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RATE OF REACTION BETWEEN BROMINE AND UNSATURATED FATTY ACIDS AS EVIDENCE OF STEREO-ISOMERISM¹

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Various studies on the action of bromine toward unsaturated fatty glycerides indicated that the rate of reaction was dependent upon several factors. Thus, the nature and dilution of the solvent has a marked effect on the rate of bromination.

Working under the same conditions with respect to solvent, the rate of bromination was applied to glycerides of oleic, linolic and linolenic acids, respectively, and to the free fatty acids of the respective glycerides. The rate of bromination was found to vary with the different unsaturated fatty acids.

The effect of ultraviolet light during the bromination was also studied, with the result that certain stereo-isomers of unsaturated acids were determined which escaped detection by the present methods of analysis.

In an investigation the authors have undertaken dealing with the stereo-isomers of linolenic acid they observed that in the bromination of the fatty acids of linseed and other oils several factors seemed to influence the addition of the bromine to the unsaturated acids as, for example, the concentration of the solvent, the composition of the fatty acids and light. A supposedly pure sample of linolenic acid was found to have an iodine number of 223 by the Wijs method and a hexabromide number of 46, while the theoretical number is 274.3. Erdman and Bedford reported an iodine number of 227 for linolenic acid. It is very difficult to explain these discrepancies but when it is considered that the *cis* modifications of stereo-isomeric compounds generally react with bromine only with difficulty, a new view of this phenomenon may be taken. The authors are certain that linolenic acid is present in linseed oil in several stereo-isomeric forms (this work is to be reported in a later paper), so that an explanation of the erratic action of the fatty acids of drying oils may be found from a stereochemical consideration of this problem.

Historical

A survey of the literature has shown that although the iodine number of oils is an important characteristic, very little work has been reported on halogenation of fatty acids aside from the methods for the determination of iodine number.

McIlhiney² reported a method of bromination which he advocated as a

¹ Presented before the Paint and Varnish Division of the American Chemical Society at its 73rd meeting, Richmond, Virginia.

² McIlhiney, *THIS JOURNAL*, 21, 1084 (1899).

substitute for the iodine methods. He used a $N/3$ solution of bromine in carbon tetrachloride, allowed the solution to stand for only two minutes, then added potassium iodide and titrated with sodium thiosulfate. After this titration was finished, neutral potassium iodate solution was added and the liberated iodine, equivalent to free hydrogen bromide, was titrated and calculated as the substitution.

Cerdeiras³ has studied the rate of reaction of the Wijs reagent on fats and fatty acids but the reaction was too rapid to draw any positive conclusions with respect to the different components of the fatty acids.

Kaufmann⁴ describes the use of bromine instead of iodine for the analysis of oils and has shown the action of bromine in a number of solvents toward highly unsaturated compounds. Thus, bromine dissolved in carbon tetrachloride was found to be less reactive than bromine in chloroform and, further, bromine in methyl alcohol saturated with sodium bromide was in most cases less reactive than in carbon tetrachloride. The latter mixture he recommended as a reagent for the analysis of oils. Kaufmann also reported some data showing a comparison of the rate of bromination of linseed oil in carbon tetrachloride and in methyl alcohol saturated with sodium bromide. The experiments were carried out in sunlight and in the dark; the results are given as the equivalent of iodine absorbed. The results reported by Kaufmann are as follows.

TABLE I
BROMINATION OF LINSEED OIL (IODINE No. 182)

Time	Carbon tetrachloride		Methyl alcohol and sodium bromide	
	Dark	Sunlight	Dark	Sunlight
2 min.	152.0	178.1
10 min.	151.2	183.0	156.2	161.3
30 min.	152.8	190.5	157.4	163.8
60 min.	156.8	194.3	160.7	164.3
24 hours	183.0	239.5 ^a	183.3	182.0
48 hours	182.0	252.0	183.5	181.6

^a No mention is made by Kaufmann as to whether any substitution had taken place to give these high values.

Kaufmann⁵ also reported the rate of bromination of cinnamic acid both in the dark and in ultraviolet light but no mention is made of the use of ultraviolet light in the experiments with linseed oil. He reported the addition of six bromine atoms to elaeostearic acid and no substitution when exposed to ultraviolet light for forty minutes.

Coffey⁶ has shown that the different constituents of linseed oil oxidize differently, so that it would appear reasonable to assume that the different constituents should brominate differently.

³ Cerdeiras, *Bull. soc. chim.*, **35**, 902-904 (1924).

⁴ Kaufmann, *Z. Untersuch. Lebensm.*, **51**, 3 (1926).

⁵ Kaufmann, *Ber.*, **59**, 1390 (1926).

⁶ (a) Coffey, *J. Chem. Soc.*, **119**, 1408 (1921); (b) **28**, 286 (1925).

Experimental Procedure

These investigators have shown that unsaturated compounds react slowly with bromine when the solution is dilute. Sunlight and ultraviolet light increase this rate of reaction, producing greater bromine absorption without necessarily causing substitution.

In order to study the action of bromine on "drying" oils, the fatty acids of poppy seed, soya bean, linseed and perilla oils were treated with a dilute solution of bromine in carbon tetrachloride for different periods of time. The amount of bromine that reacted with the fatty acids was calculated on the basis of iodine numbers. The calculation to an iodine number was made so as to have a ready reference to the ordinary iodine number of the fatty acids as determined by the Wijs method. These results were then compared with the results for oleic, linolic and linolenic acids by similar treatment. In order to determine if light produced any action between bromine and the fatty acids aside from substitution, the fatty acids were allowed to stand in contact with bromine for an hour in the dark. These solutions were then subjected to ultraviolet irradiation for definite periods of time and the amounts of additive and substitutive bromine determined.

Materials

Bromine-Carbon Tetrachloride Solution.—Two and six-tenths cc. of bromine was added to 500 cc. of Eastman Kodak Co. "sulfur free" carbon tetrachloride which was freshly distilled over phosphorus pentoxide before adding the bromine. It is necessary that extreme precaution be taken to have the liquids dry or else bromine acids will form and cause substitution with the fatty acids.

Fatty Acids.—Twenty-five g. of poppy seed, soya bean, linseed and perilla oils were each dissolved in 50 cc. of alcohol and saponified with potassium hydroxide in an atmosphere of carbon dioxide. The potassium soaps were converted into the free acids by treatment with dilute hydrochloric acid. The fatty acids were then dissolved in ethyl ether and dried over anhydrous sodium sulfate. The ether was evaporated just before using the acids.

The fatty acids from the different oils had the following iodine numbers by the Wijs method.

Poppy seed oil fatty acids.....	135.5
Soya bean oil fatty acids.....	137.0
Linseed oil fatty acids.....	186.4
Perilla oil fatty acids.....	197.1

Oleic Acid.—The oleic acid used was so-called c. p. quality. It had an iodine number of 86.0. The theoretical iodine number is 90.

Linolic Acid.—The linolic acid was so-called c. p. quality with an iodine number of 184.0; theoretical, 181.4.

Linolenic Acid.—The linolenic acid was prepared by treating 50 g. of white crystalline hexabromostearic acid with 15 g. of freshly reduced zinc dust in 100 cc. of absolute methyl alcohol saturated with dry hydrogen chloride gas. This mixture was refluxed on a water-bath and mechanically stirred for about three hours. Dry carbon dioxide and hydrogen chloride was passed into the flask during the reaction. The mixture was then treated with warm brine to remove the methyl ester from excess methyl

alcohol. The methyl ester of linolenic acid was then saponified with potassium hydroxide and the potassium salt hydrolyzed with dilute hydrochloric acid. The linolenic acid thus produced was washed several times with water until it gave no test for halogens. It was then dissolved in ethyl ether to remove any unchanged hexabromide, filtered and the ethereal solution dried with sodium sulfate. The ether was removed by distillation; yield, 90%. This linolenic acid had an iodine number of 222.8; theoretical, 274.3.

Method

In studying the reaction between bromine and the fatty acids in the dark the methods of McIlhiney and of Kaufmann were combined with some slight modifications. One g. of the fatty acid from the freshly saponified oil was dissolved in 200 cc. of the bromine-carbon tetrachloride solution and diluted to 250 cc. with carbon tetrachloride. The mixture was thoroughly shaken and placed in the dark at room temperature. At periods of 5, 10, 20 and 30 minutes, 1 hour, 24 hours and 48 hours, 25 cc. of the solution was removed, 15 cc. of a 15% solution of potassium iodide and 100 cc. of water were added and it was titrated with standard sodium thiosulfate solution in the usual manner. When the end-point was reached, 5 cc. of a 2% solution of sodium iodate was added. If the blue starch-iodide color reappeared, the titration was continued to the end-point. The amount of sodium thiosulfate used for this second titration was doubled and calculated as the substitution value.

In studying the reaction between bromine and the fatty acids due to ultraviolet irradiation, the method used was similar to the above except that the solution of fatty acids and bromine remained mixed in the dark for one hour, then 25cc. portions were placed in quartz tubes and exposed to a Cooper-Hewitt quartz Uviarc at a distance of 30 cm. (12 inches) for periods of 5, 15, 30 and 60 minutes. The titrations for additive and substitutive bromine were similar to the above. By keeping the reacting mixture in the dark for an hour the amount of bromine absorbed was in nearly all cases practically equivalent to the ordinary iodine number. As the study of the reaction under ultraviolet irradiation was not to increase the speed of the reaction but rather to determine if any increase in bromine addition took place, it was advisable practically to complete the additive reaction before exposure. Under this condition any increase in iodine number will correspond to bromine which does not react with the fatty acids in the dark.

Experimental Results

As mentioned before, instead of reporting the results as the amount of bromine absorbed, the calculations are converted to the equivalent iodine number.

The results have been presented in the following tables. Table II comprises the results of the reaction between the fatty acids of poppy seed, soya bean, linseed and perilla oils with bromine in the dark for definite periods of time. Table III shows the results of the same fatty acids and bromine after standing in the dark for one hour and then being exposed to ultraviolet light for definite periods. Table IV gives the results for oleic, linolic and linolenic acids corresponding to Table II, and Table V corresponds to Table III with the same acids. The columns headed "Addit." are equivalent iodine numbers corresponding to the amount of additive bromine, and those headed "Subst." correspond to substitutive bromine.

It is interesting to note that the amount of substitution seems to corre-

spond to the amount of hydroxy acids present in linseed oil. If this assumption is correct, then hydroxy acids may be present in poppy seed and perilla oils.

The effect of the ultraviolet light seems to increase the energy of the reacting mixture so that more bromine is absorbed by the fatty acids than under normal conditions.

TABLE II
REACTIONS IN THE DARK

Time of reaction	Poppy seed acids I no., 135.5		Soya bean acids I no., 137.0		Linseed acids I no., 186.4		Perilla acids I no., 197.1	
	Addit.	Subst.	Addit.	Subst.	Addit.	Subst.	Addit.	Subst.
5 min.	126.5	2.9	129.7	0.0	161.6	2.9	161.1	0.7
10 min.	126.5	4.4	129.7	.0	162.7	4.4	164.0	1.4
20 min.	127.1	4.4	131.0	.0	164.4	4.4	165.8	1.4
30 min.	124.0	8.8	131.0	.0	165.1	4.4	171.0	2.9
1 hour	124.7	7.5	131.6	.0	166.2	4.4	184.5	4.4
24 hours	127.2	8.8	134.0	.0	174.4	4.4	191.4	8.4
48 hours	128.4	8.8	134.4	.0	176.3	5.1	195.4	8.8

TABLE III

REACTION IN THE DARK FOR ONE HOUR; THEN EXPOSED TO ULTRAVIOLETE LIGHT

Time of reaction	Poppy seed acids I no., 135.5		Soya bean acids I no., 137.0		Linseed acids I no., 186.4		Perilla acids I no., 197.1	
	Addit.	Subst.	Addit.	Subst.	Addit.	Subst.	Addit.	Subst.
5 min.	140.8	7.5	142.0	0.0	185.0	4.9	193.7	7.4
15 min.	151.0	8.8	144.7	.0	198.2	4.9	206.6	7.4
30 min.	167.1	10.3	151.3	.0	204.1	7.8	218.2	7.4
1 hour	166.3	12.6	161.0	.0	210.3	17.5	224.7	7.4

TABLE IV

REACTIONS IN THE DARK

Time of reaction	Oleic acid I no., 86.0		Linolic acid I no., 184.0		Linolenic acid I no., 222.8	
	Addit.	Subst.	Addit.	Subst.	Addit.	Subst.
5 min.	89.5	0.0	177.8	0.0	209.8	0.0
10 min.	89.5	.0	179.5	0.0	209.0	.0
20 min.	90.3	.0	181.0	.0	210.9	.0
30 min.	90.7	.0	184.5	.0	214.4	1.2
1 hour	185.2	.0	214.4	1.2
24 hours	201.0	3.4	221.4	2.1

TABLE V

REACTION IN THE DARK FOR ONE HOUR; THEN EXPOSED TO ULTRAVIOLETE LIGHT

Time of reaction	Oleic acid I no., 86.0		Linolic acid I no., 184.0		Linolenic acid I no., 222.8	
	Addit.	Subst.	Addit.	Subst.	Addit.	Subst.
5 min.	102.2	0.0	202.5	0.0	218.0	1.5
15 min.	102.5	.0	206.0	.0	220.0	3.7
30 min.	103.9	.0	222.1	.0	242.6	4.9

Discussion of Results

An examination of the results which are summarized in the tables indicates that in the case of the so-called pure oleic and linolic acids, there are

other substances present which react with bromine under the influence of ultraviolet light to yield the high values reported. The oleic acid used was the U. S. P. grade and might possibly contain some linolic acid. Likewise, linolic acid, which is usually prepared from poppy seed or soya bean oil, is also difficult to obtain pure. In fact, Pfahler⁷ claims that up to the present time pure linolic acid has not been prepared from an oil in a way analogous to Erdmann's method for linolenic acid. The high results with linolic acid may, therefore, be due partly to the presence of a stereo-isomer of linolenic acid which is not detectable by the hexabromide method of Eibner.

In the case of linolenic acid, which was prepared from hexabromolinolenic acid by de-bromination, according to the method of Erdmann, an iodine number lower than the theoretical is shown. Pfahler⁷ also states that the iodine number of acids prepared in this manner is always lower than the theoretical and that the low value is not due to impure acid. It is known that de-bromination in many cases causes stereomutation to forms which do not readily absorb halogen.

If this view is correct, then the acids should absorb more bromine under the influence of ultraviolet light. This has actually been accomplished, as will be seen from the results as given in tabular form. The abnormally high results for oleic and linolic acids beyond that assumed for the presence of other acids are as yet unexplainable, and work has been outlined, and is in progress, in an endeavor to explain these values. Linolenic acid, however, does not reach the theoretical value of absorption, even under the influence of ultraviolet light. It does show greater absorption than by the ordinary method, which indicates the presence of stereo-isomers.

The rate of bromine addition, in the dark, shows that oleic and linolic acids react more rapidly than linolenic acid. Oleic and linolic acids, therefore, should react more slowly toward oxygen than does linolenic acid, since compounds of this order which react slowly toward bromine do react more readily toward oxygen or hydroxyl. This is actually the case.

The results obtained on the mixed fatty acids of the different oils follow in a general way the results on the individual acids, with the further interesting observation that they all absorb more bromine under the influence of ultraviolet light than in the dark. This then indicated the presence of stereo-isomers which are not determined by present methods of analysis.

This same condition holds true for the other oils which are known to yield solid hexabromides under the ordinary treatment. The additional bromine absorbed means that, just as in poppy seed oil, there is another isomer present which does not react with bromine in the ordinary way.

⁷ Pfahler, *Chem. Umschau*, 33, 65-70 (1926).

The actual percentage of these isomers which are inactive toward bromine is approximately 10 to 15% in excess of the usual amount. In some cases the percentage may be lower than this. It is perhaps fortunate that these values are not high, because such substances in oils would be very energetic toward oxygen and thus cause too rapid drying of the oil.

Further evidence to support the authors' view of the presence of stereoisomers in linseed oil is the following.

Through the courtesy of H. A. Nelson and G. F. A. Stutz of the New Jersey Zinc Company, a sample of raw linseed oil was exposed to ultraviolet light in the presence of a dry carbon dioxide atmosphere for eighteen hours. This sample after exposure showed an iodine number of 148 and a hexabromide number of 35. This confirms the work of G. F. A. Stutz,⁸ who has shown a marked decrease in the iodine number of raw linseed oil after exposure to ultraviolet light in the presence of nitrogen gas.

However, when this sample of oil was brominated in the presence of ultraviolet light for thirty minutes, the total bromine absorbed was equivalent to an iodine number of 190, as against 180 for the original raw, unexposed oil.

In the first exposure of the raw oil in the presence of carbon dioxide, the ultraviolet rays caused a mutation of the stable isomers to labile isomers. This is in accordance with the views of Stoermer⁹ and others.

In the second exposure, where the pre-exposed oil was brominated, the action of the ultraviolet rays stimulates the addition of halogen to the isomers which were stereomuted in the first exposure. It is known that labile isomers react very slowly with halogen and, furthermore, they react easily with oxygen. This too was found to be the case, for the oil from the first exposure (iodine No. 148) dried more rapidly than the raw, unexposed oil.

It is apparent, therefore, that the ultraviolet rays in thirty minutes' time could hardly have changed the labile form to that which required eighteen hours to accomplish in the other direction, but that they simply stimulated the absorption of the halogen.

Our experiments so far indicate that the optimum condition for bromination under the influence of ultraviolet light is attained just at the point where substitution begins. A small substitution value is discernible in practically all glycerides which represents hydroxy acids, even before exposure, so that this value must be considered first before assuming substitution by ultraviolet light.

Conclusions

The rate of bromination of oleic, linolic and linolenic acids is in inverse ratio to the number of double bonds, that is, oleic acid reacts the most rapidly and linolenic acid the least.

⁸ Stutz, *Ind. Eng. Chem.*, **18**, 1238 (1926).

⁹ Stoermer, *Ann.*, **342**, 1 (1905).

The rate of bromination of the mixed fatty acids of poppy seed, soya bean, linseed and perilla oils is in direct relation to the acid constituents.

Under the influence of ultraviolet light the bromination of both the so-called pure acids and the mixed fatty acids proceeds beyond the bromination in the dark, which indicates the presence of unsaturated isomers which are not shown by present methods of analysis.

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THE CONSTITUTION OF ORTHO-NITROBENZALDEHYDE AND THE INTERFERENCE OF NITRO AND NITROSO GROUPS ON THE ZEREWITINOFF METHOD FOR THE QUANTITATIVE ESTIMATION OF ACTIVE HYDROGEN

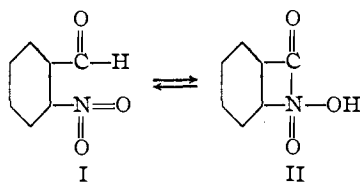
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Introduction

In connection with a study of the mechanism of the photochemical reactions of *o*-nitrobenzaldehyde and some of its condensation products, Tanasescu² has suggested recently that *o*-nitrobenzaldehyde is very probably an equilibrium mixture of the following forms:



The chief evidence brought forward in support of the cyclic hydroxy formula (II) is the quantity of methane evolved when *o*-nitrobenzaldehyde is treated with methylmagnesium iodide according to the method of Zerewitinoff.³ This quantitative method for the estimation of active hydrogens has been used very extensively and, on the whole, is eminently satisfactory.

However, Tanasescu is probably quite wrong in suggesting that *o*-nitrobenzaldehyde has the cyclic hydroxy formula (II) because the compound evolves methane when treated with methylmagnesium iodide. We do not say that a formula like (II) is impossible. If it is a likely formula it must find support in evidence other than that given by the Zerewiti-

¹ A preliminary report on this work was presented at Iowa City, May 7, 1927, before the Organic Division of the Iowa Academy of Science.

² Tanasescu, *Bull. soc. chim.*, 39, 1443 (1926).

³ Zerewitinoff, *Ber.*, 40, 2023 (1907), and numerous succeeding papers. The most recent reference is the *Z. anal. Chem.*, 68, 321-327 (1926) [*C. A.*, 21, 153 (1927)]. See also Chugaev, *Ber.*, 35, 3912 (1902), for earlier experimental work on the same method.