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THE DETERMINATION OF MOLECULAR FORMULAE OF POLYOLS BY MASS SPECTROMETRY OF THEIR TRIMETHYLSILYL ETHERS; THE STRUCTURE OF THE MACROLIDE ANTIBIOTIC FILIPIN.

B.T.Golding and R.W.Rickards,
Department of Chemistry, University of Manchester.
and M. Barber
Associated Electrical Industries Ltd., Manchester.

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A major obstacle in the determination of the structure of many polyhydroxy compounds is in establishing the correct molecular formulae. The classical methods of elemental analysis and molecular weight determination are not sufficiently precise for substances of high molecular weight, whilst direct mass spectrometry is vitiated by dehydration and decomposition at temperatures necessary to produce an adequate vapour pressure of these involatile compounds.

The problem is accentuated in the case of macrolide antibiotics.[1,2] These compounds are frequently difficult to crystallise, whilst their common tendency[1] to occlude solvent or other impurities makes even molecular weight determination by X-ray crystallography hazardous (cf.lagosin[3]). With such compounds, the molecular formula may remain in doubt at an advanced stage of the structure determination (eg. nystatin[4]), or even until the complete structure is assigned (eg. magnamycin[5]). We report here a method,

developed for polyene macrolide antibiotics but applicable in principle to any polyol, whereby exact molecular formulae can be rapidly determined by mass spectrometry of the corresponding trimethylsilyl ethers.

The silyl ethers were prepared by reaction of the macrolides in pyridine with hexamethyldisilazane and chlorotrimethylsilane at room temperature for periods up to one hour. This is an extension of the method used by Sweeley et al[6] for carbohydrates. Concentration of the reaction mixtures in vacuum afforded residues which, after evaporating several times with carbon tetrachloride, were extracted with hexane. These extracts, after concentration, were examined without further manipulation by direct evaporation into the ionisation chamber of the mass spectrometer.*

Under these conditions, lagosin[3] (I), $C_{35}H_{58}O_{12}$, and decahydrolagosin[3], $C_{35}H_{68}O_{12}$, yield deca-trimethylsilyl ethers which are thermally stable and sufficiently volatile to be introduced into the spectrometer at 160 - 180°/10^{-6}mm. The high mass regions of the spectra of these two derivatives are presented as line diagrams in Figures 1 and 2. Characteristic features are the groups of isotope peaks for the molecular ion and fragment ions, arising primarily from the presence of heavy isotopes of silicon and carbon. The molecular weights of these macrolide derivatives are readily established from the mass-to-charge ratio of the intense molecular ions. Accurate mass measurement, by reference

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* Spectra were obtained using an A.E.I. Ltd., MS9 double-focussing mass spectrometer operating with 70ev. electrons, 100 μa trap current and 6kv ion-accelerating voltage.

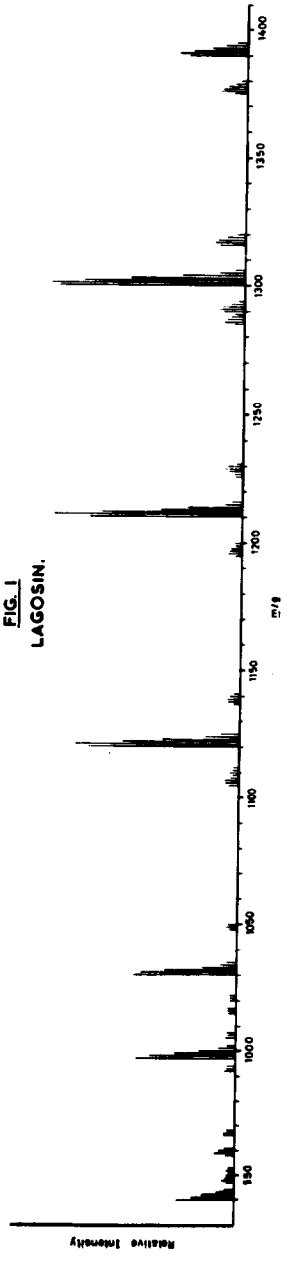


FIG. 1
LAGOSIN.

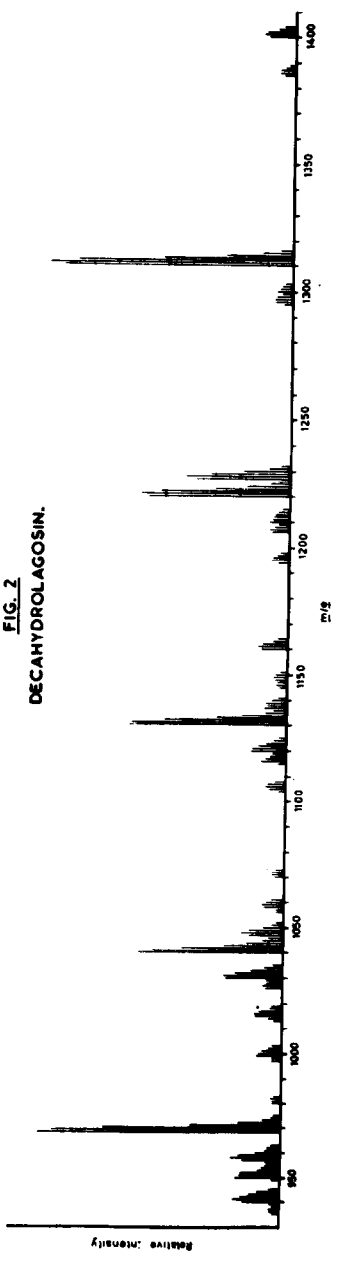
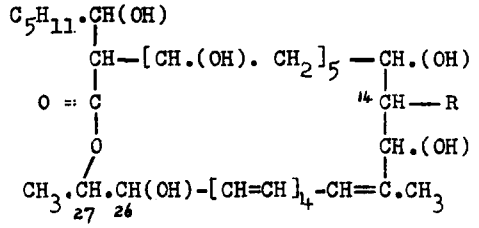


FIG. 2
DECAHYDROLAGOSIN.

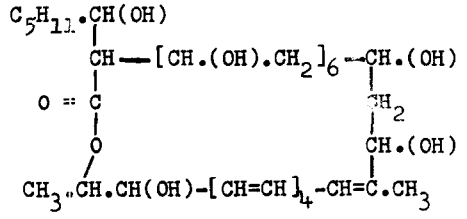
MASS SPECTRA OF DECA-TRIMETHYLSILYL ETHERS.

to appropriate peaks in the spectrum[7] of "Fluorolube" using a double-focussing mass spectrometer, permits the direct determination of molecular formulae. The lightest molecular ions of the derivatives of lagosin (I) and decahydrolagosin have m/e ratios of 1390 and 1400 respectively, corresponding to the ions $[C_{65}H_{138}O_{12}Si_{10}]^+$ and $[C_{65}H_{148}O_{12}Si_{10}]^+$ containing C^{12}, H^{1}, O^{16} and Si^{28} in full agreement with Dhar, Thaller and Whiting's structure[3] (I).



(I; R = OH).

(II;R = H).



(III).

The utility of this technique for the determination of molecular formulae of polyols is demonstrated by its application in the case of the antibiotic filipin.[8,9] This pentaene macrolide from Streptomyces filipinensis was suggested by Dhar, Thaller and Whiting[3] to be 14-deoxylagosin (II), $C_{35}H_{58}O_{11}$, because of similarities between lagosin and filipin in their infrared and ultraviolet spectra. The $C_{37}H_{62}O_{12}$ structure (III) assigned to filipin by Djerassi and his co-workers[9] was based on integration of the nuclear

magnetic resonance spectrum of the non-crystalline filipin peracetate. These techniques of absorption spectroscopy are not sufficiently accurate to permit an unambiguous decision between $C_{35}H_{58}O_{11}$ and $C_{37}H_{62}O_{12}$ formulae.

The per-trimethylsilyl ethers of filipin and its decahydro-derivative gave mass spectra in which the lightest molecular ions (corresponding to molecules lacking heavy isotopes) occurred at m/e 1302 and 1312 respectively. These values correspond to formulae $C_{62}H_{130}O_{11}Si_9$ and $C_{62}H_{140}O_{11}Si_9$ respectively, i.e. to nonasilyl ethers of C_{35} compounds. Accurate mass measurement of this molecular ion in the case of the per-trimethylsilyl ether of decahydrofilipin, by reference to the $[C_{28}F_{51}]^+$ ion (m/e 1304·9186) in the spectrum[7] of "Fluorolube", gave a value (on the C^{12} scale) of 1312·8342, compared with the calculated value of 1312·8319 for the formula $C_{62}H_{140}O_{11}Si_9$. Hence the molecular formula of filipin itself is settled absolutely as $C_{35}H_{58}O_{11}$, as in the structure[3] (II) without regard to stereochemistry.

In a recent publication,[10] Ceder and Ryhage described the deoxygenation of filipin by improvements of the procedures of Cope et al[11], obtaining a C_{35}-carboxylic acid and finally a hydrocarbon $C_{35}H_{72}$, the structures of which confirmed the carbon skeleton of structure (II). Consideration of an n.m.r. spectrum of filipin indicated[10] that the lactone ring was closed to C-27 (as in II), rather than to C-26 as was also possible from chemical evidence alone[9].

Fragmentations apparent in the mass spectra of such trimethylsilyl ethers could also yield structural information in the case of unknown macrolides.* The high mass regions

* These fragmentations will be discussed in detail elsewhere.

of the spectra of ethers of lagosin (I), filipin (II), and their decahydro-derivatives are dominated by groups of isotope peaks, separated by multiples of 90 mass units from the molecular ions and corresponding to successive eliminations of neutral trimethylsilanol units. The prominent ions at m/e M-173 in the spectra of the decahydro-derivatives arise by cleavage of the siloxyhexyl side-chain β to the macrolide carbonyl group. This fragmentation is not pronounced in ethers of the parent macrolides, where the primary ion will be stabilised by the conjugated system present. Care is needed in interpreting the lower mass regions of all these spectra, in view of the rearrangements which are known to occur during mass spectrometry of silyl ethers of simple alcohols.[12]

In view of the ease with which silyl ethers are hydrolysed to the parent polyols,[13] these derivatives are also of value in the purification of polyene macrolides. Both thin layer and column chromatographic techniques are effective, and we are currently investigating the fractionation of these volatile macrolide ethers by gas-liquid chromatography.

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