AN EXTENDED CONFORMATIONAL ANALYSIS OF DOXORUBICIN

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1. Introduction

The antibiotic doxorubicin (fig.1a) is a highly active anti-cancer agent in current clinical use [1]. It is believed that the inhibitory effect of this compound on both normal and tumour cell growth is due to its interference with RNA synthesis by binding directly to the double-stranded DNA template [2]. X-Ray fiber diffraction studies are believed to support a binding model in which intercalation between DNA base pairs, with consequent helix unwinding, plays an important role [3]. The crystallographic analysis of doxorubicin has not been determined. However, the crystal structures of daunomycin (fig.1b) as pyridine [4] and butanol [5] adducts, the *N*-bromoacetyl derivative of daunomycin [6] and 4-hydroxy-daunomycin (carminomycin) [7–9] have been determined by X-ray analysis.

Neidle and Taylor have reported in this yournal [10] an intramolecular conformational analysis of daunomycin and some corresponding congeners. Their work was prompted by the observation that the apparently flexible sugar–chromophore system, characterized by the bond rotations ϕ_1 and ϕ_2 defined in fig.2, adopts similar conformations in the crystals mentioned above. This preferred crystal conformation has been suggested to be the active biological conformation as well as a minimum energy conformation [4,11]. Neidel and Taylor have specifically calculated the conformational energy associated with rotations ϕ_1 and ϕ_2 using molecular mechanics [12]. The particular set of potential functions, e.g., atomic charge densities, used in these calculations are not given. The remainder

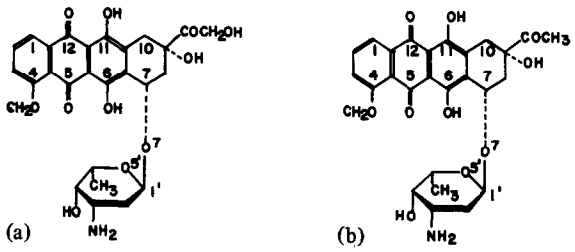

Fig.1. Chemical structure and ring-numbering of: (a) doxorubicin; (b) daunomycin.

of the molecule, that is all other bond rotations, bond lengths and angles, has been frozen into the crystal conformation of the daunomycin–pyridine structure. Only one low-conformational region in (ϕ_1, ϕ_2)-space is found in these studies. The minimum energy conformation in this region is close to those observed crystal structures. This is taken as evidence in support of the crystal conformation being the active conformation which fits a general DNA-binding model [4,13–15].

Constraining all bond rotation angles, other than ϕ_1 and ϕ_2, to the crystal structure values might significantly bias the results of the conformational analysis in favor of the crystal conformation. In particular, deletion of the 9-hydroxyl group gave rise to a second minimum energy conformation different from that corresponding to the crystal structure [10]. This new stable conformer state might also be realized with the 9-hydroxyl present by rotating this group about its C–O bond. Hence, we decided to perform a generalized conformational analysis. Doxorubicin was selected because of its biological importance, and it has not been considered in previous conformational analyses. However, as we show the intramolecular conformational preferences of daunomycin and doxorubicin are virtually identical.

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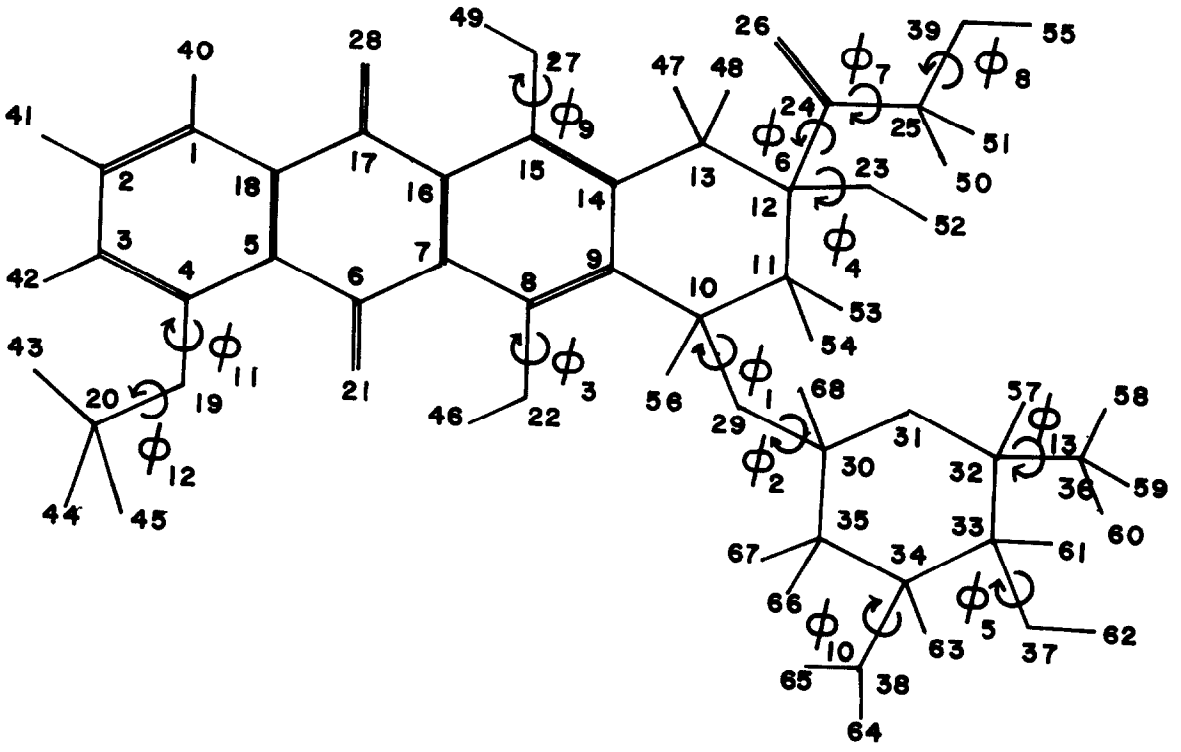

Fig.2. Arbitrary atom and torsional rotation angle numbering for doxorubicin.

2. Methods

The *N*-bromoacetyl-daunomycin crystal structure was used as the skeletal structure to determine the initial valence geometry of doxorubicin. The oxygen atom (O-39 in fig.2) and hydrogen atoms were located according to standard bond lengths and angles. The final valence geometry was determined using a consistent force field arrived at by variable metric energy minimization [16]. The crystal structure coordinates plus hydrogen and O-39 oxygen positions provided the starting point in the energy minimization. The residual charge densities used in this calculation were determined using the CNDO/2 method. In arriving at suitable CNDO/2 charges for doxorubicin, it was necessary to partition the molecule into smaller fragments whose charges were independently obtained and pieced together in order to yield a single consistent charge distribution for doxorubicin as a whole. The charge distributions for the neutral (NH_2) and charged (N^+H_3) forms of doxorubicin are given in fig.3.

The valence geometry was held fixed in all subsequent conformational analyses. In addition to bond rotations ϕ_1 and ϕ_2, bond rotations ϕ_3–ϕ_{12}, as defined in fig.2, were also considered as limited degrees of conformational freedom. Both scanning and minimizing conformational energy as a function of the ϕ_i was carried out using the CAMSEQ-II system [17] and its family of molecular mechanics potential functions [12,18]. Consistent force-field energy minimizations [16] were repeated using the minimum energy conformations found in the fixed valence geometry scan calculations as starting points. The relative zero values for the ϕ_i, $i > 2$, are chosen as *cis/trans* planar conformer states. $\phi_1 \equiv \phi_2 \equiv 0°$ is the same as that in [10].

3. Results

The first conformational scan was performed by simultaneously varying both ϕ_1 and ϕ_2 from 0–360° at 10° intervals, with all other degrees of conformational freedom fixed, for the neutral molecule. The free-space and aqueous-solvent conformational energy

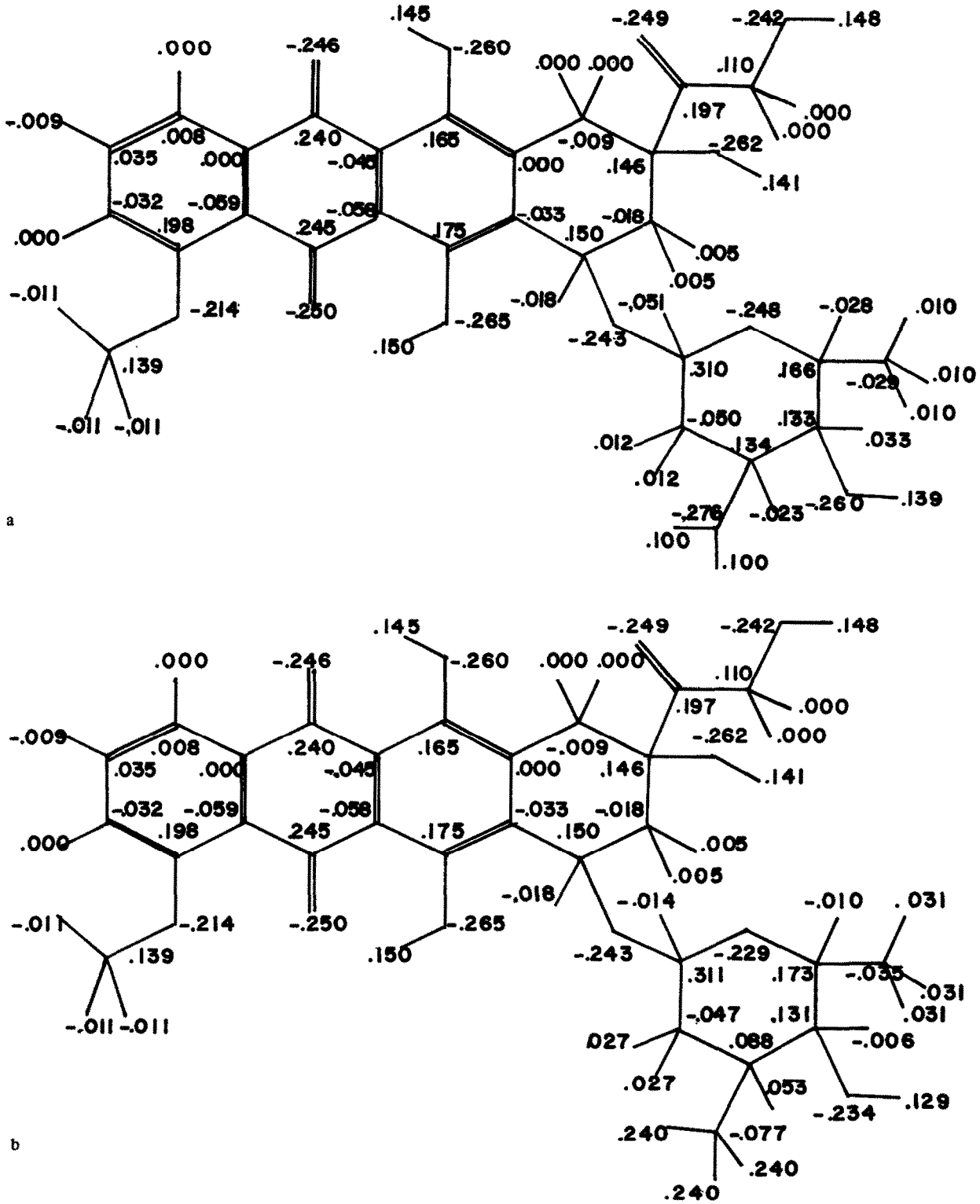

Fig.3. Atomic charge distribution on neutral (NH_2 9) and charged (N^+H_3) doxorubicin.

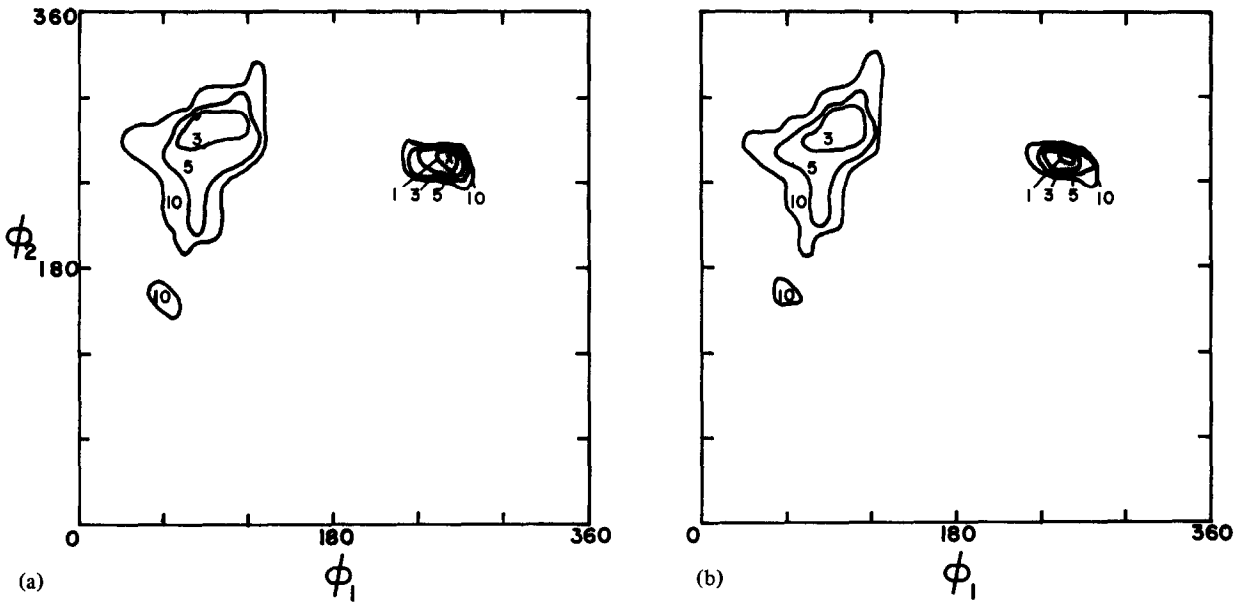

Fig.4. Conformational energy maps for ϕ_1 and ϕ_2 of neutral doxorubicin: (a) free-space map; (b) aqueous-solvent map. Energy contours are in kcal/mol.

maps are shown in fig.4. The effect of the aqueous medium on relative conformer stability is quite small. Consequently solvent contributions were neglected in subsequent neutral molecule studies. Aqueous solvent-dependent conformational studies could not be performed for the charged form of doxorubicin because the appropriate N^+H_3 solvation parameters are not available.

The $\phi_1 = 90°, \phi_2 = 280°$ minimum energy conformer corresponds to the crystal structure. However, two other energy minima are observed, one of which is the global energy minimum for the two-dimensional scan. This conformer is located at $\phi_1 = 250°, \phi_2 = 260°$ and is ~3.0 kcal/mol more stable than the 'crystal' conformer. The global minimum structure is stabilized by a hydrogen bond between (O22–H46) and the sugar ring oxygen, O31. The distance between O22 and O31 is only ~2.62 Å. However, the distance between H46 and O31 is ~1.95 Å which is longer than O · · · H distances of hydrogen bonds involving hydroxyl groups. In essence, the stability of this conformation, relative to the crystal conformer, depends upon the ability to form the (O22–H46) · · · O31 hydrogen bond.

The third conformer, which corresponds to the sugar ring being nearly parallel to the anthraquinone ring is located at $\theta_1 = 60°, \theta_2 = 160°$. This structure is >9.5 kcal/mol less stable than the global energy minimum conformation. Therefore it was neglected in subsequent calculations.

Multidimensional, fixed valence geometry energy minimization calculations were next carried out for ϕ_1 to ϕ_{13}. The starting values for glycosidic bond rotations ϕ_1 and ϕ_2 were either (90°, 280°) or (250°, 260°), while ϕ_3–ϕ_8 were individually assigned rotameric states which would be theoretically expected. The set of hypothetical starting conformer values were generated using the ROTAMER option of CAMSEQ-II. The torsional rotations ϕ_9–ϕ_{13} were always assigned initial values consistent with the *N*-bromoacetal derivative crystal conformation.

The global energy minimum conformer state is shown in fig.5a and is close to the global minimum realized from fig.4. The secondary minimum (corresponding to the crystal structure) is shown in fig.5b. The minimum energy conformations are described in table 1 along with the crystal conformations of daunomycin, carminomycin and *N*-bromoacetyl-daunomycin.

The minimum energy conformers found for the neutral form of doxorubicin were used as respective starting point in energy minimizations of the charged

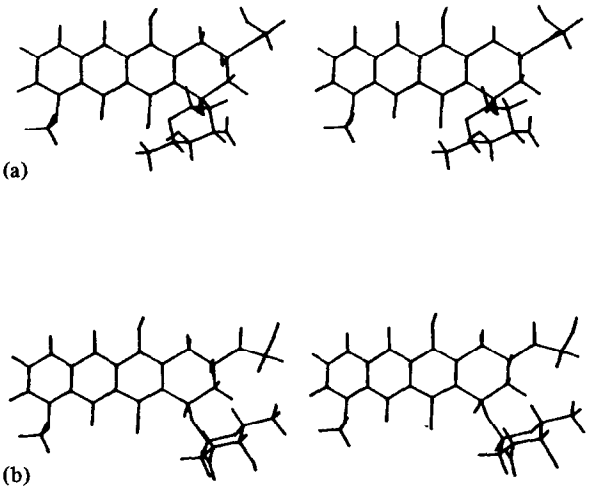

Fig.5. Stereo-stick models of: (a) the global minimum energy conformation; (b) the 'crystal' conformation.

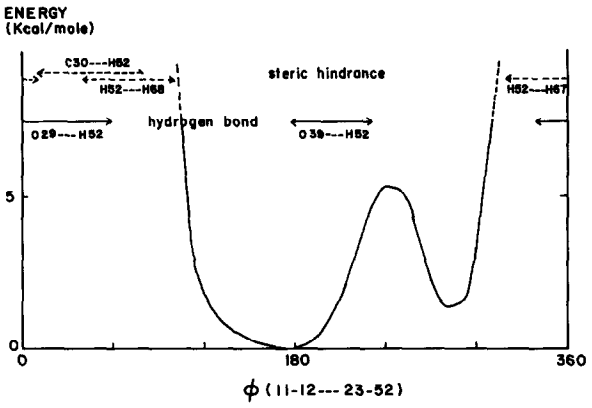

Fig.6. Dependence of the position of the H52 atom upon the conformational energy of the global minimum conformer of doxorubicin.

form (N^+H_3) of doxorubicin, and the neutral and charged form of daunomycin. The corresponding minimum energy conformer states are also presented in table 1. It can be seen that the neutral and charged forms of both doxorubicin and daunomycin are all quite similar to one another.

Table 1
Conformational features of some observed daunomycin derivatives and calculated doxorubicin and daunomycin minimum energy conformers

Compound	ϕ_1	ϕ_2	Hydrogen bonding[a]	ΔE^b (kcal/mol)
Daunomycin –pyridine xtal	125°	292°	d(O29–H52) = 2.12 Å	–
Carminomycin xtal	117°	290°	d(O29–H O23) = 2.80 Å d(O31–H O23) = 2.89 Å	–
N-bromoacethyl--daunomycin xtal	102°	281°		–
Doxorubicin-NH_2				
Conformer I	259°	257°	d(O31–H46) = 1.95 Å d(O39–H52) = 2.22 Å	0
Conformer II	77°	288°		4.6
Doxorubicin-N^+H_3				
Conformer I	259°	257°	d(O31–H46) = 2.27 Å	0
Conformer II	77°	288°		1.5
Daunomycin-NH_2				
Conformer I	258°	257°	d(O31–H46) = 2.24 Å	0
Conformer II	79°	289°		5.0
Daunomycin-N^+H_3				
Conformer I	258°	257°	d(O31–H46) = 2.26 Å	0
Conformer II	79°	288°		1.8

[a] Using atom numbering fig.2
[b] Global minimum energy conformer I has its energy set equal to zero

4. Discussion

The major finding from this work is a stable conformation in both doxorubicin and daunomycin different from the crystal conformation. This new conformation is characterized by a stablizing bifurcated hydrogen bond involving the 5-oxygen and 6-hydroxyl group of the anthraquinone ring and the 5′-oxygen of the sugar ring. Space filling molecular model building indicates that doxorubicin can intercalate between adjacent bases of double helical DNA, in this new conformation, such that the protonated sugar amino nitrogen atom can be close to a phosphate group of the polynucleotide backbone. This has been suggested as an important component to the DNA binding mechanism [4,13–15].

Unfortunately, a very large number of reasonable intercalation modes of doxorubicin (and daunomycin) can be postulated from examination of molecular models. This is in a large part a consequence of the many hydrogen bonding sites on both the drug and DNA. Our feeling is that detailed intermolecular energy calculations represent a minimum effort in order to make any comparative statements about preferred modes of intercalation.

There is one intramolecular conformational property of doxorubicin that may be relevant to DNA intercalation behavior. The conformational flexibility of the crystal conformation is much larger than that of the global minimum. This can be discerned from the energy map shown in fig.4a. The low energy contour areas about the crystal minimum are much larger than those of the global minimum. It is not unreasonable to suppose that the more flexible a conformer state, the easier it is for the molecule to adjust to the receptor site and maximize binding energy. This reasoning would support the crystal conformation as the 'active' conformer. The higher intramolecular flexibility might also explain why the $\phi_1 = 77°$, $\phi_2 = 288°$ conformer can be crystallized.

Lastly, Neidle and Taylor [10] intrinsically postulated a hydrogen bond between O29 and H52. Their conformational analyses were performed by restricting ϕ_4 to two values which imposed the O29–H52 hydrogen bond. In the case of the global energy minimum the O29–H52 hydrogen bond cannot be made due to steric hindrance involving H52· · · H67, H52· · · C30, and H52 · · · H68 as shown in fig.6. This may explain why Neidle and Taylor did not find our global intramolecular energy minimum.

Acknowledgments

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