U.D.C. 547.854.4'861.2 : 582.284

7. Toru Masuda : Application of Chromatography. XXXI*. Structure of a Green Fluorescent Substance produced by *Eremothecium ashbyii*.

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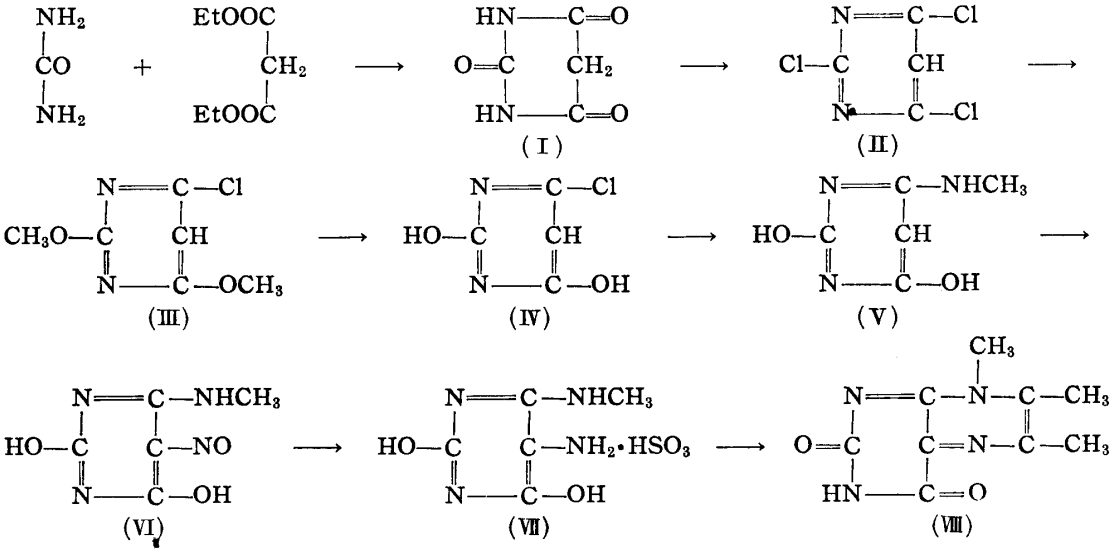
In the Part XXIX[1] of this series the author assumed that the green fluorescent substance (G compound) obtained from the mycelium of *Eremothecium ashbyii* might be an intermediate of riboflavin. From the fact that one of the photodecomposition products of G compound closely resembles 6,7-dimethyllumazine (6,7-dimethyl-2(1*H*),4(3*H*)-pteridinedione) and from other experimental results, the structure of 6,7-dimethyl-8-ribityllumazine was assigned to this substance. Thereafter, 6,7,8-trimethyllumazine was synthesized, and as the ultraviolet spectrum of the product was in complete accord with that of G compound, the site of the substituent in G compound was confirmed to be the nitrogen at 8-position.

6,7,8-Trimethyllumazine was synthesized by the following route.



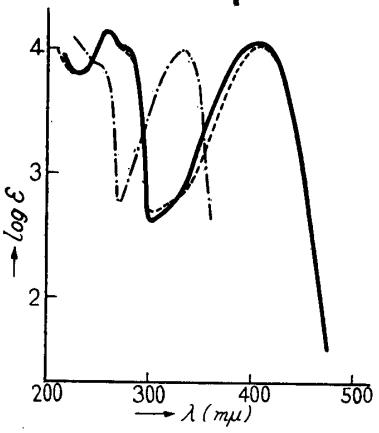
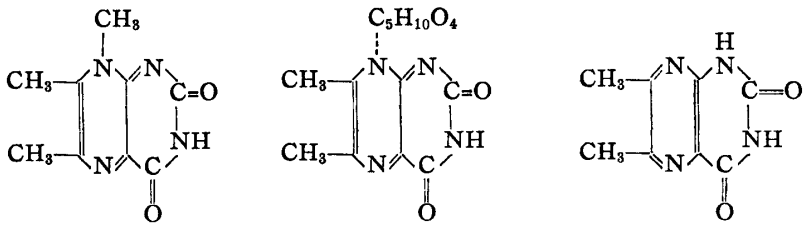


Fig. 1. Ultraviolet Absorption Spectra

6,7,8-Trimethyllumazine G Compound 6,7-Dimethyllumazine



λmax 258, 407 mμ λmax 258, 407 mμ λmax 250, 330 mμ

6,7,8-Trimethyllumazine, m.p. 300~301°(decomp.), thus obtained is readily soluble in ethanol, methanol, and pyridine, and its solution exhibits a strong green fluorescence. It is insoluble in chloroform and differs from lumiflavin, which is also a photodecom-

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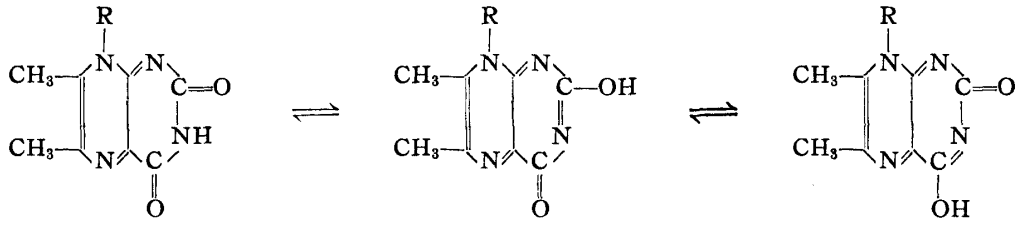
1) T. Masuda : This Bulletin, **4**, 375(1956).

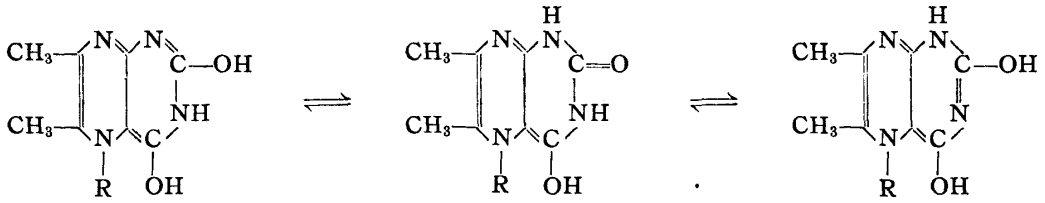
position product of riboflavin, in its properties. The ultraviolet absorption of this compound has its maxima at 258 and 407 mμ, as shown by the solid line in Fig. 1, resembling that of G compound shown by the dotted line, but is different from that (chain line) of 6,7-dimethyllumazine. This shows that the change of the site of the conjugate double bond due to the introduction of the methyl group into 8-position of 6,7-dimethyl lumazine shifted the absorption maxima to a longer wave length region.

From the close agreement between G compound and 6,7,8-trimethyllumazine in ultraviolet spectrum, the position of the ribityl group of G compound is supposed to be the nitrogen at 8-position of lumazine, but it may be possible that the absorption of 5,6,7-trimethyllumazine is also in accord with that of G compound. In such a case, however, the compound would have the structure shown by (2) in the following equation.

(1) 8-Substitution



(2) 5-Substitution



Comparison between the above two structures shows that the conjugated double bonds in them are clearly different not only in position but also in number. In addition, from the fact that riboflavin and lumiflavin have absorption maxima in the same position, difference in absorption of compounds of this series seems to be due not to the magnitude of the substituent but to the number or position of the unsaturated bond in the parent structure. It follows then that the complete agreement between G compound and 6,7,8-trimethyllumazine in the position of the absorption maxima indicates that the former is the 8-ribityl derivative of 6,7-dimethyllumazine.

The author is grateful to Mr. T. Kishi and Mrs. M. Asai for their help throughout the work, and to members in charge of elementary analysis.

Experimental

2,4,6-Trihydroxypyrimidine (I)[2] (Barbituric Acid)—160 g. of ethyl malonate is added to a EtONa solution prepared from 23 g. of metallic Na and 500 cc. of dehyd. EtOH, followed by 60 g. of dry urea, and the mixture is heated in an oil bath at 100~110° for 7 hrs. After cooling, the reaction mixture is acidified with HCl and the separated barbituric acid is collected. The yield is 86 g. or 67.2% of the theoretical.

2,4,6-Trichloropyrimidine (II)[3]—To 50 g. of barbituric acid is added dropwise a mixture of 156 cc. of $POCl_3$ and 38.5 cc. of dimethylaniline, and the whole is boiled in an oil bath for 45 mins. The reaction mixture is poured onto 800 g. of chipped ice and, after standing overnight, extracted several times with ether. The ethereal solution is dried over Na_2SO_4, the ether is distilled off, and the residue is subjected to fractional distillation. The fraction of $b.p_{34\sim35}$ 102~105° is collected. The yield is 42 g. or 58.8% of the theoretical.

2) Org. Syntheses, II, 60.
3) J. Baddiley, A. Topham : J. Chem. Soc., **1944**, 678.

4-Chloro-2,6-dimethoxypyrimidine (III)[4]—To a solution of 25 g. of 2,4,6-trichloropyrimidine in 360 cc. of MeOH is added gradually MeONa solution prepared from 6.4 g. of metallic Na and 81 cc. of MeOH, and the mixture is allowed to stand overnight. The separated NaCl is filtered off, the filtrate is concentrated, and the resulting crystals are filtered and washed with a little water; m.p. 75°. The yield is 11 g.

4-Chloro-2,6-dihydroxypyrimidine (IV)[5]—10 g. of 4-chloro-2,6-dimethoxypyrimidine is hydrolyzed by heating with 60 cc. of 20% HCl on a water bath for 2 hrs. After cooling, the reaction mixture is diluted with 70 cc. of water and the resulting crystals are filtered and recrystallized from water, m.p. 300°(decomp.). The yield is 6 g. *Anal.* Calcd. for $C_4H_3O_2N_2Cl$: N, 19.12. Found : N, 19.05.

4-Methylamino-2,6-dihydroxypyrimidine (V)—A mixture of 5 g. of 4-chloro-2,6-dihydroxypyrimidine and 70 cc. of 30% aq. solution of methylamine is heated in an autoclave at 130° (10 atm.) for 3 hrs. The reaction mixture is concentrated to remove the excess methylamine and the resulting crystals are recrystallized from water to light yellow prisms, m.p. 290°(decomp.). The yield is 2 g. *Anal.* Calcd. for $C_5H_7O_2N_2$: N, 29.87. Found : N, 30.54.

4-Methylamino-5-nitroso-2,6-dihydroxypyrimidine(VI)—To a suspension of 2 g. of 4-methylamino-2,6-dihydroxypyrimidine in 30 cc. of water is added 2 g. of $NaNO_2$ and then about 6 cc. of 4*N* AcOH is added dropwise, whereupon the mixture becomes a red clear solution and bright red needles, m.p.>350°(decomp.), soon separate out. The yield is 2.4 g. *Anal.* Calcd. for $C_5H_6O_3N_4 \cdot H_2O$: C, 31.92; H; 4.29; N, 29.78. Found : C, 32.05; H, 4.40; N, 29.63.

4-Methylamino-5-amino-2,6-dihydroxypyrimidine Bisulfite (VII)—A suspension of 0.5 g. of 4-methylamino-5-nitroso-2,6-dihydroxypyrimidine in 10 cc. of water is heated on a water bath and 1 g. of $Na_2S_2O_4$ is added, when the mixture becomes a slightly yellow clear solution. The solution is filtered while hot and cooled, whereupon light yellow needles, m.p.>350°(decomp.)(blackening at 100°), separate out. The yield is 0.3 g.

6,7,8-Trimethyllumazine (6,7,8-Trimethyl-2(8*H*),4(3*H*)-pteridinedione) (VIII)—To a solution of 300 mg. of 4-methylamino-5-amino-2,6-dihydroxypyrimidine bisulfite in 15 cc. of water is added 1 cc. of diacetyl and the mixture is heated at 80° for 30 mins. The reaction mixture is concentrated to 8 cc. under a reduced pressure and cooled, when crystals separate out, which are recrystallized from a small quantity of water to yellow needles, m.p. 300~301°(decomp.). The yield is 140 mg. *Anal.* Calcd. for $C_9H_{10}O_2N_4$(dried at 110° for 3 hrs. *in vacuo*) : C, 52.42; H, 4.89; N, 27.18. Found : C, 52.55; H, 5.17; N, 27.28.

The product exhibits a green fluorescence and it always appears as a single spot in various paper partition chromatography. The Rf values are shown in the Table. I.

Table. I.

Solvent	Rf
EtOH•BuOH•H_2O(15 : 50 : 35)	0.34
AcOH•BuOH•H_2O(1 : 4 : 5)	0.26
Benzyl alcohol	0.52
Na_2HPO_4(5% H_2O)	0.70

Summary

The green fluorescent substance (G compound) isolated from the mycelium of *Eremothecium ashbyii* was presumed to be a ribityl derivative of 6,7-dimethyllumazine and already reported in the previous paper. In the present work 6,7,8-trimethyllumazine was synthesized, and from the complete agreement of ultraviolet spectrum between this product and G compound, the position of the ribityl group in G compound was confirmed to be the nitrogen at 8-position.

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4) H. J. Fisher, T. B. Johnson : J. Am. Chem. Soc., **54**, 727(1932).
5) W. R. Boon, T. Leigh : Brit. Pat. No. 677,342.