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THE BIOSYNTHESIS OF CHROMOMYCIN-A_3

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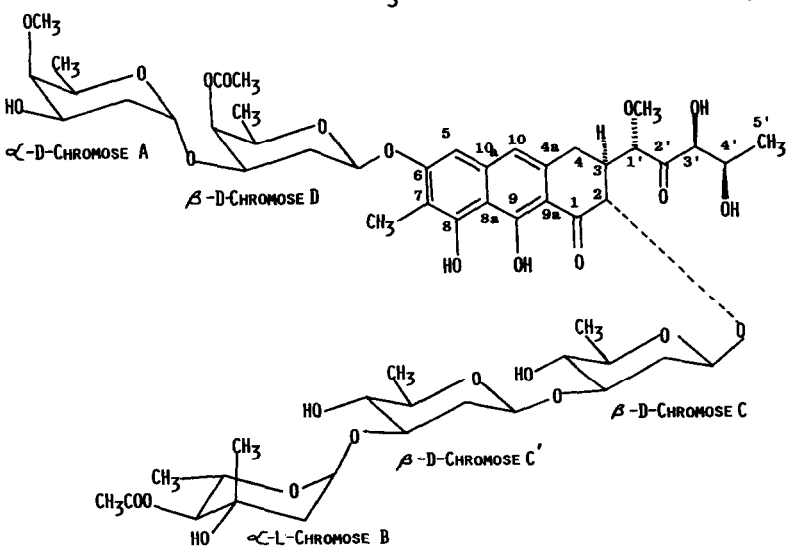
Abstract: Feedings of [2-^{13}C]- and [1,2-$^{13}C_2$]acetate to Streptomyces griseus gave chromomycin-A_3 which was analyzed by NMR. Patterns of carbon enrichments and couplings indicate that 1 is formed from separate octa- and diketides.

Chromomycin-A_3 (1)[1,2,3] is a typical member of the aureolic acid family of antitumor antibiotics. Total synthetic efforts toward 1 remain incomplete[4] and studies on the biosynthesis of 1 have been limited to the incorporation of ^{14}C-labeled precursors[5]. Of these, methionine is incorporated best, followed by serine, acetate, propionate and others. Methionine is presumed to be incorporated into O-methyl groups and the 7-methyl group of the aglycone, chromomycinone. Distribution of label in carbons 3'-5' of the side chain confirms the involvement of a polyketide origin for the aglycone[5a]. Recently published carbon-NMR spectral properties of the aureolic acids[6], new rapid methods for the isolation of the air and light sensitive antibiotics, and successes in producing relatively high antibiotic titers have permitted us to conduct the first ^{13}C-labeling experiments for this antibiotic family.

Cultures of S. griseus ATCC 13273 were grown in two-stages using a medium of dextrose(5%), soybean meal(1.5%), NaCl(0.3%), and $CaCO_3$(0.3%). Sterile solutions of sodium[1,2-$^{13}C_2$]acetate[7], and sodium[2-^{13}C]acetate[8] were added to 48 hr and 72 hr old second stage cultures, respectively. After addition of the isotopic acetates, cultures were incubated for 120 hr before being exhaustively extracted with $CHCl_3$. Purification of extracts by silica gel column



chromatography, and preparative HPLC over octadecyl silica gel ($CH_3CN:H_2O:HCOOH$ 45:55:1 v/v/v) gave 38 mg of 1 from [1,2-$^{13}C_2$]acetate, and 25 mg from [2-^{13}C]acetate which were analyzed by ^{13}C-NMR (Table I).

Table I. ^{13}C-NMR spectral properties of ^{13}C-labeled chromomycin A_3

Carbon	$C^{(m)}$[6]	[2-^{13}C]Acetate Normalized Incorporation[b]	[1,2-$^{13}C_2$]Acetate J_{CC}, Hz
1	202.1(s)	-	43.5
2	75.9(d)	1.3	44.0
3	43.8(d)	-	31.7
4	27.0(t)	1.4	34.2
4a	134.6(s)	-	65.8
10	117.1(d)	1.9	65.2
5	100.8(d)	1.4	59.5
10a	138.4(s)	-	59.5
6	159.6(s)	-	68.6
7	111.6(s)	2.4	69.5
8	165.3(s)	-	65.1
8a	108.0(s)	1.9	64.0
9	156.1(s)	-	62.7
9a	108.0(s)	1.9	63.0
7-CH_3	8.2(q)	-	-
1'-OCH_3	59.7(q)	-	-
1'	82.0(d)	1.8	42.6
2'	211.2(s)	-	43.0
3'	78.4(d)	1.5	-
4'	67.9(d)	-	39.1
5'	20.5(q)	2.4	39.3
Chromose-B			
CH_3COO	20.9(q)	2.2	59.7
	171.4(s)	-	59.7
Chromose-D			
CH_3COO	20.8(q)	2.1	59.7
	170.9(s)	-	59.7

[a]Bruker WM-360, 90.55 MHz, $CDCl_3$; Spectral width 23809 Hz, 32K data points, 30° pulse, acquisition time 1.31 s.

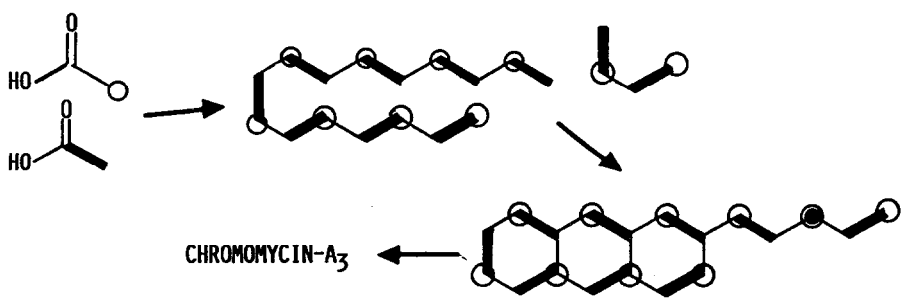
[b]Relative intensities of peaks are normalized to the 1-carbon atom signal of the aglycone.

Labeled acetates were incorporated only into the aglycone, and into the two acetate ester functional groups attached to Chromose B and D sugar moieties. Table I shows that twelve enhanced (average enrichment 0.85%) signals were displayed in the ^{13}C-NMR spectrum of the

aglycone-portion of 1 derived from sodium [2-^{13}C]acetate indicating an even distribution of label in the aglycone. The spectrum derived from 1 labeled with [1,2-$^{13}C_2$]acetate (average enrichment 1.65%) gave one enhanced singlet (C-3', 78.4 ppm), together with eleven pairs of enhanced and coupled doublets. Coupled doublets correspond to acetate units incoporated intact, while the singlet for C-3' must arise from labeled acetate split during aglycone assembly. Resonances for eighteen of twenty-one aglycone carbons were readily distinguished in ^{13}C-NMR spectra of labeled 1[6]. Those for methoxyl- and C-7 methyl, all aromatic carbons (4a, 5 - 8, 8a, 9, 9a, 10 and 10a), the two carbonyls (C-1, C-2'), C-3, and side-chain carbons C-1', C-3', and C-4' were clear and could be unambiguously measured and assigned. Those for carbons at positions 2, 4 and 5' were partially obscured. However, C-5' could be distinguished from acetate ester methyl groups which lie in close proximity at 20.5 ppm. By deduction, C-1 must be coupled to C-2 with J_{CC} 43.5 Hz, vs 64 Hz for the only other adjacent aromatic carbon atom, C-8a. Likewise, C-4, a multiplet within multiplets must be coupled to C-3 with J_{CC} 34 Hz.

Enzymatic steps involved in the biosynthesis of 1 may be grouped into the separate syntheses of sugars and the aglycone; aglycone glycosylations either with single, di- or trisaccharide moieties; and additional reactions including hydroxylation, methylation and acetylation which may occur early or late during antibiotic assembly. The observed labeling pattern obtained with [2-^{13}C]actate is normal for the biosynthesis of polyketides[5,9], and indicates that all carbons of the aglycone, except for the 7-CH_3 and the 1'-OCH_3 derive from acetate. With singly labeled acetate, peaks for C-7 and C-5' are slightly greater in intensity than the rest, indicating the possibility that separate hexa- and tetraketide units are linked together in the assembly of the aglycone. Patterns of incorporation for doubly-labeled acetate are surprising, and rule out this two-chain hypothesis. They suggest (Scheme I) that clockwise polyketide assembly begins with carbon atom 2 to form a single octaketide chain with a carboxy-terminus at carbon-2'. The intense, labeled singlet signal for C-3' clearly indicates that a break in the carbon-chain occurs between positions 2' and 3', and that the terminal three-carbons of the side-chain are derived from a separate acetoacetate unit. Ring assembly may or may not precede the linking of side-chain carbons 3'-5' by aldol condensation of the acetoacetate unit. Further experiments to probe the mechanism of chromomycin-A_3 biosynthesis are in progress[10].

Scheme I



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7. Two gm of 99 atom % sodium [1,2-$^{13}C_2$]acetate (Aldrich) was added to 2000 ml of Stage-two culture.

8. Two gm of 99 atom % sodium [2-^{13}C]acetate (Aldrich) was added to 2000 ml of Stage-two culture.

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10. Authentic Chromomycin A_3 was obtained from the Natural Products Branch of the National Cancer Institute, and was fully examined by proton- and carbon-NMR, mass spectrometry, and was employed in establishing HPLC analytical and semipreparative chromatographic procedures. The major antibiotic produced by strain ATCC 13273 was verified as chromomycin A_3 by full spectral comparison with authentic 1.

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