INTERMOLECULAR STACKING OF GILVOCARCIN V TETRAACETATE AS EVIDENCED BY NUCLEAR MAGNETIC RESONANCE STUDIES

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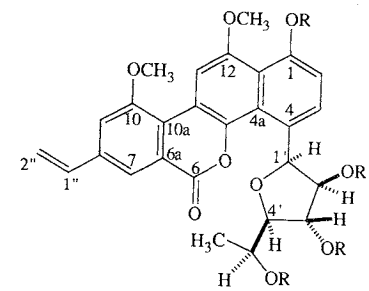
The temperature-dependent and concentration-dependent ^{1}H NMR studies as well as ^{13}C-relaxation experiments on gilvocarcin V tetraacetate strongly suggest intermolecular stacking of the antibiotic in solution.

Molecular interaction between biologically active compounds and cell constituents is a subject of current biological interest and antibiotics are no exceptions. During our chemical studies of gilvocarcin V, we were aware that the ^{1}H NMR chemical shifts of gilvocarcin V derivatives vary from time to time and the literature data are also different depending upon the authors.[1~6] We suspected this may reflect possible intermolecular interaction of the gilvocarcin molecules, because the gilvocarcin structure includes a highly aromatic benzo[d]naphthopyranone nucleus. To get insight into these observations, we undertook temperature-dependent and concentration-dependent ^{1}H NMR studies as well as ^{13}C-relaxation experiments on gilvocarcin V tetraacetate and wish to describe here NMR evidence which strongly suggests intermolecular stacking of the antibiotic in solution, thereby providing an additional support for the mechanism of its intercalation into DNA.

Fig. 1. Structures of gilvocarcin V and its tetraacetate.



Gilvocarcin V R=H
Gilvocarcin V tetraacetate R=$COCH_3$

Materials and Methods

Gilvocarcin V was provided for us by Pharmaceutical Laboratories, Kirin Brewery Co., Ltd. Gilvocarcin V tetraacetate was prepared by the method described in the literature.[6] ^{1}H and ^{13}C NMR spectra were recorded at 27°C, unless otherwise stated, on a Jeol GSX-270 spectrometer operating at 270 MHz and at 67.8 MHz, respectively. Deuteriochloroform (99.75% atom enriched, Merck) was used for the solvent throughout. ^{1}H NMR chemical shifts were reported in ppm relative to the signal of internal tetramethylsilane. ^{13}C NMR chemical shifts were calculated from the resonance frequency of the center peak (77.0 ppm) of the solvent signal. The longitudinal relaxation time (T_1) was measured by the inversion recovery method (180°-τ-90° pulse sequence).

Results and Discussion

The ^{1}H and ^{13}C NMR spectra of gilvocarcin V and its tetraacetate were analyzed and assignments were

Table 1. ^{1}H NMR data of gilvocarcin V tetraacetate.[a]

Proton	Chemical shifts and coupling constants
2-H	7.10 (d, J=8.4 Hz)
3-H	7.99 (d, J=8.3 Hz)
7-H	7.90 (d, J=2.5 Hz)
9-H	7.10 (d, J=2.3 Hz)
11-H	8.26 (s)
10-OCH$_3$	3.79 (s)
12-OCH$_3$	3.86 (s)
1′-H	6.40 (d, J=3.1 Hz)
2′-H	6.10 (dd, J=0.7, 3.2 Hz)
3′-H	5.16 (dd, J=0.7, 3.9 Hz)
4′-H	4.19 (dd, J=3.9, 6.4 Hz)
5′-H	5.39 (quintet, J=6.4 Hz)
1″-H	6.64 (dd, J=10.9, 17.5 Hz)
2″E-H	5.35 (d, J=10.9 Hz)
2″Z-H	5.84 (d, J=17.6 Hz)
1-OCOCH$_3$	2.35 (s)
2′-OCOCH$_3$	1.51 (s)
3′-OCOCH$_3$	2.15 (s)[b]
5′-OCOCH$_3$	2.32 (s)[b]

[a] The data were recorded at 27°C in a concentration of 4.9×10^{-1} M.
[b] May be interchangeable.

Table 2. ^{13}C NMR data of gilvocarcin V tetraacetate.[a]

Carbon	Chemical shift
1	145.8
2	120.0
3	127.3
4	129.6
4a	123.8
4b	141.3
6	159.7
6a	122.1
7	119.6
8	138.6
9	113.5
10	157.1
10a	122.9
10b	114.1
11	104.3
12	150.6
12a	119.5
1′	81.3
2′	77.7
3′	78.7
4′	83.1
5′	69.6
6′	16.3
1″	135.0
2″	116.2
10-OCH$_3$	55.7
12-OCH$_3$	55.7
1-CO	169.7
2′-CO	168.3
3′-CO	170.3
5′-CO	170.3
1-OCOCH$_3$	21.1
2′-OCOCH$_3$	19.9
3′-OCOCH$_3$	20.9[b]
5′-OCOCH$_3$	20.7[b]

[a] The data were recorded at 27°C in a concentration of 4.9×10^{-1} M.
[b] May be interchangeable.

made by the previous workers.[6] However, further confirmation seemed to be required for several signal assignments, and we first carried out such reinvestigation using modern NMR techniques including ^{1}H-^{1}H-COSY, ^{1}H-^{1}H-NOESY, ^{1}H-^{13}C-COSY, and correlation *via* long range coupling (COLOC). Unambiguous assignments of ^{1}H and ^{13}C NMR signals of gilvocarcin V tetraacetate were made by means of these 2D-NMR spectra and are listed in Tables 1 and 2, respectively.

The concentration-dependent ^{1}H NMR spectra of gilvocarcin V tetraacetate were measured at 27°C in concentrations of 4.9×10^{-1}, 4.9×10^{-2}, and 2.5×10^{-3} M solution and are shown in Fig. 2. The signals assigned to the protons on the aromatic ring, the protons close to aromatic ring such as 1′-H, 2′-H, and 1″-H, and two methoxy groups were remarkably shifted into more highly shielded region as the concentration was increased. In other words, the local field strength surrounding these hydrogen nuclei decreased by increasing the concentration. These alterations of the local filed may be attributed to the well-established shielding effects of aromatic rings. Other protons, *e.g.*, the methyl groups of the acetates, 4′-H, 5′-H, and 6′-H were almost unchanged. Similar tendency was observed with variable temperature ^{1}H NMR spectra which are shown in Fig. 3. By cooling the probe the chemical shifts of the aromatic protons, 10-methoxy group, and 1′-H significantly changed toward the more highly shielded region just as increasing the concentration. These observations strongly indicate that gilvocarcin nucleus is associated intermolecularly so that a part of a molecule is magnetically shielded by the aromatic nuclei of another molecule. Therefore,

Fig. 2. Concentration-dependent ^{1}H NMR spectra of gilvocarcin V tetraacetate (270 MHz, $CDCl_3$, 27℃).
(A) 2.5×10^{-3} M, (B) 4.9×10^{-2} M, (C) 4.9×10^{-1} M. Asterisked are impurity signals.

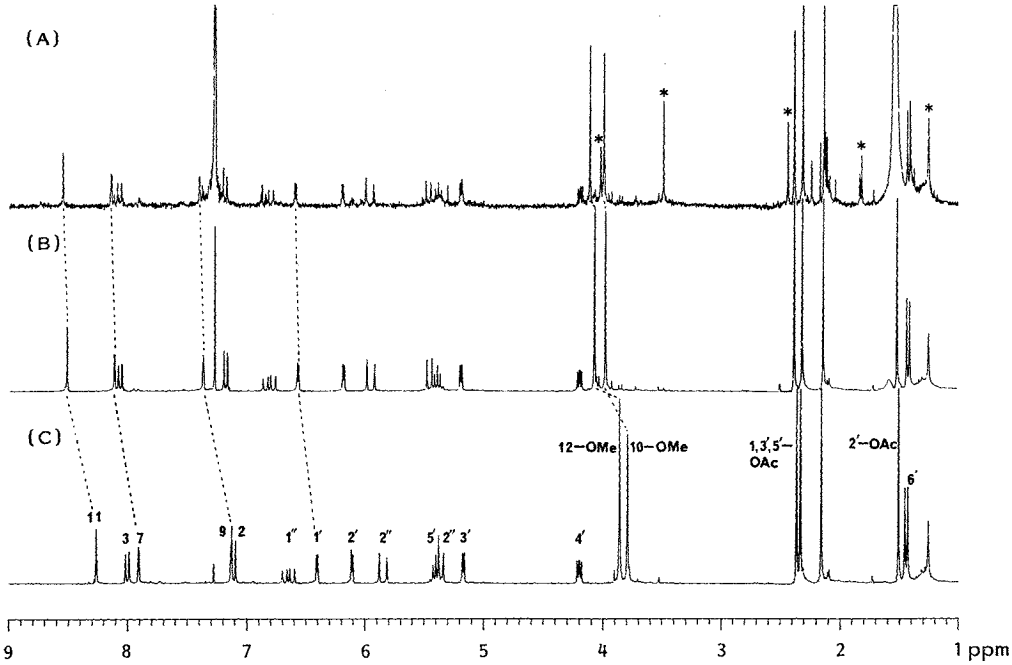
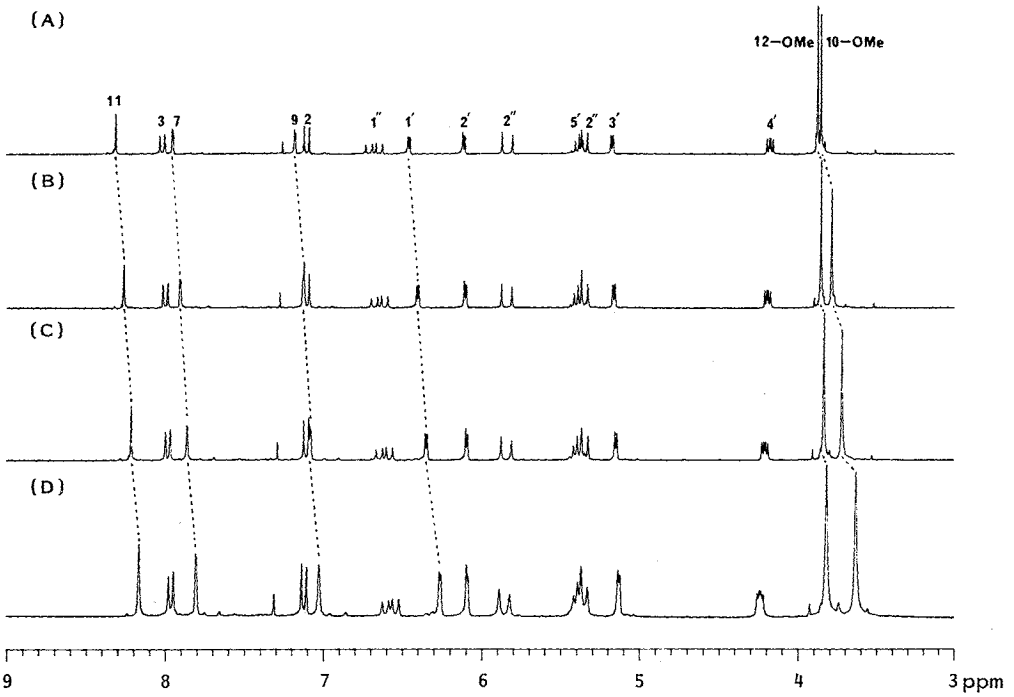


Fig. 3. Temperature-dependent ^{1}H NMR spectra of gilvocarcin V tetraacetate (270 MHz, $CDCl_3$, 4.9×10^{-1} M).
(A) 60℃, (B) 27℃, (C) 0℃, (D) −40℃. The spectrum (B) is corresponding to a high frequency region of Fig. 2(C).



as the mode of the interaction, molecular stacking is suggested between two molecules of the antibiotic through the intermolecular π-electron interaction due to the partial planarity of the molecule. However, possible multilayer stacking of the antibiotic molecules cannot be dealt with from the present results.

The above-mentioned discussion is further supported by the ^{13}C NMR relaxation studies. The T_1 values of gilvocarcin V tertraacetate were measured in two different concentrations (4.9×10^{-1} M and 1.5×10^{-1} M) and the results are summarized in Table 3. Increasing the concentration significantly shortened the T_1 values of the all signals. Although details of the ^{13}C spin-relaxation mechanism of gilvocarcin V tetraacetate are not clear, dipolar relaxation with protons generally dominates for ^{13}C nuclei in such a relatively large organic molecule, particularly for the protonated carbons. Dipole-dipole relaxation usually depends on the rate of molecular reorientation, *e.g.* molecular motion.[7] The present observation that T_1s were shortened in a higher concentration strongly suggests intermolecular association affecting the molecular motion for gilvocarcin V tetraacetate. In conclusion NMR evidences strongly suggest intermolecular stacking of the gilvocarcin V tetraacetate in solution. Recently, phenomena of intermolecular self-stacking have been reported for non-antibiotic dyes such as acridine[8] and flavocommelin.[9]

Table 3. ^{13}C Spin-lattice relaxation times (T_1s) of gilvocarcin V tetraacetate in different concentrations.[a,b]

Carbon	4.9×10^{-1} M	1.5×10^{-1} M
	T_1 (seconds)	
1	3.7	5.8
2	0.21	0.32
3	0.17	0.32
4	2.1	4.2
4a	3.4	4.9
4b	5.4	6.4
6	4.5	7.1
6a	2.7	4.3
7	0.20	0.29
8	1.9	3.5
9	0.18	0.35
10	3.2	6.4
10a	2.8	6.0
10b	3.0	5.5
11	0.18	0.24
12	3.3	6.6
12a	2.5	7.6
1′	0.22	0.28
3′	0.26	0.32
4′	0.25	0.34
5′	0.28	0.42
6′	0.57	0.60
1″	0.32	0.46
2″	0.13	0.19
1-CO	3.3	5.8
2′-CO	3.3	7.5
1-OCOCH$_3$	1.3	1.6
2′-OCOCH$_3$	1.0	1.6
3′-OCOCH$_3$	1.2	1.9
5′-OCOCH$_3$	1.1	1.5

[a] T_1s were measured by the inversion recovery method at 27°C.

[b] T_1s of the carbons at two methoxy groups, 3′-CO; 5′-CO and C-2′ were not obtained due to overlapping of the signals each other or the solvent signal.

The significance of these findings that gilvocarcin has intrinsic tendency of self-assemblage through molecular stacking is to provide further support from the molecular bases to the previous discussions that gilvocarcin V interacts with DNA through intercalation,[10,11] which is the initial cause of DNA unwinding as well as the covalent-bond formation by photoactivation.[12]

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