Pergamon

Tetrahedron 56 (2000) 2629–2639

TETRAHEDRON

Synthesis of ^{13}C-Dehydrocoelenterazine and NMR Studies on the Bioluminescence of a *Symplectoteuthis* Model

Masaki Kuse and Minoru Isobe*

Laboratory of Organic Chemistry, School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan

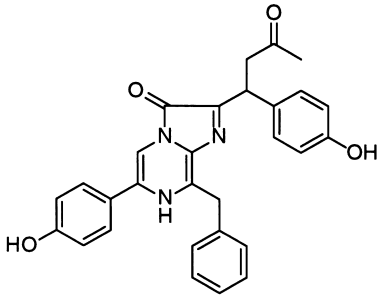
Received 24 January 2000; accepted 21 February 2000

Abstract—The bioluminescence of luminous squid (*Symplectoteuthis oualaniensis*) is presumed to be initiated by the addition of the sulfhydryl residue of a photoprotein to dehydrocoelenterazine (DCL). To clarify this step, a novel synthetic route was established to label DCL with ^{13}C. Dithiothreitol (DTT) and glutathione (GSH) were used as photoprotein models. The addition of DTT and GSH to ^{13}C-labeled DCL gave luminous chromophores. Its structures were confirmed by NMR and MS spectrometry. The DTT adduct emitted light under alkaline condition to produce an oxidized compound. Thus we succeeded in modeling the bioluminescence of a photoprotein with DTT.

Introduction[1]

Molecular mechanisms of the bioluminescence and chemiluminescence have not been well-defined, although many studies on chemi- and bioluminescence have been undertaken in recent decades.[2] We have studied a bioluminescence system of an oceanic luminous squid, *Symplectoteuthis oualaniensis* L. In 1981, Tsuji and Leisman reported that a homogenate of the luminous organ of this squid gave light in the presence of monocations such as Na^+, K^+ etc. and molecular oxygen at pH 7.8.[3] This luminous system is potentially useful as a monitor of monocations in single cells. For monitoring dications, an aequorin system is especially famous for the determination of the Ca^{2+} concentration in living cells.[4] In 1993, we succeeded in the extraction of the photoprotein, named symplectin, responsible for the bioluminescence in a 0.6 M KCl solution from the same squid *S. oualaniensis* collected in Okinawa, Japan. We have reported that this symplectin contains dehydrocoelenterazine (**1a**), a similar chromophore to coelenterazine (**4**), and that this chromophore may chemically be bound with the symplectin through a covalent bond such as thioether.[5] The major evidence for this chromophore is that the mixing of dehydrocoelenterazine (**1a**) solution into apoprotein of this squid enhanced the bioluminescence in almost the same amount of light emission as from natural symplectin, but mixing with the yellow coelenterazine (**4**) solution did not show any bioluminescence. The reddish solution of **1a** mixed with aposymplectin solution instantaneously changed into a yellowish color since the chromophore in **2** is the same as **4**; this chemical change was supported by UV and Fluorescence spectra.[4]

The connecting position of this chromophore has been suggested from an acetone adduct (**3**) to a dehydrocoelenterazine (**1a**), which was isolated as an artifact from this photogenic organ.[4]



3 (acetone adduct)

A time course of fluorescence and absorption spectra were recorded during the symplectin bioluminescence.[6] As a result of the spectra, the chromophore seemed to link with the cysteine residue of the symplectin through a thioether as illustrated in Scheme 1. As an evidence of this hypothesis, model studies on the bioluminescence were undertaken with DTT and GSH as a model for the symplectin. Firstly, the predicted carbon connecting to the sulfhydryl residue was labeled with ^{13}C, and secondly, the chemical shift changing was measured by high-field NMR.

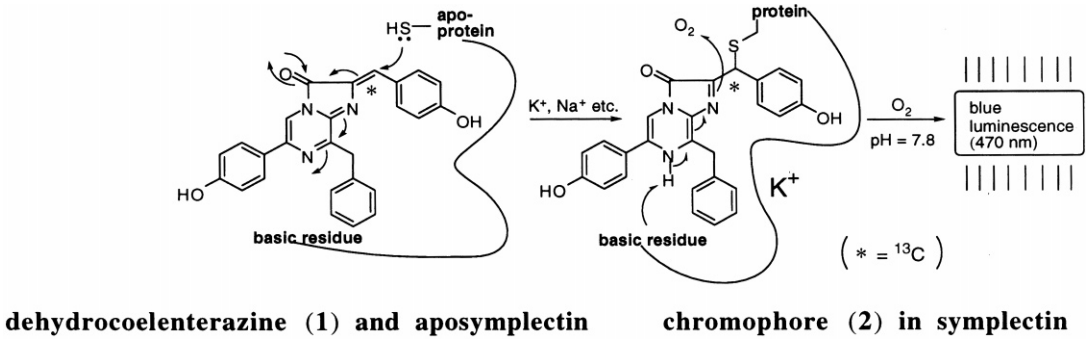
Result and Discussion

The retrosynthetic analysis of the ^{13}C-labeled dehydrocoelenterazine (**1a***) is shown in Scheme 2. (In order to indicate

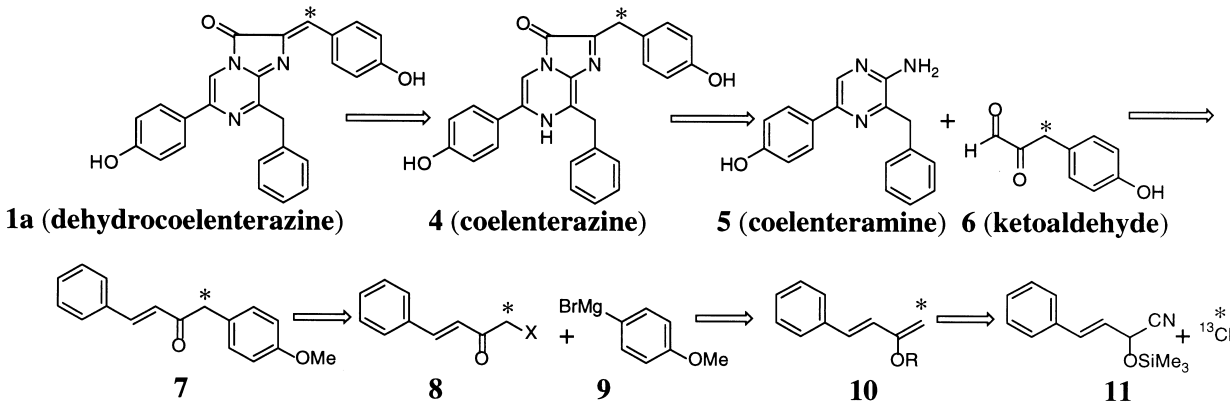
Keywords: bioluminescence; photoprotein; dehydrocoelenterazine; nuclear magnetic resonance.

* Corresponding author. Tel.: +81-52-789-4109; fax: +81-52-789-4111; e-mail: isobem@agr.nagoya-u.ac.jp

0040–4020/00/$ - see front matter
PII: S0040-4020(00)00163-0

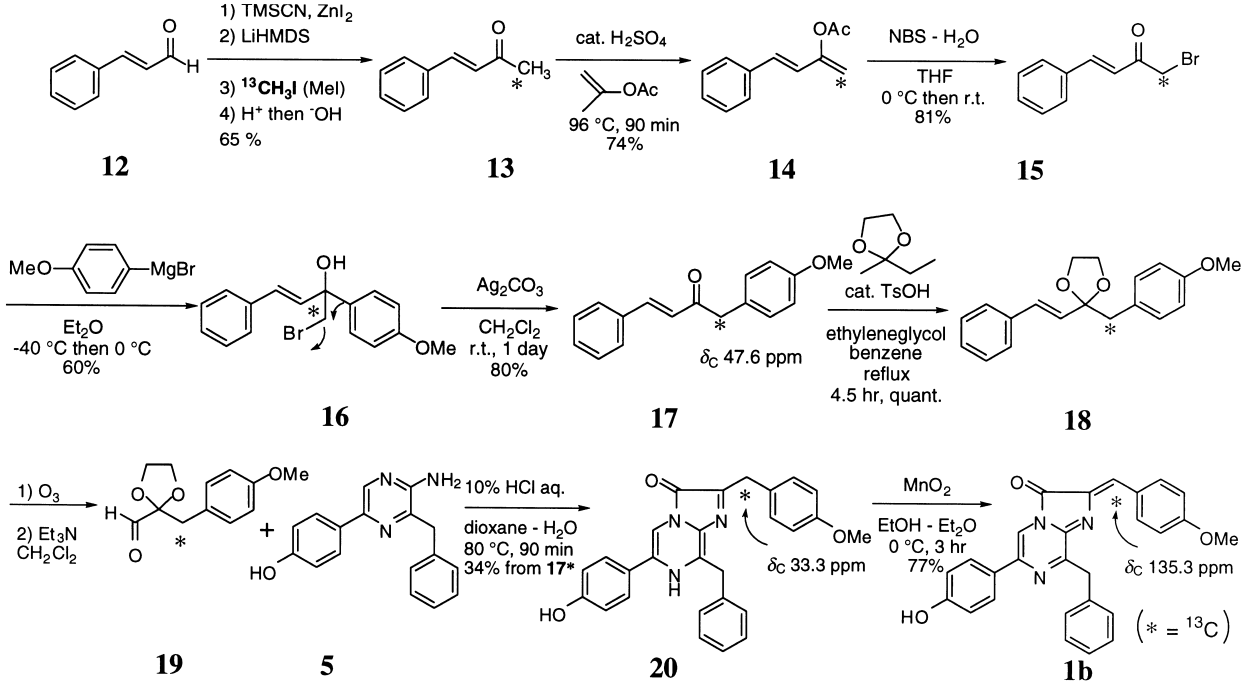


Scheme 1. Initial step in the bioluminescence of the *Symplectoteuthis* photoprotein.



Scheme 2. Retrosynthetic analysis of ^{13}C-labeled dehydrocoelenterazine (**1a**).

and to distinguish ^{13}C-labeled from natural abundance isotope compounds the asterisk is attached to the formula number such as **N*** in text.) Dehydrocoelenterazine (**1a**)[7] is obtained from coelenterazine (**4**)[8] by MnO_2 oxidation, and **4** is synthesized from coelenteramine (**5**) and ketoaldehyde (**6**) by reported procedures.[9] For the preparation of the ^{13}C-labeled compound **6***, it is necessary to establish a novel synthetic route to **6**. Ozonolysis of α,β-unsaturated



Scheme 3. Synthesis of ^{13}C-labeled and non-labeled dehydrocoelenterazine.

ketone (**7**) will afford **6**. The disconnection between benzylic and phenyl carbon gives the critical two precursors **8** and **9**. Halogenation of the diene (**10**) will give **8**. After a consideration of the availability of ^{13}C sources, we decided to use $^{13}CH_3I$. A derived cyanohydrin (**11**) from cinnamaldehyde is a precursor of **10**.

The novel synthetic route to the 2′-^{13}C-dehydrocoelenterazine analog (**1b***) is shown in Scheme 3. The cinnamaldehyde (**12**) was reacted with trimethylsilyl cyanohydrin (TMS-CN)[10] to give cyanohydrin (**11**). Methylation of **11** with $^{13}CH_3I$ and subsequent hydrolysis afforded benzalacetone (**13**) in 65% overall yield. To oxidize the methyl group of **13**, it was first converted to vinylacetate (**14***, 74%) in isopropenylacetate in the presence of catalytic amounts of conc. H_2SO_4. Oxidation of the methyl moiety of **13** was accomplished by bromination of **14** with *N*-bromosuccinimide (NBS) to give bromoketone (**15***, 81%). The critical addition of *p*-methoxyphenylmagnesium bromide[11] to **15** was carried out in diethyl ether first at −40°C and then 0°C to provide the bromohydrin (**16***) as the sole product in 60% yield. This bromohydrin (**16**) rearranged and then converted into various products at higher temperatures. The expected 1,2-rearrangement cleanly took place with silver carbonate to give α,β-unsaturated ketone (**17***, 80%). Isomeric product was unexpectedly obtained in 20% yield.[12] We found that the cinnamyl moiety of **16** had also an ability of rearrangement. A direct ozonolysis of **17** gave some degradation products presumably due to its facile enolization. The carbonyl moiety of **17** was temporally protected as the ethylene ketal (**18**). The ozonide prepared by ozonolysis of **18** was successfully reduced with triethylamine[13] to give the desired ketal–aldehyde (**19***). Because of an instability with silica gel, **19** was not isolated and was directly reacted with aminopyrazine (**5**) at 80°C in a mixture of 10% HCl water and dioxane. The coelenterazine mono-methyl ether (**20***, δ 2′-C 33.3 ppm) was isolated in 34% overall yield in 4 steps from the enone (**17***). Oxidation of **20*** with manganese dioxide in a mixture of ethanol and diethyl ether provided the dehydrocoelenterazine mono-methyl ether (**1b***, δ 2′-C 135.5 ppm) in 77% yield.

The equilibrium of the addition of DTT as a sulfhydryl compound to dehydrocoelenterazine (**1a**) and its analogs

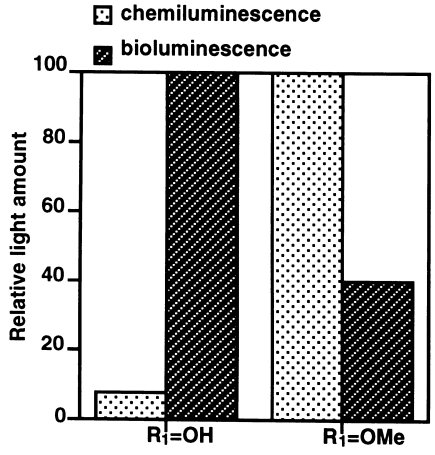
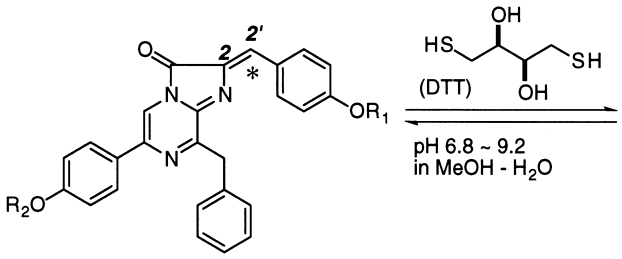
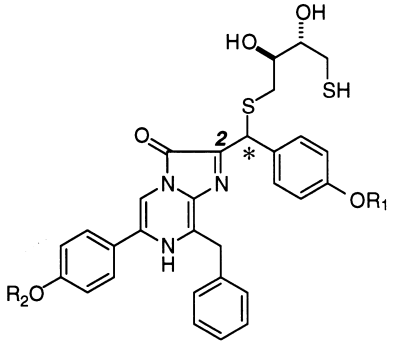


Figure 1. Relative light amount (%) of bio- and chemiluminescence of **21a** and **21b**.

(**1b**–**1d**) with natural abundance isotopes (Eq. (1), equilibrium between dehydrocoelenterazine (**1**) and DTT adduct (**21**)) was studied prior to the use of ^{13}C-labeled compounds.



1a $R_1 = H$ $R_2 = H$
1b $R_1 = Me$ $R_2 = H$
1c $R_1 = H$ $R_2 = Me$
1d $R_1 = Me$ $R_2 = Me$



21a $R_1 = H$, $R_2 = H$
21b $R_1 = Me$, $R_2 = H$
21c $R_1 = H$, $R_2 = Me$
21d $R_1 = Me$, $R_2 = Me$

(1)

The questions in Eq. (1) are the effects of the dissociation of the phenol groups in the equilibrium and also the relationship to the pK_a being 8 for the dihydromidazopyrazinone N–H proton in **21**. The dissociation of the N–H proton is the critical step for the chemiluminescence in aprotic polar solvents as DMSO with strong bases such as potassium *t*-butoxide. When an aqueous solution of the compounds **21a** or **21b** was added to a solution of aposymplectin prepared from symplectin (0.6 M KCl at pH 7.8 under air oxygen atmosphere), both of them showed a bioluminescence and the latter afforded about 40% of the light of the former. On the contrary the chemiluminescence of **21a** in DMSO with *t*-BuOK yielded less than 10% of the light amount of **21b** as shown in Fig. 1. This striking difference would be due to the dissociation of phenol extended to the 2-position of the imidazopyrazinone ring. A strong alkaline condition for the chemiluminescence caused the equilibrium to eliminate the sulfhydryl residue from **21a**.

The equilibrium between compound **21** and **1** with DTT was measured under slightly alkaline media between pH 6.8 and 9.2 since the optimum pH is known to be 7.8 for the squid symplectin. The equilibrium is monitored by means of UV–Vis spectra as shown in Fig. 2, where **A**–**D** are corresponding to **1a**–**d** (**21a**–**d**), respectively. The increase

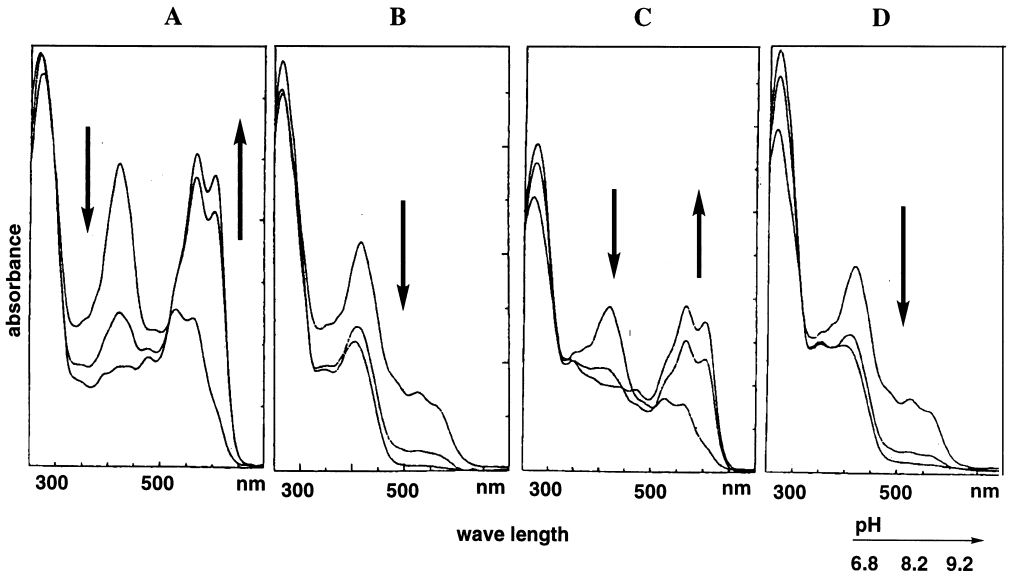


Figure 2. UV spectra **A**–**D** of the equilibrium between dehydrocoelenterazine (**1a**–**d**) and its DTT adduct (**21a**–**d**), respectively, at various pH.

in the absorbance at around 550 nm in **A** indicates the increase in the concentration of dehydrocoelenterazine (**1a**). On the other hand, the decrease in the absorbance at around 400 nm means the decrease in the amount of **21a**. (Arrows indicate the direction of increasing pH.) A similar tendency is observed with **21c** in **C**.

Those compounds (**21b** and **21d**) having methyl ether on the right phenol does not show such propensity in the spectrum **B** and **D**. No elimination of SH in **21b** and **21d** happens under these alkaline conditions. The equilibrium of **1** and **21** was not affected by the left phenol residue (**B** and **D**). As a consequence the dehydrocoelenterazine analogs having R_1=Me (**21b** and **21d**) can provide stable adducts with SH compounds. Dehydrocoelenterazine mono-methyl ether **1b*** (2′-carbon labeled with ^{13}C) is the best candidate to study the mimicking of the bioluminescence of the symplectin. Mixing **lb*** and dithiothreitol (DTT) with 1:10 molar ratio in a mixture of methanol and dichloromethane (1:1) provided the adduct **21b*** at room temperature. After stirring for 20 min the reaction mixture was acidified to pH 3.0 with 1N HCl. The adduct **21b*** is the most stable in this acidic media. Concentration of the solution of the DTT adduct (**21b***) under reduced pressure gave a yellow oil. This residue was purified by HPLC to isolate the adduct **21b*** with a Develosil® ODS-UG-5 reversed-phase column (10×250 mm) and 70% acetonitrile–water containing 0.1% TFA. As a mobile phase 70% methanol–water was not suited for this purification, since the high pressure in the column decomposed all **21b*** to DTT and **1b***. Isolated **21b*** was analyzed by NMR to afford two signals at δ 45.2 and 45.4 ppm in the ^{13}C spectra (Fig. 3); thus derived from δ 135.3 ppm. Proton NMR also showed two doublets at ca. 5.6 ppm, and a C–H COSY spectrum showed the correlation of the signals at ^{1}H NMR δ 5.67 and 5.62 ppm with the signals at δ 45.2 and 45.4 ppm, respectively, as shown in Fig. 4 (left side). We found that the addition of DTT dehydrocoelenterazine analog (**1a**) gave two diastereomers of **21b***. Isolated DTT adduct **21b** (natural abundance ^{13}C) gave m/z=590 $(M+H)^+$ by FAB-MS spectrum. The

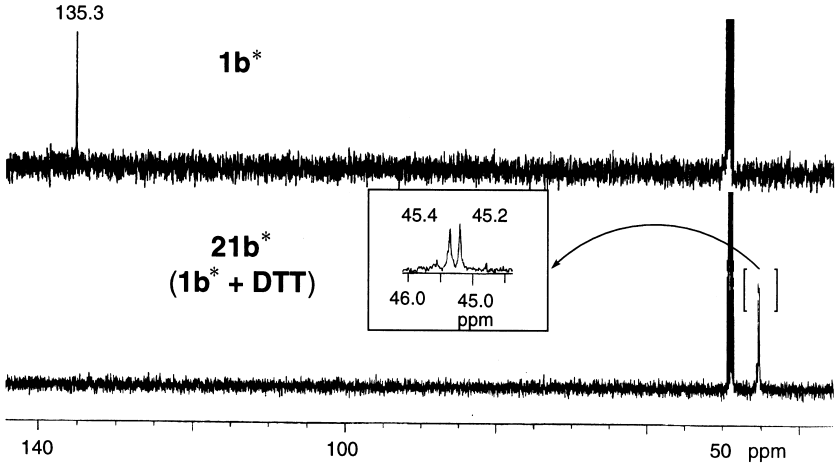


Figure 3. ^{13}C NMR spectra of adduct **21b*** of ^{13}C-labeled dehydrocoelenterazine analog **1b*** and DTT.

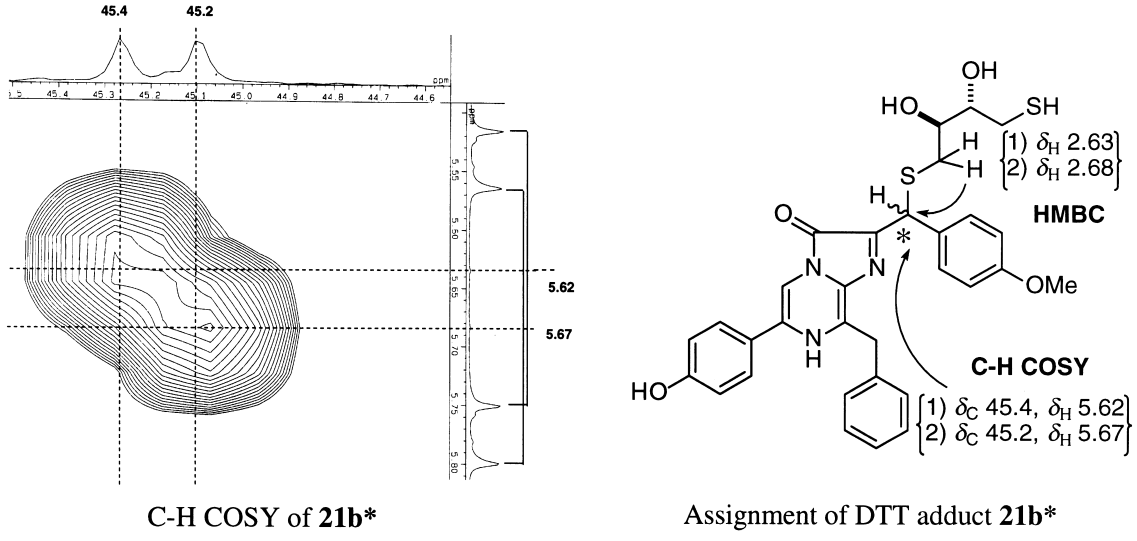


Figure 4. C–H COSY spectrum and summary of the measurement of DTT adduct **21b*** with NMR.

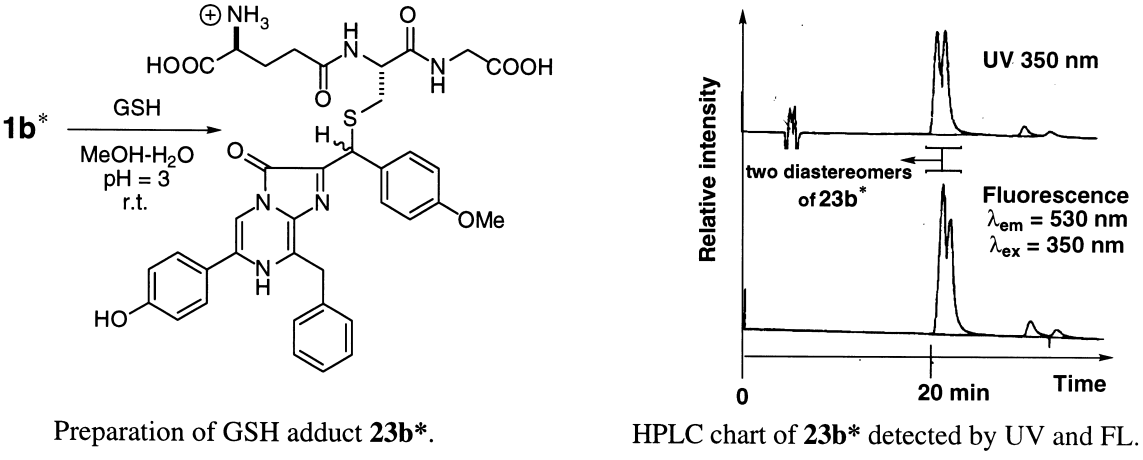


Figure 5. Structure of GSH adduct (**23***) and HPLC chart of **23b*** analyzed by reversed phase ODS colunm.

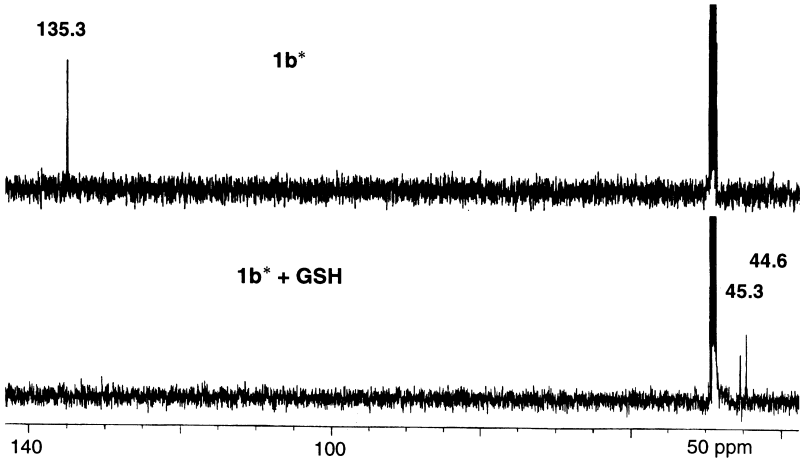


Figure 6. ^{13}C NMR of adduct **23*** of ^{13}C-labeled dehydrocoelenterazine analog **1b*** and GSH.

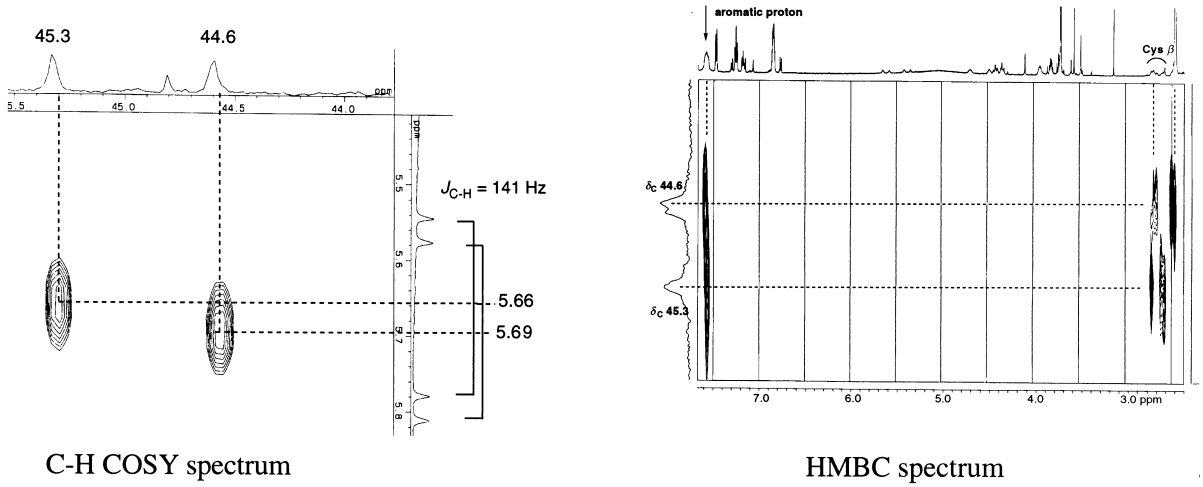


Figure 7. 2D (C–H COSY and HMBC) NMR spectrum of isolated GSH adduct **23b***.

absolute structure shown in Fig. 4 (right side) was confirmed by measurement of **21b*** with 2D NMR (HMBC and HOHAHA spectrum).

Similarly glutathione (GSH) was added to **1b*** with 1:5 molar ratio in a mixture of methanol and water (1:1) at room temperature for 50 min, then the reaction mixture was acidified to pH 3.0, as shown in Fig. 5 (left side). Concentration of the solution of the GSH adduct (**23b***) gave a yellow oil. Isolation of **23b*** was performed with reversed phase HPLC (30% acetonitrile–water containing 0.1% TFA) as shown in Fig. 5 (right side). Two peaks corresponding to the diastereomers of **23b*** appeared in the chromatogram. But the peaks could not be separated, due to only slight differences in their retention time. Thus the purified **23b*** was analyzed by NMR as the diastereomeric mixture.

Two signals at δ 44.6 and 45.3 ppm appeared in the ^{13}C NMR spectrum and two signals at δ 5.69 and 5.66 ppm in the ^{1}H NMR spectrum, respectively, as shown in Figs. 6 and 7. The correlation between the ^{13}C-labeled carbon and the *beta* protons of the cysteine residue of GSH was observed in the HMBC spectrum as shown in Fig. 7. (HMBC and HOHAHA was measured in DMSO-d_6.) These *beta* protons of cysteine were confirmed by HOHAHA spectrum as illustrated in Fig. 8. Isolated GSH adduct **23** (natural abundance ^{13}C) gave m/z=743 $(M+H)^+$ by FAB-MS spectrum.

The structure of the GSH adduct **23b*** was elucidated in

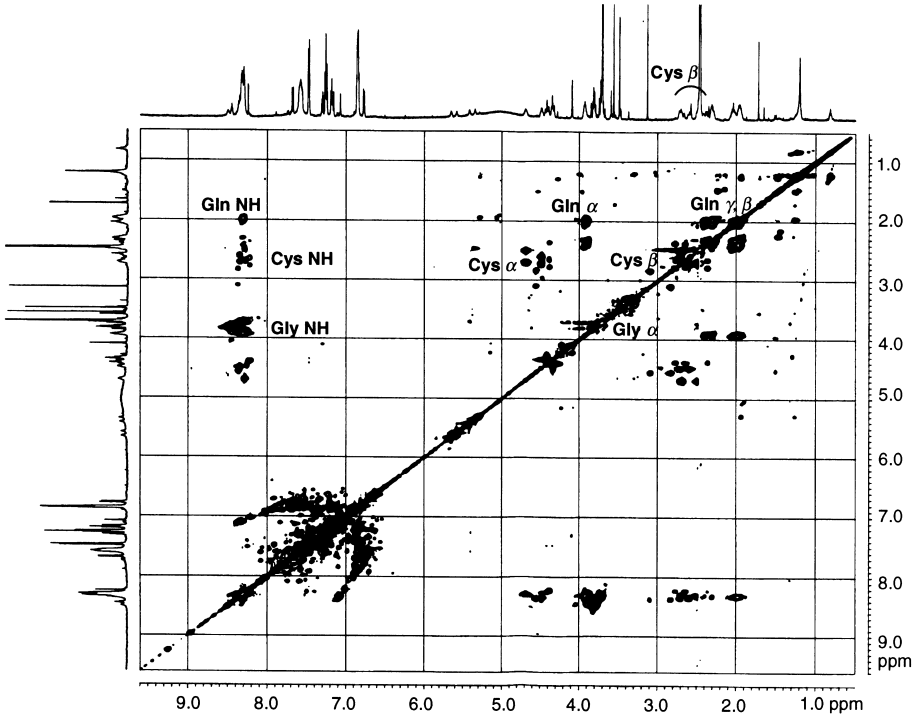


Figure 8. HOHAHA spectrum of adduct **23b*** of ^{13}C-dehydrocoelenterazine analog **1b*** and GSH.

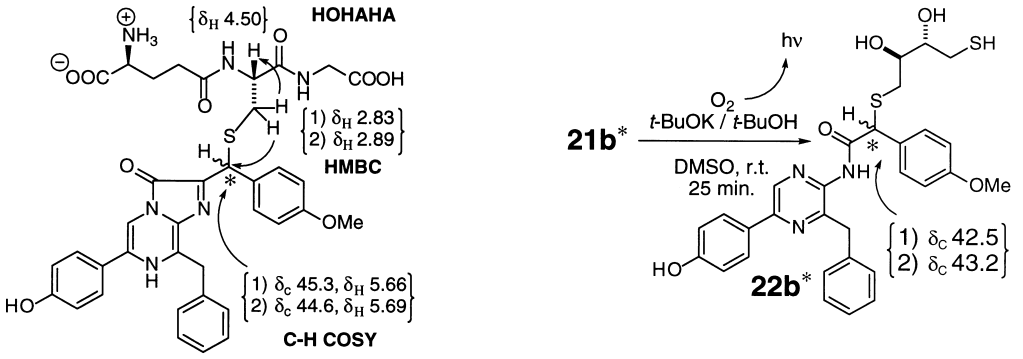


Figure 9. Summary of NMR measurements of GSH adduct (**23b***) and the chemiluminescence product **22b*** as *Symplectoteuthis* bioluminescence model.

detail as shown in Fig. 9 (left side) from a series of measurement by NMR. Undoubtedly the sulfhydryl group of the cysteine residue of GSH is connecting to the carbon labeled with ^{13}C of dehydrocoelenterazine (**1b***).

The chemiluminescence of DTT adduct **21b*** emits light (520 nm) in DMSO under basic condition (containing 10% of 1N *t*-BuOK/*t*-BuOH) as shown in Fig. 9 (right side). After the chemiluminescence was completed, the solution was neutralized with saturated ammonium chloride, then the product (**22b**) was extracted and analyzed to give *m*/*z*=577 (using natural abundance ^{13}C) by FAB-MS spectrum. In its ^{13}C NMR spectrum two sets of signals at δ 42.5, 43.2 ppm appeared, presumably derived from the two diastereomers of coelenteramide analog (**22b***). The molecular mechanism on the bioluminescence of *S. oualaniensis* may be similar to the above model studies. In the natural dehydrocoelenterazine (**1a**) which exhibits efficient bioluminescence the local pH around the phenol and dihydroimidazopyrazinone groups should be presumably controlled by the protein surfaces. To get further evidence the molecular mechanism should be studied directly with the symplectin. Now structural and cloning studies on this symplectin are also under investigation.

Experimental

All melting points were measured on Yanaco MP-S3 and uncorrected. UV spectra were taken on a JASCO U-best 50 spectrometer. IR spectra were determined on a JASCO FT/IR-7000S spectrophotometer. Proton NMR spectra were recorded on a JEOL EX 270 or GSX 270 for 270 MHz, a JEOL JNML-500 for 500 MHz or a Bruker AMX-600 for 600 MHz. Chemical shifts (δ) are given in parts per million relative to tetramethylsilane (δ 0.00) or CD_3OD (δ 3.30) or DMSO-d_6 (δ 2.49) as internal standard and coupling constants (*J*) in Hz. Carbon NMR were recorded on a JEOL EX 270 or GSX 270 for 67.8 MHz or a JEOL JNML-500 for 125.7 MHz or on a Bruker AMX-600 for 150.9 MHz. Chemical shifts are (δ) given in parts per million relative to $CDCl_3$ (δ 77.0) or CD_3OD (δ 49.0) or DMSO-d_6 (δ 45.0) as internal standard. Coupling constants (*J*) are given in Hz. The asterisks (*) represent signals of the ^{13}C incorporated carbon. Low-resolution EI mass spectra and FAB mass spectra were measured with a JEOL JMS-700 or JMS-600. High-resolution (HR) mass spectra were measured with a JEOL JMS-700. Elemental analysis and HRMS were performed by the Analytical Laboratory of this school. Fluorescence spectra were measured with a JASCO FP-777 spectrometer. Chemiluminescence was recorded on an Otsuka Electronics MCPD- 110A, or a JASCO FP-770 spectrometer without excitation beam. Chemiluminescence profiles were recorded on a Labo Science Lumiphotometer TD-1000. Light yields of the chemiluminescence were determined with a Labo Science TD-4000 Lumiphotometer, or a TS-1000 Lumiphotometer by integration of the total light emission. Ozone was produced by a Nihon Ozone Corporation Ozonator, style 0-3-2, with the voltage set at 40 V and oxygen pressure at 2 kg cm^{-2} to give approximately 0.3 g h^{-1} ozone concentration. The input oxygen was passed through a column of Drierite to ensure dryness.

Dichloromethane (CH_2Cl_2) was distilled from calcium hydride. Tetrahydrofuran (THF) or 1,4-dioxane was distilled from sodium metal in the presence of sodium benzophenone ketyl as indicator. Dimethylsulfoxide (DMSO) was distilled from calcium hydride under reduced pressure. Pyridine was dried over NaOH pellets and used without distillation. The other solvents were of reagent grade.

Analytical thin-layer chromatography (TLC) was conducted on precoated TLC plates: silica gel 60 F-254 [E.Merck (Art 5715) Darmstadt, Germany], layer thickness 0.25 mm. Silica gel column chromatography utilized Fuji Devision (BW 820-MH) or Merck (Silica Gel 60).

The HPLC analysis was carried out using a JASCO PU-980 pump system equipped with a JASCO UV-970 UV–Vis detector and JASCO 807-IT integrator. An ODS column of 5×250 mm (Develosil ODS-UG-5, Nomura Chemicals Co., Ltd) was eluted with acetonitrile–water containing 0.1% of trifluoroaceticacid (TFA) at a flow-rate of 0.5 mL min^{-1} at room temperature. The purification by HPLC was performed with an ODS column 10×250 mm (Develosil ODS-UG-5, Nomura Chemicals Co., Ltd).

Cinnam-cyanomethyl trimethylsilyl ether 11

To a suspension of cinnamaldehyde (**12**) (1.56 g, 8.0 mmol)

and ZnI_2 (catalytic amount) in CH_2Cl_2 was added TMSCN (1.18 ml, 8.8 mmol) at 0°C. Then the mixture was allowed to warm to room temperature and was stirred under argon atmosphere for 2 h. The reaction mixture was concentrated under reduced pressure to remove CH_2Cl_2. The resultant residue was in almost pure state **11** in quantitative yield. ^{1}H NMR ($CDCl_3$, 270 MHz) δ 0.27 (s, 9H), 5.13 (dd, J=5.9, 1.0 Hz, 1H), 6.21 (dd, J=16.0, 5.9 Hz, 1H), 6.82 (dd, J=16.0, 1.0 Hz, 1H), 7.3–7.43 (m 5H, Ar H) ppm. ^{13}C NMR ($CDC1_3$, 67.8 MHz), δ −0.1, 62.3, 118.4, 123.6, 127.0, 128.7, 128.8, 134.0, 135.1 ppm. EIMS m/z 205 (M^+-CN), 158 (M^+-TMS), 131 ($M^+-TMSCN$).

Cinnam-methylketone 13, 13* (^{13}C)

A solution of the cyanohydrin TMS–ether (**11**) (ca. 1.9 g, 8.0 mmol) in 9 mL of THF was added dropwise over 20 min to a solution of 8.8 mL (1.0 M hexane solution) of LiHMDS in THF (35 mL) under argon atmosphere at −78°C. After stirring this solution for 1 h, $^{13}CH_3I$ (1.0 g, 7.0 mmol) was added dropwise to the solution over 5 min through a gas-tight syringe under argon atmosphere. After being stirred for 25 min at −78°C, the reaction mixture was allowed to warm to 0°C over 1 h, then warmed up to room temperature. After 20 min later, the reaction mixture was cooled to 0°C, then slowly poured into 1N HCl (30 mL) and stirred for 1 min, then allowed to warm to room temperature, and stirred overnight at room temperature. The layers were separated, and the water layer was extracted with CH_2Cl_2 (×3). The combined organic layers were washed firstly with 1/10N HCl twice, secondly with 1N NaOH twice, and then dried over anhydrous Na_2SO_4. Evaporation under reduced pressure gave a crude product (1.12 g), and it was purified by chromatography on silica gel with diethyl ether–*n*-hexane (1:4 then 1:3) to afford **13***(^{13}C) (660 mg) in 65% (3 steps). ^{1}H NMR ($CDCl_3$, 270 MHz), δ 2.38 (d, J=127.6 Hz, 3H), 6.71 (dd, J=16.0, 1.5 Hz, 1H), 7.44 (brt, J=2.5 Hz, 3H), 7.52 (d, J=16.0 Hz, 1H), 7.55 (brd, J=9.4 Hz, 2H) ppm. ^{13}C NMR ($CDC1_3$, 67.8 MHz), δ *27.5 ppm. EIMS m/z 147 (M^+), 131 ($M^+-{}^{13}CH_3$). HRMS (EI) calcd for $^{13}C_1C_9H_{10}O$ 147.0766, found 147.0755.

The compound **13** (natural isotope abundance) was synthesized according to the same procedure from ca. 0.46 g (2.0 mmol) of the TMSCN-adduct (**11**), 2.3 mL (1.0 M hexane solution) of LiHMDS and 0.26 mL of CH_3I in 63% yield (**13**, 0.20 g). Mp 34–35°C. IR (KBr) ν_{max} 1682, 1605, 1452, 1363, 1189, 985, 735 cm^{-1}. ^{1}H NMR ($CDCl_3$, 270 MHz), δ 2.38 (s, 3H), 6.72 (d, J=16.0 Hz, 1H), 7.40 (brt, J=3.5 Hz, 3H), 7.52 (d, J=16.0 Hz, 1H), 7.55 (brd, J=9.4 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 67.8 MHz), δ 27.5, 127.1, 128.2, 128.9, 130.5, 134.4, 143.4, 198.3 ppm. EIMS m/z 146 (M^+), 131 (M^+-CH_3), 103 (M^+-CH_3CO), 77 (M^+-69). HRMS (EI) calcd for $C_{10}H_{10}O$ 146.0732, found 146.0743.

1-Acetoxy-1-cinnam-ethene 14, 14* (^{13}C)

To a solution of the cinnam-methylketone (**13***) (**^{13}C**) (620 mg, 4.2 mmol) in 10 mL of isopropenylacetate was added a few drops of conc. H_2SO_4, then the solution was refluxed over 90 min under argon atmosphere. After cooled to room temperature, the reaction mixture was poured into water (20 mL). The layer was separated, and the water layer was extracted with diethyl ether (×3). The organic layer was washed firstly with water, secondly with sat. NaCl aq., and then dried over anhydrous Na_2SO_4. Evaporation under reduced pressure gave a crude oil, and it was purified by chromatography on silica gel with diethyl ether–*n*-hexane (1:5) to afford **14*** (^{13}C) (590 mg) in 74%. ^{1}H NMR ($CDCl_3$, 270 MHz), δ 2.30 (s, 3H), 4.98 (dd, J=160.0, 2.0 Hz, 1H), 5.13 (dd, J=160.0, 2.0 Hz, 1H), 6.58 (d, J=16.3 Hz, 1H), 6.66 (dd, J=16.3, 1.5 Hz, 1H), 7.22–7.44 (m, 5H, Ar H) ppm. ^{13}C NMR ($CDCl_3$, 67.8 MHz), δ *106.2 ppm. EIMS m/z 189 (M^+), 147 ($M^+-{}^{13}CH_3CO$). HRMS (EI) calcd for $^{13}C_1$,C_{11} $H_{12}O_2$ 189.0871, found 189.0860.

The compound **14** (natural isotope abundance) was prepared according to the same procedure from 310 mg (2.1 mmol) of cinnam-methylketone (**13**), 3.0 mL of isopropenylacetate, and few drops of conc. H_2SO_4 in 69% yield (**14**, 280 mg). Mp 27–28°C. IR (KBr) ν_{max} 1757, 1640, 1607, 1448, 1372, 1210, 1022, 966, 870, 753, 694 cm^{-1}. ^{1}H NMR ($CDCl_3$, 270 MHz), δ 2.29 (s, 3H), 4.98 (d, J=2.0 Hz, 1H), 5.12 (d, J=2.0 Hz, 1H), 6.58 (d, J=16.3 Hz, 1H), 6.64 (d, J=16.3 Hz, 1H),7.21–7.43 (m, 5H, Ar H) ppm. ^{13}C NMR ($CDCl_3$, 67.8 MHz), δ 20.9, 106.1 122.6, 126.9, 128.2, 128.6, 129.9, 135.9, 151.8, 168.7 ppm. EIMS m/z 188 (M^+), 146 (M^+-CH_3CO). Anal, calcd for $C_{12}H_{12}O_2$; C, 76.57; H, 6.43%. Found C, 76.36; H, 6.41%.

Bromomethyl-cinnamketone 15, 15* (^{13}C)

To a solution of 1-acetoxy-l-cinnnam-ethene (**14***) (^{13}C) (610 mg, 3.2 mmol) in 20 mL of THF was added NBS (970 mg, 5.4 mmol) and water (94 mg, 5.4 mmol) at 0°C. After a few minutes, the reaction mixture was allowed to warm to room temperature and stirred for 45 min under argon atmosphere. Then the solution was cooled to 0°C and poured into 20 mL of sat. $NaHCO_3$ aq. then diluted with water. After extraction by diethyl ether (×3), the organic layer was washed firstly with water, secondly with brine, and then dried over anhydrous Na_2SO_4–SiO_2–anhydrous Na_2SO_4 column. Evaporation under reduced pressure gave a crude oil, and it was purified by chromatography on silica gel with diethyl ether–*n*-hexane (1:5) to afford **15*** (^{13}C) (570 mg) in 81%. ^{1}H NMR ($CDCl_3$, 270 MHz), δ 4.09 (d, J=151.4 Hz, 2H), 6.96 (dd, J=15.8, 1.0 Hz, 1H), 7.39–7.62 (m, 5H, Ar H), 7.71 (d J=15.8 Hz, 1H,) ppm. ^{13}C NMR ($CDCl_3$, 67.8 MHz), δ *33.1 ppm. EIMS m/z 227 (M^+(^{81}Br)), 225 (M^+(^{79}Br)), 146 (M^+-Br). HRMS (EI) calcd for $^{13}C_1C_9H_9OBr$ 224.9871 (^{79}Br) and 226.9850 (^{81}Br), found 224.9871 (^{79}Br) and 226.9831 (^{81}Br).

The compound **15** (natural isotope abundance) was synthesized according to the same procedure from 210 mg (1.1 mmol) of 1-acetoxy-1-cinnnam-ethene (**14**) in 6.3 mL of THF, and NBS (330 mg, 1.9 mmol) and water (34 mg, 1.9 mmol) in 75% yield (**15**, 190 mg). Mp 44–45°C. IR (KBr) ν_{max} 1694, 1613, 1496, 1449, 1388, 1333, 1157, 1069, 993, 892, 748, 688, 557 cm^{-1}. ^{1}H NMR ($CDCl_3$, 270 MHz), δ 4.09 (s, 2H), 6.95 (d, J=16.0 Hz, 1H), 7.38–7.45 (brdd J=5.4, 1.5 Hz, 3H,), 7.55–7.61 (brdd, J=6.4, 2.0 Hz, 2H), 7.70 (d, J=16.0 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$, 67.8 MHz), δ 33.0, 122.2, 128.6, 129.0, 131.1,

134.0, 145.4, 191.0 ppm. EIMS *m/z* 226 ($M^+(^{81}Br)$), 224 ($M^+(^{79}Br)$), 145 (M^+-Br). Anal, calcd for $C_{10}H_9OBr$; C, 53.36; H, 4.03%. Found C, 53.26; H, 4.02%.

2-Bromo-1-cinnnam-1-(*p*-methoxyphenyl)-ethanol 16, 16* (13C)

To a solution of bromomethyl-cinnamketone (**15***) (^{13}C) (540 mg, 2.4 mmol) in THF (18 mL) was added dropwise over a few minutes 1.4 M *p*-methoxyphenylmagnesium bromide freshly prepared in diethyl ether solution[11] (1.9 mL, 2.6 mmol) under argon atmosphere at −40°C. After being stirred for 20 min at −40°C, the reaction mixture was poured into sat. NH_4C1 aq. at 0°C. The resulting mixture was extracted with diethyl ether (×3) and the combined organic layers were washed with water (×1) and sat. NaCl aq. (×1). After being dried over anhydrous Na_2SO_4, the organic layer was evaporated under reduced pressure, then the crude products obtained. They were purified by using column chromatography on silica gel with diethyl ether–*n*-hexane (1:3), the product **16*** (^{13}C) was given in pure state (60%, 500 mg). ^1H NMR ($CDCl_3$, 270 MHz), δ 2.79 (d, *J*=2.0 Hz, 1H), 3.81 (s, 3H), 3.86 (dd, *J*=153.5, 2.0 Hz, 2H), 6.48 (dd, *J*=15.8, 3.0 Hz, 1H), 6.71 (d, *J*=15.8 Hz, 1H), 6.91 (d *J*=8.9 Hz, 2H) 7.21–7.40 (m, 5H, Ar H), 7.44 (d, *J*=8.9 Hz, 2H) ppm. ^{13}C NMR ($CDC1_3$, 67.8 MHz), δ *44.6 ppm. EIMS *m/z* 335 ($M^+(^{81}Br)$), 333 ($M^+(^{79}Br)$), 254 (M^+-Br). HRMS (EI) calcd for $^{13}C_1C_{16}H_{17}O_2Br$ 333.0446 (^{79}Br) and 335.0426 (^{81}Br), found 333.0460 (^{79}Br) and 335.0426 (^{81}Br).

The compound **16** (natural isotope abundance) was synthesized according to the same procedure from 660 mg (3.0 mmol) of bromomethyl-cinnamketone (**15**) in 20 mL of diethyl ether, and 1.4 M *p*-methoxyphenylmagnesium bromide in diethyl ether solution (2.2 mL, 3.0 mmol) in 76% yield (**16**, 750 mg). IR ($CHCl_3$) ν_{max} 3024, 2942, 1609, 1511, 1461, 1419, 1304, 1250, 1180, 1034, 971, 832 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz), δ 2.78 (s, 1H), 3.81 (s, 3H), 3.85 (d, *J*=4.9 Hz, 2H), 6.47 (d, *J*=16.0 Hz, 1H), 6.70 (d, *J*=16.0 Hz, 1H), 6.91 (d, *J*=8.8 Hz, 2H) 7.24 (t, *J*=5.4 Hz, 1H), 7.31 (t, *J*=7.8 Hz, 2H), 7.39 (d, *J*=7.3 Hz, 2H), 7.43 (d, *J*=8.8 Hz, 2H) ppm. ^{13}C NMR ($CDC1_3$, 67.8 MHz), δ 44.6, 55.3, 75.3, 113.9, 126.7, 127.0, 128.0, 128.6, 130.8, 132.0, 134.5, 136.2, 159.2 ppm. EIMS *m/z* 334 ($M^+(^{81}Br)$), 332 ($M^+(^{79}Br)$), 253 (M^+-Br). HRMS (El) calcd for $C_{17}H_{17}O_2Br$ 332.0412 (^{79}Br) and 334.0393 (^{81}Br), found 332.0422 (^{79}Br) and 334.0373 (^{81}Br).

Cinnnam-(*p*-methoxyphenyl)-ketone 17, 17* (13C)

Silver carbonate (480 mg, 1.6 mmol) was added to a solution of bromohydrin (**16***) (^{13}C) (500 mg, 1.5 mmol) in CH_2Cl_2 (500 mL) at 0°C. Then the reaction mixture was allowed to warm to room temperature, and stirred overnight at room temperature. The reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure to a volume of ca. 20 mL. The solution was diluted with AcOEt (100 mL) and the organic layer washed with sat. $NaHCO_3$ aq.(×1), water (×1), and brine (×1). After dried over anhydrous Na_2SO_4, the organic layer was evaporated under reduced pressure to give a crude oil. The residue was chromatographed on silica gel with CH_2Cl_2 as an eluent. 4-methoxyphenyl-cinnnamyl ketone **17*** (^{13}C) was given in 80% (440 mg). ^1H NMR ($CDCl_3$, 270 MHz), δ 3.80 (s, 3H), 3.87 (d, *J*=127.6 Hz, 2H), 6.77 (d *J*=16.3 Hz, 1H,), 6.88 (d, *J*=8.4 Hz, 2H), 7.18 (dd, *J*=8.9, 4.0 Hz, 2H) 7.33–7.54 (m, 5H, Ar H), 7.60 (d, *J*=16.3 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$, 67.8 MHz), δ *47.6 ppm. EIMS *m/z* 253 (M^+), 131, 122, 103, 77. HRMS (EI) calcd for $^{13}C_1C_{16}H_{16}O_2$ 253.1184, found 253.1172. Isomer **24*** (^{13}C) in 20% (220 mg). ^1H NMR ($CDCl_3$, 270 MHz), δ 3.85 (dd, *J*=127.1, 5.4 Hz, 2H), 3.87 (s, 3H), 6.50 (m, 2H), 6.95 (d, *J*=8.9 Hz, 2H), 7.20–7.45 (m, 5H, Ar H), 8.00 (d, *J*=8.9 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 67.8 MHz), δ *42.5 ppm. EIMS *m/z* 253 (M^+), 131, 118. HRMS (EI) calcd for $^{13}C_1C_{16}H_{16}O_2$ 253.1184, found 253.1156.

The compound **17** (natural isotope abundance) was synthesized according to the same procedure from 750 mg (2.2 mmol) of bromohydrin (**16**) in 750 mL of CH_2Cl_2, and silver carbonate (720 mg, 2.5 mmol) in 70% yield (**17**, 400 mg). Mp 79–80°C. IR (KBr) ν_{max} 2364, 1665, 1617, 1513, 1341, 1247, 1177, 1034, 805, 750, 691 cm^{-1}. ^1H NMR ($CDCl_3$, 270 MHz), δ 3.80 (s, 3H), 3.88 (s, 2H), 6.77 (d, *J*=16.0 Hz, 1H), 6.89 (d, *J*=8.9 Hz, 2H), 7.19 (d, *J*=8.9 Hz, 2H) 7.37–7.59 (m, 5H, Ar H), 7.62, (d, *J*=16.0 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$, 67.8 MHz), δ 47.5, 55.2, 114.2, 125.1, 126.3, 128.3, 128.9, 130.4, 130.5, 134.4, 143.2, 158.6, 197.6 ppm. EIMS *m/z* 252 (M^+), 131, 121, 103. Anal, calcd for $C_{17}H_{16}O_2$; C, 80.93; H, 6.39%. Found C,80.49; H, 6.39%. Isomer **24** (natural abundance ^{13}C) in 30% (171 mg). Mp 109–110°C. IR (KBr) ν_{max} 2362, 1679,1598, 1256, 1215, 1172, 1027, 985, 690 cm^{-1}. ^1H NMR ($CDCl_3$, 270 MHz), δ 3.87 (d, *J*=5.4 Hz, 2H), 3.88 (s, 3H), 6.46 (dt, *J*=15.8, 6,4 Hz, 1H), 6.55 (d, *J*=15.8 Hz, 1H), 6.96 (d, *J*=8.9 Hz, 2H), 7.21–7.40 (m, 5H, Ar H), 8.00 (d, *J*=8.9 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 67.8 MHz), δ 42.5, 55.5, 113.8, 123.0, 126.2, 127.4, 128.5, 129.6, 130.6, 133.3, 137.0 163.5, 196.6 ppm. EIMS *m/z* 252 (M^+), 135, 107. Anal, calcd for $C_{17}H_{16}O_2$; C, 80.93; H, 6.39%. Found C,80.79; H, 6.38%.

Cinnam-(*p*-methoxyphenyl)-1,3-dioxolane 18. 18* (13C)

A mixture of 59 mg (0.23 mmol) of cinnnam-(*p*-methoxyphenyl)-ketone (**17***) (^{13}C), 2 mg of *p*-toluenesulfonic acid, 0.2 mL of ethylene glycol, 0.2 mL of 2-ethyl-2-methyl-1,3-dioxolane, and 5.0 mL of dry benzene was heated at reflux for 4.5 h in an apparatus equipped with a water separator (Dean–Stark condenser). After having been cooled, the reaction mixture was added to 20 mL of saturated sodium bicarbonate and extracted with AcOEt (×3). The combined extracts were washed with water and saturated brine, dried over anhydrous Na_2SO_4, and evaporated to afford 69 mg (quantitative yield) of **18*** (^{13}C) in almost pure state. ^1H NMR ($CDCl_3$, 270 MHz), δ 3.03 (d, 2H, *J*=128.1 Hz), 3.77 (s, 3H), 3.79–3.88 (m, 4H), 6.11 (d, *J*=15.8 Hz, 1H), 6.65 (d, *J*=15.8 Hz, 1H), 6.81 (d, *J*=8.9 Hz, 2H), 7.21 (dd, *J*=8.9, 5.1 Hz, 2H), 7.25–7.36 (m, 5H, Ar H) ppm. ^{13}C NMR ($CDCl_3$, 67.8 MHz), δ *44.3 ppm. EIMS *m/z* 297 (M^+), 175, 122, 103. HRMS (EI) calcd for $^{13}C_1C_{18}H_{20}O_3$ 297.1446, found 297.1432.

The compound **18** (natural abundance ^{13}C) was synthesized according to the same procedure described above for **17** (37 mg) in quantitative yield (**18**, 47 mg). ^1H NMR ($CDCl_3$, 270 MHz), δ 3.03 (s, 2H), 3.78 (s, 3H), 3.75–3.91 (m, 4H), 6.11 (d, J=15.8 Hz, 1H), 6.65 (d, J=15.8 Hz, 1H), 6.81 (d, J=8.9 Hz, 2H), 7.20 (d, J=8.9 Hz, 2H), 7.23–7.38 (m, 5H, Ar H) ppm. ^{13}C NMR ($CDCl_3$, 67.8 MHz), δ 44.3, 55.2, 64.7, 108.8, 113.3, 126.7, 127.8, 128.0, 128.5, 129.2, 130.2, 131.7, 136.3, 158.3 ppm. EIMS m/z 296 (M^+), 175, 121, 103. HRMS (EI) calcd for $C_{19}H_{20}O_3$ 296.1412, found 296.1419.

2-(*p*-Methoxybenzyl)-6-(*p*-methoxyphenyl)-8-benzyl-3,7-dihydroimidazo[1,2-a]pyrazine-3-one 20, 2* (^{13}C)

Cinnam-(*p*-methoxyphenyl)-1,3-dioxolane (**18***) (^{13}C) (48 mg, 0.16 mmol) was dissolved in CH_2Cl_2 (20 mL) and the solution was cooled to −78°C. Ozone gas was bubbled through the reaction mixture until the color of the solution became blue. Nitrogen gas was bubbled through the solution to remove excess amount of ozone gas. Then triethylamine was added and stirred at room temperature for 1 h. The volatiles were evaporated under reduced pressure and the resulting residue **19*** (^{13}C) (60 mg) was used in the following reaction without further purification. A mixture of crude residue of the ozonolysis products **19*** (^{13}C) (60 mg), aminopyrazine **5** (90 mg, 0.32 mmol), 10% HC1 (1.2 mL), water (1.0 mL), and dioxane (5.0 mL) was heated at 80°C for 90 min under argon atmosphere. After cooling this mixture in an ice bath, the reaction mixture was diluted with water (15 mL) and extracted with AcOEt (20 mL×3). The organic layer was washed with brine, and dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was chromatographed on silica gel [MeOH–CH_2Cl_2 (5:95)] afford **20*** (^{13}C) in 34% yield (24 mg, 3 steps). ^1H NMR (CD_3OD, 500 MHz), δ 3.72 (s, 3H), 4.09 (d, J=128.4 Hz, 2H), 4.29 (s, 2H), 4.38 (s, 2H), 6.81 (d, J=8.8 Hz, 2H), 6.86 (d, J=8.3 Hz, 2H), 7.23 (t, J=7.4 Hz, 2H), 7.36 (d, J=7.4 Hz, 1H), 7.56 (t, J=7.4 Hz, 2H), 8.00 (d, J=8.3 Hz, 2H), 8.01 (d, J=8.3 Hz, 2H) ppm. ^{13}C NMR (CD_3OD, 125.7 MHz), δ *33.4 ppm. FABMS (NBA) m/z 439 (MH^+). HRMS (FAB/NBA) calcd for $^{13}C_1C_{26}H_{23}O_3N_3$ 438.1771, found 438.1771 (M^+).

The compound **20** (natural isotope abundance) was synthesized according to the same procedure described above in 67% yield (3 steps from **18**). UV (EtOH); λ_{max} (log ϵ), 434, 349, 265 nm. FL (EtOH) Em λ 528 nm, Ex λ 419 nm. IR ($CHCl_3$) ν_{max} 3697, 3171, 2950, 2362, 1611, 1513, 1242, 1216, 1178, 838 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz), δ 3.61 (s, 3H), 4.03 (s, 2H), 4.29 (s, 2H), 6.71 (d, J=9.0 Hz, 2H), 6.77 (d, J=8.5 Hz, 2H), 7.10 (brt, J=7.5 Hz, 1H), 7.17 (brt, J=7.5 Hz, 5H), 7.23 (brd, J=7.5 Hz, 4H) ppm. ^{13}C NMR ($CDCl_3$, 125.7 MHz), δ 33.3, 35.0, 55.7, 107.8, 114.8,114.9, 11639. 124.8, 128.2, 129.3, 129.4 129.6, 130.6, 130.8, 131.3, 131.9, 133.5, 138.1, 159.8, 160.2 ppm. FABMS (NBA) m/z 438 (MH^+). HRMS (FAB/NBA) calcd for $C_{27}H_{23}O_3N_3$ 437.1739, found 437.1716 (M^+).

Dehydrocoelenterazine mono-methyl ether 1b, 1b* (^{13}C)

Manganese (II) oxide (110 mg, 1.3 mmol) was added to the solution of methoxy coelenterazine **20*** (^{13}C) (9.0 mg, 0.02 mmol) in diethyl ether (25 mL) and ethanol (5 ml) at 0°C. The mixture was stirred at 0°C for 3 h. The solution was filtered through a pad of Celite and then concentrated under reduced pressure to afford **1b*** (^{13}C) (7 mg, 77%). ^1H NMR ($CDCl_3$, 500 MHz), δ 3.92 (s, 3H), 4.38 (s, 2H), 6.89 (d, J=8.8 Hz, 2H), 7.03 (d, J=8.8 Hz, 2H), 7.24 (brs, 1H), 7.33 (t, J=7.3 Hz, 2H), 7.50 (d, J=156.0 Hz, 1H), 7.57 (d, J=7.3 Hz, 2H), 7.65 (s, 1H), 7.72 (d, J=8.8 Hz, 2H), 8.31 (dd J=8.8, 4.7 Hz, 2H,) ppm. ^{13}C NMR ($CDCl_3$, 125.7 MHz), δ *134.6 ppm. FABMS (NBA) m/z 437 (MH^+). HRMS (FAB/NBA) calcd for $^{13}C_1C_{26}H_{21}O_3N_3$ 436.1615, found 436.1620 (M^+).

The compound **1b** (natural isotope abundance) was prepared according to the same procedure described above for **20** in 94% yield. Mp 198–199°C (decomp.). UV (EtOH) λ_{max} (log ϵ), 529 (3.77), 402 (4.12), 270 (2.20) nm. IR (KBr) ν_{max} 3250, 1697, 1586, 1509, 1446, 1404, 1263, 1155 cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz), δ 3.87 (s, 3H), 4.30 (s, 2H), 6.80 (d, J=8.8 Hz, 2H), 7.13 (d, J=9.3 Hz, 2H), 7.23 (brt, J=7.3 Hz, 1H), 7.33 (t, J=7.3 Hz, 2H), 7.49 (d, J=6.9 Hz, 2H), 7.51 (s, 1H), 7.76 (d, J=8.8 Hz, 2H), 7.91 (s, 1H), 8.40 (d, J=8.8 Hz, 2H), 9.61 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 125.7 MHz), δ 33.3, ppm. FABMS (NBA) m/z 436 (MH^+). HRMS (FAB/NBA) calcd for $C_{27}H_{21}O_3N_3$ 435.1:583, found 435.1592 (M^+).

DTT adduct 21b, 21b* (^{13}C)

Dithiothreitol (2.0 mg, 0.012 mmol) was added to a solution of dehydrocoelenterazine mono-methyl ether (**1b***) (^{13}C) (2.7 mg, 0.0062 mmol) in MeOH (1.5 mL) and CH_2Cl_2 (1.5 mL) at room temperature under argon atmosphere. After stirring for 20 min at room temperature, the reaction solution was adjusted to pH 3 by using 1N HCl aq. A few minutes later, the reaction mixture was concentrated under reduced pressure to give a DTT adduct **21b*** (^{13}C). The adduct was purified using reversed-phase HPLC (ODS-UG-5 column, mobile phase; MeCN:H_2O=1:1) ^1H NMR of imidazopyrazinone core (CD_3OD, 500 MHz), δ 4.67 (s, 2H), 5.62 (d, J=141.1 Hz, 0.5H), 5.67 (d, J=141.1 Hz, 0.5H), 6.90 (d, J=8.8 Hz, 2H), 6.94 (d, J=8.8 Hz, 2H), 7.26–7.34 (m, 4H), 7.49 (d, J=7.4 Hz, 2H), 7.54–7.58 (m, 2H), 7.62 (d, J=8.8 Hz, 2H) ppm. ^{13}C NMR (CD_3OD, 125.7 MHz), δ *45.2, *45.4 ppm.

The compound **21b** (natural isotope abundance) was prepared according to the same procedure described above from dehydrocoelenterazine mono-methyl ether (**1b**) (3.0 mg, 0.0069 mmol), DTT (1.8 mg, 0.012 mmol), MeOH (1.5 mL), and CH_2Cl_2 (1.5 mL). FABMS (NBA) m/z 590 (MH^+).

Glutathione adduct 23b, 23b* (^{13}C)

The solution of GSH (3.0 mg, 0.0046 mmol) in water (0.5 mL) was added to a solution of dehydrocoelenterazine **1b*** (^{13}C) (2.7 mg, 0.0062 mmol) in MeOH (1.0 mL) at room temperature. After stirring for 50 min at room temperature, the reaction solution was adjusted to pH 3 by using 1N HCl aq. A few minutes later, the reaction mixture was concentrated under reduced pressure to give a GSH adduct (**23b***) (^{13}C). The adduct was purified with

reverse-phased HPLC (ODS-UG-5 column, mobile phase; MeCN:H_2O=3:7). ^{1}H NMR of imidazopyrazinone core (CD_3OD, 500 MHz), δ 5.66 (d, J=141.1 Hz, 0.5H), 5.69 (d, J=141.1 Hz, 0.5H), 6.90 (dd, J=8.8, 3.9 Hz, 2H), 6.95 (d, J=8.8 Hz, 2H), 7.25–7.34 (m, 4H), 7.50 (t, J=7.4 Hz, 2H), 7.55 (dt, J=8.8, 2.9 Hz, 2H), 7.63 (dd, J=8.8, 2.9 Hz, 2H) ppm. ^{13}C NMR (CD_3OD, 125.7 MHz), δ *44.6, *45.3 ppm.

The compound **23b** (natural isotope abundance) was prepared according to the same procedure described above from dehydrocoelenterazine mono-methyl ether (**1b**) and GSH. FABMS (NBA) *m/z* 743 (MH^+), 436 (M^+-GSH), 308 (M^+-DCL).

Chemiluminescence of DTT adduct 21b, 21b* (^{13}C)

Through a solution of **21b*** (^{13}C) (ca. 1 mg.) in DMSO (1.0 mL) containing 0.10 mL of 1N *t*-BuOK in *t*-BuOH, molecular oxygen was bubbled. After 25 min later, the mixture was neutralized with sat. NH_4Cl aq., then diluted with water (5 mL). The solution was extracted with AcOEt (20 mL×3) the organic lever was washed with water (×1) and brine (×1). The solution was dried over anhydrous Na_2SO_4, then concentrated by evaporating under reduced pressure. The resultant residue (**22b**) was analyzed by NMR without further purification. ^{13}C NMR (CD_3OD, 125.7 MHz), δ *42.5, *43.2 ppm.

The compound **22b** (natural isotope abundance) was prepared according to the same procedure described above. FABMS (NBA) *m/z* 577 (M^+).

Acknowledgements

The authors are grateful for financial support from JSPS–RFTF 96L00504. They also thank Dr T. Kondo and Mr K. Koga for technical assistance in NMR measurements. M. Kuse thanks JSPS (Japan Society for the Promotion of Science) for granting a Research Fellowship for Young Scientists. We also express our thanks to Dr T. Franz for assistance in checking the manuscript.

References

1. Preliminary communication about this paper was reported in the next literature. Isobe, M.; Kuse, M.; Yasuda, Y.; Takahashi, H. *BioMed. Chem. Lett.* **1998**, *8*, 2919–2924.

2. (a) Isobe, M.; Fujii, T.; Swan, S.; Kuse, M.; Tsuboi, K.; Miyazaki, A.; Feng, M. C.; Li, J. *Pure Appl. Chem.* **1998**, *70*, 2085–2092. (b) Isobe, M.; Takahashi, H.; Usami, K.; Hattori, M.; Nishigohri, Y. *Pure Appl. Chem.* **1994**, *66*, 765–772. (c) Usami, K.; Isobe, M. *Tetrahedron* **1996**, *52*, 12061–12090. (d) Usami, K.; Isobe, M. *Tetrahedron Lett.* **1995**, *36*, 8613–8616.

3. Tsuji, F. I.; Leisman, G. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 6719–6723.

4. (a) Shimomura, O.; Johnson, F. H.; Saiga, Y. *J. Cell. Comp. Physiol.* **1962**, *59*, 223–240. (b) Shimomura, O.; Johnson, F. H. *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 2611–2615.

5. Takahashi, H.; Isobe, M. *BioMed. Chem. Lett.* **1993**, *3*, 2647–2652.

6. Takahashi, H.; Isobe, M. *Chem. Lett.* **1994**, 843–846.

7. (a) Goto, T.; Iio, H.; Inoue, S.; Kakoi, H. *Tetrahedron Lett.* **1974**, 2321–2324. (b) Inoue, S.; Taniguchi, H.; Murata, M.; Kakoi, H.; Goto, T. *Chem. Lett.* **1977**, 259–262.

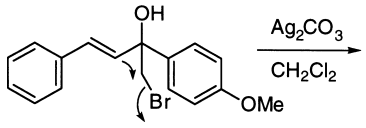
8. Recently novel synthetic routes to coelenterazines were reported in the following papers. (a) Keenan, M.; Jones, K.; Hibbert, F. *Chem. Commun.* **1997**, 323–324. (b) Hirano, T.; Negishi, R.; Yamaguchi, M.; Chen, F. Q.; Ohmiya, Y.; Tsuji, F. I.; Ohashi, M. *Tetrahedron* **1997**, *53*, 12 903–12 916.

9. Kishi, Y.; Tanino, H.; Goto, T. *Tetrahedron Lett.* **1972**, *2747*, 2750.

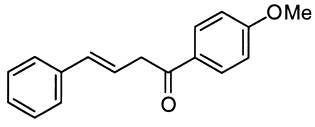
10. Gassman, P. G.; Talley, J. J. *Tetrahedron Lett.* **1978**, 3773–3776. Trimethylsilyl cyanide was prepared according to the method described in Ref. 2c.

11. The Grignard reagent was prepared from bromoanisole and magnesium turnings in diethyl ether.

12. Compound **24*** was obtained as the isomeric product in the rearrangement reaction in 20% yield from bromohydrin **16***. The spectroscopic data of **24** and **24*** were described in detail in the experimental section of this paper.



16*



24*

13. Isobe, M.; Iio, H.; Kawai, T.; Goto, T. *Tetrahedron Lett.* **1977**, 703–706. (b) Iio, H.; Isobe, M.; Kawai, T.; Goto, T. *Tetrahedron* **1979**, *35*, 941–948.