

**Figure 1.** Diagram of the oxidative sulfitolysis of carbonyl(bis(methionyl)insulin (CBM-insulin), **1**, followed by the reduction of the *S*-sulfonate **2** with dithiothreitol (DTT) to yield the sulfhydryl form **3** which in turn is oxidized in air to yield the CBM-insulin.

Ellman reagent.<sup>5</sup> The eluate was diluted to give 0.1 mg of reduced protein per milliliter, the pH was adjusted to 9.5 and the solution contained in an erlenmeyer flask coated with bovine serum albumin<sup>6</sup> was stirred in air at room temperature for 10 hr. The reoxidation mixture was lyophilized and the residue chromatographed on a Sephadex G50 (fine) column (2.5 × 150 cm) which was equilibrated and developed with 0.05 *M* NH<sub>4</sub>HCO<sub>3</sub>. The material in the main peak (*V*<sub>e</sub> of 390 ml) was lyophilized to yield 6.8–7.8 mg (75–86%) of CBM-insulin **1** which was shown to be identical with the starting material by cellulose acetate and sodium dodecyl sulfate-gel electrophoresis, circular dichroic (CD) spectrum, and immunoassay.<sup>7</sup> The rate of reoxidation of the sulfhydryls of the reduced CBM-insulin as well as the CD spectra at various stages of reoxidation are shown in Figure 2. The CD spectra indicate the presence of at least one isosbestic point located at 204 nm (Figure 2) which suggests that only two conformations, the fully reduced and the completely oxidized, contribute to the CD spectra. However, more extended studies are necessary to confirm this hypothesis.

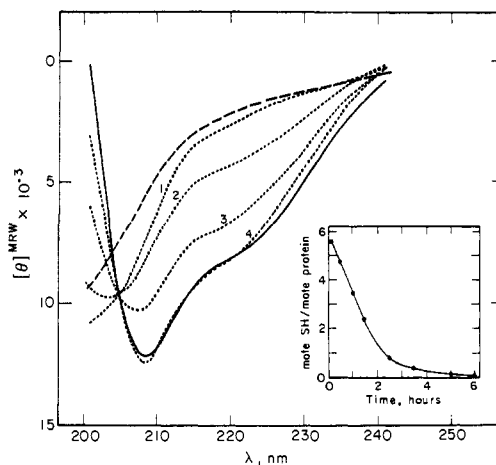
CBM-insulin which had been subjected to the above reduction and reoxidation (12.4 mg) was treated with CNBr (344 mg) in 3 ml of 70% formic acid at room temperature for 18 hr. Insulin was isolated from the reaction mixture as previously described<sup>1</sup> to give 8.4 mg (68%) of product which was identical with the native hormone in amino acid analysis, amino end groups, and CD spectrum. The product yielded a crystalline zinc complex and gave the same fragmentation pattern as insulin on chymotrypsin digestion.<sup>8</sup> This conversion

(5) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).

(6) D. F. Steiner and J. L. Clark, *Proc. Nat. Acad. Sci. U. S. A.*, **60**, 622 (1968).

(7) C. N. Hales and P. J. Randle, *Biochem. J.*, **88**, 137 (1963).

(8) W.-D. Busse and H.-G. Gattner, *Hoppe-Seyler's Z. Physiol. Chem.*, **354**, 147 (1973); D. Levy, *Biochim. Biophys. Acta*, **310**, 406 (1973).



**Figure 2.** Mean residue weight ellipticity ( $[\theta]^{MRW}$ ) as a function of wavelength at a concentration of 0.1 mg/ml in 0.05 *M* phosphate at pH 9.5 at 27°C for the *S*-sulfonate of CBM-insulin **2** (---), for CBM-insulin **1** (—), and for reoxidation mixtures of the reduced CBM-insulin **3** (.....) at various time intervals in which the re-oxidation mixture contained moles of sulfhydryls per mole of protein of (1) 5.25 at 15 min, (2) 3.5 at 60 min, (3) 2.0 at 105 min, and (4) 0.1 at 360 min. Insert. Rate of disappearance of sulfhydryls vs. time in the reoxidation of reduced CBM-insulin **3** at 0.1 mg/ml in 0.05 *M* phosphate at pH 9.5 at room temperature.

to insulin is an unambiguous demonstration that the correct pairing of disulfide bonds occurred during reoxidation of the reduced CBM-insulin.

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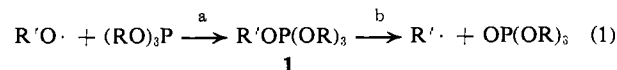
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### Evidence for the Much Greater Rate of $\beta$ -Scission Involving the *tert*-Butoxy Group Compared to Permutational Isomerization in a Cyclic Tetraalkoxyphosphoranyl Radical

Sir:

Esr<sup>1</sup> and chemical studies<sup>2</sup> have provided evidence that the oxidation reactions (eq 1) of alkoxy radicals



with trialkyl phosphites proceed *via* discrete phosphoranyl radical intermediates, **1**. The product forming step in reaction 1 is termed  $\beta$ -scission. Near-trigonal bipyramidal structures, **2**, are usually proposed<sup>1</sup> for **1**. Chemical evidence<sup>2a</sup> has been presented which shows that  $R'O\cdot$  in reaction 1 does not enter the trigonal bipyramidal intermediate **2** in a configurationally random way and by analogy to pentavalent phos-

(1) (a) G. B. Watts, D. Griller, and K. U. Ingold, *J. Amer. Chem. Soc.*, **94**, 8784 (1972); (b) P. J. Krusic, W. Mahler, and J. K. Kochi, *ibid.*, **94**, 6033 (1972); (c) A. G. Davies, D. Griller, and B. P. Roberts, *J. Chem. Soc., Perkin Trans. 2*, 993 (1972); (d) *ibid.*, 2224 (1972).

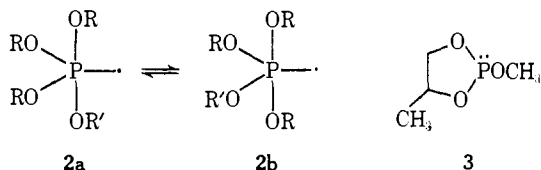
(2) (a) W. G. Bentrude and T. B. Min, *J. Amer. Chem. Soc.*, **94**, 1025 (1972); (b) W. G. Bentrude and R. A. Wielessek, *ibid.*, **91**, 2406 (1969).

phorus systems<sup>3</sup> has been presumed<sup>1,2</sup> to enter axially (2a).

What is not known in such systems with any certainty is whether  $\beta$ -scission is stereospecific (axial or equatorial only) or only stereoselective ( $k_{\beta}(\text{ax}) > k_{\beta}(\text{eq})$  or  $k_{\beta}(\text{eq}) > k_{\beta}(\text{ax})$ ) and whether some sort of permutational isomerization process such as  $2a \rightarrow 2b$  can compete with  $\beta$ -scission.<sup>4</sup> Somewhat conflicting ideas have appeared in the literature; e.g., on the basis of the relative stabilities of a series of  $R_xP(\text{OEt})_{4-x}$  as determined by esr,<sup>1b,5</sup> it has been proposed<sup>1a</sup> that pseudorotation may be necessary prior to  $\beta$ -scission which takes place only,<sup>1a</sup> or at least more rapidly,<sup>1d,5</sup> at the equatorial position.<sup>6</sup> In other instances  $\beta$ -scission from both axial and equatorial positions following rapid pseudorotational equilibration has been suggested<sup>1d</sup> with axial  $\beta$ -scission *slower* than its equatorial equivalent. Product data also have been interpreted<sup>2a</sup> in terms of a model involving a single intermediate with stereoselective  $\beta$ -scission occurring *faster* axial than equatorial and both much faster than pseudorotation.

We present here stereochemical evidence that  $\beta$ -scission of certain cyclic tetraalkoxy phosphoranyl radicals does not require a prior pseudorotation. In fact it is found that when the C-O bond of a *tert*-butoxy group is being cleaved, *pseudorotation or its permutational equivalent does not occur with a rate even competitive with  $\beta$ -scission*. Evidence will be reported elsewhere<sup>7</sup> that the same is true in *acyclic* systems in which a benzyloxy group undergoes  $\beta$ -scission.

Reaction of two different *cis/trans* ratios of the cyclic phosphite **3** (2-methoxy-4-methyl-1,3,2-dioxaphospho-



lane) with *t*-BuO $\cdot$  was found to be nearly stereospecific with retention of configuration about phosphorus. (See Table I.) The *cis/trans* ratios<sup>8</sup> of phosphite **3** and of product phosphates (7/8) were invariant with extent of reaction (10–99%). The stereochemistry of the reaction was determined by comparison with that of oxidation of **3** with N<sub>2</sub>O<sub>4</sub> which is known to be retentive.<sup>9</sup> The above ratios were followed by integration of the methyl and methoxy pmr resonances (*cis*-**3**  $\delta$

(3) See K. Mislow, *Accounts Chem. Res.*, **3**, 321 (1970); F. H. Westheimer, *ibid.*, **1**, 70 (1968); F. Ramirez, *ibid.*, **1**, 168 (1968); E. L. Muetterties, *ibid.*, **3**, 266 (1970); P. Gillespie, F. Ramirez, I. Ugi, and D. Marquarding, *Angew. Chem., Int. Ed. Engl.*, **12**, 91 (1973).

(4) A limited amount of evidence suggests that a permutational isomerization may be rapid on the esr time scale. See P. J. Krusic and P. Meakin, *Chem. Phys. Lett.*, **18**, 347 (1973). Recent results question the previously accepted mobility of ligands in PF<sub>4</sub>. See S. P. Mishra and M. C. R. Symons, *J. Chem. Soc., Chem. Commun.*, 279 (1974).

(5) A. G. Davies, R. W. Dennis, D. Griller, and B. P. Roberts, *J. Organometal. Chem.*, **40**, C33 (1972).

(6) It also is suggested<sup>1d</sup> that substituent electronegativities may be important because of certain electronic effects on scission reactions of phosphoranyl radicals.

(7) W. G. Bentrude and T. B. Min, submitted for publication.

(8) The *cis* and *trans* geometries of the isomers of **3** were established by complete pmr analysis of the ABKX spin systems (decoupled ring Me), the details of which will be reported elsewhere (H.-W. Tan, unpublished results).

(9) D. Z. Denney, G. Y. Chen, and D. B. Denney, *J. Amer. Chem. Soc.*, **91**, 6838 (1969); J. Michalski, A. Okruszek, and W. Stec, *J. Chem. Soc., D*, 1495 (1970); J. A. Mosbo, and J. G. Verkade, *J. Amer. Chem. Soc.*, **95**, 4659 (1973).

**Table I.** Stereochemistry of *tert*-Butoxy Radical Oxidation of *cis*- and *trans*-2-Methoxy-4-methyl-1,3,2-dioxaphospholane, **3**<sup>a</sup>

Cis/trans <b>3</b> (initial)	Product ratio, 7/8	Yield oxides <sup>b</sup> 7 and 8 (%)
46/54 <sup>c</sup>	45/55 <sup>b</sup> (46/54) <sup>c</sup>	72
33/67 <sup>c</sup>	33/67 <sup>b</sup> (33/67) <sup>c</sup>	71

<sup>a</sup> Reactions in degassed C<sub>6</sub>H<sub>6</sub> at 17°. *t*-BuO $\cdot$  formed by photolysis of di-*tert*-butyl peroxide, medium-pressure Hg lamp. [3] = 0.5 M and [peroxide] = 0.34 M. <sup>b</sup> By glc, sensitivity calibrated. <sup>c</sup> By pmr at 60 MHz. Consumption of **3**, 99%.

1.043 (CH<sub>3</sub>) 3.133 (CH<sub>3</sub>O); *trans*-**3**  $\delta$  0.892 (CH<sub>3</sub>), 3.128 (CH<sub>3</sub>O); **7**  $\delta$  0.748 (CH<sub>3</sub>), 3.398 (CH<sub>3</sub>O); **8**  $\delta$  0.795 (CH<sub>3</sub>), 3.412 (CH<sub>3</sub>O). The ratio 7/8 was confirmed by glc.

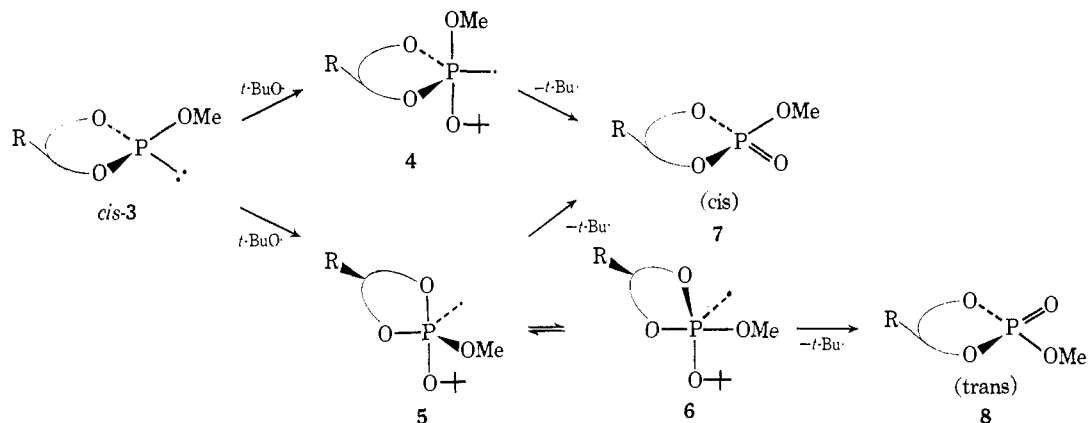
The mechanistic details of oxidation of cyclic phosphites with *t*-BuO $\cdot$  are outlined in Scheme I. Attack on the sterically least hindered faces of the pyramidal *cis* phosphite shown (axial *t*-BuO $\cdot$  introduction) leads to intermediates **4**, **5**, and the mirror image of **5** (not shown).  $\beta$ -Scission reactions of **4** or **5** yield product phosphate **7** of *retained* configuration about phosphorus. Note, however, that isomerization process **5**  $\rightarrow$  **6** gives an intermediate of similar energy to **5** but which forms on  $\beta$ -scission phosphate **8** of *inverted* configuration. (Reference 2 strongly suggests that  $\beta$ -scission can take place at apical and equatorial positions; thus **5**  $\rightarrow$  **8** should occur with ease.) Clearly, if **5**  $\rightarrow$  **6** were required prior to  $\beta$ -scission, phosphate **8** would have resulted to the extent that **5** rather than **4** were kinetically favored. Furthermore, isomerization **5**  $\rightarrow$  **6** in competition with **5**  $\rightarrow$  **7** would have resulted in at least reduced stereospecificity. Equilibration (**5**  $\rightleftharpoons$  **6**) more rapid than  $\beta$ -scission would have given the same 7/8 ratio in both experiments as required by the Hammett-Curtin principle.<sup>10</sup> In view of the propensity<sup>3</sup> of five-membered rings to be attached axial-equatorial rather than diequatorial in truly pentacovalent systems, it seems quite likely that **5** will be kinetically favored over **4**. Even statistically random attack on **3** would give **5** and its mirror image 67% of the time.

For several reasons the above systems should have a maximum propensity to undergo permutational isomerization in competition with  $\beta$ -scission. Processes analogous to **5**  $\rightarrow$  **6** are rapid in five-membered ring pentaalkoxy phosphorane systems.<sup>11</sup> Esr data for **9**<sup>1d</sup> and related phosphoranyl<sup>12</sup> can be interpreted in terms of a rapid equilibration of **9a** and **9b** on the esr time scale at -70°. (The observed triplets rule out a diequatorial ring structure like **4** for **9** which should yield a quintet.) Furthermore, the rate of  $\beta$ -scission from **9** occurs at a rate about five times slower at -60° than that for *t*-BuOP(OEt)<sub>3</sub>.<sup>1d</sup>  $\Delta G^\ddagger$  for *tert*-butoxy  $\beta$ -scission of *t*-BuOP(OEt)<sub>3</sub> at 17° is  $\sim 10.5$  kcal/mol.<sup>1a,d</sup>

(10) D. Y. Curtin, *Rec. Chem. Progr.*, **15**, 111 (1954).

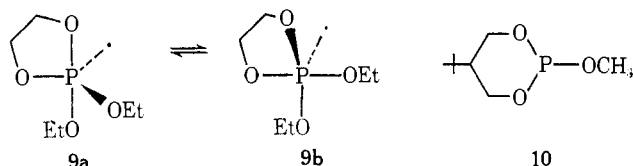
(11) For experimentally and theoretically based estimates of  $\Delta G^\ddagger$  for PF<sub>5</sub> (< 5 kcal/mol) see ref 14; P. Meakin, E. L. Muetterties, and J. P. Jesson, *J. Amer. Chem. Soc.*, **94**, 5271 (1972); R. Hoffmann, J. M. Howell, and E. L. Muetterties, *ibid.*, **94**, 3047 (1972); R. R. Holmes, *Accounts Chem. Res.*, **5**, 296 (1972). See C. H. Bushweller and H. S. Bilofsky, *Tetrahedron Lett.*, 2401 (1972), for the barrier ( $\sim 5$  kcal/mol) in homocubyltrimethylphosphorane, and D. Gorenstein, *J. Amer. Chem. Soc.*, **92**, 644 (1970), for the barrier (10 kcal/mol) to placing a five-membered ring CH<sub>2</sub> of the pentacovalent trimethyl phosphite-methyl vinyl ketone adduct (**13**) in an electronically disfavored apical position.

(12) R. W. Dennis and B. P. Roberts, *J. Organometal. Chem.*, **47**, C8 (1973); D. Griller and B. P. Roberts, *J. Chem. Soc., Perkin Trans. 2*, 1339 (1973).



Thus  $\Delta G^\ddagger$  for the isomerization  $5 \rightarrow 6$  appears to be  $>11$  kcal/mol since it is unable to compete with  $\beta$ -scission.

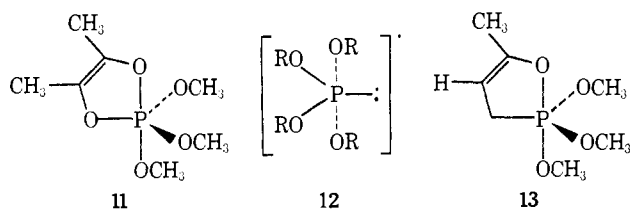
These findings may be compared with earlier studies<sup>13</sup> of the  $t\text{-BuO}\cdot$  oxidation of **10** which also proceeded



with retention at phosphorus. With **10** it could be argued, however, that the six-membered ring favors formation of **4** as is sometimes proposed<sup>14</sup> in pentacovalent phosphorus systems. Similarly the  $t\text{-BuO}\cdot$  oxidation of chiral  $n\text{-PrPMePh}$  was retentive in stereochemistry.<sup>13</sup> However, the rate of  $\beta$ -scission is not known in this system. *In none of these cases is there any evidence for permutational isomerization prior to or in competition with product formation.*

If a direct analogy is drawn between phosphoranyl radical systems and truly pentacovalent systems, then it is surprising that the apparent barrier to the process  $5 \rightarrow 6$  is so high. It is quite certain that for **11** the permutation analogous to  $5 \rightarrow 6$  has  $\Delta G^\ddagger$  several kilocalories per mole below 10 kcal/mol.<sup>11</sup>

The comparisons of barriers to permutational isomerizations for  $(\text{RO})_4\text{P}\cdot$  and  $(\text{RO})_5\text{P}$  should be of theoretical interest. It has been suggested to us by Professor Martin<sup>15</sup> that a better representation of **2** might be **12** in which the odd electron is in the apical



portion of an electron-deficient bonding system (three electrons), and a lone pair is equatorial. Such a bonding description would likely influence considerations of barriers for permutational isomerization of  $(\text{RO})_4\text{P}\cdot$ .

(13) W. G. Bentrude, J. H. Hargis, and P. E. Rusek, Jr., *Chem. Commun.*, 296 (1969).

(14) P. Gillespie, P. Hoffman, H. Khusacek, D. Marquarding, S. Pfohl, F. Ramirez, E. A. Tsolis, and I. Ugi, *Angew. Chem., Int. Ed. Engl.*, **10**, 687 (1971).

(15) J. C. Martin, University of Illinois, private communication.

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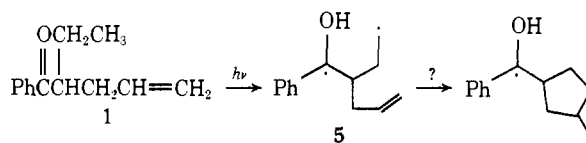
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### Photorearrangement of $\alpha$ -Allylbutyrophenone to 2-Phenyl-2-norbornanol. A Determination of 1,4-Diradical Lifetimes

Sir:

Two years ago we reported<sup>1</sup> that alkyl thiols can trap the 1,4-diradicals involved in Norrish type II photoelimination<sup>2</sup> without significantly quenching the reactive ketone triplet state.<sup>3</sup> The trapping measurements were sufficiently quantitative that diradical lifetimes could have been assigned unambiguously, except for serious discrepancies in literature values for the rate constants for reactions of alkyl radicals with thiols. Since diradicals are such important reaction intermediates in chemistry,<sup>4</sup> better estimates of their lifetimes seem highly desirable. Consequently we have prepared and irradiated  $\alpha$ -allylbutyrophenone, hoping first that  $\gamma$ -hydrogen abstraction would compete with intramolecular oxetane formation and second, that if the diradical formed by  $\gamma$ -hydrogen abstraction lived at least 1  $\mu\text{sec}$ , it might undergo measurable intramolecular cyclization to a cyclopentylmethyl structure.



Ketone **1** was prepared by treatment of the cyclohexenamine of butyrophenone with ethyl magnesium bromide and then with allyl bromide.<sup>5</sup> Upon irradiation

(1) P. J. Wagner and R. G. Zepp, *J. Amer. Chem. Soc.*, **94**, 287 (1972).

(2) R. G. W. Norrish, *Trans. Faraday Soc.*, **33**, 1521 (1937); P. J. Wagner, *Accounts Chem. Res.*, **4**, 168 (1971).

(3) R. G. Zepp and P. J. Wagner, *J. Chem. Soc., Chem. Commun.*, 167 (1972).

(4) (a) G. Jones II, *J. Chem. Educ.*, **51**, 175 (1974); (b) L. Salem and C. Rowland, *Angew. Chem. Int. Ed. Engl.*, **11**, 92 (1972); (c) L. M. Stephenson and J. I. Brauman, *J. Amer. Chem. Soc.*, **93**, 1988 (1971); (d) R. Hoffmann, S. Swaminathau, B. G. Odell, and R. Gleiter, *ibid.*, **92**, 7081 (1970).

(5) G. Stork and S. R. Dowd, *J. Amer. Chem. Soc.*, **85**, 2178 (1963).