in complex forms to the associated carbohydrate, carboxylic groups of glutamic or aspartic acids, amide groups, or oxidation products of the associated lipids. The more severe heat treatment and damage to the cashew nut discard kernel might thus explain the differences between the discard and the good grade kernel, which had received a milder heat treatment, in terms of overall protein quality and the different extent to which they responded to amino acid supplementation.

It appears that cashew nut discard meal could be of considerable value in diets, provided its production is carefully controlled to minimize heat damage. Its value in practical-type diets therefore needs to be more carefully studied.

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LITERATURE CITED

Association of Official Analytical Chemists, Official Methods of Analysis, 11th ed, Washington, D. C., 1970.
Bender, A. E., Doell, B. H., *Brit. J. Nutr.* **11**, 140 (1957).
Duncan, D. B., *Biometrics* **11**, 1 (1955).
Ford, J. E., *Brit. J. Nutr.* **19**, 277 (1965).
Kaufmann, H. P., Barve, J., *Fette Seifen Anstrichm.* **69**, 437 (1967).
Markley, K., "Soybean and Soybean Products," Interscience, New York, N. Y., 1950.
Melnick, D., Oser, B. L., *Food Technol.* **3**, 57 (1949).
Miller, D. S., *Nat. Acad. Sci.-Nat. Res. Counc. Publ.* **No. 1100**, 34 (1963).
Miller, E. L., *J. Sci. Food Agr.* **18**, 381 (1967).
Miller, E. L., Carpenter, K. J., Milner, C. K., *Brit. J. Nutr.* **19**, 547 (1965).
Neishem, M. C., Carpenter, K. J., *Brit. J. Nutr.* **21**, 399 (1967).
Nat. Acad. Sci.-Nat. Res. Counc. Publ. **No. 1100** (1963).
Piva, G., Santi, E., Ekpenyong, T. E., *J. Sci. Food Agr.* **22**, 22 (1971).
Rippon, W. P., *Brit. J. Nutr.* **13**, 243 (1958).
Roach, A. G., Sanderson, P., Williams, D. R., *Analyst* **93**, 42 (1968).
Steel, G. D., Torrie, J. G., "Principles and Procedures of Statistics," McGraw-Hill, New York, N. Y., 1960.
Wolf, W. J., *J. Agr. Food Chem.* **18**, 969 (1970).
Woodroof, J. G., *World Rev. Nutr. Diet.* **11**, 142 (1969).

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Loss of Free-Radical Signal during Induction Period of Unsaturated Lipids Containing Nitroxide Antioxidants

James S. Lin, Vega Smith, and Harold S. Olcott*

Stable free-radical nitroxides have marked antioxidant activity in unsaturated lipids. The amount of residual nitroxide can be estimated from the electron paramagnetic resonance (epr) signal. With three different nitroxides in squalene at 37° the signal gradually decreased during the induction period. Only when it was no longer or barely detectable, did active uptake of oxygen begin. At 50° with squalene and with menhaden oil, active oxidation began while the nitroxide signal was still measurable. In squalene at 37°, the three nitroxides studied, Tempol (2,2,6,6-tetramethyl-4-piperidinol-*N*-oxyl), Synvar 611 (4′,4′-dimethylspiro[5α-cholestane-3,2′-oxazolidin]-3′-yloxyl), and Synvar 614 (2-[10-carboxydecyl]-2-hexyl-4,4-dimethyl-3-oxazolidinyloxyl), had relative antioxidant activities at equivalent molarities of approximately 2:1.2:1. A simplified method for following weight gain and epr signal without transfer of sample is described.

Free radicals are involved in the autoxidation of lipids, but their half-lives are usually so short that they cannot easily be detected by electron paramagnetic resonance (epr) spectroscopy. We have taken advantage of the fact that stable free-radical nitroxides have antioxidant activity (Weil *et al.*, 1968) to study their fate during the induction period. In general the oxidation of the lipid was inhibited until the signal was no longer detectable. Our observations thus parallel those quoted by Rozantsev (1970) concerning the thermal oxidation of certain polymers: "... radical inhibitors completely block the oxidation of polyamide until they are exhausted, and, after the end of the induction period, the rate of oxidation approximates the rate of the uninhibited oxidation of the polyamide (Neiman *et al.*, 1965)."

MATERIALS AND METHODS

The nitroxides used were Synvar 611 (4′,4′-dimethylspiro[5α-cholestane-3,2′-oxazolidin]-3′-yloxyl), Synvar 614 (2-[10-carboxydecyl]-2-hexyl-4,4-dimethyl-3-oxazolidinyloxyl), and Tempol (2,2,6,6-tetramethyl-4-piperidinol-*N*-oxyl). The first two were from Synvar Associates and the third was a gift of A. Keith. Some observations on Tempol as an antioxidant appeared in a previous publication (Weil *et al.*, 1968). The substrates for antioxidant assays were squalene (Eastman) and a sample of menhaden oil that had been molecularly distilled (National Marine Fisheries Service, Seattle, Wash.). Each was further purified before use by silicic acid chromatography (Olcott and Van der Veen, 1968).

The methods used for evaluating antioxidant activity and measuring epr spectra including the following.

Method 1. Watch-glass covered 10-ml beakers containing 200 mg of substrate with and without additive were held in constant temperature draft ovens. Once or twice daily they were removed from the oven, tested for rancidity by odor, cooled to room temperature, and weighed (Olcott and Einset, 1958). At intervals an approximately 50-μl sample was transferred to a quartz tube, the epr spectra were measured with a Varian Model E-3 X-band spectrometer, and the sample was returned to the beaker. Signal intensities were determined from the height of the center line of the three-line nitroxide spectra.

Method 2. Halves of filter papers (Whatman No. 1, 4.25 cm) were rolled into small cylinders and inserted into

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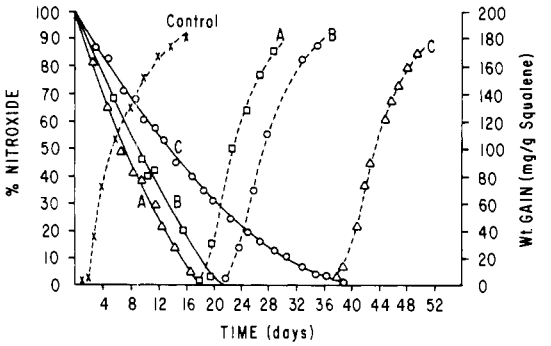

Figure 1. Effect of nitroxides in squalene on weight gain and epr signal loss at 37°: (—) signal loss; (- - -) weight gain; (control) squalene alone; (A) squalene plus Synvar 614 (48 nmol/g); (B) squalene plus Synvar 611 (45 nmol/g); (C) squalene plus Tempol (48 nmol/g).

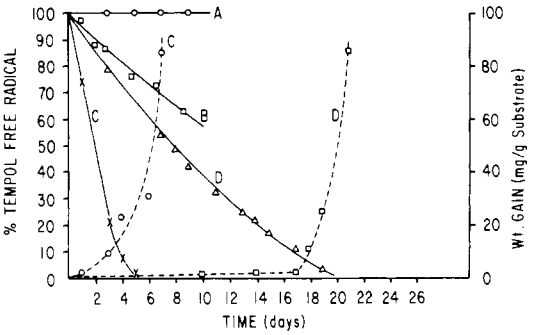

Figure 2. Effect of nitroxides on weight gain and epr signal loss in squalene and menhaden oil at 50°: (—) signal loss; (- - -) weight gain; (A) Tempol in air; (B) menhaden oil plus Tempol (15.3 μmol/g) in nitrogen; (C) menhaden oil plus Tempol (15.3 μmol/g) in air; (D) squalene plus Tempol (3.7 μmol/g) in air.

quartz tubes (length 15 cm, i.d. 3.18 mm). The tubes were dried to constant weight at 100° and 80 mg of substrate with or without additive was injected slowly into the tube from a hypodermic syringe and allowed to absorb onto the filter paper. The tubes were held in a constant draft oven. Once or twice daily they were removed from the oven, cooled to room temperature, and weighed. The epr spectrum was obtained on each tube without transfer of sample. Oxidation rates on paper were considerably faster than in bulk.

RESULTS

Calibrations of the substrates used showed that there were linear relationships between the central peak heights of the three-line epr signals and the concentrations of free radicals. Concentrations less than 10^{-1} nmol/g of substrate were undetectable.

The results obtained when samples of squalene containing each of the three nitroxides were incubated at 37° are shown in Figure 1. The lengths of the induction periods indicate that antioxidant activity of Synvar 611 was more than that of Synvar 614, but about one-half that of Tempol. Conversely, the rate of disappearance of Synvar 611 was slower than that of Synvar 614, but faster than that of Tempol. When the nitroxide radical concentration reached a critical low value, rapid lipid oxidation occurred as indicated by the gains in weight (Figure 1). Approximately 1% (5×10^{-1} nmol/g of substrate) of the Tempol free radicals remained in the squalene at the end of the induction period, but rapid oxidation began only after the signals for Synvar 611 and 614 were no longer detectable.

In contrast, at 50° both squalene and menhaden oil began to oxidize while measurable amounts of nitroxide were still present (Figure 2). Figure 2 also shows that the rate of loss of signal of Tempol in menhaden oil in nitrogen was very slow compared to that in air, and that Tempol absorbed on filter paper in the absence of substrate did not show a signal change when stored at 50° in air.

DISCUSSION

Thomas (1960) first observed that nitroxides derived from aromatic amines were antioxidants for hydrocarbons. Neiman (1965) and Rozantsev (1970) have subsequently described other examples. Thus, Likhtenshtein (1962) measured the rate of loss of diphenyl nitroxide during the oxidation of cetane at 150°. Neiman (1965) described the antioxidant capacity of the ketone corresponding to Tempol (Tempone) including the rate of loss of signal during the induction period of polycaproamide oxidation. In this case, as in the present series of studies, the autocatalytic oxidation did not accelerate until the nitroxide radical had reached a very low level.

When Tempol in purified menhaden oil was held in *nitrogen* at 50°, the very slow decrease in signal (Figure 2) may have been due to trace amounts of oxygen or peroxide that remained in the oil. The rapid loss of signal when *oxygen* was present thus legitimately reflects the reactions occurring as the lipid began to oxidize.

The mechanism by which a nitroxide radical acts as antioxidant is believed to be by direct coupling to an alkyl radical, the hypothetical first intermediate produced in the oxidation of lipids: $RNO\cdot + R\cdot \rightarrow RNOR$ (Thomas and Tolman, 1962; Brownlie and Ingold, 1967). Consequently, the rates of loss of epr signals of nitroxide shown in Figures 1 and 2 should be reflecting the rate of generation of alkyl free radicals in the oxidation mixtures.

Both a more rapid rate of loss of nitroxide and a slowed rate of loss during the latter part of induction periods have previously been reported (Neiman, 1965; Rozantsev, 1970). Each situation is probably so dependent on the individual substrate and antioxidant used, and on the temperature, oxygen pressure, and other conditions of testing as to make reaction kinetics interpretation difficult without more detailed observations.

Several investigators (Calvin *et al.*, 1969; McConnell and McFarland, 1970; Raison *et al.*, 1971) have observed losses of spin-label nitroxide signals in the presence of biological materials. Giotta and Wang (1972) suggested that such losses are due to reaction of the nitroxides with sulfhydryl groups. McConnell and McFarland (1970) report that A. Horowitz (unpublished) observed rapid reduction with ascorbic acid. Preliminary observations in this laboratory, not included in this paper, show that nitroxides disappear from oxidizing aqueous linoleate emulsions during the induction period similar to the reactions in nonaqueous systems described above. The rate of nitroxide loss was accelerated by hemoglobin. These observations suggest that lipid oxidation in biological systems may account for some of the nitroxide losses observed by others.

LITERATURE CITED

Brownlie, J. T., Ingold, K. U., *Can. J. Chem.* **45**, 2427 (1967).
Calvin, M., Wang, H. H., Entine, G., Gill, D., Ferruti, P., Harpold, M. A., Klein, M. P., *Proc. Nat. Acad. Sci. U. S.* **63**, 1 (1969).
Giotta, G. J., Wang, H. H., *Biochem. Biophys. Res. Commun.* **46**, 1576 (1972).
Likhtenshtein, G. I., Dissertation, Institute of Chemical Physics, Academy of Science, USSR, 1962; cited by Neiman, M. B., "Aging and Stabilization of Polymers," Consultants Bureau Enterprises, Inc., New York, N. Y., 1965, p 34.
McConnell, H. M., McFarland, B. G., *Quart. Rev. Biophys.* **3**, 91 (1970).

Neiman, M. B., "Aging and Stabilization of Polymers," Consultants Bureau Enterprises, Inc., New York, N.Y., 1965, p 35.
Neiman, M. B., Krinitskaya, L. A., Rozantsev, E. G., *Izv. Akad. Nauk SSSR, Ser. Khim.* 2055 (1965); cited by Rozantsev, E. G., "Free Nitroxyl Radicals," Hazzard, B. J., Translator, Ulrich, H., Ed., Plenum Publishing Co., New York, N. Y., 1970, p 113.
Olcott, H. S., Einset, E., *J. Amer. Oil Chem. Soc.* **35**, 159 (1958).
Olcott, H. S., Van der Veen, J., *Lipids* **3**, 331 (1968).
Raison, J. K., Lyons, J. M., Mehlorn, R. J., Keith, A. D., *J. Biol. Chem.* **246**, 4036 (1971).
Rozantsev, E. G., "Free Nitroxyl Radicals," Hazzard, B. J., Translator, Ulrich, H., Ed., Plenum Publishing Co., New York, N. Y., 1970.
Thomas, J. R., *J. Amer. Chem. Soc.* **82**, 5955 (1960).
Thomas, J. R., Tolman, C. A., *J. Amer. Chem. Soc.* **84**, 2930 (1962).
Weil, J. T., Van der Veen, J., Olcott, H. S., *Nature (London)* **219**, 168 (1968).

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Photochemistry of Bioactive Compounds. Multiphase Photodegradation of Basalin

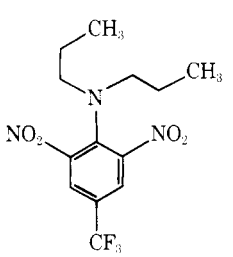
George P. Nilles and Matthew J. Zabik*[1]

The photolysis of basalin (*N*-(2-chloroethyl)-2,6-dinitro-*N*-propyl-4-trifluoromethylbenzenamine) has been studied, in solution, as a thin film and on soil. The structures of the major photoproducts have been determined and a comparison has been made between laboratory photolysis and natural sunlight photolysis for this compound. The major routes of degradation include photodealkylation, benzimidazole formation, and quinoxaline formation. At 5 ppm in solution, the rate of disappearance of basalin shows zero-order kinetics with a rate constant of 2.6×10^{-7} mol/(l. min).

It is now accepted that laboratory studies of the photochemistry of pesticides have broad significance in regard to formulating environmental usage and persistence parameters. Such studies should, of course, be conducted either in natural sunlight or with the use of irradiation devices which simulate sunlight as closely as possible in regard to spectral distribution.

The trifluralin (1) type herbicides were introduced in the early 1960's and have met with widespread acceptance and use. However, only recently has the detailed photochemistry of 1 (Crosby and Leitus, 1973) or similar compounds such as dinitramine (Newsom and Woods, 1973) been explored.

Basalin (**2**, Fluchloralin, BAS-392, *N*-(2-chloroethyl)-2,6-dinitro-*N*-propyl-4-(trifluoromethyl)benzenamine) is an experimental herbicide under development by BASF-Wyandotte Corp. Its ultraviolet spectrum shows a continuous extinction coefficient of at least 475 throughout the 290–450-nm region with a maximum at 364 nm (ϵ 2060). A study of the photoproducts from this molecule should serve to further confirm earlier findings in regard to the generality of the formation of benzimidazoles from this type of compound. In addition, the presence of the chlorinated side chain could well lead to reactions giving products more diverse than the normally substituted anilines.



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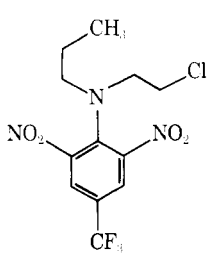

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EXPERIMENTAL SECTION

Equipment and Reagents. Gas chromatography (gc) employed a Beckmann GC-65 instrument equipped with a 6 ft × ⅛ in. i.d. glass column packed with either 3% XE-60 or 3% SE-30 on 80–100 mesh Gas Chrom Q. The oven was programmed as follows: 8 min at 105° and then heated to 175° at 2.5°/min and held at 175°. The injector temperature was 265° and the flame ionization detector was heated at 280°. The carrier gas was 99.996% pure helium at 20 ml/min. Mass spectra were run on a DuPont 21-490 instrument interfaced to the above gc. All samples were introduced *via* the gc inlet and spectra were determined at 70 eV with the source temperature at ambient. Integration was performed electronically (Houston Instruments "Omniscribe" Integrator) or by the cut and weigh method. Nmr spectra were determined on a Varian T-60 machine in carbon tetrachloride with TMS as the internal standard. Ultraviolet spectra were run on a Unicam SP 800 spectrometer in 95% ethanol. Liquid scintillation counting employed a Nuclear-Chicago "unilux" counter. Samples were counted in 10 ml of scintillation cocktail (5 g of PPO and 300 mg of POPOP/l. of toluene). Quench correction was by the internal standard method. Thin-layer chromatography used either (A) "Analtech" 250-μ, 5 × 20 cm silica G plates, (B) E. Merck 250-μ, 20 × 20 cm silica GF-254 plates, or (C) a 500-μ coating of E. Merck silica GF-254 on a 20 × 20 cm plate. All elutions were performed with chloroform. A Rayonet Srinivasan-Griffin photoreactor employing eight RPR 3000-Å and eight RPR 3500-Å lamps in an alternate configuration was used for all photolyses involving artificial sunlight. The spectral distribution of the photoreactor is shown in Figure 1. The photolysis vessel consisted of a 30 × 160 mm Pyrex vessel fitted with an air inlet and cold finger condensor which was immersed in the photolysis solution and connected to an external constant temperature bath by which means the photolysis solution was maintained at 15°. The solution was magnetically stirred during photolysis. For the kinetic studies in solution, the above vessel was replaced with a 4.5-l. Pyrex bottle. The solution was cooled and stirred in the same manner as above. The energy output of the photolamps in either vessel was measured by a YSI