An Elimination–Rearrangement of Ribulose-1,5-bisphosphate with Implications for Riboflavin Biosynthesis

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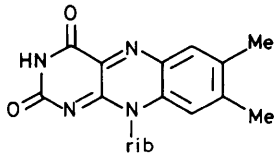
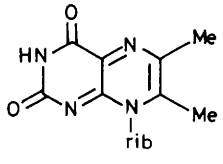
Ribulose-1,5-bisphosphate (**5**) undergoes a sequence of elimination and benzilic acid rearrangement in a process with features that parallel those of the rearrangement occurring during incorporation of pentose precursors into riboflavin (**1**).

The biogenesis of the *o*-xylene moiety of riboflavin (**1**) has not been firmly established. Although the later steps from 6,7-dimethyl-8-ribityl-lumazine (DMRL) (**2**) are well understood,[1] the origin of the four-carbon component in DMRL (**2**) which furnishes all eight carbon atoms of the *o*-xylene system of riboflavin (**1**) is still uncertain. Previously postulated four-carbon precursors such as acetoin (3-hydroxybutan-2-one)[2] and diacetyl (butane-2,3-dione)[3] have now been eliminated although rumours of their involvement still persist.[4]

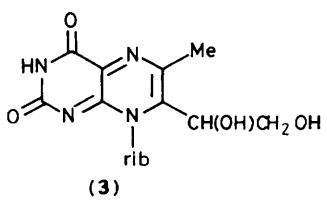
Bresler *et al.* observed that a riboflavin-requiring mutant of *Bacillus subtilis* excreted a compound identified as 6-methyl-7-(1,2-dihydroxyethyl)-8-ribityl-lumazine (MERL) (**3**) which supported the growth of a riboflavin auxotroph, albeit at a concentration 10^3 times greater than that required when riboflavin itself was supplied.[5] It was suggested that the C_5 unit of this compound was derived from the ribityl component of the established riboflavin precursor 5-amino-2,4-dioxo-6-ribitylaminopyrimidine (ADRAP) (**4**). Mechanistically and biochemically acceptable processes can be postulated whereby MERL (**3**) might be converted into DMRL (**2**) and thence into riboflavin (**1**) with loss of the hydroxymethyl group. Although the evidence supporting the precursor role of MERL (**3**) in riboflavin (**1**) biosynthesis seemed persuasive, it was not consistent with the results of Floss *et al.* who have obtained evidence that a pentose precursor or a closely related species, is converted into the C_4 unit of DMRL (**2**) with excision of C-4 and formation of a new carbon–carbon bond between C-3 and C-5 (Scheme 1).[6]



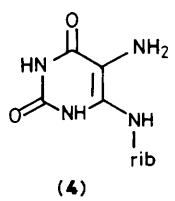
(**1**)



(**2**)



(**3**)

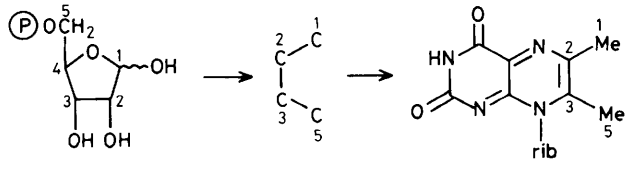


(**4**)

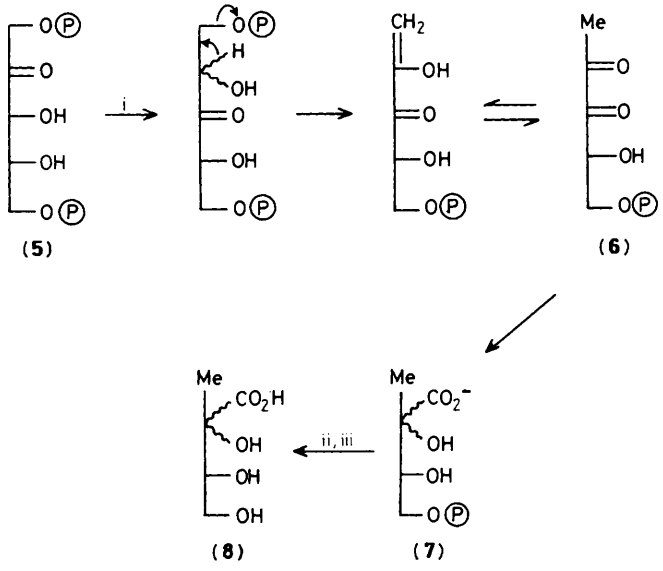
rib = ribityl

We here describe experimental evidence bearing both on MERL (**3**) formation and on the rearrangement mechanism of riboflavin (**1**) biosynthesis proposed by Floss *et al.*[6] These experiments arose out of mechanistic considerations pointing to the possible involvement of ribulose-1,5-bisphosphate (**5**), or an equivalent system, in riboflavin (**1**) biosynthesis. Thus in Scheme 2, a postulated sequence is shown whereby ribulose-1,5-bisphosphate (**5**) might be converted into the 5-phosphate of the diketone (**6**). Hydrolyis of the 5-phosphate ester followed by condensation with ADRAP (**4**) would furnish MERL (**3**). This sequence requires Lobry de Bruyn–Alberda van Ekenstein rearrangement of ribulose-1,5-bisphosphate (**5**), phosphate elimination, for which there is ample chemical[6,7] and biochemical[8] precedent, and ketonisation of the resulting enol.

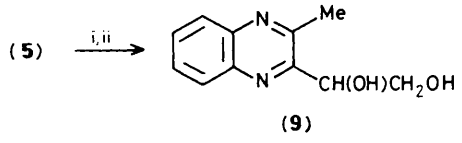
To test the validity of this proposal ribulose-1,5-bisphosphate (**5**) was incubated at pH 13 and product formation was monitored by ^{13}C and ^1H n.m.r. spectroscopy.



Scheme 1



Scheme 2. *Reagents:* i, pH 13; ii, alkaline phosphatase; iii, H^+



Scheme 3. *Reagents:* i, pH 6.2, 1,2-diaminobenzene; ii, alkaline phosphatase.

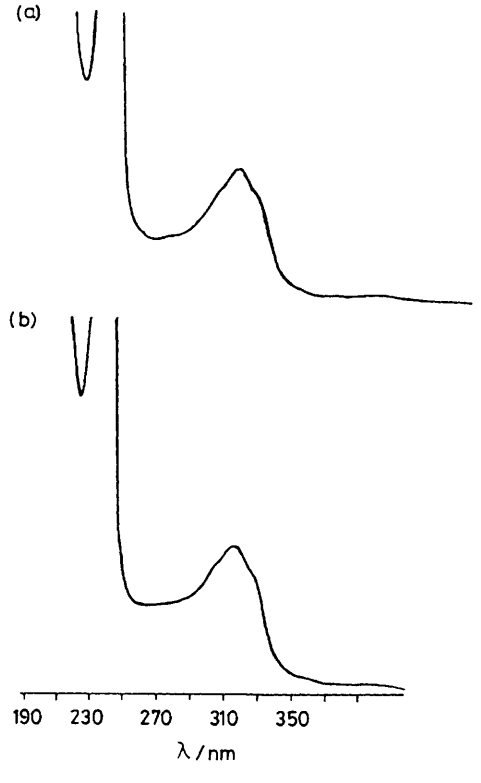
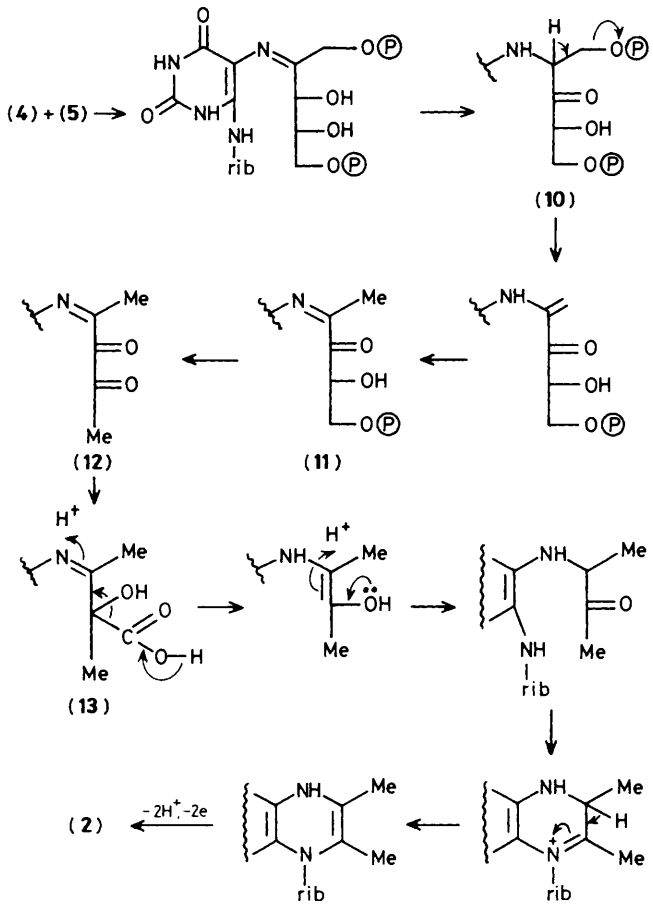


Figure 1. U.v. spectra [MeOH:H_2O–0.05% CF_3CO_2H (1:1)] of 2-(1,2-dihydroxyethyl)-3-methylquinoxaline (**9**) (a) from the incubation of ribulose-1,5-bisphosphate with 1,2-diaminobenzene at pH 6.2; (b) synthetic.



Scheme 4. rib = ribityl.

Signals attributable to newly formed methyl groups were observed but at higher field than expected for the diketone (**6**). Also, a carbonyl carbon resonance was observed in the ^{13}C n.m.r. spectrum with a chemical shift (δ 181.7) corresponding to that of a carboxylate rather than a ketonic carbonyl group. It appeared likely that formation of the expected diketone (**6**) was rapidly followed by benzilic acid rearrangement to the carboxylate (**7**). Proof was obtained by hydrolysis to the trihydroxy acid (**8**) using alkaline phosphatase. This acid was obtained as a mixture of diastereoisomeric lactones identified by comparison with an authentic sample. Comparison was made by ^1H n.m.r. and by g.l.c.–mass spectral analysis† of trimethylsilyl derivatives of both the lactone and the corresponding hydroxy acid.

When the incubation of ribulose-1,5-bisphosphate (**5**) was carried out at pH 6.2 in the presence of 1,2-diaminobenzene a complex mixture resulted which was subjected to hydrolysis with alkaline phosphatase. From the product mixture a compound was isolated by h.p.l.c. which had a retention time identical (confirmed by co-chromatography) with an independently synthesised sample of the MERL (**3**) analogue (**9**) (Scheme 3) and with a qualitatively identical u.v. spectrum (Figure 1). It therefore appears that under these conditions, the intermediate (**6**) postulated to have been formed from ribulose-1,5-bisphosphate (**5**) can be trapped as the quinoxaline (**9**). Alternatively, the elimination may occur in the Schiff

† Determined using a Kratos MS 80 mass spectrometer with g.l.c. on SE 30, He carrier gas, temperature programme 120°/3° min^{-1}/180 °C.

base formed by initial condensation between ribulose-1,5-bisphosphate (**5**) and 1,2-diaminobenzene.

Floss *et al.*[6] have proposed a radical mechanism for the rearrangement (Scheme 1) of the postulated five-carbon precursor of the *o*-xylene system in riboflavin (**1**). However, the above results suggest an alternative mechanism. Thus condensation of ribulose-1,5-bisphosphate (**5**) with ADRAP (**4**) (Scheme 4) followed by Amadori rearrangement would give the iminoketone (**10**). Phosphate elimination would then lead to the iminoketone (**11**) which has the functionality that permitted the elimination–benzilic acid sequence to occur with ribulose-1,5-bisphosphate (**5**) itself. However, in this case a β-iminocarboxylic acid (**13**) would be produced which could undergo ready decarboxylation. Formation of DMRL (**2**) would then require only condensation and two-electron oxidation. To be consistent with the results of Floss *et al.*,[6] it is only necessary to make the further postulate of enzymatic control of methyl group rather than enamino group migration during the benzilic acid rearrangement (**12**) → (**13**).

The sequence of Scheme 4 is open to considerable modification with regard to detail. However, its basic features, generation of a 1,2-diketone by elimination in a 3-oxophosphate, benzilic acid rearrangement, and decarboxylation, offers an attractive explanation, consistent with the available evidence, for the conversion of a pentose precursor into the *o*-xylene ring of riboflavin (**1**).

Ribulose-1,5-bisphosphate (**5**) is a key intermediate, as the substrate for the enzyme ribulose-1,5-bisphosphate carboxylase, in the photosynthetic carbon cycle. The degradative rearrangement demonstrated above therefore has implications for ribulose-1,5-bisphosphate (**5**) metabolism,[9,10] since phosphate elimination, as in our studies, can occur over a wide pH range,[11] encompassing the region normally regarded as 'physiological.'

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