THE CHEMICAL CONVERSION OF GENTAMINE C_{1a} INTO GENTAMINE C_2 AND ITS 6′-EPIMER

Sir:

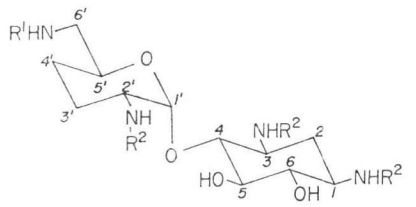
Gentamine C_2, a biologically active pseudo-disaccharide, was obtained by methanolysis of gentamicin C_2[1] and its absolute structure was determined by DANIELS[2,3]. We studied a synthetic route to 6′-C-alkyl derivatives of gentamicins and kanamycins which were active against resistant bacteria producing aminoglycoside 6′-N-acetyltransferase. In this study, we synthesized gentamine C_2 and its 6′-epimer from gentamine C_{1a} (3′,4′-dideoxyneamine) through the 5′-deaminomethyl-5′-C-formyl derivative which is a useful intermediate for the 6′-modification of aminoglycoside antibiotics.

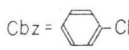
The 6′-amino group of 3′,4′-dideoxyneamine (**1**) which was synthesized by UMEZAWA *et al.*[4,5] was selectively acylated with an equimolar amount of benzyl S-4,6-dimethylpyrimid-2-ylthiocarbonate in aqueous dioxane at room temperature for 3 hours to afford 6′-N-benzyloxycarbonyl-3′,4′-dideoxyneamine monohydrate (**2**, 35.1% yield), mp 88~92°C (decomp.), $[\alpha]_D^{24}$ +73° (*c* 1, water).

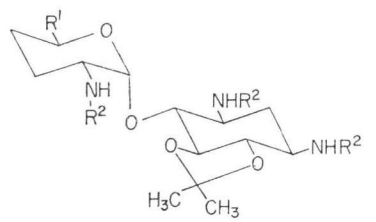
The other three amino groups of **2** were protected by reaction with *tert*-butyl S-4,6-dimethylpyrimid-2-ylthiocarbonate in aqueous dioxane at room temperature for 22.5 hours to yield 6′-N-benzyloxycarbonyl-1,3,2′-tri-N-*tert*-butoxycarbonyl derivative (**3**, 87.3% yield), $[\alpha]_D^{25}$ +35° (*c* 1, methanol). Treatment of **3** with 2,2-dimethoxypropane in anhydrous N,N-dimethylformamide in the presence of *p*-toluenesulfonic acid at 61°C for 30 minutes followed by silicic acid column chromatography developed with chloroform gave 6′-N-benzyloxycarbonyl-1,3,2′-tri-N-*tert*-butoxycarbonyl-5,6-O-isopropylidene-3′,4′-dideoxyneamine (**4**, 92.6% yield), $[\alpha]_D^{26}$ +30° (*c* 1, methanol), *m/e* 765 $(M+1)^+$. The N-benzyloxycarbonyl group in **4** was removed by catalytic hydrogenation with 5% palladium-barium carbonate in 80% aqueous ethanol under atmospheric pressure for 5 hours to give the 1,3,2′-tri-N-*tert*-butoxycarbonyl-5,6-O-isopropylidene derivative (**5**, 95.4% yield), *m/e* 631 $(M+1)^+$.

Two synthetic methods for conversion of the aminomethyl group at the 5′-position into the formyl group were designed. According to the method of DINIZO and WATT[6], SCHIFF base formation of **5** with 2-pyridinecarboxaldehyde followed by treatment with *m*-chloroperbenzoic acid afforded an oxaziran (**6**, 26.1%), *m/e* 735 M^+. Hydrolysis of the oxaziran ring with potassium hydroxide in an aqueous acetone solution containing N,N-dimethylformamide followed by silicic acid column chromatography (benzene-methyl ethyl ketone, 3: 1) gave 1,3,2′-tri-N-*tert*-butoxycarbonyl-5′-deaminomethyl-5′-C-formyl-5,6-O-isopropylidene-3′,4′-dideoxyneamine (**7**, 47.9%), mp 127~132°C (decomp.), $[\alpha]_D^{26}$ +42.5° (*c* 1, methanol), PMR ($CDCl_3$): δ 9.64 (s, CHO).

Another approach by ninhydrin oxidation[7] of **5** gave **7** in a good yield. Oxidation of an aminomethyl group in **5** with ninhydrin and sodium hydrogencarbonate in a heterogeneous mixture of chloroform and water at room temperature for 46.5 hours followed by silicic acid column chromatography (chloroform - methanol, 100: 1) afforded **7** (69.8%).



Cbz =  , Boc = $(CH_3)_3COCO-$



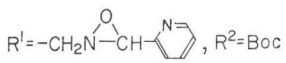
1 R^1, R^2=H

2 R^1=Cbz, R^2=H

3 R^1=Cbz, R^2=Boc

4 R^1=$-CH_2NHCbz$, R^2=Boc

5 R^1=$-CH_2NH_2$, R^2=Boc

6 R^1=  , R^2=Boc

7 R^1=$-CHO$, R^2=Boc

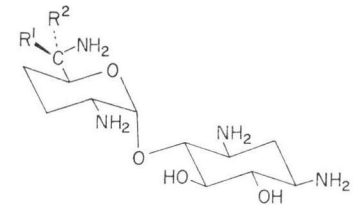
8 R^1=$-COCH_3$, R^2=Boc

9a R^1=$-CH(NH_2)CH_3$ (S), R^2=Boc

9b R^1=$-CH(NH_2)CH_3$ (R), R^2=Boc

Treatment of **7** in benzene with an excess of ethereal diazomethane overnight at room temperature followed by silicic acid column chromatography (benzene - methyl ethyl ketone, 3: 1) afforded the 5′-deaminomethyl-5′-C-ethanoyl derivative (**8**, 32.1% yield), PMR($CDCl_3$): δ 2.19 (s, $COCH_3$). Reductive amination of **8** in anhydrous methanol with ammonium acetate and sodium cyanoborohydride followed by silicic acid column chromatography (chloroform - methanol - 17% aqueous ammonia, 120: 10: 1) afforded two diastereomers: the 6′(*S*)-C-methyl derivative (**9a**, 18.5%) and the 6′(*R*)-C-methyl derivative (**9b**, 42.0%). Thin-layer chromatography of **9a** and **9b** on Silica gel G with chloroform - methanol - 17% aqueous ammonia (80: 10: 1) showed Rf 0.53 and 0.43, respectively. The N-*tert*-butoxycarbonyl groups and O-isopropylidene group in **9a** were removed in 90% trifluoroacetic acid at room temperature for 45 minutes to afford 6′(*S*)-C-methyl-3′,4′-dideoxyneamine as a dicarbonate (**10a**, 72.8% yield), which was purified by column chromatography on Amberlite CG-50 (NH_4^+) resin eluted with 0.4 N ammonia, mp 140~154°C (decomp.), $[\alpha]_D^{22}$ +54° (*c* 0.63, water), PMR (D_2O, pD 4.2): δ 1.83 (d J=7 Hz, CH_3), 6.33 (d J=3.5 Hz, anomeric). The treatment of **9b** with 90% trifluoroacetic acid followed by column chromatography on Amberlite CG-50 (NH_4^+) afforded 6′(*R*)-C-methyl-3′,4′-dideoxyneamine as a dicarbonate (**10b**, 86.9% yield) which was identical with gentamine C_2[2] in all respects.

Thin-layer chromatography of **10a** and **10b** on cellulose plates (Avicel) with butanol - ethanol - chloroform - 17% aqueous ammonia (4: 5: 2: 5) showed Rf 0.55 and 0.52, respectively. As shown in Table 1, CMR spectra of the synthetic gentamine C_2 (**10b**) and its 6′(*S*)-epimer (**10a**) at pD 4.2 are different in chemical shifts



10a R^1=H, R^2=CH_3
10b R^1=CH_3, R^2=H

Table 1. C-13 Chemical shifts of the synthetic gentamine C_2 (**10b**) and its 6′(*S*)-epimer (**10a**).

Carbon	Chemical shift (ppm)	
	10a	**10b**
1	50.6*	50.6*
2	29.2	29.1
3	49.6*	49.6*
4	78.0	78.0
5	76.2	76.1
6	73.4	73.3
1′	95.7	96.1
2′	49.6*	49.7*
3′	21.1	21.4
4′	26.1	23.2
5′	70.8	69.7
6′	51.9*	50.5*
7′	15.1	13.6

^{13}C FT NMR spectra were taken with a Varian XL-100 spectrometer in D_2O at pD 4.2. Internal reference: dioxane (67.4 ppm). Similar values with asterisks within each column may be interchanged.

Table 2. Minimum inhibitory concentrations (μg/ml) of the synthetic gentamine C_2 (**10b**) and its 6′(*S*)-epimer (**10a**).

Test organisms	**10a**	**10b**
Staphylococcus aureus FDA 209P	50	100
Staphylococcus aureus Smith	3.13	6.25
Bacillus subtilis PCI219	3.13	6.25
Bacillus cereus ATCC10702	50	>100
Mycobacterium smegmatis ATCC607	50	>100
Escherichia coli NIHJ	50	100
Escherichia coli K-12	100	100
Escherichia coli K-12 R5	>100	>100
Escherichia coli K-12 ML1629	50	100
Escherichia coli JR66/W677	100	>100
Klebsiella pneumoniae PCI602	100	>100
Shigella dysenteriae JS11910	100	>100
Salmonella typhi T-63	25	50
Salmonella enteritidis 1891	50	100
Proteus vulgaris OX19	50	100
Pseudomonas aeruginosa A3	100	100
Pseudomonas aeruginosa No. 12	>100	>100
Pseudomonas aeruginosa GN315	>100	>100

of carbons at C-4′ and C-7′. The C-13 chemical shifts of the 6-*epi*-purpurosamine B moiety in fortimicin B sulfate reported by EGAN *et al.*[9] are very similar to those of **10a**. The two synthetic products, **10a** and **10b** showed weak biological activity as shown in Table 2.

DAISHIRO IKEDA
TSUYOSHI MIYASAKA
KENZABURO YOSHIDA
KATSUHARU IINUMA
SHINICHI KONDO
HAMAO UMEZAWA

Institute of Microbial Chemistry
14-23, Kamiosaki 3-Chome,
Shinagawa-ku, Tokyo 141,
Japan

(Received September 4, 1979)

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