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Return of the lysergamides. Part VII: Analytical and behavioural characterization of 1-valeroyl-*d*-lysergic acid diethylamide (1V-LSD)

Simon D. Brandt,[1,*] Pierce V. Kavanagh,[2] Folker Westphal,[3] Benedikt Pulver,[3] Kathleen Morton,[4] Alexander Stratford,[5] Geraldine Dowling,[2,6] Adam L. Halberstadt [4,7]

[1] *School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK*

[2] *Department of Pharmacology and Therapeutics, School of Medicine, Trinity Centre for Health Sciences, St. James Hospital, Dublin 8, Ireland*

[3] *State Bureau of Criminal Investigation Schleswig-Holstein, Section Narcotics/Toxicology, Mühlenweg 166, D-24116 Kiel, Germany*

[4] *Department of Psychiatry, University of California San Diego, La Jolla, CA 92093-0804, USA*

[5] *Synex Synthetics BV, Karveelweg 20, 6222NH, Maastricht, The Netherlands*

[6] *Department of Life Sciences, School of Science, Sligo Institute of Technology, Ash Lane, Sligo, F91YW50, Ireland*

[7] *Research Service, VA San Diego Healthcare System, San Diego, CA 92161, USA*

* Correspondence to: Simon D. Brandt, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF, UK. E-Mail: s.brandt@ljmu.ac.uk

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Abstract

The psychopharmacological properties of the psychedelic drug lysergic acid diethylamide (LSD) have attracted the interest of several generations of scientists. Whilst further explorations involving novel LSD-type compounds are needed to assess their potential as medicinal drugs, the emergence of novel derivatives as recreational drugs has also been observed. 1-Valeroyl-LSD (also known as 1-valeryl-LSD, 1-pentanoyl-LSD, 1V-LSD, or "Valerie") is a new *N*[1]-acylated LSD derivative that recently appeared on the online market and it could be viewed as a higher homolog of ALD-52, 1P-LSD, and 1B-LSD. The present study included the analytical characterization and involved various methods of mass spectrometry (MS), gas- and liquid chromatography (GC and LC), nuclear magnetic resonance spectroscopy (NMR), GC solid-state infrared (GC-sIR) analysis and Raman spectroscopy. The *in vivo* activity of 1V-LSD was assessed using the mouse head twitch response (HTR), a 5-HT_{2A}-mediated head movement that serves as a behavioral proxy in rodents for human hallucinogenic effects. Similar to LSD and other psychedelic drugs, the HTR induced by 1V-LSD was dose-dependent and the median effective dose for 1V-LSD was 373 nmol/kg, which was about a third of the potency of LSD (ED_{50} = 132.8 nmol/kg). Lysergamides containing the *N*[1]-substituent typically act as weak partial agonists at the 5-HT_{2A} receptor and are believed to serve as prodrugs for LSD. 1V-LSD is also likely to be hydrolyzed to LSD and serve as a prodrug, but studies to assess the biotransformation and receptor pharmacology of 1V-LSD should be performed to fully elucidate its mechanism-of-action.

1. Introduction

The psychopharmacological properties of the psychedelic drug lysergic acid diethylamide (LSD, Figure 1A) have attracted the interest of several generations of scientists. Many studies have focused on the structure-activity relationships of LSD and related lysergamides. One of the positions on the lysergamide scaffold most amenable to chemical modification is the indole nitrogen (position *N*[1]), with 1-acetyl-LSD (1A-LSD, ALD-52) being one of the simplest examples (Figure 1A).[1,2] ALD-52 and LSD produce nearly indistinguishable effects in humans.[3-6] However, *N*[1]-substitution disrupts the interaction of lysergamides with the 5-HT_{2A} receptor (the primary target for psychedelic drugs in the brain[7]) so ALD-52 itself is unlikely to be the active species when it is administered.[8] Since ALD-52 is hydrolyzed to LSD *in vitro* and *in vivo* (after administration to rats),[8,9] it is likely acting as a prodrug.

LSD has remained a popular recreational drug since the 1960s. More recently, ALD-52 and the higher homologs 1-propanoyl-LSD (1P-LSD)[10,11] and 1-butanoyl-LSD (1B-LSD)[12-14] (Figure 1A) have been sold by online vendors in the form of blotter papers and other formulations such as powders, pellets and solutions. Similar to ALD-52, 1P-LSD and 1B-LSD undergo hydrolysis to LSD in vitro.[9] Studies in rats[8] and humans[15] have confirmed that 1P-LSD is hydrolyzed to LSD *in vivo*.

1-Valeroyl-LSD (also known as 1-valeryl-LSD, 1-pentanoyl-LSD, 1V-LSD, or "Valerie"), IUPAC name *N*,*N*-diethyl-6-methyl-1-pentanoyl-9,10-didehydroergoline-8β-carboxamide (Figure 1A), is a new N^1-acylated LSD derivative that recently appeared on the online market. The appearance of 1V-LSD as a recreational drug prompted the present investigation, which included collecting an in-depth analytical profile. The analytical characterization included various methods of mass spectrometry (MS), gas- and liquid chromatography (GC and LC), nuclear magnetic resonance spectroscopy (NMR), GC solid-state infrared (GC-sIR) analysis and Raman spectroscopy. In addition, the *in vivo* activity of 1V-LSD was assessed using the mouse head twitch response (HTR), a 5-HT_{2A}-mediated head movement that serves as a behavioral proxy in rodents for human hallucinogenic effects.[16-19] ALD-52, 1P-LSD, 1B-LSD, and 1-cyclopropanoyl-LSD (1cP-LSD) were previously found to induce the HTR[8,10,14,20] and it was hypothesized that 1V-LSD would also induce the response.

2. Experimental

2.1 Materials

All chemicals and solvents were of analytical and HPLC grade and obtained from Aldrich (Dorset, UK). DMSO-d_6 (99.8% D) was from VWR (Leicestershire, UK). 1-Valeroyl-LSD hemitartrate (2:1) powder (1V-LSD) was supplied by Synex Synthetics BV, Maastricht, The Netherlands.

2.2 Sample preparation

For the analysis of 1V-LSD base, the hemitartrate salt (2 mg) was dissolved in 2 mL demineralized water and made alkaline with one drop of NaOH (5% w/w). The solution was extracted with 2 mL diethyl ether and the ethereal phase was transferred into a new vial for analysis by GC-MS and GC-sIR.

2.3 Instrumentation

2.3.1 Gas chromatography mass spectrometry

GC-EI-MS analysis was conducted on a Finnigan TSQ 8000 triple stage quadrupole mass spectrometer coupled to a Trace GC Ultra gas chromatograph (Thermo Fisher, Waltham, USA) equipped with a fused silica DB-1 column (30 m x 0.32 mm i.d., 0.25 µm film thickness) (Agilent Technologies, Santa Clara, USA). Sample introduction was performed using a CTC CombiPAL (CTC Analytics, Zwingen, Switzerland) autosampler.

GC parameters of the analysis were as follows: injection volume: 0.5 µL, splitless; injector temperature: 280°C; carrier gas: helium; flow rate: 1.2 mL/min. The oven temperature was initially kept at 80°C for 2 min, ramped to 310°C at 20°C/min and subsequently maintained at the final temperature of 310°C for 23 min. MS

parameters were set as follows: ionization mode: EI at 70 eV; emission current: 50 µA; ion source temperature: 220 °C; scan time: 1 s; scan range: m/z 29–600.

Data Analysis was conducted using Xcalibur 4.0 Qual Browser (Thermo Fisher) and the National Institute of Standards and Technology (NIST) MS search program (version 2.3) (NIST, MD, US). EI-MS spectra were compared to EI-MS spectra libraries provided by the NIST, the European Network of Forensic Science Institutes (ENFSI), Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), as well as the designer drug library 2021 (DigiLab, SH, DE) and libraries built in-house. Kovats retention indices (RI) were calculated from the measurement of retention times obtained from the constituents of an *n*-alkane mixture. The temperature program is specified above. For calculation, logarithmic interpolation was applied between two consecutive n-alkanes.

2.3.2 Gas chromatography solid-state infrared analysis

A GC-solid phase-IR-system consisting of an Agilent GC 7890B (Agilent Technologies) equipped with a fused silica capillary DB-1 column (30 m x 0.32 mm i.d., 0.25 µm film thickness), an Agilent G4567A probe sampler (Agilent Technologies) and a DiscovIR-GC (Spectra Analysis, Marlborough, MA, US) were used for the acquisition of solid transmission IR spectra. The eluting substances were cryogenically accumulated on a spirally rotating ZnSe disk cooled by liquid nitrogen to -40 °C. IR spectra were recorded through the IR-transparent ZnSe disk using a nitrogen-cooled MCT (HgCdTe) detector.

The GC parameters were as follows: injection volume 1 µL; splitless mode; injection port temperature 240 °C; carrier gas: helium; flow rate 2.5 mL/min. Chromatographic conditions were as follows: oven temperature program: 80°C for 2 min, ramped to 290°C at 20 °C/min, and maintained for 20 min; transfer line: 280°C. Infrared conditions: oven temperature 280°C; restrictor temperature 280°C; disc temperature –40°C; dewar cap temperature 35°C; vacuum 0.2 mTorr; disc speed 3 mm/min; spiral separation 1 mm; wavelength resolution 4 cm^{-1}; IR range 650–4000 cm^{-1}; acquisition time: 0.6 s/file and 64 scans per spectrum. Data were processed using GRAMS/AI Ver. 9.1 (Grams Spectroscopy Software Suite, Thermo Fisher) followed by OMNIC Software, Ver. 7.4.127 (Thermo Fisher).

2.3.3 High mass accuracy electrospray ionization mass spectrometry

Ultrahigh-performance liquid chromatography quadrupole time-of-flight single and tandem mass spectrometry (UHPLC-QTOF-MS/MS) data were obtained from a Bruker compact Q-TOF instrument (Bruker Daltonik GmbH, Bremen, Germany) and coupled to a Dionex Ultimate 3000 UHPLC system (Thermo Fisher, Waltham, USA) equipped with a Thermo Hypersil GOLD column (2.1 x 50 mm, 1.9 µm) (Thermo Fisher). Mobile phase A consisted of aqueous buffer (10 mM NH_4HCO_2, 0.12 % formic acid) and mobile phase B was acetonitrile. The column temperature was set at 30°C, the flow rate was 0.2 mL/min) and data were acquired for 49 min. The gradient commenced at 10% B and was held for 2 min, then increased to 90% B over 31 min and returned to 10% B over a period of 11 min. The total run time was 49 min.

QTOF-MS data were acquired in positive mode scanning from m/z 50–1070. QTOF-MS parameters: drying gas temperature 190°C, drying gas flow 6 L/min, nebulizer pressure 0.9 bar, capillary voltage: 4000 V, collision gas: nitrogen, collision energy 35 eV. Sodium formate was used for calibration purposes.

2.2.4 Liquid chromatography electrospray ionization mass spectrometry

For ESI-MS analysis, 1V-LSD was dissolved in MeOH and introduced into the ESI interface via a direct insertion syringe at 3–10 µL/min depending on the signal intensity observed. The analysis was conducted on a Thermo Velos Pro (linear ion trap) spectrometer (Thermo Fisher, Waltham, USA). The mass range of the spectrometer was set to m/z 50–2,000 for the full scan. The upper limit of the mass range was adjusted to m/z $[M+H]^+$ + 50 for the MS^n experiments (up to MS^4). Wideband and non-wideband collision-induced dissociation (CID) spectra were recorded after fragmentation using helium as collision gas. The collision energy was set at an intensity at which the molecular ion peak was retained at 10% intensity of the base peak.

For the LC-ESI-MS/MS analysis, the mass spectrometer mentioned above was coupled to a Thermo Accela 1250 HPLC chromatograph (Thermo Fisher, Waltham, USA) equipped with an analytical Aqua C18 column (3 µm, 150 x 2 mm, 125 Å) (Phenomenex, Torrance, USA). Chromatographic separation was achieved at a flow rate of 100 µL/min with a gradient comprised of 0% B for 3 min, then 0% to 98% B in 14 min, 98% B held for 32 min and 98% to 0% B in 10 min, where mobile phase A was 0.25% formic acid (FA) in water and mobile phase B was 0.25% FA in methanol. The column temperature was held at 24°C. MS analysis was performed using a data-independent top 7 method in positive ion mode followed by a top 5 method in negative ion mode with the full MS and MS/MS scans acquired at unit resolution. After the acquisition of a full scan, the most seven (respectively five) intense ion species were automatically chosen as precursors for MS/MS experiments in positive and negative ion modes. Data evaluation was conducted using XCalibur 4.0 (Thermo Fisher, Waltham, USA) in conjunction with the NIST MS search program (version 2.3) and spectra compared to libraries built in-house.

2.2.6 Nuclear magnetic resonance (NMR) spectroscopy

Samples were prepared in deuterated dimethyl sulfoxide (DMSO-d_6) and ^1H (600 MHz) and ^{13}C (150 MHz) spectra were recorded on a Bruker AVANCE III 600 MHz NMR spectrometer. Spectra were referenced to residual solvent and assignments were supported by both 1D and 2D experiments.

2.4 Animal pharmacology

Male C57BL/6J mice (6−8 weeks old) were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and housed up to four per cage with a reversed light-cycle (lights on at 1900 h, off at 0700 h). Food and water were provided *ad libitum*, except during behavioral testing. Testing was conducted between 1000 and 1830 h. The HTR was assessed using a head-mounted magnet and a magnetometer detection coil.[18] Mice

were anesthetized, a small incision was made in the scalp, and a small neodymium magnet was attached to the dorsal surface of the cranium using dental cement. Following a two-week recovery period, HTR experiments were carried out in a well-lit room with at least 7 days between sessions to avoid carryover effects. Mice received intraperitoneal (IP) vehicle (saline) or 1V-LSD (0.125, 0.25, 0.5, 1, or 2 mg/kg dissolved in saline) and then activity was recorded in a glass cylinder surrounded by a magnetometer coil for 30 min. The injection volume was 5 mL/kg. Coil voltage was low-pass filtered (1 kHz cutoff frequency), amplified, digitized (20 kHz sampling rate, 16-bit ADC resolution), and saved to disk using a Powerlab/8SP data acquisition system with LabChart software ver. 7.3.2 (ADInstruments, Colorado Springs, CO, USA), then filtered off-line (40–200 Hz band-pass). To detect head twitches, events in the recordings were transformed to scalograms, deep features were extracted using the deep convolutional neural network ResNet-50, and then the images were classified using a support vector machine (SVM).[21] Head twitch counts were analyzed using one-way analyses of variance (ANOVA). *Post-hoc* comparisons were made using Tukey's studentized range method. Significance was demonstrated by surpassing an α-level of 0.05. ED_{50} values and 95% confidence intervals were calculated using nonlinear regression.

3. Results and discussion

3.1 Analytical features

The electron ionization (EI) mass spectrum of 1V-LSD is presented in Figure 1B (proposed fragmentation pathways are included in the Supporting Information) based on those suggested previously involving the lower 1-acyl homologs 1P-LSD and 1B-LSD.[10,14] Comparable to other LSD-related substances, the EI mass spectrum showed fragment clusters at m/z 151–156, m/z 161–169, m/z 178–182, m/z 191–197, m/z 205–208, and m/z 229–224. Similar to other lysergamides with an *N*,*N*-diethylamide group, the iminium ion was detected at m/z 72 together with m/z 128 and m/z 100.[10,14,20,22,23] Since 1V-LSD is a higher homolog of 1B-LSD,[14] several mass shifts of 14 Da could be observed which were specific to 1V-LSD. Examples included the molecular ion (1V-LSD: m/z 407; 1B-LSD: m/z 393), the *retro*-Diels-Alder fragment at m/z 364 (1B-LSD: m/z 350), and oxonium ions associated with the 1-acyl substituents (1V-LSD: m/z 85; 1B-LSD: m/z 71), which in turn underwent a loss of CO to form the carbenium ions at m/z 57 (1V-LSD) and m/z 43 (1B-LSD), respectively. The increase of 14 Da associated with 1V-LSD was also observed in the fragment cluster at m/z 305–308 (1B-LSD: m/z 291–294) and in ions at m/z 280 (1B-LSD: m/z 266), m/z 265 (1B-LSD: m/z 251), and m/z 249 (1B-LSD: m/z 235) (Figure 2 and the Supporting Information).

When the 1V-LSD hemitartrate salt was subjected to basification and extraction with diethyl ether prior to GC-MS analysis, it was found that hydrolysis to LSD occurred (Supporting Information). Furthermore, three more 1V-LSD isomers were detected in the GC-MS TIC trace with 1V-LSD labeled as isomer I. When the 1V-LSD salt was dissolved in methanol and subjected to GC-MS analysis without any further treatment, LSD was not detectable under the same analytical conditions. However,

all four isomers were still detectable (Supporting Information). The identity of these isomers is currently unknown though some tentative suggestions have been included as Supporting Information based on mass spectral considerations. However, these have to remain speculative at this stage. As shown in the Supporting Information, analysis of the same methanolic solution by LC-linear ion trap-MS confirmed the detection of only one isomer, which suggested that the detection of the three minor isomers were induced artificially during the GC-based procedure. No additional isomer was detected either when subjecting the methanolic sample to LC-QTOF-MS analysis. Both LC-MS procedures did also not reveal the detection of LSD as an impurity (Supporting Information).

The infrared spectrum obtained from GC solid-state analysis is shown in Figure 2 and it was found to be very comparable to the spectrum recorded for 1B-LSD previously. This included the signals recorded from to the carbonyl groups (1702 and 1639 cm^{-1}) and the absence of the indole NH signals due to 1-acyl substitution. Some minor differences between 1V-LSD and 1B-LSD included signals at 1389/1399, 1355/1369, 1327/1339, or 1236/1244 cm^{-1}. The ATR-IR spectrum recorded from the 1V-LSD hemitartrate salt has also been added to the Supporting Information section for completeness together with a Raman spectrum. For two of the three additional GC-induced 1V-LSD isomers, a corresponding GC-sIR spectrum could be recorded (Supporting Information) though they did not provide additional information as to the nature of the corresponding structures. A Raman spectrum can also be found as Supporting Information.

The electrospray ionization QTOF-MS/MS and linear ion trap tandem mass spectra recorded for 1V-LSD are presented in Figures 3A and 3B with the proposed fragmentation pathways shown as Supporting Information. Alternative species for m/z 208 and m/z 180 could also be proposed. In relation to the mass spectrum of 1B-LSD reported previously,[14] the 14 Da mass shift was reflected in the increased mass of the protonated molecule at m/z 408, m/z 365 (1B-LSD: m/z 351), m/z 307 (1B-LSD: m/z 293), and m/z 281 (1B-LSD: m/z 267). Other key ions remained the same such as 128, 208, and 223 and these were also detectable with the other lower homolog 1P-LSD.[10]

The nuclear magnetic resonance (NMR) spectroscopy data for 1V-LSD are summarized in Table 1 (full spectra are shown in the Supporting Information). The assignments were supported by HSQC and HMBC experiments and were essentially consistent with the lower homologs reported previously.[10,14] In comparison with 1B-LSD, the additional methylene unit found in 1V-LSD (1-butanoyl vs. 1-pentanoyl) was detected in the proton NMR spectrum as a multiplet at 1.45–1.36 ppm (H-26 label, Table 1). A full 1B-LSD proton and carbon NMR spectrum stacked with the corresponding 1V-LSD spectra can be found in the Supporting Information document for visual comparison. In the carbon NMR spectrum, the presence of the additional methylene carbon (C-26 label, Table 1) resonated at 21.68 ppm. In 1B-LSD, the two methylenes and the methyl group carbons related to the 1-acyl group (1-butanoyl) were detected at 37.04, 18.13, and 14.01 ppm whereas in 1V-LSD the three methylene carbons (C-24–C-26) and the methyl group (C-27) related to the 1-pentanoyl group were detected at 34.46, 26.31, 21.68, and 13.77 ppm, respectively.

3.2 Head-twitch response

1V-LSD was tested in the mouse HTR assay to determine whether it has an LSD-like behavioral profile. As shown in Figure 4, 1V-LSD produced a dose-dependent increase in HTR counts ($F(4,26) = 37.82$, $P < 0.0001$). Similar to LSD and other psychedelic drugs,[19,24] the HTR induced by 1V-LSD had an inverted U-shaped dose-response function. The median effective dose (ED_{50}) for 1V-LSD was 181 (142–232) μg/kg. Based on molar mass, the ED_{50} was determined as 373 (290-477) nmol/kg. When tested using similar experimental conditions, ALD-52 (ED_{50} = 297.2 nmol/kg), 1P-LSD (ED_{50} = 349.6 nmol/kg), and 1cP-LSD (ED_{50} = 430 nmol/kg) have about the same potency as 1V-LSD, whereas 1B-LSD (ED_{50} = 976.7 nmol/kg) is about three-fold less potent.[8,10,14,20] These results indicate that 1V-LSD administration leads to 5-HT_{2A} receptor activation *in vivo*. Lysergamides containing an N^1-substituent typically act as weak partial agonists at the 5-HT_{2A} receptor and are believed to serve as prodrugs for LSD. 1V-LSD is also likely to be hydrolyzed to LSD and serve as a prodrug, but studies to assess the biotransformation and receptor pharmacology of 1V-LSD must be performed to fully elucidate its mechanism-of-action.

In previous studies, LSD induced the HTR with an ED_{50} of 132.8 nmol/kg,[18] so 1V-LSD has about one-third of the potency of LSD in mice. However, the extent to which the relative potencies of LSD and 1V-LSD in mice can be extrapolated to humans is unclear. For LSD and a large series of structurally diverse psychedelic drugs, there is a robust correlation (r = 0.9448) between HTR ED_{50} values in mice and hallucinogenic potencies in humans;[19] however, the relationship between hallucinogen potencies in mice and humans may not extend to N^1-substituted lysergamides. If N^1-substituted lysergamides act as prodrugs then there may be considerable cross-species variation in potency depending on the identity of the enzymes mediating the hydrolysis to LSD and their expression level and pattern of expression in different tissues.

4. Conclusion

1-Valeroyl-LSD (1V-LSD), a lysergamide derived from LSD, has recently emerged as a new recreational drug. The analytical data reported in this investigation might be useful to scientists interested in the field of psychoactive drug research, to clinicians treating patients who may have ingested 1V-LSD, as well as to other stakeholders. The behavioral effects of 1V-LSD are closely reminiscent of LSD and other serotonergic psychedelics. Clinical studies are now needed to explore the activity and potency of 1V-LSD in humans and to define the qualitative nature of its effects and its abuse potential.

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Orcid

Simon D. Brandt: https://orcid.org/0000-0001-8632-5372
Pierce V. Kavanagh: https://orcid.org/0000-0002-1613-3305
Folker Westphal: https://orcid.org/0000-0003-0452-7814
Benedikt Pulver: https://orcid.org/0000-0002-7772-2111
Kathleen Morton: https://orcid.org/0000-0001-9773-8949
Geraldine Dowling: https://orcid.org/0000-0001-8344-6582
Adam L. Halberstadt: https://orcid.org/0000-0001-5096-5829

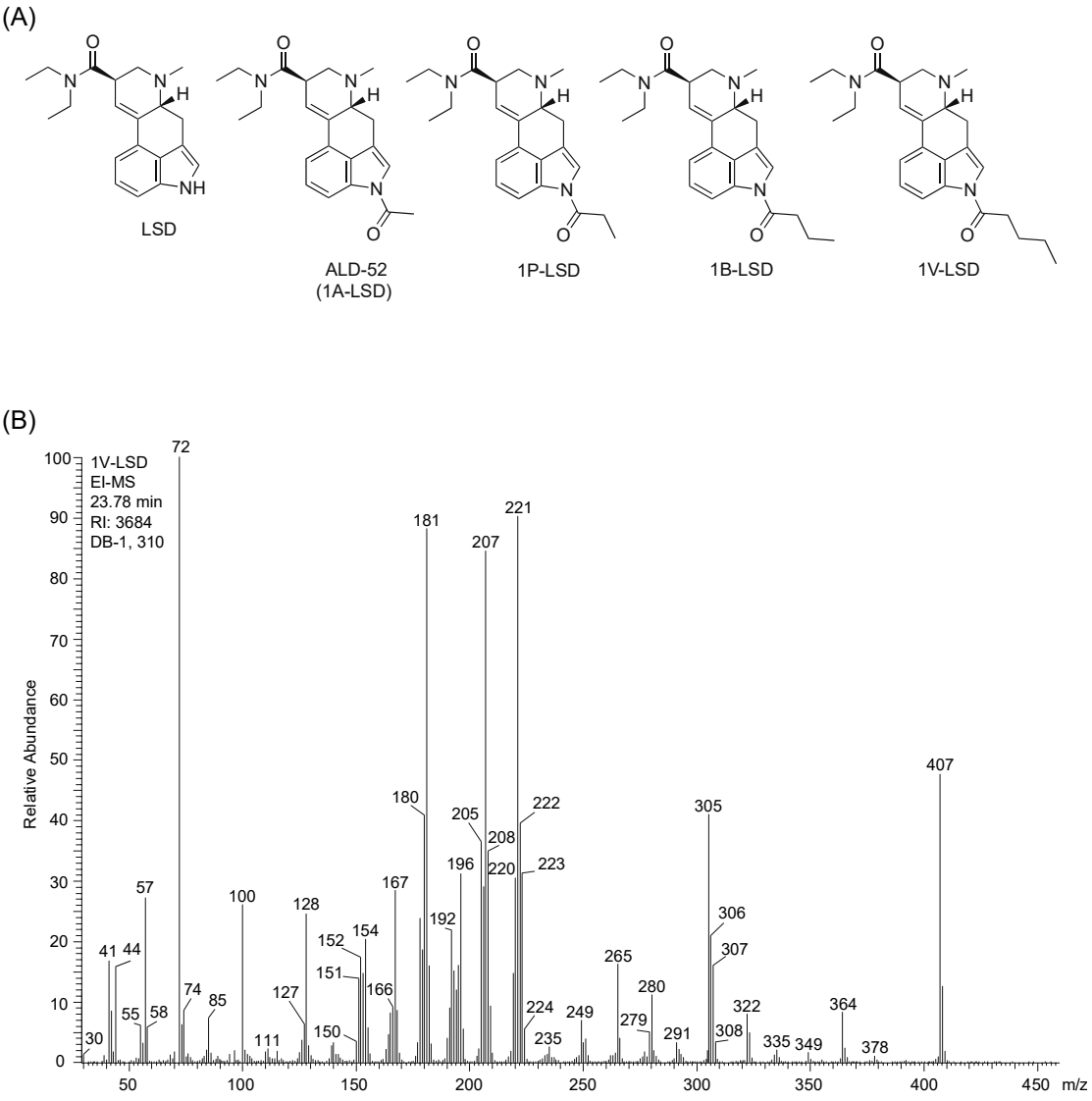


Figure 1. (A) Chemical structures of lysergic acid diethylamide (LSD) and various 1-acyl homologs. (B) Electron ionization mass spectrum of 1V-LSD.

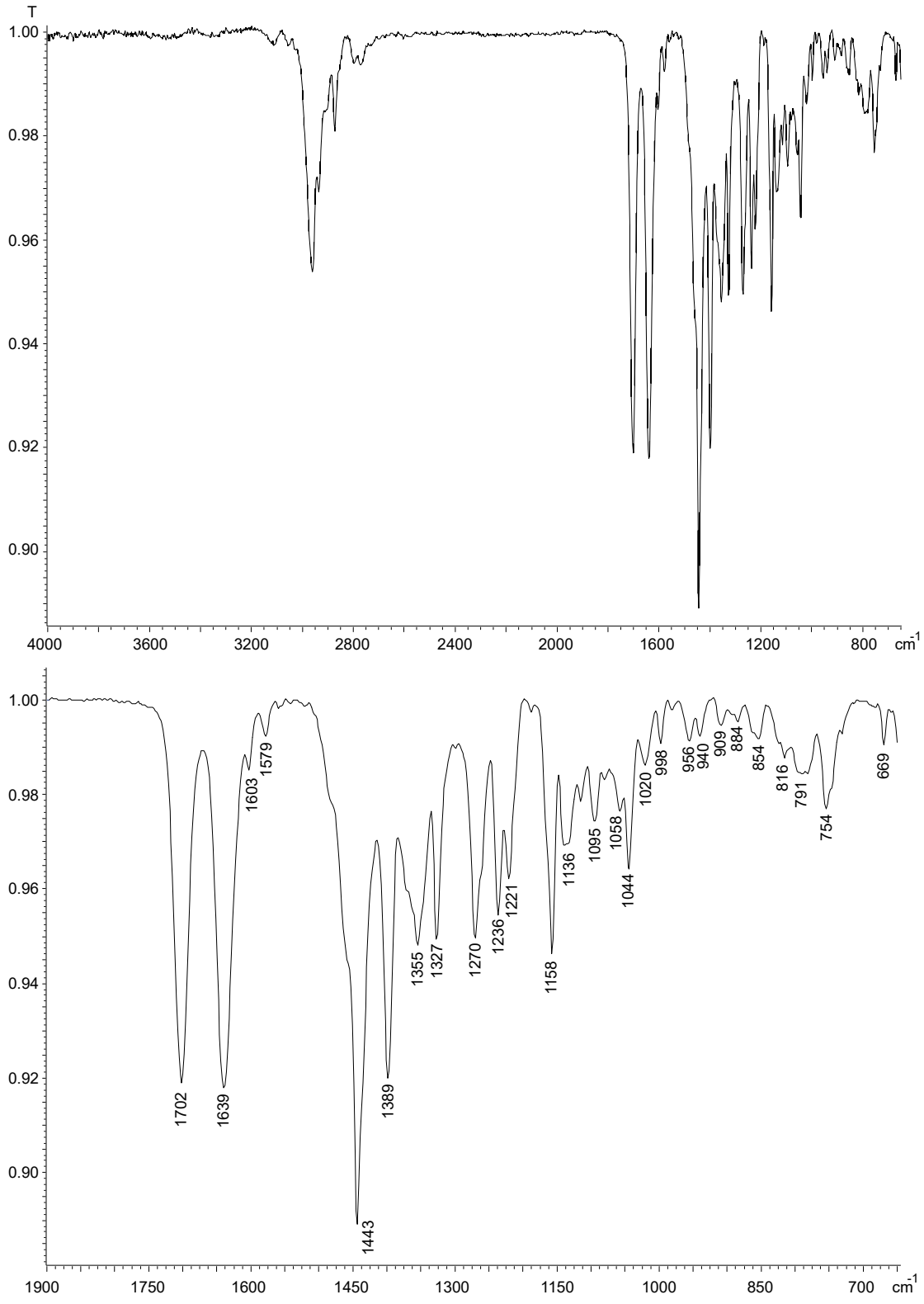
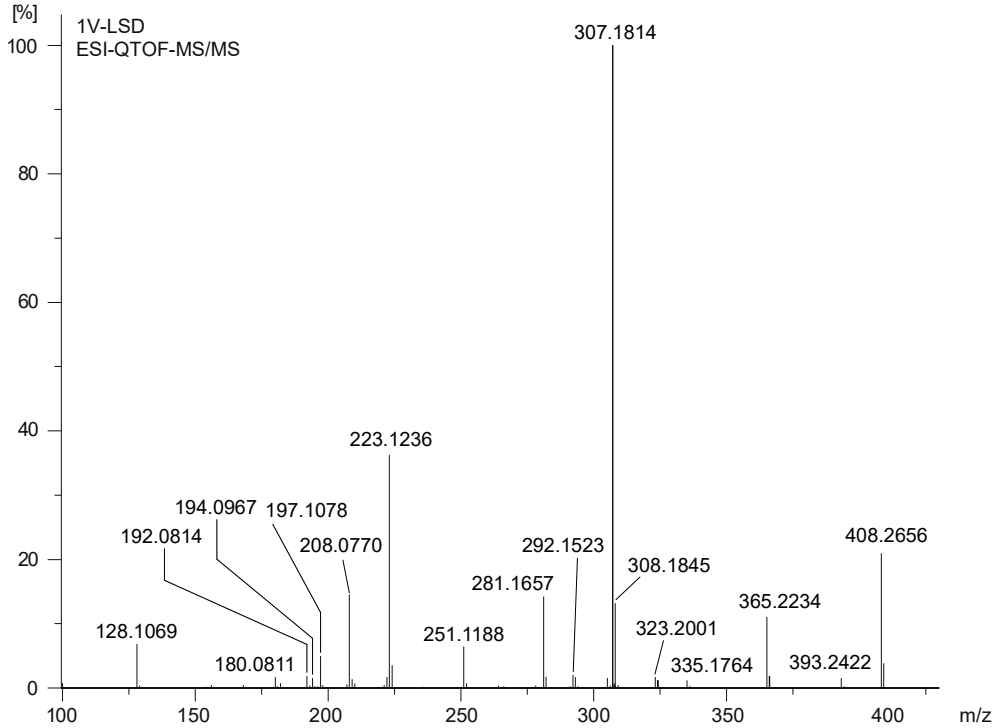


Figure 2. Gas chromatography-solid state-infrared spectrum of 1V-LSD. Top: entire scan range. Bottom: partial scan range.

(A)



(B)

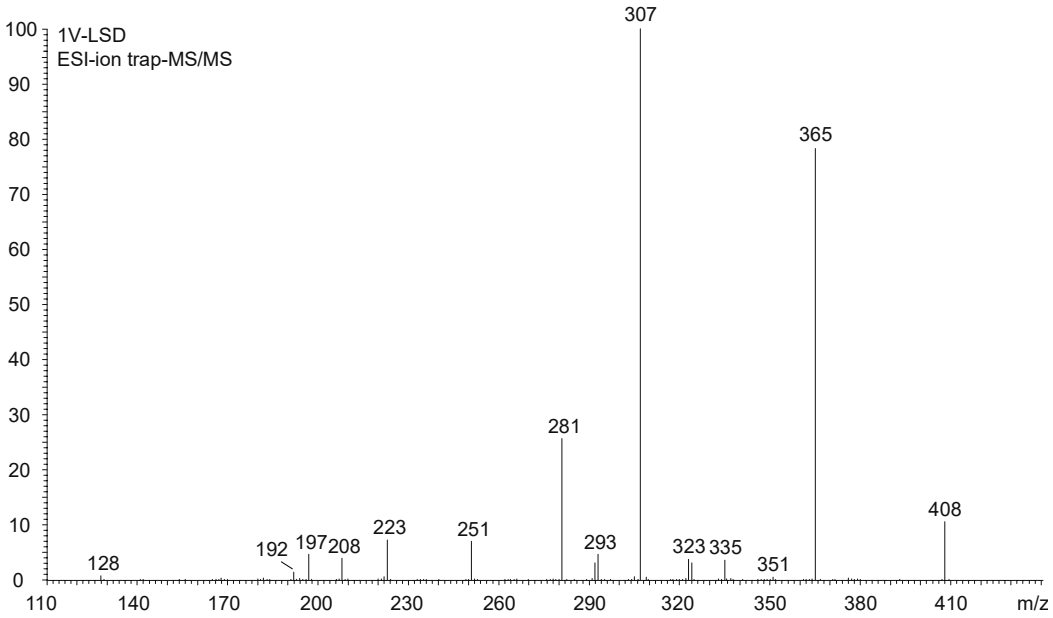


Figure 3. (A): Electrospray ionization (ESI) quadrupole time-of-flight tandem mass spectrum obtained from 1V-LSD. (B): ESI-ion trap- tandem mass spectrum of 1V-LSD.

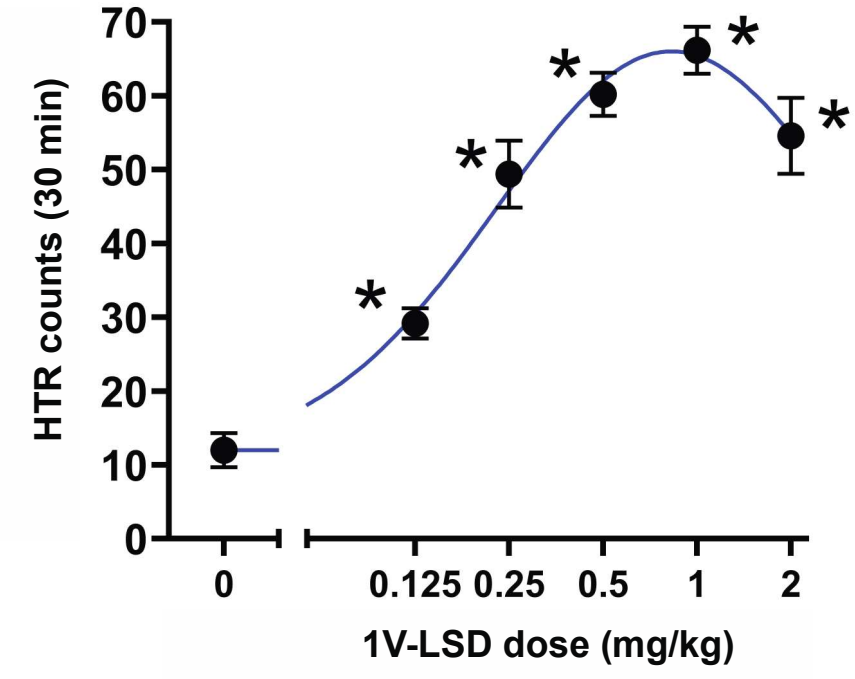
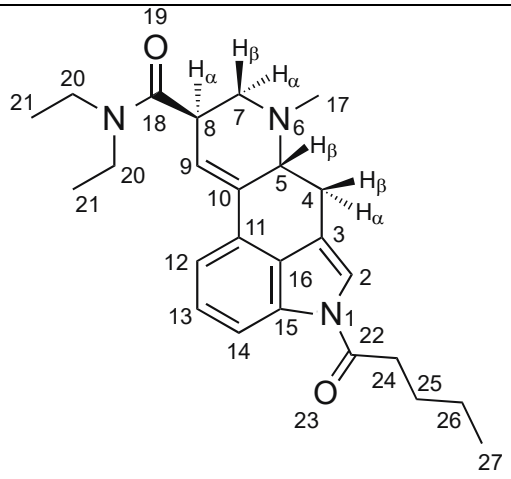


Figure 4. Dose-dependent increase of head-twitch response counts.

Table 1. ^{1}H and ^{13}C NMR data for 1V-LSD hemitartrate in d_6-DMSO at 600/150 MHz



No.	^{13}C [δ / ppm]	^{1}H [δ / ppm]
1	–	–
2	120.07	7.61 (s, 1 H)
3	116.04	–
4	26.64	2.51–2.43 (m, H-4α, 1 H) * partially coalescing with DMSO and H-17. 3.49 (dd, *J* = 15.2, 5.1 Hz, H-4β, 1 H) * partially coalescing with 2 x H-20.
5	61.82	3.08–3.06 (m, H-5β, 1 H)
6	–	–
7	55.32	3.02 (dd, *J* = 11.1, 4.7 Hz, H-7α, 1 H) 2.62 (t, *J* = 10.7 Hz, H-7β, 1 H)
8	38.90	3.84–3.81 (m, H-8α, 1 H)
9	121.83	6.35 (s, 1 H)
10	133.47	–
11	127.78	–
12	116.58	7.35 (d, *J* = 7.4 Hz, 1 H)
13	125.93	7.31 (t, *J* = 7.7 Hz, 1 H)
14	114.84	8.01 (d, *J* = 7.5 Hz, 1 H)
15	133.16	–
16	127.56	–
17	43.12	2.49 (s, 3 H) * coalescing DMSO and partially coalescing with H-4α
18	170.38	–
19	–	–
20	41.58	3.45 (q, *J* = 6.7 Hz, 2 H)
20	39.84	3.32 (AB qq, *J* = 13.9, 7.0 Hz, 2 H) *peaks are coalescing with broad water signal
21	14.83	1.19 (t, *J* = 6.9 Hz, 3 H)
21	13.07	1.07 (t, *J* = 6.9 Hz, 3 H)
22	171.84	–
23	–	–
24	34.46	2.98 (t, *J* = 7.2 Hz, 2 H)
25	26.31	1.73–1.63 (m, 2 H)
26	21.68	1.45–1.36 (m, 2 H)
27	13.77	0.94 (t, *J* = 7.3 Hz, 3 H)
TA [a]	173.30	–
TA [a]	72.02	4.25 (s, ~1.2 H)
[a] TA: Tartaric acid		