A Simplified Technique for Analysis by Alkali Isomerization[1]

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ALTHOUGH refinements in technique by various workers have improved the reliability of the original method of analysis by alkali isomerization (1), they have done little to simplify the equipment and operations which are necessary to obtain acceptable results. Use of an inert gas in the reaction vessel at 180° (2) has, in fact, made the analysis more cumbersome. One of the most objectionable features of the method however is the use of a 180° oil bath. An important step toward its elimination was made by Sreenivasan and Brown (3) in showing that potassium-t-butoxide can produce the conjugation at temperatures as low as 90°. Their findings resulted in little simplification of techniques however, since the reaction containers were either flasks fitted with air condensers (3) or sealed tubes (4).

In this paper we present a simplified technique, referred to as the "bottle method," in which the sample is isomerized with potassium-t-butoxide in a reagent bottle at 60° for 20 hrs.

Experimental

Preliminary tests showed that molar potassium-t-butoxide was capable of effecting isomerization at 60° and even lower temperatures. Absorptivity values were calculated for pure linoleic and linolenic acids after alkali isomerization with 1.1 M potassium-t-butoxide in t-butanol at 60° for 20 hrs., and these constants were used for a comparison of results with the A.O.C.S. Method Cd 7-58 (2) on three samples (Table I). These samples were a) a synthetic mixture of the methyl esters of palmitic, oleic, linoleic, and linolenic acids in proportions commonly found in soybean oil; b) soybean oil; and c) the esters obtained after methanolysis of the soybean oil.

TABLE I
Comparison of Methods

Sample analyzed	Linoleic acid		Linolenic acid	
	A.O.C.S.	Bottle	A.O.C.S.	Bottle
	%	%	%	%
Synthetic mixture of methyl esters[a]	42.4	42.6	7.44	7.36
Soybean oil	47.2	46.6	6.72	11.1
Methyl esters obtained after alcoholysis of soybean oil	47.5	48.2	6.75	7.17

[a] The mixture contained 12.4% palmitic, 30.9% oleic, 42.3% linoleic, and 7.41% linolenic acids.

For the synthetic mixture both procedures gave results consistent with the amounts of each of the polyunsaturated fatty acids known to be present. For soybean oil both procedures showed the same amount of linoleic acid present. However the potassium-t-butoxide reaction at 60° showed a much larger amount of linolenic acid than the A.O.C.S. method. On the other hand, when the soybean oil was subjected to methanolysis before analysis, this discrepancy in the amount of linolenic acid was no longer observed.

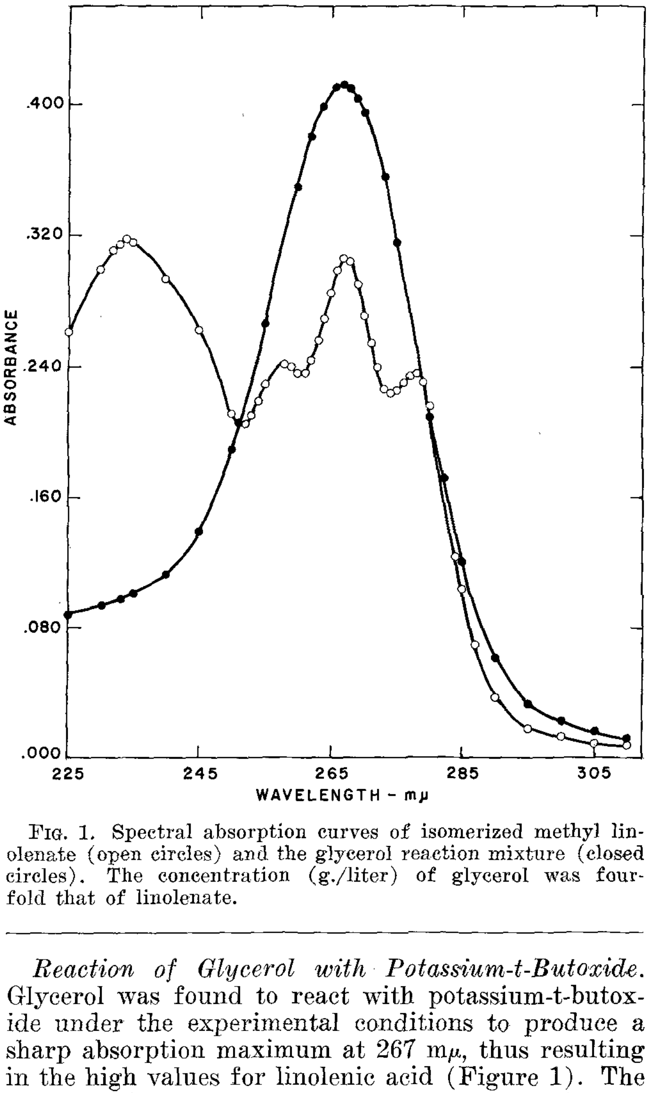

FIG. 1. Spectral absorption curves of isomerized methyl linolenate (open circles) and the glycerol reaction mixture (closed circles). The concentration (g./liter) of glycerol was fourfold that of linolenate.

Reaction of Glycerol with Potassium-t-Butoxide. Glycerol was found to react with potassium-t-butoxide under the experimental conditions to produce a sharp absorption maximum at 267 mμ, thus resulting in the high values for linolenic acid (Figure 1). The position of the absorption maximum of the glycerol reaction product was almost identical with that of the conjugated triene from isomerized linolenic acid. Its absorptivity was only about 30% as great however.

Reaction studies on the glycerol reaction product showed that the values at 268 mμ reached a maximum after 16–20 hrs. and then sharply declined (Figure 2). Because of the great variations in absorptivity after 16 hrs. it was not possible to ascertain a maximum value. The absorptivities at 233 mμ were low and increased only slightly during the 48-hr. time study.

In view of the considerable absorption of the glycerol reaction product in the region of conjugated triene in the present study, other methods of alkali isomerization were tested to observe whether they would produce a similar effect with glycerol (Table II). Absorption produced from glycerol in the A.O.C.S. method with different concentrations of KOH was negligible during standard periods and also during shorter periods of time. However the addition of glycerol to potassium-t-butoxide reagent

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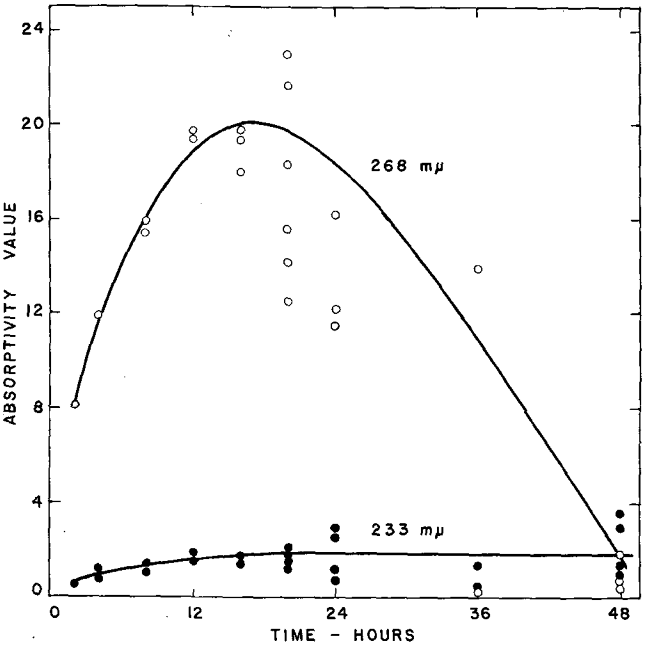

FIG. 2. Changes in absorptivity of glycerol reaction mixture with time.

resulted in an absorptivity of 4.8 at 268 mμ in the open-tube method of Sreenivasan and Brown (3) and an absorptivity of 17.6 in the bottle method. The reaction of glycerol with potassium-t-butoxide and with KOH at 60° for 20 hrs. resulted in absorptivity values which showed that potassium-t-butoxide rather than low temperature and length of isomerization was the active agent in producing the glycerol absorption at 268 mμ.

Treatment with hydrochloric acid, as described in a later section, was found to be an effective means of avoiding the glycerol effect. Tests showed that, at 2.5, 5, 10, and 30 min. after acidification, the absorptivity was reduced to the same degree.

After acidification the absorptivity declined from 17.6 to 0.7 with free glycerol and from 19.6 to 1.4 with an equivalent amount of glycerol released from triolein. The higher value for triolein probably reflected the presence of a small amount of polyunsaturated fatty acid in the triolein. Residual absorptivities seemed to be slightly higher than those obtained in the A.O.C.S. procedure. However, it appeared that, when the acidification step was included, the bottle reaction at 60° provided the basis for a simple alkaline isomerization technique for the determination of polyunsaturated fatty acids.

TABLE II

Reaction of Glycerol with Alkali in Different Methods

Method	Conditions	Absorptivity			
		Glycerol		Glycerol from triolein	
		233 mμ	268 mμ	233 mμ	268 mμ
A.O.C.S.	25 min., 6.6% KOH, 180°	.08	.12	3.2	.85
A.O.C.S.	15 min., 21% KOH, 180°	.00	.00	2.4	.53
Sreenivasan and Brown	4 hrs., 17.8% KtB,[a] 90°	1.2	4.8	4.4	5.6
Bottle	20 hrs., 15.5% KtB 60°	1.7	17.6	4.6	19.6
Bottle	20 hrs., 15.5% KtB 60° (acidified)	1.3	.7	4.7	1.4
Bottle	20 hrs., 6.6% KOH, 60°	.00	.00	....	....

[a] KtB = potassium-t-butoxide.

Isomerization of Linoleic and Linolenic Acids with Potassium-t-Butoxide. Absorptivity values of high quality methyl linoleate (Hormel) and methyl linolenate (Hormel) were calculated for the free acids after isomerization for various lengths of time (Figure 3). The absorptivity of linoleic acid rose rapidly at first and then increased slowly. The maximum value had not been reached even at the end of 72 hrs. Maximum absorptivity values for linolenic acid at 233 mμ and 268 mμ were attained much more quickly, and both maxima decreased with time. The absorptivity of isomerized linolenic acid was always greater at 233 mμ than at 268 mμ. Accordingly there is no evidence that, at 60°, conjugated diene is a precursor of conjugated triene as was observed by Sreenivasan and Brown with potassium-t-butoxide at 90° (3). From these data an isomerization period of 20 hrs. was selected as a reasonable compromise between the rising values for linoleic acid and the decreasing values for linolenic acid. The importance of timing for the reaction was therefore not as critical by this method as with the A.O.C.S. method.

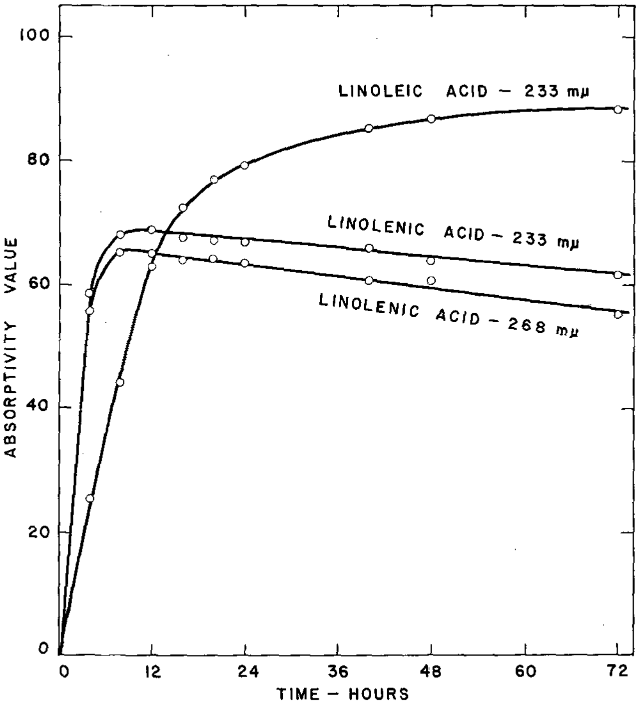

FIG. 3. Absorptivity values after isomerization for various lengths of time. Methyl linoleate and methyl linolenate both calculated to acid basis. All readings were made in absolute methanol.

A comparison is shown of the published absorptivity values (5) for the A.O.C.S. 6.6% KOH-glycol method with those for the bottle method (Table III).

TABLE III

Constants for A.O.C.S. and Bottle Methods

Acid	a_{233}		a_{268}	
	A.O.C.S. (lit.)	Bottle	A.O.C.S. (lit.)	Bottle
Linoleic	92.1	76.7	....	....
Linolenic	61.6	67.2	50.7	64.4

Since maximum precision in fat analysis by alkali isomerization can generally be expected to result from those procedures which yield the highest degree of conjugation with pure acids, the bottle method should have a slight advantage in the analysis of linolenic acid. This is desirable for the analysis of soybean oil or other oils which are low in linolenic acid.

The Bottle Method

Preparation of Isomerizing Reagent. Tertiary butanol was dried by refluxing over calcium hydride, then the fraction boiling at 82–83° was collected. Potassium sticks were cleansed by immersion, first in heptane to remove the mineral oil, and then in dry tertiary butanol. The slow reaction of the latter solvent with potassium removed the oxidized layer on the surface of the metal and thus eliminated the need for cutting it under an inert solvent. The amount of potassium (5 g. in 100 ml. of t-butanol) needed to provide approximately 17% of the alkoxide was allowed to react with t-butanol for 6 hrs. Any excess potassium, if present, was then removed by decantation or by filtration through glass wool. An aliquot was titrated with 1 M HCl, using phenolphthalein as indicator, and the solution was then diluted to 1.1 M (15.5%) potassium-t-butoxide in t-butanol.

Procedure. About 100 (±5) mg. of fat were weighed accurately into a 60-ml. glass-stoppered Pyrex reagent bottle, then blanketed with nitrogen. Following the addition of 10 ml. of the isomerizing reagent, the bottle was stoppered and swirled to insure complete mixing and placed in a forced-draft 60° oven for 20 hrs. Use of a forced-draft oven was a safety precaution to prevent accumulation of vapor in case of breakage or leakage from reaction flasks. The reaction was terminated by setting the bottles in a cold-water bath. An appropriate aliquot from the reaction mixture which had been diluted to 100 ml. with methanol or ethanol was pipetted directly into 10 ml. of 6 M HCl contained in a 100-ml. volumetric flask. The contents were swirled and 15 min. later were diluted to volume with methanol. Ethanol was not a suitable diluent after acidification since it produced a white precipitate.

Light absorption at 233 mμ and 268 mμ was measured with a Beckman DU spectrophotometer, and the absorptivity values were calculated. The absorptivity of the glycerol reaction mixture after acidification was subtracted from the observed value. A correction for background absorption with the equation from the A.O.C.S. method was made on isomerized cottonseed and corn oils. The corrected absorptivity values were then placed in the following equalities, which were derived by substituting the constants of the bottle method (Table III) in two simultaneous equations:

$$\%\ \text{linolenic acid} = 1.553\ a_{268}$$
$$\%\ \text{linoleic acid} = 1.304\ a_{233} - 1.360\ a_{268}.$$

Fat Analysis. The bottle method as finally adopted was compared with the A.O.C.S. 6.6% KOH-glycol method in the analysis of five natural oils (Table IV).

TABLE IV

Comparison of Methods Applied to Oils [a]

Oil	Linoleic acid		Linolenic acid	
	A.O.C.S.	Bottle	A.O.C.S.	Bottle
	%	%	%	%
Cottonseed	46.8 ± .11	46.6 ± .49	0	0
Corn A extract[b]	50.1 ± .10	50.8 ± .32	.54 ± .014	.89 ± .053
Corn B extract,[b] high carotene[c]	58.4	58.4	.63	1.03
Corn C extract,[b] low carotene[c]	63.4	61.9	1.45	1.78
Wheat germ	48.9 ± .34	47.7 ± .32	6.44 ± .026	7.46 ± .12
Soybean	46.9 ± .19	46.1 ± .29	6.72 ± .029	7.07 ± .01
Linseed	13.5	13.4	49.9	50.0

[a] The oils, except for corn, were obtained from commercial sources. Spectrophotometric tests showed only trace amounts of conjugated bonds. The standard errors of the mean shown are based on averages of at least five analyses. Other values show simple averages of duplicate determinations.

[b] Whole kernels of corn were ground and then extracted with purified hexane.

[c] Residual oil after molecular distillation. Corn B and Corn C had a carotene content of 1900 and 15 μg./g. of extract, respectively.

The percentage of linoleic acid found by the bottle method agreed well with the comparable values found by the A.O.C.S. method; however the percentage of linolenic acid was slightly higher with the former than with the latter procedure. It is uncertain whether these small discrepancies are caused by real differences in the two methods or whether they reflect a dissimilarity in the purity of the linoleic and linolenic acids used as standards or indicate that some of the triene measured in the bottle method is coming from factors other than linolenic acid. Fresh extracts of ground corn from three sources showed an absorption maximum in the conjugated triene region after alkali isomerization, and this was not eliminated by molecular distillation at 175° for 1 hr.

Mildly oxidized linolenic and linoleic acids, when heated in ethylene glycol either with or without KOH, have been shown to give rise to absorption bands characteristic of conjugated tetraenoic and trienoic fatty acids, respectively, thus indicating polyunsaturated fatty acids which are not present (6). However, in this study, a slightly autoxidized extract of

TABLE V

Spectrophotometric Analyses of Some Samples After Various Treatments

Sample	Treatment	Absorptivity			
		233 mμ	262 mμ	268 mμ	274 mμ
Fresh extract of ground corn A	None	.15	.053	.066	.079
	Heated [a] in t-butanol	.13	.047	.055	.063
	Bottle method	38.0	.91	.98	.77
Slightly autoxidized corn A extract (PV = 8)	None	.22	.051	.059	.070
	Heated in t-butanol	.22	.044	.051	.059
	Bottle method	38.5	.87	.94	.73
Autoxidized ethyl linoleate (PV = 78)	None	......	.074	.070	.060
	Heated in t-butanol	2.58	.088	.079	.069
	Bottle method	61.7	.92	.86	.76
Glycerol	Bottle method	......	.77	.72	.72

[a] 20 hrs. at 60°.

corn or pure ethyl linoleate autoxidized to a peroxide value of 78, when heated in t-butanol without added potassium, did not form an absorption maximum at 268 mμ (Table V). Furthermore the 20 hours of heat treatment did not produce any oxidation-conjugation in fresh extracts of corn. The samples from corn showed a maximum at 268 mμ only after heat treatment with alkali.

Gas-liquid chromatography of the methyl esters prepared from Corn C extract separated an amount of linolenic acid ester in agreement with the alkaline isomerization treatments. The experimental conditions were: flow rate, 70 ml. of helium/min.; temperature, 218°; sensitivity, 250 milliamperes; column (5 ft., ½ in. in diam.), Craig butanediol succinate. However this procedure did not clearly define a peak for methyl linolenate in the esters prepared from Corn A extract.

Summary

Potassium tertiary butoxide (1.1 M, 15.5%) in t-butanol readily isomerized linoleic and linolenic acids at 60°C. The absorptivity value for linoleic acid during isomerization rose rapidly, then slowly reached a maximum (88.5) after three days. Maximum absorptivity values for linolenic acid at both 233 mμ (68.5) and at 268 mμ (65.0) were attained at 12 hrs.; thereafter absorptivity at both wavelengths decreased with time.

Glycerol also reacted with potassium-t-butoxide under the experimental conditions to produce an absorption maximum at 267 mμ; however this absorbance was easily destroyed by treatment with hydrochloric acid.

A simple procedure has been described for isomerization with the alkoxide in a reagent bottle at 60° for 20 hrs. Analyses for linoleic and linolenic acids in five seed oils isomerized by the "bottle method" agreed well with results obtained by the A.O.C.S. KOH-glycol method.

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Acid-Catalyzed Addition of Phenols and Phenyl Ethers to Oleic Acid[1]

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OUR WORK on the addition of nitriles to unsaturated compounds (1, 2) suggested that other nucleophilic substances should add to the reactive center of oleic acid in strong acid media, that is, in the presence of carbonium ion-producing substances. In this paper we are reporting the addition of phenols and phenyl ethers to oleic acid in a sulfuric acid medium and also in the presence of an acid ion-exchange resin catalyst. We are also reporting on the structure of the reaction products based on infrared absorption spectra.

Although isolated instances of reactions between phenols and unsaturated compounds have been known for a long time, Niederl and co-workers (3) examined the reaction more closely and applied it to several types of unsaturated compounds. Later m-cresol (4) and phenol (5) were added to oleic acid in the presence of concentrated sulfuric acid. Stirton and co-workers (6) have reported the addition of aryl ethers to oleic acid, using aluminum chloride as the condensing agent. More recently Gisser and co-workers (7) published their work on the preparation of hydroxyarylstearic acid. In one instance, with sulfuric acid as the condensing agent, p-tert.-butylcatechol added to oleic acid to form 9(10)-(2,3,-dihydroxy-5-tert.-butyl)phenylstearic acid. Inasmuch as derivatives of this type are of interest as lubricant additives, we hoped to broaden the scope of the reaction to include other phenolic materials and, in the cases reported, to increase the yields.

By adaptation of the methods found successful in our earlier work with nitriles (1, 2), other phenolic materials than were previously reported have now been added to oleic acid in a sulfuric acid medium. When it became apparent that the limiting factor in the yields of products was by-product formation owing to polymerization or other reaction, different condensing agents were sought. Recently Loev and Massengale (8) used sulfonic acid cation-exchange resins as catalysts for the alkylation of phenol with olefins. While their investigations were with the relatively more reactive olefins, such as isobutylene and 1-nonene, we were able to obtain condensations with oleic acid, provided sufficient reaction time was allowed and a sufficient quantity of the appropriate resin catalyst was used. No attempt was made to examine a wide variety of resins.

Experimental

Starting Materials. Oleic acid (93.3%) essentially free of polyunsaturated acids was prepared from commercial oleic acid by one low-temperature crystallization and fractional distillation. The phenols and phenyl ethers were the best commercial grades and were used as received.

Infrared Spectra. Infrared spectra were obtained on a Perkin Elmer Model 21 double beam recording spectrophotometer. For general spectra a demountable cell consisting of sodium chloride prisms (one

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