PERGAMON

Phytochemistry 51 (1999) 657–662

PHYTOCHEMISTRY

Bisezakyne-A and -B, halogenated C_{15} acetogenins from a Japanese *Laurencia* species

Minoru Suzuki[a,*], Satoru Nakano[a], Yoshinori Takahashi[a], Tsuyoshi Abe[b], Michio Masuda[b]

[a]*Division of Material Science, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan*
[b]*Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan*

Received 8 May 1998; accepted 9 November 1998

Abstract

Two novel halogenated C_{15} acetogenins, named bisezakyne-A and -B, have been isolated along with dactylyne from an undescribed species of the red algal genus *Laurencia* collected from Japan. The structures of these compounds were elucidated by spectroscopic data. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Laurencia* species; Rhodomelaceae; Red alga; C_{15} acetogenin; Halogenated compound; Chemotaxonomy

1. Introduction

In connection with our taxonomic studies of Japanese species of the red algal genus *Laurencia* (Ceramiales, Rhodophyta) based upon morphological and chemical features as well as genetic affinities (Masuda, Abe, Suzuki, & Suzuki, 1996; Masuda, Abe, Sato, Suzuki, & Suzuki, 1997a; Masuda, Itoh, Matsuo, & Suzuki, 1997b; Abe, Masuda, Kawaguchi, Itoh, & Suzuki, 1997), we reported recently (Masuda et al., 1997b) the chemical composition of *Laurencia majuscula* (Harvey) Lucas collected from Okinawa, Japan, which contained halogenated chamigrene derivatives that have previously been found in the sea hare *Aplysia dactylomela*. Halogenated secondary metabolites of *Laurencia* have been shown to be under severe genetic control (Masuda et al., 1997a). Thus, secondary metabolite chemistry can be useful in taxonomical classification of the *Laurencia* (Masuda et al., 1996). As part of chemotaxonomic studies on the Japanese *Laurencia* species, we examined an undescribed species collected at Bisezaki, Motobu, Okinawa Prefecture and found that this species produced two novel halogenated metabolites, named bisezakyne-A (**1**) and -B (**2**), together with dactylyne (**3**). The latter had previously been isolated from the sea hare *Aplysia dactylomela* (McDonald, Campbell, Vanderah, Schmitz, Washecheck et al., 1975; Vanderah & Schmitz, 1976). We wish to report herein the isolation and structural elucidation of these novel metabolites.

2. Results and discussion

Specimens of *Laurencia* collected, were extracted with methanol, within the methanol extract then being subjected to a combination of column and thin-layer chromatographies to afford small amounts of bisezakyne-A (**1**), bisezakyne-B (**2**) and dactylyne (**3**).

Bisezakyne-A (**1**), colorless oil, $[\alpha]_D^{22}$ -7.13° ($CHCl_3$), was subjected to LR–FDMS and HR–EIMS, giving a molecular formula of $C_{15}H_{20}BrClO$. The IR spectrum showed signals due to a terminal acetylenic group at ν_{max} 3300 and 2100 cm^{-1}. The presence of a 2-penten-4-ynyl moiety was evident from the ^1H NMR spectrum (Table 1) [δ_H 2.89 (1H, d, $J=1.5$ Hz), 5.49

* Corresponding author. Tel.: +81-11-7062272; fax: +81-11-7096416.
E-mail address: misu@ees.hokudai.ac.jp (M. Suzuki)

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00102-8

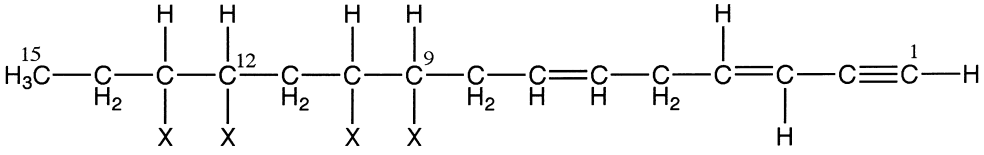
Table 1
^{13}C (100 MHz, DEPT) and ^{1}H NMR (400 MHz) and HMBC spectral data[a] for bisezakyne-A (**1**)

C[b]	^{13}C (δ)	^{1}H (δ)	*J* (Hz)	Long-range correlations
1	76.3	2.89	d (1.5)	
2	82.4			H-4
3	109.1	5.49	br dd (16.1, 1.5)	H-1, H_2-5
4	144.0	6.24	ddd (16.1, 6.4, 6.4)	H_2-5
5	30.9	2.92	m	H-4
6	128.2	5.51	m	H-4, H_2-5, H_a-8
7	126.4	5.51	m	H_2-5, H_2-8
8	29.2	2.56	ddd (13.7, 6.4, 6.4; H_a)	H-6, H-7
		2.47	ddd (13.7, 6.8, 6.8; H_b)	
9	82.2	3.83	ddd (6.8, 6.4, 3.6)	H_b-8, H_β-11
10	60.4	4.42	ddd (6.8, 3.6, 2.4)	H_b-8, H-9, H_β-11
11	39.5	2.65	ddd (15.1, 8.3, 6.8; H_α)	
		2.24	ddd (15.1, 6.4., 2.4; H_β)	
12	80.6	4.15	ddd (8.3, 6.4, 6.4)	H-10, H_2-11, H-13
13	59.0	4.05	ddd (9.9, 6.4, 2.9)	H-12, H_3-15
14	27.0	2.01	ddq (14.6, 2.9, 7.3; H_a)	H_3-15
		1.74	ddq (14.6, 9.9, 7.3; H_b)	
15	12.2	1.10	t (7.3)	H_2-14

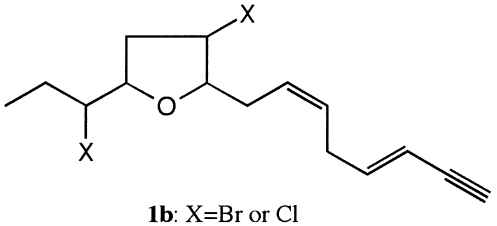
[a] Measured in chloroform-d_1.
[b] Assignments were made with the aid of the HSQC spectrum.

(1H, br dd, $J=16.1$ and 1.5 Hz) and 6.24 (1H, ddd, $J=16.1$, 6.4 and 6.4 Hz)]. The *J*-value (16.1 Hz) for H-3 and H-4 as well as the chemical shift value (δ_H 2.89) of the acetylenic proton indicated the geometry of the double bond at C-3 to be *E* (Suzuki & Kurosawa, 1987). Furthermore, the presence of an additional double bond was revealed by the signals at δ_H 5.51 (2H, m). Interpretation of the ^{1}H–^{1}H COSY spectrum permitted partial structure **1a** to be considered (Fig. 1). In the ^{13}C NMR spectrum, the chemical shift values of the methine carbons at C-9 (δ_C 82.2) and C-12 (δ_C 80.6) indicated that the oxygen atoms are attached to these carbons. Moreover, the remaining substituents at C-10 and C-13 were verified to be halogen atoms based upon the chemical shifts of the pertinent carbons at δ_C 60.4 and 59.0, respectively. Since the IR spectrum showed no absorption indicative of a hydroxyl group, bisezakyne-A, having five degrees of unsaturation, must contain one oxide ring, thus leading to the planar formula **1b** for bisezakyne-A. The formula **1b** was confirmed by the HMBC spectrum whose results are summarized in Table 1. The positions of the bromine and chlorine atoms were established by the halogen-induced ^{13}C isotope shifts (Sergeyev, Sandor, Sergeyeva, & Raynes, 1995; Raynes, Sergeyev, Sandor, & Grayson, 1997) in the ^{13}C NMR spectrum, the details of whose measurements are described in Section 3. As shown in Fig. 2, the methine carbon at C-10 (δ_C 60.4) showed an isotope shift of 8.53 ppb with relative intensities of about 3:1 induced by ^{35}Cl and ^{37}Cl, while the methine carbon at C-13 (δ_C 59.0) showed a shift of 0.86 ppb with relative intensities of about 1:1 induced by ^{79}Br and ^{81}Br, according to natural isotopic abundance of both halogen atoms; ^{35}Cl (75.711%)/^{37}Cl (24.229%) and ^{79}Br (50.686%)/^{81}Br (49.314%), respectively. Hence the chlorine atom is linked to C-10 and the bromine atom to C-13.

The relative stereochemistry, excluding that at C-13, was determined by analysis of the NOESY spectrum. NOEs were observed between H-9/H-12, H-10/H-12, H-9/H_α-11 and H_α-11/H-12, thus indicating that the stereochemistries at H-9, H-10 and H-12 on the tetrahydrofuran ring were all *cis*. Moreover, the *Z*-configuration of the double bond between C-6 and C-7 was proven by a NOE between H_2-5 and H_2-8. The remaining configuration at C-13 is suggested from consideration of a known pattern of biogenesis. It is suggested that many halogenated C_{15} acetogenins isolated from various *Laurencia* species arise from



1a: X=O, Br, Cl



1b: X=Br or Cl

Fig. 1. Partial and planar structure for bisezakyne-A (**1**).

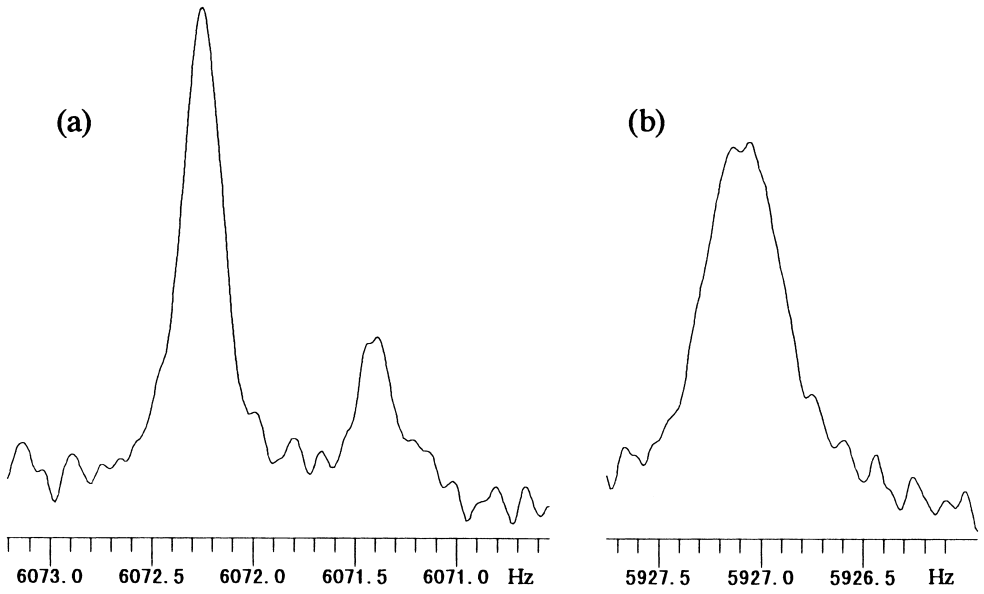
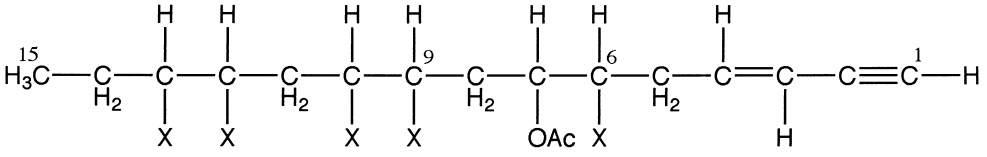


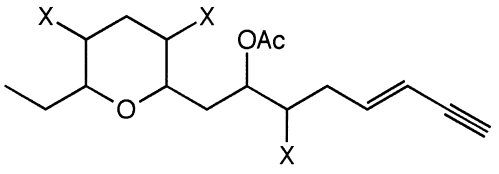
Fig. 2. The ^{13}C NMR spectral lines (halogen-isotope shifts) of the signals at δ_C 60.4 (a) and 59.0 (b) of bisezakyne-A (**1**); (a) the line separation is 0.858 Hz (8.53 ppb) with a peak intensity ratio of 3.06:1 and (b) the line separation is 0.086 Hz (0.86 ppb) with a peak intensity ratio of 1:1.008.

(6*S*,7*S*)- or (6*R*,7*R*)-laurediol (Fukuzawa, Kurosawa, & Irie, 1972) and possess (12*R*,13*S*)- or (12*S*,13*R*)-*erythro* configuration which is formed via (12*S*,13*S*)- and/or (12*R*,13*R*)-bromonium ion (Kikuchi, Suzuki, Kurosawa, & Suzuki, 1991), reflecting the (12*E*)-double bond in both precursors. Assuming that bisezakyne-A (**1**) is also biosynthesized either from (6*S*,7*S*)- or (6*R*,7*R*)-laurediol via (12*S*,13*S*)-bromonium ion, the relative configuration at C-9, C-10, C-12 and C-13 in **1** would be expected to be *R**, *R**, *R** and *S**, respectively.

Bisezakyne-B (**2**), $C_{17}H_{23}BrCl_2O_3$, also had a *trans*-2-penten-4-ynyl grouping [ν_{max} 3300 and 2100 cm^{-1}; δ_H 2.85 (1H, d, $J=2.0$ Hz), 5.56 (1H, br dd, $J=16.1$ and 2.0 Hz) and 6.23 (1H, ddd, $J=16.1$, 7.3 and 7.3 Hz)] as well as an acetoxyl group [ν_{max} 1745 and 1225 cm^{-1}; δ_H 2.10 (3H, s)]. Judging from the five degrees of unsaturation implied by the molecular formula of **2**, bisezakyne-B must be a monocyclic compound possessing one double bond, one triple bond and one carbon–oxygen double bond (an acetoxyl group). Interpretation of the ^1H–^1H COSY spectrum suggested the presence of partial structure **2a** (Fig. 3). The chemical shift value of the methine proton of C-7 (δ_H 5.35) in the ^1H NMR spectrum revealed that the acetoxyl function is attached to this carbon. In the ^{13}C



2a: X=O, Br, Cl



2b: X=Br or Cl

Fig. 3. Partial and planar structure for bisezakyne-B (**2**).

Table 2
^{13}C (100 MHz, DEPT) and ^{1}H NMR (400 MHz) and HMBC data[a] for bisezakyne-B (**2**)

C[b]	^{13}C (δ)	^{1}H (δ)	*J* (Hz)	Long-range correlations
1	77.7	2.85	d (2.0)	
2	81.6			H-4
3	112.3	5.56	br dd (16.1, 2.0)	H_2-5
4	140.5	6.23	ddd (16.1, 7.3, 7.3)	H_b-5, H-6
5	38.3	2.59	ddd (15.6, 7.3, 5.4; H_a)	
		2.47	ddd (15.6, 7.8, 7.3; H_b)	
6	62.7	3.98	ddd (7.8, 5.4, 2.4)	H_b-5
7	71.2	5.35	ddd (9.3, 2.9, 2.4)	H-6, H_b-8, H-9, H_3-Ac
8	36.5	2.07	m (H_a)	H-9
		1.78	dd (14.6, 9.3, 2.4; H_b)	
9	75.4	3.57	ddd (10.3, 2.4, 2.0)	H_a-8, H_α-11
10	61.2	4.00	m	H-9
11	43.9	2.74	ddd (14.2, 4.4, 2.9; H_β)	H-10
		2.41	ddd (14.2, 11.7, 3.4; H_α)	
12	46.3	4.11	ddd (11.7, 10.3, 4.4)	H-10, H_2-11, H_a-14
13	84.0	3.30	ddd (10.3, 8.8, 2.4)	H-12, H_2-14, H_3-15
14	26.2	2.07	m (H_a)	H_3-15
		1.54	ddq (14.2, 8.8, 7.3; H_b)	
15	9.5	1.01	t (7.3)	H_2-14
	20.6	2.13	s (Ac)	
	170.2		(Ac)	

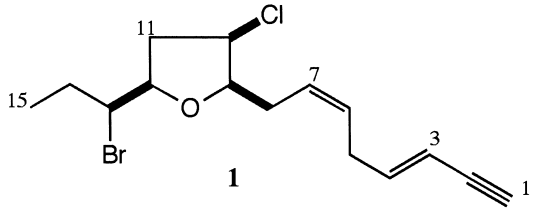
[a] Measured in benzene-d_6.
[b] Assignments were made with the aid of the HSQC spectrum.

NMR spectrum, the chemical shifts of the methine carbons at C-9 (δ_C 75.4) and C-13 (δ_C 84.0) showed that oxygen atoms are attached to these carbons. Moreover, the remaining substituents at C-6, C-10 and C-12 were proven to be halogen atoms based upon the chemical shifts of the pertinent carbons at δ_C 62.7, 61.2 and 46.3, respectively. Therefore, bisezakyne-B should have the planar formula **2b** because the IR spectrum showed no absorption due to a hydroxyl group. The planar formula **2b** was established by interpretation of the HMBC spectrum whose data are summarized in Table 2. The positions of the bromine and chlorine atoms were also determined by the halogen-induced ^{13}C isotope shifts in the ^{13}C NMR spectrum as mentioned above in the case of **1**. Both methine carbons at C-6 (δ_C 62.7) and C-10 (δ_C 61.2) showed isotopic shifts with relative intensities of about 3:1, thus proving that the chlorine atoms are linked to C-6 and C-10.

The relative stereochemistry of substituents on the tetrahydropyran ring was provisionally sustained with the aid of coupling constants. As shown in Table 2, in the ^{1}H NMR spectrum the protons on carbons C-9 to C-13 showed coupling constants of $J_{9,10}=2.0$ Hz, $J_{10,11\alpha}=3.4$ Hz, $J_{10,11\beta}=2.9$ Hz, $J_{11\beta,12}=4.4$ Hz, $J_{11\alpha,12}=11.7$ Hz and $J_{12,13}=10.3$ Hz, which are typical for protons on a tetrahydropyran ring in a chair conformation. The axial configurations of the protons at C-9 and C-13 were also confirmed by observation of NOEs between H-9/H_α-11, H-9/H-13 and H_α-11/H-13. However, the configurations at C-6 and C-7 remained unsure. Consequently, the structure of bisezakyne-B, excluding configurations at C-6 and C-7, is provisionally represented by formula **2** with relative configurations of $9R^*$, $10R^*$, $12R^*$ and $13S^*$. We are currently attempting to prepare a crystal suitable for X-ray crystallographic analysis in order to establish the absolute structure (and stereochemistry) for bisezakyne-B.

The third halogenated metabolite (**3**) from this collection was identified as dactylyne, which had previously been isolated from the sea hare *Aplysia dactylomela* (McDonald et al., 1975; Vanderah & Schmitz, 1976), by independent analyses using ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, NOESY and HMBC spectroscopy. This is due to the first report of dactylyne **3** in *Laurencia* species and these results may suggest that the sea hare may accumulate this compound for consumption of *Laurencia* species.

Bisezakyne-A (**1**) is an isomer of srilankenyne (**4**) that was previously isolated from the sea hare *Aplysia oculifera* (de Silva, Schwartz, Scheuer, & Shoolery, 1983).



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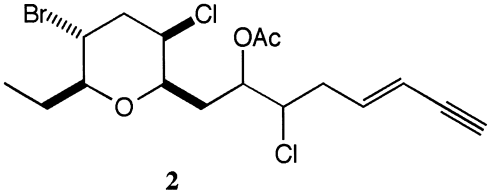

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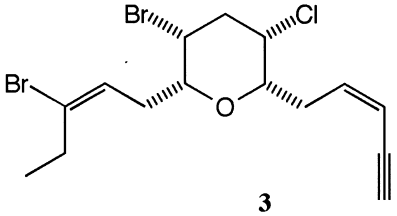

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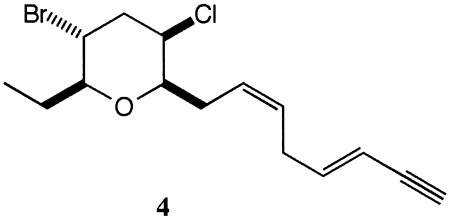

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3. Experimental

3.1. General

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz), TMS as int. standard; low and high resolution MS: 70 eV; CC: silica gel (Merck, Kieselgel 60, 70–230 mesh); Prep. TLC: silica gel plate (Merck, Kieselgel 60_{254S}).

3.2. Collection

A sample of an undescribed species of the genus *Laurencia* was collected Bisezaki, Motobu, Okinawa Prefecture, on March 27, 1997. Voucher specimens are deposited in the Herbarium of the Graduate School of Science, Hokkaido University (SAP 063774-063779).

3.3. Extraction and isolation

The partially dried alga (101.7 g) was soaked in methanol for one week. The MeOH soln was concentrated in vacuo and partitioned between Et_2O and H_2O. The Et_2O soln was washed with water, dried over anhydrous Na_2SO_4 and evaporated to leave a dark green oil (1.05 g). The extract was fractionated by Si gel column chromatography with a step gradient (hexane and EtOAc). The fraction eluted with hexane–EtOAc (9:1) was further submitted to prep. TLC with hexane–EtOAc (3:1) to give bisezakyne-A (**1**) (21 mg), bisezakyne-B (**2**) (17 mg) along with dactylyne (**3**) (17 mg).

3.4. Bisezakyne-A (*1*)

Oil; $[\alpha]_D^{22}$ −7.13° ($CHCl_3$; *c* 0.33); UV λ_{max} (EtOH) nm: 228 (ε 14400); IR ν_{max} (neat) cm^{-1}: 3320, 3020, 2100, 1440, 1290, 1225, 970 and 770; ^{1}H and ^{13}C NMR spectra, Table 1; LR–FDMS *m/z* (rel. int.): 334, 332, 330 (30:100:73) $[M]^+$; LR–EIMS *m/z* (rel. int.): 297, 295 (9:10) $[M–Cl]^+$, 253, 251 (16:12) $[M–C_6H_7]^+$, 227, 225 (14:11) $[M–C_8H_9]^+$, 215 (25), 197 (11), 187 (9), 145 (34), 129 (36), 105 (58), 81 (100), 97 (47) and 41 (44); HR–EIMS *m/z*: 295.0650. Calcd for $C_{15}H_{20}$ ^{79}BrO, 295.0698 [M–Cl].

3.5. Bisezakyne-B (*2*)

Mp 69–70°; $[\alpha]_D^{22}$ −45.1° ($CHCl_3$; *c* 0.27); UV λ_{max} (EtOH) nm: 223 (ε 14400); IR ν_{max} ($CHCl_3$) cm^{-1}: 3300, 2890, 2100, 1745, 1440, 1380, 1225, 1095 and 970; ^{1}H and ^{13}C NMR spectra, Table 2; LR–FDMS *m/z* (rel. int.) 429, 427, 425 (30:100:56) $[M+H]^+$, 392, 390, 388 (5:12:11) $[M–HCl]^+$; LR–EIMS *m/z* (rel. int.): 392, 390, 388 (2:3:2) $[M–HCl]^+$, 349, 347, 345 (11:42:33) $[M–C_2H_4OCl]^+$, 295, 293 (14:13) $[M–C_2H_5O_2Cl_2]^+$, 227 (34), 202 (75), 159 (56), 149 (50), 107 (76), 81 (62), 79 (43) and 43 (100); HR–EIMS *m/z*: 388.0464. Calcd for $C_{17}H_{22}$ ^{79}Br^{35}ClO, 388.0442 [M–HCl].

3.6. Dactylyne (*3*)

Mp 62–3°; $[\alpha]_D^{25}$ −38.2° ($CHCl_3$; *c* 0.19); ^{13}C NMR ($CDCl_3$), CH_3: δ 13.3 (C-15), CH_2: δ 29.5 (C-14), 34.2 (C-5), 35.5 (C-11) and 39.2 (C-8), CH: δ 46.1 (C-9), 54.8 (C-7), 77.2 (C-1), 79.4 (C-10), 79.9 (C-6), 111.0 (C-3) and 140.1 (C-4), C: δ 82.6 (C-2), 100.6 (C-13) and 125.4 (C-12); The ^{1}H NMR and mass spectral data were identical to those reported previously (McDonald et al., 1975).

3.7. Measurement of ^{13}C NMR isotope shifts

The ^{13}C NMR spectra in the bilevel complete decoupled mode were obtained with a JEOL JNM-EX-400 spectrometer. The operating frequency was 100.4 MHz. The spectra were acquired using a 2500 Hz spectral window and acquisition time of 52 s and the number of points acquired was 131K. The FID was zero-filled to 262K data points (digital resolution was 0.0095 Hz/Pt). The standard Lorentzian–Gaussian resolution enhancement procedure was used before Fourier transformation to achieve a better separation of ^{37}Cl/^{35}Cl and ^{81}Br/^{79}Br splittings. In the spectrum of bisezakyne-A (**1**), the signal of δ_C 60.4 ppm (C-10) showed an isotopic shift of 8.53 ppb (0.858 Hz) and a peak intensity ratio of 3.06:1, while the signal at δ_C 59.0 ppm (C-13) showed an isotopic shift of 0.86 ppb (0.086 Hz) and a peak intensity ratio of 1:1.008. On the other hand, in the spectrum of bisezakyne-B (**2**), the signals at δ_C 62.7 ppm (C-6) and δ_C 61.2 ppm (C-10) revealed isotopic shifts of 7.90 ppb (0.793 Hz) with a peak intensity ratio of 2.55:1 and 7.74 ppb (0.779 Hz) with a peak intensity ratio of 2.75:1, respectively. The isotope shift of the signal of δ_C 46.3 ppm (C-12) was not detected.

Acknowledgements

We are grateful to Ms. Eri Fukushi, GC–MS and NMR Laboratory, Faculty of Agriculture, Hokkaido University, for valuable information about ^{13}C NMR isotopic shifts. This study was supported in part by a Grant-in-Aid for Scientific Research (No. 09839001 to M. M.) from the Ministry of Education, Science, Sports and Culture of Japan.

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