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Complex Daunomycin–Butanol

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Abstract. $C_{25}H_{29}NO_{10}.HCl.C_4H_{10}O$, monoclinic, $P2_1$, $a = 17{\cdot}389\ (8)$, $b = 5{\cdot}557\ (3)$, $c = 16{\cdot}405\ (8)$ Å and $\beta = 91{\cdot}41\ (8)°$, $Z = 2$. The structure was solved by direct methods and refined to an R of 0·07 for 2611 unique diffractometer data. The conformation and the interactions present in the complex structure allow us to propose a model for intercalation with DNA.

Introduction. Daunomycin or daunorubicin is an anthracyclic antibiotic isolated from *Streptomyces peucetius* (Di Marco *et al.*, 1964; Arcamone, Franceschi, Orezzi, Cassinelli, Barbieri & Mondelli, 1964; Arcamone, Cassinelli, Orezzi, Franceschi & Mondelli, 1964). It is used in the treatment of leukaemia (Tan, Tasaka, Murphay & Karnofsky, 1967; Maral, 1977). It is assumed that its therapeutic activity is due to a particular interaction with deoxyribonucleic acid (DNA) called intercalation (Kersten, Kersten & Szybalski, 1966; Waring, 1970). This kind of interaction may be characterized by various physicochemical parameters, two of which are: a binding constant and an unwinding angle of the double helix of DNA. Preliminary X-ray studies (Courseille, Busetta, Geoffre & Hospital, 1977) of daunomycin chloride have been completed to define the interaction axes of the molecule and to specify a model of intercalation.

Daunomycin chloride crystallizes by slow evaporation of an *n*-butanol solution. Intensities of 2611 independent reflections (1813 observed) were measured on a Siemens diffractometer with Cu $K\alpha$ radiation ($\lambda = 1{\cdot}54178$ Å).

Discussion. Tables 1 and 2 give the final atomic coordinates and their standard deviations.* Interatomic distances, valency angles and torsional angles are shown in Fig. 1. Standard deviations are in the neighbourhood of 0·01 Å for distances and 1° for angles. This conformation is practically identical to that found for the crystal structure of daunomycin crystallized with pyridine and water (Neidle & Taylor, 1977). The only slight differences are found in the distances

Table 1. *Atomic coordinates* ($\times 10^4$)

	x	*y*	*z*
Cl(30)	9239 (1)	−2713 (7)	1096 (1)
C(1)	3560 (5)	−2225 (23)	2431 (5)
C(2)	4258 (6)	−1645 (21)	1907 (6)
C(3)	4482 (5)	987 (22)	1918 (5)
C(4)	4694 (5)	1810 (22)	2789 (5)
C(5)	4186 (5)	1839 (21)	4214 (6)
C(6)	3819 (6)	1833 (23)	5661 (6)
C(7)	3492 (7)	1659 (29)	7105 (6)
C(8)	3075 (8)	591 (37)	7729 (7)
C(9)	2571 (9)	−1177 (35)	7617 (6)
C(10)	2464 (7)	−2178 (31)	6828 (6)
C(11)	2833 (6)	−2299 (25)	5329 (5)
C(12)	3215 (5)	−2096 (20)	3904 (5)
C(13)	3662 (5)	−1093 (19)	3260 (5)
C(14)	4148 (5)	782 (19)	3409 (5)
C(15)	3764 (5)	784 (23)	4847 (5)
C(16)	3271 (5)	−1109 (21)	4694 (5)
C(17)	2889 (6)	−1216 (26)	6167 (6)
C(18)	3374 (6)	671 (25)	6323 (5)
O(20)	1975 (5)	−3968 (22)	6652 (5)
O(21)	2421 (5)	−4075 (19)	5168 (4)
O(22)	2770 (4)	−4002 (15)	3719 (4)
O(23)	5203 (5)	1327 (24)	1374 (6)
O(25)	4664 (4)	3728 (15)	4345 (4)
O(26)	4234 (4)	3627 (18)	5809 (4)
O(33)	5726 (5)	−69 (23)	1472 (5)
C(40)	1562 (9)	−5125 (38)	7305 (9)
O(41)	2864 (4)	−1204 (14)	2051 (4)
C(42)	2283 (5)	−2894 (23)	1900 (5)
O(43)	2465 (4)	−4411 (13)	1228 (3)
C(44)	2479 (5)	−3144 (22)	452 (5)
C(45)	1711 (5)	−2033 (21)	262 (5)
C(46)	1512 (5)	−265 (21)	930 (5)
C(47)	1537 (5)	−1589 (22)	1752 (5)
O(53)	3890 (4)	2477 (16)	1566 (4)
C(54)	2730 (6)	−5055 (25)	−179 (6)
O(55)	1095 (4)	−3681 (16)	183 (4)
N(56)	717 (4)	713 (19)	778 (5)
C(63)	5224 (8)	3339 (28)	769 (7)
C(70)	1249 (12)	−8897 (54)	4684 (16)
C(71)	712 (12)	−7310 (59)	4296 (14)
C(72)	710 (11)	−7319 (57)	3484 (10)
C(73)	182 (10)	−5881 (45)	2942 (10)
O(74)	203 (5)	−6285 (19)	2109 (5)

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 33962 (29 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

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C(8)–C(9), C(12)–C(16) and O(43)–C(44). In both structures, the lengthening of ketonic bonds C(6)–O(26) and C(11)–O(21) and the shortening of hydroxy bonds C(5)–O(25), C(12)–O(22) are associated with intramolecular chelation. In our case it was even possible to find the positions of hydrogen atoms. The characteristics of the chelation are described as follows:

O(25)–H(125)···O(26) 2·53 Å and 124°

O(22)–H(122)···O(21) 2·47 Å and 135°.

Such chelations were also suggested in the structure of *N*-bromoacetyldaunomycin (Angiuli *et al.*, 1971).

The aromatic part of the molecule is planar. The cyclohexane ring has the more stable form: the mono-planar or half-chair form (Bucourt & Hainaut, 1967). The sugar daunosamine is protonated on the amino group. It has the chair form and its mean plane is almost perpendicular to the tetracyclic plane (83°).

Fig. 2 shows the packing of the molecules and the set of hydrogen bonds necessary for the cohesion of the crystal. If we consider the aromatic daunomycinone nucleus, we notice a weak superposition of the molecules in the *b* direction. The overlapping occurs only for the hydroxy and keto groups belonging to the *B* and *C* rings. The distance is 3·51 Å. The cohesion forces are intensified in the *b* direction by a weak hydrogen bond between O(53) and O(43) of the molecules situated in a period. Although the distance between the two atoms is long (3·06 Å), the

Table 2. *Hydrogen coordinates* (×10⁴)

	x	*y*	*z*
H(101)	3492 (47)	−3986 (153)	2472 (48)
H(144)	2866 (49)	−1858 (147)	473 (49)
H(145)	1729 (47)	−1173 (146)	−277 (49)
H(146)	1886 (49)	1055 (149)	966 (50)
H(147)	1075 (56)	−2761 (152)	1756 (57)
H(247)	1470 (49)	−386 (143)	2199 (50)
H(171)	154 (51)	−7643 (148)	4478 (50)
H(271)	925 (53)	−5535 (147)	4468 (54)
H(172)	1257 (52)	−6930 (148)	3319 (53)
H(272)	620 (52)	−9099 (147)	3320 (54)
H(173)	−352 (55)	−6203 (145)	3163 (57)
H(273)	305 (52)	−4040 (143)	3065 (54)
H(102)	4709 (46)	−2612 (147)	2122 (47)
H(202)	4131 (47)	−2142 (147)	1333 (48)
H(104)	5234 (47)	1246 (152)	2933 (49)
H(204)	4672 (47)	3609 (148)	2806 (49)
H(107)	3848 (46)	3062 (146)	7215 (49)
H(108)	3159 (47)	−1202 (145)	8296 (49)
H(109)	2271 (46)	−1793 (149)	8086 (48)
H(142)	2228 (47)	−3936 (151)	2406 (49)
H(122)	2582 (48)	−4917 (150)	4267 (49)
H(125)	4601 (45)	4712 (151)	4780 (49)
H(153)	3632 (47)	4222 (151)	1424 (48)
H(155)	963 (47)	−3695 (147)	−394 (49)
H(154)	3257 (47)	−5981 (148)	6 (49)
H(254)	2297 (47)	−6309 (149)	−248 (49)
H(354)	2811 (48)	−4239 (143)	−707 (48)
H(156)	702 (47)	1620 (144)	253 (48)
H(256)	580 (46)	1796 (147)	1221 (48)
H(356)	337 (47)	−660 (146)	749 (49)
H(163)	5465 (48)	4771 (149)	1026 (50)
H(273)	4696 (47)	3712 (153)	608 (49)
H(363)	5529 (45)	2837 (152)	287 (47)
H(140)	1369 (48)	−3794 (148)	7801 (49)
H(240)	1087 (47)	−5898 (152)	7064 (49)
H(340)	1916 (46)	−6336 (147)	7559 (48)

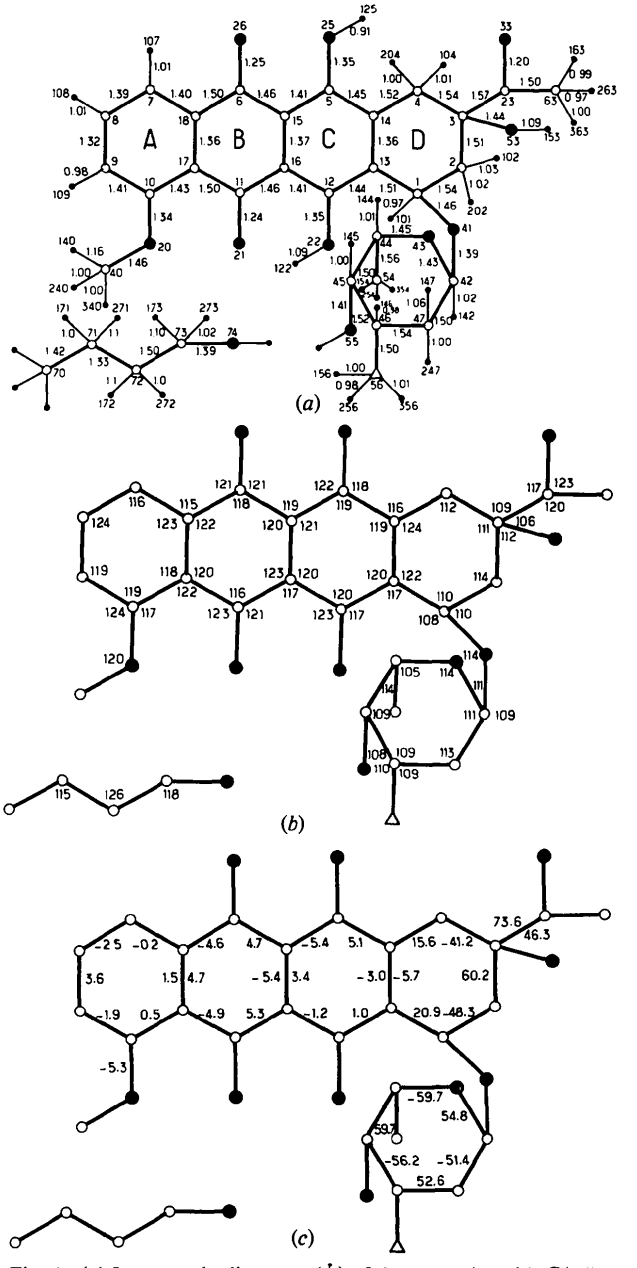

Fig. 1. (*a*) Interatomic distances (Å) of daunomycin, with C(54)–H(154) = 1·09 Å; C(54)–H(254) = 1·03 Å; C(54)–H(354) = 0·99 Å [$\tilde{\sigma}_{C-C,C-O,C-N}$ = 0·01 Å; $\tilde{\sigma}_{C-H,O-H,N-H}$ = 0·09 Å]. (*b*) Interatomic angles (°) of daunomycin [$\tilde{\sigma}$ = 1°]. (*c*) Torsion angles of daunomycin (°).

localization of H(153) on a difference map allows one to calculate a geometry which is that of a hydrogen bond. The short intramolecular distance O(53)···O(41) of 2·84 Å implies the existence of another hydrogen bond as proposed for carminomycin (Von Dreele & Einck, 1977) (homologue of daunomycin), and for the complex daunomycin–pyridine (Neidle & Taylor, 1977). We report the geometry of these different hydrogen bonds in Fig. 3. We can assume a possible bifurcated hydrogen bond for carminomycin as noted for polycarpine (Damak & Riche, 1977). It seems that the hydrogen atom is in equilibrium between an intramolecular hydrogen bond and an intermolecular one.

Along the *b* axis, the molecules are infinitely stacked and between every stack the cohesion is provided by a set of hydrogen bonds in which the daunosamine sugar, the chloride ion and the butanol are involved (Table 3). Most of the hydrogen bonds proceed from the sugar ring and it is reasonable to assume that it must also play a very important part in the biological interactions.

As the conformation and interactions remain the same for the structures of daunomycin complexed with butanol and with pyridine it is possible to assume a certain rigidity of the molecule and to suppose that at least a part of these linkages will be satisfied during the interaction with DNA.

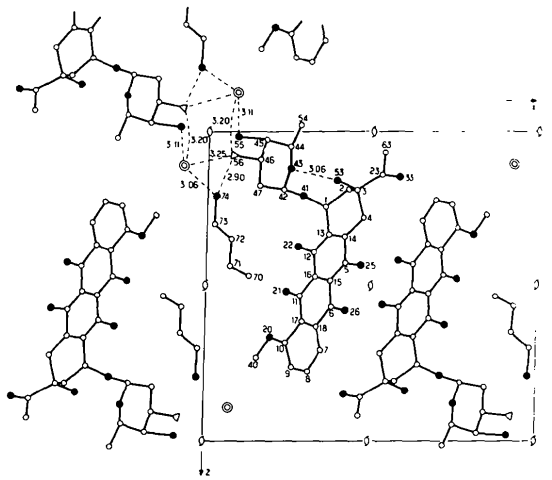

Fig. 2. Projection of the structure along *b*.

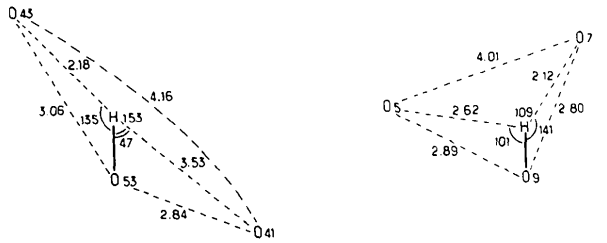

Fig. 3. Hydrogen-bond geometry (distances in Å, angles in °) (on the left: daunomycin, on the right: carminomycin).

Table 3. *Geometry of hydrogen bonds*

The hydrogen atom marked with a star was not found on the difference map.

D–H	Donor	*A*	Acceptor	*D*···*A* distance	*D*–H···*A* angle
O(53)–H(153)	(*x*,*y*,*z*)	O(43)	(*x*, *y* + 1, *z*)	3·06 Å	136°
N(56)–H(256)	(*x*,*y*,*z*)	O(74)	(*x*, 1 + *y*, *z*)	2·91	173
N(56)–H(156)	(*x*,*y*,*z*)	Cl⁻(31)	(1 − *x*, ½ + *y* − *z*)	3·20	159
N(56)–H(356)	(*x*,*y*,*z*)	Cl⁻(30)	(−1 + *x*, *y*, *z*)	3·25	155
O(55)–H(155)	(*x*,*y*,*z*)	Cl⁻(30)	(1 − *x*, −½ + *y*, −*z*)	3·11	119
O(74)–H*	(*x*,*y*,*z*)	Cl⁻(30)	(1 + *x*, *y*, *z*)	3·06	

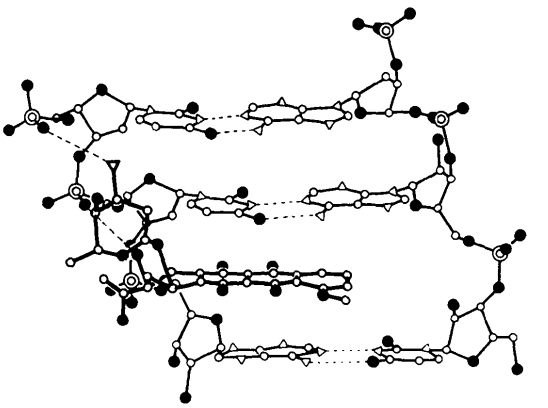

Fig. 4. Model of interactions: daunomycin–DNA fraction.

The method already suggested (Pigram, Fuller & Hamilton, 1972) of an X-ray study of DNA fibre after daunomycin interaction and the drawing of the structure of 5-iodouridylyl-(3′→5′)-adenosine–ethidium bromide complex (Tsai, Jain & Sobell, 1975) may be used to propose a perspective view of the interaction of daunomycin with a polynucleotide (Fig. 4). To satisfy at the same time the intercalation of the tetracyclic part between the pairs of bases and the hydrogen bonds of the daunosamine sugar with the phosphodiester chain, a trinucleotide must be involved.

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Structure of 1-(*N'*-Ethoxycarbonylhydrazino)phthalazinium Chloride Monohydrate

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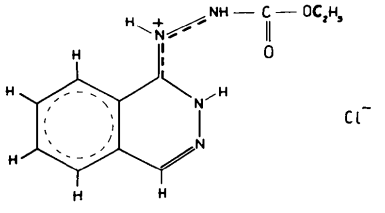
Abstract. $C_{11}H_{13}N_4O_2^+.Cl^-.H_2O$, monoclinic, $P2_1/c$, $a = 5{\cdot}110$ (1), $b = 15{\cdot}435$ (3), $c = 17{\cdot}948$ (5) Å, $\beta = 105{\cdot}13$ (2)°, $U = 1366$ Å^3, $D_m = 1{\cdot}397$, $D_x = 1{\cdot}393$ Mg m^{-3}, $Z = 4$. The structure was solved by the heavy-atom method and refined to $R = 0{\cdot}086$ for 1784 counter-collected independent reflections. In the title compound (an antihypertensive agent) water molecules and chloride anions bridge the cations *via* hydrogen bonds in a three-dimensional net.

Introduction. The title compound (I) is used (as ethoxycarbonylhydralazine or Binazin) in the treatment of hypertension because of its hypotensive and antihypertensive properties (Biniecki, Haase, Izdebski, Kesler & Rylski, 1958). This structure analysis was performed to compare its molecular geometry with those of other phthalazine derivatives which act as vasodilators: hydralazine (Stadnicka & Lebioda, 1978) and dihydralazine (Stadnicka & Lebioda, 1979).



(I)

Colourless needle-shaped single crystals of (I) were obtained by recrystallization from aqueous solution. The systematic absences *h*0*l* for *l* odd and 0*k*0 for *k* odd were found from Weissenberg photographs. A crystal with dimensions 0·12 × 0·05 × 0·12 mm was used to collect intensity data with a CAD-4 (Enraf–Nonius) diffractometer using graphite-monochromated Mo $K\alpha$ radiation. Unit-cell parameters were obtained by least-squares refinement from the setting angles of 15 reflections used in the auto-indexing procedure. All reflections in the range $2° \le \theta \le 23°$ were measured with a $\theta/2\theta$ scan and a $(0{\cdot}8 + 0{\cdot}3 \tan\theta)°$ scan range. The intensities were corrected for Lorentz and polarization effects, but not for absorption [μ(Mo $K\alpha$) = 0·296 mm^{-1}]. The fluctuation in the intensity of a standard reflection was less than 5%. 1879 independent reflections were measured and 1784 of these, with positive intensities, were used in further calculations. The structure of the hydrobromide counterpart, which is isomorphous (Chojnacki, Lebioda & Stadnicka, 1975), was solved by the heavy-atom method but not refined ($R = 0{\cdot}18$). The parameters of this structure were used as the starting values in the full-matrix least-squares refinement of the hydrochloride. Neutral-atom scattering factors (Cromer & Waber, 1965) for all atoms and anomalous-scattering corrections (Cromer, 1965) for the Cl scattering function were used. A difference map revealed 12 of the 15 H atom positions; those missing were from the ethyl group which undergoes strong thermal motion as shown in Fig. 1. The positional parameters of the H atoms were refined, except for those of the C_2H_5 group which were located from geometrical considerations (C–H = 1·08 Å, and tetrahedral valence angles). The H atoms were given isotropic thermal parameters equivalent to the aniso-

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