TABLE II

Band Progression in 1180-1350 cm^{-1} Region of IR Spectra of Glycidyl Esters in Solid State

Ester	Frequency of bands, cm^{-1}								
Miristate	1351	1328	1306	1280	1255	1228	1201		
Palmitate	1350	1328	1310	1288	1265	1242	1219	1197	
Stearate	1347	1328	1312	1292	1273	1254	1233	1213	1193

spectra showed a doublet at ca. 718 and 729 cm^{-1}, suggesting some kind of perpendicular arrangement among the hydrocarbon chain planes, as in β' forms glycerides.

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Electron Spin Resonance of Free Radicals Formed in Irradiated Fatty Acid Methyl Esters

ABSTRACT

A composite nonsymmetrical electron spin resonance signal was detected in unsaturated fatty acid methyl esters (FAME) irradiated in quartz by a γ-ray dose of 500 Krad at liquid nitrogen temperature. The signal spread over a width of ca. 20 G with a spectroscopic splitting factor value of ca. 2.038, but contained an interference from the irradiated quartz. A narrower signal($\simeq$6 G) with a spectroscopic splitting factor value of 2.0361 was detected in the unsaturated FAME irradiated by UV, and was devoid of the hyperfine structure. No signal could be detected in methyl stearate irradiated by UV under the same conditions.

The autooxidation of unsaturated fatty acids is almost universally accepted to occur via a free radical mechanism in which electromagnetic energy, among other factors, plays an important role in the initiation step (1). Since electron spin resonance spectroscopy (ESR) responds only to the presence of unpaired electrons, it provides the best direct approach for ascertaining the presence of free radicals. Free radicals have been detected by ESR during ozonization of linoleic acid (2) and in the reaction of nitrogen dioxide with unsaturated fatty acids and phospholipids (3). However free radicals could not be detected by ESR, under the most favorable conditions, during the peroxidation of arachidonate in aqueous emulsions (4). On the other hand, it was demonstrated that fish protein concentrates with added highly unsaturated fatty acids exhibited a characteristic ESR signal when lipid oxidation occurred (5).

ESR has been applied to the study of the free radicals formed in triglycerides upon high energy irradiation. The types of radicals produced were found to vary with both the substrate and temperature of irradiation (6,7). The free radicals formed in several fatty acids upon irradiation have also been investigated (8,9). No radicals could be detected by ESR in fatty acids oxidized by air, irradiated by UV, or by a γ-ray dose of less than 1.0-1.2 Mrad. This communication reports an attempt to detect and study the free radicals formed by γ or UV irradiation of some unsaturated fatty acid methyl esters (FAME).

About 200 mg of the high purity FAME, Hormel Institute, was placed in a thin-walled 4 mm OD Spectrosil quartz tube and degassed by alternative freezing and warming five times under high vacuum (5-10 μ). The tubes were sealed and then irradiated in a Dewar flask filled with liquid nitrogen (-196 C). The γ source was a Co^{60} Gamma Cell 220 designed by Atomic Energy of Canada Ltd., and the dose applied was measured by a Fricke-Miller dosimeter. In the case of UV irradiation, the Dewar flask was made of quartz and the light source was a 500 W mercury arc lamp, PEK, Model 915 L.H., Sonnyvale, Calif. The light was passed through a 5 cm wide water filter to eliminate IR light and then focused on the sample placed ca. 15 cm from the lamp. Condensation of moisture on the outer wall of the Dewar flask was prevented by passing a jet of dried air around the flask. At the end of irradiation, the sample tube was transferred quickly to the spectrometer cavity, which was precooled to -196 C in order to prevent recombination of the free radicals. The spectra were recorded by using a Varian (V-4500-10A) X-band spectrometer utilizing 100 Kc/sec modulation. The spectrometer was fitted with a Fieldial (V-FR 2503) magnetic field regulator and an Alpha (M 3093) Digital NMR Gaussmeter.

The ESR spectra of the methyl esters of oleate, linoleate and linolenate irradiated at a dose of 0.5 Mrad revealed a nonsymmetrical signal that was almost identical for the three FAME examined. The composite signal spread over ca. 20 G and had a spectroscopic splitting factor (g) value of 2.038. A hyperfine structure was apparent but variant at higher irradiation doses. A smaller and similar signal was always present in the spectra of irradiated empty quartz tubes used as blanks. This is in disagreement with the re-

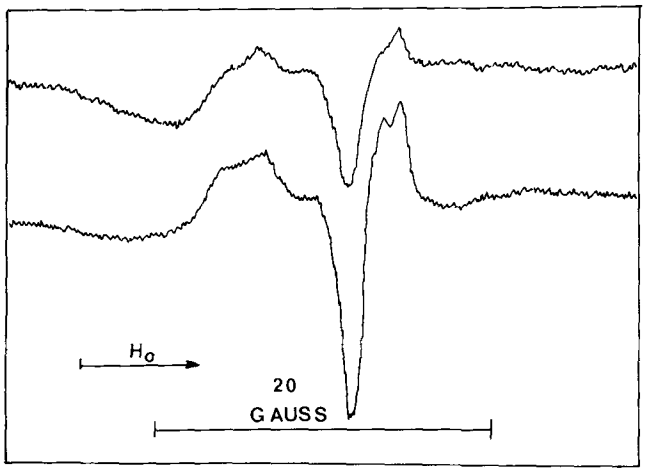

FIG. 1. ESR spectra of methyl linolenate (bottom) and Spectrosil quartz blank (top) after irradiation with 0.5 Mrad. Irradiation and recording were performed at -196 C; the curve represents first derivative of the actual absorption line.

sults of Wozniak and Krauze (9), who obtained no signal for quartz irradiated at a γ-ray dose of up to 3.5 Mrad. The signals obtained for quartz and for methyl linolenate are presented in Figure 1. UV light was used for initiating free radicals because the energy associated with γ-rays, being far in excess of all bond dissociation energies, produced an interfering signal from quartz, while UV light is less energetic and tends to rupture weaker bonds preferentially.

When the FAME samples were irradiated with the high intensity UV light for 1 or 2 hr, no signals could be detected. After 3 hr of irradiation a small signal was detectable in methyl linolenate, and after 4 hr the signal was apparent in methyl oleate and linoleate. A further increase in irradiation time brought about a small or no increase in the signal intensity. No signals representing hydrogen atoms (doublets) or methyl radicals (quartets) could be detected in the irradiated samples of unsaturated FAME. In addition, no signal was detectable in methyl stearate irradiated by UV under the same conditions. The spectra recorded for methyl linoleate after 6 hr of UV irradiation are presented in Figure 2.

The apparent g value of the signal was 2.0361, which is close to that found for the composite signal obtained in γ irradiation of the samples. This g value is considerably higher than the 2.0055 reported for the lipid signal in oxidized freeze-dried fish (5). Thus the latter value may have resulted from an interaction of the free radicals of lipids and proteins. The absence of any signal for hydrogen atoms (formed presumably during irradiation) indicates the rapid diffusion of these light and highly mobile radicals and recombination with themselves or with the parent molecules. The narrow line width observed for the signal of the organic radical, 2.5 G from peak to peak, might be attributed in part to delocalization of the unpaired electrons. Attempts to resolve the hyperfine structure of the signal were unsuccessful, thus making the interpretation of the spectra difficult. Reducing modulation amplitude or power resulted in almost complete loss of the spectra. It was evident that the signal observed was near the limit of instrument detection.

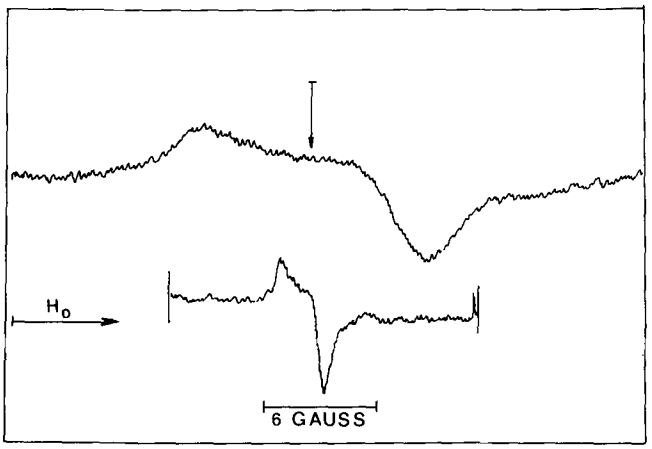

FIG. 2. ESR spectrum of methyl linoleate irradiated with UV light for 6 hr. Irradiation and recording were performed at -196 C; the curve represents first derivative of the actual absorption line; g value of the marker is 2.0361; upper curve is an expanded recording. Identical spectra for methyl oleate and linolenate, and no signal for methyl stearate, were recorded.

Results of this study provided a direct proof for the formation of free radicals upon γ and UV irradiation of some common unsaturated fatty acids. Although the presence of free radicals has been established, the site of the unpaired electrons could not be localized due to the limits of the present instrumentation. Thus the answer to this fundamental question should await further instrumentation developments.

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