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Studies on Lysergic Acid Diethylamide and Related Compounds. IX.[1] Microbial Transformation of Amides Related to Lysergic Acid Diethylamide by *Streptomyces roseochromogenes*

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Microbial transformation of the amide congeners of lysergic acid diethylamide (LSD) by *Streptomyces roseochromogenes* was examined.

Derivatives having no methylene group at the (ω-1) position of the amine alkyl chain of the amide function, lysergic acid dimethylamide (LDM) and lysergic acid diallylamide (LDA), were oxidized at the α-position of the amide nitrogen to give the corresponding N-dealkylated products, lysergic acid methylamide (LAM) and lysergic acid allylamide (LAA).

On the other hand, derivatives having a methylene group at the (ω-1) position, lysergic acid di-*n*-propylamide (LDP) and lysergic acid di-*n*-butylamide (LDB), were oxidized at the (ω-1) position to give pairs of (ω-1) hydroxylation products, 2-hydroxyLDP and epi-2-hydroxyLDP, and 3-hydroxyLDB and epi-3-hydroxyLDB, together with the corresponding ketomethyl products, 2-oxoLDP and 3-oxoLDB. The predominant formation of 2-hydroxyLDP in the case of LDP indicated that (ω-1) hydroxylation occurred stereospecifically.

Keywords——congeners of LSD; *Streptomyces roseochromogenes*; microbial transformation; (ω-1)-oxidation; α-oxidation; stereospecific hydroxylation

In the preceding papers,[1,3] we reported that lysergic acid diethylamide (LSD) (**1**) was biotransformed by *Streptomyces roseochromogenes* into lysergic acid ethylamide (LAE) (**2**), lysergic acid ethylvinylamide (LEV) (**3**), and lysergic acid ethyl-2-hydroxyethylamide (LEO) (**4**), while norlysergic acid diethylamide (norLSD) (**5**) was obtained practically as a sole product with *S. lavendulae*. We would like to emphasize that the biological formation of a vinylamide group from an ethylamide function is quite new. We also showed that LEV (**3**) was converted into LEO (**4**) together with LAE (**2**) during the reincubation of LEV (**3**) under the same conditions, but a similar experiment on LEO (**4**) resulted in recovery of the starting material, indicating that LEO (**4**) itself is not biotransformed into LEV (**3**). Although these results suggested the presence of a sequential process [LSD (**1**)$\rightarrow$LEV (**3**)$\rightarrow$LEO (**4**)], the direct formation of LEO (**4**) from LSD (**1**) could not be excluded, because the ω-oxidation of an alkyl side chain[4] is known to occur in the metabolism of foreign compounds.

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1) Part VIII: H. Ishii, T. Niwaguchi, Y. Nakahara, and M. Hayashi, *J.C.S. Perkin I*, in press. Part of this study was presented at the 19 th Symposium on the Chemistry of Natural Products ,Hiroshima, Japan, October 1975.
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3) H. Ishii, M. Hayashi, T. Niwaguchi, and Y. Nakahara, *Chem. Pharm. Bull.* (Tokyo), **27**, 1570 (1979).
4) A.M. Guarino, W.D. Conway, and H.M. Fales, *European J. Pharmacol.*, **8**, 244 (1969); J.E. Bakke, J.D. Robbins, and V.J. Feil, *J. Agric. Food Chem.*, **19**, 462 (1971); M. Keise and W. Lenk, *Biochem. Pharmacol.*, **22**, 2575 (1973); D.J. Tocco, A.E.W. Duncan, F.A. Deluna, H.B. Hucker, V.F. Gruber, and W.A. Vandenheuvel, *Drug Metabolism and Disposition*, **3**, 361 (1975).

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These considerations led us to examine the microbial transformation of several amide congeners of LSD (**1**) by *S. roseochromogenes*.

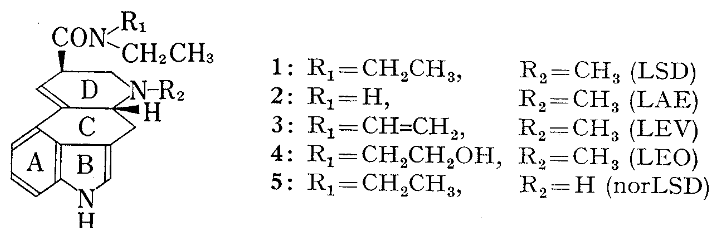

Chart 1. Structures of LSD and Its Metabolites

Materials and Methods

Analytical Methods——All melting points were observed on a microscopic hot-stage and are uncorrected. Infrared (IR) and ultraviolet (UV) spectra were obtained with a JASCO DS-701G machine equipped with a microscope and a Hitachi EPS-3T spectrometer, respectively. Nuclear magnetic resonance (NMR) spectra were measured with tetramethylsilane as an internal standard, using a Hitachi R-22 spectrometer (90 MHz). The following abbreviations are used; s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, sh=shoulder. Optical rotatory dispersion (ORD) curves and mass spectra (MS) were taken with JASCO ORD/UV-5 and JEOL JMS-01SG spectrometers, respectively. Peaks with an intensity of more than 10%, and diagnostically important peaks in the mass spectra of the metabolites are listed here.

Thin-layer chromatography (TLC) was carried out on Silica gel G plates, Merck No. 5721 glass plate (0.25 mm thick), using the following solvent systems: i) $CHCl_3$–MeOH [4 : 1 (v/v)] (solv. A); ii) acetone–$CHCl_3$ [4 : 1 (v/v)] (solv. B); iii) $CHCl_3$–hexane–MeOH [4 : 2 : 1 (v/v/v)] (solv. C). Spots on TLC were visualized by UV irradiation at 365 nm and/or spraying with Ehrlich reagent (*p*-dimethylaminobenzaldehyde in alcoholic HCl).

Identification of a metabolite was achieved by comparison with an authentic sample (TLC in solv. A, B, and C, and mass spectra and/or IR spectra).

Quantitative analysis of each metabolite was performed by developing the mixture of metabolites on a TLC plate using 10—20 μl of medium with solv. A, followed by measurement of the fluorescence intensity of each spot at λ_{ex} 330 nm and λ_{em} 410 nm with a Hitachi MPF-2A fluorescence spectrophotometer equipped with a J-201 digital integrator, a J-301 digital recorder, and a TLC accessory. The photometric yields of unchanged LSD (**1**) and metabolites were calculated from their fluorescence intensities as percentages of the total fluorescence intensity. The isolation yields are also given.

Materials——Materials which were used as substrates or authentic samples in this study were the same as those reported in the previous paper[5] except for lysergic acid diallylamide (LDA) (**13**) and lysergic acid allylamide (LAA) (**15**). These two substrates were prepared essentially according to our reported method.[5] The starting *d*-lysergic acid (**12**) was purchased from Sigma Co. Ltd. For column chromatography, alumina (Merck, Brockmann grade II—III) was used.

Lysergic Acid Dimethylamide (LDM) (6)——Colorless oil [*d*-tartrate: colorless needles, mp 200—202° (lit.[6] mp 203°)]. *Rf* values: 0.65 (solv. A); 0.26 (solv. B); 0.53 (solv. C).

Lysergic Acid Methylamide (LAM) (7)——Pale yellow oil [methanesulfonate: colorless fine needles, mp 236—238° (lit.[6] mp 234°)]. *Rf* values: 0.40 (solv. A); 0.09 (solv. B); 0.33 (solv. C).

Lysergic Acid Di-*n*-propylamide (LDP) (8)——Colorless plates, mp 196° (lit.[6] mp 195°). *Rf* values: 0.84 (solv. A); 0.94 (solv. B); 0.75 (solv. C).

Lysergic Acid *n*-Propylamide (LAP) (9)——Pale yellow oil [maleate: colorless needles, mp 210—213° (lit.[6] mp 207°)]. *Rf* values: 0.57 (solv. A); 0.39 (solv. B); 0.55 (solv. C).

Lysergic Acid Di-*n*-butylamide (LDB) (10)——Colorless needles, mp 98—99° (lit.[6] mp 97°). *Rf* values: 0.86 (solv. A); 0.75 (solv. B); 0.68 (solv. C).

Lysergic Acid *n*-Butylamide (LAB) (11)——Colorless oil [maleate: colorless needles, mp 215—217° (lit.[6] mp 216°)]. *Rf* values: 0.74 (solv. A); 0.45 (solv. B); 0.56 (solv. C).

Synthesis of Lysergic Acid Diallylamide (LDA) (13)——i) Amidation of *d*-Lysergic Acid (**12**) with Diallylamine: A solution of imidazole (35 mg) and triphenylphosphite (0.2 ml) in MeCN (10 ml) was added to a solution of *d*-lysergic acid (**12**) (50 mg) and hexamethylphosphoric triamide (HMPA) (0.2 ml) in MeC

5) Y. Nakahara and T. Niwaguchi, *Yakugaku Zasshi*, **94**, 407 (1974).
6) A. Stoll and A. Hofmann, *Helv. Chim. Acta*, **38**, 421 (1955).

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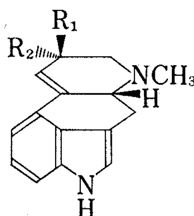

6: R_1=CONMe$_2$, R_2=H (LDM)
7: R_1=CONHMe, R_2=H (LAM)
8: R_1=CON (*n*-Pr)$_2$, R_2=H (LDP)
9: R_1=CONH (*n*-Pr), R_2=H (LAP)
10: R_1=CON (*n*-Bu)$_2$, R_2=H (LDB)
11: R_1=CONH (*n*-Bu), R_2=H (LAB)
12: R_1=COOH, R_2=H (lysergic acid)
13: R_1=CON (CH$_2$CH=CH$_2$)$_2$, R_2=H (LDA)
14: R_1=H, R_2=CON (CH$_2$CH=CH$_2$)$_2$ (isoLDA)
15: R_1=CONH (CH$_2$CH=CH$_2$), R_2=H (LAA)
16: R_1=H, R_2=CONH (CH$_2$CH=CH$_2$) (isoLAA)
17: R_1=H, R_2=COOH (isolysergic acid)
24: R_1=H, R_2=CONEt$_2$ (isoLSD)

Chart 2. Structures of LSD Congeners and Their Derivatives

(30 ml). The mixture was left to stand for 1 hr at room temperature. After addition of diallylamine (1 ml), the mixture was left to stand for a further 20 hr at room temperature, then concentrated under reduced pressure, diluted with benzene, and extracted with 1% tartaric acid aq. The aqueous layer was made basic with 1 N NaOH aq. and extracted with $CHCl_3$. The $CHCl_3$ solution was dried over anhydr. Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was dissolved in benzene–acetone [9: 1 (v/v)] and chromatographed on alumina.

ii) Lysergic Acid Diallylamide (LDA) (**13**): The first elution fraction gave colorless needles (52.5 mg), mp 123—125°, which were recrystallized from acetone. *Rf* values: 0.81 (solv. A); 0.65 (solv. B); 0.71 (solv. C). IR ν_{max}^{KBr} cm^{-1}: 3230 (NH), 1650 (amide). UV λ_{max}^{EtOH} nm (log ε): 241 (4.31), 314 (3.97). ORD (c=0.0160, MeOH) $[M]_{peak}^{24°}$ (nm): +10500° (340). MS *m/e*: 348 (M$^+$+1, 13%), 347 (M$^+$, 100%), 304 (ion A, 9%), 263 (12%), 249 (10%), 223 (ion B, 35%), 222 (38%), 221 (ion C, 67%), 207 (23%), 196 (25%), 192 (13%), 181 (24%), 167 (20%), 154 (19%). High resolution MS Calcd. for $C_{22}H_{25}N_3O$: 347.200. Found: 347.197 (M$^+$). NMR ($CDCl_3$) δ: 2.57 (3H, s, NCH$_3$), 3.98 (4H, m, NC$\underline{H}_2$CH=CH$_2$×2), 5.23 (4H, m, CH=C$\underline{H}_2$×2), 5.94 (2H, m, CH$_2$C$\underline{H}$=CH$_2$×2), 6.46 (1H, d, J=3 Hz, C$_9$–H), 6.94 (1H, s, C$_2$–H), 7.20 (3H, m, aromatic H), 8.21 (1H, br. s, NH).

iii) Isolysergic Acid Diallylamide (**14**): Subsequent elution with $CHCl_3$ gave an oily substance (3 mg). *Rf* values: 0.55 (solv. A); 0.42 (solv. B); 0.38 (solv. C). IR[7] $\nu_{max}^{CHCl_3}$ cm^{-1}: 1660 (amide). UV λ_{max}^{EtOH} nm: 242, 313. ORD (c=0.0113, MeOH) $[M]_{peak}^{24°}$ (nm): +21200° (340). MS *m/e*: 348 (M$^+$+1, 12%), 347 (M$^+$, 100%), 306 (10%), 304 (ion A, 9%), 263 (15%), 249 (12%), 223 (ion B, 37%), 222 (41%), 221 (ion C, 75%), 208 (16%), 207 (35%), 206 (18%), 196 (27%), 192 (30%), 181 (25%), 180 (15%), 179 (11%), 167 (19%), 154 (21%). High resolution MS Calcd. for $C_{22}H_{25}N_3O$: 347.200. Found: 347.199 (M$^+$).

Synthesis of Lysergic Acid Allylamide (LAA) (15)——i) Amidation of *d*-Lysergic Acid (**12**) with Allylamine: A solution of imidazole (14 mg) and triphenylphosphite (0.08 ml) in MeCN (4 ml) was added to a solution of *d*-lysergic acid (**12**) (20 mg) and HMPA (0.08 ml) in MeCN (12 ml). The mixture was left to stand for 1 hr at room temperature. After addition of allylamine (0.5 ml), the mixture was left to stand for 22 hr at room temperature, condensed under reduced pressure, diluted with benzene, and extracted with 1% tartaric acid aq. The aqueous solution was made alkaline with 1 N NaOH aq. and extracted with $CHCl_3$. The organic layer was dried over anhydr. Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was dissolved in benzene–acetone [9: 1 (v/v)] and chromatographed on alumina.

ii) Isolysergic Acid Allylamide (**16**): The first elution fraction afforded an oily substance (3 mg) (lit.[5] oil). *Rf* values: 0.82 (solv. A); 0.60 (solv. B); 0.67 (solv. C). IR[7] $\nu_{max}^{CHCl_3}$ cm^{-1}: 1650 (amide). UV λ_{max}^{EtOH} nm: 242, 313. ORD (c=0.0125, MeOH) $[M]_{peak}^{24°}$ (nm): +22600° (338). MS *m/e*: 308 (M$^+$+1, 14%), 307 (M$^+$, 100%), 264 (ion A, 10%), 249 (32%), 223 (ion B, 44%), 222 (50%), 221 (ion C, 80%), 208 (15%), 207 (36%), 196 (22%), 192 (18%), 181 (38%), 180 (13%), 179 (11%), 167 (15%), 154 (17%). High resolution MS Calcd. for $C_{19}H_{21}N_3O$: 307.168. Found: 307.170 (M$^+$).

iii) Lysergic Acid Allylamide (LAA) (**15**): Subsequent elution with benzene–acetone [5: 1 (v/v)] gave an oily substance (14 mg). *Rf* values: 0.70 (solv. A); 0.49 (solv. B); 0.50 (solv. C). MS *m/e*: 308 (M$^+$+1, 12%), 307 (M$^+$, 100%), 264 (ion A, 9%), 249 (51%), 223 (ion B, 60%), 222 (61%), 221 (ion C, 75%), 207 (36%), 196 (23%), 192 (9%), 181 (18%), 167 (14%), 154 (11%). High resolution MS Calcd. for $C_{19}H_{21}N_3O$: 307.168. Found: 307.167 (M$^+$). NMR ($CDCl_3$) δ: 2.58 (3H, s, NCH$_3$), 3.95 (2H, m, NC$\underline{H}_2$CH=CH$_2$), 5.16 (1H, double d, J_1=10.0 Hz, J_2=2.0 Hz, CH$_2$CH=C$\underline{H}_A$H$_B$), 5.21 (1H, double d, J_1=19.0 Hz, J_2=2.0 Hz, CH$_2$CH=CH$_A\underline{H}_B$), 5.90 (1H, m, CH$_2$C$\underline{H}$=CH$_2$), 6.48 (1H, d, J=3.0 Hz, C$_9$–H), 6.95 (1H, s, C$_2$–H), 6.96 (1H, br. s, CONH), 7.22 (3H, m, aromatic H), 8.09 (1H, br. s, NH).

7) The amount of material isolated was so small that we could determine only the carbonyl band.

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This material was crystallized as the hydrochloride, colorless needles, mp 180—185° (dec.), which were recrystallized from MeOH–ether. IR ν_{max}^{KBr} cm^{-1}: 3230 (NH), 2660, 2610, 2530 (quaternary amine), 1655 (amide). UV λ_{max}^{MeOH} nm (log ε): 241 (4.32), 316 (4.05). ORD (c=0.0164, MeOH) $[M]_{peak}^{22°}$ (nm): +9990° (340).

This material was also characterized as the *d*-tartrate, colorless needles, mp 113—116° (*lit.*[6]) mp 110—115°).

Structural Assignments of LDA (13) and LAA (15)——It is well known that lysergic acid (**12**) is partially epimerized on amidation by various methods.[6,8] In the previous paper,[5] we reported an improved method for the amidation of *d*-lysergic acid (**12**). LDA (**13**) and LAA (**15**) were prepared from *d*-lysergic acid (**12**) by this method. The absolute configurations of a pair of epimeric products were determined by inspection of their ORD curves (Fig. 1) the molecular optical rotation $[M]$ of the derivative belonging to the lysergic acid (**12**) series is smaller than that of the corresponding derivative belonging to the isolysergic acid (**17**) series at 340 nm.[5]

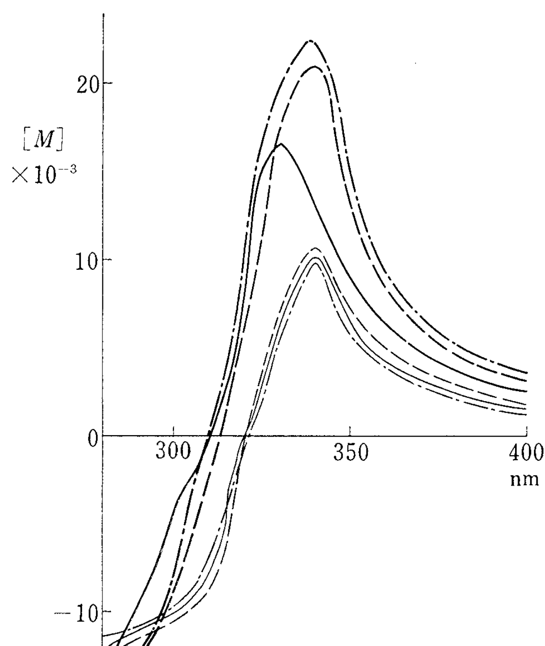

Fig. 1. ORD Curves of LSD (**1**) ——, isoLSD (**24**) ━━, LDA (**13**) ----, isoLDA (**14**) ----, LAA (**15**) ---, and isoLAA (**16**) -·-

Strain——*Streptomyces roseochromogenes* (IFM 1081) maintained at the Research Institute for Chemobiodynamics, Chiba University, was used.

Culture——Mycelia and/or spores of *S. roseochromogenes* were suspended in a soybean medium [soybean meal, 30 g; soluble starch, 20 g; K_2HPO_4, 1 g; NaCl, 1 g; city water, 1 l; pH adjusted to 7.2 with 1 N NaOH aq.], which was sterilized in an autoclave at 121° for 15 min before use. The microorganism was preincubated at 28° on a reciprocating shaker at 60 rpm for 48 hr using a Sakaguchi flask (500 ml) containing the medium (100 ml).

i) Preliminary Tests: In order to examine the yields of metabolites for each substrate, preliminary tests were performed at a scale of 1 mg of substrate using a Monod tube (15×220 mm) containing the medium (10 ml). The preincubated culture (1 ml) was inoculated into the medium (9 ml) and incubated at 28° for 48 hr at 60 rpm. After addition of a substrate solution in EtOH (1 mg/0.1 ml), the culture was further incubated until conversion of the substrate ceased. The quantities of components present in the medium were determined by densitometry of thin-layer chromatograms.

ii) Preparative Scale Experiments: In order to isolate sufficient amounts of metabolites to examine their structures, experiments were performed on a relatively large scale under the same conditions as the preliminary test using a Sakaguchi flask (500 ml) containing 100 ml of medium and 10 mg of substrate.

Isolation and Purification of Metabolites——EtOH (3 vol.) was added to the collected culture and the mixture was centrifuged at 3000 rpm. The precipitate was washed with EtOH. The washings were combined with the filtrate and evaporated to about 1/5 of the original volume *in vacuo*. The condensed solution (*ca.* pH 8) was extracted with AcOEt. The combined extract was dried over anhydr. Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was dissolved in AcOEt and extracted with 1% tartaric acid aq. The acid layer was adjusted to *ca.* pH 8 with $NaHCO_3$ and extracted with AcOEt. The AcOEt extract was dried over anhydr. Na_2SO_4 and evaporated to dryness *in vacuo*. The mixture of metabolites was separated into individual components by preparative TLC on silicic acid or by column chromatography on alumina.

Oxidation of Metabolites Having a Hydroxy Group with Jones' Reagent——Jones' reagent (CrO_3, 2.67 g; conc. H_2SO_4, 2.3 ml; distilled water, 10 ml) was added to a solution of a metabolite having a hydroxy group in acetone and the mixture was left to stand for 30 min at room temperature. After addition of $CHCl_3$ (20 ml) and H_2O (10 ml), the mixture was made basic with $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was dried over anhydr. Na_2SO_4 and evaporated to dryness *in vacuo* to give the desired oxidation product.

Reduction of Metabolites Having a Ketonic Group with Sodium Borohydride——Sodium borohydride was added to a solution of a metabolite having a ketonic group in MeOH. The mixture was stirred for 15 min at room temperature and evaporated to dryness *in vacuo*. Each product, obtained in the reaction mixture as a pair of epimeric reduction products, was isolated by preparative TLC (solv. A).

8) W.L. Garbrecht, *J. Org. Chem.*, **24**, 368 (1959); P.P. Pioch, U.S. Patent 2736728 Feb. 28 (1956) [*Chem. Abstr.*, **50**, 10803 (1956)]; A. Cerny and M. Semonsky, *Coll. Czech. Chem. Commun.*, **27**, 1585 (1962).

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Table I. Yields of Metabolites of Amide Congeners of LSD on Transformation with *S. roseochromogenes*

Substrate	Unchanged starting material	α-Oxidation product	(ω-1) Oxidation product	Metabolite *via* undefined pathway
LDM(**6**)	60%(65%)	LAM(**7**) 35%(28%)		
LSD(**1**)	25%(28%)	LAE(**2**) 37%(22%)		LEV(**3**) 2.0%(1.0%) LEO(**4**) 20%(10%)
LDP(**8**)	29%(37%)	—	2-OxoLDP(**18**) 9%(5%) 2-HydroxyLDP(**19**) 45%(38%) Epi-2-hydroxyLDP(**20**) 16%(10%)	
LDB(**10**)	51%(60%)	—	3-OxoLDB(**21**) 6%(3%) 3-HydroxyLDB(**22**) 16%(13%) Epi-3-hydroxyLDB(**23**) 10%(6%)	
LDA(**13**)	63%(70%)	LAA(**15**) 24%(15%)		

The photometric yields are based on the fluorescence intensities as percentages of the total fluorescence intensity. Figures in parentheses are isolation yields.

Results

The yields of the metabolites corresponding to each amide derivative are listed in Table I.

Experiment with Lysergic Acid Dimethylamide (LDM) (6)

Microbial transformation of LDM (**6**) (10 mg) gave a mixture showing two spots on TLC [*Rf*(solv. A): 0.65 and 0.40]. Preparative TLC (solv. A) of the mixture gave unchanged LDM (**6**) [*Rf*(solv. A) 0.65; 6.5 mg] and LAM (**7**) [*Rf*(solv. A) 0.40; 2.7 mg].

Experiment with Lysergic Acid Di-*n*-propylamide (LDP) (8)

Microbial transformation of LDP (**8**) (30 mg) provided a mixture which showed four spots on TLC [*Rf*(solv. A): 0.84, 0.65, 0.55 and 0.50]. The mixture was dissolved in benzene and chromatographed on alumina. Elution with benzene gave colorless needles (11 mg), mp 196—197°, which were identified as unchanged LDP (**8**) [*Rf*(solv. A) 0.84].

i) Lysergic Acid *n*-Propyl-*n*-2-oxopropylamide (2-oxo LDP) (18)——Elution with benzene–acetone [9:1 (v/v)] yielded an oily substance (1.5 mg). *Rf* values: 0.65 (solv. A); 0.66 (solv. B); 0.63 (solv. C). IR[7] $\nu_{max}^{CHCl_3}$ cm^{-1}: 1736 (C=O), 1635 (amide). UV λ_{max}^{EtOH} nm: 243, 313. MS *m/e*: 366 (M$^+$ +1, 20%), 365 (M$^+$, 100%), 323 (M$^+$ $-CH_2CO$, 43%), 322 (ion A, 33%), 223 (ion B, 45%), 222 (30%), 221 (ion C, 60%), 207 (29%), 196 (51%), 181 (52%), 167 (31%), 154 (49%). High resolution MS Calcd. for $C_{22}H_{27}N_3O_2$: 365.210. Found: 365.209 (M$^+$).

Subsequent elution with AcOEt gave a mixture showing two spots on TLC [*Rf*(solv. A): 0.55 and 0.50]. Each component was isolated by preparative TLC (solv. A).

ii) Lysergic Acid *n*-Propyl-*n*-2-hydroxypropylamide (2-hydroxy LDP) (19)——Treatment of the fraction corresponding to *Rf*(solv. A) 0.55 in the preparative TLC described above gave an oily substance (13 mg). *Rf* values: 0.55 (solv. A); 0.31 (solv. B); 0.52 (solv. C). MS *m/e*: 368 (M$^+$ +1, 10%), 367 (M$^+$, 100%), 324 (ion A, 8%), 268 (15%), 223 (ion B, 27%), 222 (30%), 221 (ion C, 70%), 208 (26%), 207 (49%), 196 (27%), 192 (28%), 181 (47%), 167 (19%), 154 (41%). High resolution MS Calcd. for $C_{22}H_{29}N_3O_2$: 367.226. Found: 367.226 (M$^+$). NMR

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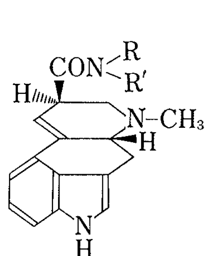
($CDCl_3$) δ: 0.86 (3H, t, J=7.0 Hz, $CH_2C\underline{H}_3$), 1.18 (3H, d, J=6.5 Hz, >$CHC\underline{H}_3$), 1.57 (2H, m, $CH_2C\underline{H}_2CH_3$), 2.56 (3H, s, NCH_3), 6.32 (1H, s, C_9–H), 6.85 (1H, s, C_2–H), 7.1—7.2 (3H, m, aromatic H), 8.53 (IH, br. s, NH).

This material was crystallized as the hydrochloride, which was recrystallized from MeOH–ether to give colorless needles (12 mg), mp 190—194° (dec.). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH or NH), 2480, 2400, 2350 (qurternary amine), 1628 (amide). UV λ_{max}^{MeOH} nm (log ε): 240 (4.28) sh, 314 (3.95). ORD (c=0.0184, MeOH) $[M]^{22°}$ (nm): +430° (500), +1940° (400), +7900° (340, peak), 0° (322), −13500° (300).



18: R=$CH_2CH_2CH_3$, R'=CH_2COCH_3 (2-oxoLDP)

19: R=$CH_2CH_2CH_3$, R'= CH_2–C(OH)(H)–CH_3 (2-hydroxyLDP)

20: an epimer of **19** at a hydroxyl group in R'. (epi-2-hydroxyLDP)

21: R=$(CH_2)_2CH_2CH_3$, R'=$(CH_2)_2COCH_3$ (3-oxoLDB)

22: R=$(CH_2)_2CH_2CH_3$, R'=$(CH_2)_2$–C(OH)(H)–CH_3 (3-hydroxyLDB)

23: an epimer of **22** at a hydroxyl group in R'. (epi-3-hydroxyLDB)

Chart 3. Structures of Metabolites of LDP (**8**) and LDB (**10**)

iii) Lysergic Acid *n*-Propyl-*n*-epi-2-hydroxypropylamide (epi-2-hydroxy LDP) (20)——Treatment of the fraction corresponding to Rf(solv. A) 0.50 gave an oily substance (3 mg). Rf values: 0.50 (solv. A); 0.26 (solv. B); 0.49 (solv. C). IR[7] $\nu_{max}^{CHCl_3}$ cm^{-1}: 1635 (amide). UV λ_{max}^{EtOH} nm: 241sh, 313. ORD (c=0.0122, MeOH) $[M]^{22°}$ (nm): +440° (500), +2150° (400), +8240° (339, peak), 0° (323), −12800° (300). MS m/e: 368 (M^+ +1, 11%), 367 (M^+, 100%), 324 (ion A, 11%), 268 (26%), 223 (ion B, 48%), 222 (55%), 221 (ion C, 72%), 208 (30%), 207 (63%), 196 (19%), 192 (11%), 181 (49%), 167 (19%), 154 (18%). High resolution MS Calcd. for $C_{22}H_{29}N_3O_2$: 367.226. Found: 367.228 (M^+). NMR ($CDCl_3$) δ: 0.86 (3H, t, J=7.0 Hz, $CH_2C\underline{H}_3$), 1.18 (3H, d, J=6.5 Hz, >$CHC\underline{H}_3$), 1.57 (2H, m, $CH_2C\underline{H}_2CH_3$), 2.56 (3H, s, NCH_3), 6.32 (1H, s, C_9–H), 6.85 (1H, s, C_2–H), 7.1—7.2 (3H, m, aromatic H), 8.58 (1H, br. s, NH).

Although the crude extract of metabolites of LDP (**8**) was carefully examined byTLC, no spot corresponding to LAP (**9**), an N-depropyl product of LDP (**8**), was observed.

Experiment with Lysergic Acid Di-*n*-butylamide (LDB) (10)

Microbial transformation of LDB (**10**) (30 mg) gave a mixture which showed four spots on TLC [Rf(solv. A): 0.86, 0.80, 0.70, and 0.65]. The mixture was dissolved in benzene and chromatographed on alumina. Elution with benzene gave colorless needles (18 mg), mp 98—99°, which were identified as unchanged LDB (**10**) [Rf(solv. A) 0.86].

i) Lysergic Acid *n*-Butyl-*n*-3-oxobutylamide (3-oxo LDB) (21)——Elution with benzene–acetone [9:1 (v/v)] gave a crude oil. Purification of this oil by preparative TLC (solv. A) afforded a pure oily substance (1 mg). Rf values: 0.80 (solv. A); 0.52 (solv. B); 0.61 (solv. C). IR[7] $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725 (C=O), 1660 (amide). UV λ_{max}^{EtOH} nm: 242, 313. MS m/e: 394 (M^+ +1, 14%), 393 (M^+, 100%), 350 (ion A, 10%), 280 (11%), 223 (ion B, 45%), 222 (49%), 221 (ion C, 65%), 208 (39%), 207 (58%), 196 (55%), 193 (12%), 192 (28%), 181 (61%), 180 (14 %), 167 (25%), 154 (30%). High resolution MS Calcd. for $C_{24}H_{31}N_3O_2$: 393.242. Found: 393.240 (M^+).

Subsequent elution with $CHCl_3$ gave an oily mixture which showed two spots on TLC [Rf(solv. A): 0.70 and 0.65]. This mixture was separated by preparative TLC (solv. A).

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ii) Lysergic Acid *n*-Butyl-*n*-3-hydroxybutylamide (3-hydroxy LDB) (22)——The component corresponding to *Rf* (solv. A) 0.70 was obtained as an oily substance (4 mg). *Rf* values: 0.70 (solv. A); 0.20 (solv. B); 0.44 (solv. C). IR[7] $\nu_{max}^{CHCl_3}$ cm^{-1}: 1635 (amide). UV λ_{max}^{EtOH} nm: 241, 313. MS *m/e*: 396 (M$^+$ +1, 11%), 395 (M$^+$, 100%), 352 (ion A, 10%), 268 (11%), 223 (ion B, 38%), 222 (46%), 221 (ion C, 72%), 208 (13%), 207 (54%), 196 (33%), 192 (11%), 181 (45%), 180 (12%), 167 (18%), 154 (20%). High resolution MS Calcd. for $C_{24}H_{33}N_3O_2$: 395.257. Found: 395.255 (M$^+$). NMR ($CDCl_3$) δ: 0.95 (3H, t, J=7.0 Hz, $CH_2C\underline{H}_3$), 1.32 (3H, d, J=6.0 Hz, >$CHC\underline{H}_3$), 2.61 (3H, s, NCH_3), 6.22 (1H, s, C_9–H), 6.78 (1H, s, C_2–H), 7.0—7.1 (3H, m, aromatic H), 8.34 (1H, br. s, NH).

iii) Lysergic Acid *n*-Butyl-*n*-epi-3-hydroxybutylamide (epi-3-hydroxy LDB) (23)——The component corresponding to *Rf* (solv. A) 0.65 was obtained as an oily substance (2 mg). *Rf* values: 0.65 (solv. A); 0.13 (solv. B); 0.42 (solv. C). IR[7] $\nu_{max}^{CHCl_3}$ cm^{-1}: 1635 (amide). UV λ_{max}^{EtOH} nm: 241, 313. MS *m/e*: 396 (M$^+$ +1, 19%), 395 (M$^+$, 100%), 365 (10%), 352 (ion A, 8%), 323 (10%), 268 (18%), 223 (ion B, 44%), 222 (46%), 221 (ion C, 65%), 208 (27%), 207 (53%), 196 (19%), 192 (10%), 181 (32%), 180 (10%), 167 (16%), 154 (14%). High resolution MS Calcd. for $C_{24}H_{33}N_3O_2$: 395.257. Found: 395.260 (M$^+$).

Although the crude extract of metabolites of LDB (**10**) was carefully examined by TLC, no spot corresponding to LAB (**11**), an N-debutyl product of LDB (**10**), was observed.

Table II. Oxidation with Jones' Reagent and Reduction with Sodium Borohydride

Starting material*	Reagent	Products
2-HydroxyLDP(**19**): 3 mg	J.R.: 0.2 ml Acetone: 2 ml	2-OxoLDP(**18**): 3 mg
Epi-2-hydroxyLDP(**20**): 2 mg	J.R.: 0.1 ml Acetone: 1 ml	2-OxoLDP(**18**): 2 mg
2-OxoLDP(**18**): 5 mg	$NaBH_4$: 5 mg MeOH: 2 ml	2-HydroxyLDP*(**19**): 2.5 mg Epi-2-hydroxyLDP*(**20**): 2.4 mg
3-HydroxyLDB(**22**): 1 mg	J.R.: 0.05 ml Acetone: 1 ml	3-OxoLDB(**21**): <1 mg
Epi-3-HydroxyLDB(**23**): 1 mg	J.R.: 0.05 ml Acetone: 1 ml	3-OxoLDB(**21**): <1 mg
3-OxoLDB(**21**): 1 mg	$NaBH_4$: 2 mg MeOH: 1 ml	3-hydroxyLDB(**22**): <1 mg Epi-3-hydroxyLDB(**23**): <1 mg

J.R.=Jones reagent.
* Small-scale experiments were necessary because of the limited supply of lysergic acid, which is a starting material for synthesis of these substrates. Identification of the products was carried out by TLC (solv. A) and mass spectrometry.

Experiment with Lysergic Acid Diallylamide (LDA) (13)

Microbial transformation of LDA (**13**) (10 mg) gave a mixture showing two spots on TLC [*Rf*(solv. A): 0.81 and 0.70]. Preparative TLC (solv. A) of the mixture gave unchanged LDA (**13**) [*Rf*(solv. A) 0.81; 7 mg] and LAA (**15**) [*Rf*(solv. A) 0.70; 1.5 mg].

Discussion

In the experiment with lysergic acid dimethylamide (LDM) (**6**), an N-demethylated product, lysergic acid methylamide (LAM) (**7**), was obtained as a sole metabolite. Such N-dealkylation of an N,N-dialkylamide group has been observed for LSD (**1**) in *S. roseochromogenes*. On the other hand, similar treatment of lysergic acid di-*n*-propylamide (LDP) (**8**) or di-*n*-butylamide (LDB) (**10**) gave three new metabolites [LDP (**8**) gave lysergic acid *n*-propyl-*n*-2-oxopropylamide (2-oxo LDP) (**18**),*n*-propyl-*n*-2-hydroxypropylamide (2-hydroxy-LDP) (**19**), and *n*-propyl-*n*-epi-2-hydroxypropylamide (epi-2-hydroxy LDP) (**20**), while LDB

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(**10**) gave lysergic acid *n*-butyl-*n*-3-oxobutylamide (3-oxo LDB) (**21**), *n*-butyl-*n*-3-hydroxybutylamide (3-hydroxy LDB) (**22**), and *n*-butyl-*n*-epi-3-hydroxybutylamide (epi-3-hydroxy LDB) (**23**)], but no N-dealkylation product [LAP (**9**) or LAB (**11**)] was obtained.

As regards the metabolites obtained from LDP (**8**), the high resolution mass spectra of the molecular ions of 2-hydroxy- (**19**) and epi-2-hydroxy- (**20**) LDP indicate the same molecular formula, $C_{22}H_{29}N_3O_2$. In the same manner, the more oxidized molecular formula, $C_{22}H_{27}N_3O_2$, was obtained for the third metabolite, 2-oxoLDP (**18**), by measurement of the high resolution mass spectrum of its parent ion. The presence of a ketonic group in the molecule of 2-oxoLDP (**18**) was recognized by the appearence of a new carbonyl band at 1736 cm^{-1} in the IR spectrum. In the NMR spectrum, 2-hydroxyLDP (**19**) and epi-2-hydroxyLDP (**20**) show a 3H doublet together with a 3H triplet in the C-methyl region, as shown in Fig. 2, suggesting that these compounds have both $CH_3CH(OH)$– and CH_3CH_2– groups in their molecules. These observations strongly suggest that both 2-hydroxyLDP (**19**) and epi-2-hydroxyLDP (**20**) are formed by the hydroxylation of LDP (**8**) at the (ω-1) position of an *n*-propyl group, indicating that these two metabolites are epimeric at the inserted hydroxy group. The validity of this view was confirmed by the finding that oxidation of both metabolites (**19** and **20**) with Jones' reagent gave the same compound (**18**) as a sole product. This oxidation product was identical with the third metabolite, 2-oxoLDP (**18**), by TLC comparison, and reduction of 2-oxoLDP (**18**) with sodium borohydride conversely produced a mixture of equal amounts of 2-hydroxyLDP (**19**) and epi-2-hydroxyLDP (**20**).

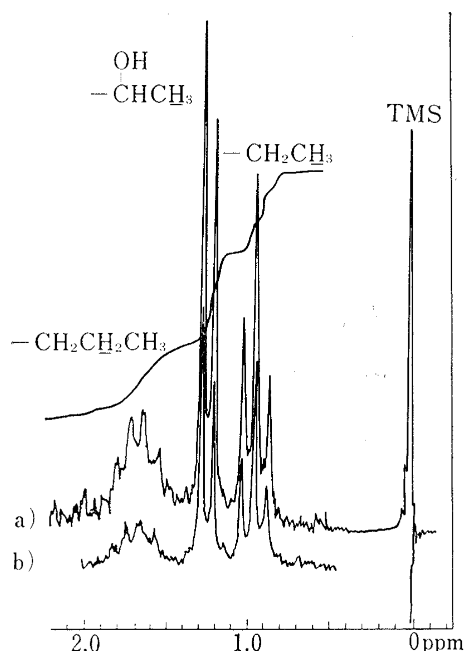

Fig. 2. NMR Spectra of 2-HydroxyLDP(**19**) (spectrum a) and 2-EpihydroxyLDP(**20**) (spectrum b) in the Region of the C-Methyl Group

In the mass spectrum, 2-hydroxyLDP (**19**) and epi-2-hydroxyLDP (**20**) show a set of diagnostically important ions at *m/e* 324, 223, and 221. As reported in the previous paper,[9] these ions are assignable to the fragment ions A, B, and C (Chart 4), produced by retro Diels–Alder fragmentation of the molecular ion (ion A), removal of an amide group situated at the C_8 position from the molecular ion (ion B), and aromatization of the ion B (ion C), whereas 2-oxo LDP (**18**) shows the ion A shifted to *m/e* 322. These observations support our proposed structures for the metabolites (**18**, **19**, and **20**). Since it is well known that derivatives of lysergic acid (**12**) readily epimerize at the C_8 position even under mild conditions, our proposal cannot be regarded as definitive. However, we could not detect any epimerized metabolite in the experiments with LSD (**1**), LDM (**6**), and LDA (**13**), suggesting that epimerization at the C_8 position did not take place during the microbial transformation.

As regards the three metabolites (**21**, **22**, and **23**) of LDB (**10**), their molecular formulae were determined to be $C_{23}H_{31}N_3O_2$ (**22** and **23**) and $C_{23}H_{29}N_3O_2$ (**21**) by measurement of their molecular ions in the high resolution mass spectra. The first two compounds (**22** and **23**) showed a 3H doublet together with a 3H triplet in the C-methyl region in the NMR spectrum; in addition, oxidation of these two compounds (**22** and **23**) with Jones' reagent gave the third metabolite (**21**), and reduction of the third metabolite (**21**) with sodium borohydride yielded a mixture of equal amounts of the first two compounds (**22** and **23**), as in the case

9) T. Inoue, Y. Nakahara, and T. Niwaguchi, *Chem. Pharm. Bull.* (Tokyo), **20**, 409 (1972).

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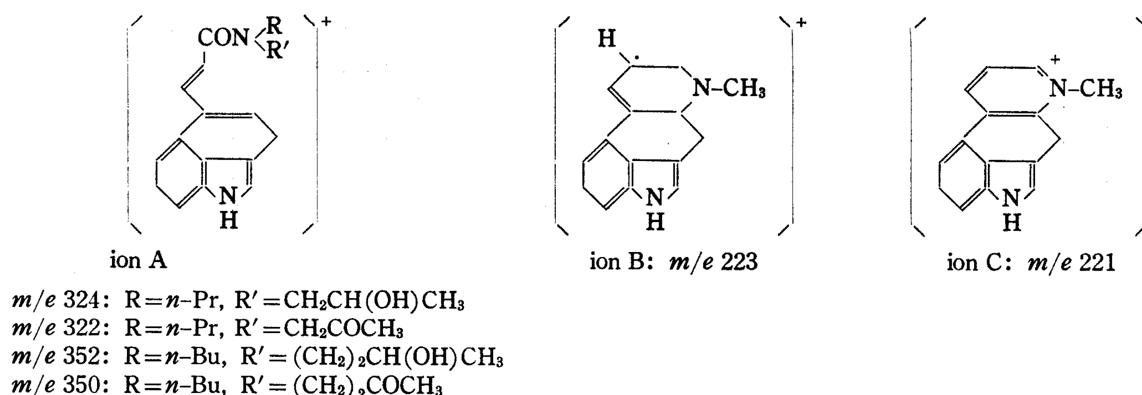

Chart 4. Diagnostically Important Fragment Ions of LSD Congeners

of the metabolites of LDP (**8**). Thus, we may conclude that these are (ω-1) oxidation products of LDB (**10**) [lysergic acid *n*-butyl-*n*-3-hydroxybutylamide (**22**), *n*-butyl-*n*-epi-3-hydroxybutylamide (**23**), and *n*-butyl-*n*-3-oxobutylamide (**21**)]. This conclusion was supported by examination of their mass spectra.

The results on microbial transformation of various amide congeners of LSD (**1**) (Table I) clearly show that the length of the alkyl chain on the amide group of a substrate is a determinant in the regulation of the mode of biotransformation. An α-oxidation takes place in the case of short N-alkyl chains [LDM (**6**) and LSD (**1**)], whereas (ω-1) oxidation is predominant in the case of longer chains [LDP (**8**) and LDB (**10**)]. It should be noted here that an apparent ω-oxidation product, LEO (**4**), was obtained as a metabolite in the experiment with LSD (**1**), but not with LDP (**8**) or LDB (**10**) under the same conditions. These findings seem to be in conflict. However, the chemical evidence that LEO (**4**) was also produced by reincubation[3] of LEV (**3**) suggests that LEO (**4**) was formed only *via* the sequential metabolic pathway, LSD (**1**)→LEV (**3**)→LEO (**4**) and not by direct ω-oxidation of LSD (**1**).

In the previous paper,[3] we reported that *S. roseochromogenes* biotransformed LSD (**1**), but not isoLSD (**24**) which is an epimer of LSD (**1**) at C_8. These findings suggest that the N-dealkylation of an amide chain by *S. roseochromogenes* may proceed stereospecifically. This is supported by the large difference in yields between the epimeric 2-hydroxylated LDP metabolites (**19** and **20**), since, if the microbial (ω-1) hydroxylation took place non-stereospecifically, the yields of both metabolites should be the same. The stereospecificity of the (ω-1) hydroxylation can be explained by the assumption that the substrate is bound at the

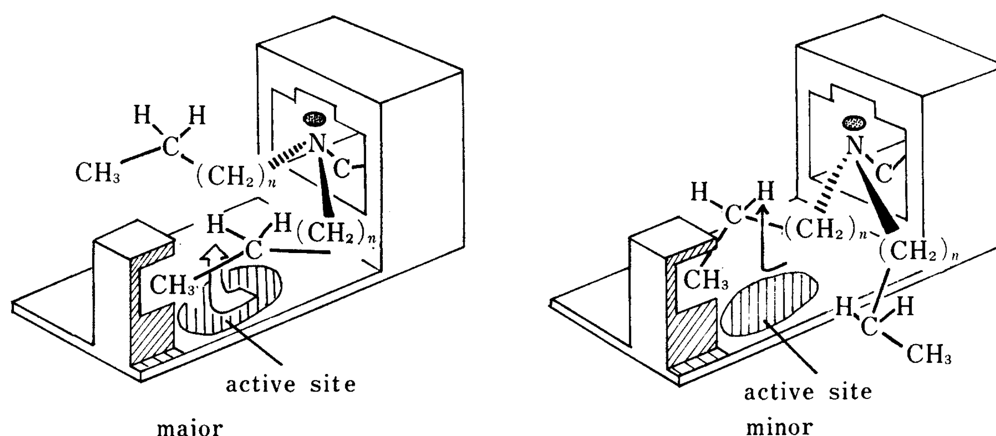

Chart 5. A Possible Model for the Microbial (ω-1) oxidation of an Amide Chain of LSD Congeners

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enzyme surface and attacked from a specific direction of the molecule. Consequently, two alkyl chains in the amide group become non-equivalent. Moreover, this concept can account for the decrease in the difference in yields between the epimeric hydroxylation metabolites of LDB (**10**) on lengthening the alkyl chain of the amide group, since the steric requirement for the attack point in the alkyl chain decreases in some extent. However, other possibilities cannot be completely excluded; for example, the stereospecific hydroxylation of an alkyl group followed by epimerization due to some epimerase, *etc.*

The mode of microbial transformation of lysergic acid diallylamide (LDA) (**13**) is of particular interest. Even though it has the same number of carbon atoms in amide chain as LDP (**8**), LDA (**13**) was oxidized at the α-methylene of the N,N-diallylamide group to give lysergic acid allylamide (LAA) (**15**) in fairly good yield. This result is in striking contrast to that for LDP (**8**). The microbial N-dealkylation of LDA (**13**) could be explained chemically by the reactivity of an allylic position. However, we may instead assume that a substrate having methylene groups at both α- and (ω-1) positions in an amide straight chain would be oxidized preferentially at the latter. An α-oxidation occurs when a substrate has no methylene group at an (ω-1) position. Accepting this view, the oxidation of LSD (**1**) at the α-position of an amide ethyl group can be regarded as an (ω-1) oxidation rather than an α-oxidation. This hypothesis rationalizes the experimental findings quite effectively.

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