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

Sir:

Triostin A is a member of quinoxaline antibiotics, which is the generic name of a group of antibiotics comprising quinomycin antibiotics and triostin antibiotics. The structure of echinomycin, in which the presence of a dithian ring cross-link was proposed, and the structure of triostin C, in which a disulfide cross-link was proposed, had been determined mainly by chemical evidences. The structures of other quinoxaline antibiotics, 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. The same conclusion has been reported with quinomycin A (echinomycin) and C. 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., 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 in deuterodimethylsulfoxide (DMSO-d$_6$), its spectral pattern indicated a symmetrical conformation of the antibiotic, e.g., the signals of solvents were measured from internal TMS.

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.
showed the presence of 50 carbons (Fig. 3-b), but the 13C spectrum of the DMSO-d6 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 CDCl₃. In DMSO-d6, 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-d6 solution are listed in Table 1. Complete assignments for the proton signals in the spectrum of the CDCl₃ solution are now under investigation by the use of
Table 2. Assignment of the $^{13}$C magnetic resonance spectra of triostin A.

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Signal, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in DMSO-d$_{6}$</td>
</tr>
<tr>
<td>N-Methylvaline</td>
<td></td>
</tr>
<tr>
<td>$\alpha$CH</td>
<td>62.7</td>
</tr>
<tr>
<td>$\beta$CH</td>
<td>27.7</td>
</tr>
<tr>
<td>CH$_{3}$</td>
<td>19.5</td>
</tr>
<tr>
<td>CH$_{3}$</td>
<td>19.7</td>
</tr>
<tr>
<td>N,N'-Dimethylcystine</td>
<td></td>
</tr>
<tr>
<td>$\alpha$CH</td>
<td>56.3</td>
</tr>
<tr>
<td>$\beta$CH$_{2}$</td>
<td>42.8</td>
</tr>
<tr>
<td>Alanine</td>
<td></td>
</tr>
<tr>
<td>$\alpha$CH</td>
<td>46.8</td>
</tr>
<tr>
<td>$\beta$CH$_{3}$</td>
<td>15.4</td>
</tr>
<tr>
<td>Serine</td>
<td></td>
</tr>
<tr>
<td>$\alpha$CH</td>
<td>50.7</td>
</tr>
<tr>
<td>$\beta$CH$_{2}$</td>
<td>64.6</td>
</tr>
<tr>
<td>Quinoxaline</td>
<td></td>
</tr>
<tr>
<td>C-2</td>
<td>143.4</td>
</tr>
<tr>
<td>C-3</td>
<td>143.4</td>
</tr>
<tr>
<td>C-9, 10</td>
<td>139.1</td>
</tr>
<tr>
<td>C-5, 6, 7, 8</td>
<td>128.4</td>
</tr>
<tr>
<td>Carbonyl</td>
<td></td>
</tr>
<tr>
<td>NCH$_{3}$</td>
<td>30.2</td>
</tr>
<tr>
<td>NCH$_{2}$</td>
<td>31.2</td>
</tr>
</tbody>
</table>

shift reagents, since some signals are overlapped with each other. However, we can distinguish the signals of the C-$\alpha$ and C-$\beta$ protons of N-methyl cystine residue by decoupling experiments; the signals of the C-$\alpha$ protons are observed at 5.7 and 6.8 ppm. The appearance 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-carbobenzoxy-L-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. The assumption that all triostin antibiotics contain the disulfide cross-link 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 $^1$H spectrum of its CDCl$_3$
 solution, was shown also in the $^1$H 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, 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), 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

References

1) KUROYA, M.; N. ISHIDA, K. KATAGIRI, J.
SHOJI, T. YOSHIDA, M. MAYAMA, K. SATO,
S. MATSUURA, Y. NIHOMI & O. SHIRATORI:
Studies on quinoxaline antibiotics. I. General
properties and the producing strains. J. Anti-
biotics, Ser. A 14: 324~329, 1961
2) KELLER-SCHIERLEIN, W.; M. LI. MIHAILOVIC
& V. PRELOG: Stoffwechselprodukte von
Actinomyceten. XV. Uber die Konstitution
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
5) DELL, A.; D.H. WILLIAMS, H.R. MORRIS,
G.A. SMITH, J. FEENEY & G.C.K. ROBERTS:
Structure revision of the antibiotic echino-
mycin. 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. Anti-
biotics 28: 332~336, 1975