THE SYNTHESES OF 1-*N*-[(*S*)-4-AMINO-2-HYDROXYBUTYRYL]GENTAMICIN C_1 AND 1-*N*-[(*S*)-3-AMINO-2-HYDROXYPROPIONYL]GENTAMICIN C_1

Sir:

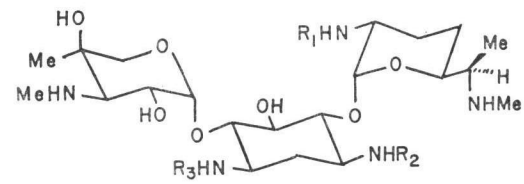
The naturally occurring aminoglycoside antibiotic butirosin has been shown to possess an *N*-(*S*)-4-amino-2-hydroxybutyryl [AHB] substituent[1] which is uniquely associated with the activity of this antibiotic against species of *Pseudomonas aeruginosa*. KAWAGUCHI and coworkers[2] synthesised an analogous derivative of kanamycin A, 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] kanamycin A (amikacin), which not only possessed anti-*Pseudomonas* activity not shown by the parent antibiotic, but also exhibited marked inhibition of organisms resistant to kanamycin A and other aminoglycoside antibiotics.[2,3] Since this time analogous derivatives of a number of other aminoglycosides have been reported, for example, of 3′,4′-dideoxykanamycin B.[4]

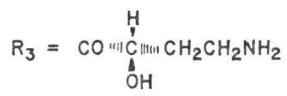
In an investigation of structure-activity relationships in the butirosin series, HASKELL and coworkers[5] prepared semisynthetic analogs in which the structure of the aminohydroxyacyl side chain was varied. Of these compounds only the lower homolog, *i.e.* the 1-*N*-3-amino-2-hydroxypropionyl [AHP] derivative, showed activity comparable to butirosin *in vitro*. In this communication we report the synthesis and *in vitro* antibacterial activity of 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]gentamicin C_1 [AHB-C_1] (**1**) and 1-*N*-[(*S*)-3-amino-2-hydroxypropionyl]gentamicin C_1 [AHP-C_1] (**2**).*

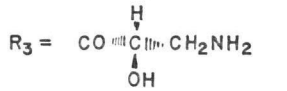
The approach taken was similar to that used by previous workers[2,4] involving sequential selective blocking of amino groups; however, we have found it convenient to use trifluoroacetyl (TFA) protecting groups, introduced using ethyl trifluorothiolacetate. The order of reactivity of the amino functions of gentamicin C_1 (**3**) to this reagent was 2′>3>1>6′ and 3″.

Treatment of a methanolic solution of **3** with one equivalent of ethyl trifluorothiolacetate at room temperature, followed by isolation of the major product by chromatography over silica gel, eluting with the lower phase of a 2:1:1 chloroform-methanol-3 N ammonium hydroxide solvent system, gave 2′-*N*-trifluoroacetylgentamicin C_1 (**4**), $C_{23}H_{42}N_5O_8F_3 \cdot H_2O$†, m.p. 108~111°C, $[\alpha]_D^{23}$ +128° (*c* 0.4, H_2O), in 69% yield. The location of the TFA group was indicated by mass spectrometry.** The molecular ion and $[M+1]^+$ peaks of **4** appeared at *m/e* 573 and 574 with intense ions at *m/e* 160, corresponding to an unsubstituted garosamine[6] fragment, *m/e* 253, consistent with a monotrifluoroacetyl purpurosamine A[6] fragment and *m/e* 191, 173, 163 and 145 indicating

Scheme 1.



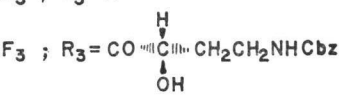
1 $R_1 = R_2 = H$; $R_3 =$ 

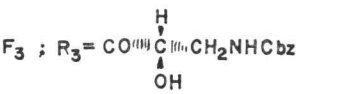
2 $R_1 = R_2 = H$; $R_3 =$ 

3 $R_1 = R_2 = R_3 = H$

4 $R_1 = COCF_3$; $R_2 = R_3 = H$

5 $R_1 = R_2 = COCF_3$; $R_3 = H$

9 $R_1 = R_2 = COCF_3$; $R_3 =$ 

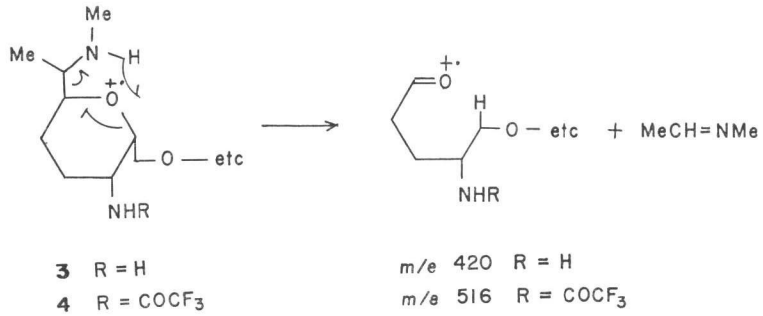
10 $R_1 = R_2 = COCF_3$; $R_3 =$ 

* Since this work was completed, compound **1** was reported in a patent (U.S. Patent 3,780,018, December 18, 1973). The preparation described therein involved reaction of gentamicin C_1 directly without protection of the 2′ and 3 amino groups. Also since this work was completed, the preparation of 1-*N*-3-amino-2-hydroxypropionyl derivatives of some kanamycins has been reported (KONDO, S.; K. IINUMA, M. HAMADA, K. MAEDA & H. UMEZAWA: J. Antibiotics 27: 90~93, 1974).

† Satisfactory elemental analyses were obtained for all compounds indicated by molecular formulae as shown.

** Mass spectra were obtained using a Varian MAT CH5 spectrometer at 70 eV with a probe temperature of 200~250°C. The direct inlet technique was used.

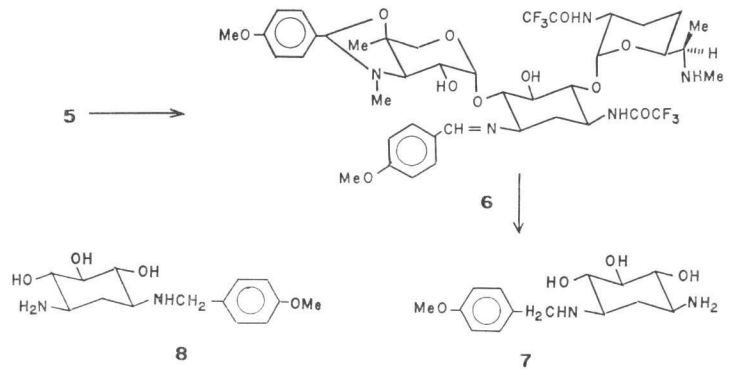
Scheme 1.



3 R = H
4 R = $COCF_3$

m/e 420 R = H
m/e 516 R = $COCF_3$

Scheme 2.

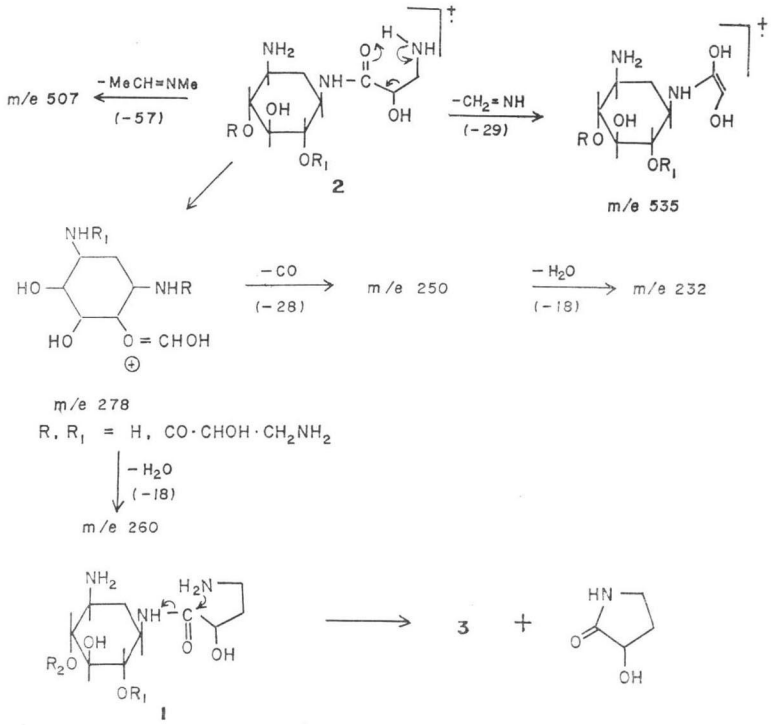


an unsubstituted 2-deoxystreptamine fragment.[7] A relatively intense $[M-57]^+$ ion at *m/e* 516 was consistent with loss of the purpurosamine A side chain, possibly as shown in Scheme 1, indicating the location of the TFA group at *N*-2′. An analogous ion is observed at *m/e* 420 in the mass spectrum of gentamicin C_1, and its composition in this latter case was confirmed by high resolution measurements. Other ions in the mass spectrum of **4**, including the expected series of pseudo-disaccharide ions, were consistent with the assigned structure.

Treatment of **4** with one equivalent of ethyl trifluorothiolacetate, followed by chromatography gave, in 42 % yield, 2′,3-di-*N*-trifluoroacetylgentamicin C_1 (**5**), $C_{25}H_{41}N_5O_9F_6 \cdot H_2O$, m.p. 121～129°C, $[\alpha]_D^{26}+121°$ (*c* 0.3, H_2O). The second TFA group in **5** was located on the 2-deoxystreptamine moiety by mass spectrometry. The series of ions identified with the 2-deoxystreptamine residue were shifted by 96 mass units appearing at *m/e* 287, 269, 259 and 241, whereas the monosaccharide ions were unshifted at *m/e* 160 and 253. The molecular ion and $[M+1]^+$ peak appeared at *m/e* 669 and 670 and the $[M-MeCH=NMe]^{+\cdot}$ ion at *m/e* 612. Differentiation between 1-*N* and 3-*N* substitution was made on the basis of experiments outlined in Scheme 2. Condensation of **5** with *p*-methoxybenzaldehyde gave the bis-*p*-methoxybenzylidene derivative **6** ($M^{+\cdot}$ at *m/e* 905) which was reduced with sodium borohydride, hydrolysed with 6 N hydrochloric acid and the product chromatographed to give a 74 % yield of 2-deoxy-1-*N*-*p*-methoxybenzyl-D-streptamine (**7**), $[\alpha]_D^{26}-44.6°$ (*c* 0.5, H_2O). This compound was identical spectroscopically and by chromatographic criteria with an authentic sample of the enantiomeric 3-*N*-*p*-methoxybenzyl derivative **8**,[2] $[\alpha]_D+44°$ (*c* 0.3, H_2O), but had the opposite sign of optical rotation.* Compound **5** was also prepared directly from **3** in 34 % yield by reaction with two equivalents of ethyl trifluorothiolacetate. Condensation of **5** with one equivalent of ethyl trifluorothiolacetate resulted in reaction at *N*-1 affording 1,3,2′-tri-*N*-trifluoroacetylgentamicin C_1, $C_{27}H_{40}N_5O_{10}F_9 \cdot H_2O$, m.p. 147 ～ 151°C, $[\alpha]_D^{26}+114°$ (*c* 0.38, H_2O), mass spectral peaks at *m/e* 160, 253 (intense), *m/e* 383, 365, 355,

* We thank Dr. I. R. HOOPER of Bristol Laboratories, Syracuse, N.Y. for an authentic sample of this material.

Scheme 3.



337 (weak).

Condensation of **5** with 1.2 equivalents of *N*-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyryloxy]succinimide[2] at room temperature *in* tetrahydrofuran solution afforded, after purification by chromatography over silica gel, the 1-*N*-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyryl] derivative **9**, $C_{37}H_{54}N_6O_{13}F_6$, m.p. 115~120°C, $[\alpha]_D^{26}$+80° (*c* 0.35, MeOH) in 51% yield. Removal of the trifluoroacetyl groups was effected by stirring **9** with 5 N ammonium hydroxide in aqueous methanol at room temperature for 3 days with TLC monitoring. The resulting product was hydrogenated in acetic acid over a 10% palladium on carbon catalyst to remove the carbobenzyloxy group affording the desired product. Purification was effected by chromatography over silica gel, eluting with the lower phase of a 1:1:1 chloroform-methanol-conc. ammonium hydroxide solvent system affording 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] gentamicin C_1 (**1**), $C_{25}H_{50}N_6O_9 \cdot 2H_2O$, in 78% yield from **9**. Data for **1** were as follows: m.p. 103~110°C, $[\alpha]_D^{26}$ +101° (*c* 0.45, H_2O), PMR (100 MHz, D_2O) δ1.01 (3H, d, J=7 Hz, $\underline{CH_3}$-CH), 1.17 (3H, s, $\underline{CH_3}$-C), 2.32 (3H, s, $\underline{CH_3}$-N), 2.47 (3H, s, $\underline{CH_3}$-N), 4.19 (1H, t, J=4.2 Hz, H-2‴), 5.08 (1H, d, J=4.5 Hz, H-1″), 5.13 (1H, d, J=4 Hz, H-1′) ppm. Irradiation at δ 1.73 ppm collapsed the triplet at δ 4.19 ppm to a singlet. The structure of **1** was confirmed by the method of KAWAGUCHI[2]. Condensation of **1** with *p*-methoxybenzaldehyde afforded the tetra-*N*-*p*-methoxybenzylidene derivative, which was reduced with sodium borohydride, and hydrolysed with acid affording 2-deoxy-3-*N*-*p*-methoxybenzyl-D-streptamine (**8**).

In a similar manner, condensation of **5** with *N*-[(*S*)-3-benzyloxycarbonylamino-2-hydroxypropionyloxy]succinimide afforded the 1-*N*-substituted compound **10**, $C_{36}H_{52}N_6O_{13}F_6 \cdot H_2O$, m.p. 125~131°C, $[\alpha]_D^{26}$+93° (*c* 0.34, MeOH). Removal of the protecting groups as described previously gave 1-*N*-[(*S*)-3-amino-2-hydroxypropionyl]gentamicin C_1 (**2**), $C_{24}H_{48}N_6O_9 \cdot H_2O$, m.p. 109~119°C, $[\alpha]_D^{26}$+98° (*c* 0.27, H_2O), PMR (100 MHz, D_2O) δ 1.10 (3H, d, J=7 Hz, $\underline{CH_3}$-CH), 1.19 (3H, s, $\underline{CH_3}$-C), 2.41 (3H, s, $\underline{CH_3}$-N), 2.49 (3H, s, $\underline{CH_3}$-N), *ca* 4.16 (2H, overlapping signals. H-2‴ and H-5‴$_q$), 5.08 (1H, d, J=4 Hz, H-1″), 5.18 (1H, d, J=3.5

Table 1. *In vitro* antibacterial activities of 1-*N*-[(*S*)]-4-amino-2-hydroxybutyryl]gentamicin C_1 (AHB-C_1), 1-*N*-[(*S*)-3-amino-2-hydroxypropionyl]gentamicin C_1 (AHP-C_1) and gentamicin C_1 (C_1)

Test organism	Resistance mechanism	Minimal inhibitor concentration (mcg/ml)*		
		C_1	AHB-C_1	AHP-C_1
S. aureus FDA 209 P		0.03	0.3	0.3
〃 Wood		0.03	0.3	0.3
S. pyogenes C		3.0	>25	17.5
〃 27		3.0	7.5	>25
〃 A Alvarez		0.75	17.5	—
E. coli LA290/R55	2″-*O*-adenylylating	>25	0.3	0.75
〃 W677/R55	〃	>25	0.75	0.3
〃 JR88	3-*N*-acetylating	>25	0.75	0.75
〃 Baker 2		0.75	0.75	3.0
〃 F 14-BK		0.75	0.75	0.75
〃 St. M. 589		3.0	3.0	0.75
〃 R5/W677	6′-*N*-acetylating	0.3	0.3	0.3
P. aeruginosa D2		3.0	17.5	17.5
〃 NRRL 3223		0.75	3.0	3.0
〃 1395		0.75	7.5	3.0
〃 Travers	3-*N*-acetylating	>25	7.5	7.5
〃 Stone 138	〃	>25	7.5	3.0
〃 Shriners 10099	Unknown	>25	>25	>25
〃 GN 315	6′-*N*-acetylating	3.0	3.0	3.0
K. pneumoniae Ad 17		0.3	0.3	0.075
〃 Ad 18		0.3	0.3	0.3
〃 Georgetown	2″-*O*-adenylylating	>25	0.75	0.3
Providence 164	2′*N*-acetylating	>25	>25	>25
P. mirabilis Harding		3.0	0.3	0.75
P. rettgeri Membel		3.0	3.0	7.5
B. subtilis 6623		0.03	0.03	0.03

* In MUELLER-HINTON broth pH 7.2.

Hz, H-1′) ppm. The mass spectrum of **2** gave relatively intense ions at *m/e* 565 $(M+1)^+$, 535 and 507; an intense series of ions at *m/e* 278, 260, 250 and 232 corresponded to the normal series of deoxystreptamine ions[7] displaced to higher mass by the additional substituent (see Scheme 3). The mass spectrum of **1** on the other hand was essentially the same as that of the parent gentamicin C_1 except for a weak ion at *m/e* 560 $[M-H_2O]^+$. These date suggest that compound **1** undergoes thermal elimination of the side chain in the mass spectrometer by the cyclic mechanism shown (Scheme 3). Such a mechanism would not be easily available to the lower homolog **2**.

The *in vitro* antibacterial activities of **1** [AHB-C_1] and **2** [AHP-C_1] against some representative organisms are shown in the Table in comparison to gentamicin C_1. In general, **1** and **2** have excellent activity and are potent inhibitors of organisms which inactivate gentamicin C_1 by 2″-*O*-adenylylation and 3-*N*-acetylation. They are however inactive against a strain of *Providence* which inactivates gentamicin by 2′-*N*-acetylation.[8] **1** and **2** have only weak activity against *S. pyogenes* strains and are relatively weak against gentamicin-sensitive *Pseudomonas* compared to gentamicin itself.

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References

1) WOO, P.W. K.; H.W. DION & Q. R. BARTZ: Butirosins A and B, aminoglycoside antibiotics. I. Structural units. Tetrahedron Letters 1971: 2617~2620, 1971
2) KAWAGUCHI, H.; T. NAITO, S. NAKAGAWA & K. FUJISAWA: BB-K8, a new semisynthetic aminoglycoside antibiotic. J. Antibiotics 25: 695~708, 1972
3) PRICE, K. E.; D. R. CHISHOLM, M. MISIEK, F. LEITNER & Y. H. TSAI: Microbiological evaluation of BB-K8, a new semisynthetic aminoglycoside. J. Antibiotics 25: 709~731, 1972
4) KONDO, S.; K. IINUMA, H. YAMAMOTO, K. MAEDA & H. UMEZAWA: Syntheses of 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-kanamycin B and -3′,4′-dideoxykanamycin B active against kanamycin-resistant bacteria. J. Antibiotics 26: 412~415, 1973
5) HASKELL, T. H.; R. RODEBAUGH, N. PLESSAS, D. WATSON & R. D. WESTLAND: The preparation & biological activity of novel amino acid analogs of butirosin. Carbohydrate Res. 28: 263~280, 1973
6) COOPER, D. J.; M. D. YUDIS, H. M. MARIGLIANO & T. TRAUBEL: The gentamicin antibiotics. II. Separation and degradation of the gentamicin C components. The purpurosamines, a new class of naturally occurring 2,6-diaminomonosaccharides. J. Chem. Soc. (C) 1971: 2876~2879, 1971
7) DANIELS, P. J. L.; M. KUGELMAN, A. K. MALLAMS, R. TKACH, H. F. VERNAY, J. WEINSTEIN & A. YEHASKEL: Mass spectral studies on aminocyclitol antibiotics. Chem. Comm. 1971: 1629~1631, 1971
8) CHEVEREAU, M.; P. J. L. DANIELS, J. DAVIES & F. LEGOFFIC: Aminoglycoside resistance in bacteria mediated by gentamicin acetyltransferase II, an enzyme modifying the 2′-amino group of aminoglycoside antibiotics. Biochemistry 13: 598~603, 1974