Biocatalytic Promiscuity of HRP in Resveratrol Oxidation: A Function Guided Design with Metal Binding

Chang Li, Xiaofei Xu, Jing Lu, Lin Wang and Yuanjiang Pan*

Department of Chemistry, Zhejiang University, Hangzhou 310027, China

panyuanjiang@zju.edu.cn

Supporting information

Table of contents

I. HPLC yields of the products in HRP catalyzed resveratrol oxidation.

Enzyme used	conversion of **1** (%)	Yield of **2** (%)	Yield of **3&4** (%)	Yield of **5** (%)	Yield of **6** (%)	Yield of **7** (%)
native HRP	95.9	52.5	35.7	7.7	-	-
HRP-Zn^{2+}	96.7	67.6	23.1	6.1	-	-
HRP-Ni^{2+}	96.3	61.7	27.4	7.2	-	-
HRP-Co^{2+}	94.1	51.2	34.2	8.8	-	-
HRP-Ca^{2+}	57.1	34.0	19.8	3.3	-	-
HRP-Cu^{2+}	>99	-	-	-	70.5[a]	48.2[a]
HRP-Fe^{2+}	>99	-	-	-	74.2[a]	51.6[a]
HRP-Mn^{2+}	>99	94.0 (91.2[a])	-	-	-	-

Table S1. HPLC yields of the products in HRP catalyzed resveratrol oxidation

[a] isolated yields.

II. UV spectra of synthesized resveratrol dimers 2, 3&4 and 5.

1, **2**, **3&4**, and **5** were prepared as 0.001 M acetone solution, and their spectra were recorded using a Shimadzu. Spectra were recorded in the range of 200 - 400 nm. The spectra were shown in Figure S1.

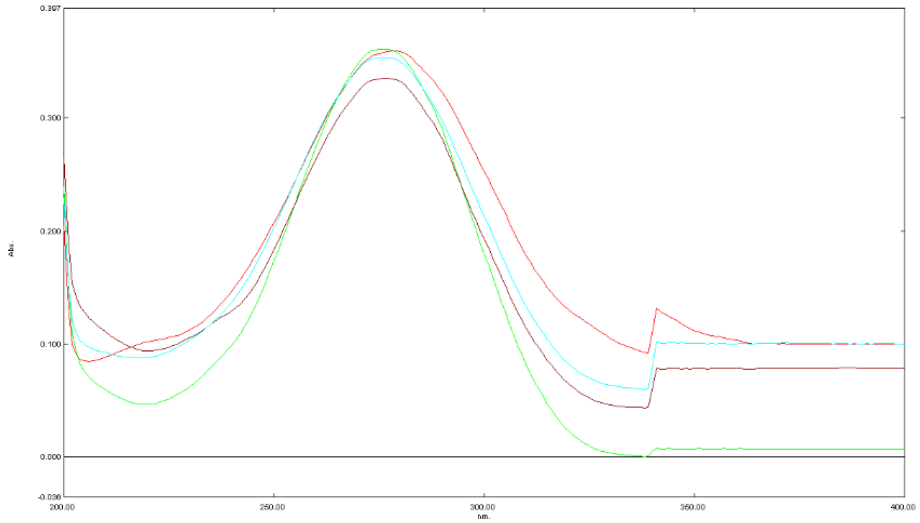


Figure S1. UV spectra of **1**, **2**, **3&4** and **5** (0.001 M acetone solution)

III. Characterization of HRP-metal complex's conformation with circular dichroism spectrometer.

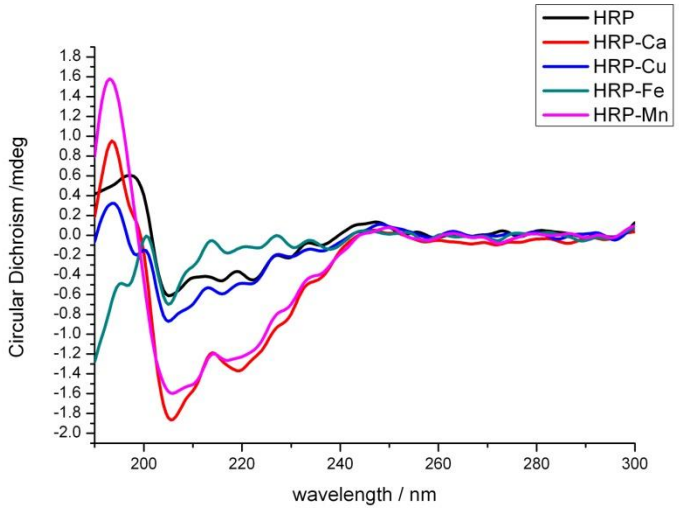


Figure S2. CD spectra of native HRP, [HRP-Ca], [HRP-Mn], [HRP-Fe] and [HRP-Cu]

enzyme/complex	α-helix	β–sheet	β–turn	random
native HRP	0.0	82.8	0.0	17.2
[HRP-Ca]	0.0	82.0	0.0	18.0
[HRP-Cu]	0.0	82.5	0.0	17.5
[HRP-Fe]	0.0	82.7	0.0	17.3
[HRP-Mn]	0.0	82.1	0.0	17.9

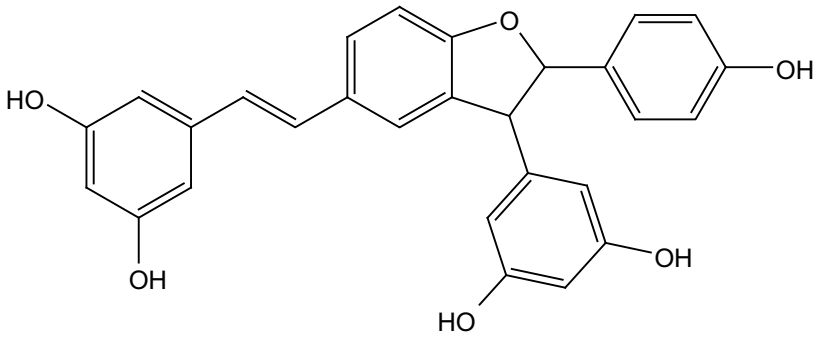
Table S2. Prediction of secondary structure constitution by Yang's method

III. Determination of the HRP-metal complex constitution with Atomic Absorption Spectrometer (AAS)

Sample	Concentration of Fe (ppm)	Concentration of binded metal (ppm)	Number of binded metal
Native HRP	13.5564	-	-
HRP-Cu	13.5782	83.1007	6.12
HRP-Fe	52.4633	52.4633	2.87
HRP-Mn	13.5773	150.5723	11.09

Table S3. AAS suggested concentration of Fe and binded metals in 2 mg/mL native HRP, [HRP-Cu], [HRP-Fe] and [HRP-Mn]

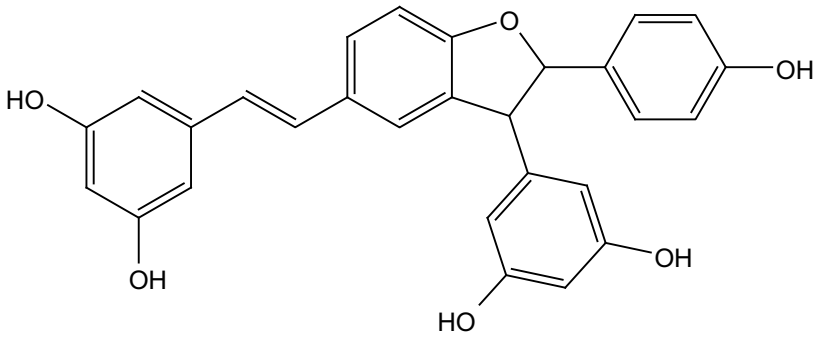
III. NMR Spectra of Synthesized Compounds



2: *trans*- δ-viniferin

proton spectrum in acetone, 500MHz

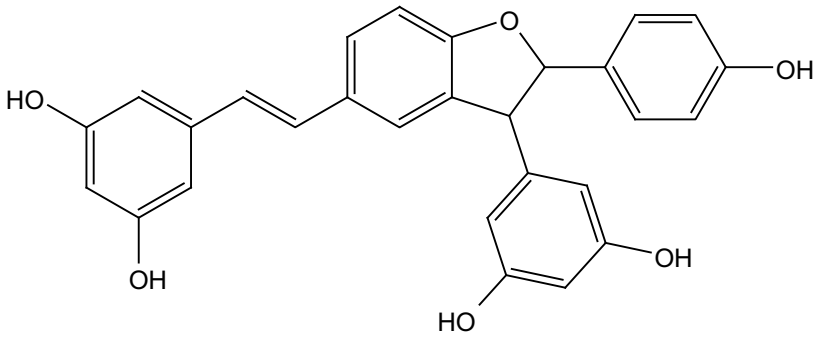
160.58
159.72
159.51
158.38
145.21
140.77
132.12
131.70
127.18
123.91
116.16
110.14
105.72
102.71
102.37
94.02
57.79
55.40



2: *trans*- δ-viniferin

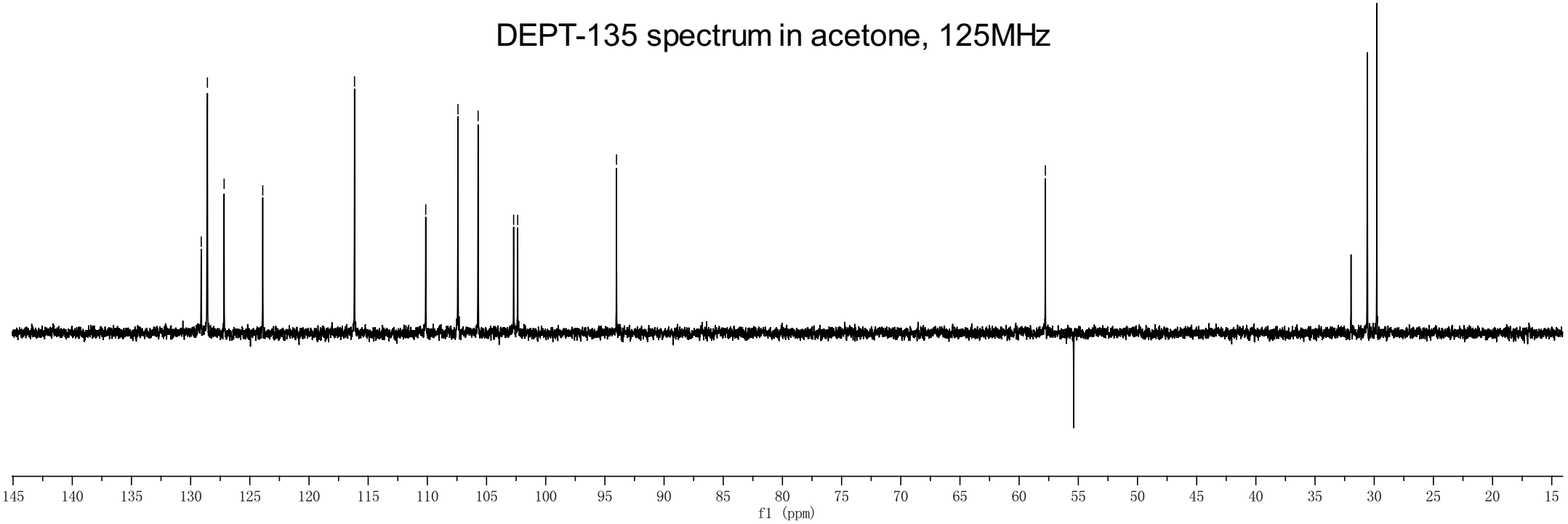
carbon spectrum in acetone, 125MHz

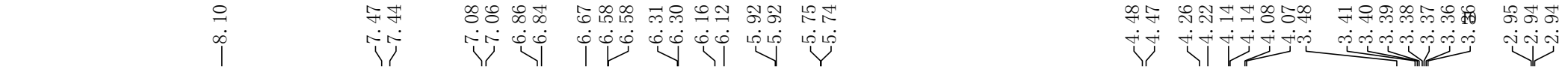
129.11
128.58
127.17
123.91
116.15
110.14
107.42
105.71
102.71
102.37
94.02
57.78

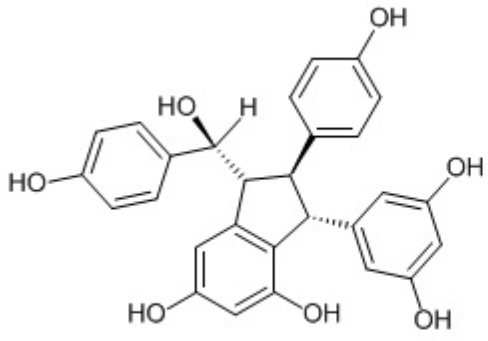


2: *trans*- δ-viniferin

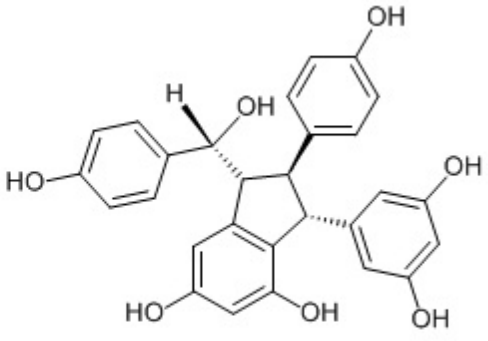
DEPT-135 spectrum in acetone, 125MHz





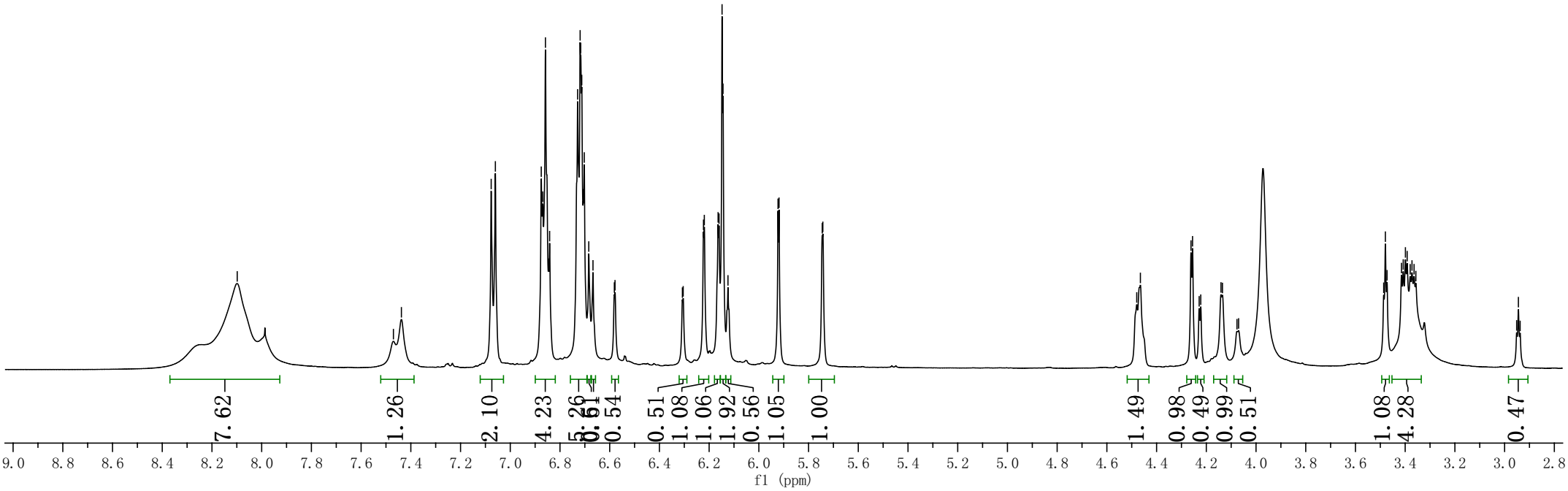


3: leachianols F

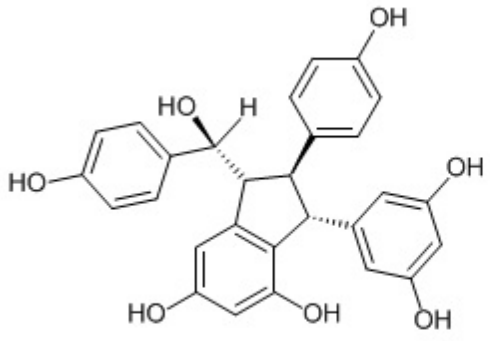


4: leachianols G

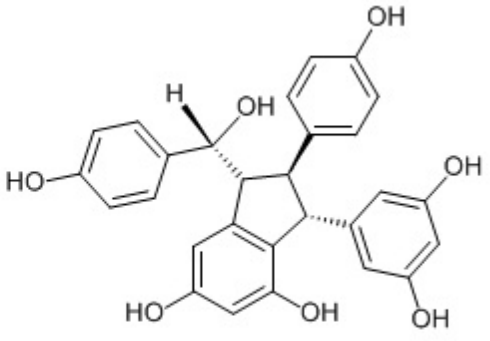
mixture of **3** and **4** (1:2)
proton spectrum in acetone, 500MHz



159.20
159.12
158.49
157.24
156.18
154.85
151.43
147.27
137.97
135.67
129.43
129.29
129.26
128.81
123.09
115.63
115.59
115.40
115.25
106.19
106.01
105.60
102.36
101.05
77.29
69.85
62.54
58.97
55.95
55.35

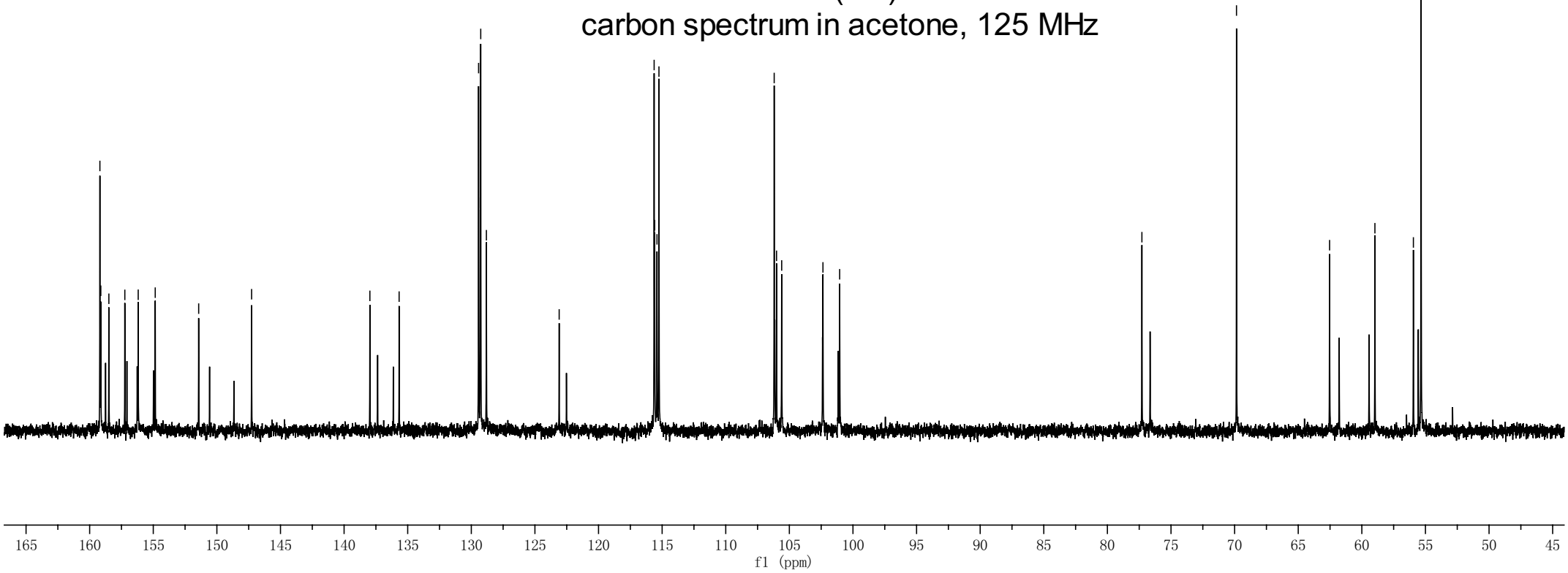


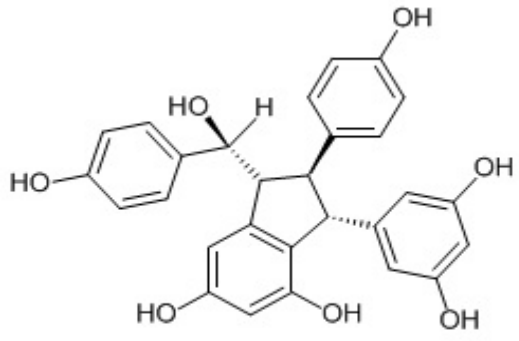
3: leachianols F



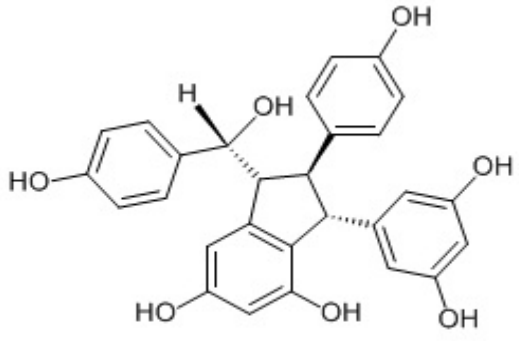
4: leachianols G

mixture of **3** and **4** (1:2)
carbon spectrum in acetone, 125 MHz



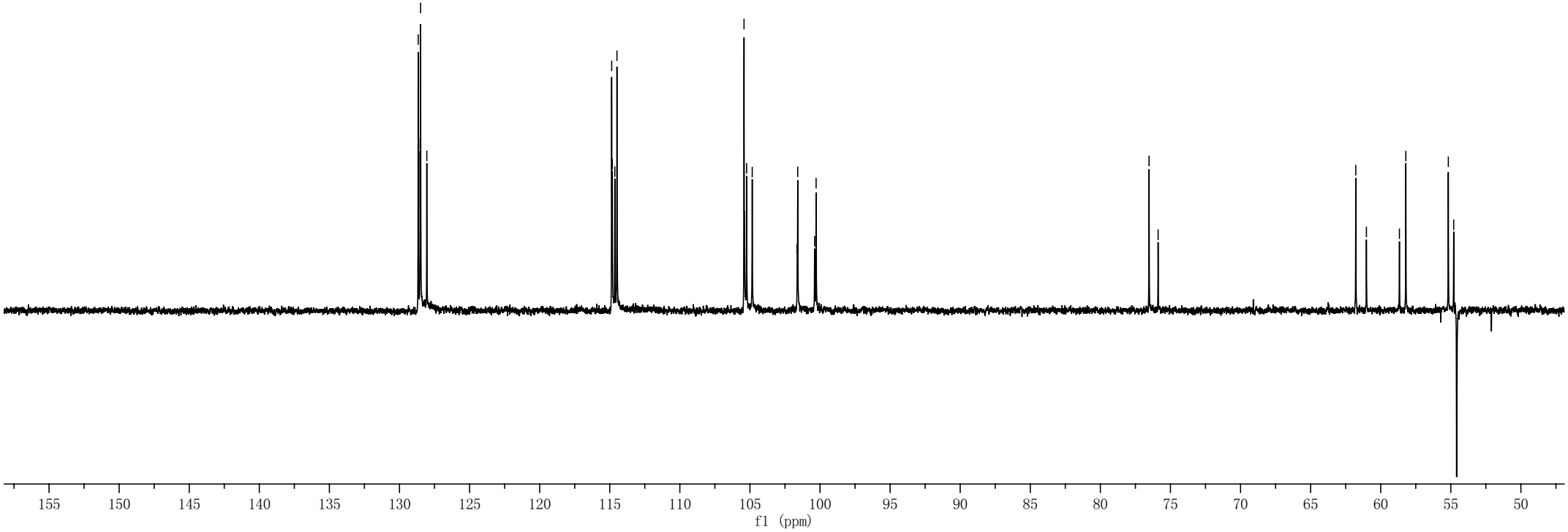


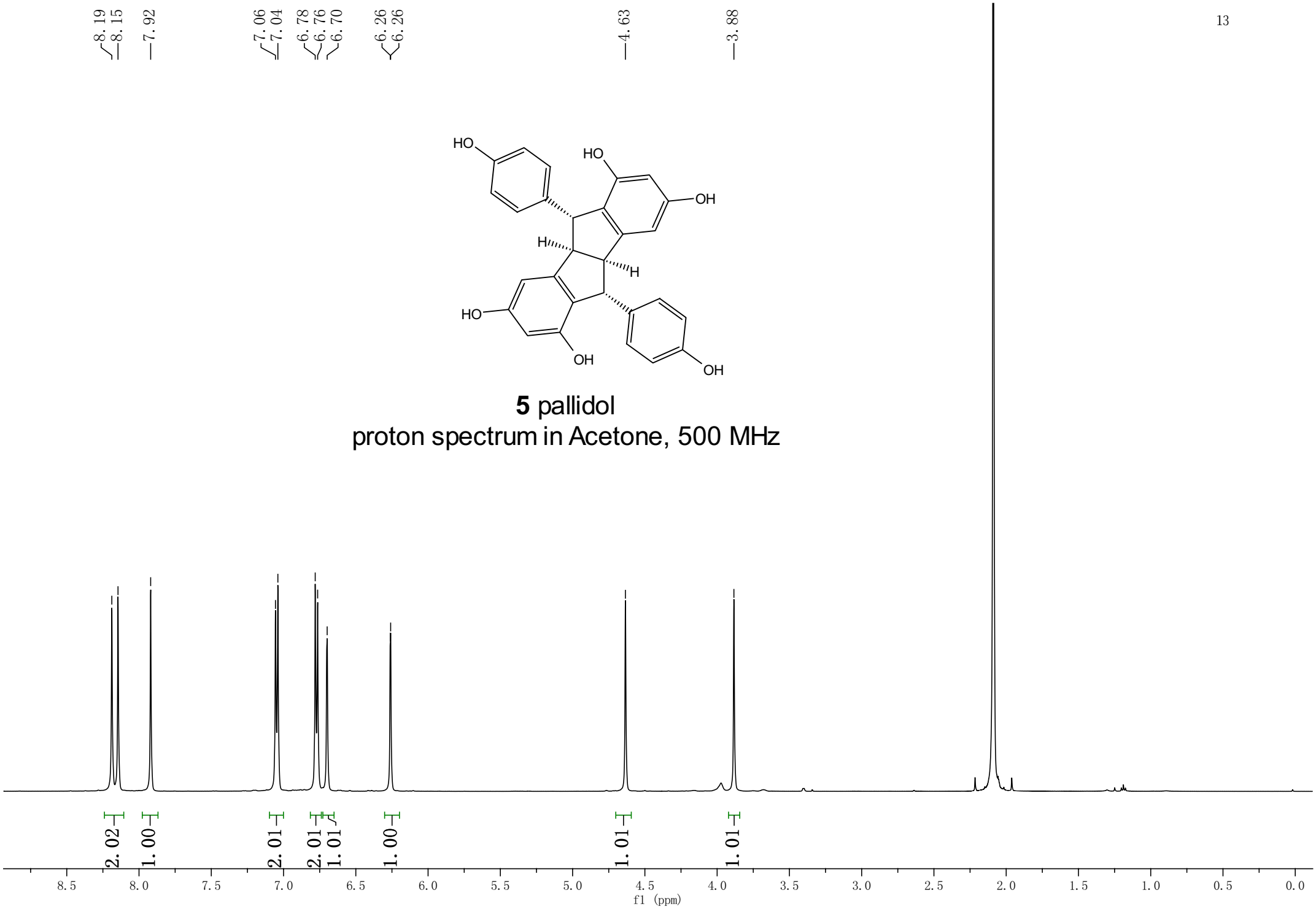
3: leachianols F



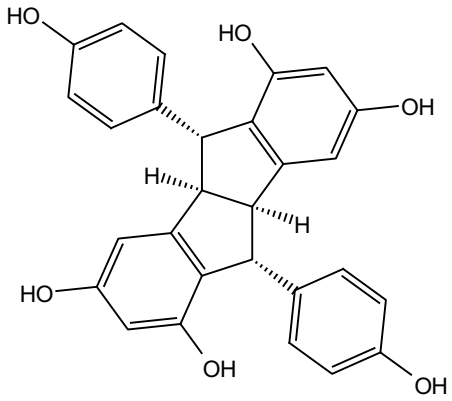
4: leachianols G

mixture of **3** and **4** (1:2)
DEPT-135 spectrum in acetone, 125 MHz

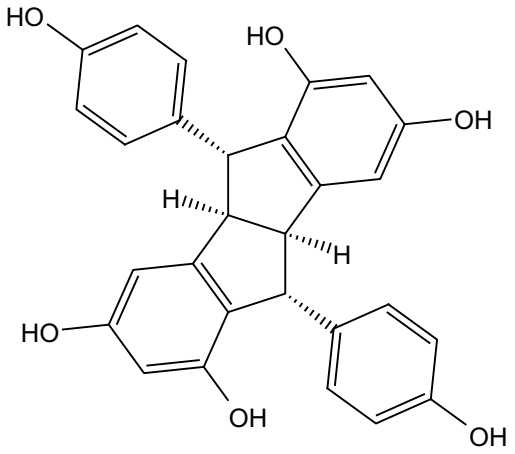




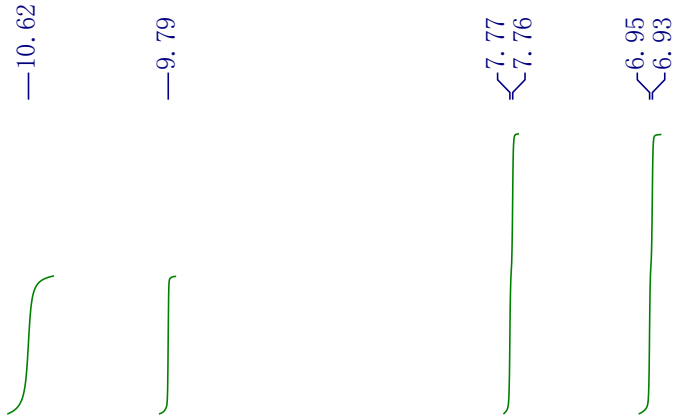
5 pallidol
proton spectrum in Acetone, 500 MHz

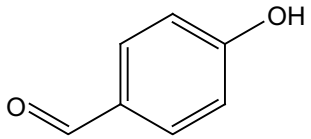


5 pallidol
carbon spectrum in Acetone, 125 MHz

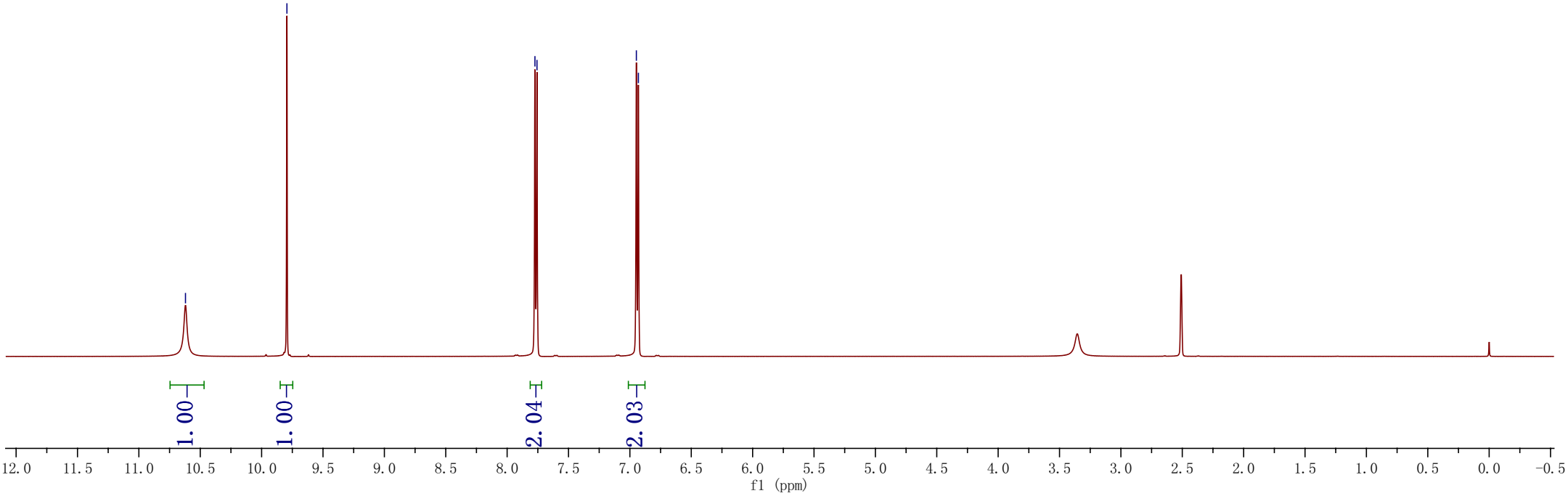


5 pallidol
DEPT-135 spectrum in Acetone,
125 MHz

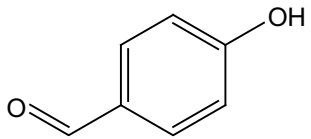




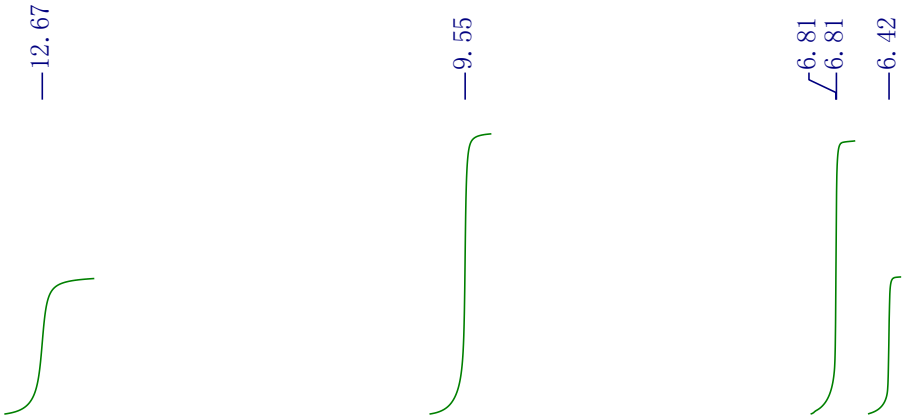
6 4-hydroxylbenzylaldehyde
proton spectrum in DMSO, 500 MHz

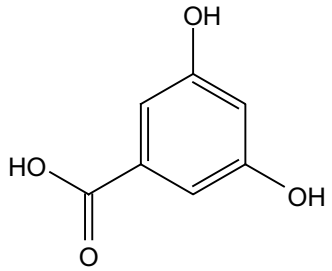


190.91
163.31
132.06
128.39
115.81

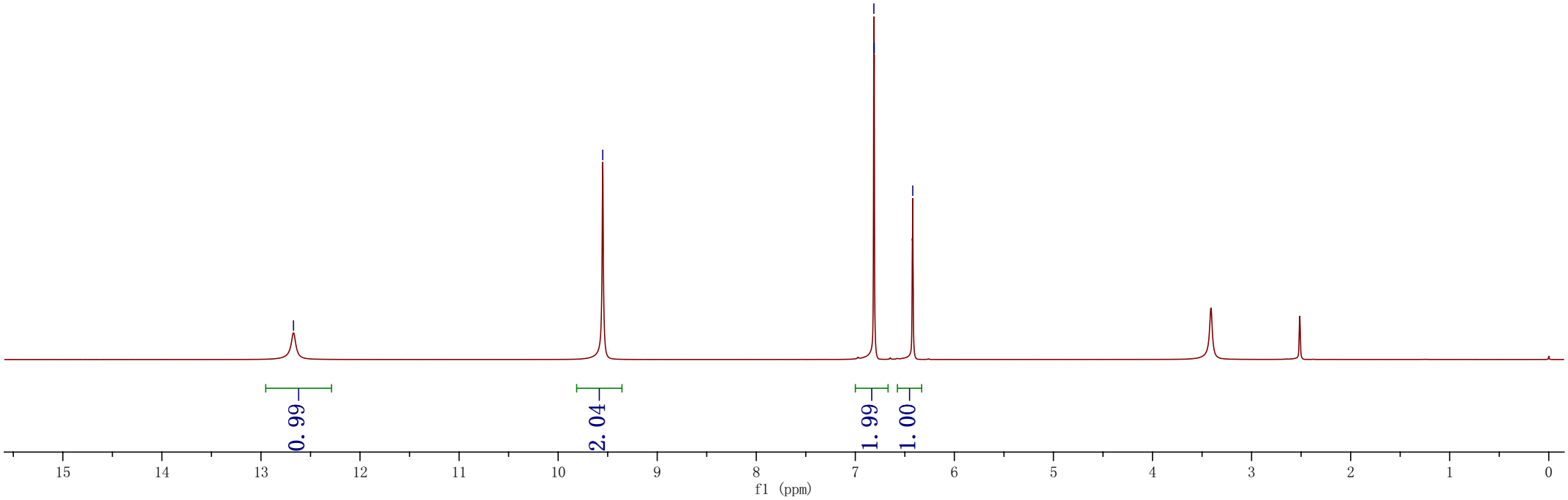


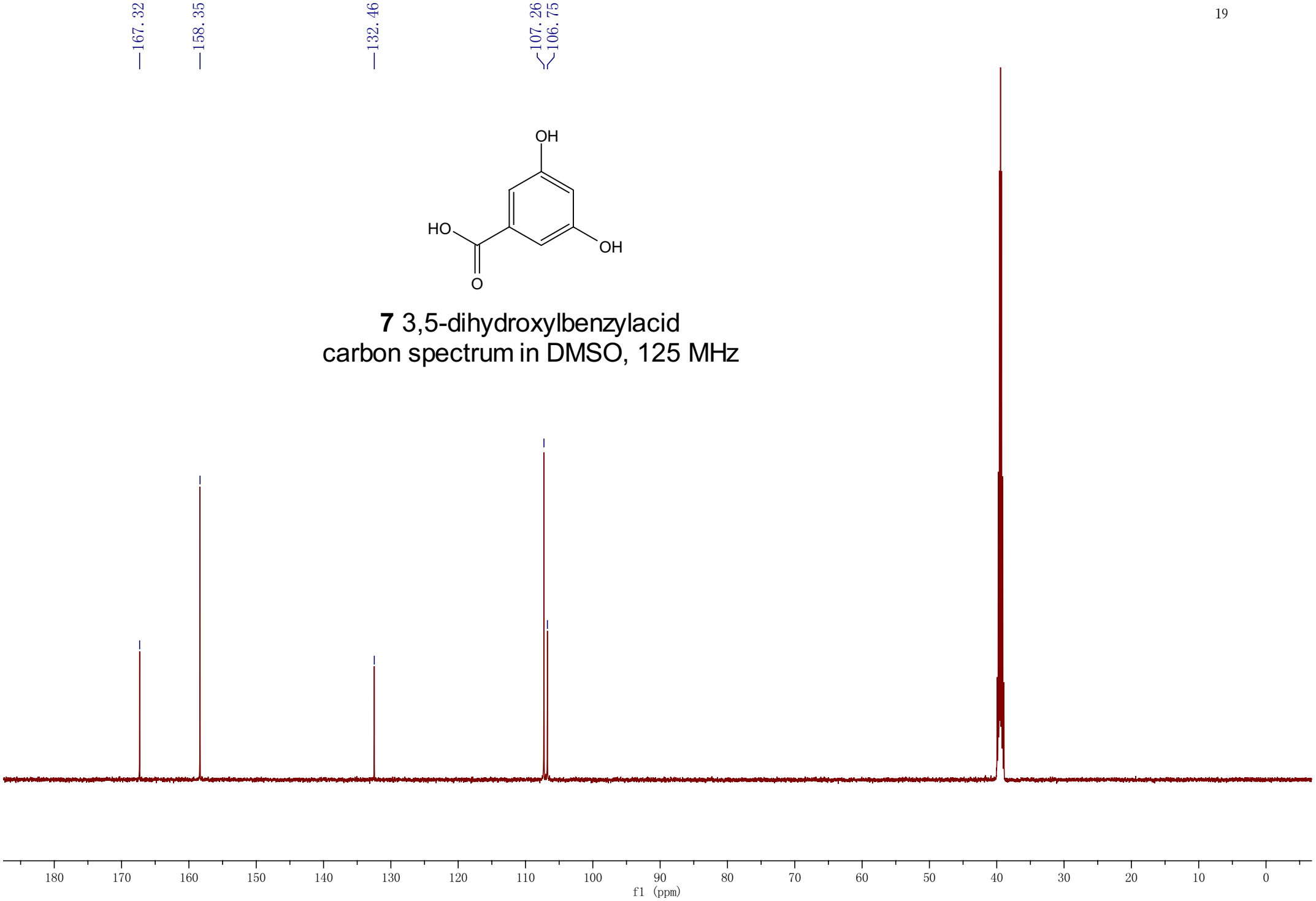
6 4-hydroxylbenzylaldehyde
carbon spectrum in DMSO, 125 MHz





7 3,5-dihydroxylbenzylacid
proton spectrum in DMSO, 500 MHz





7 3,5-dihydroxylbenzylacid
carbon spectrum in DMSO, 125 MHz