CHARACTERIZATION OF THE OLIGOMYCINS AND RELATED ANTIBIOTICS

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Data have been obtained by mass spectrometry which demonstrate that the molecular weights of the oligomycins and rutamycin are in the vicinity of 800. The mass spectra of oligomycin B and peliomycin are qualitatively the same. A degradation product from rutamycin has been characterized as an unsaturated, polyhydroxy aldehyde with a tentative molecular formula of $C_{27}H_{46}O_5$. An analogous product was obtained from oligomycin B.

A number of substances are known which affect oxidative phosphorylation and related processes in mitochondria. Among these is a group of chemically related antibiotics which includes the oligomycins, rutamycin, peliomycin, ossamycin, and venturicidin. These compounds, whose structures are unknown, are believed to inhibit the reversible transfer of a phosphoryl group from a phosphorylated high-energy intermediate to ADP in mitochondria.[1] We would like to present new data pertaining to the characterization of the oligomycins and rutamycin.

Previous work has shown that these compounds are neutral substances containing only carbon, hydrogen, and oxygen.[2] They contain hydroxyl groups which can be acetylated, and their infrared spectra indicate the presence of carbonyl groups. They are unsaturated and absorb strongly in the ultraviolet with maxima at about 225 and 230 mμ. This absorption may be due in part to the presence of a conjugated diene moiety. Published molecular weight values for the compounds are given in Table 1. We have obtained

mass spectral data which indicate that the molecular weights are, in fact, considerably greater than any of these values.

A portion of the mass spectrum of rutamycin is shown in Figure 1. It can be seen that, while a very intense peak appears at 432 ($C_{27}H_{44}O_4$),* peaks of low intensity are found at higher values. The highest peak occurs at 740. The peaks at 722 and 704

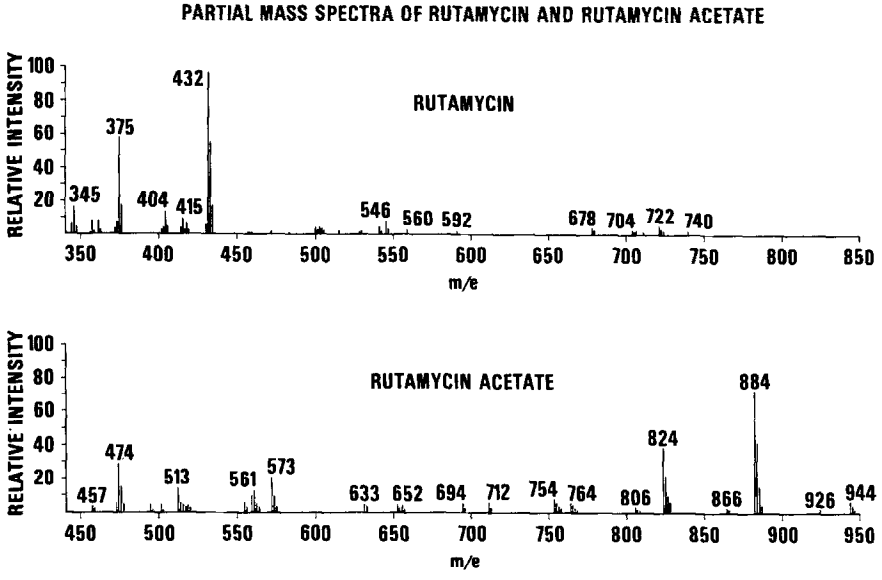

FIGURE 1. Partial Mass Spectra of Rutamycin and Rutamycin Acetate.

may be attributed to the loss of one and two molecules of H_2O from the 740 ion. While we could not be certain that the peak at 740 was due to the molecular ion, it seemed clear that, unless all the peaks at higher values were extraneous, the 432 peak was not due to the molecular ion as reported by Beechey and co-workers[3] (Table 1).

*As a matter of convenience, the symbol m/e has been omitted when quoting values from the mass spectra, but it is to be understood that these values are mass-to-charge ratios. Elemental compositions of fragment ions were determined by accurate mass measurement. Mass spectra were recorded on a CEC Model 21-110A-1 mass spectrometer using a direct inlet system with source temperatures in the range 235-280°. The ionizing potential was 70 eV.

TABLE 1. Published Molecular Weight Data

Rutamycin:	419.2 (X-ray)[2b]
	432.3231 (mass spectrometry)[3]
Oligomycin A:	397, 408, 426 (Rast)[2a]
	426 (X-ray)[2a]
	433 (isothermal distillation)[2a]
	446.3387 (mass spectrometry)[3]
Oligomycin B:	394, 416 (Rast)[2a]
	396.2 (X-ray)[2a]
	416 (isothermal distillation)[2a]
Oligomycin C:	496 (Rast)[2a]

Thermal instability could account for the molecular weight values obtained by earlier workers. An attempt to sublime a sample of rutamycin for mass spectral analysis (175°, 0.01 mm Hg, overnight) resulted in complete decomposition of the sample. A volatile product was obtained which gave a mass spectrum exhibiting a strong peak at 432, a peak of lesser intensity at 450, and none at higher values. The ultraviolet spectrum of this product is qualitatively similar to that of rutamycin.

Compelling evidence for a higher molecular weight was afforded by the mass spectrum of rutamycin acetate,* a portion of which is shown in Figure 1. Here the peaks in the high-mass range show more clearly. The highest peak is found at 944. The peaks at 884, 824, and 764 are most likely due to the loss of molecules of acetic acid. The intense 432 peak of rutamycin is shifted to 474 indicating that this fragment retains one acetate group. It is again difficult to decide whether the highest peak in this spectrum is the molecular ion. In the mass spectrum of the deuterioacetate, the 944 peak is

*The acetate derivative was prepared by treating rutamycin with acetic anhydride-pyridine overnight at room temperature.

shifted to 956. This means that the 944 ion contains four acetyl groups. Subtracting four acetyl groups, that is, 168 from 944, gives 776. Therefore, the molecular weight of rutamycin must be at least 776. The 740 peak found in the spectrum of rutamycin could arise in a reasonable manner from a molecule of mass 776 by the loss of two molecules of H_2O.

Similarly, the mass spectra of the oligomycins and the acetate derivatives of oligomycin A and B were determined. The data are summarized in Table 2. The calculated mass values for oligomycin A and B are 772 and 804, respectively. We did not have sufficient oligomycin C for the preparation of derivatives.

Comparison of the mass spectra of rutamycin and the oligomycins lends support to the proposal that the four antibiotics are closely related in structure. The spectra of oligomycin A and C are identical up to mass 446. Except for a 14-unit shift to lower mass, rutamycin shows a close correspondence to A and C in this region. The spectrum of oligomycin B, while similar, shows more

TABLE 2. Summary of Mass Spectral Data

Compound	Abundant Fragment Ion	Highest Peak Observed	Calculated Mass Value
Rutamycin	432 ($C_{27}H_{44}O_4$)	740	776
Rutamycin Acetate	474	944	
Rutamycin Acetate-d	477	956	
Oligomycin A	446 ($C_{28}H_{46}O_4$)	688	772
Oligo A Acetate	488	898	
Oligo A Acetate-d	491	907	
Oligomycin B	460 ($C_{28}H_{44}O_5$)	786	804
Oligo B Acetate	502	1014	
Oligo B Acetate-d	505	1029	
Oligomycin C	446	774	---

pronounced differences. Peliomycin may be closely related to the oligomycins. Thus, the mass spectrum of peliomycin is qualitatively the same as that of oligomycin B up to mass 702, the maximum value observed for peliomycin. Ossamycin and venturicidin differ notably from the other members of the group in that they contain nitrogen, and this prevents a direct comparison of the mass spectra.

We believe that the foregoing results demonstrate that the oligomycins and rutamycin have molecular weights considerably greater than previously reported values. We would like to point out, however, that the true molecular weights may be greater than the maximum values observed by mass spectrometry due to the uncertainty involved in identifying the molecular ion in spectra of these compounds. We believe that the differences, if any, will be equal to a molecule of H_2O or multiple thereof. It is known that compounds containing hydroxyl groups may not exhibit a molecular ion due to loss of H_2O or other fragmentation,[4] and the problem is accentuated if the compound is of high molecular weight.

Subsequent to the completion of the present study, we became aware of results obtained by Foster and Strong at the University of Wisconsin which also indicate molecular weights in the vicinity of 800 for the oligomycins.[5]

When rutamycin was refluxed for one hour in dilute methanolic sodium hydroxide solution, no starting material remained, and the product contained three components. The major component, which appeared similar to the thermal degradation product, was isolated by chromatography. The infrared spectrum of this compound, with peaks at 3571, 3425, and 1724 cm^{-1} ($CHCl_3$), indicates the presence of hydroxyl and carbonyl groups. The ultraviolet spectrum is similar to that of rutamycin with maxima at 226 and 231 mμ (EtOH). A peak in the nmr spectrum at δ 9.67 ppm ($CDCl_3$, TMS=0), which is

not exhibited by rutamycin, is attributed to an aldehyde proton. The highest peak in the mass spectrum occurs at 450, followed by a peak at 432 ($C_{27}H_{44}O_4$). A tentative formula for the degradation product is $C_{27}H_{46}O_5$. It formed a triacetate (M^+ 576) with acetic anhydride-pyridine. Treatment with $NaBH_4$ gave a dihydro derivative (M^+ 452) which does not exhibit carbonyl absorption in the infrared. Its nmr spectrum indicates the absence of an aldehyde proton; a doublet is found at δ 3.38 ppm (acetone-d_6) which is shifted to δ 3.85 ppm by acetylation. The ultraviolet spectrum was unchanged. The hydrolysis product was found to be inactive against fungi which are sensitive to rutamycin. A similar result was obtained with oligomycin B. The mass spectrum of its hydrolysis product exhibited the highest peak at 478, followed by a peak at 460.

Further characterization of these degradation products appears to be a promising approach to the structure elucidation of rutamycin and the oligomycins.

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