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

The authors are indebted to E. B. Lancaster for his interest and advice throughout the course of this study.

REFERENCES

1. Bailey, A. E., J. Am. Oil Chemists' Soc., *26*, 644 (1949).
2. Dutton, H. J., J. Am. Oil Chem. Soc., *38*, 631 (1961).
3. Scholfield, C. R., Jones, E. P., Nowakowska, Janina, Selke, E., and Dutton, H. J., J. Am. Oil Chemists' Soc., *38*, 208 (1961).
4. Aylward, F., and Rao, C. V. N., J. Sci. Ind. Res., *18B*, 131 (1959).
5. Scholfield, C. R., Nowakowska, Janina, and Dutton, H. J., J. Am. Oil Chemists' Soc., *37*, 27 (1960).
6. Dutton, H. J., Jones, E. P., Scholfield, C. R., Chorney, W., and Scully, N. J., J. Lipid Res., *2*, 63 (1961).
7. Dutton, H. J. Presented at spring meeting of American Oil Chemists' Society, St. Louis, Missouri, May 1–3, 1961.
8. Aylward, F., and Rao, C. V. N., J. Appl. Chem., *7*, 137 (1957).
9. Hilditch, T. P., and Pathak, S. P., Proc. Roy. Soc. (London), *A198*, 323 (1949).
10. Johnston, A. E., Macmillan, D., Dutton, H. J., and Cowan, J. C. Presented at spring meeting American Oil Chemists' Society, St. Louis, Missouri, May 1–3, 1961.
11. Ames, W. F., Ind. Eng. Chem., *52*, 517 (1960).

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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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reaction rates at this crossover point is related to the percentage of the monoene or triene components and is read from a theoretically established curve.

Theoretical

The kinetics of consecutive mono-molecular reactions may be represented as follows:

$$\underset{\text{(Triene)}}{A} \xrightarrow[H_2]{k_a} \underset{\text{(Diene)}}{B} \xrightarrow[H_2]{k_b} \underset{\text{(Monoene)}}{C}$$

as described by the equations:

$$A_t = A_o e^{-k_a t} \quad 1)$$

$$B_t = \frac{k_a}{k_b - k_a} A_o(e^{-k_a t} - e^{-k_b t}) + B_o e^{-k_b t} \quad 2)$$

$$C_t = 1 - (A_t + B_t) \quad 3)$$

where A_t, B_t, and C_t are the amounts of A, B, and C present at any given time (t) and A_o and B_o are the initial amounts. Under this procedure, $A_o = B_o = 0.5$, and k_a and k_b are the specific reaction rates for linolenate and linoleate, respectively. The ratio of reaction rates (K) is defined as $K = \frac{k_a}{k_b}$.

The operation of these equations for the ratio of reaction rates for $K = k_a/k_b = 2$ is illustrated in Figure 1, together with published experimental data for the hydrogenation of an equal mixture of linolenate and linoleate (2). It may be seen from these data that curves for A and C cross at the point where the level of unsaturation is 2.0 double bonds per mole, as required by theory. The necessity for the crossover point ($A_x = C_x$) existing at this level of unsaturation becomes apparent by writing two simultaneous equations, one accounting for the unsaturation and the other for composition:

$$3A_x + 2B_x + C_x = 2 \quad 4)$$

$$A_x + B_x + C_x = 1 \quad 5)$$

Eliminating B_x from these simultaneous equations:

$$A_x = C_x \text{ (Q.E.D.)} \quad 6)$$

On eliminating C_x, one obtains

$$2A_x + B_x = 1 \quad 7)$$

Substituting in equation 7, their equivalents from equations 1 and 2 and substituting for k_b its equivalent k_a/K, one obtains the implicit function

$$e^{-k_a t} \cdot \frac{2-K}{2-2K} + e^{\frac{-k_a t}{K}} \cdot \frac{1-2K}{2-2K} = 1 \quad 8)^3$$

Three solutions are readily apparent from this equation. For the specific case of K = 2 the first term of equation 8) drops out and becomes $e^{\frac{-k_a t}{2}} = \frac{2}{3}$.

Therefore, $k_a t$ is equal to −0.80994 and A_x is equal to $A_o e^{-k_a t} = 0.5 \times 0.4445 = .2222. \cong 22.22\%$. Similarly, for K = 0.5, the second term drops out and A_x is equal to 0.333. As K approaches zero, A_x approaches 0.5. These three points are used in setting up Figure 2. The remaining points establishing the curve were obtained by plotting data for values of A_x and C_x vs. t for k_a ranging from 1 to 50 and by graphically determining the crossover points. These calculations for 33 k_a's and 11 t periods each were computed by the ARS Biometrical Services, U. S. Department of Agriculture.

Procedure

An equimixture of linolenate and linoleate is hy-

[3] In the course of reviewing this manuscript, E. B. Lancaster of this laboratory suggested that $e^{-k_a t}$ in this equation might be eliminated by substituting its equivalent—$2A_x$. Equation 8 then becomes:

$$\frac{2-K}{1-K} \cdot A_x + \frac{1+2K}{2(1-K)} \cdot (2A_x)^{\frac{1}{K}} = 1$$

The three solutions discussed become readily apparent in this form.

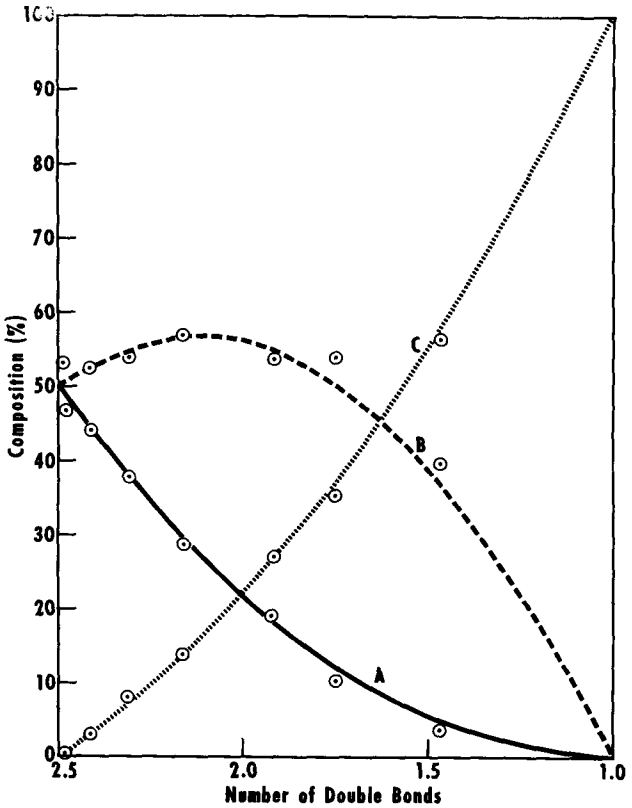

FIG. 1. Kinetics for consecutive reactions of hydrogenation $K = \frac{k_a}{k_b} = 2$ with experimentally determined points (2). A = triene, B = diene, C = monoene.

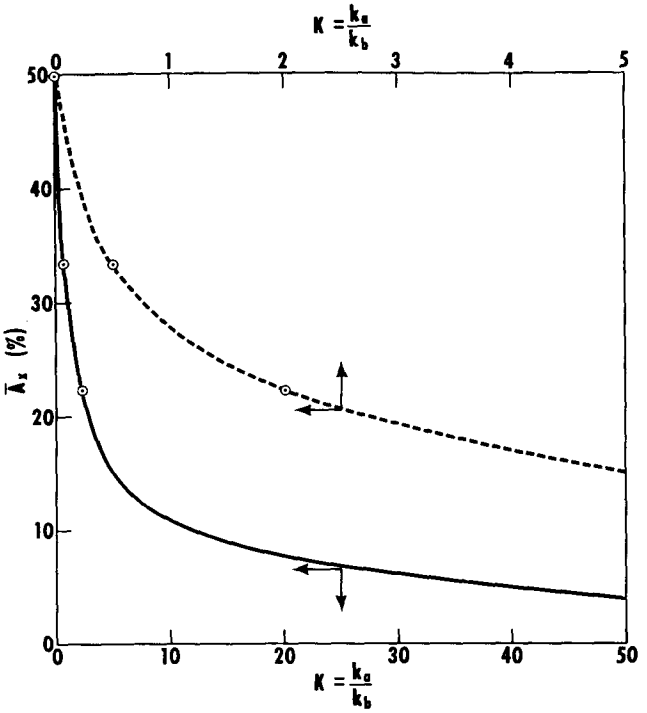

FIG. 2. The relationship of the ratio of reaction rates $K = \frac{k_a}{k_b}$ to the amount of linolenate (A_x) at the crossover point.

drogenated with 0.5 mole hydrogen per mole ester. Triene, diene, and monoene are determined on the hydrogenated esters by gas chromatography or other analytical procedures.[4]. Since triene (A_x) should equal monoene (C_x), any failure to absorb exactly 0.5 mole will be reflected in a high amount of triene and a low amount of monoene, or vice versa, but with little change in diene. The average value for monoene and triene is therefore used to indicate ($\bar{A}_x$) which equals $\frac{A_x + C_x}{2}$. The value of K corresponding to $\bar{A}_x$ is read from Figure 2.

Discussion

Since a variety of equipment may be used to carry out the hydrogenation under many conditions of temperature, pressure, stirring rates, and hydrogen dispersion and to determine when 0.5 mole of hydrogen has been absorbed, no attempt is made here to specify experimental procedures. It is believed that the general experimental design and method of handling data proposed should be applicable to a variety of hydrogenation techniques. Other analytical determinations undoubtedly will be desired such as *trans* acids by infrared spectrophotometry, conjugated dienes by ultraviolet spectrophotometry, position of bond by oxidative cleavage or mass spectroscopy.

Cognizance has not been taken in this discussion of the inevitable traces of saturates present. Their formation in appreciable amounts by any catalyst would, of course, vitiate its usefulness for hydrogenation purposes, as well as introduce errors, or invalidate the procedure outlined. Traces of saturates, when they occur, are corrected by adding their percentage to that of the oleate in the following manner: $\frac{A_x + C_x + S_x}{2} = \bar{A}_x$. The formation of diene geometric and positional isomers and their effect on relative rates of hydrogenation also have not been considered in the present attempt to develop a simple equation. Some justification for this omission may be found in the observation that during the absorption of 0.5 mole of hydrogen per mole of ester by the 50:50 linolenate-linoleate mixture, the maximum proportion of isomeric dienes in the mixture only reaches 10%.

This procedure has been applied routinely in this laboratory for evaluating the selectivity of hydrogenation catalysts, and subsequent papers will describe its use.

[4] Because residual triene is essentially unaltered during hydrogenation, the spectrophotometric determination of linolenic acid following alkali isomerization appears valid.

REFERENCES

1. Bailey, A. E., J. Am. Oil Chemists' Soc., *26*, 644 (1949).
2. Scholfield, C. R., Nowakowska, Janina, and Dutton, H. J., *Ibid.*, 39, 90 (1962).

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The Effect of Some Amino Acids on the Oxidation of Linoleic Acid and Its Methyl Ester[1]

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Manometric studies of the effect of certain amino acids on oxidation (measured as oxygen consumption) of linoleic acid, as well as the methyl esters of linoleic acid and linolenic acid dispersed in water or phosphate buffers at pH 7 to pH 5 have shown that

1. The amino acids tested (except cysteine) have a potential antioxidative effect.
2. The antioxidative capacity of different amino acids may be rather different (it is especially pronounced in the case of histidine and tryptophane).
3. Under suitable conditions extremely low amino acid concentrations may have rather strong effect.
4. The antioxidative efficiency is less pronounced than in earlier experiments with linoleate at pH>7, and decreases with decreasing pH.
5. There may be a tendency towards a prooxidative inversion with relatively high amino acid concentrations, or at low pH.
6. The antioxidative effect is enhanced and a prooxidative effect may be lowered or inverted into an antioxidative one by an addition of phosphate, or an emulsifier like Tween.
7. A strong inhibitory effect is obtained by combined addition of phosphate and emulsifier like Tween, together with the amino acid.
8. The antioxidative tendency was stronger in the case of methyl linoleate than with linoleic acid, and was also stronger with methyl linoleate than with methyl linolenate.

THERE IS an increasing interest in antioxidative substances occurring in biological material and in synergistic relationships with regard to protection against fat oxidation *in vivo* and in food. As the content of so-called primary antioxidants of phenolic type (e.g. the tocopherols) capable of breaking oxidative chain reactions often is very low, the interest is directed towards substances of other types, which may occur in biological material and may participate, e.g. as synergists, in preventing rancidification. These substances may react in different ways: by rendering prooxidative substances innocuous, or by regenerating oxidized primary antioxidants.

Amino acids have been mentioned earlier in the literature as antioxidants (3,15,17,18,34) and as components of patented antioxidant mixtures (8,19,38). But there is relatively little exact knowledge as to their effects. Amino acids are also mentioned as prooxidants (5,15,40).

Their antioxidative effect is generally suggested to be of synergistic nature, i.e., to require the presence of a primary antioxidant. Amino acids are usually supposed to function by chelating prooxidative metal traces. The prooxidative effect of copper traces is mainly due to copper in free state in the fat phase (18). In the presence of amino acids copper complexes are formed and transported into the water phase. In this way traces of copper may be rendered innocuous as catalysts. It has however also been shown (39) that copper bound to amino acids, peptides, and proteins has a strong catalyzing effect on the oxidation of ascorbic acid and that linoleate oxidation is accelerated by copper protein complexes (46). But also an inhibiting action of proteins on the prooxidative effect of metal traces in fat emulsions

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