Communications to the editors

NEOMYCINS D, E AND F: IDENTITY WITH PAROMAMINE, PAROMOMYCIN I AND PAROMOMYCIN II

Sir:

Neomycin, one of the first reported antibiotics[1], remains of medical importance[2]. With the availability of recently developed techniques of spectroscopy and separation, which allow the isolation and identification of trace components of mixtures, we have undertaken a study of the small amounts of other constituents present in commercial neomycin samples in addition to the well known neamine (neomycin A), neomycin B and neomycin C. These "extra" neomycins were found initially by ion-exclusion chromatography (Fig. 1) and by the recently reported gas-liquid chromatographic procedure[3]. They appear in varying amounts (always less than 1 %) as constituents of many commercial neomycin samples, both from American and foreign neomycin producers, including neomycin produced by strains of *Streptomyces fradiae* 3535 descendent from the original isolate of WAKSMAN and LECHEVALIER[1]. In the present report we identify three "extra" neomycins, neomycins D, E and F, as paromamine, paromomycin I and paromomycin II, respectively.

Neomycin sulfate (a commercial sample from S. B. Penick Co.) was neutralized and subjected to ion-exchange chromatography (CG 50 resin, ammonium ion form). Gradient elution with ammonium hydroxide yielded two fractions. The first contained neamine, neomycin D and neomycin F, while the second fraction contained neamine and neomycins D, F, B and E, in order of elution. The two fractions were then futher separated by ion-exclusion chromatography (Dowex 1×2 resin, hydroxide ion form); elution with water gave the individual components (Table 1).

Nuclear magnetic resonance spectra of neomycins D, E and F contained the characteristic ABX_2 multiplet[4,5] for the methylene group of deoxystreptamine. The three antibiotics were separately N-acetylated[6] for further characterization. The derivatives' nmr spectra indicated the presence of three N-acetyl groups for neomycin D, five N-acetyl groups for neomycins E and F. The mass spectrum of the N-acetyl-O-trimethylsilyl derivative[7] of neomycin D, prepared by the usual method[5], showed diagnostic peaks at *m/e* 809 (P, parent ion), 794 (P-CH_3), 420 and 373. The last peak is

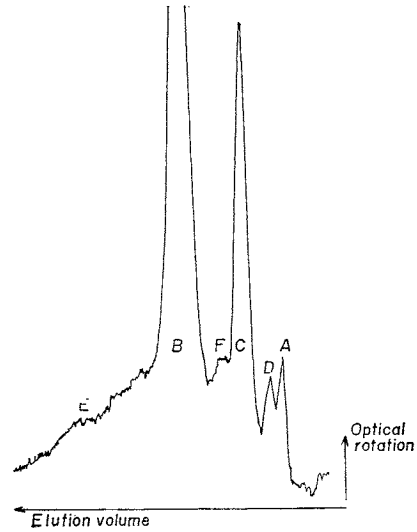
Table 1. Comparison of properties of the antibiotics and derivatives

Antibiotic	Free base	N-Acetyl derivative		TMS derivative GLC retention time [c]	
	$[\alpha]_D$ [a]	$[\alpha]^{19.5}$	Rf [b]	min.	(column temp.)
Neomycin D	+103°	+98.6°	0.29	10.7	(210°C)
Paromamine	+114°	+97.7°	0.29	10.7	
Neomycin E	+55.1°	+59.0°	0.38	18.5	(270°C)
Paromomycin I	+64°	+64.5°	0.38	18.5	
Neomycin F	+102.3°	+83.0°	0.24	23.2	(270°C)
Paromomycin II	+96°	+87.8°	0.24	23.2	

a) Rotations of paromamine, paromomycin I and paromomycin II are literature values.[8,16]
b) Paper chromatography Rf values are for the solvent system 1-butanol - pyridine - water (6 : 4 : 3).
c) GLC retention times were determined isothermally using the method described previously[3].

Fig. 1. Ion-exclusion chromatogram of neomycin sample, as described in text.

The order of elution is neomycin A (neamine), D (paromamine), C, F (paromomycin II), B, and E (paromomycin I). The location of the last component is estimated from its position in other chromatograms of samples enriched in neomycin E.



due to the deoxystreptamine fragment[7] and appears at the same position in the mass spectrum of N-acetyl-O-trimethylsilylneamine. However, the first three peaks appear 31 mass units higher than in the neamine derivative's spectrum (-OTMS=89 amu, -NHAc=58 amu) and indicate replacement of an amino group in neamine (one of those on the diamino sugar neosamine C) by a hydroxyl group in neomycin D. Mass spectra of the N-acetyl-O-trimethylsilyl derivatives of neomycins E and F were essentially identical and revealed similar differences from spectra of neomycins B and C: peaks at *m/e* 1401 (P), 1386 (P-CH_3), and 420, all 31 amu higher for the derivatives of neomycins E and F; peaks at *m/e* 665 and 389, at the same masses for derivatives of all neomycins.

The nmr and mass spectral data indicate a close resemblance of neomycin D to paromamine[8] and of neomycins E and F to paromomycins I[9] and II[10], respectively. Accordingly, a direct comparison was made between the compounds and their derivatives. Paromomycin was a commercial sample (Humatin, Parke-Davis), paromamine was obtained by hydrolysis of that sample. As seen in Table 1, the chromatographic and optical properties confirm the identification of neomycin D as paromamine and of neomycins E and F as paromomycins I and II, respectively. Moreover, the nmr spectra (100 MHz) of the N-acetyl derivatives are identical, as are the mass spectra of the N-acetyl-O-trimethylsilyl derivatives.

The discovery of the antibiotic producing properties of *Streptomyces fradiae* 3535 in 1949[1] antedates the discovery of catenulin (1952)[11], paromomycin (1956)[12], hydroxymycin (1958)[13], aminosidin (1959)[14], zygomycin A (1961)[15], and others[16]; the latter compounds have all been shown to be identical[17]. Thus, the compounds described in the present report can properly be considered as neomycins, just as kanamycin B, with a diamino sugar (neosamine C), is co-produced with kanamycins A and C, which contain monoamino sugars (6-amino-6-deoxy-D-glucose and glucosamine, respectively)[2].

The biosynthesis of neomycin has been demonstrated to involve conversion of glucose *via* glucosamine to deoxystreptamine and neosamines B and C[18], and deoxystreptamine is incorporated underivatized into the antibiotic[5,19]. The long-standing surmise[2] that the paromomycins (neomycins E and F) are biosynthesized similarly is placed on a firmer basis by the present results.

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