Xylariterpenoids A–D, four new sesquiterpenoids from the Xylariaceae fungus

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List of Supporting Information

1.General experimental procedures

Optical rotations were measured on a JASCO P-1020 polarimeter with a 1 cm cell at room temperature. UV spectra were recorded on a JASCO V-550 UV/Vis spectrometer. IR spectra were obtained using a JASCO FT/IR-480 plus spectrometer. CD spectra were obtained on a JASCO J-810 spectrophotometer at room temperature. ESIMS spectra were acquired using a FINNIGAN LCQ Advantage MAX mass spectrometer. HR-ESI-MS spectra were acquired using a Waters Synapt G2 mass spectrometer. The NMR spectra were measured with a Bruker AV-400 spectrometer. The analytical HPLC was performed on a Dionex HPLC system equipped with an Ultimate 3000 pump, an Ultimate 3000 diode array detector (DAD), an Ultimate 3000 Column Compartment, an Ultimate 3000 autosampler (Dionex, USA) and an Alltech (Grace) 2000 ES evaporative light scattering detector (ELSD) (Alltech, USA) using a reversed-phase C18 column (5 μm, 4.6 × 250 mm; Phenomenex, Gemini). The semi-preparative HPLC was carried out on a Shimadzu LC-6AD Liquid Chromatography with SPD-20A Detector (Shimadzu, Japan) using a reversed-phase C18 column (YMC Park, RP-C_{18} column, 5 μm, 10 × 250 mm). Column chromatography (CC) was carried out on silica gel (200–300 mesh) (Qingdao Haiyang Chemical Group Corporation, Qingdao, China), Sephadex LH-20 (Pharmacia) and ODS (50 μm, YMC), respectively. TLC was performed on precoated silica gel plate (SGF254, 0.2 mm, Yantai Chemical Indus-try Research Institute, China).

2.Fungus material

The strain of Xylariaceae fungi was isolated from the lichen *Everniastrum cirrhatum* (Fr.) Haleex Sipman collected in Zixi Mountain, Yunnan province, People's Republic of China, in November, 2006. The isolate was identified by one of the authors (L.-D. Guo) and assigned the accession number 63-19-7-3 in the culture collection at the Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25˚C for 5 days. Agar plugs were used to inoculate four Erlenmeyer flasks (250 mL), each containing 100 mL of potato dextrose broth (PDB). Four flasks of the inoculated media were incubated at 25˚C on a rotary shaker at 200 rpm for five days to prepare the seed culture. Fermentation was carried out in twenty Erlenmeyer flasks (500 mL), each containing 70 g of rice. Distilled H_2O (105 mL) was added to each flask, and the rice was soaked overnight before autoclaving at 120˚C for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at room temperature for 51 days.

3.Extraction and isolation

The fermented material was extracted three times with EtOAc (3×4 L), and the organic solvent was evaporated under vacuum to afford the dry crude extract (28.1 g). Then the crude extract was dissolved in 90% v/v aqueous MeOH (500 mL) and partitioned against the same volume cyclohexane to afford cyclohexane extract (C, 15.3 g) and aqueous MeOH extract (W, 12.5 g). The aqueous MeOH extract (W, 12.5 g) was separated by ODS CC (4×45 cm) eluting with MeOH–H_2O elution (30:70, 50:50, 70:30 and 100:0, v/v, each 2 L) to afford 4 fractions (W1–W4). Fraction W2 (1.8 g) was further separated by ODS CC (4 ×30 cm) eluting with MeOH–H_2O elution (20:80, 30:70, 50:50 and 100:0, v/v, each 1.8 L) to afford 8 subfractions (W2a–W2h). Subfraction W2d (66.5 mg) was purified by semi-preparative HPLC (30% MeOH-H_2O, v/v, 3.0 mL/min) to afford compound **1** (15.4 mg, t_R: 17.5 min) and compound **2** (10.8 mg, t_R: 20.3 min). Subfraction W2e (86.2 mg) was purified by Sephadex LH-20 CC (100 cm) to afford compound **3** (6.2 mg) and compound **4** (4.5 mg).

4.Spectroscopic data of 1–4

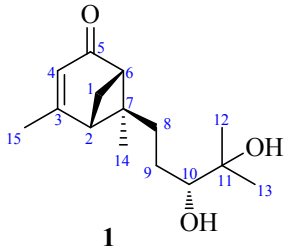
Xylariterpenoid A (1): Yellow oil; $[\alpha]_D^{25}$ – 90.7 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε): 206 (4.23), 252 (4.35); nm; CD (*c* 1.0 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 207 (+3.48), 321 (–6.65); IR (KBr) ν_{max} 3424, 2969, 1663, 1381, 1075 cm^{-1}; ESI-MS (positive) *m/z* 275 $[M + Na]^+$, 527 $[2M + Na]^+$; HRESI-TOF-MS (positive): *m/z* 275.1615 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1623); The ^1H and ^{13}C NMR, see Table 1.

Xylariterpenoid B (2): Yellow oil; $[\alpha]_D^{25}$ – 85.2 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε): 204 (4.19), 252 (4.29) nm; CD (*c* 1.0 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 215 (+5.77), 320 (–8.50); IR (KBr) ν_{max} 3420, 2971, 1760, 1663, 1379 cm^{-1}; ESI-MS (positive) *m/z* 275 $[M + Na]^+$, 527 $[2M + Na]^+$; HRESI-TOF-MS (positive): *m/z* 275.1624 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1623); The NMR data see Table 2.

Xylariterpenoid C (3): Yellow oil; $[\alpha]_D^{25}$ – 40.7 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε): 205 (4.34), 230 (4.36) nm; IR (KBr) ν_{max} 3439, 1739, 1646, 1387, 1045 cm^{-1}; ESI-MS (positive) *m/z* 251 $[M + H]^+$, 273 $[M + Na]^+$; HRESI-TOF-MS (positive): *m/z* 273.1465 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_3Na$, 273.1467); The NMR data see Table 3.

Xylariterpenoid D (4): Yellow crystals; $[\alpha]_D^{25}$ – 40.4 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε): 204 (4.15), 238 (3.74) nm; IR (KBr) ν_{max} 3440, 1645, 1385, 1257 cm^{-1}; ESI-MS (positive) *m/z* 237 $[M + H]^+$, 259 $[M + Na]^+$; HRESI-TOF-MS (positive) *m/z* 259.1667 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_2Na$, 259.1674); The NMR data see Table 4.

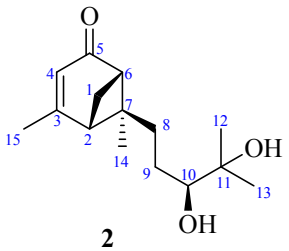
The NMR data of 1-4



1

Table 1 ^{13}C NMR (100 MHz) and ^{1}H NMR (400 MHz) data of **1** in $CDCl_3$

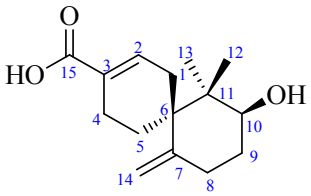
position	δ_H (J in Hz)	δ_C, mult	$^{1}H,^{1}H$-COSY	HMBC
1	2.07, d (9.1), a	40.8, CH_2	1b	2, 3, 5, 6, 7, 14
	2.80, dt (9.1, 5.3), b		1a, 2, 6	2, 3, 5, 6
2	2.45, br t (5.8)	48.6, CH	1b, 4, 6	1, 3, 4, 6, 7, 8, 15
3		170.6, qC		
4	5.73, br q (1.0)	121.5, CH	2, 6, 15	2, 6, 15
5		204.4, qC		
6	2.71, br t (5.8)	55.5, CH	1b, 2, 4	1, 2, 4, 5, 7, 8
7		57.1, qC		
8	1.73, td (12.8, 4.0), a	35.6, CH_2	8b, 9a, 9b	2, 6, 7, 9, 10, 14
	2.31, td (12.8, 4.0), b		8a, 9a, 9b	2, 6, 7, 9, 10, 14
9	1.34, m, a	26.5, CH_2	8a, 8b, 9b, 10	7, 8, 10
	1.48, m, b		8a, 8b, 9a, 10	7, 8, 10
10	3.33, dd (10.3, 1.6)	78.8, CH	9a, 9b	8, 9, 11, 12, 13
11		73.2, qC		
12	1.16, s	23.3, CH_3		10, 11, 13
13	1.21, s	26.5, CH_3		10, 11, 12
14	0.96, s	19.0, CH_3		2, 6, 7, 8
15	2.00, d (1.0)	23.6, CH_3	4	2, 3, 4, 5



2

Table 2 ^{13}C NMR (100 MHz) and ^{1}H NMR (400 MHz) data of **2** in $CDCl_3$

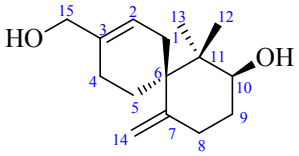
position	δ_H (J in Hz)	δ_C, mult	$^{1}H,^{1}H$-COSY	HMBC
1	2.08, d (9.5), a	40.8, CH_2	1b	2, 3, 5, 6, 7
	2.80, dt (9.5, 5.5), b		1a, 2, 6	3, 5
2	2.47, br t (5.7)	48.4, CH	1b, 4, 6	4, 6
3		170.6, qC		
4	5.74, br q (1.4)	121.5,CH	2, 6, 15	2, 6, 15
5		204.2, qC		
6	2.71, br t (5.7)	55.8, CH	1b, 2, 4	2
7		57.1, qC		
8	1.90, td (12.4, 4.5), a	35.7, CH_2	8b, 9a, 9b	2, 6, 7, 9, 14
	2.17, td (12.4, 4.5), b		8a, 9a, 9b	6, 7, 9, 14
9	1.34, m, a	26.5, CH_2	8a, 8b, 9b, 10	8, 10
	1.50, m, b		8a, 8b, 9a, 10	7, 8
10	3.36, dd (10.4, 1.8)	78.8, CH	9a, 9b	8, 9, 11, 12, 13
11		73.3, qC		
12	1.17, s	23.2, CH_3		10, 11,13
13	1.23, s	26.7, CH_3		10, 11, 12
14	0.98, s	19.0, CH_3		2, 6, 7, 8
15	2.01, d (1.4)	23.6, CH_3	4	2, 3, 4



3

Table 3 ^{13}C NMR (100 MHz) and ^{1}H NMR (400 MHz) data of **3** in $CDCl_3$

position	δ_H (*J* in Hz)	δ_C, mult	$^{1}H,^{1}H$-COSY	HMBC
1	2.17, m, a	30.2, CH_2	1b, 2	6
	2.31, m, b		1a, 2	2, 3, 6, 7
2	7.09, m	141.5, CH	1a, 1b, 4a, 4b	4, 6, 15
3		128.8, qC		
4	1.80, m, a	21.4, CH_2	2, 4b, 5a, 5b	3, 5
	2.31, m, b		2, 4a, 5a, 5b	3, 6, 15
5	1.48, m, a	25.2, CH_2	4a, 4b, 5b	6, 7
	1.95, m, b		4a, 4b, 5a	1, 3, 4
6		45.4, qC		
7		146.4, qC		
8	2.17, m, a	30.0, CH_2	9a	6, 10, 14
	2.31, m, b		9a, 9b, 14a, 14b	6, 7
9	1.47, m, a	31.9, CH_2	8a, 8b, 9b, 10	7, 10
	1.86, m, b		8b, 9a, 10	
10	3.88, dd (11.9, 4.6)	73.3, CH	9a, 9b	
11		41.9, qC		
12	0.76, s	15.1, CH_3		6, 10, 11, 13
13	1.02, s	20.4, CH_3		6, 10, 11, 12
14	4.44, br s, a	112.0,CH_2	8b, 14b	6, 8
	4.92, br s, b		8b, 14a	6, 8
15		171.7, qC		



4

Table 4 ^{13}C NMR (100 MHz) and ^{1}H NMR (400 MHz) data of **4** in $CDCl_3$

position	δ_H (J in Hz)	δ_C, mult	$^{1}H,^{1}H$-COSY	HMBC
1	2.05, m, a	28.9, CH_2	1b, 2	
	2.15, m, b		1a, 2	6, 7
2	5.63, br s	122.0, CH	1a, 1b, 4a, 4b, 15	
3		136.3, qC		
4	1.67, m, a	23.1, CH_2	2, 4b, 5a, 5b	5
	1.96, m, b		2, 4a , 5a, 5b	
5	1.50, m, a	25.4, CH_2	4a, 4b, 5b	1, 4, 6, 7
	1.87, m, b		4a, 4b, 5a	1
6		45.7, qC		
7		146.8, qC		
8	2.17, m, a	30.1, CH_2	9a, 9b	6, 7, 9, 14
	2.33, m, b		9a, 9b, 14b	7, 9, 14
9	1.45, m, a	32.0, CH_2	8a, 8b, 9b, 10	7, 8, 10, 11
	1.82, m, b		8a, 8b, 9a, 10,	7, 8, 10, 11
10	3.87, dd (11.9, 4.7)	73.5, CH	9a, 9b	8, 9, 11, 12, 13
11		42.0, qC		
12	0.73, s	15.0, CH_3		6, 10, 11, 13
13	1.01, s	20.4, CH_3		6, 10, 11, 12
14	4.55, br s, a	111.9, CH_2	14b	6, 7, 8
	4.92, br s, b		8b, 14a	6, 8
15	3.92, br s	67.2, CH_2	2	2, 3, 4

5.Quantum chemical CD calculation of 5.

The molecules of (2R, 6R)-**5** and (2S, 6S)-**5** were converted into SMILES codes before their initial 3D structures were generated with CORINA version 3.4. For each molecule, the initial 3D structure was minimized with MMFF94S force field implemented in CONFLEX version 7.0 with default parameters before it conformation space was sampled. Conformer databases were generated using CONFLEX version 7.0, with an energy window for acceptable conformers (ewindow) of 5 kcal·mol^{-1} above the ground state using the modified version of the MMFF94 force-field, a maximum number of conformations per molecule (maxconfs) of 100 and an RMSD cutoff (rmsd) of 0.5Å. After that, only one conformer was found out. And then the conformer was optimized with HF/6-31G(d) method in Gaussian09[1]. Further optimization at the B3P86/6-31G(d) level led the dihedral angles to be got. The optimized conformerwas taken for the CD calculations, which was performed with Gaussian09 (B3P86/6-311++G(2d,p)). The solvent effects were taken into account by the polarizable-conductor calculation model (CPCM, methanol as the solvent).

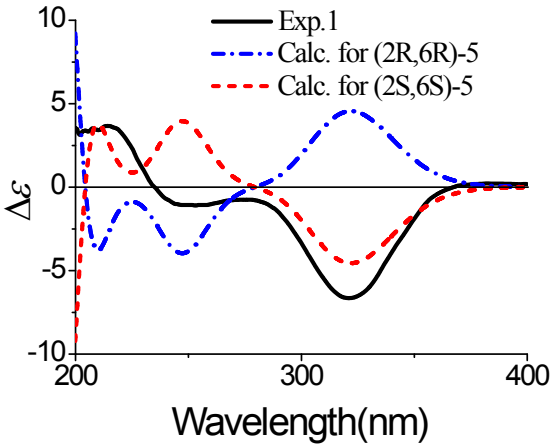


Fig. 1 Experimental CD spectra of **1** and calculated CD spectra of (2R, 6R)-**5** and (2S, 6S)-**5** (UV correction = -4 nm, band width σ = 0.3 eV)

6. X-ray Crystallographic Analysis of 4

Upon crystallization from MeOH using the vapor diffusion method, needles of **4** were obtained. Data were collected using a Sapphire CCD with a graphite monochromated Cu Kα radiation, λ = 1.54184 Å at 173.00 (10) K. Crystal data: $C_{15}H_{24}O_2$, M = 236.34, orthorhombic, space group $P212121$; unit cell dimensions were determined to be a = 6.5259(3) Å, b = 13.1402(5)Å, c = 15.6863(7) Å, α = 90.00 °, β = 90.00 °, γ = 90.00 °, V =1345.12(10) Å^3, Z = 4, Dx = 1.167 g/cm^3, F (000) = 520, μ (Cu K_α) = 0.586 mm^{-1}. 10952 reflections were collected until θ_{max} = 62.93°, in which independent unique 1950 reflections were observed [$F^2 > 4\sigma$ (F^2)]. The structure was solved by direct methods using the SHELXS-97 program, and refined by the SHELXL-97 program and full-matrix least-squares calculations.[1] In the structure refinements, nonhydrogen atoms were placed on the geometrically ideal positions by the "ride on" method. Hydrogen atoms bonded to oxygen were located by the structure factors with isotropic temperature factors. The final refinement gave R = 0.0363, R_W = 0.0909, S = 1.079, Flack = -0.1(3), and Hooft y = 0.04(16).

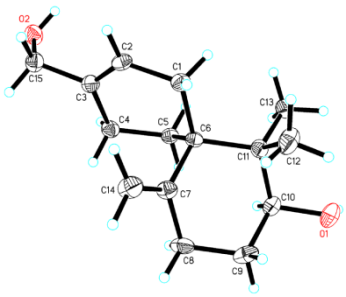


Fig.2 X-ray structure of **4**

Reference:

(1) O.Dolomanov,L.Bourhis, R.Gildea, J. Howard, H. Puschmann, *J. Appl. Cryst.,* 2009, **42**, 339-341.

7. The *in situ* dimolybdenum CD method

HPLC grade DMSO was dried with 4Å molecular sieves. According to a published procedure, a mixture of 1:1.3 diol-$Mo_2(OAc)_4$ for **1** was subjected to CD measurement at a concentration of 0.5 mg/mL. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed signs of the diagnostic bands at around 310 nm in the induced CD spectrum were correlated to the absolute configuration of C-10.

8. Preparation of (*S*)- and (*R*)-MTPA esters of 3 (3a and 3b)

A solution of **3** (1.0 mg) in pyridine-d_5 (0.5 mL) was treated with (*S*)-MTPA chloride (15 μL) under an atmosphere of nitrogen in an NMR tube. The mixture was stirred at room temperature for 4 h to obtain the (*R*)-MTPA ester (**3b**). The same procedure was used to prepare the (*S*)-MTPA ester (**3a**) with (*R*)-MTPA chloride.

The $\Delta\delta$ values ($\Delta\delta_{Hb\text{-}4}$: -0.01468; $\Delta\delta_{Ha\text{-}5}$: -0.01124; $\Delta\delta_{Ha\text{-}8}$: -0.03264; $\Delta\delta_{H\text{-}9}$: -0.17335 and -0.02243; $\Delta\delta_{H\text{-}12}$: +0.01453; $\Delta\delta_{H\text{-}13}$: +0.14367; $\Delta\delta_{H\text{-}14}$: -0.03475 and -0.03326) of the (*S*)- and (*R*)-MTPA esters of **3** (**3a** and **3b**) indicated the S configuration for C-10 in **3**.

9. Cytotoxicity assay

Five human cancer cell lines, human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and colon cancer SW480, were used in the cytotoxicity assay. All the cells were cultured in DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA), in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates,[2,3] with cisplatin and paclitaxel (Sigma, USA) as the positive controls. Cell viability after 48h treatment was detected and cell growth curve was graphed. The IC_{50} values were calculated by Reed and Muench's method.[4]

Reference:

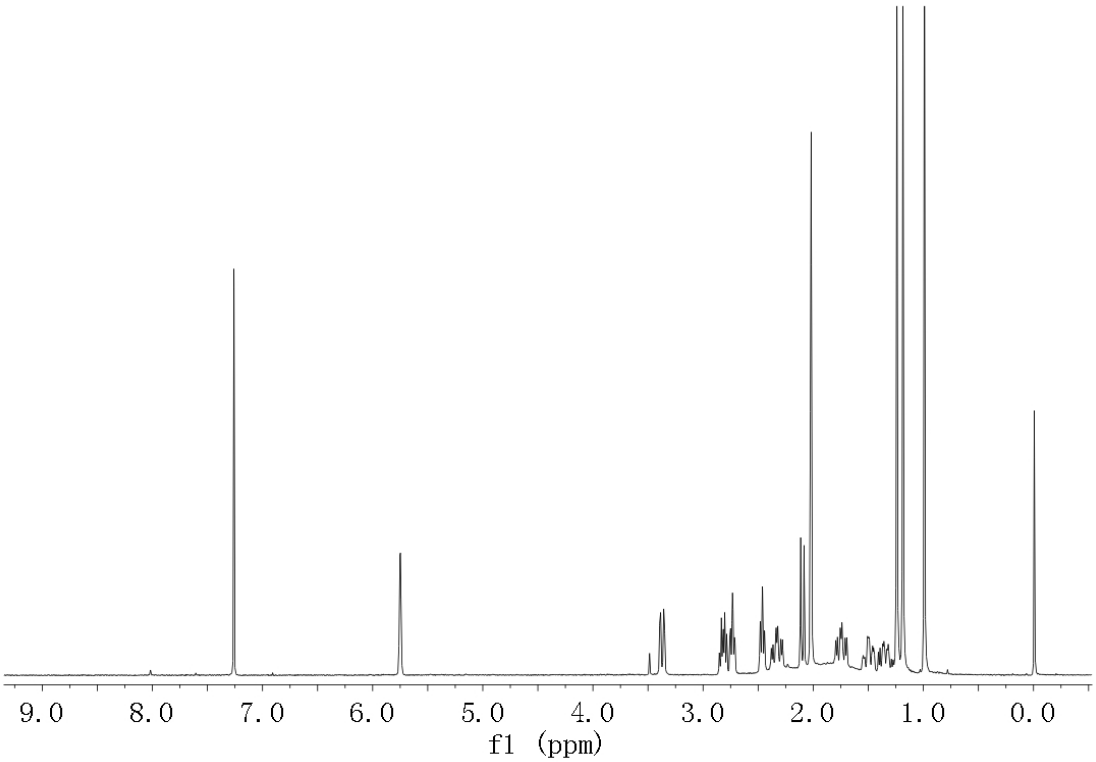
(2)T., Mosmman, *J. Immunol. Methods.*, 1983, **65**, 55-63.

(3)M. C. Alley, D. A. Scudiero, A., Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, *Cancer. Res.*, 1988, **48**, 589-601.

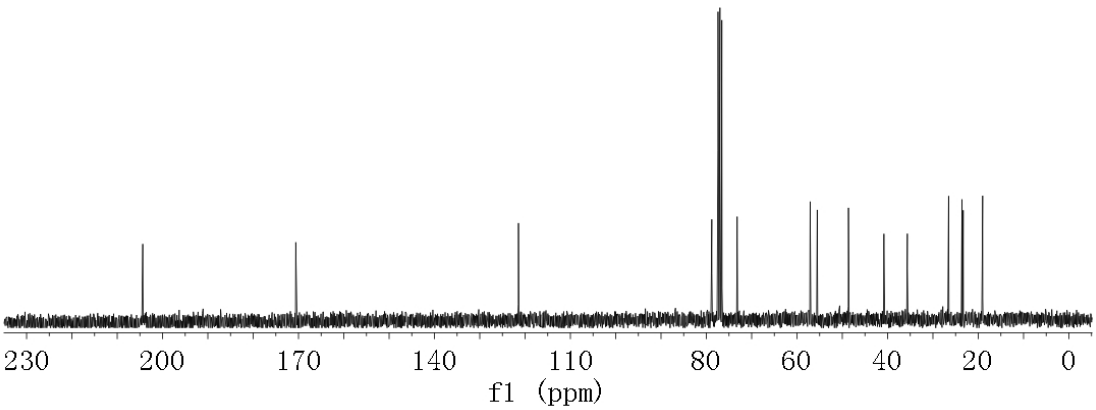
(4)L. J. Reed, H., Muench, *Am.J. Hyg.,* 1938, **27**, 493-497.

10. The 1D and 2D NMR spectra of 1–4

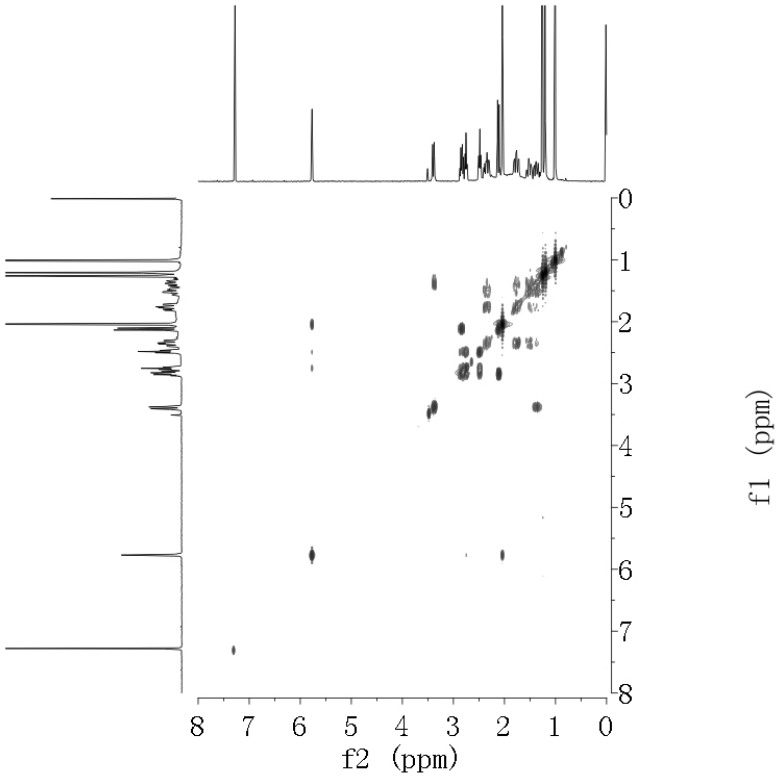
The 1D and 2D NMR spectra of xylariterpenoid A (1)



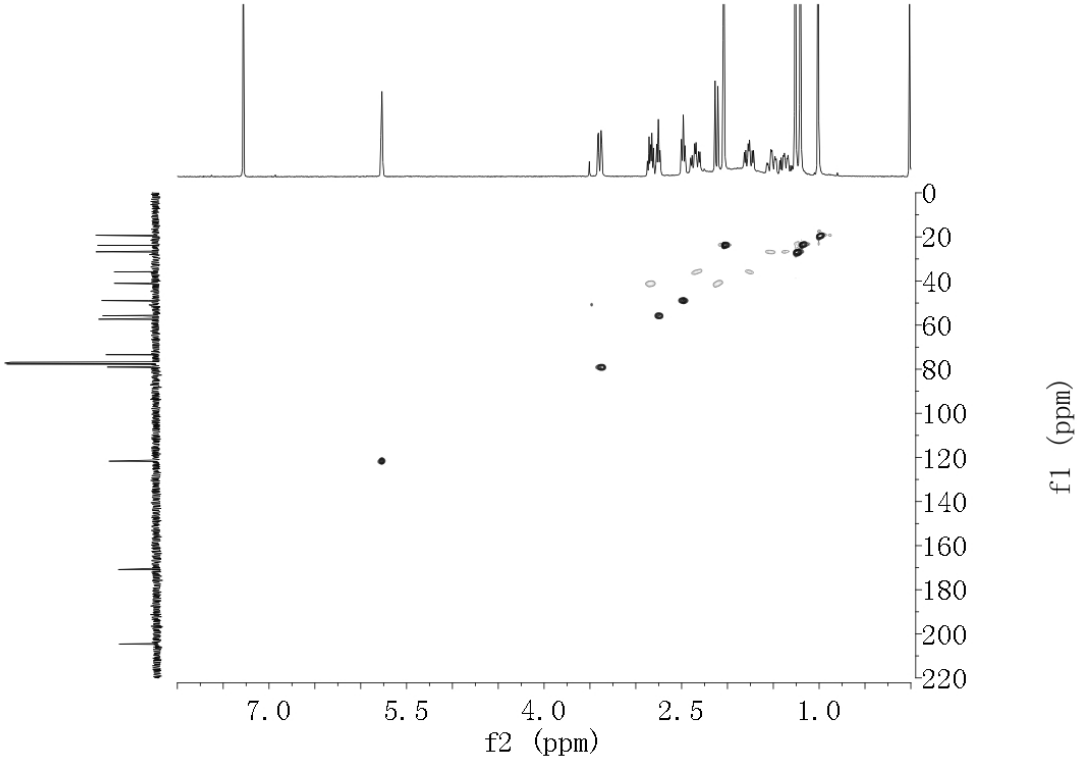
^{1}H NMR spectrum for xylariterpenoid A (**1**) (400 MHz, in $CDCl_3$)



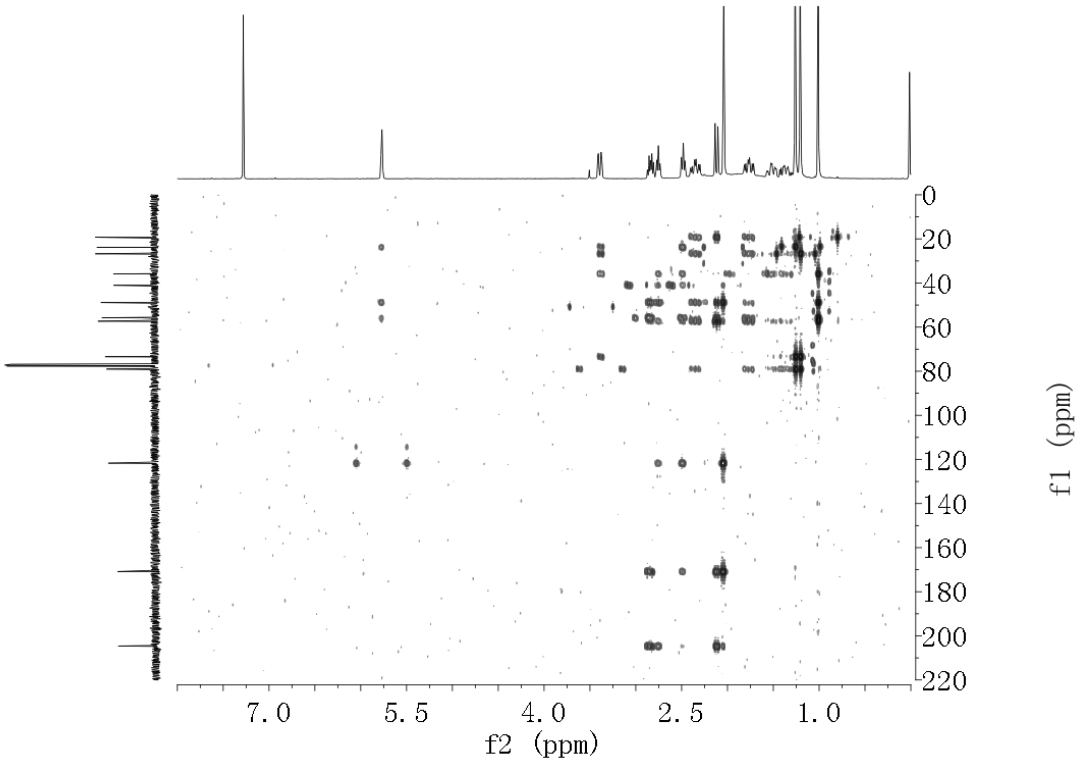
^{13}C NMR spectrum for xylariterpenoid A (**1**) (100 MHz, in $CDCl_3$)



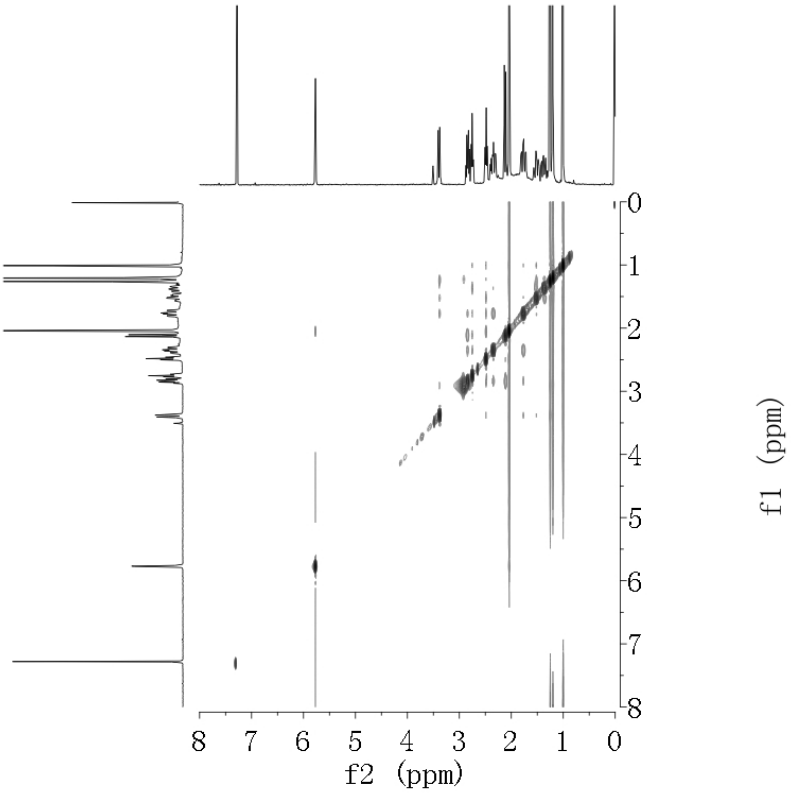
^{1}H-^{1}H COSY spectrum for xylariterpenoid A (**1**) in $CDCl_3$



HSQC spectrum for xylariterpenoid A (**1**) in $CDCl_3$

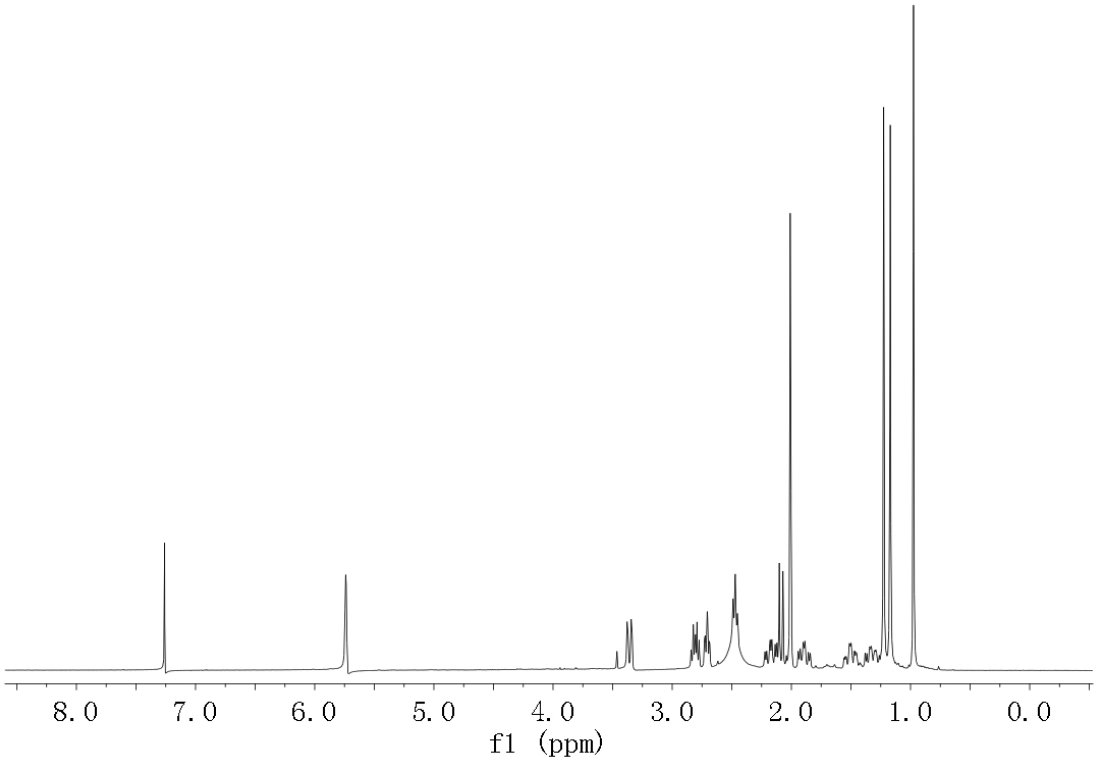


HMBC spectrum for xylariterpenoid A (**1**) in $CDCl_3$

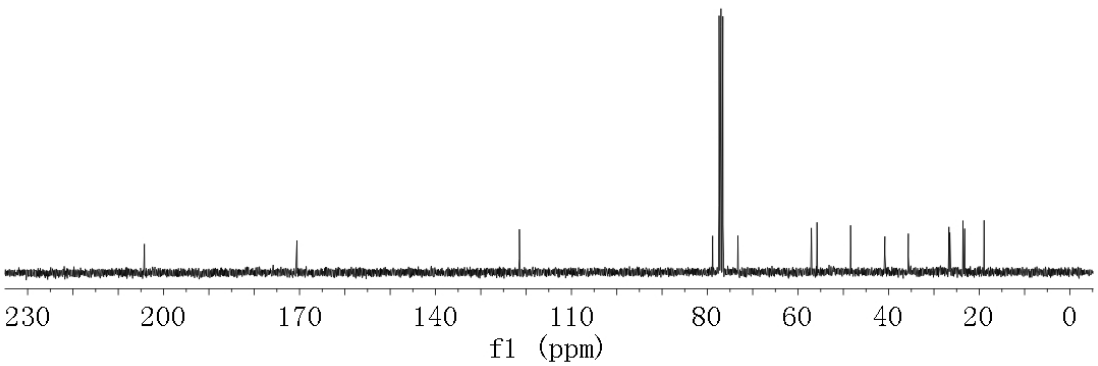


NOESY spectrum for xylariterpenoid A (**1**) in $CDCl_3$

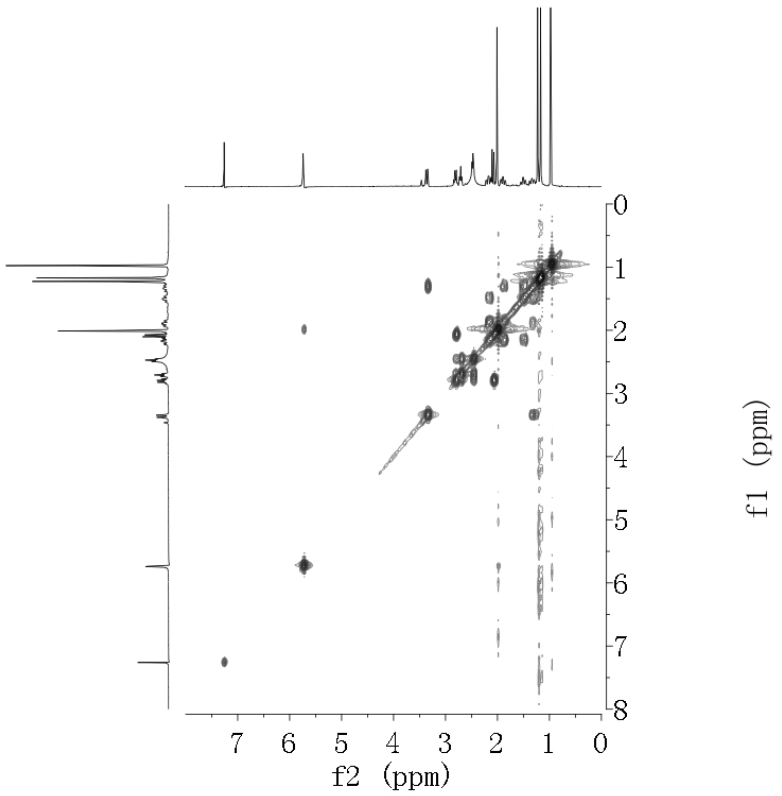
The 1D and 2D NMR spectra of xylariterpenoid B (2)



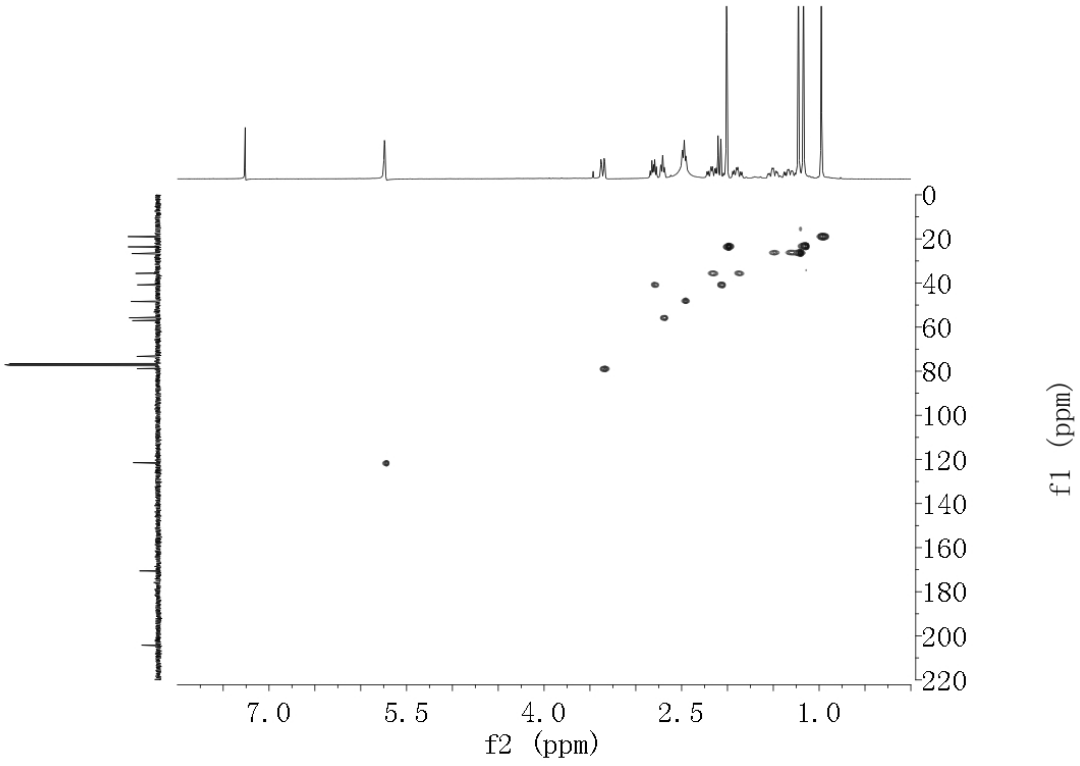
^{1}H NMR spectrum for xylariterpenoid B (**2**) (400 MHz, in $CDCl_3$)



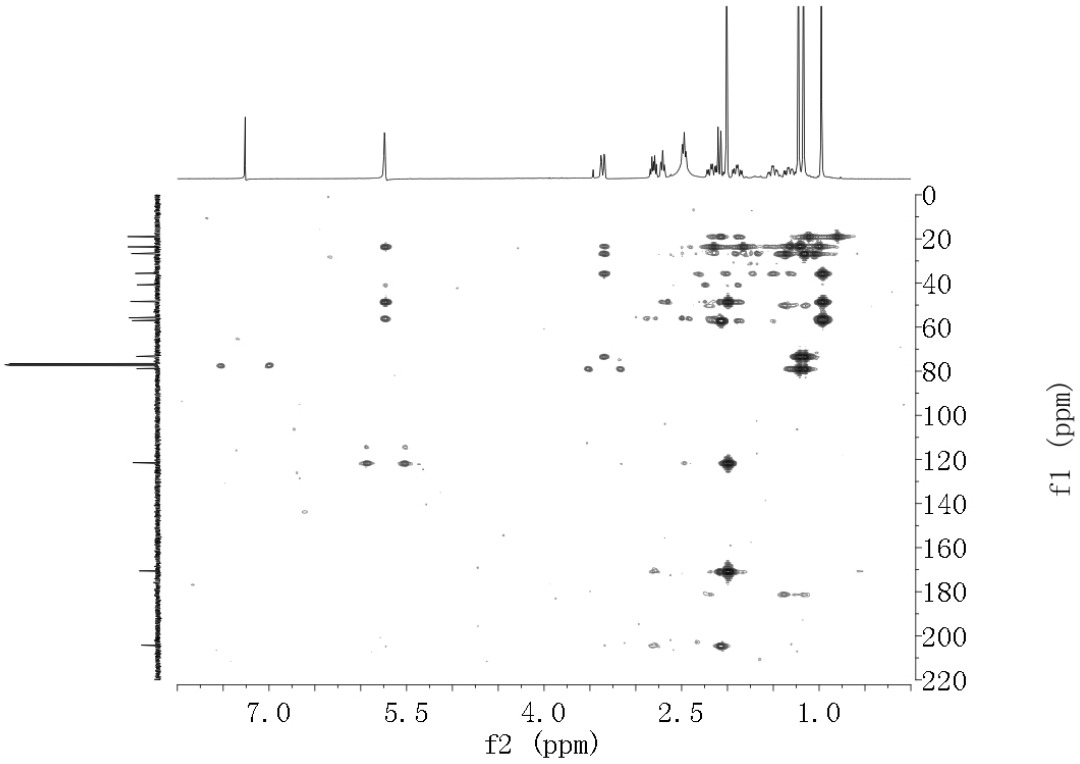
^{13}C NMR spectrum for xylariterpenoid B (**2**) (100 MHz, in $CDCl_3$)



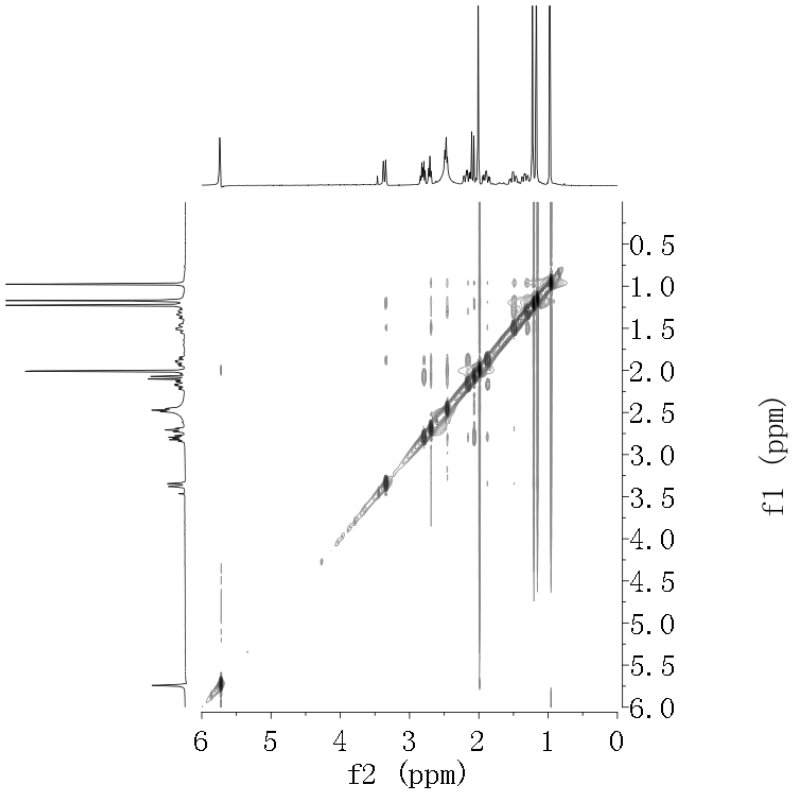
^{1}H-^{1}H COSY spectrum for xylariterpenoid B (**2**) in $CDCl_3$



HSQC spectrum for xylariterpenoid B (**2**) in $CDCl_3$

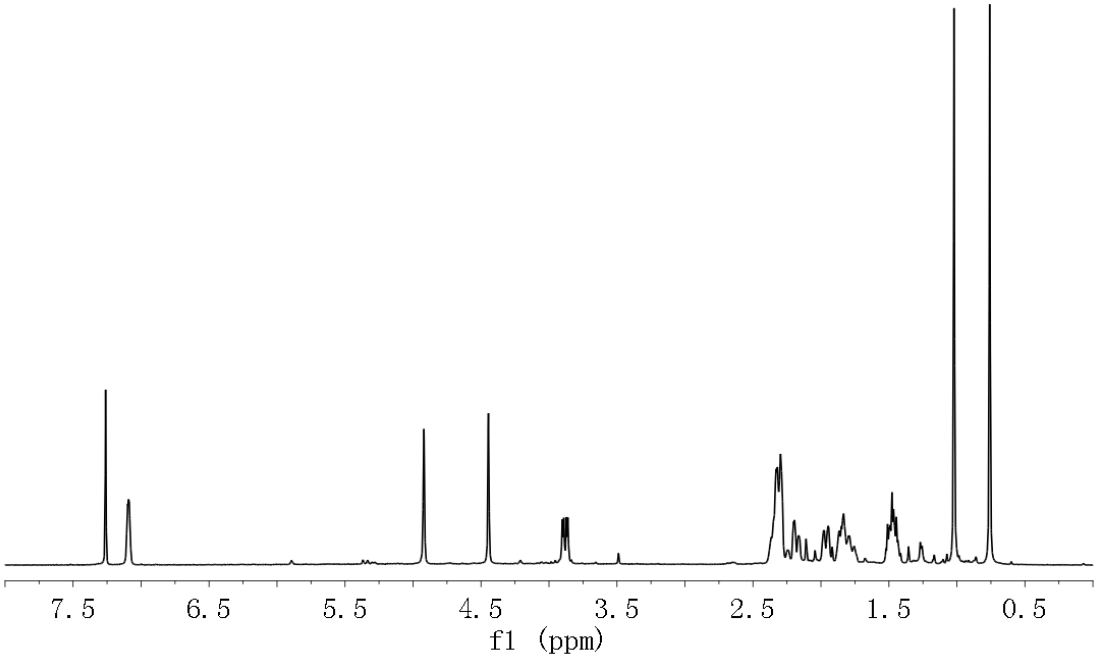


HMBC spectrum for xylariterpenoid B (**2**) in $CDCl_3$

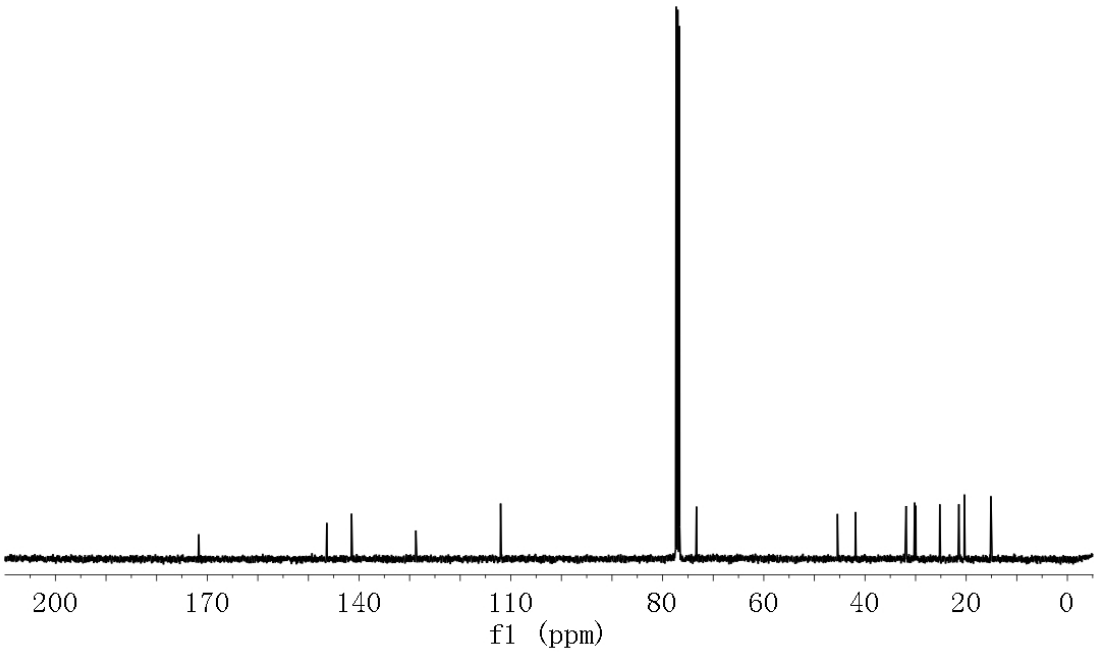


NOESY spectrum for xylariterpenoid B (**2**) in $CDCl_3$

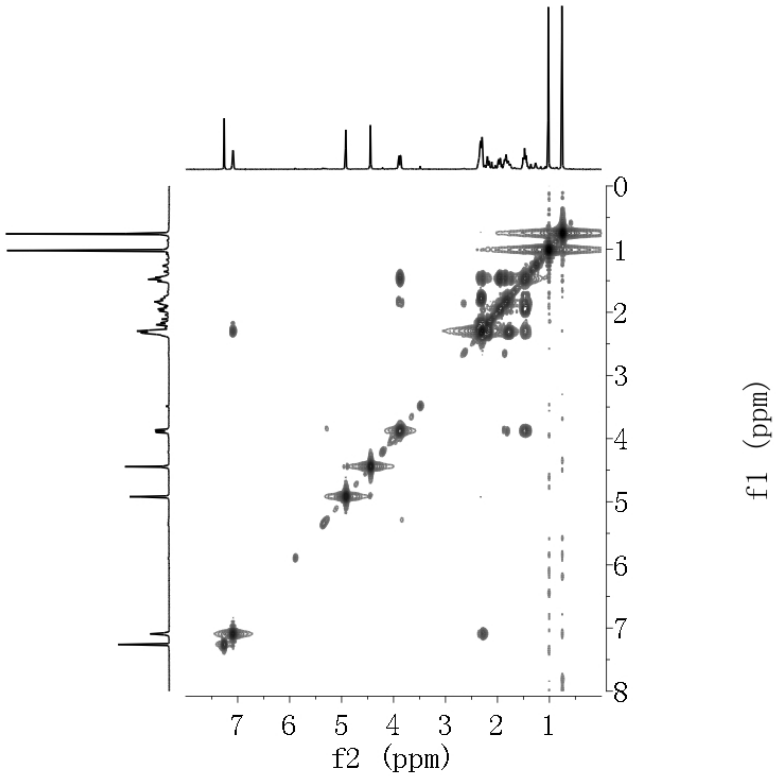
The 1D and 2D NMR spectra of xylariterpenoid C (3)



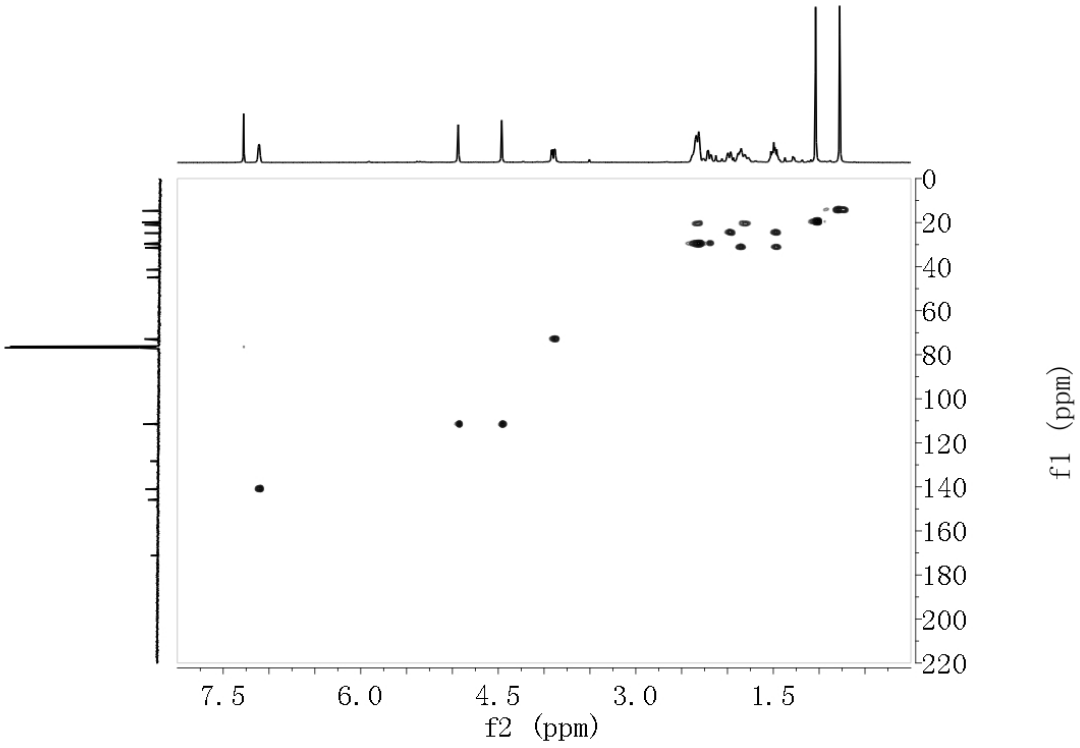
^{1}H NMR spectrum for xylariterpenoid C (**3**) (400 MHz, in $CDCl_3$)



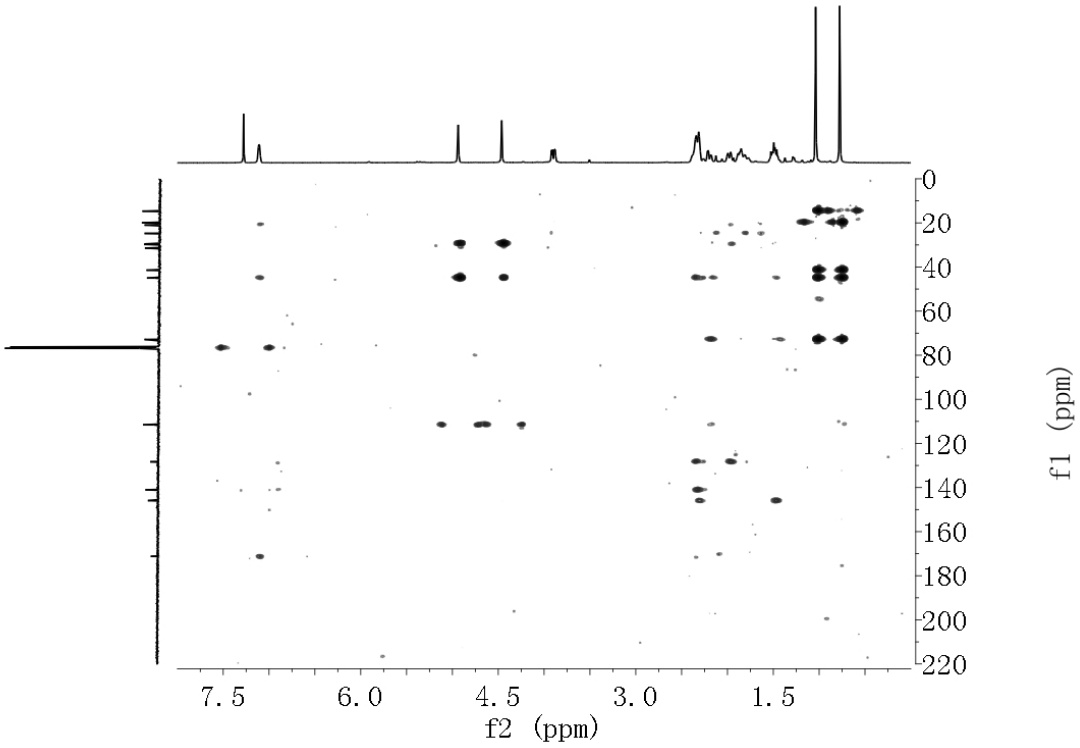
^{13}C NMR spectrum for xylariterpenoid C (**3**) (100 MHz, in $CDCl_3$)



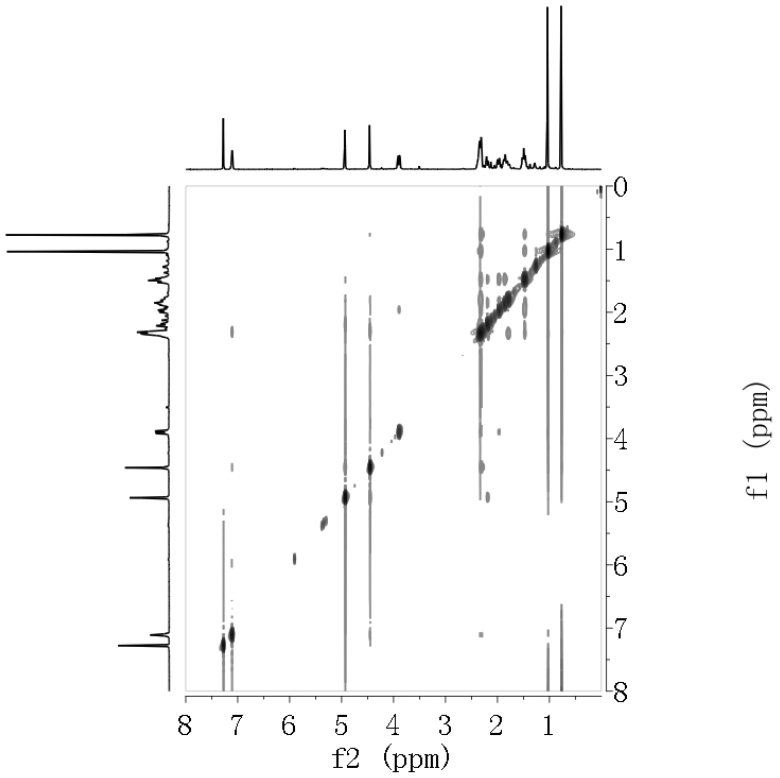
^{1}H-^{1}H COSY spectrum for xylariterpenoid C (**3**) in $CDCl_3$



HSQC spectrum for xylariterpenoid C (**3**) in $CDCl_3$

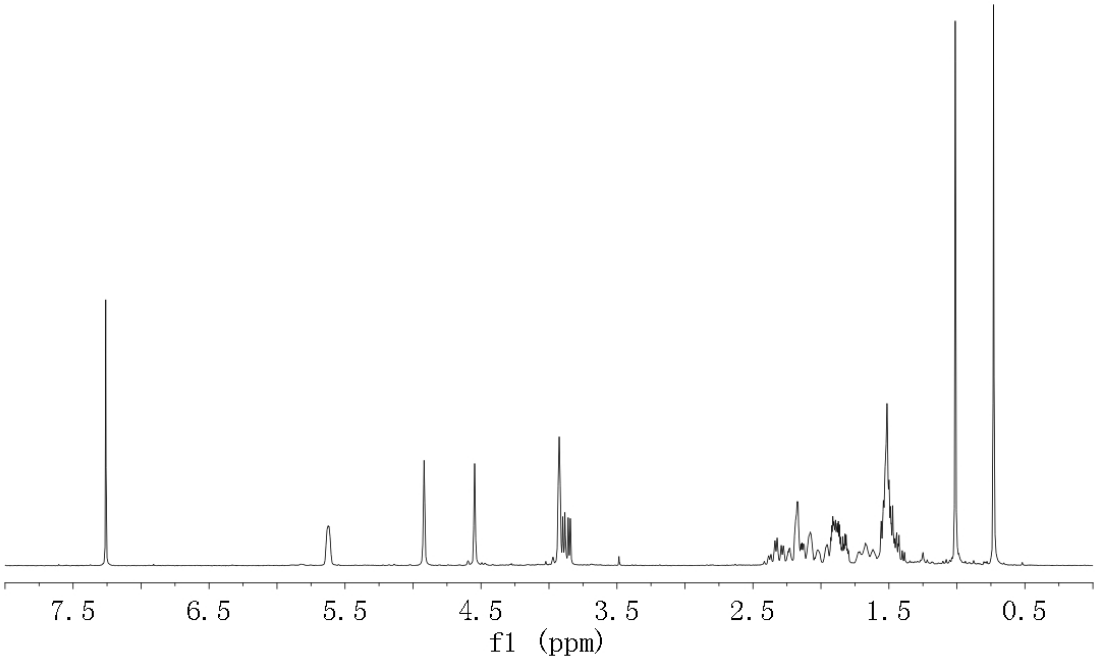


HMBC spectrum for xylariterpenoid C (**3**) in $CDCl_3$

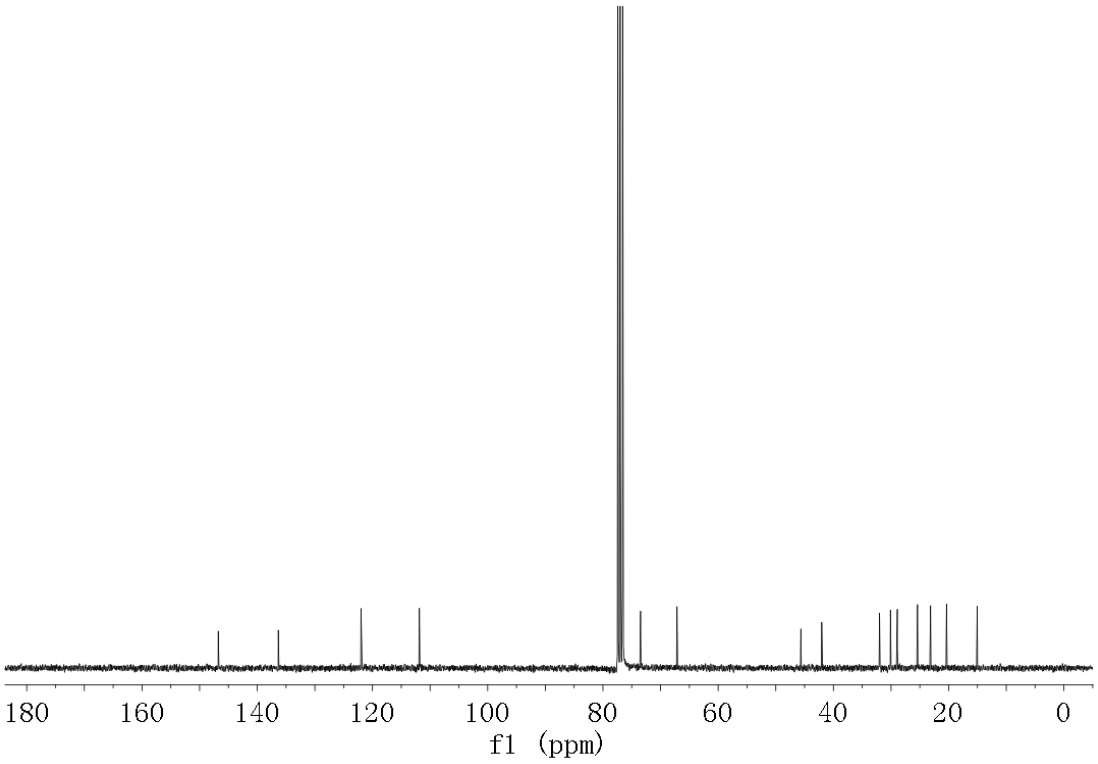


NOESY spectrum for xylariterpenoid C (**3**) in $CDCl_3$

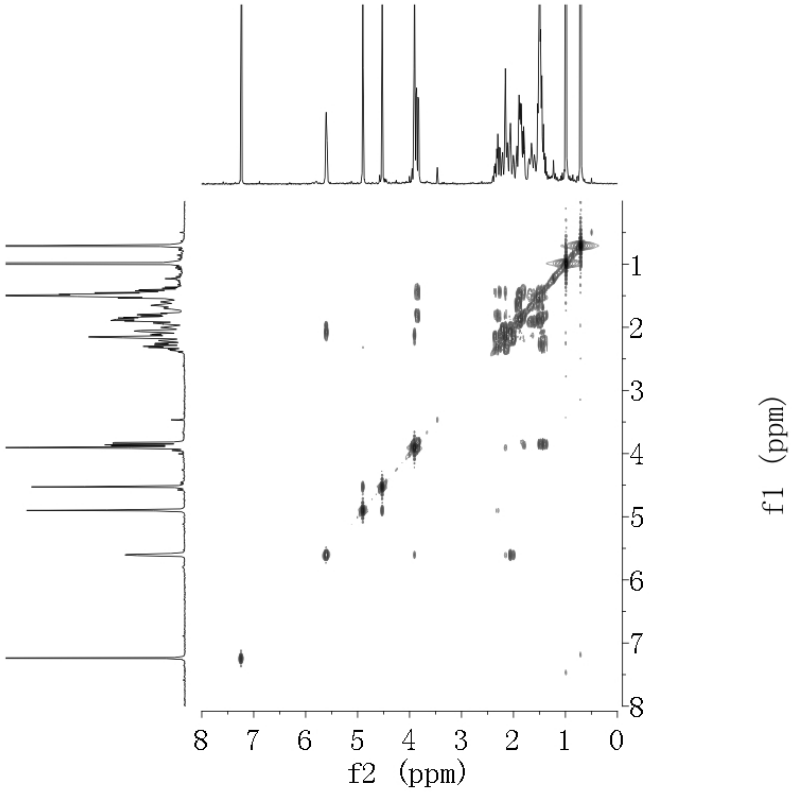
4 The 1D and 2D NMR spectra of xylariterpenoid D (4)



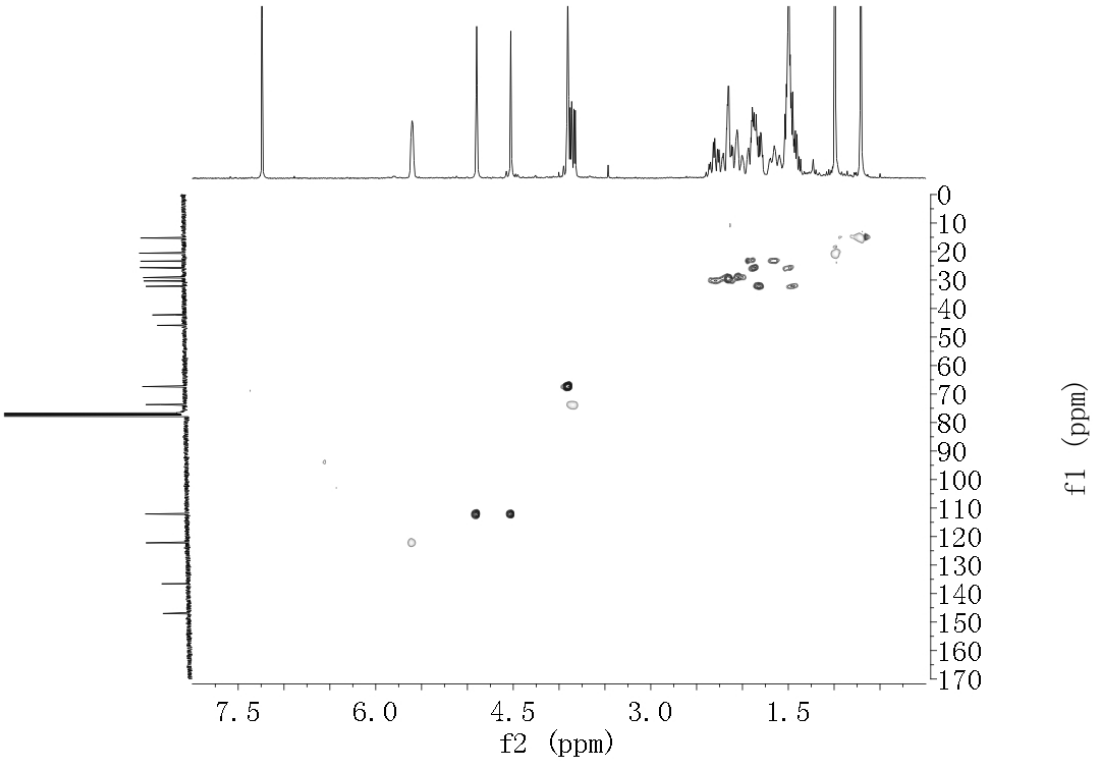
^{1}H NMR spectrum for xylariterpenoid D (**4**) (400 MHz, in $CDCl_3$)



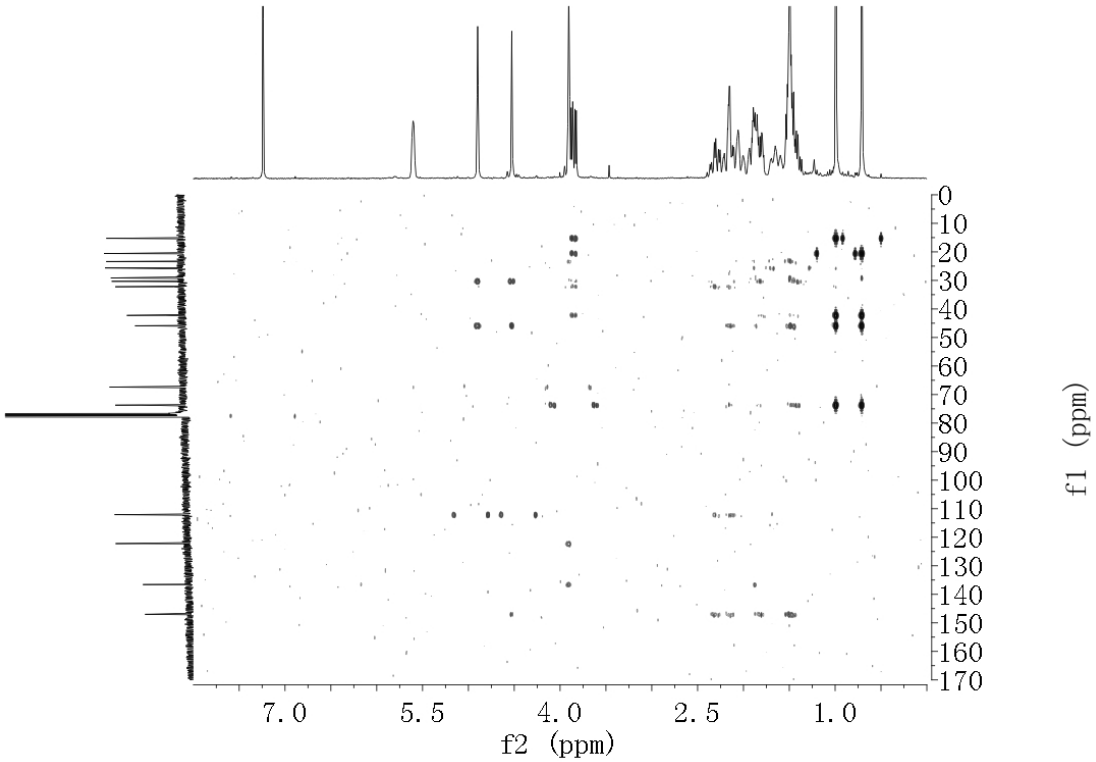
^{13}C NMR spectrum for xylariterpenoid D (**4**) (400 MHz, in $CDCl_3$)



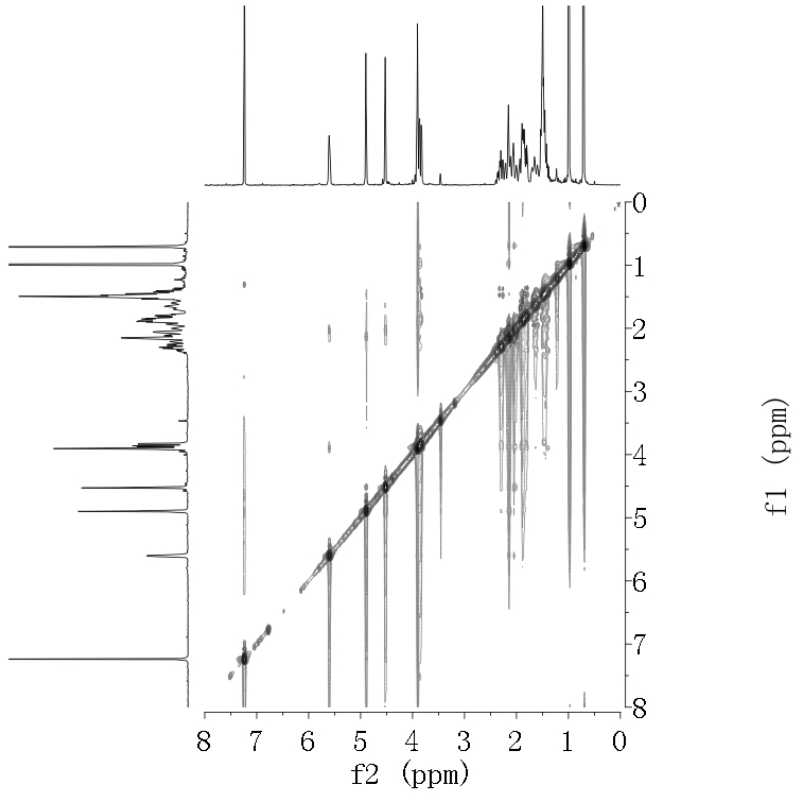
^{1}H-^{1}H COSY spectrum for xylariterpenoid D (**4**) in $CDCl_3$



HSQC spectrum for xylariterpenoid D (**4**) in $CDCl_3$



HMBC spectrum for xylariterpenoid D (**4**) in $CDCl_3$



NOESY spectrum for xylariterpenoid D (**4**) in $CDCl_3$