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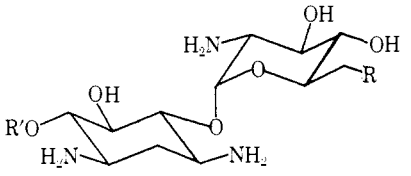
Synthesis and Biological Properties of 6′-Amino-6′-deoxygentamicin A

Tattanahalli L. Nagabhushan* and Peter J. L. Daniels

Schering Corporation, Bloomfield, New Jersey 07003.
Received February 21, 1974

Structure-activity relationship studies on aminoglycoside antibiotics possessing a paromamine moiety have shown that replacement of the 6′ primary hydroxyl group by an amino group leads to compounds with enhanced antibacterial properties. For example, neamine (1) is biologically more active than paromamine (**2**)[1] and kanamycin B (**3**) is more potent than kanamycin C (**4**).[2] It was of interest, therefore, to convert the relatively weakly active gentamicin A (**5**)[3] to its 6′-amino-6′-deoxy derivative **6** to examine its biological properties.



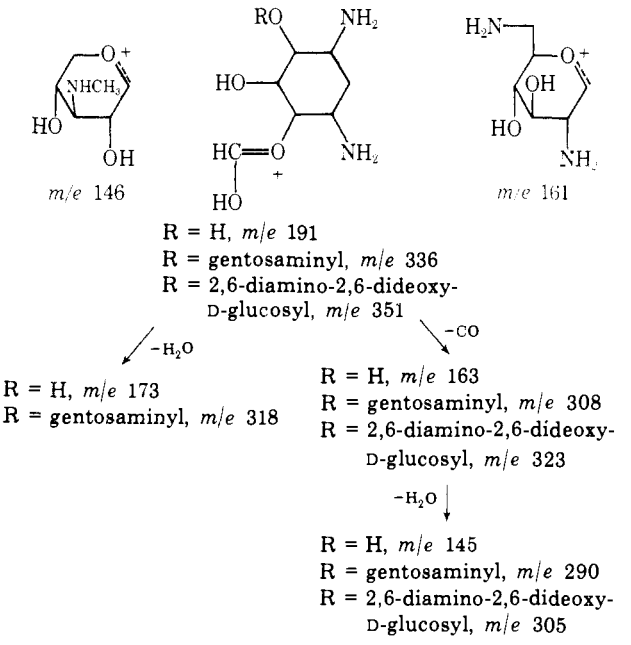
1. R = NH_2; R′ = H
2. R = OH; R′ = H
3. R = NH_2; R′ = 3-amino-3-deoxy-α-D-glucopyranosyl
4. R = OH; R′ = 3-amino-3-deoxy-α-D-glucopyranosyl
5. R = OH; R′ = 3-methylamino-3-deoxy-α-D-xylopyranosyl
6. R = NH_2; R′ = 3-methylamino-3-deoxy-α-D-xylopyranosyl

The synthesis of 6′-amino-6′-deoxygentamicin A (**6**) was accomplished by standard procedures as follows. Gentamicin A (**5**) was converted to tetra-*N*-benzyloxycarbonylgentamicin A (**7**) and then to the corresponding penta-*O*-acetyl-6′-*O*-triphenylmethyl derivative **8** in a overall yield of 73%. The triphenylmethyl group was removed with acid and the *p*-toluenesulfonyl group introduced to give penta-*O*-acetyltetra-*N*-benzyloxycarbonyl-6′-*O*-*p*-toluenesulfonylgentamicin A (**9**) in 91% yield. The *p*-toluenesulfonyl group was displaced by azide ion in near quantitative yield and the resulting compound **10** subjected to O-deacetylation (70% yield) followed by catalytic hydrogenation to give crude 6′-amino-6′-deoxygentamicin A (**6**). The compound thus obtained was purified by column chromatography on silica gel using chloroform–methanol–ammonium hydroxide (3:4:2) as the developing phase (40% yield) followed by rechromatography on Dowex 1-X2 in the hydroxyl cycle using water as the eluent to provide an analytically pure sample in 21% yield. The structure of **6** was established as follows.

Elemental analysis of **6** was consistent with the formula $C_{18}H_{37}N_5O_4 \cdot CH_3OH$ and the mass spectrum showed the $(MH)^+$ ion at *m/e* 468 in agreement with the composition. Prominent fragment ions in the mass spectrum[4] are shown in Scheme I.

The pmr spectrum of **6** showed the *N*-methyl protons at

Scheme I. Prominent Mass Spectral Fragment Ions of 6′-Amino-6′-deoxygentamicin A (6)



δ 2.5 ppm as a sharp singlet and the two anomeric signals at δ 5.33 and 5.05 ppm, each with a coupling constant of 3.5 Hz. The low-field signal is assigned to H-1′ and the doublet at δ 5.05 ppm to H-1″ by analogy with the assignments made by Lemieux and coworkers[5] for structurally closely related kanamycin B. Furthermore, the pmr spectrum of gentamicin A (**5**) was similar to that of **6** and exhibited two doublets at δ 5.22 and 5.02 ppm with coupling constants of 3.5 and 3.75 Hz, respectively. The doublet at δ 5.22 ppm was attributed to H-1′ by the INDOR response of the H-2′ resonances (quartet at δ 2.75 ppm in the normal spectrum, $J_{2',3'}$ = 9.0 Hz) obtained by monitoring the left-hand peak of the anomeric doublet centered at δ 5.22 ppm. Similarly, the doublet at δ 5.02 ppm could be assigned to H-1″ (quartet at δ 3.60 ppm for H-2″, $J_{2'',3''}$ = 9.0 Hz in the normal spectrum).

The biological properties of gentamicin A (**5**) have been reported.[3] Although both **5** and its 6′-amino-6′-deoxy congener **6** have some broad spectrum activity, neither has potent inhibitory activity *vs. Escherichia coli, Klebsiella pneumoniae*, or *Pseudomonas aeruginosa*. In particular, neither compound has activity against strains carrying gentamicin adenylylating or acetylating *R* factors or against strains carrying kanamycin phosphorylating *R* factors. However, as shown in Table I, the *in vitro* biological activity of **6** against some sensitive strain is superior to that of **5**, gentamicin C complex (Garamycin), and the structurally related kanamycin B. The most striking property of **6** is its potent *in vitro* inhibitory activity against *Staphylococcus aureus* and *Streptococcus pyogenes*.

Experimental Section

Thin-layer chromatography was performed on silica gel GF (Analtech, Inc., Newark, Del.) using chloroform–methanol–ammonium hydroxide (3:4:2) as the developing phase. Column chromatography was carried out on silica gel (60–200 mesh, J. T. Baker Chemical Co., Phillipsburg, N. J.) using the same solvent system and on Dowex 1-X2 (200–400 mesh, hydroxide form, Sigma Chemical Co., St. Louis, Mo.) with water as the eluent.

The pmr spectra were recorded using a Varian Associates XL-100 nmr spectrometer. Chemical shifts are given in δ values for solution in deuterium oxide using DSS as the internal standard.

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.

Acknowledgments. The authors wish to thank Dr. A. J. Waitz and Mr. E. L. Moss, Jr., of our Microbiology Department for the biological data and Messrs. J. Morton, J. McGlotten, and P. Bartner for spectral determinations.

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

Surendra S. Parmar,* B. R. Pandey, C. Dwivedi, and Basheer Ali

Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow-3, India, and Department of Physiology and Pharmacology, University of North Dakota School of Medicine, Grand Forks, North Dakota 58201.
Received December 13, 1973

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-

† This investigation was supported by a research grant from the Council of Scientific and Industrial Research, New Delhi, India, and the U. S. Public Health Service NIH Grants 1 T01 HL 05939-01A1 and 5 S01 RRO 5407.
* Address correspondence to this author at the University of North Dakota.