ear portion of the curve and was observed to have a value of 0.016, 0.042, 0.112, 0.208, 0.370, and 0.067 OD units at pH 8.5, 8.0, 7.5, 7.0, 6.5, and 6.0, respectively. These data indicate that DOAP is capable of causing the rapid oxidation of NADH with a pH optimum of 6.5 and is itself apparently reduced to its hydrazo form while acting as an electron acceptor.

DOAP has also been found to be an extremely potent inhibitor of bovine milk xanthine oxidase employing a spectrophotometric method[9] which measures uric acid formation at 290 nm. Assay conditions were adjusted so that the rate of increase in absorbance using a Beckman DB-G recording spectrophotometer was linearly proportional to the amount of xanthine oxidase** present. Each experimental cuvette contained 1.75 ml of 0.05 M potassium phosphate buffer, pH 7.5, 1.0 ml of a 1.6×10^{-5} M solution of xanthine, 0.01 ml of various concentrations of DOAP in 0.005 N NaOH, and 0.25 ml of a 0.5 mg/ml solution of xanthine oxidase. After a 2-min equilibration period at 25°, the enzyme was added last, the cuvette contents were rapidly mixed, and the A_{290} was recorded with time against a reference cell from which the substrate had been omitted. The ΔA_{290}/min was obtained from the linear portion of the curve of increasing extinction in the presence and absence of DOAP. A 50% inhibition of xanthine oxidase activity was found to occur at a final concentration of 3.5×10^{-8} M DOAP in the reaction mixture (Figure 6), while allopurinol under similar conditions was found to produce a 50% inhibition of xanthine oxidase activity at 6.1×10^{-6} M. Results of both a Lineweaver and Burk plot (Figure 7) as well as a Dixon plot revealed a mean K_i of 2.8×10^{-8} M for DOAP while indicating that the nature of the inhibition of xanthine oxidase by DOAP was noncompetitive. The present finding of such a high degree of potency of DOAP as a xanthine oxidase inhibitor does suggest the further investigation of this compound as a potentially useful agent in the treatment of hyperuricemia. Experiments are now in progress in our laboratory which are designed for further studies of both the chemical and biological properties of 8,8′-dioxo-6,6′-azopurine (DOAP), a member of a new class of compounds designed as the oxoazopurines.

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**Obtained from Sigma Chemical Co., St. Louis, Mo.

Synthesis and Enzymatic Activity of 1,2,4-Triazole-3-carboxamide 6′-Deoxyhomoribonucleoside-6′-phosphonic Acid and Related Compounds

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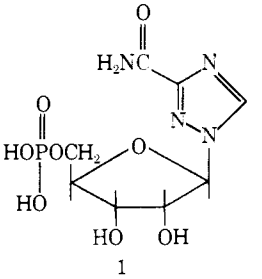
The synthesis, *via* the Wittig reaction, of the phosphonic acid corresponding to the nucleotide 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-phosphate (1) is reported. The same route is used to synthesize nucleosides with a polar substituent (carboxamide) and an ionizable group (carboxylic acid) at the position normally occupied by the phosphate group in the nucleotide. These compounds were tested as the inhibitors of the enzyme inosine 5′-phosphate dehydrogenase isolated from *Escherichia coli*. In this system, compound 1 produced 50% inhibition of this enzyme at a concentration of 6×10^{-7} M. In the same assay, the corresponding phosphonic acid 9 inhibited the dehydrogenase by 50% at 2×10^{-5} M concentration. The other compounds tested (12, 13, and 14) were not effective as inhibitors up to 3×10^{-4} M.

The conversion of certain antiviral nucleosides to the corresponding nucleotides has been shown to be a necessary process in order for these drugs to exhibit their inhibitory activity.[1,2] In our studies of the synthetic nucleoside 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide[3] (ribavirin†), which exhibits activity against both DNA and RNA viruses,[4,5] we have found evidence[6] that the corresponding 5′-phosphate 1 is the active form of this antiviral agent. This nucleotide (1) was found to be a potent competitive inhibitor of the enzyme inosine 5′-phosphate dehydrogenase.[6]



1

The syntheses of several nucleoside phosphonic acids, in which the phosphate ester oxygen is replaced by a methylene group, have been reported.[7] Hampton and coworkers studied the interactions of a number of homoadenosine-6′-phosphonic acid derivatives with adenine nucleotide utilizing enzymes and found that replacement of the phosphate ester oxygen with a methylene or substituted methylene group allows these nucleotide analogs to retain varying degrees of binding to the enzyme site.[8]

We now report the synthesis of the phosphonic acid cor-

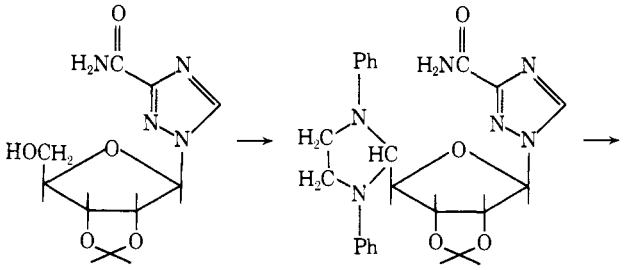
† Ribavirin is the name approved by the U. S. Adopted Names Council for this compound, previously identified as Virazole.

responding to the nucleotide 1 and the results of some enzymatic studies with this product. This isostere of 1 is inherently stable to phosphatases and thus the stepwise degradation of nucleotide → nucleoside → base is precluded. In addition, we have investigated nucleosides with a polar substituent (carboxamide) and an ionizable group (carboxylic acid) at the position normally occupied by the phosphate group in the corresponding nucleotide. Purine nucleoside derivatives similarly modified at the 5′ position have recently been reported.[9,10]

Nucleoside phosphonates have previously been obtained *via* the Wittig reaction from the appropriate nucleoside 5′-aldehydes.[7] The synthesis of the isostere related to nucleotide 1 was approached similarly.

Treatment of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide[3] with 2,2-dimethoxypropane in the presence of perchloric acid[11] afforded the isopropylidene derivative 2 in 90% yield (Scheme I). Oxidation of 2 by the procedure of Pfitzner and Moffatt[12] using pyridinium trifluoroacetate and DCC in DMSO gave the aldehyde 4, which was isolated as the crystalline 1,3-diphenylimidazoline[13,14] derivative 3 in 70% yield. Acidic hydrolysis of 3 with Dowex 50 (H^+) at room temperature selectively removed[14] the aldehyde protecting group to give the isopropylidene derivative 4 in quantitative yield. Treatment of the aldehyde 4 with diphenyl triphenylphosphoranylidenemethylphosphonate[15] provided the α,β-unsaturated phosphonate 5 in 64% yield. The nmr spectrum of 5 is consistent with the trans configuration for this compound. Hydrogenation of the vinyl phosphonate 5 proceeded readily to give the saturated phosphonate 6. Attempts to remove the phenyl groups from 6 by hydrogenolysis were not successful and this compound was converted to the dibenzyl ester 7 by transesterification[7,8c] in 95% yield. Removal of the benzyl groups from 7 by hydrogenolysis, followed by heating the phosphonic acid 8 in H_2O to remove the isopropylidene group, provided 1-(5,6-dideoxy-β-D-*ribo*-hexofuranosyl-6-phosphonic acid)-1,2,4-triazole-3-carboxamide (9).

Scheme I



2

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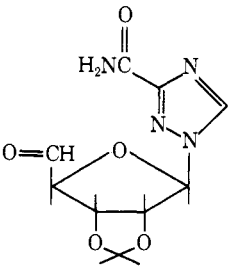
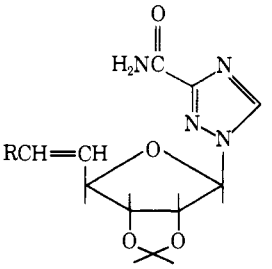
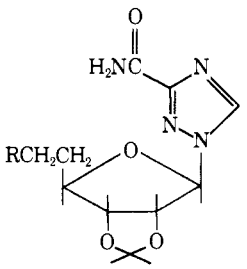


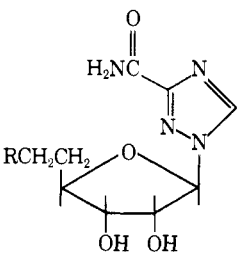
4



5, R = $(C_6H_5O)_2P{=}O$
10, R = CO_2Et



6, R = $(C_6H_5O)_2P{=}O$
7, R = $(C_6H_5CH_2O)_2P{=}O$
8, R = $(HO)_2P{=}O$
11, R = CO_2Et



9, R = $(HO)_2P{=}O$
12, R = CO_2Et
13, R = $CONH_2$
14, R = COOH

In a second series of reactions, the aldehyde 4 was treated with carbethoxymethylenetriphenylphosphorane to give the unsaturated ester 10 in 84% yield. The nmr data are in agreement with the trans configuration for 10. Hydrogenation of 10 followed by removal of the isopropylidene group gave 1-(ethyl 5,6-dideoxy-β-D-*ribo*-heptofuranosyluronate)-1,2,4-triazole-3-carboxamide (12). Formation of the amide 13 from the ethyl ester 12 on treatment with methanolic ammonia was very slow but after several days this product (13) was obtained in 64% yield. Mildly basic conditions were necessary to obtain the carboxylic acid 14 from the ester 12 since the use of 1 *N* NaOH hydrolyzed the carboxamide group in the 3 position of the triazole ring. Treatment of the ester 12 with a buffered solution (pH 10.7) afforded, after acidification, 1-(5,6-dideoxy-β-D-*ribo*-heptofuranosyluronic acid)-1,2,4-triazole-3-carboxamide (14).

These compounds were tested as inhibitors of the enzyme inosine 5′-phosphate dehydrogenase isolated from *Escherichia coli* using the assay described by Streeter, *et al.*[6] In this system, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-phosphate produced 50% inhibition of this enzyme at a concentration of 6×10^{-7} *M*. In the same assay 1-(5,6-dideoxy-β-D-*ribo*-hexofuranosyl-6-phosphonic acid)-1,2,4-triazole-3-carboxamide (9) inhibited the dehydrogenase by 50% at 2×10^{-5} *M* concentration. In a study[8a] of the interaction of a similar nucleotide analog, 6′-deoxyhomoinosine-6′-phosphonic acid, with the enzyme adenylosuccinate synthetase, Hampton suggested that the decrease in binding which resulted from replacement of the phosphate ester oxygen with the somewhat larger methylene group may be due to steric factors. Since other properties of the phosphonic acid 9 should be similar to those of the nucleotide 1, steric interference may also account for the reduction in inhibitory activity exhibited by 9 in comparison with the potent inhibitor 1.

The other nucleosides (12, 13, and 14) were tested against inosine 5′-phosphate dehydrogenase and were not effective as inhibitors up to a concentration of 3×10^{-4} *M*. These results are consistent with the observation[8a,16] that the nucleotide dianion appears to be necessary for binding to this enzyme.

These nucleosides were tested for activity in tissue culture against type 3 adeno, type 1 herpes simplex, type 13 rhino, and type 3 parainfluenza viruses. The phosphonic acid 9 and the other triazole nucleosides (12, 13, and 14) did not exhibit antiviral activity in contrast to 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-phosphate which shows activity[17] similar to that of the corresponding nucleoside.[3] The lack of antiviral activity of the phosphonic

acid 9 is consistent[6] with the relatively high concentration of this product necessary for inhibition of inosine 5′-phosphate dehydrogenase.

Experimental Section

Melting points were determined in Thomas-Hoover apparatus and are uncorrected. Nuclear magnetic resonance spectra were obtained on a Hitachi Perkin-Elmer R20-A spectrometer using DSS as internal standard. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter at 25°. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and were within 0.4% of the theoretical values. Thin-layer chromatography was performed on silica gel GF 254. Detection of the compounds on the plates was done by spraying with 10% v/v sulfuric acid in methanol followed by heating. Evaporations were carried out *in vacuo* with bath temperature below 40°.

1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (2). 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (30.0 g, 123 mmol) was suspended in a mixture of acetone (400 ml) and 2,2-dimethoxypropane (200 ml). The mixture was cooled in an ice bath and 70% perchloric acid (6 ml) was added. The mixture was kept at room temperature for 3 hr and at 5° overnight. The resulting orange solution was neutralized with 2 *N* KOH, filtered, and evaporated to dryness. The solid residue was treated with methanol and the insoluble product was removed by filtration. The methanolic solution was concentrated to a small volume and the crystalline product was collected. Recrystallization from EtOAc–MeOH gave **2** (31.0 g, 90%) with mp 153–154°; $[\alpha]_D$ 29.9° (*c* 1, MeOH); nmr (DMSO-d_6) δ 8.83 (s, 1, H-5), 6.23 (d, 1, $J_{1',2'}$ = 1.5 Hz, H-1′), 5.1 (m, 2, H-2′ and H-3′), 4.31 (m, 1, H-4′), 1.56 and 1.38 (2 s, 3 each, CH_3). *Anal.* ($C_{11}H_{16}N_4O_5$) C, H, N.

1-(2,3-*O*-Isopropylidene-β-D-*ribo*-pento-1,5-dialdo-1,4-furanosyl)-1,2,4-triazole-3-carboxamide (4). 1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (**2**, 5.68 g, 20 mmol) and dicyclohexylcarbodiimide (16.48 g, 80 mmol) were dissolved in 200 ml of DMSO. Pyridine (2 ml) and F_3CCOOH (1 ml) were then added and the mixture was stirred at room temperature for 20 hr. Then H_2O (30 ml) was added and the resulting mixture was stirred for an additional 30 min. The precipitated dicyclohexylurea (16 g) was removed by filtration and the solution was evaporated to dryness. The residue was dissolved in CH_2Cl_2 (200 ml) and *N,N′*-diphenylethylenediamine (4.24 g, 20 mmol) dissolved in ether (15 ml) was added. The resulting mixture was refluxed for 1 hr. The reaction mixture was cooled and extracted with water. The organic layer was dried over $MgSO_4$ and evaporated to a syrup. Diethyl ether was added to the syrup and the mixture was stirred at room temperature overnight. The finely divided precipitate was collected by filtration to yield 9.0 g of compound **3**. This material was recrystallized from ether–CH_2Cl_2–cyclohexane to give 6.8 g (71%) of the pure 1-[4(*R*)-(1′,3′-diphenyl-2′-imidazolidinyl)-2,3-*O*-isopropylidene-β-D-erythrofuranosyl]-1,2,4-triazole-3-carboxamide (**3**) with mp 155–156°. *Anal.* ($C_{25}H_{28}N_6O_4 \cdot H_2O$) C, H, N.

The blocked aldehyde **3** (2.39 g, 5.0 mmol) was dissolved in THF (100 ml) and H_2O (50 ml). Bio-Rad AG-50W-X8 (H^+) (25 g) was then added and the reaction mixture was stirred at room temperature for 1 hr. The resin was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was coevaporated twice with THF, suspended in $CHCl_3$, collected by filtration, and dried *in vacuo* to give a quantitative yield of the free aldehyde **4** as a chromatographically pure product that was used in the next step without further purification.

1-(5,6-Dideoxy-6-diphenylphosphono-2,3-*O*-isopropylidene-β-D-*ribo*-hex-5-enofuranosyl)-1,2,4-triazole-3-carboxamide (5). A solution of **4** (1.41 g, 5.0 mmol) and diphenyl triphenylphosphoranylidenemethylphosphonate (2.38 g, 5.0 mmol) in THF (100 ml) was stirred at room temperature for 5 hr. After this time, an additional 2.38 g (5.0 mmol) of the Wittig reagent was added and the solution was stirred at room temperature for another 20 hr and finally was refluxed for 2 hr. The solvent was removed and the residue (7.0 g) was purified by column chromatography over silica gel with 19:1 $CHCl_3$–MeOH to give **5** (1.6 g, 64%) as a homogenous syrup: $[\alpha]_D$ +19.1° (*c* 1, $CHCl_3$); nmr (DMSO-d_6) δ 8.91 (s, 1, H-5), 7.3 (m, 10, phenyl), 6.80 (d of d, 1, $J_{5',6'}$ = 17 Hz, $J_{4',5'}$ = 6 Hz, H-5′), 6.46 (d, 1, $J_{1',2'}$ < 1 Hz, H-1′), 6.14 (d of d of d, 1, $J_{6',P}$ = 22 Hz, $J_{5',6'}$ = 17 Hz, $J_{4',6'}$ = 2 Hz, H-6′), 4.8–5.4 (m, 3, H-2′, H-3′, and H-4′), 1.38 and 1.57 (2 s, 3 each, CH_3). *Anal.* ($C_{24}H_{25}N_4O_7P$) C, H, N.

1-(5,6-Dideoxy-6-diphenylphosphono-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl)-1,2,4-triazole-3-carboxamide (6). The vinyl phosphonate **5** (1.28 g, 2.5 mmol) was hydrogenated at 14 psi of hydrogen in methanol (50 ml) using 10% Pd on barium sulfate (100 mg) as catalyst for 1 hr. The solution was filtered through Celite and the solvent evaporated to give a quantitative yield of chromatographically pure product that was used in the next step without further purification.

1-(5,6-Dideoxy-6-dibenzylphosphono-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl)-1,2,4-triazole-3-carboxamide (7). A solution of sodium benzyl oxide (8.0 mmol) in dry benzyl alcohol (10 ml) was added to a solution of the diphenyl ester **6** (1.0 g, 1.94 mmol) in benzyl alcohol (3.0 ml). After 1.5 hr at room temperature, tlc on silica gel (9:1 EtOAc–EtOH) showed that the reaction was complete. Then, ether (70 ml) was added and an excess of CO_2 was introduced. The precipitated gel was dissolved by addition of EtOAc and silica gel (20 g) was added. The mixture was concentrated to obtain a fine powder that was slurried in ether and applied to a silica gel column packed in ether. Elution with ether (250 ml), 1:1 ether–EtOAc (500 ml), EtOAc (1 l.), and 9:1 EtOAc–MeOH (1 l.) successively removed phenol, benzyl alcohol, and the benzyl ester **7**. This product was obtained as a chromatographically homogenous foam (1.0 g, 95%): nmr (DMSO-d_6) δ 8.87 (s, 1, H-5), 7.37 (br s, 10, phenyl), 6.28 (br s, 1, $J_{1',2'}$ < 1 Hz, H-1′), 5.27 (m, 1, $J_{2',3'}$ = 6 Hz, H-2′), 5.04 and 4.91 (2 s, 4, benzyl CH_2), 4.54 (m, 1, $J_{2',3'}$ = 6 Hz, H-3′), 4.28 (m, 1, H-4′), 1.6–2.2 (m, 4, CH_2CH_2), 1.36 and 1.52 (2 s, 3 each, CH_3).

1-(5,6-Dideoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl-6-phosphonic acid)-1,2,4-triazole-3-carboxamide (8). A solution of the dibenzyl phosphonate **7** (0.90 g, 1.66 mmol) in EtOH was shaken on a Parr hydrogenator at 14 psi of hydrogen using Pd black (100 mg) as catalyst. After 0.5 hr the solution was filtered through Celite and the filtrate was evaporated to dryness under reduced pressure. The residue **8** (0.54 g, 90%) was chromatographically homogenous and was used in the next step without further purification.

1-(5,6-Dideoxy-β-D-*ribo*-hexofuranosyl-6-phosphonic acid)-1,2,4-triazole-3-carboxamide (9). The phosphonic acid **8** (0.5 g, 1.38 mmol) was heated with H_2O at 80° for 1.5 hr. Then, the solution was lyophylized and the product dried over P_2O_5 at 110° for 1 hr. Compound **9** (0.40 g, 91%) was obtained as an analytical sample: $[\alpha]_D$ −9.2° (*c* 0.5, H_2O); nmr DMSO-d_6–D_2O) δ 8.84 (s, 1, H-5), 5.86 (d, 1, $J_{1',2'}$ = 3 Hz, H-1′), 4.5–3.7 (m, 3, H-2′, H-3′, and H-4′), 2.1–1.2 (m, 4, CH_2CH_2). *Anal.* ($C_9H_{15}H_4O_7P$) C, H, N, P.

1-(Ethyl 5,6-dideoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hept-5-enofuranosyl uronate)-1,2,4-triazole-3-carboxamide (10). A mixture of 1-(2,3-*O*-isopropylidene-β-D-*ribo*-pento-1,5-dialdo-1,4-furanosyl)-1,2,4-triazole-3-carboxamide (**4**, 7.05 g, 25 mmol) and carbethoxymethylenetriphenylphosphorane (11.5 g, 33 mmol) in THF (500 ml) was stirred at room temperature for 5 hr. The solvent was evaporated and the residue was dissolved in a small volume of $CHCl_3$ and chromatographed on a silica gel column using 30:1 $CHCl_3$–MeOH as eluent. From this column **10** (7.4 g, 84%) was obtained as an analytically pure amorphous solid: $[\alpha]_D$ +32.4° (*c* 1, $CHCl_3$); nmr (DMSO-d_6) δ 8.9 (s, 1, H-5), 6.87 (d of d, 1, $J_{5',6'}$ = 16 Hz, $J_{4',5'}$ = 7 Hz, H-5′), 6.43 (br s, 1, $J_{1',2'}$ < 1 Hz, H-1′), 5.77 (d of d, 1, $J_{5',6'}$ = 16 Hz, $J_{4',6'}$ = 2 Hz, H-6′), 5.29 (m, 2, H-2′ and H-3′), 4.99 (m, 1, H-4′), 4.10 (q, 2, CH_2 of CH_2CH_3), 1.58 and 1.39 (2 s, 3 each, CH_3), 1.20 (t, 3, CH_3 of CH_2CH_3). *Anal.* ($C_{15}H_{20}N_4O_6$) C, H, N.

1-(Ethyl 5,6-dideoxy-2,3-*O*-isopropylidene-β-D-*ribo*-heptofuranosyluronate)-1,2,4-triazole-3-carboxamide (11). A solution of compound **10** (3.52 g, 10.0 mmol) in MeOH (150 ml) was hydrogenated at 47 psi on a Parr hydrogenator in the presence of 10% Pd on charcoal (300 mg) for 1 hr. The catalyst was filtered over Celite and the solvent was evaporated. The chromatographically homogenous product obtained (3.18 g, 90%) had the expected nmr for the hydrogenated nucleoside and was used in the next step without further purification.

1-(Ethyl 5,6-dideoxy-β-D-*ribo*-heptofuranosyluronate)-1,2,4-triazole-3-carboxamide (12). A solution of **11** (3.18 g, 9.0 mmol) in 80% aqueous acetic acid (100 ml) was heated on a steam bath for 2 hr. The solution was concentrated to dryness and the residue was coevaporated several times with ethanol. The crude product (3.0 g) was dissolved in MeOH and silica gel (18 g) was added to the solution. The mixture was evaporated to dryness and the silica gel mixture was slurried with $CHCl_3$ and applied to a silica gel column (100 g) packed in $CHCl_3$. Elution with 9:1 $CHCl_3$–MeOH provided the pure nucleoside **12** (2.1 g, 75%) as a foam: $[\alpha]_D$ +7.6°

(c 0.5, H_2O); nmr (DMSO-d_6) δ 8.87 (s, 1, H-5), 5.92 (d, 1, $J_{1',2'}$ = 3.5 Hz, H-1'), 4.50–3.75 (m, 3, H-2', H-3', and H-4'), 4.06 (q, 2, CH_2 of CH_2CH_3), 2.50–1.68 (m, 4, CH_2CH_2), 1.19 (t, 3, CH_3 of CH_2CH_3). *Anal.* ($C_{12}H_{18}N_4O_6$) C, H, N.

1-(**5,6-Dideoxy-β-D-*ribo*-heptofuranosyluronamide**)-**1,2,4-triazole-3-carboxamide** (**13**). A solution of ethyl ester **12** (0.80 g, 2.55 mmol) in methanol saturated at 0° with NH_3 (70 ml) was kept at 25° for 5 days and at 50° for 6 hr. Evaporation of the solution gave a residue that was purified by chromatography on silica gel following the same procedure indicated for the ethyl ester. Elution of the column with $CHCl_3$ (100 ml), EtOAc (200 ml), and 4:1 EtOAc–MeOH (1 l.) provided 0.46 g (64%) of **13** as an amorphous solid. The product was dried over P_2O_5 at 110° for 2 hr to give an analytical sample: $[\alpha]_D$ −1.7° (c 1, H_2O); nmr (DMSO-d_6-D_2O) δ 8.81 (s, 1, H-5), 5.87 (d, 1, $J_{1',2'}$ = 3.5 Hz, H-1'), 3.80–4.55 (m, 3, H-2', H-3', and H-4'), 2.40–1.70 (m, 4, CH_2CH_2). *Anal.* ($C_{10}H_{15}N_5O_5$) C, H, N.

1-(**5,6-Dideoxy-β-D-*ribo*-heptofuranosyluronic acid**)-**1,2,4-triazole-3-carboxamide** (**14**). A solution of the ethyl ester **12** (0.94 g, 3.0 mmol) in a pH 10.7 buffer (Na_2CO_3–$NaHCO_3$) (125 ml) was maintained at 50° for 7 hr. The solution was neutralized with Dowex 50 (H^+) under stirring. The resin was filtered and the aqueous solution was freeze-dried. The lyophilized product (0.7 g, 81%), chromatographically homogenous, was dried over P_2O_5 at 110° for 2 hr to obtain an analytical sample: $[\alpha]_D$ −5.9° (c 1, H_2O); nmr (DMSO-d_6-D_2O) δ 8.74 (s, 1, H-5), 5.80 (d, 1, $J_{1',2'}$ = 3 Hz, H-1'), 4.53–3.80 (m, 3, H-2', H-3', and H-4'), 2.50–1.70 (m, 4, CH_2CH_2). *Anal.* ($C_{10}H_{14}N_4O_6 \cdot 0.5H_2O$) C, H, N.

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3-Substituted 5,7-Dimethylpyrazolo[1,5-*a*]pyrimidines, 3′,5′-Cyclic-AMP Phosphodiesterase Inhibitors. 1†

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A series of 3-substituted 5,7-dimethylpyrazolo[1,5-*a*]pyrimidines has been synthesized and evaluated for their ability to inhibit the enzyme 3′,5′-cyclic-AMP phosphodiesterase that was isolated and purified from rabbit kidney, rabbit lung, and beef heart. The 3-bromo, 3-chloro, 3-iodo, and 3-acetyl derivatives have been found to be more potent than theophylline in their ability to inhibit these 3′,5′-cAMP phosphodiesterases.

Recently Rose and coworkers[1-3].‡ have reported a number of triazolo[2,3-*c*]pyrimidines and triazolo[4,3-*c*]pyrazines which are capable of protecting animals from a histamine-induced bronchospasm. In particular, compounds **1b** and **2b** were found to be the most potent bronchodilators in these ring systems.[4] These workers point out the structural similarities of these compounds to theophylline (**3**). Inhibition of 3′,5′-cyclic-AMP phosphodiesterase (PDE) appears to be the underlying biochemical mechanism for the pharmacological effects of theophylline,[5] compounds **1** and **2**, and their derivatives.[6] 2-Amino-7-methyl-5-*n*-propyl-*s*-triazolo[2,3-*c*]pyrimidine (**1a**) has been found in our laboratories to be approximately equal to theophylline (**3**) in inhibiting the PDE isolated from rabbit lung; however, **1a** is only 0.2 as active as **3** in inhibiting PDE isolated from rabbit kidney. In an effort to discover more potent inhibitors of PDE, and possibly compounds with superior selective pharmacological activity, we have prepared a number of 3-substituted 5,7-dimethylpyrazolo[1,5-*a*]pyrimidines and evaluated these derivatives for their ability to inhibit PDE.

Chemistry. According to the procedure of Makisumi,[7] condensation of 3-aminopyrazole (**4a**) and 3-amino-4-carbethoxypyrazole (**4b**) with acetylacetone gave 5,7-dimethylpyrazolo[1,5-*a*]pyrimidine (**5a**) and the corresponding 3-carbethoxy derivative **5b**. We have also found that a similar condensation of 3-amino-4-cyanopyrazole[8] (**4c**) and 3-amino-4-pyrazolecarboxamide[8] (**4d**) yields the 3-cyano (**5c**) and the 3-carbamoyl (**5d**) derivatives in excellent yields. The catalytic reduction of 3-cyano-5,7-dimethylpyrazolo[1,5-*a*]pyrimidine (**5c**) with palladium on charcoal catalyst affords 3-aminomethyl-5,7-dimethylpyrazolo[1,5-*a*]pyrimidine (**5e**) which was isolated as the hydrochloride salt.

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‡These authors point out that the acetylamino derivative **1b** is preferred for *in vivo* studies because of toxicological findings.