

Supplementary Data

Investigating the psychedelic hypothesis of *kykeon*, the sacred elixir of the Eleusinian Mysteries

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Supplementary Note 1: Ergot Alkaloids – Structure, Stereochemistry, and Classification.

General Description

Over 80 ergot alkaloids (EAs) have been isolated from diverse natural sources, primarily from members of the genus *Claviceps* infecting higher plants, but also from other fungal taxa occurring as symbionts or endophytes of their hosts¹⁻⁴. EAs are a structurally diverse class of alkaloids biosynthetically derived from tryptophan (Trp) and dimethylallyl pyrophosphate (DMAPP)⁵, along with their semi-synthetic derivatives and synthetic analogues. All share the ergoline ring system as their core structure⁶.

Stereochemistry

- Most EAs are methylated at N-6 and substituted at C-8^{7,8}
- They commonly feature a double bond at either C8–C9 ($\Delta^{8,9}$ -ergolenes) or C9–C10 ($\Delta^{9,10}$ -ergolenes)⁶.
- Stereocenters occur at C-5 and C-8 (or C-10), allowing up to four stereoisomers per EA⁶.
- In naturally occurring EAs, the C-5 stereocenter is invariably (*R*) configuration⁸.
- At C-8, (*R*)-epimers (i.e., (+)-*d*-lysergic acid derivatives) are designated with the suffix “-ine” and they typically exhibit high pharmacological activity at adrenergic, dopaminergic, and serotonergic receptors⁹⁻¹¹.
- The usually inactive (*S*)-epimers carry the suffix “-inine” or the prefix “iso-”¹⁰.
- C-8 epimers are interconvertible and prone to epimerization under heat, light, or extreme pH⁷.
- Historical terms like α and β epimers refer to the same C-8 stereochemistry but are inconsistent with modern nomenclature¹¹.

Structural Classes of EAs^{4,7,8}

EAs are categorized into four main biosynthetically related groups:

1. Clavines

- Ergoline structure or tricyclic precursors of ergoline with an open D ring.
- Do not yield lysergic acid upon hydrolysis.
- Subdivided into tetracyclic, tricyclic, or rearranged subclasses.

2. Lysergamides (Ergoamides)

- Simple lysergic acid derivatives (primary or secondary amides).
- Include compounds such as LSA and LSD analogues.

3. Ergopeptines

- Lysergic acid linked to three amino acids forming a tricyclic peptide.
- Classified into subgroups based on peptide composition.
- Often referred to as cyclol ergot alkaloids (CEA).

4. Ergopeptams

- Structurally similar to ergopeptines but with a bicyclic peptide.
- Classified into subgroups based on peptide composition.
- Often annotated as lactam ergot alkaloids (LEA).

(Ergopeptines and ergopeptams are collectively called peptidic EAs or ergopeptides)

Supplementary Note 2: Kykeon in Ancient Greek Texts – Variants and Cultural Context

There are many “versions” or variants of the kykeon, described in ancient Greek manuscripts beyond the Eleusinian formulation. Despite their variations, all share a common nucleus: mainly prepared by women with the inclusion of barley, barley groats or barley flour referred to in ancient Greek as *ἄλφι*, *ἄλφιτον*, or *ἄλφιτα*¹², with the resulting mixture acting as a drug, referred to in ancient Greek as *φάρμακον* (from *φέρω* “to bring” and *ἄκος* “cure/remedy”), highlighting kykeon’s dual role as both a therapeutic agent and a potentially intoxicating and ritualistic substance¹³. Such examples in ancient Greek literature, beyond the Eleusinian formulation, are the following:

1. A tonic mixed restorative potion prepared by Hecamede and given to Nestor and the wounded Machaon, when they left the fray of battle of Troy, referred to in book 11 line 624 (or 638–641 depending on edition) in Homer’s “The Illiad”. The drink includes barley-meal, goat-cheese grated into a wine (Pramnian wine) base.
2. The magic intoxicating mixed potion crafted by the demi-goddess and witch Kirke (Circe) and used to drug Odysseus and transform his companions into swine as referred to in book 10 lines 234–237, 290 and 316-317 of Homer’s “The Odyssey”. Kirke also adds some honey and pours her magic potion into it.
3. The kykeon referred by the ancient Greek poet Hipponax as a drug or remedy for his soul torments¹³.
4. Theophrastus (319 BC) refers to the drink in his “Characters” (IV, 2-3), mentioning a drunk farmer whose thyme-scented breath annoyed the Assembly in the Ecclesia.
5. Its digestive properties are mentioned in Aristophanes’ “Peace” (V. 712) (421 BC), when Hermes suggests it to the hero who had eaten too many dried fruits and nuts.

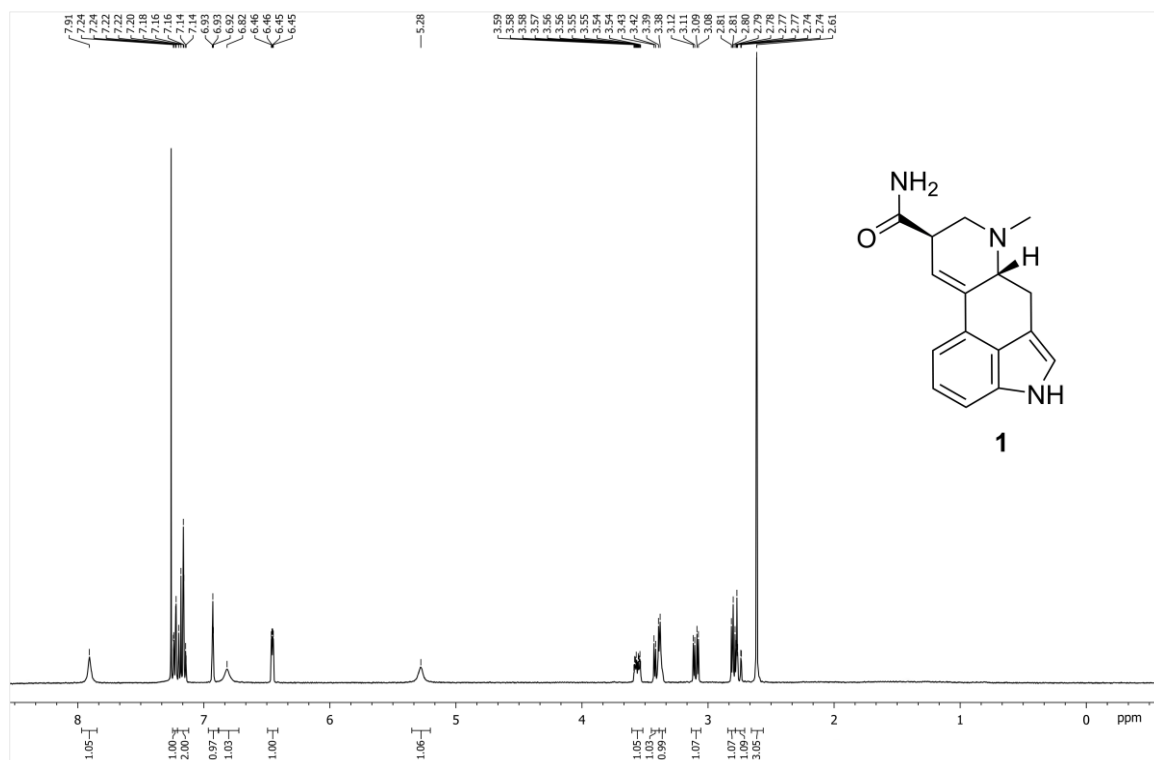


Figure S1. ^1H NMR (400 MHz) spectrum of LSA (compound **1**) in CDCl_3

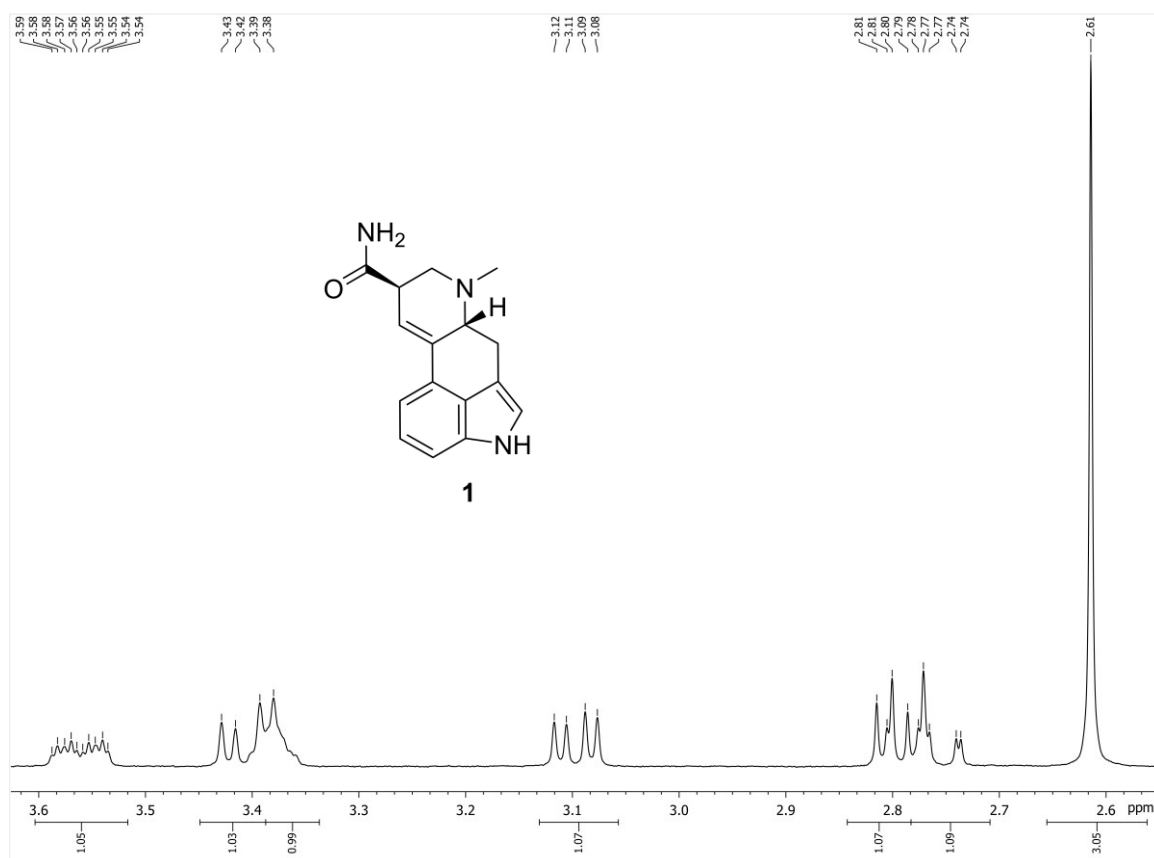


Figure S2. Expanded region of the high-field resonances of rings C and D (δ 2.6–3.6 ppm) of LSA (compound **1**) from Figure S1

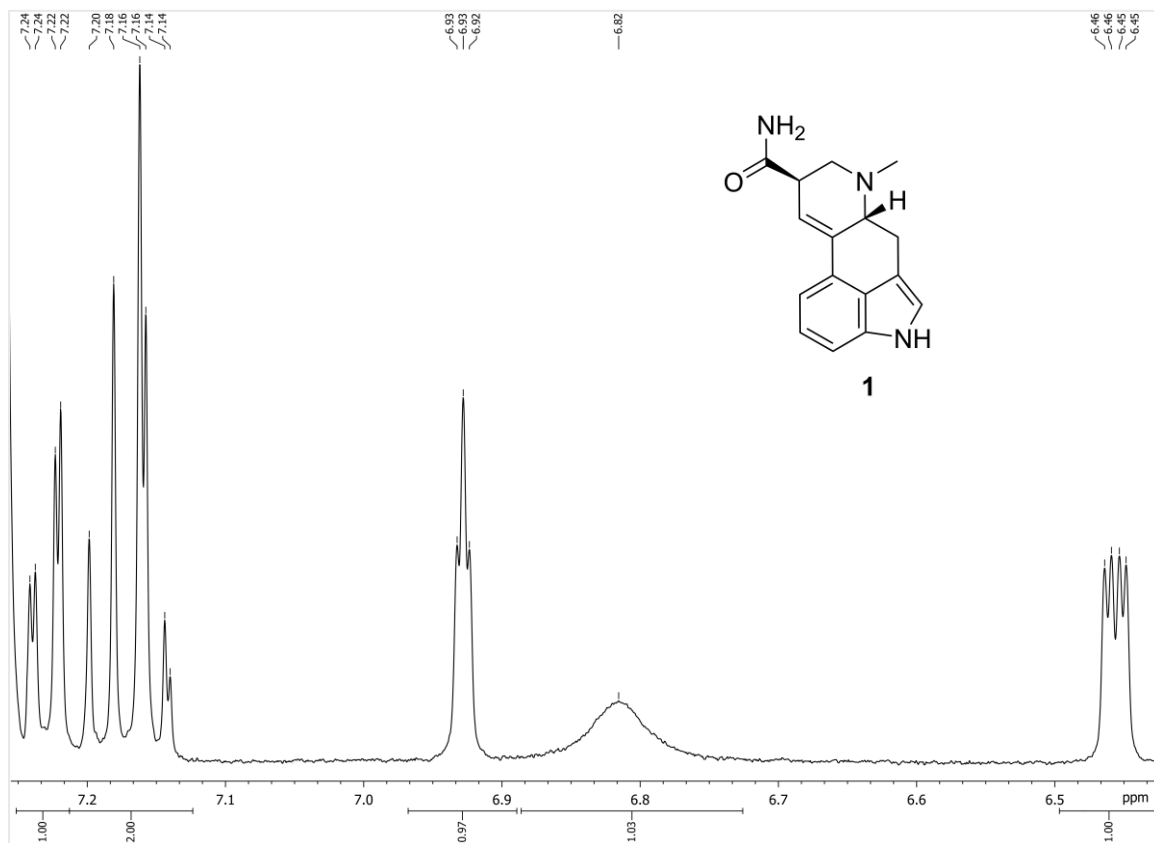


Figure S3. Expanded region of the low-field resonances (δ 6.5–7.3 ppm) of LSA (compound **1**) from Figure S1

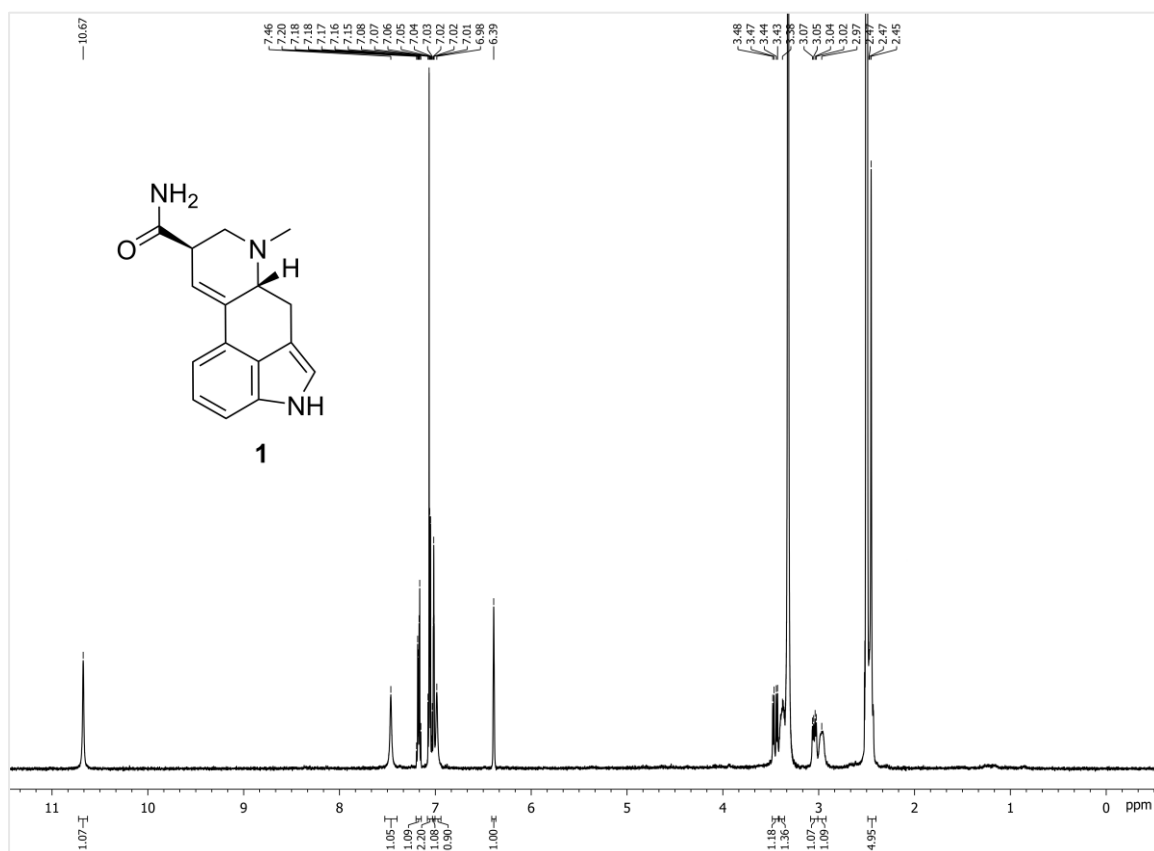


Figure S4. ¹H NMR (400 MHz) spectrum of LSA (compound **1**) in DMSO-d₆

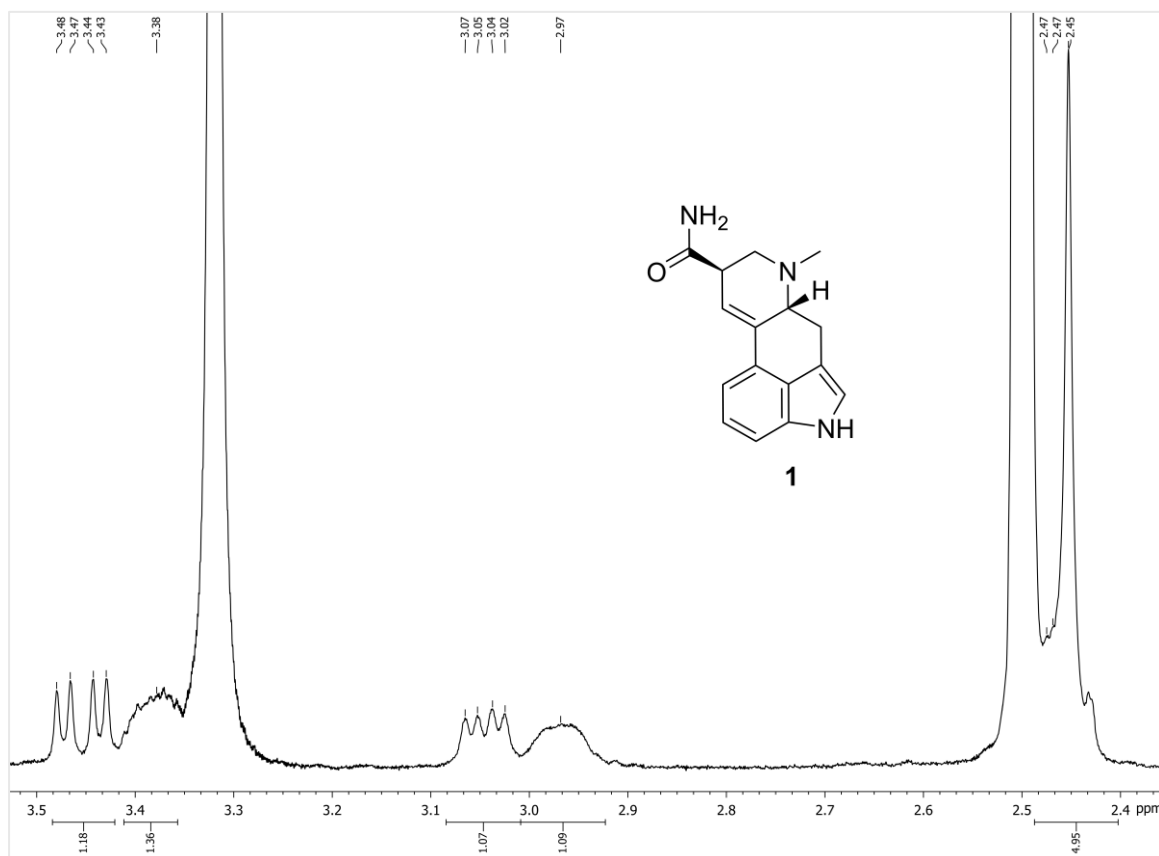


Figure S5. Expanded region of the high-field resonances of rings C and D (δ 2.4–3.5 ppm) of LSA (compound **1**) from Figure S4

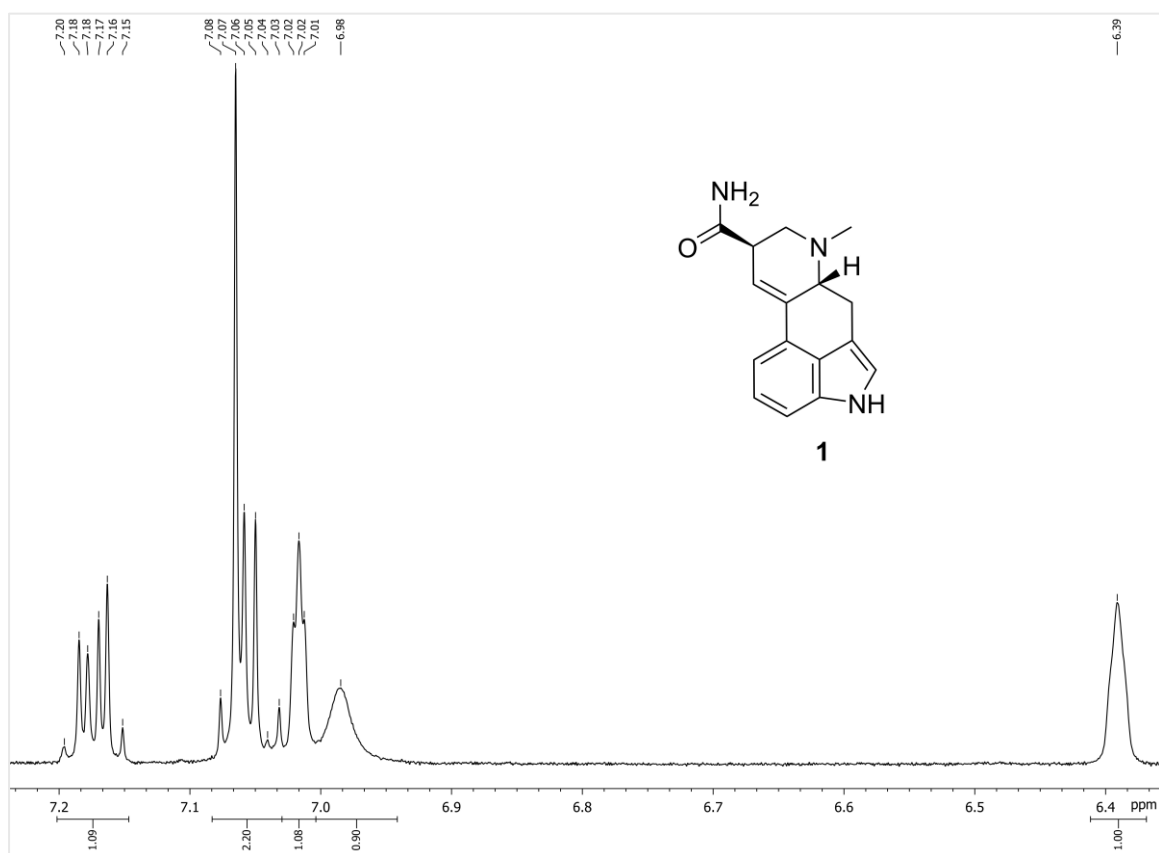


Figure S6. Expanded region of the low-field resonances (δ 6.3–7.2 ppm) of LSA (compound **1**) from Figure S4

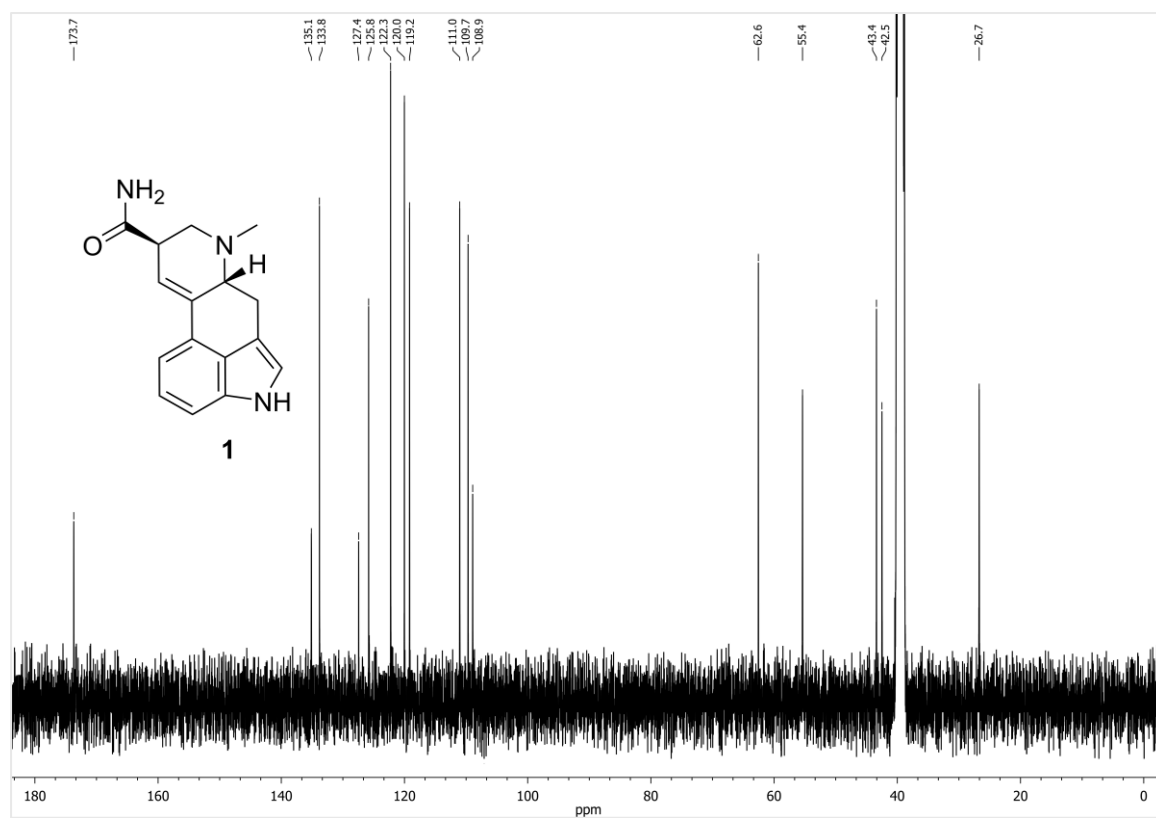


Figure S7. ¹³C NMR (100 MHz) spectrum of LSA (compound **1**) in DMSO-d₆

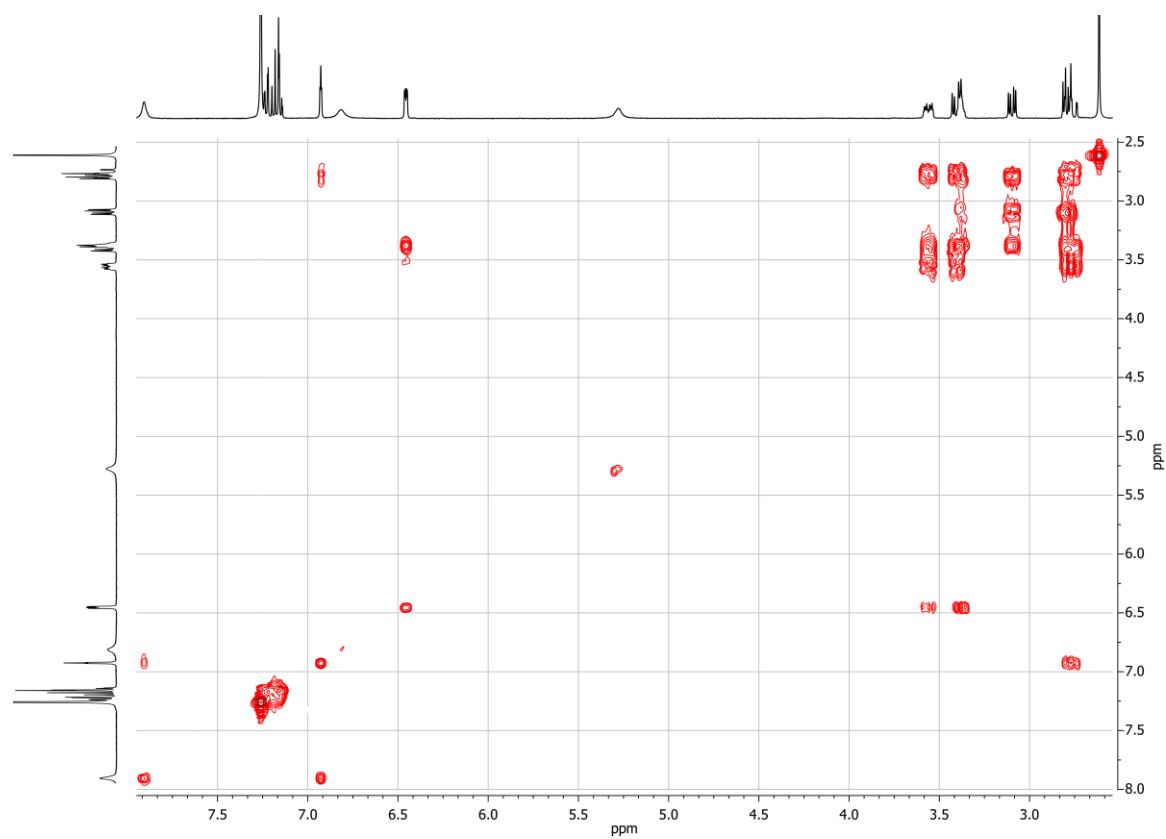


Figure S8. ¹H-¹H COSY spectrum of LSA (compound **1**) in CDCl₃

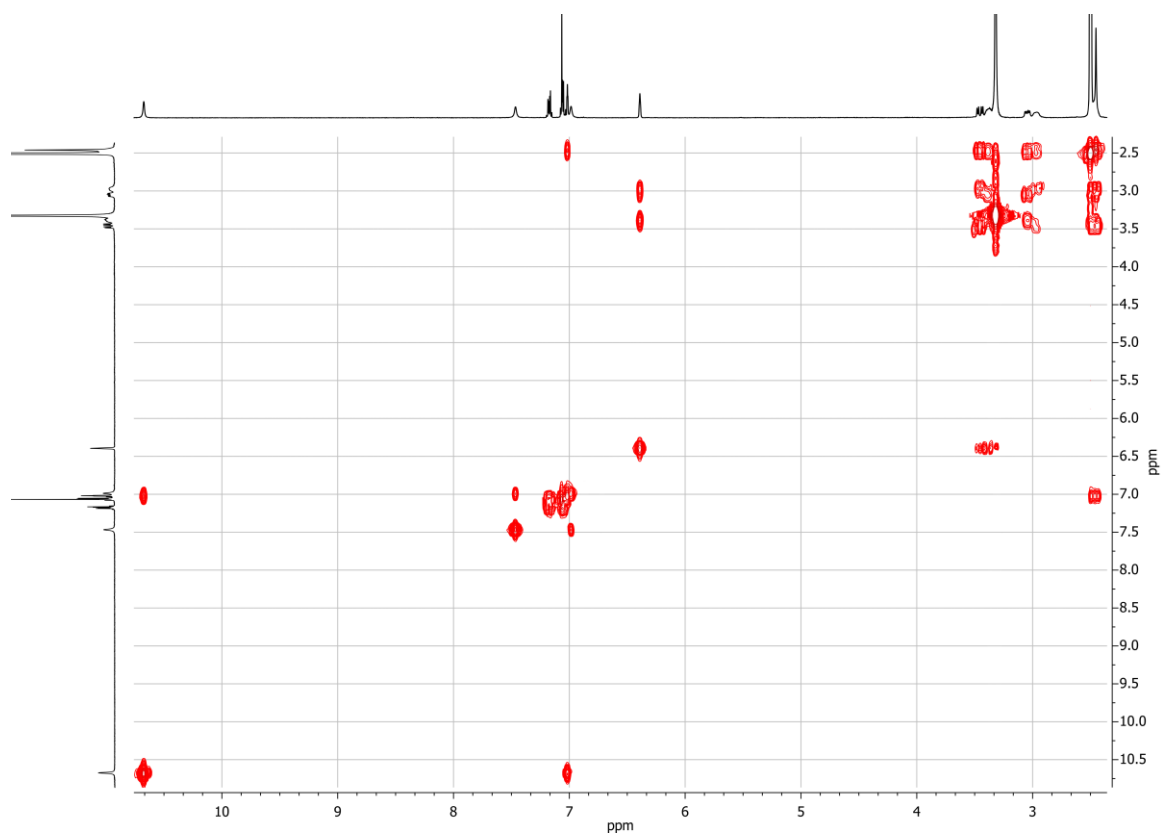


Figure S9. ^1H - ^1H COSY spectrum of LSA (compound **1**) in DMSO-d_6

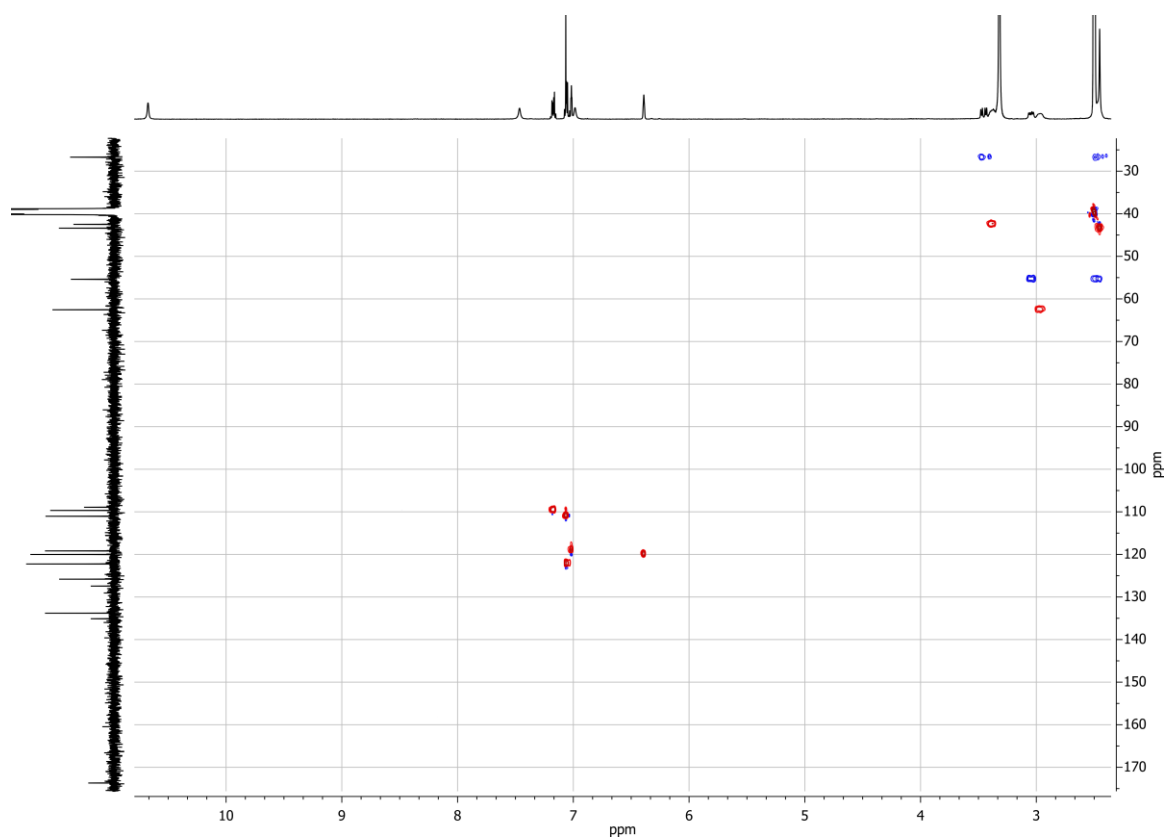


Figure S10. ^1H - ^{13}C HSQC spectrum of LSA (compound **1**) in DMSO-d_6

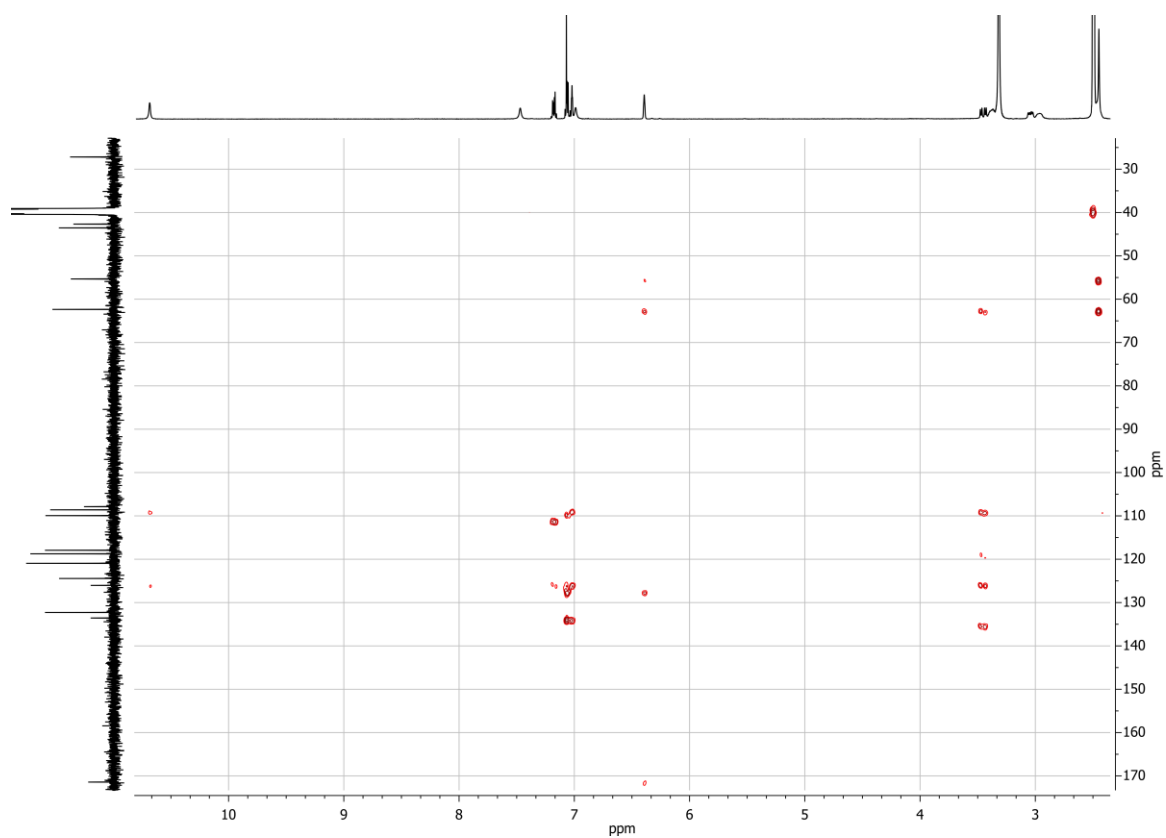


Figure S11. ^1H - ^{13}C HMBC spectrum of LSA (compound **1**) in DMSO-d_6

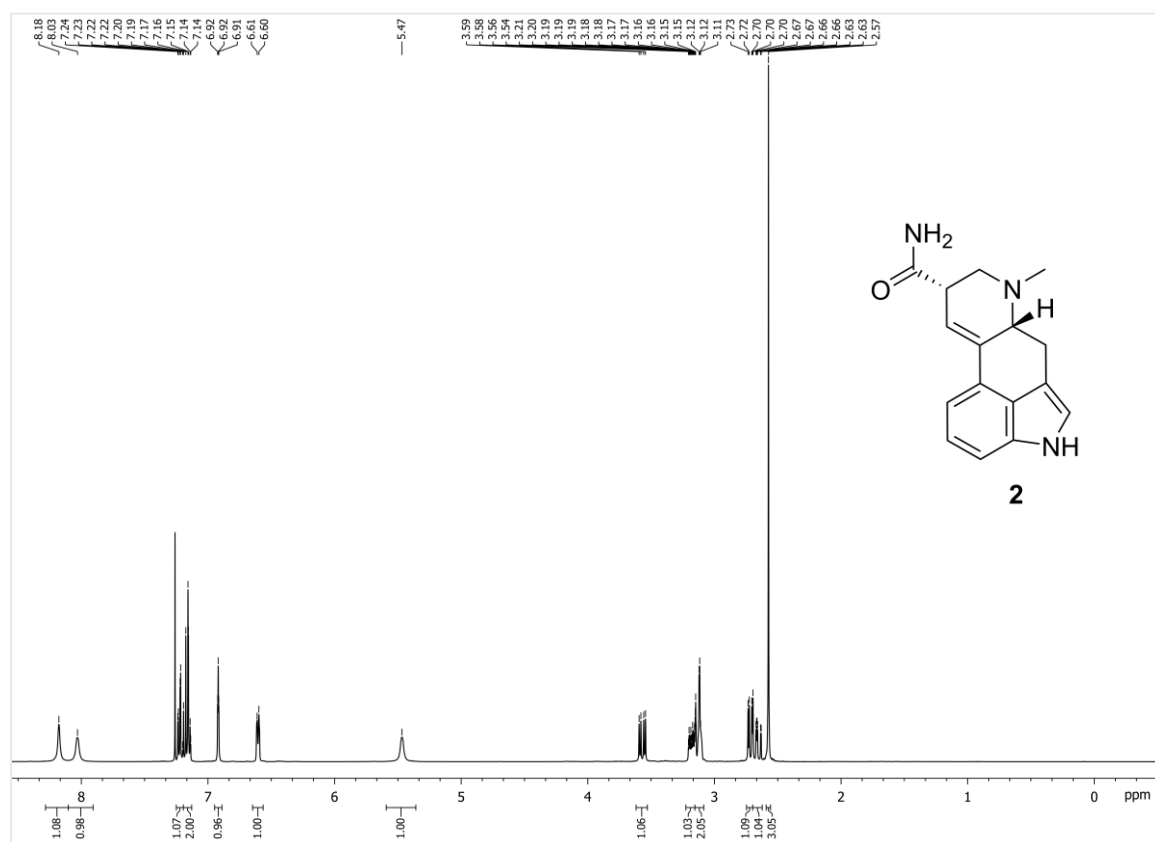


Figure S12. ^1H NMR (400 MHz) spectra of iso-LSA (compound **2**) in CDCl_3

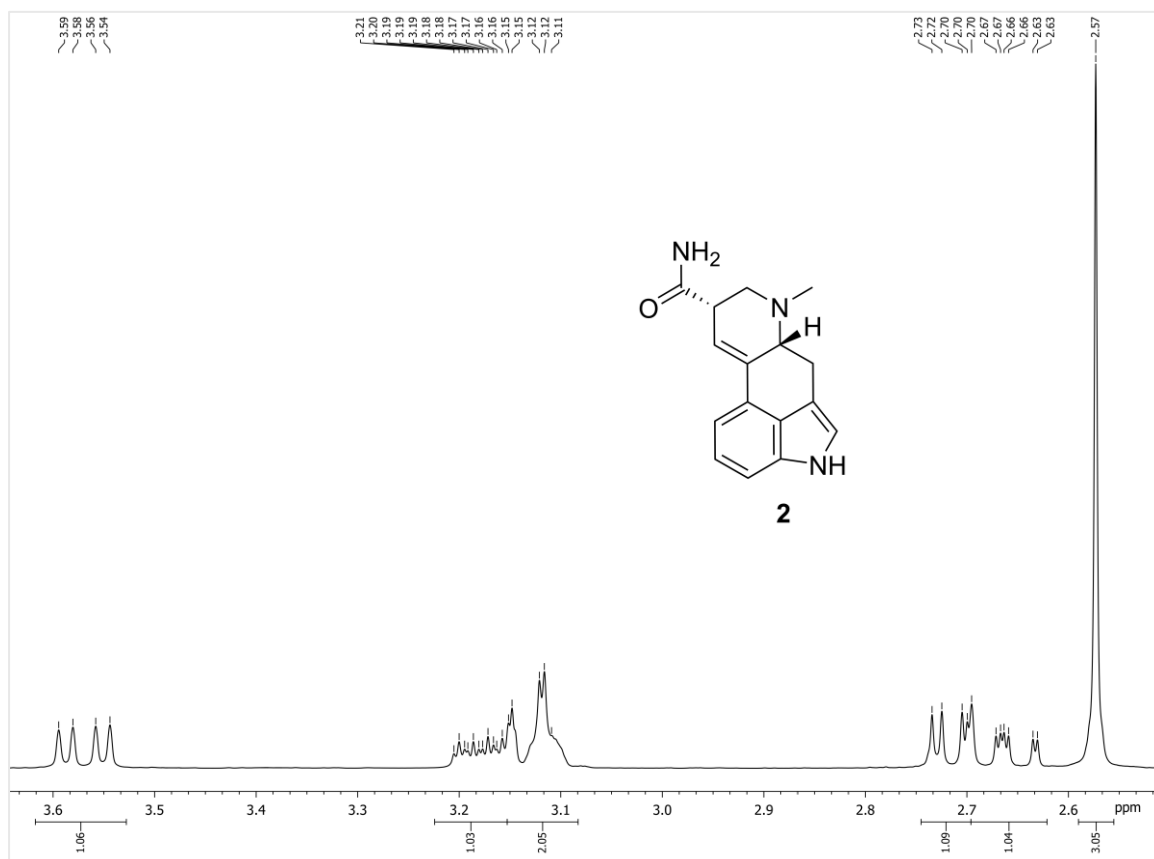


Figure S13. Expanded region of the high-field resonances of rings C and D (δ 2.5–3.6 ppm) of iso-LSA (compound **2**) from Figure S12

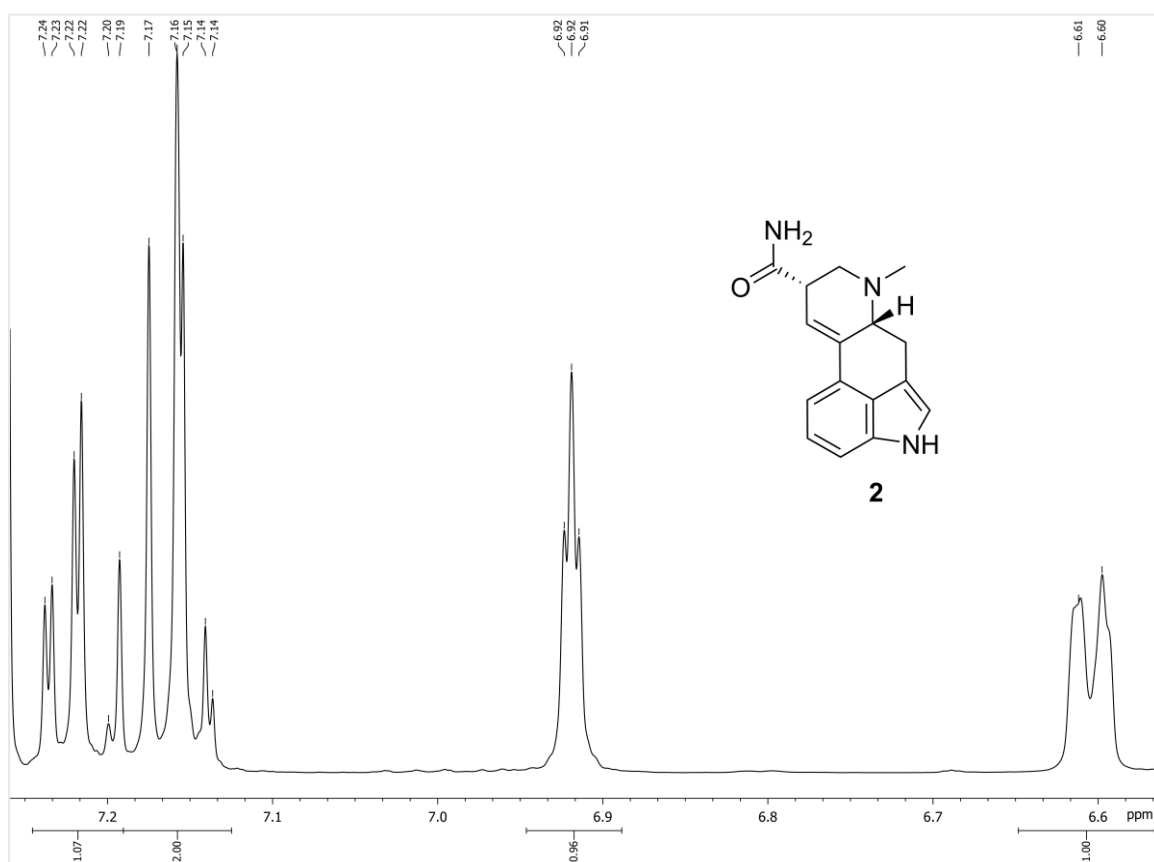


Figure S14. Expanded region of the low-field resonances (δ 6.5–7.3 ppm) of iso-LSA (compound **2**) from Figure S12

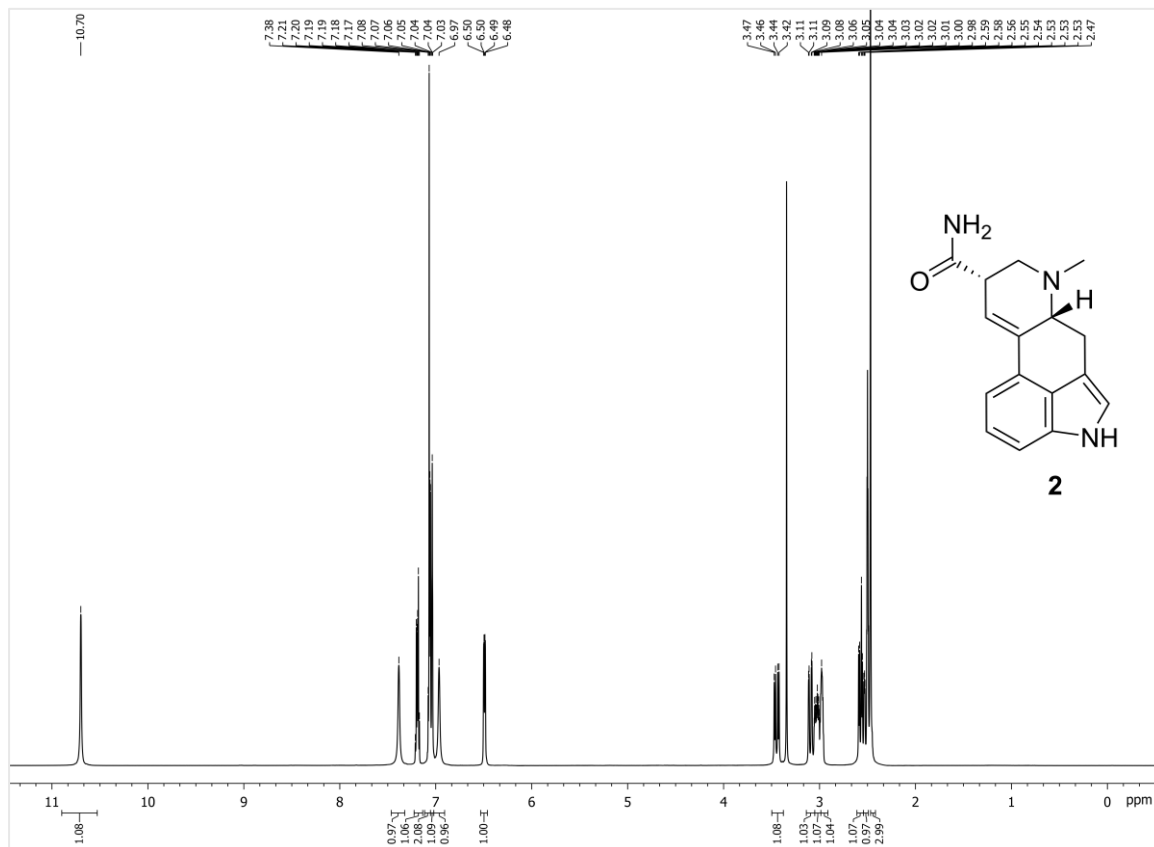


Figure S15. ¹H NMR (400 MHz) spectra of iso-LSA (compound 2) in DMSO-d₆

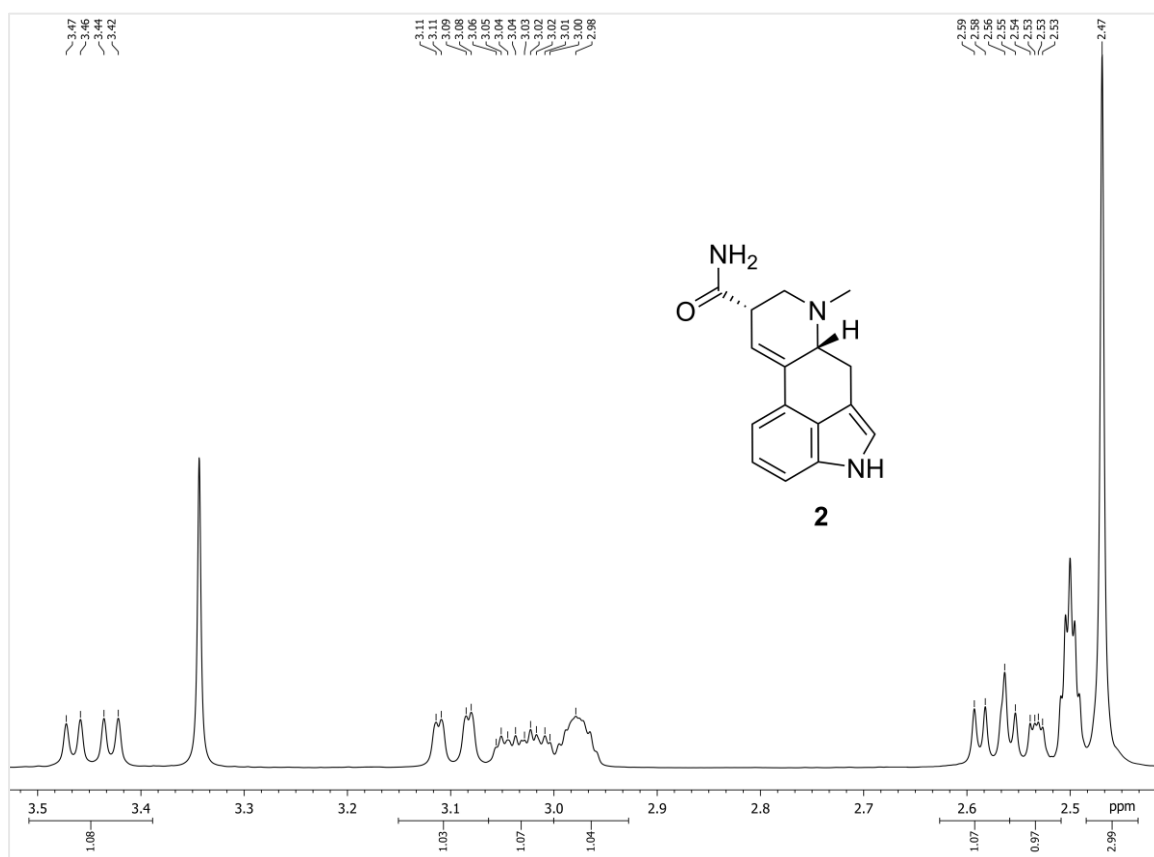


Figure S16. Expanded region of the high-field resonances of rings C and D (δ 2.4–3.5 ppm) of iso-LSA (compound 2) from Figure S15

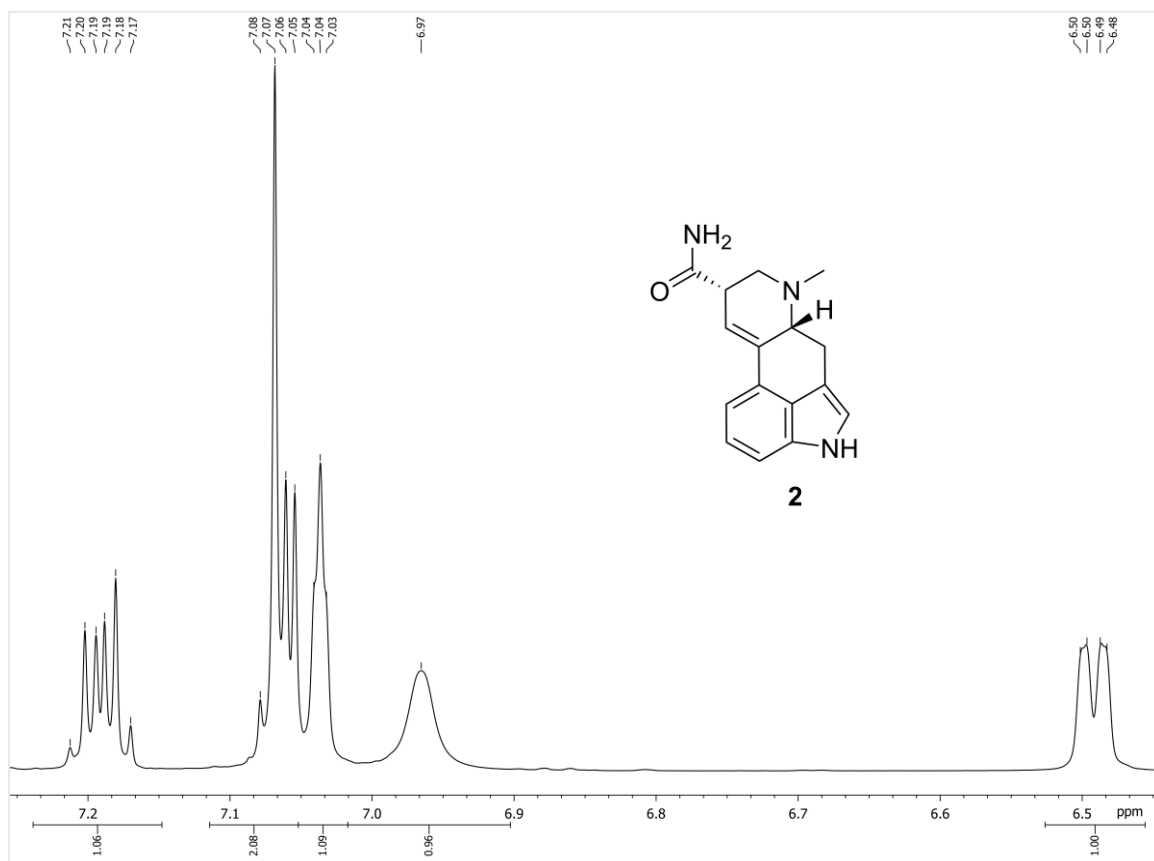


Figure S17. Expanded region of the low-field resonances (δ 6.4–7.3 ppm) of iso-LSA (compound **2**) from Figure S15

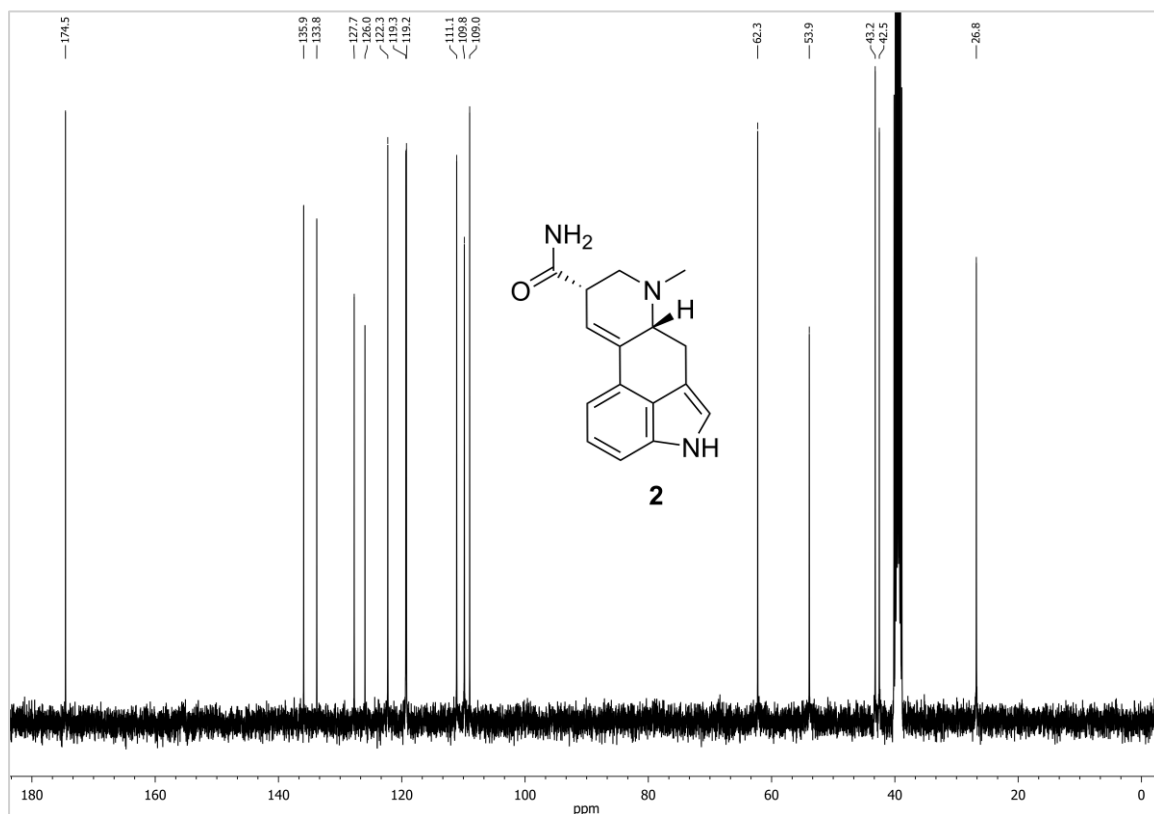


Figure S18. ^{13}C NMR (100 MHz) spectrum of iso-LSA (compound **2**) in DMSO-d_6

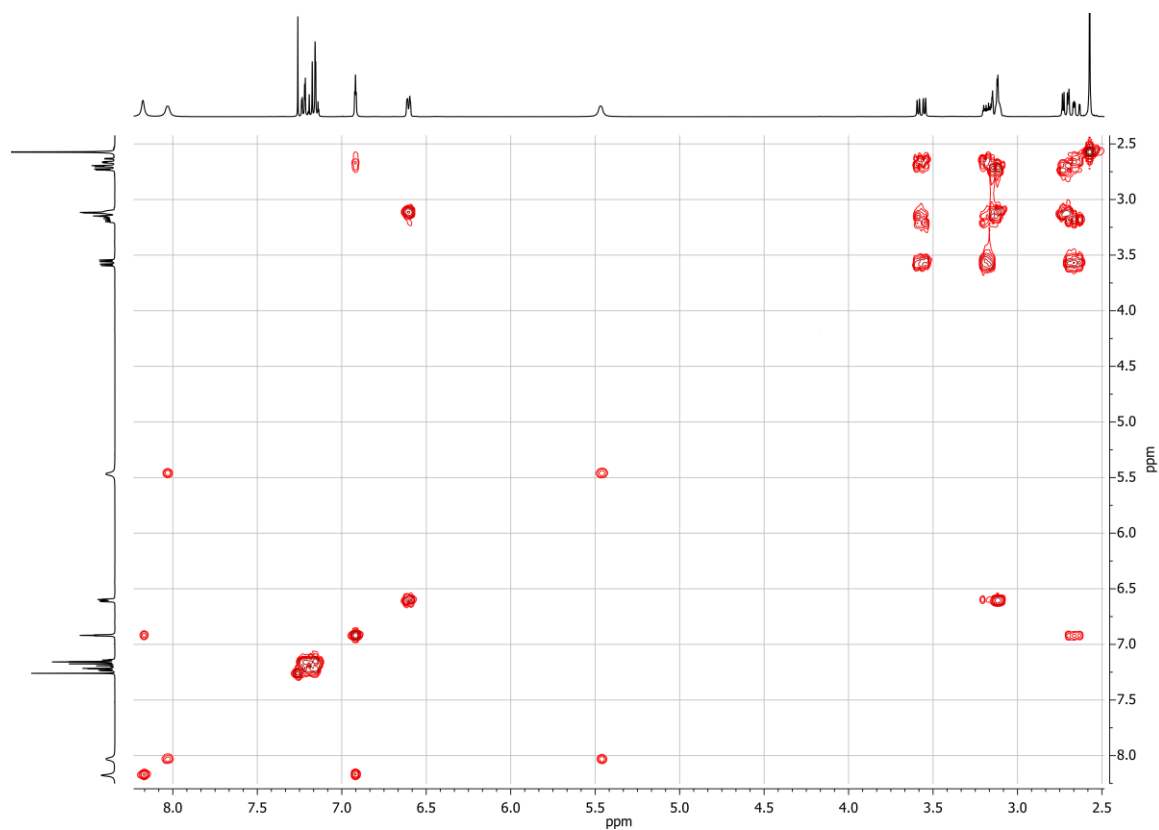


Figure S19. ^1H - ^1H COSY spectrum of iso-LSA (compound **2**) in CDCl_3

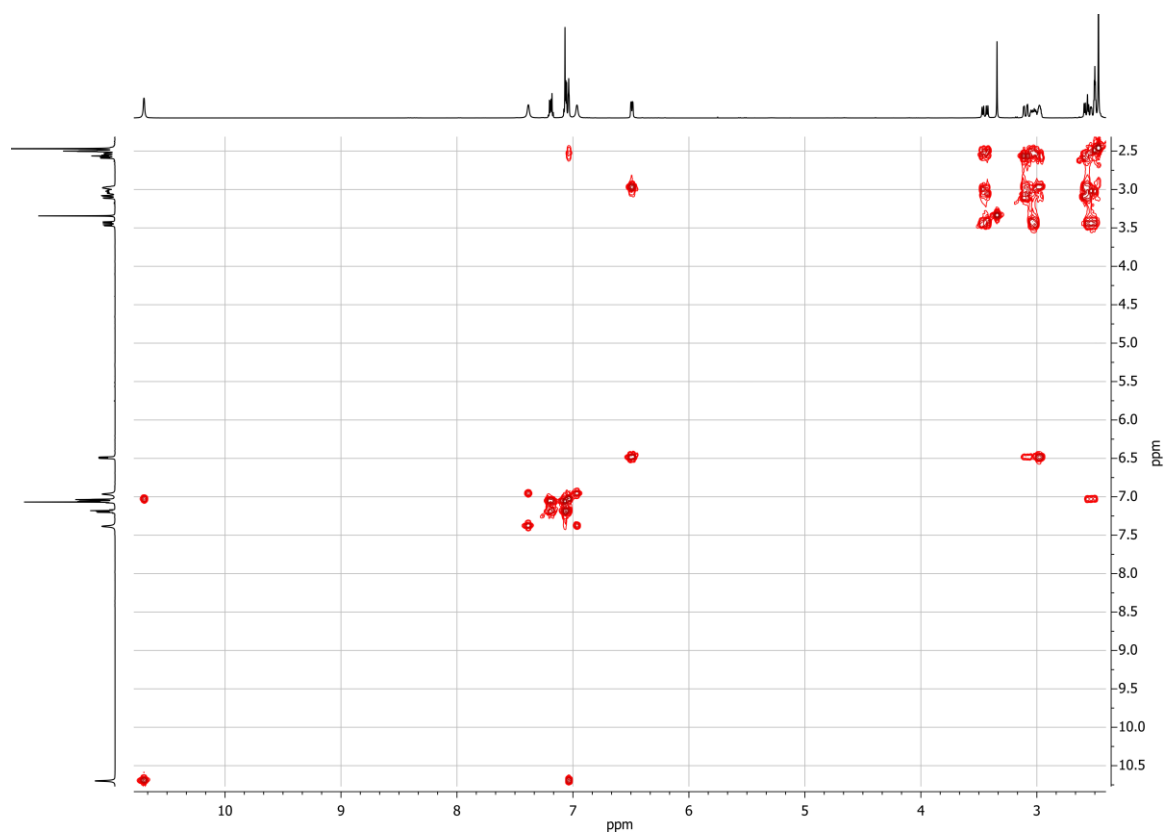


Figure S20. ^1H - ^1H COSY spectrum of iso-LSA (compound **2**) in DMSO-d_6

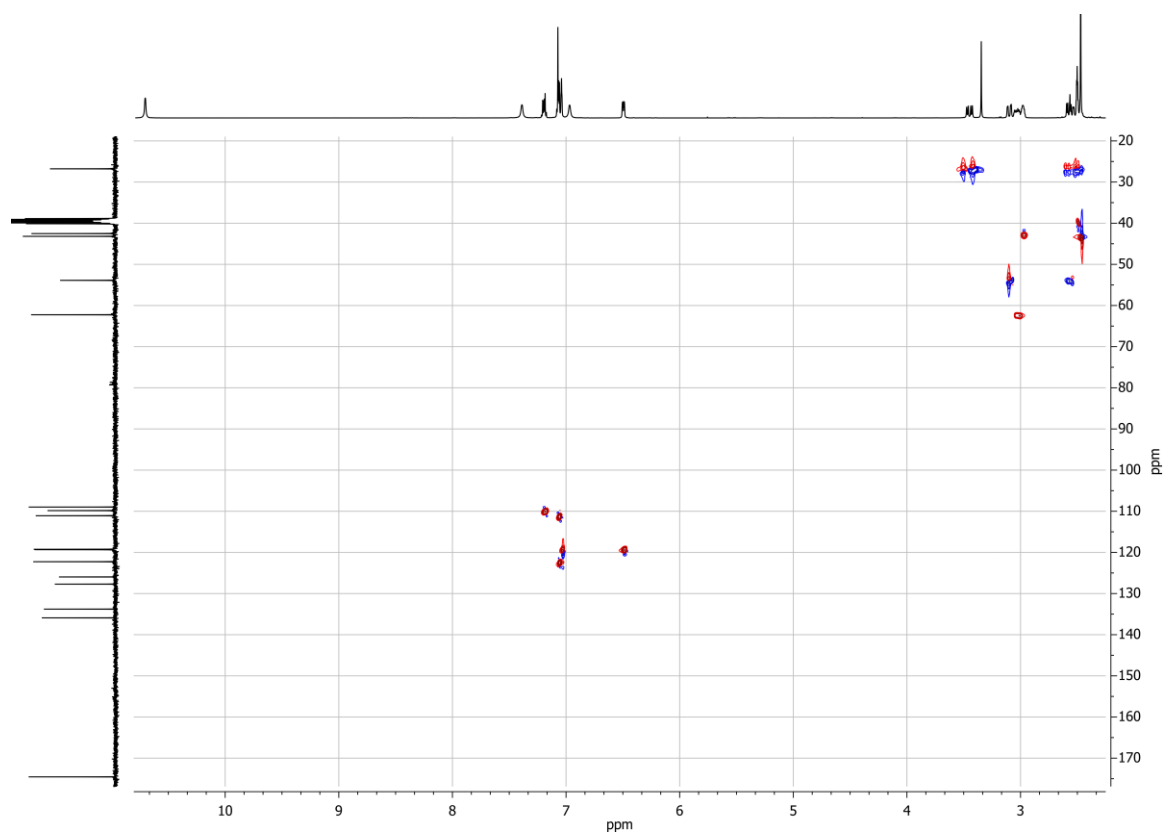


Figure S21. ^1H - ^{13}C HSQC spectrum of iso-LSA (compound **2**) in DMSO- d_6

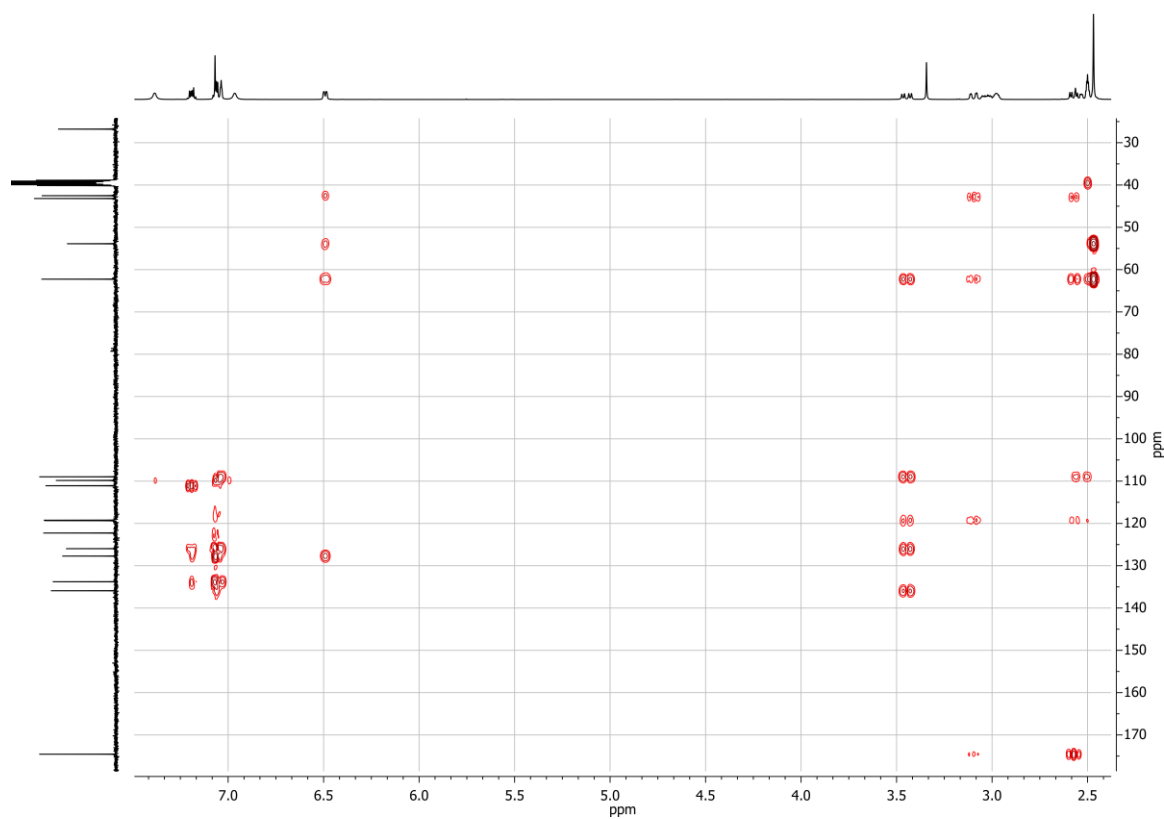


Figure S22. ^1H - ^{13}C HMBC spectrum of iso-LSA (compound **2**) in DMSO- d_6

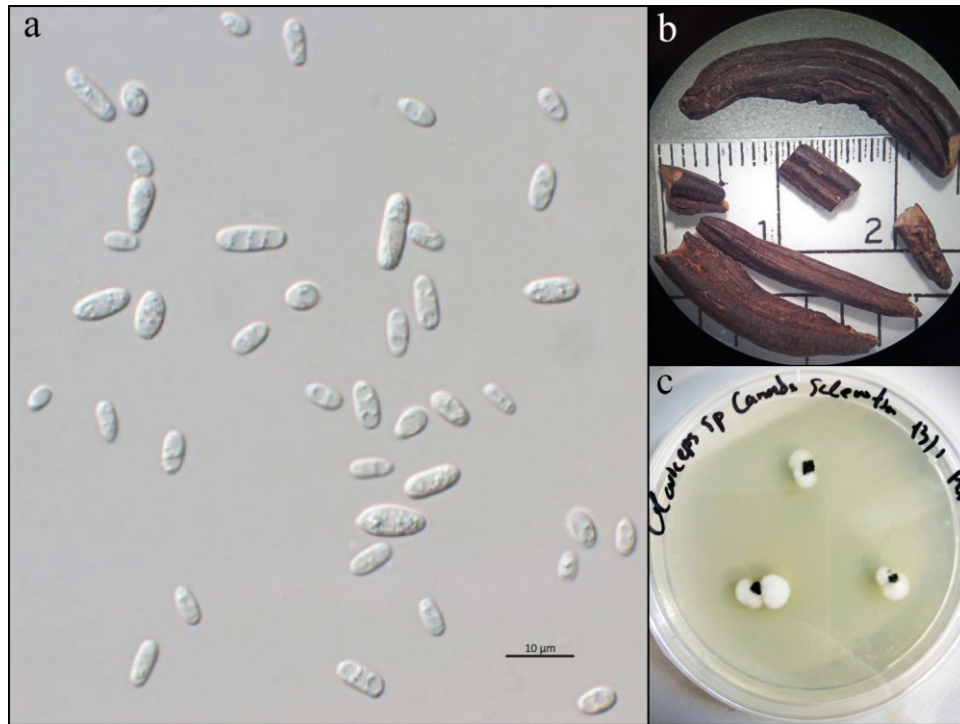
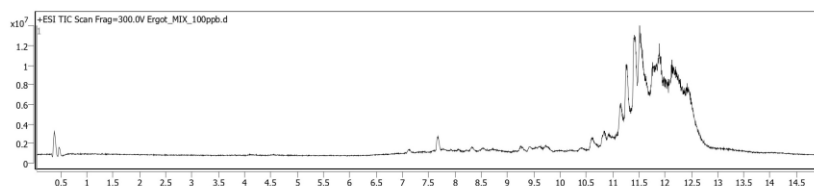


Figure S23. a) Conidia at 400× (DIC; scale bar = 10 μm), b) sclerotia (scale in cm), and c) inoculated sclerotia on PDA in a 9 cm Petri plate after 1 week. All images from strain ATHUM 10382.

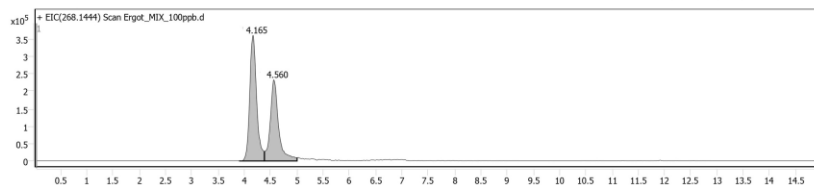
Table S1. Sequence of the ITS rDNA region of ATHUM 10382

rDNA-ITS region (5'→3'; contig length = 738 bases)
GCCCGTCGCTACTACCGATTGAATGGCTCAGTGAGGCGTCCGGACTGGCCCAGAGAGGTGG GCAACTACCACTCAGGGCCGGAAGCTCTCCAAACTCGGTCAATTTAGAGGAAGTAAAAGTC GTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAAC CCACTGTGAACTTATACCCAAAACGTTGCCTCGGCGGGCACAGCGGTACCCGAGCCCCCGC AAGGGAGGCAGAGGCGCCCGCCCGCCAGGGGACCAAACTCTTCTGTATACCCATAGCGGC ATGTCTGAGTGGATTTACAAACAAATGAATCAAACTTTCAACAACGGATCTCTTGTTCTG GCATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCAGTGAATC ATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTTCGAGCGT CATTTCAACCCTCAAGCCCTGCTTGTTGGTGGGGACCGGCTCAGCGGGTGCGGGCTTCGGCC CGCCCCGTGCCGCCCCCGAAATGGATCGGCGGTCTCGTCGCAGCCTTCTTTGCGTAGTAACA TACCACCTCGCAACAGGAGCGCGGCGCGGCCACTGCCGTAAAACGCCCAACTTTTTAGAGTT GACCTCGAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAGAC

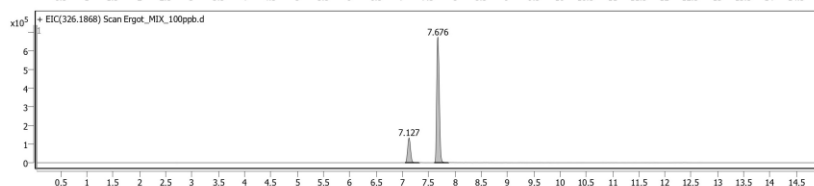
TIC of reference standards
mixed solution, 0.1 $\mu\text{g mL}^{-1}$
each in acetonitrile.
Chromatographic conditions
as described in Section 2.8.



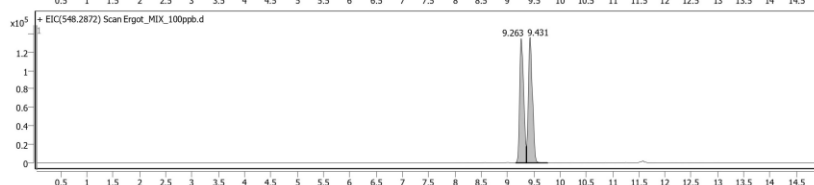
EIC for $[M+H]^+$ m/z 269.1444
Molecular formula: $\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}$
peak a: isoergine (iso-LSA)
peak b: ergine (LSA)



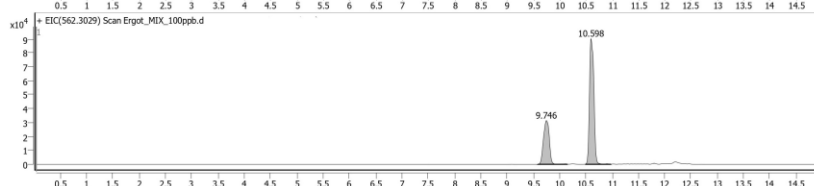
EIC for $[M+H]^+$ m/z 326.1868
Molecular formula: $\text{C}_{19}\text{H}_{23}\text{N}_3\text{O}_2$
peak a: ergometrine (Em)
peak b: ergometrinine (Emn)



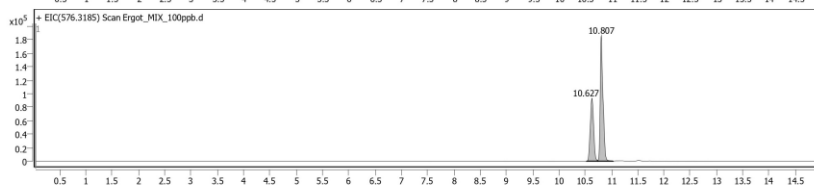
EIC for $[M+H]^+$ m/z 548.2872
Molecular formula: $\text{C}_{30}\text{H}_{37}\text{N}_5\text{O}_5$
peak a: ergosinine (Esn)
peak b: ergosine (Es)



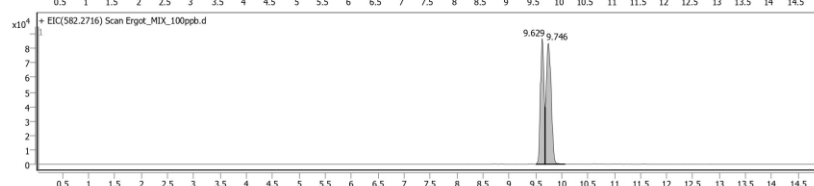
EIC for $[M+H]^+$ m/z 562.3029
Molecular formula: $\text{C}_{31}\text{H}_{39}\text{N}_5\text{O}_5$
peak a: ergocornine (Eco)
peak b: ergocorninine (Econ)



EIC for $[M+H]^+$ m/z 576.3185
Molecular formula: $\text{C}_{32}\text{H}_{41}\text{N}_5\text{O}_5$
peak a: ergokryptine (Ekr)
peak b: ergokryptinine (Ekrn)



EIC for $[M+H]^+$ m/z 582.2716
Molecular formula: $\text{C}_{33}\text{H}_{35}\text{N}_5\text{O}_5$
peak a: ergotaminine (Etn)
peak b: ergotamine (Et)



EIC for $[M+H]^+$ m/z 610.3029
Molecular formula: $\text{C}_{35}\text{H}_{39}\text{N}_5\text{O}_5$
peak a: ergocristine (Ecr)
peak b: ergocristinine (Ecrn)

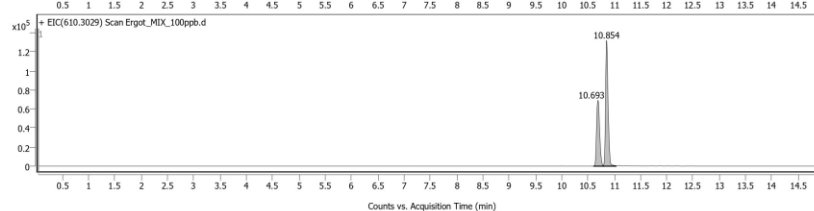


Figure S24. Total ion chromatogram (TIC) and extracted ion chromatograms (EICs) of reference standards.

Table S2. UHPLC/Q-TOF-HRMS quantitation results for LSA and iso-LSA in the extracts and related calculations

Initial lye pH	Ergot concentration in lye (% w/v)	Reaction time (min)	Dry extract yield of 10 mL aliquot (g)	LSA concentration in extract (mg g ⁻¹)	Total LSA in 10 mL aliquot (mg)	Produced LSA (mg g ⁻¹ of ergot used)	iso-LSA concentration in extract (mg g ⁻¹)	Total iso-LSA in 10 mL aliquot (mg)	Produced iso-LSA (mg g ⁻¹ of ergot used)
7	5	15	0.08	0.38	0.03	0.06	0.42	0.03	0.07
7	5	30	0.10	0.44	0.04	0.09	0.52	0.05	0.11
7	5	60	0.14	0.67	0.09	0.18	0.82	0.11	0.22
7	5	120	0.21	0.27	0.06	0.11	0.39	0.08	0.16
7	10	15	0.19	0.11	0.02	0.02	0.12	0.02	0.02
7	10	30	0.23	0.16	0.04	0.04	0.23	0.05	0.05
7	10	60	0.24	0.26	0.06	0.06	0.33	0.08	0.08
7	10	120	0.29	0.36	0.11	0.11	0.46	0.13	0.13
7	20	15	0.47	0.07	0.03	0.02	0.08	0.04	0.02
7	20	30	0.53	0.08	0.04	0.02	0.13	0.07	0.03
7	20	60	0.55	0.19	0.11	0.05	0.20	0.11	0.05
7	20	120	0.68	0.24	0.16	0.08	0.28	0.19	0.10
10.5	5	15	0.09	0.78	0.07	0.15	0.61	0.06	0.11
10.5	5	30	0.10	1.18	0.12	0.24	0.97	0.10	0.19
10.5	5	60	0.10	1.67	0.18	0.35	1.40	0.15	0.29
10.5	5	120	0.12	1.12	0.14	0.27	0.82	0.10	0.20
10.5	10	15	0.20	0.32	0.07	0.07	0.27	0.05	0.05
10.5	10	30	0.21	0.40	0.08	0.08	0.27	0.06	0.06
10.5	10	60	0.23	0.71	0.16	0.16	0.55	0.13	0.13
10.5	10	120	0.28	0.46	0.13	0.13	0.37	0.10	0.10
10.5	20	15	0.38	0.10	0.04	0.02	0.11	0.04	0.02
10.5	20	30	0.52	0.11	0.06	0.03	0.11	0.06	0.03
10.5	20	60	0.54	0.21	0.11	0.06	0.20	0.11	0.05
10.5	20	120	0.59	0.36	0.21	0.10	0.39	0.23	0.11
11.5	5	15	0.09	0.63	0.06	0.11	0.54	0.05	0.10
11.5	5	30	0.11	0.33	0.04	0.07	0.29	0.03	0.07
11.5	5	60	0.12	0.41	0.05	0.10	0.44	0.05	0.10
11.5	5	120	0.13	0.47	0.06	0.12	0.27	0.04	0.07
11.5	10	15	0.24	0.44	0.11	0.11	0.51	0.13	0.13
11.5	10	30	0.26	0.86	0.22	0.22	0.88	0.22	0.22
11.5	10	60	0.27	1.10	0.29	0.29	1.40	0.38	0.38
11.5	10	120	0.27	1.19	0.32	0.32	1.06	0.29	0.29
11.5	20	15	0.49	0.60	0.29	0.15	0.66	0.32	0.16
11.5	20	30	0.58	0.73	0.42	0.21	0.86	0.49	0.25
11.5	20	60	0.60	0.93	0.56	0.28	1.17	0.70	0.35
11.5	20	120	0.63	1.54	0.96	0.48	1.75	1.10	0.55
12.5	5	15	0.09	4.21	0.38	0.77	4.93	0.45	0.90
12.5	5	30	0.12	3.32	0.38	0.77	2.91	0.34	0.67
12.5	5	60	0.14	2.88	0.39	0.78	3.66	0.50	1.00
12.5	5	120	0.14	1.89	0.27	0.54	1.69	0.24	0.48
12.5	10	15	0.29	1.98	0.57	0.57	2.39	0.68	0.68
12.5	10	30	0.28	2.70	0.76	0.76	3.95	1.12	1.12
12.5	10	60	0.24	3.37	0.81	0.81	3.02	0.72	0.72
12.5	10	120	0.29	2.69	0.77	0.77	2.00	0.57	0.57
12.5	20	15	0.48	2.14	1.02	0.51	2.24	1.07	0.54
12.5	20	30	0.51	1.79	0.91	0.45	2.09	1.06	0.53
12.5	20	60	0.51	2.17	1.11	0.55	2.05	1.05	0.52
12.5	20	120	0.52	2.22	1.15	0.58	2.07	1.08	0.54

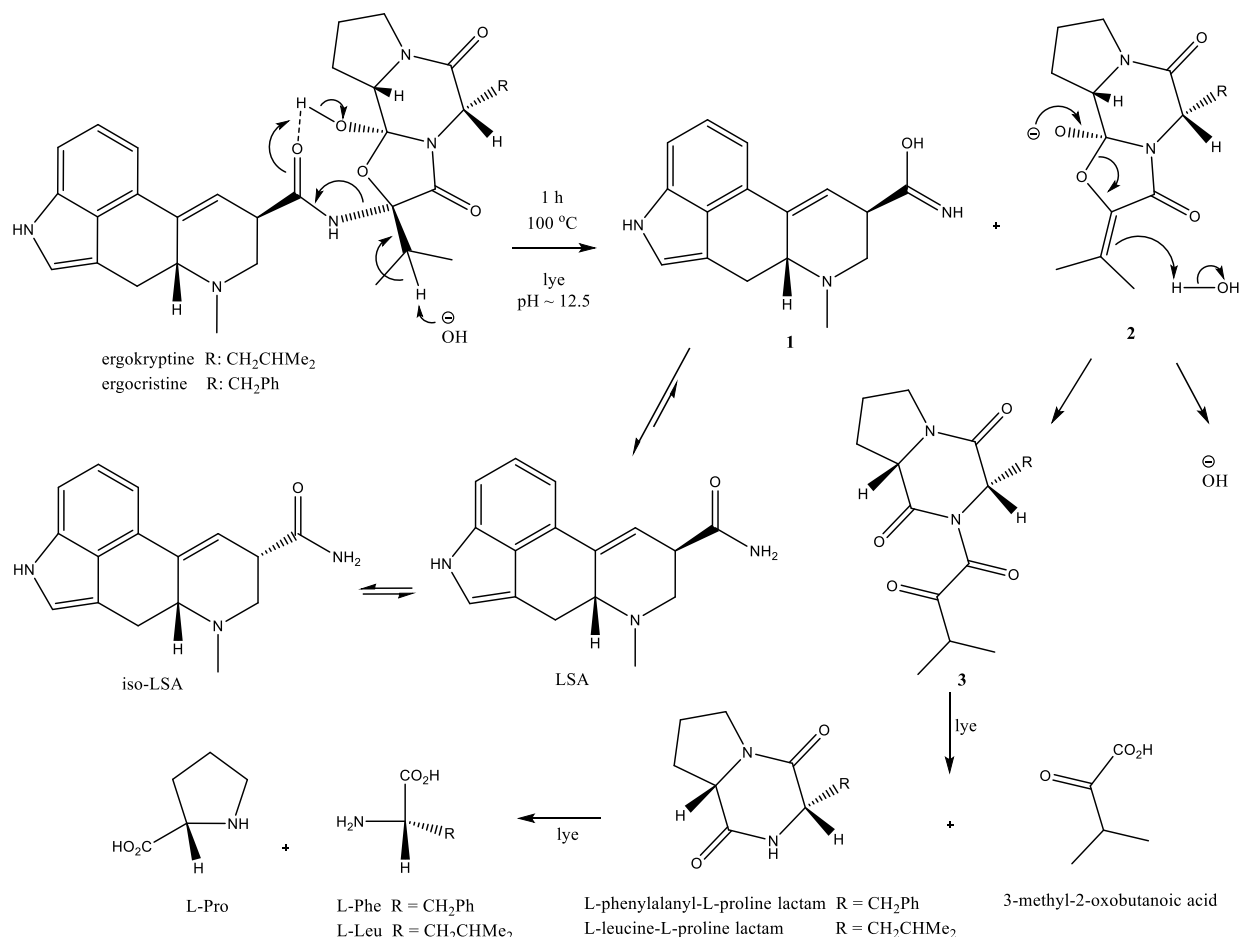


Figure S25. Proposed mechanism of the chemical transformation of the ergopeptides ergokryptine and ergocristine into LSA/iso-LSA and non-toxic secondary by-products. The lye solution hydroxide removes a proton from the isopropyl group, thereby initiating a cascade of reactions, probably more or less simultaneous, involving the intramolecular hydrogen bond and leading to the enol tautomer of LSA/iso-LSA (**1**), which rapidly rearranges to LSA and iso-LSA. The other product of this cleavage (**2**) is a reactive intermediate, which rapidly forms a stable compound (**3**) by extracting a proton from the solvent to regenerate a hydroxide ion. This stable compound should itself be easily hydrolyzed in mild base to produce 3-methyl-2-oxobutanoic acid and lactams, harmless dipeptides, which can further hydrolyze to essential amino acids. The same reactions would apply to C-8 epimers and ergopeptames (adapted and based on literature^{5,14,15})

Table S3. Results of effect tests and parameter estimates, together with their levels of significance, derived from the model equations employed for LSA.

Equation 1				Equation 2				Equation 3			
LSA	Effect Tests	Parameter Estimates			Effect Tests	Parameter Estimates			Effect Tests	Parameter Estimates	
Term	Prob > F	Estimate	Prob> t	Source	Prob > F	Estimate	Prob> t	Source	Prob > F	Estimate	Prob> t
Intercept	-	0.0289	0.5813	Intercept		0.2291	0.0001	Intercept		0.0279	0.5891
X ₁	0.0001	0.2703	0.0001	X ₁	0.0001	0.2197	0.0001	X ₁	0.0001	0.2711	0.0001
X ₂	0.2462	-0.0261	0.2462	X ₂	0.4546	-0.0192	0.4546	X ₂	0.0908	-0.0350	0.0908
X ₃	0.0395	0.0495	0.0395	X ₃	0.2425	0.0304	0.2425	X ₃	0.0431	0.0454	0.0431
X ₁ ²	0.0001	0.3693	0.0001	X ₁ *X ₂	0.8909	-0.0020	0.8909	X ₁ ²	0.0001	0.3693	0.0001
X ₂ ²	0.7599	-0.0125	0.7599	X ₁ *X ₃	0.9902	-0.0438	0.9902	X ₂ ²	0.7564	-0.0125	0.7564
X ₃ ²	0.1756	-0.0575	0.1756	X ₂ *X ₃	0.4213	0.0467	0.4213	X ₃ ²	0.1692	-0.0575	0.1692
X ₁ *X ₂	0.8203	-0.0061	0.8203								
X ₁ *X ₃	0.9838	-0.0006	0.9838								
X ₂ *X ₃	0.1863	0.0354	0.1863								

X1 : lye pH, X2 : ergot powder concentration in the lye, and X3 : reaction time

Table S4. Results of effect tests and parameter estimates, together with their levels of significance, derived from the model equations employed for iso-LSA.

iso-LSA				Effect Tests				Parameter Estimates			
Term	Prob > F	Estimate	Prob> t	Source	Prob > F	Estimate	Prob> t	Source	Prob > F	Estimate	Prob> t
Intercept	-	0.0191	0.7700	Intercept		0.2291	0.0001	Intercept		0.0158	0.8096
X ₁	0.0001	0.2727	0.0001	X ₁	0.0001	0.2197	0.0001	X ₁	0.0001	0.2823	0.0001
X ₂	0.5359	-0.0173	0.5359	X ₂	0.6549	-0.0192	0.6549	X ₂	0.2885	-0.0277	0.2885
X ₃	0.2229	0.0360	0.2229	X ₃	0.5041	0.0304	0.5041	X ₃	0.4570	0.0209	0.4570
X ₁ ²	0.0001	0.4213	0.0001	X ₁ *X ₂	0.9697	-0.0020	0.9697	X ₁ ²	0.0001	0.4213	0.0001
X ₂ ²	0.6919	-0.0203	0.6919	X ₁ *X ₃	0.4456	-0.0438	0.4456	X ₂ ²	0.6934	-0.0203	0.6934
X ₃ ²	0.1577	-0.0751	0.1577	X ₂ *X ₃	0.3699	0.0467	0.3699	X ₃ ²	0.1594	-0.0751	0.1594
X ₁ *X ₂	0.9526	-0.0020	0.9526								
X ₁ *X ₃	0.2350	-0.0438	0.2350								
X ₂ *X ₃	0.1636	0.0467	0.1636								

X1 : lye pH, X2 : ergot powder concentration in the lye, and X3 : reaction time

Table S5. Summary of fit based on the model equations applied for LSA and iso-LSA.

	LSA Summary of Fit			iso-LSA Summary of Fit		
	Equation 1	Equation 2	Equation 3	Equation 1	Equation 2	Equation 3
R ²	0.830032	0.496444	0.821693	0.781616	0.421537	0.761629
R ² Adj	0.789776	0.422752	0.795599	0.729893	0.336884	0.726745
Root Mean Square Error	0.116012	0.192239	0.114394	0.145051	0.227273	0.145894

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