Supplementary data

Two new ditetrahydrofuran lignans from aerial parts of *Artemisia sieversiana*

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ABSTRACT

Two new ditetrahydrofuran lignans, named sieverlignans A and B (**1** and **2**), together with six known ones (**3-8**), were isolated from the aerial parts of *Artemisia sieversiana*. Their structures were established on the basis of spectroscopic analysis including HRMS, NMR spectra and circular dichroism experiments. All the compounds were evaluated for their anti-neuroinflammatory effects on the lipopolysaccharides (LPS)-induced nitric oxide production in BV-2 murine microglial cells. Compound **2** exhibited the significant activity with its IC_{50} value of 11.9±0.8 μM respectively, compared to a positive control quercetin with its IC_{50} value of 16.0 ±1.1 μM.

Known compounds information:

Compound **3**: White solid; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.93 (1H, d, J = 1.9 Hz,), 6.89 (1H, d, J = 8.0, 1.9 Hz), 6.84 (1H, d, J = 8.1 Hz), 6.60 (1H, s), 6.52 (1H, s,), 5.97 (2H, s, -OCH_2O-), 4.83 (1H, d, J = 5.4 Hz), 4.42 (1H, d, J = 7.3 Hz), 4.11 (1H, m), 3.93 (3H, s), 3.90 (3H, s), 3.87 (3H, s), 3.84 (1H, m), 3.83 (1H, overlapped), 3.34 (1H, m), 3.32 (1H, m), 2.90 (1H, m). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 149.2, 148.8, 148.7, 143.5, 134.1, 133.5, 132.9, 118.5, 110.9, 109.0, 104.8, 101.4, 100.0, 87.6, 82.0, 70.9, 69.6, 56.7, 55.9, 55.9, 54.4, 50.0. Comparing these NMR data with ref. (Pablo et al. 2005), compound **3** was identified as 3,4,5′-trimethoxy-3′,4′-methylenedioxy-7,9′:7′,9-diepoxylignan.

Compound **4**: White solid; ^{1}H-NMR (500 MHz, MeOD) δ: 6.89 (1H, d, J = 1.5 Hz), 6.86 (1H, dd, J = 8.0, 1.5 Hz), 6.78 (1H, d, J = 8.0 Hz), 6.68 (2H, s), 5.93 (2H, s,), 4.74 (2H, m), 4.28 (1H, dd, J = 9.2, 6.7 Hz), 4.26 (1H, dd, J = 9.2, 6.7 Hz), 3.91 (1H, dd, J = 9.2, 3.6 Hz), 3.89 (1H, dd, J = 9.2, 3.6 Hz), 3.85 (6H, s), 3.75 (3H, s), 3.11 (2H, m).^{13}C-NMR (125 MHz, MeOH) δ: 154.6, 148.2, 147.3, 138.8, 137.0, 135.2, 120.6, 108.9, 107.5, 104.3, 102.4, 87.4, 87.3, 72.9, 72.8, 61.1, 56.6, 55.7, 55.6. Comparing these NMR data with ref. (Ahmed et al. 2002), compound **4** was identified as ashantin

Compound **5**: White solid; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.58 (2H, s), 6.57 (2H, s), 4.84 (1H, d, J = 5.4 Hz), 4.42 (1H, d, J = 7.0 Hz), 4.15 (1H, d, J = 9.5 Hz), 3.88 (2H, m), 3.86 (12H, brs), 3.84 (3H, s), 3.81 (3H, s), 3.35 (1H, m), 3.36 (1H, m), 2.90 (1H, m)。^{13}C-NMR (125 MHz, $CDCl_3$) δ: 153.3, 153.1, 137.4, 136.7, 136.6, 133.9, 102.8, 102.4, 87.7, 82.0, 70.9, 69.7, 60.8, 60.7, 56.0, 54.4, 49.9. Comparing these NMR data with ref. (Ahmed et al. 2002), compound **5** was identified as epiyangambin.

Compound **6**: White solid; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.85 (1H, d, J = 1.5 Hz), 6.81 (1H, dd, J = 1.5, 8.0 Hz),6.77 (1H, d, J = 8.0 Hz), 6.71 (2H, brs), 5.96 (2H, s), 4.46 (1H, d, J = 6.0 Hz), 4.09 (1H, dd, J = 7.0, 9.0 Hz), 4.03 (1H, dd, J = 2.0, 9.0

Hz), 3.89 (6H, s), 3.87 (3H, s), 3.81 (1H, dd, *J* = 9.0, 9.0 Hz), 3.29 (1H, dt, *J* = 9.0, 9.0 Hz), 3.07 (1H, dd, *J* = 2.0, 9.0 Hz), 3.00 (1H, m), 2.98 (3H, s).^{13}C-NMR (125 MHz, $CDCl_3$) δ: 153.1, 147.9, 147.3, 137.6, 134.9, 133.2, 119.6, 110.2, 108.1, 106.7, 103.9, 101.0, 87.8, 70.3, 69.5, 60.8, 56.8, 56.2, 52.9, 48.8. Comparing these NMR data with ref. (Ma et al. 2001), compound **6** was identified as carullignan B.

Compound **7**: White solid; ^{1}H-NMR (500 MHz, MeOD) δ: 6.64 (1H, s), 6.60 (1H, s), 6.59 (2H, s), 5.96 (2H, s), 4.64 (1H, d, *J* = 4.5 Hz), 4.60 (1H, d, *J* = 4.5 Hz), 4.16 (1H, dd, *J* = 6.7, 9.2 Hz), 4.14 (1H, dd, *J* = 6.7, 9.2 Hz), 3.82 (3H, s), 3.80 (1H, m), 3.78 (1H, m), 3.75 (6H, s), 3.03 (2H, m)。^{13}C-NMR (125 MHz, MeOD) δ: 148.6, 147.9, 143.1, 136.5, 134.6, 133.7, 131.5, 107.5, 104.2, 102.3, 99.9, 85.8, 85.0, 72.8, 72.7, 56.6, 56.0, 55.7, 55.5. Comparing these NMR data with ref. (Li et al. 2007; Greger et al. 1980), compound **7** was identified as2-(5-methoxy-3,4-methylenedioxyphenyl)-6-(4-hydroxy-3,5-dimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane.

Compound **8**: White solid; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.58 (4H, s), 4.88 (2H, d, *J* = 4.3 Hz), 3.75 (2H, d, *J* = 9.5 Hz), 3.60 (2H, m), 3.86 (12H, brs), 3.82 (6H, s), 3.32 (1H, m)。^{13}C-NMR (125 MHz, $CDCl_3$) δ: 153.4, 137.5, 134.5, 103.2, 84.0, 68.8, 60.8, 56.0, 49.4. Comparing these NMR data with ref. (Tulake et al. 2012), compound **8** was identified as diyangambin.

Contents:

Table S1:

Table S1. The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of **1** (in $CDCl_3$) and **2** (in CD_3OD (δ in ppm, J in Hz)

No.	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
1	136.6	--	137.4	--
2	101.3	6.68, s	101.1	6.57, s
3	148.9	--	150.6	--
4	135.1	--	136.2	--
5	143.4	--	145.0	--
6	106.6	6.73, s	107.4	6.62, s
7	110.1	--	87.3	4.72, d, (4.8)
8	56.9	3.29, dd, (8.5, 8.7)	55.7	3.12, m
9α	70.4	3.86, overlapped	72.7	4.25, dd, (9.0, 6.6)
9β	70.4	3.12, m		3.87, m
1′	137.5	--	135.2	--
2′	103.1	6.58，s	119.8	6.93，s
3′	153.4	--	150.5	--
4′	132.3	--	111.2	6.99, s
5′	153.4	--	150.3	--
6′	103.1	6.58，s	112.9	6.93，s
7′	87.9	4.47, d, (6.6)	87.2	4.74, d, (4.7)
8′	53.0	3.04, m	55.4	3.11, m
9′α	69.6	4.11, dd, (8.9, 6.8)	72.8	4.27, dd, (9.2, 6.6)
9′β	69.6	4.05, dd, (8.9, 1.6)		3.88, m
3,4-OCH_2O-	101.6	6.01, s	102.6	5.92, s
5-OMe	56.7	3.94, s	57.3	3.89, s
7-OMe	48.7	2.99, s	56.5	3.84, s
3′-OMe				
4′-OMe	60.8	3.84, s		
5′-OMe			56.5	3.82, s
3′,5′-OMe	56.2	3.88, s		

Table S2:

Table S2. Inhibitory effects of compounds **1**–**8** on NO production induced by LPS in BV-2 cells

Compounds	IC_{50} (μM) [a]	Cell viability[b]	Compounds	IC_{50} (μM)	Cell viability[b]
1	>100	92.3±1.1	**5**	56.3±0.6	89.9±0.3
2	11.9±0.8	97.4±0.9	**6**	>100	91.3±1.2
3	>100	98.0±1.8	**7**	37.9±1.6	92.2±0.3
4	48.6±3.4	88.1±0.5	**8**	83.8±0.5	98.7±0.8
Quercetin [c]	16.0±1.1	95.9±1.3			

[a] The values represent the mean±SD of three individual observations.

[b] Cell viability was expressed as a percentage (%).

[c] Quercetin was used as a positive control.

Figure S1:

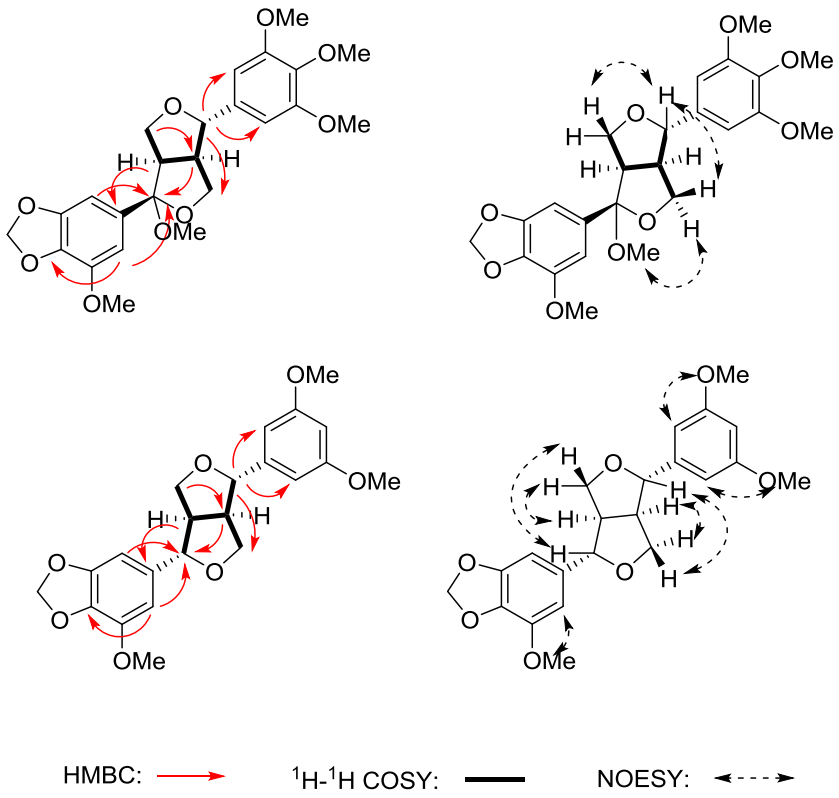


Figure S1. Key HMBC, ^{1}H-^{1}H COSY and NOESY correlations of compounds **1** and **2**.

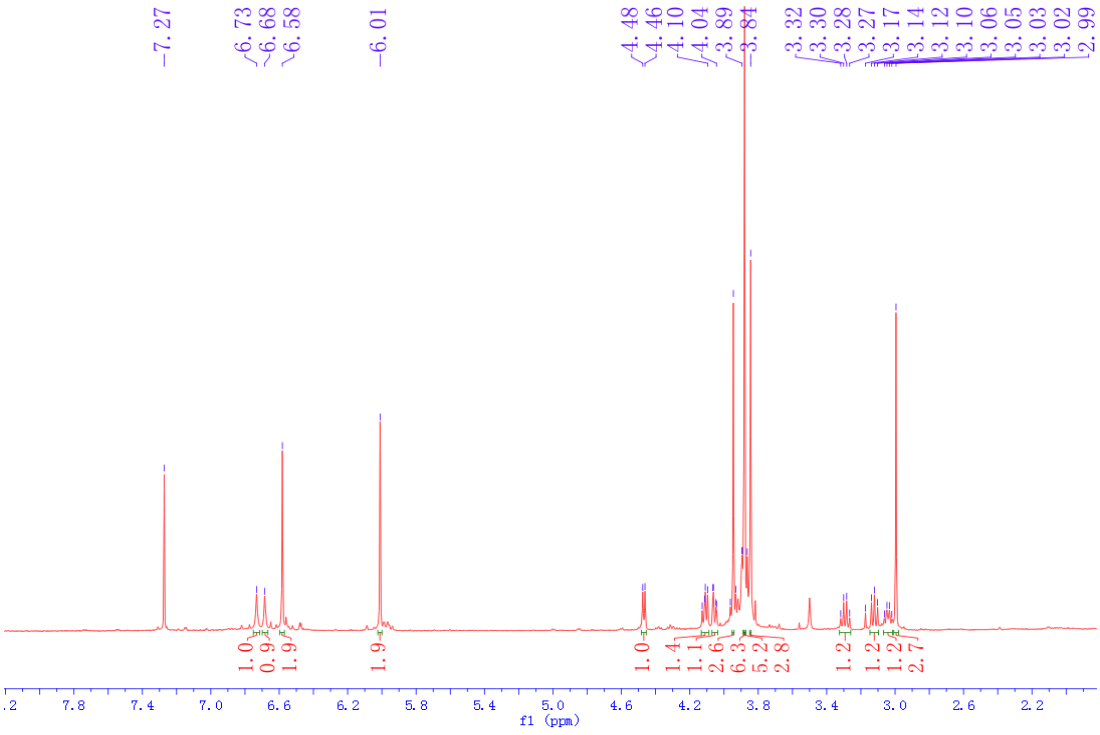


Figure S2. ^{1}H NMR spectrum of sieverlignan A (**1**) ($CDCl_3$, 500 MHz).

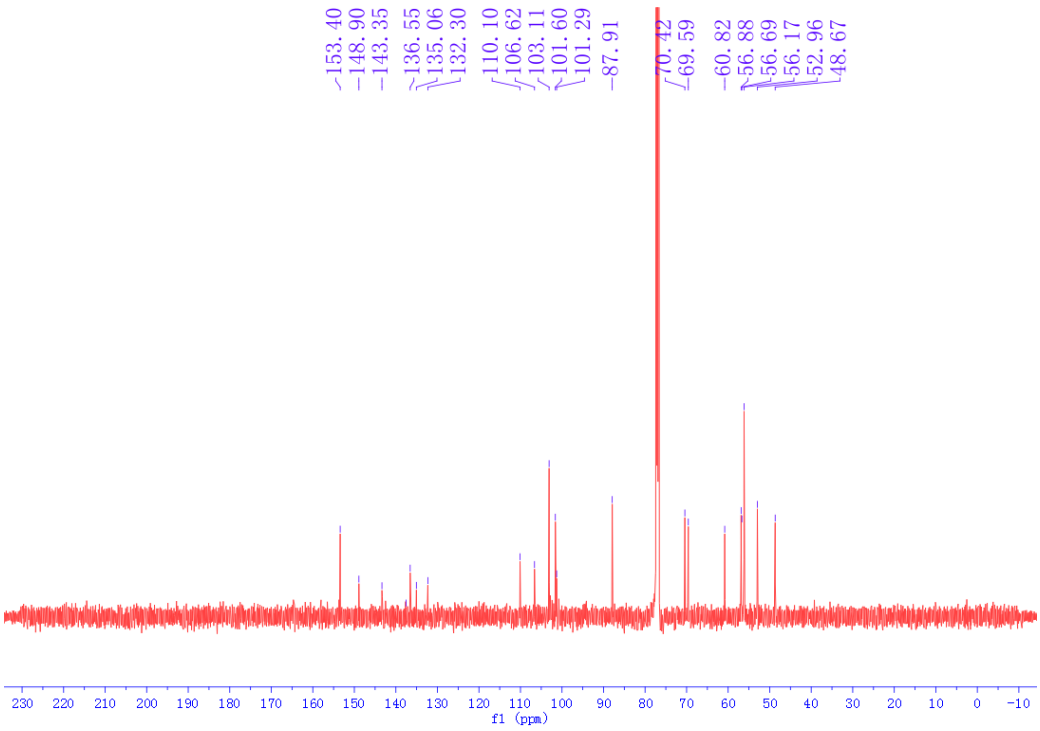


Figure S3. ^{13}C NMR spectrum of sieverlignan A (**1**) ($CDCl_3$, 125 MHz).

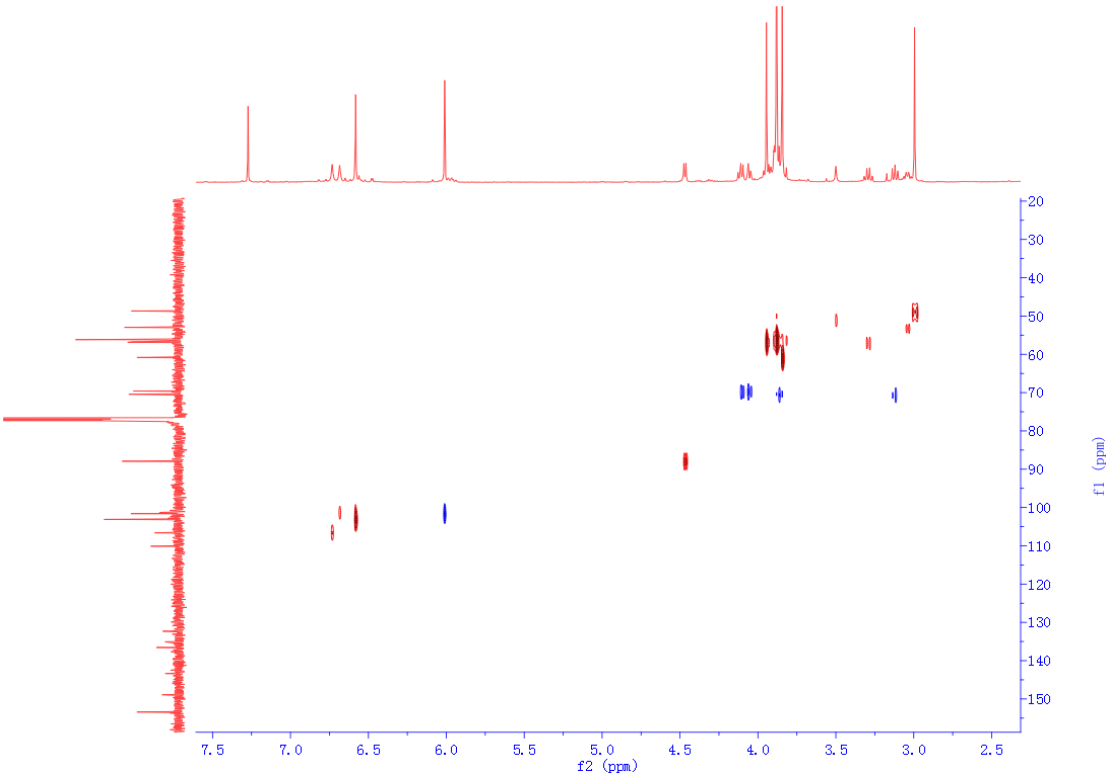


Figure S4. gHSQC spectrum of sieverlignan A (**1**).

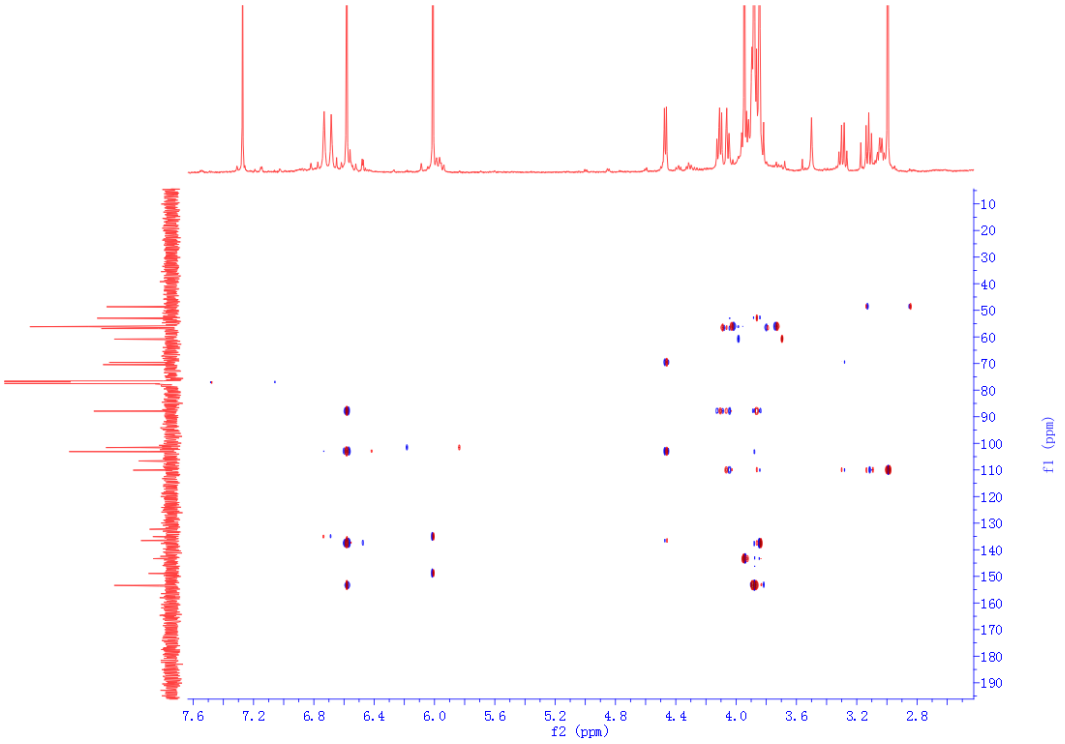


Figure S5. gHMBC spectrum of sieverlignan A (**1**).

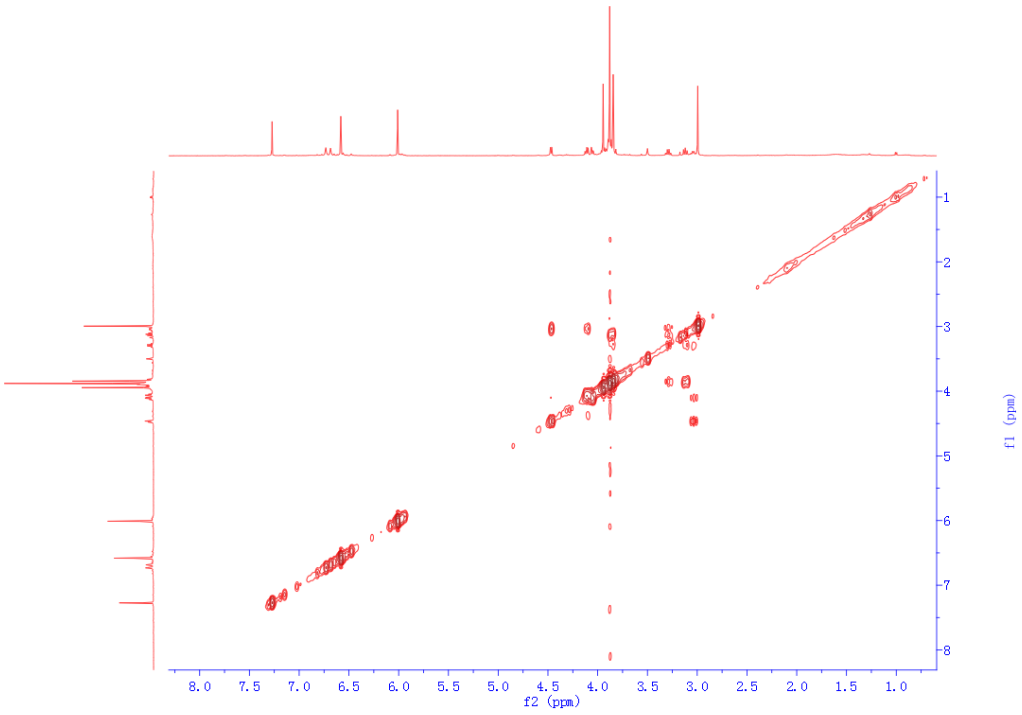


Figure S6. ^{1}H-^{1}H gCOSY spectrum of sieverlignan A (**1**).

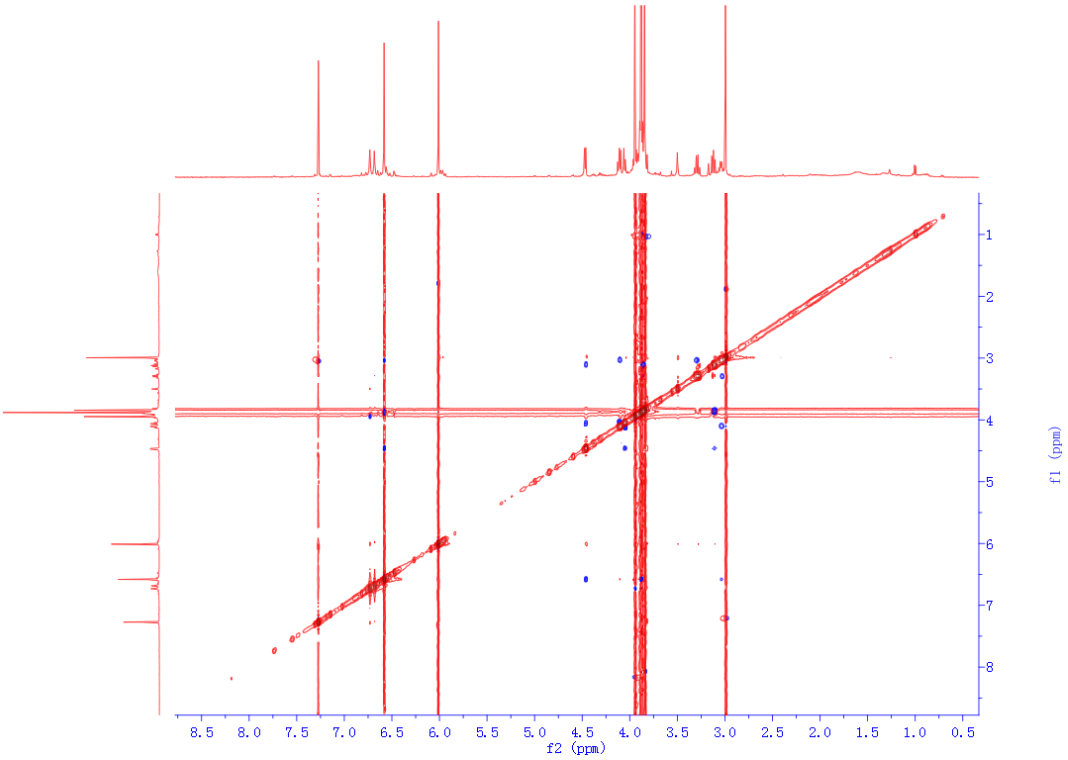
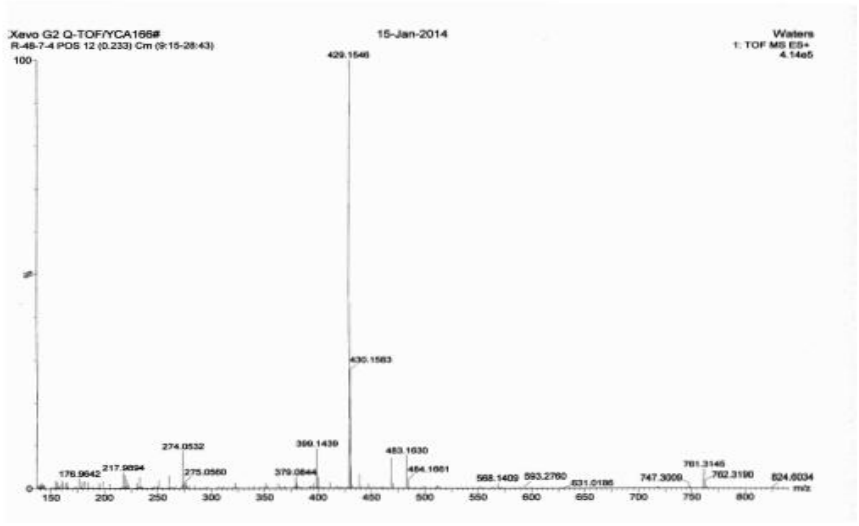


Figure S7. NOESY spectrum of sieverlignan A (**1**).

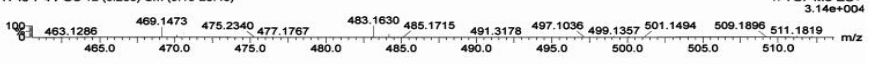


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
233 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 O: 0-30 Na: 0-1

Xevo G2 Q-TOF/YCA166#
R-48-7-4 POS 12 (0.233) Cm (9:15-28:43)
15-Jan-2014
Waters
1: TOF MS ES+
3.14e+004



Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
483.1630	483.1631	-0.1	-0.2	10.5	183.7	0.176	83.84	C24 H28 O9 Na
	483.1655	-2.5	-5.2	13.5	185.4	1.895	15.03	C26 H27 O9
	483.1596	3.4	7.0	22.5	188.0	4.482	1.13	C33 H23 O4

Figure S8. HR-ESIMS spectrum of sieverlignan A (**1**).

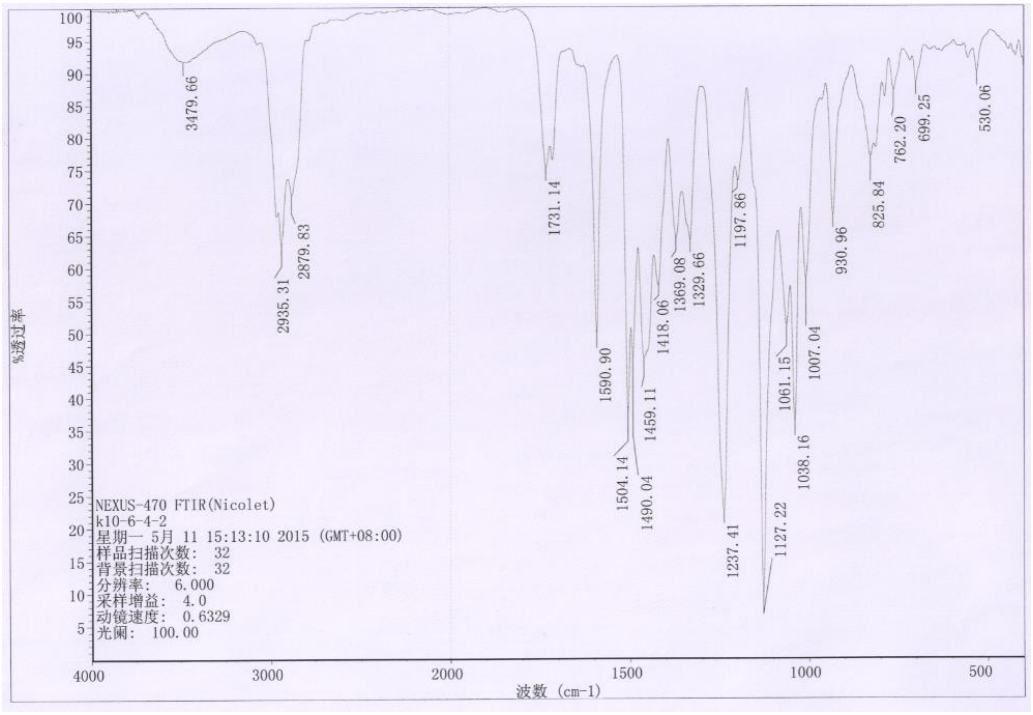


Figure S9. IR spectrum of sieverlignan A (**1**).

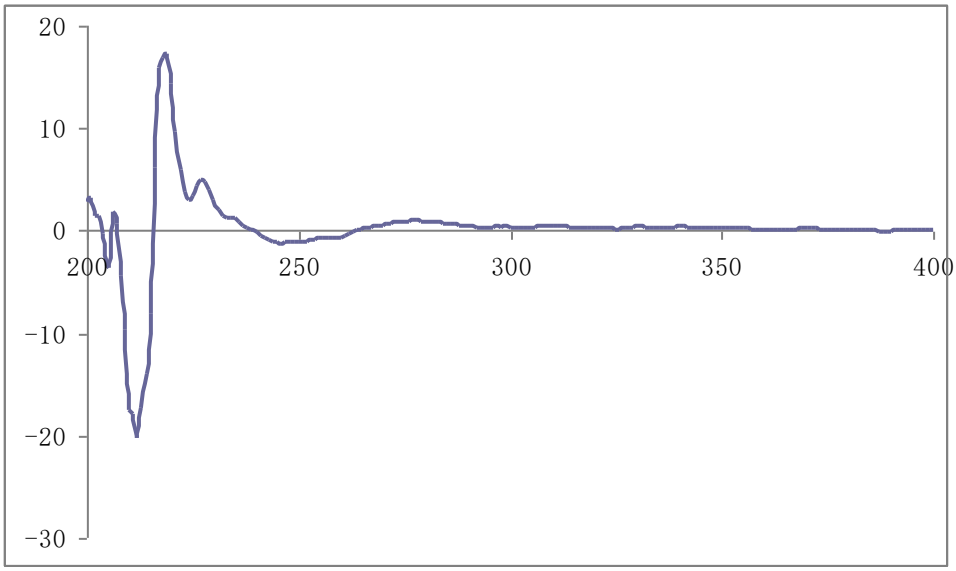


Figure S10. CD spectrum of sieverlignan A (**1**).

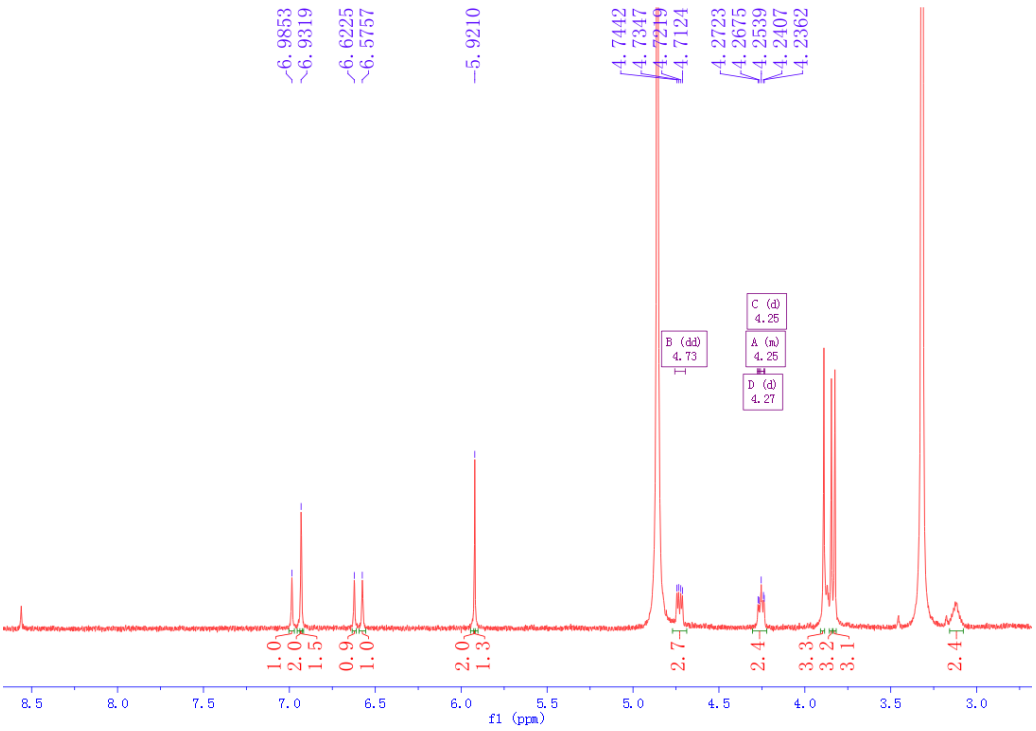


Figure S11. ^{1}H NMR spectrum of sieverlignan B (**2**) (CD_3OD, 500 MHz).

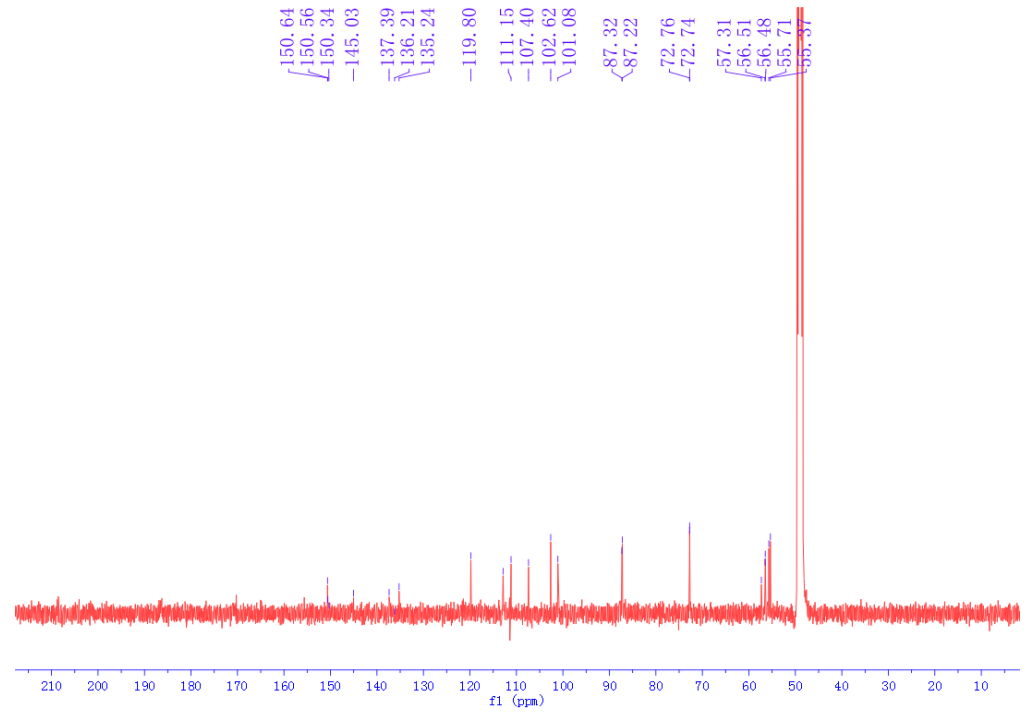


Figure S12. ^{13}C NMR spectrum of sieverlignan B (**2**) (CD_3OD, 125 MHz).

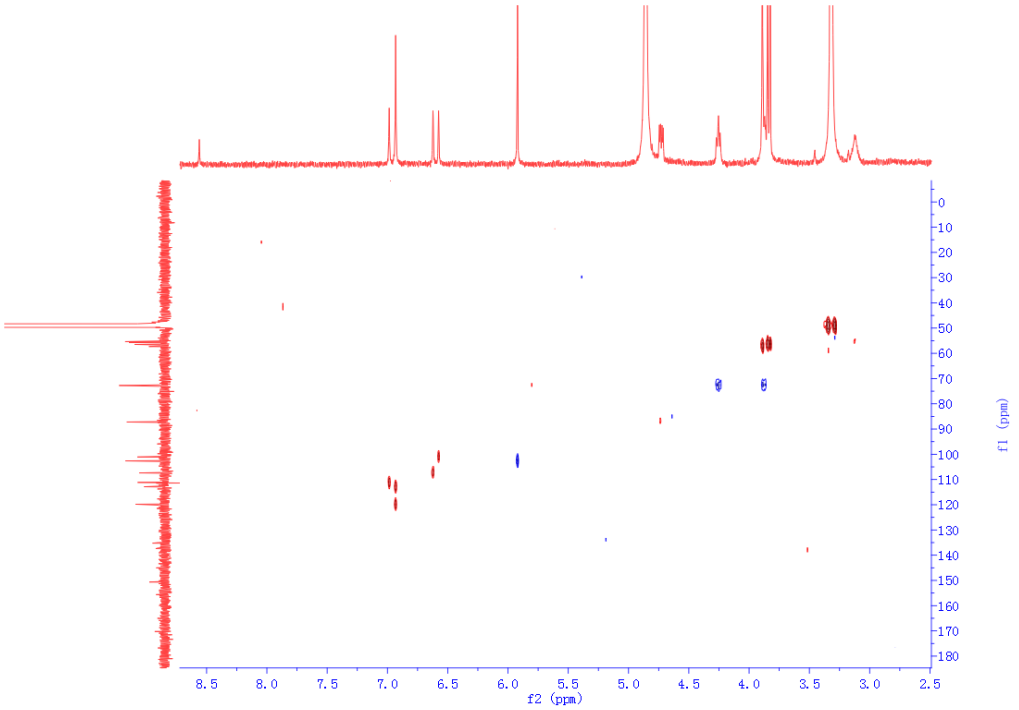


Figure S13. gHSQC spectrum of sieverlignan B (**2**).

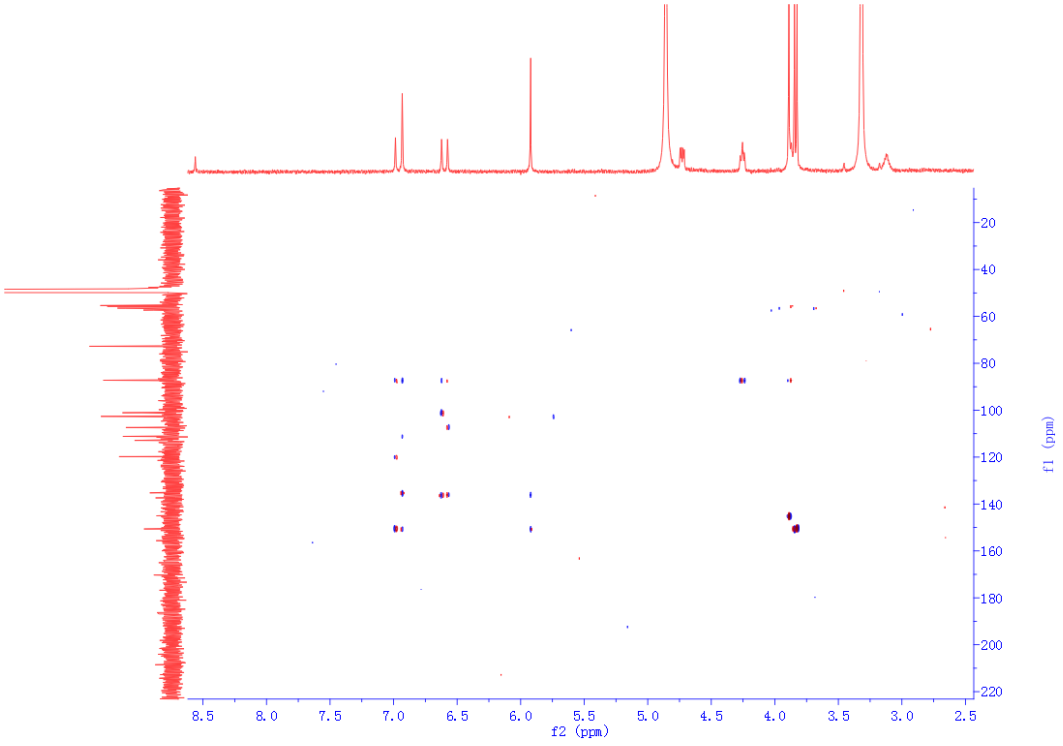


Figure S14. gHMBC spectrum of sieverlignan B (**2**).

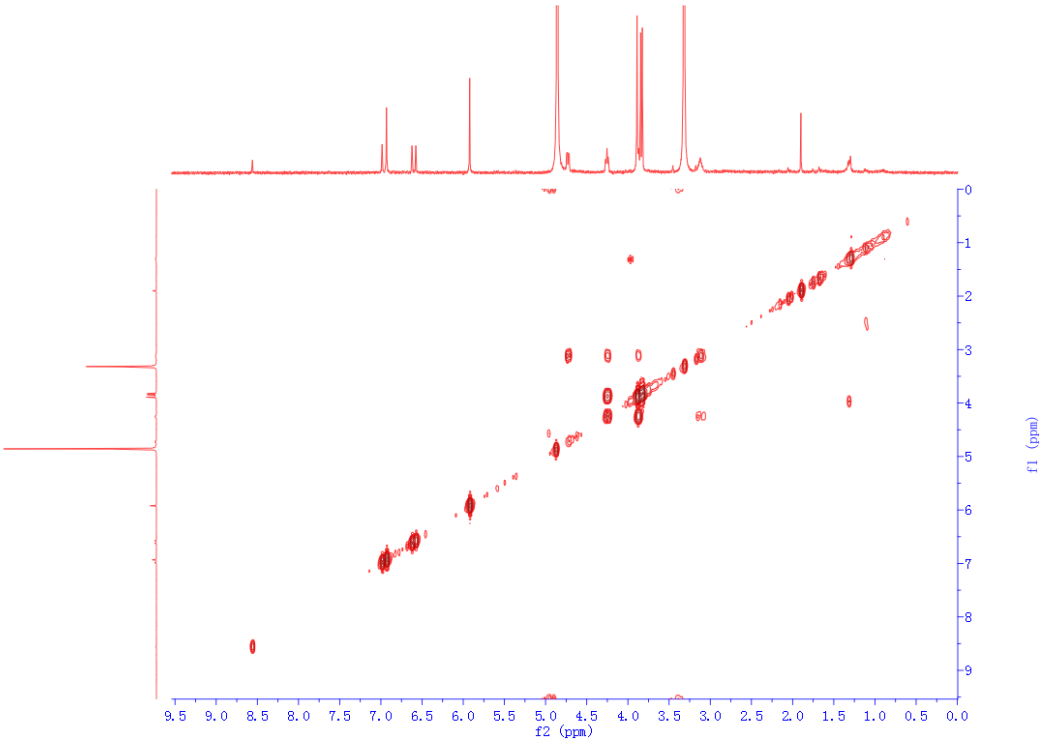


Figure S15. ^{1}H-^{1}H gCOSY spectrum of sieverlignan B (**2**).

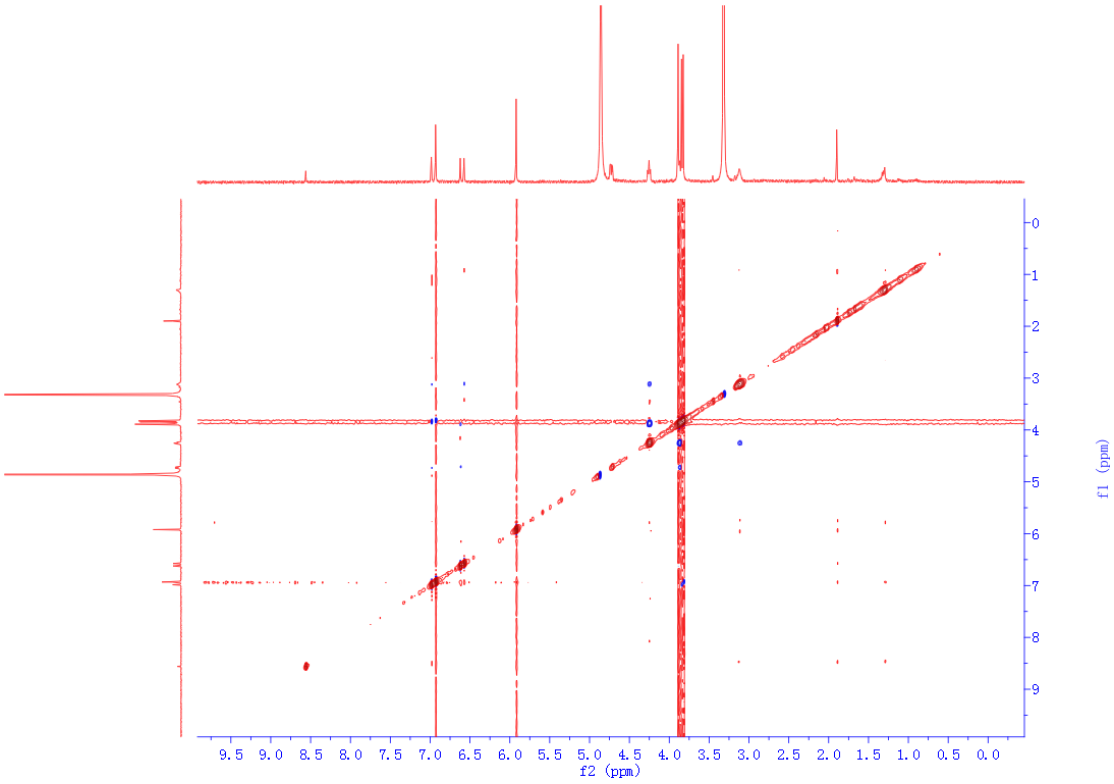


Figure S16. NOESY spectrum of sieverlignan B (**2**).

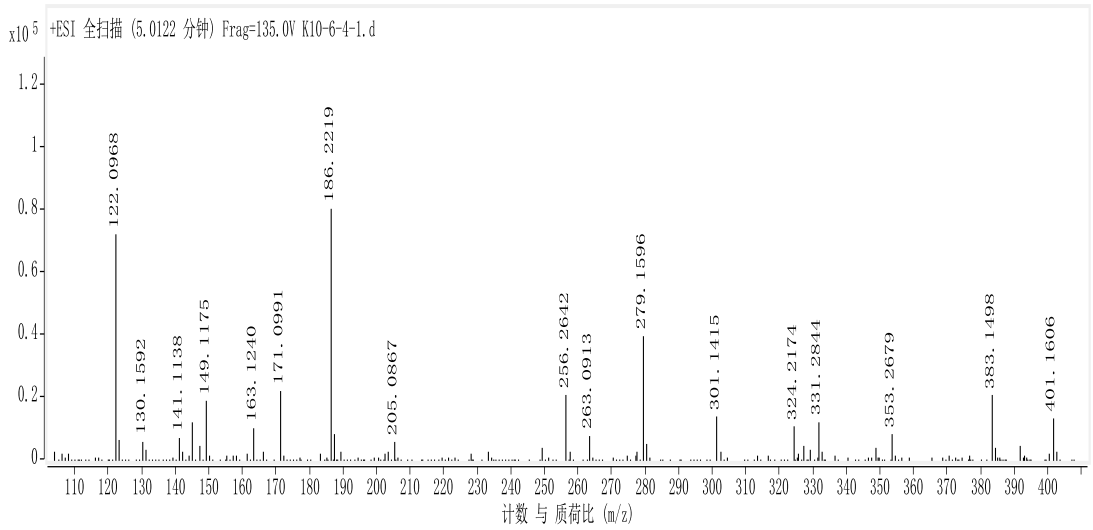


Figure S17. HR-ESIMS spectrum of sieverlignan B (**2**).

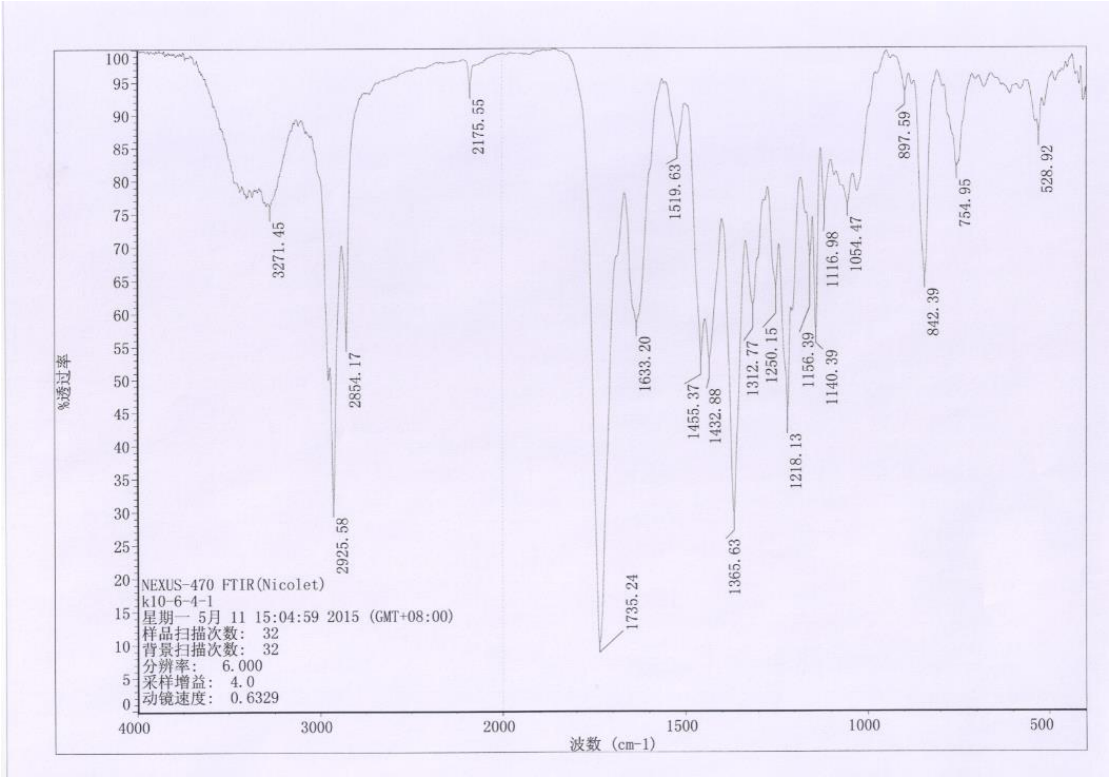


Figure S18. IR spectrum of sieverlignan B (**2**).

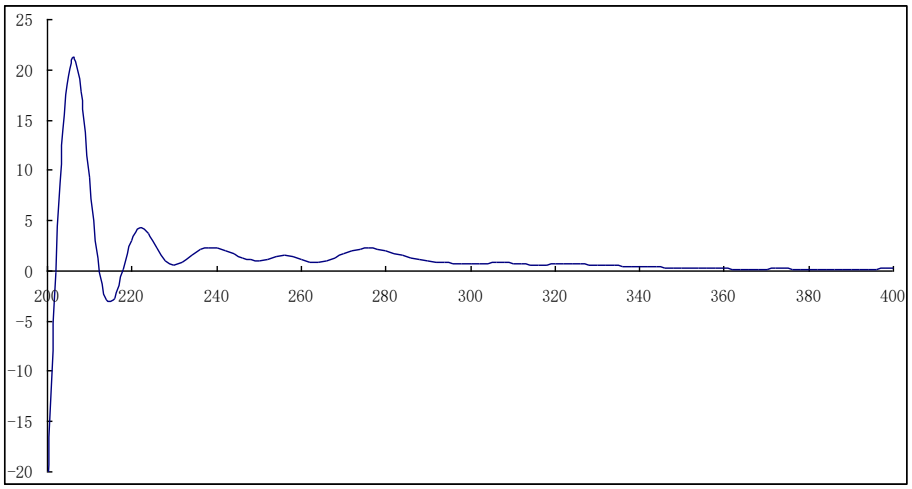


Figure S19. CD spectrum of sieverlignan B (**2**).