C, 38.26; H, 4.38; N, 15.64. Ultraviolet absorption properties were $\lambda_{max}^{pH\ 1}$ 247 mμ (ε 13,800), 294 (13,200); $\lambda_{max}^{H_2O}$ 247 mμ (ε 13,100), 294 (12,400); $\lambda_{max}^{pH\ 13}$ 258 mμ (ε 11,100), 278 (10,700). The infrared absorption spectrum showed ν_{max}^{Nujol} 1170 cm^{-1} (RSO_2O). Paper chromatography showed R_f (B) 0.24, R_f (C) 0.34.

Attempted Cyclization of 2′,3′-*O*-Isopropylidene-5′-*O*-mesyl-8-oxyguanosine.—A small amount (*ca.* 5 mg) of 2′,3′-*O*-isopropylidene-5′-*O*-mesyl-8-oxyguanosine was heated with the following reagents: (i) sodium acetate in dimethylformamide (or Methyl Cellosolve) at 100–110° for 1 hr; (ii) sodium acetate in dimethylformamide + acetic anhydride at 120–130° for 10 hr; (iii) triethylamine in tetrahydrofuran, refluxing for 1 hr; (iv) refluxing for 4 hr in tetrahydrofuran; (v) refluxing for 12 hr in water. In all these cases ultraviolet absorption of an aliquot withdrawn from the reaction mixture showed $\lambda_{max}^{H^+}$ 252–258, 287–290 mμ; $\lambda_{max}^{OH^-}$ 256–258 (sh), 298–300 mμ. Paper chromatography showed R_f (A) 0.66, R_f (B) 0.08. In the reaction in water (v) the pH of the reaction mixture became strongly acidic which may be caused by the liberated methylsulfonic acid.

N^3,5′-Cyclo-2′,3′-*O*-isopropylidene-8-oxyguanosine.—N^3,5′-Anhydro-2′,3′-*O*-isopropylidene-8-bromoguanosine[17] (4 mg) was refluxed with sodium acetate (8 mg) in acetic acid (10 ml) for 1 hr. Acetic acid was removed by distillation *in vacuo* and the residue was washed with water to remove sodium acetate. Solid material, thus obtained, showed the following ultraviolet absorption properties: $\lambda_{max}^{H^+}$ 251, 290 mμ; $\lambda_{max}^{OH^-}$ 258 (sh), 300 mμ. Paper chromatography showed R_f (A) 0.66, R_f (B) 0.08. These properties were well coincided with those observed in the above cyclization reactions.

Registry No.—I, 13591-82-7; II, 13591-83-8; III, 13591-84-9; IV, 13591-85-0; V, 13573-35-8; VI, 13591-86-1; VII, 13565-77-0; VIII, 13565-78-1; IX, 13591-87-2.

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Studies of Nucleosides and Nucleotides. XXXVI.[1] Purine Cyclonucleosides. 6.[2] Further Investigation on the Formation of 8,5′-*S*-Cyclonucleoside from Guanosine[3]

MORIO IKEHARA AND KEI MUNEYAMA

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

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2′,3′-*O*-Isopropylidene-5′-*O*-mesyl-8-bromoguanosine (II) was converted into 8,5′-anhydro-2′,3′-*O*-isopropylidene-8-mercaptoguanosine (III) *via* the 5′-*O*-mesyl derivative. The structure of III was elucidated by the desulfurization to afford 5′-deoxyguanosine, followed by the acid-catalyzed removal of the isopropylidene group. Another cyclonucleoside having the 5′,N^3-anhydro structure was obtained from compound II.

Recently, the synthesis of cyclonucleosides of purines have been reported[4–6] from our laboratory. Among these purine cyclonucleosides, 8,2′-*S*- and 8,2′-*O*- as well as 8,3′-*S*-cyclonucleosides in the adenosine series were investigated extensively as to their chemical and physical properties. Although an attempt to form an 8,5′-cyclonucleoside of adenosine failed because of a rapid cyclization to the N^3 position, a nucleoside having an 8,5′-*S* cyclo linkage could be synthesized in guanosine.[7] This fact indicated that in guanosine the cyclization to 8,5′-cyclonucleoside preceded N^3,5′ cyclization, presumably because of its lower pK value of base moiety than is the case for adenosine.[8] However, in this instance 2′,5′-di-*O*-mesyl-8-bromoguanosine was chosen as the starting material and the resulting cyclonucleoside has a mesyl group on 2′ position, which could not easily be removed. We have, therefore, reinvestigated the formation of the 8,5′-*S* cyclo linkage in 2′,3′-*O*-isopropylidene-5′-*O*-mesyl-8-bromoguanosine and, from the resulting cyclonucleoside, 5′-deoxyguanosine could be obtained by the desulfurization with Raney nickel. In the course of this study, a cyclization by product, N^3,5′-cyclo-8-bromoguanosine, was obtained.

2′,3′-*O*-Isopropylidene-8-bromoguanosine[10,11] (I) was mesylated with 1.2 equiv of mesyl chloride in pyridine to afford the 5′-*O*-mesyl derivative (II) (Scheme I). Although ultraviolet absorption properties of compound II showed a slight shift of the absorption maxima from those of 8-bromoguanosine,[7,12] a similar change observed for 2′,5′-di-*O*-mesyl-8-bromoguanosine[7] suggested that the mesylation had occurred only on the furanose moiety. An infrared absorption band at 1170 cm^{-1} also suggested this view. Since the elemental analytical data confirmed the monomesylation, the structure of compound II was established at 2′,3′-*O*-isopropylidene-5′-*O*-mesyl-8-bromoguanosine.

The cyclization reaction was carried out by refluxing with thiourea as reported previously.[7] When compound II was refluxed in dioxane or tetrahydrofuran[13] in the presence of thiourea, a compound (III) having ultraviolet absorption properties similar to those reported for 8-methylmercaptoguanosine[12,15] was obtained. From the previous studies of the purine cyclonucleosides,[6,7] the similarity in ultraviolet absorption properties of compound III with 8-methylmercaptoguanosine was as expected. Although the efforts to crystallize compound III failed, a glass obtained by the vacuum evaporation of the solvent showed an elemental

(1) Part XXXV: M. Ikehara, H. Tada, and M. Kaneko, in preparation.

(2) Part 5 of this series, same as above.

(3) This work has been presented at the Hokkaido Regional Meeting of Pharmaceutical Society of Japan, 1966.

(4) M. Ikehara and H. Tada, *J. Am. Chem. Soc.*, **85**, 2344 (1963); **87**, 606 (1965).

(5) M. Ikehara and H. Tada, *Chem. Pharm. Bull.*, **15**, 94 (1967).

(6) M. Ikehara, H. Tada, J. Muneyama, and M. Kaneko, *J. Am. Chem. Soc.*, **88**, 3165 (1966).

(7) M. Ikehara, H. Tada, and K. Muneyama, *Chem. Pharm. Bull.*, **13**, 639 (1965).

(8) N^6 acetylation of adenosine inhibited the N^3,5′ cyclization of 5′-*O*-tosyladenosine.[9]

(9) W. Jahn, *Chem. Ber.*, **98**, 1705 (1965).

(10) M. Ikehara, H. Tada, and K. Muneyama, *Chem. Pharm. Bull.*, **13**, 1140 (1965).

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(12) R. E. Holmes and R. K. Holmes, *J. Am. Chem. Soc.*, **86**, 1242 (1964).

(13) Since these solvents extremely enhanced the reaction, some interaction[14] with bromoguanine moiety could be assumed.

(14) R. A. Vitali, E. S. Inamine, and T. A. Jacob, *Arch. Biochem. Biophys.*, **112**, 313 (1965).

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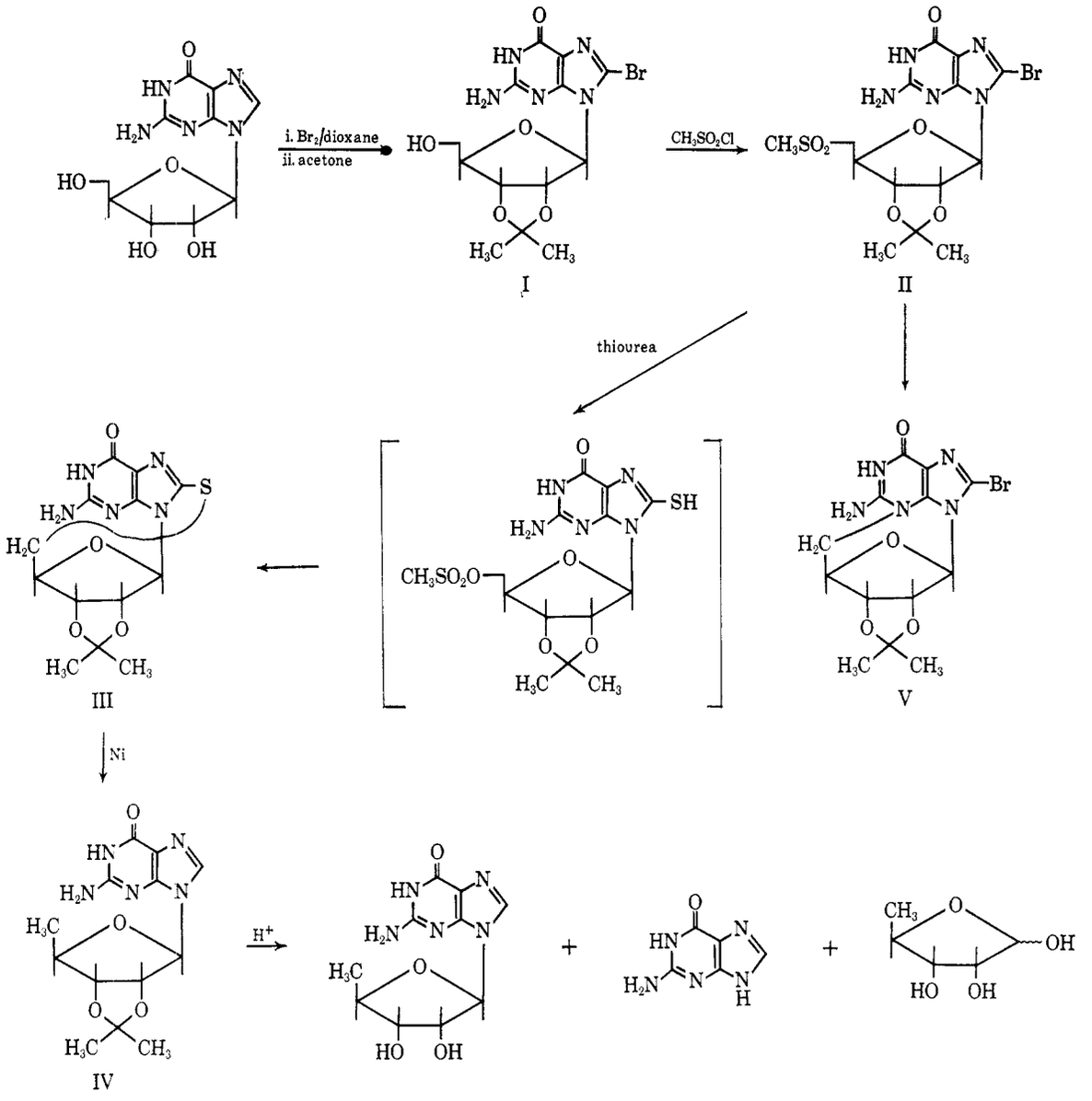
SCHEME I



analysis value consistent with 2′,3′-*O*-isopropylidene-8,5′-cyclo-8-mercaptoguanosine.

In order to decide finally the position of the cyclo linkage in compound III, it was desulfurized with Raney nickel to afford the 5′-deoxyguanosine derivative (IV). Ultraviolet absorption properties changed from $\lambda_{max}^{OH^-}$ 289 mμ to $\lambda_{max}^{OH^-}$ 269 mμ indicating the presence of 9-substituted guanine residue. The similarity in behaviors on paper chromatography to isopropylideneguanosine also supported this view. The final decision of the structure of compound IV was achieved by the removal of protecting group with dilute acetic acid accompanied by some cleavage of glycosidic linkage to afford 5′-deoxyguanosine, guanine, and 5-deoxyribose. These products were identical with the authentic samples[16] by paper chromatography in two solvent systems. Easier cleavage of nucleosidic linkage in 5′-deoxyguanosine than in the case of guanosine has been reported previously.[17] The position of the anhydro linkage in compound III was thus concluded to be in 8,5′ position.

In the course of the study of 8,5′ cyclization, we have observed that, in the recrystallization of 2′,3′-*O*-isopropylidene-5′-*O*-mesyl-8-bromoguanosine (II), a single spot having R_f (A)[18] 0.65 gradually changed to R_f (A) 0.52 by the repeated recrystallizations. The ultraviolet absorption also changed from $\lambda_{max}^{H^+}$ 263 mμ to 270 mμ. Similar changes were observed previously in the case of 2′,3′-*O*-isopropylidene-5′-*O*-tosyladenosine.[19] Absence of absorption peak at 1170 cm^{-1} in compound V and elemental analysis also supported the 5′,N^3-cyclization structure. Since it was reported that 5′-sulfonylated[20] or -phosphorylated[21] guanosine easily cyclized to form N^3,5′-cyclonucleosides, the cyclization of 2′,3′-*O*-isopropylidene-5′-*O*-mesyl-8-bromoguanosine could be expected. Moreover by the treatment with sodium acetate in acetic acid compound V gave the

(16) We are indebted to Dr. E. J. Reist of Stanford Research Institute for the gift of 5′-deoxy-5′-ethylmercapto-2′,3′-*O*-isopropylideneguanosine, which was derived to 5′-deoxyguanosine.

(17) E. J. Reist, P. A. Hart, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 1557 (1961).

(18) R_f (A) stands for R_f in paper chromatography in solvent A (water adjusted to pH 10 with ammonia; see Experimental Section).

(19) V. M. Clark, A. R. Todd, and J. Zussman, *J. Chem. Soc.*, 2952 (1951).

(20) R. E. Holmes and R. K. Robins, *J. Org. Chem.*, **28**, 3483 (1963).

(21) R. W. Chambers, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, **79**, 3747 (1957).

same N^3,5′-cyclization product obtained from 2′,3′-*O*-isopropylidene-5′-*O*-mesyl-8-oxyguanosine.[11] Considering this evidence together with elemental analytical data, the structure of V could be assigned as that of 2′,3′-*O*-isopropylidene-N^3,5′-cyclo-8-bromoguanosine. Although the possibility of the cyclization to 2-NH_2 group could not be eliminated, the N^3,5′-cyclization structure is much more likely.

When 2′,3′-isopropylidine-8-bromoguanosine (I) was tosylated in pyridine at room temperature, intermedially formed 5′-tosylate easily cyclized to give compound V. This fact may be ascribed to the large steric interaction[22] of 5′-phenylsulfonyl group with guanine moiety, which enhanced the leaving of tosyloxy group. Although introduction of a bromine atom into the 8 position of guanosine lowered the pK_a value for 0.5 units, N^3,5′ cyclization could not be avoided. This is in contrast to the expectation that the acidic nature of the base moiety makes cyclization difficult.

The formation of 8,5′-*S*-cyclonucleoside in guanosine was thus reconfirmed and another route for the synthesis of 5′-deoxyguanosine was developed (see Scheme I).

Experimental Section[23]

Paper Chromatography.—All chromatography was performed by the ascending method on Toyo filter paper no. 51 A: solvent A, water adjusted to pH 10 with ammonia; solvent B, 1-butanol–water, 86:14; solvent C, 2-propanol–ammonia–water, 7:1:2.

2′,3′-*O*-Isopropylidene-5′-*O*-mesyl-8-bromoguanosine.—2′,3′-*O*-Isopropylidene-8-bromoguanosine[11] (804 mg, 2 mmoles) was dissolved in anhydrous pyridine (30 ml). Into this mixture was added dropwise a solution of mesyl chloride (276 mg, 2.4 mmoles) in pyridine (15 ml) with cooling in an ice–salt bath. After it was kept for 2 hr at room temperature, the mixture was stored in a refrigerator for 20 hr. When this solution was poured into ice–water, a cream-colored substance precipitated. The precipitate was collected by filtration and recrystallized from a water–ethanol–acetone mixture. Crystalline material was obtained in a yield of 40% (385 mg). *Anal.* Calcd for $C_{14}H_{18}N_5O_7SBr$: C, 35.00; H, 3.75; N, 14.58. Found: C, 35.07; H, 3.94; N, 14.96. Ultraviolet absorption properties were $\lambda_{max}^{pH\ 1}$ 263, 275 (sh) mμ; $\lambda_{max}^{H_2O}$ 263, 275 (sh) mμ; $\lambda_{max}^{pH\ 13}$ 272 mμ (because of the rapid decomposition, ε values are not given). The infrared spectrum showed ν_{max}^{Nujol} 1170 cm^{-1} (mesyl). Paper chromatography showed R_f (A) 0.65, R_f (B) 0.33.

8,5′-Cyclo-2′,3′-*O*-isopropylidene-8-mercaptoguanosine.—A solution of thiourea (91 mg, 1.2 mmoles) in dioxane (100 ml) was heated at 80–90°. To this solution was added isopropylidene-mesylbromoguanosine (480 mg, 1 mmole) to form a clear solution. Soon after the end of the addition, a precipitate began to appear. The reflux was continued further 20 min; then the solvent was removed *in vacuo*. The product was extracted with ethanol, which was evaporated *in vacuo* to afford a glass (350 mg). *Anal.* Calcd for $C_{13}H_{15}O_4N_5S \cdot H_2O$: C, 43.94; H, 4.78; N, 19.72. Found: C, 44.42; H, 4.59; N, 19.38. Ultraviolet absorption properties were $\lambda_{max}^{pH\ 1}$ 273 mμ (ε 12,900), $\lambda_{max}^{H_2O}$ 274 mμ (ε 15,700), $\lambda_{max}^{pH\ 13}$ 289 mμ (ε 12,600). No infrared absorption band was observed at 1170 cm^{-1}. Paper chromatography showed R_f (A) 0.43, R_f (B) 0.39.

2′,3′-*O*-Isopropylidene-5′-deoxyguanosine.—8,5′-Anhydro-2′,3′-*O*-isopropylidene-8-mercaptoguanosine (100 mg) was refluxed for 3 hr in 50% aqueous Methyl Cellosolve with 1 spoonful of Raney nickel. The catalyst was removed by filtration; the filtrate and washing were combined and evaporated *in vacuo*. The residual hard oil was dried over phosphorus pentoxide *in vacuo* (yield 30 mg). Ultraviolet absorption properties were $\lambda_{max}^{H^+}$ 254, 268 (sh) mμ; $\lambda_{max}^{OH^-}$ 258, 269 mμ. Paper chromatography showed R_f (A) 0.65 (guanosine 0.49, isopropylideneguanosine 0.59), R_f (B) 0.63 (guanosine 0.07, isopropylideneguanosine 0.43).

Acidic Treatment of 2′,3′-*O*-Isopropylidene-5′-deoxyguanosine.—2′,3′-*O*-Isopropylidene-5′-deoxyguanosine (10 mg) was dissolved in 2% acetic acid (4 ml) and refluxed for 45 min. Evaporation of the solvent with the repeated addition of ethanol gave a glass. Paper chromatography performed in two solvent systems showed three spots each: R_f (A) 0.57 (revealed by metaperiodate spray[24] and ultraviolet absorption), 0.59 (revealed by ultraviolet absorption), and 0.62 (revealed by metaperiodate spray); R_f (B) 0.05 (revealed by ultraviolet absorption), 0.13 (revealed by ultraviolet absorption and metaperiodate spray), and 0.58 (revealed by metaperiodate spray). These products were identified by the cochromatography with the authentic samples.[16]

2′,3′-*O*-Isopropylidene-5′,N^3-cyclo-8-bromoguanosine.—(A) 2′,3′-*O*-Isopropylidene-5′-*O*-mesyl-8-bromoguanosine (100 mg) was heated in hot water (30 ml) for 5–10 min. Examination of an aliquot by the paper chromatography showed two spots corresponding to the starting material and a new product. Prolonged heating gave a substance having R_f (A) 0.52, R_f (C) 0.44, and R_f (B) 0.39. Cooling of the water solution gave a crystalline material. *Anal.* Calcd for $C_{13}H_{14}N_5O_4Br \cdot 0.5H_2O$: C, 39.69; H, 3.82; N, 17.80. Found: C, 39.67; H, 4.00; N, 17.74. Ultraviolet absorption properties were $\lambda_{max}^{pH\ 1}$ 256 mμ (ε 16,700), $\lambda_{max}^{H_2O}$ 220 mμ (ε 26,500), 270 (14,500); $\lambda_{max}^{pH\ 13}$ 251 (sh), 270 mμ (ε 10,100). An infrared band at ν_{max} 1170 cm^{-1} was not observed. (B) 2′,3′-*O*-Isopropylidene-8-bromoguanosine (402 mg, 1 mmole) was dissolved in a mixture of pyridine (15 ml) and benzene (30 ml), followed by the addition of *p*-toluenesulfonyl chloride (400 mg). After the reaction mixture was kept overnight at room temperature, the mixture was poured into ice–water. The precipitates were extracted with chloroform; the chloroform layer was washed with sodium bicarbonate solution and finally with water. Drying over sodium sulfate and evaporation of the solvent *in vacuo* gave a residue, which was recrystallized from ethanol. It was crystalline substance having the following ultraviolet absorption properties: $\lambda_{max}^{H^+}$ 257 mμ; $\lambda_{max}^{H_2O}$ 221, 270 mμ; $\lambda_{max}^{OH^-}$ 220, 270 mμ. The yield was 250 mg. This substance was completely identical with 2′,3′-*O*-isopropylidene-5′,N^3-anhydro-8-bromoguanosine obtained above.

Measurement of pK_a Value of 8-Bromoguanosine.—The pK_a values were measured by the photometrical method according to Shugar and Fox.[25] The inflection point at pH 8.7 was observed in the plot of ultraviolet absorption *vs.* pH.

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