ON THE BIOSYNTHESIS OF FLAVIPUCINE

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An antibiotic produced by a strain of *Aspergillus flavipes*, flavipucine, was first isolated by CASINOVI *et al.*[1] and subsequently identified as 6-methyl-2-(3-methyl-1-oxobutyl)-1-oxa-5-azaspiro(2,5)-oct-6-ene-4,8-dione (**1**) by FINDLAY and RADICS[2]. As a first step towards uncovering its biosynthetic pathway, we have now performed a series of ^{13}C incorporation experiments and our principal observations are reported in the present note.

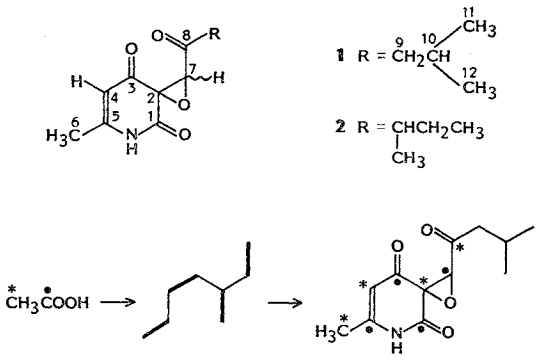
Three-day cultures of *A. flavipes* were enriched with 100 mg of sodium [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetate (90% enrichment) per 1 liter of culture broth[1]. Fermentations were terminated 4 days after inoculation and the isotope-enriched products were isolated *via* acidification (H_2SO_4, pH 3.0~3.5) and subsequent extraction with $CHCl_3$. The extraction residues (*ca.* 600 mg) were purified by repeated column chromatography (acidic alumina - benzene) to yield *ca.* 200 mg of the final products. As attested by the NMR spectra, these consisted of 78 to 82% of **1** and 22 to 18% of the biologically inactive, isomeric flavipucine **2**[2]. In experiments with mono-labeled acetate, the quantitative evaluation of the percentage ^{13}C incorporation (above the natural abundance level) was achieved by the difference, *i.e.* by subtracting the ^{13}C NMR spectrum of the unlabeled sample from those of the enriched metabolites. To allow for this technique to be applied, special care had been taken to have the spectra recorded under strictly identical experimental conditions (solute concentration, sample temperature, *etc.*). In experiments with double labeled acetate, the integral satellite intensities were related to that of the pertinent central line.

The incorporation data collected in the table show that out of the 12 carbon atoms only eight became enriched. No sizable incorporation could be detected in the isobutyl side chain

Table 1. Incorporation of sodium [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]acetates into antibiotic **1** as determined as ^{13}C unit[a].

Carbon	Chemical shift (ppm)	Incorporation (%)			J_{cc} (Hz)
		[1-^{13}C]	[2-^{13}C]	[1,2-$^{13}C_2$]	
1	168.34	0.68	—	0.17	64.14
2	59.77	—	0.47	0.13	64.21
3	186.57	0.66	—	0.22	59.27
4	106.81	—	0.42	0.20	59.42
5	156.38	0.73	—	0.17	44.97
6	20.67	—	0.52	0.16	44.87
7	68.61	0.74	—	0.17	64.14
8	203.11	—	0.51	0.14	64.21
9	49.63	—	—	—	—
10	24.07	—	—	—	—
11	22.85	—	—	—	—
12	22.54	—	—	—	—

[a] At 100 MHz, $CDCl_3$ solution. Chemical shifts are relative to internal TMS. Assignments are in agreement with ref 4. Incorporation data are above the natural abundance.



(C-9 to C-12) nor did any measurable enrichment appear in **2**, the biologically inactive co-metabolite. The data in the table also show that enrichment is generally higher when the label comes from the [1-^{13}C] carbon atom of the precursor, in agreement with the known higher biosynthetic "mobility" of the acetate methyl group[3].

The present experimental results suggest that microbiological synthesis of **1** proceeds through the condensation of two diacetate units (C-1 to C-8), followed by cyclization (involving a nitrogen atom) and the incorporation of the isobutyl side chain group. The fact that co-metabolite **2** carried no detectable label under the present experimental conditions suggests that its biosynthesis occurs at a different stage of fermentation.

Labeling experiments aimed at the elucidation of the complete biosynthetic pathway are now underway in our laboratories.

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