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Purine Nucleosides. VII. Direct Bromination of Adenosine, Deoxyadenosine, Guanosine, and Related Purine Nucleosides[1]

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Procedures have been developed for direct bromination of the acetyl derivatives of adenosine, deoxyadenosine, guanosine, and inosine to yield (after deacetylation with alcoholic ammonia) the corresponding 8-bromopurine nucleosides, 8-bromoadenosine (X), 8-bromo-2′-deoxyadenosine (XI), 8-bromoguanosine (III), and 8-bromoinosine (XVI). These 8-bromopurine nucleosides may be treated with various nucleophilic reagents to provide new and interesting 8-substituted derivatives of the naturally occurring purine nucleosides. 2′-Deoxy-8-mercaptoadenosine [XIII, prepared from 8-bromo-2′-deoxyadenosine (XI)] when treated with iodine in potassium iodide solution gave 2′-deoxy-8-iodoadenosine (XIV) which could not be prepared by direct iodination procedures. The possible biochemical importance of these derivatives is discussed.

Direct halogenation of various pyrimidine ribosides and deoxyribosides has long been known to provide the appropriate 5-halogenated pyrimidine nucleoside.[2-7] Interest in recent years in the biochemistry of nucleic acids has stimulated improved halogenation procedures[8-12] and study of the 5-halogenated pyrimidine nucleosides as therapeutic agents.[13-15] The halogenation of the naturally occurring purine nucleosides, however, has received very little attention.[16,16a] Although Suzuki and Ito[17] and Jones and Woodhouse[18] have studied the action of bromine water on adenosine and guanosine and have noted some change in the ultraviolet absorption spectra, no identifiable products were isolated. The bromination of RNA and DNA has been studied by various investigators.[18-21] Of particular interest is the fact that bromination of tobacco mosaic virus RNA has been shown to cause mutations which changed the amino acid composition of the isolated protein.[21-23]

It thus seemed worth while to investigate the bromination of purine nucleosides to determine the site of bromination and to isolate and characterize any brominated purine nucleoside derivatives. The possibility of preparing well defined, crystalline brominated purine nucleosides for incorporation into RNA and DNA seemed especially attractive for future biochemical studies.

Since 9-methylxanthine has been reported[24] to yield 8-bromo-9-methylxanthine when treated with bromine in acetic acid, it seemed possible that purine nucleosides such as xanthosine and guanosine might also brominate under similar conditions. This has now proved to be the case. When 2′,3′,5′-tri-*O*-acetylguanosine (I),[25] dissolved in glacial acetic acid containing sodium acetate, was treated with bromine in glacial acetic acid at 50–60°, a good yield of 8-bromo-2′,3′,5′-tri-*O*-acetylguanosine (II) was isolated. Deacetylation of II with methanolic ammonia provided 8-bromoguanosine (III) in above 50% over-all yield from 2′,3′,5′-tri-*O*-acetylguanosine (I). Treatment of III with refluxing *N* hydrochloric acid gave D-ribose, identified chromatographically,[26] and 8-bromoguanine which proved to be identical with an authentic sample[27] as judged by ultraviolet absorption data and the same R_f values in three different solvents (Table I). The position of substitution of the bromine atom was further confirmed by the absence of the characteristic sharp absorption peak at 8.38 δ due to H-8 of guanosine[28] when the n.m.r. spectrum was determined in dimethyl sulfoxide.

TABLE I

R_f VALUES OF 8-BROMOPURINES AND PURINE NUCLEOSIDES

Compound	Solvent[a] A	B	C
8-Bromoguanosine	0.69	0.69	0.19
8-Bromoadenosine	.64	.73	.51
8-Bromo-2′-deoxyadenosine	.65	.78	.26
8-Bromoguanine	.38	.67	.12
8-Bromoadenine	.28	.62	.24

[a] Solvent A = 5% NH_4HCO_3 in H_2O; B = EtOH:H_2O::7:3 (v./v.); C = DMF:NH_4OH:*i*-PrOH::25:10:65 (v./v.).

The preparation of 8-bromoguanosine (III) provides a useful synthetic intermediate since the bromo group can be replaced by various nucleophilic reagents. For example, III and thiourea in refluxing ethanol readily provided 8-mercaptoguanosine (IV). Alkylation of IV with dimethyl sulfate gave 8-methylthioguanosine (V). 8-(2-Hydroxyethylthio)guanosine (VI) was similarly prepared from IV and 2-bromoethanol. 8-Mercaptoguanosine (IV) also provided 8-iodoguanosine upon treatment with iodine in potassium iodide solution in the presence of sodium bicarbonate.[29] 8-Bromoxanthosine (VII) was readily prepared by treatment of 8-

(1) Supported by research grants CY-4008(C4) and CA 04008-06 from the National Cancer Institute of the National Institutes of Health, Public Health Service.

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(16a) NOTE ADDED IN PROOF.—A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, New York, N. Y., 1963, p. 34. Notation is made that guanosine can be brominated at the 8-position with dioxane bromide.

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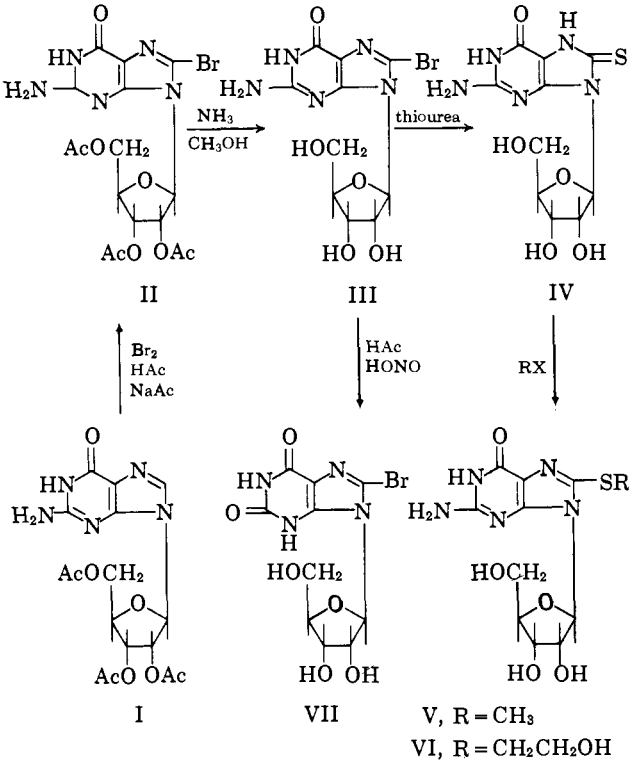
bromoguanosine (III) with glacial acetic acid and sodium nitrite. The ultraviolet absorption spectra of 8-bromoxanthosine were found to be very similar to those reported for 8-bromo-9-methylxanthine.[30]

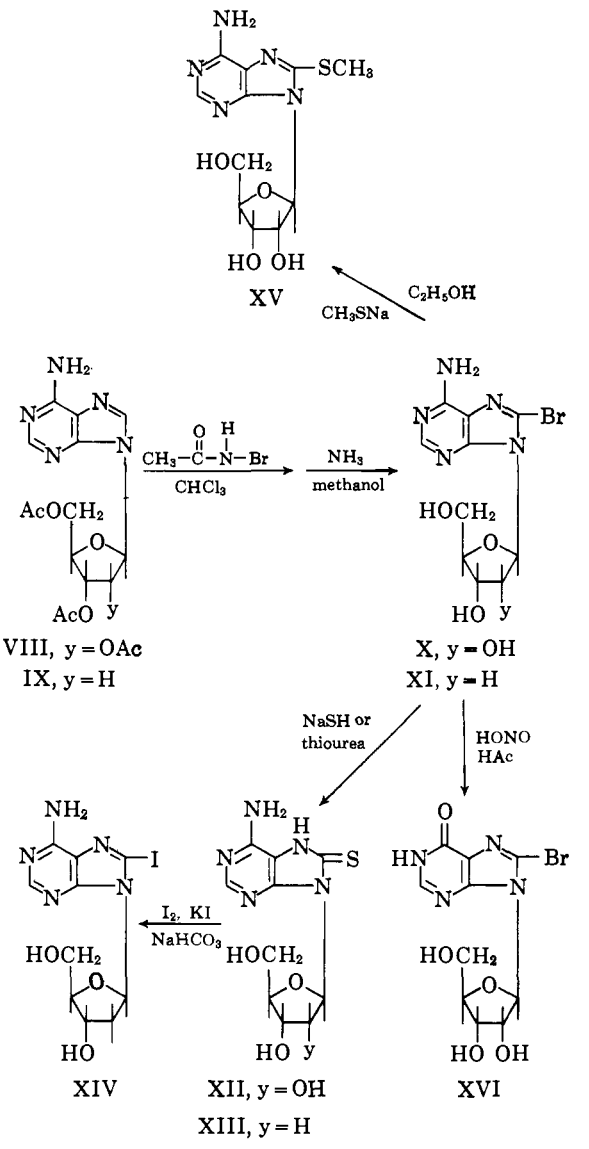
REACTION SCHEME I



Bromination of 2′,3′,5′-tri-*O*-acetyladenosine (VIII) occurred under conditions similar to those employed for the bromination of 2′,3′,5′-tri-*O*-acetylguanosine (I) to give a 59% yield of 8-bromo-2′,3′,5′-tri-*O*-acetyladenosine. It was later discovered that VIII could be brominated in a more satisfactory manner by the utilization of N-bromoacetamide in chloroform. Deacetylation of this product gave 8-bromoadenosine (X) in 80% yield. The position of the entering bromine was confirmed by acid hydrolysis to the known 8-bromoadenine[31] and D-ribose.[26] These milder conditions for bromination utilizing N-bromoacetamide were also found suitable for the direct bromination of 3′,5′-di-*O*-acetyl-2′-deoxyadenosine (IX)[32] to yield 8-bromo-2′-deoxyadenosine (XI) after deacetylation. The successful bromination of IX could not be accomplished in glacial acetic acid due to loss of 2′-deoxyribose under these conditions.

Although thiourea and refluxing ethanol converted 8-bromoadenosine (X) into 8-mercaptoadenosine (XII), the same conditions resulted in nucleoside cleavage when 8-bromo-2′-deoxyadenosine (XI) was used. However, when sodium hydrosulfide was employed, the desired 2′-deoxy-8-mercaptoadenosine (XIII) was readily obtained. Treatment of XIII with iodine in potassium iodide solution in the presence of sodium bicarbonate gave 2′-deoxy-8-iodoadenosine (XIV) which could not be successfully prepared by direct iodination procedures. Acid hydrolysis of 8-mercaptoadenosine (XII) and 2′-deoxy-8-mercaptoadenosine (XIII) each gave 8-mercaptoadenine which was identified by comparison (ultraviolet absorption data and paper chromatography)

REACTION SCHEME II



with an authentic product prepared by an independent route.[33] This provided additional proof of 8 substitution.

Sodium nitrite and glacial acetic acid converted 8-bromoadenosine (X) to 8-bromoinosine (XVI). 8-Bromoinosine was also prepared *via* direct bromination of 2′,3′,5′-tri-*O*-acetylinosine[25] with N-bromoacetamide in chloroform which gave 8-bromo-2′,3′,5′-tri-*O*-acetylinosine. Deacetylation of 8-bromo-2′,3′,5′-tri-*O*-acetylinosine gave 8-bromoinosine (XVI) identical with that prepared from 8-bromoadenosine. Further proof of the position of the bromine was found when XVI was hydrolyzed with acid to yield 8-bromohypoxanthine.[31] Further synthetic utility of these 8-bromopurine nucleosides is illustrated by the preparation of 8-methylthioadenosine (XV) from 8-bromoadenosine (X) and sodium methylmercaptide.

Electron density calculations[34,35] predict the 8-position of purine to be the most susceptible to electrophilic and free radical attack. The possibility that the bromination of the purine nucleosides may proceed *via* a free radical mechanism in nonpolar solvents is a subject under current exploration in our laboratory.

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Experimental

8-Bromo-2′,3′,5′-tri-*O*-acetylguanosine (II).—To a solution of 100 g. of sodium acetate and 100 g. of 2′,3′,5′-tri-*O*-acetylguanosine[25] in 700 ml. of glacial acetic acid was added 157 g. of bromine. The temperature was maintained between 50–60° for 36 hr., and the solution was then evaporated to dryness *in vacuo* using a steam bath as a source of heat. The residue was added to 1 l. of boiling isopropyl alcohol. The sodium acetate was filtered, the isopropyl alcohol was evaporated to dryness *in vacuo*, and the residue was recrystallized from acetone–water to yield 76 g. (64%) of product, m.p. 214–217°. An analytical sample, m.p. 216–218°, was obtained by further recrystallization from acetone–water.

Anal. Calcd. for $C_{16}H_{18}BrN_5O_8$: C, 39.3; H, 3.69; N, 14.3; Br, 16.3. Found: C, 39.4; H, 3.66; N, 14.4; Br, 16.5.

8-Bromoguanosine (III).—8-Bromo-2′,3′,5′-tri-*O*-acetylguanosine (II, 80 g.) was added to 700 ml. of methanolic ammonia (saturated at −10°), and the solution was allowed to stand for 18 hr. at room temperature. The precipitate that formed was removed by filtration. The methanolic solution was evaporated to dryness, and the residue and precipitate were combined and recrystallized twice from water to yield 49.6 g. (84%) of product which softened at 192° and slowly decomposed above 200°. The over-all yield from tri-*O*-acetylguanosine was 53–54%. 8-Bromoguanosine showed $[\alpha]^{30}D$ −25.9° (*c* 2, dimethyl sulfoxide–water, 1:1, v./v.), $\lambda_{max}^{pH\ 1}$ 261 mμ (ε 15,600), $\lambda_{max}^{pH\ 11}$ 270 mμ (ε 14,100).

Anal. Calcd. for $C_{10}H_{12}BrN_5O_5$: C, 33.2; H, 3.32; N, 19.3; Br, 16.3. Found: C, 33.2; H, 3.02; N, 19.4; Br, 16.5.

8-Bromo-2′,3′,5′-tri-*O*-acetyladenosine. Method A.—Tri-*O*-acetyladenosine (VIII, 50 g.)[25] and N-bromoacetamide[36] (50 g.) were added to 500 ml. of dry chloroform, and the solution was refluxed for 5 hr. The chloroform was removed *in vacuo*, and the dark red residue was dissolved in 1 l. of ethyl acetate. The ethyl acetate solution was extracted first with 500 ml. of an aqueous solution containing 60 g. of sodium hydrosulfite and finally with 500 ml. of a saturated sodium bicarbonate solution. The ethyl acetate extract was dried over sodium sulfate and then evaporated to dryness *in vacuo*. The red residue was recrystallized from tetrahydrofuran to yield 33.5 g. of product (yield 55%), m.p. 185–186° dec. An analytically pure sample, m.p. 187–188° dec., was obtained by one more recrystallization from tetrahydrofuran. Ultraviolet data showed $\lambda_{max}^{pH\ 1}$ 262.5 mμ (ε 18,900), $\lambda_{max}^{pH\ 11}$ 264 mμ (ε 17,500).

Anal. Calcd. for $C_{16}H_{18}BrN_5O_7$: C, 40.7; H, 3.82; N, 14.9; Br, 16.9. Found: C, 40.8; H, 4.00; N, 15.0; Br, 16.5.

Method B.—To a solution of 15 g. of sodium acetate and 15 g. of tri-*O*-acetyladenosine[25] in 100 ml. of glacial acetic acid was added 20 g. of bromine. The solution was kept between 50–60° for 36 hr. and then evaporated to dryness *in vacuo* (water aspirator). To the residue was added 500 ml. of ethyl acetate, and the mixture was filtered to remove the sodium acetate. The ethyl acetate solution was extracted with 100-ml. portions of a 10% sodium bisulfite solution until the dark red color disappeared. The solution was then extracted with two 100-ml. portions of a saturated solution of sodium bicarbonate, dried over sodium sulfate, and finally evaporated to dryness *in vacuo*. The residue was recrystallized from tetrahydrofuran to yield 8.8 g. (59%) of product, m.p. 186–187° dec. The ultraviolet absorption spectrum was identical with that of the compound prepared by method A. A mixture melting point of this product and that obtained by method A showed no depression.

Anal. Calcd. for $C_{16}H_{18}BrN_5O_7$: C, 40.7; H, 3.82; N, 14.9. Found: C, 40.5; H, 3.45; N, 14.9.

8-Bromoadenosine (X).—8-Bromo-2′,3′,5′-tri-*O*-acetyladenosine (30 g.) was added to 500 ml. of a methanolic ammonia solution (saturated at −10°), and the resulting solution was allowed to stand for 18 hr. at room temperature. The methanol was removed *in vacuo*, and the residue was recrystallized from ethanol–water to yield 17.5 g. (80%) of product which melted with slow decomposition at >200°. 8-Bromoadenosine showed $[\alpha]^{30}D$ −55° (*c* 2.5, in dimethyl sulfoxide–water, 1:1, v./v.), $\lambda_{max}^{pH\ 1}$ 262.5 mμ (ε 19,000), $\lambda_{max}^{pH\ 11}$ 264 mμ (ε 17,600).

Anal. Calcd. for $C_{10}H_{12}BrN_5O_4$: C, 34.6; H, 3.46; N, 20.2; Br, 23.1. Found: C, 34.8; H, 3.91; N, 20.2; Br, 23.0.

8-Bromo-2′-deoxyadenosine (XI).—3′,5′-Di-*O*-acetyl-2′-deoxyadenosine[32] (20 g.) and 20 g. of N-bromoacetamide were added to 200 ml. of dry chloroform. The solution was refluxed for 5 hr., during which time all solids dissolved and the solution turned dark red. The chloroform was removed *in vacuo*, and to the dark red residue was added 300 ml. of methanolic ammonia (saturated at −10°). The solution was allowed to stand at room temperature for 18 hr., and then the methanol was removed *in vacuo*, and the dark brown residue was triturated twice with 150 ml. of hot acetone. The brown residue was then dissolved in 1 l. of hot methanol; charcoal was added, and the solution was filtered through Celite. The methanolic solution was reduced *in vacuo* to approximately 100 ml. and set in the refrigerator overnight. The solid that crystallized was filtered and washed with cold methanol to yield 9.7 g. (49%) of product which slowly decomposed >195°. 8-Bromo-2′-deoxyadenosine exhibited $[\alpha]^{30}D$ −33° (*c* 0.47, in methanol), $\lambda_{max}^{pH\ 1}$ 262.5 mμ (ε 17,800), $\lambda_{max}^{pH\ 11}$ 264 mμ (ε 17,000).

Anal. Calcd. for $C_{10}H_{12}BrN_5O_3$: C, 36.3; H, 3.63; N, 21.2; Br, 24.2. Found: C, 36.0; H, 3.41; N, 21.5; Br, 23.9.

8-Mercaptoguanosine (IV).—Thiourea (4 g.) was added to a suspension of 8-bromoguanosine (III, 10 g.) in 400 ml. of absolute ethanol, and the mixture was refluxed for 5 hr. The reaction mixture was allowed to cool to room temperature and then was filtered. The residue was recrystallized from water to give 7.8 g. (92%) of product, m.p. >220° dec. For analysis a sample was dried at 110° (0.1 mm.) for 12 hr. The ultraviolet data showed $\lambda_{max}^{pH\ 1}$ 302, 285, 230 mμ (ε 19,400, 19,500, 13,000); $\lambda_{max}^{pH\ 11}$ 290 mμ (ε 21,100).

Anal. Calcd. for $C_{10}H_{13}N_5O_5S$: C, 32.8; H, 4.13; N, 22.4; S, 10.0. Found: C, 32.8; H, 4.51; N, 22.4; S, 9.5.

8-Mercaptoadenosine (XII).—Thiourea (0.6 g.) was added to a solution of 2 g. of 8-bromoadenosine (X) in 100 ml. of absolute ethanol, and the solution was refluxed for 4 hr. The ethanol was removed *in vacuo*, and the residue was recrystallized from a water–ethanol mixture to give 0.9 g. (52%) of product, m.p. 171–173° dec. The ultraviolet data showed $\lambda_{max}^{pH\ 1}$ 308, 240, 222 mμ (ε 27,600, 12,000, 14,400); $\lambda_{max}^{pH\ 11}$ 297, 230 mμ (ε 25,100, 20,900).

Anal. Calcd. for $C_{10}H_{13}N_5O_4S$: C, 40.1; H, 4.35; N, 23.4. Found: C, 39.8; H, 5.00; N, 23.8.

8-Bromoxanthosine (VII).—8-Bromoguanosine (III, 5 g.) was suspended in 100 ml. of glacial acetic acid, and then 5 g. of sodium nitrite, dissolved in 15 ml. of water, was added. The solution was stirred for 5 hr. during which time all solid material dissolved. The solution then was evaporated to dryness *in vacuo*, and the residue was dissolved in a minimum amount of hot water. After allowing the solution to cool to room temperature, the white solid that precipitated was filtered and recrystallized from water to yield 2.6 g. (52%) of product, m.p. >210° dec. The ultraviolet data showed $\lambda_{max}^{pH\ 1}$ 267, 240 mμ (ε 12,900, 19,500); $\lambda_{max}^{pH\ 11}$ 281, 252 mμ (ε 12,700, 12,000).

Anal. Calcd. for $C_{10}H_{11}BrN_4O_5$: C, 33.11; H, 3.03; N, 15.4. Found: C, 33.2; H, 3.56; N, 15.3.

8-(2-Hydroxyethylthio)guanosine (VI).—To a mixture of 8-mercaptoguanosine (IV, 10 g.) and 4.8 g. of anhydrous potassium carbonate in 150 ml. of dimethylformamide was added 4.1 g. of 2-bromoethanol. The mixture was heated with stirring at 75° for 4 hr., allowed to cool to room temperature, and then added to 1500 ml. of acetone. The pH of the solution was adjusted to 6 with glacial acetic acid; the mixture was filtered and the residue was recrystallized from water to yield 5.9 g. (51%), m.p. 128–129°. For analysis a sample was dried at 110° (0.1 mm.) for 12 hr. The ultraviolet data showed $\lambda_{max}^{pH\ 1}$ 289 (shoulder), 272 mμ (ε 12,900, 16,000); $\lambda_{max}^{pH\ 11}$ 285 mμ (ε 16,000).

Anal. Calcd. for $C_{12}H_{17}N_5O_6S$: C, 40.1; H, 4.73; N, 19.5. Found: C, 39.9; H, 4.82; N, 19.2.

8-Methylthioadenosine (XV).—To a solution of 0.7 g. of sodium in 100 ml. of absolute ethanol was added 4 ml. of methanethiol. To this solution was added 2 g. of 8-bromoadenosine (X). The solution was refluxed for 4 hr. and cooled to room temperature. Glacial acetic acid (2 ml.) was added, and the sodium acetate that precipitated was filtered. The filtrate was evaporated to dryness *in vacuo*, and the residue was recrystallized from ethanol–water to yield 1.2 g. (66%) of product, m.p. 237–238° dec. The ultraviolet data showed $\lambda_{max}^{pH\ 1}$ 281 mμ (ε 18,500), $\lambda_{max}^{pH\ 11}$ 279 mμ (ε 17,200).

Anal. Calcd. for $C_{11}H_{15}N_5O_4S$: C, 42.1; H, 4.8; N, 22.4. Found: C, 42.4; H, 5.1; N, 22.2.

8-Methylthioguanosine (V).—To a mixture of 8-mercaptoguanosine (IV, 5 g.) and 2.4 g. of anhydrous potassium carbonate in 75 ml. of dimethylformamide was added 2.2 g. of dimethyl sulfate. The mixture was heated with stirring at 70–75° for 3 hr., and the resulting solution was cooled to room temperature and poured into 1 l. of acetone. The pH of the solution was adjusted to 6 with glacial acetic acid. The mixture was filtered, and the residue was recrystallized from water to yield 3.7 g. (70%) of product, m.p. >200° dec. For analysis a sample was heated at 110° (0.1 mm.) for 12 hr. The ultraviolet data showed $\lambda_{max}^{pH\ 1}$ 289 (shoulder), 272 mμ (ε 13,500, 16,900); $\lambda_{max}^{pH\ 11}$ 284 mμ (ε 16,900).

Anal. Calcd. for $C_{11}H_{15}N_5O_5S \cdot 0.5H_2O$: C, 39.1; H, 4.73; N, 20.7. Found: C, 38.8; H, 5.00; N, 21.1.

8-Iodoguanosine.[16]—To a suspension of 5 g. of 8-mercaptoguanosine (IV) in 250 ml. of water was added 15 g. of sodium bicarbonate, 10 g. of potassium iodide, and 15 g. of iodine. The

(36) Purchased from Arapahoe Chemicals, Inc., Boulder, Colo.

solution was stirred for 7 days at room temperature, and the resulting mixture was filtered. The residue was washed with 100 ml. of a 10% potassium iodide solution and then dissolved in a minimum amount of hot water. Concentrated ammonium hydroxide was added until the solution became colorless, and then the pH was adjusted to 6 by addition of acetic acid. The solution then was allowed to cool to room temperature, and the white precipitate that formed was filtered and recrystallized from water to give 3.7 g. (57%) of product, which darkens at 190°, dec. 203–204°. For analysis a sample was dried at 110° (0.1 mm.) for 12 hr. The ultraviolet data showed $\lambda_{max}^{pH\ 1}$ 261 mμ (ε 17,600), $\lambda_{max}^{pH\ 11}$ 271 mμ (ε 16,800).

Anal. Calcd. for $C_{10}H_{12}IN_5O_5$: C, 29.4; H, 2.94; N, 17.1; I, 33.1. Found: C, 29.3; H, 3.50; N, 16.9; I, 31.5.

2′-Deoxy-8-mercaptoadenosine (XIII).—To a suspension of 8-bromo-2′-deoxyadenosine (2 g.) in 200 ml. of absolute ethanol was added 10 ml. of a 2 *M* solution of sodium in absolute ethanol saturated at 0° with hydrogen sulfide. The resulting solution was refluxed for 3 hr. and finally cooled to 0°. The pH was adjusted to 6–7 with glacial acetic acid, and the solution then was evaporated to dryness *in vacuo*. To the residue was added 20 ml. of water, and the resulting solution was allowed to stand for 2 hr. at room temperature during which time a precipitate formed. The precipitate was removed by filtration and then dissolved in hot water; the solution was treated with charcoal, filtered, and allowed to stand overnight in the refrigerator. The crystals which formed were removed by filtration to yield 1.3 g. (76%) of product, m.p. 133–134° (loss of water), sample resolidifies at 165–170°, decomposes >250°. The ultraviolet data showed $\lambda_{max}^{pH\ 1}$ 308, 242, 222 mμ (ε 27,500, 11,800, 13,000); $\lambda_{max}^{pH\ 11}$ 297, 230 mμ (ε 24,400, 21,700).

Anal. Calcd. for $C_{10}H_{13}N_5O_3S \cdot 1.5H_2O$: C, 39.1; H, 4.73; N, 20.7. Found: C, 38.8; H, 5.00; N, 21.1.

The sample was dried at 110° (0.1 mm.) for 10 hr.

Anal. Calcd. for $C_{10}H_{13}N_5O_3S \cdot 0.5H_2O$: C, 41.1; H, 4.79; N, 24.0. Found: C, 41.2; H, 5.01; N, 23.9.

2′-Deoxy-8-iodoadenosine (XIV).—To a suspension of 2′-deoxy-8-mercaptoadenosine (XIII, 3 g.) in 75 ml. of water was added 9 g. of sodium bicarbonate, 6 g. of potassium iodide, and 9 g. of iodine. The resulting mixture was stirred for 28 hr. and filtered. The residue was washed with 100 ml. of a 10% potassium iodide solution and then dissolved in 95% ethanol. The resulting solution was boiled with charcoal and filtered, and the filtrate was evaporated to dryness *in vacuo*. The residue was recrystallized from ethanol–water to yield 1.9 g. (46%) of product, m.p. 199–201° dec. The ultraviolet data showed $\lambda_{max}^{pH\ 1}$ 279 (shoulder), 271 mμ (ε 16,200, 19,200); $\lambda_{max}^{pH\ 11}$ 280 (shoulder), 269 mμ (ε 13,200, 17,000).

Anal. Calcd. for $C_{10}H_{12}IN_5O_2$: C, 31.8; H, 3.18; N, 18.6; I, 33.7. Found: C, 31.9; H, 3.72; N, 18.6; I, 33.9.

8-Bromoinosine (XVI). Method A.—To a suspension of 8-bromoadenosine (X) in 100 ml. of acetic acid was added 2 g. of sodium nitrite dissolved in 15 ml. of water. The mixture was stirred for 5 hr. during which time all solid material dissolved. The solution was then evaporated to dryness *in vacuo* and the residue was recrystallized from ethanol–water to yield 0.92 g. (46%) of product, m.p. 195–198° dec. Recrystallization from ethanol–water raised the melting point to 198–200° dec. The ultraviolet data showed $\lambda_{max}^{pH\ 1}$ 253.5 mμ (ε 15,100), $\lambda_{max}^{pH\ 11}$ 259 mμ (ε 14,400).

Anal. Calcd. for $C_{10}H_{11}BrN_4O_5$: C, 34.6; H, 3.17; N, 16.1. Found: C, 34.8; H, 3.62; N, 15.8.

Method B.—8-Bromo-2′,3′,5′-tri-*O*-acetylinosine (3 g.) was added to 150 ml. of methanolic ammonia (saturated at −10°); the resulting solution was stirred overnight and then evaporated to dryness *in vacuo*. The residue was crystallized from ethanol–water to yield 1.4 g. (63%) of product, m.p. 198–200° dec. The ultraviolet absorption spectral data were identical with those of XVI prepared by method A.

Anal. Calcd. for $C_{10}H_{11}BrN_4O_5$: C, 34.6; H, 3.17; N, 16.1; Br, 23.0. Found: C, 34.4; H, 3.55; N, 16.2; Br, 23.0.

8-Bromo-2′,3′,5′-tri-*O*-acetylinosine.—Tri-*O*-acetylinosine[25,37] (20 g.) and N-bromoacetamide (20 g.) were added to 500 ml. of dry chloroform, and the resulting solution was refluxed for 8 hr. Another 10 g. of N-bromoacetamide was added, and the solution was refluxed for an additional 10 hr. The excess chloroform was removed *in vacuo* and the dark red residue dissolved in 1 l. of ethyl acetate. The ethyl acetate solution was extracted twice with 200-ml. portions of a 10% solution of sodium hydrosulfite and then with 200 ml. of a saturated sodium bicarbonate solution. The ethyl acetate solution then was dried over sodium sulfate and evaporated to dryness *in vacuo*, and the residue was recrystallized from ethanol to yield 12.6 g. (52%) of product, m.p. 169–172°. Recrystallization from ethanol raised the melting point to 173–175°. The ultraviolet data showed $\lambda_{max}^{pH\ 1}$ 253.5 mμ (ε 14,700), $\lambda_{max}^{pH\ 11}$ 259 mμ (ε 13,900).

Anal. Calcd. for $C_{18}H_{17}BrN_4O_8$: C, 40.7; H, 3.59; N, 11.8; Br, 16.9. Found: C, 40.9; H, 3.90; N, 12.1; Br, 16.7.

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