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Periodate-Permanganate Oxidations for Determining Location and Amount of Unsaturation in Monounsaturated Fatty Acids[1]

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A MODIFIED METHOD is described for determining accurately the position of unsaturation in monounsaturated acids by oxidative cleavage. By combining Lemieux and von Rudloff's (11) periodate-permanganate oxidative cleavage method with Begemann's (3) chromatographic identification procedure, with slight modifications in technique, we have been able to obtain reproducible yields of the dibasic acids liberated on oxidative cleavage. Oleic acid and elaidic acid of high purity were employed in most of the experimental work. Over-all recovery of dibasic acids was about 92%, and control tests using high-purity azelaic acid instead of oleic acid indicate the quantitative reliability of the procedure employed by us. Isomeric impurities amounting to about 1% have been consistently detected. By replacing potassium carbonate with potassium hydroxide in the oxidation and by careful attention to experimental technique, we have been able to obtain a maximum of 92% dibasic acids from high-purity, monounsaturated fatty acid. Because of the importance of the determination of double-bond position in fat chemistry we have given more than the usual experimental detail in this paper so that anyone wishing to use the modified method will have necessary experimental details and yield data available.

Research in the field of isomerization, reduction, and conjugation of unsaturated fats, fatty acids, and esters has shown double-bond shift and has made it difficult to interpret the findings of various workers. Thus an accurate method for determining the position of unsaturation would be useful in research in this general field.

Armstrong and Hilditch (2) were among the first to employ potassium permanganate in dry acetone or acetic acid to oxidize unsaturated esters. After separation and identification of the acidic end-products they could fix the position of major unsaturation, but the exact amount of total unsaturation and the position of minor unsaturation remained doubtful, principally because of low yield, fragmentary products, and inadequate methods of separation of the acids formed.

Begemann in 1950 introduced partition chromatography for quantitative identification of the acids produced on oxidation with permanganate as well as by the ozonization technique (7). In 1953 Boelhower (4) employed Begemann's technique in an investigation of the catalytic hydrogenation of mono- and diunsaturated esters. He reported that partial reduction with nickel catalysts caused a predominant shift of double bonds away from the carboxyl group, but over-all yields were not given. Oxidation of the unreduced acids and the same acids processed with the catalyst in the absence of hydrogen rounded out a significant contribution.

Allen (1) employed ozonization to fix the position of unsaturation after partial hydrogenation of unsaturated fatty acids, coupled with the chromatographic separation techniques of Higuchi (8) and Corcoran (6). He found equal double-bond migration in both directions, explainable by partial hydrogenation and subsequent dehydrogenation. Lemieux and coworkers (11, 12, 14, 15) in 1955 proposed a novel method of oxidation applicable to mono- and diunsaturated fatty acids and esters in which a mixture of permanganate and sodium meta-periodate oxidizes the soaps in aqueous medium at room temperature. The novel feature of the method centers around the continuous regeneration of permanganate by the periodate under alkaline conditions (pH 7.5 to 9.0). After partial reduction of permanganate to manganate the latter is oxidized back to permanganate as shown by the following reaction:

$$2K_2MnO_4+NaIO_4+H_2O \rightleftarrows 2KMnO_4+NaIO_3+2KOH$$

The acidic products of oxidation were separated by Begemann's chromatographic method. Quantitative yields were reported for oleic acid, elaidic acid, 10-undecenoic acid, 9-eicosenoic acid, and linoleic acid. Less than quantitative yields were reported for methyl oleate, methyl linoleate, triolein, and erucic acid (13-docosenoic) by the addition of pyridine to the aqueous medium.

Experimental

A. Optimum Oxidizing Conditions. Oleic Acid. Upon duplicating the reaction conditions recommended by Lemieux and von Rudloff, the maximum recovery of azelaic acid was approximately 75%. Subsequently all experimental variables were investigated, but the indicated optimum conditions for oxidation did not materially increase the yield of

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TABLE I

Acids Oxidized

Acid	Iodine value	Neutral equivalent	Melting point
Oleic (A)	88.5	280.5	—
Oleic (B)	90.0	280.8	—
Oleic (C)	88.5	278.9	—
Elaidic from (A)	91.0	279.2	44
10-Undecenoic	131.4	196.5	—
9,10-Dihydroxystearic	0.9	315.0	96–98
Erucic	74.1	344.7	26–28
Vaccenic	67.2	305.5	45–46
Azelaic	0	94.4	106
Pelargonic	0	158.2	—

azelaic acid. These tests were carried out, using pure oleic acid (B in Table I) supplied by the Hormel Institute[2] of Austin, Minn. Analysis by the method of Brice (5) gave 99.37% monoethenoic acid, 0.07% diethenoic acid, and 0.60% saturates. Reagent-grade sodium meta-periodate was obtained from the G. F. Smith Chemical Co., Columbus, O. Other chemicals were C. P. and analytical grade. We established the following optimum conditions for oxidation, which are in general accord with those found by Lemieux and von Rudloff.

Temperature	25°C.
K_2CO_3	3.2 to 5.5 equivalents
$KMnO_4$	0.1 to 0.15 equivalents
$NaIO_4$	6.0 to 8.0 equivalents
Time	4 to 24 hrs.

We established moreover that the use of either sodium hydroxide or potassium hydroxide gave higher yields than potassium carbonate. When the latter was incorporated in the reaction mixture, the subsequent sulphur dioxide treatment (to destroy the oxidants) gave a solution containing some small, dark floating particles. These were not present when strong alkali was employed to form the soap of oleic acid.

When dilute potassium hydroxide (0.09 N) and not potassium carbonate was used to form potassium oleate, higher yields were obtained under optimum conditions. The amount of alkali required to attain optimum pH of 8.0 to 8.5 for regeneration of permanganate was found to be dependent on the equivalents of periodate employed. Seven equivalents of periodate solution at a pH of 5.2 required about two equivalents of alkali to raise the pH to 8.0.

B. Control Tests on Method and Technique. Utilizing optimum oxidizing conditions, we ran control tests by using pure end-products, azelaic acid with and without pelargonic acid, to ascertain if oxidation, subsequent handling techniques, and chromatographic separation caused a loss of azelaic acid. Azelaic acid was successively recrystallized from water and benzene. Pelargonic acid (98%) supplied by Emery Industries Inc., Cincinnati, O., was purified in a Podbielniak still. Neither showed impurities when analyzed by the Begemann chromatographic technique.

When azelaic acid alone was oxidized, processed, and chromatographed, three tests gave an average recovery of 98.8% (Table II). Subsequently a pelargonic acid-azelaic mixture was similarly processed, and a single test (Table II) showed azelaic acid recovery of 98.8%. The lower recovery of 89% for pelargonic was caused by loss due to its volatility; the present method was designed for dibasic acid recovery.

An additional control included the oxidation of a sample of 9,10-dihydroxystearic acid obtained from Arnold, Hoffman, and Company, Providence, R. I., which was recrystallized from a hexane-benzene mixture. Its chromatographic purity was not established. Four tests gave a total dibasic average recovery of 97.3%. Of this amount 2.0% was due to sebacic acid, either produced during oxidation or present as 10,11-dihydroxystearic acid impurity in the original acid.

C. Description of Processing Details. Oleic Acid. In order to explain the method and to clarify the calculations and data in Table III, the following description is given for the first test [first column under "Oleic Acid (A)"] with oleic acid. Introduce 157.9 mgm. (0.559 millimol) of oleic acid into 6 equivalents of alkali (37.3 ml. of 0.09 N potassium hydroxide) and add 200 ml. of water and shake well in a 500-ml. g.s. flask freed of oxygen with purified nitrogen. Mix in a separate container 7.9 equivalents of sodium meta-periodate (94.6 ml. of a solution containing 1 g./100 ml.) and 1 ml. of 0.1 M potassium permanganate. This solution at pH of 5.2 is added to the potassium oleate at pH of 12 and the total

TABLE II

Oxidation of 9,10-Dihydroxystearic Acid and Azelaic Acid and Azelaic-Pelargonic Mixture

	9,10-Dihydroxystearic acid					Azelaic acid				Azelaic-pelargonic acid mixture
Oxidation conditions										
M. mol acid × 1,000	463	451	459	472		498	493	588		987
Relative eq. KOH	4	4	5	5		1.1	1.1	1.3		1.3
Relative eq. $KMnO_4$ × 100	5	10	5	10		5	5	10		5
Relative eq. $NaIO_4$ × 10	40	40	50	50		39	39	39		39
Reaction time, hrs	8	8	8	8		24	4	4		4
Reaction vol. ml	250	250	250	250		250	250	250		250
Aliquot titer ml. × 1,000	1099	1075	1003	1106		849[a]	848[a]	950		963
Apparent degree reaction, %	95	97	83	92		—	—	—		—
Titer; column peaks, ml. × 1,000										
Monobasic acids	341	329	240	339		—	—	—		529
C_{10} dibasic acid	12	13	17	17		—	—	—		—
C_9 dibasic acid	712	716	711	737	Av.	837	834	947		422
Total	1065	1058	968	1093		—	—	—	Av.	951
Column recovery, %	97.0	98.5	96.5	98.8	97.7	98.5	98.3	99.7	98.8	98.8
Mol % recovery										
Monobasic acids	90.6	90.4	64.4	88.4	83.5	—	—	—	—	89.7
C_{10} dibasic acid	1.6	1.8	2.3	2.3	2.0	—	—	—	—	—
C_9 dibasic acid	94.4	95.2	95.4	96.0	95.3	98.7	98.8	98.9	98.8	98.8
Total dibasic acid	96.0	97.0	97.7	98.3	97.3	—	—	—	—	—
Total	186.6	187.4	162.1	186.7	180.8	98.7	98.8	98.9	98.8	188.5

[a] Titration with 0.234 N KOH instead of with 0.246 N KOH.

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[2] The mention in this article of commercial products or equipment under names of their manufacturers does not constitute endorsement by the U. S. Department of Agriculture of such firms or products.

TABLE III

Oxidation of Oleic Acid and Elaidic Acid

	Oleic acid (A)				Elaidic acid			
Oxidation conditions								
M. mol acid × 1,000	559	571	574		541	543	534	
Relative eq. KOH	6	5	5		7	6	5	
Relative eq. $KMNO_4$ × 100	10	15	10		10	10	10	
Relative eq. $NaIO_4$ × 10	79	79	79		79	79	79	
Reaction time, hrs.	10	4	4		20	20	20	
Reaction vol., ml.	500	250	250		500	500	500	
Aliquot titer ml. × 1,000	1275	1303	1410		1294	1279	1277	
Apparent degree reaction, %	89	90	94		90	92	94	
Titer; column peaks, ml. × 1,000								
Monobasic acids	414	425	475		424	414	432	
C_9 dibasic acid	809	835	857		832	835	817	
C_8 dibasic acid	9	16	15		15	9	11	
Total	1222	1276	1347	Av.	1271	1258	1260	Av.
Column recovery, %	96.8	98.3	95.6	96.9	98.3	98.3	98.8	98.5
Mol % recovery								
Monobasic acids	91.4	91.5	94.3	92.4	96.4	93.7	98.2	96.2
C_9 dibasic acid	89.4	90.0	90.3	89.9	94.7	94.6	94.7	94.7
C_8 dibasic acid	1.1	1.9	1.9	1.6	1.4	0.8	0.9	1.0
Total dibasic acid	90.5	91.9	92.2	91.5	96.1	95.4	95.6	95.7
Total	181.9	183.4	186.5	183.9	192.5	189.1	193.8	191.8

volume brought to 500 ml. Allow to stand 10 hrs. at room temperature when the pH will be about 8.7. Arrest the oxidation by adding 4–5 ml. of 5 N hydrochloric acid to make strongly acid and pass in gaseous sulfur dioxide until the free iodine color has disappeared. Add about 1 mg. of solid phenolphthalein and make alkaline with 5 N potassium hydroxide. Evaporate all but 20–25 ml. on a steam bath in a current of air. Quantitatively transfer to a separatory funnel and add 5 N hydrochloric acid to form the free acid. Saturate the solution with sodium chloride and extract with ethyl ether three successive times, with intermediate washing of each extract with 5–10 ml. of saturated salt solution. Dry with anhydrous sodium sulphate, and quantitatively separate the extract with an asbestos-prepared Gooch filter. Remove the ether at about 30°C. in a current of air to minimize the loss of pelargonic acid, while at the same time eliminating traces of hydrochloric acid. Previous workers recommend sulphuric acid addition before ether extraction, but it should be avoided as the trace amounts remaining in the ether are concentrated and have been shown to decrease dibasic acid yields. Evacuate in a desiccator, dissolve in the mobile phase, and transfer to a 25-ml. volumetric, preparatory to chromatographic separation in the manner disclosed by Begemann (3) and Boelhouwer (4).

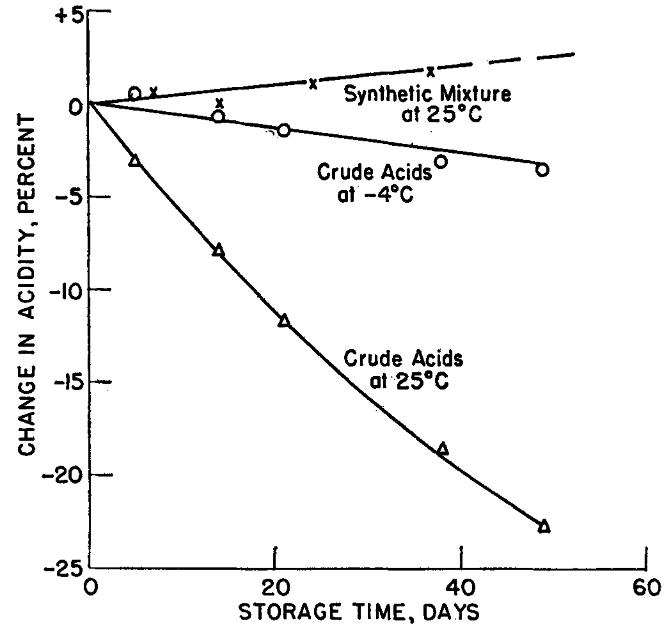

Fig. 1. Change in acidity on storage of acids resulting from the oxidation of oleic acid.

The sample should be kept at about 0°C. rather than at room temperature to minimize esterification of the dibasic acid by the methyl alcohol and ethyl alcohol present in the equilibrated, mobile, chromatographic liquid. The magnitude of this reaction was shown by tests conducted with a typical acidic product and with a synthetic mixture of pelargonic acid and azelaic acid. The percentage of change in titratable acidity on storage at 25°C. and at —4°C. may be seen in Figure 1.

An approximation of the extent of oxidation may be made by titration of a 5-ml. aliquot, provided care has been taken to reduce the loss of the relatively volatile pelargonic acid. Each mol of oleic acid oxidized should form one mol of pelargonic acid and one mol of azelaic acid corresponding to a two-fold increase in acidity. Titrations were conducted under nitrogen with a Gilmont microburette of 1-ml. capacity graduated to 0.001 ml. modified by the incorporation of a 1-ml. bulb in the ascending (right hand) side of the glass, inverted U tube. This modification expedited the titrations tremendously and permitted uninterrupted usage even when the confined mercury became dirty. Alcoholic thymol blue was selected as indicator.

If we assume no loss of pelargonic acid, titration of the 5-ml. aliquot of this sample with 0.246 N potassium hydroxide [first column under "Oleic Acid (A)," Table III] corresponded to 40.40 ml. per gram of original oleic acid for oxidation. The latter required 14.49 ml. per gram for neutralization of the 0.1579 g. employed.

$$\text{ml./g. KOH} = \frac{25/5 \times 1.275}{0.1579} = 40.40$$

Thus the increment of 25.91 ml. when divided by 28.98 ml. gave 89.4% for an approximation of the apparent degree of oxidation or percentage of theoretical yield.

$$\text{Approximate \% yield} = \frac{40.40 - 14.49}{2 \times 14.49} 100 = 89.4$$

Solvents used for chromatographic separation should be and were distilled before use. Chemical removal of

TABLE IV

Oxidation of Oleic Acid

	Oleic acid (B)				Oleic acid (C)			
Oxidation conditions								
M. mol acid × 1,000	495	528	522		521	534	528	
Relative eq. KOH	4.0	4.5	5.0		5	5	5	
Relative eq. $KMNO_4$ × 100	10	10	10		10	10	10	
Relative eq. $NaIO_4$ × 10	79	79	79		79	79	79	
Reaction time, hrs	5	5	5		11	11	11	
Reaction vol., ml	500	500	500		500	250	500	
Aliquot titer, ml. × 1,000	968	1159	1206		1152	1279	1225	
Apparent degree reaction, %	69	84	91		84	92	90	
Titer; column peaks, ml. × 1,000								
Monobasic acids	196	334	383		337	416	407	
C_{10} dibasic acid	13	12	11		—	—	—	
C_9 dibasic acid	729	783	765		764	807	785	
C_8 dibasic acid	—	—	—		9	12	9	
Total	938	1129	1159	Av.	1110	1235	1201	Av.
Column recovery, %	96.8	97.4	96.1	96.8	97.5	96.5	97.7	97.2
Mol % recovery								
Monobasic acids	48.7	77.8	90.0	72.2	79.6	93.7	93.6	88.9
C_{10} dibasic acid	1.6	1.4	1.3	1.4	—	—	—	—
C_9 dibasic acid	90.5	91.2	90.0	90.5	90.0	91.0	90.5	90.5
C_8 dibasic acid	—	—	—	—	1.2	1.3	1.1	1.2
Total dibasic acid	92.1	92.6	91.3	92.0	91.2	92.3	91.6	91.7
Total	140.8	170.4	181.3	164.2	171.0	186.0	185.2	180.5

aldehydes and acids from the alcohols and of thiophene from benzene was found unnecessary. Normal mobile and immobile phases were prepared by equilibrating 32 parts of methanol, 24 parts of ethanol, 24 parts of water, and 80 parts of benzene. However, when it was necessary to separate monobasic acid from the C_{12} dibasic acid (dodecane dianoic), we decreased the polarity of the mobile phase by increasing the methanol content and decreasing the ethanol content while at the same time increasing adsorbent height in the column. Conversely when C_8 dibasic acid (suberic) or C_7 dibasic acid (pimelic) were expected, they formed well-defined peaks by increasing the proportion of ethanol and decreasing the proportion of methanol in the mixture. Appropriate alteration of this mixture has enabled us to identify a mixture of monobasic acid containing dibasic acids from C_{13} to C_7 by using two columns.

The usual column was 14 in. long and made of 13–14 mm. internal diameter Pyrex tubing with a sealed-in fritted disk terminating in a smaller delivery tube constructed to deliver drops at the axial center. The top of the column terminated in a 150- to 200-ml. reservoir bulb with a 19 × 38-mm. ground fitting. General practice was to add 16 g. of silicic acid to 40 ml. of mobile phase in an Erlenmeyer flask and, while shaking the slurry, 4–6 ml. of immobile or water-rich phase were added. This mode of preparation of the adsorbent was found to be superior to mixing the dry silicic acid supplied by Mallinkrodt Chemical Works (chromatographic grade, 100-mesh) with immobile phase in a mortar. About half the slurry was introduced through a powder funnel, terminating below the reservoir, and air pressure was applied to pack the adsorbent. The rest of the slurry was added, and the packing repeated to obtain an adsorbent height of about 8 in. To minimize the evaporation of the eluent and consequent deposition of the acids on the glass fittings which normally conduct the eluent as drops through the beam of light, these fittings were removed from the Technicon fraction collector. Instead we mounted the column above the light beam and adjusted the bottom tip so that the individual drops actuated the device. A 5-ml. aliquot was pipetted directly onto the column, and when the liquid level coincided with the adsorbent height, the sides of the column were washed down twice with 1-ml. portions of mobile phase. Finally the reservoir was filled with mobile phase, and the pressure adjusted to deliver 2-ml. fractions at a rate of about 1–2 ml. per minute.

Titration of each 2-ml. fraction with 0.246 N potassium hydroxide when plotted gave well-defined peaks for pelargonic acid, azelaic acid, and suberic acid at effluent volumes of 20 ml., 48 ml., and 75 ml. Total net titrations for the respective peaks were 414, 809, and 9 thousandths ml. The sum of the three acid peaks was 1.222 ml. which, when divided by the titration of the sample applied to the column (1.275 ml.), showed that the recovery of acid amounted to 96.8%. This loss, which averaged 3.1% for the three samples, has not been explained and naturally was a limiting factor in attaining quantitative yields.

The millimols of pelargonic acid delivered by the column were obtained by multiplying the titration in ml. by the normality of the caustic used. This, when multiplied by the aliquot factor (25/5) of 5, gave 0.510 millimols of pelargonic acid in the total sample.

$$\text{Millimols pelargonic} = 25/5 \times 0.414 \times 0.246 = 0.510$$

The percentage recovery of pelargonic acid was found by dividing the recovered acid in millimols by the millimols of oleic acid (0.559) used for oxidation.

$$\% \text{ recovery, pelargonic acid} = \frac{0.510}{0.559} 100 = 91.4$$

In similar fashion the millimols of azelaic acid were calculated to be $\frac{25}{5} \times 0.809 \times \frac{0.246}{2}$ or 0.498, which, when divided by 0.559, gave 89.4% recovery. The suberic acid yield was 1.1% of theory. Total dibasic recovery was 90.5% of theory. Summation of both monobasic and dibasic recovery amounted to 181.9 mol %. Assuming no loss of pelargonic acid by volatilization and complete oxidation of pure oleic acid, we should get a total yield of 200%. Accordingly 181.9 divided by 200.0 gave a yield of 91% of theory.

Discussion of Data

Data are submitted on the oxidation of three different samples of high-purity oleic acid. Oleic acid

A (Table III) was prepared from olive oil by Knight *et al.* (10) by low temperature crystallization and distillation and was found to have an iodine value of 88.5 and a neutralization equivalent of 280.5. Knight claimed 96–99% purity for oleic acid prepared by his method so we conclude that sample A approximates the higher value. Before discussing the recovery data, it is important to know that they were all based on titrations with the same standard alkali; consequently errors in standardization were minimized. For example, oleic acid (A) was initially titrated in 4 ml. of equilibrated mobile phase against 0.246 N alcoholic potassium hydroxide under nitrogen in the presence of 2 ml. of 0.005% alcoholic solution of thymol blue to establish a neutralization equivalent of 280.5, and this value (not the theoretical value 282.5) was used to calculate the millimols weighed for oxidation. Fractions from the chromatographic column were titrated with the same alkali under the same conditions. Mean values of three oxidations for this oleic acid showed mol percentage recovery of 92.4% for monobasic acid, 89.9% for azelaic acid, and 1.6% for suberic acid, with the total dibasics of 91.5%.

The elaidic acid prepared at this laboratory by M. J. Danzig from oleic acid (A) gave greater total acid yield from the column of about 1.6% and gave total dibasic recovery of 95.7%. This could be interpreted to indicate better oxidation for the *trans* isomer. Further work with *trans* isomers is indicated. We believe it significant that both the *cis* and *trans* isomer show about 1% yield of suberic acid, which leads to the conclusion that this minor amount of the total unsaturation in the starting acid was actually in position 8 in the molecule. Although alkali is known to cause double-bond shift, it is considered unlikely that the free alkali-concentration of about 0.005 N under the reaction conditions at room temperature would cause this shift. It seems much more likely that the high concentration of alkali employed to saponify the olive oil from which the oleic acid was prepared caused the shift.

Oleic acids (B) and (C) supplied by the Hormel Institute were also of high purity, as previously shown, and were stated to have an iodine value of 89.8. Average recovery for three tests with the former was found to be 72.2% for monobasic acids, 1.4% for sebacic acid, and 90.5% as azelaic acid (Table IV). Total dibasic was 92.0%. The lower value for the monobasics and the wide variation in individual tests were not disturbing because both were caused by volatility loss in processing. Similarly, oleic acid sample (C) showed total dibasic recovery of 91.7%. It should be noted however that these two lots of acid showed minor unsaturation in positions C_{10} and C_8, respectively. Because all processing details were the same, we conclude that the starting oleic acids actually possessed minor unsaturation at these two positions in the carbon chain.

TABLE V

Oxidation of 10-Undecenoic Acid

Oxidation conditions				
M. mol acid × 1,000	505	517	579	
Relative eq. KOH	5.0	5.5	6.0	
Relative eq. $KMnO_4$ × 100	10	10	10	
Relative eq. $NaIO_4$ × 10	79	79	79	
Reaction time, hrs	7	7	7	
Reaction vol., ml	500	500	500	
Aliquot titer, ml. × 1,000	765	801	890	
Apparent degree reaction, %	100	103	95	
Titer; column peaks, ml. × 1,000				
Monobasic acids	12	12	11	
C_{10} dibasic acid	693	709	842	
C_9 dibasic acid	15	22	19	
C_8 dibasic acid	—	20	—	
Total	720	763	872	Av.
Column recovery, %	94.1	95.2	98.0	95.8
Mol % recovery				
Monobasic acids	2.9	2.9	2.8	2.8
C_{10} dibasic acid	84.6	84.4	89.5	86.2
C_9 dibasic acid	1.8	2.6	2.0	2.1
C_8 dibasic acid	—	2.4	—	0.8
Total dibasic acid	86.4	89.4	91.5	89.1
Total	89.3	92.3	94.3	92.0

Three relatively impure monounsaturated acids were oxidized to show that the method disclosed could be employed to determine the positions of unsaturation for acids other than oleic and that pure acids are not easy to obtain.

10-Undecenoic acid from Eastman Kodak Company, Rochester, N. Y., (No. 975 White Label) after recrystallization from acetone at —60°C. and oxidation gave a total dibasic yield of about 90% (Table V) with evidence for minor amounts of unsaturation at positions C_9 and C_8.

TABLE VI

Oxidation of Erucic Acid and Vaccenic Acid

	Erucic acid				Vaccenic acid
Oxidation conditions					
M. mol acid × 1,000	534	529	516		504
Relative eq. KOH	5	5	5		5
Relative eq. $KMnO_4$ × 100	20	30	30		10
Relative eq. $NaIO_4$ × 10	79	79	79		79
Reaction time, hrs	24	24	24		8
Reaction vol., ml	500	250	250		250
Aliquot titer, ml. × 1,000	1165[a]	1165[a]	1154[a]		816[a]
Apparent degree reaction, %	87	88	90		89
Titer; column peaks, ml. × 1,000					
Monobasic acids	375	360	349		171
C_{13} dibasic acid	716	714	707		112
C_{12} dibasic acid	27	19	15		228
C_{11} dibasic acid	—	—	—		174
C_{10} dibasic acid	—	—	—		43
Total	1118	1093	1071	Av.	728
Column recovery, %	95.9	93.8	92.8	94.2	89.2
Mol % recovery					
Monobasic acids	85.6	83.7	83.3	84.2	41.2
C_{13} dibasic acid	82.6	83.0	84.1	83.2	13.5
C_{12} dibasic acid	3.2	2.2	2.0	2.5	27.6
C_{11} dibasic acid	—	—	—	—	21.0
C_{10} dibasic acid	—	—	—	—	5.2
Total dibasic acid	85.8	85.2	86.1	85.7	67.3
Total	171.4	168.9	169.4	169.9	108.5

[a] Titration with 0.234 N KOH instead of with 0.246 N KOH.

TABLE VII

Summary of Mol % Dibasic Acid Recovery

Acid oxidized	C_{13}	C_{12}	C_{11}	C_{10}	C_9	C_8	Total
9,10-Dihydroxystearic	—	—	—	2.0	95.3	—	97.3
Oleic (A)	—	—	—	—	89.9	1.6	91.5
Oleic (B)	—	—	—	1.4	90.5	—	91.9
Oleic (C)	—	—	—	—	90.5	1.2	91.7
Elaidic	—	—	—	—	94.7	0.9	95.6
10-Undecenoic	—	—	—	86.2	2.1	0.8	89.1
Erucic	83.2	2.5	—	—	—	—	85.7
Vaccenic	13.5	27.6	21.0	5.2	—	—	67.3
Azelaic	—	—	—	—	98.8	—	98.8
Azelaic + pelargonic	—	—	—	—	98.8	—	98.8

Erucic acid (13-docosenoic), also from Eastman Kodak Company (No. 2233 Red Label), after recrystallization from 30% ethanol and oxidation showed total dibasic yield (Table VI) of 85.7% with a minor amount of unsaturation at the C_{12} position.

Vaccenic acid (11-octadecenoic) supplied by Jasonols Chemical Corporation, Brooklyn, N. Y., after recrystallization from acetone at —60°C. on oxidation (Table VI) gave 13.5% brassylic acid, 27.6% dodecadianoic acid, 21.0% nonane dianoic acid, and 5.2% sebacic acid. Thus the "pure" acid after recrystallization contained a minimum of 21% and a maximum of about 31% vaccenic acid.

Oxidation of 9,10-dihydroxystearic acid was conducted as a control on handling and processing technique as previously mentioned. King (9) and Robinson (13) have reported quantitative yield of 9,10-dihydroxystearic acid by potassium permanganate oxidation of sodium oleate. Our tests with variable ratios of periodate and permanganate gave uniform yields of dibasic acids. Total dibasic recovery of 97.3% was considered good evidence that our technique was practically quantitative and that it was not the cause of the lower yields established for oleic acid. The method could be used to establish the purity of similar isomeric compounds.

The special control tests designed to determine the effect of oxidation and handling on the end-products formed from oleic acid have shown practically quantitative recovery. These tests were crucial. Data for oxidation of a chromatographically pure sample of azelaic acid on three occasions (Table II) not only show good agreement but practically quantitative recovery. A single oxidation of a mixture of azelaic acid and pelargonic acid has also shown practically complete recovery of the dibasic acid. Consideration of these control tests and the oxidation of 9,10-dihydroxystearic acid permits us to conclude that the method as disclosed was practically quantitative but that yields for oleic acid were diminished because of some unknown factor during oxidation *per se*. All data are summarized in Table VII.

Conclusions

Periodate-permanganate oxidation of three samples of oleic acid considered to be of high purity has given reproducible but less than theoretical amounts of total dibasic acids. Recovery approximated 92% with about 90.3% of the expected azelaic acid. Recovery of other dibasic acids indicated that about 1.5% of the total unsaturation of these samples of oleic acid was present in positions 8 or 10 in the fatty acid molecule.

Oxidation of elaidic acid produced from one of the oleic acids has given total dibasic acid yield of about 96%, with a smaller amount of its total unsaturation in the same position as in the parent oleic acid.

Oxidation of high purity 9,10-dihydroxystearic acid has given essentially quantitative yield of total dibasic acids. The method described should be useful in determining the composition of similar unsaturated positional isomers.

Controls showing the effect of the method on azelaic acid and on a mixture of azelaic acid with pelargonic have shown essentially quantitative recovery of azelaic acid. Failure to establish quantitative recovery on oxidation of oleic acid must be caused by some unknown factor during oxidation *per se*. The experimental technique described was satisfactory for quantitative studies of the type undertaken.

Oxidation of moderate-purity, mono-unsaturated fatty acids, such as erucic, 10-undecenoic, and vaccenic acid, has given mixed dibasic acids corresponding to the respective positions of unsaturation.

The data indicate that the method described shows the position of minor unsaturation within about 1%.

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The Refining and Bleaching of Vegetable Oils

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A DISCUSSION of refining and bleaching of vegetable oils, as a section of this symposium on unit processes, must, of necessity, be in the nature of a review of those methods which are now in use or available to the manufacturer. Most, if not all, of these methods have been made the subject of nu-