Manipulation of the Nuclear Overhauser Effect by the Use of a Viscous Solvent: the Solution Conformation of the Antibiotic Echinomycin

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Summary In an experiment which should be of wide applicability, a viscous solvent has been used to induce relatively slow tumbling of the solute; under these conditions, negative n.O.e.'s may be observed and, in the present case, these have been used to deduce the solution conformation of echinomycin.

WITH the present sophistication of n.m.r. techniques,[1] it is clear that the nuclear Overhauser effect (n.O.e.) will play an increasingly important role in the determination of molecular structure in solution. However, the maximum positive n.O.e. (50%) is smaller than the maximum negative n.O.e. (100%), and additionally the molecular weights of many molecules of interest are such that the n.O.e. may be insignificant. It would clearly be extremely useful to be able to manipulate the size of the n.O.e.

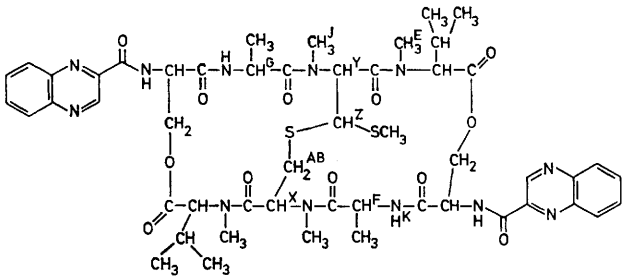
The theoretical maximum value of the n.O.e. is crucially dependent on the product $\omega\tau_c$, where ω is the Larmor precession frequency and τ_c is the molecular rotational correlation time. The maximum value, η_{max}, is given[2] by equation (1). Thus, when $\omega\tau_c = ca.\ 1{\cdot}2$, no n.O.e. will be

$$\eta_{max} = (5 + \omega^2\tau_c^2 - 4\omega^4\tau_c^4)/(10 + 23\omega^2\tau_c^2 + 4\omega^4\tau_c^4) \quad (1)$$

observable. By increasing the viscosity of the solution, it should be possible to increase τ_c significantly so that n.O.e.'s can be seen; this has been demonstrated by Bothner-By and Johner,[3] though for different reasons.

We have chosen to use the oil Voltalef 10S† as a viscous solvent. It is a polymer of chlorotrifluoroethylene and has the advantage over more obvious solvents (such as [$^2H_6$]-ethylene glycol) that (i) it is readily obtainable in high purity, (ii) it is very viscous [1550 cP at 25 °C; contrast ethylene glycol (17·0 cP) or chloroform (0·54 cP)], and (iii) it is readily miscible with all common organic solvents. It is not in itself a good solvent, but solutions of many reasonably non-polar molecules can be obtained when the oil is added to chloroform or dichloromethane solutions.

To illustrate the usefulness of the technique, we report its application to the determination of the solution conformation of echinomycin. Echinomycin (**1**) is a cyclic



(1)

octapeptide antibiotic, which acts by intercalation of both quinoxaline rings between the base pairs of DNA. It is important to know the conformation in order to be able to study the interactions of echinomycin with different DNA bases.[4] Cheung *et al.*[5] have studied the conformation by

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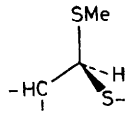
n.m.r. spectroscopy, model building, and potential energy calculations, and have deduced a set of closely related conformations in which both quinoxaline rings and both valine N–Me groups are on the same, 'upper' side of the molecule. It is further suggested that both cysteine N–Me groups and the S–Me group are on the 'lower' side; the stereochemistry of the thioacetal CH^Z carbon and the torsion angles of the cross-bridge are unknown. No n.O.e.'s were observed in chloroform at 270 MHz, probably because n.O.e. difference spectra (n.O.e.d.s.) were not determined. Indeed, using n.O.e.d.s. at 400 MHz, we have observed small positive n.O.e. enhancements. Larger negative enhancements were obtained as follows.

A 4·5 mM solution of echinomycin in $CDCl_3$ and Voltalef 10S (1:1) was prepared. This solution should have a viscosity at least 10 times that of $CDCl_3$ alone and increase η_{max} from *ca.* +30% to *ca.* −70%. No n.O.e.'s of this size were observed, but this is not unexpected since most protons are effectively relaxed by more than one neighbour. The largest n.O.e. seen was of −25%, upon irradiation of H^A (characterized by J_{AX} 11·2 Hz) and observation of H^B (characterized by J_{BX} 1·7 Hz). Spectra were run at 400 MHz on a Bruker WH 400; all n.O.e.'s were observed as n.O.e. difference spectra, with enhanced and control spectra acquired alternately to minimise the effects of spectrometer drift.‡

The four NMe singlets can be clearly seen, although the two at highest field are coincident. Irradiation of the lowest field peak gave no n.O.e.'s, while irradiation of the next lowest gave an n.O.e. to H^Y, thus identifying it as the valine *N*–methyl CH_3^E. Irradiation of the other two, coincident, peaks gave n.O.e.'s to Ala–C_αH H^F, Ala–C_αH H^G, and Cys–C_βH H^Z, identifying them as the two Cys–NMe groups. The two Ala–C_αH protons are on the 'lower' side of the molecule; we have therefore confirmed that the two Cys–NMe groups are on the 'lower' face, as is H^Z. Irradiation of the S–Me gives an n.O.e. to H^Z (the reverse n.O.e. can also be seen), confirming that the SMe is on the 'lower' face.

We have deduced that the chirality at the carbon bearing H^Z is (*S*)-(**2**); this is evidenced by the n.O.e. {Cys–NMe} → H^Z. Measurement of the ^3J coupling constant[5] shows that this part of the cross-bridge must have the conformation (**3**) or (**4**); only (**3**) is compatible with the (*S*) chirality (**2**).



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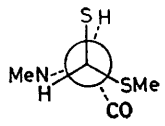

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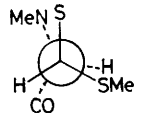
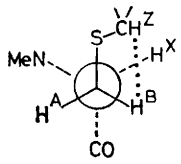


(4)

The only remaining problem is whether the methylene cross-bridge protons AB are held in the conformation (**5**) or (**6**). Irradiation of H^B gives a small n.O.e. to H^Z [as indicated by the dotted line in (**5**)], indicating the conformation (**5**). This n.O.e. is expected to be small since H^Z is efficiently relaxed by the SMe and Cys–*N*-methyl (CH_3^J) protons. In the conformation (**6**), a large n.O.e. would have been expected to the Ala–NH^K, as this proton would be relaxed relatively efficiently by H^B in conformation (**6**); however, no such n.O.e. was seen (*i.e.* n.O.e. ⩽ 0·5%). A further possible n.O.e. in (**6**) [{H^B} → H^F] is also absent.



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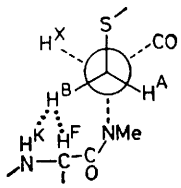

(6)

The increasing availability of n.O.e. difference spectroscopy and of highly dispersed spectra obtained at high field strengths, coupled with manipulation of the magnitude of n.O.e.'s by varying τ_c as described in the present work, provide an extremely powerful method for the determination of structures in solution. The versatility and potential of the technique is illustrated by the fact that, in preliminary work, we have obtained n.O.e.'s of up to −63% in the ^1H spectra of steroids.

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‡ For tumbling molecules, spin diffusion begins to be important. This has been considered and does not compromise the present conclusions. In this type of work, althoxgh $\omega\tau_c > 1$, it is important that $\omega\tau_c \gg 1$.

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