Isolation of 6,7-Dimethyl-8-ribityl Lumazine from a Riboflavineless Mutant of *Aspergillus nidulans*

Since the production of riboflavine in mammals is dependent on the intestinal microflora, the biosynthesis of riboflavine has been studied mostly in microorganisms. The successful isolation of a pair of pteridine derivatives, 6,7-dimethyl-8-ribityl lumazine and 6-methyl, 7-hydroxy-8-ribityl lumazine, in the flavinogenic microorganisms *Eremothecium ashbyii*[1], *A. gossypii*[2], and in others, have been reported and confirmed by isotopic labelling studies. However, the exact mechanism of conversion of these compounds to riboflavine remains to be established. PLAUT[3] has postulated that the additional four carbon unit needed in the riboflavine molecule comes from the additional molecule of 6,7-dimethyl-8-ribityl lumazine. PANICKER and SHANMUGASUNDARAM[4] have shown, using *Aspergillus nidulans* mutants deficient in riboflavine, that this lumazine compound first decomposes to give 4-ribityl-amino, 5-amino uracil and is the more immediate precursor of the vitamin, and that the two compounds are interconvertible, being in equilibrium with each other in the system.

The present note deals with the identification of the more stable 6,7-dimethyl-8-ribityl lumazine in the cultures of a riboflavine-requiring mutant of *Aspergillus nidulans*. The compound 6,7-dimethyl-8-ribityl lumazine isolated from the cultures of *E. ashbyii* and *A. gossypii* fluoresces green under UV-light and its chemical structure has been tentatively established by comparison with a pure synthetic compound.

One of the 5 non-allelic riboflavineless mutants of *Aspergillus nidulans*, designated as $ribo_1$, with green spore colour was grown in riboflavine supplemented minimal medium which consisted of the following composition: $NaNO_3$ 6.0 g, KH_2PO_4 1.52 g, KCl 0.52 g, $MgSO_4\,7H_2O$ 0.52 g, glucose 20.0 g, with traces of iron and zinc and 1.0 mg riboflavine/l. The pH was adjusted to 6.4. 2 l of this medium were sterilized and inoculated with the spore suspension of the mutant and incubated at 37°C for ten days. At the end of the incubation period, the cultures were concentrated by vacuum distillation to about 5 ml and chromatographed on acid alumina containing N-butanol and absolute ethanol using the method of PLAUT[5]. The sample was made up in a solvent composed of 1 ml water, 9 ml ethanol and 10 ml N-butanol, then allowed to pass through the adsorbent and the column was washed by an additional 10 ml of the solvent. Riboflavine was eluted from the column with 50 ml of the solvent system containing 80 ml of N-butanol and 28 ml each of ethanol and water, and 6,7-dimethyl-8-ribityl lumazine was eluted using the solvent containing 80 ml N-butanol, 28 ml ethanol and 56 ml water. The column was then washed with 50 ml of 50% (v/v) ethanol and 50 ml of 0.03 *M* ammonium hydroxide to elute 6-methyl, 7-hydroxy-8-ribityl lumazine. The eluates were concentrated to about 1 ml by per-evaporation at 3° to 5°C in a cold room. They were spotted on Whatman No. 3 filter paper along with authentic samples and chromatographed, using the solvent butanol-ethanol-water (50:15:35), and were examined under UV-light. The eluted spot corresponding to the green fluorescing authentic sample of 6,7-dimethyl-8-ribityl lumazine, having an Rf value of 0.26, was found to have an identical absorption spectrum in the UV-region in a Unicam SP-700 spectrophotometer. Violet spot for the eluted 6-methyl, 7-hydroxy-8-ribityl lumazine could not be detected. This is the first observation wherein 6,7-dimethyl-8-ribityl lumazine has been isolated from a riboflavine-requiring mutant[6].

Zusammenfassung. Die Gegenwart von 6,7-Dimethyl-8-ribityl-Lumazin, einem Vorläufer des Riboflavins in seiner Biosynthese, ist im Kulturfiltrat einer riboflavinlosen Mutante von *Aspergillus nidulans* festgestellt worden.

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Cation Transport in Normal Human Red Cells Treated with Sulfhydryl Compounds

Some of us have recently observed[1] that treatment of normal human red cells with AET (2-amino-ethylisothiouronium bromide) or cysteine, under suitable experimental conditions, modifies them in such a way that their behaviour in some in vitro hemolysis tests becomes similar to that of paroxysmal nocturnal hemoglobinuria (PNH) erythrocytes. The effect is supposedly due to the –SH groups that both substances possess. So far, a few enzymatic properties of these treated red cells have been investigated and related to those of PNH erythrocytes[2]. Since in vitro studies with K^{42} have recently shown[3] that PNH red cells have normal K^+ influx and efflux rates, we have investigated whether the treatment with AET or cysteine modifies the cation transport of normal human erythrocytes. The blood of seven healthy adults was drawn with heparin; the red cells were separated by centrifugation and washed thrice with saline. An aliquot was treated with the above-mentioned sulfhydryl compounds as previously described[1]. (The Ham's test was performed on each sample to ascertain that the PNH-like behaviour had been achieved.) Another aliquot was similarly

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