Supporting Information

A NEW MONOTERPENOID INDOLE ALKALOID FROM *UNCARIA RHYNCHOPHYLLA*

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Extraction and isolation of 2-15 The alkaloid fraction was subjected to silica gel column chromatography (CH_2Cl_2-MeOH 100:0-0:100, v/v) to obtain four fractions. Fraction A (813.3 mg) was separated on Sephadex LH-20 using CH_2Cl_2-MeOH (1:1 v/v) to obtain two subfractions (A1-A2). Subfraction A1 (213.7 mg) was purified by HPLC on a C_{18} column with 65% MeOH/H_2O as mobile phase to provide **2** (27.6 mg) and **8** (17.2 mg). HPLC of subfraction A2 (145.4 mg) on a C_{18} column with 80% MeOH/H_2O as mobile phase gave **9** (1.5 mg) and **13** (1.2 mg). Fraction B (765.3 mg) was separated by the same procedure as fraction A to furnish two subfractions (B1-B2). Separation of subfraction B2 (154.8 mg) by HPLC on a C_{18} column with 45% ACN/H_2O gave **3** (0.8 mg), **4** (4.6 mg), **5** (4.1 mg) and **6** (1.5 mg). Fraction C (867.6 mg) was separated on Sephadex LH-20 using CH_2Cl_2-MeOH (1:1 v/v) to obtain two subfractions C1 and C2. Subtraction C2 (204.4 mg) was purified by semi-preparative HPLC (C_{18} column with 50% ACN/H_2O) to afford **1** (3.6 mg), **10** (40.0 mg), **11** (2.5 mg) and **12** (6.7 mg). Fraction D (976.7 mg) was separated by the same procedure as fraction C to furnish three subfractions (D1-D3). Separation of subfraction D2 (194.3 mg) by semi-preparative HPLC on a C_{18} column with 20% ACN/H_2O gave **2** (3.5 mg), **14** (31.8 mg) and **15** (1.8 mg).

The ^{13}C NMR calculation The conformational analysis of two plausible diastereomers of **1** (*s*-isomer and *r*-isomer) were performed in the SYBYL 8.1 program using MMFF94s molecular force field. All of the conformers were fully optimized by DFT at the B3LYP/6 31 G(d) level using Gaussian16 software. The ^{13}C NMR shielding constants of **1** (*s*-isomer and *r*-isomer) were computed using GIAO technique at the B3LYP/6 31 G (2d, p) level of theory in PCM solvent continuum model with CD_3OD as a solvent.

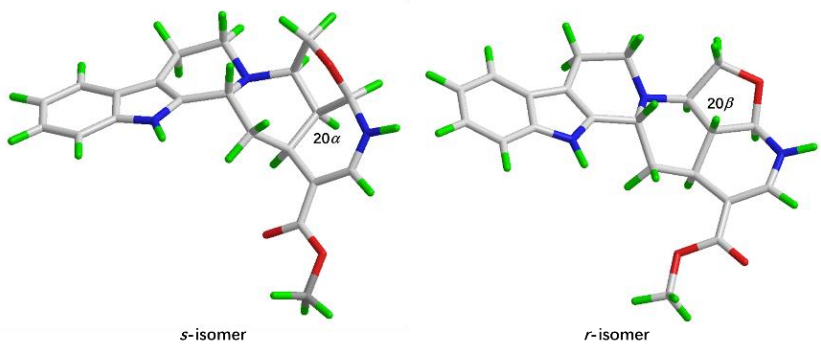


Figure S1. DFT-calculated of two isomers (*s*-isomer and *r*-isomer) of **1**, corresponding to the α- and β-orientations of H-20, respectively.

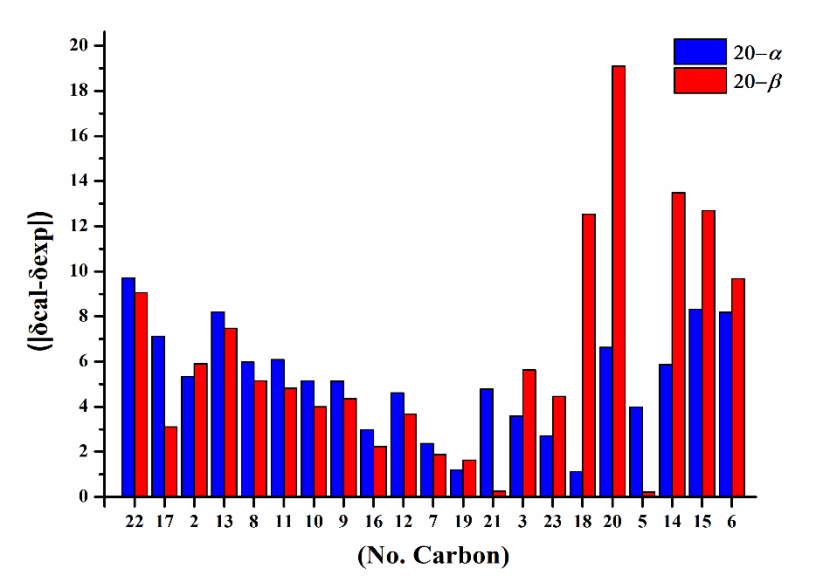


Figure S2. Comparison between calculated ^{13}C NMR chemical shifts and experimental observed shifts for **1** (*s*-isomer and *r*-isomer).

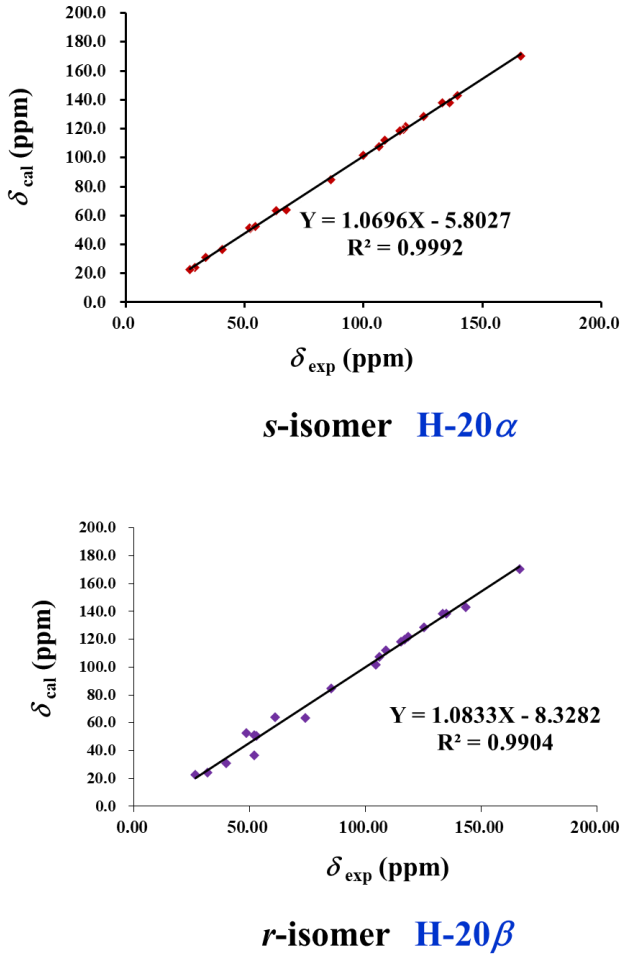


Figure S3. Regression analysis of experimental versus calculated ^{13}C NMR chemical shifts of **1** (*s*-isomer and *r*-isomer)

Table S1. Experimental and calculated ^{13}C NMR data of **1** (*s*-isomer and *r*-isomer) (δ in ppm)

No.	δexp	δcal		δscaled		corrected error ($\Delta\delta$)		*t* distribution		Probability	
		20α	**20β**	**20α**	**20β**	**20α**	**20β**	**20α**	**20β**	**20α**	**20β**
22	170.5	166.2	166.6	160.8	161.4	-9.7	-9.1	1.00	1.00	0.00	0.00
17	143.0	139.5	143.2	135.9	139.9	-7.1	-3.1	0.99	0.90	0.01	0.10
2	138.1	136.2	134.9	132.8	132.2	-5.3	-5.9	0.98	0.99	0.02	0.01
13	138.1	133.1	133.2	129.9	130.6	-8.2	-7.5	1.00	1.00	0.00	0.00
8	128.4	125.1	125.2	122.4	123.3	-6.0	-5.1	0.99	0.98	0.01	0.02
11	121.7	117.9	118.3	115.6	116.9	-6.1	-4.8	0.99	0.97	0.01	0.03
10	119.7	116.7	117.0	114.6	115.7	-5.1	-4.0	0.98	0.94	0.02	0.06
9	118.4	115.3	115.2	113.3	114.0	-5.1	-4.4	0.98	0.96	0.02	0.04
16	101.9	100.0	104.5	98.9	104.1	-3.0	2.2	0.89	0.82	0.11	0.18
12	111.9	109.0	108.9	107.3	108.2	-4.6	-3.7	0.96	0.93	0.04	0.07
7	107.4	106.5	106.0	105.0	105.5	-2.4	-1.9	0.84	0.78	0.16	0.22
19	84.8	86.2	85.3	86.0	86.4	1.2	1.6	0.69	0.75	0.31	0.25
21	63.8	67.5	61.1	68.6	64.1	4.8	0.3	0.97	0.54	0.03	0.46
3	50.8	52.4	52.8	54.4	56.4	3.6	5.6	0.93	0.98	0.07	0.02
23	51.3	52.0	52.1	54.0	55.8	2.7	4.5	0.87	0.96	0.13	0.04
18	63.4	63.2	73.9	64.5	75.9	1.1	12.5	0.68	1.00	0.32	0.00
20	36.7	40.5	52.1	43.3	55.8	6.6	19.1	0.99	1.00	0.01	0.00
5	52.4	54.5	48.7	56.4	52.6	4.0	0.2	0.94	0.54	0.06	0.46
14	31.1	33.7	40.0	37.0	44.6	5.9	13.5	0.99	1.00	0.01	0.00
15	24.3	29.1	31.7	32.6	37.0	8.3	12.7	1.00	1.00	0.00	0.00
6	22.6	27.1	26.6	30.8	32.3	8.2	9.7	1.00	1.00	0.00	0.00
Product of probabilties										7.55×10^{-36}	9.40×10^{-43}
Bayes's theorem probability (%)										99.99	0.01

Calculation of ECD Spectra The conformational analysis of **1** was performed in Sybyl 8.0 program by using MMFF94s molecular mechanic force field. All of the obtained conformers were further optimized at B3LYP/6-31+G(d) level in Gaussian 16 package, followed by the CD calculation of each single conformer by means of TDDFT methods at B3LYP/6-31+G(d) level. The overall calculated CD curves were obtained by means of Boltzmann weighting of single CD data (with a half-bandwidth of 0.3 eV). The calculated ECD spectra of **1** was subsequently compared with the experimental ones. The ECD spectra were produced by SpecDis 1.60 software.

The anti-inflammatory activity assay The inhibitory activity of the compound **1** toward NO production by RAW 264.7 cells was determined using Griess reagent. RAW 264.7 cells (ATCC) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FBS) in a humidified 5% CO_2, 95% air atmosphere at 37 °C. RAW 264.7 cells were seeded in 96-well plates at 5×10^4 cells/well for NO production. The plates were pre-treated with **1** for 60 min and then incubated for 24 h with or without 1 μg/mL of LPS (Sigma, U.S.A.). The nitrite concentration in the culture supernatant was measured by the Griess reaction. An amount of 100 μL of each culture supernatant was mixed with the same volume of Griess reagent (1% sulfanilamide in 5% phosphoric acid, and 0.1% naphthylethyl-enediamine dihydrocholoride in water) and the absorbance of the mixture at 550 nm was measured using a microplate reader. Cell viability was measured by MTT assay (Sigma-Aldrich). The absorbance at 540 nm was read using a micro-plate reader (POLAR star). In this experiment, based on the establishment of a cell model of LPS damage, the release of NO from RAW 264.7 cells was detected. Indomethacin (200 μM) was used as a positive control. As shown in Figure S1, compound **1** could inhibit the NO production induced by LPS.

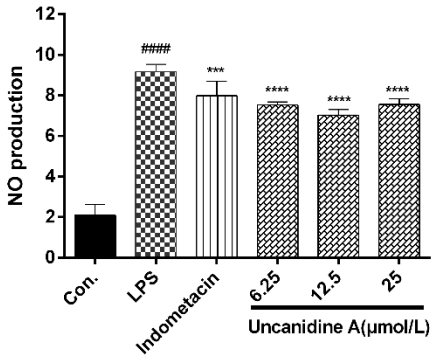


Figure S4. Effect of uncanidine A (**1**) on the NO production in LPS-activated RAW 264.7 cells.

Due to the high strained fused ring in the structure, this compound was unstable during isolation and purification, which may affect the results of bioassay. More analogues of compound **1** need to be discovered and their bioactivities need to be evaluated deeply and comprehensively in the further study.

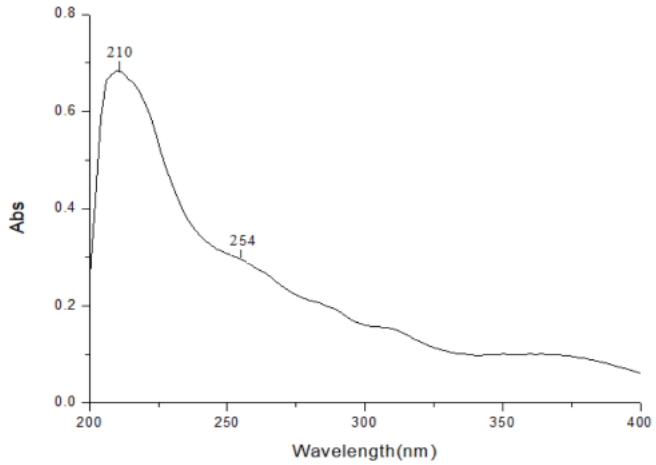


Figure S5. UV spectrum of **1** (MeOH)

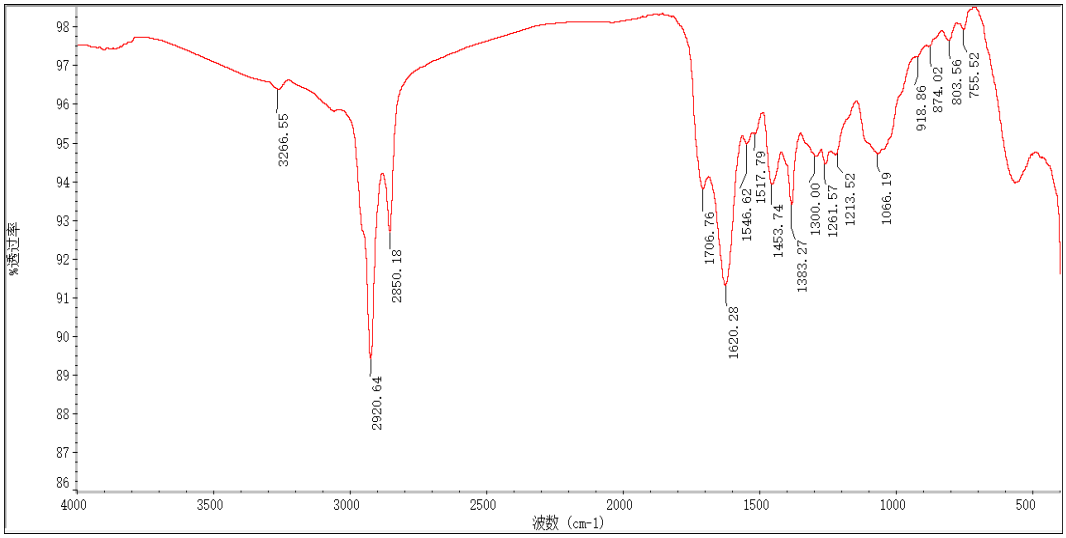


Figure S6. IR spectrum of **1** (KBr)

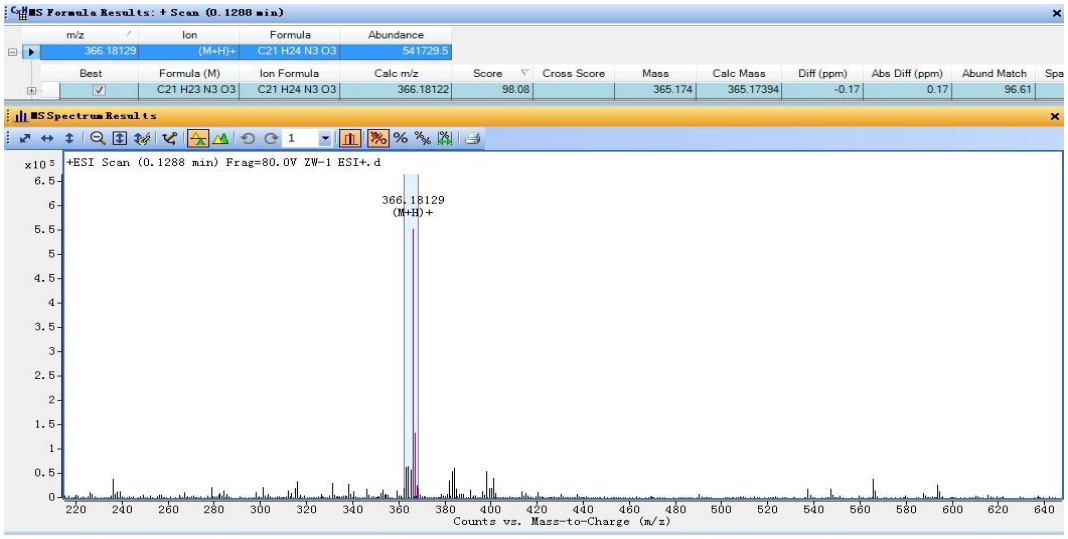


Figure S7. HR-ESI-MS spectrum of **1**

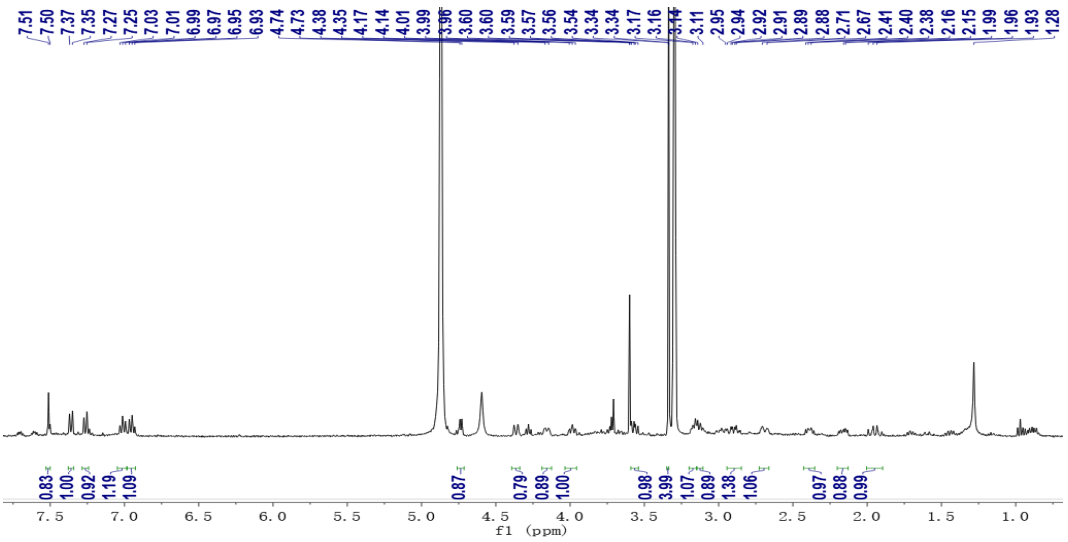


Figure S8. ^{1}H NMR spectrum of **1** (CD_3OD, 700 MHz)

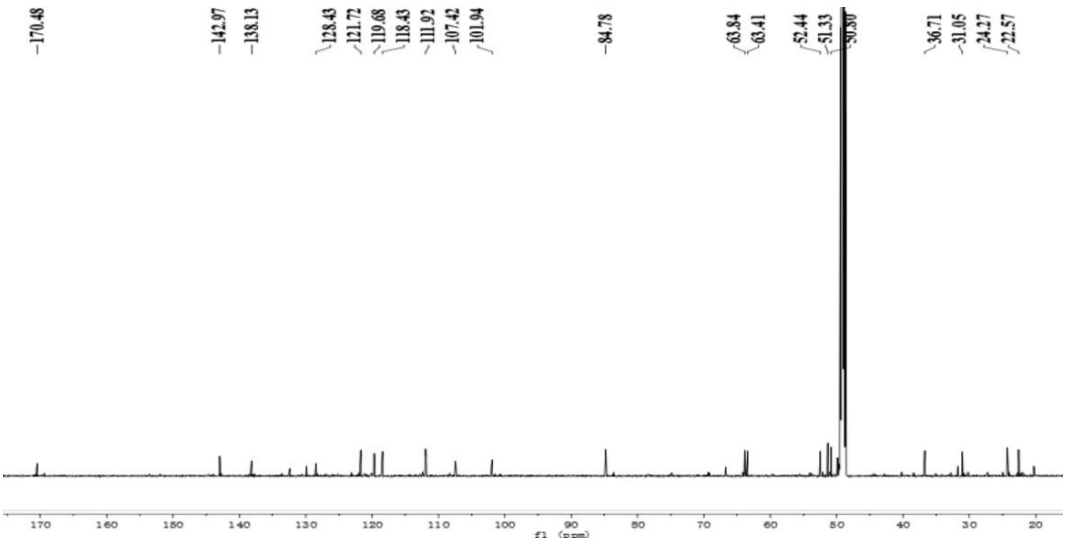


Figure S9. ^{13}C NMR spectrum of **1** (CD_3OD, 175 MHz)

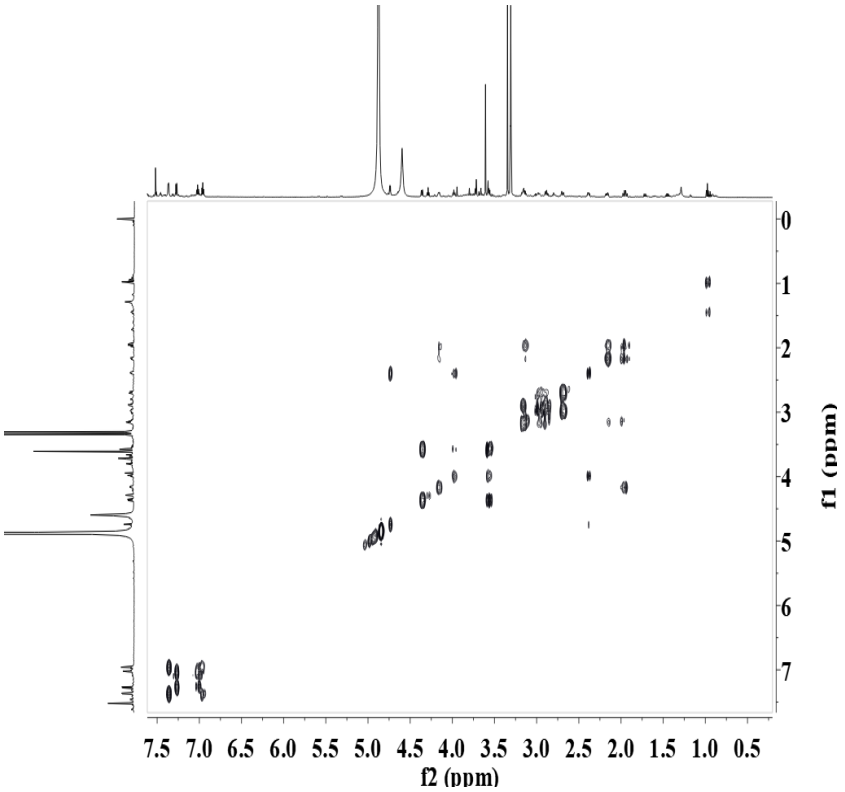


Figure S10. ^{1}H-^{1}H-COSY spectrum of **1** (CD_3OD)

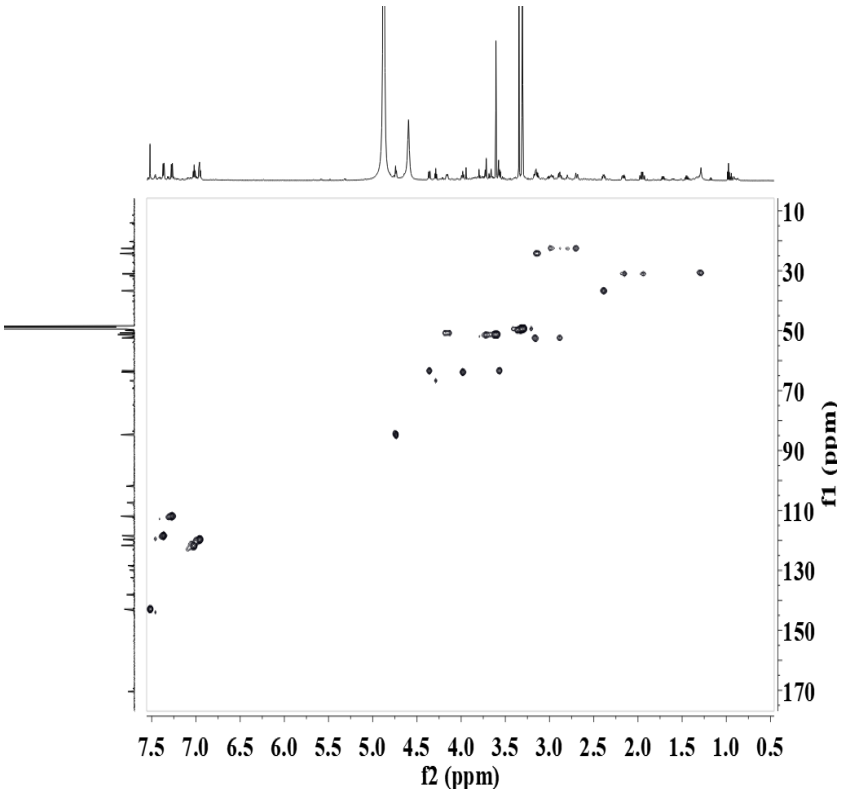


Figure S11. HSQC spectrum of **1** (CD_3OD)

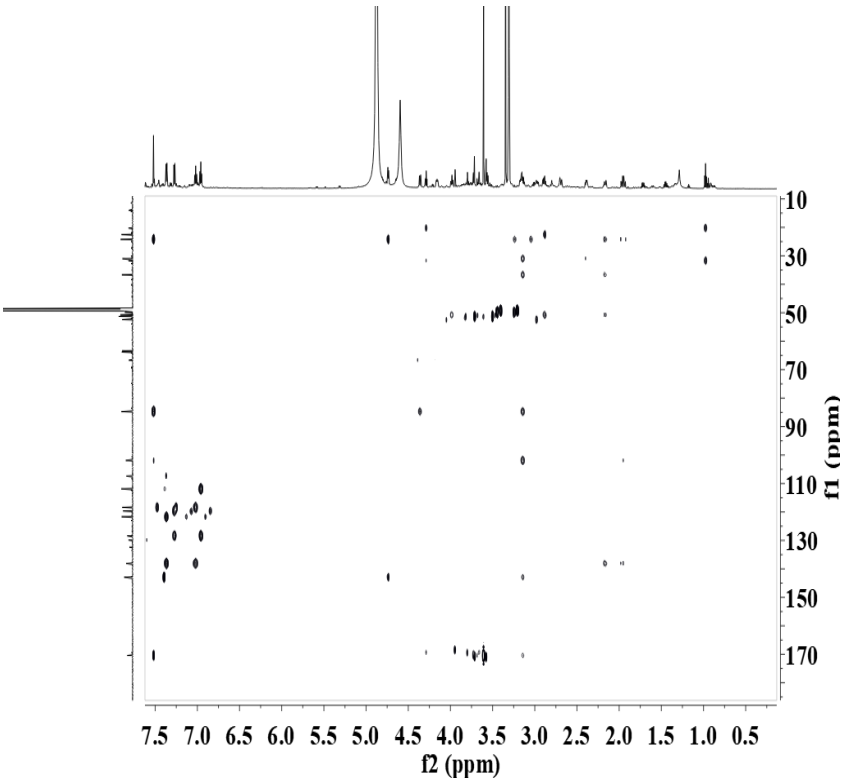


Figure S12. HMBC spectrum of **1** (CD_3OD)

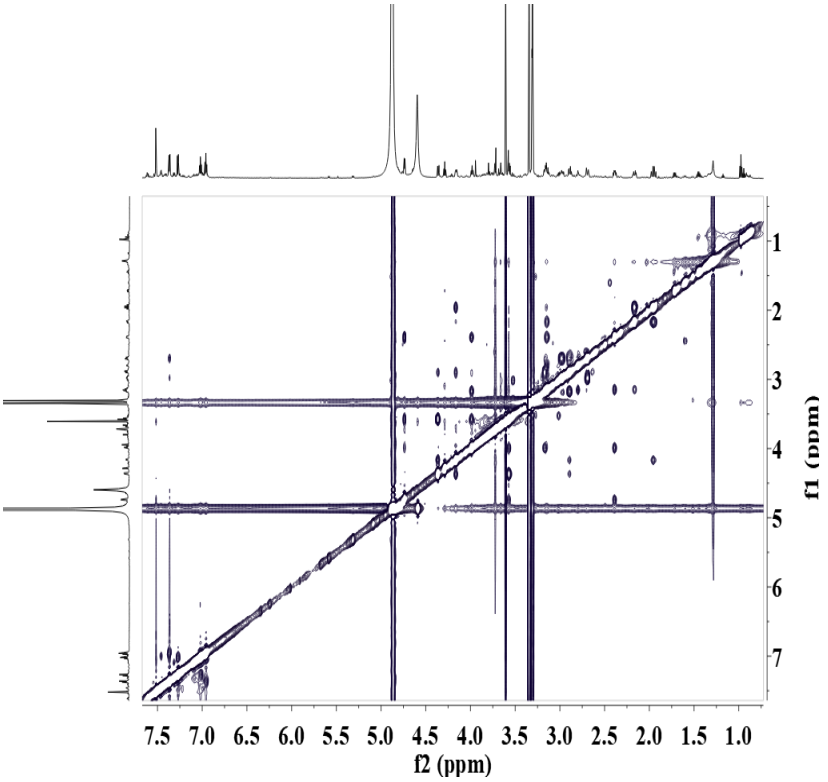
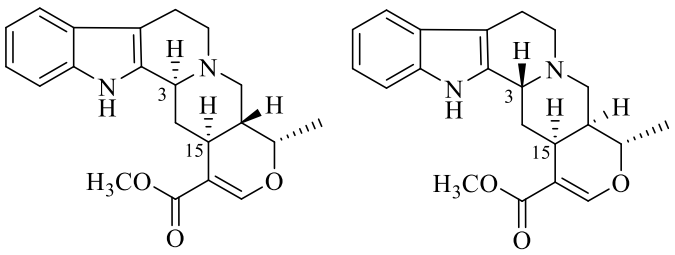


Figure S13. NOESY spectrum of **1** (CD_3OD)



ajmalicine

akuammigine

Figure S14. Chemical structures of ajmalicine and akuammigine