Table I. *In Vitro* Antibacterial Activity of 6′-Amino-6′-deoxygentamicin A (**6**), Gentamicin A (**5**), Gentamicin, and Kanamycin B

Test organism	Minimum inhibitory concn, μg/ml[a]			
	5	**6**	Gentamicin	Kanamycin B
Staph. aureus 209P	3.0	0.03	0.1	0.3
Staph. aureus 59N	37.5	0.8	0.1	0.03
Strept. C	>50	0.8	3–7.5	>25
Strept. 27	>50	0.8	3–7.5	7.5
Strept. group A cruz	>50	0.8	3.0	17.5
Strept. Alvarez	>50	0.03	3.0	>25
Bacillus subtilis 6623	0.8	0.01	<0.1	<0.01
Proteus mirabilis Harding	37.5	0.8	8.0	3.0
Salmonella Gr. B. *typhim*	17.5	3.0	2.0	3.0

[a] In Mueller Hinton broth (BBL) according to J. A. Waitz, E. L. Moss, Jr., C. G. Druge, and M. J. Weinstein, *Antimicrob. Ag. Chemother.*, **2**, 431 (1972).

The mass spectrum was obtained on a Varian MAT CH5 spectrometer at 70 eV with a probe temperature of 230–250°. The elemental analyses were within ±0.4%.

Tetra-*N*-benzyloxycarbonylgentamicin A (7). A solution of gentamicin A (11.7 g, 25 mmol) in water (25 ml) and methanol (25 ml) was cooled to 10°. Sodium carbonate (10.6 g) was added and benzyloxcarbonyl chloride (20.5 g, 120 mmol) was added with rapid stirring. After 3 hr at room temperature, the solid was isolated by filtration, washed with water, followed by ether, and dried. The crude product was recrystallized from hot aqueous dioxane to give 22.5 g of pure **7** (90%): mp 269–270° dec; ν_{max} (Nujol) 3300 (NH, OH), 1700, and 1540 cm^{-1} (NHCbz). *Anal.* ($C_{50}H_{60}N_4O_{18}$) C, H, N.

Penta-*O*-acetyltetra-*N*-benzyloxycarbonyl-6′-*O*-triphenylmethylgentamicin A (8). To a solution of **7** (6 g, 6 mmol) in dry pyridine (60 ml) was added recrystallized triphenylchloromethane (3.36 g, 12 mmol) and the mixture was heated on the steam bath for 4 hr with exclusion of moisture. The mixture was cooled in an ice–water bath and acetic anhydride (18 ml) added dropwise with stirring. The mixture was set aside at room temperature for 24 hr after which time it was poured into ice–water. After 1 hr the product was extracted into chloroform (500 ml); the chloroform solution was dried over sodium sulfate and concentrated to dryness. The residue was dissolved in ether (100 ml) and petroleum ether (250 ml) was added to precipitate a gum which was separated from the supernatant liquid by decantation. The gum was dissolved in 200 ml of hot 2-propanol, decolorized with active charcoal, and set aside. On the next day the crystals were isolated, washed with cold 2-propanol, and dried to give 6.3 g (81%) of pure **8**: mp 125–126°; ν_{max} (Nujol) 3300 (NH), 1760–1750 (OAc, NHCbz), 1540 cm^{-1} (NHCbz). *Anal.* ($C_{79}H_{84}N_4O_{23}$) C, H, N.

Penta-*O*-acetyltetra-*N*-benzyloxycarbonyl-6′-*O*-*p*-toluenesulfonylgentamicin A (9). To a cold solution of **3** (3.75 g, 2.5 mmol) in glacial acetic acid (10 ml) was added a 6.34 w/w % solution of hydrogen bromide in glacial acetic acid (3.83 g) dropwise with stirring. After 15 min, the precipitated trityl bromide was removed by filtration and the filtrate was poured into ice–water. The solid precipitate was collected by filtration, washed with water, and dried to give 3 g of product. The crude product was dissolved in dry pyridine (15 ml), *p*-toluenesulfonyl chloride (1.14 g) was added, and the mixture set aside for 24 hr at room temperature. The reaction mixture was poured into ice–water; the precipitated solid was collected, washed with water, and dried to give 3.2 g (91.5%) of essentially pure **9**. An analytical sample was obtained by recrystallizing the above product from hot 2-propanol (75% yield): mp 117–119°; ν_{max} (Nujol) 3325 (NH), 1700–1750 (OAc, NHCbz), 1540 (NHCbz), 1180 cm^{-1} (tosyl). *Anal.* ($C_{67}H_{76}N_4O_{25}S$) C, H, N, S.

6′-Amino-6′-deoxygentamicin A (6). A suspension of sodium azide (260 mg, 4 mmol) in dimethylformamide (10 ml) was heated at a steam bath. To the hot mixture water was added dropwise with swirling, until a clear solution was obtained. Compound **9** (2.34 g, 1.71 mmol) was then added and the solution heated on the steam bath for 12 hr. The solution was concentrated to dryness *in vacuo* and the residue partitioned between chloroform and water. The chloroform layer was dried, concentrated to a small volume, and added dropwise to stirred petroleum ether. The precipitate was collected, washed with ether, and dried. The dried product **10** (2.0 g) was dissolved in methanol (45 ml) and water (10 ml) and triethylamine (5 ml) were added. The mixture was set aside 72 hr at room temperature. The precipitated crystalline solid was isolated, washed with methanol, and dried to give 0.8 g of product. The mother liquor was concentrated to dryness and triturated with methanol to obtain a further 250 mg of product. The above product (1 g) was dissolved in dioxane (50 ml), water (15 ml), and 1 *N* hydrochloric acid (5 ml) and hydrogenated in the presence of 10% palladium on carbon (0.5 g) at 50 psi for 24 hr. The catalyst was removed by filtration through Celite and washed with water and the combined filtrate was concentrated to dryness. The crude product was chromatographed on 25 g of silica gel in a 2 × 35 cm column using chloroform–methanol–ammonium hydroxide (3:4:2) as the developing phase. Fractions (5 ml) were collected. Tubes 14–18 were pooled and concentrated to dryness to give 187 mg (40%) of pure **6**. An analytically pure sample of **6** was obtained by rechromatographing the above product on a 2 × 36 cm column of Dowex 1-X2 ion-exchange resin in the hydroxide cycle using water as the eluent. Fractions (4 ml) were collected. Tubes 8 and 9 were combined, concentrated to dryness, dissolved in a little methanol, and precipitated with ether to give 100 mg (21% yield) of pure **6**: $[\alpha]_D$ +130° (c 0.4, water). *Anal.* ($C_{18}H_{37}N_5O_9 \cdot CH_3OH$) C, H, N.

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3-Hydroxy-1-nitrophenyl-1*H*-pyrazolo[3,4-*b*]pyridines as Selective Inhibitors of Rat Liver Xanthine Oxidase†

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In an earlier communication[1] the synthesis of 3-hydroxy-1-nitrophenyl-1*H*-pyrazolo[4,3-*c*]pyridines, which were devoid of the nitrogen atom at position 7 of the allopurinol nucleus, was reported. These pyrazolopyridines were found to inhibit the activity of rat liver xanthine oxidase. With a view to study the structural requirements in the allopurinol nucleus for the inhibition of xanthine oxidase, we have synthesized some 3-hydroxy-1-nitrophenyl-1*H*-pyrazolo[3,4-*b*]pyridines which are devoid of nitrogen at position 5 of the allopurinol structure. In the present study the effect of these pyrazolopyridines was investigated on the activity of purine-catabolizing enzymes. These 3-hydroxy-1-nitrophenyl-1*H*-pyrazolo[3,4-*b*]pyri-

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Table I. Inhibition of Rat Liver Xanthine Oxidase by 3-Hydroxy-1-nitrophenyl-1*H*-pyrazolo[3,4-*b*]pyridines

Compd no.	% inhibition[a]				I_{50} value,[b] *M*	[(*I*)/(S)]$_{0.5}$[c]
	2×10^{-3} *M*	1×10^{-3} *M*	3×10^{-4} *M*	1×10^{-4} *M*		
6	>100	>100	85.29 ± 2.54	29.4 ± 1.15	2.5×10^{-4}	1.666
7	70.03 ± 2.06	47.03 ± 1.19	26.4 ± 0.98	8.25 ± 0.58	1.1×10^{-3}	7.333
8	47.13 ± 1.38	26.6 ± 1.20	12.86 ± 0.55	Nil	2.1×10^{-3}	14.00

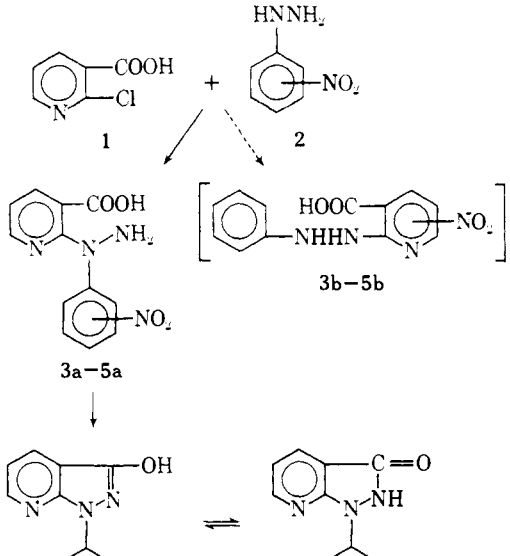
[a]All values represent the per cent inhibition with ± standard error of the mean obtained from four duplicate experiments. Molar concentrations given are the final concentrations of the test compounds used in these experiments. [b]Represents the molar concentration of the test compounds required to produce 50% inhibition of xanthine oxidase obtained graphically under the experimental conditions described in the text. [c]Represents the ratio of the concentration of the inhibitor producing 50% inhibition of xanthine oxidase activity and the concentration of the substrate (hypoxanthine) used in these experiments.

Table II. Substituted 2-[α-(Nitrophenyl)hydrazino]nicotinic Acids (**3–5**) and Substituted 3-Hydroxy-1-nitrophenyl-1*H*-pyrazolo[3,4-*b*]pyridines (**6–8**)

No.	Compound	Mp, °C	Yield, %	Molecular formula[a]
3	2-[α-(2-Nitrophenyl)hydrazino]-nicotinic acid	129	60	$C_{12}H_{10}N_4O_4$
4	2-[α-(4-Nitrophenyl)hydrazino]-nicotinic acid	265	66	$C_{12}H_{10}N_4O_4$
5	2-[α-(2,4-Dinitrophenyl)hydrazino]-nicotinic acid	190	57	$C_{12}H_9N_5O_6$
6	3-Hydroxy-1-(2-nitrophenyl)-1*H*-pyrazolo[3,4-*b*]pyridine[b]	120	50	$C_{12}H_9ClN_4O_3$
7	3-Hydroxy-1-(4-nitrophenyl)-1*H*-pyrazolo[3,4-*b*]pyridine[b]	250	53	$C_{12}H_9ClN_4O_3$
8	3-Hydroxy-1-(2,4-dinitrophenyl)-1*H*-pyrazolo[3,4-*b*]pyridine[b]	130	48	$C_{12}H_8ClN_5O_5$

[a]All compounds were analyzed for C, H, and N and analyses were within ±0.4% of the theoretical value. [b]Hydrochlorides.

Scheme I



dines were synthesized by following the methods outlined in Scheme I.

The reaction of 2-chloronicotinic acid 1 with various nitrophenylhydrazines 2 might give either the diarylamino acids **3a** or the hydrazo acids **3b**, but the product obtained could not be oxidized by treatment with mercuric oxide or with nitric acid and gave a positive Leibermann reaction (hydrazobenzene did not give this reaction). These evidences support the formation of 2-[α-(nitrophenyl)hydrazino]nicotinic acids **3a** during this reaction. The formation of these compounds was further confirmed by the presence of two characteristic bands of primary amino groups in their infrared spectra. Similar products were also identified earlier[1-3] during this type of reaction. These hydrazinonicotinic acids **3–5** on treatment with dilute HCl cyclized to yield the corresponding pyrazolopyridines **6–8**. All these pyrazolopyridines thus synthesized exhibit lactam–lactim tautomerism. The presence of a very strong peak characteristic for the carbonyl group in their infrared spectrum suggested that these compounds exist predominantly in the lactam form. These compounds were further characterized by their elemental analyses.

Results and Discussion

All these 3-hydroxy-1-nitrophenyl-1*H*-pyrazolo[3,4-*b*]pyridines were found to cause selective inhibition of rat liver xanthine oxidase. Their xanthine oxidase inhibitory property is summarized in Table I. Greater effectiveness of 3-hydroxy-1-(2-nitrophenyl)-1*H*-pyrazolo[3,4-*b*]pyridine to inhibit xanthine oxidase was observed in the present study as compared to 3-hydroxy-1-(4-nitrophenyl)-1*H*-pyrazolo[3,4-*b*]pyridine and 3-hydroxy-1-(2,4-dinitrophenyl)-1*H*-pyrazolo[3,4-*b*]pyridine. A similar degree of inhibition by these pyrazolopyridines was reflected by the I_{50} values of these compounds which were found to be 2.5×10^{-4}, 1.1×10^{-3}, and 2.1×10^{-3} *M* for the compounds possessing the 2-nitrophenyl, 4-nitrophenyl, and 2,4-dinitrophenyl moiety, respectively. The ratio of the inhibitory effectiveness of these pyrazolopyridines as calculated from the ratio of the concentration of the inhibitor exhibiting 50% inhibition (I_{50} values) to the concentration of the substrate was found to be 1.666, 7.333, and 14.00, respectively (Table I). In the present study no inhibition was observed in the activity of rat liver adenosine deaminase, guanosine deaminase, and guanine deaminase by these pyrazolopyridines when used at a concentration of 5×10^{-3} *M*.

Experimental Section

Analyses for carbon, hydrogen, and nitrogen were performed Melting points were taken in open capillary tubes and are uncorrected. Infrared spectra were obtained by using Perkin-Elmer spectrophotometer Model 137.

Nicotinic Acid 1-Oxide. Nicotinic acid 1-oxide was prepared by the oxidation of nicotinic acid with hydrogen peroxide (30%) and glacial acetic acid: mp 260–261° dec (lit.[4] mp 254–255°).

2-Chloronicotinic Acid. Nicotinic acid 1-oxide on treatment with phosphorus oxychloride gave 2-chloronicotinic acid: mp 174–178° dec.[4]

2-[α-(Nitrophenyl)hydrazino]nicotinic Acids (3–5). A mixture of 2-chloronicotinic acid (1, 7 mmol) and appropriate nitrophenylhydrazine (2, 7 mmol) in 15 ml of absolute ethanol was refluxed on a steam bath for 8 hr. The solid mass which separated out on removing the excess of ethanol under reduced pressure and on cooling was collected by filtration, washed with water, and recrystallized from ethanol. These hydrazinonicotinic acids were characterized by their sharp melting points and elemental analyses (Table II).

3-Hydroxy-1-nitrophenyl-1*H*-pyrazolo[3,4-*b*]pyridines (6–8). An appropriate 2-[α-(nitrophenyl)hydrazino]nicotinic acid (2 mmol) was added to 15 ml of water containing 1.5 ml of concentrated hydrochloric acid and the mixture was refluxed on a sand bath for 6 hr. The reaction mixture was cooled, filtered, and concentrated. The crude product which separated out was collected by filtration and recrystallized from dilute hydrochloric acid. The pyrazolopyridines thus synthesized were characterized by their sharp melting points and elemental analyses (Table II).

Biochemical Studies. Normal healthy albino rats (100–150 g) kept on *ad libitum* diet were sacrificed by decapitation. Livers were immediately removed and homogenized in 0.25 *M* ice-cold sucrose (1:9 ratio) in a Potter-Elvehjem homogenizer. The homogenates were centrifuged at 100,000g for 30 min and the clear supernatant (nonparticulate or soluble fraction) thus obtained was used for the assay of the purine-catabolizing enzymes. Adenosine deaminase activity was determined by the estimation of the disappearance of adenosine at 265 nm. Guanosine deaminase activity was determined by estimation of the disappearance of guanosine at 245 nm and guanine deaminase activity was determined by the estimation of the disappearance of guanine at 245 nm. Xanthine oxidase activity was determined by the estimation of uric acid formed from the oxidation of hypoxanthine at 290 nm. Details of the assay procedure are as reported earlier.[1]

Protein Estimation. Protein estimations were carried out by following the method of Lowry, *et al.*,[5] using bovine serum albumin as the standard. Readings were taken at 750 nm.

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Cycloserine Carbamates

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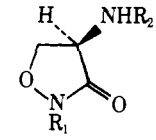
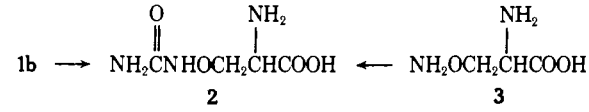
Cycloserine[1] (**1a**) is a broad-spectrum antibiotic which is known to inhibit cell wall synthesis in certain bacteria. It is a cyclic hydroxylamine which upon carbamoylation forms a derivative of *N*-hydroxyurea (HU), a known[2] anticancer agent. β-Aminooxy-D-alanine, the hydrolysis product of **1a**, and cycloserine dimer **6** are both *O*-alkylhydroxylamines which upon carbamolyation lead to HU derivatives which might eliminate molecular HU *in vivo*. It was, consequently, of interest to prepare these carbamoyl compounds and several others for screening by the National Cancer Institute.



1a, $R_1 = H$; $R_2 = H$ — **d**, $R_1 = H$; $R_2 = CONH_2$
b, $R_1 = CONH_2$; $R_2 = H$ — **e**, $R_1 = CONH_2$; $R_2 = Z$
c, $R_1 = CONH_2$; $R_2 = CONH_2$ — **f**, $R_1 = Z$; $R_2 = CONH_2$

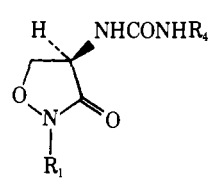
Z = carbobenzyloxy

Our earlier work[3] on carbobenzyloxy (Z) derivatives of **1a** allowed us to prepare the appropriately blocked derivatives so that carbamoylation would lead to **1e** and **1f**. Deblocking of **1e** and **1f** with HBr–HOAc and HF, respectively, afforded the desired products. The carbamoylations were carried out using potassium cyanate in aqueous alcoholic solution at pH 4 and were routinely screened of salts (KCl) by ion-exchange chromatography. Control of the pH during the reaction was crucial, since both **1b** and **1d** were sensitive to low pH and **1b** was destroyed at pH >5. The 2-carbamoyl derivative **1b** underwent very rapid ($t_{1/2} \sim$ 40 min) hydrolysis even in the cold at pH <4 to form β-ureidooxy-D-alanine (**2**). This hydroxyurea derivative was

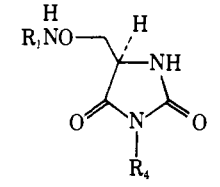


1b ⟶ $NH_2CNHOCH_2CHCOOH$ (**2**) ⟵ $NH_2OCH_2CHCOOH$ (**3**)

prepared more conveniently by direct carbamoylation of β-aminooxy-D-alanine[4] (**3**) at pH 5. The urea **1d**, which was nitroprusside† positive, was also converted into another compound at pH <4 as shown by the rapid formation of another spot (nitroprusside red) on thin-layer chromatography (tlc). Earlier workers[6] had shown that the carbamate **1g** was readily converted by HCl into the hydantoin **4b**; consequently, we expected that **1d** might form the unsubstituted hydantoin **4a** under acidic conditions with loss of the nitroprusside (blue) sensitive isoxazolidone ring.



1d, $R_1 = H$; $R_4 = H$
g, $R_1 = H$; $R_4 = C_6H_5$



4a, $R_1 = H$; $R_4 = H$
b, $R_1 = H$; $R_4 = C_6H_5$
c, $R_1 = CONH_2$; $R_4 = H$

At pH >5, **1b** dimerized to give the dicarbamoylcycloserine dimer **5**. This compound, another hydroxyurea derivative, was prepared very readily by direct carbamoylation of cycloserine dimer[7] **6**. The dimerization of **1b** is analogous to the spontaneous dimerization of 2-carbobenzyloxy-D-cycloserine previously observed in these laboratories.[8]

†Both **1b** and **1c** also give a blue nitroprusside test.[5] These ring carbamylated derivatives are the only 2-substituted cycloserines so far encountered which react with this reagent.