CHEMISTRY AND BIOCHEMISTRY OF THE NEOMYCINS. XVI

SYNTHESIS AND BIOACTIVITY OF HEXA-N-BENZYLNEOMYCINS[1]

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Hexa-N-benzylneomycin and five analogs substituted on the phenyl ring have been prepared by reductive alkylation of neomycin with the appropriate benzaldehydes. Most of the six show activity, to varying degrees, against a number of microorganisms, including *Staphylococcus aureus*, *in vitro*. Little or no activity was evidenced *in vivo* against *Staphylococcus aureus*, however.

FUJII, MAEDA and UMEZAWA[2] have reported the preparation of an extensive series of tetra-N-arylalkylkanamycin derivatives. These derivatives were less active than kanamycin against most strains of test organisms, but several of the derivatives were found to be active against kanamycin-resistant strains. In was suggested that the modification in activities was related to the lipophilicities of the derivatives. While a considerable number of biologically active derivatives of kanamycin have been prepared,[3] biologically active modifications of the structure of another important aminoglycoside antibiotic, neomycin,[4] have been limited to the semisynthetic hybrimycins[5] A and B, in which the methylene group of the deoxystreptamine moiety is substituted with a hydroxyl group, and a series of monoalkyl derivatives of neomycin, prepared by PÉNASSE, *et al.*,[6] which were reported to have greater activity against several strains of the bacteria tested.

In view of the high activity of the per-N-benzyl derivatives of kanamycin,[2] it was deemed of interest to determine whether per-N-benzyl derivatives of neomycin retain activity in a manner analogous to similar derivatives of kanamycin. A series of lipophilic hexa-N-benzylneomycins have now been prepared and tested for antibacterial activity; since the lack of absorption of neomycin from the intestine[7] is probably due to the low lipophilicity of the antibiotic, two of the derivatives were also tested for oral activity.

Discussion

The hexa-N-benzylneomycins shown in Fig. 1 were synthesized by a modification of the method of FUJII, MAEDA and UMEZAWA,[2] employing reductive coupling of the appropriate aldehyde and neomycin B in the presence of sodium borohydride in aqueous methanol. Since neomycin B has six amino groups, while kanamycin A has only four, longer reaction times and a larger excess of reagents were required for complete derivatization of neomycin and the yield of totally derivatized antibiotic was lower.

Microanalytical data (Table 4) indicated complete N-benzylation. The compounds were further characterized by melting points, R_f values on paper chromatography and thin-layer chromatography, and infrared and nmr data (Table 1). In contrast to neomycin the derivatives are rather lipophilic, being sparingly soluble in acidic water, methanol, and ethanol but very soluble in acetone and chloroform.

The antibacterial activities of the hexa-N-benzylneomycins B, determined by broth dilution assay, are shown in Table 2. In order to facilitate *in vitro* antibacterial testing the derivatives were

Fig. 1. Structures of benzylneomycins described in the present report

1, Hexa-N-benzylneomycin B; **2**, hexa-N-(*p*-chlorobenzyl)neomycin B; **3**, hexa-N-(*o*-chlorobenzyl) neomycin B; **4**, hexa-N-(*p*-methoxybenzyl)neomycin B; **5**, hexa-N-(3, 4-dimethoxybenzyl) neomycin B; **6**, hexa-N-(3, 4-methylenedioxybenzyl)neomycin B.

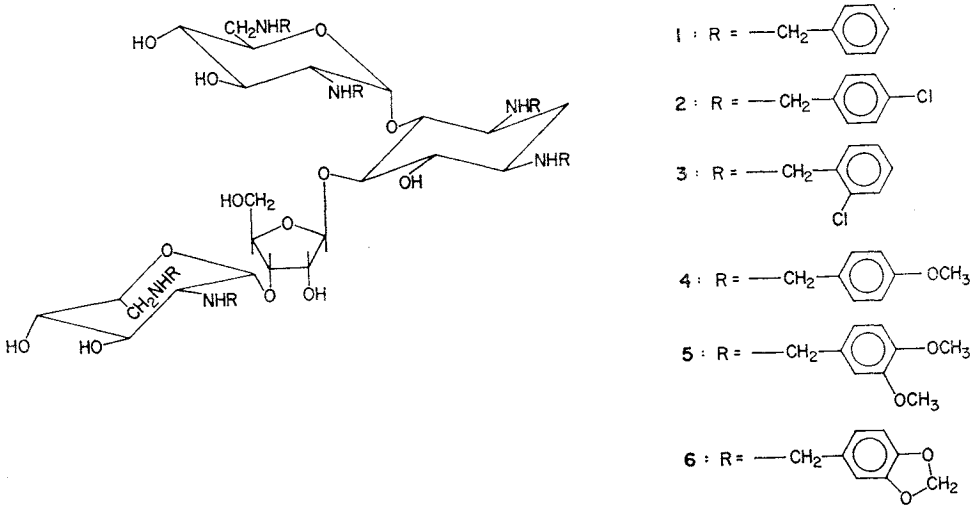

Table 1. Physical properties of hexa-N-benzylneomycins B.

Compound[a]	M.p., °C[b]	R_f						IR, cm^{-1} (KBr)[g]	NMR, δ ($CDCl_3$)[h]
		A[e]	B[e]	C[e]	D[e]	E[e]	TLC[f]		
1	93～97[c]	0.927	0.912	0.898	0.870	0.898	0.21	740,695	7.24 (s)
2	103～105[c]	0.874	0.947	0.778	0.864	0.923	0.30	802	7.23 (m)
3	92～95[c]	0.930	0.939	0.800	0.912	0.933	0.40	748	7.24 (m)
4	102～104[d]	0.896	0.896	0.905	0.880	0.901	0.18	814	6.83 (d, J=8.5 Hz), 7.16 (d, J=8.5 Hz), 3.74 (s, OCH_3)
5	104～106[c]	0.914	0.905	0.887	0.799	0.870	0.18	804,762	6.80 (m), 3.83 (s, OCH_3)
6	103～107[c]	0.863	0.913	0.671	0.770	0.898	0.20	805	6.70 (m), 5.88 (s, OCH_2O)

a) For structures, see Fig. 1. *b*) Melting points were determined on a Reichert hot-stage microscope. All compounds decompose on melting. *c*) Off-white powder. *d*) Colorless needles. *e*) Paper chromatography. Development was by the descending method on Whatman No. 1 paper. Solvent systems: A= ethanol-concd. ammonia-water (8:1:1, v/v); B=1-butanol-concd. ammonia-water, (8:1:1, v/v); C=methanol-concd. ammonia (4:1, v/v); D=1-octanol-water-concd. ammonia (5:4:1, upper layer); E=1-butanol-pyridine-water (6:4:3, v/v). *f*) Thin-layer chromatography was on PF_{254} silica gel (E. Merck AG) using the solvent system chloroform-methanol-concd. ammonia (100:15:2). *g*) Aromatic CH out-of-plane deformations. Spectra taken with a Perkin-Elmer 521 spectrophotometer. *h*) Aromatic proton absorptions, except as noted. Spectra determined on a Varian Associates A-60A spectrometer.

converted to their hydrochlorides by acidifying acetone solutions of the derivatives with 6N hydrochloric acid and evaporating the solutions to dryness.

Compounds **1** and **2** were tested for *in vivo* antibacterial activity in *Staphylococcus aureus* infected mice. Compound **1** was inactive subcutaneously at 40 mg/kg. Compound **2** showed very slight activity subcutaneously at 100 mg/kg but was inactive orally at 800 mg/kg. As a control, underivatized neomycin gave a subcutaneous CD_{50} of 0.93 (0.66～1.3) mg/kg and an oral CD_{50} of 38 (28～51) mg/kg.

Table 2. Broth dilution assays of hexa-N-benzylneomycins B

Organism	MIC (μg/ml)[a,b]					
	1	**2**	**3**	**4**	**5**	**6**
Escherichia coli UC 51	31.2	250	>500	>500	500	250
Proteus vulgaris UC 93	250	500	>500	>500	250	250
Pseudomonas aeruginosa UC 95	125	250	>500	>500	250	250
Klebsiella pneumoniae UC 57	15.6	250	>500	500	250	62.5
Streptococcus faecalis UC 3235	31.2	31.2	>500	>250	500	250
Staphylococcus aureus UC 80	3.9	31.2	31.2	125	250	7.8
Diplococcus pneumoniae UC 41	3.9	2.0	2.0	125	125	15.6
Streptococcus hemolyticus UC 152	15.6	31.2	500	>250	500	125

a) Each compound was dissolved in dimethylformamide at 10 mg/ml and diluted to 1 mg/ml with brain heart infusion broth (BHI). (For compounds **1**, **2**, **3**, **4** and **6**, which formed precipitates upon addition of the BHI, the suspensions were serially diluted. Two-fold dilutions were made with BHI. Each solution was seeded with test organism for a final suspension containing $10^4 \sim 10^5$ organism per ml. The systems were incubated for 18 hours at 37°C. *b*) The tests with 125 μg/ml or above had to be read microscopically due to precipitates.

Table 3. Yields and microanalytical data for hexa-N-benzylneomycins

Compound	Reactants, g		Product, yield		Formula	Microanalytical Data (%)					
						C		H		N	
	Neomycin	Aldehyde	g	%		Calcd.	Found	Calcd.	Found	Calcd.	Found
1	1.0	2.4	0.51	27	$C_{65}H_{82}N_6O_{13}$	67.57	67.44	7.15	7.44	7.27	7.08
2	10.0	32.0	11.1	50	$C_{65}H_{76}Cl_6N_6O_{13}$	57.32	57.15	5.62	5.76	6.17	6.18
3	1.0	3.2	0.89	40	$C_{65}H_{76}Cl_6N_6O_{13}$	57.32	57.11	5.62	5.53	6.17	6.33
4	1.0	3.1	0.52	24	$C_{71}H_{94}N_6O_{19}$	63.83	63.32	7.09	7.06	6.28	6.47
5	1.0	3.8	0.38	16	$C_{77}H_{106}N_6O_{25}$	61.02	60.75	7.05	6.95	5.54	5.69
6	1.0	3.4	0.45	20	$C_{71}H_{82}N_6O_{25}$	60.07	59.99	5.82	5.75	5.92	5.94

Experimental

Hexa-N-benzylneomycin (1)

Neomycin B (1.0 g, Upjohn Co.) and 2.4 g of benzaldehyde were dissolved in 11 ml of 90% aqueous methanol and 1.2 g of sodium borohydride was slowly added. The mixture was allowed to stand at room temperature for 24 hours, then poured into 200 ml of water. The supernatant was decanted and the precipitate was dried *in vacuo* then dissolved in a small amount of acetone, and the solution was poured into 200 ml of water. The supernatant was decanted and the precipitate was dried *in vacuo*, then dissolved in a small amount of chloroform. The resulting solution was applied to a column containing 40 g of heat-activated silica gel (Brinkmann, 0.05~0.2 mm) in chloroform. The column was eluted in 20-ml fractions with chloroform-methanol-concd. ammonia (100:15:2), and the fractions were monitored by TLC. The fractions containing only the major component were combined and evaporated, and the residue was dissolved in a small volume of acetone. The solution was poured into 200 ml of water, the supernatant was decanted, and the residue was dried *in vacuo* to yield 0.51 g (27%) of an off-white powder whose physical properties are summarized in Table 1, biological properties in Table 2, and microanalytical data in Table 3.

Substituted hexa-N-benzylneomycins B (2~6)

Reaction conditions and isolation procedures identical to those used for the synthesis of **1** were

used for the preparation of derivatives **2**~**6** from *p*-chlorobenzaldehyde, *o*-chlorobenzaldehyde, *p*-anisaldehyde, veratraldehyde, and piperonal, respectively, except that the molar amounts of reactants were adjusted appropriately. Yields and microanalytical data are presented in Table 3, physical properties in Table 1, and biological properties in Table 2.

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