BIOSYNTHESIS OF MONENSINS A AND B

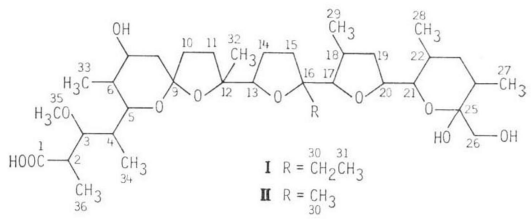
Sir:

Monensin A (I), a polyether antibiotic[1] produced by *Streptomyces cinnamonensis*, is a natural ionophore specific for sodium ions[2,3]. It is successfully used to control coccidiosis in poultry and to promote the growth of cattle.

Studies employing ^{14}C-labelled precursors have proved that molecule of **I** is assembled from five acetate, seven propionate, and one butyrate units[4]. According to a recent ^{13}C NMR study[5], carbons 7, 9, 13, 19, and 25 arise from the acetate carboxyl and carbons 1, 3, 5, 11, 17, 21, and 23 from the propionate carboxyl. Double labelling by ^{13}C and ^{18}O permitted the determination of the origin of the seven oxygen atoms in **I**.

Monensin B (**II**) differs from **I** in the size of the side chain attached to C-16 only. During its biosynthesis the butyrate unit of **I** is replaced by propionate unit. Recently, we have found that the **I**: **II** ratio is affected by addition of valine or isoleucine[6]. Using [*U*-^{14}C]valine, we found that the specific activity of **I** was 3.9 times greater than that of **II**; with [*U*-^{14}C]isoleucine, both compounds were nearly equally labelled. The next compound are tested, [1-^{14}C]*iso*-butyrate, belonged to the valine metabolic pathway. In this experiment also, the specific activity of **I** was 3.8 times that of **II**. Without tedious degradations, these results suggest a direct incorporation of *iso*-butyrate in monensin A.

A subsequent stable isotope study was based on an unambiguous assignment of the ^{13}C NMR



spectrum of **I** by two-dimensional NMR spectroscopy[7], which agrees with the partial assignment used by CANE *et al.*[4] The ^{13}C NMR spectrum of **II** was assigned by comparison with that of **I**. The signal of the C-30 methylene is missing, that of C-31 (methyl) moves from 8.2 to 23.9 ppm; and the signals of C-13, 16, and 17 (81.5, 83.8, and 86.4 ppm), are also changed with respect to **I**.

Streptomyces cinnamonensis C-100-5 (non-pigmenting strain) was cultivated as described previously[8]. The monensins A and B were isolated and separated by preparative thin-layer chromatography[8]. Labelled compounds were administered in three portions (1/2, 1/4, 1/4) at the 24th, 48th, and 72nd hour of fermentation. The doses per 50 ml flask were 17, 25, and 50 mg of sodium [1-^{13}C]butyrate, [1-^{13}C]*iso*-butyrate, and [1,2-$^{13}C_2$]acetate, respectively.

As expected, C-15 of **I** is predominantly labelled by sodium [1-^{13}C]butyrate; a lower level of C-13 enrichment was observed at C-1, 3, 5, 11, 17, 21, and 23 (see Table 1), *i.e.* at carbons arising from C-1 of propionate[5]. With **II**, only sites derived from C-1 of propionate (now also C-15) were labelled. Sodium [1-^{13}C]*iso*-butyrate produced almost the same incorporation pattern

Table 1. Incorporation of sodium [1-^{13}C]butyrate and [1-^{13}C]*iso*-butyrate in monensin.

Carbon atom[a]	Monensin A			Monensin B		
	Chemical shift[b]	Enrichment factor[c]		Chemical shift[b]	Enrichment factor[c]	
		Butyrate	*iso*-Butyrate		Butyrate	*iso*-Butyrate
1	181.2	2.2	1.4	181.3	2.3	1.5
3	82.9	3.7	2.2	82.9	2.7	1.5
5	68.2	3.0	2.4	68.2	2.0	1.4
11	33.1	2.4	1.6	33.1	2.4	1.6
15	29.8	17.3	17.9	30.4	2.1	1.4
17	84.6	3.0	2.4	86.4	2.2	1.5
21	74.5	3.8	2.6	74.6	2.0	1.2
23	35.6	3.2	1.6	35.6	1.6	1.4

[a] Numbering according to reference 9. [b] ±0.06 ppm. [c] ratios between peak heights of the observed resonances of ^{13}C-enriched and natural abundance monensin from NMR spectra recorded under identical conditions (JEOL FX-60, 15.036 MHz, $CDCl_3$, 25°C).

Table 2. ^{13}C–^{13}C coupling observed in [1,2-$^{13}C_2$]-derived **I** and **II**.

Carbon atom[a]	Monensin A[d]		Monensin B[e]	
	Chemical shifts[b] (ppm)	$^1J(^{13}C$–$^{13}C)$[c] (Hz)	Chemical shifts[b] (ppm)	$^1J(^{13}C$–$^{13}C)$[c] (Hz)
7, 8	70.4, 33.1	37.4	70.4, 33.1	37.3
9, 10	107.0, 39.1	42.4	107.0, 39.2	43.0
13, 14	82.8, 27.2	34.8	81.5, 27.3	36.3
19, 20	33.5, 76.4	35.4	33.5, 76.4	33.5
25, 26	98.2, 64.8	46.1	98.2, 64.8	44.7
15, 16	29.8, 85.8	35.4		
30, 31	30.5, 8.2	34.3		

[a] Numbered according to reference 9. [b] δ-scale, $\pm$0.06 ppm. [c] measured with digital resolution 0.6 Hz, average value. [d] enrichment 60~65%. [e] enrichment 53~64%.

in **I** and **II** as seen for [1-^{13}C]butyrate. Using double labelled acetate (Table 2), we found that five intact acetate units are incorporated in monensin B but seven of them in monensin A. The additional carbons labelled in **I** are those of butyrate origin.

Successful incorporation of *iso*-butyrate in **I** apparently demonstrates a new metabolic pathway involving its isomerization to *n*-butyrate. The corresponding reaction mechanism might be the same as suggested by ŌMURA *et al.*[10] Both *iso*-butyrate and *n*-butyrate can be converted to propionate by *S. cinnamonensis* with retention of the carboxyl label. Such an effect has been already observed in lysocellin[11], tylosin[12], and probably also in lasalocid A[13]. Butyrate synthetase also participates on the biosynthesis of monensin A. This finding explains the higher than theoretical radioactivity found by DAY *et al.*[4] for [1-^{14}C]acetate-derived monensin A.

Our results show that despite the use of simple building blocks, the biosynthesis of monensin is complex owing mainly to the multiple relationships among them, these simple precursors.

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