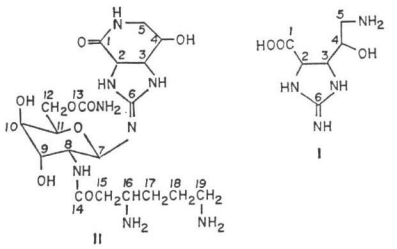
INCORPORATION OF CARBOXYL AND METHYL CARBON-13 LABELED ACETATES INTO RACEMOMYCIN A BY *STREPTOMYCES LAVENDULAE* ISP 5069

Sir:

In a previous study of biosynthetic pathways leading to the formation of racemomycin A, we observed that ^{14}C from (U-^{14}C)arginine was not located in the streptolidine moiety in this antibiotic, whereas the ^{14}C from (1-^{14}C)acetate was. However, we did not determine the distribution of labeled atoms in the metabolite. The availability of CMR spectroscopy prompted us to use ^{13}C-labeled acetate for this purpose.

Acetate was incorporated into racemomycin A as follows: Submerged cultures of *Streptomyces lavendulae* ISP 5069 were grown at 27°C on a reciprocal shaker (160 rpm) in an organic medium as described before.[2] In the first experiment, racemomycin A was labeled with sodium (1-^{13}C)acetate, and in the second with sodium (2-^{13}C)acetate (Merck Sharp and Dohme, Canada, 90 atom % ^{13}C), by adding an aqueous solution (1 ml containing 100 mg of acetate) to 100 ml of the broth after 4 hours and again after 68 hours of cultivation. Production of the antibiotic was depressed approximately 25% under these conditions. Racemomycin A was isolated by column chromatography on Amberlite IRC-50(Na^+), then on activated carbon[3] and several times on Sephadex LH-20[4]. Homogeneity of the antibiotic was examined by chromatography on Toyo-Roshi No. 51 paper with

Chart 1.



I

II

BuOH-pyridine-AcOH-H_2O-*tert.*-BuOH(15:10:3:12:4) as the solvent system and ninhydrin, RYDON-SMITH reagent and bioassay against *Bacillus subtilis* PCI 219 detecting agents.

The hydrolysate of racemomycin A hydrochloride (6 N HCl, 48 hours, 120~125°C, sealed tube) was applied to a cellulose column (1×28 cm) and developed with a solvent system of BuOH-pyridine-AcOH-H_2O-*tert.*-BuOH (75:50:191:236:548) to give two components, β-lysine and streptolidine.

Resonances of the six carbons in streptolidine (**I**) were assigned with the help of an off-resonance experiment and data from a spin-decoupling study reported by BORDERS *et al.*[5] The relative intensity of signals given by streptolidines at natural abundance and after enrichment from ^{13}C-labeled acetate are shown in Table 1. It is evident that (1-^{13}C) acetate was incorporated into the carbons at C-1 and C-5, and that (2-^{13}C) acetate was incorporated into C-2 and C-4, respectively. Contrary to expectation, the carbon at C-5 was enriched more than that of C-1 by $CH_3{}^{13}COO^-Na^+$. The carbons of β-lysine were

Table 1. Incorporation of ^{13}C-labeled acetates into streptolidine (**I**).

Assignment[a]	Chemical shift[b]	Relative intensities		
		Natural abundance	$^{13}CH_3COO^-Na^+$ labeled[c]	$CH_3{}^{13}COO^-Na^+$ labeled[d]
C-5	41.4	0.8	0.8	6.7
C-4	57.4	0.7	1.1	0.7
C-3	62.0	0.9	0.9	1.0
C-2	68.9	1.0	2.0	1.0
C-6	159.7	0.8	0.5	0.6
C-1	174.4	0.4	0.4	1.2

a) Numbering system for **I** is shown in Chart 1.

b) Spectra were recorded at a frequency of 20 MHz by means of a Varian CFT-20 spectrometer using deuterium oxide in solution. Chemical shifts were measured relative to the ^{13}C signal of dioxane and converted to ppm from TMS using δ (dioxane)=67.4 ppm.

c) 35 mg of the sample hydrochloride was measured.

d) 10 mg of the sample hydrochloride was measured.

Table 2. Incorporation of ^{13}C-labeled acetates into racemomycin A(II)*.

Assignment[a]	Chemical shift[b]	Relative intensities		
		Natural abundance	$^{13}CH_3COO^-Na^+$ labeled[c]	$CH_3{}^{13}COO^-Na^+$ labeled[d]
C-18	23.7 (23.7)	1.1	1.0	1.1
C-17	29.8 (29.8)	1.2	1.0	1.1
C-16	37.1 (36.5)	1.0	0.6	0.8
C-19	39.8 (39.7)	1.1	1.1	1.1
C-15	49.1 (48.6)	1.4	1.3	1.4
C-5	49.8	1.1	0.7	5.0
	50.1	0.9	0.7	0.7
	55.1	1.0	1.0	0.7
	61.1	1.1	1.2	1.2
C-4	61.6	1.0	3.0	0.8
	62.1	1.0	0.8	1.0
C-2	67.2[e]	—	—	—
	70.8	1.0	0.7	0.7
	74.3	1.0	1.0	0.8
C-7	79.8	0.8	0.6	0.8
C-6	158.8	0.5	0.5	0.5
C-13	163.5	0.6	0.4	0.4
C-1	170.7	0.4	0.4	0.9
C-14	172.8 (174.8)	0.8	0.8	0.7

* See footnote a and b, Table 1.

a) Some resonances of streptolidine and D-gulosamine have not yet been satisfactorily assigned.

b) ( ): Chemical shifts measured for β-lysine hydrochloride.

c) 70 mg of the sample hydrochloride was measured.

d) 20 mg of the sample hydrochloride was measured.

e) This peak overlapped the dioxane peak; the intensity is not measured.

not labeled.

CMR spectra of the two labeled samples of racemomycin A (II) were then compared with that of a sample at natural abundance (Table 2). The signals for four of the carbon atoms were enhanced by ^{13}C enrichment. Of these one at δ 67.2 ppm could be assigned to C-2 of the streptolidine moiety. Although it was partially superimposed on the dioxane peak the two signals were distinguishable, with a difference of δ 0.16 ppm. The other three signals could be assigned from their characteristic chemical shifts to C-1, C-4 and C-5 of the streptolidine moiety. This provides unambiguous evidence for the incorporation of two units of acetate.

The resonances of β-lysine[6,7], and the carbamoyl, guanido and carbonyl carbons were assigned, but, some of the resonances of streptolidine in its lactam form, and of carbamoyl D-gulosamine linked through glycosidic and amide bonds, have not yet been assigned.

KAWAMURA *et al.*[6] reported the effective use of CMR spectroscopy to identify the streptothricin antibiotics. We wish to emphasize also the value of this technique for biosynthetic studies. It has established that only two units of acetate are incorporated into racemomycin A by *S. lavendulae* ISP 5069, both of them in the streptolidine moiety. Streptolidine was reported to be synthesized from arginine by *S. noursei* JA 3890b[8] and *S. lavendulae* OP-2[9], and thus seems to be formed by at least two different pathways in the streptothricins.

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