Communications to the Editor

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Inclusion Compounds of Cyclodextrin and Azo Dyes.[1] Formation of a Liquid Crystal

An aqueous solution of orange II and γ-cyclodextrin forms a lyotropic mesophase at room temperature. Nuclear magnetic resonance and circular dichroism suggest that the mesophase is formed from the inclusion compound which has a 1:1 structure and that orange II enters γ-cyclodextrin mainly from the long axis side of the molecule. Moreover this side aligns perpendicular to the magnetic field.

Keywords——γ-cyclodextrin; orange II; liquid crystal; NMR; CD; photomicrograph with crossed polarizers; alignment in the magnetic field

In this letter, we report the finding of the lyotropic mesophase formed from the inclusion compound. When aqueous solutions of γ-cyclodextrin (cdx) and orange II (Chart 1) were mixed in the 5 mm NMR tubes, they caused the enhancement of the viscosity, and the bright substance like the apparently millet jelly grew diagonally.[2] On standing a few days, this substance was separated from a clear layer and gathered at a lower layer. It showed the thread texture and did not flow easily when the NMR tubes were tilted.

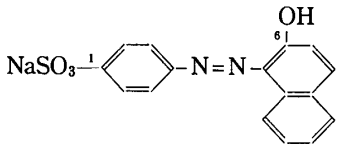

Chart 1

To interpret this phenomenon, the resultant material was examined by the optical microscopy.[3] The microscopic textures observed between crossed polarizars varied with time and somewhat with the molar ratio of γ-cdx to orange II. The sample was chosen of which the molar ratio of γ-cdx to orange II is 3/2 and on standing a few days after mixing. It showed the resemblance to the concentrated solution of a soap and had the stripes of light and darkness (Fig. 1a). On standing one day, the mesophase gathers at the edge of the cover glass and the texture like the interwinded worsted appears (Fig. 1b). The whole view becomes dark at about 60° and on cooling, the crystals like the comfits appear here and there in the texture which can be distinctly differentiated from the texture.

The oriented texture was observed in the magnetic field. When the mesophase in the solution which grew diagonally in the 5 mm NMR tubes was placed in the magnetic field, the texture was oriented apparently parallel to the magnetic field. So after standing the 5 mm tubes in the magnetic field, the integration values in ^1H nuclear magnetic resonance (NMR)

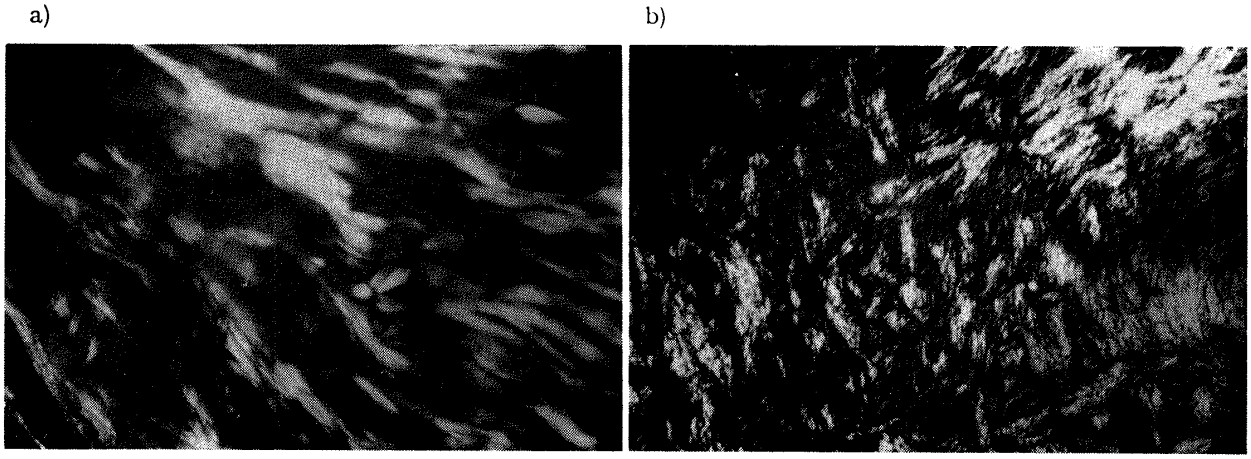

Fig. 1. Microscopic Textures exhibited between crossed Nicols at 60x Magnification

a) immediately after preparation. b) after 1 day.

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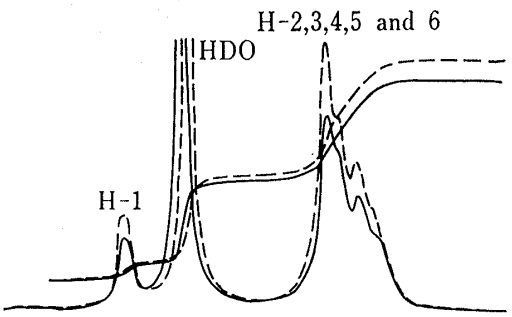

Fig. 2a. Alignment by a Magnetic Field (21138 gauss)

------: after incorporated in a magnetic field for 1 hr,
——: immediately after incorporated in a magnetic field.

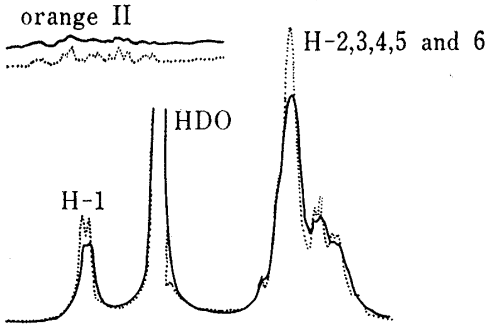

Fig. 2b. Spinning Effect

------: with spinning,
——: without spinning.

spectra of γ-cdx were measured (Fig. 2a); they showed *ca.* 15% enhancement after an hour in the region of the H-2,3,4,5 and 6 signals. The fact indicates that the axis of the cavity is preferentially aligned perpendicular to the direction of the magnetic field.[4] Moreover, about 5% decrease in the half line width of the H-1 signal was observed by spinning the tube (Fig. 2b). The fact that the sample can be spun in the magnetic field without destroying the field-induced mesophase orientation, lead us to conclude that the phase alignment is one in which the long axis of the substrate is perpendicular to the field.[5]

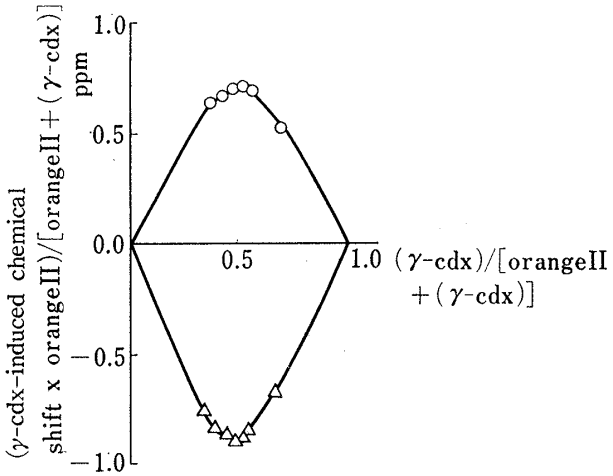

Fig. 3. Continuous Variation Plots of the γ-Cyclodextrin-induced Chemical Shifts of Orange II (66°)

C-1, ○—○; C-6, △—△.

Next, how orange II and γ-cdx in this mesophase interact was examined. As shown in Fig. 2, the ring current of azo dye molecule has obviously an effect on the proton signals of γ-cdx.[6] Namely, it seems certain that γ-cdx and orange II form a inclusion compound.

In ^{13}C NMR,[1,6] both molecules form a 1 : 1 complex as determined from continuous variation plots for the chemical shifts of the signals of C-1 and C-6 of orange II which show the largest shifts to the low and high fields, respectively, when γ-cdx is added.[7]

CD spectrum[8] also supports the formation of the inclusion compound. It shows the peak at 272 nm ($\Delta\varepsilon$ +1.5), 318 nm (+1.0), 466 nm (N-π^* transition of azo group, +3.2), 528 nm (−1.8). Methyl orange–α-cdx complex has also a large positive maximum at 421 nm (the N-π^* transition). It is obvious from the X-ray data that methyl orange in this complex is fixed in the cavity.[6] Thus azo dyes having a positive maximum in the N–π^* transition may be included mainly from the long molecular axis side. In the case of orange II–γ-cdx complex, such inclusion form may also be considered.

The following points are noted. The inclusion compound which has a 1 : 1 structure forms a liquid crystal. In the inclusion compound, orange II enters γ-cdx mainly from the long axis side of the molecule and this side aligns perpendicular to the magnetic field.

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References and Notes

1) Part IV (a); part III, M. Suzuki and Y. Sasaki, *Chem. Pharm. Bull.*, **27**, 1797 (1979).
2) Job plots was achieved so that the sum of the concentrations comes to 0.07 M. When molar ratios of γ-cdx to orange II are from 0.5 to 1.5, the above phenomenon appears.
3) The microscopic texture was viewed using the micro melting measurement apparatus with the polaroid plate ($\times$60).
4) A. Johansson and T. Drakenberg, *Mol. Cryst. Liq. Cryst.*, **14**, 23 (1971).
5) P.J. Black, K.D. Lawson, and T.J. Frautt, *Mol. Cryst. Liq. Cryst.*, **7**, 201 (1969).
6) M. Suzuki and Y. Sasaki, *Chem. Pharm. Bull.*, **27**, 609 (1979).
7) In preparation.
8) M. Suzuki and Y. Sasaki, *Chem. Pharm. Bull.*, **27**, 1343 (1979).

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Chemical Modification of Lactose. XVI.[1] Synthesis of Lacto-N-neohexaose

Reaction of 1,6-anhydro-2,2′,3,4′-tetra-O-benzyl-β-lactose (1 mol eq.) with the acetylated oxazoline of N-acetyllactosamine (5 mol eq.) gave the corresponding 1,6-anhydro-β-tetrasaccharide (**3**, 24.5%) and hexasaccharides (**8**, 53.5%). The protecting groups of **3** and **8** were removed by the following series of reactions to provide 6′-N-acetyllactosaminyllactose (**7**) and lacto-N-neohexaose (**12**), respectively: debenzylation followed by acetylation, acetolysis, and de-O-acetylation. ^{13}C-NMR spectral data for 1,6-anhydro-β-derivatives of **7** and **12** are presented.

Keywords——synthesis; human milk oligosaccharide; lacto-N-neohexaose; oxazoline glycosidation method; 6′-N-acetyllactosaminyllactose; 1,6-anhydro-β-tetrasaccharide; 1,6-anhydro-β-hexasaccharide; ^{13}C-NMR

The occurrence and the structure of lacto-N-neohexaose (**12**) in human milk were reported by Kobata and Ginsburg,[2] and the existence of more complex oligosaccharides having **12** as a partial structure has been described.[3] We now report a synthesis of **12** together with 6′-N-acetyllactosaminyllactose (**7**) as a by-product.

A mixture of 1,6-anhydro-2,2′,3,4′-tetra-O-benzyl-β-lactose (**1**)[4] (1 mol eq.) and the acetylated oxazoline of N-acetyllactosamine (**2**)[5] (3 mol eq.) in dry 1,2-dichloroethane containing 0.01 M anhyd. p-toluenesulfonic acid was stirred at 60—65° for 48 hr under nitrogen. After 48 hr, more **2** (2 mol eq.) was added and stirring was continued for further 24 hr. The mixture was neutralized and concentrated to dryness: TLC showed two spots. By column chromatography on Kieselgel 60 (Merck, 70—230 mesh) with $CHCl_3$–ether–MeOH (7: 7: 1, v/v), the products were separated into tetra- and hexasaccharide fractions. The former was re-chromatographed with $CHCl_3$–acetone (3: 1) to isolate the protected tetrasaccharide (**3**, 24.5%) as amorphous powder, $[\alpha]_D^{21}$ −10.8° ($CHCl_3$). ^{1}H-NMR ($CDCl_3$): 1.84, 1.98, 2.01, 2.06, 2.16 (21H, all s, OAc$\times$6, NAc), 5.51 (1H, s, H-1, β-Glc), 5.65 (1H, d, exchangeable with D_2O, $J_{NH,2'}$=8.5 Hz, NH), 7.20—7.44 (20H, m, aromatic protons). Hydrogenolytic debenzyl-

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