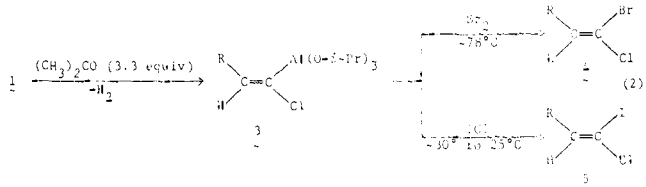
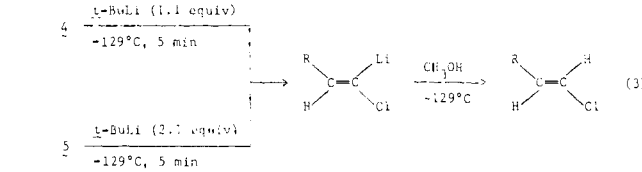


The configurations of the 1,1-dihalo olefins obtained were established through their conversion into the known *trans*-1-chloro-1-alkenes. This was accomplished by sequential treatment of **4** or **5** dissolved in a mixture of THF-ether-*n*-pentane (4:4:1)[6] and cooled to −129 °C (*n*-pentane-liquid N_2 bath) with precooled solutions of *tert*-butyllithium in *n*-pentane and methanol in ether. After workup, GLC analysis revealed the nearly exclusive formation of the *trans*-chloro-1-alkenes in >85% yields (eq 3).



A typical procedure for the preparation of **4** (R = *n*-C_4H_9) is as follows. To a solution of $LiAlH_4$ (1 M, 20 mmol) in THF cooled to −30 °C ($CaCl_2$-H_2O-dry ice)[11] was added 1-chloro-1-hexyne (20 mmol)[5] while the temperature was maintained below −25 °C during the addition. After the mixture was stirred for an additional 15 min, it was brought to 0 °C (ice bath) and stirred for 90 min. Dry acetone (66 mmol) was then added dropwise over a 20-min period while the temperature was maintained below 10 °C. After 1 hr, the reaction mixture was cooled to −78 °C and then treated dropwise with a solution of bromine (22 mmol) in methylene chloride (10 mL). The mixture was allowed to warm to room temperature in the dark and then was slowly poured into a mixture of 10% HCl (80 mL), 10% aqueous $NaHSO_3$ (10 mL), *n*-pentane (20 mL), and ice (50 g). After extraction with *n*-pentane, the combined organic extract was washed with 10% HCl and saturated aqueous sodium bicarbonate and then treated with a few crystals of 2,6-di-*tert*-butyl-*p*-cresol (BHT). Drying ($MgSO_4$) and distillation from a small amount of $CaCO_3$ afforded a 78% yield of (*Z*)-1-bromo-1-chloro-1-hexene. GLC examination on a glass capillary column (SE-30, 90 m, 80 °C)[12] revealed that the compound was 97% isomerically pure. To prevent isomerization from occurring, it is important to treat the pure 1,1-dihalo olefins distillate immediately with a few crystals of BHT.

Acknowledgment. The authors thank the National Science Foundation for support of this investigation.

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(2) For example, hydroalumination of 1-chloro-1-octyne with diisobutylaluminum hydride resulted, after hydrolysis, in a complex mixture of products. It is noteworthy that the reaction of 1-bromo-2-phenylethyne with diisobutylaluminum hydride afforded nearly exclusively phenylacetylene (Eisch, J. J.; Foxton, M. W. *J. Org. Chem.* **1971**, *36*, 3520), whereas its reaction with $LiAlH_4$ in the presence of $AlCl_3$ led to the formation of *trans*-styryl bromide in 40–60% yields after a hydrolytic workup (Kruglikova, R. I.; Kravets, L. P.; Unkovskii, B. V. *Zh. Org. Khim.* **1975**, *11*, 263).

(3) It has been reported that the reactions of 1-chloroalk-1-yn-3-ols with $LiAlH_4$ lead, after workup, to 1-chloroalk-1-en-3-ols. The trans structure has been tentatively assigned to these. Julia, M.; Surzur, J.-M. *Bull. Soc. Chim. Fr.* **1956**, 1615.

(4) Hydroalumination of the 1-chloro-1-alkynes was done using 1.0 equiv of $LiAlH_4$. Use of 0.5 equiv of $LiAlH_4$ resulted in somewhat lower yields of the α-chloroalkenylaluminum compounds.

(5) The 1-chloro-1-alkynes were prepared as follows. The hexane was stripped from a solution of *n*-butyllithium in hexane (0.21 mol, 1.72 M) under reduced pressure at 25 °C and then was replaced with THF (200 mL) at −78 °C. To dissolve the *n*-butyllithium, the reaction mixture was brought to −25 °C. Then it was cooled to −78 °C and treated sequentially with the 1-alkyne (0.20 mol) and *N*-chlorosuccinimide (0.23 mol) at −25 °C. The resulting mixture was maintained for 2 h at −25 °C and then was stirred for an additional 4 h at room temperature (Zweifel, G.; Murray, R. E., unpublished results).

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(9) Direct treatment of **1** with 2.5 equiv of bromine in methylene chloride at −78 °C also gave high yields of the simple 1-bromo-1-chloro-1-alkenes **4**. However, with α-chloroalkenylalanates containing functional groups, prior treatment with acetone was necessary to obtain high yields of **4**.

(10) Interestingly, direct iodination of **1** (R = *n*-C_4H_9) with 2.5 equiv of iodine did not produce the anticipated 1-chloro-1-iodo-1-hexene but yielded after workup a mixture of iodo-1-hexene and 1-hexyne. Also, treatment of **3** with 1 equiv of iodine afforded **5** in only modest yields.

(11) Bryan, W. P.; Byrne, R. H. *J. Chem. Educ.* **1970**, *47*, 361.

(12) GLC analyses were performed on J & W glass capillary columns.

George Zweifel,* William Lewis, Harry P. On
Department of Chemistry, University of California
Davis, California 95616
Received December 14, 1978

Biosynthesis of Vitamin B_1 in Yeast. Origin of the Thiazole Unit

Sir:

More than forty years after the elucidation of the structure of vitamin B_1 (thiamin),[1,2] the primary precursors of its thiazole unit (**5**) are still in dispute[3] and a chemically rational hypothesis of the biosynthesis of this unit has not yet been formulated. We now advance such a hypothesis and present some evidence which is consistent with it.

It is our view that, in yeast, the thiazole unit of vitamin B_1 is derived from one of the stereoisomers of the Schiff base **3**, which is generated by condensation of glycine (**1**) with a phosphoketopentose (**2**). The Schiff base **3** is converted into the thiazole derivative **4**[5] in a multistep sequence (Scheme I) comprising dehydration (or elimination of phosphate) (A), dehydration and tautomerization (B and C), and addition of sulfur (D), followed by ring closure (E) and concerted decarboxylation and dehydration. Several variants of this route are shown in Scheme I.

In support of this scheme, we now present experimental evidence which demonstrates the participation of a C_5 unit (**2**) derived from glucose in the biosynthesis of the thiazole unit of thiamin in yeast. The incorporation of C-2 and N of glycine (**1**) into the thiazole unit of thiamin in yeast, in accord with Scheme I, has been documented.[4,10]

Origin of the C_5 Unit 2. Yeast (*Saccharomyces cerevisiae*) does not utilize ribose or other pentoses[11] when these are supplied to the culture medium. Evidence for the participation of a pentose in thiamin biosynthesis was therefore obtained indirectly, by testing the mode of incorporation of glucose and fructose. These hexoses are known to be utilized and to yield pentoses in vivo. In separate experiments,[12] D-[1-^{14}C]-, D-[2-^{14}C]-, and D-[6-^{14}C]glucose and D-[1-^{14}C]fructose were administered to growing yeast cultures (*S. cerevisiae* A.T.C.C. 24903)[13] at the onset of logarithmic growth. The cells were collected when logarithmic growth had ceased and radioactive thiamin was isolated[4] by carrier dilution. Bisulfite cleavage[9] yielded the thiazole moiety (5-(β-hydroxyethyl)-4-methylthiazole) (**5**) as an oil, some of which was oxidized[14] to 5-formyl-4-methylthiazole[15] (isolated as the semicarbazone[15]) and some of which was converted, via the 5-(β-chloroethyl) derivative,[16,17] into the 5-(-β-phthalimidoethyl) derivative.[18] This

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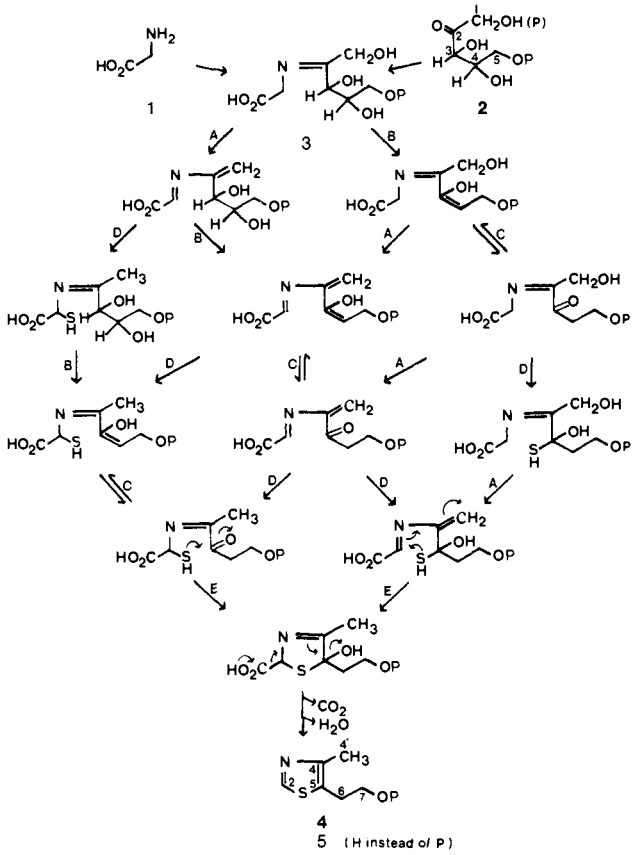
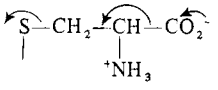
Scheme I. Biogenesis of the Thiazole Unit (4) of Vitamin B_1, in yeast, from Glycine (1) and a Phosphoketopentose (2)[a]



[a] (A) Dehydration (or elimination of phosphate) (P ≡ $-PO(OH)_2$); (B) dehydration; (C) tautomerization; (D) addition of sulfur. The S donor which, for simplicity, is represented as H_2S, is more likely to be cysteine; if so, ring closure (E) would be accompanied by the fragmentation process



was purified to constant radioactivity and was further degraded. Kuhn-Roth oxidation[19] yielded acetic acid (C-4′,C-4 of the thiazole moiety) (isolated as the α-naphthylamide[20]) which was converted into methylamine (C-4′) (isolated as *N*-methylphthalimide[21]) by a Schmidt reaction.[22] Acid permanganate oxidation[23] of the phthalimidoethyl derivative gave N-phthaloyl-β-alanine (C-5,-6,-7).

The results of these experiments are shown in Table I. Activity from D-[1-^{14}C]glucose and from D-[1-^{14}C]fructose is located almost entirely (>90%) in the *C*-methyl group (C-4′) of the thiazole unit (*N*-methylphthalimide). The remaining activity (<10%) was located at C-5, C-6, and/or C-7 (*N*-phthaloyl-β-alanine). Lack of material precluded further degradation to determine the exact location of label. It is very likely, however, that C-7 is the site of labeling. A complementary result was obtained when D-[6-^{14}C]glucose served as substrate. The label was located mostly at C-7 (~80%), while the *C*-methyl group contained the remaining activity (~20%). The label from D-[2-^{14}C]glucose was confined to the C_2 unit, C-4,C-4′ (acetyl-α-naphthylamine, 96%), and was almost equally divided between these two carbons (C-4′, methylphthalimide, 46%; C-4, 50%, by difference).

Several phosphoketopentoses (**2**), all metabolically interconvertible without skeletal rearrangement, are derivable from glucose in the course of metabolism in yeast.[24] Thus, D-ribulose 5-phosphate (**2**, 3-*R*, 4-*R*) is generated from D-glucose, via D-glucose-6-phosphate and 6-phospho-D-gluconic acid, by oxidation and decarboxylation of the latter. Generation of **2** in this way would, in accord with observation (Table I), deliver activity from [6-^{14}C]glucose into C-7 and from [2-^{14}C]glucose into C-4′ of the thiazole nucleus, but would, contrary to observation, lead to unlabeled thiazole from [1-^{14}C]glucose. D-Xylulose 5-phosphate (**2**, 3-*S*, 4-*R*) is produced by a transketolase reaction which transfers the C_2 unit, C-1,C-2, of fructose 6-phosphate (derivable from glucose or fructose) onto glyceraldehyde 3-phosphate, which is derived, primarily, from the C_3 unit, C-4,C-5,C-6, of glucose or fructose, via fructose 1,6-diphosphate. Generation of **2** by this route would deliver activity from [6-^{14}C]glucose into C-7, from [2-^{14}C]glucose into C-4, and from [1-^{14}C]glucose and [1-^{14}C]fructose into C-4′ of the thiazole nucleus. Neither the oxidative route via 6-phospho-D-gluconic acid by itself nor the transketolase pathway alone can account for the observed distribution of activity, derived from labeled hexoses, within the C_5 unit of the thiazole. However, the two pathways operating concurrently[25] will lead to a pool of labeled pentoses which, upon metabolic interconversion,[24] would show a labeling pattern corresponding to that observed in the C_5 unit of the thiazole moiety of thiamin, isolated in each of the four experiments.

Several other possibilities for the generation of **2**, e.g., a contraction of the hexose chain similar to that occurring in the biosynthesis of streptose,[27] followed by elimination of C-3 or C-4 of glucose, are less likely but cannot yet be ruled out.

Glycine (1). It was reported in 1967 that glycine (**1**) is implicated in the biosynthesis of the thiazole moiety of thiamin in yeast.[10] This report contradicted accepted dogma that the thiazole unit is derived from methionine and alanine.[28] We have recently shown[4] that published results[28] concerning the incorporation, in yeast (*S. cerevisiae* 39916, H. J. Bunker), of the *S*-methyl group of methionine into C-2 of the thiazole unit are in error. We also found that the α-carbon atom of tyrosine, reportedly the source of C-2 of the thiazole unit in bacteria,[29-31] does not serve as a precursor in yeast (*S. cerevisiae* A.T.C.C. 24903). We demonstrated that, in these two yeast strains, the methylene carbon of glycine serves as the specific precursor of C-2 of the thiazole unit of thiamin, and of no other

Table I. Incorporation of Hexoses into the Thiazole Unit of Thiamin

products	C atoms of the thiazole moiety	relative specific activity %[a] D-[6-^{14}C] glucose[b]	D-[2-^{14}C] glucose[b]	D-[1-^{14}C] glucose[b]	D-[1-^{14}C] fructose[b]
5-(β-hydroxyethyl)-4-methylthiazole[c]	all	100 ± 2	100 ± 1	100 ± 3	100 ± 2
5-formyl-4-methylthiazole	all except C-7	17 ± 1			
acetyl-α-naphthylamine	C-4, -4′		96 ± 1	92 ± 3	84 ± 2
N-methylphthalimide	C-4′	16 ± 2	46 ± 1	93 ± 2	91 ± 3
N-phthaloyl-β-alanine	C-5, -6, -7	84 ± 2		6 ± 1	5 ± 1

[a] The specific activity (disintegrations per minute/millimole) of the thiazole derivative **5**, derived from thiamin isolated by carrier dilution in each of the four experiments, is normalized to 100. The specific activity of each degradation product is expressed as percent of the molar specific activity of the thiazole derivative from which it was obtained. [b] Precursor. [c] Assayed as the phthalimidoethyl derivative.

carbon atom.[4] This evidence reinforces the findings of Linnett and Walker[10] that, in yeast (*S. cerevisiae* N.C.Y.C. 1062), the methylene group of glycine supplies C-2, and the amino nitrogen of glycine supplies the nitrogen atom of the thiazole nucleus of vitamin B_1. It is very likely therefore that an intact C-N unit, derived from glycine (**1**), enters the thiazole nucleus. Scheme I is consistent with these results.

The origin of the thiazole moiety of thiamin in bacteria differs from that in yeast in two respects. Firstly, the unit C-2,N is derived from tyrosine[29,30,32] and not from glycine. A simple modification[33] of the present biogenetic scheme can accommodate this difference. Secondly, the C_5 precursor is generated by condensation of 3-phosphoglyceraldehyde with a C_2 unit derived from C-3,C-2 of pyruvate[34] rather than from C-1,C-2 of fructose 6-phosphate. An acyloin condensation was proposed to account for the formation of the C_5 unit from these precursors. The distribution of label observed in the present work is not consistent with such a proposal.

Acknowledgment. This work was supported by the National Research Council of Canada.

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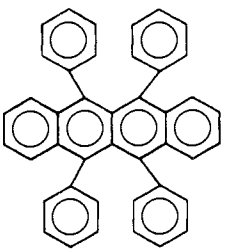
Robert L. White, Ian D. Spenser*
Department of Chemistry, McMaster University
Hamilton, Ontario, Canada L8S 4M1
Received April 6, 1979

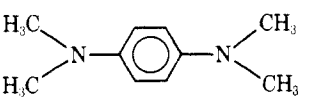
Quenching of the Fluorescent State of Rubrene Directly to the Ground State

Sir:

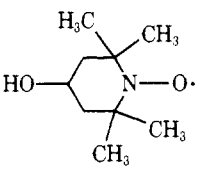
Until recently it was believed that exciplexes of aromatic hydrocarbons decay in a fashion similar to that of their parent aromatic monomers.[1] Here fluorescence and intersystem crossing account for most of the excitation energy; that is $\Phi_f + \Phi_{isc} \simeq 1.0$ is often a good rule. We report in this communication evidence in two systems where singlet quenching leads to internal conversion which is nearly quantitative. Quenching of fluorescence of rubrene (RUB) by *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) and by 4-hydroxy-2,2,6,6-tetramethylpiperidinoxy free radial (R·) has been studied.



RUB



TMPD



R·

The Stern-Volmer dependence of rubrene fluorescence quenching was determined from the steady-state spectral data. For TMPD and for R· the apparent rate constants in degassed benzene are $k_q = 6.0 \times 10^9$ and $k_q = 3.3 \times 10^9$ M^{-1} s^{-1}, respectively. In neither case was a long-wavelength new emission observed. The absorption spectra of the solutions show no evidence of complexing in the ground state. To test the possibility that quenching resulted in rubrene triplet formation, a nanosecond laser flash experiment was performed. The method used has been described previously.[2] Benzene solutions with and without TMPD were examined in 1-cm cells. The quenched sample contained TMPD such that the rubrene fluorescence was reduced by 75%. Rubrene was selectively excited at 488 nm using a Coumarin 102 dye laser pumped by a 337-nm nitrogen laser. No transient absorption attributable to a short-lived exciplex or the TMPD cation radical was observed. Also, we observed no rubrene triplet even though triplet formation as small as ~5% could have been detected. For the unquenched sample, our result is consistent with the reported rubrene fluorescence yield in benzene of 1.0.[3] We did observe a broad, structureless absorption from 400 to 450 nm which decayed with a lifetime approximately equal to that of the rubrene fluorescence. This was assigned to an absorption of the lowest excited singlet state of rubrene.[4] The experiment was repeated

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