

THE ANALYSIS OF OILS AND FATS BY GAS CHROMATOGRAPHY

IV. THE ALKALINE ISOMERISATION OF LINOLEYL ACETATE AND OCTA-DECA-9,12-DIENE

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When linoleic acid is heated with alkali, rearrangement to conjugated isomers takes place. It has been found¹ that the amount of rearrangement to these isomers is negligible at reaction temperatures up to 120° but increasing amounts of isomerisation products are formed as the reaction temperature is increased. At a reaction temperature of 234°, about 2 % of the linoleic acid has not been isomerised. When four vegetable oils were heated at 234° it was found that about 5 % of the linoleic acid originally present had not been isomerised. Other investigators²⁻⁵ have found that varying amounts (up to 6 %) of linoleic acid remain unisomerised after reaction with alkali at 180°.

It has also been shown⁶ that, when carboxylic acids are converted to the corresponding acetates and hydrocarbons, gas chromatographic retention data for the corresponding methyl esters, alcohols and acetates are very similar when they are chromatographed under the same conditions.

The purpose of the present work is to investigate the action of alkali on linoleyl acetate and on octadeca-9,12-diene and to separate the conjugated isomers by means of gas chromatography. A comparison can then be made with the amounts of conjugated isomers obtained from the corresponding acid, acetate and hydrocarbon.

EXPERIMENTAL

Preparation of derivatives

Octadeca-9,12-dienyl acetate (linoleyl acetate). Linoleic acid (puriss. Koch-Light Laboratories Ltd.) was reduced to octadeca-9,12-dienol with aluminium lithium hydride and this alcohol was converted to its acetate by reaction with acetyl chloride⁶.

Octadeca-9,12-diene. Octadeca-9,12-dienol was converted to its *p*-toluene-sulphonate and this derivative was reduced to the diunsaturated hydrocarbon using the method of DYEN, HAMMAN AND SWERN⁷.

Alkali isomerisation

The derivative (200 mg) was heated with potassium hydroxide-diethylene glycol for 1 h as described previously¹. In the case of the acetate, reaction with the alkali brought about hydrolysis and therefore, after isolation of the isomerisation products from the reaction mixture, they were converted to the acetates by reaction with acetyl chloride.

Gas-liquid chromatography

Gas chromatographic separations were carried out, as described previously⁸, using a Perkin Elmer 800 chromatograph with butanediol succinate (BDS) as the stationary liquid. The acetate derivatives were separated at a column temperature of 210° and the hydrocarbons at 150°.

RESULTS AND DISCUSSION

When octadeca-9,12-dienyl acetate and octadeca-9,12-diene are heated with potassium hydroxide-diethylene glycol it is found that isomerisation to conjugated derivatives takes place and also that the amounts of the isomerisation products increase with an increase in reaction temperature (Table I). The results obtained for the acetate and hydrocarbon are similar to those obtained previously¹ for linoleic acid under similar reaction conditions except that the amount of hydrocarbon unisomerised at 180° is greater than that found for both the acid and the acetate. This is probably due to the fact that the hydrocarbon was not very soluble in the reaction mixture and a homogeneous solution could not be obtained.

TABLE I

PERCENTAGE AMOUNTS OF PRODUCTS FROM ISOMERISATION REACTION

	Original (%)	Temperature of reaction			
		120°	150°	180°	234°
<i>Acetate</i>					
18:1 ⁰	0.9	0.9	0.9	0.7	0.8
18:2 ^{0,12}	99.1	98.0	86.7	5.0	4.5
18:2conj. <i>cis,trans</i>	—	1.1	11.0	85.5	54.2
18:2conj. <i>cis,cis</i>	—	—	1.4	3.9	13.8
18:2conj. <i>trans,trans</i>	—	—	—	4.9	26.6
<i>Hydrocarbon</i>					
18:1 ⁰	0.8	0.8	0.8	0.9	0.7
18:2 ^{0,12}	99.2	99.2	96.4	46.9	5.1
18:2conj. <i>cis,trans</i>	—	—	1.6	45.7	39.2
18:2conj. <i>cis,cis</i>	—	—	0.5	3.0	13.8
18:2conj. <i>trans,trans</i>	—	—	0.7	3.5	41.2

Retention data and ECL values of the various C₁₈ derivatives are shown in Table II. It was found that the peak assigned to the conjugated *cis,trans* acetate was broad and misshapen and at a column temperature of 190° this was resolved into two incompletely separated peaks. The corresponding hydrocarbon gave a broad misshapen peak which could not be resolved by lowering the column temperature to 120°. We have found⁶ that methyl esters and the corresponding alcohols and acetates give similar retention times and ECL values when chromatographed under the same conditions. Since there is a very close agreement between the relative retention times and ECL values found in the present investigation for the various acetates and those for the corresponding methyl esters found previously, we have tentatively identified the peaks due to the various isomerisation products of linoleyl acetate. The retention data for the hydrocarbon isomerisation products followed a similar pattern to those

TABLE II
RETENTION DATA AND EQUIVALENT CHAIN LENGTHS

	<i>Column temperature</i>				
	<i>190°</i>	<i>200°</i>	<i>210°</i>	<i>140°</i>	<i>150°</i>
	<i>Relative retention times</i>				
	<i>Acetates</i>			<i>Hydrocarbons</i>	
18:0	1.00	1.00	1.00	1.00	1.00
18:1 ⁰	1.11	1.10	1.10	1.12	1.12
18:2 ^{0,12}	1.32	1.31	1.30	1.38	1.37
18:2conj. <i>cis,trans</i>	{ 1.79 1.85	1.78	1.77	2.06	1.96
18:2conj. <i>cis,cis</i>	1.98	1.95	1.92	2.19	2.16
18:2conj. <i>trans,trans</i>	2.15	2.12	2.09	2.50	2.41
	<i>Equivalent chain lengths</i>				
18:0	18.00	18.00	18.00	18.00	18.00
18:1 ⁰	18.30	18.31	18.31	18.27	18.27
18:2 ^{0,12}	18.85	18.86	18.88	18.73	18.75
18:2conj. <i>cis,trans</i>	{ 19.80 19.91	19.88	19.95	19.65	19.62
18:2conj. <i>cis,cis</i>	20.13	20.18	20.22	19.79	19.85
18:2conj. <i>trans,trans</i>	20.38	20.44	20.50	20.09	20.16

given by the methyl esters and the acetates. Octadeca-9(*trans*),11(*trans*)-dienyl acetate and octadeca-9(*trans*),11(*trans*)-diene were prepared from the corresponding methyl ester⁰ and the retention times found for these compounds agreed with those obtained for the peaks assigned to the conjugated *trans,trans* isomerisation product.

The relative amounts of the isomerisation products formed are shown in Table III and they are similar to the amounts obtained from vegetable oils¹. At reaction temperatures of 150° and 180° the main products are the *cis,trans* isomers. At a reaction temperature of 234° there are decreases in the relative amounts of *cis,trans* isomers and these decreases are accompanied by increases in the relative amounts of the *cis,cis* isomers and larger increases in the relative amounts of the *trans,trans* isomers. These results obtained by gas-liquid chromatography are supported by the

TABLE III
RELATIVE PROPORTIONS (PER CENT) OF ISOMERISATION PRODUCTS

	<i>Temperature of reaction</i>		
	<i>150°</i>	<i>180°</i>	<i>234°</i>
<i>Acetate</i>			
18:2conj. <i>cis,trans</i>	89	91	57
18:2conj. <i>cis,cis</i>	11	4	15
18:2conj. <i>trans,trans</i>	—	5	28
<i>Hydrocarbon</i>			
18:2conj. <i>cis,trans</i>	95	87	42
18:2conj. <i>cis,cis</i>	2	6	15
18:2conj. <i>trans,trans</i>	3	7	43

results obtained by infra-red spectroscopy when the absorptions at 985 cm^{-1} and 946 cm^{-1} are compared for the samples obtained from reaction at different temperatures.

We find that, with both the acetate and hydrocarbon, there is about 5 % of unisomerised linoleic derivative at a reaction temperature of 234° . We have previously found that about 2 % of linoleic acid was unisomerised at this temperature. DANIELS AND RICHMOND² found that, when oil extracted from flour was heated at 180° with potassium hydroxide-ethylene glycol, 2.9 % of the linoleic acid was unisomerised.

SREENIVASAN *et al.*³ have shown that not all the C_{18} dienes in corn oil were the 9,12-isomers and also that 3.9 % of the linoleic acid was not conjugated after heating

TABLE IV
AMOUNTS OF OXIDATION PRODUCTS

Methyl ester ^a	Weight (%)	
	Calculated ^b	Found
$C_5\text{ mc}$	—	0.7
$C_6\text{ mc}$	37.1	34.2
$C_9\text{ mc}$	0.4	0.6
$C_7\text{ dc}$	—	0.3
$C_8\text{ dc}$	—	2.1
$C_9\text{ dc}$	62.5	58.1
$C_{10}\text{ dc}$	—	0.3
Unidentified	—	3.7

^a mc = Monocarboxylic ester; dc = dicarboxylic ester.

^b From GLC analysis of original ester, assuming normal positions of the double bonds.

for 25 min at 180° with potassium hydroxide-ethylene glycol. BEADLE, JUST, MORGAN AND REINERS⁴ found that for a number of corn oils the linoleic acid content determined by gas chromatography was higher by 2.4–6.0 % than that determined by the alkali isomerisation method.

CARTONI, LIBERTI AND RUGGERI⁵, using capillary columns, have shown that a commercial sample of linoleic acid contained 89.4 % 9,12-*cis,cis* isomer, 5.4 % 9,12-*cis-trans* isomer and 5.2 % 9,12-*trans,cis* + *trans,trans* isomers. Since it would not be expected to be able to separate these isomers on a conventional packed column, this sample of linoleic acid would be taken to be 100 % 9,12-*cis,cis*-linoleic acid when analysed on these packed columns. When this commercial sample was isomerised by heating with alkali at 180° it was found that 99.5 % of the *cis,cis* isomer had been converted to conjugated derivatives but only 61 % of the *cis,trans* isomer and 75 % of the remaining isomers had been isomerised. If this isomerised sample had been chromatographed on a conventional packed column the results would have indicated that 2.9 % of the linoleic acid had not been isomerised.

A sample of the linoleic acid used in the present investigation was oxidised with periodate-permanganate¹⁰. The main oxidation products found were hexanoic acid and azelaic acid in the relative amounts expected from the oxidation of octadeca-9,12-dienoic acid. However a number of minor products were found (Table IV). Some of these minor products may have been produced by secondary oxidation although a pure sample of azelaic acid on being treated with periodate-permanganate under the same conditions as those used for the oxidation of linoleic acid did not give any

detectable oxidation products. The difficulties of differentiating secondary oxidation products from the primary oxidation products of isomers present in amounts of about 1 % have been discussed by KUEMMEL¹⁰.

SUMMARY

Isomerisation occurs when octadeca-9,12-dienyl acetate and octadeca-9,12-diene are heated with potassium hydroxide-diethylene glycol at temperatures above 120°. The amounts and nature of the isomerisation products parallel those obtained from linoleic acid. It is found that about 5 % of both the acetate and diene are not isomerised even at 234°. It is possible that, in the original linoleic acid, some geometrical and positional isomers are present which either do not isomerise to conjugated compounds or do not isomerise at the same rate as the 9,12-*cis,cis* isomer.

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