Studies on the Mechanism of the Hydrazine Reduction Reaction: Applications to Selected Monoethylenic, Diethylenic and Triethylenic Fatty Acids of *cis* Configurations

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The hydrazine reduction of *cis* ethylenic bonds has been studied using a number of C_{16}, C_{18}, and C_{20} monoethylenic, C_{18} diethylenic, and C_{18} plus C_{20} triethylenic fatty acids. Relative reduction rates were determined and correlated with the positions of the ethylenic bonds in the fatty acids. The terminal ethylenic bond was the most reactive and among the others, reactivity was greater nearer the ends of the chain than in the center. Also, the ethylenic bonds closer to the carboxyl group showed greater reactivity than those closer to the methyl end of the chain. Differences in reactivity among the centrally located double bonds were not significant. These reactivity trends are consistent with a mechanism involving protonated diimide as an intermediate in the reduction process. In diethylenic fatty acids, the non-methylene-interrupted dienes showed slightly lower reactivity than the methylene-interrupted counterparts, which indicated that both the position of ethylenic bonds and the relative distance between two ethylenic bonds in the diene influence the rate of reaction. The information obtained from the rate studies should be useful for predicting the product distribution from partial hydrazine reductions of long-chain polyethylenic compounds.

KEY WORDS: Diimide reductions, fatty acids, hydrazine reduction, reduction of unsaturated fatty acids, structure determinations.

The use of hydrazine for the reduction of ethylenic (olefinic) bonds has been known for almost eighty years and is believed to involve the intermediacy of diimide (HN=NH, [1]), a reactive species that first excited considerable attention some twenty years ago (1,2). A range of papers describing calculations on (3-7), or properties of (8-10), diimide is evidence of a continuing interest in unravelling the chemistry of this species. The hydrazine reduction reaction has found favor for reductions of unsaturated fatty acids (11-13) because no apparent *cis-trans* isomerism or ethylenic bond positional shifts occur, in strong contrast to the results from the use of catalytic hydrogenation (14).

However, the hydrazine reduction reaction is not completely understood. In conjunction with physicochemical methods, it has mostly been used as a technique for locating and determining the position and geometry of individual ethylenic bonds in polyunsaturated fatty acids (11,12,15,16). The general approach has consisted of partial reduction of the polyethylenic acid with hydrazine to give a maximum yield of monoethylenic acids. This is followed by isolation of the monoethylenic products and determination of their olefinic bond positions by, for example, oxidative ozonolysis (17). It is generally recognized that hydrazine reduction rates vary with the molecular environment of isomeric ethylenic bonds (14,18-21), but this potentially useful selectivity in rates of reduction has never been clearly defined. We anticipated that the relative reduction rates of different monoethylenic fatty acids would be sufficiently different so as to cause significant distortions in the proportions of reduced products of polyunsaturated fatty acids (12). The method has suffered from poor reproducibility and a systematic study of the hydrazine reduction reaction for fatty acids with double bonds in various positions has never been reported. It has however been successful in determining the structures of both novel polyenoic fatty acids (15,16,22), those resulting from commercial hydrogenation of fats and oils (23,24), and in the analysis of naturally occurring cyclopropenoic fatty acids (25). More widespread application requires a study of the general principles relating ethylenic bond positions, their interactions, and their relative reduction rates. In this paper we describe such a study for a wide range of monoethylenic fatty acids, and for some selected diethylenic and triethylenic fatty acids.

MATERIALS AND METHODS

Fatty acids for kinetic experiments. Fatty acid standards were purchased from Nu-Check-Prep Inc., (Elysian, MN) and Applied Science Laboratories (State College, PA). A sample of *cis*-3-octadecenoic acid (Δ3-18:1; this shorthand notation for fatty acids is defined as Δy, Δz-n:x, where n is the number of carbon atoms in the chain, x is the number of ethylenic bonds, and the numbers following Δ denote the positions of the ethylenic bonds with respect to the carboxyl group, which is numbered as position 1) was a gift from Dr. W.E.M. Lands, University of Michigan, Ann Arbor, MI.

The uncommon fatty acid, *cis*-4-hexadecenoic acid (Δ4-16:1) was isolated from the marine alga *Glenodinium* sp. by a combination of preparative gas-liquid chromatography (GLC) and silver-nitrate-impregnated thin-layer chromatography ($AgNO_3$-TLC). Experimental details are given elsewhere (22).

A sample of 15-hexadecenoic acid was prepared by methylating 16-bromo-9-hexadecenoic acid (250 mg, technical grade, Aldrich Chemical Co., Milwaukee, WI) with refluxing 7% BF_3-MeOH (30 min) (26). The methyl ester was dissolved in ethanol (50 mL, 96%), platinum oxide (ca. 3 mg) was added and the stirred solution was hydrogenated by passing in a gentle stream of hydrogen gas for 3 hr. Analysis of the hydrogenated sample by GLC showed 58% $Br(CH_2)_{15}$ COOMe and 42% $Me(CH_2)_{14}$

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COOMe. The hydrogenated methyl ester mixture (220 mg), dissolved in anhydrous MeOH (50 mL), was added dropwise under nitrogen over a period of 30 min to a freshly prepared, stirred sodium methoxide solution (from sodium, 50 mg, and dry MeOH, 20 mL). The reaction mixture was stirred for 3 hr at 45°C, diluted with water (100 mL) and the product extracted with diethyl ether. The final product was fractionated by preparative $AgNO_3$-TLC (developed in benzene), which showed 2 bands. Analysis of the upper band (R_f 0.72) by GLC showed the presence of methyl hexadecanoate and another component, presumed to be the substitution product $MeO(CH_2)_{15}COOMe$; methyl 15-hexadecenoate (30 mg) was isolated from the lower band (R_f 0.60). Catalytic hydrogenation (PtO_2) of a small portion (ca. 1 mg) of the methyl 15-hexadecenoate yielded methyl hexadecanoate, while oxidative ozonolysis in BF_3-MeOH (17) gave the dimethyl ester of pentadecanedioic acid, thus revealing the ethylenic bond to be in the desired position. The free 15-hexadecenoic acid, [prepared by saponification (KOH/MeOH) and acidification (HCl)] had m.p. 45-46°C, and a nuclear magnetic resonance (NMR) spectrum ($CDCl_3$):δ 1.2-2.1 (m, CH_2 chain), 2.30 (t, $\underline{CH_2}COOMe$), 3.56 (s, $COOC\underline{H_3}$), 4.95 (m, $C\underline{H_2}{=}CH$) and 5.80 (m, $CH_2{=}C\underline{H}$).

Certain fatty acids for rate studies were isolated by hydrazine reduction of various commercially available polyethylenic fatty acids. The total reduced mixture was methylated and then separated into monoethylenic, diethylenic and more highly unsaturated fractions by $AgNo_3$-TLC. These fractions were analyzed by GLC and/or oxidative ozonolysis (22,27). Hydrolysis of the esters gave mixtures of monoethylenic or diethylenic fatty acids of known composition and structure, which were used for rate studies.

Reduction experiments. The hydrazine reduction reactions were carried out on approximately equimolar mixtures of two or more fatty acids. The relative reduction rates were determined under competitive conditions (28). The reductions were carried out in a 250-mL, three-necked, round-bottom flask, equipped with a water condenser, an oxygen inlet tube and a magnetic stirrer.

TABLE 1

The Relative Rates of Hydrazine Reduction for Selected Long-Chain *cis*-Monoethylenic and *cis, cis*-Diethylenic Acids

Fatty acid being reduced	Standard for comparison	Relative rates of reduction
Δ15-16:1	Δ9-16:1	5.79
Δ4-16:1	Δ9-18:1	1.50
Δ3-18:1	Δ9-18:1	1.57
Δ6-18:1	Δ9-18:1	0.91 (0.97)*
Δ12-18:1	Δ9-18:1	1.04 (1.10, 1.03)*
Δ15-18:1	Δ9-18:1	1.28 (1.30)*
Δ5-20:1	Δ11-20:1	0.92
Δ8-20:1	Δ11-20:1	0.73
Δ14-20:1	Δ11-20:1	1.02
Δ17-20:1	Δ11-20:1	1.31
Δ6,Δ9-18:2	Δ9,Δ12-18:2	1.00
Δ12,Δ15-18:2	Δ9,Δ12-18:2	1.24
Δ6,Δ12-18:2	Δ9,Δ12-18:2	0.84
Δ9,Δ15-18:2	Δ9,Δ12-18:2	0.99

*Results in parentheses are from Scholfield *et al.* (18).

The unsaturated fatty acid mixture to be reduced was dissolved in 96% ethyl alcohol (70 mL) and to this was added heptadecanoic acid (20 mg), which served as the internal standard in calculating the GLC peak areas. A slow stream of oxygen (10 mL min^{-1}) was passed over the fatty acid solution throughout the reaction period and the solution was stirred magnetically at a steady rate. The reaction flask was maintained at 40±1°C using a constant-temperature water bath. At zero time, a 0.5-mL sample was removed and 95% hydrazine (1mL;Eastman Kodak, Rochester, NY) was added. Samples (0.5 mL) were withdrawn as the reaction proceeded and quenched immediately by addition to an HCl solution (3M,1mL). Fatty acids were extracted with diethyl ether, converted to methyl esters by heating with 7% BF_3-MeOH (Applied Science) and analyzed by GLC. The relative rates of

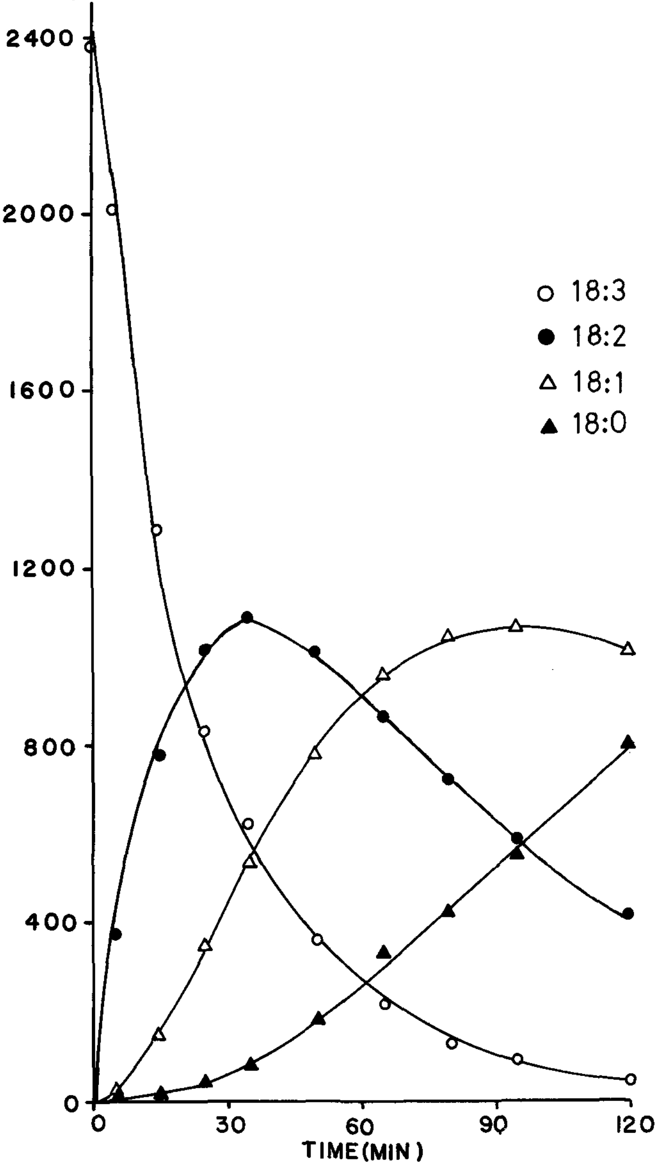


FIG. 1. The partial reduction of *cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid with hydrazine to give the total production of diethylenic, monoethylenic and octadecanoic acids with time. The quantities of acids were monitored by capillary GLC analyses of their methyl esters on a SILAR-5CP column. Quantities were standardized by the use of heptadecanoic acid as an internal standard; the peak area of methyl heptadecanoate was arbitrarily taken as 1,000 units.

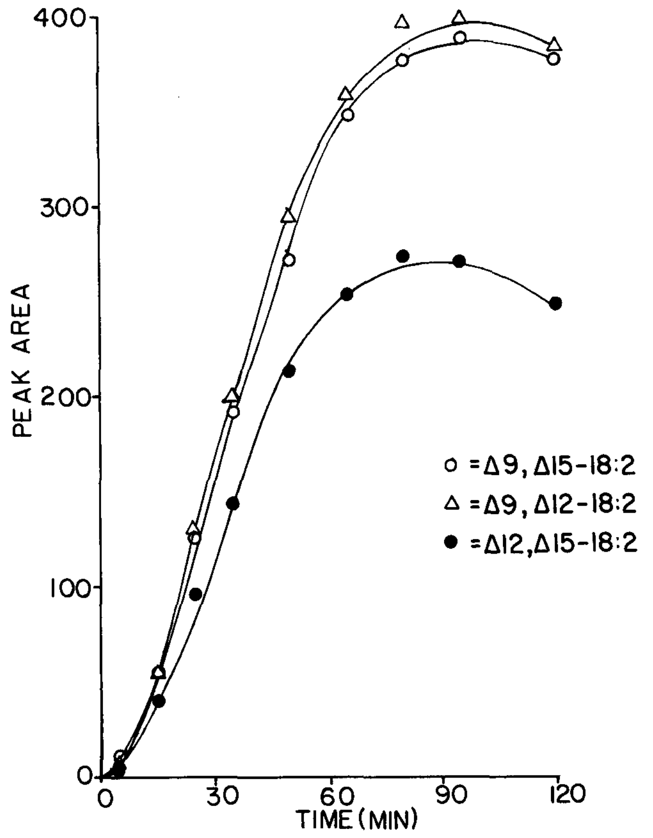


FIG. 2. The distribution of octadecadienoic acid isomers during the reduction of *cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid with hydrazine. For units, see Figure 1.

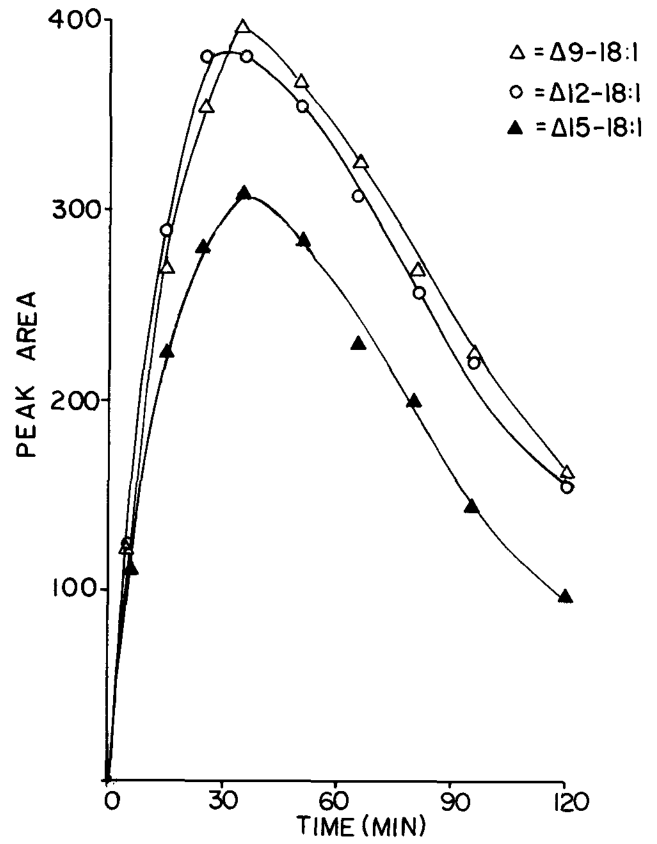


FIG. 3. The distribution of octadecenoic acid isomers during the reduction of *cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid with hydrazine. For units, see Figure 1.

reduction for monoethylenic and diethylenic acids are given in Table 1; detailed experimental results are given elsewhere (22). Typical results are presented in Figures 1-3.

Chromatography and spectroscopy. Preparative TLC (thin-layer chromatography) was carried out on "Prekote" Adsorbosil-5 silica gel plates (20 cm × 20 cm, Applied Science). The plates were cleaned prior to use by developing in ethyl acetate and activated by heating at 110°C in an oven for 30 min. Silver nitrate TLC plates were prepared by dipping the "Prekote" Adsorbosil-5 plates horizontally in a 10% solution of $AgNO_3$ in MeCN for 30 min and drying at 110°C for 60 min. The $AgNO_3$-TLC plates were streaked and developed in benzene, and the methyl ester bands were located by viewing under ultraviolet (UV) light, after spraying the plates with 2′,7′-dichlorofluorescein. Material from preparative TLC was removed by extracting three times with chloroform:*n*-hexane (1:1, v/v). Analytical GLC was performed on stainless-steel, wall-coated, open-tubular columns, 47 m × 0.25 mm i.d., coated with SILAR-5CP (Perkin-Elmer), operated in a Perkin-Elmer 3920 series gas chromatograph with flame ionization detector (FID). The column was operated isothermally at 175°C, with helium carrier gas pressure at 60 psig. Injection ports and split assemblies were held at 270°C and manifold assemblies at 250°C. A 1-mV Linear Instruments Corp. model 252A recorder (Costa Mesa, CA), including a stepping-pen integrator for area determination, was employed.

Proton NMR spectra were recorded using a Varian CFT 20 spectrometer at 80 MHz, in $CDCl_3$ solutions with tetramethylsilane as the internal standard.

RESULTS

Reactivity trends of selected monoethylenic fatty acids. The relative reaction rates of some positional isomers of *cis*-hexadecenoic, *cis*-octadecenoic and *cis*-eicosenoic acids are summarized in Table 1. In the C_{18} chain length, the reduction rates were evaluated with respect to *cis*-Δ9-18:1(oleic acid), the most common C_{18} monoethylenic fatty acid isomer in the plant and animal kingdoms. In the C_{20} chain length, relative rates were evaluated with respect to Δ11-20:1, which is the most common ethylenic bond position in the naturally occurring unsaturated C_{20} fatty acids.

If the influence of total chain length on reduction rates is not considered (i.e. whether C_{16}, C_{18} or C_{20}), it can be seen that the terminal ethylenic bond is the most easily reduced. The value obtained in this study for the ratio of rates Δ15-16:1/Δ9-16:1 = 5.8 is reasonably comparable to that of Schilling (19) who obtained a value of 8.3 for the ratio of rates Δ10-11:1/Δ9-18:1. This is the only literature value available for the rate of reduction of a terminal ethylenic bond in fatty acids. From Table 1, it is clear that the relative reduction rates reported here for Δ6-18:1, Δ12-18:1 and Δ15-18:1 are in agreement with those reported by Scholfield *et al.* (18). In the octadecenoic acid series, the Δ3 isomer showed a significantly higher reduction rate than the other isomers. As the ethylenic bond moved from Δ3 to Δ6 the relative reduction rate dropped from 1.57 to 0.91 and then increased with the proximity of the ethylenic bond to the methyl end of the fatty acid chain. A similar trend could be observed within the eicosenoic acid series in which the reduction rate dropped from Δ5 to Δ8 and then increased slowly as the unsaturation center moved toward the methyl group. Clearly the relative reduction rates presented here suggest that the ethylenic bonds closer to the two ends of the chain are reduced at a more rapid rate than are the centrally located ethylenic bonds. The differences in reactivity between these centrally located ethylenic bonds are not significant. Further, for the positional isomers evaluated, the ethylenic bonds closer to the carboxyl group (e.g. Δ3-18:1 and Δ4-16:1) showed significantly higher reactivities than those closer to the methyl group. If only steric factors are considered, a Δ3 isomer should show a similar reactivity to an ethylenic bond closer to a terminal methyl group. However, the above results indicate that a proximate carboxylate anion (see below also) does in fact influence the rate of hydrazine reduction.

A way of examining the effect of the carboxylate anion is to follow the relative hydrazine reduction rates of fatty acid methyl esters. For this purpose the reduction rate of the Δ-18:1 fatty acid methyl ester was evaluated relative to the Δ9-18:1 fatty acid methyl ester. Usually hydrazine reductions (in the hydrazine-oxygen system) are performed only on the fatty acids, for two reasons. First, a weak acid, such as a carboxylic acid, is necessary to generate active reducing species, and second, methyl esters could form the corresponding acid monohydrazide at higher temperatures (29-32). To check if this was possible under the conditions employed in this work, an ethanolic solution of methyl *cis*-9-octadecenoate and heptadecanoic acid (a combined proton source and internal standard) was stirred at 40°C for 180 min in the presence of oxygen and a large excess of hydrazine. The products were examined in detail by TLC and GLC (22), and the relative amounts of C_{18} ester and heptadecanoic acid were found to be unchanged. Thus, under these normal experimental conditions, there is no observable formation of acid hydrazides from methyl esters.

Therefore, a 50:50 mixture of methyl *cis*-3- and *cis*-9-octadecenoates with a large excess of heptadecanoic acid was reduced under the same conditions as above for 240 min. A reduction rate of 0.83 for Δ3 relative to Δ9 was obtained. The large difference in the relative reduction rates between the corresponding pair of methyl esters (0.83) and fatty acids (1.57) suggests participation by a proximate carboxylic acid group and/or carboxylate anion in the reduction step of ethylenic bonds.

Reactivity trends of diethylenic fatty acids. The relative hydrazine reduction rates of some isomeric octadecadienoic fatty acids are given in Table 1. The differences in relative reduction rates between isomers are small; nevertheless, these differences have to be taken into account when discussing the proportions of the partial hydrazine reduction products of triethylenic fatty acids (discussed in the following section). The results suggest that isomers of the 1,4-diethylenic type, commonly found in nature, are reduced in the same way as would be expected from a knowledge of rates of reduction of the individual ethylenic bonds. For example, the reactivity of Δ12,Δ15-18:2 compared to Δ9,Δ12-18:2 reflected the greater reactivities of the Δ12-18:1 and Δ15-18:1 monoethylenic fatty acids. The relative reduction rates of the structurally similar Δ6,Δ9-18:2 and Δ9,Δ12-18:2 diethylenic acids are also reasonably in agreement with the reactivities of the corresponding monoethylenic fatty acids.

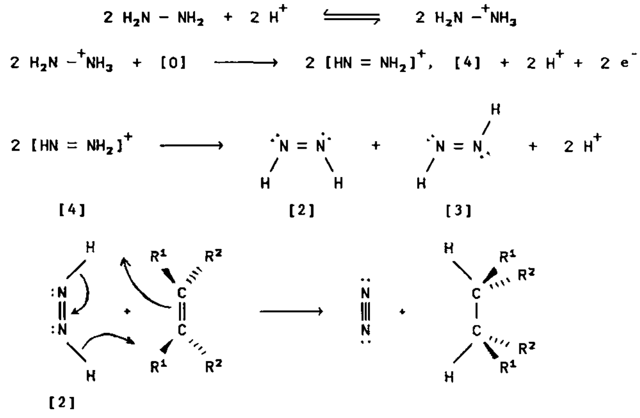
However, the two 1,7-diethylenic fatty acids, Δ6,Δ12-18:2 and Δ9,Δ15-18:2, differed from the general behavior of 1,4-dienes. Because of the presence of a Δ15 ethylenic bond (the most reactive monoethylenic isomer compared to the Δ6, Δ9, and Δ12 isomers), the Δ9,Δ15-18:2 isomer showed a reactivity comparable to that of Δ9,Δ12-18:2. From the relative rates of reduction of Δ6-18:1, Δ9-18:1, Δ12-18:1 and Δ15-18:1, the Δ6,Δ12-18:2 and Δ9,Δ15-18:2 dienes would not be expected to have the same reactivities. As expected, Δ6,Δ12-18:2 reduced more slowly than Δ9,Δ15-18:2, but the latter reduced more slowly than would be expected from the reduction rates of the corresponding monoethylenic fatty acids.

Reactivity trends of triethylenic fatty acids. Having established the reactivity trends of monoethylenic and diethylenic fatty acids, it was decided to examine the hydrazine reduction of selected triethylenic fatty acids in order to compare the experimental results with the established reactivities of the mono- and diethylenic fatty acids. Three, all-*cis*, triethylenic acids, *viz* Δ9,Δ12,Δ15-18:3, Δ6,Δ9,Δ12-18:3 and Δ11,Δ14,Δ17-20:3, were studied.

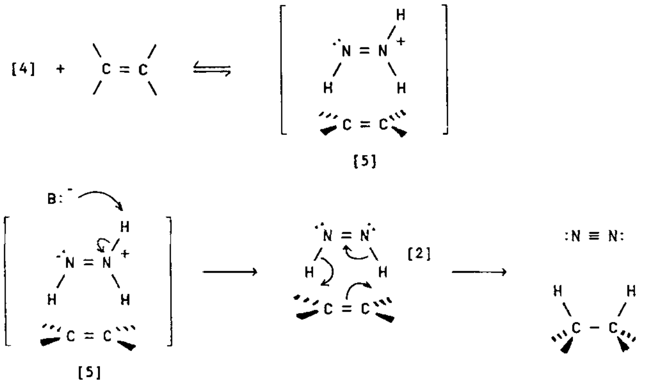
The reduction of Δ9,Δ12,Δ15-18:3 was evaluated in detail as a function of time. Figure 1 shows the course of reduction and the production of total diethylenic acids, monoethylenic acids and saturated octadecanoic acid, while Figures 2 and 3 give detailed isomer distributions of the respective diethylenic and monoethylenic acids. An actual gas-liquid chromatographic analysis of methyl Δ9,Δ12,Δ15-18:3 is reproduced elsewhere (33).

DISCUSSION

Mechanistic considerations. Specific conditions for the reduction of alkenes and alkynes by hydrazine were detailed by Aylward and Sawistowska (34) in 1962. These are: an excess of hydrazine, the presence of oxygen or a mild oxidizing agent, a polar solvent kept within a pH range of 8.5-9, and the presence of a weak proton source. Fatty acids are adequate proton sources themselves as it was shown (35) that no reduction of ethylenic bonds in a long-chain unsaturated alcohol would occur under the same conditions, unless some acid was added. Since the reductions invariably led to *cis* hydrogenation, it was assumed that the reactive intermediate (Scheme 1) was the *cis* diastereomer of diimide [2], and that this was either formed in equal amounts with the more stable *trans* diastereomer [3] (3,4), or was in rapid equilibrium with [3]. Further experimental results (1) indicated that dideuterodiimide reduced *cis* and *trans* alkenes to give *meso* and *dl* products respectively; the reaction is highly exothermic, but is subject to steric hindrance and *cis* alkenes are reduced faster than their *trans* diastereomers. It should be noted that the reduction is far more effective toward symmetrical multiple bonds (e.g. $C=C$, $C\equiv C$, $N=N$, etc.) than asymmetrical bonds (e.g. $C=O$, $C\equiv N$, etc.) (1). All of the facts have been embodied in a mechanism which has been proposed for the reaction, and is portrayed in Scheme 1 (1).



SCHEME 1



SCHEME 2

Such a mechanism scheme can be criticized on the grounds that in fact one would anticipate the *trans* diimide [3] to be the predominant species formed (3,4). It has been suggested (3,4) that it is unlikely that [3] would isomerize directly to the *cis* isomer [2] with sufficient facility to account for the ease with which hydrazine reductions of alkenes occur. The precise mechanistic pathway of this reaction is thus at present not totally clear.

The above relative rates for the hydrogenation of monoethylenic acids with hydrazine are of interest not only in their own intrinsic way, but also because they permit some comments to be made on the mechanism of the reaction. It is known that steric effects are an important factor in this reaction (1,10); however, electronic effects are said to have little effect (1), although this is not so in the case of allenes (10). It should be noted that this paper (10) is concerned with "diimide" generated by decomposition of *p*-toluenesulfonylhydrazide. The active species in this reduction may or may not be the same as when hydrazine and oxygen are used for the reduction reaction.

It is not easy to visualize how the differences in the reactions described above can be explained if the mechanism for hydrogen transfer from *cis*-diimide [2], as outlined in Scheme 1, is operating in the present set of reactions. Our results can be interpreted in two possible ways: either, the reaction sequence involves an electrophilic species that is influenced by electronic effects within the substrates, or the active reductant is generated from two different precursors in two different ways, with two different reaction rates. These two possibilities are explored in the following paragraphs.

In the first instance, the ease of hydrogenation at any position in a fatty acid chain is undoubtedly modified by steric factors. These factors are important in this reaction (1,2,10). A double bond at the center of a chain would be more sterically hindered than one toward either end of a chain, especially if there is a predominance of gauche conformations in the chain as might be expected in a polar solvent such as ethanol, but the ends of a chain should experience roughly comparable steric hindrance. However, the greater reduction rates of Δ3 and Δ4 acids versus those acids with unsaturation in the center of the chain, and the diminution in rate for a Δ3 methyl ester versus a Δ3 carboxylic acid suggest an interaction of the carboxylate anions with a reactive species that is electrophilic in nature.

Aylward and Sawistowska (34) considered the possibility that, among others, one of the intermediates in the hydrazine reduction reaction is the protonated diimide [4], formed by the oxidation either of hydrazine or of its conjugated acid, $H_2NNH_3^+$. Our results could be interpreted to support the possibility that [4] is actually directly involved in the reduction step. In general, the oxidaton step to produce [4] could occur anywhere in the solution, and most molecules of [4] would simply disproportionate or decompose without effecting any reduction—a large excess of hydrazine is used and there is no evidence that the reduction reaction is efficient in terms of hydrazine consumption. However, if a molecule of [4] is produced in proximity to a double bond, the two would interact in the normal way that π clouds interact with electrophilic species to form a loose complex [5], which could be deprotonated to form *cis*-diimide [2], in very close proximity to the alkene π cloud. Concerted, stereoselective transfer of two hydrogen atoms to the alkene would then be very rapid and exothermic, and would undoubtedly occur before the molecule of [2] could diffuse away into the reaction medium. The whole process is outlined in Scheme 2. The equilibrium constant for the formation of [5] will depend on the steric environment of the double bond, and the proximity of a carboxylate anion would improve the entropy of the reaction, leading to higher reaction rates.

Alternatively, since oxidation of hydrazine by molecular oxygen must proceed via the radical cation, $[H_2NNH_2]^{+\cdot}$ [6], which would also be electrophilic, the same arguments as were made for [4] could be applied. It is conceivable, but less likely, that [6] could also deliver two hydrogen atoms stereoselectively in a concerted and competitive process to an alkene.

The differences in reactivities of the 1,4- and 1,7-dienes appear to derive from electronic effects that can be rationalized by invoking an electrophilic species as the key intermediate. In this approach, the differences in rates between 1,4-dienes and 1,7-dienes can be explained on the basis of the differences in stability between 5- or 6-membered rings on the one hand, and 8- or 9-membered rings on the other. The relative stabilities of these rings are well-documented (36). Also, the interactions of olefinic bonds and positive centers at carbon have been studied and these are quite dependent on the number of atoms between the olefin and the cationic center (37). Therefore, the fact that 1,4-diethylenic acids are reduced more rapidly than 1,7-diethylenic acids is consistent with the mechanistic observations made previously.

Practical applications. On the basis of the results from the hydrazine reduction of mono- and diethylenic fatty acids, it is reasonable to assume that ethylenic bonds close to either end of the chain should be reduced more rapidly and that 1,7-dienes should be reduced more slowly. One of the factors that influenced the choice of oleic acid as a standard against which to measure the relative rates of reduction of other monoethylenic fatty acids is the fact that its double bond is in the center of the chain, which would give rise to the most constant steric or electronic interactions. From Figure 2 it is clear that during the entire reaction period, the amount of Δ12, Δ15-18:2 was less than either of the other two isomers. As the reaction progressed (>30 min), the rate of disappearance of dienes became rapid compared to the rate of formation of dienes (Fig. 1). Thus, the three diethylenic acid curves (Fig. 2) changed after 30 min from reflecting triene reduction to reflecting the rates of reduction of the three diethylenic isomers. Therefore, the continued lower proportion of Δ12,Δ15-18:2 could be rationalized as being due to its higher reactivity compared to the other two diethylenic isomers, as well as to its lower rate of formation. The relative proportions of Δ9,Δ12-18:2 and Δ9,Δ15-18:2 were very similar; up to about 30 min, the Δ9,Δ12 isomer was present to a slightly greater extent whereas after that time the Δ9,Δ15 isomer was the major diene by a slight amount. Based on the monoene reduction results, the Δ15 bond should be reduced the fastest and it was earlier shown that the Δ9,Δ12 and Δ9,Δ15 isomers reduce at comparable rates. Therefore, the diethylenic acid isomer distribution pattern (Fig. 2) suggests that in Δ9,Δ12,Δ15-18:3 the Δ12 bond reduces slightly faster than would be expected from the reduction rate of the Δ12-18:1 acid. This more rapid reduction of the Δ12 bond than expected could be rationalized as suggested earlier. Stabilization of an electrophilic intermediate at site of the Δ12 bond in the Δ9,Δ12,Δ15-18:3 acid could be brought about either by the Δ9 or the Δ15 double bond, whereas the comparable species formed in the reduction of either the Δ9 or the Δ15 bonds could be stabilized only from one direction.

A plot of the monoethylenic acid isomer distribution versus time from the hydrazine reduction of Δ9,Δ12, Δ15-18:3 is given in Figure 3. The proportions of the monoethylenic isomers throughout the reaction were in agreement with the reactivity of the monoethylenic and diethylenic fatty acids. The lower proportion of the Δ15-18:1 acid was obviously due to the higher reactivity of the Δ15 ethylenic bond at all times.

TABLE 2

Fatty Acid Composition of Partial Hydrazine Reduction Products of *cis*-6, *cis*-9, *cis*-12-Octadecatrienoic Acid

Fatty acid	Mole%*
18:0	1.4
Δ6-18:1†	1.8
Δ9-18:1†	2.5
Δ12-18:1†	2.1
Δ6,Δ9-18:2	7.5
Δ6,Δ12-18:2	11.9
Δ9,Δ12-18:2	10.1
Δ6,Δ9,Δ12-18:3	62.7

*Calculated from GLC (SILAR-5CP) peak area of the methyl esters; 55 mg of Δ6,Δ9,Δ12-18:3 was reduced for 60 min.

†The distribution of monoethylenic fatty acids was determined by oxidative ozonolysis of the isolated monoenes.

TABLE 3

Fatty Acid Composition of Partial Hydrazine Reduction Products of *cis*-11, *cis*-14, *cis*-17-Eicosatrienoic Acid

Fatty acid	Mole%*
20:0	20.2
Δ11-20:1	13.3
Δ14-20:1	13.9
Δ17-20:1	9.0
Δ11,Δ14-20:2	14.3
Δ11,Δ17-20:2	14.8
Δ14,Δ17-20:2	9.2
Δ11,Δ14,Δ17-20:3	5.4

*Calculated from GLC (SILAR-5CP) peak area of the methyl esters; 20 mg of Δ11,Δ14,Δ17-20:3 was reduced for 120 min.

The composition of the partial reduction products from two other triethylenic fatty acids, *viz* Δ6,Δ9,Δ12-18:3 (reduced for 60 min; Table 2) and Δ11,Δ14,Δ17-20:3 (reduced for 120 min; Table 3) gave further evidence inline with the postulates discussed in the previous sections. From the relative rates of reduction of the monoethylenic acids, Δ6-18:1, Δ9-18:1 and Δ12-18:1, it is reasonable to anticipate almost equal proportions of the three diethylenic isomers and, as well as, of the three monoethylenic isomers from the partial reduction of Δ6,Δ9,Δ12-18:3. However, the 1,7-diene isomer, Δ6,Δ12-18:2, predominated among the dienes. This is obviously due to the lower reactivity of Δ6,Δ12-18:2 compared to the other two intermediate diethylenic isomers. The distribution of hydrazine reduction products from Δ11,Δ14,Δ17-20:3 (Table 3) was similar to that of the corresponding C_{18} homolog, Δ9,Δ12,Δ15-18:3. The proportion of the 1,7-diene, Δ11,Δ17-20:2, was almost equal to that of the 1,4-diene, Δ11,Δ14-20:2. In the C_{20} chain length, the order of reactivity of the monoethylenic fatty acids was Δ17-20:1>Δ14-20:1>Δ11-20:1. From the relative rates of individual ethylenic bonds one would expect Δ11-20:1 to be the major monoethylenic isomer. However, Δ14-20:1 was slightly in excess of Δ11-20:1, and accumulations of both were much more important than of the more reactive Δ17-20:1. The higher proportion of Δ14-20:1 is most probably due to the lower reactivity of Δ11,Δ17-20:2, showing again the effect of lower rates of reduction for 1,7-dienes as compared to 1,4-dienes on the product distribution at the diethylenic acid stage of reduction.

These results show that there is a good correlation between the reactivities of both monoethylenic and diethylenic fatty acids with the reduction products of triethylenic fatty acids. The observations do not permit a conclusive answer to be offered regarding the detailed mechanism of this reaction, but they do offer a useful rationalization of the product distributions obtained from the reductions of polyunsaturated fatty acids. We suggest that the results open the way for a greater use of this reaction in controlled, partial reductions of polyene systems and that they point toward further experiments to throw light on a fascinating mechanistic problem.

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