MS data in literature reports were in excellent agreement with those for the components identified as 1-cyano-2,3-epithiopropane (Cole, 1975) and 1-cyano-3,4-epithiobutane (Kirk and MacDonald, 1974). The remaining four nitrile compounds identified in our work gave appropriate MS data for the assigned structures and spectra for three of them agreed with MS data reported by Buttery et al. (1976). These spectra and that of the remaining component (no. 14, Table I) were easily interpreted from spectra of the known related compounds and knowledge of the constituent glucosinolate precursors.

Based on GLC, three sauerkraut preparations contained the 1-cyano-3-methylsulfinylpropane in estimated amounts of 4 to 16 ppm of the whole kraut. No GLC evidence for the corresponding isothiocyanate was found. Since no compositional information was available for the cabbages from which the krauts were made, further experiments are being planned in which krauts from cabbages of known glucosinolate content will be studied.

Nitriles previously identified as hydrolysis products from the Cruciferae plants are listed in Table III.

Formation of nitriles instead of the expected isothiocyanates and goitrin from the glucosinolates by simply crushing the cabbage shows that consideration must also be given to the possible physiological effects of these compounds. In view of the long history of raw and cooked cabbage as a food staple without acute toxic effects, there is no immediate cause for alarm. However, more biological testing is warranted to properly evaluate the effects of the glucosinolates and their breakdown products and how food preparation methods affect their presence.

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LITERATURE CITED

Buttery, R. G., Guadagni, D. G., Ling, L. C., Seifert, R. M., Lipton, W., *J. Agric. Food Chem.* **24**, 829 (1976).
Cheung, K. K., Kjaer, A., Sim, G. A., *Chem. Commun.*, 100 (1965).
Cole, R. A., *Phytochemistry* **14**, 2293 (1975).
Cole, R. A., *Phytochemistry* **15**, 759 (1976).
Daxenbichler, M. E., Spencer, G. F., Kleiman, R., VanEtten, C. H., Wolff, I. A., *Anal. Biochem.* **38**, 374 (1970).
Daxenbichler, M. E., VanEtten, C. H., Northern Regional Research Center, Peoria, Ill., unpublished data, 1976.
Daxenbichler, M. E., VanEtten, C. H., Tallent, W. H., Wolff, I. A., *Can. J. Chem.* **45**, 1971 (1967).
Daxenbichler, M. E., VanEtten, C. H., Wolff, I. A., *Biochemistry* **5**, 692 (1966).
Daxenbichler, M. E., VanEtten, C. H., Wolff, I. A., *Phytochemistry* **7**, 989 (1968).
Gmelin, R., Virtanen, A. I., *S. Kimistilehti B* **34**, 15 (1961).
Josefsson, E., *Phytochemistry* **6**, 1617 (1967).
Kirk, J. T. O., MacDonald, C. G., *Phytochemistry* **13**, 2611 (1974).
Macleod, A. N., Macleod, G., *J. Sci. Food Agric.* **19**, 273 (1968).
Schultz, O. E., Gmelin, R., *Arch. Pharmacol.* **287** (59), 404 (1954).
Senti, F. R., Rizek, R. L., *Crop Sci. Soc. Am. Spec. Publ.* **No. 5**, (1974).
Spencer, G. F., Powell, R. G., Plattner, R. D., *J. Chromatogr.*, in press (1976).
Tookey, H. L., *Can. J. Biochem.* **51**, 1654 (1973).
VanEtten, C. H., Daxenbichler, M. E., *J. Agric. Food Chem.* **19**, 194 (1971).
VanEtten, C. H., Daxenbichler, M. E., Peters, J. E., Tookey, H. L., *J. Agric. Food Chem.* **14**, 426 (1966).
VanEtten, C. H., Daxenbichler, M. E., Williams, P. H., Kwolek, W. F., *J. Agric. Food Chem.* **24**, 452 (1976).
VanEtten, C. H., Daxenbichler, M. E., Wolff, I. A., *J. Agric. Food Chem.* **17**, 483 (1969).

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Cooxidation of β-Carotene by an Isoenzyme of Soybean Lipoxygenase

Christopher O. Ikediobi[1] and Harry E. Snyder*

Soybean lipoxygenase 1 (SL1) will cooxidize β-carotene while catalyzing the oxidation of linoleic acid (LH). This study established that SL1 will also cooxidize β-carotene when linoleic hydroperoxide (LOOH) is substituted for LH. Reduction of LOOH with sodium borohydride stops the cooxidation of β-carotene, and molecular oxygen is required for the cooxidation. The rate of cooxidation of β-carotene is less with LOOH than with LH. The products of the enzymatic β-carotene oxidation are essentially the same with LOOH as with LH. Rate studies indicate that LH and β-carotene are competitive inhibitors, but LOOH and β-carotene are not competitive.

Interest in the activity of soybean lipoxygenase (SL) on β-carotene ranges from practical applications to basic questions about the mechanism. Wolf (1975) recently reviewed the use of soybean flour as an aid to bleaching wheat flour for bread and pointed out that the process is currently in wide use.

Kies et al. (1969) raised a question about whether soybean lipoxygenase or some other enzyme in soy extracts causes β-carotene oxidation. That question has now been answered with the discovery of isozymes of soybean lipoxygenase (Guss et al., 1967; Christopher et al., 1970, 1972; Verhue and Francke, 1972). Several authors have found that the acid lipoxygenases, SL2 and SL3, are more active catalysts of β-carotene oxidation than the alkaline soybean lipoxygenase, SL1 (Weber et al., 1974; Arens et al., 1973). However, SL1 does catalyze the cooxidation of β-carotene in the presence of linoleic acid (LH). Since SL1 is the predominant isozyme present in soybean flour and since SL1 is much more stable than SL2 or SL3, SL1 may be the important catalyst in wheat flour bleaching by soybean flour.

Early work on the cooxidation of β-carotene by SL and LH established rates of oxidation of β-carotene and LH (Blain et al., 1953; Tookey et al., 1958). The general conclusion from these studies was that an intermediate free

Departments of Biochemistry and Biophysics and of Food Technology, Iowa State University, Ames, Iowa 50011.

[1]Present address: Department of Chemistry, Florida A&M University, Tallahassee, Fla. 32307.

radical, produced in the oxidation of LH to linoleic hydroperoxide (LOOH), was causing β-carotene oxidation. One difficulty with these experiments was the lack of a well-emulsified β-carotene preparation that would allow continuous spectrophotometric measurements of β-carotene bleaching. Ben Aziz et al. (1971) developed a technique for β-carotene emulsification that allows continuous spectrophotometric measurements. Friend (1956, 1958) and Zinsou (1971) have isolated and identified carotenoid products of the cooxidation of β-carotene by LH and SL.

A recent development that bears on the mechanism of action of SL is the discovery of nonheme iron associated with the enzyme (Chan, 1973; Roza and Francke, 1973; Pistorius and Axelrod, 1974). There is now evidence that the iron plays an essential role in lipoxygenase activity and that LOOH is required in the reaction of SL1 with LH (Finazzi-Agro et al., 1973; de Groot et al., 1975).

We have investigated the cooxidation of β-carotene using SL1, and our results have suggested a new interpretation of the role of SL1 in β-carotene bleaching.

EXPERIMENTAL SECTION

Purification of SL1. Hexane-defatted and ground cotyledons (300 g) of *Glycine max* (Amsoy 71 variety) were extracted with 3.2 l. of 0.2 M acetate buffer of pH 4.5. The soluble whey proteins were fractionated with solid ammonium sulfate. The fraction precipitating between 40 and 80% $(NH_4)_2SO_4$ saturation was subsequently purified at 5 °C by gel filtration on Sephadex G-150 (40–120 μm) with 0.2 M phosphate buffer of pH 6.8, and by ion-exchange chromatography on DEAE-Sephadex A-50-120 (40–120 μm; 3.5 ± 0.5 mequiv g^{-1}). Elution was with a linear gradient from 0.05 M phosphate buffer (pH 6.8) to 0.05 M phosphate buffer of pH 6.8 + 0.5 M sodium chloride. The column was 40 × 2.7 cm, and SL1 eluted with approximately 250 ml. The recovered SL1 solution was concentrated and lyophilized. One enzyme unit was defined as the amount of SL1 that produced a 0.001 change in absorbance at 234 nm in 1 min. The specific activity of our preparation of SL1 is 1.52×10^5 units per mg of protein.

Preparation and Identification of LOOH. LOOH was prepared and purified by the method of Gardner (1970) and Gardner and Weisleder (1972). Positive identification of the –OOH function was made by observation of a brick-red zone upon spraying a sample chromatographed on silica gel G (solvent, *n*-hexane–diethyl ether–acetic acid, 50:50:1) with freshly prepared ferrous thiocyanate (Vioque and Holman, 1962). The uv spectrum was a single strong absorption at 233–234 nm, indicative of the conjugated diene chromophore (calcd E_{max} 27 000 M^{-1} cm^{-1}). The ir spectrum (solvent CS_2) determined after esterification of LOOH with diazomethane showed a moderately strong, but slightly broadened, absorption at 3420 cm^{-1} attributed to the –OOH group. The intense absorption at 1740 cm^{-1} was due to the stretching vibration of the C═O group and the sharp absorptions at 950 and 985 cm^{-1} were those of a cis,trans-conjugated diene system. The NMR spectrum taken with a 100-MHz instrument was identical with that reported by Gardner and Weisleder (1972).

Preparation of Substrates. Substrates were prepared daily prior to enzyme assays.

Aqueous β-Carotene. Repurified (TLC) commercial β-carotene (0.5 mg) was mixed with 18 mg of Tween 80 and dissolved in 4 ml of $CHCl_3$, which was subsequently evaporated under a stream of N_2. The resulting residue was dissolved in 10 ml of 0.25% EDTA solution to give an aqueous water-clear solution of β-carotene. The exact concentration of β-carotene was determined spectrophotometrically.

Aqueous LOOH. An appropriate amount of pure LOOH was dissolved in 50 ml of 0.2 M air-saturated borate buffer (pH 9.0) to give the desired concentration of LOOH.

Aqueous LH. One milliliter of a freshly prepared stock solution of LH in 95% ethanol (10 mg ml^{-1}) was diluted to 50 ml with 0.2 M air-saturated borate buffer of pH 9.0.

Assay of the Cooxidation Reaction. All assays were performed at room temperature (25 ± 2 °C).

Spectrophotometric Assay. Two-tenths milliliter of aqueous β-carotene and 1.5 ml of aqueous LOOH or LH were pipetted separately into a 3-ml quartz cuvette. An amount of 0.2 M borate buffer of pH 9.0 necessary to bring the total reaction volume to 3 ml was added to the same cuvette. The reference cuvette containing 1.5 ml of aqueous LOOH or LH was made up to 3 ml with borate buffer. The decrease in absorbance at 452 nm, produced as a result of the enzymatic bleaching of β-carotene, was recorded automatically as a function of time in a Beckman DK-2A ratio-recording spectrophotometer. Initial velocity was computed from the linear portion of the progress curve.

Isolation of Colored β-Carotene Oxidation Products. β-Carotene (3 mg) and presolubilized LOOH (80–90 mg) were incubated with 9 mg of SL1 in 100 ml of 0.2 M phosphate buffer (pH 6.8) preequilibrated with the atmosphere. The reaction was allowed to proceed for 2–3 min at room temperature and stopped with 6 ml of 20% KOH. The colored reaction products and unreacted β-carotene were extracted with chloroform (6 × 50 ml). The chloroform extract was washed with H_2O several times, dried with Na_2SO_4, and concentrated under reduced pressure. The colored oxidation products were separated on a short column of deactivated Woelm alumina (10% H_2O) eluted and further purified by TLC on silica gel G in a variety of solvent systems (Knowles and Livingston, 1971).

Synthetic Carotenoid Standards. Dr. O. Isler of Hoffman LaRoche, Basel, Switzerland, supplied us with the following synthetic carotenoids: β-apo-8′-carotenal, cryptoxanthin (3-hydroxy-β-carotene), β-carotene monoepoxide (5,6-epoxy-β-carotene), mutatochrome (5,8-furanoid oxide of carotene), β-carotene diepoxide (5,6,-5′,6′-diepoxy-β-carotene), and aurochrome (5,8,5′,8′-difuranoid oxide of β-carotene).

RESULTS

Rates of β-Carotene Oxidation. It is known that SL1 acts on LH to produce LOOH, and if β-carotene is present, it is cooxidized to other carotenoids which are subsequently bleached completely. Since LOOH is a strong oxidizing agent, we isolated it and investigated its role in the cooxidation of β-carotene. Reacting β-carotene with LOOH in the absence of SL1 caused detectable bleaching of β-carotene, but the rate was much slower than with LH and SL1.

When LOOH and SL1 were reacted with β-carotene (in the absence of LH), we observed a cooxidation of β-carotene at approximately one-third the rate of the cooxidation with LH and SL1. This is the first report that LOOH can replace LH in the enzymatic cooxidation of β-carotene. Further experimentation with the reaction mixture containing LOOH, SL1, and β-carotene gave the results shown in Figure 1. When the LOOH concentration increased, the rate of β-carotene bleaching also increased. If either LOOH or SL1 or both were eliminated from the reaction mixture, little β-carotene bleaching occurred.

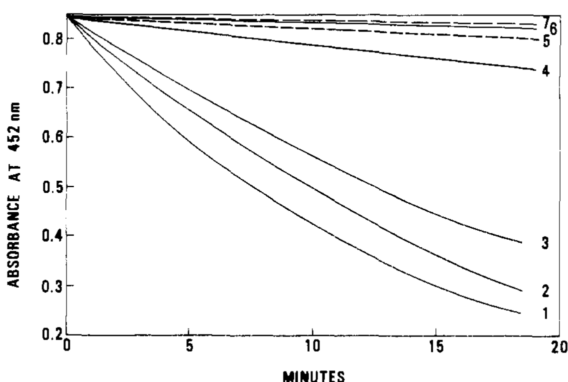

Figure 1. Assays of oxidation of β-carotene by the LOOH-SL1 system. Each reaction mixture contained 4.3 μM β-carotene and 1.84×10^4 units of SL1 (when present) in 0.2 M borate (pH 9). The LOOH concentrations were: (1) 6.8×10^{-4} M; (2) 3.4×10^{-4} M; (3) 1.7×10^{-4} M; (4) 2.72×10^{-3} M with no SL1; (5) no LOOH and no SL1; (6) no LOOH; (7) 3.4×10^{-3} M and 2.3×10^{-2} M $Na_2S_2O_4$.

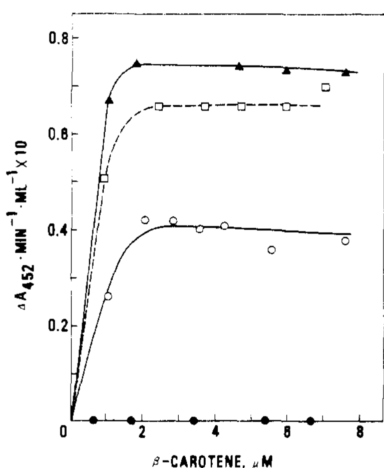

Figure 2. Effect of different concentrations of β-carotene on the rate of β-carotene bleaching in the presence of LOOH. SL1 concentration was 1.84×10^4 units/ml in 0.2 M borate (pH 9), and the reaction mixture was saturated with air (240 μM O_2). LOOH concentrations were: (○) 0.45×10^{-3} M; (□) 1.48×10^{-3} M; (▲) 2.96×10^{-3} M; (●) 0.5×10^{-3} M but reduced with $NaBH_4$.

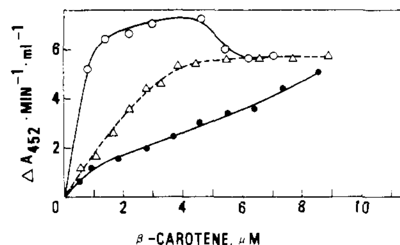

Figure 3. Effect of different concentrations of β-carotene on the rate of β-carotene bleaching in the presence of LH. SL1 concentration was 9.2×10^4 units/ml in 0.2 M borate at pH 9, and the reaction mixture was saturated with air (240 μM O_2). LH concentrations were: (○) 3.57×10^{-4} M; (△) 10.7×10^{-4} M; (●) 21.4×10^{-4} M.

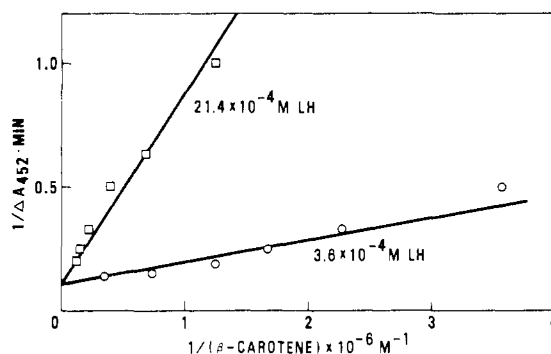

Figure 4. Lineweaver-Burke plot showing competitive inhibition of LH on β-carotene bleaching. Rates taken from Figure 3.

Table I. Inhibition of β-Carotene-Oxidizing Activity of LOOH-SL1 System by Propyl Gallate[a]

Propyl gallate $\times 10^{-3}$ M	% β-carotene-oxidizing act. remaining
0	100
0.22	34
0.45	23.2
0.67	20
0.90	17
1.12	14

[a] The concentrations of β-carotene and LOOH in the 3-ml reaction mixture were 5.4 μM and 148×10^{-3} M, respectively (0.2 M borate; pH 9.0).

Also, if the reaction mixture was made anaerobic by the addition of 2.3×10^{-2} M $Na_2S_2O_4$, no β-carotene bleaching took place. Preincubating SL1 with $Na_2S_2O_4$ caused no loss of activity and purging the assay system with N_2 stopped the β-carotene oxidation. So, the $Na_2S_2O_4$ was reacting with O_2 and was not inactivating SL1 or reducing LOOH to inhibit β-carotene bleaching.

Figure 2 gives data on the rates of β-carotene bleaching with changing β-carotene concentration at three different concentrations of LOOH. These data show that the concentration of β-carotene becomes saturating at concentrations greater than about 2 μM. Note also that reduction of LOOH with $NaBH_4$ (Christopher and Axelrod, 1971) completely stopped β-carotene bleaching.

In contrast to Figure 2, Figure 3 shows data on β-carotene bleaching in the presence of SL1 and LH rather than LOOH. As the concentration of LH increases, the rate of β-carotene bleaching decreases indicating possible competition between LH and β-carotene for a reactive site on SL1. The competitive inhibition is shown clearly in Figure 4.

The common phenolic antioxidants, propyl gallate, butylated hydroxyanisole, and butylated hydroxytoluene, inhibit β-carotene bleaching in the presence of LOOH and SL1. Table I shows data for propyl gallate inhibition. A Lineweaver–Burke plot of these data showed a pattern intermediate between competitive and noncompetitive inhibition.

Products of β-Carotene Oxidation. In addition to the rate studies of β-carotene cooxidation, the colored reaction products from a reaction mixture containing LOOH, β-carotene, and SL1 were isolated and identified. We found that SL1 was more rapidly destroyed in the presence of LOOH than in the presence of LH, and consequently a greater amount of SL1 was needed to accumulate reaction products from LOOH–β-carotene than from LH–β-carotene.

The products were identified by comparing known samples and unknowns with respect to (1) ir, visible, and uv absorption spectra (Davies, 1965); (2) mass spectra (Enzell et al., 1968, 1969); (3) cochromatography in several solvent systems; (4) partition coefficients (Subrarayan and Cama, 1968; Davies, 1965; Petracek and Zeichmeister, 1956); (5) characteristic color changes on treatment with acid (Monties and Costes, 1968; Korosy, 1956); and (6) reactions with hydroxylamine hydrochloride (Critchley et

al., 1958). The products isolated were identified as: mutatochrome, aurochrome, a β-apocarotenal, and a cis isomer of β-carotene. A fifth product was isolated in very small quantities and was tentatively identified as retrodehydro-β-carotene. These are the same products (except for retrodehydro-β-carotene) that were isolated by Zinsou (1971) and by Friend (1956, 1958) in reaction mixtures of SL, LH, and β-carotene. Zinsou also found luteochrome (5,6-epoxy-5′,8′-furanoid oxide β-carotene).

DISCUSSION

Previous investigators have attributed the cooxidation of β-carotene by SL1 and LH to a free-radical intermediate formed during the oxidation of LH to LOOH. Now, the finding that SL1 and LOOH can also cause the cooxidation of β-carotene casts doubt on the idea of a free-radical intermediate such as L· or LOO· being the cause of β-carotene oxidation. Some LOO· may be produced from LOOH, but SL1 is required for the cooxidation of β-carotene, so SL1 must be catalyzing changes in LOOH and β-carotene.

Teng and Smith (1973) reported cholesterol could be cooxidized by SL with ethyl linoleate hydroperoxide as well as with ethyl linoleate as a substrate. The oxidation with ethyl linoleate hydroperoxide was slower than with ethyl linoleate, and it was a free-radical reaction based on the oxidation products of cholesterol. We find β-carotene cooxidation is slower with LOOH than with LH, and the cooxidation is inhibited by free-radical inhibitors.

The role played by LOOH in the cooxidation of β-carotene is important, because reduction of LOOH with $NaBH_4$ effectively stopped the β-carotene oxidation. Also, with LOOH as substrate, molecular O_2 was needed for the enzymatic oxidation of β-carotene. Since the discovery of iron as an integral part of lipoxygenase, it is feasible to think in terms of a reversible oxidation and reduction of iron. de Groot et al. (1975) have proposed that LOOH and O_2 are involved in activating the enzyme which exists in a resting state binding oxygen to ferrous iron. Then the active enzyme with iron in the ferric state abstracts H from linoleic acid and becomes reduced. The lipoxygenase iron is reoxidized by a linoleic peroxy radical to continue the cycle.

Soybeans are known to possess hydroperoxidase activity that cannot be separated from lipoxygenase (Schormüller et al., 1969; Grosch et al., 1972). The hydroperoxidase activity requires the presence of guaiacol and has been called guaiacol–linoleic acid hydroperoxide-oxidoreductase. Perhaps the reaction observed by us of SL1 acting on LOOH and β-carotene and the reaction observed by Teng and Smith (1973) of SL acting on ethyl linoleate hydroperoxide and cholesterol are other examples of hydroperoxidase activity of SL.

If it is necessary for SL iron to be alternatively oxidized and reduced, then LOOH or LOO· may serve as the oxidizing agent, and cosubstrates such as guaiacol, β-carotene, or cholesterol may act as reducing agents by providing the H· abstracted by SL.

The results in this study of the competitive inhibition between β-carotene and LH are a further indication that there is a direct attack by SL1 on β-carotene to abstract a H· probably from the C_4 or C_4' carbons. This attack by SL1 followed by oxidation with molecular oxygen would yield products such as mutatochrome and aurochrome.

LITERATURE CITED

Arens, D., Seilmeier, W., Weber, F., Kloos, G., Grosch, W., *Biochim. Biophys. Acta* **327**, 295 (1973).

Ben-Aziz, A., Grossman, S., Ascarelli, I., Budowshi, P., *Phytochemistry* **10**, 1445 (1971).

Blain, J. A., Hawthorn, J., Todd, J. P., *J. Sci. Food Agric.* **4**, 580 (1953).

Chan, H. W-S., *Biochim. Biophys. Acta* **327**, 32 (1973).

Christopher, J. P., Axelrod, B., *Biochem. Biophys. Res. Commun.*, **44**, 731 (1971).

Christopher, J. P., Pistorius, E. K., Axelrod, B., *Biochim. Biophys. Acta* **198**, 12 (1970).

Christopher, J. P., Pistorius, E. K., Axelrod, B., *Biochim. Biophys. Acta* **284**, 54 (1972).

Critchley, J. P., Friend, J., Swain, T., *Chem. Ind. (London)* **18**, 596 (1958).

Davies, B. H., "Chemistry and Biochemistry of Plant Pigments", Goodwin, T. W., Ed., Academic Press, New York, N.Y., 1965, Chapter 18.

de Groot, J. J. M. C., Veldink, G. A., Vliegenhart, J. F. G., Boldingh, J., Wever, R., Van Gelder, F. B., *Biochim. Biophys. Acta* **377**, 71 (1975).

Enzell, C. R., Francis, G. W., Liaaen-Jensen, S., *Acta Chem. Scand.* **22**, 1054 (1968).

Enzell, C. R., Francis, G. W., Liaaen-Jensen, S., *Acta Chem. Scand.* **23**, 727 (1969).

Finazzi-Agro, A., Avigliana, L., Veldink, G. A., Vliegenhart, J. F. G., Boldingh, J., *Biochim. Biophys. Acta* **326**, 462 (1973).

Friend, J., *Biochem. J.* **64**, 19 pp (1956).

Friend, J., *Chem. Ind. (London)*, 598 (1958).

Gardner, H. W., *J. Lipid Res.* **11**, 311 (1970).

Gardner, H. W., Weisleder, D., *Lipids* **7**, 191 (1972).

Grosch, W., Höxer, B., Stan, H-J., Schormüller, J., *Fette, Seifen, Anstrichm.* **74**, 16 (1972).

Guss, P. L., Richardson, T., Stahmann, M. A., *Cereal Chem.* **44**, 607 (1967).

Kies, M. W., Haining, J. L., Pistorius, E. K., Schroeder, D. H., Axelrod, B., *Biochem. Biophys. Res. Commun.* **36**, 312 (1969).

Knowles, R. E., Livingston, R. L., *J. Chromatogr.* **61**, 133 (1971).

Korosy, F., *Experientia* **11**, 342 (1956).

Monties, B., Costes, C., *C. R. Hebd. Seances Acad. Sci., Ser. C*, 266 (1968).

Petracek, F. J., Zeichmeister, L., *Anal. Chem.* **28**, 1484 (1956).

Pistorius, E. K., Axelrod, B., *J. Biol. Chem.* **249**, 3183 (1974).

Roza, M., Francke, A., *Biochim. Biophys. Acta* **327**, 24 (1973).

Schormüller, J., Weber, J., Höxer, B., Grosch, W., *Z. Lebensm.-Unters.-Forsch.* **139**, 357 (1969).

Subrarayan, C., Cama, H. R., *J. Sci. Ind. Res.* **27**, 306 (1968).

Teng, J. I., Smith, L. L., *J. Am. Chem. Soc.*, **95**, 4060 (1973).

Tookey, H. L., Wilson, R. G., Lohmar, R. L., Dutton, H. J., *J. Biol. Chem.* **230**, 65 (1958).

Verhue, W. M., Francke, A., *Biochim. Biophys. Acta* **284**, 43 (1972).

Vioque, E., Holman, R. T., *Arch. Biochem. Biophys.* **99**, 522 (1962).

Weber, F., Laskawy, G., Grosch, W., *Z. Lebensm.-Unters.-Forsch.* **155**, 142 (1974).

Wolf, W. J., *J. Agric. Food Chem.* **23**, 136 (1975).

Zinsou, C., *Physiol. Veg.* **9**, 149 (1971).

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