THE BIOSYNTHESIS OF AR-5 (MYCINAMICINS) ANTIBIOTICS

Sir:

AR-5 (Mycinamicins) antibiotics, 16-membered ring macrolides, are produced by *Micromonospora* sp. These novel macrolides are potent antibiotics with unusual activity. Structures of these compounds were independently assigned by us and a group of Japanese workers.[1,2] The assignments of the signals in the ^{13}C NMR spectra have been previously reported[1]. In analogy with the origin of the carbon skeleton of the aglycone ring of rosaramicin[3,4] and tylosin[5], which are derived from two acetates, five propionates and one butyrate, we report here the biosynthesis of new 16-membered macrolide antibiotics.

For a typical incorporation study, *Micromonospora polytrota* (NRRL 12066, ATCC 31584) was inoculated into a medium containing starch (50 g), distiller's solubles (5 g) and Pharmamedia (5 g) in tap water (1 liter) and the initial pH was adjusted to 7.5. After cultivation for 48 hours at 30°C, the ^{14}C or ^{13}C labeled precursors, [1-^{13}C] Na-acetate, [2-^{13}C] Na-acetate, [1-^{13}C] Na-propionate, [1-^{13}C] Na-butyrate and L-[methyl-^{13}C] methionine were added to the culture. The fermentation was further continued for 72 hours and harvested. The harvested broth was adjusted to pH 8.5 and extracted twice with ethyl acetate. The ethyl acetate extract was washed with 5% acetic acid. The aqueous layer was removed, its pH adjusted to 8.5 and then extracted with toluene. The toluene extract contained a mixture of enriched AR-5 antibiotics, AR-5 #1 (mycinamicin I) and AR-5 #2 (mycinamicin II). For

Table 1. Incorporation of ^{14}C- and ^{13}C-labeled precursors.

	Percent ^{14}C[a]	Percent ^{13}C[b,c]
[1-*C] acetate	0.8	C-1, C-9, C11 0.2, 0.2, 0.3
[2-*C] acetate	1.6	C-2, C-10, C-12 0.4, 0.3, 0.4
[1-*C] propionate	8.8	C-3, C-5, C-7, C-13, C-15 0.6, 0.8, 0.6, 1.0, 1.0
[3-*C] propionate	8.6	—
L-[methyl-*C] methionine	30.4	OCH_3, OCH_3, $N(CH_3)_2$ 4.7, 4.9, 3.1

[a] Ethyl acetate extract, concentrated to dryness, reconstituted in $CHCl_3$ - CH_3OH (1 : 1) for radioactivity measurements.

[b] Calculated as natural abundance times the percentage increase in the intensity of the same carbon for enriched and natural abundance samples. The spectra were obtained under essentially identical conditions.

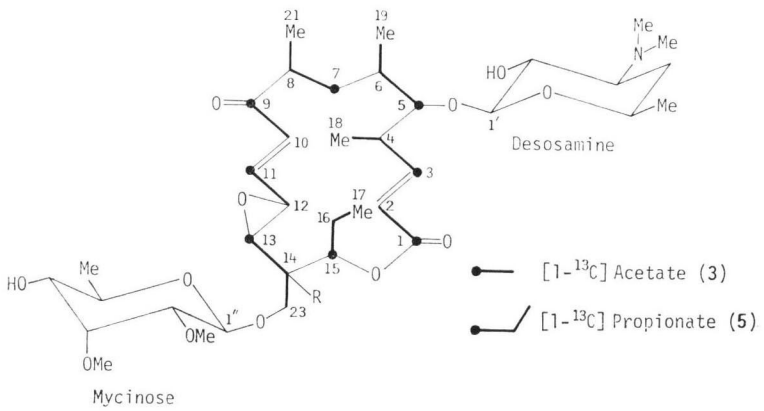
[c] One component only.

^{13}C NMR studies, further purification, on a silica-gel column using the solvent system toluene - methylene chloride - methanol - conc. ammonia (16: 4: 2: 0.1), was carried out to separate AR-5 #1 (mycinamicin I) and AR-5 #2 (mycinamicin II).

^{13}C NMR of the cold and the enriched antibiotic samples were recorded in $CDCl_3$ on a Varian XL-100-15 NMR spectrometer operating at 25.2 MHz. Percentage enrichments obtained from both components are presented in the Table 1.

When L-[methyl-^{13}C] methionine was added to the culture the dimethylamino and the methoxyl carbons of the desosamine and mycinose sugars,

Fig. 1. Biosynthesis of AR-5 antibiotics (R=H, AR-5 #1; R=OH, AR-5 #2)



respectively were strongly enriched. [1-^{13}C]-Butyrate was not incorporated into the aglycone ring. The addition of [1-^{13}C]acetate enriched carbons-1,-9 and -11, whereas, [2-^{13}C]acetate enriched carbons-2,-10 and -12. However acetate incorporation was poor and considerable distribution of the label was observed. The addition of [1-^{13}C]propionate resulted in enrichment of carbons-3,-5,-7,-13 and -15. Thus, unlike rosaramicin or tylosin which are made up of two acetates, one butyrate and five propionates, AR-5 macrolides are biosynthesized from three acetate and five propionate units. The differences lie in the absence of 22-Me and 20-CHO carbons in AR-5 and the fact that carbons-20,-19,-6 and -5 in rosaramicin and tylosin originate from a butyrate unit. The methyl carbons of the dimethylamino and methoxyl groups of desosamine and mycinose respectively, are derived exclusively from L-methionine.

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