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Long-range ^{13}C–^{1}H coupling constants ($^{3}J_{C-H}$) of monensin sodium

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Abstract—Long-range ^{13}C–^{1}H coupling constants ($^{3}J_{C-H}$) of monensin sodium in $CDCl_3$ and in CD_3OD were measured by gs-J-HMBC experiments. The $^{3}J_{C-H}$ of monensin sodium was almost the same both in $CDCl_3$ and in CD_3OD. The obtained $^{3}J_{C-H}$ suggested a small difference in the conformation in solvents from that in crystals. © 2001 Elsevier Science Ltd. All rights reserved.

Monensin (Fig. 1) is a representative of naturally occurring polyether acid ionophores, and transports cations, especially the sodium ion, through a biological membrane.[1] Monensin attracted much attention because of not only its complex structure but also the conformation when the molecule complexes cations. X-Ray crystal structure analysis was carried out on various salts of monensin and free acid,[2] and extensive data regarding the conformations in the crystals were accumulated. We have prepared various monensin derivatives to obtain more potent ionophores[3,4] and clarified the conformations in the crystals.[4] As the ionophores transport ions in the liquid phase and are always surrounded by polar and/or non-polar molecules, we are interested in the conformation in solvents. However, only limited information about the conformation of monensin in solvents has been reported.[5]

When the conformations of organic compounds in solvents are discussed, the data from NMR spectra such as NOE and/or $^{3}J_{H-H}$ are usually used. In the case of monensin, we can interpret only limited numbers of $^{3}J_{H-H}$, because of overlapping of the methylene and methine proton signals resonated at δ 1–2 ppm. Even in such case, $^{3}J_{C-H}$ should give us considerable information related to C–X–X–H dihedral angles and should reveal the conformational changes in the molecule caused by cation complexation, solvents, or substituents on their functional groups. Some methods for measurements of $^{3}J_{C-H}$ have been developed[6–10] and applied to some molecules in order to determine the conformations[11] or the configurations.[12] In addition, the higher-resolution FT-NMR machine with a highly efficient processing system has been quite popular for routine work during the last decade. Thus, the measurement of $^{3}J_{C-H}$ is much more practical than before with these machines. Here, we report $^{3}J_{C-H}$ of monensin sodium and the conformational difference between in solvents and in crystals based on the $^{3}J_{C-H}$.

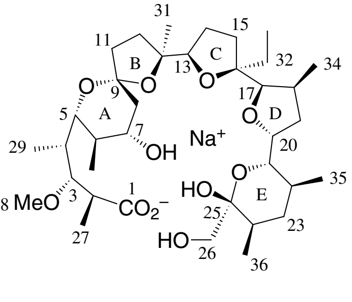

Figure 1. Chemical structure of monensin sodium.

1. Results and discussion

Some methods for measuring $^{3}J_{X-H}$ were reported such as long-range ^{13}C J resolved NMR,[6] 2D hetero half-filtered TOCSY (HETLOC)[7] and other TOCSY-based methods,[8] the methods based on the HMQC or HSQC pulse sequence,[9] and the methods based on the HMBC pulse sequence.[10] We chose the 2D version of gradient-selected HMBC method (gs-J-HMBC)[10b,13] to determine $^{3}J_{C-H}$ of monensin sodium. By the HMBC-based methods, we can theoretically detect all $^{3}J_{C-H}$ in the molecule including those between a quaternary carbon and a proton and small $^{3}J_{C-H}$ values. In addition, the gs-J-HMBC method should be a practical method for the researchers who are not extremely skillful in the operation of an NMR spectrometer.

The present experiment was carried out with JEOL Lambda-500 spectrometer. In order to determine the difference in conformation in polar and non-polar solvents, we performed gs-J-HMBC experiments both in $CDCl_3$ and CD_3OD.

Keywords: monensin; long-range ^{13}C–^{1}H coupling constants; gs-J-HMBC; conformation.

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Table 1. Assignments of ^{13}C and ^{1}H in the spectra of monensin sodium in CDCl$_3$ and in CD$_3$OD

Position	In CDCl$_3$ (ppm)		In CD$_3$OD (ppm)		Position	In CDCl$_3$ (ppm)		In CD$_3$OD (ppm)	
	δ_C	δ_H	δ_C	δ_H		δ_C	δ_H	δ_C	δ_H
1 (C)	181.2		183.3		19 (CH$_2$)	33.2	1.46, 1.63	34.3	1.61, 2.21
2 (CH)	45.0	2.47	46.0	2.46	20 (CH)	76.4	4.33	77.5	4.42
3 (CH)	83.0	3.12	83.9	3.12	21 (CH)	74.4	3.76	76.2	3.85
4 (CH)	37.4	1.99	38.7	2.07	22 (CH)	31.8	1.31	32.9	1.42
5 (CH)	68.3	3.97	69.1	3.96	23 (CH$_2$)	35.6	1.25, 1.41	36.9	1.38, 1.41
6 (CH)	34.8	2.15	36.1	2.02	24 (CH)	36.5	1.38	37.4	1.55
7 (CH)	70.4	3.82	71.6	3.89	25 (C)	98.3		99.2	
8 (CH$_2$)	33.5	1.61, 1.84	34.4	1.66, 1.95	26 (CH$_2$)	64.8	3.22, 3.91	65.8	3.30, 3.91
9 (C)	106.9		108.4		27 (CH$_3$)	16.7	1.17	16.8	1.17
10 (CH$_2$)	39.2	1.63, 1.94	40.2	1.76, 1.98	28 (CH$_3$)	57.9	3.31	58.3	3.37
11 (CH$_2$)	33.2	1.92, 2.11	34.0	1.82, 1.93	29 (CH$_3$)	11.0	1.11	11.5	1.10
12 (C)	85.2		86.8		30 (CH$_3$)	10.5	0.87	11.0	0.94
13 (CH)	82.5	3.47	83.2	3.60	31 (CH$_3$)	27.4	1.44	28.1	1.48
14 (CH$_2$)	27.2	1.50, 1.71	28.1	1.53, 1.87	32 (CH$_2$)	30.6	1.44, 1.51	31.1	1.49, 1.71
15 (CH$_2$)	29.8	1.39, 2.23	31.3	1.47, 2.33	33 (CH$_3$)	8.2	0.87	8.3	0.93
16 (C)	85.8		87.0		34 (CH$_3$)	14.5	0.83	14.9	0.93
17 (CH)	84.9	3.87	85.9	4.00	35 (CH$_3$)	16.8	0.73	17.0	0.83
18 (CH)	34.3	2.18	35.7	2.30	36 (CH$_3$)	16.0	0.78	16.5	0.85

Before starting the gs-J-HMBC experiment, we checked the assignment of all ^{1}H and ^{13}C of monensin sodium in both solvents by measuring ^{1}H and ^{13}C NMR, H–H COSY, HMQC, and HMBC ($^{2,3}J_{C\text{–}H}$=8.3 Hz) spectra (Table 1). The assignment in CDCl$_3$ was identical with the reported data.[14]

The $^{3}J_{C\text{–}H}$ of monensin sodium both in CD$_3$OD and CDCl$_3$ are indicated in Table 2. From the experiment in CDCl$_3$, 43 $^{3}J_{C\text{–}H}$ out of 84 C–X–X–H in the molecule were obtained, and 45 from that in CD$_3$OD. The other $^{3}J_{C\text{–}H}$ were not determined probably due to too small $^{3}J_{C\text{–}H}$, too low signal-to-noise ratio of the cross peaks, and/or overlapping of the cross peaks of $^{3}J_{C\text{–}H}$ with those of $^{2}J_{C\text{–}H}$. $^{3}J_{C\text{–}H}$ in CD$_3$OD and CDCl$_3$ were almost the same in most of the positions. This fact indicated that the conformations of monensin sodium in polar and nonpolar solvent are almost the same and the solvents had no essential influence on the conformation.

In Table 2, the dihedral angles obtained from X-ray crystal structure analysis[2d] and some $^{3}J_{C\text{–}H}$ calculated from the dihedral angles are also indicated. The calculated $^{3}J_{C\text{–}H}$ were obtained from the Karplus equations: $^{3}J_{C\text{–}H} = 5.7\cos^2\varphi - 0.6\cos\varphi + 0.5$ for C–O–C–H,[15] and $^{3}J_{C\text{–}H} = 3.6\cos 2\varphi - 1.0\cos\varphi + 4.3$ for C–C–C–H[16] having no oxygenated carbons on the dihedral intersection. As the Karplus equations for C–C–C–H with the oxygenated carbon(s) on the dihedral intersection have not been established, the calculated $^{3}J_{C\text{–}H}$ were not indicated for those systems. The calculated $^{3}J_{C\text{–}H}$ around C-23 are found to be different from the data obtained in our experiments as shown in Table 2. The E ring was known to be an exact chair form in the crystal. So $^{3}J_{C21\text{–}H23\alpha}$ and $^{3}J_{C25\text{–}H23\alpha}$ were expected to be large (>6 Hz) and $^{3}J_{C21\text{–}H23\beta}$ and $^{3}J_{C25\text{–}H23\beta}$ to be small (<3 Hz), but these $^{3}J_{C\text{–}H}$ obtained from our experiment were medium (around 4 Hz), except for $^{3}J_{C25\text{–}H23\alpha}$. The vicinal couplings of H-23α/H-22 and H-23β/H-22 were reported as 3.5 and 11 Hz,[5a] respectively, which supported the exact chair form of the E ring. However, those of H-23α,β and H-24 were reported unclear and we could not confirm $J_{H23\alpha,\beta\text{–}H22}$ and $J_{H23\alpha,\beta\text{–}H24}$. Thus, it is likely that the conformation of the E ring is a more or less distorted chair form or the E ring moves in the solvents.

There are two other positions showing the much larger $^{3}J_{C\text{–}H}$ than those expected from the crystal data. One is $^{3}J_{C20\text{–}H17}$ (5.4 Hz in CDCl$_3$ and 5.1 Hz in CD$_3$OD), which indicated that the dihedral angle was not around 90°. This data suggested the conformational change of the D ring. The other is $^{3}J_{C11\text{–}H13}$, which can be expected to be medium (around 2–3 Hz) from the dihedral angle in the crystal, although the $^{3}J_{C\text{–}H}$ was not calculated. The value of $^{3}J_{C11\text{–}H13}$ (7.4 Hz) in the solvents was large enough to suggest that the dihedral angle was around 0 or 180° and that the bond between the B and C rings might rotate or might be a different conformation from the crystal.

The $^{3}J_{C4\text{–}H2}$, $^{3}J_{C5\text{–}H3}$, and $^{3}J_{C29\text{–}H3}$ in the chain part at C1–C5 seemed smaller than those deduced from the corresponding dihedral angles in the crystal data. However, $^{3}J_{C\text{–}H}$ with oxygenated carbon(s) on the dihedral intersection frequently showed smaller values relative to those without oxygenated carbon(s).[17] So the $^{3}J_{C\text{–}H}$ values of the chain part were possible to indicate the same dihedral angles as crystals. The $^{3}J_{H\text{–}H}$ values (Table 3) of the chain part exhibited that H2–H3 and H4–H5 were *anti* and H3–H4 was *gauche* and $^{2}J_{C3\text{–}H2}$ (Table 4) suggested H2–O3 was *gauche*.[12] These data indicated that the conformation of C1–C5 in the solvents should be identical with that in the crystal.

In conclusion, we have demonstrated the measurement of $^{3}J_{C\text{–}H}$ of monensin, a complex but well-known molecule, and clarified that the conformations of monensin in nonpolar and aprotic CDCl$_3$ and in polar and protic CD$_3$OD were identical. At the same time, the $^{3}J_{C\text{–}H}$ together with $^{3}J_{H\text{–}H}$ and $^{2}J_{C\text{–}H}$ suggested that the conformation in solvents might be a little different from that in crystals not at chain part but at the rings, although the relation between $^{3}J_{C\text{–}H}$ and dihedral angles was not sufficiently established yet and it may be risky to discuss the conformation based on the $^{3}J_{C\text{–}H}$. The obtained $^{3}J_{C\text{–}H}$ should constitute a basic data set to compare the conformation of monensin with those of the

Table 2. $^{3}J_{C-H}$ of monensin sodium in $CDCl_3$ and CD_3OD obtained from gs-J-HMBC experiment and calculated from X-ray crystal structure analysis

C–X–X–H	$^{3}J_{C-H}$ (Hz)			X-Ray[2d] (deg)	C–X–X–H	$^{3}J_{C-H}$ (Hz)			X-Ray[2d] (deg)
	$CDCl_3$	CD_3OD	Calcd			$CDCl_3$	CD_3OD	Calcd	
C1–C2–C3–H3	1.6	1.9		−74.8	C17–C18–C19–H19α	Nd[a]	Nd[a]	0.9[d]	75.7
C2–C3–C4–H4	Nd[a]	Nd[a]		168.5	C17–C18–C19–H19β	Nd[b]	6.6	7.1[d]	−151.4
C3–C4–C5–H5	Nd[a]	Nd[a]		71.6	C18–C19–C20–H20	Nd[a]	Nd[a]		−93.6
C4–C3–C2–H2	1.0	1.3		61.5	C19–C18–C17–H17	1.4	1.7		75.0
C4–C5–C6–H6	Nd[a]	Nd[a]		64.1	C19–C20–C21–H21	4.5	4.7		−180.0
C5–C4–C3–H3	1.6	1.2		−52.4	C20–O–C17–H17	5.4	5.1	0.5[c]	84.6
C5–C6–C4–H7	4.2	4.5		174.8	C20–C19–C18–H18	6.2	5.8	7.3[d]	152.7
C6–C5–C4–H4	Nd[a]	Nd[a]		−61.3	C20–C21–C22–H22	Nd[a]	2.8		−55.6
C6–C7–C8–H8α	3.6	4.2		69.1	C21–C20–C19–H19α	4.9	5.0		−28.5
C6–C7–C8–H8β	Nd[a]	Nd[a]		−174.5	C21–C20–C19–H19β	Nd[a]	Nd[a]		96.2
C7–C6–C5–H5	1.6	1.1		−59.2	C21–C22–C23–H23α	3.6	3.2	8.9[d]	175.4
C8–C7–C6–H6	Nd[a]	Nd[a]		−172.6	C21–C22–C23–H23β	Nd[a]	3.6	1.3[d]	−68.0
C8–C9–C10–H10α	Nd[b]	Nd[b]		−79.9	C22–C21–C20–H20	Nd[a]	Nd[b]		68.0
C8–C9–C10–H10β	Nd[b]	Nd[b]		32.6	C22–C23–C24–H24	Nd[b]	Nd[b]	2.5[d]	55.3
C9–O–C5–H5	1.7	1.7	2.0[c]	−55.6	C23–C22–C21–H21	1.3	1.7		−68.5
C9–C8–C7–H7	7.4	7.6		164.9	C24–C23–C22–H22	Nd[a]	Nd[a]	1.4[d]	66.5
C9–C10–C11–H11α	2.1	Nd[b]	0.7[d]	84.4	C24–C25–C26–H26α	Nd[a]	1.0		50.6
C9–C10–C11–H11β	5.7	4.5	6.5[d]	−146.2	C24–C25–C26–H26β	Nd[a]	Nd[a]		−56.5
C10–C9–C8–H8α	3.8	Nd[b]		−53.5	C25–O–C21–H21	Nd[a]	1.6	1.3[c]	−65.0
C10–C9–C8–H8β	Nd[a]	1.0		61.6	C25–C24–C23–H23α	6.1	4.3	8.9[d]	175.8
C11–C12–C13–H13	7.4	Nd[a]		−66.2	C25–C24–C23–H23β	3.0	3.5	1.6[d]	−64.3
C12–C11–C10–H10α	Nd[b]	Nd[b]	7.4[d]	154.3	C26–C25–C24–H24	Nd[a]	3.8		−57.7
C12–C11–C10–H10β	Nd[b]	Nd[b]	0.7[d]	−88.2	C27–C2–C3–H3	3.2	2.8		48.8
C12–C13–C14–H14α	Nd[a]	Nd[b]		33.2	C28–O–C3–H3	5.7	5.7	4.9[c]	21.9
C12–C13–C14–H14β	Nd[a]	Nd[b]		−92.0	C29–C4–C3–H3	4.8	4.4		−180.0
C13–C12–C11–H11α	4.4	Nd[a]		−134.0	C29–C4–C5–H5	3.2	Nd[b]		−56.3
C13–C12–C11–H11β	2.8	6.1		−19.3	C30–C6–C7–H7	1.3	1.1		49.3
C13–C14–C15–H15α	<1.0	3.2	2.3[d]	114.2	C30–C6–C5–H5	4.5	Nd[b]		176.4
C13–C14–C15–H15β	4.3	4.1	4.5[d]	−131.0	C31–C12–C11–H11α	Nd[a]	7.1		−8.0
C15–C14–C13–H13	0.9	Nd[a]		86.0	C31–C12–C11–H11β	Nd[a]	Nd[a]		106.7
C15–C16–C17–H17	3.4	Nd[b]		161.9	C31–C12–C13–H13	1.2	1.3		60.8
C15–C16–C32–H32α	1.2	2.6		−33.2	C32–C16–C15–H15α	5.3	6.0		18.3
C15–C16–C32–H32β	Nd[a]	1.9		75.4	C32–C16–C15–H15β	Nd[a]	3.8		135.5
C16–C15–C14–H14α	Nd[a]	Nd[b]	4.1[d]	128.3	C32–C16–C17–H17	3.8	Nd[b]		−67.6
C16–C15–C14–H14β	Nd[a]	Nd[b]	1.2[d]	−102.3	C34–C18–C17–H17	6.6	6.4		−164.3
C16–O–C13–H13	2.4	2.6	0.7[c]	75.1	C34–C18–C19–H19α	Nd[a]	Nd[a]	8.1[d]	−161.5
C16–C17–C18–H18	Nd[b]	Nd[b]		−81.0	C34–C18–C19–H19β	3.9	4.3	5.4[d]	−28.6
C17–C16–C32–H32α	Nd[a]	Nd[a]		−165.6	C35–C22–C21–H21	0.7	1.1		54.6
C17–C16–C32–H32β	Nd[a]	3.7		−57.0	C35–C22–C23–H23α	Nd[b]	Nd[b]	1.9[d]	−61.1
C17–C16–C15–H15α	Nd[c]	Nd[a]		−112.6	C35–C22–C23–H23β	Nd[b]	Nd[b]	2.4[d]	55.5
C17–C16–C15–H15β	6.3	4.3		4.5	C36–C24–C23–H23α	Nd[b]	Nd[b]	2.0[d]	−60.0
C17–O–C20–H20	<1.0	2.2	2.4[c]	122.0	C36–C24–C23–H23β	Nd[b]	Nd[b]	2.0[d]	59.9

[a] Not determined because of too small *s*/*n* values including too small $^{3}J_{C-H}$ values.

[b] Not determined because of overlapping of the cross peaks.

[c] Calculated with Karplus equations $^{3}J_{C-H} = 5.7\cos^{2}\varphi - 0.6\cos\varphi + 0.5$ where φ are the corresponding dihedral angles.

[d] Calculated with Karplus equations $^{3}J_{C-H} = 3.6\cos 2\varphi - 1.0\cos\varphi + 4.3$ where φ are the corresponding dihedral angles.

Table 3. Determined H–H coupling constants of monensin sodium (Hz)

	In $CDCl_3$	In CD_3OD
J 2–3	10.2	10.1
J 3–4	2.4	2.3
J 4–5	11.0	11.5
J 5–6	2.6	2.3
J 6–7	3.9	2.3
J 6–8[a]	3.4	
J 7–8α	4.4	4.3
J 7–8β	3.4	4.1
J 13–14α	5.0	4.9
J 13–14β	10.6	10.4
J 17–18	3.5	3.6
J 18–19α	0.0	0.0
J 18–19β	6.3	6.5
J 19α–20	7.0	7.2
J 19β–20	10.2	9.9
J 20–21	4.1	4.1
J 21–22	9.8	9.9

[a] W-coupling.

derivatives. Investigation of $^{3}J_{C-H}$ of dicarboxylic monensin derivatives[3] we prepared is now in progress.

2. Experimental

Monensin sodium recrystallized from *n*-hexane–Et_2O (40 mg) was dissolved in $CDCl_3$ or CD_3OD (0.55 ml) and packed in the NMR tube (ϕ 5 mm). The gs-J-HMBC experiments were carried out with JEOL JMN Lambda-500 spectrometer at 26°C. The spectra were recorded with a data size of 4K (F_2)×128 (F_1) points for the spectral width of 2000 Hz (^{1}H) and 22600 Hz (^{13}C) with a 200 ms constant time. Ten gs-J-HMBC spectra in $CDCl_3$ were acquired with 84 scans (ca. 8 h) and those in CD_3OD with 80 scans (ca. 7.5 h) per increment with varying the pulse interval time (Δ) every 20 ms within 20–200 ms. Two-fold zero-filling was conducted for F_1 dimension and the digital resolution in F_2 were 0.49 Hz. The signal amplitudes were determined

Table 4. Determined $^2J_{C-H}$ of monensin sodium in $CDCl_3$ and CD_3OD by gs-J-HMBC experiment

	$^2J_{C-H}$ (Hz)			$^2J_{C-H}$ (Hz)	
	In $CDCl_3$	In CD_3OD		In $CDCl_3$	In CD_3OD
C1–C2–H2	6.1	5.9	C16–C15–H15α or β	2.4	
C3–C2–H2	6.3	6.3	C16–C17–H17	4.6	4.7
C27–C2–H2	2.4	3.1	C18–C17–H17	1.2	1.9
C2–C3–H3		3.0	C34–C18–H18	2.3	
C4–C5–H5	2.6	2.9	C18–C19–H19α	2.1	
C7–C6–H6	6.7	6.7	C18–C19–H19β	3.3	
C30–C6–H6	4.6		C20–C19–H19α	5.8	
C6–C7–H7		<1.0	C20–C19–H19β	3.2	
C8–C7–H7	1.8		C21–C20–H20	3.8	4.2
C7–C8–H8α	5.2		C20–C21–H21	3.5	4.2
C7–C8–H8β	1.1	2.7	C22–C23–H23α or β	3.9	
C9–C8–H8α or β	2.2	3.6	C24–C23–H23α or β	3.8	
C9–C10–H10α or β	3.0	2.6	C25–C26–H26α or β	2.1	1.4
C12–C13–H13	2.3	2.6	C33–C32–H32α		4.3
C15–C14–H14α or β	1.2		C33–C32–H32β		4.0
C14–C15–H15α or β		3.5			

from peak heights recorded using the peak-picking program of the JEOL JMN Lambda-500 data processing software. The obtained data were fitted to a sine curve by the least-squares method. In order to assign the ^1H of monensin sodium more clearly, the ^1H NMR spectra were also recorded with JEOL Eclipse 800 spectrometer.

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