

Specific ^3H -Labelling by diimide reduction of unsaturated bonds

I. Mechanism of reductions with hydrazine in aprotic solvents

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ABSTRACT

The applicability of aprotic solvents for hydrazine reductions was studied for labelling purposes and acetonitrile was found to be a favourable medium for this reaction. The reaction can be carried out in the absence of protons, but small amounts of acetic acid have a catalytic effect. Kinetic investigations have shown that oxygen supply in the medium is the rate-determining step under the conditions applied. The mechanism of the reaction is discussed. No explanation can be given of the autocatalytic and acid-catalysed acceleration of the reaction.

INTRODUCTION.

Hydrogenations of unsaturated bonds with diimide (N_2H_2) have been studied extensively and a number of interesting applications has been described⁽¹⁻³⁾. One of the simplest sources of diimide is hydrazine, from which it is supposed to be formed by oxidation with oxygen *in situ*.

In general the reaction with hydrazine is carried out in aqueous alcohols. For specific labelling of fatty acids with tritium a procedure was studied, in which hydrazine was labelled by exchange with tritiated water *in situ*. For this purpose a medium had to be found, which does not contain exchangeable hydrogen atoms. Therefore, we were interested in the applicability of aprotic solvents for hydrogenation reactions with hydrazine. The study of the optimal conditions for the reduction of methyl oleate as a model compound revealed a number of characteristic features of this reaction, which will be discussed in this article.

OPTIMAL CONDITIONS FOR THE REDUCTION OF METHYL OLEATE IN APROTIC SOLVENTS.

Experimental conditions.

For the study of reductions with hydrazine in the absence of water anhydrous aprotic solvents were prepared by passing them—after distillation—over a column of dry molecular sieve type 3A in powder form. Anhydrous hydrazine (purity 99-100 %) was prepared according to the procedure described by Audrieth and Ogg⁽⁴⁾. The hydrazine concentrations in samples were determined⁽⁵⁾ by titration with 0.1 N KIO_3 .

In reduction experiments, 10 ml samples of freshly distilled pure methyl oleate were introduced into solutions of suitable amounts of hydrazine in 60 ml solvent in a three-necked roundbottomed flask, provided with a reflux condenser and a capillary gas inlet tube, through which a continuous flow of 60 ml/min O_2 was introduced into the solution. The reaction mixture was stirred magnetically; the temperature of the surrounding heating bath was controlled with a regulating thermometer. The reaction was followed by taking 1 ml samples, which were added to 10 ml 8% HCl, followed by extraction with 5 ml light petroleum. The organic layer was washed with 10 % sodium carbonate and 3-4 times with distilled water and dried on anhydrous sodium sulfate.

The composition of the reaction mixture was determined by gas-liquid chromatography on a column of 5% polyethylene glycol adipate on Diatoport S at 200° C. The recovery of the methyl ester in the final product was checked by adding methyl palmitate as an internal standard and appeared to be quantitative.

The influence of the solvent.

Apart from alcohols and alkyl monoglycol ethers^(1, 6), experience with other solvents is scarce. Results obtained without solvents, in non-polar solvents, as e.g. hexane, and a mixture of hexane and ethanol⁽¹⁾ were not attractive. Using dioxan or glycoldimethyl ether we found a slow and incomplete reaction, probably due to the limited miscibility of hydrazine with these solvents.

Acetonitrile, dimethyl formamide and dimethyl sulfoxide on the other hand gave homogeneous solutions and were investigated further. In all cases small amounts of acetic acid were added.

Figure 1 shows results obtained in ethanol and acetonitrile. Using anhydrous hydrazine instead of aqueous hydrazine hydrate in ethanol as solvent did not improve the yield of reduced oleate. The reaction rate and the yield of reduced product were higher in acetonitrile than in ethanol. Results from reductions in acetonitrile, dimethyl sulfoxide and dimethyl formamide, under

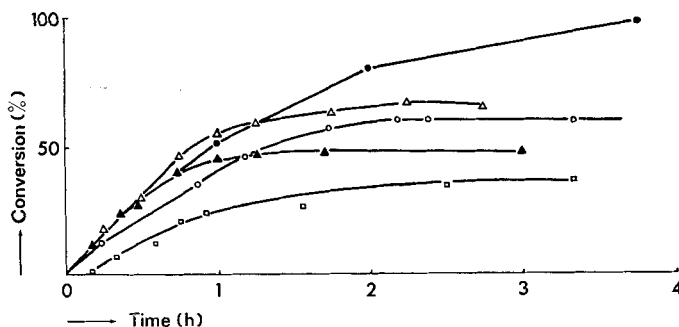


FIG. 1. Reduction of methyl oleate (10 ml) with solutions (60 ml) of hydrazine in various solvents.

- Ethanol with 400 % excess of 80 % aq. N_2H_4 at 50-60° C; 4 ml CH_3COOH
- Acetonitrile with 100 % excess of anh. N_2H_4 at 80° C; 40 μl CH_3COOH
- △ Acetonitrile with 7 % excess of anh. N_2H_4 at 80° C; 40 μl CH_3COOH
- ▲ Dimethyl formamide with 7 % excess of anh. N_2H_4 at 80° C, 40 μl CH_3COOH
- Dimethyl sulfoxide with 7 % excess of anh. N_2H_4 at 100° C, 40 μl CH_3COOH .

equal conditions, are also shown in Figure 1. Acid-free acetonitrile was chosen as the most attractive solvent.

The influence of other reaction variables.

The influence of variables, which are known to affect the reaction in other solvents ⁽¹⁾, was also investigated for the reaction in acetonitrile. The presence of O_2 appeared to be essential: no reaction took place in an atmosphere of N_2 but as soon as N_2 was replaced by O_2 , the reaction started immediately. Further it was found that a decrease in pH, caused by consumption of hydrazine during the reaction, hardly affects the reaction rate. Other experiments showed that small deviations from the flow of 60 ml O_2 /min had no influence; decreasing the flow to 30 ml/min, however, lowered the rate of the reaction considerably.

Addition of Cu^{++} -ions, which have been reported to promote the oxidation of hydrazine ^(7, 8), or of *t*-butyl hydroperoxide, did not influence the reduction reaction in the presence of O_2 .

A proton donor is not essential for the reduction (Fig. 2). Small amounts of acid, however, catalyse the reaction, optimal results being obtained with an amount of 40 μl per 10 ml oleate and per ml anhydrous hydrazine.

Varying the temperature of the reaction from 60-80° C did not influence the reaction perceptibly. Much lower conversions were found at 40° C, probably due to the limited miscibility of the reagents.

It can be concluded that the best results in the reduction of 10 g methyl oleate are obtained with 1 ml anh. N_2H_4 (7 % excess) in acetonitrile at 60-

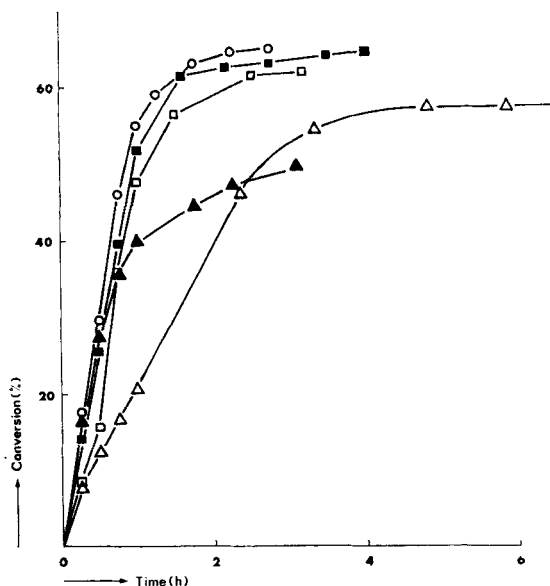


FIG. 2. Influence of catalytic amounts of anhydrous acetic acid on reduction of 10 ml methyl oleate at 80° C with 1 ml anh. N_2H_4 (7 % excess) in 60 ml acetonitrile. Amounts of acetic acid : Δ no; \square 10 μ l; \circ 40 μ l; \blacksquare 100 μ l; \blacktriangle 1 ml.

80° C in the presence of 40 μ l anh. CH_3COOH and a continuous stream of O_2 -gas of 60 ml/min.

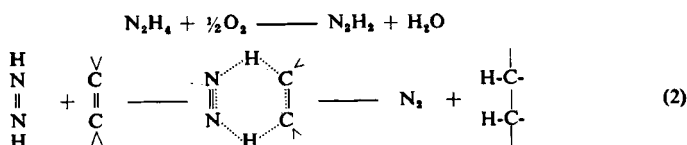
THE MECHANISM OF REDUCTIONS WITH HYDRAZINE.

Introduction.

The observation that oxygen is essential for hydrogenation of unsaturated bonds by hydrazine suggests the formation of a reducing intermediate by oxidation of hydrazine. The formation of diimide from different sources, detected at low temperatures by IR-spectroscopy, the occurrence of diimide in the mass spectrum of hydrazine and the diversity of reagents for hydrogenation of double bonds, which are all possible precursors of diimide, are arguments in favour of the occurrence of diimide as the actual reducing intermediate in this type of reactions ^(2, 3).

The exclusive occurrence of *cis*-addition in diimide reductions ⁽⁷⁾ and the influence of steric factors and inductive effects on the course of these reactions ⁽⁹⁾ are in accord with a synchronous double hydrogen transfer through a cyclic transition state in the final reduction step ^(7, 10, 11). Therefore the follow-

ing reaction scheme is generally accepted for hydrazine reductions of double bonds :



The identity of the rate-limiting step in this sequence of reactions is still in dispute^(6, 3). The identity of the reaction intermediates and the fate of *trans*-diimide, which may be formed in reaction (1) and which will not be able to take part in reaction (2), is not known either.

Kinetics of the reduction in acetonitrile.

Our experiments suggest that the initial rate of oleate conversion is zero order with respect to the substrate. More evidence for this conclusion was obtained from the results of the consecutive reactions in the hydrogenation of the methyl esters of stearolic acid (9-octadecynoic acid) and conjugated *cis, trans*-linoleic acid (9*cis*, 11*trans*-octadecadienoic acid).

From Table 1 it is seen that the conversions of methyl stearolate and the intermediate methyl oleate $\left(\frac{100 S}{O + S}\right)$ are equal. For *cis, trans*-linoleic acid, which contains two double bonds per molecule, the conversion of linoleate is twice as high as that of the intermediately formed oleic and elaidic acids $\left(\frac{100 S}{O + E + S}\right)$. The rates of the reduction reactions appear to be independent of the concentration of the substrate. Moreover, the difference in the reduction rates of different types of unsaturated bonds can only be of secondary importance (see also ref.¹²). This means that the overall reaction rate is not determined by the reaction of diimide with unsaturated substrate. This is in agreement with the calculation of Corey, Mock and Pasto⁽⁸⁾, that the hydrogen transfer reaction from diimide is fast and highly exothermic. The rate of reduction reaction (2) will therefore be determined by the formation of diimide in the medium.

The influence of pH and of the presence of acid on the rate of reductions with hydrazine in alcoholic media was ascribed by Aylward and Sawistowska^(1, 6) to the formation of hydrazonium ions (N_2H_5^+) as intermediate in the production of diimide, which will be favoured by solvation. Since cation solvation decreases⁽¹³⁾ in the order $(\text{CH}_3)_2\text{SO} > \text{HCON}(\text{CH}_3)_2 > \text{H}_2\text{O} > \text{CH}_3\text{OH} > > \text{CH}_3\text{CN}$, decreasing concentrations of hydrazonium ions are to be expected in these media. The fact, that pH and the concentration of acid are at least of secondary importance in the reaction in acetonitrile,

TABLE 1. Rate of consecutive reactions in reductions of methyl 9-octadecynoate and methyl 9*cis*, 11*trans*-octadecadienoate. O = oleate, E = elaidate, S = stearate.

Methyl 9-octadecynoate					
Time (min)	Conversion C (%)	Reaction products*			$\frac{100 S}{C(O + S)}$
		O	S	$\frac{100 S}{O + S}$	
5	2.3	2.25	0.05	2.2	0.96
10	4.4	4.2	0.18	4.0 ^s	0.92
15	6.0	5.5	0.36	6.1	1.02
25	9.5	8.3	1.2	12.6	1.32
40	16.0	13.0	2.95	18.4	1.15
55	24.0	17.5	6.4	26.9	1.12
125	48.0	24.0	24.0	50.0	1.04
175	49.0	22.6	26.4	54.0	1.10
225	49.5	22.7	26.8	54.2	1.10
285	49.5	22.6	26.9	54.3	1.10
320	49.7	22.2	27.5	55.5	1.12

Methyl 9 <i>cis</i> , 11 <i>trans</i> -octadecadienoate					
Time (min)	Conversion C (%)	Reaction products*			$\frac{100 S}{C(O + E + S)}$
		O + E	S	$\frac{100 S}{O + E + S}$	
15	7.3	7.3	—	—	—
30	15.1	13.8	1.3	8.6	0.57
45	26.0	22.6	3.4	13.0	0.50
75	32.2	26.8	5.4	16.8	0.52
105	37.7	30.5	7.2	19.1	0.51
165	41.0	31.5 ^s	9.4 ^s	23.0	0.56

* (in % of starting ester)

may indicate that hydrazonium ions are not involved in the formation of diimide under our conditions. At the same time, the high rates and the good yields of reductions in acetonitrile as compared with alcohol suggest that diimide formation from hydrazine is unfavourably influenced by intermediate formation of hydrazonium ions (cf. Fig. 1). The lower yield in acetonitrile in the presence of 1 ml anhydrous acetic acid per ml hydrazine (Fig. 2) may also be due to the formation of less diimide by an increasing concentration of hydrazonium ions.

By further experiments with hydrazine, alone or in the presence of unsaturated substrate, it was shown that the initial reaction rate is also zero-order with respect to hydrazine concentration under our conditions. This means that only oxygen supply can be the rate-determining factor under our conditions. However, even at the highest reaction rate in our experiments only 6 ml O_2/min is consumed, corresponding to an oxidation rate of 30 mmole $\text{N}_2\text{H}_4/\text{h}$, whereas a flow-rate of 60 ml O_2/min is maintained throughout the reaction. These results suggest that under our conditions in acetonitrile, the supply of oxygen is determined by the rate of oxygen transport from the gaseous phase to the site of the reaction. This is confirmed by the independence of the tritium incorporation efficiency from the concentration of all reactants, which was found in labelling experiments ⁽¹⁴⁾.

Identity of reactive intermediates.

On page 102 it was stated that a cyclic transition state is assumed to be involved in the hydrogenation step of the unsaturated substrate. Only diimide in the *cis*-configuration will be able to take part in reaction (2). Aylward and Sawistowska ^(1, 6) observed that optimally only half of the reacting hydrazine was effective in the reduction of double bonds and therefore assumed an equimolar formation of *cis*- and *trans*-diimide in the oxidation of hydrazine. This suggestion seems to be confirmed by the observation that conversions of only 60-65 % can be obtained by reduction of methyl oleate with a 7 % excess of anhydrous hydrazine in acetonitrile (Fig. 2). However, other investigators suppose that the energy barriers for *cis-trans* isomerization of diimide will be low and that a rapid equilibrium will be attained between *cis*- and *trans*-diimide ^(8, 7, 15). At the same time, disproportionation of *cis*-diimide is assumed to be responsible for the inefficiency of the reduction.

The reduction of oleate in acetonitrile in the absence of protons and without acetic acid did reveal interesting new aspects of the reaction. In some experiments a steep rise in the reaction rates was observed (Fig. 3). This sudden rise sometimes occurred during the first hours of the reduction; in other experiments the mixture could be preserved at room temperature in a nitrogen atmosphere for more than 24 h without any change, but after another night the same rise in the yield of stearate was found. It was shown by GLC, using methyl palmitate as an internal standard, that these phenomena were not due to side-reactions of methyl oleate; methyl stearate and methyl oleate together account for the total original amount of oleate. This means that a rather stable irreactive intermediate must be formed, which can be converted into an active reductant by an autocatalytic reaction in the absence of oxygen. A comparable steep rise in the yield was also obtained on addition of 40 μl acetic acid to the anhydrous reaction medium (Fig. 4). The increase in the reaction rate by the addition of catalytic amounts of acetic acid before the start of the reaction (Fig. 2) may be due to the same effect. As little as 1 μl acetic acid per ml hydrazine appears to have a pronounced effect.

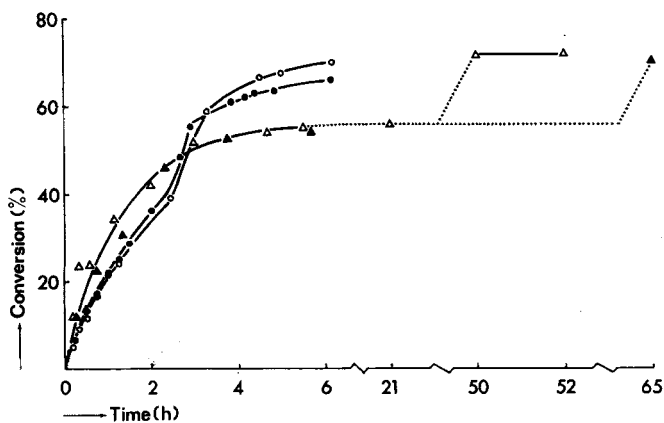


FIG. 3. Autocatalytic reactions in four reduction experiments of 10 ml methyl oleate with 1 ml anh. N_2H_4 in 60 ml acetonitrile.

Reaction conditions :

— at $80^\circ C$ in O_2 -atmosphere

- - - at $20^\circ C$ in N_2 -atmosphere.

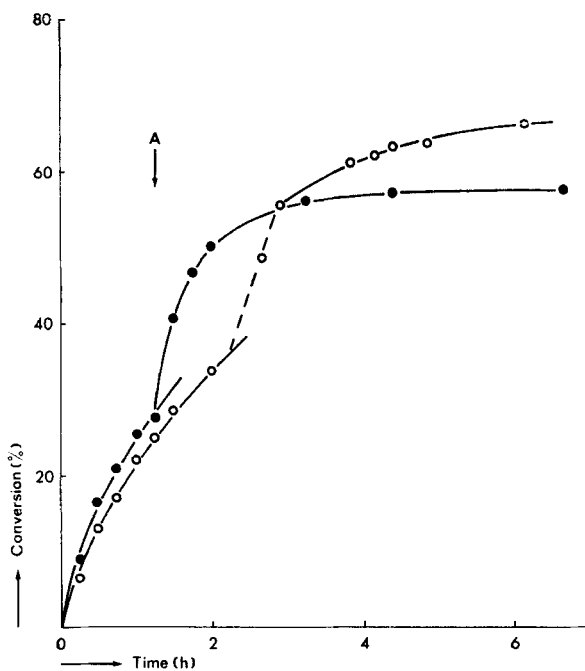
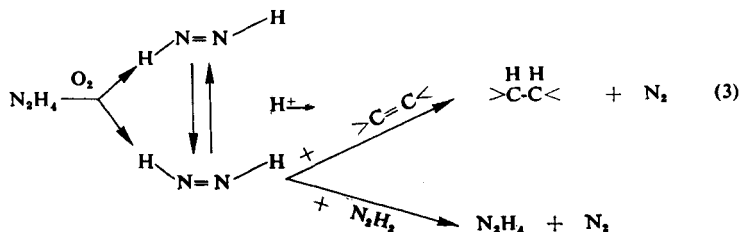


FIG. 4. Accelerating effect of $40 \mu l$ anh. CH_3COOH on the reduction of methyl oleate with anh. hydrazine.

○ Autocatalytic conversion

● CH_3COOH added after time A.

A possible interpretation of these experimental data could be based upon the formation of irreactive stable *trans*-diimide, which is converted into *cis*-diimide by an acid-catalysed or an autocatalytic reaction. Autocatalytic *cis-trans* isomerizations of azo-compounds have been reported before⁽¹⁶⁾. The disproportionation reaction of *cis*-diimide, which can be considered as a self-hydrogenation reaction, in which the same cyclic transition state will be involved, will also be absent for *trans*-diimide and can be responsible for the apparent difference in stability of *cis*- and *trans*-diimide :



To check this supposition, hydrazine was oxidized with oxygen in anhydrous acetonitrile in the absence of acetic acid until half the amount of hydrazine was consumed. At this point, the oxygen stream was replaced by nitrogen and methyl oleate was added after about 30 min. No further oxidation of hydrazine was observed and not more than 1-2 % of the oleate was converted in 1½ h. This is in agreement with complete decomposition of *cis*-diimide during hydrazine oxidation and with the possible formation of irreactive *trans*-diimide. However, addition of 40 µl anhydrous acetic acid at this moment, which should be able to convert *trans*- into *cis*-diimide, did not give a significant increase in oleate reduction. At the same time, no difference was found between the spectrophotometric determination of residual hydrazine, which is based on the formation of a hydrazone with salicyl aldehyde⁽¹⁷⁾ and which will only detect hydrazine itself, and the oxidative titrimetric determination, which will also reflect the amount of unreacted *trans*-diimide. Therefore, the occurrence of stable *trans*-diimide as the cause of autocatalytic and acid-catalysed accelerations of hydrazine reductions is highly improbable.

In one experiment, an autocatalytic acceleration was found during the hydrazine oxidation step (Fig. 5). Also in this case almost identical curves were found for the titrimetric and the spectrophotometric determination of residual hydrazine. Moreover, at the point of acceleration of the oxidation reaction, the amount of conversion (ca. 20 %) and of produced *trans*-diimide cannot be sufficient to account for the large acceleration of the oxidation reaction (ca. 40 %).

In conclusion no arguments were obtained for the occurrence of stable *trans*-diimide and its participation in the observed autocatalytic and proton-catalysed reactions in hydrazine oxidation. No other explanation can be given for these phenomena at the moment.

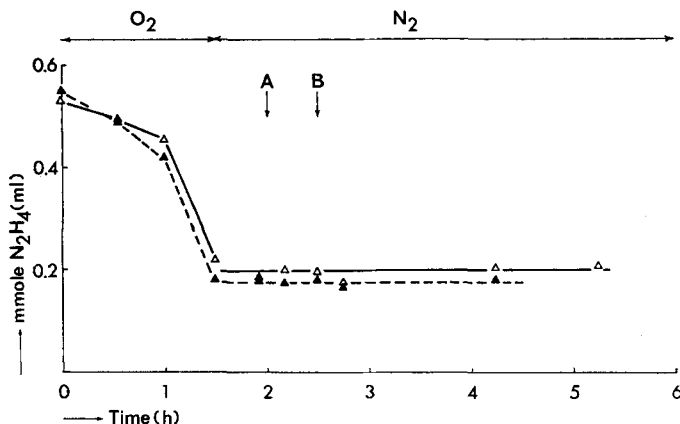


FIG. 5. Autocatalytic acceleration in oxidation of hydrazine in the absence of protons.
 \triangle Amount of N_2H_4 by titration
 \blacktriangle spectrophotometric determination of N_2H_4
 A : 1 ml methyl oleate added
 B : 40 μ l CH_3COOH added.

On the other hand it is shown by the results of Figure 5, that both the autocatalytic and acid-catalysed accelerations of reductions with hydrazine are probably connected with the hydrazine oxidation step and not with the reduction of the substrate.

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