Reactions of Conjugated Fatty Acids. VIII. Dibasic Acids by Hydrogenation and Oxidative Cleavage [1,2]

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ALKALI ISOMERIZATION of linoleic acid produces a mixture of 9,11- and 10,12-octadecadienoic acids (6). Partial hydrogenation of these isomers should produce a mixture containing a large amount of monounsaturated acids with double bonds in the 9, 10, 11, and 12 positions. Such monounsaturated acids would be useful intermediates in the production of long-chain dibasic acids for use in plasticizers and synthetic lubricants. A study was made therefore of the hydrogenation of these conjugated isomers and the position of their double bonds in resulting products.

Because conjugated linoleic acid isomers are produced by heating in alkaline solution, hydrogenation of the conjugated soaps would eliminate the isolation of the highly unstable conjugated acids. Although traces of soap in glyceride oils are known to act as catalyst poisons during hydrogenation, it has been shown by Tsuchiya (8, 9) that aqueous fatty acid soap solutions can be reduced with nickel catalysts. Preliminary experiments at this laboratory indicated that, in an ethylene glycol solution under certain conditions, only the conjugated soaps are reduced and that the reaction stops at the monoene stage without appreciable formation of saturates. A more detailed study was made of the reduction in aqueous solution rather than in ethylene glycol since it was believed that aqueous solutions would be more suitable for large-scale reactions.

The position of the double bonds in the monounsaturated fatty acid products was determined by analytical oxidation, using permanganate-periodate to give dibasic acids of varying chain length. The amounts and chain length of these dibasic acids were compared with those obtained from the same monounsaturated fatty acids by ozonization and oxidative cleavage simulating commercial practice.

Experimental

Most of the experiments were performed with safflower fatty acids since these provide a readily available product, which is high in linoleic acid and quite low in more highly unsaturated acids. The hydrogenations were carried out in a 2-liter Parr medium pressure hydrogenator equipped with a gas-dispersing type of stirrer. Two commercial nickel catalysts were used: a Rufert catalyst containing 24.34% nickel manufactured by the Seymour Chemical Company and the G-15 catalyst manufactured by the Girdler Company consisting of 27% nickel on 9% kieselguhr in 64% of hardened soybean oil.

Hydrogenation in Ethylene Glycol. The safflower oil used in this hydrogenation was conjugated by heating with sodium hydroxide in an ethylene glycol solution. Acids prepared from a portion of the soaps had an absorptivity at 233 mμ of 72.1, indicating about 75% conjugation. The remaining soaps were diluted with ethylene glycol to give a solution containing soaps equivalent to 13.5 g. of safflower oil and 2.7 g. of sodium hydroxide in 250 ml. of solution. One gram of the Rufert catalyst was added, and the solution was heated to 144°C. under nitrogen. The hydrogenator was then flushed and filled with hydrogen to 25.8 p.s.i., and the solution was stirred for 35 min. at 144° to 156° although the pressure remained constant at about 17.6 p.s.i. after 20 min.

Acids were prepared by dilution of the solution with water, acidification with sulfuric acid, and extraction into petroleum ether. The petroleum ether solution was washed until neutral and dried over sodium sulfate, and the solvent was removed. The acids had an iodine value of 77.0 and absorptivity at 233 mμ of 0.71. This iodine value corresponds to 14% saturated acids, which is approximately the amount in the original safflower oil. This percentage and the constant hydrogen pressure at the end of the reaction suggest that hydrogenation stopped after reduction of the conjugated acids to monoenes.

Hydrogenation in Aqueous Solution. A series of hydrogenations in aqueous solution was carried out, using a concentrate of conjugated linoleic acid prepared in the following manner. Safflower fatty acids in hexane were cooled to −40° and filtered to remove the saturated acids. A product was obtained from the filtrate which had an iodine value of 164.8 and contained 81.6% linoleic acid and 17.8% oleic acid. A portion of this product designated as sample A was reserved for the work described later on conjugation and hydrogenation in aqueous solution. The remainder was conjugated by heating with sodium hydroxide in ethylene glycol for 1 hr. at 200°.

The conjugated acids designated as sample B were recovered and had an absorptivity at 233 mμ of 75.5. Using Nichol's (6) value of $a_{233} = 95$ for pure alkali conjugated linoleic acid, this absorptivity corresponds to 79.5% conjugated linoleic acid. Infrared analysis indicated that substantially all this conjugation was *cis,trans*. Saturated acids were measured by Nijkamp's (7) chromatographic procedure, following permanganate-periodate oxidation of the unsaturated acids (4, 5). No saturated acids were found in either sample A or B.

Sample B was hydrogenated under a variety of experimental conditions to determine within what range of conditions all the conjugated diene could be reduced to monoene without formation of saturates. A number of these hydrogenations are outlined in Table I. After hydrogenation was carried out as described in the table, the soap solution was acidified with dilute sulfuric acid, and the acids were extracted with petroleum ether. The catalyst usually appeared in a small amount of emulsion at the interface and was removed during the washing of sulfuric acid from the petroleum ether solution. Sometimes catalyst was still present after washing and evapo-

rating the solvent; it was then necessary to filter the acids through a filter aid to remove the catalyst.

Runs 1 and 2 are duplicate preparations made several months apart, using the standard conditions outlined in Table I. These two runs produced material of low conjugation with iodine values in the monoene range. Analysis indicated that these samples contained 3% saturated acids. About one-half of this amount would result from the hardened fat added with the catalyst. Analytical alkali isomerizations (3) indicated that 2.0% of unreacted linoleic acid was still present after hydrogenation. Variations from standard conditions were made in Runs 3 through 18 to test their effect upon the products.

Runs 3, 4, and 5 show that doubling the reaction time or changing the sample size had little effect on the products. Runs 6, 7, and 8 show that the temperature may be decreased to 100°, but a decrease to 75° or an increase to 160° results in a product which still has appreciable conjugation. Run 9 indicates that the reaction proceeds normally when a higher hydrogen pressure is used. Runs 10, 11, and 12 indicate that decreasing the alkali concentration or using potassium hydroxide instead of sodium hydroxide results in a lower iodine value and a greater formation of saturated acids. On the other hand, increasing the alkali concentration leaves a greater residual conjugation. Runs 13 and 14 with smaller amounts of catalyst resulted in high residual conjugation, but Run 15 with a larger amount of catalyst gave results similar to Runs 1 and 2. Raney nickel used in Run 16 is much less active than the Rufert catalyst. In Run 17 the G-15 catalyst was satisfactory. Several later runs not listed in Table I indicated however a somewhat lower activity for this catalyst, as illustrated by Run 18 where even with a 2-hr. reaction time the iodine value and conjugation are still somewhat high.

These data show that the hydrogenation of conjugated soaps can be carried out in aqueous alkaline solutions. Bradley and Richardson (2) have shown that the same conjugation of fatty acids can also be accomplished in aqueous solutions.

TABLE I

Effect of Varying Standard Conditions upon Hydrogenation of Conjugated Linoleic Acid Soaps

Run no.	Variation from standard conditions [a]	Analysis of product	
		a233	Iodine value
1	None	0.59	91.2
2	None	0.62	92.7
3	Reaction time, 2 hr.	0.31	89.7
4	Sample, 1 g.	2.55	89.2
5	Sample, 30 g.	0.17	89.5
6	Temperature 160°	7.8	97.2
7	Temperature 100°	0.45	89.7
8	Temperature 75°	28.8	....
9	Initial hydrogen pressure 100 p.s.i.	0.27	88.6
10	NaOH, 1.75 g.	0.19	77.1
11	NaOH, 21 g.	23.8	....
12	KOH instead of NaOH	0.34	81.6
13	0.1% Ni	64	....
14	0.2% Ni	27	....
15	1.95% Ni	0.60	89.6
16	8% Raney Ni used in place of Rufert catalyst	25.5	98.1
17	G-15 catalyst used in place of Rufert catalyst	0.81	91.3
18	G-15 catalyst used in place of Rufert catalyst (2-hr. reaction time)	7.7	94.7

[a] Standard conditions are 10 g. fatty acid Sample B and 7 g. NaOH made to 250 ml. with water; 0.5% Ni based on weight of sample added as Rufert catalyst; initial H₂ pressure 40 p.s.i. at room temperature; mixture stirred 1 hr. at 140° ± 10°.

TABLE II

Effect of Temperature upon Conjugation and Hydrogenation of Linoleic Acid Soaps

Run no.	Variations from standard conditions [a]	Analysis of product	
		a233	Iodine value
19	Sample was unconjugated linoleic acid concentrate, sample A. Reaction time 2 hr.	0.38	135.5
20	Sample was unconjugated linoleic acid concentrate, sample A. Reaction time 2 hr. Temperature 155°	0.81	122.3
21	Sample was unconjugated linoleic acid concentrate, sample A. Reaction time 2 hr. Temperature 200°	16.2	104.0
22	Catalyst heated 1 hr. at 200° in aqueous alkaline solution. After cooling conjugated linoleic acid concentrate, sample B, was added and processed under standard conditions	55.3	....
23	Same as Run 22 except G-15 catalyst was used	59.3	....
24	Unconjugated linoleic acid concentrate, sample A, heated 2 hr. at 200° without catalyst. After cooling, catalyst was added and sample processed under standard conditions	0.44	89.2

[a] See Table I for description of standard conditions.

As outlined in Table II, a series of experiments was made to determine if conditions could be found to effect isomerization and hydrogenation simultaneously. Although conditions suitable for simultaneous isomerization and hydrogenation were not found, stepwise isomerization and hydrogenation in the same solution were successful. All reactions were carried out according to the standard conditions except as noted.

Runs 19, 20, and 21 were attempts to produce simultaneous conjugation and hydrogenation. Run 19 was made, using the standard conditions except that the unconjugated fatty acid sample "A" was used and the reaction time was 2 hrs. At the standard temperature (140°) the over-all reaction depends upon the rate of conjugation. Although all the linoleic acid which was conjugated was also hydrogenated, the reaction was not complete because the rate of conjugation at that temperature was quite slow. This was also true of Run 20 at 155°.

At 200°, as in Run 21, conjugation proceeds rapidly. However the catalyst has been partially inactivated by the high temperature, and the reaction is incomplete because of a slower hydrogenation of the conjugated acids.

Runs 22 and 23 confirm that the catalyst is inactivated by heating at 200° in alkaline solution. In these runs the catalyst was first heated at 200° without the fatty acid sample. Conjugated acids were then added and carried through the hydrogenation procedure. Hydrogenation was incomplete, and a large amount of conjugation remained. These experiments show that simultaneous conjugation and reduction is impractical. However the solution may be conjugated at 200° as in Run 24; then the catalyst may be added, and the hydrogenation carried out at 140° in the same solution.

Dibasic acids were prepared from some of the samples in Table I by the procedures of Lemieux and von Rudloff (5) as modified by Jones and Stolp (4). Additional modifications were made in this procedure from time to time in an attempt to improve the total yield of dibasic acids. For this reason values are only comparable when calculated to show the relative amounts of dibasic acids. Total yield of dibasic acids

was usually from 80 to 90% of theory. For comparison the best values obtained with oleic acid were about 93% of theory. The dibasic acids obtained from the various runs were similar, and the differences are not believed to be significant. The average dibasic acid composition of 12 preparations from sample B is shown in Table III. The dibasic acid composition of Run 24 in which the linoleic acid concentrate A was conjugated in aqueous solution was quite similar. This composition is also shown in Table III.

TABLE III
Composition of Dibasic Acids Obtained by Hydrogenation and Oxidation

Sample	Composition of dibasic acids, %					
	C_{13}	C_{12}	C_{11}	C_{10}	C_9	C_8
A, run 24	1.3	14.5	24.7	26.8	29.9	2.8
B	1.9	14.3	25.4	26.5	29.1	2.8
B corrected for oleic acid	2.3	17.4	31.0	32.2	13.7	3.4
Linoleic acid, run 25	0.8	15.0	26.9	29.0	24.5	3.8
Run 25 corrected for unconjugated linoleic acid	0.9	16.7	29.9	32.3	16.0	4.2
Run 26, t,t-9,11-linoleic acid	0.4	1.7	27.9	38.0	29.0	3.0

Hydrogenation of Pure Acids. Several hydrogenations were made to determine the course of the reaction with pure acids. The data in Table IV are typical of those obtained when oleic acid soap is carried through the standard hydrogenation procedure. There is a decrease of about 10% in iodine value and a corresponding increase in the saturated acid content. There is also a small but definite shift in double bond position. Very little *trans* acids are formed.

In Run 25 a combined conjugation and hydrogenation of pure linoleic acid was carried out similar to Run 24 in Table II. To 6.64 g. of linoleic acid were added 8.47 g. of sodium hydroxide and 300 ml. of water. The reactor was flushed with nitrogen and heated 2 hrs. at 194° to 214° to conjugate the linoleic acid. Acids prepared from a portion of the solution had a_{233} of 80.0; infrared analysis indicated 75% *cis,trans* conjugation. To the remainder of the soap solution, 1% nickel as the Rufert catalyst was added. The reactor was filled with hydrogen to 40 p.s.i. and stirred for 1 hr. at 124° to 138°. The hydrogenated acids had an iodine value of 89.7 and $a_{233} = 1.2$. Saturated acid content was 4.4%. An analytical alkali isomerization (3) showed that 10.1% unreacted linoleic acid was still present after hydrogenation. Infrared analysis indicated 64.3% *trans* acids calculated as elaidic acid. The composition of the dibasic acids prepared by oxidation is shown in Table III.

Another hydrogenation, Run 26, was performed on *trans,trans*-9,11-linoleic acid. To 4.3 g. of the acid were added 7 g. of sodium hydroxide, 250 ml. of water, and 1% nickel as Rufert catalyst. The apparatus was filled with hydrogen to 40 p.s.i. and heated at 130° to 140° for 1 hr. The hydrogenated acids had an iodine value of 77.6 and a_{233} of 0.26, saturated acid content was 10.4%, and infrared analysis indicated 85% *trans* acids. Composition of the dibasic acids prepared by oxidation is shown in Table III.

Hydrogenation of Large Samples. While the described procedures are quite satisfactory for small samples, the processing of large amounts of acids would require inconveniently large amounts of solutions. In adapting the process to more concentrated soap solutions, certain changes, particularly in alkali concentration, are necessary.

The most satisfactory procedure used is this. To 250 g. of safflower oil in the hydrogenator, 60 g. of sodium hydroxide in 500 ml. of water are added. The apparatus is flushed with nitrogen and heated at 210° ± 10° for 2.5 hrs. The reactor is cooled, and catalyst containing 0.5% nickel, based on the weight of the oil, is added. Also 50 to 75 ml. of water are added to reduce the alkali concentration. This addition of water seems to result in slightly lower iodine value and residual conjugation of the hydrogenated acids. The hydrogenator is filled with hydrogen to 40 p.s.i. and heated to 130°. The total pressure is then about 100 p.s.i. Hydrogen is added to a total pressure of 180 to 200 p.s.i., and additional hydrogen is added as needed for the reaction. The mixture is stirred vigorously at 140° ± 10° for 1.5 hrs. although hydrogen uptake usually stops after about 1 hr.

After cooling, the soap solution is transferred to a beaker, acidified with sulfuric acid, warmed to 70° to melt the acids, and stirred. The aqueous layer is siphoned off, and the acids are washed with water. The acids at this stage are in a thick emulsion with water. They are transferred to a separatory funnel with ethyl ether. (Petroleum ether does not break the emulsion.) They are again shaken with dilute sulfuric acid, washed with water, and dried with sodium sulfate. The solvent is removed by evaporation.

Five 250-g. lots of safflower oil were processed by minor variations of this process with G-15 catalyst to give 1,080 g. of reduced acids. The analysis of the original safflower oil is iodine value 139.6, linolenic acid 0.2%, linoleic acid 72.7%, oleic acid 15.2%, and saturated acids by difference 11.5%. Analysis of the reduced acids is iodine value 87.0, $a_{233} = 2.9$. An analytical alkali isomerization showed that 6.2% linoleic acid was still present after hydrogenation.

Dibasic acids were prepared from a 425-g. lot of these acids by an ozonization process at Emery Industries. In Table V the composition of these acids is compared with that obtained by our analytical procedure. Also for comparison, the dibasic acid composition and saturated acid content of acids prepared by a selective hydrogenation of the safflower oil glycerides are included. In this hydrogenation the glycerides were reduced at 216° to 227° and 7 p.s.i. with 0.5% nickel as the G-15 catalyst to an iodine value of 86.7.

Soybean oil was conjugated and reduced in the same way as described for the safflower oil. Analysis of the original soybean oil is iodine value 135.0, linolenic acid 8.6%, linoleic acid 49.2%, oleic acid 31.7%, and saturated acids by difference 10.5%. Analysis of the reduced acids is iodine value 85.5, $a_{233} = 1.4$, $a_{268} = 0.07$. An analytical alkali isomerization showed that 4.4% linoleic acid was still present after isomerization. For comparison, soybean oil glycerides were selectively hydrogenated at 212° to 220° and 3 p.s.i. with 0.5% nickel. This hydrogenation was carried to an iodine value of 69.4. The dibasic acid compositions obtained by oxidation of these samples are shown in Table VI.

Preparation of Di-(2-Ethylhexyl) Esters of Dibasic Acids. Since esters of long-chain dibasic acids are used as plasticizers and lubricants, the di-(2-ethylhexyl) ester of the dibasic acids made by ozonization

TABLE IV
Effect of Treating Oleic Acid Soaps with Hydrogen

Oleic acid	a_{233}	Iodine value	Saturated acids, %	Composition of dibasic acid, %		
				C_{10}	C_9	C_8
Before hydrogenation	0.16	89.2	....	0	98.2	1.8
After hydrogenation	0.10	82.4	7.5	2.7	94.6	2.7

was prepared. The ozonization reaction mixture was steam-distilled to remove short-chain monobasic acids. To 170 g. of the remaining dibasic acid mixture were added 440 ml. (100% excess) of 2-ethylhexanol. Twenty-one grams of Dowex 50 × 4 resin in the hydrogen form were used as catalyst. This mixture was stirred and heated under nitrogen for 7 hrs. at 126° to 147°. Water was removed as the 2-ethylhexanol azetrope. The catalyst was removed by filtration and excess 2-ethylhexanol removed under vacuum. The product had an acid number of 5.1. These esters solidified at −22° to −25°C. These dibasic acids prepared from safflower oil glycerides contained stearic and palmitic acids as impurities. It is believed that the comparatively high solidification point may be caused by the esters of stearic and palmitic acid rather than by the dibasic acid esters.[2]

Discussion

Although the experiments described in Table I would indicate that the reduction stops at the monoene stage, samples of oleic acid were reduced about 10% in iodine value as shown in Table IV. Thus under some conditions the formation of small amounts of saturates occurs. Also the experiments with oleic acid show that a small amount of double bond shifting occurs. This change probably accounts for the C_8 and C_{13} dibasic acids found throughout these studies.

The trans-trans-9,11-linoleic acid also was reduced to an iodine value of 77.6, and the reaction proceeded past the monoene stage to produce 10.4% of saturated acids. The dibasic acids contain a larger amount of C_{10} than of C_9 or C_{11} acids, which would indicate that, although all types of addition occur, there is more 1,4 addition than 1,2 or 3,4. This reaction is in contrast to the hydrogenation of methyl cis-10,cis-12-linoleate by Allen, in which 1,2, 1,4, and 3,4 addition took place with equal ease (1). Since 10.4% of the saturated acids is present in the hydrogenated prod-

 uct, the value of 85% trans acids accounts for nearly all the unsaturated acids. Therefore 1,4 addition as well as 1,2 and 3,4 leaves a trans bond in the molecule. This is true although formation of a cis bond by 1,4 addition would not seem to be impossible sterically as in the cis,cis isomer.

If the alkali-conjugated linoleic acid in Run 25 contained equal amounts of 9,11 and 10,12 acid (6) and if 1,2, 1,4, and 3,4 addition took place with equal ease, the composition of the dibasic acids should be C_{12} 16.7%, C_{11} 33.3%, C_{10} 33.3%, and C_9 16.7%. Actually there is an excess of azelaic acid which may be caused by preferential reduction of the other bonds; however it is more likely caused by the presence of unconjugated linoleic acid that was not reduced. In Table III the dibasic acid composition is corrected for the 10.1% of linoleic acid present in the reduced product. The dibasic acids from the conjugated acids then are consistent with equal 1,2, 1,4, and 3,4 addition. Because the conjugation of the alkali-isomerized acids is cis-trans, the 64.3% isolated trans content of the reduced acids indicates that all 1,4 addition produces a trans bond.

A similar correction may be made in the average composition of the dibasic acids prepared from the hydrogenated linoleic acid concentrate, sample B. In Table III the values are corrected for the 17.8% oleic acid in sample B; the dibasic acid composition is then in agreement with that expected for equal rates of 1,2, 1,4, and 3,4 addition.

Conjugation and hydrogenation starting with safflower and soybean oils can be accomplished in aqueous solution in a manner similar to that used with the fatty acids. Hydrogenated acids can be oxidized to long-chain dibasic acid mixtures, which contain additional azelaic acid from the oleic acid in the oils. Saturated fatty acids from the oils are also present in the dibasic acid mixtures. These saturated acids affect the properties of esters prepared from them, and it may be necessary to remove the saturated acids for some low-temperature applications.

Fatty acids prepared from selectively reduced glycerides are similar to those produced by conjugation and hydrogenation in that both can be oxidized to long-chain dibasic acids. These dibasic acids from selectively reduced oils however contain larger amounts of both C_{13} and of C_7 and C_8 dibasic acids.

Summary

Conjugated linoleic acid can be hydrogenated as sodium soap in an aqueous or ethylene glycol solution

[2] Since the preparation of this paper, T. R. Steadman and J. O. H. Peterson have reported that the di-2-ethylhexyl esters of a mixture of undecanedioic and dodecanedioic acids are solid at −65°F. [Ind. Eng. Chem., 50, 63 (1958)].

TABLE V
Composition of Dibasic Acids Obtained from Safflower Oil by Hydrogenation and Oxidation

Acids		Composition of dibasic acids, %						
Hydrogenation	Saturates (%)	C_{13}	C_{12}	C_{11}	C_{10}	C_9	C_8	C_7
As soaps, dibasic acids obtained by ozonization...............	....	5.2	13.1	19.4	19.0	30.1	3.9	3.9
As soaps, dibasic acids obtained by analytical oxidation...	9.0	4.8	14.3	22.5	21.0	33.3	4.1	0
Selectively reduced glycerides....................................	10.4	5.4	15.4	19.9	19.4	32.2	7.7	0

TABLE VI
Composition of Dibasic Acids from Soybean Oil by Hydrogenation and Oxidation

Acids		Composition of dibasic acid, %						
Hydrogenation	Saturates (%)	C_{13}	C_{12}	C_{11}	C_{10}	C_9	C_8	C_7
As soaps..	16.4	3.9	10.0	17.4	16.3	48.2	3.8	0.4
Selectively reduced glycerides....................................	21.0	8.6	14.1	14.5	17.6	32.3	10.4	2.5

with commercial nickel catalysts. Under suitable conditions the acid is reduced predominantly to monounsaturated acids with only a slight increase in saturated acids. An alkali-conjugation reaction mixture may be hydrogenated without isolating the conjugated acids. One set of conditions found suitable for hydrogenation is as follows: 10 g. of conjugated linoleic acid, 7 g. of sodium hydroxide, 250 ml. of water, and 0.05 g. of nickel placed under 40 p.s.i. hydrogen pressure and heated at 140°C. for 1 hour. Acids prepared from this reaction mixture have an iodine value of about 90. Oxidation and chromatographic analyses of the resultant dibasic acids indicate that with alkali-conjugated linoleic acid, 1,2, 1,4, and 3,4 addition of hydrogen take place with equal ease. The reduced acids contain 66% *trans* acids. With *trans,trans* conjugated linoleic acid, 1,4 addition takes place to a greater extent that 1,2 and 3,4 addition, and the reduced acids are all *trans*.

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Isolation of Ricin, Ricinine, and the Allergenic Fraction from Castor Seed Pomace from Two Different Sources[1]

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IN INVESTIGATIONS involving poisonous materials, it is necessary to curtail the handling as much as possible to avoid the associated hazards. Precaution is necessary with castor seeds because in handling them one is exposed not only to the extremely toxic protein ricin but also to the possibility of acquiring sensitivity to the allergenic fraction which has been described as a protein-polysaccharide complex. Besides ricin and the allergenic fraction, there is a mildly toxic alkaloid, ricinine. Methods for the individual isolation of one or the other of these factors in castor seeds or pomace have appeared in the literature (3, 4, 7, 10, 16). The classical work on the isolation of ricin is that of Osborne, Mendel, and Harris (15). More recent works on the isolation and purification of ricin are those of Kabat *et al.* (7) and Kunitz and McDonald (10). In spite of the fact that crystalline preparations of ricin have been reported to be homogenous, there is evidence that ricin may be a mixture of more than one component (2, 7).

The procedure used in the preparation and purification of ricin in this report is patterned after one used by Corwin of Johns Hopkins University as reported by Kabat *et al.* (7). The method of isolation of the allergenic fraction in castor seeds has been developed by Spies and Coulson (16). Their procedure was followed in this work. The principle used in the isolation of the alkaloid ricinine was essentially that of Evans (3).

Here an attempt has been made to develop a method for simultaneous extraction of these three fractions from one lot of the material. Preliminary steps in the isolation of any of these factors are to shell, grind, remove the oil, and in some cases decorticate the castor seeds. Two samples of castor seed pomace, one a commercially prepared sample and the other prepared in the laboratory, were subjected to the following procedure.

Experimental Procedure

Extraction and Isolation. The general plan followed in this work is shown in Figure 1. The different steps involved are as follows.

Extraction of Oil from Castor Seeds. The shelled castor seeds were ground, pressed, and extracted with ethyl ether. This was done only on the laboratory-prepared pomace.

Extraction of Castor Seed Pomace. The oil-free pomace was extracted with five volumes of water acidified with HCl to a pH of 3.8. This was effected by shaking the contents in a large, wide-mouthed bottle during a 24-hr. period. The contents were allowed to settle and then were filtered through linen cloth (about 45 threads per square inch). The residue was treated with 3 volumes of distilled water, shaken for 2–4 hrs. and filtered through the same cloth. A second treatment with water was given to the pomace.

The three filtrates were combined to give filtrate I. The remaining residue was termed insoluble residue I.

Filtrate I. This filtrate contains all the ricin and portions of ricinine and allergenic fractions that are soluble in cold, dilute HCl. It was evaporated to a small volume by vacuum distillation below 40°C. Ricin I was obtained by saturating the filtrate with NaCl and was then separated by filtration. The filtrate after separation of ricin I is termed filtrate II.

Filtrate II. A precipitate of ricin II was obtained by saturation of this filtrate with sodium sulfate. The filtrate after separation of ricin II is termed filtrate III.

Insoluble Residue I. The residue of castor seed pomace left after the extraction of ricin was treated with five volumes of water between 70°–80°C., thoroughly shaken for 1–2 hrs., allowed to settle, and filtered through linen cloth. The residue was washed twice more with hot water and filtered through the same cloth. The combined filtrate and washings gave filtrate IV. The residue is termed insoluble residue II.