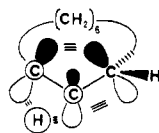


closely related to the stepwise  $1 \rightarrow [10 \text{ or } 12] \rightarrow 4$  pathways and could be favored by constraints imposed by the carbocyclic ring.

If we assume that generation from tosylhydrazones and from photolysis of **4** define the chemistry of vinylcarbenes **10** and **12**, then it seems unlikely that either is a true intermediate in the  $1 \rightarrow 4$  transformation. Thus, present results favor an effectively concerted mechanism. Experiments with isotopically labeled and optically active **1** should permit further mechanistic clarification.

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(12) Zimmerman, H. E. "Pericyclic Reactions"; Marchand, A. P., Lehr, R. E., Eds.; Academic Press: New York, 1977; Chapter 2, p 53, and references therein. The Hückel array for 1,2-H shift is depicted here:



## Biosynthesis of Riboflavin. An Unusual Rearrangement in the Formation of 6,7-Dimethyl-8-ribityllumazine

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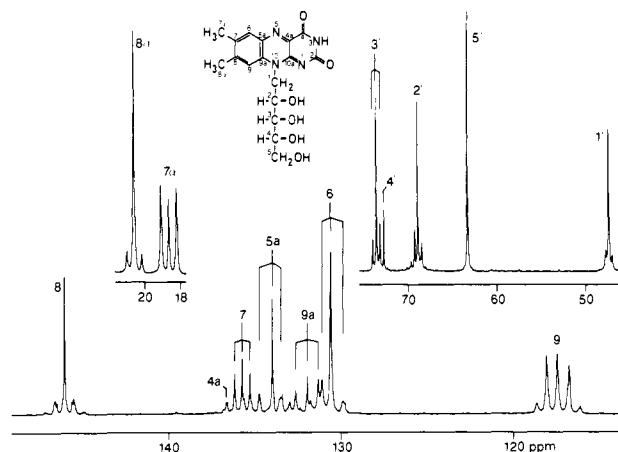
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In the course of our continuing studies<sup>1</sup> on the biosynthesis of riboflavin, we have encountered an apparently novel biochemical carbon skeletal rearrangement. Riboflavin (**3**, Scheme I) has been shown<sup>2</sup> to form by a disproportionation of two molecules of 6,7-dimethyl-8-ribityllumazine (**2**). The eight carbon atoms of the xylene ring of **3** thus originate from two biochemically identical four-carbon units, namely, carbons  $1\alpha$ , 6, 7, and  $7\alpha$  of **2**. The regiochemistry of this condensation is depicted in Scheme I through the use of an arbitrary numbering system ( $1^*$ – $4^*$ ) for the biosynthetically equivalent carbon atoms. The pyrimidinedione **1** is known to be the direct precursor of **2**, but the origin of the four carbon atoms that eventually form the pyrazine ring of **2** is not clear. Several authors have suggested<sup>3</sup> the involvement of a pentose; hence, formation of **2** would require the elimination of one carbon atom from the pentose precursor.

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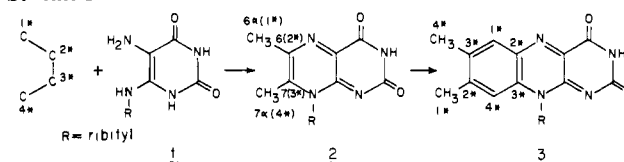
(2) For reviews see: (a) Plaut, G. W. E.; Smith, C. M.; Alworth, W. L. *Ann. Rev. Biochem.* **1974**, *43*, 899–922. (b) Brown, G. M.; Williamson, J. M. *Adv. Enzymol.* **1982**, *53*, 345–381.

(3) (a) Alworth, W. L.; Dove, M. F.; Baker, H. N. *Biochemistry* **1977**, *16*, 526–531. (b) Bresler, S. E.; Perumov, D. A.; Chernik, T. P.; Glazunov, E. A. *Genetika* **1976**, *12*, 83–91. (c) Hollander, I. J.; Braman, J. C.; Brown, G. M. *Biochim. Biophys. Res. Commun.* **1980**, *94*, 515–521. (d) Logvinenko, E. M.; Shavlovskii, G. M.; Zakal'skii, A. E.; Zakhodylo, I. V. *Biokhim. (Moscow)* **1982**, *47*, 931–936.



**Figure 1.** Selected regions from 50.3-MHz proton-decoupled <sup>13</sup>C NMR spectrum of riboflavin derived biosynthetically from [1,3-<sup>13</sup>C<sub>2</sub>]glycerol. Conditions: 60° pulse, repetition time 2 s, 11-KHz spectral width, 32K data points, solvent Me<sub>2</sub>SO-*d*<sub>6</sub>.

### Scheme I



In earlier studies, we had investigated this proposal through incorporation of <sup>13</sup>C-labeled precursors into riboflavin followed by <sup>13</sup>C NMR spectroscopic analysis of the products. In such experiments, the ribityl side chain of **3** can serve as a probe for the labeling pattern of the pentose pool, since its origin from the ribose moiety of GTP is firmly established.<sup>4</sup> The results showed that position  $1^*$  is labeled by the same precursors as carbon  $1'$  in the side chain, whereas carbon  $2^*$  corresponds to C- $2'$  and  $3^*$  to C- $3'$  in their respective labeling patterns. However, position  $4^*$  is efficiently labeled from [6-<sup>13</sup>C<sub>1</sub>]glucose even though this precursor contributes its label almost exclusively to the 5' carbon of the ribityl side chain.<sup>1b</sup> Furthermore, incorporation of [2-<sup>13</sup>C<sub>1</sub>]glycerol gave riboflavin that was highly labeled at C- $4'$  but not at position  $4^*$ ,<sup>1c</sup> thus suggesting extrusion of C-4 of a pentose or its equivalent (e.g., C-2 of a triose) in the formation of **2**. In addition, a feeding experiment with [U-<sup>13</sup>C<sub>6</sub>]glucose had indicated the transfer of C- $3^*$  and C- $4^*$  as coupled pair of carbons from the same glucose molecule.<sup>5</sup> In summary, the available evidence suggested that the carbon atoms 4 and 6 of an individual hexose molecule can give rise to the directly connected carbons  $3^*$  and  $4^*$  of **2** and **3**. This apparent paradox can be explained by the assumption of an intramolecular skeletal rearrangement. The present paper reports proof for such a process.

[1,3-<sup>13</sup>C<sub>2</sub>]Glycerol (0.2 g, 90% <sup>13</sup>C, MSD Inc.) was fed to the flavinogenic fungus *Ashbya gossypii* (ATCC 10859), and the resulting riboflavin was isolated and purified as previously described.<sup>1a,6</sup> The <sup>1</sup>H-decoupled <sup>13</sup>C NMR spectrum of this material (Figure 1) was measured at 50.3 MHz on a Bruker WP-200 spectrometer.

It was expected on the basis of previous studies that the proffered glycerol would label the ribityl moiety at C- $3'$  and C- $5'$  due to the straightforward derivation of C- $3'$ –C- $5'$  from a triose. Additionally, C- $1'$  and C- $2'$  should be labeled by the action of transketolase and oxidative decarboxylation of glucose, respec-

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(6) Bacher, A.; Mailänder, B. *J. Biol. Chem.* **1973**, *248*, 6227–6231.

tively. As can be seen in the spectrum, both the four-carbon unit and the ribityl function except C-4' are significantly enriched, in agreement with these expectations.  $^{13}\text{C}$  enrichments are in the range of 10–12% per site. The spectrum indicates extensive coupling between the 3\* and 4\* positions (approximate ratio of coupled to uncoupled signals 3:1),<sup>7</sup> whereas C-5' of the side chain shows no one-bond coupling. Thus the terminal carbons of the glycerol become directly connected via an intramolecular process during conversion into the pyrazine ring of 2, while they remain separated in the C-3'–to C-5' portion of the ribityl side chain. The low degree of coupling observed between C-2' and C-3' is due to the statistical joining of two labeled trioses in the formation of a hexose. Analogous low-level couplings are observed between the 2\* and 3\* positions.

The present experiment gives no indication of whether the rearrangement occurs at the triose or pentose level or at some other stage. Further work is required to settle this issue and to unravel the mechanism of this intriguing rearrangement.

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**Registry No.** 2, 5118-16-1; 3, 83-88-5; glycerol, 56-81-5.

(7) This ratio is only approximate due to the effects on the relaxation characteristics of an observed  $^{13}\text{C}$  by a neighboring  $^{13}\text{C}$ . These effects have been found to be substantial for quaternary carbons, but relatively small for protonated carbons (ref 5b).

## Novel Homo- and Heterometallic Coordination Macrocycles

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The bicyclic geometry of  $\text{P}(\text{OCH}_2)_3\text{P}^1$  (1) permits complexation of each of the bridgehead phosphorus atoms to separate metal carbonyl moieties.<sup>2</sup> Although 1 is prone to forming insoluble polymers with most metal carbonyl substrates, the last step in the reactions shown in Scheme I provides 40–86% yields of the novel tetramers 5–9, each of which are 20-membered rings.

Comparison of the  $^{31}\text{P}$  NMR spectra of the mononuclear metal complexes 2–4 and the dinuclear compounds 10–12 in Tables I with those of 5–9 reveals as expected two equal-intensity peaks in all three sets of compounds.<sup>3</sup> Further, the  $^{31}\text{P}$   $\delta$  values closely correspond for a given metal in the series 10–12 and 5–9, and the same is true for 2–4 and 5–9 except for the uncoordinated  $\text{PC}_3$  moiety in 2–4. The single  $^1\text{H}$  NMR resonance in this table for all three types of compounds also gives credence to the postulated cyclic nature of 5–9, since the protons in such species are expected to be chemically equivalent in contrast to an acyclic oligomer. Moreover, the proton peaks of 5–9 are broad ( $\sim 25$  Hz) symmetrical multiplets stemming from the "virtual" coupling expected from these  $\text{AA}'\text{A}''\text{A}'''\text{MM}'\text{M}''\text{M}'''\text{X}_6\text{X}_6'\text{X}_6''\text{X}_6'''$  spin systems. Efforts to obtain molecular weight data by osmometry failed owing to facile decomposition in pyridine, the only solvent found in which appropriate solubility could be achieved. In the case of 6, however,

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(2) Bertrand, R. D.; Allison, D. A.; Verkade, J. G. *J. Am. Chem. Soc.* 1970, 92, 71.

(3) The proton-decoupled  $^{31}\text{P}$  peaks of 5–9 are about 200 Hz wide and are attributable to an  $\text{AA}'\text{A}''\text{A}'''\text{MM}'\text{M}''\text{M}'''$  system, which could not be resolved.

## Scheme I<sup>a</sup>

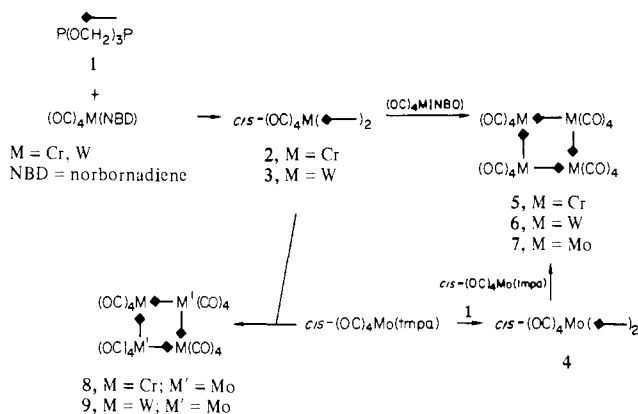


Table I. NMR Data<sup>a</sup>

compound	$^{31}\text{PO}_3$ , $\delta$	$^{31}\text{PC}_3$ , <sup>a</sup> $\delta$	$^1\text{H}$ , $\delta$
$\text{P}(\text{OCH}_2)_3\text{P}$ (1)	90.0	-67.0	4.45dd <sup>b</sup>
<i>cis</i> -(OC) <sub>4</sub> Cr(1) <sub>2</sub> (2)	157.4	-68.1	4.91 <sup>c</sup> dt <sup>c,d,e</sup>
(OC) <sub>5</sub> Cr(1)Cr(CO) <sub>5</sub> (10)	155.5 <sup>c</sup>	8.2 <sup>c</sup>	5.00dd <sup>c</sup>
<i>cyclo</i> -[(OC) <sub>4</sub> Cr(1)] <sub>4</sub> (5)	160.5	11.9	4.80m
<i>cis</i> -(OC) <sub>4</sub> Mo(1) <sub>2</sub> (4)	133.5	-68.4	4.77 <sup>c</sup> dt <sup>c,e,f</sup>
(OC) <sub>5</sub> Mo(1)Mo(CO) <sub>5</sub> (11)	131.0 <sup>c</sup>	-18.9 <sup>c</sup>	4.98dd <sup>c</sup>
<i>cyclo</i> -[(OC) <sub>4</sub> Mo(1)] <sub>4</sub> (7)	133.6	-14.6	4.85m
<i>cis</i> -(OC) <sub>4</sub> W(1) <sub>2</sub> (3)	109.9	-68.6 <sup>g</sup>	4.88 <sup>c</sup> dt <sup>c,e,g</sup>
(OC) <sub>5</sub> W(1)W(CO) <sub>5</sub> (12)	108.4 <sup>c</sup>	-36.4 <sup>c</sup>	4.82dd <sup>c</sup>
<i>cyclo</i> -[(OC) <sub>4</sub> W(1)] <sub>4</sub> (6)	112.6	-33.5	4.80m
<i>cyclo</i> -[(OC) <sub>4</sub> Cr(1)Mo(1)(CO) <sub>4</sub> ] <sub>2</sub> (8)	159.8	-11.9	4.77m
<i>cyclo</i> -[(OC) <sub>4</sub> W(1)Mo(1)(CO) <sub>4</sub> ] <sub>2</sub> (9)	111.2	-12.6	4.82m

<sup>a</sup> Positive chemical shifts are downfield from  $\text{H}_3\text{PO}_4$  external standard. Measurements were made in  $\text{CDCl}_3$  unless specified otherwise. <sup>b</sup>  $(\text{CD}_3)_2\text{CO}$ . <sup>c</sup>  $\text{CH}_3\text{CN}$ . <sup>d</sup>  $^2J_{\text{PH}} = 8.4$ ,  $^3J_{\text{PH}} = 5.3$  Hz. <sup>e</sup> The outer lines of the apparent triplet are separated by  $^3J_{\text{PH}} + ^5J_{\text{PH}}$ , but  $^5J_{\text{PH}}$  is very small as is suggested by the spectrum of *cis*-(OC)<sub>4</sub>Mo[P(OCH<sub>2</sub>)<sub>3</sub>P][P(CH<sub>2</sub>O)<sub>3</sub>P], a ligand isomer of 4 in which all the P atoms are chemically different. No splitting due to  $^5J_{\text{PH}}$  could be detected in the  $^1\text{H}$  NMR spectrum ((CH<sub>3</sub>CN)PC<sub>3</sub>-bound 1,  $\delta$  4.84 dd,  $^3J_{\text{PH}} = +5.3$ ,  $^2J_{\text{PH}} = +8.5$  Hz; PC<sub>3</sub>-bound 1, 4.46 t,  $^3J_{\text{PH}} = ^2J_{\text{PH}} = +2.6$  Hz). The  $^{31}\text{P}$  NMR spectrum and the coupling signs were obtained by INDOOR techniques described earlier.<sup>2</sup> Each of the 12  $^{31}\text{P}$  peaks is a septet owing to coupling to six equivalent protons (MoPO<sub>3</sub>, 133.3 dd,  $^3J_{\text{PP}} = -6.0$  Hz,  $^2J_{\text{PP}} = 138.0$ ; PO<sub>3</sub>, 88.0 d,  $^3J_{\text{PP}} = +20.0$  Hz; MoPC<sub>3</sub>, -13.1 dd,  $^3J_{\text{PP}} = +20.0$  Hz,  $^2J_{\text{PP}} = 138.0$ ; PC<sub>3</sub>, -69.3,  $^3J_{\text{PP}} = -6.0$  Hz). <sup>f</sup>  $^2J_{\text{PH}} = 9.0$ ,  $^3J_{\text{PH}} = 5.0$  Hz. <sup>g</sup>  $^2J_{\text{PH}} = 8.5$ ,  $^3J_{\text{PH}} = 5.5$  Hz. <sup>h</sup>  $J_{\text{WP}} = 402.8$  Hz. This value is in the range reported for other P(OR)<sub>3</sub> ligands in (OC)<sub>5</sub>WL complexes (Verkade, J. G. *Coord. Chem. Rev.* 1972, 9, 1).

the parent ion was clearly observed by FAB/MS.<sup>4</sup> Although the cyclic compounds 5–9 might be expected to show two sets of carbonyl bands in their IR spectra because of PC<sub>3</sub> and PO<sub>3</sub> coordination to the metals, this is not observed. Only 7 displays two A<sub>1</sub><sup>2</sup> peaks. This lack of band doubling is not unprecedented, however, since complete band superposition was observed in the IR spectra of 10–12.<sup>2</sup>

The 1:1 stoichiometry of the reactants in the reactions in which 5–7 are formed (see Scheme I) coupled with the high yields in which these compounds are realized are consistent with cyclic tetramer formation. The unusually high yields of these compounds probably arise primarily from the opportunity for the unligated phosphorus in the final intermediate D shown in Scheme II to cyclize into a strainless macrocyclic ring structure by reacting with a nearby reactive metal center. Because of the 90° angle between

(4) We thank Steve Vesey for these data obtained at the MCMNS Regional NSF Facility, University of Nebraska, Lincoln, NE.