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On the Mechanism of the Acid Catalysis and New Activating Agents in the Fusion Reaction of an Acylated Sugar with a Purine Derivative*

Masao SEKIYA, Teruo YOSHINO, Hisayuki TANAKA, and Yoshiharu ISHIDO
Department of Chemistry, Faculty of Science, Tokyo Institute of Technology, O-okayama, Meguro-ku, Tokyo

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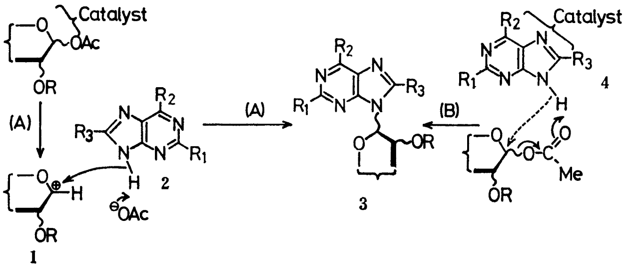
An examination of the site where the acidic catalysts might strongly interact in the fusion reaction of a purine derivative with a fully-acylated sugar led to the conclusion that a purine derivative was activated by the interaction with the catalysts, and, moreover, resulted in the discovery of a new type of activating agent other than the acidic catalysts which had previously been known in the fusion method. In the presence of an excess amount of activating agents, *e.g.*, *p*-nitrophenol, the condensation reaction of theophylline with 1,2,3,4,6-penta-*O*-acetyl-*β*-D-glucopyranose proceeded, even in the absence of *p*-toluenesulfonic acid, to give the corresponding nucleoside. Some of the activating agents were found to form molecular compounds with theophylline, and a few of the latter to afford the corresponding nucleoside similarly. A possible mechanism for the activation process of theophylline by the above agents was discussed.

In a previous paper[1] concerned with the fusion reaction of a purine derivative with an acylated sugar, an assumption concerning such a mechanism of acid catalysis was made involving two possibilities (Scheme I): A) The acidic catalyst might interact with an acylated sugar molecule to afford the C-1 carbonium ion (**1**), and **1** might then successivly be subject to the attack of a purine (**2**) to give the corresponding nucleoside (**3**). B) Alternatively, the catalyst might interact with an electron-sufficient nitrogen atom in the purine nucleus to form an activated complex (**4**), and **4** might then be subject to the reaction with an acylated sugar, accompanied by the elimination of acetic acid, thus affording **3**. In order to solve this problem, a competitive reaction of a purine derivative with a phenol for condensation with a fully-acetylated sugar in the presence of an acidic catalyst was examined; the results thus obtained will be described in the present article.



Scheme I.

Results and Discussion

To confirm the above proposed mechanism of the acid catalysis, a condensation reaction of 1,2,3,4,6-penta-*O*-acetyl-*β*-D-glucopyranose (**5a**) with theophyl-

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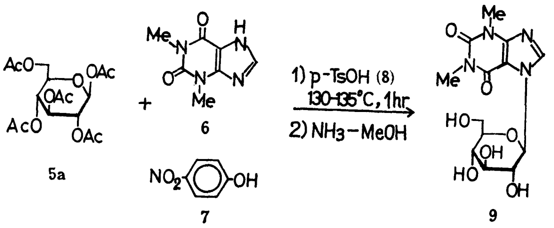
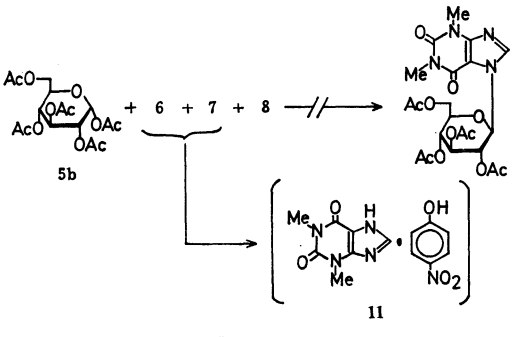
* A part of this paper was presented at the Symposium on the Charge Transfer Complexes, June 10, 1969 (Nagoya).

[1] Y. Ishido, A. Hosono, K. Fujii, Y. Kikuchi, and T. Sato, *Nippon Kagaku Zasshi*, **87**, 752 (1966).



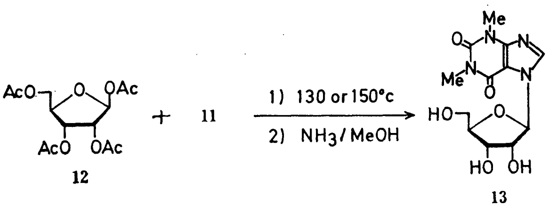
Scheme II.

line (**6**) in competition with *p*-nitrophenol (**7**), in the presence of *p*-toluenesulfonic acid (**8**) as a catalyst, was carried out as a model experiment (Scheme II). After the addition of **5a** (10 mmol) to a homogeneously prefused mixture of **6** (10 mmol), **7** (30 mmol), and **8** (0.05 mmol), the mixture was stirred at 130—135°C under atmospheric pressure and then treated as usual. 7-β-D-Glucopyranosyltheophylline (**9**) was selectively obtained in a 67% yield after the deacetylation of the corresponding tetra-*O*-acetate. This result is of much interest in view of the molar ratio of **6** to **7** and the considerably lowered reaction temperature in comparison with that (160°C) applied in an equimolar fusion reaction of **5a** with **6**.[2] No by-product other than a small amount of *p*-nitrophenyl acetate was observed by tlc or by a column chromatographic separation test on the mixture prior to the deacetylation. The reaction which proceeds *via* the mechanism A may be expected to give a mixture of acetates of *p*-nitrophenyl D-glucopyranosides (**10**) and **9**. On the other hand, the reaction *via* the mechanism B may be expected to give **9** selectively, since **8** should strongly interact with the most basic **6**. Moreover, tetra-*O*-acetate of the β-anomer of **10** (**10a**) was examined by using it in the reaction in place of **5a** in the same way as above, based on the assumption of a possible course of the formation of **9** *via* **10**; however, this examination resulted in the quantitative recovery of **10a**. Accordingly, it was concluded that the acid catalysis takes place *via* the mechanism B.



Scheme III.

The application of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (**5b**) (the α-anomer of **5a**) to the above competitive reaction, on the other hand, resulted in the formation of a molecular compound (**11**), composed of equimolar **6** and **7**, in a quantitative yield, and **5b** was recovered almost quantitatively (Scheme III). The recovery of **5b** in this case may be explained by considering that the difference in the stability of the 1-*O*-acetyl group between **5a** and **5b** arises from the anomeric effect.[3] The formation of **11** was remarkably interesting in view of the nature of the molecular compounds, whose components are generally accepted to be linked with each other by an intermolecular interaction such as polarization bonding;[4] hence, its properties were examined. Since **11** separated out on the dissolution of the resultant reaction mixture in benzene, the preparation of **11** from **6** and **7** was attempted by dissolving them in hot chloroform, benzene, and toluene respectively; the solution gave **11** in a fairly good yield in each case after having been allowed to cool at room temperature. However, the use of water, ethanol, or nitromethane as a solvent furnished no molecular compound. Moreover, considerable differences were observed in the IR spectral comparison of **11** with each component, as may be seen in Fig. 1. These differences suggest that a certain intermolecular interaction might exist between **6** and **7**, and that **6** might be activated by an intermolecular interaction such as polarization bonding. On the basis of this assumption, an equimolar fusion reaction of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**12**) (mp 82°C) with **11** (mp 148—150°C) was attempted at 130 and at 150°C respectively for 3 hr without the acidic catalyst, followed by deacetylation; this gave 7-β-D-ribofuranosyltheophylline (**13**) in 30 and 59% yields respectively (Scheme IV). The activation of **6** by **7** was thus confirmed, and the corresponding fusion reaction of **5a** with **6** in the presence of an excess amount of **7** was carried out. The acetate of **9** was also obtained in a good yield, although this reaction required a longer period than that in the presence of **8**. These results are of much interest in view of the fact that **7** exerts no catalytic effect on the fusion reaction when a catalytic amount is used, although it is acidic (p*Ka*=7.14). Thus, the possibility of the activation of a purine by a compound other than the acidic catalysts, which have been known to be active in the fusion method,[1] was established.



Scheme IV.

In view of the above results, an extensive search for compounds which might behave as activating agents for a purine in the fusion reaction with a fully-acylated sugar was carried out by means of the reaction of **5a**

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[2] T. Shimadate, *ibid.*, **83**, 212 (1962): According to this paper, the equimolar fusion reaction of **5a** with **6** takes place at 160°C in the presence of **8** or zinc chloride, respectively, and gave the equimolar compound of **9** with **6** in 7% and 3% yield, respectively.

[3] R. U. Lemieux and N. J. Chu, 133rd Meeting Amer. Chem. Soc., Abstracts of Papers, 31N (1958); R. U. Lemieux, 135th Meeting Amer. Chem. Soc., Abstracts of Papers, 5E (1959).

[4] S. C. Wallwork, *J. Chem. Soc.*, **1961**, 494.

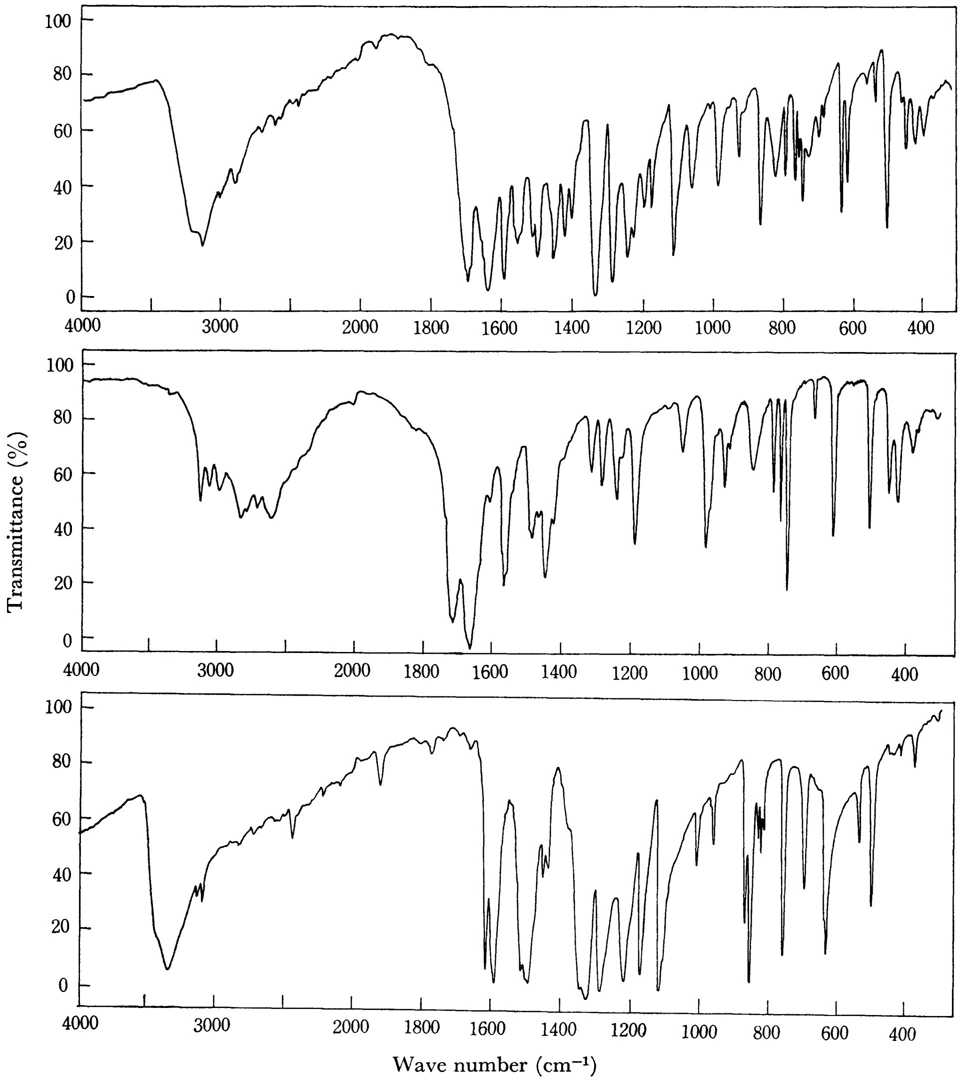

Fig. 1. The IR absorption spectra of theophylline: *p*-nitrophenol (**11**), theophylline (**6**), and *p*-nitrophenol (**7**) in turn from up to down.

with **6** in the absence of **8**. In the light of the properties of **7**, the potential activating agents were chosen as below:

I) Compounds with a nitro group as an electron-attracting group,
   nitrobenzene, *p*-chloronitrobenzene, and methyl *p*-nitrophenyl ether (*p*-nitroanisole).

II) Phenols,
- a) *o*-, *m*-nitrophenols (**14**), 2,4-dinitrophenol, and 2,4,6-trinitrophenol (picric acid)
- b) phenol, *o*-, *m*-, *p*-chlorophenols, and *p*-methoxyphenol.

III) Carboxylic acids and their derivatives,
- a) aromatic carboxylic acids,
  benzoic acid, *o*- (**15**), *m*-nitrobenzoic acid (**16**), *o*-, *m*-chlorobenzoic acids, and salicylic acid (**17**)
- b) aliphatic carboxylic acids,
  acetic acid, propionic acid, and maleic acid
- c) acid anhydrides and esters,
  succinic anhydride, phthalic anhydride, and methyl *o*-, *m*-nitrobenzoates.

IV) Acceptor in EDA complexes,
   p-benzoquinone.

V) Amides,
- a) aromatic sulfonamides,
  p-toluenesulfonamide (**18**), *N*-methyl- (**19**), *N*,*N*-dimethyl-*p*-toluenesulfonamides (**20**), *p*-chlorobenzenesulfonamide (**21**), *N*-acetyl- (**22**), and *N*-phenyl-*p*-toluenesulfonamides (*p*-toluenesulfonanilide) (**23**).
- b) carboxyamides,
  benzamide, benzanilide, and salicylamide.

Using the order of these categories, we made a general survey of the factors affecting the activation process of **6** in the condensation reaction. No activating agent was found among the compounds in Category I; this fact suggested that acidic as well as electron-attracting substituents might be required for the activation of **6**. To confirm this view, the compounds in Categories II and III were examined; the results supported the theory, although only **14**, **15**, **16**, and **17** were found to be effective. This fact, moreover, suggested that the activation process of **6** might be delicately connected with the correlation in the mutual

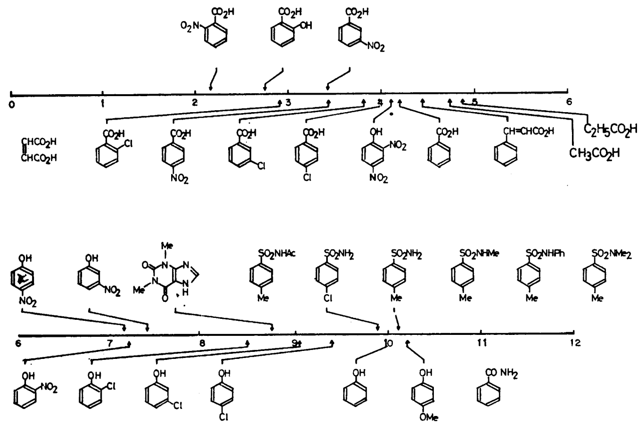

Fig. 2.

position of substituents on the aromatic nuclei, and that aromatic nuclei might be required, at least for the activation. Subsequently, an examination of *p*-benzoquinone (Category IV), which is well known as a typical acceptor in the field of electron-donor-acceptor (EDA) complexes,[4] was carried out; it was found to be ineffective. Moreover, all the sulfonamides in Category Va (**18**—**23**) were found to be effective, in contrast with the carboxyamides in Category Vb. In view of these results, a consideration was made of the factors affecting the activation of **6**: (1) The activation process is conceivably independent from the homogeneity of the reaction system, since all the attempted reactions were carried out in a completely homogeneous state. (2) The possibility of the participation of such a potential active intermediate as **10** was also denied for such compounds as 2,3,4,6-tetra-*O*-acetyl-1-*O*-*o*-nitrobenzoyl-*β*-D-glucopyranose gave no nucleoside even when treated with **6** in the presence of *o*-nitrobenzoic acid in the same way as has been described in the examination reaction of **10a** with **6**, although the formation of a transesterification product had been confirmed in the fusion reaction of **5a** with various carboxylic acids.[5] (3) In considering the possibility that the activation effect of these compounds in Categories II—V might depend on their acidity, they were arranged in the order of their p*Ka* values, as is shown in Fig. 2, and the effective and ineffective ones were placed above and under the line graduating in p*Ka* value. It was thus proved that the effectiveness of the activating agents is independent of their acidity. (4) Consequently, the activation of **6** was deduced to be brought about by such a certain intermolecular interaction as was noticed between **6** and **7**. Incidentally, an intermolecular interaction of such species of heterocyclic compounds has recently been discussed by Shefter[6] in his X-ray crystal structure analysis of the molecular compounds of *p*-chlorosalicylic acid with caffeine[6a] and with theobromine.[6b]

The nature of the intermolecular interaction in these cases was described as the polarization bonding which was proposed by Wallwork.[4] Moreover, it has been shown that **6** and caffeine are both capable of forming EDA complexes with *p*-benzoquinone in water,[7] and that the rate of the alkaline hydrolysis of methyl *trans*-cinnamate is reduced by the addition of such imidazole homologues as **6** because of the formation of EDA complexes.[8] Although the preparations of molecular compounds of all the applied agents with **6** were attempted on the basis of the above facts by dissolving equimolar amounts of them in hot chloroform, no molecular compound such as **11** was isolated except in the cases of **14**, **15**, **16**, **17**, *m*-chlorobenzoic acid, and salicylamide. In addition, *p*-nitro- and 3,5-dinitrobenzoic acid, which can not be used in the reaction because of their high melting points, also gave the corresponding molecular compounds. These results are summarized in Table 2. The IR spectra of these molecular compounds were considerably different from those of each component; the difference was especially remarkable in the specific absorption band of the N–H stretching of **6**. The absorption band was significantly simplified or sharpened by forming the corresponding

Table 1. Condensation reaction of 1,2,3,4,6-penta-*O*-acetyl-*β*-D-glucopyranose (**5a**) with theophylline (**6**) in the presence of activating agents[a]

Activating agent	Reaction temp. (°C)	Period (hr)	Yield of 7-*β*-D-glucopyranosyltheophylline tetra-*O*-acetate (%)
p-$O_2N-C_6H_4-OH$	130—135	5	35 (90[b] 67[c])
m-$O_2N-C_6H_4-OH$	130—135	11	15 (94[b])
o-$O_2N-C_6H_4-CO_2H$	150—155	3	70
o-$HO-C_6H_4-CO_2H$	160—165	3	30
p-$Me-C_6H_4-SO_2NH_2$	160	3	71
p-$Cl-C_6H_4-SO_2NH_2$	150—155	3	58
p-$Me-C_6H_4-SO_2NHMe$	155—160	4	41
p-$Me-C_6H_4-SO_2-NMe_2$	160—165	9	58
p-$Me-C_6H_4-SO_2NHPh$	170	3	50

a) All the reaction were carried out by the use of **5a**(10 mmol), **6**(10 mmol) and activating agents (30—50 mmol).
b) These reactions were carried out for 1 hr in the presence of *p*-TsOH(0.5 mmol).
c) The yield stands for that of 7-*β*-D-glucopyranosyltheophylline(**9**) obtained by deacetylation of the tetra-*O*-acetate described in footnote b with methanolic ammonia.

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5) Y. Ishido, H. Tanaka, K. Iwabuchi, and T. Sato, *Nippon Kagaku Zasshi*, **87**, 1113 (1966).

6) a) E. Shefter, *J. Pharm. Sci.*, **57**, 350 (1968); b) E. Shefter, T. E. Brennan, and P. Sackman, *Chem. Pharm. Bull.*, **19**, 746 (1971).

7) T. Okano and K. Aida, *Yakugaku Zasshi*, **87**, 1243 (1967).

8) J. A. Mollica, Jr., and K. A. Connors, *J. Amer. Chem. Soc.*, **89**, 308 (1967).

Table 2. Molecular compounds of theophylline (**6**) with some compounds[a)]

Countercomponent	Mp (°C)		Molecular compounds					
			Calcd			Found		
			C	H	N(%)	C	H	N(%)
p-O_2N-C_6H_4-OH	148—150	$C_{13}H_{13}O_5N_5$:	48.90	4.10	21.94	48.89	3.80	21.87
m-O_2N-C_6H_4-OH	150—160	$C_{13}H_{13}O_5N_5$:	48.90	4.10	21.94	49.19	3.69	22.03
o-O_2N-C_6H_4-CO_2H	160—168	$C_{14}H_{12}O_6N_5$:	48.42	3.77	20.17	48.64	3.55	20.42
m-O_2N-C_6H_4-CO_2H	200—202	$C_{14}H_{12}O_6N_5$:	48.42	3.77	20.17	48.71	3.52	20.20
p-O_2N-C_6H_4-CO_2H[b)]	191—195	$C_{14}H_{12}O_6N_5$:	48.42	3.77	20.17	48.68	3.69	20.15
o-HO-C_6H_4-CO_2H	183—184	$C_{13}H_{13}O_5N_4$:	52.83	4.43	17.60	52.70	4.13	17.84
3,5-$(O_2N)_2C_6H_3$-CO_2H[b)]	190—191	$C_{14}H_{12}O_8N_6$:	42.87	3.08	21.43	42.87	3.19	21.53
m-Cl-C_6H_4-CO_2H[c)]	182—185	$C_{14}H_{12}O_4N_5Cl$:	49.93	3.89	16.64	49.83	3.81	16.84
o-HO-C_6H_4-$CONH_2$[c)]	187—210	$C_{14}H_{14}O_4N_5$:	52.99	4.77	22.07	53.22	4.72	22.40

a) All the preparations were carried out by the use of **6**(10 mmol) and countercomponents (11 mmol), respectively, in chloroform (30—50 ml) under reflux.

b) The condensation reaction of **5a** with **6** was not attempted on account of their high melting point.

c) The corresponding molecular compounds could not affect the reaction of **5a** with **6**.

molecular compounds. Moreover, the IR spectroscopic behavior of the phenolic hydroxyl group of **7** was easily detected by examining the spectrum of the molecular compound of **7** with caffeine, which is the $N_{(7)}$-methyl derivative of **6**, in a similar manner. The shift of the O–H stretching absorption band to a lower frequency from the original band of **7** was estimated to be about 180 cm^{-1}. Although various individual variations were observed, similar tendencies were recognized in the specific IR absorption bands of all the countercomponents other than **7**. The magnitude of the activation by such intermolecular interaction is considered to be delicately affected by the correlative position on the aromatic nuclei and by the chemical properties of each functional group in view of the facts that all the homologues are not necessarily effective for the reaction in each category, and that **16** is not effective for the reaction of **6** with **5a**, but is effective for that of **6** with 2,3,4,6-tetra-*O*-acetyl-1-*O*-trichloroacetyl-β-D-glucopyranose.[9)] Moreover, there may be no correlation between the formation of a crystalline molecular compound and the activation of **6** in view of the facts that all the sulfonamides are effective for the reaction, although they gave no crystalline molecular compound, and that *m*-chlorobenzoic acid and salicylamide, which are ineffective for the reaction, afforded the corresponding molecular compounds. Furthermore, the intermolecular interaction observed among these molcular compounds was assumed to be not so strong on the basis of the facts that no formation of crystalline molecular compounds took place in such polar solvents as water, ethanol, and nitromethane, and that their UV absorption spectra could not be determined because of the dissociation into their components in the concentration of 10^{-4} mole per liter in chloroform. On the basis of these facts, **6** was deduced to be activated by the polarization bonding with the activating agents described above.

It may be concluded from these experiments that a new type of fusion method for the synthesis of purine nucleosides involving an intermolecular interaction between a purine and an activating agent was established.[10)]

Experimental

All the melting points are uncorrected. The IR absorption spectra were taken with a Hitachi-225 in a pellet of potassium bromide, while the NMR spectra were taken with a Varian T-60 in deuteriochloroform ($CDCl_3$) by the use of tetramethylsilane (TMS) as the internal standard.

Competitive Reaction of Theophylline (6) with p-*Nitrophenol (7) for the Condensation with 1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranose (5a).* Theophylline (**6**) (1.8 g, 10 mmol), *p*-nitrophenol (**7**) (4.2 g, 30 mmol), and *p*-toluenesulfonic acid (**8**) (100 mg, 0.5 mmol) were heated in an oil-bath (130—135°C) to fuse them homogeneously under stirring, and then 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**5a**) (3.9 g, 10 mmol)[11)] was added to the prefused mixture. After the addition, the mixture was stirred for 1 hr at that temperature under atmospheric pressure, and then it was allowed to cool at room temperature. The resultant mixture was dissolved in benzene (300 ml), and the solution was successively washed with a 1M aqueous sodium hydroxide solution to remove the co-produced acetic acid, unchanged **6**, and **7**, and with water, and then dried over anhydrous calcium chloride. After removing the desiccant by filtration, the organic layer was concentrated *in vacuo* to a pale yellow half-glassy sirup. The sirup was dissolved in methanol (200 ml) and was mixed with methanolic ammonia (200 ml) which had been prepared by saturating methanol with ammonia gas at 0°C. After standing in a round-bottomed flask (500 ml) with a tight stopper at room temperature overnight, the mixture was cooled in an ice-bath and then concentrated *in vacuo* to about a quarter of the original volume. The crystals thus precipitated were filtered by suction and were dissolved in water (10 ml) by warming. The solution was mixed with acetone (100—150 ml) after decoloration with active charcoal. After the solution had been stored in a refrigerator overnight, the resultant crystals were filtered and dried over phosphorus

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[9)] M. Sekiya, T. Yoshino, H. Tanaka, and Y. Ishido, unpublished data: The tetra-*O*-acetate of **9** was obtained in 55% yield.

[10)] The reaction is found to be also applicable to the other purine derivatives such as $N_{(6)}$-benzyladenine *etc.*, to which the usual fusion reaction has been inapplicable, and the results will be published elsewhere.

[11)] E. Fischer, *Ber.*, **49**, 584 (1916).

pentoxide at 110°C *in vacuo* to give 7-β-D-glucopyranosyltheophylline (**9**)12) (2.3 g, 67% yield). Mp 265—267°C. $[\alpha]^{21}_{578}$ $-3°$ (*c* 1.0, H_2O). $\lambda^{H_2O}_{max}$ 275 nm (ε 7200). The NMR spectra of the above sirup was taken prior to deacetylation; it was found to be almost as pure as 7-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)theophylline: δ ($CDCl_3$) 1.92 (3H; AcO-2′), 2.04, 2.07 (9H; AcO-3′, 4′, and 6′), 3.43, 3.60 (6H; MeN-1 and 3), 3.95—4.30 (3H; H-5′, $6'_a$, and $6'_b$), 5.2—5.7 (3H; H-2′, 3′, and 4′), 6.21 (1H; H-1′, $J_{1',2'}=10$ Hz), and 7.90 (1H; H-8) ppm.

*The Molecular Compound of Theophylline (**6**) with* p-*Nitrophenol (**7**): Theophylline*—p-*Nitrophenol (**11**).* a) On the use of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (**5b**)13) in the competitive reaction in place of **5a**, white scaly crystals were separated when the resultant reaction mixture was dissolved in benzene. The crystals were filtered and dried over phosphorus pentoxide at 110°C *in vacuo*, and a molecular compound composed of equimolar **6** and **7** (**11**) (3.1 g, 97% yield) was obtained. Mp 148—150°C. The IR spectrum of **11** is shown in Fig. 1. However, no new absorption band was found in either its UV or visible region spectrum. After the removal of the residual **7** as described in the previous experiment, the organic layer was concentrated *in vacuo* to give **5b** (3.5 g, 90% recovery). b) **11** was also obtained quantitatively by dissolving an equimolar amount of **6** (1.8 g, 10 mmol) and **7** (1.6 g, 11.4 mmol) in hot chloroform (30—50 ml), and by then allowing the solution to cool at room temperature.

*An Equimolar Fusion Reaction of the Molecular Compound (**11**) with 1,2,3,5-Tetra-O-acetyl-β-D-ribofuranose (**12**).* To 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**12**)14) (3.2 g, 10 mmol) prefused at 130°C, was added finely-powdered **11** (3.2 g, 10 mmol), after which the mixture was stirred at 130°C for 3 hr under atmospheric pressure. The resultant reaction mixture was dissolved in chloroform (150—200 ml), successively washed with a 1M aqueous sodium hydroxide solution and water in the same way as has been described in the first example of this section, and dried over anhydrous calcium chloride. The solution was, after the desiccant had been removed by filtration, concentrated *in vacuo* to a sirup; the sirup was then dissolved in methanol (100 ml) and mixed with methanolic ammonia, as has been described above. After standing in a refrigerator overnight, the solution was evaporated *in vacuo* to dryness and the residue was triturated with ethanol (10 ml). The resultant crystals were filtered and recrystallized from ethanol (*ca.* 200 ml) with the use of active charcoal to give 7-β-D-ribofuranosyltheophylline (**13**) (1.2 g, 39% yield). Mp 189.5—191°C. $[\alpha]^{25}_{D}$ $+27.1°$ (*c* 1.02, H_2O). $\lambda^{H_2O}_{max}$ 274 nm (ε 8400). This product was identified with an authentic specimen.1) **13** was also obtained in a 59% yield by carrying out this reaction at 150°C for 3 hr.

*Examination for the Potential Activating Agents by Means of the Condensation Reaction of **6** with **5a**.* The reactions of the examination were carried out with each potential compound under the conditions summarized in Table 1 or by managing to make the reaction system homogeneous; the resultant reaction mixtures were treated in the same way as has been described in the first example of this section. On the basis of the properties of the potential agents applied, the procedures for their removal from the resultant mixtures were varied as below. a) Phenols, *p*-toluene-, and *p*-chlorobenzenesulfonamide: The washing procedure of the organic solution of each resultant mixture was carried out with a 1M aqueous sodium hydroxide solution. b) Carboxylic acids: The procedure was carried out with an aqueous sodium carbonate or bicarbonate solution. c) *p*-Chloro- and *p*-methoxynitrobenzene: These compounds were removed by steam distillation. d) The other amides and acid anhydrides: These were separated by the column chromatography of the resultant reaction mixtures; that is, the mixtures were treated on a short column (d: 3 cm) packed with Mallinckrodt silicic acid (100 mesh, 45 g); the column was first eluted with chloroform to give the potential activating agents as the first, and **5a** as the second fraction. Successive elution with benzene–methanol (9: 1) gave the tetra-*O*-acetate of **9** as the third fraction; unchanged **6** was obtained by the following elution with ethanol. The third fraction thus obtained was evaporated at 30—40°C, and then again at 100°C, finally to a half-glassy sirup, and weighed. The purity of each of the above products can be checked NMR spectroscopically by a comparison of the ratio of 2′-*O*-acetyl (δ 1.92 ppm) to other *O*-acetyl (*ca.* δ 2.06 ppm) signals in the integration curve. The yields demonstrated in Table 1 were calculated from the weight and this ratio in the NMR spectra of each product. To make sure of the composition of each product, the products were examined by tlc (eluting solvent: benzene–methanol=9: 1 v/v). In the case of *p*-nitrophenol (**7**), *p*-nitrophenyl acetate (*ca.* 100 mg) was obtained as a fraction before that of **7**.

Preparation of Molecular Compounds. As has been described in the second example of this section, the potential activating agents were each dissolved in hot chloroform together with an equimolar amount of **6**, after which the solution was allowed to cool at room temperature for crystallization. The precipitated crystals were subsequently filtered and dried at 110°C *in vacuo* over phosphorus pentoxide. The IR spectra of the molecular compounds of **6** with *m*-nitrophenol (**14**), with *o*-(**15**), *m*-nitrobenzoic acid (**16**), and with salicylic acid (**17**) showed the same tendency as has been described in the case of **11**. The experimental results are summarized in Table 2. No new absorption band was, on the other hand, observed in either the UV or the visible region spectrum similar to those of **11**. Almost the same results were obtained by the use of benzene, toluene, or xylene as the solvent for the preparation.

Preparation of the Molecular Compound of Caffeine with p-*Nitrophenol (**7**).* As has been described in the previous experiment, equimolar amounts of caffeine (1.95 g, 10 mmol) and **7** (1.60 g, 11 mmol) were dissolved in xylene (50 ml) under reflux, and the resultant solution was allowed to cool at room temperature for crystallization. The needles thus precipitated were filtered and dried at 110°C over phosphorus pentoxide *in vacuo* to give the molecular compound (3.20 g, 95% yield). Mp 154.5—156°C. Found: C, 50.36; H, 4.61; N, 20.64%. Calcd for $C_{14}H_{15}N_5O_5$: C, 50.45; H, 4.54; N, 21.00%. IR (KBr): 3170 cm^{-1} (phenolic OH).

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12) E. Fischer und B. Helferich, *ibid.*, **47**, 210 (1914).

13) C. S. Hudson and J. K. Dale, *J. Amer. Chem. Soc.*, **37**, 1264 (1915).

14) H. Zinner, *Chem. Ber.*, **83**, 517 (1950).