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# New sesquiterpenoids from the rhizomes of *Acorus tatarinowii*

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## List of Supporting Information

## 1 AChE and BACE1 activity assays.

### 1.1 Chemicals and reagents

Lyophilized powder of acetylcholinesterase (AChE) from electric eel source, acetylthiocholine iodide (ATCH) and 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB) were purchased from Sigma-Aldrich (USA). TruPoint$^{TM}$ beta-secretase assay kit$^{384}$ was obtained from PerkinElmer (USA). Recombinant human beta-secretase (BACE1) was purchased from Invitrogen (USA). DMSO was obtained from ACROS (USA). Huperzine A and Ⅵa were synthesized by the Beijing institute of Pharmacology and Toxicology. 0.1M phosphate buffer (PB) with pH of 7.4 was used as a buffer throughout the AChE inhibitory assay.

### 1.2 *In Vitro* AChE Activity Assay

The AChE activity was measured using a modified 96-well microplate assay based on Ellman's method.[1] The enzyme hydrolyses the substrate (ATCH) resulting in the formation of thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 405 nm. The AChE stock solution (200 U/mL) was kept at -20°C. The further enzyme-dilution was done in deionized water. 0.7 mM DTNB and 3 mM ATCH were dissolved in the PB, respectively. The reaction mixture contained: 20 μL of 0.4 U/mL of AChE, 40 μL of test compound dissolved in PB (DMSO<1%, which was no influence on AChE activity) and 20 μL of ATCH, which incubated for 30 min at 37 °C. Then ice bath for 30 s, 10 μL of 1.0 N HCl was added to terminate reaction. After the addition of 120 μL of 0.7 mM DTNB, the absorbance was measured at 405 nm (EnSpire 2300, PerkinElmer) in 5 min.

Huperzine A was used as positive control, with $IC_{50}$ value of 22.6 nM. **1-12** did not show any significantly inhibition of AChE activity, even at the concentration $1\times10^{-3}$M.

### 1.3 *In Vitro* BACE1 Activity Assay

The assay was carried out using a homogeneous time-resolved fluorescence technique and a TruPoint$^{TM}$ beta-secretase assay kit$^{384}$ according to the manufacturer's protocol. Briefly, the test compounds in DMSO and recombinant human beta-secretase (BACE1) were first incubated in a 384-well black OptiPlate$^{TM}$ for 30

min at 20-25°C. Then, the fluorescent Eu-CEVNLDAEFK-Qsy7 substrate was added to the wells and incubated for 6 h at 20-25°C, and the reaction was terminated by adding stop solution. Relative fluorescence units (RFU) at an excitation of 340 nm and an emission of 615 nm and kinetics were measured using a micro-plate reader Envision (PerkinElmer).

Ⅵa was used as a positive control,[2] with $IC_{50}$ value of 2.5 nM. Most of the sesquiterpenoids did not show any percentage of fluorescence reduction, except that 4-epi-2-acetoxyacorenone (**2**) showed week inhibitory rate (I %) at the concentration $1\times10^{-3}$M (33.6 %).

**1.4 Statistics**

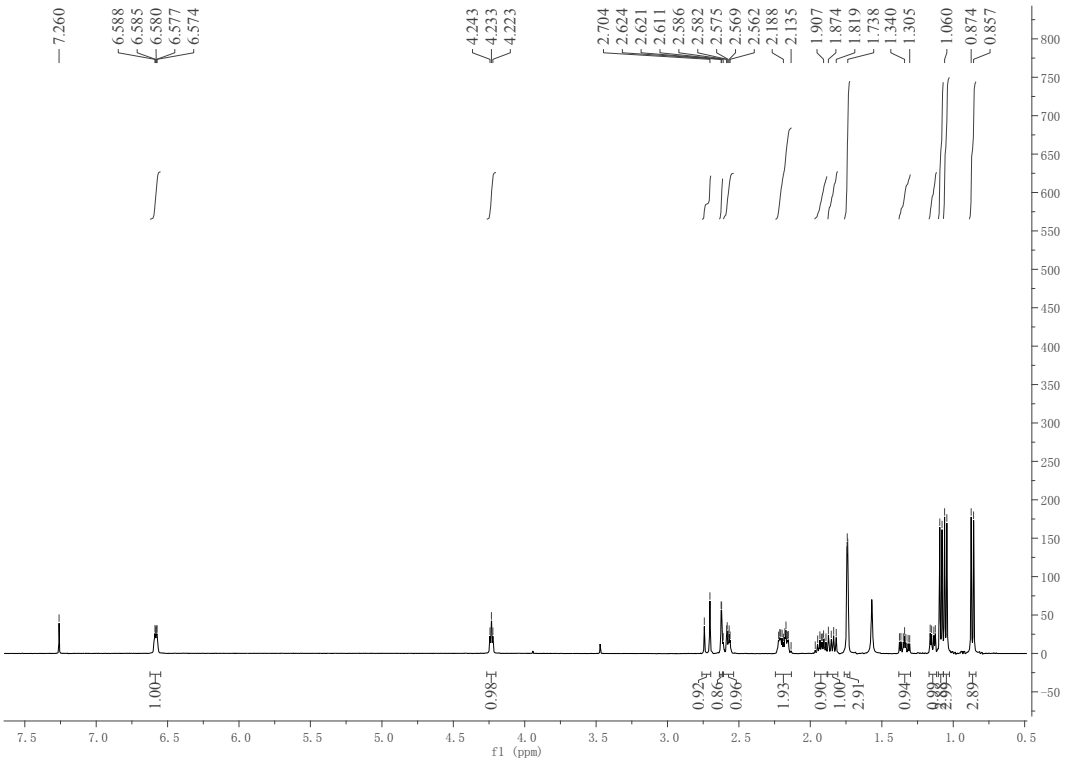
All statistical analyses were performed using Graph Pad® 5.03 for Windows. Data are shown as means ± S.D. The percent inhibition of AChE activity was calculated as follows: I % = ($E - S$) / $E$ × 100%, where $E$ and $S$ were the respective enzyme without and with the test sample, respectively. The percentage of inhibition of BACE1 activity was calculated using the following equation: I % = [1 − ($S - B$) / ($N - B$)] × 100%, where $N$ is the activity of enzyme without test sample, $S$ is the activity of enzyme with test sample and $B$ is the background without enzyme and test sample. For the determination of $IC_{50}$ concentrations, the mean % inhibition dose-response curves were fitted to the dose-response-inhibition [log (inhibitor) vs. response-Variable slope (four parameters)]. The dose-response equation: Y = Bottom + (Top − Bottom) / [1 + 10($\log IC_{50}$ − X) × Hillslope], where X is the compound concentration, Y is the I %, top and bottom are the plateaus in the units of the y-axis, Hillslope describes the steepness of the family of curves.

**Reference:**

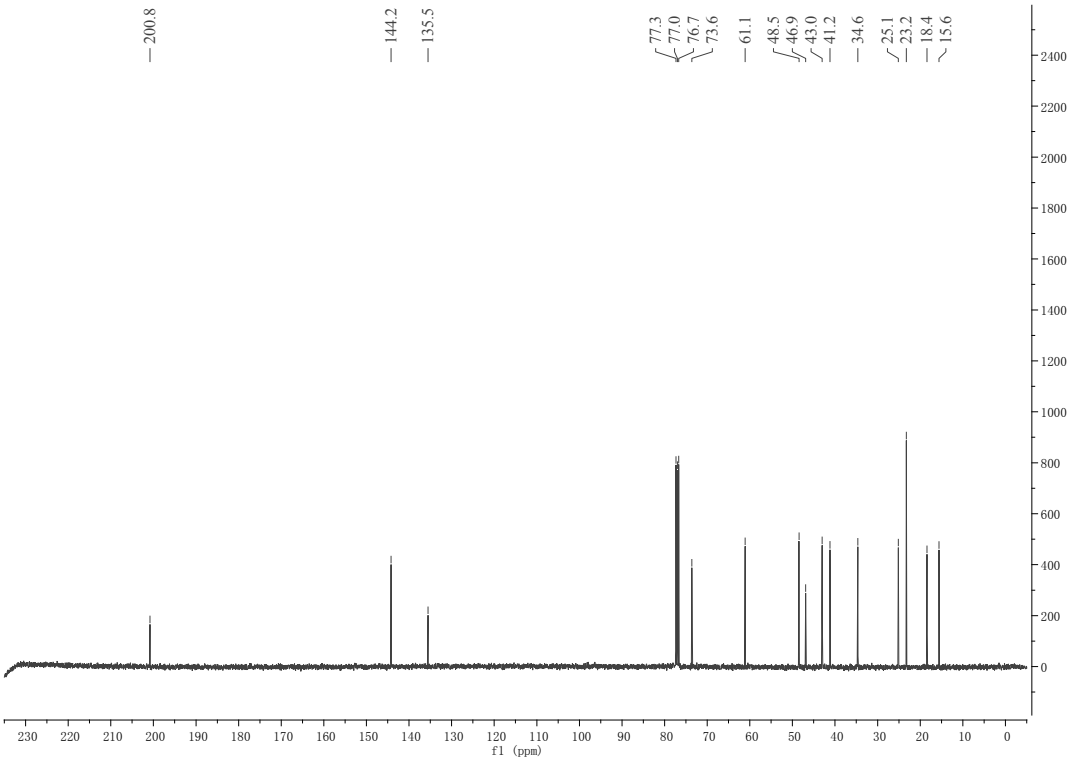
[1] G. L. Ellman, K. D. Courtney, V. Andres, R. M. Feather-Stone, *Biochem. Pharmacol*. 1961, **7**, 88-95.

[2] X. R. Cheng, Y. Zhou, W. Gu, J. Wu, A. H. Nie, J. P. Cheng, J. W. Zhou, W. X. Zhou, Y. X. Zhang, *Journal of Alzheimer's Disease*, 2013, **37**, 823-834.

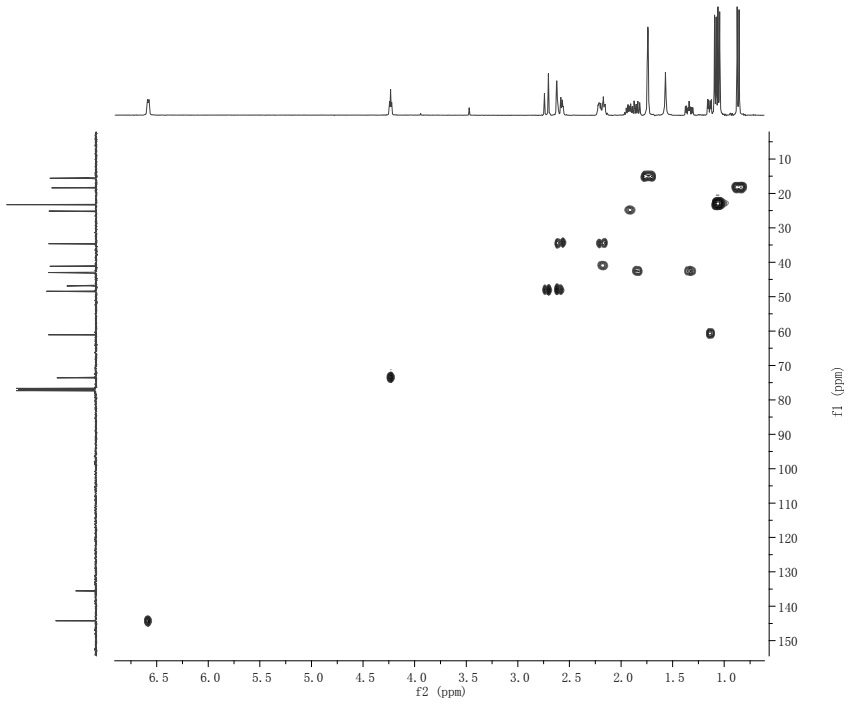
## 2 The 1D and 2D NMR spectra of 4-epi-2-hydroxyacorenone (1)



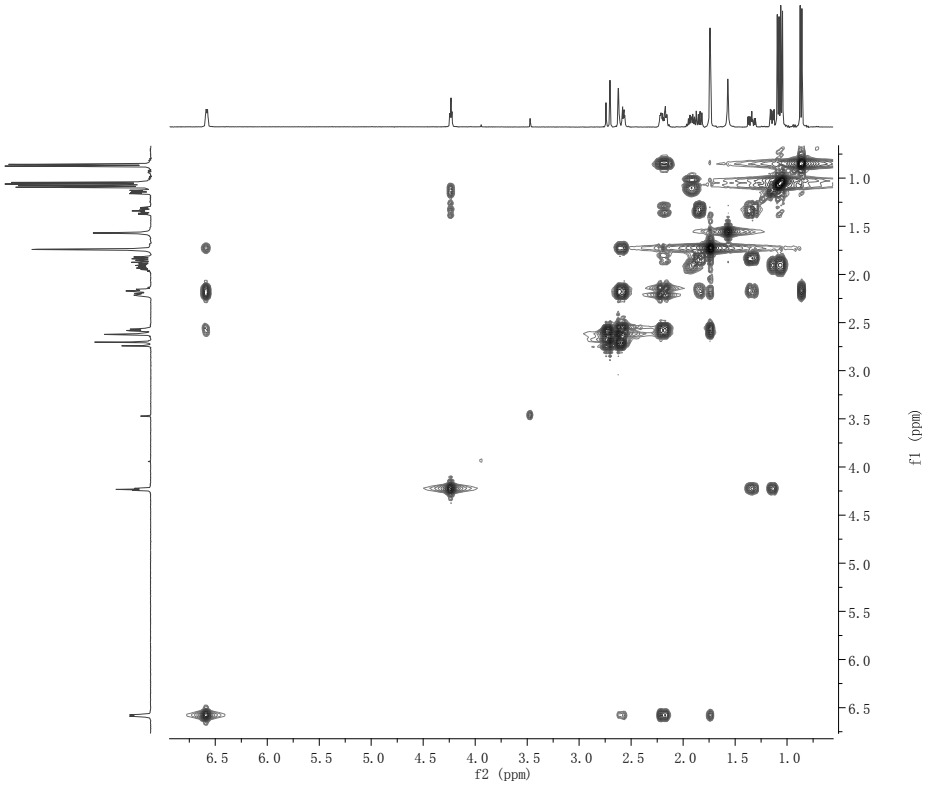

$^{1}$H NMR spectrum for 4-epi-2-hydroxyacorenone (**1**) in $CDCl_3$



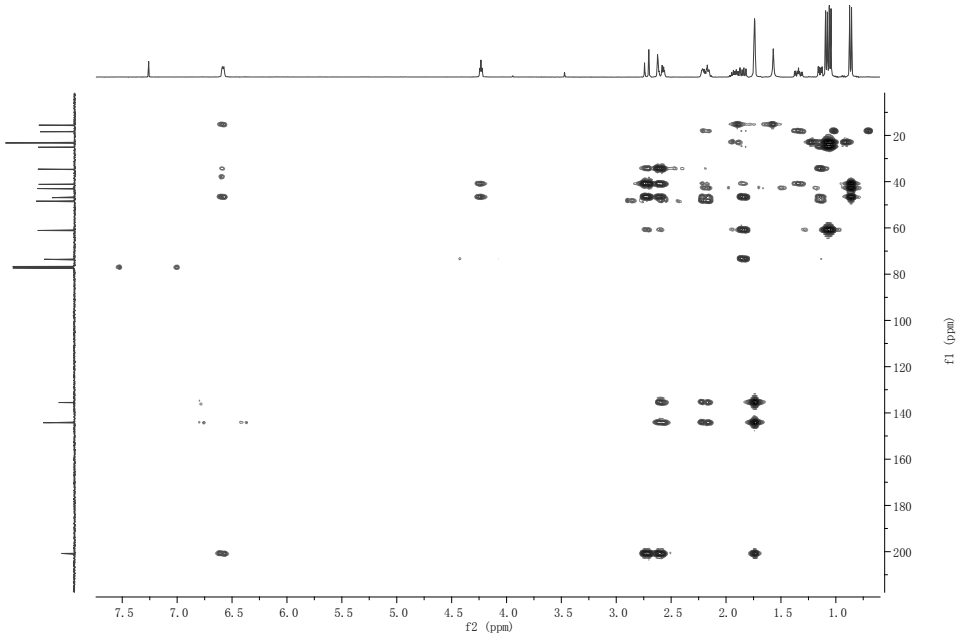

$^{13}$C NMR spectrum for 4-epi-2-hydroxyacorenone (**1**) in $CDCl_3$




HSQC spectrum for 4-epi-2-hydroxyacorenone (**1**) in $CDCl_3$




$^1H$-$^1H$ COSY spectrum for 4-epi-2-hydroxyacorenone (**1**) in $CDCl_3$

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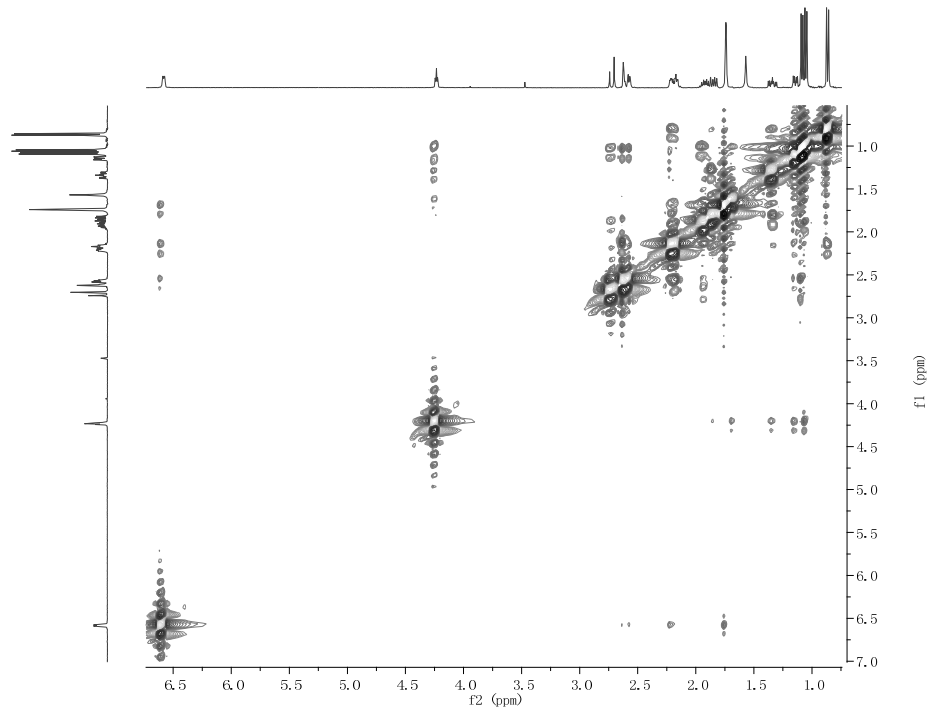
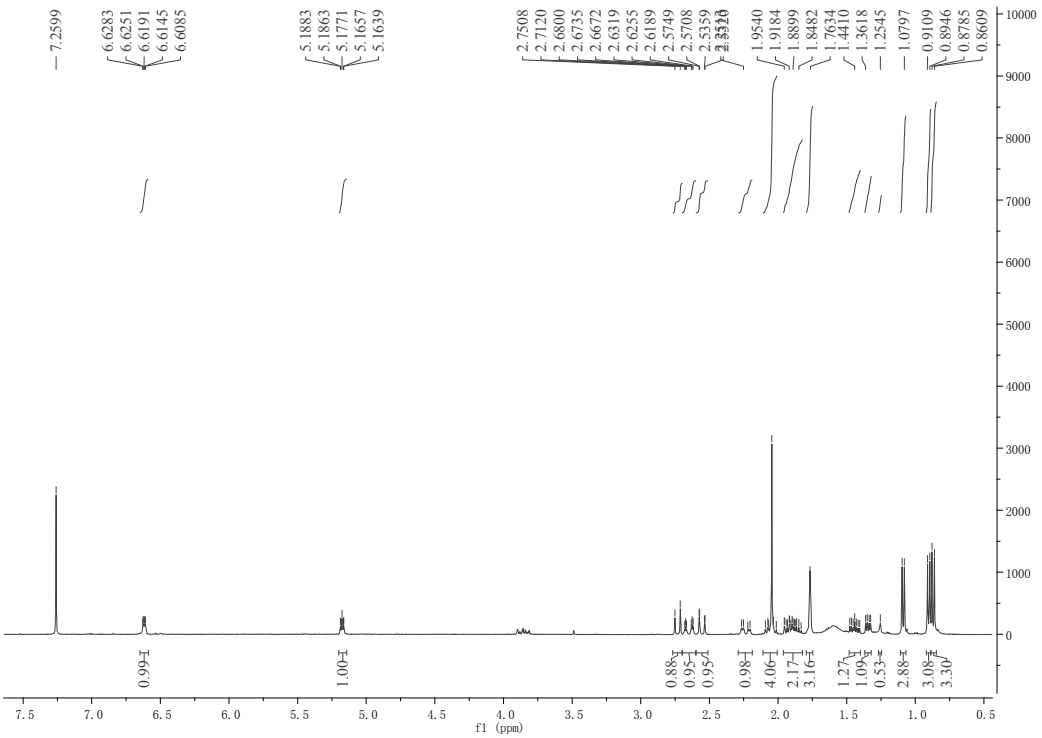
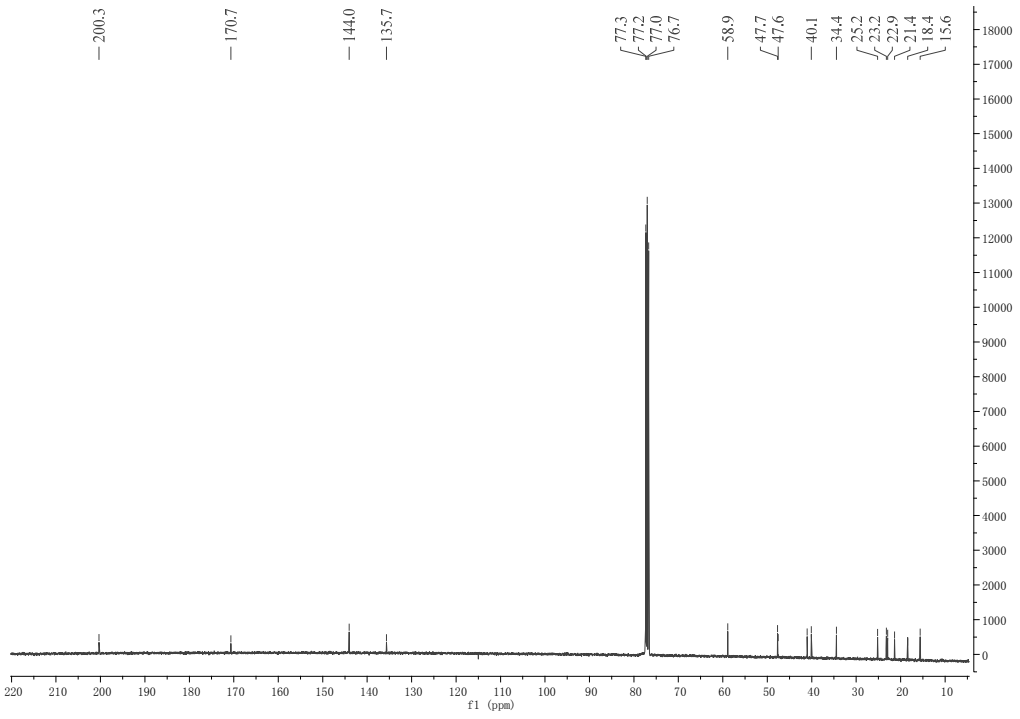



NOESY spectrum for 4-epi-2-hydroxyacorenone (**1**) in $CDCl_3$

## 3 The 1D and 2D NMR spectra of 4-epi-2-acetoxyacorenone (2)




$^{1}$H NMR spectrum for 4-epi-2-acetoxyacorenone (**2**) in $CDCl_3$

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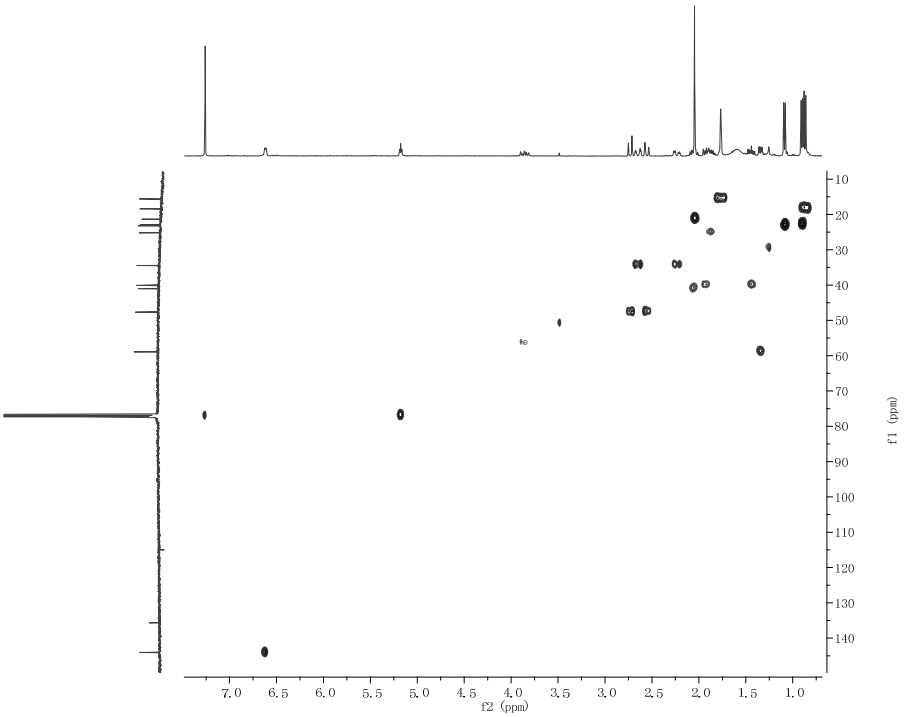


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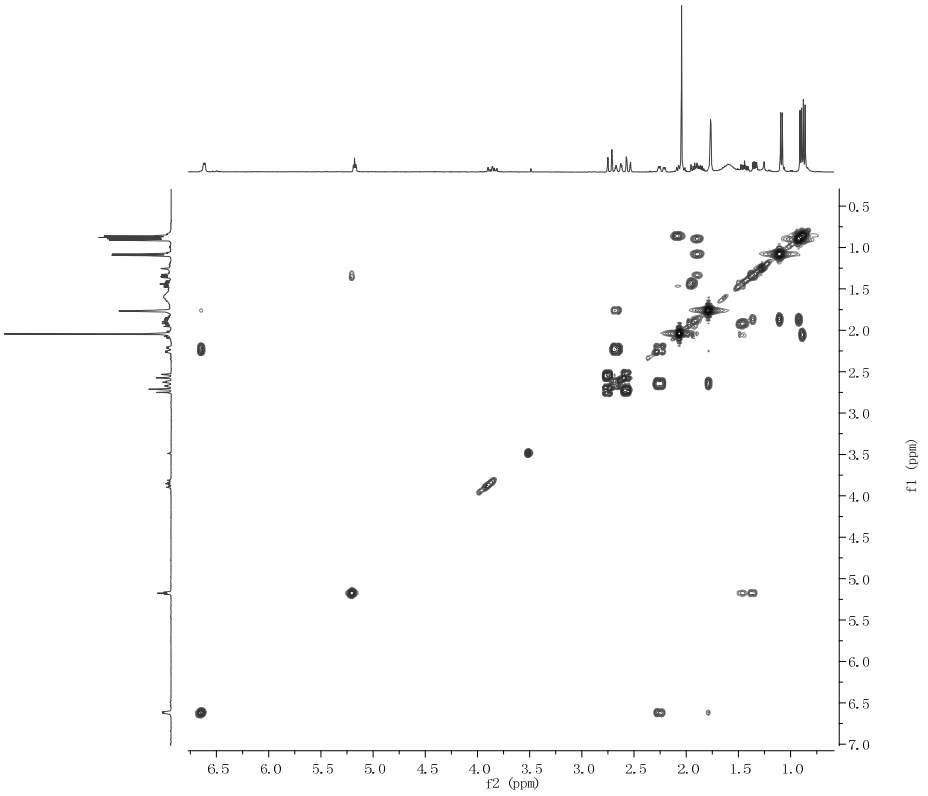


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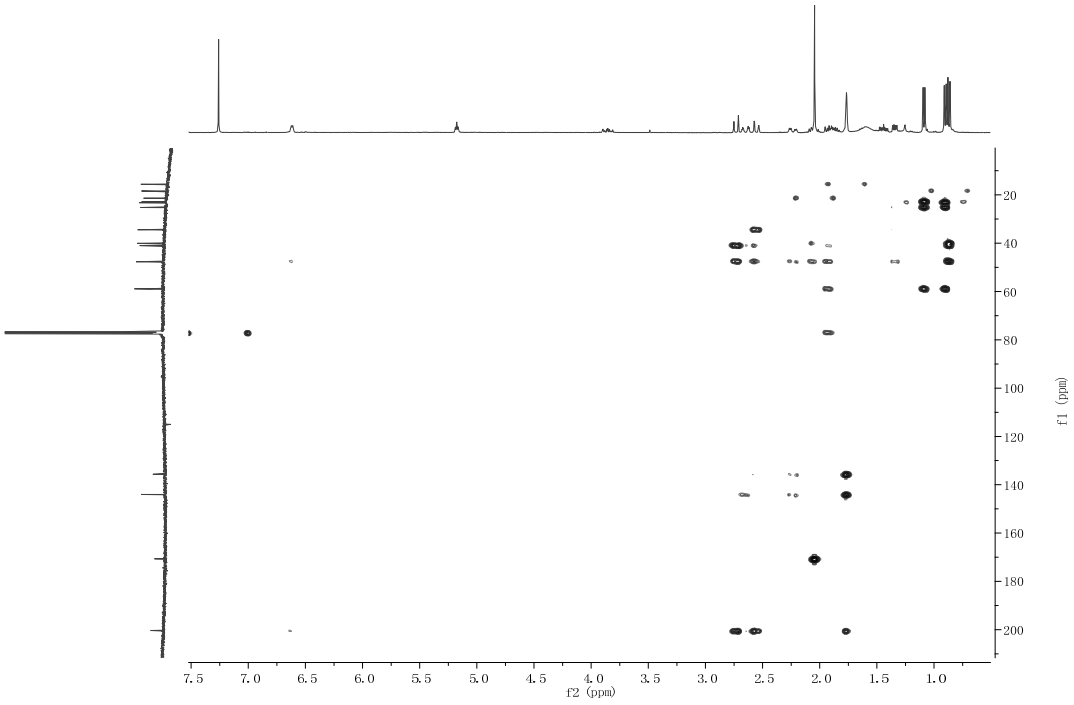
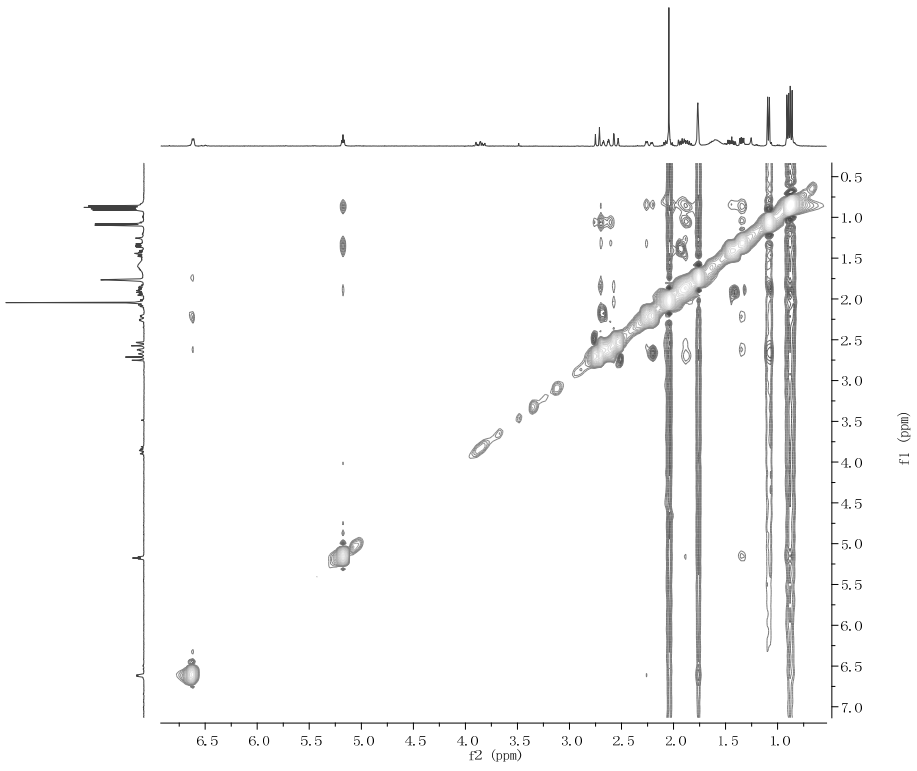
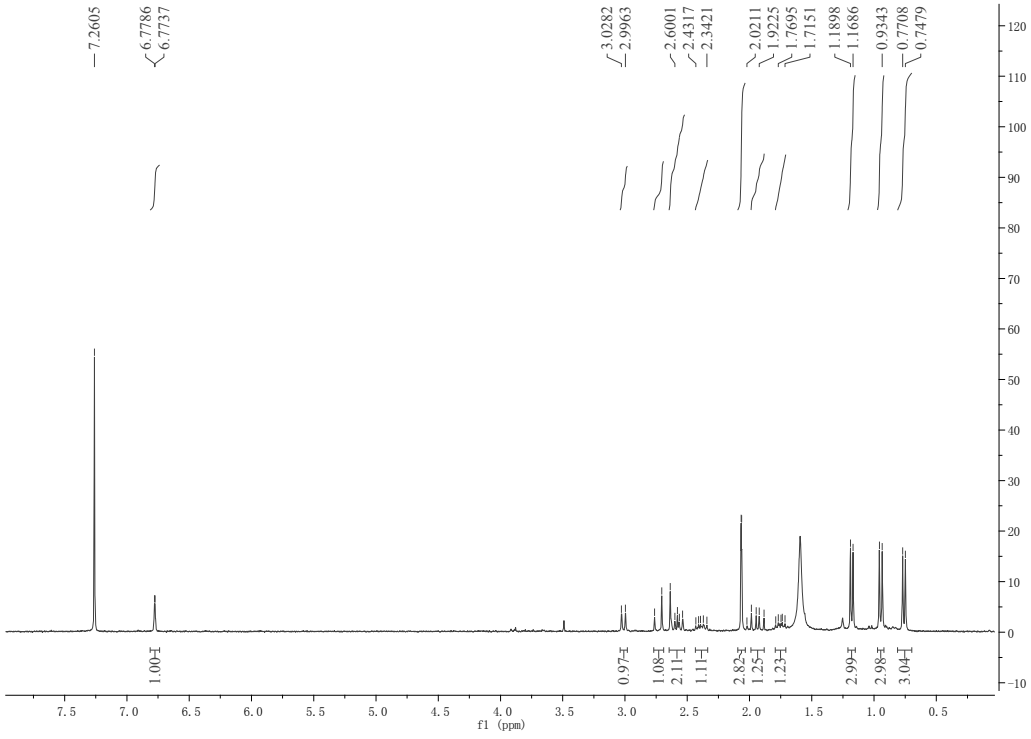



HMBC spectrum for 4-epi-2-acetoxyacorenone (**2**) in $CDCl_3$

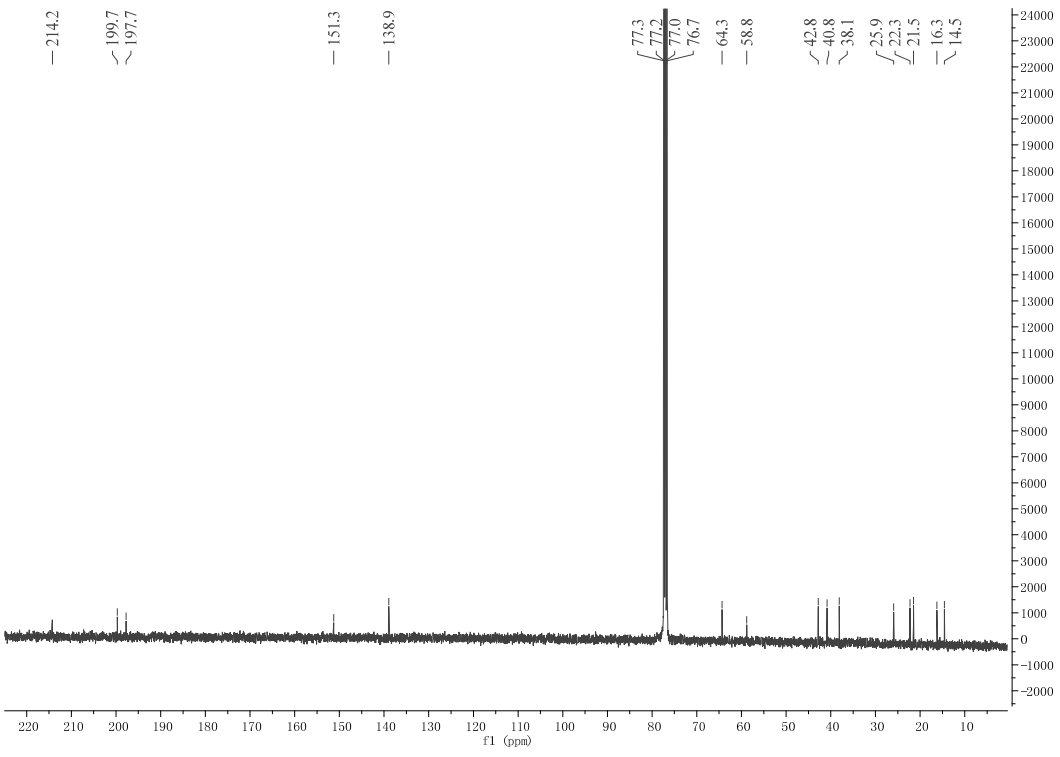



NOESY spectrum for 4-epi-2-acetoxyacorenone (**2**) in $CDCl_3$

## 4 The 1D and 2D NMR spectra of acotatarone A (3)




$^{1}$H NMR spectrum for acotatarone A (**3**) in $CDCl_3$

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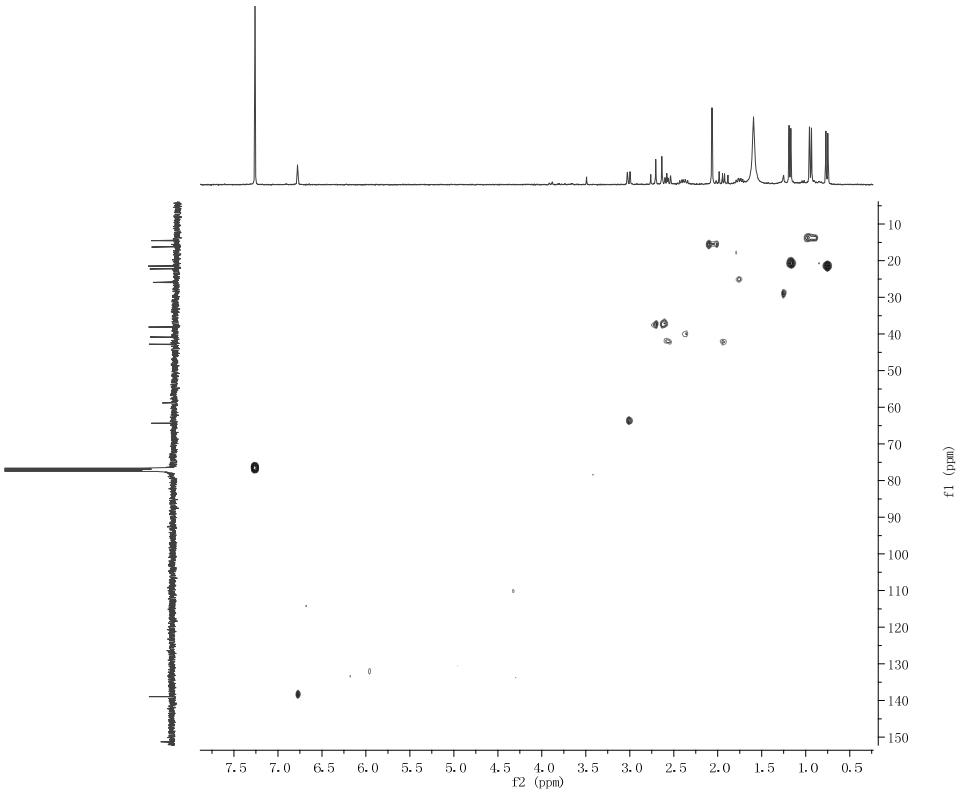


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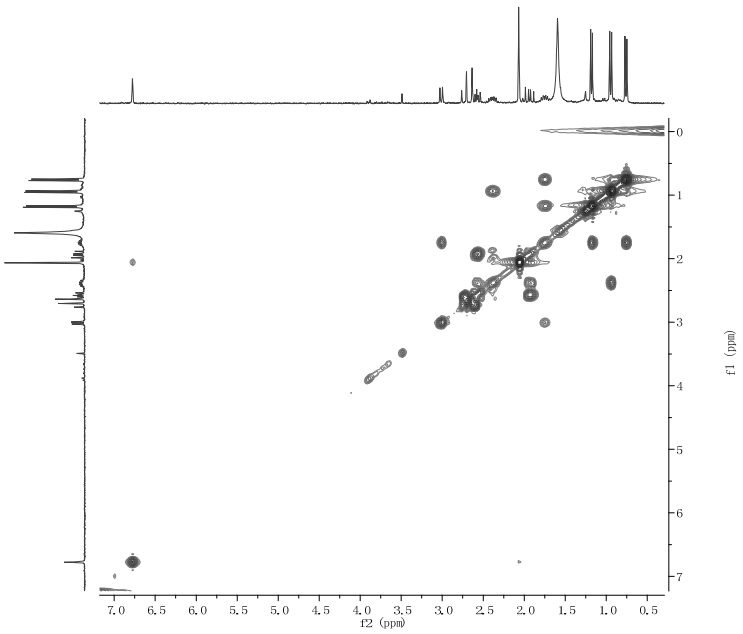


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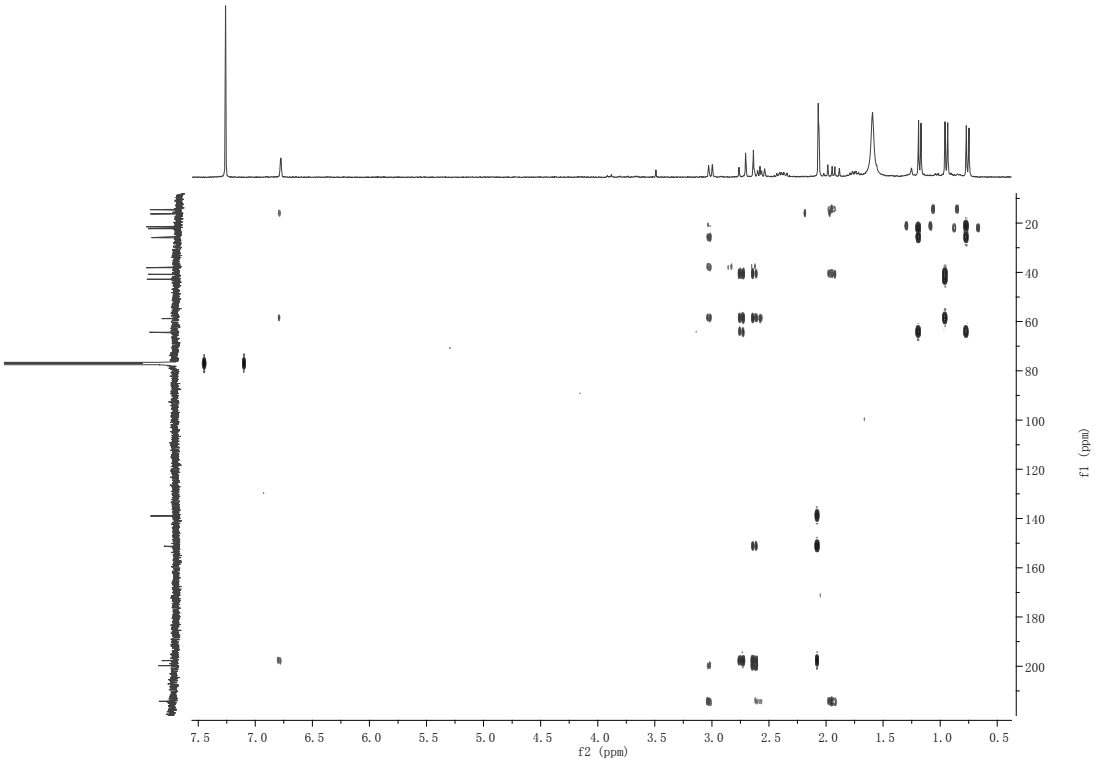
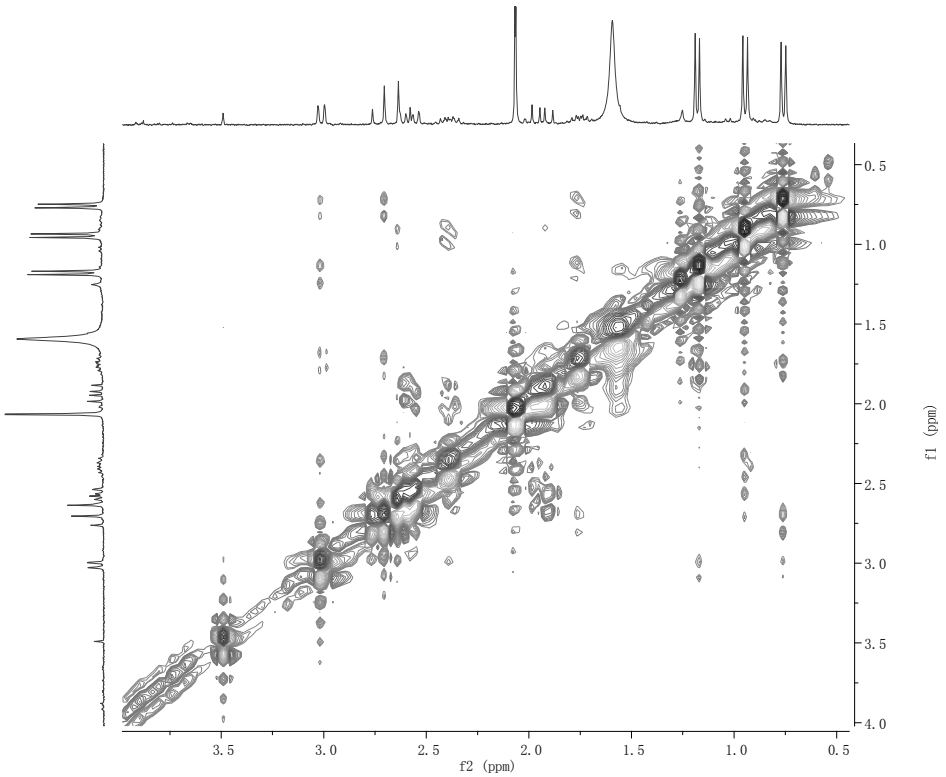
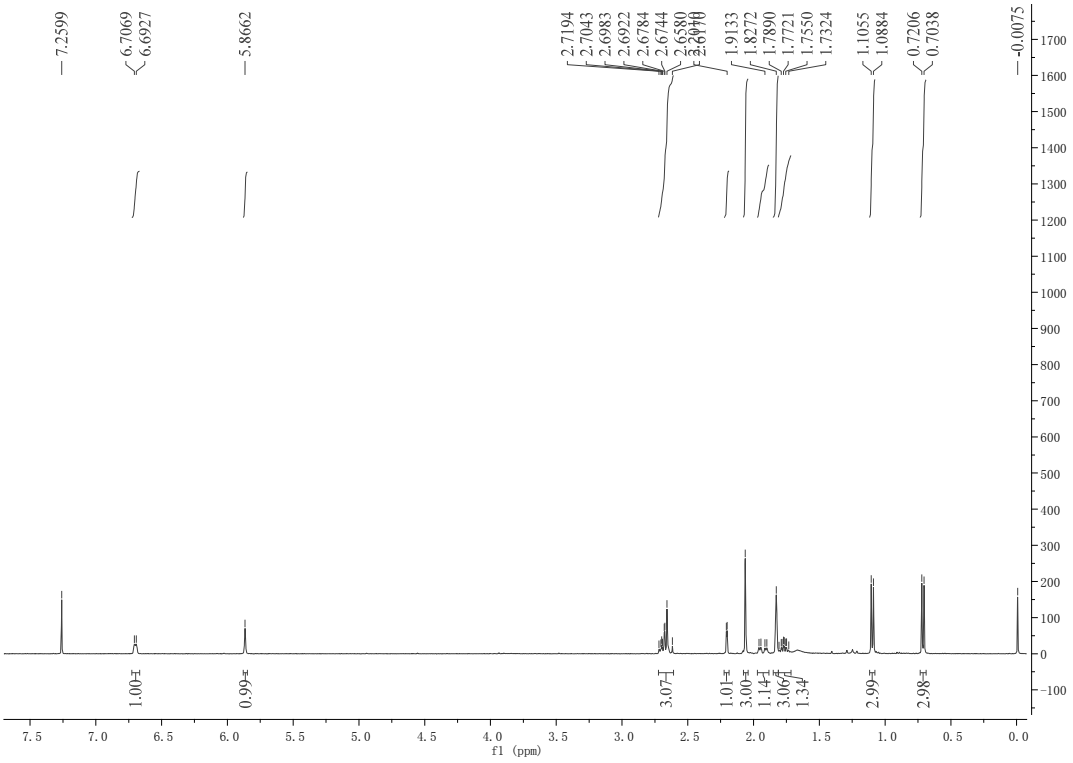



HMBC spectrum for acotatarone A (**3**) in $CDCl_3$

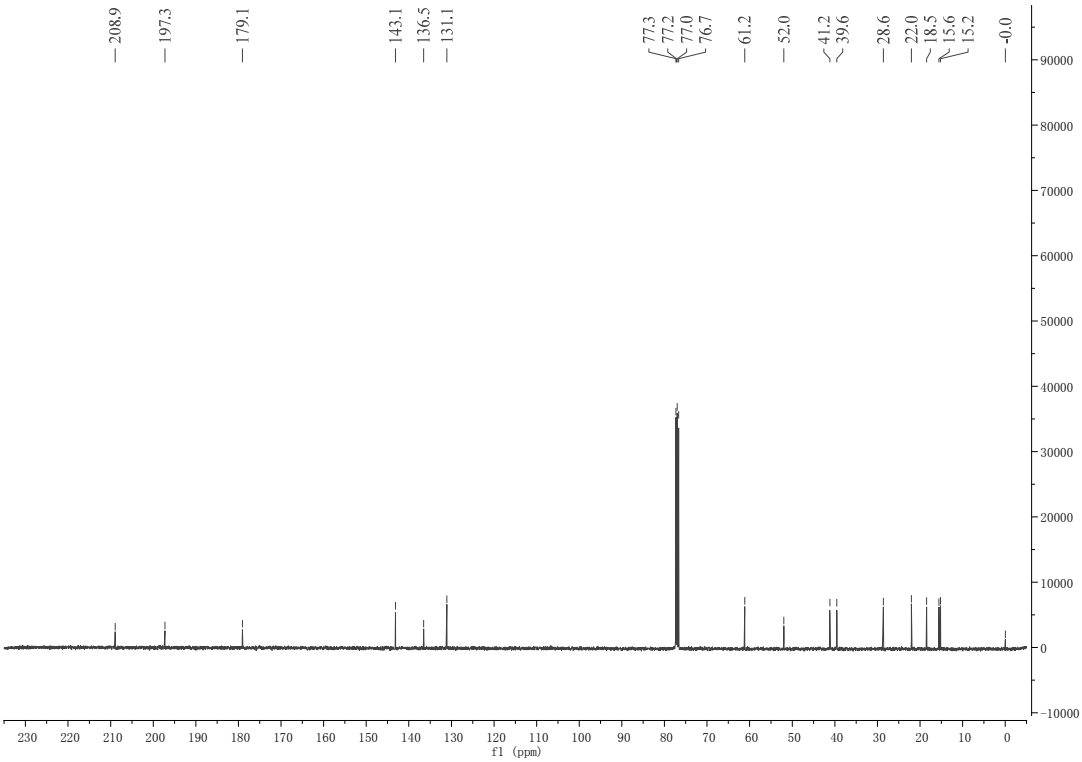



NOESY spectrum for acotatarone A (**3**) in $CDCl_3$

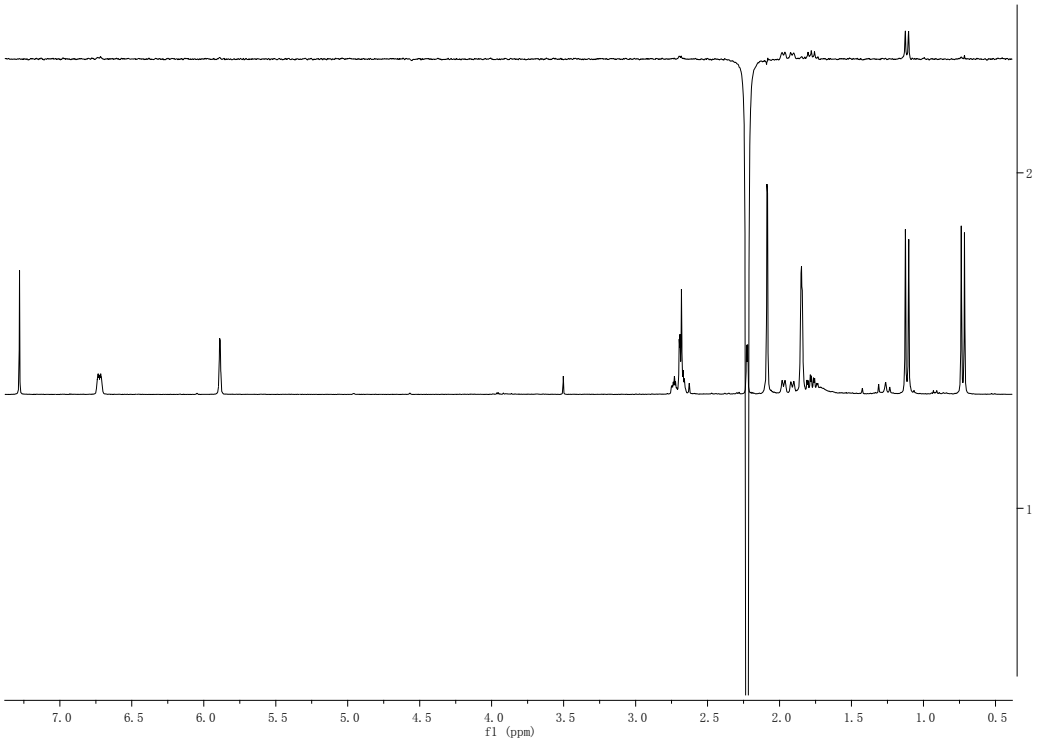
## 5 The 1D and 2D NMR spectra of acotatarone B (4)



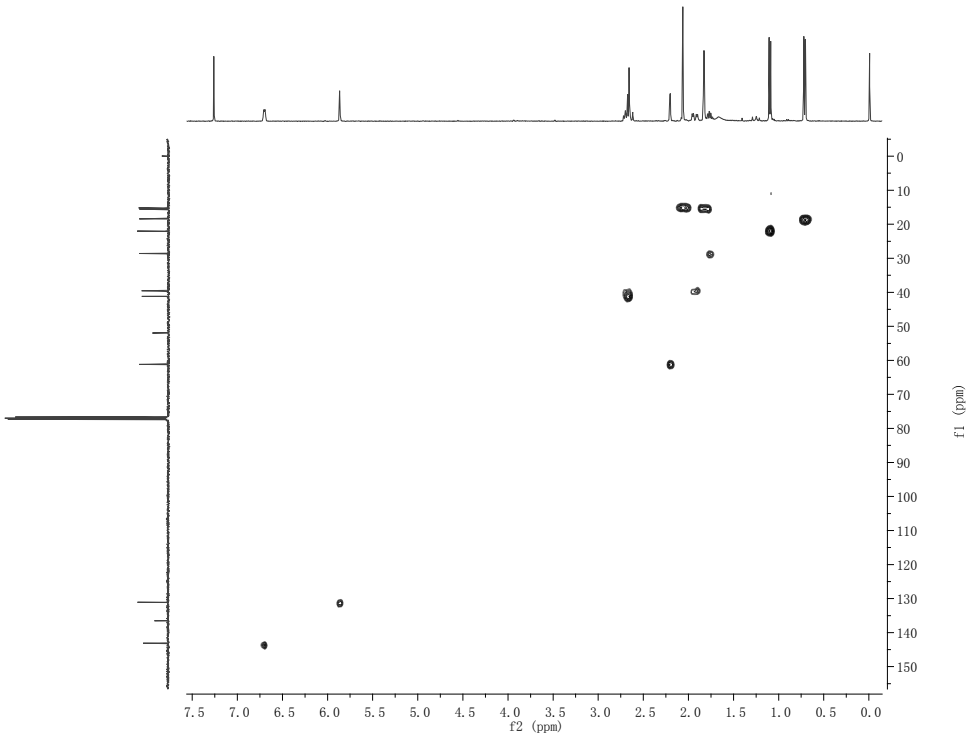

$^{1}$H NMR spectrum for acotatarone B (**4**) in $CDCl_3$



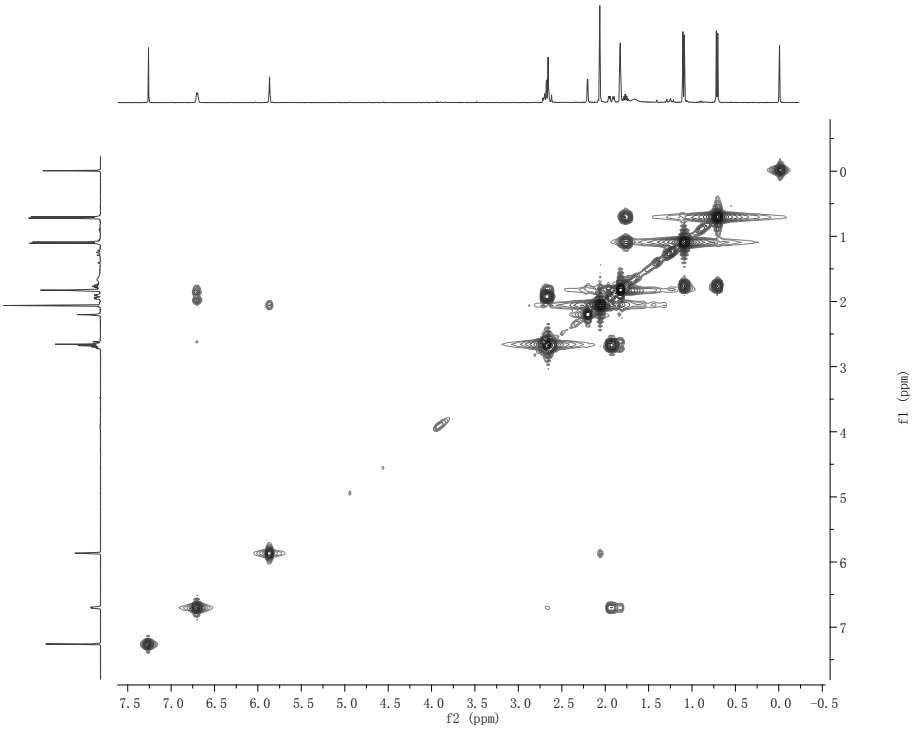

$^{13}$C NMR spectrum for acotatarone B (**4**) in $CDCl_3$



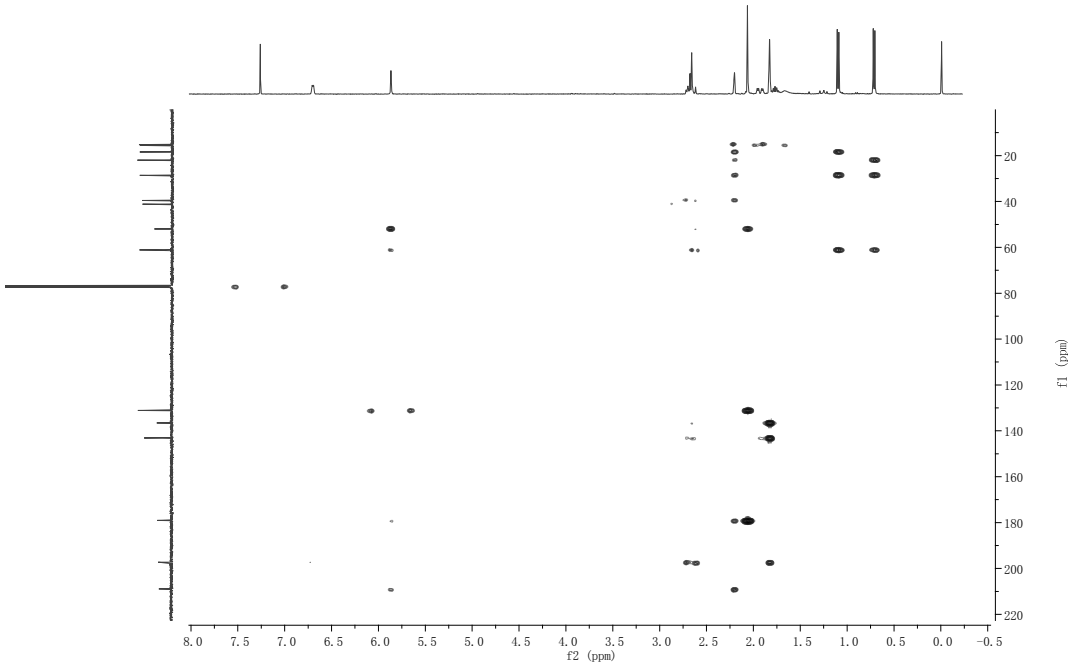

$^{1}$H NMR and 1D-NOE spectra for acotatarone B (**4**) in $CDCl_3$



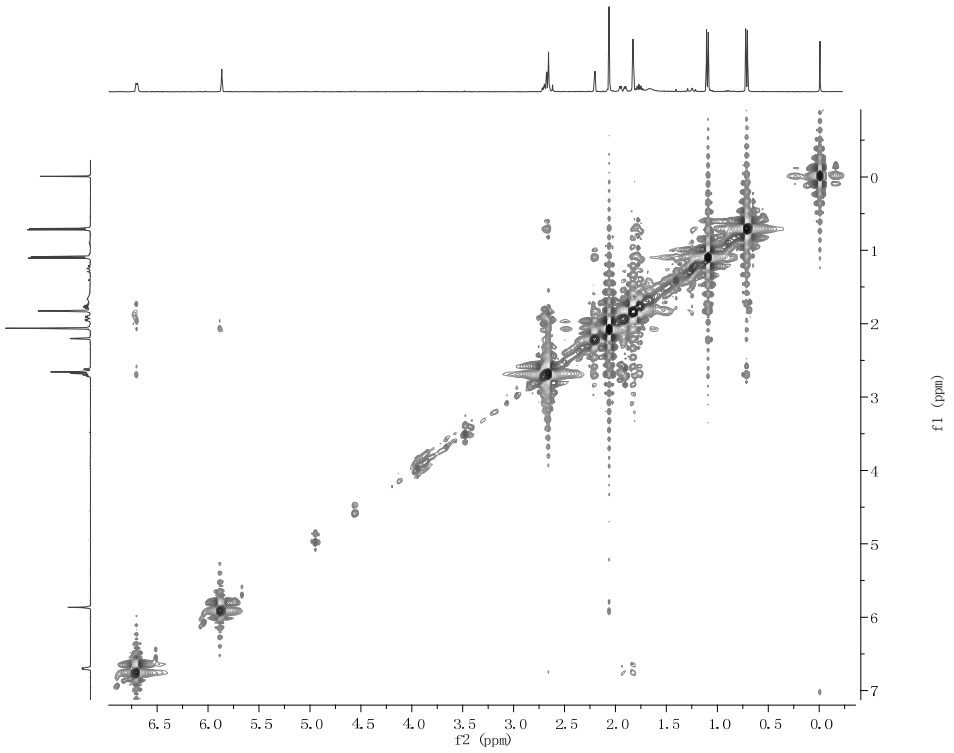

HSQC spectrum for acotatarone B (**4**) in $CDCl_3$




$^{1}$H-$^{1}$H COSY spectrum for acotatarone B (**4**) in $CDCl_3$

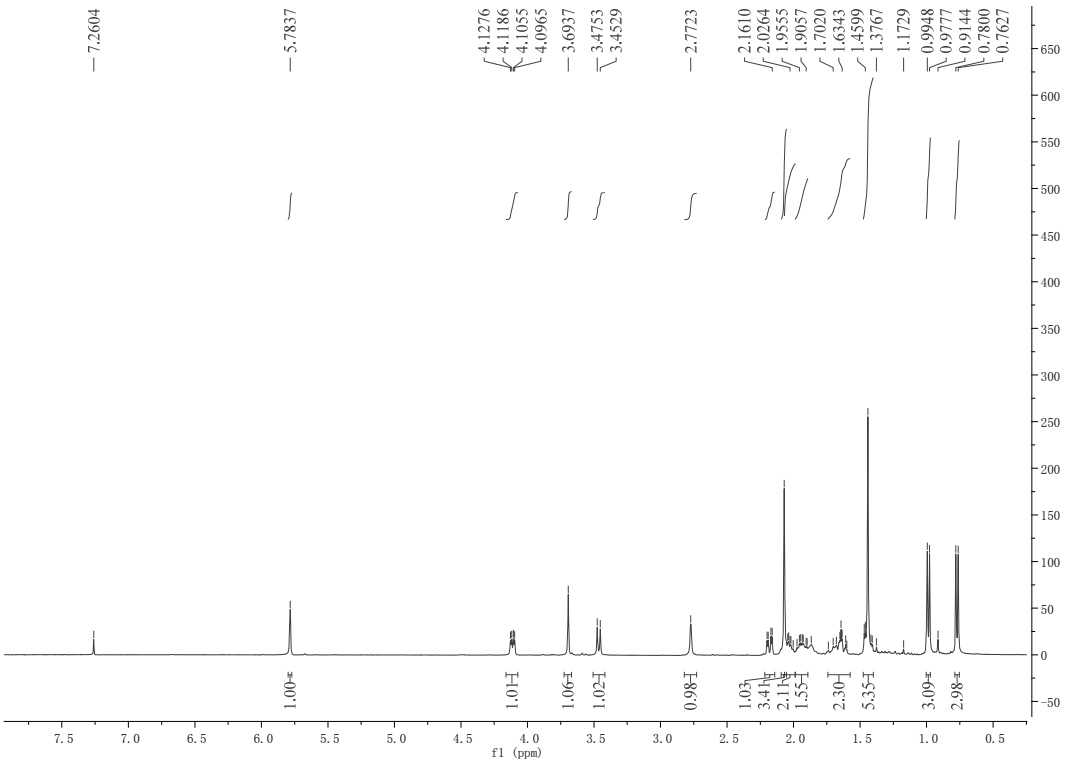



HMBC spectrum for acotatarone B (**4**) in $CDCl_3$

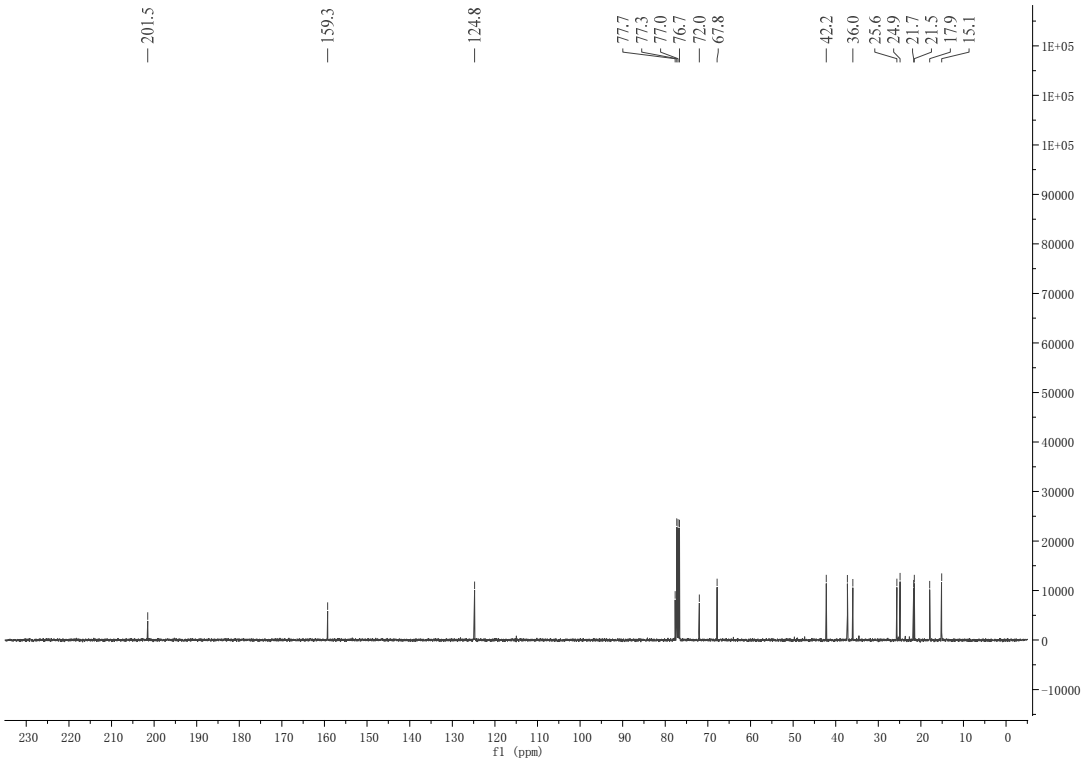



NOESY spectrum for acotatarone B (**4**) in $CDCl_3$

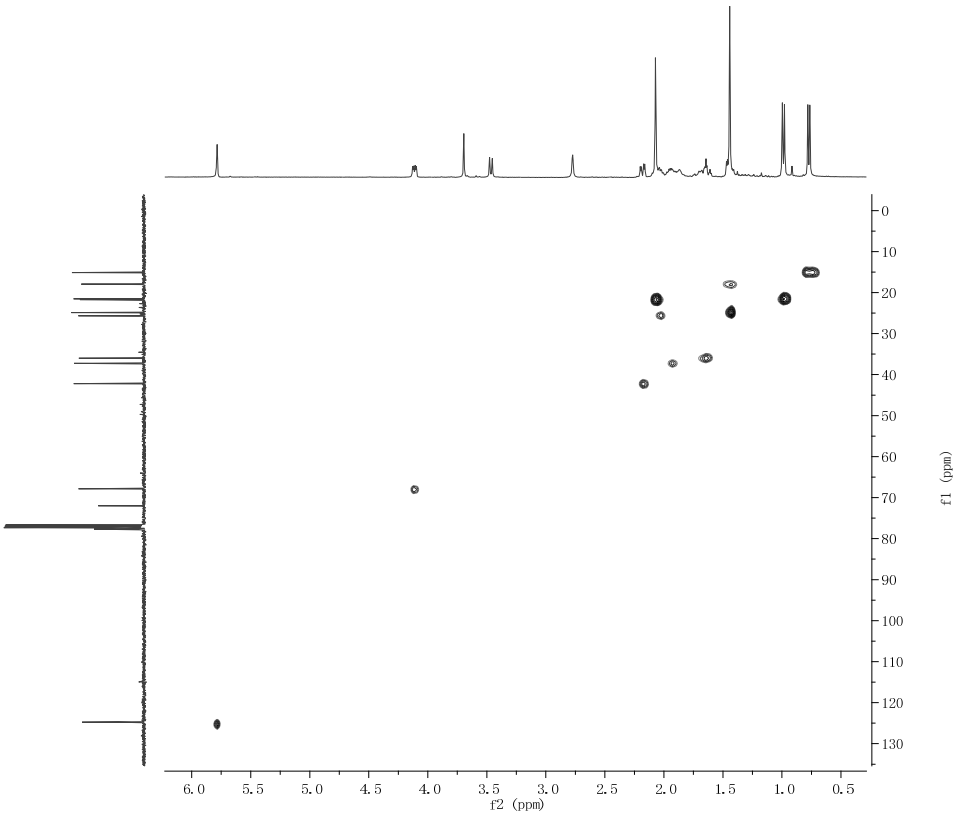
## 6 The 1D and 2D NMR spectra of tatarinowin C (5)



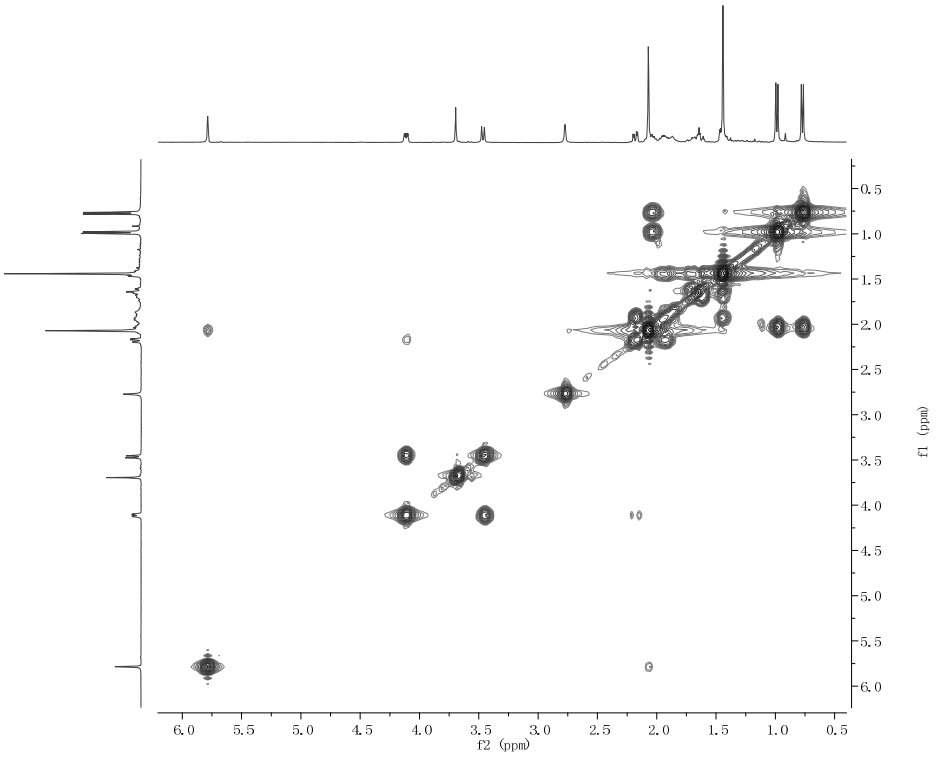

$^{1}$H NMR spectrum for tatarinowin C (**5**) in $CDCl_3$




$^{13}$C NMR spectrum for tatarinowin C (**5**) in $CDCl_3$




HSQC spectrum for tatarinowin C (**5**) in $CDCl_3$

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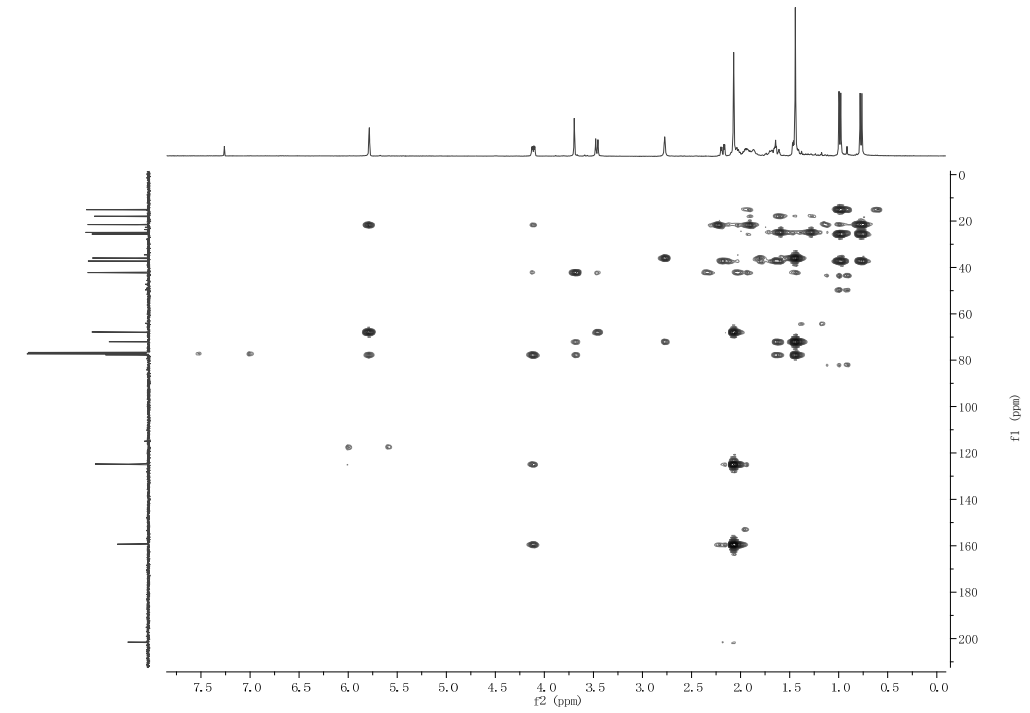
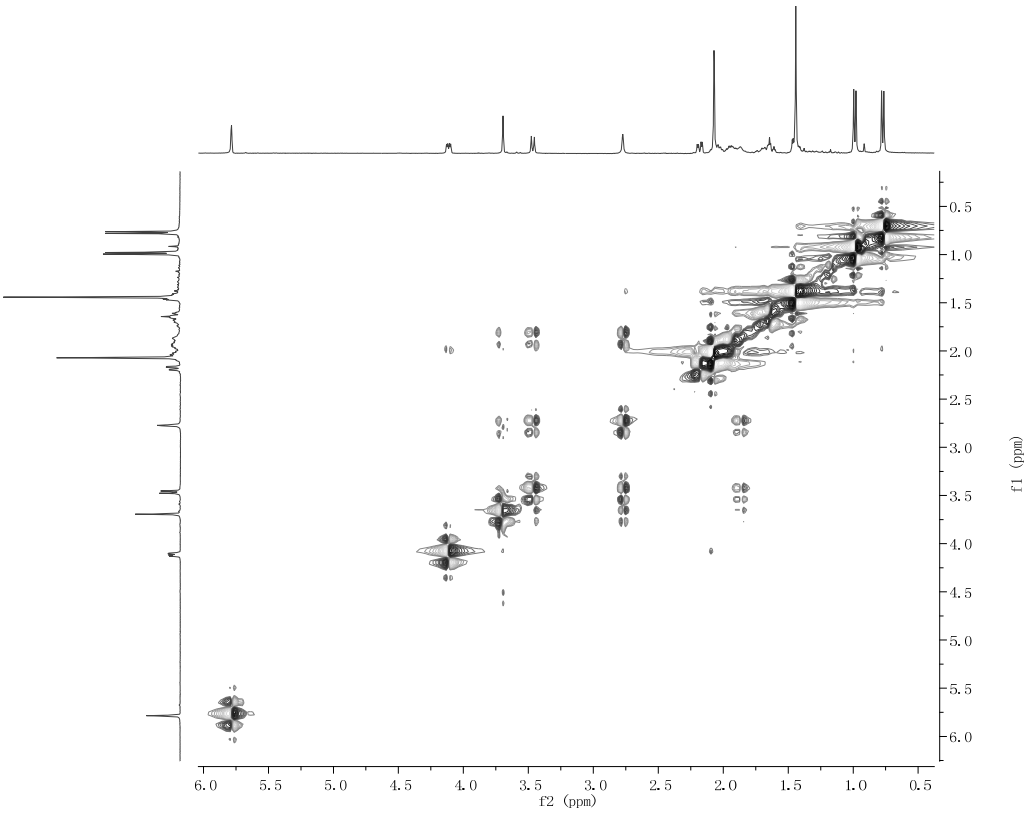
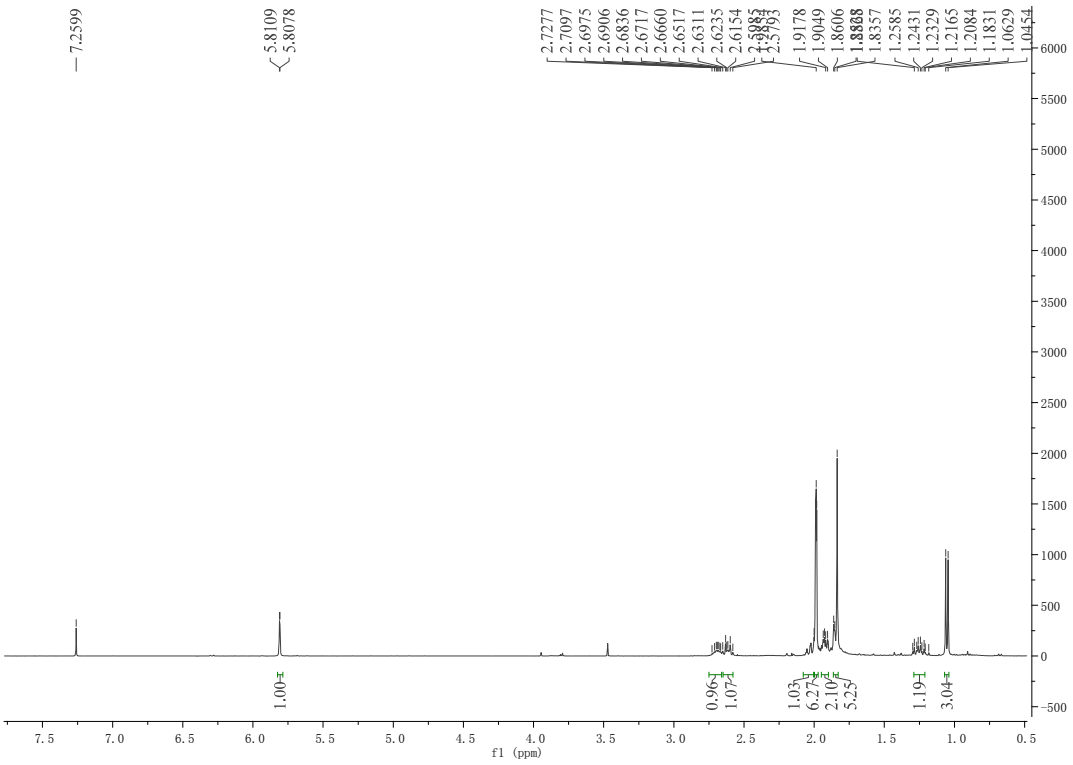



HMBC spectrum for tatarinowin C (**5**) in $CDCl_3$

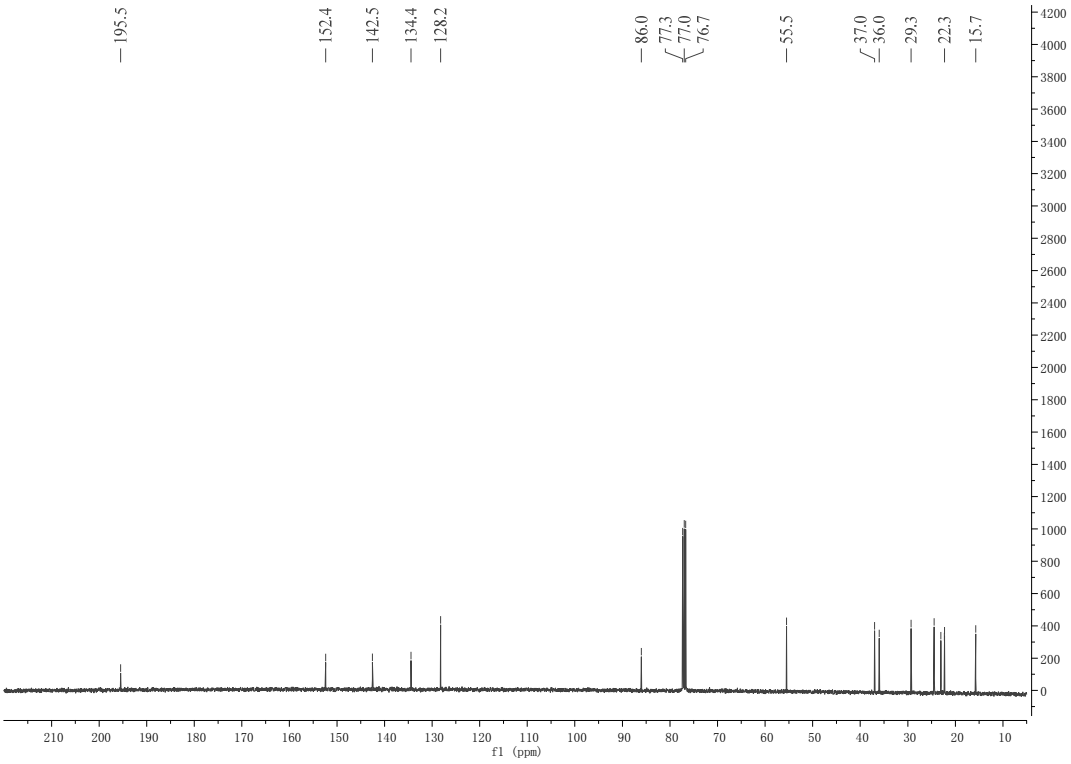



NOESY spectrum for tatarinowin C (**5**) in $CDCl_3$

## 7 The 1D and 2D NMR spectra of acotatarone C (6)




$^{1}$H NMR spectrum for acotatarone C (**6**) in $CDCl_3$

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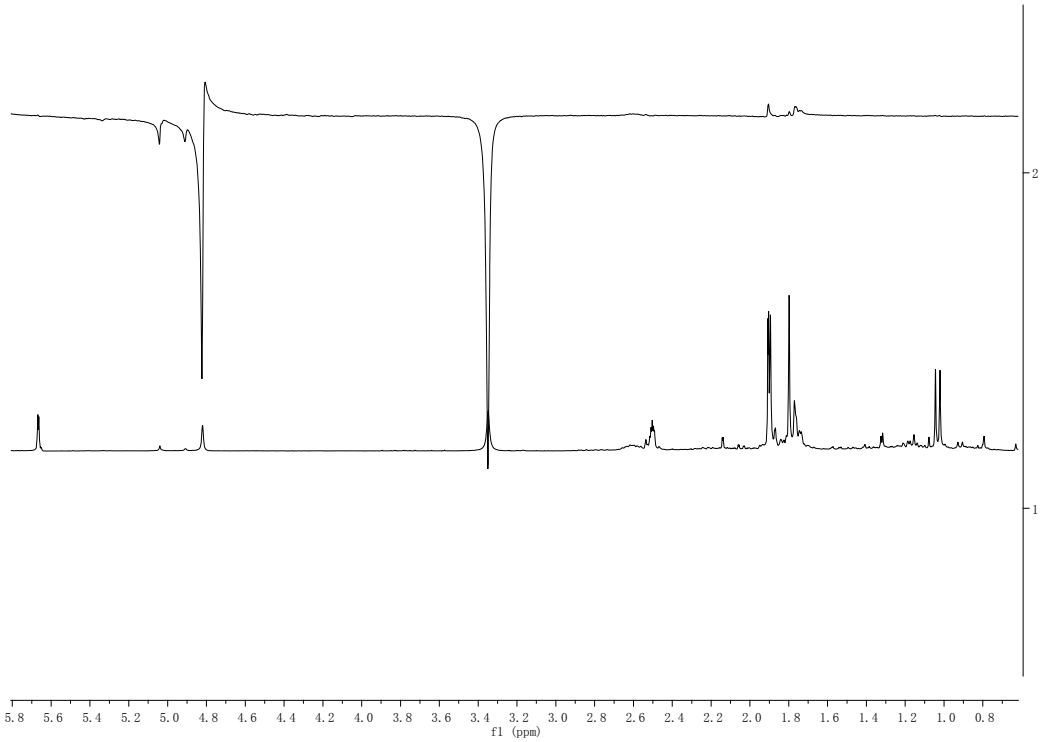
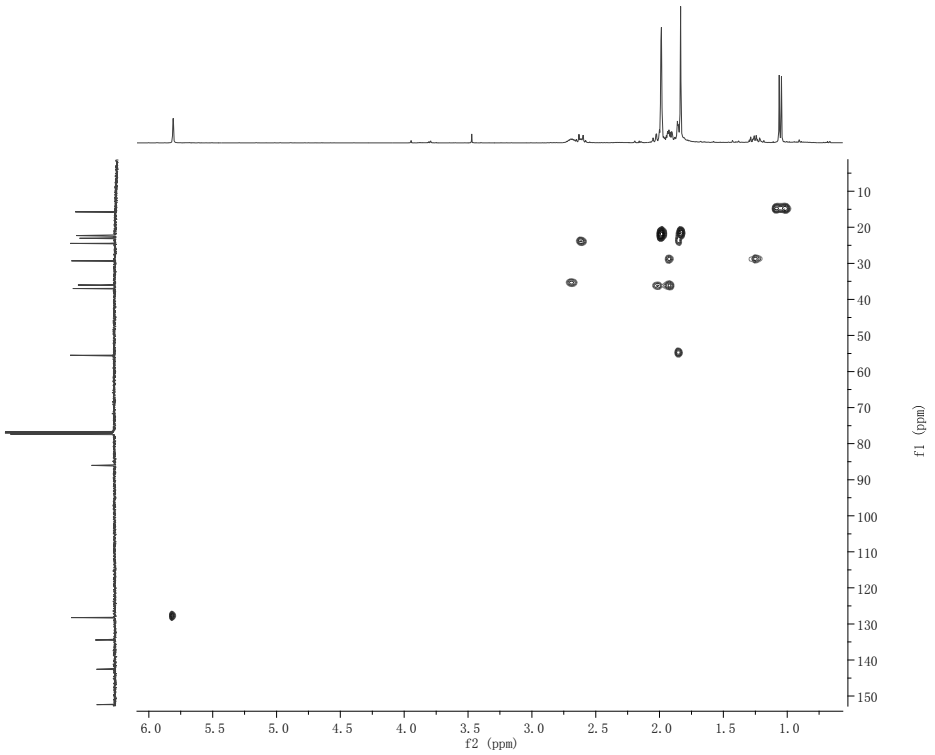
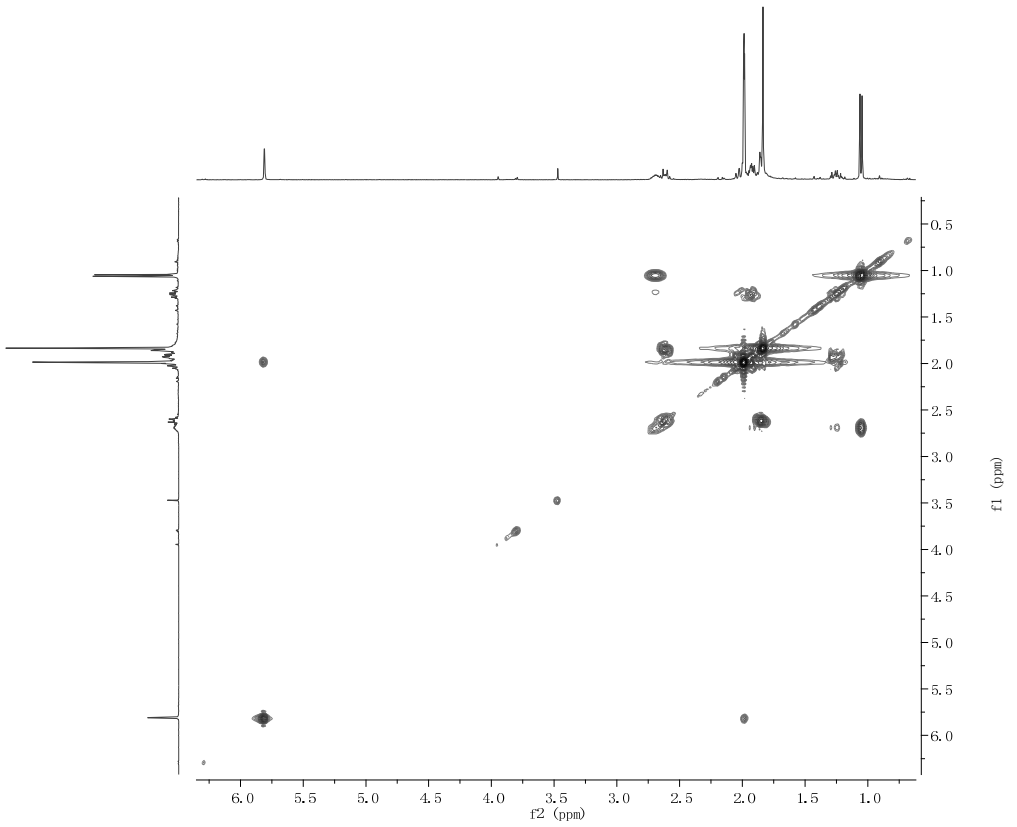


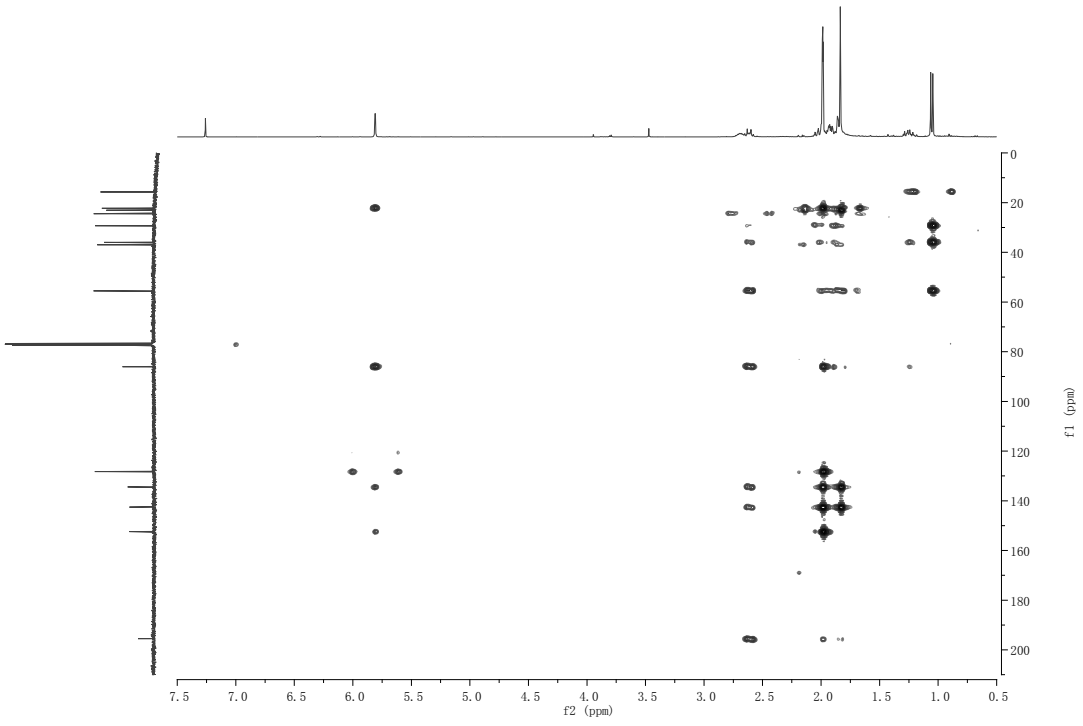

$^1$H NMR and 1D-NOE spectra for acotatarone C (**6**) in DMSO-$d_6$




HSQC spectrum for acotatarone C (**6**) in $CDCl_3$




$^{1}H$-$^{1}H$ COSY spectrum for acotatarone C (**6**) in $CDCl_3$




HMBC spectrum for acotatarone C (**6**) in $CDCl_3$