Electronic Supplementary Information (ESI)

Oleonin, the first secoiridoid with 1α-configuration from *Ligustrum lucidum*

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Contents

Experimental section

General experimental procedures. Melting point was recorded on an XT-4 micro melting point apparatus without correction. Optical rotation value was measured on a JASCO P-1020 digital polarimeter with a 0.01 dm length cell. IR spectrum (KBr) was recorded on a JASCO FT/IR-480 plus Fourier transform infrared spectrometer. UV spectrum was obtained on a JASCO V-550 UV/VIS spectrophotometer with a 0.1 dm length cell. ^{1}H (400 MHz), ^{13}C (100 MHz), and 2D NMR spectra were recorded on Bruker AV-400 spectrometer. HR-ESI-MS spectrum was measured using an Agilent 6210 LC/MSD TOF mass spectrometer. Preparative HPLC was carried out on a Varian instrument equipped with UV detector (Varian, Palo Alto, CA, USA) and a reversed-phase C_{18} column (5 μm, 20 × 250 mm; Cosmosil, Houston, TX, USA). Column chromatographies were carried out using silica gel (200–400 mesh; Qingdao Haiyang Chemical Group Corporation, Qingdao, China). TLC analyses were performed on precoated silica gel GF_{254} plates (Yantai Chemical Industrial Institute, Yantai, China). All solvents used in column chromatography and HPLC were of analytical grade (Shanghai Chemical Plant, Shanghai, China) and chromatographic grade (Fisher Scientific, New Jersey, U. S. A), respectively.

Plant Material. The fruits of *Ligustrum lucidum* Ait. (Oleaceae) were collected from Nanjing City, Jiangsu Province of China, in October of 2006, and authenticated by Prof. Min-Jian Qin (Department of Pharmacognosy, China Pharmaceutical University, Nanjing, China). A voucher specimen (No. 20060517) was deposited in the herbarium of the China Pharmaceutical University, Nanjing.

Extraction and isolation. The air-dried and powdered fruits (40 kg) of *L. lucidum* were extracted with 70 % (V/V) EtOH three times (3 × 40 L, 1 h each) under reflux. The EtOH solution was concentrated under vacuum to yield a residue, which was suspended in water and partitioned successively with petroleum ether, EtOAc and *n*-BuOH. The *n*-BuOH extract (1.2 kg) was subjected to macroporous resin D101 (3 kg) column eluted with EtOH-H_2O mixtures (0:100, 15:85, 35:65, 55:45, 95:5). The 35 % EtOH eluate (200 g) was subjected to a silica gel column eluted with gradient mixtures of $CHCl_3$-MeOH-H_2O (90:10:0.1→50:50:5) to afford 11 fractions (Fr. 1-11). Fr. 9 (20 g) was separated by ODS column using gradient mixtures of MeOH-H_2O (20:80→70:30) as eluent to yield 10 subfractions. Subfraction 10 was further purified by preparative HPLC (MeOH-H_2O, 50:50) to afford **1** (5.5 mg).

Oleonin (1). Yellow plates, mp 152-154 ºC, $[\alpha]_D^{26}$ -153 ° (*c* = 0.20, MeOH). UV (MeOH) λ_{max} 235 nm; IR (KBr) ν_{max} 3443, 2952, 1729, 1701, 1641, 1434, 1256 and 1080 cm^{-1}; CD (MeOH) 225 ($\Delta\varepsilon$ -18.20) nm; HR-ESI-MS *m/z* 293.0994 ($[M+Na]^+$, calcd for $C_{13}H_{18}O_6Na$ 293.0996).

Computational methods

Computational methods for ECD calculation

Conformational analyses were performed for all possible stereoisomers by Sybyl 8.0 program using Random Searching together with the MMFF94s molecular mechanics force field charged with MMFF94. And the conformers that differ from the most stable one by less than 6 kcal mol^{-1} had been taken into account for optimization at B3LYP/6-31G(d,p) in Gaussian 09 software.[1] All stable conformations obtained, using a window of 2 kcal mol^{-1}, were then further reoptimized at the B3LYP/6-311++G(2d,p) level. The ECD calculations were performed at the same level in Gaussian 09, and the solvent effects were considered under the PCM model. The overall ECD curves were weighted by Boltzmann distributions after a UV correction of 11 nm, and were subsequently compared with the experimental ones. The ECD curves were produced by SpecDis 1.60 software. [2]

Coordinates and optimized energies of computations (method: B3LYP/6-311++G (2d,p))

Cartesian coordinate of 1*S*,5*S*-1 optimized:

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z

1	6	0	-1.507267	0.000631	0.209972
2	6	0	-0.020694	-0.198927	0.520766
3	6	0	0.541281	1.147611	0.986273
4	6	0	0.015481	2.338326	0.167492
5	8	0	-1.425781	2.426984	0.191665
6	6	0	-2.055695	1.196865	0.078849
7	6	0	-2.351637	-1.245034	0.082363
8	8	0	-1.946362	-2.387836	0.210333
9	8	0	-3.687688	-0.949850	-0.203197
10	6	0	-4.489991	-2.135309	-0.316517
11	8	0	0.476671	2.204549	-1.183463
12	6	0	0.175147	3.386348	-1.940608
13	6	0	1.330656	1.361886	2.016246
14	6	0	1.908469	0.330472	2.973409
15	6	0	0.722177	-0.776182	-0.721120
16	6	0	2.112624	-1.354120	-0.380412
17	8	0	2.918953	-1.371813	-1.518376
18	6	0	4.211198	-1.945034	-1.261351
19	8	0	2.485012	-1.776975	0.696841
20	1	0	0.063905	-0.929361	1.324632
21	1	0	0.360363	3.288320	0.605525
22	1	0	-3.122410	1.310935	-0.108591

23	1	0	-5.507297	-1.807590	-0.536756
24	1	0	-4.142457	-2.785920	-1.123894
25	1	0	-4.494722	-2.713506	0.611693
26	1	0	0.680470	4.267161	-1.532081
27	1	0	0.534379	3.217755	-2.957211
28	1	0	-0.898670	3.590116	-1.976697
29	1	0	1.603108	2.387239	2.245567
30	1	0	1.318960	0.294675	3.887836
31	1	0	1.933228	-0.659485	2.531878
32	1	0	2.922432	0.609216	3.246158
33	1	0	0.131096	-1.587770	-1.143460
34	1	0	0.838260	-0.010401	-1.483589
35	1	0	4.764192	-1.378361	-0.507081
36	1	0	4.135842	-2.982526	-0.923954
37	1	0	4.762395	-1.916186	-2.202586

Cartesian coordinate of 1*S*,5*R*-1 optimized:

Standard orientation:

Center	Atomic	Atomic	Coordinates (Angstroms)		
Number	Number	Type	X	Y	Z

1	6	0	1.511302	0.083524	0.080418
2	6	0	0.046404	-0.344795	0.193109
3	6	0	-0.787436	0.895685	0.521141
4	6	0	-0.344472	2.127744	-0.290965
5	8	0	1.049243	2.459540	-0.060086
6	6	0	1.874996	1.352698	-0.012718
7	6	0	2.550994	-1.009741	0.128713
8	8	0	2.317842	-2.200212	0.251465
9	8	0	3.850065	-0.506413	0.015641
10	6	0	4.838825	-1.546129	0.072920
11	8	0	-1.117800	3.261871	0.086012
12	6	0	-0.850419	4.370339	-0.786538
13	6	0	-1.755721	0.987713	1.403633
14	6	0	-2.309750	-0.111823	2.295217
15	6	0	-0.420814	-1.065312	-1.110008
16	6	0	-1.873909	-1.577543	-1.023245
17	8	0	-1.989442	-2.581421	-0.061124
18	6	0	-3.321238	-3.116472	0.008722
19	8	0	-2.811574	-1.190777	-1.693174
20	1	0	-0.032943	-1.066919	1.004030
21	1	0	-0.459133	1.916711	-1.371882

22	1	0	2.926419	1.636024	-0.043533
23	1	0	4.801458	-2.091130	1.020263
24	1	0	5.811939	-1.061213	-0.020490
25	1	0	4.719589	-2.265982	-0.741658
26	1	0	-1.106588	4.137315	-1.825000
27	1	0	0.196848	4.682038	-0.747729
28	1	0	-1.475644	5.198994	-0.449921
29	1	0	-2.227126	1.956315	1.531364
30	1	0	-2.084360	0.105675	3.337382
31	1	0	-1.903974	-1.086455	2.049971
32	1	0	-3.391887	-0.156347	2.197543
33	1	0	0.231319	-1.918865	-1.285556
34	1	0	-0.351722	-0.398022	-1.965437
35	1	0	-4.054753	-2.350028	0.273497
36	1	0	-3.313004	-3.884523	0.783717
37	1	0	-3.625694	-3.569604	-0.938821

Cartesian coordinate of 1*R*,5*R*-1 optimized:

Standard orientation:

Center	Atomic	Atomic	Coordinates (Angstroms)		

Number	Number	Type	X	Y	Z
1	6	0	1.507255	0.000616	0.209975
2	6	0	0.020677	-0.198891	0.520783
3	6	0	-0.541255	1.147676	0.986258
4	6	0	-0.015429	2.338350	0.167432
5	8	0	1.425834	2.426972	0.191607
6	6	0	2.055716	1.196831	0.078816
7	6	0	2.351593	-1.245076	0.082393
8	8	0	1.946289	-2.387864	0.210382
9	8	0	3.687651	-0.949934	-0.203178
10	6	0	4.489919	-2.135418	-0.316476
11	8	0	-0.476622	2.204529	-1.183518
12	6	0	-0.175067	3.386285	-1.940719
13	6	0	-1.330601	1.362011	2.016242
14	6	0	-1.908428	0.330652	2.973459
15	6	0	-0.722211	-0.776154	-0.721093
16	6	0	-2.112662	-1.354084	-0.380382
17	8	0	-2.918964	-1.371866	-1.518362
18	6	0	-4.211199	-1.945109	-1.261331
19	8	0	-2.485065	-1.776889	0.696886
20	1	0	-0.063939	-0.929303	1.324666

21	1	0	-0.360290	3.288369	0.605427
22	1	0	3.122432	1.310866	-0.108638
23	1	0	5.507235	-1.807735	-0.536719
24	1	0	4.142366	-2.786032	-1.123843
25	1	0	4.494629	-2.713600	0.611744
26	1	0	0.898756	3.590015	-1.976829
27	1	0	-0.680354	4.267133	-1.532222
28	1	0	-0.534318	3.217663	-2.957310
29	1	0	-1.603002	2.387382	2.245541
30	1	0	-2.922383	0.609426	3.246210
31	1	0	-1.318909	0.294885	3.887879
32	1	0	-1.933206	-0.659325	2.531974
33	1	0	-0.131135	-1.587753	-1.143419
34	1	0	-0.838295	-0.010388	-1.483576
35	1	0	-4.762374	-1.916340	-2.202581
36	1	0	-4.764227	-1.378401	-0.507113
37	1	0	-4.135819	-2.982577	-0.923862

Cartesian coordinate of 1*R*,5*S*-1 optimized:

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-1.479870	0.847671	-0.058657
2	6	0	-0.418000	-0.197863	0.286013
3	6	0	0.863825	0.541386	0.678507
4	6	0	1.155623	1.739425	-0.245493
5	8	0	0.071899	2.704979	-0.246172
6	6	0	-1.178815	2.120447	-0.266402
7	6	0	-2.935498	0.451455	-0.125344
8	8	0	-3.863370	1.192043	-0.404333
9	8	0	-3.116456	-0.899378	0.180658
10	6	0	-4.499742	-1.283594	0.138847
11	8	0	2.327125	2.418052	0.194436
12	6	0	2.719547	3.419818	-0.756374
13	6	0	1.657390	0.265059	1.687973
14	6	0	1.524860	-0.863115	2.698494
15	6	0	-0.203745	-1.188492	-0.901369
16	6	0	0.848196	-2.270358	-0.571631
17	8	0	2.013005	-2.068892	-1.313048
18	6	0	3.018445	-3.054638	-1.026728
19	8	0	0.718065	-3.183562	0.220063

20	1	0	-0.776373	-0.782081	1.131630
21	1	0	1.278191	1.379600	-1.285450
22	1	0	-1.963243	2.851072	-0.465623
23	1	0	-4.540153	-2.344218	0.392246
24	1	0	-5.099444	-0.721376	0.859975
25	1	0	-4.932318	-1.137231	-0.854842
26	1	0	1.950368	4.185348	-0.889590
27	1	0	3.621872	3.893591	-0.366169
28	1	0	2.948013	2.981546	-1.733125
29	1	0	2.517346	0.908771	1.839220
30	1	0	1.370666	-0.452695	3.694391
31	1	0	0.703307	-1.532084	2.470200
32	1	0	2.442081	-1.447030	2.724143
33	1	0	-1.150999	-1.680081	-1.113255
34	1	0	0.117760	-0.657483	-1.793256
35	1	0	3.320725	-3.032960	0.023929
36	1	0	2.675075	-4.065389	-1.264110
37	1	0	3.881670	-2.816428	-1.650168

TDDFT results for the optimized conformer of compound (1*S*,5*S*)-1 (200 nm < λ < 400 nm). The TDDFT calculations are done at the level of B3LYP/6-31G(d) in the CPCM model (CH_3OH solvent: dielectric constant ε = 32.63).

No.	Excitation energy (cm^{-1})	Wavelength (nm)	Oscillator Strength *f*	Major contributions
1	41969.35	238.3	0.1313	71→LUMO (36%), HOMO→LUMO (62%)
2	42086.30	237.6	0.0009	69→LUMO (41%), 70→LUMO (54%)
3	44435.81	225.0	0.2469	71→LUMO (61%), HOMO→LUMO (33%)
4	45941.66	217.7	0.0253	HOMO→74 (81%)
5	46690.95	214.2	0.0027	69→74 (10%), 69→75 (16%), 70→74 (11%), 70→75 (16%), HOMO→75 (34%)
6	48669.44	205.5	0.0072	69→LUMO (19%), 70→LUMO (13%), HOMO→75 (46%)
7	49003.36	204.1	0.0045	38→LUMO (74%)
8	49561.50	201.8	0.0184	68→LUMO (19%), 69→LUMO (30%), 70→LUMO (27%)

[1] Gaussian 09, Revision A.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.

[2] T. Bruhn, A. Schaumlöffel, Y. Hemberger, G. Bringmann, SpecDis version 1.60, University of Wuerzburg, Germany, 2012.

Computational methods for transition states:

The transition states were obtained by using QST3 method at B3LYP/6-31+G(d) level in Gaussian 09. Vibration frequency calculations were done at B3LYP/6-31+G(d) and MP2/6-31+G(d) level of theory, and were used to characterize all of the stationary points as each minima (the number of imaginary frequencies NIMAG=1). The relative energies are thus corrected for the vibration zero-point energies. Intrinsic reaction coordinate (IRC) calculations were performed to unambiguously connect the transition states with the reactions. All these calculations were performed in gas phase.

Cartesian coordinate of TS1 optimized:

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	0.201399	-0.763796	-0.091383
2	1	0	0.117189	-1.590813	-0.813135
3	6	0	-0.891831	0.283139	-0.468688
4	1	0	-0.875549	0.523739	-1.536422

5	6	0	-0.743570	1.627106	0.279352
6	1	0	-0.825579	1.551396	1.381283
7	6	0	-0.319807	-1.298046	1.272017
8	1	0	0.166867	-2.239600	1.548209
9	1	0	-0.097720	-0.578128	2.069884
10	6	0	-1.838469	-1.443250	1.044971
11	1	0	-2.406152	-1.312613	1.972695
12	1	0	-2.064825	-2.451852	0.676578
13	6	0	-2.233838	-0.388575	-0.039604
14	1	0	-2.891963	0.372615	0.395105
15	6	0	-2.973871	-1.010280	-1.232198
16	1	0	-3.273107	-0.244034	-1.956486
17	1	0	-2.346518	-1.745324	-1.753467
18	1	0	-3.879776	-1.528490	-0.895250
19	6	0	1.603453	-0.257851	-0.115390
20	6	0	1.878892	1.080996	0.162520
21	1	0	2.913540	1.432418	0.144566
22	6	0	2.707901	-1.146787	-0.468327
23	1	0	2.405755	-2.175161	-0.761806
24	8	0	3.896514	-0.840847	-0.459329
25	8	0	0.985863	1.967311	0.412685
26	8	0	-1.292840	2.609777	-0.269778

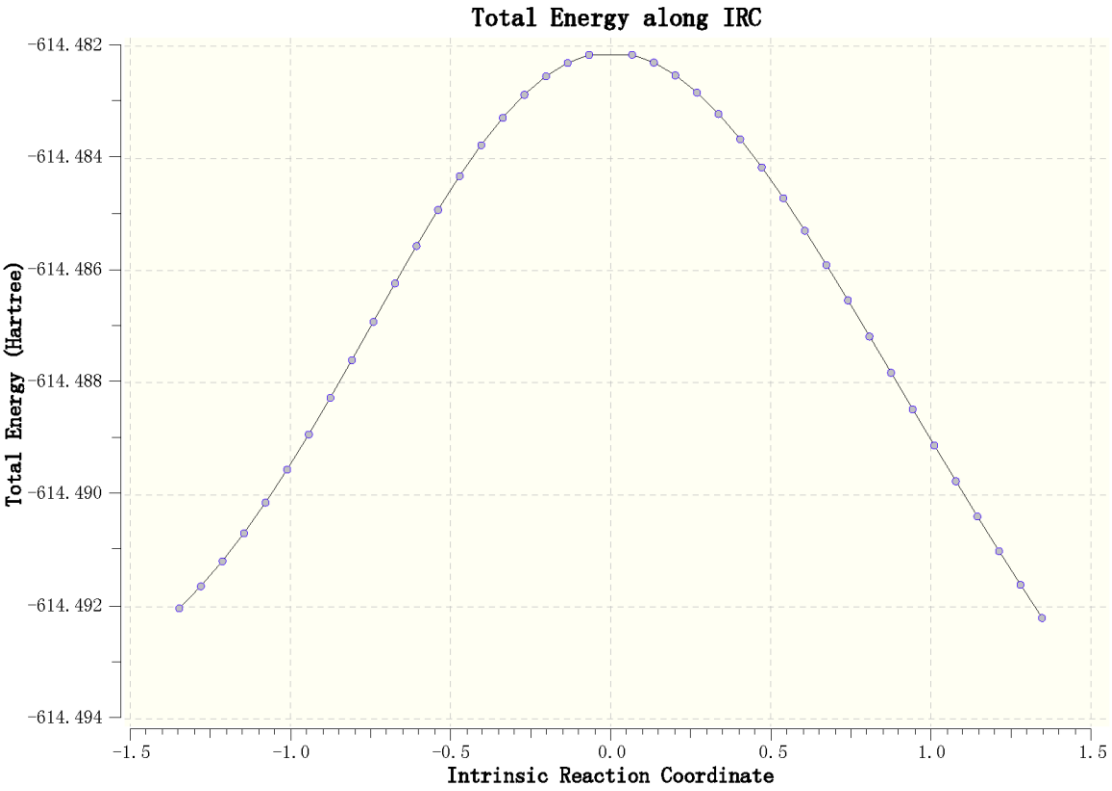
Cartesian coordinate of TS2 optimized:

Standard orientation:

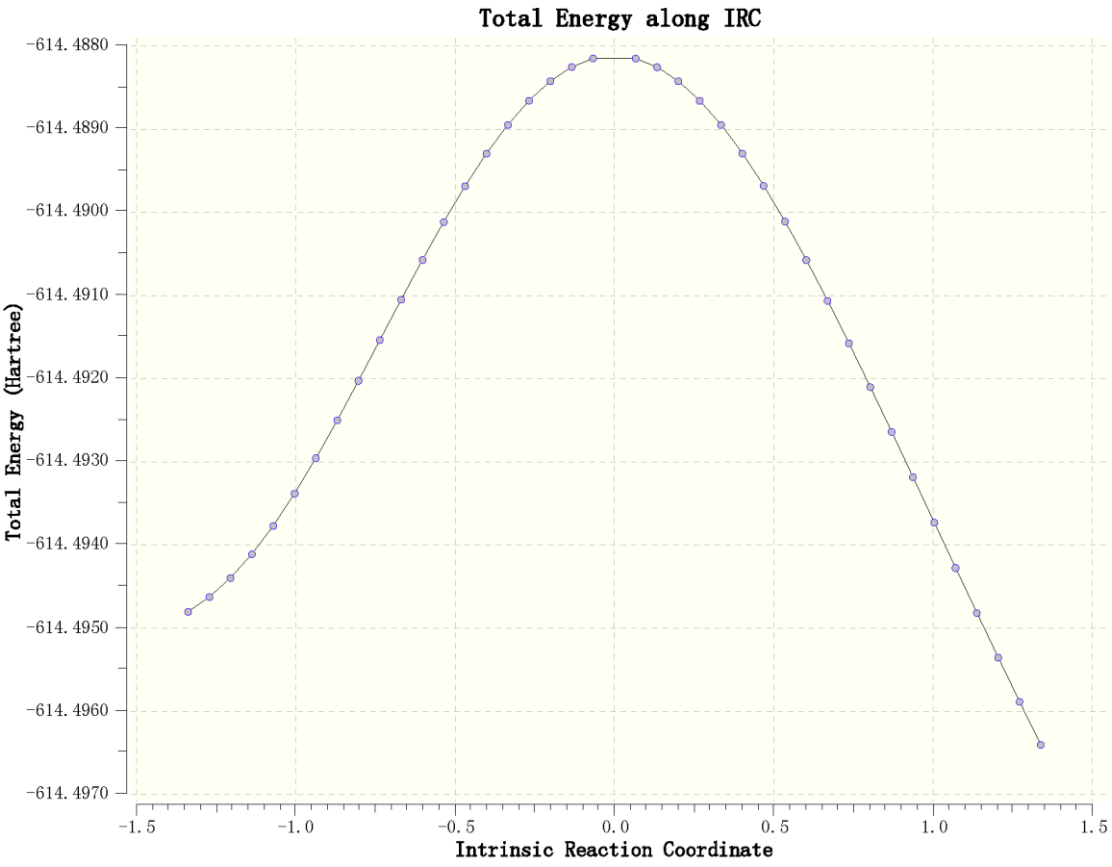
Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	0.096923	-0.710596	-0.335842
2	1	0	0.007569	-1.322851	-1.253763
3	6	0	-0.936959	0.425454	-0.487282
4	1	0	-1.008769	0.764964	-1.526968
5	6	0	-0.499645	1.668411	0.328717
6	1	0	-1.002266	2.592860	-0.005639
7	6	0	-0.454122	-1.575837	0.822779
8	1	0	-0.019798	-2.581102	0.860379
9	1	0	-0.236311	-1.070080	1.771139
10	6	0	-1.962291	-1.580856	0.533843
11	1	0	-2.559076	-1.859064	1.409167
12	1	0	-2.186442	-2.310955	-0.257128
13	6	0	-2.296410	-0.148473	0.033426
14	1	0	-2.615335	0.449376	0.895329
15	6	0	-3.411761	-0.111872	-1.016912

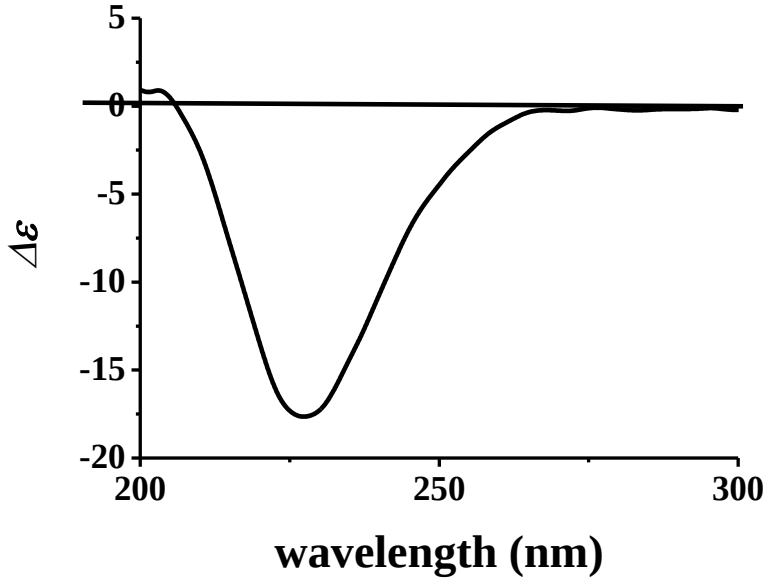
16	1	0	-3.644120	0.915638	-1.323189
17	1	0	-3.128720	-0.675313	-1.916056
18	1	0	-4.333593	-0.556725	-0.622665
19	6	0	1.518908	-0.253266	-0.265870
20	6	0	1.868698	1.097877	-0.375070
21	1	0	2.924332	1.368092	-0.445482
22	6	0	2.603601	-1.235370	-0.205263
23	1	0	2.283195	-2.297484	-0.229442
24	8	0	3.798269	-0.963035	-0.141980
25	8	0	1.033265	2.072998	-0.368644
26	8	0	-0.286825	1.531265	1.566770

IRC plots for TS1:

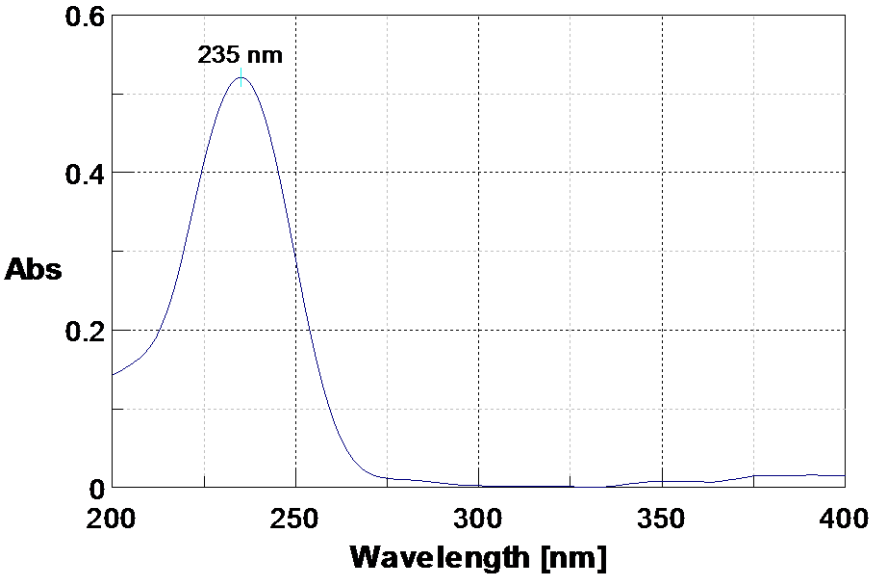


IRC plots for TS2:

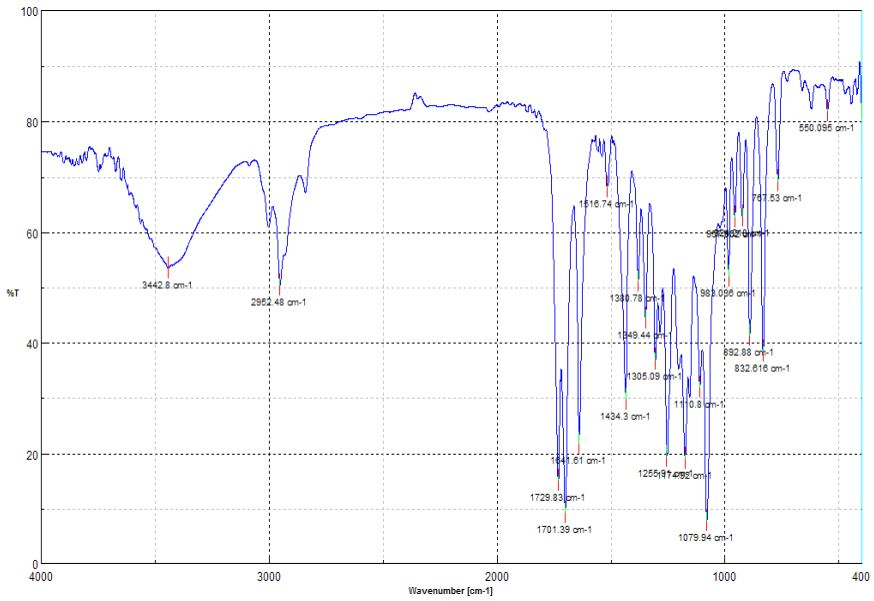




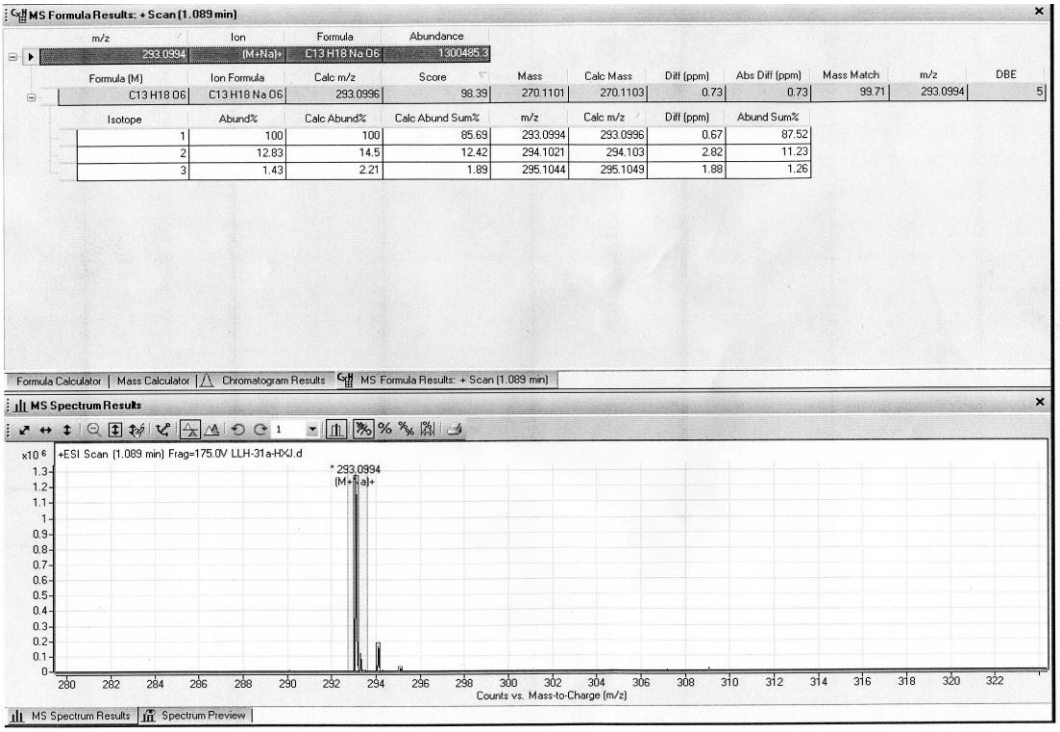
CD spectrum of oleonin (**1**) in CH_3OH



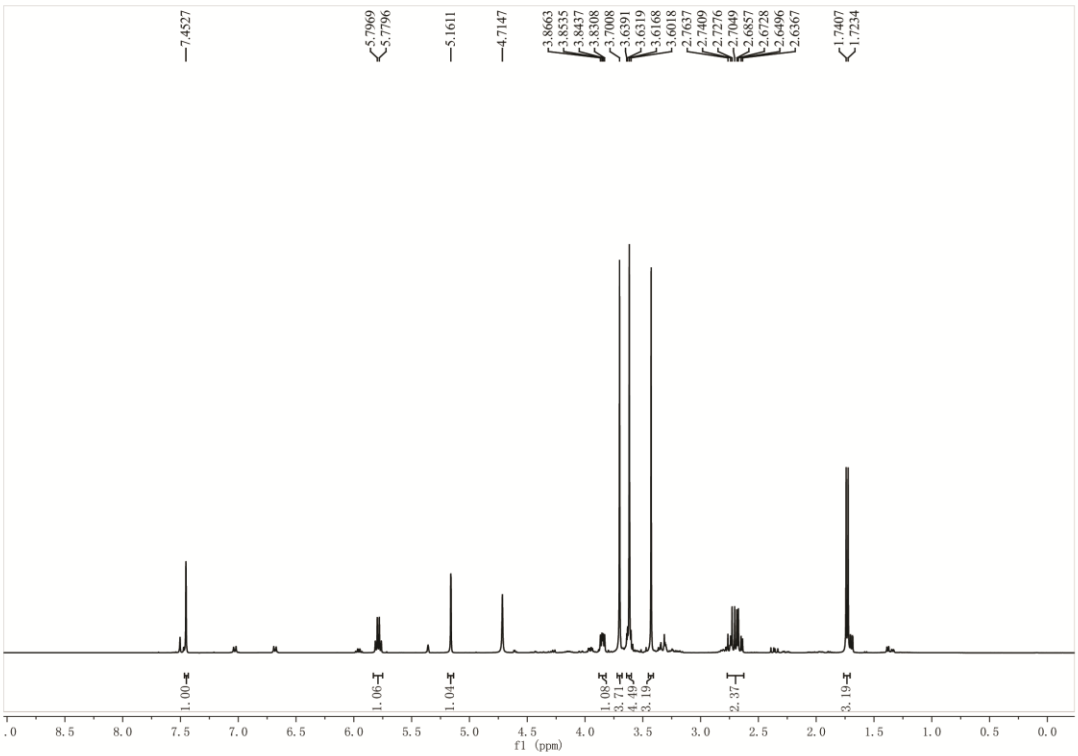
UV spectrum of oleonin (**1**) in CH_3OH



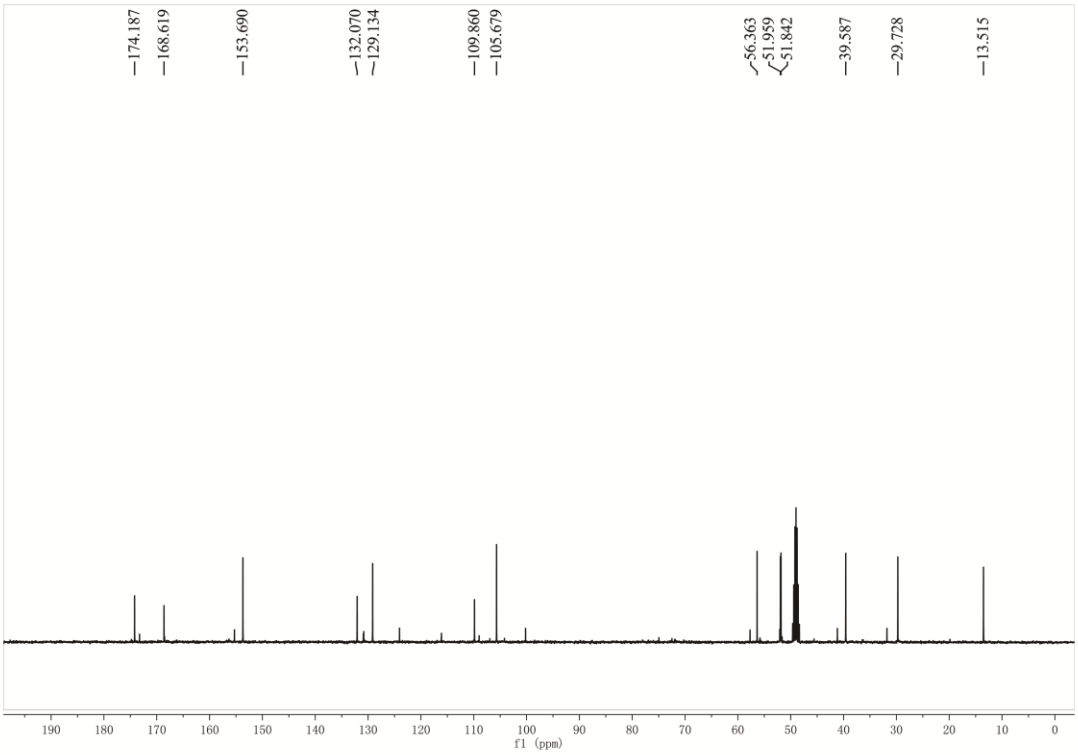
IR spectrum of oleonin (**1**)(KBr disc)



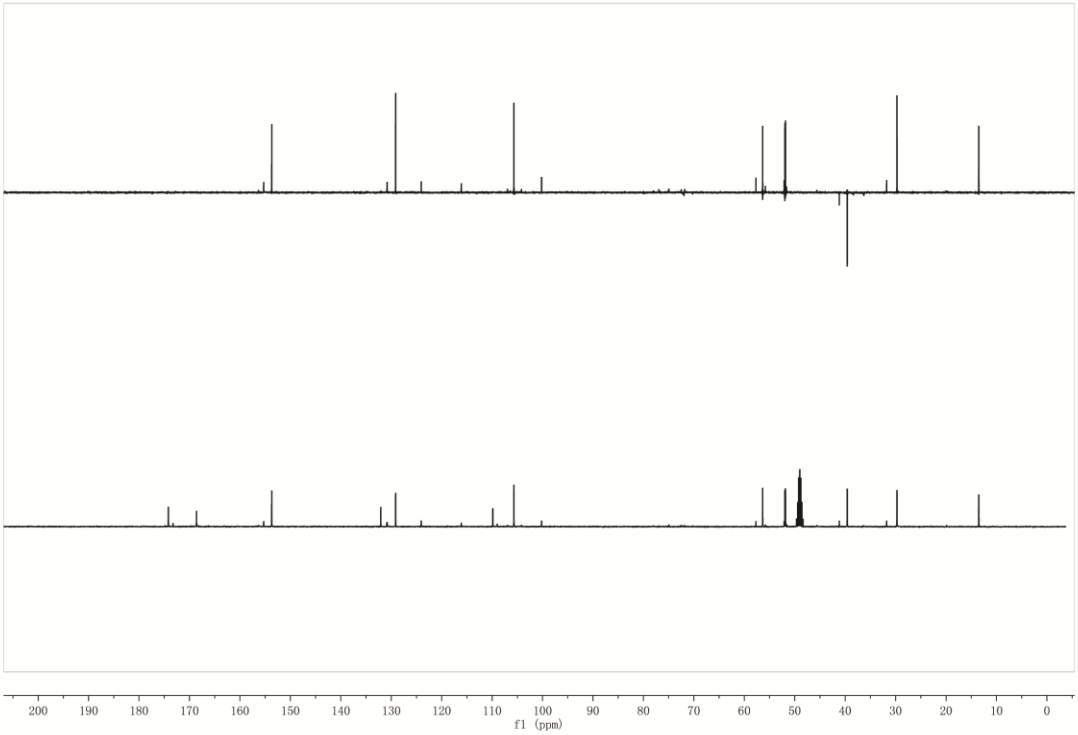
HR-ESI-MS spectrum of oleonin (**1**)



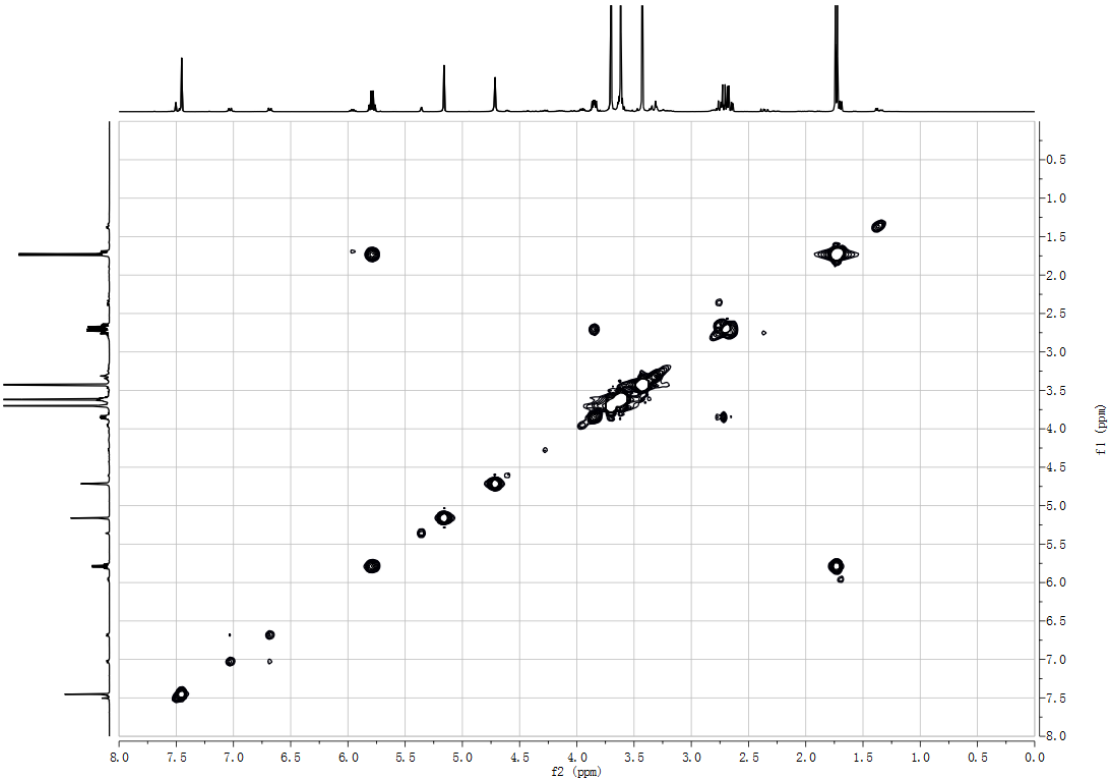
^{1}H NMR spectrum of oleonin (**1**) in CD_3OD



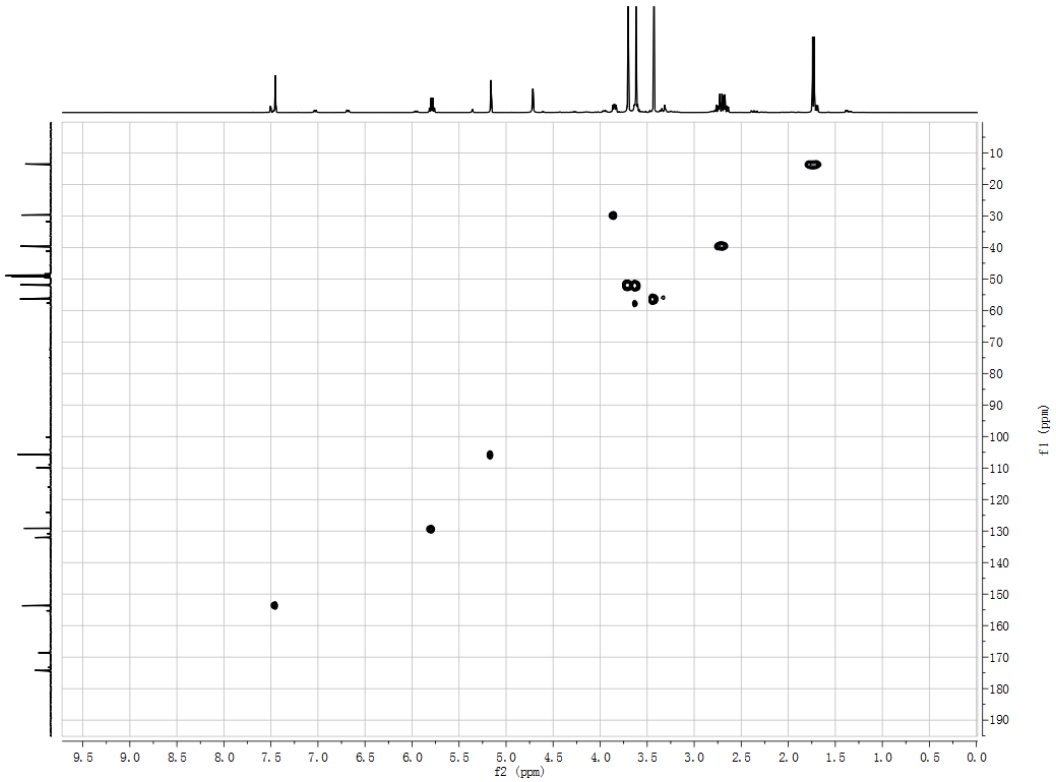
^{13}C NMR spectrum of oleonin (**1**) in CD_3OD



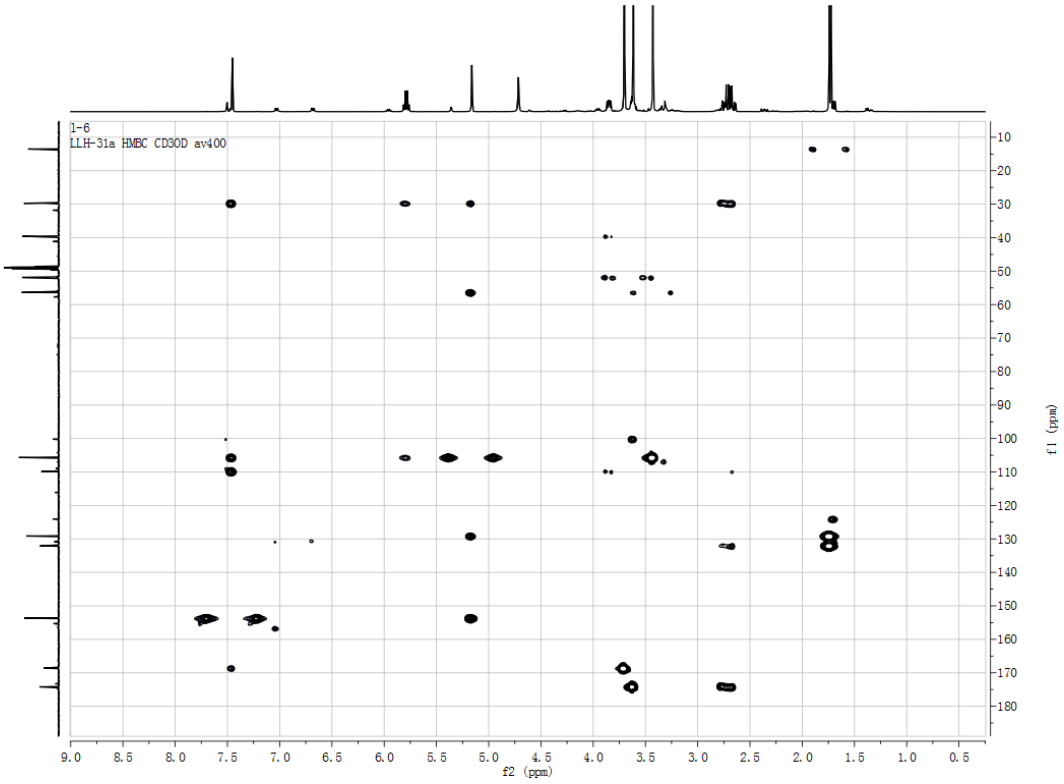
DEPT-135 spectrum of oleonin (**1**) in CD_3OD



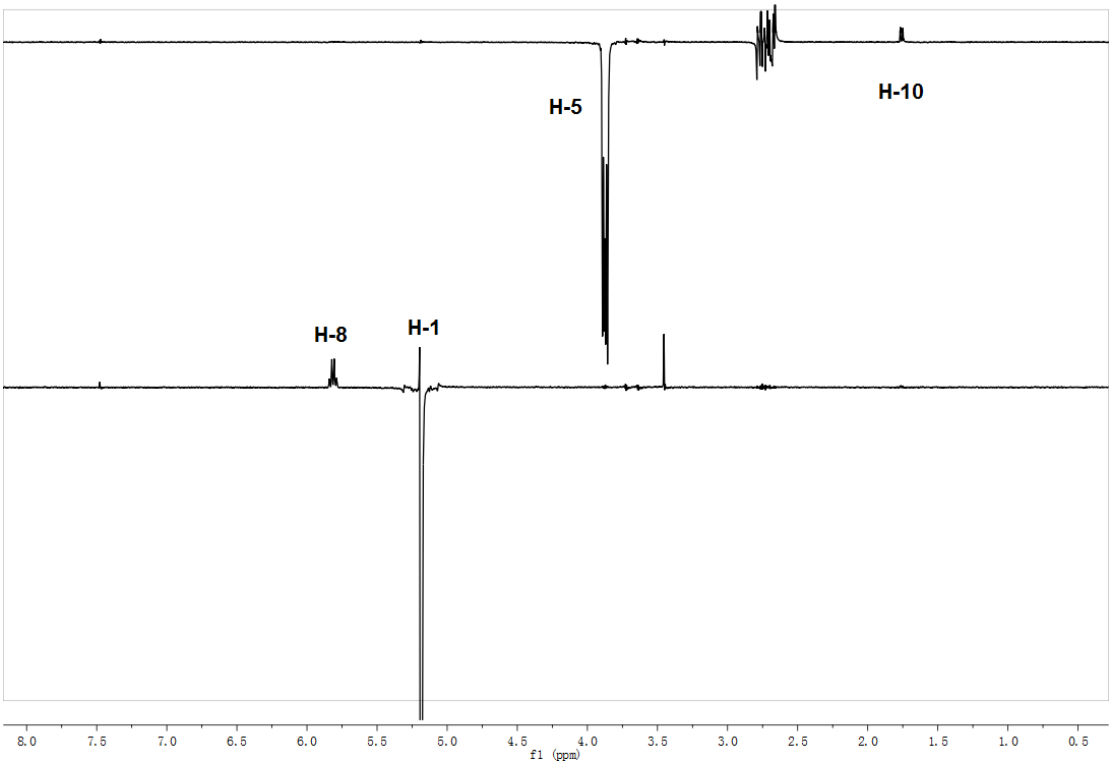
^1H-^1H COSY spectrum of oleonin (**1**) in CD_3OD



HSQC spectrum of oleonin (**1**) in CD_3OD



HMBC spectrum of oleonin (**1**) in CD_3OD



1D NOE spectra of oleonin (**1**) in CD_3OD