The Structures of the Highly Modified Peptide Antibiotics Micrococcin P_1 and P_2

By Barrie W. Bycroft and Maxim S. Gowland
(*Department of Chemistry, University of Nottingham, Nottingham* NG7 2RD)

Summary Structures for the modified peptide antibiotics micrococcin P_1 and P_2 are proposed on the basis of chemical and spectroscopic evidence, and biogenetic implications discussed.

A recent communication[1] on the structure of micrococcin P has prompted us to report the results of our, as yet incomplete, chemical and spectroscopic investigations which are part of a general programme on highly modified peptide

antibiotics, particularly those containing amino-acid residues at higher oxidation levels.[2]

The antibiotic is a mixture of two components† (*ca.* 7 : 1) which have been separated by preparative t.l.c. and designated micrococcin P_1 and micrococcin P_2 respectively. The molecular formula of the major component micrococcin P_1, $C_{48}H_{49}N_{13}O_9S_6$, followed from analytical and molecular weight data coupled with evidence from ^{13}C and ^{1}H n.m.r. spectroscopy (see Table) and fragment analysis of hydrolysates of the antibiotic and its derivatives.

TABLE. N.m.r. data for compounds (**1**) and (**5**).[a]

^{13}C Chemical shift assignments

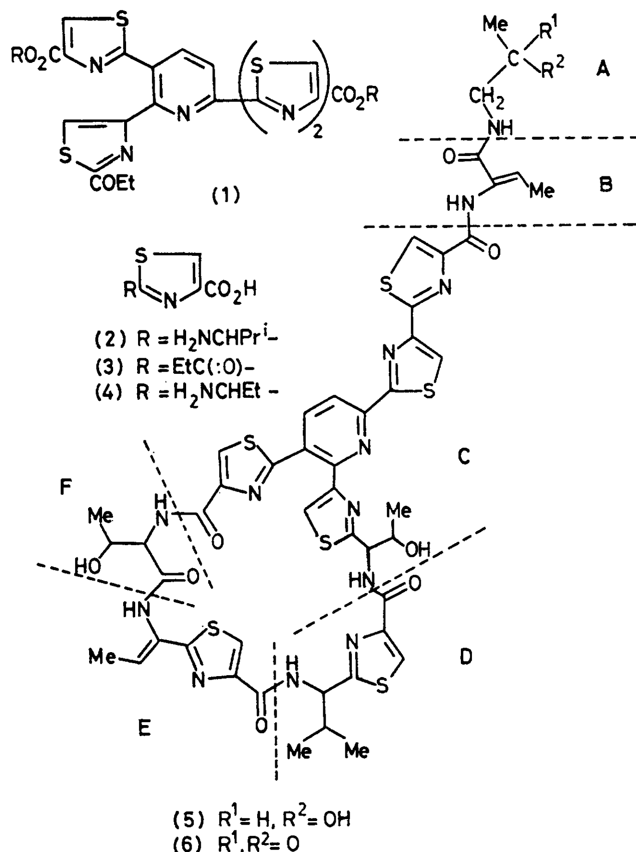
	(5)	(1)
C=O		194·3
O–C=O		168·3, 168·3
–NH–CO	170·8, 169·9, 168·8, 168·5, 166·4, 165·8	
–N=C–S– (thiazoles)	165·4, 162·7, 161·6, 161·0, 160·8, 160·3	165·9, 165·5, 163·3, 161·8
>C=N– (thiazoles, pyr. α-c)	153·8, 151·2, 150·4, 149·9, 149·7, 149·5 149·0, 148·6,	154·7, 151·0 150·0, 149·9, 147·5, 146·9
–HC= (pyr. γ-C)	140·2	140·7
–(NH)C= (ethylidene, B, E)	130·2, 129·6	
>C= (pyr. β-C	129·3	129·2
–S–CH=, –HC= (thiazoles, ethylidene, B, E)	128·7, 128·3, 125·3, 124·9, 124·4, 123·9, 121·6, 121·0	128·2, 128·2, 128·5, 120·9
–HC= (pyr. β-C)	118·6	118·7
–CHMe–O– (A, C, F)	68·0, 67·5, 66·5	
>CH–N– (C, D, F)	58·2, 56·2, 55·8	
MeO		52·5, 52·5
$-CH_2$–NH– (A)	47·4	
>CH– (D)	33·5	
$-CH_2-$		31·7
Me–C	20·4, 19·8, 19·5, 19·2, 18·9	
*Me*CH=	14·4, 13·8	
Me		7·9

^{1}H Chemical shift assignments.

	(5)	(1)
Thiazole C-5	8·25, 8·24, 8·20, 8·17, 8·02, 8·01 (s)	8·48, 8·35, 8·31, 8·23 (s)
Pyr. ring	8·45, 8·10 (d, *J* 10·1 Hz)	8·36, 8·10 (d, *J* 10·0 Hz)
MeCH=	6·47, 6·73 (q)	
>CH–N	5·21, 5·13, 4·80 (d)	
>CH–O	4·55, 4·38, 3·98 (m)	
MeO		3·98, 3·94 (s)
$-CH_2$–N	3·45, 3·15 (m)	
$MeCH_2-$		2·97 (q)
Me_2CH-	2·55 (m)	
*Me*CH=	1·85, 1·84 (d)	
*Me*CH<	1·60, 1·20, 1·20 1·20, 0·98 (d)	
$MeCH_2$		1·08 (t)

[a] The ^{13}C n.m.r. spectra were obtained using a JEOL PS100 pulsed Fourier-transform spectrometer at 25·15 MHz, and the ^{1}H n.m.r. spectra at 220 MHz, for solutions of (**1**) in $CDCl_3$ and (**5**) in $CDCl_3$–CD_3OD.

Earlier investigations on the acid hydrolysate of the micrococcin P complex had established the presence of L-threonine, D-alaninol, aminoacetone, and ammonia, as well as the structures of the important fragments (**1**)—(**3**).[3] In our hands the hydrolysis of micrococcin P_1 afforded 3 mol of ammonia, 1 mol each of L-threonine, compounds (**1**)—(**3**), and a compound which was chromatographically (g.l.c. and t.l.c.) identical with alaninol, but subsequently identified by its 2,4-dinitrophenyl (DNP) derivative as 2-hydroxypropylamine. No aminoacetone was detected, but α-oxobutyric acid, not previously observed, was trapped as its 2,4-dinitrophenylhydrazone derivative. $NaBH_4$ reduction of micrococcin P_1, conditions known to reduce didehydro-amino-acid systems,[4] followed by acid hydrolysis, afforded 1 mol each of ammonia, L-threonine, 2-hydroxypropylamine, and compounds (**1**) and (**2**). In addition 1 mol of α-aminobutyric acid‡ and the new thiazole (**4**) were identified, neither having been observed in the hydrolysate of micrococcin P_1 itself.



The products from each of these hydrolysates accounted for the total carbon, nitrogen, and sulphur content of the antibiotic. The results are also incompatible with the structure recently proposed on the basis of the ^{13}C n.m.r. data.§ The spectral data (Table) provided further confirmation for the structural units identified in the hydrolytic experiments. The noise-decoupled ^{13}C n.m.r. spectrum of micrococcin P_1 exhibited 48 signals and their assignments,

† We are extremely grateful to Dr. J. Walker for this and other unpublished information, as well as for a quantity of micrococcin.

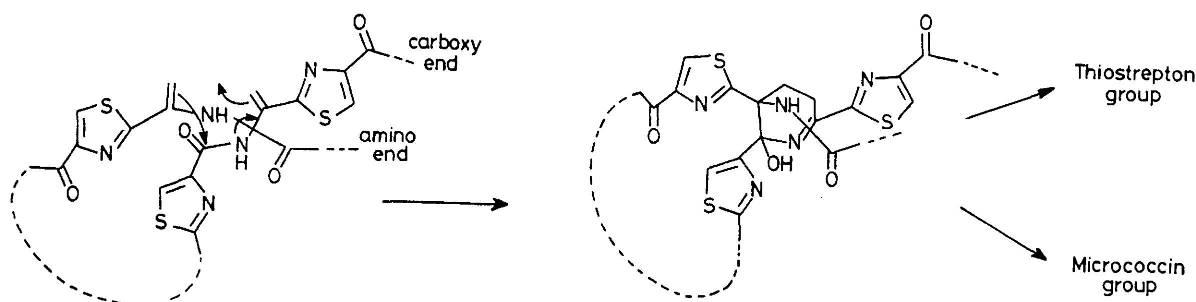
‡ The amino-acids and the other hydrolysis products were identified by g.l.c.–mass spectroscopy as their methyl ester and trifluoroacetyl derivatives.

§ This formulation would require the formation of 2 mol of threonine on the hydrolysis of both micrococcin P_1 and its reduction product, and does not accord with the isolation of α-oxo- and α-amino-butyric acids from the respective hydrolysates.

based on the off-resonance decoupled spectra and on comparison with the spectra of the hydrolysis products, model systems,[5] and the related antibiotics althiomycin,[6] thiostrepton, siomycin,[7] and nosiheptide,[8] are indicated. The ^{1}H n.m.r. spectrum showed 40 non-exchangeable protons and their assignments followed by similar comparative analysis. Particularly noteworthy were the signals corresponding to 3 -CHOHMe and 2 -NHC=CHMe residues. On the basis of the chemical evidence these were assigned to the units A, C, and F, and B and E respectively. The close similarity between the spectral data from methyl micrococcinate (**1**, R = Me) and micrococcin P_1 (Table) confirms the presence of unit C in the intact antibiotic. The formation of (**1**, R = H) on the hydrolysis of both micrococcin P_1 and its reduction product established that the propionyl group in (**1**) was derived from a -HN-CH-CHOHMe residue and not the corresponding anhydro system.

Micrococcin P_1 failed to give a DNP derivative and does not possess a free carboxylic acid group, but it readily formed a tri-*O*-acetate with pyridine-acetic anhydride. Attempts to obtain peptide fragments from partial hydrolysis of either micrococcin P_1, or its reduction product have so far been unsuccessful. However some indication of sequence could be deduced from the fact that 2-hydroxypropylamine is always released first from reduced micrococcin P_1 followed by α-aminobutyric acid and then threonine. The above observations are consistent with the structure (**5**) which contains the same sequence of structurally related units found in thiostrepton[9] and nosiheptide.[10] These latter structures have been determined by *X*-ray crystallographic methods. The minor component micrococcin P_2 afforded on hydrolysis the same products as P_1 with the exception of 2-hydroxypropylamine which was replaced by aminoacetone. The ^{1}H and ^{13}C n.m.r. data for this metabolite are consistent with the structure (6).¶

Biogenetically these structures and those of the related antibiotics are of particular interest in that they represent highly modified peptides. The thiazole residues can be formally derived from didehydrocysteine entities and the terminal units together with the didehydrobutyrine in P_1 and P_2 from threonine residues by obvious transformations. Structural analysis of the unusual unit C, coupled with the increasing evidence that many complex structures found in modified peptides result from oxidative processes and other transformations on a preformed peptide precursor,[11] lead to the intriguing possibility that the unit C may be derived from the interaction of two didehydroalanine units in a single peptide chain as illustrated in the Scheme. This proposal also provides a convenient rationale for the formation of the related tetrahydropyridine unit in thiostrepton.



SCHEME. Proposed derivation of the thiostrepton and micrococcin antibiotics from a single peptide chain.

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¶ Careful reduction of micrococcin P_2 (**6**) with $NaBH_4$ gave a compound chromatographically identical with micrococcin P_1.

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