Spirocurcasone, a Diterpenoid with a Novel Carbon Skeleton from *Jatropha curcas*

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General methods. Low and high resolution ESI-MS spectra were performed on a LTQ OrbitrapXL (Thermo Scientific) mass spectrometer. Optical rotations ($CHCl_3$) were measured at 589 nm on a P2000 Jasco polarimeter using a 10 cm microcell. UV spectra were measured on a Thermo Scientific (mod. Nanodrop 2000c) instrument. CD spectra were registered on a Jasco J-710 instrument. ^1H (700 and 500 MHz) and ^{13}C (175 and 125 MHz) NMR spectra were measured on Varian INOVA spectrometers. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H 7.26, δ_C 77.0). Homonuclear ^1H connectivities were determined by the COSY experiment. Through-space ^1H connectivities were evidenced using a ROESY experiment with a mixing time of 500 ms. Two and three bond ^1H-^{13}C connectivities were determined by gradient 2D HMBC experiments optimized for a $^{2,3}J$ of 9 Hz. Medium pressure liquid chromatography was performed on a Büchi apparatus. HPLC were achieved on a Knauer apparatus equipped with a refractive index detector and LUNA (Phenomenex) SI60 (250 × 4 mm) columns.

Collection, extraction and isolation. A ground root bark of *J. curcas* (1.0 kg) was extracted with at room temp sequentially with hexane (2 x 3 L) and EtOAc (3 x 3 L), to give the respective crude extracts which were concentrated *in vacuo*. The hexane extract (10.5 g) was fractionated by gravity column chromatography on silica gel (60 g) using a *n*-hexane/EtOAc gradient. Fractions eluted with *n*-hexane/EtOAc 9:1 were further purified by HPLC (*n*-hexane/EtOAc 9:1, flow 0.8 mL/min) to yield curcusones A (**9**, 85.2 mg), B (**10**, 99.7 mg), E (**13**, 11.5 mg). Fractions eluted with *n*-hexane/EtOAc 8:2 were further purified by HPLC (*n*-hexane/EtOAc 8:2, flow 0.8 mL/min) to yield curcusones C (**11**, 13.2 mg), D (**12**, 5.7 mg), 15-epi-4E-jatrogrossidentadion (**3**, 15.5 mg), and 4Z-jatrogrossidentadion (**1**, 8.4 mg). Fractions eluted with *n*-hexane/EtOAc 7:3 were further purified by HPLC (*n*-hexane/EtOAc 85:15, flow 0.8 mL/min) to yield multidione (**6**, 14.2 mg), 4E-jatrogrossidentadion (**2**, 19.3 mg). Fractions eluted with *n*-hexane/EtOAc 6:4 were further purified by HPLC (*n*-hexane/EtOAc 7:3, flow 0.8 mL/min) to yield hydroxyisojatrogrossidion (**4**, 12.2 mg) and 2-epi-hydroxyisojatrogrossidion (**5**, 7.1 mg). The EtOAc extract (11.2 g) of root barks was fractionated by gravity column chromatography on silica gel (60 g) using a *n*-hexane-EtOAc gradient. Fractions eluted with *n*-hexane/EtOAc 9:1 were further purified by HPLC (*n*-hexane/EtOAc 9:1, flow 0.8 mL/min) to yield spirocurcasone (**14**, 14.5 mg). Fractions eluted with *n*-hexane/EtOAc 8:2 were further purified by HPLC (*n*-hexane/EtOAc 8:2, flow 0.8 mL/min) to yield jatropholone B (**7**, 11.4 mg) and acetoxyjatropholone (**8**, 3.4 mg).

Pharmacological tests. The extracts were subjected to in vitro cytotoxicity assay applying the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Sigma-Aldrich, Taufkirchen;

Germany) method (Sarin PS, Sun D, Thornton A, Müller WEG J Natl. Cancer Inst., 1987, 78, 663-666). L5178y mouse lymphoma cells have been selected for *in vitro* toxicity testings (The cells were grown in RPMI 1640 medium (Gibco BRL, Eggenstein; Germany), supplemented with 10 mM Hepes [hydroxyethyl-piperazineethane-sulfonic acid], 10% fetal calf serum (FCS) (PAA, Cölbe, Germany) and 0.1 % gentamycin. The cells were routinely passaged twice weekly. The cells were kept in a humidified atmosphere of 95% air and 5% CO_2 at 37^{o}C.

To estimate the EC_{50} values, L5178Y, PC12 or HeLa cells were incubated for 72 hrs in the presence of different concentrations (0.1; 0.3; 1.0; 3.0, and 10.0 µg/ml) of the respective compounds. The final volumes in the assay were 200 µL. All compounds/extracts were dissolved in DMSO and stored at -20^{o}C. The viability of the cells was determined using the MTT colorimetric assay system. The evaluation was performed in 96-well plates at 595 nm using an ELISA plate reader, after overnight incubation at 37^{o}C as described (Schröder HC, Sarin PS, Rottmann M, Wenger R, Maidhof A, Renneisen K, Müller WEG. Biochem Pharmacol, 1988, 37, 3947-3952). The 50% effective concentration (IC_{50}), representing that concentration at which the growth rate of the infected cells was reduced by 50%, was estimated by logit regression as described (Weiler BE, Schröder HC, Stefanovich V, Stewart D, Forrest JMS, Allen LB, Bowden BJ, Kreuter MH, Voth R, Müller WEG. J Gen. Virol 1990, 71, 1957-1963). The means (±SD) from 10 separate experiments are given.

Computational Details. A preliminary conformational search on spirocurcasone (**14**) was performed by Simulated Annealing in the INSIGHT II package. The $CHCl_3$ solution phases were mimicked through the value of the corresponding dielectric constant. Using the steepest descent followed by quasi-Newton–Raphson method (VA09A) the conformational energy was minimized. Restrained simulations were carried out for 500 ps using the CVFF force field as implemented in Discover software (Accelrys, San Diego, USA). The simulation started at 1000 K, and then the temperature was decreased stepwise to 300 K. The final step was again the energy minimization, performed in order to refine the structures obtained, using the steepest descent and the quasi-Newton–Raphson (VA09A) algorithms successively. Both dynamic and mechanic calculations were carried out by using 1 (kcal/mol)/A˚ 2 flat well distance restraints. One hundred structures were generated. To simulate the solvent chosen for NMR analysis, a distance-dependent dielectric constant set to the value of $CHCl_3$ (ε 4.8) was used during the calculations. All optimizations were performed with the software package Gaussian 03,[1] by using the DFT functional RB3LYP and the basis set 6-31G(d). The B3LYP/6-31G(d) harmonic vibrational frequencies were further calculated to confirm their stability. Rotatory strength values for the electronic transitions from the ground state to the singly excited states for all conformers of **14** were obtained by TDDFT calculations RB3LYP/6–31G(d,p) with Gaussian 03. The excitation energies as well as the oscillator and rotatory strengths of the electronic excitation were calculated for both the conformational families using the TDDFT methodology and their ECD spectra were then simulated by overlapping the Gaussian functions for each transition. The simulated spectra of the lowest energy conformers were averaged on the basis of their Boltzmann distribution. Δε values were calculated by forming sums of Gaussian functions centred at the wavelengths of the respective electronic transitions and multiplied by the corresponding rotatory strengths. The obtained ECD spectra were UV-corrected and compared with the experimental ones.

[1] Gaussian 03-Revision B05: Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. A.; Montgomery, Jr., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian03W, Revision B05*, Inc., Wallingford CT, **2004**

Table S1. Conformational Analysis of **14** in the Gas Phase

	ΔE[a]	ΔG[b]	P%[c]
14A	0.00	0.00	65.00
14B	0.42	0.37	35.00

[a] Relative energy (kcal/mol). [b] Relative Gibbs free energy (kcal/mol). [c] Conformational distribution calculated at the at the B3LYP/6-31G(d) level in the gas phase.

Table S2. Optimized Z-Matrices of **14** in the Gas Phase (A).

1				**2**			
C	-0.070760000	-2.050208000	-0.274246000	C	-0.075803000	-2.048953000	-0.186025000
C	-3.913568000	0.046237000	0.152298000	C	-3.910849000	0.046667000	0.290581000
C	-4.810933000	0.625830000	-0.653821000	C	-4.081294000	0.301917000	1.592557000
C	-4.165775000	-0.073315000	1.638590000	C	-5.031886000	0.239315000	-0.702356000
C	0.856030000	-2.825262000	0.296794000	C	0.856893000	-2.818340000	0.382839000
C	-0.296527000	3.833596000	-0.392523000	C	-0.263921000	3.832228000	-0.417017000
O	2.106351000	2.407140000	-0.336890000	O	2.127545000	2.384919000	-0.434473000
C	1.574426000	0.077759000	1.626604000	C	1.641581000	0.115066000	1.617300000
C	3.054420000	-0.216214000	1.628428000	C	3.118380000	-0.192959000	1.581927000
C	3.581752000	-0.406334000	0.407341000	C	3.606284000	-0.420324000	0.350826000
C	2.506450000	-0.266220000	-0.606165000	C	2.501319000	-0.297833000	-0.632658000
O	2.599698000	-0.479117000	-1.799055000	O	2.555793000	-0.543938000	-1.821576000
C	4.986977000	-0.729736000	0.012673000	C	4.996042000	-0.766249000	-0.078075000
H	-0.073353000	-0.320574000	-1.477810000	H	-0.105400000	-0.344111000	-1.422688000
H	-1.347444000	-0.144187000	1.283738000	H	-1.299576000	-0.108954000	1.370763000
H	-2.308758000	2.072938000	0.085809000	H	-2.266014000	2.101371000	0.197464000
H	-2.605762000	-0.298270000	-1.488073000	H	-2.612162000	-0.282624000	-1.370447000
H	-2.637361000	-2.356110000	0.778330000	H	-2.637157000	-2.301468000	0.919173000
H	-3.476062000	-2.496864000	-0.767142000	H	-3.490372000	-2.475955000	-0.611589000
H	-1.297472000	-3.748963000	-0.772517000	H	-1.325755000	-3.747907000	-0.624765000
H	-1.310589000	-2.459780000	-1.976209000	H	-1.347628000	-2.485860000	-1.856962000
H	-4.643076000	0.720718000	-1.724113000	H	-3.289218000	0.169516000	2.324852000
H	-5.750560000	1.020733000	-0.274689000	H	-5.033948000	0.654951000	1.979180000
H	-3.403093000	0.456064000	2.224769000	H	-4.765349000	0.996672000	-1.452447000
H	-5.140077000	0.346679000	1.905433000	H	-5.235496000	-0.687188000	-1.256441000
H	-4.147701000	-1.118160000	1.972477000	H	-5.959681000	0.553199000	-0.214424000
H	1.770656000	-2.446118000	0.736906000	H	1.782160000	-2.436272000	0.797453000
H	0.734961000	-3.905890000	0.313430000	H	0.729417000	-3.897551000	0.424176000
H	0.134865000	4.102095000	-1.363491000	H	0.367207000	4.364906000	0.303164000
H	0.299414000	4.357998000	0.362904000	H	-1.287610000	4.210589000	-0.339185000
H	-1.325938000	4.201891000	-0.351151000	H	0.128499000	4.079548000	-1.409893000
H	1.351629000	0.990110000	2.195225000	H	1.444566000	1.044620000	2.167246000
H	1.010259000	-0.731800000	2.106742000	H	1.085120000	-0.675139000	2.136966000
H	3.617572000	-0.272150000	2.557204000	H	3.709240000	-0.229291000	2.494302000
H	5.402330000	0.072712000	-0.608725000	H	4.996797000	-1.689586000	-0.668935000
H	5.633461000	-0.866367000	0.884736000	H	5.401092000	0.019500000	-0.727033000
H	5.014930000	-1.641232000	-0.595771000	H	5.666449000	-0.892500000	0.777323000

Table S3. Frequencies of **14** at the B3LYP/6-31G* Level in the Gas Phase (cm^{-1}).

14A	42.2907	47.6641	58.4454	63.3436	87.2221	114.5908	120.2151
	124.547	136.468	164.525	179.3605	195.3316	197.4674	211.7265
	221.3312	258.4839	261.8258	276.0028	292.44	301.5613	322.8498
	338.7079	359.9033	366.5604	390.0987	400.6622	457.6766	477.0069
	493.1077	506.7708	530.321	561.7681	601.7547	647.7869	656.0384
	661.8678	674.4815	707.3434	724.4083	744.2789	756.6094	775.0509
	829.3904	835.0724	849.2338	862.296	901.8237	916.2502	924.4245
	933.6563	939.8286	947.8196	956.0303	987.5944	990.0369	1008.2543
	1028.8456	1030.2146	1039.3976	1046.3244	1063.1193	1072.8041	
	1077.3564	1081.5512	1086.1222	1093.1754	1102.4072	1123.4503	
	1145.4719	1166.3199	1188.3065	1220.5579	1233.6207	1250.9439	
	1260.0417	1271.1844	1293.0659	1306.6538	1311.457	1335.9208	
	1339.2221	1356.2095	1362.5401	1375.9573	1377.6923	1388.8397	
	1401.9142	1407.7623	1442.3338	1443.8204	1445.8063	1479.306	
	1486.8753	1502.6772	1503.3955	1512.0612	1514.4399	1515.6168	
	1516.4056	1517.6075	1521.6609	1528.7934	1712.5058	1716.229	
	1725.0077	1732.1592	1749.5169	1812.0345	2983.6497	3000.341	
	3016.8478	3023.6982	3040.6049	3044.8273	3045.5841	3052.2372	
	3055.9831	3073.7577	3086.1064	3090.1059	3092.992	3107.6916	
	3112.7198	3129.8892	3134.5196	3136.1322	3155.7446	3164.0639	
	3170.4858	3195.5639	3231.9488	3259.5264			
14B	36.4624	42.7754	52.9919	62.9927	89.0895	110.8221	117.4908
	127.7412	140.8402	157.1063	179.7207	194.8828	203.2207	212.2353
	221.3514	256.0121	260.7923	275.9933	293.4209	299.3921	312.9682
	340.3318	359.9515	367.2302	387.169	401.4726	452.1482	478.7054
	484.1783	510.3931	530.0829	556.1544	600.6975	648.0219	654.8333
	662.609	674.7846	708.7558	721.8678	730.8519	757.713	774.9311
	833.8788	841.665	860.0717	864.6116	902.0668	917.4658	921.9904
	923.7945	939.3075	947.5559	956.4659	983.8215	988.5362	1002.8369
	1028.9326	1029.4597	1038.0781	1043.8545	1061.2735	1071.7843	
	1077.0966	1081.0541	1086.2537	1094.5006	1101.0214	1125.0682	
	1145.5555	1166.5133	1187.4177	1222.7026	1234.4904	1246.4951	
	1256.5293	1267.6352	1276.0575	1293.6704	1311.7978	1335.6897	
	1348.6194	1356.2668	1362.1969	1376.7278	1384.4323	1391.7043	
	1404.6474	1411.0028	1439.5295	1442.0153	1443.5456	1473.267	
	1486.5035	1502.2716	1503.1142	1508.2355	1513.1116	1514.351	
	1516.4773	1516.9964	1520.5199	1523.9366	1712.5363	1715.1377	
	1730.5573	1732.8016	1749.6701	1812.1789	2986.7384	2997.2034	
	3009.0043	3016.754	3030.9268	3043.7845	3045.47	3052.3365	
	3055.9235	3073.9689	3076.3456	3083.9187	3090.8322	3107.8497	
	3112.8119	3127.5734	3134.5213	3135.4914	3162.8547	3170.6582	
	3171.6506	3195.4086	3235.7317	3260.1101			

Table S4. ^{13}C (175 MHz) and ^{1}H (700 MHz) NMR Data of Spirocurcasone (**14**) (in $CDCl_3$).

Pos.	δ_C	δ_H (mult., *J* in Hz)
1	208.8 (C)	
2	144.3 (C)	
3	153.4 (C)	7.27 (bs)
4a	33.0 (CH_2)	3.17 (bd, 18.8)
4b		2.42 (bd, 18.8)
5	199.7 (C)	
6	133.5 (C)	
7	146.6 (CH)	6.60 (bs)
8	40.9 (CH)	2.35 (bdd, 11.9, 10.8)
9	47.9 (CH)	3.07 (d, 10.8)
10	60.7 (C)	
11	145.3 (C)	
12a	36.8 (CH_2)	2.32 (ddd, 13.2, 3.5, 3.5)
12b		2.17 (ddd, 13.2, 11.9, 3.5)
13a	33.6 (CH_2)	1.84 (dddd, 13.2, 3.5, 3.5, 2.8)
13b		1.53 (m)
14	50.4 (CH)	2.11 (ddd, 11.9, 11.0, 2.8)
15	146.2 (C)	
16a	113.4 (CH_2)	4.94 (bs)
16b		4.85 (bs)
17	19.4 (CH_3)	1.72 (bs)
18a	106.7 (CH_2)	4.66 (bs)
18b		4.18 (bs)
19	10.5 (CH_3)	1.89 (bs)
20	16.1 (CH_3)	1.74 (bs)

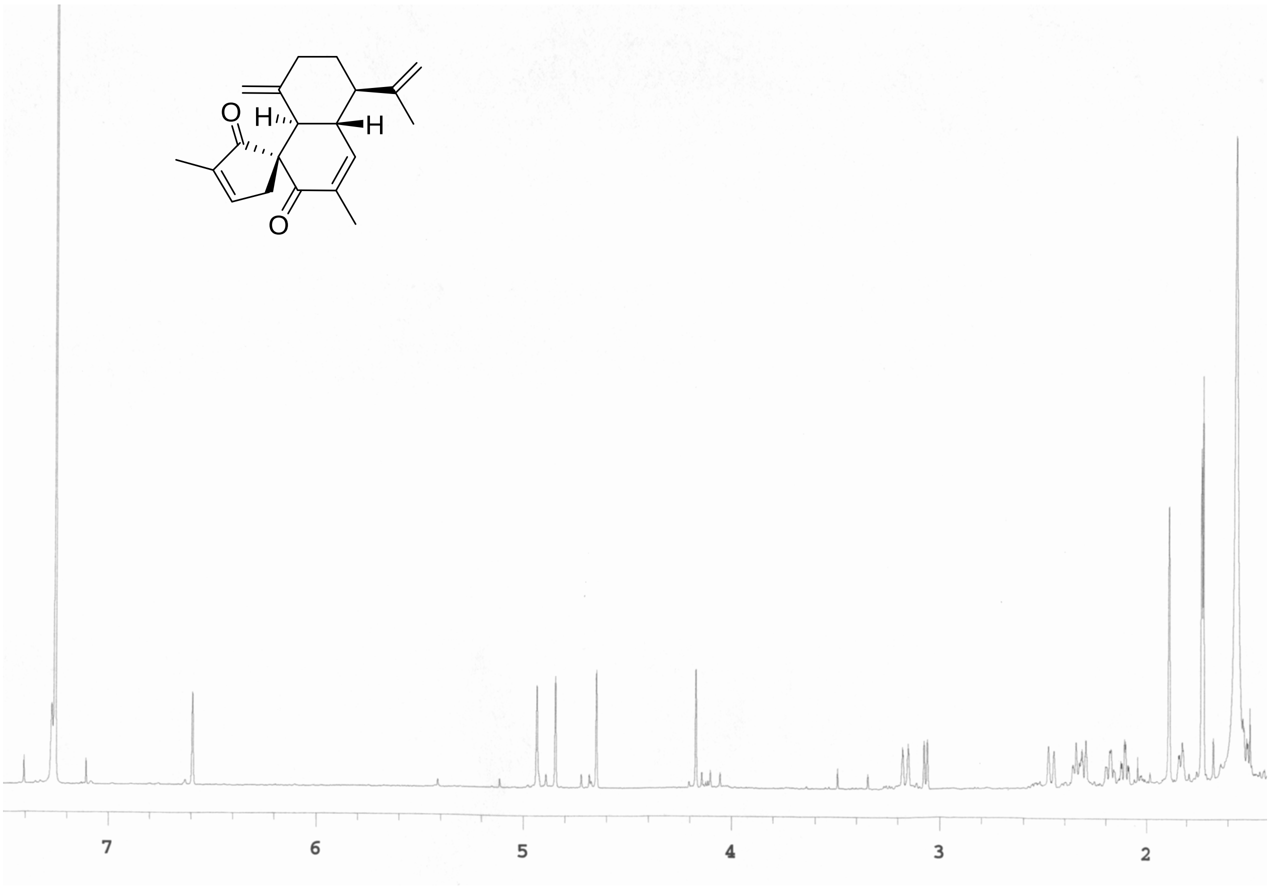
Table S5. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data of Acetoxyjatropholone (**8**) (in $CDCl_3$).

Pos.	δ_C	δ_H (mult., *J* in Hz)
1a	30.4	3.19 (dd, 16.7, 8.3)
1b		2.52 (dd, 16.7, 2.3)
2	42.7	2.63 (overlapped)
3	208.0	
4	137.5	
5	134.0	
6	145.5	
7a	33.5	2.70 (overlapped)
7b		2.67 (overlapped)
8a		1.84 (m)
8b		1.34 (m)
9	26.2	0.93 (overlapped)
10	23.3	
11	23.1	0.92 (overlapped)
12	136.0	
13	131.0	
14	150.6	
15	131.8	
16	17.3	1.30 (d, 6.9)
17a	115.6	5.26 (bs)
17b		4.69 (bs)
18	12.5	0.91 (s)
19a	74.1	4.11 (d, 11.6)
19b		3.95 (d, 11.6)
20	13.2	2.31 (bs)
OAc	20.9	2.12 (s)
	170.5	

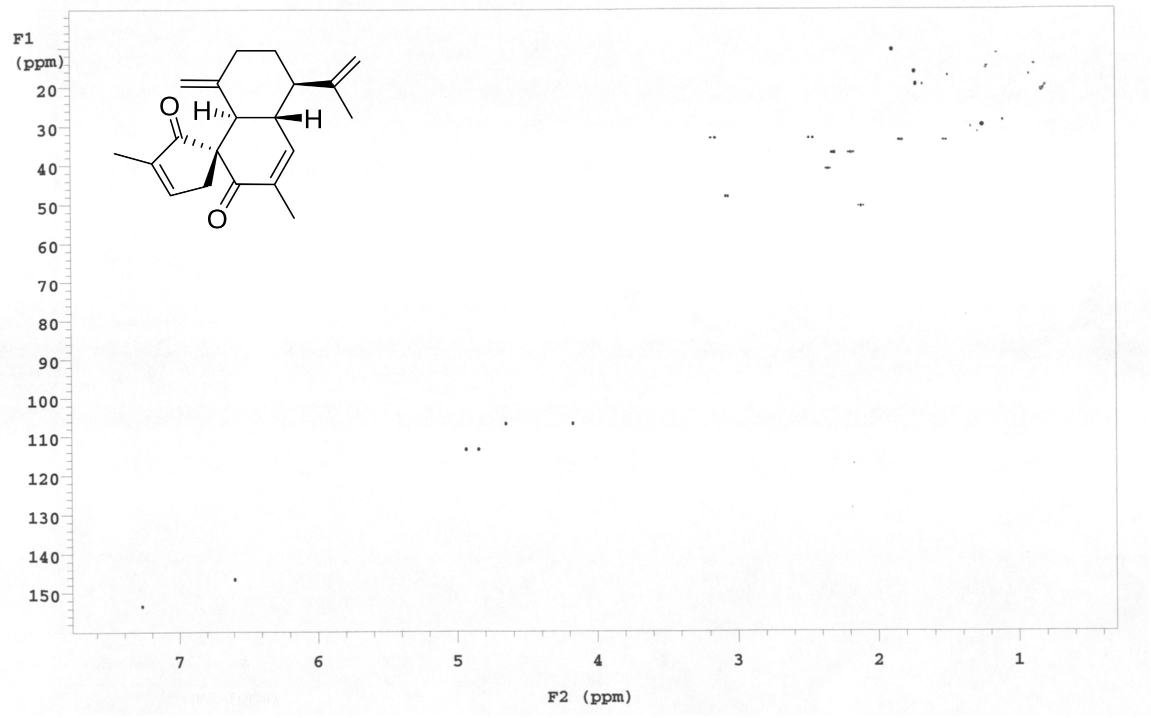
Table S6. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data of Curcusone E (**13**) (in $CDCl_3$).

Pos.	δ_C	δ_H (mult., *J* in Hz)
1	204.1	
2	145.7	
3	152.1	6.68 (bs)
4	88.9	
4-OH		4.64 (s)
5	206.3	
6	131.2	
7	139.0	5.77 (bd, 2.1)
8	45.7	2.65 (dt, 8.5, 8.5, 2.1)
9	42.8	2.37 (bd, 8.5)
10	57.4	3.32 (bs)
11	148.7	
12a	36.9	2.42 (td, 11.6, 2.5, 2.5)
12b		2.05 (m)
13a	34.4	1.74 (partially overlapped)
13b		1.60 (ddd, 9.0, 8.5, 2.5)
14	53.7	1.93 (dt, 8.5, 8.5, 2.5)
15	146.8	
16a	112.5	4.76 (bs)
16b		4.71 (bs)
17	20.2	1.65 (s)
18a	108.0	4.95 (bs)
18b		4.80 (bs)
19	10.8	1.83 (s)
20	22.0	1.75 (bs)

^{1}H NMR spectrum of spirocurcasone (in $CDCl_3$)



2D HSQC NMR spectrum of spirocurcasone (in $CDCl_3$)



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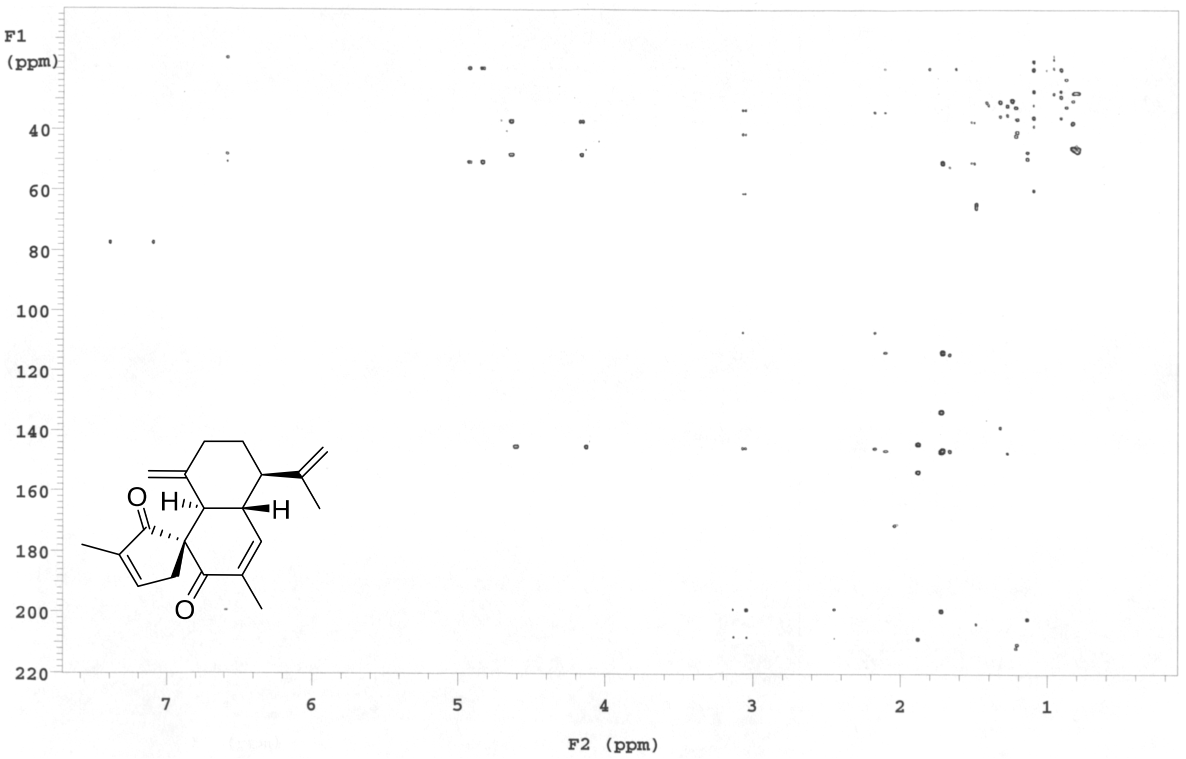
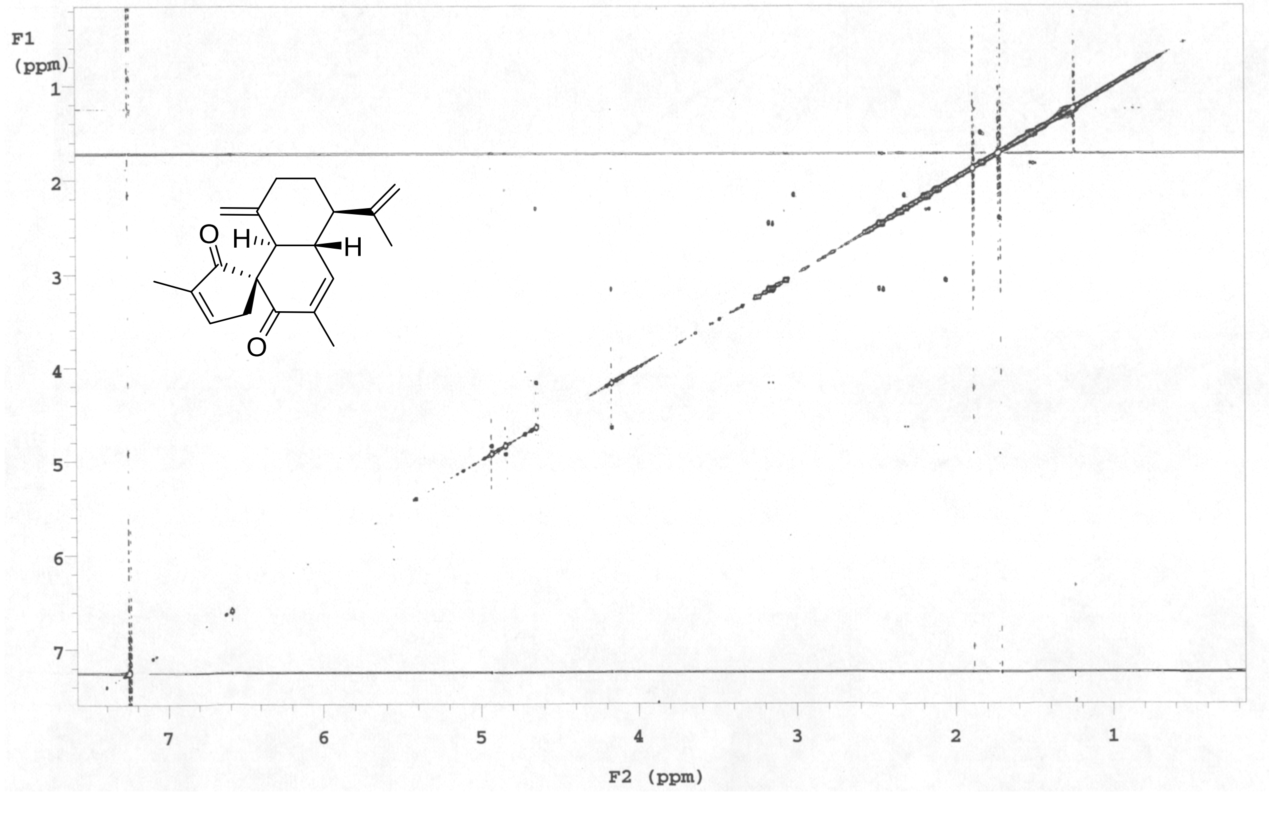
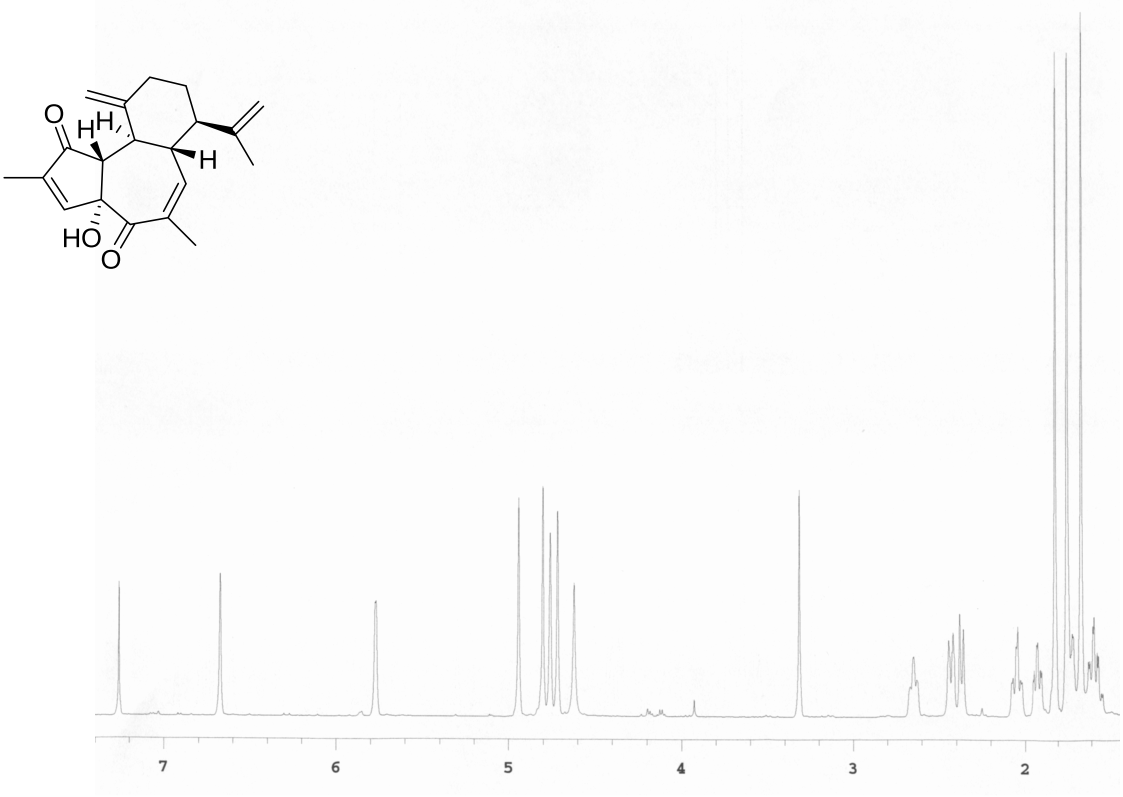


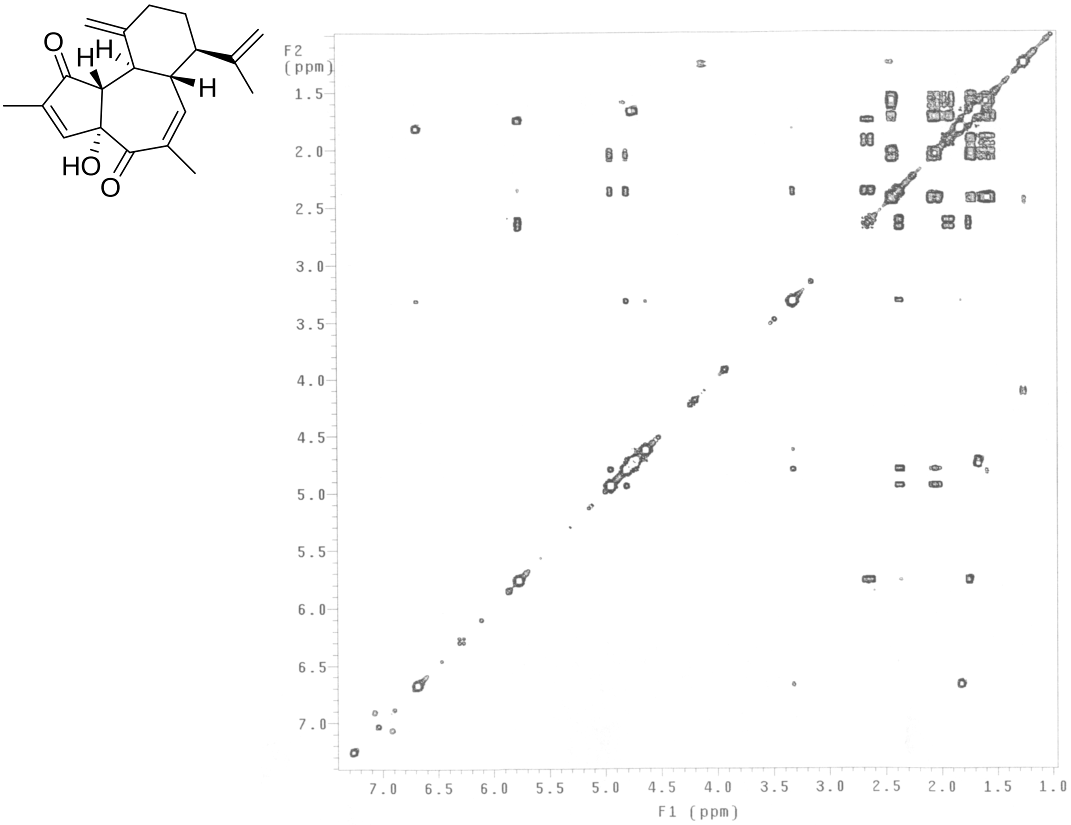
2D ROESY NMR spectrum of spirocurcasone (in $CDCl_3$)



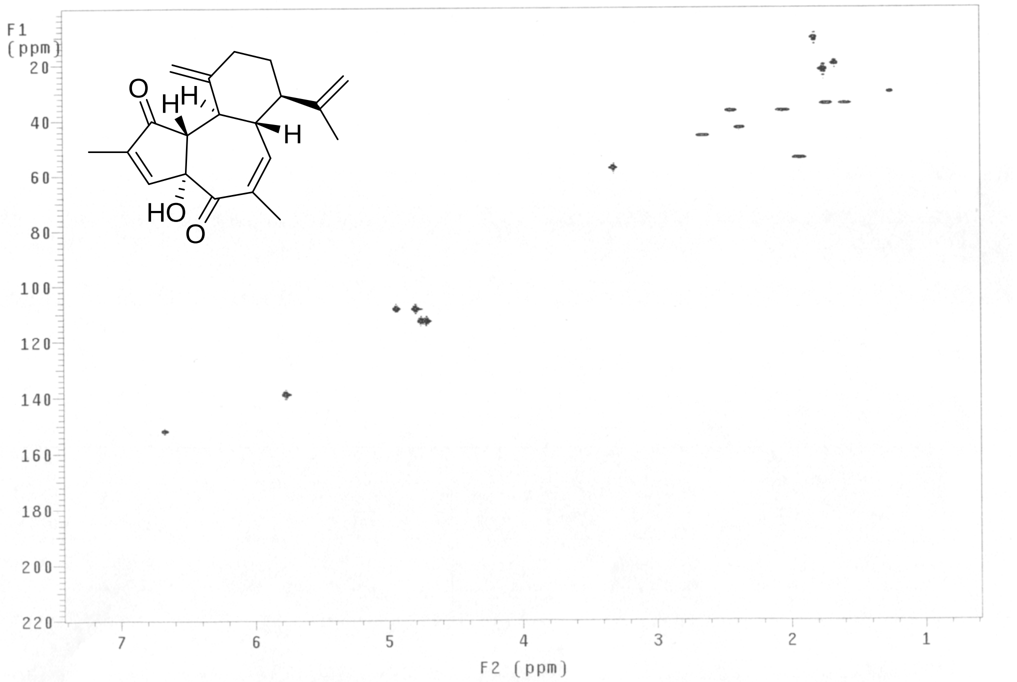
^{1}H NMR spectrum of curcusone E (in $CDCl_3$)



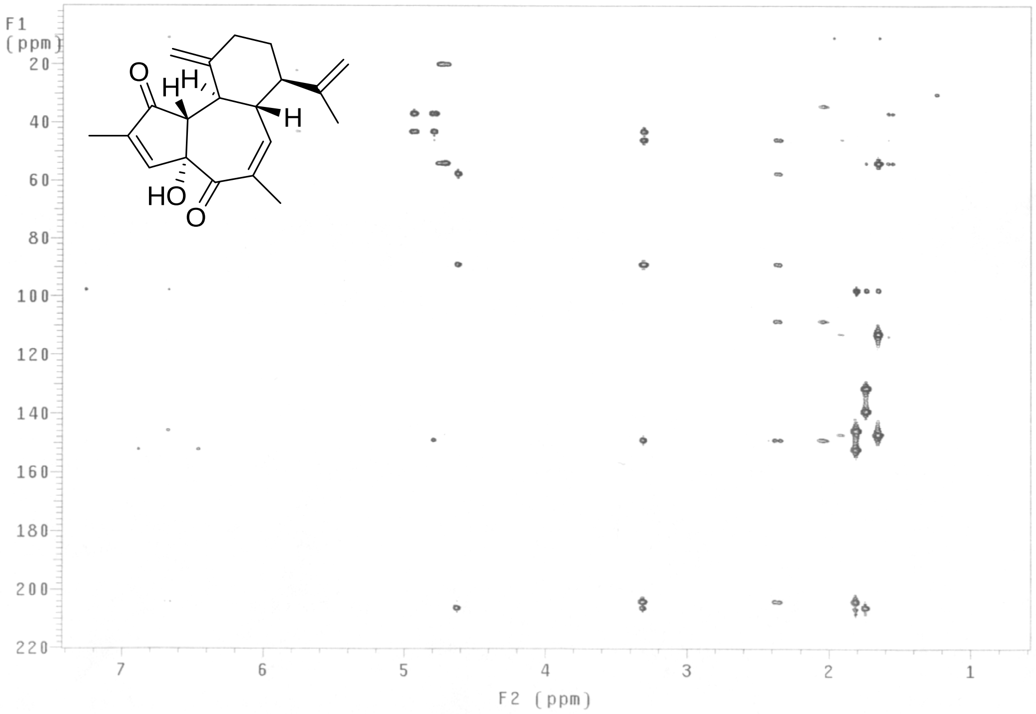
COSY NMR spectrum of curcusone E (in $CDCl_3$)



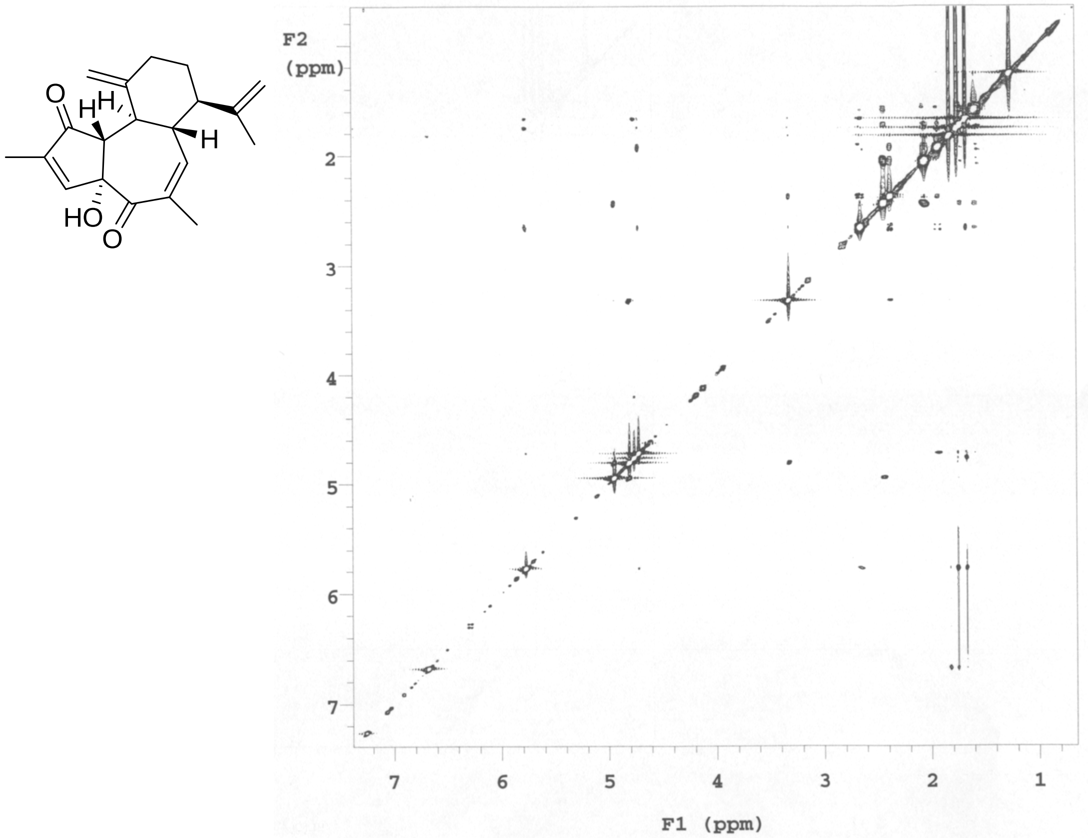
HSQC NMR spectrum of curcusone E (in $CDCl_3$)



HMBC NMR spectrum of curcusone E (in $CDCl_3$)



ROESY NMR spectrum of curcusone E (in $CDCl_3$)



^{1}H NMR spectrum of acetoxyjatropholone (in $CDCl_3$)