Supporting Information

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Neuroprotective and Antioxidant Constituents from *Curcuma zedoaria* Rhizomes

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S1. Materials and methods

S1.1. General

NG108-15 hybridoma cell line was obtained from American Type Culture Collection (ATCC). Dulbecco's Modified Eagle's Medium (DMEM), phosphate buffered saline (PBS), sodium bicarbonate, HEPE sodium salt and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich; fetal bovine serum (FBS), penicillin/streptomycin and amphotericin B were purchased from PAA Laboratories, Austria; accutase was purchased from Innovative Cell Technologies, Inc.; hydrogen peroxide (H_2O_2) was purchased from Systerm®.

Analytical thin layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated aluminium sheets (0.2 mm, Merck); High performance thin layer chromatography (HPTLC), and preparative thin layer chromatography (PTLC) were done on silica gel 60 GF_{254} precoated glass plates (0.5 mm, Merck). Spots were detected under UV (254 and 366 nm) and by spraying vanillin-H_2SO_4 or anisaldehyde-H_2SO_4 reagent followed by heating at 105ºC. Open column chromatography was done with silica gel 60 (0.043-0.063 mm and 0.063-0.200 mm, Merck) while Sephadex® LH-20 (25-100 µ, Sigma Aldrich) was used for size exclusion chromatography. HPLC was performed using Waters System equipped with binary gradient module (Waters 2545), system fluidics organizer, photodiode array detector (190-400 nm; Waters 2998) and sample manager (Waters 2767). Waters XBridge™ Prep column (C18, 5 µM, 10×250 mm) was used with Waters XBridge™ Prep column guard catridge (C18, 5 µM, 10×10 mm). The data were collected and analyzed by MassLynx software. Both 1D and 2D NMR spectra were recorded on a JEOL 400 MHz FTNMR spectrometer. Deuterated chloroform ($CDCl_3$) was used as the NMR solvent and TMS (δ 0 ppm) as the reference standard for chemical shifts. The GC-MS analyses were performed using Shimadzu QP2010 Series gas chromatography system and operated in the split-less mode at 275ºC on a DM 5MS capillary column (dimethyl polysiloxane:diphenyl 95:5, 30.0 m × 0.25 mm × 0.25 µm) with helium as the carrier gas (flow rate: 1 ml/min). The column temperature was programmed initially at 60°C, then increased to 250°C at a rate of 5°C increase per min and then held for 1 min. The total ion chromatogram obtained was auto-integrated by Chem Station and the components were identified by comparison with the accompanying spectral database (NIST 05, Mass Spectral Library, USA). IR spectra were obtained on a Perkin Elmer 1600 Series FT-IR infrared spectrophotometer using chloroform as a solvent or as KBr disc.

S1.2. Characterisation of the isolated pure compounds

***Germacrone* (1):** White amorphous solid, $C_{15}H_{22}O$, **GC MS**: RT 25.90 min, 218(M^+, 13), 175(27), 136(61), 135(85), 121(30), 107(100), 105(20), 91(31), 67(42). IR ($CHCl_3$) ν_{max} cm^{-1}: 1677. **UV** (MeOH) λ_{max} nm (log ε): 206 (1.47). **^{13}C NMR** (**Table 1**). **^{1}H NMR** (**Table 2**).

***Dehydrocurdione* (2)**: Pale yellow oil, $C_{15}H_{22}O_2$, **GC MS**: RT 27.86 min, 234(M^+, 10), 178(27), 164(53), 152(49), 121(37), 96(53), 68(100), 41(59). **IR** ($CHCl_3$) ν_{max} cm^{-1}**:** 1742, 2934, 1680, 1453, 1375. **UV** (MeOH) λ_{max} nm (log ε): 207 (1.16). **^{13}C NMR** (**Table 1**). **^{1}H NMR** (**Table 2**).

***Curcumenol* (3)**: Colourless oil, $C_{15}H_{22}O_2$, **GC MS**: RT 26.70 min, 234(M^+, 26), 189(53), 147(52), 145(30), 133(53), 121(39), 119(35), 105(100), 91(37), 55(18), 41(25). **IR** ($CHCl_3$) ν_{max} cm^{-1}: 3432, 2934, 1457. **UV** (MeOH) λ_{max} nm (log ε): 248(3.92). **^{13}C NMR** (**Table 1**). **^{1}H NMR** (**Table 2**).

***Zerumin* A (4)**: Pale yellow oil, $C_{20}H_{30}O_3$, **GC MS**: RT 24.32 min, 318 (M^+, 0) 164 (100), 137(81), 95(60), 81(70), 41(55).**IR** ($CHCl_3$) ν_{max} cm^{-1}: 3080, 1646, 890, 1686. **UV** (MeOH) λ_{max} nm (log ε): 210 (1.37). **^{13}C NMR** (**Table 1**). **^{1}H NMR** (**Table 2**).

***Isoprocurcumenol* (5)**: Colourless oil, $C_{15}H_{20}O_2$. **GC MS**: RT 29.36 min, 234(M^+, 6.08), 158(35), 121(84), 105(100), 93(60), 43(79). **IR** ($CHCl_3$) ν_{max} cm^{-1}: 3450, 1674, 1610. **UV** (MeOH) λ_{max} nm (log ε): 205(1.83). **^{13}C NMR** (**Table 1**). **^{1}H NMR** (**Table 2**).

***Curcumenone* (6)**: Colourless oil, $C_{15}H_{22}O_2$. **GC MS**: RT 28.9, 234(M^+, 13.5), 176(78), 163(29), 161(48), 149 (43), 133(37), 107(32), 91(29), 68(91), 67(75), 43(100). **IR** ($CHCl_3$) ν_{max} cm^{-1}: 1679, 1715. **UV** (MeOH) λ_{max} nm (log ε): 205(1.28). **^{13}C NMR** (**Table 1**). **^{1}H NMR** (**Table 2**).

***Procurcumenol* (7):** Colourless oil, $C_{15}H_{22}O_2$. **GC MS**: RT 18.13 min, 234(M^+, 8.9), 216(79), 123(75), 105(55), 91(41), 43(100), 41(40). **IR** ($CHCl_3$) ν_{max} cm^{-1}: 3409, 1712. **UV** (MeOH) λ_{max} nm (log ε): 204.0(1.16). **^{13}C NMR** (**Table 1**). **^{1}H NMR** (**Table 2**).

Zerumbone epoxide (**8**): Pale yellow amrphous powder, $C_{15}H_{18}O_2$. **GC MS**: RT: 16.8 min, 234(M^+, 7.04), 135(89), 121(44), 107(99), 43(100). **IR** (KBr) ν_{max} cm^{-1}: 3426, 2963, 1712. **UV** (MeOH) λ_{max} nm (log ε): 216 (2.6). **^{13}C NMR** (**Table 1**). **^{1}H NMR** (**Table 2**).

***Zederone* (9)**: Colourless crystals, $C_{15}H_{18}O_3$. **GC MS**: RT 32.09 min, 246(M^+, 35.6), 188(35), 176(35), 175(100), 161(55), 119(90), 91(55), 43(55). **IR** ($CHCl_3$) ν_{max} cm^{-1}: 2929, 1664, 1527, 1404. **UV** (MeOH) λ_{max} nm (log ε): 239 (2.08). **^{13}C NMR** (**Table 1**). **^{1}H NMR** (**Table 2**).

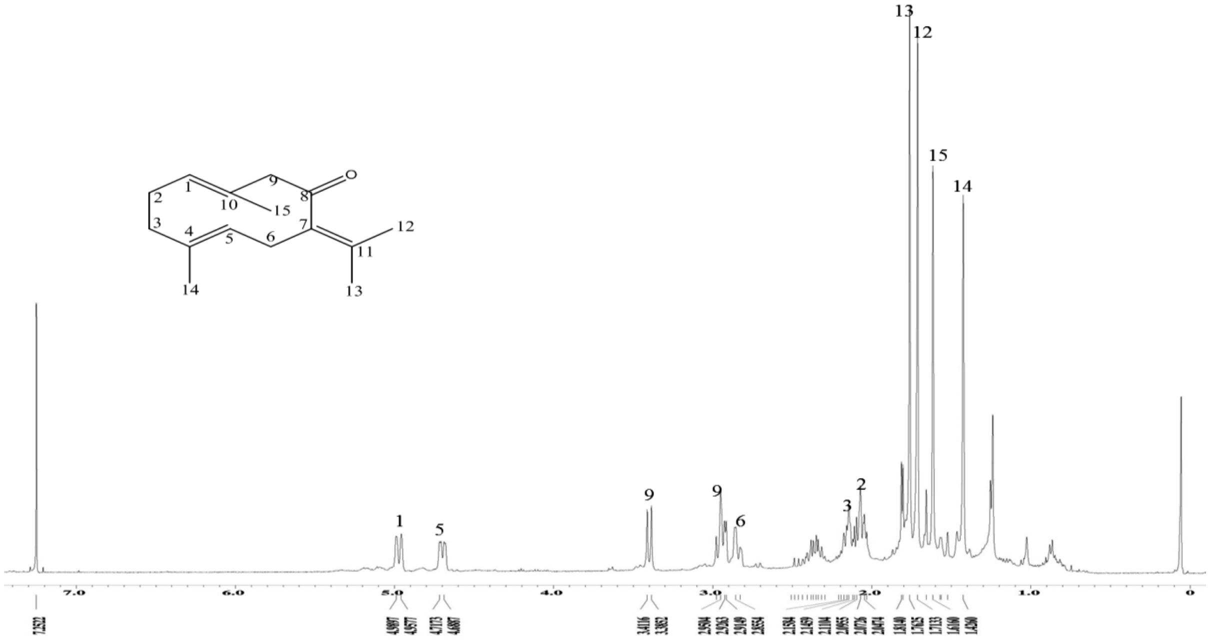
***Gweicurculactone* (10)**: Reddish crystal, $C_{15}H_{16}O_2$. **GC MS**: RT 22.3 min. 228(M^+, 100), 89(199), 157.1(22.3), 142.1(16.9), 77(9.6), 51(5.6). **IR** ($CHCl_3$) ν_{max} cm^{-1} 1004, 898, 750, 1725. **UV** (MeOH) λ_{max} nm (log ε): 218(3.66). **^{13}C NMR** (**Table 1**). **^{1}H NMR** (**Table 2**).

S2. ^{13}C (100 MHz) NMR data of isolated compounds (**1-10**) from *C. zedoaria* in $CDCl_3$

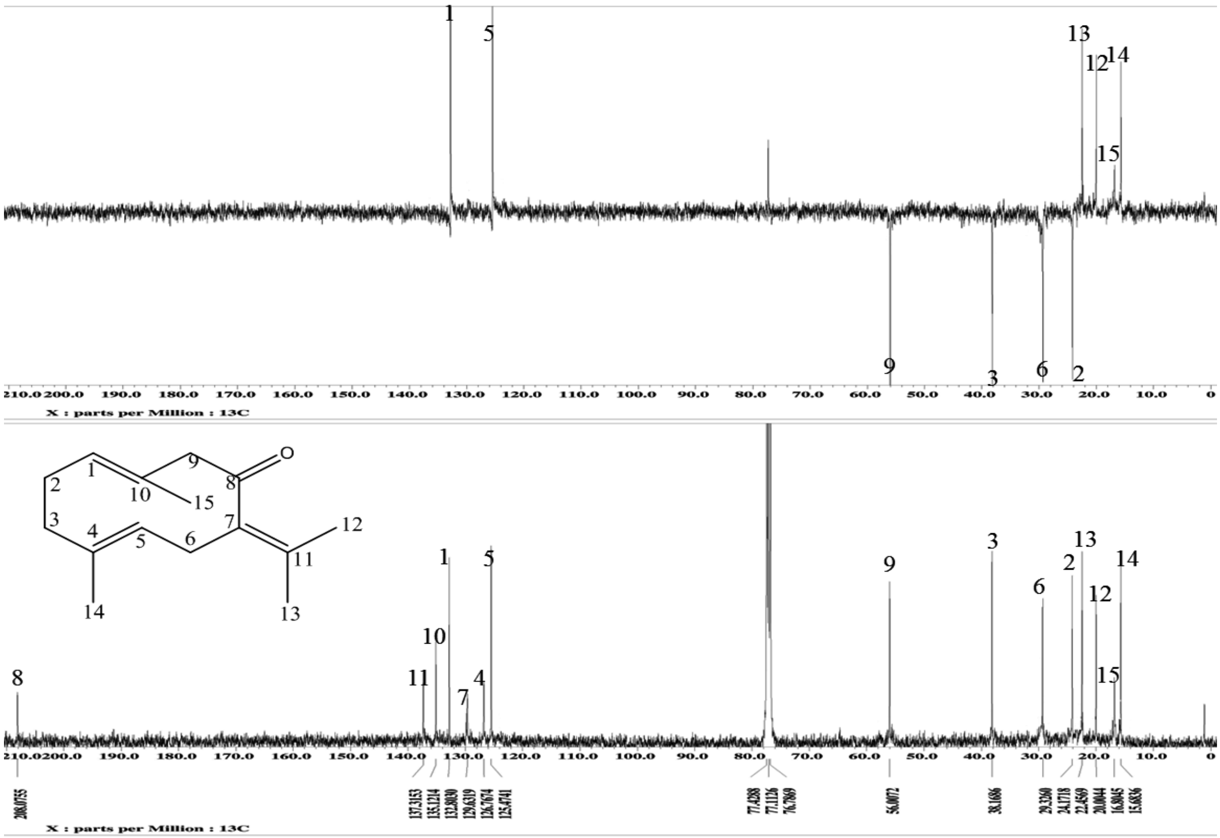
Position	**δ_C**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	132.8	133.0	51.3	39.2	51.2	24.1	50.5	42.7	131.2	144.2
2	24.0	26.4	27.6	19.3	24.7	23.4	26.9	62.5	24.7	31.9
3	38.1	34.2	31.2	42.0	28.2	44.0	39.9	61.9	38.0	33.3
4	126.0	46.4	40.4	33.6	77.4	209	80.3	38.2	64.0	43.9
5	125.4	211.1	85.8	55.4	58.9	24.2	53.9	24.7	66.6	156.6
6	29.3	43.4	37.2	24.1	39.8	28.0	28.6	147.8	192.2	117.9
7	129.0	129.3	139.2	37.9	134.5	128.1	136.9	139.5	123.2	146.2
8	208.0	207.2	101.6	148.1	203	201.9	199.2	203.0	157.2	154.8
9	56.0	57.0	125.7	56.4	53.8	49.0	129.2	128.3	41.9	116.4
10	135.1	129.9	137.2	39.6	141.3	20.2	155.1	159.5	131.1	136.5
11	137.0	137.0	122.3	24.6	143.9	147.6	136.3	36.0	122.2	103
12	20.0	21.0	22.4	159.5	21.9	23.5	21.3	15.7	138.1	170.7
13	22.4	22.1	18.9	135.7	22.8	23.5	22.4	12.1	10.3	7.7
14	15.6	18.4	11.9	29.7	24.4	30.1	23.4	24.0	15.2	20.1
15	16.8	16.3	21.0	174.3	111.6	19.1	24.3	29.8	15.8	24.8
16				193.8						
17				107.9						
18				33.6						
19				21.8						
20				14.1						

S3. ^{1}H (400 MHz) NMR data of isolated compounds (**1-10**) from *C. zedoaria* in $CDCl_3$

Position	**δ_H (*J* in Hz)**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	4.94 *d* (11.8)	5.13 *t* (8.24)	1.9 *m*	1.07/1.72 *m*	3.22 *q* (14.68)	0.43 *dt* (4.56, 7.32)	2.34 *dd* (8.7, 9.6)	1.44 *dd* (2.7, 11.4)	5.46 *d* (11.8)	2.12 *m*
2	2.08/2.35 *m*	2.10 *m*	1.9/1.9 *m*	1.52/1.59 *m*	1.21 *m*	1.64 *q* (7.32)	1.66/1.94 *m*	2.74 *d* (11.0)	2.24/2.46 *m*	1.53 *m*
3	2.15 *m*	2.0 *m*	1.9/1.9 *m*	1.21/1.42 *m*	1.39 *m*	2.47 *t* (7.36)	1.88 *m*	-	1.24/2.27 *m*	2.81/2.67 *m*
4	-	2.38 *m*	2.62 *d* (15.6)	-	-	-	-	1.34, 2.20, *m*	-	3.08, *q* (14.2)
5	4.71 *d* 11.0	-	-	1.13 *dd* (2.7, 12.8)	1.40 *m*	0.67 *q* (4.56)	1.91 *m*	1.94 *d* (13.7)	3.77 *s*	-
6	2.86 *m*	3.21/3.29 *dd* (16.48)	2.11/2.66 *d* (15.4)	1.36/1.75 *m*	2.81 *d* (14.2)	2.8 *m*	2.16 *dd* (13.2, 15.6)/2.57 *d* (15.6)	6.1 *d* (12.3)	-	6.88 *s*
7	-	-	-	2.41 *m* 2.02 *dd* (5.0, 13.2)	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-
9	3.42/2.95, *dd* (11, 3.68)	3.06/3.23 *dd* (11.44)	5.74 *brs*	1.92 *d* (11)	2.16 *s*	2.52 *d* (15.6)	5.85 *d* (1.4)	6.10 *d* (1.36)	3.66/3.70 *m*	6.73 *s*
11	-	-	-	2.55 *ddd* (2.7, 3.2, 6.3)	-	-	-	-	-	-
12	1.73 *s*	1.76 *s*	1.54 *s*	6.68 *t* (6.4)	1.92 *s*	2.07 *s*	1.73 *s*	1.20 *s*	7.04 *brs*	-
13	1.76 *s*	1.73 *s*	1.61 *s*	-	1.82 *s*	1.77 *s*	1.76 *s*	1.84 *s*	2.07 *s*	1.98 *s*
14	1.43 *s*	1.01 *d* (6.88)	1.01 *d* (6.4)	3.36 *d* (16.4)	1.24 *s*	2.12 *s*	1.86 *s*	1.28 *s*	1.30 *s*	1. 30 *d* (6.84)
15	1.62 *s*	1.63 *s*	1.79 *s*	-	4.90 *brs*	1.10 *s*	1.22 *s*	1.07 *s*	1.56 *s*	2.24 *s*
16	-	-	-	9.37 *s*	-	-	-	-	-	-
17	-	-	-	4.37/4.84 *brs*	-	-	-	-	-	-
18	-	-	-	0.87 *s*	-	-	-	-	-	-
19	-	-	-	0.81 *s*	-	-	-	-	-	-
20	-	-	-	0.73 *s*		-	-	-	-	-



S4: ^{1}H NMR spectrum of germacrone (**1**) in $CDCl_3$



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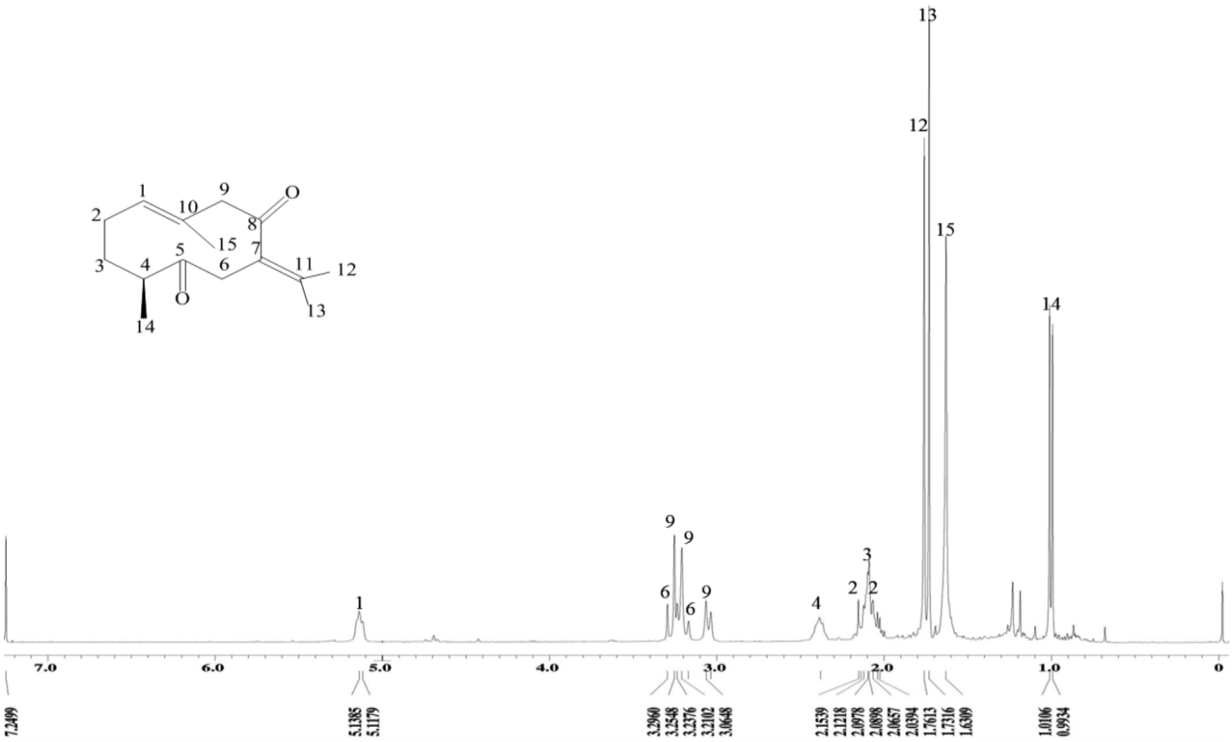

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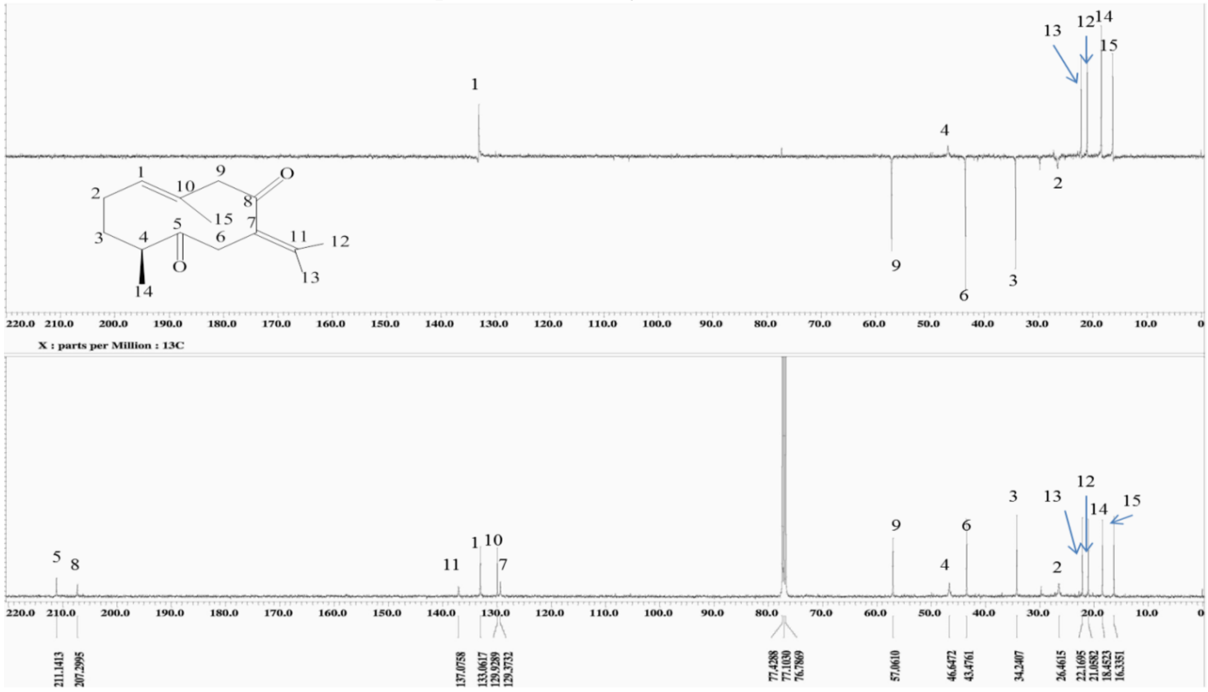

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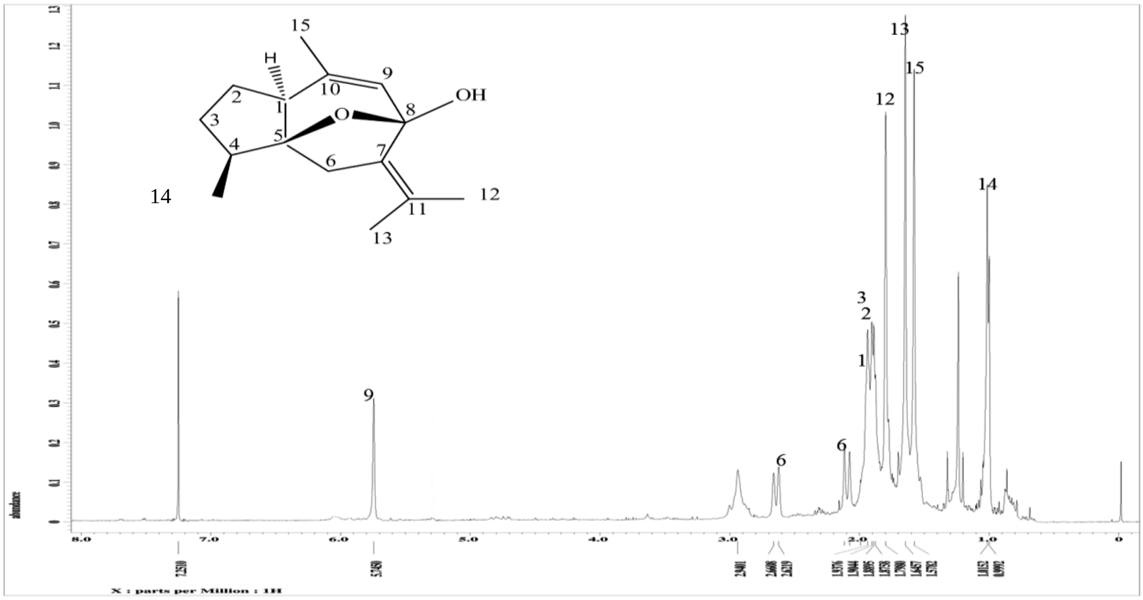
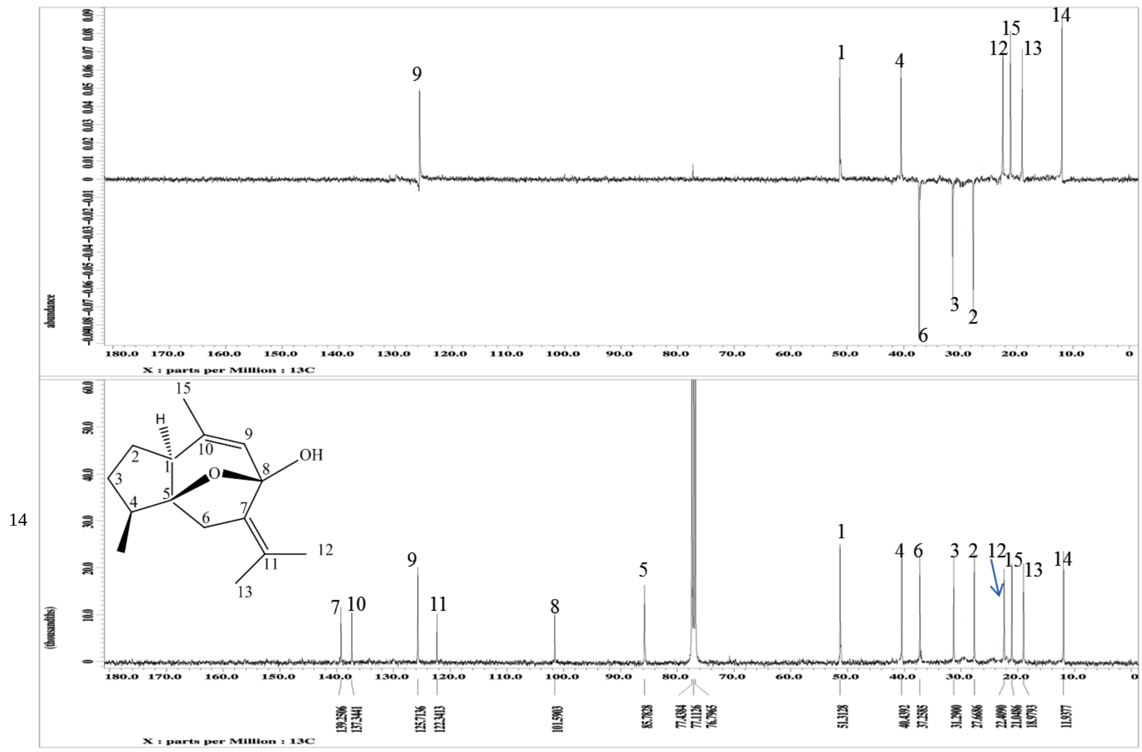


S8: ^{1}H NMR spectrum of curcumenol (**3**) in $CDCl_3$



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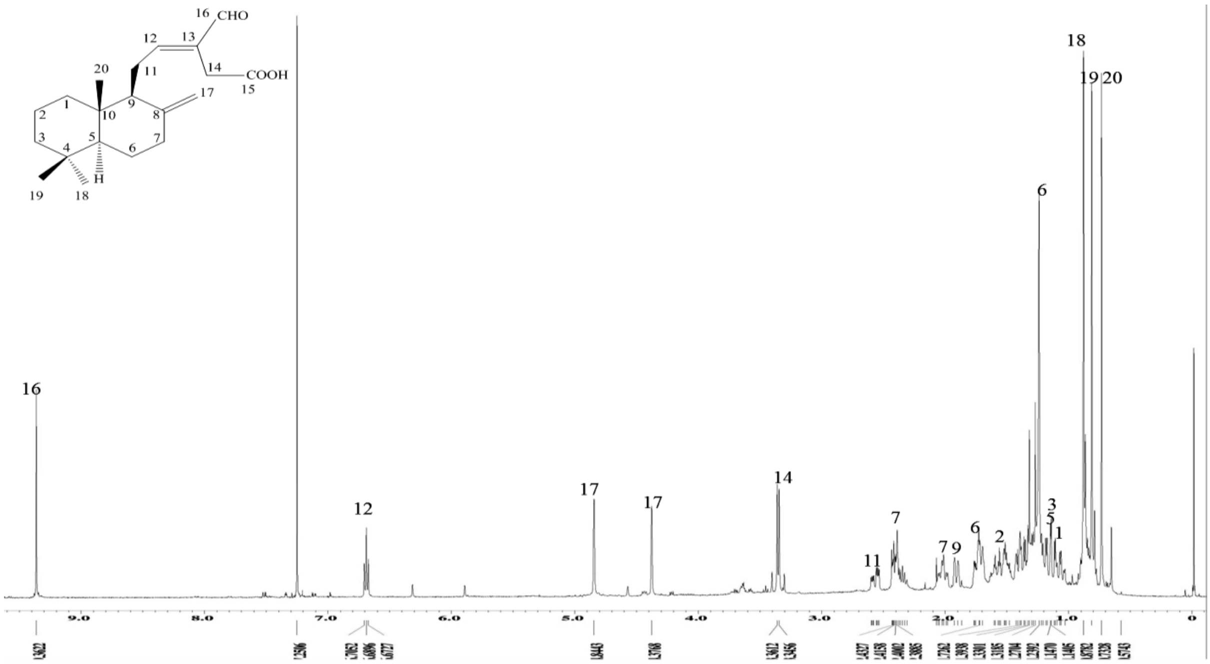

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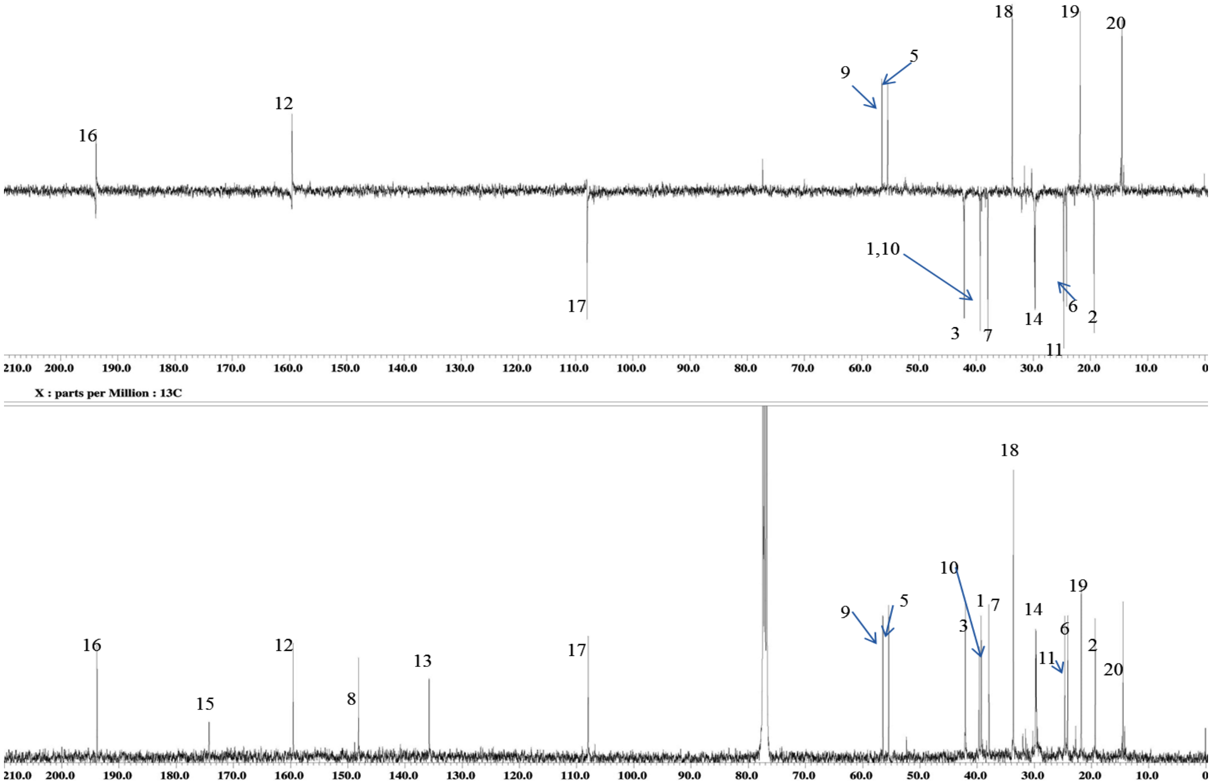

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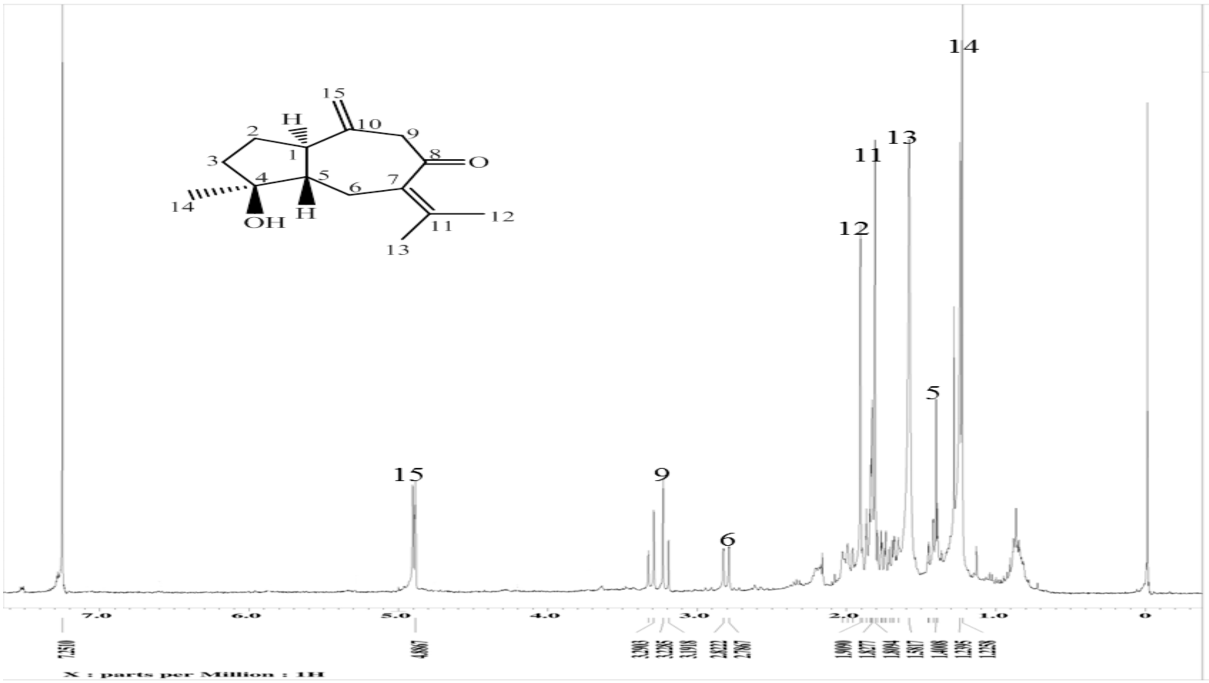

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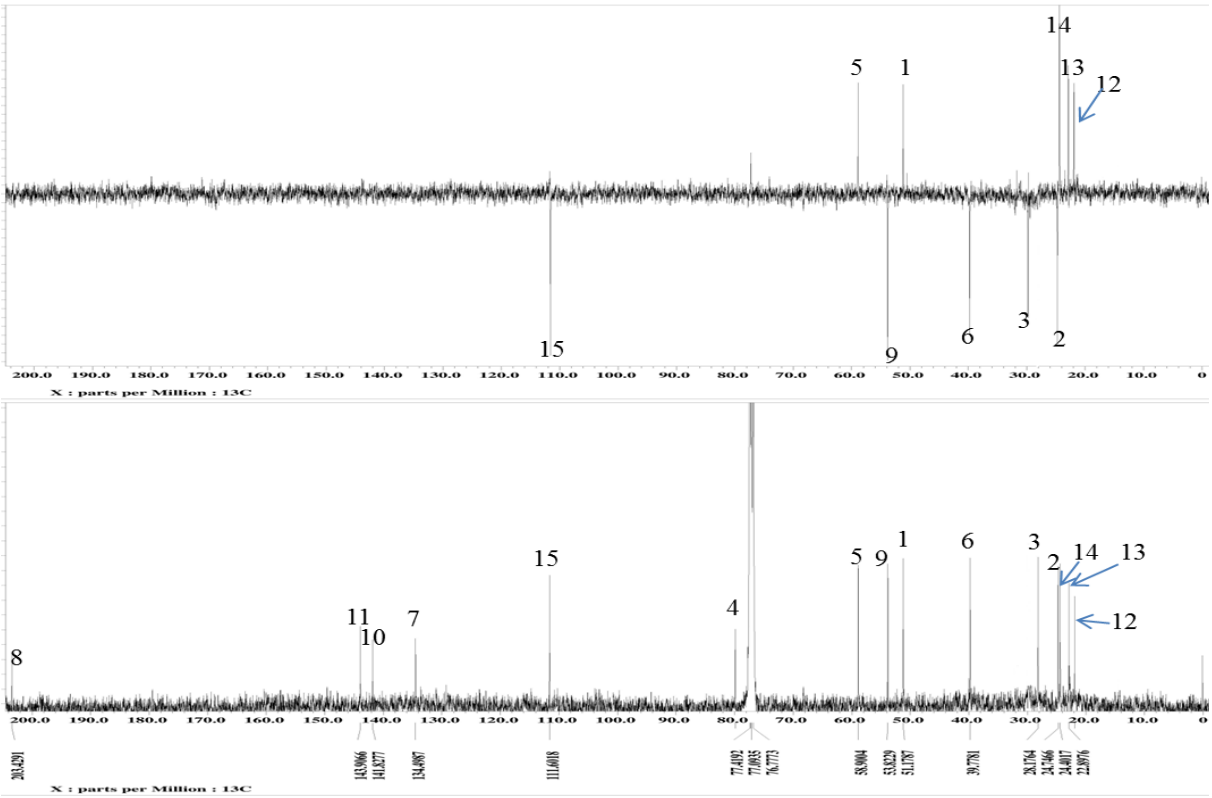

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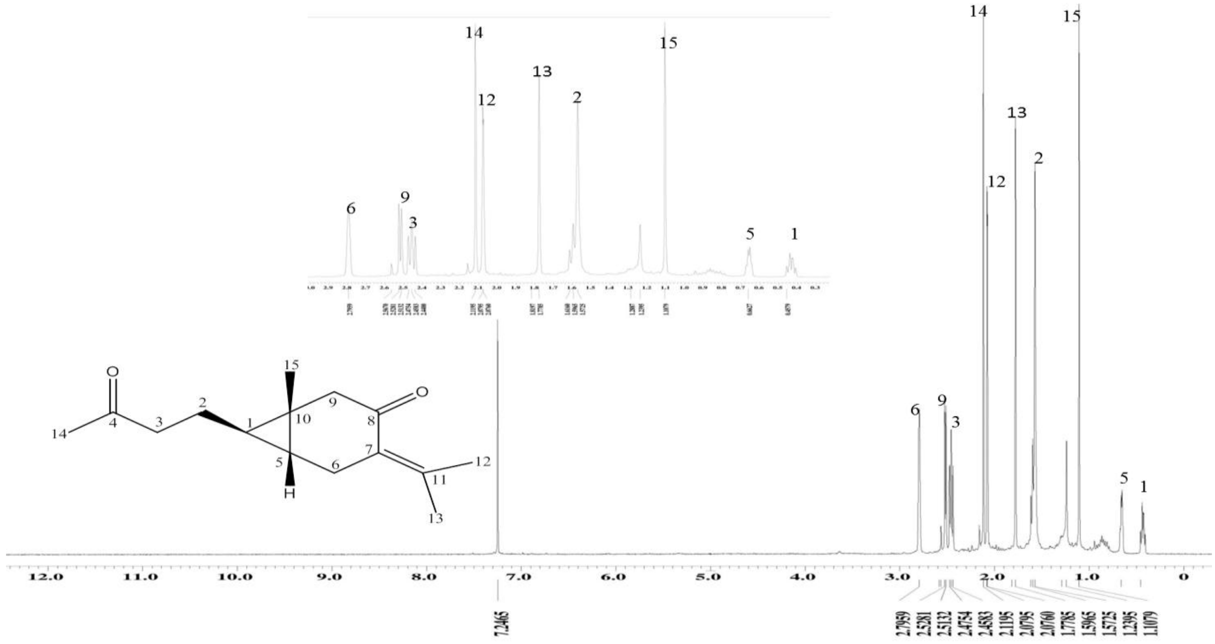

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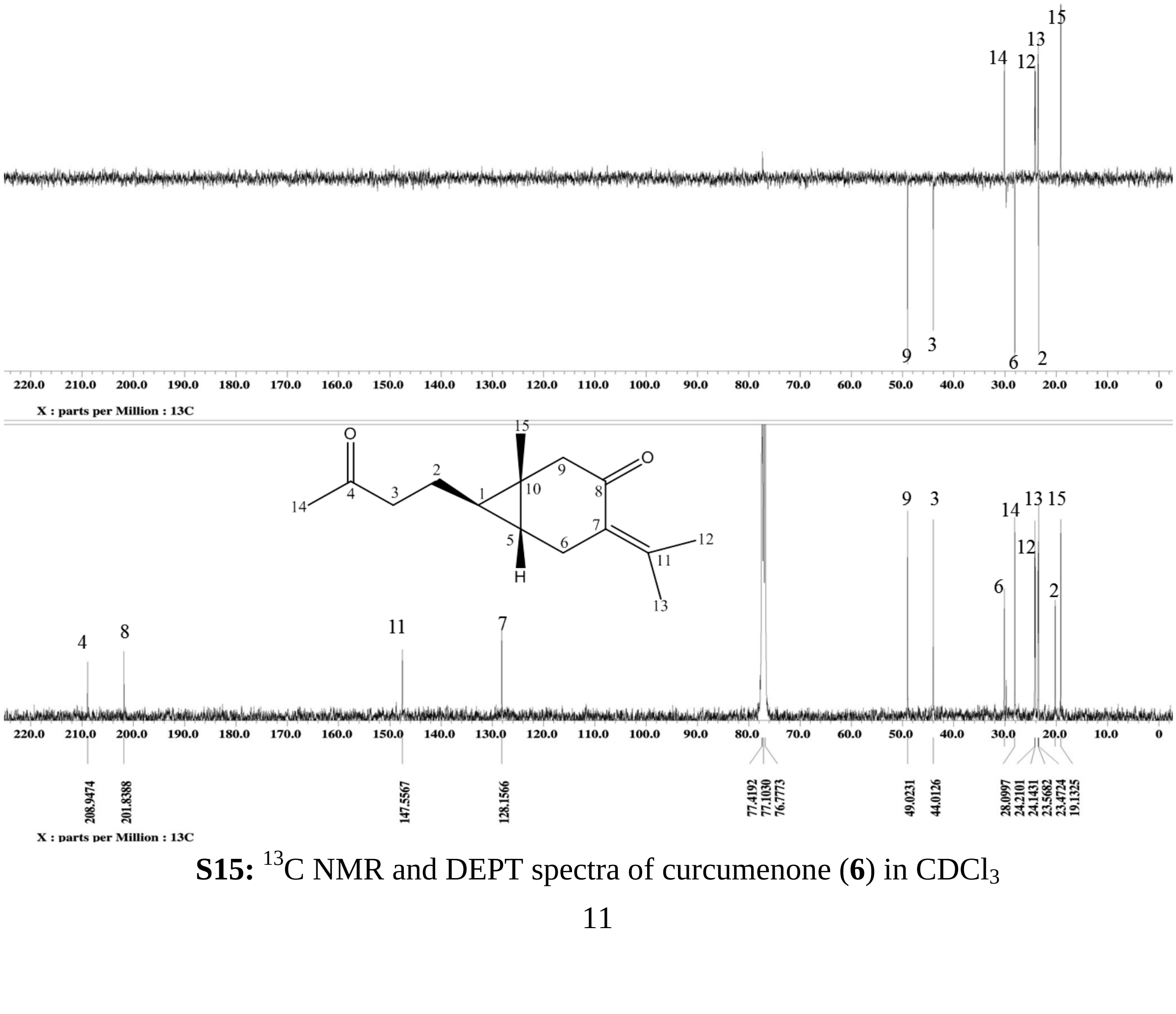

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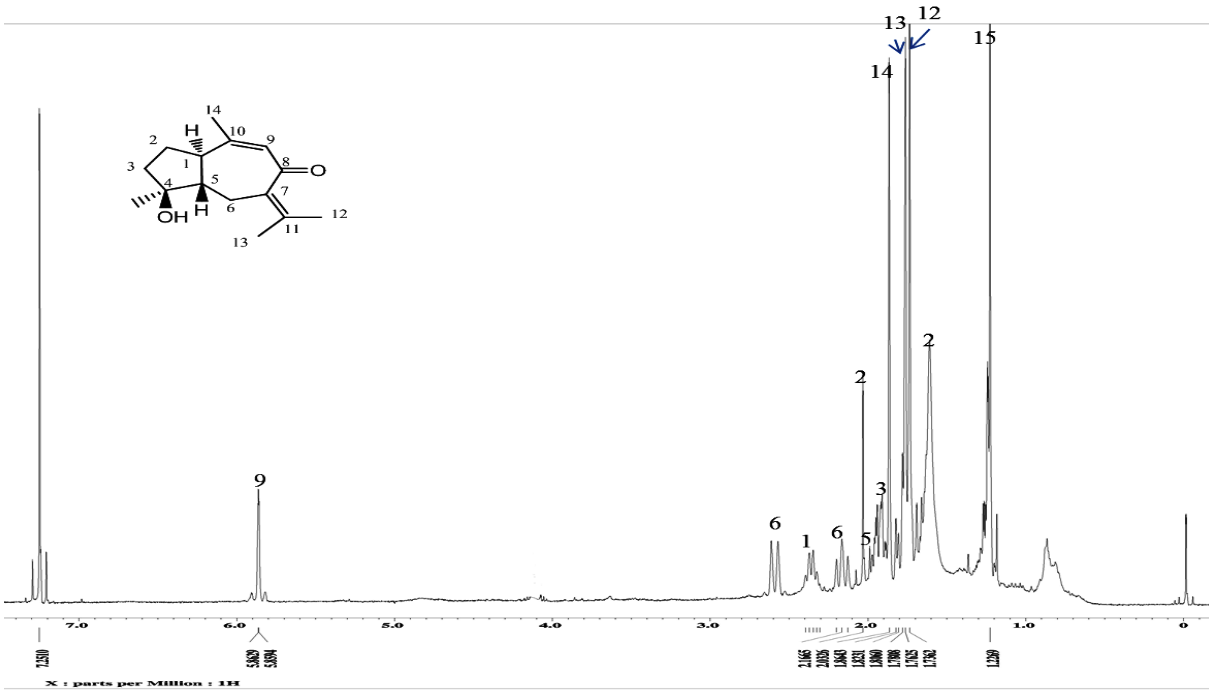
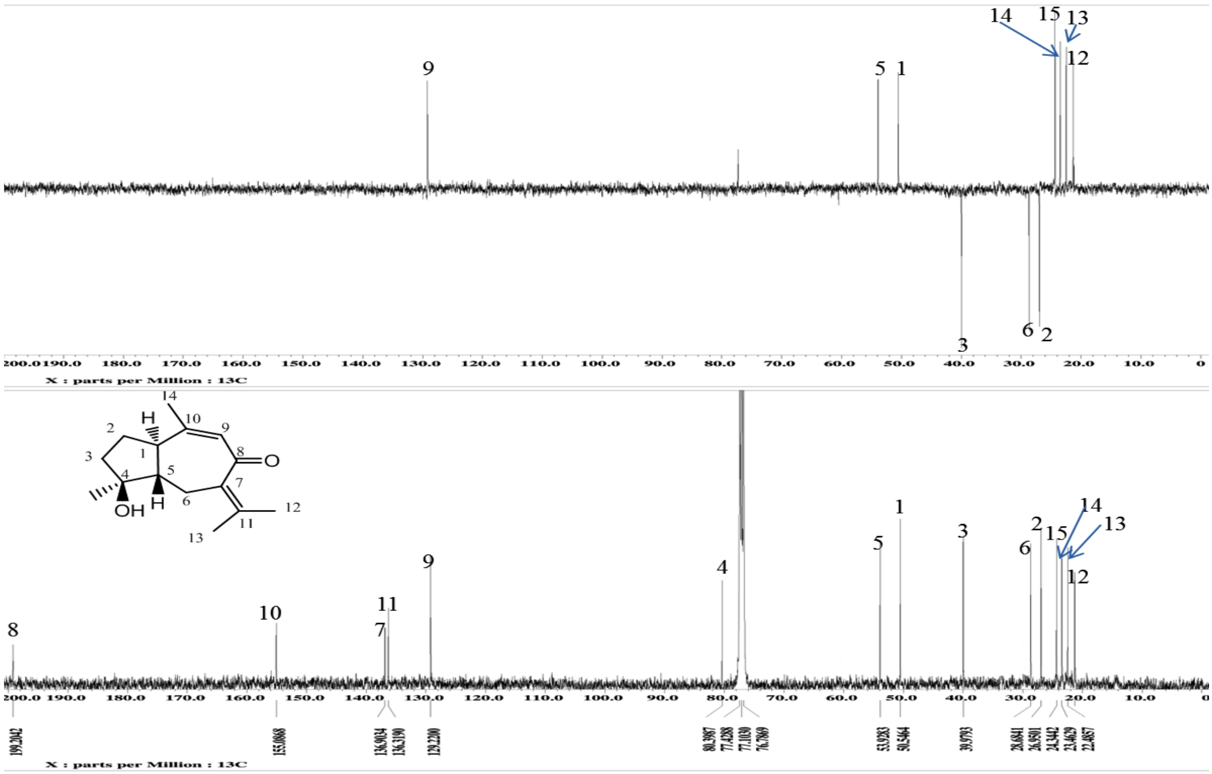


S16: ^{1}H NMR spectrum of procurcumenol (**7**) in $CDCl_3$



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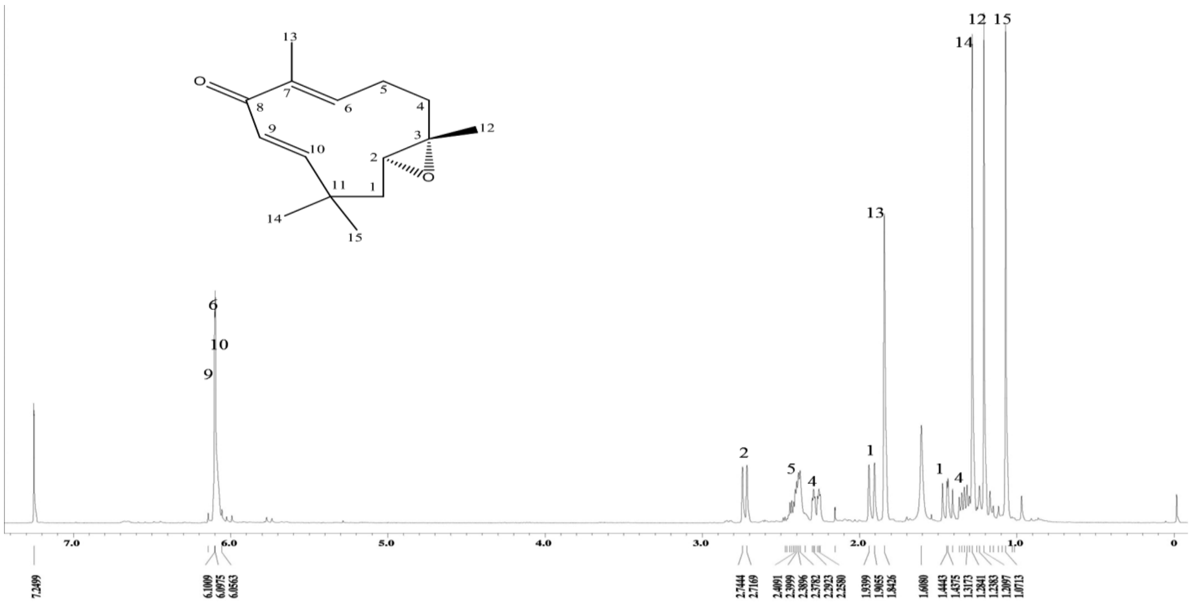
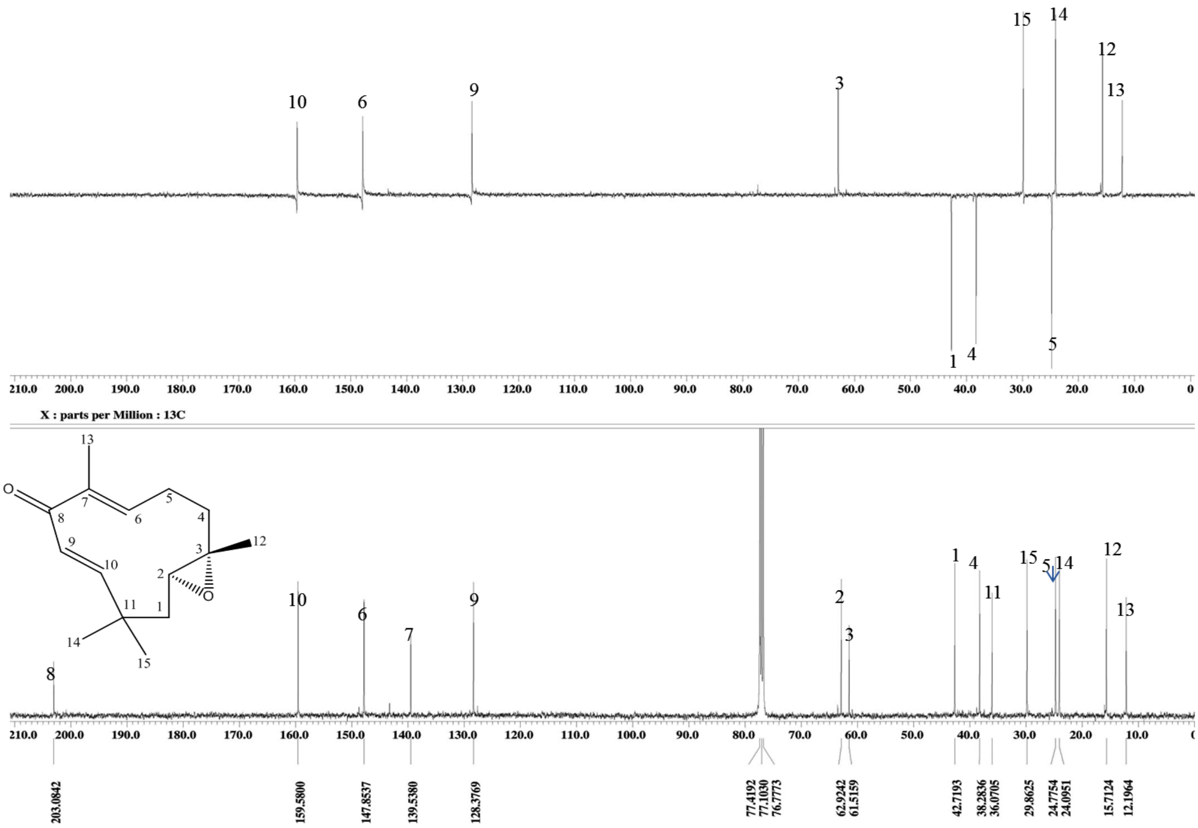
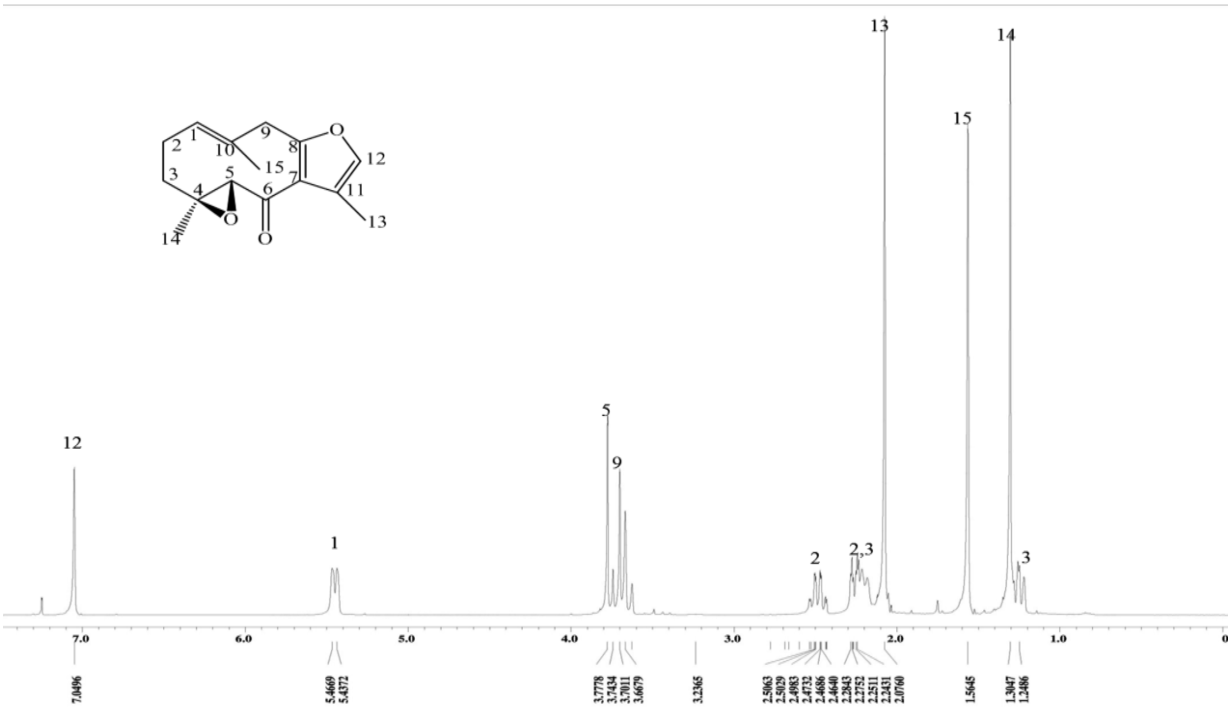


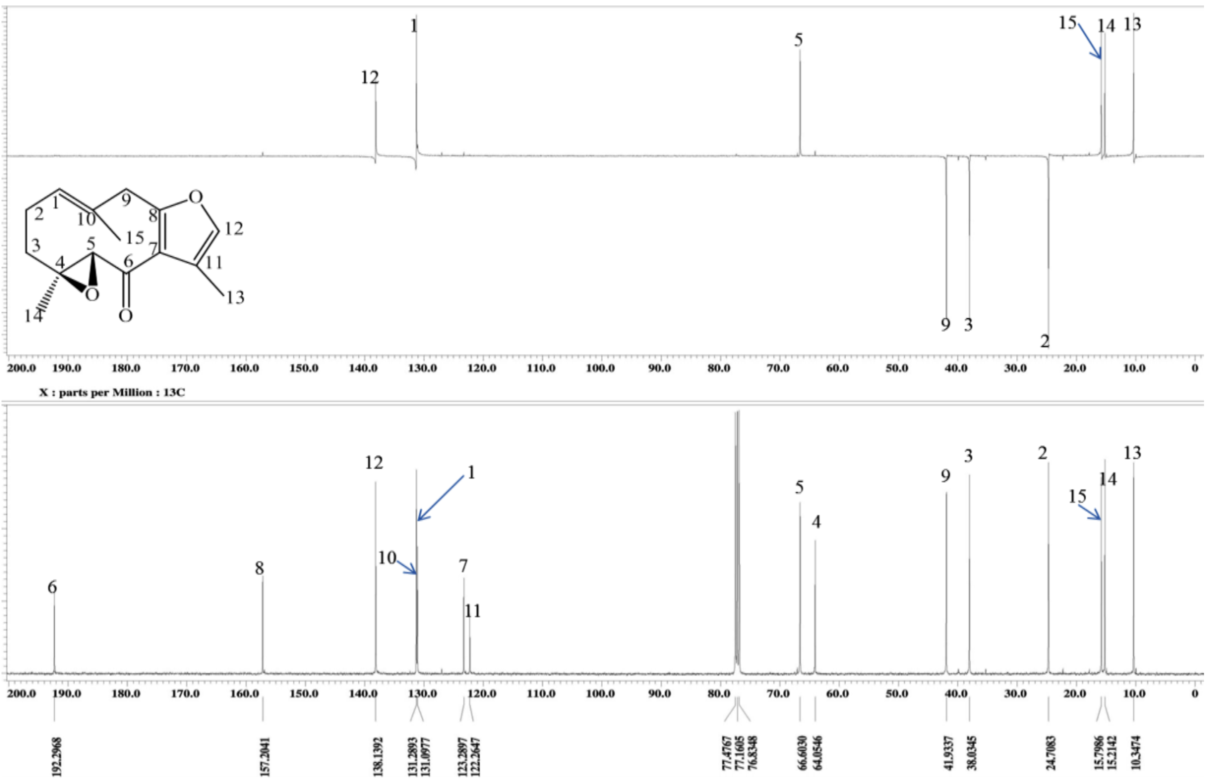
S18: ^{1}H NMR spectrum of zerumbone epoxide (**8**) in $CDCl_3$



S19: ^{13}C NMR and DEPT spectra of zerumbone epoxide (**8**) in $CDCl_3$



S20: ^{1}H NMR spectrum of zederone (**9**) in $CDCl_3$



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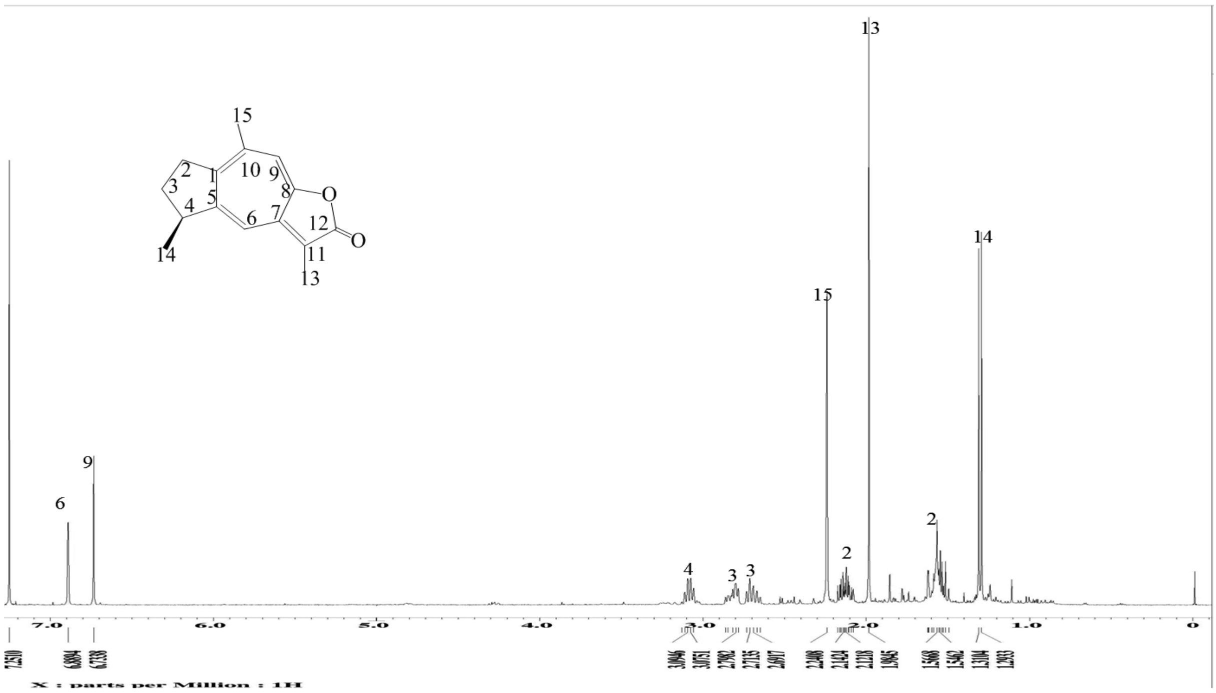

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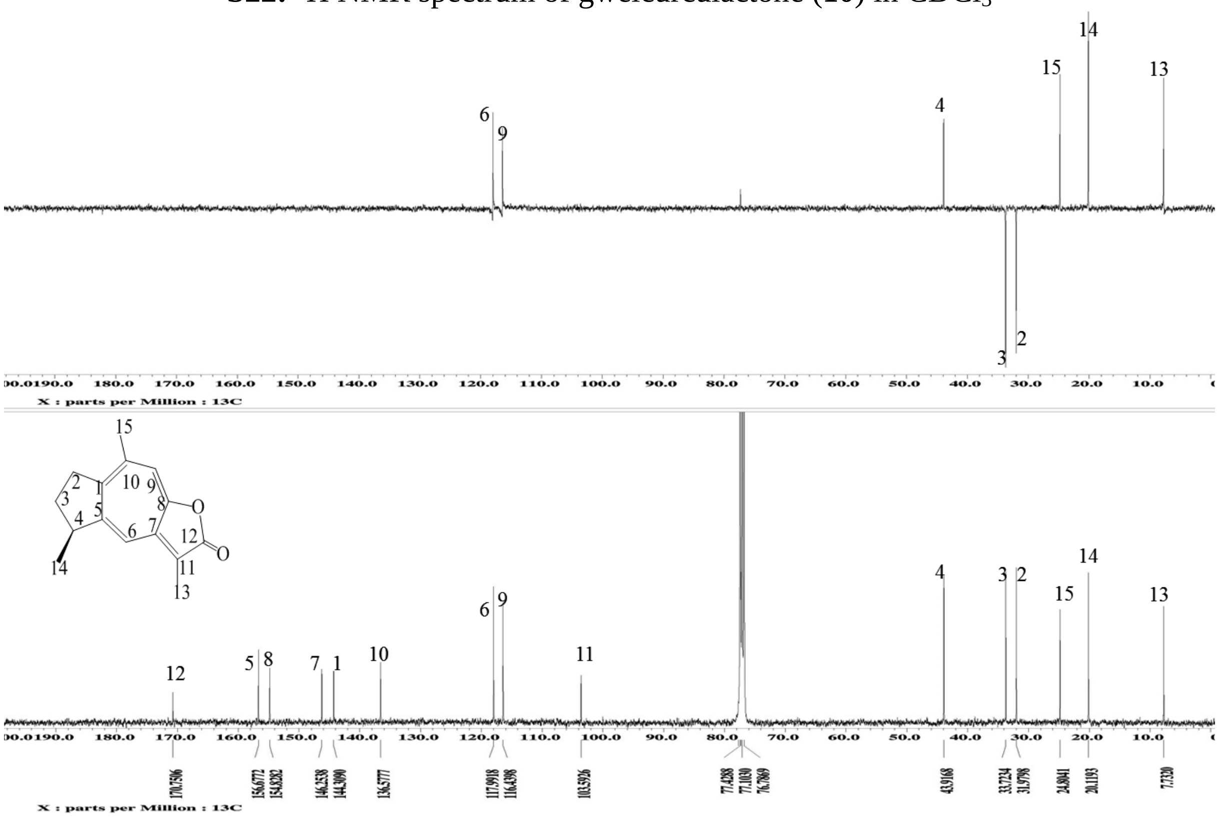

S23: ^{13}C NMR and DEPT spectra of gweicurculactone (**10**) in $CDCl_3$