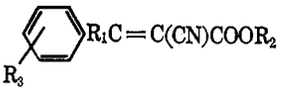
TABLE V

PHYSICAL PROPERTIES OF NEW α-CYANOCINNAMIC ESTERS OF THE TYPE 

Compd	R_1	R_2	R_3	Configuration	Mp, °C [bp °C (mm)]	Formula	Calcd, % C	Calcd, % C	Calcd, % H	Found, % H	Found, % N	Found, % N
III	H	Me	*o*-OMe	*trans*	111–112	$C_{12}H_{11}NO_3$	66.35	66.61	5.10	5.40	6.45	6.39
V	H	Me	*p*-OMe	*trans*	104–106	$C_{12}H_{11}NO_3$	66.35	66.53	5.10	5.05	6.45	6.26
VII	H	Me	*p*-N(Me)$_2$	*trans*	142–143	$C_{13}H_{14}N_2O_2$	67.81	...	6.13	...	12.17	12.03
VIII	H	Me	*p*-NO_2	*trans*	153–154	$C_{12}H_{10}N_2O_4$	58.53	58.67	4.09	4.16	11.38	11.14
XIII	Et	Me	H	Mixture	[154 (7)]	$C_{13}H_{13}NO_2$	72.54	72.46	6.09	5.98	6.51	6.76
XIV	Et	Et	H	Mixture	[163 (6)]	$C_{14}H_{15}NO_2$	73.34	73.59	6.59	6.56	6.11	5.95
XV	*i*-Pr	Me	H	Mixture	80–82 [146 (6)]	$C_{14}H_{15}NO_2$	73.34	73.63	6.59	6.60	6.11	5.96
XVI	*i*-Pr	Et	H	Mixture	73–76	$C_{15}H_{17}NO_2$	74.05	74.11	7.04	6.97	5.76	5.50
XIX	Me	Me	*p*-Me	Mixture	64–66 [168 (6)]	$C_{13}H_{13}NO_2$	72.54	72.46	5.09	6.19	6.51	6.54
XXII	Me	Me	*p*-NO_2	*trans*	153–154	$C_{12}H_{10}N_2O_4$	58.53	58.67	4.09	4.16	11.38	11.14

Acknowledgment.—The author wishes to express his gratitude to Dr. Hiroshi Midorikawa of this Institute and Dr. Toshio Miwa[21] and Dr. Wakatsu Inagaki of the Osaka City University for their valuable discussion. Thanks are also due to Mr. Jun Uzawa of this institute for running many of the nmr spectra and due to Dr. Isaburo Hori and Mr. Minoru Igarashi of this institute for supplying the samples.

(21) During the present investigation, a similar study has been made by him independently.

Purine Nucleosides. XIII. The Synthesis of 2-Fluoro- and 2-Chloroinosine and Certain Derived Purine Nucleosides[1]

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Received September 22, 1965

The preparation of 2-fluoroinosine (VI) was accomplished first, by catalytic debenzylation of 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (VIII) and secondly, by oxidative desulfurization of 2-fluoro-9-β-D-ribofuranosyl-6-purinethione. 2-Chloroinosine (III) was similarly prepared from 2-chloro-9-β-D-ribofuranosyl-6-purinethione (II). Treatment of 2-fluoroinosine with dimethylamine gave N^2-dimethylguanosine. 2-Methylamino- and 2-dimethylamino-9-β-D-ribofuranosyl-6-purinethione (IX) were converted to N^2-methyl and N^2-dimethylguanosine, respectively. These reactions provide new synthetic routes to the N-methylated guanosine nucleosides which have been shown to occur naturally in soluble RNA. 2-Methoxyinosine (XI) has been prepared from 2-methoxy-6-benzyloxy-9-β-D-ribofuranosylpurine (XII) by catalytic debenzylation. The synthesis of XII was readily accomplished from 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (VIII). The interesting derivative, 2-fluoro-6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (XVIII), was prepared by diazotization of 2-amino-6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine in the presence of fluoroboric acid. The preparation of 2,6-dichloro-9-β-D-ribofuranosylpurine (XV) has been achieved for the first time by direct diazotization of 2-amino-6-chloro-9-β-D-ribofuranosylpurine in the presence of concentrated hydrochloric acid. The importance of these new intermediates in the general syntheses of new purine nucleosides is discussed.

A recently devised route for the synthesis of N^2-substituted guanosine[2] involved the synthesis of 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (VIII) from 2-amino-6-benzyloxy-9-β-D-ribofuranosylpurine (IV). In connection with this earlier work[2] several attempts were made to prepare 2-chloro- and 2-fluoroinosine as intermediates in this investigation. The present study is a report of the successful synthesis of these most useful nucleoside derivatives.

The preparation of 2-fluoroinosine (VI) has now been accomplished in excellent yield from 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (VIII) by catalytic debenzylation. A second synthesis of 2-fluoroinosine (VI) has also been achieved from 2-fluoro-9-β-D-ribofuranosyl-6-purinethione[3] (V) and hydrogen peroxide in the presence of a small amount of dilute, aqueous ammonia. The synthesis of 2-chloroinosine (III) was also investigated. Previous attempts to prepare 2-chloroinosine from 2,6-dichloro-9-(2′,3′,5′-*O*-acetyl-β-D-ribofuranosyl)purine in our laboratory[2] have been unsuccessful. Diazotization of 2-amino-9-β-D-ribofuranosyl-6-purinethione[4] (I) in the presence of concentrated hydrochloric acid gave 2-chloro-9-β-D-ribofuranosyl-6-purinethione (II). Treatment of II with alkaline hydrogen peroxide gave 2-chloroinosine in good yield. This oxidative removal of sulfur and exchange for hydroxyl proved to be much simpler and greatly superior to the exchange of sulfur for oxygen *via* the β-hydroxyethylthio procedure employed for the conversion of 2-amino-9-(2′-deoxy-β-D-ribofuranosyl)-6-purinethione to 2′-deoxyguanosine.[5] This simple procedure provided a new synthetic route to N^2-

(1) Supported by Research Grant CA-08109 from the National Cancer Institute of the National Institutes of Health, Public Health Service.

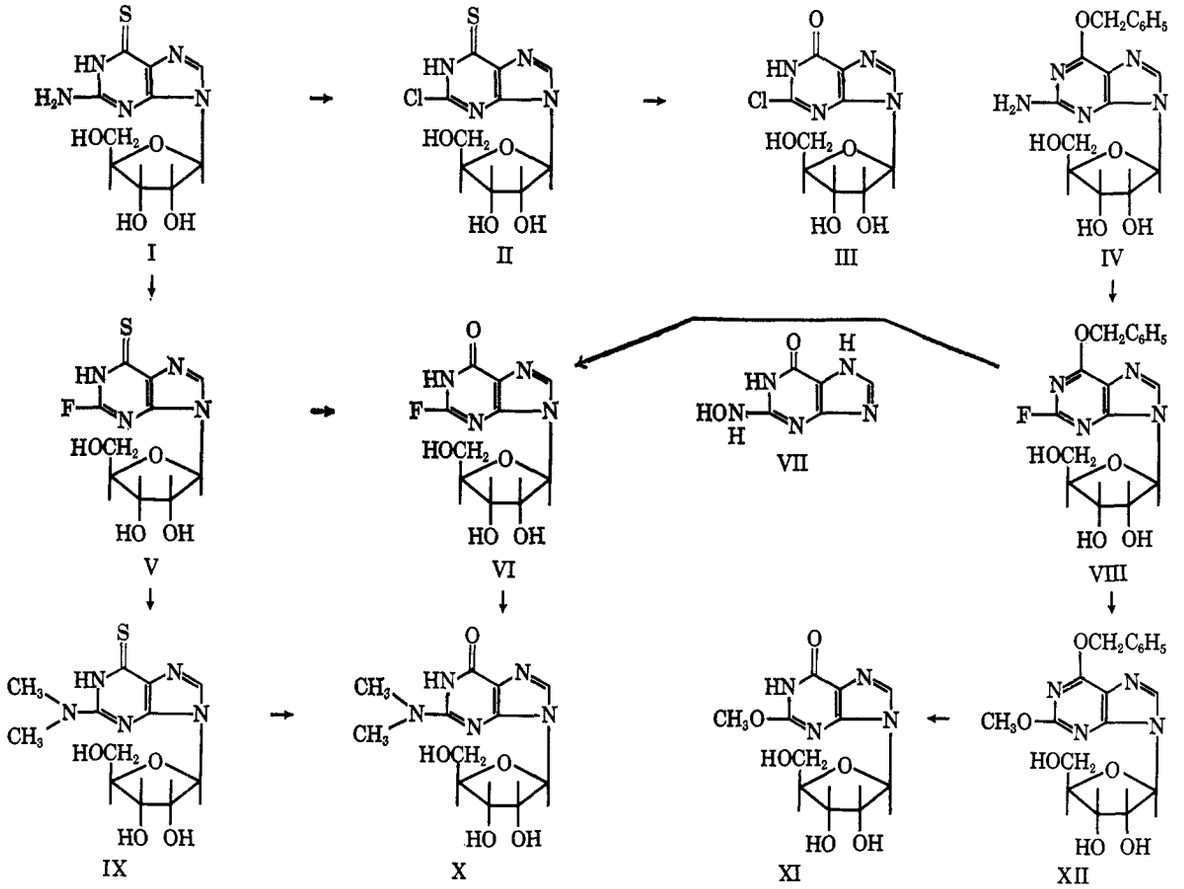
(2) J. F. Gerster and R. K. Robins, *J. Am. Chem. Soc.*, **87**, 3752 (1965).

(3) J. A. Montgomery and K. Hewson, *ibid.*, **82**, 463 (1960).

(4) J. J. Fox, I. Wempen, A. Hampton, and I. L. Doerr, *ibid.*, **80**, 1669 (1958).

(5) R. H. Iwamoto, E. M. Acton, and L. Goodman, *J. Med. Chem.*, **6**, 684 (1963).

SCHEME I



methyl- and N^2-dimethylguanosine. Treatment of 2-fluoro-9-β-D-ribofuranosyl-6-purinethione[3] (V) with methylamine and dimethylamine readily gave 2-methylamino-9-β-D-ribofuranosyl-6-purinethione and 2-dimethylamino-9-β-D-ribofuranosyl-6-purinethione (IX), respectively. Aqueous hydrogen peroxide and ammonia gave N^2-methylguanosine and N^2-dimethylguanosine (X) which were shown to be identical with the naturally occurring nucleosides isolated[6] from RNA. These methylated guanosines have recently been prepared in our laboratory by another route.[2] The ready availability of 2-fluoroinosine (VI) suggested an even more direct synthesis by replacement of the fluoro group. Treatment of 2-fluoroinosine with dimethylamine gave N^2-dimethylguanosine (X). N^2-Dimethylguanosine is of additional interest since it has recently been detected in human urine.[7]

The synthesis of 2-methoxyinosine (XI) was next investigated. Direct synthesis of XI from 2-fluoroinosine presented the difficult problem of separating 2-methoxyinosine from inorganic salts, since XI proved to be extremely water soluble. This difficulty was overcome by the preparation of 6-benzyloxy-2-methoxy-9-β-D-ribofuranosylpurine (XII) from 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine[2] and sodium methoxide. The presence of the benzyloxy group simplified the isolation problem and subsequent removal of the benzyl function was readily accomplished catalytically to leave only toluene and the desired 2-methoxyinosine (XI) (see Scheme I).

(6) J. D. Smith and D. B. Dunn, *Biochem. J.*, **72**, 294 (1959).

(7) K. Fink, W. S. Adams, F. W. Davis, and M. Nakatani, *Cancer Res.* **23**, 1824 (1963).

For the possible preparation of 2-chloroinosine from 2,6-dichloro-9-β-D-ribofuranosylpurine the synthesis of the corresponding 2′,3′,5-tri-*O*-acetate (XVI) was investigated. The synthesis of XVI has recently been reported by Montgomery and Hewson,[8] and was prepared from the fusion of 2,6-dichloropurine and tetra-*O*-acetyl-β-D-ribofuranose. Although the structure of XVI has been assumed to be β, rigorous proof was not supplied. In our own work 2,6-dichloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (XVI) was prepared by diazotization of 2-amino-6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine[9] in the presence of concentrated hydrochloric acid. This preparation of XVI which gave a product of known β configuration was shown to be identical with the product of Montgomery and Hewson,[8] by rigorous comparison of the two samples. The synthesis of 2,6-dichloro-9-β-D-ribofuranosylpurine (XV) was next explored. Previous attempts to remove the acetyl or benzoyl blocking groups of XV have been unsuccessful since replacement of halogen at position 6 occurred simultaneously.[10] Thus prior to the present work, the synthesis of 2,6-dichloro-9-β-D-ribofuranosylpurine (XV) was unreported. When 2-amino-6-chloro-9-β-D-ribofuranosylpurine[9,11] was diazotized directly in the presence of concentrated hydrochloric acid, 2,6-dichloro-9-β-D-ribofuranosylpurine (XI) was isolated in

(8) J. A. Montgomery and K. Hewson, *J. Heterocyclic Chem.*, **1**, 213 (1964).

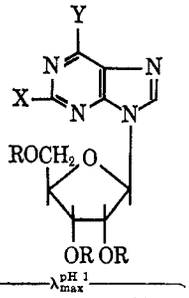
(9) J. F. Gerster, J. W. Jones, and R. K. Robins, *J. Org. Chem.*, **28**, 945 (1963).

(10) H. J. Schaeffer and H. J. Thomas, *J. Am. Chem. Soc.*, **80**, 3738 (1958).

(11) R. K. Robins, *ibid.*, **82**, 2654 (1960).

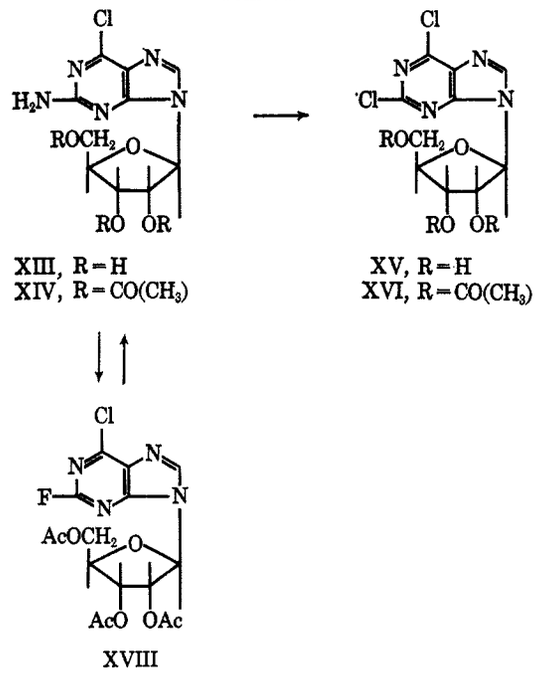
TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF VARIOUS PURINE NUCLEOSIDES



Compd	R	X	Y	$\lambda_{max}^{pH\ 1}$ mμ	(ε)	$\lambda_{max}^{pH\ 11}$ mμ	(ε)	λ_{max}^{EtOH} mμ	(ε)
XVI	Ac	Cl	Cl					273	(11,300)
								253	(5,800)
XVIII	Ac	F	Cl					269	(12,500)
II	H	Cl	SH						
III	H	Cl	OH	252	(12,000)	257	(14,000)		
VI	H	F	OH	244	(11,400)	254	(13,800)		
	H	$HNCH_3$	SH	355	(21,400)	323	(17,300)		
				269	(11,000)	278	(10,200)		
						257	(17,500)		
IX	H	$N(CH)_3$	SH	359	(20,600)	327	(15,100)		
				277	(14,800)	282 (infl)	(13,900)		
						265	(18,800)		
	H	$HNCH_3$	OH	259	(13,900)	258	(12,000)		
				282 (infl)	(7,900)	268 (infl)	(11,000)		
X	H	$N(CH_3)_2$	OH	264	(12,800)	262	(12,200)		
				293 (infl)	(5,900)	273 (infl)	(10,600)		
XII	H	CH_3O	$C_6H_5CH_2O$	267	(11,600)	265	(11,800)		
				236	(6,200)	245	(6,700)		
XI	H	CH_3O	OH	249	(8,900)	259	(10,800)	257 (infl)	(8,900)
								247	(10,400)
XV	H	Cl	Cl	274	(8,600)	274	(10,700)	275	(9,300)

SCHEME II



XIII, R = H
XIV, R = $CO(CH_3)$

XV, R = H
XVI, R = $CO(CH_3)$

XVIII

above 30% yield. Diazotization of 2-amino-6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine[9] (XIV) in fluoroboric acid gave 6-chloro-2-fluoro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (XVIII). Treatment of XVIII with alcoholic ammonia gave 2-amino-6-chloro-9-β-D-ribofuranosylpurine (XIII) as the major product. This reaction is of interest since it illustrates the increased reactivity of the fluorine group over that of chlorine which is in a more favorable position for nucleophilic substitution.[12] (See Scheme II.)

The synthesis of N^2-hydroxyguanine (VII) was accomplished by treatment of 2-fluorohypoxanthine (XIX) with hydroxylamine. 2-Fluorohypoxanthine was readily obtained from 2-fluoro-6-benzyloxypurine,[13] *via* catalytic debenzylation.

The ultraviolet absorption spectra of these new nucleoside derivatives are recorded in Table I.

Experimental Section

2-Fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine[3] (VIII).—The synthesis of this compound was improved to 30% yield by the utilization of 50% aqueous sodium hydroxide instead of concentrated aqueous ammonia for the neutralization step.

2-Dimethylamino-9-β-D-ribofuranosyl-6-purinethione (IX).—2-Fluoro-9-β-D-ribofuranosylpurine-6-thione[3] (V, 2.5 g, 0.008 mole) was dissolved in 40 ml of 50% ethanolic dimethylamine and the solution was allowed to stand at room temperature for 1 hr. At this time the solution was evaporated *in vacuo* leaving a residue. The residue was mixed with 25 ml of water and the mixture was neutralized with 25% aqueous formic acid. An oil formed and the aqueous phase was decanted and discarded. The oil was then evaporated to a foam *in vacuo* and 25 ml of ethanol was added. The foam solidified and was pulverized and triturated with ethanol and filtered. A yield of 1.9 g (70%) of crude product was obtained. This product was shown to be approximately 85% pure by comparison of ultraviolet absorption data

(12) For a more complete discussion of nucleophilic substitution in the purine ring see R. K. Robins, "The Purines and Related Ring Systems in Heterocyclic Compounds," Vol. 8, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1966, in press.

(13) M. J. Robins, Ph.D. Thesis, Arizona State University, 1964; synthesis to be published.

of an analytically pure sample. A small amount was recrystallized from water and dried at 80° (*ca.* 0.1 mm) over P_2O_5 for 6 hr to give an analytically pure sample, mp 224–226° dec.

Anal. Calcd for $C_{12}H_{17}N_5O_4S$: C, 44.1; H, 5.23; N, 21.4. Found: C, 44.1; H, 5.09; N, 20.9.

2-Methylamino-9-β-D-ribofuranosyl-6-purinethione.—To a 50% solution of ethanolic methylamine (30 ml) was added 2.5 g (0.008 mole) of 2-fluoro-9-β-D-ribofuranosyl-6-purinethione[14] (V), and the resulting solution was treated as for the preparation of IX to give 1.1 g of crude product. This product was recrystallized from water and dried at 80° (*ca.* 0.1 mm) over P_2O_5 to give 400 mg (15%) of analytically pure material.

Anal. Calcd for $C_{11}H_{15}N_5O_4$: C, 42.2; H, 4.83; N, 22.4. Found: C, 42.0; H, 5.01; N, 22.6.

2-Dimethylamino-9-β-D-ribofuranosyl-6-purinone (X). Method A.—To a solution of absolute ethanol (30 ml) and anhydrous dimethylamine (20 ml) was added 2-fluoroinosine VI, 250 mg, 0.87 mole. The resulting solution was refluxed for 30 min and then evaporated to dryness *in vacuo*. The residue was dissolved in 5 ml of hot water containing 5 drops of concentrated, aqueous ammonia. The solution was boiled and treated with charcoal, and the mixture was filtered through a Celite pad. The charcoal was then washed twice with small portions of hot water. The pH of the filtrate was adjusted to 6 with 25% formic acid and then allowed to stand at 0.5° overnight. The product crystallized from solution and was filtered. A yield of 150 mg (55%) of analytically pure colorless needles (mp 242° dec) was obtained. The sample was dried at 79° (*ca.* 0.1 mm) over phosphorus pentoxide for 3 hr for analysis, $[\alpha]^{26}D$ −35.6° (*c* 1.07, 50% dimethyl sulfoxide–ethanol).

Anal. Calcd for $C_{12}H_{17}N_5O_5$: C, 46.4; H, 5.49; N, 22.5. Found: C, 46.3; H, 5.51; N, 22.7.

Method B.—2-Dimethylamino-9-β-D-ribofuranosyl-6-purinethione (IX, 500 mg, 1.5 moles) was dissolved in 10 ml of 28% ammonium hydroxide, and 2.0 ml of 30% hydrogen peroxide was added dropwise. The temperature was maintained at 25° during the addition of the hydrogen peroxide by the use of a water bath. The solution was then stirred at room temperature for 24 hr. The solution was filtered through a Celite pad and the filtrate was evaporated to dryness *in vacuo*. The residue was slurried with ethanol and filtered from the mixture to give 380 mg (79%) of white product. The product, which was chromatographically homogeneous, was recrystallized from water and dried at 80° (*ca.* 0.1 mm) over P_2O_5 for 6 hr for analysis. The product melts with decomposition at 242°, $[\alpha]^{26}D$ −35.6° (*c* 1.07, 50% dimethyl sulfoxide–ethanol).

Anal. Calcd for $C_{12}H_{17}N_5O_5$: C, 46.4; H, 5.49; N, 22.5. Found: C, 46.46; H, 5.49; N, 22.8.

The product prepared by methods A and B was found to be identical with X previously prepared in this laboratory by another procedure.[2]

2-Methylamino-9-β-D-ribofuranosyl-6-purinone.—2-Methylamino-9-β-D-ribofuranosyl-6-purinethione (100 mg) was dissolved in 125 ml of concentrated aqueous ammonia and the solution was treated with 30% hydrogen peroxide (3 ml) as for the preparation of IX (method B). The resulting residue was recrystallized from water to give 60 mg (68%) of analytically pure, colorless needles, $[\alpha]^{26}D$ −34.6° (*c* 1.01, 50% dimethyl sulfoxide–ethanol).

Anal. Calcd for $C_{11}H_{15}N_5O_5 \cdot 1H_2O$: C, 42.0; H, 5.45; N, 22.2. Found: C, 42.3; H, 5.44; N, 22.1.

This product proved identical with that previously prepared in our laboratory by another procedure.[2]

6-Benzyloxy-2-methoxy-9-β-D-ribofuranosylpurine (XII).—2-Fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine[2] (VIII) was dissolved in 20 ml of 0.5 *N* methanolic sodium methoxide solution at room temperature and the resulting solution was allowed to stand at room temperature for 30 min. The solution was then carefully neutralized with 6 *N* hydrochloric acid and evaporated to dryness *in vacuo*. The residue was slurried in 10 ml of water and then extracted five times with 20-ml portions of warm ethyl acetate. The extracts were combined, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to give a colorless foam. A yield of 850 mg (85%) of colorless product was obtained. An analytically pure sample was obtained by recrystallization from a water–ethanol mixture.

Anal. Calcd for $C_{18}H_{20}N_4O_6$: C, 53.2; H, 5.46; N, 13.8. Found: C, 53.2; H, 5.46; N, 13.9.

2-Fluoroinosine (VI). Method A.—To a solution of 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (VIII, 2.2 g) and absolute ethanol (200 ml) was added 200 mg of 5% palladium on carbon. The mixture was hydrogenated on a Parr shaker at 46 psi of hydrogen for 6 hr and then filtered through a Celite pad. The palladium on carbon was washed four times with 10-ml portions of hot absolute ethanol. The combined washings and the filtrate were then evaporated to dryness *in vacuo* to give a white residue. The residue was then boiled with 200 ml of acetone and the solution was filtered. The volume of the filtrate was reduced to 100 ml to yield 1.5 g (78%) of analytically pure, colorless needles, $[\alpha]^{25}D$ −34.4° (*c* 1.00, H_2O).

Anal. Calcd for $C_{10}H_{11}FN_4O_5 \cdot CH_3CO(CH_3)$: C, 45.4; H, 4.98; F, 5.52; N, 16.28. Found: C, 45.3; H, 5.02; F, 5.56; N, 16.52.

The pmr spectrum of the pure compound exhibited a peak at δ 2.30 (which integrated for six protons) corresponding to the alkyl hydrogens of acetone.

Method B.—A solution of 2-fluoro-9-β-D-ribofuranosyl-6-purinethione (V, 1.5 g) in 25 ml of water containing 1 ml of concentrated ammonium hydroxide at room temperature was treated with 30% hydrogen peroxide (2.0 ml) as for the synthesis of III. The colorless foam obtained (1.1 g) was found to possess an ultraviolet absorption spectrum identical with that for VI prepared by method A.

2-Methoxyinosine (XI).—To a solution of 6-benzyloxy-2-methoxy-9-β-D-ribofuranosylpurine (400 mg, 1.03 moles) in absolute ethanol (100 ml) was added 5% palladium on carbon (100 mg). The mixture was hydrogenated on a Parr shaker at 46 psi for 5 hr and then filtered through a Celite pad. The palladium on carbon was washed four times with 5-ml portions of hot ethanol. The filtrate was evaporated to dryness *in vacuo* and the white residue was boiled with 300 ml of acetone. The acetone solution was filtered and the filtrate was evaporated *in vacuo* to a volume of approximately 20 ml. An equal amount of ether was added to the mixture and the white solid which precipitated was filtered. A yield of 150 mg (50%) of analytically pure product was obtained, $[\alpha]^{28}D$ −27.0° (*c* 1.00, H_2O).

Anal. Calcd for $C_{11}H_{14}N_4O_6$: C, 44.30; H, 4.73; N, 18.78. Found: C, 44.06; H, 4.89; N, 18.70.

The pmr spectrum exhibited a peak at δ 4.3 corresponding to the methyl hydrogens.

2-Chloro-9-β-D-ribofuranosyl-6-purinethione (II).—6-Thioguanosine (15.0 g, 0.05 mole) was dissolved in concentrated hydrochloric acid (85 ml) at −5° and the mixture was cooled to −10°. A solution of sodium nitrite (5.1 g, 0.075 mole dissolved in 15 ml of water) was added to the mixture dropwise over a a period of 30 min. The temperature of the mixture was maintained at −10° throughout the addition period. After the addition of sodium nitrite was complete the mixture was stirred at −10° for 15 min. To the mixture was added 25 g of crushed ice and the solution was neutralized wih concentrated aqueous ammonia (temperature below −10°). The mixture was allowed to stand at 0° for 1 hr, and the solid was then filtered and washed thoroughly with water and pressed dry. After drying the solid further *in vacuo* over calcium chloride a yield of 3.0 g of product (19%) was obtained. A small amount of product was recrystallized from ethanol–water and dried at 80° (*ca.* 0.1 mm) for 6 hr over P_2O_5 to give an analytically pure sample, mp 160–162° dec, $[\alpha]^{25}D$ −63.0° (*c* 1.00, 0.1 *N* NaOH).

Anal. Calcd for $C_{10}H_{11}ClN_4O_4S \cdot 0.5CH_3CH_2OH$: C, 38.8; H, 4.13; Cl, 10.40; N, 16.40. Found: C, 38.6; H, 4.15; Cl, 9.88; N, 16.73.

The presence of ethanol was verified by pmr spectrum.

2,6-Dichloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine[8] (XVI).—Finely divided 2-amino-6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine[9] (XIV, 10.0 g, 0.023 mole) was dissolved in concentrated, aqueous hydrochloride acid (80 ml) at 0°. Sodium nitrite (2.1 g, 0.03 mole in 5 ml of water) was added to the solution over a period of 20 min while the temperature was maintained at 0° for 10 min. The solution was then diluted with water (50 ml) without allowing the temperature to rise, and concentrated aqueous ammonia (60 ml) was then slowly added while the temperature was maintained at 0° or below. The reaction mixture was extracted five times with 50-ml portions of methylene chloride and the combined extracts were washed with water until the washes were neutral to pH paper. After the methylene chloride extract was dried over anhydrous magnesium sulfate the solution was evaporated to an oil *in vacuo*. The oil was dissolved in absolute ethanol and the solution was evaporated again to an oil. After the oil was dissolved in absolute ethanol a second time, a solid formed

upon standing. Filtration yielded 4.4 g (44%) of product. An analytically pure sample (mp 158°) was prepared by recrystallization of the product from ethanol, $[\alpha]^{27}D$ −3.3° (*c* 1.00, $CHCl_3$).

Anal. Calcd for $C_{16}H_{16}Cl_2N_4O_7$: C, 43.0; H, 3.58; Cl, 15.9; N, 12.55. Found: C, 42.8; H, 3.65; Cl, 16.0; N, 12.3.

2-Fluoro-6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (XVIII).—To 40 ml of 48% aqueous fluoroboric acid at 0° was added 4.3 g (0.01 mole) of 2-amino-6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine.[9] The solution was cooled to −5°. After all the solid had dissolved, sodium nitrite (1.5 g, 0.02 mole) in 5 ml of water was then added dropwise to the solution over a 30-min period. During the addition the temperature of the solution was maintained at −5°. The solution was stirred for an additional 15 min at −5°, and then 30 ml of cold aqueous ammonia was added dropwise. The temperature was again maintained at −5 to 0° during this addition. The solution was then diluted with 25 ml of water and extracted five times with 30-ml portions of methylene chloride. The methylene chloride extracts were dried over anhydrous magnesium sulfate and the solution was evaporated to a foam. A yield of 3.0 g of crude product was obtained. This product was recrystallized from isopropyl alcohol to give 1.9 g (44%) of pure material, mp 121–123°.

Anal. Calcd for $C_{16}H_{16}ClFN_4O_7$: C, 44.7; H, 3.75; N, 13.01. Found: C, 44.97; H, 4.10; N, 12.8.

Reaction of 2-Fluoro-6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (XVIII) with Methanolic Ammonia.—2-Fluoro-6-chloro-9-(2′,3′,5′-tri *O* acetyl-β-D-ribofuranosyl)purine (XVIII) was dissolved in a solution of methanol saturated with ammonia at 0°. The solution was allowed to stand at room temperature for 24 hr and was then evaporated *in vacuo* to yield an oil. The product was chromatographed on Whatman No. 1 paper using 3% ammonium chloride. Several spots were observed and the darkest of these spots was eluted with hot water and was shown to have ultraviolet absorption spectra identical with that of 2-amino-6-chloro-9-β-D-ribofuranosylpurine (XIII).[9]

2,6-Dichloro-9-β-D-ribofuranosylpurine (XV).—Finely divided 2-amino-6-chloro-9-β-D-ribofuranosylpurine (XIII, 10.0 g) was dissolved in 75 ml of 37% aqueous hydrochloric acid at −5° with stirring. A solution of sodium nitrite (5.0 g) in 8 ml of water was added dropwise to the acidic mixture over a period of 30 min. The temperature during the addition was maintained at −5 to −8°. After the addition of sodium nitrite was complete the mixture was stirred at −5° for 15 min. The cold solution was then carefully neutralized with 50% sodium hydroxide. The solution was maintained during this time at a temperature of −5°. The cold, neutral mixture was diluted with 75 ml of cold water and filtered. The cold filtrate was extracted five times with 80-ml portions of ethyl acetate. The combined extracts were dried with anhydrous magnesium sulfate and evaporated to dryness to yield 3.6 g (43%) of a colorless, solid foam. A sample was recrystallized from petroleum ether (bp 90–110°) and acetone to give colorless needles, mp 153°, $[\alpha]^{26}D$ −27.1° (*c* 1.00, absolute ethanol).

Anal. Calcd for $C_{10}H_{10}Cl_2N_4O_4$: C, 37.4; H, 3.14; Cl, 22.0; N, 17.5. Found: C, 37.3; H, 3.37; Cl, 12.9; N, 17.6.

2-Chloroinosine (III).—2-Chloro-9-β-D-ribofuranosyl-6-purinethione (II, 500 mg) was dissolved in a solution of 10.0 ml of water and 2.5 ml of concentrated, aqueous ammonia. To the solution was added 1.0 ml of 30% aqueous hydrogen peroxide. The solution was cooled in a cold water bath in order to maintain the temperature below 25°. The solution was allowed to stand at room temperature for 1 hr and then evaporated to dryness *in vacuo*. The residue was slurried with 25 ml of absolute ethanol and again evaporated to dryness. The dry residue was slurried with 75 ml of absolute methanol for 30 min and then filtered. The solid was discarded and the filtrate evaporated to dryness to yield a colorless foam. The foam was boiled with 100 ml of acetone and filtered. A yield of 330 mg (65%) of product was obtained which proved to be the ammonium salt.

Anal. Calcd for $C_{10}H_{14}ClN_5O_5$: C, 33.8; H, 5.12; Cl, 9.98; N, 19.7. Found: C, 34.1; H, 4.64; Cl, 9.68; N, 19.1.

Prolonged boiling of the product in acetone gradually decomposed the ammonium salt to yield 2-chloroinosine obtained from the acetone filtrate, which analyzed for III plus 1 mole of acetone.

Anal. Calcd for $C_{10}H_{11}ClN_4O_5 \cdot CH_3CO(CH_3)$: C, 43.0; H, 5.02; Cl, 9.85; N, 15.52. Found: C, 43.2; H, 4.72; Cl, 9.70; N, 15.63.

The presence of acetone was confirmed by pmr spectra.

2-Fluorohypoxanthine (XIX).—2-Fluoro-6-benzyloxypurine[13] (1.0 g) was dissolved in absolute ethanol (150 ml) and 5% palladium on carbon (300 mg) was added to the solution. The mixture was hydrogenated on a Parr shaker at 46 psi of hydrogen for 3 hr, the solution was then filtered through a Celite pad. The palladium on carbon was washed five times with 5-ml portions of hot ethanol. The clear, colorless filtrate was evaporated to dryness *in vacuo* to yield 590 mg (94%) of a colorless product. An analytically pure sample was obtained by recrystallization of the product from acetone.

Anal. Calcd for $C_5H_3FN_4O$: C, 39.0; H, 1.99; F, 12.3; N, 36.4. Found: C, 39.0; H, 2.12; F. 12.1; N, 36.3.

N^2-Hydroxyguanine (VII, 2-Hydroxylamino-6-purinone).—2-Fluorohypoxanthine (XIX, 200 mg) was dissolved in ethanolic hydroxylamine (50 ml) and the solution was refluxed for 2 hr. The product began to precipitate shortly after refluxing began and was filtered from the hot mixture after the 2-hr period. A yield of 120 mg (55%) of colorless product was obtained after the solid was washed and dried. For analysis the product was triturated with boiling absolute ethanol (20 ml), filtered, and dried.

Anal. Calcd for $C_5H_5N_5O_2$: C, 35.9; H, 2.99; N, 41.8. Found: C, 35.7; H, 3.13; N, 41.6.