Cyclopentyl Derivatives of 8-Azahypoxanthine and 8-Azaadenine. Carbocyclic Analogs of 8-Azainosine and 8-Azaadenosine (1)

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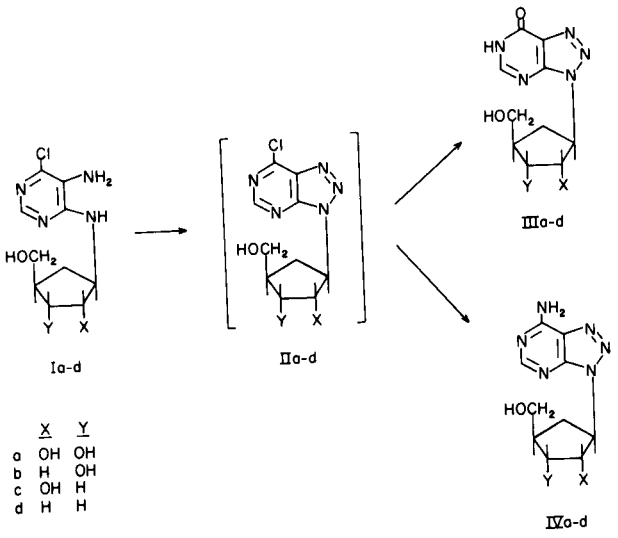
Received March 23, 1973

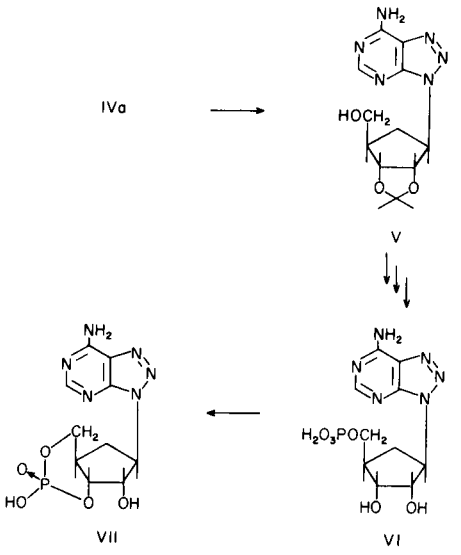
Four types of carbocyclic analogs of 8-azahypoxanthine and 8-azaadenine nucleosides have been prepared. This group of analogs is comprised of derivatives having the (±)-*as*-3-(hydroxymethyl)cyclopentyl, (±)-*trans*-3-hydroxy-*cis*-4-(hydroxymethyl)cyclopentyl), (±)-*trans*-2-hydroxy-*cis*-4-(hydroxymethyl)cyclopentyl, and (±)-*trans*-2, *trans*-3-dihydroxy-*cis*-4-(hydroxymethyl)-cyclopentyl groups at position 3 of 3,6-dihydro-7*H*-*v*-triazolo[4,5-*d*]pyrimidin-7-one and of 7-amino-3*H*-*v*-triazolo[4,5-*d*]pyrimidine. Diazotization of (5-amino-6-chloropyrimidin-4-yl-amino)cyclopentane derivatives and acidic hydrolysis, without isolation of the resulting 7-chloro-3*H*-*v*-triazolo[4,5-*d*]pyrimidines yielded the 8-azahypoxanthine derivatives (III). Treatment of unpurified 7-chloro-3*H*-*v*-triazolo[4,5-*d*]pyrimidines with anhydrous ammonia gave the 8-azaadenine derivatives (IV). The cyclopentane analogs of 8-azaadenylic acid and of 8-azaadenosine 3′,5′-cyclic monophosphate were prepared from the 8-azaadenosine analog.

Analogs of purine nucleosides in which the furanose ring has been replaced by a cyclopentane ring have a stable C-N bond in place of the glycosidic bond. For this reason, they are not subject to cleavage by enzymes of the purine salvage pathways (2,3) that metabolize preformed purine nucleosides. Because of conformational similarities between the tetrahydrofuran and the cyclopentane rings, these carbocyclic analogs might function, after activation by kinases, as active-site or allosteric inhibitors of enzymes that effect interconversions of purine nucleotides or that catalyze steps in the synthesis *de novo* of purine ribonucleotides (2-5).

Earlier reports have dealt with syntheses of the carbocyclic analogs of adenosine (6,7), 2′- and 3′-deoxyadenosine (8), several 6-substituted-purine ribonucleosides (9), adenylic acid and cyclic adenosine monophosphate (9), and guanosine and 8-azaguanosine (10). Several biochemical and biological investigations of these compounds show that replacement of the furanose ring of nucleosides with a cyclopentane ring can produce active compounds. The studies of Bennett and co-workers (11,12) have shown that the carbocyclic adenosine analog can serve as a substrate for adenosine kinase and for adenosine deaminase and as an inhibitor, presumably after phosphorylation, of an early step in the biosynthesis *de novo* of inosinic acid. The carbocyclic analog of adenylic acid is a potent inhibitor of guanylic acid (GMP) kinase (13), and an analog of vitamin B_{12} coenzyme prepared from the adenosine analog can function as a coenzyme for dioldehydrase (14).

Furthermore, several carbocyclic analogs of purine nucleosides are cytotoxic (9,12) and have antimicrobial activity (15), the carbocyclic analog of adenosine is a moderate inhibitor of *Tetrahymena pyriformis* (16), and aristeromycin, an antibiotic for certain plant pathogens (17) isolated after the synthesis of the racemic carbocyclic analog of adenosine, is an optically active form (1R, 2S, 3R, 5R) of this analog (18,19). Carbocyclic analogs of nucleosides of 8-azaadenine and 8-azahypoxanthine and





of 8-azaadenosine ribonucleotides are described in this report.

Stereospecific syntheses of the pyrimidines (Ia-c) required for the preparation of the ribofuranoside and the 2′- and 3′-deoxyribofuranoside analogs have been reported previously (6-8). *Cis*-3-(5-amino-6-chloropyrimidin-4-ylamino)cyclopentanemethanol (Id) (20) was synthesized by stereospecific routes (21) similar to those developed for Ia-c.

The 8-azainosine analog (IIIa) was obtained by diazotizing Ia in aqueous acetic acid, hydrolyzing the intermediate 6-chloro-8-azapurine (IIa) in refluxing dilute hydrochloric acid, and isolating IIIa by chromatography on a cation-exchange resin. The remaining three hypoxanthine derivatives (IIIb-d) were obtained similarly after diazotizing the pyrimidines (Ib-c) in dilute hydrochloric or aqueous acetic acid.

The 6-chloro-8-azapurine derivatives were not isolated, but examination of reaction solutions containing these derivatives by thin-layer chromatography indicated that they are easily hydrolyzed, as expected, in aqueous media. The adenine derivatives were prepared, therefore, by reducing the time allowed for the diazotization reactions, lyophilizing the solutions after neutralization, and treating the entire reaction residues with anhydrous ammonia. Yields of pure derivatives (IVa-d) were 38-76%.

The 8-azaadenosine analog (IVa) was converted to the isopropylidene derivative (V), and the latter compound was phosphorylated with phosphorus oxychloride in trimethyl phosphate (22,23). The intermediate phosphoric dichloride was hydrolyzed in dilute aqueous ammonia. The isopropylidene group was removed and the phosphate (VI) was isolated by passing the aqueous ammonia solution through a cation-exchange resin. The yields of VI in the form of the ammonium salt monohydrate were 73-93%. This form was used for biological tests and for cyclization to VII, an analog of cyclic 3′,5′-adenosine monophosphate. The cyclization was effected with dicyclohexylcarbodiimide by the method of Smith, Drummond, and Khorana (24), and VII was isolated with the aid of a basic ion-exchange resin.

The 8-azaadenosine analog (IVa) was highly cytotoxic in tests against Human Epidermoid Carcinoma cells, No. 2, (H.Ep./-2) in culture (ED_{50} = 0.3 mcg./ml.), increased lifespan by 40-70% in tests against P388 leukemia in mice, and demonstrated borderline activity against L1210 leukemia (25). The phosphate (VI) and cyclic phosphate (VII) derivatives also proved to be cytotoxic to cells in culture; the values of ED_{50} for VI *versus* H.Ep./-2 cells and for VII *versus* Eagle's KB cells were less than 1 mcg./ml. and 9.4 mcg./ml., respectively. None of the other adenine or the hypoxanthine derivatives were significantly cytotoxic to H.Ep./-2 cells in culture; values of ED_{50} were greater than 100 mcg./ml. for all except IVd (ED_{50} = 42 mcg./ml.). All of the hypoxanthine derivatives (IIIa-d), the remaining adenine derivatives (IVb-d), and the phosphate (VI) were tested against leukemia L1210 in mice at 400 mg./kg. on the Day 1 schedule (26); none were active or toxic. In addition, compounds IIId and IVb were tested at 400 mg./kg./day on the *q.d.* 1-9 schedule, and compound IIIa was administered at 200, 100, and 50 mg./kg. on Days 1, 5, and 9. There was no evidence of activity or toxicity in these tests or in tests of the cyclic phosphate (VII) at 100, 67, or 44 mg./kg./day, *q.d.* 1-7 against leukemia L1210.

EXPERIMENTAL

Melting temperatures were determined in capillary tubes heated in a Mel-Temp apparatus. Ultraviolet spectra were recorded with a Cary Model 17 or Model 14 spectrophotometer. Ultraviolet maxima are in nm; sh. = shoulder, infl. = inflection. Solutions for ultraviolet determinations were prepared by diluting a 5-ml. aliquot of a water or ethanol solution to 50 ml. with 0.1 *N* hydrochloric acid, phosphate buffer (*p*H 7), or 0.1 *N* sodium hydroxide; absorption maxima are reported as being at *p*H 1, 7, or 13, respectively. Infrared spectra were recorded with Perkin-Elmer Model 621 or 521 spectrometers from samples in potassium bromide disks; s = strong, sh. = shoulder. Mass spectra data were taken from low resolution spectra determined with a Hitachi-Perkin-Elmer RMU-7 double focusing instrument. Unless otherwise indicated, thin-layer chromatography (tlc) was performed on plates of silica gel, and spots were detected by ultraviolet light (254 nm) after spraying the chromatogram with an optical whitening agent, Ultraphor WT (BASF Colors and Chemicals, Inc., Charlotte, N. C.). The quantity applied and the developing solvent are shown parenthetically at the appropriate places in the procedures.

(±)-3,6-Dihydro-3-[*trans*-2, *trans*-3-dihydroxy-*cis*-4-(hydroxymethyl)cyclopentyl]-7*H*-*v*-triazolo[4,5-*d*]pyrimidin-7-one (IIIa).

This compound was prepared from Ia by a procedure similar

to that described below for IIId. A syrup obtained by evaporating the combined effluent and water-washings from a column of a cation-exchange resin was dissolved in ethanol and stored at 5° and then at -20°. A crystalline solid was separated by filtration, washed with ethanol-hexane, and dried *in vacuo* at 78°: yield, 563 mg. (53%) from 1.1 g. of Ia; m.p. 181-186° dec. The crude product was recrystallized from ethanol containing a small amount of water: m.p. 185-187° dec.; homogeneous by tlc (10 mcg., 5:3:2 butanol-water-acetic acid, detection by uv + Ultraphor and by basic potassium permanganate spray); ir (cm^{-1}) 1730 and 1705 (CO, split); uv max were: 256 (ϵ 10,000), 270 (infl.) at *p*H 1; 256 (ϵ 9600), 268 (infl.), 282 (infl.) at *p*H 7; 275 (ϵ 11,300) at *p*H 13.

Anal. Calcd. for $C_{10}H_{13}N_5O_4$: C, 44.94; H, 4.90; N, 26.21. Found: C, 45.09; H, 4.99; N, 26.20.

(±)-3,6-Dihydro-3-[*trans*-3-hydroxy-*cis*-4-(hydroxymethyl)cyclopentyl]-7*H*-*v*-triazolo[4,5-*d*]pyrimidin-7-one (IIIb).

A solution of 144 mg. of sodium nitrite in 5 ml. of water was added dropwise to a solution of 517 mg. of Ib, 10 ml. of water, and 0.67 ml. of 6 *N* hydrochloric acid at 0°. After the resulting solution had been stirred at 0-5° for 2.5 hours, 12 *N* hydrochloric acid (2 ml.) was added. This solution was stirred at room temperature overnight, heated under reflux for 1 hour, cooled, treated with activated carbon, and concentrated *in vacuo* to a mixture of syrup and solid. A solution of the residue in water (25 ml.) was passed through a column of 10 g. of a cation-exchange resin (Amberlite CG-120, H^+ form), and the column was washed with water (100 ml.). Concentration of the effluent and the water washings left a syrup that was crystallized by dissolving it in ethanol (8 ml.), diluting the hot solution with cyclohexane, and refrigerating. The white crystals were crystallized a second time from ethanol-cyclohexane: wt., 170 mg. (34%); m.p. 182-185°; ir (cm^{-1}) 1735 and 1705 (CO, split); uv max were as follows: 255 (ϵ 10,200), 270 (infl.) at *p*H 1; 256 (ϵ 9400), 268 (infl.), 280 (infl.) at *p*H 7; 275 (ϵ 11,500) at *p*H 13.

Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.21; N, 27.88. Found: C, 47.87; H, 5.22; N, 27.77.

(±)-3,6-Dihydro-3-[*trans*-2-hydroxy-*cis*-4-(hydroxymethyl)cyclopentyl]-7*H*-*v*-triazolo[4,5-*d*]pyrimidin-7-one (IIIc).

This compound was prepared by the procedure described for IIIb. The syrup obtained from the cation-exchange column was crystallized once from ethanol-cyclohexane: yield, 49% (245 mg. from 517 mg. of Ic); m.p. 178-181°; ir (cm^{-1}) 1725 (CO); uv max were: 255 (ϵ 9950), 270 (infl.) at *p*H 1; 256 (ϵ 8900), 268 (infl.), 280 (infl.) at *p*H 7; 277 (ϵ 11,300) at *p*H 13.

Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.21; N, 27.88. Found: C, 47.72; H, 5.19; N, 27.71.

(±)-3,6-Dihydro-3-[*cis*-3-(hydroxymethyl)cyclopentyl]-7*H*-*v*-triazolo[4,5-*d*]pyrimidin-7-one (IIId).

A solution of IId was prepared by adding 1.63 g. of sodium nitrite in 45 ml. of water to a cold (0°) solution of 5.25 g. of Id, 29 ml. of acetic acid, and 28 ml. of water and stirring the solution at 0° for 2 hours. A reaction solution consisting of 14.5 ml. of 12 *N* hydrochloric acid and the solution of IId was heated under reflux for 2 hours and evaporated *in vacuo*, and several portions of water were added and evaporated. A water (200 ml.) solution of the residue was passed through a column of 70 g. of a cation-exchange resin (Rexyn RG50, H^+ form). The effluent and the water washings (200 ml.) were combined, treated with activated carbon, and evaporated to dryness. The solid residue was triturated with cold ethanol and collected by filtration: yield, 1.5 g. (30%); m.p. 166-168°; homogeneous by tlc (10 mcg., 9:1 chloroform-methanol); ir (cm^{-1}) 1725 (CO). A specimen was recrystallized from ethanol-hexane; uv max. were: 254 (ϵ 10,000), 269 (infl.) at *p*H 1; 255 (ϵ 9400), 268 (infl.), 280 (infl.) at *p*H 7; 275 (ϵ 11,300) at *p*H 13.

Anal. Calcd. for $C_{10}H_{13}N_5O_2$: C, 51.06; H, 5.57; N, 29.77. Found: C, 51.19; H, 5.54; N, 29.55.

(±)-*trans*-3-(7-Amino-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-3-yl)-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol (IVa).

A solution of 655 mg. of sodium nitrite in 21 ml. of water was added slowly during 25 minutes to a stirred, cold (0°) solution consisting of 2.4 g. of *trans*-3-[(5-amino-6-chloro-4-pyrimidinyl)-amino]-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol (Ia), 2.9 ml. of 6 *N* hydrochloric acid, and 36 ml. of water. The solution was stirred for an additional 15 minutes, neutralized with solid sodium bicarbonate (870 mg.), and lyophilized. A mixture of the residue (containing IIa) and 100 ml. of liquid ammonia was heated in a glass-lined, stainless steel bomb at 70° for 22 hours. The residue left by evaporation of the ammonia was recrystallized from water (250 ml.); the white solid was collected by filtration, washed with water and with ethanol, and dried *in vacuo* at 78°: yield, 1.77 g. (76%); m.p. 276-277° dec.; tlc, 1 spot (applied in 0.1 *N* hydrochloric acid, 60 mcg., 5:3:2 butanol-water-acetic acid, detection by uv + Ultraphor and by basic potassium permanganate spray). A specimen for analysis was recrystallized a second time from water: m.p. 278° dec. (inserted at 100°, 2°/min. at 270°); uv max at 261-265 (ϵ 12,700) and 275 (sh.) in 0.1 *N* hydrochloric acid, 278 (ϵ 12,600) in phosphate buffer, 279 (ϵ 12,800) in 0.1 *N* sodium hydroxide; MS (direct-probe inlet temperature = 350°, P = 7-amino-*v*-triazolo[4,5-*d*]pyrimidinyl fragment) m/e 267 (M^+ + H), 266 (M^+), 265 (M^+ - H), 249 (M^+ - OH), 248 ($M^+ - H_2O$), 237, 235 ($M^+ - CH_2OH$), 219, 209, 207, 191, 189, 179, 173, 165, 163, 161, 151, 149, 144.8 (metastable, 179 → 161), 137 (P^+ + 2H, base peak), 136 (P^+ + H), 135 (P^+); 137 > 179 > 136 ~ 135 > 111.

Anal. Calcd. for $C_{10}H_{14}N_6O_3$: C, 45.11; H, 5.30; N, 31.57. Found: C, 44.90; H, 5.46; N, 31.39.

(±)-*cis*-4-(7-Amino-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-3-yl)-*trans*-2-hydroxycyclopentanemethanol (IVb).

A solution prepared from 348 mg. of sodium nitrite, 1.25 g. of Ib, 25 ml. of water, and 1.62 ml. of 6 *N* hydrochloric acid was stirred at 0° for 30 minutes, partially neutralized with saturated aqueous sodium bicarbonate to *p*H 5.4, and lyophilized. The white residue was transferred with liquid ammonia into a glass-lined, stainless steel bomb and heated with liquid ammonia (50 ml.) at 60° for 24 hours. The residue left by evaporation of the ammonia was triturated with water (15 ml.), washed with water (4 x 5 ml.), and dried *in vacuo* at 78°: wt., 738 mg. (61%); m.p. 212-216° dec. (inserted at 90°, 3°/min.); homogeneous by tlc (80 mcg., 5:3:2 butanol-water-acetic acid). A second portion of white solid was obtained from the filtrate: wt., 70 mg. (total yield, 67%); m.p. 212-216° dec. A specimen was recrystallized from ethanol-cyclohexane: m.p. 213-217° dec. (inserted at 140°, 3°/min.); uv max at 261-266 (ϵ 12,500) and 275 (sh.) in 0.1 *N* hydrochloric acid, 278 (ϵ 12,000) in phosphate buffer, 278 (ϵ 12,200) in 0.1 *N* sodium hydroxide.

Anal. Calcd. for $C_{10}H_{14}N_6O_2$: C, 47.99; H, 5.64; N, 33.59. Found: C, 47.77; H, 5.57; N, 33.38.

(±)-*cis*-3-(7-Amino-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-3-yl)-*trans*-4-hydroxycyclopentanemethanol (IVc).

This compound was prepared by the procedure described for IVb: yield, 83%; m.p. 223-225°. Recrystallization of IVc from water gave white crystals: m.p. 227-229° dec. (inserted at 100°, 4°/min.); uv max at 261-265 (ϵ 12,400) and 275 (sh.) at *p*H 1, 279 (ϵ 12,300) in phosphate buffer, 279 (ϵ 12,100) in 0.1 *N* sodium hydroxide.

Anal. Calcd. for $C_{10}H_{14}N_6O_2$: C, 47.99; H, 5.64; N, 33.59. Found: C, 48.00; H, 5.82; N, 33.44.

(±)-*cis*-3-(7-Amino-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-3-yl)cyclopentanemethanol (IVd).

This compound was prepared by the procedure described for IVa and was recrystallized from water: yield, 38%; m.p. 185-186° dec.; uv max at 261-265 (ϵ 12,300) and 275 (sh.) at *p*H 1, 278 (ϵ 12,100) at *p*H 7 and *p*H 13.

Anal. Calcd. for $C_{10}H_{14}N_6O$: C, 51.27; H, 6.02; N, 35.88. Found: C, 51.05; H, 5.86; N, 35.72.

Isopropylidene Derivative (V) of IVa.

A mixture of 1.37 g. of IVa, 500 ml. of dry acetone, and 20 g. of *p*-toluenesulfonic acid (dried *in vacuo* over phosphorus pentoxide) was stirred at room temperature for 5 hours and poured into 600 ml. of frozen, saturated aqueous sodium bicarbonate. The brei was stirred for 10 minutes and concentrated *in vacuo* to a solid which was dried further by the addition and evaporation of several portions of ethanol. The residual solid was leached with hot chloroform (4 x 200 ml.), and the chloroform extract was concentrated to dryness. Recrystallization of the residue from ethanol produced white needles which were washed with ethanol and dried *in vacuo* over phosphorus pentoxide at 78°: yield, 1.39 g. (88%); m.p. 223-225° dec. (inserted at 200°); uv max at 261-265 (ϵ 12,500) and 275 (sh.) at *p*H 1, 279 (ϵ 12,300) at *p*H 7, 278 (ϵ 12,100) at *p*H 13.

Anal. Calcd. for $C_{13}H_{18}N_6O_3$: C, 50.97; H, 5.92; N, 27.44. Found: C, 50.81; H, 5.86; N, 27.31.

(±)-*trans*-3-(7-Amino-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-3-yl)-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol 5-(Dihydrogen Phosphate) (VI).

A solution of 2 ml. of trimethyl phosphate, 0.122 ml. (1.33 mmoles) of phosphorus oxychloride, and 200 mg. (0.65 mmole) of V was prepared at -5°, stirred at this temperature for 3 hours, and poured into a solution of 50 ml. of water and 2 ml. of 2.8 *N* aqueous ammonia. The mixture was kept at room temperature for 0.5 hour and additional quantities of 2.8 *N* aqueous ammonia were added, as required, to maintain the *p*H at 6.5-7.4. The solution (final *p*H = 7.2) was passed through a column of a cation-exchange resin (10 g. of Amberlite CG-120, H^+ form). The column was washed with 200 ml. of water and with 300 ml. of 0.5 *N* aqueous ammonia. The ammonia eluate was concentrated to a low volume (*ca.* 5 ml.), diluted with ethanol (10 ml.), and cooled to -20°. The white precipitate was collected by filtration and dried *in vacuo* over phosphorus pentoxide at 78°: yield, 167 mg. (73%); m.p. 195-205° dec. (inserted at 150°); uv max at 261-266 (ϵ 12,200) and 275 (sh.) at *p*H 1, 278 (ϵ 12,200) at *p*H 7, 278 (ϵ 12,100) at *p*H 13. The infrared spectrum included broad, unresolved absorption at 3600-2500 cm^{-1}; bands at 1710, 1655, 1615, 1575, 1275, 795 cm^{-1}; and broad bands centered near 1450, 1325, 1150, 1050s, 920, 725, 510 cm^{-1}.

Anal. Calcd. for $C_{10}H_{15}N_6O_6P \cdot NH_3 \cdot H_2O$: C, 31.50; H, 5.29; N, 25.72; P. 8.11. Found: C, 31.51; H, 4.92; N, 25.30; P, 7.90.

A specimen of the monohydrate of the ammonium salt of VI, identical according to tlc (5:3:2 butanol-water-acetic acid on silica gel or cellulose) and the uv and ir spectra, was obtained in 93% yield from a larger scale run.

A water solution of a small amount of the ammonium salt was neutralized with 1 *N* hydrochloric acid, diluted with ethanol, and chilled to 5° and then to -20°. The white crystalline phosphate (VI) was removed by filtration, washed with ethanol, and dried *in vacuo* over phosphorus pentoxide at 78°; m.p. 228-229° dec. (inserted at 220°, 2°/min.); ir spectrum (1700-1500 and 1200-1000 cm^{-1} regions only) 1690s, 1615, 1555, 1200 (broad), 1130sh., 1120, 1105, 1060s, 1035s, 995.

Anal. Calcd. for $C_{10}H_{15}N_6O_6P \cdot H_2O$: C, 32.97; H, 4.70; N, 23.07; P, 8.50. Found: C, 32.67; H, 4.10; N, 23.13; P. 8.51.

(±)-*trans*-3-(7-Amino-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-3-yl)-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol Cyclic 1,5-(Hydrogen Phosphate) (VII).

A solution of 1.14 g. (3 mmoles) of VI ammonium salt monohydrate, 880 mg. (3 mmoles) of 4-morpholino-*N*,*N*′-dicyclohexylcarboxamidine, 15 ml. of water, and 75 ml. of pyridine was evaporated to dryness *in vacuo*, and five 50-ml. portions of dry pyridine were added and evaporated *in vacuo* to dry the residue. A solution of the residual gum in 300 ml. of dry pyridine was added dropwise during 3.5 hours to a refluxing solution of 1.236 g. (6 mmoles) of dicyclohexylcarbodiimide in 200 ml. of dry pyridine, and two 50-ml. portions of hot, dry pyridine used to wash the dropping funnel were added. The mixture was heated under reflux for 2 hours and concentrated to dryness *in vacuo*. A mixture of the residue and water (750 ml.) was stirred at room temperature for 0.5 hour, concentrated *in vacuo* at 35-40° to about 500 ml., and shaken with 300 ml. of ether. The entire mixture was filtered to remove dicyclohexylurea, and the aqueous layer was separated, concentrated to about 400 ml. *in vacuo*, and stirred for 15 minutes with a strongly basic ion-exchange resin (Rexyn 210, OH^- form). The resin was stirred repeatedly with small portions (10-30 ml.) of 25% acetic acid. Concentration of the aqueous acetic acid solution (300 ml.) to dryness and trituration of the syrup with ethanol afforded 355 mg. of VII. Repetition of the process of stirring the resin with 25% acetic acid gave an additional 192 mg. of VII. The aqueous solution was then stirred with a second 10-g. portion of the basic ion-exchange resin, the 2 portions of resin were combined and stirred with five 50-ml. portions of 2 *N* hydrochloric acid, and the hydrochloric acid solution was reduced to dryness *in vacuo* at 35°. Trituration of the residue with ethanol-water yielded an additional 322 mg. of VII (total, 72%). The 3 portions of product were recrystallized from water and dried *in vacuo* over phosphorus pentoxide at 78°: yield, 378 mg. (35%); progressively darkened above 260° without melting; uv max at 262-266 (ϵ 12,900) and 275 (sh.) at *p*H 1, 279 (ϵ 12,700) at *p*H 7 and at *p*H 13.

Anal. Calcd. for $C_{10}H_{13}N_6O_5P \cdot H_2O$: C, 34.69; H, 4.37; N, 24.27; P. 8.94. Found: C, 34.63; H, 4.26; N, 24.27; P, 8.86.

The ir spectrum of VII (monohydrate) differed from that of the phosphate (VI; free acid, monohydrate) especially in the region of 1200-1000 cm^{-1}; ν (1700-1500 and 1200-1000 cm^{-1} only) 1690s, 1650sh., 1605, 1560, 1220, 1190, 1155, 1120, 1075, 1050sh., 1040s, 1015, 995. The cyclic phosphate moves faster than VI (free acid) on tlc plates of silica gel developed in 3:1 *n*-propanol-water (detection by ultraviolet light before and after spraying with Ultraphor WT).

Acknowledgments.

The authors are indebted to Dr. W. C. Coburn, Jr., and members

of the Molecular Spectroscopy Section of this Institute for microanalytical and spectroscopic data. Some of the elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee. Biological testing was performed in this Institute under the supervision of Drs. F. M. Schabel, Jr., W. R. Laster, Jr., L. J. Wilkoff, and G. J. Dixon.

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(1) This work was supported by the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Contracts PH43-64-51 and NIH-NCI-C-71-2021; biological tests were carried out under terms of Contracts PH43-65-594 and NIH-NCI-C-71-2098.

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