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Kinetic and NMR Spectroscopic Studies of the Fusion Reactions of Chloropurines with 1,2,3,5-Tetra-*O*-acetyl- β -D-ribofuranoses

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A kinetic study of the fusion reaction and the reaction in dimethyl sulfoxide of 2,6-dichloropurine with 1,2,3,5-tetra-*O*-acetyl- β -D-ribofuranose was undertaken, and both of them were proved to be of the second order. The activation energy and the activation entropy of the reaction in dimethyl sulfoxide were obtained: $E_a = 38.0$ kcal/mol and $\Delta S^\ddagger = +15.3$ cal/deg·mol. The reaction was thus concluded to be bimolecular involving a considerable unimolecular character. Subsequently, the fusion reactions of 2,6-dichloro- and 2,6,8-trichloropurine with 1,2,3,5-tetra-*O*-acetyl- α - and - β -D-ribofuranose were examined by NMR spectroscopy with respect to the relative content ratios of all the materials and products in each reaction mixture; the results were found to be in good agreement with those obtained by the above kinetic study. On the basis of these results, the mechanism of the fusion reaction was discussed.

In the previous paper,¹⁾ the mechanism of the acid-catalysis and the new activating agents for the reaction of a purine with a fully-acetylated sugar were examined; it was proved that a purine is activated by the interaction with an acidic catalyst to react with an acetylated sugar. Successively, a kinetic study of the reaction of 2,6-dichloropurine (**1**) with 1,2,3,5-tetra-*O*-acetyl- β -D-ribofuranose (**2**) by means of UV absorption spectroscopy, and a study of the process of the reaction of **1** with **2** and 1,2,3,5-tetra-*O*-acetyl- α -D-ribofuranose (**3**) respectively by NMR spectroscopy, were undertaken.

Results and Discussion

*A Kinetic Study of the Reaction of 2,6-Dichloropurine with 1,2,3,5-Tetra-*O*-acetyl- β -D-ribofuranose.* No kinetic study of the synthetic reaction of glycosides by the fusion method has ever been undertaken since the discovery of the fusion reaction for the synthesis of phenyl glycosides. The reasons may be that a remarkable coloration inevitably accompanies the reaction with phenols, although it proceeds under a completely homogeneous state, and that the reaction with purines usually proceeds under a heterogeneous state in addition to a considerable coloration. Fortunately, the reaction of **1** with **2** was found to proceed auto-

catalytically^{2a)} under a completely homogeneous state, and it is accompanied by a scarce coloration which may be accepted as showing that the reaction scarcely involves decomposition of the materials or of the resultant 2,6-dichloro-9-(2',3',5'-tri-*O*-acetyl- β -D-ribofuranosyl)-purine (**4**). The appearance of the reaction system did not vary much even in the presence of an acidic catalyst provided that the reaction time was not extended much longer.^{2b)} In view of this, kinetic studies of the fusion reaction and of the reaction in dimethyl sulfoxide of **1** with **2** were undertaken by means of UV-absorption spectroscopy. It was considered possible to follow the reaction by measuring the UV spectra of the reaction mixture at different reaction times, since the spectra of **1** and **4** were found to be completely different from each other in an aqueous ethanolic sodium hydroxide solution ($\lambda_{\max}$ 258 nm³⁾ and $\lambda_{\max}$ 280 nm respectively). These spectra were confirmed to be unchanged under the present conditions even after 24 hr. An equimolar mixture of **1** and **2**, which were mingled as completely as possible,

2) a) Y. Ishido, T. Matsuba, A. Hosono, K. Fujii, T. Sato, S. Isome, A. Maruyama, and Y. Kikuchi, *ibid.*, **40**, 1007 (1967); b) Y. Ishido, A. Hosono, K. Fujii, Y. Kikuchi, and T. Sato, *Nippon Kagaku Zasshi*, **87**, 752 (1966).

3) The chloro substituent at the 6-position of **4** is known to be susceptible to hydrolysis under alkaline conditions to give 2-chloro-inosine [A. Yamazaki, T. Saito, Y. Yamada, and I. Kumashiro, *Chem. Pharm. Bull.* (Tokyo), **17**, 2581 (1969); $\lambda_{\max}^{H_2O}$ 258 nm (ϵ 11400)].

1) M. Sekiya, T. Yoshino, H. Tanaka, and Y. Ishido, *This Bulletin*, **46**, 556 (1973).

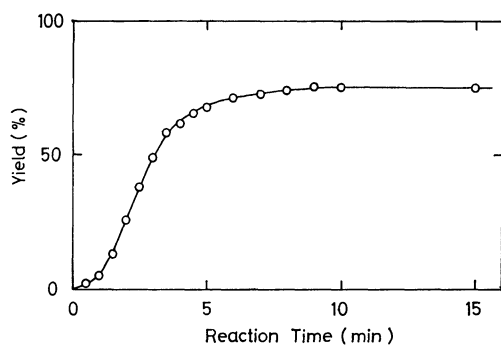


Fig. 1. A plot of the yields of the fusion reaction of **1** with 1,2,3,5-tetra-*O*-acetyl- β -D-ribofuranose (**2**).

in a capillary tube was heated at 152–154 °C for a scheduled reaction time in a micro melting-point apparatus. On the basis of the molar extinction coefficients of **1** ($\epsilon_{280\text{ nm}}$ 8670 and $\epsilon_{258\text{ nm}}$ 4150) and **4** ($\epsilon_{280\text{ nm}}$ 2440 and $\epsilon_{258\text{ nm}}$ 11800) in the same alkaline solvent, the yields of the reaction at each reaction time were calculated by the following simultaneous equations, where A , x , and y stand for the total molar extinctions observed at both of the wavelengths, molar concentration of **1**, and that of **4** after t hr respectively:

$$\begin{cases} A_{258\text{ nm}} = 4150 \cdot x + 11800 \cdot y \\ A_{280\text{ nm}} = 8670 \cdot x + 2440 \cdot y \end{cases}$$

A plot of the yields $[y/(x+y)]$ against the reaction times is shown in Fig. 1; it was thus proved that the equilibrium of the reaction was established at the yield of ca. 76%. The reaction can thus be stated to be reversible provided that the reaction is accompanied by no decomposition reaction as has been mentioned above. The reversibility of this reaction was confirmed by a tlc examination and by a UV spectral determination with respect to a mixture which had been obtained by fusing **4** with glacial acetic acid at 150 °C for 15 min. The content ratio of **1** and **4** in this mixture was determined to be 18:82 on the basis of the total molar extinctions at 258 and 280 nm in the alkaline solvent. Supposing that a stand for the initial concentration of **1**, and that the reaction is of the second order with respect to both reactants and products, the reaction was assumed to conform to the following second-order equation, where k_1 and k_2 stand for the rate constants of the reactants and products respectively:

$$dy/dt = k_1 \cdot (a-y)^2 - k_2 \cdot y^2 \quad (1)$$

The correlation between k_1 and k_2 can be concluded from the concentrations of **1** and **4** at the equilibrium of this reaction as below:

$$0.24^2 \cdot k_1 = 0.76^2 \cdot k_2 \quad (2)$$

Supposing the equilibrium constants as K , moreover, Eq. (3) can be obtained:

$$k_2 = \frac{1}{K} \cdot k_1 \quad (3)$$

When Eq. (3) was substituted into Eq. (1), the resultant equation was integrated to give Eq. (4):

$$t = \frac{2.303 \cdot \sqrt{K}}{2a \cdot k_1} \log \left| \frac{K + \sqrt{K} - (K-1) \cdot y/a}{K - \sqrt{K} - (K-1) \cdot y/a} \right| + \text{Const.} \quad (4)$$

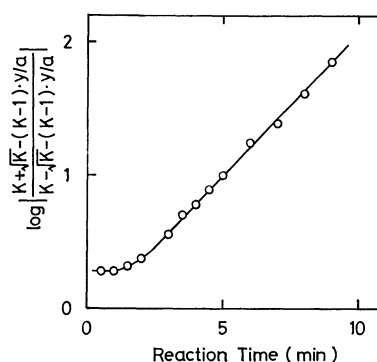


Fig. 2. A plot of $\log \left| \frac{K + \sqrt{K} - (K-1) \cdot y/a}{K - \sqrt{K} - (K-1) \cdot y/a} \right|$ vs. reaction time determined in the fusion reaction of **1** with **2**.

Moreover, y/a corresponds to the yields of the reaction, and K is $76^2/24^2$, which was obtained from Eq. (2). Thus, we can obtain the values of $\log \left| \frac{K + \sqrt{K} - (K-1) \cdot y/a}{K - \sqrt{K} - (K-1) \cdot y/a} \right| = \log \left| \frac{475 - 325 \cdot y/a}{247 - 325 \cdot y/a} \right|$ by measuring the yields at each reaction time. A plot of these values against the reaction time is given in Fig. 2. The deviation of the plots of the initial stage of the reaction from the linearity may be ascribed to the heterogeneity of the reaction mixture because it takes about 2.5 min until the mixture becomes almost homogeneous. According to the figure, it can be concluded that the fusion reaction of **1** with **2** is of the second order with respect to both reactants and products, and that the linear relationship implies that the reaction is reversible and that there is no catalytic effect of the acetic acid co-produced during the reaction.

Subsequently, the rate of the equimolar reaction in dimethyl sulfoxide was measured in the same way, since it was impossible to calculate the precise concentrations of **1**, **2**, and **4** in the fusion state; we thus obtained the activation energy and the activation entropy for the reaction. Supposing that the reaction in dimethyl sulfoxide also conforms to the second-order equation, the following relation can be expected to hold for the reaction:

$$k \cdot t = y/[a \cdot (a-y)] = \frac{y}{x+y} \cdot \frac{1}{a\{1 - [y/(x+y)]\}}$$

The values of $y/[a \cdot (a-y)]$ can be determined from the data of UV spectroscopy, which were obtained in the same way as has previously been described; these values were then plotted against the reaction times to give the two linear relationships, which were obtained at 130 and 135 °C as demonstrated in Fig. 3. The rate constants of $k_{130^\circ\text{C}}$ and $k_{135^\circ\text{C}}$ were calculated as 1.46×10^{-4} l/mol·sec and 2.50×10^{-4} l/mol·sec respectively from the slopes of these linear rate plots. According to the following equations, the activation energy (E_a) and the activation entropy (ΔS^*) of this reaction were calculated as 38.0 kcal/mol and +15.3 cal/deg·mol respectively, where K , h , R , and ΔH^* stand for the Boltzmann constant, the Planck constant, the gas constant, and the activation enthalpy respectively:

$$E_a = \frac{T_1 \cdot T_2}{T_1 - T_2} \cdot R \cdot \ln \frac{k_1}{k_2}$$

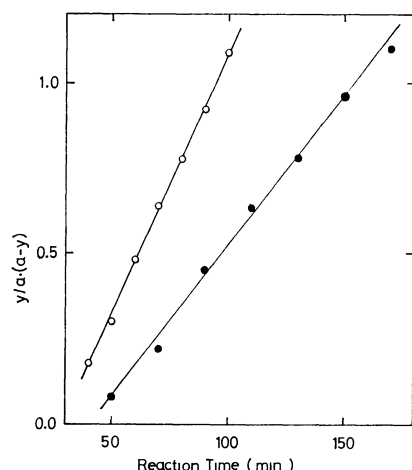


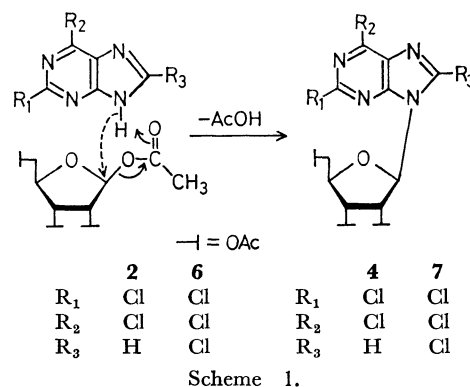
Fig. 3. A plot of $y/a(a-y)$ vs. reaction time in the reaction of **1** with **2** in DMSO at 130 °C (—●—) and at 135 °C (—○—).

$$E_a = \Delta H^\ddagger + R \cdot T$$

$$k = \frac{K \cdot T}{h} \cdot e^{\Delta S^\ddagger/R} \cdot e^{-\Delta H^\ddagger/R \cdot T}$$

On the basis of these results, the reaction of **1** with **2** in dimethyl sulfoxide was concluded to be second order, involving a considerable first-order character; *i.e.*, the reaction conceivably proceeds through a bimolecular mechanism involving a considerable unimolecular character. In this case, no catalytic effect of the co-produced acetic acid was also observed. Thus, this fact provides a new concept with respect to the acid-catalysis in the condensation reaction of purines with fully-acetylated sugars, although it has been generally accepted that the reaction may be induced by the interaction of the acidic catalyst with fully-acetylated sugars to give the corresponding C-1 carbonium ion—*i.e.*, that the rate-determining step of the reaction may be the step of the carbonium-ion formation.⁴⁾ In the autocatalytic reaction of **1** with **2**, the acidic proton of **1** must be concluded to induce the reaction in view of the lack of any catalytic effect of acetic acid. Consequently, it was deduced that the rate-determining step of the reaction must not be the step of the C-1 carbonium-ion formation; this was deduced from the facts that the reaction is of a second order and that the more weakly acidic compounds,

4) a) The fusion reaction was suggested to proceed *via* a $C_{(1)}-C_{(2)}$ orthoester-type carbonium ion on the basis of requirement of 2-*O*-acyl groups in all sugar derivatives thus far examined [T. Shimadate, *Nippon Kagaku Zasshi*, **83**, 214 (1962)]; b) The reaction was, on the other hand, considered to proceed *via* the C-1 carbonium ion, at least in the case of fully acylated D-ribofuranoses, because of the steric effect of the 3-*O*-acyl group, which may prevent the formation of such a carbonium ion, as has been described by Shimadate^{4a)} [2b]; The mechanism involving the orthoester-type carbonium ion, which had been proposed by R. U. Lemieux and W. P. Shyluk [*Can. J. Chem.*, **31**, 528 (1953)] in the acid-catalyzed condensation reaction of phenol with 1,2,3,4,6-penta-*O*-acetyl- β -D-glucopyranose, has been reinvestigated by J. I. Bose and T. R. Ingle [*Chem. Ind.*, **1967**, 1451]; they concluded that the reaction proceeded *via* the C-1 carbonium ion on the basis of the simultaneous formation of phenyl 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranoside and its β -anomer.



such as 6-chloropurine ($pK_a=7.68^5$) and 6-cyanopurine ($pK_a=6.88^6$) *etc.*, in addition to **1** and in comparison with acetic acid, also gave the corresponding nucleosides in the reaction with **2** in the absence of such acidic catalyst as *p*-toluenesulfonic acid.^{2a)} Therefore, it is now necessary to postulate another reaction mechanism. On the basis of the above results, the autocatalytic reaction was postulated to proceed *via* the mechanism depicted in Scheme 1, which implies that the rate-determining step of the reaction is the step of the elimination of acetic acid from purines and fully-acetylated sugars. Thus, usual purines may be activated by interaction with acidic catalysts to react with fully-acetylated sugars, and the corresponding nucleosides may be produced accompanied by an electron transfer such as is depicted in the Scheme. The mechanism of the acid-catalysis in the fusion reaction has recently been verified by an investigation of the reaction of 1,2,3,4,6-penta-*O*-acetyl- β -D-glucopyranose with theophylline in competition with *p*-nitrophenol and in the presence of *p*-toluenesulfonic acid;¹⁾ this reaction resulted in the selective formation of 7-(2',3',4',6'-tetra-*O*-acetyl- β -D-glucopyranosyl)-theophylline.

*An Examination on the Reaction of 2,6-Dichloropurine with 1,2,3,5-Tetra-*O*-acetyl- α - and - β -D-ribofuranose by NMR Spectroscopy.* In order to elucidate the steric course of the fusion reaction, the reactions of **1** with **2** and with

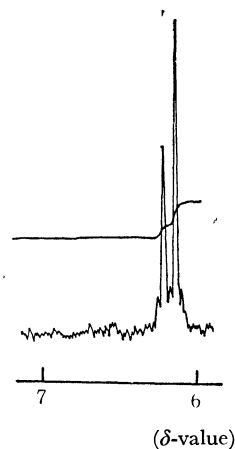


Fig. 4. The anomeric region (δ 6—7 ppm) of the NMR spectrum ($CDCl_3$, TMS) of the resultant mixture from the fusion reaction of **1** with **2** at 150 °C for 10 min *in vacuo*.

5) A. Bendich, P. J. Russel, and J. J. Fox, *J. Amer. Chem. Soc.*, **76**, 6073 (1954).

6) A. G. Sorolla, *ibid.*, **80**, 3732 (1958).

3 were examined by NMR spectroscopy. A crude mixture obtained from the fusion reaction of **1** with **2** at 150 °C for 10 min gave an NMR spectrum with the anomeric region shown in Fig. 4. The reaction was thus concluded to give **4** ($H-1'$: δ 6.24 ppm, $J_{1',2'} = 4.7$ Hz) selectively; *i.e.*, it gave a nucleoside with the same anomeric configuration as that of the fully-acetylated sugars used as the starting material. A crude mixture obtained from the same reaction after 60 min gave substantially the same spectrum, as is shown in Fig. 4. No other new anomeric proton signals could be detected, not even by an enhancement of the ratio of signal to noise. Accordingly, it can further be stated that the autocatalytic fusion reaction is not accompanied by any anomerization reaction of the nucleoside once produced in the course of the reaction.

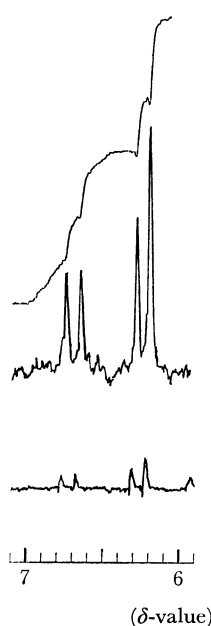


Fig. 5. The anomeric region (δ 6—7 ppm) of the NMR spectrum ($CDCl_3$, TMS) of the resultant mixture from the fusion reaction of **1** with 1,2,3,5-tetra-*O*-acetyl- α -D-ribofuranose (**3**) at 150 °C for 10 min *in vacuo*.

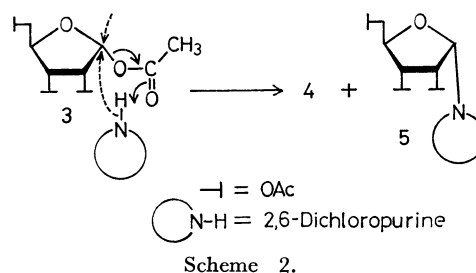
On the other hand, a crude mixture obtained from the reaction of **1** with **3** at 150 °C for 10 min gave a spectrum with the anomeric region shown in Fig. 5; this is in remarkable contrast with that of the reaction with **2**. A new doublet (δ 6.75 ppm, $J = 5.6$ Hz) appeared other than that of **4** in the spectrum. The product, which gave the new doublet, was deduced to be the α -anomer of **4** (**5**) in view of the NMR data reported with respect to the α -D-ribofuranose derivatives.⁷⁾ The relative ratio of **4** to **5** in the mixture was

7) J. D. Stevens and H. G. Fletcher, Jr., *J. Org. Chem.*, **33**, 1709 (1968); where the anomeric proton signals of α - and β -ribazole tri-*O*-acylates were reported to be observed in their NMR spectra ($CDCl_3$, TMS) as follows:

Ribazole	Chemical shift of anomeric protons in δ value ($J_{1',2'}$, Hz)	
	Acetate	Benzoate
α -anomer	6.47 (5.0)	6.70 (5.0)
β -anomer	6.08 (5.0)	6.11 (5.0)

calculated to be 59/41 from the integration curve of its spectrum.

The difference in the content ratio of the anomeric nucleosides between the above reaction mixtures were of interest in connection with the reaction mechanism, since no anomerization reaction of the nucleosides is conceivable in the autocatalytic fusion reaction. Incidentally, the fusion reactions of phenol with 2,3,4,6-tetra-*O*-acetyl-1-*O*-trichloroacetyl- β -⁸⁾ and α -D-glucopyranose⁹⁾ have been shown to give the corresponding glycosides, with the same anomeric configuration as those of the starting material, in 70 and 20% yields respectively.¹⁰⁾ Thus, the reaction described here can be explained as proceeding through a bimolecular mechanism involving a considerable unimolecular character, *i.e.*, a considerably cationic character, assuming that the conclusion in the kinetic study of the reaction of **1** with **2** in dimethyl sulfoxide is the case for the fusion reaction as well. The results obtained in this section were proved to support the results of the kinetic study strongly. Accordingly, the reaction was deduced



to proceed through the mechanism depicted in Schemes 1 and 2. Just after the elimination of acetic acid between **1** and **2** or **3**, accompanied by such electron transfer as is shown in the arrows with ordinary lines, the carbonium ion thus produced may be subjected to the nucleophilic attack of the anion of **1** to afford **4** or **5**. In any case, the reaction conceivably proceeds with a considerably strong interaction between the acidic proton of **1** and the 1-*O*-acetyl group of **2** or **3**. The concomitant formation of **4** and **5** in the fusion reaction with **3** can be explained as being brought about by the steric effect of the 2-*O*-acetyl group of the D-ribofuranosyl ring on the attacking of the resultant anion of **1** on the short-lived C-1 carbonium ion.

Subsequently, the acid-catalyzed fusion reaction of **1** with **2** was examined by the same technique in order to elucidate the effect of the acidic catalyst on the relative ratio of the anomeric nucleosides in the resultant reaction mixture. A mixture of **1**, **2**, and sulfamic acid (molar ratio = 1 : 1 : 0.02) was heated at 150 °C *in vacuo* for a scheduled period in a small test tube. The resultant mixtures, obtained at the reaction times of 1, 2, 3, 4, 5, 7, and 10 min, were each dissolved in

8) B. Helferich and B. Gootz, *Ber.*, **62**, 2788 (1929).

9) I. Karasawa and R. Onishi, *Nippon Nogei Kagaku Zasshi*, **33**, 817 (1961).

10) It was elucidated, by an examination with NMR spectroscopy, that these reactions resulted in the predominant formation of the phenyl glycosides with the same anomeric configuration as that of the fully-acylated sugars used for starting materials. The results will be published elsewhere, together with those obtained in the same way with respect to other phenyl glycoside syntheses.

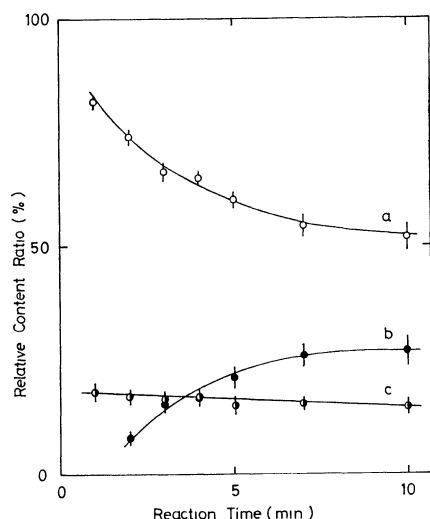


Fig. 6. The outline of the fusion reaction of **1** with **2** in the presence of sulfamic acid as a catalyst at 150 °C *in vacuo*; curves *a*, *b*, and *c* corresponds to the relative content ratio of **4**, the α -anomer of **4**(**5**), and **2** respectively.

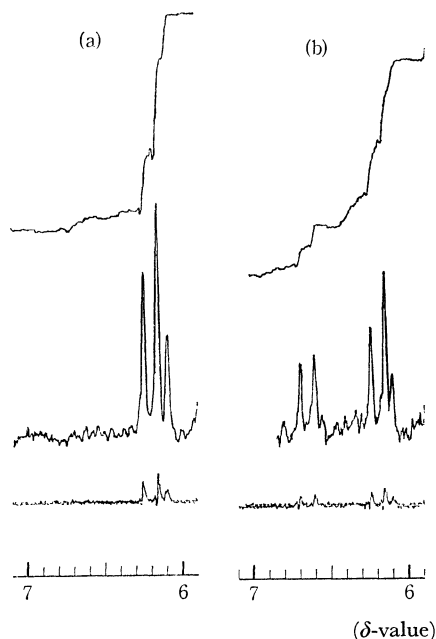


Fig. 7. The observed change in the anomer region (δ 6—7 ppm) of the NMR spectrum (CDCl_3 , TMS) in the acid-catalyzed fusion reaction of **1** with **2**; *a* and *b* corresponds respectively to the spectrum of the resultant mixture obtained after 1 and 10 min from the beginning of the reaction.

deuteriochloroform, and their NMR spectra were taken. The relative content ratios of **2**, **4**, and **5**, which were calculated from the integration curves corresponding to the anomer region of each NMR spectrum of the resultant mixtures, were plotted against the reaction times to give an outline of the reaction process such as is shown in Fig. 6. For reference, the spectra of the resultant mixtures obtained after 1 and 10 min are shown in Fig. 7. Judging from Fig. 6, **4** was predominantly produced in the initial stage of the reaction, and its content ratio decreased with the passage of time. Parallel with this decrease, the relative content ratio of

5 gradually increased, although a scarce anomerization of **2** into **3** was observed during the reaction. Incidentally, the anomerization of **4** into **5** was also detected by heating **4** with a catalytic amount of sulfamic acid under the same conditions; this gave the doublet at δ 6.75 ppm corresponding to the anomeric proton of **5**, in addition to that at δ 6.24 ppm corresponding to that of **4**. Consequently, the acid-catalyzed fusion reaction of **1** with **2** was deduced to proceed through this mechanism; *i.e.*, **1** might be activated by the interaction with an acidic catalyst, as was also confirmed recently in a different manner,¹⁾ and the β -nucleoside (**4**) formed by the reaction of activated **1** with **2** might be isomerized into its α -anomer (**5**) by the effect of the acidic catalyst.

An Examination of the Fusion Reaction of 2,6,8-Trichloropurine with 1,2,3,5-Tetra-O-acetyl-D-ribofuranoses by NMR Spectroscopy.

An autocatalytic fusion reaction of 2,6,8-trichloropurine (**6**) with **2** at 150 °C was found to give 2,6,8-trichloro-9-(2',3',5'-tri-O-acetyl- β -D-ribofuranosyl)-purine (**7**) selectively, much as in the reaction of **1** with **2**. The anomeric region of the NMR spectrum of the reaction mixture obtained by heating at the same temperature for 10 min is shown in Fig. 8;

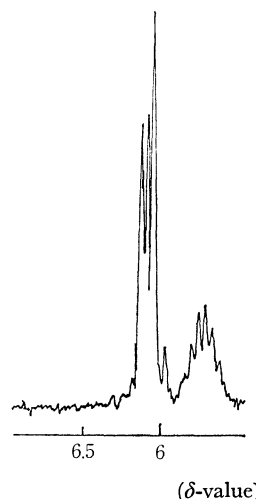


Fig. 8. The anomer region (δ 6—7 ppm) of the NMR spectrum (CDCl_3 , TMS) of the resultant mixture from the fusion reaction of 2,6,8-trichloropurine (**6**) with **2** at 150 °C for 10 min *in vacuo*.

the anomeric proton of **7** was observed, together with one of the other ring protons on its D-ribofuranosyl ring, at *ca.* δ 6.1 ppm. Accordingly, the steric course of this reaction can be concluded to be the same as that of the reaction of **1** with **2**. In contrast with the reaction of **1**, an autocatalytic reaction of **6** with **3** and the acid-catalyzed reactions of **6** with **2** and **3** were all found to give **7** predominantly. In the latter two cases, a new doublet (δ 6.60 ppm, $J=5.0$ Hz) was distinctly observed; this signal may arise from the formation of the α -anomer of **7** (**8**), in view of the data reported with respect to the α -D-ribofuranose derivatives.⁷⁾ On the basis of this assumption, the relative content ratios of **7** to **8** were calculated, from the integration curve of each NMR spectrum, as 79/21 and 81/19 respectively. In the reactions of **6**, the

relative content ratio of **8** to **7** was considerably lower than those observed in the reaction of **1** with **2** in the presence of the catalyst and in the autocatalytic and the acid-catalyzed reaction of **1** with **3**. These results can be ascribed to the steric effect of the chloro substituent at the 8-position of **6** in addition to that of the 2-*O*-acetyl group of **2** or **3**. That is, the nucleophilic attack of the anion of **6** from the α -side of the D-ribofuranosyl ring may be largely prevented by those steric effects. A bulky substituent at the 8-position of purine nucleus was also found to affect the relative content ratio of the position isomers of purine nucleosides in a condensation reaction.¹¹⁾

These facts, elucidated by the above kinetic and NMR spectroscopic investigation of the reaction of **1** or **6** with **2** or **3**, can be considered to hold for reactions involving other purine derivatives and acetylated sugars.

Experimental

The UV spectra were taken with a Hitachi EPS-3T photo-electronic spectrophotometer. The NMR spectra were taken with a JEOL JNM-C-60 spectrometer in deuterochloroform, with tetramethylsilane as the internal standard.

*Rate Determination of the Reaction of 2,6-Dichloropurine with 1,2,3,5-Tetra-*O*-acetyl- β -D-ribofuranose.* a) *The Solvent for the Determination of UV Absorption Spectra (0.1 M ethanolic aqueous sodium hydroxide solution):* In a measuring flask (500 ml), a 1 M ($f=0.887$) aqueous sodium hydroxide solution (50 ml) was mixed with ethanol (250 ml), which had then purified by distillation after having been treated with sodium hydroxide under reflux. The mixture was then diluted to the total volume of 500 ml by the addition of distilled water.

b) *The UV Spectral Data of 2,6-Dichloropurine (1) and 2,6-Dichloro-9-(2',3',5'-tri-*O*-acetyl- β -D-ribofuranosyl)-purine (4):* The UV spectra of **1** and **4** were determined in the above alkaline solution as follows:

1: $\lambda_{\text{max}}^{\text{1M NaOH/EtOH-H}_2\text{O}}$ 280 nm (ϵ 8670)

4: $\lambda_{\text{max}}^{\text{1M NaOH/EtOH-H}_2\text{O}}$ 258 nm (ϵ 11800)

Moreover, the $\epsilon_{258\text{nm}}$ of **1** ($\epsilon_{258\text{nm}}^1$) and $\epsilon_{280\text{nm}}$ of **4** ($\epsilon_{280\text{nm}}^4$) were determined as follows:

$\epsilon_{258\text{nm}}^1: 4150$ $\epsilon_{280\text{nm}}^4: 2440$

The data concerned with **4** were used as they were for the calculation of its concentration, because it was convenient to use them for the conversion of its UV spectral data in the calculations, although the data were identical with those reported with respect to 2-chloroinosine.³⁾

c) *Rate Determination of the Fusion Reaction:* Equimolar amounts of **1**¹²⁾ (0.0187 g, 0.1 mmol) and 1,2,3,5-tetra-*O*-acetyl- β -D-ribofuranose (**2**)¹³⁾ (0.0318 g, 0.1 mmol) were mingled in an agate mortar as completely as possible, and then a suitable amount of the mixture was placed in a capillary tube. The sample in the capillary tube was heated at 152—

154 °C for a scheduled period in a Micro Melting Point Apparatus (Yanagimoto Seisakusho Co., Ltd.) and then cooled immediately in an ice-bath after the reaction. The fused mixture was taken out by crushing the capillary tube, and then it was dissolved in the alkaline solvent described in a). The total coefficients of the $\epsilon_{258\text{nm}}$ and $\epsilon_{280\text{nm}}$ of the resultant solution, which had been properly diluted with the same solvent so as to enable us to determine the UV spectrum, and the yields of the reaction at each reaction time were calculated in the manner described in the text. The $y/(x+y)$ [yields] thus obtained were plotted against the reaction times to give an outline such as is shown in Fig. 1.

The reversibility of this reaction was confirmed by heating **4** (300 mg) with glacial acetic acid (0.1 ml) in a small test tube at 150 °C for 15 min in an oil-bath. The tlc examination of the resultant fusion on a plate of Wakogel B5-F (0.25 mm) [the solvent system for the development: benzene-methanol (9:1 v/v)], in comparison with the authentic **1**, **2**, and **4**, showed the formation of **1** and **2** by the reverse reaction. The spots corresponding to **1**, **2**, and **4** were easily detected by the use of a UV lamp [S. L. Light, Tokyo Machinery Co., Ltd. (2537 and 3650 Å)] or by developing with iodine or with a dilute aqueous sulfuric acid solution, followed by heating. The UV spectral determination of the resultant fusion proved it to be composed of **1** (18) and **4** (82%).

d) *Rate Determination of the Reaction of 1 with 2 in Dimethyl Sulfoxide:* The reaction rates were determined at 130 °C and at 135 °C. Dimethyl sulfoxide shows no absorption in the vicinity of either 258 or 280 nm under the conditions mentioned above. **1** and **4** were used for the determinations as a 40 mmol/l dimethyl sulfoxide solution. The reaction vessel used for the determinations was a three-necked, 100 ml, round-bottomed flask, fitted with a standard stopcock and an automatic stirrer. The temperature of the reaction bath (Mitamura Riken Kogyo Inc., Type 13—76) was regulated precisely with a thermostat (Jumo Co., Ltd., West Germany) within ± 0.2 °C. 20 ml portions of the above freshly-prepared solution of **1** and **2** was pipetted into the reaction vessel, the mixture being constantly stirred at the reaction temperature. After each scheduled reaction time, a small amount of the reaction mixture was taken out with a pipette and diluted properly with the same alkaline solvent so as to enable us to determine the UV spectra. The data thus obtained were treated in the same way as has been described in c).

NMR Examination of the Relative Content Ratios of Anomeric Nucleosides in the Mixtures Obtained by the Fusion Reactions.

a) *The Sample from the Reaction of 2,6-Dichloropurine (1) with 1,2,3,5-Tetra-*O*-acetyl- β -D-ribofuranose (2) for NMR Determination:* Equimolar amounts of **1** (1.87 g, 10 mmol) and **2** (3.18 g, 10 mmol) were sufficiently mingled in an agate mortar, and then a 200 mg portion of the mixture was put into a small glass test tube. The sample was then heated in the above-described reaction bath at 150 °C *in vacuo* for a scheduled reaction time and subsequently cooled immediately after the reaction in an ice-bath. Each of the resultant mixtures was dissolved in deuterochloroform and then subjected to an NMR determination. The deuterochloroform solution was filtered through a small cotton tampon on precipitation of some crystalline mass. In the case of the acid-catalyzed fusion reaction, a mixture of **1** (10 mmol), **2** (10 mmol), and sulfamic acid (20 mg, 0.02 mmol) was utilized after sufficient mingling in the mortar. The anomeric proton signals of 2,6-dichloro-9-(2',3',5'-tri-*O*-acetyl- β -D-ribofuranosyl)-purine (**4**)²⁾ and its α -anomer (**5**) were observed at 6.24 ($J_{1',2'}=4.7$ Hz) and 6.75 ($J_{1',2'}=5.6$ Hz) ppm respectively (cf. Figs. 4, 6, and 7).

b) *The Sample from the Fusion Reaction of 1 with 1,2,3,5-*

11) A similar steric effect of 8-halogeno substituents has recently been reported in the condensation reaction of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide with 8-chloro(or -iodo)adenine, which brought about the preferred formation of the corresponding nucleoside glycosylated at $N_{(3)}$ in the purine nucleus [C. L. Schmidt and L. B. Townsend, *J. Org. Chem.*, **37**, 2300 (1972)].

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Tetra-O-acetyl- α -D-ribofuranose (3): The sirup of **3**, which had been prepared according to the method of Zinner,¹⁴ was found to be composed of **2** and **3** in the ratio of 13/87, by a study of the integration curve of its NMR spectrum, in which a quartet (δ 6.80, H -1 of **3**) and a singlet (δ 6.28, H -1 of **2**) were observed in the anomeric region. An equimolar mixture of **1** and **3** was prepared in the way described in connection with the previous experiment, a). A 200 mg portion of this mixture was put into a small test tube and similarly subjected to a reaction. After the reaction, the resultant mixture was dissolved in deuteriochloroform and used for the NMR determination (*cf.* Fig. 5).

c) *The Sample from the Fusion Reaction of 2,6,8-Trichloropurine (6) with 2 or 3*: The mixture of **6**¹⁵ and **2** or **3** for the autocatalytic or acid-catalyzed fusion reaction was pre-

pared in the way described in a) and then treated similarly. The resultant mixtures were dissolved in deuteriochloroform, filtered with a small cotton tampon if needed, and used for the NMR determination. The anomeric proton signals of 2,6,8-trichloro-9-(2',3',5'-tri-*O*-acetyl- β -D-ribofuranosyl)-purine (**7**)^{2a} and its α -anomer (**8**) were observed at *ca.* δ 6.1 ppm, together with the other one proton on the D-ribofuranosyl ring, and at 6.60 ($J_{1',2'}=5.0$ Hz) ppm respectively (*cf.* Fig. 8).

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