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Nucleic Acid Binding Drugs. VII. Molecular-Mechanics Studies on the Conformational Properties of the Anti-Cancer Drug Daunomycin: Some Observations on the Use of Differing Potential-Energy Functions

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Abstract

The conformation of the anti-cancer drug daunomycin has been investigated in detail by potential-energy calculations. The flexibility around the ether linkage, connecting the anthracycline chromophore and the amino sugar group, has been evaluated using several types of potential-energy function. The results largely support the hypothesis that the crystallographically observed conformation is the most stable one, although considerable detailed variation with respect to potential function was found.

Introduction

We have previously reported (Neidle & Taylor, 1979) the results of a conformational analysis on the antileukaemic antibiotic daunomycin (Fig. 1), using empirical force-field methods of calculating non-bonded energies. These results indicated that the minimum-energy conformation corresponds to that observed in the crystal structures of daunomycin itself [as a pyridine adduct (Neidle & Taylor, 1977) and a butanol adduct (Courseille, Busetta, Geoffre & Hospital, 1979)], of *N*-bromoacetyldaunomycin (Angiuli *et al.*, 1971), and of 4-hydroxydaunomycin (carminomycin) (Wani, Taylor, Wall, McPhail & Onan, 1975; Pettit *et al.*, 1975; Von Dreele & Einck, 1977). The conformational analysis was performed by examining the relative dispositions of the sugar group and anthracycline chromophore, by varying the two bond torsion angles φ_1 and φ_2 and maintaining the rest of the molecular structure in the crystal geometry (Neidle & Taylor, 1977).

A recent analysis of the closely related antibiotic adriamycin (also termed doxorubicin) has cast some doubt on these findings with the location of a distinct global minimum-energy conformation (Nakata & Hopfinger, 1980). This, it is suggested, is stabilized by an intramolecular hydrogen bond between H(6) and O(5)′ (Fig. 1).

In an effort to resolve this conflict, we have systematically analysed the conformational and hydrogen-bonding energies, using a number of distinct approaches. We find that precise calculated conformations and their relative associated energies depend to a considerable extent on the particular energy formalism employed. However, in no case can we reduce the minimum-energy region found (Nakata & Hopfinger, 1980) to a global minimum stabilized by an intramolecular hydrogen bond between H(6) and O(5)′ (Fig. 1), since this would require the breaking of an already existing strong hydrogen bond [O(5)···H(6)–O(6)]. Throughout our calculations we have employed fixed bond distance and angle geometry, since the several precise crystallographic studies indicate that the bonding geometry does not vary in diverse crystallographic environments. In contrast, Nakata & Hopfinger (1980) have optimized a doxorubicin geometry which was derived from *N*-bromoacetyldaunomycin (Angiuli *et al.*, 1971) although the final geometry that they obtained has not been stated.

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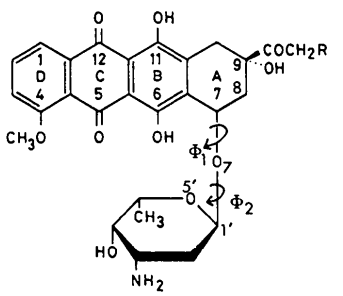

Fig. 1. Molecular structure of daunomycin (R = H). Adriamycin has R = OH. Torsion angles φ_1 and φ_2 are defined by C(8)–C(7)–O(7)–C(1)′ and C(7)–O(7)–C(1)′–O(5)′ respectively.

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We have deliberately used various types of commonly used functions and associated constants, so as to represent a broad spectrum of analysis.

A recent complete optimization of daunomycin is in substantial agreement with our results (Brown, Kollman & Weiner, 1982).

Methods

The semi-empirical energy of a particular conformation was calculated as the sum of terms representing non-bonded (N-B), torsional (TORS), electrostatic (E-S) and hydrogen-bonding potential energies (H-B):

$$V_{TOT} = V_{N\text{-}B} + V_{TORS} + V_{E\text{-}S} + V_{H\text{-}B}$$

where

$$V_{TORS} = \frac{V_o}{2}(1 \pm \cos n\varphi)$$

$$V_{E\text{-}S} = \frac{q_1 q_2}{\varepsilon r}$$

$$V_{N\text{-}B} = \frac{-A}{r^6} + \frac{B}{r^{12}}$$

or

$$= \frac{-A}{r^6} + B \exp Cr$$

$$V_{H\text{-}B} = \frac{A_H}{r^{12}} - \frac{B_H}{r^{10}}.$$

Table 1 details values used for the constants in these experiments. A value of unity for the dielectric constant (ε) was employed for the results presented here. Generally, although the total electrostatic contribution was reduced on using a distance-dependent function for ε (Table 1), no significant differences were obtained either in the positions of the minima in the maps or in their general shape. Since we were only interested in the relative energies of the molecule with respect to the crystal conformation, $\varepsilon = 1$ was chosen for convenience.

Individual atomic partial charges were taken from the study of Nakata & Hopfinger (1980), and were also calculated for specific fragments of the daunomycin molecule using the *GAUSSIAN* 76 *ab initio* quantum-chemistry programs (Pople, Binkley, Whiteside, Hariharar & Seeger, 1978) (Table 2). When studying hydrogen bonding the minimal STO-3G base was used, and the bond lengths and angles were constrained to their crystal values. Fragmentation of a molecule of the size of daunomycin is essential because of the severe limitation in the number of atomic nuclei that can be currently simultaneously considered in the *ab initio* approach, on the computer systems available to us. It is well known that results obtained by these methods show dependence on the nature of the base set used. Since, however, the overall energy of a hydrogen bond has been found (Iwata & Morokuma, 1973) to be lower for the STO-3G base set than for more extended ones, it is felt that the minimal set was adequate for the present purpose, to give an upper limit to the hydrogen-bond energy and for comparison with the empirical energy calculated. The *ab initio* method was also employed to calculate the total potential energy of a particular conformation of the daunomycin fragment. The fragment considered (Fig. 2) was taken by us to be a representative model for the intramolecular hydrogen bonding in daunomycin and anthraquinones.

Ab initio calculations were performed on the University of London CDC 7600 computer. Conformational energies and flexibilities were calculated on a

Table 1. *Definition of parameters and atom types used in non-bonded potentials*

(*a*) Parameters for 6-12 potential
Parameter set *A*
α: polarizability ($\times 10^{24}$ cm^3)
N: effective numbers of electrons
R: van der Waals radii (Å)

Type	Species	α	*N*	*R*
1	H	0·42	0·85	1·20
2	C (sp^2)	1·30	5·2	1·70
3	C (sp^3)	5·2	1·70	1·70
4	CH (aliphatic)	1·35	6·0	1·95
5	CH_2 (aliphatic)	1·77	7·0	1·95
6	CH_3 (aliphatic)	2·17	8·0	1·95
7	CH (aromatic)	2·07	6·0	1·90
8	N (sp^2-amide)	1·15	6·0	1·55
9	N (sp^3)	0·87	6·0	1·55
10	N (sp^3)	0·87	9·0	1·75
11	N^+ (imidazole)	2·03	6·0	1·65
12	O (sp)	0·85	7·0	1·52
13	O (sp^2)	0·59	7·0	1·52
14	O^- (carboxyl)	2·14	7·0	1·60
15	S (single bonds)	0·34	16·0	1·80
16	S (single bonds)	0·50	14·8	1·80
17	P	3·45	14·2	1·80

Working 6-12 formula

$$V = \frac{3}{2}\frac{eh}{\sqrt{m}}.A.\{|1{\cdot}0 - (R^6/D^6)|/D^6\}$$

$$A = \alpha_1.\alpha_2/|(\alpha_1/N_1)^{1/2} + (\alpha_1/N_2)^{1/2}|$$
$$R = R_1 + R_2$$

References

Atom type	Variable	Reference
3–9	α	Olson (1973)
16	α	Lindeberg & Wägner (1977)
17	α	Thornton & Bayley (1975)
14	α = 1·47	

All other α's from Gibson & Scheraga (1967)
All *N*'s from Scott & Scheraga (1965)
All *R*'s from Bondi (1964)

Parameter set *B*
Parameters taken from Stuper, Dyott & Zander (1979)

Table 1 (*cont.*)

(*b*) Parameters for 6-exp potential

Atom pair	A (kJ mol^{-1} Å^6)	B (kJ mol^{-1})	C (Å^{-1})
H–H	79·72	6876·0	3·76
H–C	504·08	47278·0	3·67
H–O	504·08	47278·0	3·60
H–N	504·08	47278·0	3·60
H–P	1604·53	14505·2	3·60
C–C	3125·36	386820·0	3·60
C–N	3125·36	386820·0	3·60
C–O	3125·36	205186·4	3·60
C–P	10044·00	1277969·3	3·60
N–N	3125·36	386820·0	3·60
N–O	3125·36	205186·4	3·60
N–P	10044·00	1277969·3	3·60
O–O	3125·36	176422·9	3·60
O–P	10044·00	1074599·2	3·60

Working formula
$V = -A(D^{-6}) + B\exp[-(CD)]$
Nuss, Marsh & Kollman (1979)

Parameters for torsion potential

Group	V_0 (kJ mol^{-1})	Sign	N
–C–O	4·2	+	3

Hopfinger (1973)

(*c*) Parameters for hydrogen bonding

A_H (kJ mol^{-1} Å^{12})	B_H (kJ mol^{-1} Å^{10})	R_{min}(O–O) (Å)	U_{min} (kJ mol^{-1})
50387	16799	1·9	−4·65
55845	24202	1·66	−24·78
54543	19293	1·84	−7·41

Scheraga (1974)

U_{min} is the minimum energy for the hydrogen bond defined by the minimum R_{min}(O–O) distance.

Dielectric constant
$\varepsilon = 1$ and also simple distance-dependent linear functions were tried (see Hopfinger, 1973); these are of the form:

$\varepsilon = 1$ for $r_{ij} < 3$ Å
$\varepsilon = 0{\cdot}75.\ r_{ij} - 1{\cdot}25$ for 3 Å $< r_{ij} <$ 7 Å
$\varepsilon = 4$ for $r_{ij} > 7$ Å.

All maps shown are for $\varepsilon = 1$.

PDP 11/34 computer with an interactive graphics facility.

Results and discussion

Calculations, using the Nakata & Hopfinger (1980) partial charges (obtained by CNDO/2 methods), were performed on all the daunomycin analogues examined crystallographically. Our own partial-charge calculations on carminomycin fragments gave very similar results to these, apart from differences in the region of the hydroxyl protons, which are particularly important for hydrogen-bond potentials. Since the model used by Nakata & Hopfinger for hydrogen bonding is an electrostatic one, use of different partial charges would have made a significant difference to their results. In order to preserve standardization as far as possible with their results the Nakata & Hopfinger partial charges were used, except when we specifically considered hydrogen bonding. For any one type of calculation, the results were broadly similar for all derivatives. We have concentrated on discussing those for carminomycin since its crystallographic analysis is the most accurate of the series, with H-atom positions being located and hence hydrogen-bonding geometry being determined. By contrast, the crystallographic analysis of *N*-bromoacetyldaunomycin (Angiuli *et al.*, 1971), which was used as the basis of the calculations on adriamycin (Nakata & Hopfinger, 1980), is of an order of magnitude less in precision, and therefore far less reliable.

Figs. 3–6 and Table 3 show the results of calculations on carminomycin using differing potential terms. In general, hydrogen-bond energies have been excluded from these scans. All three conformational maps show the same relatively broad minimum at $\varphi_1 \simeq 90°$, $\varphi_2 \simeq 290°$, which is the area of the crystallographically observed conformations, as well as being in agreement with our earlier results (Neidle & Taylor,

Table 2. *Individual atomic partial charges*

(*a*) Results of partial-charge calculations using *GAUSSIAN* 76, at various τ_2 values, for several atom species of the carminomycin fragment in Fig. 2.

τ_2 (°)	O(5)···H(6) distance (Å)	H(6)	O(6)	O(5)	C(6)
5	3·60	0·183	−0·244	−0·186	0·143
50	3·32	0·178	−0·255	−0·182	0·134
90	2·77	0·175	−0·263	−0·185	0·127
120	2·26	0·188	−0·263	−0·201	0·133
140	1·94	0·208	−0·272	−0·218	0·140
154	1·76	0·226	−0·286	−0·232	0·146
164	1·67	0·238	−0·297	−0·240	0·150
170	1·67	0·240	−0·301	−0·242	0·151
180	1·63	0·244	−0·304	−0·244	0·152
200	1·73	0·230	−0·289	−0·235	0·147

(*b*) Results of Nakata & Hopfinger (1980)

τ_2 (°)	H(6)	O(6)	O(5)	C(6)
53	0·150	−0·265	−0·250	0·175

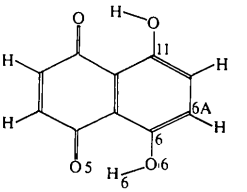

Fig. 2. Fragment used to model hydrogen bonding for *ab initio* calculation. The torsion angle τ_2 is defined by the atom sequence C(6*A*)–C(6)–O(6)–H(6).

Fig. 3. Conformational map [using the 6-12 potential function and parameter set *B* (Table 1)] for carminomycin. Angles φ_1 and φ_2 are defined in Fig. 1. The τ_1 angle is at its crystallographic position of 18°.

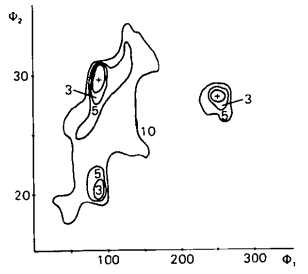

Fig. 4. Conformational map (using the 6-12 potential function and parameter set *B*), with $\tau = 180°$.

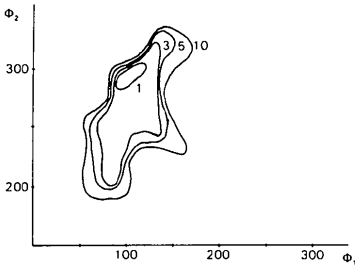

Fig. 5. Conformational map [using the 6-12 potential function and parameter set *A* (Table 1)] for carminomycin.

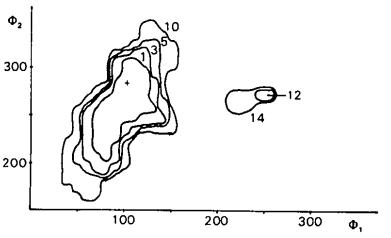

Fig. 6. Conformational map (using the 6-exp potential function) for carminomycin.

1979). However, other features of the maps are significantly distinct from one to the other. The subsidiary minimum at $\varphi_1 \simeq 90°$, $\varphi_2 \simeq 200°$ has a barrier of ~12·6 kJ mol^{-1} between it and the global minimum, in Fig. 3. In contrast, the other maps with different terms (Figs. 5 and 6), show a much broader global minimum, with the subsidiary one at most about 4·2 kJ mol^{-1} above it. The minimum at $\varphi_1 \simeq 240°$, $\varphi_2 \simeq 250°$, is in the region of the Nakata & Hopfinger global minimum.

The barrier at the (257°, 257°) position (Nakata & Hopfinger, 1980) arises from close contacts developing between the daunosamine sugar atoms HC(1)′, C(1)′ and C(2)′ and the O(9) hydroxyl group (Fig. 1). Hence the relative height of this barrier is highly dependent upon the disposition of the C(9) hydroxyl group. (φ_1,φ_2) conformation scans were carried out as a function of the C(8)–C(9)–O(9)–H(9) torsion angle (τ_1) at a number of discrete τ_1 values distinct from the crystallographic position of $\tau_1 = 18°$. The O(9)–H(9)···O(7) interaction was included in these calculations. Again the results were highly dependent upon the form of the potential function used. The energy of the hydrogen bond was obtained using the expression $V_{\text{H-B}}$ given above. The results overall indicated a retention of the features of the (φ_1,φ_2) scan when τ_1 was in the crystallographically observed position, in that the global minimum is always at the (φ_1,φ_2) crystallographic position regardless of the

Table 3. *Features of the conformational maps*

Compound	Non-bonded function used	Position of principal minimum φ_1, φ_2 (°)	Relative energy at 90°, 290° (crystallographic minimum) in kJ mol^{-1}	Relative energy at (260°, 260°) in kJ mol^{-1}	Other minima (°) and their relative energies kJ mol^{-1}
Carminomycin	'6-12' parameter set *A* (Fig. 5)	100, 290	0	>84	
	'6-12' parameter set *B* (Fig. 4)	100, 300	0	12·6	100°, 180°; 4·2
	'6-exp' (Fig. 6)	100, 280	0	50·4	
	'6-12' parameter set *B*, with $\tau = 53°$	100, 200	<2·1	16·8	
N-Bromoacetyldaunomycin	'6-12' parameter set *A*	90, 200	4·2	25·2	
	'6-12' parameter set *B*	90, 290	0	12·6	
	'6-exp'	80, 220	8·4	16·8	

Table 4. *The effect of variation in the τ_1 torsion angle on the energy of the minimum positions in the φ_1,φ_2 maps*

In all cases, the minimum was at $\varphi_1 \sim 100°$, $\varphi_2 \sim 290°$. The '6-12' parameter set *B* was used for these calculations.

τ_1 (°)	Relative energy (kJ mol^{-1})
18	12·6
30	4·2
90	5·5
120	12·6
180	6·3

values of τ_1 (Table 4). However, the energy at the position of the Nakata & Hopfinger minimum is reduced from 12·6 at $\tau_1 = 18°$ to ~6·3 kJ mol^{-1} (at $\tau_1 = 180°$, the Nakata & Hopfinger position) above the global minimum in Fig. 3. For the conditions used in Fig. 4, the barrier height at this minimum is reduced to ~42 kJ mol^{-1} above the global minimum for $\tau_1 = 180°$.

These results reinforce our view that starting with the observed carminomycin crystal conformation is the most valid approach. It is apparent that in no instance is this position less than 12·6 kJ mol^{-1} above the global minimum in energy, leading one to conclude that this minimum is not a global one, as judged by the various distinct calculations we have used. Moreover, this minimum varies significantly in energy, with a difference of 42 kJ mol^{-1} between Figs. 5 and 6. We are unable to judge which, if any, more nearly represent reality. This wide variation does, however, suggest that the quantitative results from any one empirical force-field calculation, at least on molecules of the present type, must be treated and used with some caution.

Energy calculations have also been performed with the O(6)–H(6) bond in the Nakata & Hopfinger position, with $\tau_2 = 53°$ (Table 3). Again, the minimum-energy position is at (90°, 290°), with an energy barely distinguished from that in the other carminomycin calculations.

An identical set of calculations has also been performed on the considerably less reliable molecular geometry of *N*-bromoacetyldaunomycin. Table 3 gives the results of these. In all three cases, the (257°, 257°) minimum is still above the global one, though now the former is only 12·6–25·2 kJ mol^{-1} greater in energy. Considerable differences between three conformational maps are observed in the region of the global minimum, which is actually shifted to (90°, 200°), although this is only about 8·4 kJ mol^{-1} below the energy at the (90°, 290°) position. We conclude that these calculations again broadly demonstrate a correspondence between the crystallographic and calculated minimum conformation, with the important proviso of inaccuracy in the starting-point geometry in this instance.

An important feature of the Nakata & Hopfinger global minimum molecular geometry, as compared to the crystallographic one, concerns its hydrogen bonding. In the former, it is stated that stabilization of this conformation is produced by an intramolecular hydrogen bond between the hydroxyl group at O(6) on the chromophore, and the sugar ring O atom O(5)′. We first note that this arrangement has not been observed experimentally in any of the crystal structures; in that of carminomycin (Von Dreele & Einck, 1977), where H-atom positions were observed, one intramolecular hydrogen bond is unequivocally between O(7) and O(9), with standard hydrogen-bonding geometry being observed [the O(7)···H(9)–O(9) angle is, for example, 141°]. Other intramolecular hydrogen bonding involves (*a*) carbonyl O(5) and phenolic H(6) and (*b*) carbonyl O(12) and phenolic H(11). Recent NMR data in solution fully support these assignments (Patel, Kozlowski & Rice, 1981). The alternative arrangement proposed (Nakata & Hopfinger, 1980) has an O(6)–H(6)···O(5)′ angle of about 120°. We consider this arrangement less plausible in the light of the accepted geometry of hydrogen bonding (Donohue, 1968). Furthermore, we have carried out extensive calculations on the various hydrogen-bonding possibilities (to be reported in detail elsewhere), which have further supported our rejection of the O(6)–H(6)···O(7) hydrogen bond. The results of *ab initio* calculations on the variation of energy with rotation of the H(6) atom around the C(6)–O(6) bond are shown in Figs. 7 and 8. These results reveal the presence of a pronounced energy minimum at the point of closest approach to the carbonyl atom O(5). It is reasonable to interpret this as corresponding to an O(6)–H(6)···O(5) intramolecular hydrogen bond, which is indeed observable

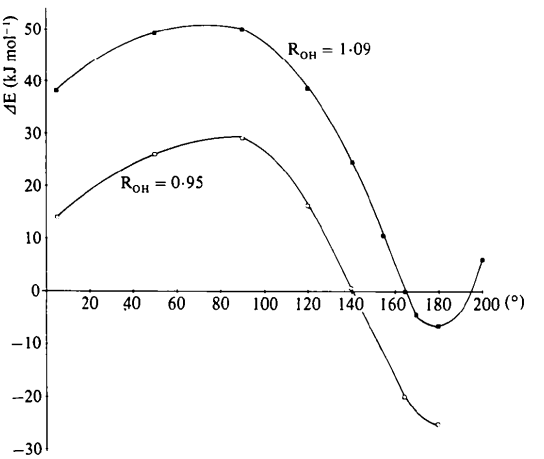

Fig. 7. Change in energy of fragment (Fig. 2) as a function of the torsion angle C(6*A*)–C(6)–O(6)–H(6) (τ_2) (Fig. 2) using *GAUSSIAN* 76. The energy at the crystal conformation has been set to zero. The change in energy shown both here and in Fig. 8, is the total of torsion and hydrogen-bond energy, and is not to be taken as representing only the latter.

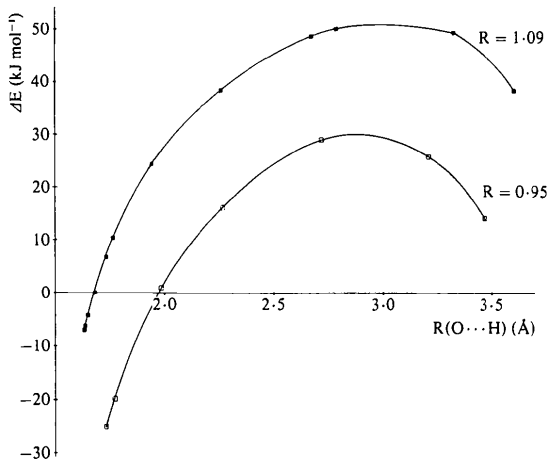

Fig. 8. Change in energy of the fragment (Fig. 2) as a function of the H(6)···O(5) distance (Fig. 2) calculated using *GAUSSIAN* 76. The energy at the crystal conformation has been set to zero.

in the crystal. The conformation of the O(6)–H(6) bond which could give rise to an O(6)–H(6)···O(5)′ hydrogen bond has $\tau_2 = 53°$ which is over 42 kJ mol^{-1} less favoured than the strong O(6)–H(6)···O(5) one. This destabilization at $\tau_2 = 53°$ has not been included in the calculation of the energies given in Table 3; its conclusion would tend to make the Nakata & Hopfinger minima of higher energy. Figs. 7 and 8 show the same general features for the O(6)–H(6) distance of 0·95 Å, compared to the perhaps more likely one of 1·09 Å. This difference in hydrogen-bond strengths, coupled with the lack of a global minimum in the region of the (260°, 260°) conformation (Table 3), further argues against the likelihood of this alternative being the stable form of the molecule.

Conclusions

It is apparent that for three different, commonly used empirical force-field energy-calculations methods, the minimum conformation found is close to that seen in the crystalline state in a variety of environments. This conformation has also now been observed when adriamycin is bound to a hexanucleotide system modelling DNA itself (Quigley, Wang, Ughetto, van der Marel, van Boom & Rich, 1980). It is further clear that quantitative interpretations of such calculations are not straightforward, and are markedly dependent on the formalism employed.

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