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Carbon-13 Nuclear Magnetic Resonance Spectral Assignments of Grayanotoxin-I

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The ^{13}C-NMR signals of grayanotoxin-I, a tetracyclic diterpene with an A-nor-B-homo-*ent*-kaurane skeleton, were assigned by means of single-frequency off-resonance decoupling, selective proton decoupling and by comparison with spectra of derivatives.

Keywords——^{13}C-NMR spectral assignments; grayanotoxins; *ent*-kaurane skeleton; lyoniol; *Leucothoe grayana*

Grayanotoxins, isolated from *Leucothoe grayana* MAX., are tetracyclic diterpenoids with an A-nor-B-homo-*ent*-kaurane skeleton. As most of them have physiological and pharmaceutical activities,[2] various workers have investigated their structure-activity relationships.[3]

In previous papers,[4] we reported the first isolation of two grayanotoxin glycosides. The ^{13}C-nuclear magnetic resonance (NMR) spectra of grayanotoxins are useful in determining the structures of the glycosides, and this report describes the assignment of the ^{13}C-NMR signals of all the carbons in grayanotoxin-I.

Experimental

Grayanotoxin-I (4),[5] -II (5),[6] -III (2),[5] -IV (6),[7] and -XVIII (10)[4] were isolated from *Leucothoe grayana* and lyoniol-B (12)[8] from *Lyonia ovalifolia* var. *elliptica*. Other compounds were prepared from the natural

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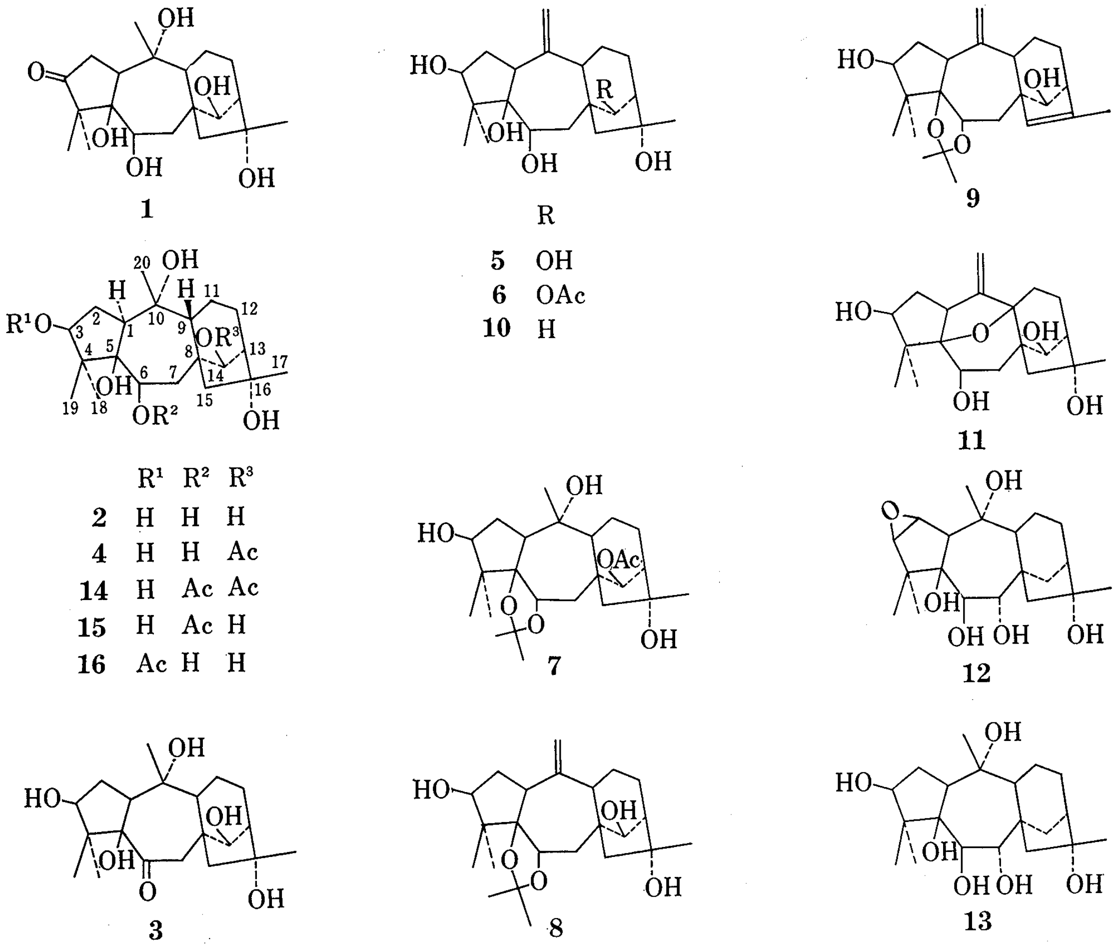

Fig. 1

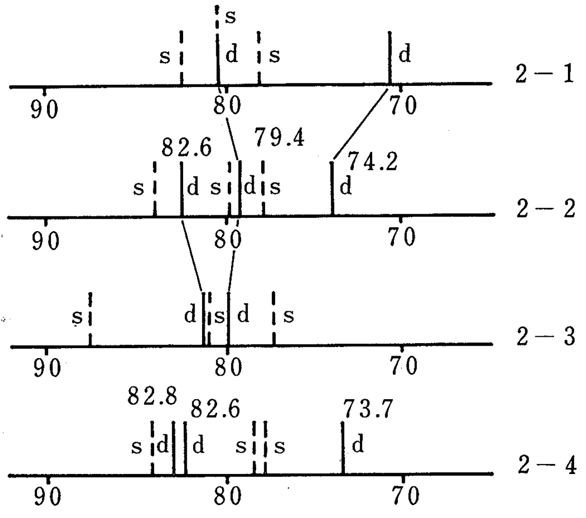

Fig. 2

compounds. Their structures were confirmed by ^{1}H-NMR, infrared (IR), mass spectrum (MS), mp and/or elemental analysis. ^{13}C-NMR spectra were measured using a 10 mm glass tube on a JEOL FX-60 spectrometer (15 MHz) with Fourier transform techniques, controlled by a JEC-98B 16K computer. The sample (about 100 mg) was dissolved in d_5-pyridine (about 1 ml) with Me_4Si as an internal reference.

Results and Discussion

The structures of grayanotoxins to be discussed in this paper are shown in Fig. 1. The ^{13}C-NMR signals were divided into 11 groups, as shown in Table I, based on the information obtained from chemical shifts and coupling patterns in off-resonance experiments. Abbreviations are s, singlet; d, doublet; t, triplet; and q, quartet. The sign 2—3 refers to the NMR spectrum of compound 3 in Fig. 2, and so on.

Table I. ^{13}C-NMR Data for Grayanotoxins and Related Compounds

Compounds	**1**	**2**	**3**	**4**	**4′**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
$-CH_3$	18.1 22.3 24.0 27.9	19.8 23.3 23.9 28.3	17.9 23.9 26.9 26.9	19.8 21.2 23.4 24.0 28.3	19.0 19.0 22.7 23.9 27.4	18.7 23.8 24.6	18.5 21.1 23.8 25.6	20.7 21.4 24.2 25.9 25.9 27.2 27.7	20.3 23.5 27.0 27.4 27.8	15.3 20.2 27.1 27.2 27.7	19.2 24.1 25.4	19.2 23.3 24.2	20.6 21.3 23.9 30.9	20.2 23.3 23.9 28.5
$-CH_2-$	22.3 27.0 38.8 44.3 61.0	22.6 27.1 35.8 44.4 60.4	20.7 26.9 36.0 50.9♯ 59.7	22.5 27.3 35.8 44.1 61.1	21.2 26.2 34.7 42.5 59.8	24.6 24.8 39.4 42.2 60.7	23.8 24.8 39.6 40.8 61.4	20.7 27.2 35.3 37.5 63.6	22.1 24.2 35.8 38.3 61.5	21.8 23.3 31.1 38.2	24.1 25.8 36.5 39.4 46.7 62.5	23.3 29.5 37.3 42.6 50.0	23.1 26.9 29.3 52.8	23.3 27.1 29.5 35.8 53.4
$\rangle CH-$	47.6 54.3 55.7	51.7 55.2 56.4	51.8♯ 51.8♯ 54.3	51.4 55.0 55.6	49.5 53.5 54.5	45.9 50.8 54.3	45.3 51.7 52.8	49.5 50.3 53.1	44.2 45.8 55.0	38.6 45.8 54.7	44.5 47.9 52.3	51.2 52.6	50.7 51.4 54.5	50.8 52[a)] 52[a)]
$-\overset{\mid}{\underset{\mid}{C}}-$	52.2 57.1	51.8 52.6	50.4 53.7	51.0 51.8	49.5 50.4	50.6 50.6	49.0 50.3	48.6 50.9	48.1 51.3	48.0 54.5	44.7 50.6	48.5 50.8	48.1 51.7	52[a)] 52[a)]
H H $-\overset{\mid}{C}\ \ \overset{\mid}{C}-$ \O/													60.0 64.4	
$-\underset{\mid}{C}H-O-$	70.8 80.4	74.2 79.4 82.6	79.9 81.2	73.7 82.6 82.8	72.3 80.8 81.0	69.5 81.0 81.2	69.3 80.8 84.0	76.1 79.0 86.2	77.1 78.3 82.8	77.3 78.4 84.2	70.6 81.2	67.9 82.1 85.3	76.0 79.1	76.8 80.3 82.8
$-\overset{\mid}{\underset{\mid}{C}}-O-$	78.0 80.4 82.5	78.1 79.7 84.6	77.5 81.0 87.6	77.9 78.6 84.4	76.5 77.0 83.0	81.2 83.9	79.5 83.4	77.3 80.4 93.5 108.0	81.0 93.5 107.4	93.5 107.3	79.5 83.5	81.2 89.1 95.6	77.1 78.0 79.0	77.8 78.0 83.7
$=CH_2$						111.9	112.5		111.2	111.5	112.2	107.4		
$=CH-$										133.5				
$=C\langle$						153.0	151.8		149.9	137.4 149.5	153.0	161.8		
$\rangle C=O$	221.3		216.1	170.2	169.7		170.3	170.6						

Proton off-resonance spectra were obtained for all compounds.
d_5-Pyridine solvent, except in the case of 4′ (d_6-DMSO).
Owing to the complex proton off-resonance spectrum, signals marked ♯ may be reversed.
a) These signals appear in the region of 51.9—52.2 ppm.
^{13}C-NMR spectra of **14**, **15** and **16** were not measured.

A. Signals in the Region of 65—90 ppm

1. Secondary Carbinyl Carbons (C-3, C-6 and C-14: Fig. 2)——The signals arising from C-3 and C-6 can readily be assigned by comparison with the corresponding spectra of keto-derivatives as follows: a signal at 82.6 ppm(d) in 2—2 disappeared in 2—1 (3-keto derivative), so it was assigned to C-3 of **2**. A signal at 74.2 ppm(d) in 2—2 was assigned to C-6 of **2** because of the absence of one doublet signal in 2—3 (6-keto derivative). The remaining signal at 79.4 ppm(d) was assigned to C-14 of **2**. These assignments were confirmed by selective proton decoupling techniques with **2**. Namely, a doublet at 82.6 ppm in the ^{13}C-NMR of **2** changed to a signlet on irradiating a signal at 3.84 ppm (C-3-H, br-s, $W_{1/2}=6$ Hz) in the ^{1}H-NMR of **2** and a doublet at 79.4 ppm in the ^{13}C-NMR of **2** changed to a singlet on irradiating a signal at 4.95 ppm (C-14-H, s) in the ^{1}H-NMR of **2**. A doublet at 82.8 ppm in the ^{13}C-NMR of **4** changed to a singlet on irradiating a signal at 6.22 ppm (C-14-H, s) in the ^{1}H-NMR of **4**. Summarizing these assignments in 2—4, peaks at 73.7, 82.6 and 82.8 ppm were assigned to C-6, -3 and -14 of **4**, respectively.

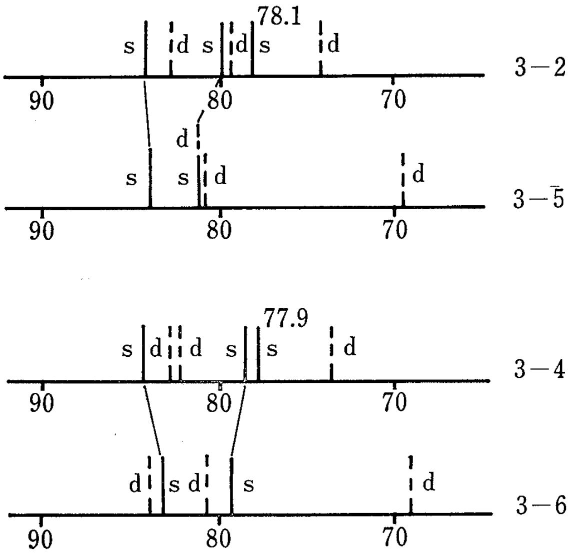

Fig. 3

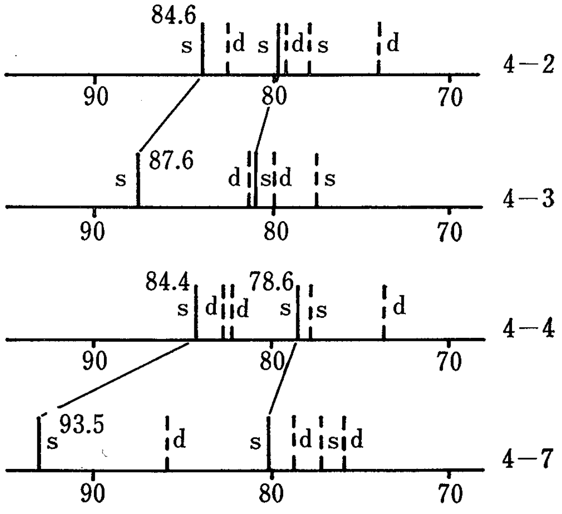

Fig. 4

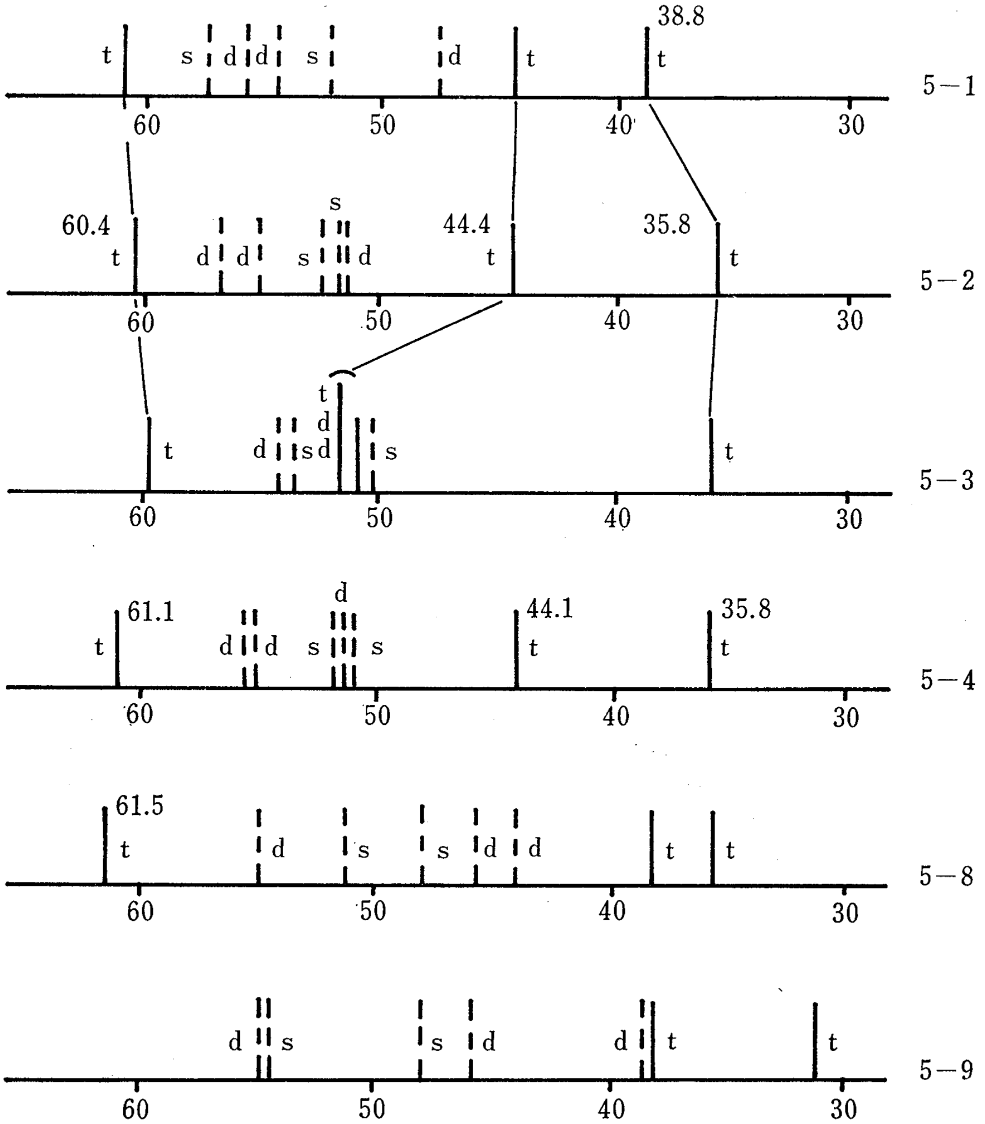

Fig. 5

2. **Tertiary Carbinyl Carbons (C-5, C-10 and C-16: Fig. 3 and 4)**——Singlet signals at the highest field of 3—2 and 3—4 (78.1 and 77.9 ppm) were assigned to C-10, since these signals were absent in 3—5 and 3—6 (Δ 10(20) compounds).

It is well known that the α-carbon of a carbonyl group is more deshielded than the β-carbon of a carbinyl carbon,[9] and a signal at 84.6 ppm(s) in 4—2 shifted 3.0 ppm downfield in 4—3 (6-keto derivative). The signal at 84.6 ppm was assigned to C-5 of **2**. A signal at 84.4 ppm(s) in 4—4 was assigned to C-5 of **4** in a similar way. The signal at 84.4 ppm in 4—4 showed a downfield shift to 93.5 ppm(s) in 4—7 (5,6-O-isopropylidene derivative). The remaining singlet at 78.6 ppm(s) in 4—4 was assigned to C-16 of **4**.

B. Signals in the Region of 30—65 ppm

1. **Methylene Carbons (C-2, C-7 and C-15: Fig. 5)**——The signal at 38.8 ppm in 5—1 is assigned to C-2, since the signal was absent in the spectrum of a deuterium-labelled derivative,[10] 2,2-d_2-3-keto-grayanotoxin-III. Only one triplet at 35.8 ppm in 5—2 moved downfield to 38.8 ppm(t) in 5—1 (3-keto derivative). Therefore, the signal at 35.8 ppm was assigned to C-2 of **2**. A signal at 60.4 ppm(t) in 5—2 remained essentially unchanged in 5—3, whereas the triplet at 44.4 ppm in 5—2 showed a downfield shift to 51—52 ppm in 5—3 (6-keto derivative). Consequently, the latter triplet was assigned to C-7 of **2**. In 5—4, a signal at 35.8 ppm was assigned to C-2 and a signal at 44.1 ppm to C-7.

In order to assign the signal of C-15, the acetonide and dehydrated derivatives (**8** and **9**) were prepared. In comparison with 5—8 and 5—9, a signal at 61.5 ppm(t) in 5—8 was absent in 5—9, so this signal was assigned to C-15 of **8**. Therefore, a signal at 61.1 ppm(t) in 5—4 was assigned to C-15 of **4**. In the spectra of **1**—**8** and **10**, the triplet signals of C-15 were observed in the region of 58—63 ppm. Carbon-15 is not a carbinyl carbon but carbinyl carbon chemical shifts generally appear in this region (for example, 61.7 ppm in 1-butanol[11]). On the other hand, the triplet signals of **11**—**13** were seen between 50 and 54 ppm. The triplet signal of **4** measured in d_6-DMSO shifted only slightly upfield to 59.8 ppm. This shows that the remarkable chemical shift of C-15 is independent of the solvent. The reason for this phenomenon is not clear.

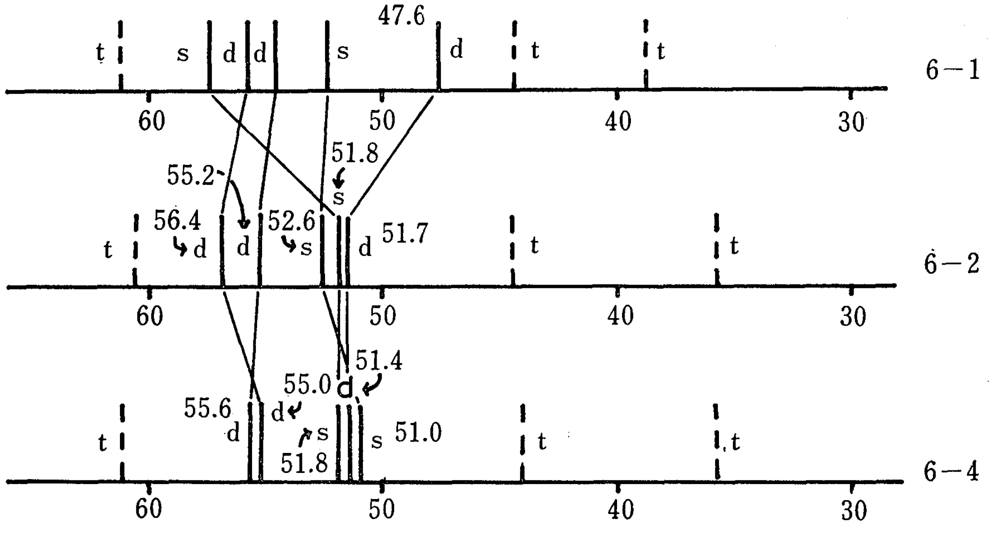

Fig. 6

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2. **Tertiary Carbons (C-1, C-9 and C-13: Fig. 6)**——A signal at 51.7 ppm(d) in 6—2 which moved upfield to 47.6 ppm(d) in 6—1 (3-keto derivative) was assigned to C-1 of **2**, because C-1 is in the γ position relative to C-3.

When comparing 6—2 with 6—4 (14-acetate of **2**), slight shifts of one singlet and one doublet (52.6⟶51.0 and 56.4⟶55.0) were observed. Since this was due to acetylation of the 14-hydroxyl group, the singlet and doublet peaks were assigned to C-8 and C-13, which are β to C-14. The remaining doublet at 55.2 ppm in 6—2 and 55.6 ppm in 6—4 were assigned to C-9. However, the assignments of C-9 and C-13 may be reversed.

3. **Quaternary Carbons (C-4 and C-8: Fig. 6)**——In the above discussion, the signal at 51.0 ppm in 6—4 was assigned to C-8 of **4**. The remaining singlet at 51.8 ppm in 6—4 was assigned to C-4 of **4**.

C. Signals in the Region of 15—30 ppm

1. **Methyl Signals (C-17, C-18, C-19 and C-20: Fig. 7)**——Initially, we examined the ^{1}H-NMR spectra. As shown in Fig. 7, a methyl signal which appears about 1.8 ppm in 7—2, 7—4, 7—14, 7—15 and 7—16 is absent in 7—6 (Δ10(20) compound). Therefore, the signal at 1.84 ppm in 7—4 was assigned to the 20-methyl group of **4**.

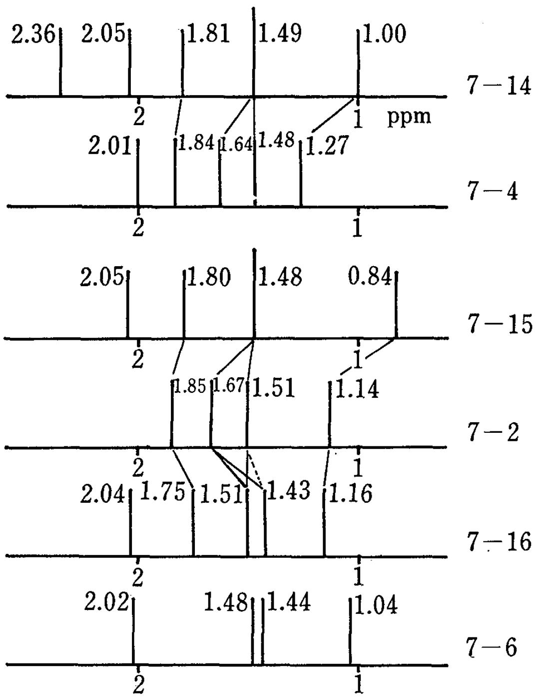

Fig. 7. ^{1}H-NMR(C_5D_5N)

Kawazoe *et al.*[12] reported that methyl proton resonances can shift to lower fields due to steric interaction with a neighboring hydroxyl group, and that such a methyl group can be identified by acetylation of the hydroxyl group; the resonance then appears at markedly higher field than that of the original hydroxyl compound. X-ray crystallographic analysis of **4**[13] and examination of its Dreiding model showed that the 18- and 19-methyl groups are close to the 6-hydroxyl group. Two signals at 1.27 and 1.64 ppm in 7—4 move to 1.00 and 1.49 ppm in 7—14 (6-acetate of **4**). Comparing 7—2 with 7—15 (6-acetate of **2**), similar shifts were observed for the two methyl groups. Therefore, these signals were assigned to the 18- and 19-methyl groups. Comparing 7—2 with 7—16 (3-acetate of **2**), a signal at 1.14 ppm in 7—2 scarcely moved in 7—16, but a signal at 1.67 ppm in 7—2 shifted to 1.43 or 1.51 ppm in 7—16. Therefore, this signal was assigned to the 19-methyl group, because it is nearer to the 3-hydroxyl group than the 18-methyl is.

Consequently, five methyl proton signals, 1.27, 1.48, 1.64, 1.84 and 2.01 ppm, in the ^{1}H-NMR spectrum of **4** were assigned to the 18-, 17-, 19-, 20- and acetyl methyl groups, respectively.

Using the single frequency selective decoupling technique, five methyl carbon signals in the ^{13}C-NMR spectrum of **4** were then assigned. The signals at 19.8, 21.2, 23.4, 24.0 and 28.3 ppm were assigned to C-19, acetyl methyl, C-18, C-17 and C-20, respectively.

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2. **Methylene Carbons (C-11 and C-12)**——Two signals at 22.5 and 27.3 ppm in the spectrum of **4** corresponded to C-11 and C-12, but an unambigous assignment could not be made.

Conclusion

The ^{13}-NMR spectral assignments of grayanotoxin-I (**4**) are as follows——C-1, 51.4 ppm; C-2, 35.8 ppm; C-3, 82.6 ppm; C-4, 51.8 ppm; C-5, 84.4 ppm; C-6, 73.7 ppm; C-7, 44.1 ppm; C-8, 51.0 ppm; C-9, 55.6 ppm; C-10, 77.9 ppm; C-11 and C-12, 22.5 and 27.3 ppm; C-13, 55.0 ppm; C-14, 82.8 ppm; C-15, 61.1 ppm; C-16, 78.6 ppm; C-17, 24.0 ppm; C-18, 23.4 ppm; C-19, 19.8 ppm; C-20, 28.3 ppm; acetyl methyl, 21.2 ppm; and acetyl carbonyl, 170.2 ppm.

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