ANTIMYCIN A COMPONENTS. II

IDENTIFICATION AND ANALYSIS OF ANTIMYCIN A FRACTIONS BY PYROLYSIS-GAS LIQUID CHROMATOGRAPHY

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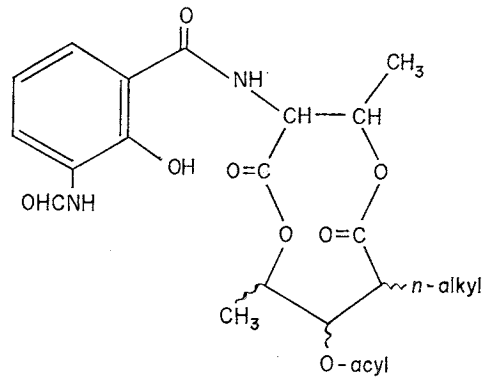
A relatively rapid method for the qualitative and semiquantitative analysis of the components present in the antimycin A complex is described. The method is based on the pyrolysis of the antimycin A complex and subsequent gas liquid chromatography of the pyrolysate. The mass spectra of isolated GLC fractions from the pyrolysate of pure antimycin A components are also used to support the chemical structure of the antimycin molecule.

In the preceding paper[1] we reported the fractionation of the antimycin A complex into its various components using countercurrent distribution. The identification of these individual fractions as well as their purity was originally based on a paper partition chromatographic method described by LOCKWOOD *et al.*[2] It became apparent to us that this method had its limitations when applied to the determination of the purity of a single antimycin A component, and we thus sought to develop a more rapid and more reliable routine method of analysis.

The structure of the antimycin molecule has been elucidated[3,4,5,6].

Because of the very small differences in the structures of the various antimycin A components it was essential to use a chromatographic step for the separation of these components or their derivatives. Straight gas liquid chromatography (GLC) was not applicable because of the thermal instability of the antimycin A molecule. This thermal instability was made use of in the development of a method based on pyrolysis of the compound and subsequent GLC of the pyrolysate.



Component	*n*-Alkyl	Acyl
A_1	*n*-hexyl	isovaleryl
A_3	*n*-butyl	isovaleryl

It was possible to demonstrate that the pyrolysate of each of the isolated pure antimycin A components showed a characteristic GLC pattern consisting of three major peaks. The unresolved antimycin A complex on the other hand yielded a pyrolysate which gave a GLC pattern showing the peaks of all the individual

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components mentioned. The method is thus suitable to characterize the antimycin A components and also allows a semiquantitative analysis of the relative amounts of the various antimycin A components which make up a particular antimycin A complex.

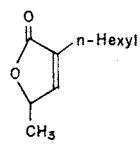
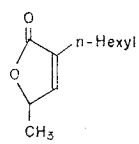
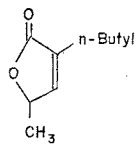
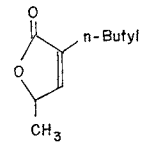
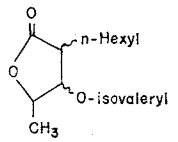
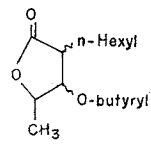
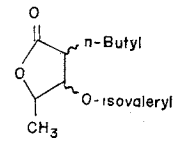
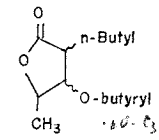
The systematic and consistent GLC patterns observed for the pyrolysates of the various pure antimycin A components which are the basis of the new method which will be described in detail.

Pyrolysis of an antimycin A component is carried out in a sealed glass tube under controlled conditions at *ca.* 550°C. The pyrolysate thus obtained shows three major peaks M_i, N_i and O_i for a component i. The systematic character of these three peak patterns is reflected in their relative retention times and also in the mass numbers of the molecular ions observed in the mass spectrum of each of the collected GLC fractions.

Two of the three peaks, N and O, are characteristic of each individual antimycin A component, while $M_{1,2}$ the peak with the shortest retention time (*RT* 5.65 min.) is observed in both antimycin A_1 and A_2; antimycin A_3 and A_4 share peak $M_{3,4}$ (*RT* 3.10 min.). The details of these thermolytic patterns are shown in Table 1; they suggest the following thermal breakdown pattern:

Table 1. Pyrolysis-gas liquid chromatography.

GLC conditions: 9 ft, 2.8 mm ID coiled glass columns
packing: 15% Se-30 on Chromosorb W 80~100 mesh
column temperature: 204°C
helium inlet pressure: 50 psi

	Antimycin A_1	Antimycin A_2	Antimycin A_3	Antimycin A_4
Peak M	5.65 min. (log: 0.752)	5.65 min. (log: 0.752)	3.10 min. (log: 0.491)	3.10 min. (log: 0.491)
Molecular ion observed mass number	182	182	154	154
Proposed structure				
Peak N	20.05 min. (log: 1.302)	14.70 min. (log: 1.167)	10.70 min. (log: 1.029)	7.80 min. (log: 0.892)
Molecular ion observed mass number	284	270	256	242
Proposed structure				
Peak O	23.15 min. (log: 1.365)	17.05 min. (log: 1.232)	12.15 min. (log: 1.085)	8.95 min. (log: 0.952)
Molecular ion observed mass number	284	270	256	242
Proposed structure	Stereochemical (or positional) isomer of pentanoic acid γ-lactone proposed for peak N series.			

Peak O, the peak with the longest retention time may be interpreted as 2-alkyl-3-acyloxy-4-hydroxy pentanoic acid -γ-lactone.

In the case of antimycin A_1 and A_2 the common 2-alkyl group is *n*-hexyl, in the case of antimycin A_3 and A_4 the common 2-alkyl group is *n*-butyl. In a similar way antimycin A_1 and A_3 lead to 2-alkyl-3-isovaleryloxy-pentanoic acid-γ-lactone while antimycin A_2 and A_4 give the corresponding 3-butyryloxy compound.

Antimycin	A_1	A_2	A_3	A_4
Peak	O_1 23.15 min.	O_2 17.05 min.	O_3 12.15 min.	O_4 8.95 min.
2-alkyl	*n*-hexyl	*n*-hexyl	*n*-butyl	*n*-butyl
3-acyloxy	isovaleryloxy	butyryloxy	isovaleryloxy	butyryloxy
Mass number of molecular ion	284	270	256	242

The second peak, N, shows in each case a mass spectrum similar to that of the corresponding peak O and has the same mass number for the molecular ions. Unfortunately it was not possible to prepare enough pyrolysate from a pure antimycin A component to collect sufficient material of each peak in order to obtain the other spectroscopic data, especially nuclear magnetic resonance spectra. A possible explanation based on the similarity of the mass spectra would bo that N is a stereochemical or positional isomer of the γ-butyrolactone structure assigned to peak O.

Antimycin	A_1	A_2	A_3	A_4
Peak	N_1 20.05 min.	N_2 14.7 min.	N_3 10.7 min.	N_4 7.8 min.
2-alkyl	*n*-hexyl	*n*-hexyl	*n*-butyl	*n*-butyl
3-acyloxy	isovaleryloxy	butyryloxy	isovaleryloxy	butyryloxy
Mass number of molecular ion	284	270	256	242

It is impossible at this time to determine whether the difference between peak O and peak N has its origin in the pyrolysis reaction or whether each antimycin A "component" itself consists already of two isomers. These isomers would then be pairs of stereoisomers in the 4-hydroxypentanoic acid moiety of the macrocyclic lactone system of the antimycin A molecule which give on pyrolysis two pentanoic acid -lactones. The loss of the oxygen function in position 3 would eliminate this stereochemical difference and thus give from both isomers the same peak M which is common for antimycin A_1 and A_2 and again for antimycin A_3 and A_4.

On the basis of the mass spectra which were obtained for each of the collected GLC fractions, the structure assigned to these peaks is 2-alkyl 4-hydroxy 2-pentanoic acid -γ-lactone.

Antimycin	A_1 and A_2	A_3 and A_4
Peak M	5.65 min.	3.10 min.
2-alkyl	*n*-hexyl	*n*-butyl
Mass number of molecular ion	182	154

The regular pyrolysis pattern is supported by the change of the relative retention times which accompanies each structural change, as shown by the excellent correlation of the differences of the logarithms of the retention times ($\Delta \log RT$) and the structural change:

Structural change	$\Delta \log RT$
n-hexyl ⟶ *n*-butyl	-0.276 ± 0.006
isovaleryl ⟶ butyryl	-0.136 ± 0.004
loss of isovaleric acid	-0.543 ± 0.009
loss of butyric acid	-0.409 ± 0.010
isomer O ⟶ isomer N	-0.061 ± 0.005

All details are shown in Table 2.

Pyrolyses of antimycin A samples were normally carried out by introducing only the sample area of the sealed tube into the heating block. In an experiment where the pyrolysate was passed through a tube section heated to 600°C, the condensate revealed only peak M thus suggesting the further thermal degradation of peaks O and N into M. Similarly the limited thermal stability of fraction O or N is also reflected in the fact that reinjection of collected fraction O or N invariably produced at least traces of the corresponding peak M.

It should be pointed out that in all the pyrolysates no peak was ever observed which could be associated in any way with the aromatic moiety of the antimycin A molecule. Surprising as this may seem, it is possible to explain this fact by the formation polymers.

We have not been able to observe any peak in the GLC of the pyrolysates which could be derived from the threonine part of the antimycin molecule. We have performed the pyrolysis of samples of ^{14}C labeled antimycin A complex, one sample labeled in the threonine part of the molecules and another in the aromatic moiety of the antimycin molecule*. Both compounds gave pyrolysis-GLC patterns normal for antimycin A complexes containing all the fractions described above. None of the ten GLC fractions collected showed any significant radioactivity.

Table 2. Δ logarithms of retention times supporting a systematic thermolytical breakdown pattern.

Structural change		$\Delta \log RT$
Isomer O ⟶ isomer N	$\log O_1 - \log N_1$	0.063
	$\log O_2 - \log N_2$	0.065
	$\log O_3 - \log N_3$	0.056
	$\log O_4 - \log N_4$	0.060
Average isomer O ⟶ isomer N		0.061 ± 0.005
Loss of isovaleric acid	$\log N_1 - \log M_1$	0.550
	$\log N_3 - \log M_3$	0.538
	$\log O_1 - \log M_1 - 0.061$	0.552
	$\log O_3 - \log M_3 - 0.061$	0.553
Average loss of isovaleric acid		0.543 ± 0.009
Loss of butyric acid	$\log N_2 - \log M_2$	0.415
	$\log N_4 - \log M_4$	0.401
	$\log O_2 - \log M_2 - 0.061$	0.419
	$\log O_4 - \log M_4 - 0.061$	0.400
Average loss of butyric acid		0.409 ± 0.010
n-hexyl ⟶ *n*-butyl	$\log M_{1,2} - \log M_{3,4}$	(0.261)
	$\log N_1 - \log N_3$	0.283
	$\log N_2 - \log N_4$	0.275
	$\log O_1 - \log O_3$	0.280
	$\log O_2 - \log O_4$	0.280
Average *n*-hexyl ⟶ *n*-butyl		0.276 ± 0.006
Isovaleryl ⟶ butyryl	$\log N_1 - \log N_2$	0.135
	$\log N_3 - \log N_4$	0.137
	$\log O_1 - \log O_2$	0.133
	$\log O_3 - \log O_4$	0.133
Average isovaleryl ⟶ butyryl		0.136 ± 0.004

* The radioactive antimycin A complexes were obtained from Dr. S. N. SEHGAL.

Since evidence for the structural difference of the pure antimycin A components is also reflected in their mass, IR, NMR and UV spectra, these analytical data of the intact antimycin A components are given below:

Mass Spectrometry

The mass spectra of each of the isolated antimycin A components show a fragmentation pattern consistent with the molecular structures reported. The patterns suggest fragmentation due to both electron impact and thermolysis. When the temperature of the direct inlet system of the mass spectrometer was kept at not more than 155°C, the molecular ion could be observed in each instance:

Component	Molecular ion
Antimycin A_1	548
Antimycin A_2	534
Antimycin A_3	520
Antimycin A_4	506

At higher inlet temperatures no molecular ions were observed.

Infrared Spectrophotometry

As may be expected for such closely related molecules, the infrared spectra of the four antimycin A components are very similar and show the typical broad absorption bands of the carbonyl stretching vibrations: Macrocyclic lactones and butyryl or isovaleryl ester are present at 1742 cm^{-1}, benzamide at 1695 cm^{-1} and formanilide at 1640 cm^{-1}, and in addition to this one also observes the aromatic ring vibration at 1610 and 1590 cm^{-1} and the second amide band at 1520 cm^{-1}. However, there are characteristic spectral differences between the two pairs of antimycin A_1 and A_2 on the one side and A_3 and A_4 on the other side. Since A_1 and A_2 share the same *n*-hexyl group while A_3 and A_4 have the *n*-butyl group in common, it is likely to associate the small differences in the 900 cm^{-1} to 1,100 cm^{-1} region with those vibration modes of the molecule which involve in particular the pentanoic acid part and the alkyl group. Fig. 1 shows the IR spectrum of the antimycin A_4 together with the 900~1,100 cm^{-1} region of the IR spectrum of antimycin A_1.

Nuclear Magnetic Resonance Spectra

As in the case of the IR spectra, the NMR spectra are again very similar but they stress the difference between A_1 or A_3 on the one side and A_2 or A_4 at the other side. The spectra of pure A_1 and A_3 show clearly a doublet δ 0.91 ppm J=5 Hz

Fig. 1. I. R. spectrum of antimycin A_4 together with the 900~1100 cm^{-1} region of the I. R. spectrum of antimycin A_1. The slight differences between the spectra are shown by arrows.

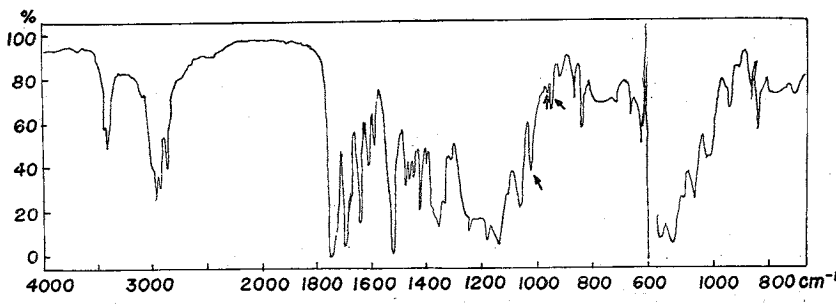
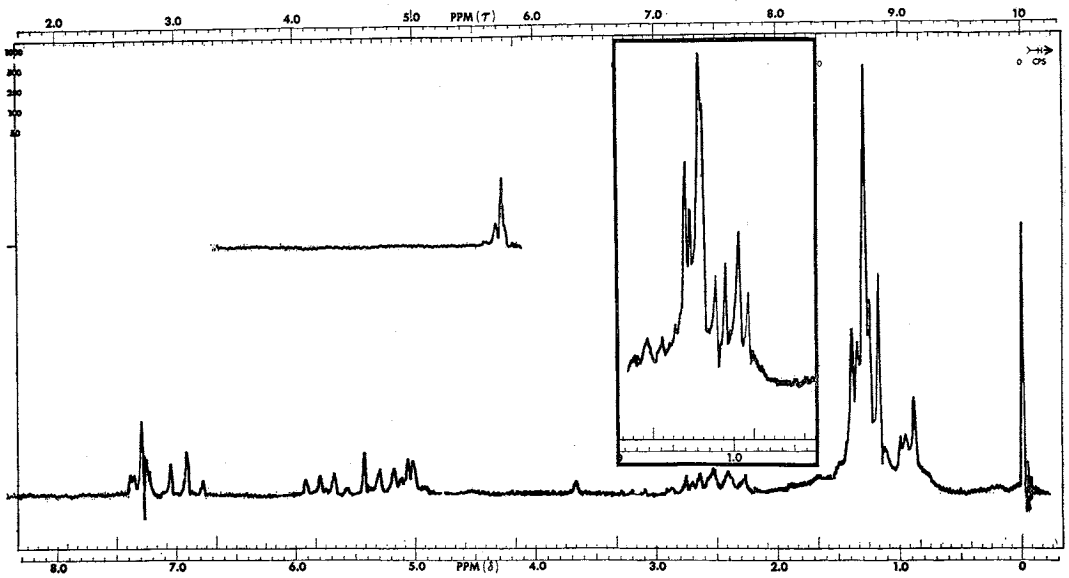


Fig. 2. N.M.R. spectrum of antimycin A_4 after D_2O exchange. This insert shows the part of the N.M.R. spectrum of antimycin A_3 δ 0.5~2.0 ppm showing the methyl doublet of the isovaleryl group at δ 0.91 ppm.

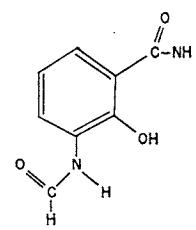


as one would expect from methyl groups of isovaleric acid. The NMR spectra of antimycin A_2 and A_4 show only the typical pattern observed for a terminal $-CH_2CH_2CH_3$ methyl group which is indistinguishable from the terminal methyl group present in the *n*-alkyl groups. Since the NMR spectra of all antimycin A components are similar, only the NMR spectrum of the newly described antimycin A_4 is shown (Fig. 2). The insert in Fig. 2 represents the part of the NMR spectrum of A_3 (δ 5~2 ppm) showing the methyl doublet $-CH<\begin{matrix}CH_3\\CH_3\end{matrix}$ of the isovaleryl group at δ 0.91 ppm. Table 3 gives the chemical shifts of all the protons in the antimycin A_4 molecule.

Table 3. N.M.R. Chemical shifts of antimycin A_4

Number of protons	Type of proton	Chemical shifts δ ppm tetramethyl silane as internal standard δ 0.00 ppm
27	Aliphatic protons	−0.7 to −2.0 ppm*
2	α methylene of butyryl group	−2.1 to −2.7 ppm multiplet
1	Tertiary proton of amino acid	
3	Tertiary carbinolic protons	−4.9 to −6.1 ppm several multiplets
1	Amide or formyl proton	−8.1 ppm
2	Both amide protons or one amide and one formyl proton	−8.6 ppm
1	Phenol proton	−12.6 ppm easily exchanged with D_2O

* In this signal the characteristic doublet of the isopropyl group (6 protons) are observed at −0.91 ppm (J=5Hz) in the NMR spectra of A_1 and A_3 (isovaleryl esters).



Ultraviolet Absorption Spectra

The UV absorption spectra of all four antimycin A components are practically identical and consistent with a common chromophor which is the aromatic moiety of the molecule. The absorption maximum at 317 mμ (ε=6000) shows a bathochromic shift to 341 mμ (ε=8000), on

making the alcohol solution alkaline with potassium hydroxide. All details are given in Table 4.

Microchemical Analysis

In the case of the newly described antimycin A_4 an elementary analysis was performed. The results are in good agreement with the proposed formula:

	C	H	N
Found	59.10 %	6.84 %	5.75 %
Calculated	59.22 %	6.76 %	5.53 %

Table 4. UV Spectra

Antimycin fraction	λ_{max}	ε
A_1	227 mμ	38,300
	317 mμ	6,000
	341 mμ (alkaline shift)	8,000
A_2	227 mμ	32,500
	317 mμ	5,580
	341 mμ (alkaline shift)	8,230
A_3	227 mμ	35,700
	317 mμ	6,200
	341 mμ (alkaline shift)	8,220
A_4	227 mμ	31,900
	317 mμ	5,500
	341 mμ (alkaline shift)	7,490

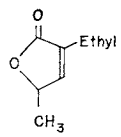
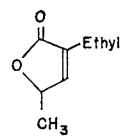
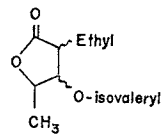
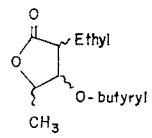
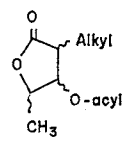
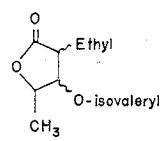
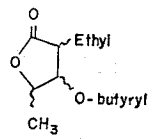
Other Components Observed in the Antimycin A Complex

It has been mentioned in the preceding paper[1] that apart from the four major components of the antimycin A complex (A_1, A_2, A_3, A_4) which have been isolated in the pure state and characterized extensively, several other minor components have been observed. Their presence had been demonstrated by paper partition chromatography although their isolation in the pure form failed due to the small quantities present in the total antimycin A complex. Thus counter-current distribution fractions could be obtained in which these antimycin A components were enriched but no more. The minor components A_5 and A_6 are considerably more polar than the four

Table 5. Pyrolysis gas liquid chromatography of counter current distribution fractions

Enriched in the more hydrophylic antimycin A fractions A_5 and A_6. GLC conditions (see Table 1).

Enriched in more lipophilic antimycin A fractions A_0. GLC conditions as in Table 1, except column temperatue.*

	Antimycin A_5	Antimycin A_6	Antimycin A_0
Peak M	1.89 min. (log: 0.276)	1.89 min. (log: 0.276)	12.75 min.* (log: 1.106) [9.1 min.]
Proposed structure			Peak is very small thus favouring the first of the two structures given below for N_0, since this would lead again to peak $M_{1,2}$ (Table 1).
Peak N	6.30 min. (log: 0.799)	4.65 min. (log: 0.667)	32.45 min.* (log: 1.511) [23.25 min.]
Proposed structure			 alkyl / acyl: a) hexyl / hexanoyl; b) butyl / heptanoyl; c) octyl / butyryl; d) heptyl / isovaleryl
Peak O	7.07 min. (log: 0.849) Stereo isomer of N_5	5.27 min. (log: 0.719) Stereo isomer of N_6	37.20 min.* (log: 1.569) [26.45 min.]
Proposed structure			Stereo isomer of N_0

* Retention times are corrected for GLC conditions given in Table 1, in [ ] brackets are actually observed retention times.

major antimycin A components, and naturally accumulate at the hydrophilic side of the counter-current distribution. Fractions in these zones contained antimycin A components which in paper partition chromatography had R_f values of 0.866 and 0.940. When subjected to pyrolysis-GLC the gas-chromatogram could easily be explained on the basis of the now familiar three peak pattern extended into a region of shorter retention times, as shown in Table 5. The strong similarity of the thermolytic pattern between the four isolated antimycin components described above seems also to apply to these new more hydrophilic components. The alkyl group again is shortened by one ethylene group from *n*-butyl to ethyl. Substitution in position 3 again is either isovaleryloxy or butyryloxy. The change of the relative retention time, expressed as $\Delta \log RT$ gives further support to the suggested thermolytic pattern:

Change	$\Delta \log RT$
n-butyl ⟶ ethyl	-0.228 ± 0.01
3-isovaleryloxy ⟶ 3-butyryloxy	-0.130 ± 0.01
loss of isovaleric acid	-0.523 ± 0.01
loss of butyric acid	-0.394 ± 0.01
isomer O ⟶ isomer N	-0.050 ± 0.01

Table 6. Antimycin A_5 and A_6

Structural change		$\Delta \log RT$
butyl ⟶ ethyl	$\log M_{3,4} - \log M_{5,6}$	0.215
	$\log N_4 - \log N_5$	0.225
	$\log O_4 - \log O_6$	0.233
	$\log N_3 - \log N_5$	0.230
	$\log O_3 - \log O_5$	0.236
Average butyl ⟶ ethyl		0.228 ± 0.013
isovaleryl ⟶ butyryl	$\log N_5 - \log N_6$	0.132
	$\log O_5 - \log O_6$	0.130
Expected value 0.137		0.131 ± 0.002
isomer 'O' ⟶ isomer 'N'	$\log O_5 - \log N_5$	0.050
	$\log O_6 - \log N_6$	0.052
Expected value 0.057 ± 0.006		0.050 ± 0.002
	Relative change may be smaller due to less accurate determinations of retention times.	
Loss of isovaleric acid Expected value 0.538	$\log N_5 - \log M_{5,6}$	0.523
Loss of butyric acid Expected value 0.405	$\log N_6 - \log M_{5,6}$	0.391

Table 7. Antimycin A_0

Correction factor 23.15/16.5=1.403

Peak M_0 12.76 (9.1) small peak should be 10× as big
$\log M_0$ calc 1.106
Peak N_0 32.45 (23.25)
$\log N_0$ 1.511
Peak O_0 37.20 (26.45)
$\log O_0$ 1.569
$\log M_0 - \log M_1$
Alkyl Hexyl $\log N_0 - \log N_1$ 0.209
$\log O_0 - \log O_1$ 0.204
Isomer O Isomer N $\log O_0 - \log N_0$ 0.058
Loss of butyric acid $\log N_1 - \log M_1$
Mass spectrum of pure antimycin in A_1 shows a trace of molecular ion 562 (A_0).

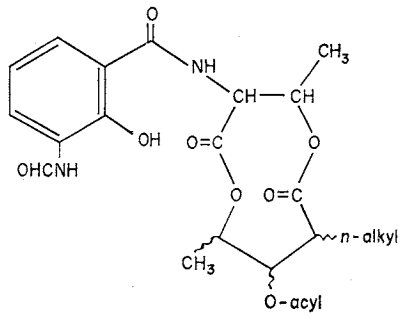
All details for obtaining the $\Delta \log RT$ are give in Table 6. The values are in agreement with those found for the four major peaks. The errors are somewhat larger since the shorter retention times are measured less accurately. The structures for antimycin A_5 and A_6 could not be confirmed by mass spectrometry because the compounds could only be obtained in the presence of certain amounts of antimycin A_4. The mass numbers of the molecular ions 492 for A_5 and 478 for A_6, are also found as fragments in the mass spectrum of pure antimycin A_4:

Mass number	Relative intensity
507	0.59
506	1.94 (molecular ion of A_4)
492	0.066
491	0.043
479	0.14
478	0.52
Base peak 43	100.00

On the lipophilic side of the counter-current distribution curve a zone was isolated which contained components less polar than A_1. The mass spectrum of the total antimycin A complex showed already a very small mass peak with mass number 562 *i. e.* 14 above A_1 (548). Pyrolysis-GLC of this enriched fraction showed again peaks with even longer retention times (Table 5). The pair of peaks (37.05 min and 32.45 min) have a $\Delta \log RT$ of -0.057 which would be in good agreement with the change of relative retention time observed for the N and O pairs in all the other cases. Calculated on the basis of $\Delta \log RT$ -0.275 going from *n*-butyl to *n*-hexyl or 0.23 going from ethyl to *n*-butyl, the retention times for the pair N_0 and O_0 are considerably shorter and there is hardly any peak at 12.5 min. It is therefore more likely to assume that the alkyl group is again *n*-butyl or *n*-hexyl but having an acyloxy group which is larger by one or two methylene groups. Pyrolysis would lead to a pair N_0 and O_0 as observed. Loss of the acyloxy group would now lead to one of the two common crotono lactones M: if the alkyl group is *n*-butyl, peak M_0 would be identical with M_3 or M_4, *i. e.* the acyl group is larger by two methyl groups; if the alkyl group is *n*-hexyl, peak M_0 would be identical with peak M_1 or M_2, *i. e.* the acyl group is larger by one methylene group.

Since these compounds were not available in either larger quantities in a pure form, observations could not be corroborated by sufficient analytical data, which means our conclusions are largely speculative.

On the basis of the pyrolysis-GLC analysis of the various pure and the enriched antimycin A fractions, we propose the presence of at least seven antimycin A components listed in increasing polarity.



Component	*n*-alkyl	acyl
A_0	a) *n*-hexyl C_6H_{13}	hexanoyl
	b) *n*-butyl C_4H_9	heptanoyl $C_6H_{13}CO$
	c) octyl C_8H_{17}	butyryl C_3H_7CO
	d) heptyl C_7H_{15}	isovaleryl C_4H_9CO
A_1	*n*-hexyl C_6H_{13}	isovaleryl C_4H_9CO
A_2	*n*-hexyl C_6H_{13}	butyryl C_3H_7CO
A_3	*n*-butyl C_4H_9	isovaleryl C_4H_9CO
A_4	*n*-butyl C_4H_9	butyryl C_3H_7CO
A_5	ethyl C_2H_5	isovaleryl C_4H_9CO
A_6	ethyl C_2H_5	butyryl C_3H_7CO

a) or b) would explain absence of peak M_0 (identical with $M_{1,2}$ or $M_{3,4}$) all four would explain mass peak of 562 observed in intact antimycin A complex.

Experimental

Pyrolysis: 2~3 mg of antimycin A are wrapped in a small piece of aluminum foil and introduced as a small plug into the bottom section of a 20~25 cm long pyrex tube with an internal diameter of 0.4 cm, closed at one end. The tube is then drawn out into a narrow section about 15 cm from the bottom. Finally the tube is alternately evacuated (10^{-2} mmHg) and rinsed with dry nitrogen at least 5~6 times, then sealed off under vacuum at the narrow part of the tube. The sample side is now introduced about 2~3 cm into a stainless steel block pre-heated to 600°C. The temperature of the block is measured with a thermo-couple. After about twelve seconds the pyrolysis sets in suddenly. The aluminum foil acts as heat shields avoiding a slow initial thermolysis. The pyrolysate condenses in the cold part of the tube. The tube is then opened, the condensate is taken up in 0.5 ml ethylacetate and now ready for injection. The GLC conditions are given in Table 1.

Collection of GLC fractions for reinjection or mass spectrometry—The fractions were collected in glass capillaries introduced into the 1/8" OD stainless steel outlet of a Perkin-Elmer model 800 gas chromatograph*). The instrument was modified for all-glass columns with on-column injection. The collected fracitons were sealed in their capillaries and submitted for mass spectrometry.

Mass Spectrometry—All mass spectra have been obtained on a Hitachi-Perkin-Elmer RMU 6 mass spectrometer (Morgan-Schaeffer Corporation, Montreal). The antimycin A components as such were introduced through the direct inlet system at 155°C. (At higher temperatures no molecular ions were observed). The ionization potential was usually 70 eV. Fractions from pyrolysates were introduced through the indirect inlet via a reservoir.

Infrared Spectroscopy—All infrared spectra were obtained on a Model 225 Perkin-Elmer infrared spectrophotometer using 10 % chloroform solutions.

NMR Spectroscopy—All NMR spectra were obtained on a Varian A-60-A NMR spectrometer using 10 % solutions in deutrochloroform with tetramethyl silane as internal standard.

Ultraviolet Spectroscopy—The ultraviolet spectra have been obtained on a Perkin-Elmer Model 350 UV-visible spectrophotometer. The final sample concentration was about 80 micrograms per ml ethanol. The spectra for the alkaline shifts were run diluting the stock solution with 0.5 N NaOH in 80 % ethanol-20 % H_2O, instead of plain ethanol. The ethanol used is the commercial methyl hydrate (10 % methanol-86 % ethanol-4 % H_2O) which is refluxed overnight with sodium borohydride, and distilled before being used for spectroscopy.

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*) It was shown by Mr. FRANK CABOT then working with the Perkin-Elmer Corporation that samples with high boiling points like steroids would condense in a narrow zone about 2~3 cm inside the 1/8" OD stainless steel vent. Recoveries are between 60 and 80 %.