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STEREOCHEMICAL CHARACTERIZATION OF HYDRATED AND DEHYDRATED CRYSTALS OF N-ACETYLNEURAMINIC ACID AS REVEALED BY THE IR,CD, AND ^{13}C CROSS POLARIZATION-MAGIC ANGLE SPINNING NMR SPECTROSCOPY[1)]

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Fine needle (dehydrated) and prism (hydrated) crystals were obtained by changing condition of recrystallization of N-acetylneuraminic acid. The anomeric configuration of both was confirmed as the β-form. We found that local conformation of N-acetyl and glycerol moieties of the fine needles is significantly altered as compared with that of the prisms.

The anomeric configuration of N-acetylneuraminic acid (NANA) residue in oligosaccharides has been shown as the α-type.[2)] In contrast, the β-anomer of NANA is more stable in the solid state (hydrated prism crystal) as revealed by the X-ray diffraction[3)] and also in aqueous solution as demonstrated by the rapid mutarotation undergoing from the α-anomer to the β-anomer.[4,5)] Fine needle crystals (dehydrated)[6)] are usually obtained besides the above-mentioned prism crystals, although the amount of the former differs significantly depending on the condition of recrystallization. Obviously, major difference between the two crystals arises from the presence or absence of water molecules in the crystals. However, it can not be ruled out that the fine needles take an α-anomeric form, as postulated previously,[7)] by taking into account of the ratio of the β- to α-anomers (9:1) in aqueous solution.[4,5)] No stereochemical data have been obtained for the fine needles in spite of considerable importance as a starting material available from commercial source.

In this paper, we aimed to analyze the stereochemical feature of the fine needles by means of the IR, CD, and ^{13}C cross polarization-magic angle spinning (CP-MAS) NMR methods. As a new means of conformational analysis in the solid state, it is emphasized that the ^{13}C chemical shifts are sensitive to change of conformation as well as a manner of hydrogen bonding.[8-13)]

Recrystallization of NANA from acetic acid-water gave the fine needles. On the other hand, recrystallization of NANA from isopropyl alcohol-water gave the fine needles and prisms in a ratio of 1:1. Further, we found that recrystalliza-

Table 1. Physical Data of N-Acetylneuraminic Acid

	Molecular formula[a)]	Mp (Decomp) °C	$[\alpha]_D^{19}$ / ° (c 1, H_2O)	IR (ν/cm^{-1}, KBr)		
				COOH	Amide I	Amide II
Fine needles	$C_{11}H_{19}NO_8$	185-187	-33.8	1723	1652	1525
Prisms	$C_{11}H_{19}NO_8 \cdot 2H_2O$	146-148	-32.0	1756	1629	1592

a) Data of satisfactory elemental analysis were obtained.

tion from dioxane-water gave the fine needles and prisms in a ratio of 1:11. Those crystals gave identical ^1H and ^{13}C NMR spectra in aqueous solution. Physical data of both forms are summerized in Table 1.

Figure 1 shows the CD spectra of the fine needles in KBr as well as of NANA in aqueous solution.[14] Obviously, the CD spectrum of the fine needles in the solid state is very similar to that of methyl β-glycoside of NANA. Thus, both the fine needles and prisms have the same β-configuration at the C-2 position.

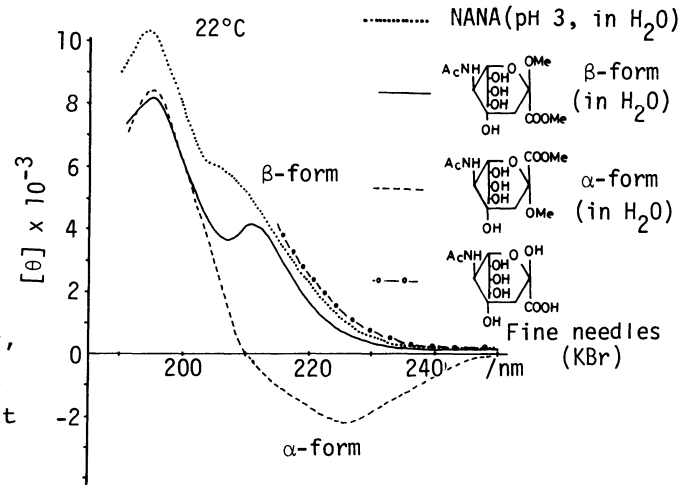

Fig. 1. CD curves of N-acetylneuraminic acid.

Figure 2 shows the ^{13}C CP-MAS NMR spectra of the prism (a), fine needles (b), and Na salt (c) (lyophilized from neutral aqueous solution), together with the high resolution NMR spectra in aqueous solution (see Table 2).[15] The assignment of peaks is straightforward in view of the data taken in aqueous solution.[16-19] It is interesting to note that the ^{13}C chemical shifts in aqueous solution are rather close to those of the hydrated crystal. Distinction of the C-1 and C-10 peaks was performed by comparing the spectra between the fine needles and Na salt in which the C-1 signal is selectively displaced downfield by 4.4 ppm with respect to that of the fine needles. The C-10 signal naturally remains unchanged.

Clearly, the ^{13}C chemical shifts of the C-11 methyl group of the fine needles and Na salt are displaced downfield by 3.7 and 3.3 ppm, respectively, as compared

Table 2. ^{13}C Chemical Shifts of N-Acetylneuraminic Acid in the Solid State and in Aqueous Solution (ppm from TMS; ± 0.4 ppm for the CP-MAS Data)

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11
Fine needles (A)	171.3	95.7	41.9	66.7	50.2	69.6	69.6	72.8	64.8	174.1	25.1
Prisms (B)	170.9	95.4	41.0	65.5	51.4	71.4	69.3	71.4	63.7	174.4	21.4
Na salts	175.7	95.2	41.7	66.1	49.8	69.1	69.1	72.2	63.7	173.1	24.7
Δ(A-B)	0.4	0.3	0.9	1.2	-1.2	-1.8	0.3	1.4	0.6	-0.3	3.7
Aqueous soln.											
pH 1.7	173.2	95.3	28.9	66.8	52.2	70.3	68.4	70.6	63.5	175.0	22.2
pH 7.7	175.0	96.6	39.0 ~39.5	67.5	52.6	70.5	68.9	70.7	63.6	176.6	22.4

with that of the prisms. These displacements are also significant as compared with the values in aqueous solution. In addition, there appears displacement of signals between the fine needles and prisms for the C-4, C-5, C-6 and C-8 by 1.2, -1.2, -1.8 and 1.4 ppm, respectively, which are larger than the experimental errors (0.8 ppm).

In the prism crystals, there exist eleven intermolecular hydrogen bonds involving the two bonds to two water molecules which play important role in fixation of N-acetyl and glycerol moieties.[3] In particular, NH and C=O groups act as proton donor and acceptor to a water molecule and hydroxyl groups of the C-20 and C-9, respectively.[3] The presence of this manner of hydrogen bonds is substantiated from the characteristic displacement of the IR absorption frequencies[20] (amide I and II, see Table 1) and also from the similarity of the ^{13}C chemical shifts of the C-11 methyl and C-10 carbonyl group with those of corresponding data (22.4 and 172.9 ppm for the methyl and carbonyl group, respectively) of chitin and N-acetyl chitosan[10] in which similar hydrogen bonds are formed.[21]

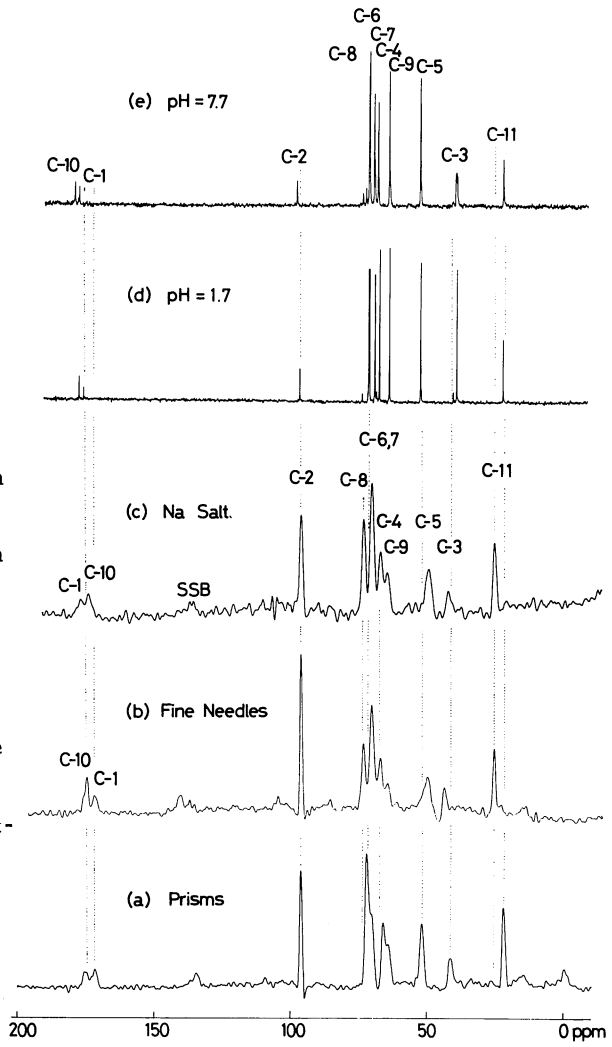

Fig. 2. 75.46 MHz ^{13}C CP-MAS NMR spectra of NANA in the solid state (a-c) together with high resolution ^{13}C NMR spectra in aqueous solution (d and e). Number of transients is 2000-3000. SSB stands for the spinning sidebands.

It is plausible that the local conformation of N-acetyl moiety of the dehydrated fine needles could be inevitably altered owing to the loss of the water molecule to which hydrogen bonds were formed. In fact, no significant hydrogen bonds as mentioned above are formed at the vicinity of N-acetyl moiety as seen from the positions of the amide I and II bands (Table 1). Major intermolecular hydrogen bonds are formed between the carboxyl groups as manifested from the infrared frequency of 1723 cm^{-1}.[20] A plausible alternative conformation of N-acetyl moiety is that the carbonyl group is weakly hydrogen bonded to the hydroxyl group of C-4, because the C-4 and C-5 ^{13}C chemical shifts are significantly changed (Fig. 3). This kind of intramolecular hydrogen bond, however, should be strongly deformed because of steric hindrance. Thus, the significant downfield shifts of the C-11 chemical shifts in the fine needles and Na salt should be caused by this change of the manner of hydrogen bonds at N-acetyl moiety (absence of a proton acceptor at NH group and weakened intramolecular OH···O=C hydrogen bond).

There exists another water molecule hydrogen-bonded to glycerol moiety in the prism crystal.[3] Therefore, further conformational change should be induced in this moiety. This view is supported by observation of small but distinguishable change of the C-6 and C-8 ^{13}C chemical shifts which might be sensitive to the change of the rotation about the C-6 - C-7 and C-7 - C-8 bonds, respectively, and/or the manner of hydrogen bondings.

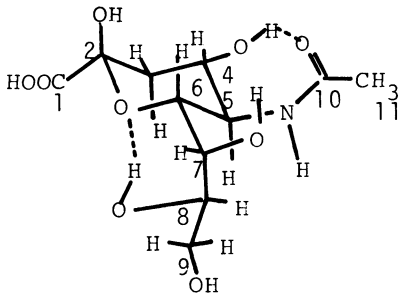

Fig. 3. Conformational model for the fine needles.

In conclusion, we have shown that conformation of N-acetyl and glycerol moieties in the fine needles is considerably altered owing to the loss of water molecules which maintain conformation of these moieties. A study of dehydrated crystals by means of the ^{13}C CP-MAS NMR method is proven to be very useful to gain insight of such situation.

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14) The [θ] values (±20%) were obtained from average of six disk samples (diameter, 1.2 cm; thickness, 0.4-0.5 mm; 3-4 mg/0.20-0.25 g KBr; density of KBr, 2.75). It was difficult to measure the CD spectrum of the prisms in KBr because of strong light scattering.
15) ^{13}C CP-MAS NMR spectra were recorded on a Bruker CXP-300 spectrometer at 75.46 MHz. Contact time and repetition time were 1 ms and 2 s, respectively. Chemical shifts are from TMS.
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19) As listed in Table 2, distinction of the anomeric configuration could be performed from the displacements of the C-2 (0.8 ppm), C-6 (1.3 ppm) and C-8 (2.2 ppm). However, the C-6 and C-8 signals are also displaced, in the solid state, by a plausible conformational change described in the text.
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