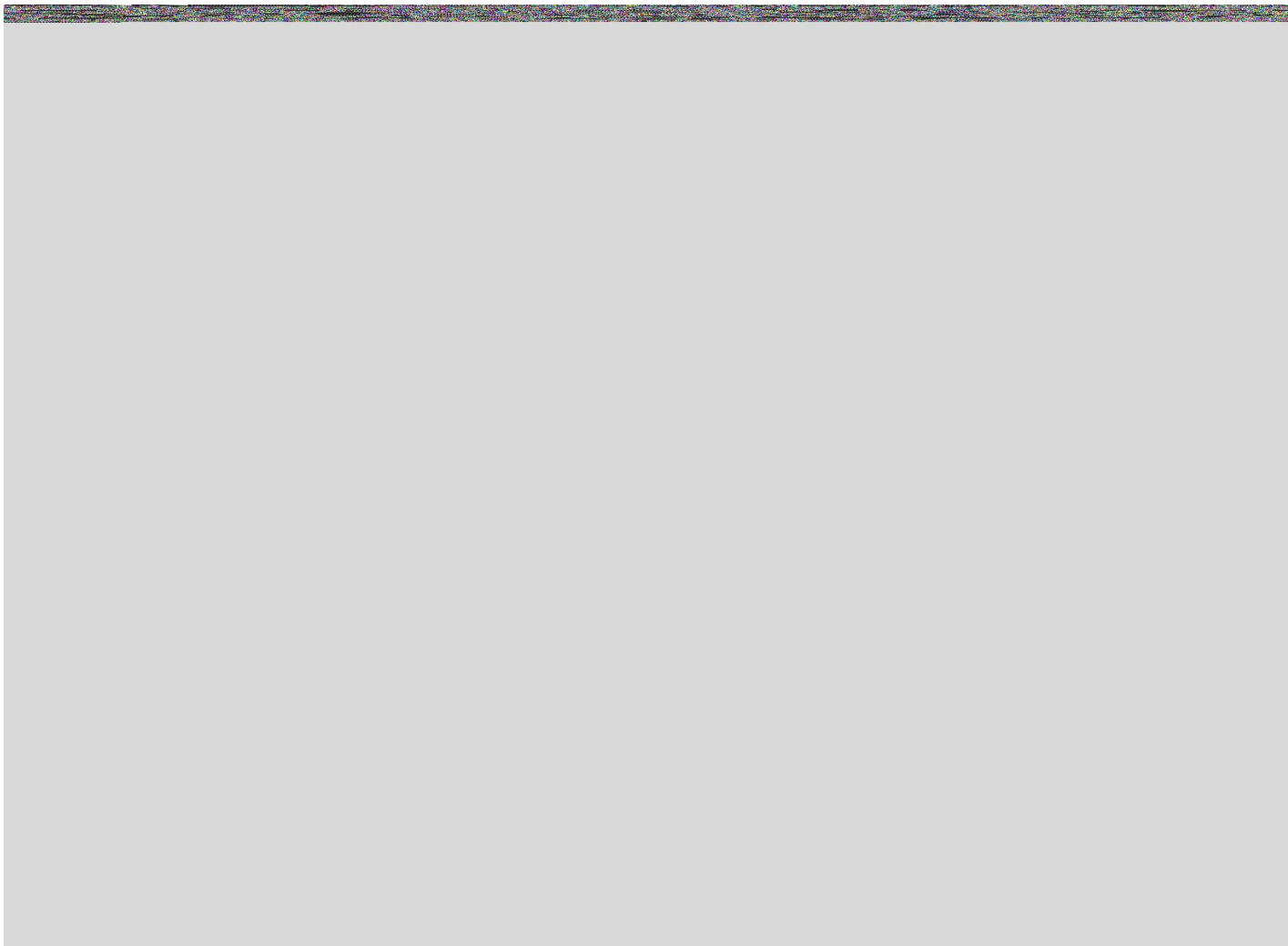
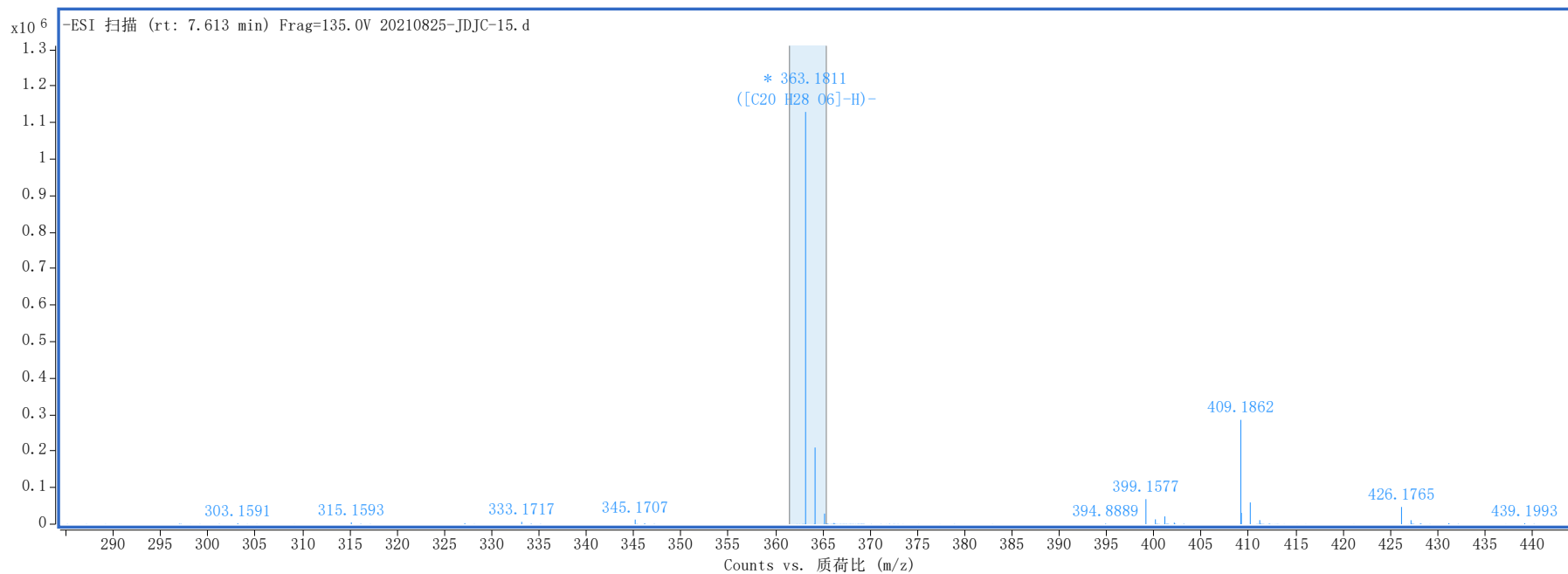


Figure S15. NOESY spectrum of 17 $\beta$ -hydroxy ingenol (**M3**) in methanol-d4



Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z
1087811.5	79.5	100	363.1813	0.2	1128967.4	100	82.5	363.1811

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M-H)-	363.1811	90.56	99.74	97.14	97.14	99.85	1128967.4	C20 H27 O6

Figure S16. HRESIMS spectrum of 19-hydroxy ingenol (**M4**)

## Rudolph Research Analytical

Monday, 07/05/2021

This sample was measured on an Autopol VI, serial number 90079,  
manufactured by Rudolph Research Analytical, Hackettstown, NJ.

LotID : JDJC-15

Set Temperature : 20.0

Temp Corr : OFF

n	Average	Std.Dev.	Maximum	Minimum
6	-18.500	0.5000	-18.000	-19.000

S.No	Sample ID	Time	Result	Scale	OR ° Arc	WLG	Lg.mm	Conc.	Temp.	Comment
1	JDJC-15	03:36:44 PM	-18.000	SR	-0.018	589	100.00	0.100	19.8	
2	JDJC-15	03:36:52 PM	-19.000	SR	-0.019	589	100.00	0.100	19.8	
3	JDJC-15	03:36:59 PM	-19.000	SR	-0.019	589	100.00	0.100	19.8	
4	JDJC-15	03:37:06 PM	-19.000	SR	-0.019	589	100.00	0.100	19.8	
5	JDJC-15	03:37:13 PM	-18.000	SR	-0.018	589	100.00	0.100	19.8	
6	JDJC-15	03:37:20 PM	-18.000	SR	-0.018	589	100.00	0.100	19.8	

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Signature

Figure S17. OR Value of 19-hydroxy ingenol (**M4**) in CH<sub>3</sub>OH

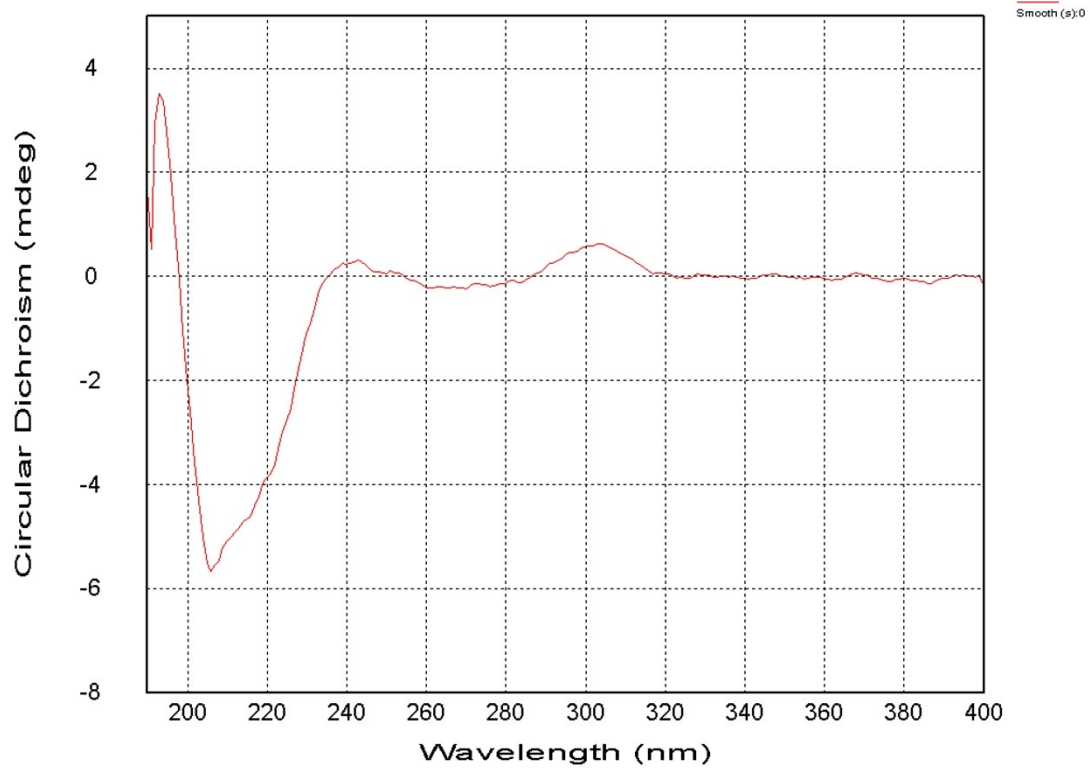


Figure S18. CD Value of 19-hydroxy ingenol (**M4**) in CH<sub>3</sub>OH

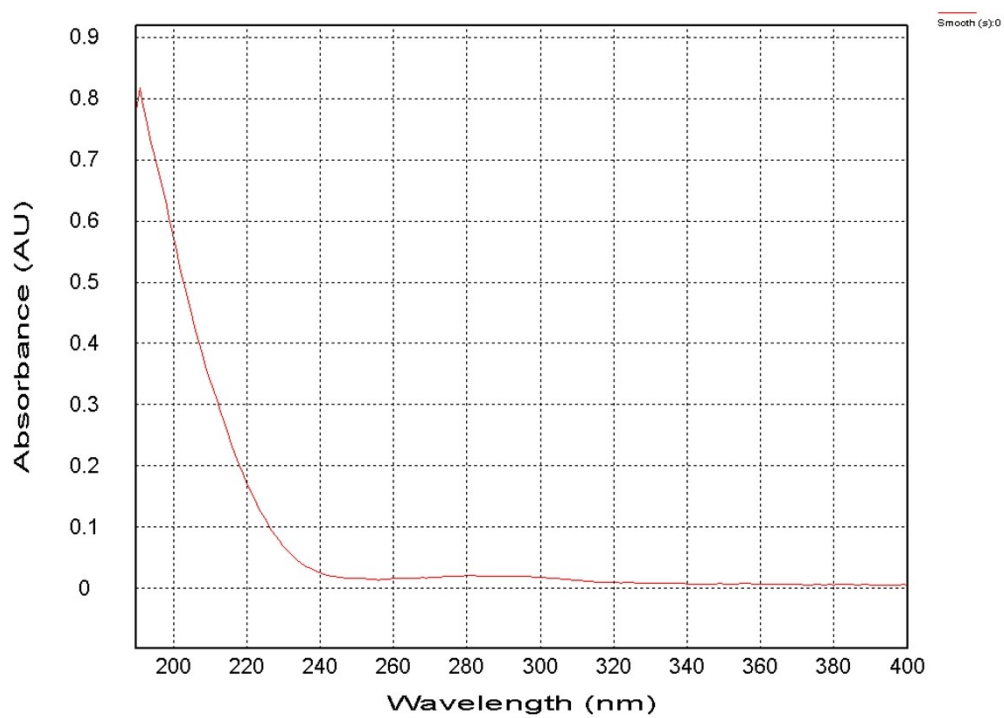


Figure S19. UV spectrum of 19-hydroxy ingenol (**M4**) in CH<sub>3</sub>OH

XSJ-JDJC-15 1H NMR (500 MHz, CD3OD)

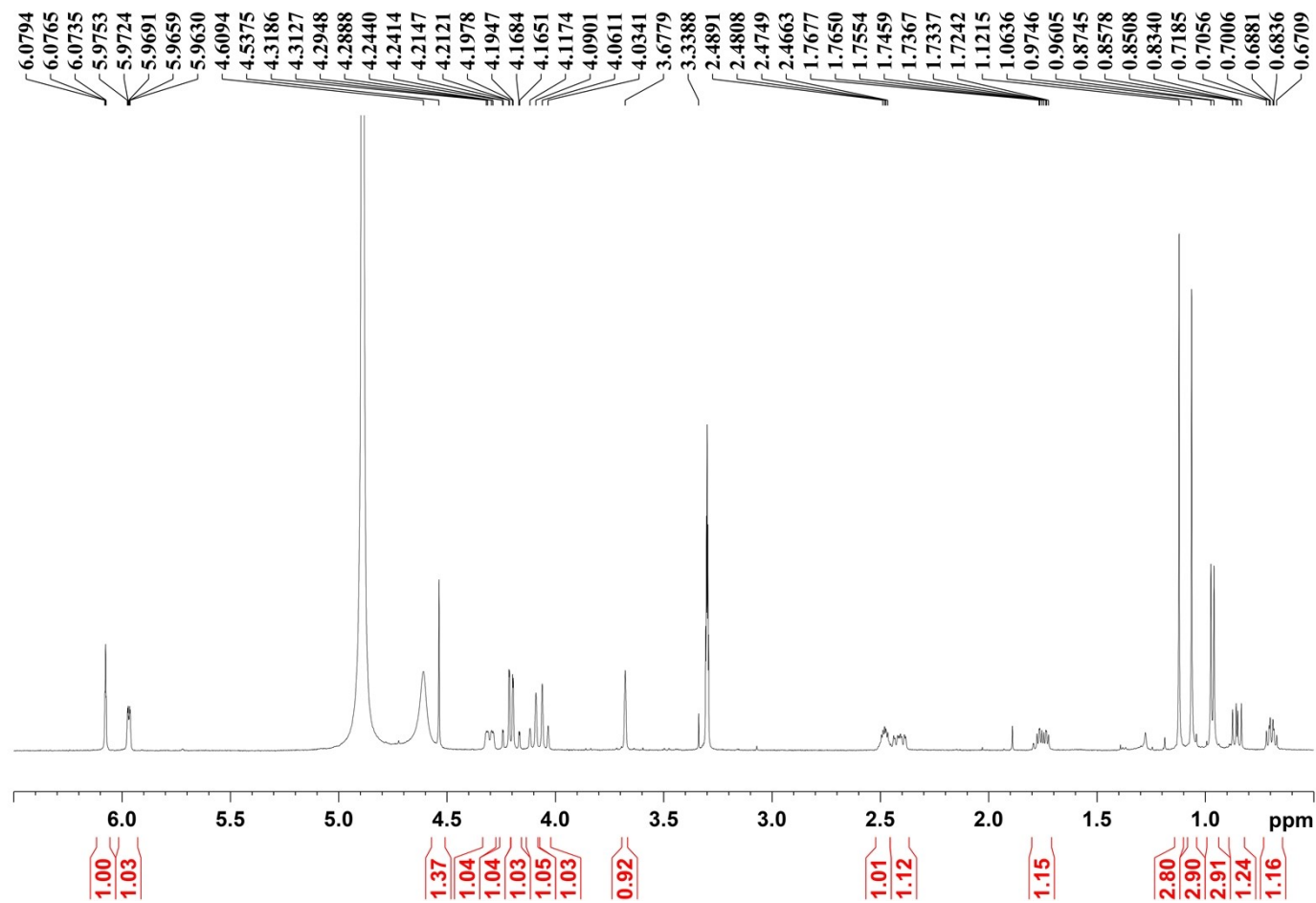


Figure S20. 1H NMR spectrum of 19-hydroxy ingenol (M4) in methanol-d4

<sup>13</sup>C and DEPT NMR for JDJC-15 (125 MHz, CD<sub>3</sub>OD)

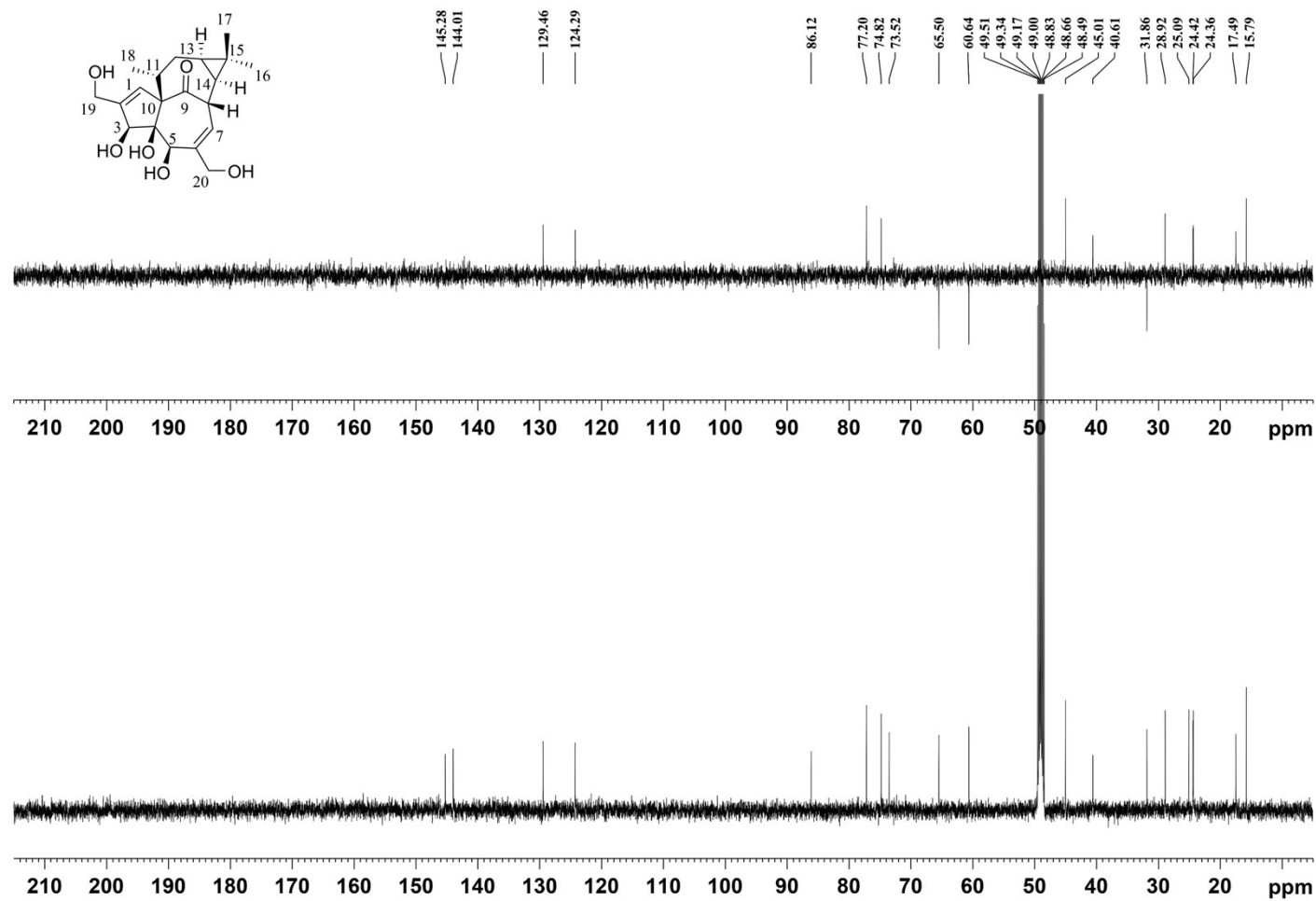


Figure S21. <sup>13</sup>C NMR and DEPT-135 spectrum of 19-hydroxy ingenol (M4) in methanol-d<sub>4</sub>



XSJ-JDJC-15 1H 1H COSY

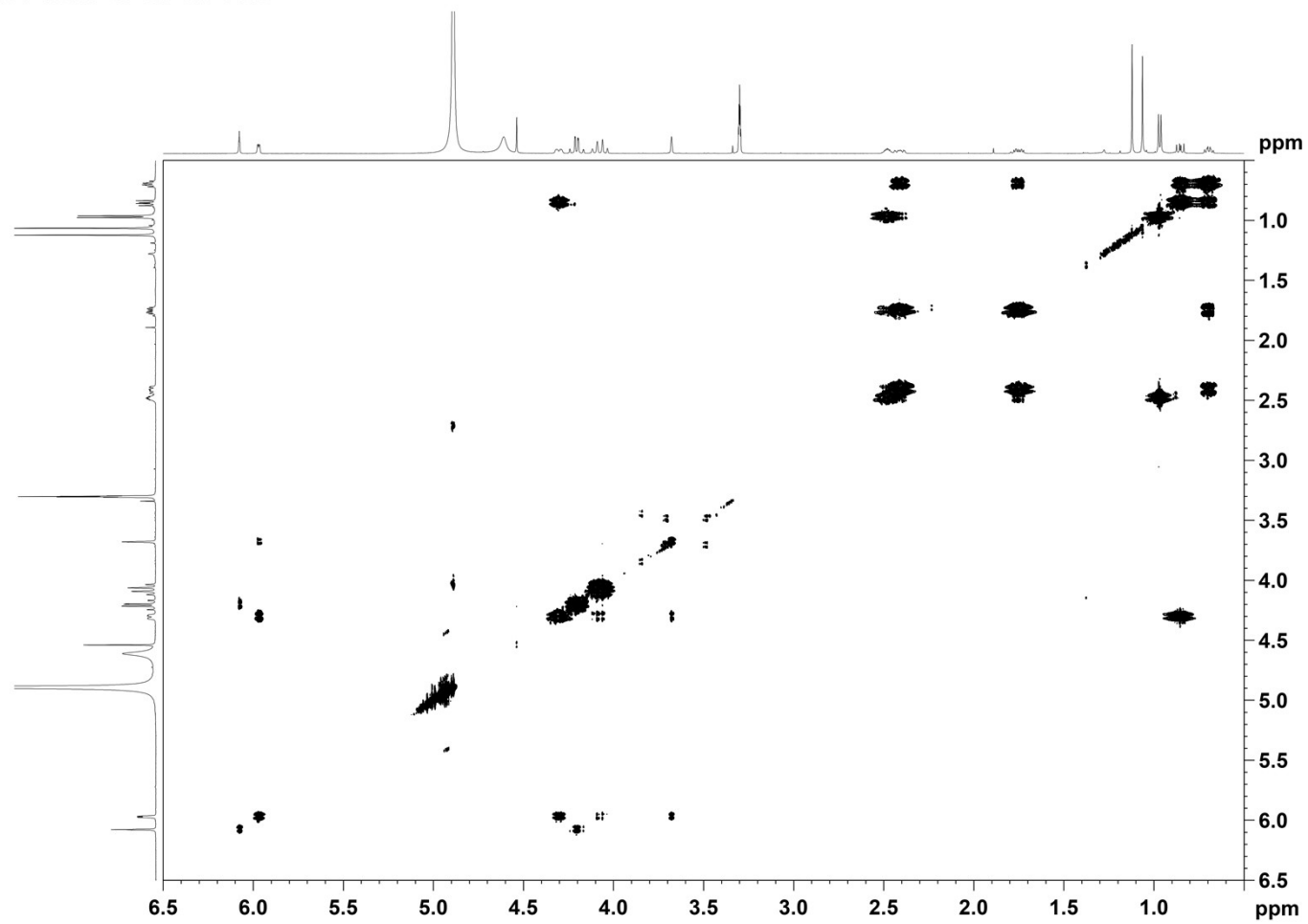


Figure S22.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of 19-hydroxy ingenol (M4) in methanol- $d_4$

XSJ-JDJC-15 HSQC

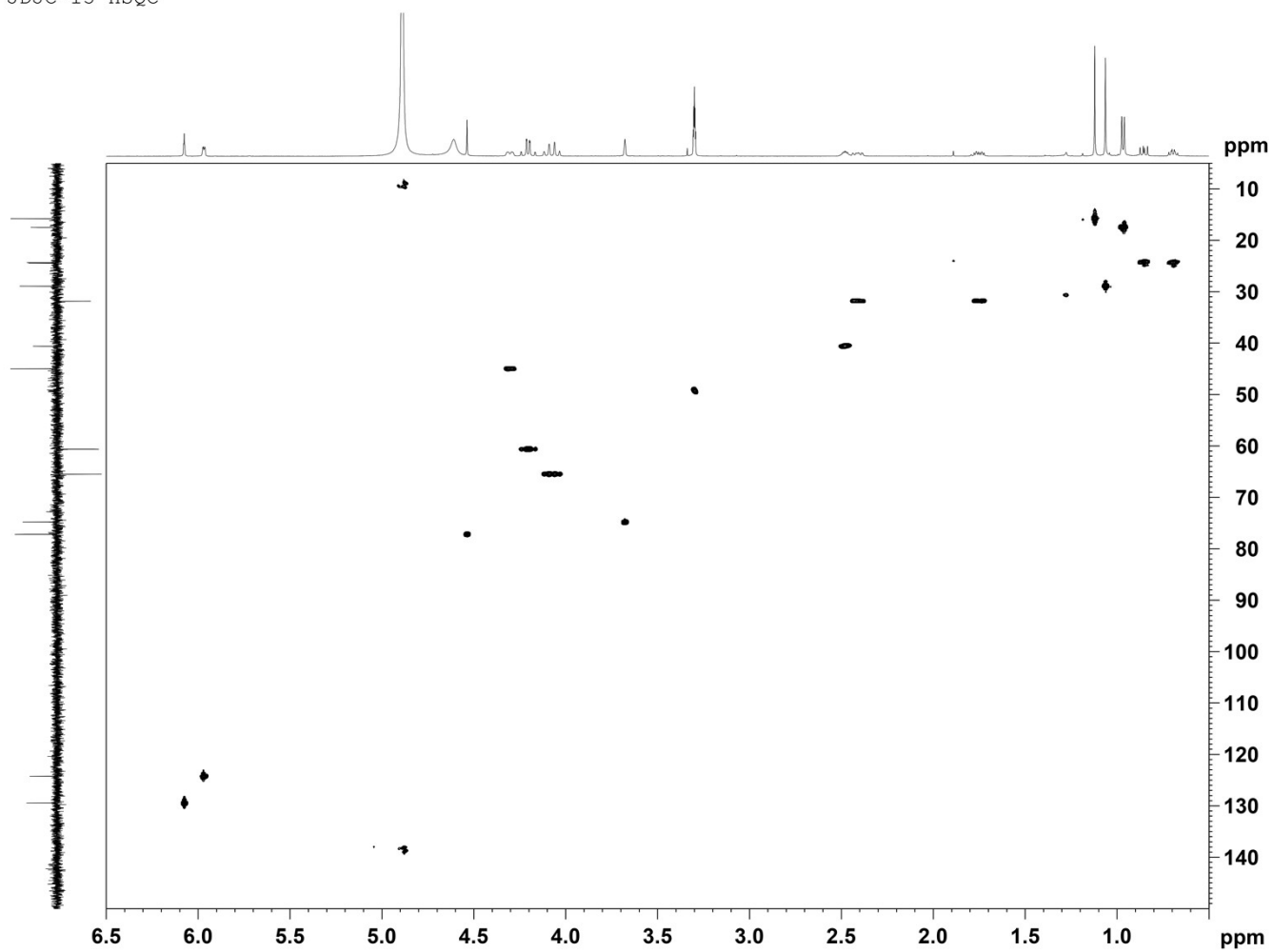


Figure S23. HSQC spectrum of 19-hydroxy ingenol (**M4**) in methanol- $d_4$

XSJ-JDJC-15 HMBC

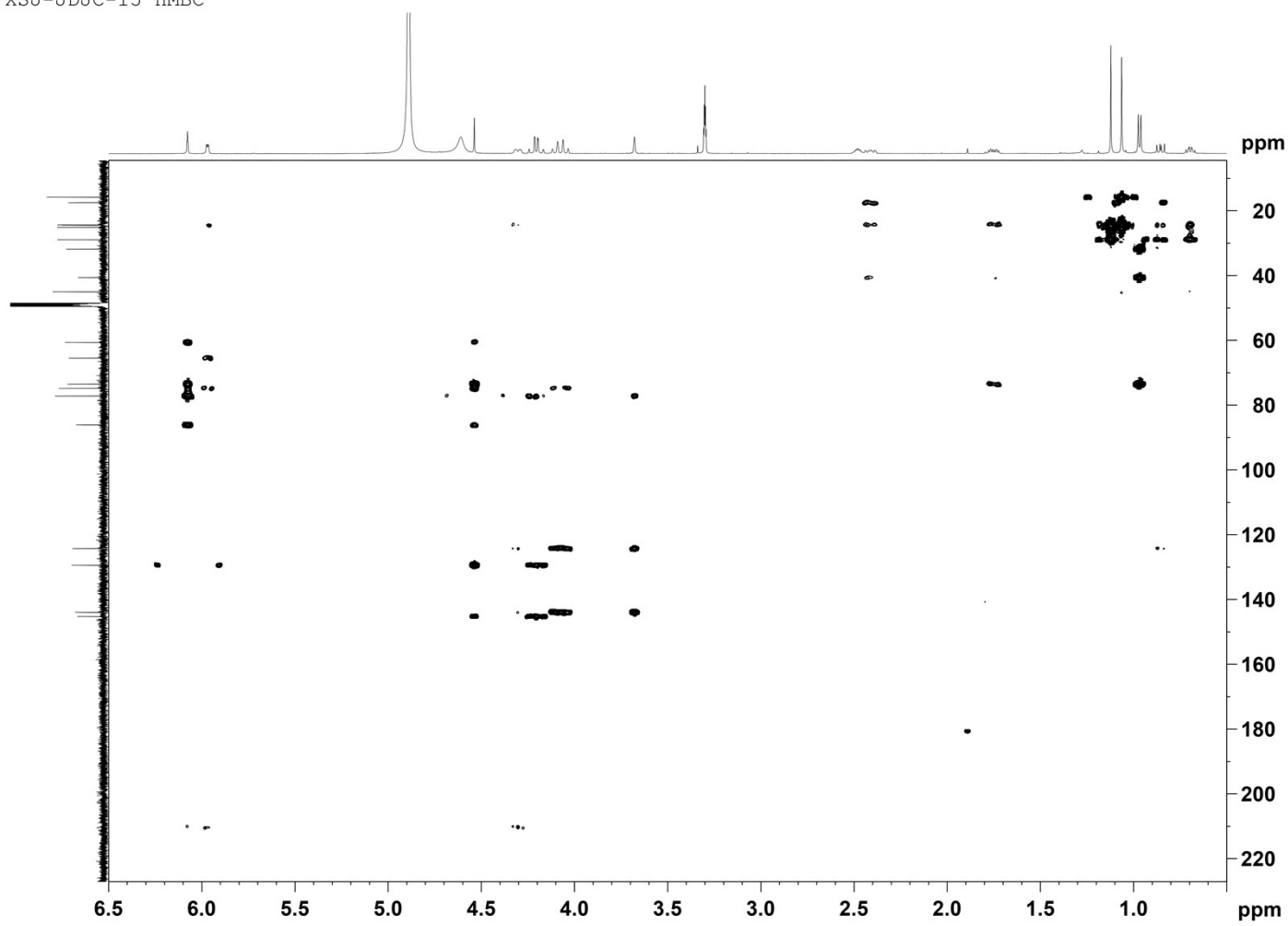


Figure S24. HMBC spectrum of 19-hydroxy ingenol (M4) in methanol-d4

XSJ-JDJC-15 NOESY

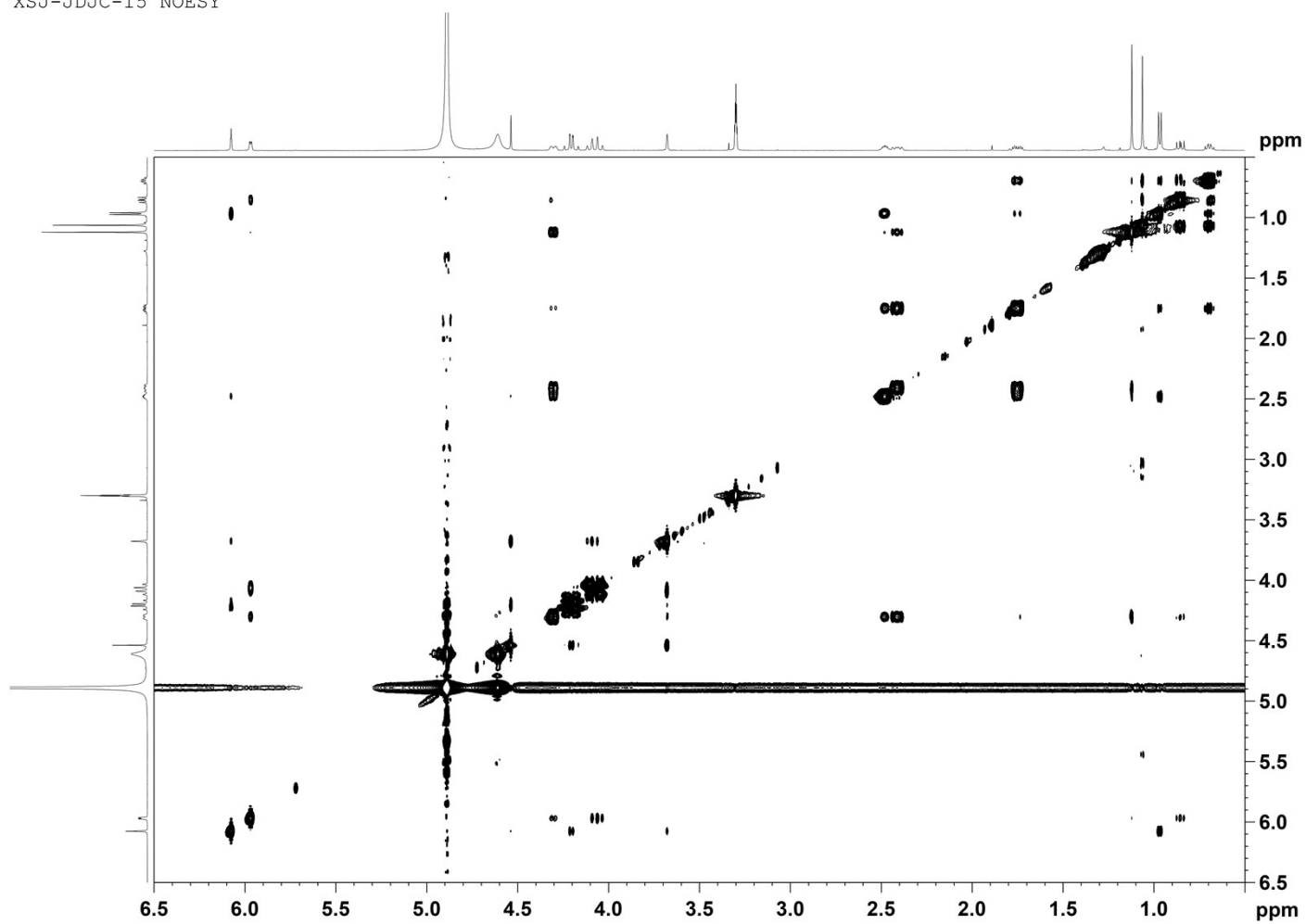


Figure S25. NOESY spectrum of 19-hydroxy ingenol (M4) in methanol-d4