



Title	網走近郊の能取で採取された紅藻 <i>Laurencia nipponica</i> Yamadaから単離されたC-15プロモエーテル,notoryneの <sup>13</sup> C NMRスペクトルの完全帰属
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Citation	北海道教育大学紀要. 第二部. A, 数学・物理学・化学・工学編, 48(1) : 13-17
Issue Date	1997-08
URL	<a href="http://s-ir.sap.hokkyodai.ac.jp/dspace/handle/123456789/1971">http://s-ir.sap.hokkyodai.ac.jp/dspace/handle/123456789/1971</a>
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# Complete Assignments of Signals of $^{13}\text{C}$ NMR Spectrum for C15-Bromoether, Notoryne Isolated from the Marine Red Alga, *Laurencia nipponica* Yamada Collected at Notoro Point near Abashiri in Hokkaido

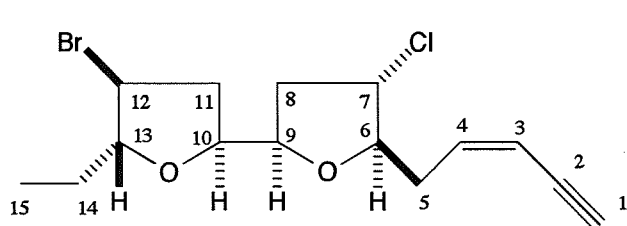
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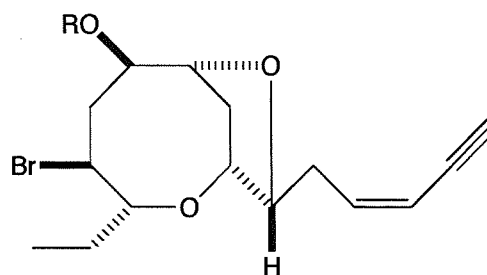
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## ABSTRACT

Notoryne (**1**), a C-15 bromoether compound isolated from the title alga, is a unique metabolite having two oxolane rings with an enyne side chain, a bromine and a chlorine. Its structure was established in 1991 by Kikuchi et al.<sup>1)</sup> as Formula 1 on the basis of chemical and spectroscopic evidence. In that report, the author described assignments for resonances of its  $^{13}\text{C}$  NMR spectrum, but those assignments were not only tentative but also partly unsettled. Recently, we have newly isolated pure notoryne from extract of the same alga and taken  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra including H-H and C-H chemical shift correlation NMR spectra to carry out complete assignments for the unsettled signals as well as to confirm the tentative assignments. The assignments were satisfactorily accomplished and are shown in Table 1 and Figure 4. Consequently, we were able to revise the some mistaken assignments coupled with assigning the unsettled signals of  $^{13}\text{C}$  NMR spectrum in the literature.



**1. notoryne**



**2. R=H, laurefucin**  
**3. R=COCH<sub>3</sub>, acetyl laurefucin**

## INTRODUCTION

C-15 bromoether, notoryne (**1**) was at first isolated as a minor metabolite along with a major one, laurefucin (**2**) from the marine red alga *L. nipponica* collected at Notoro Point near Abashiri in Hokkaido. The unique structure of notoryne having two oxolane rings with an enyne side chain, a bromine and a chlorine was established in 1991 by Kikuchi et al. as Formula **1** by means of the spectroscopic analyses including the classical proton-proton spin decoupling technique for NMR spectrum and conscientious chemical reactions.<sup>1)</sup> Therefore, the assignments for the proton resonances of the NMR spectra of notoryne are complete except for those of the five overlapping signals. However, the signals of <sup>13</sup>C NMR spectrum were not only partly unsettled but also assigned tentatively in comparison to those of similar compounds, indicating that those assignments were not reliable. Recent progress of the NMR instrument and measurement technique including two-dimensional methods make it easy to assign exact resonances of the NMR spectra. It is particularly easy to assign resonances of <sup>13</sup>C NMR spectrum on the basis of C-H chemical shift correlation NMR spectra, in the case that assignments of <sup>1</sup>H NMR spectrum are completely established. Recently, we have newly isolated pure notoryne from the extract of the same alga and taken <sup>1</sup>H and <sup>13</sup>C NMR spectra including H-H and C-H COSYs to carry out complete assignments of the <sup>13</sup>C NMR spectrum. We would like now to report here the complete assignments of the carbon resonances (Figure **1**) in the NMR spectra of notoryne with the aid of C-H chemical shift correlation NMR spectra. In addition, we will revise the some mistaken assignments in the literature.

## EXPERIMENTAL

The IR spectrum was measured on a Shimadzu IR-440 spectrophotometer and the UV spectrum on a Shimadzu UV-260 spectrophotometer. The <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained in CDCl<sub>3</sub> as the solvent by using the JEOL-EX 270MHz spectrometer and JEOL-A-400II spectrometer. Optical rotation was determined on an Atago POLAX polarimeter in CHCl<sub>3</sub> solution. Aluminum oxide (Merck, activity II-III) and silica gel (Merck, Kieselgel 60, 70-230 mesh) were used for column chromatography. The high-performance liquid chromatography (HPLC) was carried out on a JASCO TRIROTAR using Finpak SIL-C18 or Megapak SIL-C18 (JASCO).

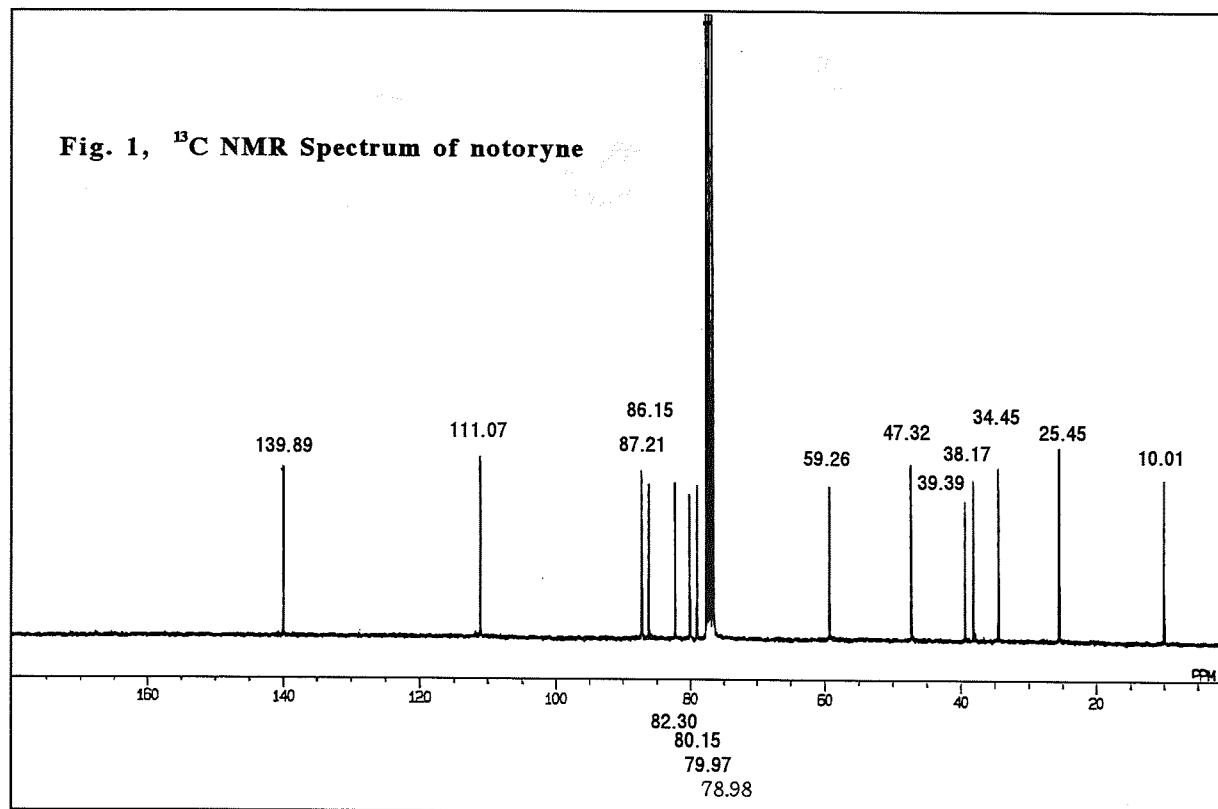
**Isolation:** The half-dried alga collected at Notoro Point were dipped in methanol for 1 week to remove water, followed by extraction with methanol two times. The combined methanol solution was evaporated under reduced pressure to give a dark green residue, which was dissolved in diethyl ether. The ether solution was washed with 1M HCl, NaOH, and saturated salt solution in this order to yield a neutral extract. After drying over Na<sub>2</sub>SO<sub>4</sub>, the ether was evaporated and the neutral portion obtained was fractionated into four by Al<sub>2</sub>O<sub>3</sub> column chromatography in this order with an elution of hexane, benzene, ethyl acetate, and ethyl acetate containing 5% methanol. The benzene fraction was chromatographed on silica-gel with an elution of mixed solvent (hexane : benzene, 1 : 1) to give a mixture of notoryne (**1**) and acetyllaurefucin (**3**). The mixture was further separated by repeated silica-gel column chromatography to provide an almost pure notoryne. Final

purification was carried out using a reverse-phase HPLC with an elution of methanol containing 10%  $\text{H}_2\text{O}$  to give a pure fraction showing one spot on TLC, whose physical properties (mp, optical rotation, IR,  $^1\text{H}$  NMR, and Rf value on TLC) were identical to those of the authentic notoryne.

## RESULTS AND DISCUSSION

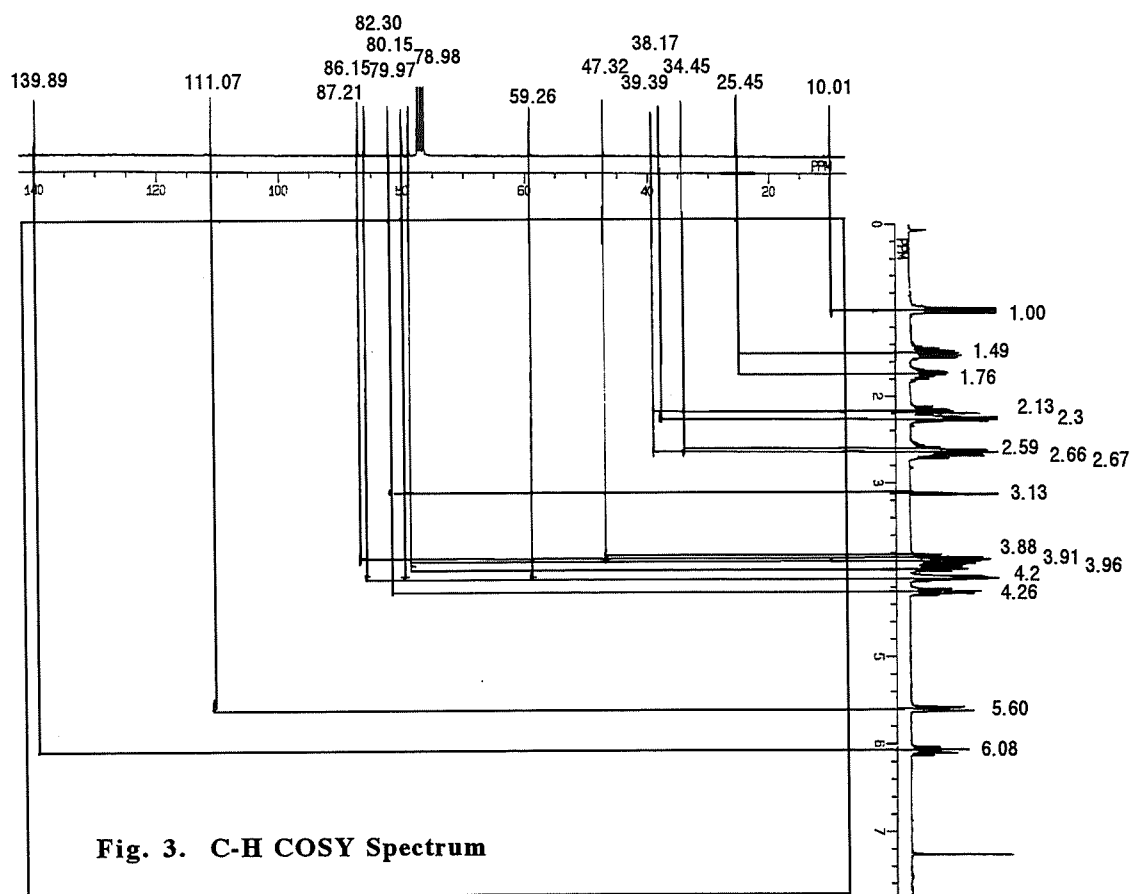
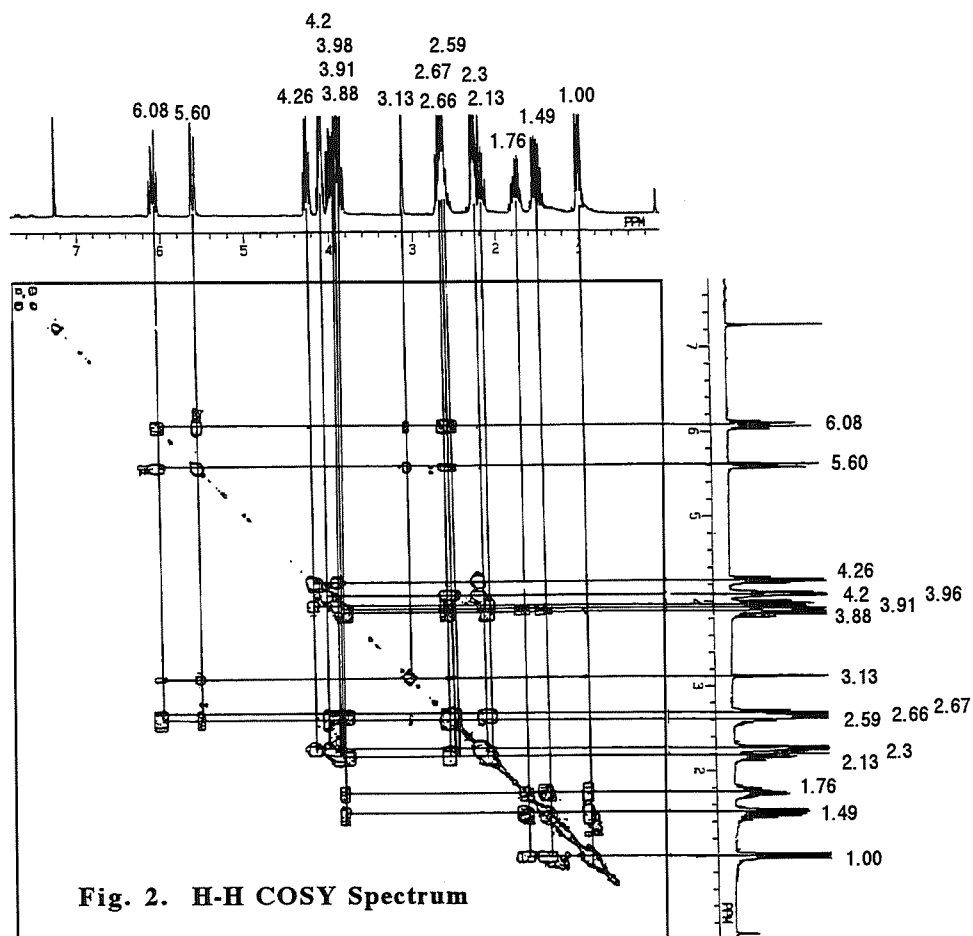
The assignments of all protons of notoryne on the basis of the proton-proton spin decoupling technique of Kikuchi<sup>1)</sup> were reconfirmed by H-H COSY spectrum (Figure 2).

The assignments of all carbon resonances (Figure 3) in the NMR spectra of notoryne were made following the acquisition of H-H COSY and C-H COSY (Figure 4) 2D chemical shift correlation NMR spectra. The starting points for the assignments were the unique resonances at  $\delta$  82.30 ppm and at  $\delta$  3.13 ppm in the  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectra attributable to the acetylenic carbon C-1 and to the proton H-1, respectively. From these resonances, pathways of connectivities of the sequence could be traced using the 2D NMR spectra.



The connectivities observed, and which permitted the assignments of the resonances of all protons and carbons to be made, are shown in Table 1.

As shown in Table 1, the assignments of the resonances of  $^{13}\text{C}$  NMR spectrum at C-1, C-2, C-5, C-6, C-7, C-8, and C-10 described in Reference 2 should be revised.

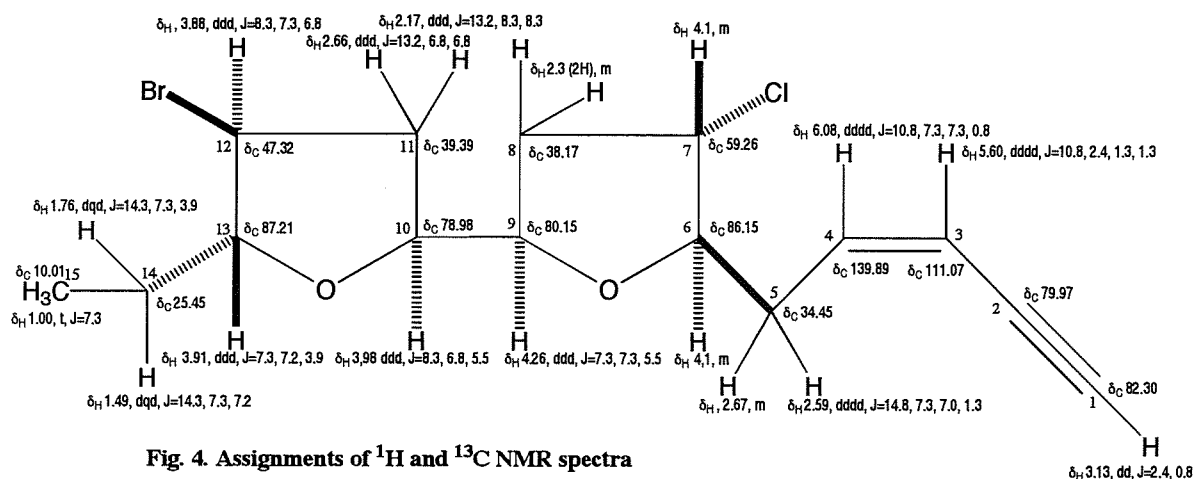


**Table 1. Assignments of  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals of notoryne (1)**

No	$^{13}\text{C}$ ( $\delta$ )	$^{13}\text{C}$ ( $\delta$ )	$^1\text{H}$ ( $\delta$ )	multiplicity	J(Hz)
1	82.30	82.1	3.13	dd	2.4, 0.8
2	79.97	79.8			
3	111.07	110.8	5.60	dddd	10.8, 2.4, 1.3, 1.3
4	139.89	139.5	6.08	dddd	10.8, 7.3, 7.3, 0.8
5	34.45	34.3	2.59	dddd	14.8, 7.3, 7.0, 1.3
			2.67	m	
6	86.15	78.8 or 79.9	4.1	m	
7	59.26	59.1	4.1	m	
8	38.17	38.0 or 39.3	2.3 (2H)	m	
9	80.15	85.9 or 87.0	4.26	ddd	7.3, 7.3, 5.5
10	78.98	85.9 or 87.0	3.98	ddd	8.3, 6.8, 5.5
11	39.39	38.0 or 39.3	2.17	ddd	13.2, 8.3, 8.3
			2.66	ddd	13.2, 6.8, 6.8
12	47.32	47.2	3.88	ddd	8.3, 7.3, 6.8
13	87.21	78.8 or 79.9	3.91	ddd	7.3, 7.2, 3.9
14	25.45	25.4	1.49	dqd	14.3, 7.3, 7.2
			1.76	dqd	14.3, 7.3, 3.9
15	10.01	10.0	1.00	t	7.3

\* our data.

# data reported in the reference 1.

**Fig. 4. Assignments of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra**

**Acknowledgment:** We would like to thank Professor K. Nabeta of the Department of Bioresource Chemistry, Obihiro University of Agriculture and Veterinary Medicine and Dr. K. Orito of the Laboratory of Organic Synthesis, Division of Molecular Chemistry, Graduate School of Engineering, Hokkaido University for the measurements of NMR spectra.

## References

- 1) H. Kikuchi, T. Suzuki, E. Kurosawa and M. Suzuki, *Bul. Chem. Soc. Jpn.*, **64**, 1763 (1991).