BIOSYNTHESIS OF OXANTHROMICIN

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Oxanthromicin (Antibiotic 16-550), a novel dimeric anthrone peroxide produced by *Actinomadura* sp. SCC 1646, exhibited good *in vitro* activity against dermatophytic fungi. The details of taxonomy, biological properties[1] and the structure[2] have been reported from our laboratories. The biosynthetic origin of aclacinomycins[3], cetocycline[4], tetracyclines[5] and of similar ring structures has been studied extensively and their overall polyketide mechanism is well established[6]. However oxanthromicin is a unique structure and we report here our studies on the biosynthesis of the basic nucleus.

Addition of ^{14}C-labeled Precursors

The fermentation was carried out either in flasks or in a 14-liter New Brunswick Scientific Laboratory fermentor according to the published procedure[1].

The optimum addition time of the labeled precursors, was studied using L-[*methyl*-^{14}C]-methionine, Na-[1-^{14}C]acetate, Na-[2-^{14}C]acetate, and Na-[1-^{14}C]propionate. The additions (conc 2.5 μCi/10 ml) were made at 24, 48 and 72 hours of fermentation. Fermentations were harvested at 96 hours, pH adjusted to 5, and extracted twice with EtOAc. The extract was concentrated 20-fold under vacuum. The amount of radioactivity incorporated was measured in a scintillation spectrophotometer. The EtOAc extract (100 μl) was chromatographed on TLC silica plates (Rf 0.33) in $CHCl_3$ - MeOH - 17% ammonia (2: 2: 1) and then scanned for radioactivity on a Nuclear Chicago radiochromatogram scanner (Model 1002).

The rate of incorporation of the precursors

Table 1. Incorporation of ^{13}C-labeled precursors into oxanthromicin.

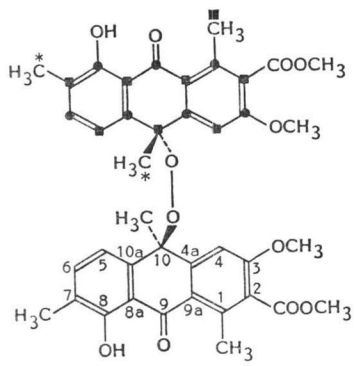
Carbon atoms	Chemical shift[b] ppm (δ)	Enrichment factor[a]			
		[1-^{13}C]-Acetate	[2-^{13}C]-Acetate[c]		L-[*methyl*-^{13}C]-Methionine
1	141.3	1.69	1.13	0.97	
2	127.0	1.20	1.51	1.12	
3	159.1	2.39	1.36	1.05	
4	117.5	0.76	0.95	1.28	
4a	150.9	1.71	1.20	0.93	
5	107.3	0.73	0.98	1.31	
6	135.9	1.61	0.85	1.11	
7	115.3	1.11	1.32	1.15	
8	160.8	2.05	1.03	0.92	
8a	122.4	1.20	1.21	1.19	
9	189.6	1.67	1.13	0.93	
9a	125.9	0.86	1.21	1.07	
10	79.8	0.94	1.33	1.23	
10a	140.6	2.13	1.22	1.10	
1-CH_3	20.8	0.85	1.17	1.09	
2-$COOCH_3$	168.1	2.31	1.20	0.86	
2-$COOCH_3$	56.1	Reference	—	—	
3-OCH_3	52.5	Reference	—	—	
10-CH_3	34.0	0.85	1.10	1.10	14.8
7-CH_3	15.6	0.79	1.14	1.03	14.6

[a] Peak heights of the enriched sample divided by the natural abundance sample obtained from spectra run under essentially identical instrumental conditions. The average of peak intensities due to OCH_3 carbons, is taken as 1.00 and the heights of other peaks are relative to 1.00.

[b] The chemical shift assignments are based upon single frequency off-resonance (SFOR) and single frequency proton-decoupled spectra.

[c] Results of two separate experiments.

Fig. 1. Biosynthesis of oxanthromicin.



■ ● CH_3COONa[^{13}C(1 or 2)acetate]
*L-[*methyl*-^{13}C]Methionine

into the EtOAc extract indicated that the optimal addition times were 24, 48 and 48 hours for L-[*methyl*-^{14}C]methionine, [1-^{14}C]acetate and [2-^{14}C]acetate, respectively. Percentage incorporations into antibiotic 16-550 at the optimum time were 4.8 for L-[*methyl*-^{14}C]methionine, 3.5 for [1-^{14}C]acetate, 3.5 for [2-^{14}C]acetate and 0.0 for [1-^{14}C]propionate.

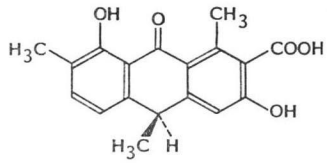
Addition of ^{13}C-Labeled Substrate

At the optimum times L-[*methyl*-^{13}C]methionine, Na-[1-^{13}C]acetate, and Na-[2-^{13}C]acetate were added individually (conc 250 mg/liter) to the culture. The fermentation broth was harvested at 96 hours, acidified to pH 5 with conc HCl, and extracted twice with EtOAc. The organic extract was concentrated to about 1 liter, washed with 0.1 N HCl, several times with H_2O, dried over $MgSO_4$ and finally evaporated to dryness. The dried material was washed with hexane to yield crude antibiotic.

The crude antibiotic was methylated with CH_2N_2 in ether for 4 hours and the excess CH_2N_2 decomposed with dil AcOH. The ether phase was separated, washed with H_2O, dried over $MgSO_4$, evaporated, and the solid residue purified by preparative TLC, on silica plates.

Proton-noise decoupled (p.n.d.) ^{13}C NMR spectra of the natural abundance ^{13}C and the enriched tetramethylated antibiotic samples were recorded in $CDCl_3$ on a Varian XL-100-15 NMR spectrometer operating at 25.2 MHz. Percentage enrichments are shown in Table 1.

The ^{14}C-labeled studies indicated that only [1-^{14}C]acetate, [2-^{14}C]acetate and L-[*methyl*-^{14}C]-methionine were incorporated into the antibiotic.



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The addition of [1-^{13}C]acetate enriched 8 carbons; C-1, C-3, C-4a, C-6, C-8, C-9, C-10a and C(2)-*C*OOCH$_3$. Similarly the addition of [2-^{13}C]-acetate* enriched 8 carbons; C-2, C-4, C-5, C-7, C-8a, C-9a, C-10 and C(1)-*C*H$_3$, whereas, L-[*methyl*-^{13}C]methionine only enriched two carbons; C(10)-*C*H$_3$ and C(7)-*C*H$_3$.

The results of the incorporation experiments are consistent with a biosynthetic pathway involving polyketide formation, alkylation, reduction and dehydration to give the monomer **2**, which is then oxidatively converted into the natural product (Fig. 1). The nature of this final conversion, which is presumably an enzyme-mediated transformation as the product is optically active, is of moderate interest.

Direct evidence for the intermediacy of **2** will require further studies with labeled substrate.

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* The reasons for the low incorporation of [2-^{13}C]-acetate (Table 1) are not fully understood. However, the results of two experiments support the enrichment of 8 carbons.

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