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Studies of Nucleosides and Nucleotides. XL.[1] Synthesis of 8,5′-O-Cyclonucleoside derived from 8-Oxyguanosine and Its Cleavage by Nucleophilic Reagents

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2′,3′-O-Isopropylidene-8-bromoguanosine was cyclized to 2′,3′-O-isopropylidene-8,5′-anhydro-8-oxyguanosine (II) by the cyclization with sodium hydride in dioxane. 8,5′-Anhydro linkage of the compound II was cleaved by various nucleophiles, such as water, hydrogen sulfide, sodium thiolacetate, sodium acetate, potassium thiocyanate and potassium cyanate in acetic acid to give 5′-substituted 5′-deoxy-8-oxyguanosine. Alkaline hydrolysis of II did not proceed smoothly.

In these five years we have reported on the synthesis and the properties of various types of purine cyclonucleosides having 8,2′-,[3-7] 8,3′-[4,8] and 8,5′-[9-12] anhydro linkages. These cyclonucleosides were proved to be useful for the transformation of structure of purine nucleosides and for the elucidation of sterical conformation of purine nucleosides.[13] However, mainly due to lack of sufficient amount of substrate cyclonucleoside, the cleavage reaction of these anhydro bonds could not be studied extensively.

Recently, we have found a novel cyclization reaction using sodium hydride in dioxane solution and synthesized 8,5′-O-cyclonucleoside derived from 8-oxyadenosine.[11] Independently from us, Nagpal and Dhar[14] reported the same cyclization reaction. The 8,5′-O-cyclonucleoside seems to be suitable for the study of the nature of the anhydro bond towards attack of various reagents, because synthetic route is sufficiently simple for obtaining a large quantity of cyclonucleoside. We therefore attempted to synthesize 8,5′-anhydro-8-oxyguanosine by the essentially same procedure, which was used successfully for adenosine, and the product was investigated on susceptibility to various nucleophiles.

When 2′,3′-O-isopropylidene-8-bromoguanosine[9,10] (I) was treated with 3 equivalents of sodium hydride in dimethylformamide (DMF), a compound having ultraviolet absorption resembled to that of 8-methoxyguanosine[15] was obtained. Behaviors in paper chromatography and elemental analytical data suggest that this compound is 2′,3′-o-isopropylidene-8,5′-anhydro-8-oxyguanosine (II). Relatively low yield (37%) of II may be due to loss during recrystallization process, since examination of reaction mixture at several reaction intervals

1) Part XXXIX: M. Ikehara, I. Tazawa and T. Fukui, *Chem. Pharm. Bull.* (Tokyo), **17**, 1019 (1969).
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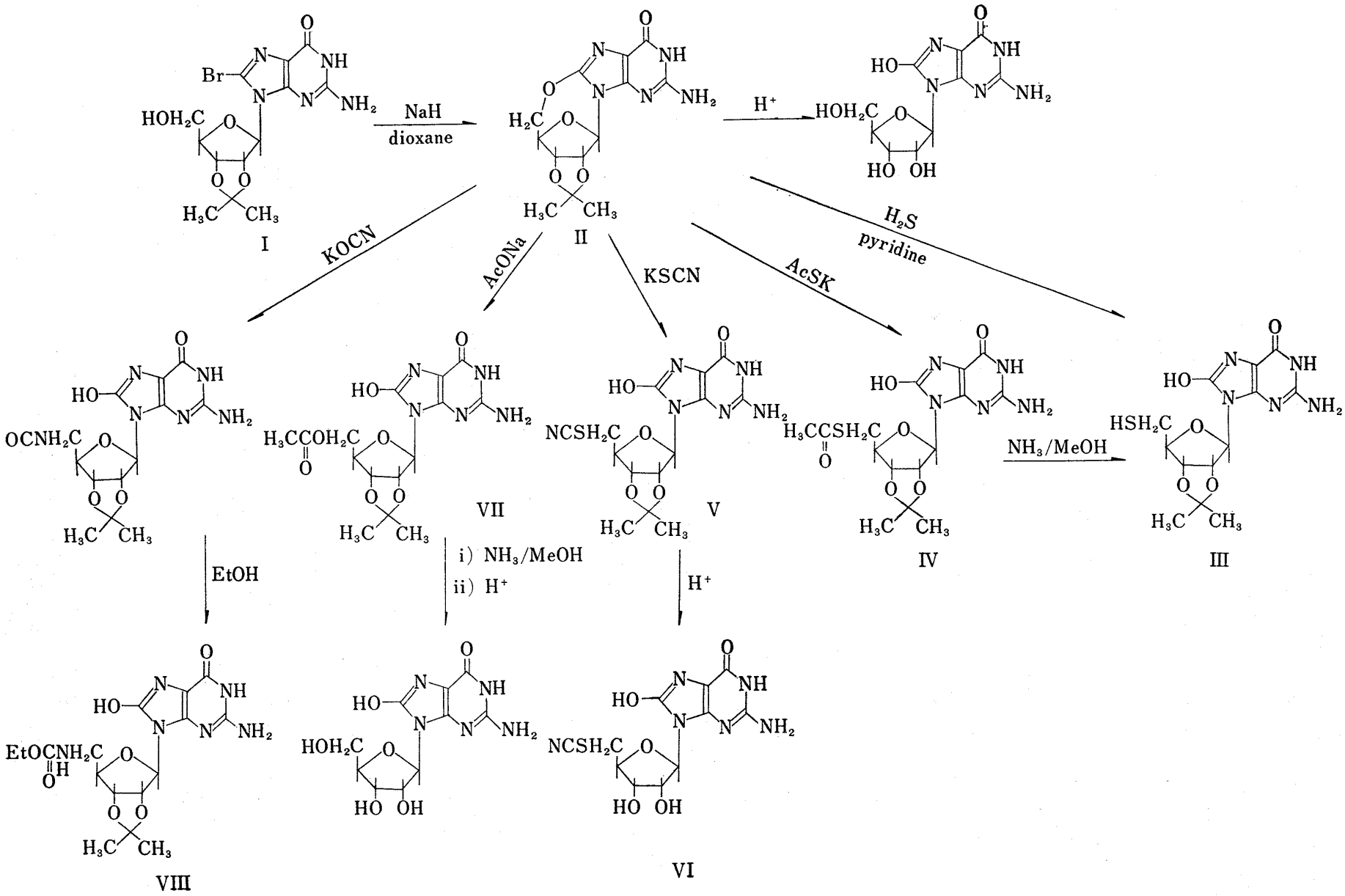
3) M. Ikehara and H. Tada, *J. Am. Chem. Soc.*, **85**, 2344 (1963); *idem, ibid.*, **87**, 606 (1965).
4) M. Ikehara and H. Tada, *Chem. Pharm. Bull.* (Tokyo), **15**, 94 (1967).
5) M. Ikehara, H. Tada, K. Muneyama and M. Kaneko, *J. Am. Chem. Soc.*, **88**, 3165 (1966).
6) M. Ikehara, H. Tada and M. Kaneko, *Tetrahedron*, **24**, 3489 (1968).
7) M. Ikehara and K. Muneyama, *J. Org. Chem.*, **34**, 3039 (1967).
8) M. Ikehara and M. Kaneko, *Chem. Pharm. Bull.* (Tokyo), **90**, 1261 (1967).
9) M. Ikehara, H. Tada and K. Muneyama, *Chem. Pharm. Bull.* (Tokyo), **13**, 639 (1965).
10) M. Ikehara and K. Muneyama, *J. Org. Chem.*, **34**, 3042 (1967).
11) M. Ikehara and M. Kaneko, *J. Am. Chem. Soc.*, **90**, 497 (1968).
12) M. Ikehara, M. Kaneko and M. Sagai, *Chem. Pharm. Bull.* (Tokyo), **16**, 1151 (1968).
13) M. Ikehara, M. Kaneko, K. Muneyama and H. Tanaka, *Tetrahedron Letters*, **1967**, 3977.
14) K.L. Nagpal and M.M. Dhar, *Tetrahedron Letters*, **1968**, 47.
15) M. Ikehara and K. Muneyama, *Chem. Pharm. Bull.* (Tokyo), **14**, 46 (1966).



showed only starting material and product cyclonucleoside. The use of 1—2 equivalents sodium hydride lowered the yield of compound II in contrast to the case of adenosine, in which 1.5 equivalents were sufficient to complete cyclization. When unprotected 8-bromoguanosine was treated with excess sodium hydride as above, no cyclization occurred even though in higher temperature (100—110°).[16] In some cases removal of 8-bromo atom was observed. These results may be ascribed to the consumption of sodium hydride by acidic NH and/or free hydroxyl groups. The possibility of a favored configuration of the sugar and the base moiety for 8,5′-cyclization in isopropylidene derivative could not be denied.

In order to investigate the mode of hydrolysis of 8,5′-cyclonucleosides, compound II was treated with 0.05N hydrochloric acid in aqueous dioxane. Examination of the reaction mixture by paper chromatography and ultraviolet absorption showed that hydrolytic removal of isopropylidene group proceeded first and it was followed by scission of anhydro-linkage. Reaction completed after 8 hr and the main product was 8-oxyguanosine. Heating in 0.05—1N sodium hydroxide showed no cleavage of the anhydro linkage even at reflux temperature for 7 hr. This result is rather unexpected, because O-anhydro linkage in pyrimidine cyclonucleosides could be easily cleaved by an alkaline treatment.[17]

8,5′-O-Cyclonucleoside (II) was then treated with hydrogen sulfide in pyridine. Reaction was carried out at 110°—120° in a sealed tube for 8 hr. By this procedure 2′,3′-O-isopropylidene-5′-deoxy-5′-mercapto-8-oxyguanosine (III) was obtained. The structure of III was confirmed as having 8-oxyguanosine chromophore, by sodium nitroprusside test and by elemental analyses. From this result it was proposed that nucleophilic attack by thiol had occurred on 5′-carbon rather than on 8-carbon atom and resulted in scission of aliphatic C–O bond. This is in contrast to our expectation that a weak nucleophile would attack 8-carbon and cleave aromatic C–O bond as in 2,5′-cyclonucleoside.[18]

Compound II was then allowed to react with another nucleophile, pottasium thiolacetate. When this reagent was used in ethanol, acetone and DMF, no reaction was observed. However, using acetic acid as solvent, reaction proceeded smoothly at 110—120°. After 8 hrs' reaction, total cleavage of 8,5′-anhydro linkage occurred. Purification of the product by repeated recrystallization gave 2′,3′-O-isopropylidene-5′-S-acetyl-5′-deoxy-5′-mercapto-8-oxyguanosine (IV). Removal of acetyl group in IV with methanolic ammonia gave compound III. Using pottasium ethylxanthogenate as nucleophile in the same condition as above, no reaction with compound II was observed.

When compound II was heated with pottasium thiocyanate in acetic acid at 80—90° for 8 hr, 5′-thiocyanate derivative (V) was obtained in 74% yield. In this case again aliphatic C–O fission is predominant to aromatic C–O and replacement of 8–H to –SCN was not observed. The structure of compound V was confirmed by elemental analytical data and ultraviolet absorption. From infrared absorption spectra an alternate structure 5′–N=C=S could be neglected. The isopropylidene group of V was removed by reflux in 30% acetic acid for 6 hr to give 5′-deoxy-5′-thiocyanate-8-oxyguanosine (VI).

The same type of nucleophilic attack on compound II occurred by treatment with sodium acetate in acetic acid. When reaction mixture was refluxed for 16 hr, 2′,3′-O-isopropylidene-5′-O-acetyl-8-oxyguanosine (VII) was obtained. This compound was identified with an authentic sample[7] and was easily derived to 8-oxyguanosine.

Finally compound II was reacted with potassium cyanate. As could be expected from the results obtained above, reaction proceeded smoothly and gave a crystalline compound VIII by repeated recrystallization from ethanol. Examination of infrared absorption spectra showed that no absorption band at 2200 cm^{-1} to be ascribed to –N=C=O group was found.

16) Treatment of 8-bromoadenosine with sodium hydride in dioxane gave a dimerized product, which involved presumably 8C-2′(or 3′)O bond (unpublished experiment by M. Kaneko).
17) For instance, N.C. Yung and J.J. Fox, *J. Am. Chem. Soc.*, **83**, 3060 (1960).
18) D.M. Brown, D.B. Parihar, A.R. Todd and S. Varadarajan, *J. Chem. Soc.*, **1958**, 3028.

From the elemental analytical data of compound VIII it was suggested that cyanate group once introduced in 5′-position further reacted with ethanol during recrystallization process to give 2′,3′-O-isopropylidene-5′-deoxy-5′-ethoxycarbamido-8-oxyguanosine (VIII).

Considering these results, 8,5′-O-anhydro linkage of cyclonucleoside II is easily cleaved in acetic acid by nucleophilic attack with various reagents such as, water, hydrogen sulfide, sodium thiolacetate, sodium acetate, potassium thiocyanate and potassium cyanate. In all cases replacement occurred exclusively on 5′-C rather than on 8-C atom. This provides a new route to introduce various substituents in 5′-position of 8-oxyguanosine. In contrast to this, in alkaline media 8,5′-O-anhydro linkage of guanosine is fairy stable and cleavage reaction without damaging base and/or sugar moiety seems to be difficult.

Extention of the cleavage reaction on other purine cyclonucleosides is under investigation in our laboratory and will be reported in subsequent papers.

Experimental[19]

Paper Chromatography——Solvent A, *n*-butanol–water, 86:14; solvent B, isopropanol–conc. ammonia–water, 7:1:2. All chromatographies were performed on Toyo filter paper No. 51A by ascending technique.

2′,3′-O-Isopropylidene-8,5′-anhydro-8-oxyguanosine——Sodium hydride (containing 50% mineral oil, 144 mg) was added to pre-cooled DMF (30 ml) at −20°. After 20—30 min, isopropylidene-8-bromoguanosine (402 mg, 1 mmole) was added at −20° and the mixture was stirred for 3—4 hr at this temperature with exclusion of moisture. After it was kept at room temperature for 2 days, yellow reaction mixture was poured in ice-water (30—40 ml) and the solution was neutralized with conc. HCl with stirring. Solvent was evaporated *in vacuo*, the residue was washed with ether and then water. Recrystallization from ethanol–water three times gave 120 mg (37%) of cyclonucleoside. *Anal.* Calcd. for $C_{13}H_{15}O_5N_5 \cdot 1/2H_2O \cdot 1/2C_2H_5OH$: C, 47.59; H. 5.38; N, 19.83. Found: C, 47.93; H, 5.24; N, 19.82. UV: $\lambda_{max}^{H^+}$ 251, 280 nm; $\lambda_{max}^{OH^-}$ 255, 270 nm (shoulder). Paper chromatography: *Rf*(A)[20] 0.47 (isopropylidene-bromoguanosine, 0.68), *Rf*(B) 0.50 (isopropylidene-bromoguanosine, 0.68.).

Hydrolysis of 2′,3′-O-Isopropylidene-8,5′-anhydro-8-oxyguanosine——i) Isopropylidene-8,5′-anhydro-8-oxyguanosine (10 mg) was dissolved in 0.1N HCl–dioxane (1:1, vol/vol) and the mixture was heated in a boiling water bath for 8 hr. Paper chromatography at several reaction intervals performed in solvent A showed change of UV $\lambda_{max}^{H^+}$ at 251 nm to 248 nm and that 280 nm to 293 nm, respectively. The final spectra were identical with those reported for 8-oxyguanosine. ii) Hydrolysis of cyclonucleoside II in 0.1N sodium hydroxide–dioxane (1:1, vol/vol) or in 1N sodium hydroxide at reflux temperature as long as 7 hr showed no change.

2′,3′-O-Isopropylidene-5′-deoxy-5′-mercapto-8-oxyguanosine——Anhydrous pyridine (30 ml) was saturated with hydrogen sulfide with cooling in ice–salt bath. Isopropylidene-cyclonucleoside (II) (321 mg, 1 mmole) was dissolved in the above solution. Reaction mixture was heated at 110—120° in a sealed tube for 8 hr. Solvent was evaporated *in vacuo* and the residue was recrystallized several times from ethanol–water. Yield was 30 mg (8.4%). *Anal.* Calcd. for $C_{13}H_{17}O_5N_5S \cdot H_2O$: C, 41.82; H, 5.09; N, 18.77. Found: C, 42.09; H, 5.20; N, 18.79. UV: $\lambda_{max}^{H^+}$ 248, 295 nm ; $\lambda_{max}^{H_2O}$ 248, 295 nm; $\lambda_{max}^{OH^-}$ 255, 280 nm. Sodium nitroprusside test for SH group was positive. Paper chromatography: *Rf*(A) 0.66, *Rf*(B) 0.57. The spot on paper chromatogram could be revealed by metaperiodate benzidine spray[21] due to the presence of SH group.

2′,3′-O-Isopropylidene-5′-S-acetyl-5′-deoxy-5′-mercapto-8-oxyguanosine——Isopropylidene-8,5′-o-cyclonucleoside (II) (321 mg, 1 mmole) and potassium thiolacetate (228 mg, 2 mmoles) were dissolved in acetic acid (40 ml) and the mixture was heated at 110—120° for 8 hr. Solvent was evaporated *in vacuo* and the residual syrup was evaporated with ethanol in order to remove traces of acetic acid. The final residue was washed with water and recrystallized from ethanol–water. S-Acetyl derivative was obtained as an amorphous solid. (*ca.* 20 mg). *Anal.* Calcd. for $C_{15}H_{19}O_5N_5S$: C, 45.34; H, 4.79; N, 17.63. Found: C, 45.36; H, 5.12; N, 16.86. UV: $\lambda_{max}^{H^+}$ 247, 295 nm; $\lambda_{max}^{H_2O}$ 247, 295 nm; $\lambda_{max}^{OH^-}$ 254, 281 nm (shoulder). Paper chromatography: *Rf*(A) 0.75, *Rf*(B) 0.64.

2′,3′-O-Isopropylidene-5′-deoxy-5′-thiocyano-8-oxyguanosine——Isopropylidene-8,5′-cyclonucleoside (II) (642 mg, 2 mmoles) and potassium thiocyanate (388 mg, 4 mmoles) were dissolved in acetic acid (70 ml). The mixture was heated at 80—90° for 8 hr. Solvent was evaporated *in vacuo*, traces of acetic acid was

19) Ultraviolet absorption spectra were taken with Hitachi EPS-2U spectrophotometer and infrared absorption spectra were taken with JASCO model DS-301 spectrophotometer. All mp were uncorrected.
20) *Rf*(A) stands for *Rf* value obtained in paper chromatography performed in solvent A.
21) M. Viscontini, D. Hoch and P. Karrer, *Helv. Chim. Acta*, **38**, 642 (1948).

removed by repeated addition and evaporation of ethanol, and the residue was recrystallized from ethanol–water. Yield was 566 mg (74%). *Anal.* Calcd. for $C_{14}H_{16}O_5N_6S$: C, 44.21; H, 4.21; N, 22.11; Found: C, 44.19; H, 4.16; N, 21.96. UV: $\lambda_{max}^{H^+}$ 247, 295 nm; $\lambda_{max}^{H_2O}$ 247, 295 nm; $\lambda_{max}^{OH^-}$ 253, 280 (shoulder) nm. IR: ν_{max}^{Nujol} 2180 cm^{-1} (–SCN).

5′-Deoxy-5′-thiocyano-8-oxyguanosine——Isopropylidene-5′-thiocyano-8-oxyguanosine (380 mg, 1 mmole) was dissolved in 30% acetic acid (70 ml) and the mixture was refluxed for 6 hr. Solvent was evaporated *in vacuo*, traces of water were removed by repeated addition and evaporation of ethanol, and the residue was recrystallized from ethanol–water. Yield was 115 mg (34%). *Anal.* Calcd. for $C_{11}H_{12}O_5N_6S$: C, 38.82; H, 3.53; N, 24.71. Found: C, 38.85; H, 3.74; N, 24.61. Paper chromatography: *Rf*(A) 0.19, *Rf*(B) 0.42. UV: $\lambda_{max}^{H^+}$ 247, 295 nm; $\lambda_{max}^{H_2O}$ 247, 295 nm; $\lambda_{max}^{OH^-}$ 253, 280 (shoulder) nm.

2′3′-O-Isopropylidene-5′-O-acetyl-8-oxyguanosine——Isopropylidene-8,5′-O-cyclonucleoside (II)(321 mg, 1 mmole) was dissolved in acetic acid (50 ml) containing anhydrous sodium acetate (100 mg, 1.2 mmole). Mixture was refluxed for 16 hr. Evaporation of the solvent gave a hard glass. UV: $\lambda_{max}^{H^+}$ 245.6, 292.5 nm; $\lambda_{max}^{H_2O}$ 245.6; 292.5 nm; $\lambda_{max}^{OH^-}$ 258, 280 nm. Paper chromatography: *Rf*(A) 0.72, *Rf*(B) 0.61. IR: ν_{max}^{Nujol} 1720 cm^{-1} (8–CO), 1720 cm^{-1} (shoulder, CH_3CO). These properties were identical with an authentic sample.[7] Isopropylidene group of the product was removed by reflux with 30% acetic acid to give 5′-O-acetyl-8-oxyguanosine.

2′,3′-O-Isopropylidene-5′-deoxy-5′-ethoxycarbamido-8-oxyguanosine——Isopropylidene-8,5′-cyclonucleoside (II)(1.284 g) and pottasium cyanate (486 mg) were dissolved in acetic acid (70 ml) and the mixture was refluxed for 8 hr. Solvent was evaporated *in vacuo*, traces of acetic acid were removed by repeated addition and evaporation with ethanol, and the residue was recrystallized from ethanol–water three times. Yield was 100 mg (24%). *Anal.* Calcd. for $C_{16}H_{22}O_7N_6 \cdot 1/3H_2O$: C, 46.15; H, 5.52; N, 20.19. Found: C, 46.01; H, 5.21; N, 20.15. UV $\lambda_{max}^{H^+}$ 248, 292 nm; $\lambda_{max}^{H_2O}$ 248, 292 nm; $\lambda_{max}^{OH^-}$ 257, 281 nm. IR: no band at 2220 cm^{-1} (–N=C=O) was found. Paper chromatography: *Rf*(A) 0.72, *Rf*(B) 0.66.