SUPPLEMENTARY MATERIAL

First report of antioxidant abeo-labdane type diterpenoid from intertidal red seaweed *Gracilaria salicornia* with 5-lipoxygenase inhibitory potential

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ABSTRACT

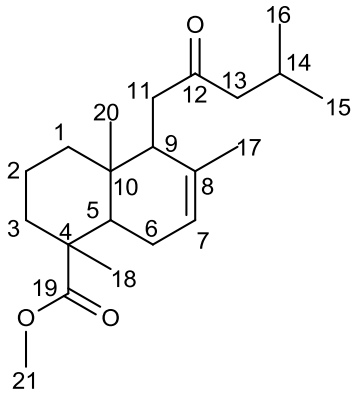
Phytochemical investigation on the biologically active compounds of seaweed *Gracilaria salicornia* {(C. Agardh) E.Y. Dawson} (family Gracilariaceae) guided to the separation of a previously unreported abeo-labdane class of diterpenoid. The compound was characterized as methyl-

16(13→14)-abeo-7-labdene-(12-oxo) carboxylate by extensive spectroscopic experiments, and comparison with the related compounds. The studied compound registered significantly greater activities against pro-inflammatory 5-lipoxygenase (IC_{50} 0.86 mg/mL) than that exhibited by non-steroidal anti-inflammatory agent ibuprofen (IC_{50} 0.92 mg/mL, $P<0.05$). Likewise, this compound exhibited comparable radical quenching (1, 1-diphenyl-2-picryl-hydrazil) activity (IC_{50} 0.66 mg/mL) as standard antioxidant agent α-tocopherol (IC_{50} 0.62 mg/mL).

Keywords: *Gracilaria salicornia* (C. Agardh) E.Y. Dawson, Gracilariaceae, 16(13→14)-abeo labdane, anti-inflammatory, antioxidant

Supplemental Figures

Table S1. NMR spectroscopic data[a] of abeo-labdane type diterpenoid isolated from red seaweed *G. salicornia*



Type of C	C. No.	^{13}C NMR	^{1}H NMR	int., mult., J in Hz[b]	COSY	HMBC
CH_2	1	38.65	1.54	t, J=5.5Hz	H-2	C-2, 10
			1.30	t, J=4.2Hz	-	-
CH_2	2	20.85	1.54	m	H-3	C-1, 10
			1.42	m	-	-
CH_2	3	38.26	2.19	t, J=5.0Hz	-	C-4, 19
			1.88	t, J=5.0Hz	-	-
C	4	42.91	-	-	-	-
CH	5	44.68	1.98	t, J=3.54Hz	H-6	C-4, 6, 10
CH_2	6	23.54	1.79	t, J=5.1Hz	H-7	-
			1.98	t, J=5.7.1Hz	-	-
CH	7	124.87	5.55	t, J=6.6Hz	-	C-5, 8
C	8	135.93	-	-	-	-
CH	9	45.18	2.21	t, J=5.5Hz	H-11	C-8, 10, 11, 12
C	10	39.27	-	-	-	-
CH_2	11	41.32	2.37	dd, J=10.9, 6.5 Hz	-	C-9, 12
			2.28	dd, J=12.7, 5.4Hz	-	-
C	12	203.27	-	-	-	-
CH_2	13	54.80	2.30	d, J=6.5Hz	H-14	C-12
CH	14	24.16	1.99	m	H-15, 16	C-12
CH_3	15	16.27	0.79	d, J=6.0Hz	-	-
CH_3	16	17.96	0.87	d, J=6.1 Hz	-	-
CH_3	17	19.60	1.14	s	-	C-8
CH_3	18	17.96	1.38	s	-	C-4
C	19	167.63	-	-	-	-
CH_3	20	10.96	0.63	s	-	C-10
CH_3	21	69.78	3.46	s	-	C-19

[a]NMR spectra were recorded using a Bruker AVANCE III 500 MHz (AV 500) spectrometer (Bruker, Karlsruhe, Germany) in MeOH as aprotic solvent at ambient temperature with TMS as the internal standard (δ 0 ppm).

[b]Values in ppm, multiplicity and coupling constants (*J*= Hz) were indicated in parentheses. Multiplicities were allocated by [135]DEPT NMR spectrum. The assignments were made with the aid of the COSY, HSQC, HMBC and NOESY experiments.

Table S2. *In vitro* bioactive potentials (antioxidant and anti-infammatory) of compound 16(13→14)abeo-labdene type diterpenoid isolated from red seaweed *G. salicornia vis-a-vis* commercially available references (α-tocopherol and ibuprofen)

Compounds	Anti-oxidant activities in IC_{50} mg/mL		Anti-inflammatory properties IC_{50} mg/mL			
	DPPH quenching	ABTS quenching	COX-1 inhibition	COX-2 inhibition	5-LOX inhibition	Selectivity index†
16(13→14)abeo-labdene	0.66 ± 0.03[a]	0.78 ± 0.04[a]	0.80 ± 0.03[a]	0.57 ± 0.02[a]	0.78 ± 0.02[a]	1.40 ± 0.02[a]
Standards	α-tocopherol		ibuprofen			
	0.62±0.04[a]	0.75±0.05[a]	0.06±0.00[b]	0.08±0.02[b]	0.92±0.11[b]	0.75±0.01[b]

The anti-oxidant and anti-diabetic activities were expressed as IC_{50} values (mg/mL).

[a-c] Column-wise values with different superscripts of this type indicate significant difference (P<0.05). Triplicate values were taken and the variance analyses (ANOVA) were carried out (using Statistical Program for Social Sciences 13.0) for means of all parameters to examine the significance level (P<0.05).

Results were expressed as mean ± SD (n = 3).

† Selectivity index = IC_{50}anti-COX-1/ IC_{50}anti-COX-2

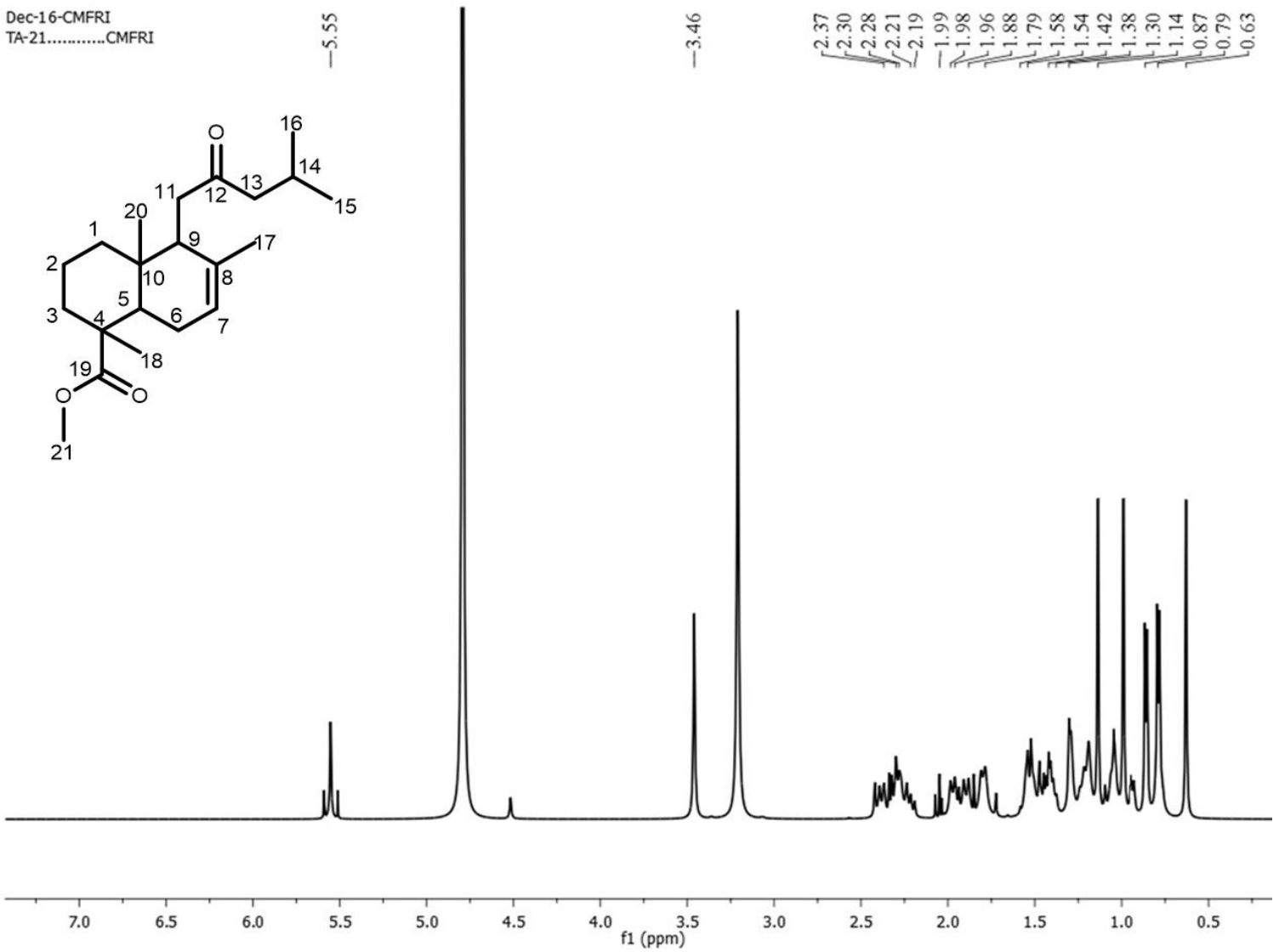


Fig. S1 ^{1}H NMR spectrum of methyl-16(13→14)-abeo-7-labdene-(12-oxo) carboxylate

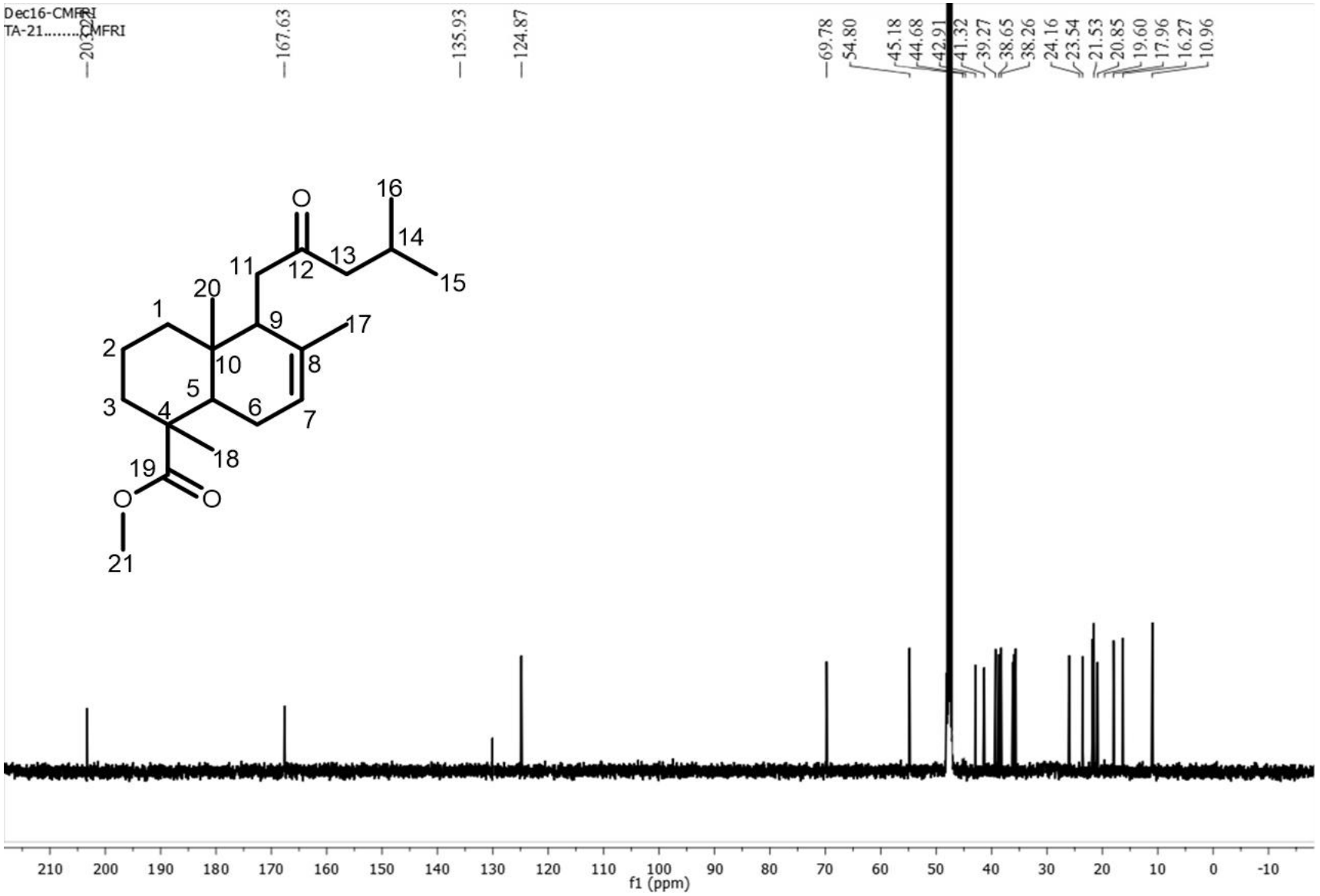


Fig. S2. ^{13}C NMR spectrum of methyl-16(13→14)-abeo-7-labdene-(12-oxo) carboxylate

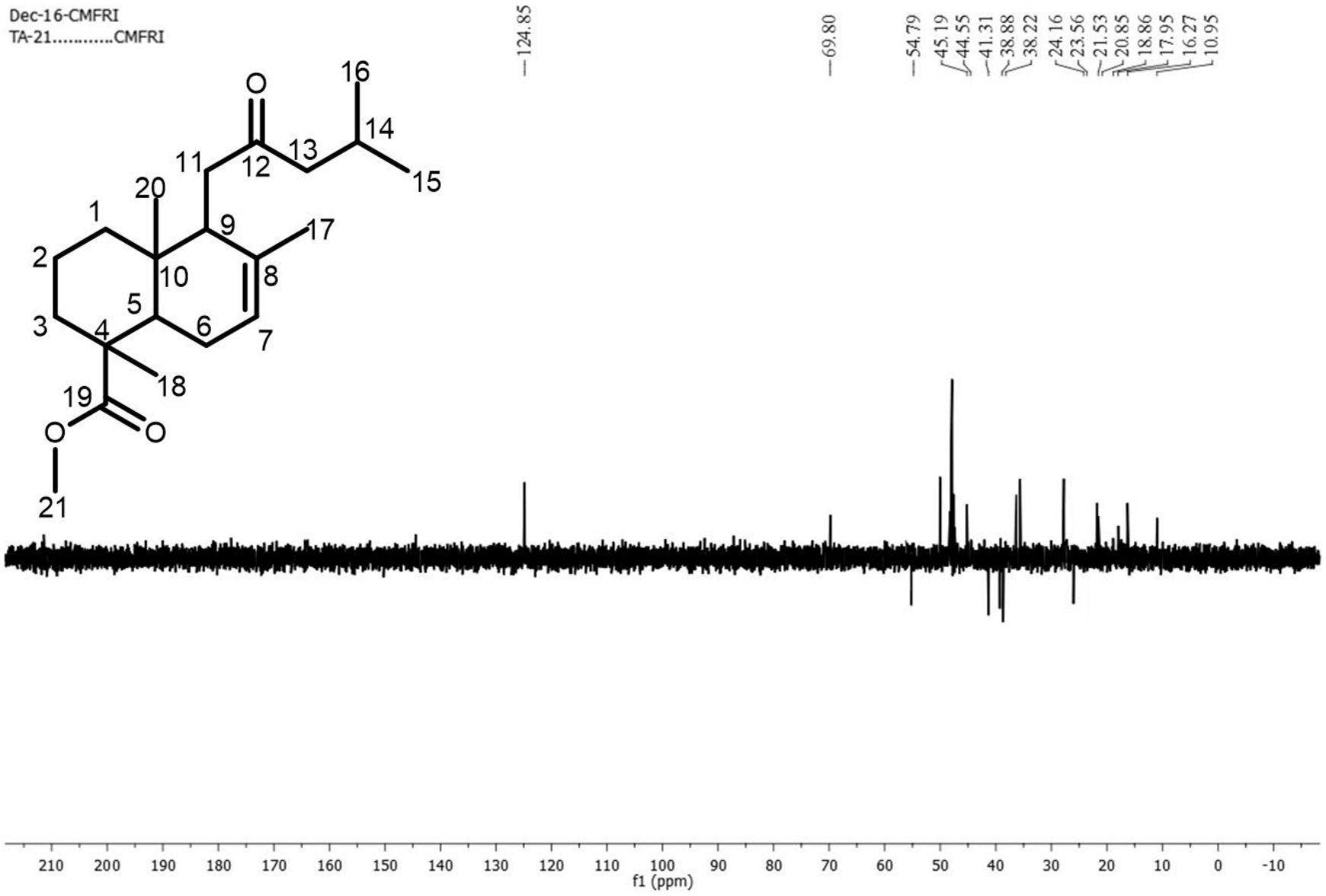

Dec-16-CMFRI
TA-21.....CMFRI
124.85
69.80
54.79
45.19
44.55
41.31
38.88
38.22
24.16
23.56
21.53
20.85
18.86
17.95
16.27
10.95
O
16
14
13
15
11
12
20
1
9
17
2
10
8
5
3
4
6
7
18
19
O
O
21
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Fig. S3. DEPT spectrum of methyl-16(13→14)-abeo-7-labdene-(12-oxo) carboxylate

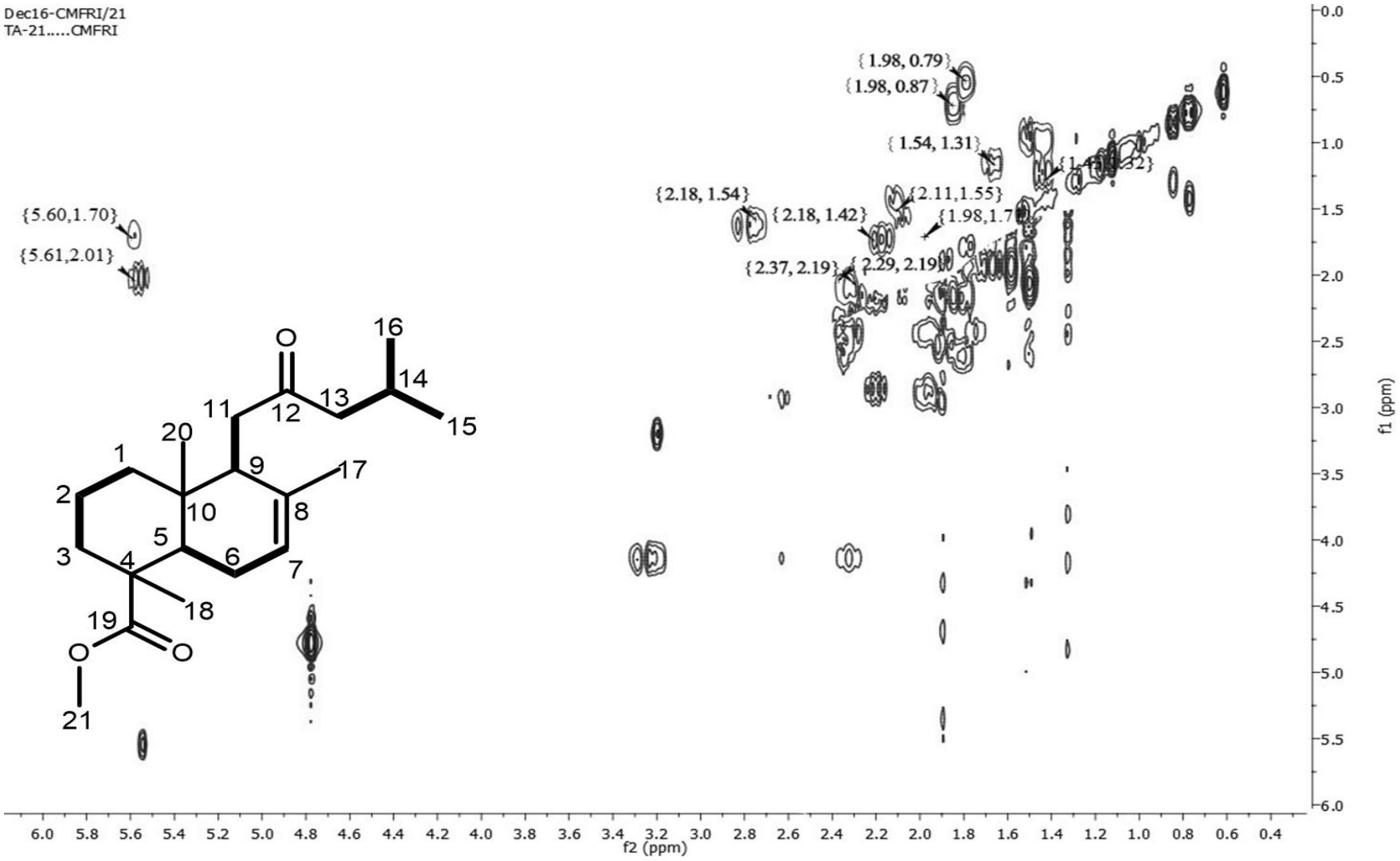


Fig. S4. ^{1}H-^{1}H COSY spectrum of methyl-16(13→14)-abeo-7-labdene-(12-oxo) carboxylate

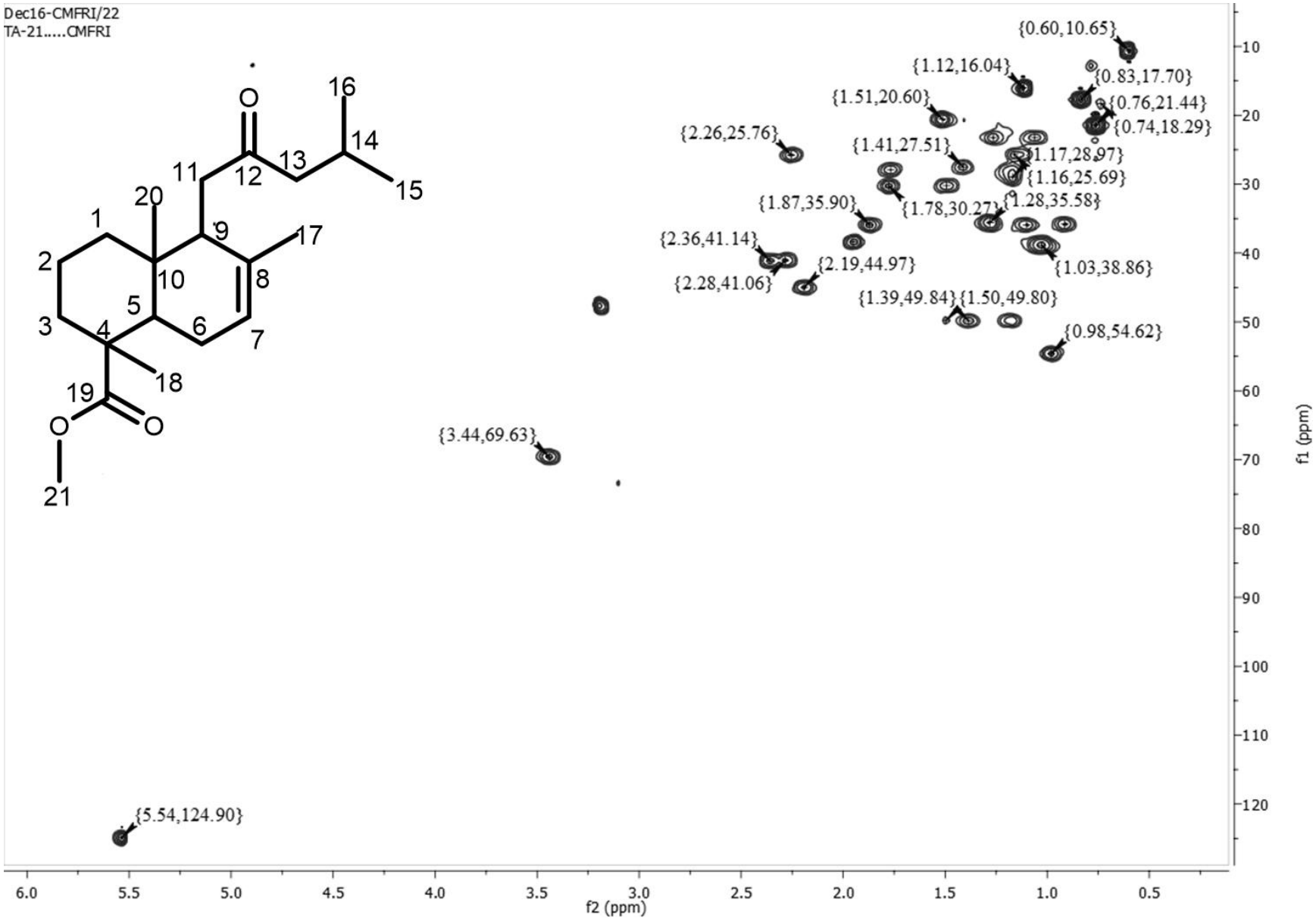


Fig. S5. HSQC spectrum of methyl-16(13→14)-abeo-7-labdene-(12-oxo) carboxylate

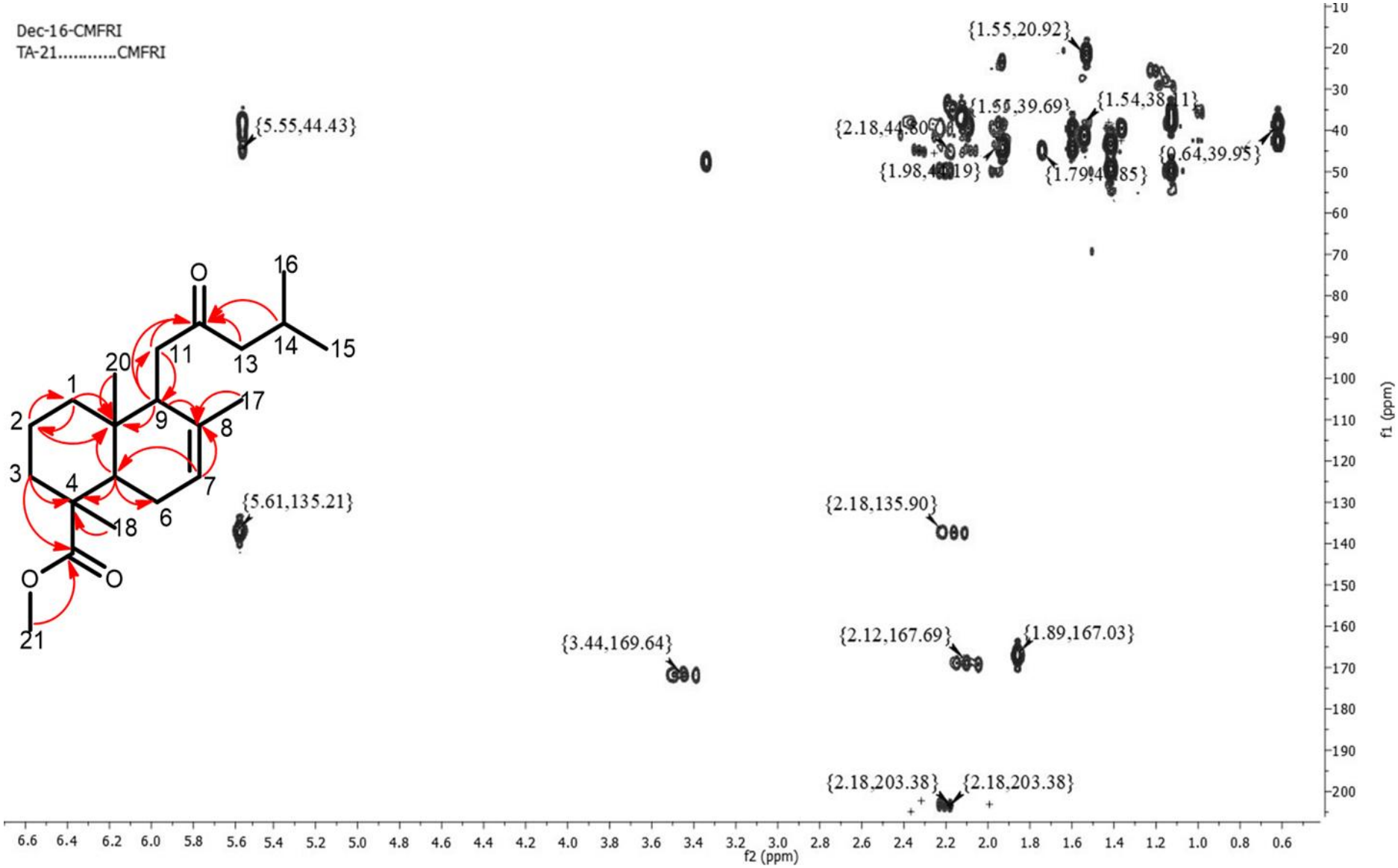


Fig. S6. HMBC spectrum of methyl-16(13→14)-abeo-7-labdene-(12-oxo) carboxylate

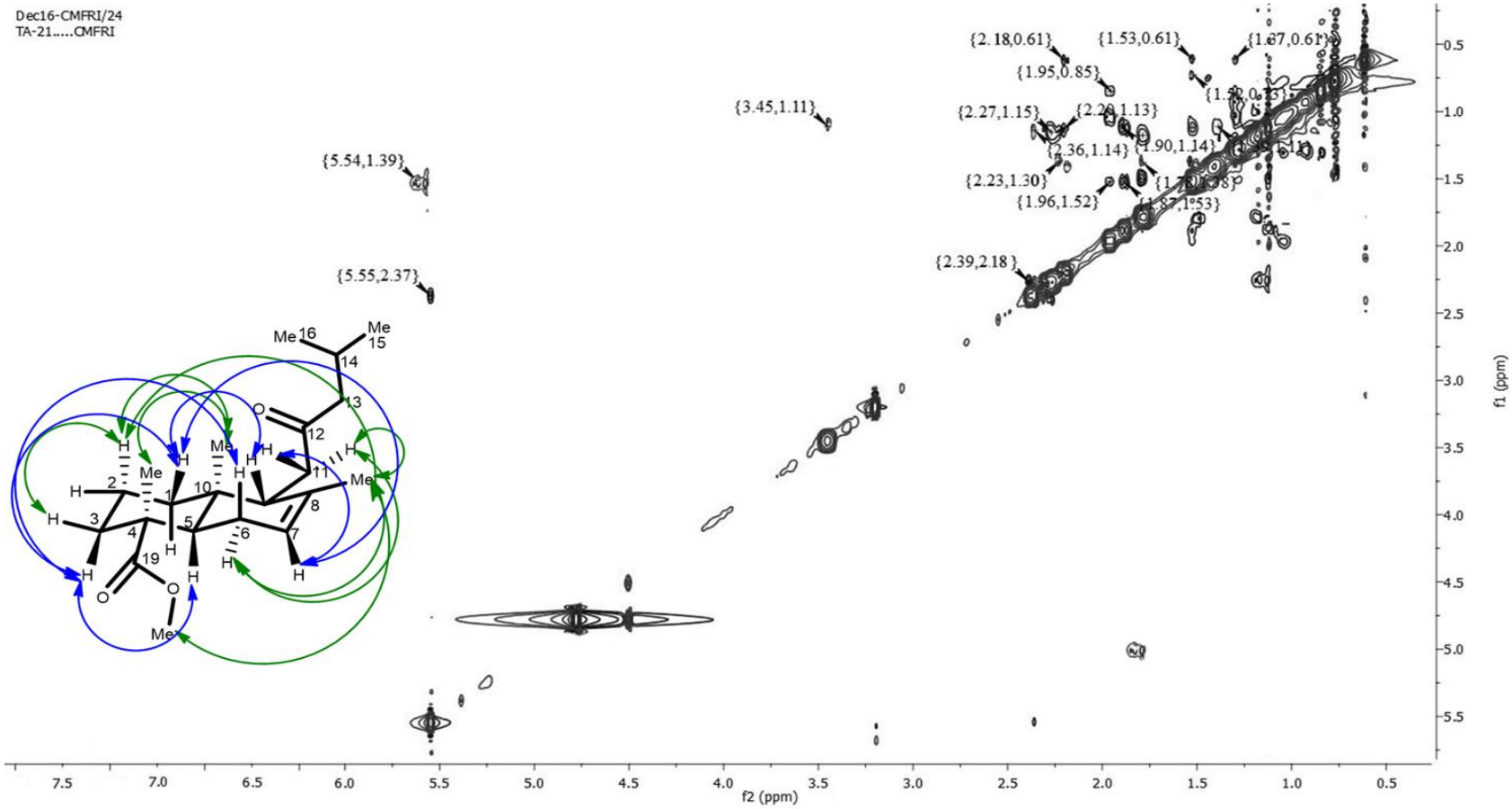


Fig. S7. NOESY spectrum of methyl-16(13→14)-abeo-7-labdene-(12-oxo) carboxylate

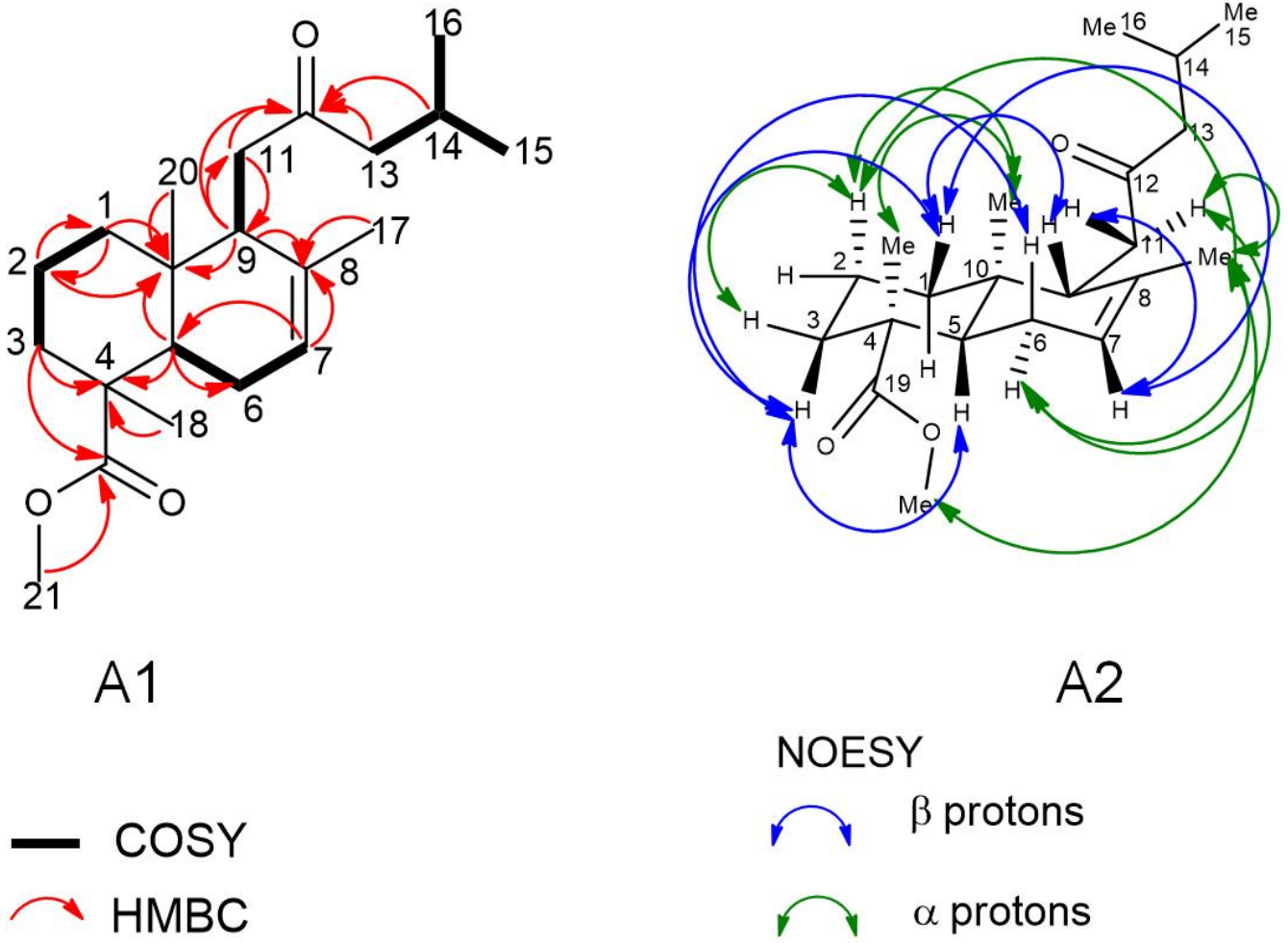


Fig. S8. (**A1**) ^{1}H-^{1}H COSY (bold face bonds), selected HMBC (red coloured double barbed arrows) and (**A2**) NOESY (coloured arrows) correlations of methyl-16(13→14)-abeo-7-labdene-(12-oxo) carboxylate

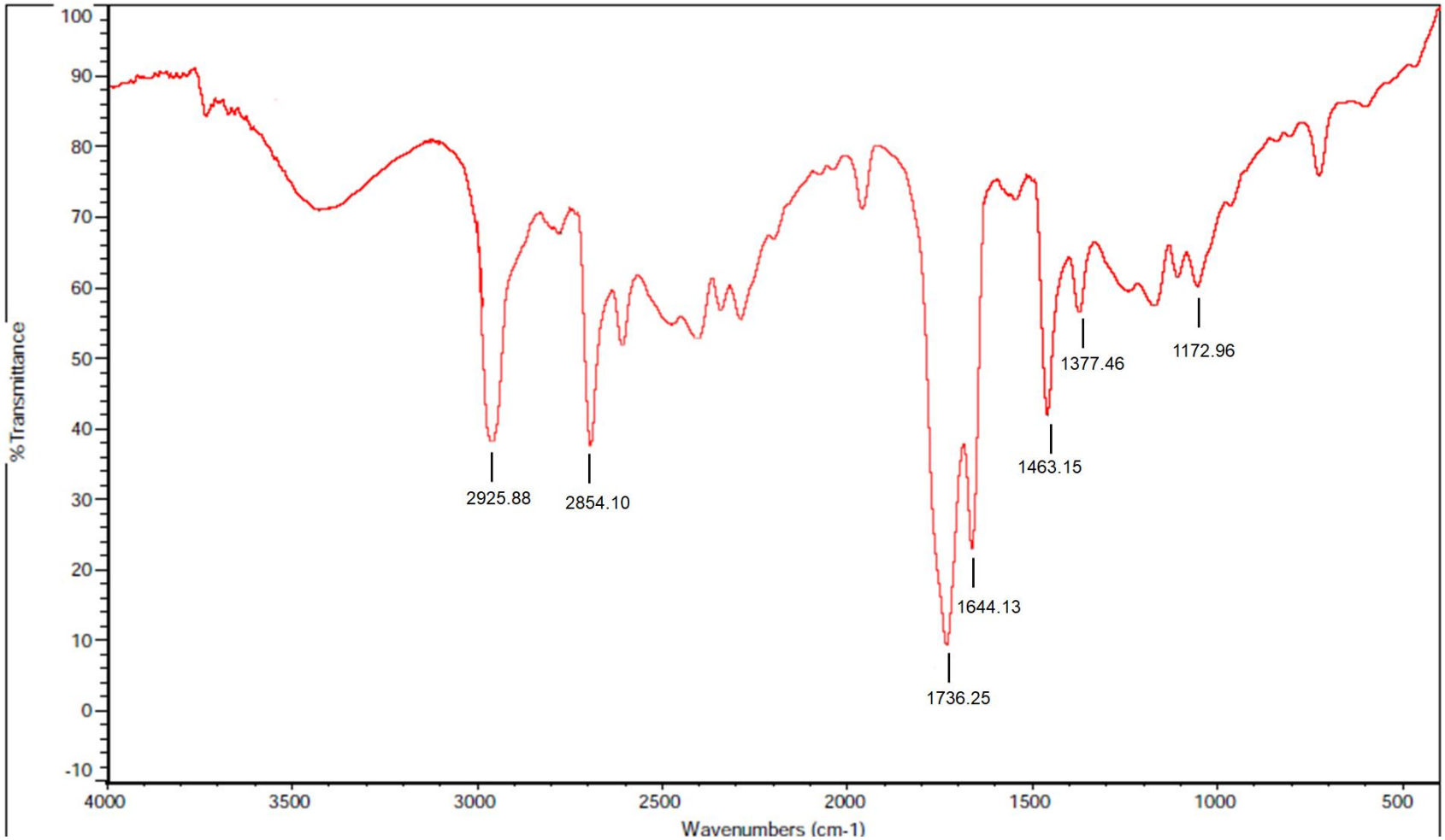


Fig. S9. FTIR spectrum of methyl-16(13→14)-abeo-7-labdene-(12-oxo) carboxylate

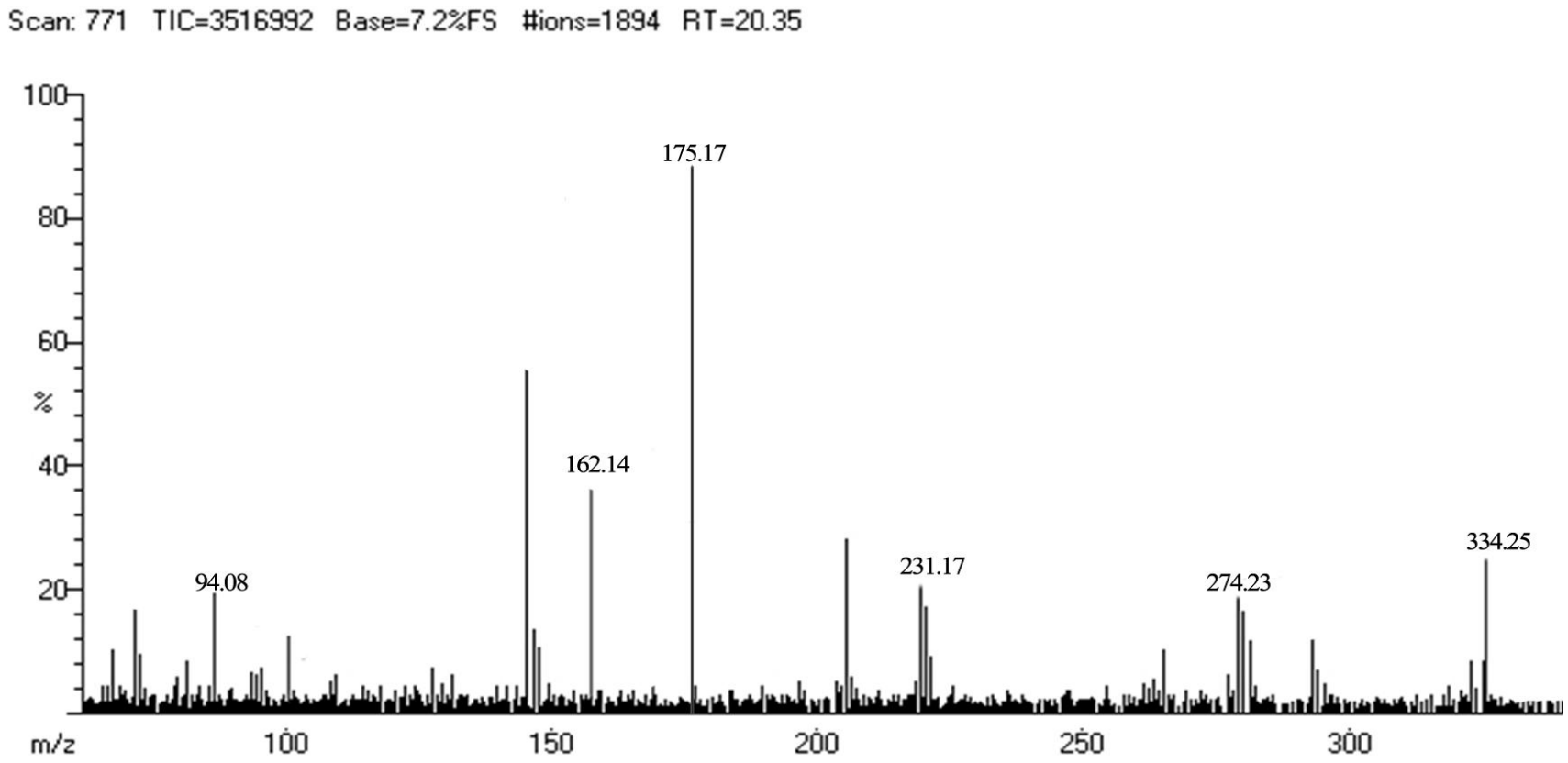


Fig. S10. GC-MS spectrum of methyl-16(13→14)-abeo-7-labdene-(12-oxo) carboxylate

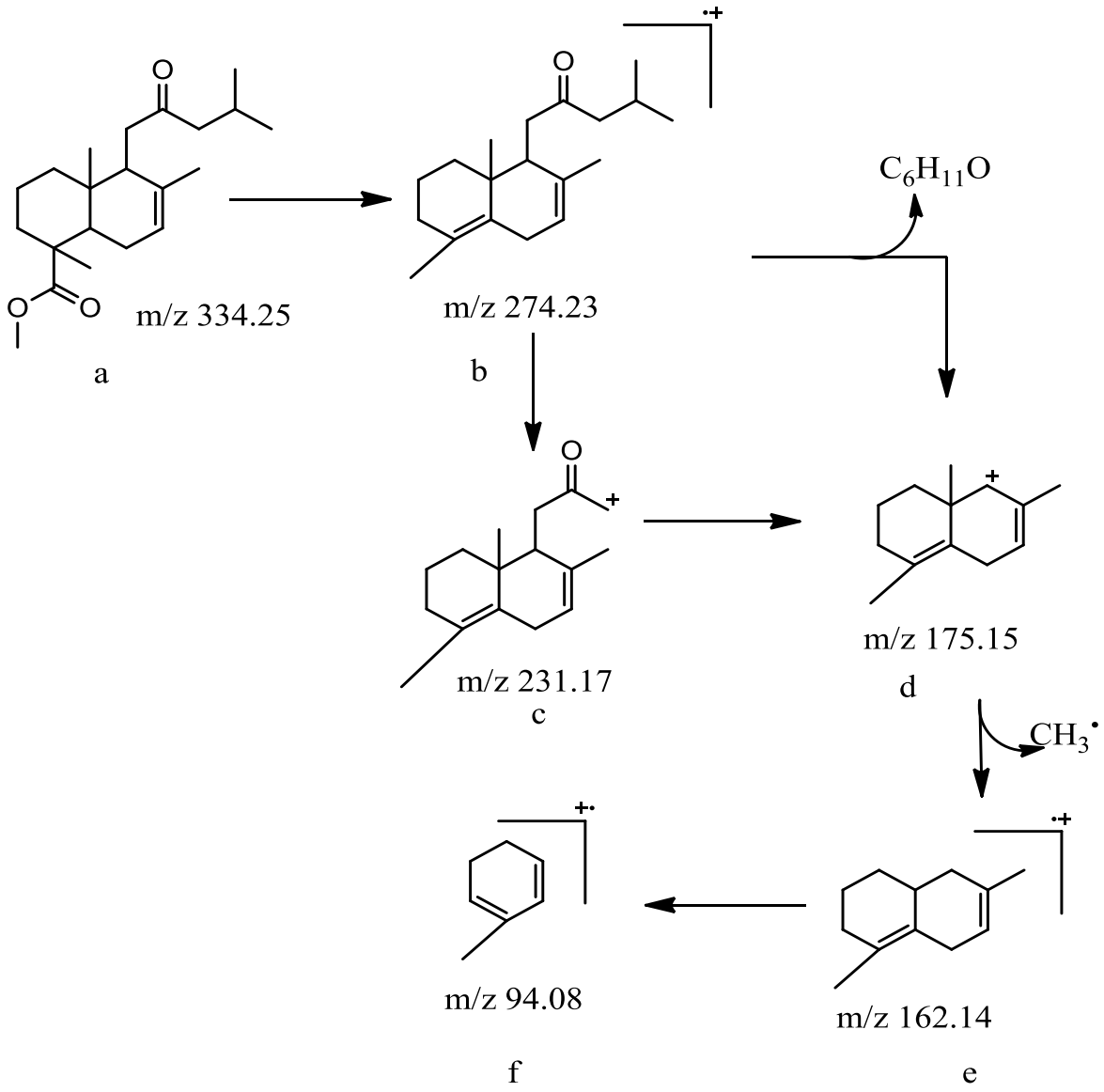

m/z 334.25
a
m/z 274.23
b
$C_6H_{11}O$
m/z 231.17
c
m/z 175.15
d
$CH_3^{\bullet}$
m/z 162.14
e
m/z 94.08
f

Fig. S11. Tentative mass fragmentation pattern of methyl-16(13→14)-abeo-7-labdene-(12-oxo) carboxylate