

SUPPLEMENTARY MATERIAL

Piptolinic acids F–J, five new lanostane-type triterpenoids from *Piptoporus betulinus*

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ABSTRACT:

Five new lanostane triterpenoids, named piptolinic acids F–J (**1–5**), as well as seven known analogues (**6–12**), were isolated from methanolic extract of the fruiting bodies of *Piptoporus betulinus*. Compounds **1–4** are 24-methyl-lanostane triterpenoids, while compound **5** is a 3,4-*seco*-lanostane derivative. Their structures were established on the basis of extensive spectroscopic analysis (1D, 2D NMR, and HRESIMS). Cytotoxicity evaluation indicated that compound **6** exhibited moderate cytotoxic activity against human melanoma cell line A-375 (IC₅₀ = 42.8 μM) and human renal carcinoma cell line 786-O (IC₅₀ = 56.5 μM).

Keywords: *Piptoporus betulinus*; Polyporaceae; Lanostane triterpenoid; Cytotoxicity; A-375; 786-O

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Table S1. ^1H and ^{13}C NMR data of compounds **1** and **2**^a (in CD_3OD)

position	1		2	
	δ_{H} (mult, J , Hz)	δ_{C}	δ_{H} (mult, J , Hz)	δ_{C}
1	2.26 (m), 1.57 (m)	28.4	1.85 (m), 1.79 (m)	28.7
2	2.24 (m), 1.77 (m)	24.5	2.26 (m), 1.72 (m)	24.8
3	3.59 (brs)	74.2	3.61 (brs)	75.5
4		41.5		41.8
5		176.6		173.0
6	6.00 (s)	125.5	6.16 (s)	126.0
7		202.5		186.4
8	3.08 (s)	52.8		139.0
9		141.8		165.6
10		39.8		43.6
11	5.55 (d, 5.7)	118.1	2.46 (2H, m)	23.1
12	2.17 (m), 1.84 (m)	34.7	1.93 (m), 1.64 (m)	28.4
13		45.3		47.4
14		45.3		47.1
15	2.31 (m), 1.89 (m)	47.0	2.44 (m), 2.17 (d, 14.4)	44.6
16	4.02 (t, 7.2)	76.1	4.07 (t, 7.3)	77.0
17	2.05 (m)	55.0	2.07 (m)	54.4
18	0.83 (3H, s)	15.2	0.79 (3H, s)	16.8
19	1.41 (3H, s)	29.6	1.44 (3H, s)	27.9
20	2.41 (m)	47.1	2.40 (m)	47.3
21		178.3		179.2
22	2.06 (m), 1.74 (m)	30.3	2.09 (m), 1.77 (m)	30.6
23	2.02 (2H, m)	32.0	2.07 (m), 2.00 (m)	32.2
24		155.2		155.5
25	2.25 (m)	33.5	2.26 (m)	33.8
26	1.01 (3H, d, 7.0)	20.9	1.03 (3H, d, 6.8)	21.1
27	1.03 (3H, d, 6.7)	20.8	1.04 (3H, d, 6.9)	21.0
28	1.20 (3H, s)	25.8	1.25 (3H, s)	27.1
29	1.23 (3H, s)	29.5	1.32 (3H, s)	28.0
30	0.89 (3H, s)	17.1	1.17 (3H, s)	23.9
31	4.75 (s), 4.72 (s)	106.0	4.77 (s), 4.74 (s)	106.2

^a Recorded at 400 or 100 MHz for ^1H and ^{13}C , respectively.

Table S2. ¹H and ¹³C NMR data of compounds **3–5**^a

position	3 ^b		4 ^b		5 ^c	
	δ_{H} (mult, <i>J</i> , Hz)	δ_{C}	δ_{H} (mult, <i>J</i> , Hz)	δ_{C}	δ_{H} (mult, <i>J</i> , Hz)	δ_{C}
1	2.02 (m), 1.44 (m)	31.2	2.34 (m), 1.84 (m)	31.8	1.72 (2H, m)	34.0
2	2.02 (m), 1.81 (m)	27.3	2.29 (m), 1.93 (m)	27.1	2.32 (m), 1.96 (m)	30.6
3	3.64 (brs)	75.5	3.77 (brs)	75.8		178.5
4		38.6		41.6		148.8
5	2.00 (m)	45.0		152.3	2.19 (m)	48.3
6	1.71 (m), 1.60 (m)	19.0	6.18 (d, 6.2)	119.5	1.75 (m), 1.55 (m)	25.3
7	2.13 (m), 2.01 (m)	27.1	5.76 (d, 6.2)	117.2	1.98 (m), 1.38 (m)	28.0
8		135.0		141.3		140.3
9		135.8		147.8		130.9
10		37.9		41.9		41.6
11	2.12 (m), 2.04 (m)	21.5	5.87 (d, 5.9)	120.6	2.11 (m), 1.96 (m)	22.8
12	2.21 (m), 2.01 (m)	30.2	2.74 (d, 8.3), 2.51 (m)	37.5	1.74 (m), 1.50 (m)	30.3
13		46.7		45.4		45.3
14		49.3		49.7		51.7
15	2.42 (m), 1.70 (m)	44.1	2.48 (m), 2.00 (d, 13.1)	44.7	1.65 (m), 1.26 (m)	31.7
16	4.54 (t, 7.0)	77.2	4.56 (t, 6.9)	77.2	2.00 (2H, m)	27.1
17	2.81 (dd, 11.1, 7.0)	57.8	2.88 (dd, 11.2, 6.9)	57.8	2.07 (m)	48.4
18	1.18 (3H, s)	18.2	1.16 (3H, s)	18.2	0.83 (3H, s)	16.7
19	1.06 (3H, s)	19.8	1.41 (3H, s)	36.6	0.97 (3H, s)	22.8
20	2.96 (m)	49.2	2.98 (m)	49.0	2.24 (m)	49.4
21		179.3		179.1		180.6
22	2.66 (m), 2.45 (m)	32.1	2.67 (m), 2.47 (m)	31.9	1.52 (2H, m)	33.7
23	2.54 (m), 2.40 (m)	33.7	2.54 (m), 2.39 (m)	33.7	1.98 (m), 1.92 (m)	27.0
24		156.6		156.5	5.11 (t, 7.1)	124.9
25	2.30 (m)	34.6	2.30 (m)	34.6		133.0
26	1.00 (3H, d, 6.9)	22.5	1.00 (3H, d, 6.8)	22.5	1.67 (3H, s)	25.9
27	1.01 (3H, d, 6.8)	22.3	1.01 (3H, d, 6.8)	22.3	1.59 (3H, s)	17.8
28	0.94 (3H, s)	23.1	1.46 (3H, s)	29.2	4.91 (s), 4.71 (s)	114.5
29	1.24 (3H, s)	29.5	1.33 (3H, s)	32.6	1.78 (3H, s)	23.5
30	1.41 (3H, s)	25.8	1.40 (3H, s)	27.1	0.98 (3H, s)	25.7
31	5.00 (s), 4.86 (s)	107.5	5.00 (s), 4.86 (s)	107.5		

^a Recorded at 400 or 100 MHz for ¹H and ¹³C, respectively; ^b in pyridine-*d*₅; ^c in CD₃OD.

Figure S1. ^1H NMR spectrum of piptolinic acid **1** in CD_3OD

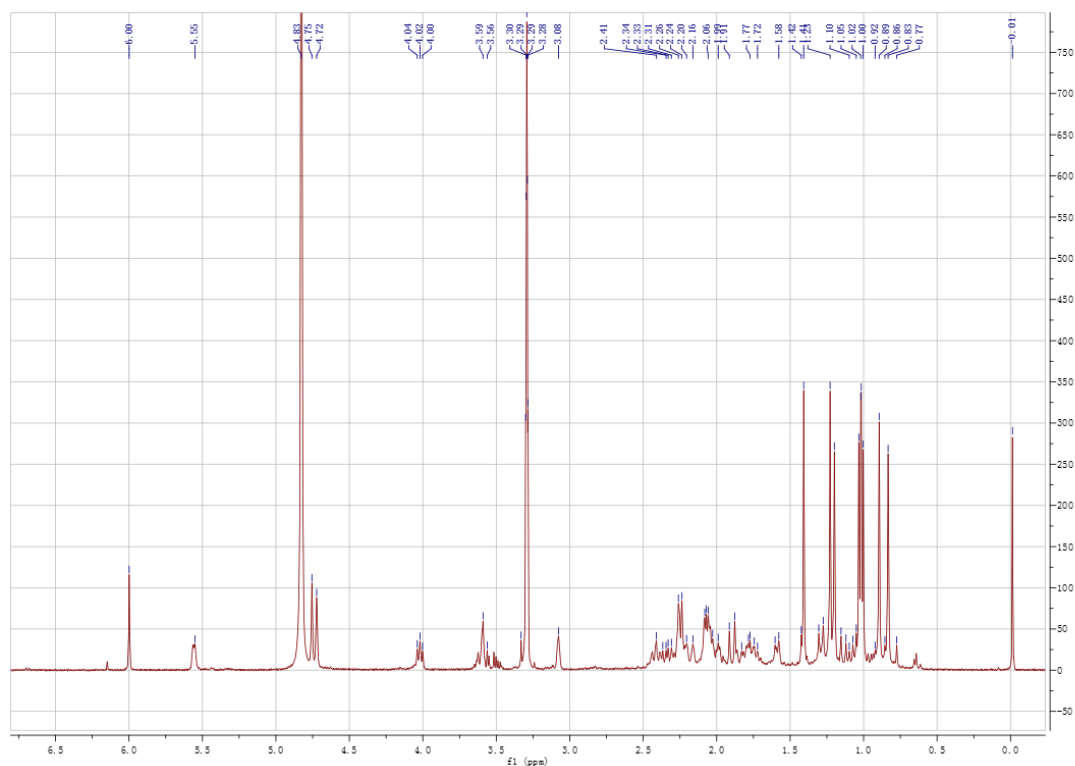


Figure S2. ^{13}C NMR spectrum of piptolinic acid **1** in CD_3OD

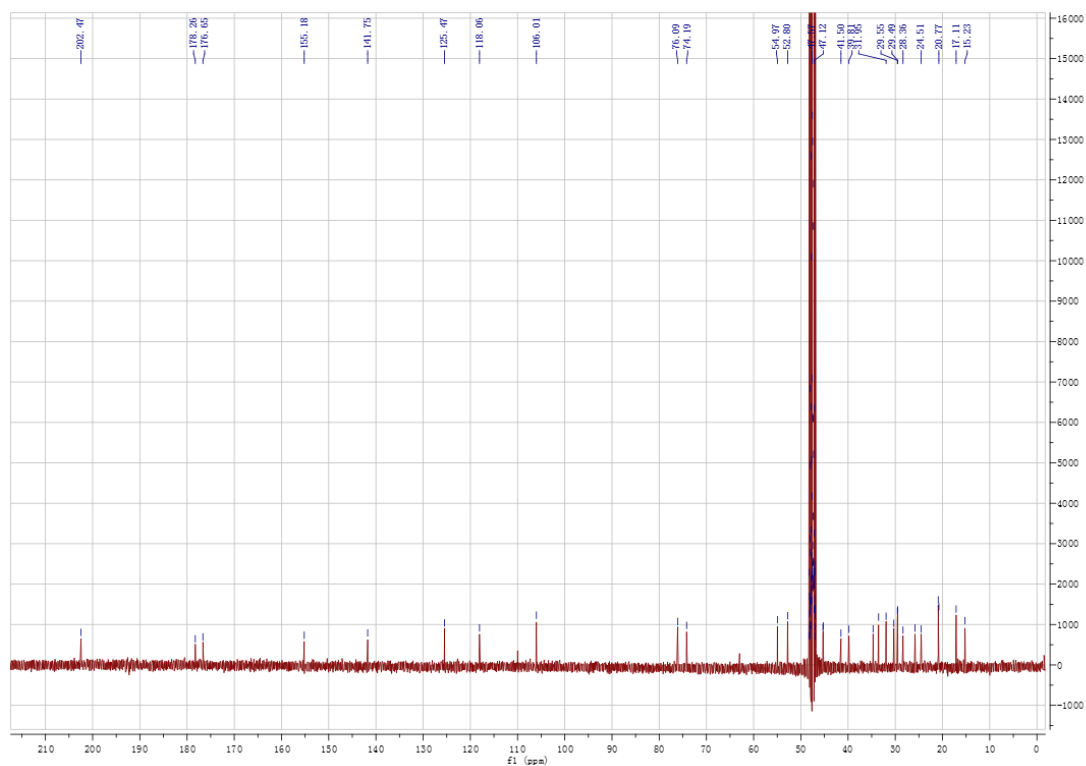


Figure S3. ^1H - ^1H COSY spectrum of piptolinic acid F (**1**) in CD_3OD

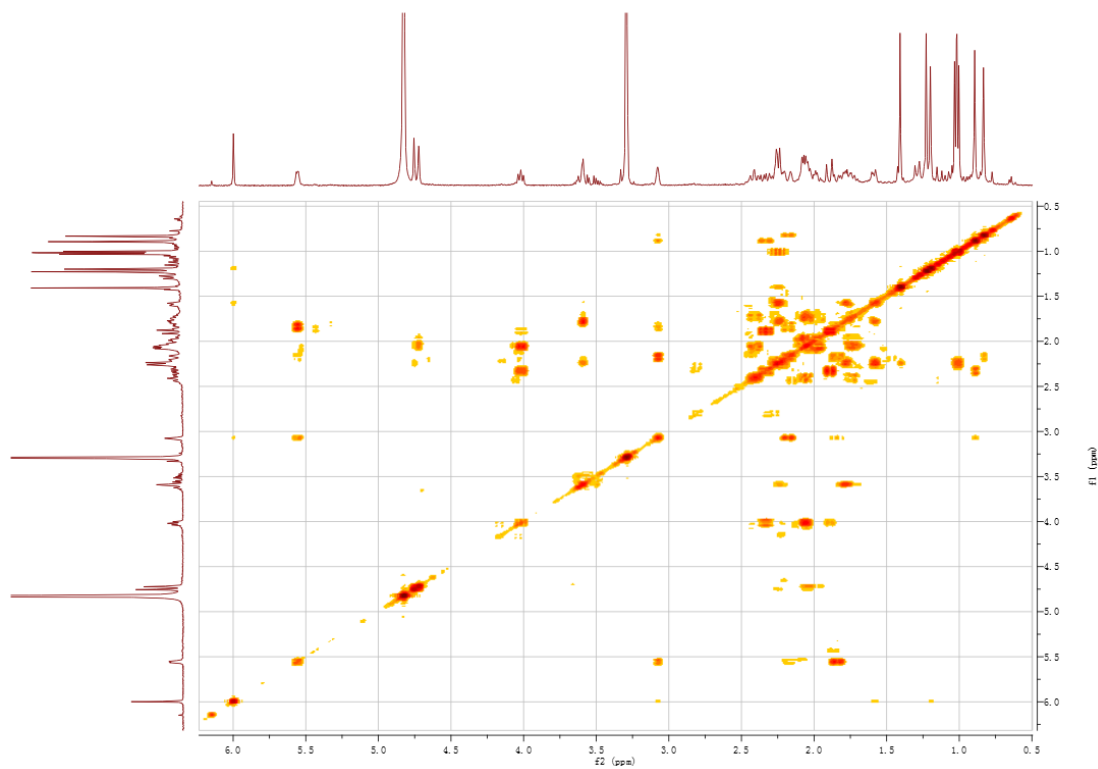


Figure S4. HSQC spectrum of piptolinic acid F (**1**) in CD_3OD

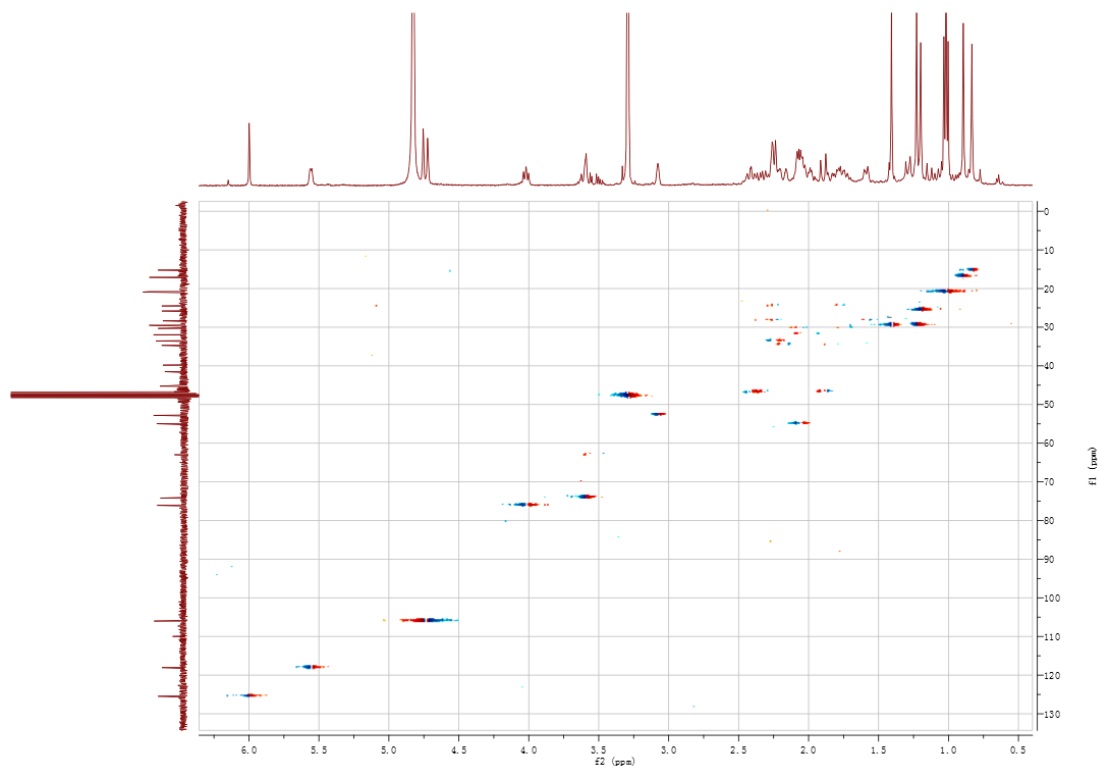
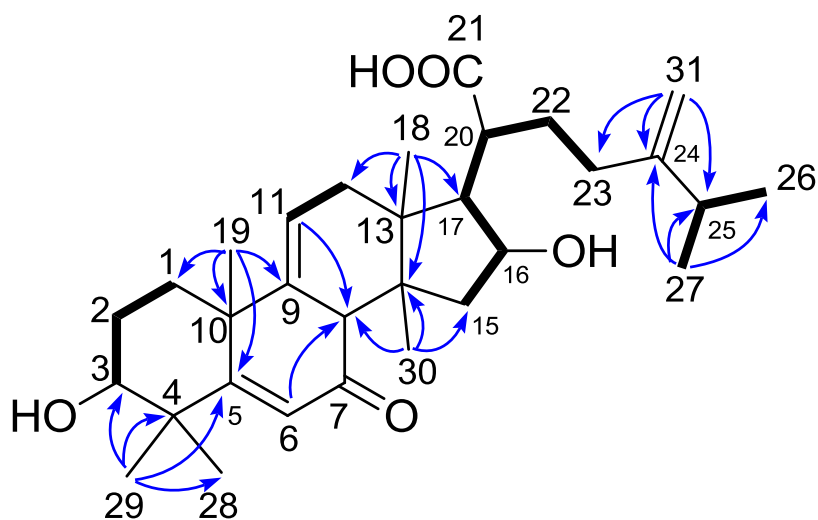
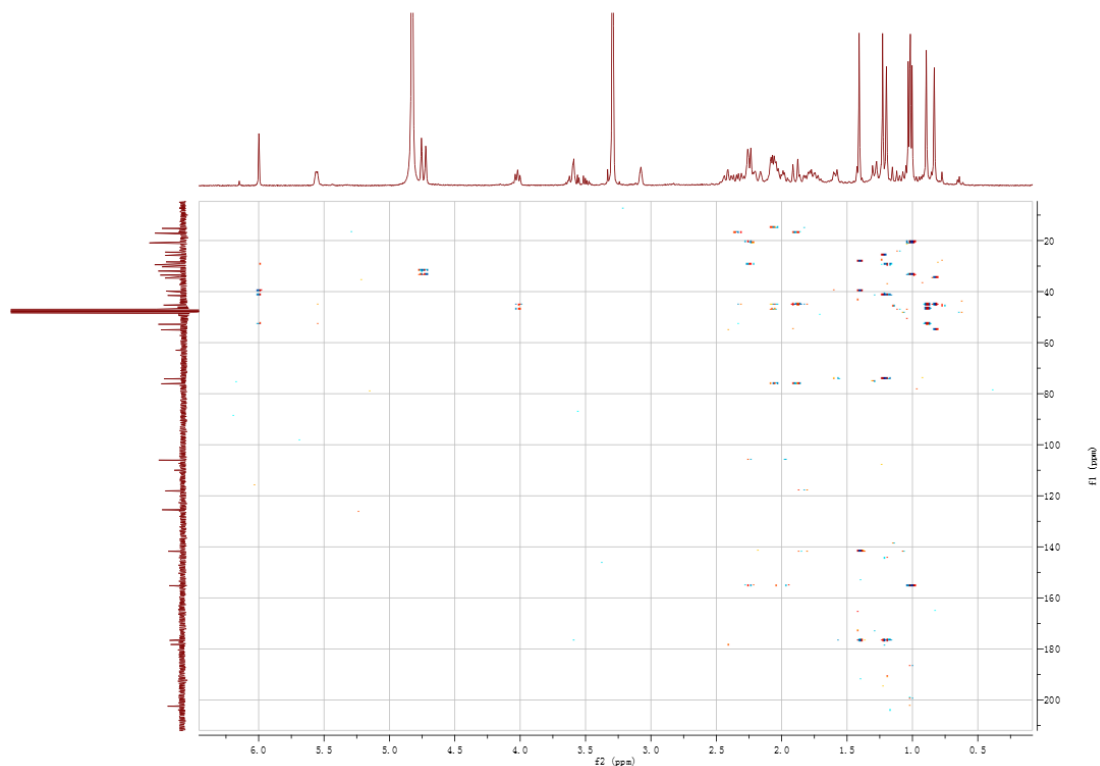
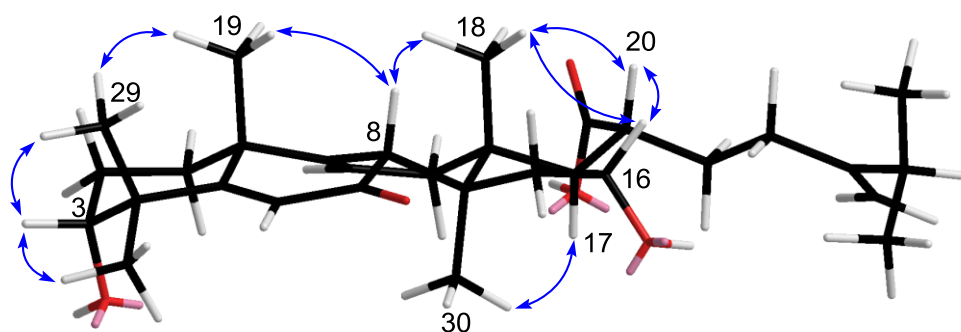
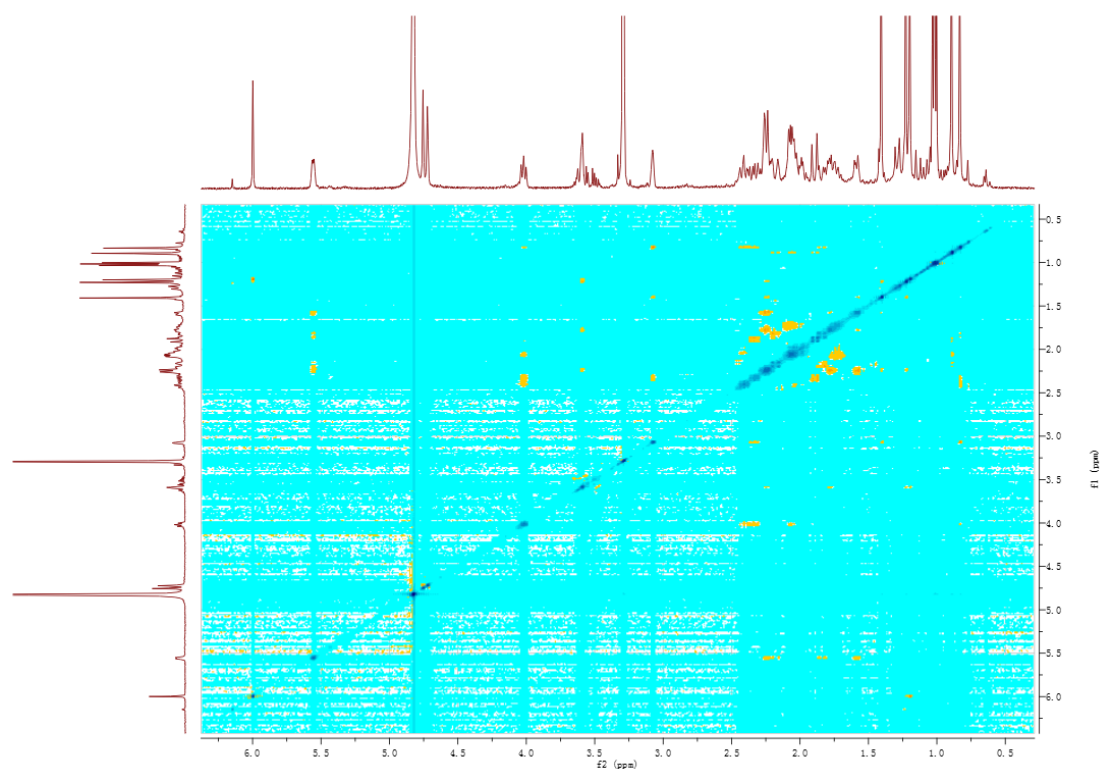


Figure S5. HMBC spectrum of piptolinic acid F (**1**) in CD₃OD



Key ^1H - ^1H COSY (—) and selected HMBC correlations (H→C) of **1**

Figure S6. NOESY spectrum of piptolinic acid F (**1**) in CD₃OD



Key NOESY correlations (H \leftrightarrow H) of **1**

Figure S7. HRESIMS spectrum of piptolinic acid F (1)

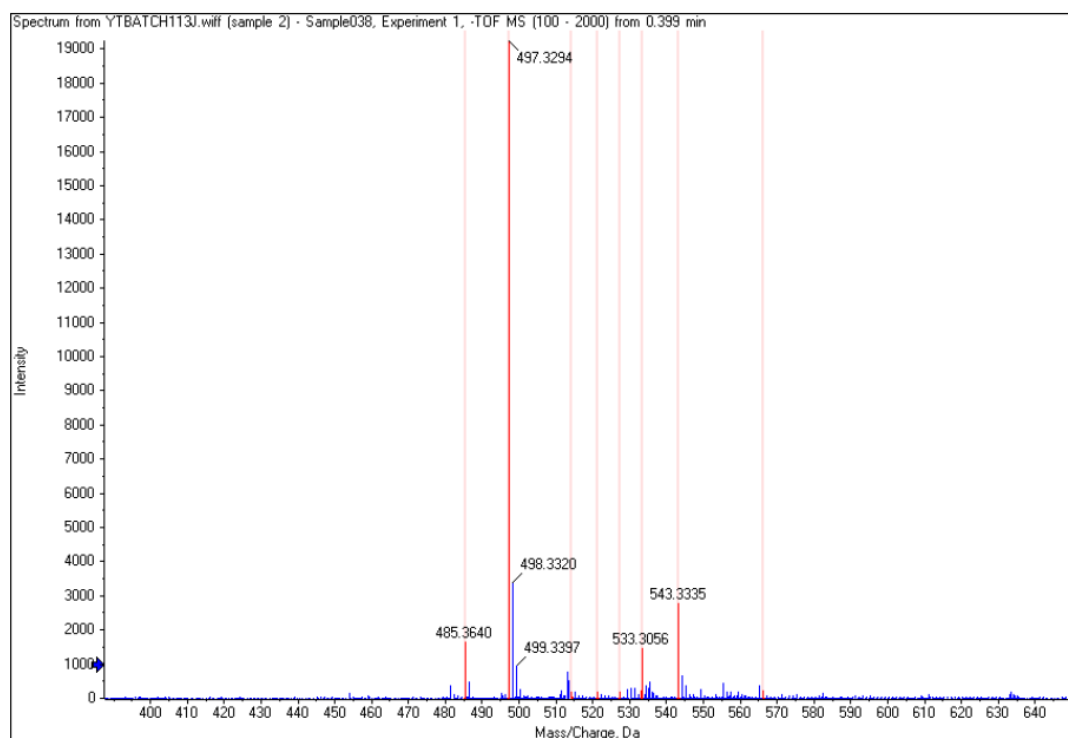


Figure S8. IR (KBr disc) spectrum of piptolinic acid F (1)

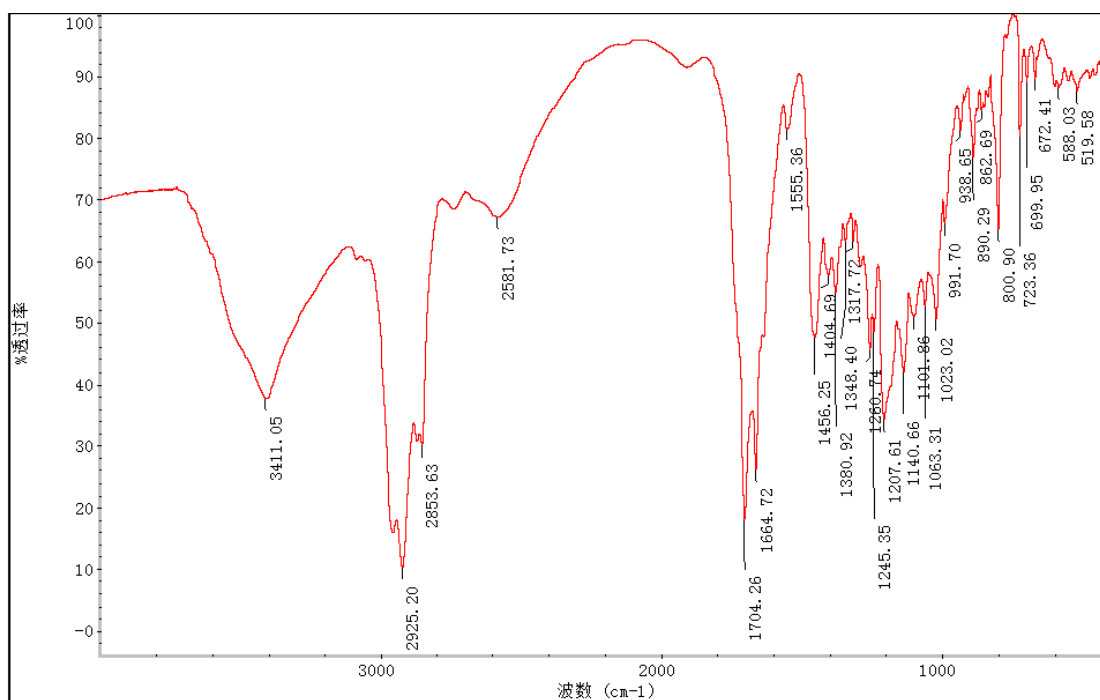


Figure S9. ^1H NMR spectrum of piptolinic acid G (**2**) in CD_3OD

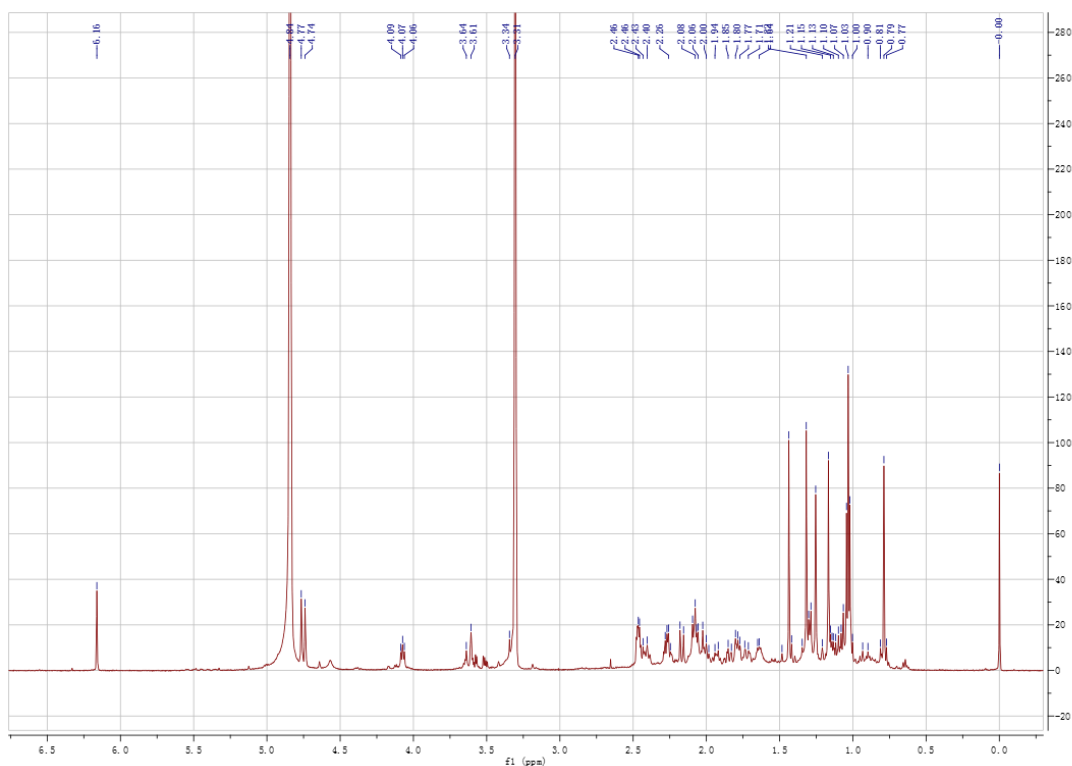


Figure S10. ^{13}C NMR spectrum of piptolinic acid **2** in CD_3OD

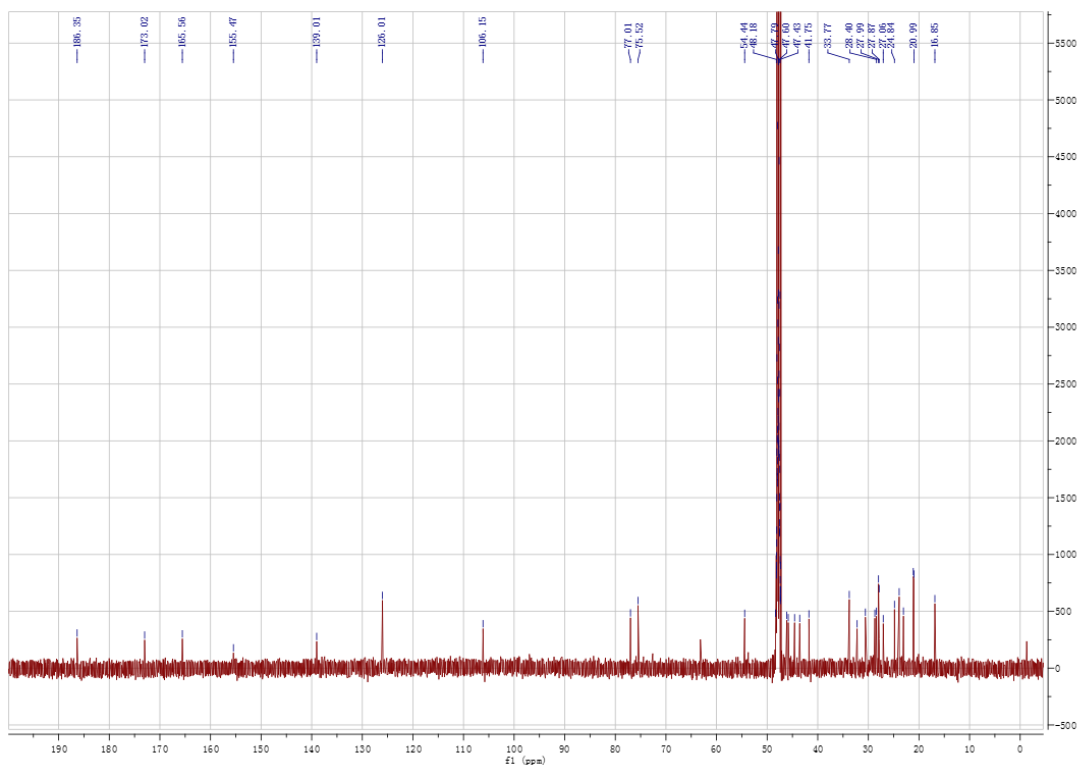


Figure S11. HRESIMS spectrum of piptolinic acid G (**2**)

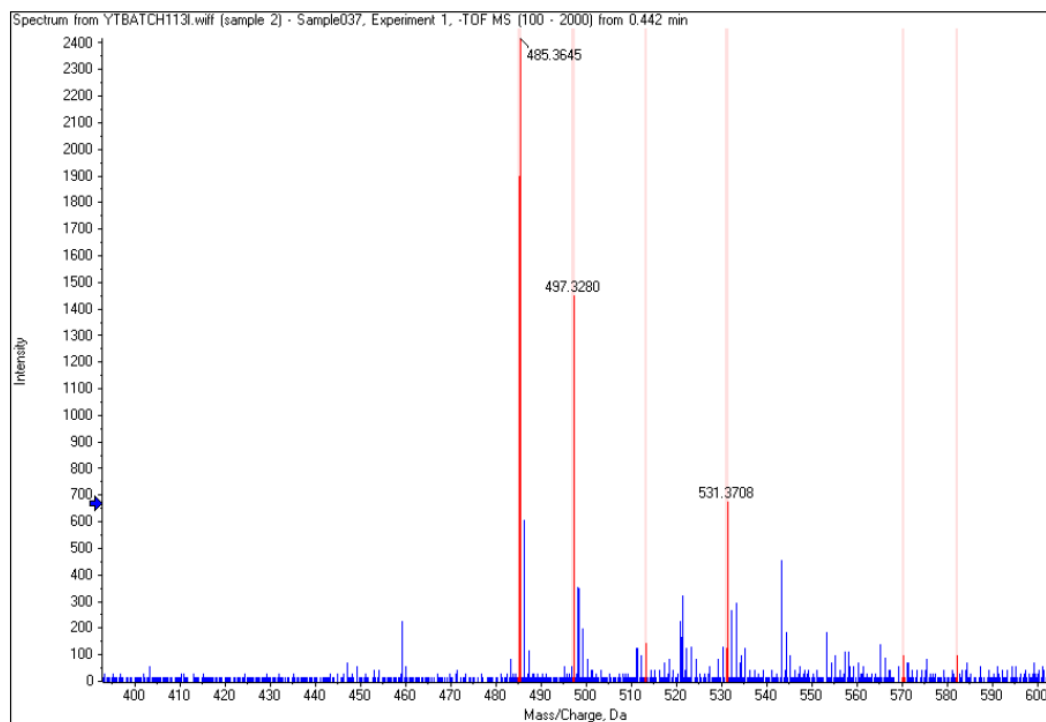


Figure S12. ^1H NMR spectrum of piptolinic acid H (**3**) in $\text{C}_5\text{D}_5\text{N}$

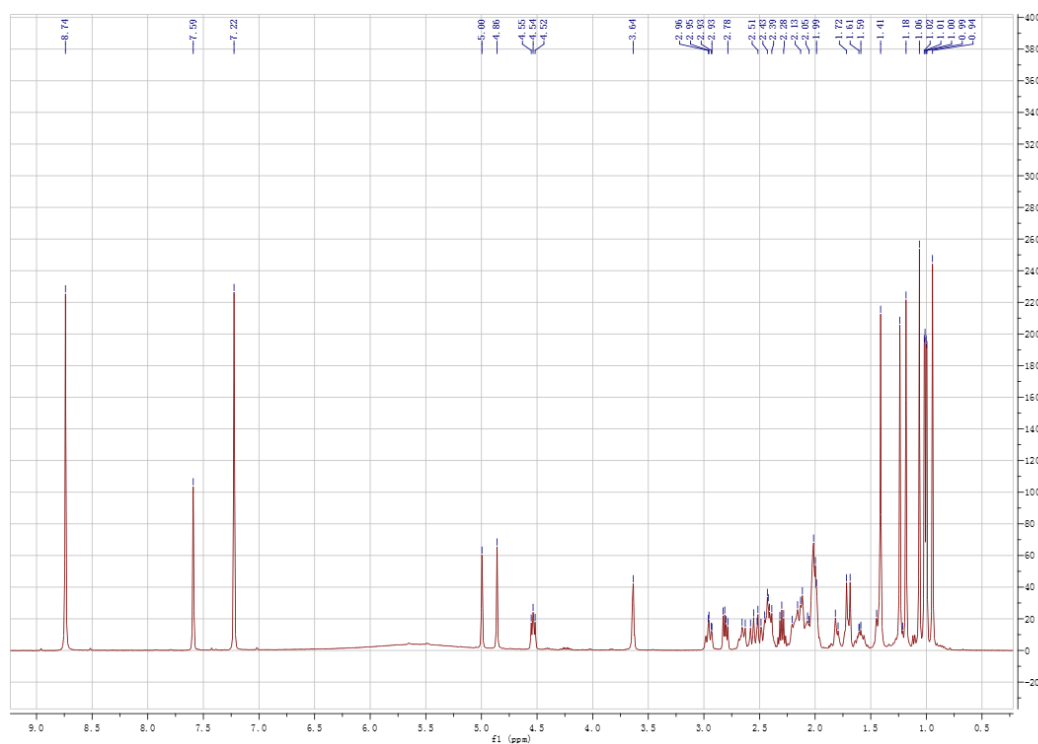


Figure S13. ^{13}C NMR spectrum of piptolinic acid H (**3**) in $\text{C}_5\text{D}_5\text{N}$

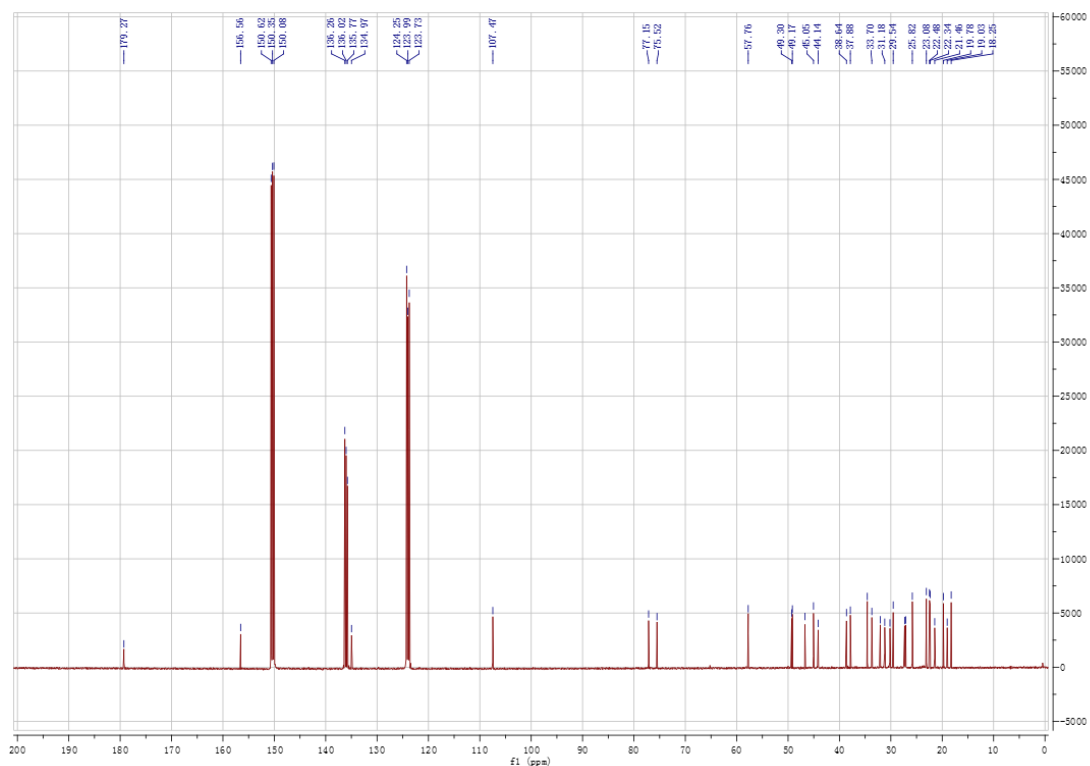


Figure S14. HRESIMS spectrum of piptolinic acid H (**3**)

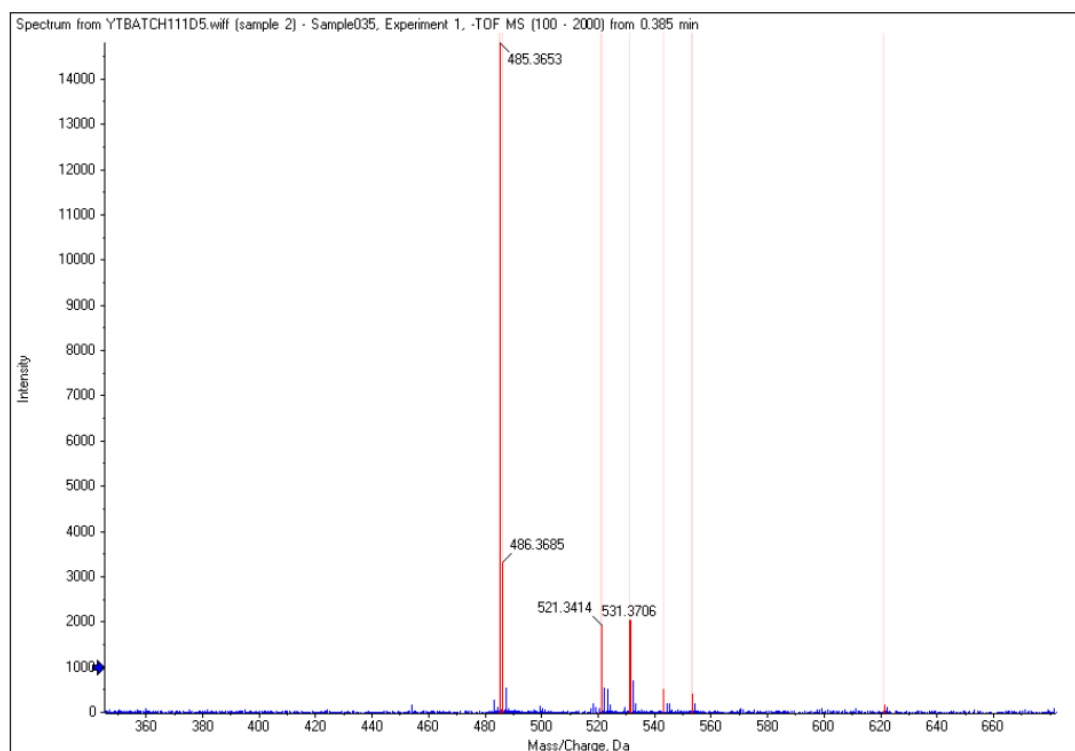


Figure S15. ^1H NMR spectrum of piptolinic acid I (**4**) in $\text{C}_5\text{D}_5\text{N}$

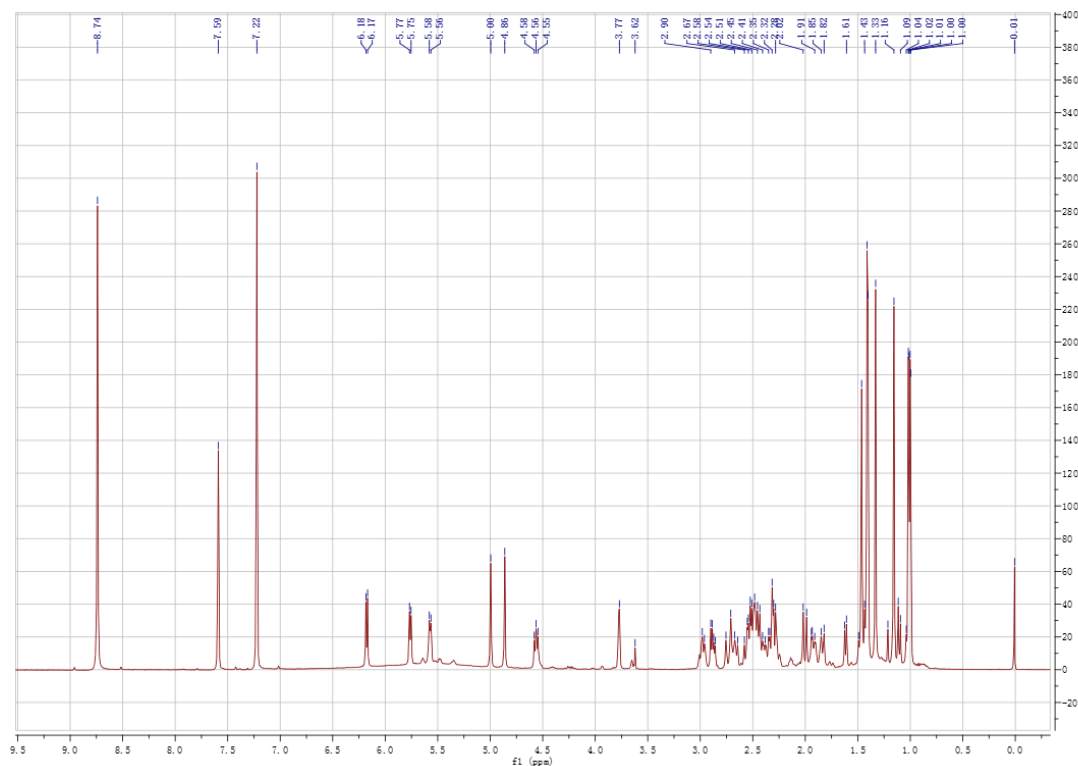


Figure S16. ^{13}C NMR spectrum of piptolinic acid I (**4**) in $\text{C}_5\text{D}_5\text{N}$

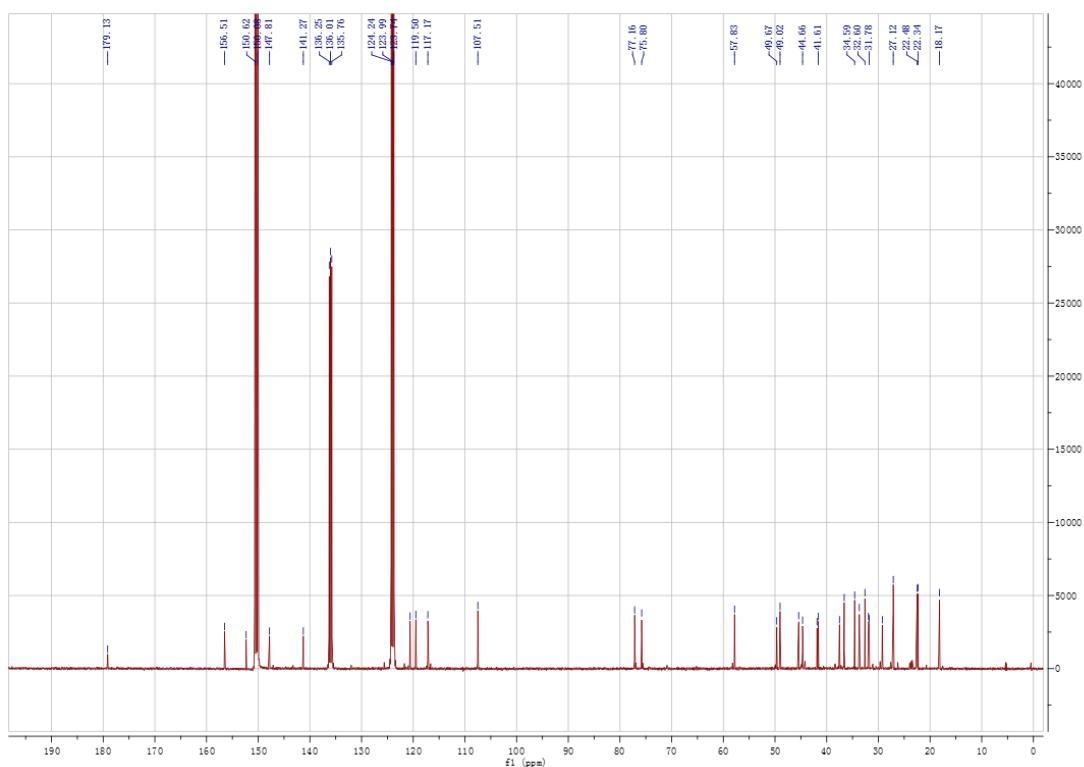


Figure S17. HRESIMS spectrum of piptolinic acid I (**4**)

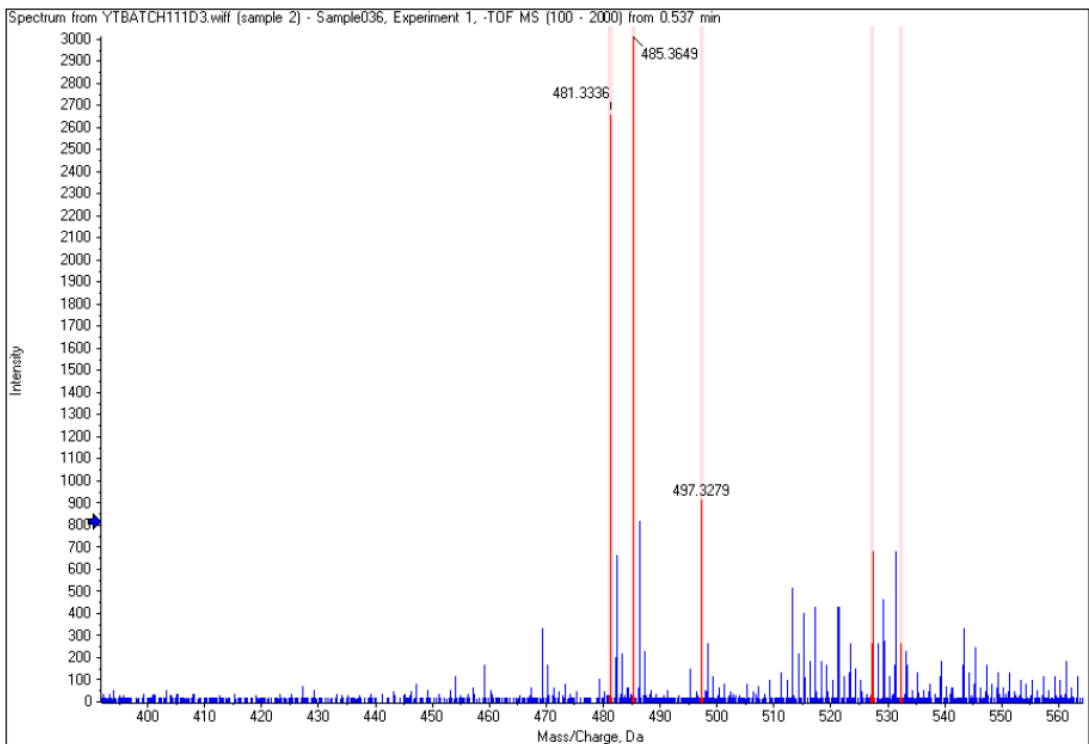


Figure S18. ^1H NMR spectrum of piptolinic acid J (**5**) in CD_3OD

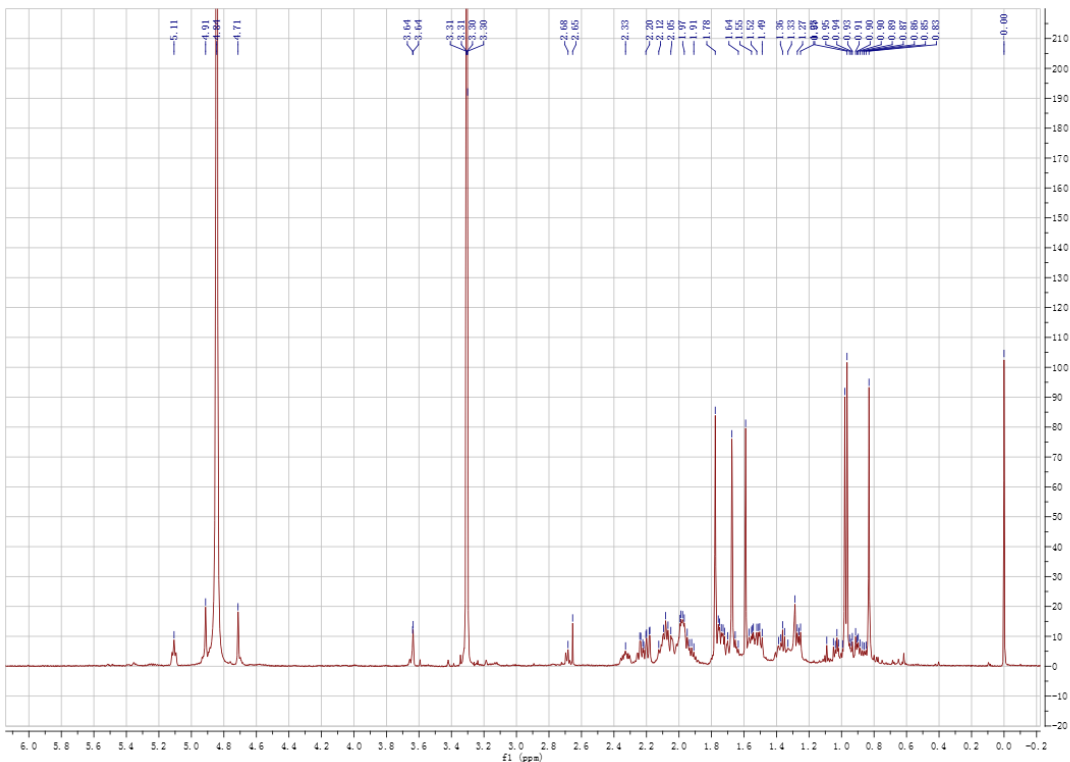


Figure S19. ^{13}C NMR spectrum of piptolinic acid **5** in CD_3OD

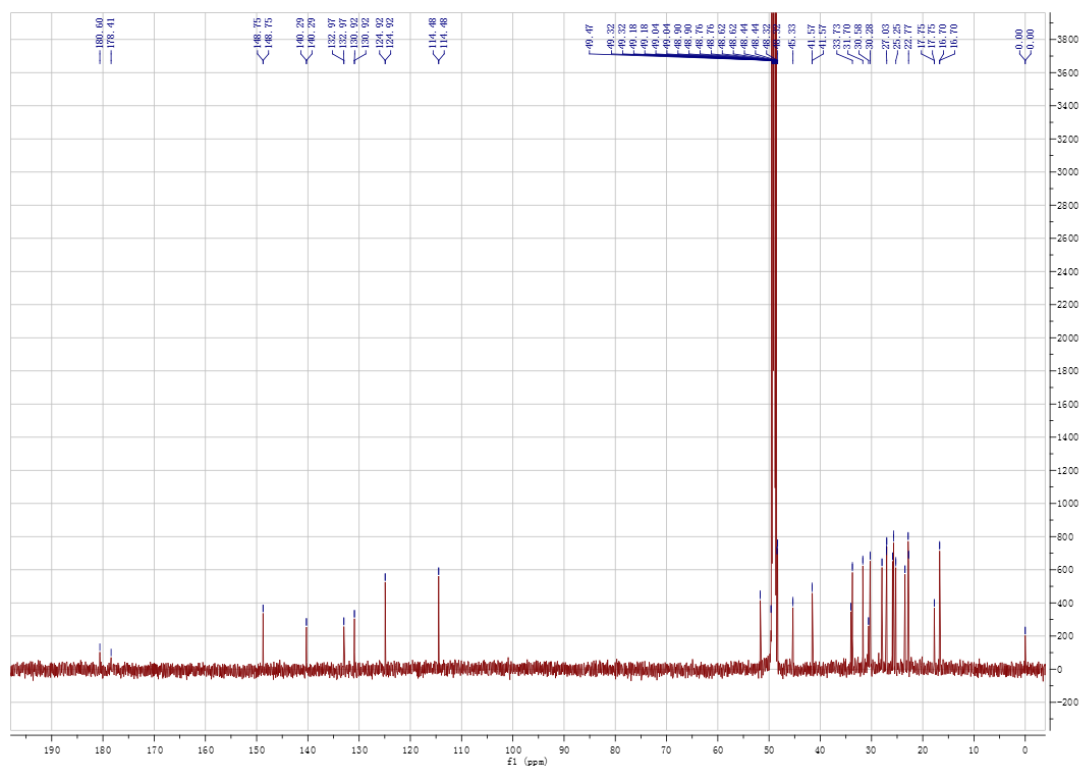


Figure S20. ^1H - ^1H COSY spectrum of piptolinic acid **5** in CD_3OD

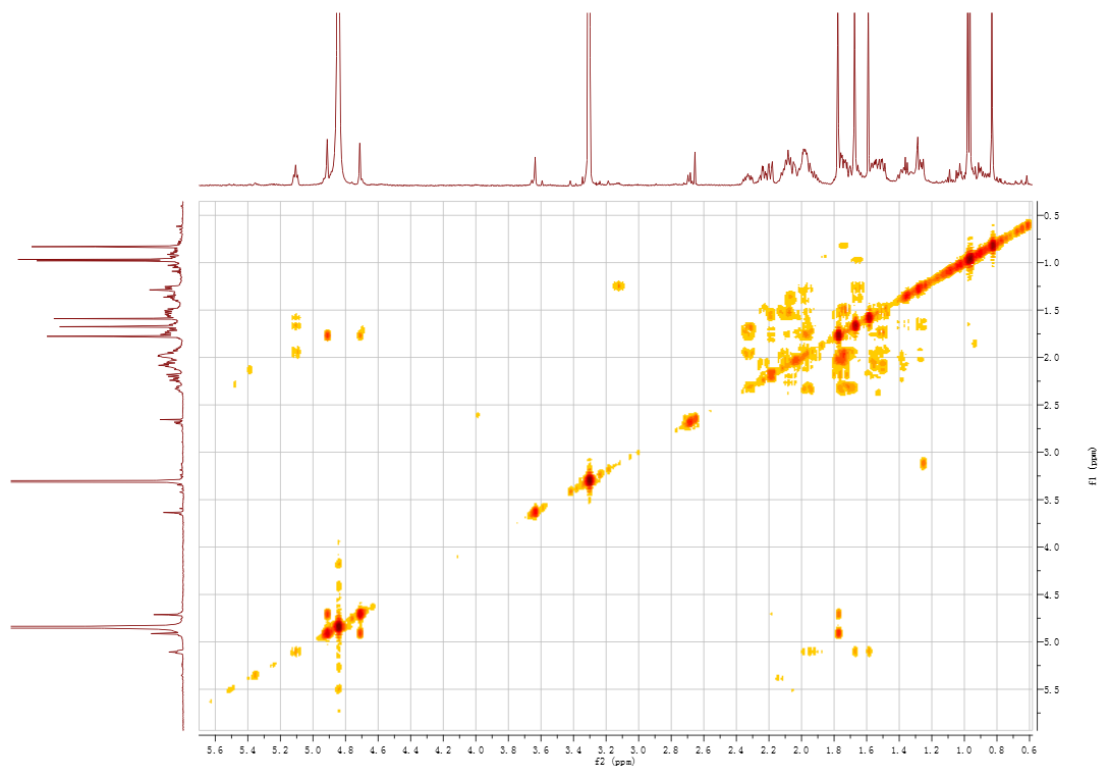


Figure S21. HSQC spectrum of piptolinic acid J (**5**) in CD₃OD

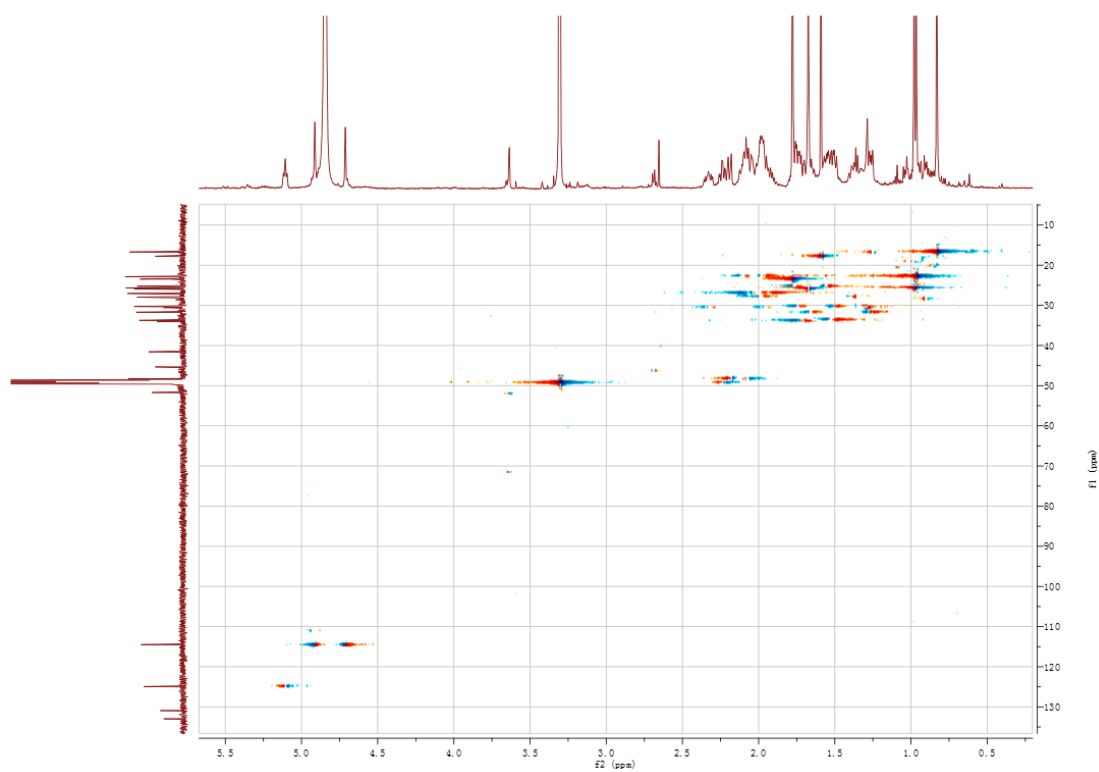
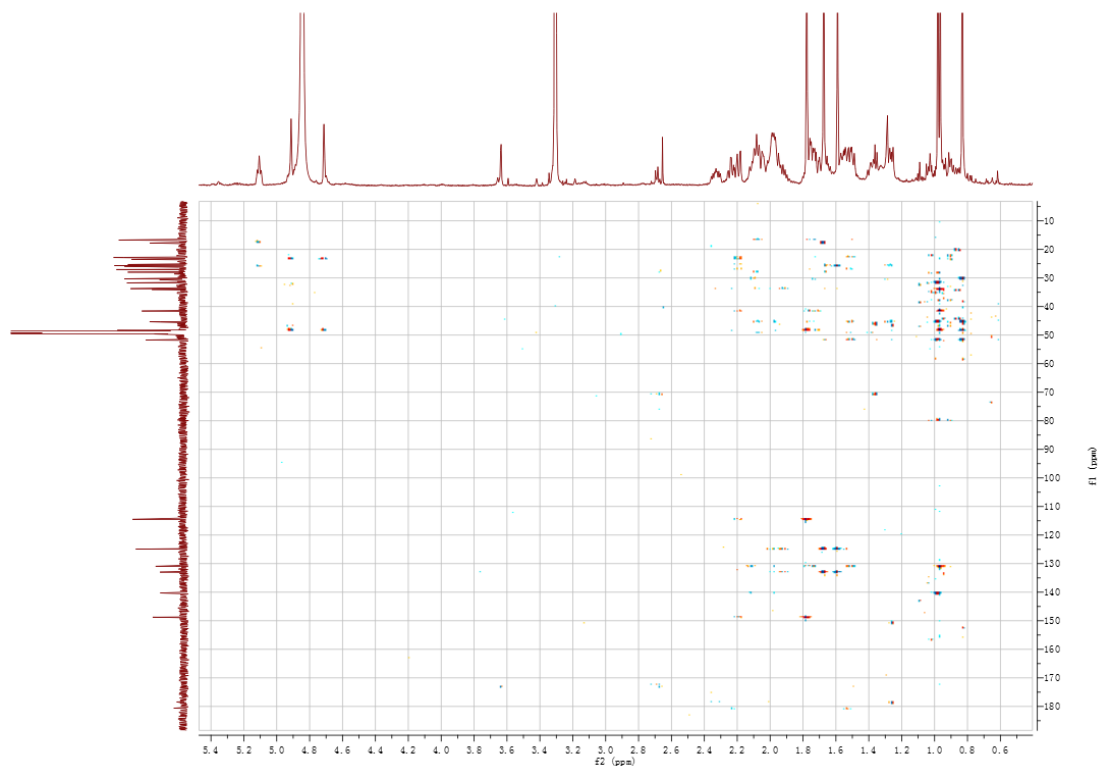
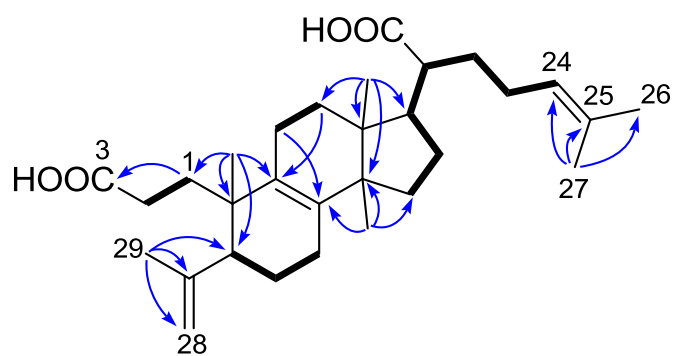


Figure S22. HMBC spectrum of piptolinic acid J (**5**) in CD₃OD





Key ^1H - ^1H COSY (—) and selected HMBC correlations (H \rightarrow C) of **5**.

Figure S23. HRESIMS spectrum of piptolinic acid J (**5**)

