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Isomerization of Linoleic, Linolenic, and Other Polyunsaturated Acids with Potassium Tertiary Butoxide and Its Application in the Spectrophotometric Estimation of These Acids

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THE RECENT METHODS (1–5) of estimating polyunsaturated acids are based on the observations that conjugated polyunsaturated acids have definite absorption peaks in the ultraviolet region of the spectrum (6, 7, 8) and that acids having double bonds isolated by only one methylene group can be converted to the conjugated isomers by heating with alkali in water, alcohols, or ethylene glycol (9, 10, 11). Heating the unsaturated acids with potassium hydroxide in ethylene glycol is the most widely accepted procedure for effecting such a conjugation. The mechanism of isomerization has been shown to be due to the attack of the base on the methylene group between the double bonds (12, 13, 14) and subsequent allylic rearrangement of the carbanion to a conjugated system. Thus with linoleic acid both the conjugated acids I and II have been isolated (14):

$\longleftarrow$ $CH_3(CH_2)_4-CH=CH-CH_2-CH=CH-(CH_2)_7COOH$

Linoleic Acid

$\longrightarrow$ Attack of base $CH_3(CH_2)_4\cdot CH=CH-\underset{\ominus}{CH}\cdot CH=CH-(CH_2)_7COOH$

Linoleic Acid Carbanion

$\longrightarrow$ Rearrangement with 9,10 double bond $CH_3(CH_2)_4CH=CH-CH=CH-CH_2(CH_2)_7COOH$ – I

Conjugated Diene

$\longrightarrow$ Rearrangement with 12,13 double bond $CH_3(CH_2)_4CH_2-CH=CH-CH=CH(CH_2)_7COOH$ – II

Conjugated Diene

It is generally assumed that hydroxide ion ($OH^\ominus$) is the base that brings about this isomerization. It occurred to us that, under the anhydrous conditions of isomerization in ethylene glycol, the conjugated base of the solvent, $HOCH_2-CH_2-O^\ominus$, is likely to be the active base responsible for the rearrangement. It follows logically that a more powerful base should be able to bring about the reaction at a lower temperature and possibly take it to completion. In fact, Abu Nasr and Holman (15) have shown that by use of sodamide in liquid ammonia conjugation of polyunsaturated acids can be effected at as low a temperature as —32°C. However under these conditions the isomerization occurred to a limited extent with such side reactions as the formation of amides. Further considerations on these lines led us to believe that a tertiary base could bring about these isomerizations with less side reaction. This paper reports the results of the attempts to isomerize linoleic, linolenic, and arachidonic acids with potassium tertiary butoxide as the base.

Experimental

Materials Used

Linoleic Acid Concentrate. This was prepared by Mrs. Orians in this laboratory by low temperature crystallization of corn oil fatty acids: I. V., 170.4; about 82% linoleic acid.

Linolenic Acid Concentrate. A concentrate obtained from linseed oil fatty acids was recrystallized from pentane at —70°: I. V., 260.0; about 90% linolenic acid.

Methyl Arachidonate Concentrate I. This was prepared by Robert Hook in this laboratory by the urea adduct procedure from methyl esters of beef suprarenal phosphatides: I. V., 305.0; about 78% arachidonate and 10% pentaenoate.

Methyl Arachidonate Concentrate II. This was prepared by M. F. White (16) by fractional distillation of a concentrate obtained by a chromatographic procedure: I. V., 318.4; mean mol. wt., 317.9; preconjugated diene, 4.6%; $k_{10.36\mu}$, 0.229.

Pure Linoleic Acid, Matthews *et al.* (17). This was prepared by 12 recrystallizations of debromination linoleic acid: I. V., 181.0; polybromide no., 102.9;

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mol. wt., 280.9; n^{20} 1.4699; m.p., —5.2 to —5.0°; preconjugated diene, 0.2%; preconjugated triene, nil; nonconjugated triene. nil. $K_{10.36\mu}$, 0.171.

Pure Linolenic Acid, Matthews *et al.* (17). This was prepared by 14 recrystallizations of debromination linolenic acid: I. V., 273.8; hexabromide no., 96; mol. wt., 278.1; n^{20}, 1.4800; m.p., —11.3 to —11.0°; preconjugated diene, 0.4%; preconjugated triene, nil; preconjugated tetraene, nil. Nonconjugated diene, nil. Nonconjugated tetraene, nil; $k_{10.36\mu}$, 0.193.

Olive, Cottonseed, and Linseed Oils. These were commercial unrefined oils; soybean oil was an alkali-refined specimen.

Methanol. This was of the same purity as for the standard spectrophotometric procedure.

Tertiary Butanol. Technical-grade, fractionally distilled and the fraction distilling at a constant boiling point of 83° was collected and used.

Potassium. This was Baker's Purified Lump.

Description of Method

Apparatus. Isomerization was carried out in a 150- x 25-mm. Pyrex test tube, connected to a 750-mm. air condenser by means of a 24/40 ₸ joint. The heating bath was a two-liter Pyrex beaker containing mineral oil, the height of which was so adjusted that it was about the same or slightly higher than the level of the liquid inside the tubes when about 7 tubes were inserted into the bath.

Reagent. A piece of potassium was cleaned while immersed in heptane by shaving off the sides and the desired quantity was weighed. The weighings were all done in heptane and are accurate to ± 0.1 g. The weighed pieces were lifted out, heptane was allowed to drain, and then they were transferred to a 125-ml. stoppered flask containing 100 ml. of tertiary butanol. The flask was covered with a liter beaker and allowed to stand over-night. This procedure was found to be the most convenient for dissolving the desired quantities of potassium, which reacts very slowly. For convenience the concentrations will be referred to in terms of the weight of potassium dissolved in 100 ml. of tertiary butanol, *e.g.*, 5% of potassium-tertiary butanol means 5 g. of potassium dissolved in 100 ml. of tertiary butanol. The actual concentrations of the 5% reagent may be variously expressed as follows:

As KOH			As Potassium Tertiary Butoxide		
g./100 ml. of reagent	g./100 g. of reagent	Molar concentration	g./100 ml. of reagent	g./100 g. of reagent	Molar concentration
7.3	8.9	1.3	14.5	17.8	1.3

Procedure. The isomerization tube was flushed out with nitrogen, and 10 ml. of the reagent were added. About 100 mg. of the sample (80 mg. with arachidonate) were weighed in a small Pyrex cup and dropped into the tube. The air condenser was also flushed out with nitrogen and fitted onto the tube. The free end of the condenser was covered with a small test tube. The whole assembly was then transferred to the heating bath, which had been previously brought to the desired temperature (92–95° with 5% of potassium-tertiary butanol reagent). The solution began boiling after about three minutes of heating, and the time of reaction was recorded from here on. When a number of tubes were inserted, it was necessary to raise the flame of the burner to maintain the bath temperature at about 95°. It was found desirable to keep the bath temperature about 5° higher than the boiling point of the solution so as to assure a gentle and free boiling. The temperature of isomerization, the boiling point of the reagent, was noted by suspending a thermometer in a tube containing 10 ml. of the reagent and a small Pyrex cup, heated in the same bath. A blank experiment was conducted alongside. Two minutes after the contents of the tube started boiling, it was lifted and swirled to insure good mixing. After reacting for the desired period (4 hrs. in the finally recommended method), the tubes were lifted out and dipped into a water bath so as to be quickly cooled to room temperature. The solution was transferred to a 100-ml. volumetric flask with purified methanol in the usual way. After suitable dilutions the optical density measurements were made with a Beckman Model DU spectrophotometer.

Effect of Concentration and Time of Reaction. A study of isomerization of linoleic acid was carried out with 2, 3, 4, and 5% potassium-tertiary butanol solutions for periods varying from 1 to 10 hrs., and the results are shown in Figure 1. The boiling points of the reagents were 84°, 85–86°, 86–88°, and 88–90°, respectively. Concentrations above 5% could not be prepared in a homogenous solution form; with 6% the reagent caked up and did not give a clear solution even at reflux. It is thus obvious that 5% is near the saturation point. With linolenic and arachidonate concentrates a time study with the 5% reagent was conducted, and the results are represented in Figures 2 and 3. In order to fix a time of reaction

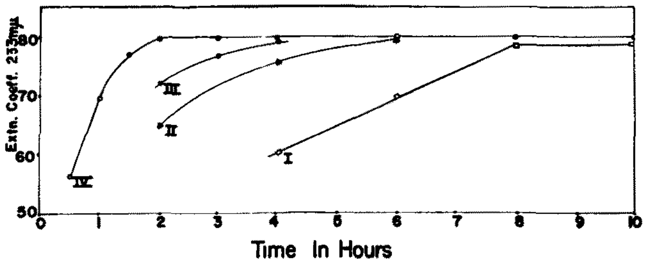


FIG. 1. Effects of time and concentration on the isomerization of linoleic acid concentrate by potassium tertiary butoxide.

I. . . . 2% potassium in tertiary butanol
II. . . . 3% potassium in tertiary butanol
III. . . . 4% potassium in tertiary butanol
IV. . . . 5% potassium in tertiary butanol

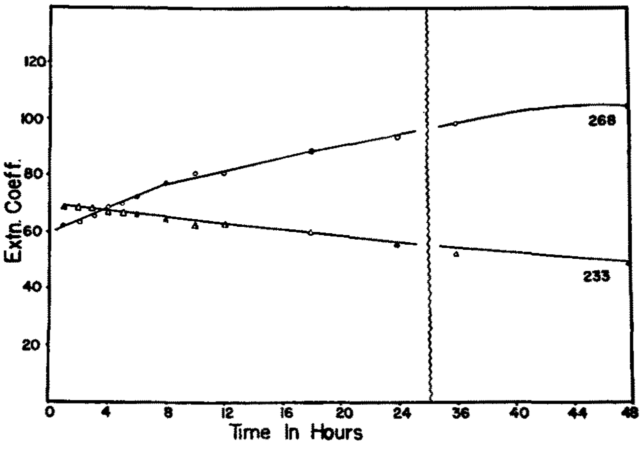


FIG. 2. Effect of time on the isomerization of linolenic acid concentrate by 5% potassium-tertiary butanol reagent.

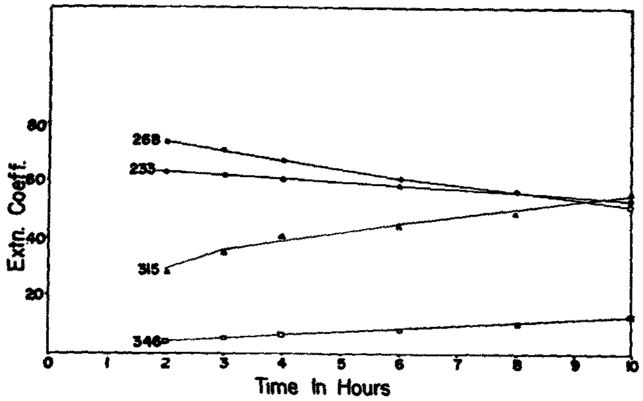


FIG. 3. Effect of time on the isomerization of methyl arachidonate concentrate I by 5% potassium tertiary butanol reagent.

a comparative study by the standard 6.6% potassium hydroxide-glycol and 21% potassium hydroxide-glycol methods was carried out on these concentrates. The results, together with those obtained for three different periods of reaction by the 5% potassium-tertiary batanol method, are given in Table I.

TABLE I

Extinction Coefficients at Wavelengths Maxima for Polyunsaturated Acid Concentrates

Concentrate	Wave-length mμ	Method				
		6.6% KOH glycol	21.0% KOH glycol	5% Potassium-t-butanol		
		25 min.	15 min.	2 hours	4 hours	6 hours
Linoleic acid	233	75.5	75.3	79.6	79.5	79.4
	268	1.3	2.6	—	2.3	—
Linolenic acid	233	63.6	50.7	69.1	67.7	66.7
	268	47.3	81.4	63.1	68.3	74.2
	315	1.6	3.2	—	2.9	—
Methyl arachidonate (I)	233	53.8	39.1	63.4	60.5	58.5
	268	43.8	41.3	74.0	67.1	61.0
	315	21.2	56.0	29.0	41.9	45.0
	346	2.7	9.6	3.7	6.8	8.8

Studies with Pure Acids. From the above study a time of reaction of 4 hrs. was chosen finally in order that the complete determination could be carried out in the course of one day. Pure linoleic and linolenic acids and the methyl arachidonate concentrate II were examined by these methods: the standard 6.6% potassium hydroxide-glycol 25 minutes, the 21% potassium-hydroxide-glycol 15 minutes, and the present 5% potassium-tertiary butanol 4 hrs. The results are given in Table II.

TABLE II

Extinction Coefficients for Pure Acids Isomerized by Different Methods

Acid	Wave-length of maxima mμ	6.6% KOH glycol 25 min.	21.0% KOH glycol 15 min.	5% Potassium-t-butanol 4 hours
Linoleic acid	233	90.9, 91.0	91.9, 91.3	94.1, 94.3, 93.5
Linolenic acid	233	59.1, 59.6	47.1, 47.1	62.7, 63.1, 63.9
	268	50.2, 50.9	90.3, 91.0	73.5, 74.2, 74.9
Methyl arachidonate (II)[a]	233	54.9, 55.5	39.2, 39.2	62.0, 62.8, 62.6
	268	47.8, 48.2	41.0, 41.4	68.9, 68.8, 68.7
	315	23.8, 24.3	59.2, 59.2	38.7, 38.4, 39.2
	346	2.5, 2.5	9.2, 9.2	5.2, 5.3, 5.6

[a] Concentrate containing about 10% pentaenoate, presented here because the absorption spectrum of this was taken along with the other acids and supposedly (16) contains only C_{20} and possibly C_{22} acids and no C_{18} acids.

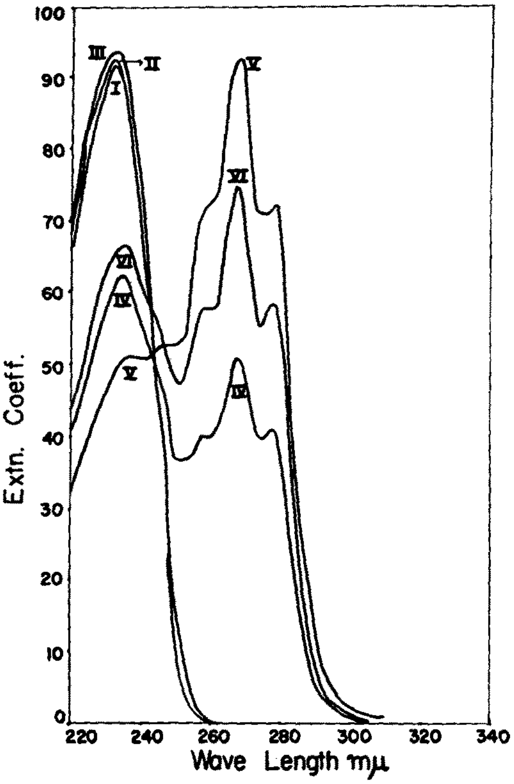


FIG. 4. Absorption curves for pure linoleic and linolenic acids isomerized by different methods.

Linoleic Acid	I. . . .	6.6% KOH-glycol
	II. . . .	21.0% KOH-glycol
	III. . . .	5.0% K-t-butanol
Linolenic Acid	IV. . . .	6.6% KOH-glycol
	V. . . .	21.0% KOH-glycol
	VI. . . .	5.0% K-t-butanol

The absorption spectra from 200–360 mμ were taken for these acids and are recorded in Figures 4 and 5. As a pure sample of arachidonic acid was not available, only results obtained with two concentrates of methyl arachidonate are reported, mainly to show the extent of isomerization obtained by the present method as compared to the other two methods.

Calculations. The average k_{233} value for linoleic acid from Table II by the proposed method is 94.0, and the k_{233} and k_{268} values for linolenic acid are 63.2 and 74.2, respectively. Substituting these in two simultaneous equations, the following relationships are derived for calculating the contents of linoleic and linolenic acids.

$$\% \text{ linoleic acid} = 1.064\ k_{233} - 0.906\ k_{268}$$

$$\% \text{ linolenic acid} = 1.348\ k_{268}$$

Some typical samples of oils such as olive, cottonseed, soybean, and linseed, containing linoleic and linolenic

TABLE III

Analysis of Samples of Oils by Different Methods[a]

Sample of oil	Component acid	Method		
		6.0% KOH glycol	21.0% KOH glycol	5% Potassium-t-butanol
Olive oil	Linoleic	4.9, 5.1	5.8, 5.7	5.5, 5.5
	Linolenic	0.5, 0.5	0.7, 0.6	1.3, 1.4
Cottonseed oil	Linoleic	47.2, 47.8	49.8, 49.7	49.5, 49.2
Soybean oil	Linoleic	27.8, 27.8	29.8, 29.7	30.6, 30.6
	Linolenic	5.7, 5.8	5.6, 5.4	6.6, 6.4
Linseed oil	Linoleic	13.1, 13.3	14.8, 15.1	14.0, 14.3
	Linolenic	37.8, 38.0	35.8, 35.6	38.4, 38.0

[a] Results expressed as % acid in the sample.

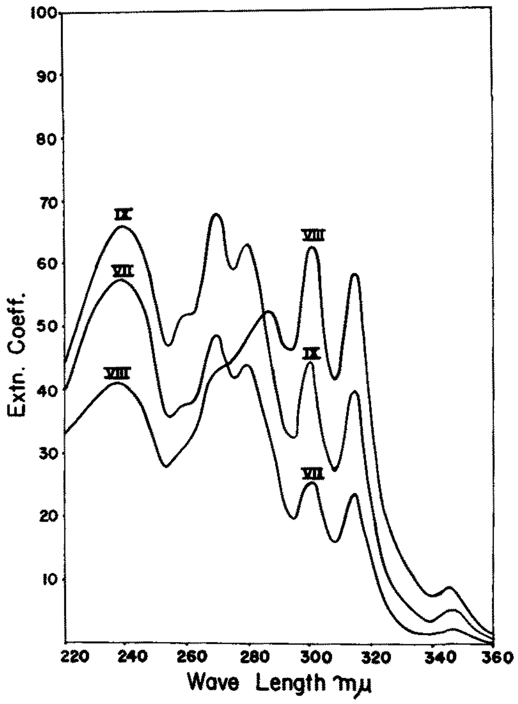


FIG. 5. Abstorption curves for methyl arachidonate concentrate II isomerized by different methods.

VII. . . . 6.6% KOH-glycol
VIII. . . . 21.0% KOH-glycol
IX. . . . 5.0% K-t-butanol

acids in varying proportions, were analyzed by all the three methods mentioned above and the results are given in Table III.

Results and Discussion

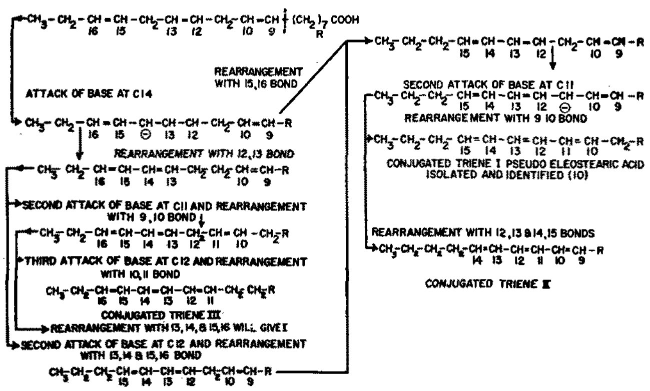
The time and concentration study on linoleic acid concentrate shows that with increasing concentration of the reagent the time required to attain a steady state of maximum possible conjugation is reduced greatly. It must be pointed out that, unlike similar studies using KOH-glycol reagent (3, 18) where the temperature remained constant at 180°, the temperature in the present study rises from 83°–90° at the several concentrations. Therefore this great reduction in time for attaining optimum conditions is due to both the concentration of the reagent and the higher temperature with the probability of the latter exerting a greater influence. With the 5% potassium-t-butanol reagent the optimum state is attained after 2 hrs. of reaction and thereafter stays constant even up to 10 hrs. of reaction, thereby demonstrating the remarkable stability of the conjugated diene to a powerful base and the absence of side reactions such as polymerization. The use of tertiary amyl alcohol (b.p. 100°) suggests itself as an appropriate substitute for t-butanol as a means of raising the temperature of reaction. Unfortunately with this alcohol, even with 2% potassium, a turbid solution was obtained. Studies with this reagent on linoleic acid concentrate showed that the extinction coefficient at the end of 2 hrs. (k_{233}, 78.3) was about the same as with the 5% potassium-t-butanol reagent (79.2). The use of this alcohol was not investigated further because of the uncertainty about the amount of active reagent present when turbid solutions are pipetted.

With linolenic acid concentrate the k_{268} value still rises even after 48 hrs. of isomerization while the k_{233} values decline steadily, a fact which is in accordance with observed facts that such conjugated trienoic acids as α- and β-eleostearic acids have low absorption in the diene region (6, 20, 21, 22). A similar situation is observed with the methyl arachidonate concentrate, which contained about 10% pentaenoate, where the k_{315} and k_{346} values, respectively, show a steady rise even after 10 hrs. of isomerization with a powerful base while the corresponding absorption coefficients in the diene and triene regions decline steadily. This is also in accordance with the observed fact that parinaric acid, the naturally occurring conjugated tetraenoic acid, has a low absorption in the diene and triene regions, respectively (8). The reason why still considerable k_{233} and k_{268} values are obtained with these polyunsaturated acids (triene, tetraene, pentaene) will be discussed later. A point of interest is that in the early stages of isomerization the k_{233} value for linolenic acid concentrate is higher than the k_{268} value, and with arachidonate the k_{315} value rises above those of k_{233} and k_{268}, respectively, after 10 hrs. of isomerization only, pointing out thereby that with these acids the isomerization occurs stepwise. In other words, with linolenic acid the conjugated diene is formed first, and this isomerizes later to the conjugated triene. Similarly with arachidonic acid, the isomerization proceeds through the conjugated diene and triene, respectively.

A glance at Table I reveals that with linoleic and linolenic acid concentrates the k_{233} and k_{268} values, respectively, are of a reasonably high order at the end of only 2 hrs. as compared to the corresponding values by the 6.6% KOH-glycol and 21.0% KOH-glycol methods. With methyl arachidonate the k_{315} and k_{346} values, which are of primary interest, are higher than those by the 6.6% KOH-glycol method and well below those by the 21% KOH-glycol method. In order that the accuracy of estimating such polyunsaturated acids as arachidonic, pentaneoic, etc., by the proposed method may be high and at the same time that it should not be too time-consuming, a period of reaction of 4 hrs. is finally settled upon as at this time the absorption coefficients with all polyunsaturated acids at all wave length maxima are reasonably high. The point is appreciated better when one looks at the absorption curves of the pure acids (Figures 4 and 5). With linoleic acid there is not much to choose between the three methods. Surprisingly enough, the k_{233} values here are maximum at about the same level. The curves overlap each other, excepting at the maximum which also occurs at the same wavelength 233 mμ. The extinction coefficients recorded by previous investigators for some alkali-conjugated isomers of linoleic acid vary from 93.5–122 (12, 14, 19, 23, 24, 25) although the generally accepted value for calculating preconjugated diene is 114 (1, 19). Assuming this value, the proportions of conjugated diene in the isomerized pure linoleic acid are 79.8% (6.6% KOH glycol), 80.4% (21% KOH-glycol), and 82.5% (5% potassium-t-butanol), respectively. This confirms the previously made point that the optimum conditions (maximum possible conjugation) are attained at about the same level of conjugation. The presence of considerable quantities of non-isomerized acid demonstrates that these isomerizations are equilibrium reactions subject to the usual laws of chemical kinetics, with the formation of the carbanion from the original acid as the rate-controlling step.

With linolenic acid the absorption curves by the potassium-t-butanol method parallels that by the 6.6%

CHART 1
STEPWISE BASE CATALYZED ISOMERIZATION OF LINOLENIC ACID



1. Similar steps can be written for the formation of conjugated trienes I, II, and III and a fourth conjugated triene, 12,13, 14,15, 16,17, by the initial attacks of base on methylene groups at carbons 11 and 17, respectively. Some of the intermediate dienes in the latter case (initial attack at C 17) require a fourth attack of base before conjugated trienes are formed.

2. In most of the steps above it is presumed that the rearranged carbanion picks up a proton from the solvent, and therefore the structure of the final product is written.

3. Only those rearrangements leading to the formation of conjugated trienes are presented, the point borne in mind being that with increasing time of reaction the absorption at 268 mμ increases, attesting to the formation of these trienes.

KOH-glycol method in both the diene and triene regions while this similarity is restricted to the triene region only with the 21% KOH-glycol method. With all methods the triene peak is at 268 mμ. However the peak in the diene region is shifted slightly towards longer wavelengths with all the methods: 6.6% KOH-glycol, $k_{235} = 62.1$; 5% potassium-t-butanol, $k_{236} = 66.8$; 21% KOH-glycol, $k_{238} = 51$. While well-defined peaks occur in this region with the first two methods, there is only a slight hump with the last method. This point has been noted by previous investigators (3) of this method, and the reason for this anomaly is not clear. The k_{233} values (Table II) by the 6.6% KOH method and the proposed method agree well although the latter is slightly high, but the corresponding value by the 21% KOH method is much lower, thus showing the presence of larger proportions of conjugated diene by the first two methods. The k_{268} value by the proposed method is intermediate between those of 6.6% KOH and 21% KOH methods, respectively. It is known that the conjugated triene formed by alkali isomerization is similar to β-eleostearic acid (10, 12). By use of the recently reported (21, 26) k_{268} and k_{233} values, 202.0 and 26.1, respectively, for β-eleostearic acid and the reported k_{233} value (1, 19) for conjugated diene, 114.0, the approximate proportions of conjugated diene and triene in the final isomerized linolenic acid can be calculated with the following results:

Method	Conj. diene	Conj. triene	Total conjugated	Residual acid
	%	%	%	%
6.6% KOH-glycol	47.0	25.0	72.0	28.0
21% KOH-glycol	32.0	44.9	76.9	23.1
5% potassium-t-butanol	47.7	36.7	84.4	15.6

From these values it is obvious that as far as the extent of conjugation (total conjugated acids produced) is concerned, the proposed method is superior to the others, and there is not much to choose between the 6.6% KOH and 21% KOH methods. However in the respective proportions of conjugated diene and triene the differences are marked, thereby revealing that large quantities of dienes are produced by all the methods, but their further conversion to the conjugated trienes proceeds at different rates with different methods. The active base is the same with the first two methods, but the proportion of triene is higher with the second, thereby demonstrating the effect of concentration. The proposed method competes well with the 21% KOH-glycol method in this respect although the content of conjugated triene is slightly less. This marked ability to cause excellent conjugation is obviously due to the higher basicity of the tertiary butoxide. It is realized that, in order to appreciate this point, it is necessary to isolate the conjugated diene intermediate from partially isomerized linolenic acid and to study the isomerization of this compound by the three methods. Here again the problem is a complicated one as a number of such intermediate dienes are possible, as shown in Chart I.

It is clear from Chart I that some of the possible dienes require more steps to be isomerized to the conjugated triene than others. Thus with different dienes the rates of isomerization to the trienes will be different, a fact which again will be influenced by the geometry *(cis, trans)* of the intermediate diene. These points explain the fact why a steady state has not yet been reached with the linolenic acid concentrate with the proposed reagent even after 48 hrs. of reaction. In any case, the fact that the k_{268} value shows a tendency to rise even at such long periods proves abundantly that side reactions such as polymerization, which tend to destroy the conjugated polyenoic acids, do not occur in the proposed method. Thus the previously calculated (15.6%) residual acids with this method are likely to be unconverted linolenic acid as against the known presence of cyclic isomers with both alkali-treated linolenic and eleostearic acids (11, 12, 21) by the other methods. Thus the number of possible intermediate dienes and the formation of cyclic isomers render any such kinetic study (23) of isomerization of linolenic and other polyenoic acids at such a high temperature as 180° meaningless.

With methyl arachidonate, which contains some pentaenoate, there is again the same parallel behavior of the curves with the 6.6% KOH-glycol and 5% potassium-t-butanol methods, and both these have well-defined peaks in all the regions of maxima. With the 21% KOH-glycol method however the parallelism is broken in the triene region, where again there is a slight hump, which has been recorded by previous investigators (3), although no explanation has been given for this apparent anomalous behavior. With all three methods the peak in the diene region occurs at 238 mμ: 6.6 KOH-glycol, $k_{238} = 58.4$; 21% KOH glycol, $k_{238} = 41.0$; 5% potassium-t-butanol, $k_{238} = 54.9$. In the triene region the peak is shifted to 270 mμ with both the 6.6% KOH ($k_{270} = 48.7$) and 5% potassium-t-butanol ($k_{270} = 67.7$) methods while a maximum is reached at 286 mμ ($k_{286} = 52.6$) with the 21% KOH-glycol method. However with all the methods the peaks at the tetraene and pentaene regions occur at the expected wavelengths 315 mμ and 346 mμ, respectively. The k_{233} and k_{268} values by the 5% potassium-t-butanol method are higher than those by the other two methods while the k_{315} and k_{346} values lie in between those by the other methods. In the absence of the constants for pure arachidonic and pentaenoic acids at the different maxima it is not possible to calculate the proportions of diene, triene, tetraene, etc., present in the final isomerized product. From the high k values in all these regions by the proposed method, it can be concluded that the extent of conjugation is fairly high. The high k values in all these regions by all the methods confirms again the point made earlier that isomerization with the polyunsaturated acids occurs stepwise.

That isomerization of such polyenoic acids as linolenic, arachidonic, and pentaenoic is still proceeding even after long periods of reaction, holds forth the promise that it is possible to reach a steady state of maximum isomerization with these acids with suitable modifications of the proposed method.[3] Further studies are under way in this laboratory both towards such an ideal state and toward reducing the time required for isomerization.

With the samples of oils analyzed,[3] the proportions of linoleic and linolenic acids as estimated by the three methods lie in the same range. Duplicate values are presented in order that the reproducibility and accuracy of the three methods can better be appreciated. In general, the values obtained by the proposed method are slightly high, which is probably because the extent of conjugation is better with the tertiary butoxide reagent. The proposed method has the advantages that the isomerization is conducted at a lower temperature (90°) when such side reactions as polymerization are not likely to occur and the estimation is done at such a time when at least with one acid, linoleic acid, the isomerization has reached a steady state. With linolenic acid it is still occurring as against the decline in conjugation with the latter acid, in particular, at the times of reaction by the other methods. Thus it is possible that the values by the proposed method are likely to be nearer the truth.

Summary

It has been shown that potassium tertiary butoxide isomerizes such unsaturated fatty acids as linoleic, linolenic, arachidonic, and pentaenoic at a temperature of 90°. With 5% potassium-t-butanol reagent conjugation of linoleic acid attains a maximum at the end of 2 hrs. and remains steady thereafter even up to 10 hrs. while with linolenic, arachidonic, and pentaenoic acids it is still occurring at the end of such long times of reaction as 48 and 10 hrs., respectively. This continuance of reaction at the end of such long periods with these higher unsaturated acids demonstrates that the isomerization with the tertiary butoxide reagent at 90° is not complicated by side reactions, such as polymerization and deisomerization. All the methods studied give high absorption coefficients in the lower regions of maxima—233 mμ with linolenic, 233 mμ and 268 mμ with arachidonic acids—showing thereby that isomerizations of these acids occur stepwise. Based on this study, a method using 5% potassium-t-butanol as reagent, at 90° with a time of reaction of 4 hrs., has been proposed for the estimation of linoleic and linolenic acids. The k_{233} value for linoleic acid (94.0) obtained by this method compares very well with those by the 6.6% KOH-glycol and 21% KOH-glycol methods while with linolenic acid the k_{233} value (63.2) is higher and k_{268} value (74.2) lies intermediate between those by the other methods. The proposed method is shown to estimate the linoleic and linolenic acid contents of several typical oils with about the same degree of accuracy as either the 6.6% KOH-glycol or 21% KOH-glycol methods.

Appendix. When this paper was ready to be submitted, a note (27) describing the use of tertiary butoxide reagent on the kinetic study of isomerization of linoleic and linolenic acids came to our attention. Extinction coefficients at 235 mμ and 268 mμ are reported for linolenic acid and the values (61.4, 74.2) obtained by isomerization with a molar solution of the reagent at 99.5° and 180 minutes' time of reaction are about the same order as those reported in this paper. It is of interest to note that these authors have also noted that no destruction of the product occurs at this temperature and time of heating.

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[3] It may be pointed out that the proposed method does not require a costly, thermostatically-controlled heating bath as the temperature of isomerization is the same as the boiling point of the reagent, which stays practically constant at 89°.

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22nd Annual Review of the Literature on Fats, Oils, and Detergents. I., M. M. Piskur. This journal, vol. 33, p. 206, 1st column, second paragraph: remove complete reference to Steinacker.