

# Bleaching of Vegetable Oils. II. Conversions of Methyl Oleate and Linoleate

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## ABSTRACT

Methyl oleate and linoleate were treated with 10% acid activated clay at 90-100 C for 1-50 hr with and without admission of air. Positional and geometric isomers of fatty acid esters were found. Polar compounds were detected having one or more functional groups with respect to the starting esters. Preparative thin layer chromatography and gas liquid chromatography were used in isolating the compounds, while IR, NMR, mass spectroscopy, and gas liquid chromatography analysis were employed for identification. The unsaturation of the isolated isomers was present at carbon atoms 5-11. The polar compounds were, among others, 9- and 10- keto octadecanoic methyl esters, isomeric keto octadecenoic methyl esters, isomeric epoxy octadecanoic methyl esters, 9- and 10-hydroxy octadecanoic methyl esters, and some mono methyl ester dicarboxylic acids. It may be concluded that geometric, as well as positional, isomerization occurs and that small amounts of compounds with one or more functional groups are formed when unsaturated fatty acids were treated with acid-activated clay.

## INTRODUCTION

A previous publication (1) described the optimal conditions under which conjugated compounds were formed in soybean oil, triolein and trilinolein during bleaching. The higher the percentage of bleaching earth is, the greater is the amount of conjugated compounds formed. In the present publication further experiments are described in which methyl oleate and methyl linoleate were used as standard compounds for the isolation and identification of the nonvolatile compounds formed during bleaching. Preparative thin layer chromatography (TLC) and gas liquid chromatography (GLC) were used for the isolation of the compounds; and IR, NMR, mass spectroscopy, and GLC were employed for the identification. For the determination of the position of the double bonds, ozonolysis was used in combination with GLC.

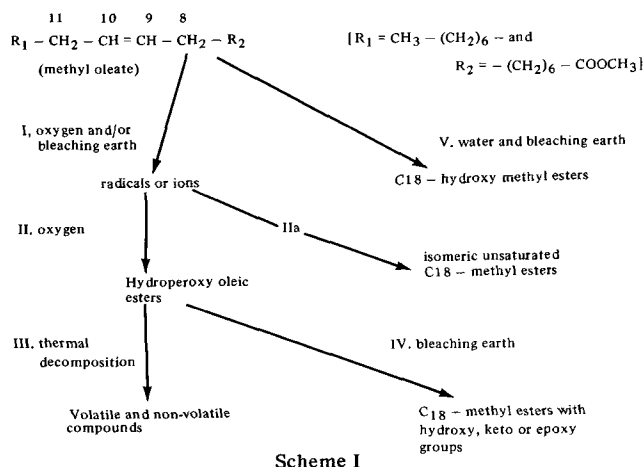
Position and geometric isomers of methyl oleate and linoleate respectively were observed, as well as polar compounds which have one or more functional group with respect to the starting esters. These compounds were present in the ester after bleaching or have been adsorbed by the bleaching earth.

## MECHANISM

During bleaching of oils containing unsaturated fatty acids, three different mechanisms for the formation of the compounds formed in the oils are possible: (a) oxidation of the unsaturated fatty acid esters by oxygen absorbed in the bleaching earth or dissolved in the oil; (b) reactions of the bleaching earth with the unsaturated fatty acid esters, and (c) reactions under the influence of the bleaching earth with the oxidized compounds formed.

Little is known about the reactions under the influence of the bleaching earth (b and c) (2, 3, 4). This is in contrast with the oxidation of the unsaturated fatty acids (a) (5-11).

With the aid of the oxidation reactions, a simplified reaction scheme (Scheme I) is given for the possible



formation of compounds during the treatment with bleaching earth with, for example, methyl oleate scheme. In Scheme I, reaction step I gives the origin of the expected compounds from the formation of radicals, positive or negative ions. The radicals or ions can produce isomeric unsaturated C<sub>18</sub>-methyl esters (step IIa). The radicals or ions also can react with oxygen via peroxide radicals or ions to the four corresponding hydroperoxides (8, 9, 10, and 11 hydroperoxy oleic ester, respectively, step II). Volatile and nonvolatile compounds may be formed from the hydroperoxides through thermal decomposition (step III). The bleaching earth can have a catalytic influence on this decomposition. Under influence of the bleaching earth, the hydroperoxides also can produce C<sub>18</sub>-esters with a hydroxy, keto, or epoxy group (2), i.e., through water elimination (step IV). Beside the formation of oxidized compounds via hydroperoxides, water addition to the double bond is possible with the formation of hydroxy compounds by the influence of the acid activated clay with the water absorbed in it (step V).

The formed hydroxy compounds also can be dehydrated to conjugated C<sub>18</sub>-methyl esters through the action of bleaching earth. In addition to the reactions mentioned, hydrogen transfer may take place. This may lead to a more unsaturated or saturated fatty acid ester. Hydrogen transfer has been observed at a higher temperature (220 C), besides the formation of dimers and trimers (12).

It also is to be expected that, with di- and tri-unsaturated fatty acid esters, more compounds may be formed, because the initial reactions take place more readily (formation of conjugated allyl radicals or ions) than with oleic methyl ester.

## EXPERIMENTAL PROCEDURES

### Materials

Methyl oleate was, according to GLC and TLC, 99.5% pure and contained 0.4% methyl linoleate. Methyl linoleate was 97.0% pure and contained 2.8% methyl oleate. Methyl oleate and methyl linoleate were obtained from J.B.A. Stroink, Unilever Research Laboratory, Vlaardingen, The Netherlands. Bleaching earth was an acid activated clay

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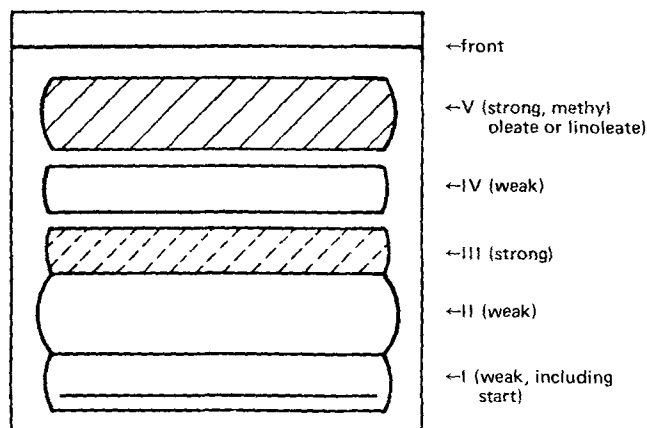


FIG. 1. Thin layer chromatography of the filtrate of methyl oleate and methyl linoleate, (ether-petroleum ether-acetic acid, 15:85:2).

(Tonsil Standard FF) of Südchemie at München, West Germany.

#### Bleaching experiments

The experiments were done in cylindrical, double-walled reaction vessels of 100 ml in the same manner as described in the previous publication (1). The whole reaction mixture was filtered in one run through a glass filter (Jena 3G3) in a cylindrical plexiglas enclosure applying a nitrogen pressure of 2-5 atmospheres.

The filtrate and a filter cake were obtained. The filter cake contained the oil, with compounds, which had been adsorbed in the bleaching earth and which was recovered through extraction with acetone (adsorbed fraction). The filtrate and the adsorbed fraction were maintained under nitrogen at 0 C before examination. Methyl oleate was treated with 10 wt% earth at 90 and 110 C with or without admission of air or under vacuum between 1-50 hr. Methyl linoleate was treated with 10 wt% earth at 90 C under admission of air for 50 hr.

#### Isolation and identification methods

**TLC.** TLC was carried out on glass plates (20 x 5 and 20 x 20 cm, respectively), coated with Silica Gel G-HR (Macherey, Nagel and Co.). If necessary, the silica gel contained 20% silver nitrate. Coat thickness was 0.3 mm for analytical purposes and 1.0 mm for preparative purposes. For preparative TLC, the mixture of compounds was deposited on the plates as an acetone solution (15-20%). After elution the compounds were made visible with an ethanol solution of 2,7-dichloro-fluorescein (0.2%) and viewed under a Camag Universal U.V. lamp. After prepara-

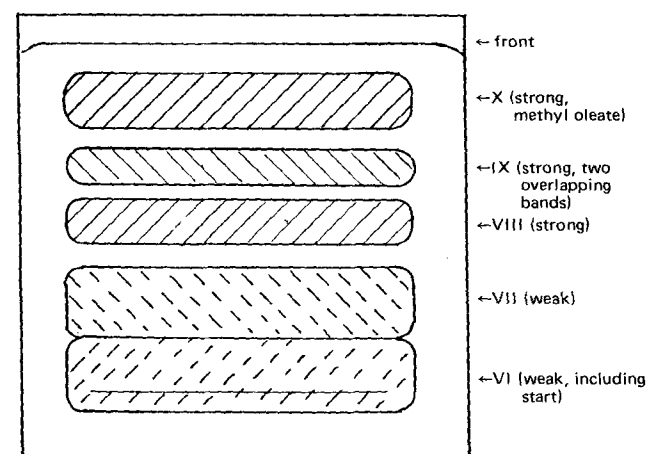


FIG. 2. Thin layer chromatography of adsorbed fraction of methyl oleate (ether-petroleum ether-acetic acid, 35:65:2).

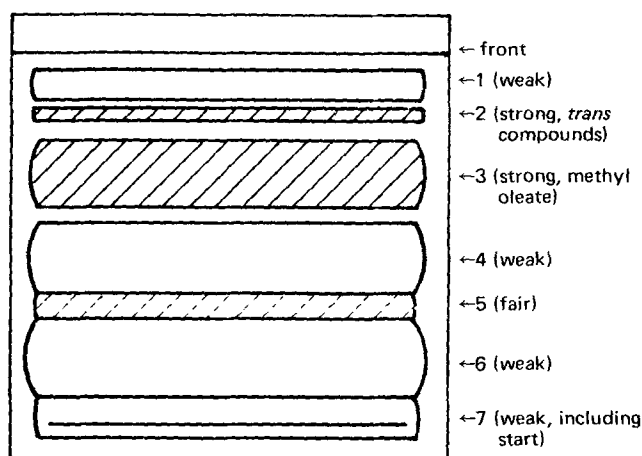


FIG. 3. Argentation thin layer chromatography of fraction V of the filtrate of bleached methyl oleate (benzene-petroleum ether, 9:1).

tive TLC the compounds, distributed over bands on the TLC (See Fig. 1-4), were scraped off the plates. The corresponding bands of several plates were put together, and the compounds present in these bands were isolated from the silica gel by extraction with distilled acetone. The acetone solutions of the extracted bands were concentrated to a few milliliters with a stream of nitrogen.

**GLC.** The GLC analyses were carried out with a Hewlett Packard 5750 chromatograph with a flame-ionization detector on stainless steel columns (2 m x 3 mm) containing the polar stationary phase, 10% polyethylene glycol adipate (PEGA) on Gaschrom Q, or the nonpolar stationary phase, 15% silicone polymer (SE-30) on Chromosorb W. The carrier gas was nitrogen. The relative retention times of the compounds in the fractions were determined and compared with methyl oleate. The isomeric fatty acids were, moreover, identified by comparing the retention times of their methyl esters with the retention times of known fatty acid methyl esters. Peak areas were measured with a planimeter and the amounts of the observed and isolated fatty acid methyl esters were calculated by comparing the area of the esters with that of a standard amount of methyl oleate.

Preparative GLC was carried out with the HP 5750 chromatograph, coupled with a preparative attachment (HP 5795 A), consisting of an injection unit, a collection unit, and a control unit. This attachment offers the possibility of automatically injecting and collecting up to six fractions. The stainless steel column (2 m x 6 mm) contained 20% SE-30 on Chromosorb W; helium flow 120 ml/min; split level 1/10. The collection traps were small vigreux traps. The traps were cooled with liquid nitrogen. Sometimes a

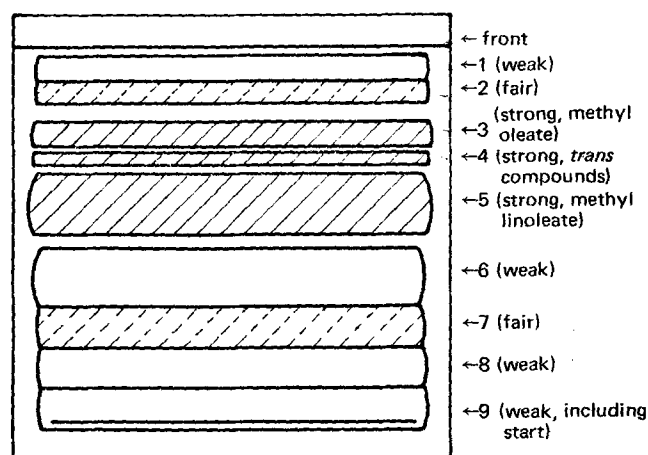


FIG. 4. Argentation thin layer chromatography of fraction V of the filtrate of bleached methyl linoleate (benzene-ether, 9:1).

TABLE I  
Isomeric Unsaturated Fatty Acid Methyl Esters Formed from  
Methyl Oleate and Characterized by Aldehydes  
Formed Upon Ozonolysis

Band number	Total (mg) <sup>a</sup>	Aldehydes and the amounts of the corresponding esters (mg) <sup>a</sup>						
		C <sub>5</sub> -	C <sub>6</sub> -	C <sub>7</sub> -	C <sub>8</sub> -	C <sub>9</sub> -	C <sub>10</sub> -	C <sub>11</sub> -
1	0.37 <sup>b</sup>	—	0.013	0.018	0.019	0.10	0.011	0.008
2	4.41 <sup>b</sup>	—	—	0.37	0.46	2.67	0.54	—
3	164.8	—	—	trace	trace	164.8	—	—
4	5.52 <sup>b</sup>	—	2.30	0.15	—	2.89	—	—
5	1.0	0.05	0.48	0.02	0.01	0.45	—	—
6	0.20	0.003	0.005	0.004	0.057	0.130	—	—
7	1.64	—	—	0.02	0.04	1.58	—	—
Total mg	178.0	0.05	2.80	0.58	0.59	171.8	0.55	0.008
Total %		0.03	1.57	0.33	0.33	96.5	0.31	0.004

<sup>a</sup>Calculated by gas liquid chromatography.

<sup>b</sup>Not fully unsaturated fatty acid ester, see Table IV.

part of the isolated compounds was not condensed through fog formation; in this case the traps were kept at 100 C. After isolation, the compounds were transferred from the traps with spectroscopic grade tetrachloromethane to small 1 ml test tubes 15 cm long, inside diameter 3 mm. From each isolated compound (preserved as CCl<sub>4</sub> solution), the purity and the quantity were determined with GLC.

*Ozonolysis in combination with GLC.* A technique of reductive ozonolysis, described by Beroza and Bierl (13), was applied for the determination of the position of the double bonds in the fatty acid methyl esters. In it, 25-100 μg fatty acid methyl ester was ozonized in 1 ml *n*-pentane at -40 - -70 C. The ozonides were reduced with triphenylphosphine to aldehydes, and possibly to dialdehydes, and aldehydic esters. The mixture was concentrated to 50-100 μl at room temperature in a small-sized test tube using a stream of nitrogen. Of this solution a sample was injected on the PEGA column of GLC. The column temperature was kept at 60, 70, 90 and 100 C, respectively for 15 min and then programmed with 8 C/min-185 C. The aldehydes formed during the ozonolysis were identified by comparing their retention times with the retention times of a mixture of known aldehydes (C<sub>5</sub> to C<sub>10</sub> aldehydes) at the isotherm part of the program. The amounts reported were based upon the total amount of the peak areas of the aldehydes formed and were corrected for the theoretical relative

sensitivity factors of the aldehydes according to van der Plank (14). The weights reported (Tables I and II) correspond with the fatty acid methyl esters from which the aldehydes were formed.

*IR spectra.* Spectra were obtained with a Hitachi EPI-G2 spectrometer. The measurements were carried with 0.5-1.0 mg of the compound in KBr micropellets (0.5 x 5 mm) with the aid of a beam condenser, according to a modification of the method of Widmark (15). The per cent unsaturation with *trans* configuration was determined with the formula: %T = K(A 10.36/A 8.55) - k (16), where %T = percentage *trans*-isomer, A 10.36 and A 8.55 are the absorbance values of the peaks at 10.36 (isolated *trans* band) and 8.55 μ (ester group), and K and k are constants found from the absorbance ratios of samples with known percentages (%T). The absorbances at 10.11 μ (conjugated *trans-trans*) and 10.55 μ (conjugated *cis-trans*) were used to determine the corresponding compounds. Minimum amount of observed compounds with *trans* configuration was 5%.

*UV spectra.* UV spectra in *n*-hexane (Merck) as solvent were recorded by a self-registering Cary-Model 14 spectrophotometer. Absorption maxima were measured at 225-235 nm (dienes) and 258-268 nm (trienes). The per cent of trienes was determined by comparing the UV absorption maximum at 258-268 nm with that of β-elaostearic acid

TABLE II  
Isomeric Unsaturated Fatty Acid Methyl Esters Formed from  
Methyl Linoleate and Characterized by Aldehydes  
Formed upon Ozonolysis

Band number	Total (mg) <sup>a</sup>	Aldehydes and the amounts of the corresponding esters (mg) <sup>a</sup>						
		C <sub>5</sub> -	C <sub>6</sub> -	C <sub>7</sub> -	C <sub>8</sub> -	C <sub>9</sub> -	C <sub>10</sub> -	C <sub>11</sub> -
1	0.05 <sup>b</sup>	—	—	—	—	—	—	—
2	0.93 <sup>c</sup>	—	0.08	0.09	0.005	0.32	0.06	0.005
3	11.47	—	trace	0.73	0.093	10.64	—	—
4	1.99	—	0.43	0.09	trace	1.40	0.07	—
5	411.5	trace	400.0	—	—	11.4	—	—
6	4.45	—	3.91	trace	trace	0.53	trace	—
7	0.71 <sup>d</sup>	trace	0.31	trace	trace	0.13	—	—
8	0.28	trace	0.03	0.003	0.002	0.24	0.003	—
9	0.28	—	0.06	—	—	0.019	0.03	—
Total mg	432.0	trace	401.8	0.91	0.10	24.65	0.16	0.005
Total %		trace	93.0	0.21	0.02	5.7	0.37	0.001

<sup>a</sup>Calculated by gas liquid chromatography.

<sup>b</sup>Too little to be ozonized.

<sup>c</sup>Not fully unsaturated fatty acid ester, see Table V.

<sup>d</sup>0.26 of possible unsaturated C<sub>10</sub> aldehyde present.

TABLE III  
Methyl Oleate and Methyl Linoleate Treated for 50 hr at 90 C  
with 10 wt% Bleaching Earth in Air

Characteristic data	Methyl oleate		Methyl linoleate	
	Before bleaching	After bleaching	Before bleaching	After bleaching
Percentage free fatty acids	0.045	3.8	0.014	2.4
100% E (U.V.) at $\lambda = 2200-2300$ 1 cm	510	1000	300	4700
100% E (U.V.) at $\lambda = 2650-2750$ 1 cm	20	700	55	2550
Percentage trienes	0.01	0.37	0.03	1.35
Peroxide value	25	52	48.1	198 <sup>a</sup>

<sup>a</sup>Corresponds with ca. 3.0% methyl linoleate hydroperoxide.

$E_{1\text{ cm}}^{100\%} = 187800, \lambda = 268\text{ nm}$ .

*NMR spectra.* NMR spectra were obtained with a Varian A-60 or A-100 M Hz spectrometer. The analyses were carried out with 1-15 mg of the compound in  $\text{CCl}_4$  solution.

*Mass spectra.* Mass spectra were measured with the AEI-MS 902 or AEI-MS 12 double focusing mass spectrometer. The ionizing current was 100 mA, the electron beam energy 70 eV, and the sample intake system was kept below 150 C.

*Peroxide values.* These were determined by modifying the AOCS method Cd 8-53 (17) and the percentage free fatty acids by the AOCS method Ca 5<sub>a</sub>-4<sub>a</sub> (18).

## RESULTS AND DISCUSSION

The experiments with methyl oleate and linoleate at 90 C during 50 hr under admission of air were used for the determination of the amounts of position and geometric isomers. The experiment with methyl oleate at 90 C during 50 hr under admission of air also was used for the determination of the polar compounds with one or more functional groups.

Some characteristic data, obtained from bleached methyl oleate and methyl linoleate after filtration, are shown in Table III. GLC spectra at isotherm and programmed temperatures on the PEGA (190 C and 80-190 C) and SE-30 (230 C and 100-300 C) columns showed peaks of many compounds and were complex. For these reasons, the TLC separations on silica gel were carried out first (Fig. 1

and 2) after which the separated fractions were studied in more detail using GLC on PEGA and SE-30 columns.

The retention times of the compounds in fractions V of the filtrate of methyl oleate and methyl linoleate, respectively, are equal, or not much different from known  $\text{C}_{18}$  methyl esters. This information and the  $R_f$ -value of the fraction on the TLC chromatogram point to the presence of esters other than the starting ester, which only differ in the number or place or configuration of the double bonds in the fatty acid chain.

Gas chromatograms of the fractions I-IV and VI-IX of filtrate and adsorbed fraction of methyl oleate show peaks of compounds which have shorter retention times than methyl oleate (volatile oxidized compounds). Most of the compounds present, however, have longer retention times, which point to compounds with polar groups.

*Position and geometric isomers of methyl oleate and linoleate.* Position and geometric isomers of methyl oleate and linoleate were separated by using TLC on silica gel/silver nitrate. A separation of the compounds was obtained according to the position and configuration (19-21). This TLC produced 7 bands for fraction V of methyl oleate (Fig. 3) and 9 bands of fraction V of methyl linoleate (Fig. 4). For the qualitative and quantitative analyses of the methyl esters in the extracted bands, GLC was carried out on the polar PEGA column at 185 C and on the nonpolar SE-30 column at 230 C. The amounts of the methyl esters with different fatty acid chain and number of double bands in the different bands of fraction V of methyl oleate and linoleate have been determined (see Tables IV and V).

TABLE IV  
Fatty Acid Methyl Esters in the Different Thin Layer  
Chromatography Bands of Fraction V of Methyl Oleate<sup>a</sup>

Band number	Esters (mg) in the bands					Total amount <sup>b</sup>	
	C <sub>16:0</sub>	C <sub>18:0</sub>	C <sub>18:1</sub>	C <sub>18:2</sub>	C <sub>18:3</sub>	mg	%
1	0.19	0.017	0.14	0.024	--	0.37	0.21
2	0.38	--	4.03	--	--	4.41	2.47
3	trace	--	164.8	--	--	164.8	92.0
4	0.20	--	4.25	1.07	trace	5.52	3.11
5	0.01	--	0.41	0.61	trace	1.02	0.66
6	0.02	--	0.18	--	trace	0.20	0.12
7	--	--	1.64	--	--	1.64	0.94
Total mg	0.83	0.017	175.53	1.76	trace	178	
Total %	0.47	0.01	98.2	0.99	trace		

<sup>a</sup>Figure 3.

<sup>b</sup>Calculated from gas liquid chromatography data.

TABLE V  
Fatty Acid Methyl Esters in the Different Thin Layer  
Chromatography Bands of Fraction V of Methyl Linoleate<sup>a</sup>

Band number	Esters (mg) in the bands					Total amount <sup>b</sup>	
	C <sub>16:0</sub>	C <sub>18:0</sub>	C <sub>18:2</sub>	C <sub>18:2</sub>	C <sub>18:3</sub>	mg	%
1	0.012	0.011	0.004	0.008	0.0095	0.05	0.01
2	0.27	0.11	0.29	0.045	0.21	0.93	0.22
3	—	—	11.47	—	—	11.47	2.66
4	—	—	0.39	1.48	—	1.88	0.44
5	—	—	—	411.5	—	411.5	95.4
6	—	—	—	4.45	—	4.45	1.03
7	—	—	—	0.71	—	0.71	0.16
8	—	—	—	0.14	0.15	0.29	0.06
9	—	—	0.03	0.25	—	0.28	0.06
Total mg	0.28	0.12	12.2	418.6	0.37	431.6	
Total %	0.06	0.03	2.8	96.9	0.03		

<sup>a</sup>Figure 4.

<sup>b</sup>Calculated from gas liquid chromatography data.

The position of the double bonds of the fatty acid esters in the different extracted bands was determined by ozonolysis, followed by GLC on the polar PEGA column. Tables I and II report the composition of the aldehydes in the different bands and the amounts of the corresponding fatty acid methyl esters which produced the aldehydes. In addition to the number and place of the double bonds in the fatty acid chain of the unsaturated methyl esters, the percentage of *trans* configuration of the double bonds was determined with IR spectroscopy. When bleaching methyl oleate, a *trans* percentage of 47% was found in band 2, of 13% in band 5, and of 6% in band 7 (10.36  $\mu$ , isolated *trans* bond). When bleaching methyl linoleate, *trans* percentages of 21% (10.18 and 10.55  $\mu$ , *cis-trans* bonds) and 13% (10.36  $\mu$ , isolated *trans* bond) are found in band 2 and 59% (10.36  $\mu$ , isolated *trans* bond) in band 4. In the other investigated bands (i.e., those with more than 0.7 mg compounds), no compounds with double bonds with *trans* configuration were observed.

*Polar compounds with one or more functional groups.* Polar compounds with one or more functional groups in the

fractions III and IV of the filtrate and in the fractions VIII and IX of the adsorbed fraction of methyl oleate, which are present in larger amounts, were isolated with preparative GLC on the nonpolar SE-30 column. Table VI shows the isolated and identified compounds of these fractions. The structure elucidation of these compounds was carried out by IR, NMR, and mass spectroscopy. These polar compounds are C<sub>18</sub> methyl esters with 1 or 2 hydroxy, keto, or epoxy groups or mono methyl ester dicarboxylic acids.

The preceding publication concerning the bleaching of vegetable oils reported the influence of different process parameters under which more or less conjugated and oxidized compounds are formed during bleaching. The bleaching was followed by determination of the peroxide value and the UV absorption. In addition to the isolation and identification of the polar compounds mentioned in Table VI, the influence of some process parameters on the formation of the identified polar compounds now has been determined. The process variables were temperature (90 and 110 C), bleaching time (1 and 50 hr at 90 C), and oxygen admission (with and without evacuation before and

TABLE VI

Isolated and Identified Compounds in Filtrate and Adsorbed Fraction of Methyl Oleate

	Nr	% <sup>a</sup>	R <sub>t</sub> <sup>b</sup>	Isolated compounds
Filtrate	IV-1	0.06	1.35	Four isomeric epoxy octadecanoic methyl esters (?)
	IV-2	0.3	1.74	9- and 10-keto octadecanoic methyl ester
	III-1	0.2	1.74	9- and 10-keto octadecanoic methyl ester
	III-2	0.25	2.1	Four isomeric keto octadecanoic methyl esters
	IX-1	0.001	1.4	Four isomeric epoxy octadecanoic methyl esters (?)
	IX-2	0.002	1.8	9- and 10-keto octadecanoic methyl esters
Adsorbed fraction	IX-3	0.003	2.2	Four isomeric keto octadecanoic methyl ester
	VIII-1	0.005	<0.1	Octanedioic acid mono methyl ester
	VIII-2	0.006	<0.1	Nonanedioic acid mono methyl ester
	VIII-3	0.005	<0.2	Decanedioic acid mono methyl ester
	VIII-4	0.002	<0.25	2-decene-1-carboxylic acid 10 methyl ester
	VIII-5	0.001	<0.5	2-undecene-1-carboxylic acid 11 methyl ester
	VIII-6	0.003	1.9-2.1	9- and 10-hydroxy octadecanoic methyl ester
VIII-7	0.004	≈4.0	Four isomeric diketo octadecanoic methyl esters	

<sup>a</sup>Percentages of the compounds in the filtrate and the adsorbed fraction calculated with respect to the total amount of original methyl oleate.

<sup>b</sup>Reported relative retention times determined on the SE-30 column, as compared with methyl oleate.

TABLE VII  
Comparison of Amounts of Polar Compounds Formed from Methyl Oleate during Bleaching at Different Process Parameters

Formed polar compounds	Relative retention times on SE-30 column	Filtrate						Adsorbed fraction						
		Experiment no. <sup>a</sup>						Experiment no.						
		0	1	2	3	4	5	6	1	2	3	4	5	6
mono keto octadecanoic esters	1.7-1.8	- <sup>b</sup>	++ <sup>c</sup>	++	= <sup>d</sup>	+ <sup>e</sup>	=	+	++	++	+	+	=	+
mono keto octadecenoic esters	2.1-2.2	-	++	++	++	++	+	++	++	++	++	++	+	++
mono hydroxy octadecanoic esters	1.9-2.1	-	++	++	++	++	+	++	++	++	++	++	+	++
mono epoxy octadecanoic esters (?)	1.3-1.4	=	++	++	=	+	+	=	++	++	=	+	=	+

<sup>a</sup>0 = unbleached methyl oleate, 1 = bleached methyl oleate at 90 C during 50 hr under admission of air, 2 = bleached methyl oleate at 90 C during 50 hr under nitrogen, 3 = bleached methyl oleate at 90 C during 1 hr under nitrogen, 4 = bleached methyl oleate at 90 C during 50 hr under nitrogen (bleaching earth and methyl oleate were evacuated a few times before being used), 5 = bleached methyl oleate at 90 C during 50 hr under vacuum, and 6 = bleached methyl oleate at 110 C during 1 hr under nitrogen.

<sup>b</sup>- = absent.

<sup>c</sup>++ = present; ca. same amount as in experiment 1.

<sup>d</sup>= = present in small amount (<30% of amount in experiment 1).

<sup>e</sup>+ = present in smaller amount (30-70% of amount in experiment 1).

during the experiment). This research has been done by fractionation of the filtrates and adsorbed fractions from these different experiments with preparative TLC in the manner previously described. The fractions so obtained were subjected to GLC on an SE-30 column. The relative retention times and amounts of some of the observed compounds formed were compared (Table VII). These data showed that the isolated compounds were formed in experiments with different process conditions.

When bleaching during short times under nitrogen and especially under evacuation, mostly smaller amounts of the isolated compounds were formed. From these data it can be concluded that probably the oxygen and water in the bleaching earth also react with the methyl oleate under the influence of the bleaching earth.

Research into the isolated and identified polar compounds and the position and geometric isomers also has been carried out in the same manner with an unbleached sample of methyl oleate. This experiment, with methyl oleate as an example, has been done to exclude the presence of the detected compounds in the observed amounts in the starting methyl oleate.

It was found that only a small part or none of the observed polar compounds and positional isomers were present in the starting methyl oleate. For the polar compounds see, for example, experiment o in Table VII. The percentages of the present geometric isomers were ca. 20% of the percentages found in the bleached methyl oleate.

The results showed that small amounts of C<sub>18</sub> methyl esters with one or two hydroxy, keto-, or epoxy-groups and some mono methyl ester dicarboxylic acids were formed from methyl oleate during bleaching at normal process temperatures (90-120 C) and reaction times (mostly 20-50 min.). These compounds were adsorbed partly by the bleaching earth, but the largest amounts remained in the bleached methyl oleate (see Table VI). Also isomerization took place when treating unsaturated fatty acid esters with acid activated clay during longer times at 90 C. The amounts were limited, and the esters with the double bond, beside that of the starting ester, were greater in relation to the other isomeric esters formed.

The observed position and geometric isomeric fatty acid esters arise through a shift of the double bonds along the

fatty acid chain by way of the fatty acid radicals or ions mentioned in the suggested scheme. The radicals or ions can, at the same time, be the first reaction steps towards the formation of the isolated oxidized compounds according to the given scheme. The observed conjugated and polyunsaturated fatty acid esters (Table III) can be explained through dehydration of the hydroxy fatty acid ester formed under influence of the bleaching earth. It also is possible that a small amount of hydrogen transfer contribute to the formation of the polyunsaturated esters, because a trace of saturated fatty acid esters was formed from methyl oleate, as well as from methyl linoleate.

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