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66. Fumiyoshi Ishikawa, Akihiko Nomura, Tohru Ueda, Morio Ikehara, and Yoshihisa Mizuno: A New and Convenient Synthesis of 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose from Guanosine; Some Observations on Solvolysis of 2',3',5'-Tri-O-benzoylguanosine.*1

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1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (I) is a key intermediate in the synthesis of ribonucleosides; purine, pyrimidine, and related nucleosides are usually synthesized by condensation of mercury salts of the corresponding bases with 2,3,5-tri-O-benzoylribofuranosyl chloride derived easily from (I).[1]

Weygand and Wirth were the first to prepare (I) from adenosine[2] and later from guanosine.[3] One year later, Fletcher, Ness, and Diehl described a method for synthesis of (I) from D-ribose which consisted of five steps shown in Chart 1.[4]

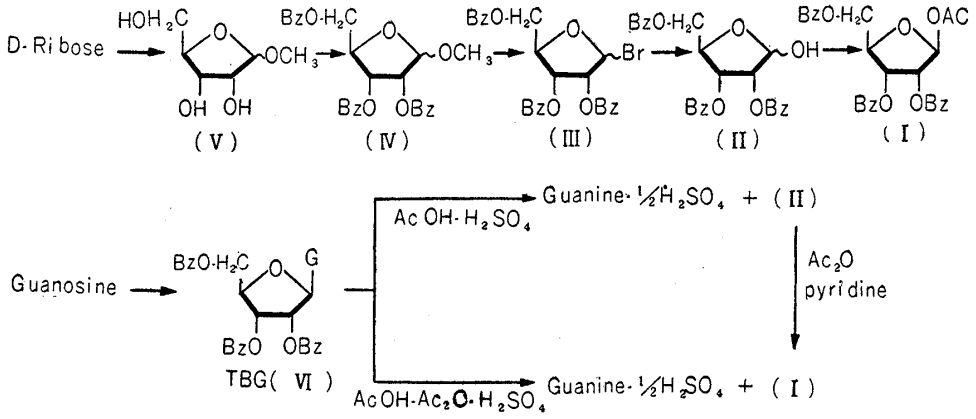

Chart 1.

In 1955, Kissman, Pidacks, and Baker[5] prepared (I) by a similar but improved procedure, without isolating the intermediates (V) to (II). The procedure has been replaced by the one proposed by Recondo and Rinderlenecht[6] which involved acetolysis of methyl 2,3,5-tri-O-benzoylribofuranoside to 1-O-acetyl derivative. The overall yield (57%) of (I) by this method[6] is fairly good. However, the synthesis of (I) from guanosine is still in use, especially in cases where guanosine is more easily available.

In connection with investigations on potential anticancer agents of nucleoside and nucleotide series, a large quantity of (I) was required. As reported before,[7] guanosine is now easily obtained in a large quantity and, therefore, Weygand and Sigmund's synthesis[3] was repeated in this laboratory with some modifications. In this case, a mixture of

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*1 Part of this work was presented at the 11th and 12th Annual Meeting of the Pharmaceutical Society of Japan (1958, 1959).

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1) J. Davoll, B. A. Lowy: J. Am. Chem. Soc., **73**, 1650(1951).
2) F. Weygand, F. Wirth: Chem. Ber., **85**, 1000(1952).
3) F. Weygand, W. Sigmund: *Ibid.*, **86**, 160(1953).
4) R. K. Ness, H. W. Diehl, H. G. Fletcher: J. Am. Chem. Soc., **76**, 763(1954).
5) H. K. Kissman, C. Pidacks, B. R. Baker: *Ibid.*, **77**, 18(1955).
6) R. E. Recondo, H. Rinderlenecht: Helv. Chim. Acta, **42**, 1171(1959).
7) Y. Mizuno, K. Nakamura, T. Ueda: Yakugaku Zasshi, **77**, 683(1957).

glacial acetic acid and sulfuric acid was employed as a hydrolytic agent of 2′,3′,5′-tri-O-benzoylguanosine (VI) in place of a mixture of isopropyl ether and sulfuric acid. (VI) was dissolved in a mixture of acetic acid and 10*N* sulfuric acid (9:1) and heated at 100° for several minutes until the precipitation of guanine sulfate was completed. 2,3,5-Tri-O-benzoylribofuranose (II) was isolated from the reaction mixture and acetylated with a mixture of acetic anhydride and pyridine to yield (I).

As shown in Table I, the maximum yield in this procedure was only 30%, an average of 25%. The low and erratic yield may be ascribed to difficulty in controlling the reaction condition and to the occurrence of unfavorable side reactions. In fact, sometimes benzoic acid was isolated from the reaction mixture, formed by hydrolysis of O-ester linkage.

It is noteworthy that in several cases, a nitrogen-free, crystalline compound (needles, m.p. 111~113°, $[\alpha]_D^{22}$ +10.4°) was isolated, which had almost the same infrared absorption pattern as that of (I) but had a different melting point and elemental analytical values (corresponding to $C_{28}H_{24}O_9 \cdot H_2O$ or $C_{28}H_{26}O_{10}$). The properties and the mode of its formation[8] suggested that this compound may be a hydrated form of (VII) or α-anomer of (I). To ascertain this possible assignment, following series of reactions were tested to convert the compound (VII) to (VIII), according to Fletcher's description.[9] However, the end pro-

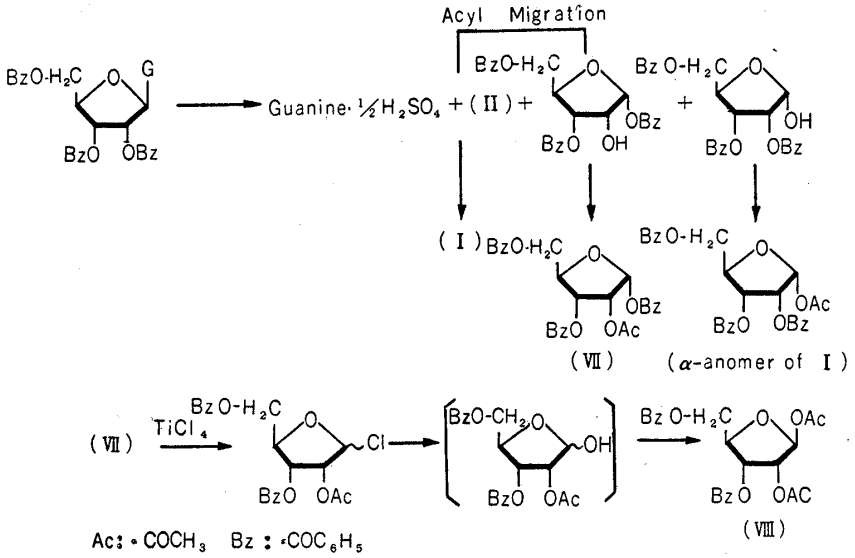

Chart 2.

duct of the reaction sequence was found to be the same as the starting material (mixed m.p. of two compounds, 111~113°). This fact led to another assumption that the compound of m.p. 111~113° might be 1,1,4-tri-O-acetyl-2,3,5-tri-O-benzoylribose, because the elemental analytical data also corresponded to this structure. However, final conclusion is reserved for further investigations.

Meanwhile, Joseph, Schaub, and Baker[10] found that methyl 2,5-di-O-benzoyl-3-phthalimido-3-deoxy-D-ribofuranoside is converted to 1-O-acetyl compound in a mixture of glacial acetic acid and acetic anhydride containing a small amount of conc. sulfuric acid. Their finding suggests that 2′,3′,5′-tri-O-benzoylguanosine might suffer the same type of acetolysis with the above reaction mixture, although no literature could be found describing such a type of acetolysis. This view was fully borne out by the following experiments: 2′,3′,5′-Tri-O-benzoylguanosine (VI) was dissolved in a mixture of acetic

8) R. K. Ness, H. G. Fletcher: J. Am. Chem. Soc., **76**, 1663(1954).
9) *Idem*: *Ibid.*, **78**, 4710(1956).
10) J. P. Joseph, R. E. Schaub, B. R. Baker: *Ibid.*, **77**, 5905(1955).

anhydride and acetic acid by heating at 70° and, after cooling to 15~20°, conc. sulfuric acid was added in small portions with stirring, and the stirring was continued for few hours until the precipitation of guanine sulfate was completed. After removing guanine sulfate by filtration, (I) was isolated. The maximum yield of (I) from this procedure was 45% (average, 20%) and these results showed that yield of (I) is quite erratic. In these reactions, besides pure and crystalline (I), a large quantity of vitreous, chloroform-soluble material was obtained. Upon triturating with ethanol, a part of the vitreous residue solidified which gave, after recrystallization from acetone, needle-like crystals, m.p. 223°. When *p*-toluenesulfonic acid was substituted for sulfuric acid in this reaction, (I) was not isolated at all or obtained in a poor yield, accompanied by a large amount of the compound of m.p. 223°. The reason has not been clarified as yet.

In order to improve the yield of (I) and to devise a procedure which would give constant results, many attempts were made and following procedure was found to be satisfactory. (VI) was first dissolved in glacial acetic acid with heating, then conc. sulfuric acid was added after cooling, and finally acetic anhydride was added. The order of adding last two reagents was just reversed from that of the foregoing experiments. The results obtained are shown in Table III. The maximum yield of this experiment was 50% and an average was 40%.

By introducing the direct acetolysis procedure, five steps of Kissman's method was reduced to two steps and, in addition, the yield of (I) was comparable to that of Kissman's. It is also noteworthy that the direct acetolysis is one of rare cases[6,10,11] in which glycofuranosides easily undergo acetolysis without cleavage of the furanoside ring and perhaps the first observed in N-glycofuranosides. The success of this experiment suggested the use of the vitreous matter, obtained by chloroform extraction, as such to prepare the ribonucleosides in the hope that the overall yield of (I) from guanosine might be further increased. The results of the experiments along this line will be reported in due course.

Experimental

2',3',5'-Tri-O-benzoylguanosine (VI)—(VI) was prepared from guanosine according to the method of Fox.[12] Yield, 85~95%.

1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (I)—i) Reaction Nos. 1~3 in Table I: 2',3',5'-Tri-O-benzoylguanosine (VI) was dissolved in a mixture of AcOH and 10*N* H_2SO_4 (9:1), and the mixture was heated under reflux on a water bath until the precipitation of guanine sulfate was completed. After standing at room temperature, precipitate was filtered off (yield of guanine sulfate, 45~60%) and the filtrate was poured into ice-water (500 cc.). The mixture was subjected to $CHCl_3$ extraction (5×50 cc.), the combined extract was washed with $NaHCO_3$ solution and H_2O, and dried over anhyd. Na_2SO_4. The dried filtrate was concentrated under a reduced pressure to a syrup, which was acetylated with Ac_2O (15 cc.) and pyridine (40 cc.). After 3 or 4 days, acetylated mixture was concentrated to a syrup below 40°, poured into $CHCl_3$-H_2O (100 cc.:50 cc.), and the organic layer

Table I.

Reaction No.	TBG (g.)	AcOH-10*N* H_2SO_4 (9:1) (cc.)	Guanine sulfate (g.)	Guanine sulfate (%)	Yields of (I) (g.)	Yields of (I) (%)
1	10	50	2	60	2.5	29.6
2	20	100	4	60	4.2	26.2
3	10	50	1.5	45	1.5	17.9

TBG: 2',3',5'-Tri-O-benzoylguanosine.

11) E. J. Reist, L. Goodman, R. R. Spenser, B. R. Baker: J. Am. Chem. Soc., **80**, 3962(1958); R. E. Schaub, L. Goodman, B. R. Baker: *Ibid.*, **80**, 4692(1958); E. J. Reist, L. Goodman, B. R. Baker: *Ibid.*, **80**, 5775(1958).

12) J. J. Fox, I. Wempen, A. Hampton, I. L. Doerr: *Ibid.*, **80**, 1669(1958).

was separated. The aqueous layer was further extracted with $CHCl_3$ (5×30 cc.), the combined extract was washed with $NaHCO_3$ solution (3×20 cc.) and H_2O, and dried over anhyd. Na_2SO_4. After the solvent was removed, toluene was added to the residue and distilled to remove even a trace of pyridine. The residue thus obtained was chromatographed on alumina with $CHCl_3$ and all the evaporated eluate gave (I) by recrystallization from EtOH. It was pure enough to use for the synthesis of 2,3,5-tri-O-benzoylribofuranosyl chloride (I), m.p. 128~130°, $[\alpha]_D^{22}$ +41.2° (c=1.92, $CHCl_3$). Yield, 18~30%. *Anal.* Calcd. for $C_{28}H_{24}O_8$: C, 66.65; H, 4.79. Found: C, 66.37; H, 4.69. IR ν_{max}^{Nujol} cm^{-1}: 1070 (lactol ether); 1734, 1231 (carbonyl of acetate); 1720, 1285, 1117 (carbonyl of benzoate); 900, 882, 858 (C-H).

A needle-like substance, m.p. 111~113°, was isolated from the mother liquor of recrystallization of (I). $[\alpha]_D^{22}$ +10.4° (c=1.02, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{24}O_9 \cdot H_2O$ or $C_{28}H_{26}O_{10}$: C, 64.37; H, 5.02. Found: C, 64.06; H, 5.01. IR ν_{max}^{Nujol} cm^{-1}: 1070 (lactol ether); 1750, 1211 (carbonyl of acetate); 1730, 1287, 1112 (carbonyl of acetate); 883, 872 (C-H).

ii) Reaction Nos. 4~7 in Table II: (VI) was dissolved in a mixture of AcOH and Ac_2O by heating and cooled to the temperature specified in the sixth column in Table II. To this solution, conc. H_2SO_4 was added dropwise with stirring. Precipitated guanine sulfate was filtered off (yield ca. 60%),

Table II.

Reaction No.	TBG (g.)	AcOH (cc.)	Ac_2O (cc.)	conc. H_2SO_4 (cc.)	Reaction temp. (°C)	Time of stirring (hr.)	Guanine sulfate (g.)	Guanine sulfate (%)	Yield of (I) (g.)	Yield of (I) (%)
4	5	60	4	2	8~14	2	1.5	90	1.0	24.8
5	10	120	8	4	13~15	2	2.0	60	2.5	29.6
6	10	120	12	4	14~20	2	0.5	15	1.2	14.3
7	5	80	4	2	17~23	2	1.4	84	1.6	44.0

the filtrate was poured into 10 volumes of ice-water, and extracted with $CHCl_3$, which was washed with $NaHCO_3$ solution and H_2O, and dried over anhyd. Na_2SO_4. It was filtered and concentrated to a syrup under a reduced pressure which was chromatographed on alumina with $CHCl_3$. The eluate was evaporated and recrystallized from EtOH to scale-like crystals, m.p. 128~130° (yield, 14~44%). On standing the syrup in EtOH for several days, a part of it converted to a solid, sparingly soluble in EtOH and was recrystallized from Me_2CO to needles, m.p. 223~224°; $[\alpha]_D^{22}$ −59.0° (c=2.07, $CHCl_3$). *Anal.* Found: C, 69.13; H, 4.30; mol. wt. (Rast), ca. 700. IR ν_{max}^{Nujol} cm^{-1}: 1727, 1280, 1115 (carbonyl of benzoate); 1068 (lactol ether).

iii) Acetolysis with *p*-toluenesulfonic acid: A solution of 2 g. of (VI) dissolved in a mixture of AcOH and Ac_2O with heating was cooled and *p*-toluenesulfonic acid was added in small portions. Upon working up as mentioned above (Reaction Nos. 4~7) a compound of m.p. 223~224° was obtained solely.

iv) Standard procedure. Reaction Nos. 8~10 in Table III: (VI) was dissolved in AcOH with heating and, after cooling to the temperature indicated in Table III, conc. H_2SO_4 was added with stirring.

Table III.

Reaction No.	TBG (g.)	AcOH (cc.)	Ac_2O (cc.)	conc. H_2SO_4 (cc.)	Reaction temp. (°C)	Time of stirring (hr.)	Guanine sulfate (g.)	Guanine sulfate (%)	Yield of (I) before crystn. (vitreous) (g.)	Yield of (I) after crystn. (g.)	Yield of (I) after crystn. (%)
8	2	25	2	1	12~14	3	0.7	100	2.4	0.5	29.6
9	2	25	2	1	17~18	3	0.7	100	1.6	0.8	47.6
10	2	25	3	1	22~23	3	0.7	100	1.3	0.5	29.6

Then, Ac_2O was added dropwise and stirring was continued for 3 hr., during which guanine sulfate precipitated. Guanine sulfate was filtered (yield, almost quantitative) and the filtrate was poured into ice-water to separate solid material, which was collected on a filter. After washing with dil. $NaHCO_3$ solution and H_2O, the filter cake was dried over P_2O_5 at reduced pressure. A $CHCl_3$ solution of this material was chromatographed on alumina with $CHCl_3$. Scale-like crystals, m.p. 129~130°. Yield, 30~48%. In these experiments the compound of m.p. 223~224° was also obtained.

Attempted Conversion of (VII) to (VIII)—To a solution of 0.4 g. of (VII) in benzene (0.8 cc.), a solution of $TiCl_4$ (0.24 cc.) in benzene (40 cc.) was added at 20°. After standing at room temperature for 20 min., $CHCl_3$ was added, the mixture was washed with cold water and cold dil. $NaHCO_3$ solution, and dried over anhyd. Na_2SO_4. After evaporation of solvent, residual syrup was dissolved in pyridine (3 cc.) and Ac_2O (0.12 cc.). The mixture was left standing for 2 days and the solvent was removed. The trace of pyridine was distilled off with toluene. The residue was crystallized from EtOH, m.p. 112~113°.

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Summary

Acetolysis of 2′,3′,5′-tri-O-benzoylguanosine with glacial acetic acid, acetic anhydride, and sulfuric acid gave rise to 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in a fairly good yield (overall yield from guanosine was 50%). This method will afford a most convenient procedure for preparing 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose which is a key intermediate in the synthesis of ribonucleosides and ribonucleotides.

The direct acetolysis is one of the rare cases in which N-glycosides are easily acetolyzed without cleavage of the furanoside ring.

Some observations on hydrolysis and acetolysis of 2′,3′,5′-tri-O-benzoylguanosine were also described.

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67. Mitsuru Uchiyama*1 and Tyunosin Ukita*2 : Protection of Mammalia from the Poisoning of Radioactive Strontium. IV.*3 Aluminum Citrate.

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It was pointed out by Copp, *et al.*[1] that feeding of mice with low-phosphorus diet results in increasing excretion of administered strontium. In the previous paper of this series,[2] the effect of dihydroxyaluminum aminoacetate (DAA) on elimination of radioactive strontium was reported. In the case of DAA-treatment, the aluminum contained in DAA fed to mice with normal food was liberated in gastrointestinal canal and made the phosphates in the food insoluble to inhibit its gastrointestinal absorption. By this process the food containing DAA caused the same result as a low-phosphorus diet.

On the other hand, it has been indicated[3] that some kind of organic acids, i.e. citric and acetic acids, stimulated the excretion of strontium even when they were administered orally. Further, several organic acids, i.e. citric, tricarballylic, and lactic acids, are known to have the ability of forming chelate compounds with strontium and of stimulating excretion of the metal.[4] It may be possible to expect an additional effect in this line for aluminum salts of these acids. This paper deals with the result of the research on above-described effect of these salts.

Aluminum citrate, aluminum tricarballylate, and aluminum lactate were synthesized

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*3 Part III. M. Uchiyama : Yakugaku Zasshi, **78**, 254(1958).
1) D. H. Copp, *et al.* : UCRL-1464(1951) (C. A., **46**, 11398(1952)).
2) M. Uchiyama : Yakugaku Zasshi, **78**, 251(1958).
3) S. Akiya, M. Uchiyama : Seikagaku, **28**, 154(1956).
4) J. Schubert, H. D. Wallace, Jr. : J. Biol. Chem., **183**, 157(1950); J. Fried, M. White Rosenthal, J. Schubert : Proc. Soc. Exptl. Biol. Med., **92**, 331(1956); Y. Ito, *et al.* : This Bulletin, **6**, 34, 92(1958).