Nuclear Magnetic Resonance Conformational Study of Daunomycin and Related Antitumour Antibiotics in Solution. The Conformation of Ring A

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The conformational properties of daunomycin (**1**) and several analogues (**2**)—(**16**) have been investigated by ^{1}H n.m.r. in different solvents, $CDCl_3$, D_2O, DMSO, dioxane, and pyridine. From H,H three-bond and long-range coupling constants, the geometry of ring A in each solvent has been determined, through a Karplus–Altona equation, which includes a correction for the substituent electronegativity. Conformers $^9H_8$, $^8H_9$, ^9S, S_9, and S_8 have been found in solution, and in several cases, especially in DMSO, more than one conformer is present at equilibrium. The equilibrium is always fast compared with n.m.r. times and the relative populations of each conformer have been calculated from experimental and model coupling constants by using a least-squares procedure. Two factors have been recognized to be responsible for the shape of ring A: the intramolecular hydrogen-bond 9–OH ••• O(7) and steric interactions between *peri* substituents on the A and B rings. Evidence for this hydrogen-bonding has been obtained by dilution experiments, and, for the *peri* interactions, by evaluating the substituent effects on the conformational preference. Other intramolecular hydrogen bonds have been proved not to exist in solution. The influence of the solvents on the shape of ring A has also been studied. All the results are discussed and compared with those obtained in the solid phase. Daunosamine moiety has also been analysed, and the conformation of the sugar ring is always $^{1'}c_{4'}$ (L) in all the solvents examined.

Quite a number of papers have appeared concerning daunomycin (daunorubicin) and the closely related compound adriamycin (doxorubicin), both highly active anticancer agents.[1] The mechanism of the antimitotic activity is generally accepted to occur *via* the interference of these drugs with the nucleic acids synthesis by binding to the DNA template.[1,2] *X*-Ray crystal analyses of daunomycin[3–5] and studies of daunomycin–DNA or daunomycin–oligonucleotide complexes both in the solid state[6,7] and in solution[8] were performed. Conformational analyses by semi-empirical calculations were also published,[9–11] but no sound conformational study in solution of these drugs and their derivatives or of the analogues carminomycin and nogalamycin[1,2] have appeared yet. As the importance of the medium for conformational studies is well known, this lack may be attributed to the difficulty of obtaining a complete n.m.r. analysis for these compounds. Actually they show very broad n.m.r. spectra, especially in the charged form and in water solution, due to strong intermolecular interactions.[12] From the earliest n.m.r. data, some conformational features were deduced for *N*-acetyldaunomycin[1,13] in $CDCl_3$, while the neutral form of daunomycin showed a similar orientation for 7- and 1′-H in pyridine.[14] † The conformation of ring A, bound or not bound to nucleic acids, has been the subject of speculation.[16–18] From an incomplete and rough analysis of daunomycin in water, wrong conclusions have been drawn[17] and then reported by other authors[11,18] (see Results section).

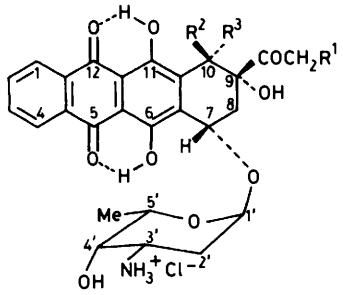
Here we report the results of an n.m.r. investigation on daunomycin (**1**), adriamycin (**2**), 10*R*- and 10*S*-methoxydaunomycin (**3**) and (**4**), acetyldaunomycin (**5**), their aglycones and analogues (**6**)—(**13**). Also the results on 4-demethoxy-6-deoxy- and -11-deoxy derivatives (**14**)—(**16**)[19,20] are presented.

As the biological activity and probably also the cardiotoxicity of these drugs seems to be affected by modification of the shape of ring A, the 10-methoxy isomers are interesting compounds, because they show different steric and biological properties.[1] The cardiotoxicity in particular is claimed[21] to be related to the ability to generate oxygen radicals during the redox cycling, and there is evidence that the reductive glycosidic cleavage of daunomycin occurs *in vitro* through the formation of the tautomer of 7-deoxydaunomycinone.[22] Recently the polarographic reduction, which leads to the loss of the amino sugar, has been found[23] to be under steric control, as the conformation of ring A strongly affects the kinetics of the sugar moiety detachment.

From an *X*-ray study of daunomycin with a self-complementary DNA fragment,[7] it has been found that the positively charged amino group does not interact with the phosphates, as previously proposed,[2,16] but rather sits in the centre of the minor groove of the helix. It is ring A which provides proper interactions with DNA bases (especially through 9-OH) thus performing the important anchoring function.

For these reasons, the determination of the preferred conformation of ring A in different solvents is the main object of this study, carried out by the use of several aglycone models of rigid and flexible structure, and by correlation of proton couplings with the geometry of the ring. The existence of molecular aggregates and of intramolecular hydrogen bonding, as possible stabilizing factors of particular conformations, were taken into account. We were particularly interested in the results for aqueous solutions, especially at low concentration, but other solvents were also used, for instance $CDCl_3$ for hydrogen-bonding studies and dimethyl sulphoxide (DMSO) to correlate data between aglycones, insoluble in water, and antibiotics.

‡ The interpretation of the data in acetone solution led to incorrect conclusions about the conformation of the amino sugar.[14] Recently it has been proved that daunomycin and adriamycin react with acetone to give *N,O*-isopropylidene derivatives.[15]



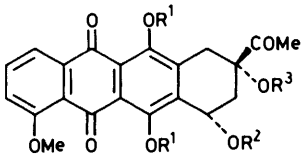
(1) $R^1 = R^2 = R^3 = H$

(2) $R^1 = OH$, $R^2 = R^3 = H$

(3) $R^1 = R^3 = H$, $R^2 = OMe$

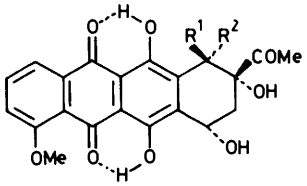
(4) $R^1 = R^2 = H$, $R^3 = OMe$

(5) 3′-*N*-acetyl-(1)



(6) $R^1 = H$, $R^2, R^3 = >CMe_2$

(7) $R^1 = R^2 = OMe$, $R^3 = H$

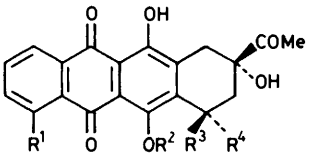


(8) $R^1 = R^2 = H$

(9) $R^1 = OMe$, $R^2 = H$

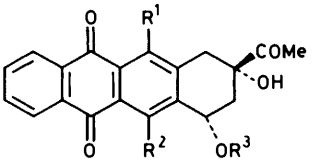
(10) $R^1 = H$, $R^2 = OMe$

(11) $R^1 = H$, $R^2 = OCOMe$



(12) $R^1 = R^2 = R^4 = H$, $R^3 = OH$

(13) $R^1 = R^4 = OH$, $R^2 = Me$, $R^3 = H$



(14) $R^1 = OH$, $R^2 = R^3 = H$

(15) $R^1 = R^3 = H$, $R^2 = OH$

(16) $R^1 = OH$, $R^2 = H$, R^3 = daunosaminyl

Experimental

N.m.r. spectra were recorded with a Bruker CXP-300 and a Varian XL-100-15 spectrometer. Chemical shifts are in δ values from internal Me_4Si and from external 3-(trimethylsilyl)-propane-1-sulphonic acid, sodium salt hydrate in D_2O (DSS) for D_2O solutions. Concentrations are variable, depending on the best condition for the analysis; they are reported in each Table. ^1H Spectra were completely analysed by using the PANIC program, included in the Aspect-2000 computer library. Also, first-order spectra were simulated in order to correct small coupling constants from line-broadening effects. ^1H Chemical shifts are accurate to within ±0.01 p.p.m. and coupling constants to within ±0.05 Hz unless specified in the Tables; for the sugar moiety J values are accurate to within ±0.1 Hz. Some examples of spectra are given in Figures 1—3.

The assignment of hydrogen signals was done by the usual decoupling techniques; in particular the assignment of H-8eq *versus* H-8ax and of H-10eq *versus* H-10ax were made through the four-bond coupling $J_{8,10}$, which involves only equatorial protons (**W** geometry). Interactions between diaxial and equatorial–axial protons are zero for all compounds, except for (**6**), where ring A is locked and $J_{8ax,10eq}$ 0.7 Hz was also detected. The assignment in this case is straightforward from the values of the two couplings. For the particular case of (**12**) in DMSO and dioxane, we used the trend of $^4J_{8,10}$ with temperature together with the chemical-shift difference between 10-CH_2, compared with that in $CDCl_3$, and with the other compounds. The detection of 4′-OH and the assignment of phenolic hydroxy groups and of the aromatic protons 1-H *versus* 3-H were performed by n.O.e. experiments. For the details of n.O.e. experiments see the accompanying paper. On irradiation of 4-OMe, strong n.O.e. effects were observed on the adjacent 3-H of daunomycin (**1**) in D_2O and *N*-acetyldaunomycin (**5**) in $CDCl_3$: the n.O.e. is negative (11%) in D_2O* and positive (21%) in $CDCl_3$. Since 1-H gave always a broader signal than 3-H, the assignment for the other compounds follows from these experiments directly by comparison of the two signals. The detection of 4′-OH, when hidden by other absorptions as in the case of (**5**), was made by observing the 'saturation transfer' effect on 4′-OH from the phenolic OH protons.† The attribution of 6-OH *versus* 11-OH for (**5**) was made by irradiation of 7- or 1′-H (2.7% and 3.3% n.O.e. on 6-OH respectively).† In DMSO, the n.O.e. experiments gave very low intensities, because the increased viscosity of the solution leads to an increase in correlation times. Therefore the assignment in this solvent was performed by 'single frequency selective heteronuclear decoupling,' following the same procedure already applied for daunomycinone.[24]

All chemical-shift and coupling-constant values are reported in Tables 1—4.

The torsional angles of Table 5, θ = H(7)–C(7)–C(8)–H(8eq)‡ and θ′ = H(7)–C(7)–C(8)–H(8ax), were calculated from $^3J_{7,8}$ by using a Karplus equation modified by Altona,[25] which includes a correction for the electronegativity of the substituents. The β-substituent effect on $^3J_{7,8}$, due to the different groups at O(7) (OH, OCH_3, OCH_2, OCH), is neglected, because it was found to be small, *i.e.* 0.1—0.2 Hz.[25] In the case of angles lower than 90°, θ and θ′ values were compared with those obtained through a standard Karplus equation: $^3J = K\cos^2\theta + C$, where the parameters K and C were obtained as

* The aggregation causes an increase in correlation times. Studies are in progress in D_2O.

† See n.O.e. and saturation transfer experiments of the accompanying paper.

‡ Equatorial and axial in the text stand for pseudo-equatorial and pseudo-axial.

Table 1. ^{1}H Chemical-shift values of daunomycin and analogues[a]

	(1)				(2)		(3)		(4)		(5)			
	$CDCl_3$[b]	pyr[c,b]	DMSO	D_2O	DMSO	D_2O	DMSO	D_2O	DMSO	D_2O	$CDCl_3$	diox[e]	pyr	DMSO
1-H	8.05	8.02	7.74	7.53	7.84	7.45	7.93	7.49	7.82	7.40	8.05	8.00	8.08	7.90
2-H	7.79	7.68	7.79	7.71	7.86	7.66	7.67	7.40	7.86	7.60	7.79	7.76	7.70	7.90
3-H	7.40	7.38	7.52	7.43	7.60	7.37	7.93	7.22	7.60	7.33	7.40	7.45	7.38	7.64
7-H	5.31	5.42	4.85	4.82	4.90	4.82	4.94	4.94[d]	4.89	5.11	5.28	5.15	5.46	4.93
H(8eq)	2.36	2.76	2.15	2.22	2.18	2.28	2.07	2.18[d]	2.47	2.68	2.32	2.32	2.81	2.20
H(8ax)	2.09	2.40	2.03	2.10	2.11	2.06	2.42	2.55[d]	2.33	2.42	2.12	2.04	2.50	2.10
H(10eq)	3.24	3.45	2.86	2.92	2.97	2.95	4.84	4.80[d]	4.74	5.04	3.26	3.11	3.51	2.92
H(10ax)	3.00	3.36	2.79	2.67	2.87	2.68					2.96	3.01	3.47	2.95
1′-H	5.51	5.83	5.24	5.48	5.28	5.43	5.37	5.50	5.36	5.59	5.49	5.33	5.81	5.22
H(2′eq)	1.66	2.08	1.68	1.96	1.71	1.98	1.64	1.99[d]	1.76	2.05	1.89	*q*	2.19	1.42
H(2′ax)	1.75	2.22	1.87	2.00	1.90	2.03	1.87	2.05[d]	1.91	2.11	1.74	*q*	2.43	1.83
3′-H	3.13	4.10	3.37[f]	3.66	3.32[f]	3.68	3.4[f]	3.62	3.35[f]	3.7[f]	4.16	*q*	4.74[d]	3.97[f]
4′-H	3.47	3.68	3.62	3.81	3.61	3.83	3.56	3.82	3.64[f]	3.85	3.64	*q*	3.98	3.39
5′-H	4.09[f]	4.53	4.18	4.26	4.21	4.21	4.18	4.20	3.96	4.26	4.24	4.28	4.70	4.18
5′-Me	1.35	1.52	1.15	1.28	1.16	1.29	1.14	1.29	1.18	1.31	1.29	1.18	1.49	1.12
4-OMe	4.09	3.92	3.92	3.93	3.97	3.90	4.00	3.84	3.96	3.92	4.08	3.98	3.93	3.98
9-COMe	2.41	2.54	2.27	2.43	4.63[l]	4.79[l]	2.38	2.52	2.15	2.46	2.40	2.32	2.57	2.26
6-OH	14.05	*h*	14.05		14.01		13.95		13.86		13.99	14.06	14.56	13.97
11-OH	13.32	*h*	13.23		13.21		13.49		13.36		13.30	13.25	13.69	13.26
9-OH	4.73	*h*	5.50		5.49		5.5[n]		6.25		4.43	4.68	6.22[d,n]	5.52
4′-OH	*g*	*h*	5.12		5.57		5.5[n]		5.50		1.92	*q*	*q*	4.74
3′-NH	*g*	*h*	7.96[i]		8.05[i]		7.89[i]		8.00[i]		5.75	6.54	8.37	7.55
Others					4.90[m]		3.39[o]	3.54[o]	3.63[o]	3.68[o]	1.93[p]	1.77[p]	1.00[p]	1.76[p]

[a] Measured at 300 MHz (25 °C; δ from internal Me_4Si, except for D_2O solutions, where an external DSS reference was used). Accuracy within 0.01 p.p.m., unless specified. Concentration: 1 mg ml^{-1} for $CDCl_3$ solutions; 1 and 5 mg ml^{-1} in D_2O for (**1**), (**4**) and (**2**), (**3**) respectively; 30 and 15 mg ml^{-1} in DMSO for (**1**), (**2**) and (**3**)—(**5**) respectively; *ca.* 0.2 and 3.5 mg ml^{-1} for (**5**) in dioxane and in pyridine respectively. [b] Unprotonated form. [c] Pyridine. [d] Measured at 40 °C. [e] Dioxane. [f] Partially overlapped by OMe or water signals. [g] δ 1.5—1.8 (3′-NH_2 + 4′-OH). [h] δ 5.5—6.8 (OH + NH_2 + H_2O). [i] NH_3^+ signal. [l] CH_2(14) signal. [m] 14-OH signal. [n] Broad. [o] 10-OMe signal. [p] 3′-NCOMe signal. [q] Not detected.

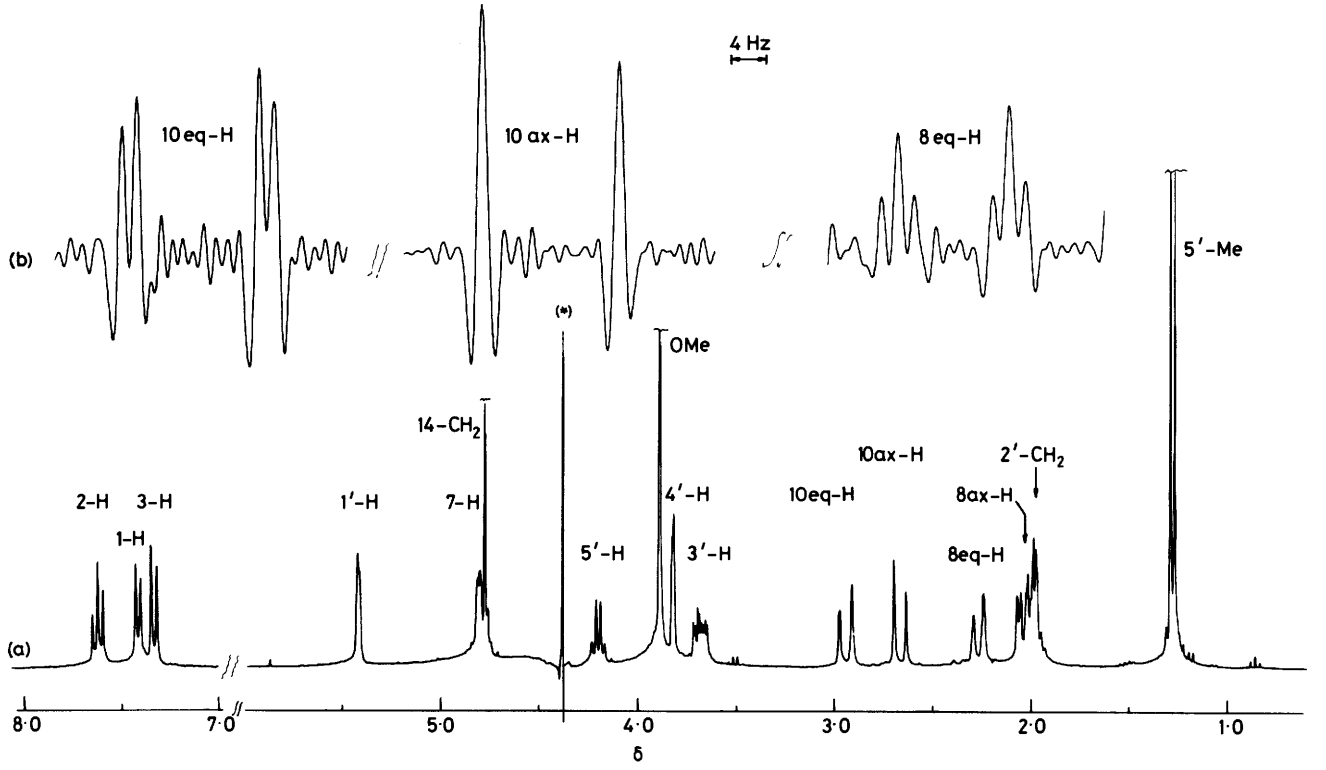


Figure 1. (a) ^{1}H N.m.r. spectrum of adriamycin (**2**) (doxorubicin) in D_2O (5 mg ml^{-1}) at 300 MHz and 40 °C. The peak marked * is the residual HDO signal after 'solvent suppression'. (b) Expanded plot of H(10ax), H(10eq), and H(8eq) with resolution enhancement

Table 2. ^{1}H Chemical-shift values for daunomycinone analogues[a]

	(6)			(7)			(8)			(9)			(10)			(12)		
	$CDCl_3$	diox	DMSO	$CDCl_3$	diox	DMSO	$CDCl_3$	diox	DMSO	$CDCl_3$	diox	DMSO	$CDCl_3$	diox	DMSO	$CDCl_3$	diox	DMSO
1-H	8.03	7.97	7.89	7.71	7.66	7.75	8.05	7.99	7.90	8.06	8.00	7.89	8.07	7.99	7.89	8.36[i]	8.35[i]	8.32[i]
2-H	7.77	7.75	7.89	7.63	7.61	7.50	7.79	7.76	7.90	7.80	7.77	7.89	7.82	7.76	7.89	7.85[l]	7.85[l]	8.00[l]
3-H	7.39	7.45	7.66	7.25	7.31	7.61	7.40	7.45	7.63	7.41	7.46	7.61	7.42	7.45	7.61			
7-H	5.51	5.43	5.33	4.92	4.86	4.78	5.34	5.24	5.04	5.32	5.23	5.12	5.15	5.00	4.89	5.41	5.20	5.06
H(8eq)	2.67	2.59	2.62	2.44	2.36	2.28	2.35	2.22	2.18	2.25	2.12	2.03	2.33[e]	2.32	2.37	2.35	2.26[e]	2.28[e]
H(8ax)	1.94	1.90	1.95	1.88	1.89	2.00	2.16	2.05	1.96	2.75	2.49	2.33	2.23[f]	2.17	2.20	2.19	2.16[f]	2.10[f]
H(10eq)	3.21	3.15	3.04	3.27	3.13	3.08	3.19	3.09	2.98	4.66	4.74	4.80	4.94	4.73	4.68	2.94	3.32[g]	3.32[g]
H(10ax)	2.79	2.82	2.79	3.04	2.98	2.91	2.94	2.91	2.86							3.10	2.68[h]	2.51[h]
4-OMe	4.09	3.99	4.00	4.02	3.93	3.93	4.10	4.00	3.98	4.10	4.00	4.01	4.09	3.99	3.98			
9-COMe	2.41	2.34	2.36	2.42	2.29	2.24	2.42	2.32	2.32	2.46	2.33	2.33	2.39	2.18	2.16	2.41	2.27	2.22
10-OMe										3.50	3.41	3.38	3.65	3.60	3.59			
6-OH	13.81	13.80	13.84[c]				13.96[c]	14.03	14.00[c]	13.91[c]	13.89	13.86[c,d]	13.99[c]	14.04	13.9[c,d]	13.94	13.65	13.47[d]
11-OH	13.22	13.10	13.12[c]				13.26[c]	13.21	13.28[c]	13.52[c]	13.49	13.48[c,d]	13.59[c]	13.43	13.5[c,d]	13.32	13.31	13.36[d]
9-OH	1.47[b]	1.43[b]	1.41[b]	5.04	4.84	5.53	4.54	5.07	6.13	4.20	4.87	5.95	3.87	4.84	6.18	3.88	4.51	5.88
7-OH	1.15[b]	1.06[b]	1.05[b]				3.71	4.73	5.35	3.61	4.54	5.2[d]	4.28	4.37	5.18	4.27	4.27	5.28

[a] Measured at 300 MHz (25 °C; δ from internal Me_4Si); accurate within 0.01 p.p.m., unless specified. Concentration *ca.* 10 mg ml^{-1} except for dioxane solutions and for (**12**) in all solvents. [b] Isopropylidene methyl groups. [c] Measured at 100 MHz (39 °C). [d] Broad. [e,f] 8-H *trans* (*e*) and *cis* (*f*) to 7-H respectively. [g,h] 10-H *trans* (*g*) and *cis* (*h*) to 9-OH respectively. [i,l] Two protons: (*i*) 1-H and 4-H; (*l*) 2-H and 3-H.

Table 3. H–H Coupling-constant values (Hz) for daunomycin (1), adriamycin (2), 10-methoxy and *N*-acetyl analogues (3)—(5)[a]

	(1)				(2)		(3)		(4)		(5)			
	$CDCl_3$[b]	pyr[b]	DMSO	D_2O	DMSO[g]	D_2O[g]	DMSO	D_2O	DMSO	D_2O	$CDCl_3$	pyr[g]	diox	DMSO
7,8eq	2.5	2.9	3.0	2.4	3.0	2.5	1.5	1.6	7.9	7.4	2.3	3.2	2.5	3.5
7,8ax	4.1	4.7	5.6	5.0	5.7	5.1	5.8	5.3	8.4	7.5	4.2	5.0	4.7	5.7
8eq,8ax	14.9	14.5	14.6	15.0	14.6	15.0	15.3	15.3	13.0	13.0	14.8	14.5	14.6	14.5
10eq,10ax	19.0	18.0	18.1	18.3	18.2	18.6					18.7	18.4	18.7	18.2
8eq,10eq	2.0	~1.7[c,d]	1.6	2.0	1.6	2.0	1.3	1.5	1.7	1.5	2.0	1.7	1.8	1.5
7,10ax	≤0.7[c]	≤0.7[c]	≤0.5[c]	≤0.7[c]	*f*	*f*	*f*	*f*	*f*	*f*	0.7	~0.5	*f*	*f*
1′,2′eq[d]	1.0[e]	1.0	1.0	1.3	1.0	1.2	1.0	1.2	1.0	1.2	1.1	1.3	*f*	1.2
1′,2′ax	4.1[e]	4.0	3.7	3.9	3.9	3.8	3.7	3.9	3.8	3.8	4.1	4.0	*f*	3.9
2′eq,2′ax	13.3[e]	12.5	12.5	13.1	12.5	13.0	12.6	13.0	12.5	13.0	13.6	12.9	*f*	12.5
2′ax,3′	12.2[e]	12.5	12.9	13.0	13.0	13.0	13.0	13.0	13.1	13.0	12.8	12.9	*f*	12.9
2′eq,3′	5.2[e]	~4.5[d]	4.7	4.7	4.9	4.8	4.5	4.7	4.8	4.8	5.0	4.8	*f*	4.5
3′,4′	2.8	~2.5[d]	3.0	2.8	3.2	2.7	~2.5[d]	2.8	~2.7[d]	2.8	2.7	2.8	*f*	2.5
4′,5′	1.5	1.5	1.4	1.4	1.5	1.3	1.3	1.4	1.3	1.4	1.2	1.4	*f*	1.4
3′,NH											8.5	8.0	*f*	6.5
2′eq,4′[d]	1.0	*f*	1.0	1.0	1.0	1.1*f*	0.8	1.0	1.0	0.8	0.9	1.0	*f*	0.8
1′,5′	*f*	*f*	≤0.5[c]	*f*	*f*	*f*	≤0.5[c]	*f*	*f*	*f*	≤0.5[c]	*f*	*f*	*f*
1′,4′	*f*	*f*	*f*	*f*	*f*	*f*	≤0.5[c]	*f*	*f*	*f*	1.0	0.7	*f*	*f*
4′,OH	*f*	*f*	~5.0[d]		*f*	*f*	*f*		6.0		5.0	*f*	*f*	5.0

[a] Accuracy within ±0.05 Hz unless specified; within 0.2 Hz for the sugar moiety. Concentration: 30 mg ml^{-1} for (1) and (2) in $CDCl_3$, DMSO, and pyridine; 10—15 mg ml^{-1} for (3)—(5) in DMSO and $CDCl_3$. *ca.* 5 mg ml^{-1} for D_2O solutions and for (5) in pyridine. Temperature 25 °C unless specified. *J* Values for ring D protons are not reported because not significant. $J_{4',OH}$ 5.0—6.0 Hz in DMSO for (1) and (4); $J_{3',NH}$ 8.0—8.5 for (5). [b] Unprotonated form. [c] Detected by decoupling experiments. [d] The low accuracy (within 0.3 Hz) for this coupling, except for (5) in $CDCl_3$, is due to the broad signals, caused by the presence of several interactions. [e] From $CDCl_3$–benzene (*ca.* 1:1). [f] Not measured. [g] Temperature *ca.* 40 °C.

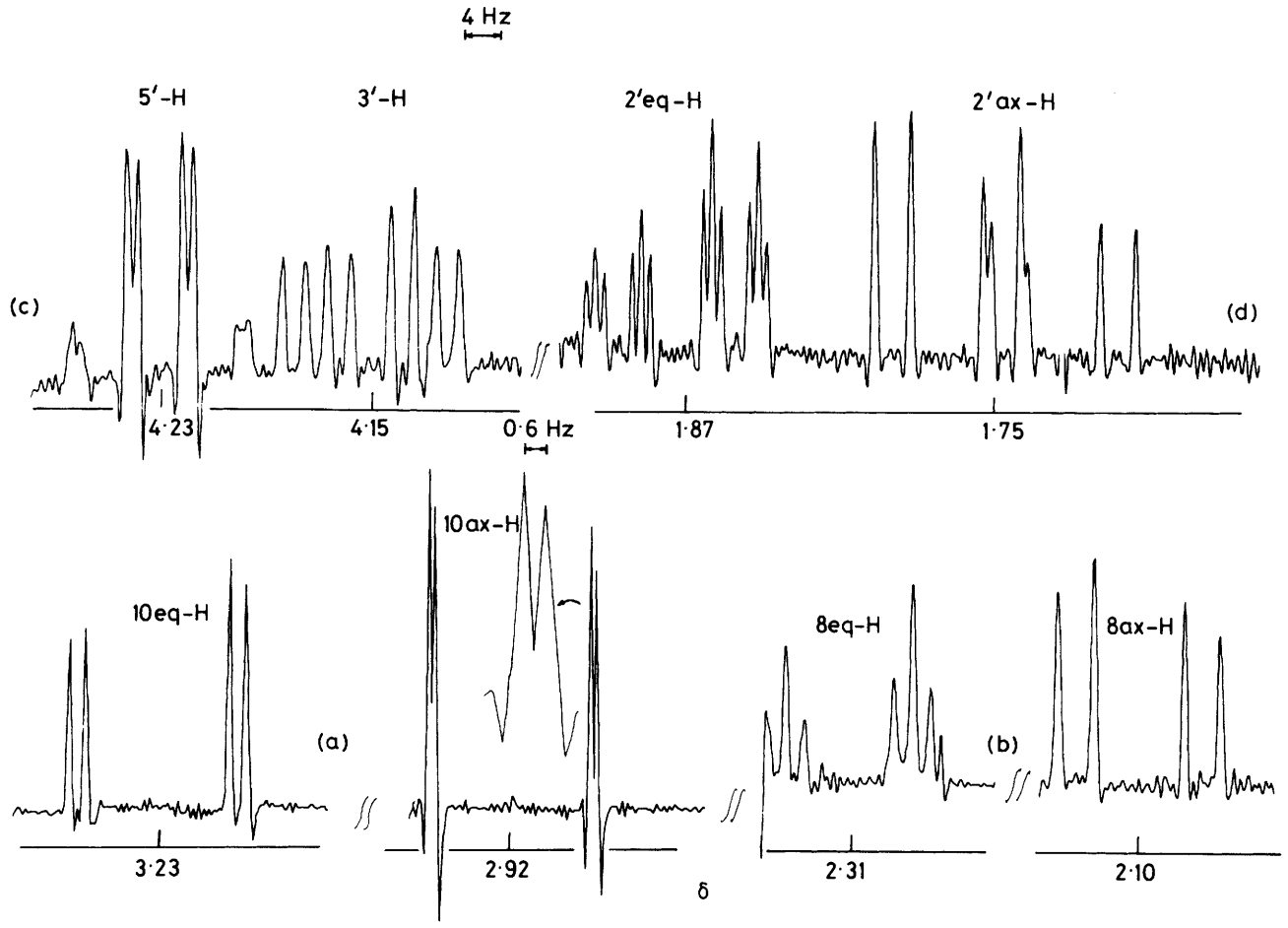


Figure 2. ^1H N.m.r. spectrum of *N*-acetyldaunomycin (5) in $CDCl_3$ (10 mg ml^{-1}) at 300 MHz and 25 °C with resolution enhancement: (a) 10-CH_2, (b) 8-CH_2, (c) 5′-H and 3′-H, (d) 2′-CH_2 expanded patterns (full spectrum is reported in the accompanying paper)

Table 4. H–H Coupling-constant values (Hz) for daunomycinone analogues[a]

	(6)			(7)			(8)			(9)		
J	$CDCl_3$	diox	DMSO	$CDCl_3$	diox	DMSO	$CDCl_3$	diox	DMSO	$CDCl_3$	diox	DMSO
7,8eq	3.3	3.3	3.3	2.3	2.3	2.8	1.9	2.2	2.9	1.6	1.8	1.9
7,8ax	2.8	2.8	2.6	3.6	3.7	4.9	4.9	4.8	4.7	5.2	4.8	4.8
8eq,8ax	14.0	14.1	14.0	14.6	14.7	14.9	14.7	14.4	14.3	14.7	15.0	15.0
10eq,10ax	18.3	18.4	18.3	18.3	17.8	16.9	18.7	18.7	18.4			
8eq,10eq	2.1	2.0	1.9	2.0	1.8	1.1	2.3	1.9	1.9	1.5	1.4	1.3
8ax,10eq	0.7	~0.5[e]	~0.5[e]	0.0	0.0	*b*	0.0	*b*	*b*	0.0	*b*	0.0
7,10ax	0.0	0.0	*b*	0.5[f]	*b*	*b*	0.9	~0.5[e]	*b*			
8ax,OH[c,d]				0.0	0.0	0.0	1.2[d]	*b*	*b*	~0.8[c,e]	*b*	*b*

	(10)						(11)[21]	(13)[20]		(14)[20]		(15)[20]		(16)[20]
				dioxane										
J	$CDCl_3$	DMSO	MeOH				DMSO	$CDCl_3$	DMSO	$CDCl_3$	DMSO	$CDCl_3$	DMSO	DMSO
7,8eq	5.5[g]	7.6	7.3	7.0	7.0[m]	6.9[n]	6.2[g]	2.2	2.9	1.7	5.8[g]	2.2	2.9	6.2[g]
7,8ax	6.0[h]	8.5	7.8	8.1	7.7	7.4	8.2[h]	4.8	4.4	4.5	9.7[h]	4.9	4.6	8.8[h]
8eq,8ax	14.1	13.1	13.2	13.2	13.2	13.3	1.6	2.0	1.1[o]	2.0	1.2[o]	2.2	1.0[o]	0.7[o]
10eq,10ax														
8eq,10eq	0.7[i]	1.7	1.5	1.5	1.4	1.3								
8ax,10eq	0.0	0.0	*b*	0.0										
7,10ax	0.0													
8ax,OH[c,d]	0.8[d,l]	0.0	*b*	0.0	0.0	0.0								

[a] Accuracy within ±0.05 Hz, concentration: *ca.* 10 mg ml^{-1}, at 25 °C, unless specified. Couplings of 5—6 Hz were found between 7-OH and 7-H. [b] Not detected. [c,d] Coupling (*c*) with 7-OH, (*d*) with 9-OH. [e] Accuracy within 0.2 Hz. [f] From concentrated solution (50 mg ml^{-1}). [g,h] Coupling of 8-H *trans* (*g*), and *cis* (*h*) to 7-H. [i,l] Coupling involving (*i*) H(8eq) of the S_9 form, (*l*) H(8ax) of the $^9H_8$ form. [m,n] At 65 and 90 °C respectively. [o] These values could not be used, because they were not corrected[20] for line-broadening effects.

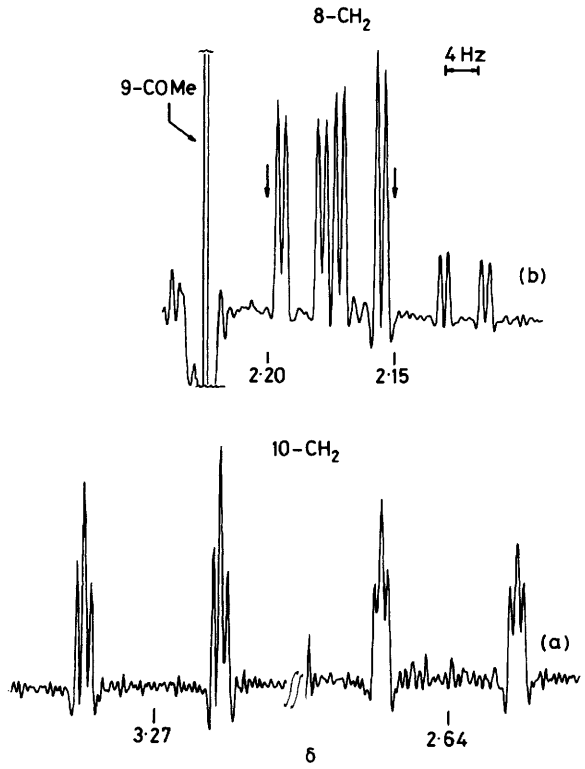


Figure 3. ^1H N.m.r. spectrum of 7-*epi*-4-demethoxydaunomycinone (**12**) in DMSO (10 mg ml^{-1}) at 300 MHz and 85 °C, with resolution enhancement: (a) 10-CH_2, (b) 8-CH_2 patterns

follows: $C = 1.40$ Hz,[25] since $J_{90°}$ is predicted and experimentally found as positive; $K = 6.4$ Hz, deduced from the experimental *J* values of (**6**) and from θ and θ′, whose value, measured with Dreiding models for the rigid structure of (**6**), was 60°. An estimate of the accuracy of the method can be obtained, due to the condition that each couple of angles either add or subtract to 120°. The fit is considered to be good (error <10%) when (θ + θ′) or (θ′ − θ) is 116—118°.[25] For values lower than 106°, the results are not considered to be reliable and *J* values (averaged couplings) are an indication that more than one conformer is present at the equilibrium.

The population of the conformers was calculated through the usual equations;[26] for example with an equilibrium of three conformers: A ⇌ B ⇌ C, we have equations (1)—(3) with

$$^3J_{obs} = \alpha_A J_A + \alpha_B J_B + \alpha_C J_C \quad (1)$$

$$^3J'_{obs} = \alpha_A J'_A + \alpha_B J'_B + \alpha_C J'_C \quad (2)$$

$$^4J_{obs} = \alpha_A {}^4J_A + \alpha_B {}^4J_B + \alpha_C {}^4J_C \quad (3)$$

the constraint $\alpha_A + \alpha_B + \alpha_C = 1$. $^3J_{obs}$, $^3J'_{obs}$, and $^4J_{obs}$ are the experimental coupling constants $J_{7.8eq}$, $J_{7.8ax}$, and $^4J_{8.10}$, and α_A, α_B, and α_C the molar fractions of conformers A, B, and C, respectively. When the coupling constants of each conformer are known, the molar fractions of the three conformers can be deduced. In the case of an equilibrium of two half-chair and one skew conformations [compounds (**4**), (**10**), (**11**), (**14**), and (**16**)], $^9H_8 \rightleftharpoons {}^8H_9 \rightleftharpoons S_9$, we employed the parameters in equations (4)—(9), obtained from the Karplus–Altona equation.[25] Alternatively, for J_A and J'_A of the $^9H_8$ conformer, the

$$J_A = J_{7eq.8eq}\ (^9H_8) = 2.57 \quad (4)$$

$$J_B = J_{7ax.8ax}\ (^8H_9) = 10.88 \quad (5)$$

$$J_C = J_{7.8}\ trans\ (S_9) = 8.19 \quad (6)$$

$$J_{A'} = J_{7eq.8ax}\ (^9H_8) = 3.17 \quad (7)$$

$$J'_B = J_{7ax.8eq}\ (^8H_9) = 5.64 \quad (8)$$

$$J'_C = J_{7.8}\ cis\ (S_9) = 7.82 \quad (9)$$

Table 5. Rotational angles θ and θ′ (°) for (1)—(15)[a]

		(1)[b]	(2)	(3)	(4)	(5)[b]	(6)	(7)	(8)	(9)	(10)	(12)	(13)	(14)	(15)
$CDCl_3$	θ	71				72	64	72	78	80	50[f]	42	73	79	73
	θ′	43				43	53	47	38	37	138[f]	159	39	41	38
$CDCl_3$	θ[c]	70				69	60	68	72	79			69	77	69
	θ′[c]	48				49	60	54	44	39			43	46	42
D_2O or dioxane	θ	72[d]	71	80	38	71[e]	64	72	73	78	40				
	θ′	38[d]	37	36	146	40[e]	53	46	39	39	150				
DMSO	θ	66[f]	66[f]	80	35	62[f]	64	68	67	78	36		67	49	68
	θ′	34[f]	34[f]	33	152	34[f]	55	39	40	39	153		42	160	40

[a] Values obtained from $J_{7,8}$ through a Karplus–Altona equation including a correction for substituent electronegativity.[25] θ and θ′ are defined as in Table 6. The estimated error, unless specified, is <10% (see Experimental section). [b] In pyridine, θ = 67 and 65°, θ′ = 40 and 38°, for (1) and (5) respectively. [c] Values obtained from a standard Karplus equation, where coefficient K was experimentally determined from (6). [d] D_2O; calculation as in [c] gave: (1) (67°, 41°); (2) (66°, 40°); (3) (80°, 34°). [e] Dioxane. [f] Values unreliable for a single conformer (see Experimental section).

Table 6. Rotational angles (°) for the conformations of ring A described in the text[a]

		S_8	$^9H_8$	^9S	$B_{7,10}$	X-Ray[3–5]		
H(7eq)–C(7)–C(8)–H(8eq)	= θ[b]	60	70	90	70	85.0[c]	65.7[d]	67.5[e]
H(7eq)–C(7)–C(8)–H(8ax)	= θ′	60	50	30	50	36.8	53.3	50.6
H(7ax)–C(7)–C(8)–H(8eq)	= θ′	60	50	30	50			
H(7ax)–C(7)–C(8)–H(8ax)	= θ′	180	170	150	170			
C(6)–C(6a)–C(7)–H(7eq)	= ψ	30	40	60	10	60.3		36.8
C(11)–C(10a)–C(10)–H(10ax)	= ψ	60	80	90	110	78.9		73.0
H(8eq)–C(8)–C(9)–C(10)	= φ	165	175	180	120	178.7	176.5	179.1
C(8)–C(9)–C(10)–H(10eq)	= φ′	165	175	180	160	179.9	159.5	161.7

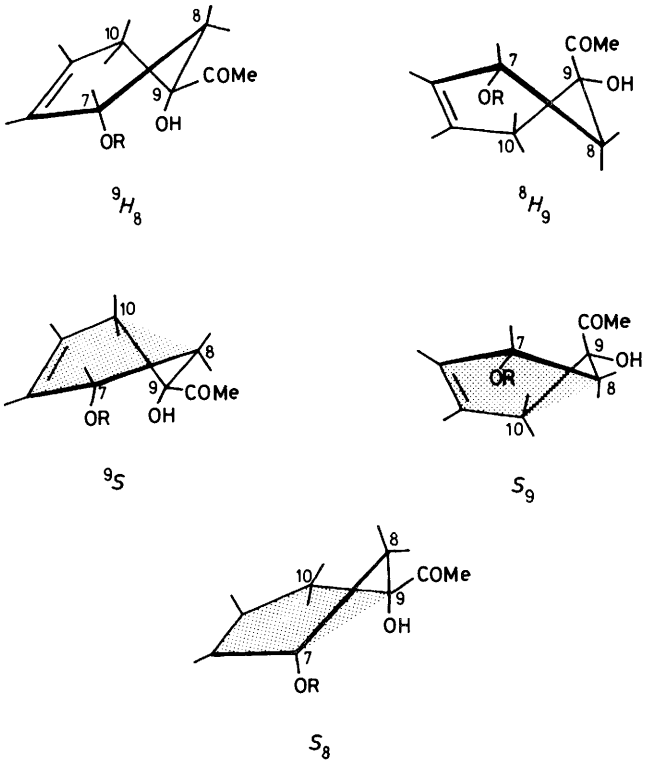
[a] Values deduced from those calculated for cyclohexene by Bucourt and Hainant,[30] and from X-ray atomic co-ordinates.[3–5] The accuracy is estimated within 5°. Conformations ^8S, $^8H_9$, and S_9 display the same angles as S_8, $^9H_8$, and ^9S respectively. [b] All rotational angles are given without sign. [c] From refs 3—5 respectively.

Table 7. Coupling-constant values (Hz) and conformer populations (%) at different temperatures for 7-*epi* derivative (12)

Solvent	$CDCl_3$	DMSO		DMSO		DMSO		DMSO		DMSO		DMSO		Dioxane	
Temperature (°C)	25	25		40		60		~70		85		105		25	
$^9H_8$ α %	50	18		15		9		11		15		6		31	
^9S α %	50	9		18		31		32		32		45		29	
S_9 α %		27		20		12		13		9		3		25	
$^8H_9$ α %		46		47		48		45		44		46		15	
J	exp[a]	exp[a]	calc[b]	exp	calc	exp	calc	exp	calc	exp	calc	exp	calc	exp[a]	calc[b]
$J_{7,8}$ *cis*	6.8[e]	4.9	5.0	5.0	5.1	5.2	5.3	5.3	5.4	5.3	5.3	5.5	5.5	6.0	6.1
$J_{7,8}$ *trans*	9.5[e]	4.3	4.2	4.6	4.5	4.9	4.9	5.1	5.2	5.5	5.5	5.6	5.6	6.5	6.5
$J_{8,10}$[c]	2.6	0.5	0.6	0.7	0.8	1.0	0.9	1.1	1.0	1.2	1.1	1.4	1.3	1.5	1.4
$J_{8,10}$[d]	0.0	1.5	1.6	1.4	1.5	1.3	1.3	1.3	1.2	1.2	1.1	1.1	1.0	1.1	1.0
$J_{7,10}$ *cis*	0.9	≤0.5	0.7	≤0.5	0.7	0.6	0.7	*f*	0.7	0.7	0.8	0.7	0.8	*f*	0.8
$J_{7,10}$ *trans*	1.6	≤0.5	0.4	≤0.5	0.5	0.7	0.6	*f*	0.6	0.8	0.7	1.0	0.8	0.9	1.0

[a] Accuracy within ±0.05 Hz. [b] Calculations were performed by considering the equilibrium $^9H_8 \rightleftharpoons {}^9S \rightleftharpoons S_9 \rightleftharpoons {}^8H_9$ (see Experimental section). [c,d] These interactions involve two couples of protons in *cis* relationship: (*c*) 8-H *cis* to 7-H and 10-H *cis* to 9-OH; (*d*) 8-H *trans* to 7-H and 10-H *trans* to 9-OH. Since the interaction between axial protons was proved to be zero, the coupled nuclei, for each conformer, are always equatorial. An additional coupling constant of 0.5 Hz between 9-OH and H(8ax) was detected, only in $CDCl_3$, by decoupling experiment. [e] Calculated values: 6.7 and 9.5 Hz. [f] Not measured.

experimental couplings in $CDCl_3$ of (7) can be used (2.3 and 3.6 Hz). The values of ^4J parameters were taken from the experimental $^4J_{8,10}$ of (7), (9), and (12) in $CDCl_3$ and (10) in DMSO. The probable errors of the molar fractions were calculated from the experimental uncertainties of $J_{obs.}$, as ±2%, by using the error propagation law. Taking into account the uncertainties of the parameters used, a more realistic estimate of the error is *ca.* 10%. In the case of the 7-*epi* derivative (12) in DMSO and in dioxane, an equilibrium of four conformers was considered, $^9H_8 \rightleftharpoons {}^9S \rightleftharpoons S_9 \rightleftharpoons {}^8H_9$, and a fourth equation, involving $^4J_{8,10}$, was added, as the protons at C-8 and C-10 show an additional coupling. The probable errors of the molar fractions were calculated as ±5%. However, we shall consider, for the data of Table 7, the trend of variations with temperature, rather than absolute values. $^5J_{7,10}$ values were not employed for the calculation, because these small couplings involve additional experimental errors; the data reported in Table 7 are just to show that the trend of the relative populations is also consistent with the variations of these couplings.



Scheme. The conformational nomenclature for ring A is that generally used for carbohydrates.[27] Dotted areas represent coplanarity

Results

The values of the coupling constants for the sugar ring given in Table 3 show that the shape of daunosamine moiety is constant for all compounds in the solvents examined. The preferred conformation is the same chair $^1C_4$ (L) reported[1,13] for *N*-acetyldaunomycin (**5**) in $CDCl_3$. Variable-temperature measurements, performed with (**5**) from −70 to +70 °C, confirm the stability of this form.

The conformation of ring A was determined by using H,H coupling constants $^3J_{7,8}$, $^4J_{8,10}$ and occasionally $^5J_{7,10}$ (Tables 3 and 4). From $J_{7,8}$ values, rotational angles θ and θ′ were obtained and reported in Table 5. Since a consistent set of ^3J values, for the fragment CH(7)–CH_2(8), is now available from compounds (**1**)—(**16**), the geometry of this ring in solution can be accurately determined. An inspection of Table 5 shows that the results in $CDCl_3$ are very satisfactory, firstly because the sums of θ and θ′ values are 116—118°, which is correct with the projected H–C–H angles of 118°;[25] secondly because the variation of J_{eqeq} *versus* J_{eqax} for (**6**) with respect to (**7**) follows the predicted trend,[25] *i.e.* $J_{eqeq} < J_{eqax}$ for θ = θ′ = 60°, $J_{eqeq} > J_{eqax}$ for θ = 70° and θ′ = 50°. This is good evidence for the reliability of the method used.

(a) *Chloroform Solution.*—^3J Values indicate that the isopropylidene derivative (**6**) has a ring A conformation (θ = 64°, θ′ = 53°) very close to the skew S_8 form (in all the solvents), whereas trimethoxydaunomycinone (**7**), daunomycin (**1**) in the unprotonated form, and the *N*-acetyl derivative (**5**) show preferred conformations, with angles in the range 71—72° (θ) and 43—47° (θ′), which correspond to the half-chair $^9H_8$ (Tables 5 and 6). Daunomycinone (**8**) and the 10*R*-methoxy derivative (**9**) show values of 78—80 and 37—38° correct for a half-chair $^9H_8$ slightly distorted toward the skew ^9S or for a fast equilibrium between them. The distortion may reasonably be induced by 1,3-diaxial interactions between O(7) and O(9), as the intramolecular hydrogen bond 9−OH ··· O (7) for (**8**) and (**9**) is weaker than for (**5**) and (**7**). Furthermore, for (**9**) an additional 1,3-diaxial interaction, OMe(10)–H(8ax), contributes to this distortion, so that (**9**) will hold the same conformation in all the solvents.

These results are also supported by three long-range couplings $^4J_{8eq10eq}$, $^4J_{8ax,10eq}$, and $^5J_{7,10ax}$. This latter gives, when detected, precious information on the geometry of ring A, as it depends, besides the bond order, upon the angles from the aromatic plane to the direction of 7-H (ψ) and 10-H (ψ′) bonds respectively, with a maximum value for angles of 90°.[28] In the skew S_8 form, these angles are 30 and 60°, respectively, which agree with the absence of coupling found for the isopropylidene daunomycinone (**6**). Both ψ and ψ′ increase from form S_8 to the half-chair $^9H_8$, and to the skew ^9S (Table 6), in agreement with the observation of increasing coupling from (**6**) to (**7**) (0.5 Hz) and to (**8**) (0.9 Hz). A model of ring A with a high population of the ^9S form is given by the 7-*epi* derivative (**12**) in $CDCl_3$ (see later for the discussion of this compound), which shows $J_{7,10}$ 1.6 Hz, thus confirming the above trend. The four-bond coupling between H(8eq) and H(10eq), already large for (**1**) and (**5**)—(**7**) (2.0 Hz),* increases further to 2.3 Hz for (**8**), and to 2.6 Hz for (**12**). This proves the perfect **W** geometry of the four bonds connecting H(8eq) and H(10eq) in structure ^9S (coplanarity leading to maximum coupling[28]), and agrees with the preferred conformation of daunomycinone (**8**). Finally (**6**) displays a small but clearly detectable coupling between H(8ax) and H(10eq) (0.7 Hz), whereas this interaction was proved to be absent in the other compounds. This shows the high sensitivity of these long-range couplings to even small conformational changes of ring A, and is additional evidence for the difference between forms S_8 and $^9H_8$.

The conformational properties of 10*S*-methoxydaunomycinone (**10**) differ from those of the corresponding stereoisomers (**9**), as the steric interactions between the equatorial 10-OMe group and the *peri* 11-OH in the $^9H_8$ form are so strong as to flip the ring. In $CDCl_3$, the values of $J_{7,8}$ do not correspond to reasonable θ and θ′ angles for a single conformation, but indicate, together with the small $J_{8,10}$ (0.6 Hz), that more than one conformer is present at the equilibrium, which is fast compared with n.m.r. time scale. These values also disagree with the boat $B_{7,10}$ conformation, previously suggested.[21,29] The calculation of $J_{7,8}$ for a dynamic mixture of forms $^9H_8$ and S_9 (1:1), by using the parameters of Karplus–Altona equation[25] and the experimental values of (**7**), as model for the $^9H_8$ form, gave values of 5.24 and 6.01 Hz, which agree very well with the experimental ones (5.5 and 6.0 Hz). Moreover the detection of a small coupling between H(10) and one proton at C(8) confirms the above equilibrium: this coupling belongs to H(8eq) of form S_9. If one considers a value of *ca.* 1.5 Hz for a 'pure' S_9 structure [see (**10**) in DMSO, Table 3], the experimental coupling of 0.7 Hz agrees with the calculation (see Experimental section). Another small interaction, involving 9-OH and H(8ax), is significant, if one compares (**8**), (**9**) with (**10**) and (**12**) (Tables 4 and 7, see also the discussion of hydrogen bonding). The flexibility of ring A in this compound was also proved by heating to 65 °C: a slight increase of the three couplings to 6.1, 6.2, and 0.8 Hz indicated a slightly increased population of form S_9, which is in line with the results obtained in the other solvents. Low-temperature experiments were not performed, as the barrier to the ring inversion is so low[30] that the equilibrium is still fast at −90 °C.

* The lower value observed for (**9**) must be attributed to the substituent effect[28] at C-10, which causes variations at the C-10 bond angles.

(b) *Dioxane, Dimethyl Sulphoxide (DMSO), and Pyridine Solution.*—In order to examine the factors which contribute to the stabilization of the various conformations, other solvents were considered. ^3J Values can be compared each other, as vicinal couplings are known to have a negligible solvent dielectric constant dependence,[31] which is confirmed by the constant values obtained for (**6**). For positive ^4J (such as $J_{8eq,10eq}$), a small reaction field effect with the polarity of the solvent could be expected,[31] which can be estimated not to be larger than 10% [observe the values for (**6**)]. Of the solvents, DMSO has the advantage of allowing the correlation between glycosides and aglycones, but may involve some complications, because of the strong interactions with the solute molecules. Actually, $J_{7,8}$ values are similar to those observed in $CDCl_3$, but in some cases the sum ($\theta + \theta'$) is far from 118° (Table 5). This might be due to bond angle distortions, or to a fast equilibrium of different conformations. The interactions of DMSO or dioxane with phenolic hydroxy protons cannot be responsible for bond angle distortions, because isopropylidene compound (**6**) displays almost constant values for all couplings. The interaction with 9-OH instead may induce some deformation effects. For instance, trimethoxydaunomycinone (**7**) is the only case where a relevant decrease (50%) of $J_{8,10}$ was observed, together with a positive increment (less negative) of $J_{10eq,10ax}$: the *exo* orientation of 9-OH, induced by hydrogen-bonding with the solvent, leads to a rotation of the 9-COMe group around the bond C(9)–C(13), that pushes the methyl toward H(10eq). The consequent steric compression on this proton, added to the steric interaction by the *peri* methoxy group, induces a distortion of H(10eq), which results in an increase of the bond angle H–C(10)–H. This is expected to give a positive shift (less negative) of the geminal coupling,[32] and negative shift (less positive) of $J_{8eq,10eq}$, as the distortion tends to move H(10eq) out of the **W** geometry.[28] The above interpretation is supported by the results in dioxane, which does not induce any change in *J versus* $CDCl_3$: dioxane probably does not bind to (**7**) because of steric hindrance, as appears from the shift of 9-OH compared with those of the other compounds (Table 2). Therefore, we can conclude that the preferred conformation in these solvents, for (**7**), as for (**8**) and (**9**), is still the half-chair $^9H_8$, even though in DMSO the fitting of $J_{7,8}$ is lower than in dioxane. If we consider, for DMSO, a small contribution of the skew S_9 form (for instance 10%), the calculated averaged couplings for (**7**) and (**8**) become closer to the experimental values. This is in line with the results obtained in the same solvent for acetyldaunomycin (**5**) and for the active substances (**1**) and (**2**). In fact the values of $J_{7,8}$ for (**1**), (**2**), and (**5**) in DMSO gave unreliable θ and θ' angles (Table 5), but are better in agreement with a fast equilibrating mixture (*ca.* 75:25) of forms $^9H_8$ and S_9. Calculation performed by using the values of (**5**) in $CDCl_3$ for the $^9H_8$, and Altona[25] parameters for the S_9 conformation gave $J_{7,8}$ = 3.77 and 5.10 Hz. Calculations involving also the other half-chair $^8H_9$ gave physically meaningless results. In pyridine and in dioxane, the position of the equilibrium for (**1**) and (**5**) seems intermediate between those in DMSO and in $CDCl_3$ (80—90% of form $^9H_8$). No appreciable change was observed with temperature, but the trend of $J_{8,10}$ in these solvents (1.5 Hz* in DMSO, 1.7—1.8 Hz in pyridine and in dioxane, 2.0 Hz in $CDCl_3$) agrees with the results from the three-bond couplings.

10*R*-Methoxydaunomycin (**3**), like aglycone (**9**), shows a constant preferred conformation in all the solvents examined, *i.e.* a half-chair $^9H_8$, slightly distorted towards the ^9S. This probably occurs in order to decrease the interaction between 10-OMe and H(8ax): the high preference for an axial orientation by the methoxy group at C-10 is responsible for the constant shape of ring A in those derivatives.

10*S*-Methoxydaunomycinone (**10**) in DMSO was previously used as a model for the S_9 conformation; the same preference was found for the corresponding antibiotic (**4**). They show indeed a very good fitting for $J_{7,8}$ between calculated and experimental values, *i.e.* 7.8 and 8.2 *versus* 7.9 and 8.5 (**4**) *versus* 7.6 and 8.5 Hz (**10**), respectively. The rotational angles show an almost 'pure' S_9 form ($\theta' - \theta = 177°$). For the half-chair conformation reported by Malatesta *et al.*,[23] $J_{7,8}$ values should be 5.6 and 10.8 Hz.[25] The S_9 structure is also in agreement with (i) the values of $J_{8,10}$, which indicate an equatorial orientation of both 8-H and 10-H, and (ii) the axial orientation of 10-methoxy group, which leads to the same low-field shift of H(8ax) observed for (**3**) and (**9**), with respect to daunomycin (**1**) and daunomycinone (**8**). In dioxane and in methanol, the S_9 form is still preferred, but other conformers must be present. If we consider an equilibrium of the three forms $^9H_8$, $^8H_9$, and S_9, calculations (see Experimental section) gave *ca.* 70 and 85%, respectively of the skew form. With DMSO values, the same procedure gave populations of 98 and 90% of form S_9, for (**4**) and (**10**), respectively, in agreement with the results above for this solvent. The lower values of $J_{8,10}$ in dioxane and in methanol also prove the existence of an equilibrium. An increase in temperature to 90 °C leads to a slight increase of conformers S_9 (already observed in $CDCl_3$) and $^9H_8$, at the expense of the other one. The same equilibrium must be considered for 10*S*-acetoxydaunomycinone (**11**) in DMSO; with the *J* values reported by Malatesta *et al.*,[23] we obtained the following populations: 44% S_9, 38% $^8H_9$, and 17% of $^9H_8$.

(c) 7-epi *Stereoisomers.*—The flexibility of ring A is also expected to increase when the substituent at C-7 changes configuration, with respect to (**1**). We elected to study, as an example of what occurs in this case, 7-*epi*-4-demethoxydaunomycinone (**12**).[33,34] This compound shows $J_{7,8}$ values (Table 7) in $CDCl_3$ consistent with a preferred conformation intermediate between forms $^9H_8$ and ^9S or with a fast equilibrium (*ca.* 1:1) between them.† We are inclined towards the first hypothesis, because of the large values of $^4J_{8,10}$ and $^5J_{7,10}$ (Tables 6, 7), and because an increase in temperature to 70 °C did not induce any change in $CDCl_3$, whereas all coupling constants varied significantly in dioxane and in DMSO (Table 7). In the latter solvents, both $J_{7,8}$ and $J_{8,10}$ indicate that ring inversion occurs and leads to a complex equilibrium. Calculations for systems of two and also three conformers did not fit the experimental results, especially for DMSO, but a good fit was obtained, at five different temperatures, if we consider the following equilibrium: $^9H_8 \rightleftarrows {}^9S \rightleftarrows S_9 \rightleftarrows {}^8H_9$.

The results are given in Table 7. Variable-temperature measurements showed an increasing population, with temperature, of the ^9S conformer from *ca.* 9 to *ca.* 45%, at the expense of S_9, while the others remain approximately constant. An interpretation of these data is not easy. The preference in $CDCl_3$ for the skew form ^9S must be the result of a compromise between the opposite effects of the *peri* O(7)–OH(6) interaction, and of a certain degree of 7–OH · · · O(6) hydrogen-bonding (see chemical-shift values, Table 4), which prevents ring inversion in $CDCl_3$. Breaking of the hydrogen bond in DMSO and in dioxane should instead induce the ring inversion. However, the new structures, $^8H_9$ and S_9, do not seem to be energetically more favoured than the other ones, as the 1,3-diaxial interaction between 7–OH and 9–COMe is not compensated by the

* Small couplings of *ca.* 0.5 Hz for the other couple H(8,10) are not detectable, because of the lower resolution in these solvents.

† For a pure $^9H_8$ structure[34] we should have expected $J_{7,8}$ values of 10.9 and 5.6 Hz, corresponding to θ = 50° and θ' = 170°.

formation of the intramolecular hydrogen bond. Actually they appear the most populated in DMSO at room temperature, whereas at 100 °C the conformer preferred in $CDCl_3$, ^9S, becomes relatively more stable. This could be tentatively explained by the formation of an hydrogen bond between DMSO and 9-OH, because the equatorial hydroxy, in the S_9 and $^8H_9$ conformers, is more accessible to interactions with the solvent. At high temperature this intermolecular interaction breaks and the population of conformer ^9S, which is probably thermodynamically less stable, increases. Dioxane reflects, for this compound also, an intermediate position between $CDCl_3$ and DMSO solution, and probably it is just the similar energy of the four conformers that leads to the complex equilibrium observed experimentally.

(d) *6-Deoxy and 11-Deoxy Analogues.*[19,20]—The absence of the hydroxy at C-11 does not affect, as expected, the conformation of ring A. $J_{7,8}$ Values of (**15**) are the same as for daunomycinone (**8**). For the 6-deoxy isomer (**14**) also, the shape of ring A remains constant in $CDCl_3$, whereas in DMSO solution the stability of the half-chair $^9H_8$ decreases. Calculations performed with the values of $J_{7,8}$ reported by Penco *et al.*[20] for (**14**) and (**16**) are in agreement with a fast equilibrium of three conformers $^9H_8 \rightleftarrows {}^8H_9 \rightleftarrows S_9$. The population of the $^9H_8$ form is reduced to *ca.* 10% for both compounds, while the population of the other conformers varies between 75—50 ($^8H_9$) and 15—40% (S_9), respectively. On the other hand, an increase in the size of the group at C–6, as for the 6-methoxy derivative (**13**), does not induce any further distortion on ring A, with respect to (**8**), because the methoxy group can easily assume orientations (out of the aromatic plane), such as not to interfere with the C–7 substituent. The four-bond couplings $J_{8,10}$ reported[20] for these compounds (smaller than expected), could not be used to optimize the results obtained from $J_{7,8}$, because they were not corrected[20] for line-broadening effects. Data in water were not reported for 4-demethoxy-6-deoxydaunorubicin; it should be interesting to know whether the half-chair form $^9H_8$ is still preferred as in $CDCl_3$.

(e) *Aqueous Solution.*—The experiments in D_2O gave unexpected results, because they appeared to be more similar to those in chloroform than in DMSO solution. Daunomycin (**1**) and adriamycin (**2**) showed the same long-range coupling ($J_{8,10}$ 2.0 Hz) as in $CDCl_3$, and $J_{7,8}$ values gave rotational angles in good agreement ($\theta + \theta' = 110°$) with a single conformation. The preferred conformer is a half-chair $^9H_8$ here also, with a slight deformation of θ' angle. This deformation, also suggested by the small negative increment of the geminal coupling[32] (J_{8ax8eq}) in D_2O *versus* DMSO, might be a consequence of possible hydrogen-bonding between water and the carbonyl group at C-9. 10*R*-Methoxydaunomycin (**3**) showed a constant shape of ring A in all the solvents examined, whereas the 10*S* isomer (**4**) appeared in large amount (90%) in the S_9 conformation. The small population (*ca.* 10%) of the half-chair $^9H_8$, calculated from $J_{7,8}$ values, is in fact in agreement with the decrease of $J_{8,10}$ with respect to DMSO solution.

These substances are known to exist as aggregates in aqueous solution. Although coupling-constant values were obtained from diluted solution (8.6×10^{-3}M) in order to avoid broadening, association is still present. Dilution measurements, performed for (**1**) down to 2.8×10^{-5}M, indicate a constant shape of the signals. Also an increase in temperature up to 60 °C, for 10^{-3} and 10^{-2}M solutions of (**1**) and (**2**), did not lead to any conformational change. On the other hand, the similar results obtained in $CDCl_3$ confirm that aggregation does not affect the conformational properties of ring A. Dilution and variable-temperature experiments were performed in $CDCl_3$ for (**1**) and (**5**) and show that coupling constants at the monomer concentration (10^{-3}M) are the same as those given in Table 4. Therefore, the preferred conformation here reported must be referred to the monomeric species.

These results disagree completely with those published by Kollman *et al.*[1,17] They have deduced, from the splitting of H-7 and H-8, that the preferred conformation of ring A for daunomycin in D_2O and in pyridine is the half-chair $^8H_9$, and that this conformation should be maintained upon binding to dinucleotides. Probably these authors did not consider that the spectra may be second order and that, even for first-order spectra, errors in the determination of small couplings (1—3 Hz) may be large, when an appropriate correction for line broadening is not performed. This could explain the discrepancy in $J_{7,8}$ values. However it is not possible to deduce, even from their $J_{7,8}$ values, on the basis of Karplus–Altona equation,[25] any conformational equilibrium where conformer $^8H_9$ prevails. Therefore we think that the experimental results presented[17] are insufficient and anyhow inconsistent with their conclusions.

(f) *Comparison with X-Ray Studies.*[3–7]—The conformation of ring A in the solid state is a slightly flattened half-chair $^9H_8$ as also appears from the rotational angles θ and θ' that we have obtained from *X*-ray atomic co-ordinates[3–5] and reported in Table 6. The analyses of daunomycin in the protonated form,[4,5] showed that C-8 deviates most significantly from the mean plane of the other five atoms (+0.58 Å), as in carminomycin.[2] On the other hand, for the *N*-bromoacetyl derivative,[3] the out-of-plane atom is C-9 (+0.7 Å). The shape of ring A thus appears as an half-chair $^9H_8$ conformation slightly distorted toward the skew forms: S_8 for daunomycin and carminomycin, and ^9S in the case of the *N*-bromoacetyl derivative. This is not surprising, if we consider the packing of the molecules in the crystals; however it is interesting to observe that in the hexanucleotide–daunomycin complex[7] ring A seems to be a less distorted half-chair (C-9 is 0.3 Å removed from the plane of the unsaturated ring system) and probably similar to the conformation in solution ($CDCl_3$ or D_2O).

Discussion

It is now possible to examine the factors which contribute to stabilize the various conformers. For the half-chair $^9H_8$, the preferred conformation of the antimitotic substances (**1**)—(**3**) and of most daunomycinones, we can recognize two stabilizing factors: (a) the intramolecular hydrogen-bonding between 9-OH and O(7); (b) the relatively low steric interactions between substituents at C-7,10 and the *peri* hydroxy groups of ring B.

Other intramolecular hydrogen-bonding such as 6-OH ••• O(5′) and 9-OH ••• O(1′) have been postulated.[5,10] We can exclude that both these hydrogen bonds, involving the sugar ring oxygen, occur in solution, because they imply preferred orientations of the sugar moiety, which are not consistent with the results of n.O.e. experiments (see following paper).

(a) *Evidence for the Intramolecular Hydrogen Bond involving* 9-OH.—This was obtained by dilution-shift experiments performed in $CDCl_3$ for compounds (**1**) (free base), (**5**), and (**7**)—(**10**). The most significant result, because of the large range of concentrations available, comes from trimethoxydaunomycinone (**7**), which shows a constant value for 9-OH signal from 3×10^{-1} to 4×10^{-3}M. The absence of dilution effects was also observed for (**1**) from 5.4×10^{-2} to 5×10^{-4}M, while (**5**) in the same concentration range shows a small upfield shift of 0.06 p.p.m. In the latter case, comparison with 4′-OH and NH signals is however significant, as they move upfield by *ca.* 0.5 p.p.m. The low value of the shift for 4′-OH (δ 1.92) and the

dilution experiments indicate that this proton, as the NH_2 group, is not involved in intramolecular hydrogen-bonding. Daunomycinones (**8**)—(**10**) display small dilution shifts for both 9- and 7-OH: actually the proton at O(7) might interfere in the interaction with 9-OH; in addition the existence for (**10**) of a dynamic equilibrium, with 50% of form S_9 where 9-OH is pseudoequatorial, should decrease the strength of the intramolecular hydrogen bond. This appears more clearly, if one compares coupling constant and shift values of 9- and 7-OH, for compounds (**8**)—(**10**) and (**12**) (Tables 2, 4, and 7).

A four-bond coupling of 0.8 Hz between 9-OH and one proton at C-8 was detected for (**10**) and indicates that bonds connecting these protons are approximately coplanar;[28] a **W** geometry between 9-OH and H(8ax) can be reached for conformer $^9H_8$, when the O(9)–H(9) bond is oriented toward the *cis*-10-methoxy group. The orientation is opposite to that required to form the hydrogen bond with O(7). This coupling is also present in 7-*epi* derivative (**12**), but absent in (**8**) and (**9**), which instead shows a 1 Hz interaction between 7-OH and H(8ax): the **W** geometry can be reached here when 7-OH is preferentially oriented toward the aromatic ring, thus allowing the possibility of a hydrogen bond with O(6). The upfield shift of 9-OH with the concomitant low-field shift of 7-OH (both *ca.* δ 0.5) observed for (**10**) and (**12**), with respect to (**8**) and (**9**), indicate, for the former ones, a weaker interaction 9-OH ••• O(7), and some degree of hydrogen-bonding involving 7-OH. This latter, when 7-OH is equatorial or in the skew forms, might interact with O(6) of the *peri* hydroxy group. The same trend observed for (**10**) and for 7-*epi* derivative (**12**), in $CDCl_3$, confirms the above results, which lead to the conclusion that intramolecular hydrogen bond 9-OH ••• O(7) occurs in (**1**), (**5**), (**7**) and, to a lesser extent, also in (**8**) and (**9**). In the case of (**10**) and (**12**) this hydrogen bond appears to be absent, in agreement with the preferred conformations deduced for all these compounds in the preceding paragraph. DMSO partially breaks the 9-OH ••• O(7) hydrogen bond, and also interferes in the strong chelation of the phenolic hydroxy groups, as it appears from the broadening of the proton signals of 6,11-OH in this solvent. 9-OH does not broaden, but shifts 1 p.p.m. to low field for all compounds. As a consequence conformer $^9H_8$ loses stability in DMSO, and ring inversion should become easier, thus leading to some contribution of the S_9 form. This is actually what occurs for daunomycinones (**7**), (**8**), and especially for antibiotics (**1**), (**2**), and *N*-acetyldaunomycin (**5**). Dioxane interacts with both phenolic and alcoholic hydroxys groups, but is not as strong as DMSO in breaking the hydrogen-bonding; in addition, its larger dimensions compared with DMSO probably do not allow, through steric hindrance, much interference with 9-OH. In fact, the preferred conformations in dioxane are approximately the same as in $CDCl_3$. Therefore, not surprisingly, even in D_2O the shape of ring A is very similar to that in chloroform. Water is a weaker donor than dioxane and probably the intramolecular hydrogen bond 9-OH ••• O(7) still exists in aqueous solution.

Preliminary results for 9-deoxydaunorubicin showed that the conformation of ring A in $CDCl_3$ is the same $^9H_8$ form observed for daunomycin (**1**). Actually, with the absence of the 9-OH group, the 1,3-diaxial interaction between O(7) and O(9) vanishes, and thus the equatorial orientation of 9-COMe favours the half-chair $^9H_8$ conformation. For daunomycin, the increase of energy due to the 1,3-diaxal interaction is largely compensated by intramolecular hydrogen-bonding.

(b) *Interaction between* peri *Substituents.*—This can be studied by considering 10-methoxy stereoisomers and 6-deoxy- and 11-deoxy-daunomycin analogues. Dreiding model examination shows that the steric hindrance is more important when the substituents on ring A are equatorial. Experimentally this is proved by the preferred axial orientation of the 10-methoxy group, in both 10*R* and 10*S* isomers. It is significant that the constant shape of ring A is observed in all solvents for 10*R* compounds (**3**) and (**9**). The axial preference at C-10, in the case of 10*S* isomers (**4**) and (**10**), is strong enough to break the intramolecular hydrogen bond 9-OH ••• O(7), leading to ring inversion. The resulting skew conformation S_9 shows an acceptable *peri* interaction for the 7-substituent (ψ 60°).

The high stability of the half-chair $^9H_8$ for daunomycin, adriamycin, and related aglycones could thus be explained by the axial preference of the oxygen substituent at C-7, which decreases the *peri* interaction. The axial orientation of C-7 substituent allows even the presence of a bulky group at C-6 as in the 6-methoxy derivative (**16**), which shows the same conformation as daunomycinone (**8**). However, the *peri* interaction does not appear to be the determining factor for the stability of form $^9H_8$, as shown by the results for 6-deoxydaunorubicin analogues. Although for these compounds steric hindrance vanishes, the preferred conformation in $CDCl_3$ is still the same as that of daunomycinone. Only a strong donor solvent like DMSO, so strong as to break the 9-OH ••• O(7) hydrogen bond, can lead to ring inversion. When this hydrogen bond is structurally impossible, as in 7-*epi* derivatives, or is broken by the solvent, the $^9H_8$ form loses stability. The hypothesis of an hydrogen bond between 6-OH and the oxygen of the sugar ring, proposed[19] for daunomycin on the basis of Nakata calculations,[10] has insufficient experimental evidence, and is not consistent both with the results above reported and with the conformational preference of the sugar moiety. The shift variations of 7- and 1′-H, observed[19] for 4-demethoxy-*N*-trifluoroacetyldoxorubicin with respect to 6-deoxy analogues, are easily explained by a steric deshielding effect of the 6-OH group. This effect also remains for 7-H in DMSO, but not for 1′-H. The different trend in DMSO could be interpreted by the strong interactions of this solvent with the chelated hydroxys, which might induce small conformational change at the glycosidic bond, moving 1′-H out of the influence of 6-OH. However, the presence in DMSO of different conformers for ring A makes difficult an interpretation of chemical-shift values in this solvent.

(c) *The Other Conformations of Ring A.*—The other half-chair conformation of daunomycin $^8H_9$ has been predicted[11] to be quite stable, even if somewhat lower in energy than the $^9H_8$ form. The present results show that this prediction is unfulfilled. Conformation $^8H_9$ was never found experimentally for compounds (**1**)—(**9**), either as preferred conformer, or even as a minor population of a conformational equilibrium. We interpret these results as a loss of stability of form $^8H_9$, due to the absence of the intramolecular hydrogen bond 9-OH ••• O(7), and because of the unfavourable interactions between the equatorial O(7) substituents and the *peri* 6-OH group. Actually, only the 7-*epi* derivative (**12**) and 6-deoxy analogues (**14**) and (**15**) show the presence of this conformer in DMSO, with a population of at least 50%. In contrast, a conformation which appears frequently is the skew form S_9. It is the predominant conformer in the case of 10*S*-methoxydaunomycin (**4**) and of aglycone (**10**). This structure has the advantage, with respect to $^8H_9$, that the interactions between *peri* substituents become acceptable (Table 6). When there is a hydroxy at C-7, the interaction with the *peri* 6-OH group may even be favoured in $CDCl_3$ solution, because of the possibility of an intramolecular hydrogen bond 7-OH ••• O(6), as probably occurs for (**10**).

Conclusions.—The high stability of the half-chair conformation, $^9H_8$, for ring A of daunomycin, adriamycin, and related aglycones was proved to be due to two stabilizing factors: the

intramolecular 9-OH · · · O(7) hydrogen bond and the relatively low steric interaction between C-7 substituents and the *peri*-hydroxy group of ring B. Strong donor solvents such as DMSO, which partially break the hydrogen bond, decrease the stability of form $^9H_8$. As a consequence some contribution of the skew conformation S_9 appears [*ca.* 25% for (**1**), (**2**), (**5**), *ca.* 10% for (**7**) and (**8**)], whereas the half-chair $^8H_9$ was never found for these compounds, either as preferred conformer, or even as a minor population of the conformational equilibrium. The position of the equilibrium, which is fast on the n.m.r. time scale, does not change appreciably with temperature. Of the solvents, chloroform and water surprisingly show the same effect: the compounds exist as 'pure' half-chair $^9H_8$; dioxane and pyridine are intermediate between $CDCl_3$ and DMSO, dioxane being close to the former one.

The flexibility of ring A increases when the C-7 substituent changes configuration (**12**) with respect to daunomycin, when a substituent with the *S* configuration is placed on C-10 (**4**), (**10**), or when the 6-hydroxy is absent (**14**), (**16**). This proves the importance of the *peri* interactions. 10*R* Substituents (**3**), (**9**) do not affect the conformation of the ring, as they are axially oriented. For stereoisomers (**4**), (**10**) the axial preference of the 10-methoxy group is responsible for the ring inversion. 10*S*-Methoxydaunomycin (**4**) is always (D_2O, DMSO) a skew form S_9, its aglycone (**10**) is an equilibrium mixture of $^9H_8$ and S_9 (*ca.* 1:1) in $CDCl_3$, while in the other solvents the S_9 conformer predominates (*ca.* 90%). The high flexibility of ring A is reflected in the complex conformational equilibrium $^9H_8 \rightleftharpoons S_9 \rightleftharpoons {}^8H_9$ we found for (**10**)—(**12**), (**14**), and (**16**) in dioxane and in DMSO, and in the sensitivity to temperature variations. For the 7-*epi* derivative (**12**), a fourth conformation, the skew ^9S, is present, with high population in $CDCl_3$ and depending on temperature in the other solvents. The 6-deoxy analogues (**14**)—(**16**) show a conformational preference in DMSO for the forms $^8H_9$ (75—50%) and S_9 (15—40%), while the half-chair $^9H_8$ is only present in *ca.* 10%. This latter form however is the only one present in $CDCl_3$, thus showing the importance of the hydrogen bond 9-OH · · · O(7). Other intramolecular hydrogen bonds, such as 6-OH · · · O(5′) and 9-OH · · · O(5′), suggested by other authors, have been proved not to exist in solution. The conformation of daunosamine moiety is constantly $^1C_4$ (L), for all compounds, at the temperatures and in the solvents examined.

Acknowledgements

This work was supported by C.N.R. Progetto Finalizzato Chimica Fine e Secondaria. All samples were kindly supplied by Farmitalia Carlo Erba, whose courtesy we acknowledge.

References

1 F. Arcamone, 'Doxorubicin Anticancer Antibiotics' in 'Medicinal Chemistry,' Academic Press, New York, 1981, vol. 17.
2 S. Neidle, 'The Molecular Basis for the Action of Some DNA-Binding Drugs' in 'Progress in Medicinal Chemistry,' eds. G. P. Ellis and G. B. West, Elsevier/North Holland Biomedical Press, 1970, vol. 16.
3 R. Angiuli, E. Foresti, L. Riva di Sanseverino, N. W. Isaacs, O. Kennard, W. D. S. Motherwell, D. L. Wampler, and F. Arcamone, *Nature New Biol.*, 1971, **234**, 78.
4 S. Neidle and G. Taylor, *Biochim. Biophys. Acta*, 1977, **479**, 450.
5 C. Courseille, B. Busetta, S. Geoffre, and M. Hospital, *Acta Crystallogr.*, 1979, **B35**, 764.
6 W. J. Pilgram, W. Fuller, and L. D. Hamilton, *Nature New Biol.*, 1972, **235**, 17.
7 G. J. Quigley, A. H. J. Wang, G. Ughetto, G. van der Marel, J. H. van Boom, and A. Rich, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 7204.
8 D. R. Phillips and G. C. K. Roberts, *Biochemistry*, 1980, **19**, 4795; D. J. Patel, S. A. Kozlowski, and J. A. Rice, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 3333; M. Spinelli and J. C. Dabrowiak, *Biochemistry*, 1982, **21**, 5862; H. Fritzsche, H. Triebel, J. B. Chaires, N. Dattagupta, and D. M. Crothers, *ibid.*, p. 3940; J. B. Chaires, N. Dattagupta, and D. M. Crothers, *ibid.*, p. 3933; J. M. Neumann, J. A. Cavailles, M. Herve, S. Tran-Dinh, B. Langlois d'Estaintot, T. Huynh-Dinh, and J. Igolen, *FEBS Lett.*, 1985, **182**, 360.
9 S. Neidle and G. L. Taylor, *FEBS Lett.*, 1979, **107**, 348.
10 Y. Nakata and A. J. Hopfinger, *FEBS Lett.*, 1980, **117**, 259.
11 S. C. Brown, P. A. Kollman, and P. K. Weiner, *Biochim. Biophys. Acta*, 1982, **717**, 49.
12 J. B. Chaires, N. Dattagupta, and D. M. Crothers, *Biochemistry*, 1982, **21**, 3927; I. J. Lennan, R. E. Lenkinski, and Y. Yanuka, *Can. J. Chem.*, 1985, **63**, 1233.
13 F. Arcamone, G. Cassinelli, G. Franceschi, P. Orezzi, and R. Mondelli, *Tetrahedron Lett.*, 1968, 3353; F. Arcamone, G. Cassinelli, G. Franceschi, R. Mondelli, P. Orezzi, and S. Penco, *Gazz. Chim. Ital.*, 1970, **100**, 949.
14 R. H. Iwamoto, P. Lim, and N. S. Bhacca, *Tetrahedron Lett.*, 1968, 3891.
15 R. Mondelli, A. Arnone, G. Fronza, G. Cassinelli, B. Gioia, and A. Vigevani, *J. Chem. Res. (S)*, 1984, 180.
16 D. W. Henry, *Am. Chem. Soc. Symp. Ser.*, 1976, **30**, 15; T. Momose and Y. Ozaki, *Chem. Pharm. Bull.*, 1976, **24**, 1631.
17 M. E. Nuss, T. L. James, M. A. Apple, and P. A. Kollman, *Biochim. Biophys. Acta*, 1980, **609**, 136.
18 J. W. Lown and H.-H. Chen, *Can. J. Chem.*, 1981, **59**, 3212.
19 S. Penco, F. Angelucci, F. Arcamone, M. Ballabio, G. Barchielli, G. Franceschi, A. Suarato, and E. Vanotti, *J. Org. Chem.*, 1983, **48**, 405.
20 S. Penco, F. Angelucci, M. Ballabio, G. Barchielli, A. Suarato, E. Vanotti, A. Vigevani, and F. Arcamone, *Heterocycles*, 1984, **21**, 21.
21 S. Pan and N. R. Bachur, *Mol. Pharmacol.*, 1980, **17**, 95; 1981, **19**, 184, and references quoted; H. H. Doroshow and J. Reeves, *Biochem. Pharm.*, 1980, **30**, 259.
22 D. L. Kleyer and T. H. Koch, *J. Am. Chem. Soc.*, 1983, **105**, 2504; 1984, **106**, 2380.
23 V. Malatesta, S. Penco, N. Sacchi, L. Valentini, A. Vigevani, and F. Arcamone, *Can. J. Chem.*, 1984, **62**, 2845.
25 C. A. G. Haasnoot, F. A. A. M. De Leeuw, and C. Altona, *Tetrahedron*, 1980, **36**, 2783 and references quoted.
24 A. Arnone, G. Fronza, R. Mondelli, and A. Vigevani, *Tetrahedron Lett.*, 1976, 3349.
26 R. J. Abraham, 'The Analysis of High Resolution NMR Spectra,' Elsevier, Amsterdam, 1971.
27 J. C. P. Schwarz, *J. Chem. Soc., Chem. Commun.*, 1973, 505.
28 M. Barfield and S. Sternhell, *J. Am. Chem. Soc.*, 1972, **94**, 1905; M. Barfield, A. M. Dean, C. J. Fallick, R. J. Spear, S. Sternhell, and P. W. Westerman, *ibid.*, 1975, **97**, 1482.
29 S. Penco, F. Gozzi, A. Vigevani, M. Ballabio, and F. Arcamone, *Heterocycles*, 1979, **13**, 281.
30 R. Bucourt and D. Hainaut, *Bull. Soc. Chim. Fr.*, 1967, 4564.
31 M. Barfield and M. D. Johnston, Jr., *Chem. Rev.*, 1973, **73**, 53.
32 G. E. Maciel, J. W. McIver, Jr., N. S. Ostlund, and J. A. Pople, *J. Am. Chem. Soc.*, 1970, **92**, 4151.
33 K. Krohn and K. Tolkiehn, *Chem. Ber.*, 1979, **112**, 3453.
34 V. Prikrylova, P. Sedmera, J. V. Jizba, J. Vokoun, H. Lipavska, M. Podojil, and Z. Vanek, *Collect. Czech. Chem. Commun.*, 1984, **49**, 313.

Received 13*th December* 1985; *Paper* 5/2192