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THE FORMATION OF RIBOFLAVIN FROM 6,7-DIMETHYL-8-RIBITYLLUMAZINE IN ACID MEDIA‡

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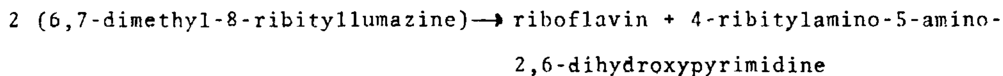
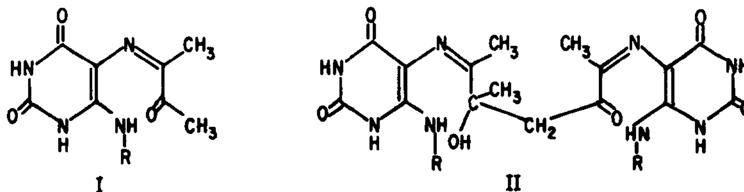
The presence of an enzyme capable of catalyzing the formation of riboflavin from 6,7-dimethyl-8-ribityllumazine has been demonstrated in a number of micro-organisms (1). The stoichiometry of the reaction has been established in studies with highly purified riboflavin synthetase from yeast (2).



2 (6,7-dimethyl-8-ribityllumazine)⟶ riboflavin + 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine

It was shown by Rowan and Wood (3) that formation of riboflavin from 6,7-dimethyl-8-ribityllumazine can also occur in good yield non-enzymically when the precursor is refluxed under nitrogen in phosphate buffer at pH 7.3. They have proposed that the chemical transformation occurs by a mechanism which involves an opening of the pyrazine ring of the lumazine between nitrogen atom No. 8 and carbon No. 7 initiated by nucleophilic attack. In a subsequent aldol condensation two molecules of this substance (I) are suggested to condense to form a dimeric intermediate (II) which is thought to cyclize to riboflavin by a mechanism involving the 6-methyl group and carbon atom No. 6 of the initial lumazine.



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It has been established spectrophotometrically that under alkaline conditions ring opening to form (I) occurs, preceded by hydration of the pyrazine ring of the lumazine, reactions which are readily reversible upon acidification (4). Formation of the suggested α-methyl ketone in alkaline solution is consistent with recent results (Beach and Plaut, unpublished data); namely, that the NMR spectrum of 6,7-dimethyl-8-ribityllumazine exhibits a significant change in the position of absorption of the 7-methyl group absorbing at 2.88 ppm in neutral solution and shifting upfield to 1.37 ppm in alkaline solution. Acidification of the latter reestablished the identical spectrum seen at neutrality. In addition, if the reaction is done in D_2O the hydrogen atoms of the 7-methyl group, but not the 6-methyl group, exchange rapidly, resulting in loss of absorption at 1.37 ppm and 2.88 ppm in alkaline and acid solution, respectively.

It is doubtful, however, whether a ring opening reaction to form compound I is involved as the first step of the enzyme catalyzed reaction (5). Furthermore, the need for at least an alternative mechanism not involving formation of compound I also arises from the demonstration that hydrogen-deuterium exchange at the 7-methyl group of 6,7-dimethyl-8-ribityllumazine and its derivatives occurs very rapidly in acid solution and the present finding (Table I) that chemical formation of flavin in 40-50% yield from the lumazine can take place in 0.1 M HCl, i.e., conditions which oppose hydration and opening of the pyrazine ring.

A mechanism for the acid catalyzed formation of riboflavin from 6,7-dimethyl-8-ribityllumazine is suggested below in which the neutral form (III) of the lumazine is converted first to a cationic species (IV). The occurrence of cationic forms of the lumazine in acid solution is supported by spectrophotometric data (4). The subsequent reversible transformation of compound (IV) to (V) could explain the experimentally observed acid catalyzed hydrogen exchange at the 7-methyl group of the lumazine (Beach and Plaut, unpublished data). These compounds are then visualized to condense to form a dimeric intermediate (VI). The latter is thought to be transformed to species in which one pyrazine ring is opened (VII-IX), followed by ring closure at positions corresponding to the 6-methyl group and carbon atom No. 6 of the initial lumazine (X); and, finally, cleavage to riboflavin (XI) and 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine (XII)

Table I

Compound	Visible and ultra violet spectra (mμ)[a]		Paper chromatography (R_f)[b]		
	λmax	λmin	Solvent (A)	Solvent (B)	Solvent (C)
Authentic riboflavin	440 370 264	 395 310	0.61	0.24	0.39
Isolated riboflavin	440 370 264	 395 305	0.62	0.25	0.39
Authentic 2,10-dihydro-4,6,8-trihydroxy-10-ribityl-2-oxopyrimidino-(5,4-g) pteridine	425 286 242	 310 265	0.11	0.02	0.11
Isolated[c] 2,10-dihydro-4,6,8-trihydroxy-10-ribityl-2-oxopyrimidino-(5,4-g) pteridine	425 282 242	 310 265	0.11 (0.35 trace)	0.02 (0.11 trace)	0.11 (0.28 trace)

[a] Absorption spectra were determined in 5% acetic acid in a Cary Model 14 spectrophotometer.

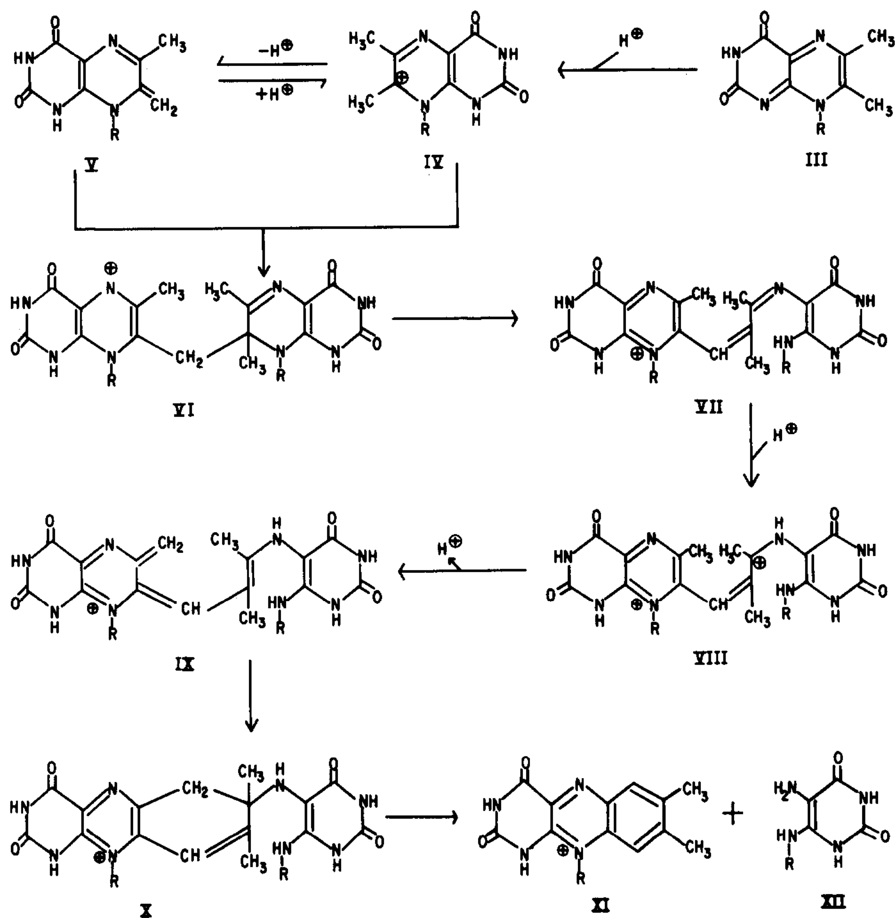
[b] A: isobutyric acid-1 N ammonia-0.1 M EDTA (250:150:4); B: (n-butylalcohol-glacial acetic acid-H_2O (200:30:75); C: n-butyl alcohol-100% ethanol-H_2O (500:175:360).

[c] The second product of the reaction identified here has been reported to be formed from 4-ribitylamino-5-amino-2,6-dihydroxypyrimidine (6).

Methods

6,7-Dimethyl-8-ribityllumazine (82 mmoles/ml 0.1 M HCl) under a nitrogen atmosphere in a sealed vial was heated at 90° C for 40 hr. in the dark.

Products of the reaction mixture were separated by first extracting the flavin from the aqueous phase with water saturated benzyl alcohol, the aqueous phase retaining 2,10-dihydro-4,6,8-trihydroxy-10-ribityl-2-oxopyrimidino-(4,5-g) pteridine and most of the lumazine. The pteridines in the aqueous phase were separated by column chromatography on Dowex 50W X 4 (2) while the flavin, extracted from the benzyl alcohol layer with water, was purified further on a column of Lloyd's reagent (7). The substances thus isolated were characterized by their absorption spectra and by chromatography on Whatman #3 (Table I).



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