(22) M. Caplow, *J. Am. Chem. Soc.*, **87**, 5774 (1965).
(23) A. F. Hegarty, T. C. Bruice, and S. J. Benkovic, *Chem. Commun.*, 1173 (1969).
(24) (a) T. C. Bruice and A. F. Hegarty, *Proc. Natl. Acad. Sci. U.S.A.*, **65**, 805 (1970); (b) A. F. Hegarty and T. C. Bruice, *J. Am. Chem. Soc.*, **92**, 6561 (1970); (c) *ibid.*, **92**, 6568 (1970); (d) *ibid.*, **92**, 6575 (1970); (e) A. F. Hegarty, R. F. Pratt, T. Giudici, and T. C. Bruice, *ibid.*, **93**, 1428 (1971).
(25) (a) R. B. Guchhait, S. E. Polakis, P. Dimroth, E. Stoll, J. Moss, and M. D. Lane, *J. Biol. Chem.*, **249**, 6633 (1974); (b) R. B. Guchhait, S. E. Polakis, D. Hollis, C. Fenselaw, and M. D. Lane, *ibid.*, **249**, 6646 (1974); (c) S. E. Polakis, R. B. Guchhait, E. E. Zwergel, and M. D. Lane, *ibid.*, **249**, 6657 (1974).
(26) R. Griesser, H. Sigel, L. D. Wright, and D. B. McCormick, *Biochemistry*, **12**, 1917 (1973).
(27) (a) G. M. Maggiora, *J. Theor. Biol.*, **41**, 523 (1973); (b) *ibid.*, **43**, 51 (1974).
(28) The stated aim of the authors, ref 5, was the unequivocal identification of the major product of the reaction of biotin methyl ester with methyl chloroformate as 1′-*N*-methoxycarbonyl biotin methyl ester. Because the only interest was the elucidation of the structure, no special precautions were taken in the data collection. Some significant discrepancies between bond lengths as calculated from the positional parameters, Table I, and as listed in Table II, ref 5, exist.
(29) C. S. Chen, R. Parthasarathy, and G. T. DeTitta, *J. Am. Chem. Soc.*, in press.
(30) C. E. Bowen, E. Rauscher, and L. L. Ingraham, *Arch. Biochem. Biophys.*, **125**, 865 (1968).

Structure of the Antibiotic Griseoviridin[1]

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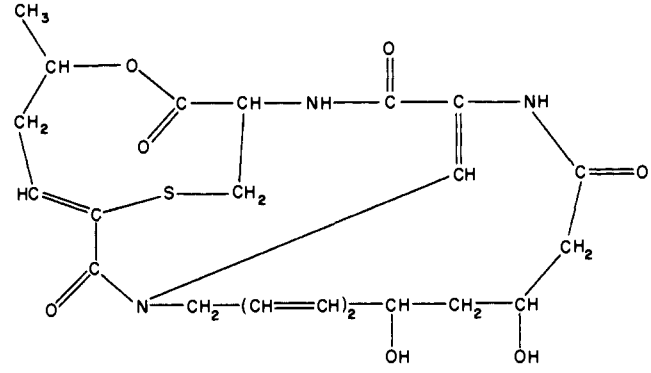
Contribution from the Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6, and Department of Energy, Mines and Resources, Ottawa, Canada K1A 0G1. *Received July 21, 1975*

Abstract: The three-dimensional structure of griseoviridin methanolate, $C_{22}H_{27}O_7N_3S{\cdot}CH_3OH$, was determined by x-ray crystallography. The substance crystallizes in the monoclinic space group $P2_1$ and the unit cell dimensions are a = 10.559 (1), b = 13.066 (3), and c = 9.433 (1) Å, β = 99.00 (1)°. Intensity data were collected with a diffractometer and the structure was solved by a combination of Patterson superposition and tangent refinement techniques. Contrary to a previous proposal the structure contains an oxazole ring. The relative contributions of three canonical structures are assessed on the basis of bond lengths in that ring. It is estimated that the uncharged species contributes 80% to the structure while delocalizations of oxygen electrons to the ring nitrogen and to a conjugated amide oxygen account for an additional 10% each. The molecular structure is quite rigid and most atoms lie in one of four major planes. There are several short intramolecular contacts which give rise to some unusual bond angles and torsion angles.

Griseoviridin is a broad-spectrum antibiotic with inhibitory activity in vitro toward various pathogenic bacteria and fungi.[3] It has also been shown to inhibit bacterial protein synthesis in cell-free systems and poly-U-directed poly(phenylalanine) synthesis in yeast and in human tonsil cell-free systems.[4] It was isolated from *Streptomyces griseus*[5] and its chemical structure has been the subject of intensive investigations by Ames, Bowman, and their colleagues[6-8] as well as by de Mayo, Stoessl, and their collaborators.[9-12] On the basis of degradative and spectroscopic studies the latter group proposed **1** for the structure of griseoviridin.[11] An x-ray analysis was undertaken in order to establish unambiguously the structure and complete stereochemistry. We report here the results of this work which revealed that the correct structure is **2**. It should be pointed out that the Canadian workers considered the skeleton of **2** a plausible one but rejected it in favor of **1**.[11]



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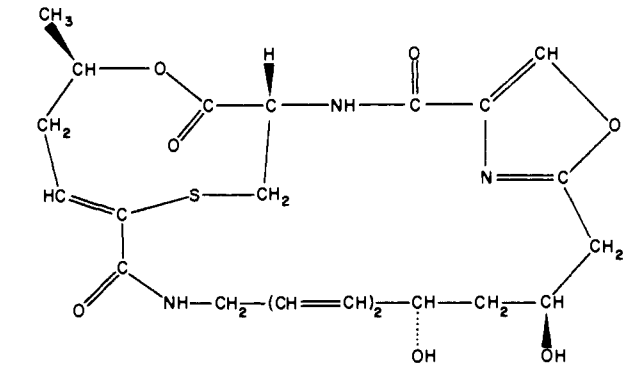

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Experimental Section

Colorless crystals of griseoviridin methanolate, $C_{22}H_{27}O_7N_3S{\cdot}CH_3OH$, were sent to us by Drs. A. Stoessl and P. de Mayo. Precession photographs showed them to be monoclinic and the space group $P2_1$ was indicated by systematic absences of $0k0$ reflections for k odd. A crystal with dimensions 0.29 × 0.29 × 0.14 mm was mounted on a Picker four-circle diffractometer equipped with a Mo target and a graphite monochromator. The cell parameters were obtained from a least-squares fit of the diffractometer angles 2θ, ϕ, and χ for 42 independent reflections, using Mo $K\alpha_1$ radiation (λ 0.70926 Å) and assuming a triclinic cell. The crystal data are as follows: a = 10.559 (1), b = 13.066 (3), and c = 9.433 (1) Å; β = 99.00 (1)°; V = 1285.4 Å^3; d_x = 1.316 g cm^{-3}; Z = 2; $F(000)$ = 540; μ (Mo $K\alpha$) = 1.8 cm^{-1}.

The moving-crystal–moving-counter technique (θ–2θ scan) was used to collect the intensity data. The 2θ scan rate was 2° min^{-1} and the scan width was varied according to the angular dispersion (2.5–3.0°). Background counts were measured for 30 s on each side of the scan. Three reference reflections were monitored every 50 measurements; their intensities decreased by 3.3% during the course of data collection. A net count of 18 was determined as threshold intensity below which reflections were considered unob-

Table I. Final Coordinates and Temperature Parameters[a]

Atom	x	y	z	U_{11}	U_{22}	U_{33}	$2U_{23}$	$2U_{13}$	$2U_{12}$
				Nonhydrogen Atoms[b]					
S(1)	6171 (1)	5000 (0)	7586 (2)	38 (1)	37 (1)	45 (1)	−10 (2)	−8 (1)	1 (2)
C(2)	5762 (6)	3851 (5)	6612 (7)	45 (4)	31 (3)	46 (4)	−5 (5)	−14 (6)	30 (6)
C(3)	5496 (6)	2991 (5)	7255 (7)	48 (4)	36 (3)	47 (4)	−2 (6)	−18 (6)	28 (6)
C(4)	5256 (7)	2843 (5)	8755 (7)	67 (4)	31 (3)	51 (4)	17 (6)	−40 (6)	6 (6)
C(5)	3874 (7)	3029 (5)	8870 (7)	71 (5)	35 (3)	37 (3)	17 (6)	−15 (6)	−7 (7)
O(6)	3677 (4)	4146 (3)	8742 (4)	59 (3)	36 (2)	34 (2)	10 (4)	1 (4)	9 (4)
C(7)	3203 (6)	4568 (5)	7482 (6)	39 (3)	46 (4)	30 (3)	7 (5)	1 (5)	11 (6)
C(8)	3583 (5)	5676 (4)	7491 (6)	41 (3)	33 (3)	31 (3)	−15 (5)	−1 (5)	16 (5)
C(9)	4827 (6)	5808 (5)	6879 (6)	45 (3)	25 (3)	42 (3)	7 (5)	10 (5)	7 (5)
N(10)	2613 (5)	6316 (4)	6672 (5)	55 (3)	34 (3)	21 (2)	−7 (4)	6 (4)	39 (5)
C(11)	2097 (5)	7121 (4)	7229 (6)	30 (3)	32 (3)	30 (3)	5 (5)	16 (5)	3 (5)
C(12)	1406 (5)	7825 (5)	6157 (6)	26 (3)	36 (3)	33 (3)	−10 (5)	10 (4)	9 (5)
N(13)	1343 (4)	7660 (4)	4680 (5)	40 (3)	33 (3)	30 (2)	−2 (4)	12 (4)	15 (5)
C(14)	768 (5)	8459 (4)	4094 (6)	33 (3)	28 (3)	35 (3)	−11 (5)	6 (5)	13 (5)
O(15)	430 (4)	9141 (3)	5057 (4)	51 (3)	38 (2)	32 (2)	−5 (4)	1 (4)	32 (4)
C(16)	870 (6)	8724 (5)	6361 (6)	46 (4)	41 (4)	32 (3)	−8 (5)	1 (5)	29 (6)
C(17)	455 (6)	8720 (5)	2541 (6)	47 (4)	29 (3)	37 (3)	16 (5)	4 (5)	35 (6)
C(18)	739 (5)	7838 (4)	1589 (6)	40 (3)	29 (3)	30 (3)	14 (5)	18 (5)	16 (5)
C(19)	−163 (6)	6932 (5)	1604 (6)	32 (3)	47 (4)	39 (3)	−2 (6)	19 (5)	1 (6)
C(20)	297 (5)	5952 (4)	1013 (6)	44 (3)	31 (3)	26 (3)	−1 (5)	12 (5)	−17 (5)
C(21)	1475 (6)	5544 (4)	1962 (6)	52 (4)	28 (3)	31 (3)	19 (5)	17 (5)	10 (6)
C(22)	2609 (6)	5461 (5)	1588 (6)	61 (4)	32 (3)	29 (3)	4 (5)	7 (5)	3 (6)
C(23)	3794 (6)	5135 (5)	2491 (6)	50 (3)	31 (3)	38 (3)	0 (6)	5 (5)	7 (6)
C(24)	4901 (6)	5072 (5)	2034 (6)	46 (3)	40 (3)	43 (3)	−2 (6)	12 (5)	23 (7)
C(25)	6172 (6)	4739 (5)	2864 (7)	43 (3)	53 (4)	51 (4)	−8 (6)	26 (6)	3 (6)
N(26)	6193 (5)	4660 (4)	4412 (5)	35 (3)	37 (3)	47 (3)	−7 (5)	2 (4)	0 (4)
C(27)	5736 (6)	3834 (5)	4995 (7)	29 (3)	34 (3)	54 (4)	−20 (6)	7 (5)	18 (5)
C(28)	3519 (8)	2719 (6)	10293 (7)	101 (6)	46 (4)	41 (4)	11 (7)	−6 (7)	−25 (9)
O(29)	2618 (4)	4132 (4)	6480 (5)	56 (3)	46 (3)	51 (3)	10 (5)	−27 (4)	−5 (5)
O(30)	2197 (5)	7275 (4)	8525 (4)	69 (3)	67 (3)	30 (2)	−8 (4)	22 (4)	67 (5)
O(31)	613 (5)	8265 (3)	168 (4)	78 (3)	42 (3)	37 (2)	19 (4)	41 (4)	47 (5)
O(32)	−767 (4)	5260 (3)	942 (4)	54 (2)	40 (3)	50 (2)	−8 (4)	5 (4)	−6 (4)
O(33)	5285 (5)	3094 (4)	4302 (5)	73 (3)	40 (3)	53 (3)	−28 (4)	6 (5)	−15 (5)
C(34)	8115 (9)	7113 (9)	5792 (13)	66 (6)	101 (8)	140 (9)	68 (14)	−21 (12)	−51 (11)
O(35)	6881 (5)	6809 (5)	5059 (7)	73 (4)	63 (4)	96 (4)	0 (6)	10 (6)	−39 (6)

Hydrogen Atoms[c]

Atom	x	y	z	Atom	x	y	z	Atom	x	y	z
H(31)	524	240	644	H(171)	−42	895	231	H(241)	492	515	98
H(41)	543	217	905	H(172)	78	946	234	H(251)	647	421	236
H(42)	587	321	930	H(181)	167	758	188	H(252)	682	518	279
H(51)	328	268	827	H(191)	−45	681	256	H(261)	662	523	496
H(81)	364	597	835	H(192)	−118	712	98	H(281)	354	204	1029
H(91)	515	651	696	H(201)	62	612	9	H(282)	275	293	1038
H(92)	448	571	574	H(211)	141	523	281	H(283)	398	296	1104
H(101)	250	624	580	H(221)	271	561	66	H(311)	100	793	−36
H(161)	56	903	728	H(231)	376	500	347	H(321)	−43	487	54

[a] Esd's in parentheses. [b] The coordinates were multiplied by 10^4 and the thermal parameters by 10^3. The thermal parameters are expressed as $\exp[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{23}klb^*c^* + 2U_{13}hla^*c^* + 2U_{12}hka^*b^*)]$. [c] The coordinates were multiplied by 10^3.

served. There were 2371 unique reflections with $2\theta < 50°$ and 2011 (85%) of them had intensities above the threshold. The intensities were corrected for Lorentz and polarization factors. Absorption effects were considered insignificant and corrections were not applied.

In our initial attempts to solve the structure we used the direct methods programs of the X-RAY System.[13] Several starting sets, each consisting of six reflections, were used to initiate a series of iterative tangent procedures. E maps calculated from tangent refinements where the phase estimates converged and R_E values were relatively low showed one high peak whose x and z coordinates agreed with those obtained from the highest peak in the $(E^2 - 1)$ Patterson map. These coordinates (with $y = 0.5$) were assumed to correspond to the sulfur atom site. Other peaks in the E maps appeared to have molecular significance, but their identification as atom sites was seriously hampered by several factors. Firstly, all major peaks were mirrored across the $y = 0.5$ plane containing the sulfur atom, indicating that the enantiomorph definition was inadequate. Secondly, as it later became apparent, 14 of the 35 atoms had y coordinates in the range 0.4–0.6, close to the pseudomirror plane. Finally, the proposed structure (**1**) did not contain a rigid small ring which could be readily identified.

The structure was finally solved by a combination of Patterson techniques and direct methods. Two $(E^2 - 1)$ Patterson maps were manually superimposed with the sulfur atom site of one map on the origin of the other. Significant peaks which coincided were assumed to be due to S–O, S–N, or S–C vectors. A molecular fragment consisting of seven contiguous atoms [subsequently labeled C(12), N(13), C(14), O(15), C(17), C(18), and O(31)] was selected on the basis of reasonable bond lengths and angles. These seven atoms and the sulfur atom were used to calculate structure factors and to initialize a partial-structure tangent calculation.[14] An E map revealed 26 of the 35 nonhydrogen atoms; the remaining nine atoms were then found in a difference Fourier map.

Atomic parameters were refined by block-diagonal least squares.[15] All scattering factors were taken from the "International Tables for X-Ray Crystallography" [16] and the sulfur and oxygen curves were corrected for anomalous dispersion. All hydrogen atoms, except those on the solvent methanol, were located on difference Fourier maps. Their contributions were included in structure factor calculations (with $B = 3.5$ Å^2) but their parameters were not refined. Throughout the refinement the function $\Sigma w(|F_o| - |F_c|)^2$ was minimized and a factor of 0.8 was applied to all shifts. The following weighting scheme was used during the final stages:

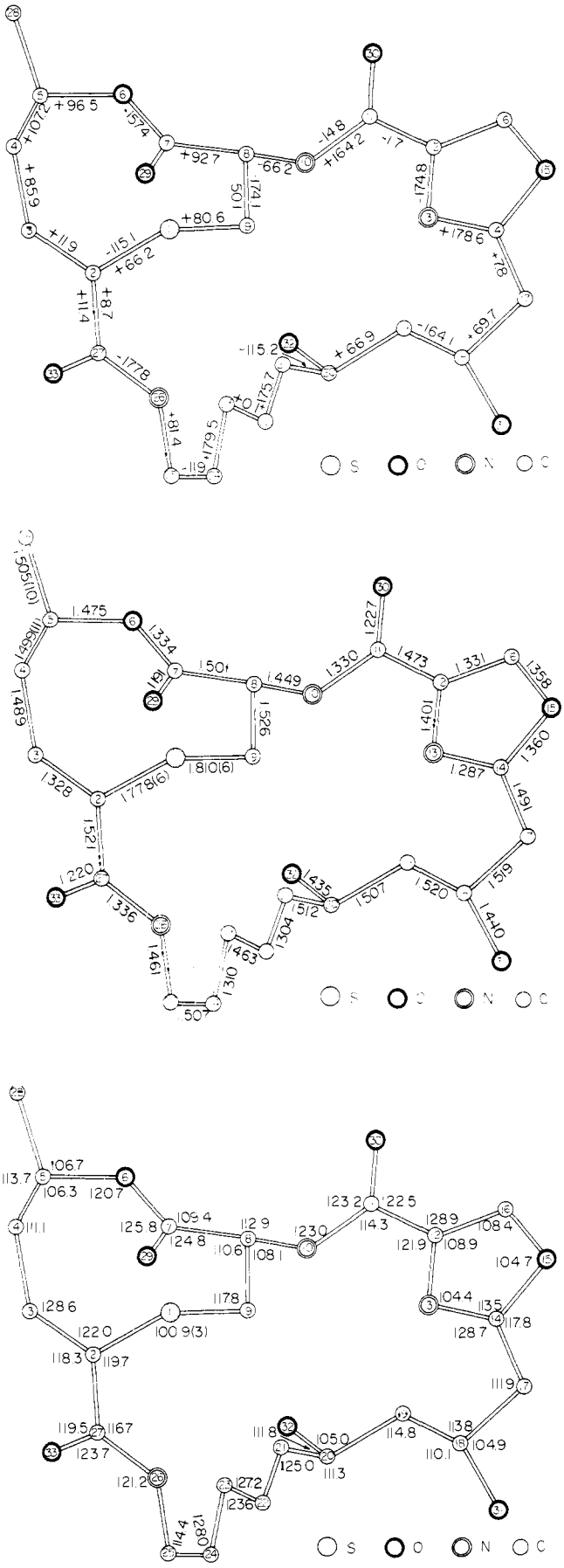


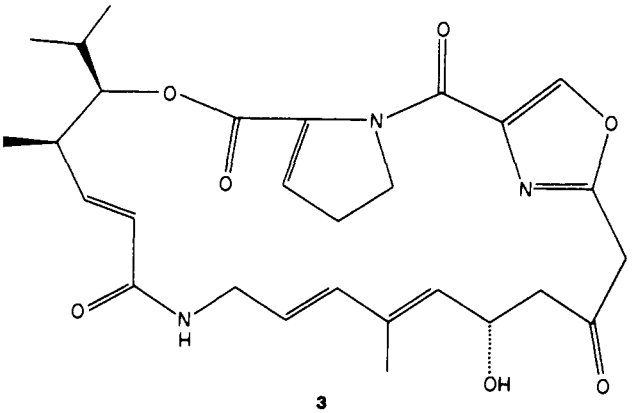
Figure 1. Molecular geometry. Top: torsion angles in degrees (their esd's are 0.4–0.7°). Middle: bond lengths in ångströms (unless otherwise indicated their esd's are 0.007–0.009 Å). The C–O bond length in the solvent methanol is 1.431 (12) Å. Bottom: bond angles in degrees [except at S(1) esd's are 0.4–0.6°].

$w = w_1w_2$, where $w_1 = |F_o|/18$ for $|F_o| \leq 18$, $w_1 = 18/|F_o|$ for $|F_o| > 18$; and $w_2 = \sin^2\theta/0.04$ for $\sin^2\theta < 0.04$, $w_2 = 1$ for $\sin^2\theta \geq 0.04$. The 100 reflection appeared to suffer from extinction effects and it was given zero weight. After the final cycle the average parameter shift equalled 0.09 σ and the largest one 0.57 σ. The index R ($\Sigma|\Delta F|/\Sigma|F_o|$) is 0.063 and the weighted index R' ($\Sigma w\Delta F^2/\Sigma wF_o^2$) is 0.059 for 2089 reflections, including unobserved reflections for which $|F_o| < |F_c|$. A final difference Fourier map was featureless except for unresolved positive regions in the vicinity of the methanol hydrogen atoms.

Results and Discussion

The final coordinates and temperature parameters, as well as their estimated standard deviations, are listed in Table I. The precise molecular geometry can be seen in Figure 1 which gives torsion angles, bond lengths, and bond angles. A stereoscopic view of griseoviridin (Figure 2) shows the conformation of the molecule, while Table II gives details of several mean planes.

This x-ray analysis revealed that griseoviridin is one of the extremely rare natural products containing an oxazole ring.[17] Apart from several small alkaloids the most complex naturally occurring substance known so far has been ostreogrycin A. This antibiotic had been isolated from *Streptomyces ostreogriseus* and its structure (but not stereochemistry) was elucidated by Todd and his colleagues.[18,19] The stereochemistry was recently revealed by an x-ray analysis of virginiamycin M,[20] which is known to be identical with ostreogrycin A.[4] The structure (**3**) is strikingly similar to



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that of griseoviridin and it is interesting to note that the stereochemistry at the two common asymmetric centers [C(5) and C(20)] is the same. This shows that the tentative inclusion of griseoviridin in group A of the streptogramin family of antibiotics (which was done in spite of its supposedly very different structure)[4] is indeed justified.

Molecular Geometry. One of the most striking conformational features is the fact that 28 of the 33 atoms lie in planar regions. There are four such regions associated, respectively, with an α,β-unsaturated amide, a lactone, an amide conjugated with an oxazole ring, and a conjugated diene. The maximum deviations from these planes range from 0.02 to 0.20 Å. The molecule is quite rigid, with few degrees of freedom, and one might assume that its conformation in solution is probably rather similar to the one found in the solid state. This is of relevance to the geometry of the "chloramphenicol area" in bacterial ribosomes to which griseoviridin is known to bind.[21] There are only three ring atoms, C(5), C(9), and C(19), which do not lie in any of these four planes and can therefore act as "hinges". It is noteworthy that the endocyclic bond angles at these atoms are somewhat abnormal, the first one being smaller and the other two larger than usual. The bond angle at O(6) is also larger than normal. In senkirkine, where there are two lactone groups in a 12-membered ring, the corresponding an-

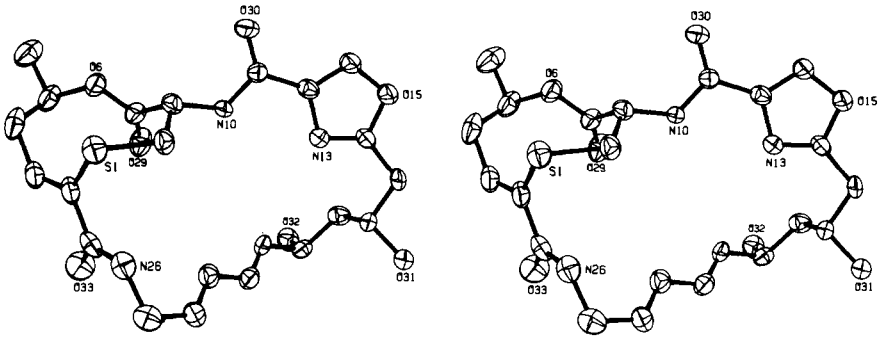


Figure 2. Stereoscopic view of griseoviridin; the thermal ellipsoids correspond to 50% probability.

Table II. Major Planes in the Molecule[a]

Plane 1[b]		Plane 2[b]		Plane 3[b]		Plane 4[b]	
Atom	Δ, Å	Atom	Δ, Å	Atom	Δ, Å	Atom	Δ, Å
S(1)	0.007	O(6)	0.002	N(10)	−0.024	C(20)	−0.028
C(2)	−0.053	C(7)	−0.017	C(11)	0.005	C(21)	0.017
C(3)	−0.156	C(8)	0.004	C(12)	0.063	C(22)	0.033
C(4)	0.001	O(29)	0.004	N(13)	0.049	C(23)	−0.009
C(25)*	−0.043			C(14)	0.000	C(24)	0.008
N(26)	−0.116			O(15)	−0.007	C(25)	−0.020
C(27)	−0.015			C(16)	0.008	N(26)*	−0.347
O(33)	0.131			C(17)	−0.065		
O(35)*	0.088			C(18)*	0.122		
				O(30)	−0.031		
				O(31)*	−0.205		

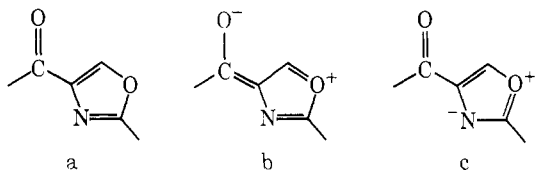
Dihedral angles (deg) between planes: 1,2 29.3; 1,3 48.5; 1,4 84.3; 2,3 47.3; 2,4 79.4; 3,4 53.4

[a] Atoms marked with an asterisk were not included in the calculation of the plane. [b] Plane 1: $0.9344X - 0.3175Y + 0.1613Z - 4.1158 = 0$. Plane 2: $0.9115X - 0.2348Y - 0.3377Z + 1.6622 = 0$. Plane 3: $0.8727X + 0.4881Y + 0.0095Z - 5.6108 = 0$. Plane 4: $0.1567X + 0.9357Y + 0.3162Z - 7.5723 = 0$.

gles are 115.5 and 116.1°.[22] There are abnormalities within the planar regions as well. In the *cis*-ethylene system S(1)-C(2)=C(3)-C(4) the interaction between the sulfur atom and one of the hydrogen atoms attached to C(4) is relieved in two ways: there is a very appreciable twist about the double bond (+11.9°) and the bond angle at C(3) is increased to 128.6°. The resulting S···H distance is 2.89 Å. There is also a transannular interaction between S(1) and C(7) with a distance of 3.171 Å, resulting in a slight displacement of the latter atom from the lactone plane. These nonbonded interactions in the nine-membered ring, caused by the fact that two groups of atoms are forced into near coplanarity, further increase the high strain which is known to exist in medium-ring compounds.

The conjugated amide system is not quite planar either, as evidenced by the C(3)=C(2)-C(27)=O(33) torsion angle of +11.4°. This might be attributed to an interaction between O(33) and the hydrogen atom attached to C(3); the distance between those atoms (2.22 Å) is rather short. The twist also relieves the interaction between the sulfur atom and N(26) and the hydrogen atom attached to it (S··· N 3.031 Å, S···H 2.61 Å). A similar situation can be observed near the other peptide bond. The C(8)-N(10)-C(11)=O(30) torsion angle is −14.8° and the distance between O(30) and the hydrogen atom attached to C(8) is 2.31 Å. Another manifestation of this distortion is the value of +164.2° for the endocyclic torsion angle C(8)-N(10)-C(11)-C(12). It should be noted that both peptide bonds are in the more stable trans conformation.

The geometry of the oxazole ring is of interest because of the paucity of x-ray data on such systems. Apart from the structure of virginiamycin M[20] we are aware of only two reports which give geometrical details of structures incorporating an oxazole ring, the results of Albano et al.[23] being much less precise than those of Ambats and Marsh.[24] The bond lengths observed in the present structure allow us to estimate the relative contributions of various resonance forms. In addition to the uncharged partial structure a, the canonical structures b and c may also be expected to contribute. On the other hand, the three other canonical structures in which the negative charge resides on one of the ring carbon atoms are not likely to be as significant. In our estimation the relative contribution of the three structures shown above is approximately 80:10:10 and this is based on the following arguments. (a) The C(11)-C(12) bond length (1.473 Å) corresponds to a bond number of 1.10.[25a] (b) The two C-O bonds in the ring are, as expected, of equal length. (c) These bond lengths (1.359 Å) are very similar to those found in furan (1.37 Å) where the total contribution of structures with a positively charged oxygen atom was calculated to be 23%.[25b] (d) The C(12)-C(16) bond length (1.331 Å) is not likely to correspond to less than 90% double bond character. (e) The C(12)-N(13) bond length of 1.401 Å agrees very well with the length of 1.404 Å found for a single $C(sp^2)$-$N(sp^2)$ bond in *N,N,N',N'*-tetramethylbenzidine.[26] (f) The N(13)-C(14) bond length of 1.287 Å corresponds to a bond order of 1.9.[27]



a b c

Dewar and Turchi have recently carried out MINDO/3 calculations on various oxazole derivatives. One of them, 5-methoxyoxazole-4-carboxamide (**4a**),[28] is quite similar to the oxazole system present in griseoviridin. A comparison of the geometry calculated by them and the one found by us shows some significant differences, notably in the ring C-C

Table III. Distances and Angles for Hydrogen Bonds

	Distances, Å		Angles, deg	
	D···*A*	H···*A*	*D*-H···*A*	H-*D*···*A*
N(26)-H(261)···O(35)(x,y,z)	2.941	2.08	146	23
O(31)-H(311)···O(30)($x,y,-1+z$)	2.772	1.96	170	7
O(32)-H(321)···O(31)($\bar{x},-\frac{1}{2}+y,\bar{z}$)	2.823	2.20	140	30

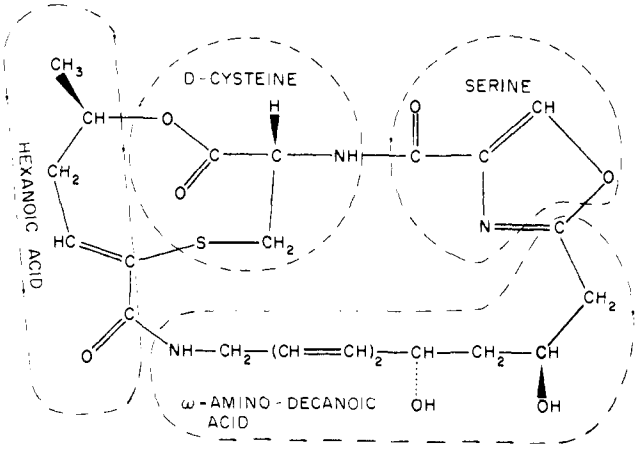


Figure 3. Biogenetic precursors of griseoviridin.

bond (calculated 1.399 Å) and the adjacent C-N bond (calculated 1.427 Å). On the basis of the calculated geometry and the distribution of formal charge it appears that, in contrast to the situation in griseoviridin, there is some contribution from structure **4b.**

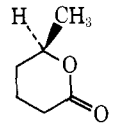
4a **4b**

The conjugated diene system is planar but the C(21)-C(22) bond and the C(23)-C(24) bond are both significantly shorter than the C=C bond lengths found in butadiene (1.341 Å)[29] while the C(21)-C(22)-C(23) bond angle is appreciably larger than the expected value of 123.3°. In view of this it is surprising that the central bond C(22)-C(23) has exactly the same length as that in butadiene. No obvious explanation of these observations comes to our minds. The C(23)-C(24) double bond almost eclipses the C(25)-N(26) bond. Consequently, the bond angles at C(24) and C(25) are each several degrees larger than normal, thus increasing the C(23)···N(26) distance to 2.941 Å.

It is interesting to note that in spite of all the interactions mentioned above no bond is stretched beyond its normal length. This shows again that distortions of bond angles and torsion angles are energetically more favorable than bond stretchings. On the other hand, where angular distortions are more difficult to achieve, as in a cyclobutane ring, a C-C bond may be stretched to the extreme length of 1.628 Å.[30]

In view of the fact that many macrocyclic antibiotics form complexes with various metal ions it should be pointed out that there is a large (~4.3 × 7.5 Å) cavity in the center of the griseoviridin molecule. There are several oxygen and nitrogen atoms to which a metal ion could conceivably be coordinated. However, we are not aware of any metal-binding studies involving griseoviridin.

Absolute Configuration. Chemical degradations have established unambiguously the absolute configuration of griseoviridin. de Mayo and Stoessl[9] found that basic hydrolysis of perhydrodethiogriseoviridin diacetate yielded (+)-δ-caprolactone (**5**). The *R* configuration of this lactone was



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subsequently deduced from a comparison with the (−)-δ-caprolactone to which Kuhn and Kum[31] and later Kuo et al.[32] assigned the *S* configuration and with the (+)-δ-caprolactone from colletodiol for which the positive Cotton effect predicted the *R* configuration.[33] This establishes the 5*R* configuration of griseoviridin. An additional and independent proof rests on an isolation from griseoviridin of D-cysteine[7] from which the configuration at C(8) may be deduced. There is, therefore, no doubt that the absolute configuration used in the structure refinement and shown in the figures is correct.

Hydrogen Bonding. There are four intermolecular hydrogen bonds in the crystal structure. One of the two amide nitrogen atoms and all three hydroxyl groups (i.e., including that in the solvent methanol) donate their protons. The geometry of three of these bonds is given in Table III. The last one appears to have a somewhat unfavorable geometry. However, a hydrogen bond with very similar H···*A*, *D*-H···*A*, and H-*D*···*A* values was recently encountered in the much more precisely determined structure of 3′-*O*-methylarabinosylcytosine.[34] The fourth bond is formed by a donation of the proton attached to O(35) to O(33) in the molecule at ($1-x,\frac{1}{2}+y,1-z$). Although we were unable to locate the proton involved in this hydrogen bond the evidence for the existence of such a bond is fairly convincing: the O(35)···O(33) distance is 2.974 Å, the C(34)-O(35)···O(33) angle is 114.6°, and the O(35)···O(33)=C(27) angle is 125.9°. The bond is not very strong which probably accounts for the strong thermal vibration of the proton.

Biosynthesis. On the basis of the structure revealed by this analysis we can identify the fragments which are the biogenetic precursors of griseoviridin, viz. D-cysteine, serine, an unsaturated ω-aminodecanoic acid, and a δ-hydroxyhexanoic acid. The configuration of the serine cannot be ascertained, the asymmetric nature of the α-carbon having been destroyed. The four fragments are indicated in Figure 3.

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Supplementary Material Available: Listing of observed and calculated structure factors (10 pages). Ordering information is given on any current masthead page.

References and Notes

(1) Issued as N.R.C.C. No. 15221.
(2) (a) National Research Council; (b) Dept. of Energy, Mines and Resources.
(3) J. Ehrlich, G. L. Coffey, M. W. Fisher, M. M. Galbraith, M. P. Knudsen, R. W. Sarber, A. S. Schlingman, R. M. Smith, and J. K. Weston, *Antibiot. Annu.*, 790 (1954–1955).
(4) D. Vazquez, "Antibiotics", Vol. III, J. W. Corcoran and F. E. Hahn, Ed., Springer-Verlag, New York, N.Y., 1975.
(5) Q. R. Bartz, J. Standiford, J. D. Mold, D. W. Johannessen, A. Ryder, A. Maretzki, and T. H. Haskell, *Antibiot. Annu.*, 777 (1954–1955).
(6) D. E. Ames, R. E. Bowman, J. F. Cavalla, and D. D. Evans, *J. Chem. Soc.*, 4260 (1955).
(7) D. E. Ames and R. E. Bowman, *J. Chem. Soc.*, 4264 (1955).
(8) D. E. Ames and R. E. Bowman, *J. Chem. Soc.*, 2925 (1956).
(9) P. de Mayo and A. Stoessl, *Can. J. Chem.*, **38**, 950 (1960).
(10) M. C. Fallona, T. C. McMorris, P. de Mayo, T. Money, and A. Stoessl, *J. Am. Chem. Soc.*, **84**, 4162 (1962).
(11) M. C. Fallona, P. de Mayo, T. C. McMorris, T. Money, and A. Stoessl, *Can. J. Chem.*, **42**, 371 (1964).
(12) M. C. Fallona, P. de Mayo, and A. Stoessl, *Can. J. Chem.*, **42**, 394 (1964).
(13) J. M. Stewart, G. J. Kruger, H. L. Ammon, C. Dickinson, and S. R. Hall, The X-RAY System of Crystallographic Programs, University of Maryland, 1972.
(14) J. Karle, "Crystallographic Computing", F. R. Ahmed, Ed., Munksgaard, Copenhagen, 1970, p 37.
(15) These and all subsequent calculations were carried out with the help of the NRC programs written by Ahmed, Hall, Pippy, and Huber. The ORTEP-II program of C. K. Johnson was used for drawing Figure 2.
(16) J. A. Ibers and W. C. Hamilton, Ed., "International Tables for X-Ray Crystallography", Vol. IV, Kynoch Press, Birmingham, England, 1974.
(17) M. J. S. Dewar and I. J. Turchi, *Chem. Rev.*, **75**, 389 (1975).
(18) G. R. Delpierre, F. W. Eastwood, G. E. Gream, D. G. I. Kingston, P. S. Sarin, Lord Todd, and D. H. Williams, *J. Chem. Soc. C*, 1653 (1966).
(19) D. G. I. Kingston, Lord Todd, and D. H. Williams, *J. Chem. Soc. C*, 1669 (1966).
(20) F. Durant, G. Evrard, J. P. Declercq, and G. Germain, *Cryst. Struct. Commun.*, **3**, 503 (1974).
(21) M. Barbacid, A. Contreras, and D. Vazquez, *Biochim. Biophys. Acta*, **395**, 347 (1975).
(22) G. I. Birnbaum, *J. Am. Chem. Soc.*, **98**, 6165 (1974).
(23) V. Albano, P. L. Bellon, F. Pompa, and V. Scatturin, *Ric. Sci.*, **33**, 1143 (1963).
(24) I. Ambats and R. E. Marsh, *Acta Crystallogr.*, **19**, 942 (1965).
(25) (a) L. Pauling, "The Nature of the Chemical Bond", 3rd ed, Cornell University Press, Ithaca, N.Y., 1960, p 237; (b) p 303.
(26) K. Yakushi, I. Ikemoto, and H. Kuroda, *Acta Crystallogr., Sect. B*, **27**, 1710 (1971).
(27) A. Lofthus, *Mol. Phys.*, **2**, 367 (1959).
(28) M. J. S. Dewar and I. J. Turchi, *J. Chem. Soc., Perkin Trans. 2*, submitted for publication.
(29) K. Kuchitsu, T. Fukuyama, and Y. Morino, *J. Mol. Struct.*, **1**, 463 (1968).
(30) G. I. Birnbaum, *Acta Crystallogr., Sect. B*, **28**, 1248 (1972).
(31) R. Kuhn and K. Kum, *Chem. Ber.*, **95**, 2009 (1962).
(32) C. H. Kuo, D. Taub, R. D. Hoffsommer, N. L. Wendler, W. H. Urry, and G. Mullenbach, *J. Chem. Soc., Chem. Commun.*, 761 (1967).
(33) J. MacMillan and T. J. Simpson, *J. Chem. Soc., Perkin Trans. 1*, 1487 (1973).
(34) G. I. Birnbaum, E. Darżynkiewicz, and D. Shugar, *J. Am. Chem. Soc.*, **97**, 5904 (1975).

Internal Rotations of Side Chains and Backbone in Luteinizing Hormone-Releasing Hormone (LH-RH). Analysis of Carbon-13 Spin–Lattice Relaxation Times[1a]

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Abstract: We have analyzed the ^{13}C spin–lattice relaxation times obtained for LH-RH in aqueous solution in terms of contributions from both overall and internal motions of backbone and side chains. The method of analysis is based on a model of stochastic rotational diffusion about bonds. It assumes that these rotations about individual bonds are *independent* of and *uncorrelated* with rotations about all other bonds. We have calculated minimum and maximum dimensions for the LH-RH monomer and computed the corresponding T_1 values for *overall* molecular tumbling. For this T_1 range, corresponding to $2 \times 10^{-10} \leq \tau_{mol} \leq 2 \times 10^{-9}$, the *internal* motions of the backbone must be assumed slower than the overall molecular motion in order to simulate the observed near equality in NT_1 values for the α-carbons in positions 3–8 of LH-RH. For the side chains, the observed NT_1 values are good qualitative monitors of internal rotations about bonds whose relaxing carbons are more than one bond removed from C_α.

The purpose of this study is to gain qualitative insight into the types and rates of motion which can occur in linear peptides of intermediate molecular weight ($\simeq$1000) and which can produce the spin–lattice relaxation times (T_1) observed by carbon-13 nuclear magnetic resonance spectroscopy. T_1 values have been measured and can be analyzed in terms of both overall molecular and internal motion in both cyclic and linear molecules.[2-11]

The carbon-13 (^{13}C) spin–lattice relaxation times of luteinizing hormone-releasing hormone (LH-RH) (Figure 1) have been measured in aqueous solution and effective correlation times have been reported.[12] We now analyze the T_1 data in terms of rates of both overall and internal molecular motions. The latter comprise rotation about single bonds, both in the backbone and in side chains.

The method of analysis is essentially that of Levine and co-workers.[3,10,11] Because LH-RH is a linear and therefore potentially flexible peptide, it was necessary to explore the effect of both isotropic and anisotropic overall molecular motion on the calculated T_1 values of the backbone and side chains. We have used various methods to estimate the maximum and minimum dimensions plausible in LH-RH for fully extended and compact conformations and examined the effect of varying these size parameters on the calculated rates of internal motion for the side chains of the various residues.

Methods. Levine et al.[3,10,11] have presented a method which extends previous treatments[13-16] and permits the calculation of dipolar relaxation times when nuclei are reorienting in a magnetic field as a result of multiple internal motions in a molecule. The treatment applies to all values of rotational correlation times (τ). The relaxation times of nuclei in chains attached to bodies undergoing overall isotropic[10] and anisotropic[3] motion have been considered. The formulation assumes that the motions about each individual bond are *independent* and *uncorrelated* with the motions