2-Amino-2-deoxy-1,2-O,N-carbonyl-3,4,6-tri-O-acetyl- D-glucopyrane (II)——The compound (I) (500 mg.) was dissolved in trifluoroacetic acid (4 ml.) and the solution was refluxed for 15 min. at 70°. After the reaction, it was evaporated *in vacuo*, the resulting syrup was dissolved in $CHCl_3$, washed with cold $NaHCO_3$ aqueous solution and then cold H_2O successively. The $CHCl_3$ solution was dried and evaporated to syrup, which was crystallized gradually as needles, yielding 200 mg. (58%). It was recrystallized from Me_2CO-Et_2O, melted at 170°, $[\alpha]_D^{22}$ +33° (c=2, $CHCl_3$). *Anal.* Calcd. for $C_{13}H_{17}NO_9$: C, 47.11; H, 5.18; N, 4.23. Found: C, 47.23; H, 4.84; N, 4.24.

2-Amino-2-deoxy-1,2-O,N-carbonyl-3,4,6-tri-O-acetyl-D-glucopyrane was prepared from the compound (I) by the treatment with titanium tetrachloride[5] in $CHCl_3$, giving m.p. 170°,*3 $[\alpha]_D^{22}$ +33°*3 (c=1.5, $CHCl_3$). No melting point depression was observed on admixture of the above two crystals and their IR spectra[8] were identical.

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Summary

On treating with trifluoroacetic acid, 2-benzyloxycarbonylamino-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose afforded 2-amino-2-deoxy-1,2-O,N-carbonyl-3,4,6-tri-O-acetyl-D-glucopyrane.

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*3 m.p. 174~175°, $[\alpha]_D$+50.3°, were recorded by S. Konstas, I. Photaki and L. Zervas.

8) The infrared spectrum of the compound (II) showed the bands due to oxazolidone ring. cf. R. Mecke Jr., R. Mecke sen: Chem. Ber., **89**, 343 (1956).

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Masamichi Tsuboi, Shigesada Higuchi, Yoshimasa Kyogoku, Kimiko Matsuo,*1 and Akiyoshi Wada*2: Actinomycin Bound to Deoxyribonucleic Acid in Solution.

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Biological activity of actinomycin D is now correlated with its ability to bind deoxyribonucleic acid (DNA),[1~3] probably by complexing specifically to guanine residue.[4,5] The purpose of this note is to present a piece of information on the actinomycin D-DNA complex in solution, on the basis of the results of our recent two preliminary experiments.

First, the melting temperature (T_m) of DNA has been examined according to the method of Doty, Marmur and Sueoka,[6] in solutions with and without actinomycin D.

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As may be seen in Fig. 1, T_m is markedly elevated by the addition of actinomycin D. The amount of elevation (ΔT_m) is as great as 15° under the condition in which Goldberg, *et al.*[5] examined the metachromasis of actinomycin D on binding to DNA (see Fig. 1, A and B). ΔT_m is slightly smaller under the condition similar to that in which Mandel[7] examined the melting temperatures of spermine-DNA complexes, but it is still several times as great as that in the case of spermine. Thus, it has been shown that actinomycin D has an ability to stabilize the double-helix conformation of DNA, and that it is much greater than the similar ability of spermine.

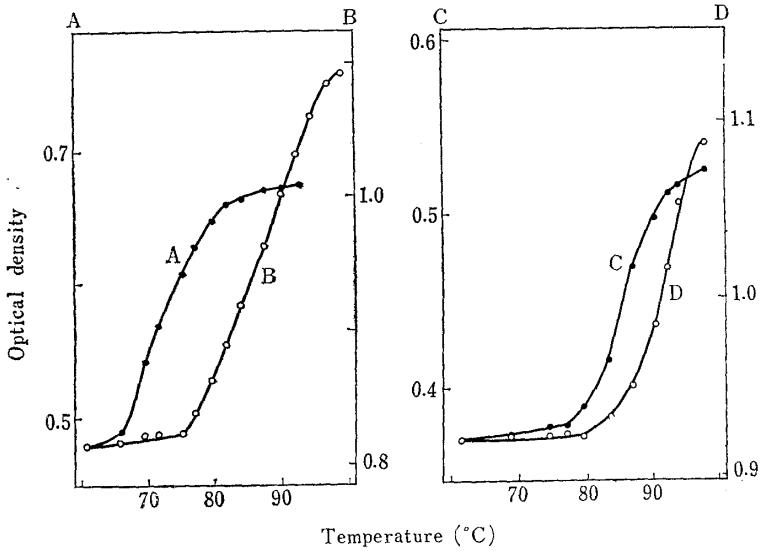

Fig. 1. Optical Density of Calf-thymus DNA Solution at 260 mμ plotted against Temperature

A : DNA concentration=21.8 μg./ml., in 0.01*M* Tris-0.01*M* NaCl solution at pH 7.4.
B : The same solution as described in A plus 31.8 μg./ml. actinomycin D.
C : DNA concentration=25 μg./ml., in 0.15*M* NaCl- 0.015*M* sodium citrate solution at pH 6.9.
D : The same solution as described in C plus 37.5 μg./ml. actinomycin D.
optical path length=10mm.

Secondly, flow dichroism of an actinomycin D-DNA solution has been observed. The apparatus and the method of our flow-dichroism observation were previously detailed by one of the authors.[8] The result of the present observation is given in Fig. 2. As may be seen in the figure, the solution gives absorption bands in the 260 mμ region and in the 445 mμ region (see the curve along solid circles in Fig. 2.). In both of these two regions, the solution shows higher absorption intensity for the radiation polarized perpendicular to the direction of the flow ($D_\perp$) than that for the radiation polarized parallel to it ($D_{/\!/}$). The absorption in 260 mμ region is primarily due to the base residues of DNA. The perpendicular flow-dichroism ($D_\perp - D_{/\!/} = 0.063$, $(D_\perp - D_{/\!/})/D = 0.14$) observed here indicates that the DNA molecule tends to orient preferably with the base plane perpendicular to the direction of the flow. Similar perpendicular flow-dichroism was previously observed by one of us[9] for DNA solutions without actinomycin D, and it was interpreted on the basis of the Watson-Crick type double-helical conformation of the DNA molecule. Therefore, the present observation is understandable by considering that such a double-helical conformation of the DNA molecule is preserved in the acti-

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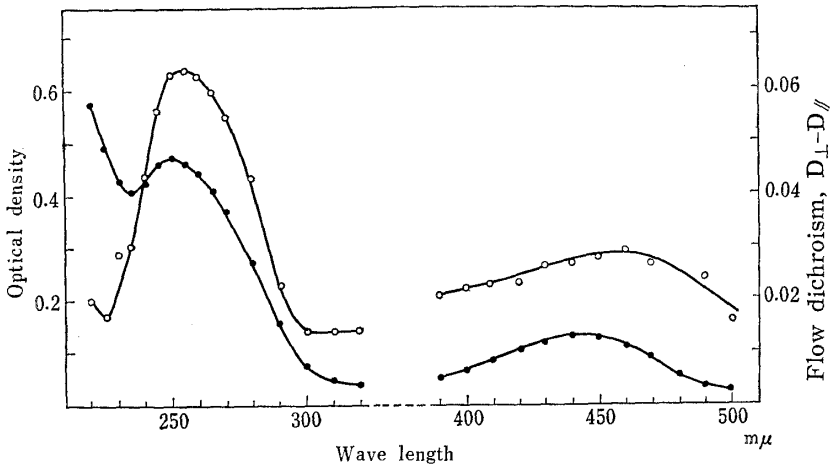

Fig. 2. Optical Density (Solid circles, D) and Flow Dichroism (Open circles, $D_{\perp}-D_{//}$) of a mixed Solution of Calf-thymus DNA (107 μg./ml.) and Actinomycin D (100 μg./ml.), in 0.01*M* Tris-0.01*M* NaCl solution at pH 7.4

optical path length=1.4 mm. velocity gradient of the flow=2190 sec^{-1}.

nomycin D-DNA complex. The 445 mμ absorption is entirely due to the actinomycin D chromophore. The perpendicular flow-dichroism ($D_{\perp}-D_{//}=0.028$, $(D_{\perp}-D_{//})/D=0.22$) observed here indicates that, in the complex in question, actinomycin D chromophore is placed so that its transition moment corresponding to the 445 mμ absorption is oriented almost perpendicular to the direction of the flow.

On the basis of our flow-dichroism observation, a model of actinomycin D-DNA complex, in which the actinomycin D molecule is placed with its chromophore plane almost parallel to the plane of the base-pairs of DNA, is suggested. Intercalation of actinomycin D chromophore between successive base-pairs, however, would cause a lowering of T_m of DNA in contradiction to the result of our observation. Thus, we have reached a conclusion that, in the model, actinomycin D chromophore would be placed aside the base-pairs (specifically guanine-cytosine base-pairs) with its plane almost parallel to the plane of the base-pairs. This conclusion is in accord with the model of actinomycin C_1 (D) binding to DNA proposed by Hamilton *et al.*[10] on the basis of their X-ray diffraction and molecular model building studies. It may now be suggested that the proposed model would be valid not only for the actinomycin D-DNA complex in the solid state but also for that in solutions.

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