Supporting Information

The natural product antroalbol H promotes phosphorylation of liver kinase B1 (LKB1) at threonine 189 and thereby enhances cellular glucose uptake

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Running title: *AH enhances cellular glucose uptake through T189 of LKB1*

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Keywords: sesquiterpene, fungi, glucose metabolism, liver kinase B1 (LKB1), AMP-activated protein kinase (AMPK), glucose transporter type 4 (GLUT4), diabetes, glucose homeostasis, Basidiomycete, energy sensing

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Table S1. ^{1}H (400 Hz) and ^{13}C NMR (100 Hz) NMR data in $CDCl_3$ for antroalbol H

Position	δ_C type	δ_H mult. (*J* in Hz)
1	40.1, CH_2	2.72, d (14.8)
		2.33, d (14.8)
2	211.9, C	
3	73.5, CH	4.01, t (8.0)
4	34.1, CH_2	2.14, m
5	26.1, CH_2	1.86, m
		1.55, m
6	55.6, C	
7	82.1, C	
8	214.1, C	
9	33.4, CH_2	2.66, m
		2.38, m
10	36.1, CH_2	1.78, m
		1.63, m
11	38.2, C	
12	28.0, CH_3	1.01, s
13	25.1, CH_3	1.12, s
14	26.4, CH_3	1.36, s

Table S2. Antibodies used for western blotting

Antibody	Vendor	Cat. No.	Fold Dilution
Rabbit anti-AMPKα	Cell Signaling Technology	2532	1000
Rabbit anti-phospho-AMPKα (Thr172)	Cell Signaling Technology	2535	1000
Rabbit anti-phospho-AMPK β (Ser182)	Cell Signaling Technology	4186	1000
Rabbit anti- AMPKβ1/2	Cell Signaling Technology	4150	1000
Rabbit anti-phospho-Acetyl-CoA Carboxylase (Ser79)	Cell Signaling Technology	11818	1000
Rabbit anti- Acetyl-CoA Carboxylase	Cell Signaling Technology	3662	1000
Rabbit anti-Akt	Cell Signaling Technology	9272	1000
Rabbit anti-phospho-Akt (S473)	Cell Signaling Technology	4060	1000
Rabbit anti-phospho-Akt (T308)	Cell Signaling Technology	13038	1000
Rabbit anti- LKB1	Santa Cruz Biotechnology	Sc-374300	1000
Rabbit anti-phospho-LKB1 (T189)	Cell Signaling Technology	3054	1000
Rabbit anti- phospho-LKB1 (S307)	Merck-Millipore	09-478	1000
Rabbit anti-phospho-LKB1 (S428)	Cell Signaling Technology	3482	1000
Rabbit anti-CaMKII	Cell Signaling Technology	3362	1000
Rabbit anti-phospho-CaMKII (T286)	Cell Signaling Technology	12716	1000
Mouse anti-N-cadherin	Cell Signaling Technology	13116	1000
Mouse anti-GLUT4	Abcam	Ab654	1000
Mouse anti-β-Actin	Sigma	A5316	8000

Alexa Fluor ® 488-conjugated AffiniPure goat anti-mouse lgG (H+L)	Jackson ImmunoResearch Laboratories Inc.	A5316	2000
CyTM 3-conjugated AffiniPure goat anti-mouse lgG (H+L)	Jackson ImmunoResearch Laboratories Inc.	A5316	2000

Table S3. siRNA nucleotide sequence.

Genes	**siRNA sequence**
Control	UUCUCCGAACGUGUCACGUTT
AMPKα	AUGAUGUCAGAUGGUGAAUUU
LKB1	CGGUCAAGAUCCUCAAGAAUU

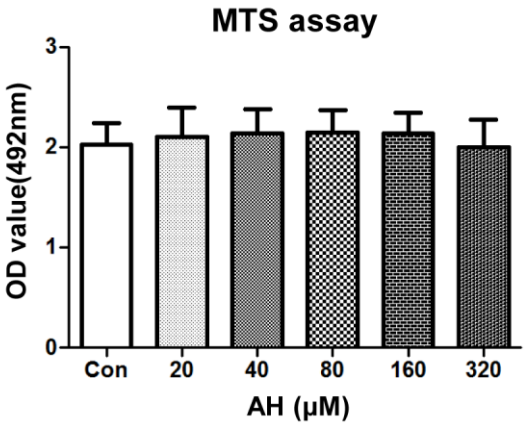

Figure S1. Effects of antroalbol H on cell viability in 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with 20 - 320 μM AH for 24 h, the cytotoxicity was detected by MTS assay. Results were represented as means ± SEM (n = 6).

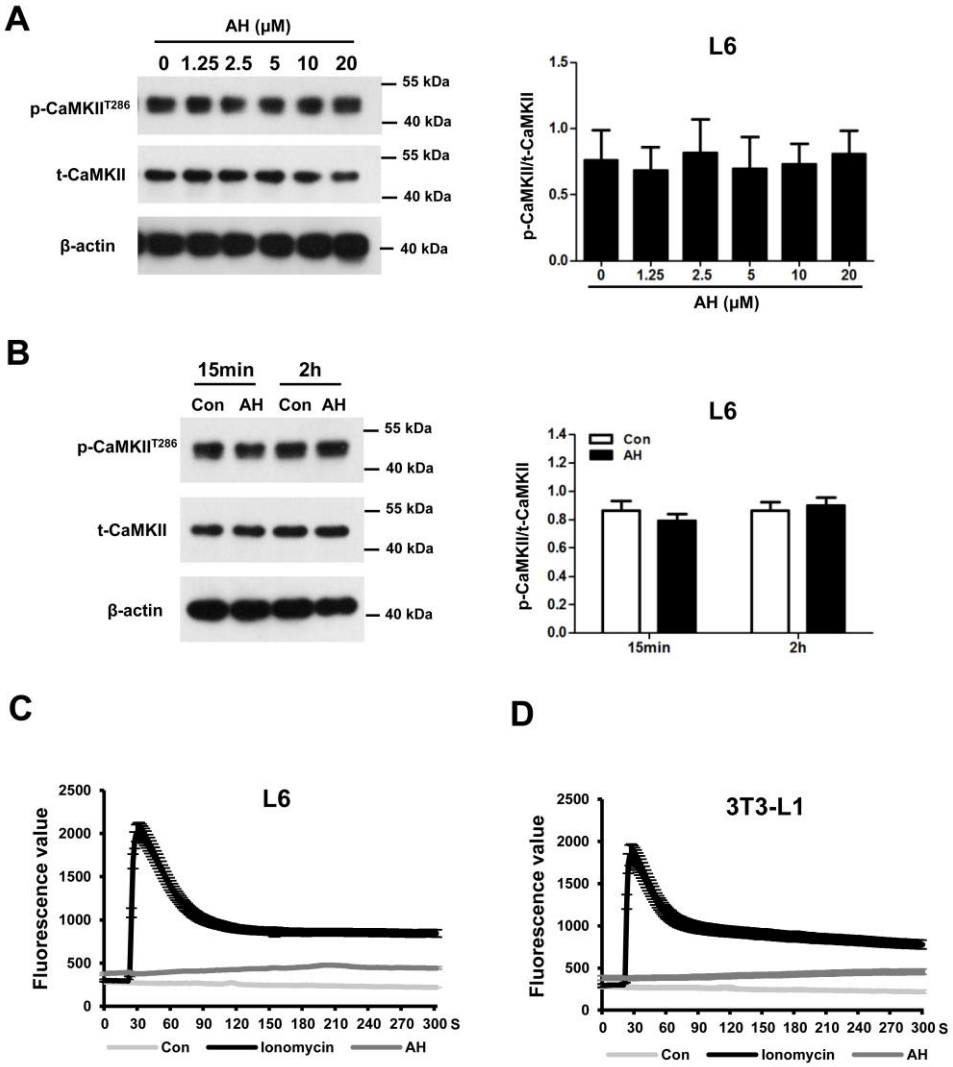

Figure S2. Antroalbol H does not change the phosphorylation of CaMKII and Ca^{2+}. (A and B) Immunoblots of p-CaMKII^{T286}, t-CaMKII and β-actin in L6 myotubes. L6 myotubes were treated with 0-20 μM AH for 24 h or 10 μM AH for indicated hours. Results were represented as means ± SEM (n = 3). (C and D) Ca^{2+} signal assay in L6 myotubes and 3T3-L1 adipocytes. Cells were grown on 24-well plate and washed three times with Ca^{2+}-containing resting buffer (145 mM NaCl, 5 mM KCl, 2.6 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM D-glucose, and 10 mM HEPES, pH 7.4). Loading with 10 μM Fluo 3-AM was for 30 min at 37 ℃. Washed plate were mounted in fluorescence microscope and recorded of fluorescence (excitation490 nm, emission 530 nm) was initiated about 30 seconds prior to addition of 0.1% DMSO (Con), 1 μM ionomycin or 10 μM AH. Results were represented as the net increase value of fluorescence in 15-20 cells per condition (n = 3).

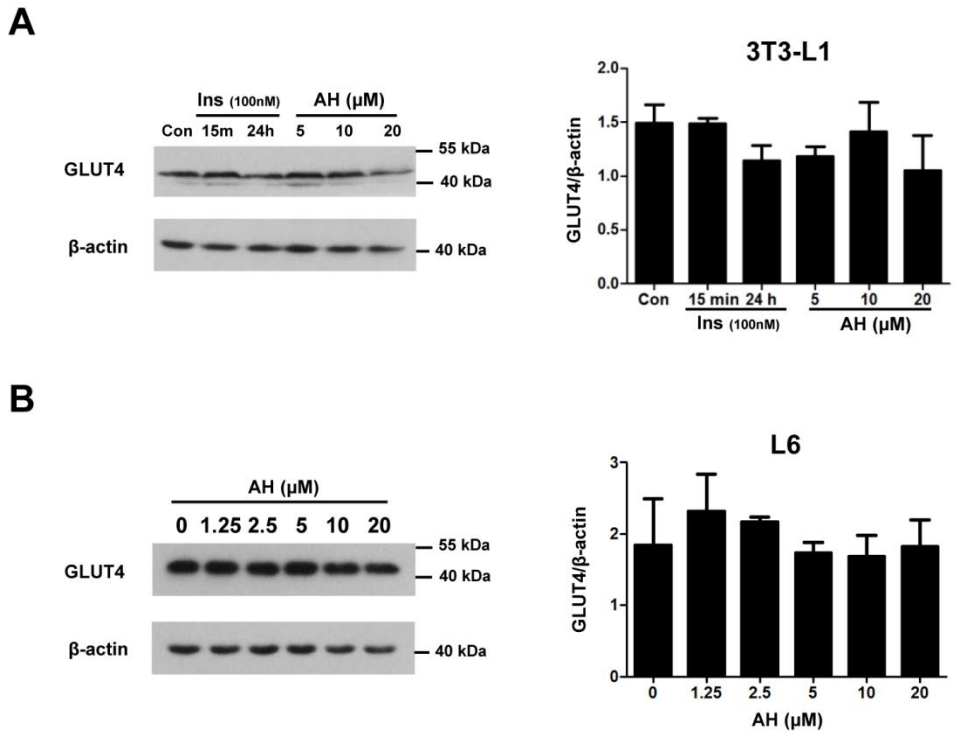

Figure S3. Antroalbol H does not change the protein levels of GLUT4. (A) 3T3-L1 adipocytes were treated with 5-20 μM AH for 24 h or 100 nM insulin for indicated times, then immunoblots of GLUT4 and β-actin were performed. (B) L6 myotubes were treated with 0-20 μM AH for 24 h, GLUT4 protein levels also assayed by western blotting. Results were represented as means ± SEM (n = 3).

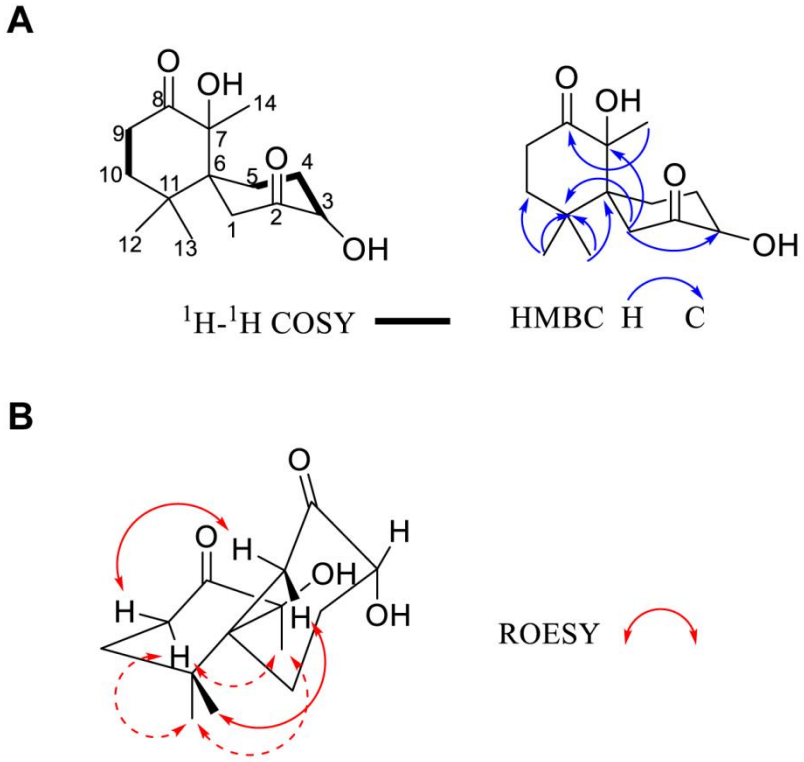

Figure S4. Key 2D NMR correlations of antroalbol H. (A) ^{1}H-^{1}H COSY and key HMBC correlations of antroalbol H. (B) Key NOESY correlations of antroalbol H.

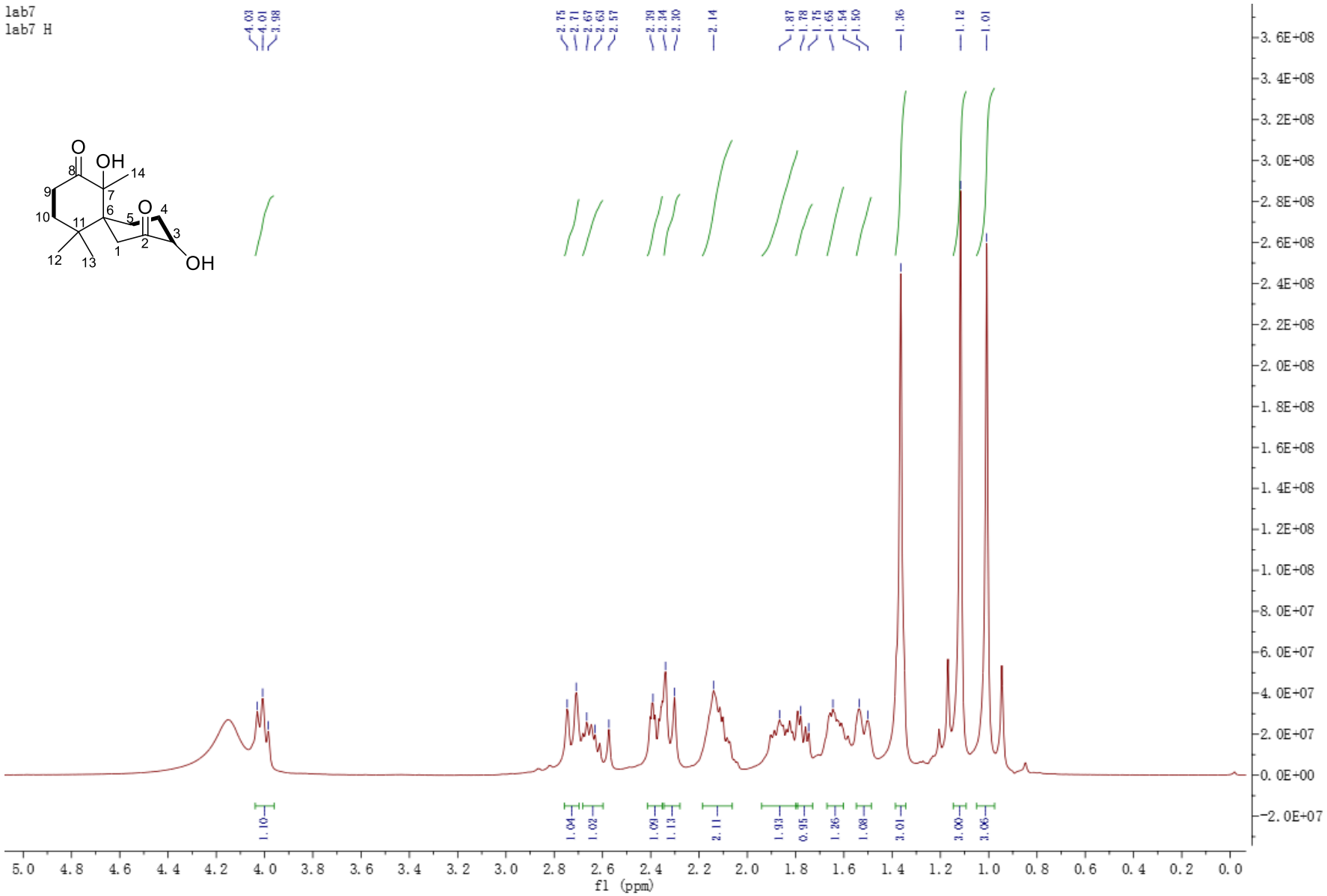

Figure S5. ^{1}H NMR (400MHz, $CDCl_3$) spectrum of antroalbol H

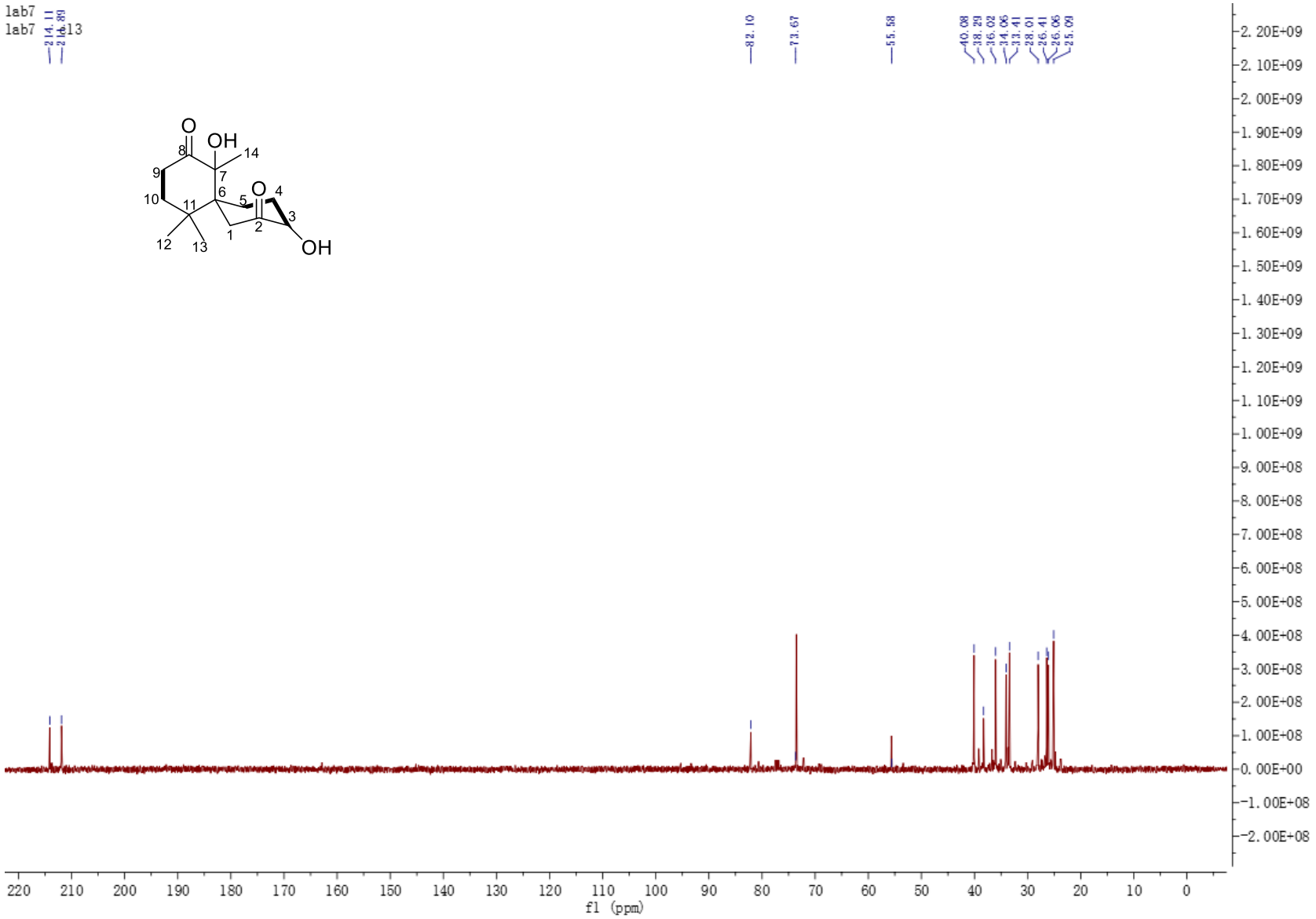

Figure S6. ^{13}C NMR (400MHz, $CDCl_3$) spectrum of antroalbol H

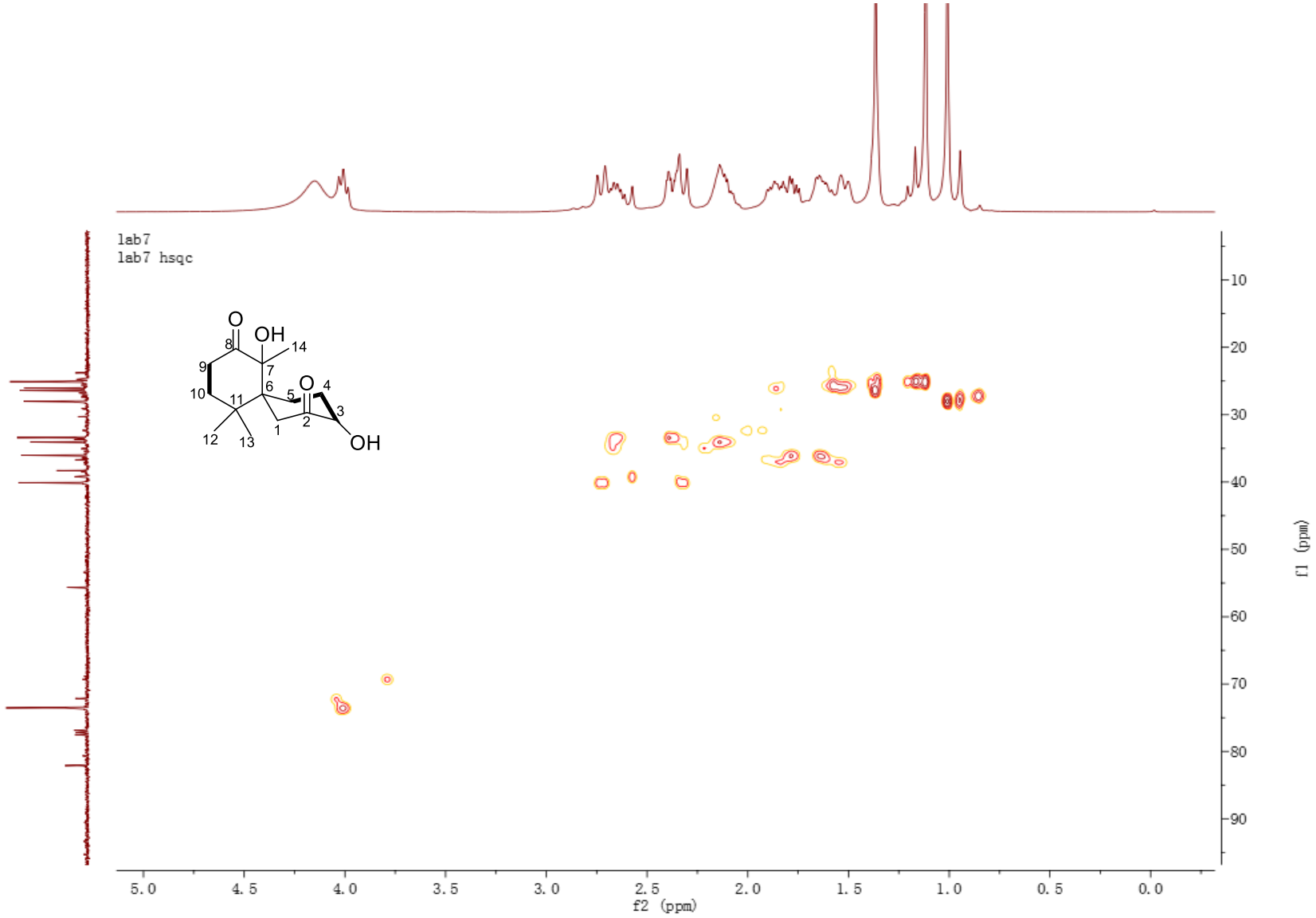

Figure S7. HSQC spectrum of antroalbol H

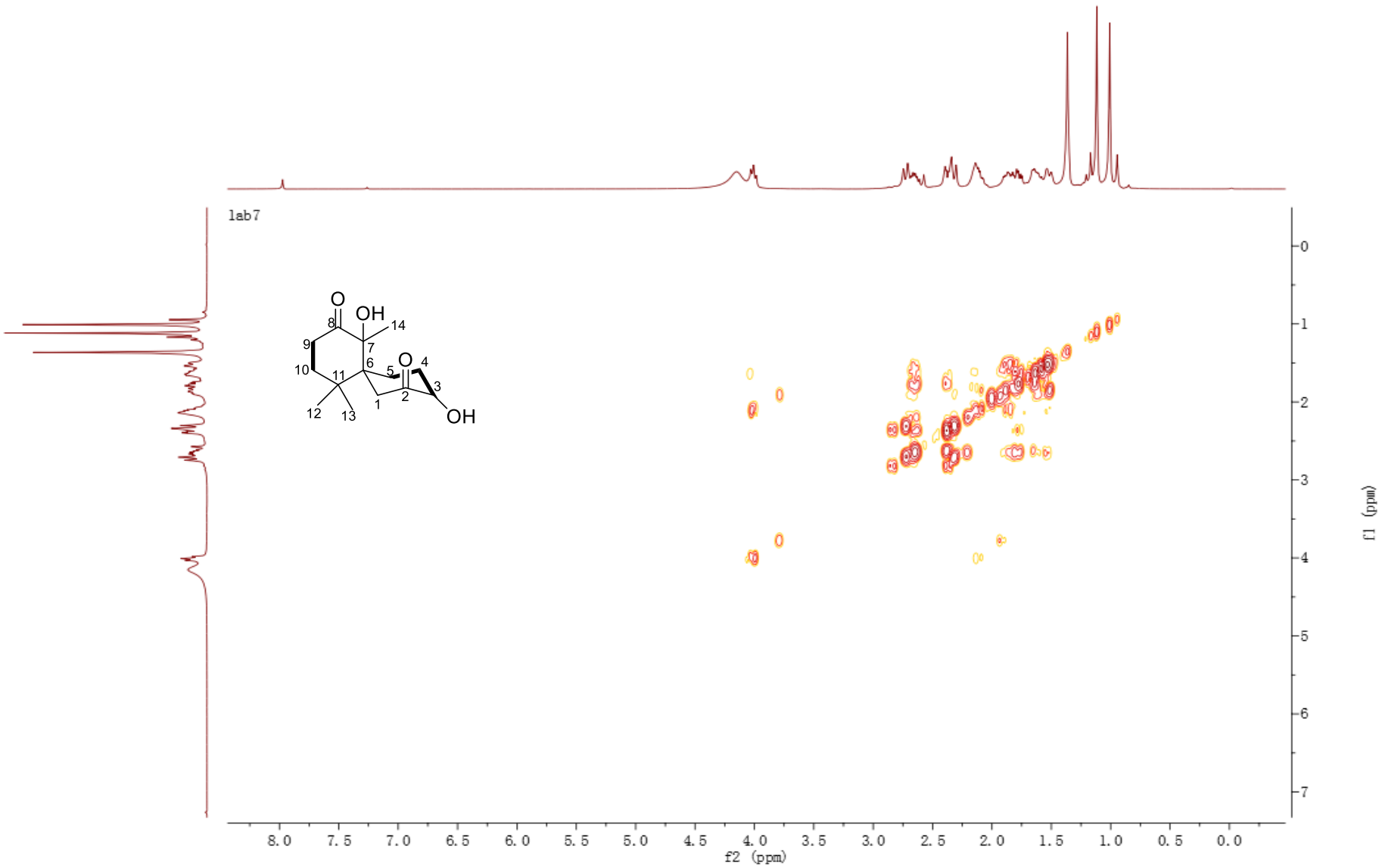

Figure S8. ^{1}H-^{1}H COSY spectrum of antroalbol H

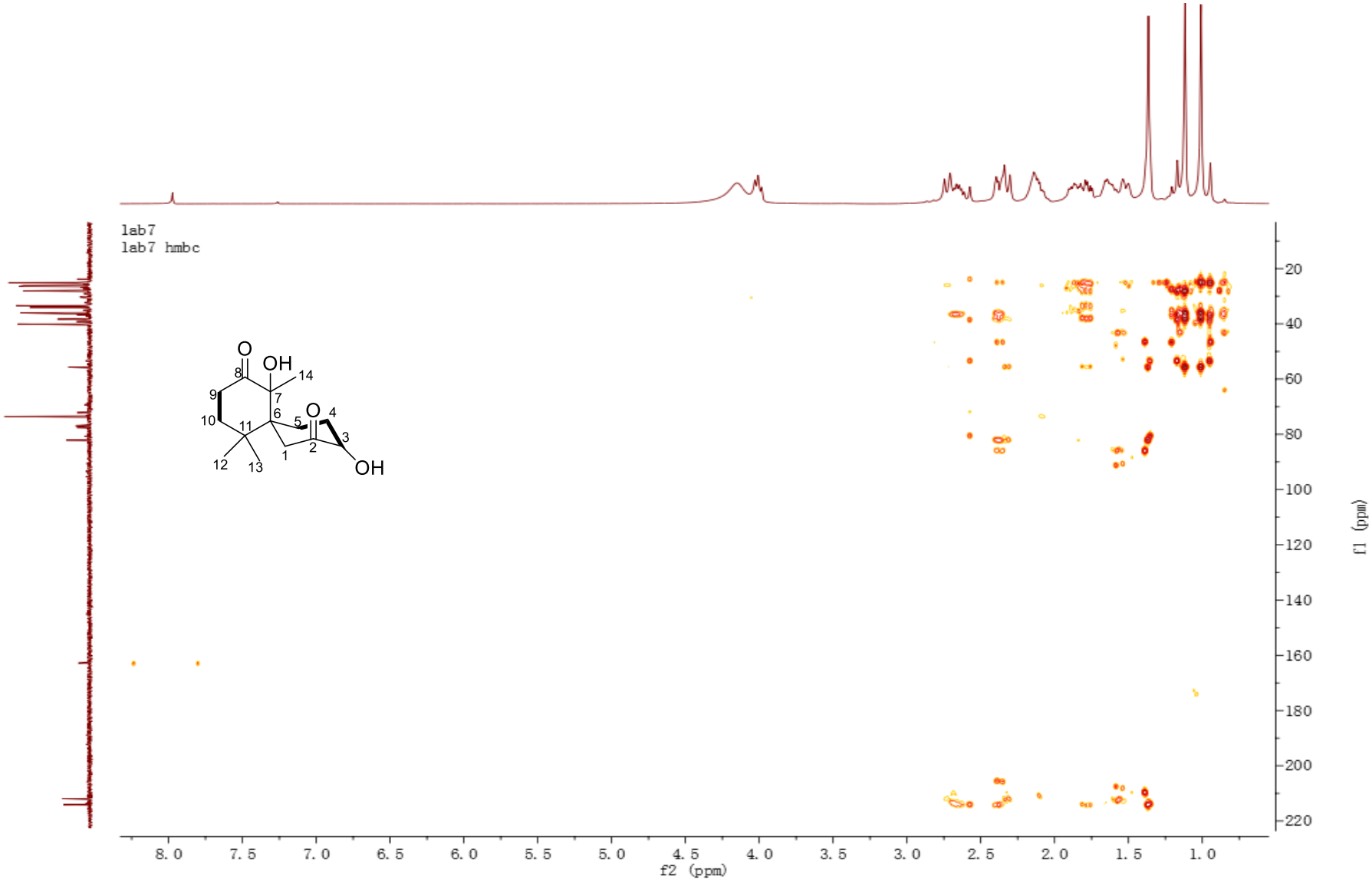

Figure S9. HMBC spectrum of antroalbol H

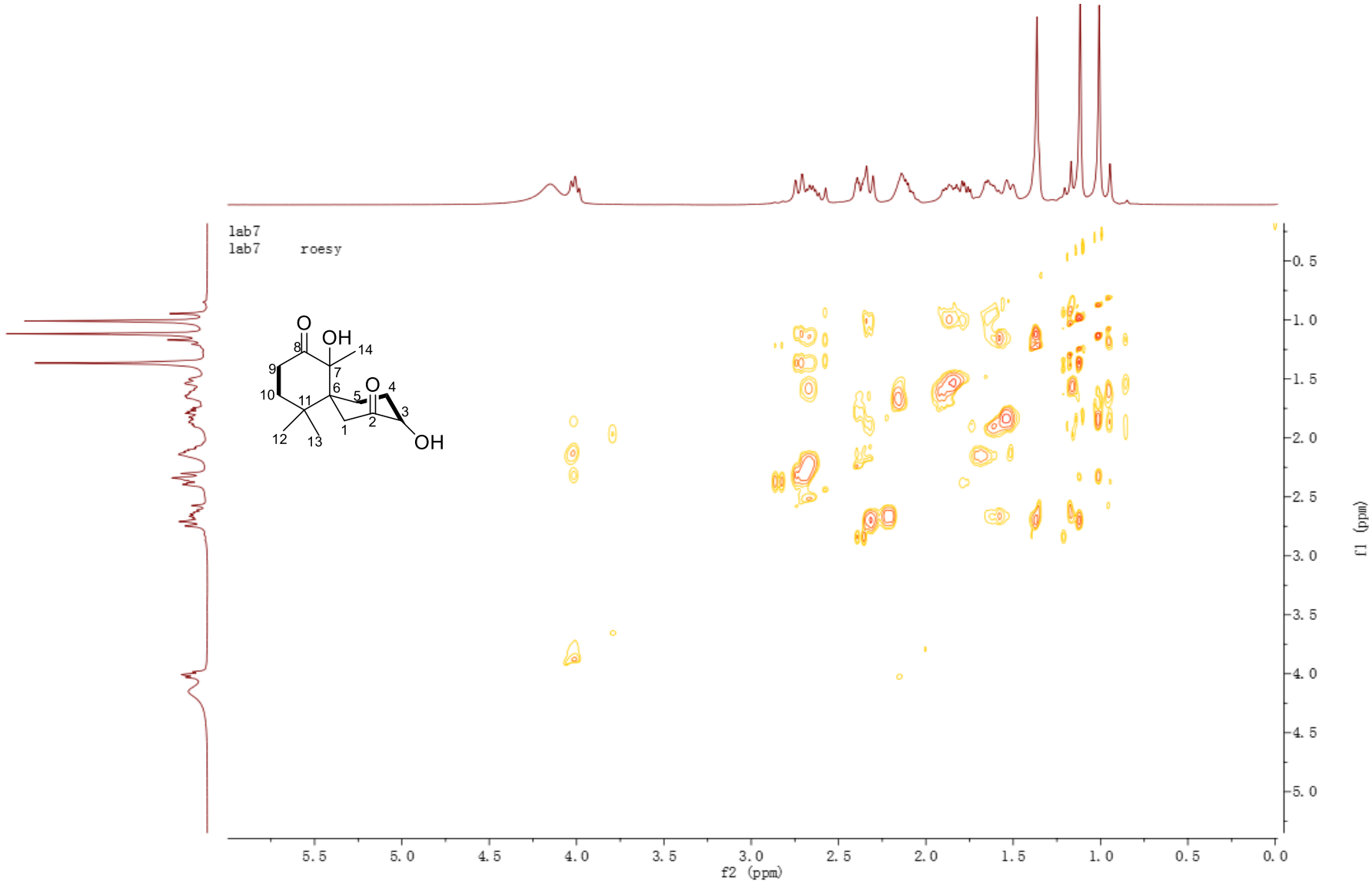

Figure S10. ROESY spectrum of antroalbol H

Elemental Composition Report **Page 1**

Single Mass Analysis
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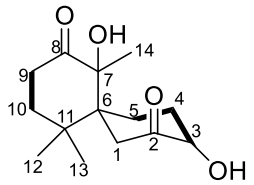
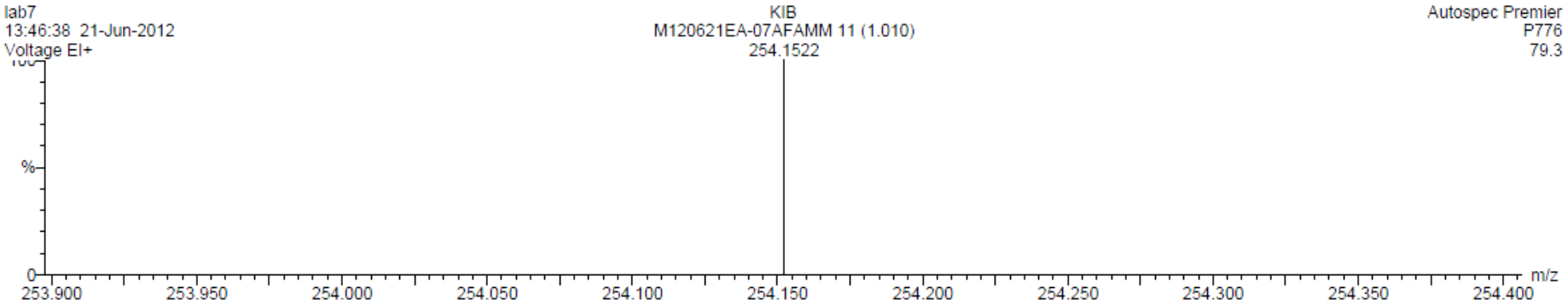
Selected filters: None



Monoisotopic Mass, Odd and Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 51 closest results for each mass)
Elements Used:
C: 0-200 H: 0-400 O: 1-4



Minimum:				-10.0		
Maximum:		100.0	10.0	120.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
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Figure S11. HR-ESI-MS spectrum of antroalbol H

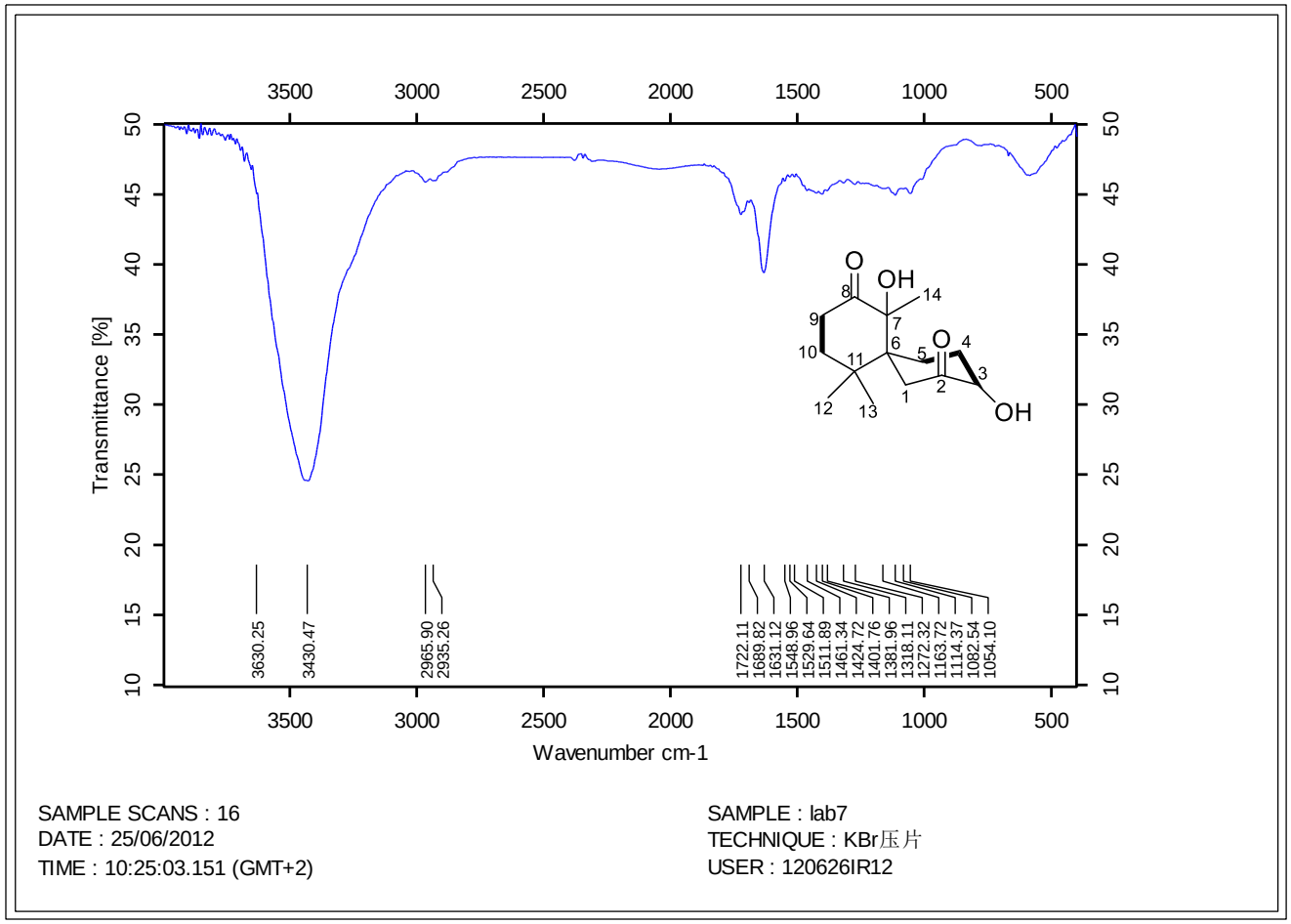

Figure S12. IR spectrum of antroalbol H