

[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY, HARVARD UNIVERSITY]

The Absorption of Oxygen by Linseed Oil in the Presence of Catalysts of Different Oxidation-Reduction Potentials

BY BACON F. CHOW

A. Introduction

Chow and Kamerling¹ recently suggested that in the absence of other catalysts the rate of oxygen absorption of oleic acid catalyzed by ferricyanide is apparently independent of the chemical structures of the catalyst but dependent on its oxidation-reduction potential E_0 . In this paper an attempt was made to study quantitatively the various factors governing the oxygen absorption of linseed oil as catalyzed by ferri-, tungsti- and molybdicyanides. It was found that the rate is dependent not only on concentrations of hydroxyl ion and the oxidized form of the catalyst but is inversely proportional to the reduced form. An empirical equation was formulated to account for the results.

B. Experimental Procedure

The absorption of oxygen was measured with the conventional Warburg² absorption apparatus, following the usual procedure, at a temperature of $25.0 \pm 0.1^\circ$. The charge consisted of 0.1 cc. of raw linseed oil and 1.0 cc. of catalysts of specified concentrations in buffers, and 1.2 cc. of the same buffer.

The composition of the buffers, made up with water to 50 cc., and the P_{H^+} 's of the resulting solutions were as follows.

TABLE I

Mixture	P_H
25.0 cc. of 0.824 M K_2HPO_4 + 21.6 cc. of 1.65 N NaOH	12.0
25.0 cc. of 0.824 M K_2HPO_4 + 12.25 cc. of 1.65 N NaOH	11.6
33.3 cc. of 0.33 M K_2HPO_4 + 16.7 cc. of 0.33 M KOH	10.8
20 cc. 0.1 N NaOH + 30 cc. of borate solution ^a	10.0
0.1 M $Na_2B_4O_7$	9.2
25.8 cc. 1 M KH_2PO_4 + 24.2 cc. 1 N NaOH	8.0

^a Borate solution: 12.4 g. H_3BO_3 + 100 cc. N NaOH.

Only one sample of raw linseed oil has been used in this report because the purpose of this paper is to study the relative and not the absolute rates of oxygen absorption under different experimental conditions. Furthermore, the concentration of linseed oil was kept constant by using a saturated solution of linseed oil and the partial pressure of oxygen was maintained at the atmospheric condition.

(1) Chow and Kamerling, *J. Biol. Chem.*, **104**, 69 (1934).

(2) Warburg, *Biochem. Z.*, **152**, 51 (1924).

C. The Empirical Formula

For the sake of the convenience of presentation, the empirical equation is given at this point.

It was found that for one catalyst, the rate of oxygen absorption was given by

$$-\frac{dO_2}{dt} = k' \left[(OH^-) \frac{(A)^2}{(A^-)} \right]^{1/2} \quad (1)$$

where k' is the rate constant for a particular catalyst, (A) and (A^-) are the concentrations of the catalyst in the oxidized and reduced states, respectively. Thus at constant $(A)^2/(A^-)$ ratio, the rate is given by

$$-dO_2/dt = k'' [(OH^-)]^{1/2} \quad (2)$$

On the other hand, if the hydroxyl-ion concentration is kept constant by a buffer, we have

$$-\frac{dO_2}{dt} = k''' \left[\frac{(A)^2}{(A^-)} \right]^{1/2} \quad (3)$$

D. Experimental Results

Effect of Hydrogen-Ion Concentration.—Measurements of the rate of oxygen absorption were made in buffers of different P_H 's, keeping the term $(A)^2/(A^-)$ constant. This was done by adding solid reduced catalyst equivalent to 5% of the

TABLE II
EFFECT OF HYDROGEN-ION CONCENTRATION
 $[(A)^2/(A^-)]^{1/2} = 0.56$

$-dO_2/dt$, cc. $\times 10^{-3}$ per hour	P_H	k'' (calcd.) $\times 10^4$
	$K_3Fe(CN)_6$	
8.0	12.4	49
4.0	11.6	45
2.1	10.8	44
1.3	10.0	50
0.57	9.1	45
.29	8.0	52
	Av. $k'' =$	48
	$K_3W(CN)_6$	
6.0	10.8	125
1.43	9.1	111
0.56	8.0	101
	Av. $k'' =$	112
	$K_3Mo(CN)_6$	
24.0	10.8	500
5.8	9.1	450
2.3	8.0	410
	Av. $k'' =$	453

oxidized form. Since in general much less than 5% of (A^-) is produced, the ratio is but slightly changed and its cube root still less. In this way a straight line is obtained during the first part of the experiment whose slope gives the rate of absorption. The results for potassium ferri-, tungsti- and molybdicyanide in different buffers are collected in Table II.

Typical data for the rate of oxygen absorption by linseed oil catalyzed by potassium ferricyanide in different buffers are shown in Fig. 1 for purposes of illustration.

Effect of the Concentration of Catalyst.—A series of experiments using ferricyanide as catalyst whose concentration varied from 0.91 *M* to 0.00091 *M* was performed. In each experiment, 5% of ferrocyanide was present. The results are given in Table III.

TABLE III

TO DETERMINE THE EFFECT OF VARYING CONCENTRATIONS OF THE CATALYST, FERRICYANIDE, IN *P_H* 10.8

The rate of oxygen absorption is in mm. $\times 10^3$ per hour.		
$-dO_2/dt$	(A)	$k''' \times 10^3$
10.2	9.1×10^{-1}	3.9
8.9	4.6×10^{-1}	4.3
4.9	9.1×10^{-2}	4.1
2.1	9.1×10^{-3}	3.8
0.72	9.1×10^{-4}	2.8

Av. $k''' = 3.8$

The average value of k''' is 3.8×10^{-3} , from which k' can be calculated by the equations (1) and (3). This gives $k' = 4.4 \times 10^{-2}$.

Effect of the Ratios of Ferricyanide to Ferrocyanide.—A series of experiments was carried out by adding varying amounts of ferrocyanide to 0.0091 *M* ferricyanide, in the phosphate buffer *P_H* 10.8. By virtue of equation (3), k''' can be calculated. The results are given in Table IV.

TABLE IV

TO DETERMINE THE EFFECT OF THE ADDITION OF FERROCYNANIDE TO 0.0091 *M* FERRICYANIDE AT *P_H* 10.8

The rate of oxygen absorption is in mm. $\times 10^3$ per hour.		
$-dO_2/dt$	(A ⁻) in molarity	$k''' \times 10^3$
1.90	4.6×10^{-4}	3.3
1.34	2.3×10^{-3}	4.1
1.21	4.6×10^{-3}	4.6
0.67	6.8×10^{-3}	2.9
.55	8.7×10^{-3}	2.6

Av. $k''' = 3.5$

The average value of k''' is 3.5×10^{-3} from which we get $k' = 4.1 \times 10^{-2}$.

Effect of the Variation both in the Ratio of $(A)^2/(A^-)$ and in their Concentrations.—A series of experiments was carried out with different

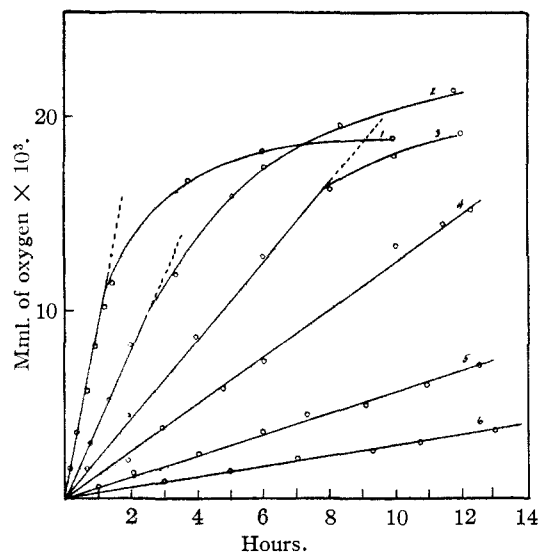


Fig. 1.—Showing the rate of oxygen absorption by linseed oil catalyzed by $K_3Fe(CN)_6$ in different buffers: Curve 1, *P_H* = 12.4; Curve 2, *P_H* = 11.6; Curve 3, *P_H* = 10.8; Curve 4, *P_H* = 10.0; Curve 5, *P_H* = 9.1; Curve 6, *P_H* = 8.0.

concentrations of both ferricyanide and ferrocyanide. The results are recorded in Table V.

The validity of the empirical equation (1) can best be verified by the constancy of k' . In

TABLE V

TO DETERMINE THE EFFECT OF VARYING THE CONCENTRATIONS OF $K_3Fe(CN)_6$ AND $K_4Fe(CN)_6$

The rate of oxygen absorption is in mm. $\times 10^3$ per hour.

(a) Total concentration of $K_3Fe(CN)_6 + K_4Fe(CN)_6$ 0.0091 *M*

$-dO_2/dt$	(A) in molarity	(A ⁻) in molarity	$k''' \times 10^3$
1.7	8.6×10^{-3}	4.5×10^{-4}	3.1
1.08	6.8×10^{-3}	2.3×10^{-3}	4.0
0.76	4.6×10^{-3}	4.6×10^{-3}	4.5
.27	2.3×10^{-3}	6.8×10^{-3}	3.0

Av. $k''' = 3.7$

Therefore $k' = 4.3 \times 10^{-2}$

(b) Total concentration of $K_3Fe(CN)_6 + K_4Fe(CN)_6$ 0.0455 *M*

1.8	4.1×10^{-2}	4.6×10^{-3}	2.5
1.5	3.4×10^{-2}	1.1×10^{-2}	3.3
1.1	2.3×10^{-2}	2.3×10^{-2}	3.9
0.58	1.1×10^{-2}	3.4×10^{-2}	3.9
.27	2.3×10^{-3}	4.3×10^{-2}	5.3

Av. $k''' = 3.8$

$k' = 4.4 \times 10^{-2}$

TABLE V (Concluded)

(c) Total concentration of $K_3Fe(CN)_6 + K_4Fe(CN)_6$,
0.218 M

$-dO_2/dt$	(A) in molarity	(A ⁻) in molarity	$k''' \times 10^3$
3.4	2.1×10^{-1}	1.1×10^{-2}	2.2
2.7	1.6×10^{-1}	5.5×10^{-2}	3.4
2.2	1.1×10^{-1}	1.1×10^{-1}	4.6
0.72	5.5×10^{-2}	1.6×10^{-1}	2.7
.31	1.1×10^{-2}	2.1×10^{-1}	3.8

$$\text{Av. } k''' = 3.4$$

$$k' = 3.9 \times 10^{-2}$$

(d) Total concentration of $K_3Fe(CN)_6 + K_4Fe(CN)_6$,
0.455 M

	(A) in molarity	(A ⁻) in molarity	$k' \times 10^3$
8.9	4.3×10^{-1}	2.2×10^{-2}	4.4
5.0	3.9×10^{-1}	6.8×10^{-2}	3.9
3.8	3.4×10^{-1}	1.1×10^{-1}	3.7
3.1	2.3×10^{-1}	2.3×10^{-1}	5.0

$$\text{Av. } k''' = 4.3$$

$$k' = 5.0 \times 10^{-2}$$

Table VI the average k' values obtained by different methods are tabulated in the second column.

TABLE VI
Method

Method	$k' \times 10^2$
By the variation of P_H using $K_3Fe(CN)_6$ as catalyst	4.8
By the variation of concentrations of $K_3Fe(CN)_6$	4.4
By the variation of the ratio of $K_3Fe(CN)_6/K_4Fe(CN)_6$	4.1
By the variation of both the ratio and concentration of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$	4.4

Equation (1) shows that the rate of oxygen absorption is proportional to the product of concentration of the catalyst and the ratio of the oxidized form to the reduced. The appearance of the concentration of the reducing agent in the denominator of the rate expression makes it plausible that a preliminary oxidation-reduction equilibrium is reached.

If we assume that the rate is proportional to $e^{(nF/RT)E_0}$, then equation (3) can be transformed into

$$-\frac{dO_2}{dt} = k [e^{(nF/RT)E_0} (A)e^{ln(A)/(A^-)}]^{1/3} \quad (4)$$

where $k''' = k [e^{(nF/RT)E_0}]^{1/3}$, n , F , R , T have their usual meanings.

Collecting the exponents of e and substituting the value of E for $E_0 + \frac{RT}{nF} \ln \frac{(A)}{(A^-)}$, we get

$$-\frac{dO_2}{dt} = k_0 [(A)e^{(nF/RT)E}]^{1/3} \quad (5)$$

Experimental verification of equation (4) is complicated by the arbitrary standard, the "normal" hydrogen electrode to which all E_0 's are

referred. Thus to test the assumption, it is best to compare the experimental ratios of rates of two catalysts with the calculated value. Thus at constant hydroxyl ion concentration and $(A)^2/(A^-)$ ratio, we can rewrite equation (4) as follows

$$-\left(\frac{dO_2}{dt}\right)_T / -\left(\frac{dO_2}{dt}\right)_F = [e^{(nF/RT)(E_{0T} - E_{0F})}]^{1/3} \quad (4a)$$

where subscripts T and F denote tungsticyanide and ferricyanide, respectively. Similarly, the ratios of the rates of oxygen absorption catalyzed by molybdicyanide and ferricyanide can be calculated. The cases of methylene blue and indophenol are complicated by the fact that the concentrations of the reduced forms are not known. However, the experimental results of both dyes are of the right order of magnitude and direction (see Fig. 2).

TABLE VII

THE RELATION BETWEEN THE RATE AND E_0 The rate of oxygen absorption is in $\text{mm.} \times 10^3$ per hour.

Catalyst	$-dO_2/dt$	E_0 , volt	Ratio of rates of absorption	$[e^{(nF/RT)(E_0 - E_{0F})}]^{1/3}$
$K_4Fe(CN)_6$	44	0.45	1	1
$K_4W(CN)_6$	125	.53	2.8	2.5
$K_2Mo(CN)_8$	500	.72	11.4	38

Table VII shows a rough correlation between E_0 and the rate. The choice of E_0 given in Table VII needs a slight comment, since E_0 of the polyvalent ions is greatly affected by the salt concentration. According to Conant and Pratt's³ measurement the average values of E_0 for molybdicyanide and tungsticyanide at the range of P_H between 8 and 11, are +0.72 v. and +0.53 v., respectively. Collenberg⁴ gave the normal potential of molybdicyanide and tungsticyanide as +0.839 v. and +0.569 v., when referred to the normal hydrogen electrode. Both values of Collenberg are higher than those of Conant and Pratt. However, the latter's values are used because their experimental conditions are more comparable with those reported in this paper.

E. Inhibitors

It is to be pointed out that inhibitors must not reduce the catalysts appreciably and the ones, such as hydroquinone, etc., which are very powerful in inhibiting the other chain reactions cannot be used in this reaction. In spite of this limitation the following compounds show marked inhibiting effects.

(3) Conant and Pratt, *THIS JOURNAL*, **48**, 3229 (1926).(4) Collenberg, *Z. physik. Chem.*, **109**, 353 (1924).

TABLE VIII

TO DETERMINE THE EFFECT OF INHIBITORS IN 0.0046 *M* SOLUTION OF $K_3Fe(CN)_6$

Names	Concn. of inhibitors in molarity	Absorption of O_2 in mml. $\times 10^{-3}$				$-\frac{dO_2}{dt}$ in mml. $\times 10^2$ per hr.
		2 hrs.	5 hrs.	10 hrs.	20 hrs.	
None	0.00	4.4	9.1	17.0	23.8	2.2
Acetoxime	4.5×10^{-4}	1.1	2.9	6.3	12.1	0.62
	4.5×10^{-5}	1.7	4.2	7.6	16.1	.81
α -Aminoiso-butyric acid	4.5×10^{-4}	2.4	5.6	10.7	21.4	1.1
	4.5×10^{-5}	2.8	6.3	12.5	23.0	1.3
<i>dl</i> -Serine	4.5×10^{-4}	1.6	3.9	7.8	16.5	0.78
Arginine	4.5×10^{-4}	1.8	4.5	9.4	18.8	.93
Histidine	4.5×10^{-4}	1.3	3.8	8.1	15.2	.80
Cinnamic acid	4.5×10^{-4}	2.2	5.5	11.2	21.4	1.14
	9.1×10^{-4}	2.2	4.9	9.8	19.2	1.02
Ethanolamine	9.1×10^{-5}	3.6	8.5	16.5	..	1.7

A series of the derivatives of aniline and phenol has been tried, *e. g.*, *p*- or *m*-nitroaniline, *p*-aminobenzoic or *p*-sulfanilic acid; *p*- or *o*-carboxylphenol. The rate of oxygen absorption was cut down 40%, under the same experimental conditions.

From Table VIII, acetoxime is a fairly powerful inhibitor. The presence of 5×10^{-8} mole of acetoxime cuts the rate to half. All the amino acids show definite inhibition, though there is no apparent relation between their structures and power of inhibition. Ethanolamine is not a very powerful inhibitor. It is interesting that cinnamic acid which possesses a conjugated double bond apparently reduced the rate considerably. Lastly, it is to be mentioned that dimethylaniline, which is a good inhibitor for the oxygen absorption by oleic acid, is a catalyst for the oxygen absorption by linseed oil. This is probably due to the fact that dimethylaniline being more soluble in linseed oil than in water goes into the linseed oil phase. Francke⁵ has shown that dimethylaniline is a good catalyst for the oxygen absorption.

F. Discussion of Results

Equation (5) shows two peculiarities. First, the cubic root in the kinetic equation is justified only by the results. Second, the rate is proportional not only to the concentration of the catalyst but also to the potential of the system,⁶

(5) Francke, *Ann.*, **498**, 129 (1932).

(6) Studies on the relation between the rate and the potential of homogeneous solutions are in progress. Preliminary experiments on the nitrogen evolution of hydrazine in the presence of an oxidizing agent show that the rate is also related to the potential of the reagent, such as $K_3Fe(CN)_6$, $K_3W(CN)_6$, etc.

which is a measure of the free energy. The importance of the relationship, if true, is obvious.

However, it must be pointed out that although equation (1) can be formulated from equation (5), it must not be taken as a proof for the correctness of the assumption. In a chain reaction the concentration of an inhibitor⁷ also appears in the denominator. It is possible that the reduced form of the catalyst may function in the chain breaking process.

G. The Secondary Oxygen Absorption

It was observed in the experiments with potassium molybdicyanide and tungsticyanide in the alkaline buffer of *P*H 9 or greater that the oxygen absorption would occur at the normal rates only to be followed by a tremendous increase.

An experiment was performed to follow the

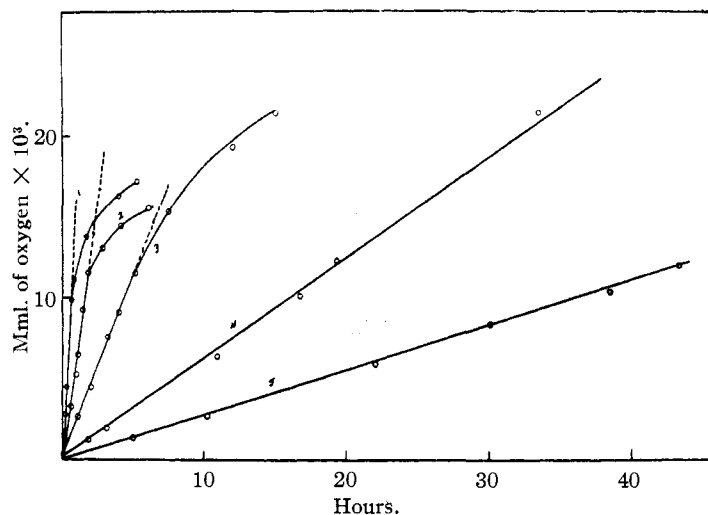


Fig. 2.—Showing the rate of oxygen absorption by linseed oil with catalysts of different oxidation-reduction potentials: 1, molybdicyanide; 2, tungsticyanide; 3, ferricyanide; 4, indophenol; 5, methylene blue.

rates of oxygen absorption and of the reduction of molybdicyanide. The results showed that the normal rate of oxygen absorption as required by equation (1) continued until about 80% of the molybdicyanide was used up. Then the rate of oxygen absorption gradually increased to a maximum. An electrometric titration with ferrocyanide showed no presence of molybdicyanide at this point. The oxygen absorption continued for several hours at the maximum rate and gradually decreased to zero in not less than thirty hours.

It is not clear just why the absorption took place after molybdicyanide was used. How-

(7) Jeu and Alyea, *THIS JOURNAL*, **65**, 575 (1933).

ever, the following experiments point to the possibility of peroxide formation. (1) This secondary absorption occurred most readily only in the alkaline solutions where molybdicyanide is metastable. In *PH* 7.0 only the normal oxygen absorption was obtained within twenty hours. (2) Preliminary experiments showed that linseed oil absorbed oxygen at a great rate in the presence of hydrogen peroxide and peroxidase.

The "complex" is greatly deactivated by heat. A sample which showed a rate of absorption at the maximum was heated in nitrogen for half an hour over the steam-bath, whereupon it absorbed only about 1/300 as fast.

The author is greatly indebted to Prof. James

B. Conant for invaluable suggestions and to Prof. G. B. Kistiakowsky for reading and criticizing the manuscript.

H. Summary

1. An empirical equation relating the rate of oxygen absorption of linseed oil to the potential of the catalysts has been obtained.

2. A series of inhibitors has been found. Acetoxime is the most powerful one. The presence of 5×10^{-8} mole of acetoxime will lower the rate to about one-half.

3. A "secondary" oxygen absorption has been found when molybdicyanide or tungsticyanide were used as catalysts in *PH* 9 or greater.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

The Preparation of Pure Eleostearic Acids from Chinese Wood Oil

BY ARTHUR W. THOMAS AND JAMES CLAUDE THOMSON

The following prescriptions are proposed for the isolation of eleostearic acids from tung oil,¹ the operations being performed in a dimly lighted room using a black cloth to envelop the glass apparatus.

α -Eleostearic Acid

Saponify 25 g. of tung oil with 100 cc. of 10% solution of potassium hydroxide in alcohol by gentle boiling under a reflux condenser for one hour. Remove the condenser and insert in the flask a three-holed rubber stopper containing a tube for entry of carbon dioxide gas, a siphoning tube and a dropping funnel. Fill the flask with carbon dioxide. Decompose the soaps by addition of 200 cc. of hot molar sulfuric acid through the funnel in small portions. Allow to stand for five minutes. Siphon off aqueous layer under carbon dioxide pressure. Add 250 cc. of boiling water through funnel and agitate to wash the fatty acids. Siphon off aqueous layer as before and repeat until sulfate ion is absent (at least 6 washings are required). Remove stopper, add water at about 0°, to solidify the fatty acids. Immerse the flask in an ice bath for thirty minutes. Filter off the frozen nodules in a Buchner funnel which is in a carbon dioxide bath. Wash with very cold water and suck dry. Transfer the nodules to a beaker filled with carbon dioxide. Add about 1.5 g. 90% ethyl alcohol per gram of nodules to dissolve them by heating to about 35°. Store this solution at 10° overnight, filter crystals

(1) The authors are indebted to Mr. Sheo Loh who personally collected the specimen of tung oil near Hangchow in Chekkiang Province, China and shipped it in glass bottles, protected from air and light, to New York. They thank Prof. Chen Yung and Mr. Tang I for securing seeds of *Aleuritis Fordii*.

in a Buchner funnel in carbon dioxide atmosphere and wash four times with 5 cc. of 90% alcohol at room temperature. Using the same proportion of 90% alcohol, redissolve the crystals, store for two hours at 10° and filter. Repeat the recrystallization operation but obtain the crystals this time by immersion of the beaker for thirty minutes in a bath of cracked ice, always maintaining the atmosphere of carbon dioxide.

β -Eleostearic Acid

Treat "Wood oil butter"² in a manner similar to that just described with the following exceptions. Melt the solidified tung oil or "wood oil butter" by heating at 80° for about one hour. Saponify 25 g. with 200 cc. of the alcoholic potash, boiling carefully and very gently³ for one and one-half hours. Use 300 cc. of hot molar sulfuric acid for decomposition of the soaps. Use 2 g. of 90% alcohol per gram of crystals. Let stand for three instead of ten hours at 10° for the first crystallization. Use a thirty-minute period in cracked ice for the first and second recrystallizations.

The melting points of separate batches of β -eleostearic acid prepared at different times ranged from 70.1 to 71.0° while the preparations of α -eleostearic acid melted always at 48.0°.

The spectrographic characterization of these acids will be submitted in a subsequent paper.⁴

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(2) This product as used by the authors was prepared by the contact of the above mentioned tung oil sample with a trace of sulfur.

(3) The relatively insoluble potassium- β -eleostearate induces bumping and frothing.

(4) Dingwall and Thomson, *THIS JOURNAL*, **56**, 899 (1934).