

# RESEARCH PAPERS

## STUDIES OF RICINOLEIC ACID AND A TURBIDIMETRIC METHOD OF EVALUATING THE BACTERICIDAL ACTION OF SOLUTIONS OF PHENOLS IN POTASSIUM RICINOLEATE

PART I. THE EFFECTS OF HEAT AND AGEING UPON RICINOLEIC ACID

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THE object of these studies was threefold: (1) To trace the effects of heat on the acid value of samples of the fatty acids, obtained by hydrolysis of castor oil, and known as Ricinoleic Acid B.P. (2) To ascertain the value of a nephelometric method of evaluating bactericidal values, from the point of view of the reproducibility of results. (3) The application of this method to investigate the effects of using ricinoleic acids of different acid values to solubilise insoluble phenols in potassium ricinoleate solutions.

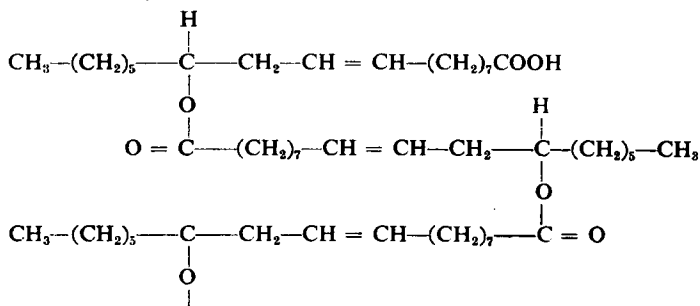
### INTRODUCTION.

The objects of the work described in Part I were to prepare a ricinoleic acid of high acid value, to trace the effects of heats and ageing on this acid, to investigate the relationship between refractive index and acid value, and to prepare a series of potassium soaps of ricinoleic acid for further investigations. Work by Maquenne<sup>1</sup>, Goldsobel<sup>2</sup>, and Vernon and Ross<sup>3</sup> has established the formula of ricinoleic acid as



Several workers, Koszowski<sup>4</sup>, Rider<sup>5</sup>, and Valette and Salvant<sup>6</sup>, and Brown and Green<sup>7</sup> have described attempts to obtain a pure sample of the acid. The latter two workers concluded that even by using a pure methyl ester it was difficult to obtain an acid of more than 95 per cent. purity. They attributed this difficulty to the ease with which ricinoleic acid forms polyricinoleic acids.

This formation of polyricinoleic acid has been described by Chowdhury *et al.*<sup>8</sup>, Jakes and Hökl<sup>9</sup>, and Esafor and Shpadi<sup>10</sup> and the explanation is that the COOH group of one molecule reacts with the OH group of another molecule to give a chain-like formation of from 2 to 6 molecules of ricinoleic acid, i.e.



As a result of the formation of these poly-acids or estolides the acid value of ricinoleic acid will be lower than that of a pure ricinoleic acid. This estolide formation would also explain the variation of acid value in commercial samples of ricinoleic acid and the drop in acid value which occurs when samples of ricinoleic acid are stored. Esafor and Shpadi<sup>10</sup> reported that these estolides were formed when ricinoleic acid was heated at under 200°C., but that prolonged or more vigorous heating resulted in the breakdown of the estolides and the formation of other polymerisation and thermal degradation products.

## EXPERIMENTAL

### 1. PREPARATION OF A RICINOLEIC ACID OF HIGH ACID VALUE

Since heat promotes the formation of estolides of ricinoleic acid a method of preparing the acid in the cold was attempted. The method adopted was the saponification of castor oil to give potassium ricinoleate and the conversion of this soap to ricinoleic acid with hydrochloric acid. A supply of castor oil was stored in a refrigerator and from time to time samples of this oil were taken and filtered free of any solid particles and used in the experiments.

A refrigeration temperature of 4°C., however, introduced a complication in that at such a temperature the acid formed a semi-solid from which the last traces of moisture were difficult to remove. The solubilising power of the soap (Berry and Bean<sup>11</sup>) also presented a difficulty in that, if alcoholic potassium hydroxide was used in the preparation, some of the alcohol would be solubilised by the soap and find its way into the final acid from which it would be difficult to remove except by the use of heat. This solubilising effect also hindered the removal of the unsaponifiable matter since any solvent used in its extraction would also be solubilised by the soap.

The acidification of the soap with hydrochloric acid in the cold resulted in a small portion of the soap remaining unchanged, with the result that the process of washing the acid free of chloride produced extremely stable emulsions from which the acid was difficult to separate.

The scheme evolved was to prepare acids in the cold (a) making no effort to remove the unsaponifiable matter, using aqueous potassium hydroxide (b) in a similar manner using aqueous and alcoholic potassium hydroxide and removing the unsaponifiable matter, and then to repeat the experiments using a minimum amount of heat.

Acid values were determined by the method of the British Pharmacopœia 1932.

(a) *Cold Aqueous Potassium Hydroxide; Unsaponifiable Matter not Removed.* 6 g. of potassium of hydroxide was dissolved in 75 ml. of distilled water and mixed with 25 g. of castor oil; the mixture was stored at 4°C. and shaken frequently. Even after 6 months' storage at 4°C. a considerable portion of the oil remained unsaponified in this mixture. A portion of the mixture, after 3 weeks' storage, was filtered through a filter paper wetted with distilled water, which served to remove the majority of the unsaponified oil and gave a filtrate with only slight opalescence. The filtrate was acidified with hydrochloric acid, thoroughly

shaken and left for 48 hours for the acid to separate. This mixture separated into 3 distinct layers, a clear amber layer of acid, a layer of thick white emulsion and a lower aqueous layer. The acid layer was separated off and washed with distilled water until the washings were free from chloride. An emulsion layer formed at each washing, and considerable amounts of acid were lost in discarding this layer each time. The acid was dried by gentle heat under reduced pressure. Acid value of product, 168.6.

(b) *Cold Aqueous Potassium Hydroxide; Unsaponifiable Matter Removed.* A portion (25 ml.) of the soap solution from experiment (a) was placed in a separator, 25 ml. of ether were added and the majority of this was solubilised by the soap so a further 25 ml. were added, after shaking and allowing to stand the mixture separated into 3 layers and the emulsion layer was discarded with the ethereal extract, further portions of ether were added to complete the extraction of the unsaponifiable matter. The ether solubilised by the soap solution was removed by gentle warming under reduced pressure. Addition of hydrochloric acid converted the soap to acid which was washed with water and dried with anhydrous sodium sulphate. Acid value of product, 166.5.

(c) *Cold Alcoholic Potassium Hydroxide; Unsaponifiable Matter Removed.* 25 g. of castor oil was mixed with 75 ml. of 95 per cent. alcohol in which 6 g. of potassium hydroxide was dissolved, and the mixture was left at 4°C., and shaken frequently, for 7 days. The unsaponifiable portion was extracted with light petroleum (this being less soluble in water than ether, separation of the layers was easier), 6 quantities, each of 25 ml., being used. The resulting soap solution was divided into 2 portions. The first was treated with hydrochloric acid and left at room temperature for several days, then the liberated ricinoleic acid was washed free of chloride and dried with anhydrous sodium sulphate. Acid value of product, 180.8.

The second portion was refluxed for 30 minutes with hydrochloric acid. The liberated ricinoleic acid was washed and dried at 100°C. under reduced pressure. Acid value of product, 174.7.

(d) *Hot Aqueous Potash; Unsaponifiable Matter not Removed.* 50 g. of castor oil was refluxed for 2 hours with 150 ml. of aqueous potassium hydroxide when saponification was shown to be complete by the absence of an oily layer. The resulting soap solution was divided into 2 equal portions. The first was acidified with hydrochloric acid and refluxed for 30 minutes. The resulting acid was separated, washed free of chloride and dried at 100°C. Acid value of product, 165.7.

The remainder of the soap solution was acidified with hydrochloric acid at 0°C. and kept at 4°C. for 7 days. The ricinoleic acid so formed was washed free of chloride and dried with anhydrous sodium sulphate. Acid value of product, 164.8.

(e) *Hot Alcoholic Potassium Hydroxide; Unsaponifiable Matter not Removed.* When alcoholic potassium hydroxide was used, refluxing for 30 minutes was sufficient to complete the saponification. As in experiment (d) the soap solution was divided into two portions, both

of which were converted to acid, one by 30 minutes' refluxing and the other in the cold. Acid value of acid obtained, by refluxing 181.6; in the cold 164.8.

An attempt was made to repeat these results. Acid value of acid obtained, by refluxing 157.9; in the cold 179.5.

(f) *Hot Alcoholic Potassium Hydroxide; Unsaponifiable Matter Removed.* A soap solution was prepared as above (e) and the unsaponifiable matter extracted with 6 lots of light petroleum. As in the previous experiments, half the soap solution was acidified with hydrochloric acid, refluxed for 30 minutes and then the liberated acid separated off, washed free of chloride and dried at 100°C.; the second half of the soap solution after acidification was left at room temperature for 7 days before the liberated acid was washed and dried with anhydrous sodium sulphate. Acid value of acid prepared, by refluxing 178.4; in the cold 177.8.

(g) *Summary of Acid Values.*

(i) *Cold Saponification.*

Aqueous potassium hydroxide, unsaponifiable matter not removed	...	...	...	...	...	...	168.6
Aqueous potassium hydroxide, unsaponifiable matter removed	...	...	...	...	...	...	166.6
Alcoholic potassium hydroxide, unsaponifiable matter removed:							
(a) cold acidification	...	...	...	...	...	...	180.8
(b) hot acidification	...	...	...	...	...	...	174.7

(ii) *Hot Saponification.*

Aqueous potassium hydroxide, unsaponifiable matter removed:							
(a) cold acidification	...	...	...	...	...	...	164.8
(b) hot acidification	...	...	...	...	...	...	165.7
Alcoholic potassium hydroxide, unsaponifiable matter not removed:							
(a) cold acidification	...	...	...	...	...	...	179.5
(b) hot acidification	...	...	...	...	...	...	157.9
Alcoholic potassium hydroxide, unsaponifiable matter removed:							
(a) cold acidification	...	...	...	...	...	...	177.8
(b) hot acidification	...	...	...	...	...	...	178.4

(h) *Preparation of Stock Potassium Ricinoleate Solution.* 250 g. of potassium hydroxide was dissolved in 3 l. of alcohol (90 per cent.) and 1000 g. of castor oil was added. This mixture was refluxed on a water-bath for 30 minutes. The unsaponifiable matter was removed from the soap solution with light petroleum (50° to 60°C.). The alcohol and solubilised light petroleum were removed from the soap solution by diluting it with an equal quantity of water and evaporating the resulting solution down to half its volume under reduced pressure. The resulting solution was made up to 5 l. to give an approximate 1 in 5 solution of potassium ricinoleate.

RICINOLEIC ACID. PART I

Over a period of 18 months, samples of this soap solution were taken, from time to time, and converted to ricinoleic acid by acidification with hydrochloric acid. The acid values of the acids so prepared were (a) 174.5, (b) 165.8, (c) 179.0, (d) 181.2, (e) 183.4, (f) 186.4. This latter sample was prepared by mixing the soap solution and hydrochloric acid at 0°C. and allowing the reaction to proceed at 4°C. for 14 days, with frequent shaking; after washing free from chloride it was dried by two separate treatments with anhydrous sodium sulphate.

2. EFFECTS ON ACID VALUE OF HEATING SAMPLES OF RICINOLEIC ACID AT 150°C. AND 160°C.

Samples of some of the acids prepared above were placed in open or closed containers and subjected to a temperature of 150°C. in a hot-air oven for varying lengths of time, cooled in a desiccator and the acid values determined immediately the samples were cool. One sample in an open container was heated in an autoclave and after cooling the condensed moisture was removed, the acid was dried and then its acid value determined.

Table I is a summary of results obtained : —

TABLE I  
EFFECT OF HEAT TREATMENT ON THE ACID VALUE OF RICINOLEIC ACID

Initial Acid Value	Heat Treatment			Final Acid Value
	Time (hr.)	Temperature °C.	Container	
174.5	1	150	Sealed	164.1
	3	150	Sealed	140.5
	1	150	Sealed in presence of anhydrous sodium sulphate	171.8
	3	150	Sealed in presence of anhydrous sodium sulphate	144.4
	7½	150	Sealed	133.4
	3	150	Open, in autoclave	172.7
	7½	150	Open	121.7
	8½	150	Open	93.5
	2	160	Open	137.5
	165.8	1	150	Open
2		150	Open	155.2
3		150	Open	153.3
4		150	Open	141.6
5		150	Open	128.7
6		150	Open	111.7
7		150	Open	94.9
186.4		4½	150	Open
	4¾	150	Open	152.1
	5½	150	Open	142.0
183.4	½	150	Open	184.5
	1	150	Open	182.6
	1½	150	Open	178.7
	2	150	Open	175.7
	2½	150	Open	170.2
	3	150	Open	168.0
	3½	150	Open	161.5
	4	150	Open	154.6
	4½	150	Open	150.3
	5	150	Open	144.3
	5½	160	Open	108.1

It was noted that a gradual darkening in colour accompanied the decrease in acid value of any one sample of original acid, although the acid of acid value 164.1 obtained by heating the acid of acid value 174.5

for 1 hour was considerably lighter in colour than the original acid of acid value 165.8.

### 3. EFFECTS OF AGEING ON ACID VALUE

Samples of some of the acids prepared above were stored in closed containers for 12 months or more at room temperature and then their acid values were determined. Table II is a summary of the results obtained.

TABLE II  
DECREASE OF ACID VALUE ON STORAGE

Initial Acid Value	Time Months	Final Acid Value	Decrease
174.4	19	158.0	16.4
164.2	18	147.0	17.2
165.8	12	152.4	13.4
156.8	12	144.9	11.9
155.2	12	143.7	11.5
153.3	12	149.4	3.9
141.6	12	132.1	9.5
128.5	12	120.1	8.4
111.7	12	104.8	6.9
94.9	12	92.8	2.0

The logarithms of the reductions in acid value occurring after 12 months' storage were plotted against the initial acid values and a graph showing close approximation to a straight line was obtained. The long

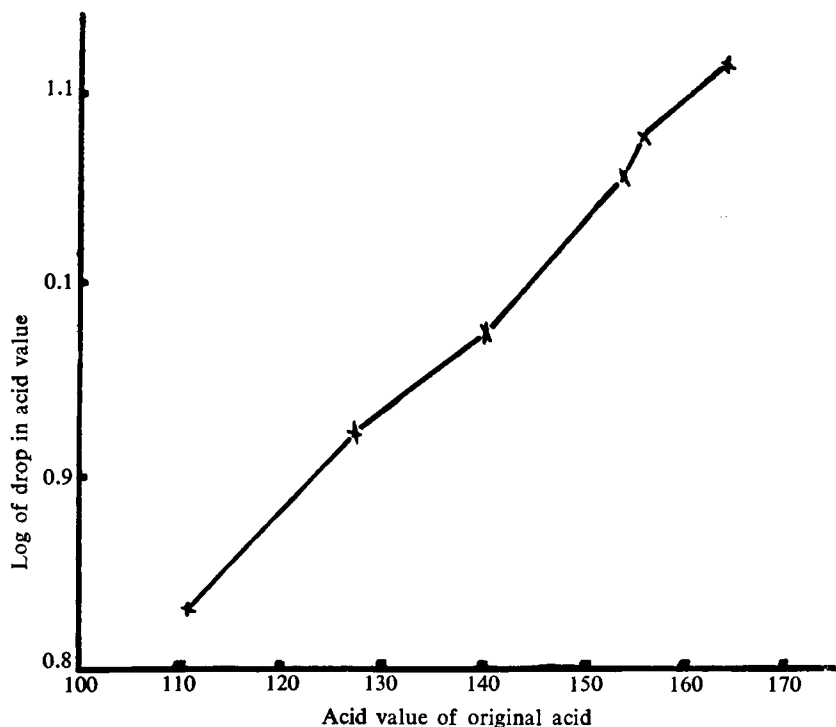


FIG. 1. Plot of logarithm of drop in acid value after 12 months' storage, against original acid value of ricinoleic acid.

RICINOLEIC ACID. PART I

period of storage, 12 months, made it difficult to obtain any confirmation of these results.

4. REFRACTIVE INDEX AND ACID VALUE

The British Pharmacopœia 1932 stipulates 40°C. as the temperature for the determination of the refractive index of ricinoleic acid; this temperature was therefore maintained for these measurements, which were made with an Abbé Refractometer. The refractive indices of three series of acids were measured. The acids of each series were obtained from the same original acid, the reduction of acid value had been brought about by:—Series I—heat treatment and 18 months' ageing; Series II—heat treatment and 12 months' ageing; Series III—heat treatment only. The refractive index and acid value for each acid were determined on the same day or within three days of each other. Table III summarises the results.

TABLE III  
THE RESULTS OF ACID VALUE AND REFRACTIVE INDEX DETERMINATIONS

Series I		Series II		Series III	
Acid Value	Refractive Index	Acid Value	Refractive Index	Acid Value	Refractive Index
160.9	1.4620	152.4	1.4623	183.4	1.4611
157.9	1.4611	149.4	1.4624	184.5	1.4614
147.0	1.4613	144.9	1.4621	182.6	1.4615
		143.7	1.4630	178.7	1.4619
		132.1	1.4650	175.1	1.4621
		120.1	1.4640	170.2	1.4624
		104.8	1.4649	168.0	1.4627
		92.9	1.4650	161.5	1.4630
				154.6	1.4630
				150.3	1.4634
				144.3	1.4639
				108.1	1.4656

Using the last two figures of the refractive indices in Series I and II as abscissæ and acid values as ordinates these results were plotted in Figure II.

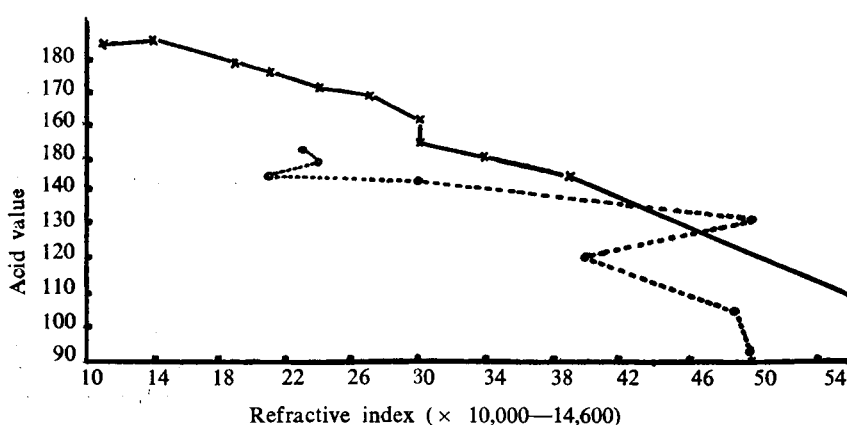


Fig. 2. Showing relationship between acid value and refractive index for 2 series of acids.

## DISCUSSION

Results showed that the preparation of a ricinoleic acid of high acid value from castor oil is possible. The complete saponification of the glycerides in the oil is best accomplished by the use of alcoholic potassium hydroxide, and there is no indication that moderate heating at this stage in any way affects the final acid value. The removal of unsaponifiable matter, difficult on account of the solubilising effects of the soap solution is recommended to ensure the production of an acid of high acid value, but it is not always essential. The soap was converted to ricinoleic acid by addition of hydrochloric acid; the acids of highest acid value (170 to 185) were obtained when this reaction was done in the cold and allowed at least 7 days at 4°C. for complete conversion. Emulsion difficulties were experienced and slight loss of acid resulted when the acid was washed free of chloride. It was possible to convert samples of potassium ricinoleate into ricinoleic acids of similar acid values to that of the original acid used to make the soap, even after the soap solution had been stored for over 12 months. The acid value appeared to depend upon the amount of heat used in converting the soap to acid.

The heating of ricinoleic acid at 150°C. in an open container resulted in a regular drop in acid value of something less than 10 units of acid value per hour, although slight difficulty was experienced in stopping this reduction at a level between acid values of 140 and 125. Heating at 160°C. produced a more rapid fall in acid value.

Twelve months' storage of several ricinoleic acids resulted in falls in acid value, the higher values suffering a greater drop than lower values. A straight line relationship was indicated when the logarithm of the decrease in acid value over 12 months was plotted against the acid value of the original acid. If this drop in acid value was due to the formation of estolides, then such a proportionate drop in acid value is to be expected as the concentration of individual molecules of ricinoleic acid available for estolide formation would be greater in the acids of higher acid values. The long time interval (12 months) did not permit of confirmation of the relationship found for one series of acids.

Results of refractive index measurement showed that the drop in acid value resulting from heat treatment was proportional to the increase in refractive index. A similar proportionality was not as clearly defined in the case of acids whose acid values had dropped through ageing. No similarity was found between the refractive indices of acids of the same acid value but prepared by different means.

## SUMMARY

1. A method of preparing a ricinoleic acid of high acid value from castor oil has been described and the desirability of not using heat in the conversion of the potassium ricinoleate to ricinoleic acid has been shown.
2. It has been shown that the storage of ricinoleate soap solutions does not result in any drop in acid value of the acid prepared from the soap.



## RICINOLEIC ACID. PART I

3. The drop in acid value of ricinoleic acid when heated at 150°C. has been traced.

4. The drop in acid value of ricinoleic acid when stored for 12 months has been shown to be proportional to the original acid value in the case of one series of acids.

5. It has been shown that the use of refractive index as a measurement of the acid value of ricinoleic acid has very restricted application.

This work forms part of a thesis submitted for the degree of Doctor of Philosophy in the University of London.

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