Supporting Information

Bovistol B, bovistol D and strossmayerin: Sesquiterpene metabolites from the culture filtrate of the basidiomycete *Coprinopsis strossmayeri*

Short title - Sesquiterpene metabolites from *Coprinopsis strossmayeri*

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S1 Text. Internal transcribed spacer region of *Coprinopsis strossmayeri*.

>CBS177.39_ITS
GTTGTAGCTGGCTCTACCCGAGCATGTGCACGCCCCGTCACCTTTATCTTTCCACCTGTGCACA
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GGTCGGCTTGCTTCGAATTTCCAGGTCTATGAACCCCTTTACGTACCCCAAACGAATGTTAAGGA
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GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG
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TTCTCAACCTCACCAACTTTTGTTGTGTGAAGGCTTGGATTTGGAGGTGTGCAGGTCCACATTTT
TTTAGTGGTCTGCTCCTCTGAAATGTATTAGTGGGTTAGGCCCCCTAATCTATTGGTGTGATAATT
ATCTACACCGTGGATTTGGGAAAGCTACATTTAGACCTGCTTCTAACTGTCCTCACAGGGGACAA
CATTTGACAATTTTGACCTCAAATCAGGTAAGACTACCCGCTGAACTTAAGCATA

S2 Text. Terpene cyclase protein sequences used in phylogenetic tree construction.

>Omp1
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>Omp2
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>Omp10
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>Cop1
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>Cop2
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>Cop3
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>C.s_contig36
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>C.s_contig56
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>C.s_contig69
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NAEGAQWAQSAMILDALRHAEDGLDYLRLLKNQTVFNFCAIPATMAIATLELCFMNPAMFQRNIKIRK
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>C.s_contig81
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NPGKLARDFWARCIPDCGPGAQARFKETLGLFFEAVSIQARSRDEGVIPDLESYIDVRRDTSGCKPC
WALIEYALDIDLPDFVVEHPVIEALNQGTNDLVTWSNSRGDTHNMIVILMEHHGHTLQSAIDYVGDLC
QQTIDTFVYNKNNLPSWGPEIDDMVARYVKGLQDWIVGSLHWSFQTHRYFGKEGQAIKQHRLVKLLP
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LPRLFFGFLFPYSH-

>C.s_contig98
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>C.s_contig109
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>C.s_contig158_GGS
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>C.s_contig158_Cyc
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LIFASLWLTASSFMNLESDIARHNYKSSLEAVKGMFETPIGPLTAAIVSTFGIYLIGSILYLDPWHMIHSF
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>C.p_cyc1
MRIPNVFLSYLRQVAVDGTLSSCSGVKSRKPVIAYGFDDSQDSLVDENDEKILEPFGYYRHLLKGKSA
RTVLMHCFNAFLGLPEDWVIGVTKAIEDLHNASLLIDDIEDESALRRGSPAAHMKYGIALTMNAGNLVY
FTVLQDVYDLGMKTGGTQVANAMARIYTEEMIELHRGQGIEIWWRDQRSPPSVDQYIHMLEQKTGG
LLRLGVRLLQCHPGVNNRADLSDIALRIGVYYQLRDDYINLMSTSYHDERGFAEDITEGKYTFPMLHS
LKRSPDSGLREILDLKPADIALKKKAIAIMQDTGSLVATRNLLGAVKNDLSGLVAEQRGDDYAMSAGLE
RFLEKLYIAE-

S3 Text. Genomic DNA sequences of genes comprising the putative gene cluster for the biosynthesis of 1.

>FAD oxidoreductase
ATGGTTTCTCCAAAGAACTTCCTCTTTACGGTCGCTTCTACGGCATTGCTCGCCCATGCAGCCCC
TGCCGCTGATGGTGGAAGGTTGGCCGCAATCGCTGCTTGCGCGTTAGTCGGTGTGAGGCCCAG
CCTCGAGCCAGCCTTTGCTGGTGAGGAATCTGTTCAAAAGCCCTATAAACTTAGGCTAATACCTT
CATCTTTAGGCTCTTTACAGTACACCAACGACATCTCCCATTGGTCAAACGCTAGCAGTCTTCCTT
CGTTGTGTAGCGCTCAGCCAAATACGCCACAAGAAATCCAGACATTGGTACGCTATGCTACTATT
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CAGTGAGTCCCCTCCTAATTGCCATGGTTTCATCTTTAATTCTTCCGCGTCCAGGTGAAAGGCAA
AGGTCACACCAACGTCAACAATGCGAGCTCTACATACGGTCTACAAATTGCCATGACCCAATTCA
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GGTGTACACGGCCTTGGATCCGCACGGTGTCATCGTAGTTGGCGGCAGAGCTCCTGGAGTTGG
TATGTGGAAAGCATTACCTACGATTCGACTGAAAAACTAATCGAGTTCCTCAGGTGTGGCCGGAC
TGACCCTCAGCAGCGGATATGCCTGGTTCACCAACGAATATGGATTCGCGATCGACAACACTCT
CGCCGCTGGTACGCTTATTTTGACTAATCTCGAAGCTTATCAGAGCTTATCTGAATCGGTACCAG
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GGTCTGCAAGTAGGTGCCTTTTATTCGGCTGCTTCGCAGCATGCTTATGACGTTACAGGGCGGT
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CCGGTGCTAGCGGTACCGTCACCACAAGATCGTTCCTTGATTTCATCCTCACGAACGACGTTCC
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CTCATGTTCAACCTCACCGTCGAATATCATACTGCTGTTTGGGACACTGCAGTCGCCCTTGGTAC
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TTCACAACATTCATTCTAATTACGCCGTCCTTATCTTCATCAGCTTTGCTTACTTATCTTTATTAAC
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>sesquiterpene cyclase
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>aldo-keto reductase
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CACCATCAGTGCGGAGGGACCTCTTCCGGCCGAGGTTGTTGAGGCATTTGACGTCACCTACAAG
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CTACTAA

>P450-dependent oxidoreductase
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TCATCGCCCTCCTTGCAAGCAAGCCAAAGAAGGGAAAACGACCTCCTGGTCCTCGTGGTCTGCC
ACTGGTATGTAAACTCTTATAATTCAGCACTAGTTCTCGGGGATTTACGTCGCATAGGTTGGCAA
TGTTTTCCAATTGTCTGAAGAGAATTGGATTACCTTCACCGAGTGGAAGTTCAAGTATGGTCCCA
TCGTCGGCCTGAACCTCGGTGGTAAGAACACCATCGTCCTCAACACCCACAAAGTAGCATCAGA
TCTCCTCGACAAGCGCGCATCTATCTATTCCGATAGTGAGTCGTTGCCTTCCTCTAAAGTCCTCC
CGTCTCATGCCAATAATTCACGATAGGACCTCGATTCATTGTCGCGCAGGAGATGCTTTGTGGTG
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CTCGTCCAACGTCTCGTCAAGGCAGCTTATCCCGGAGAGCACTTGGTCGAATTCTTCCCCTTCCT
TAACAAACTTCCTGATTGGATTGCCAAGTGGAAACCTTGGGCTGCCGAGTGGCACCGAAAGGAT
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GCGCCCGGTAGATCCTATCGGTCTCCAACATCAGTCTACGGAAGACGACGTCTACGAGGGTTAC
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TGAGGAATTCAAACCCGAGCGATTCCTTGACGAAGATGGTCAACTCAAGCCCACTCATCCCGCT
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CCAACAACACCCTCTTCATTGATATGGCCCATGTCCTTTGGGCTTGCTCGATTGAAAAGGCAAAG
AACCCTGACGGGACCGATATCAACTATGACTCCTATGCCAACCACAACGAGGGTCTCGTCGTGT
ACGTTGTTCTTTCTCTTGACTTCGATTTAGGATACTGATTATTCTCAATTCGTGTAGTCGTCCTGC
CTACTTCAAGAATGTTATCAAGCCCCGGTTCCCCGCGGCGGCCCAGATAGTTGCAGAGCAGAAG
GAGCGCCTCATTTCTGCTGCCAGCGAAGGCAAAACAATCAGAATTTCTGGAACTTCATTCCAGGA
TGTTTAA

S1 Table. ^{13}C and ^{1}H NMR data of bovistol B (1) in d_4- MeOH.

position	δC (ppm)*	δH-ppm (J, Hz)	Key HMBC
1	150.0		
2	119.1		
3	133.3		
4	124.5		
5	142.1		
6	43.1	2.83/2.52(d/d, 15.8, 15.8)	C5, C7, C9, C10, C11,
7	45.0		
8	40.1	2.70/2.55 (d/d, 16.2, 16.2)	C1, C5, C7, C9, C10, C11,
9	127.4		
10	70.7	3.40(s)	C11, C7, C6, C8
11	24.9	1.16(s)	C10, C7, C6, C8
12	15.0	2.13(s)	C3, C4, C5
13	32.8	2.80(t, 8.3)	C2, C3, C4, C14
14	61.8	3.50(m)	C13, C3
15	20.6	2.77/2.36 (ddt, 16.1, 4.6)	C2, C1, C3, C15’, C2’
1’	198.9		
2’	80.6		
3’	31.6		
4’	144.9		
5’	158.5		
6’	43.8	2.94/2.39(d/d, 17.8, 17.8)	C5’, C7’, C9’, C10’, C11’,C1’
7’	43.7		
8’	40.7	2.57/2.39 (d/d, 17.0, 17.0)	C5’, C7’, C9’, C10’, C11’,C1’
9’	135.1		
10’	70.4	3.41(s)	C11’, C7’, C6’, C8’
11’	24.9	1.14(s)	C10’, C7’, C6’, C8’
12’	113.3	5.29/5.25 (s/s)	C4’, C3’, C5’
13’/14’	12.9	1.38/0.26 (ddd, 16.7, 6.5.5.2)	C3’, C4’, C2’, C14’/C13’
13’/14’	5.31	1.13/0.80(ddd, 16.7, 6.5, 5.2)	C3’, C4’, C2’, C13’/C14’
15’	30.0	2.19/1.87 (ddt, 16.1, 4.6,)	C1’, C15, C2, C2’, C3’

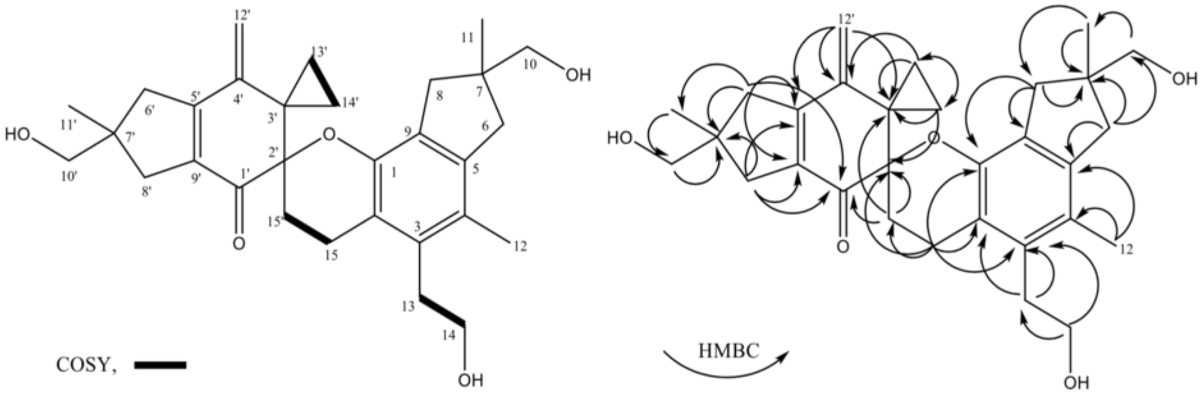
S2 Table. ^{13}C and ^{1}H NMR data of D (2) in d_4- MeOH.

position	δC (ppm)*	δH-ppm (J, Hz)	Key HMBC
1	150.2		
2	119.0		
3	133.8		
4	124.4		
5	141.3		
6	44.8	3.36/2.75(d/d, 15.1, 15.1)	C5, C7, C9, C10, C11,
7	45.1		
8	41.6	3.23/2.76 (d/d, 15.2, 15.2)	C1, C5, C7, C9, C10, C11,
9	126.6		
10	182.2		
11	26.3	1.37(s)	C10, C7, C6, C8
12	15.3	2.14(s)	C3, C4, C5
13	32.8	2.81(OL[1])	C2, C3, C4, C14
14	61.5	3.50(m)	C13, C3
15	20.5	2.78/2.36 (ddt, 4.6, 16.1)	C2, C1, C3, C15’, C2’
1’	198.9		
2’	80.8		
3’	31.8		
4’	144.4		
5’	158.5		
6’	43.7	2.94/2.39(d/d, 17.8, 17.8)	C5’, C7’, C9’, C10’, C11’,C1’
7’	43.8		
8’	40.5	2.56/2.39 (d/d, 17.0, 17.0)	C5’, C7’, C9’, C10’, C11’,C1’
9’	135.1		
10’	70.3	3.42(s)	C11’, C7’, C6’, C8’
11’	24.8	1.15(s)	C10’, C7’, C6’, C8’
12’	113.4	5.29/5.25 (s/s)	C4’, C3’, C5’
13’/14’	13.7	1.31/0.25 (ddd, 16.7, 6.5.5.2)	C3’, C4’, C2’, C14’
13’/14’	5.4	1.12/0.80(ddd, 16.7, 6.5, 5.2)	C3’, C4’, C2’, C13’
15’	29.9	2.19/1.88 (ddt, 4.6, 16.1)	C1’, C15, C2, C2’, C3’

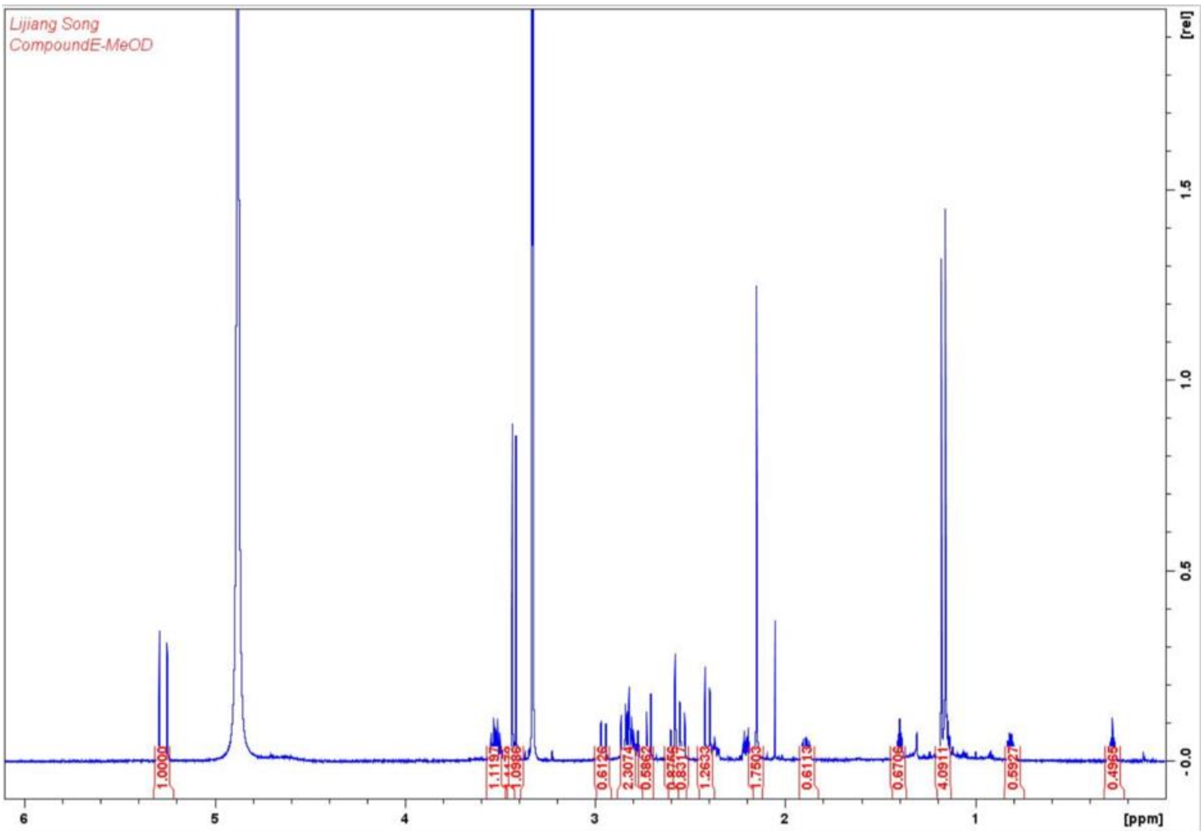
S3 Table. ^{13}C and ^{1}H NMR data of strossmayerin (7) in d_4- MeOH.

position	δC (ppm)*	δH-ppm (J, Hz)	Key HMBC
1	147.4		
2	120.9		
3	131.3		
4	123.8		
5	141.4		
6	42.8	2.80/2.51(d/d, 15.5, 15.5)	C4, C5, C7, C8, C9, C10, C11,
7	45.1		
8	40.1	2.78/2.51(d/d, 15.5, 15.5)	C1, C5, C6, C7, C9, C10, C11,
9	125.6		
10	70.4	3.42(s)	C6, C7, C8, C11
11	24.6	1.14(s)	C6, C7, C8, C10
12	14.2	2.02(s)	C3, C4, C5
13	27.1	2.63(t, 6.0)	C2, C3, C4, C14
14	65.9	3.90(t, 6.0)	C13, C3, C15
15	65.6	4.70 (s)	C1, C2, C3, C13, C14

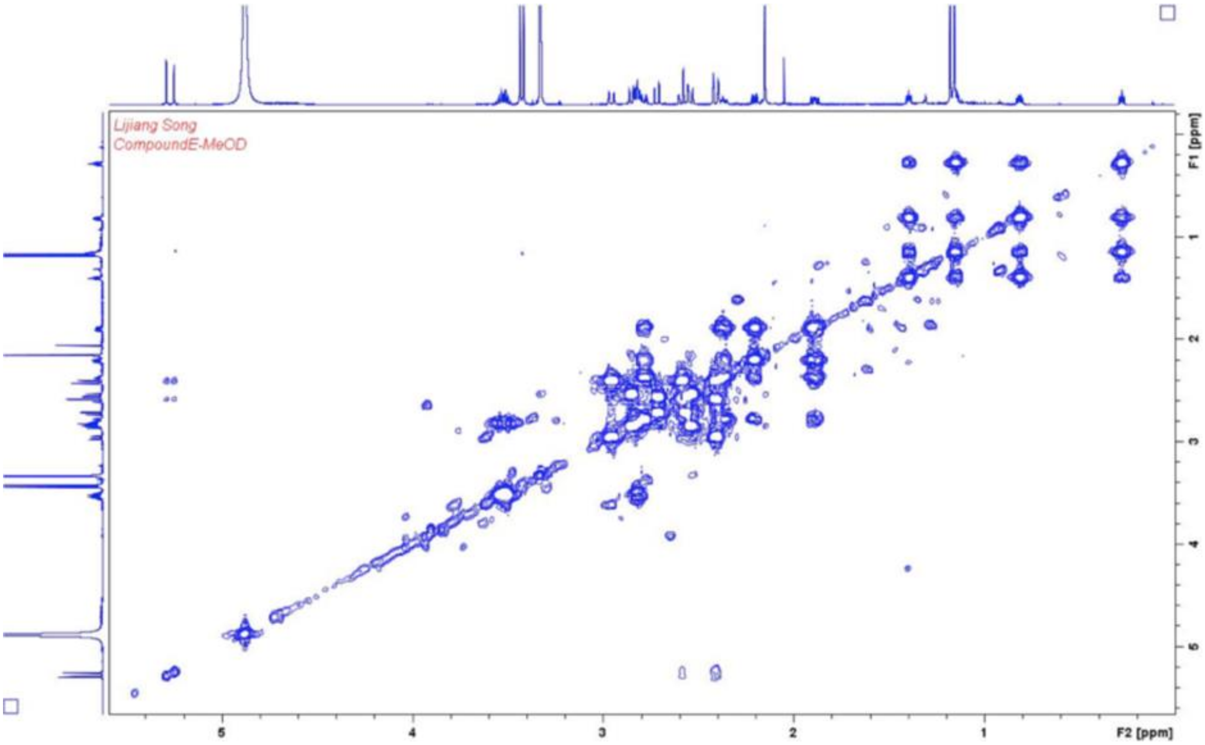
[1] Over-lapping signal



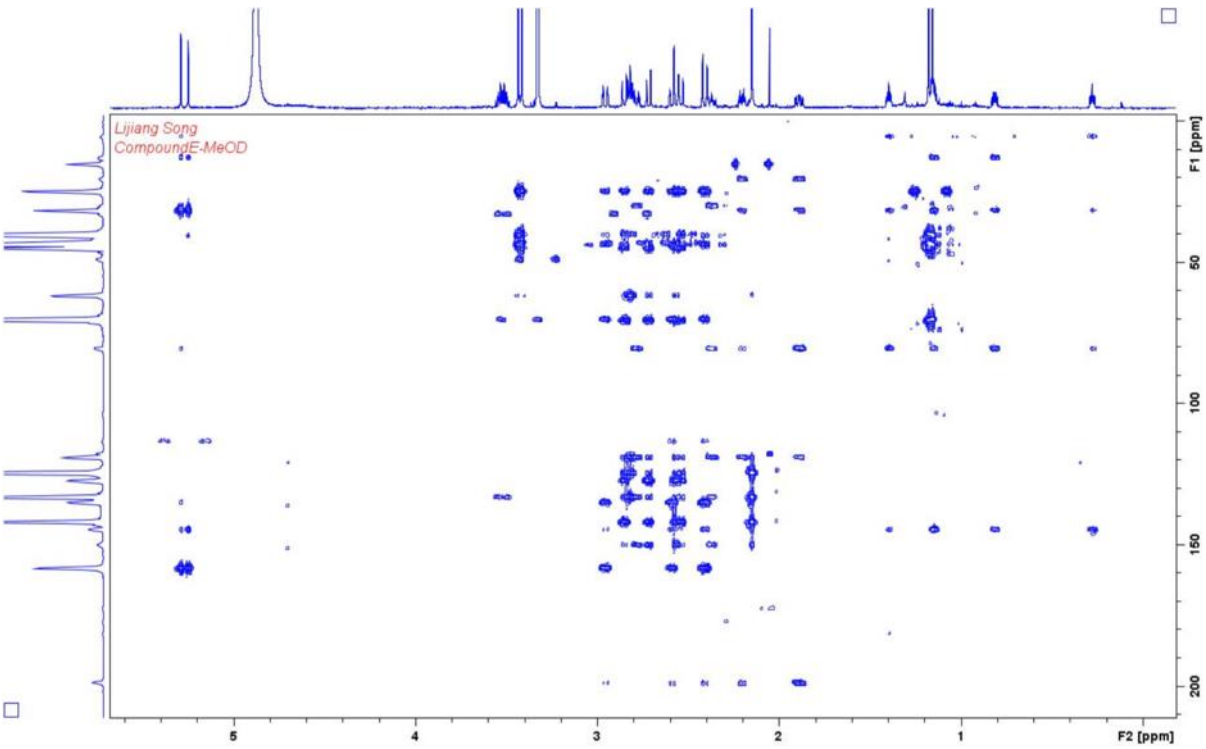
S1 Figure. Key 2D NMR correlations of bovistol B (1) showing COSY HMBC in d4- MeOH.



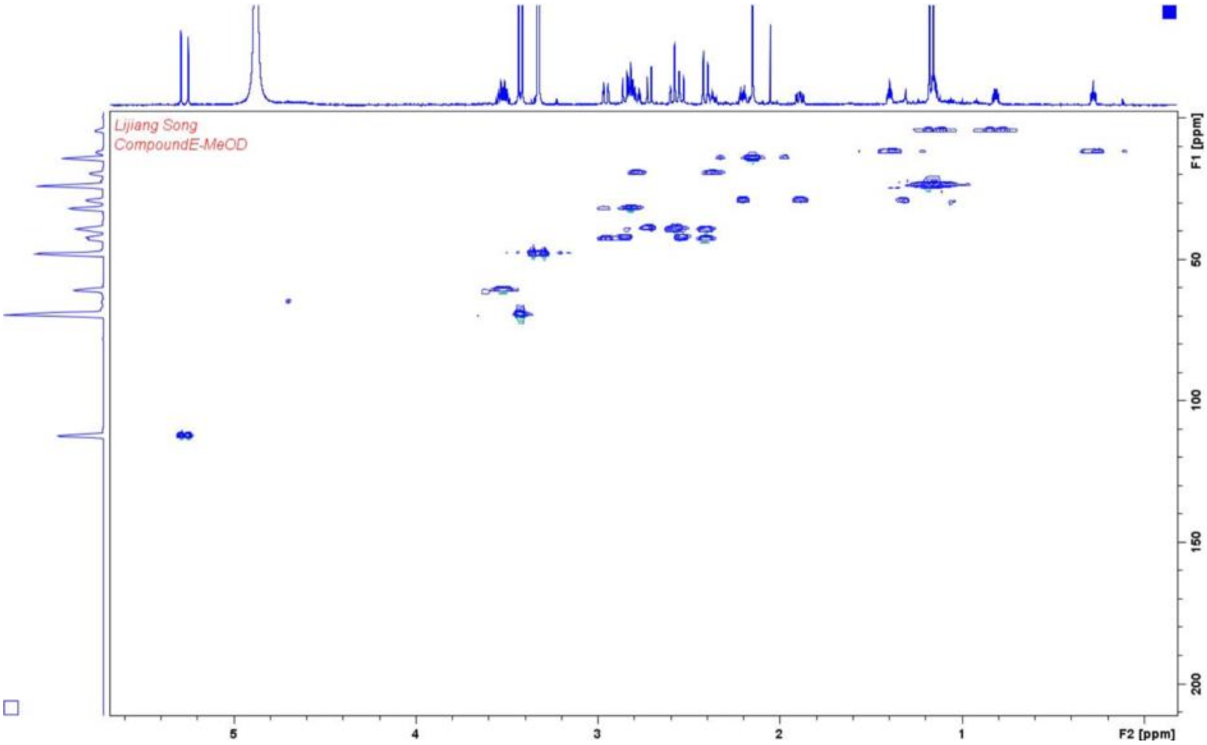
S2 Figure. 1H NMR spectrum of bovistol B (1) in d4- MeOH.



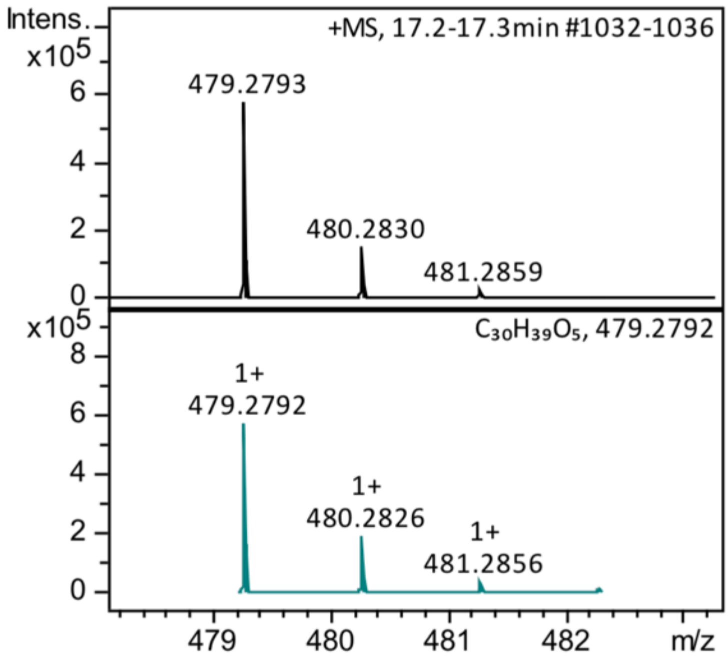
S3 Figure. COSY NMR spectrum of bovistol B (1) in d4- MeOH.



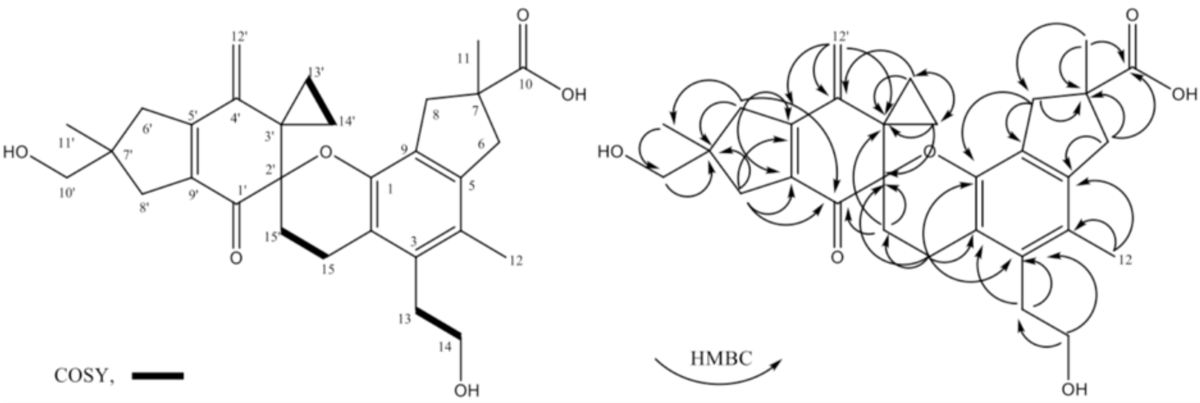
S4 Figure. HMBC NMR spectrum of bovistol B (1) in d4- MeOH.



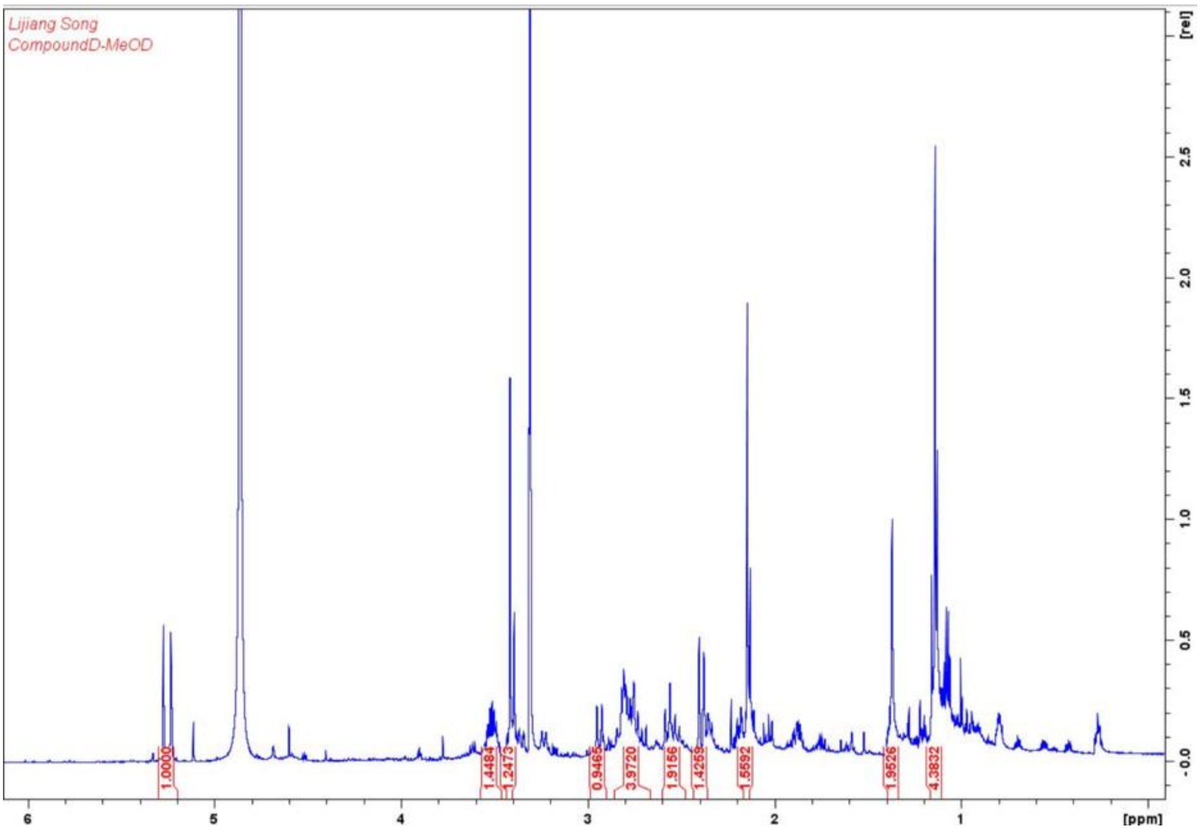
S5 Figure. HSQC NMR spectrum of bovistol B (1) in d4- MeOH.



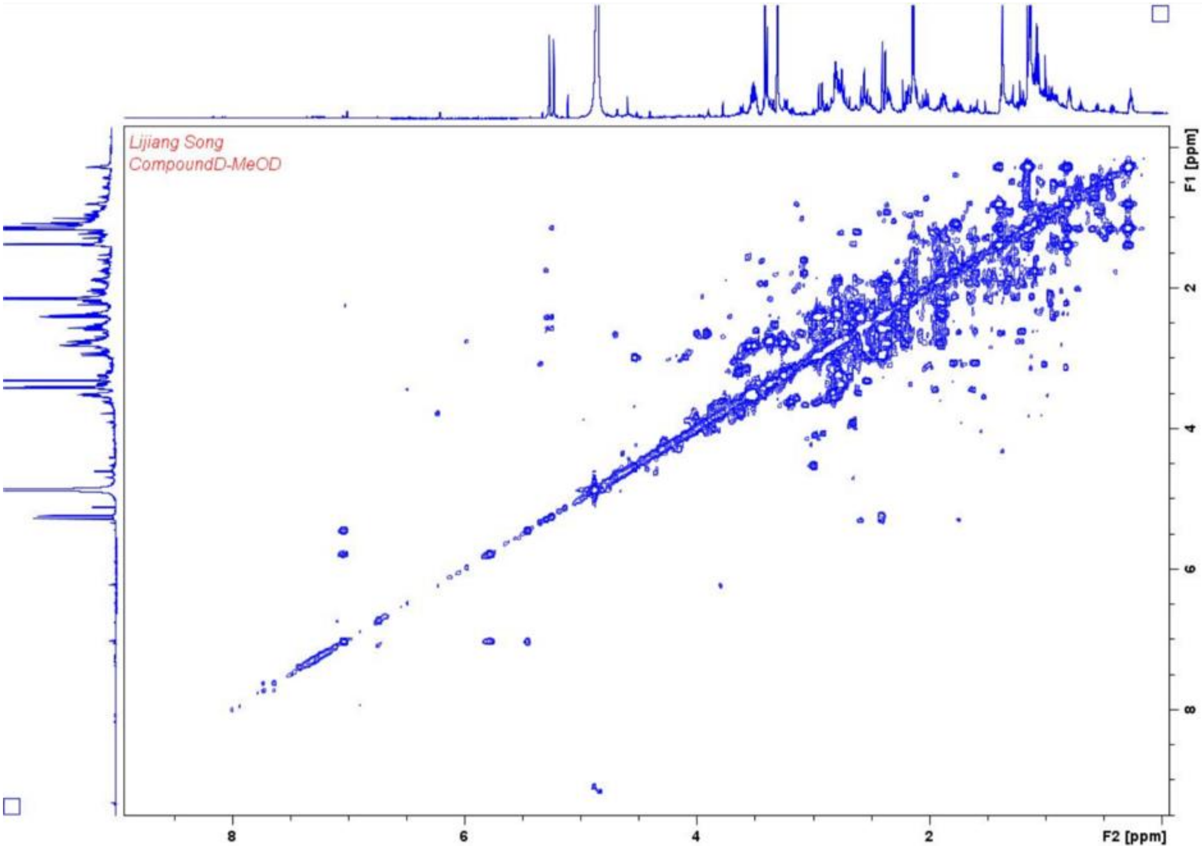
S6 Figure. HRMS of bovistol B (1). Top panel: measured spectrum, bottom pannel: simulated spectrum.



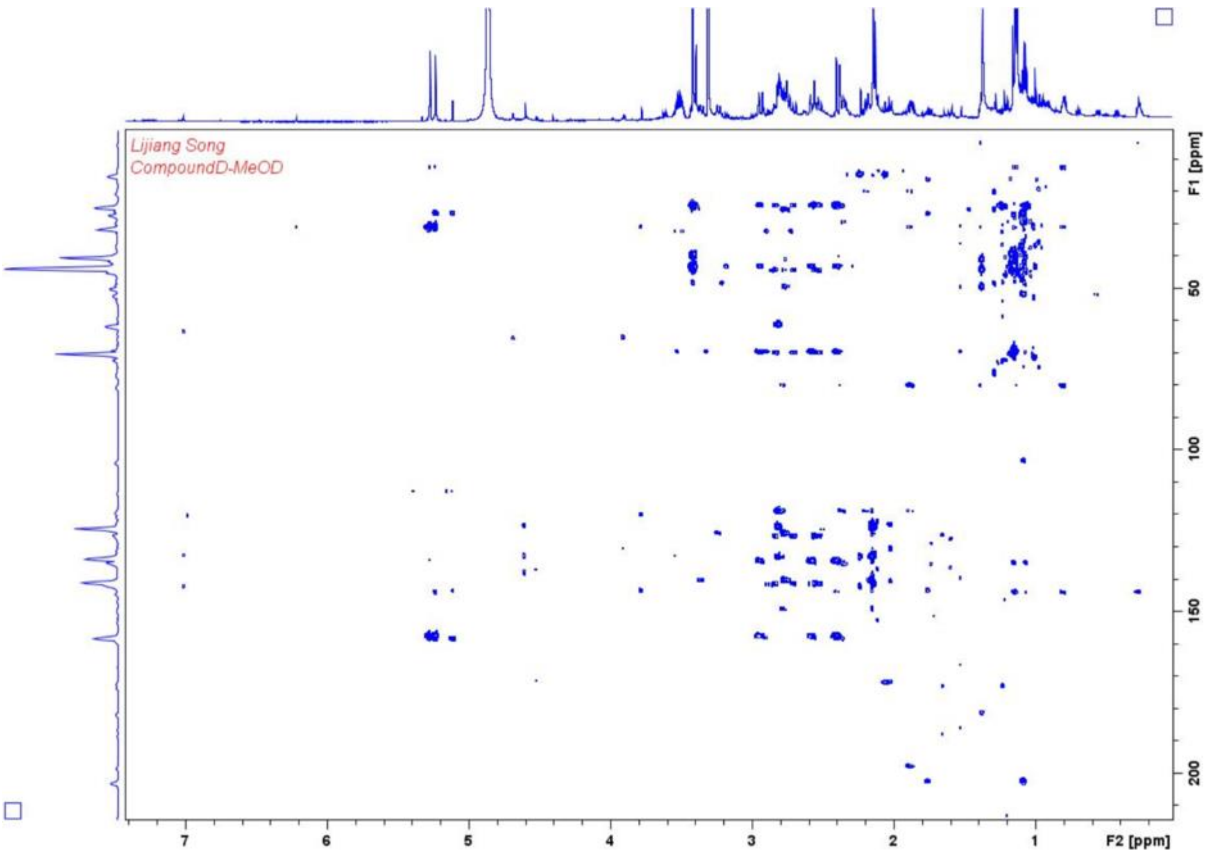
S7 Figure. Key 2D NMR correlations of D (2) showing COSY HMBC in d_4- MeOH.



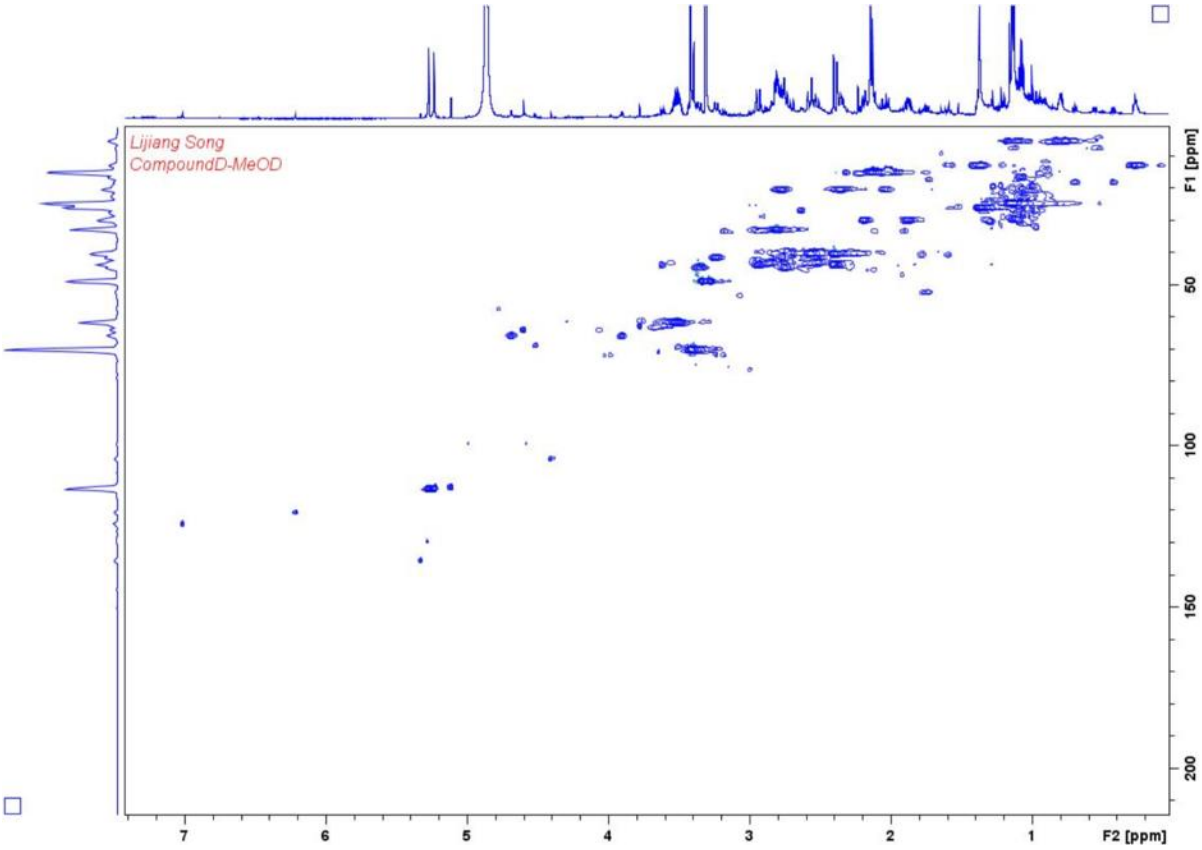
S8 Figure. ^{1}H NMR spectrum of D (2) in d_4- MeOH.



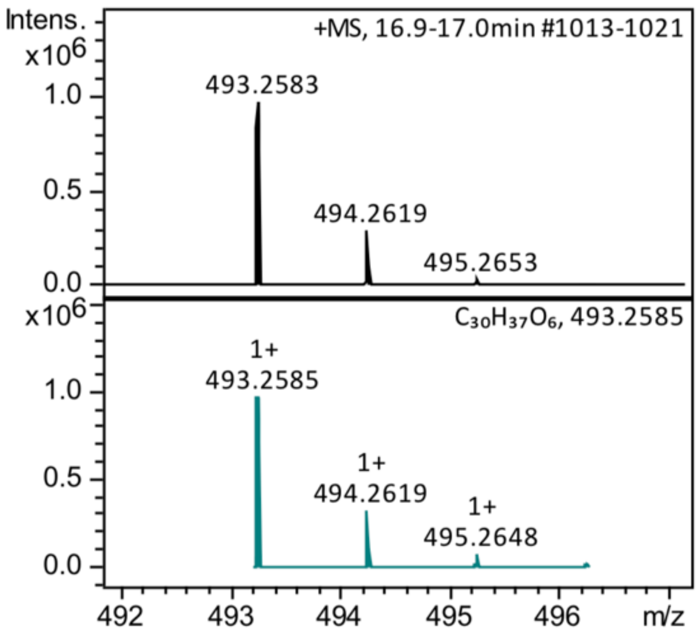
S9 Figure. COSY NMR spectrum of D (2) in d_4- MeOH.



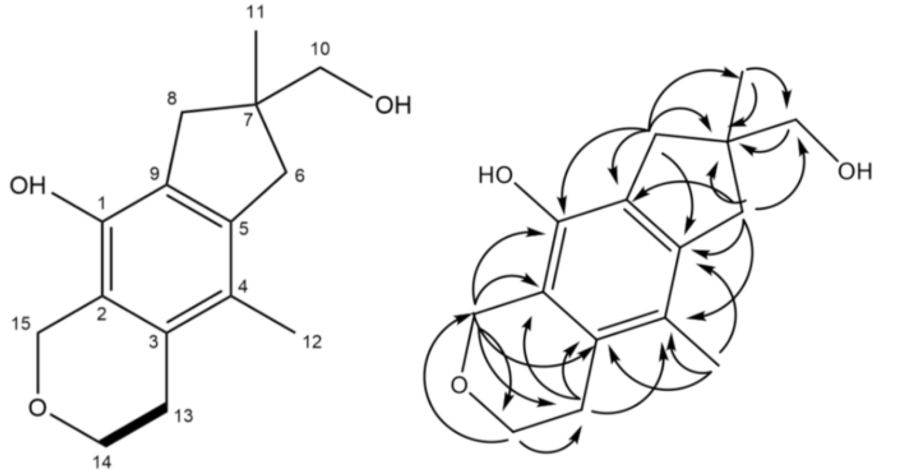
S10 Figure. HMBC NMR spectrum of D (2) in d_4- MeOH.



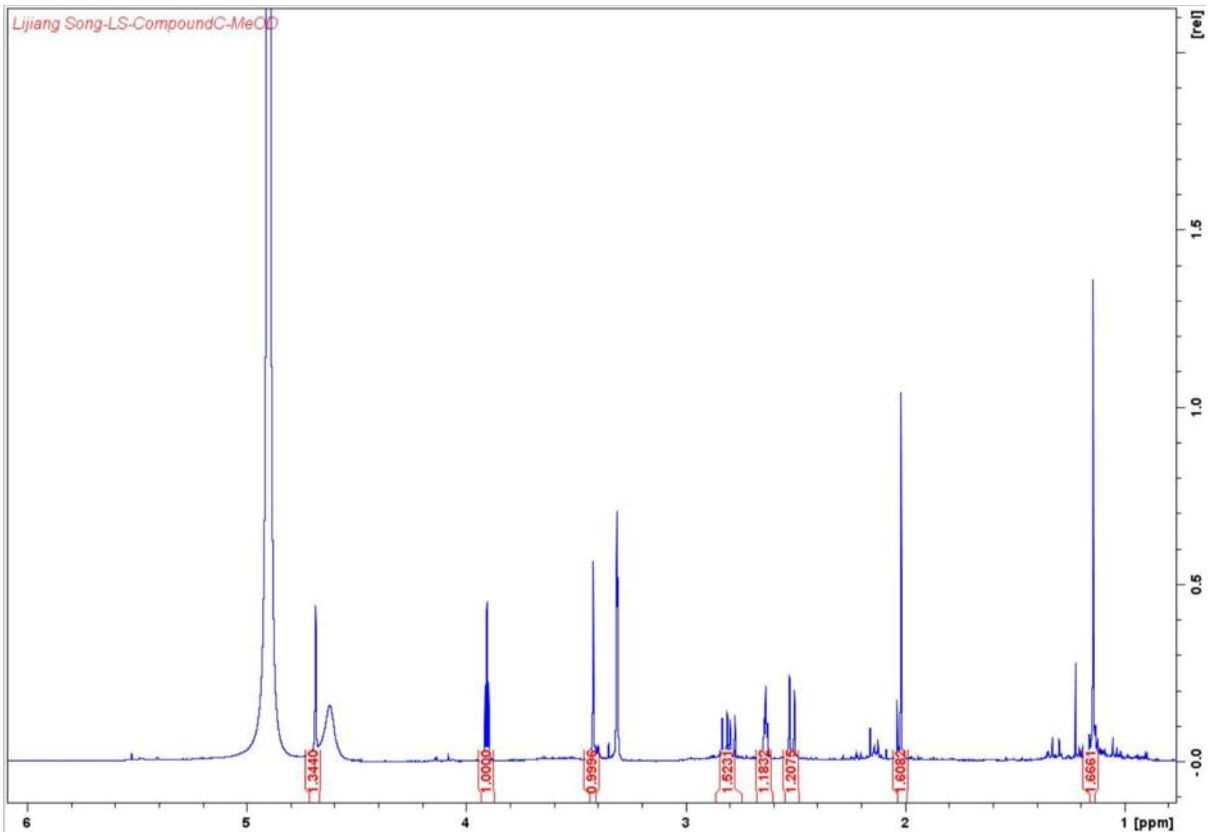
S11 Figure. HSQC NMR spectrum of D (2) in d_4- MeOH.



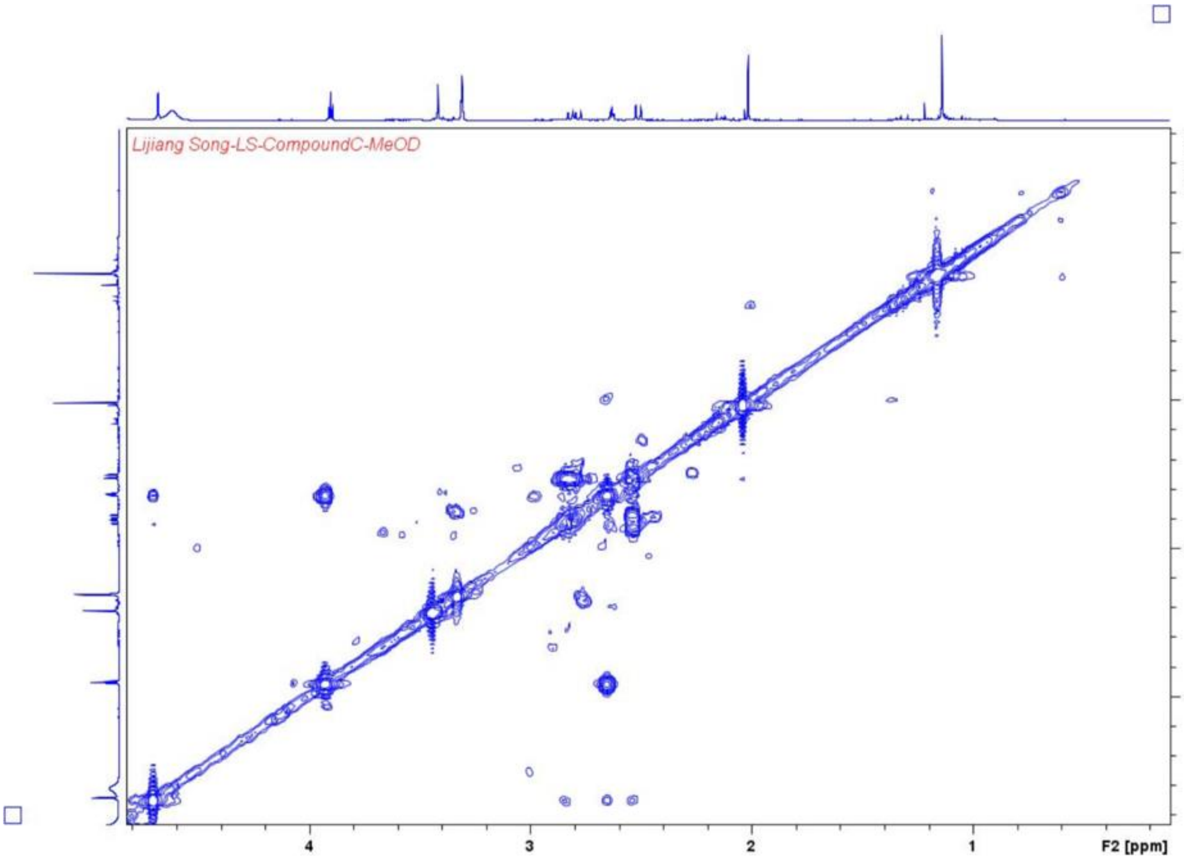
S12 Figure. HRMS of D (2). Top panel: measured spectrum, bottom pannel: simulated spectrum.



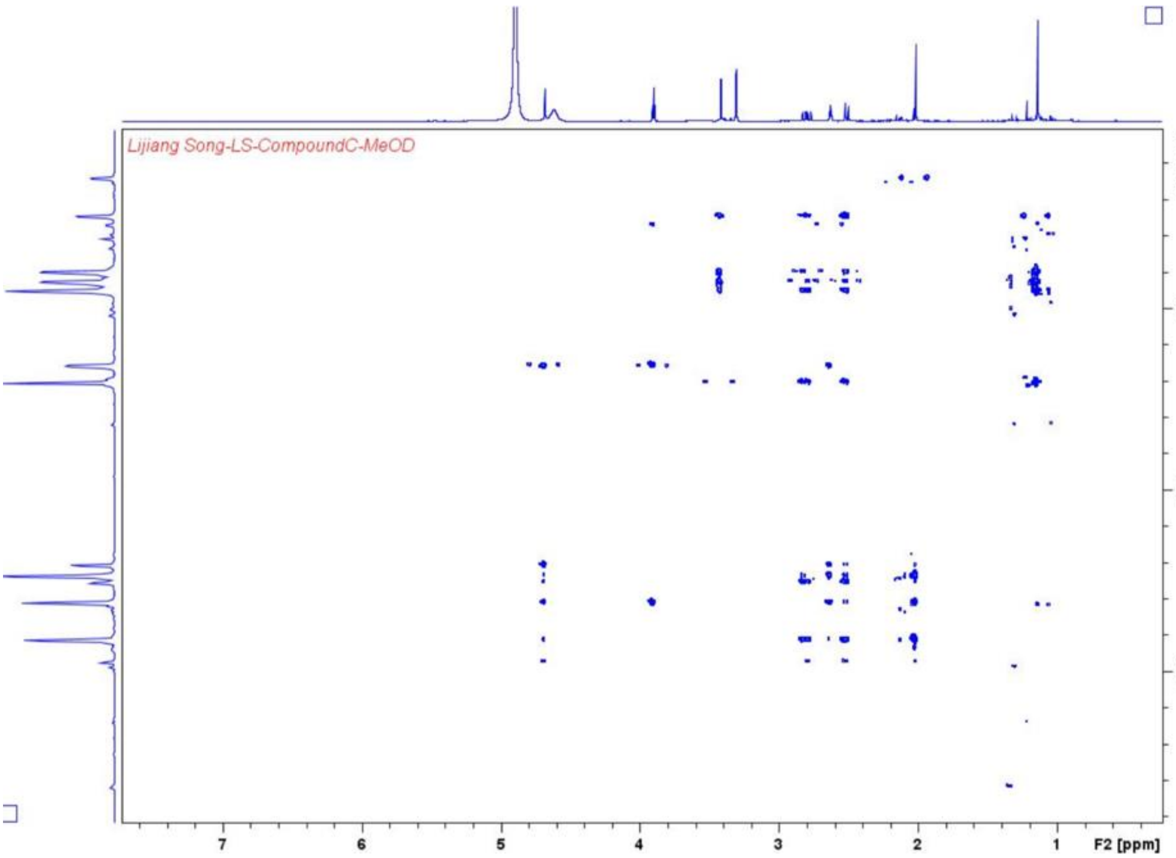
S13 Figure. Key 2D NMR correlations of strossmayerin (7) showing COSY HMBC in d4- MeOH.



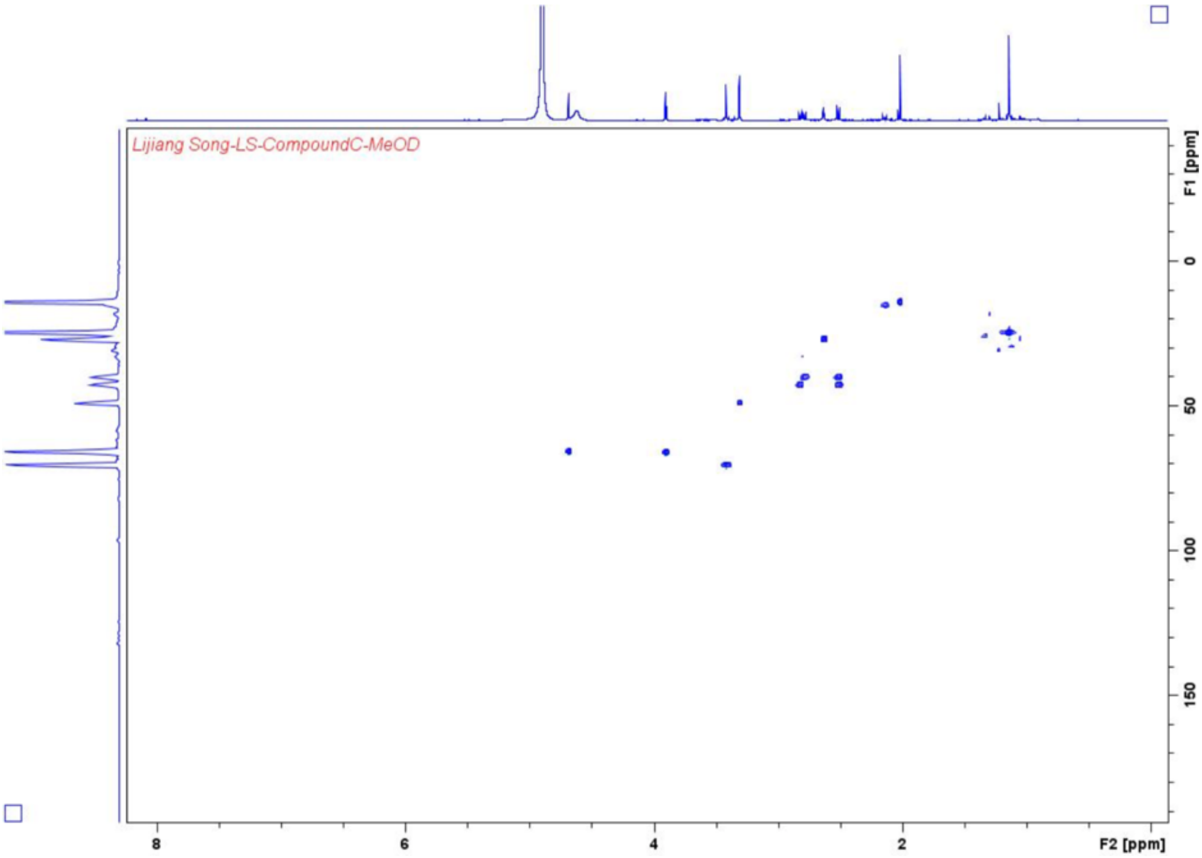
S14 Figure. 1H NMR spectrum of strossmayerin (7) in d4- MeOH.



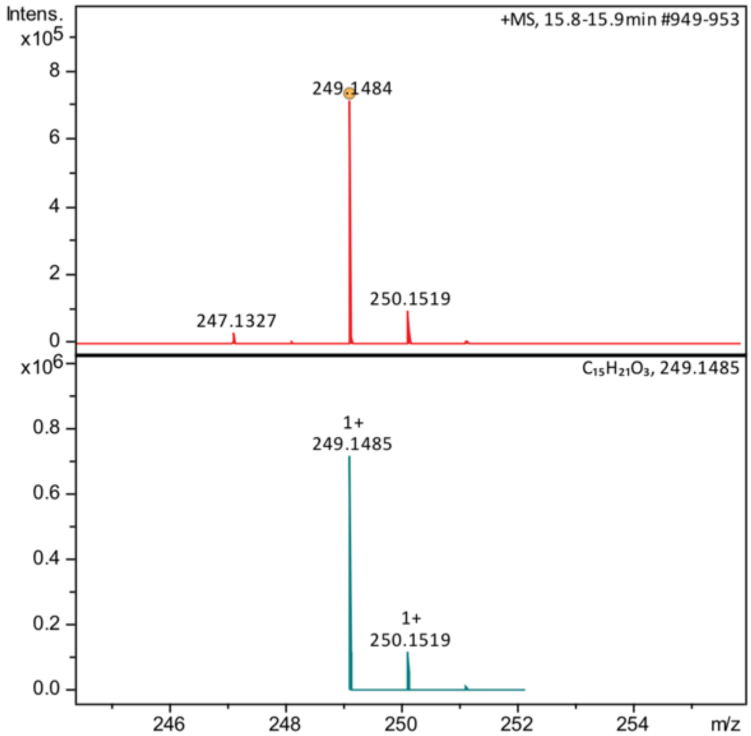
S15 Figure. COSY NMR spectrum of strossmayerin (7) in d4- MeOH.



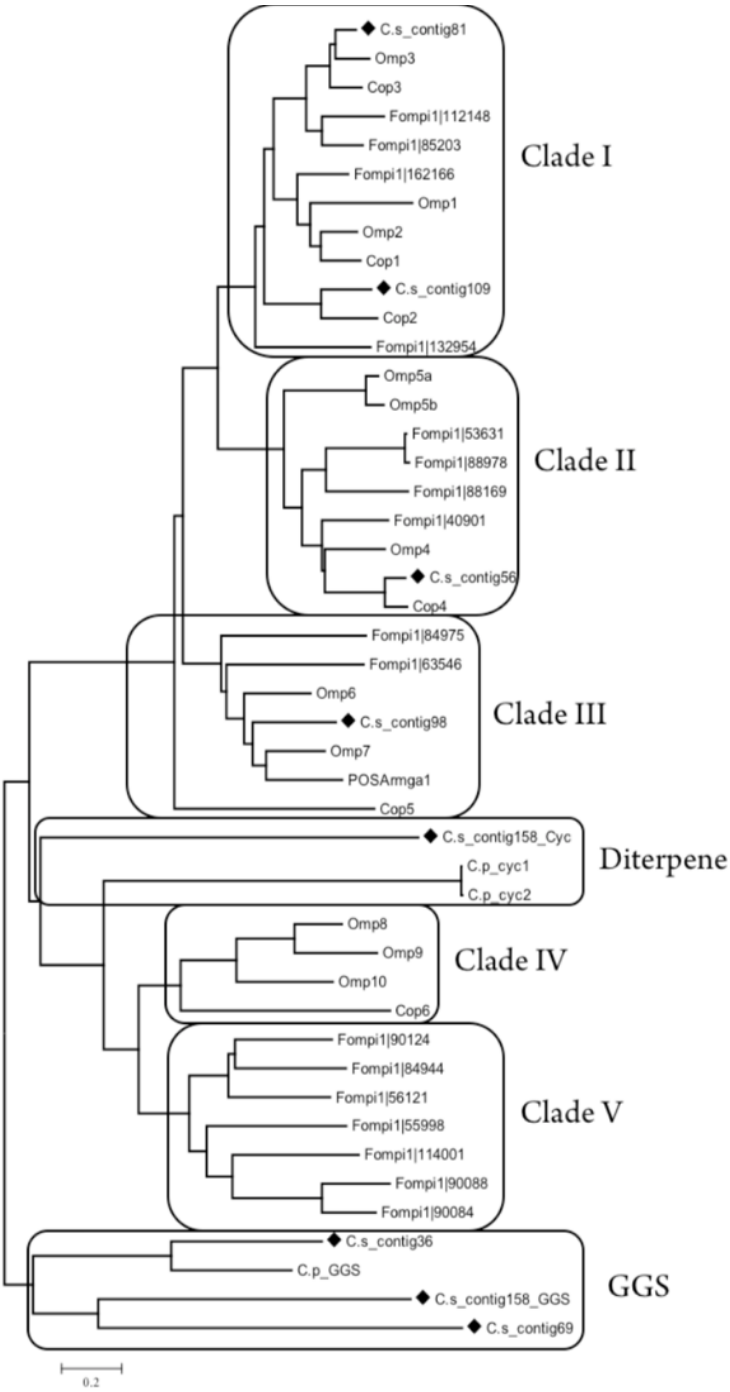
S16 Figure. HMBC NMR spectrum of strossmayerin (7) in d4- MeOH.



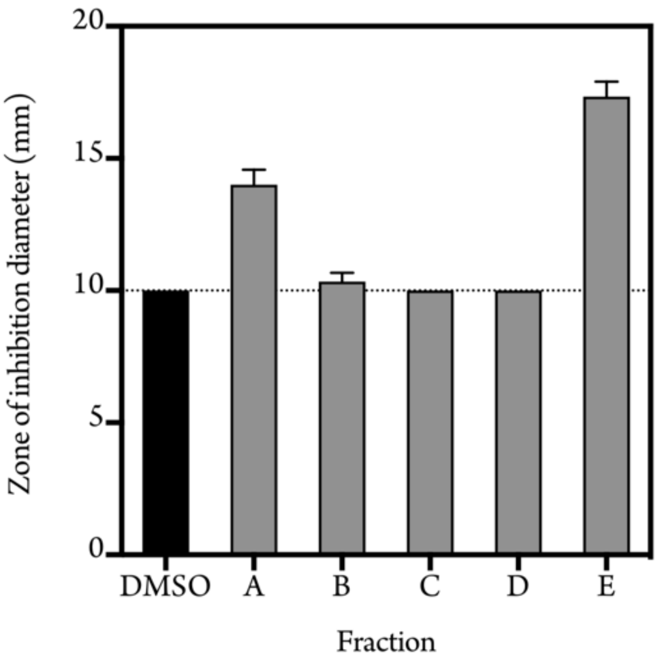
S17 Figure. HSQC NMR spectrum of strossmayerin (7) in d4- MeOH.



S18 Figure. HRMS of D (2). Top panel: measured spectrum, bottom pannel: simulated spectrum.



S19 Figure. Phylogenetic analysis of terpenoid cyclase homologues from *Coprinopsis strossmayeri* (C.s). Genes from *C. strossmayeri* have been compared to characterised sesquiterpene synthases from *Coprinopsis cinerea* (Cop), *Omphalotus olearius* (Omp), *Armillaria gallica* (Armga), and *Fomitopsis pinicola* (Fompi1) [32], and the GGS and diterpene cyclase sequences from *Clitopilus passeckerianus* (C.p). Sesquiterpene clades are indicated in the unrooted neighbor-joining phylogram and accession numbers, contigs, or gene references are indicated aside species abbreviations. The evolutionary history was inferred using the Neighbor-joining method [20]. The optimal tree with the sum of branch length = 20.43 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site [33]. The analysis involved 46 amino acid sequences. All positions containing gaps and missing data were eliminated. There was a total of 210 positions in the final dataset. Evolutionary analyses were conducted in MEGA 5.0 [21].



S20 Figure. Antimicrobial activity of fractions A-E against *Bacillus subtilis*. Fractions A-E were tested at 1 mg/mL for antimicrobial activity against *B. subtilis* ATCC 6633, resulting zones of inhibition were compared to the DMSO solvent control. *P* values were generated by ANOVA using the Dunnett's Test for multiple comparisons to one control. Data show the mean ± SEM (n = 3). This revealed significant inhibition in fractions A and E (both *P* <0.0001).