Notes

STRUCTURE OF AKLAVINONE, A DNA BINDING ANTHRACYCLINE ANTIBIOTIC

S. K. ARORA

Drug Dynamics Institute
University of Texas
Austin, Texas 78712, U.S.A.

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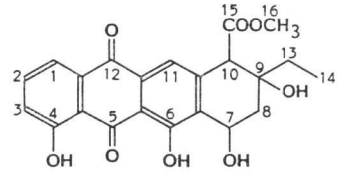
Among the anthracycline antibiotics, aclarubicin (aclacinomycin) is a relatively new antibiotic that shows strong anti-neoplastic activity. Aklavinone[1] (Fig. 1) is a natural antibiotic that can be bioconverted into aclarubicin by mutant strains of *Streptomyces galilaeus*[2]. Since the configuration of ring A and its substituents in anthracycline antibiotics is important from the point of biological activity, structural studies on aklavinone were carried out to reveal the structure and to compare the conformation of ring A and its substituents with that of the aglycone of daunorubicin (daunomycin), nogalamycin, and steffimycin.

Red needle shaped crystals of aklavinone were obtained from a mixture of methanol and dichloromethane. The crystals belong to the monoclinic space group $P2_1$ with cell dimensions of $a=6.827(1)$, $b=13.747(2)$, $c=19.630(3)$ Å, $\beta=90.65°$, $Z=4$ (2 mol/asymmetric unit) and $Dm=1.32\ g\ cm^{-1}$. A crystal with dimensions $0.3\times0.15\times0.15$ mm was used for data collection. Intensities of 3,394 reflections $2\theta\leq53.0°$ were measured using Mok_α ($\lambda=0.71069$ Å) radiation on a Syntex $P2_1$ diffractometer equipped with a graphite monochromator and a Syntex LT-1 inert-gas (N_2) low temperature delivery system (−110°C), omega scan technique, a variable scan rate (2.0~6.0°), a scan range of 2.0° and a scan to background ratio of 1.0. Only reflections with $I\geq3\sigma$ (I), 1,685, were considered observed. Intensities were corrected for Lorentz and polarization effects, but no absorption correction was applied.

The structure was solved by direct methods program MULTAN[3] with E's≥1.5. The first E map revealed 40 of 60 non-hydrogen atoms in the asymmetric unit. The structure was first refined isotropically and then anisotropically to an R factor of 0.083. At this stage all the hydrogen atoms, except the methyls, were located from the subsequent difference Fourier maps. Further refinement using anisotropic temperature factors for non-hydrogen atoms and isotropic temperature factors for hydrogen atoms reduced R to the final value of 0.069. The function minimized was $\Sigma(Fo-Fc)^2$ and the scattering factors used were those of CROMER and MANN[4].

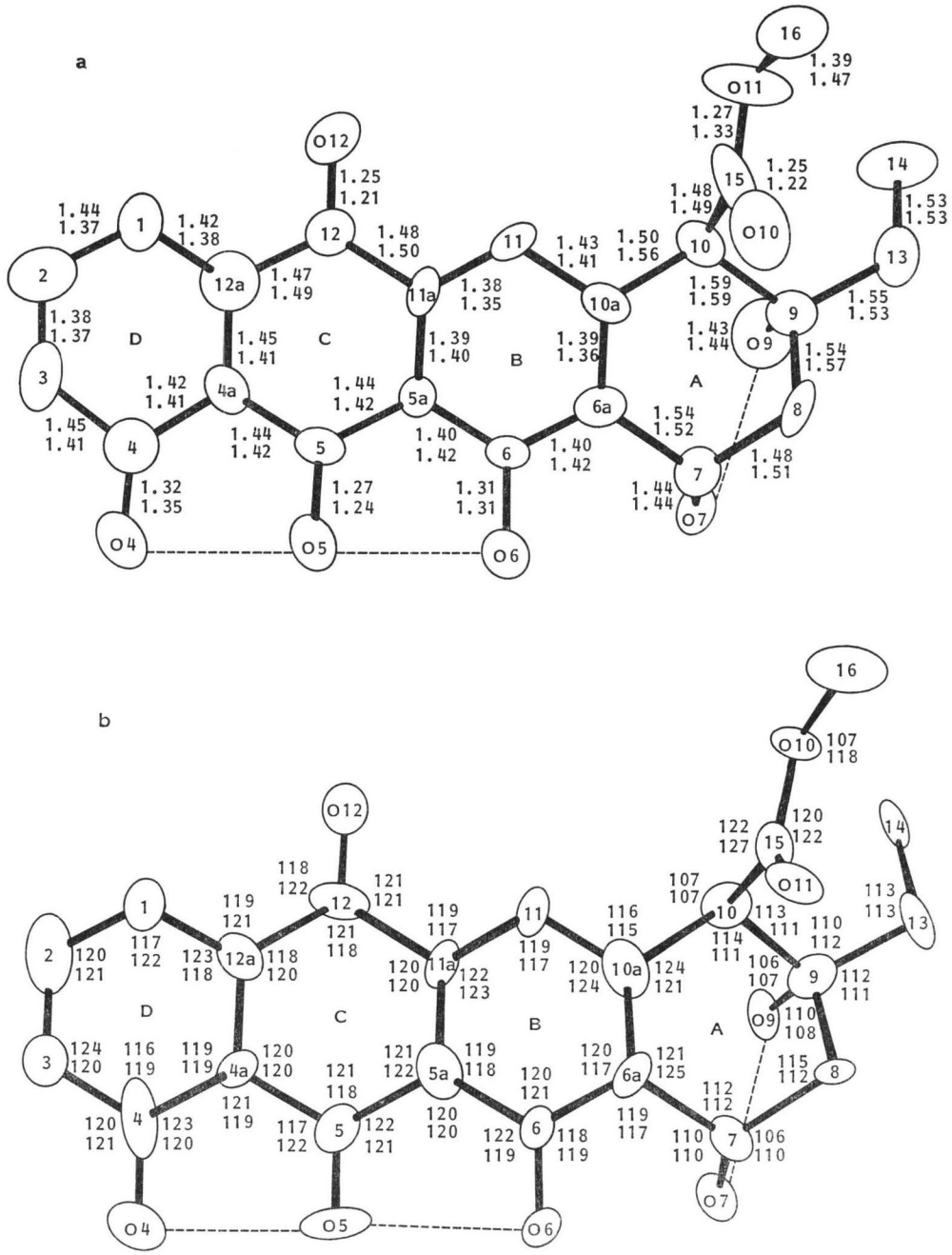
Fig. 1. Chemical formula of aklavinone.



Figs. 2a and 2b show the stereochemistry of the two independent molecules in the asymmetric unit. While most of the bond distances in the two independent molecules are similar, a few seem to differ. The average bond lengths and angles in the aklavinone agree with similar bonds and angles in daunorubicin[5], carminomycin[6], nogalamycin[7], and steffimycin B[8]. The configuration in ring A is 7*S*,9*R*,10*R*.

The two molecules in the asymmetric unit have similar conformation, as shown in Figs. 2a and 2b. Rings B, C and D as expected are planar. Table 1 compares the torsion angles in ring A with those of other anthracyclines. These torsion angles indicate that in aklavinone, like in other anthracyclines, the conformation of ring A is half chair. The O(9) is axial, while C(13) is equatorial similar to daunorubicin and carminomycin. The carbomethoxy group which is essential for binding of type II anthracyclines to DNA, is axial. This orientation is similar to that observed in nogalamycin[7], the dihedral angle C(9)-C(10)-C(15)-O(11) having a value of 124° (aklavinone) and 115° (nogalamycin). Both C(8) and C(9) atoms are equally displaced (−0.62 Å and 0.62 Å) from the mean plane of the other four atoms of ring A. This is different from the other anthracyclines where the maximum deviation of C(9) (duanorubicin[5], carmino-

Fig. 2. ORTEP drawing of two independent molecules depicting stereochemistry of two molecules.



mycin[6]) or C(8) (nogalamycin[7], *N*-bromoacetyldaunomycin[8]) is observed.

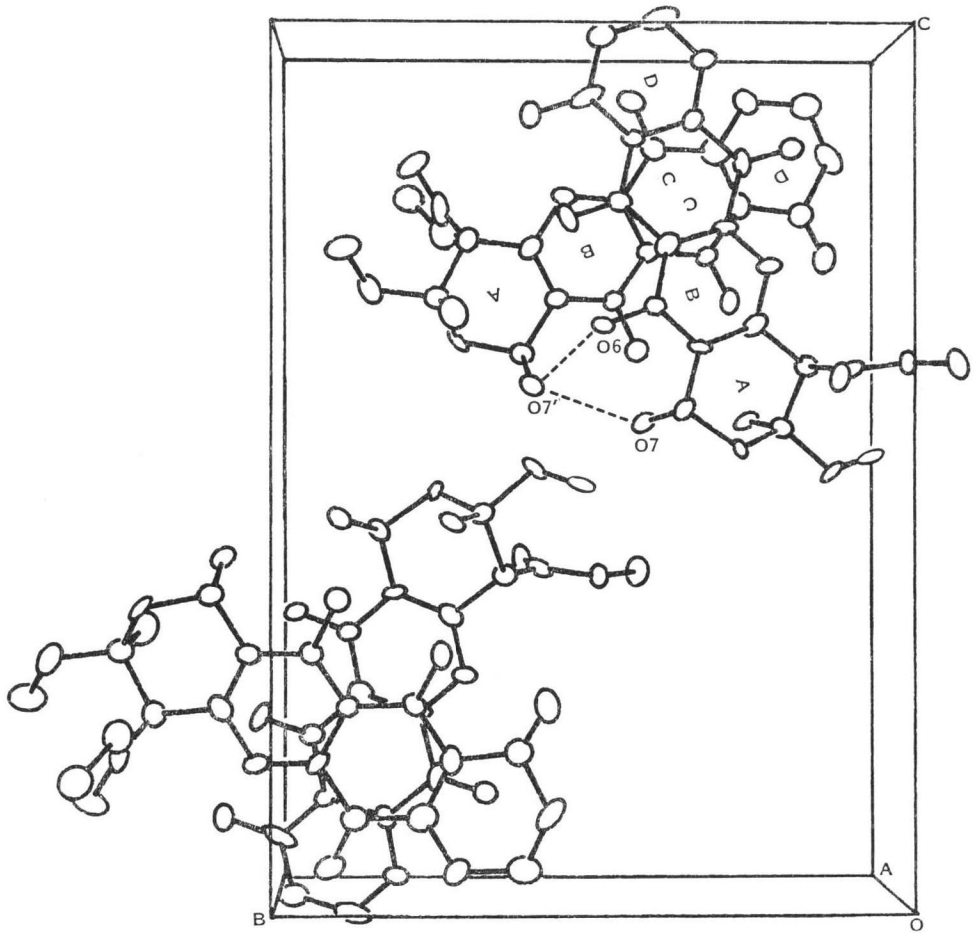
Fig. 3 shows the packing of molecules down the *a* axis. The two independent molecules stack over each other with a separation of 3.4 Å but the stacking observed in this study is a little different from that observed in nogalamycin. While the angle between the long direction of the two independent molecules is different in aklavinone and nogalamycin, the ring that overlaps is the same, *i.e.*, ring C.

Table 2 gives the atoms involved in the hy-

Table 1. Selected torsion angles (deg): Comparison of the selected common torsion angles in aklavinone (I and II), steffimycin B, daunorubicin, carminomycin and nogalamycin (I and II).

Atoms	Aklavinone		Steffi-mycin B	Dauno-rubicin	Carmino-mycin	Nogalamycin	
	I	II				I	II
C(6a)-C(7)-C(8)-C(9)	−48	−44	−50	−48	−48	−39	−47
C(7)-C(8)-C(9)-C(10)	62	64	60	58	62	60	63
C(8)-C(9)-C(10)-C(10a)	−40	−49	−37	−38	−45	−48	−47
C(9)-C(10)-C(10a)-C(6a)	10	18	7	14	18	19	18
C(10)-C(10a)-C(6a)-C(7)	5	2	3	−4	−5	−2	−4
C(10a)-C(6a)-C(7)-C(8)	13	11	17	20	20	8	18

Fig. 3. Packing of the molecules in the unit cell.



drogen bonding and the distances. There are three intramolecular hydrogen bonds, O(4)→O(5), O(6)→O(5), O(9)→O(7), and two intermolecular hydrogen bonds involving O(7)→O(7′) and O(7′)→O(6), with the arrow indicating the probable direction of proton donation.

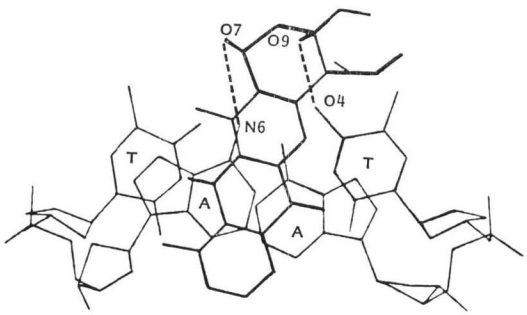
Previous studies have indicated that four parts of anthracycline antibiotic molecules are important for interaction with DNA: (a) A planar ring system for intercalation into the base pairs of DNA, (b) orientation of O(9) on ring A, *i.e.*, axial, (c) orientation of the carbo-

Table 2. Hydrogen bonds.
The arrow denotes probable direction of proton donation.

Hydrogen bonding	Distance (Å)
O (4) → O (5)	2.506
O (6) → O (5)	2.531
*O (7′)→ O (6)	2.881
O (9) → O (7)	2.692
O (7) → O (7′)	2.769
O (4′)→ O (5′)	2.503
O (6′)→ O (5′)	2.556
O (9′)→ O (7′)	2.758

* Prime refers to molecule II.

Fig. 4. Possible interactions of aklavinone with d (A–T).



methoxy group at C(10) with respect to ring A and (d) the amino sugar or sugars. Doxorubicin, daunorubicin and carminomycin possess (a) (b) and (c), while nogalamycin contains (a), (c) and (d) (even though amino sugar is at the other end). In the case of aclarubicin all four groups are present. Aklavinone, being the aglycone of aclarubicin, contains the first three groups, of which (b) and (c) are in the active orientation. COLLIER *et al.*[10] have done molecular modeling of the interaction of nogalamycin with the dinucleotide d(A–T) with the orientation of the carbomethoxy group at C(10) changed to accomodate the molecule. Thus what we see in aklavinone is that, other than not having three sugars which are present in aclarubicin, it has the conformation which is suitable for maximum interaction with DNA. The length of DNA required may be only two base pairs, preferably d(A–T). Fig. 4 shows possible interaction of aklavinone with DNA [d(A–T)$_2$], with O(7) and O(9) of the antibiotic forming hydrogen bonding with N(6) of adenine and O(4) of thymine of the base pair below, some what similar to daunorubicin-d(ATGCAT)[11] complex.

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