(1973).
(50) L. K. Hanson, M. Gouterman, and J. C. Hanson, *J. Am. Chem. Soc.*, 95, 4822 (1973).
(51) R. Countryman, D. M. Collins, and J. L. Hoard, *J. Am. Chem. Soc.*, **91**, 5166 (1969).
(52) L. J. Randonovich, A. Bloom, and J. L. Hoard, *J. Am. Chem. Soc.*, **94**, 2073 (1972).
(53) See discussion following A. Tulinsky, *Ann. N.Y. Acad. Sci.*, **206**, 47 (1973).
(54) J. L. Hoard, *Science*, **174**, 1295 (1971).
(55) J. P. Collman, R. R. Gagne, C. A. Reed, W. T. Robinson, and G. A. Rodley, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 1326 (1974).
(56) J. L. Hoard and W. R. Scheidt, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 3919 (1973).
(57) M. F. Perutz, *Nature (London)*, **237**, 995 (1972).
(58) W. H. Woodruff, T. G. Spiro, and T. Yonetani, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 1065 (1974).
(59) M. Perutz, *Nature (London)*, **228**, 726, 734 (1970).
(60) R. G. Little and J. A. Ibers, *J. Am. Chem. Soc.*, **96**, 4452 (1974).
(61) D. W. Smith and R. J. P. Williams, *Struct. Bonding (Berlin)*, **7**, 1 (1970).
(62) J. M. Rifkind in "Inorganic Biochemistry", Vol. 2, G. L. Eichhorn, Ed., Elsevier, New York, N.Y., 1973, p 832.
(63) G. R. Schonbaum, A. Romans, R. Felton, and N. T. Yu, unpublished data.
(64) G. R. Schonbaum, *J. Biol. Chem.*, **248**, 502 (1973).
(65) G. Rakshit and T. G. Spiro, *Biochemistry*, **13**, 5317 (1974).

Molecular and Crystal Structure of Streptonigrin

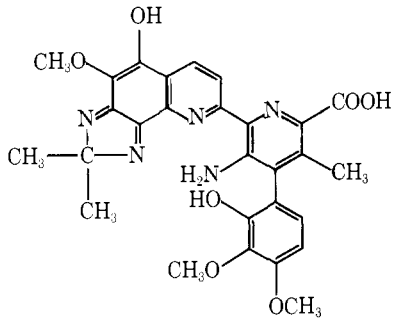
Yuan-Yuan H. Chiu and William N. Lipscomb*

Contribution from the Gibbs Chemical Laboratory, Harvard University, Cambridge, Massachusetts 02138. *Received August 16, 1974*

Abstract: The chemical structure assigned by Rao, Biemann, and Woodward is confirmed in an X-ray diffraction study of streptonigrin cocrystallized with ethyl acetate. Rings A, B, and C, including the carboxyl group on ring C, are very nearly coplanar. This configuration is partially maintained by a short, probably bent, hydrogen bond (2.65 Å) between the NH_2 of ring C and the N atom within ring B. The space group is $P2_12_12_1$, and there are four molecules of streptonigrin and four molecules of ethyl acetate in a unit cell having dimensions a = 13.676, b = 30.663, and c = 6.821 Å. For the 1677 independent X-ray diffraction maxima the value of $R = \sum||F_o| - |F_c||/\sum|F_o|$ is 0.098.

Streptonigrin, isolated by Rao and Cullen[1] as a metabolite of streptomyces flocculus, is an antibiotic. Showing activity against tumor,[2-4] against lymphoma,[5] and also against several viruses,[6] streptonigrin has a number of undesirable side effects[3] including severe bone marrow depression, nausea, vomiting, diarrhea, and alopecia. It causes chromosomal damage[7,8] when added to cultures of human leucocytes, inhibits DNA synthesis in tissue culture cells[9-11] and in bacteria,[9,12-14] and inhibits partial RNA and protein synthesis.[9] Streptonigrin also causes single strand breaks[7,8] on DNA upon reduction and subsequent reaction with oxygen.[14] At least two types of binding, reversible and irreversible (upon dialysis), occur when streptonigrin interacts with calf thymus DNA.[13] Reduction of streptonigrin is not required for binding, and association appears to occur primarily with the dCMP portion of DNA.

The bacteriocidal activity of streptonigrin is similar to that of mitomycin which also has an aminoquinone fragment,[15] a structural feature common to several closely related antitumor agents.[15,16] The possibility that the semiquinone form of streptonigrin is involved in its activity has been examined by electron spin resonance methods.[10,17,18]



A derivative, isopropylidene azastreptonigrin, has significant antitumor activity in animals only at 100 times the normal dosages of streptonigrin.[19] Even at 10^5 times the dosages, there is no antibactericidal effect or cytotoxicity in tissue cultures, no effect on DNA synthesis, and no activity against virus.[6,20] It also lacks the reduction and subsequent oxygenation reactions of streptonigrin, thus demonstrating the critical nature of the aminoquinone function region of streptonigrin.[19] Moreover, since the methyl ester of streptonigrin has $\frac{1}{100}$th the activity of streptonigrin in tissue culture cells,[20] $\frac{1}{500}$th the activity against Meloney leukemia virus replication, and $\frac{1}{3}$rd the activity in inhibiting the DNA polymerase of C-type RNA virus,[6] the carboxyl group probably is another chemical or stereochemical site of interest.

We report a crystal structure study of streptonigrin in which the aminoquinone function is intact. The three-dimensional ring orientations may be of value in elucidating its interactions with DNA or RNA, and may aid in interpreting later steps in its complex sequence of reactions in bacteria and in tumors.

Structure Determination. Fragile, dark brown, thick plates were grown as single crystals of streptonigrin cocrystallized with the solvent, ethyl acetate. The crystal data are given in Table I. The unit cell dimensions were determined from Weissenberg photographs on which Al powder diffraction lines were superimposed. Their values and errors were obtained by least squares by minimizing

$$\sum_{n=1}^{N} w_n(\sin^2 \theta_{obsd} - \sin^2 \theta_{calcd})^2$$

where the weights w_n are $1/\sin^2 \theta_{obsd}$, and N = 41 reflections. The experimental density was determined by suspension in a solution of n-heptane and carbon tetrachloride (Table I). Omission of the four ethyl acetate molecules led to an unreasonably low calculated density of 1.182 g/cm^3.

The structure was eventually solved from data taken from multiple level Weissenberg photographs of one crystal mounted about c (hkL for $0 \leq L \leq 5$) and a second crystal mounted about a (Hkl for $0 \leq H \leq 5$), using Cu Kα radiation which had been filtered through Ni foil. Aside from the extremely intense 002 and 022 reflections (>300), which

Table I. Crystal Data

Compd	$C_{25}H_{22}O_8N_4 \cdot CH_3COOC_2H_5$
Mol wt	594.6
Crystal system	Orthorhombic
Unit cell	a = 13.676 ± 0.005 Å
	b = 30.663 ± 0.009 Å
	c = 6.821 ± 0.002 Å
Systematic absences	$h00$ for h odd
	$0k0$ for k odd
	$00l$ for l odd
Space group	$P2_12_12_1$
Density	D_{exp} = 1.387 g/cm³
	D_{calcd} = 1.381 g/cm³ for Z = 4
$F(000)$	1248 electrons
μ (Cu Kα)	9.04 cm⁻¹

Table II. Final Positional Parameters of the Streptonigrin and Ethyl Acetate Molecules with Standard Deviations in Parentheses

Atoms	x	y	z
C(1)	0.4935 (7)	0.5177 (3)	0.4967 (21)
C(2)	0.5960 (8)	0.5020 (4)	0.4981 (24)
C(3)	0.6744 (8)	0.5356 (4)	0.5228 (22)
C(4)	0.6514 (8)	0.5775 (4)	0.5384 (24)
C(5)	0.5510 (8)	0.5934 (4)	0.5259 (22)
C(6)	0.4737 (8)	0.5615 (4)	0.5092 (19)
C(7)	0.3735 (8)	0.5741 (4)	0.4959 (23)
C(8)	0.3026 (8)	0.5431 (4)	0.4816 (20)
C(9)	0.3270 (7)	0.4987 (3)	0.4822 (20)
N(10)	0.4219 (6)	0.4865 (3)	0.4866 (18)
C(11)	0.2574 (7)	0.4621 (4)	0.4781 (20)
C(12)	0.2776 (8)	0.4175 (4)	0.4648 (21)
C(13)	0.2013 (8)	0.3865 (3)	0.4814 (19)
C(14)	0.1043 (8)	0.4011 (3)	0.5052 (20)
C(15)	0.0844 (8)	0.4450 (4)	0.5090 (22)
N(16)	0.1627 (6)	0.4753 (3)	0.4911 (17)
C(17)	0.2252 (9)	0.3394 (4)	0.4658 (22)
C(18)	0.2124 (10)	0.3165 (4)	0.2865 (20)
C(19)	0.2339 (11)	0.2719 (5)	0.2755 (24)
C(20)	0.2752 (10)	0.2500 (4)	0.4309 (20)
C(21)	0.2903 (9)	0.2727 (4)	0.6071 (20)
C(22)	0.2678 (9)	0.3171 (5)	0.6223 (21)
O(23)	0.5328 (6)	0.6333 (3)	0.5410 (16)
O(24)	0.7286 (6)	0.6070 (3)	0.5593 (15)
N(25)	0.7661 (7)	0.5200 (3)	0.5176 (21)
O(26)	0.6156 (6)	0.4627 (3)	0.4820 (18)
N(27)	0.3735 (7)	0.4037 (3)	0.4318 (18)
C(28)	0.0239 (9)	0.3674 (4)	0.5367 (23)
C(29)	−0.0091 (8)	0.4674 (4)	0.5181 (21)
O(30)	−0.0855 (6)	0.4475 (3)	0.5238 (20)
O(31)	−0.0081 (5)	0.5102 (2)	0.5111 (17)
O(32)	0.2818 (8)	0.3381 (3)	0.7945 (16)
O(33)	0.3297 (9)	0.2536 (3)	0.7740 (18)
O(34)	0.3014 (8)	0.2068 (3)	0.4314 (17)
C(35)	0.7370 (13)	0.6268 (5)	0.7497 (30)
C(36)	0.4287 (14)	0.2382 (5)	0.7541 (36)
C(37)	0.2827 (15)	0.1814 (5)	0.2623 (34)
C(38)	0.5437 (20)	0.3850 (8)	−0.1769 (32)
C(39)	0.5026 (19)	0.3699 (7)	−0.0066 (54)
O(40)	0.5432 (14)	0.3236 (5)	0.0429 (35)
C(41)	0.5170 (24)	0.2991 (9)	0.1946 (43)
C(42)	0.5639 (16)	0.2571 (6)	0.2290 (38)
O(43)	0.4893 (19)	0.3205 (7)	0.3607 (35)

suggested that the structure has a large planar part perpendicular to the crystallographic c axis, the remaining reflections were comparatively weak. Moreover, all reflections were badly smeared by diffuse streaking and varied in size as a function of θ and of μ (the equiinclination angle).[21] Therefore difficulty was encountered in solving the structure, and in its later refinement. In the following three paragraphs we describe briefly our unsuccessful attempts to solve the structure, our use of the MULTAN program to achieve a solution, and then refinement of a new set of X-ray data obtained by automatic scanning of the Weissenberg photographs.

Visual estimates of X-ray intensities were made on the Weissenberg photographs with the aid of a standard scale prepared from the same crystal. This great leap backward was believed to be necessary because the diffuseness, streaking, and variable size made counter measurements unreliable. After corrections for Lorentz and polarization factors, a list of 1652 unique reflections was obtained after minimizing[22]

$$R = \sum_{hi,\,hj} w_{hij}(\ln S_i I_{hi} - \ln S_j I_{hj})^2$$

where S_i is the scale factor for the ith set, I_{hi} is the intensity of I_h of set i, w_{hij} is $(\sigma_{hi}^2 + \sigma_{hj}^2)^{-1}$, and σ_{hi} is the statistical error in I_{hi}. The value of

$$R_C = \sum_h |I_{hi} - I_{hj}| / \sum_h |I_{hi} + I_{hj}|$$

was 0.081 over all observed data. These data were scaled initially by Wilson's method, and later by least-squares procedures. A three-dimensional sharpened Patterson function indicated that a large planar portion of the molecule was at $z = 0$ and at $z = \frac{1}{2}$, not at $z = \frac{1}{4}$ and $z = \frac{3}{4}$. While the orientation of six-membered rings was clearly indicated, the structure was not obtained from this Patterson function, from the application of superposition techniques,[23,24] or from the use of vector search methods[25] using two fused aromatic rings as the fragment. Also, probably owing to the unusual intensity distribution, the symbolic addition method[26] did not yield the structure. In using this method we calculated normalized squared structure factors

$$E_H^2 = F_H^2 / (\epsilon \sum_{j=1}^{N} f_{jH}^2)$$

where F_H^2 has been corrected for thermal motion, f_{jH} are atomic scattering factors, N is the number of atoms per unit cell, and ϵ is 2 for $h00$, $0k0$, and $00l$ reflections and ϵ is 1 otherwise. The extremely intense 002 and 022 reflections were not included in this procedure. Of the remaining centrosymmetric reflections most were weak, and one having E about 1.9 was chosen for origin determination. Indications of relationships among several starting symbolic sets were all weak, and further expansion of these by the tangent formula to include E's greater than 1.3 gave nearly centric phases and yielded uninterpretable maps. Tangent refinement[27] and F_o syntheses failed to improve these maps.

The MULTAN program,[28,29] which is a related multiple solution method, finally yielded the structure. The starting set was

h	k	l	E	ϕ
11	6	1	3.03	$\pm\pi/4$
8	19	1	3.16	$\pm\pi/4$
8	18	1	3.31	$\pi/4$
12	6	2	2.86	$\pm\pi/4$, $\pm 3\pi/4$
3	23	2	2.61	$\pm\pi/4$, $\pm 3\pi/4$

where 8,18,1 determines the enantiomorph, and 11,6,1 and 8,19,1 determine the origin. Reiterative application of the weighted tangent formula to 347 reflections having E greater than 1.3 (omitting 002 and 022) led to phases. A map based on E values having the second highest figure of merit[29] was interpretable, yielding 26 atoms which were in or near the plane at $z = \frac{1}{2}$ and which were consistent with the Patterson function. (The correct structure could not be found when E values greater than 1.5 were included.) The value of

$$R = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$$

was 0.46 when these 26 atoms were included with a thermal

Table III. Thermal Parameters Expressed as $10^4 \times \exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + \beta_{12}hk + \beta_{13}hl + \beta_{23}kl)]$

Atom	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	24 (6)	14 (1)	139 (30)	3 (5)	18 (30)	3 (14)
C(2)	53 (7)	11 (1)	221 (36)	7 (5)	−39 (39)	−2 (14)
C(3)	45 (7)	14 (1)	229 (41)	−3 (7)	−1 (39)	−29 (16)
C(4)	48 (7)	11 (1)	332 (47)	−10 (6)	−26 (40)	−3 (16)
C(5)	54 (8)	12 (1)	252 (51)	1 (6)	−42 (39)	3 (16)
C(6)	43 (7)	13 (1)	139 (33)	4 (5)	1 (38)	3 (15)
C(7)	62 (7)	11 (1)	216 (37)	6 (6)	33 (39)	−12 (16)
C(8)	47 (7)	11 (1)	195 (37)	3 (5)	31 (40)	−19 (14)
C(9)	38 (6)	11 (1)	139 (32)	6 (5)	25 (36)	−9 (15)
N(10)	49 (5)	11 (1)	215 (29)	5 (5)	−22 (29)	−6 (12)
C(11)	35 (6)	11 (1)	150 (33)	3 (5)	21 (29)	8 (14)
C(12)	38 (7)	12 (1)	232 (41)	4 (5)	−4 (33)	−14 (13)
C(13)	49 (7)	10 (1)	207 (34)	−2 (6)	−19 (36)	6 (14)
C(14)	47 (7)	12 (1)	161 (34)	−4 (5)	8 (39)	5 (16)
C(15)	40 (7)	13 (1)	186 (34)	0 (5)	−43 (36)	−3 (15)
N(16)	39 (5)	11 (1)	177 (30)	5 (4)	−23 (29)	15 (11)
C(17)	53 (7)	13 (1)	235 (39)	0 (6)	16 (39)	13 (15)
C(18)	78 (9)	12 (1)	196 (42)	0 (7)	−28 (40)	−13 (16)
C(19)	91 (11)	16 (2)	279 (49)	16 (8)	−81 (50)	−41 (18)
C(20)	71 (10)	13 (2)	162 (42)	9 (8)	8 (35)	15 (14)
C(21)	61 (9)	13 (2)	212 (46)	6 (7)	−20 (37)	7 (14)
C(22)	54 (9)	14 (1)	167 (38)	−6 (6)	−8 (33)	5 (14)
O(23)	70 (7)	11 (1)	336 (33)	0 (4)	−19 (27)	9 (11)
O(24)	50 (5)	17 (1)	278 (29)	−8 (4)	9 (26)	4 (11)
N(25)	45 (6)	16 (1)	383 (38)	−14 (5)	−39 (38)	−42 (19)
O(26)	48 (5)	13 (1)	420 (36)	2 (4)	−13 (29)	14 (12)
N(27)	51 (6)	10 (1)	350 (40)	9 (5)	28 (28)	−16 (13)
C(28)	57 (8)	12 (1)	277 (56)	−8 (6)	−62 (42)	8 (17)
C(29)	38 (6)	15 (1)	167 (34)	10 (6)	28 (37)	−9 (16)
O(30)	52 (5)	16 (1)	456 (38)	2 (5)	−27 (31)	−10 (14)
O(31)	53 (5)	14 (1)	314 (29)	13 (4)	6 (28)	6 (12)
O(32)	109 (8)	16 (1)	266 (31)	8 (6)	−34 (32)	0 (12)
O(33)	114 (9)	15 (1)	397 (40)	11 (7)	−61 (36)	11 (13)
O(34)	113 (8)	11 (1)	357 (35)	10 (5)	−85 (32)	1 (12)
C(35)	75 (11)	23 (3)	445 (66)	−7 (9)	42 (57)	−12 (24)
C(36)	105 (13)	20 (2)	724 (95)	27 (9)	−140 (64)	52 (24)
C(37)	144 (18)	18 (2)	564 (71)	13 (12)	−68 (78)	−76 (22)
C(38)	208 (24)	43 (5)	342 (75)	10 (21)	−50 (86)	38 (39)
C(39)	189 (29)	31 (4)	1124 (160)	38 (100)	−45 (139)	61 (61)
O(40)	238 (19)	33 (3)	876 (12)	−8 (12)	−125 (100)	48 (32)
C(41)	299 (41)	54 (7)	679 (137)	−54 (27)	454 (115)	−95 (50)
C(42)	173 (20)	23 (3)	701 (100)	63 (13)	33 (81)	44 (31)
O(43)	396 (32)	52 (4)	819 (106)	39 (23)	203 (125)	−41 (40)

parameter B of 3.6 Å^2. An electron density map based on phases calculated from these atoms then yielded the fourth ring (atoms 18-22, and 32-34), but two possible positions related by a pseudomirror at $z = \frac{1}{2}$ were present for all atoms except 20. Inclusion of these atoms yielded $R = 0.34$ for the eventually correct structure, and $R = 0.36$ for the structure based on positions related by this pseudomirror. A new electron density map then yielded all 43 nonhydrogen atoms of streptonigrin and ethyl acetate. At this stage the scattering factor of N was assigned to the acetyl C(42) and the O(43) of ethyl acetate. Several cycles of least-squares refinements of scale, positional, and isotropic thermal parameters reduced R to 0.20, where we minimized

$$\Sigma w[|F_o|^2 - |F_c|^2]^2$$

in which $w = |F_o|^{-2}$. The atomic identifications of the acetyl group were based upon bond distances and thermal parameters in these refinements. Further refinement using anisotropic thermal parameters yielded $R = 0.146$, and

$$R_w = \Sigma w[|F_o|^2 - |F_c|^2]/\Sigma w|F_o|^2 = 0.052$$

At this stage, an Optronics film scanner, and computer program,[30] became available. Depending on the μ and Υ angle, different sizes of rectangular boxes were chosen as the reflection spot scan areas. Entire films of all levels were scanned at 100 μ intervals. Then the peak of optical density for each reflection was located, and all optical density greater than or equal to $\frac{1}{3}$ of this peak height around a given center was added to give the total intensity of that reflection. An average optical density in the region of each reflection, but far enough away to contain no appreciable contribution from the reflection, was taken as background. Appropriate background corrections were then made, reflections common to multiple films of the same level were scaled together, and Lorentz and polarization corrections were made. The four asymmetric units of each level were correlated to one unique (average) asymmetric unit. Finally, all data of different levels about the two axes were correlated to a single list of 1677 unique reflections having an R_c value of 0.064. Assuming isotropic thermal parameters for each nonhydrogen atom, we refined these new measurements of X-ray data to an R value of 0.14. Having anisotropic thermal parameters and scale and positional parameters in three matrices, several cycles of refinement led to an R value of 0.114. At this stage a difference electron density map revealed all H atoms except for those bonded to C(36) and C(37) of the streptonigrin molecule and those of the solvent molecule. Assignment of a fixed thermal parameter of 3.6 Å^2 and of fixed positions for H atoms then led upon refinement of all other parameters to an R value of 0.108. After another difference electron density, and introduction of disordered methyl hydrogens bonded to C(36) and C(37), a similar further refinement yielded the final value of $R = 0.098$. A final difference electron density map from which all atoms were subtracted yielded no peaks higher than 0.25 e/Å^3.

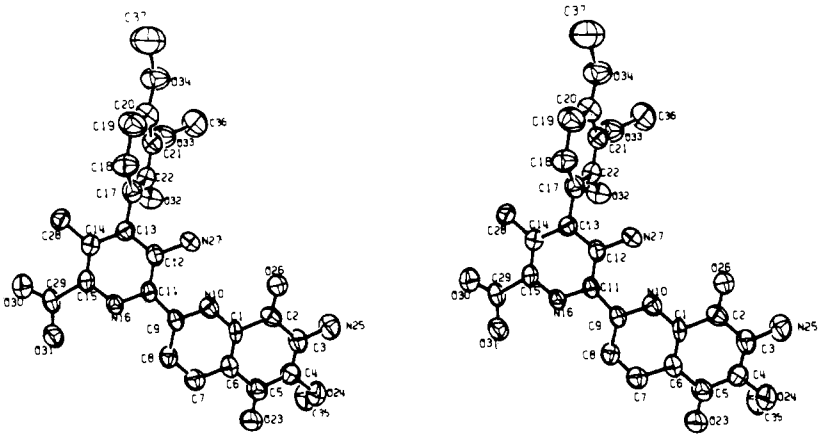


Figure 1. Stereoscopic showing of the molecule and numbering scheme for streptonigrin.

Table IV. Positional Parameters of the Hydrogen Atoms[a]

Atoms	Bonded to	Pop.	x	y	z
H(44)	C(8)	1	0.234	0.550	0.470
H(45)	C(7)	1	0.358	0.604	0.472
H(46)	C(18)	1	0.147	0.326	0.188
H(47)	C(19)	1	0.215	0.258	0.105
H(48)	N(25)	1	0.770	0.492	0.423
H(49)	N(25)	1	0.807	0.534	0.622
H(50)	O(31)	1/2	0.041	0.526	0.445
H(51)	O(31)	1/2	−0.032	0.523	0.643
H(52)	C(35)	1	0.808	0.645	0.758
H(53)	C(35)	1	0.671	0.640	0.812
H(54)	C(35)	1	0.765	0.602	0.862
H(55)	N(27)	1	0.420	0.435	0.453
H(56)	N(27)	1	0.375	0.368	0.416
H(57)	C(28)	1	0.048	0.339	0.556
H(58)	C(28)	1	−0.023	0.376	0.656
H(59)	C(28)	1	−0.016	0.365	0.393
H(60)	O(32)	1/2	0.300	0.312	0.899
H(61)	O(32)	1/2	0.355	0.328	0.831
H(62)	C(36)	1/2	0.475	0.258	0.826
H(63)	C(36)	1/2	0.461	0.237	0.898
H(64)	C(36)	1/2	0.436	0.207	0.827
H(65)	C(36)	1/2	0.432	0.207	0.671
H(66)	C(36)	1/2	0.448	0.229	0.620
H(67)	C(36)	1/2	0.475	0.262	0.680
H(68)	C(37)	1/2	0.342	0.168	0.179
H(69)	C(37)	1/2	0.310	0.152	0.265
H(70)	C(37)	1/2	0.233	0.155	0.294
H(71)	C(37)	1/2	0.209	0.176	0.231
H(72)	C(37)	1/2	0.235	0.198	0.165
H(73)	C(37)	1/2	0.303	0.196	0.133

[a] Positional parameters of hydrogens were obtained from successful difference Fourier maps.

Table V. Bond Distances (Å) of the Streptonigrin and Ethyl Acetate Molecules with Standard Deviations[a] in Parentheses

Bonds	Bond distances	Bonds	Bond distances
C(1)–C(2)	1.48 (1)	C(14)–C(28)	1.52 (1)
C(1)–C(6)	1.37 (1)	C(15)–N(16)	1.38 (1)
C(1)–N(10)	1.37 (1)	C(15)–C(29)	1.50 (1)
C(2)–C(3)	1.49 (1)	C(17)–C(18)	1.42 (2)
C(2)–O(26)	1.24 (1)	C(17)–C(22)	1.40 (2)
C(3)–C(4)	1.33 (1)	C(18)–C(19)	1.40 (2)
C(3)–N(25)	1.34 (1)	C(19)–C(20)	1.38 (2)
C(4)–C(5)	1.46 (1)	C(20)–C(21)	1.41 (2)
C(4)–O(24)	1.40 (1)	C(20)–O(34)	1.37 (1)
C(5)–C(6)	1.45 (1)	C(21)–C(22)	1.40 (2)
C(5)–O(23)	1.25 (1)	C(21)–O(33)	1.39 (2)
C(6)–C(7)	1.43 (1)	C(22)–O(32)	1.35 (2)
C(7)–C(8)	1.36 (1)	O(24)–C(35)	1.44 (2)
C(8)–C(9)	1.40 (1)	C(29)–O(30)	1.21 (1)
C(9)–N(10)	1.35 (1)	C(29)–O(31)	1.31 (1)
C(9)–C(11)	1.47 (1)	O(33)–C(36)	1.44 (2)
C(11)–C(12)	1.40 (1)	O(34)–C(37)	1.42 (2)
C(11)–N(16)	1.36 (1)	C(38)–C(39)	1.37 (4)
C(12)–C(13)	1.42 (1)	C(39)–O(40)	1.56 (3)
C(12)–N(27)	1.40 (1)	O(40)–C(41)	1.33 (4)
C(13)–C(14)	1.41 (1)	C(41)–C(42)	1.46 (3)
C(13)–C(17)	1.49 (1)	C(41)–O(43)	1.36 (4)
C(14)–C(15)	1.37 (1)		

[a] Standard deviations were calculated from the errors of atomic coordinates.

Results and Discussion

Positional parameters are given for nonhydrogen atoms (Table II). We also give their thermal parameters (Table III) and hydrogen atom coordinates (Table IV). Bond distances (Table V) and angles (Table VI) exclude the hydrogen atoms. We note that bond distances and angles of the solvent molecule are generally less accurate, probably because of their relatively large thermal parameters. The C–H distances varying from 0.9 to 1.2 Å are also comparatively unreliable.

The properly numbered molecular structure of streptonigrin is shown as a stereoview in Figure 1 and with bond distances in Figure 2. Details of molecular packing viewed along the c axis are shown in Figure 3. Almost three quarters of the streptonigrin molecule lies nearly in the same plane. The direction cosines of the plane normal to the two fused rings with respect to the real crystallographic axes are [−0.0541, −0.0589, 0.9968], therefore the fused rings make an angle of 85° with the $+c$ axis. The direction cosines of the plane normal to ring III and the carboxyl group are [0.0774, 0.0031, 0.9970], therefore, this plane also makes an angle of 85° with the $+c$ axis. However, the direction cosines of the plane normal to ring IV are [0.9184, 0.2452, −0.3106]; thus this plane makes an angle of 162° with respect to the $+c$ axis.

Bond distances in ring A (Figure 2) seem very appropriate for a quinoid structure. The single C-C bonds in this ring, and elsewhere in the molecule, fall in the range 1.45–1.50 Å appropriate for sp^2 σ bonds. Also distances in the aromatic rings are reasonable, and the difference in C–O distances within the carboxyl group is sufficient to allow assignment of the site of proton attachment. The intramolecular hydrogen bond between N(10) and the amino N(27) is 2.65 Å, rather short for an N···N distance in a hydrogen bond. It appears that the hydrogen bond itself is bent: N(10)-H(55) = 1.16 Å, N(27)···H(55) = 1.60 Å, and ∠N(10)-H(55)···N(27) = 148°. However, the hydrogen

Table VI. Bond Angles (deg) of the Streptonigrin and Ethyl Acetate Molecules with Standard Deviations in Parentheses[a]

Angles		Angles	
C(2)–C(1)–C(6)	120 (2)	C(13)–C(14)–C(15)	118 (2)
C(2)–C(1)–N(10)	117 (2)	C(13)–C(14)–C(28)	119 (2)
C(6)–C(1)–N(10)	123 (2)	C(15)–C(14)–C(28)	123 (2)
C(1)–C(2)–C(3)	117 (2)	C(14)–C(15)–N(16)	123 (2)
C(1)–C(2)–O(26)	121 (2)	C(14)–C(15)–C(29)	126 (2)
C(3)–C(2)–O(26)	122 (2)	N(16)–C(15)–C(29)	111 (2)
C(2)–C(3)–C(4)	120 (2)	C(11)–N(16)–C(15)	120 (2)
C(2)–C(3)–N(25)	115 (2)	C(13)–C(17)–C(18)	121 (2)
C(4)–C(3)–N(25)	125 (2)	C(13)–C(17)–C(22)	121 (2)
C(3)–C(4)–C(5)	123 (2)	C(18)–C(17)–C(22)	118 (2)
C(3)–C(4)–O(24)	117 (2)	C(17)–C(18)–C(19)	120 (2)
C(5)–C(4)–O(24)	120 (2)	C(18)–C(19)–C(20)	121 (2)
C(4)–C(5)–C(6)	118 (2)	C(19)–C(20)–C(21)	118 (2)
C(4)–C(5)–O(23)	121 (2)	C(19)–C(20)–O(34)	125 (2)
C(6)–C(5)–O(23)	122 (2)	C(21)–C(20)–O(34)	116 (2)
C(1)–C(6)–C(5)	122 (2)	C(20)–C(21)–C(22)	121 (2)
C(1)–C(6)–C(7)	117 (2)	C(20)–C(21)–O(33)	123 (2)
C(5)–C(6)–C(7)	122 (2)	C(22)–C(21)–O(33)	116 (2)
C(6)–C(7)–C(8)	120 (2)	C(17)–C(22)–C(21)	121 (2)
C(7)–C(8)–C(9)	121 (2)	C(17)–C(22)–O(32)	119 (2)
C(8)–C(9)–N(10)	120 (2)	C(21)–C(22)–O(32)	120 (2)
C(8)–C(9)–C(11)	126 (2)	C(4)–O(24)–C(35)	115 (2)
N(10)–C(9)–C(11)	114 (2)	C(15)–C(29)–O(30)	123 (2)
C(1)–N(10)–C(9)	120 (2)	C(15)–C(29)–O(31)	116 (2)
C(9)–C(11)–C(12)	128 (2)	O(30)–C(29)–O(31)	121 (2)
C(9)–C(11)–N(16)	113 (2)	C(21)–O(33)–C(36)	115 (2)
C(12)–C(11)–N(16)	120 (2)	C(20)–O(34)–C(37)	119 (2)
C(11)–C(12)–C(13)	120 (2)	C(38)–C(39)–O(40)	110 (3)
C(11)–C(12)–N(27)	120 (2)	C(39)–C(40)–C(41)	126 (3)
C(13)–C(12)–N(27)	120 (2)	O(40)–C(41)–C(42)	120 (4)
C(12)–C(13)–C(14)	119 (2)	O(40)–C(41)–O(43)	117 (4)
C(12)–C(13)–C(17)	119 (2)	C(42)–C(41)–O(43)	114 (4)
C(14)–C(13)–C(17)	122 (2)		

[a] Standard deviations were calculated from the errors in the atomic coordinates.

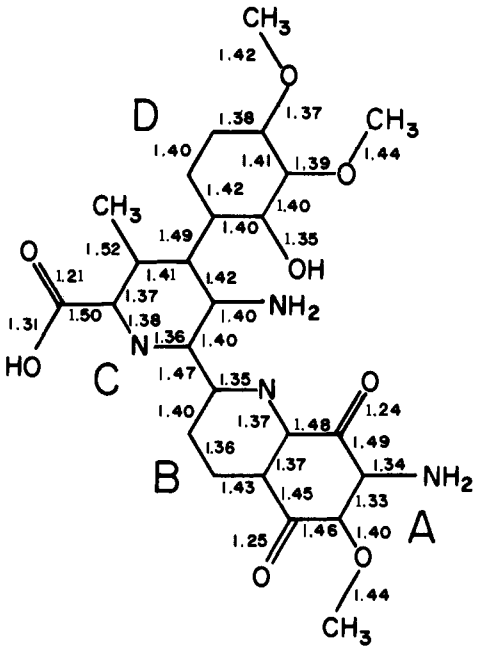


Figure 2. Bond distances (Å) in the streptonigrin molecule. The quinoid structure of ring A partially fixes the aromatic preference in B. Ring C is somewhat influenced by the ring N atom, while ring D is fully aromatic within the standard deviations of about 0.02 Å for this ring (Table IV). Ring D is tilted out of the plane of this figure (see Figure 1).

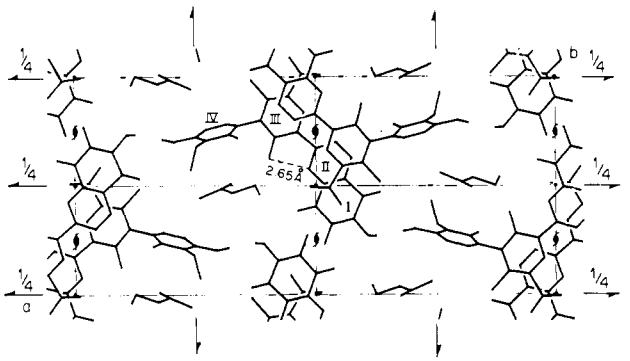


Figure 3. The packing of streptonigrin and ethyl acetate molecules in the unit cell.

atom is not well located in our study. Nevertheless, this intramolecular hydrogen bond is most likely responsible for the near coplanarity of ring C with rings A and B.

Some intermolecular contacts are suggestive of $\pi\cdots\pi$ or $\mathbf{n}\cdots\pi$ interactions. Ring A is near the carboxyl group of a symmetry-related molecule. A carboxyl oxygen O(30) is almost at the midpoint between quinone rings of different molecules related by the c-axis translation and is 3.4 Å from the ring. If this is an $n\cdots\pi$ interaction, it is surely weak. Also ring B is 3.4 Å from a parallel ring C of an adjacent molecule related by a 2_1 parallel to c. Again, this may be a weak $\pi\cdots\pi$ interaction. All intermolecular contacts in the entire crystal are in the usual ranges of van der Waals distances for first row atoms.

Ordinarily the discovery of a large nearly coplanar region (rings A, B and C of streptonigrin) in a molecule which interacts with DNA produces a theory based upon intercalation, for example of the type shown in the elegant study[31] of the 1:2 complex of actinomycin D with deoxyguanosine. However, intercalation is not an important mode of binding of streptonigrin to calf thymus DNA, as shown[13] by lack of hypochromic spectral shift, and lack of changes in T_m or viscosity. Single strand breaks do occur, however, and preferential binding of streptonigrin occurs during the S phase of the cell cycle of L929 mouse fibroblasts. This result and preferential binding to denatured or acid-treated DNA suggest that streptonigrin binds to portions of DNA that are not in the double helical conformation. Hence we forego the suggestion of intercalation of streptonigrin into the DNA structure. It therefore seems clear that one must look to local reactivity in the streptonigrin molecule, most probably in the aminoquinone region.[15]

Acknowledgment. We wish to thank the National Institutes of Health Grant 06920 for support of this research. We are also indebted to R. B. Woodward for supplying the sample, and J. L. Crawford, M. M. Woolfson, R. E. Marsh, and G. N. Reeke, Jr. for the computer programs.

Supplementary Material Available. The complete list of observed and calculated structure factors will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.50 for photocopy or $2.50 for microfiche, referring to code number JACS-75-2525.

References and Notes

(1) K. V. Rao and W. P. Cullen, *Antibiot. Annu.*, 950 (1959–1960).
(2) J. J. Oleson, L. A. Calderella, K. J. Mjos, A. R. Reith, R. S. Thle, and I. Toplin, *Antibiot. Chemother. (Basel)*, 11, 158 (1961).
(3) W. L. Wilson, C. Labra, and E. Barrist, *Antibiot. Chemother. (Basel)*, 11, 147 (1961).
(4) T. J. McBride, J. J. Oleson, and D. Woolf, *Cancer Res.*, **26A**, 727 (1966).
(5) See "Single Agents in Cancer Chemotherapy", R. B. Livingston and S.

K. Carter, IFI/Plenum, New York, N.Y., 1970, pp 389–392.
(6) M. A. Chirigos, J. W. Pearson, T. S. Papas, W. A. Woods, H. B. Wood, Jr., and G. Spahn, *Cancer Chemother. Rep.*, **57**, 305 (1973).
(7) M. M. Cohen, *Cytogenetics*, **2**, 271 (1963).
(8) M. M. Cohen, M. W. Shaw, and A. P. Craig, *Proc. Natl. Acad. Sci. U.S.*, **50**, 16 (1963).
(9) N. S. Mizuno, *Biochim. Biophys. Acta*, **108**, 394 (1965).
(10) H. L. White and J. R. White, *Mol. Pharmacol.*, **4**, 549 (1968).
(11) C. W. Young and S. Hodas, *Biochem. Pharmacol.*, **14**, 205 (1965).
(12) M. Levine and M. Barthwick, *Virology*, **21**, 568 (1963).
(13) N. S. Mizumo and D. P. Gilboe, *Biochim. Biophys. Acta*, **224**, 319 (1970).
(14) H. L. White and J. R. White, *Biochim. Biophys. Acta*, **123**, 648 (1966).
(15) K. V. Rao, K. Biemann, and R. B. Woodward, *J. Am. Chem. Soc.*, **85**, 2532 (1963).
(16) L. D. Hamilton, W. Fuller, and E. Reich, *Nature (London)*, **198**, 538 (1963).
(17) J. R. White and H. L. White, *Antimicrob. Agents Chemother.*, 495 (1964).
(18) J. R. White and H. H. Dearman, *Proc. Natl. Acad. Sci. U.S.*, **54**, 887 (1965).
(19) W. B. Kremer and J. Laszlo, *Cancer Chemother. Rep.*, 51, 19 (1967).
(20) N. S. Mizuno, *Biochem. Pharmacol.*, **16**, 933 (1967).
(21) Examples of these photographs appear in the Ph.D. Thesis of Y.-Y. Chiu, Harvard University, 1975.
(22) (a) P. G. Simpson, Ph.D. Thesis, Harvard University, 1963; (b) A. D. Rae, *Acta Crystallogr.*, 19, 683 (1965); (c) A. D. Rae and A. B. Blake, *ibid.*, **20**, 5861 (1966).
(23) S. Raman and W. N. Lipscomb, *Z. Kristallogr., Kristallgeom., Kristallphys., Kristallchem.*, 116, 314 (1961).
(24) P. G. Simpson, R. D. Dobrott, and W. N. Lipscomb, *Acta Crystallogr.*, **18**, 169 (1965).
(25) (a) C. E. Nordman and S. K. Kumra, *J. Am. Chem. Soc.*, **87**, 2059 (1965); (b) C. E. Nordman, *Trans. Am. Crystallogr. Assoc.*, **2**, 29 (1966).
(26) J. Karle and I. L. Karle, *Acta Crystallogr.*, **21**, 849 (1966).
(27) J. Karle, *Acta Crystallogr., Sect. B.*, **24**, 182 (1968).
(28) G. German, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. B*, **26**, 274 (1970).
(29) G. German, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, **27**, 368 (1971).
(30) J. L. Crawford, PDP11 computer program SCAN10 for scanning Weissenberg photographs, Harvard University, 1974.
(31) (a) S. C. Jain and H. M. Sobell, *J. Mol. Biol.*, **68**, 1 (1972); (b) H. M. Sobell and S. C. Jain, *ibid.*, **68**, 21 (1972).