Conformational Analysis of 9-Deoxydaunorubicin in Solution. The Application of a Quantitative Transient ^{1}H Nuclear Overhauser Effect

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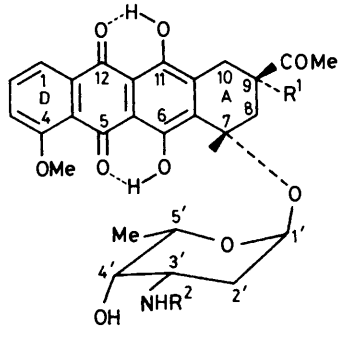
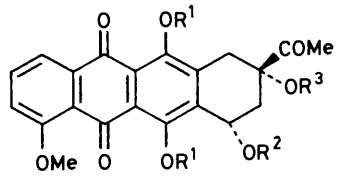
The preferred conformation of 9-deoxy-*N*-trifluoroacetyldaunorubicin has been determined by ^{1}H and ^{13}C n.m.r. in $CDCl_3$ solution. The conformation of ring A was easily defined as a pure half-chair $^9H_8$, from the values of proton coupling constants, and on the basis of a preceding study of daunomycin derivatives. The orientation of the sugar with respect to the aglycone moiety has been obtained by quantitative transient nuclear Overhauser experiments (n.O.e.). The interproton distances have been deduced from the cross-relaxation rates determined by measuring the time development of n.O.e.s, after selective inversion of single resonances. The experimental points were fitted to the theoretical curves through a non-linear least-squares procedure. The overall isotropic motion was proved by ^{13}C T_1 measurements of all protonated carbons; the interproton reference distance $r_{4'-5'}$, deduced from the correlation time value, is in satisfactory agreement with those measured by *X*-ray analyses of daunomycin and carminomycin. The glycoside linkage geometry 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). The results have been compared with those obtained through molecular mechanics calculations, performed with the Allinger MMP2 program.

In recent years much effort has been devoted to elucidate the mechanism of action of the antitumour anthracyclines; however, questions on the chemical properties responsible for the antitumour efficacy still await a satisfactory answer. The main target of these antibiotics in responsive biological systems is considered to be cell DNA, whose conformation and function are thought to be impaired as a result of drug intercalation.



(**1**) R^1 = OH, R^2 = H
(**2**) R^1 = OH, R^2 = COMe
(**3**) R^1 = R^2 = H
(**4**) R^1 = H, R^2 = $COCF_3$



(**5**) R^1 = R^2 = Me, R^3 = H

The *X*-ray study of the crystalline complex daunorubicin-hexanucleotide d(CpGpTpApCpG) carried out by Quigley *et al.*[1] gives detailed information on the structure of the complex: the drug is intercalated in the CG sequences and the intercalation of the chromophore is stabilized *inter alia* by possible hydrogen bonding between N(3) and N(2) of the second guanine and the axially oriented hydroxy group of C(9). On the other hand the crystal structure of daunorubicin (daunomycin) (**1**) hydrochloride reveals the importance of 9-OH in determining the molecular conformation of the drug. The increased stability of the $^9H_8$ half-chair conformation of ring A is considered to be promoted by a 9-OH · · · O(7) hydrogen bond,[2,3] whose presence was indicated by n.m.r. results[3] and from the value of the distance in the solid phase between the two oxygen atoms.[4]

The existence of this hydrogen bond in solution was shown by ^{1}H n.m.r. studies,[3,5] which demonstrated the glycoside linkage geometry for *N*-acetyldaunomycin (**2**) in $CDCl_3$ and the preferred conformation of ring A for daunorubicin (**1**) and several derivatives in different solvents. In order to confirm the importance of 9-OH, for the shape of ring A and the geometry of the chromophore–sugar bonds, the DNA-complex stability, and the biological activity, we have studied 9-deoxydaunorubicin (**3**).[6] This analogue, although showing an apparent association constant for the complex with native calf-thymus DNA very close (2.2×10^6 l mol^{-1}) to that of daunorubicin (1.9×10^6 l mol^{-1}), is not cytotoxic: LD_{50} on HeLa cells 1200 μg ml^{-1}, compared with 15 μg ml^{-1} for (**1**).

Herein we report the results of a conformational study in solution of 9-deoxy-*N*-trifluoroacetyldaunorubicin (9-deoxy-NTFD) (**4**). We used this derivative in order to operate in $CDCl_3$ and to compare the results with *N*-acetyldaunomycin (**2**). The preferred orientation of the sugar with respect to the aglycone moiety has been determined for (**2**) by transient nuclear Overhauser experiments (n.O.e.),[5] which were shown to give interproton distance values with an an accuracy comparable with that of *X*-ray analyses.

Table 1. ^{1}H Chemical shifts (δ) and H–H coupling constants (Hz) of 9-deoxy-*N*-trifluoroacetyldaunomycin (**4**)[a]

Chemical shifts						Coupling constants			
1-H	8.05	1′-H	5.51	4-OMe	4.08	7,8eq	2.6	1′,2′eq	1.1[b]
2-H	7.78	2′eq-H	1.89	9-COMe	2.35	7,8ax	3.3	1′,2′ax	4.1
3-H	7.39	2′ax-H	1.80	6-OH	13.97	8eq,8ax	14.1	2′eq,2′ax	13.1
7-H	5.15	3′-H	4.34	11-OH	13.36	8eq,9	2.6	2′eq,3′	5.0
8eq-H	2.39	4′-H	3.63	4′-OH	1.98	8ax,9	13.1	2′ax,3′	12.7
8ax-H	1.68	5′-H	4.15	3′-NH	6.28	9,10eq	5.2	3′,4′	2.8
9-H	3.11	5′-Me	1.31			9,10ax	11.8	4′,5′	1.3
10eq-H	3.34					10eq,10ax	18.7	2′eq,4′	1.0[b]
10ax-H	2.63					8eq,10eq	1.7	1′,4′	1.0[b]
						7,10ax	0.6	3′,NH	8.9
								4′,OH	8.7

[a] Measured at 300 MHz and 25 °C in $CDCl_3$. Chemical shifts taken from the $CHCl_3$ peak set at δ 7.26; accurate to within ±0.01 p.p.m. Coupling constant values are accurate to within ±0.05 Hz unless specified. Values for ring D protons, identical to those of (**2**), are not reported. [b] Accurate to within ±0.1 Hz.

Table 2. ^{13}C Chemical shifts (δ) and relaxation times T_1 for the protonated carbons of 9-deoxy-NTFD (**4**)[a]

	δ	δ[b]	T_1/s
C-1	(118.36)	118.0	0.41 ± 0.03
C-2	135.70	135.3	0.52 ± 0.2
C-3	(119.82)	118.0	0.46 ± 0.1
C-7	(69.34)	69.2	0.50 ± 0.05[c]
C-9	41.59	74.3	0.38 ± 0.1
C-1′	99.43	98.6	0.52 ± 0.1
C-3′	46.28	46.1	0.56 ± 0.06
C-4′	(69.24)	65.5	0.50 ± 0.05[c]
C-5′	66.34	65.5	0.61 ± 0.09
C-2′	28.44	27.8	*d*
C-8	(31.19)	35.2	0.24 ± 0.06
C-10	(30.05)	31.1	*d*
4-OMe	56.72	55.9	0.95 ± 0.05
9-COMe	25.24	23.8	0.30 ± 0.12
5′-Me	16.77	16.3	0.85 ± 0.07

[a] Measured at 75.4 MHz and 25 °C in $CDCl_3$ (18 mg ml^{-1}). Chemical shifts (δ), in p.p.m. from Me_4Si, are accurate to within ±0.01 p.p.m.; similar values in parentheses may be interchanged. [b] Chemical shifts of daunomycin (**1**) hydrochloride in dimethyl sulphoxide.[8] [c] Averaged values of C-7 and C-4′, whose signals overlap. [d] Not measured.

Experimental

N.m.r. spectra were recorded with Varian XL-300 and Bruker CXP-300 spectrometers. Chemical shifts are in δ values from internal Me_4Si. A water-free $CDCl_3$ solution of (**4**) (18 mg ml^{-1}) were degassed by five freeze–thaw–pump cycles directly in a 5 mm n.m.r. tube, which was then sealed and used for all ^{1}H and ^{13}C experiments. $CDCl_3$ (Merck) was pretreated with CaO (12 h) in order to eliminate traces of acid and water, then distilled under vacuum. The proton spectrum was analysed by using the PANIC program included in the Aspect-2000 computer library of the Bruker spectrometer. ^{1}H and ^{13}C Chemical shifts are accurate to within ±0.01 p.p.m., coupling constants to within ±0.05 Hz, unless specified in Table 1.

The assignment of hydrogen signals was done by 2D n.m.r.; COSY[7] spectra were acquired by using a 45° observe pulse, 256 f.i.d.s. zero-filled to 1 024 points, weighted with a sine-bell function, and transformed in absolute value.

The small H–H interactions ($^4J_{8,10}$, $J_{4',5'}$, $^5J_{7,10}$, and $^4J_{2',4'}$) not detected by COSY, were determined by the usual decoupling techniques. The values of coupling constants were obtained from resolution-enhanced monodimensional spectra; those of $J_{4',OH}$, $J_{3',NH}$, and $J_{3',4'}$ from a dilute solution (5 mg ml^{-1}). The assignment of H-8eq *versus* H-8ax and of H-10eq *versus* H-10ax was made through the four-bond $J_{8,10}$ coupling which involves only equatorial protons.[3] ^{1}H Chemical shift and coupling constant values are reported in Table 1.

The assignment of ^{13}C signals, which could not be done through heteronuclear shift-correlated 2D n.m.r. because of the low solubility of (**4**) in $CDCl_3$ and also in DMSO, follows from a comparison with the spectra of daunomycin (**1**).[8] The assignments for (**1**) were performed by heteronuclear decoupling techniques,[8] and the correlation with (**4**) was straightforward (Table 2). ^{13}C Relaxation times (T_1) were measured by inversion recovery; the values given in Table 2 were obtained from a set of six spectra, 2 064 scans, recycling delay 10 s, by a non-linear least-squares procedure, included in the Varian software.

NOESY[9] spectra were obtained in the phase-sensitive mode with a mixing time of 300 ms, by using standard NOESY pulse sequence and phase cycling.[10] In these spectra 2 × 256 f.i.d.s. are transformed with a line-broadening of 6 Hz. Transient and steady-state n.O.e.[11] and saturation-transfer[12] experiments were performed following the procedure reported in our previous paper.[5] The cross-relaxation rates (σ_{ij}) and the relaxation rates (ρ_i) were calculated by a non-linear least-squares fit of all experimental points to equation (1),[13] by using

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

our own program based on standard algorithms. In equation (1) $[T]$ is the matrix formed by the eigenvectors of the relaxation rates matrix, and $[D]$ is the diagonalized relaxation matrix.

Molecular mechanics calculations were performed using the MMPMI program[14] (a version of MMP2)[15] on an IBM PC-AT computer, equipped with 640 Kb of memory. The rotational angles φ and ψ were obtained with a geometrical program developed in our laboratory, by using the interproton distances from n.O.e. data. Coupling constants were calculated through a Karplus equation modified by Altona, which includes a correction for the electronegativity of the substituents.[16]

Results and Discussion

(a) *Conformation of the Sugar and of the* A *Ring.*—The shape of the daunosamine moiety does not vary for the α-glycosides studied, both in solution[3] and in the solid phase.[4] Also for 9-deoxy-NTFD (**4**) the preferred conformation is the chair $^1C_4$(L). This follows from the coupling constants given in Table 1; in particular the high value of the diaxial interaction between 2′ax- and 3′ax-H is evidence of the high population of this

Table 3. Cross-relaxation rates (s^{-1}) and interproton distances (Å) for 9-deoxy-*N*-trifluoroacetyldaunomycin (**4**)

i, *j*	$\sigma_{i,j}$	$r_{i,j}$
H(1′), H(7)	0.2019 ± 0.0070[a]	2.18 ± 0.04[b]
H(1′), OH(6)	0.0171 ± 0.0024	3.28 ± 0.12
H(7), H(8ax)	0.1070 ± 0.0108	2.43 ± 0.07
H(7), H(8eq)	0.1027 ± 0.0201	2.43 ± 0.11
H(5′), H(8eq)	0.0852 ± 0.0100	2.51 ± 0.08
H(5′), H(4′)	0.1091 ± 0.0080	2.42[d]
H(5′), H(9)	0.0355 ± 0.0039	2.91 ± 0.09
H(9), H(8eq)	0.0605 ± 0.0129	2.67 ± 0.13
H(3′), H(4′)	0.0856 ± 0.033	2.51 ± 0.19

[a] Standard deviation. [b] Uncertainties calculated from the standard deviations of σ value through the error propagation law. [d] Reference value, obtained from the average of T_1 and *X*-ray measurements and molecular mechanics calculations.

conformer. The 1,3-diaxial relationship between 3′- and 5′-H, which cannot be deduced from the coupling constants, is shown by the significant n.O.e. effect obtained by steady-state (2.6%) and NOESY experiments.

The preferred conformations of ring A found for a series of daunomycin derivative are the half-chairs $^9H_8$, $^8H_9$ and the skew forms ^9S, S_9, and S_8. The coupling constants of all the protons on ring A for 9-deoxy-NTFD (**4**) are reported in Table 1. The values of $J_{7,8}$ (2.7 and 3.3 Hz) indicate a pure half-chair $^9H_8$ which is the conformation also found for trimethoxydaunomycinone (**5**) in $CDCl_3$ ($J_{7,8}$ 2.3 and 3.6 Hz).[3] This is confirmed by the couplings between 9-H and the geminal protons at C-8 and -10. The slightly lower values calculated[16] for some of the interactions involving 9-H (see later, Table 4), might be due to the use of a non-appropriate substituent contribution for the COMe group. Similar difficulties have been found for the nitrile group.[16] Further information on the geometry of ring A comes from the four- and five-bond couplings ($J_{8eq,10eq}$ and $J_{7,10ax}$). The latter (0.6 Hz) is the same as for (**2**) (0.7 Hz) and (**5**) (0.5 Hz), whereas the values of 1.7 Hz for $J_{8,10}$ differ from that expected (2.0 Hz)[3] for the half-chair $^9H_8$. In the daunomycinone series, a value <2 Hz is indicative of ring inversion with some population of $^8H_9$ or S_9 at equilibrium; higher values instead are characteristic of the skew form ^9S, which involves the perfect coplanarity of the four bonds connecting 8eq- and 10eq-H. Since a dynamic mixture of conformers in the case of 9-deoxy-NTFD (**4**) can be excluded upon considerations of other couplings; the lower value of $J_{8,10}$ must be explained by a substituent effect, due to the absence of the hydroxy group at C-9. Actually a positive increment in $^4J_{H,H}$ over σ bonds was predicted[17] and found[18] experimentally, when an electronegative substituent (inductive effect) is directly bonded to the central carbon of a propane fragment in a W arrangement. The half-chair $^9H_8$ conformation is also consistent with the interproton distances of 7-H with 8ax-and 8eq-H respectively, determined from n.O.e. data (Table 3).

We have identified two main stabilizing factors for the half-chair $^9H_8$ conformation in the daunomycinone series: (i) intramolecular hydrogen-bonding between 9-OH and O(7) and (ii) relatively low steric interactions between substituents at C-7, -10 and the *peri* hydroxy groups of the vicinal ring. Factor (ii) holds for the 9-deoxy analogues, while factor (i) disappears. However, with the hydrogen bond, the 1,3-diaxial interaction between O(7) and O(9) substituents also vanishes and consequently it is reasonable that the half-chair $^9H_8$, with an equatorial orientation of COMe group, is the most stable and thus the preferred conformer in solution. PCILO calculations[19] on 9-deoxydaunomycin gave a 0.69 kcal mol^{-1} energy difference between the $^9H_8$ and $^8H_9$ conformations.

(b) *Glycosidic Linkage Geometry.*—We have performed phase-sensitive NOESY and steady-state experiments in order to find the principal n.O.e. interactions. Some spectra are reported in Figure 1. The most important interactions, in order to define the geometry at the glycosidic bond, involve 5′-, 8eq-, and 9-H. Enhancements of 2.5 (8eq-H) and 2.4% (9-H) upon irradiation of 5′-H are shown in Figure 1b. In the same experiment significant effects are also observed on 4′-H (7.6%), 5′-Me (1.7%), 3′-H (2.6%) and on the aromatic 1-H; this latter is due to the perturbation of 4-OMe which is close to the irradiation frequency. The reverse experiments are in agreement with these results. Another sequence of nuclei is significant, *i.e.* 1′-H, 7-H and the chelated 6-OH. Great care has been taken to eliminate traces of acid, in order to decrease as much as possible the chemical exchange of the hydroxy protons with water. The exchange rate of 6-OH with water, measured by saturation transfer experiments as for *N*-acetyldaunomycin,[5] was indeed found to be negligible (8%) relative to its own relaxation rate. In the spectrum reported in Figure 1c, a strong n.O.e. effect (13.2%) was measured for 7-H following irradiation of 1′-H, but a significant enhancement (4.3%) was also observed for 6-OH. Similar results were obtained on irradiation of 7-H, whereas lower effects appear on irradiation of the hydroxy proton.

Transient n.O.e. experiments were then performed by inversion of 9-, 7-, 5′-, 1′-, and 4′-H, and some of the results are reported in Figures 2—4. The experimental points were fitted, through a non-linear least-squares procedure, to equation (1) (see Experimental section). We have used a 'two-spin approximation,' as this was proved[5] adequate for the analogous spin systems of *N*-acetyldaunomycin (**2**). The additional proton (9-H) of 9-deoxy-NTFD is expected to affect the relaxation parameters ρ_i, but not the cross-relaxation rates, σ_{ij}, which thus allow a reliable determination of the interatomic distances. The curves in Figure 2 show the time development of n.O.e.s for 8eq- and 5′-H, following inversion of 9-H. This leads to the relaxation parameters $\sigma_{8eq,9}$ and $\sigma_{5',9}$. The inverse experiment, given in Figure 3, confirms these results. The inversion of 5′-H leads to good curves for both 8eq- and 4′-H, although the former is partially overlapped by the COMe group and the latter is close to the irradiation frequency. The curve of 9-H was not considered, because this proton gives a large signal, which is more difficult to integrate. The values of $\sigma_{5',8eq}$ and $\sigma_{4',5'}$ were thus obtained. Figure 4 shows the development of the n.O.e.s of 6-OH and 7-H following inversion of 1′-H; although the maximum enhancement of the hydroxy proton is low, the cross-relaxation rate $\sigma_{1',6}$ has been determined with great accuracy, owing to the small width of the 6-OH signal. The value of $\sigma_{1',7'}$ containing the steric relationship between 1′- and 7-H, is obtained from the curve in Figure 3, and from an inverse experiment not here reported. All the relaxation data are given in Table 3.

The interproton distance ratios were obtained by the usual equation (2)[11] given for extreme narrowing conditions and

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

isotropic molecular motions, where τ_{eff} is the effective correlation time. The assumption that the motion of different interatomic vectors is described by a single correlation time has been tested by ^{13}C T_1 measurements. From the results reported in Table 2, it can be seen that ^{13}C NT_1 values for all protonated carbons, excluding methyl groups, are equal within experimental error. This means that the correlation time is the same

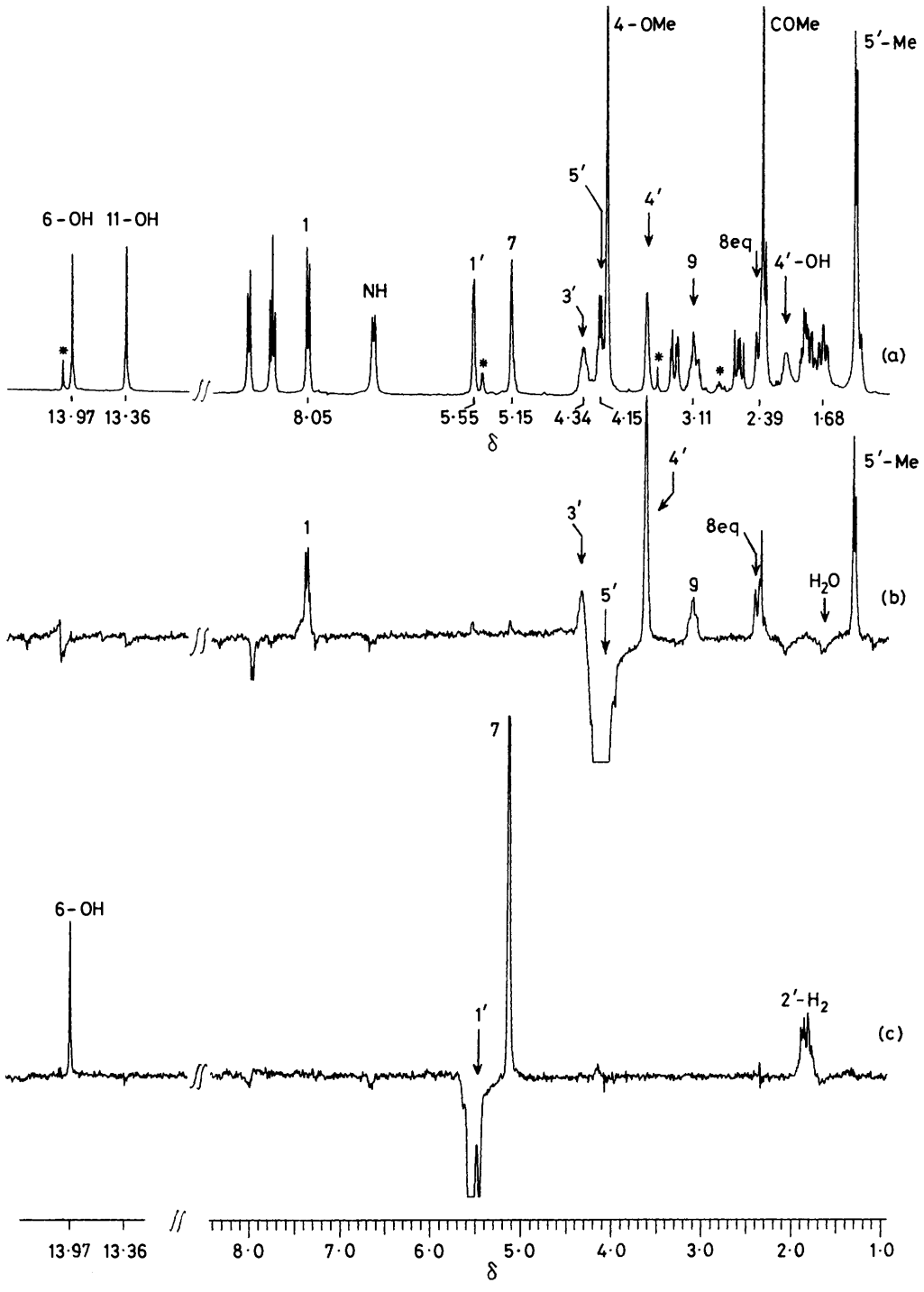


Figure 1. ^{1}H N.m.r. spectrum (a) and steady-state n.O.e. difference spectra (b), (c) of (**4**) in $CDCl_3$. Irradiation of 5′-H (b) and 1′-H (c)

for all the interatomic vectors and thus the overall motion of the molecule is isotropic. Consequently internal motions such as rotation or ring inversion are either negligible or do not contribute to the relaxation process.

The correlation time derived by equation (2) is equal to 4.9×10^{-11} s, assuming a predominant contribution from directly attached protons and a C–H distance of 1.1 Å. This value has been used to calculate an interproton reference distance ($r_{4',5'}$ 2.52 Å), which was then compared with those obtained by molecular mechanics calculations (2.41 Å) and by *X*-ray analyses of daunomycin (2.38, 2.33 Å)[4] and carminomycin (2.45 Å).[20] We have adopted $r_{4',5'}$ as a reference distance, because the conformation of the sugar moiety, daunosamine, is constant for all daunomycin analogues studied.[5] The values of $r_{4',5'}$ are scattered around an average value of 2.42 Å, which is actually equal to that found by molecular mechanics calculations. As T_1 measurements are considered not to be very accurate, owing to the low signal to noise ratio, we prefer to use as a reference the average of all the above experimental results ($r_{4',5'}$ 2.42 Å), to which the interproton distances given in Table 3 are referred.

The interatomic distances listed in Table 3, as calculated by

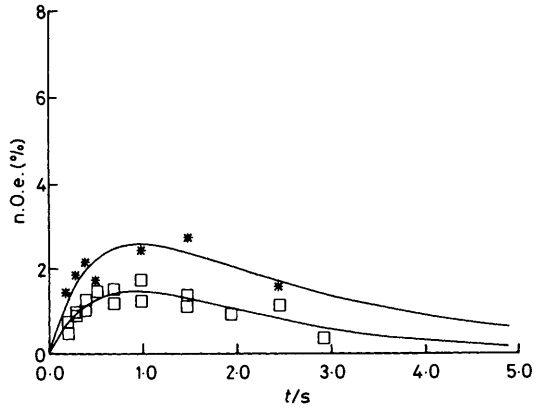


Figure 2. Experimental transient n.O.e.s on 8eq-H (*) and 5′-H (□) following inversion of 9-H

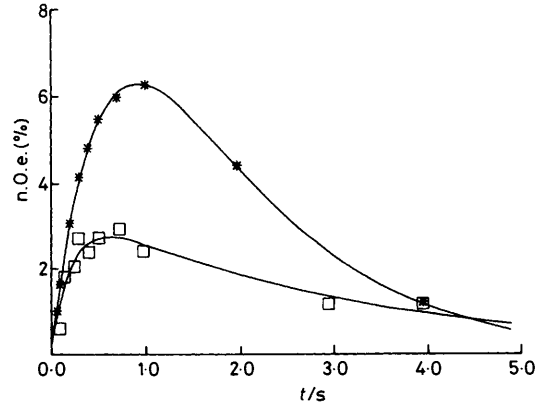


Figure 3. Experimental transient n.O.e.s on 4′-H (*) and 8eq-H (□), following inversion of 5′-H

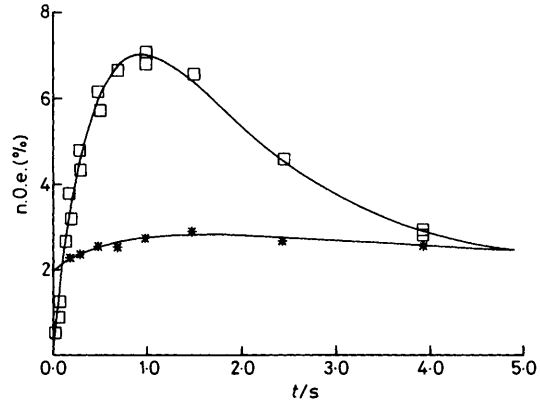


Figure 4. Experimental transient n.O.e.s on 6-OH (*) and 7-H (□) following inversion of 1′-H

equation (2), rely on the assumption that 9-deoxy-NTFD is a rigid molecule. However, the dihedral angles φ and ψ represent an internal degree of freedom, which may in principle lead to the presence of internal movements around the glycoside bond. In particular, the molecule may be present as a mixture of differently populated conformations in fast exchange between each other and even in the presence of one predominant conformer oscillations around the potential-energy minimum may still be present. In these cases the distances must be considered as averages $\langle r_{ij}^{-6} \rangle^{-1/6}$.

Let us first consider the simplest model which consists in 9-deoxy-NTFD kept fixed in a rigid conformation and look for the values of φ and ψ angles which best fit the interatomic

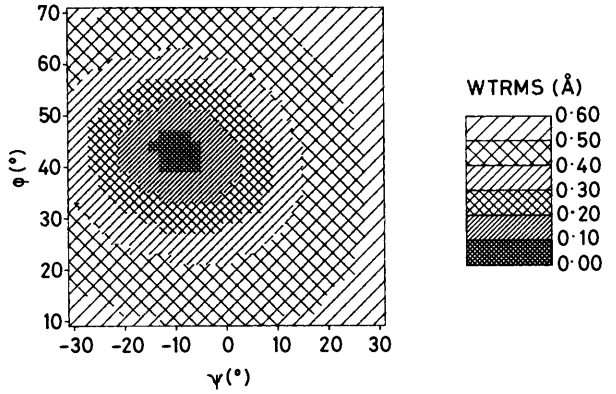


Figure 5. Weighted root mean square (WTRMS) of deviations between computed and experimental distances, as functions of φ and ψ angles for 9-deoxy-NTFD (**4**). The following distances have been considered: $r_{5',8eq}$, $r_{5',9}$, $r_{1',7}$, and $r_{1',6}$

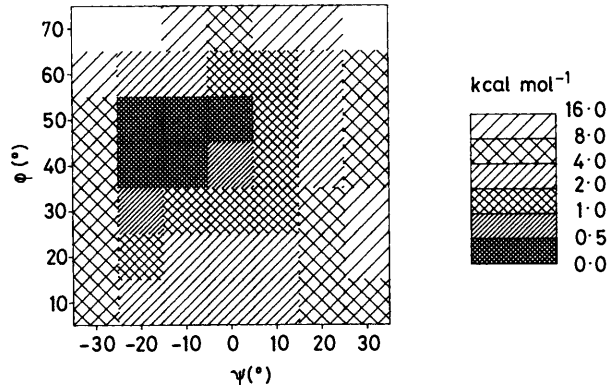


Figure 6. Energy map for $^9H_8$ conformer of (**4**), in φ,ψ space as calculated by molecular mechanics. The energy is given relative to the minimum

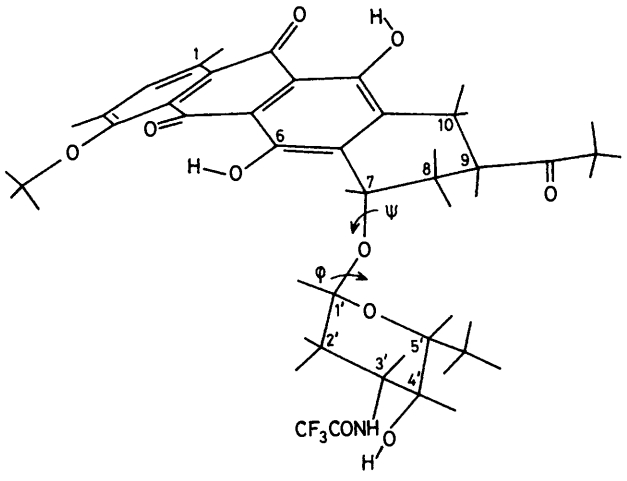


Figure 7. Preferred conformation of 9-deoxy-NTFD (**4**) in $CDCl_3$ solution

distances. A starting geometry was created by standard bond lengths and angles, with a half-chair conformation $^9H_8$ for ring A and a chair conformation $^{1'}C_4$ for the sugar moiety. This structure was then optimized allowing all atoms to move freely. The resulting geometry is in agreement with the proton coupling constants given in Table 1. We then rotated the sugar moiety, changing systematically the torsional angles φ and ψ, in order to find the best agreement between the experimental and

Table 4. Rotational angles (°) and calculated coupling constants (Hz) for the final geometry of (**4**)

φ^a		ψ^b	
Exp[c]	Calc.	Exp[c]	Calc.
42 ± 7	40[d]	−10 ± 7	−20[d]
	47[e]		−36[e]

i,*j*	I_{ij}[f]	θ_{ij}[g]
7,8eq	3.0	66
7,8ax	3.2	50
8eq,9ax	2.4	66
8ax,9ax	12.3	176
9ax,10eq	4.5	51
9ax,10ax	12.0	169
1′,2′eq	2.3	65
1′,2′ax	3.9	51
2′eq,3′ax	4.0	57
2′ax,3′ax	11.7	174
3′ax,4′eq	1.7	57
4′eq,5′ax	0.1	57

[a] φ = H(1′)–C(1′)–O(7)–C(7). [b] ψ = C(1′)–O(7)–C(7)–H(7). [c] From n.O.e. experiments. [d] From molecular mechanics calculations. [e] From the values of the angles C(7)–O(7)–C(1′)–O(5′) and C(8)–C(7)–O(7)–C(1′) obtained by Neidle[2] through semiempirical energy calculations. [f] Calculated from the Karplus–Altona equation.[14,16] [g] Dihedral angles defined by *ij* protons, for instance $\theta_{7,8eq}$ = H(7)–C(7)–C(8)–H(8eq), as calculated by molecular mechanics.

calculated interatomic distances (Figure 5), which occurs for φ 42° and ψ −10°. From Figure 5 it can be seen that there exists a narrow region in φ,ψ plane which fits with the distances of Table 3. Although the n.O.e. data alone can not exclude the presence of a mixture of conformations, the internal consistency of data certainly indicates the presence of a major conformer. The conclusion is also confirmed by semiempirical energy calculations,[2] where an extensive map of the potential energy for 9-deoxy-NTFD shows the presence of two minima: the global minimum is located in the same region as that derived from our n.O.e. experiments, while the secondary minimum is of far too high an energy to be populated. We also report here the energy map as derived from our molecular mechanics calculations, for the sake of completeness (Figure 6).

The values of the torsional angles for the final geometry, given in Figure 7, and those of the corresponding coupling constants, calculated from the Karplus–Altona equation,[16] are reported in Table 4. The comparison between experimental and calculated φ and ψ values shows very good agreement.*

The preferred conformation of the 9-deoxyanalogue is the same as that of *N*-acetyldaunomycin (**2**).[3,5] Consequently we must conclude that the 9-OH group is not important for the geometry of the glycoside site, which is instead determined by the steric interactions of the sugar with the aglycone moiety. The shape of ring A in 9-deoxyanalogue, as a pure $^9H_8$ half-chair, is a consequence of the equatorial conformation of the 9-COMe group, and of the pseudo-axial orientation of the C(7)–O(7) bond which gives a minimum interaction with O(6) on the vicinal ring. Also *N*-acetyldaunomycin is found to be a pure $^9H_8$ conformer, despite the presence of 9-OH in the axial orientation, but in this case the unfavourable 1,3-diaxial interaction between O(7) and O(9) is compensated by the 9-OH ··· O(7) hydrogen bond. Moreover ψ is the same for (**2**) and (**4**); this means that the orbitals of O(7) are already oriented to give the maximum interaction with 9-H. Thus we can say that this hydrogen bond gives a contribution to the significant conformational stability of the $^9H_8$ half-chair ring A in the drug.

This study was the necessary background to an investigation of daunomycins in aqueous solvents in order better to approach the physiological conditions, even though it is questionable whether the environment at the intercalation sites of a polynucleotide, such as DNA, is closer to an aqueous than to a less polar solution. Preliminary results on 9-deoxydaunorubicin hydrochloride in D_2O (work in progress) indicate a conformation similar to that found in $CDCl_3$.

* Neidle[2] found, by using semiempirical energy calculations, a slightly different ψ angle.

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