Structures of Some of the Minor Aminoglycoside Factors of the Nebramycin Fermentation

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The structures of some of the minor factors of the nebramycin complex of antibiotics are elucidated through a combination of physical and chemical methods.

Nebramycin, a complex of aminoglycosides elaborated by *Streptomyces tenebrarius*, includes a number of factors showing broad-spectrum antibiotic properties. The structures of five of these aminoglycosides have been elucidated, largely through chemical and degradative studies.[1-3] More recently, these factors have been studied using ^{13}C[4,5] and ^{15}N[6] nuclear magnetic resonance (NMR) spectroscopies. Other laboratories have used ^{13}C NMR and mass spectrometry to assign structures to closely related aminoglycosides.[7-9] In the present paper, we discuss the use of these physical methods, in conjunction with improved techniques of isolation and purification, in elucidating the structures of eight minor factors isolated by various methods from the nebramycin fermentation medium.

Results

Structures of the nebramycin factors and derivatives to be discussed appear in Chart I. Elucidation of the structures of factor **2**,[3,5] factors **4**, **5**, and **5′**,[2] and factor **6**[1] has been discussed previously. Factors **8–10** were shown to be identical with the previously known aminoglycosides nebramine,[2] lividamine,[10] and neamine,[11] respectively, by chromatographic and spectrometric comparisons with authentic samples. Primary screening of bioactivity has shown that all factors have significantly less antimicrobial activity than do apramycin and tobramycin.

A portion of the mass spectral data collected in the course of this work is presented in Table I. Most of the factors gave satisfactory electron-impact mass spectra (EIMS) which could be interpreted by analogy to results with similar compounds.[8] A scheme which can be used to understand the major fragments observed in these spectra appears in Figure 1. Peaks at *m/e* 191, 163, 145, and 173 were very common (cf. fragments g–j, Figure 1). A peak at *m/e* 203 was common to tobramycin and kanamycin B and their derivatives; in the spectrum of tobramycin, accurate mass measurement allowed this fragment to be assigned the empirical formula $C_8H_{15}N_2O_4$. In factors **8–10**, which lack the second glycosidic unit at the 6-hydroxyl, the *m/e* 203 peak was weak or absent. We formulate this fragment as k (Figure 1), though of course the specific structure of the ion cannot be elucidated on the basis of the above evidence. It is interesting that an amine at carbon 3″ (i.e., $R_3 = NH_2$, Figure 1) seems to be essential to the origin of this ion, which is absent in the spectra of factors **3** and **12**.

A major fragmentation pathway results from disruption of the glycoside attached to O(4) of the deoxystreptamine unit, leading to fragments c–f. Analogous fragmentation of the other glycoside did not appear to be important in most cases. The *m/e* values of fragments a–f were useful in characterizing the substitution patterns throughout the molecules. While (M + 1) peaks were detectable in some cases, only field-desorption mass spectrometry (FDMS) yielded reliable molecular weight determinations, especially for the carbamate derivatives. The latter compounds yield EIMS which are very similar to those of the parent aminoglycosides. Finally, while apramycin (factor **2**) gave an EIMS which could be interpreted in terms of a fragmentation pattern analogous to that shown in Figure 1, the oxygenated factor **7** yielded only a FDMS. As a consequence, mass spectrometry could not be used to aid in the location of the extra oxygen atom of this factor.

^{13}C and ^{15}N NMR data for the nebramycin factors are collected in Table II. Peak assignments are based largely on previous work.[4-6] We regard the assignments of carbon resonances in the chemical-shift regions of 70–75 ppm in alkaline solutions and 65–75 ppm in acidic solutions to be tentative. Resonances of nitrogens 1, 3, 7′, and 4″ have been assigned on the basis of detailed titration experiments that are reported later. The variation in the chemical shifts of N(6′) in factors **3**, **4**, **5**, **5′**, **6**, **8**, **11**, and **12** is due to the very high pK_a of this nitrogen,[6] a fact not recognized until after many of these spectra were measured. Thus, variation of the pH of the solution from 9.5 to 11 effects a chemical-shift change of almost 2 ppm in this resonance.

Discussion

Factor 3. The molecular weight of factor **3**, as measured by FDMS, is 1 mass unit higher than that of factor **5** (kanamycin B). Such a circumstance would result if one of the amine functions of kanamycin B was replaced by hydroxyl. The ^{15}N NMR spectrum, in which only four nitrogen resonances were observed, supports this formulation. The EIMS shows that fragments b–f are all displaced 1 mass unit to higher mass, indicating that the 3-amino-3-deoxyglucose unit of kanamycin B has been replaced by a hexose. Therefore, factor **3** is identical with the previously reported aminoglycoside NK-1012-1.[12]

The ^{13}C NMR spectrum supports this conclusion. The peak at 55.1 ppm in the spectrum of kanamycin B, which has been assigned[4] to the 3″ carbon, has been replaced by a resonance in the 70–75-ppm region in the spectrum of factor **3**. Such a change is consistent with the replacemment of NH_2 with OH. When peaks characteristic of the 2-deoxystreptamine and 2,6-diamino-2,6-dideoxyglucose portions are subtracted from the ^{13}C NMR spectrum of factor **3**, the remaining six resonances accord well with the spectrum of the α-glucosidic moiety of methyl β-maltoside.[13] Additional NMR support of the proposed structure (Chart I) of factor **3** are the ^{15}N chemical shifts.[6]

Factor 7. Careful comparison of the ^1H NMR spectra of factors **2** and **7** led to the conclusion that the latter factor was 3′α-hydroxyapramycin (Chart I).[14] Other physical data are in full accord with this proposal. Thus, the FDMS of factor **7** indicates that its molecular weight is 555, i.e., 16 mass units higher than apramycin. The ^{13}C NMR spectrum contained 21 resonances, 3 of which occurred at chemical shifts typical of anomeric carbons. Furthermore, 12 of the carbon resonances accorded well with those assigned to the 4-amino-4-

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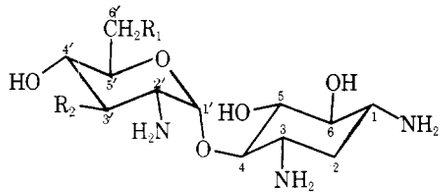
Table I. Mass Spectra of the Nebramycin Factors

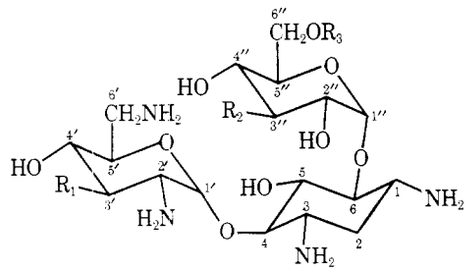
Factor	3	5	5′	6	8	9	10	11	12	13
Mol wt: FDMS[a]	484	483	510	467		307		510	468	510
EIMS (M + 1)	485	484		468	307[c]	308	323		469	
a	161	161	145	145	145	146	161	145[b]	145	145[b]
b	163	162	162[b]	162				162	163	162
c	353	352	352[b]	352				352	353	352
d	325	324	324[b]	324				324	325	324
e	307	306	306[b]	306				306	307	306
f	335	334		334				334	335	

[a] The most intense peak in the FDMS occurs at M + 1. [b] Due to the facile loss of CONH during the EIMS process, peaks incorporating this moiety are not observed. [c] $C_{12}H_{27}N_4O_5$.

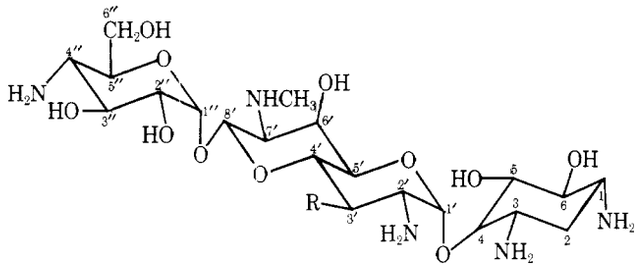
Chart I



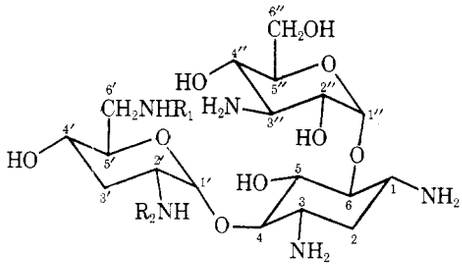
factor 8 (nebramine), $R_1 = NH_2$; $R_2 = H$
9 (lividamine), $R_1 = OH$; $R_2 = H$
10 (neamine), $R_1 = NH_2$; $R_2 = OH$



factor 3, $R_1 = OH$; $R_2 = OH$; $R_3 = H$
4, $R_1 = OH$; $R_2 = NH_2$; $R_3 = CONH_2$
5 (kanamycin B), $R_1 = OH$; $R_2 = NH_2$; $R_3 = H$
5′, $R_1 = H$; $R_2 = NH_2$; $R_3 = CONH_2$
6 (tobramycin), $R_1 = H$; $R_2 = NH_2$; $R_3 = H$
12, $R_1 = H$; $R_2 = OH$; $R_3 = H$



Factor 2 (apramycin), R = H
7, R = OH



factor 11, $R_1 = H$; $R_2 = CONH_2$
13, $R_1 = CONH_2$; $R_2 = H$
Cbz-tobramycin A, $R_1 = CBz$; $R_2 = H$
Cbz-tobramycin B, $R_1 = H$; $R_2 = CBz$
Acetyltobramycin, $R_1 = COCH_3$; $R_2 = H$

deoxyglucose and 2-deoxystreptamine portions of apramycin. The remaining 9 resonances of the ^{13}C NMR spectrum of factors **2** and **7** are compared in Figure 2. It is seen from this comparison that the CH_2-3′ resonance of apramycin is replaced by a new peak in the 72–74-ppm region. In addition, peaks assigned to C-2′ and C-4′ [5] are shifted downfield by 4–6 ppm. Such changes are consistent with oxygenation at carbon C-3′.[4,5,15] It is also noted in Figure 2 that the remaining carbon resonances are relatively unchanged in the two factors, a result which is consistent only with an equatorial hydroxyl at C-3′. An axial hydroxyl at this position, for example, should lead to a shielding effect at C-1′. The ^{13}C data are therefore in full accord with the proposed structure[14] of factor **7**.

The ^{15}N NMR spectra of factors **2** and **7** are also characteristic of the differences in their structures. Of the five resonances of each spectrum, only one shows a significant change in chemical shift. This large upfield shift associated with hydroxylation is typical of the γ effect of an oxygen atom.[6]

Factor 11. Field-desorption mass spectrometry indicated that the molecular weight of factor **11** was the same as that of factor **5′**, suggesting that these two aminoglycosides were isomeric. This hypothesis was supported by the presence of peaks characteristic of a carbonyl group in both the infrared (1660 cm^{-1}) and the ^{13}C NMR (161.2 ppm) spectra. The positions of these peaks, however, differed significantly from those of the known carbamates, factors **4** and **5′**. In fact, the stretching frequency in the infrared spectrum seemed in better accord with those of ureido groups.[15] On these bases, it was concluded that factor **11** was a ureido derivative of tobramycin.

The EIMS of factor **11** was essentially the same as that of tobramycin and was therefore not useful in locating the carbonyl substituent. Comparison of the ^{13}C spectra of factor **11** and tobramycin, however, suggested that the 2-deoxystreptamine and 3-amino-3-deoxyglucosidic moieties of these two factors were identical. In contrast, resonances assigned to C-1′ and C-3′ in the spectrum of tobramycin were significantly shielded in that of factor **11**. It was therefore inferred that the N(2′) of factor **11** was attached to a carbonyl group. This conclusion was supported by the ^{13}C NMR spectrum of factor **11** in acidic solution (pH 3). Figure 3 shows that C-1′ and C-3′ of factor **11** are significantly deshielded relative to tobramycin, consistent with the absence of a β-protonation effect[4,5] due to the nonbasic nature of N(2′).

On the basis of our present evidence, we believe that factor **11** is the *N*(2′)-ureido derivative of tobramycin. In repeated attempts to measure the ^{15}N spectrum of this factor, however, only five resonances were observed. The missing resonance is believed to be that due to the $CONH_2$ group. Possibly the difficulty in observing this signal is due to one of the "nulling" mechanisms known to complicate ^{15}N NMR spectroscopy[16] or to the presence of trace amounts of paramagnetic ions.[17] The very limited amount of this factor has precluded further studies.

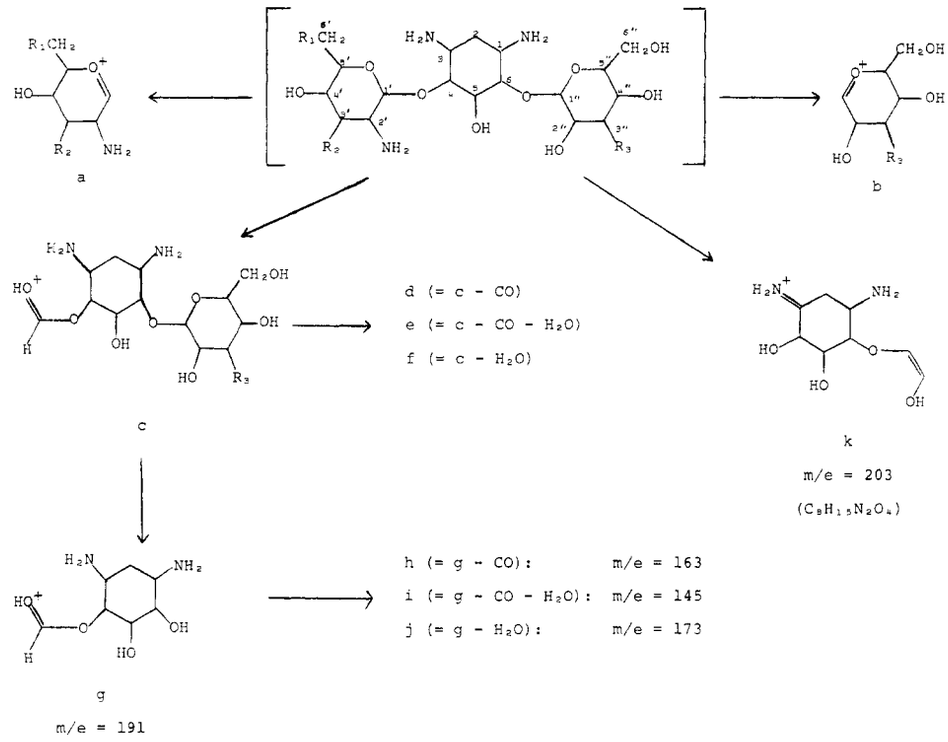


Figure 1. Representative electron-impact mass spectral fragmentation of the tricyclic nebramycin factors.

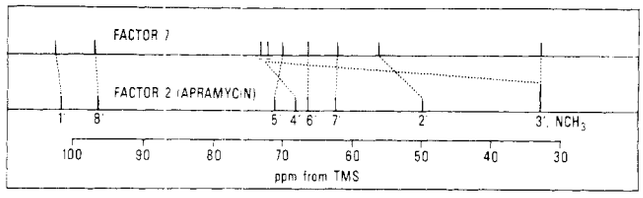


Figure 2. Comparison of the ^{13}C NMR spectra of the octose portions of apramycin and factor **7**.

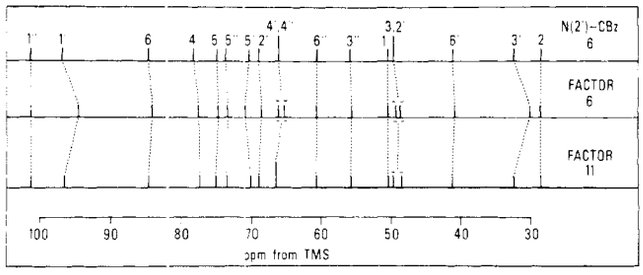


Figure 3. Comparison of the ^{13}C NMR spectra of *N*(2′)-carbobenzoxytobramycin, tobramycin (factor **6**), and factor 11 in acidic solution (pH 3).

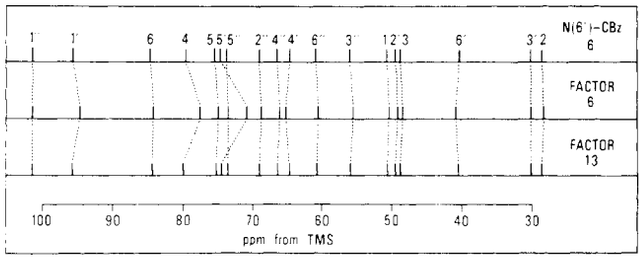


Figure 4. Comparison of the ^{13}C NMR spectra of *N*(6′)-carbobenzoxytobramycin, tobramycin (factor **6**), and factor **13** in acidic solution (pH 3).

Factor 12. Both FDMS and EIMS indicate a molecular weight of 468 for this factor. Comparison of its spectra to those of the other members of the nebramycin complex suggests that this compound is a deoxygenated factor **3**. Thus, the ^{15}N NMR spectra of factors **3** and **12** both show four resonances, one appearing at a high-field position characteristic of an aminomethyl group.[6] The four resonances of the spectrum of factor **12** correspond well with the chemical shifts of the resonances of nitrogens 1, 3, 2′, and 6′ of tobramycin and nebramine, suggesting that this portion of the molecule is similar in these three factors. Both factors **3** and **12** show b fragments with *m/e* 163, indicating the presence of a hexosoyl moiety. Comparison of the ^{13}C NMR spectra of factors **3, 6,** and **12** confirms the assignment of the 3′-deoxy factor **3** structure to the last compound.

Factor 13. The molecular weight of this aminoglycoside is also shown by FDMS to be the same as that of factors **4, 5′,** and **11**. The presence of a carbonyl group is demonstrated by the ^{13}C NMR spectrum (resonance at 162.2 ppm) and by an infrared band at 1660 cm^{-1}; as indicated above, the latter datum is characteristic of a urea. Factor **13** was therefore proposed to be a ureidotobramycin.

Comparison of the ^{13}C spectrum of this factor with that of tobramycin shows that these two compounds must be very similar structurally. The only significant differences between the ^{13}C spectra of these factors are in the chemical shifts of carbons 4, 1′, 2′, and 6′. In acidic milieu (Figure 4), the resonances of the two methylenes (2 and 3′) in factor **13** and tobramycin have very similar chemical shifts, indicating that the ureido group cannot be located at positions 1, 3, or 2′. The chemical shift differences at carbons 4, 1′, and 2′ in these factors therefore cannot result from substitution at these positions. Possibly these changes result from differing conformations around the ether bonds linking the 2-deoxystreptamine and 2,6-diamino-2,3,6-trideoxyglucose moieties of these aminoglycosides.

Because of the absence of the β-protonation shift[4,5] at carbon 5′ when factor **13** is dissolved in acidic solution (Figure 4), we propose that this factor is 6′-ureidotobramycin. In support of this hypothesis is the close correspondence in the ^{13}C NMR spectra of factor **13**, *N*(6′)-carbobenzoxytobramy-

Table II. ^{13}C and ^{15}N Chemical Shifts[a] of the Nebramycin Factors (ppm)

Carbon resonances	Factor[c] 2	3	4	5	5′	6	7	8	9	10	11	12	13	N(6′)-Acetyltobramycin[c]	6′-Cbz-tobramycin A[c]	2′-Cbz-tobramycin B[c]
1	51.1	51.2	51.4	51.2	51.5	51.2	51.1	51.1	51.3	51.2	51.0	51.2	51.3	51.4	51.5	51.2
2	36.6	36.3	36.2	36.2	36.5	36.5	36.6	36.6	36.6	36.5	36.3	36.4	36.5	36.6	36.8	36.5
3	50.3	50.2	50.1	50.1	50.1	49.9	50.1	50.3	50.5	50.1	50.2	50.3	50.1	50.2	50.5	50.5
4	87.8	87.6	87.1	86.7	87.4	87.3	88.1	87.7	88.1	88.0	87.3	87.0	88.2	88.7	89.0	88.3
5	76.8	75.2	75.0	75.2	75.1	75.3	76.8	76.8	76.9	76.8	75.0	75.3	75.3	75.4	75.6	75.1
6	78.4	88.8	88.3	88.5	88.6	88.7	78.3	78.5	78.4	78.3	89.3	89.0	88.7	89.0	89.2	89.2
1′	101.6	101.2	100.9	100.7	100.7	100.4	102.4	100.8	101.0	101.6	98.0	100.3	100.9	101.4	101.7	98.2
2′	49.8	56.2	56.1	56.1	50.4	50.2	56.1	49.9	50.0	56.1	49.7	49.9	50.6	50.7	50.9	50.9
3′	32.9	72.2	72.2	72.3	35.9	35.8	72.2	35.9	35.8	72.2	33.5	35.7	35.6	35.8	36.3	33.4
4′	67.9	72.7	73.0	72.9	67.1	67.0	73.1	67.0	65.5	73.9	66.6	67.0	67.0	67.3	67.3	66.7
5′	71.0	74.4	74.3	74.2	74.5	74.5	70.1	74.6	74.4	74.4	74.7	72.3	73.3	73.1	73.8	74.4
6′	66.2	42.4	42.3	42.3	42.6	42.6	66.3	42.5	61.7	42.5	42.3	42.4	41.8	41.3	42.8	42.5
7′	62.3						62.1									
8′	96.4						96.9									
NCH_3	32.9						32.9									
1″	95.3	101.2	100.6	100.7	100.4	100.7	95.9				100.8	101.3	100.8	100.9	101.1	100.9
2″	71.7	74.0	72.5	72.6	72.6	72.6	71.7				72.5	74.2	72.7	73.0	73.5	73.2
3″	74.2	72.5	55.0	55.1	55.2	55.2	74.3				55.0	72.5	55.3	55.2	55.5	55.2
4″	53.2	70.1	70.2	70.1	70.3	70.2	53.1				70.2	70.2	70.3	70.6	70.6	70.3
5″	73.4	74.0	71.1	72.9	71.0	73.0	73.4				73.1	73.9	73.0	73.0	73.0	72.7
6″	61.6	61.0	64.6	61.2	64.6	61.3	61.7				61.3	61.0	61.3	61.7	61.7	61.1
Others			$CONH_2$: 159.8		$CONH_2$: 159.9						$CONH_2$: 161.2		$CONH_2$: 162.2			
Nitrogen resonances																
1	8.2	7.7	7.6	7.9	7.9	8.0	8.0	7.9			7.6	7.9	7.9			
3	9.1	9.1	9.1	9.3	9.6	9.4	8.4	9.3			9.1	9.3	9.6			
2′	7.5	−1.0	−0.9	−0.8	7.5	7.4	−1.0	7.1			68.0[b]	7.3	7.4			
6′	–	−8.5	−8.5	−7.9	−7.5	−6.8	–	−7.7			−8.0	−7.0	52.3			
7′	−1.1	–	–	–		–	−1.2	–								
3′	–	–	1.1	1.2	1.3	1.3	–	–			1.0		1.3			
4′	0	–	–	–		–	0	–								
Others			$CONH_2$: 52.0		$CONH_2$: 52.3											

[a] ^{13}C chemical shifts are relative to external Me_4Si; ^{15}N shifts are relative to external NH_4Cl. All chemical shifts are measured at ca. pH 10. [b] This peak may be an instrumental artifact. [c] Registry no.: **2**, 37321-09-8; **3**, 31077-70-0; **4**, 51736-76-6; **5**, 4696-76-8; **5′**, 51736-77-7; **6**, 32986-56-4; **7**, 56283-52-4; **8**, 34051-04-2; **9**, 36019-33-7; **10**, 3947-65-7; **11**, 64332-33-8; **12**, 64332-34-9; **13**, 64332-35-0; N(6′)-acetyltobramycin, 61083-42-9; 6′-Cbz tobramycin, 50721-30-7; 2′-Cbz tobramycin, 64332-36-1.

cin, and N(6′)-acetyltobramycin. The structure of the last derivative has been determined through ^{15}N NMR spectroscopy.[6]

As in the case of factor **11**, the ^{15}N NMR spectrum of factor **13** showed five, rather than the expected six, resonances. The high-field resonance typical of the aminomethyl group was not present, confirming the hypothesis that this nitrogen had been acylated.

Carbobenzoxytobramycins A and B. Treatment of tobramycin in aqueous tetrahydrofuran (THF) with 1 equiv of *N*-(benzyloxycarbonyloxy)succinimide, followed by extensive chromatography, led to the isolation of two isomeric monocarbobenzoxylated derivatives of tobramycin. It is evident from Table II and Figures 3 and 4 that the ^{13}C NMR spectra of these compounds accord well with those of factors **11** and **13**. On this basis, carbobenzoxytobramycins A and B were identified as 6′-Cbz and 2′-Cbz derivatives, respectively.

***N*(6′)-Acetyltobramycin.** Treatment of an aqueous THF solution of tobramycin with 1 mol equiv of acetic anhydride led to the formation of a monoacetamide. From ^{15}N NMR spectroscopy, it was immediately evident that this derivative was N(6′)-acetyltobramycin.[6] Thus, of the five nitrogen resonances in the ^{15}N spectrum, only that assigned to N(6′) was significantly deshielded relative to tobramycin. Also in accord with the proposed structure is the ^{13}C NMR spectrum, which accords well with those of factor **13** and Cbz-tobramycin A (vide supra).

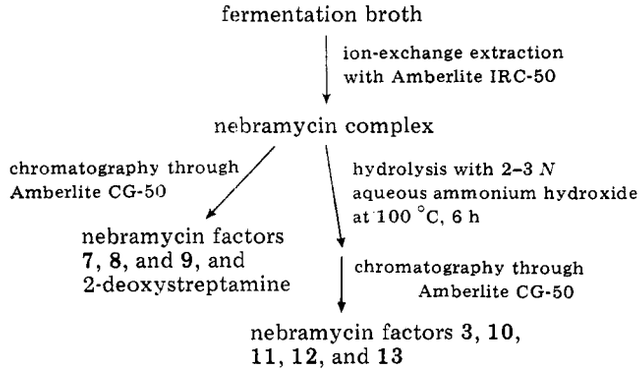
Experimental Section

^{13}C (25.03 MHz) and ^{15}N (10.09 MHz) NMR spectra were measured on a JEOL PFT-100 NMR spectrometer interfaced to an EC-100 data system. Full-proton decoupling was used in all measurements. The conditions of collection and transformation of spectra would be expected to lead to maximum line-broadening increments of 0.7 Hz for ^{13}C and 2 Hz for ^{15}H NMR spectra. Electron-impact mass spectra were obtained by direct ion-source introduction using a Varian-MAT Model 731 mass spectrometer at an ionizing energy of 70 eV. The same instrument was used to determine field-desorption spectra from carbon dendrite emitters.

Isolation and Purification of Nebramycin Minor Factors. The recovery of the crude nebramycin complex by ion-exchange extraction from the fermentation broth has been reported previously.[2] The separation of the complex has also been reported[2] and was accomplished by chromatography through Amberlite CG-50 resin.

Some of the new compounds, which include factors **7**, **8**, and **9**, and 2-deoxystreptamine, were isolated by direct chromatographic separation of the complex. Factors **3**, **10**, **11**, **12**, and **13** were separated from

Scheme I
Isolation and Purification of Nebramycin Minor Factors



the complex after mild basic hydrolysis. This hydrolysis was carried out with a 3–5% aqueous solution of the complex and 2–3 N aqueous ammonium hydroxide at 100 °C. The chromatography columns were eluted with aqueous ammonium hydroxide gradients. The direct separation of the nebramycin complex gave the following order of elution: nebramycin factors 7 and 9, 2-deoxystreptamine, and nebramycin factor 8. The separation of the hydrolyzed complex gave another elution sequence: nebramycin factors 13, 3, 11, 12, and 10.

The chromatography fractions were identified by thin-layer chromatography. Thin-layer chromatography was performed on silica gel (60 F254, EM Laboratories, Inc.) using either a mixture of methanol/chloroform/28% aqueous ammonia in the volume ratio of 3:1:2 or an aqueous solution containing 1.5 mol of sodium acetate, 1 mol of sodium chloride, and 100 mL of *tert*-butyl alcohol per liter of solution.

Identical chromatography fractions were combined, freeze-dried, and dependent on the TLC result, either purified or rechromatographed. The overall isolation and purification sequence of the nebramycin minor factors is illustrated in Scheme I.

Nebramycin Factor 3. The combined and freeze-dried chromatography fractions were decolorized with carbon and crystallized from methanol. A white solid, $[\alpha]^{20}_D$ + 136°, was obtained. Hydrolysis with 3 N aqueous hydrochloric acid at 90 °C gave neamine, which was identified by thin-layer chromatographic comparison with an authentic sample.

Nebramycin Factor 7. The crude material was purified by crystallization from aqueous 1-propanol. A white solid, $[\alpha]^{20}_D$ +170°, mp >265 °C dec, was obtained. Anal. Calcd for $C_{21}H_{41}N_5O_{12}$: C, 45.39; H, 7.44; N, 12.61; O, 34.55. Found: C, 45.10; H, 7.53; N, 12.37; O, 34.82.

Nebramycin Factor 8. The freeze-dried chromatography fractions were decolorized with carbon and crystallized from methanol. A white solid, $[\alpha]^{20}_D$ +110°, mp >225 °C dec, was obtained. Anal. Calcd for $C_{12}H_{26}N_4O_5$: C, 47.05; H, 8.55; N, 18.27. Found: C, 47.07; H, 8.35; N, 17.92.

Nebramycin Factor 9. The dried material was decolorized with carbon and crystallized from a methanol/ethanol mixture. Recrystallization from methanol afforded a white solid, $[\alpha]^{20}_D$ +94°, mp 222–224 °C dec. Anal. Calcd for $C_{12}H_{25}N_3O_6$: C, 46.90; H, 8.20; N, 13.67. Found: C, 47.18; H, 8.46; N, 13.65.

Nebramycin Factor 10. The combined dried material was decolorized with carbon and crystallized from methanol. A white solid, $[\alpha]^{20}_D$ +123°, mp >300 °C dec, was obtained.

Nebramycin Factor 11. The crude material was decolorized with carbon and crystallized from methanol. A white solid, $[\alpha]^{20}_D$ +123.5°, was obtained. Hydrolysis with 3 N aqueous hydrochloric acid at 90 °C gave tobramycin, which was identified by TLC comparison with an authentic sample.

Nebramycin Factor 12. This compound was purified by crystallization from methanol. An almost white solid was obtained.

Nebramycin Factor 13. Crystallization from a methanol/ethanol mixture yielded an off-white solid.

2-Deoxystreptamine. The freeze-dried chromatography fractions were decolorized with carbon and crystallized from methanol. The white solid had no optical rotation and was identified by its spectral data and TLC comparison with an authentic sample.

Carbobenzoxytobramycins A and B. A solution of 25 g (0.054 mol) of tobramycin in aqueous THF was cooled to −3 °C. To this solution was added 14.7 g (0.059 mol, 1.1 mol equiv) of *N*-(benzyloxycarbonyloxy)succinimide in THF in five portions. The reaction was warmed to room temperature and allowed to stir for 90 min. The solution was concentrated and extracted with chloroform and 1-butanol. The extracted aqueous solution was loaded onto a Bio-Rex 70 (NH_4^+) column and eluted with a NH_4OH gradient; yields: 7.5 g (23%) of Cbz A; 0.6 g (2%) of Cbz B.

***N*(6′)-Acetyltobramycin.** A solution of 5 g (0.011 mol) of tobramycin in 6% THF/water was cooled to 0 °C. To this solution was added 1.1 g (0.011 mol) of acetic anhydride. The reaction mixture was maintained at −4 °C for 16 h and then allowed to come to room temperature for 24 h. The solution was concentrated and loaded onto a Bio-Rex 70 (NH_4^+) column which was eluted with a NH_4OH gradient; yield: 1 g (18%) of *N*(6′)-acetyltobramycin.

Registry No.—*N*-(Benzyloxycarbonyloxy)succinimide, 13139-17-8.

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