Synthesis of 6-Methyl-7-hydroxy-8-ribityllumazine and its Identification with a Blue-violet Fluorescent Substance from *Ashbya gossypii*[1]

During a search for possible intermediates of riboflavin biosynthesis, 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine was condensed with various keto and hydroxy compounds, and carboxylic acids. One such product, 6,7-dimethyl-8-ribityllumazine, was found to be identical with a green fluorescent compound present in the riboflavin-producing organism *Ashbya gossypii* (1). Addition of formate-C^{14} to growing cultures of *A. gossypii* leads to strong labeling of carbon-2 of 6,7-dimethyl-8-ribityllumazine. Studies of the kinetics of incorporation of label from simple organic substances (e.g., formate-C^{14}, glycine-C^{14}) into 6,7-dimethyl-8-ribityllumazine and riboflavin make it likely that the lumazine derivative is an intermediate compound in riboflavin biosynthesis. In these investigations it was also observed that a pronounced incorporation of label from formate-C^{14} occurred into a compound which fluoresces blue-violet. This substance is light-sensitive, and loses fluorescence upon reduction with sodium dithionite; the fluorescence can be restored upon oxidation with molecular oxygen. A compound was prepared by the chemical condensation of 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine (I) and pyruvic acid (II) (or ethyl pyruvate) (Fig. 1) corresponding in all of these properties, in paper chromatography (Table I), and light-absorption spectra (Fig. 2), to the isolated natural compound.

The following characteristics of light absorption have been observed with the synthetic and natural compounds: in 0.1 *N* H_2SO_4, minima at 248 mμ and 299 mμ, maxima at 281 mμ and 326 mμ, molar absorbancy at 329 mμ is 12,000; in 0.1 *M* phosphate buffer at pH 6.7, minima at 266 mμ and 305 mμ, maxima at 289 mμ and 345 mμ, molar absorbancy at 345 mμ is 13,000; in 0.1 *N* NaOH, minima at 273 mμ and 307 mμ, maxima at 287 mμ and 350 mμ, molar absorbancy at 350 mμ is 13,000. In all cases there is pronounced end absorption at wavelengths shorter than 240 mμ.

The isolation of the natural compound will be described elsewhere. The preparation of the synthetic material is as follows: a solution of 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine (I) (2.2 ml.) prepared from 100 mg. of 4-ribitylamino-5-nitroso-2,6-dihydroxypyrimidine (1) is mixed with 4 ml. of a solution of 700 mg. of sodium pyruvate (II). The reaction mixture is adjusted to pH 4 and heated at 80° for 20 min. The reaction mixture is acidified with formic acid and passed through a column (1 × 14 cm.) of coarse (<30 mesh) Nuchar C. The column is washed with water and the

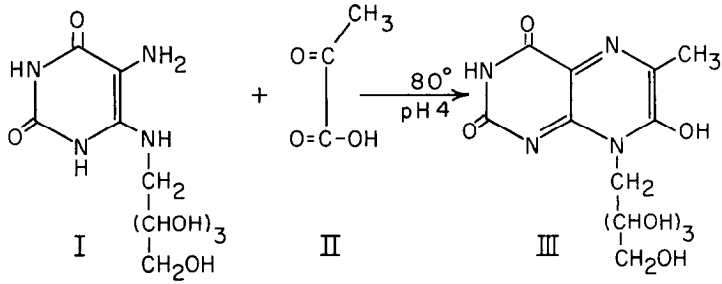

FIG. 1. The chemical synthesis of 6-methyl-7-hydroxy-8-ribityllumazine.

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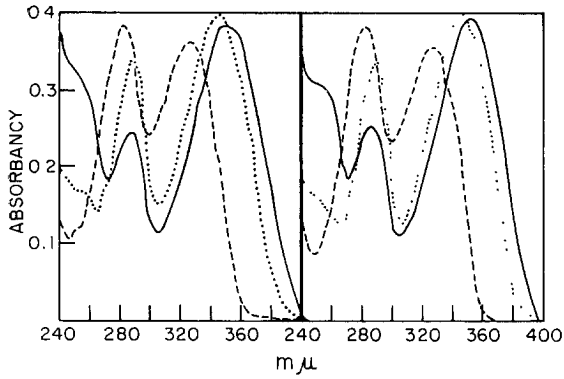

FIG. 2. Absorption spectra of 6-methyl-7-hydroxy-8-ribityllumazine. The graph on the left is the *synthetic* product and on the right the *natural* material, both solutions contained 10 μg. per ml. —— 0.1 *N* NaOH; . . . 0.1 *M* phosphate, pH 6.7; - - - 0.1 *N* H_2SO_4

TABLE I

Comparison of Natural and Synthetic 6-Methyl-7-hydroxy-8-ribityllumazine by Paper Chromatography

Solvent	R_f	
	Natural compound	Synthetic compound
Isopropyl ether–formic acid (6:4)	0.15	0.15
Isobutyric acid–1 *N* NH_4OH (6.3:3.7)	0.35	0.33
Butanol–ethanol–H_2O (5:1.5:3.5)	0.21	0.21
t-Butanol–NH_3–H_2O (6:0.5:3.5)	0.30	0.28
n-Butanol–acetic acid-H_2O (4:1:5)	0.12	0.10
H_2O saturated with isoamyl alcohol	0.91	0.90

substance is eluted with 30% aqueous pyridine. The eluate is concentrated to dryness and the substance is crystallized and recrystallized from 80% ethanol. Yield: 34 mg. of 6-methyl-7-hydroxy-8-ribityllumazine (III); m.p. 267° (dec.).

The synthetic material is probably also identical with the "V" substance which has been isolated from *Eremothecium ashbyii* by Masuda (2). Although the structure shown by chemical synthesis here is not in agreement with that first proposed for the "V" compound (3), it is consistent with Masuda's recent proposal for this substance (4).

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Influence of Dietary Fat on Oxidation of Cholesterol by Liver Mitochondria[1]

The number of reports concerning the serum cholesterol lowering effect of unsaturated fat in man have prompted investigations into the effects of these fats on cholesterol metabolism. Balance studies in man have indicated increased fecal excretion of bile acids (1) and of sterols (2) on diets high in unsaturated fat.

Rats fed unsaturated fat for relatively long periods of time synthesize cholesterol at a greater rate than do animals fed saturated fat (3–5), although no difference is observed after feedings of three-day duration (6).

Bile acids are the major products of cholesterol catabolism (7) and the liver is the apparent site of this degradation (8). We have, therefore, studied the influence of dietary fats upon the oxidation of cholesterol by liver mitochondria.

The substrate used was cholesterol-26-C^{14} since *in vitro* experiments have demonstrated that oxidation of the terminal carbon atoms of the cholesterol side chain to carbon dioxide accompanies normal catabolism to bile acids (9, 10).

In our experiments three groups of Wistar rats were fed diets consisting of rabbit chow (normal), rabbit chow plus 20 per cent of a commercially available shortening (relatively saturated, iodine number 70) and rabbit chow augmented with 20 per cent corn oil (unsaturated, iodine number 130). The rats were allowed unlimited access to the diet. After 40 days the animals were killed and a liver mitochondrial preparation was made (11). The mitochondrial preparation (1 ml.) was mixed with 1 ml. of a solution containing ATP (25 mg.), DPN (5 mg.), AMP (8 mg.), glutathione (15 mg.), penicillin G (2,000 U) and streptomycin phosphate (1 mg.) and to this were added 5 ml. of boiled supernatant fluid and 5 ml. of a cholesterol-26-C^{14} emulsion in 0.25 *M* Tris HCl (pH 8.5). The supernatant fluid from a whole liver homogenate was mechanically freed of floating fat before boiling to precipitate proteins. The resulting suspension was incubated at 37°C. for 18 hr. and the evolved $C^{14}O_2$ was trapped in alkali, precipitated as $BaC^{14}O_3$ and counted as a thixotropic gel in a Tri-Carb Liquid Scintillation Counter. In the absence of mitochondria no oxidation of cholesterol-26-C^{14} was observed after 18 hr. of incubation. Results of five runs are tabulated in Table I.

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