CYCLODEPSIPEPTIDES

By D. W. Russell

(Twyford Laboratories, Twyford Abbey Road, London, N.W.10)*

1. Introduction

An earlier Review[1] described the chemistry of homeomeric peptides, acid hydrolysis of which gives amino-acids only. Heteromeric peptides yield other products as well as amino-acids. One well-defined group of heteromeric peptides comprises a number of cyclic compounds, in which the ring is composed entirely of residues of amino- and hydroxy-acids joined by amide and ester bonds. These are cyclodepsipeptides. Their chemistry has been described in a recent detailed review which also discusses their classification and nomenclature.[2] Here we need note only that they are sometimes referred to as "peptolides".[3] Current interest in these compounds arises from the fact that many possess marked biological activity (see Section 7).

2. Occurrence, Isolation, and Physical Properties

Cyclodepsipeptides have been isolated from bacteria, actinomycetes, and fungi (Table 1), but not as yet from algae or higher organisms. Since biological activity has provided the main motive for their isolation, and such activity is not always exhibited, the present picture of their distribution may be distorted.

Most natural cyclodepsipeptides are neutral compounds, insoluble in water and soluble in organic solvents. For their isolation, the appropriate micro-organism is grown in a nutrient solution, and the aqueous culture filtrate is discarded. The dried organisms are then extracted with an organic solvent, which dissolves both cyclodepsipeptides and lipids. Further purification is by chromatographic or differential solubility techniques. One organism may produce several closely related cyclodepsipeptides, when isolation of any but the major component may be very difficult.

The pure compounds are crystalline and optically active, and have moderately high melting points. Physical criteria are inadequate to assess their purity. Thus, two synthetic diastereoisomers had almost the same m.p., undepressed in admixture, and were indistinguishable in specific rotation, *X*-ray powder diagram, thin-layer chromatography, and infra-

* Present address: Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia.

[1] H. D. Springall and H. D. Law, *Quart. Rev.*, 1956, **10**, 230; see also J. P. Greenstein and M. Winitz, "Chemistry of the Amino-acids", John Wiley & Sons, Inc., London, 1961, vol. 2, pp. 763—1295 (peptide synthesis) and 1512—1687 (peptide structural analysis).

[2] G. Losse and G. Bachmann, *Z. Chem.*, 1964, **4**, 204, 241.

[3] E. Schröder and K. Lübke, *Experientia*, 1963, **19**, 57.

TABLE 1. *Natural occurrence of cyclodepsipeptides*

Taxonomic group	Species	Cyclodepsipeptide	Approximate yield*
Bacteria	*Serratia marcescens*	Serratamolide	15
	Pseudomonas tabaci	Wildfire toxin	5
	Bacillus mesentericus	Esperin	—†
Actinomycetes	*Streptomyces* PRL 1642	(Amidomycin)‡	500
		Valinomycin	50
	S. fulvissimus	Valinomycin	30
Fungi	*Fusarium scirpi*	Enniatin A	1000
	F. orthoceras var. *enniatinum*	Enniatin A	900
	Fusarium ETH 4363	Enniatin B	500
	ETH 1574	Enniatin B	1700
	Pithomyces chartarum	Sporidesmolides I—III	200
		Pithomycolide	—
	P. maydicus	Sporidesmolide IV	200
	P. cynodontis	Angolide	100
	P. sacchari	Angolide	100
	Isaria cretacea	Isariin	250
	Giberella baccata	Enniatins	—

* In mg./l. of culture.
† A dash indicates that no figure for yield is available.
‡ See text.

red and mass spectra.[4,5] This illustrates the care needed to establish homogeneity. Quantitative amino-acid analysis is a valuable adjunct to physical measurements.

The infrared spectra of cyclodepsipeptides show strong absorptions due to ester (1755—1715 cm.$^{-1}$) and amide (1680—1635 cm.$^{-1}$) carbonyl groups. The amide II band (1575—1500 cm.$^{-1}$) is observed only when the group –CO·NH– is present; its absence is therefore strong, though not conclusive, evidence that the amino-acid residues are all *N*-alkylated. The presence of primary amino-acid residues is indicated by the N–H stretching band (3360—3260 cm.$^{-1}$).

Determination of molecular weights of these compounds, by methods which measure colligative properties of their solutions, has sometimes given misleadingly low results (see enniatins and valinomycin, section 3).

3. Structure Determination

A few simple reactions usually suffice to define a cyclodepsipeptide structure. As with homeomeric peptides, much information is gained from

[4] M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin, and K. Ku. Khalilulina, *Zhur. obshchei. Khim.*, 1965, **35**, 1399; M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and A. A. Kiryushkin, *Tetrahedron Letters*, 1963, 1927.
[5] W. S. Bertaud, M. C. Probine, J. S. Shannon, and A. Taylor, *Tetrahedron*, 1965, **21**, 677.

total and partial hydrolysis. The sensitivity of cyclodepsipeptide ester bonds to alkali affords the opportunity for *specific* partial chemical hydrolysis, which is rarely possible with homeomeric peptides.

Total Acid Hydrolysis.—Vigorous acid hydrolysis cleaves all ester and amide bonds, giving a mixture of amino- and hydroxy-acids. Table 2 lists

TABLE 2. *Products of acid hydrolysis of cyclodepsipeptides*

$R^1 \cdot CH(R^2) \cdot CO_2H$

R^1	R^2	Name	Abbreviation	Isomer
H	NH_2	Glycine	Gly	—
Me	NH_2	Alanine	Ala	L
	NHMe	*N*-Methylalanine	MeAla	L
	OH	Lactic acid	Lac	L
CH_2OH	NH_2	Serine	Ser	L
Pr^i	NH_2	Valine	Val	D, L
	NHMe	*N*-Methylvaline	MeVal	L
	OH	α-Hydroxyisovaleric acid	HyV	D, L
Bu^i	NH_2	Leucine	Leu	D, L
	NHMe	*N*-Methyl-leucine	MeLeu	L
	OH	α-Hydroxyisocaproic acid	HyC	L
Bu^s	NH_2	Isoleucine	Ile	L
		Alloisoleucine	aIle	D
	NHMe	*N*-Methylisoleucine	MeIle	L
$HO_2C \cdot CH_2$	NH_2	Aspartic acid	Asp	L
$HO_2C \cdot CH_2 \cdot CH_2$	NH_2	Glutamic acid	Glu	L

$R \cdot CHOH \cdot CH_2 \cdot CO_2H$

R	Name
$n\text{-}C_7H_{15}$	D-β-Hydroxydecanoic acid
$n\text{-}C_9H_{19}$	D-β-Hydroxydodecanoic acid
C_6H_5	D-β-Hydroxy-β-phenylpropionic acid

those acids which have been identified in cyclodepsipeptide hydrolysates. The common abbreviations are given and will be used where appropriate in this Review. Each abbreviation standing alone represents the intact acid; substitution of a hydrogen atom in the amino- or hydroxyl group, or of a hydroxyl in the carboxyl group, is indicated by a dash.[6] Thus:

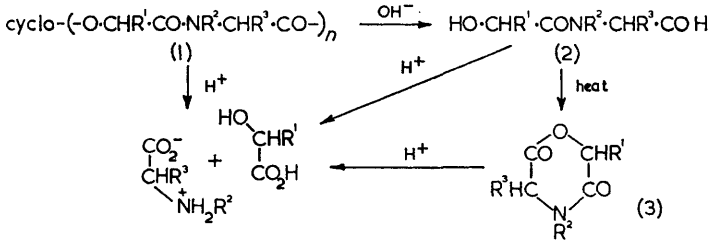
$$\begin{aligned} \text{Val} &= {}^{+}H_3N \cdot CHPr^i \cdot CO_2^- \\ \text{-Val} &= \text{-}NH \cdot CHPr^i \cdot CO_2H \\ \text{Val-} &= H_2N \cdot CHPr^i \cdot CO\text{-} \\ \text{-Val-} &= \text{-}NH \cdot CHPr^i \cdot CO\text{-} \end{aligned}$$

[6] R. Schwyzer, J. Rudinger, E. Wünsch, and G. T. Young, "Peptides", ed. G. T. Young, Pergamon Press, London, 1963, pp. 261—269.

The α-hydroxy-acids are hydroxyl analogues of common α-amino-acids with aliphatic side chains. Like the amino-acids, they are liberated in high yield by acid hydrolysis. Part or all of the β-hydroxy-acids, on the other hand, may be dehydrated under hydrolytic conditions. Thus, acid hydrolysates of pithomycolide contain not only β-hydroxy-β-phenylpropionic acid, but also cinnamic acid derived from it.[7] The α-amino- or -hydroxy-acids may have the L- or the D-configuration. Only D-β-hydroxy-acids have so far been found, and the *N*-methylamino-acids are exclusively L, facts which may have some unrecognised biochemical significance.

Amino-acids can be identified by chromatography, but most workers have prudently isolated individual amino-acids from hydrolysates in order to characterise them. Stereochemical configuration can then be determined with the polarimeter. Enzymic methods have occasionally been used. No enzymic method exists for determining the configuration of most hydroxy-acids, and their isolation is imperative. Stereoisomeric amino-acids can be distinguished by vapour-phase chromatography;[8] extension of this technique to hydroxy-acids would greatly decrease the amount of a cyclodepsipeptide needed for structure determination.

Partial Alkaline Hydrolysis.—Cyclodepsipeptides of type (1), partially hydrolysed by alkali, give *N*-(hydroxyacyl)amino-acids (2). These, when distilled, lactonise to 2,5-dioxomorpholines (3), which can be further hydrolysed by acid to the amino- and hydroxy-acids.



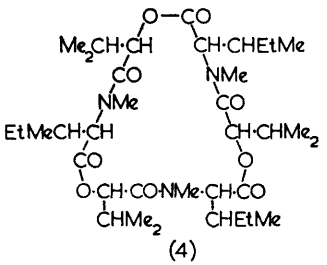
Cyclodepsipeptides which contain two or more amino-acid residues directly linked together are hydrolysed by alkali to stable, crystalline *N*-(hydroxyacyl)-peptides. The sequence of amino-acid residues in these is established by standard methods of peptide structural analysis.[1]

The results of total and partial hydrolysis generally lead unequivocally to a unique structure, provided the molecular weight is known. Other degradation techniques will be noted in connection with individual compounds.

Structures of Naturally-occurring Cyclodepsipeptides.—*Enniatins.* The long-known antimicrobial properties of cultures of *Fusarium* species

[7] L. H. Briggs, L. D. Colebrook, B. R. Davis, and P. W. LeQuesne, *J. Chem. Soc.*, 1964, 5626.
[8] B. Halpern and J. W. Westley, *Biochem. Biophys. Res. Comm.*, 1965, **19**, 361.

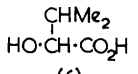
prompted a search for the active compounds.[9] One result was the isolation of the first cyclodepsipeptides, enniatins A [10] and B.[11] Enniatin A (4; ≡ 5, $n = 3$) $(C_{12}H_{21}O_3N)_n$, gave on total acid hydrolysis D-α-hydroxyisovaleric



(4)

cyclo-(-D-HyV-L-MeIle-)$_n$

(5)



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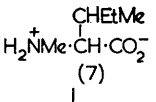
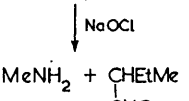
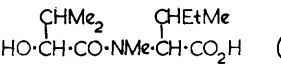


(7)



(8)



(9)

acid (6) and an amino-acid, $C_7H_{15}NO_2$. The latter was oxidised to methylamine and (+)-2-methylbutyraldehyde (8); it was therefore the *N*-methyl derivative (7) of either L-isoleucine or D-alloisoleucine, which have the same configuration about the β-carbon atom. Its assignment to the L series followed from the rule that L-amino-acids have a more negative specific rotation in acid than in neutral solution; the identification was confirmed by synthesis.[10]

Partial alkaline hydrolysis gave the *N*-(hydroxyacyl)amino-acid (9), distillation of which yielded its lactone (5; $n = 1$). Acid hydrolysis of this lactone afforded hydroxy-acid (6) and amino-acid (7).

Because of a wrong value for the molecular weight, enniatin A was formulated as (5; $n = 2$).[10] This compound, later synthesised,[12] differed from the natural antibiotic; the correct structure (4; ≡ 5, $n = 3$) was established by synthesis.[13,14]

Structure (10; $n = 2$), assigned on like evidence to enniatin B,[11] was similarly corrected to (10; $n = 3$).[15,16]

cyclo-(-D-HyV-L-MeVal-)$_n$

(10)

[9] E. Gäumann, S. Roth, L. Ettlinger, Pl. A. Plattner, and U. Nager, *Experientia*, 1947, **3**, 202.
[10] Pl. A. Plattner and U. Nager, *Helv. Chim. Acta*, 1948, **31**, 2192.
[11] Pl. A. Plattner and U. Nager, *Helv. Chim. Acta*, 1948, **31**, 665.
[12] M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin, and V. T. Ivanov, *Tetrahedron*, 1963, **19**, 581.
[13] M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin, and V. T. Ivanov, *Bull. Acad. Sci. U.S.S.R., Chem. Sci.*, 1963, 1055.
[14] P. Quitt, R. O. Studer, and K. Vogler, *Helv. Chim. Acta*, 1963, **46**, 1715.
[15] M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin, and V. T. Ivanov, *Bull. Acad. Sci. U.S.S.R., Chem. Sci.*, 1963, 526; *Tetrahedron Letters*, 1963, 885.
[16] Pl. A. Plattner, K. Vogler, R. O. Studer, P. Quitt, and W. Keller-Schierlein, *Helv. Chim. Acta*, 1963, **46**, 927.

The antibiotic "baccatin A" is a mixture of enniatins A and B.[17] Several related compounds have not been completely characterised.[18]

Valinomycin and amidomycin. The Actinomycetes are a rich source of antibiotics; two active cyclodepsipeptides have been isolated from this group. The first, valinomycin $(C_{18}H_{30}O_6N_2)_n$,[19] gave on total acid hydrolysis L-lactic acid, D-α-hydroxyisovaleric acid, and DL-valine in molar ratio 1:1:2. Partial alkaline hydrolysis gave L-lactyl-L-valine and D-α-hydroxyisovaleryl-D-valine, which lactonised readily. Molecular-weight determination indicated the cyclo-octadepsipeptide structures (11; $n = 2$) or (12).[20] However, synthetic (11; $n = 2$) differed from the natural antibiotic,[21] and structure (12) was excluded by redetermination of the molecular weight,[22] which showed that valinomycin was a cyclododecadepsipeptide. It thus contained three lactyl-valyl and three α-hydroxyisovaleryl-valyl sequences, of which three arrangements are possible. Synthesis of what was considered intuitively as the most likely structure gave (11; $n = 3$), identical with valinomycin.[23]

cyclo-(-L-Lac-L-Val-D-HyV-D-Val-)$_n$

(11)

cyclo-(-L-Lac-L-Val-L-Lac-L-Val-D-HyV-D-Val-D-HyV-D-Val-)

(12)

cyclo-(-D-HyV-D-Val-)$_n$

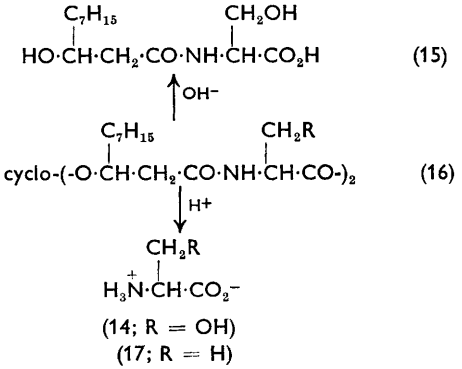
(13)

Amidomycin, $(C_{10}H_{17}O_3N)_n$, from a closely related organism,[24] gave on total acid hydrolysis only D-α-hydroxyisovaleric acid and D-valine. Partial alkaline hydrolysis followed by lactonisation yielded the dioxomorpholine (13; $n = 1$). Structure (13; $n = 4$), suggested for amidomycin,[25] was excluded by synthesis.[26] Subsequently the organism failed to form amidomycin and instead produced valinomycin.[27] Alteration in their biosynthetic capabilities is an occasional and frustrating feature of work with micro-organisms.

Serratamolide. From alkali-treated extracts of *Serratia*, Cartwright

[17] J. Guérillot-Vinet, L. Guyot, J. Montégut, and L. Roux, *Compt. rend.*, 1950, **230**, 1424; G. E. Hall, *Chem. and Ind.*, 1960, 1270.
[18] M. Lacey, *J. Gen. Microbiol.*, 1950, **4**, 122; A. H. Cook, S. F. Cox, and T. H. Farmer, *J. Chem. Soc.*, 1949, 1022.
[19] H. Brockmann and G. Schmidt-Kastner, *Chem. Ber.*, 1955, **88**, 57.
[20] H. Brockmann and H. Geeren, *Annalen*, 1957, **603**, 216.
[21] M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, and N. A. Aldanova, *Tetrahedron Letters*, 1963, 351.
[22] H. Brockmann, M. Springorum, G. Träxler, and I. Höfer, *Naturwiss.*, 1963, **22**, 689.
[23] M. M. Shemyakin, N. A. Aldanova, E. I. Vinogradova, and M. Yu. Feigina, *Tetrahedron Letters*, 1963, 1921.
[24] W. A. Taber and L. C. Vining, *Canad. J. Microbiol.*, 1957, **3**, 953.
[25] L. C. Vining and W. A. Taber, *Canad. J. Chem.*, 1957, **35**, 1109.
[26] M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, and N. A. Aldanova, *Tetrahedron Letters*, 1963, 351; *Zhur. obshchei. Khim.*, 1964, **34**, 1798.
[27] J. C. Macdonald, *Canad. J. Microbiol.*, 1960, **6**, 27.

isolated serratamic acid,[28] $C_{13}H_{25}NO_5$, which gave on acid hydrolysis L-serine (14) and D-β-hydroxydecanoic acid. The postulated *N*-(hydroxyacyl)amino-acid structure (15) was confirmed by synthesis.[29] Extraction of *Serratia* cultures with organic solvents gave serratamolide, $C_{26}H_{46}N_2O_8$, a neutral compound which on partial alkaline hydrolysis gave serratamic acid.[30] The ready formation of *OO'*-ditritylserratamolide (trityl = $Ph_3C\cdot$) suggested the presence of two primary hydroxyl groups. Structure (16; R = OH), thus indicated, was supported by reduction of the hydroxyl groups to give dideoxyserratamolide, which was (16; R = H) since its acid hydrolysate contained alanine (17) but no serine (14). The correctness of structure (16; R = OH) has been confirmed by synthesis.[31] Whether serratamic acid occurs free in *Serratia* or whether it is wholly an artifact of alkaline extraction is unknown.



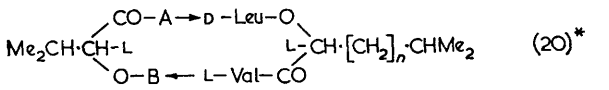
Sporidesmolides. The toxic pasture fungus, *Pithomyces chartatum*,[32] produces (non-toxic) cyclodepsipeptides,[33,34] chiefly sporidesmolide I, $C_{33}H_{58}N_4O_8$.[35] Partial alkaline hydrolysis of sporidesmolide I gave sporidesmolic acids A (18) and B (19), two *N*-(L-α-hydroxyisovaleryl)-dipeptides. Their structures were deduced by total and partial acid hydrolysis, and confirmed by Dakin–West reaction. This converts the carboxyl group into a methyl ketone. Acid hydrolysates of the reaction product therefore lack the *C*-terminal amino-acid, which was found to be leucine and *N*-methyl-leucine in sporidesmolic acids A and B respectively.

[28] N. J. Cartwright, *Biochem. J.*, 1955, **60**, 238.
[29] N. J. Cartwright, *Biochem. J.*, 1957, **67**, 663.
[30] H. H. Wasserman, J. J. Keggi, and J. E. McKeon, *J. Amer. Chem. Soc.*, 1962, **84**, 2978.
[31] (*a*) M. M. Shemyakin, Yu. A. Ovchinnikov, V. K. Antonov, A. A. Kiryushkin, V. T. Ivanov, V. I. Shchelekov, and A. M. Shkrob, *Tetrahedron Letters*, 1964, 47; (*b*) V. K. Antonov, V. I. Shchelekov, M. M. Shemyakin, I. I. Tovarova, and O. A. Kiseleva, *Antibiotiki*, 1965, **4**, 387.
[32] R. L. M. Synge and E. P. White, *Chem. and Ind.*, 1959, 1546.
[33] E. P. White, *New Zealand J. Agric. Res.*, 1958, **1**, 859.
[34] J. Done, P. H. Mortimer, A. Taylor, and D. W. Russell, *J. Gen. Microbiol.*, 1961, **26**, 207.
[35] D. W. Russell, *J. Chem. Soc.*, 1962, 753.

One mol. of each sporidesmolic acid was obtained from sporidesmolide I, which was formulated as the cyclohexadepsipeptide (20; A = D-Val, B = L-MeLeu, $n = 0$).[35]

L-HyV-D-Val-D-Leu
(18)

L-HyV-L-Val-L-MeLeu
(19)



(20)*

L-HyV-L-Val-L-Leu
(21)

* The arrows → or ← indicate the direction of the peptide bond, –CO·NR– and –NR·CO– respectively.

Impure sporidesmolic acid A, from the total sporidesmolide fraction, contained isoleucyl residues resistant to Dakin–West reaction, and the presence of a higher homologue, sporidesmolide II, was suspected, in which isoleucine replaced D-valine.[35] On a growth medium containing isoleucine the fungus produced no sporidesmolide I, but a homologue (20; A = D-aIle, B = L-MeLeu, $n = 0$) was isolated.[5,36,37]

Sporidesmolide III, $C_{32}H_{56}N_4O_8$, had the mass spectrum expected for *N*-demethylsporidesmolide I, and on total acid hydrolysis gave L-α-hydroxyisovaleric acid, DL-valine, and DL-leucine (2:2:2). The partial alkaline hydrolysate, treated with carboxypeptidase, yeilded sporidesmolic acid A (18). The other product of alkaline hydrolysis was therefore the isomer (21) which, having an L-L-dipeptide sequence, was degraded by the enzyme. Accordingly, sporidesmolide III is (20; A = D-Val, B = L-Leu, $n = 0$).[38]

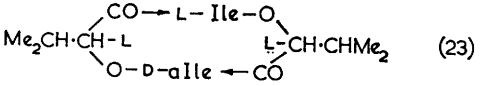
Sporidesmolide IV, $C_{34}H_{60}N_4O_8$,[39] an isomer of sporidesmolide II, contained a residue of L-α-hydroxy-β-methylvaleric acid. Its structure is (20; A = D-Val, B = L-MeLeu, $n = 1$).[40]

The structures assigned to the sporidesmolides have been confirmed by synthesis.[4,41]

Angolide. The finding that sporidesmolides occur as crystals on the surface of *P. chartarum* spores[36] prompted a study of related fungi, among which another spore-surface cyclodepsipeptide, angolide, was discovered.[39]

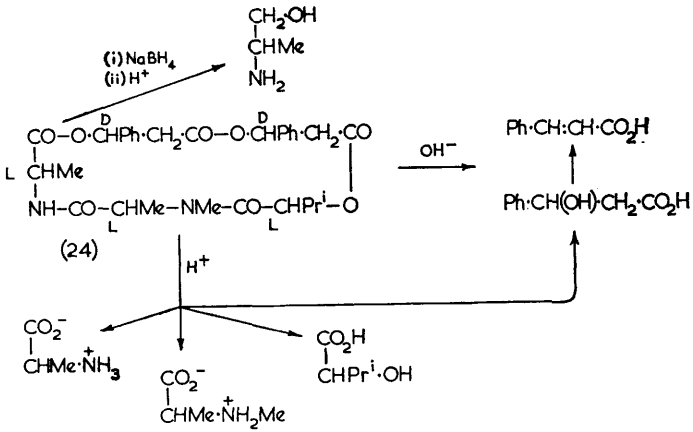
[36] W. S. Bertaud, I. M. Morice, D. W. Russell, and A. Taylor, *J. Gen. Microbiol.*, 1963, **32**, 385.
[37] D. W. Russell, Abstr. 2nd Meeting Fed. European Biochem. Soc., 1965, No. A9.
[38] D. W. Russell, C. G. Macdonald, and J. S. Shannon, *Tetrahedron Letters*, 1964, 2759.
[39] E. Bishop, H. Griffiths, D. W. Russell, V. Ward, and R. N. Gartside, *J. Gen. Microbiol.*, 1965, **38**, 289.
[40] E. Bishop and D. W. Russell, *Biochem. J.*, 1964, **92**, 19P.
[41] (*a*) M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and A. A. Kiryushkin, *Tetrahedron*, 1963, **19**, 995; (*b*) A. A. Kiryushkin, Yu. A. Ovchinnikov, and M. M. Shemyakin, *Tetrahedron Letters*, 1965, 143; Yu. A. Ovchinnikov, A. A. Kiryushkin, and M. M. Shemyakin, *ibid.*, p. 1111.

Angolide, $C_{22}H_{38}N_2O_6$, yielded on total acid hydrolysis L-α-hydroxyisovaleric acid and an amino-acid, $C_6H_{13}NO_2$, which was optically almost inactive, but which on oxidation gave (+)-2-methylbutyraldehyde. It was therefore a mixture of L-isoleucine and D-alloisoleucine.[42] Partial hydrazinolysis of angolide ruptured the ester bonds and gave the hydrazides of the diastereoisomers, L-α-hydroxyisovaleryl-L-isoleucine and L-α-hydroxyisovaleryl-D-alloisoleucine. Molecular-weight determination showed[43] angolide to be a cyclotetradepsipeptide, which accordingly had structure (23), a conclusion supported by mass spectrometry[44] and confirmed by synthesis.[45]



(23)

Pithomycolide. This unusual cyclodepsipeptide, $C_{30}H_{36}N_2O_8$, gave on total acid hydrolysis cinnamic acid, L-α-hydroxyisovaleric acid, L-alanine, and *N*-methyl-L-alanine. Partial alkaline hydrolysis liberated both cinnamic acid and D-β-hydroxy-β-phenylpropionic acid, in a total yield approaching 2 mol., the former acid being presumably derived from the latter by dehydration. Borohydride reduction of pithomycolide, followed by acid hydrolysis, gave *N*-methylalanine and 2-aminopropanol. As the latter must have arisen by reduction of an ester-linked alanine residue, the structure of pithomycolide could be completely defined as (24).[7] This structure awaits synthetic confirmation.



[42] W. S. Fones, *J. Amer. Chem. Soc.*, 1954, **76**, 1377.
[43] D. W. Russell, *J. Chem. Soc.*, 1965, 4664.
[44] C. G. Macdonald and J. S. Shannon, *Tetrahedron Letters*, 1964, 3113.
[45] A. A. Kiryushkin, Yu. A. Ovchinnikov, and M. M. Shemyakin, *Khim. Prirod. Soedinen.*, 1965, 58; *Tetrahedron Letters*, 1964, 3313.

Isariin. This neutral cyclodepsipeptide, $C_{33}H_{59}N_5O_7$, yielded on total acid hydrolysis glycine, L-alanine, D-leucine, L-valine, and D-β-hydroxydodecanoic acid (1:1:1:2:1). Partial alkaline hydrolysis gave isariic acid, $C_{33}H_{61}N_5O_8$, for which the partial structure (25) was derived by partial acid and enzymic hydrolysis.[46]

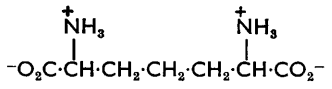
$CH_3{\cdot}[CH_2]_8{\cdot}CHOH{\cdot}CH_2{\cdot}CO$-Gly-(L-Ala, L-Val, D-Leu,)-L-Val

(25)

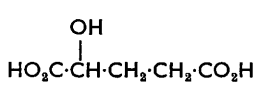
The order of the residues in parentheses is unknown

Of the two remaining cyclodepsipeptides of natural origin, one is an amino-acid, the other a carboxylic acid. They are present in the culture filtrates after growth of the source organisms.

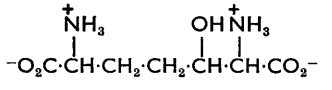
Wildfire toxin. The bacterium *Pseudomonas tabaci* causes wildfire disease in tobacco plants. Laboratory cultures produce a complex α-amino-acid, wildfire toxin, $(C_{10}H_{16}O_6N_2)_n$.[47] This on total acid hydrolysis gave lactic acid of undetermined configuration, and an amino-acid, $C_7H_{14}N_2O_5$, named tabtoxinine. Both nitrogen atoms of tabtoxinine were present as amino-groups in the α-position with respect to carboxyl groups, and tabtoxinine was thought to be a hydroxyl derivative of 2,6-diaminopimelic acid (26).[48] When both amino-groups were replaced by hydroxyl, the product gave on oxidation with periodate and then bromine water a compound, believed to be (27) because of its further oxidation to succinic acid (28). However, the structure suggested[48] for tabtoxinine (29) is incorrect,[49] and the structure of the important natural product, wildfire toxin, therefore remains unknown.



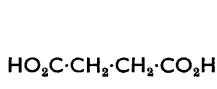
(26)



(27)



(29)

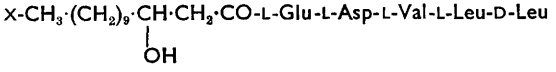


(28)

Esperin. This antibiotic is a dicarboxylic acid, $C_{39}H_{69}N_5O_{11}$, which with alkali gave the tricarboxylic hydroxy-acid, esperinic acid, $C_{39}H_{67}N_5O_{12}$. Esperin or esperinic acid gave on total hydrolysis one mol. each of L-Asp, L-Glu, L-Val, L-Leu, D-Leu, and 2-tridecanoic acid; the last was presumed

[46] L. C. Vining and W. A. Taber, *Canad. J. Chem.*, 1962, **40**, 1579.
[47] D. W. Woolley, R. B. Pringle, and A. C. Braun, *J. Biol. Chem.*, 1952, **197**, 409; D. W. Woolley, G. Schaffner, and A. C. Braun, *J. Biol. Chem.*, 1955, **215**, 485.
[48] D. W. Woolley, G. Schaffner, and A. C. Braun, *J. Biol. Chem.*, 1952, **198**, 807.
[49] J. M. Stewart and D. W. Woolley, *J. Amer. Chem. Soc.*, 1956, **78**, 5336; J. M. Stewart, *ibid.*, 1961, **83**, 435.

to arise from a β-hydroxy-tridecanoic acid residue by dehydration. Structures (30) or (31) were suggested for esperinic acid, of which esperin would be the lactone with the hydroxyl group esterified by the ω-carboxyl group of aspartic or glutamic acid.[50] Shemyakin and his colleagues have however reported that both synthetic isomers (30) and (31) differ from esperinic acid.[51]

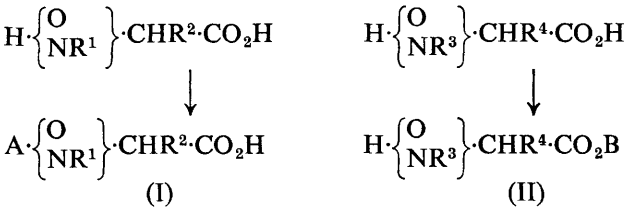


(30) X = L; (31) X = D

5. Synthesis

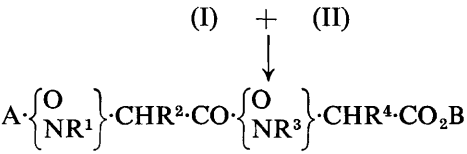
In the synthesis of cyclodepsipeptides the general problem is to condense together, in a definite sequence, several compounds each with a carboxyl group and either an amino- or an hydroxyl group. The solution lies in the four operations of peptide synthesis.[1,52]

(*a*) Protection of one functional group of each reactant:



(I) (II)

(*b*) Condensation of the protected compounds:



(*c*) Removal of protecting group A or B and condensation of further units as in (*b*) to lengthen the chain.

(*d*) Final removal of both protecting groups.

Preparation of cyclodepsipeptides requires the further intramolecular condensation of the free carboxyl with the free amino- or hydroxyl group of the product from (*d*). Attempts thus to cyclise depsipeptides with a terminal hydroxyl group, *e.g.*,

[50] T. Ito and H. Ogawa, *Bull. Agric. Chem. Soc. Japan*, 1959, **23**, 536.

[51] A. A. Kiryushkin, P. W. Kostezky, Yu. A. Ovchinnikov, and M. M. Shemyakin, Proc. 9th Mendeleev Congr. Pure and Appl. Chem., Section of Chemistry and Technology of Natural Products, Kiev, May 1965, p. 42.

[52] H. N. Rydon, "Peptide Synthesis", R.I.C. Lecture Series, 1962, No. 5; E. Schröder and K. Lübke, "The Peptides", Academic Press, London, 1965, vol. 1, "Methods of Peptide Synthesis".

$$HO{\cdot}CHR^1{\cdot}CO[NR^2{\cdot}CHR^3{\cdot}CO{\cdot}O{\cdot}CHR^1{\cdot}CO]_n{\cdot}NR^2{\cdot}CHR^3{\cdot}CO_2H,$$

have been unsuccessful, whereas the isomers with a terminal amino-group, *e.g.*, $HNR^2{\cdot}CHR^3{\cdot}CO[O{\cdot}CHR^1{\cdot}CO{\cdot}NR^2CHR^3{\cdot}CO]_n{\cdot}O{\cdot}CHR^1{\cdot}CO_2H$, cyclise readily.[53] For this and other reasons,[54] the first bond formed in stage (*b*) has been an ester; subsequent condensations, including the final cyclisation, then involve the easier amide bond formation.

Synthetic Techniques.—*Protecting groups.* The most widely used amino-protecting groups are benzyloxycarbonyl (Z = $PhCH_2{\cdot}O{\cdot}CO$–)[6] and *p*-nitrobenzyloxycarbonyl [Z(NO_2)].[6] They are removed by catalytic hydrogenolysis or by hydrogen bromide in acetic acid at room temperature. Their use excludes carboxyl group masking with benzyl esters, which are also cleaved by catalytic hydrogenolysis.

$$Z{\cdot}Cl + H_2\overset{+}{N}Me{\cdot}CHPr^i{\cdot}CO_2^- \xrightarrow[]{\text{LiOH, 10°; 74\%}} Z{\cdot}NMe{\cdot}CHPr^i{\cdot}CO_2H\ ^{16}$$

Most alkyl esters are also unsuitable, because their removal with alkali would cleave the depsipeptide ester bonds. t-Butyl esters are almost exclusively used, being readily split by toluene-*p*-sulphonic acid in benzene, or by hydrogen bromide in acetic acid. They are commonly prepared by the acid-catalysed addition of isobutene to the carboxyl group. Considerations governing the choice of protecting groups have been discussed.[54,55]

$$ZO{\cdot}CHPr^i{\cdot}CO_2H \xrightarrow[10°,\ 92\%]{CH_2{:}CHMe_2,TosOH} ZO{\cdot}CHPr^i{\cdot}CO_2CMe_3 \xrightarrow[64\%]{H_2,Pd} HO{\cdot}CHPr^i{\cdot}CO_2CMe_3\ ^{12}$$

One synthesis of enniantin A exploited the conversion of *N*-methyl-amino-acids into *N*-nitroso-derivatives, the *N*-methylamino-group being regenerated by the action of hydrogen chloride in benzene.[56]

$$H_2\overset{+}{N}Me{\cdot}CHBu^i{\cdot}CO_2^- \xrightarrow[85\%]{C_5H_{11}ONO} ON{\cdot}NMe{\cdot}CHBu^i{\cdot}CO_2H \xrightarrow[\text{with HyV-OR}]{\text{condensation}}$$

$$ON{\cdot}NMe{\cdot}CHBu^i{\cdot}CO{-}HyV{-}OR \xrightarrow[79\%]{HCl/C_6H_6,12°} Cl^-H_2\overset{+}{N}Me{\cdot}CHBu^i{\cdot}CO{-}HyV{-}OR,\ R = p\text{-nitrobenzyl.}$$

Condensation. To form a peptide bond between components $R^1{\cdot}CO_2H$ and $H_2N{\cdot}R^2$ or $HNMe{\cdot}R^2$, the carboxylic acid is converted into a deriva-

[53] M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, and N. A. Aldanova, *J. Gen. Chem. U.S.S.R.*, 1964, **34**, 1809.
[54] M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, N. A. Aldanova, Yu. A. Ovchinnikov, and A. A. Kiryushkin, *J. Gen. Chem. U.S.S.R.*, 1964, **34**, 1796.
[55] K. Lübke, Dissertation, 1961, Freie Universität Berlin.
[56] P. Quitt, R. O. Studer, and K. Vogler, *Helv. Chim. Acta*, 1964, **47**, 166.

tive $R^1 \cdot CO \cdot X$.[1,52] X is an electron-withdrawing group, its presence facilitating nucleophilic attack on the carbonyl carbon. Ester bonds are formed similarly but, the secondary hydroxyl group being weakly nucleophilic, choice of X is limited to those with strongly electronegative character. *N*-Methylamino-esters, presumably for steric reasons, are much less reactive towards activated carboxyl groups –CO·X than the corresponding primary amino-compounds.[57] Since *N*-methylamino-acids are common components of cyclodepsipeptides, this fact too restricts the choice of carboxyl-activating groups. The variety of condensation methods used, or advocated, for the synthesis of homeomeric peptides[58] finds no place in cyclodepsipeptide synthesis. Most syntheses of the complex natural products use only two techniques. The first is a mixed anhydride method,[59] the anhydride being formed by the action of benzenesulphonyl chloride on the carboxyl component.[60] The anhydride is allowed to react without isolation, with the hydroxyl component. The second is the classical acid

$$Z(NO_2) \cdot NH \cdot CHBu^t \cdot CO_2H \xrightarrow[\text{pyridine, } 0^\circ]{Ph \cdot SO_2Cl,} Z(NO_2) \cdot NH \cdot CHBu^t \cdot CO \cdot O \cdot SO_2 \cdot Ph$$

$$\xrightarrow{HO \cdot CHPr^i \cdot CO_2CMe_3} Z(NO_2) \cdot NH \cdot CHBu^t \cdot CO \cdot O \cdot CHPr^i \cdot CO_2CMe_3 \quad (80\% \text{ overall}).$$

$$Z(NO_2)\text{–Val} \xrightarrow[\substack{(2)\ \text{MeLeu-HyV-OCMe}_3 \\ 85\%}]{(1)\ PCl_5} Z(NO_2)\text{–Val–MeLeu–HyV–OCMe}_3$$[41a]

chloride method,[61] otherwise largely abandoned in peptide synthesis.[52] The chloride is formed, usually with phosphorus pentachloride, and used without delay to avoid decomposition.[52]

Cyclisation. Simultaneous removal of the protecting groups from a protected, open-chain benzyloxycarbonyl depsipeptide t-butyl ester with hydrogen bromide in acetic acid gives the hydrobromide of the open-chain depsipeptide with a terminal amino-group. This is converted into the acid chloride, which is treated with one equivalent of base in high dilution, whereupon intramolecular reaction, *i.e.*, cyclocondensation, is favoured as against intermolecular condensations.[52] The moderate yields could probably be improved.[62]

[57] P.-A. Jacquenoud, *Chimia*, 1960, **14**, 373; R. L. Huguenin and R. A. Boissonas, *Helv. Chim. Acta*, 1961, **44**, 213.
[58] T. Wieland, "Peptides", ed. G. T. Young, Pergamon Press, London, 1963, pp. 59—68.
[59] T. Wieland and R. Sehring, *Annalen*, 1950, **569**, 122.
[60] J. H. Brewster and C. I. Ciotti, *J. Amer. Chem. Soc.*, 1955, **77**, 6214.
[61] E. Fischer and E. Otto, *Ber.*, 1903, **36**, 2106.
[62] M. Fridkin, A. Patchornik, and E. Katchalski, *J. Amer. Chem. Soc.*, 1965, **87**, 4646.

Z-D-*a*Ile-L-HyV-L-Ile-L-HyV-OCMe$_3$

↓ HBr/HOAc, 80%

H[D-aIle-L-HyV-L-Ile-L-HyV]$^+$ Br$^-$

↓ 1. SO_2Cl_2; 2. Et_3N, 28%

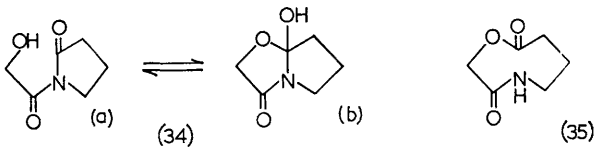
(23)[45]

O-L-Leucyl-β-hydroxypropionic acid (32) with thionyl chloride in high dilution undergoes bimolecular condensation to the serratamolide analogue (33).[63]

$2H_2N{\cdot}CHBu^i{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$ (32) ⟶ $NH{\cdot}CHBu^i{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CO$ / $CO{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}CO{\cdot}CHBu^i{\cdot}NH$ (33)

Racemisation. Strongly activating X groups such as Cl or SO_3Ph would be expected to produce some racemisation, but this is not excessive.[12] The specific rotations of synthetic cyclodepsipeptides are close to, or identical with, those of the natural products, and the biological activities (section 7) of the natural and synthetic compounds show good qualitative and quantitative agreement.

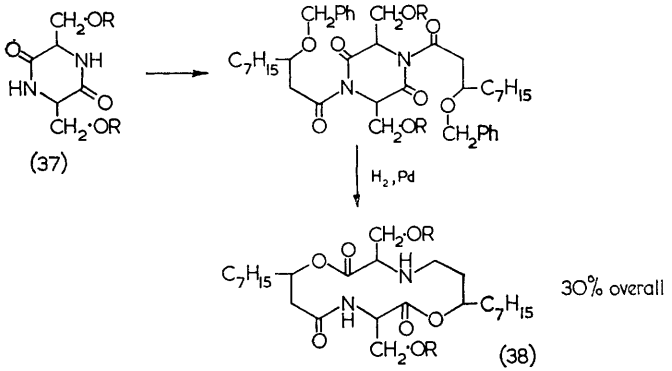
Hydroxyacyl incorporation. A very interesting synthesis of the diacetyl derivative of serratamolide (16; R = O·COMe) was based on the observed tautomerism of β-hydroxyacyl lactams of the type (34).[64] Although the cyclol (34b) did not rearrange to the cyclodepsipeptide (35), it was reasoned that formation of a larger ring might be sterically favoured.



Catalytic hydrogenolysis of the benzyloxycarbonyloxyacylpiperidone (36) indeed gave a quantitative yield of the cyclodepsipeptide (37).[65] For the serratamolide synthesis, the dioxopiperazine (37; R = H) from L-serine

[63] C. H. Hassall, T. G. Martin, and J. A. Schofield, *Tetrahedron Letters*, 1964, 3741.
[64] M. M. Shemyakin, V. K. Antonov, A. M. Shkrob, Yu. N. Shchelekov, and L. B. Senyavina, *Tetrahedron Letters*, 1962, 701; M. M. Shemyakin, V. K. Antonov, A. M. Shkrob, V. I. Shchelekov, and Z. E. Agadzanyan, *Tetrahedron*, 1965, **21**, 3537.
[65] V. K. Antonov, A. M. Shkrob, and M. M. Shemyakin, *Tetrahedron Letters*, 1963, 439.

was *O*-acetylated and the product (37; R = Ac) was *N*-acylated with D-β-benzyloxydecanoyl chloride. Removal of the *O*-benzyl groups was accompanied by spontaneous rearrangement of the product to *OO'*-diacetylserratamolide (38; R = Ac).[31a]



6. Mass Spectrometry

Since classical methods may give inaccurate results (see sections 2 and 3), one obvious application of mass spectrometry to cyclodepsipeptide chemistry is the accurate determination of molecular weights. Studies of compounds of known structure have also laid the basis for elucidating unknown structures. The primary fragmentation routes established are as follows.[66]

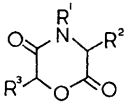
(a) The "COX" Route.—In a cyclodepsipeptide ion, of molecular weight M, loss of the elements of an ester or amide bond, *viz.*, –COO–, –CONH–, or –CONMe–, may occur; fragment ions of mass $M-44$, $M-43$, or $M-57$ respectively then appear in the mass spectrum. This route is particularly characteristic of cyclotetradepsipeptides.[66] Thus, a strong peak in the spectrum of angolide (23) appears at $M-44$.[44]

(b) The "Morpholinic" Route.—Fragmentation may liberate ions, or neutral molecules, of a 2,5-dioxomorpholine (39) from adjacent amino- and hydroxy-acid residues, or of a 2,5-dioxopiperazine (40) from two adjacent amino-acid residues.[66] 2,5-Dioxomorpholines also result from *pyrolysis* of cyclodepsipeptides in the mass spectrometer, particularly when the amino- and hydroxy-acid residues alternate, so that the magnitude of the peaks is strongly temperature-dependent.[67] In the mass spectrum of enniatin B

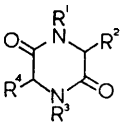
[66] (*a*) N. S. Wulfson, V. A. Puchkov, V. N. Bochkarev, B. V. Rozinov, A. M. Zyakoon, M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin, E. I. Vinogradova, M. Yu. Feigina, and N. A. Aldanova, *Tetrahedron Letters*, 1964, 951; (*b*) N. S. Wulfson, V. A. Puchkov, B. V. Rozinov, A. M. Zyakoon, M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin, and V. T. Ivanov, *ibid.*, 1965, 2793.

[67] V. A. Puchkov, N. S. Wulfson, B. V. Rozinov, Y. V. Denisov, M. M. Shemyakin, Yu. A. Ovchinnikov, and V. T. Ivanov, *Tetrahedron Letters*, 1965, 543.

(10; $n = 3$) a peak at mass 213 is attributable to the ion of the dioxomorpholine (10; $n = 1$).[66]

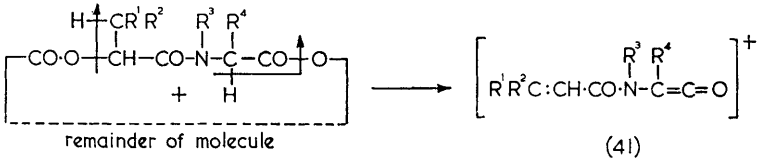


(39)



(40)

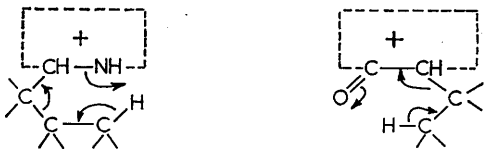
(c) The "Acylaminoketen" Route.—Rupture at two ester functions separated by an amide function gives an acylaminoketen ion (41).[66] A



(41)

strong peak, mass 209, in the spectrum of enniatin A (4) is due to (41; $R^1 = R^2 = R^3 = Me$; $R^4 = Bu^s$).[66b]

(d) Side-chain Fission.—Fission of branched-chain alkyl groups[65a] gives large fragment ions. Peaks at $M-42$ and $M-56$ in the mass spectra of sporidesmolides arise in this way, *i.e.*,



Routes (*a*) to (*d*) give ions which themselves further fragment,[66,68b] thus providing structural information. Details cannot be discussed here, but one other development deserves mention. The findings are quoted directly: "If the primary act or one of the subsequent fragmentation stages of molecular ion disintegration affords a linear ion or ion-radical wherein the positive charge is localised on the *C*-terminus and the *N*(*O*)-terminus is protected (for instance by acylation, or by the presence of an unpaired electron) then further rupture of the amide (ester) bonds leads to *consecutive elimination* of the amino(hydroxy-) acid residues from the *C*-terminus with transfer of the positive charge along the chain."[69] Similar results for homeomeric peptide chains have been reported (see ref. 70 and references

[68] (*a*) F. W. McLafferty, *Analyt. Chem.*, 1959, **31**, 82; (*b*) C. G. Macdonald, J. S. Shannon, and A. Taylor, *Tetrahedron Letters*, 1964, 2087.

[69] N. S. Wulfson, V. A. Puchkov, B. V. Rozinov, Yu. V. Denisov, V. N. Bochkarev, M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin, E. I. Vinogradova, and M. Yu. Feigina, *Tetrahedron Letters*, 1965, 2805.

[70] E. Bricas, J. van Heijenoort, M. Barber, W. A. Wolstenholme, B. C. Das, and E. Lederer, *Biochemistry*, 1965, **4**, 2254.

therein). In favourable cases, therefore, mass spectrometry can yield a complete amino- and hydroxy-acid sequence.

7. Biological Activities

Many cyclodepsipeptides are antibiotics. The activity is low and none has found clinical application. Activity does not entirely depend upon the cyclic structure, for the open-chain analogue of enniatin B, L-MeVal-(D-HyV-L-MeVal)$_2$-D-HyV, is weakly active. This activity is unaltered by changes in the extent of *N*-methylation. Since the removal of even one *N*-methyl group from enniatin B (10; $n = 3$) drastically reduces the activity,[71] the mechanism of antimicrobial action probably differs in cyclic and open-chain compounds.

The size of the macrocycle is important. The cyclotetradepsipeptide (10; $n = 2$) has only one-tenth of the activity of enniatin B (10; $n = 3$);[16,71] the cyclo-octadepsipeptide (10; $n = 4$) is highly active but has a narrower antibiotic spectrum than enniatin B.[72]

The substituents on the cyclodepsipeptide ring influence activity. The effect of removing one *N*-methyl group has been mentioned. No further change is produced by removing the other *N*-methyl groups.[71] Replacement of *N*-methyl-L-valine by *N*-methyl-L-isoleucine, as in enniatin A (4), considerably enhances activity;[72,73] its replacement by *N*-methyl-L-leucine gives an inactive product.[74]

The configuration of the amino- and hydroxy-acid residues has a marked influence. Inversion of one valine residue in valinomycin (11; $n = 3$) reduces activity; inversion of three such residues abolishes activity. The activity of valinomycin is relatively independent of the nature of the amino-acid side chains. It is strongly influenced by the structure of the hydroxy-acid residues; almost any change in their configuration or side-chain structure abolishes activity. Curiously, replacement of one ester bond by an amide (L-Lac replaced by L-Ala) has little effect.[74]

More than in the enniatin series, the size of the valinomycin macrocycle is of crucial importance. The cyclo-octa- and cyclohexadeca-depsipeptides (11; $n = 2$ and 4 respectively) are completely inactive,[74] as is the analogous cyclotetradepsipeptide, angolide (23).[39]

Finally, activity also depends upon the arrangement of amino- and hydroxy-acid residues. The sporidesmolides (20) and their synthetic analogues, in which these residues do not alternate, are inactive.[39,72,74]

Valinomycin uncouples oxidative phosphorylation in isolated mitochondria,[75] an effect mediated by induction of an energy-dependent

[71] R. O. Studer, P. Quitt, E. Böhni, and K. Vogler, *Monatsh.*, 1965, **96**, 461.
[72] M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin, G. L. Zhdanov, and I. D. Ryabova, *Experientia*, 1963, **19**, 566.
[73] Pl. A. Plattner, U. Nager, and A. Boller, *Helv. Chim. Acta*, 1948, **31**, 594.
[74] M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, N. A. Aldanova, N. F. Loginova, I. D. Ryabova, and I. A. Pavlenko, *Experientia*, 1965, **21**, 548.
[75] W. C. McMurray and R. W. Begg, *Arch. Biochem. Biophys.*, 1959, **84**, 546.

accumulation of potassium ions.[76] Other cyclodepsipeptide antibiotics similarly affect ion-transport, their effectiveness being roughly proportional to their antibiotic activity.[74] They may therefore exert antibiotic action through an effect on ion-transport in micro-organisms.

Wildfire toxin produces chlorotic patches on tobacco leaves, similar to those produced by methionine sulphoximine. In *Chlorella* the toxin acts as a competitive inhibitor of methionine metabolism.[77] Symptoms of toxic wilt are produced in tomato shoots by enniatin A (4), which affects the permeability of protoplasts,[78] and in *Chenopodium* leaves by sporidesmolic acids (18) and (19). The latter also inhibit the 2,4-dichlorophenoxyacetic acid-stimulated elongation of barley coleoptile sections, their potency being similar to that of maleic hydrazide. The parent compound, sporidesmolide I (20; A = D-Val, B-L-MeLeu, $n = 0$) is inactive.[79]

8. Biosynthesis

Exogenous L-amino-acids are equally good precursors of corresponding L- *and* D-residues in sporidesmolide I[80] and valinomycin,[27] as in actinomycin.[81] Exogenous D-amino-acids are poor precursors.[27] The effect may be due to racemisation of L-amino-acids after, and perhaps because of, their "activation" for peptide or ester bond formation. L-Valine is also a precursor[80] for L-α-hydroxyisovaleryl residues in sporidesmolide I and for D-α-hydroxyisovaleryl residues in valinomycin.[27] The intermediate stages in cyclodepsipeptide biosynthesis are completely unexplored.

The preponderance in naturally-occurring cyclodepsipeptides of residues of leucine, isoleucine, and valine and their derivatives, suggests that biosynthesis is related to accumulation of branched-chain α-keto-acids. Synthesis of secondary metabolites may result from the accumulation of biochemical intermediates that occurs when a nutrient in the culture medium becomes exhausted.[82] The relationship of *Pithomyces* cyclodepsipeptide biosynthesis to sporulation[34,36,39] may indicate that such accumulation can also occur locally in an organism, perhaps as a consequence of biochemical changes taking place during the process of cell differentiation.

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