Molecular Structure, Absolute Stereochemistry, and Interactions of Nogalamycin, a DNA-Binding Anthracycline Antitumor Antibiotic

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Abstract: The crystal and molecular structure of a potent antitumor anthracycline antibiotic, nogalamycin ($C_{39}H_{47}NO_{16}$), has been determined, the absolute stereochemistry established, and a possible mode of interaction with DNA postulated. The orthorhombic unit cell, space group $P2_12_12_1$ with the dimensions a = 14.853 (3), b = 15.961 (3), and c = 33.788 (6) Å, contains eight molecules (two molecules/asymmetric unit). Intensity data for 6120 independent reflections were collected. The structure was solved by repeated use of direct methods and refined isotropically to an R factor of 0.11 for 2998 reflections. The configuration in ring A is 7*S*,9*S*,10*R*. The glucopyranose has the α-L configuration. The molecules in the asymmetric unit are related by a noncrystallographic 2-fold axis and have similar conformations. Ring A adopts a half-chair conformation, while the nogalose and glucopyranose have normal chair conformations. The carbomethoxy group at C(10) is axial. Both water molecules are involved in hydrogen bonding. The molecule possibly intercalates into DNA with the amino sugar and nogalose interacting in wide and narrow grooves, respectively.

Nogalamycin (Figure 1), an anthracycline antibiotic produced by *Streptomyces nogalater*, has high activity against Gram-positive microorganisms and is an antitumor agent[1-3] which has considerable activity against KB cells in vitro. It belongs to a group of useful antibiotics that interact with nucleic acids[4] by binding and inhibiting both DNA replication and transcription. The nature of binding has been suggested to be intercalative from several physiochemical and biological studies.[5-7] Recently, a study on template specificity of DNA binding by nogalamycin using fluoroscence polarization has been reported.[8] However it seems that hydrogen binding and electrostatic interactions also play an important role in anthracycline antibiotics binding to nucleic acids.

A great number of structural analogues of nogalamycin have been prepared recently[9] and their biological activity has been studied. The con and dis isomer of 7-O-alkyl derivatives, which differ slightly in structure are quite different in their biological and chemical activities. The dis isomer were more inhibitory toward DNA and RNA synthesis than the con isomers.[10] A study by DuVarney et al.[11] on anthracyclines has pointed out an interesting feature; the stereochemistry of a carbomethoxy group at position C(10) (which is also present in nogalamycin) is very important for DNA binding.

Wiley et al.[12] had proposed possible structures for nogalamycin from chemical degradation and spectral studies, but without knowledge of absolute stereochemistry. Hence, the complete structure of nogalamycin had not been established. A crystallographic study of nogalamycin was carried out to establish the absolute stereochemistry, reveal the molecular structure, and propose models for the probable mode of interaction with DNA.

Table I. Crystal Data for Nogalamycin

$C_{39}H_{47}NO_{16}\cdot H_2O$	V = 8010.0 Å
M_r = 805.8	d_{measd} = 1.35 g cm^{-3}
orthorhombic, $P2_12_12_1$	d_{calcd} = 1.34 g cm^{-3}
a = 14.853 (4) Å	temp = 22 °C
b = 15.961 (4) Å	radiation Mo Kα (λ = 0.71069 Å)
c = 33.788 (9) Å	$((\sin\theta)/\lambda)_{max}$ = 0.54 Å^{-1}
Z = 8	

Experimental Section

Data Collection. Red needle-shaped crystals of nogalamycin (kindly supplied by Dr. P. F. Wiley of Upjohn) were grown from a 3:1 mixture of methanol and methylene chloride after long period of unsucessful attempts. The crystal data are given in Table I. A crystal with the dimensions of 0.2 × 0.2 × 0.3 mm was used for data collection and measurement of cell constants. Intensities of 6120 unique reflections with $2\theta < 45°$ were measured at room temperature using MoKα radiation (λ = 0.71069 Å) on a Syntex $P2_1$ diffractometer equipped with a graphite monochromator, using a θ–2θ scan technique, a variable scan rate (1.0–29.3 min), a scan range of 2.0° and a background to scan ratio of 0.8. A total of 2998 reflections greater than $3\sigma(I)$ was considered observed. These reflections were monitored every 100 reflections and no significant deterioration in check reflections was observed. Lorentz and polarization corrections were applied to the data. The unit cell parameters were determined by least-squares fitting of the settings of 20 reflections having 2θ range of 10–25°.

Structure Determination and Refinement. Since there were two molecules (114 non-hydrogen atoms) in the asymmetric unit, the process of solving the structure turned out to be tedious. Attempts to solve the structure with the direct methods program MULTAN78[13] met with failure initially. Most of the E maps displayed a "chicken wire net" pattern. Finally, by variance in the number of E's (400 > 1.5) and the number of starting reflections (5), an E map showed two fragments (30 atoms in all) in the asymmetric unit which resembled the planar parts of the nogalamycin molecules, but attempts to develop these fragments by difference Fourier technique failed. At this stage it was concluded that most probably the orientation of the fragment was correct, but the position in the cell was not; i.e., the fragment needed to be translated in the unit cell. When translation function (using the option 3 in MULTAN) was applied, 22 more chemically reasonable peaks appeared in the E map. The remaining 60 atoms and two water molecules were located by difference Fourier.

The initial R factor with all 114 atoms in was 0.37. Four cycles of isotropic least-squares refinement reduced R to 0.11 for 2998 observed

(1) Bhuyan, B. K.; Dietz, A., *Antimicrob. Agents Chemother.* **1965**, 836–844.
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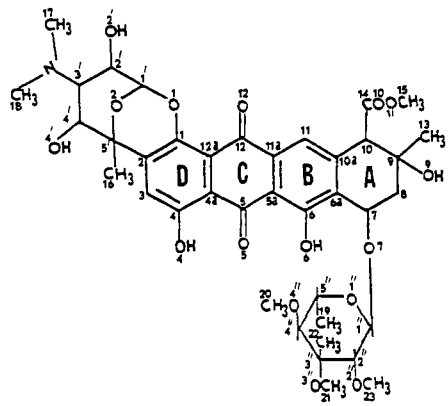

Figure 1. Chemical formula of nogalamycin.

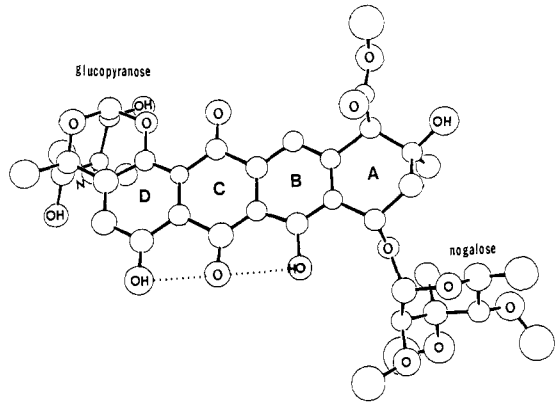

Figure 2. ORTEP drawing of molecule I depicting the stereochemistry.

reflections. The mean value of isotropic temperature factors was 5.2 Å^2. Because of the limited amount of data, no refinement of anisotropic thermal parameters was carried out. The refinement was based on Fo, with the quantity minimized being $\sum(F_o - F_c)^2$. The scattering factors used were those of Hanson et al.[14] The final atomic coordinates for the two molecules in the asymmetric unit are given in Table II.

Results and Discussion

Geometry. The standard deviations in the bond distances and angles are of the order of 0.02 Å and 1.0°, respectively. While some of the bond distances for the same bond in the two molecules are similar, others differ significantly. One of the reasons could be that because the two molecules are related by a noncrystallographic 2-fold axis, the refinement of coordinates might have been hindered. Chidester et al.[15] observed similar differences in CC 1065. With few exceptions, the average of bond lengths of the two molecules in nogalamycin agree with the similar bonds in daunomycin hydrochloride[16] and carminomycin.[17]

Absolute Stereochemistry. The absolute stereochemistry of nogalamycin was not previously known. Its chemical structure was originally published by Wiley et al.[12] with considerable uncertainty from the known stereochemistry of nogalose and considering that the amino sugar could have either α-L or α-D configuration. They predicted that the configuration of ring A was either 7*S*,9*S*,10*R* or 7*R*,9*R*,10*S*. Looking at other anthracyclines, the latter configuration seemed more reasonable. From their CD studies, Brockmann et al.[18] assigned the configuration 7*S*,9*R*,10*S* and analogues were assigned by using this configuration. The crystal structure of 7-con-O-methylnogarol was studied by Eckle et al.,[19] but the study did not reveal the absolute stereochemistry. Figure 2 shows the stereochemistry of nogalamycin. The configuration in ring A is 7*S*,9*S*,10*R* (different from other anthracyclines) and the glucopyranose has the α-L configuration. This establishes the absolute stereochemistry of nogalamycin.

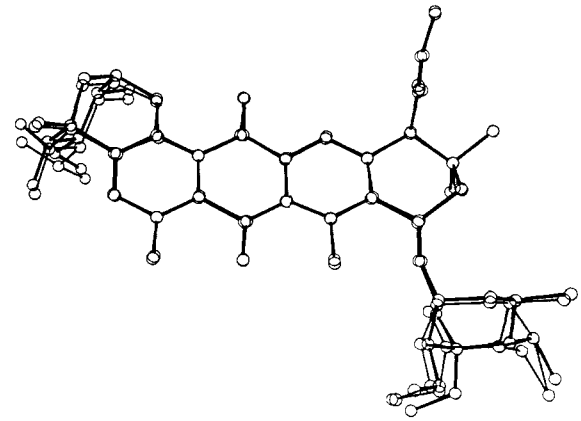

Figure 3. Comparison of the conformation of two molecules in the asymmetric unit.

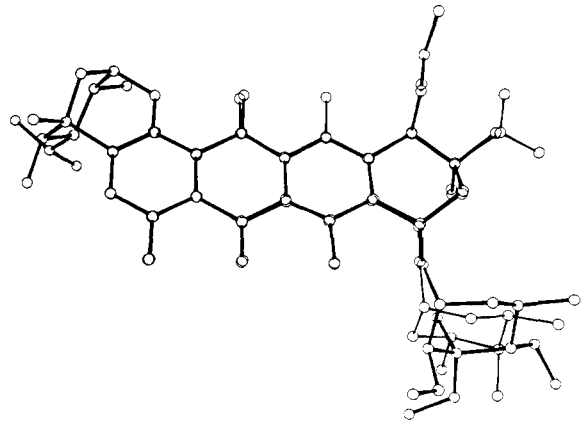

Figure 4. Comparison of the conformations of nogalamycin and daunomycin (thin bonds).

Conformation. As shown in Figure 3, the two molecules in the asymmetric unit (related by an approximate noncrystallographic 2-fold axis) have similar conformations. Rings B, C, and D are planar to within a root-mean-square deviation of 0.02 Å. The amino sugar and the nogalose are on the same side of the plane through rings B, C, and D. The O(9) is equatorial while methyl C(13) is axial. The carbomethoxy group at C(10) is axial, which is essential for DNA binding.[11] The plane of the carbomethoxy group is almost perpendicular to the least-squares plane through atoms C(6a), C(7), C(8), C(10), and C(10a). The torsion angles around ring A, starting from bond C(6a)–C(10a) and going in a clockwise direction are −2°, 19°, −48°, 60°, −39°, 8 (molecule I) and −4°, 18°, −47°, 63°, −46°, 18° (molecule II). Thus, the conformation of ring A is a half chair.[20] This is similar to the confirmation of ring A as observed in daunomycin and carminomycin. In nogalamycin, the out-of-plane atom is C(9), displaced by 0.65 Å from the mean plane of the other five atoms of ring A. This is similar to (*N*-bromoacetyl)daunomycin,[21] but different from duanomycin hydrochloride[16] and carminomycin,[17] where it is the C(8) atom that is displaced most. The torsion angles are given in Table III.

The nogalose ring adopts a chair conformation and is attached axially to ring A via O(7). This conformation is similar to that

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Table II. Fractional Coordinates ($\times 10^4$) and Standard Deviations for Non-Hydrogen Atoms in Nogalamycin

	molecule I			molecule II		
atoms	x/a	y/b	z/c	x/a	y/b	z/c
C(1)	3757 (13)	−1200 (11)	3789 (5)	1558 (16)	−1204 (14)	4827 (6)
C(2)	3898 (14)	−1085 (12)	3403 (6)	1412 (19)	−1202 (16)	5243 (8)
C(3)	4079 (15)	−0282 (14)	3238 (6)	1267 (18)	−0395 (16)	5420 (8)
C(4)	4094 (15)	0404 (14)	3490 (7)	1261 (17)	0355 (15)	5181 (7)
C(4a)	3967 (13)	0306 (12)	3888 (5)	1360 (16)	0263 (14)	4763 (6)
C(5)	3968 (13)	1064 (11)	4156 (5)	1381 (18)	1082 (16)	4506 (8)
C(5a)	3901 (13)	0980 (12)	4572 (6)	1508 (16)	0992 (14)	4111 (7)
C(6)	3965 (14)	1618 (13)	4838 (6)	1510 (18)	1680 (15)	3845 (7)
C(6a)	3886 (14)	1531 (13)	5257 (6)	1520 (15)	1631 (14)	3447 (6)
C(7)	4069 (14)	2308 (13)	5507 (6)	1499 (15)	2428 (13)	3204 (6)
C(8)	4130 (15)	2052 (13)	5948 (6)	1289 (16)	2257 (14)	2751 (7)
C(9)	3433 (16)	1368 (13)	6082 (6)	1859 (15)	1545 (13)	2602 (6)
C(10)	3590 (15)	0577 (12)	5844 (6)	1646 (15)	0718 (14)	2813 (6)
C(10a)	3679 (15)	0728 (13)	5392 (6)	1612 (15)	0859 (13)	3281 (6)
C(11)	3616 (14)	0057 (13)	5143 (6)	1603 (15)	0127 (13)	3502 (6)
C(11a)	3718 (14)	0163 (13)	4733 (6)	1539 (17)	0201 (15)	3916 (7)
C(12)	3719 (15)	−0589 (14)	4491 (6)	1566 (16)	−0599 (14)	4169 (7)
C(12a)	3820 (12)	−0507 (10)	4047 (5)	1492 (15)	−0525 (12)	4594 (6)
C(13)	2526 (16)	1658 (14)	6061 (6)	2813 (16)	1721 (14)	2565 (6)
C(14)	4414 (19)	0128 (15)	5978 (7)	0721 (16)	0337 (14)	2683 (6)
C(15)	4965 (25)	−0969 (19)	6354 (9)	0089 (27)	−0693 (24)	2303 (11)
C(16)	4883 (15)	−1822 (14)	2866 (6)	0533 (20)	−1933 (19)	5789 (8)
C(17)	0740 (25)	−2164 (23)	3163 (9)	4630 (17)	−2278 (16)	5405 (7)
C(18)	1454 (22)	−3280 (19)	2786 (9)	3902 (23)	−3259 (21)	5823 (9)
C(19)	3655 (20)	4516 (18)	6422 (8)	2343 (23)	4661 (21)	2333 (9)
C(20)	1348 (21)	5507 (18)	6251 (8)	4490 (26)	5727 (25)	2590 (9)
C(21)	1099 (17)	4985 (15)	4991 (7)	4891 (33)	4472 (30)	3807 (14)
C(22)	1452 (18)	3544 (15)	5565 (7)	4291 (18)	3369 (16)	3160 (8)
C(23)	3906 (22)	5023 (19)	4840 (8)	2313 (25)	4975 (24)	4040 (10)
C(1′)	3556 (15)	−2690 (13)	3698 (6)	1826 (17)	−2732 (15)	4926 (7)
C(2′)	2634 (15)	−2859 (14)	3519 (6)	2679 (17)	−2918 (15)	5061 (7)
C(3′)	2389 (14)	−2200 (13)	3203 (6)	2980 (14)	−2259 (13)	5400 (6)
C(4′)	3157 (14)	−2073 (13)	2913 (6)	2211 (17)	−2150 (16)	5695 (7)
C(5′)	4008 (18)	−1897 (16)	3131 (7)	1397 (17)	−1996 (15)	5483 (7)
C(1″)	3603 (16)	3694 (14)	5400 (7)	2210 (17)	3781 (16)	3329 (7)
C(2″)	2816 (13)	4225 (12)	5227 (5)	3021 (16)	4130 (14)	3536 (6)
C(3″)	2063 (16)	4384 (14)	5512 (6)	3797 (19)	4218 (17)	3257 (8)
C(4″)	2468 (16)	4724 (14)	5908 (16)	3508 (17)	4696 (14)	2883 (7)
C(5″)	3189 (17)	4171 (15)	6039 (7)	2712 (20)	4217 (18)	2702 (8)
N	1535 (17)	−2400 (14)	2970 (6)	3820 (14)	−2429 (13)	5618 (6)
O(1)	3528 (8)	−1953 (7)	3946 (3)	1794 (9)	−2005 (8)	4667 (4)
O(2)	4246 (9)	−2587 (8)	3398 (4)	1128 (9)	−2613 (8)	5233 (5)
O(4)	4218 (10)	1147 (9)	3315 (4)	1112 (13)	1072 (12)	5356 (5)
O(5)	4141 (10)	1775 (9)	4002 (4)	1282 (11)	1753 (10)	4694 (5)
O(6)	4167 (9)	2418 (8)	4696 (4)	1444 (11)	2493 (9)	4013 (4)
O(7)	3318 (8)	2858 (7)	5451 (3)	2298 (10)	2876 (9)	3260 (4)
O(9)	3707 (11)	1171 (9)	6493 (4)	1514 (12)	1418 (11)	2196 (5)
O(10)	4206 (13)	−0560 (11)	6174 (5)	0895 (13)	−0311 (11)	2462 (5)
O(11)	5164 (14)	0252 (12)	5902 (5)	0031 (14)	0627 (12)	2781 (5)
O(12)	3647 (9)	−1291 (9)	4653 (4)	1540 (13)	−1268 (11)	3981 (5)
O(2′)	1998 (10)	−2928 (9)	3833 (4)	3328 (11)	−2975 (10)	4769 (4)
O(4′)	2945 (10)	−1436 (9)	2653 (4)	2472 (11)	−1512 (10)	5973 (4)
O(1″)	3917 (10)	4063 (9)	5766 (4)	1998 (11)	4199 (10)	2949 (4)
O(2″)	3152 (10)	5020 (9)	5116 (4)	2809 (12)	4928 (11)	3670 (5)
O(3″)	1522 (10)	5047 (9)	5379 (4)	4512 (14)	4776 (14)	3429 (6)
O(4″)	1787 (12)	4731 (11)	6198 (5)	4255 (14)	4817 (13)	2621 (6)
O(W1)	2369 (18)	0335 (16)	1669 (7)			
O(W2)	3838 (17)	−0260 (15)	2113 (7)			

adoped by daunosamine as observed in daunomycin and carminomycin. The torsion angles differ by a maximum of 4°. The amino sugar (glycopyranose) has a chair conformation as indicated by the torsion angles, which, starting with a bond C(1′)–C(2′) and going clockwise, are −51°, 48°, −52°, 60°, −62°, and 58° (molecule 1) and −50°, 46°, −49°, 61°, −61°, and 57 (molecule II). Figures 4 and 5 show a comparison of the conformation of nogalamycin with that of daunomycin and carminoymcin after least-squares fitting of the aromatic B, C, and D rings. The major difference is in the relative disposition of sugar (nogalose in this study, and daunosamine in daunomycin and carminomycin). The torsion angle C(8)–C(7)–O(7)–C(1″) has a value of 109° and 99° (molecules I and II) in nogalamycin, while the values in daunomycin and carminomycin are 117° and 125°, respectively.

Packing and Hydrogen Bonding. Figures 6 and 7 show the packing of the molecules down the *a* and the *b* axis, respectively. The molecules are shaped like "⌐——¬" and are positioned in the cell with anthracycline plane parallel to the *c* axis and perpendicular to the *a* axis. The two molecules in the asymmetric unit stack with the planar parts parallel and are separated by a distance of 3.49 Å. Figure 8 shows the intermolecular hydrogen bonds. The intermolecular hydrogen bonds between the two molecules in the asymmetric unit are O(2′)–O(1) (3.19 Å), O(2′)–O(12) (2.78 Å). The water molecules are involved in extensive intermolecular hydrogen bonding with distances varying from 2.83 to 3.06 Å. The intramolecular hyrogen bonding involves O(4)→O(5) (2.56 Å), and O(6)→O(5) (2.61 Å), with the arrow indicating the probable direction of proton donation.

Table III. Selected Torsion Angles (deg): Comparison of the Selected Common Torsion Angles in Nogalamycin (I and II), Carminomycin, and Daunomycin

atoms	nogalamycins		carminomycin	daunomycin
	I	II		
C(6a)-C(7)-C(8)-C(9)	−39	−47	−48	−48
C(7)-C(8)-C(9)-C(10)	60	63	62	58
C(8)-C(9)-C(10)-C(10a)	−48	−47	−45	−38
C(9)-C(10)-C(10a)-C(6a)	19	18	18	14
C(10)-C(10a)-C(6a)-C(7)	−2	−4	−5	−4
C(10a)-C(6a)-C(7)-C(8)	8	18	20	20
C(6a)-C(7)-O(7)-C(1″)	−137	−134	−119	−114
C(8)-C(7)-O(7)-C(1″)	105	99	117	125
C(7)-O(7)-C(1″)-C(2″)	164	158	167	167
C(7)-O(7)-C(1″)-O(1″)	−73	−75	−70	−67
C(1″)-C(2″)-C(3″)-C(4″)	50	51	50	56
C(2″)-C(3″)-C(4″)-C(5″)	−51	−57	−54	−61
C(3″)-C(4″)-C(5″)-O(1″)	58	63	59	61
C(4″)-C(5″)-O(1″)-C(1″)	−61	−60	−61	−59
C(5″)-O(1″)-C(1″)-C(2″)	54	53	55	56
O(1″)-C(1″)-C(2″)-C(3″)	−53	−48	−50	−54

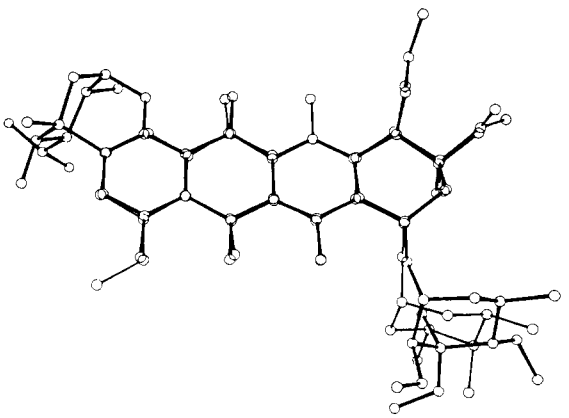

Figure 5. Comparison of the conformations of nogalamycin and carminomycin (thin bonds).

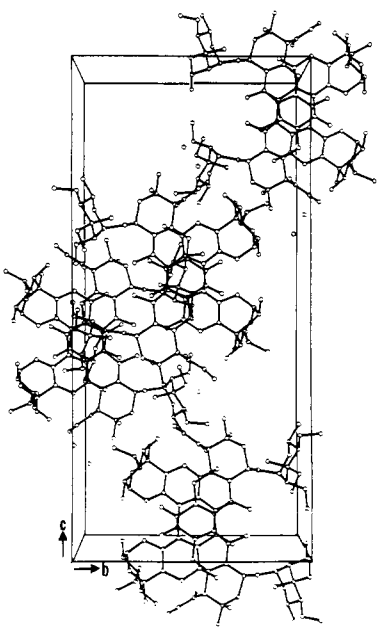

Figure 6. Packing of the molecules in the unit cell with *a* axis vertical.

Model for Interaction with DNA. Li et al.,[22] from their studies on the effect of nogalamycin on L1210 cells, have shown that the antibiotic strongly inhibits RNA and DNA synthesis. Circular

(22) Li, L. H.; Kuentzel, S. L.; Murch, L. L.; Pschigoda, M.; Kreuger, W. C. *Cancer Res.* **1979**, *39*, 4816–4822.

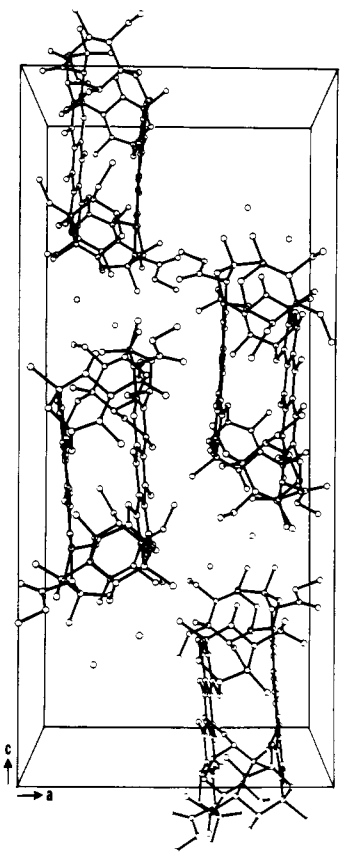

Figure 7. Packing of the molecules in the unit cell with *b* axis vertical.

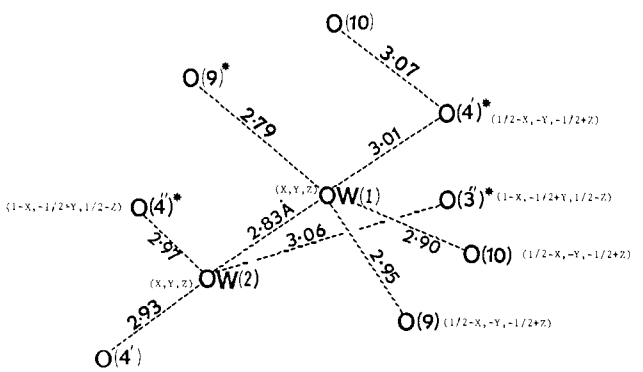

Figure 8. Intermolecular hydrogen bonding. The star refers to molecule II.

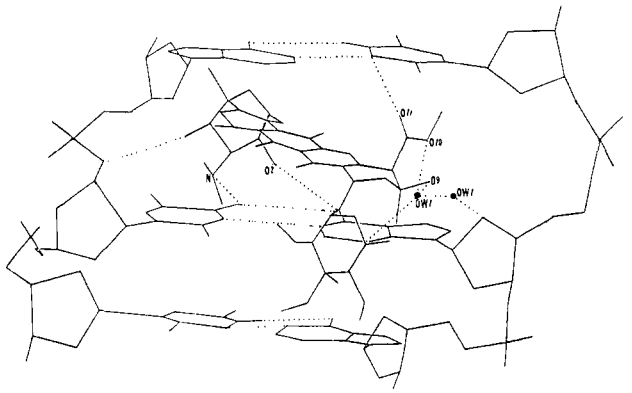

Figure 9. Possible mode of interaction of nogalamycin with DNA. Only base pairs AT, TA, and TA are shown.

dichroism studies[9] have shown that nogalamycin binds firmly to calf thymus DNA. This result is also supported by the results from the melting point increase technique. The DNA unwinding angle has a value of 8.1°.

Only one study[23] of an anthracycline (daunomycin) and DNA (CpGpTpApCpG) by crystallography has been done. It confirmed that daunomycin intercalates into DNA and that the amino sugar lies in the narrow groove. Fluorescence studies[8] have shown that nogalamycin has a preference for the A-T rather than G-C base pair. A study by DuVarney et al.[11] on anthracyclines revealed that stereospecificity of the carbomethoxy group at C(10) is very important for antitumor activity and DNA binding; i.e., it must be axial. Using the above information, along with the stereochemistry and molecular geometry of nogalamycin found in the present study and making use of Kendrew and CPK models of antibiotic and DNA, we have postulated a possible model for nogalamycin–DNA interaction in Figure 9. The base pairs AT, TA, and TA are shown. It has been assumed that water molecules are involved in antibiotic–DNA interaction. The primary interaction causing the binding of nogalamycin to DNA is of course, intercalation. Rings A and D will stick out (as in daunomycin), and the amino sugar and nogalose will lie in the wide and narrow grooves, respectively. The secondary forces of interaction will be hydrogen bonding involving (a) O(11) of the carbomethoxy group with O(2) of thymine in the top base pair, (b) the hydoxyl O(9), O(10), and backbone in the narrow groove through water molecules, and (c) O(2′) and nitrogen of amino sugar with thymine of the middle base as well as with the backbone in the wide groove. The nogalose may be involved in hydrogen bonding (since the oxygens of methoxyls are involved in hydrogen bonding in the crystal structure).

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Registry No. Nogalamycin, 1404-15-5.

Supplementary Material Available: Bond lengths, angles, and isotropic thermal parameters (2 pages). Ordering information is given on any current masthead page.

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Detailed Binding Sites of the Antibiotics Vancomycin and Ristocetin A: Determination of Intermolecular Distances in Antibiotic/Substrate Complexes by Use of the Time-Dependent NOE

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Abstract: The binding site of the antibiotic vancomycin for the peptide cell-wall analogue Ac-D-Ala-D-Ala has been studied by nuclear Overhauser effect difference spectroscopy (NOEDS). Intramolecular nuclear Overhauser effects (NOEs), observed between protons of the antibiotic in the ^{1}H spectrum of the vancomycin/Ac-D-Ala-D-Ala complex, confirm the formation of a carboxylate binding pocket in the bound state of the antibiotic. This pocket is not present in the X-ray structure of a compound, CDP-I, closely related to vancomycin; it appears to be induced by binding. The spectrum of the complex shows three intermolecular NOEs that define further the overall picture of the binding site, and one establishes that the hydrophobic side chain of N-terminal *N*-methylleucine is folded in to form a pocket that accommodates the carboxyl group of Ac-D-Ala-D-Ala. Additionally, by measuring the rate of buildup of the NOE, it is possible to measure intermolecular as well as intramolecular distances. This technique has been applied to a complex of ristocetin A with Ac_2-L-Lys-D-Ala-D-Ala, and intermolecular proton–proton distances have been determined. These studies also confirm the recent proposal that the N-terminal amino acid of ristocetin A has the *R* absolute configuration. Relative to the binding of Ac-D-Ala-D-Ala, the lysine residue is more weakly bound to both vancomycin and ristocetin, and its binding site less precisely defined.

In earlier work, we have determined the X-ray structure and conformation of a compound, CDP-I, obtained by conversion of a primary amide in the antibiotic vancomycin to a carboxyl group.[1] This structure was initially interpreted as representing the structure and conformation of vancomycin, except for the conversion noted above.[1] However, it has been shown subsequently that two other changes occur in the conversion of vancomycin to CDP-I. One is that the ring bounded by curved arrows in Figure 1 is rotated through ca. 180°;[2a] this brings both chlorine atoms to the same side ("top face") in CDP-I. The second is that the third amino

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