BIOSYNTHESIS OF THE ANTIBIOTIC OKILACTOMYCIN

Sir:

Okilactomycin is a novel polyketide antibiotic produced by *Streptomyces griseoflavus* subsp. *zamamiensis* subsp. nov. which shows antitumor activity against Ehrlich ascites carcinoma *in vivo* and weak antimicrobial activity against Gram-positive bacteria. A unique 13-membered ring with the intra-ether bridge forming a tetrahydro-γ-pyrone ring with the exo-methylene at the β-position for okilactomycin has been reported by a combination of spectroscopic and X-ray crystallographic studies.[1,2] In the present paper, we describe the determination of the biosynthetic origin of the carbon atoms of okilactomycin molecule using ^{13}C-labeled precursors.

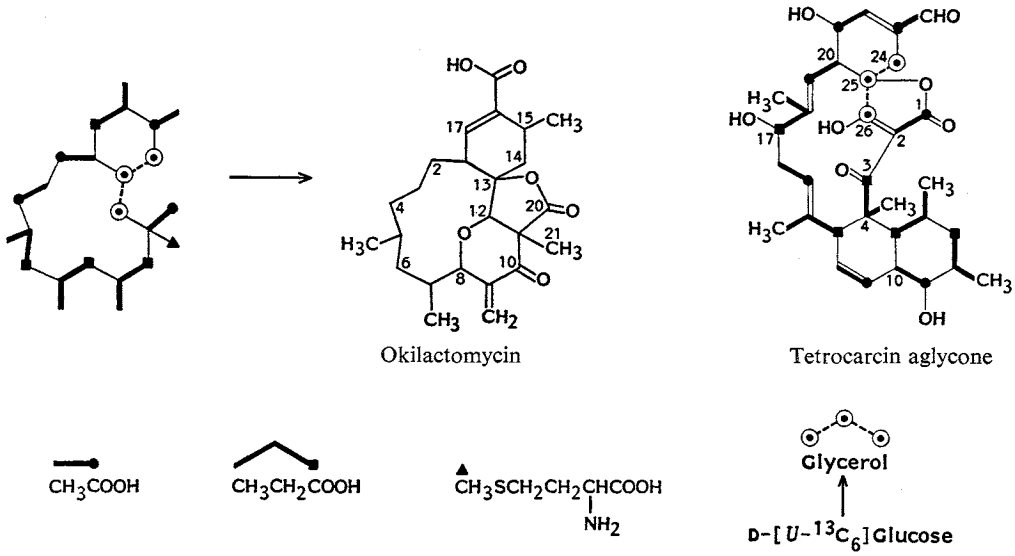
The ^{13}C precursors (0.1~0.2%, w/v), 99% enriched [1-^{13}C]acetate, [2-^{13}C]acetate, [1-^{13}C]-propionate, L-[*methyl*-^{13}C]methionine and 75% enriched D-[*U*-$^{13}C_6$]glucose were fed to a 3-day fermentation broth (medium; dextrin 2.5%, glucose 2.5%, soybean meal 1.5%, Pharmamedia 1.5%, KH_2PO_4 0.006%, K_2HPO_4 0.025%, $CoCl_2 \cdot 6H_2O$ 0.0004%, pH 7.0) and the cultivations were continued at 27°C for 3 days. ^{13}C-Labeled okilactomycins (3~10 mg) were isolated by solvent extraction, followed by silica gel and Sephadex LH-20 column chromatography from each broth filtrate (1 liter). The ^{13}C NMR spectrum of okilactomycin labeled with [1-^{13}C]-propionate showed a strong enrichment for four carbons (δ 45.0 (C-6), 82.4 (C-8), 191.9 (C-10), 141.0 (C-17)). The ^{13}C spectrum of okilactomycin labeled with [1-^{13}C]acetate exhibited enrichment for seven carbons (δ 32.2 (C-2), 37.6 (C-4), 45.0 (C-6), 82.4 (C-8), 191.9 (C-10), 27.2 (C-15), 171.3 (C-20)). Furthermore the feeding of [2-^{13}C]acetate showed incorporation with ^{13}C for eight carbons (δ 30.1 (C-5), 45.0 (C-6), 34.6 (C-7), 82.4 (C-8), 142.0 (C-9), 121.6 (C-22), 20.5 (C-23), 23.5 (C-24)) which correspond to

Table 1. ^{13}C abundances in okilactomycins enriched with ^{13}C-labeled compounds.

Carbon atom	Chemical shift (ppm)	Relative enrichment			
		[1-^{13}C]-Acetate	[2-^{13}C]-Acetate	[1-^{13}C]-Propionate	L-[*methyl*-^{13}C]-Methionine
1	42.2	1.1	3.0	1.1	1.2
2	32.2	4.3	1.2	1.2	1.1
3	23.4	1.0	3.6	1.1	1.1
4	37.6	5.6	1.3	1.4	1.3
5	30.1	1.2	2.3	1.1	1.2
6	45.0	2.9	2.0	9.1	1.0
7	34.6	1.5	2.5	0.9	1.2
8	82.4	2.5	1.6	5.8	1.1
9	142.0	1.3	1.6	1.4	1.1
10	191.9	1.8	1.4	9.8	1.2
11	52.2	1.6	2.8	1.4	1.1
12	83.5	1.2	0.9	1.4	1.3
13	84.8	1.0	0.5	1.0	1.0
14	33.5	1.4	1.2	1.1	0.9
15	27.2	3.2	1.0	1.0	1.0
16	133.0	0.6	1.3	0.5	0.7
17	141.0	1.0	0.9	7.1	1.1
18	172.2	0.3	0.6	0.7	0.6
19	20.3	1.0	3.0	0.9	1.0
20	171.3	2.6	0.8	0.3	1.1
21	25.7	1.3	1.2	1.3	5.4
22	121.6	1.3	2.2	1.0	1.0
23	20.5	1.1	1.9	1.0	1.1
24	23.5	1.2	2.1	1.1	1.0

Relative enrichment: Intensity of carbon atom of enriched okilactomycin/intensity of carbon atom of unenriched okilactomycin.

Fig. 1. Biosynthetic building units of okilactomycin and tetrocarcin aglycone.



propionate-derived carbons, in addition to four carbons (δ 42.2 (C-1), 23.4 (C-3), 52.2 (C-11), 20.3 (C-19)) corresponding to carbon derived from C-2 of acetate, as shown in Table 1. The incorporation of the methyl carbon of acetate into positions which originate from three carbons of propionate units in okilactomycin indicates that this building unit is formed through the tricarboxylic acid cycle *via* succinyl-CoA and methylmalonyl-CoA, as shown in our biosynthetic studies[3] on macrolide antibiotics. A weak incorporation of the carboxyl carbon of acetate into the carboxyl carbons (C-6, C-8 and C-10) of propionate unit in okilactomycin molecule also was observed. Thus, okilactomycin molecule is built up from four propionate units, four acetate units and four carbons unlabeled with [^{13}C]acetate and [^{13}C]propionate. The feeding experiment with L-[*methyl*-^{13}C]methionine showed a strong enrichment for the methyl carbon (C-21) at δ 25.7. It is a common observation that *C*-methyl groups on a polyketide chain in streptomycete molecules are derived biosynthetically from the methyl of propionate, rather than from methionine. The okilactomycin producing strain therefore shows an unusual metabolism. The evidence for the origin of the remaining three carbons at C-12, C-13 and C-14 was obtained from the feeding experiment of 75% enriched D-[*U*-^{13}C$_6$]-glucose. The observation of the ^{13}C-^{13}C coupling pattern for C-12 (d, $^1J_{CC}$=40 Hz), C-13 (dd, $^1J_{CC}$=37 Hz, $^1J_{CC}$=40 Hz) and C-14 (d, $^1J_{CC}$=37 Hz) indicates that these carbons arise from an intact glycerol unit derived *via* glucose (Fig. 1). A similar biosynthetic pathway has been reported in an unusual macrolide chlorothricin.[4] Okilactomycin producing strain, *S. griseoflavus* also produces tetrocarcin A,[5] an antitumor antibiotic as a by-product. Tetrocarcin A contains structurally similar C_3 unit as okilactomycin. It has been reported that the aglycone of tetrocarcin A biosynthetically consists of seven acetates, five propionates and an unknown C_3 unit.[6] The results of okilactomycin biosynthesis suggests that the C_3 unit (C-24, C-25 and C-26) of tetrocarcin A aglycone is also derived from glycerol as a direct precursor.

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