p-AMINOPHENYLALANINE AND threo-p-AMINOPHENYLSERINE; SPECIFIC PRECURSORS OF CHLORAMPHENICOL[1]

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The structure of chloramphenicol (Fig. 1) suggests a biogenetic relationship to the phenylpropanoid amino acids. Vining and Westlake (1964) showed that chloramphenicol-producing cultures of Streptomyces sp. 3022a fed specifically labeled D-glucose yielded chloramphenicol and protein phenylalanine with a similar distribution of label in the C_6-C_3 skeletons. Shikimic acid, although poorly utilized by Streptomyces sp. 3022a, was efficiently incorporated into the p-nitrophenylserinol moiety of the antibiotic as well as into protein phenylalanine and tryosine. As reported earlier by Gottlieb et al. (1962) there was no conversion of the phenylpropanoid amino acids to chloramphenicol without prior degradation. These data suggest that, although the C_6-C_3 skeleton of chloramphenicol is assembled via the shikimic acid pathway, a branch point occurs prior to completion of the amino acids. Evidence presented in this paper establishes L-p-aminophenylalanine and threo-p-aminophenylserine as specific precursors of the p-nitrophenylserinol moiety of chloramphenicol.

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MATERIALS AND METHODS - Streptomyces sp. 3022a (Upjohn Culture collection No. UC 2374) was used in experiments where glycerol-1,3-^{14}C, D-glucose-2-^{14}C, and L-p-aminophenylalanine-α-^{14}C were administered. Streptomyces venezuelae (Parke Davis culture collection No. 04828) was used in the remaining experiments. The composition of the media and methods of cultivation were similar to those reported by Sala and Westlake (1966). Techniques for administering radioactive compounds to growing cultures and determining their incorporation into chloramphenicol have been described (Vining and Westlake, 1964). Methods used to synthesize specifically labeled compounds will be described elsewhere. Atom percent excess ^{15}N was measured with a Consolidated Electrodynamics Corporation Model 21-620A mass spectrometer after Dumas combustion of the sample (Barsdate and Dugdale 1965) and calculated as described by Rittenberg (1946).

RESULTS - In our previous work (Vining and Westlake, 1964) D-glucose-2-^{14}C was the most efficient precursor of chlorampehnicol tested and radioactivity resided mainly in the p-nitrophenylserinol moiety. L-p-Aminophenylalanine-α-^{14}C and DL-p-aminophenylserine-carboxyl-^{14}C are now shown to be superior to D-glucose-2-^{14}C (Table I). Degradation of the chloramphenicol produced from these precursors located all of the radioactivity in the aminomethine and hydroxymethyl carbons respectively of the p-nitrophenylserinol moiety. Incorporation of p-aminophenylalanine entirely via the α-oxo acid is excluded since both isotopes of L-p-aminophenylalanine-α-^{14}C; α-^{15}N were incorporated (Table II). Further evidence that L-p-aminophenylalanine is a true intermediate in chloramphenicol biosynthesis was obtained from a trapping experiment. Carrier p-aminophenylalanine (35 mg) was added to the soluble fraction extracted with hot water from the mycelium of a 5 day old culture grown in the presence of glycerol-1,3-^{14}C (82.8 μc; 0.77 μc/mmole). Amino acids were separated by chromatography on a column of anion exchange resin (Dowex-1, acetate form), then fractionated by partition chromatography on

TABLE I

Incorporation of radioactivity into chloramphenicol from ^{14}C-labeled compounds administered to cultures of Streptomyces sp.

Compound Administered		Chloramphenicol		
	μc/mmole	μc/mmole	Dilution[1]	Percent Incorporation
D-Glucose-2-^{14}C	14.9	0.456	34.8	1.09
L-p-Aminophenylalanine-α-^{14}C	6.60	3.50	1.9	19.7
DL-p-Nitrophenylalanine-α-^{14}C	11.5	0.015	767	0.09
DL-threo-p-Aminophenylserine-carboxyl-^{14}C	7.60	0.870	8.7	4.03
DL-erythro-p-Aminophenylserine-carboxyl-^{14}C	7.60	0.128	59.4	0.48
DL-threo-p-Nitrophenylserine-carboxyl-^{14}C	9.00	0.024	375	0.18
D-threo-p-Aminophenylserinol-hydroxymethyl-^{14}C	6.93	0.002	3,470	0.03
D-threo-p-Nitrophenylserinol-hydroxymethyl-^{14}C	10.2	0.023	449	0.13

[1]Specific activity of precursor - specific activity of chloramphenicol.

sheets of Whatman No. 3 MM paper. p-Aminophenylalanine (6.3 mg) eluted from the chromatograms and rechromatographed to constant specific activity was radioactive (60 mμc/mmole). Assuming that p-aminophenylalanine present in the mycelium had a specific activity at least as high as that of chloramphenicol (1.56 μc/mmole) produced in this experiment the minimum concentration of amino acid was 710 μg per g of dried mycelium.

TABLE II

Dilution of ^{14}C and ^{15}N in chloramphenicol from cultures fed L-p-aminophenylalanine-α-^{14}C; α-^{15}N

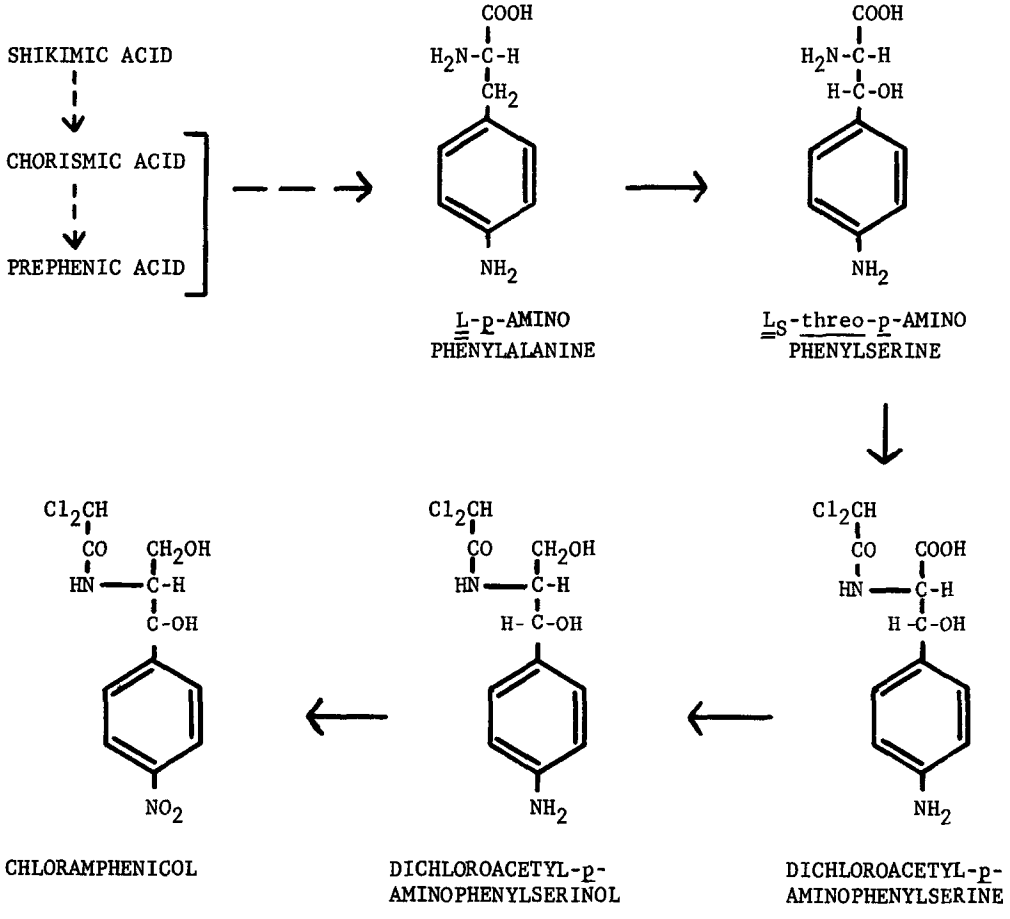
p-Aminophenylalanine					
^{14}C	^{15}N	^{14}C		^{15}N	
μc/mmole	Atom % Excess	μc/mmole	Dilution	Atom % Excess	Dilution
12.3	57.4	3.49	3.52	9.1	6.3

The poor incorporation of radioactivity from DL-p-nitrophenylalanine-α-^{14}C into chloramphenicol suggests that oxidation of the p-amino group is not the next step in the biosynthetic pathway. Since DL-threo-p-amino-phenylserine-carboxyl-^{14}C was incorporated with low dilution of specific activity a trans β-hydroxylation of L-p-aminophenylalanine is the more probable reaction. The small incorporation of erythro-amino acid is attributed to the presence of some threo-isomer in the preparation used. L-threo-p-Amino-phenylserine has the same configuration at both asymmetric centers as chloramphenicol.

Although extensively metabolized DL-threo-p-nitrophenylserine-carboxyl-^{14}C was also a poor precursor of chloramphenicol. This evidence, together with the absence of the p-nitro substituted amino acid in cultures to which ^{14}C-labeled DL-threo-p-aminophenylserine was fed,

FIGURE 1

HYPOTHETICAL PATHWAY FOR BIOSYNTHESIS OF CHLORAMPHENICOL



indicate that further modification of the propanoid moiety precedes oxidation of the p-amino group. Reduction of the carboxyl group of p-aminophenylserine as the succeeding step in the pathway is rendered unlikely by the poor incorporation of ^{14}C-labeled D-threo-p-amino- and p-nitrophenylserinol. The remaining possibility is acylation of the α-amino group. Identification of α-N-Dichloracetyl-Ls-p-aminophenylserinol as a trace metabolite in cultures of a chloramphenicol-producing strain of Streptomyces venezuelae (Stratton and Rebstock, 1963) suggests that oxidation of the p-amino group may be the terminal reaction and the

pathway shown in Fig. 1 is put forward as the most probable route for chloramphenicol biosynthesis.

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