

The Crystal and Molecular Structure of Naphthyridinomycin, C₂₁H₂₇N₃O₆, a Broad Spectrum Antibiotic

BY JURGEN SYGUSCH, FRANÇOIS BRISSE AND STEPHEN HANESSIAN

Département de Chimie, Université de Montréal, C.P. 6210 Montréal, P.Q., H3C 3V1, Canada

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The crystals of naphthyridinomycin, C₂₁H₂₇N₃O₆, belong to the orthorhombic system, $a=11.038$ (1), $b=19.560$ (2), $c=9.255$ (1) Å and the space group is $P2_12_12_1$. The structure was solved by the symbolic addition procedure. Anisotropic least-squares refinement was concluded with a final $R_w(|F|^2)$ value of 0.070 for 1904 measured reflexions. None of the bond distances and angles differ significantly from the expected values. A novel type of intramolecular hydrogen bonding is observed, between a hydroxyl group and a tertiary amine.

Introduction

Recently a new broad spectrum antibiotic, naphthyridinomycin, was isolated from a streptomycete (Kluepfel, Baker, Piattoni, Sehgal, Sidorowicz, Singh & Vézina, 1975). The antibiotic exhibited *in vitro* inhibition of RNA and protein biosynthesis as well as cell wall formation. Microanalytical data suggested the empirical formula C₇H₉NO₂. The molecular formula C₂₁H₂₇N₃O₆ was obtained by high resolution mass spectrometry. The crystal structure determination of this antibiotic was then undertaken and a preliminary communication (Sygusch, Brisse, Hanessian & Kluepfel, 1974) revealed the peculiar conformation of the molecule. The purpose of this paper is to report the crystallographic studies in more detail.

Experimental

The pure antibiotic was obtained as ruby red crystals by recrystallization of naphthyridinomycin in warm

ether. Precession photographs indicated that the crystals were orthorhombic, space group $P2_12_12_1$ as indicated by the systematic absences ($h00$, $h \neq 2n$; $0k0$, $k \neq 2n$; $00l$, $l \neq 2n$). The unit-cell dimensions, determined on a Picker FACS-I diffractometer by a least-squares fit to the settings of 12 reflexions, are presented below together with other relevant crystal data.

Crystal data

C₂₁H₂₇N₃O₆, F.W.417.45, $F(000)=864$, m.p.108–110° (dec). Orthorhombic, $P2_12_12_1$, $Z=4$, $a=11.038$ (1), $b=19.560$ (2), $c=9.255$ (1) Å; $V=1998.8$ Å³, $\rho_{\text{obs}}=1.39$ g cm⁻³ (floatation), $\rho_{\text{cal}}=1.387$ g cm⁻³, $\mu(\text{Cu } K\alpha)=8.6$ cm⁻¹, $\lambda(\text{Cu } K\alpha)=1.54178$ Å, $T=20$ (1)°C.

Intensities for 1904 unique reflexions with $2\theta \leq 124^\circ$ were measured using graphite-monochromatized Cu $K\alpha$ radiation and the θ - 2θ scan technique with a 1° min^{-1} scan speed. Backgrounds were measured for 40 s on each side of the reflexion. The average intensity of three reference reflexions, monitored every 30 reflexions, decreased uniformly by about 10% of its initial value during the data collection. All the intensities were corrected for background and placed on a common scale. The data were then corrected for Lorentz, polarization and absorption effects. To correct for secondary extinctions, $dA^*/d\mu$ was evaluated for all reflexions (Åsbrink & Werner, 1966). The standard deviation for the net intensity of a reflexion was calculated according to $\sigma(I)=[I_T + (t/80)^2 B + (0.03 I_N)^2]^{1/2}$, I_T being the total count, B the total background, I_N the net count and t the scanning time over one reflexion. Of the 1904 measured reflexions, only 42 were such that $I \leq 2.0\sigma(I)$.

Structure determination and refinement

The structure was solved by the symbolic addition method using 195 reflexions with normalized structure factors $|E| \geq 1.50$. The first 30 peaks from the E map yielded the entire structure. Two cycles of full-matrix isotropic least-squares refinement based on $|F|$

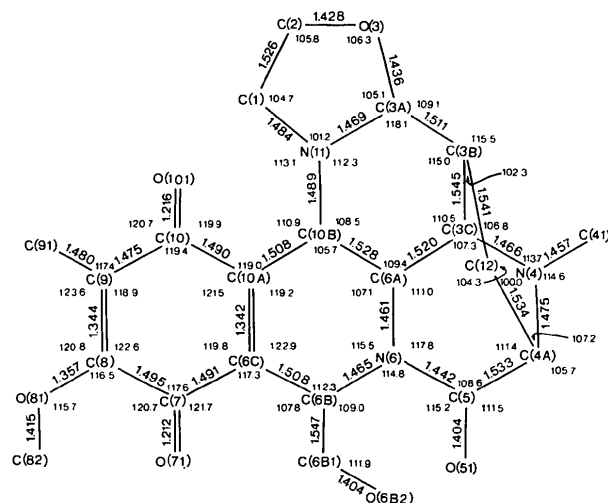


Fig. 1. Schematic diagram showing the numbering of the atoms and the bond distances and angles.

reduced $R(|F|) = \sum \Delta F / \sum F_o$ to 0.165. The N and O atoms were identified by consideration of their thermal parameters. Two cycles of block-diagonal anisotropic refinement led to $R(|F|) = 0.076$. Two successive difference Fourier syntheses revealed all the H atoms. From then on the refinement was continued with $|F|^2$

coefficients. Two cycles of refinement with isotropic H temperature factors reduced $R(|F|^2)$ to 0.078. Each reflexion was assigned a weight w inversely proportional to the variance derived from the value of $\sigma(J)$: $w = 1/\sigma^2(F)$. Reflexions with $I \leq 2.0\sigma(J)$ were excluded from the refinement. Anisotropic refinement for all

Table 1. Fractional coordinates, thermal parameters and their e.s.d.'s

The thermal parameters are the coefficients of the expression: $T = \exp[-2\pi^2(U_{11}a^{*2}h^2 + \dots + 2U_{12}a^*b^*hk + \dots)]$. The coordinates are in fractions of unit-cell edges $\times 10^5$ for O, N and C atoms and $\times 10^4$ for the H atoms. The thermal parameters are given $\times 10^4$ for O, N and C and $\times 10^3$ for H atoms. The standard deviations refer to the least significant digit.

	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i> ₁₁	<i>U</i> ₂₂	<i>U</i> ₃₃	2 <i>U</i> ₁₂	2 <i>U</i> ₁₃	2 <i>U</i> ₂₃
C(1)	20195 (19)	47353 (9)	14639 (19)	741 (11)	643 (10)	494 (8)	-115 (4)	-40 (5)	-6 (4)
C(2)	20895 (25)	48657 (11)	30918 (22)	1039 (17)	796 (12)	564 (10)	-195 (6)	-36 (6)	-15 (5)
O(3)	17316 (15)	42377 (7)	37567 (14)	1017 (10)	734 (7)	414 (6)	-114 (4)	-61 (3)	-8 (3)
C(3A)	18125 (18)	37200 (9)	26517 (19)	631 (10)	660 (9)	435 (8)	-31 (4)	-61 (4)	5 (4)
C(3B)	11147 (17)	30946 (9)	31407 (17)	664 (10)	589 (8)	415 (8)	3 (4)	-40 (4)	24 (4)
C(3C)	-2525 (15)	31055 (9)	28058 (17)	609 (10)	456 (7)	385 (7)	2 (3)	13 (4)	6 (3)
N(4)	-6580 (15)	23917 (7)	27627 (16)	714 (9)	491 (6)	492 (7)	-24 (3)	35 (3)	30 (3)
C(4A)	3427 (18)	20835 (8)	19359 (20)	754 (11)	456 (7)	545 (9)	21 (4)	24 (4)	16 (4)
C(41)	-8387 (24)	20925 (11)	42024 (23)	1040 (16)	663 (10)	596 (11)	-20 (6)	80 (6)	94 (5)
C(5)	934 (17)	22616 (8)	3496 (19)	653 (10)	450 (8)	522 (8)	-6 (4)	22 (4)	-19 (3)
O(51)	-9676 (14)	19444 (7)	-1594 (15)	873 (9)	611 (7)	613 (7)	-110 (3)	5 (3)	-36 (3)
N(6)	1139 (13)	29965 (6)	1923 (14)	592 (7)	427 (6)	397 (6)	-3 (3)	3 (3)	-10 (3)
C(6A)	-4762 (14)	34045 (7)	13101 (16)	491 (8)	442 (7)	404 (7)	2 (3)	1 (3)	-9 (3)
C(6B)	-396 (16)	32423 (8)	-12923 (17)	599 (9)	487 (8)	392 (7)	-29 (4)	-9 (4)	-25 (3)
C(6B1)	11790 (19)	31737 (9)	-20990 (20)	747 (11)	575 (9)	478 (8)	-2 (4)	48 (4)	-33 (4)
O(6B2)	20985 (12)	35650 (6)	-14261 (15)	628 (7)	669 (7)	622 (7)	-8 (3)	51 (3)	-23 (3)
C(6C)	-4271 (14)	39828 (8)	-13454 (17)	529 (8)	513 (8)	403 (7)	-32 (3)	-11 (3)	7 (3)
C(7)	-9339 (16)	42399 (9)	-27338 (17)	594 (10)	646 (9)	413 (8)	-37 (4)	-34 (4)	17 (4)
O(71)	-11267 (16)	38577 (8)	-37483 (14)	1102 (11)	728 (7)	463 (7)	-69 (4)	-105 (4)	0 (3)
C(8)	-12421 (17)	49830 (9)	-28309 (19)	575 (9)	682 (9)	497 (9)	4 (4)	-39 (4)	64 (4)
O(81)	-17089 (14)	52061 (8)	-41069 (15)	810 (9)	924 (9)	570 (7)	20 (4)	-92 (3)	99 (4)
C(82)	-8742 (29)	52502 (14)	-52670 (24)	1213 (20)	998 (16)	525 (10)	-18 (7)	-33 (7)	106 (6)
C(9)	-11394 (18)	54051 (9)	-17026 (20)	629 (10)	600 (9)	593 (10)	19 (4)	-47 (4)	43 (4)
C(91)	-14699 (27)	61474 (11)	-17466 (29)	1133 (19)	647 (11)	863 (16)	116 (6)	-124 (7)	47 (5)
C(10)	-6938 (17)	51369 (8)	-3052 (19)	623 (9)	464 (8)	530 (8)	8 (4)	-27 (4)	3 (4)
O(101)	-6129 (16)	55079 (6)	7481 (15)	1131 (11)	475 (6)	591 (7)	25 (3)	-57 (4)	-31 (4)
C(10A)	-3211 (14)	44066 (8)	-2105 (17)	517 (8)	453 (7)	414 (7)	-13 (3)	-14 (3)	2 (3)
C(10B)	663 (15)	41221 (7)	12300 (15)	578 (9)	449 (7)	342 (7)	-3 (3)	-5 (3)	-21 (3)
N(11)	14057 (13)	40622 (7)	13231 (15)	570 (8)	530 (7)	403 (6)	-43 (3)	-33 (3)	3 (3)
C(12)	15272 (18)	24098 (10)	24631 (22)	644 (10)	642 (9)	628 (11)	57 (4)	-21 (5)	45 (4)
H(1-1)	2900 (17)	4690 (11)	1102 (27)	38 (11)	92 (14)	90 (15)	-8 (5)	2 (6)	-5 (7)
H(1-2)	1558 (23)	5115 (9)	906 (25)	119 (19)	49 (11)	73 (14)	-10 (6)	-30 (7)	8 (6)
H(2-1)	2876 (40)	5015 (19)	3402 (33)	279 (50)	193 (29)	96 (20)	-97 (17)	-52 (13)	22 (11)
H(2-2)	1330 (28)	5209 (16)	3257 (40)	78 (19)	152 (26)	165 (30)	4 (9)	14 (11)	19 (12)
H(3A)	2743 (16)	3638 (12)	2539 (25)	28 (11)	149 (20)	107 (19)	-12 (6)	-6 (6)	50 (9)
H(3B)	1202 (20)	3062 (9)	4184 (23)	85 (15)	59 (12)	68 (13)	-13 (5)	0 (6)	0 (6)
H(3C)	-704 (15)	3355 (8)	3466 (18)	59 (11)	35 (8)	46 (10)	-11 (4)	6 (5)	-5 (4)
H(4A)	322 (23)	1601 (9)	2081 (25)	125 (20)	33 (9)	82 (15)	6 (5)	0 (8)	4 (6)
H(41-1)	-56 (21)	2111 (12)	4818 (23)	85 (15)	111 (18)	54 (12)	-5 (7)	-5 (6)	9 (7)
H(41-2)	-1080 (25)	1644 (10)	4105 (22)	158 (23)	58 (11)	44 (11)	-5 (7)	1 (8)	8 (5)
H(41-3)	-1484 (26)	2376 (12)	4729 (25)	138 (21)	75 (15)	70 (16)	2 (7)	34 (8)	3 (6)
H(5)	781 (18)	2061 (8)	-272 (20)	74 (12)	33 (9)	58 (11)	0 (4)	16 (5)	-5 (4)
H(51)	-1468 (27)	1948 (13)	572 (18)	154 (22)	109 (18)	21 (10)	-19 (8)	-24 (6)	-1 (5)
H(6A)	-1359 (17)	3434 (7)	1135 (18)	83 (12)	19 (7)	33 (9)	0 (4)	11 (5)	-3 (4)
H(6B)	-624 (18)	2959 (9)	-1815 (20)	61 (11)	54 (10)	51 (11)	-8 (4)	-9 (5)	8 (5)
H(6B1-1)	1447 (20)	2682 (8)	-2196 (27)	76 (13)	28 (8)	117 (18)	0 (5)	6 (7)	-2 (6)
H(6B1-2)	1099 (19)	3364 (10)	-3155 (19)	79 (14)	80 (12)	32 (9)	2 (6)	2 (5)	-10 (5)
H(6B2)	1844 (28)	3677 (14)	-453 (24)	135 (22)	129 (22)	43 (12)	-7 (9)	5 (7)	-5 (7)
H(82-1)	-338 (28)	4813 (12)	-5251 (35)	171 (31)	68 (16)	130 (24)	2 (8)	23 (12)	10 (8)
H(82-2)	-1274 (30)	5371 (17)	-6071 (29)	129 (24)	191 (29)	66 (16)	-6 (11)	-1 (9)	36 (10)
H(82-3)	-189 (33)	5644 (22)	-4925 (40)	182 (36)	183 (36)	136 (29)	-7 (15)	25 (16)	7 (16)
H(91-1)	-1912 (36)	6229 (17)	-2631 (32)	177 (32)	168 (26)	97 (22)	27 (12)	-44 (11)	-1 (11)
H(91-2)	-744 (37)	6373 (17)	1844 (44)	196 (32)	161 (32)	149 (31)	38 (15)	16 (15)	13 (13)
H(91-3)	-1784 (50)	6325 (13)	-772 (47)	394 (60)	69 (18)	181 (38)	67 (14)	42 (21)	11 (11)
H(100)	-245 (15)	4359 (8)	1977 (18)	42 (10)	49 (9)	47 (10)	-3 (4)	2 (4)	4 (4)
H(12-1)	1963 (24)	2112 (11)	3220 (21)	120 (19)	84 (14)	36 (10)	10 (7)	-8 (6)	-1 (5)
H(12-2)	2077 (17)	2495 (9)	1627 (25)	55 (12)	58 (11)	90 (15)	-3 (5)	-7 (6)	9 (6)

atoms was carried out until all shift to σ ratios were less than 0.3. At the end of the refinement $R_w(|F|^2) = [\sum w(F_o^2 - F_c^2)^2 / \sum wF_o^4]^{1/2} = 0.070$, $R(|F|^2) = \sum (F_o^2 - F_c^2) / \sum F_o^2 = 0.044$ and the $|F|^2$ equivalent of the standard deviation of an observation of unit weight, $\sigma_{|F|^2} = [\sum w(F_o^2 - F_c^2)^2 / (m - n)]^{1/2} = 2.65$. The final value of the secondary extinction coefficient, g , was $0.79 (21) \times 10^4$. A final difference electron density map showed no significant residual density; the extreme values were $\pm 0.09 \text{ e } \text{Å}^{-3}$. The scattering factors for C, N and O were taken from Cromer & Waber (1965) and from Stewart, Davidson & Simpson (1965) for the H atoms.

Results and discussion

The fractional atomic coordinates and thermal parameters are listed in Table 1.* The bond distances and bond angles which are presented in Fig. 1 were obtained from a least-squares program to be described (Sygusch & Brisse, 1976) which refines the librational, translational, librational-coupling tensors (**L**, **T** and **S**) as well as local librational modes θ . The program also

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31372 (13 pp., 1 microfiche). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

permits the refinement of the atomic coordinates, the scale factor and the secondary extinction coefficient. The average estimated standard deviations for bond lengths and bond angles are 0.005 Å and 0.3° respectively. The average distances in the molecule are compared in Table 2 with the values given by Sutton (1965). The average angle around the Csp^3 atoms is 109.5° , in keeping with the tetrahedral coordination of these atoms. The average angle around the N atoms is 112.0° .

Table 2. Comparison of average bond distances

Bond type	Number	Average distance	Sutton's values
Csp^3-Csp^3	9	1.532 Å	1.537 (5) Å
Csp^3-Csp^2	3	1.499	1.506 (5)
Csp^3-Nsp^3	9	1.476	1.472 (5)
Csp^3-OH	2	1.404	1.426 (5)
Csp^2-O	2	1.214	1.215 (5)
Csp^2-O-CH_3	1	1.357	1.358 (5)

A stereoscopic view of the molecule is shown in Fig. 2. Naphthyridinomycin is essentially a substituted quinone ring fused to an unusual alkaloid possessing three tertiary amines. Conjugation of the quinone ring is not present as shown by the bond distance $C(6C)-C(10A)$ and $C(8)-C(9)$ of 1.344 and 1.342 Å respectively, values significantly different from

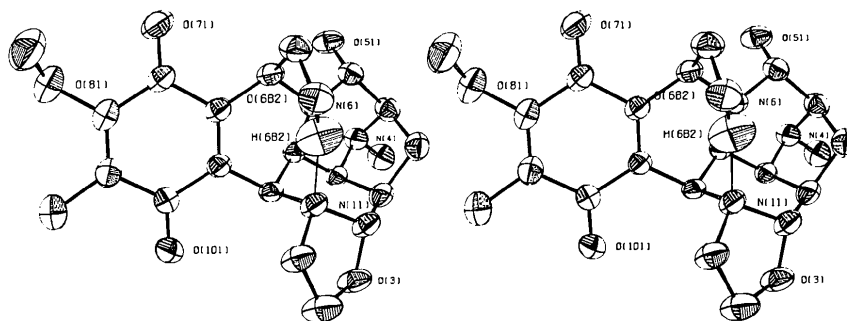


Fig. 2. Stereoscopic pair representing one molecule of naphthyridinomycin.

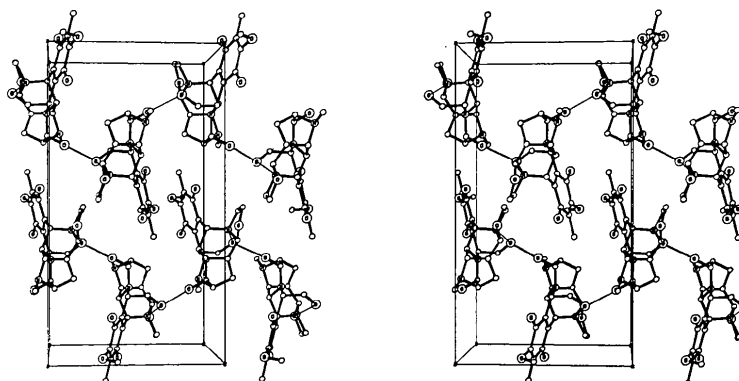


Fig. 3. Molecular packing and hydrogen bonding.

the remaining ring bonds averaging 1.488 Å. The quinone ring is slightly puckered as revealed by the following torsional angles: $C(8)-C(7)-C(6C)-C(10A) = 3.7$ (5), $C(6C)-C(7)-C(8)-C(9) = 4.3$ (5) and $C(8)-C(9)-C(10)-C(10A) = 2.2$ (5)°.

A novel structural feature present in the compound is an intramolecular hydrogen bond between a hydroxyl group and a tertiary amine: $O(6B2)-H(6B2) \cdots N(11)$. The relevant bond distances are: $O(6B2)-H(6B2) = 0.97$ (2), $O(6B2) \cdots N(11) = 2.828$ (3) and $H(6B2) \cdots N(11) = 1.87$ (4) Å. The $O(6B2)-H(6B2) \cdots N(11)$ bond angle is 169 (3)°.

Molecular packing

The hydrogen-bonding scheme and the molecular packing are shown in Fig. 3. There are no van der Waals contacts less than 3.50 Å and the only intermolecular hydrogen bond present is defined* by $O(51)-H(51) \cdots O'(6B2)$. The relevant bonds have the lengths: $O(51)-H(51) = 0.87$ (3), $O(51) \cdots O'(6B2) = 2.774$ (4) and $H(51) \cdots O'(6B2) = 2.03$ (4) Å. The bond angle $O(51)-H(51) \cdots O'(6B2)$ is 142 (4)°. The packing is characterized by two antiparallel chains extending in the **a** direction and held together by van der Waals interactions. Each chain unit consists of two molecules related to each other by the 2_1 axis parallel to the **a** direction and when repeated in the chain direction form a zigzag type of network.

As the $O(6B2)$ atom already participates in an intramolecular hydrogen bond, the additional formation of an intermolecular hydrogen bond with $O(6B2)$ produces a bifurcated hydrogen-bonding scheme. A *PCILO* (Diner, Malrieu & Claverie, 1969) energy calculation was performed to examine whether the intramolecular hydrogen bond is related to the formation of the intermolecular hydrogen bond. The conformational energy was calculated as a function of the rotation of the hydroxyl group about the $C(6B)-C(6B1)$ bond.

The energy was calculated at 30° intervals and at each of these steps the hydroxyl proton $H(6B2)$ was rotated by 90° intervals until the lowest energy was arrived at. The result of these calculations is shown in Fig. 4. Near the minima of the energy curve, the proton was displaced by 30° steps. The lowest energy calculated corresponds well to the position found by X-ray diffraction. A second minimum, 0.9 kcal mol⁻¹ higher, at 240° corresponds to a potential hydrogen bond between the atoms: $O(6B2)-H(6B2) \cdots O(71)$. That the absolute energy minimum about $C(6B)-$

* The unprimed atoms belong to the *xyz* symmetry position, while the primed atom belongs to the position: $\frac{1}{2} + x, \frac{1}{2} - y, \bar{z}$.

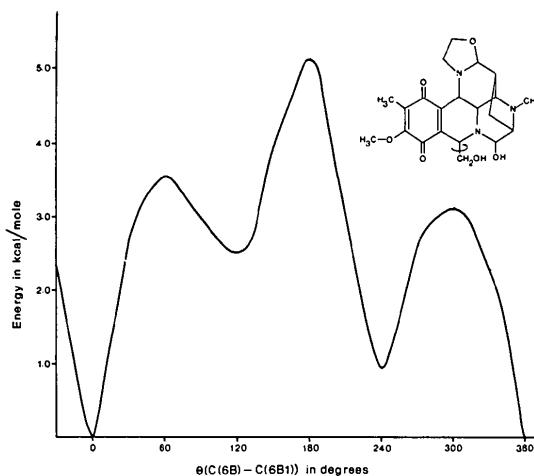


Fig. 4. Variation of potential energy as a function of the torsional angle $\theta[C(6B)-C(6B1)]$. The zero reference point was taken to be the crystallographic position. The rotation is positive in the clockwise direction when viewing from $C(6B)$ to $C(6B1)$.

$C(6B1)$ is not influenced by the intermolecular hydrogen bond implies that local conformational factors are indeed responsible for the energy minimum.

Although the biological mode of action of this compound is not known at this time, a noteworthy feature of the naphthyridinomycin molecule is the electron-rich $N(6)$, $N(11)$ and $O(6B2)$ cavity which can be created by a 180° rotation of the $H(6B2)$ proton around the $C(6B1)-O(6B2)$ bond. From the above calculation this rotation requires less than 2.0 kcal mol⁻¹.

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