Some Michael-Type Reactions with Adenine

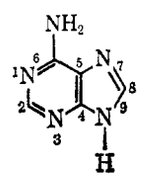
E. P. LIRA[1] AND C. W. HUFFMAN

Growth Sciences Center, International Minerals and Chemical Corporation, Libertyville, Illinois

December 21, 1965

The reaction of adenine (I) with ethyl acrylate, acrylonitrile, 2-vinylpyridine, and bis(2-chloroethyl)vinylphosphonate under alkaline and/or neutral conditions afforded only the 9-N adducts in yields of 27–98%. Several conversion products of ester IV and nitrile V were prepared. Evidence is presented which suggests, in the case of nitrile V, that the 9-N isomer is the stable adduct under these conditions. Some nmr spectral properties of IV, V, XII, XIII, and XIV are reported. The influence of some 9-N substituents on the chemical shift of the 8-hydrogen of the purine ring is discussed.

Our interest in the chemistry of a wide variety of purine and pyrimidine derivatives[2-7] led to our preparation of some adenine (I, 6-aminopurine) derivatives *via* Michael-type addition reactions. Although a



I

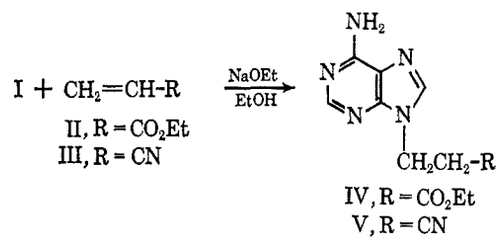
number of synthetic methods exist for the preparation of all the N-adenine derivatives,[8-19] utilization of this particular procedure has only recently been reported[20] in the purine series (6-chloropurine).

West,[21] in a closely related system, allowed ethyl acrylate (II) and acrylonitrile (III) to react under alkaline conditions with 4-chloropyrrolo[2,3-*d*]pyrimidine to afford the 7-carbethoxyethylated and cyanoethylated products, respectively, in excellent yields. In addition, the cyanoethylation of uracil and a few nucleosides has recently been reported by Chambers,[22] Yoshida and Ukita,[23] and Ofengand[24] in an attempt to modify selectively pseudouridine in transfer ribonucleic acid (t-RNA).

The base-catalyzed Michael addition reaction is usually thermodynamically controlled; *i.e.*, the product is in equilibrium with the reactants.[25] Therefore, it seems likely that the reported products in the above papers and in this report are the expected thermodynamically stable isomers. However, in most of the previously reported alkylations of adenine (I),[10,11,13-15] the distribution of N isomers probably was determined by kinetic control.

Lewin[26] has indicated, in the case of the equilibrium reaction between formaldehyde and adenine (I), that the 6-hydroxymethylamino derivative is the stable product under acidic and neutral conditions while the 9-N isomer is stable under alkaline conditions. He did not isolate any specific adduct but based his conclusions on a pH variation method and on a spectrophotometric investigation.[26]

In our work, the isolation of 6-amino-9-β-carbethoxyethylpurine (IV) and 6-amino-9-β-cyanoethylpurine (V)[20] was uniquely realized in high yields (>90%) when adenine (I) was allowed to react with ethyl acrylate (II) and acrylonitrile (III), respectively, under base-catalyzed conditions. Nitrile V has been prepared recently by the reaction of ammonia with 6-chloro-9-β-cyanoethylpurine.[20] Our physical properties for V are similar to those described in that report.

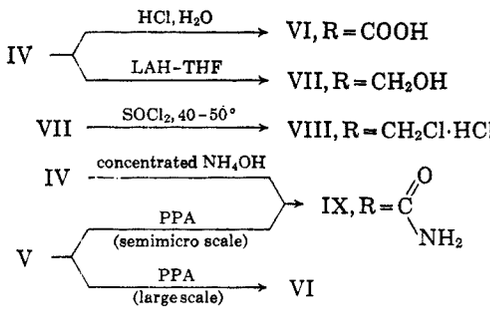


An attempt to prepare the dicarbethoxyethylated and dicyanoethylated adenines gave some important information on the stabilities of IV and V. When IV was treated with either a catalytic amount (0.1 equiv) or larger quantity (0.7 equiv) of alcoholic sodium ethoxide and 3 equiv of ethyl acrylate (II), only starting material was recovered. The reaction of V in the presence of 1 equiv of alcoholic sodium ethoxide and 3 equiv of acrylonitrile (III) gave a complete reversal to adenine (I). The retrogression of V was also noticed under a variety of alkaline conditions. Our data indicate IV to be more stable than V under these conditions.

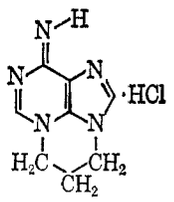
The reaction mixture from the neutral reaction of adenine and three equivalents of acrylonitrile in dimethylacetamide (DMA) was not processed, but was evaluated by paper chromatography. The R_f values in three solvent systems indicated the only product was V.

(1) To whom correspondence should be sent.
(2) H. Q. Hamzi and F. Skoog, *Proc. Natl. Acad. Sci. U. S.*, **51**, 76 (1964).
(3) S. Okumura, H. Kusaka, and T. Takematsu, *J. Biochem.* (Tokyo), **49** 133 (1961), and preceding papers.
(4) C. O. Miller, *Ann. Rev. Plant Physiol.*, **12**, 395 (1961).
(5) C. Skinner, E. O. Leonard, W. Shive, *Arch. Biochem. Biophys.*, **92**, 33 (1961), and preceding papers.
(6) D. S. Letham, J. S. Shannon and I. R. McDonald, *Proc. Chem. Soc.*, 230 (1964).
(7) Z. Sormova, O. Melichar and F. Sorm, *Collection Czech. Chem. Commun.*, **25**, 2889 (1960).
(8) D. N. Ward, J. Wade, E. F. Walborg, and T. S. Osdene, *J. Org. Chem.*, **26**, 5000 (1961).
(9) H. Lettre and H. Ballweg, *Ann.*, **633**, 171 (1960).
(10) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951).
(11) N. J. Leonard and J. A. Deyrup, *ibid.*, **84**, 2148 (1962).
(12) J. A. Montgomery and C. Temple, *ibid.*, **83**, 630 (1961).
(13) J. W. Jones and R. K. Robins, *ibid.*, **84**, 1914 (1962).
(14) B. C. Pal, *Biochemistry*, **1**, 558 (1962).
(15) C. J. Abshire and L. Berlinguet, *Can. J. Chem.*, **42**, 1599 (1964).
(16) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **28**, 2304 (1963).
(17) N. J. Leonard and T. Fujii, *J. Am. Chem. Soc.*, **85**, 3719 (1963).
(18) N. J. Leonard and T. Fujii, *Proc. Natl. Acad. Sci. U. S.*, **51**, 73 (1964).
(19) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **85**, 193 (1963).
(20) B. R. Baker and P. M. Tanna, *J. Org. Chem.*, **30**, 2857 (1965).
(21) R. A. West, *ibid.*, **28**, 1991 (1963).
(22) R. W. Chambers, *Biochemistry*, **4**, 219 (1965).
(23) M. Yoshida and T. Ukita, *J. Biochem.* (Tokyo), **57**, 818 (1965).
(24) J. Ofengand, *Biochem. Biophys. Res. Commun.*, **18**, 192 (1965).
(25) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt and Co., New York, N. Y., 1959, p 394.
(26) S. Lewin, *J. Chem. Soc.*, 400 (1964).

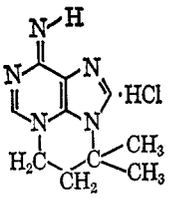
SCHEME I



A number of conversion products of IV and V have been prepared (Scheme I) and characterized. Ester IV was easily hydrolyzed to acid VI in 81% yield. Acid VI was prepared recently from 6-chloro-9-β-carboxyethylpurine and ammonia.[20] Ester IV was reduced with lithium aluminum hydride in tetrahydrofuran (THF) to the corresponding alcohol VII[27] in 53% crude yield. The melting point (215–217.5°) of VII was *ca.* 20° higher than that of the reported melting point (194–198°).[27] However, the ultraviolet spectral properties of VII were similar to those reported.[21] The alcohol VII was treated with thionyl chloride with the hope of preparing an internally cyclized chloride X similar to Leonard and Deyrup's pyrotricanthine chloride (XI).[11] Instead, we obtained mainly 6-amino-9-γ-chloropropylpurine hydrochloride (VIII).[27]



X



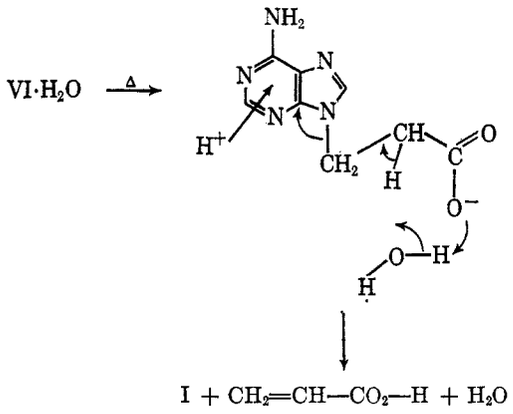
XI

Ester IV easily underwent ammonolysis with concentrated ammonium hydroxide to yield the corresponding amide IX in 70% yield. Some acid VI (20%) was also isolated. Amide IX had been prepared initially on a semimicro scale by the polyphosphoric acid (PPA) treatment of nitrile V. These conversions offered additional evidence that IV and V were the same N isomer. When the PPA reaction was conducted on a much larger scale (31×) only VI was isolated.

All of the prepared compounds exhibited a range of ultraviolet absorption maxima[28] at pH 1.0 and 10.0 of between 258.0 and 262.0 mμ. In addition, the range of differences of the absorption minima from pH 1.0 to 7.0 was between 0.5 and 4.5 mμ. The nuclear magnetic resonance spectra[29] of compounds IV, V, and XII–XIV indicated that the range of chemical shift values for the methylene adjacent to the functional group was 2.78–3.40 ppm; for the methylene attached to the purine ring was 4.43–4.68 ppm; for the 6-amino group was 7.25–7.95 ppm; and for the 2- and 8-hydrogens of the purine ring was 7.99–8.35 ppm. The range of Δδ between the 2- and 8-hydrogens for IV and V was 0.5–3.0 cps. The spectral correlations of Baker, *et al.*,[30] Leonard and Deyrup,[11] and Townsend, *et al.*,[31] and the dissociation constant of ester IV (3.70)[11] indicated that these compounds were the 9-N isomers.

An unambiguous proof of structure was attempted on VI using a variety of decarboxylation procedures. When VI monohydrate was heated to *ca.* 250° at atmospheric pressure and then to 300° under reduced pressure (0.3 mm) the only solid sublimate isolated proved to be adenine. A similar experiment was performed under continuous vacuum (0.3 mm) and in this case only VI could be identified in the sublimate. A possible explanation of this difference may be associated with the water of hydration. The low pressure technique rapidly removes the water, whereas under atmospheric pressure a water-catalyzed[32] reverse Michael reaction occurs (see Scheme II).

SCHEME II



We have circumstantial evidence, *i.e.*, high yield of a single product and the ease of reversal of V, that our reaction conditions are reversible. Indeed, the specificity of these reactions is in interesting contrast to the normal base-catalyzed alkylation reaction which usually gives a mixture of 3-N, 7-N and 9-N isomers.[11,20,33] In addition, the 9-N isomer was the major product of an essentially neutral reaction. Under these conditions, I is normally alkylated at 3-N[13–15,17] with small amounts of 1-N and 7-N products.[14,17] Therefore, our results confirm Lewin's conclusion[26] for alkaline reaction conditions and are in disagreement with his results for a neutral system.

Adenine has been allowed to react with 2-vinylpyridine under essentially neutral conditions (DMA) to yield 49% of 6-amino-9-β-pyrid-2-ylethylpurine (XII) (Scheme III). The nmr spectrum of XII in deuterated dimethyl sulfoxide (DMSO-*d*) indicated the Δδ[31] between the 2-hydrogen and the 8-hydrogen was 14 cps. In addition, 6-amino-9-β-phenethyl-

(27) J. H. Lister and G. M. Timmis, *J. Chem. Soc.*, 327 (1960).

(28) Spectra were determined in aqueous solutions using a Beckman DK-2A ratio recording spectrophotometer.

(29) Spectra were determined in deuterated dimethyl sulfoxide with tetramethylsilane as an internal standard at 60 Mc/sec on a Varian Associates A-60 spectrometer at Simon Research Laboratory, Elgin, Ill., by Dr. W. Simon.

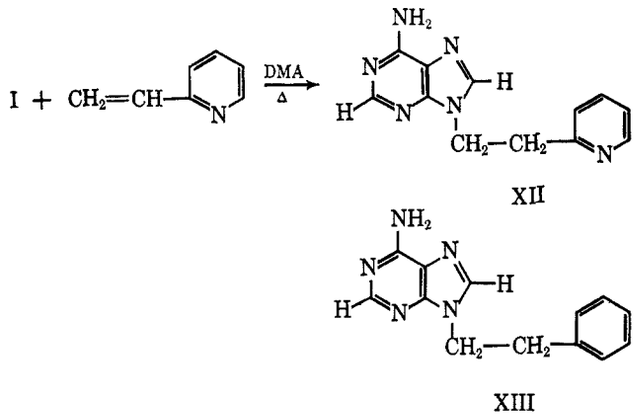
(30) B. R. Baker, R. E. Schaub, and J. P. Joseph, *J. Org. Chem.*, **19**, 638 (1954).

(31) L. B. Townsend, R. K. Robins, R. N. Leoppky, and N. J. Leonard, *J. Am. Chem. Soc.*, **86**, 5320 (1964).

(32) We thank Dr. B. R. Baker for the suggestion that the hydrate might be a zwitterion that undergoes β elimination while the anhydrous acid may not be, thus containing formal COOH and adenine groups which may not undergo β elimination.

(33) Unpublished results of authors and see experimental for preparation of 6-amino-9-β-phenylethylpurine (XIII).

SCHEME III

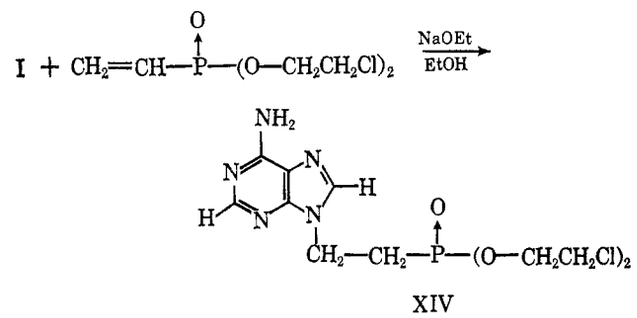


XII

XIII

purine (XIII)[34] also showed approximately the same $\Delta\delta$, *i.e.*, 16 cps. Although these values suggest these substances are 3-N isomers[31] the restrictions noted by Townsend, *et al.*,[31] have to be considered. It is evident that the pyridine and benzene ring affect the chemical shift of the 8-hydrogen. It is not known at this time whether the 8-hydrogen is shielded or deshielded.

The use of vinylphosphonium compounds in Michael-type reactions has been reported recently by Keough and Grayson.[35] The base-catalyzed reaction of I with bis(β-chloroethyl) vinylphosphonate gave a 27% yield of adduct XIV. The reaction was much slower



XIV

than any of the previous reactions. Interestingly, in this case the nmr spectrum in deuterated DMSO indicated that the $\Delta\delta$ between the 2-hydrogen and the 8-hydrogen was 0; *i.e.*, both were in the same magnetic environment. This can be rationalized on the bases of an anisotropic effect[36] of the P→O bond on the 8-hydrogen, or else by some small contribution due to hydrogen bonding between the 8-hydrogen and the oxygen.[37]

We are currently investigating the potential synthetic utility of the retrogression of nitrile V.

Experimental Section[38]

6-Amino-9-β-carbethoxyethylpurine (IV).—To a 3-l. three-necked round bottom flask equipped with a stirrer, thermometer, addition funnel, distillation head, condenser, receiver, and drying tube was added adenine (81.2 g, 0.6 mole), absolute ethanol (2100 ml), and benzene (250 ml); *ca.* 700 ml of solvent was distilled azeotropically. Small pieces of sodium (600 mg) were added, and, after the evolution of hydrogen had ceased, ethyl acrylate (192 ml, 1.8 mole) was added slowly to the stirred reddish solution. The solution was heated at reflux overnight. Solvent (1 l.) was distilled and the remaining solution was cooled. The resulting solids were slurried with additional cold absolute ethanol (200 ml) and filtered to afford light brown solids. The solids were reslurried in ethanol (300 ml), filtered, and dried to afford pure ester IV (139 g, 98%), mp 167–168°. Ester IV had a pK_a of 3.70.[39] The product had the following infrared absorptions (KBr): 5.8, 6.0, 6.1, 6.2, 8.3 μ.

Anal. Calcd for $C_{10}H_{13}N_5O_2$: C, 51.06; H, 5.57; N, 29.8. Found: C, 51.26; H, 5.50; N, 30.4.

6-Amino-9-β-cyanoethylpurine (V).[20]—The general procedure followed in the preparation of ester IV was used. Adenine (1.36 g, 0.01 mole) was treated with acrylonitrile (1.6 g, 0.03 mole, 2.0 ml) in the presence of 0.00087 mole of sodium ethoxide. The mixture was heated at the reflux temperature for 24 hr and then cooled to 0°. The resulting slightly yellowish material was isolated by filtration, washed with 5 ml of cold ethanol (three times), and dried to yield V (1.7 g, 90%), mp 250–255°. Paper chromatography using solvent systems A, B, and C[40] indicated that V was the only product. It was contaminated with trace amounts of adenine. Repeated recrystallization from water raised the melting point from 250–255° to 258–261° (lit.[20] mp 247–250°). The product had the following infrared absorptions (KBr): 3.0, 3.3, 4.5 (minor), 6.1, 6.3 μ.

Anal. Calcd for $C_8H_8N_6$: C, 51.06; H, 4.28; N, 44.7. Found: C, 51.17; H, 4.23; N, 45.0.

Reaction of Adenine and Acrylonitrile in DMA.—A solution of I (1.35 g, 0.01 mole) in 100 ml of dry DMA (dimethylacetamide) was prepared and then acrylonitrile (2.0 ml, 0.03 mole) was added slowly. The solution was heated to boiling for 1 hr, cooled to 130°, and heated for 3.5 hr. An additional 2 ml of acrylonitrile was added and heating was continued for 4 days. Paper chromatography in solvent systems A, B, and D indicated that nitrile V was the only product.

6-Amino-9-β-carboxyethylpurine (VI).[20]—To a one-necked 200-ml round bottom flask equipped with a reflux condenser was added ester IV (2.35 g, 0.01 mole) and 3 *N* hydrochloric acid (75 ml). The solution was heated at reflux for 3 hr and neutralized to pH 10 with sodium hydroxide pellets (8.5 g, 0.21 mole). Addition of concentrated hydrochloric acid resulted in a precipitate forming at pH 6–7. The final pH of the mixture was 3. The acidified mixture was cooled covernight and the resulting solids (1.81 g, 81% crude yield) were collected and dried: mp 280–288°. A portion of this crude material was dissolved in sodium carbonate solution, decolorized with charcoal, and filtered, the filtrate was acidified to pH 3, and the mixture was cooled. The solids were removed by filtration and dried for the analytical sample: mp 284–288° (lit.[20] mp 279–280°). Acid VI had the following infrared absorptions (KBr): 3.2, 5.9, 6.1, 6.7, 8.0 and 10.5 μ.

Anal. Calcd for $C_8H_9N_5O_2$: C, 46.37; H, 4.38; N, 33.8. *Anal.* Calcd for $C_8H_9N_5O_2 \cdot H_2O$: N, 31.1. Found for crude sample: N, 30.7. Found for analytical sample: C, 46.11; H, 4.54; N, 33.6.

6-Amino-9-γ-hydroxypropylpurine (VII).[27]—To a flame-dried 3-l. three-necked round bottom flask equipped with reflux condenser and drying tube was added THF (1400 ml) followed by powdered $LiAlH_4$ (10 g). The first few grams of $LiAlH_4$ gave a considerable evolution of gas (H_2). The resulting mixture was stirred for 2 hr under 1 atm of nitrogen. Dry, powdered ester IV (50 g, 0.21 mole) was added slowly over a 60-min period. The greenish mixture was heated at reflux for *ca.* 11 hr and at 40° for 32 hr. Water (20 ml) was added cautiously to the reaction mixture, and after stirring for 90 min the solids were separated by filtration. The filtrate was reduced in volume and

(34) C. L. Leese and G. M. Timmis, *J. Chem. Soc.*, 4107 (1958).

(35) P. T. Keough and M. Grayson, *J. Org. Chem.* **29**, 631 (1964).

(36) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," D. H. R. Barton and W. Doering, Ed., Pergamon Press Inc., New York, N. Y., 1959, pp 14–21.

(37) Reference 36, pp 66–71.

(38) The melting points were determined with a Fisher-Johns melting point apparatus or a Thomas–Hoover capillary melting point apparatus and are corrected. The microanalyses were performed by Micro-Tech Laboratories, Inc., Skokie, Ill., or by our analytical department. The infrared spectra were determined on either a Perkin-Elmer Infracord or a Perkin-Elmer Model 521 spectrophotometer. Chromatograms were developed by the ascending technique with Whatman No. 1 paper. The solvent systems employed were A, *n*-butyl alcohol–acetic acid–water (4:1:1); B, isopropyl alcohol–acetic acid–water (69:1:30); C, dimethylacetamide–concentrated ammonium hydroxide–isopropyl alcohol (25:10:65); and D, 5% aqueous ammonium sulfate–isopropyl alcohol (95:5).

(39) Obtained spectroscopically by our analytical department.

(40) Eastman Chromatogram Sheet, Type K301R2 (silica gel).

cooled to afford impure reddish brown alcohol VII (11 g): mp 208–213°. Additional crude aldohol (10.7 g) was isolated from the filtrate and the solids. The total yield of crude VII was 21.7 g (53.5%). Repeated recrystallization from absolute ethanol (after two charcoal treatments) afforded yellowish VII (9.3 g, 23%): mp 211–214°. Analytically pure VII, mp 215–217.5° (lit.[37] mp 194–198°), had the following infrared absorptions (KBr): 3.1 (OH), 6.0, 6.2, 6.3, 9.2, 9.4, 9.7, and 14.2 μ.

Anal. Calcd for $C_8H_{11}N_5O$: C, 49.7; H, 5.73; N, 36.2. Found: C, 49.9; H, 5.87; N, 35.9.

6-Amino-9-γ-chloropropylpurine Hydrochloride (VIII).[37]—To a 500-ml three-necked round bottom flask equipped with gas inlet tube, addition funnel, and condenser with drying tube was added thionyl chloride (69 ml) under an atmosphere of nitrogen. Alcohol VII (4.45 g, 0.023 mole) was added slowly to the stirred acid chloride and allowed to react at 35–40° for 1.5 hr. An additional 32 ml of thionyl chloride was added and the mixture was stirred overnight at 50°. The red-orange mixture was cooled to room temperature, diluted with benzene, stirred for 2 hr, and then absolute ethanol (13.5 ml) was added slowly. The resulting mixture was stirred for 3 hr and then cooled in freezer overnight. The solids were removed by filtration under an atmosphere of nitrogen, washed with dry benzene, and dried to yield 5.0 g of crude hydrochloride VIII: mp >320°. Paper chromatography in solvent systems A and C indicated one major product (>95%) and one trace component (<5%). Crude VIII was recrystallized from absolute ethanol (five times) to yield the analytical sample which had the following infrared absorptions (KBr): 3.0, 3.3, 5.9, 6.2, 6.6, 7.1, and 8.2 μ.

Anal. Calcd for $C_8H_{10}ClN_5 \cdot HCl$: C, 38.72; H, 4.47; Cl, 28.58; N, 28.19. Found: C, 38.93; H, 4.53; Cl, 28.39; N, 28.39.

6-Amino-9-β-carbamidoethylpurine (IX).—A mixture of nitrile V (1.6 g, 0.0085 mole) and PPA (polyphosphoric acid, 20 g) was prepared in an open 125-ml erlenmeyer flask and heated at 125° for 3 hr with occasional stirring. Enough water was added to make the mixture fluid and the solution was added rapidly to ice. The resulting mixture was stirred until a solution occurred and was neutralized to pH 7 with 50% sodium hydroxide and cooled at 32° overnight. The entire mass solidified, and, therefore, was warmed to room temperature followed by the addition of a small amount of water. The solids were separated by filtration to afford impure IX (0.75 g, 43%), mp 255–260°, some sublimation from 250°. A mixture melting point with the starting material was depressed. The solids were recrystallized twice from hot dimethylacetamide to yield analytically pure IX, mp 275–281°, sublimation from 240°; a mixture melting point with the corresponding acid, mp 282–287°, was depressed to 263–270°. The infrared spectrum had the following absorptions: 3.0, 3.2, 5.9, 6.0, 6.2, 6.3, 7.0, 7.5, 8.0 and 8.3 μ.

Anal. Calcd for $C_8H_{10}N_6O$: C, 46.59; H, 4.85; N, 40.7. Found: C, 46.78; H, 4.86; N, 40.4.

6-Amino-9-β-pyrid-2-ylethylpurine (XII).—A solution of adenine (16 g, 0.118 mole) and dry dimethylacetamide (700 ml) was prepared under an atmosphere of nitrogen in a 1-l. three-necked flask equipped with reflux condenser with drying tube, syringe septum, and gas inlet tube. 2-Vinylpyridine (43 ml) was added and the mixture was heated at 145–155° for 18 hr, cooled to room temperature, and allowed to stand for 3 days. Paper chromatography in solvent system A indicated the presence of unreacted adenine and one major product.

Vacuum distillation of the solvent afforded a tannish red solid which was extracted with boiling water. The extract was decolorized with charcoal, reduced in volume (1/3), and cooled. The highly hydrated solids were removed by filtration, dried for 3 hr on the filter paper, and then dried in a vacuum oven at 30° for 2 hr, and finally at 70° overnight to yield XII (13.8 g, 49%): mp 146–149°. Recrystallization from absolute ethanol gave analytically pure XII: mp 146–147°. Paper chromatography of the filtrate (water crystallization) in solvent system A indicated adenine was the major constituent. A trace of XII was also present. The product had the following infrared absorptions (KBr): 5.9, 6.2, 6.7 μ (aromatic C–H).

Anal. Calcd for $C_{12}H_{12}N_6$: C, 60.3; H, 5.03; N, 34.9. Found: C, 60.1; H, 5.10; N, 35.1.

6-Amino-9-β-phenethylpurine (XIII).[34]—To a 2-l. three-necked round bottom flask, equipped with condenser and drying tube, stirrer, and addition funnel was added 1000 ml of absolute ethanol and small pieces of sodium (3.5 g, 0.15 mole). Dry adenine (20.2 g, 0.15 mole) was added and the mixture was heated to the reflux temperature. β-Phenylethyl bromide (27.8 g, 0.15 mole) was added dropwise over 14 min. The resulting yellow solution was heated at the reflux temperature for 4 hr, then cooled, and stirred overnight. The solution was reduced to *ca.* 25% of the original volume and neutralized with concentrated hydrochloric acid. The resulting mixture was cooled over the weekend and filtered to yield 22.3 g of solids (A). Fractional crystallization from water gave pure XIII (3.42 g, 9.5%): mp 172–173.5° (lit.[34] mp 179–180°). A second fraction, 5.70 g, was obtained. Its ultraviolet spectrum indicated it was a mixture. A third fraction, 1.0 g, mp 277–282°, gave an ultraviolet spectrum which indicated it to be the 3-N isomer. The spectral properties of this fraction were λ_{max}, pH 1, 274.0 mμ (ε 12,520), pH 7, 271.0 mμ (ε 9730), and pH 10, 271 mμ (ε 9520). The pH 1 λ_{min} (237.5 mμ) − pH 7 λ_{min} (244.5 mμ) was −7.0 mμ.

Bis(β-chloroethyl)-β-adenin-9-ylethylphosphonate (XIV).—The general procedure followed in the preparation of ester IV was used. Adenine (20.25 g, 0.15 mole) was treated with bis-(β-chloroethyl)vinylphosphonate (100 g, 0.43 mole) in the presence of 0.02 mole of sodium ethoxide. The mixture was heated at reflux temperature 6 days; *ca.* 400 ml of solvent was removed by distillation; and the remaining mixture was cooled overnight. The solids were removed by filtration and oven-dried to afford crude XIV (33.8 g, 61%): mp 154.5–166°. The solids were extracted with absolute ethanol and the extract was reduced in volume (to 200 ml) and allowed to cool overnight. The resulting solids, removed by filtration, were pure XIV (15.1 g, 27%): mp 169.5–171.5°. Analytically pure XIV, mp 180.5–181.5°, was prepared by recrystallization (five times) from absolute ethanol. The product had the following infrared absorptions (KBr): 5.9, 6.2, 6.3, 8.0 (P=O), 9.1–9.2, 9.5, 9.7, 10.2 μ (P–O–C). Paper chromatography in solvent system A indicated only one product. A silver nitrate test was negative.

Anal. Calcd for $C_{11}H_{16}Cl_2N_6O_3P$: C, 35.88; H, 4.38; Cl, 19.25; N, 19.02, P, 8.42. Found: C, 35.76; H, 4.48; Cl, 19.33; N, 19.34; P, 8.44.

Acknowledgment.—The authors are grateful to Messrs. G. James, B. Page, and R. Singer who performed much of the experimental work and to Mr. L. W. Ferrara and his staff for most of the analytical results. We wish to thank Dr. W. Simon of Simon Research Laboratory, Elgin, Illinois, for his help in interpreting the nmr spectra and Drs. F. Greer, M. Hamer, and R. P. Mariella for their stimulating discussions.