seems of interest to note that Que and co-workers[16] did not observe ring cleavage with [Fe(salen)(Bucat)]$^-$, although in this complex, too, the catecholate appears to be stabilized relative to the semiquinone form. This may be due to the fact that the tetragonal salen ligand does not allow catechol and oxygen to be bound to the iron in adjacent positions, thus preventing reactions between these two components other than electron exchange.[16] (Vice versa, this may be the reason for the nonheme nature of this class of dioxygenases.) Further, neither ferric semiquinone nor monodentate catecholate[16] complexes were found to yield ring-cleavage reactions with O_2. Proposed mechanisms involving such species[4,16] now seem less likely since we here show that chelated catecholate in [Fe(NTA)(Bucat)]$^{2-}$ does undergo the dioxygenase reaction.

Ferric semiquinones, however, appear to serve as intermediates of the alternative reaction pathway, iron(III)-catalyzed catechol autoxidation (eq 1a; M. G. Weller and U. Weser, unpublished results). The formation of these complexes (e.g., tris(3,5-di-*tert*-butylsemiquinone)iron(III)) is favored by resonance stabilization. Such stabilization is lost with "hard" ligands present in mixed complexes. In our studies, the hard phenolate ligands in pyrocatechase (Tyr residues of the protein)[14] are replaced by the hard NTA. Such "hardening" of a nonheme ferric center, both in the enzyme and model system, finally leads to catechol ring cleavage upon reaction with O_2. Mechanistic studies of the oxygenation are under way.

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Registry No. 1 (R = *t*-Bu), 1020-31-1; **5a**, 22802-86-4; **5b**, 22961-94-0; Fe, 7439-89-6; [Fe(NTA)(Bucat)](pipH)$_2$, 81770-38-9; H_3NTA, 139-13-9.

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Biosynthesis of Riboflavin. Incorporation of D-[1-^{13}C]Ribose

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The terminal step of riboflavin (**3**) biosynthesis consists of the transfer of four carbon atoms from one molecule of 6,7-dimethyl-8-ribityllumazine (**2**) to a second molecule of **2**. This reaction is catalyzed by the enzyme riboflavin synthase and has been studied in considerable detail (for a review see ref 1). The biosynthetic precursor of **2** is 5-amino-6-ribitylamino-2,4-(1*H*,3*H*)-pyrimidinedione 5′-phosphate (**1**),[2-4] which in turn arises from guanosine triphosphate (GTP).[5] The formation of the pyrazine ring of **2** from **1** requires the addition of four carbon atoms; duplication of this four-carbon moiety by riboflavin synthase ultimately produces the xylene moiety of the riboflavin molecule.[1]

Table I. Relative ^{13}C Abundance in Riboflavin Tetraacetate Derived from Riboflavin Biosynthetically Labeled by Feeding D-[1-^{13}C]Ribose to *Ashbya gossypii*

carbon	chemical shift, ppm[a]	rel ^{13}C abundance, %
2	159.3	0.9
4	154.4	0.8
4a	136.1	0.9
5a	134.6	3.3
6	133.0	10.3
7	137.0	0.8
8	148.1	3.7
9	115.5	1.1
9a	131.2	0.6
10a	150.7	0.6
7-Me	19.4	0.9
8-Me	21.4	9.1
1′	45.0	19.4
2′	69.4	2.9
3′	70.5	0.6
4′	69.0	1.3
5′	61.9	1.1
$\underline{C}H_3CO$	20.3, 20.7, 20.8, 21.0	av 1.0[b]
$CH_3\underline{C}O$	169.7, 169.8, 170.3, 170.6	av 0.9

[a] Relative to $Me_4Si = 0$. [b] Standard.

Various hypotheses for the origin of the four-carbon moiety involved in the formation of **2** have been proposed. Thus, diacetyl or acetoin,[6,7] a tetrose,[8] a pentose,[9] or a hexose[10] have been discussed as the ultimate source of these four carbon atoms. More recently, it has been suggested that one molecule of the ribitylaminopyrimidine **1** (R = H) donates its ribitol moiety, which is subsequently inserted into a second molecule of **1** to form the pyrazine ring.[11,12] We decided to reexamine this issue by feeding ^{13}C-labeled precursors and analyzing the product **3** by ^{13}C NMR spectroscopy.

It had been shown earlier that the isotope from [1-^{14}C]ribose is incorporated into one or both of the methyl groups of **3** and into carbon atoms 6 and/or 9.[9] However, the incorporated isotope could not be traced to individual atoms in these experiments.

We therefore studied the incorporation of [1-^{13}C]ribose into riboflavin by the flavinogenic fungus *Ashbya gossypii*. The organism was grown in 200 mL of complete medium[13] containing glucose (10 g/L) until the onset of flavinogenesis. D-[1-^{13}C]Ribose (250 mg, 90% ^{13}C, Los Alamos Stable Isotope Resource) was added, and the culture was incubated for another 24 h. Riboflavin was isolated and purified by column chromatography. Acetylation ($HClO_4$/acetic anhydride)[14] gave the 2′,3′,4′,5′-tetraacetate (**4**), which was purified by column chromatography and crystallization from water (65 mg). ^{13}C NMR spectra of **4** were recorded in $CDCl_3$ on a Varian XL-200 Fourier transform spectrometer. The signal assignments for **3** and **4** (Table I) rest firmly on chemical shift and multiplicity arguments,[15] the spectral analysis of specifically ^{13}C- and ^{15}N-labeled flavins[16,17] and isoalloxazines,[18] the

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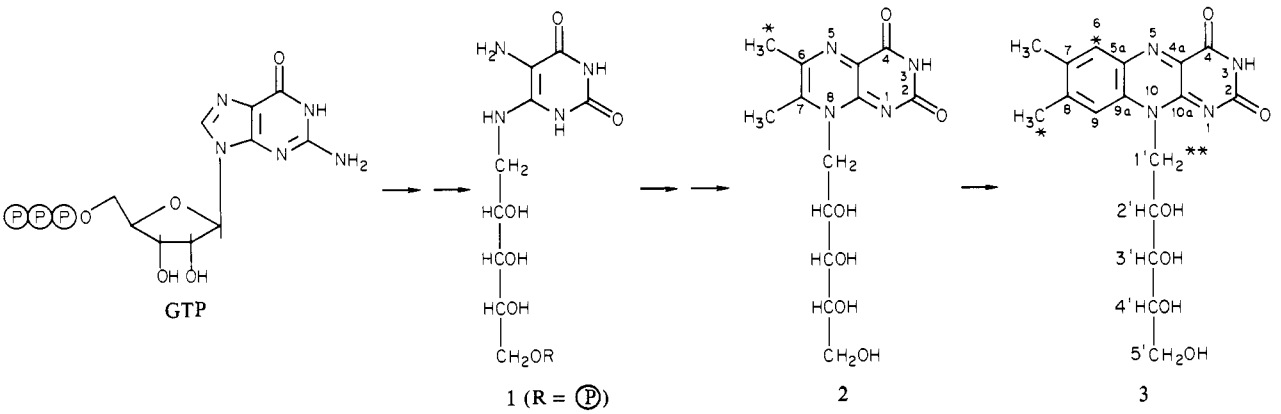
Scheme I. Biosynthesis of Riboflavin[a]



[a] The single asterisks illustrate the regiochemistry of the riboflavin synthase reaction. They also coincide with the major sites of labeling of the xylene moiety of **3** from [1-^{13}C]ribose. The double asterisk indicates the major site of labeling in the ribitol moiety of **3**.

^{13}C NMR spectra of [2′-^{2}H]- and [3′-^{2}H]riboflavin[19] obtained from Dr. William M. Moore, Utah State University, and a determination of carbon–carbon connectivities by analysis of the two-dimensional double-quantum coherence ^{13}C NMR spectrum[20] of **4** biosynthesized from [U-$^{13}C_6$]glucose.

The ^{13}C distribution in **4** derived from [1-^{13}C]ribose is shown in Table I. The majority of the ^{13}C is found in three positions, with secondary labeling evident in three additional carbon atoms. The isotope is efficiently incorporated into position 1′ of the ribityl side chain. This is expected since it has been shown that the ribitol moiety of riboflavin is derived from the ribose moiety of GTP.[3,21] In the heterocyclic moiety, carbon atom 6 and the 8-methyl group show the same ^{13}C abundance within experimental error. However, the enrichment at these positions is significantly lower than that at C-1′.

These results lead to the following conclusions: (i) In the last biosynthetic step catalyzed by riboflavin synthase, the 6-methyl group of **2** gives rise to carbon atom 6 and the 8-methyl group of **3**. This is in agreement with the regioselectivity of the enzyme as suggested earlier on the basis of in vitro studies with deuterium-labeled **2**.[22]

(ii) The isotope from [1-^{13}C]ribose is efficiently incorporated into the 6-methyl group but not into the 7-methyl group of the lumazine **2**. It follows that symmetrical molecules such as diacetyl are ruled out as intermediates in the generation of the four-carbon unit, because any symmetrical intermediate would lead to an even distribution of the label between the two methyl groups.

(iii) Since carbon 1′ of the ribitol moiety is labeled significantly more heavily than any atom in the aromatic ring, it appears that although a pentose does contribute to the generation of the four-carbon moiety, the ribitol moiety of **1** may not be a direct precursor of this four-carbon unit as has been suggested.[11,12] If two molecules of **1** were to react to give one molecule of **2** in a manner similar to the conversion of two molecules of **2** into one molecule of **3**, the final product **3** would have to contain equal amounts of ^{13}C at C-1′, C-6, and the 8-methyl group. The finding that this is not the case is in line with earlier results on the incorporation of guanosine into riboflavin by a purine mutant, which indicated that the ribose moiety of GTP contributes to the ribitol moiety but not to the isoalloxazine ring of **3**.[21]

Studies with other ^{13}C-labeled precursors are underway in order to further delineate the specific origin of this four-carbon moiety.

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Electron-Transfer Pathways in the Reduction of d^6 and d^7 Organoiron Cations by $LiAlH_4$ and $NaBH_4$

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The activation by transition metals of unsaturated ligands toward reduction by main-group hydrides has been used extensively during the last two decades.[1,2] Recently, attention has focused on the homogeneous reduction of coordinated CO by borohydrides[3]

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