Characterisation of eight Antibiotics of the Quinomycin Group by Field Desorption Mass Spectrometry

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Summary The characterisation of a number of new antibiotics of the quinomycin class (containing various novel chromophores) is reported; characterisation was achieved by field desorption mass spectroscopy, using the desorption of cationised samples.

QUINOXALINE antibiotics are derived from various species of *Streptomycetes*. The history of their discovery and their biological properties have been thoroughly reviewed by Katagiri *et al.*[1] The basic skeleton of the quinoxaline antibiotics is characterised by two quinoxaline-2-carboxamide chromophores linked to a cross-bridged dilactone cyclic peptide. In one family of quinoxaline antibiotics, the quinomycins, the cross-bridge is a dithioacetal, as is the case in the antibiotic echinomycin (Figure).

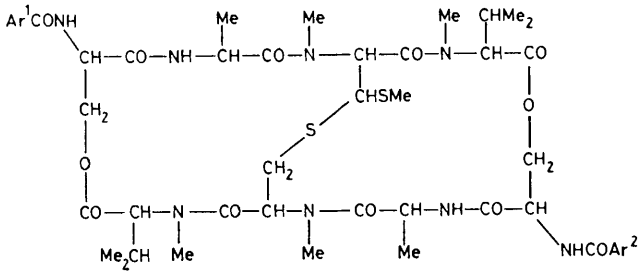


FIGURE.

Waring and Wakelin[2] have proposed a model of bifunctional intercalation for the DNA echinomycin complex. More recently, Lee and Waring[3] have compared the binding properties of different naturally occurring, semisynthetic and synthetic derivatives of the quinoxaline antibiotics.

Pulse-labelling studies with the triostin-producer *Streptomyces* S-2-210-L have shown that all the constituent amino-acids have L-precursors, and that L-tryptophan and quinoxaline-2-carboxylic acid can be used as precursors for the chromophoric moieties.[4,5] Addition of quinoline-2-carboxylic acid to the culture medium of *Streptomyces* sp. 732 and *Streptomyces* S-2-210-L led to the production of new derivatives with quinoline chromophores replacing the quinoxaline rings.[6] The production of new derivatives of echinomycin with quinazolin-4-one-3-acetyl residues has also been reported.[7,8] We now report the characterisation by field desorption mass spectrometry (F.D.M.S.) of 8 antibiotics of the quinomycin group which have been produced by directed biosynthesis; 6 of these antibiotics are new.

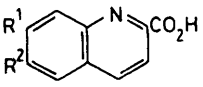
The new derivatives have been biosynthesised in order to study the role of the chromophores in intercalation of the antibiotics into DNA. Their molecular weights were determined by F.D.M.S. since conventional electron impact (E.I.) fails to produce molecular ions from these antibiotics; however, 'in beam' E.I. was used successfully in the structure revision of echinomycin.[9] Full details of the preparation and isolation of the antibiotics will be reported elsewhere,[10] but briefly, 1 litre cultures of *Streptomyces echinatus* A8331 were grown in a maltose minimal medium,[6] and supplemented at inoculation time with 0·75 mmol of one of the various analogues (**1**)—(**4**) (Table).

TABLE. Substrates added to cultures of *Streptomyces echinatus* A8331, and molecular weights of identified products.

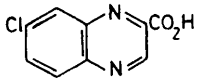
Substrate	Antibiotic acronym (mol. wt.)[a]	Antibiotic acronym (mol. wt.)[b]
(**1**)	1QN (1099)	2QN (1098)
(**2**)	1QCl (1134)	2QCl (1168)
(**3**)	1TP (1105)	2TP (1110)
(**4**)	1QnM (1113)	2QnM (1126)

[a] The molecular weights in column 2 are for the structures with one substituted chromophore and one quinoxaline substituent. [b] The molecular weights in column 3 are for the structures with two substituted chromophores.

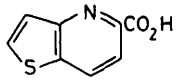
The mycelium was collected after 10 days of fermentation, and extracted with chloroform and acetone. In the cases of the substrates (**2**)—(**4**), the culture filtrate was additionally extracted with ethyl acetate. The combined extracts were partially purified by preparative t.l.c., and the pure antibiotics isolated by preparative h.p.l.c.



(1) $R^1 = R^2 = H$
(4) $R^1 = H$, $R^2 = Me$



(2)



(3)

The antibiotics thus obtained were subjected to field desorption mass spectrometry.[11,12] Determination of the molecular weights makes it possible to distinguish between the antibiotics with one or two of the substituted chromophores. Because of the asymmetry of the molecule (Figure), there are in each case potentially two antibiotics which contain one substituted chromophore and one quinoxaline ($Ar^1 \neq Ar^2$). These antibiotics have the same molecular weights and are not distinguished in the present study.

Molecular weights were determined using a Kratos MS 50 mass spectrometer equipped with a combined E.I./F.D. source. The field desorption wires were activated with benzonitrile. The mass marker was calibrated with tris-(perfluoroheptyl)triazine using the instrument in the E.I. mode. The sample was dissolved in $CHCl_3$ and applied to the wire with a syringe. Desorbed samples could be cationised with Li^+,[13,14] by applying a saturated methanolic solution of lithium chloride to the wire, prior to the application of the sample. The source temperature was 125 °C above ambient and desorption occurred at an emitter current of 18—20 mA.

In general, the above procedure gave rise to both $M^{+\cdot}$ and $(M+Li)^+$ ions, thus permitting an unequivocal determination of the molecular weights (Table). In the case of 2QCl it was not possible to observe $M^{+\cdot}$ and $(M+Li)^+$ at the same time, but in a spectrum taken without cationisation, the ion $(M+H)^+$ was observed.

The present results attest to the power of field desorption mass spectrometry in the determination of molecular weights of relatively involatile compounds of biological interest. This is especially true when the compounds are available in only small quantities, as may be the case in directed biosynthesis or mutasynthesis. Our F.D. sample size was typically 10—15 μg.

We thank the Association of Commonwealth Universities, La Fondation Jean-Louis Levesque (D. G.), the S.R.C., the M.R.C. and the Cancer Research Campaign for financial support. We thank Drs. J. Nüesch and K. Scheibli (Ciba–Geigy, Basel) for the strain of *Streptomyces echinatus* A8331, and Dr. H. P. Schultz (University of Miami), Dr. S. Gronowitz (University of Lund), and I.C.I. Pharmaceuticals (Alderley Edge, U.K.) for samples of (**2**), (**3**), and (**4**) respectively.

(*Received*, 20*th October* 1980; *Com.* 1128.)

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