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Lampteromyces Bioluminescence

3. Structure of Lampteroflavin, the Light Emitter in the Luminous Mushroom, *L. japonicus*

DUANGCHAN UYAKUL, MINORU ISOBE,[1] AND TOSHIO GOTO

Laboratory of Organic Chemistry, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya 464, Japan

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The structure of lampteroflavin, the light emitter in the luminous mushroom (*Lampteromyces japonicus*), was determined to be a new riboflavin α-D-riboside, its structure being elucidated on the basis of fast atom bombardment tandem mass spectrometry, NMR, and CD studies. © 1989 Academic Press, Inc.

Bioluminescence in fungi was generally reviewed by Wassink in at least 17 species of the basidiomycetes such as *Armillaria mellea, Pleurotus olearius* (*Clitocybe illudens*), *Omphalia flavida,* and *P. japonicus* (*Lampteromyces japonicus*) (*1*). There was little information concerning the mechanism of fungal bioluminescence. Hence, this study was conducted to elucidate the structure of the light emitter in the luminous mushroom.

In this study on the luminous mushroom (*L. japonicus*), we have isolated lampteroflavin (Structure 1) as the light emitter. Lampteroflavin is the only fluorescent component which shows a fluorescence spectrum identical to the bioluminescence spectrum of the mushroom, maxima being 524 nm. The structure of **1** has already been assigned to be 5′-riboflavinyl pentoside in our previous report (*2*). The pentose connected to riboflavin remained unidentified, but it was assigned to be furanose from ^{1}H NMR.[2] This communication describes the pentose identification and the stereochemistry of lampteroflavin by means of FAB–MS/MS (fast atom bombardment tandem mass spectrometry), NMR, and CD spectroscopy.

FAB–MS/MS of lampteroflavin showed *m/z* 509 (M + 1), 377 (riboflavin + 1), 376 (riboflavin), and 243 (isoalloxazine ring). The difference between the *m/z* 509 and the *m/z* 376 suggested the presence of a pentose corresponding to *m/z* 133 ($C_5H_9O_4$). In this study, we report on hexaacetate of lampteroflavin to observe the

[1] To whom correspondence should be addressed.

[2] The chemical shift of the C-4″ position did not change much between the lampteroflavin free form (δ 4.15 ppm) and the acetate form (δ 4.26 ppm), while the C-5″ position of the free form δ 3.75, 3.69 ppm, changed to δ 4.18, 4.34 ppm, respectively. The acetylation of the C-5″ hydroxy group affected the change of the chemical shifts by as much as 0.5–1.0 ppm, thus indicating a furanoside.

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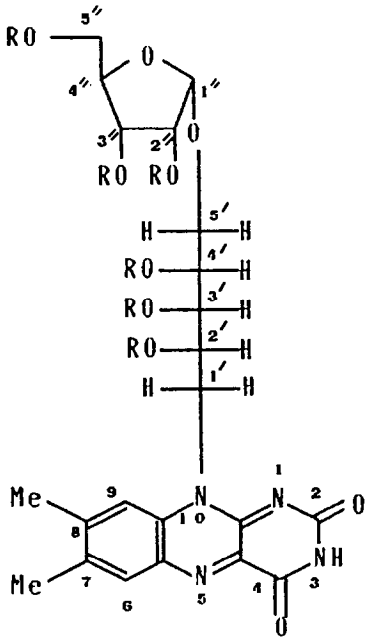


Lampteroflavin (1)
R=H MW 508 R=Ac MW 760

STRUCTURE 1

ion at m/z 761 (M + 1), 503, and 259. The latter ion, m/z 259, was employed as the precursor ion for He collision to confirm that the m/z 259 peak was derived from pentose acetate. In fact the tandem mass spectra showed the daughter ions at m/z 199 $(M - 60)^+$, 157 $(M - 60 - 42)^+$, 139 $(M - \{60 \times 2\})^+$, 97 $(M - \{60 \times 2 - 42\})^+$, and 43 $(C_2H_3O)^+$, confirming the presence of pentose acetate. This fragmentation pattern was compared with those of the authentic pentose acetates (ribose, arabinose, xylose, and lyxose) when the peak of m/z 259 was collided with He at various amounts. The relative intensity of those daughter ions varied. The fragmentation pattern of lampteroflavin acetate was similar to that of ribose acetate as shown in Fig. 1. So we assumed that the pentofuranoside of lampteroflavin was ribofuranoside.

The presence of ribose was further confirmed by acid hydrolysis of lampteroflavin and subsequent acetylation. 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[3] and ribose. 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 the mixture of EtOAc and hexane (1.5 : 1) as a developing solvent. The mixture of α and β-anomers of acetyl furanosides and acetyl pyranosides was then separated by HPLC (Develosil 60-5 silica gel column)

[3] Confirmed with a HPLC ODS column using 25% MeOH–H_2O as eluant, monitoring with uv at 254 nm and fluorescence at Em_{max} 524 nm and at Ex_{max} 445 nm; ^1H NMR of the acetate and benzoate forms in $CDCl_3$.

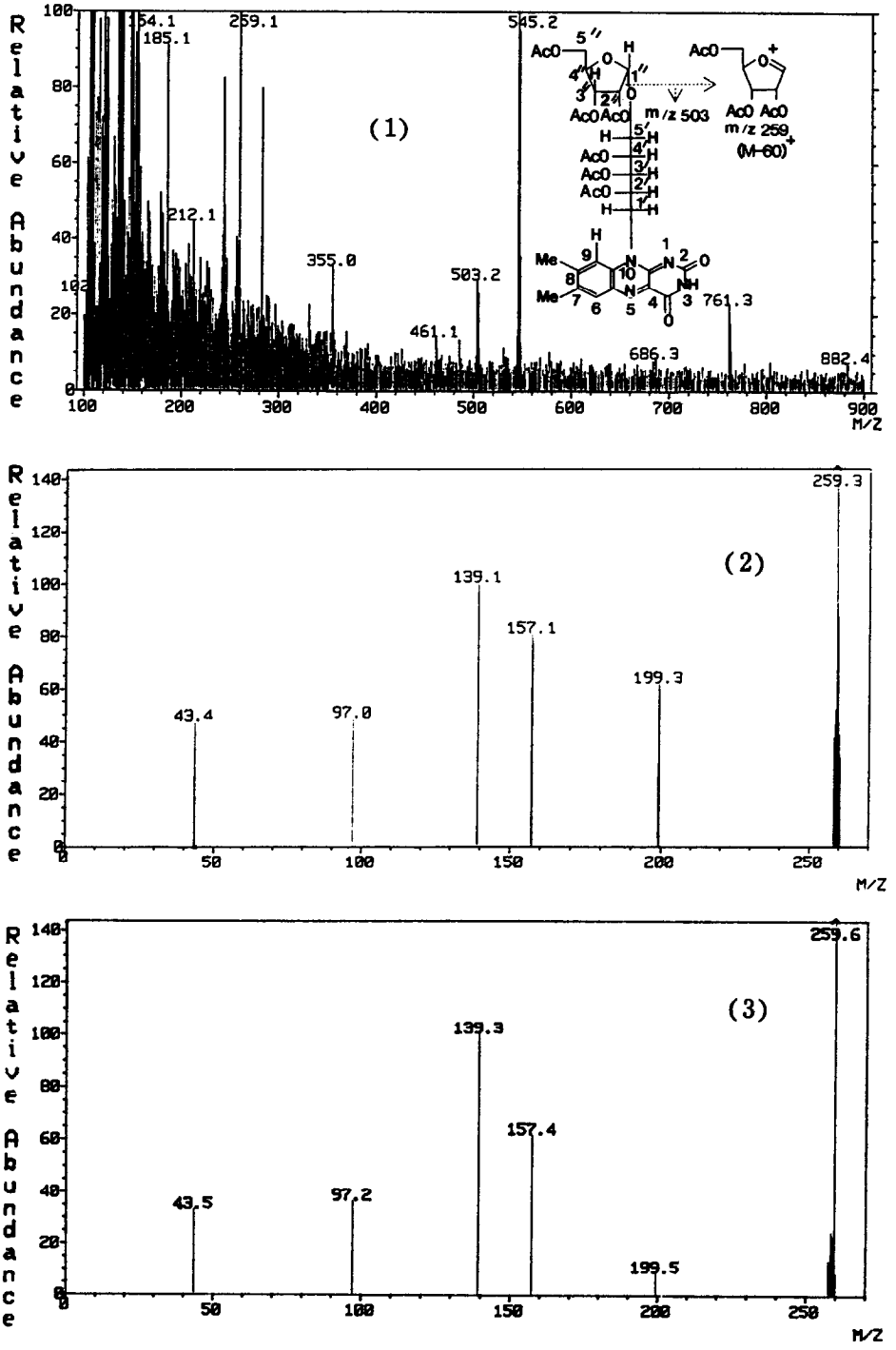


FIG. 1. FAB–MS (1) and FAB–MS/MS (2) spectra of lampteroflavin acetate in comparison with that of ribofuranoside acetate (3) (helium collision at *m/z* 259).

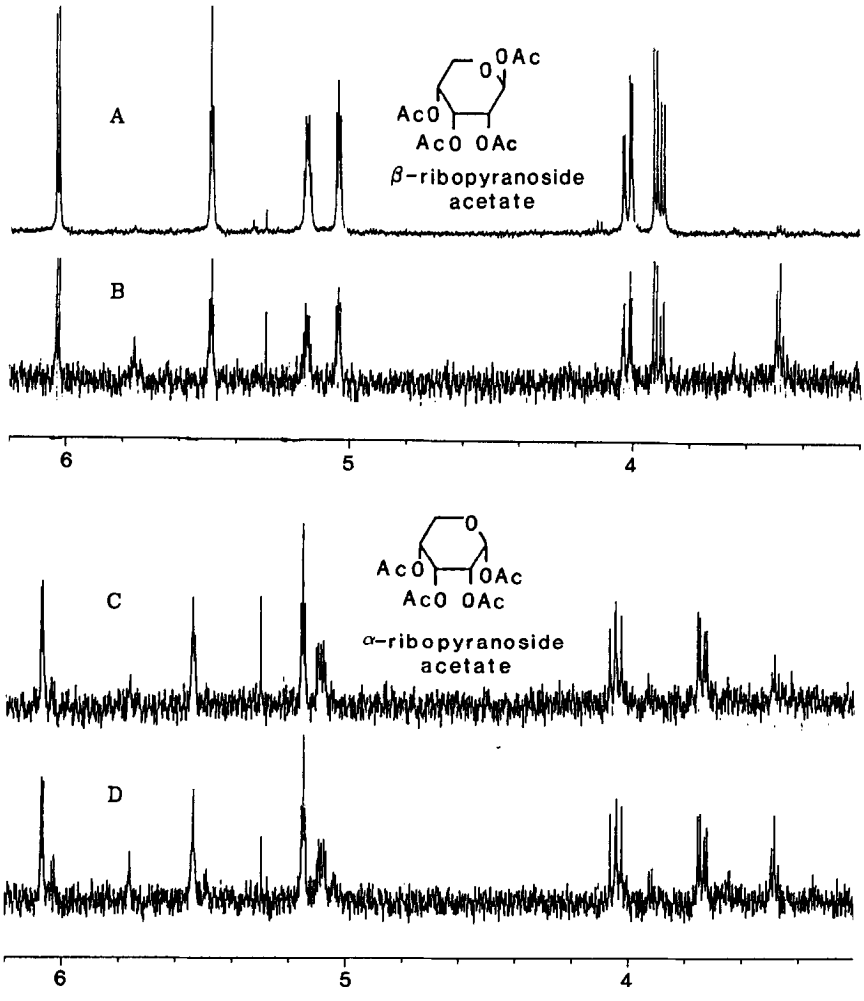


FIG. 2. The 500-MHz NMR spectra showing the identity of acetates of lamperoflavin hydrolysate and ribose after HPLC separation (A, β-ribopyranoside; C, α-ribopyranoside; B and D, lampteroflavin hydrolysate).

using a mixture of EtOAc and hexane (19 : 100) as an eluant to obtain α- and β-pyranosides in ca. 100 μg each. Similarly, authentic D- and L-ribose were acetylated and separated to identify the above pentopyranosides by ^{1}H NMR as shown in Fig. 2 (*3*) and by CD (Fig. 3). The CD spectra of the L- and D-form of β-ribopyranoside acetate in acetonitrile deduced the ribose in lampteroflavin to be D-form.

Whether the glycosidic bond of ribose was an α- or β-linkage of lampteroflavin remained to be determined. In its ^{1}H NMR spectrum in D_2O, H-1″ appeared at δ 5.17 ppm (d, J = 4.0 Hz). The glycoside was assignable as the α-linkage due to the following: (i) irradiation of H-1″ at δ 5.30 (d, J = 4.2 Hz) of lampteroflavin hexaacetate enhanced its H-3″ at δ 5.16 (dd, J = 7.1, 3.9 Hz) in the 500-MHz NOE

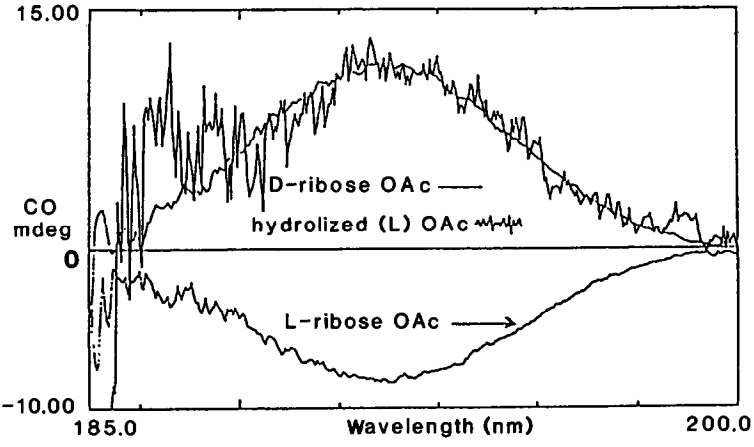
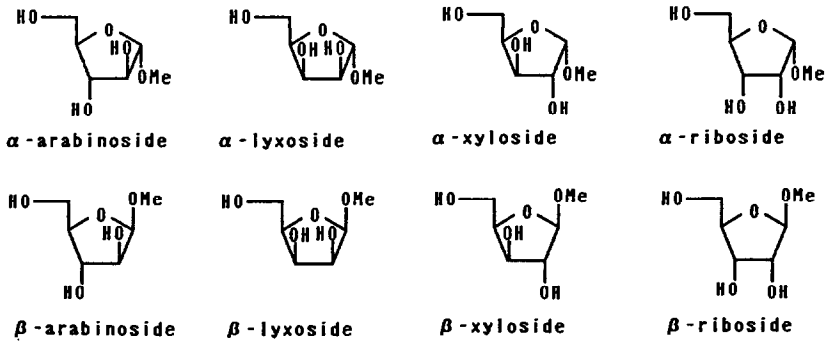


FIG. 3. Comparison of CD spectra of acetates of lampteroflavin hydrolysate and D- and L-β-ribopyranoside.

TABLE 1

Comparison of the Chemical Shift of ^{13}C NMR of Pentofuranosides

furanoses \ assignment		1	2	3	4	5	Me
arabinose	α-methyl	109.2	81.8	77.5	84.9	62.4	56.0
	β-methyl	103.1	77.4	75.7	82.9	62.4	56.3
lyxose	α-methyl	109.2	77.0	72.2	81.4	61.5	56.9
	β-methyl	103.3	73.2	71.0	82.1	62.7	56.7
xylose	α-methyl	103.0	77.8	76.2	79.3	61.6	56.7
	β-methyl	109.7	81.0	76.0	83.6	62.2	56.4
ribose	α-methyl	103.1	71.1	69.8	84.6	61.9	55.5
	β-methyl	108.0	74.3	70.9	83.0	62.9	55.3
lampteroflavin		103.2	71.5	70.3	86.2	62.8	-
(difference)		(0.1)	(0.4)	(0.5)	(1.6)	(0.9)	(-)



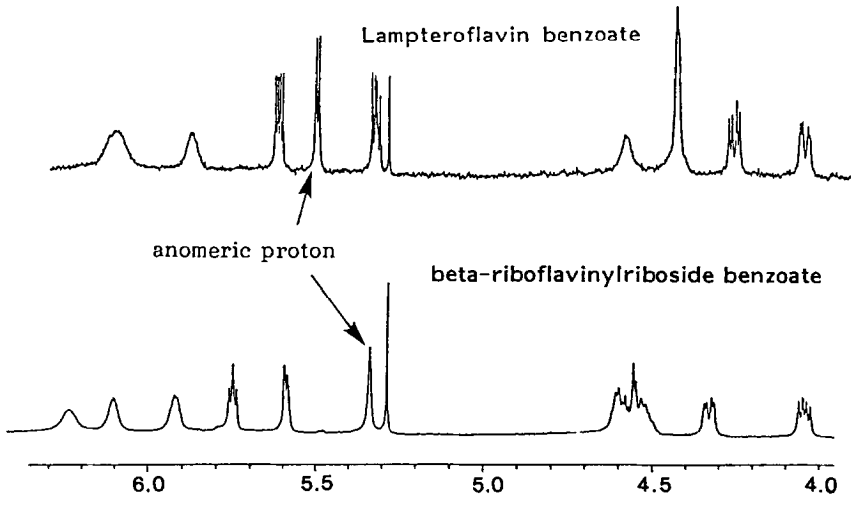


FIG. 4. The 500-MHz NMR spectra of lampteroflavin benzoate and β-riboflavinylriboside benzoate.

difference spectrum, and (ii) similar anomeric protons in propyl α- and β-ribofuranosides appeared to be doublets (J = 4.3 Hz) with the α-linkage, but singlets with the β-linkage. The α-linkage was further confirmed by ^{13}C NMR studies. The chemical shifts of the 1,2-*cis* configuration of methyl pentofuranosides are reported to appear at a higher field than that of the 1,2-*trans* configuration as shown in Table 1. ^{13}C NMR of the sugar moiety of lampteroflavin is also shown in Table 1. Among all the possible methyl pentofuranosides, lampteroflavin showed chemical shifts quite similar to those of methyl α-ribofuranoside, the different chemical shifts being shown in parentheses. The chemical shifts of the anomeric signal (C-1″) of lampteroflavin at δ 103.2 ppm and C-2″ at δ 71.5 ppm suggested the 1,2-*cis* configuration. When the chemical shifts of the anomeric signal of methyl α- and β-D-ribofuranoside (δ 103.1 and δ 108.0 ppm, respectively) (*4*) were compared with that of lampteroflavin (δ 103.2 ppm), the linkage between ribose and riboflavin of lampteroflavin was concluded to be α-linkage.

Finally, we attempted to prove the structure of lampteroflavin by chemical synthesis. Recently, 5′-riboflavinyl β-D-ribofuranoside perbenzoate was synthesized in comparison with lampteroflavin benzoate. Both showed similar spin systems in ^1H NMR spectra as shown in Fig. 4. The distinguishable signal between the α- and the β-form was the pattern of the anomeric signal; natural α-glycoside showed a doublet signal (J = 4.5 Hz), while the synthetic β showed a singlet signal.[4]

Lampteroflavin was determined to be 5′-riboflavinyl α-D-ribofuranoside. It is of interest that lampteroflavin is the first demonstration of riboflavinyl ribofurano-

[4] The assignment of the anomeric α- and β-position in D-ribose *O*-glycosides was also the case in propyl 2,3,5-tribenzylribofuranosides, the α-isomer appearing at ^1H NMR δ 5.01 ppm (d, J = 4.3 Hz), the β-isomer at 5.01 ppm (singlet), and propyl 2,3-propylidene-5-benzoylribofuranosides at δ 5.04 ppm (d, J = 4.3 Hz, α), 5.12 ppm (s, β).

side besides a 5′-riboflavinyl α-glucopyranoside (*5*). Synthetic studies of lampteroflavin are currently under investigation. This conclusion will lead us to explore the mechanism of the mushroom bioluminescence.

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