Configurational and Conformational Studies on the Group A Peptide Antibiotics of the Mikamycin (Streptogramin, Virginiamycin) Family

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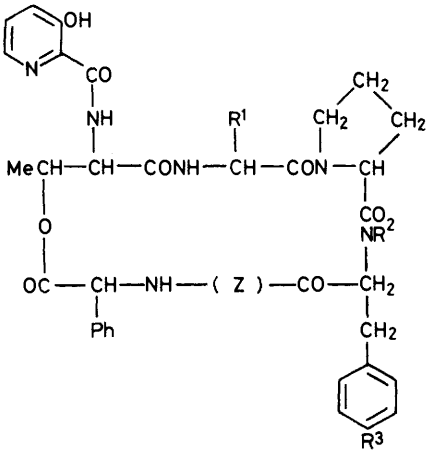
The crystal conformations of griseoviridin (3) and ostreogrycin A (5), which are shown to bear a close resemblance over a large part of the macrocycle, have been compared with those adopted in solution. It is concluded, on the basis of ^{13}C and ^{1}H n.m.r. as well as i.r. data, that one predominant solution conformer exists for both molecules which is essentially the same as that adopted in the crystal lattice. An earlier claim that ostreogrycin A is stabilised by an intramolecular hydrogen bond is reviewed. Spectral data on madumycin (A2315) are used to confirm the proposed structure (6) and comparisons with compounds (3) and (5) have allowed tentative assignments of the relative configuration and the ring conformation. Absolute configurational assignments are made for compounds (5) and (6) and the significance of the molecular geometry of the group is discussed.

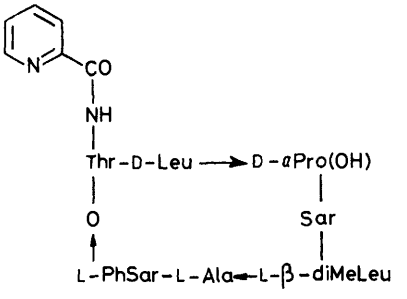
THE antibiotics of the mikamycin, virginiamycin, or streptogramin families are usually isolated as complexes of synergistic compounds.[1-3] The complexes consist of two or more components belonging to two major groups which have been designated A and B. The antibiotics in group A exhibit a marked synergism with those in group B with respect to their activity against Gram-positive bacteria, and a number of the complexes have found clinical application.

The primary site of action for these antibiotics is at the ribosome and it is well established [2] that they interfere with proton biosynthesis. However, neither the precise mechanism of this inhibition nor the mechanism of the synergism is fully understood.

Unfortunately the literature relating to these compounds is complicated by the plethora of synonyms which exist for the individual members of each family.† This is particularly apparent in group B; for example, mikamycin B, streptogramin B, vernamycin B_{α}, pristinamycin 1A, and PA 114B1 are all identical [3] (1; R^1 = Et, R^2 = Me, R^3 = NMe_2, Z = 4-oxopipecolic acid residue). The majority of this group are cyclic heterodetic peptides with the well established general structure (1); an exception is etamycin (viridogrisein), which possesses the larger peptide lactone structure (2), but clearly belongs within the same group. The group A antibiotics are less numerous and can formally be considered as highly modified cyclic depsipeptides. To date, the following have been characterised: griseoviridin



(1)



(2)

(3),[4,5] ostreogrycin G (4),[6] osteogrycin A (5),[7,8] madumycin II (6),[9] which is probably identical with A2315,‡ [10]

† There have been several attempts to clarify the nomenclature,[1-3] but each has standardised on a different antibiotic series. This has led to the absurd situation that in a recent publication essentially the same article appeared twice but under a different title. In this paper the most common nomenclature is employed and no attempt has been made to adopt one particular system.

‡ Madumycin II and A2315 have not been compared, but on the basis of the physical and spectral data reported it appears likely that they are identical. In order to avoid further confusion over nomenclature, they are assumed to be identical throughout this paper although the work was conducted on A2315.

[1] M. Arai, S. Nakamura, Y. Sakagami, K. Fukuhara, and H. Yonehara, *J. Antibiotics*, 1956, Ser. A9, 193; K. Watanabe, *ibid.*, 1961, Ser. A14, 14; N. Tanaka, in 'Antibiotics Vol. III,' eds. J. W. Corcoran and F. E. Hahn, Springer-Verlag, Berlin, 1975, p. 487.

[2] D. Vazquez, in 'Antibiotics Vol. I,' eds. D. Gottlieb and P. D. Shaw, Springer-Verlag, Berlin, 1967, p. 387; also in 'Antibiotics Vol. III,' eds. J. W. Corcoran and F. E. Hahn, Springer-Verlag, Berlin, 1975, p. 521.

[3] P. Crooy and R. De Neys, *J. Antibiotics*, 1972, **25**, 371.

[4] G. I. Birnbaum and S. R. Hall, *J. Amer. Chem. Soc.*, 1976, **98**, 1926.

[5] B. W. Bycroft and T. J. King, *J.C.S. Perkin I*, 1976, 1996.

[6] D. G. I. Kingston, P. S. Sarin, Lord Todd, and D. H. Williams, *J. Chem. Soc.* (*C*), 1966, 1856.

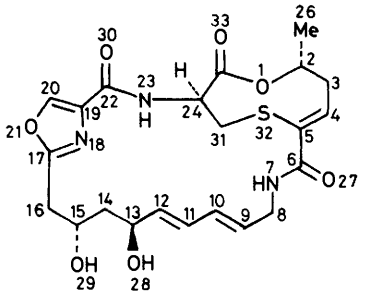
[7] D. G. I. Kingston, Lord Todd, and D. H. Williams, *J. Chem. Soc.* (*C*), 1966, 1669; G. R. Delpierre, F. W. Eastwood, G. E. Gream, D. G. I. Kingston, P. S. Sarin, Lord Todd, and D. H. Williams, *ibid.*, p. 1653.

[8] F. Durant, G. Evrard, J. P. Declercq, and G. Germain, *Cryst. Struct. Comm.*, 1974, **3**, 503.

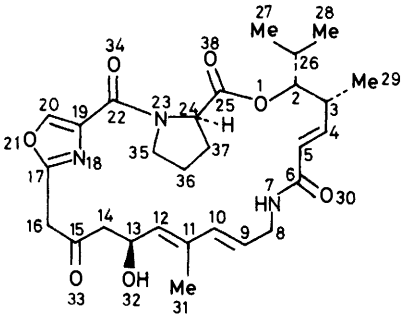
[9] M. G. Brazhnikova, M. K. Kudinova, N. P. Potapova, T. M. Filippova, E. Borowski, J. Zielinski, and J. Golic, *Bioorg. Khim.*, 1976, **2**, 149.

[10] R. L. Hamill and W. M. Stark, U.S.P. Appl. 276,546/1972 (*Chem. Abs.*, 1974, **81**, 2390y), and personal communication.

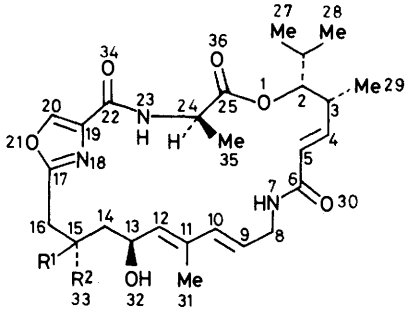
and madumycin I (7) [9] [stereochemistry not defined for (6) and (7)].* All contain a substituted aminodecanoic acid residue and the unusual oxazole system, presumably derived from a cyclised didehydroserine residue.[5]



(3)



(4)
(5) $\Delta^{24,37}$



(6) $R^1 = H$, $R^2 = OH$
(7) $R^1R^2 = >O$

Although the general structures of both groups are now well established, the conformational aspects of these molecules have received little attention. No crystallographic data are available on any of the group B antibiotics and only recently has the solution conformation of a number of this group been studied by n.m.r.[11,12]

Our interest in griseoviridin led us to consider a detailed investigation of the configurational and conformational aspects of the group A antibiotics. With the exception of the crystal structures of griseoviridin (3) [4,5] and ostreogrycin A (virginiamycin) (5),[8] which provided detailed information concerning the crystal conformation of these molecules, little consideration had been given to this group. This is surprising in view of the current interest concerning the possible relationship between the topology of cyclic peptides and their biological activity.[13] Also the restraints imposed by the planar structures present in the molecules severely limits the number of possible conformers, thus making analysis relatively easy.

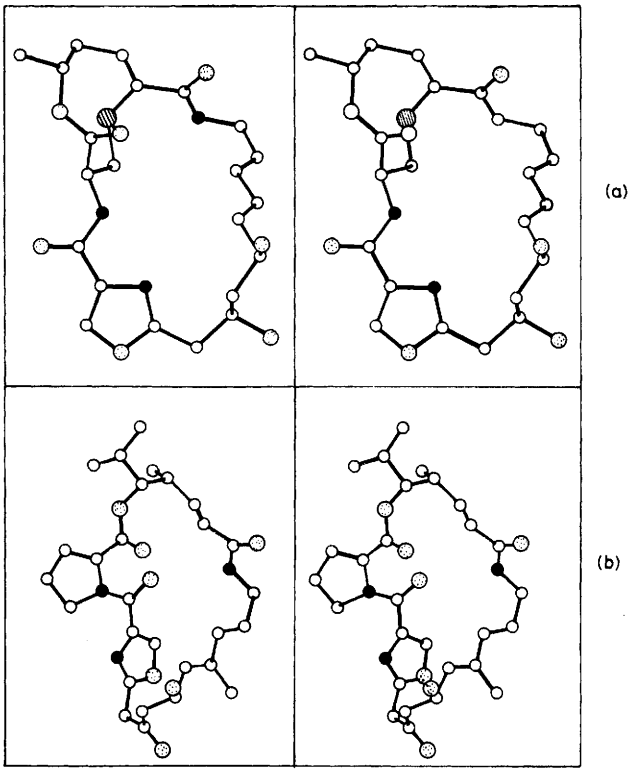


FIGURE 1 Crystal conformation of griseoviridin and ostreogrycin A,‡ perspective drawing by program pluto (Dr. S. Motherwell, Cambridge Crystallographic Data Centre, University Chemical Laboratories, Cambridge)

A comparison of the crystal conformations of griseoviridin and ostreogrycin is informative; the crystal structures (perspective drawings) for these molecules are shown in Figures 1(a) and (b). For griseoviridin, the *X*-ray analysis confirmed that the two double bonds of

* The molecules have been numbered so that the macrocycle atoms are comparable.

‡ Computed for the enantiomeric molecule from the atomic co-ordinates reported in ref. 8.

[11] D. W. Urry, in ' Spectroscopic Approaches to Biomolecular Conformation,' ed. D. W. Urry, American Medical Association, Chicago, 1970, p. 263.

[12] M. J. O. Anteunis, R. E. A. Callens, and D. K. Tavernier, *European J. Biochem.*, 1975, **58**, 259.

[13] See V. F. Bystrov, S. L. Portnova, A. Balashoya, S. A. Koz'min, Y. D. Gavnilov, and V. A. Afanasev, *Pure Appl. Chem.*, 1973, **36**, 19; Yu. A. Ovchinnikov and V. T. Ivanov, *Tetrahedron*, 1974, **30**, 1871 and 1975, **31**, 2177; B. W. Bycroft, in ' Amino Acids, Peptides, and Proteins,' Chem. Soc. Specialist Periodical Report, 1973, vol. 5, p. 351; 1974, vol. 6, p. 381; 1975, vol. 7, p. 320; 1976, vol. 8, p. 310.

the decanoic acid residue possessed the *trans*-geometry and revealed that the two amide and the ester linkages were also *trans*. No intramolecular hydrogen bonds were observed and the absolute configuration of the molecule followed from the known D-configuration of the cysteine residue.

Similarly the *X*-ray data [8] for ostreogrycin A confirmed the *trans*-geometry of the double bonds and established the *trans*-stereochemistry for the amide and ester links, but only the relative configuration of (5) followed from the crystallographic analysis and corresponds to that observed in (3) at centres C-2 and C-13. However there is reasonable circumstantial evidence that the absolute configuration is as indicated in (5) (see later). It was originally suggested [8] that ostreogrycin A possessed an intramolecular hydrogen bond between N-7 and O-34 (3.35 Å). This interatomic distance is larger than the norm for a hydrogen bond (2.70—3.10 Å) in cyclic peptides, and even these values are dependent on the angles between the planes of the amide units.[14] The calculated hydrogen–oxygen distance, assuming a standard amide N–H bond (1.00 Å), is greater than 2.50 Å and it therefore seems unlikely that this is a significant interatomic distance. The interaction, if any, is probably a weak one imposed by the geometry of the molecule and presumably plays little part in stabilising the crystal conformation.

TABLE 1

Torsion angles (°) for the ring atoms of griseoviridin and ostreogrycin computed to the nearest degree

Angle		Griseoviridin [5]	Ostreogrycin A †
1–2–4		−74	−82
2–3–5		+89	+115
3–4–6		−172	+177
4–5–7	Acrylamide	−170	+164
5–6–8		−178	+174
6–7–9		+81	+111
7–8–10		−12	−11
8–9–11		−179	−175
9–10–12	Diene	+180	+171
10–11–13		+176	−172
11–12–14		−118	−115
12–13–15		+71	+74
13–14–16		−166	−120
14–15–17		+70	+56
15–16–18		+7	−84
16–17–19	Oxazole-	+179	+174
18–19–23	2-carboxamide	−1	−19
19–22–24		+165	+178
22–23–25		+124	−47
23–24–1		−148	+147
24–25–2		−159	+176
25–2–3		+99	+103

* The sign of the angle follows the right-hand rule (W. Klyne and V. Prelog, *Experientia*, 1960, **16**, 521). † Computed for the enantiomeric molecule from the atomic co-ordinates reported in ref. 8.

The torsion angles for the ring atoms of griseoviridin and ostreogrycin A are listed in Table 1. The acrylamide, diene, and oxazole-2-carboxamide systems in both molecules show some distortion from planarity. In both the acrylamide units there is some distortion about the 5,6-bond. The diene system of griseoviridin is essentially planar, while the same feature is ostreogrycin A shows deviations about the 10,11- and 11,12-bonds. Also the oxazole-2-carboxamide system for this molecule is twisted about the 19,22-bond.

In griseoviridin the amide carbonyl is coplanar with the oxazole ring but the amide group itself is non-planar. In relation to our interest in the stereochemistry of didehydroamino-acid systems, it is noteworthy that the amide group of the 2,3-didehydropropoline residue is coplanar with the carbon–carbon double bond, while the carbonamide group is twisted *ca.* 45° from this plane.

The most striking feature that emerges from the comparison of the 25-membered macrocyclic systems in both molecules is the close similarity in the regions of the acrylamide and aminodecanoic acid residues. The planar systems in these regions are hinged at C-2, C-8, and C-15, and the torsion angles (Table 1) from O-1 through to C-15 are apparently unaffected by the different substituents.

From C-15 to C-25 the conformations differ substantially, notably in the orientation of the 5-carboxamide oxazole unit to the rest of the molecule. In griseoviridin the sulphur bridge from the D-cysteine locks the molecule, whereas in ostreogrycin A the same region is controlled by the geometry of the didehydroproline.

The solution conformation of cyclic peptides and depsipeptides has been intensively investigated over the past decade by a wide variety of physicochemical methods,[13,15] and it is evident that the predominant solution conformer frequently differs from that adopted in the crystal lattice. In an earlier paper [5] we described evidence, principally n.m.r. data, which led us to conclude that the solution conformation of griseoviridin (3) was essentially the same as that found in the crystal structure. We now report further results which substantiate these initial conclusions concerning griseoviridin and also provide evidence for the solution conformations of ostreogrycin A (5) and madumycin (A2315) (6).

Table 2 lists the ^{13}C n.m.r. data for compounds (3), (5), and (6) in $CDCl_3$ and $(CD_3)_2N{\cdot}CHO$ at 21 °C. The expected number of signals was observed for each compound in the proton noise-decoupled spectra. No doubling or coalescing of any signals was detected on warming to 45 °C or on cooling to −23 °C, which strongly implied that under these conditions one conformer predominates for each molecule. The chemical shift values and the observed multiplicities, obtained from continuous wave decoupling experiments with irradiation at the resonance frequency corresponding to Me_4Si and that corresponding to δ 10.00, accorded with the structures (3), (5), and (6). The assignments were

[14] See C. Ramakrishnan and N. Prasad, *Internat. J. Protein Res.*, 1971, **3**, 209; B. Pullman and A. Pullman, *Adv. Protein Chem.*, 1974, **28**, 348, and references cited therein.

[15] F. A. Bovey, A. I. Brewster, D. J. Patel, A. E. Tonelli, and D. A. Torchia, *Accounts Chem. Res.*, 1972, **5**, 193; C. M. Deber, V. Madison, and E. R. Blout, *ibid.*, 1976, **9**, 106.

made by comparisons within the series and with model systems, *e.g.* cysteine, alanine, and didehydroamino-acid [16] derivatives. It was possible to assign the singlets due to the ester carbonyls (C-25) in all three molecules, as well as the ketone carbonyl (C-15) in ostreogrycin A (5). The remaining singlet assignments are only tentative. There is little change in the spectra of (5) and (6) in $CDCl_3$ or $(CD_3)_2CHO$, which implies the conformations are essentially the same in polar and non-polar solvents.

The extremely good correlation between madumycin (A2315) (6) and ostreogrycin A (5) from C-2 to C-13 and C-26 to C-31, as well as between griseoviridin (3) and (6) from C-14 to C-20, supports the structure (6). In addition it offers strong circumstantial evidence that the geometry of the molecule and the relative configuration at the centres C-2, C-3, C-13, and C-15 in (6) are the same as in (3) and (5). Detailed assignments of the high resolution ^1H n.m.r. spectra for (3),[5] (5),[7] and (6)[9] had previously been made in the course of the structural investigations, and it was possible to use and extend these data in order to provide information concerning the solution conformation of these molecules. The temperature dependences of the NH-23 and NH-7 signals for (3), (5), and (6) in $(CD_3)_2N{\cdot}CHO$, expressed as gradients, are 0.002 0 and 0.004 5, NH-23 absent and 0.003 0, and 0.007 5 and 0.003 5 p.p.m. K^{-1}, respectively. The significant upfield shifts of these signals, with the possible exception of the NH-23 signal in griseoviridin, suggested that these protons are not hydrogen-bonded, an observation which found further support in their ready exchange with D_2O.

The i.r. spectra of (3), (5), and (6) in $CHCl_3$ showed broad bands in the NH region centred at 3 398, 3 360, and 3 408 cm^{-1}, respectively. Unfortunately these values, in the main, fall between those for free NH groups and those of hydrogen-bonded groups and do not provide conclusive evidence.

TABLE 2

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

	Griseoviridin	Ostreogrycin A		Madumycin (A2315)	
	$[(CD_3)_2NCHO]$	$[(CD_3)_2NCHO]$	$(CDCl_3)$	$[(CD_3)_2NCHO]$	$(CDCl_3)$
C-2	71.70 (d)	81.17	81.34 (d)	82.27	82.22 (d)
C-3	38.06 (t)	37.84	37.60 (d)	36.90	36.78 (d)
C-4	141.81 (d)	143.74	143.56 (d)	141.46	140.58 (d)
C-5	*	124.79	123.21 (d)	125.33	124.60 (d)
C-6	*	*	*	*	*
C-8	45.32 (t)	45.15	45.55 (t)	44.74	43.39 (t)
C-9	135.70 (d)	133.27	131.28 (d)	133.86	134.18 (d)
C-10	131.22 (d)	125.79	124.97 (d)	125.79	124.6 (d)
C-11	130.05 (d)	*	*	*	*
C-12	137.42 (d)	134.03	133.79 (d)	135.84	135.61 (d)
C-13	51.28 (d)	65.20	65.73 (d)	65.85	67.19 (d)
C-14	36.43 (t)	40.17	40.53 (t)	36.55	36.14 (t)
C-15	66.40 (d)	201.57	200.87 (s)	66.92	67.48 (d)
C-16	40.93 (t)	49.29	47.83 (t)	40.41	40.58 (t)
C-17	*	*	*	*	*
C-19	*	*	*	*	*
C-20	145.31 (d)	146.20	145.38 (d)	145.09	145.02 (d)
C-22	*	*	*	*	*
C-24	70.52 (d)	*	*	47.60	47.31 (d)
C-25	171.81 (s)	166.25	167.71 (s)	172.28	172.33 (s)
C-26	20.64 (q)	30.23	30.17 (d)	30.17	30.17 (d)
C-27		19.71	19.59 (q)	19.83	19.71 (q)
C-28		18.95	18.89 (q)	19.00	19.06 (q)
C-29		12.75	12.75 (q)	13.50	13.04 (q)
C-31	38.36 (t)	12.40	12.20 (q)	10.64	10.47 (q)
C-35		51.46	50.58 (t)	18.60	18.59 (q)
C-36		30.12	29.94 (t)		
C-37		126.37	125.67 (d)		

* Singlets tentatively assigned as follows:

129.41, 131.75	C-5 and C-19	133.27, 136.31	131.28, 135.78, 136.89	C-11, C-19, and C-24	134.39, 136.32	134.15, 135.61	C-11 and C-19
159.82, 163.27, 163.91	C-6, C-17, and C-22	137.27, 157.65, 161.05, 161.46	156.19, 160.11, 160.93	C-6, C-17, and C-22	160.12, 161.59, 165.86	160.05, 162.27, 166.25	C-6, C-17, and C-22

The principal conformational parameter which can be obtained from ^1H n.m.r. data of peptides is the torsion angle (θ) between the NH amide hydrogen and the α-proton of an amino-acid residue. This is derived from the corresponding vicinal spin–spin coupling constant. A number of Karplus equations [17] with coefficients adjusted for the electronegativity of substituents have been employed to express this relationship. The equation used in this investigation is that due to Bystrov *et al.*,[18] and the coupling constants and relevant calculated θ values are shown in Table 3. In these

[16] B. W. Bycroft and N. Whittaker, unpublished results.
[17] M. Karplus, *J. Amer. Chem. Soc.*, 1963, **95**, 2870.
[18] V. F. Bystrov, V. T. Ivanov, S. L. Portnova, T. A. Batashova, and Yu. A. Ovchinnikov, *Tetrahedron*, 1973, **29**, 873.

calculations the NH(7)–CH_2(8) unit was considered to be equivalent to a glycine residue. Clearly the information is limited since there are only two amide linkages within the molecules (one in ostreogrycin A). However, coupled with the ^{13}C n.m.r. data, the information relating to the NH hydrogen bonds, and an examination of Corey–Pauling–Koutlum models, the evidence is consistent with (3) and (5) retaining, in solution, conformations which are very similar to those exhibited in the crystal lattice (see Figure 1). It can also be inferred that madumycin II (6) has a solution conformation similar to those of both griseoviridin and ostreogrycin A over the region C-1 to C-16 but closer to that of griseoviridin from C-16 to C-25.

We had pointed out earlier that the formation of an intramolecular hydrogen bond from NH-23 to O-27 or any other oxygen in griseoviridin (3) is unlikely without gross distortion of the molecular framework.[5] This hydrogen is caged within the molecule and the most probable explanation for the low temperature factor is that the amide is shielded from the solvent interactions. On the other hand the formation of an intramolecular hydrogen bond between NH-7 and O-34 in ostreogrycin A would require only a small conformational change. The evidence is rather ambiguous; the n.m.r. data are not clear cut, whereas the NH stretching frequency suggests a hydrogen bond. It is possible that the crystal conformation which is stabilised by intermolecular hydrogen bonds [8] undergoes a small conformation change on dissolution resulting in the formation of a weak intramolecular hydrogen bond. The uncertainty with respect to the interpretation of the above evidence on the nature of NH bonds illustrates the extreme caution that is necessary in drawing any diagnostic conclusions from this sort of data alone.

In the group A series only the absolute configuration of griseoviridin has been established definitively. The proline residue in ostreogrycin G (4) is known to possess the D-configuration [8] and since it co-occurs with ostreogrycin A, the relative configuration of which is based on *X*-ray data, it is likely that the configurations at the C-2, C-3, and C-13 centres are the same in both molecules. No stereochemical assignments have been made for any of the centres in either of the madumycins (6) and (7).

In connection with this and other investigations we required a sensitive and accurate method for determining the chirality of α-amino-acids. To this end, we examined the g.l.c. analysis of *N*-trifluoroacetylamino-acid esters on *N*-lauroyl-L-valin-(t-butyl)amide as the stationary phase [19] and found this to be an extremely effective method of identifying individual isomers.

Hydrolysis of madumycin II (6) afforded a hydrolysate which was shown by paper chromatography to contain alanine and traces of glycine. Derivatisation with diazomethane followed by trifluoroacetic anhydride afforded the *N*-trifluoroacetyl methyl esters, which were analysed by g.l.c. The results (Figure 2), clearly established the presence of D-alanine in the hydrolysate and coupled with information presented earlier provided strong evidence that madumycin II possesses the absolute configuration incated in (6).

During the chemical investigation on ostreogrycin A (5), partially racemised D-proline was isolated from the hydrolysate of a hydro-derivative of (5).[6] We repeated this experiment using the hydrogenation conditions previously described and examination of the proline in the hydrolysis products employing the g.l.c. method outlined above gave a ratio of 7 : 3 for the D- and L-isomers, respectively. The considerable enantiomeric excess of the D-isomer must have resulted from a preferential addition of hydrogen to the *si-re*-face of the didehydroproline residue. Examination of the model of ostreogrycin A (see Figure 1) clearly suggests that for

TABLE 3

Comparison of NH–CH torsion angle (θ)

	(*a*) NH(7)–CH_2(8)				(*b*) NH(23)–CH(24)			
	Crystal		Solution *		Crystal		Solution *	
	Ring torsion angle (°)	θ (°)	J_{NHCH} †/Hz	θ	Ring torsion angle (°)	θ (°)	J_{NHCH}/Hz	θ (°)
Griseoviridin (3)	81	21, 141	12.0	0, 120	124	−176	7.5	±155
Ostreogrycin A (5)	111	51, 171	10.0	76, 196	−47			
Madumycin (A2315) (6)			12.0	0, 120			8.0	±160

* Solution data calculated from J_{NHCH} (±0.5 Hz) by using the Bystrov modification of the Karplus equation; θ values *ca.* ±10°.
† This value corresponds to $J_{NHCH_2} + J_{NHCH_2}$ and θ values derived from the analysis of a three-spin system, assuming the second torsion angle is equivalent to θ + 120°.

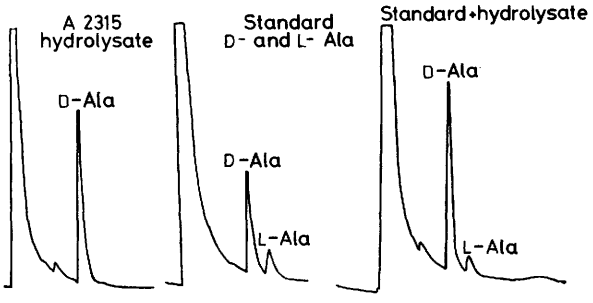
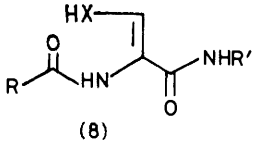


FIGURE 2 G.l.c. of alanine from hydrolysis of A-2315, derivatised as *N*-trifluoroacetyl methyl ester

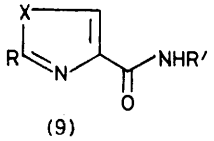
[19] B. Feibush, *J.C.S. Chem. Comm.*, 1971, 544; R. Charles, U. Beitler, B. Feibush, and E. Gil-Av, *J. Chromatog.*, 1975, **112**, 121.

such an occurrence ostreogrycin A has to possess the absolute configuration shown (5). There is considerable precedent for the assumption that in heterogeneous catalytic hydrogenation the hydrogen is delivered from the least hindered face of the αβ-unsaturated amino-acid unit,[20] although the mechanism of the process appears to be more complex than originally envisaged.[21,22] It is also assumed that there is no substantial conformational change due to the interaction of the substrate with the catalyst.

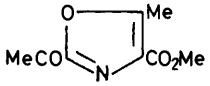
This evidence should not be considered as conclusive, but it does provide a working basis for some tentative observations. It appears that compounds (3)—(6) are a series of closely related molecules with the same relative and absolute configuration. The D-configuration of the amino-acid residues in (3), (4), and (6) is particularly noteworthy. Some years ago we pointed out the common occurrence of *D*- and didehydro-amino-acids in microbial metabolites and suggested that they might be related in some way.[23] It is interesting that the antibiotics of this series contain, linked to a D-amino-acid, the oxazole system (9; X = O), which may



(8)



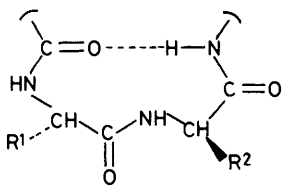
(9)



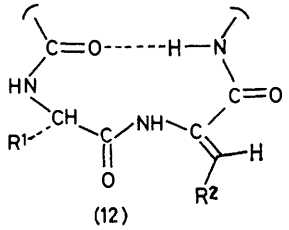
(10)

be formally considered as a cyclised acyldidehydroseryl derivative (8; X = O). The oxazole system (9) is so far unique to this class of peptide antibiotics, although the oxazole (10) has been isolated as a degradation product of berninamycin A and is believed to be present in the intact antibiotic.[24] The corresponding thiazole (9; X = S), presumably derived from a didehydrocysteinyl group, occurs in a number of antibiotics.[25]

It is now clear that for certain peptide antibiotics such as gramicidin S, tyrocidine, and related compounds which contain only D- and L-amino-acids, the D-residue is formed directly from the L-isomer by a simple racemising enzyme.[26] For structurally more complex peptides which contain both amino-acids at higher oxidation levels and with the D-configuration, there is still speculation as to whether these structures have a common biogenetic origin. However there is increasing evidence of a structural relationship, as illustrated by the co-occurrence of ostreogrycin G (4) and A (5) in the same organism. The presence of these units undoubtedly influences the geometry of the molecule. The β-turn or loop (11) is now a well characterised feature of cyclic



(11)



(12)

peptides and depsipeptides,[11,13] and several examples are known where the D-residue is replaced by a didehydro-amino-acid (12).[27]

The importance of the topochemistry of peptide antibiotics is already well recognised [13] and the above correlation of configuration and conformation may be of value to further structure–activity investigations.

EXPERIMENTAL

^{1}H N.m.r. 100 MHz spectra were recorded with tetramethylsilane as internal reference, and temperature studies were conducted at four different temperatures between 250 and 318 K. ^{13}C N.m.r. spectra were obtained at 25.15 MHz using a JEOL PS100 pulsed Fourier transform spectrometer and pulsed programmer, interfaced to a Nicolet 1085 20K computer. The pulse width was 4 μs (30°) and free induction decays were sampled using 8K data points over a spectral width of 6024 Hz.

G.l.c. was performed on a Pye 104 instrument with flame ionisation detector equipped with a silanised glass column (520 × 0.2 cm) packed with GasChrom Q (100—120 mesh) coated with 2% *N*-lauryl-L-valin-(t-butyl)amide. The gas flow (N_2) rate was 60 ml min^{-1} and the oven temperature 73 °C. Paper chromatography was carried out by the ascending technique on Whatman No. 1 paper using the solvent systems butan-1-ol–acetic acid–water (4 : 1 : 5) and butan-1-ol–acetone–water–diethylamine (10 : 10 : 5 : 2).

Hydrolysis of A2315 (Madumycin II).—A2315 (madumycin II) (10 mg) was heated in a sealed tube at 110 °C for 12 h with 6N-hydrochloric acid (2.5 ml). The cooled solution was extracted with ethyl acetate and evaporated to dryness. Paper chromatography in the above systems indicated the presence of alanine with traces of glycine. The residue was dissolved in trifluoroacetic acid (100 μl) and trifluoroacetic anhydride (100 μl) added. After 5 min at 20 °C, the solvents were removed by a stream of nitrogen. The residue was dissolved in methanol (100 μl) and treated

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[25] See B. F. Anderson, D. C. Hodgkin, and M. A. Viswamitra, *Nature*, 1970, **225**, 233; P. Brookes, R. J. Clark, A. T. Fuller, M. P. V. Mijovic, and J. Walker, *J. Chem. Soc.*, 1960, 925; B. W. Bycroft and R. Pinchin, *J.C.S. Chem. Comm.*, 1975, 121.

[26] See F. Lipmann, *Accounts Chem. Res.*, 1973, **11**, 361.

[27] H. Yoshioka, T. Aoki, H. Goko, K. Nakatsu, H. Sakakibara, T. Take, A. Nagata, J. Abe, T. Wakamiya, T. Shiba, and T. Kaneko, *Tetrahedron Letters*, 1971, 2043; B. W. Bycroft, *J.C.S. Chem. Comm.*, 1972, 660.

with ethereal diazomethane. The solvents were evaporated off after 15 min and the derivatised hydrolysate was taken up in acetone (100 μl) for injection onto the g.l.c. column (see Figure 2).

Hydrolysis of 'Hydro A' Ostreogrycin A.—'Hydro A' prepared from ostreogrycin A (10 mg) under the conditions described by Todd *et al.*, was heated in a sealed tube at 110 °C for 12 h with 6N-hydrochloric acid (4 ml). The cooled solution was extracted with ethyl acetate and evaporated to dryness. Paper chromatography indicated the presence of proline as the major component with traces of glycine, serine, and alanine. Derivatisation of the residue as in the preceding experiment and g.l.c. analysis showed the presence of D- and L-proline in the ratio 70 : 30, respectively.

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