Tetrahedron Vol. 49, No. 41, pp. 9267-9276, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Mass Spectrometric Studies on Chemiluminescence of Coelenterate Luciferin Analogues

Takashi Hirano, Sadatoshi Nishibuchi, Michiaki Yoneda, Kazuo Tsujimoto, and Mamoru Ohashi*

Department of Applied Physics and Chemistry, The University of Electro-Communications, Chofu, Tokyo 182, Japan

(*Received in USA* 19 *February* 1993; *accepted* 22 *April* 1993)

Abstract: Electron ionization (EI) and liquid secondary ionization mass spectra (LSIMS) of 30 coelenterazine analogues were examined by use of linked scanning at constant *B/E* and the fragmentation rules upon EI and LSIMS have been established. On the basis of these results the structures of intermediates in the chemiluminescence of these compounds have been deduced as monooxygenated compounds and the possible mechanisms of chemiluinescence of these compounds are discussed.

INTRODUCTION

The luminescent protein aequorin isolated from the jellyfish *Aequorea victoria* by Shimomura and Johnson in 1962 consists of apoaequorin (apoprotein), coelenterazine (chromophore), and molecular oxygen.[1] The binding of calcium ion to the protein triggers the reaction of the originally dioxygenated protein to yield carbon dioxide, coelenteramide, and light. On the other hand, Goto and his co-workers elucidated the structure of *Vargula* luciferin isolated from sea fire fly, which bioluminesces in the presence of *Vargula* luciferase.[2] Coelenterazine and *Vargula* luciferin have the common structure of a 3,7-dihydroimidazo[1,2-a]pyrazin-3-one ring system and chemiluminesce in an aprotic solvent under oxygen.[3] In order to clarify the mechanism of the chemiluminescence of those compounds we have characterized a series of reactions of coelenterazine analogues which lead to the excited state amide products.[4]

In this paper, we report here the establishment of the fragmentation pathways of colenterazine analogues under electron ionization (EI) and under Xe ion bombardment. Applying mass spectrometric measurements to the analysis of intermediates in the chemiluminescence reactions of these compounds, we could identify the oxgenated products. From this result, the possible structures of the intermediates in the chemiluminescence are deduced.

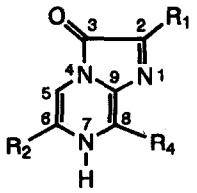
RESULTS AND DISCUSSION

EI Mass Spectra Under in-beam EI conditions,[5] each of the coelenterazine analogues **1-25** gave the ions $M^{+\bullet}$(**a**), $[M-H]^+$(**a'**), $[M-CO]^{+\bullet}$(**b**), $[M-H-CO]^+$(**b'**), $[M-CO-R_1CN]^{+\bullet}$(**c**), $[M-H-CO-R_1CN]^+$(**c'** and **c"**), $[M-CO-R_1CN-HCN]^{+\bullet}$(**d**), and $[M-H-CO-R_1CN-R_4CN]^+$(**e**). As typical spectra, the EIMS of 2-methyl (**1**) and 2-phenyl (**2**) derivatives are shown in Fig. 1(A) and 2(A), respectively. Table 1 summarizes the abundances of the

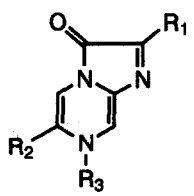
Dedicated to Professor Carl Djerassi on the occasion of his seventieth birthday.

Compound	NM[a]	R_1	R_2	R_4
1	149	CH_3	H	H
2	221	Ph	H	H
3	225	CH_2Ph	H	H
4	241	$C_6H_4OCH_3$	H	H
5	279	$C_6H_4CF_3$	H	H
6	225	CH_3	Ph	H
7	287	Ph	Ph	H
8	301	CH_2Ph	Ph	H
9	315	$(CH_2)_2Ph$	Ph	H
10	329	$(CH_2)_3Ph$	Ph	H
11	355	$C_6H_4CF_3$	Ph	H
12	241	CH_3	C_6H_4OH	H
13	303	Ph	C_6H_4OH	H
14	317	CH_2Ph	C_6H_4OH	H
15	331	$(CH_2)_2Ph$	C_6H_4OH	H
16	345	$(CH_2)_3Ph$	C_6H_4OH	H
17	331	CH_3	C_6H_4OH	CH_2Ph
18	393	Ph	C_6H_4OH	CH_2Ph
19	407	CH_2Ph	C_6H_4OH	CH_2Ph
20	421	$(CH_2)_2Ph$	C_6H_4OH	CH_2Ph
21	435	$(CH_2)_3Ph$	C_6H_4OH	CH_2Ph
22	423	$CH_2C_6H_4OH$	C_6H_4OH	CH_2Ph
23	255	CH_3	$C_6H_4OCH_3$	H
24	317	Ph	$C_6H_4OCH_3$	H
25	331	CH_2Ph	$C_6H_4OCH_3$	H

[a] NM: Nominal mass



1 -25



26 -30

Compound	NM[a]	R_1	R_2	R_3
26	163	CH_3	H	CH_3
27	166	CH_3	H	CD_3
28	225	Ph	H	CH_3
29	228	Ph	H	CD_3
30	301	Ph	Ph	CH_3

Scheme 1. Structures of Coelenterazine analogues.

major ions in EIMS of **1-25**. The elemental compositions of these fragment ions **a-e** of **7** were confirmed by high resolution mass measurements as shown in Table 3, indicating that the losses of hydrogen, carbon monoxide, benzonitrile, and hydrogen cyanide were dominant processes under EI conditions. In order to elucidate the fragmentation processes, the metastable ion spectra in the linked scans at constant B/E and B^2/E of several compounds were examined. The collision induced dissociation (CID)-MS/MS spectra of the major ions of **7** (nominal mass 287) were also obtained for this purpose. Under CID conditions the molecular ion $M^{+\bullet}$ (**a**, m/z 287) yields $[M-H]^+$ (**a'**, m/z 286), $[M-CO]^{+\bullet}$ (**b**, m/z 259), and $[M-CO-PhCN]^{+\bullet}$ (**c**, m/z 156), while the **a'** ion yields $[M-H-CO]^+$ (**b'**, m/z 258), $[M-H-CO-PhCN]^+$ ((**c'** and **c"**), m/z 155), and $[M-H-CO-PhCN-HCN]^+$ (**e**, m/z 128). The fragmentations of **b** to **c** and **b'**, of **c** to (**c'** and **c"**) and $[M-CO-PhCN-HCN]^+$ (**d**, m/z 129), of **b'** to (**c'** and **c"**) and **e**, of **c'** to **e**, and of **e** to $C_6H_5^+$ (m/z 77) were also confirmed by CID-MS/MS. These results reveal the fragmentation pathways of coelenterazine analogues as illustrated in Scheme 2. To establish these fragmentation processes, 7-methyl and 7-trideuteriomethyl derivatives of **1** (**26** and **27**), **2** (**28** and **29**), and **7** (**30**) were also synthesized and their EIMS were examined (Table 2). The typical EIMS are shown in Fig. 1(B and C) and 2(B and C). Each EIMS of **28-30** barely showed the $[M-H]^+$ ion comparing with that of 7-H derivatives indicating that the hydrogen at position 7 of coelenterazine analogues **1-25** is eliminated upon EI. The formation of the abundant ions **c** and **d** is common to 7-methyl (trideuteriomethyl) derivatives **28-30** and 7-H derivatives **1-25** indicating that the structures of the **c** and **d** ions include the N7-R_3 (R_3= CH_3, CD_3, and H) bond of these coelenterazine analogues. The observation of the $[M-R_3-CO-R_1CN-HCN]^+$ ion (**e**) instead of [M-

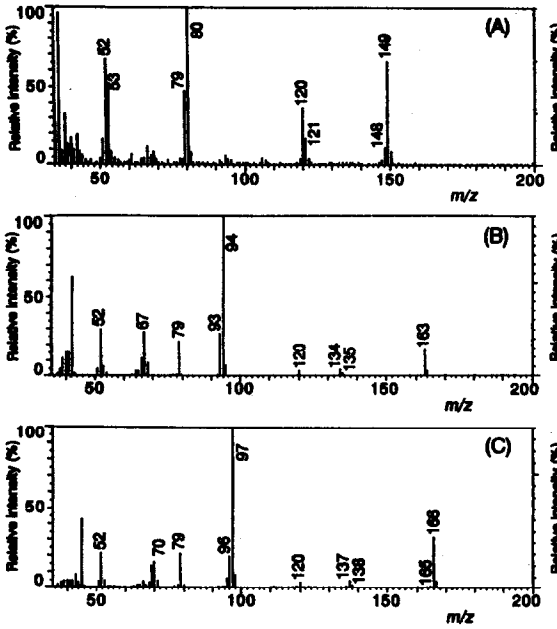


Figure 1. In-beam EI mass spectra of **1** (A), **26** (B), and **27** (C).

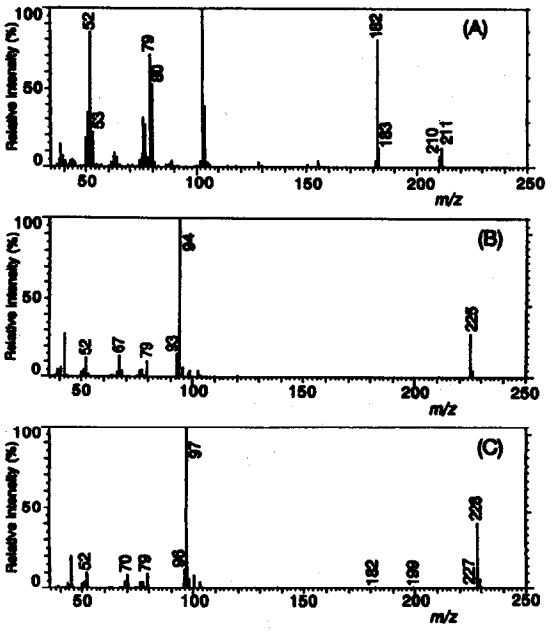
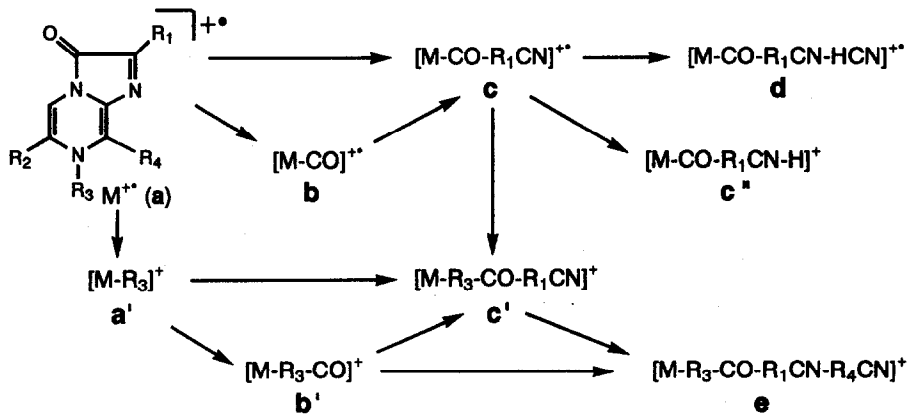


Figure 2. In-beam EI mass spectra of **2** (A), **28** (B), and **29** (C).



Scheme 2. Fragmentation pathways of coelenterazine analogues **1-30** under EI conditions.

Table 1. Characteristic ions (*m/z*) and relative peak intensities (%) in in-beam EI mass spectra of coelenterazine analogues **1-25**

Compound	$M^{+\bullet}$ a	$[M-H]^+$ a'	$[M-CO]^{+\bullet}$ b	$[M-H-CO]^+$ b'	$[M-CO-R_1CN]^{+\bullet}$ c	$[M-H-CO-R_1CN]^+$ c' and c"	$[M-CO-R_1CN-HCN]^{+\bullet}$ d	$[M-H-CO-R_1CN-R_4CN]^+$ e
1	149 (67)	148 (12)	121 (18)	120 (37)	80 (100)	79 (48)	53 (48)	52 (68)
2	211 (12)	210 (7)	183 (12)	182 (81)	80 (54)	79 (72)	53 (23)	52 (85)
3	225 (72)	224 (2)	197 (9)	196 (40)	80 (63)	79 (38)	53 (21)	52 (32)
4	241 (11)	240 (14)	213 (17)	212 (92)	80 (34)	79 (100)	53 (17)	52 (76)
5	279 (20)	278 (4)	251 (5)	250 (31)	80 (100)	79 (80)	53 (13)	52 (84)
6	225 (100)	224 (4)	197 (16)	196 (34)	156 (83)	155 (44)	129 (26)	128 (49)
7	287 (54)	286 (4)	259 (14)	258 (48)	156 (92)	155 (35)	129 (25)	128 (100)
8	301 (100)	300 (4)	273 (16)	272 (47)	156 (60)	155 (41)	129 (16)	128 (83)
9	315 (41)	314 (3)	287 (6)	286 (8)	156 (46)	155 (33)	129 (10)	128 (48)
10	329 (52)	328 (3)	301 (4)	300 (7)	156 (58)	155 (34)	129 (15)	128 (53)
11	355 (64)	354 (2)	327 (5)	326 (14)	156 (100)	155 (38)	129 (16)	128 (58)
12	241 (77)	240 (13)	213 (25)	212 (71)	172 (74)	171 (39)	145 (40)	144 (100)
13	303 (42)	302 (20)	275 (18)	274 (48)	172 (50)	171 (17)	145 (13)	144 (100)
14	317 (98)	316 (22)	289 (13)	288 (62)	172 (51)	171 (24)	145 (10)	144 (100)
15	331 (50)	330 (4)	303 (10)	302 (10)	172 (49)	171 (21)	145 (10)	144 (48)
16	345 (72)	344 (5)	317 (8)	316 (12)	172 (69)	171 (28)	145 (17)	144 (66)
17	331 (94)	330 (36)	303 (23)	302 (20)	262 (23)	261 (100)	---	144 (8)
18	393 (78)	392 (18)	365 (13)	364 (25)	262 (30)	261 (100)	---	144 (11)
19	407 (88)	406 (15)	379 (9)	378 (13)	262 (24)	261 (100)	---	144 (17)
20	421 (69)	420 (10)	393 (10)	392 (9)	262 (20)	261 (70)	---	144 (17)
21	435 (21)	434 (2)	407 (2)	406 (2)	262 (10)	261 (36)	---	144 (7)
22	423 (30)	422 (36)	395 (5)	394 (26)	262 (24)	261 (96)	---	144 (22)
23	255 (100)	254 (5)	227 (20)	226 (32)	186 (72)	185 (15)	159 (27)	158 (47)
24	317 (93)	316 (7)	289 (15)	288 (44)	186 (100)	185 (13)	159 (22)	158 (77)
25	331 (100)	330 (7)	303 (20)	302 (41)	186 (43)	185 (16)	159 (11)	158 (74)

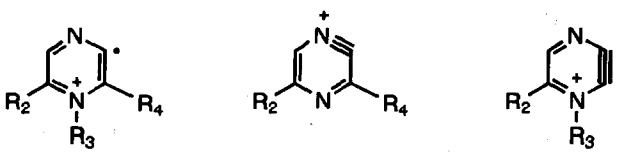
Table 2. Characteristic ions (*m/z*) and relative peak intensities (%) in in-beam EI mass spectra of coelenterazine analogues **26-30**

Compound	$M^{+\bullet}$ a	$[M-H]^+$	$[M-CO]^{+\bullet}$ b	$[M-H-CO]^+$	$[M-R_3-CO]^+$ b'	$[M-CO-R_1CN]^{+\bullet}$ c	$[M-R_3-CO-R_1CN]^+$ c'	$[M-CO-R_1CN-H]^+$ c"	$[M-CO-R_1CN-HCN]^{+\bullet}$ d	$[M-R_3-CO-R_1CN-HCN]^+$ e
26	163 (17)	---	135 (2)	134 (4)	120 (3)	94 (100)	79 (23)	93 (27)	67 (29)	52 (30)
27	166 (32)	165 (1)	138 (2)	137 (5)	120 (3)	97 (100)	79 (21)	96 (20)	70 (16)	52 (21)
28	225 (28)	---	---	---	182 (1)	94 (100)	79 (10)	93 (16)	67 (14)	52 (13)
29	228 (41)	227 (1)	---	199 (1)	182 (1)	97 (100)	79 (10)	96 (12)	70 (9)	52 (11)
30	301 (46)	---	---	---	---	170 (100)	155 (2)	169 (34)	143 (2)	128 (14)

Table 3. High-resolution mass measurement of **7**.

Observed mass	Caluculated mass	Formula	Assignment	
287.1037	287.1059	$C_{18}H_{13}N_3O$	$M^{+\bullet}$	(a)
286.0989	286.0980	$C_{18}H_{12}N_3O$	$[M-H]^+$	(a')
259.1070	259.1109	$C_{17}H_{13}N_3$	$[M-CO]^{+\bullet}$	(b)
258.1032	258.1031	$C_{17}H_{12}N_3$	$[M-H-CO]^+$	(b')
156.0662	156.0687	$C_{10}H_8N_2$	$[M-CO-PhCN]^{+\bullet}$	(c)
155.0594	155.0609	$C_{10}H_7N_2$	$[M-H-CO-PhCN]^+$	(c' and c")
129.0531	129.0578	C_9H_7N	$[M-CO-PhCN-HCN]^{+\bullet}$	(d)
128.0499	128.0500	C_9H_6N	$[M-H-CO-PhCN-HCN]^+$	(e)
77.0408	77.0391	C_6H_5		

$H\text{-}CO\text{-}R_1CN\text{-}HCN]^+$ in the spectra of **26-30** is the evidence that the formation of $[M\text{-}H\text{-}CO\text{-}R_1CN\text{-}R_4CN]^+$ (e) in **1-25** and that of $[M\text{-}R_3\text{-}CO\text{-}R_1CN\text{-}HCN]^+$ (e) in **26-30** result from the dissociation of the N7-H bond and the N7-$CH_3(CD_3)$ bond, respectively. Observed intense peaks of both $[M\text{-}R_3\text{-}CO\text{-}R_1CN]^+$ (c') and $[M\text{-}H\text{-}CO\text{-}R_1CN]^+$ (c") suggests that the formation of the $[M\text{-}H\text{-}CO\text{-}R_1CN]^+$ ion (c' and c") from **1-25** upon EI requires the cleavage of both the N7-H bond and a C-H bond of the pyrazine ring. From these results the fragmentation pathways of coelenterazne analogues are revealed as shown in scheme 2 and it is concluded that the hydrogen at 7-position dissociates mainly to give the $[M-H]^+$ ion and the losses of CO, alkylnitrile, and hydrogen cyanide are the main fragmentation processes. The dominant formation of the ions **c**, **c'**, and **c"** is explained by the stabilities of these ions. Possible structures of **c**, **c'**, and **d** are illustrated as **31**, **32**, and **33**, respectively, which contain the stable 6π aromatic ring systems.



31 **32** **33**

Positive and Negative LSIMS Spectra: Under the ionization of coelenterazine analogues by Xe ion bombardment in m-nitrobenzylalcohol (NBA) as the matrix, the protonated molecule MH^+ along with the molecular ion $M^{+\bullet}$ and with the fragment ions similar to those of EIMS was observed in the positive mode. Results obtained from the FAB CID-MS/MS spectra of $M^{+\bullet}$ and of MH^+ showed that the fragmentation involving the losses of CO and alkylnitrile occur not from MH^+ but from $M^{+\bullet}$ in a way similar to that of EIMS. Figure 3 shows the LSIMS spectrum of compound **7** as a typical example. The negative LSIMS spectra of all the analogues using NBA as the matrix exhibited only deprotonated molecules $[M-H]^-$.

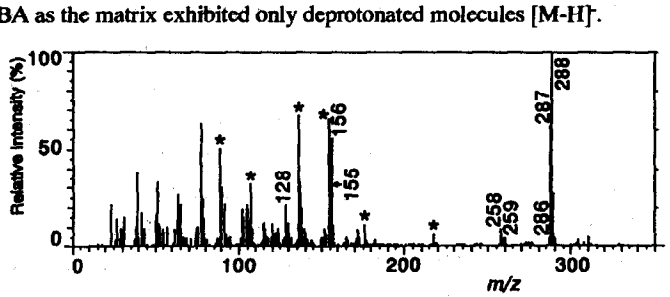
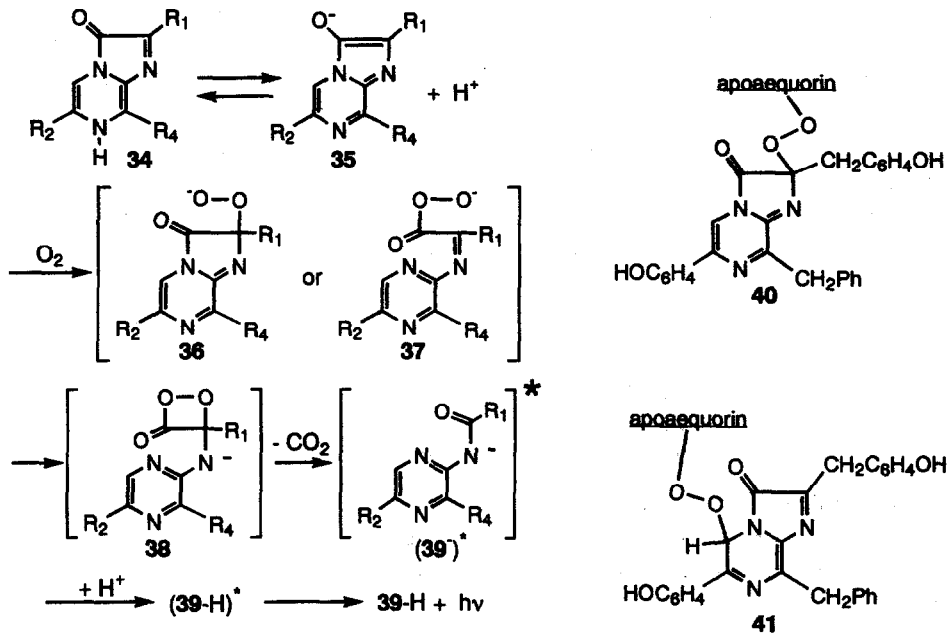


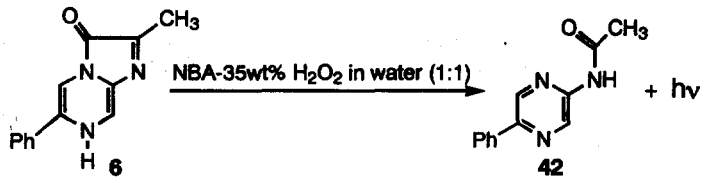
Figure 3. Positive ion LSIMS spectrum of **7** using a NBA matrix. Matrix ions are indicated with asterisks.

Negative Ion LSIMS Spectra under Chemiluminescent Conditions: Concerning bio-luminescence of *Vargula* luciferin, Goto and co-workers proposed the oxidation processes that lead to the excited state of oxyluciferin[6] as shown in scheme 3. In accord with this mechanism Shimomura and Kishi proposed the bioluminescence mechanism of aequorin in which the coelenterazine binds to apoaequorin through the -O-O- bonding at the 2-position of the chromophore (structure **40**).[7] On the other hand, recently Teranishi and Isobe et al. proposed a new structure of aequorin in which the coelenterazine binds to apoaequorin through the -O-O- bond at the 5 position (structure **41**).[8] We have also investigated a series of reactions for the chemiluminescence of coelenterazine analogues and proposed the mechanism similar to that of Goto.[4b] All the proposed mechanism have assumed the reaction to proceed via a dioxetanone intermediate (structure **38**), although the intermediate has not been detected yet.



Scheme 3. Chemi- and bio-luminescence of *Vargula* luciferin

To get some information on the structure of the oxygenated intermediates, we analyzed the chemiluminescence reaction mixtures obtained by H_2O_2 oxidation of the several coelenterazine analogues using negative ion LSIMS in the NBA matrix. As the typical example, when a solution of **6** was mixed with a 35 wt% H_2O_2 aqueous solution, the solution emitted light for 10-12 hours. The negative ion LSIMS spectrum of this reaction mixture of **6** (nominal mass 225) is shown in Figure 4a. The oxygenated ion $[M+O-H]^-$ at m/z 240 was clearly observed along with the ions at m/z 224 and m/z 212 which correspond to the deprotonated molecules of **6** and of the coelenteramide analogue **42**, respectively. The structure of the ion at m/z 212 has been confirmed by direct comparison of the daughter ion spectrum obtained by the linked scanning at constant *B/E* of this ion with that of coelenteramide analogue **42** synthesized authentically.[9] After the reaction was completed, only the peak at m/z 212 was observed, which indicated that the coelenteramide analogue **42** is the stable product of the chemilumi-



nescent reaction. A similar spectrum of **5** is also shown in Figure 5a.

Concerning the structure of the oxygenated ion $[M+O-H]^-$, the linked scanning at constant *B/E* of this ion was examined. Observed fragmentations of the $[M+O-H]^-$ ions of several compounds are summarized in Table 4 and the LSIMS *B/E* linked scan spectra of the $[M+O-H]^-$ ions of **5** and **6** are shown in Figure 5b and 4b, respectively. A dominant formation of the ion $[M-H]^-$ due to loss of oxygen from each $[M+O-H]^-$ ion suggests that the structure of oxygenated ion maintains the imidazo[1,2-a]pyrazin-3-one ring such as **B** and **C**. The fragmentation due to decarbonylation can be explained by the decomposition of ion **B**, and the elimination of CO_2 from the $[M+O-H]^-$ ions of **2**, **5**, **6**, and **11** may indicate that the carboxylate ion **A** is also contained in the reaction mixture. Formation of the ion $[P-O-CO-R_1CN]^-$ or $[P-CO_2-R_1CN]^-$ for **6** may derive from ion **A** or **B**, but not from **C**. From these results, we propose that the observed $[M+O-H]^-$ ion is a mixture of **A**, **B**, and probably **C** depending upon the structure of coelenterazine analogue, from which we can deduce the structure of

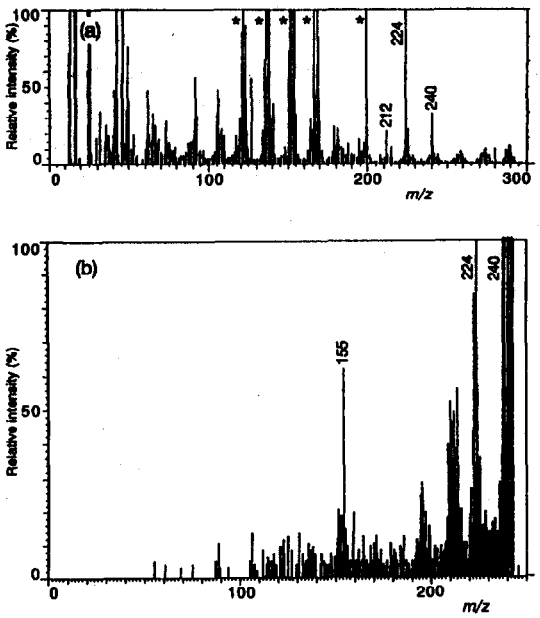


Figure 4. Negative ion LSIMS spectrum of the reaction mixture of **6** with H_2O_2 using NBA matrix (a) and the *B/E* linked scan spectrum of the $[M+O-H]^-$ ion (b). Matrix ions are indicated with asterisks.

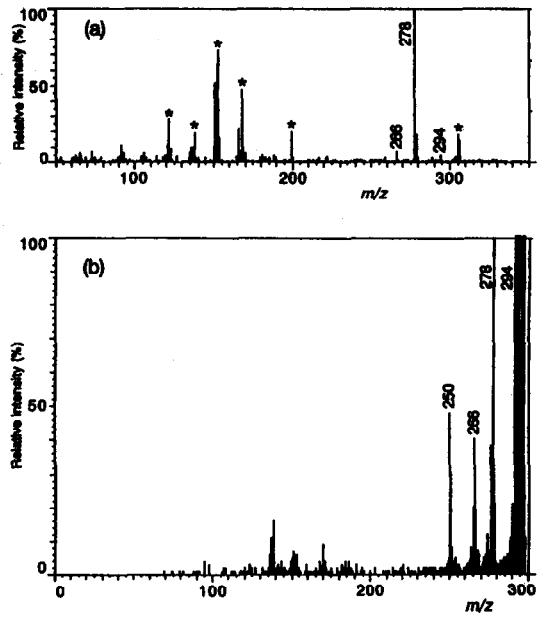
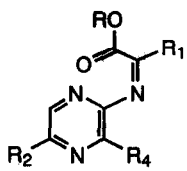


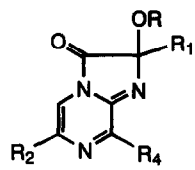
Figure 5. Negative ion LSIMS spectrum of the reaction mixture of **5** with H_2O_2 using NBA matrix (a) and the *B/E* linked scan spectrum of the $[M+O-H]^-$ ion (b). Matrix ions are indicated with asterisks.

Table 4. Metastable ion spectra of the $[M+O-H]^-$ ions of coelenterazine analogues (m/z, relative peak intensities (%) in parentheses)

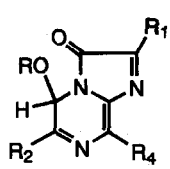
Compound	$[M+O-H]^-$ Parent ion (P)	$[P-O]^-$	$[P-CO]^-$	$[P-CO_2]^-$	$[P-O-CO-R_1CN]^-$ or $[P-CO_2-R_1CN]^-$
2	226	210 (100)	198 (50)	182 (40)	---
5	294	278 (100)	266 (40)	250 (50)	---
6	240	224 (100)	212 (50)	196 (30)	155 (70)
10	344	328 (100)	---	---	---
11	370	354 (100)	342 (30)	326 (80)	---



A ; R= -
A' ; R= H
A" ; R= OH



B ; R= -
B' ; R= H
B" ; R= OH



C ; R= -
C' ; R= H
C" ; R= OH

the oxygenated products as **A '**, **B '**, and **C '**. However, since organic peroxides give the ions due to loss of a oxygen under negative ion FAB which is a soft ionization technique similar to LSIMS,[10] the peroxide structures **A"**, **B"**, and **C"** are also conceivable for the oxidation products. The oxidation reactions of *Vargula* luciferin have been investigated by Goto in detail[11] and it has been established that the addition of a hydroxy radical to coelenterate luciferin analogues takes place mainly at the 2 position on treatment with the Fenton reagent to give **B '**. Our conclusion is in accord with this Goto's results and with the proposed structures of intermediates (Scheme 3, **36** and **37**) of bioluminescence reaction of *Vargula* luciferin.[6] Although the accurate assignment of the structure of oxygenated intermediate is now in progress, our mass spectrometric approach may give the significant information of the mechanism on chemiluminescence of those coelentrerazine analogues

Conclusion: The fragmentation pathways of coelenterazine analogues upon EI or Xe ion bombardment have been established by CID-MS/MS, linked scans at constant B/E and B^2/E and deuterium labelling experiments. The negative ion LSIMS spectra of the reaction mixture of a selected analogue with H_2O_2 under the chemiluminescence conditions exhibit the ion of deprotonated molecule $[M-H]^-$, the corresponding coelenteramide analogue and the oxygenated ion $[M+O-H]^-$. The oxygenated ion $[M+O-H]^-$ was presumed as the ion related to the intermediates of the chemiluminescence reactions and its structure was deduced based on the linked scan at constant B/E. Three possible structures depending on the substituent at the 2 position were deduced in accord with the previous proposals.[6-8] Thus, the mass spectrometric investigation has proved to be a potent tool for elucidation of the structures of unstable intermediates in a series of reactions under chemiluminescence conditions.

EXPERIMENTAL

Materials. The preparation of compounds **1**, **2**, **6**, **18**, **19**, coelenterazine **22**, and **26** are reported in the literature,[12-15] and the synthesis of **20** and **21** was reported previously.[4a] Other celenterate luciferin analogues were also prepared by the procedure reported by Goto and Kishi. [12,13]

Mass spectrometry. In-beam EI mass spectra and positive- and negative-ion LSIMS spectra as well as B/E and B^2/E constant linked scan spectra were obtained with a Hitachi M-80B mass spectrometer with a Hitachi M-0101 mass data system. In-beam EIMS was carried out at 70 eV of the ionizing electron energy and the ion accelating voltage was 3 kV. For LSIMS measurment, m-nitrobenzyl alcohol (NBA) was used as a matrix and the SIMS gun (xenon ions) was operated at 8 kV. High resolution measurement of **7** under EI was carried out on the JEOL JMS-D300 with a JMA-2000 mass data system. EIMS and FAB collision induced dissociation (CID)-MS/MS was performed on a Finnigan MAT TSQ-700 and argon was used as the collision gas.

Negative Ion LSIMS Spectra under Chemiluminescent Conditions. Coelenterazine analogue **6** (ca. 1 mg) was dissolved in the 1:1 mixture of m-nitrobenzyl alcohol (NBA) and 35wt% H_2O_2 aqueous solution. After 47 min LSIMS spectrum of this reaction mixture was obtained under the conditions noted above and was illustrated in Figure 4. The light intensity of the reaction mixture was recorded with a Labo Science Model TD-8000 Lumiphotometer and the chemiluminescence was observed over 10-12 hours. By the same procedure, the negative ion LSIMS spectrum of the reaction mixture of **5** with H_2O_2 was obtained after 20min and was shown in Figure 5.

Acknowledgements-The authors thank Messers F. -Q. Chen, Y. Gomi, K. Kitahara, I Mizoguchi, T. Takahashi, and M. Ishimaru of the University of Electro-Communications for supply of samples. This work was supported by Grant-in-Aid for Scientific Research in Priority Areas from the Ministry of Education, Science and Culture (Nos. 02250102, 03236104, and 04220105).

REFERENCES

1. O. Shimomura, F. H. Johnson, and Y. Saiga, *J. Cell. Comp. Physiol.*, **1962**, *59*, 223.
2. O. Shimomura, T. Goto, Y. Hirata, *Bull. Chem. Soc. Jpn.*, **1957**, *30*, 929; Y. Kishi, T. Goto, Y. Hirata, O. Shimomura, and F. H. Johnson, *Tetrahedron Lett.*, **1966**, 3427; Y. Kishi, T. Goto, S. Eguchi, Y. Hirata, E. Watanabe, and T. Aoyama, *Tetrahedron Lett.*, **1966**, 3437.
3. T. Goto, *Pure Appl. Chem.*, **1968**, *17*, 421.
4. (a) F. Q. Chen, Y. Gomi, T. Hirano, M. Ohashi, Y. Ohmiya, and F. I. Tsuji, *J. Chem. Soc., Perkin Trans. 1*, **1992**, 1607; F. Q. Chen, Y. Gomi, M. Ohashi, Y. Ohmiya, and F. I. Tsuji, *J. Chem. Soc., Chem. Commun.*, **1991**, 1307. (b) T. Hirano, Y. Gomi, T. Takahashi, K. KItahara, F. -Q. Chen, I. Mizoguchi, S. Kyushin, and M. Ohashi, *Tetrahedron Lett.*, **1992**, *33*, 5771.
5. Because of the thermal instability and low volatility of coelenterazine analogues, it is difficult to obtain reproducible spectra of these compounds under conventional direct EI conditions.
6. T. Goto, " Marine Natural Products" ed. by P. Scheuer, Academic Press, **1980**, p. 179.
7. B. Musicki, Y. Kishi, and O. Shimomura, *J. Chem. Soc., Chem. Commun.*, **1986**, 1566.
8. K. Teranishi, M. Isobe, T. Yamada, and T. Goto, *Tetrahedron Lett.*, **1992**, *33*, 1303.
9. T. Goto, S. Inoue, S. Sugiura, K. Nishikawa, M. Isobe, and Y. Abe, *Tetrahedron Lett.*, **1968**, 4035.
10. H. M. Fales, E. A. Sokoloski, L. K. Pannell, P. Quan-long, D. L. Klayman, A. J. Lin, A. Brossi, and J. A. Kelley, *Anal. Chem.*, **1990**, *62*, 2494.

11. T. Goto, " New Synthetic Methodology and Functionally Interesting Compounds" ed. by Z. Yoshida, Kodansha, Tokyo, **1986**, p. 285.
12. Y. Kishi, H. Tanino, and T. Goto, *Tetrahedron Lett.*, **1972**, 2747; S. Inoue, S. Sugiura, H. Kakoi, K. Hashizume, T. Goto, and Y. Kishi, *Chem. Lett.*, **1975**, 141.
13 T. Goto, S. Inoue, and S. Sugiura, *Tetrahedron Lett.*, **1968**, 3873; S. Inoue, S. Sugiura, H. Kakoi, and T. Goto, *Ibid*, **1969**, 1609; S. Sugiura, S. Inoue, and T. Goto, *Yakugaku Zasshi*, **1970**, *90*, 423, 431.
14. F. McCapra and M. J. Manning, *J. Chem. Soc., Chem. Commun.*, **1973**, 467.
15. K. Hori, J. E. Wampler, J. C. Matthews, and M. J. Cormier, *Biochemistry*, **1973**, *12*, 4463.