CONFIGURATION OF THE N-METHYLISOLEUCINE IN THE ACTINOMYCINS

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SUMMARY: The addition of L-isoleucine, D-isoleucine or L-alloisoleucine to the culture medium of Streptomyces antibioticus or S. chrysomallus led to the synthesis of new actinomycins. In all three cases hydrolysates of the actinomycin mixtures contained N-methylalloisoleucine. Evidence revealed that the N-methylamino acid possesses the L-configuration at the α-carbon.

When DL-isoleucine was provided to actinomycin-producing Streptomyces, new antibiotic components were formed (1-3). It was reported that the organism synthesized N-methylisoleucine which replaced N-methylvaline in the actinomycin peptides (1). The stereochemical configuration of the N-methylisoleucine in actinomycin has not been determined previously. Since commercial samples labeled as DL-isoleucine have frequently been found to be a mixture of L-isoleucine and D-alloisoleucine (4), it was decided to re-examine this question employing the four diastereoisomers of isoleucine.

Experimental: L-alloisoleucine was kindly supplied by Dr. M. Rabinowitz and L-isoleucine as well as D-alloisoleucine were obtained from Dr. T. Otani, both of the National Institutes of Health. D-isoleucine was purchased from Sigma Chemical Corp., St. Louis, Missouri. All four compounds were found to be homogeneous by the criteria of paper chromatography, high voltage electrophoresis and amino acid analysis on the automatic amino acid analyzer.

S. antibioticus, Strain 3720, has been employed previously (2,3,5,6); S. chrysomallus NRRL 2250 was provided by Dr. Alex Ciegler and Dr. T.G. Pridham of the Northern Utilization Research and Development Division, U.S.D.A. Conditions for cultivation of these organisms in glutamic acid-galactose-mineral salts medium have been described elsewhere (3,5,6). One of the four diastereo-

isomers of isoleucine (100 to 250 μg per ml) was provided to a given culture after 16 to 24 hrs incubation. Following an additional period of cultivation, the actinomycin mixture synthesized was isolated, purified, and hydrolyzed in 6 N HCl (6). The hydrolysates were examined by high voltage electrophoresis (6) (Gilson Mfg. Co., 4% formate, 3 hrs, 4800 volts) or by a combination of high voltage electrophoresis in one dimension and ascending paper chromatography (butanol:acetic acid:water, 4:1:5) in the second dimension. The electrophoretic mobility of various amino acids relative to that of sarcosine (1.00) was: D-valine, 0.90; L-isoleucine, 0.87; L-threonine, 0.83; L-proline, 0.80; N-methyl-DL-valine, 0.72; N-methyl-DL-isoleucine, 0.71, and N-methyl-DL-alloisoleucine, 0.68. R_f values were: sarcosine, 0.33; L-threonine, 0.34; L-proline, 0.42; D-valine, 0.54; D-isoleucine, 0.63; N-methyl-DL-valine, 0.60; N-methyl-DL-isoleucine, 0.67; N-methyl-DL-alloisoleucine, 0.68.

Results: When L-isoleucine, D-isoleucine, or L-alloisoleucine was supplied to either S. antibioticus or S. chrysomallus, hydrolysates of actinomycin mixtures were found to contain N-methylalloisoleucine (Fig. 1). This amino acid was absent from hydrolysates under standard incubation conditions or when D-alloisoleucine was provided to the cultures. The N-methylalloisoleucine (actinomycin) coelectrophoresed and cochromatographed with an authentic sample of the amino acid. N-Methylvaline and N-methylisoleucine did not separate by means of high voltage electrophoresis. However, they were resolved by high voltage electrophoresis followed by paper chromatography in the second dimension. It was thus possible to ascertain that N-methylalloisoleucine and not N-methylisoleucine was present in actinomycin hydrolysates. In addition, an unidentified ninhydrin-positive compound was detected in the actinomycins produced in the presence of L- or D-isoleucine or L-alloisoleucine. The compound had the same electrophoretic mobility as isoleucine. Further study will be required to establish its identity.

The actinomycin mixture (ca 100 mg), produced by S. antibioticus in the presence of D-isoleucine, was hydrolyzed in 6 N HCl. The hydrolysate was

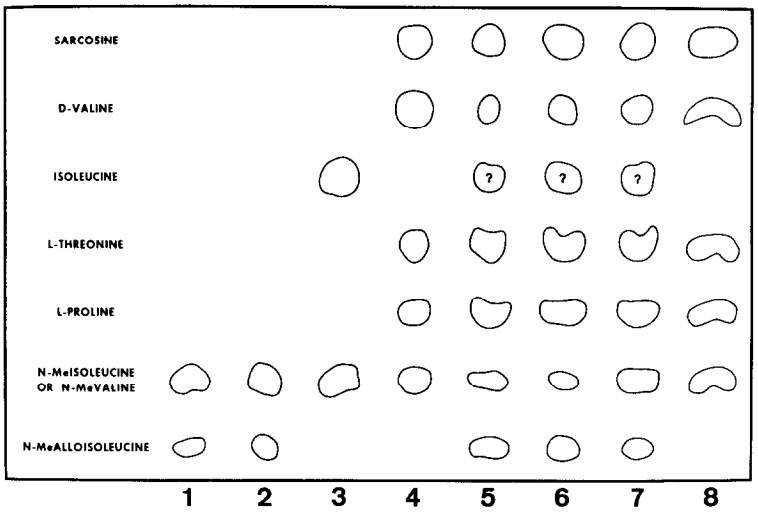

FIG. 1: Diagram of actinomycin hydrolysates (S. antibioticus) resolved by high voltage electrophoresis as described in the text. Standards: 1. N-methyl-DL-alloisoleucine and N-methyl-DL-valine, 2. N-methyl-DL-alloisoleucine and N-methyl-DL-isoleucine, 3. N-methyl-DL-valine and N-methyl-DL-isoleucine (not separated) and D-isoleucine.

Hydrolysates of actinomycin mixtures produced in glutamic acid-galactose-mineral salts medium containing: 4. No addition, 5. L-isoleucine, 6. L-alloisoleucine, 7. D-isoleucine, and 8. D-alloisoleucine. The ninhydrin-reactive compound (?) may be isoleucine.

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subjected to preparative paper chromatography (Toyo Roshi No. 525, 30 x 60 cm) with the solvent system; N-butanol:acetic acid:water (4:1:2) by descending chromatography for 15 hrs. Amino acids were detected with ninhydrin and p-nitrobenzoyl chloride (for N-methylamino acids). Zone 1 (containing methyl-alloisoleucine) and zone 2 (containing methylvaline and valine) were cut out and extracted with acidic aqueous methanol. The respective extracts were then subjected to ion-exchange resin chromatography carried out on a Dowex 50 x 4 (200 to 400 mesh) column, 1.66 x 90 cm, with 0.2 M pyridine-acetic acid buffer, pH 3.5. Methylalloisoleucine from the extract of zone 1, and methyl-valine and valine from zone 2 were separated. The respective fractions were adsorbed on a Dowex 50 x 8 (NH_4 type) column and eluted with 1 N NH_4OH. Lyophilization of the respective eluates yielded methylalloisoleucine (650 μg) methylvaline (550 μg) and valine (250 μg) as analytically pure preparations.

Identification of methylalloisoleucine was made by a mixed run on an amino acid analyzer with an authentic specimen (Aminex A-4 column, 75 x 0.8 cm, 0.2 M sodium citrate buffer, pH 3.25, rate 0.49 ml/min). A sample of methylalloisoleucine (actinomycin) 210 μg, was mixed with methylalloisoleucine (authentic) 425 μg. A single peak was found at a retention time of 3 hrs, 38 min. The optical rotary dispersion in 0.5 N HCl was determined with methylalloisoleucine ($[\phi]_{260}$ + 781, $[\phi]_{226}$ + 3795 (peak), $[\phi]_{214}$ 0) (e, 0.0650, 0.5 N HCl). The positive Cotton effect indicated that the amino acid possesses the L-configuration at the α-carbon (7). It is concluded that both N-methyl-L-alloisoleucine and N-methyl-L-valine are present in actinomycins produced by S. antibioticus in the presence of D-isoleucine. The former amino acid was first shown to be a constituent of the quinoxaline antibiotics (8).

Discussion: The evidence suggests that N-methyl-L-alloisoleucine is synthesized when either L-isoleucine, D-isoleucine or L-alloisoleucine is provided in the culture medium. This possibility has only been established in the case of D-isoleucine. As D-isoleucine shares a common keto acid intermediate with L-alloisoleucine, it is conceivable that D-isoleucine is oxidatively deaminated to yield l-α-keto-β-methylvaleric acid which gives rise to L-alloisoleucine by a transamination reaction (Fig. 2). The direct methylation of L-alloisoleucine would yield N-methyl-L-alloisoleucine. The conversion of L-isoleucine to L-alloisoleucine is more complex. Oxidation or transamination of L-isoleucine would lead to synthesis of d-α-keto-β-methylvaleric acid. The keto acid may then racemize by enolization to the dl-keto acid; on transamination this would give a mixture of L-isoleucine and L-alloisoleucine (9). Methylation appears to be specific only for the L-alloisoleucine. Meister (9) observed that Lactobacillus arabinosus grew, in an otherwise complete medium, almost as well on L-alloisoleucine as L-isoleucine. Hydrolysates of the cell protein contained L-isoleucine in both cases. The enzymatic conversion of L-alloisoleucine was not demonstrated directly. The conversion of L-alloisoleucine to L-isoleucine by L. arabinosus was considered to pro-

ceed as shown in Fig. 2. By analogy we suggest that the synthesis of N-methyl-L-alloisoleucine from L-isoleucine, D-isoleucine or L-alloisoleucine may take place as outlined (Fig. 2).

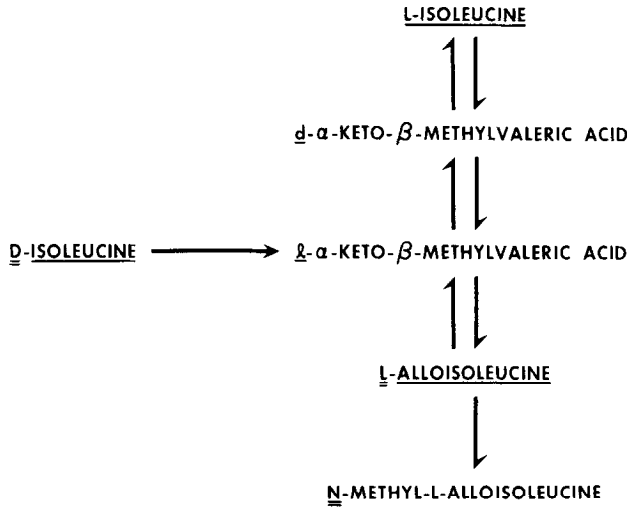

FIG. 2: Possible mechanism for synthesis of N-methyl-L-alloisoleucine.

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