Supporting Information

Alotaketal A and B, Sesterterpenoids from the Marine Sponge *Hamigera* sp. that Activate the cAMP Cell Signaling Pathway

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Experimental Section

General Experimental Procedures.

Optical rotations were measured using a Jasco P-1010 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-600 spectrometer with a 5 mm CPTCI cryoprobe. ^{1}H chemical shifts are referenced to the residual benzene-d_6 signal (δ 7.16 ppm) and ^{13}C chemical shifts are referenced to the benzene-d_6 solvent peak (δ 128.39 ppm). Low resolution ESI +/- were recorded on Bruker Esquire LC ion trap mass spectrometer equipped with an electrospray ion source. The solvent for ESI-MS experiments was methanol. The sample solution concentration was 10μM. It was infused into the ion source by a syringe pump at flow rate of 10 μL/min. High resolution ESI+ were recorded on a Micromass LCT time-of-flight (TOF) mass spectrometer equipped with an electrospray ion source. The samples were dissolved in MeOH. The working solutions were 20μM. Flow rate: 20μL min-1; sample cone: 90V; source temperature: 120 ºC; desolvation temperature: 120 ºC. For accurate mass measurement, arg-ser-arg was used as reference compound. The mass of arg-ser-arg was used as lock mass. Merck Type 5554 silica gel plates and Whatman MKC18F plates were used for analytical thin layer chromatography. Sephadex ™ LH-20 column packed and elueted with 100% MeOH was used for size separation chromatography. Reversed-phase HPLC purifications were performed on a Waters 600E System Controller liquid chromatography attached to a Waters 996 photodiode array detector. All solvents used for HPLC were Fisher HPLC grade.

Extraction of sponge: The frozen sponge (24g) was extracted repeatedly with MeOH (3 x 150mL) at room temperature. The combined methanolic extracts were concentrated *in vacuo* to afford 285 mg of brown solid. 38.2 mg of this extract were chromatographed on a Sephadex LH-20 column in 100% MeOH as eluent to give 3 fractions A-C (A: 15.0mg ; B: 16.0mg ; C: 7.2mg). Pure samples of alotaketal A (**2**) (5.3mg) and alotaketal B (**3**) (2.1mg) were obtained from fraction B (16.0mg) via C_{18} reversed-phase HPLC using a CSC-Inertsil 150A/ODS2, 5 μm 25 x 0.94 cm column, with 8:2 Acetonitrile/H_2O as eluent over 50 min (flow rate 2 mL/min).

Alotaketal A (**2**): Isolated as a white amorphous solid; $[\alpha]^{25}_{D}$ = -38.9 (c 0.01, MeOH); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; positive ion HRESIMS $[M+Na]^{+}$ *m/z* 421.2355 (calcd for $C_{25}H_{34}O_4Na$).

Alotaketal B (**3**): Isolated as a white amorphous solid; $[\alpha]^{25}_{D}$ = -10.0 (c 0.01, MeOH); ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 2; positive ion HRESIMS $[M+Na]^{+}$ *m/z* 523.3036 (calcd for $C_{30}H_{44}O_6Na$).

Table 1. NMR data for alotaketal A (**2**). recorded in C_6D_6 at 600 MHz

Position #	**^{1}H(δ)[a]**	**^{13}C (δ)[a]**	**HMBC [a b]**
1	4.36 (1H, dd, J = 4.9, 6 Hz)	63.6	3, 5, 9
2	6.32 (1H, dq, *J* = 6, 1.2 Hz)	139.6	1, 3, 4, 6
3		139.0[c]	
4		197.7	
5a	2.47 (1H, dd, *J* = 4.9, 15.6 Hz)	38.6	1, 4, 6
5b	2.39 (1H, dd, *J* = 13.2, 15.6 Hz)	38.6	1, 4, 6, 7
6	2.06 (1H, dt, *J* = 4.9, 13.2 Hz)	34.0	5, 7
7		142.5	
8	5.56 (1H, s)	125.1	6, 7, 9, 10, 22
9		97.2	
10a	2.34 (1H, d, *J* = 13.2 Hz)	44.1	8, 9, 11, 12, 23
10b	2.29 (1H, d, *J* = 13.2 Hz)	44.1	8, 9, 11, 12, 23
11		141.5	
12a	2.31 (1H,m)	40.7	10, 11, 13, 14, 23
12b	2.20 (1H, t, *J* = 12.6 Hz)	40.7	10, 11, 13, 14, 23
13	4.85 (1H, ddd, *J* = 3, 8.4, 12.6)	68.5	9, 12, 14, 15
14	5.48 (1H, d, *J* = 8.4 Hz)	126.8	13, 16, 24
15		139.2[c]	
16	1.98 (2H, t, *J* = 7.2 Hz)	39.7	14, 15, 17, 18, 24
17	1.55 (2H, q, *J* = 7.2 Hz)	26.3	15, 16, 18, 19
18	1.94 (2H, t, *J* = 7.2 Hz)	38.0	16, 17, 19, 20, 25
19		145.9	
20	4.80 (1H, bs)/4.81 (1H, bs)	110.9	18, 19, 25
21	1.71 (3H, s)	16.4	2, 3, 4
22	3.53 (2H, m)	63.8	6, 7, 8
22-OH	0.53 (1H, t, *J* = 5.4 Hz)		7, 22
23	4.87 (1H, bs)/ 4.89 (1H, bs)	111.4	10, 11, 12
24	1.68 (3H, s)	17.1	14, 15, 16
25	1.62 (3H, s)	22.8	18, 19, 20

[a] Spectra recorded in C_6D_6 at 600 MHz

[b] Proton resonances correlated to the carbon resonances listed in the δ ^{13}C NMR column

[c] May be interchanged

Table 2. NMR data for alotaketal B (**3**). recorded in C_6D_6 at 600 MHz

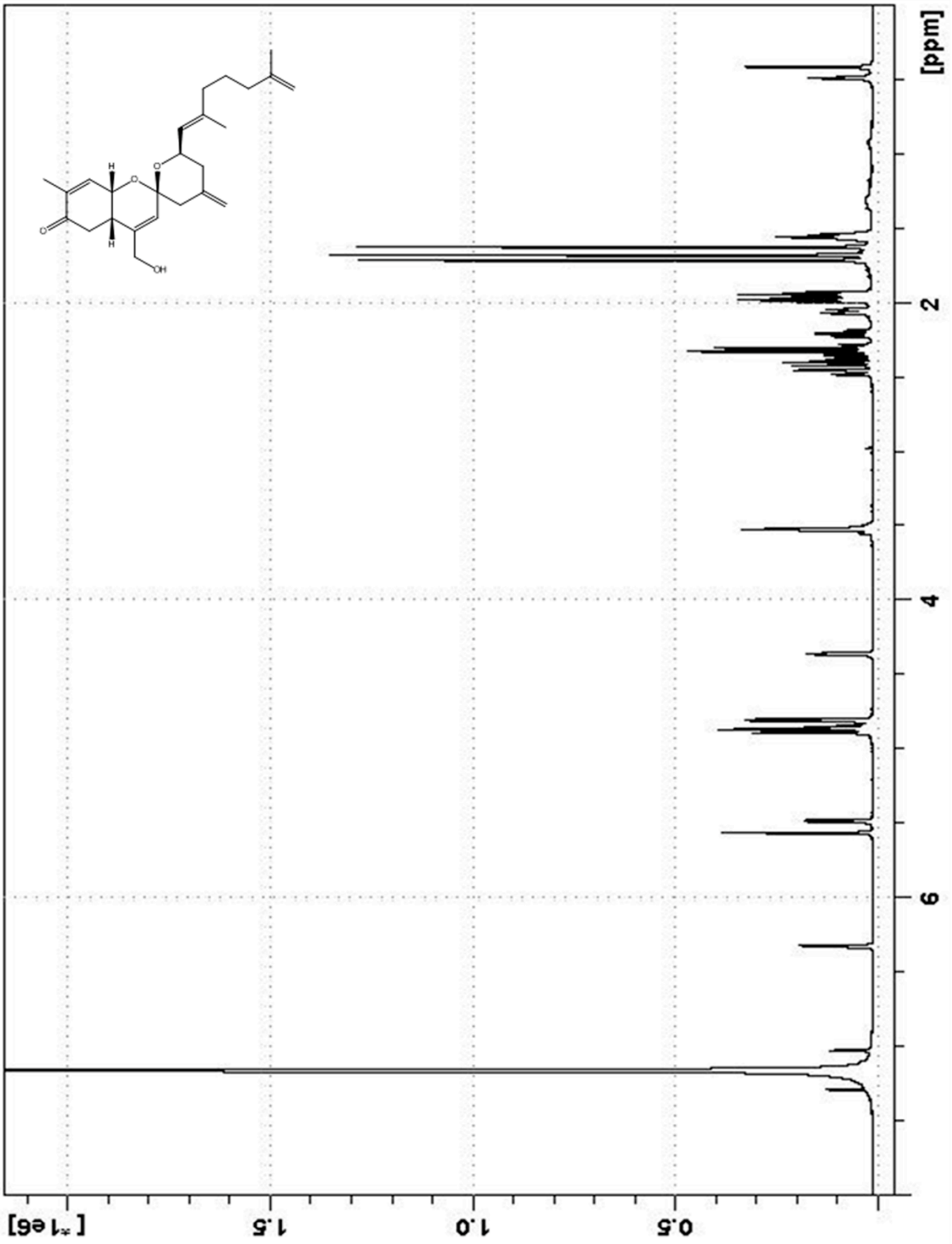
Position #	**^{1}H(δ)[a]**	**^{13}C (δ)[a]**	**HMBC [a b]**
1	4.33 (1H, bs,)	63.4	2
2	6.32 (1H, d, *J* = 6 Hz)	139.6	4, 6, 21
3		138.9[c]	
4		197.7	
5	2.37 (2H, bd, *J* = 7.8 Hz)	38.4	1, 4, 6, 7
6	2.00 (1H, m)	34.0	2, 5, 7
7		142.5	
8	5.43 (1H, s)	125.3	6, 9, 22
9		96.9	
10a	3.07 (1H, d, *J* = 15 Hz)	40.5	9, 11, 12
10b	1.23 (1H, d, *J* = 15 Hz)	40.5	8, 9, 23
11		77.4	
12a	1.95 (1H, m)	43.9	11, 23
12b	1.24 (1H, m)	43.9	13
13	5.15 (1H, t, *J* = 10.8 Hz)	63.9	15
14	5.45 (1H, d, *J* = 8.4 Hz)	126.5	12, 16, 24
15		139.1[c]	
16	2.01 (2H, t, *J* = 7.2 Hz)	39.7	14, 15, 17, 18
17	1.58 (2H, q, *J* = 7.2 Hz)	26.4	15, 16, 18, 19
18	1.96 (2H, t, *J* =7.2 Hz)	38.0	16, 17, 19, 20, 25
19		145.8	
20	4.81 (1H, bs)/4.82(1H, bs)	110.9	18, 25
21	1.83 (3H, s)	16.6	2, 4
22	3.51 (2H, m)	63.7	7, 8
22-OH	0.51 (1H, t, *J* = 5.4 Hz)		
23	1.48 (3H, s)	27.6	10, 11, 12
24	1.77 (3H, s)	17.2	14, 15, 16
25	1.63 (3H, s)	22.79	18, 19, 20
26		172.4	
27	2.12 (2H, m)	45.0	26, 29,30
28	2.12 (1H, m)	25.6	26, 27, 29, 30
29	0.89 (3H, d, J = 5.4 Hz)	23.1	27, 28, 30
30	0.89 (3H, d, J = 5.4 Hz)	23.1	27, 28, 29

[a] Spectra recorded in C_6D_6 at 600 MHz

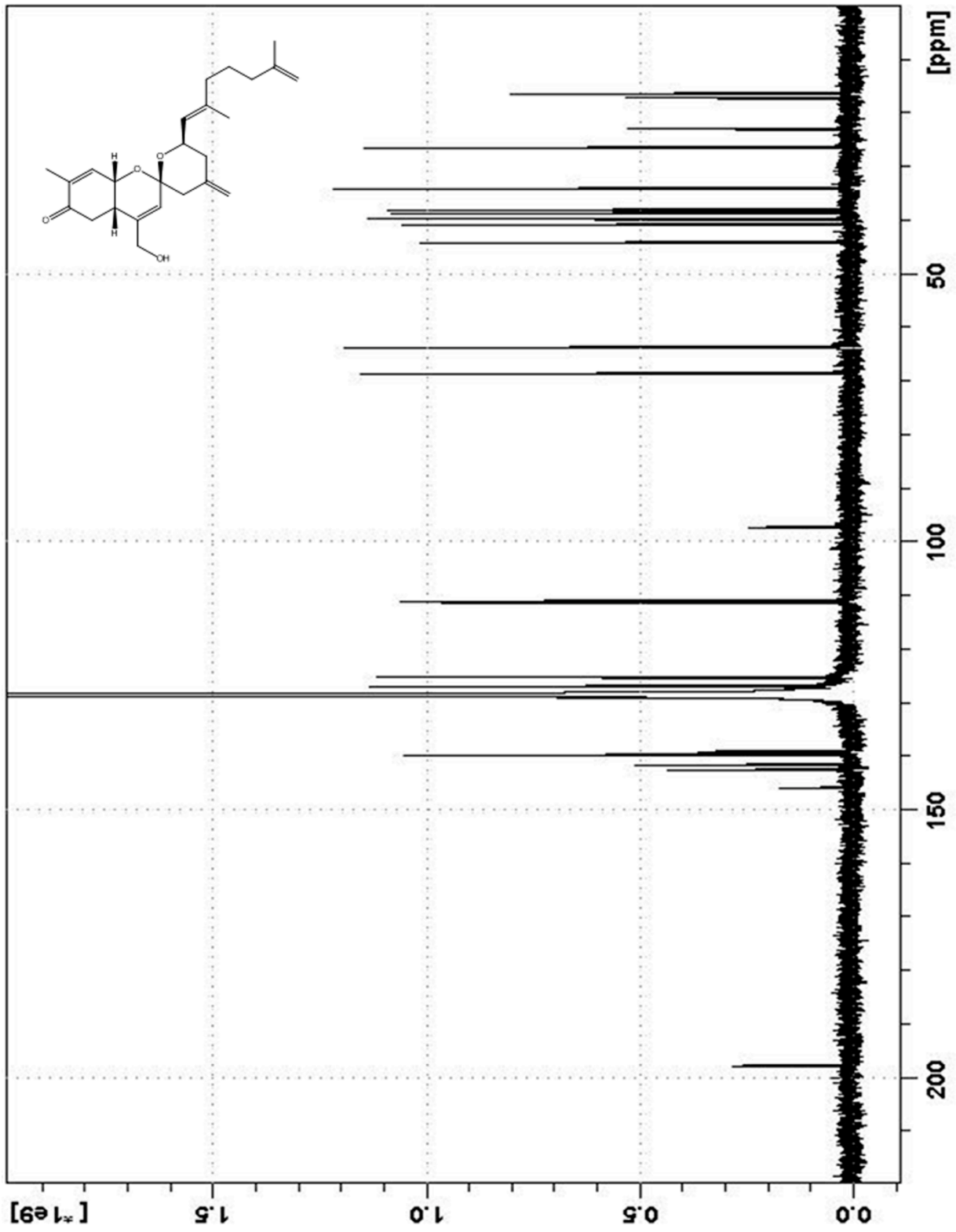
[b] Proton resonances correlated to the carbon resonances listed in the δ ^{13}C NMR column

[c] May be interchanged

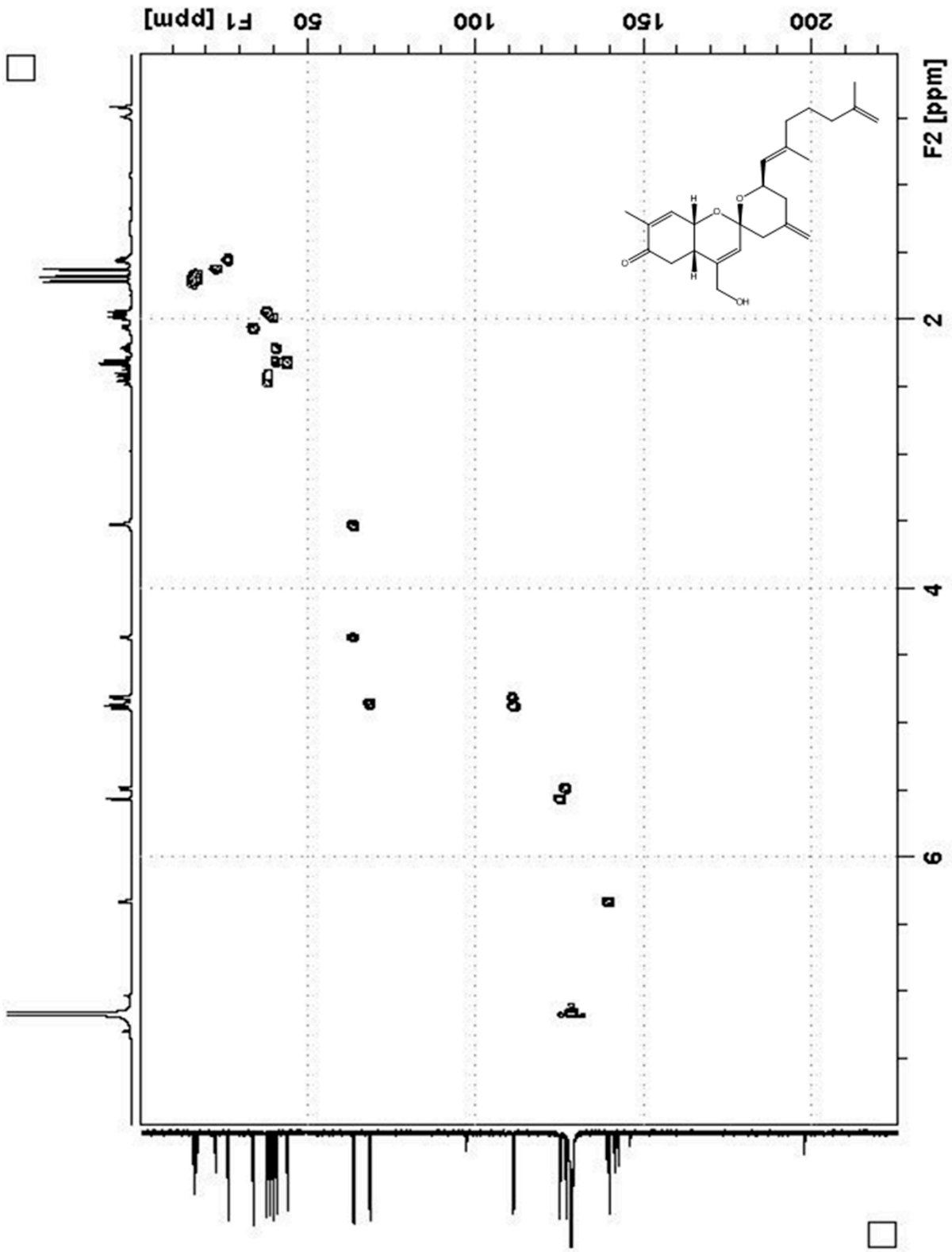
^{1}H NMR spectrum of alotaketal A (**2**) recorded in C_6D_6 at 600 MHz



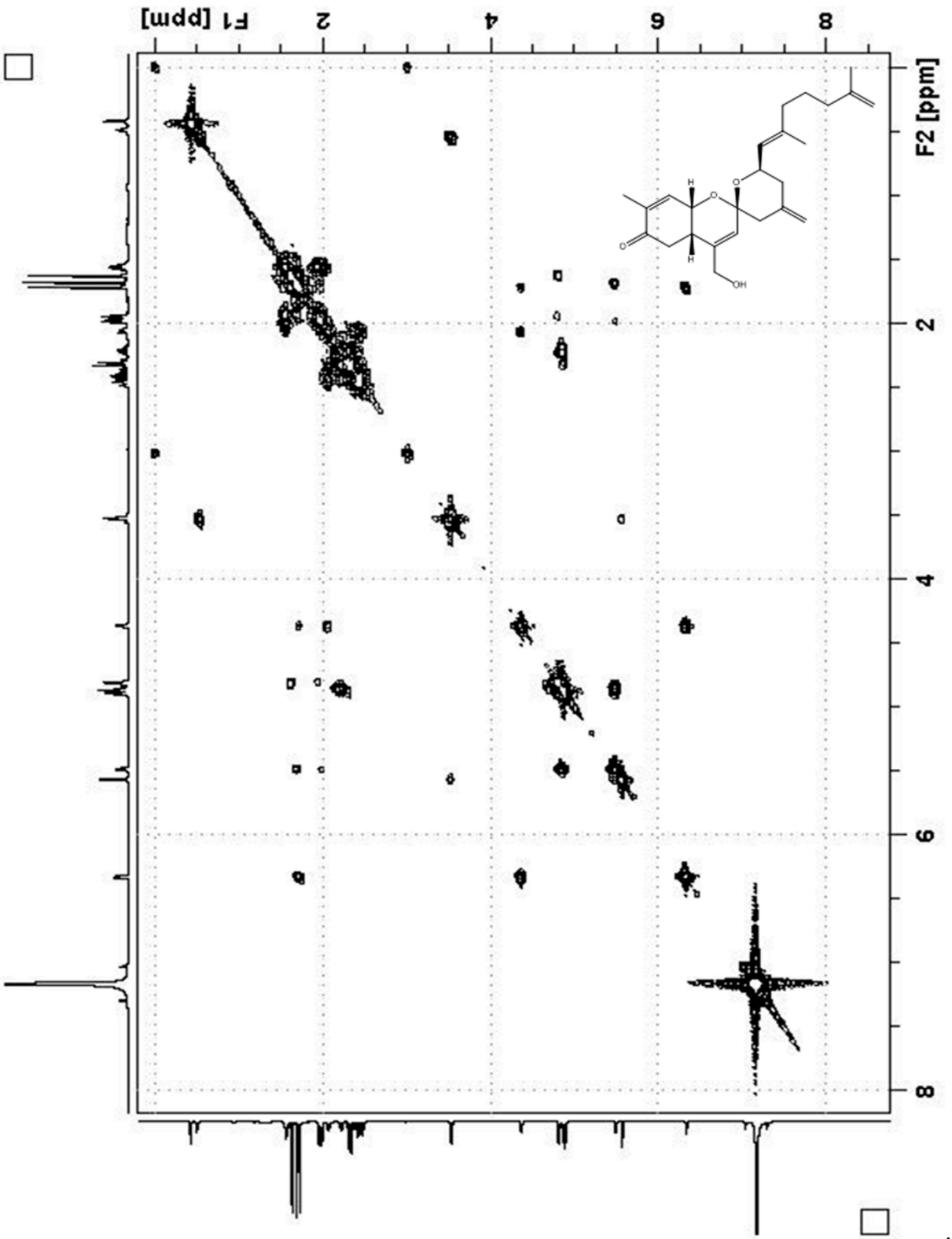
^{13}C NMR spectrum of alotaketal A (**2**) recorded in C_6D_6 at 600 MHz



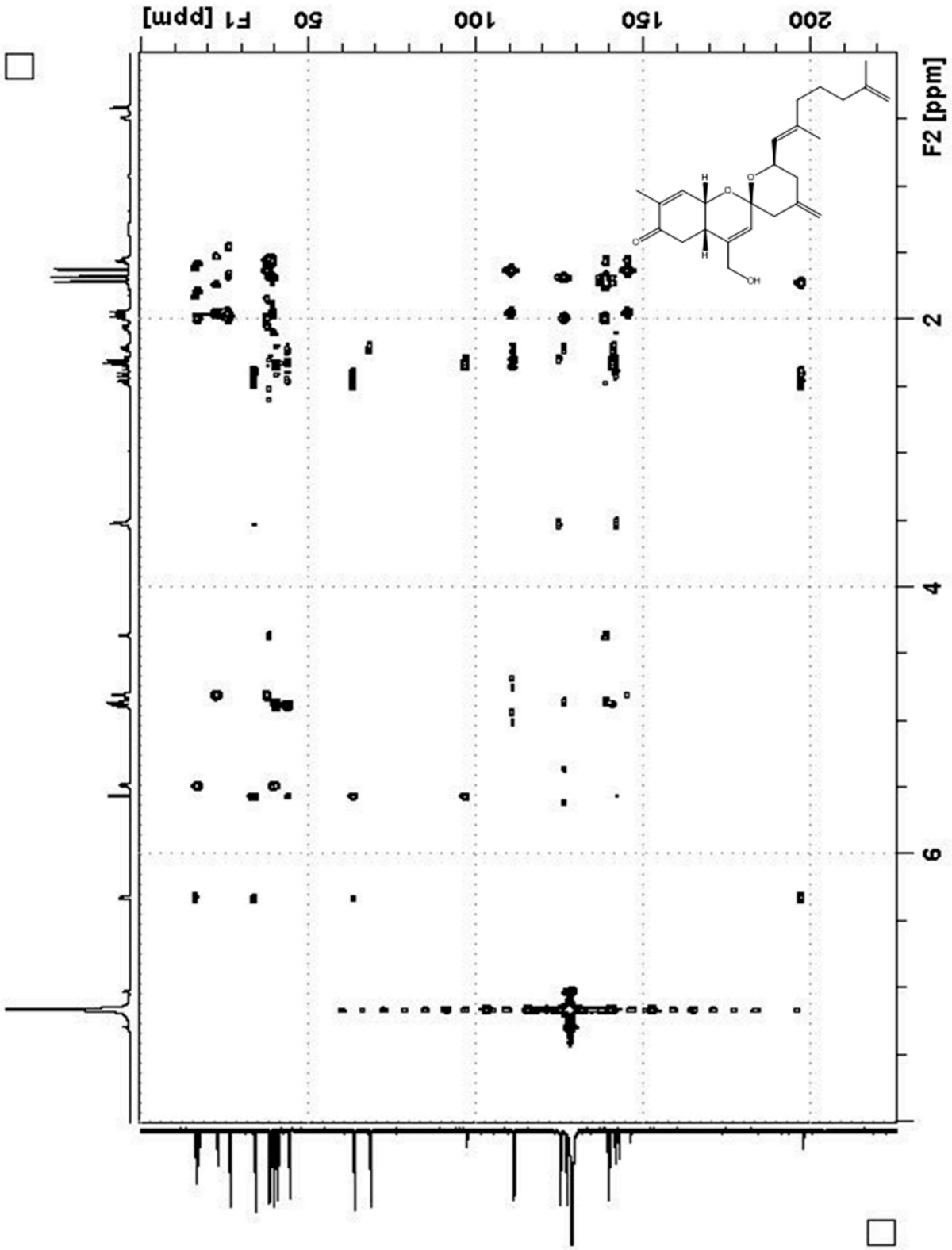
HSQC spectrum of alotaketal A (**2**) recorded in C_6D_6 at 600 MHz



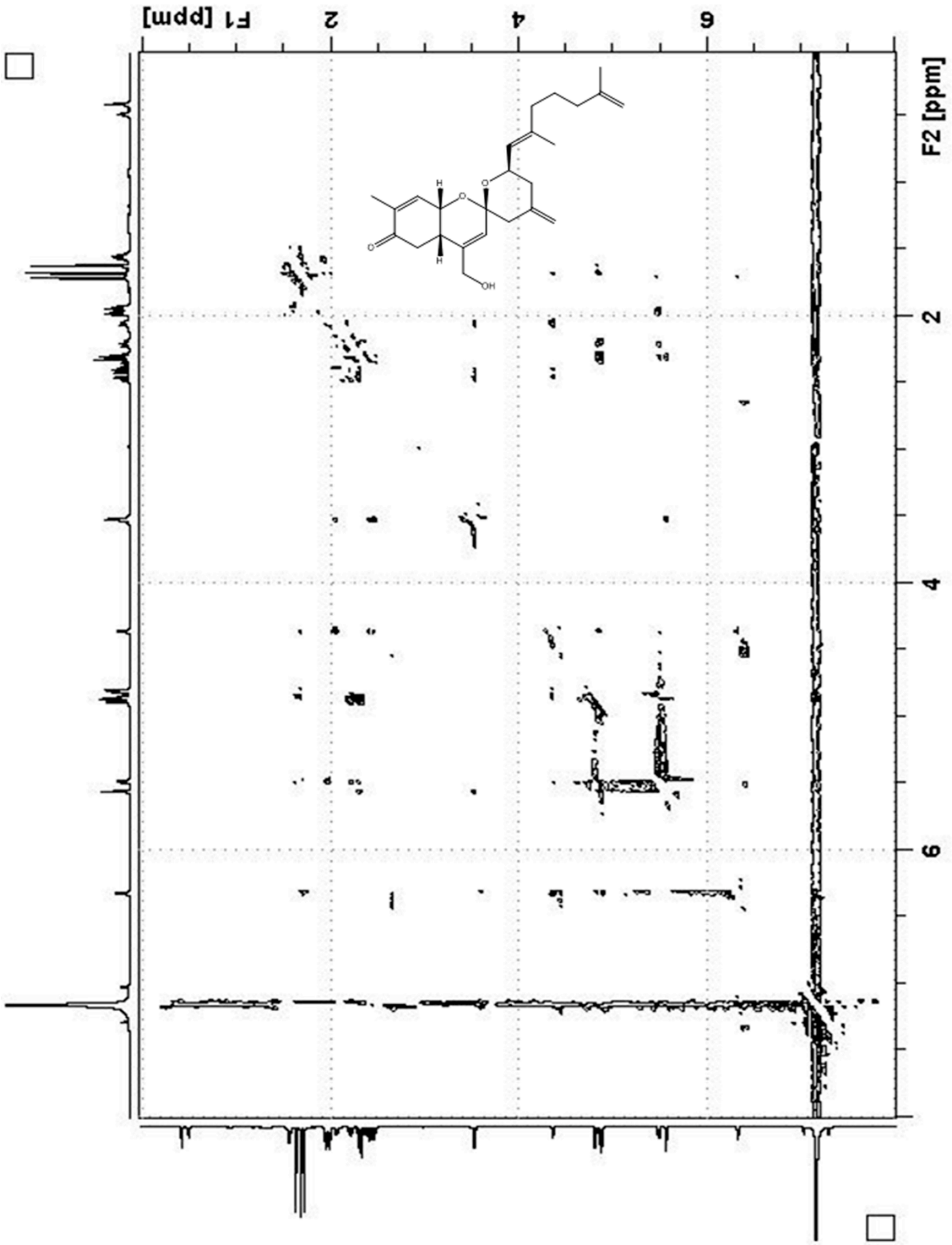
COSY spectrum of alotaketal A (**2**) recorded in C_6D_6 at 600 MHz



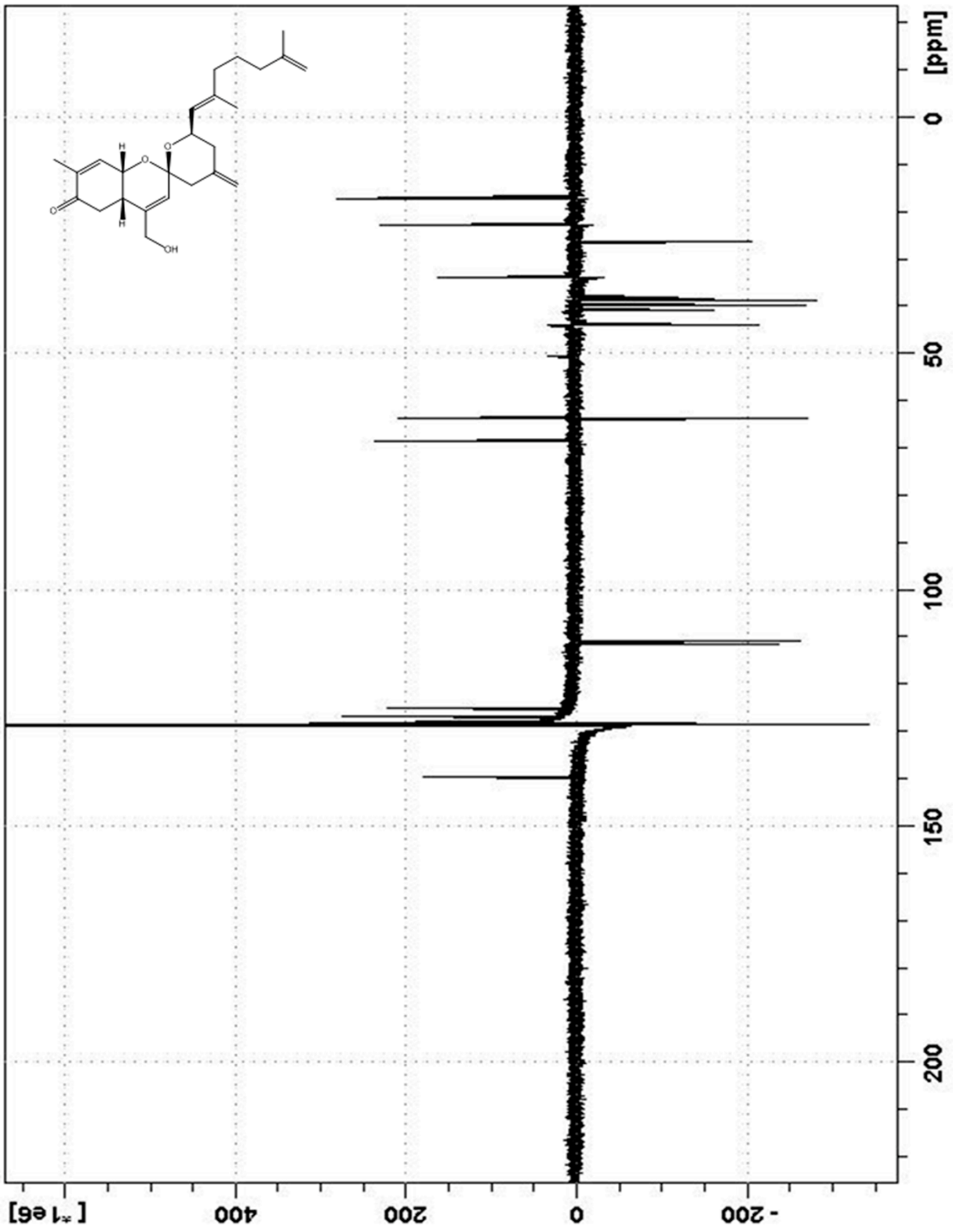
HMBC spectrum of alotaketal A (**2**) recorded in C_6D_6 at 600 MHz



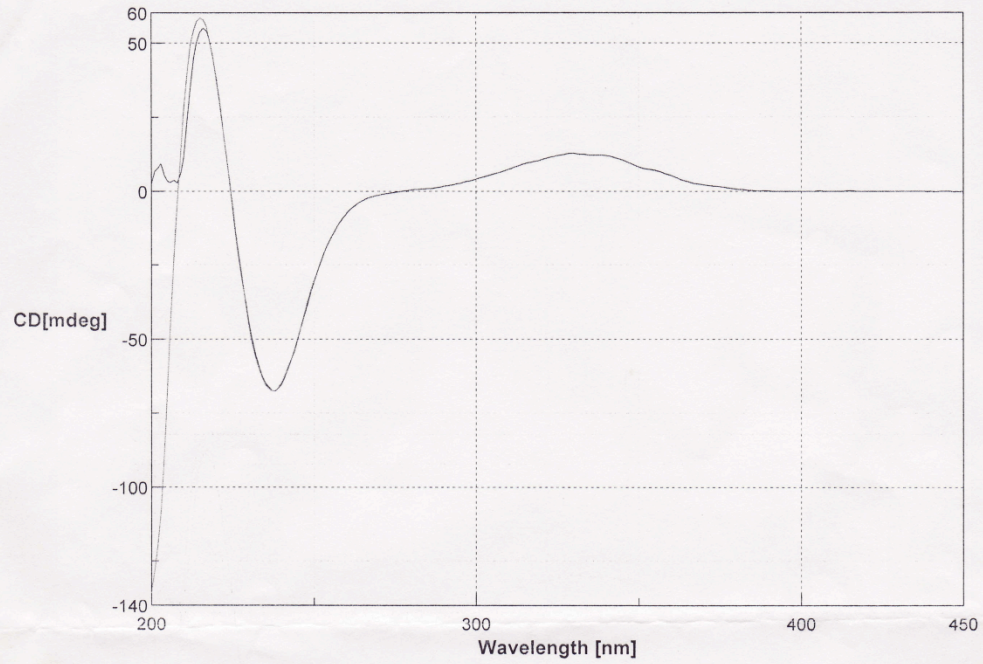
ROESY spectrum of alotaketal A (**2**) recorded in C_6D_6 at 600 MHz



DEPT 135º spectrum of alotaketal A (**2**) recorded in C_6D_6 at 600 MHz



CD spectrum of alotaketal A (**2**) recorded in MeOH.



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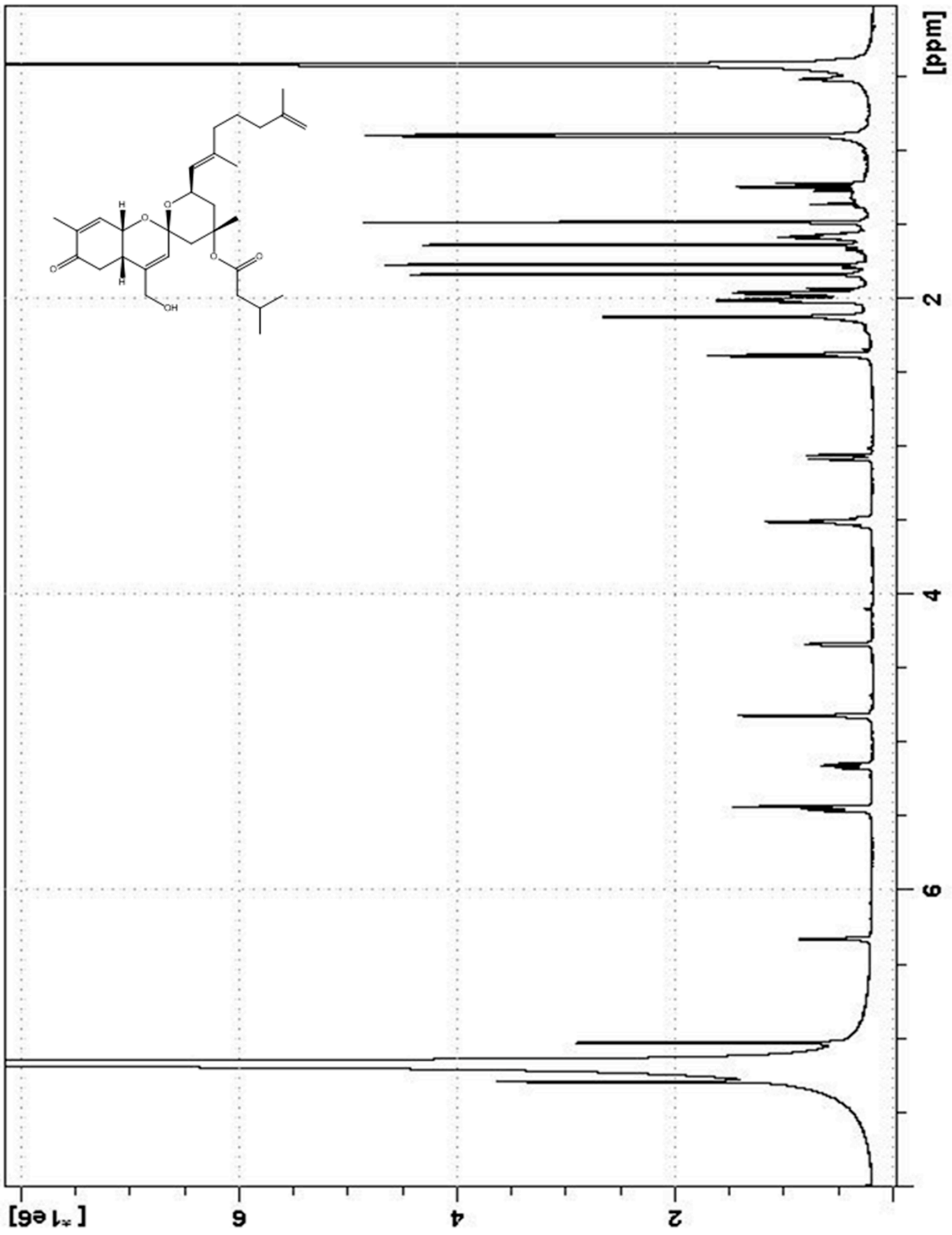
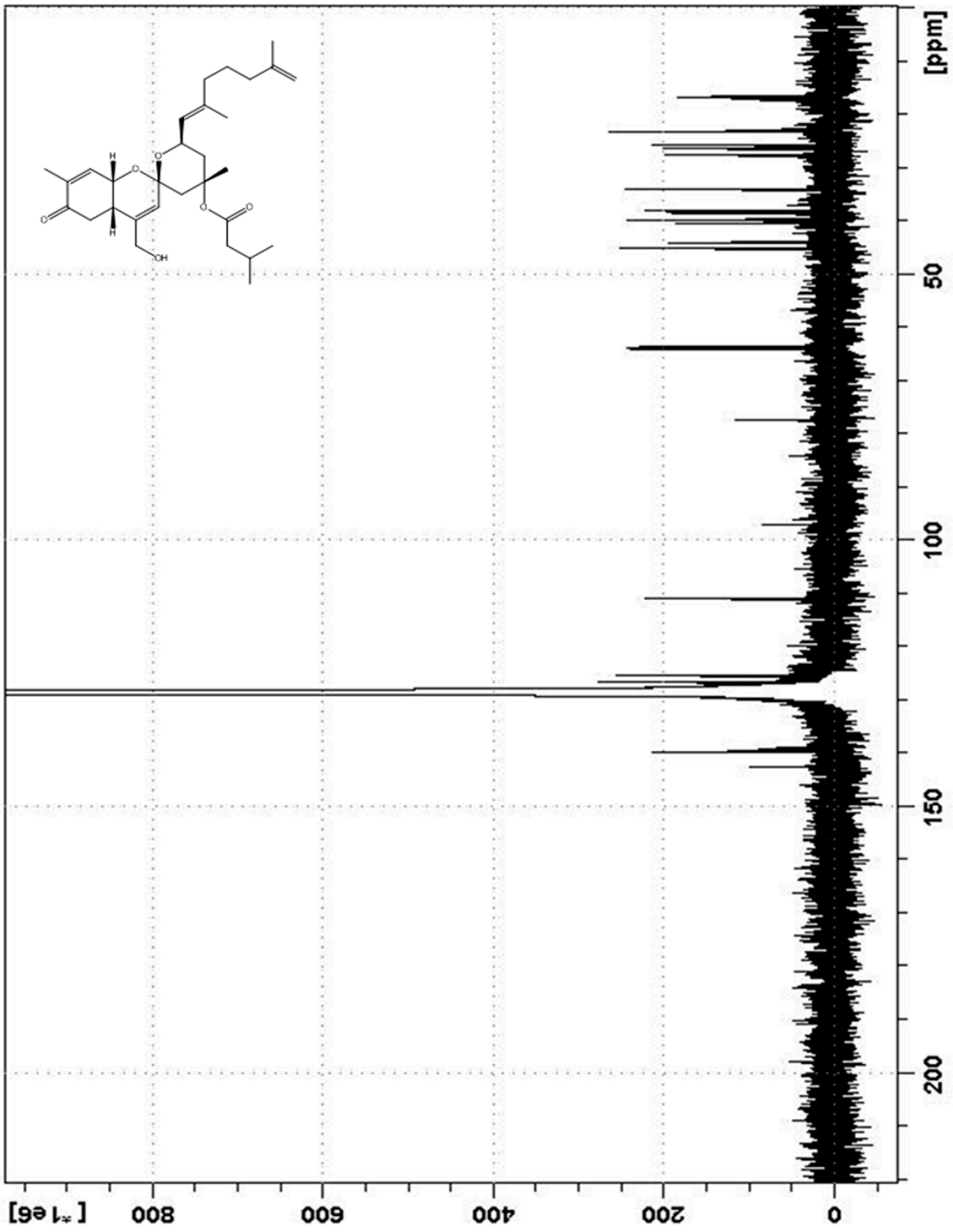
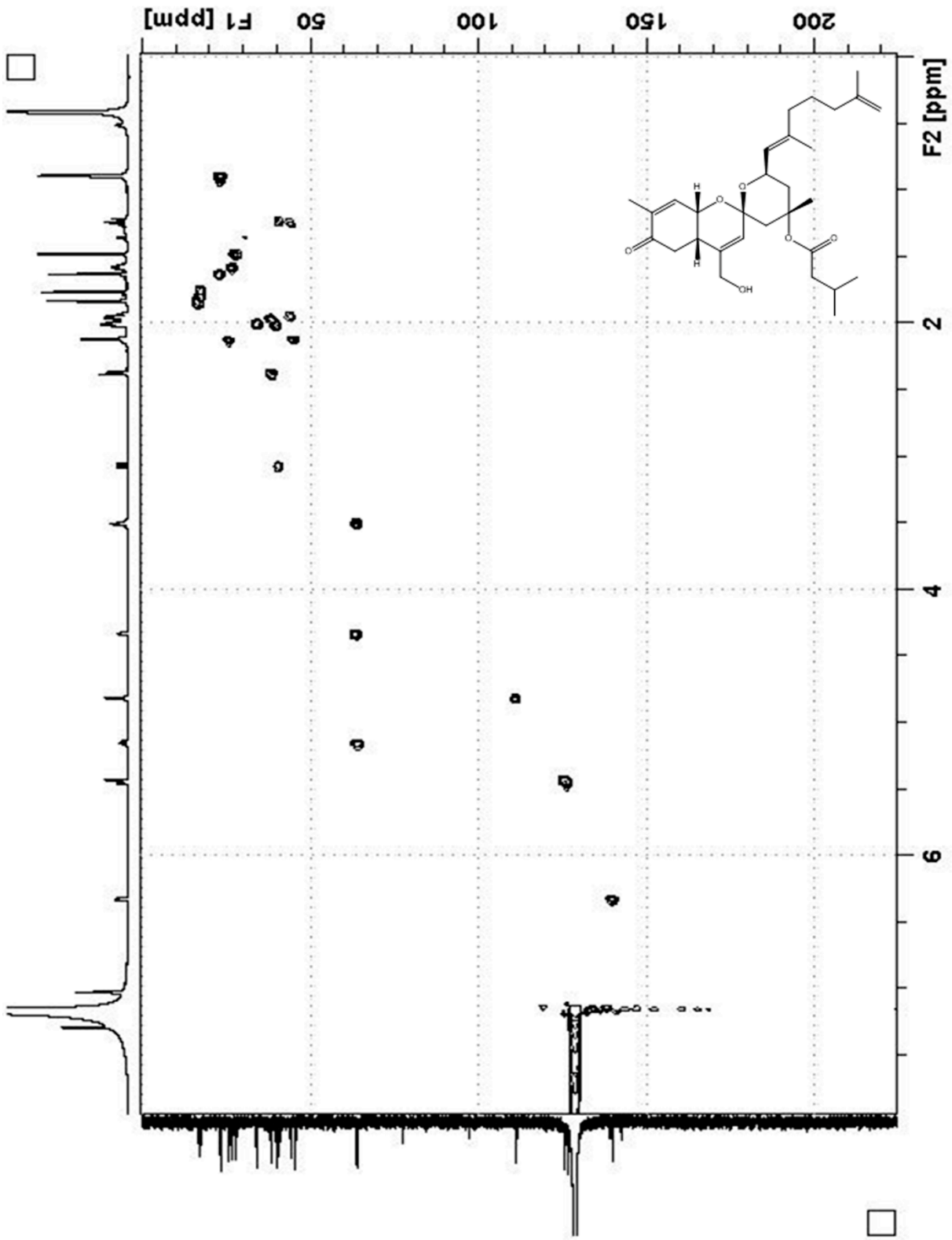
^{1}H NMR spectrum of alotaketal B (**3**) recorded in C_6D_6 at 600 MHz



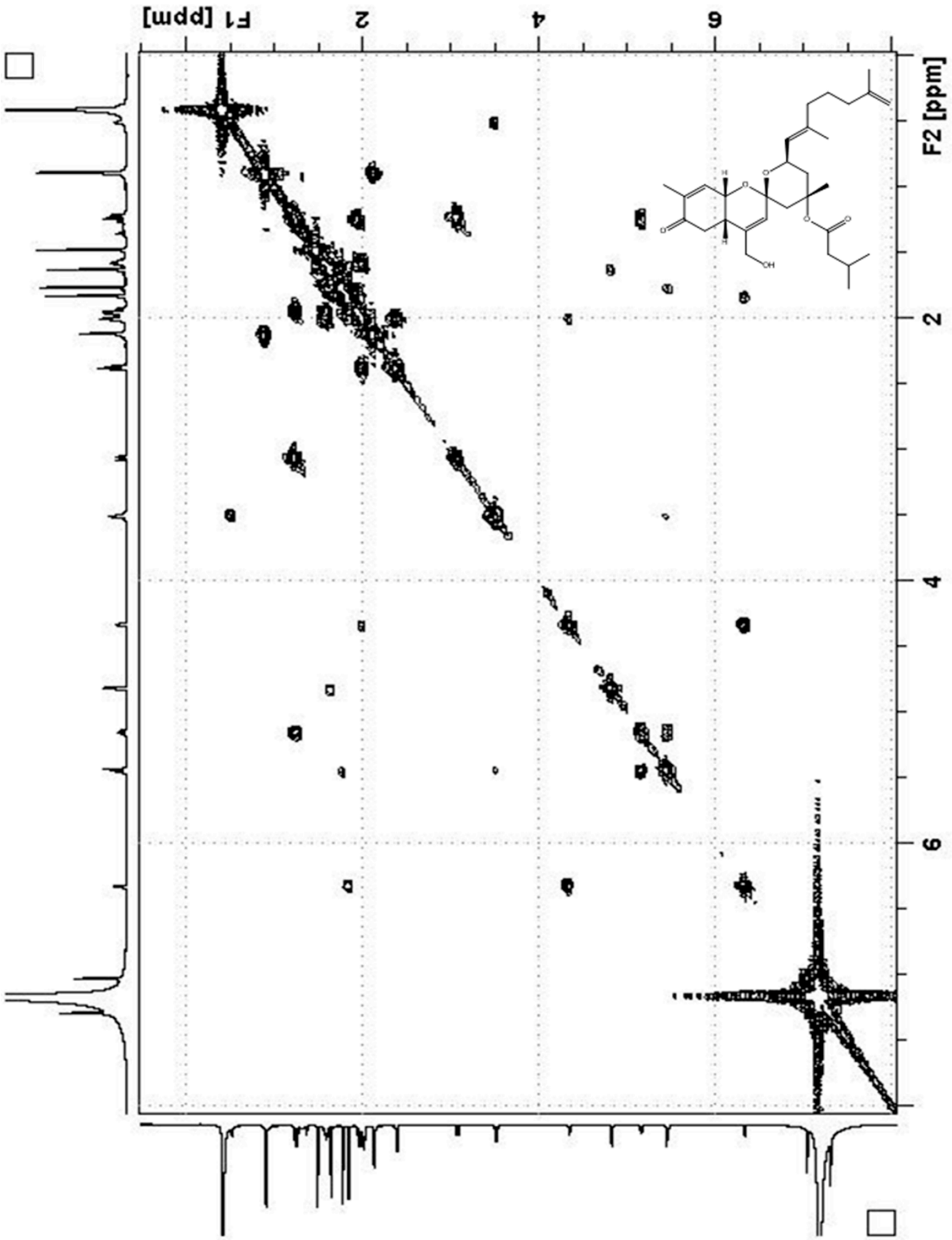
^{13}C NMR spectrum of alotaketal B (**3**) recorded in C_6D_6 at 600 MHz



HSQC spectrum of alotaketal B (**3**) recorded in C_6D_6 at 600 MHz



COSY spectrum of alotaketal B (**3**) recorded in C_6D_6 at 600 MHz



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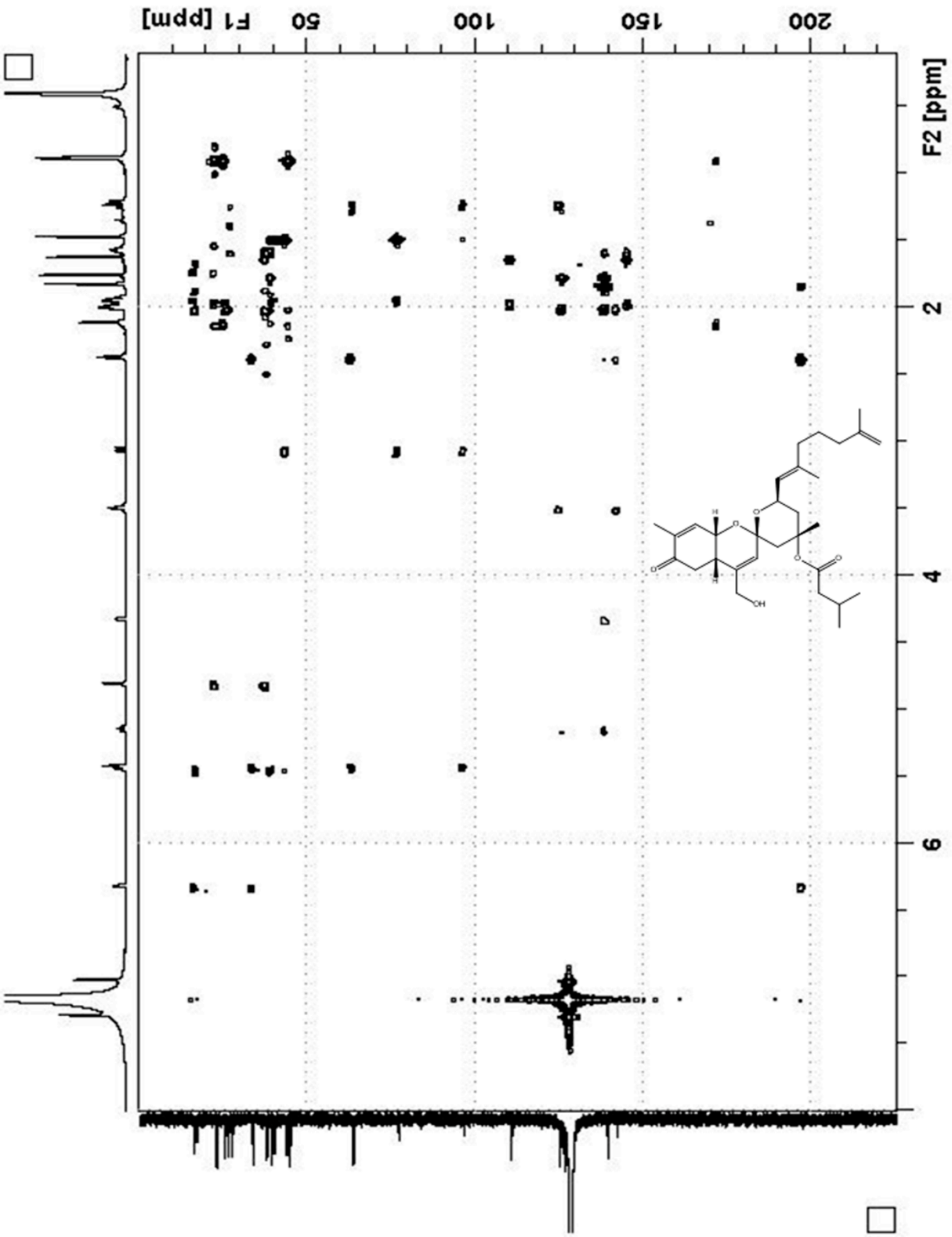
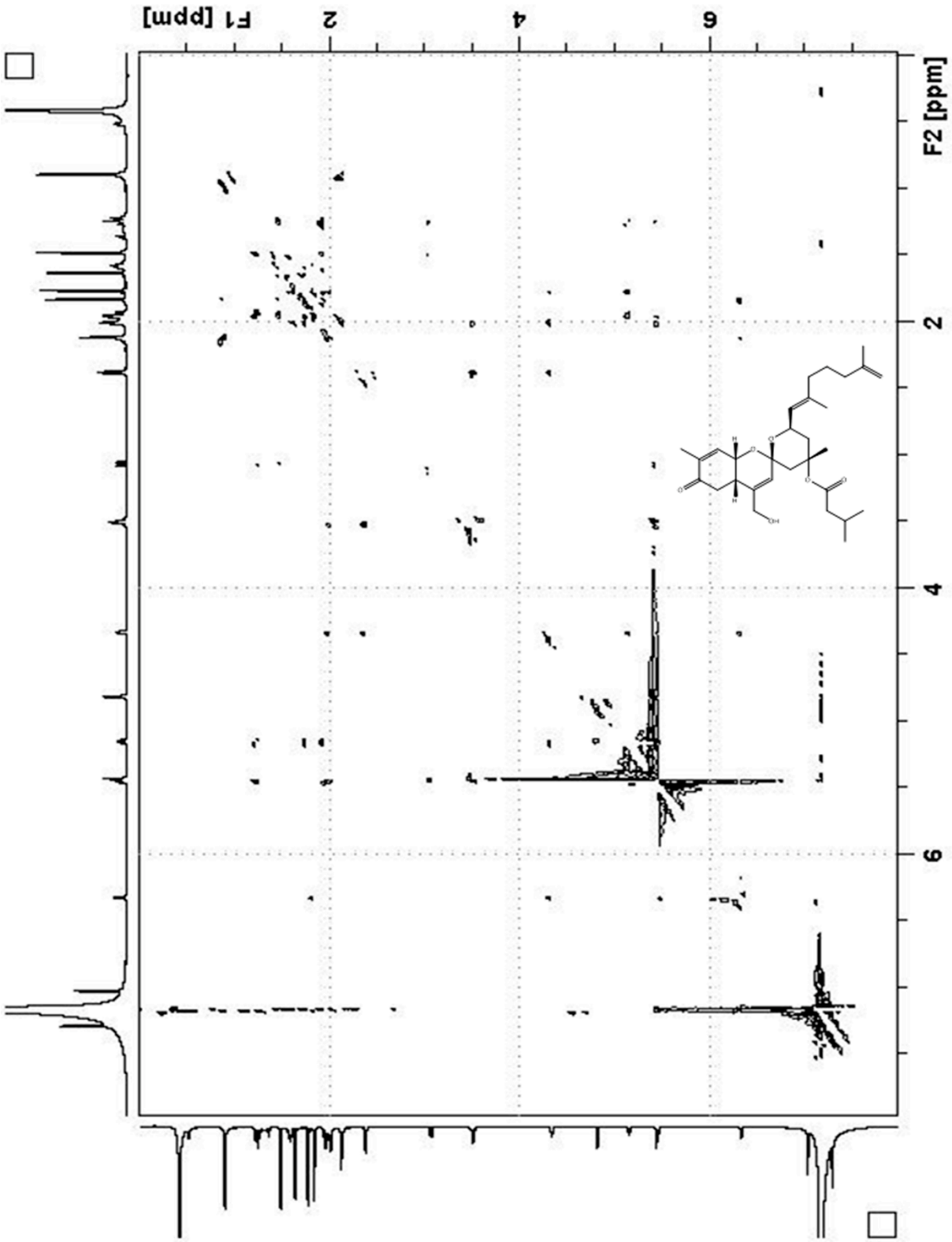
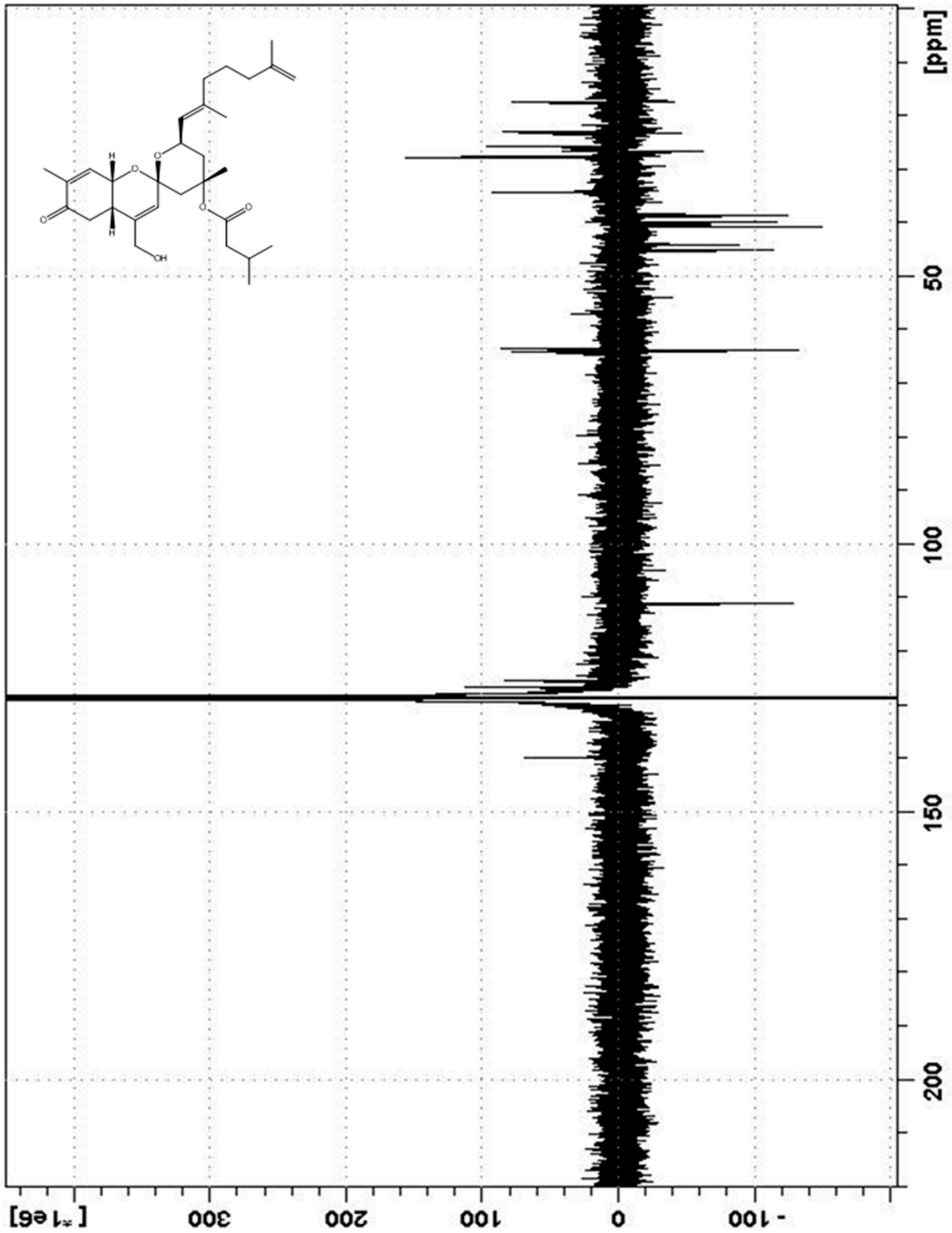


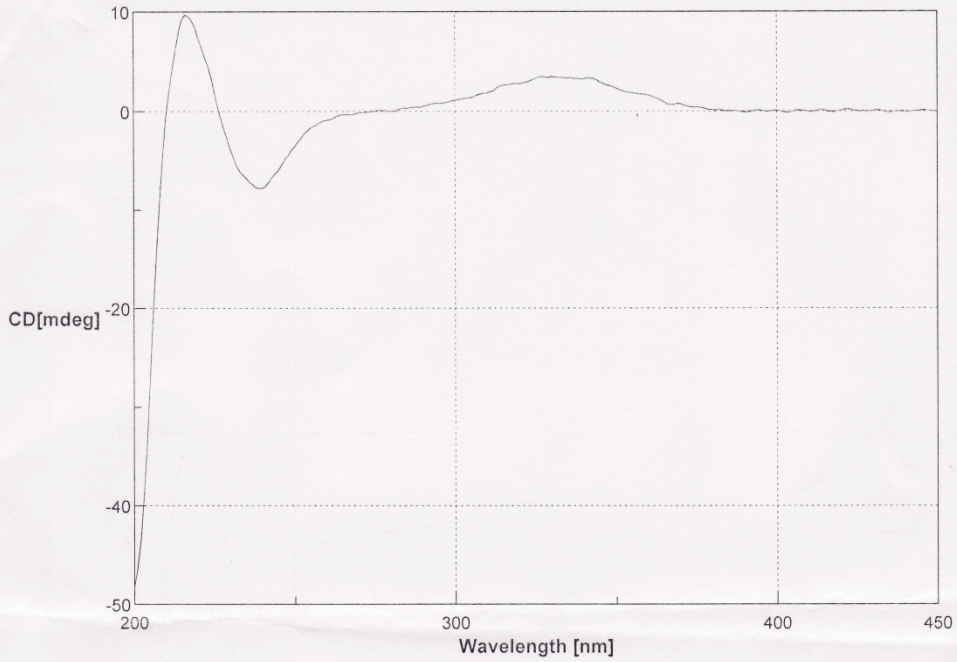
ROESY spectrum of alotaketal B (**3**) recorded in C_6D_6 at 600 MHz



DEPT 135º spectrum of alotaketal B (**3**) recorded in C_6D_6 at 600 MHz



CD spectra of alotaketal B (**3**) recorded in MeOH.



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Solvent	MeOH
Temperature	20.2 C
Sample name	03398B3
Operator	Roberto
Comment	

Materials and Methods for cAMP signaling assay:

HEK-pHTS-CRE Cell Line Derivation

HEK293 cells were transfected with the pHTS-CRE plasmid (Biomyx, San Diego, CA) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Stable clones were generated by growing the cells in HG-DMEM (10% FBS, 1 x pen-strep) with 200 μg/ml hygromycin (Invitrogen) as a selection agent. Clones were then assessed for responsiveness to forskolin induction in a luciferase assay. One clone was selected based on its high sensitivity to forskolin and favorable growth characteristics (HEK-pHTS-CRE).

Luciferase Assay

HEK-pHTS-CRE cells were plated in 96 well flat bottom white polystyrene plates (BD Falcon, Mississauga, ON) at a density of 5 x 10^4 cells/well and incubated overnight at 37°C in a 5% CO2 atmosphere. The next day, the medium was aspirated, cells were washed with PBS (Invitrogen), and 100 μl of sample (extract in media) was added. Following a 5-h incubation at 37°C in a 5% CO2 atmosphere, the samples were removed and wells washed with PBS (Invitrogen). A luciferase assay was then performed using the Bright-Glo luciferase assay kit (Promega, Madison, MI) according to the manufacturer's instructions. Luminescence was measured using a Tecan Infinite M1000 luminometer (Tecan, Männedorf, Switzerland).

FigureS-I2. Dose response curve for the crude extract of *Hamigera* sp.

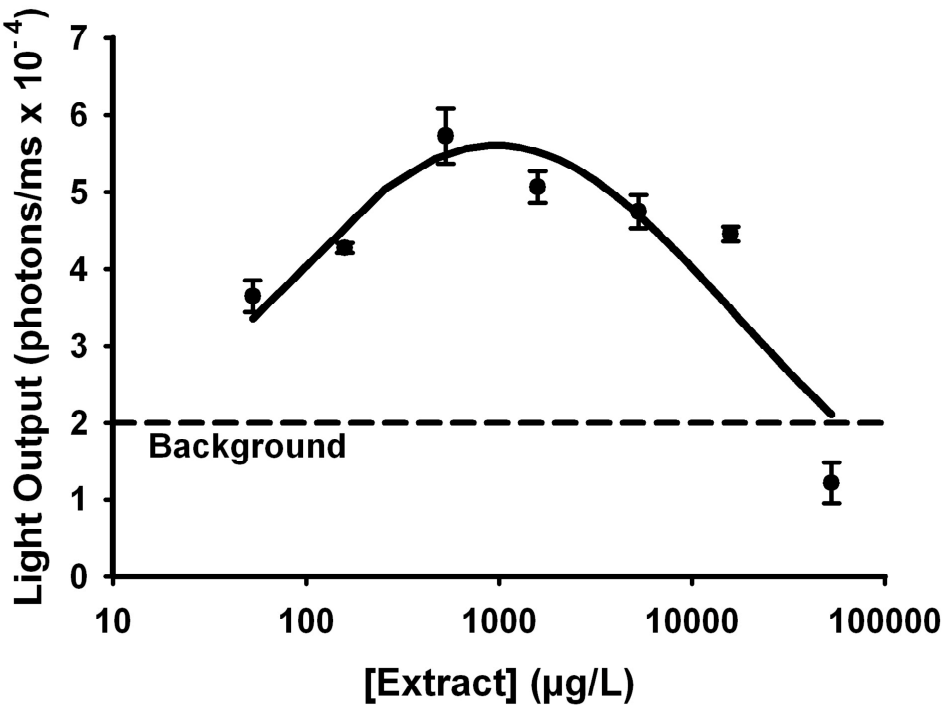

Figure SI-3. Dose response curve for forskolin (**1**).