

TABLE II  
GLC Data on Methyl Esters from Selected Calenduleae Oils

Species	Methyl ester composition, %						
	GLC						UV <sup>a</sup>
	16:0	18:0	18:1	18:2	18:3 conj.	Other	18:3 conj.
<i>Calendula officinalis</i> (2) <sup>b</sup> .....	5	2	5.5	34	53	0.5	59
<i>Chrysanthemoides monilifera</i> (3) <sup>b</sup> .....	6	7	16	37	33	2	38
<i>Chrysanthemoides incana</i> .....	10	5	17	56	11	1	16
<i>Osteospermum amplexans</i> .....	4	4	10	52	29	1	30
<i>Osteospermum microphyllum</i> .....	9	6	24	39	20	1	21
<i>Osteospermum spinescens</i> .....	6	4	8	46	34	1	39

<sup>a</sup> Determined on oil.

<sup>b</sup> Number of samples included in the average figures shown.

dimorphecolic acid, the isomer having the hydroxyl group at position 13 and the unsaturation at positions 8 and 10. Our survey analyses do not distinguish between isomers of this type, nor do they detect the small amounts of epoxy compounds reported by Morris et al. (12) in *Dimorphotheca*.

The conjugated trienoic acid in *Calendula officinalis* oil was shown by McLean and Clark (9) to be the 8,10,12 isomer and by Chisholm and Hopkins (1) to have the *trans,trans,cis* configuration. Although no definitive evidence has been obtained, it is reasonable again (4) to suggest that the conjugated trienes here reported may be the 8,10,12 isomer which arises in one way or another in the plant by dehydration of dimorphecolic acid. However, should this be the mechanism, it would involve the unexpected transformation of the 12,13 double bond from *trans* to

*cis* to make the configuration like that in *Calendula*. Since Hopkins and Chisholm (7) report that at least one genus can produce two different conjugated trienoic acids, the question can be resolved only by further investigation.

In any case, although effects of soil and climate on composition cannot be discounted, present analyses indicate the existence of a wealth of genetic stock from which oilseeds rich in either dimorphecolic acid or conjugated trienoic acid might be developed.

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## Autoxidation of Fatty Materials in Emulsion. II. Factors Affecting the Histidine-Catalyzed Autoxidation of Emulsified Methyl Linoleate<sup>1</sup>

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### Abstract

Several factors which affect autoxidation of methyl linoleate in emulsion have been examined. Data are presented which indicate: 1) In the presence of histidine, the ionic (anionic) emulsifiers examined promote autoxidation of emulsified methyl linoleate, but nonionic emulsifiers do not. 2) The concentration of an emulsifier affects the rate of oxygen absorption. 3) Inorganic salts (0.1 M or less) such as sodium chloride, sodium acetate and sodium sulfate affect oxygen absorption of emulsified methyl linoleate prepared with either ionic or nonionic emulsifiers. In histidine-catalyzed autoxidation there is a suppressing effect in the case of the ionic and a promotional effect in the case of the nonionic. In uncatalyzed autoxidation, these salts have a promotional effect in ionic emulsions and none in nonionic emulsions. 4) Sodium phosphate buffers completely suppress autoxidation due to histidine catalysis, but do not suppress the normal uncatalyzed autoxidation of emulsified methyl linoleate. 5) The pro-oxidative effects of

histidine and histidine-metal ion complexes on emulsified unsaturated materials is not limited to polyolefins but also includes mono-olefinic compounds.

### Introduction

IN A PREVIOUS paper (7) the pro-oxidative effects of histidine and metal ions on the autoxidation of linoleate esters in emulsion were reported. Although in that report cognizance was given to other factors which also affect autoxidation in emulsion, data and details were then incomplete.

One such factor then under investigation was whether the type and concentration played any role in the autoxidation process other than aiding in emulsion formation. The question as to whether emulsifiers have a promotional, retarding, or neutral effect upon autoxidation processes has not been studied in detail. However, Marcuse (6) observed that the Tweens and several related nonionic emulsifiers generally retarded pro-oxidative effects and enhanced antioxidative ones in proportion to emulsifier conen.

Another such factor then under investigation was the effect of inorganic salts which are often introduced as components of buffer mixtures. It is general knowledge that inorganic salts are used in many

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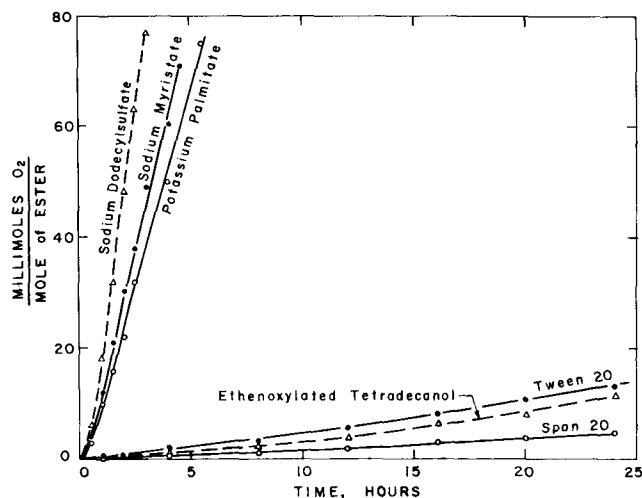


FIG. 1. Effect of 6 emulsifiers on the histidine-catalyzed autoxidation of methyl linoleate.

instances to break emulsions, but with very few exceptions (5) the effects of inorganic salts upon autoxidation processes in emulsions have not been examined in detail.

A third factor of interest was the effect of the structure of the emulsified fatty materials. Although the fruitful history of autocatalytic and chemical oxidation of both mono- and diolefins is well known, it sheds little light on our problem, for in most instances this work has been carried out in media (8,9) other than emulsions. One notable exception in the area of chemical oxidation of emulsified mono-olefins, however, is the work of Coleman and Swern (1). Otherwise fruitful information on the autoxidation of emulsified mono-olefinic compounds is indeed sparse.

Although there are doubtlessly many other factors which possibly affect and influence autoxidation in emulsion, this paper is limited to the factors mentioned above.

### Experimental

**Materials.** The preparation of methyl linoleate and the purity and sources of other reagents used such as L-histidine, emulsifiers, water and inorganic salts have already been described (7). Methyl oleate was prepared from pure oleic acid isolated from the fatty acids of olive oil (4).

**Procedure.** Methyl linoleate or oleate, emulsifier, and redistilled water were emulsified for 15 min in a Virtis 45 homogenizer. One ml of this emulsion was placed in a Warburg flask having a volume of ca. 20 ml. In the sidearm of the flasks were placed aqueous solutions of histidine, inorganic salts and other reactants, or water. When the contents of the sidearm were added to the emulsion in the main body of the flask, the concn of the reactants in the resulting emulsified mixture were as follows: 0.1 M methyl linoleate or oleate; 0.01 M histidine; 0.001, 0.005, 0.025 or 0.1 M inorganic salts; and 0.002, 0.004 or 0.008 M emulsifier. [The average mol wt of the nonionic emulsifiers, ethenoxyated tetradecanol, polyoxyethylene sorbitan monolaurate (Tween 20) and sorbitan monolaurate (Span 20) were calculated to be 875, 1226, and 346, respectively; those of the ionic emulsifiers are readily available.] The flask-manometer assemblies were flushed with oxygen for 5 min, then transferred to a Warburg bath which was maintained at 30°C in the dark. The flasks were equilibrated for 10 min, sealed and sidearm contents mixed with emul-

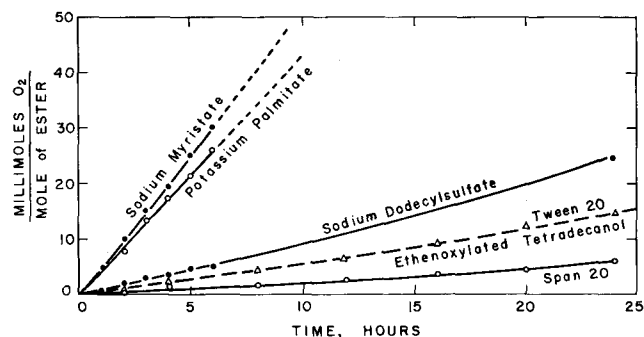


FIG. 2. Effect of 6 emulsifiers on the uncatalyzed autoxidation of methyl linoleate.

sion. Readings on manometers containing Brodie solution were recorded regularly.

### Results

**Effect of Emulsifiers.** In investigating the effects of emulsifiers upon oxygen absorption of emulsified methyl linoleate, both ionic and nonionic types were examined. The ionics included sodium dodecylsulfate, potassium palmitate and sodium myristate. The nonionic emulsifiers examined have been listed previously. With the exception of Span 20, 0.001 M concn of emulsifiers generally yielded stable reproducible emulsions. However, a 0.002 M concn was adopted as standard as offering a greater margin of stability. Emulsions prepared with this concn of Span 20 contained some oil droplets as large as 2–3  $\mu$  in size as compared with only an occasional droplet as large as 1–2  $\mu$  in size in emulsions prepared with the other five emulsifiers.

The curves in Figure 1 show the variation with time of the comparative quantities of oxygen adsorbed in emulsions prepared with various emulsifiers and containing histidine at the 0.01 M level. It is immediately apparent that the rates of oxygen absorption of emulsions prepared with the ionic emulsifiers (sodium dodecylsulfate, sodium myristate and potassium palmitate) greatly exceeded those prepared with the nonionic emulsifiers (ethenoxyated tetradecanol, Tween 20, and Span 20). It is also apparent that emulsions prepared with different ionic emulsifiers absorbed oxygen at different rates. It is quite possible that this difference in rates may be traceable, at least in part, to structural dissimilarity of the ionic emulsifiers. This suggestion is supported by the fact that emulsions prepared with the soaps, sodium myristate and potassium palmitate, absorbed oxygen at very nearly the same rate. This rate is approximately one half of that of the emulsions prepared with the detergent, sodium dodecylsulfate, although the oil particle size in the emulsions was, for all practical purposes, the same.

A similar disparity existed in emulsions prepared with nonionic emulsifiers even though the rate of oxygen absorption was slow in all cases. Compounds containing polyoxyethylene groups (ethenoxyated tetradecanol and Tween 20) yielded emulsions which absorbed oxygen at a higher rate than those prepared with Span 20 which has no polyoxyethylene content. Here it is suggested that the more hydrophobic nature of Span 20 results in emulsions containing larger oil droplets and consequently a smaller oil-water interface at which autoxidation can take place or at which reactants can pass from one phase to the other.

In experiments where no histidine was added to the emulsion (Fig. 2), the rate of oxygen absorption by

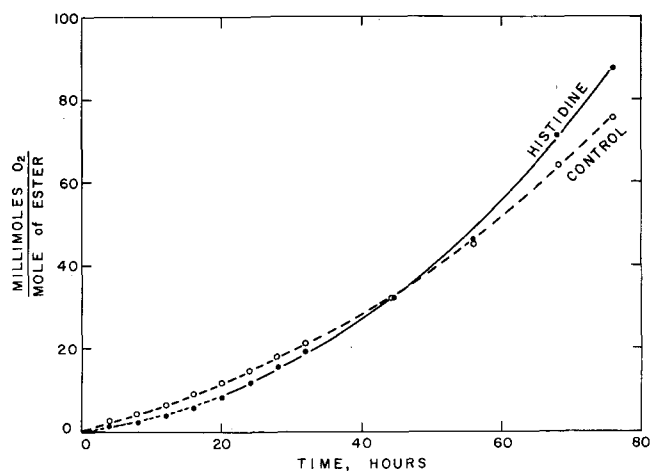


FIG. 3. Autoxidation of linoleate in 0.002 M ethenoxyated tetradecanol emulsion showing antioxidative (.....) and pro-oxidative (—) action of histidine.

emulsions prepared with sodium myristate and potassium palmitate far exceeded that of the emulsion prepared with sodium dodecylsulfate. This was the reverse of what occurred when histidine was present. By comparison with sodium dodecylsulfate, the two soaps (sodium myristate and potassium palmitate) appear to have a pro-oxidative action by themselves but retard catalysis by histidine. A plausible explanation of these observations, however, must await further investigation.

The relative rates of oxygen absorption of emulsions prepared with nonionic emulsifiers (ethenoxyated tetradecanol, Tween 20 and Span 20) were found in the absence of histidine (Fig. 2) to be very similar to those obtained with 0.01 M histidine present (Fig. 1). Actually, in the initial stages of autoxidation, histidine had a slight antioxidative action, but as soon as a small amount of oxidation had taken place, histidine became mildly pro-oxidative. This is best seen in Figure 3. Compared to the uncatalyzed control, the rate of oxygen absorption in emulsions prepared with ethenoxyated tetradecanol was slower in presence of histidine for the first 20 hr and more rapid subsequently. The same change in the action of histidine from antioxidative to pro-oxidative was observed in emulsions prepared with Tween 20 and Span 20 but at a later period in the oxidation.

Ethenoxyated tetradecanol and sodium dodecylsulfate appeared to be representative of the nonionic and ionic emulsifiers, respectively, both in histidine-catalyzed and normal uncatalyzed reactions. Since autoxidation in uncatalyzed control emulsions prepared with these two emulsifiers occurred at a low rate and since both emulsifiers were known to be of high purity, they were chosen for use in subsequent experiments.

**Effect of Emulsifier Concentration.** In exploratory experiments, the rate of oxygen absorption in linoleate emulsions prepared with 0.002 M concn of an emulsifier was equal and in an occasional experiment superior to that in emulsions with 0.001 M emulsifier. However, as the concn was increased from 0.002 to 0.008 M in emulsions containing sodium dodecylsulfate and 0.01 M histidine, the rate of autoxidation was retarded as shown in Figure 4. Similar retarding effects were noted in emulsions prepared with the same emulsifier, but which contained no histidine. Retardation was also noted in nonionic emulsions prepared with ethenoxyated tetradecanol but only at

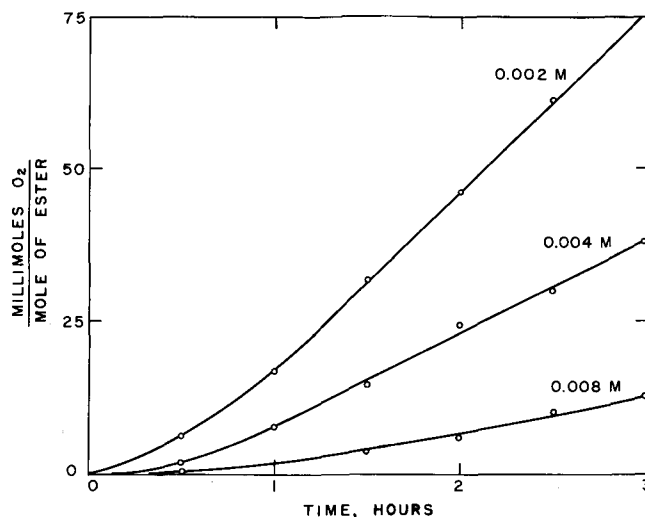


FIG. 4. Effect of 3 concn sodium dodecylsulfate on the histidine-catalyzed autoxidation of linoleate in emulsion.

the 0.008 M level when histidine was present and at the 0.004 M level in uncatalyzed emulsions. Little further decrease in oxygen uptake was observed at the 0.008 M level.

**Effect of Inorganic Salts.** The effect of various concn (0.001, 0.005, 0.025 and 0.1 M) of sodium chloride, sodium acetate, sodium sulfate and sodium phosphates were investigated in emulsions whose pH was adjusted to pH 7.3. In the histidine-catalyzed autoxidation of methyl linoleate in emulsions prepared with sodium dodecylsulfate (Fig. 5), oxygen absorption was found to be inversely proportional to salt concn in the range of 0.001–0.1 M. The decrease in oxygen uptake was roughly exponential with small initial concn of salt causing the greatest proportional decrease. For example, after a 3-hr oxidation, 0.005 M sodium chloride was found to have caused a 50% decrease in oxygen uptake. Increasing the salt concentration 20-fold (0.1 M) resulted in a further decrease in oxygen uptake of only 30%. Sodium acetate and sodium sulfate had a similar inhibiting effect on oxygen absorption. Although high concn of salt would be expected to affect emulsion stability, the particle size of oil droplets remained constant in these experiments.

The same concn of salts did not have this inhibiting effect on autoxidation under other emulsion conditions. In sodium dodecylsulfate emulsions, not catalyzed by histidine, there was a small gradual increase in rate of autoxidation with increasing salt concn. Salt in concn of 0.1 M or less had no effect on oxygen absorption in uncatalyzed linoleate emulsions prepared with ethenoxyated tetradecanol. However, when methyl linoleate with an initial peroxide value of 15 was emulsified with this emulsifier and its autoxidation catalyzed with histidine, the rate of oxygen uptake increased with the sodium chloride concn as shown in Figure 6. The "control" curve corresponds to oxygen absorption in uncatalyzed nonionic ethenoxyated tetradecanol emulsion with or without salt. The presence of 0.01 M histidine increased the oxygen absorbed in 24 hr to 2.6 times that of control while the combined effect of histidine and 0.1 M sodium chloride was 3.7 times that of the control.

A buffer mixture of sodium dihydrogen phosphate and sodium monohydrogen phosphate (pH 7.3) was investigated at concn up to 0.1 M in emulsions prepared with sodium dodecylsulfate. The variation of the oxygen absorbed with increasing phosphate ion

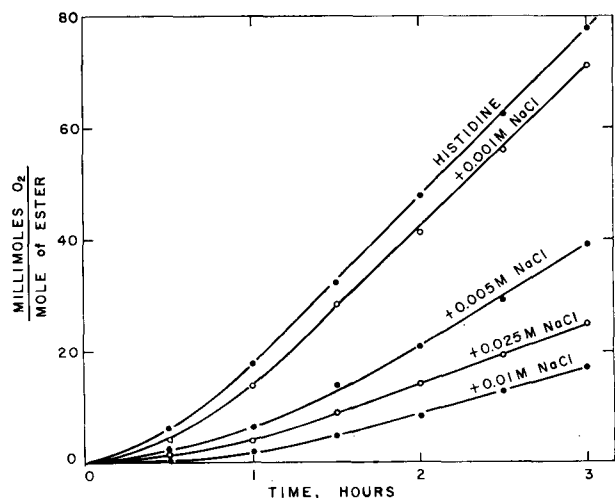


FIG. 5. Effect of sodium chloride on the histidine-catalyzed autoxidation of methyl linoleate in 0.002 M sodium dodecylsulfate emulsion.

concn after 3 hr autoxidation is shown in Figure 7. The catalytic effect of 0.01 M histidine is reduced 82% by 0.005 M phosphate and completely suppressed by a 0.1 M phosphate. In the absence of histidine, there was a small, gradual increase in oxygen absorption with increasing phosphate concn, similar to that observed with other sodium salts.

*Autoxidation of Emulsified Mono-olefinic Ester.* Because of the powerful pro-oxidative effect of histidine-metal ion complexes on emulsified linoleate esters, the question arose as to whether these effects were peculiar to polyolefinic compounds. To resolve this question, methyl oleate was chosen because of its structural similarity to methyl linoleate.

Table I shows the comparative differences in the amount of oxygen absorbed by emulsified methyl oleate under various experimental conditions. In the 22-hr oxidation period relatively little oxygen (1.3 mmole/mole methyl oleate) was absorbed in the absence of histidine. When 0.01 M histidine was added to the emulsion there was a 6-fold increase in oxygen absorption in the same period of time. When trace quantities of iron ( $2 \times 10^{-4}$  M) in the form of ferric chloride were added to the emulsion there was a 32-fold increase in oxygen absorption. When both histidine (0.01 M) and trace quantities of iron ( $2 \times 10^{-4}$

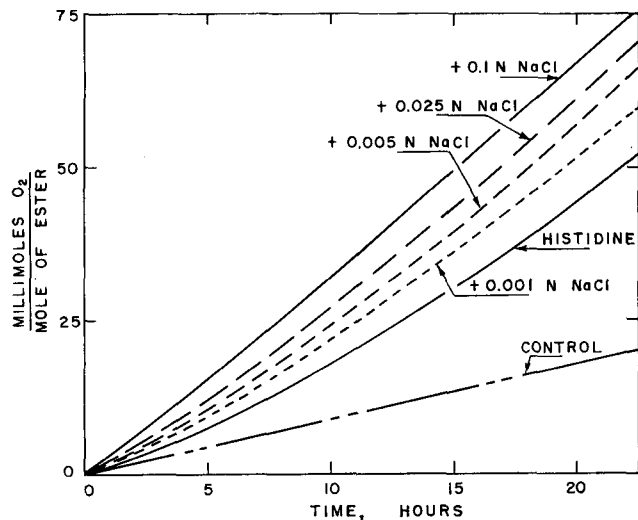


FIG. 6. Effect of histidine alone and with sodium chloride on the autoxidation of methyl linoleate in 0.002 M ethenoxy-tetradecanol emulsion.

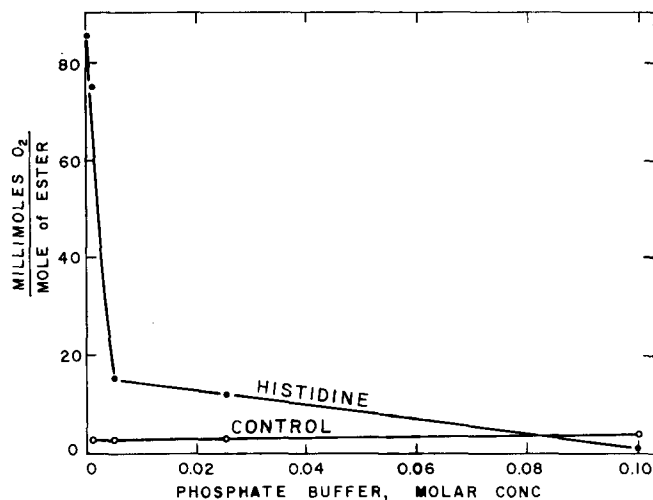


FIG. 7. Effect of  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  buffers (pH 7.3) on 3-hr oxygen uptake of methyl linoleate in 0.002 M sodium dodecylsulfate emulsion.

M) were added to the emulsion, there was an 80-fold increase in oxygen absorption above that of the emulsion alone; a 13-fold increase above that of the emulsion to which only histidine was added; and a 2.5-fold increase above that of the emulsion to which only iron was added. When the concn of  $\text{Fe}^{+++}$  was decreased to  $2 \times 10^{-5}$  M and the 0.01 M histidine was maintained, a decrease in oxygen absorption was noted.

Although under similar experimental conditions emulsified methyl linoleate absorbed oxygen more rapidly, nevertheless, the point seems quite clear that this powerful pro-oxidative effect of histidine-metal ion complexes is not peculiar to diolefinic compounds alone. The results of a detailed study of the effects of histidine-metal ion complexes on the autoxidation of emulsified mono-olefinic compounds, will be the subject of a subsequent publication.

## Discussion

The results of this investigation have shown that the histidine-catalyzed autoxidation of emulsified methyl linoleate or oleate is a highly specialized reaction which takes place only under certain limited conditions. Since the ionic nature of the emulsifier is critical, it appears likely that the pro-oxidative interaction is dependent primarily on factors peculiar to the oil-water interface.

The known facts of surface chemistry provide a basis for describing the oil-water interface in a methyl linoleate emulsion (2). In the oil droplets, polar groups are oriented at the interface toward the water phase. This includes carbomethoxy, hydroperoxide (initially present or formed in the reaction) and the hydrophilic group of the emulsifier. When an anionic emulsifier such as sodium dodecylsulfate is used, the linoleate boundary will have a negative electrical charge due to the ionic sulfate group. A second charged layer of positive ions exists on the aqueous side of the interfacial boundary. In an uncatalyzed autoxidation, this will be composed of sodium and hydrogen ions. In catalyzed reactions, ferric ions (or other transition metal ions), present as trace impurities or deliberately added, will also be concn at the interface while dipolar histidine will be oriented with its amino and imidazole groups adjacent to the metal ions. Dissolved oxygen is distributed uniformly within each phase and may also be present at

TABLE I  
Autoxidation of Methyl Oleate in Emulsion, Prepared with Sodium Dodecylsulfate, at 30C

Histidine M	Fe <sup>+++</sup> M	Mmole O <sub>2</sub> /mole methyl oleate 22 hr
0	0	1.3
0.01	0	8.0
0	2 × 10 <sup>-4</sup>	42.0
0.01	2 × 10 <sup>-4</sup>	104.0 <sup>a</sup>
0	2 × 10 <sup>-5</sup>	4.4
0.01	2 × 10 <sup>-5</sup>	61.0

<sup>a</sup> Calculated value based upon the rate of oxygen absorption at the thirteenth hour of oxidation.

the interface complexed by the ferric ion.

The catalysts and reactive groups are thus present at the interface in high concn and oriented in positions which should be highly favorable to energy transfer. This is in accord with experience where a rapid rate of autoxidation is obtained. The myristate and palmitate soaps would be expected to behave quantitatively alike since each would have a carboxyl group oriented at the boundary and exerting the same electrostatic force. The sulfate group of sodium dodecylsulfate would exert a force which was similar in type of effect but different in amount.

From a consideration of the nature of the interface, we would expect that increasing the concn of the emulsifier, sodium dodecylsulfate beyond an optimum amount (0.002 M) or by adding sodium salts such as sodium chloride, would increase the concn of sulfate and sodium ions at the boundary. It is suggested that these would act as a barrier hindering contact between the catalyst and reacting groups in the autoxidation. This would explain the retarding effects of increased emulsifier or salt concn. In case of the sodium phosphates, part of the suppressing effect can be attributed to the increase in concn of the sodium ions at the boundary as with other salts. However, since the suppression of catalysis is so complete, it is

believed that the phosphate ion also interacts with the catalyst rendering it inactive (7).

When we employed a nonionic emulsifier such as the polyoxyethylene ether of tetradecanol, the rate of the histidine-catalyzed reaction differed only slightly from the uncatalyzed rate. This would be expected for in this emulsion, there is no ionized charge at the interface. As a result metal ions and histidine will be distributed randomly throughout the bulk aqueous phase and the chance of a metal-complex catalyzed reaction at the interface would be slight. The fact that histidine becomes mildly pro-oxidative only when some oxidation has taken place may be explained by an alternative mechanism proposed by Ingold (3) in the discussion of metal catalysis of lipids in emulsion. It was suggested that since hydrated metal ions (catalyst) are present only in the water phase, they may exert their effect in this phase by reacting with water-soluble radicals (e.g., ·OH, ·OOH) or oxidation products (e.g., hydroperoxide) which would be more soluble in water than the original substrate. This is in accord with our observation that histidine has a pro-oxidative action in nonionic emulsions only when oxidation products are present.

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## The Elaidinization of Methyl Oleate with Mercaptans<sup>1,2</sup>

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### Abstract

Methyl oleate is transformed into methyl elaidate by mercaptans. The equilibrium mixture, containing 77% elaidate, can be obtained from either isomer. A concomitant, although much slower reaction, is the addition of the sulfhydryl group to the double bond of either ester. The effects of numerous reaction conditions on the extent and rate of the isomerization are given. The reaction is presented as an incipient addition of the mercaptan to the double bond, as a reversible thiyl radical attack on one of the unsaturated carbon atoms.

### Introduction

DURING RATE STUDIES on the addition of methyl mercaptan and  $\beta$ -mercaptopropionic acid to methyl sterculate and sterculene in dilute solutions, methyl oleate was used as a blank to check the reactivity of a common unsaturated ester to sulfhydryl addition (6). Very little addition of the mercaptans to methyl oleate was observed; a routine check, however, of the IR spectrum of the products from a blank run

showed a new band in the methyl oleate spectrum at 10.33  $\mu$  indicative of the *trans* double bond (12,13). Since the elaidinization of oleic acid or oleates is usually carried out with selenium or sulfur at high temp (3,8,9,16) or with oxides of nitrogen at lower temp (4,5,10,11), the sulfhydryl catalyzed isomerization in dilute solution seemed sufficiently novel to warrant its investigation. The reaction was also of interest because of its biological implications; the proteins involved in lipid transport and metabolism often contain sulfhydryl groups (1).

### Experimental

**Materials.** Technical grade methyl oleate (518 g) was distilled to yield only the C<sub>18</sub> esters. These were saponified and the acids crystallized from ethanol (2 liters) at 10C, 0C and -15C. Each fraction was recrystallized from petroleum ether (Skellysolve B) at -15C to yield oleic acid. After esterification with freshly prepared 7% BF<sub>3</sub> in methanol (2 liters) at room temperature for 24 hr, the product was distilled to yield 250 g methyl oleate bp 140°/0.4 mm, n<sub>D</sub><sup>20</sup> 1.4487 [lit. 1.4484 (13)].

Elaidic acid, mp 44-6C [lit. 44 (13)], obtained from an isomerization run was similarly converted to the

<sup>1</sup> Presented at the AOCS meeting in Minneapolis, 1963.

<sup>2</sup> Contribution No. 858 of the Agricultural Experiment Station, University of Arizona.