Revised Constitution, Absolute Configuration, and Conformation of Griseoviridin, a Modified Cyclic Peptide Antibiotic

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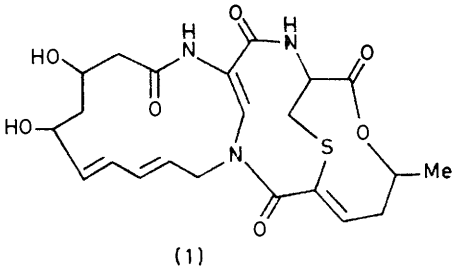
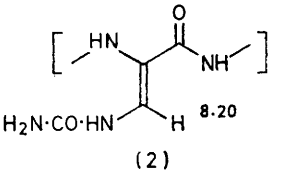
Chemical and detailed ^1H and ^{13}C n.m.r. and mass spectral studies lead to the revised structure (8) {9,11-dihydroxy-22-methyl-6,23-dioxa-26-thia-2,17,27-triazatricyclo[17.5.2.1^{4,7}]tetracosa-4,7(27),12,14,19-pentaene-3,18,24-trione} for griseoviridin, a metabolite of *Streptomyces griseus*. The known chemistry of the antibiotic is shown to be compatible with the proposed formulation. *X*-Ray analysis on griseoviridin methanol solvate by direct methods confirmed these assignments, and, with the evidence of the antibiotic's hydrolysis to D-cystine, gave the absolute configuration of the molecule. The n.m.r. data suggest that griseoviridin adopts in solution one predominant conformation, probably the same as in the crystal lattice. The relationship of griseoviridin to other related cyclic microbial peptides and possible biogenetic implications are discussed.

In 1954 two new antibiotics, griseoviridin and viridogrisein, were isolated from *Streptomyces griseus*.[1] Although individually of rather low biological activity, they exhibited together a strong synergism showing enhanced activity against Gram-positive bacteria.[2] Similar synergistic effects have been observed with antibiotics which were subsequently shown to possess related structures, *e.g.* the ostreogrycin and related groups.[3] Viridogrisein was subsequently identified with etamycin,[4] and extensive and thorough chemical investigations over a ten year period, initiated by Bowman [5,6] and extended by de Mayo,[7] led to the formulation (1) for griseoviridin by the latter group.[8]



(1)



(2)

Our interest in this novel antibiotic stemmed initially from our investigations on the tuberculostatic antibiotics viomycin [9] and capreomycin.[10] The similarity of the chromophoric unit (2) in these metabolites to that proposed for griseoviridin was of considerable interest, since it offered the opportunity for structural modifications along the lines already developed for the viomycin group.[11] Furthermore the presence of a D-cysteine

[1] Q. R. Bartz, J. Standiford, J. D. Mold, D. W. Johannessen, A. Ryder, A. Maretzki, and T. H. Haskell in 'Antibiotics Annual,' Medical Encyclopedia, Ind., New York, 1955, p. 777.

[2] L. E. Anderson, J. Ehrlich, S. H. Sun, and P. R. Burkholder, *Antibiotics Chemother.*, 1956, **6**, 100.

[3] J. Bessell, K. H. Fantes, W. Hewitt, P. W. Muggleton, and J. P. R. Toothill, *Biochem. J.*, 1958, **68**, 24P; 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*), 1966, 1653.

[4] J. C. Sheehan, H. G. Zachau, and W. B. Lawson, *J. Amer. Chem. Soc.*, 1958, **80**, 3349; R. B. Arnold, A. W. Johnson, and A. B. Mauger, *J. Chem. Soc.*, 1958, 4466.

[5] D. E. Ames, R. E. Bowman, J. F. Cavalla, and D. D. Evans, *J. Chem. Soc.*, 1955, 4260.

[6] D. E. Ames and R. E. Bowman, *J. Chem. Soc.*, 1955, 4264; 1956, 2925.

[7] P. de Mayo and A. Stoessl, *Canad. J. Chem.*, 1960, **38**, 950; M. C. Fallona, T. C. McMorris, P. de Mayo, T. Money, and A. Stoessl, *J. Amer. Chem. Soc.*, 1962, **84**, 4162.

[8] M. C. Fallona, P. de Mayo, T. C. McMorris, T. Money, and A. Stoessl, *Canad. J. Chem.*, 1964, **42**, 371.

[9] B. W. Bycroft, *J.C.S. Chem. Comm.*, 1972, 660; 'Chemistry and Biology of Peptides,' ed. J. Meienhofer, Ann Arbor Science Inc., Ann Arbor, 1972, p. 665, and references cited therein.

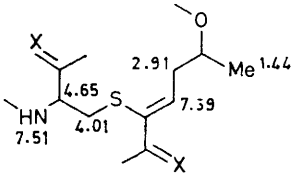
[10] B. W. Bycroft, D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson, and T. Webb, *Nature*, 1971, **231**, 301; B. W. Bycroft and N. Whittaker, in preparation.

[11] B. W. Bycroft, D. Cameron, L. R. Croft, A. Hassanali-Walji, A. W. Johnson, and T. Webb, *J.C.S. Perkin I*, 1972, 827; B. W. Bycroft, A. Hassanali-Walji, A. W. Johnson, J. D. Hardstone, and N. R. Panes, unpublished results.

residue adjacent to an αβ-didehydro-amino-acid system bore some resemblance to the structural features we have recently observed in althiomycin.[12]

Our findings from a cursory examination of both the physical and the chemical properties accorded with the original observations. The molecular formula $C_{22}H_{27}N_3O_7S$ was confirmed by an accurate mass determination, and acidic hydrolysis gave D-cystine, traces of glycine, and 5-hydroxy-2-oxohexanoic acid. Reduction with Raney nickel afforded the perhydrodethiogriseoviridin, which on hydrolysis gave 5-hydroxyhexanoic acid, two molecules of alanine, and 10-aminodecanoic acid. It had already been pointed out that these four fragments accounted for all the carbon and nitrogen atoms in griseoviridin.[8] This fact, coupled with scarcity of material, led us to consider a detailed examination of the spectral data from the antibiotic as a means of confirming the original structural assignments.

In the light of the molecular formula, the 100 MHz ^1H n.m.r. spectrum [solvent $(CD_3)_2N{\cdot}CDO$] of griseoviridin must contain signals for 27 protons. A correlation of the integration from this spectrum with that obtained after



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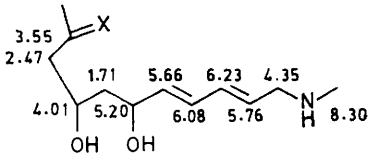


(4)

the addition of D_2O established that there were 23 non-exchangeable protons, presumably bound to carbon. Two of the four exchangeable protons gave well-defined signals at δ 8.30 (t, *J* 6.0 Hz) and 7.51 (d, *J* 7.5 Hz). A further broad signal was observed at δ 7.30, but that of the fourth proton remained undetected, presumably dispersed under other signals. A detailed analysis of the ^1H n.m.r. spectra of griseoviridin by employing double-resonance techniques proved rewarding. The results, together with the known chemistry of the antibiotic permitted assignment of the signals and also allowed the structural units (3) and (4) to be identified.

The important sequence which allowed a complete assignment for the structure of the 10-aminodecanoic acid unit (4) is illustrated. Irradiation at δ 4.35 caused the triplet at 8.30 to collapse to a singlet and the one-proton resonance centred at δ 5.76 (a pair of triplets, *J* 14.5 and *ca.* 4 Hz) was simplified to a doublet (*J* 14.5 Hz). The broad two-proton signal at δ *ca.* 4.35 can thus be assigned to $NH{\cdot}CH_2{\cdot}CH$:. The signal at δ 5.76 shows coupling to a one-proton resonance centred at δ 6.23 which appears as a pair of doublets (*J* 14.5 and 9.5 Hz). The smaller coupling (*J* 9.5 Hz) of this signal is to a further olefinic proton signal centred at δ 6.08, which is also a pair of doublets (*J* 14.5 and 9.5 Hz). This proton is in turn coupled (14.5 Hz) to a proton the resonance of which is centred at δ 5.66, a pair of doublets (*J* 14.5 and 8.0 Hz). The chemical shifts of these protons together with the large coupling constants pointed conclusively to the presence of two *trans*-double bonds.

Irradiation at δ 5.20 (one-proton resonance) perturbed the signal centred at δ 5.66 and at the same time simplified a high-field two-proton signal at δ 1.71. This methylene group was shown to be also linked to a single proton, the resonance of which was hidden under an envelope of signals between δ 3.90 and 4.30. Irradiating at δ 4.01 perturbed the 1.71 signal and caused partial collapse of the signals centred at δ 3.55 (pair of doublets, *J* 14.3 and 7.0 Hz) and 2.47 (pair of doublets, *J* 12.5 and 7.0 Hz), thus allowing the assignments shown.

These observations were not consistent with structure (1), in that the 10-amino-group of the decanoic acid fragment clearly possessed an amide hydrogen atom. Since it was already established that griseoviridin formed a diacetate,[5] the four exchangeable hydrogen atoms could be assigned to the two hydroxy-functions and the two amides associated with the cysteine and the 10-aminodecanoic acid residues. The remaining C_3 unit must therefore possess the structure (5) without any hydrogen on the nitrogen function. The low-field signal at δ 8.02 associated with this unit was comparable with those observed for the structural unit (2) in viomycin and capreomycin and for the oxazole unit (6) present in ostreogrycin (7). Indeed the general structure of this last molecule [3] bore considerable resemblance to that of griseoviridin, and summation of the present evidence led us to the alternative formulation (8) (with chirality defined only at the D-cysteine residue).

This structure had originally been considered but rejected on the grounds that the molecule was not sufficiently basic to contain an oxazole ring.[7] Although griseoviridin was known to form a well defined hydrochloride and perchlorate,[6] it had been claimed [13] that, on treating with these concentrated acids, a transannular displacement of one of the hydroxy-groups by an amide occurred with formation of the imino-lactone salt (9).

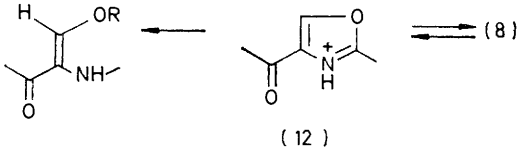
We prepared the hydrochloride, hydrobromide, and perchlorate of griseoviridin; careful analysis revealed that water was not lost in their formation. In addition the ^1H n.m.r. spectrum of the hydrochloride was virtually identical with that of the parent compound, indicating that little molecular reorganisation had occurred. One of the main reasons for advocating a complex reaction with acids was that it had not been possible to regenerate the antibiotic from its salts with aqueous base. Indeed we showed that potentiometric titration

[12] B. W. Bycroft and R. Pinchin, *J.C.S. Chem. Comm.*, 1975, 121.

[13] M. C. Fallona, P. de Mayo, and A. Stoessl, *Canad. J. Chem.*, 1964, **42**, 394.

of the hydrochloride in water required 2 mol. equiv. of alkali, but on back-titration of this solution only 1 mol. equiv. of acid was consumed. The resulting alkaline solution exhibited a u.v. maximum at *ca.* 270 nm and a low-field ^{1}H n.m.r. signal at δ 8.10. After neutralisation the solution gave an intense purple colour with iron(III) and readily reduced solutions of ammoniacal silver nitrate, copper(II) acetate, and Tillman's reagent,[14] properties which were not exhibited by griseoviridin itself.

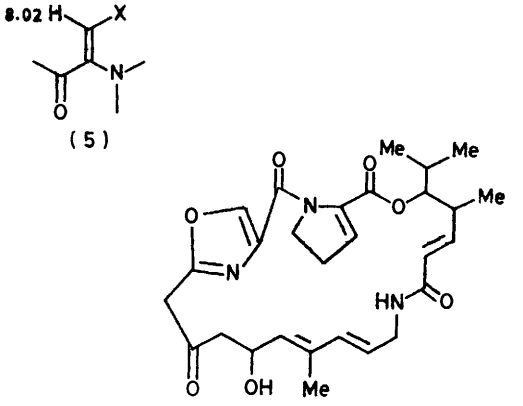
These observations were reminiscent of our earlier findings with the αβ-didehydroserine system (10) in benzyl penaldate [11,15] and the deureido-derivatives of viomycin [9] and capreomycin.[10] In view of our experience with these compounds no attempt was made to isolate the compound corresponding to (10) from griseoviridin. However it is possible that a crystalline derivative (11) had been prepared [6] earlier but its significance not appreciated. Unfortunately lack of material did not allow us to repeat this work.



(10) R = H

(11) R = $CH_2 \cdot CO \cdot C_6H_4$ Ph-*p*

A summation of the above observations found an interpretation for griseoviridin in terms of the formation of an oxazolium salt (12) which opens on treatment with aqueous base to structure (10) as illustrated. Furthermore we concluded that it should be possible to regenerate the intact antibiotic by using a non-aqueous base. This proved to be the case: treatment of the hydrochloride with dry pyridine gave griseoviridin in reasonable yield.



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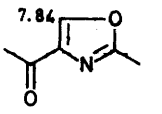

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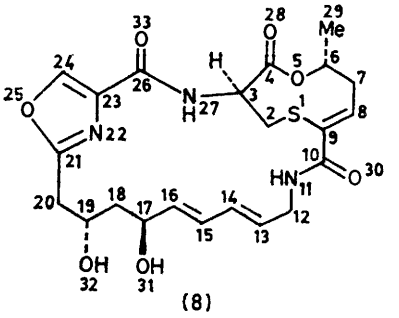

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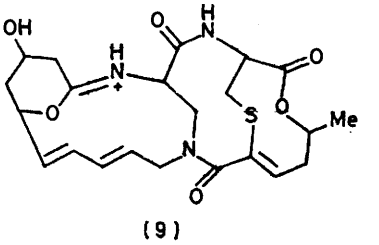


(9)

The ^{13}C n.m.r. spectrum of griseoviridin was obtained at 25.15 MHz by using a pulsed Fourier transform system with proton noise decoupling and off-resonance decoupling. All the resonances for the 22 carbon atoms were apparent in the noise-decoupled spectrum. The

TABLE 1

^{13}C N.m.r. spectrum of griseoviridin (δ in p.p.m. from Me_4Si)

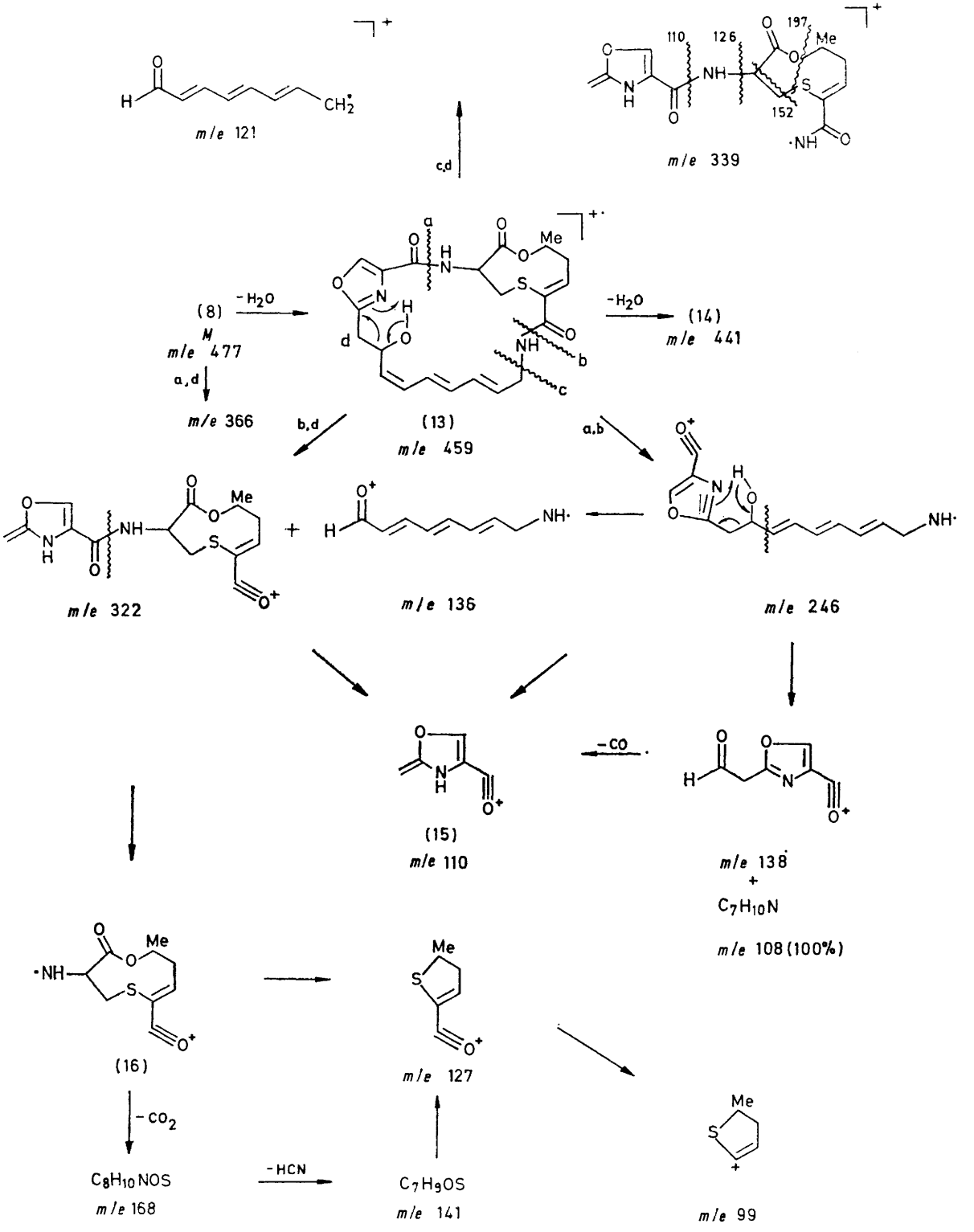
2	3	4	6	7
38.36 (t)	70.52 (d)	171.81 (s)	51.28 (d)	83.06 (t)
8	9	10 *	12	13
141.81 (d)	129.41 (s)		45.32 (t)	135.7 (d)
14	15	16	17	18
131.22 (d)	130.05 (d)	137.42 (d)	71.69 (d)	36.43 (t)
19	20	21 *	23	24
66.40 (d)	40.93 (t)		131.75 (s)	145.31 (d)
26 *	29			
	20.64(q)			

* Carbon atoms 10, 21, and 26 gave unassigned singlets at 163.91, 163.27, and 159.82.

[14] H. Von Euler, H. Hasselquist, and O. Ceder, *Annalen*, 1953, **581**, 198.

[15] 'The Chemistry of Penicillin,' eds. H. T. Clarke, J. R. Johnson, and Sir Robert Robinson, Princeton University Press, New Jersey, 1949, pp. 486, 501, and 716.

multiplicities of the signals, obtained from continuous wave decoupling experiments with irradiation at the resonance frequency of Me_4Si and that corresponding to δ 10.00, agreed with structure (8). However on the basis of the ^{13}C n.m.r. data alone, it is impossible to distinguish between structures (1) and (8). The C–Me signal centred at δ 20.64 appeared as the only quartet in the spectra. The five triplets and ten doublets were tentatively assigned on the basis of their chemical shifts and by comparison with model systems, *e.g.*



SCHEME 1

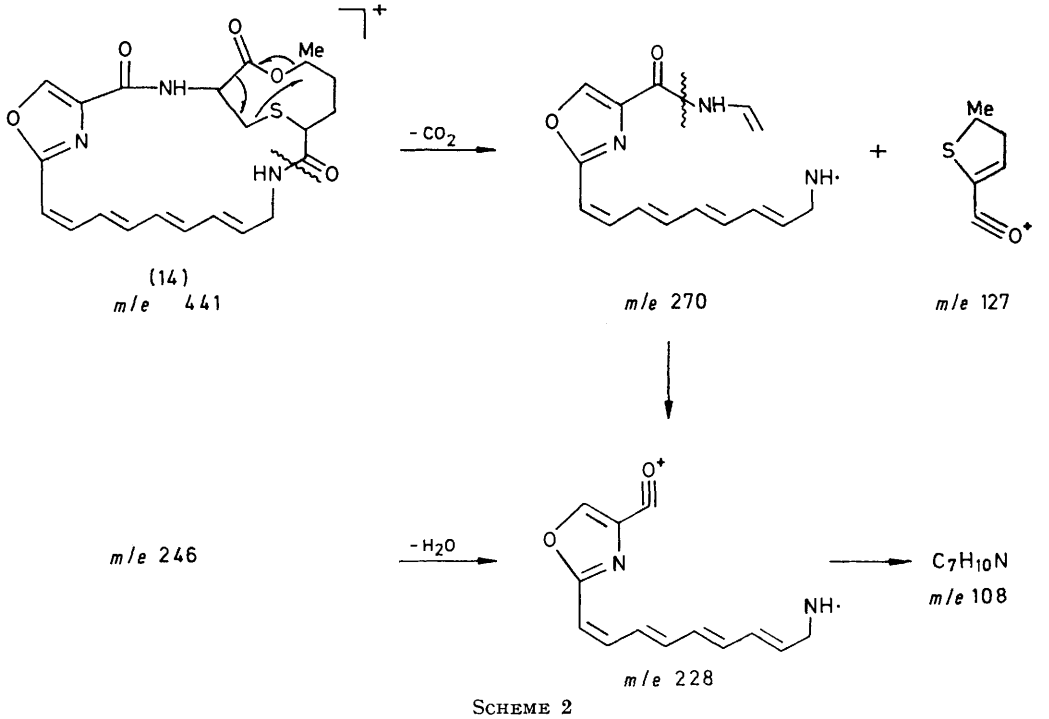
cysteine [12] and didehydro-amino-acid [16] derivatives (Table 1). Of the six remaining singlets, the low field signal at δ 171.81 was allocated to the lactone carbonyl and the two signals at δ 129.41 and 131.75 to either C-9 or C-23.

TABLE 2

Exact mass measurements on the principal peaks in the mass spectrum of griseoviridin, and shifts on deuteriation

Obs.	Abundance (%)	Composition	Calc.	$M+3$	$M+4$
477.1593	97.3	$C_{22}H_{27}N_3O_7S$	477.1625	+2	+3
459.1472	77.8	$C_{22}H_{25}N_3O_6S$	459.1465	+2	+3
441.1349	95.6	$C_{22}H_{23}N_3O_5S$	441.1359	+1	+2
366.1139	18.6	$C_{17}H_{22}N_2O_5S$	366.1226	+2	+3
339.0900	52.3	$C_{14}H_{17}N_3O_5S$	339.0890	+2	+3
322.0630	35.5	$C_{14}H_{14}N_2O_5S$	322.0624	+1	+2
270,1237	42.9	$C_{15}H_{16}N_3O_2$	270.1242	+1	+2
246.0981	18.8	$C_{13}H_{14}N_2O_3$	246.1004	+2	+2
228.0893	32.1	$C_{13}H_{12}N_2O_2$	228.0899	+1	+1
197.0558	50.0	$C_8H_9N_2O_4$	197.0562	+1	+2
168.0480	39.9	$C_8H_{10}NOS$	168.0483	0	+1
152.0601	27.4	$C_7H_8N_2O_2$	152.0586	+1	+2
141.0369	56.2	C_7H_9OS	141.0374	0	0
138.0205	80.5	$C_6H_4NO_3$	138.0192	0	0
136.0762	32.1	$C_8H_{10}NO$	136.0762	+1	+1
127.0212	39.9	C_6H_7OS	127.0217	0	0
121.0646	42.9	C_8H_9O	121.0653	0	0
110.0252	72.9	$C_5H_4NO_2$	110.0243	+1	+1
109.0175	82.8	$C_5H_3NO_2$	109.0164	+1	+1
108.0823	100.0	$C_7H_{10}N$	108.0814	+1	+1
99.0269	75.00	C_5H_7S	99.0269	0	0

Table 2, and formalistic interpretation of the fragmentation patterns [20] are summarised in Schemes 1 and 2. At temperatures above 180 and below 200 °C the mass spectrum showed an intense molecular ion at *m/e* 477, together with strong peaks at *m/e* 459 and 441 corresponding to the loss of one and two molecules of water. Metastable transitions were observed at 441.5, 423.5, and 407.5 in accord with the transformatoins (8) ⟶ (13), (13) ⟶ (14), and (8) ⟶ (14) respectively. The informative fragmentation processes stemmed essentially from the ions (13) and (14) and a satisfying correlation between the processes observed and those which were already well established for cyclic peptides [17-19] was possible. The key fragmentations of (13) are controlled by a McLafferty-type rearrangement (d) followed by typical amide cleavages (a, b, and c), which lead to the principal fragment ions illustrated in Scheme 1. The proposed McLafferty rearrangement (d) is supported by the shifts of the appropriate peaks on deuteriation. After brief exchange with D_2O (*ca.* 0.5 h) the molecular ion in the mass spectrum of griseoviridin showed an increase of only three mass units. However, prolonged contact with D_2O in acetone (*ca.* 12 h) afforded the expected increase of four mass units. These spectra with their shifts of the principal fragment ions (Table 2) provided important conformation of the proposed fragmentation processes. Furthermore, it was



SCHEME 2

The mass spectra of natural and synthetic cyclic peptides have provided many examples of the power of mass spectrometry in structural analysis.[17-19] The principal fragment ions from griseoviridin are listed in

possible to deduce that the cysteine amide proton is the least labile of the exchangeable hydrogen atoms, as already observed in the ^1H n.m.r. investigation.

The only evidence for direct fragmentation of the mole-

[16] B. W. Bycroft and N. Whittaker, in preparation.
[17] J. H. Jones, *Quart. Rev.*, 1968, **22**, 302.
[18] M. M. Shemyakin, *Pure Appl. Chem.*, 1968, **17**, 313.
[19] E. Lederer, *Pure Appl. Chem.*, 1968, **17**, 489.
[20] T. W. Bentley and R. A. W. Johnstone, *Adv. Phys. Org. Chem.*, 1970, **8**, 157.

cular ion, other than the loss of H_2O, resided in the small peak at *m/e* 366 which corresponded to the loss of species (15) together with a hydrogen atom. The subsequent breakdown of this ion leads to fragment ions illustrated in Scheme 1. An alternative breakdown pattern operates on the ion (14) (*m/e* 441). In this case the McLafferty rearrangement is blocked and it is proposed that (14) undergoes ring opening with concerted elimination of carbon dioxide (Scheme 2). Similar fragmentations have been observed in the mass spectra of several

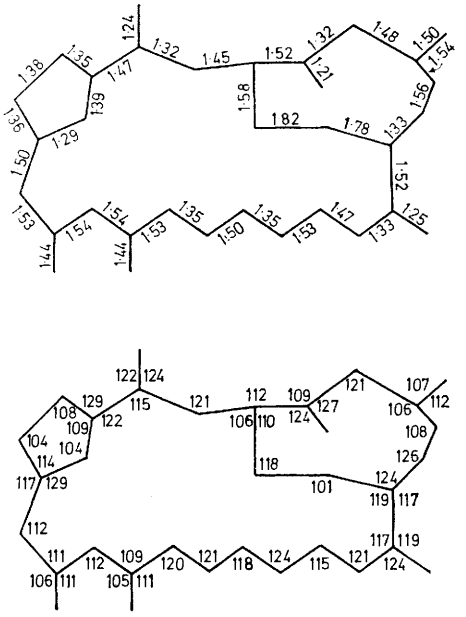


FIGURE 1 Bond lengths (Å) and angles (°) of griseoviridin

cyclic depsipeptides [21,22] as well as in that of a reduced derivative of the related antibiotic ostreogrycin [23] (7). The subsequent breakdown of the *m/e* 270 ion is unexceptional and leads to ions illustrated in Scheme 1.

Thus the mass spectral data are consistent with structure (8), and in terms of structural features the ions (15) (*m/e* 110) and (16) (not itself detected but substantiated by the breakdown ions *m/e* 168, 141, 127, and 99), are particularly significant, since they provide further conformation for the oxazole and enethiol ether units

An *X*-ray analysis of griseoviridin methanol solvate confirmed the gross structure (8), and since the absolute configuration of the cysteine residue was already known, the chirality at C-6, C-17, and C-19 is established as *R*, *S*, and *R*, respectively. Bond lengths and angles are given in Figure 1 and the conformation in the crystal lattice is shown by means of the stereoscopic drawings in Figure 2.† The *trans*-geometry for the two double bonds in the decanoic acid residue, originally suspected from the ^1H n.m.r. study, was confirmed. Furthermore,

TABLE 3

Atomic co-ordinates of griseoviridin with standard deviations in parentheses

Atom	*x/a*	*y/b*	*z/c*
S(1)	0.741 4(2)	0.121 5(5)	0.382 9(2)
C(2)	0.816 1(9)	0.038 6(7)	0.515 6(8)
C(3)	0.747 9(9)	0.052 2(7)	0.641 9(8)
C(4)	0.749 8(10)	0.164 9(7)	0.679 3(8)
O(5)	0.625 0(7)	0.206 7(5)	0.631 4(6)
C(6)	0.611 6(11)	0.318 9(7)	0.613 7(10)
C(7)	0.619 3(11)	0.338 3(8)	0.471 1(10)
C(8)	0.778 7(11)	0.323 8(7)	0.451 7(9)
C(9)	0.839 5(9)	0.236 3(6)	0.423 9(8)
C(10)	0.001 1(10)	0.237 1(7)	0.425 9(8)
N(11)	0.058 7(8)	0.154 9(6)	0.378 8(7)
C(12)	0.214 7(10)	0.147 2(8)	0.380 6(9)
C(13)	0.300 5(9)	0.113 1(8)	0.508 9(8)
C(14)	0.246 4(11)	0.108 0(7)	0.620 2(8)
C(15)	0.346 1(9)	0.074 8(7)	0.738 6(9)
C(16)	0.301 4(9)	0.067 6(6)	0.853 6(8)
C(17)	0.402 4(9)	0.026 5(7)	0.970 6(8)
C(18)	0.662 4(10)	0.427 7(7)	−0.017 9(8)
C(19)	0.657 2(8)	0.337 0(6)	0.075 1(8)
C(20)	0.753 2(9)	0.248 8(7)	0.044 2(9)
C(21)	0.909 7(8)	0.275 5(6)	0.076 4(7)
N(22)	0.968 2(7)	0.355 3(5)	0.135 2(6)
C(23)	0.115 7(9)	0.338 9(6)	0.141 4(7)
C(24)	0.137 4(10)	0.248 6(8)	0.086 0(8)
C(25)	0.004 9(7)	0.205 8(5)	0.043 1(6)
C(26)	0.222 1(9)	0.409 6(7)	0.210 3(8)
N(27)	0.166 3(7)	0.489 5(6)	0.262 2(7)
O(28)	0.853 5(7)	0.208 0(5)	0.737 7(6)
C(29)	0.469 0(11)	0.349 5(9)	0.647 6(11)
O(30)	0.073 0(7)	0.312 5(5)	0.472 0(7)
O(31)	0.409 9(6)	0.096 8(5)	0.076 8(6)
O(32)	0.514 2(6)	0.295 8(5)	0.062 4(7)
O(33)	0.353 0(7)	0.393 5(6)	0.219 9(7)
Methanol			
C(1)	0.085 1(16)	0.412 4(13)	0.811 9(12)
O(2)	0.006 8(9)	0.441 1(6)	0.688 3(8)

the two amide linkages were also shown to possess the *trans*-configuration. No intramolecular hydrogen bonds were observed and no significant intermolecular interactions were detected.

The torsion angles relating to the diene, oxazole-2-carboxamide and 2-thioacrylamide systems, as well as the NH–C_αH torsion angles are given in Table 4. The principal units are essentially planar with the exception of the 2-thioacrylamide system, which is slightly twisted about the C(10)–N(11) bond.

The solution conformation of cyclopeptides has been intensively investigated over the past decade by a wide variety of physicochemical methods.[24-27] Frequently the predominant solution conformer differs from that adopted in the crystal lattice.[26,27] The more informative

† Before our *X*-ray analysis was completed a preliminary communication describing the crystal structure of griseoviridin appeared (G. I. Birnbaum and S. R. Hall, *Acta Cryst.*, 1975, **A31**, 351). We are grateful to Dr. G. I. Birnbaum for discussions at the 10th International Congress of Crystallography, Amsterdam, 1975, which were materially helpful in hastening our solution of the *X*-ray structure.

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studies have principally involved the application of n.m.r. techniques,[25,26] and since considerable n.m.r. data on griseoviridin had been amassed, these were extended to cover the solution conformation. The line shapes and intensities of the signals in both the ^1H and ^{13}C n.m.r. spectra [solvent $(CD_3)_2N{\cdot}CDO$] were essentially independent of temperature (10—50 °C), which strongly implied that under these conditions one conformer predominates. The temperature dependence of the chemical shifts for the NH protons of the cysteine and 10-aminodecanoic acid residues expressed as gradients were 0.002 0 and 0.004 5 p.p.m. K^{-1}, respectively. The

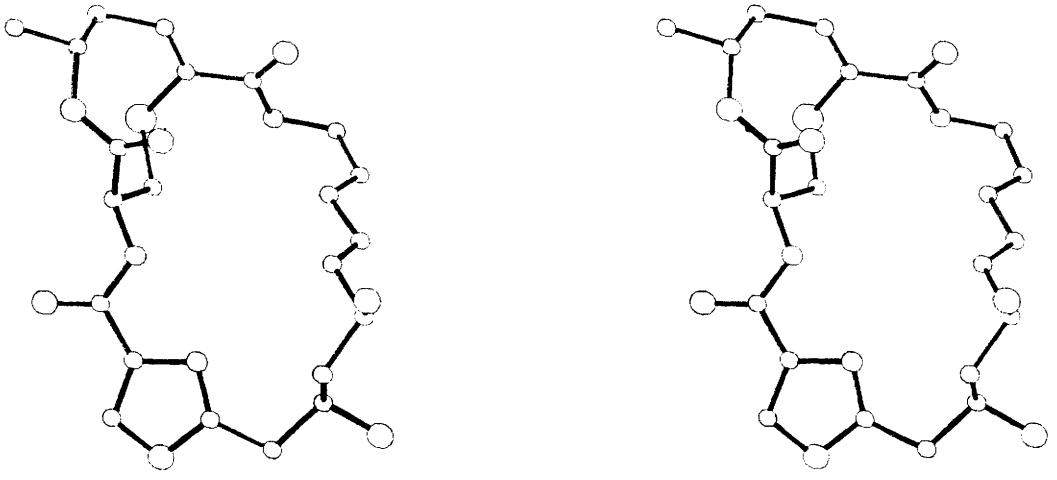

FIGURE 2

smaller upfield shift for the cysteine amide proton suggested that the proton is either intramolecularly hydrogen bonded or solvent shielded, an observation which found support in the previously mentioned slow exchange for this proton with D_2O.

TABLE 4

Torsion angles (°) * relating to the principal structural units

π-Systems	
C(13)–C(14)–C(15)–C(16)	180
C(8)–C(9) C(10)–N(11)	−170
C(24)–C(23)–C(26)–N(27)	−175
Amide units	
C(26)–N(27)–C(3)–C(4)	−124 †
C(10)–N(11)–C(12)–C(13)	81

* The sign of the angle follows the right-hand rule (W. Klyne and V. Prelog, *Experientia*, 1960, **16**, 521). † This angle corresponds to ϕ in the conformational nomenclature of peptides; see IUPAC–IUB Commission on Biochemical Nomenclature, *J. Mol. Biol.*, 1970, **52**, 1; *J. Biol. Chem.*, 1970, **245**, 6489.

The principal parameter provided by ^1H n.m.r. data is the vicinal spin–spin coupling constant of the α-proton and the NH hydrogen atom in each amino-acid residue, which provides a measure of the corresponding torsion angle (θ). A number of Karplus equations [28] with coefficients adjusted for the electronegativity of substituents have been employed to express this relationship.[24] The equation used in this study is due to Bystrov *et al.*[29] The appropriate coupling constants and calculated θ values are shown in Table 5. Since there are only two amide linkages within the molecule the amount of information is limited. However, if it is reasonably assumed that the planar rigid structures associated with the diene, oxazole-2-carboxamide, and 2-thioacylamide systems are not disrupted on dissolution, then the conformational mobility of the molecule is considerably restricted. The calculated θ values are in tolerable agreement with those observed in the crystal. A Corey–Pauling–Koutlum model for griseoviridin indicated that the formation of an intramolecular hydrogen bond to the cysteine amide proton is highly unlikely without gross distortion of the molecular framework. This hydrogen atom is caged within the centre of the molecule

TABLE 5

Comparison of NH–C_αH dihedral angles (θ) for griseoviridin in crystal and solution state

	Crystal		Solution *	
	Torsion angle (°)	θ	$J_{NH,C\alpha H}$/Hz	θ
C(26)–N(27)–C(3)–C(4)	124	−176	7.5 ± 0.5	−155 ± 8
C(10)–N(11)–C(12)–C(13)	81	21 141	12.0 ± 1.0 †	0 ± 10, 120 ± 10

* Solution data calculated from $J_{NH,C\alpha H}$ (Hz) by using the Bystrov modification [29] of the Karplus equation. † This value corresponds to $J_{NH,C\alpha H(1)} + J_{NH,C\alpha H(2)}$ and θ values derived from the analysis of a three-spin system, assuming the second torsion angle is equivalent to θ + 120°.

and the most probable explanation for the low temperature factor is that the amide is shielded from solvent interactions. Thus, on the basis of the n.m.r. data, it appears that the conformation adopted in solution is essentially the same as that in the crystal lattice.

As that of a natural antibiotic, the structure (8) for griseoviridin is of considerable interest. It is clearly related to oestreogrycin (7), as well as to the recently

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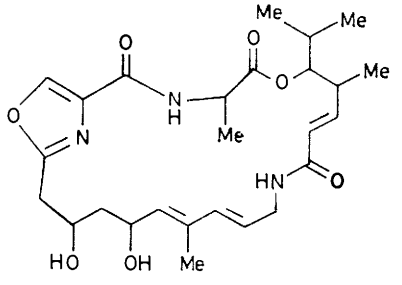
reported antibiotic A-2315 (17).[30] Polypeptide antibiotics, in general, are often associated with amino-acids of the D-configuration [31] and at higher oxidation levels.[32] The oxazole system in structures (6), (7), and (17) can be considered as a cyclised didehydroserine residue. The presence of both types of structure in (6) and (17) in



(17)

adjacent positions, as well as of the two didehydro-amino-acid systems observed in (7), is noteworthy. The importance of these units to the topochemical features of biologically active peptides [24-27] and to structure–activity correlations [33] has already been pointed out.*

EXPERIMENTAL

Mass spectra were determined with an A.E.I. MS9 high resolution spectrometer. 100 MHz ^{1}H N.m.r. spectra were recorded with tetramethylsilane as internal reference, and temperature studies were conducted at four different temperatures between 0 and 50 °C. ^{13}C N.m.r. spectra were obtained with a JEOL PS100 pulsed Fourier transform spectrometer at 25.15 MHz. M.p.s were determined with a Kofler hot-stage apparatus.

When substances are stated to be identical, their identity has been established by comparison of m.p. and mixed m.p. (if applicable), and where appropriate comparison of i.r., n.m.r., and mass spectra, as well as behaviour on paper and thin-layer chromatography.

Griseoviridin.—The compound readily crystallised from methanol; m.p. 161—163° (decomp.) (Found: C, 54.2; H, 6.4; H, 8.1; S, 6.2%; M^+, 477.1593. Calc. for $C_{22}H_{27}N_3O_7S$,MeOH: C, 54.25; H, 6.15; N, 8.25; S, 6.3%; M, 477.162 5); $[\alpha]_D^{22}$ −232° (0.2 in MeOH); for ^{1}H and ^{13}C n.m.r. data see text.

Acetylation of griseoviridin in pyridine with acetic anhydride at room temperature (24 h) gave the diacetate, m.p. 136—140° (decomp.); M^+ 561; $\nu_{max.}$ 1 795 cm^{-1}; τ [$(CD_3)_2N{\cdot}CDO$] 7.60 (3 H, s) and 7.71 (3 H, s).

Salts of Griseoviridin.—(*a*) The hydrochloride was prepared as described [6] and crystallised as well defined needles from ether–methanol. It possessed no definite m.p. and gradually decomposed above 170 °C (Found: C, 51.5; H, 5.6; Cl, 7.0; N, 8.0; S, 5.9. Calc. for $C_{22}H_{27}N_3O_7S$,HCl: C, 51.4; H, 5.5; Cl, 6.9; N, 8.2; S, 6.2%); $\nu_{max.}$ (KBr) 3 540, 3 470, 1 715, 1 659, 1 610, and 1 580 cm^{-1}; ^{1}H n.m.r. data were essentially the same as those of the free base.

(*b*) Griseoviridin was dissolved in concentrated hydrobromic acid (48% w/v) at 0 °C and the solution was evaporated at room temperature to give an oil which crystallised on addition of a small quantity of methanol. Recrystallisation from methanol–acetone afforded *griseoviridin hydrobromide* (no definite m.p. below 300 °C but extensive decomposition) (Found: C, 47.0; H, 4.9; N, 7.4; Br, 14.0. $C_{22}H_{27}N_3O_7S$,HBr requires C, 47.3; H, 5.1; Br, 14.3; N, 7.5; S, 5.7%).

(*c*) Griseoviridin was dissolved in dioxan and treated with perchloric acid (70%) as described by Ames and Bowman.[6] The perchlorate crystallised from methanol as long needles which decomposed rapidly when heated above 190 °C (Found: C, 46.1; H, 5.1; Cl, 5.9; N, 7.1. Calc for $C_{22}H_{27}N_3O_7S$,$HClO_4$: C, 45.7; H, 4.9; Cl, 6.1; N, 7.3; S, 5.5%); $[\alpha]_D^{20}$ −181° (MeOH).

Regeneration of Griseoviridin from its Hydrochloride.—Dry pyridine (2 ml) was added to griseoviridin hydrochloride (40 mg) and the mixture gently warmed until the salt had dissolved. On cooling, crystals of griseoviridin (25 mg) were deposited which after recrystallisation from methanol were identical with an authentic sample.

Hydrolysis of Griseoviridin Hydrochloride.—Titration of griseoviridin hydrochloride in water required 2 mol. equiv. of (0.1M) sodium hydroxide; on back-titration of this solution with (0.1M) hydrochloric acid only 1 mol. equiv. was consumed. The solution showed $\lambda_{max.}$ (0.1M-NaOH) 221 (ε 28 000) and 272 nm (21 500), $\lambda_{max.}$ (neutral) 218 (27 500), τ ($NaOD$–D_2O) 1.92 (−O−CH=C).

Acidic Hydrolysis of Griseoviridin.—Griseoviridin (150 mg) was hydrolysed with degassed 5M-hydrochloric acid in a sealed tube at 100 °C. The hydrolysate was filtered and extracted exhaustively with ethyl acetate. The aqueous layer was oxidised by bubbling air through the solution. Paper chromatography (two-dimensional procedures [34]) revealed the presence of cystine and small amounts of serine and glycine. The residue left after the evaporation of the solvent was chromatographed on a column of Amberlite ion-exchange resin (IRA-400) with first water and then 10% acetic acid as eluant. Evaporation of the acetic acid eluate gave D-cystine, $[\alpha]_D$ +209° (*c* 0.2 in M-HCl).

Evaporation of the ethyl acetate extract gave a gum which on treatment with 2,4-dinitrophenylhydrazine in ethanol afforded an orange-brown precipitate. Chromatography on silica ($CHCl_3$) and crystallisation from methanol gave the 2,4-dinitrophenylhydrazone of (+)-(*R*)-2-oxo-1,5-hexanolide, m.p. 222—226° (lit.,[7] 223—226°), M^+ 308.

Crystal Structure Determination.—Griseoviridin crystallises from methanol as a monosolvate. Oscillation and Weissenberg photographs were taken about the *a* axis. For intensity measurements a crystal was mounted about the *a* axis on a Hilger and Watts four-circle diffractometer. Cell parameters were refined by a least-squares method using the positions of 12 reflections. Intensity data were collected (Mo-K_α radiation) by the ω—2θ scan method over the range θ 0—30°. The intensities of 3 standard reflections monitored every 100 reflections remained constant throughout data collection. 2 170 Reflections with a net count $>3\ \sigma$ were considered observed and were used in the

* *Note added in proof:* Full crystallographic data together with a discussion of the structure of griseoviridin have recently been reported (G. I. Birnbaum and S. R. Hall, *J. Amer. Chem. Soc.*, 1976, **98**, 1976).

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refinement. Lorentz and polarisation, but no absorption corrections were made.

Crystal Data.—$C_{22}H_{27}N_3O_7S,CH_3OH$, $M = 509.26$, monoclinic, $a = 9.426(3)$, $b = 13.068(6)$, $c = 10.552(3)$ Å, $\beta = 99.03(2)°$, $D_c = 1.32$ g cm^{-3}, $U = 1\ 284$ Å^3, $D_m = Z = 2$, $F(000) = 584$. Space group $P2_1$ from systematic absences. Mo-K_α radiation, $\lambda = 0.710\ 7$ Å, $\mu = 1.804$ cm^{-1}.

The structure was solved by direct methods employing the program MULTAN.[35] It proved necessary to generate several sets of E maps before one revealed the positions of 13 sensibly connected atoms including a five-membered ring. Several rounds of structure factor calculations followed by Fourier maps enabled the location of the remaining 22 atoms. Initially five cycles of block-diagonal least-squares refinement of atomic positions and isotropic temperature factors were carried out, after which R was 9.4%. Four further cycles of anisotropic refinement reduced R to 6.5%, at which stage the refinement was terminated. Observed and calculated structure factors and thermal parameters are available as Supplementary Publication No. SUP 21819 (21 pp.) *. Final atom positional parameters are listed in Table 3. Apart for the program MULTAN, all computations were carried out by use of the *X*-Ray '70 system.[36]

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* For details of Supplementary Publications see Notice to Authors No. 7, *J.C.S. Perkin I*, 1975, Index issue.

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