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Determination of Linoleic and Linolenic Acids by Isomerization at Low Temperatures

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A catalyst system suitable for low temperature conjugation isomerization has been applied to the analysis of some linoleic and linolenic oils. Isomerization was carried out at 25°C and 60°C in 1,4-dioxane catalyzed by the potassium alkoxide of triethylene glycol monomethyl ether (KTGM). Reference curves for the absorptivities at 233 mμ and 269 mμ as a function of the time of isomerization were determined with the methyl esters of linoleic and linolenic acid. An isomerization time of 60 min was chosen for the analysis of some vegetable oils, and the calculated content of di- and triunsaturates was compared to that obtained by GLC.

Previous investigations by Ugelstad *et al.*[1–4] of the effect of the solvent on the base catalyzed conjugation isomerization of polyunsaturated fatty acid compounds revealed that isomerization might be carried out with sufficient rates even at room temperature. These catalysts include potassium *t*-butoxide in butanol with additions of dimethyl sulfoxide, dimethylformamide or tetramethyl urea,[1] potassium *t*-butoxide in diethyl ether with addition of various dimethoxy polyethylene glycols,[2] and some potassium methoxy polyethylene glycolates, dissolved in diethyl ether.[3]

The cocatalytic effect of the additives was ascribed to a specific solvation of the K^+ ion, thus rendering the RO^- ion in a more active state. In alcoholic solution, the ability of the additives to form hydrogen bonds with the OH groups, thus reducing the deactivating effect of alcohol on the butoxide ion, was also thought to play a dominant role.

In the potassium methoxy polyethylene glycolates the solvating oxygen atoms and the catalytically active $-O^-$ group are situated on the same molecule (self solvating catalysts), thus giving a more favourable entropy of solvation. This intramolecular solvation of the potassium ion probably accounts for the high reactivity of these ion pairs, the catalytic activity of which seems to be relatively independent of the solvent.[3,4]

When analyzing a fatty acid compound for the content of polyunsaturated acids by spectrophotometry, conjugation of the double bonds is usually

brought about by treating the compound with 6.6 – 21 % solutions of KOH in ethylene glycol at 180°C. The object of the present work has been to ascertain whether or not the self solvating catalysts may conveniently be used for analytical purposes at low temperatures. So far, investigations have been limited to linoleic and linolenic acid oils, although results obtained in this laboratory indicates that hexa-, penta- and tetraenoic acids may also be determined in this way.

EXPERIMENTAL

Reagents. Methyl linoleate, *puriss.* quality, was obtained from Fluka. Purity as determined by GLC was about 99 %. Content of trienoate was approximately 0.3 %.

Methyl linolenate, *puriss.* from Fluka. Purity as determined by GLC was about 99 %. Content of dienoate was approximately 0.3 %. Oils were commercial "on shelf" samples used without further refining. Methanol, Merck *p.a.*

1,4-Dioxane, "für Chromatographie" quality from Merck, was distilled from sodium wire to remove traces of water.

Triethylene glycol monomethyl ether (TGM) was Fluka, pract. grade, used without further purification.

Preparations. Potassium methoxy triethylene glycolate (KTGM) was prepared by reacting 30 g of TMG with excess potassium (15 g) in 450 ml dioxane at reflux temperature for 8 h. The cooled solution was filtered and 10 ml portions were transferred to nitrogen purged ampules which were sealed off. During the whole procedure, care was taken to exclude air by operating in nitrogen atmosphere. The concentration of KTGM, determined by titration with 0.1 N HCl, was found to be 0.3 mol/l.

Methyl esters of the various oils were prepared by reacting the glycerides for 2 h with 0.5 % NaOH in 100 % excess abs. methanol at reflux temperature. Following separation, neutralization and washing, water was removed in vacuum at 100°C.

Analysis. GLC analysis were performed on a Perkin Elmer Model F 11 equipped with a 50 m long, 0.25 mm wide PEGA coated column, operated at 170°C. Spectrophotometric determinations were carried out on a Hilger & Watts Ultrascan recording spectrophotometer. Samples were diluted in *p.a.* methanol and run against blanks containing the same amount of catalyst solution as did the sample.

Procedure. Isomerization reactions were carried out in 25 ml Erlenmeyer flasks fitted with glass stoppers. The flasks were equipped with horizontal closed-end glass tubes fused to the flasks half way up the wall. A micro beaker containing 40 – 50 mg of oil was placed in the tube. The flask was then thoroughly flushed with nitrogen, and 10 ml of catalyst solution (0.3 M KTGM in dioxane) was transferred under nitrogen blanket from the ampule to the bottom of the flask which was then placed in a thermostated bath. At zero time, the micro beaker was tipped into the catalyst solution and the flask was shaken vigorously. After the desired time isomerization was stopped by addition of 10 ml of methanol.

RESULTS AND DISCUSSION

Fig. 1 gives absorptivity *versus* time curves for methyl linolenate at 269 mμ (a°_{269}) and 233 mμ ($a^{\circ}_{T,233}$), and for methyl linoleate at 233 mμ (a°_{233}), obtained by isomerization at 60°C. Corresponding results for an isomerization temperature of 25°C are given in Fig. 2.

All absorptivities are corrected for the 99 % purity of the methyl esters, but no attempt was made to correct for the small amount of dienoate in the linolenate or for the trienoate in the linoleate.

It is seen that even at 25°C, isomerization takes place at a considerable rate. For comparison, the absorptivity constants obtained by various other

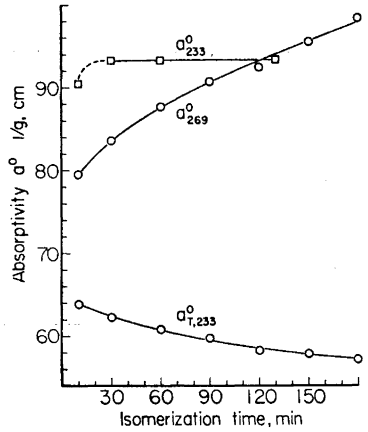

Fig. 1. Absorptivities as a function of time of isomerization. Linolenic acid methyl ester ($a°_{269}$, $a°_{T,233}$) and linoleic acid methyl ester ($a°_{233}$). 40–50 mg oil, 10 ml 0.3 M KTGM in 1,4-dioxane. Temperature 60°C.

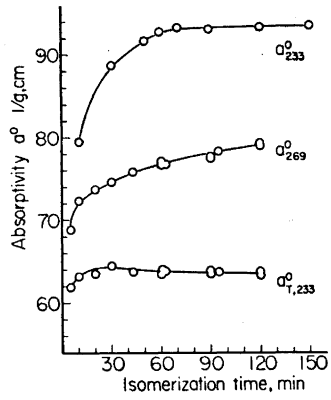

Fig. 2. Absorptivities as a function of time of isomerization. Linoleic acid methyl ester ($a°_{269}$, $a°_{T,233}$) and linoleic acid methyl ester ($a°_{233}$). 40–50 mg oil, 10 ml 0.3 M KTGM in 1,4-dioxane. Temperature 25°C.

methods are shown in Table 1 along with some of those found in the present work. The literature values are calculated to acid basis, and should therefore be divided by 1.05 when compared to the constants obtained in this work.

From the curves in Figs. 1 and 2 an isomerization time of 60 min was chosen at both 25°C and 60°C for the following analysis. Shorter reaction times may be used, although accuracy will probably decrease due to the increasing steepness of the curves at lower absorptivities. This is especially noticeable for the $a°_{233}$ values at isomerization times below 30 min.

Table 1. Comparison of absorptivity constants obtained by various methods of isomerization.

Method	Conditions	$a°_{269}$	$a°_{T,233}$	$a°_{233}$
A.O.C.S., Brice *et al.*[5]	6.6 %KOH/ethyl. glyc., 25 min, 180°C	50.7	61.6	92.2
Notevarp and Fyrst [6]	18 % KOH/ethyl. glyc., 8 min, 180°C	89	48	94
White and Quackenbush [7]	1.1 M potassium-*t*-butoxide/*t*-butanol, 20 h, 60°C	64.4	67.2	76.7
This work [a]	0.3 M KTGM/dioxane, 60 min, 25°C	76.8	63.8	92.7
This work[a]	0.3 M KTGM/dioxane, 60 min, 60°C	87.8	60.9	93.2

[a] Calculated to methyl ester basis.

Table 2. Analysis of known mixtures of methyl linolenate and methyl linoleate. About 50 mg methyl esters isomerized at 60°C for 60 min in 10 ml 1,4-dioxane containing 0.3 mol/l KTGM.

Weighed composition		Analyzed composition	
% linolenate	% linoleate	% linolenate	% linoleate
11.8	88.2	12.4	88.6
21.0	79.0	22.0	78.4
46.5	53.5	46.7	52.7
79.2	20.8	78.5	19.9
87.8	12.2	87.2	12.9

The following formulas for the calculation of the percentages of trienoate and dienoate in a sample are easily derived:

$$\% \text{ trienoate} = 100\ (a_{269}/a^{\circ}_{269})$$
$$\% \text{ dienoate} = (100\ a_{233} - (\% \text{ trienoate})a^{\circ}_{T,233})/a^{\circ}_{233}$$

where a_{269} and a_{233} are the experimental observed absorptivities for the sample.

Inserting the values obtained after 60 min isomerization in the above expressions one obtains:

Isomerization temperature = 60°C
$$\% \text{ trienoate} = 1.14\ a_{269}$$
$$\% \text{ dienoate} = 1.07\ a_{233} - 0.74\ a_{269}$$

Isomerization temperature = 25°C
$$\% \text{ trienoate} = 1.30\ a_{269}$$
$$\% \text{ dienoate} = 1.08\ a_{233} - 0.90\ a_{269}$$

The above equations were used to calculate the composition of five known mixtures of methyl linolenate and methyl linoleate from absorptivity values obtained by isomerization at 60°C. The results are given in Table 2. The average deviation in relative per cent from the weighed values is less than 3 %, the highest deviation for a single value beeing 5.8 % for the lowest value of linoleate.

Table 3 gives the results of the isomerization of some vegetable oils and their methyl esters at 25°C and 60°C. For comparison, Table 3 also includes the GLC values obtained on the methyl esters.

Spectra of the unisomerized oils revealed that most oils contained 0.1 % or less of conjugated acids. Exceptions were 0.2 % conjugated dienoate in the linseed oils and 0.5 % conjugated trienoate in the groundnut oils. These values were estimated using the absorptivity value of 190 for α-eleostearic acid given in Ref. 8. Due to the low content of trienoate in the groundnut oils, correction for the absorptivity of the preconjugated was made. The absorptivity used to calculate the content of trienoate was taken as the difference between the observed absorptivity for the isomerized oil and the absorptivity due to preconjugation. The percentages of trienoate found in the

Table 3. Content of di- and triunsaturates in various oils as determined by isomerization at 25°C and 60°C. 40 – 50 mg oil isomerized for 60 min in 10 ml 1,4-dioxane containing 0.3 mol/l KTGM. Last column gives composition as determined by GLC.

Oil	Per cent as methyl esters					
	Isomerization 25°C		Isomerization 60°C		GLC	
	Triene	Diene	Triene	Diene	Triene	Diene
Linseed oil methyl ester	48.2	14.1	48.2	14.4	49.2	14.6
Linseed oil methyl ester	48.3	14.4	48.6	14.0		
Linseed oil methyl ester			48.0	14.2		
Linseed oil methyl ester			48.5	14.3		
Average values			48.4	14.2		
Linseed oil			48.2	14.1		
Soybean oil methyl ester	8.8	52.4	8.5	52.8	8.4	53.1
Soybean oil methyl ester	8.5	52.7	8.6	52.7		
Soybean oil			8.7	52.5		
Sunflower seed oil methyl ester	0.4	64.1	0.5	62.8	0.35	63.4
Sunflower seed oil			0.5	62.6		
Groundnut oil methyl ester	0.9	23.7	0.9	22.8	0.4[a]	23.1
Groundnut oil			0.85	21.8		
Rapeseed oil methyl ester			9.4	14.7	9.6	14.3
Rapeseed oil			9.5	14.5		

[a] Conjugated not included.

groundnut oils are thus the sum of the preconjugated and the amount calculated as stated above. Due to difficulties of identification, the GLC value does not include preconjugated.

The results presented in the preceding tables indicate that the low temperature analytical isomerization operates within limits of accuracy and reproducibility that will probably be sufficient for many purposes.

The low temperatures employed in the present analysis offer the advantage of reducing the effect of side reactions, such as polymerization, cyclization and possible oxidative degradations as compared to the standard prodecures. If side reactions, that are second order with respect to the polyunsaturates, take place, results may depend on the composition of the oil. Thus, if reference absorptivities are obtained from pure substances, and a given amount of oil is isomerized, the percentage found for a given polyunsaturate will show a higher positive relative deviation from the true value the lower the content of the unsaturate.

Preliminary experiments have indicated that if the necessary reference absorptivities are determined, the low temperature isomerization may probably be extended to include the higher polyunsaturates present in, *e.g.*, fish oils or certain human lipids. In that case, the advantage of a low isomerization temperature with respect to side reactions would probably be more pronounced.

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