A STUDY OF
HYDROXYLATED FATTY ACIDS

by

WILLIAM HERMAN DAVIS

A Thesis

Presented to the Department of Biochemistry
and the Graduate Division of the University of Oregon
in partial fulfillment
of the requirements for the degree of
Master of Arts

May, 1936

APPROVED:

[redacted]

Major Adviser

[redacted]

For the Graduate Committee
of the College of Medicine

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I. Purpose of the Work

The original purpose of this work was to attempt to effect a complete separation of the ricinoleic acid from the other non-hydroxylated fatty acids of castor oil with the aim in view of obtaining a method of separating hydroxylated from non-hydroxylated fatty acids quantitatively for general use. The fatty acids of castor oil were chosen for study because of the large percentage of ricinoleic, a hydroxylated acid, combined in the esters of castor oil.

II. History of Ricinoleic Acid and Attempts at Purification

Ricinoleic acid was discovered by Bussy and Lecanu and given its name by Saalmuller (2). Its formula $C_{18}H_{34}O_3$ was established by Bouis. The crude acid, obtained by saponification of the oil is a very viscous oil of specific gravity 0.9509 at 15.5° C., solidifying at -6°C. to -10° C. The pure acid melts at 4-5° C. It is completely miscible with alcohol and ether. It decomposes on distillation even at low pressures. Its decomposition products on distillation are undecylic acid and oenanthylaldehyde. The acid displays optical activity, having an optical isomeride. It is believed that the acid undergoes polymerization on standing. The acid absorbs two atoms of halogen at its double bond.

The insolubility of hydroxy-acids in petroleum ether would afford, it would logically seem, a means of separation of hydroxylated fatty acids. But this rule does not apply. The mixed fatty acids of castor oil are just as soluble in petroleum ether as is the oil itself--soluble in equal volumes of petroleum ether.

Bussy and Lecanu (9), credited with the discovery of ricinoleic acid, 1857, obtained glycerine and two acids by the saponification of castor oil, one a liquid, one a solid melting at 130° C.

Saalmuller, (9) saponifying with alkali, followed by liberation of the free fatty acids by excess mineral acid, dissolved the recovered acids in alcohol, then cooled to -10° C., the solid acids crystallizing out. These were separated by filtration. An acid identical to Bussy's solid acid, melting at 70° C. was recovered, which seemed similar to palmitic, except that it had a higher melting point.

Saalmuller called the liquid acid ricinoleic. His purification process was to treat with ammonia, then treating the ammonium salt thus formed with barium chloride, producing the barium salt. The barium salt was crystallized from alcohol and decomposition with hydrochloric acid left ricinoleic acid, which was syrupy in consistency, almost colorless in appearance. It was oxidizable on exposure to the air. He established the formula as $C_{33}H_{36}O_6$.

Hossenkampf (9) prepared the acid by fractional precipitation of the sodium and potassium soaps with calcium chloride. In the first fraction calcium salts of the solid acids precipitated. The next fraction contained supposedly pure calcium ricinoleate.

Krafft (9) 1888 prepared the acids of castor oil, then cooled them to 0° C. The acids melting as the temperature was gradually raised were pressed out. A solid fraction melting at room temperature was thought to be pure ricinoleic acid. He analyzed the recovered acid for elemental

composition, comparing to theoretical with the following results:

			theoretical
C:	72.37%	72.45%	72.48%
H:	11.51%	11.58%	11.41%

Analysis of the prepared silver salt gave the following results:

			theoretical
C:	53.28%		53.33%
H:	11.51%		8.15%
Ag:	26.54%	26.49%	22.66%

The barium salt gave the following composition:

			theoretical
Ba:	18.65%	18.78%	18.74%

Juillard (13) 1895, showed that Krafft's method gave a product containing stearic and dihydroxystearic acids. Juillard attempted to purify the ricinoleic acid by filtering the crude acids at ordinary temperature to remove the insoluble fatty acids, followed by the preparation of the barium soaps, purified by recrystallization from alcohol. Ricinoleic acid is very soluble in alcohol at 50°C., but the barium stearate and barium dihydroxystearate and barium oleate are insoluble and separate out. By cooling the solution to 5°C. the barium ricinoleate separates out.

Haller (13) 1902 alcoholized castor oil and fractionated the resulting ester.

Myddleton, Berchem, and Barrett (13) 1927, prepared ricinoleic acid from castor oil by separating the lead soaps

of the stearic and dihydroxystearic acids by their insolubility in ether. The ricinoleic and other unsaturated acids were recovered from their soluble lead soaps and either converted into lithium salts and fractionally crystallised or into methyl esters and fractionally distilled.

Halvorsen (6) 1925, and Kozlowski (8) 1926, purified ricinoleic acid for bacteriological research. The acids were prepared by saponification, allowed to stand for two weeks, then decanted from the sediment formed. The sodium soap is then formed. Barium chloride is added and the ricinoleate converted to the barium salt. This was separated by filtration, treated with alcohol. The contaminating oleate, stearate, and dihydroxystearate were removed by keeping the alcohol at 50°C. when the ricinoleate stayed in solution. On cooling to 5° C. the barium ricinoleate was recovered. The alcohol treatment was repeated thus obtaining the ricinoleate in a much purer state.

The pure acid is obtained by treating the soap with 10% hydrochloric acid. The advantage of the treatment with hydrochloric acid is that the barium is recovered as chloride and can be used again without purification. Then the fatty acid is treated with 10% sulfuric acid to remove traces of barium, being followed by centrifugation to remove the precipitated barium sulfate. The soap that was then prepared by action of sodium hydroxide had the theoretical iodine value for sodium ricinoleate. T.H. Rider in his review of the

attempts previous to his own to obtain ricinoleic acid pure has explained this result as not necessarily a criterion of purity since a mixture of contaminating saturated acid and one more unsaturated than ricinoleic might result in a mixture having the iodine value of ricinoleic acid. Fahrion and Panjutin, and Rappoport (13) in 1930 described methods for the purification of castor oil by extraction with petroleum ether.

T.H. Rider (13) in 1931 found on the basis of the fact that a 1% solution of sodium ricinoleate clouds on standing in the cold if contaminated by saturated fatty acids that none of the earlier attempts to obtain pure ricinoleic acid had succeeded in their preparation, in freeing their product of these contaminating factors, not even the lead soap method giving a complete separation.

The solid acids appear to precipitate quantitatively by this treatment in the cold, but the liquid acids become so viscous as to make filtration impossible. Rider found that an alcoholic solution of the castor oil fatty acids, on chilling precipitates the solid acids quantitatively and the remaining liquor could be filtered. The soap solutions prepared from these fatty acids remained clear even when frozen.

Rider separated the solid stearic acid from the dihydroxystearic acid by addition of ether, the former being soluble. The dihydroxystearic acid obtained by chilling the

ether solution was crude. A solid compound, not showing the presence of many carboxyl groups, remaining undissolved on the addition of the ether, on saponification and re-acidification gave an equal weight of dihydroxystearic acid. This was evidently an anhydride of dihydroxystearic acid formed during the chilling in alcoholic solution. The ratio of dihydroxystearic to stearic acid was 2:1, indicating approximate concentration of 1.7 and 0.8% in the castor oil.

The stearic and dihydroxystearic acids having been removed by the above method the ricinoleic acid remains contaminated by oleic and linolic acids.

The methyl esters of ricinoleic, oleic, and linolic acids boil at approximately the same temperature (225, 212, and 208° at 10 mm. Hg. respectively) so that methylation followed by fractionation does not give satisfactory separation. If acetylation follows methylation, however, the boiling point of the methyl acetyl ricinoleate is raised about 35 degrees, so that by a single distillation Rider claims the ricinoleic acid can be obtained in the pure state. He prepared chemically pure ricinoleic acid in this manner having the theoretical iodine and acetyl values.

The research staff of the Department of Biochemistry at the University of Oregon Medical School undertook to check this work. (1935--Hafner). The acids were obtained by saponification of the oil. After having been washed and

dried, the acids were methylated with absolute methyl alcohol, using dry hydrogen chloride as catalyst. After separation of the excess alcohol, washing, and drying of the acids, the methyl esters were treated with a mixture of acetic anhydride and pyridine (two volumes of each), left to stand for several days; the excess anhydride and acetic acid were distilled off, and the product fractionated under a fraction of a mm. Hg., on an oil bath. The higher boiling fraction was preserved, saponified with potash and the free acid, after washing with water and drying over sodium sulfate anhydrous was preserved. The determinations were run on the purified acid, favorable results in which would be considered criteria of purity--the acetyl value and the neutral equivalent. The method of determining acetyl values introduced by Professor West and co-workers (14) was employed. The neutral equivalent was determined by refluxing a weighed sample of acid to be studied with a measured amount of standard alcoholic potash, approximately 0.5 N. for 60 minutes on the water-steam bath. This method is believed to destroy any internal esters which may be formed of the acids. 5 cc. butyl alcohol are added to prevent hydrolysis of the soap and the mixture is titrated with standard acid from which procedure the neutral equivalent is calculated according to the following formula:

$$\frac{\text{weight sample} \times 1000}{\text{cc. alkali} \times \text{N. alk.}}$$

The results of the above procedure are as follows:

	Acetyl Value	Theoretical
First fraction: (Chiefly contaminants)	124.8	144
	124.5	
	124.9	
Second fraction: (Purest)	135.8	
	135.1	
	135.0	
	Neutral Equivalent	
Second fraction:	307	298
	307	
	312	
	312	

These results indicated that the method does not give the sharp separation of the ricinoleic acid from the other fatty acids that it purports to. Evidently the difference in boiling point between the methyl, acetyl ricinoleate and the other methyl esters was not sufficient to permit fractionation.

As a consequence we undertook to devise a procedure in which a larger molecule could be incorporated into the ricinoleic acid molecule which would very materially change the boiling point of that compound from that of the contaminating esters so that a separation could be effected. The linkage was to be through the hydroxyl group.

III. The Phthallylation Procedure

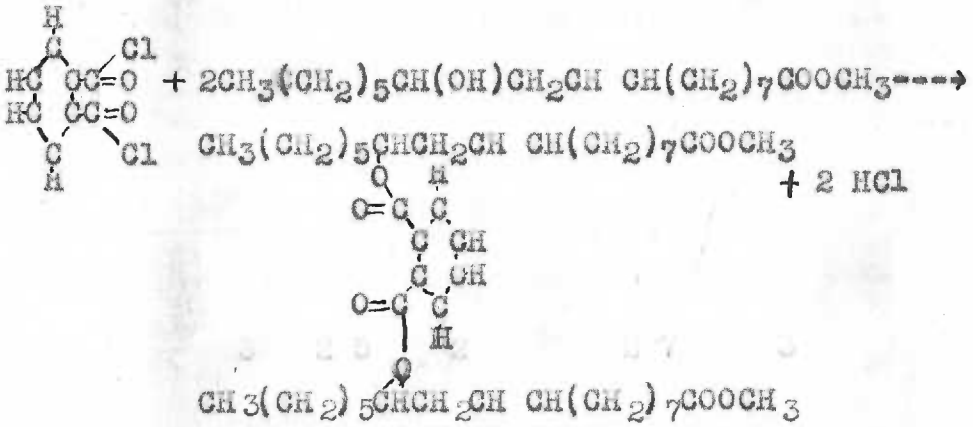
The acids, obtained by saponification of castor oil were methylated to prevent decomposition in the subsequent distillation. Then phthallyl chloride was used to react with two molecules of ricinoleic acid, producing a di-ricinolylphthallate, a large molecule. The methyl esters of the contaminating acids were to be separated by the subsequent fractional distillation, the phthallylated compound remaining behind. Then this compound was decomposed by saponification with alkali, the pure ricinoleic acid regenerated by addition of excess mineral acid, washed, dried, and tested for purity by the acetyl and neutral equivalent value determinations. A 200 gm. sample of castor oil was saponified, using 50 cc. of 68% sodium hydroxide solution in a liter of water. The separated soap was acidified with hydrochloric acid and the mixture of free fatty acids and aqueous solution was heated on the water bath to complete the separation of the fatty acid from the aqueous phase. The liberated fatty acids were then washed with warm water till free of mineral acid (neutral to litmus) , dried over sodium sulfate anhydrous, and separated by filtration.

The acetyl value and neutral equivalent value were determined at this time as a primary standard. The results:

Weight sample	Neutral Equivalent	Theoretical for Ricinoleic
0.6063 gm.	302	298
0.6075 gm.	302	
	Acetyl Value	
0.5867 gm.	128.8	144
0.6101 gm.	129.8	
0.6007 gm.	129.2	

The fatty acids prepared were mixed with 600 cc. anhydrous methyl alcohol, dry hydrogen chloride was passed in from a generator (sodium chloride plus sulfuric acid) and the mixture was refluxed for 12 hours on a water bath. The excess alcohol was removed by distillation, the residue was washed three times with equal volumes of water and dried with sodium sulfate, anhydrous. After separation the acetyl value of the ester was determined.

Phthallylation was the next step. The reaction to be effected was:



Because of the immiscibility of pyridine and phthallyl chloride, it was necessary to use an additional solvent, chloroform. The pyridine was retained to neutralize the hydrochloric acid liberated in the reaction, and thus exert a mass action effect.

50 gm. of the methyl esters were mixed with 100 cc. pyridine and 200 cc. dry chloroform in the reaction vessel. A stirring apparatus was connected and 30 gm. (22 cc.) phthallyl chloride in 30 cc. dry chloroform allowed to drop into the flask from a dropping funnel, the reaction flask being immersed in an ice bath, and stirred vigorously. 50 minutes were required for addition of the phthallyl chloride. Stirring was continued for 70 minutes with the bath still in place (½ gallon). After about 3/4 of the chloride had been added, the solution became a deep red color (phthallyl pyridium chloride?).

The mixture was stirred in the bath for three hours, and, following removal of the bath, for two hours more at room temperature. No heat was evolved, apparently. After three hours the excess phthallyl chloride was decomposed with 50 cc. cold water. The mixture was left over night.

The mixture was placed in a separatory funnel, the excess pyridine separated, and the remaining solution washed with hydrochloric acid several times to rid of pyridine. The product was then washed several times with distilled

water till neutral to litmus. The product was dried with sodium sulfate anhydrous for 30 minutes, then filtered, transferred to a distilling flask, and the chloroform was distilled off at atmospheric, and finally at reduced pressure. The product was pale yellow, showing that the red colored substance was not the product sought.

An oil bath was then used and the product distilled. The pressure was reduced with the vacuum pump to 1.5 mm Hg., the temperature of the bath rose to 220° C., and the distillate was obtained at 160° C. It was a yellow liquid which deposited crystals in the condenser tube. Solubility determinations, physical properties, etc. of the crystalline substance showed it to be phthallic acid. The residue in the flask was very dark-colored. The apparatus used was specially constructed to have few connections, and the condensing tube was made short. No jacket was employed.

In the next procedure, the same quantities of ester and reagent were used, but the mixture was left in the bath only three hours and 58 minutes were required for the addition of the reagent mixture. After washing with hydrochloric acid to remove pyridine, and with cold water to remove hydrochloric acid, the mixture was washed with water on the hot plate to remove phthallic acid. The product was dried over sodium sulfate as before and extracted with chloroform, after the removal of which solvent the lower boiling fraction

was distilled at a pressure of 0.6 mm. Hg.

The distillate was treated with 15 cc. of 60% potassium hydroxide and heated on the water bath for about two hours. The acids were regenerated by addition of excess hydrochloric acid, and were washed several times with boiling water, first to remove mineral acid, and second, to remove any contaminating phthallic acid (till neutral to litmus). The acids were extracted with chloroform, dried over sodium sulfate, and the chloroform removed by distillation, the acids being preserved in the vacuum desiccator. A neutral equivalent and acetyl value determination were run on the regenerated acids remaining behind, which theoretically were phthallylated hydroxy-acid, regenerated and treated as outlined above for the acids of the distillate.

methyl ester:

Weight sample	Acetyl value:	
0.5487 gm.	113.5	
0.5618 gm.	113.8	
0.5718 gm.	114.8	
	Free regenerated acid:	
0.6293 gm.	146.0	Theoretical for ricinoleic acid:
0.5982 gm.	144.9	
0.6383 gm.	144.9	144
	Neutral equivalent:	
0.6290 gm.	283	
0.6157 gm.	288	298

It was conjectured that the explanation for the low neutral equivalent value lay in the impurity of the phthallyl chloride reagent used.

A new batch of fatty acids was prepared from castor oil giving the following chemical constants:

Weight sample	Neutral equivalent
0.6412 gm.	327
0.6433 gm.	---
	Acetyl value
0.6559 gm.	124.1
0.6571 gm.	124.4
0.6697 gm.	124.5

The next procedure consisted of phthallylating the castor oil fatty acids directly, not following methylation. 25 gm. of acids were mixed with 100 cc. chloroform as before and 50 cc. pyridine, the mixture was placed in the reaction flask, mechanical stirring was attached, and the flask was immersed in an ice bath. 15 gm. of phthallyl chloride were added in chloroform dropwise. The mixture was stirred for 3 hours, then washed, dried, etc. as before. The product was preserved in the vacuum desiccator, for use for attempting to separate the phthallylated from the unphthallylated by solubility methods.

Another sample of acids was phthallylated directly as above. The product resulting from phthallylation was

treated by methylation as before. A precipitate formed at the beginning of the procedure, which dissolved on heating. The excess alcohol was distilled off, the product was treated as in earlier procedures, and the low fraction boiled at 160° C. at 0.7 mm. Hg. No phthallic anhydride came over in this procedure. The acids from both distillate and residue were regenerated after saponification, washed, dried, and preserved. Acetyl and neutral equivalent values were run on both.

Acids of distillate:

Weight sample	Neutral equivalent	
		Theoretical for mixed acids other than ricinoleic
0.6491 gm.	283.2	
0.6477 gm.	282.0	
		283
	Acetyl Value	
1.0775 gm.	126.8	
0.9856 gm.	128.7	
1.0234 gm.	127.9	0

Acids of residue:

	Neutral equivalent	
		Theoretical ricinoleic.
0.5594 gm.	250.5	
0.5904 gm.	254.8	298
	Acetyl value	
0.7885 gm.	165.0	
0.7597 gm.	165.4	
0.8492 gm.	164.3	144

It was thought that an impurity was introduced by the phthallyl chloride, creating the high acetyl value. The low neutral equivalent of the ricinoleic fraction was thought to be explainable by the presence of phthallic acid.

The next procedure was a repetition of the methylation, followed by phthallylation with the acid chloride. Extreme precautions were taken to wash out contaminating pathallic acid from the regenerated acids following the separation by distillation. Acetyl values and neutral equivalents:

Acids of distillate:

Weight sample	Neutral Equivalent	Theoretical for acids other than ricinoleic
0.4182 gm.	293.7	
0.4513 gm.	290.9	283
	Acetyl value	
0.8440 gm.	77.3	
1.1560 gm.	----	0

Acids of residue:

Weight sample	Neutral equivalent	Theoretical for ricinoleic
0.6691 gm.	211.8	
0.6595 gm.	218.7	298
	Acetyl value	
1.0489 gm.	177.3	
1.0319 gm.	177.8	
1.0933 gm.	177.4	144

It was decided to attempt to wash out the phthallic acid with hot water due to the appreciable solubility of that compound in water at 100° C., and its practical insolubility at room temperature. A new piece of apparatus was devised for this, the "separatory flask"--a flask with a long crooked spout leading out from the bottom. The advantage was that the flask could be heated on a hot plate, and subsequent complete separation was easily accomplished.

A 50 gm. sample of fatty acids of castor oil was methylated as before, and phthallylated. The washing was done by means of the separatory flask, and the wash water was boiling each time just before separation. The product was distilled fractionally as before after drying. The unphthallylated fraction distilled at 155° C. at a pressure of 0.7 mm. Hg. The chemical constants:

Acids of distillate:

Weight sample	Neutral equivalent	Theoretical for acids other than ricinoleic
0.8053 gm.	282.9	
0.7769 gm.	285.7	283
	Acetyl value	
0.9894 gm.	98.2	
1.0130 gm.	99.0	
1.1051 gm.	98.9	0

Acids of residue:

Weight sample	Neutral equivalent	Theoretical for ricinoleic
0.8053 gm.	239.4	
0.7769 gm.	243.3	298
	Acetyl value	
1.2679 gm.	157.5	
1.1046 gm.	159.4	
1.1426 gm.	159.0	144

The explanation for the results was that the separation from the hydroxy-acids of the non-hydroxy-acids was incomplete, contamination of the residue by phthallic acid, and possibly addition of hydroxyl groups by the reagent.

Another determination was run using a 50 gm. sample the procedure varying only in that the mixture was made alkaline with dilute sodium bicarbonate solution after treatment with hydrochloric acid to remove pyridine, to remove any phthallic acid into its salt.

It was believed that possibly the phthallic acid forming at the higher temperatures was the result of oxidation. To prevent this all subsequent distillations were carried out in a medium of carbon dioxide from a Kipp generator. By a capillary tube leading to the bottom of the distilling flask and control clamps a fine stream of carbon dioxide was maintained even at the lowest pressures at which distillation was carried out. The carbon dioxide was first

passed through ethylene glycol. All rubber-to-glass contacts were sealed with yellow shellac. With the use of this bit of tecnique, phthallic anhydride still was formed during the distillation.

The acids of the distillate as well as those of the residue were exhaustively washed in the separatory flask (one liter) fifteen times each with boiling water. The chemical constants on this sample showed a rise in the neutral equivalent of the distillate acids possibly to be explained on more satisfactory removal of phthallic acid, but the hydroxyl values remained high also.

Acids of the distillate:

Weight sample	Neutral equivalent	Theoretical for acids other than ricinoleic
0.5925 gm.	250.9	
0.6017 gm.	250.6	283
	Acetyl Value	
1.0779 gm.	144.7	
1.0970 gm.	145.1	0

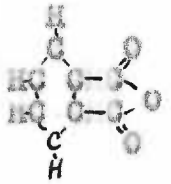
Acids of the residue

Weight sample	Neutral equivalent	Theoretical for ricinoleic
0.7046 gm.	296.6	
0.6615 gm.	264.1	298

Weight sample	Acetyl value	Theoretical for ricinoleic
1.0527 gm.	154.5	
1.1139 gm.	154.2	144

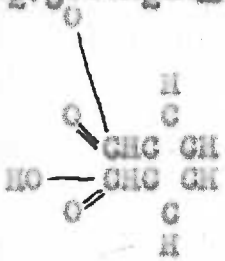
The color of the residual acids from this last run were much lighter than from any earlier procedure, likely due to the use of the reducing atmosphere.

The next attempt was to esterify the ricinoleic-methyl ester with phthallic anhydride, which could be obtained pure. Only one molecule of ricinoleate would react with the anhydride, but it was thought that the resulting molecule would be large enough to permit a satisfactory quantitative separation. The reaction:



$+ \quad CH_3(CH_2)_5CH(OH)CH_2CH{=}CH(CH_2)_7COOCH_3 \longrightarrow$

$CH_3(CH_2)_5CHCH_2CH{=}CH(CH_2)_7COOCH_3$



50 gm. of mixed methyl esters were mixed with 100 cc. pyridine and 50 gm. phthallic anhydride (50% excess) in a refluxing flask. Chloroform was not needed for homogenization. The mixture was refluxed for five hours. The pyridine was then removed with several treatments of water and

then with dilute hydroxhloric acid. The excess phthallic acid was washed out with several treatments of boiling water, the phthallylated esters (as well as the unphthallylated) extracted with chloroform, dried, and preserved. The resulting product was re-methylated in order to esterify the free carboxyl formed in the reaction (which see). The resulting mixture was fractionally distilled, removing the chloroform first under atmospheric and finally under reduced pressure. The apparatus was evacuated to 0.55 mm. Hg., carbon dioxide was passed in, and the low boiling fraction, distilling at 105 deg. was collected in an ice bath. The distillate and residue were separately saponified with 60% potassium hydroxide, the acids were regenerated, followed by repeated washings with boiling water, dried over sodium sulfate, and preserved. The chemical constants:

Acids of distillate:

Weight sample	Neutral equivalent	Theoretical for acids other than ricinoleic
0.7134 gm.	296.6	
0.6471 gm.	300.1	283
	Acetyl value	
1.0202 gm.	90.5	
1.1114 gm.	90.0	0

Acids of residue:

Weight sample	Neutral equivalent	Theoretical for ricinoleic
0.6732 gm.	249.6	
0.6520 gm.	250.6	298
	Acetyl value	
0.7656 gm.	140.9	
0.6440 gm.	132.4	
0.6134 gm.	-----	144

The reason ascribed for the low neutral equivalent was contamination with phthalic acid. To attempt to remove this contaminant, the recovered acid from the residue was treated with 60% potassium hydroxide, forming the soap, the acid was regenerated, and the product was successively washed a dozen times. After extraction, drying, removal of the solvent, etc. the neutral equivalent and acetyl values were determined, giving the following results:

Weight sample	Neutral equivalent	Theoretical for ricinoleic
0.5850 gm.	294.6	
0.4854 gm.	291.6	298
	Acetyl value	
0.6665 gm.	145.1	
0.5824 gm.	144.6	
0.7674 gm.	143.1	144

These values are the closest to theoretical yet obtained. This was the clearest looking residue yet obtained also. It was attempted to duplicate this procedure, but rather than to methylate both before and after, it was undertaken to phthallylate first, then to methylate both the ricinolyl acid group and the carboxyl formed in the phthallyl radical before distillation. A 50 gm. sample of fatty acids of castor oil was so treated. In the distillation, the low fraction boiled at 150° C. Saponification constants:

Acids of distillate:

Weight sample	Neutral equivalent	Theoretical for acids other than ricinoleic
0.5619 gm.	288.0	
0.6033 gm.	298.4	283

Acids of residue:

Weight sample	Neutral equivalent	Theoretical for ricinoleic
0.5447 gm.	246.0	
0.5255 gm.	243.7	298
	Acetyl value	
0.7684 gm.	152.6	
0.5430 gm.	151.1	
0.5927 gm.	151.2	144

The procedure was very unsatisfactory. It was impossible to obtain a pressure less than one mm. Hg. at the boiling point of the low fraction. Decomposition

was very apparent. Much phthallic anhydride distilled over. It must have been a decomposition product as there could have been no free phthallic acid in the mixture as methylation was the last procedure. It seemed that the order of treatment did make a difference in the procedure. It was planned, therefore, after trying another method which had been conceived, to repeat the treatment with phthallic anhydride, by methylating, phthallylating, and then remethylating.

This next procedure employed naphthalic anhydride, a homologue of phthallic anhydride. The castor oil fatty acids were naphthylated directly. 25 gm. of acids and 50 gm. reagent in 200 cc. pyridine were used. The mixture, placed on the hot plate, was very heterogeneous at first. Very little anhydride went into solution. After about an hour's refluxing, however, the reagent went into solution almost completely, and the refluxing went on smoothly for 5 hours. On cooling, much of the solid began to separate. So the mixture was re-heated, and, following homogenization, the pyridine was distilled off. The solid product was treated with chloroform to extract fatty matter. The the chloroform extract was treated with petroleum ether. This latter extract was dried over sodium sulfate anhydrous. The solvent was evaporated off, and the fatty substance distilled under a carbon dioxide atmosphere of 0.6 mm. Hg. The low-boiling fraction, distilling at 145° C.

was found to comprise all of the distillate. Hence, it was assumed that no naphthalation took place in the initial procedure.

The distillate, however, was believed to consist of unchanged methyl esters of castor oil fatty acids. These were treated with 25 gm. of phthallic anhydride in 100 cc. pyridine and refluxed on the hot plate for 5 hours. The product, after removal of pyridine was washed with several treatments of boiling water, followed after drying, by methylation. Fractional distillation was then done. The low-boiling fraction was collected at 0.6 mm Hg. (carbon dioxide atmosphere) at 140° C. After separate saponification, recovery, and drying, etc. both samples were placed in the vacuum desiccator. The acids of the residue showed a thick crystalline deposit assumed to be phthallic acid. After rewashing the acids 12 times more with boiling water to remove this contaminant, the crystalline product still was present in the acid after drying and evacuation to 0.88 mm. Hg.

The next procedure was the repetition of the phthallylation followed by methylation, but as before, on attempted distillation, much phthallic anhydride distilled over, and no separation was effected.

Our next procedure was to carry out Rider's procedure

of methylation and acetylation of acids purified by our phthallylation method. Two batches of our recovered acid with acetyl values 158.8 and 154.4 were treated with 2 volumes of acetic anhydride. The mixture was refluxed for 5 hours. Then the excess acetic anhydride and acetic acid were distilled off on the oil bath, the pressure was reduced to 0.75 mm. Hg., and the product distilled off. It boiled at 153° C. A light amber distillate was collected, amounting to about half of the original volume of the acid used. The apparatus was then re-evacuated, the pressure was reduced to 0.7 mm. Hg., and the second half of the distillate was collected, boiling at 147° C. It was believed that the second distillate would tend to be purer. The separate fractions were saponified on the water bath with 60% potassium hydroxide, the acids were regenerated, and were washed repeatedly with boiling water. The acids were extracted, dried, and preserved. Neutral equivalents and acetyl values were determined:

Weight sample	Neutral equivalent		Theoretical for ricinoleic
0.4808 gm.	1st. Fraction:	276.0	
0.5602 gm.		278.3	298
0.6060 gm.	2nd. Fraction:	284.7	
0.5180 gm.		286.6	

Weight sample	Acetyl value		Theoretical for ricinoleic
0.5854 gm.	1st. fraction:	149.0	
0.4910 gm.		149.0	144
0.5618 gm.	2nd. fraction:	147.5	
0.4932 gm.		149.2	

36 grams of pure acid were recovered from the 60 grams of crude acid.

IV. The Use of Other Reagents

The purpose of the next experiment was to attempt to tie one or more molecules of ricinoleic acid to the phosphate radical through the hydroxyl group through the use of phosphorus trichloride. The reaction:

$$POCl_3 + CH_3(CH_2)_5CH(OH)CH_2CH{=}CH(CH_2)_7COOCH_3 \longrightarrow$$

$$CH_3(CH_2)_5CHCH_2CH{=}CH(CH_2)_7COOCH_3$$

$$\Big|$$

$$HO-\overset{\overset{O}{\|}}{P}-OH$$

$$O$$

Approximately 25 gm. of the methyl esters were mixed with 75 cc. pyridine. The phosphorus trichloride was mixed with an equal volume of pyridine and placed in the reaction flask. The flask was immersed in an ice-salt bath, a stirring mechanism was attached, and the ester in pyridine was added dropwise. Two hours later, 50 cc. of water were added to decompose the excess phosphorus trichloride. 25 gm. barium carbonate were added (40% excess) to the reaction mixture. The mixture was shaken till no further gas pressure was developed, and the reaction was neutral. The precipitate was separated by centrifugation, the supernatant fluid separated, and the residue washed three times with dioxane. The combined washings and original solution were heated on the water bath to remove the solvents at reduced pressure. The remaining solute (should be non-hydroxylated

fatty acid esters were washed in water, extracted with chloroform, dried over sodium sulfate anhydrous, and the chloroform was removed by distillation. The chemical constants of the recovered acids from the precipitate:

Weight sample	Neutral equivalent	Theoretical for ricinoleic
0.4143 gm.	175.2	298
0.5011 gm.	176.3	
	Acetyl value	
0.5368 gm.	212.2	144
0.5267 gm.	213.6	

The next trial involved the use of butyric anhydride. 12.5 gm. of methylated castor oil acids were mixed with two parts by weight of butyric anhydride and 50 cc. pyridine, and the mixture was refluxed for seven hours on the hot plate. Then the excess butyric anhydride, butyric acid, and pyridine were distilled off at atmospheric pressure and finally at reduced pressure (0.6 mm. Hg.) A fraction boiling at 140-195° C. at this pressure was collected, and another boiling at 160-163° C. Both fractions were separately saponified with 65% sodium hydroxide and 15 cc. water on the hot bath for five hours, after which time the acids were regenerated, washed with water four times, and dried over sodium sulfate, anhydrous. The chemical constants:

	Weight sample	Neutral equivalent	Theoretical for acids other than ricinoleic
Sample 1:	0.3919 gm.	248.0	
	0.3893 gm.	258.5	283
		Acetyl value	
	0.3830 gm.	133.5	
	0.4325 gm.	130.8	
	0.4107 gm.	130.3	0

Sample 2:		Neutral equivalent	Theoretical for ricinoleic
	0.4050 gm.	213.3	
	0.4320 gm.	213.1	298
		Acetyl value	
	0.4090 gm.	152.6	
	0.4190 gm.	149.0	
	0.4480 gm.	149.0	144

It is naturally to be thought that the separation of the ricinoleic fraction into the second fraction was not complete, from the high acetyl value of the first sample.

The neutral equivalents of the two samples were redetermined, since it was thought that the values obtained were due to a technical error:

	Weight sample	Neutral equivalent	Theoretical for acids other than ricinoleic
Sample 1:	0.2634 gm.	284.0	
	0.2338 gm.	281.1	283

	Weight sample	Neutral equivalent	Theoretical for ricinoleic
Sample 2:	0.1788 gm.	293.2	
	0.2392 gm.	289.4	298

In the next procedure, the butyration procedure was repeated. Three fractions were separated by distillation as follows:

The first boiling at 178-194° C. at 1.5 mm. Hg.

The second boiling at 173-177° C. at 0.85 mm. Hg.

The third boiling at 170-180° C. at 1.2 mm. Hg.

Determination of physical constants gave acetyl values of approximately 10 in the first two fractions. It was thought that this showed the procedure to be very faulty.

Benzoylation was next attempted. Benzoyl chloride was used as benzoylating agent. 5 gm. of castor oil fatty acids were mixed with 200 cc. of one normal sodium hydroxide and the mixture was kept agitated by a mechanical stirring device. 8 cc. (200 % excess) of benzoyl chloride were added dropwise into the mixture. The reaction vessel was in a bath at 5-6° C. during the addition of the benzoylating agent, which required 10 minutes. The mixture was diluted with a liter of water. Excess acid was added till the mixture was acid to Congo paper. A heavy white precipitate formed which was believed to be benzoic acid. This was soluble in hot water. A small amount of yellow oily substance floated on the liquid, about equal in volume to the fatty acids started with. The oily substance was

washed four times in hot water, separated, taken up in ethyl ether, dried over sodium sulfate anhydrous, and the ether was taken off on a hot water bath. A yellowish oily substance, liquid at 0° C. remained. The acetyl value of the recovered substance:

Weight sample	Acetyl value	Theoretical for ricinoleic
0.3964 gm.	82.4	
0.3908 gm.	83.0	144
	Neutral equivalent	
0.3819 gm.	288.2	
0.3698 gm.	284.1	298

The last treatment undertaken to separate the ricinoleic fraction from the other fatty acids of castor oil involved the use of propionic anhydride, after the method previously used for the homologues of propionic anhydride. The mixture was refluxed over an air blanket on a hot plate for eight hours. After distillation of the solvents the product was fractionally distilled, and three fractions were collected boiling at the following temperatures under the stated conditions:

Fraction	Bath Temp.	Distilling Temp.	Pressure	Vol. Collected
I	190-198° C.	130-140° C.	1.2 mm.	42 cc.
II	193-200° C.	135-155° C.	0.95mm.	25 cc.
III	200-203° C.	140-160° C.	1.00mm.	15 cc.

The acids were regenerated by saponification followed by acidification, washed, and dried as in other procedures, and the physical constants were determined. All results on all fractions gave values which were of absolutely no significance and which lent emphasis to the conclusion that the method was unsatisfactory. This experiment terminated the work on the attempted separation of the ricinoleic from the other acids of castor oil.

Of the work on castor oil discussed up to this point, one set of results is encouraging. The first run employing phthallic anhydride as phthallylating agent, in which the fatty acid mixture was methylated, then phthallylated, and then remethylated gave both neutral equivalent and acetyl values closely approximating the theoretical values for ricinoleic acid. This sample was washed approximately twice as many times as other samples.

We feel that these results lend significance to the methylating, phthallylating, and remethylating procedure with phthallic anhydride under the conditions which we employed. We feel that with improved methods of washing, by means of which virtually all of the contaminating phthallic acid may be removed, this procedure may be expected to be helpful in bringing about the quantitative separation of the ricinoleic acid from the other acids of castor oil.

V. Auto-oxidation of Linseed Oil and its Acids--Introduction

We next turned our attention to a study of the rate of auto-oxidation of linseed oil and its acids, largely because we felt that work of this kind is important, little is as yet known about it, and it ties in with the study we had carried out up to this time.

Aas (1) reports the results of a study of the auto-oxidation of oils. During the early stage of the reactions he says the decrease in the iodine value is not accompanied by a corresponding increase in the molecular weight. The weight passes through a maximum and then starts to drop. He determined the number of oxygen atoms absorbed per double bond. He gives the following values:

Cod liver oil:	1.85-1.97	
Linseed oil:	1.81	(uncertain)
Oleic acid :	3.06	
Olive oil :	3.65-3.86	

Ellis (5) studied the auto-oxidation of elaidic, oleic, and stearic acids. He says that in a mono-ene the oxygen uptake is negligible at ordinary temperatures, but that it is possible to accelerate the oxidation of oleic and elaidic acids by the use of a catalyst and increase of temperature. But above 75° C. even stearic acid is oxidized. Hence it is necessary to stay below 75° C.

He found that oleic acid plus cobalt oleate (1%) at summer temperatures reduced the iodine value in a short time. The product was an oil contaminated by oleic acid. Hence elaidic acid was used. Elaidic acid is obtainable in the crystalline state, and does not absorb oxygen in the crystalline state, but does absorb oxygen slightly above its melting point.

He mixed elaidic acid in solution with cobalt elaidate and circulated oxygen through it. The results were not so good as when the oil was finely dispersed. The most satisfactory method, he found, was to disperse the elaidic acid on fine sand--one part acid to 80 parts sand. The amount of oxygen used was recorded on a drum.

He found that without the use of a catalyst there is an induction period lasting 1-2 days below 55° C., disappearing at 70° C. The period is reduced if the acids are heated to 70° C. and then cooled to 55° C. The rate of oxidation increases with time in the absence of a catalyst. Hence, it is auto-catalytic. With a catalyst as cobalt, the rate falls from the start. 20 gm. of acid oxidized at 74° C. absorbs 300 cc. of oxygen per hour. He found a high initial rate of oxidation and then a uniform rate for several days.

He found that the amount of oxygen absorbed was always in excess of that required for the initial oxidation process.

He experimentally determined the amount of unoxidized acid remaining when the experiment stopped and so arrived at the amount of oxygen used in the primary process. 20% by weight of oxygen was absorbed by the oxidized part of the acid. If 3 atoms of oxygen were used in the oxidation, the oxygen uptake would be 17%.

Stearic acid melts at 69° C. Therefore it was necessary to work above this temperature with this acid. Without a catalyst, stearic acid was found to take up only insignificant amounts of oxygen, but with cobalt stearate the oxidation was considerable. There was much difference in the rate at 75° C. and 80° C. But longer periods were required. Large amounts of water and carbon dioxide were found which suggested degradation of the chain.

Hamilton and Olcott (7) studied the auto-oxidation of oleic acid, methyl oleate, and oleyl alcohol. They believe that one molecule of oxygen is absorbed at the double bond. The nature of the compound is uncertain--peroxides, aldehydes, hydroxides, water and carbon dioxide occur.

The amount of oxygen absorbed was measured and the amount of carbon dioxide and water evolved measured by the increase in weight of ascarite and dehydrite. Aldehydes, hydroxyl, acid value, were determined by analysis of weighed samples taken from the original sample. The oleic acid was prepared from olive oil. After saponification the soaps

were washed with ether to remove unsaponifiable. The acids were subjected to a lead salt precipitation to remove the unsaturated acids. The data were computed on a molar basis. Iodine numbers were used to study the rate of destruction of double bonds. The moles of double-bonds destroyed per mole of original substance and of peroxide, water, carbon dioxide, aldehyde, hydroxide, free carboxyl, total carboxyl, and oxygen absorption per molecule of original substance were calculated.

Neither dihydroxystearic nor oxido-oleic acid absorbed any appreciable amount of oxygen in the experiment. The iodine number was the same in samples of rancid oleic acid before and after removal of peroxides. Comparison of the reactions showed that oleic acid, methyl oleate, and oleyl alcohol did not react just alike. Oleyl alcohol absorbs five atoms of oxygen per molecule. Oleic acid and methyl oleate absorb four atoms per molecule. Methyl oleate and oleyl alcohol produce two times as much peroxide as oleic acid. Oleic acid liberates two to four times that liberated by oleyl alcohol or methyl oleate.

There may be a reaction between a formed peroxide group and the free carboxyl group of oleic acid in which carboxyl is destroyed and hydroxyl formed. They believe that aldehydes are transient products.

They found that carbon dioxide production occurs throughout. The ratio of carbon dioxide per mole dou-

ble bond destroyed increases as the oxidation proceeds. It was at most only 0.5 molecule per molecule double bond destroyed, however.

The parallelism between the double bond destroyed, water formed, and the peroxides suggested to them that one molecule of unsaturated compound reacts with two molecules of oxygen to give one molecule of water and a peroxide-containing group.

VI. The Auto-oxidation of Linseed Oil and the Acids of Linseed Oil

Our experiments consisted of allowing in one case linseed oil, in the other the acids obtained by the saponification of the oil to auto-oxidize at a fixed temperature by passing a steady stream of air through the reaction tube containing the oil or acids. At definite intervals after starting the procedure, approximately ten gram samples of the oil or acids were withdrawn from the reaction tube. The samples taken during the run of the oil were saponified with 60% potassium hydroxide, the free acids were regenerated, washed with water several times, dried over sodium sulfate, anhydrous, and preserved in the cold. Samples of the determination run on the free acids were washed and dried directly. The rate of auto-oxidation was followed by iodine value, neutral equivalent value, and acetyl value determinations run on the material used at the outset and on each sample withdrawn.

The apparatus consisted of a large (approximately three gallon) copper vessel with an outer water jacket of copper. In this was immersed a tube approximately 18" long and 2" in diameter filled about 1/3 with glass wool and up to this level with distilled water. The reaction tube was of 12" length and 1½" diameter. This was immersed in the

bath about 2" beyond the upper level of the oil or acids. A glass tube, connected with a source of air under slight, fixed pressure passed through a rubber stopper to within 1 cm. of the bottom of the first tube. A second glass tube connected the top of the first tube with the reaction tube. The glass tubing connecting the two tubes ran to within one cm. of the bottom of the reaction tube. A third piece of glass tubing permitted egress of air from the reaction tube through the rubber stopper. Both tubes were well stoppered. The first (water-containing) tube was used as a humidifier.

The bath was kept at a constant temperature by an ingenious thermostatic device the use of which was explained in Science magazine. It was assembled by another laboratory worker for use in his project, and we subsequently made use of the same apparatus. It consists of a glass bulb of approximately 10 cm. diameter which is immersed in the bath. This bulb connects with a vertically-directed piece of glass tubing which is sealed by a mercury trap. A glass bead rides on the mercury. Within this tube is a smaller tube the end of which is completely shut off when the glass bead, riding on the mercury is pressed against the opening in the end of the inner tube. City gas enters from the jet by rubber tubing into the outer tubing above the mercury seal. A tiny pore is made in the inner tube permitting a very small amount of city gas to enter the inner tube when

the bead is compressed by the mercury, permitting a very tiny flame to continue, the inner tube being connected by rubber tubing to the gas burner under the constant temperature bath. The end of the inner tube at the outlet is sealed into the outer tube, keeping the system air tight. When the mercury seal is broken, city gas entering the outer tubing all passes into the inner tubing and thus to the burner, which then burns brightly, heating the constant temperature bath. The seal is set to just compress the bead, and cut down the gas flowing to the burner at the temperature at which the bath is to be held. When the temperature falls approximately ½ degree C. below this optimum, due to contraction of the gases in the bulb the seal is broken, and the burner burns more brightly. When the temperature exceeds the optimum by approximately ½ degree C., the seal compresses the bead against the end of the inner tube, and the burner flame becomes very low, permitting the bath to cool very slightly. In this manner, through the additional use of an electric stirring machine, the temperature of the bath was kept constant within the limits which will be discussed below.

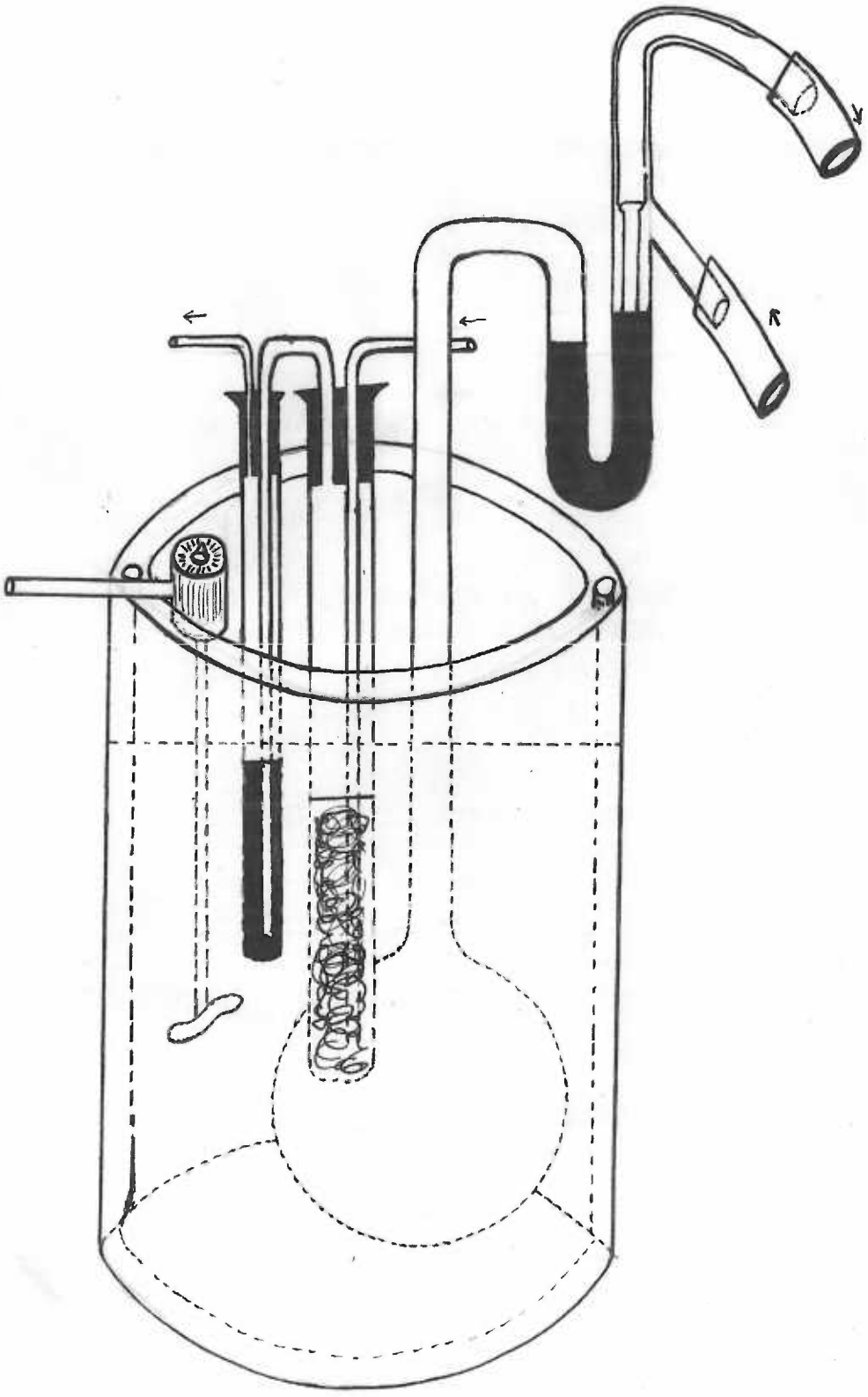


Diagram of auto-oxidation apparatus.

The acetyl and neutral equivalent values were run as in the earlier work. The iodine value was done according to the method of Rosenmund and Kuhnheim, as modified by Yasuda (15). The reagent used is pyridine sulfate dibromide. Measured quantities are run into glass-stoppered flasks containing approximately 50 mg. of the sample weighed to 0.1 mg. The reaction is permitted to go on at room temperature for 30 minutes, after which the bromide in the excess reagent is made to react with potassium iodide added in excess (5 cc. of 10% solution) liberating an equivalent quantity of iodine which is determined by standard thiosulfate. From this by subtraction from the average of triplicate blank titrations, the iodine value is determined. The accuracy of the method was first shown by running the determinations on triplicate samples of olive oil in which experiments values for olive oil lying within the limits of the theoretical were determined. The original workers found that the excess of halogen used had no effect on the values obtained when working with the acids of linseed oil. Up to 1330% excess had no untoward effect.

In the first run, the acids of linseed oil, prepared in the same way as the acids of castor oil had been prepared were studied. 75 cc. of the combined acids were placed in the reaction tube. The bath was maintained at 50-51° C. Samples were taken at 1,2,3,4,5,6,7,8,10, 13,

24, 34 hours after oxidation was started. The air passed through the humidifier tube and reaction tube at the same fixed rate throughout the experiment.

The second run consistcd of the same treatment of a 200 cc. sample of linseed oil. The bath was kept at 50-52° C. throughout, and the air flowed through the humidifier and reaction tube at the same fixed rate. It was attempted to make the rate identical in the two runs by counting the bubbles of air entering the reaction tube per unit time.

The constants determined on the samples taken showed the following results:

LINSEED OIL ACIDS---Oxidized at 50° C.

No. Hrs.	Neutral equivalent		Average	Acetyl Value			Average
0	322.3	318.3	320.3	4.6	3.4	4.0	4.0
1	332.9	344.4	338.7	2.6	2.5		2.6
2	331.3	333.5	334.4	1.7	2.1	2.4	2.1
3	338.1	341.2	339.7	1.9	2.0	2.6	2.1
4	332.7	329.2	331.0	1.5	2.0	2.0	1.9
5.	325.2	340.5	315.0	2.7	2.0	2.2	2.3
6	311.6	316.7	314.2	3.6	4.3	3.4	3.8
8	309.7	310.4	310.1	47.2	38.7		43.0
10	318.9	309.0	313.9	45.1	42.7	46.8	44.8

No. Hrs.	Neutral Equivalent		Average	Acetyl value			Average
13	294.3	298.6	296.5	55.0	67.6		61.3
24	253.8	234.8	244.3	115.4	120.2	111.5	115.7
32	212.7	209.6	211.2	162.5	158.0		160.3

Number Hours	Iodine Value			Average.
0	166.5	168.2		167.4
½	136.7	121.3	117.5	126.5
2.	148.7	145.8		147.3
3	140.6	139.3	133.7	137.9
4	151.3	151.4	144.1	148.9
5	151.7	148.3	145.6	148.5
6	155.4	146.2	139.1	146.9
8	141.8	134.6	134.2	134.4
10	115.8	119.2	123.9	119.6
13	131.2	129.1		130.1
24	109.7	99.6	106.9	102.1
32	99.2	94.9	101.5	98.5

It is to be pointed out that the neutral equivalent, acetyl, and iodine values remained practically constant during the first 6-8 hours of oxidation. Subsequent to that, the neutral equivalent started abruptly to reduce, suggestive of dissolution of the molecule. At the same

time, the acetyl value started to rise rapidly, which suggests that the molecule was becoming hydroxylated as it was being broken up. The iodine value dropped at a rapid rate at the same time, showing a comparable decrease in unsaturation as the increase in hydroxylation continued. This set of conditions prevailed as long as we carried out the experiment. From this run we conclude that at this temperature, the oil is slow in starting auto-oxidation, but once under way, it progresses rapidly, and is characterized by a dissolution of the molecule (falling neutral equivalent), hydroxylation (increasing acetyl value), and decrease in unsaturation (decrease in iodine value).

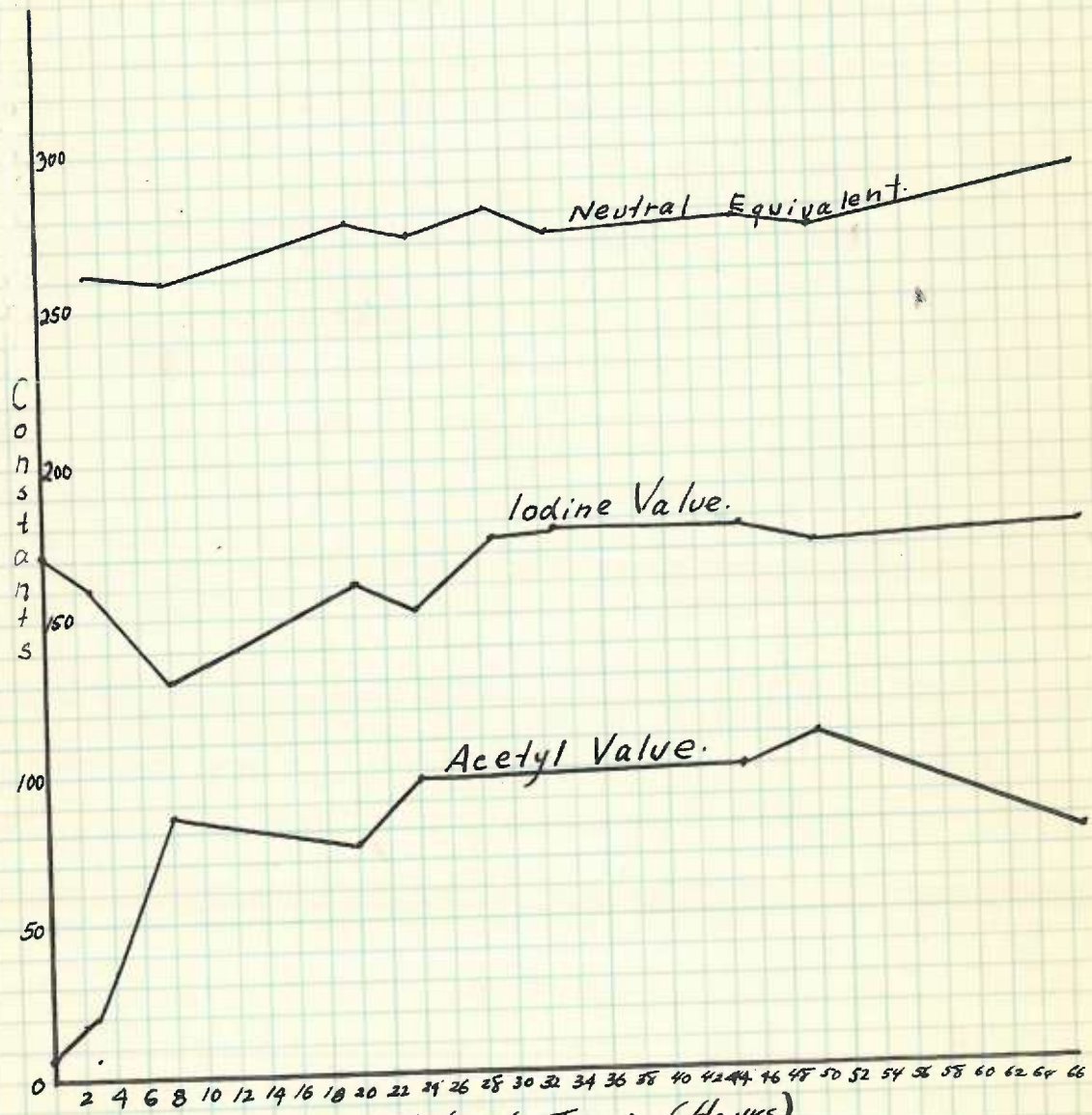
LINSEED OIL---Oxidized at 49-52° C.

No. Hrs.	Neutral equivalent		Average	Acetyl value			Average
0	199.9	202.4	(sapon. val)	6.5	7.1	6.8	6.8
3	257.3	265.2	261.1	20.7	18.5	17.6	19.0
8	263.0	256.9	259.0		85.1	85.8	85.5
20	277.2	278.0	277.6	74.0	78.7	76.3	76.3
24	271.2	275.1	273.1	98.7	95.9		97.0
29	282.6	279.8	281.0				
33	271.1	273.8	272.4				
45	276.6	277.3	272.4	100.0	99.6	99.9	99.8
50	273.1	273.5	273.3	108.8	112.2	109.7	110.0
67	296.2	285.9	291.1	76.5		76.4	76.4

No. Hrs.	Iodine Value			Average
0	169.6	173.2	171.0	171.8
3	158.7	161.3		160.1
8	129.3	132.1	127.8	130.0
20	160.6	161.5		161.0
24	152.7	154.2	150.4	152.7
29	175.9	170.2	173.3	173.0
33	180.1	171.9	175.6	175.6
45	179.4	176.7	172.5	176.0
50	175.6	169.0	170.2	171.5
67	175.2	173.0		174.1

Constants.

Linseed Oil -- Oxidized at 49-52°C.



This reaction was carried on for 35 hours longer than the first one. It will be noted that the reaction started more abruptly, with a rapid drop in the iodine value and rise in the acetyl value. After eight hours, however, the iodine value started to rise again, indicative of increased unsaturation, and at the same time the acetyl value dropped correspondingly. After 20 hours the iodine value, acetyl value and neutral equivalent remained stationary, followed by a slight rise in acetyl value and drop in iodine and neutral equivalent values after 45 hours, and a terminal lowering of the acetyl value and rise of the neutral equivalent and iodine value.

In this run, after the initial oxidation, there was very little change of the molecule as is indicated by the slight and corresponding changes in all three chemical constants. It is to be concluded, it seems, that in these two series, the acids were slower to start auto-oxidation, but, once started, continued progressively, whereas the oil started more abruptly, and then soon practically all auto-oxidation ceased.

This terminated our work on hydroxylated fatty acids.

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Typed by: William H. Gavin