Chemistry of *trans*-Resveratrol with Singlet Oxygen: [2+2] Addition, [4+2] Addition, and Formation of the Phytoalexin Moracin M

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Supporting Information

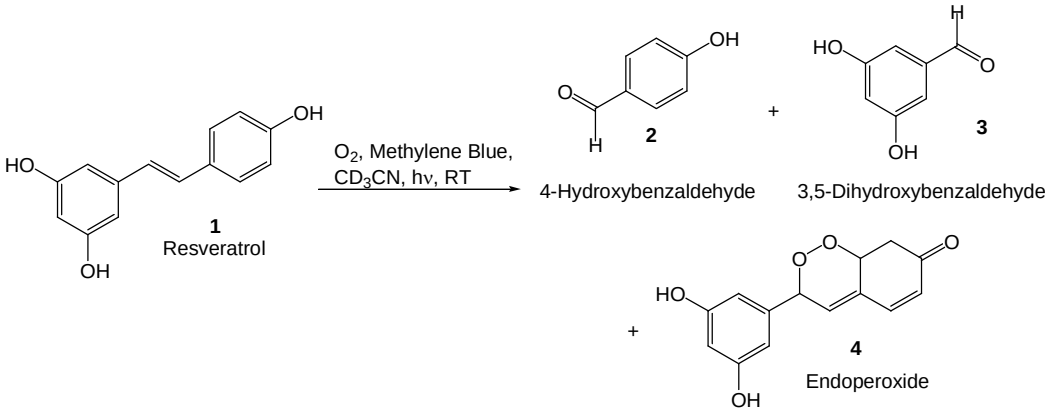
Contents

1. General information:

All reagents were obtained commercially and used without further purification unless stated otherwise. NMR spectra were acquired on a Bruker Avance II 400 MHz at 25ºC, if not stated otherwise. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra were recorded at 100 MHz. Chemical shifts in NMR spectra are expressed in ppm, and all coupling constants are expressed in Hertz (Hz). Low resolution MALDI mass spectra were obtained on an Applied Biosystems Voyager-DE STR.

2. Chemistry of *trans*-resveratrol with singlet oxygen and a one-pot, two-step synthesis of moracin M:

trans-Resveratrol (5.2 mg, 21.9 mmol) was dissolved in CD_3CN to make a 1 mL solution and placed into an NMR tube. A small amount of the sensitizer methylene blue was added and molecular oxygen was bubbled into the solution. The solution was irradiated using a 200W tungsten-halogen lamp (fitted with a glass filter that cuts off light below 493 nm) under a continuous slow stream of oxygen for 10 hours. The ^{1}H and ^{13}C NMR spectra of the crude reaction mixture were obtained. Analyses of the spectra reveal the product mixture shown in Scheme 1.



Scheme 1. Composition of the crude product mixture of the reaction of singlet oxygen and resveratrol.

The observed product mixture can be rationalized by two competing reactivity pathways: (1) a [2+2] cycloaddition to form an unstable dioxetane which cleaves to form aldehydes **2** and **3** and (2) a [4+2] cycloaddition to form the endoperoxide **4** (see text, Scheme 1). An unidentified minor secondary photoproduct forms from the endoperoxide.

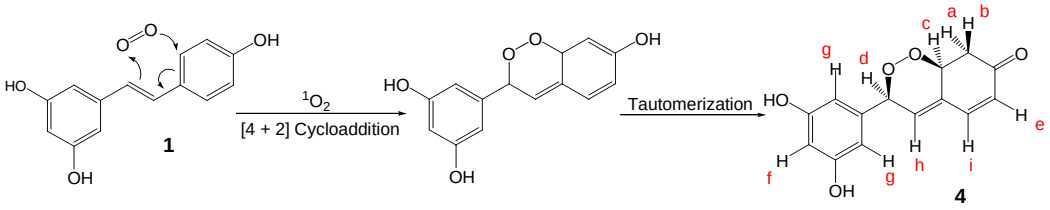
Heating the crude endoperoxide (**4**) to 80°C for 8 h leads to loss of H_2O and rearrangement to Moracin M (**5**) (see text, Scheme 2). Pure Moracin M (2.1 mg, 38% yield) was isolated from the crude product mixture by flash column chromatography (60Å 230-400 mesh silica gel, 1:1 Hexanes/EtOAc).

Moracin M (5): ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.59 (s, 1H), 9.44 (s, 2H), 7.38 (d, *J* = 8.4 Hz, 1H), 7.08 (d, *J* = 0.9 Hz, 1H), 6.92 (d, *J* = 2.0 Hz, 1H), 6.73 (dd, *J* = 8.4 and 2.1 Hz, 1H), 6.67 (d, *J* = 2.2 Hz, 1H), 6.20 (t, *J* = 2.2 Hz, 1 H); **^{13}C NMR** (100 MHz, DMSO-*d*6) δ: 158.73, 155.66, 155.21, 153.91, 131.60, 121.04, 120.73, 112.40, 102.60, 102.26, 101.48, 97.40. **^{1}H NMR** (400 MHz, CD_3OD) δ: 7.35 (d, *J* = 8.4 Hz, 1H), 6.92 (d, *J* = 0.9 Hz, 1H), 6.90 (d, *J* = 2.1 Hz, 1H), 6.76 (d, *J* = 2.2 Hz, 1H), 6.74 (dd, 8.4 and 2.1 Hz, 1H), 6.24 (t, *J* = 2.2 Hz, 1H); **^{13}C NMR** (100 MHz, CD_3OD) δ: 160.08, 157.38,

156.97, 156.27, 133.95, 123.20, 122.16, 113.40, 104.08, 103.67, 102.36, 98.62. **LRMS (MALDI)** Calculated for $C_{14}H_{10}O_4$ $[M+H]^+$: 243.06. Found: 243.09.

3. Characterization of the endoperoxide [4+2] cycloaddition adduct:

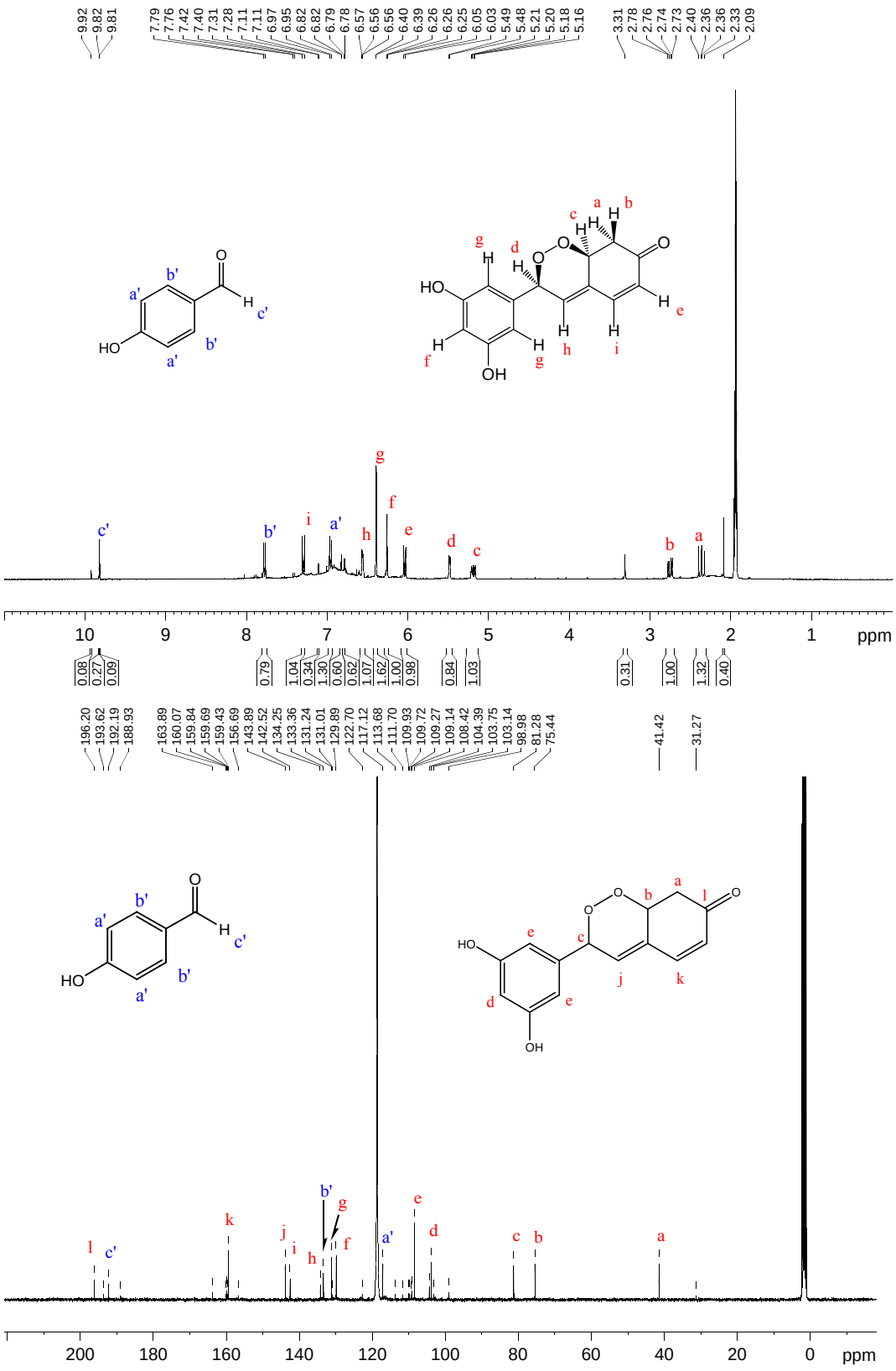
After irradiation of resveratrol, a relatively stable endoperoxide can be observed. Because all our attempts to purify this endoperoxide by column chromatography led to decomposition, we identified its structure via NMR of the crude reaction mixture. The ^1H, ^{13}C, and COSY NMR spectra (see below) are consistent with the structure shown below (one enantiomer of the racemic endoperoxide [4+2] cycloaddition adducts is shown for easier analysis of the ^1H NMR spectrum).

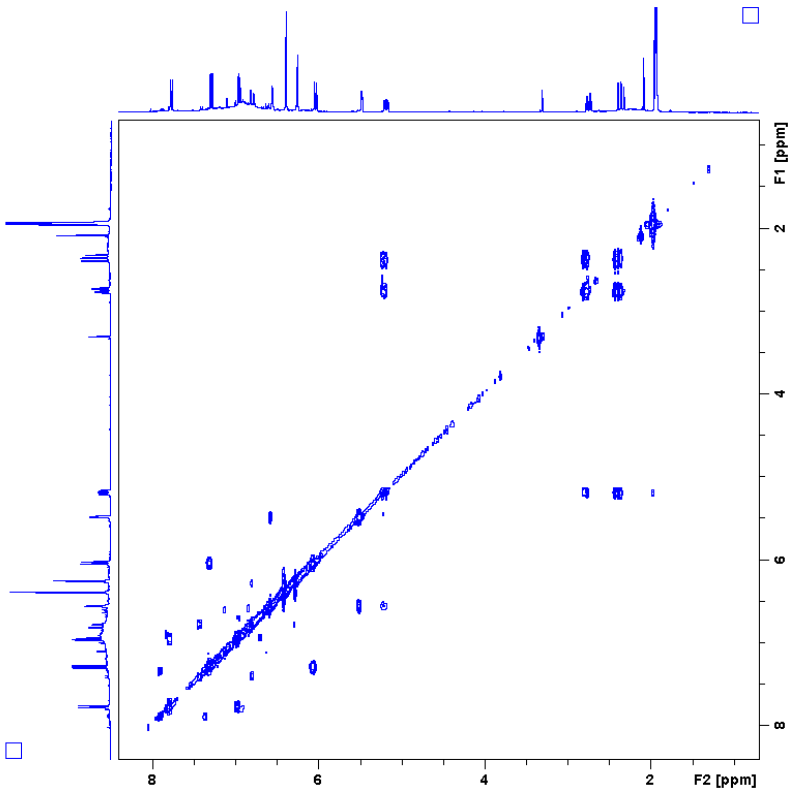


The ^1H NMR spectrum shows that the proton signals of the resorcinol ring of **1** undergo only very minor changes, while their splitting pattern remains the same: The product **4** has a triplet (labeled H_f triplet above) at 6.26 ppm (1H, J = 2.3 Hz) while this proton appears as a triplet (1H, J = 2.3 Hz) at 6.18 ppm in the starting material. Likewise, the product has a doublet (labeled the H_g doublet above) at 6.39 ppm (2H, J = 2.3 Hz) while the starting compound has the same doublet at 6.49 ppm (2H, J = 2.3 Hz). In contrast, the aromatic proton signals of **1** on the other ring are no longer present in the product. Likewise, the signals for the *trans* vinyl protons of **1** have disappeared. The ^{13}C NMR spectrum shows one carbonyl carbon and three aliphatic carbons—two corresponding to carbons that are bonded to an oxygen (the peaks at 71.44 and 81.28 ppm, which are labeled carbons b and c respectively) and the other to a simple methylene carbon (the peak at 41.42 ppm, labeled carbon a). Based on these data, we conclude the *trans*-resveratrol has undergone a [4+2] cycloaddition to form the endoperoxide with the indicated structure. The remainder of the NMR data of compound **4** confirms this structural assignment. For example, H_a and H_b are diastereotopic. Thus, H_a is a doublet of doublet at 2.36 ppm with coupling constants of 15.1 Hz (coupling to H_b) and 13.3 Hz (coupling to H_c). Likewise, H_b is a doublet of doublet with coupling constants of 15.1 Hz (coupling to H_a) and 6.1 Hz (coupling to H_c). The COSY spectrum also shows coupling among H_a, H_b, and H_c. Furthermore, it shows that H_e and H_i are coupled as are H_d and H_h.

^1H, ^{13}C and COSY spectra of the crude reaction mixture are shown on the following pages. It should be noted that most of compound **3** (3,5-dihydroxy benzaldehyde) precipitates from the crude reaction mixture. Hence compounds **2** and **4** are shown on the NMR spectra below. Nevertheless, compound **2** and the small amount of compound **3** remaining in the mixture can readily be separated by flash column chromatography (1:1 ethyl acetate:hexane).

^{1}H, ^{13}C, and COSY NMR Spectra of Crude Product Mixture in CD_3CN





4-Hydroxybenzaldehyde (2): ^{1}H NMR (400 MHz, CD3CN) δ: 9.82 (s, 1H), 7.77 (d, *J* = 8.6 Hz, 2H), 6.96 (d, *J* = 8.6 Hz, 2H); **^{13}C NMR** (100 MHz, CD_3CN) δ: 192.17, 164.14, 133.37, 130.94, 117.14.

3,5-Dihydroxybenzaldehyde (3): ^{1}H NMR (400 MHz, CD_3CN) δ: 9.81 (s, 1H), 7.32 (broad singlet, 2H), 6.82 (d, *J* = 2.3, 2H), 6.57 (t, *J* =2.3, 1H). **^{13}C NMR** (100 MHz, CD_3CN) δ: 193.64, 160.07, 140.34, 109.73, 109.13.

[4+2] Endoperoxide Adduct (4): ^{1}H NMR (400 MHz, CD_3CN) δ: 7.29 (d, *J* = 9.8 Hz, 1H), 6.56 (m, 1H), 6.39 (d, *J* = 2.3 Hz, 2H), 6.26 (t, *J* = 2.3 Hz, 1H), 6.04 (d, *J*= 9.8 Hz, 1H), 5.48 (d, *J* = 4.2 Hz, 1H), 5.19 (dd, *J* = 13.3 and 6.1 Hz, 1H), 2.75 (dd, *J* = 15.1 and 6.1 Hz, 1H), 2.36 (dd, *J* = 15.1 and 13.3 Hz, 1H); **^{13}C NMR** (100 MHz, CD_3CN) δ: 196.20, 159.43, 143.89, 142.52, 134.25, 131.24, 129.89, 108.42, 103.75, 81.28, 75.44, 41.42.

4. Singlet oxygen luminescence quenching experiments for resveratrol:

Singlet oxygen luminescence quenching experiments were carried out with a nanosecond pulsed Nd:YAG laser at an excitation wavelength of 532 nm (New Wave Research Mini-Lase II). Singlet oxygen luminescence was observed with a liquid nitrogen cooled Ge photodiode detector (Applied Detector Corp. Model 403HS). A combination of several band-pass filters are used to remove undesired radiation outside the NIR emission of singlet oxygen. A Schott color glass filter (model RG850; cut-on 850 nm; Newport, USA) is taped to the sapphire entrance of the detector to block any additional ultraviolet and visible light from entering. The port opening to the detector contains the remaining filters. The long wave pass filter (silicon filter model 10LWFw1000; Newport, USA) transmits in the range of 1100–2220 nm and blocks in the range of 800–954 nm. A band pass filter

(model BP-1270-080-B*; CWL 1270 nm; Spectrogen, USA) blocks in the UV, visible, and IR regions and only transmits in the range of 1200–1310 nm with maximum transmission of 60% at 1270 nm. All $^1O_2$ luminescence experiments were run in deuterated acetonitrile in air-saturated solutions. A 2 ml methylene blue solution in a fluorescence cell was used as photosensitizer to produce singlet oxygen. Small aliquots (5 – 20 μL) of the CD_3CN stock solutions of resveratrol were added to the methylene blue solution. 3-5 decay traces were averaged for each time-resolved measurement. $^1O_2$ luminescence decay signals were recorded on a 500 MHz oscilloscope (LeCroy 9350 CM) and fitted to a first order exponential function on Origin 7.0.

Sample plots for the rate of singlet oxygen removal, k_T, of resveratrol in various solvents are shown below. In CD_3CN and CD_3OD at room temperature, the k_T values were determined to be 1.60 x 10^6 $M^{-1}sec^{-1}$ (see text for figure) and 1.54 x 10^6 $M^{-1}sec^{-1}$ (Figure 1 below), respectively. In CD_3CN at 0°C, the k_T is 1.20 x 10^6 (Figure 2). The k_T of resveratrol in D_2O at pH 10.0 is 3.73 x 10^8 $M^{-1}sec^{-1}$ (Figure 3).

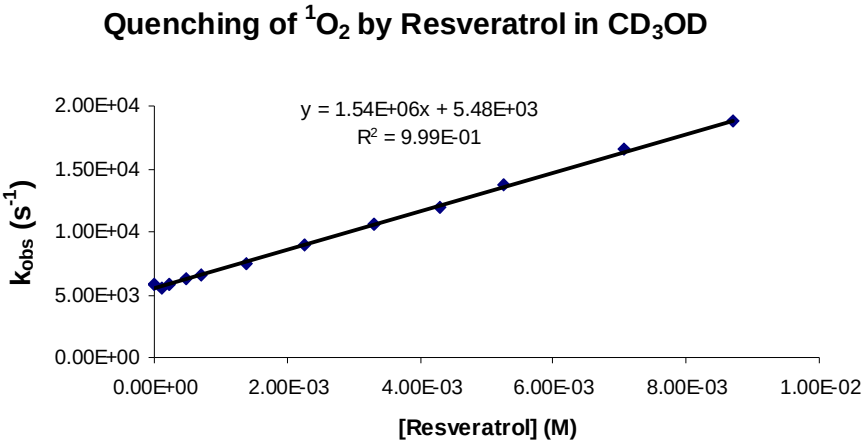


Figure 1. Singlet oxygen removal by resveratrol in CD_3OD.

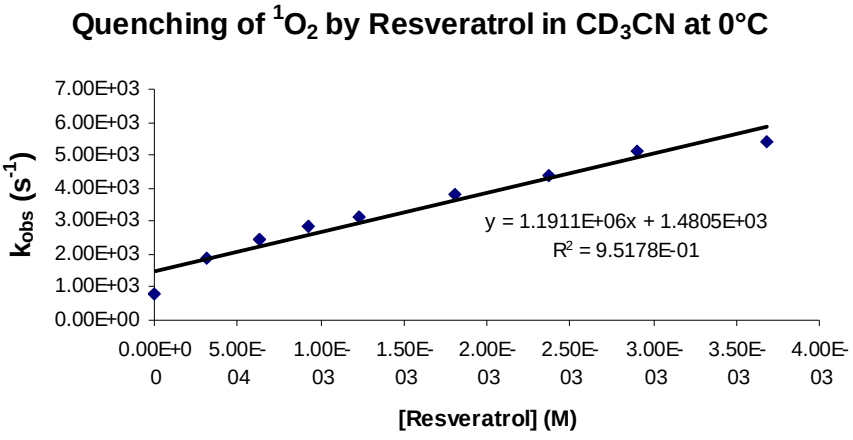


Figure 2. Singlet oxygen removal by resveratrol in CD_3CN at 0°C.

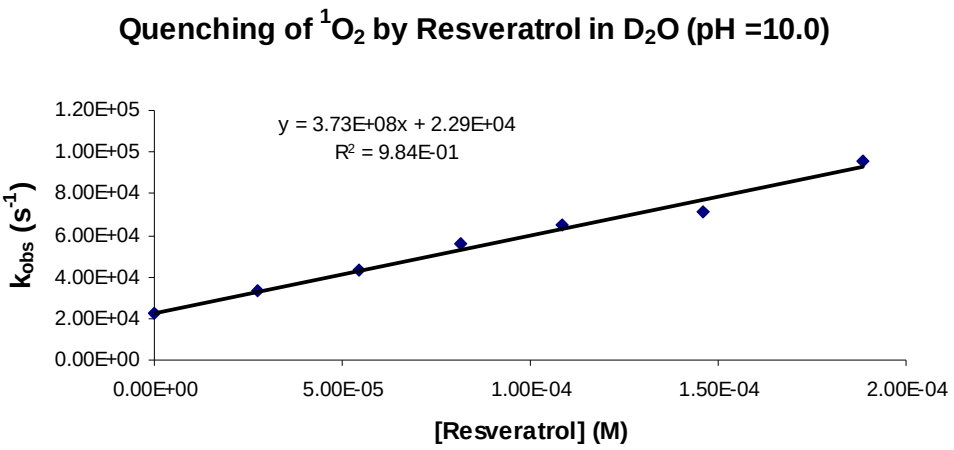


Figure 3. Singlet oxygen removal by resveratrol in D_2O at pH 10.0.

5. Determination of the rate constant of singlet oxygen quenching by reaction with resveratrol (k_r):

The rate constant of the reaction of resveratrol with singlet oxygen, k_r (**1**), was determined via competition experiments using a reference compound whose k_r is known. k_r (**1**) can then be obtained using the equation of Higgins et al[1].

$$\frac{k_r\,(\text{Substrate})}{k_r\,(\text{Reference})} = \frac{\log\,([\text{Substrate}]^f / [\text{Substrate}]^o)}{\log\,([\text{Reference}]^f / [\text{Reference}]^o)}$$

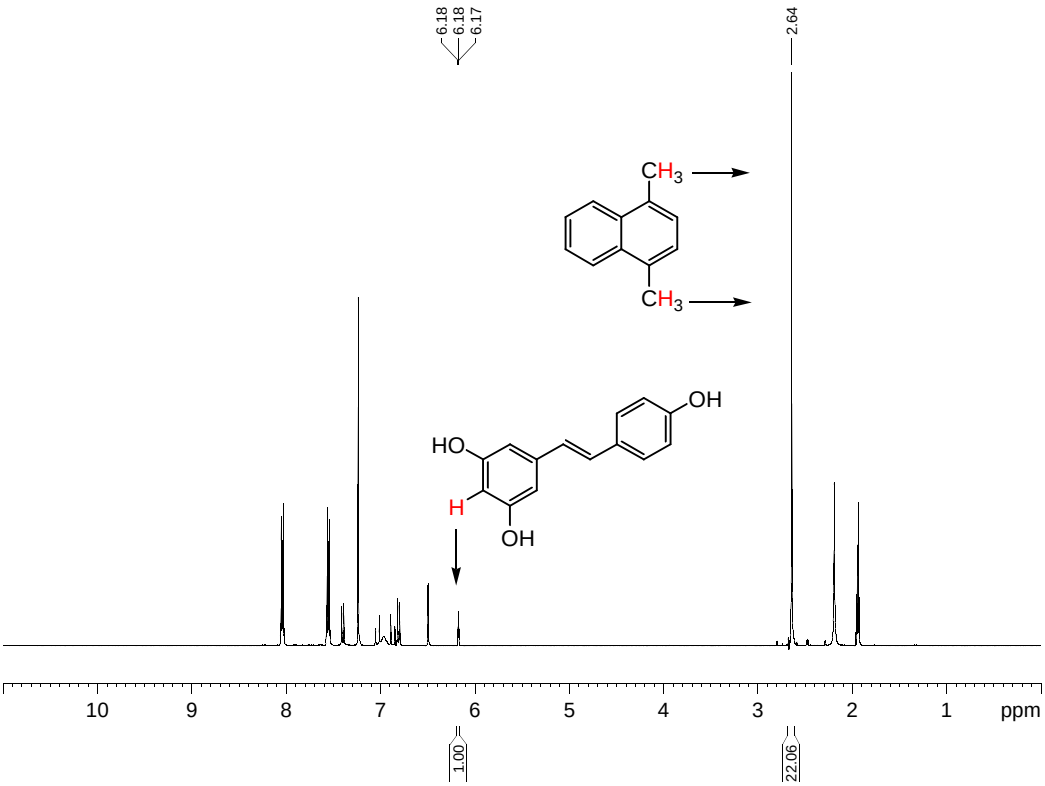
We chose to use 1,4-dimethylnaphthalene (1,4-DMN) as the reference compound. 1,4-DMN is known to interact with singlet oxygen by chemical reaction only, hence its total singlet oxygen quenching rate, k_T, is equal to k_r. Using singlet oxygen luminescence quenching experiments, we measured the k_r of 1,4-DMN to be 2.9 x 10^5 $M^{-1}sec^{-1}$ in CD_3CN at room temperature, which made it a suitable competitor for resveratrol which has a similarly low reaction rate with singlet oxygen. However, the endoperoxide formed upon reaction of singlet oxygen with 1,4-DMN has a half-life of five hours at room temperature[2]. Thus, competition experiments were performed at 0°C, a temperature wherein no decomposition of the endoperoxide was observed even over a period of five hours. The k_r of 1,4-DMN in CD_3CN at 0°C was determined via singlet oxygen luminescence quenching experiments to be 2.7 x 10^5 $M^{-1}sec^{-1}$.

The competition experiments to determine k_r (**1**) were carried out in oxygen-saturated CD_3CN solutions at 0°C. 1.0 mL solutions containing different molar ratios of resveratrol and 1,4-DMN (from 1:1.5 to 1:5.2 resvartrol/1,4-DMN), and a small amount of the sensitizer methylene blue were irradiated in an NMR tube using a 200 W Oriel tungsten-halogen lamp. A glass filter was employed to cut off light below 493 nm. The oxidized products were analyzed using ^1H NMR.

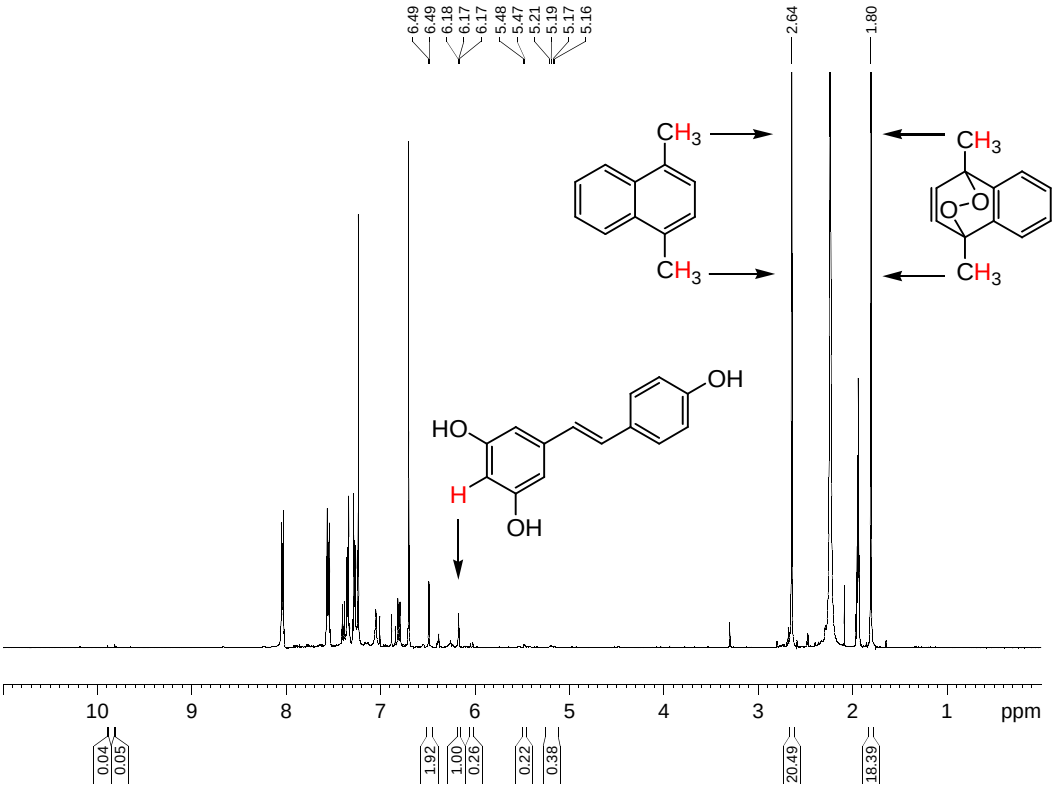
[1] Higgins, R.; Foote, C. S.; Cheng, H. *Adv. Chem. Ser.* **1968**, *77*, 102-117.

[2] Adam, W.; Prein, M. *Acc. Chem. Res.* **1996**, *29*, 275-283.

^{1}H NMR Spectrum of a 1:5 Mixture of Resveratrol to 1,4-DMN Before Reaction with Singlet Oxygen



^{1}H NMR Spectrum of a 1:5 Mixture of Resveratrol to 1,4-DMN After Reaction with Singlet Oxygen



The values for the initial and final concentrations of resveratrol and 1,4-DMN can be obtained from the integration values of representative peaks of resveratrol, 1,4-DMN, and 1,4-DMN endoperoxide present in the spectra before and after irradiation. A sample calculation for k_r(**1**) is shown below.

$$k_r\,(\mathbf{1}) = \left(\frac{\log\,([\mathbf{1}]^f / [\mathbf{1}]^o)}{\log\,([1,4\text{-DMN}]^f / [1,4\text{-DMN}]^o)} \right) k_r\,(1,4\text{-DMN})$$

$$= \left(\frac{\log\,([1.00 / \{(20.49+18.39) / 6\}] / [1.00 / (22.06 / 6)])}{\log\,(20.49 / [20.49 / (20.49 + 18.39])} \right) 2.7 \times 10^5\ M^{-1}sec^{-1}$$

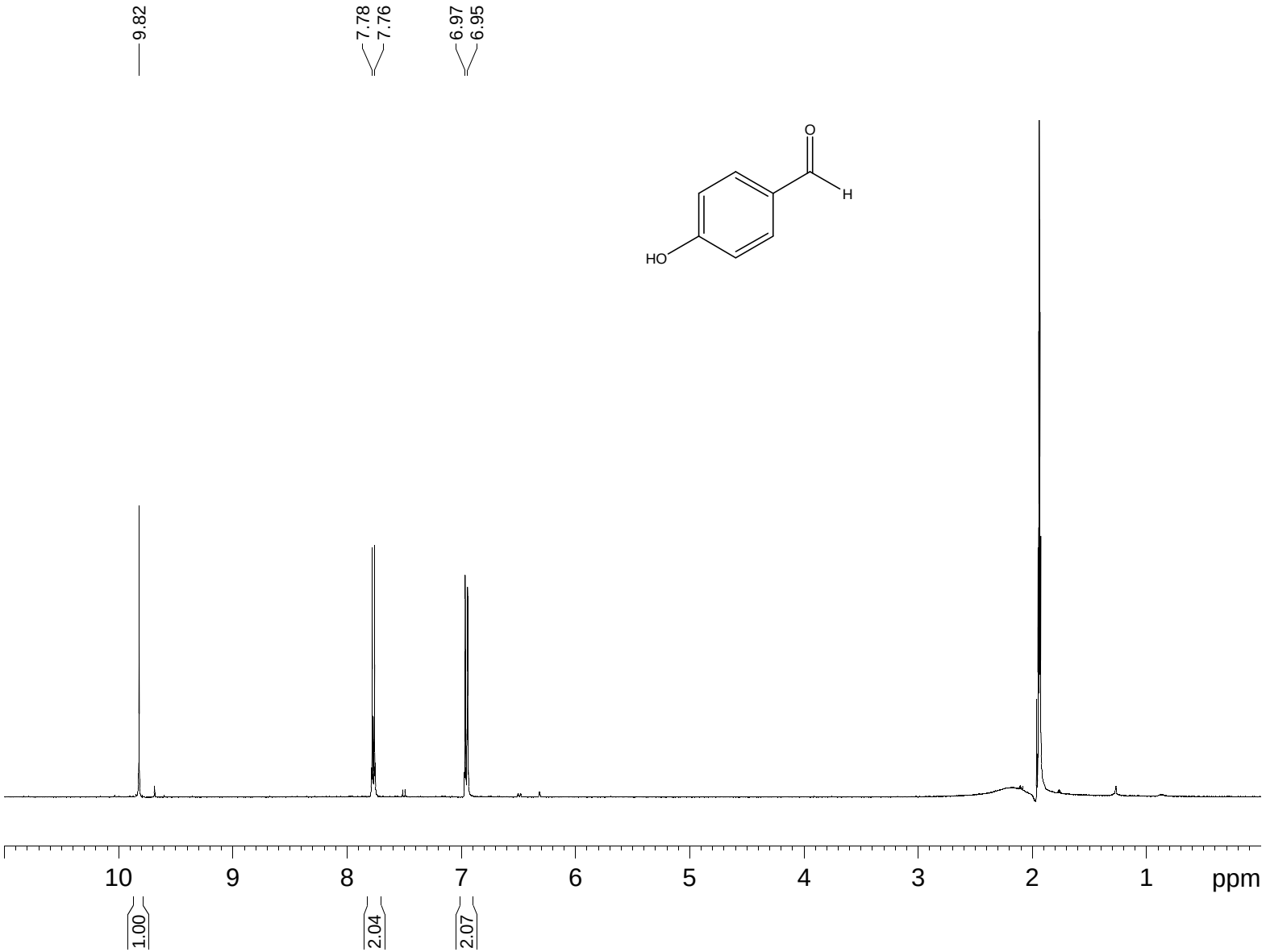
$$= 3.0 \times 10^5\ M^{-1}sec^{-1}$$

The values obtained for k_r(**1**) in four different experiments using different mole ratios of resveratrol to 1,4-DMN are shown in Table 1 below. Averaging the values, we obtained a k_r (**1**) in CD_3CN at 0°C of $3.0 \times 10^5\ M^{-1}sec^{-1}$.

Experiment	Compound	Concentration M x 10^{-3}	Mole Ratio (**1**:DMN)	Irradiation Time (h)	k_r (**1**) x 10^{-5} ($M^{-1}sec^{-1}$)
1	**1**	8.8	1 : 5.2	2	3.0
	1,4-DMN	45.4			
2	**1**	10.5	1 : 4.3	2	2.7
	1,4-DMN	45.4			
3	**1**	13.1	1 : 1.5	2	3.0
	1,4-DMN	19.2			
4	**1**	7.0	1 : 1.5	1	3.1
	1,4-DMN	10.2			

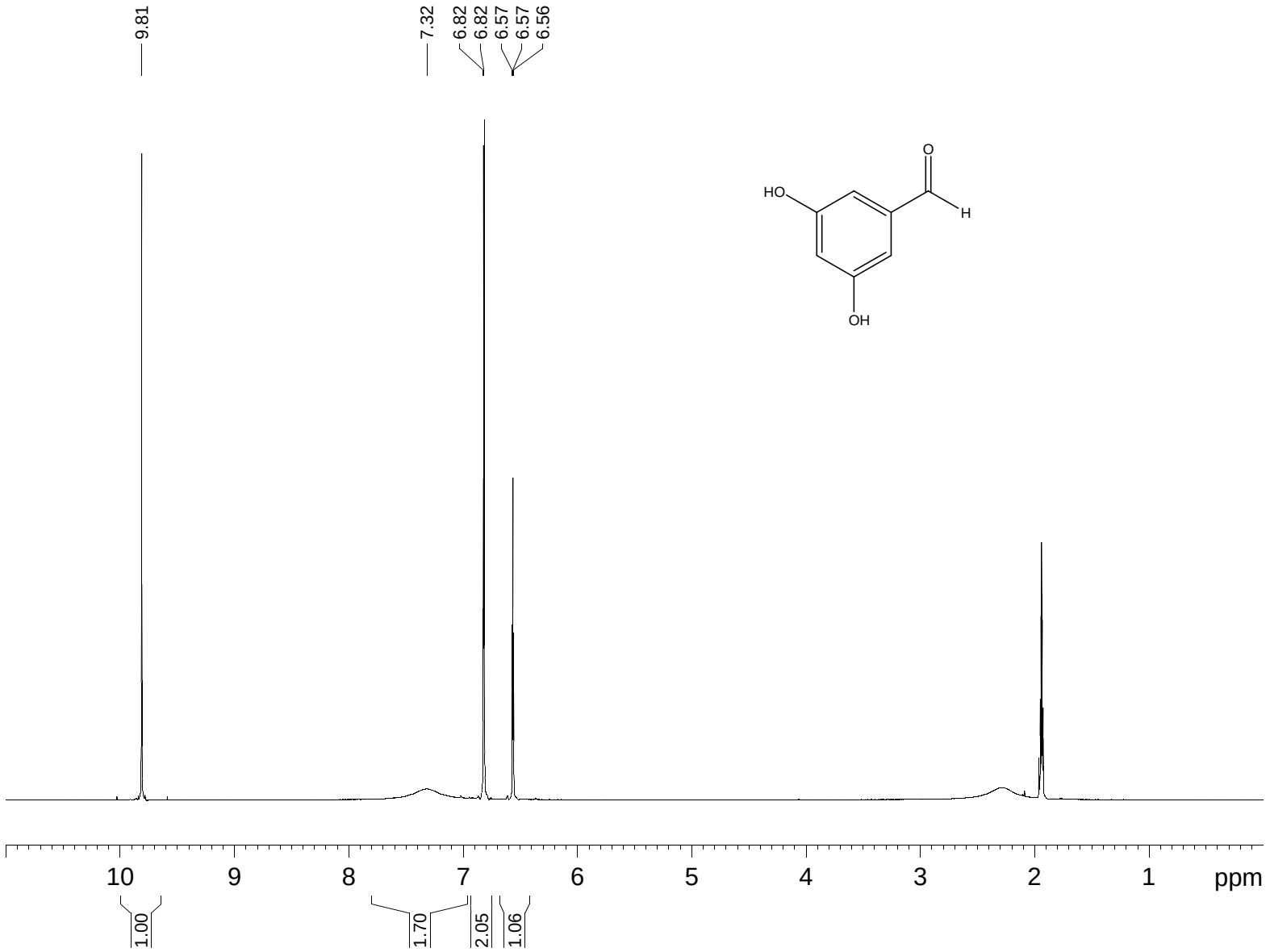
Table 1. Singlet oxygen removal of resveratrol in CD_3CN at 0°C by chemical reaction only.

^{1}H NMR Spectrum of 4-Hydroxybenzaldehyde (**2)** in CD_3CN

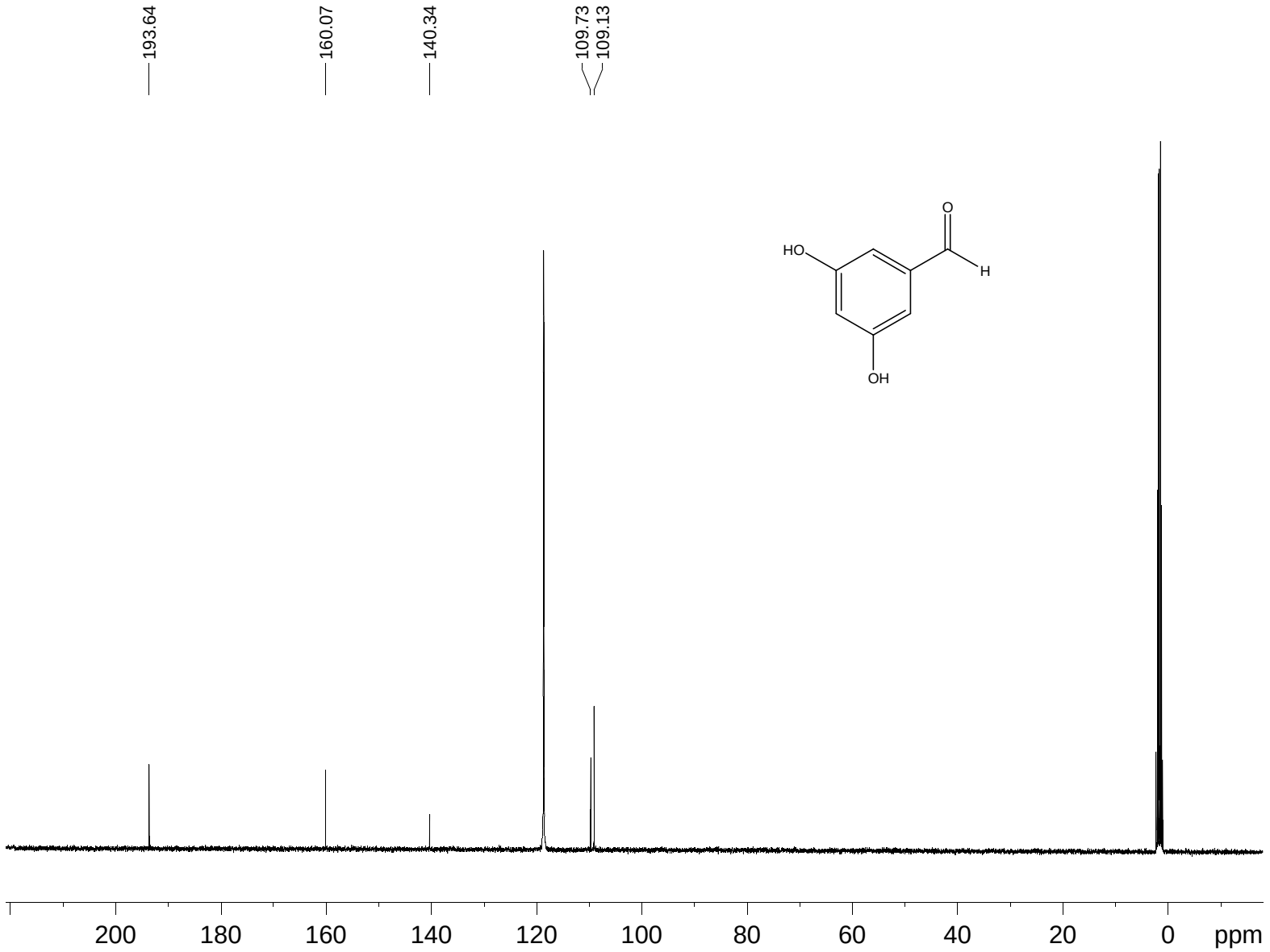


^{13}C NMR Spectrum of 4-Hydroxybenzaldehyde (**2)** in CD_3CN

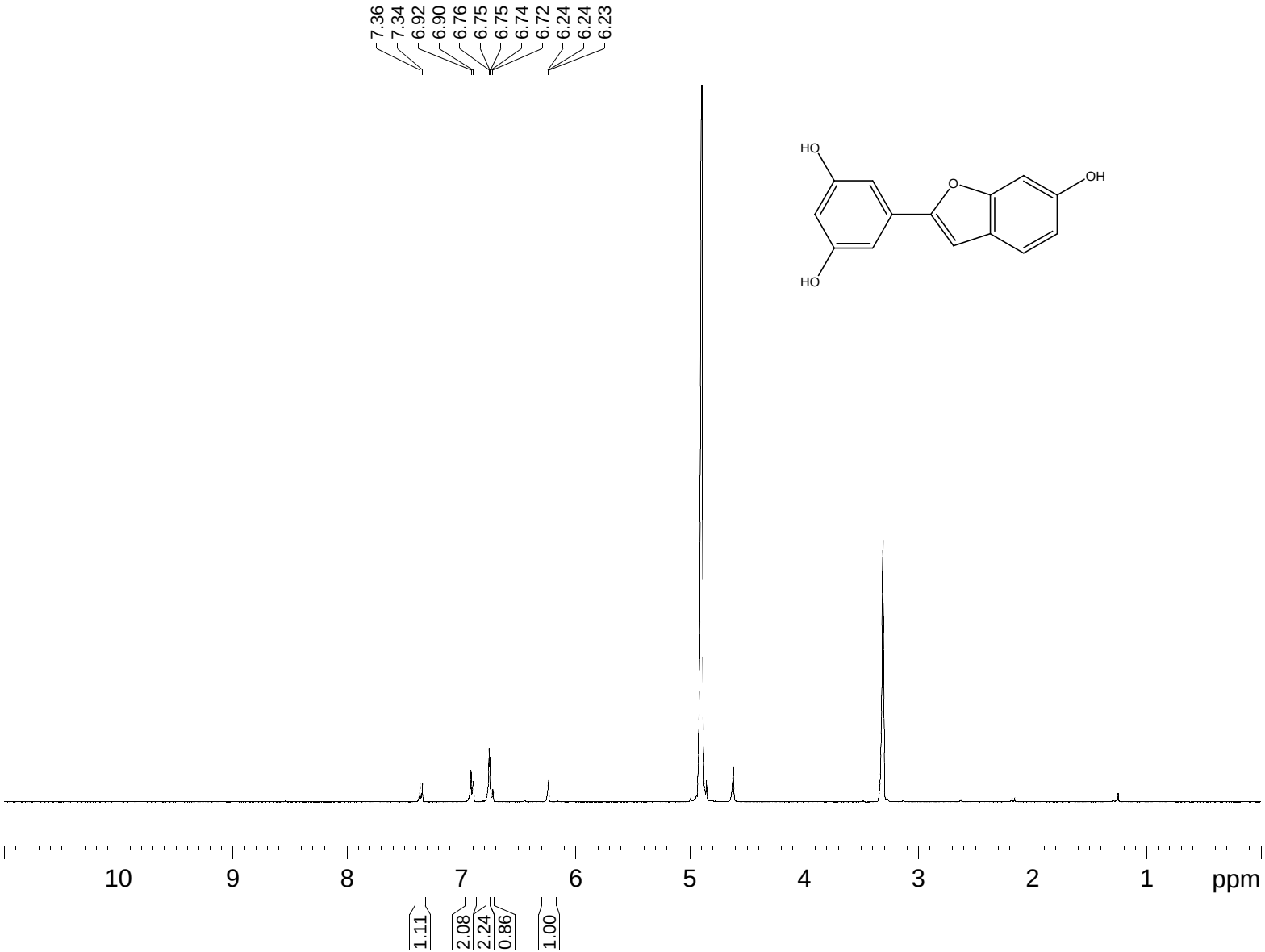
^{1}H NMR Spectrum of 3,5-Dihydroxybenzaldehyde (**3)** in CD_3CN



^{13}C NMR of 3,5-Dihydroxybenzaldehyde (**3)** in CD_3CN

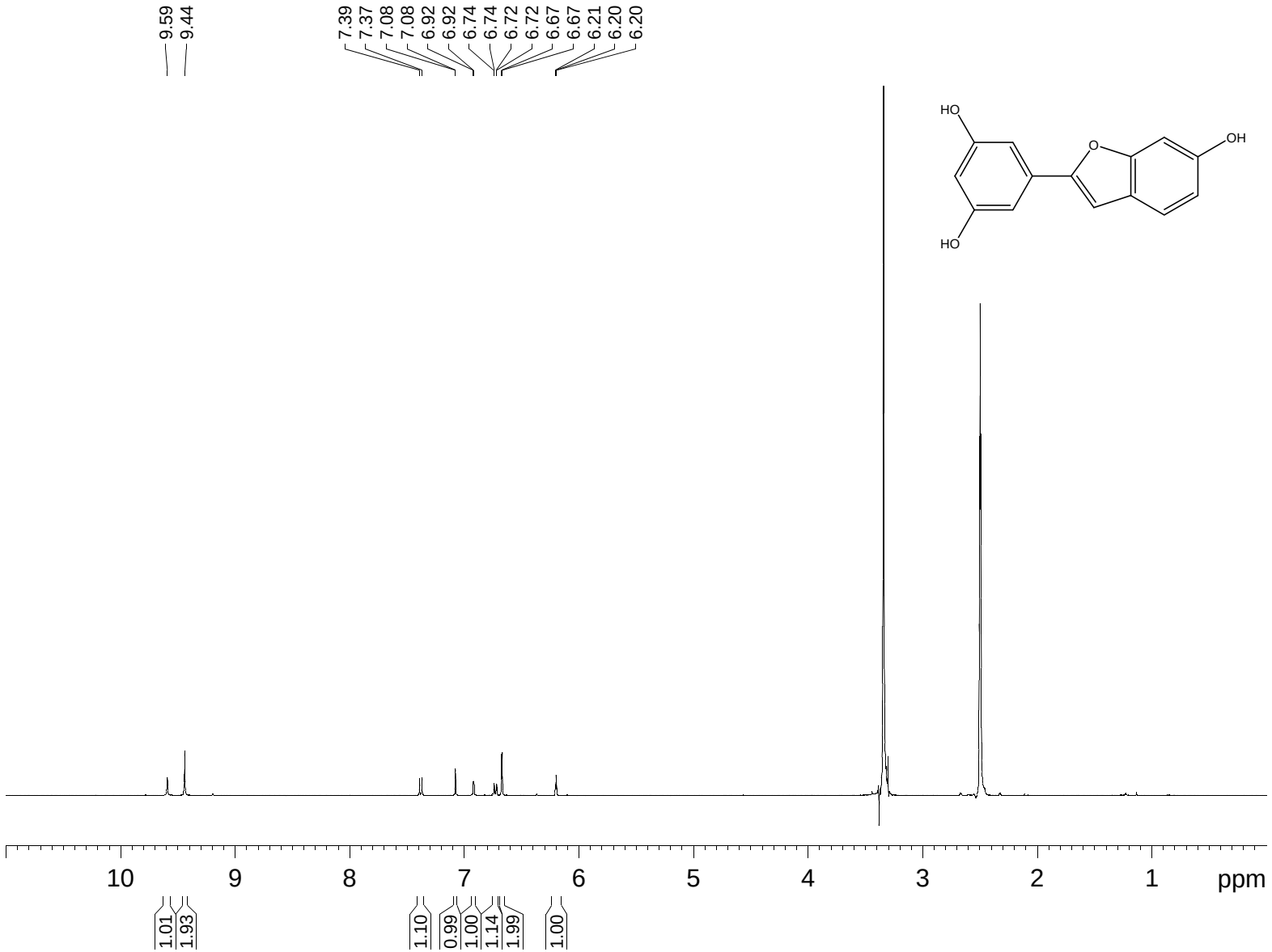


^{1}H NMR Spectrum of Moracin M (**5**) in MeOD

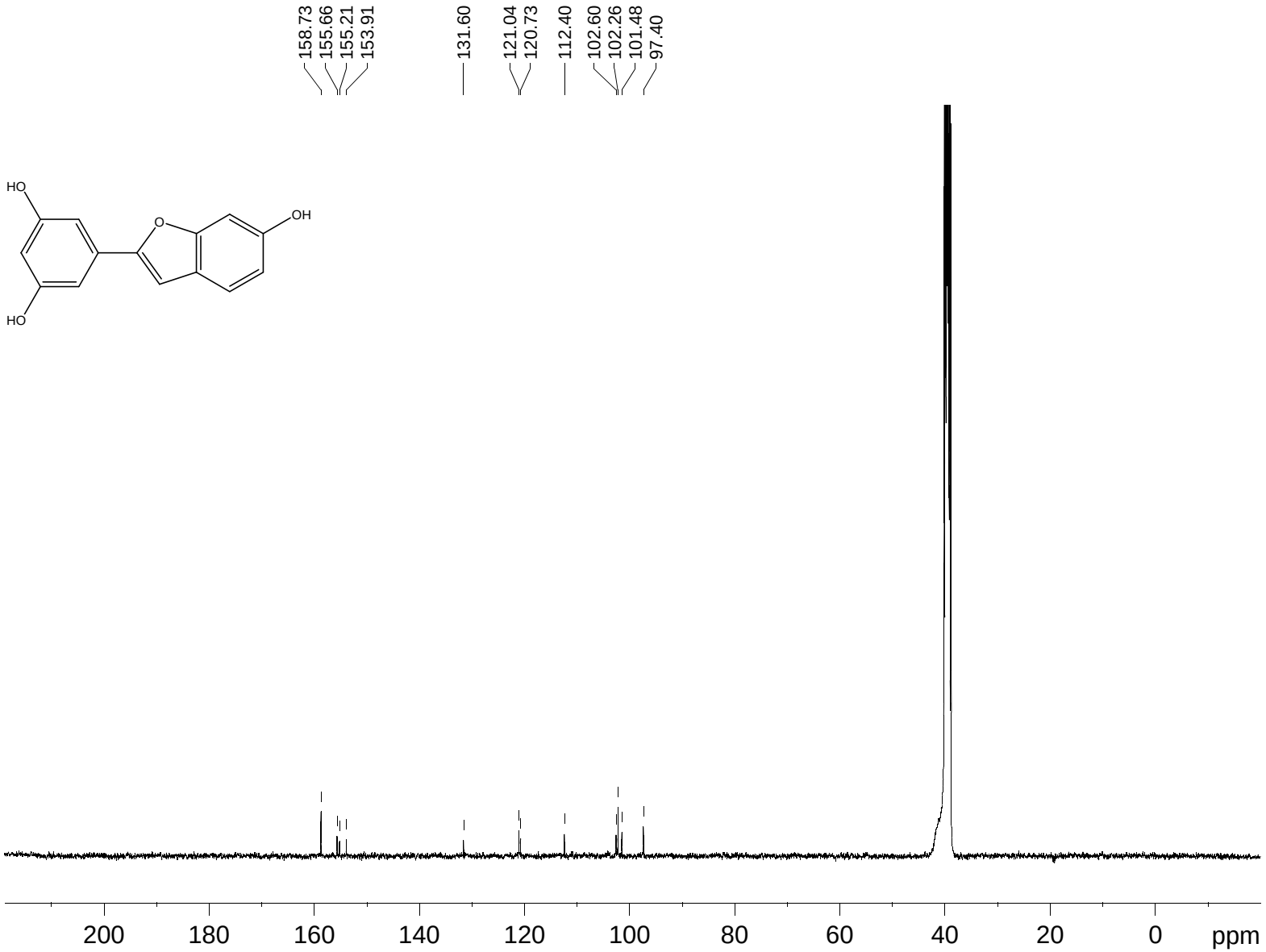


^{13}C NMR of Moracin M (**5**) in MeOD

^{1}H NMR of Moracin M (**5**) in DMSO



^{13}C NMR of Moracin M (**5**) in DMSO



MALDI Mass Spectrum of Moracin M (**5**)