BIOSYNTHESIS OF THE SPERMIDINE MOIETY OF GLYCOCINNAMOYLSPERMIDINE ANTIBIOTIC CINODINE

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We have reported in this journal[1] the biosynthesis of the glycocinnamoyl moieties of the antibiotic cinodine (Fig. 1), the broad-spectrum antibiotic discovered and identified at the Lederle Laboratories[2,3]. The glycocinnamoyl moieties were found to originate directly from D-glucosamine and L-tyrosine by isotope incorporation studies. The biosynthesis of the rest of the molecule, *i.e.*, the spermidine moiety was also studied.

Shaker-flask fermentations were carried out with *Nocardia* sp. strains LK-2558, LK-1034 and KL-5228 in media containing meat solubles and mineral salts as described in the previous paper. Carbon-14 labeled amino acids and amines were obtained from New England Nuclear Co.

A study comparing the incorporation efficiencies of arginine, citrulline, ornithine and putrescine was conducted at doses of 13 μmol which are about half of the total μmol of cinodine produced in 25 ml medium. Results shown in Table 1 indicate that all the amino acids involved in the urea cycle were incorporated into cinodine with good efficiencies. Ornithine and guanido-labeled arginine were incorporated to a degree comparable to tyrosine, the most efficiently incorporated amino acid studied previously. Citrulline and uniformly-labeled arginine were incorporated to a lesser degree than tyrosine. The high incorporation efficiency of [*guanido*-^{14}C]arginine indicated that the guanido carbon (C_6) in the molecule was readily taken up in the cinodine synthesis, probably present as the guanidine side chain in the hexose (Ring A) as well as the ureido groups in the pentoses (Rings B and C). Precedence of arginine being the donor of the guanidine side chain in antibiotics has been reported for streptomycin[4]. In two studies the ratio of incorporation efficiencies of guanido-labeled and uniformly-labeled arginine was found to be constant at about 1.4. The constant ratio indicates that in the biosynthesis of cinodine, the arginine molecule was metabolized to the guanido and ornithine portions and that the former was incorporated more efficiently than the latter, which has to go through decarboxylation to form putrescine before it is incorporated into the spermidine molecule. The L-ornithine generated *in situ* probably was used elsewhere in addition to the putrescine pathway.

Fig. 1. Cinodine (a glycocinnamoylspermidine antibiotic).

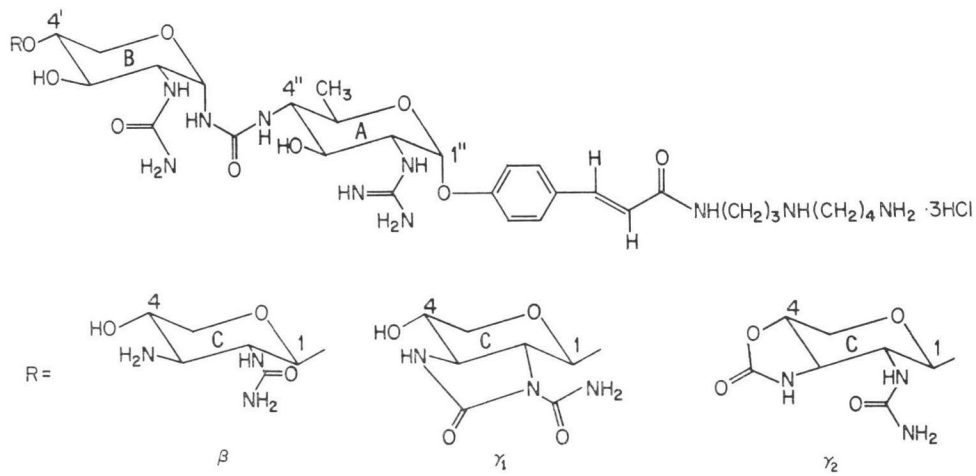

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Table 1. Incorporation of urea cycle-related compounds into cinodine.[a]

Compound	Dose/flask		% Incorporation
	mg[b]	μCi	
L-[*Guanido*-^{14}C]arginine·HCl	2.74	10.93	12.6
L-[*U*-^{14}C]arginine·HCl	2.74	10.85	8.66
L-[*Ureido*-^{14}C]citrulline	2.28	12.23	8.31
L-[*U*-^{14}C]Ornithine	2.19	9.53	12.2
[1,4-^{14}C]Putrescine·2HCl	2.05	11.00	4.44

[a] All compounds were added on the 3rd day after inoculation and cultures harvested on the 7th day.

[b] Amount corresponds to 13 μmol.

From Table 1, the efficiency of incorporation of C_6 in [*guanido*-^{14}C]arginine was 12.6%. If the same efficiency holds for C_6 of [*U*-^{14}C]arginine, the amount of carbon-14 incorporated due to C_6 would be one sixth of the total (1.37 μCi), or 0.228 μCi. Since the total carbon-14 yield observed in the incorporation of [*U*-^{14}C]-arginine was 0.94 μCi, the contribution from $C_{1\sim5}$, by difference, would be 0.712 μCi.

Since ornithine has to be decarboxylated before it can enter the cinodine molecule, it is assumed that C_1 does not contribute to the radioincorporation. Hence, only $C_{2\sim5}$ contribute to the yield of 0.712 μCi. Each carbon atom, therefore, was incorporated at 0.18 μCi, and the efficiency is calculated to be 9.95% with $C_{2\sim5}$ treated as one unit. The ratio of incorporation of C_6 and $C_{2\sim5}$ is therefore 12.59: 9.95 or 1.27, which was 87.6% of the experimental value (1.45).

The above calculations show that $C_{2\sim5}$ of arginine, which later made up the putrescine molecule, should incorporate at 9.95% if putrescine is generated *in situ*. Exogenous putrescine was incorporated at only 4.44%, *i.e.* 44.6% of the endogenous compound.

Results from Table 1 show that the ureido-labeled citrulline molecule was incorporated less efficiently than the arginine molecule. Preliminary results indicate that the labeling pattern with ureido-labeled citrulline may be identical to those obtained with guanido-labeled arginine. It is possible that citrulline is transaminated to arginine before incorporation. The complete labeling study and other related studies will be reported at a later date. Conversions of this nature, *i.e.*, from guanido- to ureido-function have been observed in microbial systems and are used to prepare citrulline from arginine[5].

Table 2. Incorporation yields of labeled compounds into cinodine[a].

Compound	μmol	μCi	% Incorporation
[*U*-^{14}C]Spermidine	25	9.0	5.1
[1,4-^{14}C]Putrescine·2HCl	25	9.1	0.71
L-[*U*-^{14}C]Methionine	13	10.2	11.9
[^{14}C]Urea	25	10.0	1.7
	25[b]	10.0	2.2
[1,2-^{14}C]Acetate,	50	10.6	1.74
sodium salt	100	10.6	0.92
[2-^{14}C]Propionate,	25	9.5	0.95
sodium salt	50	9.5	0.79

[a] All compounds were added on the 3rd day after inoculation and cultures harvested on the 7th day unless otherwise designated.

[b] Added on the 4th day and cultures harvested on the 7th day.

The effectiveness of other potential precursors for labeling the antibiotic are summarized in Table 2. Studies on L-methionine as a precursor showed that the molecule was incorporated at an efficiency of 12%, similar to that of L-ornithine. The efficient incorporation indicates that the synthesis of the spermidine portion in the cinodine molecule follows the usual polyamine synthesis pathway. That spermidine is an immediate precursor was also confirmed by a study in which about 25 μmol of spermidine was used and 5.13% was incorporated (Table 2).

Urea, which is the product of the urea cycle, has been studied similarly. Incorporation efficiency was only 20% of that observed for arginine in the same study. L-Proline, another five-carbon amino acid which is related to glutamic acid metabolism but more distantly to the urea cycle, was found to give very low incorporation efficiency (1.5%), suggesting that this compound is not directly involved in the biosynthesis of cinodine. Acetate and propionate were found to only poorly label the antibiotic.

References

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