dextrins which originate probably as residues from the more complex configurations.

An insoluble amylose fraction is obtained from corn starch by the Macerans enzyme conversion. Potato starch yields negligible quantities of this amylose.

The fraction of gelatinized corn starch more soluble in alcohol–water mixtures has been characterized by the high yield of Schardinger dextrins which can be obtained from it.

ARGO, ILLINOIS RECEIVED DECEMBER 18, 1941

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE CORN PRODUCTS REFINING COMPANY]

On the Multiple Amylose Concept on Starch. III. The Isolation of an Amylose in Crystalline Form

BY RALPH W. KERR AND G. M. SEVERSON

In a preceding paper[1] a method was described for fractionating a corn starch paste into two components by the addition of butanol plus methanol. The less soluble portion of the paste, some 45% of the total, was shown to differ from the more soluble portion, principally in its higher conversion limit[1] with beta-amylase and its higher alkali labile number as determined by the method of Schoch and Jensen.[2] These figures are given again for reference in Table II. From this study and from a study of the characteristics of a component amylose, provisionally called gamma-amylose,[3] it was concluded that corn starch is composed of at least three major constituent amyloses, two of them precipitating in the less soluble fraction. The purpose of the present work is to present two experiments which further confirm this conclusion.

It was shown that the relatively insoluble gamma-amylose collected with the alcohol precipitate as might be anticipated, but it was indicated that the balance of this latter fraction, probably originally soluble, would be found to have a conversion limit well above 70% and an alkali labile number slightly higher than the parent corn starch.

Direct fractionation of the less alcohol-soluble part to decide the point in question has presented certain obstacles. Leaching the product with a limited amount of water gives an unmanageable, gelatinous mass. The use of larger volumes of water, higher temperatures, higher *p*H's, etc., shows that little of this product can be redispersed unless conditions are used which lead to a solution of appreciable quantities of gamma-amylose as well. From this solution it has been impossible as yet to precipitate, fractionally, the originally more insoluble gamma-amylose. Therefore, indirect methods were investigated to answer the question.

Alcohol Fractionation of Potato Starch Pastes

It occurred to us, first, to investigate potato starch, wherein no gamma-amylose is found, so that if a component exists which is comparable to the third corn constituent predicted, the difficulties involved in separating gamma-amylose from this constituent would be avoided.

Accordingly, potato starch pastes were fractioned by methods employed previously on corn starch[1] with the following results

TABLE I

FRACTIONATION OF POTATO STARCH PASTES WITH ALCOHOLS

	Yield, %	Alkali no.	beta-Amylase conversion limit, %	Phosphorus, %
Main fraction sols.	73.5	5.2	56.2	0.088
First wash of insolubles	2.6			
From recrystn. of less soluble	1.8			
Less sol. fraction	22.1	12.9	89.0	.014
Parent starch		6.0	63.6	.081

As might be anticipated, the precipitated fraction is much more readily soluble than the corresponding precipitate from corn starch. Indeed, at boiling temperature, even with double the ratio of water to solids, which was used to redissolve completely the precipitated potato fraction, the corn fraction did not redissolve completely. Furthermore, the potato fraction, when redissolved, remained clear and limpid when

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[1] R. W. Kerr, O. R. Trubell and G. M. Severson, *Cereal Chem.*, **19**, 64 (1942).

[2] T. J. Schoch and C. Jensen, *Ind. Eng. Chem., Anal. Ed.*, **12**, 531 (1940).

[3] R. W. Kerr and O. R. Trubell, *Cereal Chem.*, **18**, 530 (1941).

TABLE II

ALKALI NUMBER AND CONVERSION LIMIT OF FRACTIONS OF CORN STARCH

		Yield, %	% of water extractives precipitating	Alkali number	Conversion limit	% P
Corn starch (dioxane extracted)				11.7	59.4	0.020
Alcohol more soluble		55.6		5.7	56.6	.013
Alcohol less soluble		44.4		20.0	65.3	.009
gamma-Amylose		11.55		42.0	48.1	
Crystalline amylose, water extract	First	4.1	79.5	35.0	93	
	Second	1.2	81.6	30.2	91	
Water extract, spontaneously ppt.	First	4.8	94.0	35.4	86	
	Second	1.2	84.7	28.9	86	
Butanol precipitate (Schoch)		22		22	81	

cooled and allowed to stand twenty-four hours, whereas the corn fraction gelled to an opaque mass as soon as cooled to room temperature. An additional observation was made, important to the work which follows, that the precipitated fraction of potato starch showed more definite signs of crystalline structure, was of smaller volume in relation to solids, and was much more readily filterable.

Summing up our data, it would seem that the third constituent predicted in corn starch had been found in potato starch. (It will be recalled that the alkali number for whole corn starch is about 10 to 11.) However, the tacit assumption has been made that starches are made up of several units, each of which is about the same, whether it is found in potato starch or in corn starch. While such an assumption may be justifiable in limited form, we were not inclined to rest entirely on its validity to settle this question. It has been noted, for example, that the more alcohol-soluble fraction of potato starch shows a high content of phosphorus, whereas the more alcohol-soluble fraction of corn starch showed only 0.009% phosphorus. If this difference can exist, it is quite possible that even more fundamental differences may exist between one component and its counterpart in another starch. Therefore, a more direct experiment was sought.

Butanol Crystallization of the Water Extract of Starch.—In a review[1] of several of the more common methods for preparing amyloses, attention was directed to the water extraction of corn starch at temperatures just under or at its gelatinization point. It was found that this very soluble fraction gave a very high conversion limit with barley diastase. A quantity was prepared at 1% concentration by procedures given in the Experimental Section, and alcohols were added to determine whether it belonged to the less alcohol soluble or more alcohol soluble fraction. It was surprising to find that small additions of butanol alone precipitated very readily the major portion of this amylose fraction. Indeed, a 1% solution of the amylose fraction spontaneously precipitated with no addition of alcohol whatsoever if left to stand. However, several important differences were noted in the two precipitations. The alcohol precipitate was crystalline in nature, was of small volume with respect to solids, and readily filterable on filter paper. The spontaneously precipitated material was bulky and flocculent, and when centrifuged settled as a gelatinous mass. Quantitatively, the results are summarized in Table II.

Whereas 5.1% of the original weight of starch appears in the filtrate when corn starch is extracted one hour at 70° in a 2.5% suspension and filtered, only 79.5% of the solids present precipitate when the solution is concentrated to 1 g. per 100 cc., saturated with butanol and refrigerated for four days. After the above precipitation nothing further is precipitated by adding methanol to the liquors to the extent of 15% methanol. The 1% solution above, however, spontaneously precipitates 94% of its solids when refrigerated four days. Both solutions were at *p*H 4.9.

The "conversion limit" with barley diastase of crystalline amylose was determined according to previously given procedures,[1] with the exception that the conversion was carried out at an amylose concentration of 1 g. per 250 cc., to minimize spontaneous precipitation. This value was found to be 93%. The conversion limit for the spontaneously precipitated material, determined under the same conditions, is 86%.

While both precipitates are colored blue by iodine, the solubles after butanol precipitation are colored purple by iodine, those from the spontaneous precipitation a light blue.

It would appear, then, that butanol crystallization is a more effective way to purify this amylose than spontaneous precipitation.

The crystals, precipitated by butanol, are rectangular platelets, the longer diameter being about double the shorter (Fig. 3). They are very thin and transparent as first formed, and but slightly anisotropic. Larger crystals after separation and drying are more markedly so. The product even after removal of butanol, gives a very sharp "V" type X-ray diffraction pattern[4] in contrast to our more alcohol-soluble fraction, for example, which is completely amorphous. From the manner of preparation and from the fact that the product, as first separated, contains butanol, it is suggested that crystallization is the result of an orientation induced by the butanol and that the product may be a complex between amylose and butanol. It is to be noted, however, that after removal of all butanol from the product, preferably by washing in methanol and drying at low temperatures *in vacuo*, the platelets still retain their structure and crystalline properties.[4]

The platelets, as precipitated, may be redissolved in hot water, from which they may be recrystallized by saturating with butanol and cooling. When dried, however, they do not redissolve very readily in water but rather agglutinate into masses of gel particles. The final product which is discussed below and which was used for photographing and X-ray studies was recrystallized two additional times after the first butanol crystallization.

The alkali number, determined according to the method of Schoch and Jensen, is 35.

From the exceptionally high beta-amylase "conversion limit"[1] for this product, estimated at 93% from the reaction rates as shown in Fig. 1 and from the fact that 90% of this conversion follows the course of a first order reaction as shown in Fig. 2, it is concluded that the configuration of the amylose is essentially a linear arrangement of 1,4-alpha glucosidic linked glucopyranose units. The fact that the conversion does not go entirely to completion as a first order reaction may be explainable as due to retrogradation effects which were distinctly visible at the end of the conversion graphed in Fig. 2. In this latter conversion the rate was reduced by using one-quarter of the amount of beta-amylase used in the conversion graphed in Fig. 1.

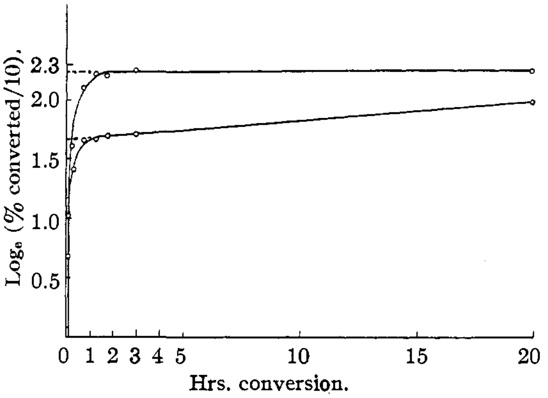

Fig. 1.—Estimation of conversion limits of corn starch (A) and its crystalline amylose (B) with beta-amylase.

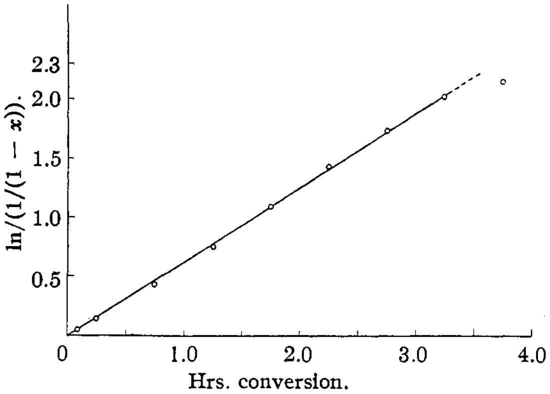

Fig. 2.—Conversion rate of corn, crystalline amylose into maltose with beta-amylase.

Both the limit of conversion and the alkali number of crystalline amylose are higher than was anticipated at the beginning of this investigation for that part of starch which precipitates with gamma-amylose in our alcohol less soluble fraction. Thus, while it is evident that if crystalline amylose is found to differ from gamma-amylose, the main purpose of the present work in showing that the less alcohol soluble fraction can be subdivided, has been accomplished, it would now appear that we had not obtained the total fraction sought but only a constituent of it. It would appear that there are other components also present in this fraction, intermediate in character between crystalline amylose and the constituents of the more alcohol-soluble portion.

A similar conclusion follows also from a consideration of the yields of these fractions obtained. The total percentage of corn starch water soluble at 70° is approximately 7% (see Experimental Section), about 80% of which latter is crystallizable with butanol. Hence, a 5.5% yield of

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(4) D. French and R. E. Rundle, private communication.

crystalline amylose plus an estimated 10% gamma-amylose content[3] leaves about 30% of corn starch unaccounted for in the less alcohol-soluble fraction.

It was felt that if the less alcohol-soluble fraction of potato starch could be subdivided, that is, if it were found that potato starch contains a component corresponding to corn, crystalline amylose, our theory as to the composition of the starches would be materially strengthened.

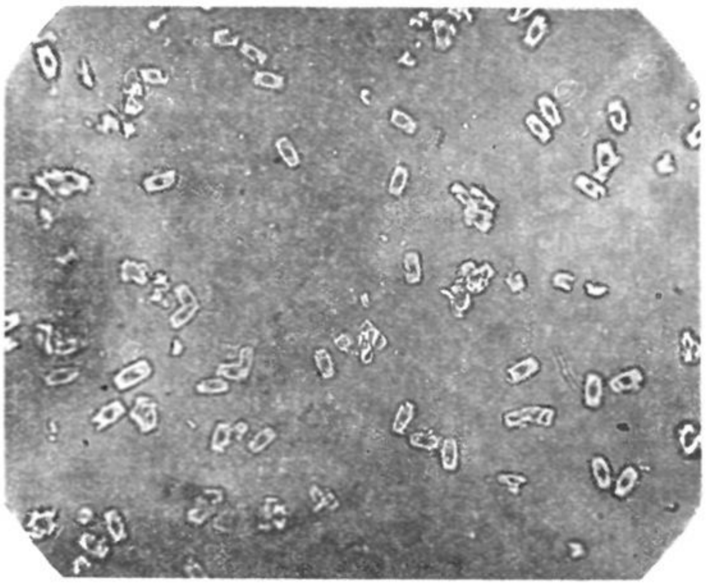

Fig. 3.—Crystalline amylose (corn starch).

Accordingly, potato starch was extracted with warm water in much the same way that corn starch was treated except that because of the more fragile nature of potato starch only thirty minutes of extraction at 65° and *p*H 5.3 was employed: 6.3% of the weight of the starch was extracted, of which 85.4% precipitated when treated with butanol in accordance with procedures given for corn starch.

This precipitate proved to be crystalline platelets likewise, but more elongated and less rectangular than those obtained from corn starch. Under low magnification they appear as thick needles which group themselves together in star-like clusters. The limit of conversion of this product was found to be 97% and the alkali number, 21.3. It follows, therefore, that the less alcohol soluble fraction of potato starch can be subdivided and that potato, crystalline amylose, is quite likely the most linear member of this fraction. Why the alkali numbers of the various potato fractions should be on a lower level in spite of their higher convertibility as compared to corresponding corn starch fractions is not clear unless the lower alkali numbers are indicative of greater molecular weights or unless combined phosphorus in the case of the amorphous potato fractions retards the alkaline degradation. It is believed significant, however, that the alkali numbers for the products: starch, alcohol less-soluble fraction, crystalline amylose, stand qualitatively in the same ratio to each other for the two starches. For potato they are 6.0, 12.9 and 20.8, respectively. For corn starch they are 11.7, 20 and 35, respectively.

Relation of Crystalline Amylose to Other Amyloses.—It is not yet certain whether crystalline amylose, in its spontaneously precipitated form, differs from originally insoluble gamma-amylose after alkali solution and reprecipitation. They resemble each other superficially, at least, and it is possible that earlier preparations of gamma-amylose contained crystalline amylose.

However, there is reason to believe that gamma-amylose differs from crystalline amylose. First, an experiment, detailed in the Experimental Section, shows that when corn starch is extracted twice with water at 70° a 4.7% yield of gamma-amylose is still obtained from this residue. This yield is practically the same as that obtained from corn starch from which crystalline amylose has not been extracted.

Second, the alkali number of gamma-amylose is definitely higher than that of crystalline amylose, being approximately 42 and, third, the limit of beta-amylase conversion is lower. But it should be pointed out, however, that low conversion limits of insoluble products or products tending to become insoluble are not entirely trustworthy data. Whereas the limit of gamma-amylose was determined[1] at 48%, using our usual concentration of 2 g. of the solubilized amylose per 250 cc. of the conversion mixture, values as high as 70% have been obtained recently by reducing the gamma-amylose concentration to 1 g. per 250 cc. This increase with dilution is in marked contrast to the behavior of whole corn starch, whose conversion limit remains practically constant at about 60% over an even wider concentration range.

It is suggested, then, that the relatively high conversion limit for gamma-amylose, as now obtained, indicates that a considerable portion of the molecule of this constituent is linear also. It may differ from crystalline amylose, however, in being sufficiently shorter in actual chain length to compensate for the effect of a small amount of branching and yet yield a substantially higher alkali number. For there is some evidence that the amount of alkaline degradation products formed in a given time (as lactic acid, in the alkali number determination) is not only influenced by configuration of the carbohydrate, but by chain length as well.[2] It is admitted that further work is needed to establish the relationship between the two amyloses as given and to eliminate the possibility of other differences in composition and constitution.

In contrast, at the other extreme from crystalline amylose, our amorphous, more alcohol-soluble fraction, with an alkali number of only 5.7 and a low beta-amylase conversion limit of 56.6%, is most complex in structure. This complexity arises principally from branching of the molecules through 1,6-alpha glucosidic linked side chains according to the view now generally proposed.

Thus, it would appear that corn starch is made up of a series of molecules which can be grouped as follows. A few are perfectly linear in constitution, while some on the other extreme are most highly branched. In between are those that are relatively long with few branches, and others shorter with an increased number of branches. An additional major difference to be pointed out between corn and potato starch is that the latter apparently contains a larger ratio of the more highly branched fraction (see Table I).

It is apparent that crystalline amylose is but one of the simpler constituents appearing in the butanol-precipitable fraction of whole corn starch pastes, reported by Schoch.[5] We find that this fraction, representing approximately one-quarter of total corn starch has a lower alkali number, 22, and a lower limit of conversion, 81%, than the constituent crystalline amylose for which we have found an alkali number of 35 and a conversion limit of 93% (see Table II).

An additional experiment indicates that crystalline amylose is a constituent of the cotton-adsorbable fraction of corn starch paste reported by Pacsu.[6]

Cotton fibers which had been triply washed in distilled water were stirred intermittently for about eighteen hours in a 0.33% solution of crystalline amylose before the latter was precipitated with butanol in the isolation procedures. Then the cotton was filtered and washed on the filter with distilled water until the wash water was no longer colored blue by iodine. After drying it was found the cotton had adsorbed approximately 1% amylose based on the dry weight of the cotton. The cotton stained blue when allowed to soak in a dilute solution of iodine. The iodine solution retained its original yellow-brown color.

Experimental

Water Extraction and Butanol Precipitation.—550 grams of commercial corn starch (moisture 10.66%) was extracted by gentle stirring in 20 liters of distilled water at 70° and *p*H of 6.6 for one hour. It was filtered by gravity, giving 15.5 l. of crystal clear filtrate with a dry solids content of 25.59 g. The filtrate was evaporated to 2.6 l. *in vacuo* and adjusted to *p*H 4.9 with hydrochloric acid.

The filtrate was divided into two equal portions; to the first, while still warm (about 50°), 130 cc. of butanol was stirred in and the mixture allowed to come to room temperature with stirring over a period of several hours. Precipitation began almost immediately. Being sure the solution was saturated with butanol, it was then refrigerated for four days with occasional stirring. The precipitate was centrifuged, washed twice with 400 cc. of ice-cold water saturated with butanol, then twice with butanol, filtered and dried. The original centrifugate plus the first wash showed a total soluble content of 2.62 g. To the former, methanol was added until the content of the methanol was 15% by volume. Nothing further precipitated although the mixture was allowed to stand several days.

The other half of the starch filtrate was allowed to come to room temperature and refrigerated for four days. A voluminous flocculent precipitate formed which was centrifuged and rewashed in the centrifuge by adding a volume of water equal to that removed by centrifuging, about 1020 cc., and recentrifuging. 0.561 gram of solids were found soluble in the first centrifugate and 0.255 g. in the first wash. The insolubles were then washed twice with 20% methanol, finally twice with methanol and then dried.

The starch residue from the first water extract was heated with 12 l. of water for an additional hour at 70° and *p*H of 6.6 with gentle stirring and a second filtrate of 10.95 l. collected which showed a total solids content of 7.818 g. Evaporated to 780 cc. *in vacuo* and adjusted to *p*H 4.8, it was divided into two equal portions which were separately treated by procedures comparable to those outlined above. Butanol precipitation left behind as solubles 0.721 g., from which it is estimated that 81.6% of the solids had been precipitated. But this time 0.600 g. was left soluble after a spontaneous precipitation, indicating only 84.7% of the product had been precipitated from the second water extract.

In a duplicate run, instead of a second hot water extraction being made as above, 10 l. of cold water was added to the starch, extracted once, and with occasional stirring allowed to stand overnight with 200 cc. of toluene added to it; 9.5 liters of cold water extract was obtained which contained 6.84 g. of solubles, indicating that about 87% of the

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(5) T. J. Schoch, *Cereal Chem.*, **18**, 121 (1941).

(6) E. Pacsu and J. W. Mullen, THIS JOURNAL, **63**, 1168 (1941).

second hot water extract has been rendered soluble in the first hot water extraction, but was held in the water phase of the mass of partially gelatinized starch. The volume of the washed paste was measured and found to be 2.9 l., containing 2.57 l. of water phase. Total grams soluble at 70° is $25.59 + 6.84 + 1.85 = 34.28$ g. per 550 g. corn starch at moisture 10.66%.

gamma-Amylose from Water Extracted Starch.—Five hundred grams of corn starch, which had been twice extracted for one hour with water at 70°, in accordance with the above procedures, was taken up in 2 l. of water to which was added sufficient sodium hydroxide in solution to give a normality of 0.667 N sodium hydroxide. The volume was about 6 liters. It was stirred at room temperature for about an hour, during which time the paste became more or less clear. The solution was then carefully neutralized to pH 6.0 with hydrochloric acid over a period of several hours, and 400 cc. of barley diastase (≎ to extract from 100 g. of ground barley) added. The volume was now 7.5 liters. The mixture was allowed to be converted at 55° for twenty-four hours at pH 6.0 under toluene.

The insolubles were separated by centrifuging, washed twice in the centrifuge with a volume of water equal to the centrifugate removed, and then dissolved in 0.667 N sodium hydroxide to a total volume of one liter. After thirty minutes at room temperature, with stirring, the solution was centrifuged and the centrifugate was neutralized to pH 4.8 with hydrochloric acid. The insolubles were then centrifuged, washed several times in the centrifuge with water, then with increasing concentrations of alcohol until finally 100% ethanol was used. It was washed twice with ethyl ether before drying in a vacuum desiccator over sulfuric acid; yield, 23.7 g. per 500 g. of dry starch.

Summary

Procedures have been given for the isolation of a crystalline amylose from corn starch.

Some of the properties of the amylose are given which relate it to gamma-amylose and which show that it is a constituent of other corn amylose fractions recently reported.

Its configuration is given as a linear arrangement of glucopyranose units.

Corn starch is now believed to be made up of a series of components, a small percentage of which are crystallizable, relatively long in chain length and linear in constitution, others complicated with some branching, and the remainder very highly branched and quite amorphous in nature.

While no product corresponding to gamma-amylose has been obtained from potato starch, the latter yields a crystalline fraction when its pastes are precipitated with alcohols. This fraction contains a crystalline amylose which can be isolated by procedures similar to those used with corn starch.

An additional difference found between corn and potato starch is that potato starch contains a larger ratio of the more highly branched, amorphous fraction.

ARGO, ILLINOIS RECEIVED FEBRUARY 9, 1942

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

The Rearrangement of Phenyl Allyl Ethers. VII. The Isomeric Ethyl p-(α- and γ-methylallyloxy)-benzoates[1]

BY WALTER M. LAUER AND PAUL A. SANDERS[2]

The behavior upon pyrolysis of the two pairs of isomeric ethers, (1) the ethyl p-(α- and γ-ethylallyloxy)-benzoates and (2) the ethyl p-(α- and γ-propylallyloxy)-benzoates, has been described in earlier papers[3] in the present series. Rearrangement products involving an attachment of the β-carbon atom of the allyl group to the aromatic nucleus were produced along with the normal rearrangement products in the case of the γ-substituted allyloxybenzoates, while only normal inversion of the allyl group occurred in the case of the α-substituted allyloxybenzoates. The present study deals with the simplest isomeric pair in this homologous series and the charts indicate the transformations which were carried out.

Pyrolysis of ethyl p-(γ-methylallyloxy)-benzoate, I, gave ethyl 3-(α-methylallyl)-4-hydroxybenzoate, III. It should be noted, however, that this rearrangement product might result from either a normal or abnormal rearrangement process (or both), since attachment of the γ-carbon atom or the β-carbon atom of the allyl group to the aromatic nucleus would result in the formation of the pyrolysis product which was actually obtained.

The isomeric ethyl p-(α-methylallyloxy)-benzoate, II, likewise, gave the pyrolysis product, IV, which would be expected on the basis of a normal

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[1] Paper VI, THIS JOURNAL, **61**, 3047 (1939).

[2] Abstract of Ph.D. Thesis submitted in June, 1940.

[3] Lauer, *et al.*, THIS JOURNAL, **61**, 3047 and 3043 (1939).