NOTE

^{13}C-NMR STUDIES ON THE BIOSYNTHESIS OF AURODOX (ANTIBIOTIC X-5108)

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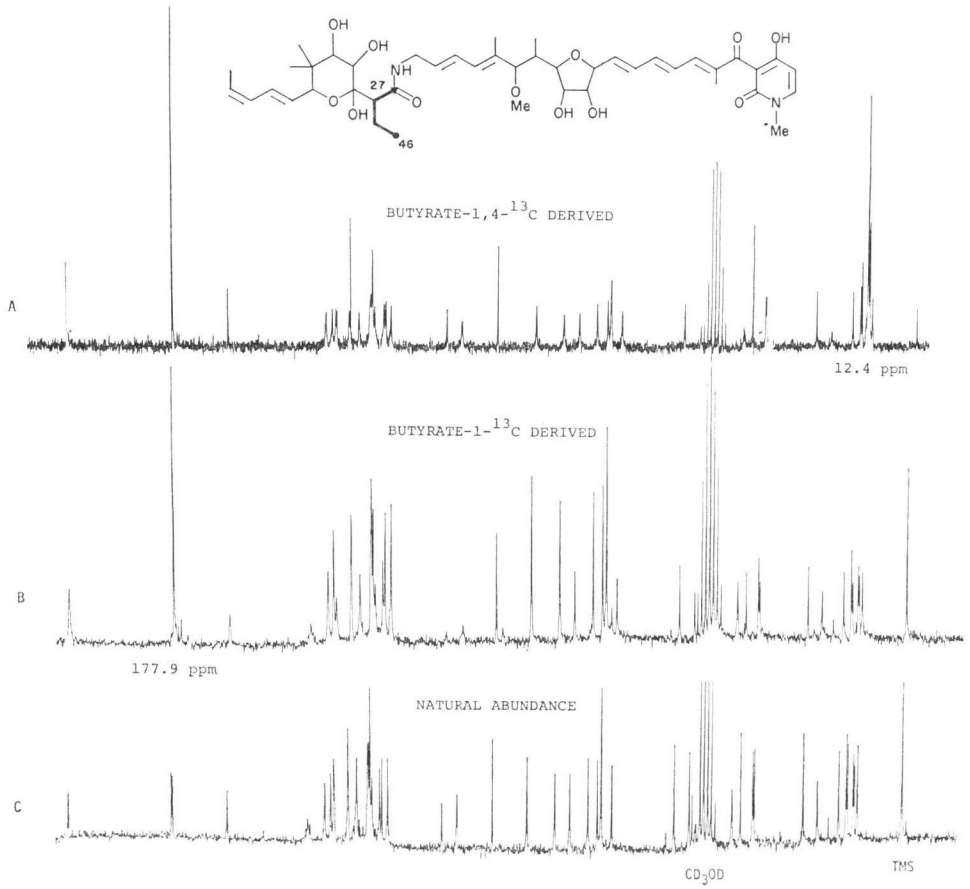
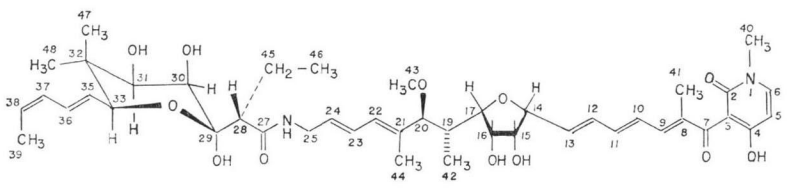
Biosynthetic studies[1] using ^{14}C-labelled substrates have shown that aurodox (**I**) is assembled by *Streptomyces goldiniensis* from acetate, propionate, and butyrate units, and a C_1 donor such as methionine or glycine. Chemical degradations of ^{14}C-aurodox derived from these substrates suggest that the backbone of the antibiotic contains carbons from both a butyrate and a propionate unit, and that four of the five branched methyls are formed by transmethylation, including the geminal methyls at C-32.

In order to gain further insight into the biosynthesis of the antibiotic, ^{13}C-precursors (at 1.0 g/liter) have been used as substrate in *S. goldiniensis* fermentations to yield acetate-1-^{13}C,

Fig. 1. ^{13}C-NMR Spectra of aurodox in deuteromethanol (CD_3OD).
A. Butyrate-1,4-^{13}C derived aurodox.
B. Butyrate-1-^{13}C derived aurodox.
C. Natural abundance.
The proton decoupled ^{13}C-NMR FOURIER Transform spectra were recorded on a Bruker HFX-90 spectrometer at 22.63 MHz, C_6F_6 was used as a spectrometer lock.
Chemical shifts are reported in ppm downfield from dissolved tetramethyl silane (TMS).





I Aurodox (X-5108)

-2-^{13}C; propionate-1-^{13}C, -3-^{13}C; butyrate-1-^{13}C, -1,4-^{13}C and methionine-methyl-^{13}C derived aurodox. Comparison of the FT-proton noise decoupled ^{13}C-NMR spectrum of natural abundance with that of butyrate-1-^{13}C derived antibiotic (Fig. 1) indicated 3.5-fold primary enrichment of the downfield peak at 177.9 ppm assigned to C-27 (the amide carbonyl group of the antibiotic molecule). The spectrum of butyrate-1,4-^{13}C derived aurodox revealed two major enrichment sites at $\delta=177.9$ (8~10 fold enrichment) and at $\delta=12.4$ ppm (3~4 fold enrichment), the latter due to C-46 (the CH_3 of the ethyl group at C-28). These results confirm our previous finding that an intact butyrate unit is incorporated into aurodox to constitute the C-46, C-45, C-28 and C-27 part of the molecule. This is one of the few examples of an intact butyrate unit being incorporated into a polyketide to form a C-ethyl group; the polyether antibiotics lasalocid and monensin are other known examples[2,3]. Propionate is also incorporated as an intact unit as shown by an 8-fold enrichment at $\delta=202$ ppm (C-7), and a 5.5-fold enrichment at $\delta=12.1$ ppm

Fig. 2. ^{13}C-NMR Spectra of aurodox in deuteromethanol (CD_3OD).
A. Propionate-3-^{13}C derived aurodox.
B. Propionate-1-^{13}C derived aurodox.
C. Natural abundance.

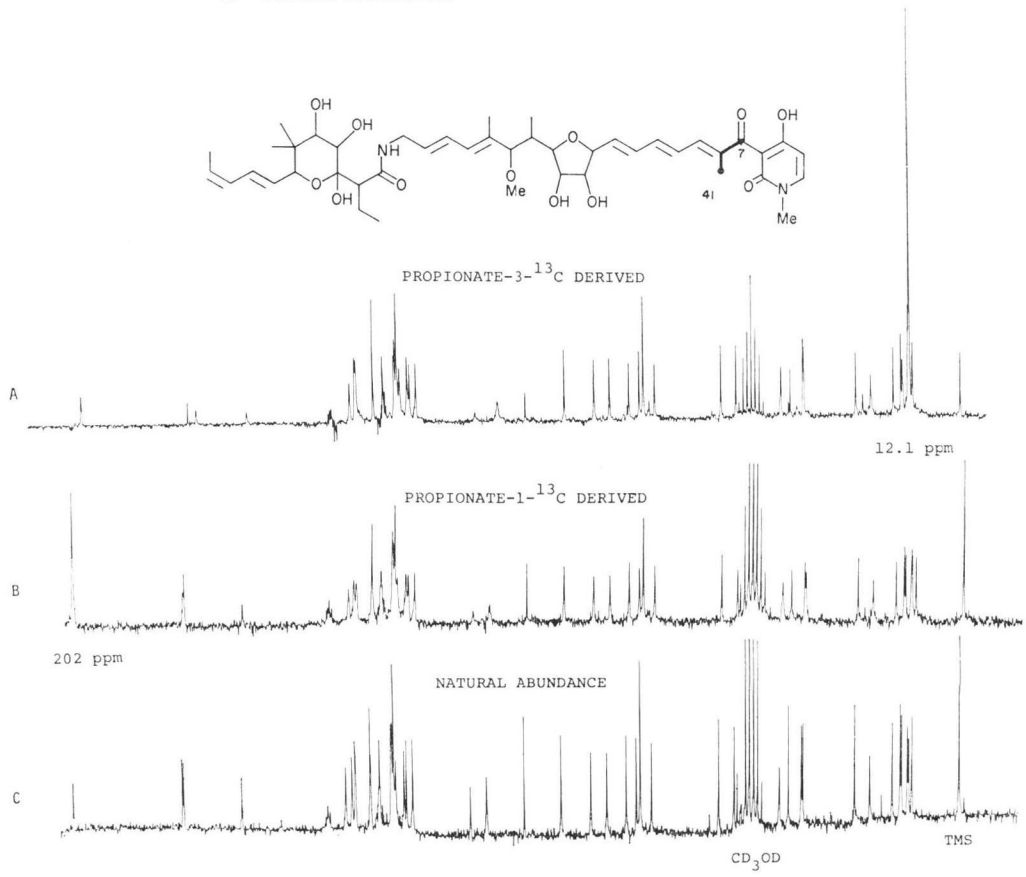


Table 1. Incorporation of acetate-1-^{13}C into aurodox as determined by ^{13}C-NMR.

Chemical shift ppm TMS	Enrichment ×natural abundance	Assignment	Functional group
178.2	2.1	C-2	N–C(=O)–
165.0	7.3	C-4	=C–OH
141.4	5.7	C-6	=CH–N
140.0	8.5	C-9, C-36	
135.9(2C)	4	C-11, C-38	
131.1(2C)	10.6	C-13	* =CH–
127.7	11.7	C-22	
126.3	9.8	C-24	
101.0	4.7	C-29	–O–C(–)–OH
92.1	9.8	C-15	
85.3	9.7	C-17	
77.4	6.9	C-20	–CH(–)–O
75.0	9.2	C-31	
74.1	3.9	C-33	

* Signals in the bracket are assigned as a group.

Table 2. Incorporation of acetate-2-^{13}C and methionine-CH_3-^{13}C into aurodox as determined by ^{13}C-NMR.

Chemical shift ppm TMS	Enrichment ×natural abundance	Assignment	Functional group
Actate-2-^{13}C derived			
135.9	2.6	C-21	=C(–)–Me
133.9	3.4	C-10, C-37	
130.6(2C)	5.7	C-12	* =CH–
130.2	4.5	C-23	
128.3	6.5	C-35	
81.8	6.0	C-14	
74.1	2.6	C-16	–CH(–)–O
71.5	3.3	C-30	
56.3	2.3	C-43	O–CH_3
42.1	2.0	C-25	N–CH_2–
40.0	4.3	C-32	–C(Me)$_2$–
37.0	5.2	C-19	–CH(Me)–
13.7	5.5	C-39	C–CH_3
Methionine-CH_3-^{13}C derived			
56.3	33	C-43	O–CH_3
36.6	24	C-40	N–CH_3
24.7	11	C-42	
15.9	12	C-44	C–CH_3
12.4	14	C-47	
11.3	20	C-48	

* Signals in the bracket are assigned as a group.

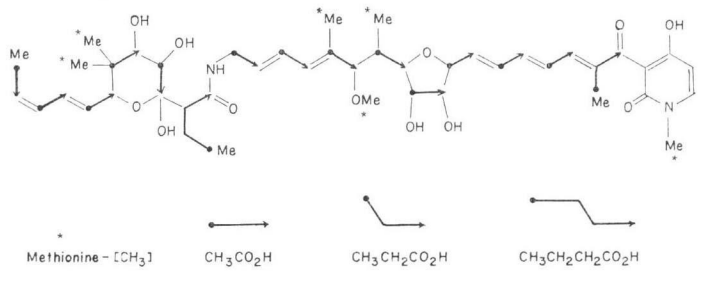
(C-41) in aurodox derived from propionate-1-^{13}C and -3-^{13}C respectively (Fig. 2).

The extent of enrichment by acetate-1-^{13}C, acetate-2-^{13}C and methionine-methyl-^{13}C is shown in Table 1 and Table 2. Carbons bearing oxygen, methine and methyl carbons are assigned on the basis of their chemical shift. Rigorous assignment of individual carbon atom resonances will require further study, but an outline of the incorporation pattern for the antibiotic can now be proposed. The spectrum of acetate-1-^{13}C derived aurodox had 16 signals enriched (C-2, 4, 6, 9, 11, 13, 15, 17, 20, 22, 24, 29, 31, 33, 36, and C-38), whereas acetate-2-^{13}C derived aurodox exhibited 14 enrichment peaks at C-10, 12, 14, 16, 19, 21, 23, 25, 30, 32, 35, 37, 39, and C-43. From the methionine-methyl-^{13}C labelling experiment the O-methyl (C-43), N-methyl (C-40) and four C-methyls (C-42, 44, 47 and C-48) were all found to be enriched.

The labelling experiments carried out so far account for 39 out of 44 carbons of the aurodox molecule. Of the five unenriched carbons, C-8, C-45, and C-28 can be expected to arise from propionate and butyrate, leaving only C-3 and C-5 to be accounted for. The carbon skeleton of the antibiotic molecule appears to be formed by the joining of two polyketide fragments joined by an amide bond. One fragment is derived from 5 acetate and one butyrate unit, the other from one propionate and 8 acetate units (Fig. 3). The sequence of C-methylation as well as O- and N-methylation remains to be determined.

The biosynthetic origin of the pyridone moiety is unclear. Although C-2, C-4 and C-6 can be derived from the carbonyl group of acetate, feeding of acetate-2-^{13}C failed to enrich C-3 and

Fig. 3. Hypothetical biosynthetic origin of the aurodox carbon skeleton.



C-5 as would be expected if intact acetate units were incorporated into the pyridone moiety. Previously, we have shown that cadaverine-1,5-^{14}C, and L-aspartic acid-U-^{14}C were poorly incorporated into aurodox (about 0.5%), and quinolinic acid-6-^{14}C and nicotinic acid-7-^{14}C were not incorporated at all. In this connection, it is of interest to note that the pyridone moiety of the antibiotic tenellin was found to be derived from an acetate unit and the alanine moiety of phenylalanine[4].

Acknowledgements

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References

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