Quantitative Determination of Double Bond Positions in Unsaturated Fatty Acids After Oxidative Cleavage[1]

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Abstract

The position and amt of unsaturation in fatty acids have been determined, especially in pure fractions of partially hydrogenated fats. In developing a quantitative method for determination of ethylenic bonds in monounsaturated and polyunsaturated fatty acids several procedures were combined. Key features include oxidative cleavage; recovery of cleaved acids as salts; and their conversion to methyl, ethyl or butyl esters for programmed gas-liquid chromatographic analysis. Monobasic analyses closely agree with the corresponding dibasic analyses, except neither malonic nor propionic acid has been quantitatively estimated. Analyses are shown for cleavage of high purity oleic, linoleic and linolenic acids; for *cis* and *trans* monoenates; and for conjugated and nonconjugated dienoates. Demonstrated are the accuracy, precision and applicability of the procedure to a wide range of pure fractions isolated after both heterogeneous and homogeneous partial catalytic hydrogenation of polyunsaturated fatty acids.

Introduction

IN NATURALLY OCCURRING fatty acids and oils knowing the position of all ethylenic bonds has long been recognized as extremely important. With the advent of chemical treatments, such as hydrogenation of commercial oils, it became evident that ethylenic bonds not only migrate to other positions in the molecule but form geometric isomers as well. Consequently, the determination of positional unsaturation for all geometric isomers has assumed greater importance from both a fundamental and a practical viewpoint in understanding those changes which alter the characteristics of treated oils.

Historically, this determination has been investigated by oxidative cleavage with either ozone or permanganate (2,12) followed by identification of the split products as acids. Although these and earlier studies were not quantitative, they did show degradation of the parent molecule. Mild oxidation with a solution of metaperiodate and potassium permanganate constituted a significant improvement (15,18). Quantitative cleavage was claimed, but only the more tractable dibasic acid products were estimated by these and other workers (11). Later studies in this field (10,14) utilized periodate-permanganate, and one (14) in an attempt to improve quantitation used a technique that minimizes loss of the monobasic acids. Subbaram (23) also used periodate-permanganate to cleave the *cis* and the *trans* fractions after partial hydrogenation of the esters of petroselenic, oleic and erucic acids. From the dibasic acid analysis by gas liquid chromatography (GLC) he found no perferential ethylenic bond movement for either the *cis* or the *trans* fraction.

Others (8) partly hydroxylated the unsaturated fatty acids with performic acid, hydrogenated the unreacted ethylenic bonds, cleaved the dihydroxy compounds with periodate-permanaganate, and analyzed the acidic products by GLC. This procedure was applied to a large number of polyunsaturated acids, but probably it would not be applicable to partially hydrogenated polyenoates if both small and large amt of unsaturation were present at any two bonds positions.

Ozonization of monoethnoids in methanol (1) when coupled with oxidative cleavage by performic acid, showed near quantitative results for the more tractable dibasic acids produced from oleic acid. Improvements in ozonization (16) techniques, plus reductive cleavage, have given good results for polyethnoid acids; but the compounds produced are relatively unstable; and this method does not appear applicable to complex mixtures in which ethylenic bonds are present at many carbon positions.

Now investigators realize the difficulty of total recovery of all cleavage products and their estimation. For example, short chain monobasic acids and their methyl esters are not only appreciably soluble in aqueous solution but are highly volatile as well. Malonic acid presents a special problem partly because of its instability in strong acid solutions.

Subsequent to our presentation, a comprehensive paper by Tulloch and Craig (24) appeared that described their independent and parallel development of methodology. Major attention was devoted to double bond locations in naturally occurring oils. Our study was undertaken to assist in the interpretation of various problems associated with partial hydrogenation (both heterogeneous and homogeneous) of unsaturated commercial oils and their fatty esters; our goal was the quantitative determination of ethylenic bonds in fractions obtained from complex mixtures of geometric isomers containing monoenates, dienoates and trienoates. Isolation of such fractions (of high purity with respect to the number and configuration of unsaturated bonds) has been accomplished by countercurrent distribution (CCD) separation techniques (19–22) and by liquid-liquid columns (13). The importance of these and other means of separation (4) cannot be overemphasized; otherwise perfection in determination of ethylenic bonds in an impure fraction would lead to confusing results. Even so, a pure monoenoate fraction containing isomers from partial hydrogenation often contains 24–26 individual cleavage components.

Experimental

Materials

Oleic acid was prepared from olive oil by the procedure of Rubin (17) and the usual analyses indicated 99+ purity. Linoleic and linolenic acids were purchased from The Hormel Institute, Austin, Minn. The history of other fractions analyzed is given in the references cited. Other chemicals were reagent grade.

Procedure

The procedure we have adopted attempts the recovery of all cleaved acidic products and their conversion to esters for analysis by GLC. We utilized alkaline periodate-permanganate (11) to cleave the unsaturated acid, and the salts of the acids were con-

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TABLE I

Cleavage Analysis, Mol Per Cent of Pure Oleic Acid and Oleic Acid Containing Internal Standards

Components	Control		Control plus standards	
	Calculated	Found	Calculated	Found
From oleic acid:				
Monobasic acid	50.0	49.2	50.0	49.4
Dibasic acid	50.0	50.8	50.0	50.6
From oleic and standards:				
Monobasic from oleic	.....	.....	34.9	33.0
C_{10} monobasic added	.....	.....	15.1	16.0
C_9 dibasic added	.....	.....	15.1	16.3
C_9 dibasic from oleic	.....	.....	**34.9**	34.7

verted directly (BF_3 in alcohols) to *N*-butyl, ethyl or methyl esters for analysis by temp-programmed GLC.

A. *Oxidative Cleavage.* To 500 ml of water in a 1-liter round-bottom flask containing 1.5 mmol of potassium hydroxide add 0.141 g (0.5 mmol) of oleic acid and shake well under inert (N_2) atmosphere. Add 85 ml (3.97 mmol) of a 1% solution of sodium metaperiodate (G. F. Smith Chemical Co., Columbus, Ohio) (25) and after mixing thoroughly, adjust the pH to 8 with potassium carbonate solution. Add a solution of 1 ml of 0.1*M* potassium permanganate in 400 ml of water, with stirring, to the oleic acid solution. The total oxidant represents a 100% excess of which permanganate accounts for ca. 10%. After ca. 2 hr, raise the pH to ca. 9 and allow oxidation to proceed for a total of ca. 24 hr; acidify with hydrochloric acid and reduce the oxidant with gaseous sulphur dioxide. Make alkaline with KOH solution and evaporate most of the water on a steam bath in a current of air. Transfer the salts to a volumetric flask (50 or 100 ml) and make to volume.

If esters rather than acids are to be cleaved, saponify in the reaction flask with 100% excess potassium hydroxide in 95% ethanol and remove the alcohol by means of a rotary evaporator. The salts of *trans* acids may not be completely soluble, and it may be necessary to increase the pH to 8.5 and the total volume to 1.5 liters. For cleavage of dienoic acids, double the oxidants and increase the volume to 1.5 liters. For trienoic acids, triple the oxidant and increase the volume to 2.5 liters. In addition, for dienoic and trienoic acids initiate the reaction at 5–10C and add one-half of the permanganate at the start and the rest when the mixture approaches room temp.

B. *Processing Salts of Cleaved Acids.* Prepare esters from 1) original dry mixed salts, 2) salts after steam distillation of their volatile acids, and in some cases, 3) mixed salts after an intial ether extraction of the cleaved acids.

For steam distillation, transfer 10–20 ml of the final solution from A to a 100-ml round-bottom standard taper flask, acidify at 0C with dilute hydrochloric acid, treat with a few ml of gaseous sulphur dioxide to insure absence of oxidants, make alkaline with dilute potassium hydroxide and reacidify with dilute H_2SO_4. Collect 15–20 ml of condensate in a 25-ml round-bottom standard taper flask under dilute potassium hydroxide. Wash down the conventional, all-glass delivery part of the distillation apparatus with diethyl ether and after neutralizing the free acid in the nonvolatile fraction, evaporate the water in both flasks in a current of air on a steam bath.

TABLE II

Mol Per Cent Monobasic and Dibasic Acids from Cleavage of High Purity Fatty Acids

Component	Oleic	Linoleic	Linolenic
Monobasic acids:			
C_3	....	....	92.1
C_4	....	1.1	4.0
C_5	....	4.8	....
C_6	....	90.7	2.3
C_7	0.8	0.3	0.1
C_8	2.9	0.8	....
C_9	96.3	0.5	1.5
C_{10}	....	1.8	....
Dibasic acids:			
C_6	....	1.0	1.3
C_7	....	1.6	1.5
C_8	2.6	6.0	5.3
C_9	96.9	91.3	90.3
C_{10}	0.5	0.1	1.6

Ether extraction to recover the cleaved acids is usually neither advisable nor necessary unless the analyses of the esters of original salts and of the steam distillation fractions disagree. If necessary, transfer a 20-ml aliquot of A to a conventional type all-glass continuous liquid-liquid extractor, make acid at 0C with dilute HCl, treat with a few ml of sulphur dioxide, make alkaline with dilute potassium hydroxide and reacidify with dilute H_2SO_4. Next, saturate the solution with sodium chloride. The round-bottom ether reservoir flask contains a slight excess of potassium hydroxide to neutralize the ethereal acidic solution as it returns to the reservoir. Special control tests with mixtures containing valeric and succinic acid show complete extraction in five hr, but allow extraction to proceed for 7–8 hr. After water removal, either esterify the acids (now as salts) directly or, in rare instances, steam distill them like the original mixed salts and then esterify the fractions.

C. *Preparation of Esters.* Prepare esters directly from the dry salts of both (1) the original solution from A and (2) the volatile and the nonvolatile fractions after steam distillation of A. Make the methyl, ethyl or butyl esters with the aid of a 10–15% solution of boron trifluoride in each of the three alcohols. The choice of ester made depends on the acids expected from cleavage. For example, the ethyl esters from 10 ml of the original solution A are made by evaporation to *apparent* dryness; then add a few ml of 95% ethanol and gaseous carbon dioxide to react with the slight excess of alkali; remove the alcohol and water with a rotary evaporator at or below room temp. Add a few ml (2–4 ml) of a solution of gaseous HCl in absolute ethanol (15/85 by wt) to free the acids. Then introduce ca. 2 ml of a solution of boron trifluoride in absolute ethanol (15/85 by wt) and reflux for ca. 10 min. After cooling, remove most of the alcohol with a rotary evaporator at or below room temp, add 5–10 ml of water and neutralize the excess acid with a saturated solution of sodium bicarbonate. Transfer to a small separatory funnel, add sodium chloride to saturation and extract twice with 5–10 ml of ether. Combine the extracts and wash twice with 3 ml of water. Dry with anhydrous sodium sulphate and remove most of the ether with a rotary evaporator at or below room temp.

Control tests show near quantitative recovery for all monoethyl esters, except propionic, and for all diethyl esters, except malonic. Preparation of monomethyl and dimethyl esters of short chain acids (if present) gave much poorer recoveries. Recovery of diethyl malonate from control acid mixtures was never greater than 80%, presumably because of its chemical instability in strong acids at reflux temperature. [We have made additional checks on the recovery of diethyl malonate from the control diacid mixtures. Possibly near quantitative recovery may be obtained by omitting the HCl, reducing the esterification temp to 50C and prolonging reaction time to five hr.]

To avoid loss of short chain monobasic esters we resorted to making *N*-butyl esters. The procedure differed chiefly in the means for removal of butyl al-

cohol: after esterification, add water, neutralize with bicarbonate and remove the butyl alcohol by several *gentle* agitations of the separatory with a half-saturated solution of sodium chloride. Our maximum recovery of propionic acid from control mixtures as butyl esters was 80–85%. All other acids gave near quantitative recovery. The use of dibutyl esters for dibasic acid analysis was impractical because those esters of the acids containing more than eight carbons elute from the gas chromatograph at excessively high temperatures.

D. *Ester Analysis by Temperature-Programmed Gas Chromatography.* The esters (usually less than one drop) were analyzed with a modified Model 350B temp programming "Aerograph" equipped with two 4–6 ft ¼ in. O.D. aluminum columns at a helium flow rate of ca. 100 ml/min. As substrate we have used 25% neopentyl glycol succinate (NPGS), 25% silicone SF 96 and 25% diethylene glycol succinate (DEGS) on 60–80 mesh or 45–60 mesh chromosorb W treated with dichlorodimethyl silizane. We prefer the NPGS substrate if butyl propionate is expected. The manually integrated peak areas for mono- and diesters were separately normalized to represent wt percentage and recalculated to mol percentage.

There is some overlapping of the mono- and dibasic ester peaks regardless of the alcohol used for ester preparation so that in a mixture of esters containing a large number of mono- and diesters sometimes DEGS or silicone SF 96 columns were used to alter the coincident peaks. Steam distillation minimized this difficulty considerably or entirely.

Control tests on weighed mixtures of methyl, ethyl or butyl monobasic esters (of known high GLC purity) gave peak areas directly proportional to the wt of each homologue. Similar mixtures for dibasic esters also gave linear wt response. In a four or five component mixture the peak areas for three individual chromatograms agreed within ca. ±1.5% of the mean. A mixture containing 20.0% of a component by wt would give a mean area of 19.7–20.3%. However, with mixtures of mono- and dibasic esters (whether methyl, ethyl or butyl) the response for monoesters (as a class) was from 1–4% greater than for the diesters.

Results and Discussion

Check on Cleavage of High Purity Oleic Acid. To verify the completeness of cleavage and at the same time to determine the efficiency of recovery of cleaved acids, we oxidized 0.3115 g of our purest oleic acid (17) and processed exactly half of the resulting solution in the three ways cited under Experimental B. To the other half was added a standard solution containing 0.0411 g of C_{10} monobasic acid and 0.0349 g of C_6 dibasic acid, after which this half was processed like the first. GLC analysis of the ethyl esters of the standard solution of the internal standards gave 50.7% for the C_{10} monoester and 49.3% for the C_6 diester, and these values agreed with the calculated theoretical values of 49.7 and 50.3%, respectively.

GLC analysis for the control half gave an average of 3.4% C_8 monobasic acid (with no corresponding C_{10} dibasic acid) and an average of 2.8% C_8 dibasic (with no corresponding C_{10} monobasic acid). These artifacts have been found by other workers (1) and may be explained by peroxide formation and ethylenic bond shift with the formation of a hydroxyl group. For discussion purposes we have combined these mono- and dibasic artifacts with the expected acids. Table I shows our average results for the control half and for the half containing the added internal standards. The cleaved acids produced from oleic acid were in good agreement with calculated values for both parts of the solution. The analysis of the part containing the internal standards also agrees with the calculated composition, and the results obtained by the three processing procedures were substantially the same.

Individual comparisons of peak areas (calculated to mol %) of pairs of components with calculated values eliminate normalizing assumptions and are a severe test of the results.

	Calculated	Found
Monoacids from oleic	66.6	63.8
C_{10} monoacid added	33.4	36.2
C_{10} monoacid added	50.0	50.9
C_6 diacid added	50.0	49.1
Dibasic acids from oleic	69.8	68.1
C_8 diacid added	30.2	31.9

These comparisons are in fair agreement and indicate that the amt of cleaved acids from oleic are slightly low.

Application to High Purity Fatty Acids. High pur-

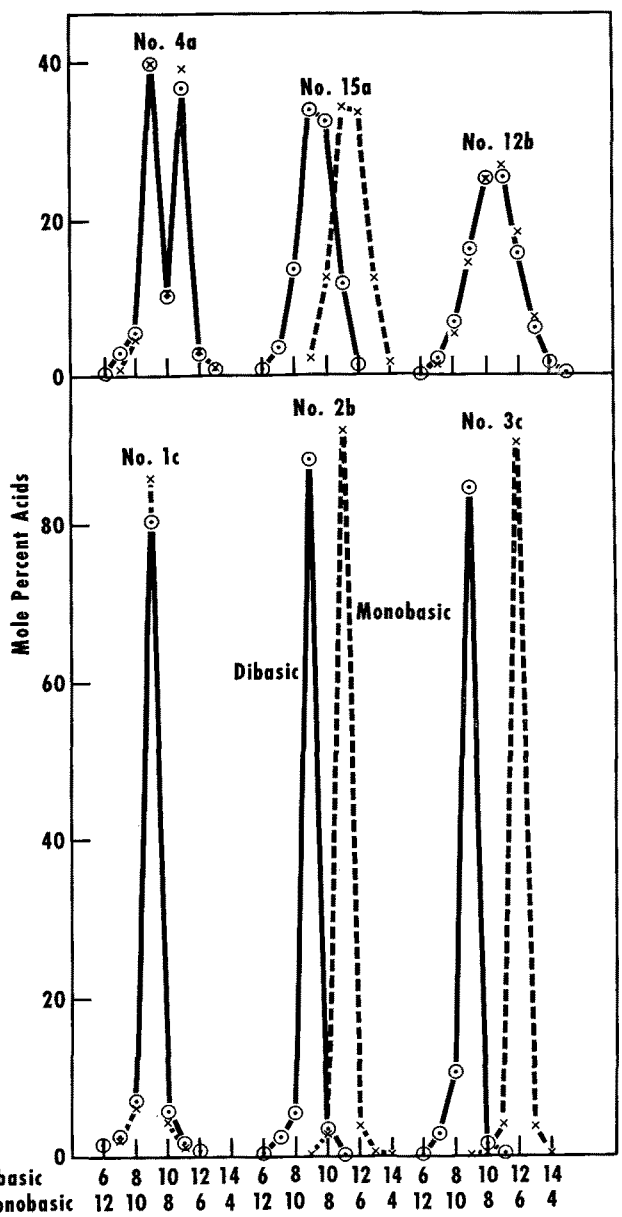

FIG. 1. Monobasic and dibasic data after cleavage analysis of some typical unsaturated acids.

TABLE III

Heterogeneous Hydrogenation Samples (Mol Percentage Monobasic and Dibasic Acids)

Sample no.	Material	Acid type	Carbons												
			3	4	5	6	7	8	9	10	11	12	13	14	15
	Partial deuteration of methyl oleate with platinum														
1a	*cis* at 20% D_2	Di				0.5	1.2	3.2	90.7	2.7	0.6	1.1			
		Mono					0.9	3.3	92.8	2.2	0.8				
1b	*trans* at 20% D_2	Di				3.6	6.5	24.0	39.8	17.7	2.9	4.7	0.8		
		Mono					3.9	20.8	45.4	25.4	4.5				
1c	*cis* at 40% D_2	Di				1.5	2.6	7.1	80.5	5.7	1.8	0.8			
		Mono					1.2	4.3	86.1	6.3	2.1				
1d	*trans* at 40% D_2	Di				5.1	7.6	22.4	36.2	19.8	4.9	3.2	0.8		
		Mono					2.3	16.6	41.8	29.8	9.5				
	Geometric dienoates; their *cis* and *trans* fractions; Hhydrogenation with platinum														
2a	Castor acids	Di			0.3	1.0	5.6	19.6	62.8	7.5	2.6	1.0		0.6	
		Mono	0.4	0.8	4.9	49.5	25.4	11.8	6.2	0.6	0.4				
2b	9,11,*trans,trans*	Di				0.3	2.3	5.5	88.3	3.5	0.1				
		Mono	0.2	0.2	0.6	3.8	92.0	3.0	0.2						
3a	Conj. *trans,trans*	Di		0.2	0.7	1.5	9.7	26.1	40.9	16.9	2.5	0.7	0.5	0.3	
		Mono	1.0	1.0	4.2	20.4	41.2	23.4	7.0	1.0	0.3	0.5			
3b	Conj. *cis,trans*	Di		0.2	0.4	1.5	7.6	28.0	49.1	10.3	2.5	0.4			
		Mono	0.7	0.9	4.2	18.0	46.8	22.3	5.6	1.1	0.4				
3c	Nonconj. *cis,trans*	Di				0.3	2.9	10.6	84.7	1.3	0.2				
		Mono	0.5	0.4	3.7	90.6	4.0	0.5	0.2	0.1					
4a	*cis* from *cis,cis*	Di				0.5	3.3	5.5	39.7	10.2	36.7	3.0	0.8	0.3	
		Mono		0.4	1.1	3.6	38.9	10.6	39.8	4.6	1.0				
4b	*trans* from *cis,cis*	Di				0.7	4.1	7.4	18.9	45.2	14.4	7.2	1.6	0.5	
		Mono		1.0	1.8	6.1	15.5	47.4	18.4	7.3	1.9	0.6			
5a	*cis* from *trans,trans*	Di		0.8	0.5	1.3	4.9	11.1	41.6	19.8	12.4	5.2	1.9	0.5	
		Mono		0.3	5.0	7.0	18.8	28.4	23.0	11.5	4.2	1.8			
5b	*trans* from *trans,trans*	Di			1.0	0.9	1.9	6.2	14.5	30.3	23.2	15.0	5.8	1.2	
		Mono	1.1	0.5	2.9	18.8	22.9	28.1	18.3	6.0	1.4				
6a	*cis* from *cis,trans*	Di		0.4	0.4	1.5	5.7	10.5	32.1	25.1	15.5	6.5	1.6	0.4	0.3
		Mono		0.6	2.5	8.8	18.0	26.4	31.6	8.2	2.9	1.0			
6b	*trans* from *cis,trans*	Di	0.3	0.3	0.6	1.9	5.5	14.4	22.4	23.5	19.1	9.1	2.2	0.7	
		Mono	0.6	0.5	2.8	8.9	22.6	26.0	21.0	12.2	3.4	1.4	0.6		
	Geometric dienoates; their *cis* and *trans* fractions; hydrogenation with nickel														
7a	*cis* from *cis,trans*	Di			0.3	1.1	5.0	10.4	34.3	26.0	16.3	5.3	1.3		
		Mono	0.7		1.4	6.2	17.1	27.8	32.2	9.4	3.8	1.4			
7b	*trans* from *cis,trans*	Di				1.2	5.8	14.5	23.0	25.0	21.3	7.5	1.7		
		Mono	0.9	0.6	2.0	7.6	23.1	24.3	20.3	17.0	2.8	0.9	0.5		
8a	*cis* from *trans,trans*	Di				1.4	6.3	12.2	25.0	27.0	18.3	8.1	1.7		
		Mono	1.2			8.0	20.6	27.8	25.3	13.2	3.9				
8b	*trans* from *trans,trans*	Di				0.7	4.8	12.3	25.6	23.3	20.6	11.5	1.2		
		Mono	2.1		1.9	9.6	23.2	25.2	22.7	11.2	3.4	0.7			

ity oleic acid was cleaved and ethyl esters were prepared in three ways: first, from the dry salts; secondly, from both fractions after steam distillation, and thirdly, from both fractions after initial ether extraction and steam distillation. The results were in agreement, but those for the second procedure (steam distillation) were more concordant as shown in Table II.

High purity linoleic acid was also cleaved under normal conditions as well as with an excess of total oxidant larger than normal. A portion of the mixed acids was steam distilled and both butyl and ethyl esters were prepared from the volatile fraction; methyl and ethyl esters were made from the nonvolatile fraction. Another portion was initially ether extracted and similarly processed. The nonvolatile fractions of each contained appreciable, but low, amt of malonic acid as well as some oxalic and succinic acids, which are not included in normalized results shown in Table II.

High-purity linolenic acid was also cleaved and processed like linoleic acid. The malonic acid values are excluded from the normalized results in Table II. For reasons unknown our results for the cleavage of linoleic and linolenic acids exhibited fragmentation. Perhaps pure polyunsaturates would be more amenable to the methods proposed by Gunstone and Sykes (8) or by Privett and Nickell (16). Despite this fragmentation our process gave reproducible cleavage data with dienoates in which ethylenic bonds were widely distributed throughout the carbon chain.

Application in Hydrogenation Studies. Investigation of heterogeneous and homogeneous partial hydrogenation of mono- and polyunsaturated oils, esters and acids has been significantly advanced by the development of high-resolution techniques that are commensurate with the complexity of positional and geometric isomers formed. Our procedure for quantitative identification of ethylenic bond positions in the separated fractions has contributed essential information and has resulted in a more explicit understanding of the changes occurring in hydrogenation.

Tables III and IV show the analyses (mol %) for the recovered di- and monobasic acids for 15 different samples and demonstrate the agreement on a variety of materials. Since it is difficult to visualize and compare mentally the appropriate mono- and dibasic acid data in tabular form, six of the analyses are plotted as Figure 1.

A. *Heterogeneous Partial Hydrogenation.* In one investigation pure methyl oleate was progressively deuterated (3) and catalyzed by platinum. Pure *cis* and *trans* monoenates were isolated at four levels of deuteration. Analyses for each isomeric fraction at the 20% (sample 1a and 1b) and at the 40% (sample 1c and 1d) levels of reduction are listed in Table III. To show the agreement of dibasic and monobasic values we plotted the data for sample 1c (*cis* fraction at 40%) in Figure 1. For example, the amt of dibasic acid at positions 8,9 and 10 should agree with the monobasic values for positions 10,9 and 8, respectively. In Figure 1 sample 1c shows good agreement, and the data for samples 1a, 1b and 1d (Table III) are also in substantial agreement. The *trans* isomers show a slight shift of the ethylenic bonds toward the carboxyl group.

In another investigation concerning partial hydrogenation of conjugated *cis,cis;* conjugated *cis,trans*; and conjugated *trans,trans* dienoates, dehydrated castor oil esters were resolved by CCD and these dienoates were then partly hydrogenated with both platinum and

TABLE IV
Homogeneous Hydrogenation Samples (Mol Percentage Monobasic and Dibasic Acids)

Sample no.	Materials	Acid type	Carbons													
			3	4	5	6	7	8	9	10	11	12	13	14	15	16
		Monoenate fraction, partial hydrogenation linolenic acid, hydrazine														
9	Monoenates	Di					0.7	0.5	43.4	0.8	0.6	37.3		0.1	16.6	
		Mono	3.6		0.1	40.6	0.5	0.5	53.9	0.3	0.5					
10	Monoenates after isomerization	Di					0.1	0.5	42.6	0.9	0.2	40.3			15.4	
		Mono	4.5			44.7	0.2	0.4	50.2							
		Partial hydrogenation with cobalt carbonyl														
11a	*cis* from soybean oil ester	Di					0.6	2.5	82.1	4.3	7.3	2.6	0.6			
		Mono				2.0	5.5	4.7	86.3	1.5						
11b	*trans* from soybean oil ester	Di					0.5	3.7	16.7	36.3	33.9	5.5	1.8	0.9	0.7	
		Mono				2.7	27.5	38.1	25.3	6.4						
12a	*cis* from linoleate	Di			0.4	0.6	2.1	7.0	21.1	21.6	21.0	17.8	6.2	1.8	0.4	
		Mono	1.3	2.0	6.3	20.1	22.6	21.4	19.1	5.5	1.5	0.2				
12b	*trans* from linoleate	Di			0.5	0.5	2.1	6.9	16.1	24.8	25.1	15.6	6.1	1.8	0.5	
		Mono		1.6	7.4	18.2	26.7	24.6	14.4	5.3	1.6	0.2				
13a	*cis* from linolenate	Di					0.8	3.4	12.4	12.8	15.2	17.6	15.1	11.9	7.0	3.8
		Mono	2.9	8.9	15.6	19.4	18.2	14.2	15.5	4.0	1.1	0.2				
13b	*trans* from linolenate	Di					0.8	3.5	9.4	15.3	15.1	20.6	14.7	12.0	6.3	2.3
		Mono	1.8	8.0	15.8	22.5	19.8	16.4	11.1	3.9	0.7					
		Partial hydrogenation with iron carbonyl														
14a	Monoenate from linoleate	Di		2.2	1.8	3.2	5.4	10.2	16.6	19.0	15.8	16.9	4.3	2.1	1.2	1.3
		Mono	1.5	2.8	6.7	15.6	21.8	21.4	15.3	7.0	3.1	1.8	1.2	1.3	0.3	0.2
14b	*cis* from linoleate	Di			0.7	1.4	2.4	5.2	17.2	20.2	21.8	16.2	5.8	3.3	2.3	3.5
		Mono		1.3	2.7	12.2	22.3	23.7	21.4	6.9	3.6	2.5	1.6	1.8		
14c	*trans* from linoleate	Di		1.4	0.9	1.6	2.8	8.4	16.6	20.9	19.9	14.2	7.6	2.7	1.9	1.1
		Mono	0.4	0.6	4.3	12.8	21.4	22.8	18.5	9.3	3.6	1.9	1.1	2.1	0.9	0.3
15a	Conj. dienoate from linoleate	Di			0.8	0.9	3.5	13.4	33.5	32.5	11.9	1.6			1.0	0.9
		Mono	3.0	1.7	12.4	33.3	34.1	12.7	2.1	0.7						
15b	Conj. dienoate complex	Di		2.8	3.1	0.9	3.6	13.4	33.7	28.4	10.5	2.8	0.8			
		Mono		3.1	12.7	33.4	32.6	12.8	3.9	0.8	0.7					
15c	Decomposed conj. dienoate complex	Di		2.0	1.9		6.0	12.8	34.9	29.9	10.2	2.3				
		Mono	2.0	2.0	11.1	33.4	34.1	13.6	2.9	0.9						

nickel catalysts. The original dehydrated castor oil (sample 2a) esters show concordant results on cleavage, which indicate predominantly 9,12 dienoate. Sample 2b, a pure 9,11-*trans,trans* dienoic acid prepared in this laboratory, shows not only good agreement of the acid types, but also 90% unsaturation at carbons 9 and 11 (Fig. 1). The importance of quantitative recovery of both mono- and dibasic acids is well illustrated by sample 2b. The C_9 dibasic (azelaic) values alone would not locate the second ethylenic bond at carbon 11, but quantitative recovery of the C_7 monobasic (heptanoic) acid confirmed the presence and location of the second bond.

Analyses of a conjugated *trans,trans* dienoate fraction (sample 3a), a conjugated *cis,trans* dienoate fraction (sample 3b) and of a nonconjugated *cis,trans* dienoate fraction (sample 3c), all from sample 2a, gave concordant results for the mono- and dibasic acid analyses and proved that samples 3a and 3b were conjugated. The plot of sample 3c (Fig. 1) showed ca. 90% hexanoic and azelaic acid and confirmed ethylenic bonds at carbons 9 and 12.

After all three conjugated dienoates were partially hydrogenated with a platinum catalyst, pure *cis* and pure *trans* monoenates were isolated. Cleavage data of the *cis* (sample 4a) and *trans* isomers (sample 4b) from the *cis,cis* dienoate were good. The plot for sample 4a (Fig. 1) conclusively shows that partial hydrogenation of the conjugated *cis,cis* dienoate did not cause appreciable ethylenic bond shift. Observation of the data for the *trans* isomer (sample 4b) shows that the ethylenic bonds did shift predominantly to carbon 10. When the *trans,trans* dienoate (sample 3a) was similarly hydrogenated, the *cis* (sample 5a) and *trans* isomers (sample 5b) had maximum amt of ethylenic bonds at carbons 9 and 10, respectively, with generally good agreement between mono- and dibasic acids. When the *cis,trans* dienoate (sample 3b) was similarly treated, the pure *cis* monoenate (sample 6a) contained ca. 30% of its unsaturation at carbon 9; whereas, the *trans* isomer (sample 6b) had ca. 25% of its ethylenic bonds at carbon 10. Excellent agreement of mono- and dibasic values was obtained.

Two of the three conjugated dienoates (from sample 2a) were partially hydrogenated a second time but with a nickel catalyst. Data on the cleavage analysis of the *cis* (sample 7a) and the *trans* isomers (sample 7b) from the *cis,trans* dienoate (sample 3b) still show maximum unsaturation at carbon 9 and carbon 10, respectively. Excellent agreement of monobasic and dibasic acids was obtained. Cleavage of the isomers derived from the *trans,trans* dienoate (sample 3a) are also listed in Table III. The positions of maximum unsaturation for the *cis* (sample 8c) and *trans* isomers (sample 8b) are doubtful but seem to be reversed as compared with the previous pattern. Agreement of mono- and dibasic values was only moderate.

B. *Homogeneous Partial Hydrogenation.* Another study at this laboratory included partial hydrogenation of polyunsaturated acids with hydrazine (21). Linolenic acid (from linseed oil) was partially hydrogenated and the monoenates (sample 9) isolated by CCD. This *cis*-9, *cis*-12 and *cis*-15 mixture was then isomerized with nitrous oxide (9) to give the corresponding *trans* (sample 10) isomers. Cleavage of each by our procedure gave the results listed in Table IV. Agreement between *mono*- and dibasic values for the chief components was poor because of our inability to recover all the propionic acid. Normalization automatically gives high values for hexanoic and pelargonic acids. The dibasic percentages are considered reliable. Data for the *cis* isomers at carbons other than 9, 12 and 15 total ca. 3%; an indication that the original linolenic acid was ca. 97% pure. Comparison of the principal dibasic acids shows excellent agreement, an indication that hydrazine reduction did not cause bond migration.

Homogeneous partial hydrogenation with cobalt carbonyl has also been investigated here, and some of the analyses of pure fractions from this study are included. Analyses of *cis* (sample 11a) and *trans* monoenate (sample 11b) fractions after partial hydrogena-

tion of soybean oil esters show good agreement between mono- and dibasic acids (5). Most of the ethylenic bonds (85%) in the *cis* (sample 11a) fraction were at carbon 9, whereas the *trans* (sample 11b) fraction had high unsaturation at carbons 10 and 11. Similarly, partial hydrogenation of methyl linoleate and separation of the products gave pure *cis* (sample 12a) and pure *trans* (sample 12b) fractions. When cleaved, each gave mono- and dibasic acids in like amounts. The plot of both sets of data for sample 12b in Figure 1 shows about equal amt of ethylenic bonds at carbons 10 and 11. Treatment of methyl linolenate in the same manner also gave pure *cis* (sample 13a) and pure *trans* monoenates (sample 13b) which on cleavage analysis showed maximum unsaturation (Table IV) at carbon 12. Mono- and dibasic data were in fair agreement. The distributional pattern was somewhat abnormal, manifesting a break or distortion at carbon 10 or 11.

Iron carbonyl (6,7) has also been investigated as a catalyst in homogeneous partial hydrogenation of polyunsaturated esters, and we have included the application of our procedure to several fractions. Table IV contains the results of cleavage of three fractions from methyl linoleate; a mixture of *cis* and of *trans* monoenate (sample 14a), a pure *cis* fraction (sample 14b) isolated from a monoenate mixture, and a pure *trans* fraction (sample 14c) from a monoenate mixture. The mixture had generally good agreement of the mono- and diacids, with carbon 10 showing the greatest amt of ethylenic bonds. Similar results for the *cis* (sample 14b) and for the *trans* fractions (sample 14c) show the greatest unsaturation at carbons 11 and 10, respectively.

The application of our procedure to the analysis of conjugated dienoates formed during partial hydrogenation of methyl linoleate is illustrated by sample 15. The conjugated dienoate (sample 15d) and a complex of this dienoate with iron carbonyl (sample 15b) were isolated after hydrogenation, and the form of their ethylenic bond distribution is practically the same. Figure 1 depicts the dibasic and monobasic results for sample 15a. The iron carbonyl-dienoate complex, as separated by CCD, was decomposed chemically (sample 15c) and after cleavage the same ethylenic bond distribution was obtained. All three fractions were principally 9,11- and 10,12-conjugated dienoates, and the amt of monobasic and dibasic acids were highly comparable.

Precision of Data. We have determined the precision of our procedure by statistical treatment of all dibasic percentages greater than 1.5% with the corresponding monobasic values.

For those sample obtained after heterogeneous hydrogenation the standard deviation was ±1.97%. The 95% confidence limits for either mono- or dibasic acids were ±3.92%; for the mean of the mono- and dibasics, ±2.77%.

For those samples obtained after homogeneous hydrogenation the standard deviation was ±1.86%. The 95% confidence limits for either mono- and dibasic acids were ±3.67%; for the mean of the mono- and dibasics, ±2.60%.

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Rearrangement of Epoxy Fatty Esters to Keto Fatty Esters

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Abstract

The rearrangement of fatty epoxyesters to ketoesters was studied. The isomerization is carried out in nonaqueous media and is catalyzed by acids. Esters containing one epoxy group per fatty acid chain are isomerized to the corresponding ketones in 70–90% yields using boron fluoride etherate catalyst in boiling dioxane. Conversion to ketonic products is measured either by chromatographic separation followed by IR analysis or by oximation. Principal byproducts are hydroxy derivatives. Fatty esters containing more than one epoxy group/fatty acid chain give low ketone yields.

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Introduction

RECENT INVESTIGATIONS at this laboratory involving the acid catalyzed hydration of epoxides of fatty acids and esters indicated that a considerable amt of ketonic material could be obtained as byproduct. This result was not totally unexpected, for the rearrangement of epoxides to ketones with the aid of catalyst and/or heat has been well documented (1). However, information concerning the isomerization of epoxides of fatty materials was noticeably absent, and an investigation in this field seemed worthwhile; since ketoacids and esters had been previously prepared only by multi-stepped processes (2).

The optimum conditions for rearrangement of epoxides to ketones were determined using methyl 9,10-