Conformational Analysis of *N*-Acetyldaunomycin in Solution. A Transient ^{1}H Nuclear Overhauser Effect Study of the Glycosidic Linkage Geometry

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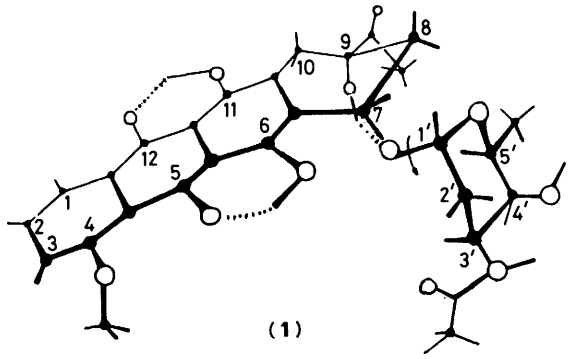
The preferred conformation of the sugar with respect to the aglycone moiety has been determined for *N*-acetyldaunomycin in solution, by transient nuclear Overhauser experiments (n.O.e.). The time development of proton n.O.e.s has been measured after the selective inversion of single resonances and the whole curves have been analysed through a non-linear least-squares procedure. Calculations performed by a 'two-spin approximation' allowed values of the cross-relaxation rates (σ) between close protons to be extracted. This method was shown to give a good estimate of σ, from which the interproton distance ratios have been obtained, with an accuracy comparable to that of an *X*-ray method. The preferred conformation of the glycosidic linkage has also been expressed in terms of the rotational angles φ = H(1')–C(1')–O(7)–C(7) and ψ = C(1')–O(7)–C(7)–H(7), and the results have been compared with those from *X*-ray analyses and from energy-minimum calculations.

In the preceding paper[1] we reported on a conformational study in solution of ring A of the anticancer agent daunomycin, and of its analogues. In order to determine the conformational preference of the glycosidic linkage, we have explored the possibility offered by n.m.r. techniques, in particular the proton nuclear Overhauser effect (n.O.e.).[2] We are interested in the quantitative aspect of these experiments, whose final object is to obtain interproton distances values with reasonable accuracy, and we report here on the results obtained with *N*-acetyldaunomycin (**1**) on performing steady-state and transient n.O.e. experiments.[2–6] Chloroform solutions were examined at first, because we preferred to avoid possible complications arising from strong self-association, as occurs in water, and strong interactions with solvents such as, for instance, DMSO.



(1)

Experimental

N.O.e. experiments were performed with a Bruker CXP-300 spectrometer at 25 °C on a sample of *N*-acetyldaunomycin in $CDCl_3$ solution and in $CDCl_3$ after exchange with D_2O. Chemical shifts are in δ values from internal Me_4Si. Solutions of 4 mg per 0.5 ml were degassed with four freeze–pump–thaw cycles directly in n.m.r. tubes, which were then sealed. N.O.e and saturation transfer effects were measured by difference spectroscopy by acquiring both on- and off-resonance spectra and the enhancements were calculated from heights and integrated intensities of the peaks. Steady-state n.O.e.[2] and saturation transfer[7] experiments were performed with a long (4 s) and weak decoupler pulse. The selective inversion pulse for transient n.O.e. experiments[2,4,6] was performed with a decoupler pulse length of 25 ms. The decoupler pulse length was optimized in order to achieve the best inversion compatible with selectivity. The inversion factor is >90%, except for 5'-H (75%) because of its larger width; this has been taken into account in the initial conditions of the fitting procedure. To average out instrumental instabilities through the whole n.O.e. experiments, an automation microprogram has been used, where several cycles of eight-scan acquisitions for on-resonance and off-resonance irradiations have been performed. The average duration of a transient n.O.e. experiment (8—10 data points) was *ca.* 10 h. The cross-relaxation rates (σ_{ij}) and the relaxation rates (ρ) were calculated by a non-linear least-squares fit to equation (4) of all the experimental points, with a program developed in our laboratory, based on standard algorithms. We report here only σ_{ij} values which carry structure information (Table 1). The simulation of transient n.O.e.s in Figure 2 was carried out with the same procedure. Selective spin–lattice relaxation times[2] of protons (T_1^s) were measured from the recovery of the inverted signal in the on-resonance spectra of transient n.O.e. experiments. Non-selective spin–lattice relaxation times[2] of protons (T_1^{ns}) were performed by using a standard inversion recovery pulse sequence. For all the experiments, a relaxation delay of 8 s was used. The analysis of the decay curves was done by using the three-parameter least-squares fitting program included in the ASPECT 2000 computer library.

Chemical-shift assignments are described in the accompanying paper.[1]

Results and Discussion

(a) *Steady-state N.O.E. and Saturation Transfer Experiments.*—In the spectra reported in Figure 1b,c, strong positive n.O.e. effects were measured for 1'-H (15%) and for 7-H (13%) following irradiation of 7-H and 1'-H respectively; significant enhancements (2.5—3.3%) were also observed for 6-OH in both experiments. This ruled out an *anti* relationship between 7-H and 1'-H (also disfavoured by steric hindrance) and suggested that they should be close to each other. Actually, 1'-H and 7-H are both vicinal to methylene groups, which greatly contribute

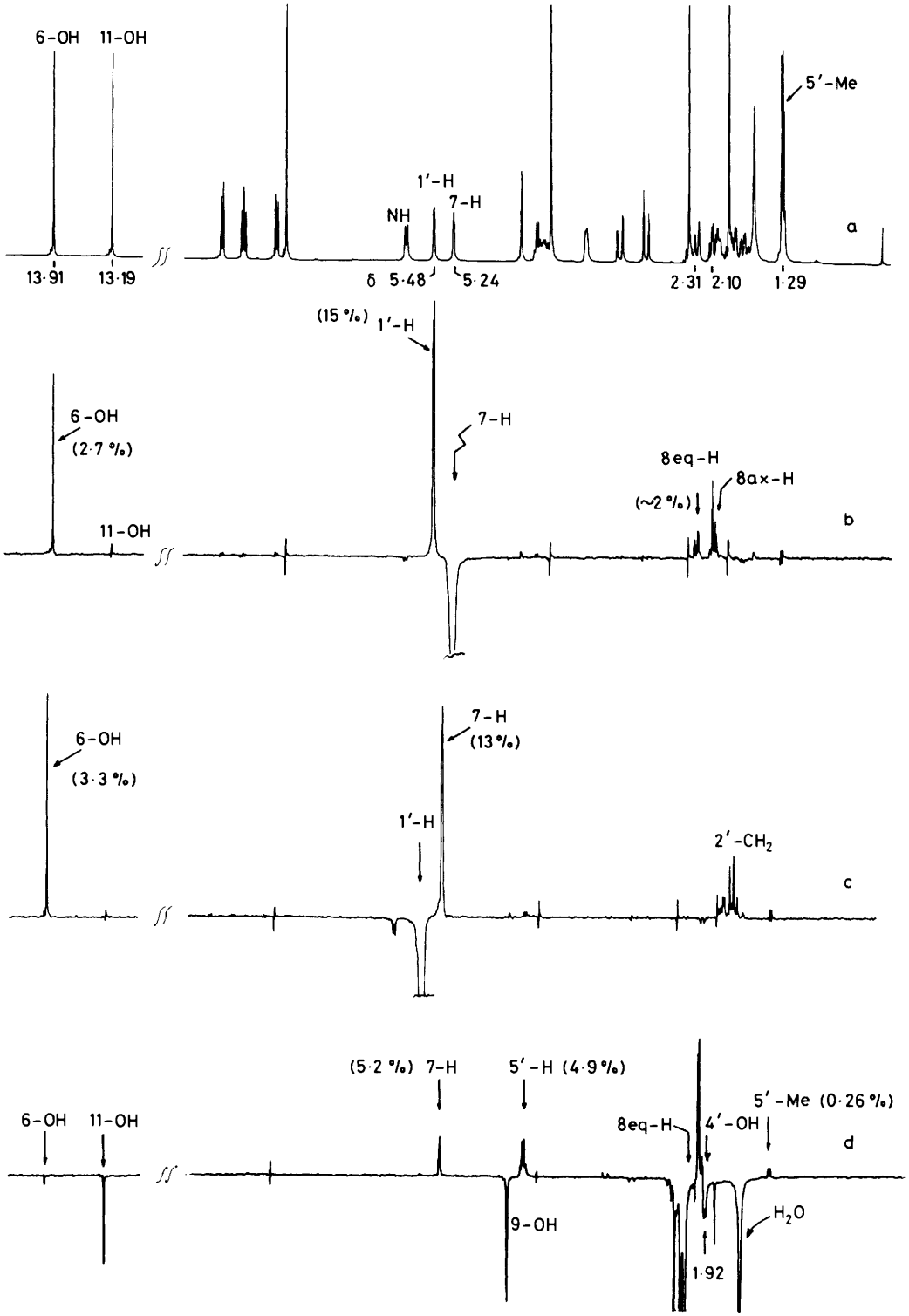


Figure 1. N.m.r. spectrum (a) and steady-state n.O.e. difference spectra (b—d) of *N*-acetyldaunomycin in $CDCl_3$. Irradiation of 7-H (b), 1'-H (c). and 8eq-H (d)

to their relaxation, thus reducing the contribution to the relaxation of 7-H by 1'-H (and *vice versa*), which consequently also reduces the n.O.e. effects. The interesting results obtained for 6-OH show that 1'-H is also close to the hydroxy proton at C-6. Another series of experiments involves the irradiation of 5'-Me, 8eq-H, and 5'-H. Of the aglycone moiety, 8eq-H is the only nucleus which is perturbed (n.O.e. 0.9%) upon irradiation of the sugar methyl group.

The inverse experiment (irradiation of 8eq-H, Figure 1d) is more interesting because it leads, beside a small enhancement of 5'-Me (0.26%), to a significant n.O.e. effect on 5'-H (4.9%). In the same experiment, the hydroxy protons and water signals become negative for saturation transfer[7] from 4'-OH, which is close to the irradiation frequency. This made it possible to obtain the chemical shift of 4'-OH (δ 1.92). The saturation transfer effect also occurs upon irradiation of 11-OH, 6-OH, or H_2O.

It is interesting to observe that the intensity of this effect is stronger for 11-OH than for 6-OH, as is clearly visible also in Figure 1d. The exchange rates of the chelated hydroxys with water (K_w), relatively to their own relaxation time (T_1^{ns}), were determined in this experiment, upon saturation of water.[7] The observed intensity of the OH signal (I_{obs}) is related to the exchange rate by the equation: $I_{obs} = I_o/(1 + K_w T_1^{ns})$, where I_o is the intensity without saturation. The measurements gave $K_w \cdot T_1^{ns} = 0.02$ for 6-OH and $K_w \cdot T_1^{ns} = 0.09$ for 11-OH; since the hydroxy protons showed the same relaxation times ($T_1^{ns} = 1.24$ for 6-OH, $T_1^{ns} = 1.34$ for 11-OH), it means that chemical exchange with the mobile protons present in solution is less efficient for 6-OH. This agrees with the chemical-shift and line-width values of the chelated hydroxy protons: the 6-OH signal is sharper and at lower field than 11-OH. All these results are evidence of the stronger hydrogen bond involving 6-OH,[8] but the lower K_w value for 6-OH can also be explained with protection of this group by the sugar moiety.

Qualitatively, the most important results obtained from the steady-state n.O.e. experiments can be summarized as follows: the orientation of the sugar with respect to the aglycone must be such as to leave (i) 1′-H close to 7-H, (ii) 5′-H close to 8eq-H but not to 8ax-H, and (iii) 1′-H relatively close to 6-OH. However, the use of equilibrium n.O.e.s to obtain more precise results became difficult, as the number of the interacting spins is high, and not all the necessary n.O.e. effects for a multispin system[2] could be measured with sufficient accuracy.

(b) *Transient N.O.E. Experiments.*—In order to calculate the interproton distances from steady-state n.O.e.s, the spin–lattice relaxation times (T_1) of each pair of protons in question can be used, but relaxation contributions, arising from chemical exchanges, paramagnetic substances, *etc.*, must also be taken into account.[2] In addition, the complete elimination of oxygen from the solution is always difficult. All these problems are less severe for 'time-dependent' and 'transient n.O.e.' experiments.[2,5] In order to obtain a higher frequency selectivity, we adopted the 'transient' method, *i.e.* n.O.e.s generated by selective 180° pulses.

The interpretation of this experiment is based on the Bloch equations (1), modified by Solomon[9] for a system of two

$$\begin{aligned} d I_z^{(i)}/dt &= -\rho_i(I_z^{(i)} - I_o^{(i)}) - \sigma_{ij}(I_z^{(j)} - I_o^{(j)}) \\ d I_z^{(j)}/dt &= -\rho_j(I_z^{(j)} - I_o^{(j)}) - \sigma_{ij}(I_z^{(i)} - I_o^{(i)}) \end{aligned} \quad (1)$$

dipolar coupled spins i and j where I_z are the values of the longitudinal magnetizations at a given moment, I_o are the values of I_z at equilibrium, ρ_i and ρ_j are the relaxation rates, and σ_{ij} the cross-relaxation rate.

Defining the time development of the n.O.e. $\eta_i(t) = (I_z^{(i)} - I_o^{(i)})/I_o^{(i)}$ and then rewriting equations (1) in terms of $\eta_i(t)$ gives (2) which can be written in matrix notation and extended to

$$\begin{aligned} d\eta_i(t)/dt &= -\rho_i\eta_i(t) - \sigma_{ij}\eta_j(t) \\ d\eta_j(t)/dt &= -\rho_j\eta_j(t) - \sigma_{ij}\eta_i(t) \end{aligned} \quad (2)$$

$$[d\eta(t)/dt] = -[\Gamma]\cdot[\eta(t)] \quad (3)$$

a multispin system {equation (3) where $[\Gamma]$ is the matrix representing the relaxation rates ρ_i and the cross-relaxation rates σ_{ij}}. The general solution[6] of the system is given by equation (4) where $[T]$ is the matrix formed by the eigenvectors

$$[\eta(t)] = [T]\exp[-Dt][T]^{-1}[\eta(t)]_{t=0} \quad (4)$$

of $[\Gamma]$, and $[D]$ is the diagonalized relaxation matrix $[\Gamma]$.

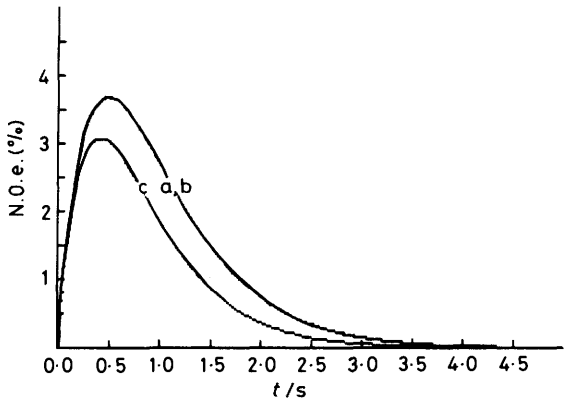


Figure 2. Simulation of transient n.O.e. experiments: a, two-spin system with $\sigma_{ij} = 0.1\ s^{-1}$; b, linear three-spin system with $\sigma_{ij} = \sigma_{jk} = 0.1\ s^{-1}$ and $\sigma_{ik} = 0.0\ s^{-1}$; c, triangular three-spin system with $\sigma_{ij} = \sigma_{ik} = 0.1\ s^{-1}$. $\sigma_{ik} = 0.8\ s^{-1}$. In all cases $\rho_i = \rho_j = \rho_k = 2.0\ s^{-1}$

$[\eta(t)]_{t=0}$ Are the initial conditions and are determined by the type of experiment performed. It is then possible to analyse the complete dependence of the n.O.e.s generated by the selective 180° pulse, by a least-squares fitting procedure, optimizing the relaxation matrix $[\Gamma]$. This leads to the cross-relaxation rates σ_{ij}, which are related to the interproton distances r_{ij} by equation (5), given for extreme narrowing conditions and iso-

$$\sigma_{ij} = \frac{\hbar^2\gamma^4}{2r_{ij}^6}\tau_{eff} \quad (5)$$

tropic molecular motions[2] where τ_{eff} is the effective correlation time. It is thus possible to obtain relatively accurate distance ratios, from equation (6), providing that the correlation times of the interproton vectors are equal.

$$r_{ij}/r_{ki} = (\sigma_{ki}/\sigma_{ij})^{1/6} \quad (6)$$

From equation (3) it can be seen that the initial gradient of $\eta(t)$ is equal to $2\sigma_{ij}$, regardless of the number of nuclei involved. The cross-relaxation parameters σ_{ij}, which carry most of the structure information, can thus be obtained also from the initial gradient of the curve by using a linear least-squares fitting.[10] However, the useful range of t in which equation (4) is linear is often very small; consequently this method may lead to an underestimate of the parameters. A better approach is to analyse the whole curve of the time development of n.O.e., with a 'two-spin approximation,' where the number of parameters to be optimized is reduced to three, the relaxation rates ρ_i and ρ_j and the cross-relaxation rate σ_{ij}. The other spins are assumed to act as an external source of relaxation, affecting only the values of ρ_i and ρ_j. In order to estimate the limit of validity of this approximation, we performed a set of calculations, which simulate transient n.O.e. experiments assuming different geometries. Three cases are reported in Figure 2: (a) two spins, (b) three spins in a linear sequence ($\sigma_{ij} = \sigma_{jk} \gg \sigma_{ik}$), and (c) three spins in a triangle geometry ($\sigma_{ij} = \sigma_{jk} < \sigma_{ik}$), as expected for daunomycin. The identity of the two curves in Figures 2a and b shows that the linear three-spin system is a favourable geometry, the relative distances between adjacent spins can be determined easily and accurately, and the whole curve can be treated with the 'two-spin' procedure. A multispin system with a linear geometry can well be analysed in the same way. In the case of a triangle geometry (Figure 2c), the deviation from the multispin treatment is evident in the second part of the curve, but the approximation is negligible in the first part up to *ca.* 200 ms. Therefore we conclude that, for geometries close to

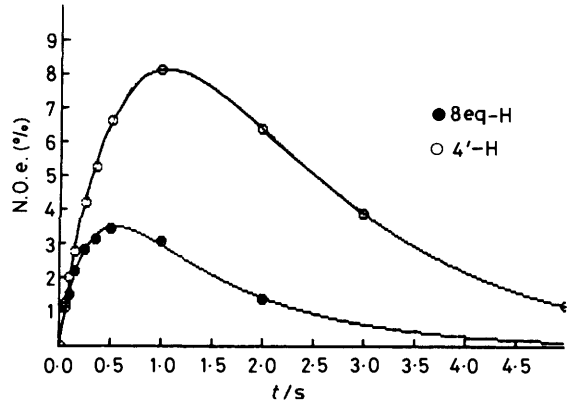


Figure 3. Experimental transient n.O.e.s on 4′-H (○) and 8eq-H (●) following inversion of 5′-H

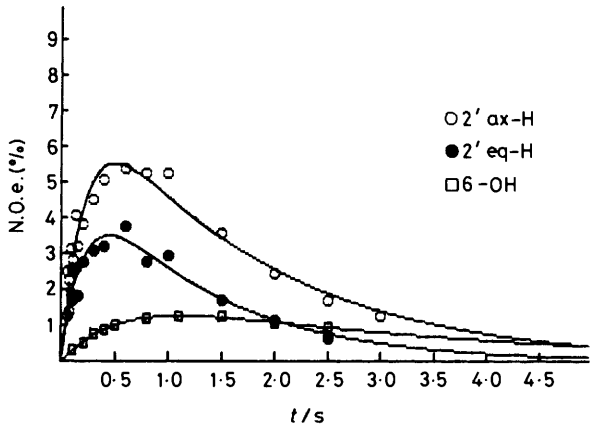


Figure 5. Experimental transient n.O.e.s on 2′ax-H (○), 2′eq-H (●), and 6-OH (□) following inversion of 1′-H

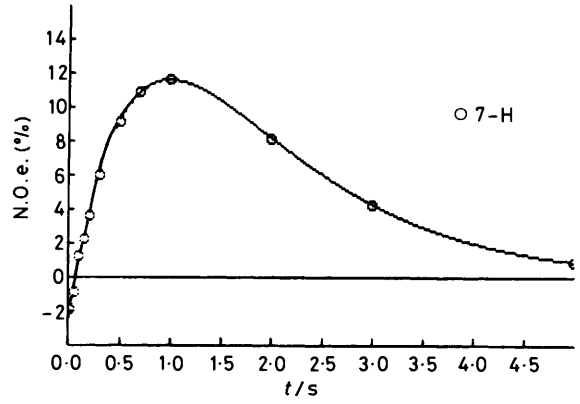


Figure 4. Experimental transient n.O.e.s on 7-H following inversion of 1′-H. The initial negative values are due to a slight perturbation of 7-H, which is close to 1′-H

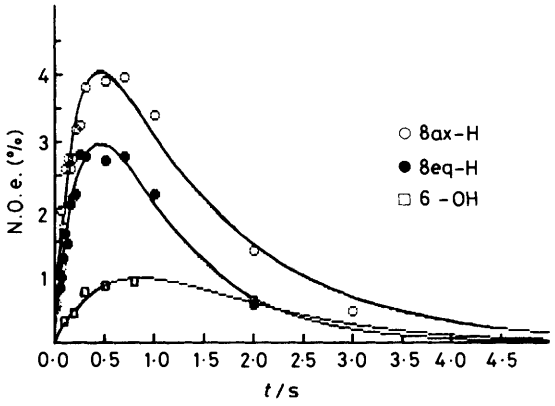


Figure 6. Experimental transient n.O.e.s on 8ax-H (○), 8eq-H (●), and 6-OH (□) following inversion of 7-H

Table 1. Cross-relaxation rates (s^{-1}) and interproton distance (Å)

Protons i–j	Cross-relaxation rates σ_{i-j}	Distance ratios $r_{i-j}/r_{4'-5'}$	Interproton distances r_{i-j} N.O.e.	*X*-Ray[11–13]		
H(1′)–H(2′ax)	0.1769 ± 0.0151[a]	0.940 ± 0.015[b]	2.21 ± 0.03[b,c]	2.34[e]	2.47[f]	2.40[g]
H(1′)–H(2′eq)	0.1207 ± 0.0107	1.002 ± 0.016	2.35 ± 0.04	2.39	2.55	2.14
H(1′)–H(7)	0.1859 ± 0.0042	0.933 ± 0.005	2.19 ± 0.01	2.20	2.22	2.20
H(1′)–OH(6)	0.0171 ± 0.0004	1.388 ± 0.007	3.26 ± 0.02	3.15		
H(7)–H(8ax)	0.1411 ± 0.0125	0.977 ± 0.016	2.30 ± 0.04	2.33	2.38	2.22
H(7)–H(8eq)	0.0948 ± 0.0051	1.044 ± 0.011	2.45 ± 0.03	2.42	2.46	2.54
H(7)–OH(6)	0.0175 ± 0.0022	1.383 ± 0.031	3.25 ± 0.07	3.42		
H(5′)–H(8eq)	0.1073 ± 0.0046	1.022 ± 0.010	2.40 ± 0.02	2.59	2.54	2.59
H(5′)–H(4′)	0.1224 ± 0.0010	1.000	2.35[d]	2.33	2.38	2.57

[a] Standard deviations. [b] Uncertainties calculated from the standard deviations of σ values through the error propagation law. [c] A more realistic estimate of the error, which also takes into account the uncertainty in the reference distance value ($r_{4'-5'}$), is within ±0.1 Å. [d] Reference value, obtained by the average of *X*-ray[11,12] results. [e–g] Calculated from *X*-ray atomic co-ordinates; refs. 11—13, respectively. As the standard deviation reported by Courseille *et al.*[11] for C-H distances is ±0.09 Å, a reasonable estimated deviation for interproton distances in the crystal phase is larger than ±0.1 Å.

those expected for daunomycin, the 'two-spin approximation' leads to acceptable estimates of cross-relaxation rates, and thus to a reliable determination of the relative interatomic distances. A complete discussion on the 'two-spin approximation' has been done by Dobson *et al.*,[5] in the case of time-dependent n.O.e. experiments, *i.e.* by using a saturation method. Also these authors concluded that fitting the experimental time course of $\eta(t)$ in a multispin system by a 'two-spin' treatment generally gives a good estimate of σ, but less satisfactory values of ρ.

Transient n.O.e. experiments following inversion of 5′-H, 1′-H, and 7-H were performed in $CDCl_3$ after exchange with D_2O and the results are reported in Figures 3 and 4. The experimental points were fitted to equation (4) through a non-linear least-squares procedure, by using a 'two-spin approxi-

mation,' and the cross-relaxation parameters (σ_{ij}) thus obtained are given in Table 1. Figure 3 shows the time development of n.O.e.s on 4′-H and 8eq-H, following inversion of 5′-H. The maximum enhancements are different, as expected, from those obtained by steady-state experiments, because in the transient method the n.O.e. growth is dampened by the still efficient relaxation process. Although 4′-H and 8eq-H gave very different maximum enhancements, the cross-relaxation rates are similar ($\sigma_{4'-5'} = 0.107$, $\sigma_{5'-8eq} = 0.120$ s^{-1}). The initial points of the experiment reported in Figure 4 are negative, because the inversion of 1′-H induces a slight perturbation of 7-H, which resonates 70 Hz apart from 1′-H. This effect was taken into account in the initial conditions of the fitting procedure. The inverse experiments, performed by selective irradiation of 8eq-H, 4′-H, and 7-H led to similar results. Figures 5 and 6 show the n.O.e. development for 2′-CH_2 and 8-CH_2 following inversion of 1′-H and 7-H respectively. Although the spin geometry of the latter two experiments is the most unfavourable because the dominant relaxation process is the strong interaction between geminal protons, the values obtained are satisfactory because they gave interatomic distance ratios in good agreement with *X*-ray results.[11,12] This confirms what is predicted in Figure 2.

The interproton distance ratios were obtained from equation (6) assuming as a reference the distance between 4′-H and 5′-H. This reference is justified by the fact that the sugar moiety has the same chair conformation $^1C_4$(L) in solution[1] and in the solid phase.[11,13] All values with their standard deviations are given in Table 1. For a better discussion and comparison with *X*-ray analyses, we have also reported the distance values referred to $r_{4'5'} = 2.35$ Å, which is the average between the results (2.33 Å[11] and 2.38 Å[12]) of two *X*-ray analyses.

The sugar moiety has in principle a greater conformational mobility than the aglycone. This may be tested by ^{13}C T_1* measurements, which was not possible in our case because of the very low solubility of the compound. (The maximum concentration in $CDCl_3$ and also in DMSO is *ca.* 10 mg ml^{-1}). On the other hand, the errors due to the uncertainty in correlation-time values are small, given the r^{-6} dependence of σ: calculations based on equation (5) show that an increase of τ by a factor of 2 leads to a decrease of only 12% in distance. However, the agreement with *X*-ray data, and the similarity of σ values for the corresponding distances in the fragments CH_2(8)–CH(7) and CH_2(2′)–CH(1′), allowed us to confirm the validity of the assumption that different interproton vectors like H(1′)–H(2′eq) and H(7)–H(8eq) or H(1′)–H(2′ax) and H(7)–H(8ax) have the same effective correlation time, even though situated in different parts of the molecule. In other words, the supposed slightly greater mobility of the sugar compared with the aglycone moiety does not appreciably affect the values of distances.

The most important interproton distance for the determination of the conformational preference of the sugar moiety is $r_{5'-8eq}$ which is 2.40 Å. Calculations, performed from *X*-ray atomic co-ordinates,[11–13] gave slightly larger distances, 2.6, 2.5, and 2.6 Å, respectively. But we must take into account that large deviations are reported for *N*-bromoacetyldaunomycin,[13] while the more accurate values of daunomycin hydrochloride[11,12] correspond to molecules with a slightly different conformation for ring A.[1] Therefore the preferred orientations of the sugar with respect to the aglycone moiety are better described by the rotational angles H(1′)–C(1′)–O(7)–C(7) = φ (rotation about the anomeric carbon to the glycoside bond) and C(1′)–O(7)–C(7)–H(7) = ψ (rotation about the aglycone carbon to glycoside oxygen bond). The value of the H(5′)–H(8eq) distance allows a few combinations of φ and ψ. Also $r_{1'-7} = 2.19$ Å is not *per se* selective. However, fortunately we could measure n.O.e. effects between the strongly chelated 6-OH and both 1′-H and 7-H. The time dependence of n.O.e.s for 6-OH following inversion of 1′-H and 7-H are reported in Figures 5 and 6. The cross-relaxation parameters are expected to be independent of exchange mechanism. The exchange rate of 6-OH with water (K_w), as measured by saturation transfer experiments, was found to be small (2%, relative to its own relaxation rate). The σ values reported in Table 1 show that the distances of 7-H and 1′-H from 6-OH are very similar.

* ^{13}C T_1 Values of daunomycin were measured in D_2O and are similar for all protonated carbons, in particular $T_1 = 0.06$ s for C-1′, C-5′, C-3′, and C-7 and for the aromatic secondary carbons. In this solvent, however, the molecule is not in the extreme narrowing limit, owing to strong self-association.

Table 2. Relaxation parameters of 7-H and 1′-H

	7-H	1′-H
$\Sigma\sigma$[a]	0.44 ± 0.02[b]	0.50 ± 0.02 s^{-1}
R[c]	1.12 ± 0.03	1.06 ± 0.03 s^{-1}
$\Sigma\sigma/R$	0.39 ± 0.03	0.47 ± 0.03
(T_1^{ns})[d]	0.69 ± 0.02	0.68 ± 0.02 s
$(T_1^{ns})^{-1} - R$	0.33 ± 0.08	0.41 ± 0.08 s^{-1}

[a] The sum is the total contribution of the interactions with neighbour protons. [b] Standard deviations. [c] Relaxation rates obtained by selective T_1 measurements: $R = 1/T_1$s. [d] Non-selective relaxation times.

As we have obtained for 1′-OH and 7-H the cross-relaxation parameters arising from the dipolar interactions of all neighbouring protons, we can compare the sum of these σ values with the difference $(T_1^{ns})^{-1} - R$, obtained by separate measurements of non-selective and selective relaxation times (see Experimental section and Table 2). The agreement is satisfactory taking account of the error involved in the determination of relaxation times. Since each value was obtained by independent experiment, we think that this result is evidence for the good quality of the experiments performed for the determination of the σ parameters. Furthermore we can also deduce from $\Sigma\sigma/R$, whose values are lower than the theoretical[2] 0.5, that small impurities of paramagnetic substances, as for instance oxygen from non-perfect degassing of samples, are critical for measurements of relaxation rates in $CDCl_3$ solution, but do not affect the cross-relaxation parameters σ.

Inspection of Table 1 shows that the interproton distances determined by n.O.e. are similar to those obtained from *X*-ray co-ordinates, but have a better accuracy. If we consider the C–C distances of crystal analyses, the accuracy is comparable. As the difference in conformation of ring A for the solid phase, although slight, is significantly reflected in the values of $r_{5'.8eq}$ and $r_{1'.6}$, it is more appropriate to consider the rotational angles φ and ψ. The values are reported in Table 3 together with those obtained from *X*-ray analysis[11–16] and from energy-minimum calculations.[17–19] The ±5° range for φ and ψ was determined from the uncertainty in the interproton distances of ±0.1 Å. This small range results from the good accuracy of n.O.e. experiments. Actually the φ and ψ values thus obtained rely on the assumption that the molecule exists preferentially as a single conformation, rather than as a fast equilibrium between different conformers. Generally both crystal and solution structures should be viewed as 'averaged' structures about which small fluctuations can take place. In particular, n.O.e. interproton distances are not arithmetic, but $(\langle r^{-6}\rangle)^{-1/6}$ means. However, in the present case a good fit of φ and ψ to the n.O.e. distances, functions of these angles, could not be reached, if the magnitude of internal motion was large. Therefore we must conclude that the internal motions are small, in order to accommodate a difference of only ±0.1 Å between experimental and calculated interproton distances. This is also justified by

Table 3. Rotational angles φ and ψ about the glycosidic bond[a]

	φ (°)	ψ (°)
N.O.e.[b]	40 ± 5	−5 ± 5
Daunomycin *X*-ray[11]	52[c]	13[c]
Daunomycin *X*-ray[12,17]	52	5
N-Br-acetyl *X*-ray[13]	41[d]	−18[d]
Daunomycin *X*-ray[14,e]	42[c]	−28[c]
Carminomycin *X*-ray[15]	50[d]	−3[d]
Nogalamycin *X*-ray[16]	47	−15
	45	−21
Calculations[17]	50	−27
Calculations[18]		
Conformer I (glob.min.)	17	138
Conformer II	49	−41
Calculations[19]	52	−24

[a] φ = H(1′)–C(1′)–O(7)–C(7), ψ = C(1′)–O(7)–C(7)–H(7). These rotational angles were obtained with a geometrical program (HILDE[20]), by using the interproton distance ratios from n.O.e. data, and mean values of bond angles and bond lengths from the literature.[21] φ and ψ angles from the literature were obtained from the values reported in refs. 16, 17, 19 for the angles C(7)–O(7)–C(1′)–O(5′) and C(8)–C(7)–O(7)–C(1′) or C(6a)–C(7)–O(7)–C(1′), respectively. [b] This work. [c] Quoted by Brown *et al.*[19] [d] Quoted by Neidle *et al.*[17] [e] Daunomycin–d(CpGpTpApCpGp) complex.

the large number of polar groups and by the presence of the 9-OH ··· O(7) intramolecular hydrogen bond (see ref. 1) which anchors the sugar moiety, precluding rotation around the O(7)–C(7) bond.

The comparison with φ and ψ reported in Table 3 needs comment. A simple examination with models allows a great number of structures to be discarded as too overcrowded, limiting the interval of possible angles to the values: $-20° \leqslant \varphi \leqslant +90°$ and $-50° \leqslant \psi \leqslant +30°$. However, the conformation with φ = 17° and ψ = 138° has been found[18] surprisingly to correspond to a global energy minimum, with *ca.* 3 kcal mol^{-1} less than the crystal structures. It is not clear from the recent study of McLeuman and Lenkiski[22] whether a similar conformation has been deduced for adriamycin–Yb^{III} complex in methanol solution. This conformation can certainly be excluded for $CDCl_3$ solution, because it requires an H(1′)–H(7) distance of more than 3 Å, and the values of H(5′)–H(8eq) and H(1′)–H(6) are larger than 4 Å, which is unacceptable from the n.O.e. results. In addition, the existence of the 9-OH ··· O(7) hydrogen bond is also evidence against this structure. The other minimum-energy conformations predicted by different authors[17–19] are also not in agreement with n.O.e. results; however, it must be observed that the low-energy contour areas about the energy minimum are large enough (for instance,[17] $+15° \leqslant \varphi \leqslant +60°$ and $+22° \leqslant \psi -45°$) to include all the crystal structures, the preferred conformation in solution here determined, and many others.

The φ values for the crystal structures of *N*-bromoacetyldaunomycin,[13] nogalamycin,[16] and daunomycin–d(CpGpTpApCpGp) complex[14] are the closest ones to that obtained from the n.O.e. conformation. It can be noted that these structures, which have the same conformation of ring A (half-chair $^9H_8$ slightly distorted toward the skew ^9S), show also a ring A shape which is similar to that of acetyldaunomycin in chloroform solution.[1] Actually, small distortions of ring A, as occur in the crystal structures of daunomycin, are expected to affect φ more than ψ, provided that the hydrogen bond 9-OH ··· O(7) locks the C(7)–O(7) bond. However, the ψ values in the solid phase are more scattered than the φ ones. This is explained by the difference in hydrogen-bond strengths, in particular 9-OH ··· O(7), due to the crystal packing and to the inclusion in the cell of molecules of water and solvents, such as butanol,[11] pyridine,[12] and acetone.[13] In the latter structure,[13] for instance, an intermolecular hydrogen bond involving O-9 and O-4′ has been postulated. In the case of the complex daunomycin–hexanucleotide structure,[14] the hydroxy on C-9 is probably involved in strong hydrogen bonds with the ring nitrogens of the underlying guanine, because O-9 is within hydrogen-bonding distance (2.6 and 2.9 Å) of N-3 and N-2 of guanine G2. *X*-Ray analysis of nogalamycin shows two molecules in the asymmetric unit of the crystal cell, which both have the same ring A conformation of daunomycin, but an opposite configuration at C-9. In both molecules 9-OH is equatorial and consequently the intramolecular hydrogen bond 9-OH ··· O(7) cannot occur.

Carminomycin, which is 4-hydroxydaunomycin, shows the most accurate[23] *X*-ray analysis[15] of the whole series, with hydrogen-atom positions being located and hence hydrogen-bonding geometry being determined.

The conformation of ring A is similar to daunomycin,[11,12] but it is particularly significant that the ψ angle has the closest value (−3°) to that obtained by n.O.e. experiments (−5°). Actually, the optimum geometry for this hydrogen bond is compatible with $-10° \leqslant \psi \leqslant 0°$,[17] which are the values found for acetyldaunomycin in solution.

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