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LAMPTEROFLAVIN, THE FIRST RIBOFLAVINYL ALPHA RIBOFURANOSIDE AS LIGHT EMITTER IN THE LUMINOUS MUSHROOM, *L. japonicus*[1]

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Abstract Lampteroflavin (1, Lf) was isolated from the suspension of alive gills of the luminous mushroom, *Lampteromyces japonicus*. It was hydrolyzed into riboflavin (2, Rf) and D-ribose, which were identified by means of HPLC, NMR, CD and FAB-MS/MS. The structure was concluded to be 5'-riboflavinyl α-D-ribofuranoside.

Introduction *TSUKIYO-TAKE* 月夜茸, or "moon night mushroom" (*Lampteromyces japonicus*), is one of the Japanese luminous mushrooms. It is a poisonous mushroom with sizes 15-30 cm. It causes serious diarrhea, since people eat it due to the similar outlook to edible mushroom, Hiratake when young and Mukitake when matured. It grows on a dead trunk of beech tree in mountain area only 1-2 weeks in a year from late September until early October. The gill of the fruiting body was first described to be luminous by Kawamura in 1915.[2] In 1970, some fluorescent compounds were reported to be related to the bioluminescence. Endo et al. isolated illudin S (Em_{max} 550 nm), ergosterol, ergosterol peroxide and ergosta-4,6,8(14),22-tetraen-3-one (Em_{max} 530 nm) from the fruiting body and mycelia of the mushroom (*Lampteromyces japonicus*)[3]. The fluorescence emission maximum were reported to be 530-550 nm which were similar to the bioluminescence peak of luminous fungi, *Armillaria mellea, Collybia velutipes,* and *Omphalia flavida*.[4]

Light emission by organisms *(bioluminescence)* required at least three different substances. One is an enzyme, luciferase, which catalyzes oxidation of a low molecular weight substrate, luciferin. Molecular oxygen is the third requirement for this oxidation. Luciferin is usually oxidized to oxyluciferin in its electronically excited state. An excited electronic state intermediate or product must be a fluorescent molecule and consequently emits light when it returns from the excited state to the ground state. Or the excited state molecule transfers its energy to a light emitter, which gives light corresponding to the fluorescence spectrum. The luminous gills in nature were not strong enough to measure its bioluminescence spectrum with an ordinary spectrofluorometer. Enhancement of the luminescence intensity by exposing the gills to oxygen gas enabled the measure-

ment. The bioluminescence spectrum being maximum at 524 nm was identical with that of the luminous mycelia being cultured from a single colony isolated from the spore culture of the mushroom.[5] The bioluminescence spectrum, having a maximum at 524 nm suggested that the chromophore of the light emitter should be isoalloxazine such as riboflavin, FMN (flavin mononucleotide), FAD (flavin adenine dinucleotide) and lumiflavin.

Chemical studies have been concerned with the identification of the fluorescent components involved in the light-emitting reaction. Most of the efforts toward extraction and purification of the mushroom constituent have been directed to elucidate the structure of the fluorescent compounds. We report here the details of structure elucidation of lampteroflavin, a new fluorescent compound composed of α-glycosidic linkage between riboflavin and D-ribose.

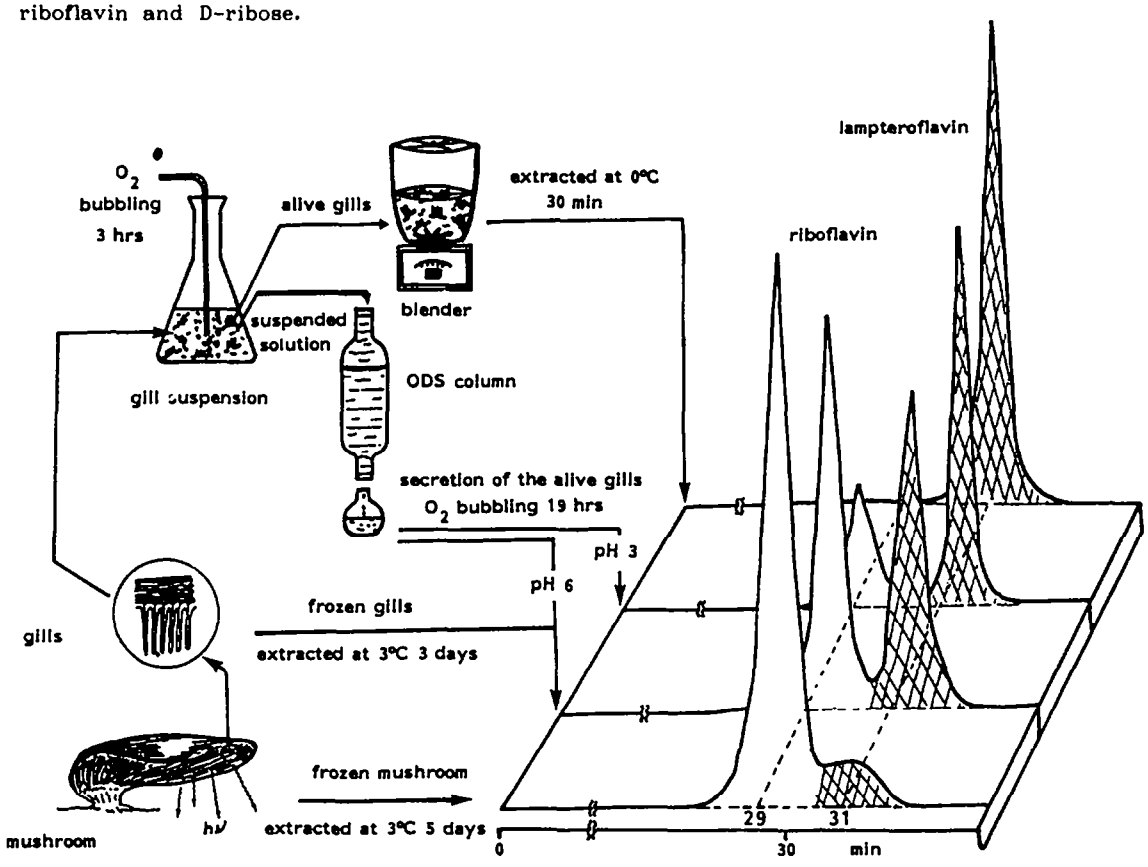


Fig. 1 Extraction of Lampteroflavin, source and analysis

Materials, Mushroom Since the mushroom was not available in off-season, the mushrooms were frozen after harvest and stocked at -30°C. In this case the whole body of the mushroom was pulverized in liquid nitrogen and extracted with organic solvents such as acetone, hexane and dichloromethane. Little and weak fluorescent compounds, however,

were detected from HPLC analysis with Develosil silica gel 60-5 column (4x250 mm) using a mixture of dichloromethane-methanol as an eluant, monitoring with UV (254 nm) and fluorescence (Ex/Em 445/524 nm) detector. We switched the extraction method by using cold water instead of organic solvent. An aqueous extract of the frozen mushroom gave a strong green fluorescent compound which was identified to be riboflavin (Rf) through HPLC analysis [using Develosil ODS-5 column (4x250 mm), and a mixture of methanol-water as an eluant]. It was isolated in less than 1 mg from 5 kg (whole body). Riboflavin showed its fluorescence maximum at 524 nm, which was identical with the bioluminescence, spectrum of the mushroom *vide supra*. Thus, riboflavin was temporarily concluded to be the light emitter of the mushroom.[5]

Light Emitter In our further studies has been found another new fluorescent compound which was named "lampteroflavin" (Lf).[6] This compound seemed to be closely related to riboflavin, but its identification was very difficult due to instability. For example, it was impossible to isolate Lf from frozen mushrooms (whole body) with cold water. Lf could be detected occasionally in the HPLC analysis right after the riboflavin (Rf) peak as a shoulder. After partial purification with an ODS column, Lf was eluted after Rf and the ratio of Lf:Rf was ca. 1:>12. This ratio was improved into (Lf:Rf) 1:1.2 when the frozen gills (luminous part of the mushroom) were utilized instead of the whole body. We have developed a new method by using alive gills instead of the frozen gills. The fresh gills were suspended in water with aeration at 17-20°C overnight and the medium was concentrated with an ODS column (28 x 310 mm) to give a mixture of the fluorescent compounds Lf:Rf in a ratio of 1:1.24. Lampteroflavin was isolated from this medium in about 100 μg from 6 kg fresh gills (corresponding to 50 kg of whole mushroom body). When the fresh gills were suspended in dilute hydrochloric acid solution (pH 3) instead of neutral aqueous solution, the ratio Lf:Rf highly improved to 1:0.3 (**Fig.1**). Hence, we obtained pure lampteroflavin capable to study structural elucidation.

NMR, UV, FL & MS Spectra of Lampteroflavin Lampteroflavin was apparently soluble in water though riboflavin was sparingly soluble in water (less than 1 mg in 15 ml). Both flavins were analyzed with 500 MHz ^{1}H NMR to show Me signals around δ2.5 ppm and two aromatic H's around δ7.9 ppm which were obviously known as flavin. Lampteroflavin showed the same signals of a ribityl group as riboflavin and 6 more additional signals. The proton nmr data are summarized in **Table 1**. One of them (δ5.17 ppm) was assigned to be an anomeric proton (H-1"), since its chemical shift did not change a lot after acetylation. The appropriate double resonance experiments with the ^{1}H NMR of riboflavin acetate, lampteroflavin, and its acetate led us consider that the 6 protons should belong to a pentofuranosyl group connecting to riboflavin with an acetal linkage.[6] There were two methylene groups at C-1' and C-5' which could be assigned interchangeably even in riboflavin itself judging from the value of 0.5 ppm difference between free form and acetate form for primary alcohol. However, this problem was solved by comparing the

chemical shifts with those of lampteroflavin and its acetate. We found that the chemical shifts of one of the methylene groups of lampteroflavin (δ=4.01 and 3.84 ppm for the free form, δ=3.95 and 3.82 ppm for the acetate form) did not change significantly. This fact suggested that this methylene group should be at C-5' position which further connected to the pentofuranosyl group. Connectivity among the flavin chromophore, the ribityl group and the pentofuranosyl group was concluded from nuclear Overhauser

Table 1 Proton NMR data of Lampteroflavin and its acetate

H	lampteroflavin[1]	NOE[3]	lampteroflavin acetate[2]	NOE[3]	difference LfOAc-Lf
1'	5.08(m, 12,3)	a d	5.68(m)	a C d	0.60
1'	4.88(m, *)	a	5.51(m)	a b	0.63
2'	4.38(m, 6.7,3)	d	5.68(m)	a b C d	1.30
3'	4.01(dd, *)	a c d	5.51(m)		1.50
4'	4.15(m, 11.7 6.6, 3.3)	a c e	5.37(m, 4.2,3.1)		1.22
5'	4.01(brd, 11.7)	a b c d	3.95(dd, 11.6,4.2)	a B	-0.06
5'	3.84(brd, 11.7)	b	3.82(dd, 11.6,3.1)	a	-0.02
1"	5.17(d, 4.0)	A b d	5.30(d, 4.2)	A b	0.13
2"	4.15(m)	(c) e	4.94(m, 7.1,4.2)		0.79
3"	4.07(m)	a b e	5.16(dd, 7.1,3.9)	a	1.09
4"	4.15(m, 5.0, 3.3, 3.3)	(c)	4.26(m, 3.9,3.6, 2.9)		0.11
5"	3.75(brd, 13.0)	c d	4.18(dd, 12.0,3.6)		0.43
5"	3.89(brd, 13.0)	c d E	4.34(dd, 12.0,2.9)		0.65
7-Me	2.57(s)	a B e	2.57(s)		0
8-Me	2.45(s).	C	2.45(s)		0
6	7.84(s)	D	7.57(s)	c	-0.27
9	7.89(s)	D	8.05(s)	c D	0.16
3	*		8.36(s)		
4'-Ac			2.22(s)		
3'-Ac			2.10(s)		
2'-Ac			1.71(s)		
2"-Ac			2.14(s)		
3"-Ac			2.13(s)		
5"-Ac			2.32(s)		

1) measured in D_2O, t-BuOH (1.27ppm) as standard
2) measured in $CDCl_3$, TMS (0.0ppm) as standard
3) Capital letters = irradiated signals and small letters = effected signals
* not readable ( ): data may be interchangable

effect by irradiating certain protons such as H-1", H-5' or aromatic proton. Its UV and fluorescence spectra were identical to the riboflavin spectra, showing UVmax (in H_2O) at 265, 368 and 445 nm, fluorescence emission maximum at 524 nm, and excitation maximum at 445 nm as shown in **Fig.2.** The molecular formula of lampteroflavin was established as $C_{22}H_{29}N_4O_{10}$ (mw 508) from high resolution fast atom bombardment mass spectrum (FAB-MS) and tandem mass spectrum (FAB-MS/MS). FAB-MS/MS of lampteroflavin at m/z 509 (M+1) gave daughter ions at m/z 377 (riboflavin + 1), 376 (riboflavin) and 243 (isoalloxazine ring). The difference between the m/z 509 and m/z 376 revealed the presence of dehydroxy-pentose. This observation was supported by FAB-MS/MS of lampteroflavin acetate; m/z 761 (M+1), 503 (riboflavin triacetate + 1) and 259 (deacetoxy-pentose acetate).

Physical Data of Lampteroflavin

UV_{max} (in H_2O):
265, 368, 445 nm
FL (in H_2O):
Em_{max} 524 nm, Ex_{max} 445 nm
MS (FAB positive):
m/z 509.1913 (M+1)
calcd for $C_{22}H_{29}N_4O_{10}$ 509.1882
FAB-MS/MS: (precursor m/z 509) to yield
m/z 377, 376 ($M-C_5H_{10(11)}O_4$),
243(chromophore)
HPLC: column ODS (0.4 x 25 cm),
solvent 25% MeOH/H_2O,
flow rate 0.8 mL/min,
eluted at 31 min

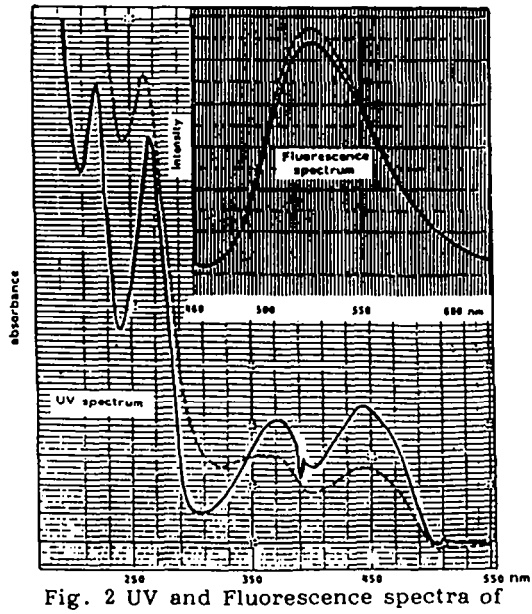


Fig. 2 UV and Fluorescence spectra of
Riboflavin ————
Lampteroflavin- - - -

Tandem Mass Spectra of Lampteroflavin and Its Acetate Further stereochemical information of lampteroflavin was attempted to obtain from the tandem mass spectrum of its acetate by focusing the fragment ion at m/z 259 (**Fig. 3 (5)**). Its He collision spectrum **(6)** was extensively compared with tandem mass spectra of the authentic acetates which were synthesized from four pentoses including ribose **(1)**, arabinose **(2)**, xylose **(3)** and lyxose **(4)**. Those FAB-MS/MS spectra as well as that of lampteroflavin acetate **(6)** gave daughter ions of m/z 199 (M-60), 157 (M-60-42), 139 (M-{60x2}), 97 (M-{60x2 - 42}) and 43 (C_2H_3O) as shown in **Fig. 3.** The fragmentation pattern of lampteroflavin acetate **(6)** was quite similar to that of ribose acetate[7] **(1)** judging from the peak intensity of the order m/z 139 > 157 > 199 ~ 97. Thus, we could temporary assign that ribose should

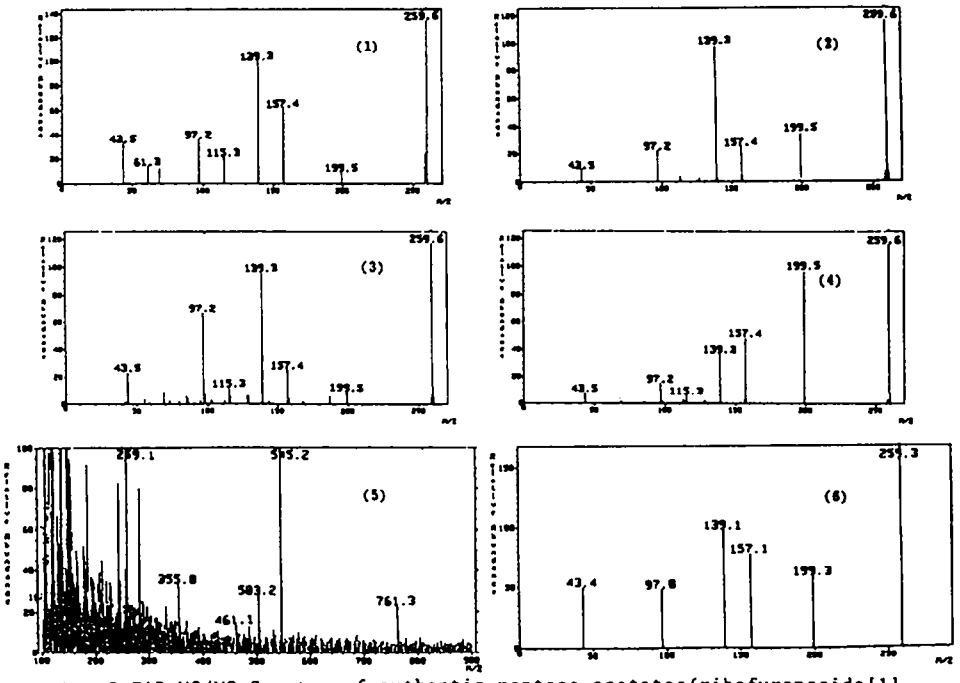


Fig. 3 FAB-MS/MS Spectra of authentic pentose acetates(ribofuranoside[1], arabinofuranoside[2], xylofuranoside[3] and lyxopyranoside[4]) compared with lampteroflavin acetate[6] using m/z 259 as a precusor ion for He collision. FAB-MS of lampteroflavin acetate[5].

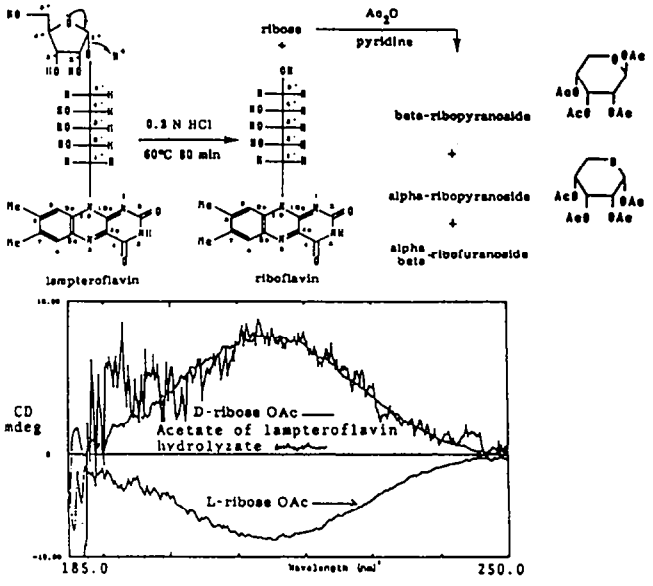


Fig. 4 Comparison of CD spectra of acetates of lampteroflavin hydrolyzate and D, L beta-ribopyranoside.

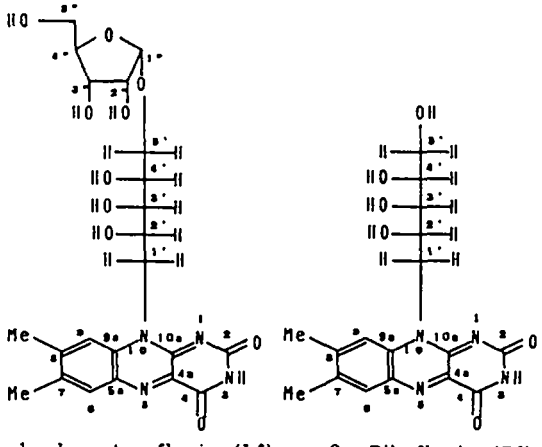
be the pentose of lampteroflavin. This conclusion was the first example applying tandem mass spectrometry to identify the stereoisomers in trace amount. Further study should be awaited to make this method for general utility.

Acid Hydrolysis of Lampteroflavin to Identify D-Ribose The presence of ribose was finally confirmed by acid hydrolysis and acetylation of lampteroflavin after we obtained 10 mg quantity of lampteroflavin. When lampteroflavin (ca 5 mg) was heated in 0.2 N HCl at 60°C for 80 min, the glycosidic bond was cleaved into riboflavin[8] and D-ribose (isolated as acetate: see experimental part for detail). CD spectrum of this acetate was compared with authentic peracetates of D- and L-ribose. The positive sign around 215

Table 2 ^{12}C NMR of Lampteroflavin (Lf), Riboflavin (Rf) and Methyl α-D-Ribofuranoside

C	Rf	Lf	Lf-Rf
4	159.7	162.6	2.9
2	155.2	158.9	3.7
10	150.6	151.7	1.1
8	145.8	147.7	1.9
4a	136.5	139.9	3.4
7	135.5	136.1	0.6
5a	133.8	133.5	-0.3
9a	131.9	132.1	0.2
6	130.5	130.5	0.0
9	117.2	118.4	1.2
3'	73.5	74.8	1.3
4'	72.6	72.7	0.1
2'	68.7	70.9	2.2
5'	63.2	70.6	7.4
1'	47.1	49.3	2.2
8α	20.6	22.2	1.6
7α	18.6	20.1	1.5
1"	103.1‡	103.2	0.1
2"	71.1‡	71.5	0.4
3"	69.8‡	70.3	0.5
4"	84.6‡	86.2	1.6
5"	61.9‡	62.8	0.9

taken in D_2O (tert-butanol as standard at 31.2 ppm)
‡ =Methyl α-D-Ribofuranoside



1 Lampteroflavin (Lf) 2 Riboflavin (Rf)

Table 3
^{13}C NMR data of pentofuranosides, comparison between 1,2-cis and 1,2-trans configuration

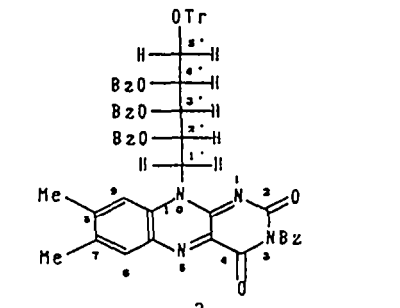
sugar	Methyl glycoside assignment	C-1		C-2	
		1,2-cis	1,2-trans	1,2-cis	1,2-trans
riboside	α	103.1		71.1	
	β		108.0		74.3
arabino-side	α		109.2		81.8
	β	103.1		77.4	
xyloside	α	103.0		77.8	
	β		109.7		81.0
lyxoside	α		109.2		77.0
	β	103.3		73.2	
average		103.1	109.0	74.9	78.5
lampteroflavin		103.2		71.5	

nm was identical to that of D-ribose acetate as shown in **Fig. 4**. This experiment led us to conclude that the ribose was identified to be D configuration.

NMR Spectroscopy to prove α Configuration The α-glycosidic bond was assigned from ^{13}C NMR studies (**Table 2**). The reported chemical shifts of methyl pentofuranosides were arranged and grouped into the "1,2-cis" configuration which appeared at higher field than that of "1,2-trans" configuration as shown in **Table 3**. The chemical shifts of the anomeric signal (C1") of lampteroflavin at δ103.2 ppm and C2" at δ71.5 ppm, suggested the 1,2-cis configuration. When the chemical shift of the anomeric signal of methyl-α and β-D-ribofuranoside (δ103.1 and δ108.0 ppm respectively)[9] were compared with that of lampteroflavin, the linkage between ribose and riboflavin of lampteroflavin was assigned to be α-linkage. The α-linkage was confirmed by irradiation of H-1" at δ5.30 (d, J=4.2 Hz) effected its H-3" at δ5.16 (dd, J=7.1, 3.9 Hz) in 500 MHz NOE difference spectrum of lampteroflavin acetate (**Table 1**).

CD Studies on Sugar Moiety In the early stage of the structural studies on lampteroflavin, only limited amount of Lf was available; thus, chemical degradation could not be achieved. We anticipated circular dichroism (CD) spectroscopic method to obtain stereochemical information about the pentose moiety. Lampteroflavin (isolated from mushroom gills) was benzoylated and its CD spectrum was measured (the spectrum being abbreviated "L"). CD Spectrum of tribenzoates of D-riboside (1-β-methoxy-2,3,5-tribenzoylribofuranoside) and D-arabinoside (1-α-acetoxy-2,3,5-tribenzoylarabinofuranoside) were measured and utilized as the authentic spectrum as shown in **Fig. 6** and **Fig. 7**, respectively. 5'-Trityl-3,2',3',4'-tetrabenzoylriboflavin (3, "T" being abbreviated this CD spectrum) was synthesized based upon such a working hypothesis that CD spectrum of the representative sugar could be obtained after subtracting the other non-sugar moiety T from the whole structure.[10] In fact, the representative sugar of lampteroflavin benzoate showed negative cotton effect identical with arabinofuranoside at 210-250 nm as shown in **Fig. 5** ("L-T"). In order to confirm the working hypothesis, β-D-ribofuranosyl riboflavin benzoate (4, "R" being abbreviation of its CD spectrum) and α-D-arabinofuranosyl riboflavin benzoate (5, "A" being abbreviated of its CD spectrum) were synthesized by coupling the bromobenzoyl derivatives of D-ribose and D-arabinose with tritylbenzoyl derivative[11] of riboflavin. Since the representative sugar of 4 ("R-T") and of 5 ("A-T") gave the same CD spectrum which showed the same sign as their authentic D-ribofuranoside and D-arabinofuranoside as shown in **Fig. 6** and **Fig. 7**, respectively.

The pentose of lampteroflavin should have been concluded to be D-arabinose since it gave the same negative sign in CD spectrum as the corresponding benzoate. However, the ^1H NMR of L and A were not identical. This disagreement might be due to the difference with anomeric isomerism between them or a higher unexpected special interaction in CD spectra. So far the CD spectroscopic method particularly of its additivity could



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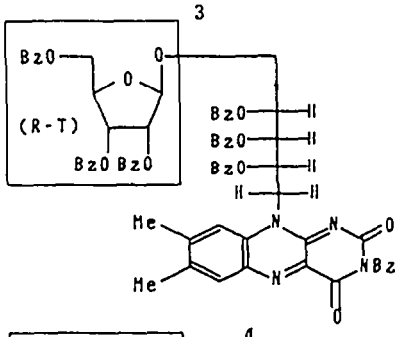

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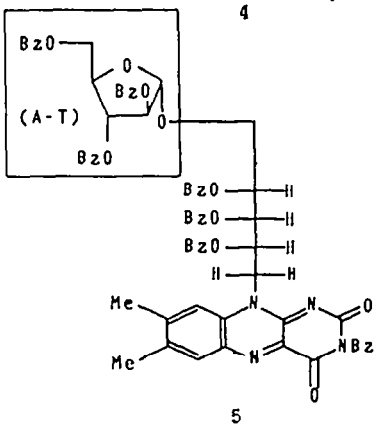

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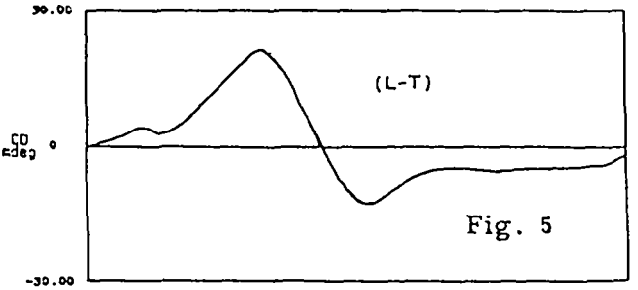

Fig. 5

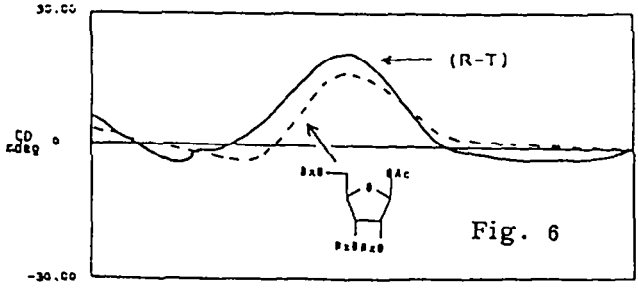

Fig. 6

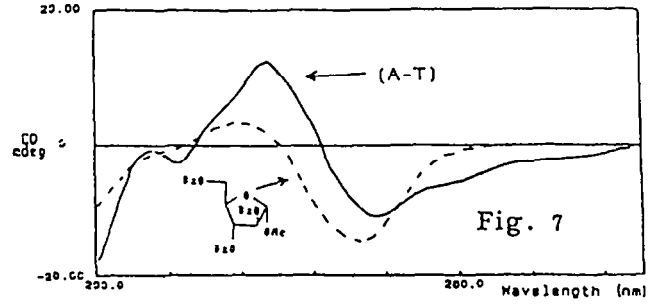

Fig. 7

CD spectra in CH_3CN

not be applied for the determination of the current system. The conformation of lampteroflavin benzoate might not be a linear structure so that the exciton(s) could have interactions between the remote ones to each other. These suggest the folding structure of lampteroflavin analogs. Further studies are awaited.

Overview of Lampteromyces Bioluminescence

1. The light intensity of moon-night-mushroom, *Lampteromyces japonicus*, varied from time to time as well as from place to place. The difference of the light intensity was due to the different pH on the gill surface, which is usually acidic. The difference could be corrected to almost identical strength by washing the luminous gills with water.

2. Lampteroflavin was secreted from the luminous cells onto their surface, where it seemed to be hydrolyzed with an enzyme to furnish riboflavin. The pentose moiety was analyzed with ultra micro analyses by FAB-MS/MS and NMR. Finally it was concluded to be ribose through milligram scale analysis after the acid hydrolysis of lampteroflavin.
3. Experiments utilizing alive luminous gills from the mushroom implied that lampteroflavin was only the green fluorescent component (Em_{max} 524 nm) existed both outside and inside of the gills. In addition, the fluorescence spectrum of lampteroflavin was identical to the bioluminescence spectrum of the mushroom. Therefore, we concluded that lampteroflavin should be participated in the mushroom bioluminescence as the light emitting species.
4. Since the structure of lampteroflavin was confirmed through chemical synthesis (to be published later), the enzymatic system will be disclosed by using this substance as a counterpart for the studies of the bioluminescence mechanism.

Conclusions

Lampteroflavin was determined to be 5'-riboflavinyl α-D-ribofuranoside. It is of interest that lampteroflavin is the first demonstration of riboflavinyl ribofuranoside besides a 5'-riboflavinyl α-glucopyranoside.[12-18] The absolute configuration of lampteroflavin was proved by chemical synthesis.[19]

The finding of lampteroflavin suggests some necessary re-investigation on the other components in microorganism and plants which were reported to produce riboflavin[20]. On the other hand this finding will lead us to a new field of further studies on lampteroflavin as the active principle in biological functions for which riboflavin (vitamin B_2) has been responsible.

Experimental

Proton and carbon nuclear magnetic resonance spectra were recorded on FT NMR machine; Jeol JNM-FX100, FX200 and GX500 spectrometers. All spectra were dissolved in D_2O or $CDCl_3$ and chemical shifts are reported as delta values in parts per million relative to t-butanol (^1H 1.27 ppm in D_2O) or tetramethylsilane (^1H and ^{13}C 0.00 ppm in $CDCl_3$) as internal standard. Mass spectra were recorded on JMS DX-300 and DX-705L instruments. Ultraviolet spectra were measured with Jasco Uvidec 505 UV/Vis recording digital spectrophotometer and UV/Vis spectrophotometer 660. Fluorescence spectra were recorded on Jasco FP770 spectrofluorometer, Hitachi MPF-2A fluorescence spectrophotometer, Jasco FP-550A spectrofluorometer. CD spectra were recorded on Jasco J-500E and J-600 spectropolarimeters. HPLC analyses were performed with an assembly of Jasco Tri Rotar-V or Twincle pump with UV detectors, Uvidec-100-V or Uvidec-100, at 254 nm. They were often monitored with a fluorescence detector, Jasco FP-110C at excitation wavelength 445 nm and emission wavelength 524 nm. Occasionally they were detected with refractive index detector, Shodex RI SE-11 or Jasco 830-RI Intelligent RI detector. Another assembly system was Water Associates PrepLC/System 500 with a special stainless column (20 x 600 mm) packed with Micro beads silica gel ODS-W, 5D(100-200), Fuji-Davison Chemical Ltd. For semi-preparative scale of separation and purification we used a stainless column (20 x 250 mm) Develosil ODS-10, or a glass column (28 x 310

mm) Develosil Lop ODS-30. For the analytical purposes we used stainless column (4 x 250 mm or 4 x 150 mm) Develosil ODS-5 for reversed phase chromatography (elusion was isocratically using methanol:water 25:75) and stainless column (4 x 250 mm or 4 x 150 mm) Develosil silica gel 60-5 or 100-5 for normal phase chromatography (elusion was isocratically using EtOAc:Hexane 1.5:1). Develosil columns were supplied by Nomura Chemical Co, Ltd.

Bioluminescence spectra

The mushroom, *L. japonicus*, was collected in Yamagata and Gifu prefectures, Japan. The spores were suspended in sterile water and cultivated at 23°C for 1-2 weeks in potato sucrose agar (potato 20%, sucrose 2%, agar 2% in distilled water) containing acromycin (0.015%). An isolated single colony was inoculated to 180 mL potato sucrose media (potato 20%, sucrose 2% in distilled water) in Sakaguchi flasks and shaken at 23 °C at 120 rpm for two weeks. The bioluminescence spectrum was measured with a high sensitivity fluorescence spectrophotometer by passing oxygen through a suspension of washed mycelia (ca. 1.5 mL in 5.0 mL distilled water). This measurement was also performed with the gills pilled from the fruiting body of the mushroom.

Isolation of the green fluorescent substances (riboflavin and lampteroflavin).

Five kilograms of fresh gills were separated from fruiting body (40 kg) and well washed with water. It was then immersed in diluted hydrochloric acid solution pH 3 (40.0 L) and oxygen was introduced into the suspension for 15 hours at 19-21°C. The suspension was filtered through celite and the filtrate (60 L) containing lampteroflavin was concentrated by the first adsorbing on an ODS column, washing with water, and eluting with methanol. The methanol eluate, showing green fluorescence, was evaporated under reduced pressure at low temperatures to nearly dryness. The residual oil was repeatedly purified by two ODS columns (20 x 250 mm and 4 x 250 mm, at 5.0 and 0.8 mL/min, respectively with methanol:water 25:75 as an eluant) to obtain riboflavin ca. 300 μg and lampteroflavin ca. 1 mg. ^1H NMR: **Table 1**, ^{13}C NMR : **Table 2**, UVmax (in H_2O) 265, 368 and 445 nm, fluorescence emission maximum at 524 nm and excitation maximum at 445 nm, high resolution FAB-mass spectrum m/z 509.1913 (M+1, calcd for $C_{22}H_{29}N_4O_{10}$ 509.1882). FAB-MS/MS 509, 377, 376 and 243.

Acetylation of lampteroflavin

The green fluorescent substances containing riboflavin (Rf) and lampteroflavin (Lf) after partial purification with ODS columns were acetylated with a mixture of acetic acid and acetic anhydride (1:1) using 6M $HClO_4$ as catalyst at 10°C for 1.6 hour. Water was added and the aqueous solution was extracted repeatedly with $CHCl_3$. The $CHCl_3$ solution was washed with water and evaporated to dryness under reduced pressure. The residue was dissolved in $CHCl_3$ and applied to a Develosil silica gel 100-5 column (4 x 150 mm) using a mixture of $CHCl_3$:MeOH:EtOAc 82:1:5 as an eluant. ^1H NMR of Rf-OAc: 5.68 (m), 5.46 (m), 5.42 (m), 4.43 (dd, J=12.5, 2.5 Hz), 4.25 (dd, J=12.5 Hz), 2.57 (s), 2.45 (s), 7.57 (s), 8.04 (s), 8.34 (s), 2.29 (s), 2.22 (s), 2.08 (s), 1.75 (s); ^1H NMR of Lf-OAc see **Table 1**.

Acidic hydrolysis of lampteroflavin

Lampteroflavin ca. 5 mg was dissolved in 200 μL of distilled water and 50 μL of 1N HCl was added to the solution, the mixture was kept at 60°C for 80 min. The completed hydrolysis of lampteroflavin to be riboflavin was examined by HPLC with an ODS column (4 x 250 mm), using 25% methanol-water as a solvent system as mentioned before. Riboflavin and lampteroflavin were eluted at 28 min and 31 min, respectively. The hydrolysates were evaporated and acetylated with acetic anhydride in pyridine at room temperature overnight. Ribose acetates were first separated by silica gel TLC using a mixture of EtOAc:Hexane 1.5:1 as a developing solvent. The mixture of α and β anomer of acetyl furanosides and acetyl pyranosides were then separated by HPLC (Develosil 60-5 silica gel column) using a mixture of EtOAc:Hexane 19:100 as an eluant to obtain α- and β-pyranosides in ca. 100 μg each. Both of them were measured ^1H NMR and CD spectra in comparison to authentic α- and β-ribopyranoside acetates (**Fig. 7**). ^1H NMR (β-ribopyranoside): 6.03 (d, J=5.1 Hz), 5.49 (t), 5.15 (m), 5.04 (m), 4.02 (dd, J=12.3 Hz), 3.91 (dd, J=12.3 Hz); ^1H NMR (α-ribopyranoside): 6.07 (d, J=3.8 Hz), 5.54 (m), 5.15 (t), 5.08 (m), 4.04 (dd, J=11.4 Hz), 3.74 (dd, J=11.4 Hz).

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