PRESENCE OF ISOMERS IN QUINOMYCIN E*

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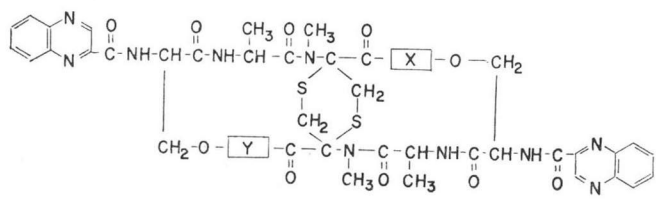
Quinomycin E is a member of quinomycin antibiotics, whose members hitherto-isolated are quinomycins A(echinomycin), Bo, C, D, B and E. The structure of echinomycin, in which a dithian ring cross-link was proposed, had been determined mainly by chemical evidences.[1] The structures of other members had been deduced as formula I in Fig. 1, in which arbitrary pairs of three kinds of N-methyl amino acid residues* are substituted at two replaceable parts, on the basis of their constituent differences in composition and analogy to the behavior of echinomycin.[2]

Recent studies[3,4] based on proton and carbon nuclear magnetic resonance and mass spectrometry carried out with quinomycins A (echinomycin) and C have resulted in revising their proposed structures in part: the dithian ring cross-link was modified to a thioacetal cross-link. As this revised part was considered to be common in all members, the structures of quinomycin antibiotics should be now presented as in formula **II**. This revision, however, gave rise to a new problem. The two replaceable parts are not equivalent in the revised formula (**II**), though they were equivalent in the former (**I**). Therefore, two possible positional isomers should be considered, when the substituents are a pair of different residues. Therefore, quinomycins Bo, D and E are now suspected to be a mixture of two positional isomers, although they were homogeneous on

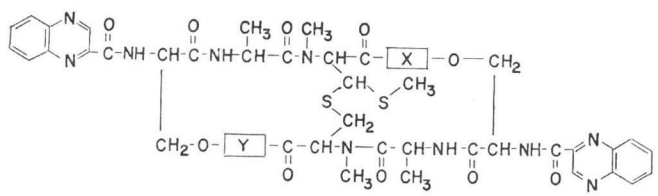
Fig. 1. Structures of quinomycin antibiotics.

Echinomycin (Quinomycin A): X=Y=MeVal
Quinomycin Bo: X = MeVal, Y = DimeAile;
X = DimeAile, Y = MeVal
Quinomycin C : X = Y = DimeAile
Quinomycin D : X = MeVal, Y = MeAile;
X = MeAile, Y = MeVal
Quinomycin B : X = Y = MeAile
Quinomycin E : X = MeAile, Y = DimeAile;
X = DimeAile, Y = MeAile



I

Originally proposed structure

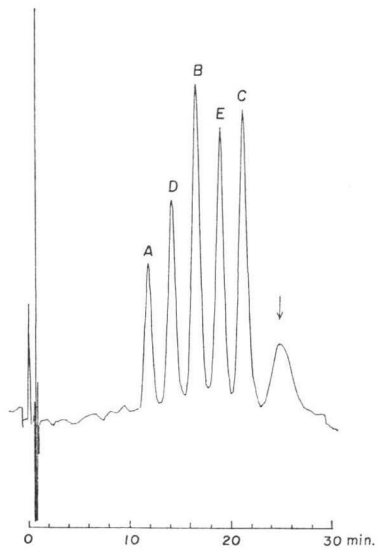


II

Revised structure

Fig. 2. High performance liquid chromatogram of quinomycins A, D, B, E and C.

Apparatus: Varian LC 8500
Column: Micropak CH-10, 25 cm×0.21 cm i.d.
Mobile phase: linear gradient, MeCN/H_2O 35/65 →MeCN 0.7%/min.
Flow rate: 1.0 ml/min.
Temperature: room temperature
Detector: UV (254 nm), 0.04 AUFS
Sample solvent: MeOH
↓ : impurity from solvent



* In this paper the following abbreviations are used: MeVal: N-methyl-L-valine; MeAile: N-methyl-L-alloisoleucine; DimeAile: N, γ-dimethyl-L-alloisoleucine.

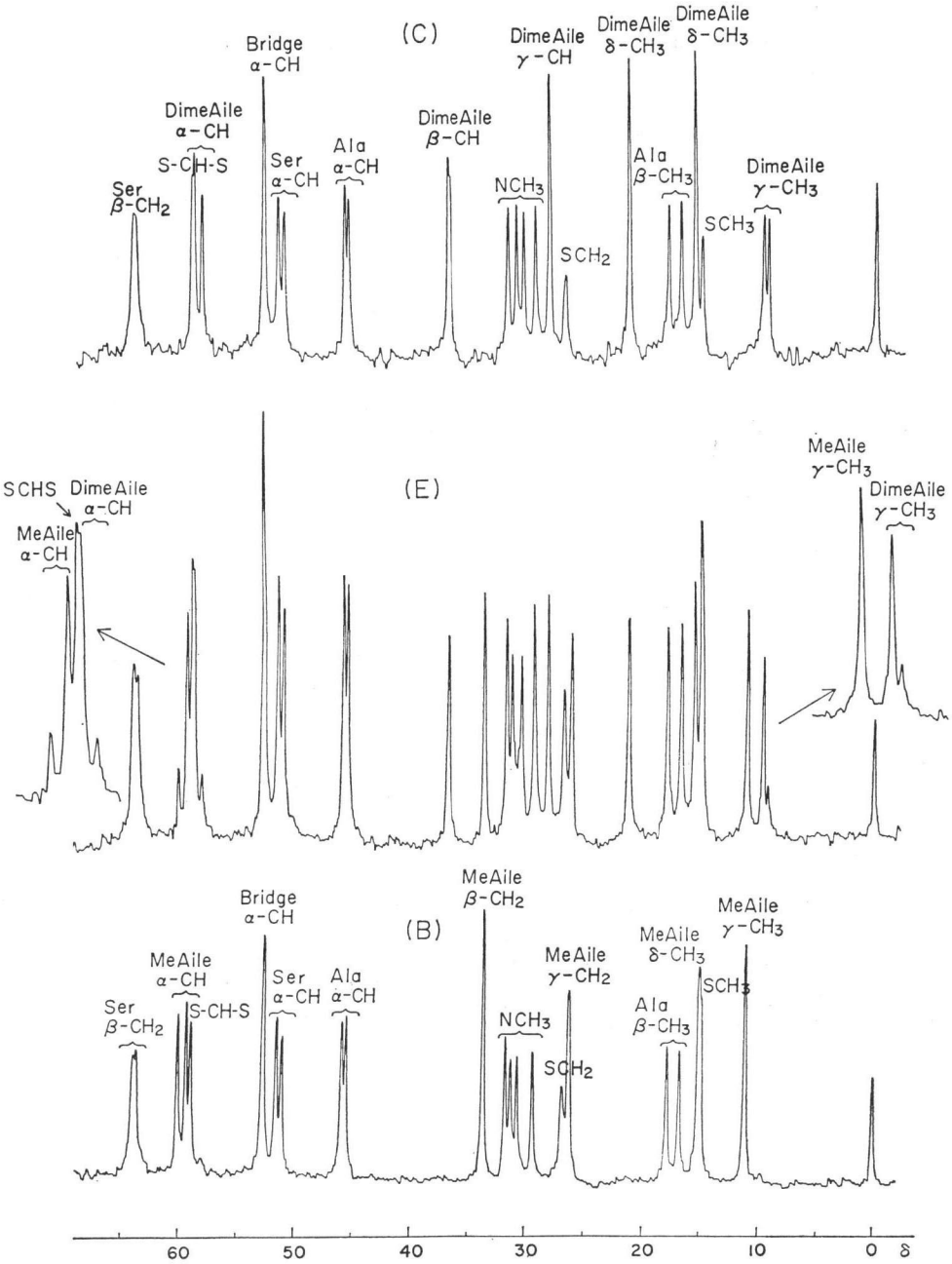
Fig. 3. ^{13}C Spectra of quinomycins C, E and B in $CDCl_3$.

Spectra were recorded on a JEOL-PFT-100 pulse-Fourier transform NMR spectrometer locked on the D resonance of the solvent. Chemical shifts are read in ppm relative to the internal TMS.

(C) Quinomycin C: 100 mg in 1.2 ml, accumulated 21,000 times.
Assignments for Ser α-CH and Bridge α-CH are based on those by MARTIN *et al.*[4]

(E) Quinomycin E: 100 mg in 1.2 ml, accumulated 54,000 times.
The spectra on the both sides are expanded ones of the region indicated by arrows.

(B) Quinomycin B: 73 mg in 1.2 ml, accumulated 45,000 times.



circular thin-layer chromatography.[2]

We first attempted further resolution of quinomycin antibiotics by means of high performance liquid chromatography. When a mixture of quinomycins A, D, B, E and C was subjected to a column of Micropack CH-10 with a mixture of acetonitrile and water as a mobile phase, separation of these antibiotics was observed (Fig. 2). Similar results were also given on columns of Micropack Al-10, Hitachi gel #3010 and Woeln neutral alumina. However, further resolution of quinomycin D or E was not achieved in any case.

The next attempt was made by comparison of ^{13}C magnetic resonance spectra of quinomycins B, E and C, in whose structures the substituents at the two replaceable parts are considered to be: X=Y=MeAile in quinomycin B; X=MeAile, Y=DimeAile and/or X=DimeAile, Y=MeAile in quinomycin E; and X=Y=DimeAile in quinomycin C. The spectra measured on $CDCl_3$ solutions are shown in Fig. 3. The assignments of signals of quinomycin C, which has already been reported,[4] and of quinomycin B, which was assigned in reference to those of quinomycins A and C,[3,4] are described in the figure. In the spectrum of quinomycin E, all the signals corresponding to those observed in the spectra of quinomycins B and C were given. In respect to the signals due to DimeAile residues in quinomycin C, the signals of γ-CH_3 and α-CH were given as pairs of signals (the former at 9.45 and 9.8 ppm, and the latter at 59.05 and 59.75), whereas others were given as single signals. Similarly, the signals due to α-CH of MeAile residues in quinomycin B were given as a pair (60.35 and 61.1 ppm). The slight differences in chemical shifts of these pairs caused by two chemically identical residues should be attributed to positional unequivalence. Demonstration of these pairs in the spectrum of quinomycin E indicated that both of MeAile and DimeAile residues were present at the two replaceable parts, providing the evidence that the possible two isomers (X=MeAile, Y=DimeAile and X=DimeAile, Y=MeAile) were present in the preparation of quinomycin E. However, the two peaks of each pair were not equal in intensity: the ratio of the intensity was approximately estimated as 4:1, and the sum of the intensity of the peak pairs seemed to correspond to one carbon atom. This meant that the two isomers were present in a ratio of approximately 4: 1 and not in equal quantities.

Thus, the presence of two positional isomers in quinomycin E, as anticipated, was proved, although they were not separable by our chromatographic experiments. These isomers are relatively large molecules with features somewhat near to symmetrical appearance. These isomers result from the presence of the -SCH_3 group and its effect is quite small in contrast to the large molecular size. It would be reasonable to consider that separation of such positional isomers is quite difficult by the techniques used at present. Though measurement with quinomycins Bo and D could not be made because of lack of their available preparations, occurrence of similar isomers as in quinomycin E is thought to be likely.

References

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