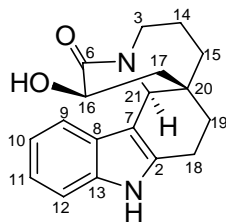


Kopsihainanines A and B, two unusual alkaloids from *Kopsia hainanensis*

Jia Chen, Jian-Jun Chen, Xiaojun Yao, and Kun Gao*

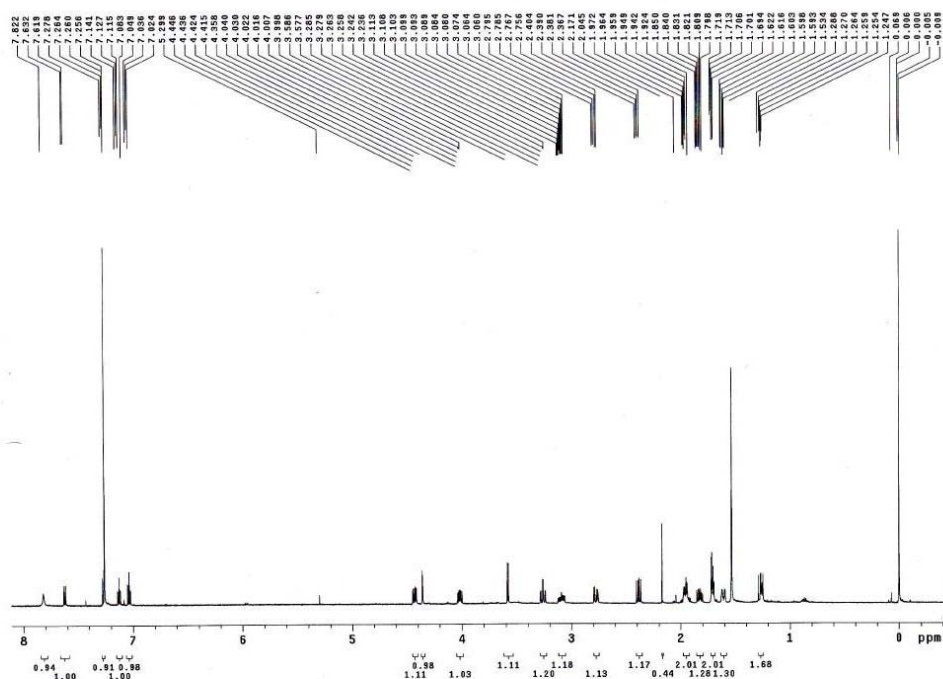
*State Key Laboratory of Applied Organic Chemistry, College of Chemistry and
Chemical Engineering, Lanzhou University, Lanzhou 730000, People's Republic of
China*

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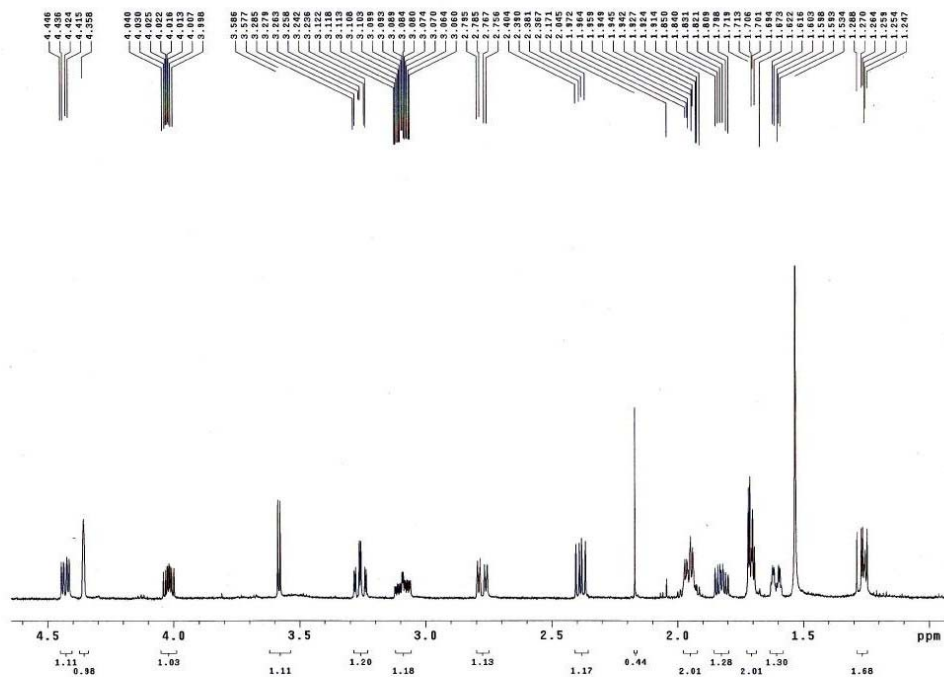


Structure of kopsihainanine A (1)

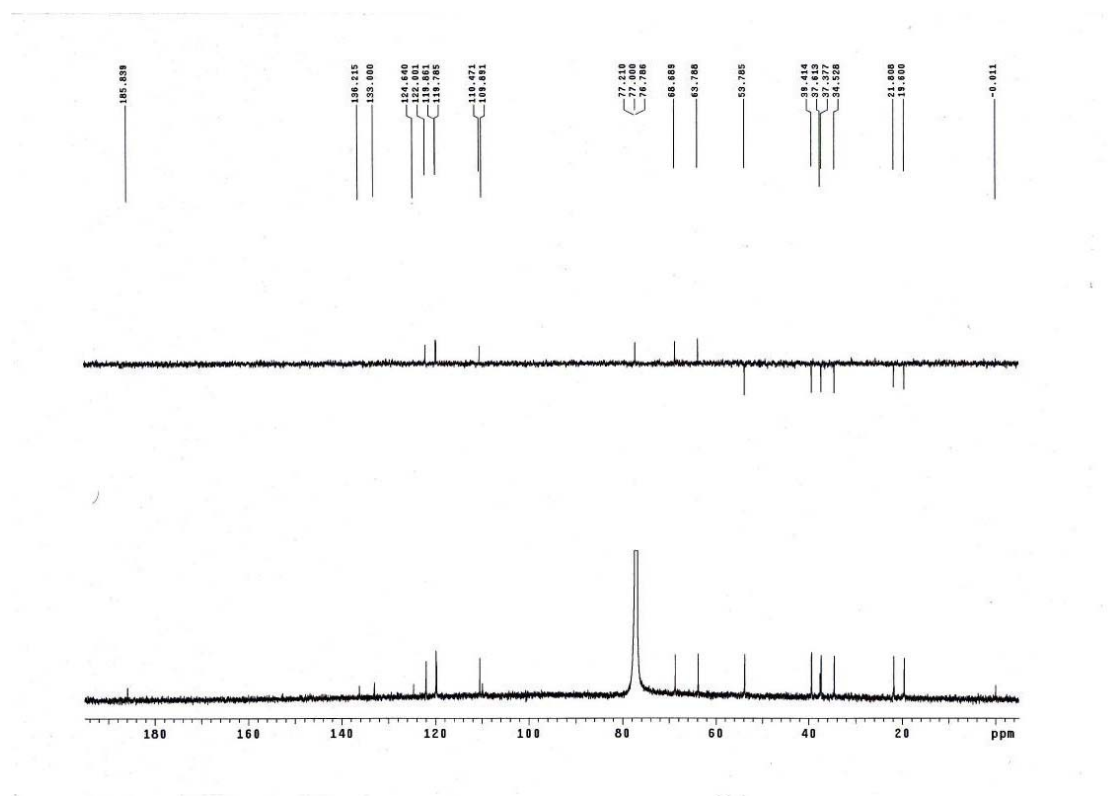
Kopsihainanine A: ^1H NMR in CDCl_3 (Varian Mercury-600)



Kopsihainanine A: ^1H NMR in CDCl_3 (Varian Mercury-600)

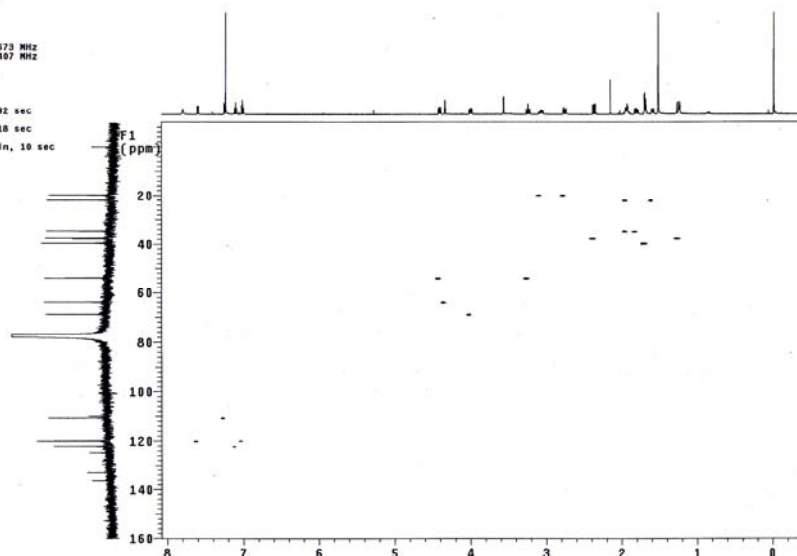


Kopsihainanine A: ^{13}C NMR in CDCl_3 (Varian Mercury-600, 125 MHz)



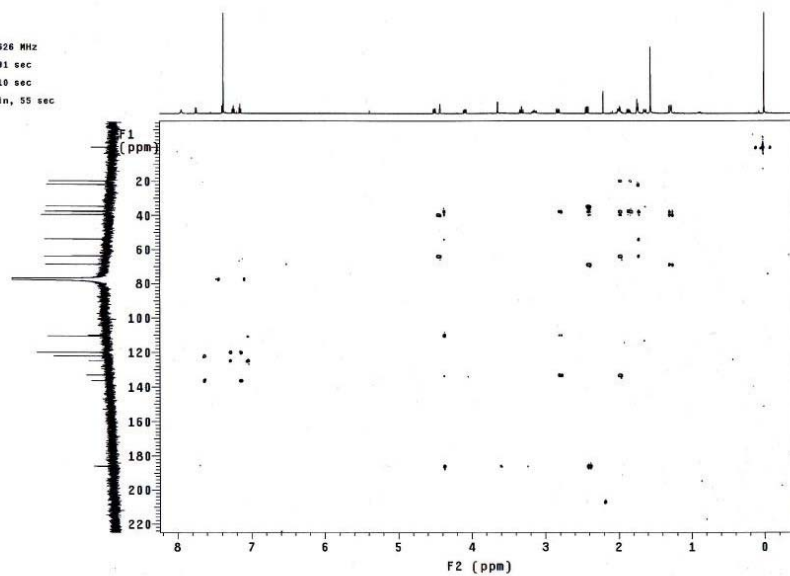
Kopsihainanine A: HSQC in CDCl₃

STANDARD PROTON PARAMETERS
Archive directory: /export/home/lilusong/vnmrsys/data
Sample directory:
Pulse Sequence: gHSQC
Solvent: CDCl₃
Temp: 25.0 C / 298.1 K
User: l-14-07
File: KF4-3-gHSQC
INOVA-600 "LZ0600"
Relax. delay 1.000 sec
Acq. time 0.200 sec
Width 5115.7 Hz
2D Width 25641.0 Hz
84 repetitions
2 x 256 increments
OBSERVE W1, 598.8438873 MHz
DECOUPLE C13, 150.8421407 MHz
Power 45 dB
on during acquisition
off during delay
GASP-1 modulated
DATA PROCESSING
Gauss apodization 0.092 sec
F1 DATA PROCESSING
Gauss apodization 0.018 sec
F1 size 2048 x 4096
Total time 11 hr, 36 min, 10 sec

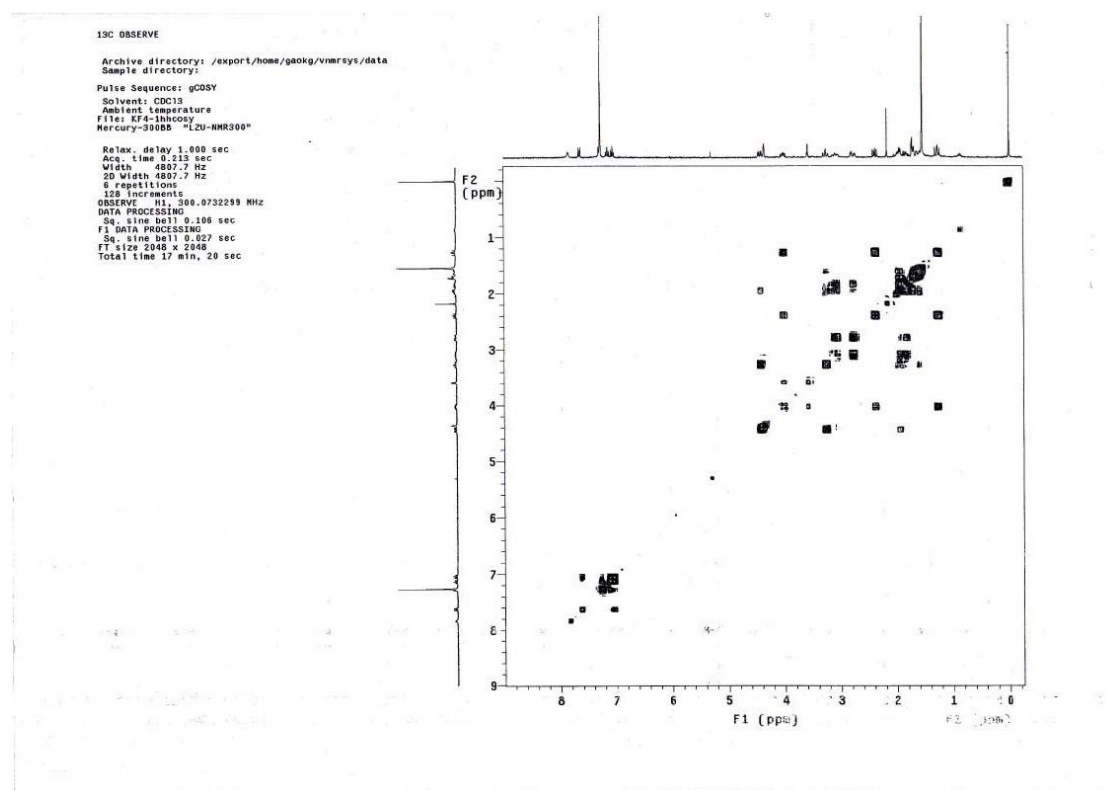


Kopsihainanine A: HMBC in CDCl₃

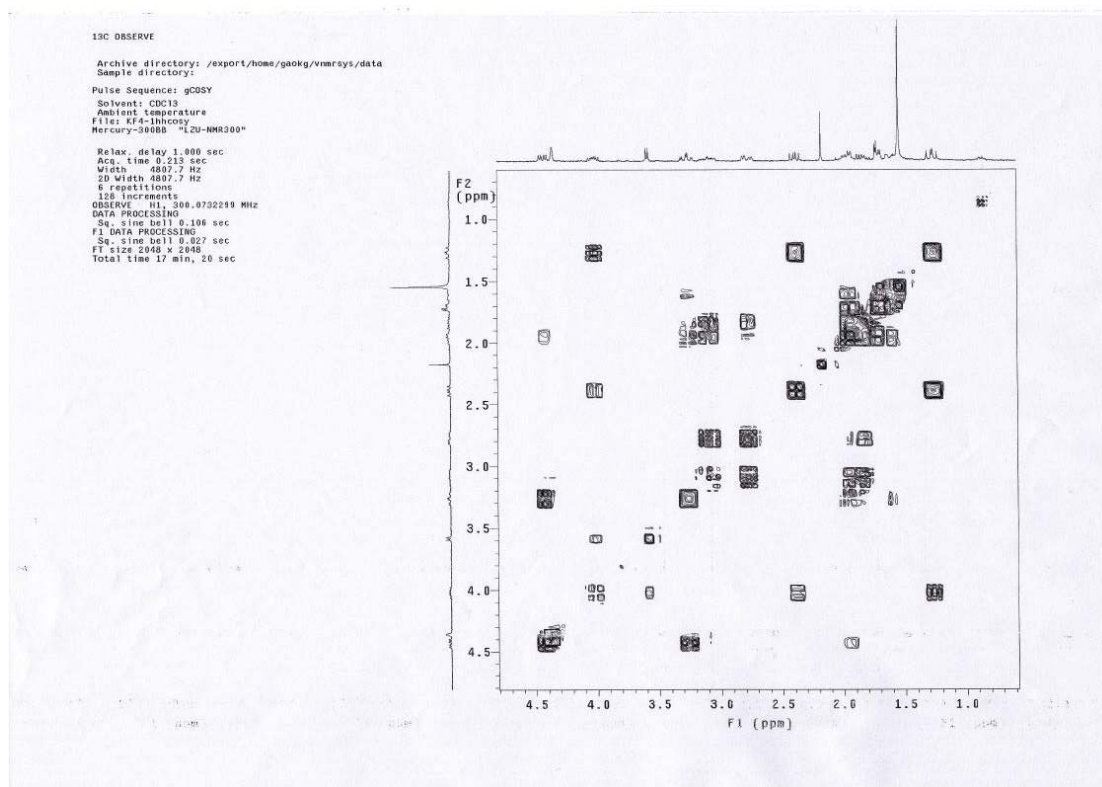
STANDARD PROTON PARAMETERS
Archive directory: /export/home/llusong/vnmr/sys/data
Sample directory:
Pulse Sequence: gHMBC
Solvent: CDCl₃
Temp: 25.0 C / 298.1 K
User: j-14-87
File: KF4-3-a-gHMBC
INOVA-600 "LZ0600"
Relax. delay 1.000 sec
Acq. time 0.197 sec
Width 5207.0 Hz
2D Width 36199.1 Hz
256 repetitions
256 increments
OBSERVE F1, 599.8438626 MHz
DATA PROCESSING
Gauss apodization 0.091 sec
F1 DATA PROCESSING
Gauss apodization 0.010 sec
F1 size 2048 x 4096
Total time 23 hr, 20 min, 55 sec



Kopsihainanine A: ^1H - ^1H COSY in CDCl_3

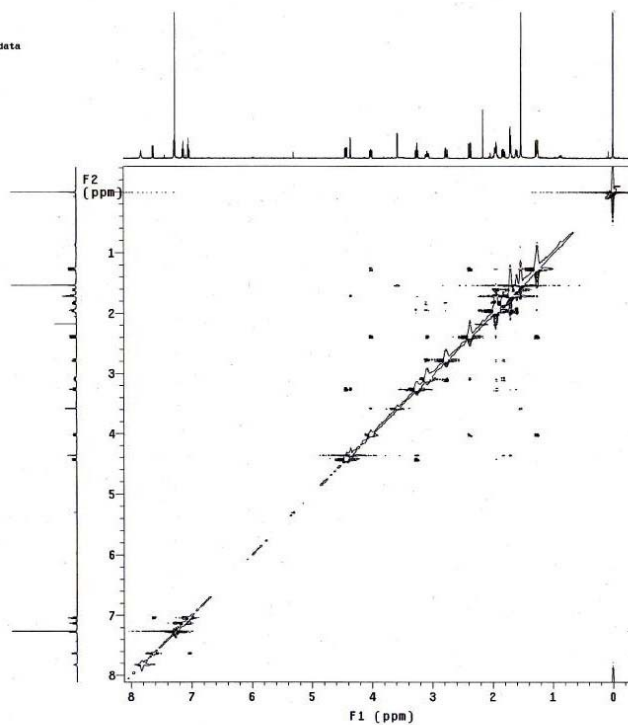


Kopsihainanine A: ^1H - ^1H COSY in CDCl_3

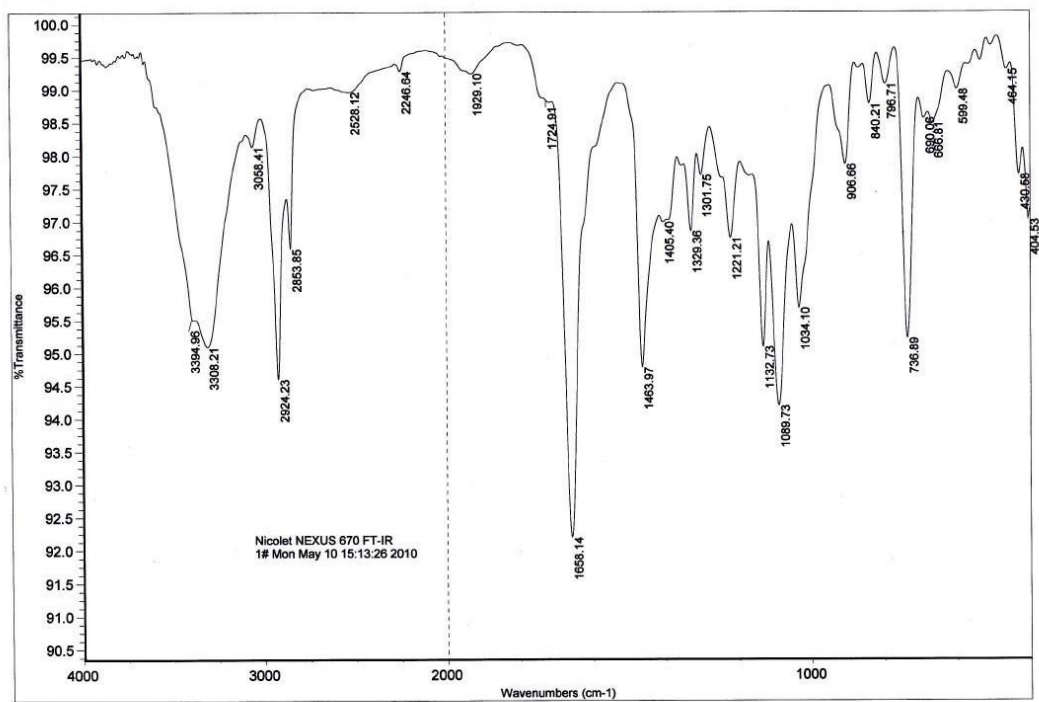


Kopsihainanine A: ROESY in CDCl₃

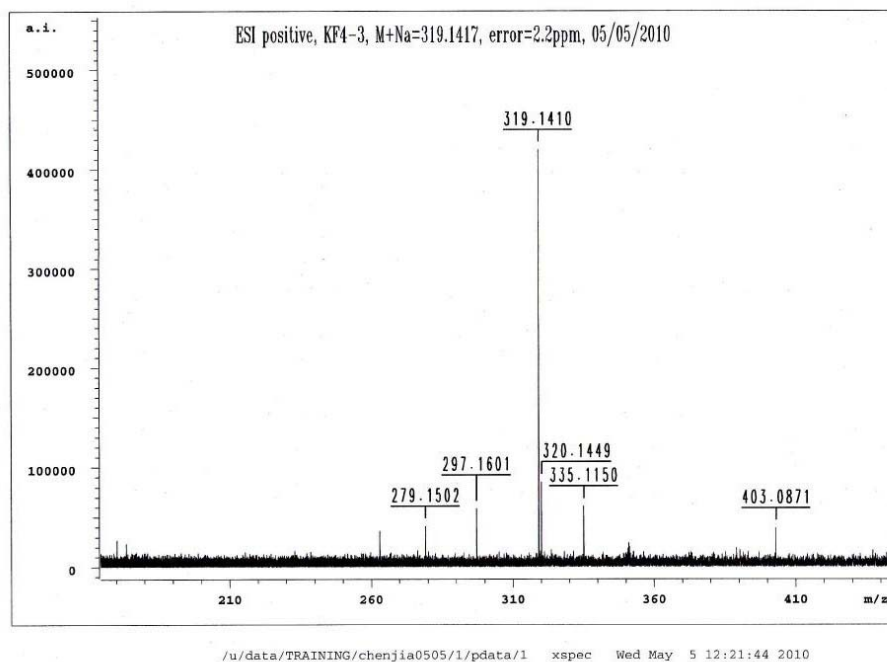
STANDARD PROTON PARAMETERS
Archive directory: /export/home/llusonga/vnmrsys/data
Sample directory:
File: PROTON
Pulse Sequence: RDESY
Solvent: CDCl3
Temp: 25.0 C / 298.1 K
INOVA-600 "LZU600"
Relax. delay 2.000 sec
Mixing 0.200 sec
Acq. time 0.200 sec
Width 5119.7 Hz
2D Width 5119.7 Hz
64 repetitions
2 x 256 increments
OBSERVE F1: 599.8438672 MHz
DATA PROCESSING
Sg. sine bell 0.200 sec
Shifted by -0.200 sec
F1 DATA PROCESSING
Sg. sine bell 0.148 sec
Shifted by -0.148 sec
F1 size 4096 x 4096
Total time 22 hr, 15 min, 4 sec

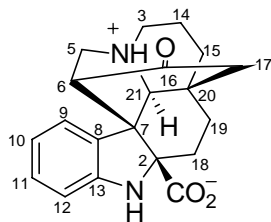


Kopsihainanine A: IR



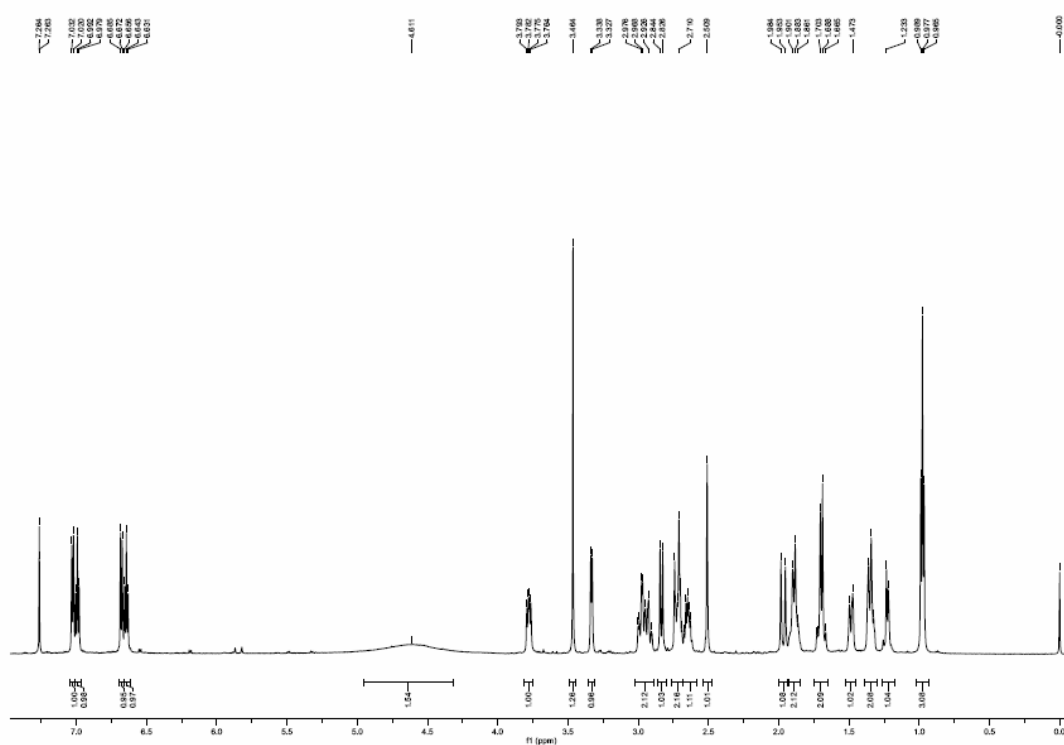
Kopsihainanine A: HRESIMS



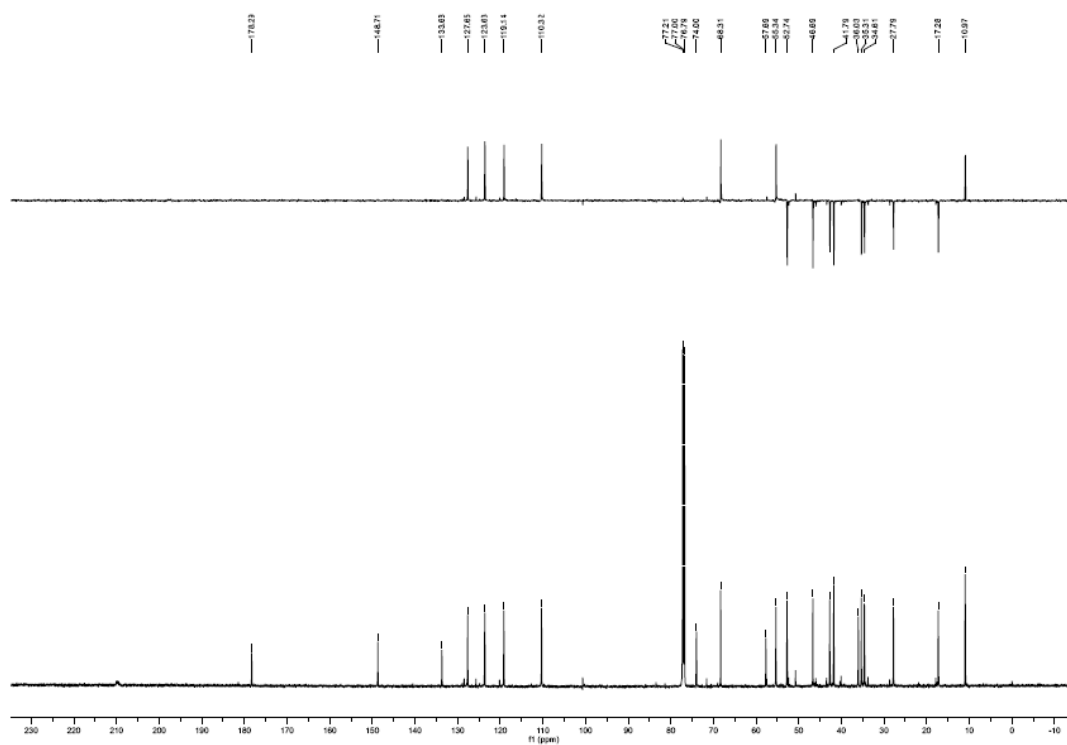


Structure of Kopsihainanine B (2)

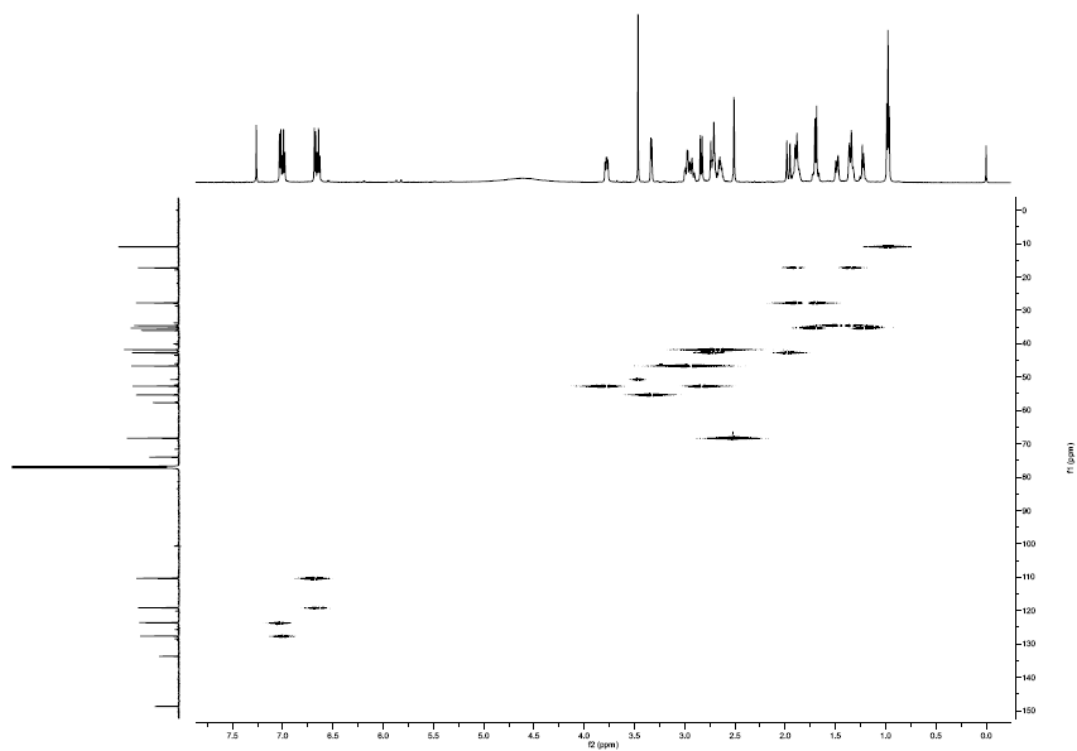
Kopsihainanine B: ¹H NMR in CDCl₃ (Varian Mercury-600)



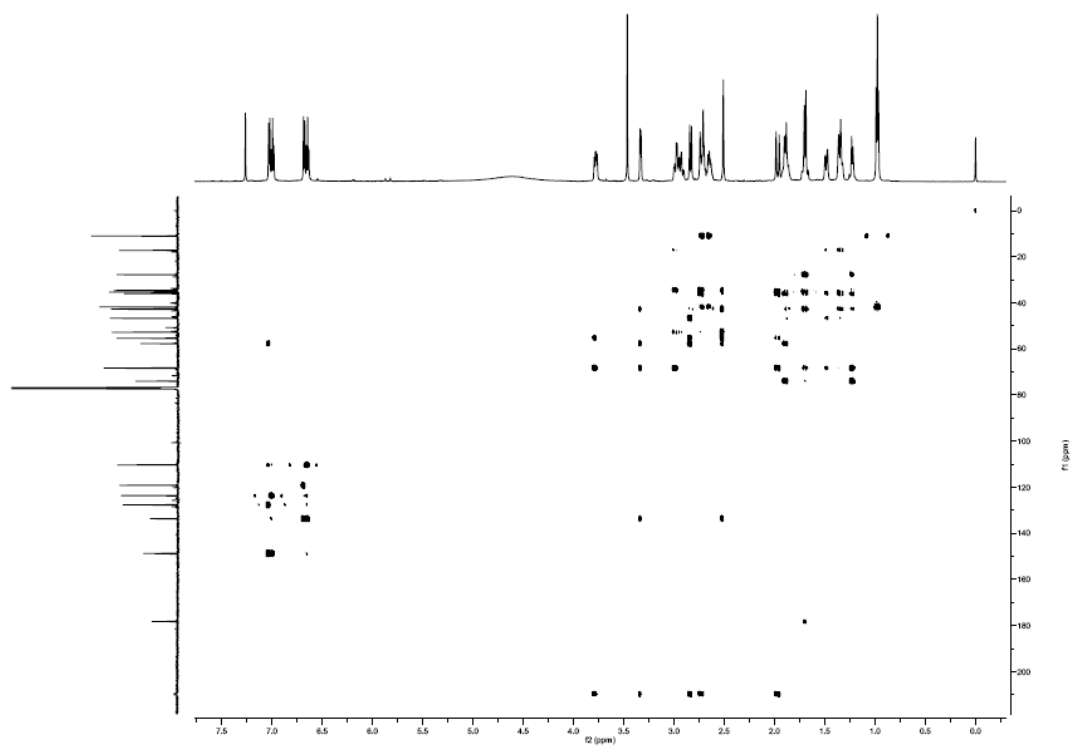
Kopsihainanine B: ^{13}C NMR in CDCl_3 (Varian Mercury-600, 125 MHz)



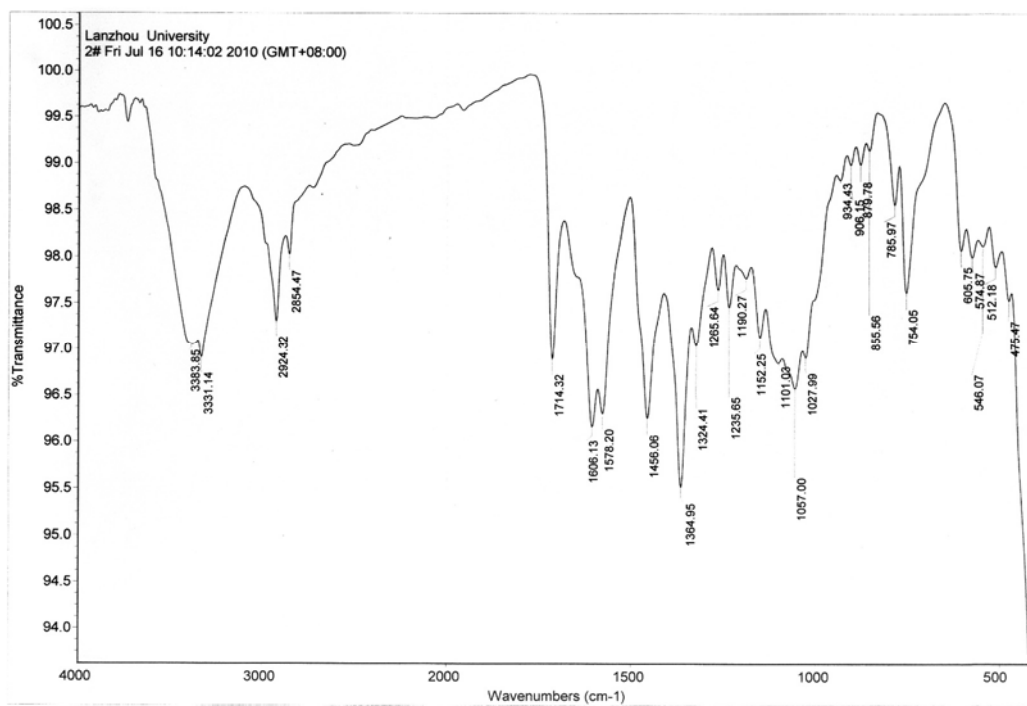
Kopsihainanine B: HSQC in CDCl₃



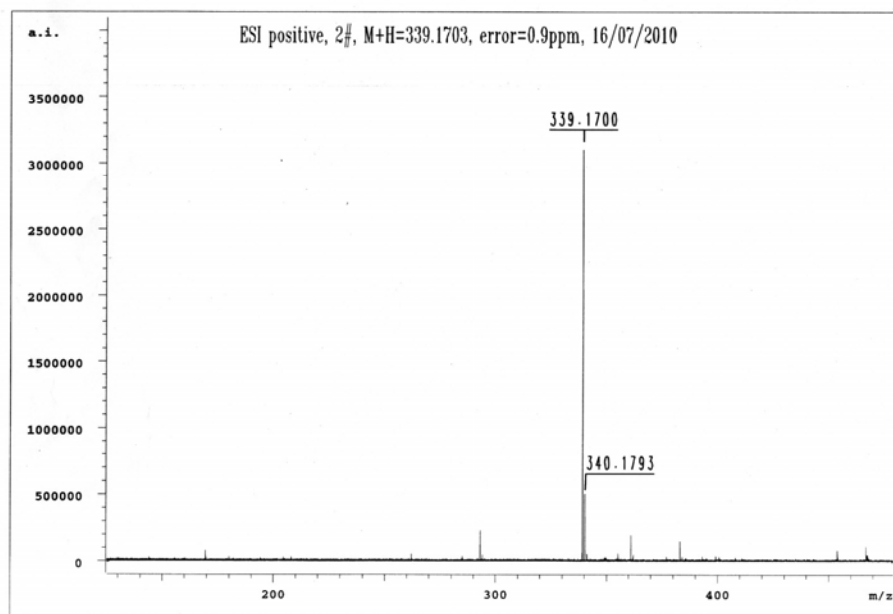
Kopsihainanine B: HMBC in CDCl₃



Kopsihainanine B: IR



Kopsihainanine B: HRESIMS



/u/data/TRAINING/chenjia0716/2/pdata/1 xspec Fri Jul 16 09:44:29 2010

Table S1: Cartesian coordinates of calculated Kopsihainanine A (1)

Atoms	x (Å)	y (Å)	z (Å)	Atoms	x (Å)	y (Å)	z (Å)
C	2.9467	0.6849	0.121	H	-2.5166	-2.5919	-1.3954
C	1.9399	-0.2144	-0.4052	C	-2.9904	0.8696	-1.1908
C	0.7004	0.5258	-0.3982	H	-3.8472	1.4515	-0.7615
C	0.9775	1.8047	0.1043	H	-2.7865	1.2808	-2.2153
N	2.3346	1.9044	0.4261	C	-3.3893	-0.5898	-1.3138
H	2.7846	2.7023	0.7847	H	-3.9012	-0.9149	-0.3691
C	-0.6388	0.1638	-0.9171	H	-4.127	-0.7014	-2.1498
H	-0.6009	0.3476	-2.0382	C	-1.2525	-1.5949	0.6212
C	-0.0215	2.8809	0.291	O	-1.4461	-2.7675	0.9748
H	-0.2259	3.0076	1.3898	C	-1.2264	-0.4605	1.6481
H	0.3757	3.8617	-0.0866	C	4.2842	0.2792	0.2539
C	-1.31	2.5518	-0.4463	C	2.3114	-1.5051	-0.7882
H	-2.1292	3.2101	-0.0545	C	4.6072	-1.0103	-0.1378
H	-1.1744	2.7899	-1.5351	H	5.6471	-1.3581	-0.0455
C	-1.7424	1.0927	-0.3207	C	3.6368	-1.8896	-0.6516
C	-2.0383	0.7339	1.1387	H	3.9424	-2.9038	-0.9482
H	-1.8197	1.6046	1.8101	H	1.5579	-2.2003	-1.1874
H	-3.1286	0.4983	1.2658	H	5.0457	0.9605	0.6546
N	-1.0374	-1.2415	-0.7465	H	-0.1543	-0.165	1.8356
C	-2.2049	-1.5265	-1.5818	O	-1.814	-0.8653	2.8725
H	-1.8815	-1.4348	-2.6555	H	-1.5975	-1.8	3.0049

Microplate assay for AChE activity.

The compounds **1** and **2** were tested for AChE inhibiting activity by Ellman's method in 96-well microplates. Briefly, 140 μL of 0.1M sodium phosphate buffer (pH = 8.0), 20 μL sample solution and 15 μL enzyme solution were mixed and incubated at 4°C for 20 mins. 10 μL of 0.075 mM DTNB was added and the reaction was then started by adding 10 μL of 0.01 mM ATCI. After incubating the reaction solution at 37°C for 20 mins, the optical densities were measured in a 96-well plate reader at 405 nm immediately. A blank positive control was set up by adding 20 μL Physostigmine (100 μM in phosphate buffered saline) instead of 20 μL sample solution. Blanks were set up by adding 20 μL buffer solutions instead of 20 μL sample solution. Experiment control was set up by adding 15 μL buffer solutions instead of 15 μL enzyme solution in order to deduct sample background. The experiments were performed on triplicates. The inhibition rate (%) was calculated by the following equation:

$$\text{Inhibition\%} = \frac{(\text{Blank} - \text{Blank positive control}) - (\text{Experiment} - \text{Experiment control})}{(\text{Blank} - \text{Blank positive control})} \times 100\%$$

The concentrations of test compounds that inhibited the hydrolysis of substrates (acetylthiocholine and butyrylthiocholine) by 50% (IC_{50}) were determined by monitoring the effect of increasing concentrations of these compounds in the assays on the inhibition values. The IC_{50} values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, U.S.A.).