

TABLE II

Repellency (%) of the Seed Oils at Different Concentrations to Adult *Tribolium castaneum* Herbst

Seed oils	Rate of application repellent (%)			
	10	5	2.5	1
<i>Solanum khasiana</i>	85	70	60	44
<i>Solanum indicum</i>	80	70	58	49
<i>Datura alba</i>	90	80	70	45
<i>Glycosmis pentaphylla</i>	90	75	60	51
<i>Heliotropium supinum</i>	60	47	36.6	20
<i>Semicarpus anacardium</i>	80	70	65	35
<i>Cardiospermum belicacabum</i>	90	80	70	45
<i>Cardiospermum canescens</i>	80	75	60	50
<i>Polyalthia longifolia</i>	80	70	57	40
<i>Annona squamosa</i>	88.8	78.6	60.9	44

P > 0.05 in all cases (significant).

family, only *Glycosmis pentaphylla* was found to be active and it had the highest repellent activity. *Polyalthia longifolia* and *Annona squamosa* of Annonaceae family also showed high repellent activity at 1% concentration.

From the above results (Table II), we observe that in all cases there is a gradual fall in the activity of oil with decrease of the concentration (P > 0.05). All oils listed in Table II, except *Heliotropium supinum* and *Semicarpus anacardium*, seem to be good repellents to adult *Tribolium castaneum* Herbst, at 1% concentration (P > 0.05).

#### ACKNOWLEDGMENT

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## ✿ An ESR Study of Radical Intermediates Formed by $\gamma$ -Radiolysis of Tripalmitin and Dipalmitoyl Phosphatidylethanolamine

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#### ABSTRACT

Radicals resulting from the  $\gamma$ -radiolysis at 77 K of neat tripalmitin and neat dipalmitoyl phosphatidylethanolamine were investigated by electron spin resonance spectroscopy (ESR). Analyses of the spectra of these complex lipids were aided by ESR studies of their components phosphorylethanolamine, palmitic acid and hexadecane. Concentrations of various radicals were followed as a function of temperature after  $\gamma$ -irradiation at 77 K. Both tripalmitin and dipalmitoyl phosphatidylethanolamine show anion radicals formed



by electron addition to the ester groups (-O-C-) and aliphatic side-chain radicals (-CH<sub>2</sub>-CH-CH<sub>2</sub>-) which are thought to be the result of deprotonation of the original positive hole. Computer analyses of the spectra as the temperature increases show loss of the anion radicals with an increase in a new radical produced by the abstraction of a hydrogen atom from the  $\alpha$  carbon on an acyl side chain. In the case of dipalmitoyl phosphatidylethanolamine, a radical of the phosphorylethanolamine portion of the molecule is detected as an intermediate. Mechanisms for the formation and decay of the free radical intermediates are discussed.

#### INTRODUCTION

The investigation of free radical mechanisms of chemical reactions induced by radiolysis of lipids is important to the understanding of radiation damage to biological systems. By identifying radicals produced by irradiation, and by monitoring their decay, predictions of the products formed

by radiolysis can be made. This is important to studies of product analyses since many of the low yield products might be overlooked unless a specific search for them is instituted. Due to the complexity of many of the naturally occurring lipids, the best initial approach for their study is to investigate model compounds. In previous work (1-3), we have investigated a number of model compounds such as various esters, carboxylic acids and a small triglyceride triacetin. Since radiation produces anions and cations as primary intermediates, to simplify the systems further, the anionic pathways were investigated separately (2). For example, electron addition to esters was suggested to result in two reactions: (a)  $\beta$  scission of the anion radical in which an alkyl radical and carboxylic acid are formed; (b) hydrogen atom abstraction of a parent molecule by the anion radical producing a hydrogen abstracted radical and an unstable diol.

In this work we apply knowledge gained in these previous investigations by ourselves and other workers to a detailed electron spin resonance (ESR) study of the radicals formed following  $\gamma$ -irradiation of the phospholipid, dipalmitoyl phosphatidylethanolamine (DPPE) and compare these to the radicals present in the triglyceride tripalmitin (TP). The radical reactions resulting from the radiolysis of phospholipids have not been investigated previously, although some product analyses studies have been reported

## RADICALS IN IRRADIATED LIPIDS: ESR

(4-7). We have reported a preliminary investigation of the tripalmitin irradiation (2). Mechanisms are presented in this report to account for the radicals observed, and other mechanisms are proposed to describe the disappearance of the radicals and to predict what the most likely final products will be. Other lipids and lipid components that were investigated in this work as model compounds include palmitic acid, hexadecane and phosphorylethanolamine.

## EXPERIMENTAL

The neat samples were flushed with nitrogen and sealed under vacuum in quartz tubes. Radiolysis was performed at 77 K or at room temperature with a cobalt-60  $\gamma$  source (Wayne State University). Doses were 0.2-1 Mrad. The spectra of the irradiated samples were obtained using a Varian E-line Century ESR spectrometer. The spectra were stored on computer memory as the samples were annealed from 108 K to above room temperature. Spectra of the complex triglyceride and phospholipid were simulated by combining spectra of the components. Computer subtraction of component radical spectra from the more complex spectra was used as an aid for determining individual radical concentrations. Details of the computer analysis of the spectra have been reported previously (8-10).

Chemicals were obtained from Sigma Chemical Company and were used without further purification.

## RESULTS AND DISCUSSION

## Phosphorylethanolamine

Gamma irradiation of neat polycrystalline phosphorylethanolamine at 77 K results in the spectra shown in Figure 1. Although the low temperature spectrum appears to be very different from the high temperature spectrum, the difference is believed due to the changes in the conformations of the major radical that occur on annealing rather than production of new radical species. This change in conformation is verified when the spectrum of the warmed sample is taken again at low temperature (Fig. 1D). It is nearly identical to the initially obtained low temperature spectrum. The radical is stable and persists to at least 373 K. The total spectral width is ca. 59 G between the major end components. If the broadening observed in the wings of the spectrum is included, the spectrum extends over 84 G. This broadening is believed due to unresolved nitrogen couplings. The width does not change as the sample is annealed from 77 K to 373 K. The only radical which is likely to be formed which would show the conformational change with temperature is A. Previous work on similar systems (discussed below) also suggests A is the most likely radical. The most probable mechanisms for the formation of A are shown below (reactions 1a and 1b).

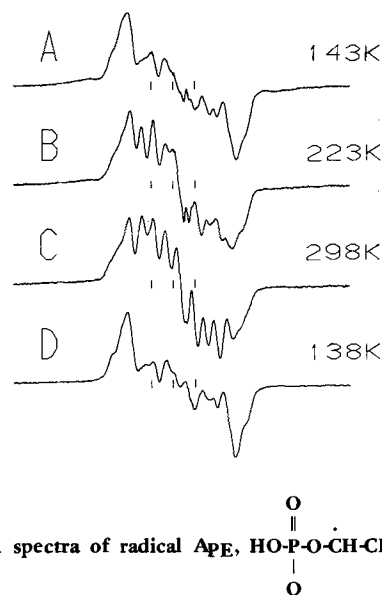
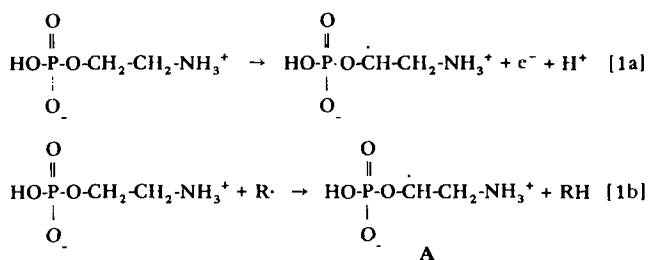


FIG. 1. ESR spectra of radical A,  $\text{HO-P-O-CH-CH}_2\text{-NH}_3^+$ , produced by the  $\gamma$ -irradiation at 77 K of neat polycrystalline phosphorylethanolamine and subsequent annealing to the temperatures shown in the figure. The change in the spectra as the temperature increases is shown to be reversible by the spectrum in D which was obtained at 138 K after the sample had been warmed to 298 K.

In reaction 1a, electron loss is followed by deprotonation of the cation at the carbon alpha to the phosphate group. In reaction 1b, hydrogen atom abstraction occurs at the carbon alpha to the phosphate. Although the mechanisms are different, the final radical product is the same in both reactions. The hydrogens on the carbon beta to the phosphate group are deactivated by the positive charge on the adjacent amino group. The spectra observed are consistent with the spectrum that would be expected from radical A. Analysis of Figure 1C suggests that there are two protons of ca. 21 G splitting and one proton splitting and one phosphorus splitting which average to 7.5 G. The nitrogen splittings are poorly resolved but are observed as broadening in the wing components.

Samuni and Neta (11) investigated hydroxyl radical abstraction reactions with aqueous solutions of phosphorylethanolamine. They found that only radical A was produced at acid and neutral pH values when the amino group would be protonated ( $a_\alpha^H = 19$  G,  $a_\beta^H = 13$  G (2),  $a^P = 4.9$  G,  $a^N = 9.5$  G). They proposed that the  $\beta$ -carbon would be the major abstraction site at alkaline pH when the amino group would no longer be positively charged, but they were unable to detect this species.

Nelson and Symons (12) found that the carbon alpha to the phosphate group in several alkyl phosphates was the major electron loss center during radiolysis of neat samples. Deprotonation at this site followed the electron loss. With neat samples of alkyl phosphates, reactions of electrons with the parent molecule were also observed. Although many reaction possibilities of this type exist for phosphorylethanolamine, we were able to detect a phosphoryl type radical ( $a^P = 660$  G) only when the irradiation was done at room temperature (1 Mrad). The phosphoryl radical was a minor radical component even under these conditions. We note that Fouse and Bernhard (13) have reported evidence for phosphoryl radical formation using X-irradiated single crystals of the phosphorylethanolamine.

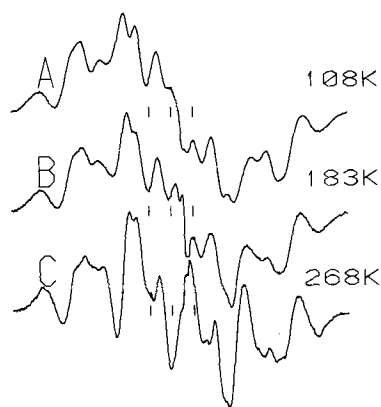


FIG. 2. ESR spectra of radical  $\text{CHD}$ ,  $-\text{CH}_2-\dot{\text{C}}\text{H}-\text{CH}_2-$ , produced by the  $\gamma$ -irradiation at 77 K of neat hexadecane and subsequent annealing to the temperatures shown in the figure. The spectral changes in A-C are believed to be caused by conformational changes in the radical species.

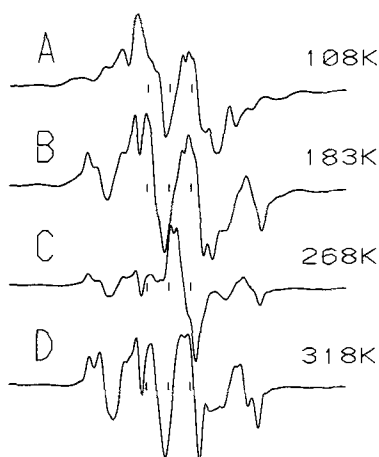
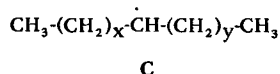


FIG. 3. ESR spectra of radicals produced by the  $\gamma$ -irradiation at 77 K of neat palmitic acid and subsequent annealing to the temperatures shown in the figure. (A) The anion radical  $\text{Bp}_A$  and the side-chain radical  $\text{Cp}_A$  are the major radical species. (B)  $\alpha$ -Carbon radical  $\text{Dp}_A$  (stable conformation) can be detected as well as radicals  $\text{Bp}_A$  and  $\text{Cp}_A$ . (C) Acyl radical (singlet,  $g = 2.0006$ ) with  $\text{Cp}_A$  and  $\text{Dp}_A$ . Radical  $\text{Bp}_A$  has disappeared. (D) Only radical  $\text{Dp}_A$  remains.

### Hexadecane

The spectra obtained for neat hexadecane  $\gamma$ -irradiated at 77 K are shown in Figure 2. The spectrum is due to the type C radical and has been reported previously (14).

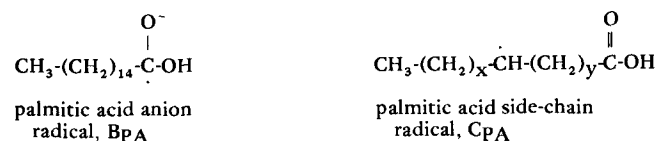


We employed it here for the analysis of spectra of lipids which contain long alkyl side chains. In these analyses, the spectra found at the various temperatures were double integrated and used to determine the side chain radical concentration in other lipids. At 108 K, the total width of the multiline spectrum is 158 G. The changes in the spectrum that are observed with annealing are predominantly due to changes in conformation rather than the

generation of other radical species. At 268 K there is a 22 G splitting from one  $\alpha$ -proton and splittings due to four  $\beta$ -protons which average to 33 G. The individual  $\beta$ -proton splittings likely vary from this value. The total spectral width is 154 G at 268 K. The radical disappears with further warming.

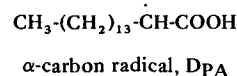
### Palmitic Acid

Figure 3 contains the spectra of neat polycrystalline palmitic acid (PA) that had been  $\gamma$ -irradiated (0.2 Mrad) at 77 K. At 108 K, a broad multiline spectrum extending 158 G was obtained. Computer analysis shows that 70% of the spectrum can be attributed to two radicals—the anion radical B (20%) and the alkyl side-chain radical (50%).

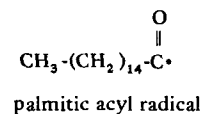


The anion is formed by electron addition to the carboxyl carbon of the fatty acid, while the side-chain radical can be formed by hydrogen atom abstraction or by ionization (electron loss) followed by deprotonation. The anion radical spectrum is a doublet of ca. 26 G splitting ( $g = 2.003$ ). Most of the remaining lines are produced by the side-chain radical as can be seen by comparing the 108 K hexadecane spectrum with the palmitic acid spectrum obtained at 108 K.

The remaining 30% of the radicals are not resolved sufficiently for identification. However, an approximation of the spectrum of the remaining radicals can be produced by subtracting the portion of the spectrum due to the anion radical and the side-chain radical. The resulting spectrum suggests that the third major radical present at 108 K is the  $\alpha$ -carbon radical  $\text{Dp}_A$  which will be discussed in more detail later.

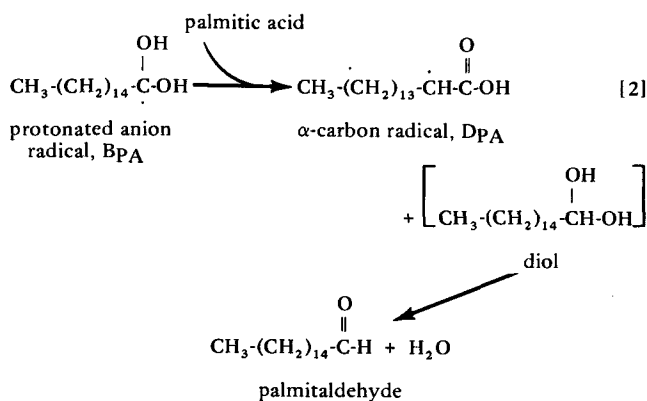


As the palmitic acid sample is annealed, the anion radical disappears. By 268 K, the anion radical has been largely replaced by the  $\alpha$ -carbon radical  $\text{Dp}_A$  and the acyl radical.



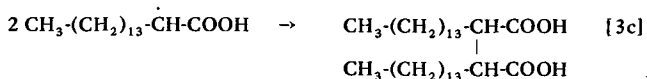
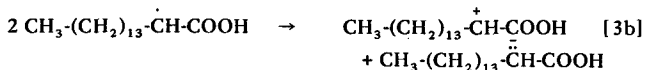
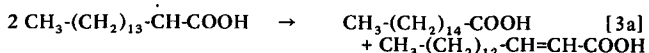
The acyl radical can arise by loss of  $\text{OH}^-$  from the anion radical or by loss of water from the protonated anion radical. The acyl radical spectrum consists of a singlet ( $g = 2.0006 \pm 0.0005$ ). The identity of this radical has been discussed in detail by Fautitano et al. (14). The acyl radical disappears with further warming while the  $\alpha$ -carbon radical  $\text{Dp}_A$  concentration continues to increase (Figure 3D). Work on model compounds has shown that type D radicals can be formed by hydrogen atom abstraction from

the parent carboxylic acid by other radicals. An example of this is shown in reaction 2.



The palmitic acyl radical would also be expected to abstract from the parent palmitic acid in a similar reaction to that shown above. In fact, product analysis could not distinguish the two pathways, since the products from both are identical, i.e., DpA and palmitaldehyde. Recall that water was also formed in the production of the acyl radical.

The  $\alpha$ -carbon radical DpA shows coupling to three protons ( $a_{\alpha}^H = 20$  G,  $a_{\beta}^{\text{CH}_2} = 32$  G;  $g = 2.003$ ). The resolved structure in the end components in Figure 3D are caused by the anisotropy of the  $\alpha$ -hydrogen and are characteristic of such hydrogens. The radical is a very stable species and persists up to at least 370 K. Likely termination reactions involving radical DpA would be hydrogen atom or electron disproportionation (reactions 3a and 3b) and dimer formation (3c), or combination with other radicals.



Wu and Howton (15) have shown that the  $\alpha$ ,  $\alpha'$  dimer, analogous to that formed in reaction 3c, is a product in the  $\gamma$ -radiolysis of the homolog, stearic acid.

### Tripalmitin

When neat tripalmitin was  $\gamma$ -irradiated at 77 K, the spectra in Figure 4 were obtained. We have previously reported a preliminary investigation of the tripalmitin (2). Here we report a detailed analysis of the radicals present in  $\gamma$ -irradiated tripalmitin (TP) and compare them to the radicals present in the phospholipid, dipalmitoyl phosphatidylethanolamine (DPPE).

At 77 K, computer analysis shows that the tripalmitin anion radical, B<sub>TP</sub>, and the tripalmitin side-chain radical, C<sub>TP</sub>, are responsible for ca. 80% of the total radical spectrum. For triglycerides, electron addition can occur at either the 1' or 2' ester sites to form the anion radicals. Although the structures are different for these two anion radicals, the spectra cannot be distinguished, due to broad linewidths obtained for the polycrystalline samples. At 77 K, the anion radical produces a 21 G doublet with  $g =$

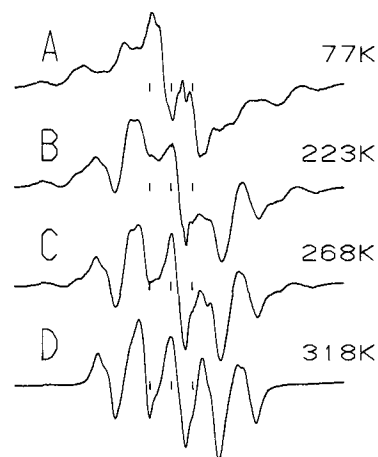
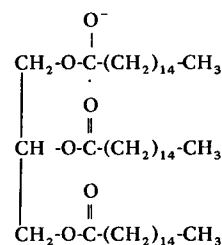


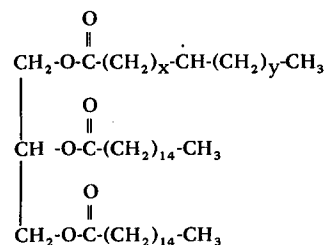
FIG. 4. ESR spectra of radicals produced by the  $\gamma$ -irradiation at 77 K of neat tripalmitin and subsequent annealing to the temperatures shown in the figure. (A) The anion radical B<sub>TP</sub> and the side-chain radical C<sub>TP</sub> are the major species (B) and (C) The stable conformation of the  $\alpha$ -carbon radical D<sub>TP</sub> builds in as the anion radical B<sub>TP</sub> disappears. (D) The side-chain radical C<sub>TP</sub> is lost and only radical D<sub>TP</sub> remains.

2.003. B<sub>TP</sub> is the structure of the 1' anion species for tripalmitin.



tripalmitin 1'-anion radical, B<sub>TP</sub>

The total width of the tripalmitin spectrum (ca. 158 G at 77 K) results from the presence of the type C side-chain radicals in the sample. Whereas the side-chain radicals can form on any of the three acyl side-chains in a triglyceride molecule, all the radicals of type C structure should produce the same spectrum. C<sub>TP</sub> is the structure of the 1'-side-chain radical species for tripalmitin.

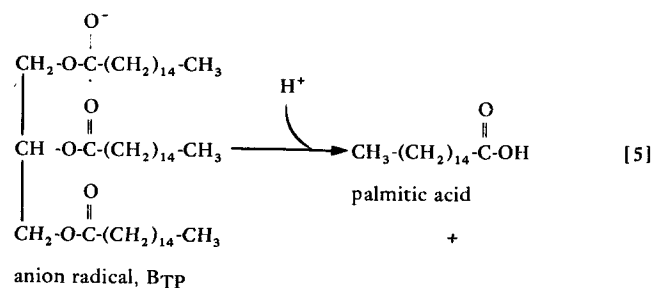
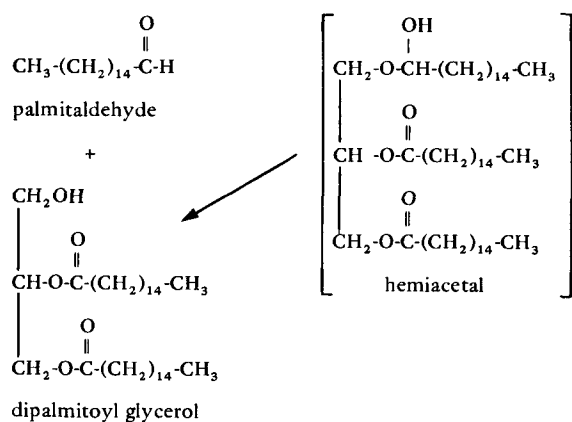
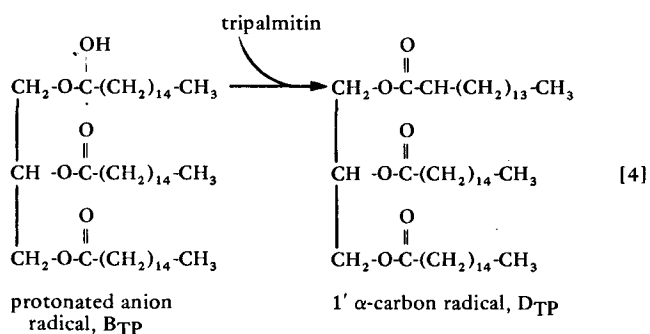


tripalmitin 1'-side-chain radical, C<sub>TP</sub>

The end portions of the spectra are very similar for hexadecane, palmitic acid, and tripalmitin.

As the tripalmitin is annealed, the anion disappears. This is shown in Figure 5 where the anion radical has disappeared by 183 K. We believe the likely modes of decay of the anion radical are abstraction of a hydrogen atom from

an  $\alpha$ -carbon atom of the acyl side chain of a parent molecule of tripalmitin (reaction 4) and  $\beta$ -scission producing palmitic acid and propane diol diester radical (reaction 5).



In agreement with the first mechanism (reaction 4), the results in Figure 5 show that the  $\alpha$ -carbon radical builds in as the anion disappears.

The propanediol diester radical expected from reaction 5 was not identified in the tripalmitin spectra. An explanation may be found in our previous study or triacetin where a small amount of propanediol diacetate radical was detected (2).

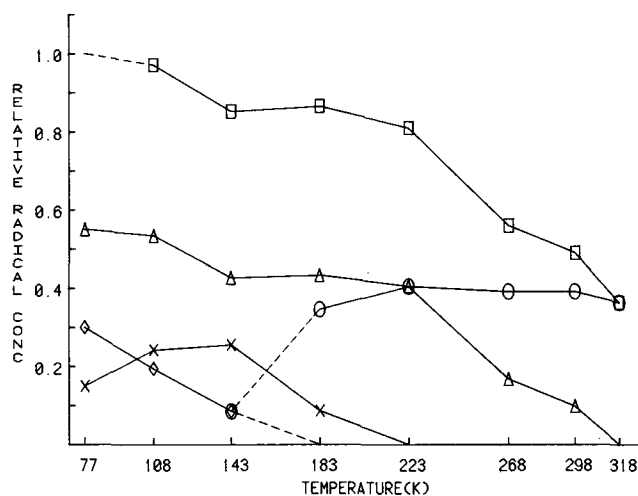
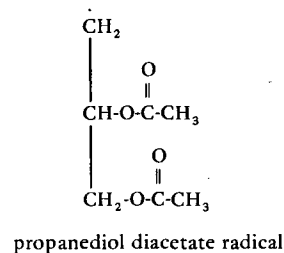
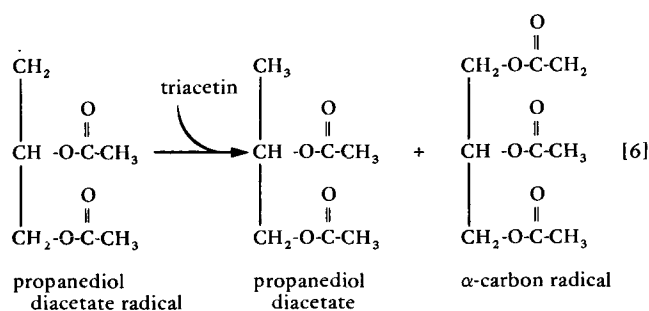


FIG. 5. Relative radical concentration as a function of temperature after the radiolysis of neat tripalmitin at 77 K (0.2 Mrad). The tripalmitin spectra were analyzed by computer double integration and subtraction techniques. Values are reported as the fraction of total radicals present at 77 K. ( $\square$ — $\square$ ): Relative total radical concentration. By 318 K, only 50% of the initial radical concentration remains. Radical signal persists until the sample melts. ( $\Delta$ — $\Delta$ ): Side-chain radical  $C_{TP}$ . The concentration is determined from spectral wing components.  $C_{TP}$  is a primary species and its concentration changes little until temperatures above 223 K. By 318 K,  $C_{TP}$  can no longer be detected. ( $\diamond$ — $\diamond$ ): Anion radical  $B_{TP}$ . The concentration was determined in the spectra after  $C_{TP}$  was subtracted out.  $B_{TP}$  is formed by electron addition during radiolysis and has decayed away by 183 K. ( $\circ$ — $\circ$ ): The stable conformation of the  $\alpha$ -carbon radical  $D_{TP}$ . The concentration appears to increase as the sample is annealed. By 318 K, only the  $D_{TP}$  remains. As the sample melts  $D_{TP}$  disappears. ( $\times$ — $\times$ ): All other unidentified radicals which remain after  $B_{TP}$ ,  $C_{TP}$  and  $D_{TP}$  have been subtracted from the spectra.



The radical is very reactive and readily abstracts a hydrogen atom from a parent molecule to form propanediol diacetate and a hydrogen abstracted radical (reaction 6).



In triacetin, the propanediol diester radical produced a quartet spectrum resulting from two protons with a 21 G

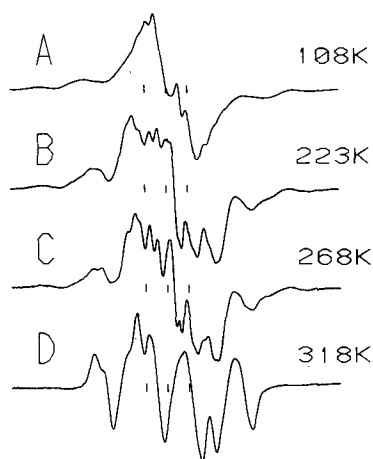


FIG. 6. ESR spectra of radicals produced by the  $\gamma$ -irradiation of neat polycrystalline dipalmitoyl phosphatidylethanolamine and subsequent annealing to the temperatures shown in the figure. (A) Anion radical  $B_{DPPE}$  and side-chain radical  $C_{DPPE}$  are the major radicals. (B) The appearance of the phosphorylethanolamine type radical  $A_{DPPE}$  in the center of the spectrum. (C) Increased resolution of radical  $A_{DPPE}$  and  $\alpha$ -carbon radical  $D_{DPPE}$ . (D) Radicals  $A_{DPPE}$  and  $C_{DPPE}$  have disappeared and only radical  $D_{DPPE}$  can be detected.

splitting and one proton with a 28 G splitting. This quartet can be easily masked when radical C, the side chain alkyl radical, or the  $\alpha$ -carbon radical D are present as they are in the case of tripalmitin. It is thus likely that the propanediol diester radical reacts so rapidly with parent molecules that its concentration does not build up sufficiently to be detected. Thus either reaction 4 or reaction 5 and a reaction analogous to 6 could account for the increase in the  $\alpha$ -carbon radical,  $D_{TP}$ , as the anion decays. Whether abstraction by the anion or  $\beta$ -scission is the predominant reaction depends on the structure of the anion. In studies of model compounds, for example, it was found that the methyl formate anion radical is involved only in the abstraction reaction, whereas methyl propionate is able to undergo both abstraction and  $\beta$ -scission reactions (2).

Product analysis studies have shown that propanediol diester, caproaldehyde, and dicaproyl glycerol are major breakdown products in the radiolysis of tricaproin (16). Product yields suggest that 70% of the tricaproin anion radical decays by reaction 5 and 30% by reaction 4. Presumably the tricaproin and tripalmitin would have similar reactions under identical conditions.

The acyl radical is an easily detected radical and is sufficiently stable so that it has been detected in many cases. The absence of the acyl singlet can be taken as good evidence that triglyceride anion radicals do not decay to any great extent by acyl radical production.

From Figure 5, it can be seen that the side-chain radical  $C_{TP}$  decays above 223 K without the appearance of any new radical species. As with the  $\alpha$ -carbon radical,  $D_{PA}$ , the likely termination reactions are hydrogen atom disproportionation, electron disproportionation and combination with other radicals.

When the irradiated tripalmitin is annealed to 318 K, only the  $\alpha$ -carbon radical,  $D_{TP}$ , is detected in the spectrum (Fig. 4D). This radical has two protons with splittings of 22 G and one proton with a splitting of 41 G. As with the spectrum of radical  $D_{PA}$ , the spectrum of  $D_{TP}$  has end com-

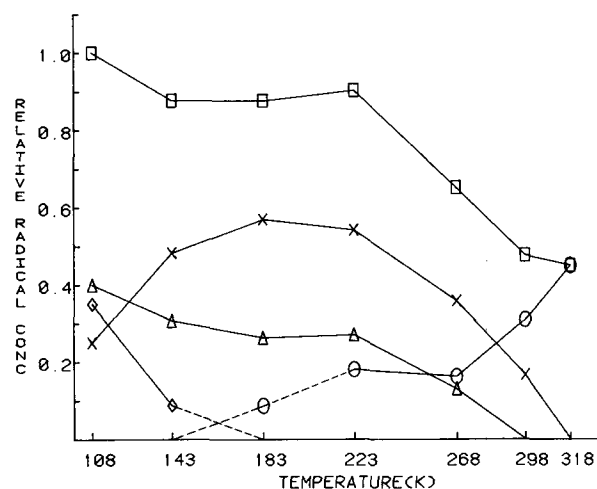


FIG. 7. Relative radical concentration as a function of temperature after the radiolysis of neat polycrystalline dipalmitoyl phosphatidylethanolamine at 77 K (0.2 Mrad). The DPPE spectra were analyzed by computer double integration and subtraction techniques. Values are reported as the fraction of total radicals present at 77 K. ( $\square$ — $\square$ ): Relative total radical concentration. By 318 K, less than 50% of the initial radical concentration remains. As the sample melts all of the radical signal disappears. ( $\Delta$ — $\Delta$ ): Side-chain radical  $C_{DPPE}$ . The concentration is determined from the spectral wing components.  $C_{DPPE}$  appears to be a primary species—the maximum concentration is reached at 77 K (unchanged at 108 K). By 298 K,  $C_{DPPE}$  is no longer detectable. ( $\diamond$ — $\diamond$ ): Anion radical  $B_{DPPE}$ . The concentration was determined in spectra after radical  $C_{DPPE}$  was subtracted out.  $B_{DPPE}$  forms by electron addition during radiolysis and has decayed away by 183 K. ( $\circ$ — $\circ$ ): Stable conformation of the  $\alpha$ -carbon radical  $D_{DPPE}$ . By 318 K, only radical  $D_{DPPE}$  remains detectable in the spectrum, and  $D_{DPPE}$  is present until the sample melts. ( $\times$ — $\times$ ): All radicals which remain after  $B_{DPPE}$ ,  $C_{DPPE}$ , and  $D_{DPPE}$  have been subtracted from the spectra. From 183 K to 268 K the major radical is believed to be the phosphorylethanolamine type radical,  $A_{DPPE}$ .

ponents caused by the  $\alpha$ -hydrogen anisotropy. Previous work with model compounds that produce radicals having the



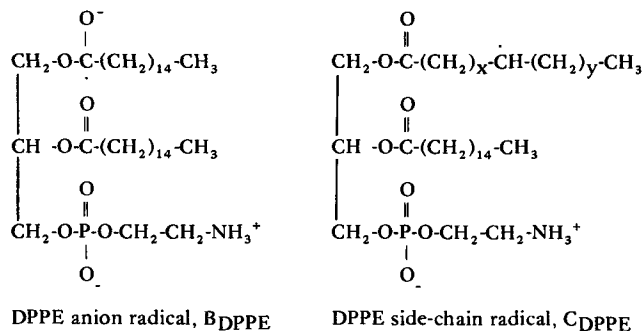
structure  $-C\dot{C}H-CH_2-R$  has shown that a variety of conformations exist for these species (18). The more stable conformation produces a spectrum of the type shown in Figures 2D and 4D having a total spectral width of at least 80 G ( $g = 2.003$ ). At low temperatures a less stable form may also be present, and the resulting spectrum is produced by a combination of these conformations. The less stable conformation has been shown in the work with model compounds to produce a triplet of ca. 50 G width. As the sample is annealed, all of the radical converts irreversibly to the more stable conformation. In the case of more complex spectra, such as those obtained with irradiated tripalmitin, where several radicals with overlapping spectra are present, it is difficult to determine the relative contribution to the spectrum of the triplet produced by the less stable conformation of the radical  $D_{TP}$ . Thus the increase in the amount of the  $\alpha$ -carbon radical,  $D_{TP}$ , can come from two general sources: (a) the conversion of the unstable conformation to the easily detectable stable form; (b) the formation of new  $D_{TP}$  by radical abstraction of a hydrogen atom from the  $\alpha$ -carbon of parent molecules of tripalmitin.

Radical  $D_{TP}$  decays at temperatures above 318 K, likely by undergoing reactions analogous to those described for the  $\alpha$ -carbon radical  $D_{PA}$  of palmitic acid (reactions 3a, 3b

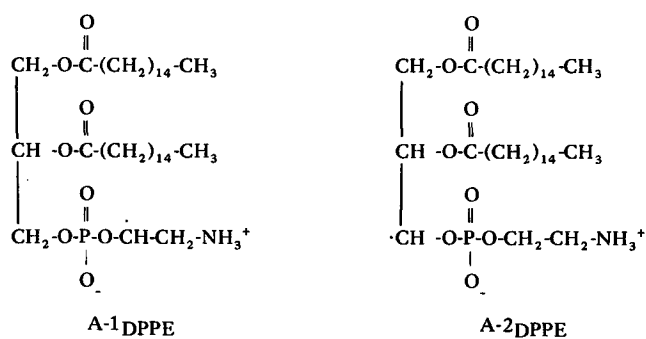
and 3c). Merritt and Vajdi (19) have shown that the  $\alpha$ ,  $\alpha'$  dimer is one product of tripalmitin radiolysis (25 Mrad, 25 C).

### Dipalmitoyl Phosphatidylethanolamine

The spectra in Figure 6 were obtained for irradiated neat polycrystalline dipalmitoyl phosphatidylethanolamine (DPPE). Figure 7 shows the relative amounts of the major radicals at various temperatures. The major identifiable radicals present at 77 K are the anion radical  $B_{DPPE}$  (24 G doublet) and the side-chain radical  $C_{DPPE}$  which extends over 157 G.



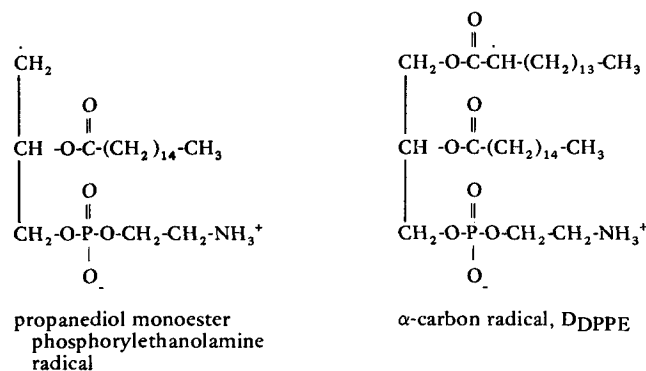
As the sample is annealed, irreversible changes occur in the spectra. By 223 K, the major radicals present are  $C_{DPPE}$ , the  $\alpha$ -carbon radical  $D_{DPPE}$ , and a radical producing a spectrum similar to that observed for the phosphorylethanolamine radical  $A_{PE}$ . The center lines present in the DPPE spectrum at 223 K are clearly absent in the TP spectra. Due to the complexity of the DPPE spectra, it is not possible to obtain accurate splitting constants, but the spectrum at 268 K suggests one phosphorus with a 5 G splitting and other hydrogens which sum to 48 G. For DPPE, there are two carbons alpha to the phosphate group, and thus there are two possibilities for radicals analogous to  $A_{PE}$ .



Since a somewhat reduced width is observed in the DPPE spectrum as compared to the phosphorylethanolamine spectrum, this suggests that radical A-2 $D_{DPPE}$  also may be formed.

It can be seen by examining Figure 7 that the anion radical  $B_{DPPE}$  decays rapidly as the sample is annealed. By 183 K, radical  $B_{DPPE}$  is no longer detectable. The x-x-x curve can be seen to increase as the anion radical decreases in concentration. The x-x-x curve represents all radicals other than the anion radical  $B_{DPPE}$ , the side-chain radical  $C_{DPPE}$ , and the stable conformation of the  $\alpha$ -carbon radical

$D_{DPPE}$ . From 183 K to 268 K, the major identifiable radical in the x-x-x curve is the  $A_{DPPE}$  radical(s) described above. Other likely radicals that are not identified are the two forms of the propanediol monoester phosphorylethanolamine radical and the unstable conformation of the  $\alpha$ -carbon radical  $D_{DPPE}$ .



By 318 K, only the stable conformation of the  $\alpha$ -carbon radical  $D_{DPPE}$  can be detected. The spectrum is produced by two protons averaging a 32 G splitting and one proton having a 21 G splitting. This signal also disappears with further annealing. Again the termination reactions that are most likely are of the type described for palmitic acid's  $\alpha$ -carbon radical  $D_{PA}$ .

### Comparison of Tripalmitin and Dipalmitoyl Phosphatidylethanolamine

The major radicals identified after irradiation at 77 K for both TP and DPPE are the anion radicals  $B_{TP}$  and  $B_{DPPE}$  which result from electron addition to ester functional groups, the alkyl side chain radicals  $C_{TP}$  and  $C_{DPPE}$  which are suggested to arise principally by electron loss followed by deprotonation. For radiation doses of 0.25 Mrad, the total number of spins for TP and DPPE is  $4 \times 10^{20}$  spins/mol at 110 K; thus they have similar radiation sensitivities. Other radicals are likely present at 77 K, but they are not sufficiently resolved for identification at these low initial temperatures.

As the samples are annealed, the major radicals present differ for the two compounds. For TP, the disappearance of the anion radical  $B_{TP}$  coincides with the increase in concentration of the  $\alpha$ -carbon radical  $D_{TP}$ . For DPPE, the phosphorylethanolamine type radical  $A_{DPPE}$  builds in as the anion radical  $B_{DPPE}$  fades. The side chain radicals  $C_{TP}$  and  $C_{DPPE}$  appear to be of equal stability as expected since both persist until ca. 300 K. The radical  $A_{DPPE}$  disappears by 318 K, while the  $\alpha$ -carbon radical  $D_{DPPE}$  builds in for the DPPE sample. The final spectra indicate that the radicals  $D_{TP}$  and  $D_{DPPE}$  are the most stable and are the only identifiable radicals that are present at 318 K.

Handal and Nawar (4,17) compared some of the products formed by the  $\gamma$ -irradiation of DPPE and TP (50 Mrad, ambient temperature). In all cases there appeared to be significantly more breakdown in the triglyceride than in the phospholipid. For example,  $>560 \mu\text{mol}$  of palmitaldehyde (the major aldehyde formed in both cases) per mole of palmitic acid were found for TP while only  $7.5 \mu\text{mol}$  of palmitaldehyde were found for DPPE. If the palmitaldehyde arises primarily from the most probable radical path-

way (reaction 4), this large difference would not be expected from results obtained in this study. Clearly more work is needed in this area to clarify these very interesting results.

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## ✿ Determination of Trace Metal Content in Corn Oil by Atomic Absorption Spectroscopy

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#### ABSTRACT

Atomic absorption spectrophotometry is used for the determination of Cu, Fe, K, Na, Ni and Zn in industrial refined corn oil. The results of five different analytical techniques are compared. Fair agreement is obtained between the char-ashing method and the dilution procedure. Char ashing is a sensitive and useful technique, if handled correctly. Dilution, coupled to carbon-rod atomization, is a reliable method. To eliminate errors, due to sample loss, a proper injection technique is required. Extraction methods suffer from incomplete recoveries, owing to deficient break up of the organometallic compounds in oil.

#### INTRODUCTION

Small amounts of metals in edible oils are well known to have serious deterioration effects on the stability of these oils (1). The altered oil characteristics are expressed in changes of color, odor and flavor. Copper and iron in particular will greatly reduce the oxidative stability of oil (2). Therefore, exact determination of trace metal contents is very important in evaluating deteriorating effects.

Due to its high sensitivity and specificity, atomic absorption spectrometry has been used extensively to study trace wear metals in used engine oils (3).

Despite the established procedures and instrumental refinements, the determination of metals in vegetable oils is still a problem. This is especially due to extremely low metal concentrations in refined edible oils.

Trace metal contents of oils can be performed using

aqueous or organic matrices (4). For aqueous matrices, techniques such as char ashing, wet ashing, acid extractions and chelation are commonly used. Direct analysis of metals in oils requires dilution of the sample by an appropriate organic solvent. Depending on sample volume, sensitivity, detection range and the specific metal concentration, either a conventional burner or a carbon-rod atomizer can be employed (5).

Several workers (6-8) compared ashing, extraction and solvent dilution and discussed the advantages and disadvantages of each. Dry ashing (9) is simple and direct, although several elements are partially lost during heating. Wet ashing (10) causes no loss of inorganics but the risk of contamination and explosion (if perchloric acid is used) (11) is higher. Various extraction methods, including acid extraction by hydrochloric acid (12) or nitric acid (13) and acid/EDTA extraction (12) have been used.

Diluting the sample with methyl-isobutyl-ketone (MIBK) (14) reduces the metal concentration, but has some practical advantages: it is fast and simple, with little chance of contamination. Because of the very low metal contents, enrichment procedures using ashing or extraction methods may be more sensitive than diluting in addition with direct analysis.

The methods used in this study, which reports the levels of several trace elements in a typical commercial salad-grade corn oil, are described below. The metals under investiga-