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8. Morio Ikehara and Kei Muneyama : Studies of Nucleosides and Nucleotides. XXX.*1 Syntheses of 8-Substituted Guanosine Derivatives.

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Recently we have reported[1] a method for the synthesis of 8-bromoguanosine directly from guanosine by means of dioxane-bromine. Starting from this easily accessible intermediate (I), various substituents were introduced to the 8-position of guanosine.*3 The reactivity of these substituents towards nucleophiles was investigated in order to synthesize cyclonucleosides[2] in the guanosine series.[1]

8-Bromoguanosine (I) was converted to 8-mercaptoguanosine (II) by the method of Holmes and Robins[3] and methylated with methyl iodide to afford 8-methylthioguanosine (IIIa). Although compound (IIIa) was obtained previously[3] by the use of dimethyl sulfate, methylation with methyl iodide provided a synthesis of simpler handlings.

8-Ethylthioguanosine (IIIb) was also obtained directly by reaction of compound (I) with ethylmercaptide in ethanol.

8-Methylmercaptoguanosine was then oxidized either with N-chlorosuccinimide[4] or hydrogen peroxide[5] to afford 8-methylsulfonylguanosine (IV), because of the high susceptibility of alkylsulfonyl group to nucleophiles.[4] The use of hydrogen peroxide in limited amount was superior to the N-chlorosuccinimide in the yield of sulfonyl derivative. The structure of compound (IV) was confirmed by the elemental analytical data and spectral properties. It showed strong absorption band at 1325 cm^{-1} in infrared spectra corresponding to that of sulfone. The intense solubility of this compound in water is noteworthy. When compound (IV) was heated at 80° in 0.1*N* hydrochloric acid for 4 hours, 8-methylsulfonylguanine (V) was obtained. In this case hydrolytic cleavage of the nucleotidic linkage was much easier than in the case of 8-bromoguanosine. These experiments showed that structure (IV) is correct and no N-oxide derivative was obtained. Treatment of IV with 0.1*N* sodium hydroxide at 100° for 3 hours also gave 8-methylsulfonylguanine. The reaction of 8-methylsulfonylguanosine with *t*-butoxide[6] was then investigated. If the replacement of methylsulfonyl group by the sugar hydroxyl group occur, as in the case of methyl-β-D-glucosyl sulfone,[7] the formation of cyclonucleoside would be expected. When compound (IV) was kept at 30° for 40 hours in dimethyl sulfoxide with excess sodium *t*-butoxide, a substance having $\lambda_{max}^{H^+}$ 256, 284 mμ; $\lambda_{max}^{OH^-}$ 286 mμ was obtained, accompanied with a small amount of 8-methylsulfonylguanine (V). The former compound contains sulfur and ribose group, but no 8-methylsulfonylguanine moiety. From the ultraviolet absorption properties of this compound, the cleavage of guanosine ring in the pyrimidine part could be deduced. If a bathochromic shift of ca. 10~20 mμ, which is generally observed by

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*1 Part XXIX. M. Ikehara, I. Tazawa : J. Org. Chem., in press.

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*3 After the completion of this work, we were aware of the report of R. E. Holmes and R. K. Robins (J. Am. Chem. Soc., **87**, 1772 (1965)).

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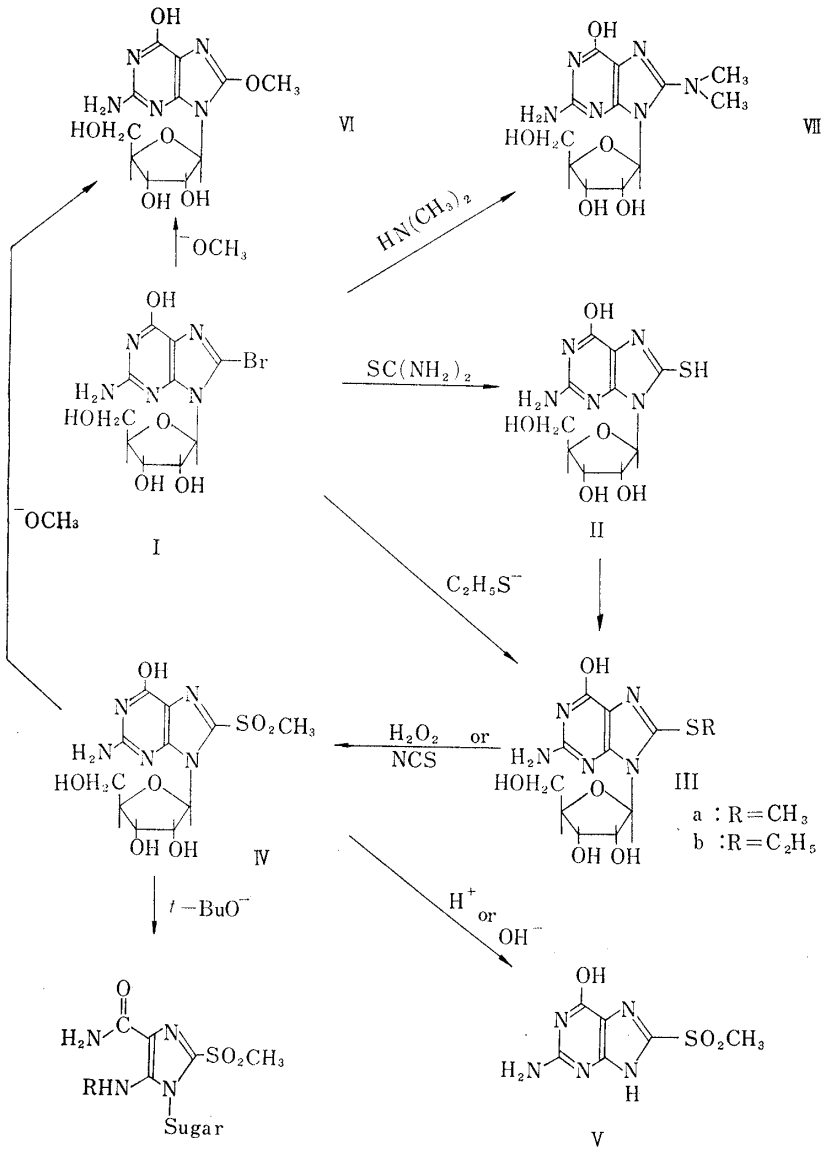

Chart 1.

the substitution of methylsulfonyl group at 8-position of guanosine, was added to the absorption spectra of 4-aminoimidazole carboxamide 1-ribose[8] ($\lambda_{max}^{H^+}$ 267, 240 mμ; $\lambda_{max}^{OH^-}$ 267 mμ), the observed absorption could be well explained.*4 The pKa value of 8-methylsulfonylguanosine (8.3) is much higher than that of guanosine (9.2[10]~9.5[11]). The easy dissociation of N^1-H of compound (Ⅳ) would render the pyrimidine ring more susceptible to the nucleophilic attack of *t*-butoxide. Under similar reaction conditions guanosine did not react with *t*-butoxide.

When 8-methylsulfonylguanosine reacted with sodium methoxide in methanol at 130~150°, 8-methoxyguanosine (Ⅵ) was obtained. The structure of Ⅵ was confirmed by the comparison with the sample obtained from 8-bromoguanosine by the similar

*4 Further study of this cleavage is in progress together with the elucidation of the reaction of 8-hydroxyguanosine with nitrous acid, in which rapid cleavage of the guanine ring has also been observed.[9]

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procedure. 8-Dimethylaminoguanosine (Ⅶ) was also obtained easily by heating I with dimethylamine in methanol at 120~130° for 5 hours. The structure of compound (Ⅵ) and (Ⅶ) was confirmed by elemental analysis.

Comparing the reactivity of 8-bromo (I) and 8-methylsulfonylguanosine (Ⅳ), nucleophilic substitution smoothly occurred in I as expected in the case of aromatic halogeno compounds activated by ortho or para electron attracting groups.[12] However in the case of Ⅳ, strong electron-attracting nature of methylsulfonyl group at position 8 caused the attack of nucleophile on N^1 or N^3, and subsequent cleavage of pyrimidine ring would be expected. Moreover, acidic hydrolysis easily cleaved Ⅳ at glycosidic linkage whereas 8-hydroxypurine ribonucleoside is resistant to such cleavage.[13]

Experimental

Paper Chromatography——Solvent A : Water adjusted to pH 10 with ammonia; solvent B : *t*-BuOH–water, 86:14. All paper chromatography were carried out by the ascending technique.

8-Methylthioguanosine——8-Mercaptoguanosine (315 mg.) was dissolved in 3 ml. of 0.4*N* NaOH (1.2 equiv.). An aqueous solution (9 ml.) containing methyl iodide (156.2 mg.) was added portionwise with cooling and vigorous stirring. After the addition was completed (ca. 10 min.), the reaction mixture was stirred further at room temperature for 2 hr. Precipitations were collected by filtration and recrystallized from water (yield 150 mg., 46%). *Anal.* Calcd. for $C_{11}H_{15}O_5N_5S\cdot H_2O$: C, 38.04; H, 4.90; N, 20.18. Found : C, 37.96; H, 5.10; N, 20.52. UV[*5] : $\lambda_{max}^{H^+}$ 273 mμ; $\lambda_{max}^{H_2O}$ 273 mμ; $\lambda_{max}^{OH^-}$ 285.5 mμ. Paper chromatography : Rf (A) 0.57, Rf (B) 0.20. This specimen was identical with a sample synthesized according to the literature.[3]

8-Methylsulfonylguanosine——a) 8-Methylthioguanosine (170 mg.) was dissolved in 1 ml. of acetic acid and 0.1 ml. of 30% hydrogen peroxide was added. The reaction mixture was heated at 30° for 48 hr. After evaporation of the solvent *in vacuo*, the residue was recrystallized from a small amount of water (yield 133 mg., 74%). *Anal.* Calcd. for $C_{11}H_{15}O_7N_5S$: C, 36.56; H, 4.16; N, 19.39. Found : C, 36.49; H, 4.37; N, 19.74. UV $\lambda_{max}^{pH\,1}$ 273 (ε 1.35×10^4), 285 (shoulder) mμ; $\lambda_{max}^{pH\,7.1}$ 272 (ε 1.19×10^4), 285 (shoulder) mμ; $\lambda_{max}^{pH\,13}$ 298 mμ (ε 1.28×10^4). IR : ν_{max}^{Nujol} 1325 cm^{-1} (RSO_2–). Paper chromatography : Rf (A) 0.70; Rf (B) 0.07. pKa obtained by the method of Shugar and Fox[14] : 2.0, 8.3.

b) 8-Methylthioguanosine (329 mg.) was dissolved in 80 ml. of 80% methanol and 320 mg. of N-chlorosuccinimide was added. After 3 hr. at 40~50°, the reaction mixture was poured into an aqueous solution of sodium bicarbonate (202 mg.). Succinimide was removed by filtration and filtrate was evaporated *in vacuo*. Treatment of this solution with Dowex 50 (H^+ form) resin gave severe losses in the yield of the desired product. The spectral and paper chromatographic behavior showed the product to be identical with that obtained above.

8-Ethylthioguanosine——Sodium metal (120 mg.) was dissolved in 100 ml. of anhydrous ethanol, followed by the addition of ethylmercaptan (0.7 ml., 9 equiv.). To this solution was added 8-bromoguanosine (362 mg.) and the mixture refluxed for 6 hr. The reaction mixture was turbid throughout the reaction time. Acetic acid (0.33 ml.) was added to the reaction mixture and the precipitates were filtered. The filtrate evaporated to a glass (100 mg.). UV : $\lambda_{max}^{H^+}$ 274 mμ; $\lambda_{max}^{H_2O}$ 274 mμ; $\lambda_{max}^{OH^-}$ 287 mμ. These values are well coincided with those of methylthioguanosine. Paper chromatography : Rf (B) 0.27.

Acid Hydrolysis of 8-Methylsulfonylguanosine——8-Methylsulfonylguanosine (90 mg.) was dissolved in 10 ml. of 0.1*N* hydrochloric acid and heated at 80° for 4 hr. When the reaction mixture was evaporated *in vacuo*, a solid material was obtained. Recrystallization from water gave 8-methylsulfonylguanine. *Anal.* Calcd. for $C_6H_7O_3N_5S\cdot H_2O$: C, 29.15; H, 3.64; N, 28.34. Found : C, 29.26; H, 2.97; N, 28.58. UV : $\lambda_{max}^{H^+}$ 261, 281 mμ; $\lambda_{max}^{H_2O}$ 261, 285 mμ; λ_{max}^{-} 254.5, 292 mμ. Paper chromatography : Rf (A) 0.56, Rf (B) 0.04.

Alkaline Hydrolysis of 8-Methylsulfonylguanosine——Ca. 10 mg. of 8-methylsulfonylguanosine was heated at 70~80° for 8 hr. in 0.1*N* sodium hydroxide solution (5 ml.). The ultraviolet absorption of the reaction mixture gradually changed to $\lambda_{max}^{H^+}$ 272, 286 (shoulder) mμ and $\lambda_{max}^{OH^-}$ 272 (shoulder), 295 mμ.

*5 Ultraviolet absorption spectra were taken with an Hitachi EPS-2U automatic recording spectrophotometer. Infrared spectra were taken with a JASCO DS-301 spectrophotometer.

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Further heating at 100° for 3 hr. showed the absorption of 8-methylsulfonylguanine. Paper chromatography : Rf (A) 0.56; Rf (B) 0.04.

Reaction of 8-Methylsulfonylguanosine with Sodium *t*-Butoxide in Dimethyl Sulfoxide——8-Methylsulfonylguanosine (70 mg.) was heated in an incubator at 30° in 20 ml. of dimethylsulfoxide in the presence of 340 mg. of sodium *t*-butoxide for 40 hr. Acetone (100 ml.) was added into the reaction mixture and the precipitate was collected by filtration. The white solid was washed well with anhydrous ethanol. UV : $\lambda_{max}^{H^+}$ 256, 284 (shoulder) mμ; $\lambda_{max}^{H_2O}$ 256, 284 (shoulder) mμ; $\lambda_{max}^{OH^-}$ 286 mμ. This sample contained sulfur and sugar residue. Paper chromatography : Rf (A) 0.72 and 0.58; Rf (B) 0.75 and 0.25. Solid material was dissolved in a small amount of water and treated with excess IRC 50 (H^+ form) resin in order to remove traces of sodium ion. Paper chromatographical separation (solvent A) gave a substance having Rf 0.72 and 8-methylsulfonylguanosine (Rf 0.55). UV of the spot of Rf 0.72; $\lambda_{max}^{H^+}$ 246, 285 mμ; $\lambda_{max}^{H_2O}$ 242.5, 288 mμ; $\lambda_{max}^{OH^-}$ 277 mμ. 8-Methylsulfonylguanine showed no change by the reaction with *t*-butoxide in an analogous condition.

Acid Hydrolysis of 8-Bromoguanosine——A small amount of 8-bromoguanosine was heated with 0.1*N* hydrochloric acid in a boiling water bath for 2 hr. After evaporation of the solvent *in vacuo* a solid material (8-bromoguanine) was obtained. *Anal.* Calcd. for $C_5H_4ON_5Br \cdot 1.5H_2O$: C, 25.10; H, 2.09. Found : C, 25.55; H, 2.30. UV : $\lambda_{max}^{H^+}$ 248.5, 269 (shoulder) mμ; $\lambda_{max}^{H_2O}$ 248.5, 271 (shoulder) mμ; $\lambda_{max}^{OH^-}$ 253, 279 mμ. Paper chromatography : Rf (A) 0.32.

8-Dimethylaminoguanosine——Dry dimethylamine (17 g.) was absorbed into 15 ml. of anhydrous methanol, followed by the addition of 181 mg. of 8-bromoguanosine. The reaction mixture was heated at 150~160° for 5 hr. in a sealed tube. Solvent was evaporated *in vacuo* and the residue was recrystallized from water (yield 73 mg., 44%). *Anal.* Calcd. for $C_{12}H_{18}O_5N_6$: C, 44.17; H, 5.52; N, 25.79. Found : C, 43.83; H, 5.78; N, 25.72. UV : $\lambda_{max}^{H^+}$ 261.5, 282 (shoulder) mμ; $\lambda_{max}^{H_2O}$ 265 μ; $\lambda_{max}^{OH^-}$ 267 mμ. Paper chromatography : Rf (A) 0.62; Rf (B) 0.27.

8-Methoxyguanosine——a) Sodium metal (100 mg.) was dissolved in anhydrous methanol (40 ml.), followed by the addition of 181 mg. of 8-bromoguanosine. The mixture was refluxed for 6 hr. at 140~150°. The solvent was evaporated *in vacuo* and the residue was recrystallized from water (yield 79 mg., 51%). *Anal.* Calcd. for $C_{11}H_{15}O_6N_5 \cdot H_2O$: C, 39.82; H, 5.13; N, 21.15. Found : C, 39.93; H, 5.04; N, 21.05. Paper chromatography : Rf (A) 0.65; Rf (B) 0.06. UV : $\lambda_{max}^{H^+}$ 248, 282 mμ; $\lambda_{max}^{H_2O}$ 248, 282 mμ; $\lambda_{max}^{H^-}$ 250.5, 267.5 (shoulder) mμ.

b) 8-Methylsulfonylguanosine (90 mg.) was dissolved in 6 ml. of anhydrous methanol containing 40 mg. of sodium metal. After heating of this solution at 130~150° for 5 hr., sodium was removed by the treatment with IRC 50 (COO^-) resin. Evaporation of the solvent gave a hard glass, having UV properties : $\lambda_{max}^{pH\,2}$ 255, 281.5 mμ; $\lambda_{max}^{pH\,10}$ 247, 279.5 mμ. Paper chromatography : Rf (A) 0.66; Rf (B) 0.55. These values were identical with 8-methoxyguanosine obtained obove.

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Summary

Starting from 8-bromoguanosine, 8-methylsulfonylguanosine, 8-dimethylaminoguanosine and 8-methoxyguanosine were synthesized. The reaction of 8-methylsulfonylguanosine with acid, alkali and *t*-butoxide were investigated. In the former two cases cleavage of nucleoside linkage was observed. In the latter case cleavage of pyrimidine ring probably occurred.

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