ESTIMATION OF THE MOLECULAR WEIGHTS AND MOLECULAR FORMULAE OF OLIGOMYCIN-A, RUTAMYCIN & AUROVERTIN BY MASS SPECTROMETRY

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Lardy and his co-workers first introduced the use of antibiotics as inhibitors of oxidative phosphorylation; oligomycin (Lardy, Johnson & McMurray, 1958), aurovertin (Lardy, Connelly & Johnson, 1964), and rutamycin (Lardy, Witoncky & Johnson, 1965). The use of these antibiotics has revealed many new features of the oxidative phosphorylation system and some significance has been ascribed to the ratio of the moles of oligomycin to grams of mitochondrial (or sub-mitochondrial fragment) protein which is required for inhibition of oxidative phosphorylation (see Lardy et al. (1965) and Lee & Ernster (1966) for further references). A knowledge of the molecular weight of the oligomycins and rutamycin is essential for this ratio to be significant. We have measured the molecular weights and estimated the molecular formulae of oligomycin-A, rutamycin and aurovertin. The values which we have obtained do not agree with those previously reported.

Oligomycin-A, rutamycin and aurovertin were admitted to an A.E.I. MS.9 mass spectrometer, using a direct insertion probe. Precise mass measurements were performed on the molecular ions, at a resolving power of 10,000. A possible range of error of ± 5ppm was allowed for the instrument.

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The only possible combinations of carbon, hydrogen and oxygen for oligomycin-A, rutamycin and aurovertin, whoses masses lie within ± 5ppm of the measured values are shown in Table 1. Masamune et al. (1958), Thompson, Hoehn & Higgins (1961) and Baldwin, Weaver, Brooker, Jacobsen, Osborne & Nash (1964), have shown respectively that oligomycin-A, rutamycin and aurovertin, only contain carbon, hydrogen and oxygen.

Table 1. Comparison of the experimental and computed molecular weights of oligomycin-A, rutamycin and aurovertin

	Molecular weight (C=12)		Δ mass	Molecular formula corresponding to computed mass
	measured	computed*		
Oligomycin-A	446.3387	446.3396*	-0.0009(-2ppm)	$C_{28}H_{46}O_4$
Rutamycin	432.3231	432.3239*	-0.0008(-2ppm)	$C_{27}H_{44}O_4$
Aurovertin	476.2047	476.2046†	+0.0001(<1ppm)	$C_{25}H_{32}O_9$

* Beynon & Williams (1963). Mass and abundance tables.

† computed value obtained by calculation.

It can be seen from the data in Table 1 that the measured molecular weight of oligomycin-A is 446.3387, which corresponds to a molecular formula of $C_{28}H_{46}O_4$, with an accuracy of 2ppm. This mass contrasts with the molecular weight of 424.56 ($C_{24}H_{40}O_6$) obtained by elemental analysis and depression of freezing point (Rast) reported by Masamune et al. (1958). The molecular weight and molecular formula of rutamycin as determined by mass spectrometry

are 432.3231 and $C_{27}H_{44}O_4$. Thompson et al.(1961) reported $C_{25}H_{42}O_6$ on the basis of elemental analysis. The molecular formula of aurovertin was reported by Baldwin et al.(1964) to be $C_{26}H_{34}O_6$. Mass spectroscopic analysis gives $C_{25}H_{32}O_9$. These differences between the molecular weights obtained by mass spectrometry and the less reliable classical methods suggest that the molecular weights of oligomycin-B and -C quoted by Masamune et al.(1958) may also be in error.

Oligomycin-A was the gift of Professor E. E. van Tamelen. The purity of the sample was examined by thin-layer chromatography on cellulose plates (MN-polygram CEL300/UV_{254}). A series of aqueous propanol mixtures, 10 - 90%(v/v) were used as eluants. No contaminants were observed. Rutamycin was donated by the Lilly Research Laboratories. Its purity was confirmed by thin-layer chromatography. Aurovertin was given by the Pitman-Moore Division of the Dow Chemical Company.

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