The existence of fish without an oxygen-carrying pigment provided the obvious extension for such studies. The icefish (*Chaenocephalus aceratus*) has a large blood volume and circulates relatively large volumes through the gills in unit time. Both the ventilation and cardiac pumps operate at low pressures and thus help maintain a low total O_2 consumption[55].

From this survey it is apparent that fish respiratory studies have already gained a great deal from the comparative approach which is one of its most rapidly advancing areas. Nevertheless, the importance of detailed investigations using single species must not be neglected. The advantages of the two approaches are now more generally appreciated and in some laboratories both are going on simultaneously. It is important that communication between workers of the two sorts should be encouraged so that the advantages of the comparative approach are gained as rapidly as possible. In this way we can expect a continuing expansion of studies on fish respiration, especially as they have become significant in relation to work on pollution and underwater exploration.

Zusammenfassung. Es wird ein Überblick über neuere Forschungen gegeben, die das weite Arbeitsgebiet des Mechanismus der Fischatmung betreffen. Nachdruck wird auf den vergleichenden Standpunkt einer solchen Forschungsarbeit gelegt, wobei gezeigt wird, dass die Wahl besonderer Spezies ein förderndes Licht auf allgemein wichtige Probleme werfen kann, die bei Kiemenuntersuchungen sowohl auf der Makrostrukturstufe wie auf der Ultrastrukturstufe auftreten. Vom physiologischen Standpunkt aus gesehen, erweisen sich einige Arten auf Grund ihrer Lebensweise für experimentelle Untersuchungen ihres Sauerstoffverbrauchs besonders geeignet. Die strukturelle Anordnung des Pumpenmechanismus anderer Arten erleichert wiederum äusserst günstig die Direktmessung des Ventilationsvolumens und anderer mit dem Gasaustausch zusammenhängender Parameter.

[56] G. M. HUGHES, *Comparative Physiology of Vertebrate Respiration* (Heinemann, Garstedt 1963).
[57] G. M. HUGHES, Folia morph., in press (1970).

SPECIALIA

Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Ответственность за короткие сообщения несёт исключительно автор. – El responsable de los informes reducidos, está el autor.

The Monamycins, a New Family of Cyclodepsipeptide Antibiotics

An earlier communication[1] described the isolation, from cultures of *Streptomyces jamaicensis*, of monamycin, a crystalline preparation which inhibited the growth of various Gram-positive bacteria at high dilution. We have reinvestigated the production and isolation of monamycin and have examined further the chemical and biological properties of what has now been identified as a new family of antibiotics.

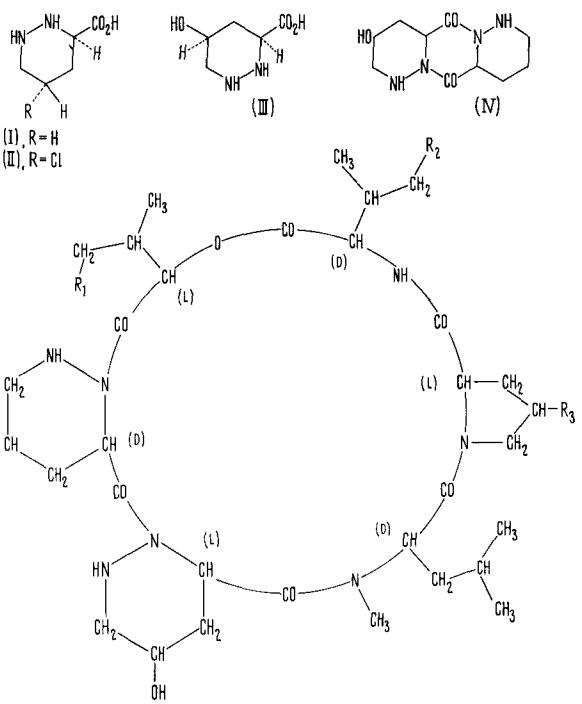
It has been established that monamycin production in deep culture is favoured by a medium based on neopeptone and glucose. Using a New Brunswick Fermacell 100 l fermenter, low aeration (0.25 v/v/min) and agitation rates, short fermentation times (42 h), low phosphate concentration and high carbon-nitrogen ratios gave optimum yields (150 mg/l). The crude mixture of crystalline antibiotics, which was obtained from a pentane extract of the culture fluid, was separated by recrystallization into 2 fractions. One consisted of compounds containing non-ionic chlorine, the other of non-chlorinated antibiotics. Extensive countercurrent distribution studies of each of these fractions (5500 and 7000 transfers), using the system ethyl acetate, cyclohexane, methanol, water (41:35:35:10) led to the isolation of pure peptides, or concentrates in which particular components predominated. The molecular formulae of the individual antibiotics were established by high-resolution mass spectrometry. The amino-acid and hydroxy-acid composition of acid hydrolysates was determined by isolation of individual components, by g.l.c. (for hydroxy-acids) and amino-acid analysis.

In addition to the acids L-proline, D-Valine, N-methyl-D-leucine, L-isoleucic acid and L-α-hydroxyisovaleric acid, all of which have been identified as metabolites of micro-organisms, the unusual amino-acids D-isoleucine (an abnormal isomer of the isoleucine group),[2] *trans*-4-methyl-L-proline, and 4 new C_5-amino-acids were present in the hydrolysates of the monamycins. Of the 4 new C_5-amino-acids[3], two, piperidazine-3-(*R*)-carboxylic acid (I) and 5-(*S*)-chloropiperidazine-3-(*R*)-carboxylic acid (II) have been identified as members of the D_8-series and a

[1] C. H. HASSALL and K. E. MAGNUS, Nature *184*, 1223 (1959).
[2] M. BODANSZKY and D. PERLMAN, Science *163*, 352 (1969).
[3] C. H. HASSALL, R. B. MORTON, Y. OGIHARA and W. A. THOMAS, Chem. Commun., *1079*, (1969).

third, 5-(*S*)-hydroxypiperidazine-3-(*S*)-carboxylic acid (III) of the L$_8$-series; the fourth, available in traces only, and presumed to be the anhydroderivative of (III) was not isolated as a pure compound but was shown to be derived from the acid (III).

The sequence of the acids in the major cyclohexadepsipeptide, monamycin D$_1$ (mol. wt. 691), has been determined from the evidence of partial hydrolysis, and through a high-resolution mass spectrometry study of the methyl ester of the linear peptide formed by treatment of the cyclic compound with mild alkali. It is of interest that diketopiperazines such as (IV) involving 1 or 2 piperidazine carboxylic residues, were isolated with dipeptides and a tripeptide from the mixture formed by partial, acid-catalysed hydrolysis. The high-resolution NMR-spectra (100 MHz and 220 MHz) of both monamycin D$_1$ and its degradation products were particularly informative and were in accord with the assignments of structure and configuration based on alternative evidence. The molecular structure of monamycin D$_1$ is shown in formula (V).



(I), R=H
(II), R=Cl

(III)

(IV)

Monamycin D$_1$(V), R$_1$=CH$_3$, R$_2$=H, R$_3$=CH$_3$, R$_4$=H.
(Substitutions R$_1$=H or CH$_3$; R$_2$=H or CH$_3$; R$_3$=H or CH$_3$; R$_4$=H or Cl)
(V)

Other members of the monamycin family resemble D$_1$ in being derived from 1 hydroxy-acid and 5 amino-acids. Quantitative studies have distinguished 9 non-chlorinated monamycins and 6 chlorinated compounds (Table). N-methylleucine and 5-hydroxypiperidazine carboxylic acid (III) occur in all compounds, but substitutions of the other acids give rise to different molecular species; these substitutions are summarized in formula (V). The level of antibacterial activity of the monamycins varies with these differences in structure. Thus monamycin I, the least active member of the family, has approximately one-sixth of the activity of monamycin D$_1$.

Studies on the mode of action of the monamycins are, as yet, exploratory. Concentrations (0.5–2.0 µg/ml) which were bacteriostatic for *Staphylococcus aureus*, led to partial lysis, but at bactericidal levels (> 20 µg/ml) extensive degradation of the cell wall occurred rapidly. The monamycins form specific complexes with potassium ions; this may relate to their antibacterial activity as proposed for other cases of cyclic peptides and depsipeptides[4]. Low concentrations of monamycin cause lysis of erythrocytes in aqueous suspensions. The acute toxicity of monamycin in tests on rats may be attributed, at least in part, to this process. This toxicity makes it unlikely that the monamycins will find use in human therapy[5].

Zusammanfassung. Die Analyse von Monamycin, einem Antibiotika-Komplex, führte zur Identifizierung der 15 Cyclohexadepsipeptidkomponenten. Für Monamycin D$_1$ wurde Strukturformel V abgeleitet.

K. Bevan, J. S. Davies, M. J. Hall,
C. H. Hassall, R. B. Morton, D. A. S. Phillips,
Y. Ogihara and W. A. Thomas

Chemistry Department,
University College of Swansea,
Singleton Park, Swansea (U.K.), 18 August 1969.

[4] M. Pinkerton, L. K. Steinrauf and P. Dawkins, Biochem. biophys. Res. Commun. *35*, 512 (1969).

[5] We are grateful to the National Research Development Corporation for financial support and to the S.R.C. for two research studentships (K.B. and D.A.S.P.).

Amino-acid and hydroxy-acid composition of the monoamycins

Compound	A	B$_1$	B$_2$	B$_3$	C	D$_1$	D$_2$	E	F	G$_1$	G$_2$	G$_3$	H$_1$	H$_2$	I
Formula	$C_{33}H_{53}N_7O_8$	$C_{33}H_{55}N_7O_8$			$C_{34}H_{55}N_7O_8$	$C_{34}H_{57}N_7O_8$		$C_{35}H_{57}N_7O_8$	$C_{35}H_{59}N_7O_8$	$C_{33}H_{54}ClN_7O_8$			$C_{34}H_{56}ClN_7O_8$		$C_{35}H_{58}ClN_7O_8$
Mol. wt.	675	677			689	691		703	705	711			725		739
Amino-acids															
N-Me-Leu	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4-Me-Pro	+	+			+	+	+	+	+	+			+	+	+
Pro			+	+							+	+			
Ile			+				+	+	+		+			+	+
Val	+	+		+	+	+				+		+	+		
$C_5H_8N_2O_2$	+				+			+							
$C_5H_{10}N_2O_2$		+	+	+		+	+		+						
$C_5H_{10}N_2O_3$	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
$C_5H_9ClN_2O_2$										+	+	+	+	+	+
Hydroxy-acids															
Isoleucic				+	+	+		+	+			+	+		+
α-HO-Isovaleric	+	+	+				+			+	+			+	