On the Biosynthesis of the Antibiotic Myxovirescin A_1 by *Myxococcus virescens*

W. Trowitzsch,* K. Gerth, V. Wray, and G. Höfle

GBF, Gesellschaft für Biotechnologische Forschung mbH., Mascheroder Weg 1, D-3300 Braunschweig, W. Germany

^{13}C N.m.r. spectroscopy has been used to deduce the labelling patterns of the antibiotic myxovirescin A_1 derived from ^{13}C-labelled acetate, methionine, and glycine which are all incorporated during biosynthesis by *Myxococcus virescens*.

We have recently reported the structure of myxovirescin A_1, a novel macrocyclic antibiotic from *Myxococcus virescens* strain Mx v 48.[1] It shows activity against many Gram-negative bacteria where it appears to interfere with cell wall synthesis. We now report results on the biosynthesis of myxovirescin A_1.

Preliminary studies with ^{14}C-labelled precursors indicated that acetate, methionine, and glycine were incorporated during biosynthesis of the antibiotic while propionate was not. Thus we have fed ^{13}C enriched acetate (both singly and doubly labelled), [methyl-^{13}C]methionine, and [1-^{13}C]glycine to *Myxococcus virescens* during the late growth phase; the antibiotic has been separated and purified as previously described.[2] Subsequent use of ^{13}C n.m.r. spectroscopy has allowed the position of incorporation of the various precursors to be deduced as shown in Figure 1.

The proton noise decoupled ^{13}C n.m.r. spectrum of myxovirescin A_1 (see Table 1), obtained after feeding with [1,2-^{13}C]acetate, indicated the incorporation of thirteen intact acetate units (2.5% enrichment). Eleven of these formed a polyketide chain from C-1 to C-22 without branching to C-31 or C-33, while the remaining two intact units formed the section C-26 to C-36. Addition of [methyl-^{13}C]methionine[3] to the nutrition medium caused intensity enhancement of four carbon signals in the ^{13}C n.m.r. spectrum attributable to C-29, C-30, C-34, and C-37 (30% enrichment). Thus all methyl groups apart from C-32 are derived from methionine. A similar experiment with [1-^{13}C]glycine caused signal enhancement of the methine signal from C-23 (33% enrichment) and indicated that only one unit of glycine was incorporated per molecule of myxovirescin.

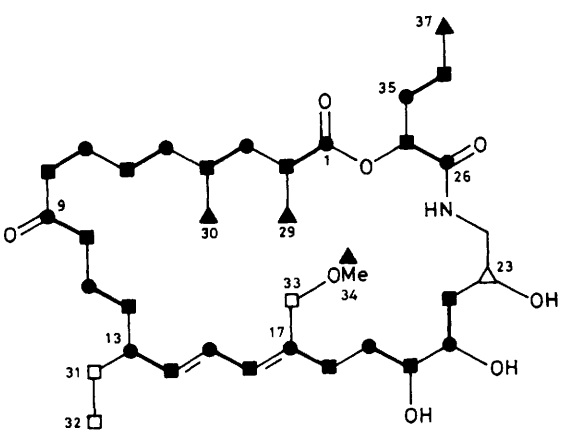

Figure 1. Schematic representation of the biosynthetic incorporation of [1-^{13}C]acetate (●), [2-^{13}C]acetate (■), [methyl-^{13}C]-methionine (▲), and [1-^{13}C]glycine (△) into myxovirescin A_1. [2-^{13}C]Acetate incorporation at C-31, C-32, and C-33 (□) is discussed in the text.

Table 1. ^{13}C N.m.r. data[a] for the incorporation of [1,2-^{13}C]acetate, [methyl-^{13}C]methionine, and [1-^{13}C]glycine into myxovirescin A_1.

Carbon[g]	δ/p.p.m.[b]	J(CC)/Hz	Carbon	δ/p.p.m.	J(CC)/Hz
1	176.01	57.1	19	30.42	38.3
2	37.18		20	73.24	
3	40.95	34.9	21	71.65	38.5
4	30.42		22	36.02	
5	36.44	34.7	23	69.05[c]	
6	26.49		24	45.39[d]	
7	23.76	35.1	26	171.14	55.8
8	42.55		27	73.62	
9	212.50	39.0	29	17.52[e]	
10	43.13		30	19.76[e]	
11	22.08	35.2	31	28.42[f]	
12	34.69		32	11.87[f]	
13	45.27	43.6	33	71.08[f]	
14	139.64		34	58.38[e]	
15	125.91	56.4	35	34.01	34.0
16	130.05		36	18.18	
17	134.60	43.2	37	13.73[e]	
18	30.20				

[a] Data were recorded on a Varian XL-100 n.m.r. spectrometer at 25.16 MHz. [b] In $CDCl_3$ relative to Me_4Si and, unless otherwise stated, the shifts for [1,2-^{13}C]acetate incorporation are given. [c] Enriched carbon atom in the [1-^{13}C]glycine experiment. [d] Unenriched carbon atom derived from glycine. [e] Enriched carbon atoms in the [methyl-^{13}C]methionine experiment. [f] Enriched but appear as singlets in the [1,2-^{13}C]acetate experiment. [g] Signal assignments have been reported by us previously (ref. 1) and are independent of the biosynthetic work.

Re-examination of the spectrum of the [1,2-^{13}C]acetate feeding experiment indicated that the signal from C-33 was a singlet and was enriched to approximately the same extent as those carbon atoms arising from intact acetate units. Similarly the singlet signals from C-31 and C-32 were enriched but to a smaller extent than C-33 (1% enrichment). In order to clarify these points and to determine the orientation of acetate incorporation, feeding experiments with both singly labelled acetates were performed.

[1-^{13}C]Acetate gave an unambiguous spectrum in which thirteen signals were enhanced (37% enrichment) corresponding to the intact units (Figure 1) with no other signal showing enrichment. With [2-^{13}C]acetate, however, the results were more complex. As expected, signals corresponding to C-2 of intact acetate units were observed together with that from C-33 with the same intensity (9% enrichment), indicating that C-33 arises from C-2 of a cleaved acetate moiety. Somewhat surprisingly, all the C-1 atoms of incorporated acetate units were enriched to about 3%. This result can only be explained by randomization of the [2-^{13}C]-acetate label in a manner similar to that described elsewhere.[4-6] Myxovirescin A_1 is produced late in the growth phase and presumably randomization occurs from recycling of acetate in the citric acid cycle.

In the same [2-^{13}C]acetate feeding experiment C-31 and C-32 were also enriched to about 3%, confirming the result from doubly labelled acetate. Thus C-31 and C-32 are derived from C-2 of acetate by a different and unknown biosynthetic pathway. During our investigation of other members of the myxovirescin family we observed an analogous 13-methyl substituted compound[7] which suggests a stepwise construction of the 13-ethyl substituent in myxovirescin A_1.

In conclusion, we have been able to demonstrate by ^{13}C n.m.r. spectroscopy the origin of all the carbon atoms in myxovirescin A_1. Presumably, glycine acts as the starter unit of the polyketide chain C-22 to C-1, which is then alkylated[8] at methylene (C-2, C-4) and acetate carbonyl (C-13, C-17) carbon atoms and oxidized (C-20). At what stage the hydroxy acid unit is incorporated, and which bond is formed first, ester or amide, is not yet known.

Received, 18th July 1983; Com. 955

References

1 W. Trowitzsch, V. Wray, K. Gerth, and G. Höfle, *J. Chem. Soc., Chem. Commun.*, 1982, 1340.
2 K. Gerth, H. Irschik, H. Reichenbach, and W. Trowitzsch, *J. Antibiot.*, 1982, **35**, 1454.
3 The [^{13}C]acetates and [1-^{13}C]glycine were commercial products. [Methyl-^{13}C]methionine was synthesized with 82% enrichment from L-methionine and [^{13}C]methyl iodide according to: D. Dolphin and K. Endo, *Anal. Biochem.*, 1970, **36**, 338.
4 T. J. Simpson and J. S. E. Holker, *Phytochemistry*, 1977, **16**, 229.
5 A. E. Chalmers, A. E. De Jesus, C. P. Gorst-Allman, and P. S. Steyn, *J. Chem. Soc., Perkin Trans.* 1, 1981, 2899.
6 A. E. De Jesus, W. E. Hull, P. S. Steyn, F. R. van Heerden, R. Vleggaar, and P. L. Wessels, *J. Chem. Soc., Chem. Commun.*, 1982, 837.
7 W. Trowitzsch, V. Wray, K. Gerth, and G. Höfle, unpublished results.
8 T. C. Feline, R. B. Jones, G. Mellows, and L. Phillips, *J. Chem. Soc., Perkin Trans.* 1, 1977, 309.