Isomerization of Unsaturated Fatty Acids and their Esters with Acetic or Inorganic Acid

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It is generally accepted, that the isomerization of fatty materials includes the following events:

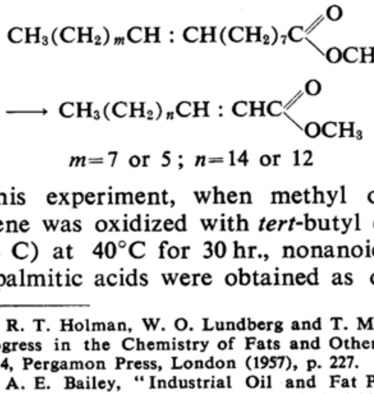
i) Formation of conjugated double bonds in the fatty molecule by heating with alkali; that gives rise to cis → trans isomerization[1].

ii) Shifting of double bonds during catalytic hydrogenation[2].

iii) Elaidinization with selenium or nitrous acid[3].

There are many literatures which describe the reaction of unsaturated fatty materials with concentrated sulfuric acid[4], and a synthesis of hydroxy acids or production of sulfated or sulfonated oils and of " iso-oleic " acid was also given. But no detailed description of analysis of " iso-oleic " acid was shown in previous literatures. Furthermore, it is not possible to find any information which refers to the reaction of unsaturated fatty materials with aqueous sulfuric acid solution at moderate temperatures.

Besides these matters the author[5] found the isomerization of oleate or palmitoleate of the following scheme, followed by oxidative fission with *tert*-butyl chromate (type C)*:



$$CH_3(CH_2)_mCH:CH(CH_2)_7C\begin{array}{l}{}^{\diagup\!\!\diagup}O\\{}_{\diagdown}OCH_3\end{array}$$

$$\longrightarrow CH_3(CH_2)_nCH:CHC\begin{array}{l}{}^{\diagup\!\!\diagup}O\\{}_{\diagdown}OCH_3\end{array}$$

$m=7$ or 5; $n=14$ or 12

In this experiment, when methyl oleate in benzene was oxidized with *tert*-butyl chromate (type C) at 40°C for 30 hr., nonanoic, azelaic and palmitic acids were obtained as oxidation products. On the other hand, it was found, that oleate in benzene/acetic acid or benzene/acetic acid/sulfuric acid was isomerized partly into 2-octadecenoate. In the oxidation of palmitoleate under almost the same condition as that of oleate, the author recognized the formation of myristic, lauric, capric, heptanoic and azelaic acid. From these experimental results, he proposed the β-oxidative action of *tert*-butyl chromate, accompanied by the isomerization shown in the above scheme.

It seems very noticeable that the isomerization of unsaturated fatty materials in acid medium proceeds even at moderate temperature. From this point of view, the action of acetic acid and inorganic acids, including aqueous sulfuric, phosphoric and boric acids, towards sesame oil fatty acid was studied. It was observed that the isomerization (conjugation) of triethylenic fatty acids of sesame oil constituents occurs by the treatment of above reagents except boric acid. The examination of ultraviolet absorption spectra of fatty acid was convenient for this purpose.

Experimental

Sample.—*Methyl Oleate*[6].—Commercial olive oil was saponified with alcoholic potash, and the mixed fatty acids obtained were treated by means of lead salt-alcohol method. The resulted liquid acid had an iodine value of 93.1, and a 10% solution of this acid in acetone was cooled to $-20°C$ and filtered. The cake of oleic acid was collected after maintaining the filtrate at $-60°C$. This acid (I. V. 90.1) was esterified in the usual manner, and the ester obtained was distilled under reduced pressure. Methyl oleate thus prepared had the following properties: b. p.$_5$ 180°C; d_4^{25} 0.8712; n_D^{25} 1.4469; acid value 0.17; saponification value 183.9; I. V. 86.4.

Ozonization of this oleate in chloroform gave no palmitic acid. This indicates the absence of 2-octadecenoate in the specimen.

Methyl Palmitoleate. — Sperm oil fatty acid was fractionated into solid and liquid acid by means of lead salt-alcohol method. The liquid acid obtained was esterified and the ester was distilled in vacuo. Methyl palmitoleate thus obtained had the following properties: b. p.$_4$ 160∼165°C; d_4^{25} 0.8748; n_D^{25} 1.4481; A. V. 3.45; S. V. 201.5; I. V. 86.1. Ozonolysis of this palmitoleate gave a complex mixture

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[1] R. T. Holman, W. O. Lundberg and T. Malkin ed., "Progress in the Chemistry of Fats and Other Lipids", Vol. 4, Pergamon Press, London (1957), p. 227.

[2] A. E. Bailey, "Industrial Oil and Fat Products", 2nd ed., Interscience Publishers, New York (1951), p. 687.

[3] A. E. Bailey, ibid., p. 910.

[4] For instance, R. M. Koppenhoefer, *J. Am. Leather Chem. Assoc.*, **34**, 622 (1939); T. N. Mehta et al., *J. Indian Chem. Soc. Ind. & News Ed.*, **15**, 111 (1952); **16**, 81 (1953); **18**, 195 (1955); K. Nishizawa et al., *J. Chem. Soc. Japan, Ind. Chem. Sec.* (*Kogyo Kagaku Zasshi*), **34**, 836 (1927); **49**, 12 (1946).

[5] Y. Watanabe, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **80**, 310 (1959); ibid., **80**, 1187 (1959).

* *tert*-Butyl chromate (type C) was prepared according to the method of Oppenauer and Oberrauch (*Anal. asoc. quim. Argentina*, **37**, 247 (1949)).

[6] E. G. Ball ed., "Biochemical Preparations", Vol. 2, Wiley, New York (1952), p. 100.

of saturated monocarboxylic acids. Paper chromatographic analysis of the mixed acids, according to the method of Inouye et al.7) gave the result that the major components of this mixture were nonanoic and heptanoic acids, and the minor were myristic and capric (trace) acids. This means, therefore, that palmitoleate contains a small amount of oleate and a trace of myristate and caprate as contaminants. Ultraviolet and infrared absorption spectra of palmitoleate revealed, that there is neither conjugated diethylenic and more highly conjugated polyethylenic acid esters nor trans type acid esters in the sample.

Sesame Oil Fatty Acid. — Saponification of commercial sesame oil (d_4^{25} 0.9097; n_D^{25} 1.4718; A. V. 0.14; S. V. 187.7; I. V. 118.9; unsaponifiable matter 1.11%) with alcoholic potash, gave the mixed fatty acids. This had the following properties: d_4^{25} 0.8983; n_D^{25} 1.4627; A. V. 185.0; I. V. 120.0; content of conjugated diene 0.55%; conjugated triene 0%. Ultraviolet absorption spectrum of this mixed acids is shown in Fig. 1. The content of linoleic and linolenic acids in this sesame oil fatty acid, estimated by the A. O. C. S. standard method8), was 20.0 and 14.2%, respectively.

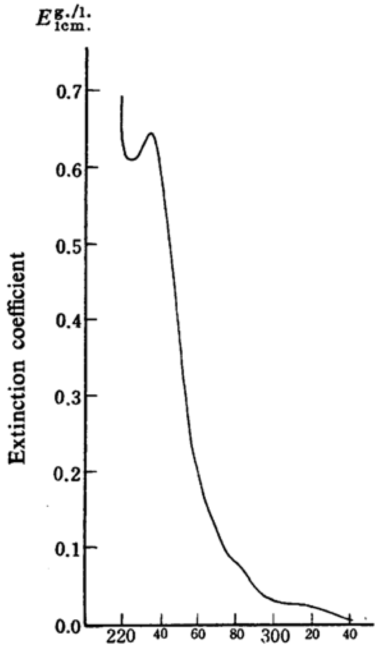

Fig. 1. U. V. absorption spectrum of sesame oil fatty acid.

Ultraviolet and Infrared Absorption Spectrum.— All ultraviolet and infrared absorption spectra mentioned in the present paper were measured by using Hilger's UVISPEK and H-800 spectrophotometer, respectively.

Purified ethyl alcohol was used as a solvent for ultraviolet spectroscopy instead of isoöctane.

Isomerization of Double Bond to α, β-Conjugated Position to Ester Carbonyl Group5).—*Methyl Oleate.* — Twenty grams of methyl oleate were added to the mixture of 90 g. of benzene, 36 g. of *tert*-butyl alcohol, 15 g. of acetic acid, and 3.0 g. of acetic anhydride, and the whole was kept at 40°C for 30 hr. with stirring. On the other hand, the mixture of 8 g. of oleate, 26 g. of benzene, 12 g. of acetic acid and 0.1 g. of concentrated sulfuric acid was kept at 70°C for 20 hr. with stirring. After each reaction time mentioned above was over, the reaction mixture was washed with water, 3% aqueous sodium bicarbonate solution, and finally again, water successively dried over anhydrous sodium sulfate, and a mixture of fatty esters was recovered after distilling off the volatile materials.

The fatty esters thus obtained were ozonized at 0°C using chloroform as a solvent. A mixture of monocarboxylic acids was obtained after the oxidative decomposition of the ozonized material, followed by extraction with petroleum ether. Paper chromatographic analyses of samples, derived from different isomerization condition were carried out using the same method as mentioned above. It was recognized that the chromatogram showed the presence of two kinds of fatty acid, namely nonanoic (major) and palmitic (minor) acids. When the mixtures of both of the monocarboxylic acids were fractionally crystallized from methyl alcohol at 0°C, an acid (m. p. 60~63°C) was precipitated in each case. This acid was identified as palmitic acid by the preparation of its *p*-bromophenacyl ester (m. p. 84~84.5°C) and mixed melting point determination with the known specimen.

Methyl Palmitoleate. — Eight grams of methyl palmitoleate were added to the mixture of 32 g. of benzene and 5.4 g. of acetic acid, and the whole was kept at 70°C for 30 hr. with stirring. The reaction product was treated in a similar way as in the case of oleate, and the result obtained was as follows. Monocarboxylic acids obtained were heptanoic, nonanoic, capric (trace) and myristic acids. The amount of myristic acid in this case seemed to be larger than that of the sample derived from the starting material (palmitoleate itself); because, the larger and more intense spot of myristic acid on the paper chromatogram was observed in the case of isomerized sample than in the case of the starting material although almost the same quantity of each sample was analyzed by means of paper chromatography mentioned above. However, the conclusive evidence, that the amount of myristic acid increased after isomerization followed by ozonization was not so remarkable.

Formation of Conjugated Triethylenic Acid in Sesame Oil Fatty Acid. — *Blank Test.* — Fifteen grams of sesame oil fatty acid and 80 g. of water in a flask provided with a gas-inlet tube, a stirrer, and a reflux condenser, were maintained at 90°C with constant stirring, under nitrogen atmosphere, for 3~30 hr. Contents of conjugated diene and triene in samples calculated from the equation shown in standard method were shown in Table I.

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7) Y. Inouye, O. Hirayama and M. Noda, *Bull. Agr. Chem. Soc. Japan*, **20**, 200 (1956); *J. Agr. Chem. Soc. Japan* (*Nippon Nogei-kagaku Kaishi*), **31**, 568 (1957).

8) American Oil Chemists' Soc., Tentative Method Cd 7-48; *J. Am. Oil Chemists' Soc.*, **35**, 593 (1958).

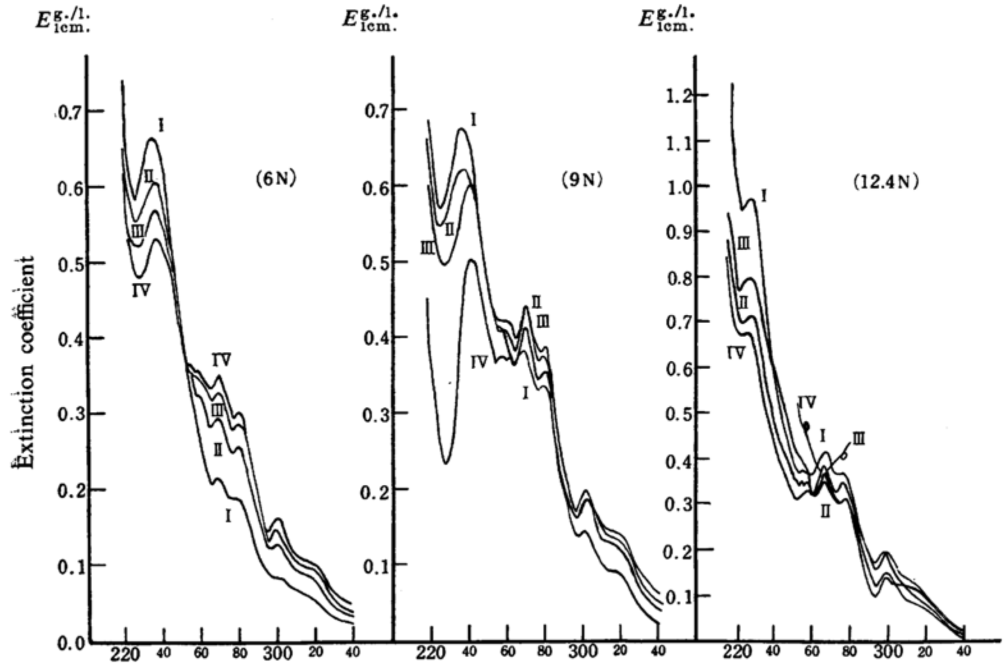

Fig. 2. U. V. absorption spectra of sesame oil fatty acid isomerized with aqueous sulfuric acid solutions. I 3 hr., II 10 hr., III 20 hr., IV 30 hr.

TABLE I. CONTS. OF CONJUGATED DIENE AND TRIENE (BLANK TEST)

Time, hr.	3	10	20	30
Diene, %	0.70	0.89	1.17	1.79
Triene, %	0	0	0.0026	0.0026

Aqueous Sulfuric Acid Solution.—The concentrations of sulfuric acid solutions used for isomerization were 6 N (d=1.18), 9 N (d=1.26) and 12.4 N (d=1.35), respectively. Each time, 30 g. of sesame oil fatty acid and 300 g. of aqueous sulfuric acid solution were treated similarly as in the case of the blank test. These isomerization experiments were carried out at 90°C. After each reaction time (Table II), fatty acid was taken out from the reaction vessel, and washed thoroughly with water and dried, and the ultraviolet absorption spectrum (Fig. 2) was measured, and the contents of diene and triene in it were calculated. The results were shown in Table II.

TABLE II. CONTS. OF CONJUGATED DIENE AND TRIENE (TREATMENT WITH SULFURIC ACID SOLUTION)

Time, hr.	Concn. of aq. H_2SO_4	3	10	20	30
Diene, %	6 N	0.54	0.48	0.45	0.42
	9 N	0.55	0.50	—	—
Triene, %	6 N	0.010	0.056	0.039	0.052
	9 N	0.053	0.062	0.086	0.085
	12.4 N	0.062	0.062	0.072	0.084

When sesame oil fatty acid was treated with 6 N sulfuric acid at room temperature for 30 hr., no remarkable change of the ultraviolet absorption spectrogram was observed.

Phosphoric Acid. — Phosphoric acid (300 g.) and sesame oil fatty acid (60 g.) were treated quite similarly as in the case of sulfuric acid solution at 90°C. In this case, remarkable coloration of phosphoric acid was observed during the reaction. After the reaction, the fatty acid was recovered similarly as in the case of sulfuric acid solution. The results obtained were shown in Table III. Ultraviolet absorption spectra of isomerized acids were shown in Fig. 3. Physical properties of recovered fatty acids were shown in Table IV.

TABLE III. CONTS. OF CONJUGATED DIENE AND TRIENE (TREATMENT WITH PHOSPHORIC ACID)

Time, hr.	3	6	10	15	20	30
Diene, %	0.61	0.65	0.62	0.76	0.61	0.71
Triene, %	0.0619	0.0711	0.0776	0.1066	0.0882	0.1040

TABLE IV. PHYSICAL PROPERTIES OF ISOMERIZED SESAME OIL FATTY ACID AFTER TREATMENT WITH PHOSPHORIC ACID AT 90°C

Time, hr.	d_4^{25}	n_D^{25}	A. V.	I. V.
3	0.8924	1.4636	185.1	117.2
15	0.8920	1.4632	186.6	118.9
30	0.8936	1.4630	188.8	117.3

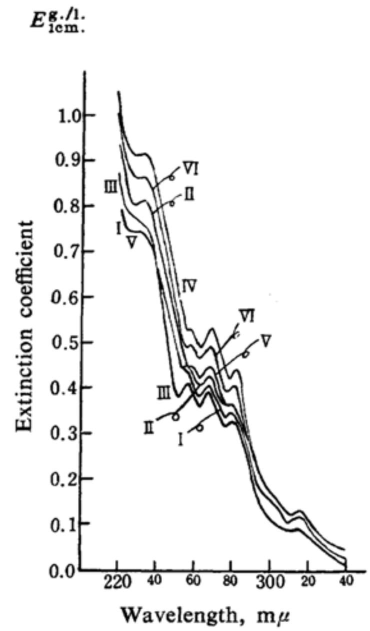

Fig. 3. U. V. absorption spectra of sesame oil fatty acid isomerized with phosphoric acid.
I 3 hr., II 6 hr., III 10 hr., IV 15 hr., V 20 hr., VI 30 hr.

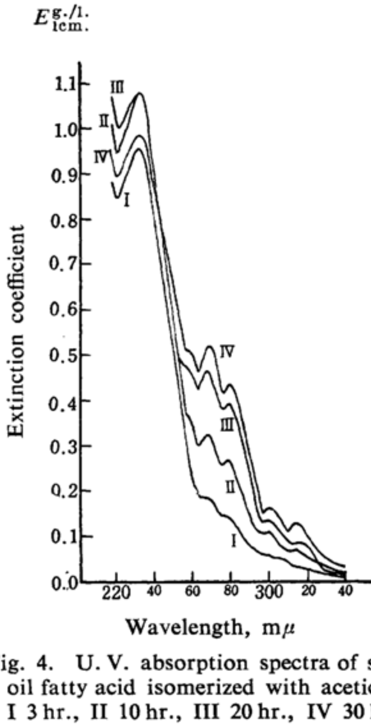

Fig. 4. U. V. absorption spectra of sesame oil fatty acid isomerized with acetic acid.
I 3 hr., II 10 hr., III 20 hr., IV 30 hr.

Glacial Acetic Acid. — Glacial acetic acid (300 g.) and sesame oil fatty acid (30 g.) were mixed and the solution was maintained at 90°C under nitrogen atmosphere, with stirring, for 30 hr. In the course of the reaction, 30 cc. portion of the solution was taken out, poured into water, extracted with ether and the ethereal solution was washed thoroughly with water, and dried over anhydrous sodium sulfate. After evaporation of the solvent, isomerized material was recovered, and its ultraviolet absorption spectrum was measured (Fig. 4). The contents of conjugated diene and triene calculated were shown in Table V.

TABLE V. CONTS. OF CONJUGATED DIENE AND TRIENE (TREATMENT WITH GLACIAL ACETIC ACID)

Time, hr.	3	10	20	30
Diene, %	0.78	0.88	0.88	0.81
Triene, %	0.0066	0.054	0.096	0.112

Treatment with Boric Acid. — At 90°C, sesame oil fatty acid (30 g.), boric acid (90 g.) and water (410 g.) were treated similarly as in the case of the blank test. Even after 30 hr., the fatty acid was so stable that the contents of conjugated diene and triene in it were essentially the same as in the case of the blank test.

Discussion

It will be noticeably demonstrated that the isomerization of unsaturated fatty materials occurs at moderate temperature (30~90°C) in the acid medium mentioned above. In the case of monoethylenic fatty esters, namely methyl oleate and palmitoleate, the double bond migrates to α, β-conjugated position to ester carbonyl group. Although the isolation of isoöleate or isopalmitoleate was unsuccessful, the formation of α, β-unsaturated esters was definitely supported by the identification of a shorter-chain saturated fatty acid, which was obtained after ozonolysis of isomerized products. The identification of palmitic and myristic acids has important meaning in this aspect. These facts support the β-oxidation mechanism of oleate and palmitoleate with *tert*-butyl chromate (type C).

In the case of sesame oil fatty acid, it is sure, that conjugation of triethylenic system in fatty acid molecule proceeds, when it was treated with acetic, phosphoric or aqueous sulfuric acid solution at 90°C. But there was no appreciable formation of a conjugated diethylenic system in such treatment, because the contents of conjugated diene of sesame oil fatty acid were almost constant throughout the whole various conditions. It is certain that the formation of conjugated triethylenic acid proceeds neither in the experiment of the blank test nor with the treatment with boric acid at 90°C. Also, the same result was obtained in the case of the treatment with 6 N sulfuric acid at room temperature. The smallest content found (0.0066%) of conjugated triene was obtained in the treatment of the fatty acid with acetic acid at 90°C for 3 hr. The highest content observed (0.112%) was obtained in the isomerization with acetic acid at 90°C for 30 hr. This value (0.112%) corresponded to 0.784% for linolenic acid present in sesame oil fatty acid.

According to Privett and Quackenbush[9], no phosphorylation occurred, when ethyl linoleate was treated with a catalytic amount of phosphoric acid (below 1%) in a sealed tube, under vacuum at 100°C for 36 hr. They have refered nothing to the isomerization of linoleate. However, in the case of the treatment of fatty acid with a large amount of phosphoric acid at 90°C described in the present paper, the author recognized the isomerization of fatty acid as above. But the physical properties of the isomerized products shown in Table IV seemed to be virtually constant, the same interpretation as for no phosphorylation can be relied on.

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[9] O. S. Privett and F. W. Quackenbush, *J. Am. Oil Chemists' Soc.*, 31, 225 (1954).