CHROMATOGRAPHIC ANALYSIS OF NEOMYCIN

ISOLATION AND IDENTIFICATION OF MINOR COMPONENTS

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(Received for publication September 2, 1974)

The presence of a number of minor components was demonstrated in commercial samples of neomycin by column chromatography on a weakly acidic ion-exchange resin (Amberlite CG-50). Some of these components were isolated by ion-exclusion chromatography and identified. The identification was based on chromatographic and nmr data and on mass spectrometry of volatile derivatives. The sequence of elution from the Amberlite column was : paromamine, mono-*N*-acetylneamine (LP-A), paromomycin II, a diamino-dideoxyhexosyl-*myo*-inositol (component G), paromomycin I, mono-*N*-acetylneomycin C (LP-C), neamine, mono-*N*-acetylneomycin B (LP-B), neomycin C and neomycin B. The LP-B fraction also contained a component (K) whose gross structure corresponds to a neomycin, in which the neosamine C linked to the deoxystreptamine subunit is missing. The presence of the components LP-A, G and K has not previously been reported.

Neomycin is a mixture of basic water-soluble antibiotics produced during fermentation of *Streptomyces fradiae*[1]. The main components are neomycin B (**1**) and neomycin C (**2**). Their structure was elucidated in the period 1950~1964[2]. The presence of small amounts of another component, with a much lower activity, neomycin A, or neamine (**3**) was revealed very early during the study of neomycin[3]. Later CHILTON *et al.*[4] reported the presence of neomycin LP-B (**4**) and LP-C (**5**) in crude neomycin. These components are mono-*N*-acetyl derivatives of neomycin B and C, respectively. They were also found in commercial samples[5]. Recently, other minor components, isolated from commercial samples, were identified as paromamine (**6**), paromomycin I (**7**) and paromomycin II (**8**)[6].

During isolation of pure neomycin B, which was needed for other purposes, the presence of other related compounds was detected. Relative amounts of several of the known components in commercial samples were estimated.

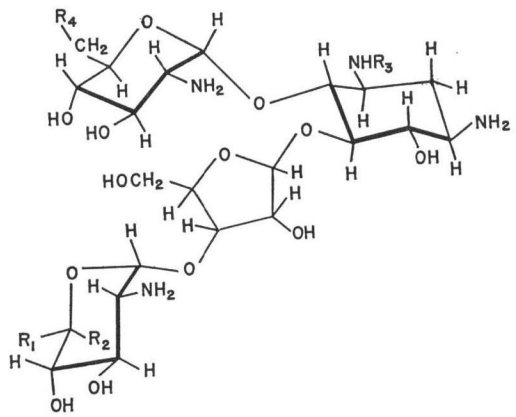
A rather large sample (100 g) of commercial neomycin (U-XZ 336) was chromatographed on a carboxylic cation-exchange resin (Amberlite CG-50)[7]. The fractions of the column were analysed by paper chromatography[8] and by chromatography on a strongly basic column (Dowex 1-X2)[9,10], using a conductimetric method of detection[11]. Chromatography on the basic Dowex column, sometimes referred to as ion-exclusion chromatography, was also used for purification of several minor components. The identification of most of the components was based on Rf values and retention volumes in several chromatographic systems, on nmr spectra, on chromatography of derivatives, and on mass spectrometry of their *O*-trimethylsilyl (TMS)-*N*-acetyl derivatives[12].

Experimental

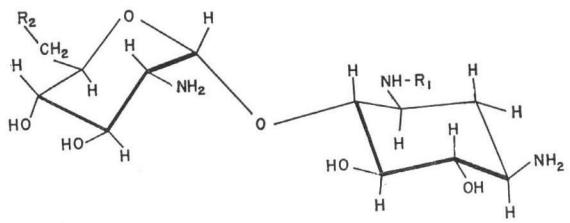
General

All column effluents were evaporated under reduced pressure at a temperature of 40°C.

Paper chromatography of the free bases and of the sulfates was performed on Whatman No. 1 paper according to MAJUMDAR and MAJUMDAR[8]. The solvent system consisted of methyl ethyl ketone - *t*-butanol - methanol - 6.5 N ammonium hydroxide (16 : 3 : 1 : 6). Thin-layer chromatography was performed on Merck precoated silica gel F-254 plates using 15% aqueous KH_2PO_4 as eluant[13]. Ninhydrin-$SnCl_2$[14] was used for detection. Paper chromatography of *N*-acetyl derivatives was performed on Whatman No. 4 paper with the system *n*-butanol - water - pyridine (86 : 16 : 2)[15]. Samples for nmr were freeze-dried twice from D_2O. Spectra of the D_2O solutions were run on a Varian A-60 spectrometer with sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal standard. Peak positions are expressed in δ values. *N*-Acyl derivatives for mass spectrometry and for paper chromatography[15] were obtained by suspending 10 mg of the free base in a mixture of 2 ml methanol and 0.4 ml acetic or propionic anhydride. After 15 hours at room temperature, the solvent was evaporated and the major part of the anhydride was removed by adding 1 ml of benzene or toluene and by evaporating under reduced pressure. The last traces of anhydride were decomposed by dissolving the residue in a mixture of 2 ml methanol and 2 ml methanol saturated with ammonia. The solutions were kept in a refrigerator for 4 hours, then evaporated and dried *in vacuo* over P_2O_5. The *N*-acyl derivatives were *O*-silylated by treating for 2 hours with 0.5 to 1 ml Trisyl-Z (a commercial trimethylsilylating agent obtained from the Pierce Chemical Company), containing *N*-trimethylsilylimidazole in pyridine (1.5 meq/ml). *O*, *N*-Acetyl derivatives for mass spectrometry were prepared on 10 mg of the free base by acetylation with pyridine-acetic anhydride. Mass spectra of these derivatives were run on an AEI-12 spectrometer by direct insertion in the ion source,



1 : R^1=H, R^2=CH_2NH_2, R^3=H, R^4=NH_2 (neomycin B)
4 : 〃 〃 R^3=Ac, R^4=NH_2 (neomycin LP-B)
7 : 〃 〃 R^3=H, R^4=OH (paromomycin I)
2 : R^1=CH_2NH R^2=H, R^3=H, R^4=NH_2 (neomycin C)
5 : 〃 〃 R^3=Ac, R^4=NH_2 (neomycin LP-C)
8 : 〃 〃 R^3=H, R^4=OH (paromomycin II)



3 : R^1=H, R^2=NH_2 (neomycin A or neamine)
6 : R^1=H, R^2=OH (paromamine)
9 : R^1=Ac, R^2=NH_2 (neomycin LP-A)

operated at a temperature of 170~230°C, according to the volatility of the derivative. Specific rotations were corrected for moisture content, determined by drying for 3 hours at 70°C under high vacuum and over P_2O_5.

Ion-exclusion column chromatography

Ion-exclusion chromatography was carried out on the strongly basic ion-exchange resin Dowex 1-×2 (200~400 mesh) in the OH^- form, using carbon dioxide-free water as eluant. Technical details on the conductimetric monitoring are given in a previous publication[16]. For analysis of the Amberlite column fractions, a column with a diameter of 3 cm and a height of 25 cm and a flow rate of 310 ml/hour was used. The sample weight was 4 mg. Commercial neomycin samples (15 mg) were analysed on a 3×27 cm column with a flow rate of 300 ml/hour. Preparative separation was performed on a 3×65 cm column with a flow rate of 370 ml/hour. The sample weight varied from 20 to 30 mg.

Amberlite CG-50 column chromatography of commercial neomycin

The Amberlite CG-50 type I (100~200 mesh) resin in the acid form was suspended in water. Ammonium hydroxide was added to the stirred suspension until a stable pH of 9~9.5 was obtained. A solution of 100 g neomycin sulfate (U-XZ336) in 4,500 ml water was adjusted to pH 9 with aqueous ammonium hydroxide and charged (300 ml/hour) on a column (4.2×90 cm) containing 1,350 ml of the equilibrated resin. Ammonium sulfate was washed out with 4,400 ml water. Charging and washing effluents were discarded. The resin bed was eluted with 0.2 N (9,000 ml) and 0.25 N (8,100 ml) aqueous ammonium hydroxide at a flow rate of 104 ml/hour. Both effluents were collected in 450-ml fractions. The last fraction of the 0.25 N effluent contained pure neomycin B. The remaining part of this component was removed from the resin by elution with 1 N ammonium hydroxide. The fractions were evaporated to dryness and the residue was dissolved in water, filtered or centrifuged and was again evaporated to dryness. The residue was triturated with absolute ethanol, and after addition of ether, the precipitate was isolated and dried *in vacuo* over P_2O_5. Fractions, containing small amounts of material, were isolated by freeze-drying. Chromatography of the U-TR032, S-52001, and R-7S1251 samples was performed as described for the U-XZ336 sample. The entire 0.2 N effluent, however, was collected as one fraction.

Crystallization of neomycin B free base

The heavy syrup obtained on evaporation of the combined neomycin B fractions was dissolved in 120 ml boiling methanol. After standing for 24 hours at room temperature the crystalline neomycin B was isolated. The optical rotation $[\alpha]_D^{25}+82°$ (*c* 1, 0.2 N H_2SO_4) was in agreement with the value reported by FORD *et al.*[17].

Isolation of component J (neomycin LP-B)

The free base of component J (750 mg) purified by ion-exchange chromatography was dissolved in absolute methanol (25 ml), and the solution was filtered. Addition of four volumes of anhydrous ether yielded an amorphous precipitate (500 mg), $[\alpha]_D^{25}+44°$ (*c* 1, 0.2 N H_2SO_4). Nmr spectrum (D_2O, DSS) δ 2.03 (3H, s, CH_3CON), 5.0, 5.41, 5.6 ppm (3H, anomeric protons).

Component J, *N*-acetyl derivative

Component J (350 mg) was suspended in a mixture of 10 ml pyridine and 3 ml Ac_2O, and kept for 48 hours at room temperature. The residue obtained on evaporation of the reaction mixture was triturated with anhydrous ether. The precipitate was isolated, dried and dissolved in a mixture of 12 ml anhydrous methanol and 12 ml methanol saturated with ammonia. After standing for 12 hours at 0°C, the precipitate was isolated and crystallized from water-acetone, yielding 200 mg of the *N*-acetylated derivative, which showed the same Rf value in paper chromatography[15] as that observed for hexa-*N*-acetylneomycin B. These physical constants, mp 194~200°C, $[\alpha]_D^{25}+55°$ (*c* 1, water), even as the nmr and mass spectrum of this compound are identical to those reported for hexa-*N*-acetylneomycin B[4].

Analysis. Calculated for $C_{35}H_{58}N_6O_{19}$ (hexa-*N*-acetylneomycin) : C, 48.49; H, 6.74; N, 9.70
Found : C, 48.19; H, 6.96; N, 10.01

Isolation and identification of the inositol of component G

The crude component G (10 mg), obtained by ion-exclusion chromatography of fraction 16, was *N*-acetylated by the usual procedure (Ac_2O/MeOH). The reaction product was dissolved in 1.5 ml 4 N HCl and was heated at 90°C in a sealed tube for 2 hours. The hydrolysate was evaporated to dryness and azeotropically distilled with absolute ethanol. The residue was taken up in 2 ml water and applied to a column of 5 ml Dowex 50W-×8 (100~200 mesh, acid form). The resin was washed twice with 5 ml water, and the combined column effluents were evaporated. The residue was dried *in vacuo* over P_2O_5 and silylated with Trisyl-Z at 60°C for 1 hour. The *O*-TMS derivative showed in glc (3% OV-1 on Gas-Chrom Q 100~200 mesh, 183°C) a retention time which was identical to that of hexa-*O*-TMS-*myo*-inositol and different from that observed for the *O*-silylated *scyllo*-inositol

Results and Discussion

A. Isolation and Identification of Components

Chromatographic analysis of the 0.2 N ammonium hydroxide effluent of the Amberlite column (Fig. 1) indicated the presence of two components (D and E) in fraction 6, which were separated by ion-exclusion chromatography. Component D, which was found only in this fraction, was identified as paromamine (**6**) by its retention volume on ion-exclusion chromatography (Fig. 2) and by its Rf value on paper chromatography (Fig. 3). Further confirmation of the identity of component D with **6** was obtained from the mass spectrum of its *O*-TMS-*N*-acetyl derivative.

Component E was found in fractions 6~11 (Fig. 1). Its nmr spectrum showed the presence of one *N*-acetyl group (2 ppm) and a 1H doublet (5.4 ppm) in the region of the anomeric protons. *N*-Acetylation of E yielded a compound which was shown to be identical to tetra-*N*-acetylneamine by paper chromatography[15], nmr and mass spectrometry of its *O*-TMS derivative. This suggested that E is a mono-*N*-acetylneamine. By mass spectrometry of the *O*-

Fig. 1. Amberlite CG-50 column chromatography of neomycin sulfate (U-XZ336).

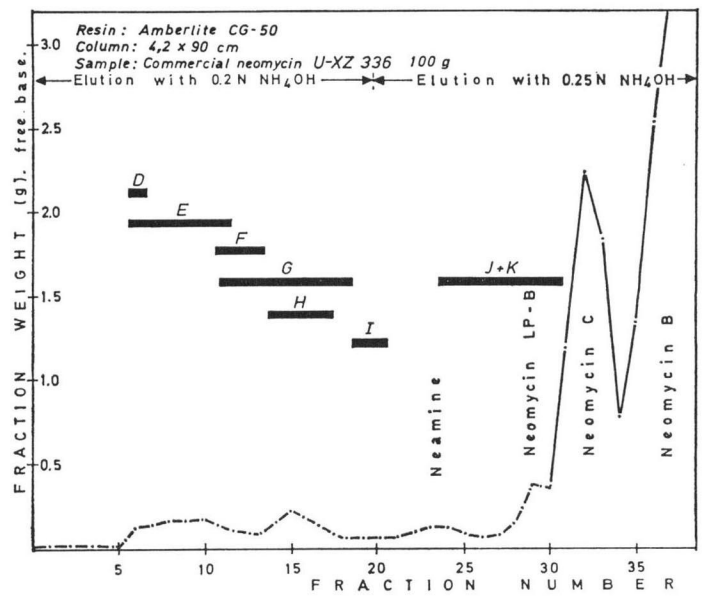
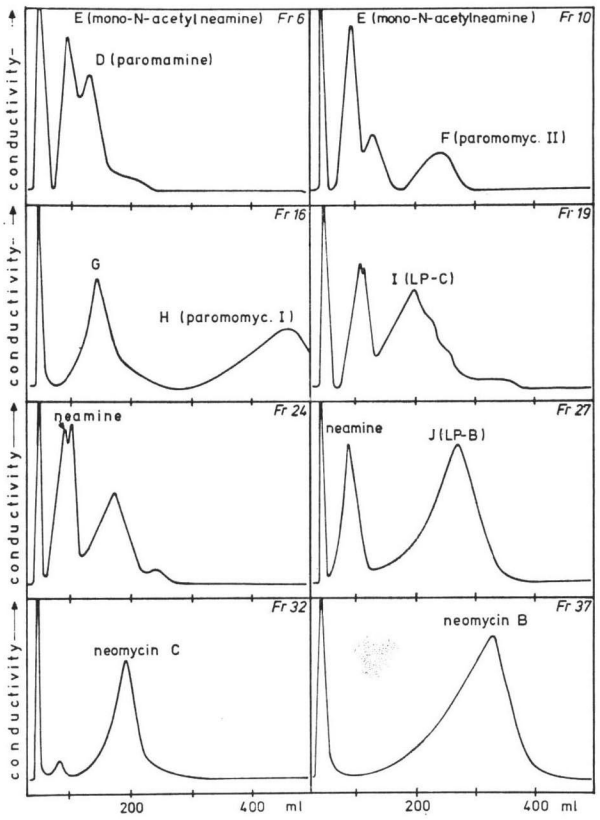


Fig. 2. Analytical ion-exclusion chromatography of the most representative fractions of the column chromatography (Fig. 1) (Dowex 1-×2; 3×2.5 cm; flow rate 310 ml/hour; sample weight 4 mg)



TMS-*N*-propionyl derivative of E, it could be shown that the *N*-acetyl group of E is located in the deoxystreptamine fragment (see under B). It has been shown[4] that the acetyl group of the mono-*N*-acetylneamine fragment of the LP-neomycins (**4** and **5**) is located on the C-3 amino function of the deoxystreptamine moiety. Since LP-neomycins were isolated from a commercial sample of neomycin[5], we assumed that the same amino group is acetylated in component E, for which structure **9** is proposed.

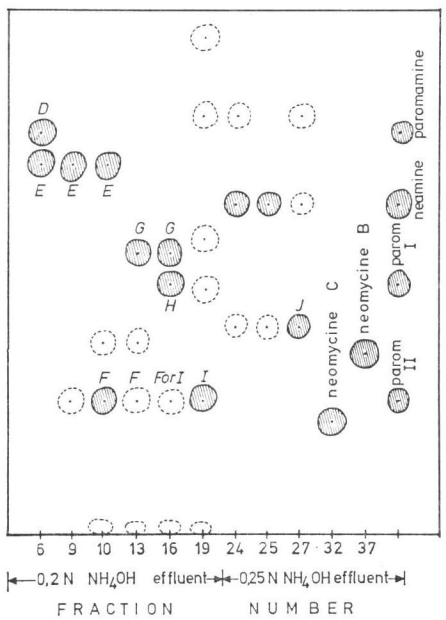
Ion-exclusion and paper chromatography (Figs. 2 and 3) indicate that component F is a major part of fractions 11~13 and component H of fractions 14~17. These components were isolated by ion-exclusion chromatography and identified as paromomycin II (**8**) and paromomycin I (**7**) respectively, by comparison with authentic samples in these chromatographic systems and by mass spectra of the *O*-TMS-*N*-acetyl derivatives.

Component G was found in fractions 11~18 (for isolation and structure see under B).

Both paper and ion-exclusion chromatography (Figs. 2 and 3) showed that fractions 19 and 20 were very complex and contained at least five components, which were only partly separated on the Dowex 1-×2 resin. Thus the main component of this mixture (designated as component I) could not be obtained in a pure state. However, its identity with mono-*N*-acetylneomycin C (neomycin LP-C) (**5**) was established. *N*-Acetylated I showed a spot in

paper chromatography[15] with an Rf value identical to that of hexa-*N*-acetylneomycin C. Since neomycin C itself was not present in fractions 19 and 20, the hexa-*N*-acetylneomycin spot in the *N*-acetylated mixture can be due to neomycin LP-C (**5**). This suggestion was confirmed by examination of the mass spectra of the *O*-TMS-*N*-acetyl and *N*-propionyl derivatives of component I (see under B).

Fig. 3. Chromatography on Whatman No. 1 paper of the most representative fractions of the column chromatography (Fig. 1) (system: methyl ethyl-keton - *tert*-butanol - methanol - 6.5 N ammonium hydroxide, 16:3:1:6; revelation ninhydrin).



The other mono-*N*-acetylneomycin (neomycin LP-B) (**4**) was found in fractions 24~31. The identification of component J as **4** was also based on chromatographic and nmr data, together with examination of the mass spectra of the *O*-TMS-*N*-acetyl and *N*-propionyl derivatives. Determination of the location of the acetyl group on the C-1 or C-3 amino group of the deoxystreptamine moiety was not possible by the method used. It is very likely that this function is on the C-3 amino group, as has been proved by CHILTON[4] for the mono-*N*-acetylneomycin B (**4**) isolated from a crude neomycin. The structure of component K, which was also present in these fractions, will be discussed under B.

The last two 0.2 N ammonium hydroxide fractions (19 and 20) and the early 0.25 N fractions (21 and 22) showed several peaks in the ion-exclusion chromatography with retention volumes similar to that observed for neamine. Paper chromatography, however, indicated that neamine was not present in these fractions. Separation of these components by preparative ion-exclusion chromatography failed, and further efforts to elucidate their structure have not yet been undertaken.

Neamine itself was found in fractions 23~30 (mainly fractions 23, 24 and 25), neomycin C in fractions 31~34, and neomycin B in the last 0.25 N fractions and in the 1 N ammonium hydroxide effluent.

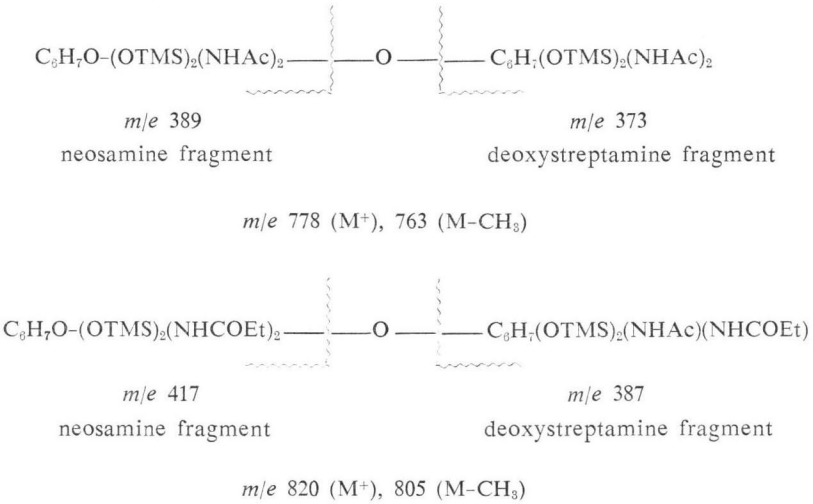
Our results confirm those of HESSLER *et al.*[6] who identified three 'extra-neomycins' as paromamine paromomycin II and paromomycin I in a commercial sample. The presence of the mono-*N*-acetyl derivatives of neamine, neomycin B and neomycin C, however, was not mentioned in that study. The sequence of elution from the Amberlite column (neamine, paromamine, paromomycin II, neomycin B and paromomycin I) was different from that found in the present study. According to our experience with this Amberlite resin it is rather strange that no neomycin C was found while neomycin B was present in the column effluent, that neamine, which contains four amino functions, was eluted before paromamine, which contains only three amino functions, and that neomycin B was eluted before paromomycin I. Their

results may be explained by the assumption that mono-*N*-acetylneamine and neomycin LP-C were erroneously identified as neamine and neomycin B, respectively because the components were apparently characterized by mass spectrometry of their *O*-TMS-*N*-acetyl derivatives.

B. Structure Determination of Components E, G, I, J and K

The mass spectrum of the *O*-TMS-*N*-acetyl derivative of component E showed diagnostic peaks at *m/e* 778 (M^+), 763 ($M-CH_3$), 389 (neosamine fragment ion) and 373 (deoxystreptamine fragment ion) (Scheme I). Preferential cleavage of the glucosidic bonds, leaving the charge on the oxygen of the pyranose- or the furanose ring is the usual fragmentation pattern of (pseudo) di- or oligosaccharides upon electron impact[12]. The spectrum of the *O*-TMS-*N*-propionyl derivative of component E showed peaks at *m/e* 820 (M^+), 805 ($M-CH_3$), 417 (neosamine fragment ion) and 387 (deoxystreptamine fragment ion) (Scheme I). These data indicate that the *N*-acetyl function is present in the deoxystreptamine moiety of the *N*-propionyl derivative, since its fragment-ion (*m/e* 387) is 14 mass units higher than that of the corresponding fragment in the spectrum of the *N*-acetyl derivative, whereas the difference for the neosamine C fragment ion is 28 mass units.

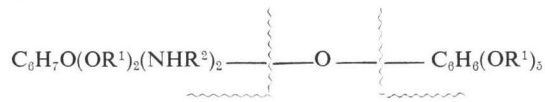
Scheme I. Diagnostic peaks observed in the mass spectrum of the *O*-TMS-*N*-acetyl and *O*-TMS-*N*-propionyl derivatives of component E.



Similar differences were found between the mass spectra of the *O*-TMS-*N*-acetyl and *O*-TMS-*N*-propionyl derivatives of the mono-*N*-acetyl derivatives of the neomycins B and C (components I and J). Detailed fragmentation patterns are given in a previous paper[18].

Component G was isolated from the fractions 11～18 by ion-exclusion chromatography. The mass spectra of its *O*-TMS-*N*-acetyl, *O*-TMS-*N*-propionyl and the *O*-acetyl-*N*-acetyl derivatives indicated the presence of a diamino-dideoxyhexose moiety (*m/e* 389 for the *O*-TMS-*N*-acetyl, 417 for the *O*-TMS-*N*-propionyl and 329 for the *O*-acetyl-*N*-acetyl derivatives). The difference of 28 mass units of the molecular ions of the *N*-acetyl and the *N*-propionyl derivatives (M^+ 928 and 956) showed the absence of primary of secondary amino groups in the other part of the molecule. The *m/e* values observed for this part in the three derivatives indicated the absence of deoxystreptamine and the presence of inositol moiety. The main

Scheme II. Diagnostic peaks observed in the mass spectra of volatile derivatives of component G.

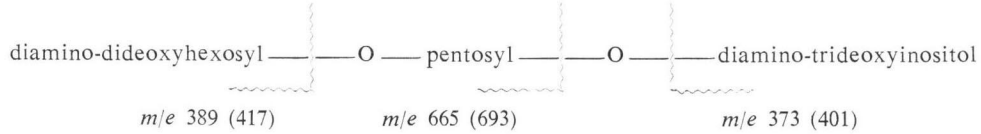


R^1=TMS, R^2=Ac : *m/e* 928 (M^+), 913 ($M-CH_3$), 389 (fragment A), 433 (fragment B-TMSOH).
R^1=TMS, R^2=COEt : *m/e* 956 (M^+), 941 ($M-CH_3$), 417 (fragment A), 433 (fragment B-TMSOH).
R^1=R^2=Ac : *m/e* 718 (M^+), 659 ($M-CH_3CONH_2$), 658 ($M-CH_3COOH$), 329 (fragment A), 373 (fragment B).

diagnostic peaks of the mass spectra are summarized in Scheme II. The inositol was isolated from the hydrolysate of *N*-acetyl G. Glc on a 3% OV-1 column of its TMS derivative showed the same retention time as that observed for hexa-*O*-TMS-*myo*-inositol. This chromatographic system is known to separate 6 of the isomers of inositol[19]. Sufficient amounts of component G were not available for further structure elucidation.

The presence of small amounts of another compound (component K), which was not separated from J (neomycin LP-B), by ion-exclusion and paper chromatography, was also observed while taking the mass spectra of this component. It was found that the *O*-TMS-*N*-acyl derivatives of component K are more volatile than the corresponding derivatives of component J and that the two components are separated by progressive heating of the sample in the mass spectrometer. The mass spectra of component K thus obtained led to the sequential arrangement given in Scheme III.

Scheme III. Diagnostic peaks observed in the mass spectra of the *N*-acetyl-*O*-TMS derivative of component K and in that of its *N*-propionyl-*O*-TMS derivative (*m/e* values in parenthesis).



m/e 1054 (1110), (M^+) 1039 (1095) ($M-CH_3$)

C. Analysis of Other Neomycin Samples

The chromatography of three other neomycin samples was carried out prior to that of the U-XZ336 sample. During separation of these samples on the Amberlite resin the 0.2 N ammonium hydroxide effluent was collected as one fraction. The 0.25 N effluent, however, was fractionated as reported for the U-XZ336 sample. The 0.2 N ammonium hydroxide effluents and the 0.25 N ammonium hydroxide fractions of the three other neomycin samples were also analyzed by paper chromatography[8] and by ion-exclusion chromatography. We found that almost all the components found for the U-XZ336 neomycin were present in the other three samples. Results obtained for the four neomycin samples are summarized in Table 1. The free base recovered from 0.2 N effluent varied from 1.3 to 3.3%. This fraction contains the components D to I and at least six other unidentified components. The determination of the exact amount of neamine was not possible because of the presence of 'neamine-like' substances, which cannot be separated by ion-exclusion chromatography. Therefore, the percen-

tages given in Table 1 are indicated as 'neamine+'. It can be seen that the amount of 'neamine+' is less than 1 % in three of the samples and 2.5 % in the U-TR032 sample. Significant differences were also observed for the amount of LP-B in the four samples.

Table 1. Comparison of the results obtained by chromatography of the four neomycin samples

	U-TRO32	S-52001	R-7S1251	U-XZ336
0.2 N NH_4OH effluent[a]	3.2	1.3	3.3	2.3
'Neamine+'[a,b]	2.7	0.3	0.7	0.6
LP-B fraction[a,c]	3.5	0.4	1.9	0.5
Neomycin C[a]	4.9	22.7	5.3	6.4
Neomycin B[a]	53.4	43.2	52.9	61.2
(%C×100)/(%C+%B)				
calculated from the % of free bases	8.4	34.5	9.1	9.5
determined by analytical ion-exclusion chromatography	12.2	39.0	12.5	12.0

a Values given are the percentages of free bases calculated from the amount of neomycin sulfate subjected to chromatography.
b 'Neamine+' is the sum of neamine and neamine-like substances eluted in the early 0.25 N NH_4OH effluents.
c These fractions also contain small amounts of component K.

Fig. 4. Ion-exclusion column chromatography of commercial neomycin (resin Dowex 1-×2 OH^- form; 200~400 mesh, column size 2.5×27 cm; sample weight 15 mg; flow rate 300 ml/hour).

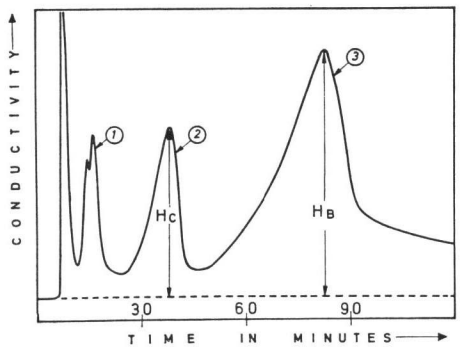
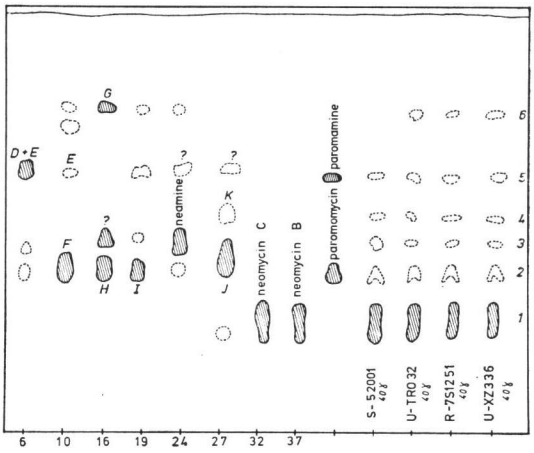


Fig. 5. Thin-layer chromatography on Merck precoated silica gel F-254 plates of the most representative fractions of the column chromatography (Fig. 1) and of the four commercial neomycins (system: 15% aqueous KH_2PO_4; revelation ninhydrin-$SnCl_2$).



It should be noted that the percentages given in Table 1 are the amounts of the free bases recovered after Amberlite chromatography of 100 g batches of neomycin sulfate. From the percentages of neomycins C and B, the values (% C×100)/(% B+% C) were calculated for comparison with the results obtained by analytical ion-exclusion chromatography. A typical ion-exclusion elution diagram, shown in Fig. 4, shows only three peaks. The first, designated as the neamine peak, contains, in addition to neamine, the components D (paromamine) and E (LP-A) and other unidentified neamine-like substances. Components G+J and F are hidden under the ascend and descend of the neomycin C peak, and components J+K and H under the ascend and descend of the neomycin

B peak. The relative amounts of neomycins B and C, expressed as (% C×100)/(% B+% C), can be determined from the relative heights of the last two peaks[20]. Table 1 shows the comparison of the relative amounts of neomycin C obtained by Amberlite and ion-exclusion chromatography. In all samples, however, the ion-exclusion method seem to overestimate the amount of neomycin C. This may be due to the presence of minor components and peak tailing.

The fractions obtained by Amberlite column chromatography of the U-X2336 sample were also analyzed by thin-layer chromatography[13]. We observed that this chromatographic system (Fig. 5) was less specific for certain components but had more separating power for others. Spot 1 corresponds to neomycins B and C, spot 2 to paromomycins I and II and the mono-*N*-acetylneomycins B and C (LP neomycins), spot 3 to neamine, spot 4 to component K, spot 5 to paromamine and mono-*N*-acetylneamine, and spot 6 to component G and other components. Chromatography of the four neomycin samples indicated that they all contained similar components, except for S-52001, which lacked component G. It is interesting to note that there is a correlation, in the thin-layer chromatographic system, between the number of amino groups and the Rf values on tlc of the different compounds : for the neomycins B and C (six amino groups), LP neomycins and paromomycins (five amino groups), neamine and component K (four amino groups), paromamine and component E (three amino groups), and component G (two amino groups). We believe that the separating power observed in this chromatographic system is mainly due to the presence of a polycarboxylic resin, which is used as a binder in the Merck precoated Silica gel plates.

Biosynthetic Significance of Components K and G

As the biosynthesis of neomycin has been partly elucidated by RINEHART and coworkers[2,21~23] it is of interest to consider how the components G and K fit into the biosynthetic scheme proposed by these authors. Although the determination of the structure of component K is limited to the sequence and the gross structure of its three subunits, it seems reasonable to assume that the diamino-trideoxyinositol, the pentose and the diamino-dideoxyhexose fragments are neosamine B (or C), D-ribose and deoxystreptamine, respectively, and that the nature and the location of the glycosidic linkages are identical to those of neomycin. Thus component K may be considered as a neomycin B (or C) which lacks the neosamine C subunit linked to the deoxystreptamine.

The structure of component K brings us to the final stage of the biosynthesis, *i.e.* the assembly of the subunits. There are several indications that neobiosamine is formed at a relatively early stage of the biosynthesis[21,23]. From this point there are two possibilities[23]; the biosamine may combine with deoxystreptamine followed by the linkage of neosamine C, or it may combine with neamine. Up to this moment it is not known which of the two pathways is followed. The presence of component K in commercial samples indicates that the linkage of neobiosamine with deoxystreptamine may occur.

A neomycin which lacks the neosamine B unit linked to D-ribose has been isolated from *Streptomyces ribosidificus*[24,25]. This compound is known as ribostamycin.

Component G can be considered as an analogue of neamine if one assumes that its diamino-dideoxyhexose fragment is neosamine C. Although *myo*-inositol is a good precursor for streptamine[26,27], it has been demonstrated[23] that *S. fradiae* did not use *myo*-inositol in the biosyn-

thesis of deoxystreptamine. Thus component G must be considered as an error occurring during biosynthesis of neamine. Neosamine is linked to *myo*-inositol, which is present in the fermentation medium, instead of to deoxystreptamine.

It is possible that additional indications for a biosynthetic scheme may be inferred from the structures of some of the unidentified components, especially those of the neamine-like substances found in the early 0.25 N ammonium hydroxide effluents.

Acknowledgement

We are indebted to L. VERLOOY for technical assistance during the course of this work and to Prof. L. ANDERSON, University of Wisconsin, for a sample of *scyllo*-inositol.

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