A Novel Protecting Group for the Synthesis of 7α-D-Pentofuranosylhypoxanthines[1]

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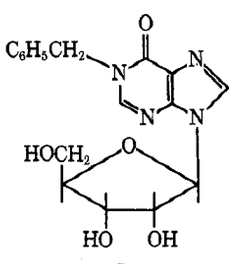
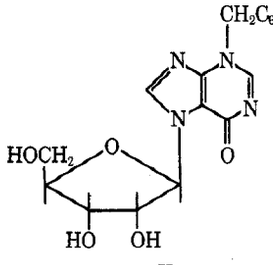
A new protecting group, the 1-oxy-2-picolyl (OP) group, has been studied and found to be quite stable both to alkaline or acid treatment but readily removable by treatment by acetic anhydride at room temperature. It was found that this protecting group was useful in cases where the benzyl protecting group does not work well. By the use of this protecting group, 3-benzyluracil (XII) and 7-benzylhypoxanthine (XIX) have been prepared which were not obtainable by direct alkylation of their respective parent heterocycles. This OP group has been successfully applied to the synthesis of 7-α-D-arabinofuranosylhypoxanthine (XXIV).

The benzyl group is a useful protecting group for hydroxyl, sulfhydryl, amino, and imino functions.[2] Removal of the benzyl blocking group is usually achieved by catalytic hydrogenation or by sodium and liquid ammonia treatment. However, difficulties have been reported[3] in removing nitrogen-linked benzyl groups from purine nucleoside derivatives. Montgomery, *et al.*,[3c] reported that catalytic debenzylation of 1-benzylinosine (I) and 3-benzyl-7-ribofuranosylhypoxanthine (II) was slow and incomplete resulting in a low yield of the debenzylation product. Anderson and coworkers[4] have also reported that 1-benzyl-3-carbomethoxypyrrole could not be debenzylated even by sodium and liquid ammonia. Obviously, the use of benzyl blocking groups is precluded for compounds containing functional groups vulnerable to hydrogenation, *e.g.*, 5-iodo-2′-deoxyuridine.



I

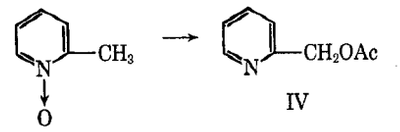


II

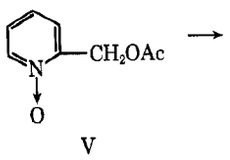
In addition to the benzyl group, a number of protecting groups, *viz.*, propenyl,[5] pivaloyloxymethyl,[6] and cyanoethyl,[7] have been used to block heterocyclic nitrogen and direct the entering group. None of these, however, are stable enough both to acid and base.

There is an urgent need for a blocking group whose removal and stability are compatible with the base lability and vulnerability to reduction of pyrimidine nucleosides and the acid lability of purine nucleosides. Such a protecting group will be especially useful for the synthesis of oligonucleotides containing both purine and pyrimidine nucleosides.

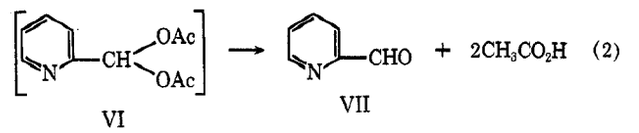
Kobayashi[8] has shown that 2-picoline 1-oxide (III) was converted in acetic anhydride to 2-pyridylmethyl acetate (VI, eq 1). Boekelheide[9] reported a similar type of rearrangement with 2-pyridylmethyl acetate 1-oxide (V, eq 2) which was converted to the aldehyde



III IV (1)



V



VI VII (2)

VII by way of the diacetate VI. Compound V may be viewed as a derivative of acetic acid whose hydroxyl function is protected by the 1-oxy-2-picolyl group, which can be removed by treatment with acetic anhydride and subsequent mild hydrolysis. These qualities suggest that the 1-oxy-2-picolyl (OP) group may be useful as an easily removable blocking group for amino or imino functions. Model studies were carried out therefore with uracil (VIII) and hypoxanthine to prepare 3-benzyluracil (XII)[10] and 7-benzylhypoxanthine (XIX).[14] The benzyl derivatives were not obtainable by the direct alkylation of their respective parent heterocycles.[11]

Alkylation of the tetraethylammonium salt of uracil (VIII) with 1 equiv of 1-oxy-2-picolyl chloride (IX)[17]

(1) Presented in part at the 2nd National Meeting of Heterocyclic Chemistry at Nagasaki, held in November of 1969.

(2) J. F. W. McOmie, "Advances in Organic Chemistry, Methods and Results," Vol. 3, R. A. Raphael, E. C. Taylor, and H. Wynberg, Ed., Interscience Publishers, New York, N. Y., 1963, p 191.

(3) (a) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **28**, 2304 (1963); (b) *J. Amer. Chem. Soc.*, **87**, 5442 (1965); (c) *J. Org. Chem.*, **31**, 1413 (1966); (d) M. Rasmussen and N. J. Leonard, *J. Amer. Chem. Soc.*, **84**, 5439 (1967).

(4) H. J. Anderson and S. J. Grifinths, *Can. J. Chem.*, **45**, 2227 (1967).

(5) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **30**, 3235 (1965).

(6) M. Rasmussen and N. J. Leonard, *J. Amer. Chem. Soc.*, **89**, 5439 (1967).

(7) E. P. Lira, *J. Heterocycl. Chem.*, **5**, 863 (1968).

(8) G. Kobayashi and S. Furukawa, *Chem. Pharm. Bull.*, **1**, 454 (1953).

(9) V. Boekelheide and W. J. Linn, *J. Amer. Chem. Soc.*, **76**, 1286 (1954).

(10) Alkylation of uracil may afford mainly 1-substituted uracil. Benzylation is no exception.[11] With excess alkylating agent, 1 substitution may be accompanied by formation of 1,3-disubstituted uracil.[12] Although relative amounts of 1- and 1,3-disubstituted uracil formed may depend on the reaction conditions, in any conditions appreciable amount of 3-substituted uracil was not formed on direct alkylation.[12] 3-Benzyluracil has been prepared by an indirect method.[13]

(11) (a) B. R. Baker and G. B. Chleda, *J. Pharm. Sci.*, **54**, 25 (1965); (b) B. R. Baker and T. J. Schwan, *J. Med. Chem.*, **9**, 73 (1966).

(12) A. R. Martinez and W. W. Lee, *J. Org. Chem.*, **30**, 317 (1965).

(13) J. B. Johnson and J. H. Derby, *Amer. Chem. J.*, **40**, 444 (1909).

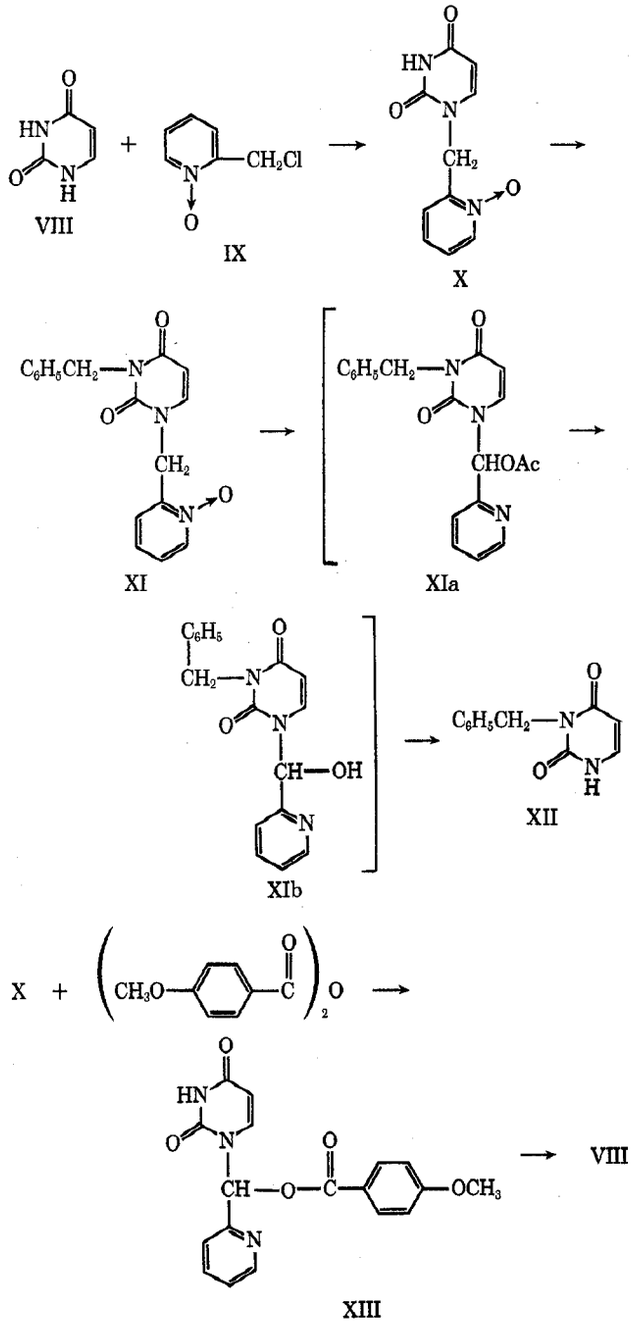
(14) (a) R. K. Robins in "Heterocyclic Compounds," Vol. 8, R. C. Elderfield, Ed., Wiley, New York, N. Y., 1967, p 372. (b) 7-Benzylhypoxanthine has been prepared by two independent (indirect) methods.[15,16]

(15) J. A. Montgomery and K. Hewson, *J. Org. Chem.*, **26**, 4469 (1961).

(16) J. A. Montgomery and C. Temple, Jr., *J. Amer. Chem. Soc.*, **83**, 630 (1961).

(17) P. T. Sullivan, M. Kester, and S. J. Norton, *J. Med. Chem.*, **11**, 1171 (1968).

in DMF afforded a 69% yield of 1-(1-oxy-2-picolyl)-uracil (X). Alkylation of the sodium salt of X with benzyl chloride gave the 1,3-disubstituted uracil XI in fair yield. The structure of X and XI was based upon elemental analysis and spectral properties. The condi-



tions required for complete rearrangement of XI in acetic or anisic anhydride are listed in Table I. As shown, XI was completely rearranged in acetic anhydride under mild conditions (36°, 44 hr) to the corresponding acetate XIa, which in turn was easily hydrolyzed to 3-benzyluracil (XXII) in mild alkaline solution. When X was treated with anisic anhydride, the rearranged product XIII was isolated and characterized. The latter, as expected, was converted into uracil by mild alkaline treatment.

Alkylation of adenine with 1-oxy-2-picolyl chloride (IX)[17] afforded a 40% yield of 3-(1-oxy-2-picolyl)adenine (XV) along with 20–25% of the 9-substituted isomer.[14] Deamination of XV with nitrosyl chloride gave

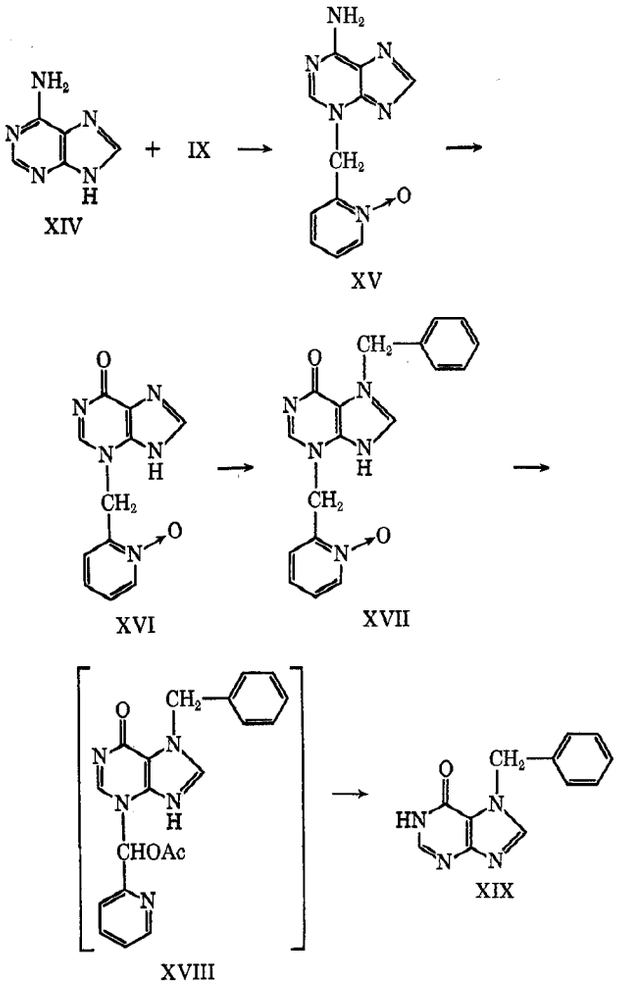
TABLE I

CONDITIONS REQUIRED FOR COMPLETE REARRANGEMENT OF 3-BENZYL-1(1-OXY-2-PICOLYL)URACIL (XI) WITH ANHYDRIDES

Anhydrides[a]	Temp, °C	Time[b]
$(p\text{-}CH_3OC_6H_4CO)_2O$	99 (fusion)	5 min
$(CH_3CO)_2O$	140 (reflux)	5 min
$(CH_3CO)_2O$	60	6.5 hr
$(CH_3CO)_2O$	36	44.0 hr

[a] Uracil derivative (XI) and anhydride employed were 2 and 8.4 mmol, respectively. [b] Time required for disappearance of the starting material on tlc (silica gel, solvent system $CHCl_3$–EtOH 35:5).

an almost quantitative yield of the hypoxanthine derivative XVI. Alkylation of the latter with benzyl chloride gave the 3,7-disubstituted derivative XVII. Treatment of XVII with acetic anhydride at room temperature for 13 hr afforded the rearranged intermediate XVIII, which was not isolated but was treated with aqueous acetic acid to give 7-benzylhypoxanthine (XIX) in 76% yield.

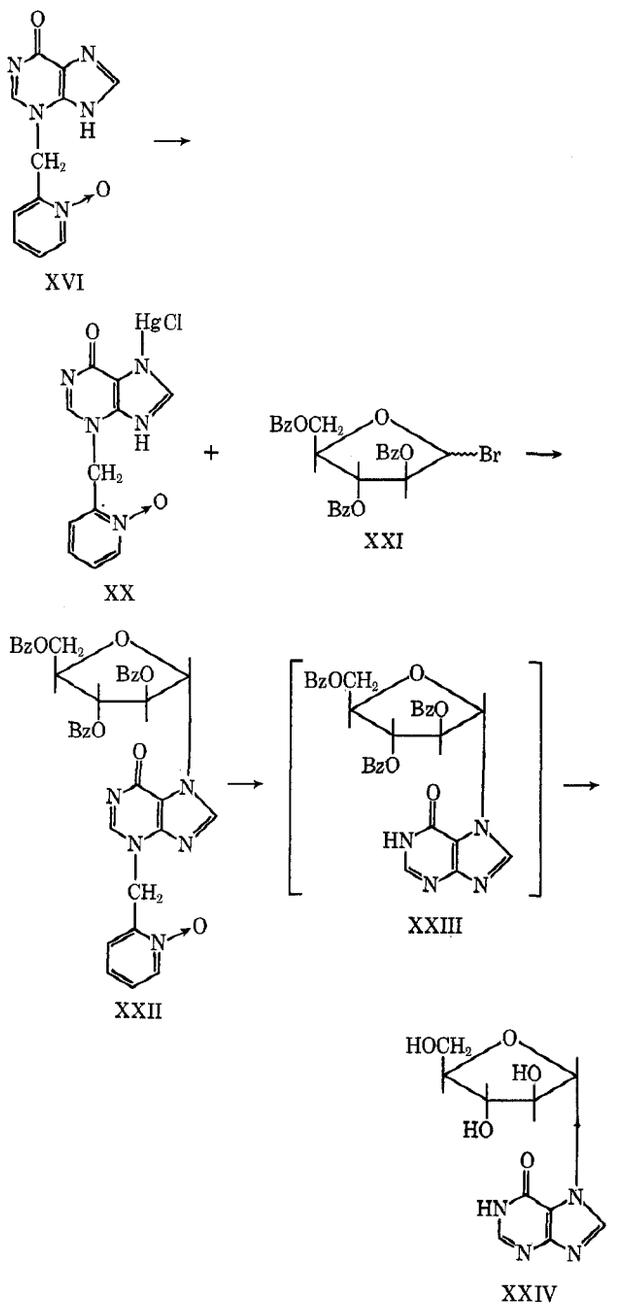


Synthesis of 7-α-D-arabinofuranosylhypoxanthine (XXIV), a nucleoside closely related to 7-α-D-ribofuranosyladenine (XXV) (a degradation product of pseudovitamin B_{12}),[18] has been recently achieved.[3c] This method, however, could not conveniently provide the large quantities of XXIV needed for our future project:

(18) J. A. Montgomery and H. J. Thomas, *J. Amer. Chem. Soc.*, **85**, 2672 (1963).

a conversion of XXIV into XXV by way of the corresponding 2',6-anhydro derivative.

In view of the successful synthesis of XIX using the OP protecting group, we tried the method in the synthesis of XXIV.



The chloromercuri derivative XX of XVI was prepared and treated with with 2,3,5-tri-*O*-benzoyl-D-arabinofuranosyl bromide (XXI) to give 3-(1-oxy-2-picolyl)-7-(2,3,5-tri-*O*-benzoyl-D-arabinofuranosyl)hypoxanthine (XXII) in 70% yield. After purification by preparative tlc, the blocked nucleoside was treated with acetic anhydride at room temperature for 40 hr to afford the tribenzoate XXIII, which was dissolved in methanolic sodium methoxide to give a 50% overall yield (based on XX) of crystalline 7-α-D-arabinofuranosylhypoxanthine (XXIV) which showed properties (melting point, mixture melting point, uv, nmr, and tlc) identical with those of an authentic sample prepared according to Thomas and Montgomery.[3c]

Experimental Section

General.—The infrared spectra were determined using Nihonbunko 137B spectrophotometer. Ultraviolet spectra were determined using a Hitachi YM 11-26 spectrophotometer, and nuclear magnetic resonance spectra were determined with a Varian Model A-60 spectrometer in deuteriochloroform. The chemical shifts are reported in parts per million downfield from tetramethylsilane as an internal standard. Paper chromatography was carried out by ascending technique. Unless otherwise stated, solvents were removed in a rotary evaporator by a water aspirator (*ca.* 12 mm).

1-(1-Oxy-2-picolyl)uracil (X).—Uracil (VIII, 2.91 g, 26 mmol) was dissolved in 300 ml of water containing tetraethylammonium hydroxide prepared from 5.5 g (26 mmol) of tetraethylammonium bromide by passing the salt through a column (Dowex-1, OH^- form). The solution was concentrated to dryness and the residue was dried completely *in vacuo*. To a solution of the tetraethylammonium salt of VIII in 100 ml of DMF was added with stirring 1-oxy-2-picolyl chloride[17] (IX, 3.71 g, 26 mmol) dissolved in 50 ml of DMF. Stirring was continued overnight at room temperature. The solution was concentrated to dryness and crystallized from 80% aqueous ethanol to give 3.91 g (69%) of X: mp 242° dec; R_f in BuOH–H_2O (84:16) 0.54; uv $\lambda_{max}^{pH\ 2}$ 260, $\lambda_{max}^{pH\ 11}$ 257, $\lambda_{min}^{pH\ 2}$ 230, $\lambda_{min}^{pH\ 11}$ 237 mμ. The uv spectral behavior was characteristic for a 1-substituted uracil.

Anal. Calcd for $C_{10}H_9N_3O_3$: C, 54.80; H, 4.11; N, 19.18. Found: C, 54.84; H, 4.16; N, 19.23.

3-Benzyl(1-oxy-2-picolyl)uracil (XI).—1-(1-Oxy-2-picolyl)-uracil (X) (1.4 g, 6.4 mmol) was dissolved in 30 ml of absolute methanol containing 0.32 mg (14 mg-atoms) of sodium metal. To the solution was added 1.77 g of benzyl chloride dissolved in 30 ml of absolute methanol. The solution was heated with stirring at 55–60° for 10 hr. The solution was filtered and the filtrate was concentrated to dryness. The residue was triturated with *n*-hexane to remove benzyl chloride and collected by filtration. Crystallization from ethanol afforded an analytical sample: mp 141°; yield 711 mg (36%); uv $\lambda_{max}^{pH\ 2}$ 260, $\lambda_{min}^{pH\ 2}$ 233, $\lambda_{max}^{pH\ 11}$ 236 mμ; R_f in BuOH–H_2O (84:16).

Anal. Calcd for $C_{17}H_{15}N_3O_3$: C, 66.00; H, 4.89; N, 13.57. Found: C, 65.63; H, 4.83; N, 13.62.

3-Benzyluracil.—3-Benzyl-1-(1-oxy-2-picolyl)uracil (XI) (200 mg) was dissolved in acetic anhydride (3 ml). The solution was heated under reflux for *ca.* 5 min and, after cooling, the solvent was removed *in vacuo*. The residue was dissolved in 5 ml of methanol containing 16 mg of sodium and the solution was kept overnight at room temperature. The solution was neutralized with a resin IRC (H^+) and filtered. The filtrate was concentrated to dryness and the residue was crystallized from water, yield 52 mg (40%), mp 175–176°. A mixture melting point with 1-benzyluracil[19] was depressed about 10°.

An experiment on the same scale was carried out at room temperature for 40 hr. The ir spectrum of the residue obtained by removal of excess acetic anhydride showed the presence of acetyl group (1770 cm^{-1}) and the absence of the absorption due to *N*-oxide: nmr δ 2.17 (s, 3, $COCH_3$), 3.44 (s, 1, NCHO).

The residue was dissolved in water and the water was removed *in vacuo*. During this process hydrolysis of XIa took place to give 3-benzyluracil (XII),[13] which was crystallized from water, 100 mg (80%), mp 175–176°.

Isolation of the Rearranged Product XIII.—A mixture of 438 mg (2 mmol) of 1-(1-oxy-2-picolyl)uracil (X) and 2.4 g of anisic anhydride was heated at 100° for 5 min. After cooling, the mixture was dissolved in chloroform. The product was isolated over a silica gel column (eluting system $CHCl_3$–EtOH 38:2). The eluate containing XIII was collected and concentrated *in vacuo* to dryness. Crystallization from ethanol afforded an analytical sample (236 mg, 33%): R_f in water (adjusted to pH 10) 0.62; ir 1730 cm^{-1} (CO of *p*-methoxybenzoate); mp 173–174°.

Anal. Calcd for $C_{18}H_{15}N_3O_4$: C, 61.19; H, 4.25; N, 11.90. Found: C, 61.16; H, 4.47; N, 11.86.

Preparation of Uracil from XIII.—Compound XIII (100 mg) was dissolved in 5 ml of 2.0 *N* methanolic sodium methoxide. The solution was kept at room temperature overnight and then neutralized with a resin (IRC, H^+ form). The solution was filtered and the filtrate was concentrated to dryness. The residue was crystallized from water to give uracil in quantitative yield. Structural confirmation rests upon uv spectral properties.

(19) H. J. Wheeler and J. B. Johnson, *Amer. Chem. J.*, **42**, 30 (1909).

3-(1-Oxy-2-picolyl)adenine Hydrochloride.—To a solution of 9.5 g of adenine (XIV) in 520 ml of dimethylacetamide (DMA) was added 9.5 g of 2-picolyl chloride 1-oxide (IX). The solution was kept at 65° for 2 days and then at 75° for 2 days. The solution was concentrated to dryness and the residue was triturated with 70 ml of water. A solid deposited which was collected by filtration and recrystallized from aqueous ethanol to afford an analytical sample: yield 40%; mp 245–246°; uv $\lambda_{max}^{pH\ 1}$ 263, 275 mμ (sh).

Anal. Calcd for $C_{11}H_{10}N_6O \cdot HCl$: C, 47.23; H, 3.96; N, 30.05; Cl, 12.76. Found: C, 47.34; H, 3.95; N, 29.91; Cl, 12.57.

3-(1-Oxy-2-picolyl)adenine (XV).—The above hydrochloride (3.7 g) was dissolved in 250 ml of water. The solution was adjusted to pH 8 with ammonia upon which a solid deposited which was collected by filtration and recrystallized from aqueous ethanol: yield 3.0 g (95%); mp 285–288°; uv $\lambda_{max}^{pH\ 2}$ 276, $\lambda_{max}^{pH\ 12}$ 262 mμ.

Anal. Calcd for $C_{11}H_{10}N_6O$: C, 54.54; H, 4.16; N, 34.70. Found: C, 54.62; H, 4.00; N, 34.73.

3-(1-Oxy-2-picolyl)hypoxanthine (XVI).—To a suspension of 1 g of XV in 20 ml of DMF was added with stirring 1 ml of nitrosyl chloride[20] at −5 to −10°. After 30 min, another 1 ml of nitrosyl chloride was added at the same temperature. The solid dissolved gradually with evolution of nitrogen. After 1 hr, complete solution resulted. The reaction mixture was then kept at room temperature. During this time a solid deposited which was collected by filtration. The filtrate was concentrated to deposit a further crop. The combined crops were washed with a small volume of cold ethanol and dried: yield 1.04 g (97%); R_f in BuOH–H_2O (84:14) 0.11; uv $\lambda_{max}^{pH\ 1}$ 256, $\lambda_{max}^{pH\ 12}$ 259 mμ.

To a suspension of 1.79 g (6.4 mmol) of the hydrochloride of XVI in 25 ml of methanol was added 6.4 ml of 1 *N* methanolic sodium methoxide at 0°. A solid deposited and was collected by filtration. Crystallization from aqueous ethanol afforded an analytical sample: yield 1.02 g (66%); mp 271–273°; uv $\lambda_{max}^{H_2O}$ 258, $\lambda_{max}^{pH\ 2}$ 256, $\lambda_{max}^{pH\ 12}$ 259 mμ.

Anal. Calcd for $C_{11}H_9N_6O_2$: C, 54.32; H, 3.73; N, 28.80. Found: C, 54.30; H, 3.371; N, 28.60.

3-(1-Oxy-2-picolyl)-7-benzylhypoxanthine (XVII).—3-(1-Oxy-2-picolyl)hypoxanthine hydrochloride (364.8 mg, 1.5 mmol) was treated with 379.5 mg (3 mmol) of benzyl chloride in 6 ml of dimethylacetamide in the presence of 414 mg (3 mmol) of potassium carbonate. The mixture was kept at 85° for 21 hr. After completion of the reaction (R_f value of product in BuOH–H_2O 86:14, 0.60), inorganic material was removed by filtration. The filtrate was concentrated to dryness *in vacuo* and the residue was triturated with ether and then crystallized from a mixture of ethanol and acetone. Prism-like crystals were obtained: yield 262 mg (52.4%); mp 215°; uv λ_{max}^{EtOH} 266, $\lambda_{max}^{pH\ 2}$ 261, $\lambda_{max}^{pH\ 11}$ 259 mμ.

Anal. Calcd for $C_{18}H_{15}N_5O_2$: C, 64.85; H, 4.54; N, 21.01. Found: C, 65.13; H, 4.46; N, 20.83.

7-Benzylhypoxanthine (XIX).—3-(1-Oxy-2-picolyl)-7-benzylhypoxanthine (100 mg) was dissolved in 7 ml of acetic anhydride. The solution was kept at 23° (room temperature) with stirring for 13 hr. The solvent was removed at 45° (bath temperature) *in vacuo* to afford crude product, which was crystallized from aqueous ethanol: 52 mg (76.7%); mp 277–279°; uv $\lambda_{max}^{pH\ 2}$ 255, $\lambda_{max}^{pH\ 11}$ 265, λ_{max}^{EtOH} 258 mμ.

Anal. Calcd for $C_{12}H_{10}N_4O$: C, 63.70; H, 4.46; N, 24.77. Found: C, 63.57; H, 4.21; N, 24.91.

Chloromercuri-3-(1-oxy-2-picolyl)hypoxanthine (XX).—To a suspension of 572 mg (2.35 mmol) of 3-(1-oxy-2-picolyl)hypoxanthine (XVI) in 60 ml of water was added 2.35 ml of 1 *N* sodium hydroxide solution. To the solution was added with stirring a solution of 638 mg (2.35 mmol) of mercuric chloride in 40 ml of water to give a precipitate which was collected by filtration, washed successively with water, ethanol, and ether, and dried, yield 904 mg (85%).

3-(1-Oxy-2-picolyl)-7-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosylhypoxanthine (XXII).—A mixture of 884 mg of chloromercuri-3-(1-oxy-2-picolyl)hypoxanthine and 884 mg of Celite in 70 ml of dry xylene was azeotropically dried by distilling 30 ml of the solvent. To the mixture was added with stirring at reflux temperature a solution of 960 mg of 2,3,5-tri-*O*-benzoyl-D-arabinosyl bromide (XXI)[21] in 13 ml of dry xylene. Stirring was continued for 70 min. After cooling the gray mixture was filtered and the insoluble material was washed with three 15-ml portions of chloroform. Xylene was removed *in vacuo* to leave a gummy residue which was dissolved in chloroform (20 ml). The chloroform solution was then washed with 80 ml of 30% potassium iodide, then with water, and dried (sodium sulfate). After filtration, the filtrated was concentrated to dryness *in vacuo* and the residue was washed with 20 ml of ether to remove sugar, crude yield 773 mg.

7-α-D-Arabinofuranosylhypoxanthine (XXIV).—Compound XXII (200 mg) was dissolved in 10 ml of acetic anhydride. The solution was kept at room temperature for 18 hr, after which the solvent was removed at 40° *in vacuo*. The residue was purified by tlc (silica gel, 30 g), yield 120 mg (72%). 7-(2,3-5-Tri-*O*-benzoyl-α-D-arabinosyl)hypoxanthine (XXII) was dissolved in 4 ml of methanol containing 0.1 ml of 1 *N* methanolic sodium methoxide solution. The solution was heated for 30 min and then treated with 10 ml of water and neutralized with a resin, Dowex 50W (H^+ form). The resin was filtered and washed with aqueous methanol. The combined washings and filtrate were concentrated to dryness *in vacuo*. The residue was dissolved in 13 ml of water. The aqueous solution was extracted with equal volume of chloroform and the aqueous layer was concentrated to dryness. The residue was crystallized from water, mp 173–175°, and a mixture melting point with an authentic sample showed no depression, $[\alpha]^{22}D$ 28° (*c* 0.34, H_2O). Literature[36] R_f values in three different systems were identical with those of an authentic sample.

	R_f	
Solvent system	XXIV	Authentic sample
n-BuOH–H_2O (86:14)	0.10	0.10
n-PrOH–H_2O (3:1)	0.20	0.21
H_2O (pH 10 with NH_4OH)	0.77	0.77

Registry No.—X, 32298-59-2; XI, 32298-60-5; XII, 28734-85-2; XIII, 32298-62-7; XV, 32298-63-8; XV HCl, 32298-64-9; XVI, 32298-65-0; XVII, 32298-66-1; XIX, 6991-06-6; XXIV, 10280-02-1.

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(20) H. Siegel and H. Brintzinger, *Helv. Chim. Acta*, **48**, 433 (1965).

(21) R. K. Ness and H. G. Fletcher, *J. Amer. Chem. Soc.*, **80**, 2008 (1958).