dine–sulfur trioxide[7] (135 g, 0.85 mol) in DMF (700 mL) was added to a solution of glucose (156 g, 0.87 mol) dissolved in DMF (1 L) over 3 h at 25 °C. The mixture was stirred for another hour and concentrated in vacuo (0.1 mmHg) at 35–40 °C to remove DMF and pyridine. The oily residue was dissolved in water (1 L), adjusted to pH 7.5 (2 N KOH), and concentrated again to remove pyridine. The process was repeated until neutrality was permanent (2–3 times). The aqueous solution (400 mL) contained 0.5 mol of G-6-S[14] (58% yield) and was used directly for the enzymatic syntheses.

Glucose 6-Phosphate. Some methods for the preparation of G-6-P are summarized in Table I. The procedure for $POCl_3$–lutidine was as follows. Triethyl phosphate (250 mL) and freshly distilled $POCl_3$ (18 mL, 200 mmol) were mixed at 0 °C. To this solution was added slowly 2,6-lutidine (20 mL, 200 mmol) with stirring over 15 min at 0 °C followed by addition of glucose (36 g, 200 mmol). The reaction mixture was stirred at 25 °C for 5 h. Aliquots were taken, diluted 10 times with water, and heated on a steam bath for 20 min. G-6-P was determined enzymatically.[14] This assay showed that 32% of the glucose had been converted to G-6-P. If chloroform (80 mL) was used instead of triethyl phosphate, and glucose dissolved in DMF (36 g in 200 mL) was added slowly (over 1 h) to the $POCl_3$–lutidine solution, a 28% reaction yield was observed in 5 h.

Synthesis of (*S*)-Benzyl-α-d_1 Alcohol. To a 1-L solution containing G-6-S (0.2 mol), $MgSO_4$ (3 mmol), NAD (0.16 mmol), β-mercaptoethanol (2 mmol), and 20 U each of immobilized G-6-PDH from *L. mesenteroides* (based on NAD and G-6-S as substrates, 10 mL of gel) and horse liver alcohol dehydrogenase (HLADH, based on benzaldehyde and NADH as substrates, 0.5 mL of gel)[2] was added slowly benzaldehyde-α-d_1 (17.1 g, 0.16 mmol)[2] over 3–4 days. The solution was kept under argon at pH 7.6.[2] After 8 days, the reaction was complete and (*S*)-benzyl-α-d_1 alcohol was isolated as described previously:[2] 15 g, 130 mmol, 81% yield, 95% ee. The turnover numbers (TN) and residual activities were as follows: NAD, 1000, 90%; G-6-PDH, 1.6×10^6, 80%; HLADH, 1×10^7, 78%. The TN for NAD is based on the quantity of NAD added at the beginning of the reaction, not the quantity lost during the reaction.

Synthesis of *threo*-D_s(+)-Isocitrate. A 1-L solution containing G-6-S (0.2 mol), α-ketoglutarate (0.15 mol), $NaHCO_3$ (0.2 mol), $MgCl_2$ (5 mmol), $MnCl_2$ (1 mmol), NADP (0.1 mmol), β-mercaptoethanol (2 mmol), and 80 U each of immobilized G-6-PDH from yeast (based on NADP and G-6-S as substrates, 3 mL of gel) and isocitrate dehydrogenase (ICDH, 12 mL of gel) was kept under CO_2 at pH 7.6 with stirring for 5 days, and isocitrate was isolated as its barium salt as described previously.[2] The solid (38 g) contained 94% of *threo*-D_s(+)-isocitrate (91.5 mmol), corresponding to 61% yield. The TN and residual activities were as follows: NADP, 1500, 84%; G-6-PDH, 5×10^6, 81%; ICDH, 1×10^6, 84%.

Registry No. G-6-S, 79084-12-1; G-6-P, 79101-58-9; NAD, 53-84-9; NADP, 53-59-8; NADH, 58-68-4; NADPH, 53-57-6; (*S*)-benzyl-α-d_1 alcohol, 3481-15-0; benzaldehyde-α-d_1, 3592-47-0; barium *threo*-D_s-(+)-isocitrate, 79120-64-2; α-ketoglutarate, 64-15-3.

Decomposition of Triphenyl Phosphite Ozonide in the Presence of Spin Traps

William A. Pryor* and C. K. Govindan

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803

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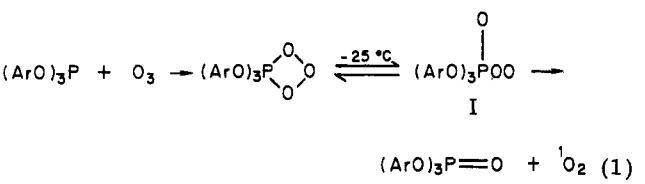
The decomposition of triphenyl phosphite ozonide (TPPO) at or above −30 °C in the presence of α-phenyl-*N*-*tert*-butylnitrone (PBN) leads to a peroxyl radical spin adduct, providing experimental evidence for the existence of a biradical during the decomposition of TPPO. Benzoyl-*tert*-butyl nitroxide also is formed during the decomposition. Singlet oxygen generated at −78 °C from TPPO and methanol–pyridine oxidizes nitrones to the corresponding acyl nitroxides. This oxidation is inhibited by β-carotene. Spin adducts are not observed from the reaction of TPPO with olefins at temperatures below the decomposition temperatures of TPPO.

Introduction

Triphenyl phosphite ozonide (TPPO), formed during the low-temperature ozonation of triphenyl phosphite, is a well-known thermal source for singlet oxygen.[1] On the basis of a study of the effects of substituents on the kinetics of decomposition, Stephenson and McClure[2] proposed that at temperatures above −25 °C TPPO exists in equilibrium with the "ring-opened"[3] form (I), which subsequently loses singlet oxygen (eq 1). The epoxidation of certain olefins[4,5] by TPPO has been attributed to a biradical form of intermediate (I) by Murray et al.[4]



Bartlett et al.[6] have reported that TPPO reacts with olefins at low temperatures, where it is thermally stable, to give products that resemble those from the reaction of singlet oxygen with olefins. However a detailed study of the distribution of products from different olefins led

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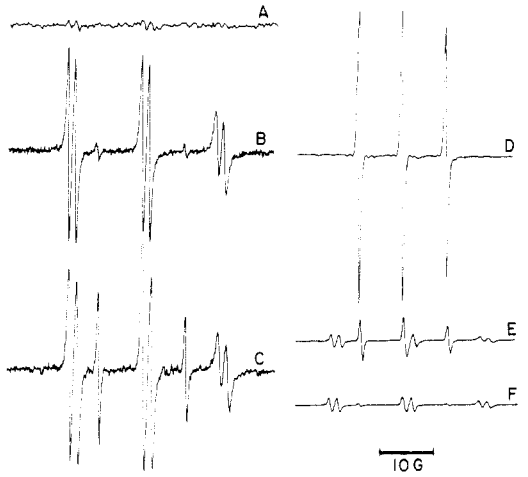
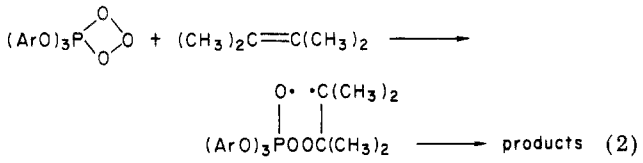


Figure 1. ESR spectra recorded at −70 °C on TPPO–PBN mixtures under various conditions. (A) TPPO–PBN mixture, after mixing at −78 °C. (B) ESR spectrum of the peroxyl radical spin adduct obtained during the decomposition of TPPO at −15 °C in the presence of PBN. (C) Spectrum obtained after the addition of methanol and pyridine at −78 °C to a sample of TPPO–PBN mixture that has been warmed at −5 °C to allow most of the TPPO to decompose. (D) Spectrum recorded after the addition of methanol and pyridine to TPPO–PBN mixture at −78 °C. (E) Spectrum obtained on the addition of methanol and pyridine to TPPO–PBN mixture containing β-carotene. The mole ratio of β-carotene to TPPO is 0.2. (F) Same as in E, but the β-carotene–TPPO mole ratio is 0.4.

Bartlett and Chu to suggest[7] that the direct bimolecular reaction of TPPO with olefins gives biradicals such as (II) (eq 2). Phenols inhibit this reaction, but it was suggested that they do so by electron transfer to TPPO rather than by scavenging biradicals like II.



II

We here report the first study of the decomposition of TPPO in the presence of spin traps.[8,9] In brief, our results are as follows. When TPPO is allowed to decompose in the presence of α-phenyl-*N*-*tert*-butylnitrone (PBN), peroxyl radical spin adducts are observed. When singlet oxygen is generated at −78 °C from TPPO and methanol–pyridine[10] in the presence of nitrones, the ESR spectrum of the acyl nitroxide (an oxidation product of the spin trap) is observed. No spin adducts are observed from the direct reaction of TPPO with olefins.

Results and Discussion

When TPPO, prepared by standard procedures, is treated with a precooled solution of PBN and the ESR spectrum recorded at −70 °C, no spin adducts are observed (Figure 1A). When the TPPO–PBN mixture is allowed to warm to 20 °C and deoxygenated with a stream of nitrogen or argon, only a very weak, unidentifiable ESR signal is observed. However when the TPPO–PBN mixture is warmed to −15 °C until at least some oxygen has evolved, cooled back to −78 °C, and deoxygenated, the ESR spectrum shown in Figure 1B is obtained. The same spectrum is obtained if the mixture is held at −30 °C for 20–30 min. This spectrum shows a strong triplet of doublets ($a^N = 13.2_5$ and $a^H = 1.2_5$ G) in excellent agreement with parameters reported for peroxyl radical spin adducts of PBN.[11–14] This spin adduct was stable from −78 to 0 °C; however, as the temperature was raised above 0 °C, the signal gradually disappeared as expected for the PBN–peroxyl radical adduct.[13,14] Some oxidation of PBN to benzoyl-*tert*-butyl nitroxide (PBNOx)[15] is indicated by the presence of a weak triplet ($a^N = 7.8_0$ G) in Figure 1B. The decomposition of TPPO at −15 °C in the presence of 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) failed to give peroxyl spin adducts; this is not surprising since peroxyl radical adducts of DMPO are too unstable to be observable.[12,13]

In order to determine if these ESR spectra result from the reaction of singlet oxygen with PBN, singlet oxygen was generated from TPPO both at −78 °C and at −30 °C by the addition of methanol and pyridine.[10] When the solution is deoxygenated and ESR recorded at −70 °C, a strong spectrum of PBNOx is observed. Control experiments showed that the preformed PBN–peroxyl radical spin adduct is stable in the presence of methanol and pyridine (Figure 1C) from −78 to 0 °C, whereas the intensity of the PBNOx signal was found to decrease with rise in temperature. Thus the peroxyl radical spin adduct obtained during the decomposition of TPPO at −15 °C (or at −30 °C) probably does not arise from a reaction of singlet oxygen with PBN.[16]

The following control experiments indicate that the reaction of the solvent with ozone or other reagents with PBN is not the source of the observed spin adduct. (a) Dichloromethane was saturated with ozone and allowed to stand at −78 °C for several minutes; the excess ozone was flushed out and a precooled solution of PBN was added. Formation of spin adducts was not observed from −78 to 20 °C. (b) A solution of triphenyl phosphite in methanol–pyridine was mixed with preozonated dichloromethane and then treated with PBN. No ESR signals were detected in the temperature range of interest. A direct reaction between TPPO and PBN at −78 °C to produce the spin adduct also may be ruled out, since only weak signals are produced even after incubating TPPO–PBN mixtures for 24 h (although oxygen evolution still occurred on warming these samples, indicating the presence of unreacted TPPO).

A speculative mechanism for the formation of the peroxyl radical spin adduct can be suggested. Trapping of biradical form of I by PBN would lead to biradical III, which may undergo β scission to give the stable spin adduct IV (eq 3). Alternatively, I may undergo β scission[17] before

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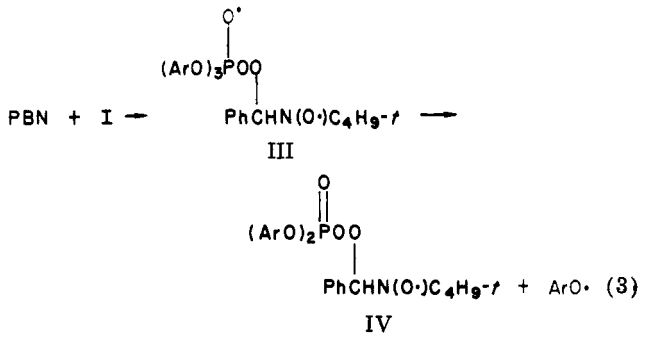
(16) (a) One possible pathway for the production of peroxyl radicals is the reaction of singlet oxygen with PBN to produce 2-methyl-2-nitrosopropane that undergoes photolysis or thermolysis to produce *tert*-butyl radicals that then react with oxygen to give *tert*-butyl peroxyl radicals. However, we have found no evidence for the production of 2-methyl-2-nitrosopropane in our system. Previous work by Harbour et al.[16b] also has shown that the photosensitized oxidation of PBN does not yield free radicals. (b) Harbour, J. R.; Issler, S. L.; Hair, L. M. *J. Am. Chem. Soc.* **1980**, *102*, 7778.



it loses singlet oxygen and the resulting phosphoranyl peroxyl radical may be spin trapped. Regardless of the detailed mechanism involved, our detection of a peroxyl radical spin adduct during the decomposition of TPPO provides experimental evidence supporting a biradical "ring-opened" form during the decomposition of triaryl phosphite ozonides, since the direct extrusion of singlet oxygen from TPPO would not be expected to produce peroxyl radical spin adducts.

There have been two previous studies on the reaction of singlet oxygen with nitrones. Harbour et al.[16b] recently reported the photooxidation of nitrones in aqueous solution at ambient temperatures; they observed oxygen uptake with all the nitrones studied but detected no ESR signals with PBN and only a very weak hydroxyl radical spin adduct with DMPO. They also showed that singlet oxygen reacts much faster with PBN than with DMPO, but no product studies were done. Ching and Foote[18] reported that 4,5,5-trimethyl-1-pyrroline *N*-oxide, a nitrone structurally very similar to DMPO, does not react with singlet oxygen at −63 °C in chloroform. Our results show that both PBN and DMPO are oxidized by singlet oxygen in low yields and the initial products of oxidation at −78 and −60 °C are the corresponding acyl nitroxides, which are converted to nonradical products as the temperature of the reaction mixture is raised above −15 °C. Signals from DMPO are very weak compared to PBN, indicating that DMPO is less readily oxidized by singlet oxygen, in agreement with the report of Harbour et al.[16b]

Quenching Experiments with β-Carotene. The addition of β-carotene, a well-known singlet-oxygen quencher,[19] to TPPO–spin trap mixtures before the addition of methanol and pyridine was found to inhibit the formation of PBNOx, confirming that singlet oxygen is the oxidizing agent in this system (compare Figures 1D and 1E). An almost complete inhibition of the formation of PBNOx is observed when the mole ratio of β-carotene to TPPO is 0.4 (Figure 1F). Considering that the rate constant[16] for reaction of singlet oxygen with PBN is 1.4×10^8 M^{-1} s^{-1} and the rate constant[18] for quenching of singlet oxygen by β-carotene is 1.3×10^{10} M^{-1} s^{-1}, the total inhibition of PBN–singlet oxygen reaction by this high ratio of β-carotene to PBN seems reasonable. In addition to the triplet due to PBNOx, another triplet of doublets ($a^N = 13.2_5$ and $a^H = 1.2_5$) is present in Figures 1E and 1F. The ESR parameters for this new spin adduct are again in agreement with a PBN–peroxyl radical spin adduct. We believe that this peroxyl radical spin adduct arises from the oxidation of β-carotene, a readily oxidizable compound. The quenching of singlet oxygen involves an energy transfer mechanism, and "no appreciable" consumption of β-carotene occurs during the quenching process.[20] However, there have been several studies[21] on the photooxidation of β-carotene and its model compounds that clearly demonstrate that small yields of cyclic peroxides and allylic hydroperoxides are produced. If a small amount of oxidation occurs (either via the reaction of an excited state of β-carotene with oxygen or by an ene reaction to give allylic hydroperoxides that subsequently decompose to peroxyl radicals), the peroxyl radical from this singlet oxygen induced oxidation would be detectable by the sensitive ESR technique used here. Note that a high concentration of singlet oxygen is generated over a short period of time in our system. The oxidation of polyenes by singlet oxygen generated in situ from TPPO is also known.[22] Thus the proposal that the peroxyl radical spin adducts seen in Figures 1E and 1F are formed from the oxidation of β-carotene seems reasonable.

The formation of DMPOx also was found to be completely inhibited by the addition of β-carotene. ESR signals were not detected when singlet oxygen was generated from TPPO in the presence of DMPO and β-carotene at −78 °C by the addition of methanol and pyridine.

The formation of DMPOx in the cumene hydroperoxide–hematin–DMPO system[23,24] has been attributed to the base-catalyzed decomposition of the cumylperoxyl radical–DMPO spin adduct.[23] In order to determine if PBNOx is formed by a base-catalyzed decomposition of the PBN–peroxyl radical spin adduct in our system, we did the following experiment. A TPPO–PBN mixture was prepared at −78 °C and then warmed to −5 °C until oxygen evolution stopped; it was then cooled back to −78 °C and methanol/pyridine was added. In this control experiment, the formation of PBNOx could not be detected and the peroxyl radical spin adduct was still present, confirming that singlet oxygen is the major source of PBNOx in our experiments. The observation of a peroxyl radical spin adduct on the addition of methanol and pyridine to the TPPO–PBN–carotene mixture also indicates that the base-catalyzed decomposition of the PBN–peroxyl radical spin adduct is not the source of PBNOx. This conclusion is further supported by the report of Rosen et al.[23b] that the cumylperoxyl radical spin adduct of α-(4-pyridyl 1-oxide)-*N*-*tert*-butylnitrone is stable toward base-catalyzed decomposition.

Spin-Trapping Experiments on the Direct Reaction of TPPO with Olefins. In an attempt to see if the radicals produced from the direct reaction of TPPO with olefins could be spin trapped, several olefins (trimethyl- and tetramethylethylene, *cis*- and *trans*-3-hexene, and 1,4-pentadiene) were incubated with TPPO–PBN mixtures for various periods of time at −50, −60, and −78 °C. Spin

(17) (a) The phenoxyl radical has never been reported to be spin trapped, possibly because it undergoes dimerization. Hindered phenoxyl radicals react with oxygen to give the cyclohexa-2,5-dienone-4-peroxyl radical, but the unsubstituted phenoxyl radical does not appear to undergo this reaction.[17b] In any case, even if the peroxyl radical spin adduct that we observe were produced from the phenoxyl radical, this still would indicate that a "ring-open" form of TPPO was produced and underwent β scission. (b) Mahoney, L. R. *J. Am. Chem. Soc.* **1967**, *89*, 1895.

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adducts were not detected in these cases, even though complete disappearance of TPPO was indicated in some cases by the absence of oxygen evolution on warming. This result suggests that biradicals like II are very short lived and decay to stable products before they can be spin trapped. This observation is in agreement with the suggestion of Bartlett and Chu[7] that hindered phenols act as electron donors to TPPO rather than intercepting biradicals like II, since radicals that can be intercepted by phenols might also be expected to form spin adducts.

Conclusion

The decomposition of TPPO mainly gives singlet oxygen and the production of III probably is a minor pathway. This is evidenced by the brisk oxygen evolution when TPPO–PBN mixtures are rapidly warmed to room temperature. Since the peroxyl spin adduct is unstable and since PBN reacts with singlet oxygen to produce nonradical products, an exact estimate of the ratio of the yields of singlet oxygen and spin adducts is not possible. However, our results combined with the kinetic evidence of Stephenson and McClure[2] confirm the biradical mechanism by which TPPO decomposes to singlet oxygen. The formation of PBNOx in many previous photooxidation studies[12,13] can be explained by our finding that nitrone spin traps are oxidized to acyl nitroxides by singlet oxygen at low temperatures.

Experimental Section

General Procedures. ESR spectra were recorded on a Varian E-109 ESR spectrometer. Low-temperature experiments were done with a quartz Dewar insert. The temperature was controlled by varying the flow of nitrogen (precooled by passing through a stainless steel coil immersed in liquid nitrogen) through the insert. Ozone was generated by using a Welsbach Model T-23 ozonator. Triphenyl phosphite (Aldrich) was washed with base and distilled in vacuo. Commercially available PBN (Eastman) was recrystallized from hexane before use. DMPO obtained from Spin Trap Producers (Guelph, Canada) was used without further purification. Dichloromethane was washed with sulfuric acid and distilled water, dried over $CaCl_2$, distilled from CaH_2, and stored over molecular sieves.

Spin Trapping: TPPO–PBN Experiments. TPPO was made by the slow addition of 8 mg of triphenyl phosphite dissolved in 0.1 mL of dichloromethane to a saturated solution of ozone in dichloromethane (0.3 mL) at −78 °C, with continued bubbling of ozone. After the addition was complete, excess ozone was flushed out with nitrogen and a precooled solution of 5 mg of PBN in 0.1 mL of dichloromethane was added. The sample was then warmed in a −15 °C bath for 10–15 min and cooled back to −70 °C, and the ESR spectrum was recorded after deoxygenation. To establish the stability of the peroxyl radical spin adduct, we added methanol and pyridine (0.1 mL, 1:1) at −78 °C to a TPPO–PBN sample that had been warmed at −5 °C until oxygen evolution almost stopped.

Quenching Experiments with β-Carotene. A blank experiment was done as follows. To a TPPO–PBN mixture prepared as described above was added 0.1 mL of precooled methanol and pyridine. The mixing of the reagents was effected by a stream of nitrogen and the sample was transferred to a −65 °C bath. After 30 min, the sample was deoxygenated with N_2 again and the ESR spectrum was recorded at −70 °C. In the β-carotene experiments, 0.1 mL of a solution of β-carotene of the required concentration was added before the addition of methanol and pyridine and treated exactly as the blank. Volumes were made equal by addition of dichloromethane.

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Registry No. 1, 79101-59-0; ozone, 10028-15-6; TPPO, 29833-83-8; $(PhO)_3P$, 101-02-0.

Enzymatic Conversion of α-Keto Aldehydes to Optically Active α-Hydroxy Acids Using Glyoxalase I and II[1]

Mark A. K. Patterson,[2] Richard P. Szajewski,[3] and Geroge M. Whitesides*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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α-Keto aldehydes (RCOCHO, R = CH_3, $CH_3CH_2CH_2$, Ph, p-CH_3OPh, p-ClPh) have been converted to optically active α-hydroxy acids ($RCHOHCO_2H$) by the combined action of glutathione and the (immobilized) enzymes glyoxalase I (GX-I, EC 4.4.1.5) and glyoxalase II (GX-II, EC 3.1.2.6). The reaction seems to provide a practical if specialized method for synthesizing 1–10-g quantities of product with enantiomeric excesses (ee) in the range 75–99%. Efforts to increase the scale of the reacton are accompanied by decreases in the ee of the product and in the turnover number reached by the enzymes.

Introduction

This paper describes the conversion of α-keto aldehydes to optically active acids, using a catalytic system comprised of two enzymes, glyoxalase I[4-13] (GX-I, EC 4.4.1.5) and glyoxalase II[14,15] (GX-II, EC 3.1.2.6), and the cysteine-containing cofactor glutathione (GSH, γ-L-Glu-L-Cys-Gly) (eq 1). GX-I accepts a number of sterically unhindered α-keto aldehydes as substrates but does not accept substances that have large substituents close to the keto

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