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Studies of Nucleosides and Nucleotides. XXXVII.[1] Synthesis of 8-Oxyguanosine Nucleotides and Uric Acid 9-Riboside 5'-Phosphate

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8–Oxyguanosine 5'–mono–, 5'–di– and 5'–tri–phosphate were synthesized from 2',3'–O–isopropylidene–8–oxyguanosine by the phosphorylation with P^1–diphenyl, P^2–morpholinopyrophosphoro chloridate, follwed either by acidic removal of the protecting group, reaction with inorganic phosphate in the presence of DCC, or by the reaction of its morpholidate with pyrophosphate. 5'–Monophosphate was hydrolyzed by the catalysis with snake venom 5'–nucleotidase. 8–Oxyguanosine 2',3'–cyclic phosphate, which was obtained by the direct phosphorylation method from 5'–O–acetyl–8–oxyguanosine, was completely resistant against pancreatic RNase and RNase T_1.

Deamination of 8–oxyguanosine and its monophosphate was investigated. In the presence of excess nitrous acid a rapid loss of ultraviolet absorption was observed. With the use of limited amount of nitrous acid, uric acid 9–riboside 5'–monophosphate was obtained.

During the course of our studies on the synthesis of 8–substituted purine nucleosides,[3-5] we have found a versatile method for the introduction of oxy function into 8–position of purine nucleosides.[6] Using this method, 8–oxyguanosine and uric acid 9–riboside could be synthesized from 8–bromoguanosine. The phosphorylation of these nucleosides would afford nucleotides, which serve as the substrates of enzymes, such as snake venom 5'–nucleotidase, polynucleotide phosphorylase, RNA polymerase, pancreatic RNase and RNase T_1. Furthermore, chemically synthesized uric acid 9–riboside 5'–monophosphate could be compared with the specimen synthesized enzymatically.[7]

In this report the synthesis of 8–oxyguanosine 5'–mono–, di–, tri–phosphate, 2',3'–cyclic phosphate and uric acid 9–riboside 5'–monophosphate is described. Some of the enzymatic reaction of these nucleotides with 5'–nucleotidase, pancreatic RNase and RNase T_1 are also included.

2',3'–O–Isopropylidene–8–bromoguanosine (I), obtained by an improved procedure from guanosine,[8] was refluxed in acetic acid with sodium acetate[6] to afford 2',3'–O–isopropylidene–8–oxyguanosine (IIa). In addition to this compound, second nucleoside (IIb) having the same ultraviolet absorption properties with IIa was obtained. Since compound IIb was converted to IIa by the brief treatment with methanolic ammonia, IIb was shown to be 5'–O–acetyl derivative of IIa, which was formed during the reflux of IIa in acetic acid. 2',3'–O–Isopropylidene–8–oxyguanosine (IIa), thus obtained, was phosphorylated with P^1–diphenyl, P^2–morpholinopyrophosphoro chloridate.[9] Since 8–oxyguanosine is thought to be labile

1) Part XXXVI: M. Ikehara and K. Muneyama, *J. Org. Chem.*, **34**, 3042 (1967).
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7) D. Hatfield, R.A. Greenland, H.L. Stewart, and J.B. Wyngaarden, *Biochim. Biophys. Acta*, **91**, 163 (1964).
8) M. Ikehara and K. Muneyama, *J. Org. Chem.*, **34**, 3039 (1967).
9) M. Ikehara and E. Ohtsuka, *Chem. Pharm. Bull.* (Tokyo), **11**, 961 (1963).

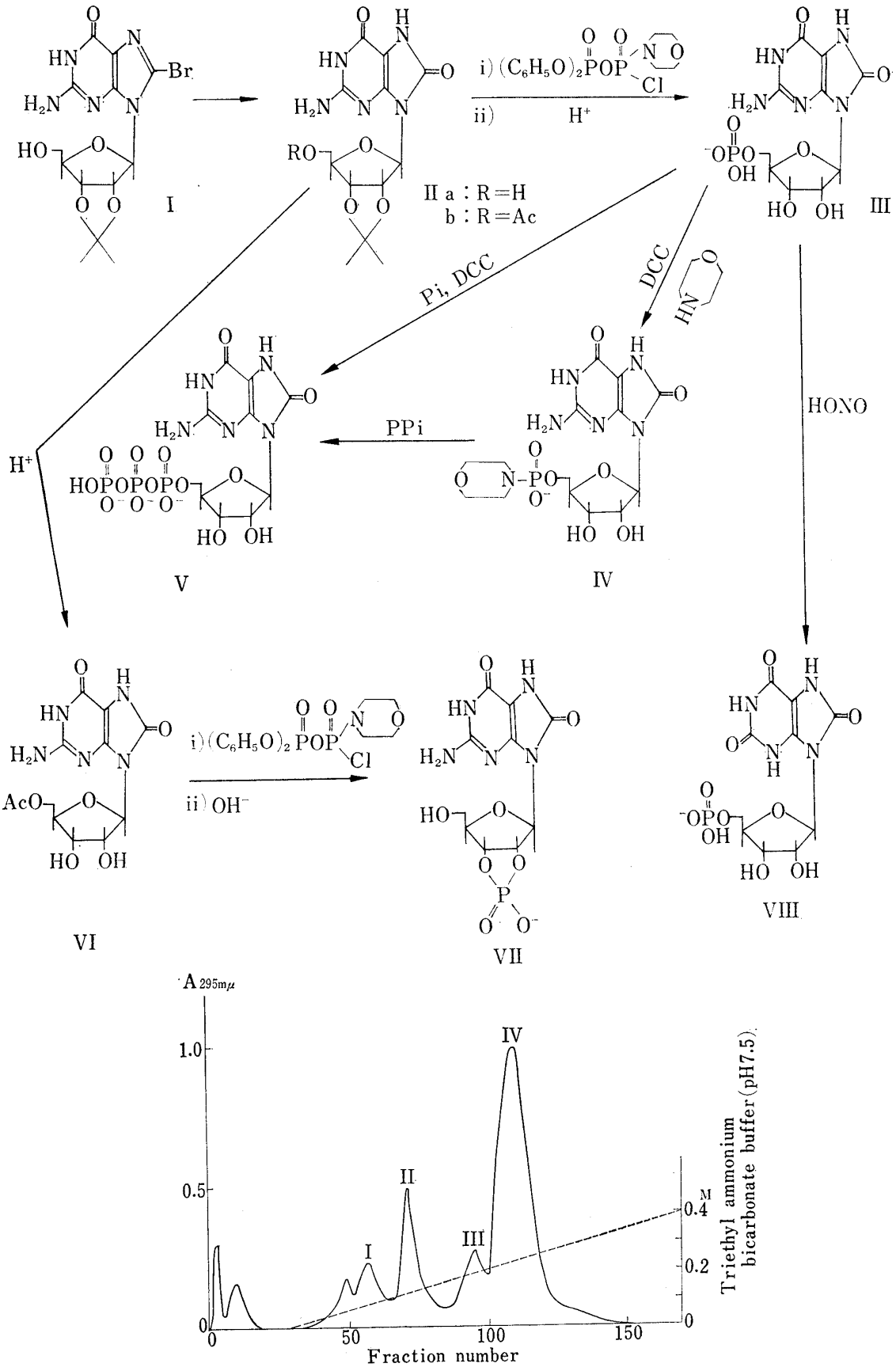

Fig. 1. DEAE–Cellulose Column Chromatography of 8–Oxyguanosine 5′–Polyphosphates

peak I: 8–oxyguanosine 5′–phosphoromorpholidate, II: monophosphate, III: diphosphate, IV: triphosphate

against alkaline treatment, mild acidic hydrolysis for the removal of proptecting groups both on 2′,3′–hydroxyl and on phosphate group would be preferable. By the purification with ion–exchange column chromatography 8–oxyguanosine 5′–monophosphate (III) was isolated in a pure state in the yield of 40%. The structure of this nucleotide was confirmed by ultraviolet absorption properties, paper chromatography, paper electrophoresis and elemental analysis.

8–Oxyguanosine 5′–monophosphate (III) was then derived to polyphosphate either by the method described by Khorana[10] or by the morpholidate method.[11,12] Separation of the reaction products by DEAE–cellulose column chromatography gave rise to 5′–di– (IV) and tri–phosphate (V) in the yield of 46% and 61% respectively (see Fig. 1). Elemental analysis and ultraviolet absorption properties, as well as paper chromatography and paper electrophoresis, showed the structure of these polyphosphates to be correct. The enzymatic reaction using these polyphosphates as substrate for polynucleotide phosphorylase and RNA polymerase will be reported separately.[13]

Starting from the compound IIb, acidic removal of the isopropylidene group afforded 5′–O–acetyl–8–oxyguanosine (VI), which was phosphorylated by the procedure reported previously[14] to give 2′,3′–cyclic phosphate (VII). Compound VII was purified by the DEAE–cellulose column chromatography in 63% yield. When 8–oxyguanosine 2′,3′–cyclic phosphate (VII) was incubated either with pancreatic RNase or RNase T_1, none of these substrates could be hydrolyzed to give 3′–phosphate. These results suggested that in the pancreatic RNase reaction, –NH–CO–NH– group in the imidazole portion of the purine ring could not replace $-N^1H-C^2O-N^3H-$ of pyrimidine nucleoside,[15] whereas these groups thought to be in a similar configuration.[16] In the RNase T_1 reaction, in which guanosine 2′,3′–cyclic phosphate is the normal substrate, 8–oxy function exerted an inhibitory effect for the cleavage of cyclic phosphate in VII. The cause of this inhibitory effect may be due to the steric hindrance for the adaptation of compound VII to the enzyme active site or to the difference in the optimal pH for the activity of this enzyme to VII and guanosine 2′,3′–cyclic phosphate. Since the apparent pK_{a2} value for 8–oxyguanosine (8.9)[17] is not largely different from that of guanosine (9.2), the former explanation will be preferable.[18]

In order to obtain uric acid derivative, the deamination reaction of 2′,3′–O–isopropylidene–8–oxyguanosine (IIa) with nitrous acid was investigated. In contrast to our expectation, when the excess amount of nitrous acid was used in dilute acetic acid, the ultraviolet absorption of compound IIa diminished rapidly (see Fig. 2) and the desired uric acid derivative could not be obtained. In order to avoid this degradation, the amount of sodium nitrite used was limited strictly to one equivalent and 2 N sulfuric acid was used for the reaction media. The ultraviolet absorption properties of uric acid 9–riboside, thus obtained, were similar to those reported by Holmes and Robins[20] for this compound. 8–Oxyguanosine 5′–mono-

10) M. Smith and H.G. Khorana, *J. Am. Chem. Soc.*, **80**, 1141 (1958).
11) J.G. Moffatt and H.G. Khorana, *J. Am. Chem. Soc.*, **83**, 649 (1961).
12) J.G. Moffatt, *Canad. J. Chem.*, **42**, 603 (1964).
13) M. Ikehara, H. Tazawa, T. Fukui, and K. Murao, in preparation.
14) M. Ikehara and I. Tazawa, *J. Org. Chem.*, **31**, 819 (1966).
15) H. Witzel in "Progress in Nucleic Acid Research," Vol. II, J.N. Davidson and W.E. Cohn, Ed., Academic Press, New York, 1963, p. 221.
16) M. Ikehara and H. Tada, *J. Am. Chem. Soc.*, **87**, 606 (1965).
17) K. Muneyama, unpublished data.
18) In the case of xanthosine 2′,3′–cyclic phosphate, RNase T_1 catalyzed the hydrolysis only in the pH range lower than 7. However, pK_a of xanthosine is 6.0[19] and it suggests that around pH 7 xanthosine is in dissociated form which is not favorable for the adaptation of this substrate to the enzyme active site (private communication from Dr. T. Ueda).
19) E. Chargaff and J.N. Davidson, Ed., "The Nucleic Acid," Vol. L, 1956, p. 459.
20) R.E. Holmes and R.K. Robins, *J. Am. Chem. Soc.*, **87**, 1772 (1965).

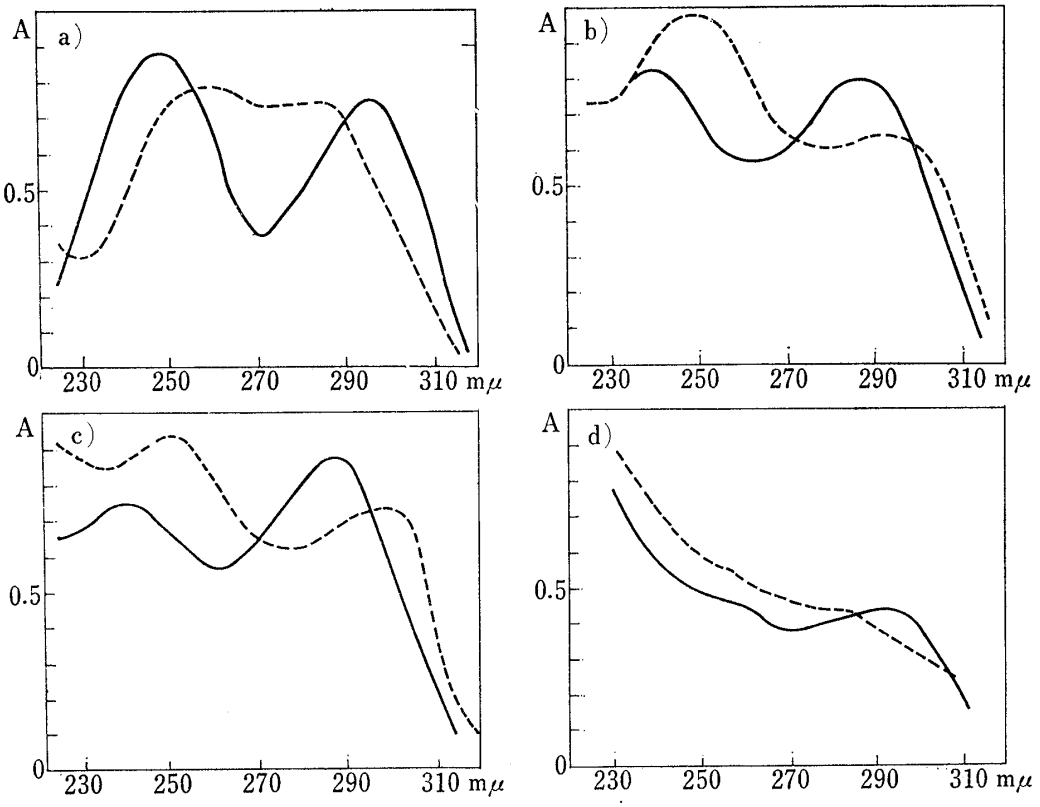

Fig. 2. Change of Ultraviolet Absorption Spectra in the Reaction of 2′,3′-O-Isopropylidene-8-oxyguanosine with Nitrous Acid

a) starting material, b) after 15 min in the presence of one equiv. nitrous acid, c) after 30 min, d) 30 min after addition of excess nitrous acid
On the ordinate optical density of the reaction mixture was plotted.

phosphate (III) was then subjected to the deamination reaction in 2 N sulfuric acid with one equivalent of nitrous acid. Purification on the DEAE-cellulose column gave a peak corresponding to uric acid mononucletide (VIII), which was characterized by paper chromatography and paper electrophoresis. These properties were identical with those reported previously.[7] The true mechanism with which 8-oxyguanosine and its phosphate degrade in the presence of nitrous acid has not been elucidated as yet. The recent report of Shapiro[21] on the mechanism of the degradation of guanosine in the bromination reaction should be recalled with much interest.

Experimental[22]

2′,3′-O-Isopropylidene-8-oxyguanosine——Anhydrous sodium acetate (2 g) was dissolved in glacial acetic acid (50 ml) by slight warming. Isopropylidene-8-bromoguanosine[8] (1.608 g, 4 mmoles) was added into the mixture. After solid material was totally dissolved by heating, the reaction mixture was refluxed for 1 hr. Acetic acid was removed by vacuum distillation and the residue was extracted twice with ethyl acetate (100 ml). Ethyl acetate solution was evaporated *in vacuo* to give a residue. Examination of the residue by paper chromatography showed the presence of three compounds. The *Rf* values were 0.73, 0.60, and 0.12 in solvent B[23] and 0.65, 0.51, and 0.10 in solvent C. Ultraviolet absorption properties of these substances were similar to 8-oxyguanosine. While faster moving two spots could not revealed by metaperiodate spray,[24]

21) R. Shapiro and S.C. Agarwal, *Biochem. Biophys. Res. Commun.*, **24**, 401 (1966).
22) Ultraviolet absorption was taken with Hitachi PPS-2U automatic recording spectrophotometer and Shimadzu QR-50 spectrophotometer.
23) Solvent used for paper chromatography: A, isopropanol-1% ammonium sulfate, 2:1; B, *n*-butanol-water, 86:14; C, isopropanol-ammonia-water, 7:1:2; D, *n*-butanol-acetic acid-water, 5:2:3; E, *n*-propanol-1% ammonia, 2:1.
24) M. Viscontini, D. Hoch, and P. Karrer, *Helv. Chim. Acta*, **38**, 642 (1955).

the slowest one consumed metaperiodate. Co-chromatography with an atuthentic samples of 2′,3′-O-isopropylidene-8-oxyguanosine,[8] 2′,3′-O-isopropylidene-5′-O-acetyl-8-oxyguanosine[8] and 8-oxyguanosine[8] showed their structure to be correct. Although 8-oxyguanosine could be removed by the recrystallization from water, the former two compounds could not be separated by the recrystallization. The mixture was then treated with anhydrous methanol saturated with dry ammonia at 0°. After the reaction mixture was kept in a refrigerator overnight, ammonia methanol was evaporated. Recrystallization from water (*ca.* 40 ml) gave pure 2′,3′-O-isopropylidene-8-oxyguanosine in 70% yield. *Anal.* Calcd. for $C_{13}H_{17}O_6N_5 \cdot H_2O$: C, 43.69; H, 5.32; N, 19.61. Found: C, 43.49; H, 5.26; N, 19.82. This sample was identical with an authentic specimen.[8]

8-Oxyguanosine 5′-Monophosphate——i) Freshly prepared P^1-diphenyl, P^2-morpholinopyrophosphoro chloridate[9] (from morpholinophosphoro dichloridate[25] (204 mg, diphenyl phosphate (250 mg) and 2,6-lutidine (227 μl)) was combined with a solution of isopropylidene-8-oxyguanosine (170 mg, 0.5 mmole) in dioxane (1 ml) and DMF (0.5 ml). After the stirring at room temperature for 48 hr, the solvent was removed by vacuum distillation and the residue was refluxed in 10% acetic acid (6 ml) for 1 hr. The reaction mixture was extracted with chloroform (20 ml × 5) and adjusted to pH 9 with 1 N lithium hydroxide. After extraction with ether (10ml × 5) and neutralization with dilute hydrochloric acid, the whole solution was adsorbed on activated charcoal (*ca.* 1 g). Elution with 50% ethanol containing 2% ammonia (100 ml × 2), followed by the evaporation of the solvent to 5 ml, gave nucleotidyl material (TOD_{295}[26] 2070). This was adjusted to pH 9 with ammonia and applied to a column (1.2 × 30 cm) of Dowex I X8 (chloride form, 100—200 mesh) resin. After washing with water, the column was eluted stepwise with 0.02 M lithium chloride + 0.003 N hydrochloric acid (500 ml), 0.04 M lithium chloride + 0.003 N hydrochlric acid (1500 ml) and 0.05 M lithium chloride + 0.003 N hydrochloric acid (600 ml). Fractions (each 10 ml) appeared in the second peak was pooled, neutralized with 1 N lithium hydroxide and evaporated *in vacuo* to a small balk. Anhydrous methanol (10 ml) and acetone (100 ml) was added to the residue. Resulting precipitate was collected by centrifugation and washed with acetone and ether successively. Drying over phosphorus pentoxide in 3 mm at room temperature gave 62.8 mg of 8-oxyguanosine 5′-monophosphate as a white powder (yield 31.9%). Paper chromatography: *Rf* (A) 0.19 (guanosine 5′-monophosphate, 0.16). Paper electrophoresis[27]: R_{GMP} 1.0. ε (P) = 12000. Purity estimated photometrically on the weight basis was 75.3%. UV: $\lambda_{max}^{H^+\ H2O}$ 248, 295 mμ; $\lambda_{max}^{OH^-}$ 250, 285 mμ.

ii) From 2 mmoles of isopropylidene-8-oxyguanosine phosphorylation was carried out as described in i). After charcoal treatment, nucleotidyl material (TOD_{295} 15420) obtained was applied to a column (3.5 × 40 cm) of DEAE-cellulose (carbonate form) and eluted with 0—0.20 M triethylammonium bicarbonate in linear gradient. Fractions were collected in 28 ml each. Peak III (fraction No. 71—106) was evaporated *in vacuo* and triethylamine was totally removed by repeated evaporation with a small amount of water. The residue was dissolved in water (*ca.* 100 ml) and stirred with Dowex 50 (Na^+, 50 ml) for 1 hr in order to convert it to the sodium salt. Evaporation of the solution to dryness gave 451.6 mg(52.9%) of 8-oxyguanosine 5′-monophosphate disodium salt. This sample was identical with that obtained in i).

Enzymatic Digestion of 8-Oxyguanosine 5′-Monophosphate——8-Oxyguanosine 5′-monophospate (1 μmole), 1 M ammonium carbonate (0.02 ml), 0.05 M Tris-HCl (pH 8) (0.01 ml), enzyme solution (containing 0.2 mg of *Trimeresurus flavoviridis* venom) and water (0.05 ml) were incubated at 37° for 24 hr. The same incubation was carried out with guanosine 5′-monophosphate. Examination of the incubation mixture by paper electrophoresis showed the essentially total cleavage of the both substrates to nucleoside and inorganic phosphate.

8-Oxyguanosine 5′-di- and tri-Phosphate——i) 8-Oxyguanosine 5′-monophosphate dilithium salt (79. 6 mg) was combined with 85% phosphoric acid (0.24 g) in a small amount of pyridine, followed by the addition of tri-*n*-butylamine (1 ml). Pyridine was added to the total volume of 4 ml to obtain a clear solution. Dicyclohexylcarbodiimide (2.4 g) was added and the mixture was kept at 25° for 38 hr under strict exclusion of the moisture. Dicyclohexylurea was removed by filtration, filtrate and washings were combined and adsorbed on the activated charcoal. Washing of the charcoal with water and elution with 50% ethanol containing 2% ammonia gave nucleotidyl material (TOD_{295} 560 units), which was applied to a column (1.3 × 40 cm) of DEAE-cellulose (carbonate form). After the water wash, the column was eluted with 0.3 M triethylammonium bicarbonate (500 ml) and water (500 ml) by the linear gradient technique. Fractions were collected in each 10 ml. 8-Oxyguanosine 3′,5′-cyclic phosphate (fraction No. 10—21, TOD_{295} 16, 4.3%), 5′-monophosphate (No. 23—45, TOD_{295} 122, 32.3%), 5′-diphosphate (No. 50—70, TOD_{295} 175, 46.4%) and 5′-triphosphate (No. 70—87, TOD_{295} 64, 17.0%) were obtained. Paper chromatography: oxy-GDP, *Rf* (D) 0.16; oxy-GTP, *Rf* (D) 0.13. Paper electrophoresis: oxy-GDP, R_{GMP} 1.26; oxy-GTP, R_{GMP} 1.32 at pH 7.5 and oxy-GDP, R_{GMP} 1.96; oxy-GTP, R_{GMP} 2.19 at 3.5.[28]

25) M. Ikehara and E. Ohtsuka, *Chem. Pharm. Bull.* (Tokyo), **11**, 435 (1963).
26) TOD_{295} stands for optical density measured at 295 mμ multiplied by the volume (ml) of the solution.
27) Achieved at pH 7.5 in 0.05 M triethylammonium bicarbonate, 20 V/cm for 1 hr.
28) Performed in 0.05 M ammonium acetate at 20 V/cm for 1 hr.

ii) 8-Oxyguanosine 5′-monophosphate (57.4 mg) was dissolved in a mixture of water (1 ml) and *tert*-butanol (1 ml), followed by the addition of morpholine (0.034 ml). Into the mixture, which was heated to the reflux temperature, was added dropwise a solution of dicyclohexyl carbodiimide (82.4 mg) in *tert*-butanol (1.5 ml). The reflux was continued until all of the monophosphate disappeared by conversion to the morpholidate (estimated by the paper electrophoresis at pH 7.5, R_{GMP} 0.77). If necessary appropriate amount of DCC and morpholine were added and refluxed further (total 4 hr). Dicyclohexyl urea was filtered, filtrate and washings were combined, and *tert*-butanol was evaporated *in vacuo*. The residual aqueous solution was extracted three times with ether. The water layer was evaporated to dryness. After drying by evacuation over P_2O_5, the residue was dissolved in methanol and precipitated by the addition of ether. 8-Oxyguanosine 5′-phosphoromorpholidate, thus obtained, was dried by the repeated evaporation with pyridine. Into the residue was added a solution of bistri-*n*-butyl ammonium pyrophosphate (prepared from tetrasodium pyrophosphate, 223 mg, 0.5 mole) in dimethylsulfoxide (0.6 ml). The reaction mixture was kept at room temperature for 2—4 days under stric exclusion of the moisture. Reaction was stopped by the addition of water (10 ml) and the mixture was directly applied to a column (1.5×25 cm) of DEAE-cellolose (carbonate form). After the water wash, elution was carried out by linear gradient of water (700 ml) and 0.4 M triethylammonium bicarbonate (700 ml). Fractions were collected in each 10 ml and the amount of nucleotide was estimated by the optical density at 295 mμ. As shown in Fig. 1, diphosphate (TOD 24.7, 9.1%) and triphosphate (TOD 153.3, 56.4%) were obtained in the peak IV and V. *Rf* values in paper chromatography and R_{GMP} values in paper electrophoresis were same as in i). Phosphorus analysis for 8-oxy-GTP (ε_{295} 9700); base: acid labile P: total P=1.0: 2.15:3.16.

8-Oxyguanosine 2′,3′-Cyclic Phosphate——5′-O-Acetyl-8-oxyguanosine[8] (170 mg, 0.5 mmole) was dissolved in dioxane (1 ml), followed by the addition of a solution of P^1-diphenyl, P^2-morpholinopyrophosphoro chloridate (1 mmole) in dioxane (2 ml). Reaction mixture was stirred at room temperature for 38 hr under exclusion of moisture. 0.02 N aqueous ammonia (100 ml) was added into the mixture, which was kept at 30° overnight. The reaction extent estimated by paper electrophoresis was 80—90% at this stage. Extraction with ether (20 ml×3) and concentration of the water layer *in vacuo* gave nucleotidyl material (TOD_{295} 6350, TOD_{260} 5200), which was applied to a column (2.5×20 cm) of DEAE-cellulose (carbonate form). After the water wash, elution was carried out by the linear gradient of water (500 ml) and 1.5 M triethylammonium bicarbonate (pH 7.5, 500 ml). Each 10 ml fractions were collected. 8-Oxyguanosine 2′,3′-cyclic phosphate was obtained in the second peak (TOD_{295} 2587, 61.6%). Paper chromatography: *Rf* (C) 0.32. Paper electrophoresis: R_{HOGMP} 0.81.

When this sample was hydrolyzed in 0.1 N hydrochloric acid at room temperature for 1 hour, 8-oxyguanosine 2′-(or 3′-)monophosphate (paper chromatography: *Rf* (A) 0.51, $R_{2',3'cyclic\,P}$ 2.08; *Rf* (D) 0.26, $R_{2',3'cyclic\,P}$ 0.65. Paper electrophoresis at pH 7.5: R_{HOGMP} 1.00) was obtained.

Hydrolysis of 8-Oxyguanosine 2′,3′-Cyclic Phosphate catalyzed by Pancreatic RNase and by RNase T_1——i) 8-Oxyguanosine 2′,3′-cyclic phosphate (5 OD units at 295 mμ) was incubated with RNase I[29] (10 μg) in 0.5 M phosphate buffer (pH 7.4, 0.1 ml) at 37° for 24 hr. Uridine 2′,3′-cyclic phosphate (5 OD units at 260 mμ) was incubated in the same condition. Blank test without enzyme was also carried out. Reaction mixture was applied to paper electrophoresis (pH 7.5) and 3′-phoshate was detected under irradiation with an UV lamp. 8-Oxyguanosine 2′,3′-cyclic phosphate was totally resistant to the enzyme, whereas uridine 2′,3′-cyclic phosphate was completely hydrolyzed to 3′-phosphate.

ii) 8-Oxyguanosine 2′,3′-cyclic phosphate (5 OD units at 295 mμ) was incubated with RNase T_1[29] (1 μg) in 0.01 M Tris-HCl buffer (pH 7.0, 0.1 ml) at 37° for 24 hr. A blank test without enzyme and incubation of guanosine 2′,3′-cyclic phosphate in the same condition were carried out. 8-Oxyguanosine cyclic phosphate was resistant to the enzyme, whereas guanosine 2′,3′-cyclic phosphate was totally hydrolyzed.

Deamination Reaction of 2′,3′-O-Isopropylidene-8-oxyguanosine——i) 2′,3′-O-isopropylidene-8-oxyguanosine (34 mg) was disolved in 2 M sulfuric acid (2 ml). Into the mixture was added dropwise a solution of sodium nitrite (6.9 mg, 1 equiv.) in water (0.2 ml) with cooling in an ice bath. Aliquots were withdrawn every 15 min and UV absorption was taken. Initial spectrum having $\lambda_{max}^{pH\,2}$ 243.5, 293 mμ (243.5/293=1.16); $\lambda_{max}^{pH\,11}$ 254, 284 mμ converted gradually to the final (1 hr) spectrum having $\lambda_{max}^{pH\,2}$ 241, 287 mμ (241/287=0.89); $\lambda_{max}^{pH\,11}$ 250, 293 mμ. The spectra taken in the final period were identical with those reported for uric acid 9-riboside.[20] This spectrum did not change even after 20 hr at room temperature. However, addition of 0.4 ml of above nitrite solution into this mixture caused the diminishing of UV absorption.

ii) The same experiment as in i) was carried out with 1.5 equiv. of sodium nitrite. After 1 hr, UV absorption decreased to about half of the initial absorption and after 20 hr at room temperature only a small absorption at 273 mμ was observed (see Fig. 2).

9-β-D-Ribofuranosyluric Acid 5′-Monophosphate——8-Oxyguanosine 5′-monophosphate diammonium salt (50 mg) was dissolved in 2 N sulfuric acid (2 ml) and the mixture was cooled to 0—5° in an ice bath. A solution of sodium nitrite (4.5 mg) dissolved in water (1.3 ml) was added gradually. After 20 min reaction was stopped by dilution with 100 ml of water. The nucleotidyl material was adsorbed on the activated

29) This was the gift from Dr. Okazaki of Sankyo Co., to whom our thanks are due.

charcoal, which was eluted by 50% ethanol containing 2% ammonia. The eluates were concentrated *in vacuo* to 10 ml (TOD_{260} 552, TOD_{287} 781) and applied to a column (1.5×25 cm) of DEAE-cellulose (bicarbonate form). After the water-wash, the column was eluted with 0—0.27 M triethylammonium bicarbonate (total 2 liter) by linear gradient technique. Fractions were collected in each 10 ml Uric acid 9-ribotide appeared in the second major peak (fraction No. 135—155), which was evaporated *in vacuo* to give 8 mg of white powder. Ultraviolet absorption: $\lambda_{max}^{pH\,2}$ 240.5, 289 mμ; $\lambda_{max}^{pH\,11}$ 246.5, 292 mμ; $\lambda_{min}^{pH\,2}$ 265 mμ; $\lambda_{min}^{pH\,11}$ 272 mμ. Paper chromatography: *Rf* (E) 0.07 (GMP 0.09), *Rf* (B) 0.27. Paper electrophoresis: R_{HOGMP} 1.0 (at pH 7.5).

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