CHARACTERIZATION OF NOVEL ANTIBIOTICS OF THE TRIOSTIN GROUP BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY

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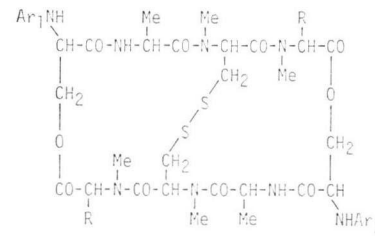
Fast atom bombardment mass spectrometry has been used to determine the molecular weights of a number of new antibiotics of the triostin group which have both the natural quinoxaline-2-carboxyl chromophores replaced by substituted analogues.

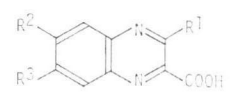
The quinoxaline antibiotics are cyclic depsipeptides characterized by the presence of two heterocyclic aromatic chromophores and a cross-bridge, which is either a dithioacetal (quinomycins) or a disulphide (triostins). In the naturally-occurring members of this class the chromophores are both quinoxaline-2-carboxyl (shown as the parent acid in **1**). There are natural variants in the class due to differences in the amino acid composition of the peptide. For example, triostin A contains two units of *N*-methylvaline whereas in triostin C these are replaced by *N*-γ-dimethylalloisoleucine (Fig. 1). The history, biological properties and mode of action of the quinoxaline antibiotics have been thoroughly reviewed by KATAGIRI *et al.*[1] and WARING.[2]

Experiments involving directed biosynthesis, with *Streptomyces triostinicus*, have led to the isolation of novel members of the triostin group containing various chromophores.[3] These antibiotics have now been characterized by fast atom bombardment (FAB) mass spectrometry.[4~6] This technique permits the determination of the molecular weights of a wide range of molecules of biological interest which are often too involatile and/or thermally labile for analysis by other mass spectrometric methods. Such information is obtained in a more facile and routine manner by FAB than by field desorption mass spectroscopy, which was used previously for the characterization of new antibiotics of the quinomycin group.[7]

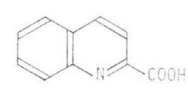
Fig. 1. The structure of the triostin group of antibiotics.



Triostin A $Ar_1 = Ar_2 = 1$, $R = CH(Me)_2$
Triostin C $Ar_1 = Ar_2 = 1$, $R = CHMeCH(Me)_2$



1 $R^1 = R^2 = R^3 = H$
2 $R^1 = NH_2$, $R^2 = R^3 = H$
3 $R^1 = R^3 = H$, $R^2 = Br$
4* $R^1 = R^3 = H$, $R^2 = Cl$
5 $R^1 = R^2 = H$, $R^3 = Cl$



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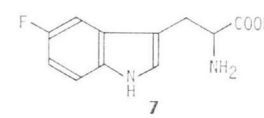

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* Supplied to cultures as the methyl ester. See reference 3.

Experimental

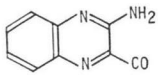
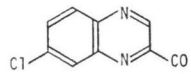
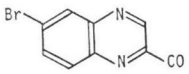
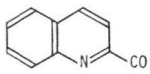
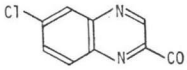
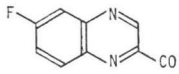
Cultures of *Streptomyces triostinicus* ATCC 21043 were supplemented separately with the derivatives of quinoxaline-2-carboxylic acid **2**~**5** and with quinoline-2-carboxylic acid **6**. These compounds stimulate the biosynthesis of triostins containing **2**~**6** in place of either one or both units of the natural chromophore **1**. Full details of the preparation and isolation of these novel triostins are given elsewhere.[3] Each antibiotic was rigorously examined for purity by TLC and HPLC, and characterised by UV spectroscopy and 400 MHz ^{1}H NMR.[3] In addition, a further analogue of triostin A has been produced in which the quinoxaline-2-carboxyl moieties are fluoro substituted. This was obtained by supplementing a culture of the same organism with DL-5-fluorotryptophan **7** (unpublished work).

FAB mass spectra were recorded on a Kratos MS 50 instrument fitted with a standard FAB source and a high field magnet which allows the instrument to be operated at full sensitivity up to 3,300 daltons. Samples (*ca.* 10 nmole) were dissolved in a matrix (2 μl) of 1 : 1 (v/v) α-thioglycerol and diglycerol. The solution was bombarded with a beam of neutral Xe atoms at an energy of 8 keV. In all but one case the antibiotics which were analysed were analogues of triostin A and all of them were disubstituted with a novel chromophore (*i.e.* $Ar_1 = Ar_2$).

Results

For each triostin sample an abundant $(M-H)^-$ ion is observed in the negative ion mode. The molecular weights of the antibiotics obtained from the mass spectra are recorded in the Table 1.

Table 1. Molecular weights determined by FAB mass spectrometry for analogues of triostin A disubstituted with a novel chromophore.

Compound fed	Chromophore	Molecular weight	Compound fed	Chromophore	Molecular weight
2		1,116	**5**		1,154
3		1,242	**6**		1,084
4*		1,154	**7**		1,122

* In this experiment a quantity of the disubstituted triostin C was also isolated and its molecular weight determined as 1,210.

Discussion

These results provide evidence for the identity of a number of new antibiotics belonging to the triostin group which have been prepared by directed biosynthesis. It is important to note that our early efforts to obtain FAB mass spectra of quinoxaline antibiotics, using glycerol as the matrix material, were unsuccessful; but that we can now reliably obtain spectra of these relatively non-polar compounds using a mixture of α-thioglycerol and diglycerol as a matrix. However, once a suitable matrix is found for a particular type of compound, the determination of molecular weights up to 3,000 daltons by FAB mass spectroscopy is virtually routine. In view of the small sample size required (*ca.* 1 nmole) the technique is well fitted to monitor experiments aimed at the modification of antibiotics, or other metabolites, (provided the modification involves a change in molecular weight) such as those involving directed biosynthesis, mutasynthesis, or isotopic enrichment.

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