Judging from the results mentioned above, the writers hesitate to think the chemical route for synthesizing riboflavin reported by Birch, *et al.* and Cresswell, *et al.* as possible pathway for the biosynthesis of riboflavin.

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## On the Mechanism of Biosynthesis of 6-Methyl-7-hydroxyribolumazine

Reports hitherto published on the biosynthesis of 6-methyl-7-hydroxyribolumazine (I) may be summarized as follows: (1) (I) is probably formed by the oxidative removal of the 7-methyl group from 6,7-dimethylribolumazine (II), for prolonged air-oxidation of (II) in an alkaline medium gives (I) (Korte, *et al.*[1]), (2) the diaminouracil derivative produced by the enzymic decomposition of (II) condenses with an aldol compound and the side-chain at the 7-position of the product (III) is cut off to give (I) (Mitsuda, *et al.*[2]), or (3) 6,7-dimethylribolumazime (II) receives a nucleophilic attack at the 7-position and, on being heated with pyruvate, a four-carbon compound is split off from the pyrazine ring and a pyruvate enters the position in its place, yielding (I) (Rowan, *et al.*[3]).

The present authors, however, do not agree with the second view based on the experimental data[4] obtained by allowing the enzyme of *Eremothecium ashbyii* to act upon the condensation product (III) of the above-mentioned diaminouracil compound with the aldol compound.

Similar enzymic reactions were effected, using 4-ribitylamino-5-aminouracil as a substrate in the presence of various carbon donors. Unlike the case of acetoin, when pyruvic acid was added, a purple fluorescent spot was detected by paper partition chro-

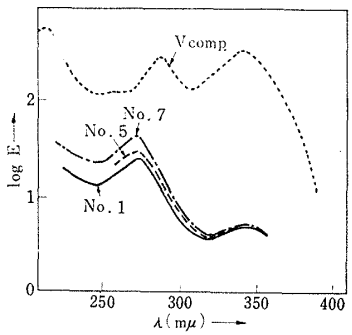
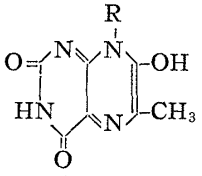



Fig. 1. Ultraviolet Absorption Spectra
..... $\log E^{1\%}_{1cm.}$ of V Comp. $\log E^{x\%}_{1cm.}$ of purple fluorescent product
——— (No. 1. Substrate+pyruvic acid+enzyme solution)
- - - - (No. 5. Substrate+pyruvic acid+formic acid+enzyme solution)
— — — (No. 7. Substrate+pyruvic acid+heat-treated enzyme solution)

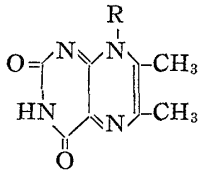
1) F. Korte, H. U. Aldag, G. Ludwig, W. Paulus, K. Störiko: Ann., **619**, 70 (1958).
2) H. Mitsuda, F. Kawai, J. Suzuki: Vitamins (Kyoto), **23**, 415 (1961).
3) T. Rowan, H. C. S. Wood, P. Hemmerich: Proc. Chem. Soc., **1961**, 260.
4) M. Asai, S. Kuwada: Part XLIV of a series entitled "Application of chromatography," This Bulletin, unpublished data.

matography, but the ultraviolet spectrum of the product, as shown in Fig. 1, was considerably different from that of authentic 6-methyl-7-hydroxyribolumazine (I). From this fact, the present writers do not agree with the third view either.

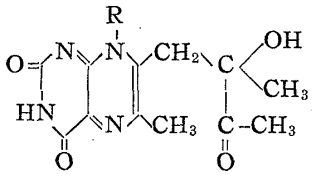
It was observed that when 6,7-dimethylribolumazine (II) was subjected to the enzymic reaction at a pH causing dissociation of the OH group (above ca. pH 4), 6-methyl-7-hydroxyribolumazine (I) was formed in a short time (30~60 min.) and its quantity increased with rise of pH value (pH 6.4, 7, 7.8). On the contrary, (I) was not produced in the control experiment without an enzyme.



(I)



(II)

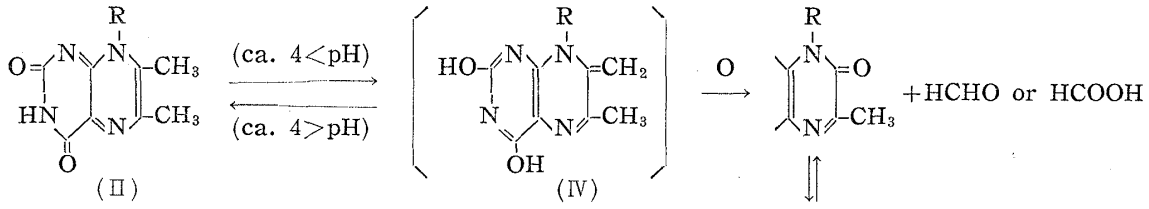


(III)

It should be noted that the formation of formaldehyde and formic acid besides riboflavin and 6-methyl-7-hydroxyribolumazine (I) was detected when the same reaction was carried out at pH 7.

On the other hand, when a basic solution of 6,7-dimethylribolumazine was subjected to air-oxidation in a dark place for four days and the product was examined by paper partition chromatography, the spot of 6-methyl-7-hydroxyribolumazine (I) was detected, as reported by Korte, *et al.*[1]

From these facts, it is assumed that the enzymic conversion of 6,7-dimethylribolumazine (II) to 6-methyl-7-hydroxyribolumazine (I) proceeds as shown in Chart 1.



(II) (IV) (I) R=ribityl

Chart 1.

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