CHEMICAL
&
PHARMACEUTICAL BULLETIN

Vol. 13 No. 6 June 1965

[Chem. Pharm. Bull. 13(6) 639~642 (1965)]

UDC 612.398.145

83. Morio Ikehara, Hiroshi Tada, and Kei Muneyama : Studies of Nucleosides and Nucleotides. XXV.*1 Purine Cyclonucleosides. 2. Synthesis of 5′-Deoxyguanosine *via* an 5′,8-Cyclonucleoside.

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In the previous paper of this series,[1] we have reported the formation of 2′,8-anhydro linkage in a 9-β-D-glycosylpurine. On the basis of investigation by many workers[2,3] with pyrimidine nucleosides, cyclonucleoside (anhydronucleoside) linkages of the 3′,8- or 5′,8-positions of purine nucleosides would be expected to form. The latter possibility has now been substantiated with guanosine by conversion of an 5′,8-cyclonucleoside to 5′-deoxyguanosine.

In order to introduce easily replaceable substituent into 8-position of guanosine, bromination by means of dioxane-bromine was investigated.[4] 8-Bromoguanosine (I) was obtained in a crystalline form, m.p. >180° in 65% yield with chemical and optical properties consistent with those reported by Robins.[5] Compound (I) was then mesylated with mesyl chloride in pyridine. Although 3.3 equivalents of mesyl chloride were used, the only product isolated as crystalline form was the dimesyl derivative (II). This was confirmed by elemental analyses and spectrophotometric behavior.*3 One of the mesyl group should be on the 5′-position because of the facile cyclization of compound (III) to cyclonucleoside salt, although whose structure has not so far been wholly established. Accordingly, another mesyl group was assumed to be on 2′- or 3′-positions.

In order to replace 8-bromo atom by a mercapto group, compound (III) was refluxed in methyl cellosolve with 1.2 equivalents of thiourea for 4.5 hours. During this time the ultraviolet maximum shifted from 264 mμ to 270 mμ (8-mercaptoguanosine has a maximum at 300 mμ). Elemental analyses and ultraviolet absorption properties were consistent with the mono-mesyl anhydronucleoside structure (IV). Compound (IV) was desulfurizated with Raney nickel to 2′(or 3′)-monomesylated deoxyguanosine (V). The direct comparison of this deoxycompound after treatment with sodium methoxide with

*1 Part XXIV of this series. M. Ikehara, H. Tada : J. Am. Chem. Soc., 87, 606 (1965).

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*3 Although a slight bathochromic shift was observed in the mesyl derivative, the mesylation of base moiety was excluded by the following experiments and by the fact that 8-bromo-2′,3′-O-isopropylideneguanosine had the similar optical properties (Unpublished experiment by K. Muneyama).

1) M. Ikehara, H. Tada : J. Am. Chem. Soc., 85, 2344 (1963) ; *Ibid.*, 87, 606 (1965).
2) J. J. Fox, I. Wempen : Advance. Carbohydrate Chem., 14, 283 (1959).
3) A. M. Michelson : "The Chemistry of Nucleosides and Nucleotides," Academic Press, N. Y., (1963), pp. 15, 68.
4) A. M. Michelson briefly reported the bromination of guanosine, "The Chemistry of Nucleosides & Nucleotides," Academic Press. N. Y., (1963), p. 34.
5) R. E. Holmes, R. K. Robins : J. Am. Chem. Soc., 86, 1242 (1964).

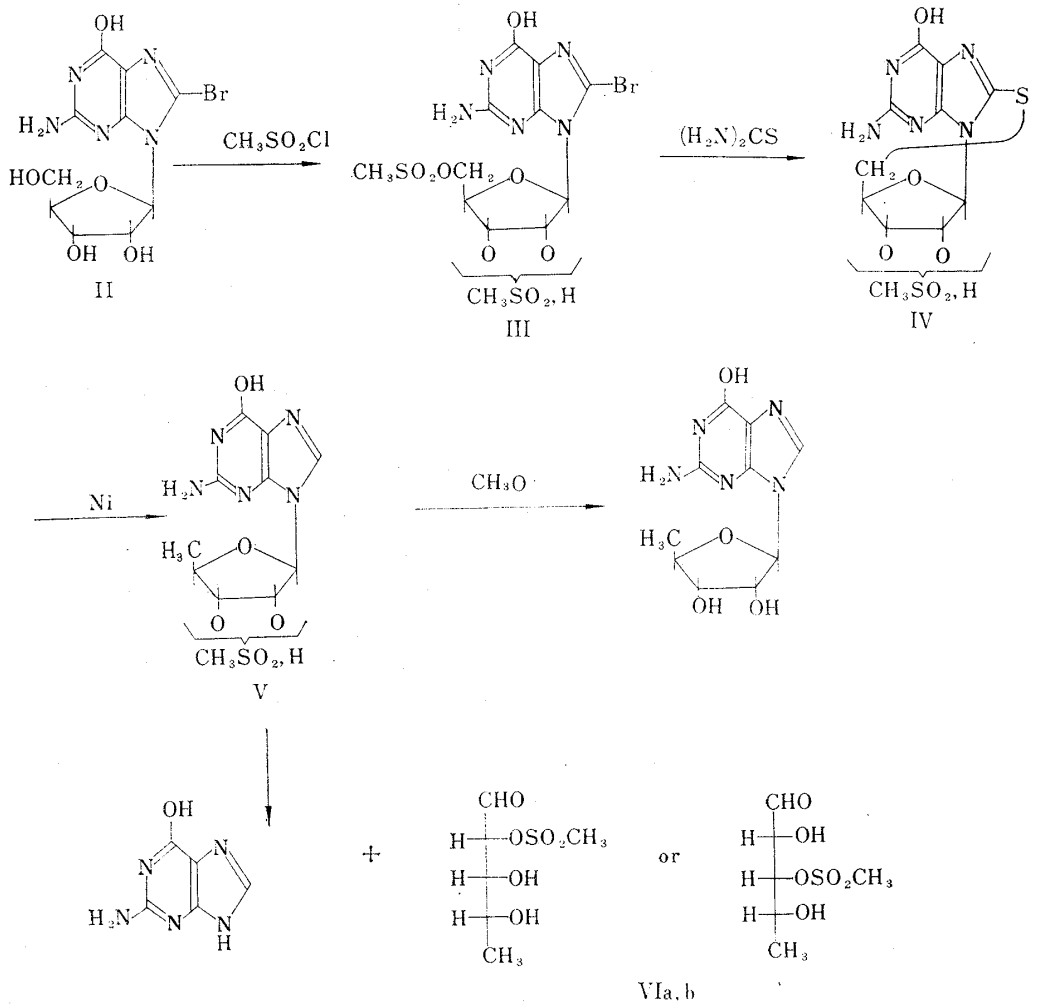

Chart 1.

an authentic 5′-deoxyguanosine synthesized from 5′-deoxy-5′-ethylmercapto-2′,3′-di-O-acetylguanosine*4 confirmed the structure of V as 2′(or 3′)-mesylated 5′-deoxyguanosine. Thus, the position of the anhydro linkage in IV was established as 5′,8 and one of the mesyl groups of compound (II) must be on 5′-position.

The final decision of the position of the other mesyl group of III was elucidated by the hydrolytic cleavage of nucleosidic linkage of V. Compound (V) was hydrolyzed either by *N* sodium hydroxide or by *N* hydrochloric acid at room temperature. Alkaline hydrolysis of V gave guanine and a deoxysugar, which was tentatively assigned as 5′-deoxy-D-arabinose. Hydrolysis of V with *N* hydrochloric acid gave guanine and a mesylated sugar (VI a or b). When VIa (VIb) was treated with metaperiodate[6] one mole of oxidant per mole was consumed without the liberation of acid. This suggested the structure of mono-mesylated 5′-deoxysugar as 2′-O-mesyl-5′-deoxy-D-ribose, VIa. The facile alkaline cleavage of monomesyl-5′-deoxyguanosine (V) could be explained by this structure, assuming elimination of methanesulfonic acid from $C^{1'}$ and $C^{2'}$. On this basis the structure of III is the 2′,5′-dimesyl derivative of 8-bromoguanosine. It is noteworthy that reaction of III with thiourea, replacement of 8-halogene substituent was followed by rapid formation of the 5′,8-rather than an 2′,8-anhydronucleoside. The

*4 This was the generous gift from Dr. E. J. Reist of Stanford Research Institute, to whom our thanks are due.

6) I. Akiya, S. Okui: Yakugaku Zasshi, **71**, 865 (1951).

higher reactivity of the 5′-rather than the 2′-mesyloxy leaving group (in contrast to the situation in pyrimidine nucleosides[7]) might be explained by the steric acceleration effect[8] of the 2-amino group of purine moiety.

From the above experiments, the formation of an anhydro linkage between 5′- and 8-position of guanosine is firmly established.

Experimental*5

Paper Chromatography——Solvent A, water (pH 10.0); solvent B butanol-water, 86:14; solvent C, iso-PrOH-ammonia (28%)-water, 7:1:2. All paper chromatographies were performed by ascending technique.

8-Bromoguanosine——Guanosine (5.66 g., dried over phosphorus pentoxide at 5 mm. Hg for 5 hr. at 80°) was dissolved in methyl cellosolve (600 ml.) with warming at 60～80° and stirring. Calcium carbonate (16 g.) was added into the solution followed by the addition of bromine (1.6 ml.) dissolved in dioxane (50 ml.), dropwise during 30～60 min. Stirring was continued for 20 hr. at 30～40°. Calcium carbonate was removed by filtration of the hot reaction mixture. The filtrate and washings (methyl cellosolve) were combined and evaporated *in vacuo* to a small volume. EtOH (500 ml.) was added to cause precipitation. Precipitates were collected by filtration and recrystllized from water. White needles were obtained in 52～65% yield. The sample colorized at 180° and then decomposed gradually. Ultraviolet absorption properties : $\lambda_{max}^{0.1NHCl}$ 261, $\lambda_{min}^{0.1NHCl}$ 223; $\lambda_{max}^{H_2O}$ 259, $\lambda_{min}^{H_2O}$ 223; $\lambda_{max}^{0.1NNaOH}$ 269, $\lambda_{min}^{0.1NNaOH}$ 233 mμ. Paper chromatography : Rf 0.16, guanosine 0.08 (solvent B). *Anal.* Calcd. for $C_{10}H_{14}N_5O_6Br \cdot H_2O$: C, 31.56; H, 3.68; N, 18.24. Found : C, 31.95; H, 3.70; N, 19.02.

8-Bromo-2′,5′-di-O-mesylguanosine——8-Bromoguanosine (2.173 g., dried over phosphrus pentoxide at 70～80° at 5 mm. for 5 hr.) was dissolved in dry pyridine (50 ml.). The pyridine solution was evaporated to 2/3 volume in order to remove the traces of water (occasionally a small amount of bromoguanosine precipitated by this procedure). Mesyl chloride (2.267 g., 3.3 equivalents) dissolved in 20 ml. of dry pyridine was added dropwise into the solution at −15° during 30 min. The reaction mixture was kept in a refrigerator under exclusion of moisture for 12 hr. After standing at room temperature for 1 hr., 20 ml. of anhydrous EtOH was added and the reaction maintained at room temperature for 1 hr. The solution was poured into ice-water and the brownish precititates were collected by filtration. Recrystallization from ethanol-water gave yellow crystalls, m.p. 184° (decomp.), yield, 37～42%. Infrared absorption : Strong absorption at ν_{max}^{NaCl} 1170 cm^{-1} (aliphatic sulfonate). Ultraviolet absorption properties : $\lambda_{max}^{0.1NHCl}$ 264, $\lambda_{min}^{0.1NHCl}$ 225; $\lambda_{max}^{H_2O}$, 264, $\lambda_{min}^{H_2O}$ 226, $\lambda_{max}^{0.1NNaOH}$ 273, $\lambda_{min}^{0.1NNaOH}$ 236 mμ. Paper chromatography : Rf 0.53 (solvent A), 0.32 (solvent B). *Anal.* Calcd. for $C_{12}H_{12}N_5O_9SBr \cdot H_2O$: C, 26.86; H, 3.35; N, 13.06. Found : C, 26.45; H, 3.28; N, 13.06.

Cyclization of 8-Bromo-2′,5′-di-O-mesylguanosine——8-Bromo-2′,5′-O-mesylguanosine (20 mg.) was heated in 30 ml. of acetonylacetone at 100～110° for 4 hr. Light yellow mixture turned to dark red at the end of the reaction. Ultraviolet absorption of this solution : $\lambda_{max}^{0.1NHCl}$ 254, $\lambda_{min}^{0.1NHCl}$ 230; $\lambda_{max}^{H_2O}$ 254, $\lambda_{min}^{H_2O}$ 230; $\lambda_{max}^{0.1NNaOH}$ 254, $\lambda_{min}^{0.1NNaOH}$ 230 mμ. Infrared absorption : λ_{max}^{Nujol} 9.8 (RSO_2O^-) and 8.5 μ (RSO_2O^-). Paper chromatography : Rf 0.35 (solvent B), 0.02 (solvent).

When silver nitrate solution was added to the reaction mixture, a white-yellow precipitates appeared, which showed the presence of ionic sulfonate.

2′-O-Mesyl-8-mercapto-5′, 8-anhydroguanosine——8-Brome-2′, 5′-di-O-mesylguanosine (518 mg.) and thiourea (91 mg., 1.2 equiv.) were dissolved in methyl cellosolve (50 ml.) and refluxed for 5 hr. Solvent was removed by vacuum distillation and the residue was recrystallized from ethanol-water (80:20, v/v). A yellow crystalline material, m.p. 205° (decomp.) was obtained in the yield of 32%. Ultraviolet absorption properties : $\lambda_{max}^{0.1NHCl}$ 269, $\lambda_{min}^{0.1NHCl}$ 227; $\lambda_{max}^{H_2O}$ 269, $\lambda_{min}^{H_2O}$ 277; $\lambda_{max}^{0.1NNaOH}$ 281, $\lambda_{min}^{0.1NNaOH}$ 239 mμ. Infrared absorption : ν_{max}^{NaCl} 1170 cm^{-1} (strong, RSO_2O^-). *Anal.* Calcd. for $C_{11}H_{13}O_6N_5S_2 \cdot H_2O$: C, 34.39; H, 3.65; N, 18.18. Found : C, 34.19; H, 4.07; N, 18.49.

A part of the above reaction mixture was heated in *N* NaOH (1 ml.) at 30° for 30 min. and at 40° for 40 min. Ultraviolet absorption bands appeared at 283 mμ and showed no change from the starting material (This excluded the possibility of compound (Ⅳ) as the thiuronium salt).

Desulfurization of 8-Mercaptoguanosine with Raney Nickel——A small amount of 8-mercaptoguanosine was suspended in methyl cellosolve (5 ml.) and refluxed with Raney nickel (one small spoonful) for

*5 Ultraviolet absorption spectra were taken with Beckman DK-Ⅱ or Hitachi EPS-2U automatic recording spectrophotometer. Infrared absorption spectra were taken with Jasco DS-301 spectrophotometer. All melting points are uncorrected.

7) N. C. Yung, J. J. Fox : J. Am. Chem. Soc., 83, 3060 (1961).
8) T. Okamoto, J. F. Bunnett : J. Am. Chem. Soc., 78, 5357, 5363 (1956).

5 hr. Examination of reaction mixture showed $\lambda_{max}^{0.1N HCl}$ 257; $\lambda_{max}^{H_2O}$ 256; $\lambda_{plateau}^{0.1N NaOH}$ 258~271 mμ. Paper chromatography: Rf 0.15, guanosine 0.15, 8-bromoguanosine 0.28 (solvent C); Rf 0.61, guanosine 0.61 and 8-bromoguanosine 0.54 (solvent A).

2'-O-Mesyl-5'-deoxyguanosine——2'-O-mesyl-8-mercapto-5',8-anhydroguanosine (100 mg.) was dissolved in methyl cellosolve-water (1:1, v/v) (30 ml.) and refluxed for 3 hr. with Raney nickel (50 mg.). The reaction mixture had ultraviolet absorption properties: $\lambda_{max}^{0.1N HCl}$ 257, $\lambda_{min}^{0.1N HCl}$ 236; $\lambda_{max}^{H_2O}$ 256, $\lambda_{min}^{H_2O}$ 227; $\lambda_{max}^{0.1N NaOH}$ 258~271, $\lambda_{min}^{0.1N NaOH}$ 239 mμ, similar to guanosine. Paper chromatography: Rf 0.08, guanosine 0.15, 8-bromoguanosine 0.22 (solvent C). No 2'- or 3'-deoxysugar was detected by the Dische's reagent.[9]

5'-Deoxyguanosine——2'-O-Mesyl-5'-deoxyguanosine (10 mg.) was dissolved in anhydrous methanol (30 ml.) containing 200 mg. of sodium metal. After allowing to stand at room temperature, 4 g. of IRC-50 (H^+) was added and shaken for 5 hr. Paper chromatography of the reaction mixture: Rf 0.06, R_{Ad}*6 0.36 (same as that reported by Reist, *et al.*[10]) (solvent B), Rf 0.20, adenosine 0.46, 5'-deoxyguanosine 0.20 (solvent C). All the spots consumed metaperiodate.

Hydrolysis of 2'-O-Mesyl-5'-deoxyadenosine——a) 2'-O-Mesyl-5'-deoxyguanosine (15 mg., 0.044 mole) was dissolved in *N* HCl for 7 days, followed by the heating at 80° for 4 hr. The reaction mixture was shaken with IRA (OH^-) and the pH of the solution was adjusted to 7.0. Resin was removed by filtration and the filtrate was concentrated *in vacuo* to 1~2 ml. Sodium metaperiodate (0.25*M*, 2 ml.) was added and the total volume of the solution was adjusted to 10 ml. by the addition of 33% sodium chloride solution. 1/5 of the above solution was added with 5 ml. of water, 5 ml. of boric acid-sodium perborate buffer(0.1*M*) and 100 mg. of potassium iodide. Titration with arsenic trioxide (0.1*N*) solution showed 0.04 mole/mole of metaperiodate consumption. Titration with 0.01*N* sodium hydroxide of another 1/5 of the above reaction mixture showed that acid was not formed from this oxidation.

b) A small amount of 2'-O-mesyl-5'-deoxyguanosine was dissolved in 1 ml. of *N* sodium hydroxide solution and kept at room temperature for 14 hr. After removing sulfonate with IRA-410 (Cl^-), reaction mixture showed absence of sulfur by sodium nitroprusside or lead acetate after the fusion with sodium. Color test with Dische's reagent was yellow (no 2'- or 3'-deoxy sugar). Paper chromatography: Rf 0.76 (metaperiodate consuming) and 0.31 (ultraviolet absorbing). D-Ribose 0.87, Guanosine (alkaline treated) 0.53 (solvent A).

The authors wish to thank Mrs. T. Tohma and Miss A. Maeda of this Faculty for the elemental analyses.

Summary

8-Bromoguanosine was directly obtained from guanosine by means of dioxane-bromine bromination. Mesylation of 8-bromoguanosine gave 2',5'-di-O-mesyl derivative, which was converted to 2'-O-mesyl-8-mercapto-5',8-anhydroguanosine by the treatment with thiourea. Desulfurization of 5',8-cyclonucleoside and subsequent removal of mesyl group gave 5'-deoxyguanosine. Acid hydrolysis of 2'-O-mesyl-5'-deoxyguanosine gave 2'-O-mesyl-5'-deoxyribose.

(Received November 13, 1964)

*6 R_{Ad} stands for Rf value divided by Rf of adenosine.

9) Z. Dische: J. Biol. Chem., **181**, 379 (1949).
10) E. J. Reist, F. A. Hart, L. Goodman, B. R. Baker: J. Org. Chem., **26**, 1557 (1961).