The accumulation of indole-3-glycerol by tryptophan auxotrophs of *Escherichia coli*

In previous studies from this laboratory[1] an unidentified indole derivative was discovered as a product which accumulated in the culture fluids of several tryptophan-requiring mutants of *Escherichia coli*, strain B. Substances with similar properties were subsequently and independently described in studies with tryptophan auxotrophs of other strains of *Escherichia coli*[2-4], *Salmonella typhimurium*[5] and *Glomerella cingulata*[6]. On the basis of nutritional and metabolic patterns of the mutants, the substance was implicated as a possible intermediate in the conversion of anthranilic acid to indole. The discovery by YANOFSKY[7] of indole-3-glycerol phosphate as an intermediate in the enzymic conversion of anthranilic acid has offered a clue for the identification of the accumulated product. In confirmation of YANOFSKY's suggestion[7], the compound has been identified as indole-3-glycerol.

The compound was isolated from cultures of strain B-42, a tryptophan auxotroph of *E. coli* which could also use indole, but not anthranilic acid, for growth. The organism was grown with aeration for 3 days in 6 l of a mineral salts-glucose medium containing tryptophan (2 μg/ml) and anthranilic acid (10 μg/ml). After removal of the bacteria by centrifugation, the compound was removed from the fluid by adsorption to charcoal (Darco 20 × 40). The charcoal was washed copiously with water and then with 10 and 25% ethanol. Elution from the charcoal was achieved with 95% ethanol. Following concentration to a small volume, the compound was precipitated by the slow addition of water and chilling. Repeated precipitations from alcoholic solution by water eventually yielded a chromatographically pure tan powder. Analysis gave the following percentages: C, 64.9; H, 6.53; N, 6.73—calculated for $C_{11}H_{13}NO_3$: C, 63.7; H, 6.28; N, 6.75.

Identification was based on the reported properties for indole-3-glycerol phosphate[7] and on a comparison with authentic indole-3-glycerol which was obtained by the action of alkaline phosphatase on a sample of the phosphate (generously supplied by Dr. YANOFSKY). The following properties served as criteria for identification.

1. *Ultraviolet absorption:* Maximum at 280 mμ; minimum at 240 mμ with optical density 30% that of maximum; optical density ratios, 290/260 = 1.1, 260/280 = 0.62; a narrow shoulder in the range of 270–276 mμ and a small secondary peak at 287 mμ.

2. *Color reactions*: Violet with xanthydrol; red-purple with $FeCl_3$ reagent[8]; red with concentrated HCl (1:1); and blue with BRATTON-MARSHALL[9] reagents for diazotizable amines (maximum absorption of color at 625 mμ).

3. *Hydrolysis:* Alkaline hydrolysis (0.1 *N* NaOH) yielded indole and acid hydrolysis (0.1 *N* HCl) completely destroyed the compound.

4. *Periodate oxidation* yielded indole-3-aldehyde which was identified by ultraviolet absorption, paper chromatography and the urorosein reaction[10].

5. *Paper chromatography:* R_F, 0.93 in methanol, ethyl acetate, water (1:2:1); 0.85 in butanol saturated with water; and 0.71 in *iso*propanol, ammonia, water (70:1:1).

Of the eight independently isolated mutants which have been found to accumulate indole-3-glycerol, half of these (strains B-42, B-47, B-48 and B-110) will grow on indole as well as tryptophan and the others (strains B-49, B-81, B-84 and B-88) will respond only to tryptophan.

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