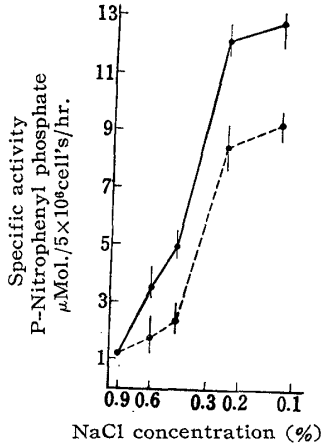

Fig. 1. Fragility of Membrane of Sensitive and Resistant Strains of Yoshida Sarcoma in Heterotonic Saline Solution

•—• : Original susceptible strain
•---• : Resistant strain (average of 5 experiments)

As shown in Fig. 1, the enzyme activity of the supernatant of the original strain is greater than that of the resistant in a concentration below 0.6% of the salt.

Since the activity of acid phosphatase of the homogenates of the two tumor strains has been determined to be quite same each other, cytolysis of the cells of the resistant strain seems to be less advanced than that of the original in this condition of experiment. This result does not demonstrate the difference in permeability of the cell membrane in the natural state but may suggest the presence of difference in chemical or physical construction of the membrane of these two different lines of tumor cells.

The author wishes to express his gratitude to Dr. Y. Sakurai for his guidance in this experiment. This experiment was in part supported by the grant, CY 2799, N.C.I., N.I.H., U.S. Public Health Service, to that the author is much indebted.

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UDC 547.457'854

Tyunosin Ukita, Hikoya Hayatsu, and Yutaka Tomita[*1] : A Reinvestigation of the Condensation Reaction of Acetobromoglucose with Chloromercuri-4-ethoxy-2(1*H*)-pyrimidinone to 1-(Tetra-O-acetyl-*β*-D-glucopyranosyl)-4-ethoxy-2(1*H*)-pyrimidinone.

(*Faculty of Pharmaceutical Sciences, University of Tokyo*[*1])

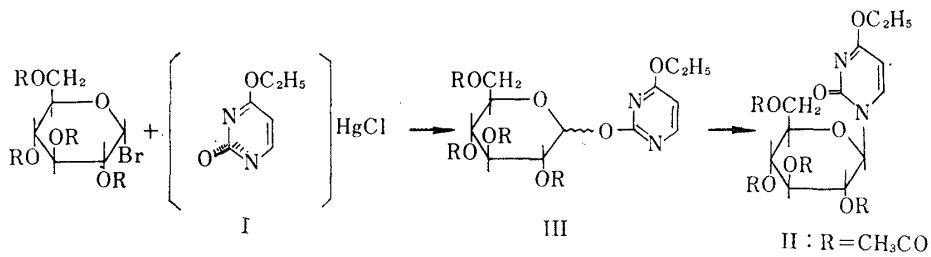
Concerning the synthesis of 1-(tetra-O-acetyl-*β*-D-glucopyranosyl)-4-ethoxy-2(1*H*)-pyrimidinone (II), Fox, *et al.*[1] reported a method which involves the condensation of chloromercuri-4-ethoxy-2(1*H*)-pyrimidinone (I) with acetobromoglucose in xylene.

For the purpose of obtaining the compound (II), the present authors followed their method and found that their procedure was lacking in reproducibility. This report deals with several new observations obtained in further investigation of the above reaction.

*1 Hongo, Tokyo (浮田忠之進, 早津彦哉, 冨田 裕).

1) J. J. Fox, N. Yung, I. Wempen, I. L. Doerr : J. Am. Chem. Soc., **79**, 5060 (1957).

According to the procedure reported by Fox, *et al.*,[1] 4-ethoxy-2(1*H*)-pyrimidinone[2] was converted to its chloromercuri salt and the salt was obtained as an analytically pure compound with almost quantitative yield.



After reaction of this chloromercuri compound (I) with acetobromoglucose under the conditions precisely duplicating Fox's experiment,[3] the xylene solution was cooled, filtered and worked up as given in Experimental part of this report to furnish a syrup which, on trituration with ethanol, gave a crystalline product. This product (III) (yield 20%) melted at 106~110° and the melting point was raised to 111~112° by subsequent recrystallization from ethanol. The melting point, however, was much lower than that (200~203°) reported by Fox, *et al.* for a product obtained from the reaction mixture.

On further treatment with ether of a syrup obtained from the filtrate of the crystals, a small amount (yield 1.5%) of another product having melting point of 193~198° was isolated. This was further purified by recrystallization from ethanol to give needles melting at 202~203° and the needles were identified by mixed fusion and comparison of ultraviolet absorptions with an authentic 1-(tetra-O-acetyl-β-D-glucopyranosyl)-4-ethoxy-2(1*H*)-pyrimidinone (II) synthesized by Hilbert-Jansen's method.[4]

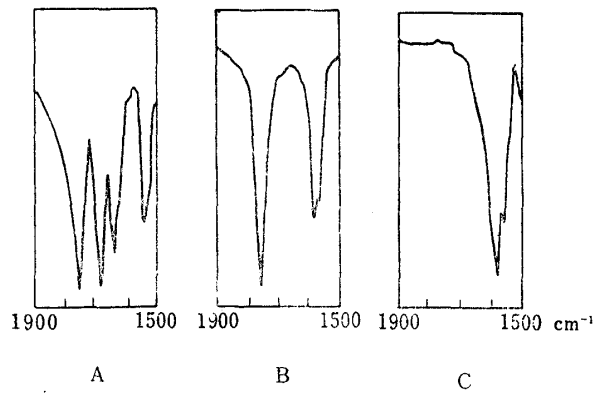


Fig. 1. Infrared Spectra of N-Glucoside (II), O-Glucoside (III) and 2,4-Diethoxypyrimidine in 1500~1900 cm^{-1} Region

A : N-Glucoside (II) (KBr)
B : O-Glucoside (III) (KBr)
C : 2,4-Diethoxypyrimidine (Cap.)

O-Glucoside (III) lacks amide-carbonyl absorption at 1640 and 1675 cm^{-1} present in the spectrum of N-glucoside (II). III absorbs at 1578 and 1590 cm^{-1} which probably correspond to the 1578 and 1587 cm^{-1} hetero-aromatic ring vibrations present in the spectrum of 2,4-diethoxypyrimidine.

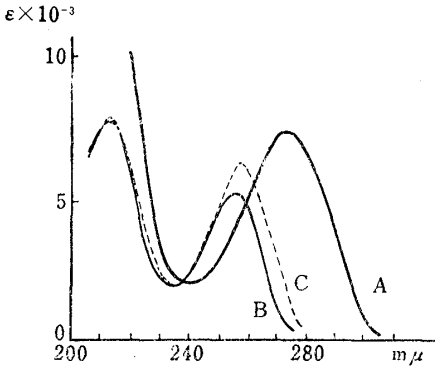


Fig. 2. Ultraviolet spectra of N-Glucoside (II), O-Glucoside (III) and 2,4-Diethoxypyrimidine

A : N-Glucoside (II)
B : O-Glucoside (III)
C : 2,4-Diethoxypyrimidine
Solvent; EtOH

2) G.E. Hilbert, E.F. Jansen : *Ibid.*, **57**, 552 (1935).
3) The mixture was kept at boiling point of xylene for 42 minutes, although the reaction occurred even at lower temperature.
4) G.E. Hilbert, E.F. Jansen : J. Am. Chem. Soc., **58**, 60 (1936).

The major product (III) gave an infrared spectrum having absorptions at 1578 and 1590 cm^{-1} (hetero-aromatic ring) and lacking those at 1620~1700 cm^{-1} range (amide carbonyl) (Fig. 1.), and an ultraviolet spectrum showing a twin peak similar in both wave lengths and molecular absorption coefficients to that of 2,4-diethoxypyrimidine (Fig. 2). The N-glucoside (II) showed a remarkable difference from III in its ultraviolet spectrum which gave single peak at 274 mμ (bathochromic shift by 19 mμ compared with the absorption maximum of III at 255 mμ). The compound (III) was found unstable in alkali. Thus, on treatment of III with dilute alkali at room temperature, quantitative liberation of the base, 4-ethoxy-2(1*H*)-pyrimidinone, was observed by paper chromatography.

From the above observations and from analysis, the major product (III) must have the O-glucoside structure of 2-[(tetra-O-acetyl-D-glucopyranosyl)oxy]-4-ethoxypyrimidine, whose configuration at glycosidic linkage is assumed to be β, as the starting material, acetobromoglucose, has α-configuration.[5]

The condensation reaction was performed under several different conditions, *i.e.*, with varied reaction time and amounts of the starting materials, and the results were summarized in Table I. In all cases the major product was not the N-glucoside (II) but the O-glucoside (III) and its maximum yield was 51.5%. Thus, this procedure was found not available for the synthesis of the N-glucoside (II).

Table I. Duplications of Condensation Reaction of Chloromercuri-4-ethoxy-2(1*H*)-pyrimidinone with Acetobromoglucose in Boiling Xylene

Experiment No.	Reactants used		Reaction period (Refluxing period)	Products isolated[a]	
	Chloromercuri salt (g.)	Acetobromoglucose (g.)	(min.)	O-Glucoside (g.) (% of yield)	N-Glucoside (g.) (% of yield)
1	4.0	4.38	50	2.58 (51.5)	—
2[b]	3.75	4.10	42	0.95 (20)	0.071 (1.5)
3	1.0	1.10	50	0.125 (10.7)	0.075 (6.4)
4	1.0	1.10	40	0.23 (18)	—
5	1.0	1.10	5	0.41 (33)	—
6	0.7	0.77	40	0.15 (17)	0.04 (4.5)
7	0.5	0.55	50	0.23 (39)	—

a) Amounts listed are those of crude crystals before recrystallization.
b) This experiment is the one described in Experimental.

As to the reason of the erratic occurrence of the N-glucoside in this reaction, rearrangement of III to II in boiling xylene under coexistence of mercuric halogenide was thought probable, because Wagner and Pischel recently communicated a similar rearrangement of 2-[(tetra-O-acetyl-D-glucopyranosyl)oxy]pyridine to the corresponding N-glucoside by treatment of the former with mercuric bromide in boiling xylene.[6,7]

The poor and irregular yield of II in our case should be attributed to the amount of mercuric bromide which was not enough to complete the rearrangement reaction. Indeed, when the O-glucoside (II) was boiled in xylene with three mole equivalents of mercuric bromide, transglycosidation reaction occurred and the N-glucoside (II) was obtained in a yield of 30~40%.

5) L.T. Haynes, F.H. Newth: Adv. in Carbohydrate Chem., **10**, 207 (1956).
6) G. Wagner, H. Pischel: Naturwissenschaften, **48**, 454 (1961).
7) *Idem*: Archiv der Pharmazie, **295**, 373 (1962).

The sequence of this transglycosidation reaction was investigated by observing ultraviolet absorption curves of the inorganic-salt-free and chloroform soluble fraction of the reaction mixture at intervals, and the result was summarized in Table II. The yield of the reaction product reached maximum within the first 30 minutes of the reaction.

Table II. Reaction Sequence in Transglycosidation of O-Glucoside to N-Glucoside Pursued by Ultraviolet Spectrum (Ratio of O.D. at 275 mμ to 255 mμ) and by Yield of N-Glucoside isolated

Reaction period (min.)	0 (O-glucoside)	10	30	120	240	(N-glucoside)
O.D. at 275 mμ / O.D. at 255 mμ	0.08	0.53	0.96	0.87	0.80	(1.85)
Yield of N-glucoside isolated (%)	—	10.5	34.5	30	30	—

From the mother liquor of the product of the transglycosidation reaction, on removal of the solvent, a syrup was obtained which showed ultraviolet absorption maximum at 259~260 mμ. The infrared spectrum of this syrup gave no absorption near 1600 cm^{-1} (aromatic ring) but a weak one at 1690 cm^{-1} (amide carbonyl). As a further treatment of this side-product with mercuric bromide did no longer give the N-glucoside (II), and as II itself was unaffected by treatment with mercuric bromide, this syrup must be derived directly from the O-glucoside (III) by unkown side reaction.

In order to synthesize the N-glucoside (II) from acetobromoglucose and the mercuric salt (I) by one-step reaction, these starting materials were reacted in boiling xylene in the presence of an excess amount of mercuric bromide and from the reaction mixture the desired N-glucoside (II) was obtained in a yield of 34.3%.

As for the configuration at the glycosidic linkage, that of the N-glucoside (II) has already been established unequivocally as β.[8] Furthermore, after completion of this work, Ulbricht[9] reported a similar rearrangement of glucosyl residue of O-glucosyl-cytosine pentaacetate to the corresponding N-glucoside and assigned its glycosidic configuration as β-type. These results suggest that in this type of transglycosidation the β-anomeric structure at the glycosidic linkage is kept unchanged during the reaction.

Experimental*2

Chloromercuri-4-ethoxy-2(1*H*)-pyrimidinone (I)——To a stirred solution containing 2.00 g. (14.3 m mol.) of 4-ethoxy-2(1*H*)-pyrimidinone[2] (colorless prisms, m.p. 167~168°) and 14.3 m moles of NaOH was added dropwise an ethanolic solution of 3.89 g. (14.3 m mol.) of $HgCl_2$. The precipitate appeared was collected and washed successively with cold H_2O, cold EtOH and Et_2O. The colorless powder, upon drying overnight over P_2O_5 *in vacuo*, weighed 5.14 g. (yield 96%). *Anal.* Calcd. for $C_6H_7N_2O_2HgCl$: C, 19.21; H, 1.88; N, 7.47. Found: C, 19.09; H, 1.61; N, 7.01. Similar experiments were carried out three times giving the chloromercuri salt in respective yield of 97, 96 and 94.5%.

Condensation of Chloromercuri-4-ethoxy-2(1*H*)-pyrimidinone with Acetobromoglucose A Reinvestigation of Fox's procedure——A suspension of 3.75 g. of the chloromercuri salt (I) in 220 ml. of dry xylene was heated under stirring, and 65 ml. of the solvent was distilled off to remove traces of moisture. To the hot mixture (bath temperature, 95°) were added 4.10 g. of freshly prepared acetobromoglucose and 45 ml. of xylene and the mixture was heated under stirring. During 10 minutes' heating the bath temperature rose to 140°. The chloromercuri salt rapidly disappeared and within a few minutes an essentially transparent solution resulted. The mixture was then refluxed for 42 min. in a bath maintained at 175~180°. The reaction mixtsre was cooled, left standing overnight, filtered and the filtrate treated with 1 L. of petr. ether to precipitate a white mass. The mass was collected,

*2 All melting points are uncorrected.

8) J. J. Fox, I. Wempen: Adv. in Carbohydrate Chem., **14**, 314 (1959).
9) T. L. V. Ulbricht: Proc. Chem. Soc., **1962**, 298.

dissolved in 50 ml. of $CHCl_3$ and filtered to remove insoluble mercuric salts. The $CHCl_3$ solution was washed successively with 30% aqueous KI (20 ml.×5) and H_2O (20 ml.×3), and dried over Na_2SO_4. Evaporation of the solvent *in vacuo* left a syrup, which was taken up in MeOH. A residue obtained on removal of MeOH was then evaporated with added Me_2CO and finally dissolved in 5 ml. of EtOH. Upon standing overnight, colorless crystals separated out, which were collected by filtration. The filtrate was treated separately as shown below. The crude crustals (m.p. 106~110°) were recrystallized from EtOH affording colorless needles of O-glucoside, 2-[(tetra-O-acetyl-β-D-glucopyranosyl) oxy]-4-ethoxypyrimidine (III), melting at 111~112°. *Anal.* Calcd. for $C_{20}H_{26}O_{11}N_2$: C, 51.07; H, 5.57; N, 5.95. Found: C, 51.31; H, 5.67; N, 6.05. UV λ mμ (ε): λ_{max}^{EtOH} 211 (7880), 255 (5440); λ_{min}^{EtOH} 232 (1900). (cf. 2,4-diethoxypyrimidine: λ_{max}^{EtOH} 212 (7850), 258 (6440); λ_{min}^{EtOH} 232 (1690)).

Solvent was removed from the filtrate and the residue was again evaporated with Me_2CO *in vacuo* leaving a glassy material, to which was added a few ml. of Et_2O. Upon scratching, a small amount of crystals appeared, which were collected and dried to give 71 mg. (yield 1.5%) of N-glucoside, 1-(tetra-O-acetyl- β-D-glucopyranosyl)-4-ethoxy-2(1*H*)-pyrimidinone (II), m.p. 193~198°. The crystals were recrystallized from EtOH to colorless needles melting at 202~203°. A mixed fusion of II with an authentic specimen (m.p. 202~203°) prepared by Hilbert-Jansen's method[4] showed no depression of the melting point. UV mμ (ε): λ_{max}^{EtOH} 274 (7560); λ_{min}^{EtOH} 238 (2000) (reported[1] λ_{max}^{EtOH} 274; λ_{min}^{EtOH} 243).

The mother liquor gave an additional crystals of O-glucoside (III) by seeding the O-glucoside and keeping in a refrigerator for 2 days; m.p. 97~102°. The total yield of crude III was 950 mg. (yield 20%).

Evaporation of the final mother liquor gave a syrup (1 g.), the ultraviolet absorption spectrum (in EtOH) of which was identical with that of O-glucoside (III).

Transglycosidation of O-Glucoside (III) to N-Glucoside (II)——A solution of 500 mg. of O-glucoside (III) and 1.2 g. of mercuric bromide in 20 ml. of hot xylene was refluxed for 2.5 hr. After removal of the solvent *in vacuo*, the residue was washed with petr. ether and dissolved in ca. 10 ml. of $CHCl_3$, and an insoluble material was removed by filtration. The filtrate was washed successively with 30% KI and H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was repeatedly evaporated with EtOH and Me_2CO, and then triturated with Et_2O giving colorless crystals of N-glucoside (II). The crystals were collected and dried to afford 190 mg. of II, m.p. 194~197°. An additional crop of II (22 mg., m.p. 199~200°) was obtained from the mother liquor (total yield, 42.4%). By recrystallization from EtOH, the melting point was raised to 202~203°, and showed no depression on admixture with authentic N-glucoside (II).[4]

Pursuit of Reaction Sequence in Transglycosidation——Eight hundred milligrams of O-glucoside (III) (m.p. 111~112°) and 2.0 g. (3.2 equivalents) of $HgBr_2$ were refluxed in 40 ml. of dry xylene. Aliquots (10 ml.) were withdrawn after 10, 30, 120 and 240 min. and each evaporated to dryness *in vacuo* and the residue dissolved in $CHCl_3$. The $CHCl_3$ solution was treated as usual with aqueous KI and evaporated to dryness. Ultraviolet absorption spectrum of a small part of the residual syrup in EtOH was measured by Cary Model 11 spectrophotometer and the ratio of optical density at 275 mμ to that of 255 mμ was calculated to follow the reaction (see Table II). The syrups obtained as above were treated with EtOH or Et_2O giving crystalline N-glucoside (II). The yields and melting point of the product (II) isolated from each aliquot were as follows. The first aliquot: 21 mg. (10.5%), 190~199°; the second: 69 mg. (34.5%), 192~198°; the third: 60 mg. (30%), 195~200°; the final: 60 mg. (30%), 198~200°.

These results are summarized in Table II.

Condensation of Chloromercuri-4-ethoxy-2(1*H*)-pyrimidinone with Acetobromoglucose in the Presence of Mercuric Bromide——To an azeotropically dried suspension of 500 mg. of chloromercuri-4-ethoxy-2(1*H*)-pyrimidinone in 36 ml. of xylene was added 550 mg. of acetobromoglucose under stirring, and the mixture heated in an oil bath. When the bath temperature reached to 140°, reaction started smoothly and within a few minutes the mixture became essentially transparent. After refluxing for about 10 min. (bath temperature, 170°), heating was interrupted and the mixture was cooled to about 100°, added with 2.0 g. of $HgBr_2$ and again refluxed for 90 min. From the mixture, xylene was removed by evaporation under a reduced pressure, and the residue treated with petr. ether. The petr. ether was decanted and discarded, and the residue dissolved in $CHCl_3$. The $CHCl_3$ solution was treated as usual and evaporation of $CHCl_3$ gave a syrup which was repeatedly evaporated with EtOH and Me_2CO. The residue (650 mg.) was triturated with Et_2O to give colorless crystals of N-glucoside (II), which was collected and washed with Et_2O. Dried material weighed 184 mg., m.p. 199~200°. An additional crop (31 mg., m.p. 193~199°) was obtained from the mother liquor. Total yield: 34.3%.

The authors are indebted to Mr. D. Ōhata of the Medical Institute of the Sasaki Foundation for carrying out the micro-analyses.

Summary

The condensation reaction of acetobromoglucose with chloromercuri-4-ethoxy-2(1*H*)-pyrimidinone (I), which had been reported by Fox, *et al.*[1] to yield 1-(tetra-O-acetyl-*β*-D-glucopyranosyl)-4-ethoxy-2(1*H*)-pyrimidinone (II), was reinvestigated. It was confirmed that the main product of the reaction is not the N-glucoside (II) but the O-glucoside, 2-[(tetra-O-acetyl-D-glucopyranosyl)oxy]-4-ethoxypyrimidine (III). II was obtained as a minor product of the reaction. The O-glucoside (III) was converted to the N-glucoside (II) by the action of mercuric bromide in boiling xylene in a yield of 30~40%. This transglycosidation reaction was followed by ultraviolet absorption spectrum and by the yield of II isolated from the reaction mixture. A direct synthesis of II by the condensation of acetobromoglucose with I in the presence of mercuric bromide was described.

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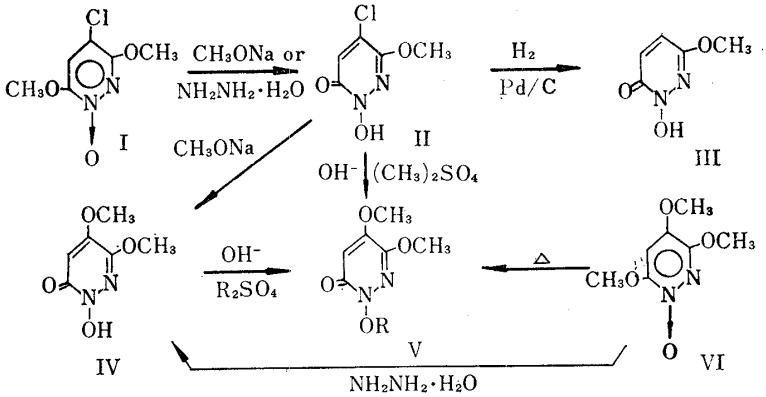
Takanobu Itai and Shozo Kamiya : Potential Anti-cancer Agents. XII.*1 Synthesis of 4-Azido-3,6-dimethoxypyridazine Derivatives.

(*National Institute of Hygienic Sciences**2)

In a previous paper of this series,[1] 4-nitro-3,6-dialkoxypyridazine 1-oxides were reported to have cancerostatic and also bacteriostaic actions *in vitro* except 4-nitro-3,6-dibutoxypyridazine 1-oxide. Therefore, it was of interest for us to synthesize 4-azido-3,6-dimethoxypyridazine 1-oxide for the examination of its biological activity.

The reaction of 4-chloro-3,6-dimethoxypyridazine 1-oxide[2] (I) was examined firstly in order to synthesize 3,6-dimethoxy-4-azidopyridazine 1-oxide (XII). When I was heated with sodium azide in hydrated ethanol at 100° for 3 hours in a sealed tube, 1-hydroxy-3-methoxy-4-chloro-6(1*H*)-pyridazinone (II) was produced in 35% yield, and 60% of the starting material was recovered unchanged. The structure of II was proved as follows.



Va : R=CH_3 Vb : R=C_2H_5

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1) T. Itai, S. Sako : This Bulletin, **9**, 149 (1961).

2) T. Itai, H. Igeta : Yakugaku Zasshi, **75**, 966 (1955).