SEPARATION OF AMINOGLYCOSIDIC ANTIBIOTICS BY GAS-LIQUID CHROMATOGRAPHY

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Eighteen aminoglycosidic antibiotics and their degradation products, containing 2-deoxystreptamine as a common moiety, were separated by gas-liquid chromatography. The largest compounds examined included the pentacyclic lividomycins A and B. These compounds were separable from each other either as pertrimethylsilyl derivatives or N-trifluoroacetyl pertrimethylsilyl derivatives on a single column of OV-1. The relative retention times are discussed in terms of structure.

During the past decade, gas-liquid chromatography (GLC) has become one of the most important analytical tools for the investigation of carbohydrates and related compounds, particularly after the introduction of volatile trimethylsilyl (TMS) derivatives. However, the literature concerning GLC analysis of carbohydrate antibiotics contains relatively few examples. Margosis[1] reported the GLC behavior of the dicyclic TMS-lincomycins, and Iwasa and coworkers[2] separated the tricyclic TMS-validamycins A and B on a column of SE-30. In the field of aminoglycosidic antibiotics containing 2-deoxystreptamine as a common moiety, Tsuji and Robertson[3] were the first workers to apply GLC to the separation of the tetracyclic TMS-neomycins B and C, employing a column of OV-1. Very recently, the same authors[4] reported the GLC behavior of the tricyclic TMS-kanamycins and the tetracyclic TMS-paromomycins. Retention times of TMS-paromomycins and TMS-paromamine were recorded by Hessler and coworkers[5].

Independent of them, we have studied the GLC behavior of a wide variety of aminoglycosidic antibiotics and their partial degradation products. The compounds examined included compounds from the monocyclic 2-deoxystreptamine (**1**) up to the pentacyclic lividomycins A and B (**17** and **18**), the latter two being the largest molecules of this class hitherto found in nature[6].

Experimental

Materials: Lividomycins A and B (**17**, **18**) were supplied from Dr. Oda of Kowa Kagaku Ltd., Tokyo*. A sample of lividomycin D (**16**) was obtained from fermentation broth of *Streptomyces microsporeus* nov. sp., and identified with lividomycin D by the structural study carried out in this laboratory[7].

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* We wish to express our thanks to Dr. Oda for supplying authentic samples of lividomycins A and B.

3′-Deoxyparomamine (**3**)[6] was prepared by partial methanolysis of antibiotic SF-767 A[7], which was identical with lividomycin B. Standard preparations of kanamycins A, B and C (**6**, **7**, **8**) were supplied from Kawasaki Factory of this company. 6″-Amino-kanamycin (**9**) and 3′-amino-kanamycin (**10**) were synthesized from kanamycin (**6**)[8,9]. Ribostamycin (**5**)[10] and destomycin A (**11**)[11] used were isolated in this laboratory, and are authentic. Respective mixtures of neomycins B and C and paromomycins I and II were obtained commercially, and each components were separated by column chromatography over Dowex 1×2[12]. Other compounds were prepared in this laboratory by the known procedures.

GLC Conditions: A Hewlett-Packard Gas Chromatograph, Model 402 equipped with dual flame ionization detectors was used throughout the study. The columns were of 0.4×120 cm U-shaped glass tubes, packed with 0.7 % OV-1* on Gas-Chrom Q (100∼120 mesh). The carrier gas was helium at a flow rate of 60 ml/min. For mono- and dicyclic compounds (**1**∼**4**), GLC was carried out at an oven temperature of 230°C, with tricaprylin as an internal standard. As the volatility decreases with increasing molecular weight, oven temperatures for GLC of tricyclic (**5**∼**11**), and tetra- and pentacyclic (**12**∼**18**) antibiotics were raised to 270°C and 300°C, respectively, using trilaurin as a common standard.

Preparation of Pertrimethylsilyl Derivatives: One mg or less of a compound to be analyzed was placed in a small vial, wetted with water, and 0.1 ml of TMS-PZ (a reagent of Tokyo Kasei Kogyo, Tokyo) was added. The resulting solution stood at room temperature for 20 minutes, then was evaporated to dryness. The residue was dissolved in 0.1 ml of pyridine, and 50 μl of N-trimethylsilyldiethylamine was added. The stoppered vial was heated at 70∼80°C for 20 minutes. One μl of the solution was used for injection.

Preparation of N-Trifluoroacetylpertrimethylsilyl Derivatives: To 1 mg of a compound in a small vial were added 0.5 ml of methanol and excess S-ethyl trifluorothioacetate[6]. The mixture stood at room temperature overnight, then was evaporated to dryness. The residue was dissolved in 0.1 ml of TMS-PZ, and 1 μl of the resulting clear solution was injected for analysis after standing for 20 minutes.

Table 1. Retention times of aminoglycosidic antibiotics and their degradation products in the form of pertrimethylsilyl derivatives.

Compound	Number of rings	Retention time (min.)	Relative value
2-Deoxystreptamine (**1**)[1]	1	0.5	0.07
Paromamine (**2**)[1]	2	5.9	0.82
3′-Deoxyparomamine (**3**)[1]	2	3.4	0.47
Neamine (**4**)[1]	2	6.8	0.95
Ribostamycin (**5**)[2]	3	6.7	0.31
Kanamycin A (**6**)[2]	3	8.2	0.38
Kanamycin B (**7**)[2]	3	7.4	0.34
Kanamycin C (**8**)[2]	3	6.4	0.29[4]
6″-Amino-kanamycin A (**9**)[2]	3	10.4	0.48
3′-Amino-kanamycin A (**10**)[2]	3	5.5	0.25
Destomycin A (**11**)[2]	3	11.5	0.53
Neomycin B (**12**)[3]	4	11.2	3.11
Neomycin C (**13**)[3]	4	13.4	3.72
Paromomycin I (**14**)[3]	4	8.6	2.39
Paromomycin II (**15**)[3]	4	10.9	3.02
Lividomycin D (**16**)[3]	4	6.6	1.88
Lividomycin A (**17**)[3]	5	35.5	9.87
Lividomycin B (**18**)[3]	5	22.7	6.30

1) Oven temperature, 230°C. Relative values were determined with an internal standard of tricaprylin, assigned retention time of 1.00.
2) Oven temperature, 270°C. Relative values were determined with an internal standard of trilaurin, assigned retention time of 1.00.
3) Oven temperature, 300°C. Relative values were determined with an internal standard of trilaurin, assigned retention time of 1.00.
4) There is a discrepancy in the order of elution of kanamycin C between TSUJI's report and ours. TSUJI and ROBERTSON[4] assigned a peak that was eluted after kanamycin A to kanamycin C. On the contrary, the kanamycin C preparation that was isolated in this laboratory was eluted before kanamycins A and B under GLC conditions similar to those employed by TSUJI and ROBERTSON.

Results and Discussion

Table 1 summarizes the retention times of peaks shown by the pertrimethylsilyl derivatives of aminoglycosidic antibiotics and their degradation

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* Besides OV-1, we have tested SE-30, OV-210 and QF-1 as stationary phases; none was superior to OV-1.

Fig. 1. Programmed gas chromatogram of a mixture containing neamine (**4**), ribostamycin (**5**), kanamycin A (**6**), neomycin B (**12**) and lividomycin B (**18**) in the form of pertrimethylsilyl derivatives.

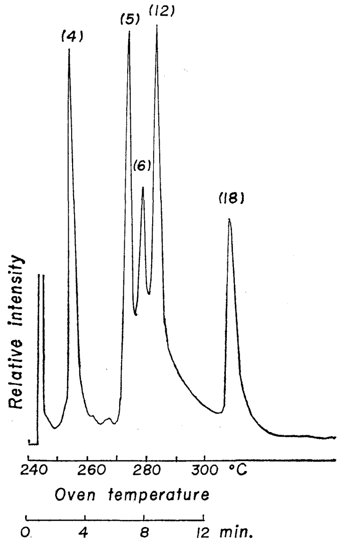
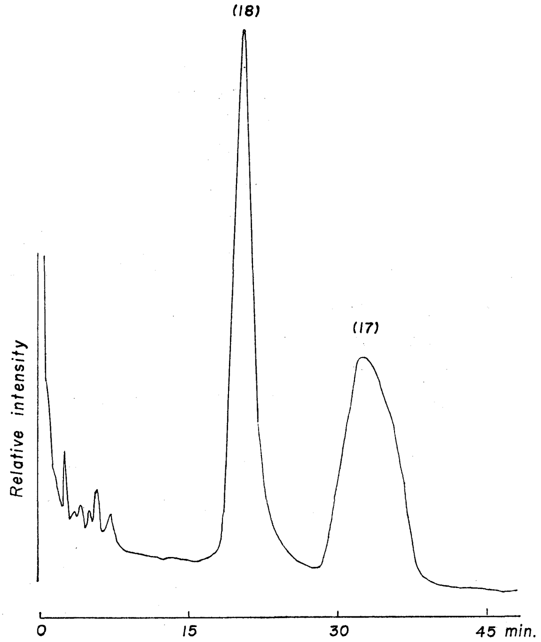


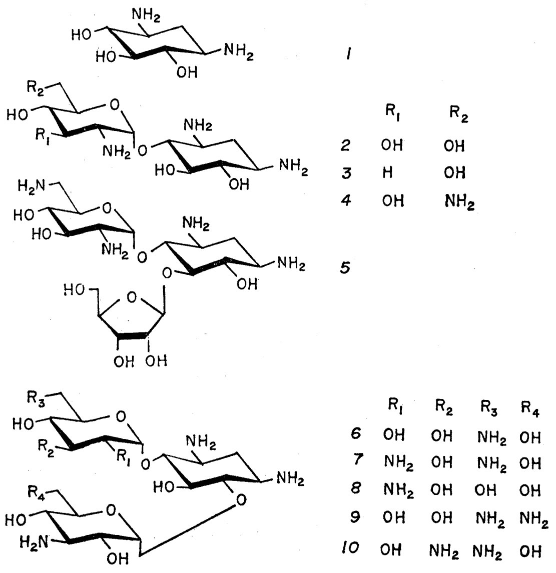
Fig. 2. Isothermal gas chromatogram of a mixture of TMS-lividomycins A (**17**) and B (**18**) at 310°C.



products. Figs. 1 and 2 illustrate the qualitative chromatograms of artificial mixtures of several antibiotics. The structures of the parent compounds are shown below. Although no effort was made to ascertain the structures of the TMS-derivatives, pertrimethylsilylation of amino and hydroxyl groups was assumed, by analogy to the results obtained by TSUJI and ROBERTSON[3,4].

It is generally recognized that the order of elution from the nonpolar OV-1 column is proportional to the volatility of the eluted compound, which is closely related to molecular weight among the structural analogues. This is clearly seen in Table 1, in which the monocyclic, dicyclic, tricyclic, tetracyclic and pentacyclic compounds are eluted in this order. To our best knowledge, this is the first report of GLC of pentacyclic TMS-derivatives (**17** and **18**). The close relationship between the number of rings and the retention time suggests that the number of the sugar moieties bound in an aminoglycosidic antibiotic can be estimated from the relative retention time.

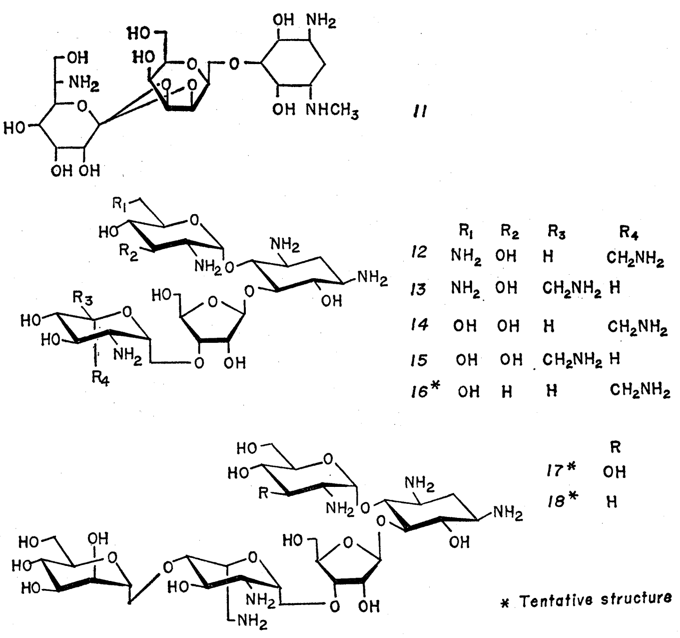
Chart 1



	R_1	R_2
2	OH	OH
3	H	OH
4	OH	NH_2

	R_1	R_2	R_3	R_4
6	OH	OH	NH_2	OH
7	NH_2	OH	NH_2	OH
8	NH_2	OH	OH	OH
9	OH	OH	NH_2	NH_2
10	OH	NH_2	NH_2	OH

Chart 2



Conversion of compounds **2**, **14** and **17** into their respective 3′-deoxy compounds (**3**, **16** and **18**) is accompanied by a remarkable decrease in the retention times. With regards to the effect of amino groups on the retention time, introduction of the terminal amino group into paromamine (**3**), paromomycins I and II (**14** and **15**), and kanamycin A (**6**) seems to retard the elution, as shown in the increased retention times of neamine (**4**), neomycins B and C (**12** and **13**) and 6′′-amino-kanamycin (**9**). On the other hand, substitution of an amino group at carbon 2′ or 3′ of kanamycin A (**6**) appears to accelerate the elution, as the retention times of kanamycin B (**7**) and 3′-amino-kanamycin A (**9**) were decreased.

Table 2. Retention times of aminoglycosidic antibiotics in the form of N-trifluoroacetyl-pertrimethylsilyl derivatives.

Antibiotic	Number of rings	Retention time (min.)	Relative value
Ribostamycin (**5**)[1]	3	4.5	0.21
Kanamycin A (**6**)[1]	3	7.9	0.36
Neomycin B (**12**)[2]	4	7.3	2.03
Paromomycin I (**14**)[2]	4	7.5	2.08

1) See footnote 2 in Table 1.
2) See footnote 3 in Table 1.

In the hope of finding a new derivative of antibiotic applicable to GLC, we have examined GLC of pertrimethylsilylethers of N-trifluoroacetyl (TFA) derivatives, whose retention times are shown in Table 2. Selective N-trifluoroacetylation of the antibiotics with S-ethyl trifluorothioacetate was demonstrated by an infrared band at 1708 cm^{-1} characteristic of the trifluoroacetamide group, and by four F^{19} NMR peaks, ascribable to four TFA groups, for derivatives of ribostamycin (**5**) and kanamycin A (**6**)*. Furthermore, the mass spectrum of N-TFA-pertrimethylsilyl ribostamycin showed a peak at *m/e* 1,255 (1,270−15), suggesting that the remaining active hydrogens in **5** were all silylated.

Comparison of the relative retention times shown in Tables 1 and 2 reveals that the N-TFA derivatives are eluted before the corresponding N-TMS derivatives. In this connection, it is interesting to see that the hexa-N-TFA derivative of neomycin B (**12**) is eluted before the penta-N-TFA derivative of paromomycin I (**14**), while the reverse is true for the form of N-TMS derivatives.

As is well established, GLC has the advantages of rapidity over solid-liquid or liquid-liquid chromatography, ease of quantitation and high sensitivity. Considering

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* The chemical shifts calculated from the F^{19} peak of trifluoroacetamide in water were −0.23, −0.06, 0.00 and +0.16 ppm in **5**, and −0.14, −0.07, +0.07 and +0.22 ppm in **6**.

those advantages, coupled with the data presented here, it may be reasonably concluded that GLC will provide a facile means to analyze aminoglycosidic antibiotics, which are often produced as mixtures of compounds of similar structures. Of the two series of derivatives described in this paper, the N-TFA derivatives merit further attention, since fluoro compounds may be analyzed with much higher sensitivity by utilizing an electron capture detector. This is currently being investigated in this laboratory.

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