Memoirs of the Faculty of Engineering, Nagoya University
Vol. 30, No. 2. (1978)

STUDIES ON THE REACTION OF FATS AND OILS —— HYDROLYSIS, AUTOXIDATION, HYDROGENATION, ETC.

KAZUO FUKUZUMI

Department of Applied Chemistry

(received October 30, 1978)

Abstract

Studies of the reactions of fats and oils mentioned above consist of those on fat splitting, surface-active agents, autoxidation, hydrogenation, fatty acid derivatives, etc.

[1] Fat-Splitting Reactions and Surface-Active Agents

Eight new fat-splitting agents (acid type surface-active agents) which were superior to those usually used were prepared. On the mechanism of fat splitting, two theories which were inconsistent with each other had been presented; one: the hydrolysis of fats was a surface reaction and the other: a homogeneous reaction in oil phase. New mechanism that three stepwise reactions are performed in oil layer and interface; oil layer (on which the reaction less depends than in the former case) and interface; and water layer, respectively, was proposed. The mechanism was demonstrated theoretically and experimentally, and both theories mentioned above were able to be explained by this mechanism reasonably.

Besides, several new superior detergents were prepared.

[2] Autoxidation Reactions of Fats (Involving the papers on Atherosclerosis and Cancer)

In the autoxidation of highly unsaturated fatty acid esters at low temperature (about 0℃), extremely high peroxide values (m.eq./kg.) which had been never reported in the field of fats, for example about 1×10^4, were estimated, and it was made clear that monomeric dihydroperoxides, which were separated in the concentrated state, could be formed. It was also first confirmed that at low temperature conjugated dienes were easily formed, but conjugated triene and so on scarcely. Trimers were first separated from the oxidized polymers of highly unsaturated fatty acid esters.

The existence of *trans*, *trans* conjugated diene hydroperoxides (a kind of lipoperoxides) in atherosclerotic artery and the tissus of cancer

was first shown. From the results of the studies and the other literature, so-called "lipoperoxide hypothesis" as the causes of the generation of atherosclerosis and cancer was first presented, by which many phenomena about these diseases were explained reasonably. It was first found that the DNA radicals were formed by the reaction of DNA with lipoperoxide. From the resemblance of such DNA radicals to the radicals in cancer tissues, it was suggested that cancer itself might be DNA radicals.

The difference in the effect of antioxidants on the autoxidation of geometric and position isomers of lipids was first made clear. It was found that photosensitizing dyes, phenothiazine derivatives, ion exchange resins, and ethyleneimine polymers were useful antioxidants. Tocopherols as antioxidants were examined, and nucleic acids were first shown as effective synergists.

Besides new quantitative methods for hydroperoxides by infrared spectroscopy and NMR shift reagent were reported.

In the same composition systems the TBA test could be utilized to estimate the variation of lipoperoxides, but not in the different systems. Even in the latter case, the TBA test could be used to evaluate lipoperoxides as long as the special treatment takes place.

[3] Hydrogenation Reactions of Fats and Some Other Compounds

In the heterogeneous catalytic hydrogenation of highly unsaturated fatty acid esters, it was clarified that precise results were hardly obtained by ultraviolet spectroscopy and gas liquid chromatography. The hydrogenation of conjugated tetraenoate took place first, and conjugated triene and conjugated diene were not detected in the reaction mixture. Conjugated diene in polyenes was hydrogenated faster than nonconjugated polyenes.

The reaction systems having the characteristic that selectivity was 100% and almost no trans isomers were formed were found by us, of which the discovery had been desired eagerly but it had been almost impossible. They were the new transfer hydrogenation systems using $RuCl_2(PPh_3)_3$ and $RuH_2(PPh_3)_4$ as homogeneous catalysts, methyl linoleate as a hydrogen acceptor, and 2-propanol and hydrocortizone as hydrogen donors, respectively. Almost no studies on the homogeneous transfer hydrogenation of simple olefins except for ours had been published. Especially such studies of ours on unsaturated fatty acid esters were the first in the world. Besides, by using general organic compounds as hydrogen acceptors, many new effective hydrogen donors such as indoline etc. were found, the reaction mechanism was first clarified, and many new informations were obtained.

[4] Fatty Acid Derivatives and Others

Gas liquid chromatography for highly unsaturated fatty acid esters was reported almost earlist.

Reactivity for the side chain of heterocyclic compounds containing nitrogen with carboxylic acids as catalysts was investigated. It was shown that eliminations from the compounds having quinolyl group as an electron-withdrawing group took place easily, whose mechanism was proposed. The elimination with carboxylic acid catalysts had never

been reported.

The olefin metathesis reactions catalyzed by the WCl_6-cocatalyst system were performed. The metathesis of olehins with functional groups had not been studied in detail. That of methyl oleate, a kind of these olefins, was carried out, and good results were obtained. Besides new superior catalyst systems and solvents were found, and the reaction mechanism was made clear.

CONTENTS

1. Introduction

As the reactions of fats and oils, hydrolysis, autoxidation, hydrogenation, etc. are important. In the industry of fats and oils, hydrolysis is available to produce fatty acids and glycerol from fats as fat splitting; autoxidation to dry paint and varnish and to recognize the deterioration of edible oils; and hydrogenation to manufacture margarine, shortening, stabilized edible oil, and the raw material of soap. Besides, autoxidation has the connection with lipoperoxides, which are found in the artery of atherosclerosis and in the tissues of cancers. By the way the existence of lipoperoxides in the tissues of cancers has been first discovered in the world by us.

[1] In the Twitchell fat-splitting, studies on new superior fat-spliting agents and correct fat-splitting mechanism were performed. The investigations on new excellent detergents also took place.

[2] Studies on the autoxidation of pure highly unsaturated fatty acid esters had never been undertaken. Therefore they were carried out. Thogh the investigation showing the existence of lipoperoxides in atherosclerotic artery had been published, the properties of the lipoperoxides had not been given. So studies on lipoperoxides in connection with atherosclerosis and cancer were performed, and the connection between lipoperoxides and the causes of atherosclerosis and cancer was discussed. The investigation on the reaction of lipoperoxides with DNA was first carried out. And then new superior antioxidants were studied. Besides, whether the TBA test could be utilized to estimate the variation of lipoperoxides was ascertained.

[3] Studies on heterogeneous catalytic hydrogenation of pure highly unsaturated fatty acid esters had never been carried out, so they took place. In the hydrogenation of fatty oils, the higher the selectivity, the larger the isolated *trans* double bonds. The discovery of the reaction systems having characteristic that the selectivity is 100% and the amount of isolated trans isomers is trace had been desired eagerly from the viewpoint of edible oils and the raw material of soap. In practice, the attainment had been thought impossible. Therefore homogeneous transfer hydrogenation of fatty acid esters was first studied to attain the desire. It was done on the basis of informations on the transfer hydrogenation of general organic compouuds, of which investigations were performed in our laboratory.

[4] Gas liquid chromatography for highly unsaturated fatty acid esters took place almost earliest. Studies on the reaction of side chain of heterocyclic compounds containing nitrogen with carboxylic acids as catalysts were first undertaken. The metathesis of olefins such as especially unsaturated fatty acid esters was carried out in detail to find new superior catalyst systems and to discuss the reaction mechanism.

2. Fat-Splitting Reactions and Surface-Active Agents

2. 1. Introduction

In Twitchell's fat-splitting process, the ability of fat-splitting agents (acid type surface-active agents) is the most important. Eight new fat-splitting agents, such

as dodecyl phenol sulfonic acid and its halogen (Cl, I) derivatives, α-chlorodibutyl naphthalene sulfonic acid, tri- and tetra-butyl naphthalene sulfonic acids, fat-splitting agent prepared from naphthalene, β, β-dinaphthyl, butanol, sulfonic acid, etc., and fat-splitting agent prepared from naphthalene, diphenyi, butanol, sulfonic acid, etc. were prepared to estimate the ability of fat-splitting.

In the usual Twitchell fat-splitting process the reaction is performed at about 100℃ under atmospheric pressure with vigorous stirring. For kinetic studies of the stepwise nature of the hydrlysis a slower rate of reaction was desirable. Accordingly experiments were conducted to determine the degree of fat splitting at 35℃ without agitation of the mixture, and the reaction mechanism was discussed.

Subsequently, the detergency properties of neutral type of fat-splitting agents mentioned above were investigated, since these compounds had not yet been used as detergents.

2. 2. Fat-Splitting Agents

Dodecyl phenol sulfonic acid and its halogen derivatives, such as chloro- and iodo-derivatives, were prepared, and the fat-splitting power of these substances was compared with that of fat-splitting agents usually used, such as dodecyl benzene sulfonic acid and Kontaktspalter.[1] These new fat-splitting agents are superior to usual ones. The fat-splitting power of dodecyl benzene sulfonic acid is increased by introducing the hydroxyl group into benzene ring, and further by introducing chlorine or iodine. α-Chlorodibutyl naphthalene sulfonic acid, synthesized by the treatment of α-chloronaphthalene and butanol with conc. sulfuric acid, was used as a new fat-splitting agent.[2] The fat-splitting power of this sulfonic acid is superior to that of usual fat-splitting agents. The fat-splitting ability of tri- and tetra-butyl naphthalene sulfonic acids, prepared from naphthalene, butanol, and conc. sulfuric acid, is superior to that of dibutyl naphthalene sulfonic acid, the well-known powerful fat-splitting agent.[3] Fat-splitting power of sulfonic acids prepared from naphthalene, β, β-dinaphthyl, butanol, sulfuric acid, etc. and prepared from naphthalene, diphenyl, butanol, sulfuric acid, etc. is superior to that of fat-splitting agents usually used.[4,5] That is, eight new fat-splitting agents mentioned above are superior to usual ones.

2. 3. Fat-Splitting Reactions[6~12]

For the first time it has been demonstrated that, at 35℃, splitting of a fat by the Twitchell process occurs in a stepwise way. Coconut oil in contact with 1 N sulfuric acid containing the sulfonic acid, corresponding to 1% by the weight of the oil, was about 90% split in 15 to 30 days, depending on the area of contact of the two layers. The diglyceride concentration reached a maximum during the early days of the reaction and then decreased somewhat. Monoglyceride concentration appeared to reach a maximum more slowly and then continued at that level as the concentration of free fatty acids and glycerol steadily increased.[7,8]

From kinetic studies of the stepwise nature of the hydrolysis, the following new mechanism was proposed, and demonstrated theoretically and experimentally.[9~12] Reaction (a) is performed in oil layer and interface; reaction (b) in oil layer (on which the reaction less depends than reaction (a)) and interface; and reaction (c) in water layer; where reactions (a), (b), and (c) are shown below, respectively.

(a) Triglyceride + H_2O $\longrightarrow$ Diglyceride + Fatty acid

(b) Diglyceride + H_2O $\longrightarrow$ Monoglyceride + Fatty acid

(c) Monoglyceride + H_2O $\longrightarrow$ Glycerol + Fatty acid

This mechanism could explain reasonably two theories which had been presented. These old theories were inconsistent with each other, that is, one: the hydrolysis of fat was a surface reaction and the other: it was a homogeneous reaction in oil phase. Consequently, these old theories had explained only a part of phenomena, respectively.

2. 4. Surface-Active Agents[5, 13~17]

Tetrabutyl naphthalene sulfonate[14] and surface-active agent prepared from naphthalene, β, β-dinaphthyl, butanol, sulfuric acid, etc.,[15] which are new detergents, have more excellent surface-active properties than dibutyl naphthalene sulfonate, which is the principal ingredient of Nekal. For polybutyl naphthalene sulfonate, within the limits of the research, the capillary activity becomes greater with increasing butyl groups introduced into the naphthalene ring. The capillary activity becomes more excellent, when the lipophilic property of butyl aryl sulfonate is increased by using the bimolecular condensation product of aromatic hydrocarbon, such as β, β-dinaphthyl, together with naphthalene.

2. 5. The Miscellaneous[18~23]

Cation exchange resin together with sulfonic acid having lipophilic group can be used as excellent catalysts in fat splitting.[21]

It has been ascertained that certain fat-splitting agents, such as Kontakt and Divulson D, are a little superior to oleic acid etc. as flotation agents.[22]

Dodecyl phenol sulfonate and its halogen derivatives are equal or a little superior to butyl p-hydroxybenzoate as antiseptics for soy.[23]

3. Autoxidation Reactions of Fats (Involving the Papers on Atherosclerosis and Cancer)

3. 1. Introduction

A great many papers on the autoxidation of fatty acid esters, such as oleate, linoleate, and linolenate, in pure state, had been published. But studies on the autoxidation of pure highly unsaturated fatty acid esters had never been performed, because the separation of pure highly unsaturated fatty acid esters from fish oils was difficult, and they were extremely unstable. So studies on these fatty acid esters carefully took place.

The existence of lipoperoxides in atherosclerotic artery had been shown, and it had been given that the more serious atherosclerosis, the larger amount of lipoperoxides. But the properties of the lipoperoxides had not been investigated. Therefore studies on lipoperoxides in connection with not only atherosclerosis but also cancer took place, because over 40 years old persons in general suffer from these diseases. And then the relationship between lipoperoxides and the causes of atherosclerosis and cancer was investigated on the base of our studies and other literature. Though it is well-known that DNA is attacked by radioactive rays, the reaction of lipoperoxides with DNA had never been studied. So the investigation of this reaction first took place in connection with cancer.

Though many papers on the antioxidants for the autoxidation of fatty oils had

appeared, to obtain new excellent antioxidants the studies were undertaken.

Even if the TBA test can be used to evaluate the variation of lipoperoxides in the same composition systems, it would not be done in the different composition systems. To be able to utilize the TBA test even in the latter case, the investigation of the special treatment in the test was carried out.

3. 2. Autoxidation of Highly Unsaturated Fatty Acid Esters[24~36]

Studies on the autoxidation of pure highly unsaturated fatty acid esters had never been performed. So they took place.

A comparison of the autoxidation of conjugated and unconjugated methyl docosahexaenoates in the dark at about 0℃ revealed that hydroperoxides were obtained more readily from the unconjugated precursor.[25] Autoxidation at 0 to 2℃, in the dark, of a mixture of methyl pentaenoates and hexaenoates derived from cuttle-fish oil showed that at relatively high levels of autoxidation, the hydroperoxyl groups appeared as expected at α-positions relative to double bonds.[26] Also although appreciable amounts of conjugated diene appeared, very little conjugated triene and no conjugated tetraene were observable. On the basis of infrared spectra and other measurements, it was suggested that dihydroperoxides were also formed. In another study the autoxidation of highly purified methyl docosahexaenoate derived from cuttle-fish oil was conducted at 33 to 35℃.[27] In this higher temperature range, stronger evidence for the formation of dihydroperoxides was obtained, and it was found that a higher proportion of the conjugated dienes had a *trans*, *trans* configuration. Appreciable amounts of polymer were also generated.

A concentrate of monomeric dihydroperoxides has been obtained by partition chromatography of autoxidized methyl docosahexaenoate, which were characterized by means of peroxide value measurements, molecular weight determinations, and ultraviolet and infrared spectra.[28,29]

Cuttle-fish oil, which contains considerable amounts of highly unsaturated fatty acid esters, was autoxidized at 35℃ in scattered sunlight to a high peroxide value, and the product was permitted to decompose in the dark at 35℃ under nitrogen, with and without the addition of metallic soaps. During decomposition, hydroperoxide groups were largely converted to OH groups, with concomitant decrease in the amount of conjugated diene.[30] Aldehydes were also formed, but the residual *cis* unsaturation did not vary appreciably. In the presence of iron or copper soaps, the changes were qualitatively similar, but occurred more rapidly and extensively.

In two studies of polymerization in autoxidation products of methyl esters of highly unsaturated fatty acids, the polymeric products obtained from methyl esters of cuttle-fish oil fatty acids, and also those obtained in the autoxidation of a nearly pure methyl docosahexaenoate at 35℃ were characterized.[31] It was postulated that the polymers of the autoxidation products involved oxygen-containing rings.[32] In yet another experiment, methyl docosahexaenoate was autoxidized in sunlight at 35℃ for 90 hours and then alkali-isomerized.[33] The derivatives of the alkali-isomerization varied in molecular weight from 440 to 1600. In another study, the dimer concentrates obtained from autoxidized methyl docosahexaenoate were found to contain dimers with direct carbon-to-carbon linkages as well as carbon-oxygen-carbon linkages.[34] Trimers were first isolated.

3. 3. Relationship among Lipoperoxide, Atherosclerosis, and Cancer

3. 3. 1. Relationship between Lipoperoxide and Atherosclerosis

Atherosclerosis is caused by deposits of lipids in the arteries. Lipids containing oxidized unsaturated fatty acids[37] and protein form complexes which have strong linkages unable to be separated by solvent extraction.[38] If oxidized lipids, lipoperoxides, form the complexes with protein of arterial wall, deposits of cholesterol and its esters of fatty acids may occur around the complexes. In this case oxidized lipids, lipoperoxides, may be detected in the lipids of arteries. It was already reported that lipoperoxides are contained in the aorta of subjects suffering from atherosclerosis. But the properties of the lipoperoxides were not shown.[39] Therefore lipids were extracted from atherosclerotic artery with methanol-chloroform (1:2) at room temperature. Fatty acids, and then methyl esters were obtained from the lipids. All procedures were performed at room temperature under the atmosphere of nitrogen. Fatty acids composition was calculated from the results of ultraviolet spectrum analysis. It became clear that *trans*, *trans* conjugated diene hydroperoxides exist in the methyl ester from the infrared spectrum. No hydroperoxides were found in the lipids of normal artery.[40]

Highly unsaturated acids in the lipids of arteries are concentrated in phospholipids. And also, more highly unsaturated acids are more easily oxidized. Therefore, in this case, oxidized lipids, lipoperoxides, may be concentrated in the phospholipids contained in the lipids of atherosclerotic arteries. From this point of view, lipids of atherosclerotic abdominal aorta were dialysed to separate the residue (phospholipids concentrates) and the dialysate. And then these fatty acids were obtained. From ultraviolet and infrared spectra, compositions and properties of these fatty acids were made clear. It was ascertained that oxidized lipids, lipoperoxides, were concentrated in the phospholipids contained in the lipids of the atherosclerotic aorta, and that the *trans*, *trans* conjugated diene hydroperoxide existed in the oxidized lipids.

After extracting the lipids from the atherosclerotic aorta, further extraction was performed to obtain residue which then was hydrolysed with alkali, and the amount of fatty acids in the residue was estimated. Thus, it was first demonstrated that the atherosclerotic aorta contained lipid-protein complexes. From the infrared spectrum it became clear that oxidized lipids, lipoperoxides, containing *trans* isomers existed in the lipids of the lipid-protein complexes.[41]

From the results of our studies mentioned above and the other literature, as the cause of atherosclerosis outbreak, so-called "lipoperoxide hypothesis" that, "lipoperoxides form the complex with the protein in aorta vessel, and then the lipids, such as cholesterol or cholesterol esters, are deposited around the complex in the artery" was first presented.[42,43] Many phenomena about atherosclerosis can be explained by "lipoperoxide hypothesis".[42,43]

3. 3. 2. Relationship between Lipoperoxide and Cancer

In general, carcinogenic agents have a close connection with protein and combine with it. Lipids having oxidized unsaturated fatty acids, lipoperoxides, and protein form complexes. Because of their strong linkges, the complexes are not decomposed by solvent extraction. Would be lipoperoxides form complexes with nucleoproteins, the status of cell-division may probably change. It is thus assumed that the

cancerous cells may be produced. In such a case, cancerous tissues may contain lipoperoxides. From this point of view, lipids were extracted from cancerous tissues, such as gastric scirrhous carcinoma, gastric adenocarcinoma, rectal adenocarcinoma and mammary carcinoma indicated the picture of carcinoma simplex tissues. Then fatty acids and their methyl esters were obtained. All procedures were carried out at room temperature under the atmosphere of nitrogen without exposing samples to direct sunlight. From ultra-violet spectrum, fatty acids composition was calculated. It was ascertained that *trans*, *trans* conjugated diene hydroperoxides exist in the methyl esters obtained from cancerous tissues, and that *trans*, *trans* nonconjugated dienoic acid also exists in some such esters from the results of infrared spectrum analysis.[44]

There has been the suggestion that all carcinogenic compounds are able to concern themselves in reactions leading to produce free radicals. Besides, it has been recognized that lipids is oxidized through catalysis by the free radicals. But it can be hardly assumed that cancer of lung may be generated by taking lipoperoxides directly through trachea. If the substances containing free radicals such as cigarrette smoke enter into the lung, lipids of the tissue may be oxidized with free radicals as catalysts. In such a case, carcerous tissue in lung may contain lipoperoxides. From this point of view, lipids were extracted from the tissue of bronchial carcinoma, a kind of cancer of lung, and fatty acid methyl esters of the lipids prepared. All procedures were performed at room temperature under the stream of nitrogen. The methyl esters of fatty acids of lipids from cancerous pleural fluids were also obtained. Then acids compositions were calculated from ultraviolet spectrum. It was made clear that *trans*, *trans* or *cis*, *trans* conjugated diene hydroperoxide was contained in the methyl esters of fatty acids obtained from the lipids of all samples from the infrared spectrum.[45]

Taking our studies mentioned above and other literature into consideration, so-called "lipoperoxide hypothesis" that "cancer is generated owing to lipoperoxides accumulated in a living body" was first presented. Many phenomena about cancer can be understood by "lipoperoxide hypothesis".[46~48]

3. 3. 3. Relationship of Lipoperoxide to the DNA Radical and Cancer[49,50]

The autoxidation reaction of lipids in the presence of nucleic acids was investigated, and it was first confirmed by the ESR measurement that the DNA radical or the RNA radical is generated in the reaction. It was also found that the radical present in cancer tissues is similar to the DNA radical mentioned above. This radical is different from the other ones which might exist in the tissues. Therefore, cancer itself might be DNA radicals.

3. 3. 3. 1. Introduction

It was reported that many chemical carcinogens and/or their intermediates may either be free radicals themselves or else may be activated by free radicals,[51] and the resulting, highly reactive intermediates can rapidly react covalently with DNA, and so can be potent frameshift mutagens.[51,52] But no paper has been given about nucleic acid free radicals generated by chamical carcinogens and/or their intermediates. On the one hand, it is well known that nucleic acids free radicals are produced by irradiation with γ-rays etc.[53~69] But no paper has been published about the reaction of lipoperoxides with deoxyribonucleic acid (DNA) or ribonucleic

acid (RNA). On the other hand, it is also known free radicals exist in cancer tissues, and cancer cells have greater free-radical character than normal ones,[70~72] But almost no studies have taken place on free-radicals in cancer tissues in detail.

In the mechanical degradation of polymers, free-radicals are produced, as follows. (A) Polyethylene and polypropylene form free-radicals when cut under benzen.[73] (B) Electron spin resonance (ESR) spectra showed that grinding of elastomers produced radicals at room temperature.[74] Animal tissues contain a lot of polymers, and so free-radicals in tissues must be measured as they are, without treatments such as the use of homogenizer.

Therefore, the autoxidation reaction of lipids in the presence of nucleic acids was investigated by using ESR spectroscopy. Moreover the free-radicals in the gastric cancers and the normal human beings tissues, and those in the Ehrlich's ascites tumors, the Ehrlich's carcino-sarcoma, and the normal tissues of mice, have been measured with ESR as tissues are, without treatments except drying.

3. 3. 3. 2. Experimental Procedure

Materials: Chemical experiments were performed by K. Fukuzumi and N. Ikeda, Nagoya University. Methyl linoleate was prepared from sufflower oil through a urea adduct procedure, and it was of 99 % purity as determined by GLC. It contained no conjugated acids shown in UV spectra, no isolated *trans* double bonds in IR spectra, no natural antioxidants such as tocopherols, and no heavy metals, such as copper and iron, in atomic absorption spectra.

The DNA with low molecular weight was a commercial high grade reagent degraded from herring. Its UV spectrum had an absorption band at 262 nm, and its absorptivity was 0.0259 (cm^{-1}. μg^{-1}. ml). The typical DNA absorbs at 260 nm, and the absorptivity is 0.020 (cm^{-1}. μg^{-1}. ml). Therefore, the DNA with low molecular weight had considerably lower molecular weight than the typical.[63] The DNA with high molecular weight was a commercial high grade reagent highly polymerized from cod sperm. It absorbed at 258 nm, and the absorptivity is 0.0163 (cm^{-1}. μg^{-1}. ml). Then, it had considerably higher molecular weight than the typical.[63]

RNA was a commercial high grade reagent from yeast. Its UV band at 261 nm showed the absorptivity 0.0247 (cm^{-1}. μg^{-1}. ml). The absorptivity at 260 nm for the typical RNA is 0.022 (cm^{-1}. $\mu g.^{-1}$ ml). So the commercial RNA had practically the typical molecular weight.[63]

The above-mentioned nucleic acids were negative for the biuret and xanthoproteic reactions.

Egg albumin was a commercial high grade reagent, and of course, positive for the biuret and xanthoproteic reactions.

β-NAD (β-nicotinamide adenine dinucleotide), β-NADH (β-nicotinamide adenine dinucleotide, reduced form, disodium salt), NADP (nicotinamide adenine dinucleotide phosphate), NADPH (nicotinamide adenine dinucleotide phosphate, reduced, sodium salt), and ATP (adenosine-5-triphosphate) were also commercial high grade reagents.

Ehrlich's ascites tumors, carcino-sarcoma, and normal tissues of mice (each ♀, ddy, 5-6 weeks old) were prepared by H. Kodama and T. Hiraiwa, Toyama Chemical Co. The abdomen of the mouse mentioned above was injected with Ehrlich's ascites tumor cells. In the ascites after the lapse of 6 days from injection, 10^8/ml of cancer cells existed. This ascites was diluted five times with physiological saline

solution, and then each 0.2 ml of the diluted ascites was injected into the abdomens of the same kind of mice as described above. After the lapse of 6 days from the injection, these were used as the Ehrlich's ascites tumor-bearing mice. Each ascites was centrifuged at a speed 2,000 rpm for 10 minutes, and the residue was used as the Ehrlich's ascites tumor tissue.

Each 0.1 ml of the ascites diluted twice instead of five times with physiological saline solution was injected under the skin of the mice. After the lapse of 12-13 days from the injection, these were used as Ehrlich's carcino-sarcoma-bearing mice.

Gastric cancers, such as adenocarcinoma scirrhosum and carcinoma scirrhosum, and normal tissues were obtained through K. Nakamura, Tokyo Jikeikai Medical College. On the one hand, pathological examinations on the tissues mentioned above took place by J. Torimi, Tokyo Jikeikai Medical College. On the other hand, these tissues wrapped with polyethylene were immediately refrigerated but not dried.

Autoxidation: Methyl linoleate containing 2 weight % of DNA, RNA, egg albumin, β-NAD, β-NADH, NADP, NADPH, or ATP, with or without 0.05 weight % of a carcinogen 3,4-benzpyrene, placed in glass dishes to an oil depth of 1.03 mm was autoxidized at 36±0.5℃. Besides, the experiments were also performed by using 0.1 weight % of the DNA with low molecular weight or RNA.

Free-Radical Determination: Free-radicals were measured at room temperature about not only the autoxidized samples but also the cancer tissues etc. in nondried state or dried (drying at room temperature under 10^{-3} mmHg for 15 hours) with a Nihon Denshi-JES-ME-1 X type apparatus for electron spin resonance spectra determination.

3. 3. 3. 3. Results and Discussion

Fig. 1 shows the ESR spectrum of the DNA with low molecular weight, because the autoxidized methyl linoleate itself does not show any peak in the ESR spectrum under this determination condition. The g-value for the DNA radical with low molecular weight is 2.0035, strikingly resembles that for DPPH, 2.0035, and larger

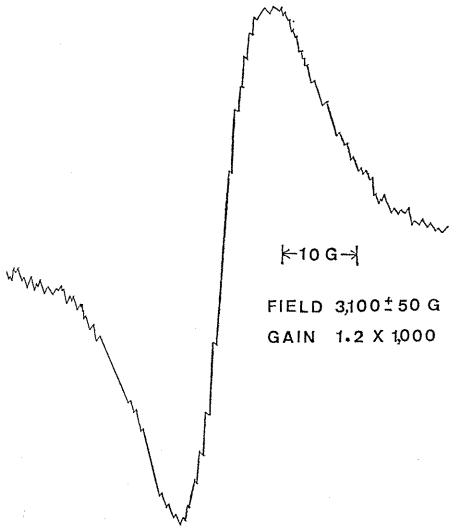


Fig. 1. ESR spectrum of the DNA with low molecular weight (2%) in the autoxidized methyl linoleate.

than that for free electron, 2.0023, but the shape of the spectrum for the DNA is quite different from that for DPPH.

The ESR spectrum of the DNA with high molecular weight in the autoxidized methyl linoleate is given in Fig. 2. The g-value for the DNA radical with high molecular weight is 2.0042.

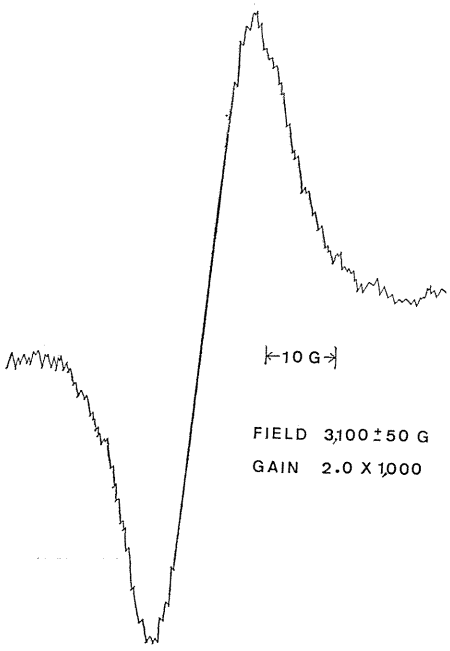


Fig. 2. ESR spectrum of the DNA with high molecular weight (2%) in the autoxidized mathyl linoleate.

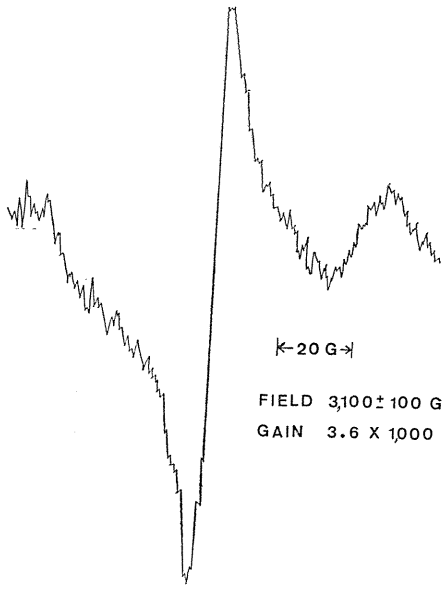


Fig. 3. ESR spectrum of RNA (2%) in the autoxidized methyl linoleate.

Fig. 3 shows the ESR spectrum of RNA in the autoxidized methyl linoleate. The g-value for the RNA radical is 2.0049, and larger than that for the DNA radical with low molecular weight, 2.0035 or that with high molecular weight, 2.0042.

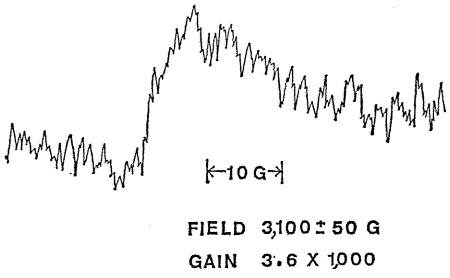


Fig. 4. ESR spectrumof egg albumin (2%) in the autoxidized methyl linoleate.

The ESR spectrum of egg albumin in the autoxidized methyl linoleate is shown in Fig. 4. The g-value for the egg albumin radical is 2.0071, and larger than that for the RNA radical, 2.0049.

Fig. 5 gives the radical concentration of the DNA with low molecular weight in the autoxidized methyl linoleate with or without 3, 4-benzpyrene. As the autoxidation of methyl linoleate proceeds, the weight gain of each sample increases, which is mainly due to the absorption of oxygen. By the addition of 3, 4-benzpyrene, the formation of the radical is appreciably accelerated at the start, and subsequently retarded.

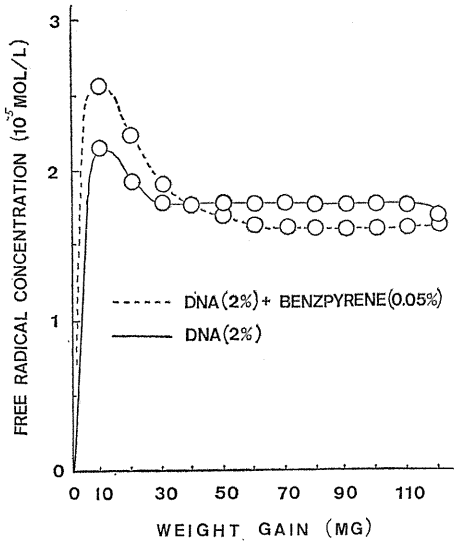


Fig. 5. Radical concentration of the DNA with low molecular weight in the autoxidized methyl linoleate with or without 3, 4-benzpyrene.

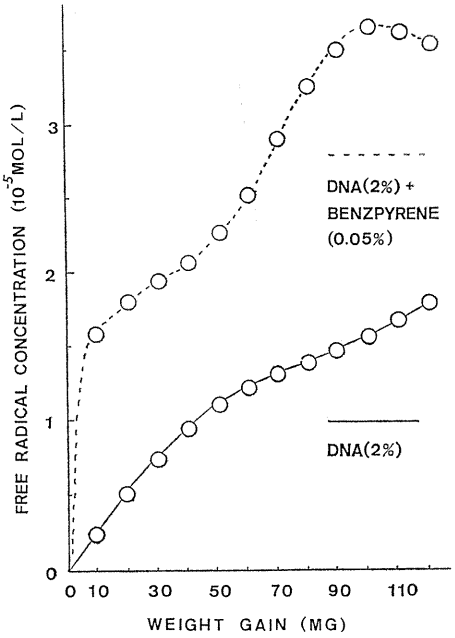


Fig. 6. Radical concentration of the DNA with high molecular weight in the autoxidized methyl linoleate with or without 3, 4-benzpyrene.

Fig. 6 shows the radical concentration of the DNA with high molecular weight in the autoxidized methyl linoleate with or without 3, 4-benzpyrene. By the addition of 3, 4-benzpyrene, the formation of the radical is remarkably accelerated.

The radical concentration of RNA in the autoxidized methyl linoleate with or without 3, 4-benzpyrene is given in Fig. 7. The effect of the addition of 3, 4-benzpyrene on the formation of the radical is similar to the previous case of the DNA with low molecular weight.

Fig. 8 shows the radical concentration of egg albumin (2%) in the autoxidized methyl linoleate. The concentration of the radical was low under the experimental condition.

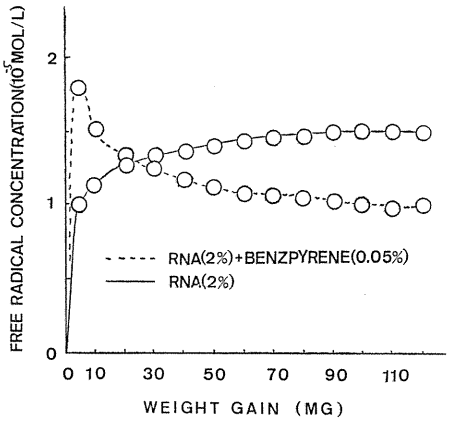


Fig. 7. Radical concentration of RNA in the autoxidized methyl linoleate with or without 3, 4-benzpyrene.

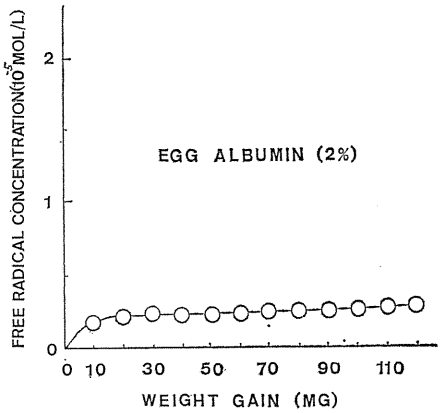


Fig. 8. Radical concentration of egg albumin in the antoxidized methyl linoleate.

The ESR spectra of β-NAD, β-NADH, NADP, NADPH, and ATP (2%) in the autoxidized methyl linoleate were also measured. Only the β-NADH, NADPH, and ATP radicals are detectable, but these radical concentrations are low as much as that of egg albumin in Fig. 8. The g-values for the β-NADH, NADPH, and ATP radicals are 2.0107, 2.0178, and 2.0092, respectively.

It is well-known that lipoperoxides have free radicals. But no radicals are found by usual ESR measurements, because the concentration of radicals is extremely low and lipoperoxides are polar substances. When 0.05% 3, 4-benzpyrene is used, its radical also is not found by this ESR measurement. Organic bases in DNA or RNA contain amino group. Therefore, this hydrogen may be abstracted by the peroxy radical, ROO•, a electrophilic radical in lipoperoxides. The DNA with low molecular weight more easily generates radicals than that with high, especially in the early stage of the autoxidation, because the steric hindrance is lower. As is well known, the yield of free radicals induced by γ- or x-irradiation on DNA, as determined by ESR, also varied inversely with the molecular weight.[67,69]

Table 1 shows the g-values and the mean contents for the radicals in the various tissues of normal mice, and those in the cancer and other various tissues of carcinoidal mice.

The g-values for radicals in the Ehrlich's ascites tumor, the carcino-sarcoma, and the human cancer (for example stomach cancer) tissue were almost all about 2.003.

The g-values for radicals in the normal tissues of the normal and the carcinoidal mice, such as liver, spleen, stomach, kidney, small intestine, and colon, were not 2.003 but 2.004-2.005. In the case of normal mice, the g-values for radicals in the stomach, the kidney, and the colon tissue were about 2.005 and those in the liver, the spleen, and the small intestine were about 2.004. In the Ehrlich's ascites tumor and the Ehrlich's carcino-sarcoma mice, the g-values for radicals in organ tissues (such as stomach, liver, etc.) were all about 2.004, except for the colon one. However the g-value for radicals in blood was 2.007 for the normal mice, but 2.006 for the Ehrlich's carcino-sarcoma ones. Consequently, though the g-values for

Table 1. The g-Value and the Mean Contents for the Radicals in Various Tissues of Normal Mice, and Those in the Cancer and Other Various Tissues of Cancer-Bearing Mice

Tissue	Normal mice		Ehrlich's ascites tumor-bearing mice		Ehrlich's carcino-sarcoma-bearing mice	
	g-Value	Content	g-Value	Content	g-Value	Content
Ascites tumor			2.00329(10) ±0.00005	5.700(10) ±0.772		
Carcinosarcoma					2.00324(15) ±0.00003	6.386(15) ±0.328
Liver	2.00438(10) ±0.00003	3.395(10) ±0.534	2.00417(10) ±0.00005	4.671(10) ±1.088	2.00431(10) ±0.00004	3.734(10) ±0.793
Spleen	2.00421(10) ±0.00004	3.029(10) ±0.581	2.00394(10) ±0.00004	8.140(10) ±2.223	2.00406(9) ±0.00005	4.984(10) ±1.190
Stomach	2.00479(6) ±0.00018	2.082(10) ±0.586	2.00437(9) ±0.00005	3.544(10) ±1.227	2.00433(10) ±0.00004	5.672(10) ±0.951
Kidney	2.00474(10) ±0.00014	4.351(10) ±0.704	2.00402(9) ±0.00005	6.361(10) ±3.685	2.00436(7) ±0.00003	6.104(10) ±1.006
Small intestine	2.00414(4) ±0.00008	1.560(10) ±0.675	2.00410(9) ±0.00006	1.654(10) ±0.448	2.00414(7) ±0.00004	1.941(10) ±0.786
Colon	2.00531(9) ±0.00005	3.220(10) ±0.540	2.00477(9) ±0.00034	2.553(10) ±0.816	2.00526(8) ±0.00003	1.707(10) ±0.437
Blood	2.00704(3) ±0.00010	5.317(3) ±0.679			2.00611(3) ±0.00010	7.896(3) ±1.289

Content: (10^{-6}mole/l).
The number in the round brackets, ( ), is that of samples.

radicals in the cancer tissues are all 2.003, those in the normal tissues for the carcinoidal mice show a tendency to decrease in comparison with the normal mice, for example in stomach etc., from 2.005 to 2.004.

The contents of radicals in the normal tissues for the carcinoidal mice tend to increase in comparison with the normal mice, except for the colon tissue. The contents of radicals in the organ tissues for the normal mice are of the narrow range of $1.6\text{-}5.3\times10^{-6}$ mole/l, and vary little among those contents of radicals in the same tissues. The contents of radicals in the normal tissues for the carcinoidal mice are of the rather wide range of $1.7\text{-}8.1\times10^{-6}$ mole/l, and are rather variable among those in the same tissues.

The ESR spectra of RNA, DNA, one of nondried gastric cancer tissues (adenocarcinoma scirrhosum, ♀, 70 years old), and one of dried Ehrlich's ascites tumors were shown in Fig. 9.

It is well-known that lipoperoxides have free radicals. But no radicals for lipoperoxides are found with ESR in this experimental condition. Also no radicals are found for nondried normal tissues of human beings and mice, and nondried Ehrlich's ascites tumors. But radicals for nondried gastric cancer tissues (adenocarcinoma scirrhosum), dried Ehrlich's ascites tumors, and dried normal tissues of mice can be measured with ESR, as given in Fig. 9 partly. The g-values for the

RNA radical, the DNA radical with high molecular weight, and that with low molecular weight are 2.0049, 2.0042, and 2.0035, respectively, as mentioned above. Besides, the g-values for the nondried tissue of gastric cancer (adenocarcinoma scirrhosum) and for dried Ehrlich's ascites tumor are 2.0040 and 2.0033, respectively. In the same kind of mice as have the tumor, their dried normal stomaches show smaller amounts of radicals than their dried tumors. But the g-value of this normal stomach radical is 2.0048 instead of 2.0033, as shown in Table 1.

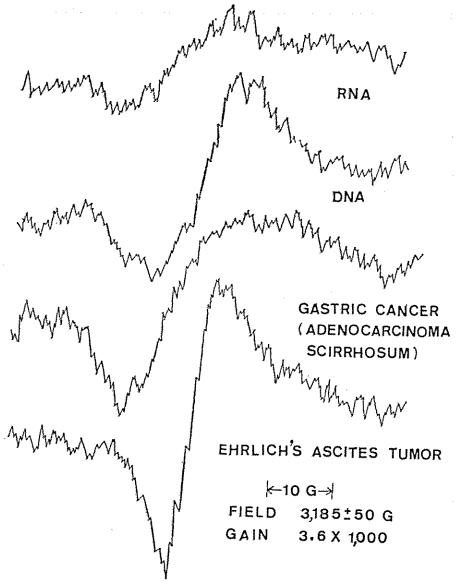


Fig. 9. ESR spectra of RNA and DNA (0.1%) treated with the autoxidized methyl linoleate, the nondried tissue of gastric cancer (adenocarcinoma scirrhosum), and dried Ehrlich's ascites tumor.

It was confirmed that the gastric cancer tissues (nondried state) radicals are almost the same in the shape and the g-value as the DNA radical formed by treating the DNA with low molecular weight with lipoperoxides, and that the Ehrlich's ascites tumors (dried state) radicals are also almost the same in shape and g-value as the DNA radical, as shown in Fig. 9. The protein (for example, egg albumin), β-NADH, NADPH, and ATP radicals which might exist in the tissues are of a small amount and different from the gastric cancer tissue, Ehrlich's ascites, and Ehrlich's carcinosarcoma radicals, in the g-value, because the g-values for the cancer radicals are all 2.003-2.004, and those for the albumin, β-NADH, NADPH, and ATP radicals are 2.007, 2.011, 2.018, and 2.009, respectively, as described before. Therefore, lipoperoxide might attack directly RNA and DNA considerably to generate the RNA and the DNA radical, especially the DNA radical with low molecular weight. This stage might be cancer. Therefore, it is suggested that cancer itself might be the DNA radical. Virus mainly consists of DNA, so the above-mentioned is not always contrary to "virus hypothesis" about cancer.

3. 4. Antioxidants in the Autoxidation of Fatty Acid Esters

Many studies had been published on the effect of antioxidants on unsaturated fatty acid esters but the differences of the effects of antioxidants on geometric isomers had never been investigated. Methyl *cis*-9, *cis*-12-octadecadienoate and its *trans* isomer methyl *trans*-9, *trans*-12-octadecadienoate were used as methyl nonconjugated dienoates, and BHA, BHT, PG, NDGA, 4, 4′-dihydroxy-3, 5, 3′, 5′-tetra-tert-butyl diphenyl methane, L-thyroxine sodium salt, α-tocopherol and sesamol were used for this purpose. The differences of the effects of antioxidants on both geometric isomers were investigated by determining the induction period using the weighing method. Also determined were the infrared and ultraviolet spectra, peroxide values, conjugated diene contents, isolated trans double bond contents and

molecular weights for the controls and the samples containing antioxidants. The *cis*, *cis* isomer was more easily autoxidized and had a shorter induction period than the *trans*, *trans* form. By the end of the induction period, no isolated *trans* double bond forms in the *cis*, *cis* isomer, but a considerable amount of isolated *trans* double bond decreased in the *trans*, *trans* isomer. In general, the effects of antioxidants, except NDGA, on the *cis*, *cis* isomer were larger than the *trans*, *trans* form.[75]

The effect of antioxidants on the autoxidation of methyl conjugated *cis*, *trans*-octadecadienoates was evaluated by estimating the induction period by measuring the increase in weight with time. Peroxide values and molecular weights were also used to determine extent of oxidation. UV and IR absorptions were measured to determine conjugated dienes and isolated *trans* double bonds. Antioxidants, such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate (PG) and sesamol, lengthened the induction period as much as seven to twelve times. After autoxidation to a weight gain of 10 mg per 1.5 g, the antixidant containing samples had higher molecular weights and lower diene contents than the control samples. The induction periods were shorter, and the peroxide values lower with or without antioxidants for the conjugated dienoates than for the nonconjugated dienoates. Effect of antioxidants might be explained by the formation of a hydrogen bond of the hydroxyl of the antioxidant and π-electrons as well as the inhibition of the chain-reaction.[76]

The sensitizing dyes for photo which are cyanine homologues have been used as medicine and cosmetics. Their uses are widespread. It was expected that these dyes might serve as antioxidants by a radical termination mechanism such as peroxy radicals added to the many conjugated double bonds which exist in the dyes. Determination of the induction period by the weighing method confirmed that some sensitizing dyes for photo are available as antioxidants in the autoxidation of methyl linoleate. Therefore, these dyes may serve as new types of antioxidants.[77~79]

The effect of new antioxidants, such as phenothiazine derivatives, on the autoxidation of methyl linoleate was evaluated by estimating the induction period using the weighing method. Peroxide values (iodometry and IR[80]), refractive indices, mol wts, and UV and IR spectra were measured to investigate the extent of the autoxidation. The induction period evaluated from the weighing method gives almost the same value as that from the peroxide values. It was shown that some new phenothiazine derivatives are remarkably effective antioxidants, and, besides, the mechanism for the autoxidation of methyl linoleate containing phenothiazine derivatives as antioxidants is probably of the same type as that for the substrate alone. The reaction mechanism of phenothiazine derivative antioxidants by determining the electron spin resonance spectra for the antioxidants in the autoxidation of methyl linoleate was also investigated. Then, the following mechanism was proposed. That is, within the induction period, these inhibitors hold stable nitroxide radicals ($>NO\cdot$) in the reaction between the antioxidant amino radical ($>N\cdot$), produced by the reaction of the antioxidant with $ROO\cdot$ or O_2, and the peroxy radical ($ROO\cdot$). Besides, the more superior the phenothiazine derivative antioxidant, the more inactive the antioxidant makes oxygen and the peroxy radical for the methyl linoleate autoxidation and also for the antioxidant oxidation.[81]

The antioxidant activity of ion exchange resins and ethyleneimine polymer was determined in methyl linoleate free from natural antioxidants and metals. Superior antioxidant activities were recognized in an ion exchange resin with NH groups and

in the ethyleneimine polymer. The antioxidant activities in these heterogeneous reaction systems increased in linear proportion to the amount present. These antioxidant activities in the heterogeneous reaction system were mainly owing to their ability to donate hydrogen to the peroxy radicals produced in the autoxidation of methyl linoleate. The antioxidant radicals produced in these cases were very stable and generally couldn't participate in the increase of the induction periods in the autoxidation of methyl linoleate by terminating the peroxy radicals. These results have never been obtained in the homogeneous reaction system but are characteristic in the heterogeneous one.[82]

The antioxidant effects of ion exchange resins and ethyleneimine polymer on the autoxidation products of methyl linoleate in a heterogeneous reaction system are discussed. Results from analyses of the various autoxidation products from linoleate samples with and without the antioxidants showed that the addition of the antioxidants did not change the original autoxidation mechanism of methyl linoleate. However, the antioxidants did retard the autoxidation in response to their antioxidant activity and, compared with a linoleate control, changed the yields of some autoxidized products such as an increased amount of conjugated diene hydroperoxides in linoleate samples with added ion exchange resins.[83]

An attempt to quantitate the tocopherol content in the autoxidized methyl linoleate was made by the iron (Ⅲ) chloride-2, 2-bipyridine method. But the quantitation was very difficult owing to the interference of hydroperoxides. This interference was also observed in t-butyl and cumene hydroperoxides, but not in di-t-butyl and dicumene peroxides. Such hydroperoxides as autoxidized methyl linoleate, t-butyl and cumene hydroperoxides catalyzed the Emmerie-Engel color reaction by itself as if it contained tocopherols. This result is contradictory to the earlier studies that the peroxides in fats inhibited the color development in the Emmerie-Engel procedure. Tocopherols in autoxidized methyl linoleate were quantitatively determined when hydroperoxides were removed by the potassium iodide treatment.[84]

The antioxidant activities of *dl*-α-tocopherol(α-T), *d*-γ-tocopherol (γ-T), and *d*-δ-tocopherol(δ-T) were determined in methyl linoleate free from natural antioxidants and metals. The relative antioxidant activities of tocopherols, at the equivalent mole concentration, were in the order α-$<\gamma$-$<\delta$-T as generally recognized. The parts of tocopherols effectively used as antioxidants were merely 13% for α-T, 46% for γ-T, and 77% for δ-T. Tocopherols added to methyl linoleate were absolutely consumed during the induction period, and the consumption rate was in the order α-$>\gamma$-$>\delta$-T. The rate of the formation of tocopheroxyl radicais was in the order α-$>\gamma$-$>\delta$-T. In the experiments oxidizing tocopherols by PbO_2 etc., the mobility of hydroxyl hydrogen of tocopherols and the stability of tocopheroxyl radicals were in the order α-$>\gamma$-$>\delta$-T. These data showed that the ability as hydrogen donors was in the order α-$>\gamma$-$>\delta$-T. However, tocopherols were consumed by direct air oxidation in the order α-$>\gamma$-$>\delta$-T. These results suggest that superior hydrogen donors are not always excellent antioxidants. Because, the substituent effect of antioxidants, as shown in tocopherols, can increase not only the ability of antioxidant as hydrogen donor but also the unstability of antioxidant against air.[85]

The study of antioxidant effects on the autoxidation products of substrates is very important in elucidating the autoxidation reaction mechanism. The antioxidant effects of tocopherols on the autoxidation products of methyl linoleate were discussed. Results from analyses of the various autoxidation products from linoleate samples with and without tocopherols showed that the addition of tocopherols did not alter

the original autoxidation mechanism of methyl linoleate. However, tocopherols did reatard the formation of autoxidation products, derived from methyl linoleate samples, according to their individual antioxidant activities. Therefore, that the antioxidant activities of tocopherols were in the order of α-<γ-<δ-T did not result from the difference of the autoxidation products among methyl linoleate samples with and without added tocopherols.[86]

Nucleic acids acted as synergists with tocopherols in inhibiting the oxidation of methyl linoleate. DNA and RNA enhanced the activity of tocopherols to different extents in the order α>γ->δ-tocopherol. Nucleic acids decreased the rates of consumption of tocopherol in the presence of oxidizing methyl linoeate. Nucleic acids also decreased the rate of oxidation of tocopherols by PbO_2. The synergistic effect of nucleic acids seemed to be caused by hydrogen bond formation with tocopherols which protected tocopherols from direct air oxidation.[87]

3. 5. The Miscellaneous

3. 5. 1. The 2-Thiobarbituric Acid Reaction of Autoxidized Unsaturatted Fatty Acid Esters[88]

It has been postulated that malonaldehyde etc. are the compounds responsible for the TBA test. But the suggestion that the TBA test could be used as an estimation of lipoperoxides has been often accepted. Therefore the relationship of the TBA value to the peroxide value has been exactly investigated. Methyl oleate, methyl linoleate, and methyl linolenate were used as the substrates, which were 94, 99, and 86 % pure by GLC, respectively. In the autoxidation of methyl linoleate, the variation of the absorptivity at 532 nm in the TBA reaction with oxidation time is similar to that of the peroxide value with the time, but in methyl linolenate the maximum points of their variations don't appear at the same time. The absorptivity at the maximum point in methyl linolenate is ten and hundred times larger than that in methyl linoleate and that in methyl oleate, respectively. Consequently, in the same compsition systems the TBA test can be utilized to estimate the variation of lipoperoxides during the early stage of autoxidation, but not in the different systems. Even in the latter case, the TBA test can be used to evaluate lipoperoxides as long as the special treatment given in this study takes place.

3. 5. 1. 1. Introduction

Wilbur et al.[89] proposed that the TBA reaction could be used as an estimation of oxidative products of unsaturated fatty acids. Patton et al.[90,91] postulated that malonaldehyde was the compound responsible for the red TBA color test and the material was present in rancid fat. But many papers[92~94] have been reported that a brilliant red color with an absorption maximum at 532 nm is produced by the reaction of TBA with dienals as well as with malonaldehyde. Besides there is a report that the TBA reactive material is not malonaldehyde.[95]

Whatever the TBA reactive material is, it is the fact that TBA reacts with the oxidative products of lipids. The TBA test is more sensitive than the peroxide one for measuring oxidative deterioration in fats, and has the advantage over the peroxide test, because it may be conducted on the intact sample and does not require fat extraction from fat-containing foods or tissues. Therefore, to investigate the utility of the TBA method for estimating the extent of the autoxidation of fats,

the TBA reaction to autoxidized unsaturated fatty acid esters has been studied. That is, the absorptivity at 532 nm due to a red color pigment in the TBA test has been compared with the peroxide value which provides a direct measure for the extent of the autoxidation of fats.

3. 5. 1. 2. Experimental Procedures

Materials: Methyl linolenate used as a substrate for autoxidation was prepared from methyl esters of linseed oil fatty acids by separating them by the urea adduct formation. Peroxides and pigments in the ester were removed by transfering it to a chromatography column containing silicic acid, and eluting with n-hexane. Thus obtained methyl linolenate was found to be 83.4 % pure by GLC analysis. Methyl linoleate (99.9 % pure by GLC analysis) and methyl oleate (95.3 % pure by GLC analysis) were obtained from sufflower oil fatty acids and olive oil fatty acids, respectively, by the same method as mentioned above. A part of methyl linolenate was a commercial product and 99.9 % pure by GLC analysis. Soybean oil removed metallic ions and natural antioxidants from a commercial product was also used.

Autoxidation: Methyl linoleate (1.5000-1.5005 g) was put into a beaker 4.1cm in diameter. Each beaker was accurately weighed and then placed in an oven at a constant temperature of 36.5±0.5℃. Of course, the depth of the oil layer for each sample was kept constant 1.03 mm.

TBA Reaction: Many reports have been published about the TBA test. The study was performed by the experimental method mentioned in Sinnhuber's review[96].

TBA Solution: 1g of TBA is dissolved in 7.5 ml of 0.1 N NaOH and diluted to 100 ml with distilled water.

Trichloroacetic Acid-HCl Reagent: 50 ml TCA solution (25 %) and 30 ml HCl (0.6N) are mixed with 420 ml of distilled water.

Antioxidant Solution: 0.3 g butylated hydroxyanisole (BHA) is dissolved in 5.4 g propylene glycol, and 0.3 g butylated hydroxytoluene (BHT) is dissolved in 4.0 g of warm Tween 20.

The sample (20-50 mg) is introduced into a test-tube and accurately weighed. Three drops of antioxidant solution and 1.5 ml of TBA solution are added to the tube. 8.5 ml of trichloroacetic acid-HCl reagent is then added. The tube is flushed with N_2 and then the cap is tightly closed. Another tube containing the same quantity of reagents, but without the sample, is used as a blank. The tubes are heated in a boiling water bath for 30 minutes. The tubes are immediately cooled to room temperature in water. Approximately 3 ml of chloroform is added and contents are centrifuged for 20 minutes at 3,000 RPM. The aqueous clear pink color solution is used for the absorptivity measurement at 532 nm. The yellow chromogen at 453 nm is measured by the same procedure as mentioned above, except that it is heated at 50℃ instead of 100℃.

3. 5. 1. 3. Results and Discussion

Fig. 10 shows the changes in the peroxide values, the absorptivties of the TBA colors (532 nm and 453 nm), and that at 272 nm due to α, β, γ, δ-unsaturated carbonyl compounds, during the autoxidation of methyl linolenate. The peak of the peroxide value appeared at the 7th day from the start of the experiment, and somewhat later than that of the absorptivity at 532 nm in the TBA color. After the peak both the peroxide value and the absorptivity decreased in a similar pattern. The absorptivity

at 453 nm was extremely weaker than that at 532 nm, and its reproducibility was bad, so it is not available. The peak of the absorptivity at 272 nm due to α, β, γ, δ-unsaturated carbonyl appeared later than that of the TBA color (532 nm). After the peak, the absorptivities at 272nm decreased rapidly, and showed a different pattern from peroxide values and the absorptivities of the TBA color (532 nm).

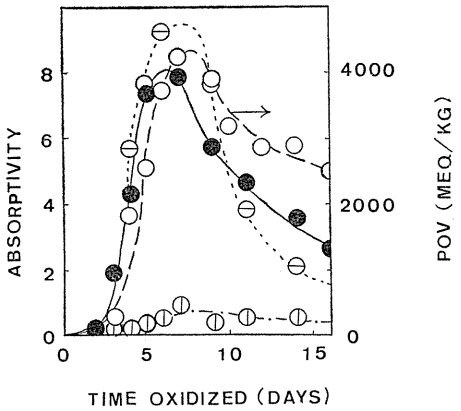


Fig. 10. Changes of POV, α, β, γ, δ-unsaturated carbonyl and the TBA absorptivity (532 nm and 453 nm) in the autoxidation of methyl linolenate.
— — —○ ; POV.
------⊖ ; α, β, γ, δ-Unsaturated carbonyl absorptivity (272 nm) × 10.
———● ; TBA absorptivity (532 nm).
-----⦶ ; TBA absorptivity (453 nm).

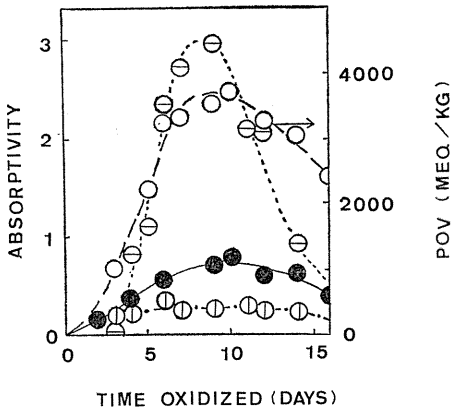


Fig. 11. Changes of POV, α, β, γ, δ-unsaturated carbonyl and the TBA absorptivity (532nm and 453 nm) in the autoxidation of methyl linoleate.
— — —○ ; POV.
------⊖ ; α, β, γ, δ-Unsaturated carbonyl absorptivity (272 nm) × 10.
———● ; TBA absorptivity (532 nm).
-----⦶ ; TBA absorptivity (453 nm).

The similar changes for methyl linoleate are shown in Fig. 11. Its absorptivity at 532 nm in the TBA color was extremely weaker than in the case of methyl linolenate. The peak of the peroxide value and that of the absorptivity at 532 nm appeared at the same time, the 10 th day from the start of the experiment. Before and after the peak, they showed a similar pattern, each other. The absorptivity at 453 nm in the TBA color was not available as shown in methyl linolenate. The pattern for the absorptivities of 272 nm due to α, β, γ, δ-unsaturated carbonyl was different from those for peroxide values and the absorptivities in the TBA color (532 nm), especially after the peak. There seems to be a little in the contribution of α, β, γ, δ-unsaturated carbonyl to the absorptivity of 532 nm in the TBA color in the both cases of methyl linoleate and methyl linolenate.

Fig. 12 shows the changes in the peroxide value, the absorptivities in the TBA colors (532 nm and 453 nm), and the absorptivity at 223 nm due to α, β-unsaturated carbonyl, during the autoxidation of methyl oleate. The absorptivity at 532 nm in the TBA color could be detected, though it was extremely weak. The absorptivity

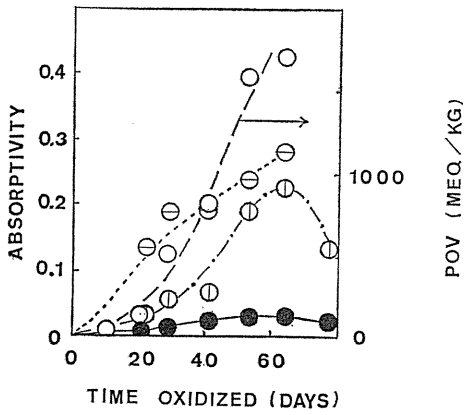


Fig. 12. Changes of POV, α, β-unsaturated carbonyl and the TBA absorptivity (532 nm and 453 nm) in the autoxidation nf methyl oleate.
— — —○ ; POV.
------⊖ ; α. β-Unsaturated carbonyl absorptivity (223 nm).
———●; TBA absorptivity (532 nm).
-----⦶ ; TBA absorptivity (453 nm).

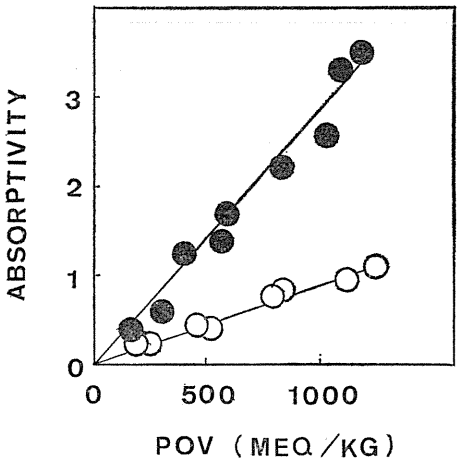


Fig. 13. Relationship between POV and the TBA absorptivity (532 nm) in the autoxidation of methyl linolenate and methyl linoleate.
●; Methyl linolenate.
○ ; Methyl linoleate × 10.

at 453 nm appeared appreciably. The maximum of the absorptivity appeared earlier than that of peroxide value. The change in the absorptivity at 223 nm due to α, β-unsaturated carbonyl showed the different pattern from from those in the TBA color and peroxide value.

In the cases of methyl linolenate and methyl linoleate, the absorptivity at 532 nm in the TBA color showed the similar pattern to peroxide value especially in the early stage of autoxidation. Therefore the relationship between peroxide values and the TBA absorptivities (532 nm) in the autoxidation of methyl linolenate and methyl linoleate is shown in Fig. 13, from which Equations (1) and (2) were obtained in the cases of methyl linolenate and methyl linoleate, respectively.

$$\text{POV} = 318 \times \text{TBA absorptivity} \quad (1)$$

$$\text{POV} = 11,300 \times \text{TBA absorptivity} \quad (2)$$

In the case of methyl linolenate or methyl linoleate, by determining the TBA absorptivity (532 nm), the peroxide value can be evaluated by Equation (1) or Equation (2).

Fig. 14 shows the relationship between peroxide values and the TBA absorptivities (532 nm) in the autoxidation of the mixtures of methyl linolenate and methyl linoleate. The ratios of methyl linolenate to methyl linoleate are 3 : 1, 1 : 1, and 1 : 4. In these cases, Equations (3), (4), and (5) were obtained, by which the peroxide values can

be estimated by determining the TBA absorptivities (532 nm), respectively.

$$\text{Linolenate : linoleate}=3:1 \quad \text{POV}=315\times\text{TBA absorptivity} \tag{3}$$

$$\text{〃} \quad =1:1 \quad \text{POV}=436 \quad \text{〃} \tag{4}$$

$$\text{〃} \quad =1:4 \quad \text{POV}=667 \quad \text{〃} \tag{5}$$

But in Equation (5), the deviation from the found value becomes large, when the peroxide value is larger than 800.

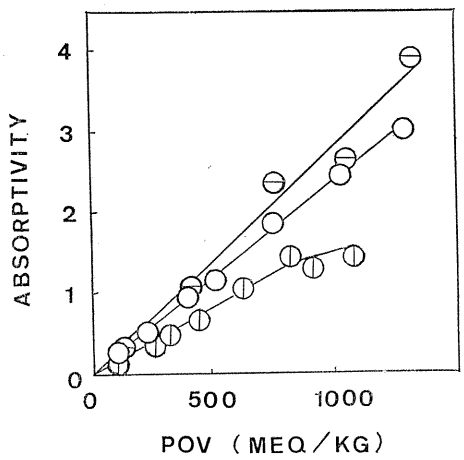


Fig. 14. Relationship between POV and the TBA absorptivity (532 nm) in the autoxidation of the mixture of methyl linolenate and methyl linoleate.
⊖ ; Linolenate : linoleate=3 : 1.
○ ; Linolenate : linoleate=1 : 1.
◐ ; Linolenate : linoleate=1 : 4.

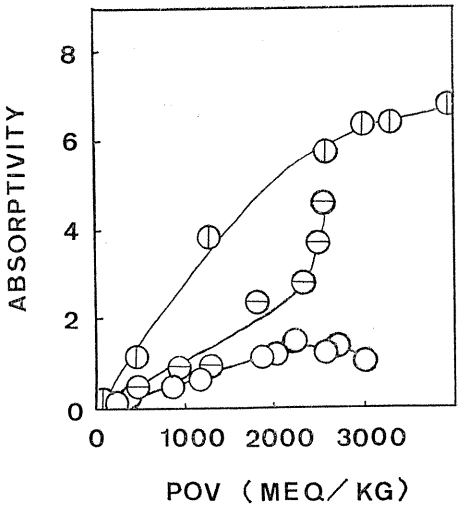


Fig. 15. Relationship between POV and the TBA absorptivity (532nm) in the autoxidation of soybean oil and the mixture of methyl oleate and methyl linolenate or methyl linoleate.
○ ; Soybean oil.
◐ ; Oleate : linolenate=1 : 1.
⊖ ; Oleate : linoleate=1 : 1×10.

The similar plots to Fig. 14 about the mixture of methyl oleate and methyl linolenate and that of methyl oleate and methyl linoleate (the ratios of fatty acid methyl esters are both 1 : 1) are shown in Fig. 15. Studies of the autoxidation of methyl linolenate, methyl linoleate, and methyl oleate by Kenaston et al.[97] showed that the TBA values for methyl linolenate were 30-80 times as large as those for methyl linoleate at the same peroxide values. They reported that these values had almost no relation to peroxide values but to the amount of aldehydes, and that methyl oleate did not give the TBA values. But Dahle et al.[98] showed a linear relationship between the TBA value and the peroxide value for methyl esters of polyunsaturated fatty acids except for methyl linoleate. A direct comparison between the peroxide value and the TBA value was found in the early stage of the autoxidation of a fish oil by Sinnhuber et al.[99]

In this study, the TBA value (532 nm) was relative to the peroxde value for methyl linolenate or methyl linoleate, and was not necessarily relative to the amount of unsaturated carbonyls. Extremely slight TBA absorptivity (532 nm) was detected for methyl oleate. The TBA value extremely changes with the unsaturation of fatty acids. The TBA values among the substrates having different composition of fatty acids are not available for the comparison of the degree of autoxidation.[98] But for the mixture of methyl linolenate and methyl linoleate, even if it consists of any ratio, in the early stage of autoxidation, a linear relationship between the TBA value and the peroxide value is given as follows.

$$\mathrm{POV} = a \times \text{TBA absorptivity} \tag{6}$$

The values of "a" at various compositions of methyl linolenate and methyl linoleate have been poltted in Fig. 16. The plots of "a" values are found below the straight line gotten by connecting the value of methyl linolenate and that of methyl linoleate. There is a very good reason for it. That is, the rate of the autoxidation of methyl linolenate, is faster than that of lmethyl linoleate, so in the mixture, methyl linoenate is more predominantly autoxidized and shows extremely stronger TBA absorptivity (532 nm) than methyl linoleate. If the composition of the mixture is determined, from the curve "a" in Fig. 16, the value of "a" can be estimated. By measuring the absorptivities (532 nm) of both mixtures having different composition, respectively, both peroxide values can be evaluated from Equation 6 substituted "a" by the estimated values, respectively, and then the comparison of the degree of autoxidation between the mixtures becomes available. In fact, in soybean oil, a linear relationship between the TBA value and the peroxide value is obtained, and in this case the found value of "a" is 1.7×10^3. Soybean oil used contained 7.9 % linolenic acid, 60.6 % linoleic acid, 21.4 % oleic acid, and 10.6 % other fatty acids. The ratio of linolenic acid and linoleic acid is 11: 89, and "a" obtained from Fig. 16 is approximately the same as the found value mentioned above.

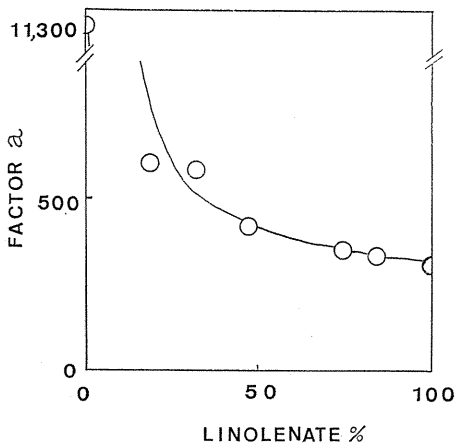


Fig. 16. Plot of methyl linolenate % in the mixture of methyl linolenate and methyl linoleate vs. factor a.
POV=a x (TBA absorptivity).
a value for methyl linolenate : 318.
a value for methyl linoleate : 11,300.

In the mixture of methyl linolenate and methyl oleate or that of methyl linoleate and methyl oleate, a linear relationship between the TBA value and the peroxide value can be observed at the early stage of autoxidation. In this case the rate of autoxidation of methyl oleate is extremely slow in comparison with that of methyl linolenate or that of methyl linoleate, so the peroxide value and the TBA absorptivity (532 nm) are almost due to the autoxidation of methyl linolenate or that of methyl linoleate in the mixture. Therefore for the mixture, the value of

"a" in Equation 6 shows the approximation of "a" for methyl linolenate or that of "a" for methyl linoleate.

In the TBA reaction, not only the typical pink pigment at 532 nm, but also a yellow pigment at 453 nm is formed. The compounds responsible include epihydrin aldehyde,[100] glyceraldehyde,[100,101] alkenal,[93,94] and alkanal.[93,94] There are few reports that the yellow pigment was used as a measure for the autoxidation of lipids. In this study, the formation of the 453nm pigment was not used as a measure of methyl linolenate oxidation or methyl linoleate one. The absorptivity at 453 nm was weaker than that at 532 nm, and its reproducibility was worce. But in the case of methyl oleate, the 453 nm pigment is more readily formed than the 532 nm pigment. The absorptivity at 453 nm has no special relation to the peroxide value, so it is not used as a measure of methyl oleate oxidation.

Consequently, in the case of lipids with the same composition, the TBA test can be used as a measure of the autoxidation of lipids, but even if in the case of lipids with the different composition, it can also be used as the measure by converting the absorptivities (532 nm) to the corresponding peroxide values, respectively, by the aid of Fig. 16 and Equation 6.

3. 5. 2. New Analytical Methods for Autoxidized Fatty Acid Esters etc.

The quantitative determination of hydroperoxides by infrared spectroscopy was first investigated, and a new analytical method was proposed.[80] The study on quantitative analyses of hydroperoxides and alcohols by NMR shift reagent was also performed.[102] The accumulation of lipoperoxide in animal tissue and blood was examined.[103]

The investigation on the autoxidation of soaps of unsaturated fatty acids in aqueous colloidal solution was carried out, and it was confirmed that the autoxidation mechanism in these cases is almost the same as that in the liquid phase of fatty acid esters.[104,105] The studies on the autoxidation of common unsaturated fatty acid esters instead of highly unsaturated ones mentioned at 3. 2. also took place.[106~108] Prooxidant effects of inorganic chromium compounds in the autoxidation of methyl linoleate were investigated, and new informations were given.[109]

4. Hydrogenation Reactions of Fats and Some Other Compounds

4. 1. Introduction

There are a great many papers, about heterogeneous hydrogenation, using heterogeneous catalyst and hydrogen gas. In industrial hydrogenation, this type has been generally used. Studies on the hydrogenation of pure highly unsaturated fatty acid esters, which had never been published until our investigations, were also grouped into this category. By these studies many informations were given.

Of course, a good many papers have been published about homogeneous hydrogenation, using transition metal complexes as catalysts and hydrogen gas. Many papers have been also presented about heterogeneous transfer hydrogenation, using heterogeneous catalysts and organic compounds as hydrogen donors. But there are relatively few studies about homogeneous transfer hydrogenation, using transition metal complexes as catalysts and organic compounds as hydrogen donors.

Almost no studies have been published about homogeneous transfer hydrogenation

of simple olefins, especially fatty oils, except for our studies. The merit of using homogeneous catalysts such as transition metal complexes is that in general high selectivity can be obtained. The demerit of using such catalysts is that the separation of reduction products from catalysts and the recycle of catalyst are difficult. Nowadays the demerit can be overcome by using transition metal complexes having appreciably high molecular weight ligands as catalysts. In the homogeneous transfer hydrogenation with these catalysts, high selectivity is maintained and besides the separation of reaction products from catalysts may get easier. In the hydrogenation of fatty oils, the higher selectivity and the lower amount of trans isomers had been desired eagerly in the edible oils and fats and the soap industry. Generally the higher the selectivity, the larger the amount of isolated trans double bonds. Therefore, attaning the desire had been thought impossible. The studies on the homogeneous transfer hydrogenation of fatty oils had never been performed as mentioned above, and so took place in order to attain the desire, after those of general organic compounds.

4. 2. Hydrogenation of Highly Unsaturated Fatty Acid Esters

Studies on the hydrogenation of pure highly unsaturated fatty acid esters had never been undertaken, owing to the difficult isolation and the low stability of these fatty acid esters. But such studies were first carried out to obtain new informations.[110~114] The isomerization during the hydrogenation of methyl docosahexaenoate was investigated, and it was shown that the composition of fatty acids during the hydrogenation can not be exactly determined by GLC or the ultraviolet absorption method.[110] The study on hydrogenation of conjugated polyenes, such as methyl conjugated eicosapentaenoate[111] and methyl conjugated octadecatetraenoate[112] was first carried out. During the hydrogenation the amount of the conjugated tetraene was decreased and no other conjugated polyenes appeared.[112] The migration of double bonds in the hydrogenation of methyl eicosapentaenoate was determined.[113] In the hydrogenation of conjugated diene concentrates obtained from methyl docosahexaenoate catalyzed by nickel-diatomaceous earth catalysts, the rate of hydrogenation of the conjugated diene was faster than that of nonconjugated polyenes.[114]

4. 3. Heterogeneous Transfer Hydrogenation[115]

Transfer hydrogenation has the same meaning as conjugated hydrogenation. Relatively many studies on heterogeneous transfer hydrogenation using hetrogeneous catalysts and organic compounds as hydrogen donors had been carried out. Conjugated hydrogenation of methyl linoleate and methl conjugated octadecadienoate using nickel-diatomaceous earth as a heterogeneous catalyst and various alcohols as hydrogen donors was investigated.[115] The selectivity in the hydrogenation with primary alcohol is inferior to that with secondary alcohol. By using the former, alcoholysis of fatty acid esters also occurs. In the conjugated hydrogenation of conjugated dienes, cis, trans conjugated dienes were decreased, and trans, trans ones increased, in the early stage. And then both conjugated dienes were hydrogenated at the same rate.[115]

4. 4. *Homogeneous Transfer Hydrogenation*

4. 4. 1. *Introduction*

In general homogeneous catalysts show higher selectivity and better reproducibility and accelerate the rate of reactions under milder conditions, in comparison with heterogeneous catalysts.

The study on the homogeneous transfer hydrogenation of fatty oils had never been undertaken. As such a kind of study, the homogeneous transfer hydrogenation of fatty acid esters catalyzed by $Ni(acac)_2$ was first investigated to obtain hydrogenated products in our laboratory.[116] Though in the studies on the homogeneous hydrogenation using fatty oils and hydrogen gas, Itatani and Bailar showed that hydrogen might be lost from solvents,[117~119] this was only a sign of the homogeneous transfer hydrogenation of fatty oils. For simple olefins except for fatty oils, studies on the homogeneous transfer hydrogenation using alcohol[120,121] and formic acid[122] as hydrogen donors were only performed at almost the same time that our study[116] was carried out or after the study. However, there are a good many papers on the homogeneous transfer hydrogenation of double bonds in α, β-unsaturated carbonyl compounds.[120,121,123~129]

The studies on the homogeneous transfer hydrogenation of simple unsaturated compounds using homogeneous catalysts, such as $RuCl_2(PPh_3)_3$,[130~153] toot place, and then those of fatty acid esters[154~161] were carried out. As mentioned in 4. 1., the attainment of 100 % selectivity and a trace of trans isomers had been desired eagerly, but it had been thought impossible. As described later, in our laboratory the desire was first attained by the homogeneous transfer hydrogenation of methyl linoleate catalyzed by $RuCl_2(PPh_3)_3$ and $RuH_2(PPh_3)_4$ and donated with hydrogen by isopropyl alcohol and hydrocortisone, respectively.

Homogeneous transfer hydrogenations can be generalized as in the following equation.

$$DH_x + nA \xrightarrow[\text{Solvent}]{\text{Homogeneous catalyst}} nAH_x + D$$

DH_x : hydrogen donor ; A : acceptor ; Homogeneous catalyst : $RuCl_2(PPh_3)_3$, $RhH(PPh_3)_4$, $RuH_2(PPh_3)_4$, etc. ; Solvent : toluene etc.

4. 4. 2. *Homogeneous Transfer Hydrogenation of Simple Unsaturated Compounds*[130~153]

Studies on the homogeneous transfer hydrogenation of simple unsaturated compounds in our laboratory also include those on hydrogenolysis.

Hydrogen transfer from a number of organic compounds to olefins in a homogeneous system was studied. In the case of hydrogen transfer to 1, 5-cyclooctadiene, using dichlorobis(triphenylphosphine)iron (Ⅱ) as the catalyst, dihydroxylbenzenes, such as pyrocatechol and hydroquinone, were much superior to *prim*- and *sec*-alcohols, 1-dodecanethiol, and cyclohexene as hydrogen donors.[131] Hydrogen transfer from alcohols to olefins was studied to find effective homogeneous catalysts and convenient reaction conditions, and it was found that $RhH(PPh_3)_4$ had the highest catalytic activity among the complexes which were tried. Spectroscopic measurements (ir, uv, and gc-mass spectrum), hydrogenation by molecular hydrogen, and isotope effects were studied. Kinetic studies were carried out at 60-100℃ using the Rh(Ⅰ) complex

as a catalyst, 2-propanol as a hydrogen donor, cycloheptene as a hydrogen acceptor, and toluene as a solvent. The hydrogen transfer reaction is inferred to proceed in the follwing order: (1) the release of triphenylphosphine from $RhH(PPh_3)_4$, (2) coordination of 2-propanol to the metal, (3) hydrogen trnsfer from 2-propanol to the metal with the oxidative addition to form a trihydride complex, (4) coordination of cycloheptene to the metal, and (5) transfer of hydrogen of the trihydride complex to cycloheptene to form cycloheptane. These data are compatible with the expression rate $=K_1K_2k_5[D]\,[Rh]_{total}/([P]+K_1)$, where [D], $[Rh]_{total}$, and [P] are donor, catalyst, and phosphine concentration, respectively, The rate-determining step of this reaction was considered as the transfer of hydrogen from alcohol to a Rh(Ⅰ) species.[133]

Hydrogen transfer from 1, 4-dioxane to olefins catalyzed by $RhCl(PPh_3)_3$ occurred. Stoichiometric amounts of paraffins and dioxene were formed from olefins and dioxane. After the reaction, the dioxane complex, $RhCl(PPh_3)_2(C_4H_8O_2)$, was isolated. The large value of the kinetic isotope effect, $R_H/R_D=3.1$, in the use of octadeuteriodioxane and the kinetic result showed that the rate-determining step of the reaction is the dehydrogenation, that is, the formation of the monohydride complex, $RhClH(C_4H_7O_2)(PPh_3)_2$, by the oxidative addition of dioxane by the cleavage of a C-H bond.[134] In the hydrogen transfer from organic compounds to olefins catalyzed by $RhCl(PPh_3)_3$, some cyclic amines were found much more reactive than oxygenated and hydroaromatic compounds such as primary and secondary alcohols, tetralin, etc. Reactivity decreased in the order indoline>pyrrolidine>tetrahydroquinoline > piperidine > 2, 3-butanediol > dioxane > cyclohexanol > isopropyl alcohol. Indoline and tetrahydroquinoline gave stoichiometrically indole and quinoline, respectively.[137] The mechanism of hydrogen transfer from indoline to cycloheptene in toluene catalyzed by $RhCl(PPh_3)_3$ has been studied. The rate data of the reaction can be accommodated by the rate expression of the form, rate $=a[D][C]_0/(b+[L])$ where $[C]_0$,[D], and [L] are the concentration of the catalyst, indoline, and triphenylphosphine, respectively. The rate-determining step of the reaction is inferred to be the dehydrogenation of indoline, that is, the hydrogen transfer from the amine to a Rh(Ⅰ) complex to form a hydride complex by oxidative addition.[138]

The dihydride complex, $RuH_2(PPh_3)_4$, has been found to be an excellent catalyst for the hydrogen transfer from 2-propanol to olefins. For cyclohexene in toluene solution, the rate law at 80℃ was:

$$\text{Rate}=\frac{0.04[C]_0\,[D]}{1+24\,[P]}$$

where $[C]_0$, [D] and [P] are the concentration of the catalyst, 2-propanol and triphenylphosphine, respectively. It is pressumed that active intermediates of the reaction are not Ru(Ⅳ)-species but Ru(0)-species. The rate-determining step of the reaction is inferred to be the dehydrogenation of 2-propanol, that is, the hydrogen transfer from the alcohol to Ru(0)-complex to form a hydride complex by oxidative addition.[139]

A considerable amount of benzene was formed in the reaction of $RhCl(PPh_3)_3$ and $RhH(PPh_3)_4$ with amines, alcohols, hydroaromatic comounds, and dioxane. For example, about eight or ten molecules of benzene were formed from a molecule of $RhCl(PPh_3)_3$ or $RhH(PPh_3)_4$, respectively, when these complexes were heated at 100℃ for 6h in pyrrolidine. In the reaction of $RhCl(PPh_3)_3$ in pyrrolidine, diphenylphosphine and biphenyl also were detected; a bright yellow complex which was

tentatively identified as $RhCl(PPh_3)_2$ (pyrrolidine) was isolated as a reaction intermediate. The isolated complex gave benzene by heating in inert selvents. The hydrogen transfer from indoline, tetrahydroquinoline, and dioxane was confirmed by the quantitative analysis of reaction mixtures.[153]

In the hydrogen transfer from organic compounds to aldehydes and ketones, $RuH_2(PPh_3)_4$ was found to have an excellent catalytic activity under mild conditions. Ethers, hydroaromatic compounds, tertiary amines, and alcohols showed hydrogen donating ability, and the ability decreased in the order 2, 5-dihydrofuran>tri-n-propylamine>benzyl alcohol>cyclohexanol>ethyl alcohol> tetralin $\approx$ 1, 2-dihydronaphthalene>dioxane. The mechanism of hydrogen transfer from alcohols to the aldehydes was investigated. The data of the reaction can be accommodated by the rate expression of the form rate $= k[D][Cat]_0/(1+K[RCHO])$, where [D], $[Cat]_0$, and [RCHO] are alcohol, catalyst, and aldehyde concentrations, respectively. The kinetic isotope effect, $R_H/R_D=0.9$, and other data suggest that the rate-determining step of the reaction is the coordination of the alcohols to the complex. The process of the hydrogen transfer from alcohols to aldehydes on the metal is also proposed.[143] Aromatic hydrocarbons, such as indan and ethylbenzene, were dehydrogenated and reduced aldehydes under mild conditions in the presence of $RuH_2(PPh_3)_4$. It was also found that indan and isobutylbenzene reduced cycloheptene under more drastic condition in the presence of $RhCl(PPh_3)_3$. The mechanism of hydrogen transfer from indan, tetralin, and dioxane to an aldehyde catalyzed by $RuH_2(PPh_3)_4$ was investigated, and found to be different from that of the reduction of aldehydes by alcohols. The transfer hydrogenation by the aprotic substances occurs via dihydride complexes, and the overall rate law was rate $= a[DH_2][cat.]_0/(1+b[DH_2]+c[RCHO])$ where $[DH_2]$, $[cat.]_0$, and [RCHO] are hydrogen donor, added catalyst, and aldehyde concentrations, respectively. The rate-determining step of the reduction by the aprotic hydrogen donors is the hydrogen transfer from the donors to the catalytic species.[144]

The dehydrogenation of 2-propanol by chloral was found to occur in the presence of several transition metal complexes to give acetone and tetrachlorohydroquinone. Some of these reactions seem to be explained by a mechanism that requires both the donor and the acceptor to coordinate simultaneously on the central metal of the catalyst, and hydrogen atoms to be transferred directly from the former to the latter without forming hydride complexes. The dehydrogenation of tetralin and 2, 5-dihydrofuran, which are unsaturated compounds, was not influenced by the addition of the metal complexes.[135] Noble metal salts were found to catalyze the homogeneous transfer hydrogenation of nitrobenzenes to anilines in good yields using indoline as a hydrogen donor.[145] It is shown that in the presence of Pd(II) salts the carbon-halogen bond of aryl halides is cleavaged to give the corresponding aryl compounds and hydrogen halides by hydrogen transfer from organic compounds.[148]

Aralkyl- and aryl-oxygen bonds of ethers, esters, and alcohols, and aralkyl- and aryl-nitrogen bonds of amines, an imide and an amide were hydrogenolyzed by the hydrogen transfer from organic compounds such as indoline in the presence of some noble metal salts. In these reactions, not only benzyl group but phenethyl and phenyl group were removed.[149]

4. 4. 3. Homogeneous Transfer Hydrogenation of Fatty Acid Esters[154~161]

4. 4. 3. 1. Experimental Procedure

Materials: Methyl linoleate was prepared from safflower oil. Conjugated linoleate was obtained by the reaction with potassium hydroxide. Methyl *trans*-9, *trans*-12-octadecadienoate was prepared by the isomerization of methyl linoleate by sodium nitrite. Methyl oleate and methyl elaidate were prepared by esterification of refined oleic and elaidic acids with methyl alcohol, respectively. Complex catalysts were synthesized by the methods previously reported.[133,134,137,139,142] The hydrogen donors and the solvent were purified by distillation.

An Example of Transfer Hydrogenation: Methyl linoleate (0.2 M), a hydrogen donor (0.4 M), and a catalyst (20 mM) were in a pyrex glass tube with toluene as a solvent. The tube was sealed under vacuum using liquid nitrogen and a vacuum line. The sealed tube was heated for 3 hr with continuous oscillation in a polyethylene glycol bath kept at the designated temperature ±1℃. The reaction mixture was submitted to gas liquid chromatographic analysis which was performed at 220℃, using 15 μl of dibenzyl ether as an internal standard. A 2 m×6 mm stainless steel column packed with 12 % diethylene glycol succinate on Diasolid L was used. Volatile compounds were removed from the reaction mixture under reduced pressure. The residue was dissolved in carbon tetrachloride and then submitted to IR analysis using the peak at 968cm^{-1} as elaidate, to measure the amount of trans double bonds. Although the residue contained dibenzylether, indoline, and indole, it was confirmed that they did not influence the IR analysis.

Other transfer hydrogenations were carried out in a similar way.

4. 4. 3. 2. Results and Discussion

Results

As substrates, methyl esters of *trans*-9, *trans*-12-octadecadienoic acid (*t*-9, *t*-12-diene), conjugated octadecadienoic acids (conj. dienes), *cis*-9-octadecenoic acid (oleate), and *trans*-9-octadecenoic acid (elaidate) were chosen in addition to linoleate. Conj. dienes were shown to consist of 90 % of cis, trans and 10 % of trans, trans isomers by GLC analysis.

As hydrogen donors, isopropyl alcohol, indoline, dioxane, tetrahydroquinoline, tetralin, 2, 5-dihydrofuran, L-ascorbic acid, L-arabinose, sucrose, and naturally occurring materials, such as steroids, terpenes, and alkaloids, were examined. Alcohol has been used in many studies as a representative hydrogen donor.[116,133,139,140,143]

Hydrogen Transfer from Isopropyl Alcohol: It is well known that isopropyl alcohol gives olefins hydrogen to yield acetone,[133,139,140,143] but it is inferred that the hydrogen-donating power of the alcohol is lower than indoline.[137,142] Therefore, the hydrogen transfer from isopropyl albohol was examined only with those catalysts which were active in the reaction with indoline or had been reported to be active in the transfer hydrogenation of oils. In the transfer hydrogenation of methyl linoleate catalyzed by $RuCl_2(PPh_3)_3$, and donated with hydrogen by isopropyl alcohol, 100 % selectivity and a trace of trans isomers were first obtained as shown in Table 2. As described before, the attainment of 100 % selectivity and a trace of trans isomers had been desired eagerly, but it had been thought impossible.

In the table, conversion shows the total yield of the hydrogenation products, and

Table 2. Homogeneous Transfer Hydrogenation Catalyzed by $RuCl_2(PPh_3)_3$[a]

Substrate	Temp. (℃)	Yield of prod.[b] (%)			Conv.[c] (%)	Select.[d] (%)	Trans[e] (%)	
		S	M	CD			A	B
Linoleate[1)]	140	0	68	15	68	100	Trace	
t, t-Diene	160	0	32	29	32	100	44	39
Conj. dienes	140	0	96	4	96	100	51	53
Oleate	140	0	100	0			Trace	
Elaidate	140	0	100	0			80	80
Linoleate[2)]	100	14	65	0	79	82	94	88
t, t-Diene	100	14	86	0	100	86	62	72
Conj. dienes	100	10	90	0	100	90	51	56
Oleate	100	38	62	0			46	74
Elaidate	100	13	87	0			64	74
Linoeate[3)]	140	0	88	0	88	100	50	44
t, t-Diene	140	0	100	0	100	100	55	55
Conj. dienes	140	0	74	26	74	100	32	43
Oleate	140	0	100	0			53	53
Elaidate	140	0	100	0			50	50
Oleate[4)]	140	0	100	0			0	0
Elaidate	140	0	100	0			101	101

1) : isopropyl alcohol, 2) : indoline, 3) : glucose, 4) : none.

[a]A hydrogen donor (0.4 M), methyl linoleate (0.2 M), and a catalyst (20 mM) were heated at 140℃ (100, 160℃) for 3 hr in toluene.

[b]S= methyl stearate. M= monoenes. CD= conjugated dienes.

[c]Conv.= conversion, given by $\frac{[\text{Stearate}]+[\text{Monoenes}]}{[\text{C}_{18}\text{-esters}]}\times 100.$

[d]Select.= selectivity, given by $\frac{[\text{Monoenes}]}{[\text{Stearate}]+[\text{Monoenes}]}\times 100.$

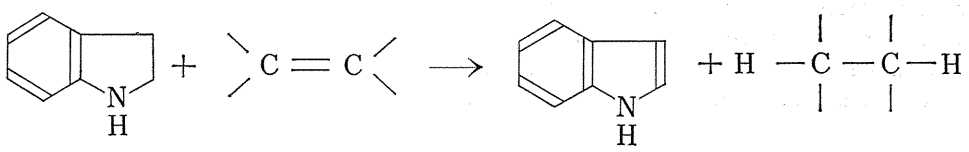
[e]Percent trans (A) $=\frac{[\text{Trans isomers as elaidate}]}{[\text{C}_{18}\text{-esters}]}\times 100$

Percent trans (B) $=\frac{[\text{Trans isomers as elaidate}]}{[\text{Monoenes}]+[\text{Nonconjugated dienes}]\times 2}\times 100.$

selectivity represents the percentage of monoenes in the hydrogenation products. Trans percent (A) implies the supposed yield of trans isomers based on elaidate, and trans percent (B) exhibits the percentage of isolated trans double bonds in all the isolated double bonds in the reaction mixtures. The former notation has been used in most reports on the hydrogenation of oils, but the latter seems to be more useful in scientific discussions. As the rough tendency, selectivity decreased, and the trans percent (B) increased with an increment in conversion. Therefore, the comparison of selectivity and trans percent (B) at constant conversion is desirable to discuss the selectivity to cis-monoenes.

Hydrogen Transfer from Indoline: It was confirmed that in the hydrogen

transfer from indoline to olefins, the stoichiometric amount of indole and paraffins were formed and the following reaction proceeded without side reactions.[137,142] In the catalytic reaction between indoline and methyl linoleate, monoenes, stearate, isomerized dienoates and indole were formed as given in Table 2 and the following equation.



The total amount of these C_{18}-esters, including residual linoleate, in the reaction mixtures was found to be equal to the amount of the initial linoleate within experimental error. The amount of the indole formed also equaled the theoretical within experimental error.

The results obtained by using glucose as a hydrogen donor are shown in Table 2.

Table 3. Homogeneous Transfer Hydrogenation by Various Hydrogen Donors

Hydrogen donor	React. cond.	Yield of prod.[b] (%)			Conv.[c] (%)	Select.[d] (%)	Trans[e] (%)	
		S	M	CD			A	B
Dioxane	a, A	0	42	28	42	100	34	34
Tetrahydro-quinoline	a, B	0	38	6	38	100	23	15
Tetralin	a, A	7	28	38	35	80	37	45
2, 5-Dihydrofuran	a, A	0	26	51	26	100		
L-Ascorbic acid	a′, A	0	70	0	70	100	25	19
L-Ascorbic acid	a′, B	0	35	36	35	100	26	28
L-Arabinose	a′, A	12	59	7	71	83	Trace	
Sucrose	a′, A	0	33	49	33	100	21	30
Sucrose	a′, B	0	48	26	48	100	24	25

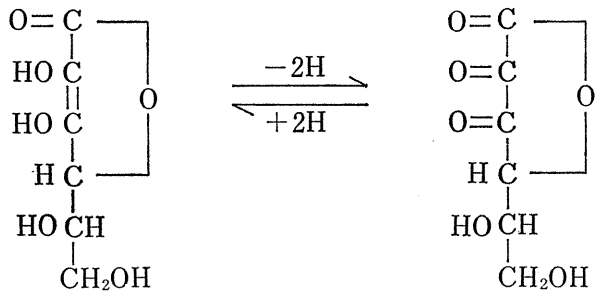
a′: methyl linoleate (0.1 M), a hydrogen donor (0.2 M), and a catalyst (10 mM) were heated in tetrahydrofuran at 140℃ for 3 hr.
A: $RuH_2(PPh_3)_4$, B: $RuCl_2(PPh_3)_3$.
Other notations in this table are the same as those in Table 2.

Various Hydrogen Donors: A part of the results given by using dioxane, tetrahydroquinoline, tetralin, 2, 5-dihydrofuran, L-ascorbic acid, L-arabinose, and sucrose as hydrogen donors is summarized in Table 3.

The combination of hydrogen donors and catalysts was determined by reference to the literature and our experience. Dioxane[137,143,144] and 2, 5-dihydrofuran[140,143] have been reported as hydrogen donors in transfer hydrogenation. In the reduction by 2, 5-dihydrofuran, the analysis of trans isomers could not be performed, because the IR spectra of the reaction mixtures showed a broad peak at 960-1000 cm^{-1}.

The fact that the total amount of C_{18}-esters and that of 2, 5-dihydrofuran and furan diminished suggests that a Diels-Alder type reaction occurred.

Then we examined some naturally occurring polyhydroxy compounds including L-ascorbic acid, which is known to function as a reducing agent to give L-dehydroascorbic acid in vivo. In the reduction of linoleate with this reductant catalyzed by $RuH_2(PPh_3)_4$, conversion, selectivity, and trans percent (B) were 70, 100, and 19 %, respectively. In any case, with this hydrogen donor the selectivity was 100 %. In these reactions L-dehydroascorbic acid was identified in the product mixture by TLC analysis. This result shows that endiol structure of the reductant was transformed to diketone form by the hydrogen transfer.



The L-arabinose-$RuH_2(PPh_3)_4$ system showed high conversion and very low trans percent (B), but the selectivity was not complete. Sucrose also reduced linoleate. It is worth noting that in almost all reactions with conversions of less than 70 %, significant amounts of conjugated dienes were detected. Among the reactions given in Table 3, the L-ascorbic acid-$RuH_2(PPh_3)_4$ system seems to be best.

When L-ascorbic acid was used as a hydrogen donor, the selectivity to monoenes was 100 % in every case, and trans percent (B) was about 30 % in the reduction of dienes. Oleate and elaidate were hardly reduced in the presence of the acid even at 140℃. This means that L-ascorbic acid not only gives no hydrogen to monoenes but also inhibits the hydrogen transfer from $RuH_2(PPh_3)_4$ to monoenes. This may be responsible for its complete selectivity to monoenes. In the reaction of the monoenes, *trans* percent (B) was nearly equal and about 40 %, showing that the ratio of *cis* to *trans* was about 3 : 2. In the absence of hydrogen donors, the reaction of oleate and elaidate gives *trans* percent (B) of 67 % and 71 %. This result demonstrates that the *cis* to *trans* ratio in the absence of hydrogen donors was about 1 : 2.

Hydrogen Transfer from Natural Products[160,161]: In the transfer hydrogenation of linoeate catalyzed by $RhH(PPh_3)_4$, eleven steroids, six terpenes, and an alkaloid were examined as hydrogen donors, and the results are summarized in Table 4. In the table, the conversion shows the total yield of the hydrogenation products, and the selectivity represents the percentage of monoenes in the hydrogenation products. *Trans* percent, (A) implies the supposed yield of *trans* isomers based on elaidate, and *trans* percent (B) exhibits the percentage of isolated *trans* double bonds in isolated double bonds in the reaction mixtures.

In Table 5, the hydrogen-giving ability of the natural products examined is given from the convesion shown in the preceding table.

Table 4. Homogeneous
Transfer Hydrogenation of Methyl Linoleate Catalyzed by $RhH(PPh_3)_4$[a]

Hydrogen donor	Yield of products[b] (%)			Conv.[c]	Select.[d]	Trans[e] (%)	
	S	M	CD	(%)	(%)	A	B
Ergosterol	0	95	2	95	100	238	236
Borneol	8	87	0	95	93	37	38
Menthol	0	93	0	93	100	35	33
Testosterone	0	93	0	93	100	59	56
Cholesterol	0	92	5	92	100	18	19
β-Sitosterol	3	89	0	92	96	54	52
Stigmasterol	3	85	6	88	97	171	175
Stanolone	0	84	4	84	100	58	54
Androsterone	2	66	25	68	98	64	78
β-Cholestanol	0	65	27	65	100	29	36
Lanosterol	0	63	22	63	100	28	30
D-Limonene	0	22	52	22	100	Trace	
β-Citronellol	3	18	53	21	84	5	7
Methyltestosterone	0	19	67	19	100	8	16
Geraniol	2	12	51	14	84	7	8
Abietic acid	0	13	35	13	100	11	9
Hydrocortizone	0	12	48	12	100	Trace	
Nicotine	0	11	71	11	100	Trace	

[a-e]Notations in this table are the same as those in Table 2.

Table 5. Hydrogen-Donating Ability in the Homogeneous Transfer Hydrogenation of Linoleate Catalyzed by $RhH(PPH_3)_4$

Ergosterol ≈ borneol > menthol ≈ testosterone ≈ cholesterol ≈ β-sitosterol > stigmasterol > stanolone > androsterone > β-cholestanol > lanosterol ≫ D-limonene ≈ β-citronellol > methyltestosterone > geraniol ≈ abietic acid ≈ hydrocortizone ≈ nicotine.

It is infered that in the reactions of the steroids except for methyltestosterone and hydrocortizone, the β-hydroxyl group attached to C_3 or C_{17} gives two hydrogen atoms to linoleate to form ketones. The hydrogen-donating power of primary alcohols seems to be weaker than that of secondary alcohols, because the conversion in the reaction of β-citronellol or geraniol was lower than that in the reaction of borneol, menthol, or most of the steroids examined. Consequently, in the hydrogen transfer from hydrocortizone, the hydroxyl group attached to C_{11} is assumed to donate hydrogen prior to the one attached to C_{21}. It is considerable that D-limonene gave dehydrogenation products having a benzene ring, but the products are not identified as yet. Nicotine which has N-methylpyrrolidine ring is speculated to be dehydrogenated at the pyrrolidine part, for N-methylpyrrolidine hydrogenated cycloheptene.[137] The selectivity to monoenes was complete or almost complete in any case but for the reaction of β-citronellol and geraniol.

Generally, the values of *trans* percents increase with increasing conversion as described in the previous paper.[156~158] Therefore, it is noteworthy that the *trans* percents were 18-19 % even at the conversion of 92 % in the reaction of cholesterol which showed the complete selectivity to monoenes. According to the definition of *trans* percent (B), the value of this parameter cannot exceed 100 %, but the values in the reactioes of ergosterol and stigmasterol were abnormally large and exceeded 100 %. As described earlier, isolated *trans* double bonds show a peak at 968 cm^{-1} and ergosterol has a *trans* double bond at 22 position. Therefore, in the reaction of this steroid, the value of *trans* percents which is evaluated from the peak, does not represent the nature of fatty acid esters in the reaction mixture. Similarly in the reduction by stigmasterol which has a *cis* double bond at 22 position, the value of *trans* percents may not show the amount of *trans* double bonds of the esters, because the isomerization of *cis* bond of this steroid to *trans* bond cannot be denied.

Various natural products described earlier, were examined as hydrogen sources in the reduction of linoeate catalyzed by $RuH_2(PPh_3)_4$, and the results are shown in Table 6 and Table 7. The catalytic activity of this catalyst was higher than that of $RhH(PPh_3)_4$ except for the reduction by borneol, menthol, and lanosterol. Hydrogen-donating ability of steroids was high and the conversion in the reaction of some steroids was almost100 % under the reaction condition described previously. To clarfy the order of the hydrogen-giving power of these hydrogen donors and to raise the selectivity to monoenes by lowing the conversion, the reactions in which the concentration of these steroids was reduced to 0.2 M were also carried out.

Table 6. Homogeneous
Transfer Hydrogenation of Methyl Linoleate Catalyzed by $RuH_2(PPh_3)_4$[a]

Hydrogen donor	Yield of products[b] (%)			Conv.[c] (%)	Select.[d] (%)	Trans.[e] (%)	
	S	M	CD			A	B
β-Cholestanol	30	70	0	100	70	24	35
Stigumasterol	20	80	0	100	80	60	75
Ergosterol	12	88	0	100	88	201	228
Testosterone	11	87	2	98	89	33	38
Cholesterol	25	72	2	97	74	17	23
Stanolone	34	61	0	94	64	31	43
Andorosterone	39	52	7	91	57	34	54
β-Sitosterol	10	80	0	90	89	49	49
Borneol	11	73	4	84	87	40	42
Lanosterol	0	74	14	74	100	34	35
Methyltestosterone	0	61	28	61	100	11	13
D-Limonene	1	56	31	57	98	9	23
Hydrocortisone	0	47	41	47	100	Trace	
β-Citronellol	2	35	63	37	95	25	72
Menthol	3	29	57	32	91	38	74
Nicotine	0	32	58	32	100	Trace	
Geraniol	0	24	66	24	100	8	17

[a-e]Notations in this table are the same as those in the previous table.

Table 7. Homogeneous
Transfer Hydrogenation of Methyl Linoleate Catalyzed by $RuH_2(PPh_3)_4$[a]

Hydrogen donor	Yield of products[b] (%)			Conv.[c]	Select.[d]	Trans[e] (%)	
	S	M	CD	(%)	(%)	A	B
β-Cholestanol	39	38	13	77	49	32	35
Stigmasterol	38	37	17	75	49	74	141
Ergosterol	24	33	17	58	58	116	137
Testosterone	8	56	31	64	87	18	27
Cholesterol	32	39	22	71	54	14	24

[a]The designated donor (0.2 M) and linoleate (0.2 M) were heated in toluene in the presence of $RuH_2(PPh_3)_4$ (20 mM) at 140℃ for 3hr.
[b-e]Notations in this table are the same as those in the preceding table.

Table 8. Hydrogen-Donating Ability in the Homogeneous Transfer Hydrogenation of Linoleate Catalyzed by RuH_2 $(PPh_3)_4$

β-Cholestanol ≈ stigmasterol > ergosterol > testosterone ≈ cholesterol > stanolone > androsterone ≈ β-sitosterol > borneol > lanosterol > methyltestosterone > *D*-limonene > hydrocortizone > β-citronellol > menthol ≈ nicotine > geraniol.

In Table 8, the hydrogen-donating ability of the natural products examined is shown, which is estimated from the conversion expressed in the preceding tables. Abietic acid hardly reduced linoleate. The selectivity to monoenes was not so good in the reactions in which conversion was higher than 74 %. The lowering of the concentration of hydrogen donors such as β-cholestanol, stigmasterol, ergosterol, testosterone, and cholesterol made unexpectedly the selectivity worse in most cases, as shown in Table 7.

The values of *trans* percents were also not improved by the reduction of the amount of the steroids described above. The abnormal values of *trans* contents in the case of stigmasterol and ergosterol can be explained by the double bond situated at 22-position of these steroids which is *trans* or can isomerize to *trans* geometry, as mentioned before.

Perhaps, it may be worth noting that moderate conversion, complete selectivity, and low *trans* percents were realized in the reduction by methyltestosterone and hydrocortizone.

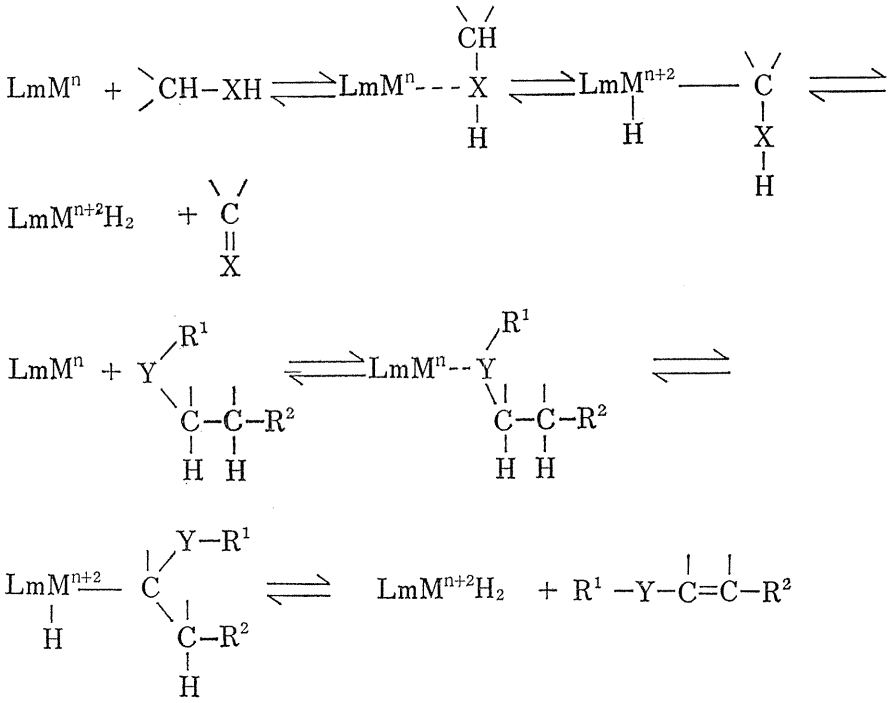
The kinetic studies on the homogeneous transfer hydrogenation of a variety of olefins were carried out and the reaction mechanism was proposed.[133,134,138~140,143] In the case of using cycloolefin as a hydrogen acceptor, it has been reported on the basis of the reaction kinetics that the coordination of hydrogen donor to the homogeneous catalyst proceeds prior to that of the hydrogen acceptor. The observed kinetic isotope effect distinctly shows that a hydrogen transfer step is rate-determining. The hydrogen donor coordinates to the complex catalyst at a heteroatom, and then makes a oxidative addition to it at the C-H bond adjacent to the heteroatom, followed by a reductive elimination.

Discussion

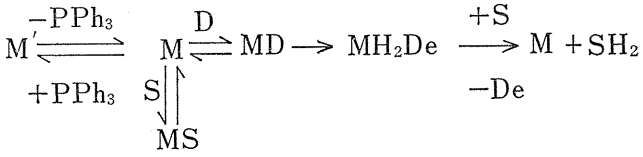
The mechanism for the homogeneous transfer hydrogenation of olefins is summarized as shown in Scheme 1.

In the case of using unsaturated fatty acids esters as hydrogen acceptors, the olefins mentioned above may be substituted by the esters. But in the geometric and position isomers of the unsaturated esters, the rate of their hydrogenations is

Scheme 1. Reaction Mechanism of Homogeneous Transfer Hydrogenation



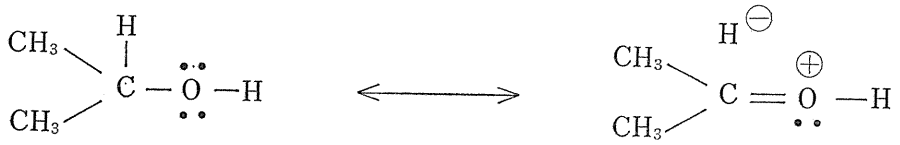
M^n : n-valent transition metal; Lm : ligand; X : $O<$, $-N<^{R}$; Y : $>C=O$, $H>C=C<^{H}$, $O<$, or $-N<^{R}$.



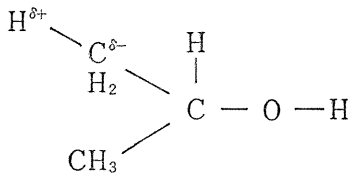
M′ : complex added; M : active species ; D : hydrogen donor ; De: dehydrogenated hydrogen donor ; S : olefin ; SH_2 : hydrogenated olefin.

different from each other, though the same catalyst and the same hydrogen donor are used.[157,159] This can be realized from the following points of view: (1) the total electron charge of the central transition metal should be zero, by Pauling's electroneutrality principle, except for the case that there are extreme difference among the electronegativities of atoms; (2) electrons of *cis*-double bonds have larger electron-donating ability than ones of *trans*-double bonds.[159]

In the case of using methyl linoleate as a hydrogen acceptor, isopropyl alcohol as a hydrogen donor, and $RuCl_2(PPh_3)_3$ as a catalyst, it has become practically possible that the selectivity is 100%, the conversion 68%, and the amount of isolated *trans* double bond almost zero; which is thought impossible.[157,159] The possible mechanism has been proposed as follows.[159] Isopropyl alcohol as a hydrogen donor has the following resonance hybrid owing to the lone pairs of oxygen.

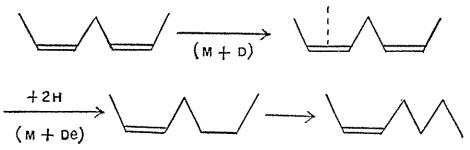


But the OH group has high electronegativity, so the electron desity for the hydrogen of the CH_3 groups in this case may be smaller than that for the hydrogen of usual CH_3 groups.



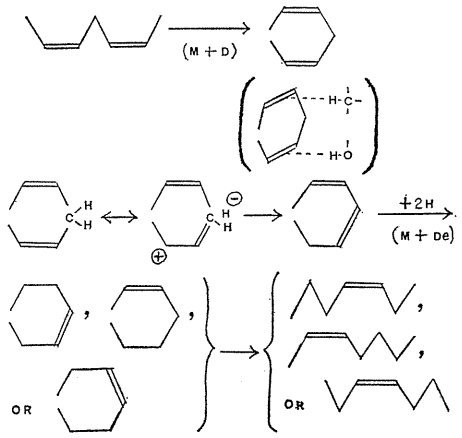
The selectivity in the case of using isopropyl alcohol as a hydrogen donor may be larger on account of donating two hydrogen atoms per a molecule.[155] This hydrogen donor coordinates to the complex catalyst by the lone pair of oxygen, the hydrogen acceptor also does, and then the hydrogen transfer may be carried out. At that time, the mutual affinity between the hydrogen of these methyl groups and π electrons of cis double bonds, which have electron donating ability, may operate. The affinity may act favorably for hydrogen transfer, and inhibit the formation of *trans* isomers from *cis* double bonds, as shown in scheme 2.

Scheme 2. Reaction Mechanism (I) of Obtaining cis-Monoene from Nonconjugated Diene



M : metal complex.
D : hydrogen donor.
De : dehydrogenated donor.

Scheme 3. Reaction Mechanism (II) of Obtaining cis-Monoene from Nonconjugated Diene



So, the selectivity is 100 % and the amount of isolated *trans* double is almost zero. In the case of methyl oleate, no hydrogen transfer occurs.[157,159] It may be realized by the lack of favorable conditions for hydrogen transfer mentioned above.

The electron density for the hydrogen of the OH group in isopropyl alcohol is small, and besides that for the hydrogen of the CH_3 groups also decreases mentioned before. That is, the both hydrogens may be large as $\delta+$ (especially the hydrogen of the OH group). π electrons of double bonds in *cis*, *cis* nonconjugated diene are electron-donating, so they have the affinity to the both hydrogens mentioned above, respectively, as given in Scheme 3.

Especially the hydrogen bond forms between a hydrogen of the OH group and π electrons of a double bond. Therefore, from the configuration between isopropyl alcohol and *cis*, *cis* nonconjugated diene, it is realized that the reaction shown in Scheme 3 may occur. So, the attainment of 100 % selectivity and a trace of isolated *trans* double bonds is easily explained. The reaction mechanism for other systems, in which 100 % selectivity and trace of isolated *trans* double bonds have been attained, is also similar.

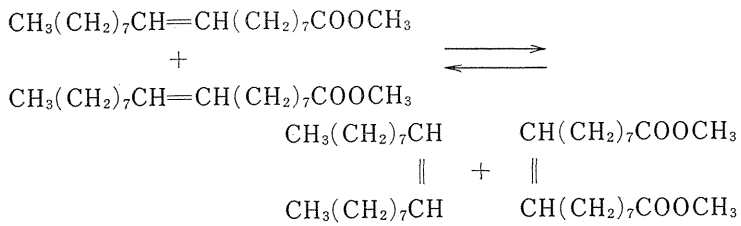
5. Fatty Acid Derivatives and Others[162~179,184~191]

Gas liquid chromatography for highly unsaturated fatty acid esters, such as methyl octadecatetraenoate, methyl eicosapentaenoate, and methyl docosahexaenoate, was investigated almost earliest.[162~164]

It was shown that the reaction of the side chain of heterocyclic compounds containing nitrogen, such as 1-(p-nitrophenyl)-2-(2-quinolyl) ethyl acetate, with carboxylic acids as catalysts proceeded. Elimination reactions of acetic acid from the compounds having quinolyl group as an electron-withdrawing group mentioned above took place easily, whose mechanism was proposed.[165~170] The elimination with carboxylic acid catalysts had never been reported.

The studies on the olefin metathesis reactions catalyzed by the WCl_6-cocatalyst

system were carried out.[171~179,184] The metathesis of olefins with functional groups had not been studied in detail. That of metyl oleate, a kind of these olefins, took place.[179] The metathesis of methyl oleate gives 9-octadecene and dimethyl 9-octadecenedioate.



The unsaturated dicarboxylic acid ester is significant as raw material for the preparation of unsaturated polyesters and polyamides, as well as for the synthesis of certain flavoring agents, from technological view points.[183] The reactivities of the WCl_6-cocatalyst systems which were found to be effective for the 2-heptene metathesis were examined in this reaction. Tetra-n-butyltin, tetraethyltin and triethylaluminum were found to be effective as cocatalysts with WCl_6 for this reaction. It is worth noting that nBu_4Sn is less toxic and easier to handle than Me_4Sn[180~183] and also less hazardous than Et_3B. In the WCl_6-Et_3Al system, the yield and the selectivity were low as compared to those in the WCl_6-Et_4Sn and WCl_6-nBu_4Sn systems, owing to the side reaction between methyl oleate and triethylaluminum. The WCl_6-Ph_4Sn, WCl_6-Ph_4Pb, and WCl_6-Ph_3Bi systems were inactive for this reaction under these reaction conditions. The presence of the considerable amount of ester groups may hinder the phenylation of WCl_6 by arylmetals and/or the reductive elimination of Ph and Cl from phenyltungsten intermediates. n-Butyl-lithium, lithium aluminum hydride, or sodium borohydride in conjunction with WCl_6 did not catalyze this reaction. Probably these reagents were consumed by the reactions with the ester. Diethylzinc did not form the active catalyst with WCl_6. On the other hand, the $WOCl_4$-nBu_4Sn system efficiently catalyzed this reaction.

The optimum reaction conditions are as follows. It is presumed that the optimum conditions for the metathesis of methyl oleate are different from those for the metathesis of alkene having no functional group, since methyl oleate contains ester group. The optimnm reaction conditions for this reaction were searched using WCl_6-nBu_4Sn.

In the reactions at 100℃, good yield was obtained in the extremely wide range of Sn/W ratio, *viz*: 2-8. This optimum range agrees with the one in the metathesis of 1-octene catalyzed by the WCl_6-$CH_3COOnPr$/nBu_4Sn system. The optimum methyl oleate/WCl_6 ratio was less than 30 under this reaction condition. The yield significantly decreased with an increase in the methyl oleate/WCl_6 ratio. The fairly low optimum olefin/WCl_6 ratio is attributable to the poisoning effect of ester group. A lowering in temperature decreased the yield intensively. This effect was observed more remarkably at the methyl oleate/WCl_6 ratio of 30 than at the ratio of 15. This result also shows that heating promotes the metathesis while the ester group inhibits this reaction. The best result was obtained at 100℃. The yield and

the selectivity both decreased at 120℃. Over-heating may induce the catalyst decomposition and the side reactions.

Effect of ester group of methyl oleate is described below. The addition of methyl oleate and methyl stearate provided a good metathesis yield by depressing the various side reactions such as double bond migration and oligomerization/ploymerization of olefins in the metathesis of 1-octene at elevated temperature, smilarly to the addition of alkyl acetates. These additives also depressed the Friedel-Crafts reaction in the 2-heptene metathesis in benzene at high temperature. These facts indicate that in spite of the high reaction temperature, the relatively high selectivity is obtained in the metathesis of methyl oleate, owing to the existence of its ester group. After all, it is concluded that the action of ester group in methyl oleate molecule is essentially the same as those of esters as additives.

References

1) K. Fukuzumi and S. Ozaki, J. Chem. Soc. Japan, Ind. Chem. Sec., **52**, 154 (1949).
2) K. Fukuzumi and S. Ozaki, J. Chem. Soc. Japan, Ind. Chem. Sec., **54**, 645 (1951).
3) K. Fukuzumi and S. Ozaki, J. Chem. Soc. Japan, Ind. Chem. Sec., **54**, 727 (1951).
4) K. Fukuzumi, S. Ozaki and Y. Yamada, J. Chem. Soc. Japan, Ind. Chem. Sec., **55**, 405 (1952).
5) K. Fukuzumi, S. Ozaki and Y. Yamada, J. Chem. Soc. Japan, Ind. Chem. Sec., **56**, 114 (1953).
6) K. Fukuzumi and M. Mizuta, J. Chem. Soc. Japan, Ind. Chem. Sec., **59**, 610 (1956).
7) K. Fukuzumi and Y. Koyama, J. Amer. Oil Chemists' Soc., **34**, 500 (1957).
8) K. Fukuzumi and Y. Koyama, J. Chem. Soc. Japan, Ind. Chem. Sec., **60**, 1455 (1957).
9) K. Fukuzumi and T. Hisatsune, J. Chem. Soc. Japan, Ind. Chem. Sec., **60**, 1458 (1957).
10) K. Fukuzumi and T. Hisatsune, J. Chem. Soc. Japan, Ind. Chem. Sec., **60**, 1460 (1957).
11) K. Fukuzumi, J. Chem. Soc. Japan, Ind. Chem. Sec., **57**, 448 (1954).
12) K. Fukuzumi and T. Hisatsune, J. Chem. Soc. Japan, Ind. Chem. Sec., **60**, 1462 (1957).
13) K. Fukuzumi and S. Ozaki, J. Chem. Soc. Japan, Ind. Chem. Sec., **51**, 56 (1948).
14) K. Fukuzumi, S. Ozaki and Y. Yamada, J. Chem. Soc. Japan, Ind. Chem. Sec., **54**, 728 (1951).
15) K. Fukuzumi and Y. Hirai, J. Chem. Soc. Japan, Ind. Chem. Sec., **55**, 403 (1952).
16) K. Fukuzumi, M. Mizuta and Y. Toyama, Bulletin of Industrial Research Institute of Nagoya, No. **7**, 36 (1954).
17) K. Fukuzumi, K. Ishiguro, K. Tachibana and Y. Toyama, Bulletin of Tooa Synthetic Chemical Co., **8**, 57 (1959).
18) K. Nishizawa and K. Fukuzumi, J. Soc. Chem. Ind., Japan, **49**, 191 (1946).
19) K. Fukuzumi, J. Oil Chemists' Soc., Japan, **1**, 181 (1952).
20) K. Fukuzumi, Memois Facul. Eng. University of Nagoya, **4**, 107 (1952).
21) K. Fukuzumi and Y. Koyama, J. Chem. Soc. Japan, Ind. Chem. Sec., **59**, 612 (1956).
22) K. Fukuzumi, J. Soc. Chem. Ind., Japan, **50**, 117 (1947).
23) K. Fukuzumi, J. Chem. Soc. Japan, Ind· Chem. Sec., **54**, 643 (1951).
24) R. T. Holman, "Progress in the Chemistry of Fats and Other Lipids", Vol. 9, W. O. Lundberg and P. Jarvi, "Peroxidation of Polyunsaturated Fatty Compounds", p. 379, 1971, Pergamon Perss, New York.
25) K. Fukuzumi and T. Wakita, J. Chem. Soc. Japan, Ind. Chem. Sec., **66**, 1846 (1963).
26) K. Fukuzumi, S. Ito and T. Hatachi, J. Japan Oil Chemists' Soc., **12**, 348 (1963).
27) K. Fukuzumi and I. Ando, J. Japan Oil Chemists' Soc., **12**, 351 (1963).
28) K. Fukuzumi, T. Miyakawa and H. Morohira, J. Chem. Soc. Japan, Ind. Chem. Sec.,

67, 2070 (1964).
29) K. Fukuzumi, T. Miyakawa and H. Morohira, J. Amer. Oil Chemists' Soc., **42**, 717 (1965).
30) K. Fukuzumi and Y. Ota, J. Japan Oil Chemists' Soc., **12**, 392 (1963).
31) K. Fukuzumi and K. Shibata, J. Japan Oil Chemists' Soc., **12**, 396 (1963).
32) K. Fukuzumi and T. Miyakawa, J. Chem. Soc. Japan, Ind. Chem. Sec., **66**, 1320 (1963).
33) K. Fukuzumi and K. Ishida, J. Chem. Soc. Japan, Ind. Chem. Sec., **67**, 324 (1964).
34) K. K. Fukuzumi and T. Maruyama, J. Chem. Soc. Japan, Ind. Chem. Sec., **68**, 308 (1965).
35) K. Fukuzumi, S. Ito and S. Nakanishi, J. Japan Oil Chemists' Soc., **12**, 89 (1963).
36) K. Fukuzumi, Y. Iwata and M. Takada, J. Chem. Soc. Japan, Ind. Chem. Sec., **66**, 1675 (1963).
37) K. Fukuzumi, J. Japan Oil Chemists, Soc., **10**, 143 (1961).
38) K. Fukuzumi, J. Japan Oil Chemists' Soc., **9**, 649 (1960).
39) J. Glavind, S. Hartmann, J. Clemmensen, K. E. Jessen and H. Dam, Acta Pathol. Microbiol. Scand., **30**, 1 (1952).
40) K. Fukuzumi and T. Tanaka, J. Japan Oil Chemists' Soc., **10**, 659 (1961).
41) K. Fukuzumi and Y. Iwata, J. Japan Oil Chemists' Soc., **12**, 93 (1963).
42) K. Fukuzumi, J. Japan Oil Chemists' Soc., **14**, 119 (1965).
43) K. Fukuzumi, Fette. Seifen. Anstrichmittel, **71**, 953 (1969).
44) K. Fukuzumi and T. Takagi, J. Japan Oil Chemists' Soc', **10**, 643 (1961).
45) K. Fukuzumi, Y. Iwata and K. Kawashima, J. Japan Oil Chemists' Soc., **12**, 165 (1963).
46) K. Fukuzumi, J. Japan Oil Chemists, Soc., **14**, 54 (1965).
47) K. Fukuzumi, Fette. Seifen. Anstrichmittel, **71**, 104 (1969).
48) K. Fukuzumi, Fette. Seifen. Anstrichmittel, **72**, 853 (1970).
49) K. Fukuzumi, llth World Congress of the International Society for Fat Research, Göteborg, Sweden, June, 1972.
50) K. Fukuzumi, llth International Cancer Congress, Florence, Italy, October, 1974.
51) V. L. Packer and J. Walton, Chemtech., **7**, 276 (1977).
52) B. N. Ames, W. E. Durston, E. Yamasaki and F. D. Lee, Proc. Nat. Acad. Sci U. S. A., **70**, 2281 (1973).
53) G. V. Abagyan and P. Yu. Butyagin, Biofizika, **10**, 763 (1965).
54) P. Alexander, J. J. Lett and M. G. Ormerod, Biochim. Biophys. Acta, **51**, 207 (1961).
55) J. W. Boag and A. Müller, Nature, **183**, 831 (1959).
56) C. Dorlet, A. vande Vorst and A. J. Bertinchamps, Nature, **194**, 767 (1962).
57) A. Ehrenberg, L. Ehrenberg and G. Löfroth, Nature, **200**, 376(1963).
58) V. P. Golikov, V. P. Trofimov and A. E. Kalmanson, Radiobiologiya, **9**, 346 (1969).
59) W. Gordy, Rad. Res., Suppl., **1**, 491 (1959).
60) J. N. Herak and V. Galogaza, Proc. Nat. Acad. Sci. U. S. A., **64** 8, (1969).
61) A. J Hoff and D. C. Konings-berger, Int. J. Radiat. Biol., **17**, 459 (1970).
62) G. Löfroth, L. Ehrenberg and A. Ehrenberg, Abhandl. Deut. Akad. Wiss. Berlin, Kl. Med., **1964**, 345.
63) J. E. Logan, N. A. Mannell and R. J. Rossiter, Biochem. J., **51**, 470 (1952).
64) M. G. Ormerod , Intern. J. Radiation Biol., **9**, 291 (1965).
65) Shen-Pei-Guen, L. A. Blumenfeld, A. E. Kalmanson and A. G. Pasynski, Biofizika, **4**, 263 (1959).
66) H. Shields and W. Gordy, Proc. Nat. Acad. Sci. U. S. A., **45**, 269 (1959).
67) A. van de Vorst and J. Duchesne, Compt. Rend., **257**, 1877 (1963).
68) A. van de Vorst and D. Krsmanovic-Simic, Compt. Rend., Ser.D., **262** , 2288 (1966).
69) A. van de Vorst, F. Villee and J. Duchesne, Intern. J. Radiation Biol., **9**, 269 (1965).
70) L. N. Ferguson, Chem. Soc. Rev., **4**, 289 (1975).
71) R. A. Passwater, Amer. Laboratory, **5**(6), 10 (1973).
72) H. M. Swartz, Adv. Cancer Res., **15**, 227 (1972).
73) R. J. Salloum and R. E. Eckert, J. Appl. Polym. Sci., **17**, 509 (1973).

74) K. L. De Vries, D. K. Roylance and M. L. Williams, Polym. Prepr. Amer. Chem. Soc., Div. Polym. Chem., **12**, 580 (1971).
75) K. Fukuzumi and N. Ikeda, J. Amer. Oil Chemists' Soc., **46**, 64 (1969).
76) K. Fukuzumi and N. Ikeda, J. Amer. Oil Chemists' Soc., **47**, 369 (1970).
77) K. Fukuzumi and N. Jkeda, J. Amer. Oil Chemists' Soc., **48**, 384 (1971).
78) K. Fukuzumi and N. Ikeda, Photosensitizing Dye, **1972**, No. 82, 1.
79) K. Fukuzumi and N. Ikeda, Reports of Sanyo Technology, **26**, 1 (1972).
80) K. Fukuzumi and E. Kobayashi, J. Amer. Oil Chemists' Soc., **49**, 162 (1972).
81) K. Fukuzumi, N. Ikeda and M. Egawa, J. Amer. Oil chemists, Soc., **53**, 623(1976).
82) N. Ikeda and K. Fukuzumi, J. Amer. Oil Chemists' Soc., **53**, 618 (1976).
83) N. Ikeda and K. Fukuzumi, J. Amer. Oil Chemists' Soc., **54**, 355 (1977).
84) N. Ikeda and K. Fukuzumi, J. Japan Oil Chemists' Soc., **26**, 212 (1977).
85) N. Ikeda and K. Fukuzumi, J. Japan Oil Chemists' Soc ., **26**, 343 (1977).
86) N. Ikeda and K. Fukuzumi, J. Japan Oil Chemists' Soc., **26**, 350 (1977).
87) N. Ikeda and K. Fukuzumi, J. Amer. Oil Chemists' Soc., **54**, 360 (1977).
88) K. Fukuzumi, N. Ikeda and K. Fujii, 14th World Congress of International Society for Fat Research, Brighton, England, September, 1978.
89) K. M. Wilbur, F. Bernheim and O. W. Shapiro, Arch. Biochem., **24**, 305 (1949).
90) S. Patton and G. W. Kurtz, J. Dairy Sci., **34**, 669 (1951).
91) S. Patton, M. Keeney and G. W. Kurtz, J. Amer. Oil Chemists' Soc., **28**, 391 (1951).
92) S. Patton and G. W. Kurtz, J. Dairy Sci., **38**, 901 (1955).
93) G. A. Jacobson, J. A. Kirkpatrick and H. E. Goff, Jr., J. Amer. Oil Chemists' Soc., **41**, 124 (1964).
94) R. Marcuse and L. Johansson, J. Amer. Oil Chemists' Soc., **50**, 387 (1973).
95) L. D. Saslaw, L. M. Corwin and V. S. Waravdebar, Arch. Biochem. Biophys. **114**, 61 (1966).
96) R. O. Sinnhuber and T. C. Yu, J. Japan Oil Chemists', Soc., **26**, 259 (1977).
97) C. B. Kenaston, K. M. Wilbur, A. Ottolenghi and F. Bernheim, J. Amer. Oil Chemists' Soc., **32**, 33 (1955).
98) L.K. Dahle, E. D. Hill and R. T. Holman, Arch. Biochem. Biophys., **98**, 253 (1962).
99) T. C. Yu and R. O. Sinnhuber, J. Amer. Oil Chemists' Soc., **44**, 256 (1967).
100) A. J. Koning aud M. H. Silk, J. Amer. Oil chemists' Soc., **40**, 165 (1963).
101) S. Satton, Food Res., **25**, 554 (1960).
102) N. Ikeda and K. Fukuzumi, J. Amer. Oil chemists' Soc., **51**, 340 (1974).
103) Y. Iwata and K. Fukuzumi, Reports of Higher Industrial Technical School of Toyota, **1973**, No. 6, 73.
104) K. Fukuzumi and T. Go, J. Chem. Soc. Japan, Ind. Chem. Sec., **69**, 260 (1966).
105) S. Nanya and K. Fukuzumi, J. Chem. Soc. Japan, Ind. Chem. Sec., **72**, 589 (1969).
106) N. Ikeda and K. Fukuzumi, J. Japan Oil Chemists' Soc., **27**, 21 (1978).
197) N. Ikeda and K. Fukuzumi, J. Japan Oil Chemists' Soc., **27**, 26 (1978).
108) N. Ikeda and K. Fukuzumi, J. Japan Oil Chemists' Soc., **27**, 33 (1978).
109) N. Ikeda and K. Fukuzumi, J. Amer. Oil Chemists' Soc., **54**, 105 (1977).
110) K. Fukuzumi and T. Yatsuo, J. Chem. Soc. Japan, Ind. Chem. Sec., **66**, 1324 (1963).
111) K. Fukuzumi, Y. Iwata, and M. Suzuki, J. Chem. Soc. Japan, Ind. Chem. Sec., **67**, 919 (1964).
112) K. Fukuzumi and T. Miyakawa, J. Chem. Soc. Japan, Ind. Chem. Sec., **67**, 2074 (1964).
113) K. Fukuzumi, S. Ito and H. Yamamoto, J. Chem. Soc. Japan, Ind. Chem. Sec., **67**, 1240 (1964).
114) K. Fukuzumi and K. Tomiyasu, J. Chem. Soc. Japan, Ind. Chem. Sec., **68**, 954 (1965).
115) K. Fukuzumi and H. Kato, IXth World Congress of the International Society for Fat Research, Rotterdam, the Netherlands, September, 1968.
116) S. Nanya, M. Hanai and K. Fukuzumi, J. Chem. Soc. Japan, Ind. Chem. Sec., **72**, 2005 (1969).
117) J. C. Bailar, Jr. and H. Itatani, J. Amer. Chem. Soc., **89**, 1592 (1967).

118) H. Itatani and J. C. Bailar, Jr., J. Amer. Chem. Soc., **89**, 1600 (1967).
119) H. Itatani and J. C. Bailar, Jr., J. Amer. Oil Chemists' Soc., **44**, 147 (1967).
120) G. Grezoio, G. F. Pregalia and R. Ugo, Inorg. Chim. Acta, **3**, 89 (1969).
121) I. S. Kelomnikov, I. L. Nakshunova, F. Prukhnik, N. A. Belikova and M. E. Vol' pin, Izv. Nauk USSR, Ser. Khim., **5**, 1180 (1972).
122) M. E. Vol' pin, V. P. Kukolev, V. O. Chernyshev and I. S. Kolomnikov, Tetrahedron Lett., 4435 (1971).
123) Y. Sasson and J. Blum, Tetrahedron Lett., 2167 (1971).
124) S. L. Regen and G. M. Whitesides, J. Org. Chem., **37**, 1832 (1972).
125) Y. Sasson and J. Blum, J. Org. Chem., **40**, 1887 (1975).
126) Y. Sasson and G. L. Rempel, Can. J. Chem., **52**, 3825 (1974).
127) J. Trocha-Grimshaw and H. B. Henbest, Chem. Commun., 544 (1967).
128) H. B. Henbest and J. Trocha-Grimshaw, J. Chem. Soc. Perkin I, 601 (1974).
129) R. Noyori, I. Umeda and T. Ishigami, J. Org. Chem., **37**, 1542 (1972).
130) T. Nishiguchi and K. Fukuzumi, Chem. Commun., 139 (1971).
131) T. Nishiguchi and K. Fukuzumi, Bull. Chem. Soc. Japan, **45**, 1656 (1972).
132) T. Nishiguchi and K. Fukuzumi, J. Amer. Chem. Soc. **94**, 8916 (1972),
133) H. Imai, T. Nishiguchi and K. Fukuzumi, J. Org. Chem., **39**, 1622 (1974).
134) T. Nishiguchi and K. Fukuzumi, J. Amer. Chem. Soc., **96**, 1893 (1974).
135) T. Nishiguchi, A. Kurooka and K. Fukuzumi, J. Org. Chem., **39**, 2403 (1974).
136) T. Nishiguchi and K. Fukuzumi, J. Organometal. Chem., **80**, C 42 (1974).
137) T. Nishiguchi, K. Tachi and K. Fukuzumi, J. Org. Chem., **40**, 237 (1975).
138) T. Nishiguchi, K. Tachi and K. Fukuzumi, J. Org. Chem., **40**, 240 (1975).
139) H. Imai, T. Nishiguchi, M. Kobayashi and K. Fukuzumi, Bull. Chem. Soc. Japan, **48**, 1585 (1975).
140) H. Imai, T. Nishiguchi and K. Fukuzumi, Chem. Lett., 807 (1975).
141) T. Nishiguchi, H. Imai and K. Fukuzumi, J. Catal., **39**, 375 (1975).
142) T. Nishiguchi, H. Imai, K. Hirose and K. Fukuzumi, J. Catal., **41**, 249 (1976).
143) H. Imai, T. Nishiguchi and K. Fukuzumi, J. Org. Chem., **41**, 665 (1976).
144) H. Imai, T. Nishiguchi and K. Fukuzumi, J. Org. Chem., **41**, 2688 (1976).
145) H. Imai, T. Nishiguchi and K. Fukuzumi, J. Org. Chem., **42**, 431 (1977).
146) H. Imai, T. Nishiguchi and K. Fukuzumi, Chem. Lett., 655 (1976).
147) H. Imai, T. Nishiguchi, M. Tanaka and K. Fukuzumi, Chem. Lett., 855 (1976).
148) H. Imai, T. Nishiguchi, M. Tanaka and K. Fukuzumi, J. Org. Chem., **42**, 2309 (1977).
149) T. Nishiguchi, H. Imai and K. Fukuzumi, Chem. Lett., 1113 (1977).
150) T. Nishiguchi, H. Sakakibara and K. Fukuzumi, Chem. Lett., 649 (1976).
151) T. Nishiguchi, A. Ohki, H. Sakakibara and K. Fukuzumi, J. Org. Dhem., **43**, 2803 (1978).
152) T. Nishiguchi, K. Tanaka and K. Fukuzumi, Transition Met. Chem., **3**, 191 (1978).
153) T. Nishiguchi, K. Tanaka and K. Fukuzumi, J. Org. Chem., **43**, 2968 (1978).
154) K. Fukuzumi, T. Nishiguchi, T. Tagawa and H. Imai, J. Japan Oil Chemists' Soc., **25**, 164 (1976).
155) K. Fukuzumi, T. Nishiguchi, T. Tagawa and H. Imai, Bull. ASAHI GARASU-Industrial Technological Association, **28**, 309 (1976).
156) T. Nishiguchi, T. Tagawa, H. Imai and K. Fukuzumi, J. Amer. Oil Chemists' Soc., **54**, 144 (1977).
157) T. Tagawa, T. Nishiguchi and K. Fukuzumi, J. Japan Oil Chemists' Soc., **27**, 70 (1978).
158) T. Tagawa, T. Nishiguchi and K. Fukuzumi, J. Amer. Oil Chemists' Soc., **55**, 332 (1978).
159) K. Fukuzumi, T. Nishiguchi and T. Tagawa, Bull. ASAHI GARASU-Industrial Technological Association, **31**, 351 (1977).
160) T. Nishiguchi, T. Tagawa and K. Fukuzumi, J. Japan Oil Chemists' Soc., **27**, 501 (1978).
161) K. Fukuzumi, T. Nishiguchi and T. Tagawa, 14th World Congress of the International Society for Fat Research, Brighton, England, September, 1978.

162) S. Ito and K. Fukuzumi, J. Chem. Soc. Japan, Ind. Chem. Sec., **65**, 1963 (1962).
163) S. Ito and K. Futuzumi, J. Japan Oil Chemists' Soc., **12**, 272 (1963).
164) S. Ito and K. Fukuzumi, J. Japan Oil Chemists' Soc., **12**, 278 (1963).
165) H. Hirata and K. Fukuzumi, NIPPON KAGAKU KAISHI, **1974**, 1929.
166) H. Hirata, H. Tanaka and K. Fukuzumi, NIPPON KAGAKU KAISHI, **1975**, 1004.
167) H. Hirata, K. Imaeda, H. Tanaka and K. Fukuzumi, J. Japan Oil Chemists, Soc., **24**, 246 (1975).
168) H. Hirata, M. Hayakawa and K. Fukuzumi, NIPPON KAGAKU KAISHI, **1976**, 921.
169) H. Hirata and K. Fukuzumi, J. Japan Oil Chemists' Soc., **25**, 207 (1976).
170) H. Hirata and k. Fukuzumi, J. Japan Oil Chemists' Soc., **25** 617 (1976).
171) T. Takagi, T. Hamaguchi and K. Fukuzumi, Chem. Commun., 838 (1972).
172) T. Takagi, K. Ichikawa, T. Hamaguchi, K. Fukuzumi and M. Aoyama, J. Japan Oil Chemists' Soc., **24**, 377 (1975).
173) T. Takagi. K. Ichikawa, K. Fukuzumi and T. Hamaguchi, J. Japan Oil Chemists' Soc., **24**, 518 (1975).
174) K. Ichikawa, T. Takagi and K. Fukuzumi, Bull. Chem. Soc. Japan, **49**' 750 (1976).
175) K. Ichikawa, T. Takagi and K. Fukuzumi, J. Japan Oil Chemists' Soc., **25**, 136 (1976).
176) K. Ichikawa, T. Takagi and K. Fukuzumi Transition Met. Chem., **1**, 54 (1976).
177) K. Ichikawa and K. Fukuzumi, J. Org. Chem., **41**, 2633 (1976).
178) K. Ichikawa, O. Watanabe, T. Takagi and K. Fukuzumi, J. Catal., **44**, 416 (1976).
179) K. Ichikawa and K. Fukuzumi. J. Japan Oil Chemists' Soc., **25**, 779 (1976).
180) P. B. van Dam, M. C. Mittelmeijer and C. Boelhouwer, Chem. Commun., 1221 (1972).
181) E. Verkuijlen and C. Boelhouwer, Chem. Commun., 793 (1974).
182) P. B. van Dam, M. C. Mittelmeijer and C. Boelhouwer, J. Amer. Oil Chemists' Soc., **51**, 389 (1974).
183) P. B. van Dam, M. C. Mittelmeijer and C. Boelhouwer, Fette. Seifen. Anstrichmittel, **76**, 264 (1974).
184) K. Ichikawa, O. Watanabe and K. Fukuzumi, Transition Met. Chem., **1**, 183 (1976).
185) K. Fukuzumi and T. Miyakawa, J. Chem. Soc. Japan, Ind. Chem. Sec., **67**, 2065 (1964).
186) T. Takagi and K. Fukuzumi, J. Japan Oil Chemists' Soc., **13**, 520 (1964).
187) T. Takagi, M. Kobayashi and K. Fukuzumi, J. Chem. Soc. Japan, Ind. Chem. Sec., **70**, 1117 (1967).
188) T. Takagi, M. Kobayashi and K. Fukuzumi, J. Japan Oil Chemists' Soc., **17**, 489 (1968).
189) T. Takagi, K. Fukuzumi and S. Nanya, J. Japan Oil Chemists' Soc., **18**, 193 (1969).
190) T. Takagi and K. Fukuzumi, J. Chem. Soc. Japan. Ind. Chem. Sec., **73**, 327 (1970).
191) T. Takagi, M. Yamazaki, K. Fukuzumi and Akiya, J. Japan Oil Chemists' Soc., **22**, 357 (1973).