Tetrahedron Letters No. 33, pp 2787 - 2790, 1974. Pergamon Press. Printed in Great Britain.

THE BIOSYNTHESIS OF METACYCLOPRODIGIOSIN AND UNDECYLPRODIGIOSIN [1,2]

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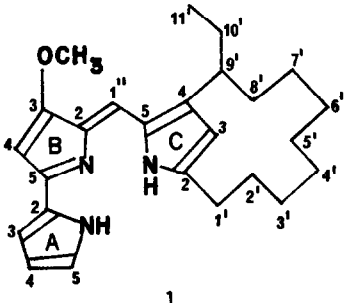
(Received in UK 4 June 1974; accepted for publication 26 June 1974)

In recent ^{13}C studies[3,4] we have shown that prodigiosin (3), the red pigment produced by Serratia marcescens, is biosynthesized from acetate, alanine, serine, proline, and methionine, by a process which is unique among naturally occurring polypyrroles. We are now reporting on the biosynthetic origin of two related tripyrrole metabolites, metacycloprodigiosin (1)[5,6] and undecylprodigiosin (2)[7], both of which are isolated from Streptomyces longisporus ruber M-3.



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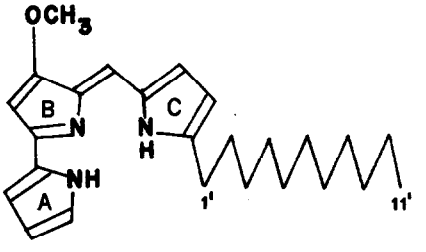

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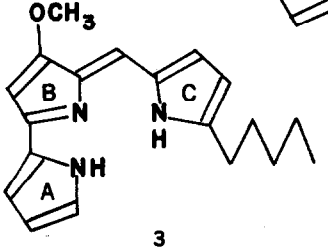

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S. Longisporus ruber were grown in the usual way[5,7] in a soymeal-mannitol medium with the labeled substrate added after four days of fermentation and prior to pigmentation. Feeding of [1-^{13}C]-proline and [1-^{13}C]- and [2-^{13}C]-acetate established the close similarity among the biosynthetic patterns of 1, 2, and 3[4] in accord with the presence of the common methoxybipyrrole unit in all three metabolites (rings A and B). The complete labeling pattern of 1 and 2, illustrated in Figure 1, was deduced by ^{13}C Fourier transform nmr. The cmr chemical shifts[8]

and the percentage of incorporation of the labeled substrates at the various sites are summarized in the Table. The high level of incorporation of labeled proline at B5 (22-23%) in both 1 and 2 indicates that ring A and carbon B5 are derived from an intact proline unit. As in the case of 3, the B3 and B4 atoms of 1 and 2 are clearly derived from an acetate unit.

With [3-^{13}C]-serine, incorporation in 1 and 2 was found at the 1" position as observed in the case of 3.[4] However, at the same time, there was substantial conversion of serine to acetate (as shown by the incorporations at A3, A4, B4, C3, 11' and 9'), and to the "one carbon" pool (as shown by the incorporation at the methoxyl carbon).

Table

	Chemical Shifts[a]		Percent excess of ^{13}C in the labeled experiment[b,c]				
	Undecyl-prodigiosin (1)	Metacyclo-prodigiosin (2)	[1-^{13}C]-acetate	[2-^{13}C]-acetate	[1-^{13}C]-DL-proline	[2-^{13}C]-glycine	[3-^{13}C]-DL-serine
A2	131.2	131.6		1(1)[c]			
A3	115.7	115.0		1(1)			1
A4	112.8	112.6		4(4)			2
A5	125.6	125.0	4(3)[c]				
B2	141.4	140.4				2	
B3	171.9	171.7	2(1)				
B4	98.0	98.1		5(4)			2
B5	163.2	162.1	1(1)		22(23)[c]		
C2	147.4	147.2	3(2)				
C3	111.4	111.9		6(6)		(d)	2 (d)
C4	124.0	144.0	3(3)				
C5	131.2	130.2				3	
1"	118.8	116.1				2	1
-OMe	61.0	61.0				6	2
11'	16.8	15.3		4(3)			1
10'	25.4		3				
9'	34.7	42.1		4			1
8'		36.9	3(1)				
1'	30.1			(4)			
2'-8'		[f]					
1'-7'; 10'	[e]						

[a] Chemical shifts are given relative to HMDS (hexamethyldisilane) and are calculated from the solvent peak $CHCl_3$ (δ_c 80.0).

[b] Percent excess of label is measured from peak heights and is rounded off to the nearest 1%.

[c] Values in parentheses are derived from undecylprodigiosin.

[d] In the case of undecylprodigiosin, enriched signals were observed at B2, C5, 1" and OMe for the glycine feedings and at A3, A4, B4, C3, 1", OMe, 11' and 9' for the serine feedings. However, since very small samples were isolated, quantitative data was difficult to obtain.

[e] Unassigned peaks for carbon atoms 1'-7' and 10' in 1: 32.8, 30.7, 30.3, 29.4, 27.9, 27.5, 25.4.

[f] Unassigned peaks for carbon atoms 2'-8' in 2: 32.4-32.1.

With [2-^{13}C]-glycine, incorporations of the label in 1 and 2 were observed at B2, 1", C5, and -OMe.[9] The appearance of glycine label at B2 and 1" parallel findings in the biosynthesis of methylamylprodigiosin, (3).[10] The appearance of [2-^{13}C]-glycine at C5, representing a major difference between the biosynthesis of 1 and 2 compared to 3, is in full accord with the different mode of acetate incorporation observed in the formation of pyrrole ring C in 1 and 2, as outlined below.

The pattern of labeling of [1-^{13}C]-acetate and [2-^{13}C]-acetate as outlined in the Table is readily explained by the involvement in the biosynthesis of a polyacetate chain of 14 atoms incorporating three carbon atoms of ring C as shown in Figure 1.[11] The other carbon atom (at the C5 position) and the nitrogen atom of the ring C pyrrole are derived from glycine as noted above. The remaining eleven-carbon section of the polyacetate chain constitutes the alkyl group at the 2-position in ring C of undecylprodigiosin (2), while in metacycloprodigiosin (1), this alkyl side chain appears to have undergone oxidative cyclization at C4 to form the meta-bridged fused ring system.

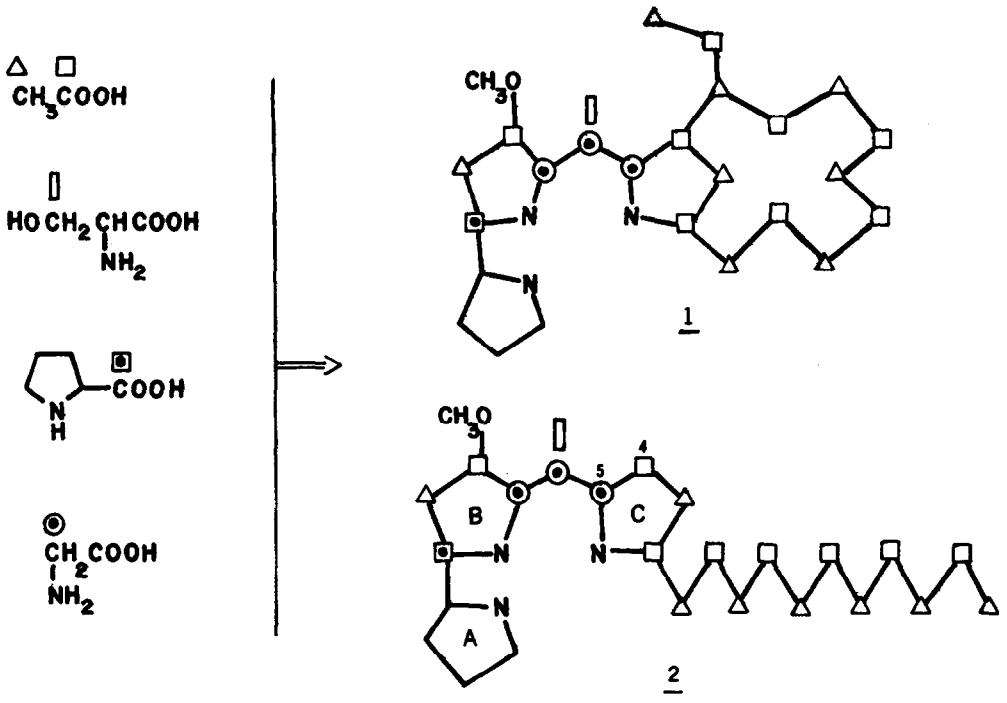

Figure 1.

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8. For details of the ^{13}C assignments, see: R.J. Cushley, R.J. Sykes, C.K. Shaw, and H.H. Wasserman, Carbon-13 Fourier Transform NMR. IX, submitted for publication.

9. As in the case of serine, a significant conversion of glycine label to the "one carbon" pool, and thence to the methoxyl carbon was observed.

10. The well-established glycine-serine interconversion may account for the involvement of glycine at B2 and 1", since labeled glycine would effectively provide doubly-labeled serine. See ref. 12, pp. 172,277.

11. In contrast to the results obtained with 3, where no incorporation into ring A was found[3], [2-^{13}C] acetate caused labeling at position A4, with smaller levels being found at A3 and A2, while [1-^{13}C] acetate labeled A5 with a smaller incorporation at B5 in both 1 and 2. While the high level of incorporation of [1-^{13}C] proline provides evidence that the ring A carbon B5 moiety is derived from an intact proline molecule, labeling of ring A by acetate indicates partial involvement of the TCA cycle. Thus, acetate enters the TCA cycle and exits as glutamate which cyclizes to proline by the well known biosynthetic pathway.[12]

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Acknowedgement. This research was supported by Grants AI- 04798 and RR-00356 from the National Institutes of Health, U.S. Public Health Service.

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