The Journal of the American Oil Chemists' Society

Volume 27 SEPTEMBER, 1950 No. 9

Kinetics of Alkali Isomerization of Linoleic, Linolenic, and Arachidonic Acids

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IN this paper the alkali isomerization of linoleic, linolenic, and arachidonic acid systems is discussed in the light of recent experimental findings and the general mechanism of prototropic change. It is shown that relatively simple reaction mechanisms can be devised from existing knowledge on debromination acids although these acids are usually contaminated with geometrical isomers of other than the pure cis-cis configuration (1). The limited amount of debromination data available, together with the lack of isomerization data on the configurationally pure isomers of the various unsaturated acids, prevents quantitative checks of some important points in the theory. However many qualitative correlations are possible.

It is also evident that a considerably greater knowledge of the various isomers produced during isomerization will be required before a complete treatment of linolenic and arachidonic acids will be possible. Therefore the present paper is to be considered only as a step towards placing the subject on a firmer theoretical basis; it should be understood that much work remains to be done.

Rate Expressions for Linoleic Acid. For the purpose of this discussion, it is sufficiently accurate to represent a prototropic shift by the following rapidly consecutive bimolecular reactions:

$$R_1{-}CH_2{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}CH_2{-}R_2 + OH^- \xrightarrow{\text{(slow)}} [R_1{-}CH_2{-}CH{=}CH{-}CH{-}CH{=}CH{-}CH_2R_2]^- + HOH$$

$$[R_1{-}CH_2{-}CH{=}CH{-}CH{-}CH{=}CH{-}CH_2{-}R_2]^- + HOH \xrightarrow{\text{(fast)}} R_1{-}CH_2{-}CH{=}CH{-}CH{=}CH{-}CH_2{-}CH_2{-}R_2 + OH^-$$

where the velocity constant is represented as $k = k'(OH^-)$. The isomerizations are in general carried out in a large excess of alkali, and the hydroxyl ion concentration remains essentially constant. Accordingly the rate of each prototropic change in linoleic acid can be represented by a first-order rate expression.

The following system,

$$R_1\overset{1}{C}H_2CH = CH - \overset{2}{C}H_2 - CH = CH - \overset{1}{C}H_2R_2,$$

exemplified by linoleic acid, contains three methylenes which might reasonably be involved in a prototropic shift. In methylenes of type 1 a doube-bond shift can occur from only one direction, whereas in methylenes of type 2 a double-bond shift can occur in either direction. When a shift occurs toward methylenes of type 1, an unconjugated system is produced in which the double bonds are separated by two methylenes. A shift in either direction toward methylene 2 would produce conjugated systems. In line with the fact that diene conjugated systems (2) and 1,5-dienes (3) are not affected appreciably by alkali under conditions employed in the usual alkali-isomerization experiments, it is assumed as a first approximation that prototropic changes which occur in the linoleic, linolenic, and arachidonic systems are not reversible. Furthermore, since treatment of 9,12-linoleic acid with alkali gives rise to approximately equal amounts of 9,11 and 10,12 position isomers (4), it is considered that the end groups R_1 and R_2 will have no influence on the direction of shift. These considerations allow the mechanism to be expressed simply as follows:

Let u = conc. of 9c,12c-linoleic acid at time t (u_o = initial conc. = 1 when expressed as mole fraction)
C_1 = 9c,11t[2]-linoleic acid (formed as result of shift toward methylene 2)
C_2 = 10t,12c[2]-linoleic acid (formed as result of shift toward methylene 2)
A_1 = 8,12-linoleic acid (formed as result of shift toward methylene 1)
A_2 = 9,13-linoleic acid (formed as result of shift toward methylene 1)
$C_T = C_1 + C_2$ = total conc. of conjugated diene at time t.

k_1 and k_2 are rate constants for the first and second type of shift, respectively.

$$\text{I)}\ u \xrightarrow{k_2} C_1$$

$$\text{II)}\ u \xrightarrow{k_2} C_2$$

$$\text{III)}\ u \xrightarrow{k_1} A_1$$

$$\text{IV)}\ u \xrightarrow{k_1} A_2$$

The rate of change of total conjugated diene with time can be expressed as

$$(1)\quad \frac{dC_T}{dt} = 2k_2 e^{-(2k_2+2k_1)t}$$

For later reference, it is important to note that the initial slope approaches $2k_2$ since the exponential term approaches unity as t approaches 0. The integrated form of equation 1 is as follows:

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[2] In a previous paper (4) the following rules were proposed to account for the configurational changes occurring during alkali isomerization:

Rule I. When the bond involved in the shift is trans, the new bond formed assumes either the cis or trans configuration with essentially equal probability.

Rule II. When the bond involved in the shift is cis, the new bond formed assumes predominantly the trans configuration.

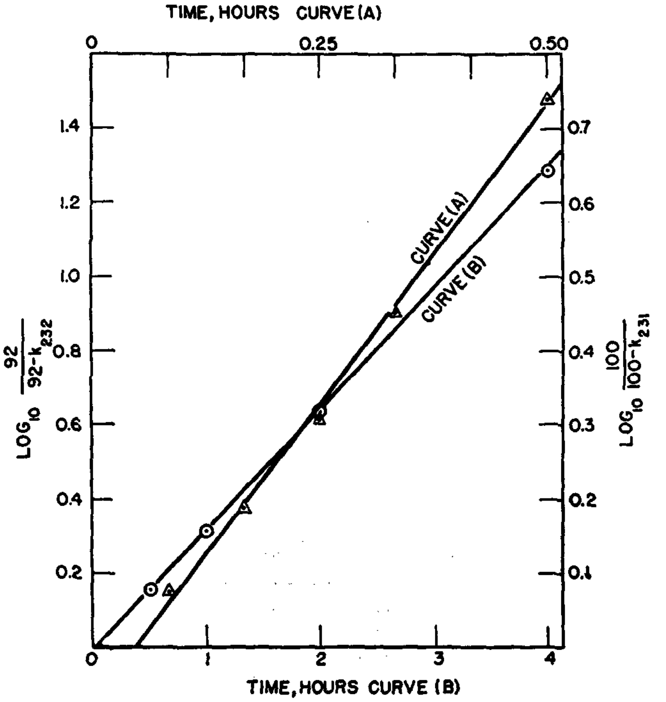

FIG. 1. Curve A represents a plot of $\log_{10} \frac{92}{92 - k_{232}}$ versus time in hours for the alkali isomerization of debromination linoleic acid at 180° in glycerin.

Curve B represents a plot of $\log_{10} \frac{100}{100 - k_{231}}$ versus time in hours for the alkali isomerization of linoelaidic acid at 180° in glycerin.

$$(2)\quad C_T = \frac{k_2}{k_2 + k_1}(1 - e^{-Kt})$$

where $K = 2k_2 + 2k_1$. From equation 2 it immediately follows that as t approaches infinity, C_T approaches $\frac{k_2}{k_2 + k_1}$. Experimentally, the amount of conjugated diene has been followed by observing the change in specific absorption coefficient. Therefore the limiting value of C_T is most conveniently expressed as $\frac{k_{232}\text{ (limit)}}{k_{232}\text{ (av.)}}$, where k_{232} (av.) represents the average absorption coefficient of the conjugated isomers present and k_{232} (limit) is the value at which k_{232} levels off during alkali treatment (3). The value for k_{232} (av.) is 95, as determined by Nichols, Herb, and Riemenschneider (4); consequently $C_T = \frac{k_{232}}{95}$. Likewise k_{232} (limit) for crystallization linoleic acid (5) is taken to be 92. Equation 2 can now be expressed as follows:

$$(3)\quad Kt = 2.303 \log \frac{92}{92 - k_{232}}$$

By using the experimental data of Brice and Swain[3] on debromination linoleic acid and plotting the right hand side of equation 3 against t, a straight line is obtained (Fig. 1, Curve A), the slope of which is equal to K. The value of K was found to be 7.46 by the method of least squares. Accordingly $k_2 = 3.61$ hr^{-1} and $k_1 = 0.116$ hr^{-1}. More accurate values for the rate constants could be found by using crystallization linoleic acid since debromination linoleic acid is not a pure geometrical isomer (1). It will be noted that the plotted line in Figure 1, Curve A, does not go through the origin as it should theoretically. This is partly due to a small uncertainty in the initial time inherent in the experimental procedure, and accordingly the initial point is not included in determining the line of regression.

In equation 3 the constant K is actually equal to the product of the hydroxyl ion concentration and another constant, L, in line with the pseudo unimolecular character of the constant. Therefore equation 3 can be written as

$$(3a)\quad (OH^-) = \frac{2.303}{Lt} \log \frac{92}{92 - k_{232}}$$

From equation 3a it is obvious that a plot of the hydroxyl ion concentration (OH^-) against $\log \frac{92}{92 - k_{232}}$ would give a straight line and that its slope would allow calculation of L. Figure 2, Curve A, shows the application of equation 3a to data of this type obtained on ethyl linoleate in ethylene glycol at the Northern Regional Research Laboratory (6). A value of 310 was obtained for L, as compared with 223 obtained in glycerin by using equation 3. The calculation for the glycerin case was made as follows: The glycerin contained 11% KOH, therefore

$$0.11 \times \frac{17}{56} = 0.0334 = (OH^-).$$

$$L = \frac{K}{(OH^-)} = \frac{7.46}{0.0334} = 223.$$

Effect of Geometrical Configuration on Rate as Applied to Linoleic Acid. So far, no mention has been made concerning the effect of geometrical configuration on the rate at which various methylenes shift. Naturally occurring linoleic acid has been prepared by fractional crystallization of corn and cottonseed oil acids (7). Previous investigators have assumed that it is of the cis-cis configuration. By elaidinization of the naturally occurring acid with selenium, Kass and Burr (8) were able to isolate an isomer melting at 28-29°, which was presumably of the trans-trans configuration. In this laboratory it has been found that the isomer melting at 28-29° is isomerized at a considerably slower over-all rate than debromination linoleic acid, which is approximately of the same composition as naturally occurring linoleic acid.[4] In determining the individual rate constants from spectroscopic data, information is required regarding the various conjugated isomers formed during the isomerization of trans-trans linoleic acid. According to the rule regarding the expected isomerization products (4) of the trans-trans isomers, essentially an equal mixture of trans-trans and cis-trans conjugated acids would be formed. If we consider the average absorption coefficients of trans-trans isomers and cis-trans isomers to be 113 and 95, respectively (4), then

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[3] In all experimental data discussed, the isomerization conditions are those described in Brice and Swain's analytical method (2) with the exception of Melvin's work (6) on the effect of alkali concentration.

[4] From this point on, the trans-trans configuration is assigned to Kass and Burr's isomer melting at 28-29°, and the cis-cis configuration is assigned to naturally occurring linoleic acid and debromination linoleic acid. The extent to which the assigned configurations are justified is discussed later.

in applying equation 2 to trans-trans linoleic acid, the value for k_{231} (av.) would be 104. During alkali isomerization k_{231} for trans-trans linoleic is 100. Therefore the following expression is used in determining K for the trans-trans acid:

$$(4)\ Kt = 2.303 \log \frac{100}{100 - k_{231}}$$

where $k_{231} = 104\ C_T$. It is interesting to note that the limiting value of C_T is $\frac{100}{104} = 0.962$, which is close to the limiting value 0.968 for cis-cis linoleic acid. The ratio $\frac{k_2}{k_1} = 25$ for the trans-trans acid whereas $\frac{k_2}{k_1} = 30$ for the cis-cis acid. As determined from Figure 1, Curve B,[5] the values of the individual rate constants for the trans acid are $K = 0.372\ hr.^{-1}$, $k_2 = 0.18\ hr.^{-1}$ and $k_1 = 0.0071\ hr.^{-1}$. No experimental information is as yet available for the cis-trans type of linoleic acid. It seems reasonable however that the rate constants for conjugation should be somewhere between the values for all trans and all cis acids.

Another interesting question arises in the case of cis-trans linoleic acid. By using the rules for predicting configuration of conjugated isomers (4) from 9c,12t-linoleic acid, for example, three isomers would be expected, namely 9c,11c-, 9c,11t-, and 10t,12t-linoleic acid. If the rate of shift were greater in one direction than another however, the predominant products would be more limited. For example, if the double bond of the cis configuration shifted more rapidly toward the methylene than did that of the trans configuration then 10t,12t-linoleic acid would be the predominant product. The possibility of a difference in rate of shift according to the configuration of the bond is discussed further in connection with linolenic acid. Preparation of a cis-trans linoleic acid is now being attempted in order to settle this question.

Rate Expressions for Linolenic Acid. In the preceding discussion a method was introduced in which various shifts in the linoleic acid system were assigned separate rate constants. A similar treatment of the linolenic acid system requires a new set of rate constants in which a new type of methylene is encountered in the intermediate stage of reaction, that is, one which is surrounded by a double bond on one side and two conjugated bonds on the other. The various rate constants for linolenic acid are designated k_1, k_2, and k_3 for the first, second, and third type shift respectively. At this point it is necessary to discuss a special property of each type of methylene in relation to the prototropic change, that is, when a hydrogen ion is removed from the methylene, the resulting anion can resonate between two or more structures in each case, as shown in Table I. As the number of double bonds increases, the number of resonance structures increases, and a resultant increase in resonance stabilization would be expected in each case. Therefore the relative order of magnitude of the rate constants would be $k_3 > K_2 > k_1$ where each inequality is large. The relation $k_2 > k_1$ is already evident by analogy with the linoleic acid system. It is shown later that experimental data on linolenic acid strongly indicates the relation $k_3 \gg k_2$

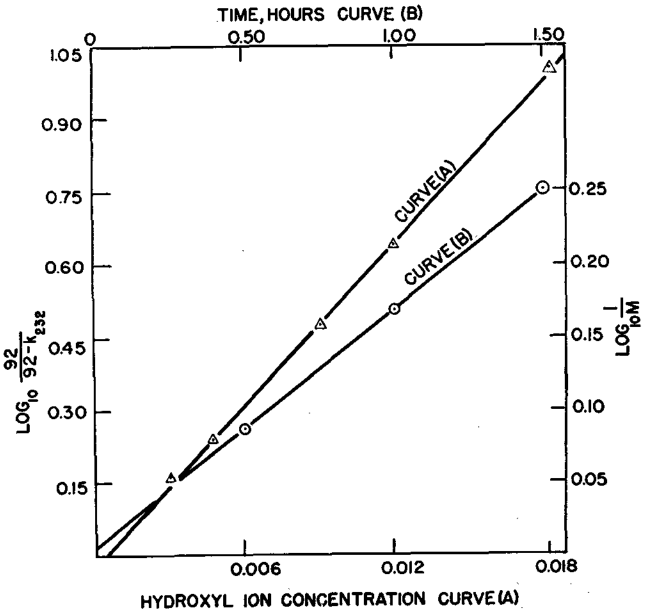

Fig. 2. Curve A represents a plot of log $\frac{92}{92 - k_{232}}$ versus the hydroxyl ion concentration for the alkali isomerization of ethyl linoleate at 180° in ethylene glycol.

Curve B represents a plot of $\log_{10} \frac{1}{M}$ versus time in hours (M = Mol fraction of pseudoeleostearic acid) at 180° in glycerin.

One other consequence of resonance as applied to the type 3 methylene needs elaboration. Considering, for example, 9,12,14-linolenic acid, an intermediate conjugated isomer, and the various resonating structures of the anion shown in Table I, it is evident that a proton removed from carbon atom 11 could be replaced on either of carbon atoms 9, 11, 13, or 15, giving rise to 10,12,14-, 9,11,13- 9,11,14- and 9,12,14-linolenic acids respectively. Since the triply conjugated structures would be stable, the above structures would eventually give rise to essentially equal amounts of the 10,12,14- and 9,11,13-linolenic acids. Extending this argument to the intermediate 10,12,15-linolenic acid, it would then be predicted that the conjugated triene produced during alkali isomerization of cis-cis-cis-linolenic acid would be predominantly composed of 2 parts of 10,12,14-linolenic acid, 1 part of 9,11,13-linolenic, and 1 part of 11,13,15-linolenic acid.

Further complications occur in the linolenic acid system with regard to the conjugated triene formed. It has been shown that absorption, at a given wave length, of conjugated triene acid such as pseudoeleostearic and β- and α-eleostearic decreases with time under the usual conditions of alkali isomerization. This effect is probably due either to cyclization or polymerization. Data on heating pseudoeleostearic acid indicate that the first-order rate constant, k_0, might be included in the reaction mechanism to account for this side reaction (Figure 2, Curve B). To a good approximation the same rate constant would also apply to α- and β-eleostearic acid.

Keeping in mind this discussion, it is now possible to propose a mechanism for alkali isomerization of the linolenic acid system, in which as an approximation, shifts of type 1 are neglected, and an equation

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[5] The authors are indebted to R. M. Speck, who provided the data shown in Fig. 1, Curve B.

TABLE I
Classification of Methylenes Involved in Prototropic Shifts

METHYLENE, TYPE	Assigned rate constant	Anion resonating structures
(1) $\mathrm{R_1{-}CH_2{-}CH{=}CH{-}R_2}$	k_1	$\mathrm{R_1{-}\bar{C}H{-}CH{=}CH{-}R_2}$ $\mathrm{R_1{-}CH{=}CH{-}\bar{C}H{-}R_2}$
(2) $\mathrm{R_3{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}R_4}$	k_2	$\mathrm{R_3{-}CH{=}CH{-}\bar{C}H{-}CH{=}CH{-}R_4}$ $\mathrm{R_3{-}CH{=}CH{-}CH{=}CH{-}\bar{C}H{-}R_4}$ $\mathrm{R_3{-}\bar{C}H{-}CH{=}CH{-}CH{=}CH{-}R_4}$
(3) $\mathrm{R_5{-}CH{=}CH{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}R_6}$	k_3	$\mathrm{R_5{-}CH{=}CH{-}CH{=}CH{-}\bar{C}H{-}CH{=}CH{-}R_6}$ $\mathrm{R_5{-}CH{=}CH{-}\bar{C}H{-}CH{=}CH{-}CH{=}CH{-}R_6}$ $\mathrm{R_6{-}\bar{C}H{-}CH{=}CH{-}CH{=}CH{-}CH{=}CH{-}R_6}$ $\mathrm{R_5{-}CH{=}CH{-}CH{=}CH{-}CH{=}CH{-}\bar{C}H{-}R_6}$

for cyclization or polymerization is introduced. To simplify discussion of the various position isomers which might be formed during alkali isomerization of linolenic acid, it is helpful to use the following representation. For double bonds the numeral 1 is used and single bonds are represented by zeros. A conjugated system $c = c - c = c$ where the double bonds are in the 9,11 position is represented as 0101. Likewise an unconjugated system such as 9,12-linoleic acid is 1001. Other examples are illustrated below.

Mechanism:

(1) $1001001 \xrightarrow{k_2} 1000101$ (9,11,15-linolenic acid)

(2) $1001001 \xrightarrow{k_2} 1001010$ (10,12,15-linolenic acid)

(3) $1001001 \xrightarrow{k_2} 1010001$ (9,13,15-linolenic acid)

(4) $1001001 \xrightarrow{k_2} 0101001$ (9,12,14-linolenic acid)

Production of conjugated triene

(5) from (2) $1001010 \xrightarrow{k_3} 0101010$ (10,12,14-linolenic acid)

(6) from (2) $1001010 \xrightarrow{k_3} 1010100$ (11,13,15-linolenic acid)

(7) from (4) $0101001 \xrightarrow{k_3} 0101010$ (10,12,14-linolenic acid)

(8) from (4) $0101001 \xrightarrow{k_3} 0010101$ (9,11,13-linolenic acid)

The mechanism can now be summarized as follows:

Let u = amount of unreacted linolenic acid at time t (u_0 = initial conc. = 1).

C_1, etc. = conjugated diene at time t.

C_{5+6} = conjugated triene in which 10,12,14 and 11,13,15 are taken together.

C_{5+6} (cyclic) = combined cyclic or polymerized product from triene of 5 and 6.

$K = 2k_3$

$$u \xrightarrow{k_2} C_1$$

$$u \xrightarrow{k_2} C_2 \xrightarrow{K} C_{5+6} \xrightarrow{k_0} C_{5+6} \text{ (cyclic)}$$

$$u \xrightarrow{k_2} C_3$$

$$u \xrightarrow{k_2} C_4 \xrightarrow{K} C_{7+8} \xrightarrow{k_0} C_{7+8} \text{ (cyclic)}$$

The rate of change of conjugated diene [C_T (diene) $= C_1 + C_2 + C_3 + C_4$] from linolenic acid with time is then given by the expression,

$$\text{(5)} \quad \frac{dC_T\text{(diene)}}{dt} = 4k_2e^{-4k_2t} - 2K\frac{k_2}{K-4k_2}e^{-4k_2t} + 2K\frac{k_2}{K-4k_2}e^{-Kt}$$

The integrated form of equation 5 then gives the total amount of conjugated diene present after time t as follows:

$$\text{(6)} \quad C_T\text{(diene)} = 0.5 + \frac{n}{2n-8}e^{-4k_2t} - \frac{2}{n-4}e^{-nk_2t} - e^{-4k_2t}$$

$$\text{where } n = \frac{K}{k_2}$$

By setting $\frac{dC_T}{dt} = 0$ in equation 5, the time at which the maximum amount of conjugated diene is produced is given by

$$(7)\quad t_{\text{max.}} = \frac{1}{(4-n)\,k_2} \ln \frac{(8-n)}{n}$$

From equation 7 it is evident that when n is greater than 8 the curve for conjugated diene has no maximum. Available experimental data on debromination linolenic acid (Table II), indicate that the conjugated diene curve is similar to that obtained with linoleic acid and that neither goes through a maximum but levels off at a value which no longer changes with time. The data in Table II show that the diene reaches a maximum value and drops off slowly. The slow diminution in diene however can be attributed to the k_1 type shift, which was neglected as a first approximation. Therefore it is perfectly consistent to ignore this slight decrease in diene with time at present and consider that the curve has leveled off. The slow decrease in diene is discussed again in connection with triene formation and the effect of alkali concentration on the products formed during the alkali isomerization of linolenic acid. Accordingly in the present treatment it will be considered that $K > 8k_2$. In Table II the value used for the average absorption coefficient of triene, namely 155, could range anywhere from 150 to 200 without altering the nature of the diene curve significantly.

TABLE II

Alkali Isomerization of Linolenic Acid-Glycerin at 180°—The Amount of Conjugated Diene Produced as a Function of Time

t(hrs.)	k_{232}	k_{268}	Mol. fraction[a] of conj. diene
0.0834	54.8	38.9	0.522
0.1667	62.8	57.0	0.581
0.250	62.7	55.6	0.581
0.333	61.9	53.9	0.576
0.417	61.2	53.4	0.570
0.500	60.6	52.6	0.564

[a] In calculating the mole fraction of conjugated diene, the overlap of conjugated triene in the 232 mμ. region was taken into account. Simultaneous equations used were $20.6\,x + 95\,y = k_{232}$; and $155\,x = k_{268}$.

Another point of interest is the determination of $\frac{k_2\,(\text{linoleic})}{k_2\,(\text{linolenic})}$. A knowledge of this ratio would allow use of the linoleic rate constant in the linolenic problem. The ratio can be determined by finding the initial slopes of equations 1 and 5 from which it is observed that

$$\frac{\dfrac{dC_T(\text{linoleic})}{dt}}{\dfrac{dC_T(\text{linolenic})}{dt}} = \frac{k_2(\text{linoleic})}{2k_2(\text{linolenic})}$$

Data on debromination linoleic acid and debromination linolenic acid indicates that $\frac{k_2\,(\text{linoleic})}{k_2\,(\text{linolenic})}$ is roughly unity.[6] Data at lower temperatures would be required for determining the value more accurately.

In considering n to be large, two approximate equations can be obtained from equation 6, namely:

$$(8)\quad C_T(\text{diene}) = 0.5 + \frac{8-n}{2n-8}\,e^{-4k_2t}$$

and

$$(9)\quad C_T(\text{diene}) = 0.5\,(1 - e^{-4k_2t})$$

Equation 9 could also be derived by using the steady state approximation.

In deciding between the applicability of equations 8 and 9, satisfactory experimental data on pure cis-cis-cis-linolenic acid at temperatures lower than 180° would be required.

The proposed mechanism gives the following expressions for the change of conjugated triene with time and the amount of conjugated triene after time t.

$$(10)\quad \frac{dC_T(\text{triene})}{dt} = 2K\frac{k_2}{K-4k_2}\left(e^{-4k_2t} - e^{-Kt}\right) - K_0C_T(\text{triene})$$

$$(11)\quad C_T(\text{triene}) = \frac{\beta e^{-4k_2t}}{k_0-4k_2} - \frac{\beta e^{-Kt}}{k_0-K} + \left(\frac{\beta}{k_0-K} - \frac{\beta}{k_0-4k_2}\right)e^{-k_0t}$$

$$\text{where } \beta = \frac{2Kk_2}{K-4k_2}$$

When $K \gg k_2$, equation (11) reduces to:

$$(12)\quad C_T(\text{triene}) = \frac{2k_2}{k_0-4k_2}\left(e^{-4k_2t} - e^{-k_0t}\right)$$

Of course, equation 12 can also be derived by applying a steady state approximation to the proposed mechanism.

By substituting m for $\frac{k_2}{k_0}$ in equation 12 and setting the first derivative equal to zero, the maximum amount of triene in terms of m and k_2 is found to be

$$(13)\quad t = \frac{m}{k_2\,(4m-1)} \ln 4m$$

In testing the validity of equations 12 and 13 by spectroscopic data, it is necessary to know the correct value of the average absorption coefficient at a given wave length of the conjugated triene present in alkali isomerized linolenic acid. By choosing the wave length 268 mμ., any overlapping of conjugated diene could be neglected; also this choice would give us the advantage of working at the maximum point of absorption for the alkali-isomerized material.

The actual amount of conjugated triene present would then depend on the absorption coefficient at 268 mμ. and the fraction of each isomer present in the mixture. An equal mixture of pseudoeleostearic acid and α-eleostearic acid, for example, would have a k_{268} of approximately 155. This value was used in Table II as an approximation to the composition of the conjugated triene in alkali-isomerized linolenic acid. As previously mentioned, a relatively large error as a result of this approximation would not have changed the essential character of the conjugated diene curve. Naturally before equations 12 and 13 can be checked quantitatively, absorption data on the true conjugated triene components of alkali-isomerized linolenic acid would be required.

It may be noted in equation 12 that since $4k_2$ (linolenic) is equal to or greater than 14.44 [$4 \times k_2$ (linoleic)], after about 20 minutes the amount of conjugated triene present decreases exponentially and is represented by the term $\frac{2k_2}{k_0 - 4k_2}\,e^{-k_0t}$. By using k_0 as determined with pseudoeleostearic acid and k_2 as determined with linoleic acid, it is found that the slope of the curve at this point, —0.22, is somewhat

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[6] Data by Brice and Swain were used for this conclusion (2).

greater than calculated from the isomerization data (2) on debromination linolenic acid. This relatively small difference in slope can be attributed to the k_1 type of shift, as was the slow decrease in conjugated diene after it rapidly reached an almost constant value. In other words, conjugated dienes of the type

$$R_1CH=CH-CH=CH-CH_2-CH_2-CH=CH-R_2$$

react slowly by the k_1 type shift followed by a rapid k_3 shift to give conjugated triene or by a k_1 shift alone to give a form in which the double bond is separated from the conjugated diene systems by three methylenes. Depending on the experimental conditions, the contribution of the k_1 shift can be negligible or appreciable since this rate constant is a function of the temperature and concentration of alkali. As shown previously, at a given temperature, the rate constant is proportional to the alkali concentration. It follows therefore that the relative amounts of diene and triene could be varied for a given temperature and time of reaction by varying the alkali concentration. This conclusion is substantiated by the work of Melvin (6) on the isomerization of ethyl linolenate in ethylene glycol at 180°, where significant increases in triene and decreases in diene at a given time of heating are observed with increasing alkali concentration.[7]

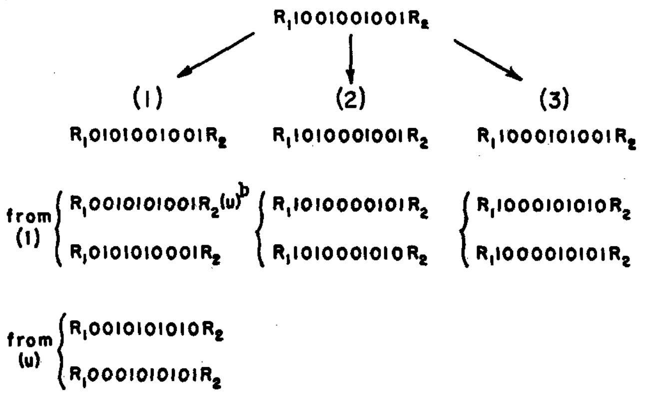
It is true that the maximum amount of triene predicted by equation 12 is greater than that observed experimentally provided k_{268} (av.) is chosen as 155. A value of k_{268} (av.) $= 120$ would be required for close agreement. It is possible that a cyclization step occurs simultaneously with the formation of triene as a side reaction. Further study on this point will be required. Hydrogenation studies of the reaction mixture at various time intervals would provide valuable information on this point.

Rate Expressions for Arachidonic Acid. Without including any new assumptions, it is possible to extend the treatment of linoleic and linolenic acid to arachidonic acid, an eicosatetraenoic acid in which the double bonds are believed to be in the 5:6, 8:9, 11:12, and 14:15 positions (9). A complete discussion of the arachidonic acid isomerization will not be attempted at present. Only those features which allow certain pertinent tests of the theory in general are considered. In Table III structures of the possible position isomers arising from the alkali isomerization of arachidonic acid are shown. In making the table, the same assumptions used for linolenic acid were applied, namely, a) type 1 shifts are neglected, b) double bonds separated by two methylenes cannot move toward one another, c) conjugated systems are stable and the double bonds remain in position with one exception, which is a result of resonance of the intermediate anion and is illustrated by the following equation:

$$0010101001 \longrightarrow 0010101010 + 0001010101$$

TABLE III

Position Isomers[a] of Arachidonic Acid Formed During Alkali Isomerization



[a] One-half the isomers are shown; the other half are obtained by exchanging R_1 and R_2.

[b] Because k_3 is large, this form would represent an unstable intermediate.

It is worth emphasizing that without the resonance concept it is difficult to understand the formation of tetraene on the basis of a prototropic shift mechanism. In fact, the same type of reasoning which led Kass (10) to predict that only the 10,12,14-conjugated position isomer is formed in linolenic acid would not satisfactorily explain the formation of tetraene from arachidonic acid. As in linolenic acid, a new rate constant k_4 must be introduced for forms of the type

$$R_1 0010101001 R_2 \xrightarrow{k_4} R_1 0010101010 R_2 + R_1 0001010101 R_2.$$

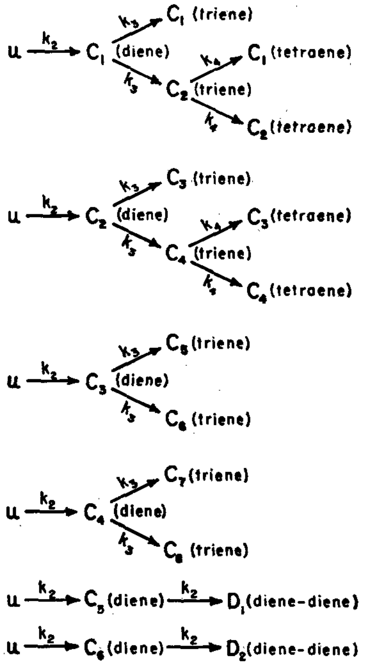
As a result of an increase in the number of resonating forms, it is reasonable that $k_4 \gg k_3$ in analogy with $k_3 \gg k_2$. The latter consideration is used in limiting the forms in Table III, since the form $R_1 0101001001 R_2$ would give predominantly

$$R_1 0010101001 R_2 \text{ and } R_1 0101010001 R_2$$

and only small amounts of

$$R_1 0101000101 R_2 \text{ and } R_1 0101001010 R_2.$$

The mechanism for arachidonic acid can now be represented as follows:



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[7] Actually the decrease in diene and increase in triene are substantially greater than that expected on the basis of calculations using k_1 and k_2 of linoleic acid. However the medium effect produced by change in KOH concentration is not known at this point. Likewise the rate expressions would hardly be expected to hold at extremely high KOH concentrations, particularly where, because of the common ion effect, the limit of solubility of the soaps is reached. Experiments carried out at low hydroxyl concentrations for longer periods when the ionic strength of the medium is held constant would provide a more suitable test of the theory.

Equations for the polymerization or cyclization which occurs with the conjugated triene are not included for the time being because the concern is with derived expressions for conjugated diene and tetraene in which there is no apparent need for such correction. The letters D_1 and D_2 are used for representing the concentration of dienes of the type 1010000101, which is henceforth referred to as a diene-diene. The expression diene-diene was chosen because these forms have two conjugated diene systems on one molecule and because this fact must be taken into account in calculating the amount of this component present. The rate of change of diene-diene with time and the amount present after a time t are given by the following expressions

$$\frac{dD_T}{dt} = \frac{2k_2}{5}e^{-k_2t} - \frac{2k_2}{5}e^{-6k_2t} \tag{14}$$

$$D_T = \frac{1}{15}e^{-6k_2t} - \frac{2}{5}e^{-k_2t} + \frac{1}{3} \tag{15}$$

Corresponding expressions for the amount of diene with time are

$$\frac{dC_T(\text{diene})}{dt} = \frac{12}{5}k_2e^{-6k_2t} - \frac{2}{5}k_2e^{-k_2t} \tag{16}$$

$$C_T(\text{diene}) = \frac{2}{5}e^{-k_2t} - \frac{2}{5}e^{-6k_2t} \tag{17}$$

Equation 16 shows that the initial slope of conjugated diene for arachidonic acid should be equal to the initial slope of conjugated diene for linoleic acid if k_2 (linoleic) = k_2 (arachidonic). A test of this type could also be used in deciding the validity of the steady state approximation since the initial slope could range from $2k_2$ to $6k_2$, depending on the extent to which the latter is valid. Therefore if the initial slope is close to $2k_2$, it is a strong indication that the steady state approximation is valid. Likewise a demonstration of no maximum in the conjugated diene curves would provide additional support.

The expressions for conjugated tetraene are:

$$\frac{dC_T(\text{tetraene})}{dt} = k_2e^{-6k_2t} \tag{18}$$

$$C_T(\text{tetraene}) = \frac{1}{6} - \frac{1}{6}e^{-6k_2t} \tag{19}$$

The initial slope for tetraene would therefore be k_2 (linoleic) or k_2 (arachidonic), provided k_2 (linoleic) = k_2 (arachidonic) and the steady state approximation hold.

In view of the fact that no pure crystallization arachidonic acid is available and that only a small amount of experimental data is available on debromination arachidonic acid, the theory cannot be accurately tested at present. The experimental evidence available on debromination arachidonic acid, however, is in qualitative agreement with the general features of the theory.

Effect of Geometrical Configuration on Rate as Applied to Linolenic Acid. It has been shown that the rate at which shifting of double bonds takes place in linoleic acid is dependent on their configuration. A type 2 shift was assigned three different constants k_2 (cis-cis linoleic acid), k_2 (cis-trans linoleic acid), and k_2 (trans-trans-linoleic acid), in order of decreasing magnitude. Available experimental evidence indicates that k_2 (cis-cis-linoleic acid) is approximately 20 times k_2 (trans-trans-linoleic acid. The value for k_2 (cis-trans linoleic acid) was considered to be something between these values although it cannot be estimated until a cis-trans linoleic acid is available. It is reasonable to expect that similar differences would hold in respect to the linolenic acid rate constants of type 2. From this it is immediately apparent that the relative proportions of position isomers vary considerably, depending on the geometrical configuration of the linolenic acid isomerized. A variation in the proportions of position isomers would cause relatively insignificant changes in the calculated amounts of diene and triene, as determined by the spectroscopic method. As already noted however, debromination linolenic acid data lead to the conclusion that the amount of conjugated diene produced levels off at approximately 0.6 (mole fraction) whereas under the experimental conditions used for isomerization a value of 0.5 for cis-cis-cis linolenic acid would be expected. Matthews, Brode, and Brown (1) have suggested that debromination linolenic acid contains appreciable amounts of geometrical isomers other than the cis-cis-cis variety. This suggestion is correlated with the discrepancy mentioned above by the following explanation.

First, a cis-trans type linoleic acid is considered. According to the rules previously mentioned, the formation of two parts of trans-trans, one part of cis-cis, and one part of cis-trans conjugated isomers would be expected. If a trans bond shifted much more slowly than a cis bond, however, the predominant product would be a trans-trans linoleic acid. By applying this assumption to the system 9t,12c,15t-linolenic acid, it is obvious that alkali isomerization would give rise to almost 100% conjugated diene, which would resist further conjugation. Similarly the conjugated diene of cis-cis-trans linolenic acid would level off at values appreciably greater than 0.5 (mole fraction), provided k_2 (cis-cis-cis linolenic acid) is not tremendously greater than k_2 (cis-trans-cis linolenic acid). It could also be argued that certain isomers, such as cis-trans-cis and trans-trans-cis, would give diene values leveling off at less than 0.5. For the moment however we are interested only in explaining how the value would be different from 0.5. Provided the explanation is correct, one could further infer that debromination linolenic acid has a significant proportion of the cis-cis-trans and trans-cis-trans type isomers. The preparation of cis-trans linoleic acid and and study of its isomerization products should definitely settle this question. Recently (5), data on pure linolenic acid obtained by chromatographic separation from linseed oil indicated that the limiting value is close to 0.5.

At present the leveling off of conjugated diene at a value higher than 0.5 (mole fraction) is considered sufficient indication that a cis bond shifts faster than a trans bond. This fact, combined with the fact that the activity of a given methylene is largely dependent on the configuration of the double bonds surrounding it, leads to the conclusion that each type of linolenic acid system — cis-cis-cis, trans-trans-trans, cis-cis-trans, trans-cis-trans, cis-trans-cis, and trans-trans-cis — would give a different pair of curves representing the formation of conjugated diene and triene with time. Therefore when the rate of isomerization and nature of isomerized products of cis-trans linoleic acid are known, it will be possible to construct fairly accurate theoretical curves for the production of conjugated diene and triene for different geometrical isomers of linolenic acid. Consequently it will be possible to assign a geometrical configuration to a particular linolenic acid, once its diene and triene time curves have been determined. With linoleic acid, determination of configuration is possible by combining a knowledge of the geometrical isomers formed and certain theoretical predictions of the geometrical isomers produced from a given configuration. In the case of linoleic acid the available experimental evidence is in good agreement with the conclusion that naturally occurring linoleic acid is of the cis-cis configuration and that Kass's linoelaidic acid is of the trans-trans configuration. Altogether it seems highly probable that adequate experimental evidence combined with the theory presented would allow accurate assignment of geometrical configuration to the various isomers of linoleic acid, linolenic acid, and arachidonic acid.

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[Received May 9, 1949]

Pilot-Plant Fractionation of Cottonseed. III. Process Development of Differential Settling[1, 2]

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ABSTRACT

Chemical engineering data are presented to show the pilot-plant process development of cottonseed fractionation employing the differential settling principle. The purpose of the process is to produce a cottonseed meal fraction essentially free of pigment glands and hulls, and a second fraction in which the pigment glands are concentrated sufficiently to serve as a raw material if pharmaceutical or other industrial use is developed for the glands or the pigments. The non-lipids fraction will make available a meal of high nutritive value and a source of industrial protein.

Unit operations involved, including machinery and other equipment required, and proposed flow diagrams for commercial application are discussed. In brief the unit operations are as follows: material preparation; disintegration for proper size reduction of cottonseed flakes (either defatted or undefatted) in solvent slurries; separation by tank differential settling or by centrifugal differential settling at 62 times gravity; meal recovery to recover separated fractions by either centrifuging at 1450 times gravity or by pressure filtration; desolventization of solvent-damp meal; and oil and solvent recovery.

IT has long been felt by the cottonseed industry that the nutritional and industrial value of cottonseed meal could be materially improved by removal of the glands which contain gossypol and other pigments of cottonseed.

The pigments of cottonseed can be removed from the flakes by two general methods. One method is to extract the flakes with an organic solvent in which the pigments are soluble, for example, acetone, ethyl ether, isopropanol, and methyl alcohol. Although by this method a meal low in gossypol is produced, the oil-solvent mixture (miscella) contains most of the pigments and other non-oil compounds which complicate recovery of the oil (4).

The second general method is to detach the pigment glands intact from the flakes or meal by disintegration of the flakes or meal in a solvent slurry (3) followed by separation (fractionation) of these two components. This method takes advantage of the high mechanical strength of the glands and of their natural detachability from the remainder of the kernel tissue as well as of the fact that these glands are not affected by certain solvents, such as the low-boiling petroleum cuts and some chlorohydrocarbons.

Utilizing this second general method, a mixed-solvent flotation process of cottonseed fractionation was developed first on a laboratory scale (3) and then on a pre-pilot-plant scale (8). This process employed a mixture of the two solvents, hexane and tetrachlorethylene, in a proportion to give a resulting specific gravity of 1.378. At this specific gravity the pigment glands tended to float and the meal and hulls to sink since the solvent specific gravity was intermediate to that of the cottonseed components being separated. The pre-pilot-plant scale development proved adequate for producing sufficient pigment glands and meal (essentially free of glands) for early nutritional studies (2). But the method was not considered commercially feasible because it required a high-boiling solvent such as tetrachlorethylene, whose removal from the oil or the meal calls for high vacuum or high temperatures. The use of high vacuum is costly; and the use of high temperatures lowers the value of the oil by color fixation (6) and the nutritive value of the meal by decreasing the protein solubility (1). Moreover, a 3-component system of oil, hexane, and tetrachlorethylene is formed, complicating the recovery of both solvents and oil.

Consequently a second fractionation process was developed. It is called "differential settling" and has been developed on a laboratory-scale (7) and a pilot-plant-scale using the "disintegration and separation" method. Differential settling overcomes the inherent disadvantages of the mixed-solvent flotation process primarily by the use of only one solvent, com-

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[1] Report of a study made under the Research and Marketing Act of 1946.

[2] Presented at the spring meeting of the American Oil Chemists' Society, held in New Orleans, La., May 1949.

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