Relative Reduction Rates of Fatty Acid Isomers by Hydrazine[1]

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Abstract

Mixtures of fatty acids and of hydrocarbons have been reduced by hydrazine in ethyl alcohol solution at 50 C. Samples were removed during reduction for analysis and the relative reduction rates calculated by digital computer. *cis*-15-Octadecenoic acid is reduced 1.3 times as fast as its *cis*-9-isomer. No differences were found among *cis*-6,9- and -12-octadecenoic acids. *cis*-3-Octadecene was also reduced 1.3 times as fast as the *cis*-9-isomer and *cis*-1-octadecene 8.6 times as fast. Elaidic acid was reduced 1.27 times as fast as oleic.

Introduction

Previous work has shown that although reduction rates of unsaturated fatty acids by hydrazine are roughly proportional to the number of double bonds in the acid, changes in position and geometric configurations of double bonds also affect the rates of reduction (1,2,10,14,15). Although these differences in rates are small compared to those between monounsaturated and some polyunsaturated esters in catalytic hydrogenation, they are large enough to affect the composition of reduced products. The relative reduction rates with hydrazine for various monounsaturated acids also are not the same as those for catalytic hydrogenation, such as the micro vapor-phase hydrogenation rates reported by Mounts and co-workers (11).

When partial hydrazine reduction is used to determine position and configuration of double bonds in polyunsaturated acids (13,15), especially in mixtures of polyunsaturated acids, a knowledge of relative rates of reduction of double bonds aids interpretation of data. Privett and Nickell (13) have noted that selectivity of hydrazine for certain double bonds may complicate such interpretation. In a study of monoenes from hydrazine reduction of mixtures of linolenic acid geometrical isomers, we found that calculating the composition of the trienoic acid mixture from analysis of the monoenoic acids, assuming equal reactivity for all double bonds, did not produce reasonable values. We suggested this difficulty might be caused, in part, by faster reduction of double bonds with certain positions or configuration (15); results in the present paper support our interpretation.

Experimental Procedure

Hydrazine Reductions

Reductions were carried out in a closed flask to prevent release of hydrazine fumes. Air was drawn through the flask under slightly reduced pressure from the vacuum system. Before passing through the flask, the air was drawn through a flow meter and through a 95% ethanol bubbler to minimize evaporation of ethanol in the reaction flask. A magnetic stirrer was used to stir the solution in the flask, which was immersed in a 50 C constant temperature bath. Samples were withdrawn as the reaction proceeded and added immediately to dilute hydrochloric acid. Free acids were extracted into diethyl ether, washed until neutral and freed of solvent under nitrogen. For analysis these acids were esterified with methanol and perchloric acid catalyst (20).

In a typical reduction 168 mg of *cis*-15-octadecenoic acid (16) and 138 mg of *cis*-9-octadecenoic acid were dissolved in 16 ml of 95% ethanol. A 3 ml sample was removed at zero time and 3 ml of 95% hydrazine was added. With this larger ratio of hydrazine to fatty acid the reduction was more rapid than in some of our previous work (4,17,18). Samples were removed after 15,30 and 60 min and the reaction was stopped after 100 min.

Most analyses were by gas liquid chromatography (GLC) with the following columns and conditions: (a) a capillary 200 ft × 0.01 in. DEGS column at 165 C and 45 psi inlet pressure; (b) a capillary 200 ft × 0.01 in. 100% cyanoethyl silicone column (General Electric experimental nitrile silicone 238–149–99) at 200 C with 20 psi inlet pressure (9); (c) a capillary 150 ft × 0.01 in. polyphenyl ether column at 190 C and 40 psi inlet pressure (8); (d) a packed 4 ft × ¼ in. OD 11% EGSS-X glass column at 170 C. A radium D ionization detector with argon gas was used with all columns. Any variations in these procedures are noted in the descriptions of the individual experiments.

With the polyphenyl ether column it was sometimes necessary to use correction factors as described for the individual experiments to correct area percentage to mole percentage. Uncertainty in these correction factors may limit the accuracy of reaction rates based on measurements with the polyphenyl ether column. No similar difficulty was experienced with other columns.

Calculation of Relative Reaction Rates

Relative reaction rates were calculated (as previously) by plotting fatty acid composition against extent of reaction (18). Rate constants were determined by a digital computer so that the sum of the squares of the differences between calculated and analytically determined compositions was a minimum.

15- vs 9-Octadecenoic Acid

The 15-octadecenoic acid was isolated by argentation chromatography (16). After reduction, fractions were analyzed both on the capillary DEGS and the packed EGSS-X columns. Results agreed well and were averaged for rate calculations. Two experiments gave excellent agreement for the reaction rate ratios with values for k_{15}/k_9 of 1.2968 and 1.3046 or an average value of 1.30. Results of one of the experiments are illustrated in Figure 1.

6- vs 9-Octadecenoic Acid

Since methyl petroselenate and oleate are not separated by GLC, an alternate analytical procedure was followed. 1-^{14}C Oleic acid was added to petroselenic acid so that each mg of the mixture contained approximately 1 μc. Since a negligible weight of oleic acid is added, it gives no GLC response. After reduction by hydrazine and conversion to methyl esters,

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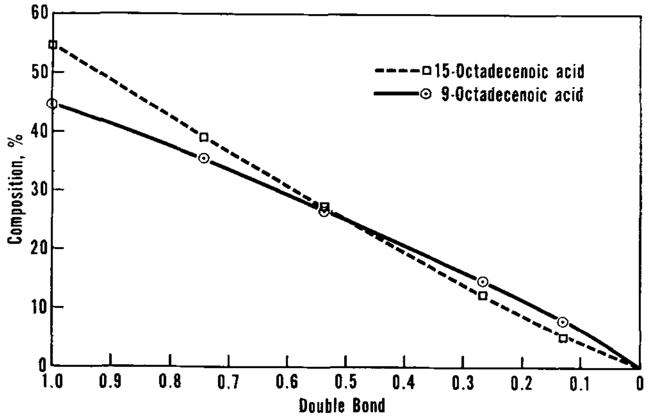

FIG. 1. Reduction of a mixture of 15- and 9-octadecenoic acids by hydrazine showing per cent of each acid remaining plotted against amount of double bond remaining. Points are experimental results. Lines are calculated for k_{15}/k_9 = 1.305.

the samples, taken during the reduction described above, were separated and analyzed by gas radiochromatography in a Cary-Loenco instrument (12). This apparatus provides for thermal conductivity detection of mass peaks and contains a 275 cc spherical ion chamber in the detector oven, in tandem, to monitor the effluent for radioactivity. Separations were made on a 15% EGSS-X (6 ft × ¼ in.) aluminum column at 175 C. Reduction of petroselenate is indicated by the thermal conductivity analysis and of oleate, by the radioactivity analysis. The value for k_6/k_9 was 0.97.

12- vs 9-Octadecenoic Acid

The acids for this reaction were prepared by partially reducing linoleic acid with hydrazine. After the methyl esters were prepared, the monoene fraction was isolated by chromatography on a rubber column (6) to yield a mixture of methyl 12- and 9-octadecenoates. Samples from hydrazine reduction of this monoene fraction were analyzed on the polyphenyl ether column. While this substrate has given good separations of isomeric *cis*-octadecenoates (8), it does not separate stearate from oleate. Stearate was measured on the packed EGSS-X column. Comparison with standard mixtures has shown that with the polyphenyl ether column, corrections must be applied to the measured areas. Correction factors were 1.068 (S + O) and 0.940 (Δ12). A value for k_{12}/k_9 of 1.10 resulted.

Since the accuracy of this value is limited by uncertainty in the gas chromatographic analyses another experiment was run in which a mixture of *cis*-12-octadecenoic acid and 1-^{14}C oleic acid was reduced and samples analyzed in the same way as with petroselenic acid. The *cis*-12-octadecenoic acid was prepared from partially hydrazine-reduced linoleic acid by low temperature crystallization from acetone, using a process based on one described by Privett (private communication). Ten samples were taken during the reduction and a good fit with a value for k_{12}/k_9 of 1.027 was obtained.

3- vs 9-Octadecene

Methyl 15- and 9-octadecenoates were reduced to their alcohols by lithium aluminum hydride. Tosyl esters were formed with *p*-toluenesulfonyl chloride and these were again reduced with lithium aluminum hydride to hydrocarbons.

To 150 mg of 3-octadecene and 128 mg of 9-octadecene in 16 ml of ethanol, 0.1 ml of acetic acid was added to supply the acid groups necessary for reduction (3). In reduced fractions the best separation of octadecane from the octadecenes was made on the 100% cyanoethyl-silicone column at 165 C with 5 to 10 psi. To separate 3-octadecene from 9-octadecene and octadecane, both DEGS at 130 C and 20 psi and polyphenyl ether at 125 C and 45 psi were used and the values were averaged. 9-Octadecene was measured by difference after 3-octadecene and octadecane had thus been determined. A value for k_3/k_9 of 1.31 was obtained.

1- vs 9-Octadecane

The reduction was similar to that of 3- and 9-octadecene except that the starting mixture was 78% 1-octadecene. The polyphenyl ether column was employed to separate octadecane and 9-octadecene from 1-octadecene and 100% cyanoethyl silicone, to separate octadecane from the octadecenes. The value for k_1/k_9 was 8.6.

9,15-Octadecadienoic Acid

This acid was isolated by argentation countercurrent distribution of the dienoic methyl esters from hydrazine-reduced linseed acids (4). Analyses for 9,15-octadecadienoate, 9- and 15-octadecenoates and stearate were by GLC on both the capillary DEGS and the packed EGSS-X columns. Average values were used. In two experiments when k_{15}/k_9 in the monoene was taken as 1.30 as determined above, values for k_{15}/k_9 in the diene were 1.219 and 1.203.

9,12-Octadecadienoic Acid

Stearate and linoleate were measured on the packed EGSS-X column. The stearate oleate combination and 12-octadecenoate were separated on the capillary polyphenyl ether column and corrections were applied to measure areas as described for the 9- and 12-monoene reduction. Relative rates were as follows:

$$\begin{tikzcd}
 & 9 \arrow[dr, "1.00"] & \\
9,12 \arrow[ur, "1.06"] \arrow[dr, "1.06"'] & & St \\
 & 12 \arrow[ur, "0.94"'] &
\end{tikzcd}$$

Elaidic vs Oleic Acid

Three reductions were made of mixtures of elaidic and oleic acids. Stearate in the reduced samples was measured by GLC on the EGSS-X column and elaidate by infrared absorption. Oleate was determined by difference. With this procedure it was clear that elaidate reduced faster than oleate. However, values for $k_{elaidate}/k_{oleate}$ of 1.75, 1.15 and 1.94 showed that the analytical precision was not high enough to give good reproducibility for the rate calculations. Two reductions were made with elaidic acid and 1-^{14}C oleic acid in the same way as described for petroselenic and oleic acid. Values for $k_{elaidic}/k_{oleic}$ were 1.21 and 1.34. These values are believed to be more reliable than those based on GLC and infrared measurements. Results of one of the radiochemical experiments are illustrated in Figure 2.

Results and Discussion

The rate of reduction of double bonds by hydrazine or other diimide-producing reagents depends upon the structure of the unsaturated compound. For example, Garbisch et al. (5) using *p*-toluenesulfonyl hydrazine found a difference of 38,000 in the relative reduction rate of bicyclo (2.2.1) heptene and 1,2-dimethylcyclohexene. However, the variations in rates of reduction of straight-chain fatty acids caused by changes in location and configuration of double bonds are much smaller.

In our previous work on reduction of linolenic acid (18) we found that any difference in reactivity of the double bonds was small. The composition of the monoenate suggested a slightly greater reactivity for the 15 double bond relative to the 9 and 12. Results reported here confirm this conclusion. Two independent hydrazine reductions of mixed 15- and 9-*cis*-octadecenoic acids gave an average value for k_{15}/k_9 of 1.30 with excellent agreement between the two runs. With 3- and 9-octadecenes our value for k_3/k_9 of 1.31 was in agreement with the value for acids. These values for reactivity of the double bond in the 3 position compared with a double bond farther removed from the end of the chain are in good agreement with Asinger and his co-workers' (1) data for undecenes where $k_{cis}/k_{cis\text{-}5} = 1.25$.

When the double bond is in the 1 position, reactivity is much greater. With 1- and 9-octadecenes our value for k_1/k_9 of 8.6 is in good agreement with that of Schilling (14) for $k_{10\text{-undecenoic}}/k_{\text{oleic}}$ of 8.3 and of Asinger et al. (1) for $k_{1\text{-undecene}}/k_{cis\text{-}5\text{-undecene}}$ of 9.3.

No significant difference was found in reactivity between oleic acid and isomers with double bonds in the 6 and 12 position. Very small changes in the gas chromatographic correction factors used with the polyphenyl ether column caused appreciable changes in the relative rates calculated. The value for k_{12}/k_9 of 1.027 obtained with radioactive oleic acid is more reliable and does not differ significantly from one.

In the 9,15-dienoic acid, the 15 double bond is also reduced faster than the 9. An average value for k_{15}/k_9 of 1.21 for double bonds in the diene resulted. It is doubtful if this value differs significantly from that for the monoene.

In the 9,12-dienoic acid, the value for k_{12}/k_9 is 1.00 for double bonds in the diene. This value is also uncertain because of the correction factors used with the polyphenyl ether column, but there is no reason to believe that the relative rates differ from one in either the 9,12-diene or the mixture of 9- and 12-monoenes. It may also be questionable if the overall reduction rate for double bonds in the diene is greater than in the monoene, but we find the value for $k_{\text{linoleate}}/k_{\text{monoene}}$ of 2.19 in agreement with Schilling (14) who found in three experiments values for $k_{\text{linoleic}}/k_{\text{oleic}}$ of 2.2, 2.4 and 2.5.

Since completion of the rest of this work we have obtained additional evidence that $k_{\text{linoleate}}/k_{\text{monoene}}$ is slightly greater than two. Ten samples of methyl linoleate have been reduced by potassium azodicarboxylate (19), a dimide forming reagent, under a variety of experimental conditions to establish a suitable procedure for partial reduction of polyunsaturated isomers and location of *cis* and *trans* double bonds (13,15). No samples were taken during the reduction; the rate ratios are based only on the final composition and so are less accurate than those in the rest of the paper. However, an average value was found for $k_{\text{linoleate}}/k_{\text{monoene}}$ of 2.16 with a range from 1.7 to 2.8.

In linolenic acid the faster disappearance of triene is mainly caused by more rapid reduction of the 15 double bond. In previous work (18) we reported relative rates for linolenate-diene-monoene-stearate of 3.46, 2 and 1. Schilling (14) reported for $k_{\text{linolenic}}/k_{\text{oleic}}$ 3.3. If all 15 double bonds are assumed to have a rate of 1.3 relative to 1.0 for 12 and 9 double bonds, the apparent overall rate for linolenate-diene-monoene-stearate will be 3.12, 2.04 and 1.00. For the 50:50 linolenate-linoleate mixture we used (18), rates will be 3.25, 2.00 and 1.00.

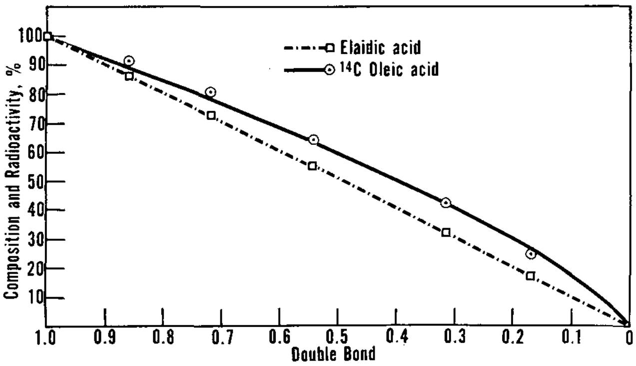

FIG. 2. Reduction of a mixture of elaidic and 1-^{14}C oleic acids by hydrazine showing per cent of each acid remaining plotted against amount of double bond remaining. Points are experimental results. Lines are calculated for $k_{\text{elaidic}}/k_{\text{oleate}} = 1.34$.

Previous results leave some uncertainty as to the effect of *cis* or *trans* configuration on rate of hydrazine reduction. Aylward and Rao (2) stated that under comparable conditions, rates of hydrogenation of elaidic and oleic acids were almost identical and Schilling (14) found *trans* double bonds were hydrogenated practically with equal velocity as the *cis* double bonds. Garbisch et al. (5) using *p*-toluenesulfonyl hydrazine, a diimide-producing reagent which should give results comparable to hydrazine, reported similar rates for *cis* and *trans* 2-pentene relative to cyclohexene. However, Asinger et al. (1) found *trans* undecenes were reduced more rapidly than the corresponding *cis* isomers. Hünig et al. (7) gave several examples of more rapid *trans* double bond reduction by diimide. They suggested that *cis* substituents are more strongly affected than *trans* by the change in bond angle in the transition state and so react more slowly. Mikolajczak and Bagby (10) wrote that *trans* double bonds reduced more rapidly than *cis* in *cis,trans* conjugated dienoic fatty acids. Our results with oleic and elaidic acids, and by two independent analytical techniques, consistently showed faster reduction for elaidic acid. We believe that the results with radioactive oleic acid are more reliable. The average values for $k_{\text{elaidic}}/k_{\text{oleate}}$ of 1.27 obtained by this method agree well with the Asinger et al. (1) undecene values for *trans*-4/*cis*-4 of 1.28 and *trans*-5/*cis*-5 of 1.32.

Our best values may be summarized as indicating a value of 1.30 for *cis*-15- compared to *cis*-9-octadecenoic acid; the same value for *cis*-3- compared to *cis*-9-octadecene, the hydrocarbons from these acids; equal reactivity for *cis*-6-, 9-, and 12-octadecenoic acids and 1.27 for elaidic compared to oleic acid.

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