STRUCTURE AND CONFORMATION OF FOURTEEN ANTIBIOTICS OF THE QUINOXALINE GROUP DETERMINED BY ^{1}H NMR

M. P. Williamson, D. Gauvreau,* D. H. Williams and M. J. Waring*

University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K.
*Department of Pharmacology, Medical School, Hills Road, Cambridge, CB2 2QD, U.K.

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Nuclear magnetic resonance has been employed to characterize fourteen new antibiotics belonging to the quinoxaline group, produced by feeding aromatic acids to *Streptomyces echinatus*. Twelve of the antibiotics are the expected substituted quinomycins and adopt conformations very similar to that of echinomycin. This is discussed in relation to their different DNA-binding characteristics. The other two antibiotics are triostins, supporting the proposal that triostins serve as biosynthetic precursors of the quinomycins.

The quinoxaline antibiotics are powerful antimicrobial agents which show significant inhibitory activity towards a variety of tumors. The history of their discovery, biological properties, and mode of action have been thoroughly reviewed by Katagiri *et al.*[1] and Waring[2]. They are heterodetic cyclic depsipeptides characterized by the presence of quinoxaline-2-carboxyl chromophores and a sulfur-containing cross-bridge. Typical members of the class are **1**, where the cross-bridge X is either a thioacetal **2** (quinomycins) or a disulfide **3** (triostins). They act by intercalating into DNA, and various members of the class show different preferences for binding to particular nucleotide sequences.[3] Varied sequence preferences have been observed with some of the derivatives described here.[4] In order to study the origins of this effect more systematically, fourteen new members of the class have been prepared and studied by NMR. Twelve are shown to be quinomycins, which adopt solution conformations essentially identical to that of echinomycin,[5,6] while the other two are triostins.

Full details of the directed biosynthesis of the antibiotics will be reported elsewhere.[7] A summary of the synthesis, and a characterization of some of the antibiotics by mass spectrometry, has already

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Ar¹CONH          Me      Me          Me CHMe2
   CH-CO-NH-CH-CO-N-CH-CO-N-CH-CO          I
   CH2              /             O
   O              X               CH2
   CO-CH-N-CO-CH-N-CO-CH-NH-CO-CH          II
   Me2CH Me      Me    Me       NHCOAr²
                        1

 -CH2-S-CH(-)-SMe           -CH2-S-S-CH2-
        2                         3
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Table 1. Antibiotic nomenclature.

Substituent chromophore	Antibiotic acronym
4 (Quinoline-2-carboxyl)	QN
5 (7-Chloroquinoxaline-2-carboxyl)	QCl
6 (Thieno(3,2-b)pyridine-5-carboxyl)	TP
7 (6-Methylquinoline-2-carboxyl)	QnM

4 R = H 5 R = Cl 6

7 R = Me 8 R = H

Fig. 1. 400 MHz ^1H NMR spectrum of 2QnM, roughly 0.4 mM in $CDCl_3$.

The figures I, II, refer to the two halves of the molecule as indicated in **1**, and the letters α, β refer to the α-CH and β-CH protons of each amino acid residue. 2QnM has $Ar^1=Ar^2=$**7**, and the signals labelled "Aromatics' and '6-Me' arise from the 6-methyl-quinoline-2-carboxyl residues **7**.

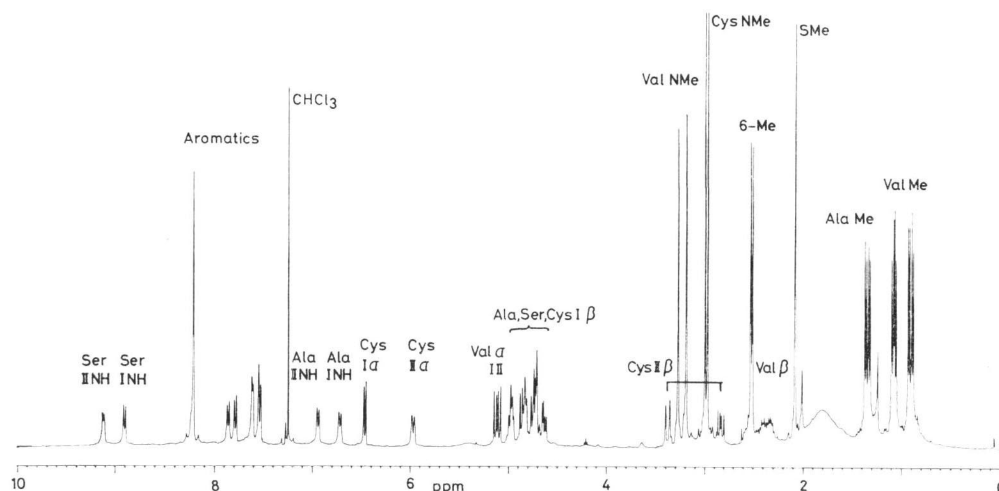


Table 2. Representative coupling constants.

	Echi	2QN	2QC1	2TP	2QnM		Triostin	2QnMb
Ala I NH	7.3	7.7		7.9		Cys p $\beta\beta'$	14.8	14.5
Ala II NH	7.1	7.7	8.4	7.9		Cys n $\beta\beta'$	15.0	15.1
Ser I NH	7.7	8.2	7.2	8.4	8.3	Cys n $\alpha\beta$	6.7	8.5
Ser II NH	6.3	6.4	5.9	6.7	6.2	Cys n $\alpha\beta'$	7.9	7.3
Ser I β	5.2, 11.1			5.0, 11.2	4.9, 11.5	Ser n $\beta\beta'$	11.2	7.6
Cys II $\alpha\beta$	1.7	1.4	1.6	1.8	1.5	Ser n $\alpha\beta$	5.9	4.4
Cys II $\alpha\beta'$	11.2	10.7	11.0	10.7	10.6	Ser n $\alpha\beta'$	1.4	0.5
Cys II $\beta\beta'$	16.1	16.1	16.3	16.1	16.0	Ala p NH	5.7	6.4
Cys I $\alpha\beta$	8.7	9.0	8.7	9.1	9.0	Ala n NH	9.0	8.6

appeared.[8] Briefly, one of the aromatic acids **4**~**7** was added to a culture of *Streptomyces echinatus* A8331 to stimulate the biosynthesis of a quinomycin containing either one or two of these chromophores in place of the natural quinoxaline-2-carboxyl moiety **8**. The nomenclature used is indicated in Table 1; the disubstituted antibiotics are referred to as 2QN, *etc.*, while the monosubstituted derivatives, in which one chromophore is the natural quinoxaline-2-carboxyl moiety **8**, are referred to as 1QN, *etc.* Because of the asymmetry of the quinomycin cross-bridge **2**, there are for each substituent chromophore two quinomycin antibiotics which contain one substituted chromophore and one quinoxaline ($Ar^1 \neq Ar^2$); these were not separable by HPLC, but were clearly distinguished by NMR. In addition to these twelve quinomycins, two minor components were isolated, designated 2QC1b and 2QnMb, which were later shown by NMR to be triostins.

Samples (containing roughly 1 mg in 400 μl $CDCl_3$) were examined at ambient temperature and spectra were collected in 8k to 32k data points using a Bruker WH 400 spectrometer. Some representative spectra are shown in Figs. 1~3. All spectra could be completely assigned, except for a few over-

lapping multiplets in the 4.5 ~ 5.0 ppm region. Chemical shifts and coupling constants for all derivatives have been measured, and (except for 2QClb and 2QnMb) are very similar to those in echinomycin, especially for the cross-bridge protons. Whereas chemical shifts may be expected to vary slightly from sample to sample due to different ring currents and concentration-dependent effects, coupling constants should be very similar for each sample if they have essentially the same conformation. This has been found to be true: in all derivatives, coupling constants were no more than 0.6 Hz different from those in echinomycin, except for Ser and Ala αCH-NH couplings which occasionally differed by as much as 1.3 Hz (Table 2).

Assignments were made by comparison with echinomycin, and by difference decoupling.[9] The signals from the two asymmetric halves of echinomycin resonate at different frequencies, and were assigned to the appropriate half of the molecule by comparison with the data listed in reference 5. With the monosubstituted derivatives, the two possible isomers were not usually present in equal amounts; thus the molar ratio of the isomer in which Ar^1 is **8** (the normal chromophore) and Ar^2 is the substitute chromophore to its counterpart where Ar^1 is the substitute and Ar^2 is **8**, is for 1QN 2: 3, for 1QCl 1: 1, for 1TP 1.2: 1, and for 1QnM 1: 3.4. This allows a confident assignment of the resonances to the appropriate half of the molecule, and reveals that the arbitrary assignments of the valine resonances in reference 5 should be reversed. Fig. 2 shows the NMR spectrum of the mixture of the two 1QnM isomers, in which the unequal proportions of the two isomers can be clearly seen.

The spectra of 2QClb and 2QnMb were found to be radically different from those of all the other derivatives. 2QClb could not be separated from the quinomycin 2QCl, but was characterized by a number of low intensity resonances, most significantly a triplet at 5.72 ppm coupled to multiplets at 3.40 and 3.29 ppm, which could all be assigned to a triostin structure comprising about 25% of the total sample. 2QnMb was purified, and its spectrum lacked the S-Me signal, besides having peaks at positions characteristic of a triostin. Although each amino acid yielded two sets of signals (as in quinomycins), they were not present in equal amounts and one set of signals increased in intensity at the ex-

Fig. 2. NMR spectrum of 1QnM.

The sample is a mixture of the two possible 1QnM isomers, and there are thus two pairs of signals from each type of amino acid. Signals arising from the 1QnM isomer in which Ar^1=**7** and Ar^2=**8** are 3.4 times more intense than signals from the other isomer in which Ar^1=**8** and Ar^2=**7**.

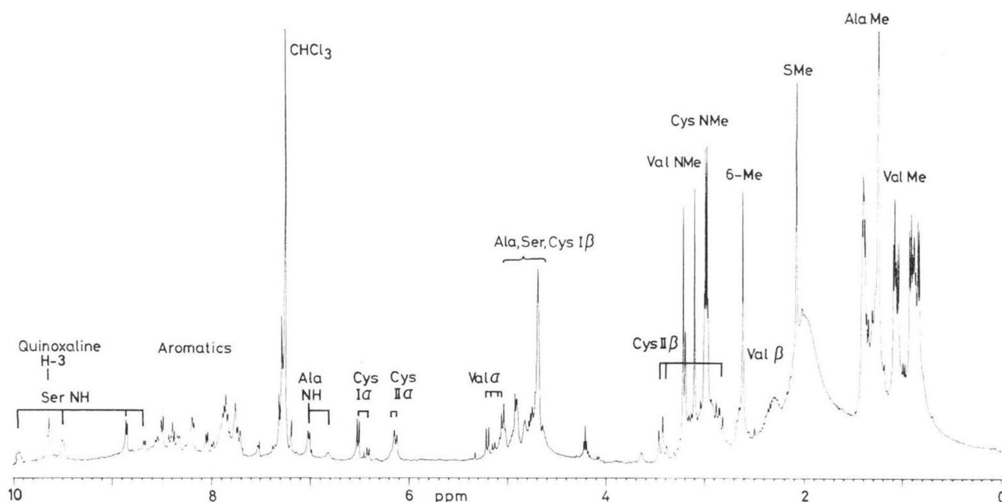
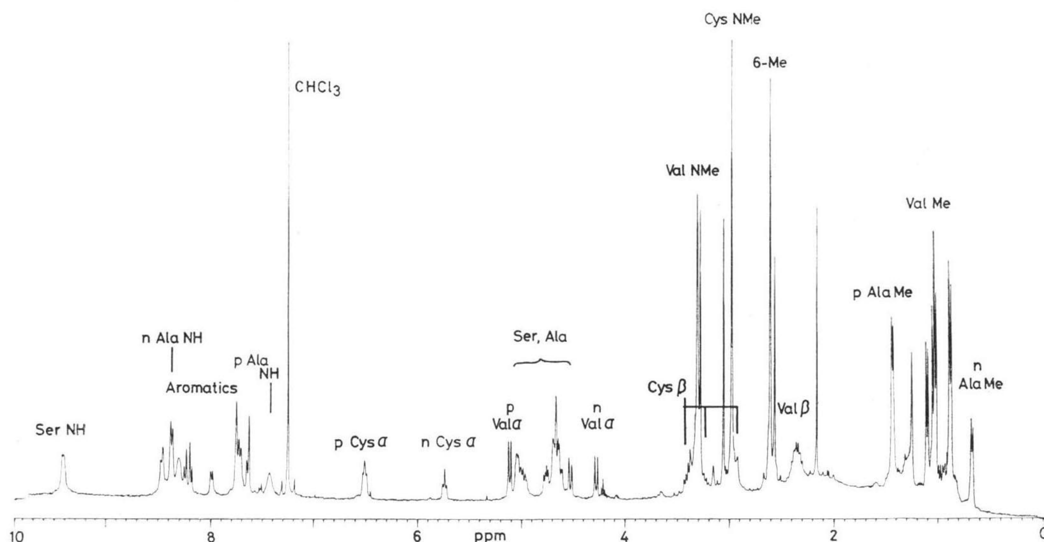


Fig. 3. NMR spectrum of 2QnMb.

This is a triostin, and the letters n and p refer to the two conformations of triostins designated "polar" and "non-polar" respectively.[10]



pense of the other set on adding CCl_4 to the solution. These facts suggest a triostin structure (cross-bridge **3**), in which the two sets of signals arise from two slowly-interconvertible conformations. The spectrum of 2QnMb has been completely assigned and is illustrated in Fig. 3, where signals arising from the two distinct conformations are indicated by the letters n and p. Comparison of chemical shifts and coupling constants with those in triostin A[10] indicates that the molecule adopts a very similar conformation to triostin (Table 2). There are differences in the Ser-Val fragment, reflecting freer rotation of the valine side-chain, but chemical shifts and coupling constants in the central portion of the molecule are closely similar, especially for the 'n' conformation. The molecular weight of 2QnMb has been found to be 1,112 (G. BOJESEN, personal communication), in agreement with the triostin structure.

We consider that the production of triostins by *S. echinatus*, hitherto only known to produce quinomycins, provides the first evidence for the proposal[11] that echinomycin may be biosynthetically derived from triostin by *S*-methylation and subsequent rearrangement.

Further work on the nucleotide sequence-specificity of these antibiotics is in progress, but FOX *et al.*[4] have already shown that 1QN and 2QN differ markedly in their specificity from echinomycin. Our results therefore serve to reinforce their conclusions, that the chromophores take an active part in binding to DNA, and do not merely act as the prongs of 'staples' to locate the peptide portion of the antibiotic onto the DNA.

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