STRUCTURE CONFIRMATION OF TRIOSTIN A BY ^{1}H AND ^{13}C MAGNETIC RESONANCE

Sir:

Triostin A is a member of quinoxaline antibiotics,[1] which is the generic name of a group of antibiotics comprising quinomycin antibiotics and triostin antibiotics. The structure of echinomycin,[2] in which the presence of a dithian ring cross-link was proposed, and the structure of triostin C,[3] in which a disulfide cross-link was proposed, had been determined mainly by chemical evidences. The structures of other quinoxaline antibiotics,[4] *i.e.*, quinomycins Bo, C,D,B and E, and triostins A, Bo, and B, had been deduced on the basis of analogy to echinomycin and triostin C, respectively.

Recently, the proposed structure of echinomycin has been revised in part by evidence based on ^{1}H and ^{13}C nuclear magnetic resonance and mass spectrometric experiments: the dithian ring cross-link is modified to a thioacetal cross-link.[5] The same conclusion has been reported with quinomycin A (echinomycin) and C.[6]

This fact led us to re-examine the structure of triostin A (Fig. 1) by ^{1}H and ^{13}C magnetic resonance experiments.

To our surprise, the ^{1}H spectrum measured for the $CDCl_3$ solution showed a pattern which may be interpreted by the presence of an asymmetrical conformation of the antibiotic, *e.g.*, each proton of chemically equivalent quinoxaline rings gave pairs of signals as in the case of echinomycin[5] (Fig. 2-b). However, when spectrum was measured for solution in deutero-dimethylsulfoxide (DMSO-d_6), its spectral pattern indicated a symmetrical conformation of the antibiotic, *e.g.*, the pairs of signals given above were reduced to single signals (Fig. 2-a). Similarly, the ^{13}C spectrum measured for the $CDCl_3$ solution

Fig. 1. Structure of triostin A

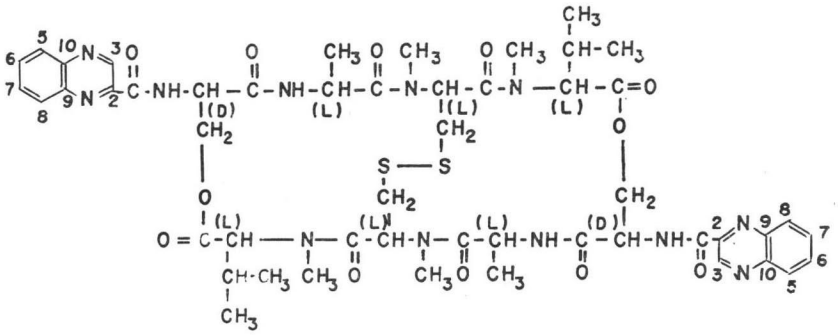

Fig. 2. ^{1}H Magnetic resonance spectra of triostin A.

a) DMSO-d_6 solution, b) $CDCl_3$ solution. Spectra were recorded on a JEOL-PS-100 spectrometer operated at 100 MHz. About 40 mg of the samples was dissolved in the solvents. Chemical shifts were measured from internal TMS.

*Indicates the signals of solvents;
**Indicates those of water.

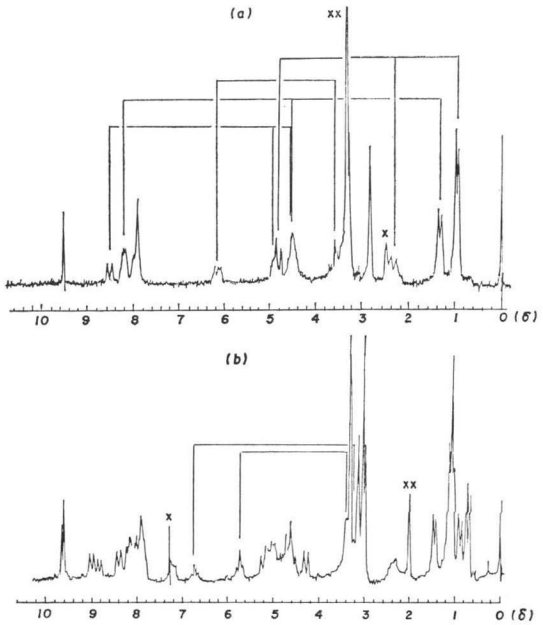

Fig. 3. Proton decoupled ^{13}C magnetic resonance spectra of triostin A.
a) DMSO-d_6 solution, b) $CDCl_3$ solution. Spectra were recorded on a JEOL-PFT-100 pulse FOURIER transform nmr spectrometer operated at 25 MHz.
About 100 mg of samples was dissolved in 1.5 ml of solvents. Each spectrum was accumulated 6000 times. Chemical shifts were measured from internal TMS.

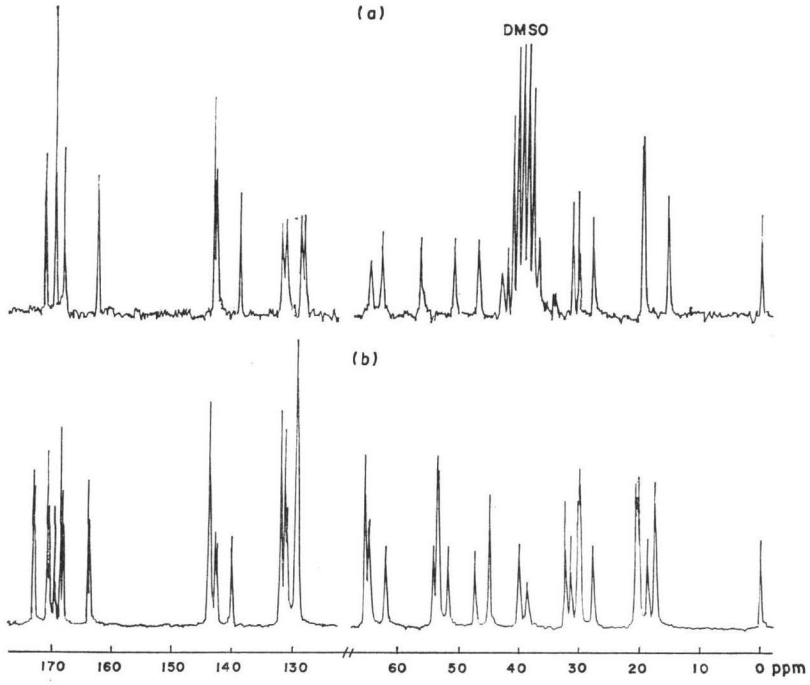

Table 1. Assignment of the ^1H magnetic resonance spectrum of triostin A in DMSO-d_6.

Assignment		Signal, ppm
N-Methylvaline	αCH	4.86
	βCH	2.37
	γCH_3	0.97
N, N'-Dimethylcystine	αCH	6.22
	βCH_2	3.62
Alanine	αCH	~4.6
	βCH_3	1.34
	NH	~8.2
Serine	αCH	4.98
	βCH_2	~4.6
	NH	8.53
Quinoxaline	CH-3	9.51
	CH-5,6,7,8	7.9~8.3
	NCH_3	2.89
	NCH_3	3.34

showed the presence of 50 carbons (Fig. 3-b), but the ^{13}C spectrum of the DMSO-d_6 solution gave the signals of only 25 carbons, just a half of the above (Fig. 3-a). These facts indicate that the molecule of triostin A is constructed from two chemically equivalent halves, but each half does not take a equivalent conformation in $CDCl_3$. In DMSO-d_6, however, interconversion of the two conformations must be rapid enough to be averaged out in the measurement of the nmr spectra, or each half must take the same conformation.

The assignments of the signals observed in the ^1H spectra were made mainly by spin-spin decoupling experiments and by the comparison with the spectra of model compounds such as quinoxaline-2-carboxilic acid methyl ester, quinoxaline-2-carboxyl-D-serine methyl ester, N,N'-dimethyl-L-cystine dimethyl ester and N-carbobenzoxy-N-methyl-L-valine. The assignments of all the signals in the spectrum of the DMSO-d_6 solution are listed in Table 1. Complete assignments for the proton signals in the spectrum of the $CDCl_3$ solution are now under investigation by the use of

Table 2. Assignment of the ^{13}C magnetic resonance spectra of triostin A.

Assignment		Signal, ppm	
		in DMSO-d_6	in $CDCl_3$
N-Methylvaline	αCH	62.7	61.9 65.2
	βCH	27.7	27.7 29.7
	γCH_3	19.5	18.6 20.0
	γCH_3	19.7	20.3 20.6
N,N'-Dimethylcystine	αCH	56.3	53.2 53.5
	βCH_2	42.8	38.5 39.9
Alanine	αCH	46.8	44.8 47.1
	βCH_3	15.4	17.4 17.5
Serine	αCH	50.7	51.7 54.0
	βCH_2	64.6	64.8 65.2
Quinoxaline	C-2	143.4	143.8 143.8
	C-3	143.4	143.7 143.7
	C-9, 10	139.1	140.0 140.1
		142.9	142.4 142.6
	C-5, 6, 7, 8	128.4	129.4 129.4
		129.1	129.6 129.6
		131.4	130.9 131.2
		132.1	132.0 132.0
Carbonyl		162.7	163.5 163.8
		168.1	167.9 168.3
		169.6	169.3 170.1
		169.6	170.4 170.5
		171.2	172.6 172.8
	NCH_3	30.2	30.0 30.2
	NCH_3	31.2	31.4 32.3

shift reagents, since some signals are overlapped with each other. However, we can distinguish the signals of the C-α and C-β protons of N-methyl cystine residue by decoupling experiments: the signals of the C-α protons are observed at 5.7 and 6.8 ppm. The appearence of these signals proves the existence of the cystine bridge in the molecule.

The signals observed in the ^{13}C magnetic resonance spectra were assigned by partial decoupling experiments, and by comparison to the spectra of model compounds such as quinoxaline-2-carboxylic acid methyl ester, quinoxaline-2-carboxyl-D-serine methyl ester, L-alanine methyl ester hydrochloride, N,N'-dimethyl-L-cystine dimethyl ester and N-carbobenzoxyl-N-methyl-L-valine. The assignments of all the signals are listed in Table 2. Each of the two signals of NCH_3 was only assignable to either N-methylvaline or N,N'-dimethylcystine residue. Similarly, complete assignments could not be made for the four carbons of quinoxaline ring (C-5, 6, 7, 8) and five carbonyl carbons.

These results proved the presence of all fragments in the proposed structure of triostin A and symmetrical arrangement of the fragments, indicating the validity of the proposed structure of triostin A (Fig. 1) based on degradation experiments.[3] The assumption that all triostin antibiotics contain the disulfide cross-link[4] will also be true. The interesting problem on the conformation of triostin A, especially the asymmetrical conformation in $CDCl_3$, is now under investigation. The result will be published elsewhere.

We also examined the nmr spectra of echinomycin (quinomycin A) as a reference of triostin A. The asymmetrical structure

of the antibiotic which has already been reported in the ^1H spectrum of its $CDCl_3$ solution,[5,6] was shown also in the ^1H spectrum of the DMSO-d_6 solution, the presence of S-CH_3 being indicated. The revised part of the structure of echinomycin, *i.e.*, the dithiane ring cross-link being modified to a thioacetal cross-link,[5,6] should be true for all quinomycin antibiotics, because the structures of quinomycins Bo, C, D, B and E, reported by two of the present authors (OTSUKA and SHOJI),[4] had been deduced on the basis of their constituent differences and analogy to the behavior of echinomycin.

Acknowledgement

The authors wish to thank to several colleagues in Shionogi Research Laboratory, especially to Dr. K. TORI and Mrs. Y. YOSHIMURA for a part of nmr measurements and Dr. K. INOUYE and Mr. M. SHIN for the model compounds. Thanks are also due to Mr. N. HIGUCHI, Osaka University, for his assistance in the measurements of nmr spectra.

HIDEO OTSUKA
JUN'ICHI SHOJI

Shionogi Research Laboratory, Shionogi & Co., Ltd. Fukushima-ku, Osaka, 553 Japan

KEIICHI KAWANO
YOSHIMASA KYOGOKU

Institute for Protein Research, Osaka University, Suita, Osaka, 565 Japan

(Received September 6, 1975)

References

1) KUROYA, M.; N, ISHIDA, K. KATAGIRI, J. SHOJI, T. YOSHIDA, M. MAYAMA, K. SATO, S. MATSUURA, Y. NIINOMI & O. SHIRATORI: Studies on quinoxaline antibiotics. I. General properties and the producing strains. J. Antibiotics, Ser. A 14: 324~329, 1961
2) KELLER-SCHIERLEIN, W.; M. LI. MIHAILOVIC & V. PRELOG: Stoffwechselprodukte von Actinomyceten. XV. Über die Konstitution von Echinomycin. Helv. Chim. Acta 42: 305~322, 1959
3) OTSUKA, H. & J. SHOJI: The structure of triostin C. Tetrahedron 21: 2931~2938, 1965
4) OTSUKA, H. & J. SHOJI: Structural studies on the minor components of quinoxaline antibiotics. Tetrahedron 23: 1535~1542, 1967
5) DELL, A.; D.H. WILLIAMS, H.R. MORRIS, G.A. SMITH, J. FEENEY & G.C.K. ROBERTS: Structure revision of the antibiotic echinomycin. J. Amer. Chem. Soc. 97: 2497~2502, 1975
6) MARTIN, D.G.; S.A. MIZSAK, C. BILES, J.C. STEWART, L. BACZYNSKI & P.A. MEULMAN: Structure of quinomycin antibiotics. J. Antibiotics 28: 332~336, 1975