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Hydrogenation of Linolenate. IV. Kinetics of Catalytic and Homogeneous Chemical Reduction[1]

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Kinetics for consecutive reactions of octadecatrienoate to octadecadienoate to octadecenoate have been studied with the aid of radioisotopic tracers and gas chromatography. Evidence for a triene to monoene shunt has been obtained. Similarly, the chemical reduction with hydrazine has been studied, but no evidence for this anomalous behavior was obtained. Methods to determine reaction rates from these kinetic measurements are discussed.

BAILEY summarized in 1949 the kinetic information available on catalytic reduction of triglyceride oils containing linolenic acid (1). First order equations for estimating the relative reaction rates of oleic, isolinoleic, linoleic, and linolenic acids were adapted by him. Under "nonselective" conditions, the ratio of reaction rates of linolenate to linoleate of 1.7 was observed, whereas under "selective" conditions a ratio of 2.5 was found. Consideration of reaction rates led him to conclude that a large portion of linolenate was directly reduced to oleate not stopping at the linoleate stage. No further publications on the rates of hydrogenation of linolenate have appeared since Bailey's summary.

In this work C^{14}-labeled fatty acid methyl esters were used to study the kinetics of catalytic reduction. Newly developed procedures for monitoring gas chromatography for labeled compounds (2) were exploited to estimate the specific activity of individual esters, to measure the rates of hydrogenation of linolenate and linoleate and to study the steps of conversion of linolenate to octadecenoate, i.e., the "oleate shunt." These kinetics for heterogeneous catalysis were compared with those for the homogeneous chemical reduction of linolenate by hydrazine. This single-phase chemical reaction, in contrast to heterogeneous catalysis, is characterized by either minimal, or no shift, in position (3) or geometric configuration of double bonds (4). Also, in contrast to catalytic hydrogenation and its varying order of reaction, first order kinetics are observed for chemical reduction; no evidence is apparent for the oleate shunt in homogeneous phase reduction. The reaction rates for the chemical reduction of octadecatrienoate, octadecadienoate and octadecenoate appear in approximately the same ratio as the number of double bonds present.

Experimental

An equimixture of methyl linolenate and methyl linoleate by weight was hydrogenated in all the experiments described. This procedure permitted relative reaction rates for the two esters to be determined under identical conditions in each experiment. It minimized variations caused from run to run by uncontrolled differences in conditions such as catalyst concentration or activity, pressure, stirring, and inhibitors.

Catalytic hydrogenations presented are of two types: a) those in which C^{14}-labeled linolenate is added to the equimixture and b) those in which C^{14}-labeled linoleate is added.

Methyl linolenate and methyl linoleate were isolated in gas chromatographically pure state by the preparative countercurrent distribution technique (5). Linolenate randomly labeled with C^{14} was isolated by countercurrent distribution from soybeans grown in an atmosphere containing $C^{14}O_2$ (6). Linoleate, carboxy labeled with C^{14}, was obtained from Nuclear-Chicago Corp.

Catalytic hydrogenation experiments were carried out on a 0.6-g. scale with approximately 3 microcuries of added labeled ester. A 50-ml. flask with slightly rounded bottom and a magnetic stirrer comprised the reactor. A temperature of 140°C., hydrogen gas at atmospheric pressure, and 0.5% of a commercial catalyst (electrolytically reduced nickel on kieselguhr) were used. The uptake of hydrogen was followed manometrically. Samples were removed through a rubber serum cap seal with a hypodermic needle and syringe at appropriate intervals of hydrogen absorption. At each sampling, approximately 50 mg. were removed, weighed in the syringe and made to 1 ml. in pentane-hexane solvent. After the catalyst settled out, 20 μl. of solution containing approximately 1 μl. of esters was injected into a 7-ft. gas chromatographic column packed with 20% (w/w) of polyethylene glycol succinate on 80- to 100-mesh Chromosorb. A conventional gas chromatograph with thermal conductivity detector was used. Eluent solutes were

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[2] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

collected at the detector outlet into vials of scintillation solvent at 30- or 60-sec. intervals, and the radioactivity of the solutes was determined by a liquid scintillation spectrometer (2). The percentage composition of the separated fatty acid esters and their corresponding radioactivity plotted from scintillation spectrometer data were determined by measuring areas under Gaussian-type curves. "Relative specific activities" were then calculated as the ratio of the per cent of radioactivity to the weight per cent for the corresponding ester component.

Hydrazine reductions were carried out at 50°C. in a flask, open to the air and provided with a magnetic stirrer. To 665 mg. of linoleic and linolenic acid mixture, containing 3.3 microcuries of carboxy-labeled linoleic acid, were added 6.5 ml. ethanol and 1.6 ml. hydrazine hydrate. Samples were removed from the flask after 0.25-, 0.50-, 1-, 2-, 4-, and 8-hr. reaction times and were acidified with hydrochloric acid. Fatty acids were recovered from the mixtures by ether extraction and were converted to their methyl esters by diazomethane. Gas chromatographic analysis and radioactive assay of the individual esters were determined as described.

Kinetic data were handled as proposed by Bailey (1), by plotting the fatty acid composition data against the extent of reaction rather than against time. As he points out, for this analysis the first order equations are valid regardless of whether or not the reactions proceed in true first order fashion. They become invalid only if the relative reaction rates change. However, instead of using iodine value as Bailey proposed, an equivalent, the average number of double bonds remaining, was plotted.

Theoretical curves for the composition were calculated using the equations for first order consecutive reactions and assuming a value for $K = k_a/k_b$, the ratio of reaction rate of linolenate (triene) to that for linoleate (diene). Equations describing this simple system

$$\begin{array}{ccccc} A & \xrightarrow[H_2]{(k_a)} & B & \xrightarrow[H_2]{(k_b)} & D \\ \text{(Triene)} & & \text{(Diene)} & & \text{(Monoene)} \end{array}$$

are to be found in textbooks of physical chemistry and are

$$A_t=A_o\, e^{-k_a t} \qquad 1$$
$$B_t=A_o \quad B_o\, e^{-k_b t}+(k_a/k_b-k_a) \quad (e^{-k_a t}-e^{-k_b t}) \qquad 2$$
$$D_t=1-(A_t+B_t) \qquad 3$$

Initial conditions, as pointed out, for these experiments are

$$A_o=B_o=0.5.$$

Theoretical curves for the analysis of radioactivity were calculated by these same equations. In experiments in which labeled linolenate is added $A_o^* = 0.5$ and $B_o^* = 0$ where the asterisk indicates that the substance is radioactive. When tagged linoleate is added $A_o^* = 0$ and $B_o^* = 0.5$.

Since nonconformity to this simple kinetic pattern became evident during the research, it was necessary to consider the formation of isolinoleate and the oleate shunt according to the scheme:

$$\begin{tikzcd} & \text{Linoleate} \arrow[dr] & \\ \text{Linolenate} \arrow[ur] \arrow[rr] \arrow[dr] & & \text{Oleate} \\ & \text{Isolinoleate} \arrow[ur] & \end{tikzcd}$$

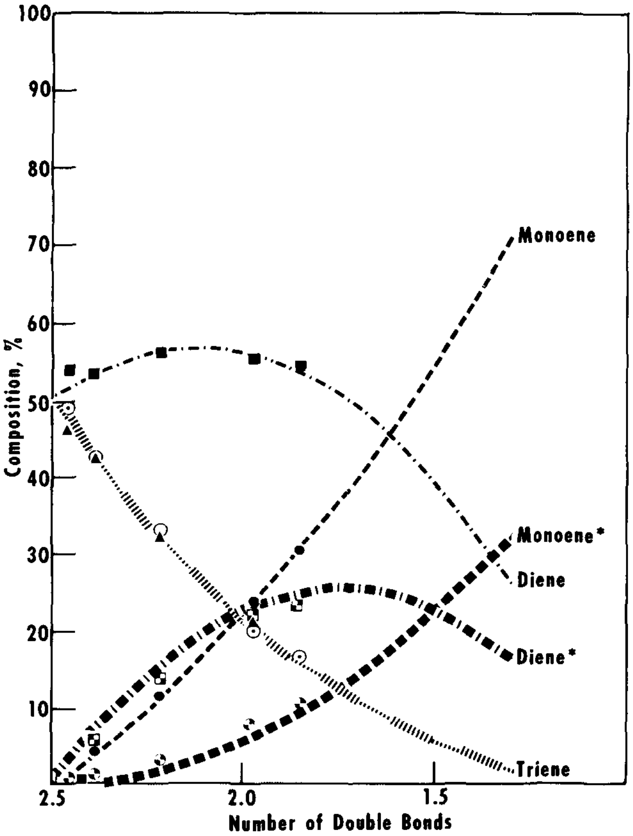

FIG. 1. Catalytic hydrogenation of a linolenate-linoleate mixture with C^{14}-labeled linolenate calculated on the basis of a three-component system (Exp. 1).

Since no stearate was found in our reduced samples and since no saturates, oleate, or isolinoleate were in the original sample, k_d, D_o, and C_o are zero. Therefore, Bailey's equations describing this system can be simplified as follows:

Let A_o = % linolenate in original mixture
F_b = % fraction of A going to linoleate
F_c = % fraction of A going to isolinoleates
F_d = % fraction of A going to oleates
B_o = % linoleate in original mixture

At any given time, t,
A_t = % linolenate in mixture
B_t = % linoleate in mixture
C_t = % isolinoleate in mixture
D_t = % oleate in mixture

Let k_a = relative reaction rate of linolenate
k_b = relative reaction rate of linoleate
k_c = relative reaction rate of isolinoleate

Then
$$A_t = A_o e^{-k_a t}$$
$$B_t = B_o e^{-k_b t}+A_oF_b\left(\frac{k_a}{k_b-k_a}\right)(e^{-k_a t}-e^{-k_b t})$$
$$C_t = A_oF_c\left(\frac{k_a}{k_c-k_a}\right)(e^{-k_a t}-1)$$
$$D_t = 100-(A_t+B_t+C_t)$$

In these experiments $A_o = B_o = 50$. In calculating the radioactive substances $A_o = 50$, $B_o = 0$ for tagged linolenate; $A_o = 0$, $B_o = 50$ for tagged linoleate.

Results

Induction periods and rates of hydrogenation varied greatly from run to run. For example, in the

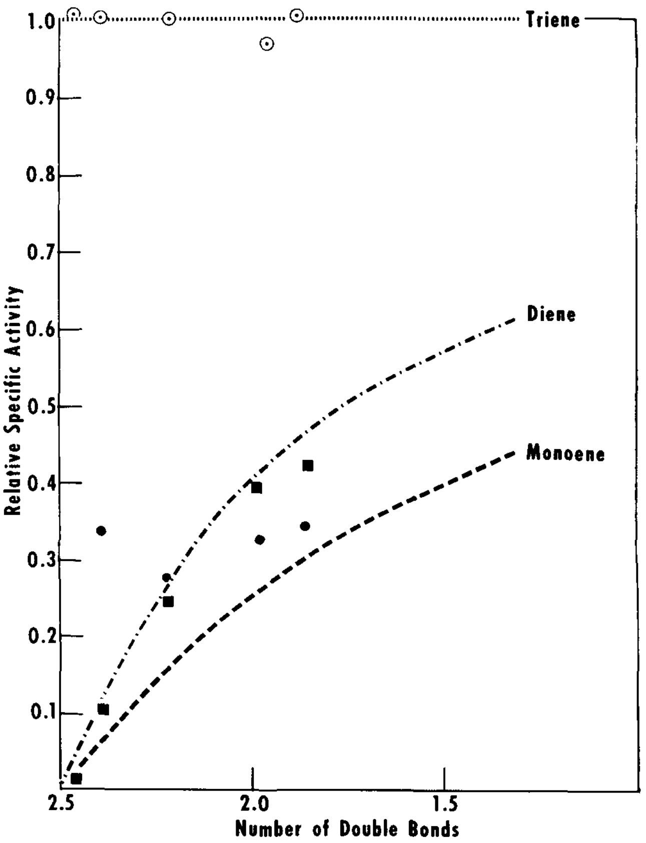

FIG. 2. Theoretical and experimental specific activities for the data of Fig. 1 (Exp. 1).

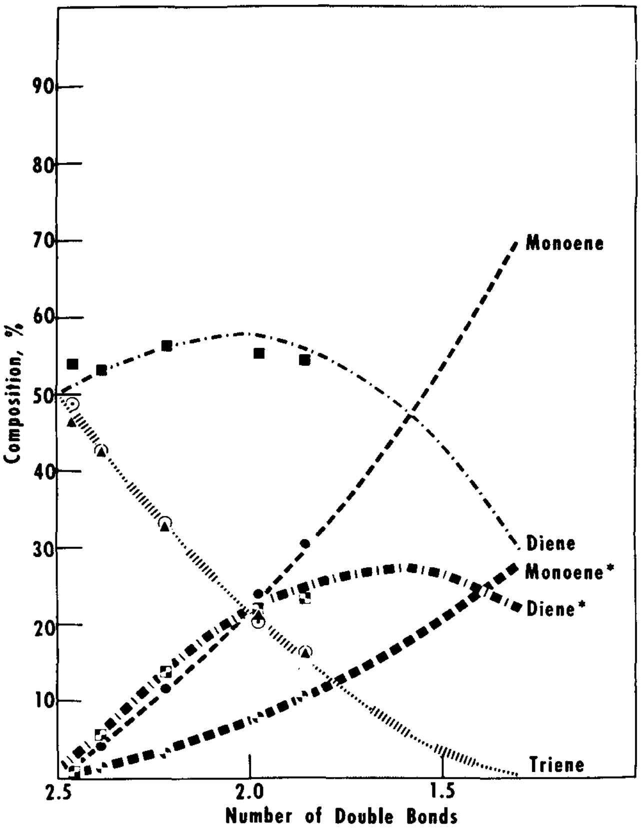

FIG. 3. Catalytic hydrogenation of a linolenate-linoleate mixture with C^{14}-labeled linolenate calculated on the basis that isolinoleate is formed and an oleate shunt occurs (Exp. 1).

experiment with radioactive methyl linolenate, there was an induction period of 25 min. followed by a reaction time of 45 min. In the experiment with radioactive methyl linoleate, the induction period was 15 min. followed by a reaction period of only 40 min. although the reduction was carried somewhat further.

After the induction period the rate of hydrogen absorption was constant; that is, the reaction was zero order for hydrogen absorption.

Catalytic Hydrogenation Using Randomly Labeled Linolenate. In Fig. 1 the experimental composition *versus* the number of bonds remaining per mole ester found in experiment 1 is compared with calculated curves based upon simple consecutive reactions: Linolenate $\xrightarrow{k_a}$, Linoleate $\xrightarrow{k_b}$, Oleate where $k_a/k_b=2$. The experimental data fit the calculated curves rather well although, and significantly, all values for radioactive monoene are above the corresponding theoretical curve. In Fig. 2 the relative specific activities for diene and monoene esters are plotted against the remaining double bonds. Relative specific activity is defined as per cent of total radioactivity divided by weight per cent. The relative specific activity of the dienes fit the curve, but for the monoenes a significant deviation from the calculated values is found, particularly in the early stages of the hydrogenation. This constitutes evidence for an oleate shunt; that is, a direct reduction of linolenate to monoene as suggested by Bailey (1).

In order to fit the experimental specific activities of the monoene to a calculated curve more closely, it is necessary to consider both the oleate shunt and the formation of isolinoleates. However, when this is done, it becomes necessary to assume a new value for k_a/k_b. Since Bailey found the reaction rate of isolinoleate to be only slightly greater than that of oleate, we have assumed $k_c = 0$ over the range of reduction considered in these experiments. Values for k_a/k_b, F_c, and F_d must then be obtained by a trial and error

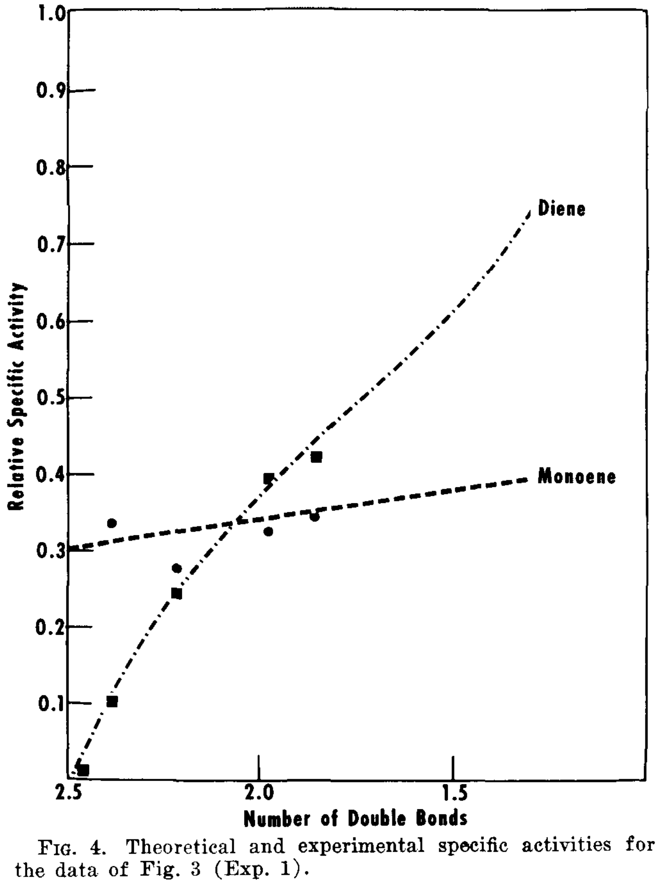

FIG. 4. Theoretical and experimental specific activities for the data of Fig. 3 (Exp. 1).

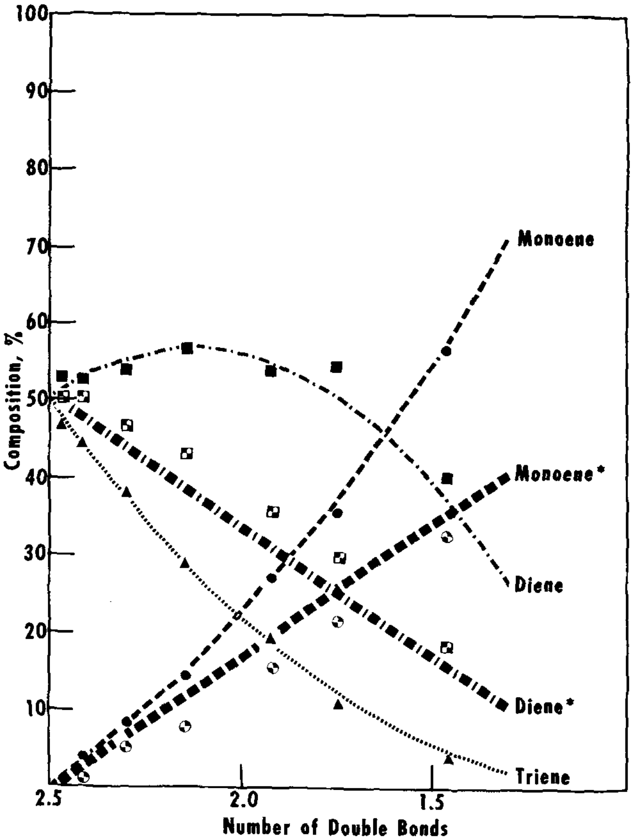

FIG. 5. Catalytic hydrogenation of a linolenate-linoleate mixture with C^{14}-labeled linoleate calculated on basis of a three-component system (Exp. 2).

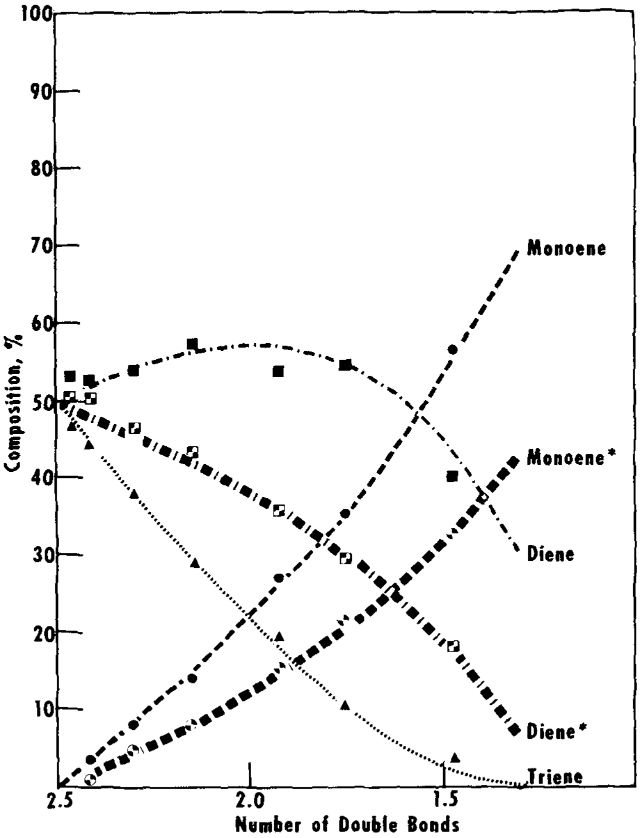

FIG. 7. Catalytic hydrogenation of a linolenate-linoleate mixture with C^{14}-labeled linoleate calculated on basis that isolinoleate is formed and an oleate shunt occurs (Exp. 2).

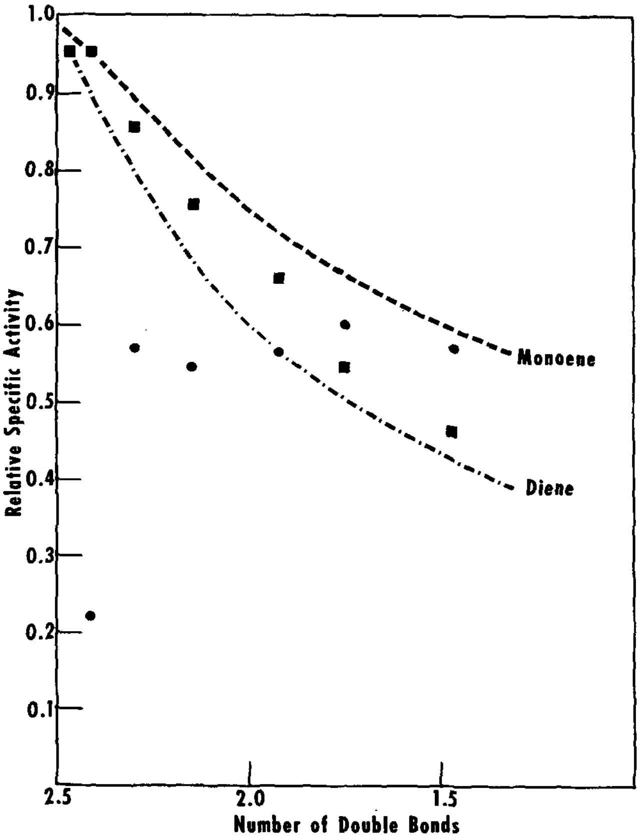

FIG. 6. Theoretical and experimental specific activities for the data of Fig. 5 (Exp. 2).

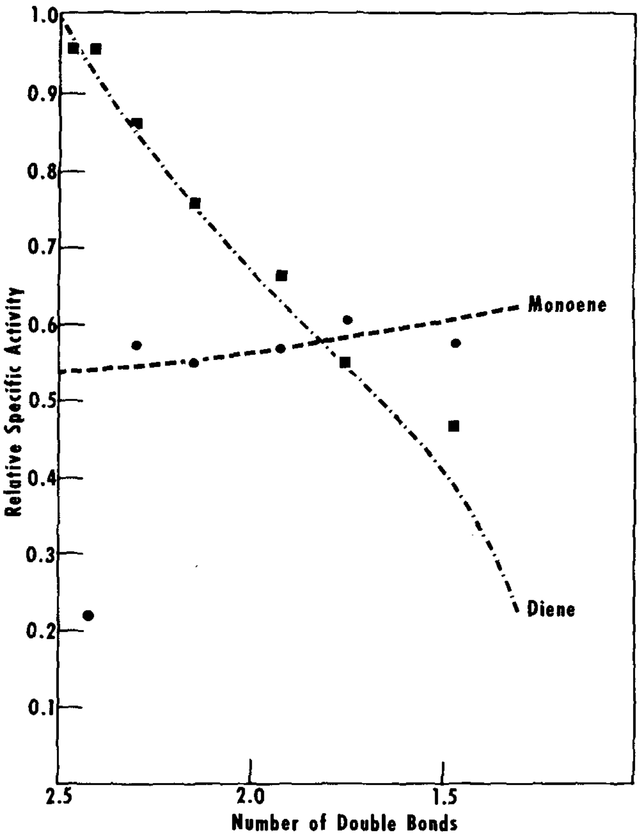

FIG. 8. Theoretical and experimental specific activities for the data of Fig. 7 (Exp. 2).

process so that both composition and specific activity curves fit the experimental values. In this experiment values $k_a/k_b = 2.5$, $F_b = 0.5$, $F_c = 0.325$, $F_d = 0.175$ satisfactorily fit the data shown in Fig. 3 and 4.

Catalytic Hydrogenation Using Carboxy C^{14}-Labeled Linoleate. In Fig. 5 the experimental composition found in experiment 2 is compared with calculated curves based upon the same simple consecutive reactions used in Fig. 2, and in Fig. 6 the specific activities for diene and monoene are plotted against the remaining double bonds. As in the preceding experiment, data fit the curves rather well for $k_a/k_b = 2.0$, except for the amount of radioactive monoene and the specific activity of the monoene. These deviate from the calculated curves in the opposite direction, and it is necessary again to take into account the oleate shunt and formation of isolinoleates. Values of $k_a/k_b = 3.0$, $F_b = 0.3$, $F_c = 0.41$, $F_d = 0.29$ fit the experimental data shown in Fig. 7 and 8.

Hydrazine Reduction Using Carboxy C^{14}-Labeled Linoleate. Semilogarithmic plots of total linolenate and radioactive linoleate *versus* time found in experiment 3 are given in Fig. 9 and confirm preliminary observations that over a wide range of the reaction the first order law is obeyed. The specific reaction rate k_a for triene determined from a least square line through the origin in Fig. 9 is 0.744. A value for $k_b = 0.38$ for diene was obtained the same way. However, a value for $k_b = 0.429$ obtained from the cross-over point (7) for the linolenate and monoene curves gives a better fit for the composition curves, and this value was used in Fig. 10 and 11. The absolute values of these rates probably have no signifiicance in themselves and could be changed by changing either the stirring rate or the amount of air dispersed in the solution. However, their ratio of 1.73 provides a satisfactory fit for the experimental points of chemical analysis in Fig. 10 and of radioactive analysis in Fig. 11.

Since the reaction in this experiment was carried to a point where less than one double bond remains, and since hydrazine reduction has much less selectivity between mono- and polyunsaturated esters than does catalytic hydrogenation, it was necessary to take into account the formation of stearic acid in the consecutive reactions. A value of k_d equal to one-half that of k_b would be expected if there were no selectivity. This value was used with the values for k_a and k_b which were calculated from data in Fig. 9. Because of the small amounts of monoene and stearate, especially in the first samples, it was not possible to calculate accurate values of specific activity. For this reason curves similar to those of Fig. 2 and 4 are not presented. However, the values obtained for specific activity in later samples gave no evidence of an oleate shunt. The ratio of rates calculated from the data of Fig. 10 for triene:diene:monoene are 3.46, 2, and 1, respectively.

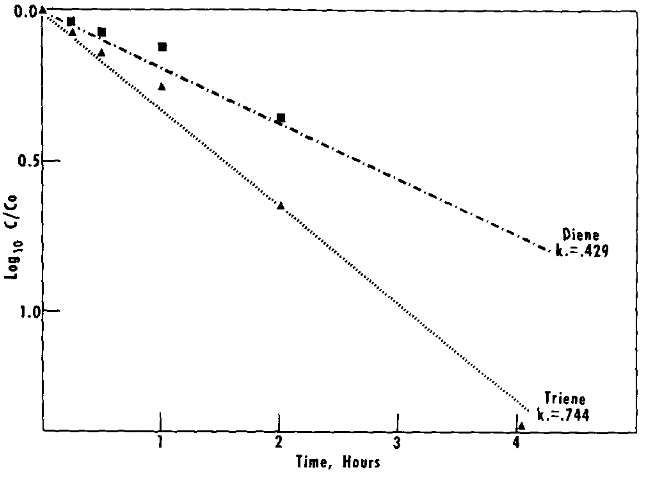

FIG. 9. Time course for hydrazine reduction of the linolenate-linoleate mixture with C^{14}-labeled linoleate (Exp. 3).

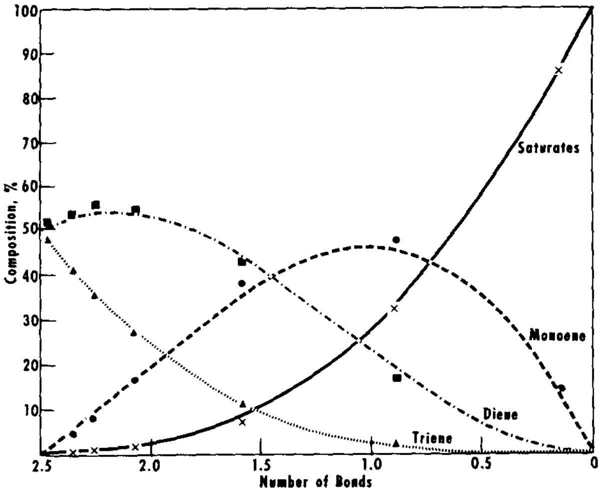

FIG. 10. Hydrazine reduction of the linolenate-linoleate mixture—chemical analyses (Exp. 3).

Discussion

Radioactive isotopic techniques frequently provide unique answers to problems of mechanism. In our work direct evidence from two types of tracer experiments has been obtained for the existence of an oleate shunt in catalytic reduction. When tagged linolenate is added, radio activity finds it way in the early stages into the monoene fraction in larger amounts than would be predicted by the simple theory of consecutive reactions. One explanation is no shunt path actually exists—but rather when a linolenate molecule is once adsorbed on the catalyst surface and reduced, there is a greater probability that this same molecule of diene will be reabsorbed and reduced to monoene than there is that a diene randomly located in the oil solution will be absorbed and reduced. Another explanation is that the shunt proceeds through a conjugated linolenate intermediate. Although we found no shunt in the hydrazine reduction of linolen-

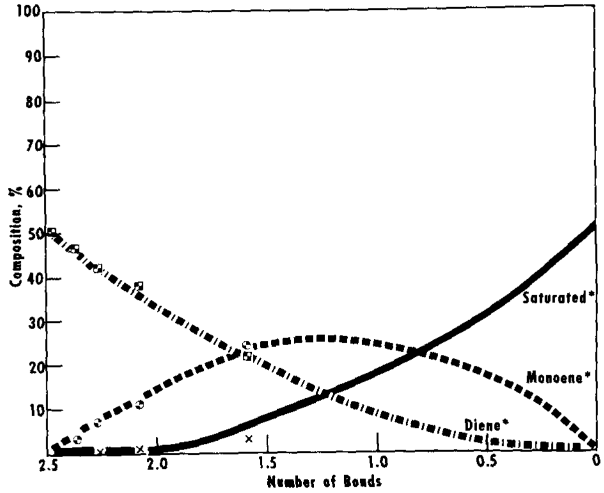

FIG. 11. Hydrazine reduction of the linolenate-linoleate mixture—radiochemical analyses (Exp. 3).

ate, Aylward and Rao (8) found that with eleostearic acid two molecules of hydrogen are added simultaneously. Also, Hilditch and Pathak (9) found that in catalytic hydrogenation two molecules of hydrogen are added simultaneously to methyl eleostearate.

The relative reaction rates and the course of linolenate reduction in the two experiments are different although the temperature, pressure, catalyst concentration, and a lot of catalyst are the same. The differences are believed to be caused by uncontrolled variation in catalyst activity since, as stated under Results, the induction periods and reaction rates are also different in the two reactions.

The determination of reaction rates k_a and k_b, and subsequently their ratio (k_a/k_b), is greatly facilitated by the radioactive tracers. For example, in the hydrazine reduction with tagged linoleate as illustrated in Fig. 9, when first order kinetics are followed, the slope of a semilogarithmic plot of percentage triene *versus* time gives k_b directly and the slope of a similar plot of radioactive diene gives k_b directly.

It can be shown that in a system undergoing simple consecutive reactions of the type $A \longrightarrow B \longrightarrow C$ if the inital amounts of the two components are equal and if the ratio of reaction rates falls in the range between 1 and 3, two opportunities are provided to measure the ratio of reaction rates without resort to isotopic tracers: Since over a considerable period of time component B remains nearly constant in amount, the slope of the curve for the formation of component C is indeed the rate of reaction for component B.

$$\frac{dc_c}{d_t} = \frac{dc_b}{d_t} = k_b$$

The slope of the initial portion of curve C is the reaction rate.

Another method (7) for determining the ratio of rates without resort to radioactive tracers is based upon stopping the reaction of the equimixture at the point of addition of 0.5 mole of H_2 per mole ester or the point of crossover of curves C and B. This procedure is easy to perform experimentally and is in routine use at this laboratory (10).

A third method for estimating the ratio of rates for consecutive reactions has recently been described by Ames (11). It involves eliminating the time variable from the differential equations describing the rates and solving the resulting differential equation by using the general theory of homogenous differential equations. This ratio is obtained in an implicit function involving the original linolenate concentration, the final linolenate concentration, and the final concentration of linoleate formed from the linolenate. Although these methods cannot give exact values for k_a/k_b when the oleate shunt and formation of isolinoleate occur, they give an approximation which is valuable for screening purposes.

Acknowledgment

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Hydrogenation of Linolenate. V. Procedure of Evaluating Hydrogenation Catalysts for Selectivity[1]

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Equations for determining the ratio of hydrogenation rates for linolenate and linoleate acyl groups are derived from kinetic theory. They are based upon the analysis for linolenate after absorption of 0.5 mole of hydrogen by an equal mixture of linoleate and linolenate. This method finds routine application in the evaluation of hydrogenation catalysts for selectivity.

TO EVALUATE hydrogenation catalysts for selectivity with respect to linolenate and linoleate acyl groups has required the development of a simple routine procedure for determining the ratio of reaction rates. Measurement of this ratio has usually involved periodic sampling and analysis, plotting the concentration of components against time or percentage reaction, and obtaining the best fit for the experimental data by more or less empirically adjusting constants for specific reaction rate (1). Radioactive tracers have been employed at this laboratory to study the mechanism of catalytic hydrogenation and to facilitate the calculation of reaction rates (2), but their use does not simplify experimental technique.

The present procedure, applicable to triglycerides or monoesters, involves hydrogenating an equal mixture of linolenate and linoleate and from the results, determining the ratio of reaction rate constants. Compared to determination of reaction rates on the single pure components, this design of experiment minimizes variations such as concentration and activity of catalyst, temperature, and pressure. In brief, the method consists of reducing the equal mixture of linolenate and linoleate (2.5 average double bonds per mole) with 0.5 mole of hydrogen (to yield 2.0 average double bonds per mole) at which point the monounsaturated components formed must equal the triunsaturated components remaining. The ratio of

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[1] Presented at spring meeting, American Oil Chemists' Society, May 1–3, 1961, St. Louis, Mo.

[2] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.