TABLE IV

Effect of Tempering on Hardness of Cocoa Butter C[a]

Sample	Wt. applied	*d/D*	Hardness index
	kg.		
Bars tested as received from manufacturer	0.7	0.181	29.8
	1.2	0.229	31.7
Cocoa butter melted by heating to 38°C., melt seeded on solidification, tempered for 5 hr. at 27°C., cooled for 10 min. at 5°C. to release from mold.	0.7	0.181	29.8
	1.2	0.232	30.8
Cocoa butter melted by heating to 50°C., solidified, and held for 5 hr. at 5°C.	0.2	0.134	15.6
	0.7	0.252	15.2
	1.2	0.331	15.0

[a] Tests made at 15°C., 3/8-in. ball used, and load put on ball for 60 sec.

TABLE V

Hardness Values of Several Fat and Wax Products[a]

Product	Test temperature	Hardness index
	°*C.*	
Rosin	30	860
Carnauba wax	30	420
Sugar cane wax	30	320
Crude candelilla wax	30	260
Rice bran wax	30	210
Tristearin	30	170
Hydrogenated cottonseed oil	30	140
Hydrogenated jojoba wax	30	65
Ceresin wax	30	25
Paraffin	30	24
Chocolate liquor	25	19
Beeswax	30	17
Cocoa butter (aged)	25	15
Commercial candy fats other than cocoa butter	25	4–12

[a] Ball and load sizes varied to suit the individual products. Test load applied for one minute in all cases.

Even well tempered samples of cocoa butter tended to show differences, as is evident from the curves in Figure 5.

Several of the conditions recommended in carrying out the hardness tests were not investigated extensively. The recommendation that the thickness of the test sample at the point of indentation be at least 10 times the depth of the indentation is based on the observation that this thickness always yielded reproducible results with the fats and waxes which were tested. It appears to be larger than necessary. The thickness recommended is a convenient one; also it conforms with the specifications of the A.S.T.M. test for determining the Brinell hardness of metals.

On the basis of considerations similar to the above it was recommended that the distance of the center of the identation from the edge of the sample or edge of another indentation be at least two and one-half times the diameter of the indentation.

With regard to the reproducibility of results, hardness values obtained under identical conditions have generally been found to deviate no more than two or three percentage units from an average value. For example, successive measurements of the hardness of sugar cane wax, made at 30°C. and using a 3/16-in. ball, 4.2 kg. weight, and a test time of 60 seconds, gave the following hardness values: 235, 238, 245, 242, and 242. The average for the series is 240, and the maximum deviation is 2.1%.

To show the versatility of the instrument which has been described, hardness values determined for a variety of fat and wax products are recorded in Table V. It is believed that this is the first time that the hardness of these compounds has been shown on a common scale. Unless noted otherwise, the hardness of each sample was determined after heating it to a few degrees above its melting point, pouring the melt into the mold, cooling the sample to room temperature, and then determining the hardness.

Summary

Heretofore a good method for measuring the hardness of fats and waxes has not been available. Instruments currently used to measure hardness (not consistency) are relatively inaccurate and give results based on an arbitrary scale.

A new instrument and technique have been devised for measuring the hardness of fats and waxes. They are essentially an adaptation of the Brinell hardness test used for metal and alloys. In determining the hardness of a fat or wax, a perfectly round steel ball having a diameter as small as 0.1250 in. or as large as 0.5000 in. is pressed with a force of 200 g. to about 6 kg. onto the surface being tested. The hardness index, in terms of kilograms per square centimeter, is determined from the slight impression produced. The index is relatively independent of ball size, test load, and other test conditions, provided these are confined to certain ranges.

Hardness indices have been determined for products ranging from poorly tempered cocoa butter to rosin. Differences in hardness observed have varied over 1500-fold. The hardness of cocoa butter has been found to vary over seven-fold, depending on thermal history.

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Ion Exchange Resin Catalyst Stability in in-situ Epoxidation[1]

W. WOOD and J. TERMINI, The Permutit Company, New York, New York,
Division of Pfaudler Permutit Inc., Rochester, New York

THE expanding epoxide applications within the last decade as plasticizers and chemical intermediates have sparked the development of improved epoxidation processes. Evidence of this development is the use of a solid ion exchange resin catalyst in the *in-situ* epoxidation process. Vital to the success of this process is the stability of the resin catalyst to physical breakdown when exposed to the highly oxidizing conditions of epoxidation.

Factors affecting resin catalyst stability have been evaluated in our laboratory in an effort to extend resin life, thereby making this process more attractive for epoxidation. This paper presents a method for evaluating resin stability and shows how metal contamination and cross-linking of the ion exchange resin, the concentration of hydrogen peroxide, the operating temperature, and the use of a complexing agent affect resin stability. The epoxidation process will first be reviewed briefly to provide background

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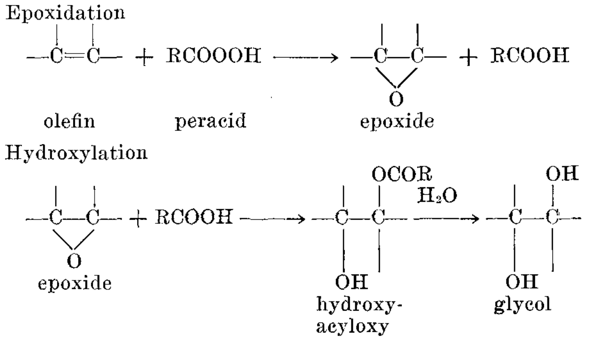
[1] Presented at the fall meeting, American Oil Chemists' Society, Cincinnati, O., September 30, October 1–2, 1957.

information on the resin epoxidation process and to illustrate why resin stability is essential.

Epoxidation

The development and chemistry of the epoxidation process and the diverse application of epoxides are reported in the literature (1, 2, 3, 13, 14) and patents (4, 6, 15, 16) and will be discussed only briefly.

Chemistry. Epoxidation is the reaction of an organic peracid with a double bond to form an oxirane compound. Hydroxylation is the further reaction of the epoxide ring to produce a 1,2-glycol derivative.



$$\text{—C=C—} + RCOOOH \longrightarrow \text{—C—C—}(\text{O}) + RCOOH$$

$$\text{—C—C—}(\text{O}) + RCOOH \longrightarrow \text{—C(OCOR)—C(OH)—} \xrightarrow{H_2O} \text{—C(OH)—C(OH)—}$$

Perbenzoic acid was first found to be suitable for epoxidation (12), but today peracetic and performic acids are now being used almost exclusively for the commercial preparation of more than 25 million lbs. per year of epoxides. Unsaturated compounds readily available for epoxidation include fatty acids, fatty esters, fats and oils, terpenes, aliphatic and alicyclic olefins. Soybean oil is most frequently used. Although these compounds all contain carbon-to-carbon double bonds, they are chemically different and in many cases permit the production of epoxides with unique physical and chemical properties, such as heat and light stability, plasticizer permanence, and other characteristics. These properties permit epoxides to be used as plasticizers for vinyl polymers, stabilizers for chlorinated resins, chemical intermediates for organic synthesis, insecticides, lubricants and lubricant additives, surface-active agents, and for applications extending to other fields (7, 8).

Resin Catalyst in-situ Epoxidation Process. Preformed peracetic solution, made by reacting concentrated hydrogen peroxide with glacial acetic acid, could be used for epoxidation. However, when using the preformed peracid, it is necessary to buffer the free mineral acid catalyst used and to operate at a reaction temperature of less than approximately 25°C. to minimize opening of the epoxy ring. The result is high hydrogen peroxide consumption and a long reaction time. These limitations and the problem of safely handling preformed peracids prompted further development.

In-situ techniques for hydroxylation (13) and epoxidation (11) were investigated. The process is based on the addition of hydrogen peroxide to an aliphatic acid solution of olefinic material in the presence of an acid catalyst. The hydrogen peroxide reacts *in situ* with the organic acid to form the peracid which immediately converts the double bond to an epoxy ring. *In-situ* epoxidation reduces hydrogen peroxide consumption to slightly more than stoichiometric and eliminates the necessity of preparing the costly and hazardous preformed peracid. The *in-situ* epoxidation catalyst may be either a strong mineral acid, such as sulfuric acid, or an acid cation exchanger. The sulfuric acid type of catalyst has a tendency toward undesirable epoxy ring opening and polymerization. Neutralization of the liquid catalyst also forms inorganic salt contaminants which are detrimental to epoxide quality (2). The recently developed resin catalyst process avoids these disadvantages. Under proper conditions this resin process increases epoxy yields, simplifies the operation, and, in many cases, produces a better epoxide with lower viscosity, greater temperature stability, and better plasticizer compatibility.

The resin process may be a batch process in which the resin catalyst is separated from the reaction products and re-used, or it may be a continuous process based on continuously passing the reaction mixture through a fixed bed of ion exchange catalyst. Recently an "expendable resin" process was developed which eliminates the need for catalyst recycling by using a lower catalyst concentration. The literature (1, 3, 4, 5) presents a detailed description of these procedures.

The resin catalyst epoxidation process has merited considerable study. Resin attack has been experienced with continued use under certain conditions (9), particularly for fixed resin catalyst bed operation. The highly oxidizing conditions occurring in the epoxidation reaction result in decross-linking of the ion exchange resin and in fragilation, causing swelling and even ultimate dissolution of the resin catalyst and making efficient column operation with a fixed resin catalyst bed very difficult. The loss of resin stability with each successive batch epoxidation interferes with filtration and reduces the epoxide yield (10). Techniques for effectively extending resin catalyst life will be discussed after reviewing the data on stability.

Experimental

Catalyst Grade Permutit QH of principally −20 to +40-mesh size was used for these tests. It is an acid cation exchanger developed specifically as a catalyst in epoxidation and other acid-catalyzed reactions. This resin is a polystyrene polymer which has been cross-linked with divinyl benzene to render an insoluble bead (Figure 1), which is then sulfonated to impart cation exchange properties. A special acid treatment converts the beads to the hydrogen form and simultaneously reduces the metal contamination to usually less than 0.005% of the dry resin weight and allows this resin to be used directly for catalysis.

Resin Stability Test. Hydrogen peroxide attack of the resin catalyst in epoxidation may be simulated by a resin stability test developed in our laboratory. Approximately 10 ml. of hydrogen-form resin (tapped volume measured with a 10-ml. graduate) is contacted with hydrogen peroxide in an Erlenmeyer flask for 3 hrs. at 60° to 80°C. as maintained by a constant-temperature water bath. The mixture is transferred quantitatively to a porous-bottomed porcelain crucible, and the resin is rinsed free of excess peroxide.

The degree of resin breakdown is indicated by determining the increase in resin volume (extent of resin decross-linking) and the loss in dry resin weight (decross-linking and solubilization). The dry resin weight is obtained by heating at 130°C. to constant weight.

Variables. The variables investigated included the following:

1. *metal contamination:* adding of iron, copper, zinc, nickel, or manganese [Metal contamination (based on dry resin weight) ranged from less than 0.005% for the commercially available Catalyst Grade Permutit QH to as high as 0.9% when contaminant was added.];
2. *hydrogen peroxide concentration:* 35 ml. of 50% H_2O_2 or 10% H_2O_2;
3. *reaction temperature:* 60°C. to 80°C.;
4. *resin cross-linking:* 10% and 15%; and
5. *sequestering agent:* 0.1 to 0.4 g. of tetrasodium ethylenediamine tetra-acetate.

Discussion

Metal Contamination. Hydrogen peroxide decomposition and the resulting attack on the ion exchange resin are accelerated in the presence of metal contaminants. The maximum allowable metal content for resin stability will depend on the type of metal contaminant, operating temperature, and peroxide concentration. Tables I and II show clearly the effect of different metals. Iron is by far the most detrimental to resin stability, and copper almost equally as active. Nickel, zinc, and manganese promote resin breakdown but to a considerably lesser extent.

TABLE I
The Effect of Hydrogen Peroxide Concentration on Resin Catalyst Stability
(10 ml. of resin catalyst with 35 mg. of metal contamination reacted 3 hrs. with 35 ml. of H_2O_2)

Contaminant[a]	60°C. Reaction		80°C. Reaction	
	10% H_2O_2	50% H_2O_2	10% H_2O_2	50% H_2O_2
	% resin swelling[b]		*% resin swelling*[b]	
Control	1	4	6	22
Fe	dis.	dis.	dis.	dis.
Cu	5	360	14	dis.
Zn	1	5	6	28
Ni	2	3	6	25

[a] Control Permutit QH contains less than approximately 0.005% iron contamination based on dry resin weight. The 35 mg. of metal contaminant added are equivalent to about 0.9% of dry resin weight.
[b] dis. means resin sample dissolved completely while the percentage figures denote resin volume increase.

For the most active contaminant, iron, the maximum allowable content for negligible resin breakdown at 60°C. and 50% H_2O_2 is roughly less than 0.027% and preferably less than 0.01% of the dry resin weight (Table III). Significantly, larger quantities (as high as 0.9%) can be tolerated for zinc, nickel, and possibly manganese. The relatively good stability of the control resins shows vividly the necessity for using a practically metal-free catalyst and for avoiding metal contamination from equipment and other reactants during epoxidation.

Hydrogen Peroxide Concentration. Increasing the hydrogen peroxide concentration for the conditions studied increases decross-linking and solubilization of the resin catalyst. Table I compares the effects of hydrogen peroxide concentration on resin breakdown. However, since 50% H_2O_2 is more efficient than the 10% H_2O_2 for epoxidation, rigid control of metal contamination would be the more practical plant control method to prevent resin breakdown.

TABLE II
The Effect of Reaction Temperature on Resin Stability
(10 ml. of resin with 35 mg. of metal contamination reacted 3 hrs. with 35 ml. of H_2O_2)

Contaminant	10% H_2O_2			50% H_2O_2	
	60°C.	70°C.	80°C.	60°C.	80°C.
	% resin swelling			*% resin swelling*	
Control	1	2	6	4	22
Fe	dis.	dis.	dis.	dis.	dis.
Cu	5	8	14	360	dis.
Zn	1	3	6	5	28
Ni	2	3	6	3	25
Mn	–	–	–	4	40

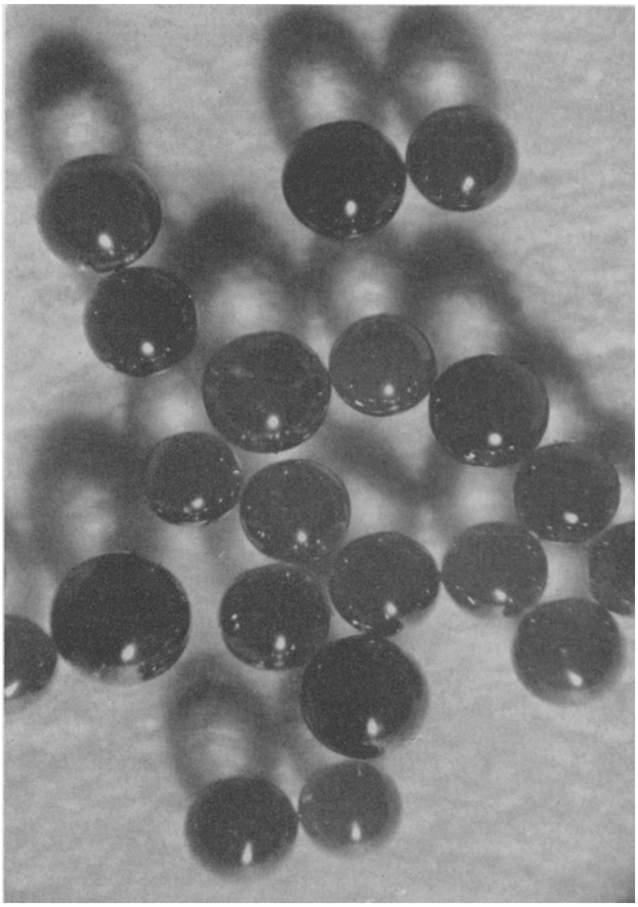


FIG. 1. Photomicrograph of catalyst grade Permutit QH resin beads.

Temperature. As expected and shown in Table II, increasing the temperature from 60°C. to 80°C. increased resin breakdown. Higher temperatures increase hydrogen peroxide decomposition and the resulting resin decross-linking. The temperature range for plant epoxidations generally ranges from 60°C. to 75°C. with perhaps as high as 80°C. for the expendable resin process. Operating at lower temperatures would minimize resin breakdown, but it would also extend the reaction time and decrease the epoxidation efficiency.

Resin Catalyst Cross-linking. The 15% cross-linked resin is more resistant to resin degradation than the 10% cross-linked resin, particularly when iron contamination is approximately 0.01% or less. Increasing resin cross-linking from 10% to 15% is not very effective in counteracting the detrimental effect of high iron concentrations. This again indicates that metal contamination is a dominant factor although greater resin cross-linking is helpful.

TABLE III
The Effect of Iron Contamination on Resin Stability
(10 ml. of resin heated with 35 ml. of 50% H_2O_2 at 60°C. for 3 hrs.)

Contamination[a]	Resin volume increase
% Fe	*%*
Control (0.001%)	3
0.0275	13
0.1375	180
0.2080	dis.

[a] Calculated on dry weight of resin.

TABLE IV
Effect of Resin Cross-Linking on Resin Catalyst Stability
(10 ml. of resin of 10% and 15% cross-linking reacted with 35 ml. of 50% H_2O_2 at 80°C. for 3 hrs.)

Contaminant[a]	Cross-linking 5%	Cross-linking 10%	Cross-linking 15%	Cross-linking 10%	Cross-linking 15%
	% resin swelling			*% resin wt. loss*[b]	
Control	dis.	22	34	1.8	3.6
Fe: 0.01	–	400	70	32	10.5
0.026	–	dis.	dis.	99	92
Cu 0.9	–	dis.	dis.	–	–
Ni 0.9	–	25	30	–	–

[a] Calculated on dry weight of resin.
[b] Change in weight of dry resin. Resin before and after dried at 130°C. to constant weight.

Complexing Agent. A complexing agent, such as tetrasodium ethylenediamine tetra-acetate, was evaluated because of its ability to complex iron and other metals. Elimination of the free metal contaminant by a complexing agent was expected to retard peroxide decomposition and resin breakdown. A complexing agent will limit and reduce the extent of resin breakdown, as shown in Table V by the smaller losses in resin weight with increasing dosages. Again, with high iron contamination, the effectiveness of a complexing agent is not significant, and a larger quantity of sequesterant appears necessary to reduce the quantity of the resin being solubilized.

Nature of Resin Degradation. During the degradation studies a special test was made to help explain the nature of resin breakdown in a metal-contaminated hydrogen peroxide system. The resin (100 g.) containing 0.1% copper was heated to 80°C. with a small quantity of water, and 100 ml. of 50% H_2O_2 were added slowly over a 2-hr. period. The mixture was then filtered, and a sample of the filtrate was dried at 70°C. over-night. One gram of the dried filtrate was dissolved in water and titrated with 0.1 N NaOH. The change in pH was followed with a Model H-2 Beckman pH Meter.

Decross-linking was evidenced when the resin became highly swollen and 80% solubilized. The titration curves shown in Figure 2 of the water-soluble portion of the decross-linked resin and the standard undegraded resin indicate some desulfonation and formation of weaker acid groups.

Summary

The highly oxidizing conditions of epoxidation cause decross-linking of the ion exchange resin catalyst, resulting in swelling of the resin with its eventual dissolution if uncontrolled.

Minimum metal contamination (particularly iron), low hydrogen peroxide concentration, low reaction temperature, high cross-linked resin catalyst, and the addition of a complexing agent all contribute to maintaining resin catalyst stability. Metal contamination is by far the most detrimental factor to resin stability.

TABLE V
Effect of Complexing Agent on Resin Catalyst Stability
(10 ml. of resin containing 35 mg. of iron reacted with 35 ml. of 10% H_2O_2 at 80°C. for 3 hrs. in presence of tetrasodium ethylenediamine tetra-acetate)

Complexing agent	Permutit QH resin Control	Permutit QH resin Contaminated
grams	*% resin weight loss*[a]	
0	2.8	100
0.1		76
0.2		64
0.3		56
0.4		46

[a] Resin samples became partially soluble. Therefore dry resin weight loss (percentage solubilized) rather than volume change used to indicate breakdown.

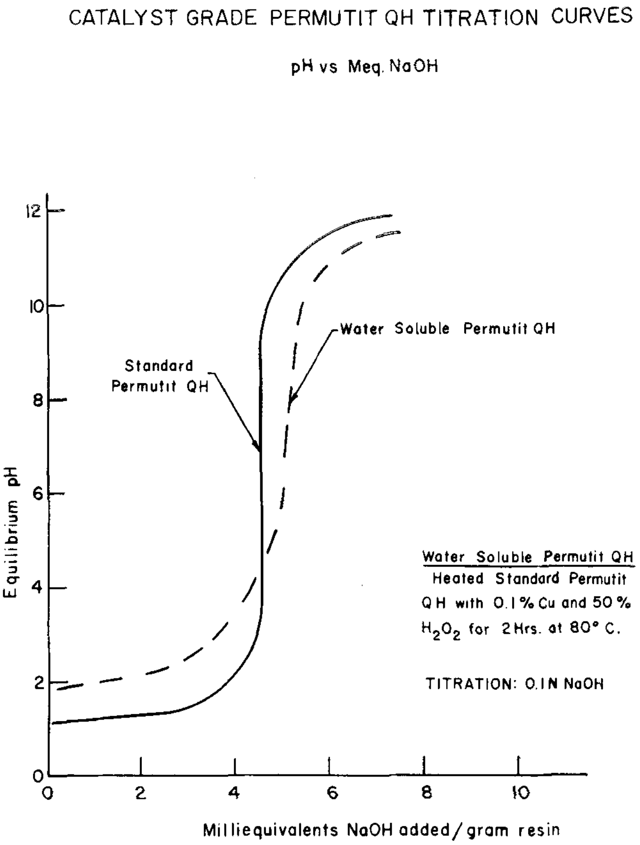


FIG. 2. The difference in titration curves of the water-soluble degraded resin and standard resin indicates some desulfonation and formation of weak acid groups.

Operating with essentially a metal-free system is most important. This can be obtained by using a resin with less than 0.005% metal contamination and by avoiding metal contamination from raw materials and equipment being used for epoxidation.

A minimum of metal contamination and perhaps a lower operating temperature provide the most practical methods for controlling resin breakdown and for improving the efficiency of the ion exchange resin catalyst in *in-situ* epoxidation processes. Avoiding resin breakdown is particularly essential for the fixed resin catalyst bed and for the resin recycle batch processes. Even for the expendable resin process where resin stability is not too critical, control of metal contamination and operating temperature should result in a trouble-free and a more economical operation.

Acknowledgment

The authors wish to express their gratitude to John Byer for his assistance during the experimental work at The Permutit Company's laboratory in Birmingham, N. J. Appreciation is also expressed to M. E. Gilwood, director of research, for permission to publish this paper and his helpful suggestions.

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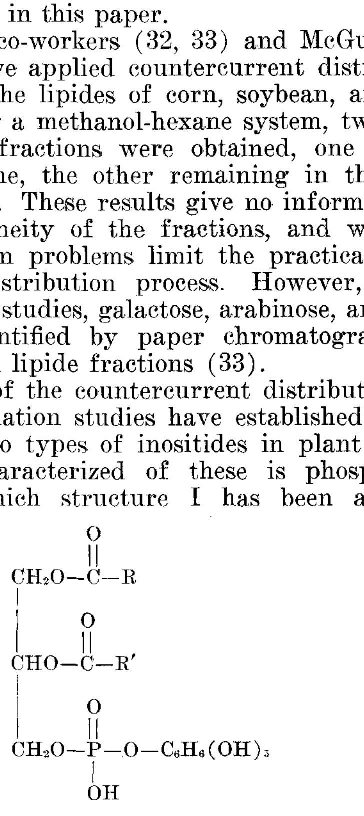
Biochemistry of the Sphingolipides. X. Phytoglycolipide, a Complex Phytosphingosine-Containing Lipide from Plant Seeds[1,2]

HERBERT E. CARTER, WALTER D. CELMER,[3] DIMITRIS S. GALANOS,[4] R. H. GIGG,[4] WILLIAM E. M. LANDS,[5] JOHN H. LAW,[6] KATHERINE L. MUELLER, TEISHI NAKAYAMA,[4] H. H. TOMIZAWA,[7] and EVELYN WEBER, Division of Biochemistry, Noyes Laboratory of Chemistry, University of Illinois, Urbana, Illinois

THE INOSITOL-CONTAINING PHOSPHATIDES have been the subject of many investigations since Klenk and Sakai (20) first reported the presence of inositol in soybean lipides. Progress has been slow, partly because of the lack of satisfactory fractionation methods and of adequate criteria of purity; nevertheless some useful information has been obtained as to the nature and number of the inositol-containing lipides of corn and soybean (11–13, 17, 18, 24–26, 32–35, 37). Folch (12, 13), Woolley (37), and others (18, 24, 26) have obtained purified fractions of higher inositol content by solvent-fractionation procedures. These materials were reported to contain fatty acids, glycerol, inositol, and phosphate; certain preparations had, in addition, carbohydrate components and tartaric acid. The only nitrogenous substances detected were ethanolamine and serine, which is of some interest in the light of the results to be presented in this paper.

Dutton and co-workers (32, 33) and McGuire and Earle (22) have applied countercurrent distribution techniques to the lipides of corn, soybean, and flaxseed. By using a methanol-hexane system, two inositol-containing fractions were obtained, one moving with the hexane, the other remaining in the early methanol tubes. These results give no information as to the homogeneity of the fractions, and with this system emulsion problems limit the practical extension of the distribution process. However, in the course of these studies, galactose, arabinose, and mannose were identified by paper chromatography in various inositol lipide fractions (33).

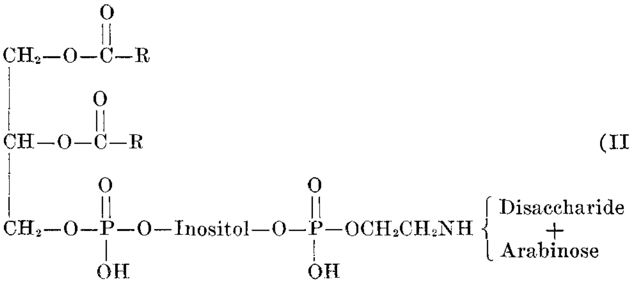
The results of the countercurrent distribution and solvent-fractionation studies have established the occurrence of two types of inositides in plant lipides. The better characterized of these is phosphatidyl inositol, to which structure I has been assigned.



$$\begin{array}{l} CH_2O{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}R \\ | \\ CHO{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}R' \\ | \\ CH_2O{-}\underset{\displaystyle OH}{\underset{|}{\overset{\displaystyle O}{\overset{\|}{P}}}}{-}O{-}C_6H_6(OH)_5 \end{array} \qquad (I)$$

Phosphatidyl inositol has been isolated from several plant sources [soybean (25), wheat germ (11), peas (35)], and a phosphatidyl inositol-like material with a phosphorus to inositol ratio of 1.8 has been obtained in a partially purified form from soybean (32).

The more complex carbohydrate-containing inositides have been less well-characterized. Folch (13) fractionated soybean phosphatides by a chloroform-ethanol technique and obtained a fraction which contained inositol, carbohydrate, glycerol, primary amine (unidentified) fatty acids, and phosphoric acid. Hawthorne and Chargaff (18), by a similar solvent-fractionation procedure, obtained an inositol lipide, which on hydrolysis gave an organic phosphate containing inositol, galactose, and arabinose. More recently Malkin and Poole (24) have purified similar fractions further and have tentatively assigned structure II



$$\begin{array}{l} CH_2{-}O{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}R \\ | \\ CH{-}O{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}R \\ | \\ CH_2{-}O{-}\underset{\displaystyle OH}{\underset{|}{\overset{\displaystyle O}{\overset{\|}{P}}}}{-}O{-}\text{Inositol}{-}O{-}\underset{\displaystyle OH}{\underset{|}{\overset{\displaystyle O}{\overset{\|}{P}}}}{-}OCH_2CH_2NH\left\{\begin{array}{c}\text{Disaccharide}\\ +\\ \text{Arabinose}\end{array}\right. \end{array} \qquad (II)$$

to the main constituent. It should be noted that none of these materials were established to be homogeneous.

Some years ago in our laboratories a procedure was devised for preparing a purified inositol lipide mixture[8] essentially devoid of lecithin, cephalin, sterols, and non-lipide contaminants (1). Countercurrent distribution studies on this material, using a methanol-

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[1] The soybean phosphatide work was done under contract with the U. S. Department of Agriculture and was authorized by the Research and Marketing Act. The contract was supervised by Herbert J. Dutton and John C. Cowan of the Northern Utilization Research and Development Division of the Agricultural Research Service, Peoria, Ill. The corn phosphatide work was supported in part by research grants (B574–C2, 3, 4) from the National Institutes of Health, United States Public Health Service. Part of the material in this paper was taken from the theses submitted by Walter D. Celmer, William E. M. Lands, John H. Law, and H. H. Tomizawa to the Graduate College of the University of Illinois in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry.

[2] A preliminary report of this work has been published (4). For number IX in this series see reference (2).

[3] Present address, Chas. Pfizer and Sons, Brooklyn, N. Y.

[4] Postdoctorate Research Associate, Department of Chemistry.

[5] Present address, Department of Biological Chemistry, University of Michigan, Ann Arbor, Mich.

[6] Present address, Converse Memorial Laboratory, Harvard University, Cambridge, Mass.

[7] Present address, University of Washington, School of Medicine, Seattle, Wash.

[8] In this paper the term inositol lipide (IL) will be used to designate the mixture of inositol-containing lipides which were obtained from a variety of plant seed phosphatides by this procedure.