

[CONTRIBUTION FROM THE IOWA AGRICULTURAL EXPERIMENT STATION]

The Significance of the "V" X-Ray Diffraction Patterns of Starches¹

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The most familiar and easily obtained types of crystalline X-ray powder pattern exhibited by starches are those designated "A" and "B" by Katz and van Itallie.³ These can be produced from native granules, from retrograded starches, and from materials prepared by slow evaporation of water from starch solutions at various temperatures.⁴ Recently, accurate comparative studies of these two types of pattern have shown a line-for-line similarity and have suggested that the configurations of starch responsible for them are very nearly the same.⁵

Another entirely different modification of starch is responsible for Katz's "V" (Verkleisterung) pattern, which in the present paper is considered in the light of modern developments in the theory of starch structure. Katz and Derksen⁶ believed that this modification, usually prepared by precipitation of starch from pastes or solutions with alcohol, represents an intermediate step between the amorphous conditions of the pasted starch and the B configuration, which finally results from precipitation at ordinary temperatures when sufficient water is present.

It has always been uncertain as to exactly what conditions are necessary for obtaining good V patterns. Samec, Katz and Derksen⁷ state that slight variations in method are very effective in influencing the perfection (sharpness) of V diffraction rings. Aged solutions may even give B patterns instead of V with alcohol precipitation. The present results clarify this situation by distinguishing five variations of V pattern and indicating conditions for securing these.

Experimental

Types of V Pattern.—Since the facts cited here are based on observations too numerous to detail minutely, it is con-

(1) Journal Paper No. J-922 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 639. Supported in part by a grant from the Corn Industries Research Foundation.

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(3) J. R. Katz and T. B. van Itallie, *Z. physik. Chem.*, **A150**, 90 (1930).

(4) J. R. Katz and J. C. Derksen, *ibid.*, **A165**, 228 (1933).

(5) R. S. Bear and D. French, *THIS JOURNAL*, **63**, 2298 (1941).

(6) J. R. Katz and J. C. Derksen, *Z. physik. Chem.*, **A167**, 129 (1933).

(7) See p. 331 of M. Samec, J. R. Katz and J. C. Derksen, *ibid.*, **A158**, 321 (1932).

venient to divide the information according to the type of pattern as follows below. Photographs of the patterns were obtained with a gas-type diffraction tube with copper target and a thin nickel filter. For the purposes of the survey described herein, 3-cm. separations between specimen and film were employed. While experience in this Laboratory and that of Samec, Katz and Derksen⁷ indicates that the type of starch is not too important, in what follows it may be assumed, unless otherwise stated, that the more convenient solubilized (commercial, potato) starch was used.

1. Corresponding to the gummy, bulky starch precipitates obtained when freshly prepared solutions are precipitated with alcohol at room temperatures, one finds that the X-ray patterns of this material are diffuse and "amorphous." They consist of three broad rings, whose widths and relative intensities are somewhat variable.

Figures 1a and 1b show typical examples. The latter, from synthetic potato polysaccharide prepared by R. B. Baldwin of this Laboratory according to the method of Hanes,⁸ is one of the most distinct patterns of this type observed, though another practically identical pattern resulted from synthetic muscle polysaccharide after Cori and Cori.⁹ Presumably the synthetic polysaccharides offer a more homogeneous population of molecules; hence their patterns are more representative of what might be expected in the way of width of ring from purer starch components.

An important feature of the preparation of these amorphous V materials is the necessity of kneading the gummy precipitates with alcohol in order to extract water, which will in time cause the starch to retrograde to the B modification.

2. Starches which give poor V patterns after alcohol precipitations in the cold can be made to yield excellent ones if properly treated. The following typical procedure illustrates how this was ordinarily accomplished. A 4% solution of starch (prepared with hot water on soluble starch, or by autoclaving at 120° with potato or corn starches) was heated to 70° and three to four volumes of hot alcohol added with vigorous shaking and stirring. At sacrifice of yield the resultant granular precipitate was immediately centrifuged off, washed with hot alcohol, and then dried in an evacuated calcium chloride desiccator. Patterns obtained in such cases show principally three almost unbelievably sharp diffraction rings, as can be seen from Fig. 2a.

One of the peculiar things about these V patterns is the fact that in spite of extreme sharpness indicative of good crystallinity, most dried preparations fail to yield much more than the *three* strong rings. A fourth moderately intense ring has been mentioned by Samec, Katz and Derksen⁷ as being observed with certain wheat, potato and amyloextrin (Meyer¹⁰) preparations. In the present

(8) C. S. Hanes, *Proc. Roy. Soc. (London)*, **B129**, 180 (1940).

(9) G. T. and C. F. Cori, *J. Biol. Chem.*, **135**, 733 (1940).

(10) A. Meyer, "Untersuchungen über die Stärkkörner," Jena, 1895.

investigations this ring was, in fact, found with an amyloextrin sample (Fig. 2b) and also in soluble-starch V material (originally exhibiting but three rings) by photographing them after remoistening with absolute alcohol and sealing in thin-walled glass capillaries.

Occasionally even fairly dry preparations yield other rings, but the new ones frequently can be identified with known rings of the A and B patterns. These spurious rings are particularly strong in precipitates which slowly deposit in the alcoholic liquors from the above described centrifugation or in materials that have not been dried thoroughly. It is evident that in dealing with V materials one must be on the alert for such evidences of retrogradation.

3. Another type of powder pattern with rings which are fairly narrow, though not as sharp as with the last described preparations, is that obtained from starch-iodide. Two ways have been used to photograph patterns of this material:

Pure starch-iodide was secured by flocculating a blue starch-iodide solution with ammonium sulfate, centrifuging, briefly washing with water, and then drying in air. The black flakes thus obtained yielded photographs (Fig. 3b) consisting of two fairly strong rings at very nearly the same positions as the innermost and outermost rings of the sharp V patterns described above. In the position where the intermediate ring would be expected could be seen only a faint diffuse indication of doubtful character.

When corn or potato starch granules are treated with potassium triiodide solutions until no more iodine is taken up, then briefly washed to remove excess iodide and iodine, the resultant black powders exhibit patterns which are identical with those of the respective native granules, except that the usual A and B patterns have overlaid upon them the two strong rings of the starch-iodide (Fig. 4). Waxy maize granules, which become only brown with application of potassium triiodide, do not show the starch-iodide rings and their patterns do not contain any evidence for the presence of iodine (Fig. 5).

Several other observations are of interest in connection with iodine coloration of granules and their X-ray patterns. Potato starch granules kept in 15% sulfuric acid for three months lost their ability to form starch-iodide readily, coloring only lightly brown though retaining the normal B pattern of the native granule. However, when gently warmed in iodine-iodide solution on a microscope slide, these granules were observed to become violet to blue before dissolving. Amyloextrin spherocrystals prepared according to the method of Meyer,¹⁰ while exhibiting the A type of X-ray pattern in a typical case, were scarcely able to take up iodine until dissolved, the solution then becoming red-brown.

4. It may be significant that corn and potato starch granules when moistened with glycerol show an effect similar to that observed when these granules are colored with iodine. In the patterns of the glycerol-treated samples, however, only the outermost of the three typical V rings is observed clearly, so that it becomes doubtful whether this is to be termed a manifestation of V characteristics or not. The position of this ring is, nevertheless, characteris-

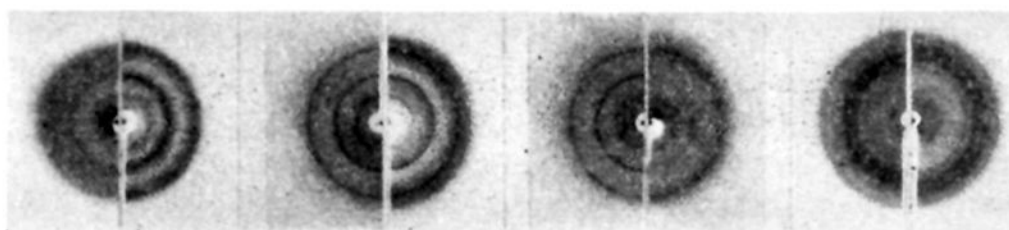
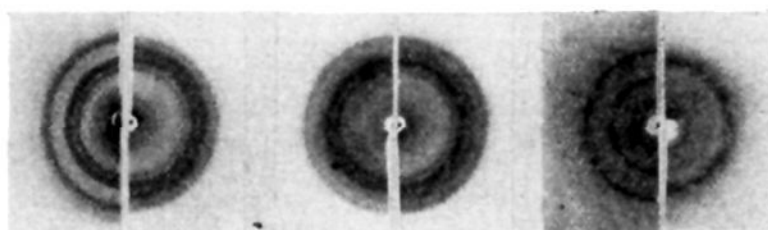


Fig. 1.

a b
Fig. 2.a b
Fig. 3.a b
Fig. 4.a b
Fig. 5.a b
Fig. 6.a b
Fig. 7.

Figs. 1-7.—The figures illustrate various types of V diffraction pattern obtained with starches. They are numbered as follows: 1, the diffuse patterns obtained when starch is precipitated from solution in the cold with alcohol, a using soluble starch, b with synthetic potato polysaccharide; 2, sharp patterns obtained by precipitation of warm solutions by means of ethanol, a using soluble starch, b with Meyer's amyloextrin; 3, comparison of a, the sharp V pattern of alcohol precipitate, with b, that obtained from starch-iodide coagulated with $(\text{NH}_4)_2\text{SO}_4$; 4, comparison of a, the pattern of ordinary cornstarch granules, with b, that of granules blackened with iodine; 5, comparison of a, obtained from iodine-treated waxy maize starch granules with b, a similar photograph of iodized ordinary cornstarch granules; 6, comparison of a, the pattern of ordinary cornstarch granules with b, that of glycerol-treated granules; 7, comparison of V patterns formed by precipitates of starch using ethanol, a, and tertiary butanol, b

tic of the V condition, and it is too distinct to be classified as a halo of the amorphous or liquid type (Fig. 6).

5. In attempts to examine the role of alcohol in the formation of the sharp V patterns, a number of simple liquids capable of acting as starch precipitants were used instead of the usual ethanol. Details of the methods employed need not be repeated except to state that in all cases precipitations were made at as elevated temperatures (up to about 70°) and carried out as rapidly as was convenient. Care was exercised to prevent long contact of the preparations with water, and they were dried before X-ray examination. Methanol, *n*-propanol, *n*-butanol, and acetone readily yielded V preparations whose patterns were indistinguishable (as examined with 3-cm. photographs) from those of samples formed by use of ethanol. In spite of the fact that the butanol is relatively insoluble in water, it readily forms V material, a fact which is of interest in connection with a method of starch fractionation devised by Schoch.¹¹

(11) Cf. p. 127 of T. J. Schoch, *Cereal Chem.*, **18**, 121 (1941). Somewhat after the experiments reported in this paper, through courtesy of Dr. Schoch, his *n*-butanol precipitated fractions of corn

The only two starch precipitating agents tested during these experiments and found unable to yield the usual V material were the tertiary butyl and amyl alcohols. Products of these tertiary alcohols gave practically the same pattern, however, with characteristic departures from the normal (Fig. 7). In these instances very sharp innermost and outermost representatives of the three V rings were present, but only a moderately diffuse halo occupied the place of the usually sharp intermediate interference. In addition the diffractions were markedly shifted closer to the central undiffracted beam, a displacement in the direction of increased interplanar spacings. The outer sharp ring was attended by diffuseness, which may or may not have been due to the essential V configuration.

Quantitative Data.—Table I lists the observed positions of the diffractions found with representatives of the various types of V material. The figures, obtained from 3-cm. photographs, are given in terms of the interplanar spacings, d , calculated from the first order Bragg relation $d = \lambda / (2 \sin \theta)$, where λ is the wave length of the X-rays (1.54 Å. for Cu radiation) and θ is half the angle between the undiffracted and diffracted beams in each case. While this relation may not apply to the more diffuse halos, it offers a convenient means for expressing the experimental results.

TABLE I

PRINCIPAL DIFFRACTION Material and reagent	Temperature of preparation ^a	RINGS OF STARCH V PATTERNS Interplanar spacings, Å.		
Sol. st., ethanol ^{a,b}	R. T.	12	7.0	4.5
Sol. st., ethanol	H. T.	11.5	6.61	4.28
Sol. st.-iodide, (NH ₄) ₂ SO ₄	R. T.	11.9	..	4.35
Native corn or potato granules, KI ₃ ^c	R. T.	12.1	..	4.27
Native corn or potato granules, glycerine ^c	R. T.	4.45
Sol. st., tert.-butanol	H. T.	13.3	..	5.00

^a Sol. st. = soluble starch; R. T. = room temperature; H. T. = about 70°. ^b The rings of this material are very diffuse. For other samples listed in the table only sharp rings are given. ^c Figures given for the treated native granules do not include the A- and B- pattern backgrounds.

Discussion

Though from the diffraction standpoint the V patterns described here are unusually simple and potato starch were subjected to X-ray examination. These preparations, containing almost half by weight of the alcohol, when radiated sealed in a glass capillary yielded patterns containing many sharp diffractions, but bearing, nevertheless, unmistakable V pattern characteristics. This material, after being freed of alcohol by oven drying, became identical to the usual dried V preparations, showing the three strong characteristic diffractions and traces of evidence of retrogradation. It is clear that the alcohol departs from the structure without extensive alterations or rearrangement of the starch chains, since intermediate stages, disclosed by changes of diffraction intensifications, were observed. Study of diffraction-rich V patterns may be expected to further greatly the investigation of this starch modification.

and devoid of sufficient information to permit detailed consideration of the actual structures producing them, the conditions under which they arise are interesting enough to warrant a brief discussion of what a study of V starch material may hold for the theory of starch structure.

Relation of V Configuration to Straight and Branched-Chain Concepts.—The experience of Samec, Katz and Derksen,⁷ as well as of this Laboratory, indicates that all starchlike materials except glycogen are capable of yielding A, B and V crystalline modifications. This seems to apply to synthetic as well as natural products.¹² Meyer and Bernfeld¹³ consider that glycogen is incapable of crystallizing because of excessive chain branching and the shortness of the unbranched chain segments. From this point of view the ability of starches to form any of the crystalline modifications is a property of unbranched chains. This is not to say that branched molecules cannot crystallize; rather must they possess sufficiently long straight-chain portions in order to do so, or to become involved with unbranched molecules in crystalline precipitates.

Relation to Iodine Color.—One gains the impression from the experiments quoted above, in the section on the type of V pattern exhibited by starch-iodide, that A and B starch modifications are incapable of absorbing iodine. Such iodine colors as may be observed with native granules and other forms of A or B solid are probably due solely to starch chains or fragments that may at the moment be unattached and able to form a configuration similar to that of the alcohol-precipitated V modification; gentle warming (of the acid treated granules) or actual dispersion (gelatinization) of the A or B crystallites frees more of the chains to permit development of iodine coloration or deepen that already present.

Relation to Schardinger Dextrin Formation.—Katz and Derksen¹⁴ have pointed out a similarity between the powder pattern of a form of the α -Schardinger dextrin¹⁵ and the V starch pattern. Just which form of " α -diamylose" (the old name) is meant to be used in this comparison is not clear from the paper of Katz and Derksen, though judging from the results of Hess, Trogus and Ulmann and unpublished work of this labora-

(12) R. S. Bear and C. F. Cori, *J. Biol. Chem.*, **140**, 111 (1941).

(13) K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **23**, 890 (1940).

(14) J. R. Katz and J. C. Derksen, *Z. Physik. Chem.*, **A158**, 337 (1932).

(15) K. Freudenberg and R. Jacobi, *Ann.*, **518**, 102 (1935).

tory the hexagonal modification No. 1,¹⁶ is probably the one involved.

While it would be surprising to find that exact comparisons between the powder patterns of molecules of as different size as starch and the α -dextrin are valid, there seem to be other reasons for suggesting a relation between V material and Schardinger dextrans. Freudenberg, Schaaf, Dumpert and Ploetz,¹⁷ starting from models for these dextrans, noted peculiarities which are suggestive for the mode of absorption of iodine by the dextrans and starch. The present demonstration of the relation between iodine color and V pattern supplies a link between these considerations and an observed configuration of starch, and indirectly adds weight to the comparison of the V starch modification with the crystalline dextrans.

Relation of V Patterns to Helical Models for Starch.—The models of Freudenberg, *et al.*,¹⁷ for starch are helices whose interiors are essentially hydrocarbon and are presumed to supply the absorptive power for iodine, causing thus the characteristic blue color. While at present the X-ray information is too meager to permit either confirmation or denial, it is true that the following two types of observation may be in support of such concepts.

1. Cylindrical helices arranged in closest packing possible should show hexagonal arrangement in a plane perpendicular to helix axes. The general diffuseness of atom arrangement in such a system would probably cause all spacings, except those at right angles to spirals and perhaps along them, to be weak. V patterns obtained from dry ethanol-precipitated material do in fact possess chiefly but three or four strong diffractions. It may be suggestive that the innermost two of these possess fairly accurately the spacing ratio $\sqrt{3}$ (*i. e.*, $11.5/6.61 = 1.74$), characteristic of hexagonal arrangements, though whether this is but a coincidence remains to be determined.

2. All of the starch-precipitating molecules which produce the unusually sharp 3-ring diffraction patterns are either small or possess one rather thin dimension. The tertiary alcohols, which cause departures in the direction of longer spacings, are all thicker molecules because of their "bushy" hydrocarbon portions. Such results

(16) K. Hess, C. Trogus and M. Ulmann, *Z. physik. Chem.*, **B21**, 1 (1932). This is also the hexagonal form of "cyclohexaamylose" described by D. French and R. E. Rundle, *THIS JOURNAL*, **64**, in press (1942).

(17) K. Freudenberg, E. Schaaf, G. Dumpert and T. Ploetz, *Naturw.*, **27**, 850 (1939).

would be in agreement with incorporation of the alcohol molecules within starch helices, in a manner similar to that supposed by Freudenberg for iodine, though this interpretation is not a necessary one at present.

Relation to Structure and Swelling Properties of the Starch Granule.—Recent investigations of the Schardinger dextrin yields obtained from starches by action of the appropriate bacterial enzyme have tended to disclose rather remarkably high conversions.^{17,18} The inference has been that the original starches must have contained a considerable fraction of material predisposed to be easily converted into the ring-shaped dextrin molecules; perhaps the original starch molecules are helices, it has been thought. Consideration of the action of the amylases in the degradation of starch led Hanes¹⁹ even earlier to suggest a helical model for starch.

Notwithstanding these suggestions, recent studies of granule structure by optical²⁰ and X-ray⁵ methods have failed to disclose the presence of unusually contracted starch chains. On the other hand, quantitative study of the remarkable tangential swelling of typical starch granules has indicated that an active contraction of radially oriented starch chains is required to explain the changes taking place during gelatinization.²¹ It is also true that the V modifications, described in this paper as most likely to be related to helical or other contracted configuration, are only obtained by precipitation from solution or from otherwise more or less free starch chains. It would appear, therefore, that if connection is to be made between iodine color, Schardinger dextrans, and helical models for starch, it is not to be sought with granular or retrograded material, but rather with freshly dispersed starch molecules in solution.

Apparently the "Verkleisterung" patterns are appropriately named. It seems clear, also, that the further study of the starch modifications exhibiting these patterns is of considerable importance.

Summary

1. Five varieties of the V type of starch X-ray diffraction powder pattern are described, and conditions for the production of these are given.

(18) E. B. Tilden and C. S. Hudson, *THIS JOURNAL*, **61**, 2900 (1939).

(19) C. S. Hanes, *New Phytologist*, **36**, 101, 189 (1937).

(20) A. Frey-Wyssling, *Naturw.* **28**, 78 (1940); *Ber. Schweiz. Bot. Ges.*, **59**, 321 (1940).

(21) R. S. Bear and E. G. Samsa, *Ind. Eng. Chem.*, in press.

2. It is pointed out that in the study of the V modifications of starch are to be sought important connections between iodine-color, Schardinger dextrin formation, and the behavior of starch molecules during the gelatinization process. Re-

lation of these properties to current ideas of straight-, branched-, and helical-chain starch molecules is briefly discussed with reference to the X-ray evidence.

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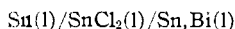
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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CARNEGIE INSTITUTE OF TECHNOLOGY]

A Thermodynamic Study of the Tin-Bismuth System

BY HARRY SELTZ AND F. J. DUNKERLEY

In previous publications¹ electromotive force studies of the thermodynamic properties of several lower melting binary metal systems have been described. In this paper the results of similar measurements on the tin-bismuth system are reported, with cells of the type



Experimental

"Chempure" tin, containing less than 0.01% of impurities, and electrolytic bismuth² were used for the electrodes. The stannous chloride was prepared by passing dry hydrogen chloride over pure molten tin and then resubliming the solid chloride in a stream of carbon dioxide. Since stan-

nous chloride, boiling at 623°, has an appreciable vapor pressure at the temperature of the measurements and since, furthermore, this electrolyte should be entirely in the stannous state, a special design of Pyrex glass cell, Fig. 1, was used for all measurements. Tungsten lead wires were sealed at "A" into two 10-mm. glass tubes bent into the shape of a "J." The short arms of these tubes were connected by a 2-cm. length of tubing to form the electrolyte bridge "B" of the cell. A horizontal boat-shaped tube, "C," was sealed to one of the legs of the cell, and this was connected to a Hyvac pump. In setting up the cell about 2 g. of tin-bismuth alloy of the desired composition was fused under a degassed molten eutectic of potassium and lithium chlorides. After cooling and reweighing, to ensure that no loss had occurred, the alloy button was placed in leg "D" of the cell, which was then sealed at "E." Pure solid tin and solid stannous chloride were placed in the side tube "C" and the entire cell was thoroughly evacuated. Then heat was applied with a burner until they melted, and they were held at this temperature under vacuum for a sufficient time to degas and to ensure complete reduction of the tin in the electrolyte to the stannous condition. The cell was then tilted and the tin and stannous chloride were run into the heated leg "F" of the cell, and finally the electrolyte was run over on the alloy electrode, filling the cell to above the connecting arm. The vacuum was maintained until no further gassing occurred and then the side tube was removed by sealing at the constriction "G." By this technique the space above the electrolyte was filled with stannous chloride vapor and the electromotive force measurements were made with the cell immersed in a fused salt bath to a point above the top of the short legs.

The cells came to equilibrium rapidly at each temperature and the electromotive force was steady and reproducible to ± 0.03 millivolt. After completion of a run with an alloy it was removed from the cell and reweighed. The maximum loss in weight in the several runs was not greater than 1 mg. Measurements were made over a range of about 100°, this range being covered two or three times with each alloy.

In Table I the experimental electromotive force values at 608.1°K. are listed for the several compositions, along with the slopes of the e. m. f.-temperature curves. The activities, a_1 , and the relative heat contents, \bar{L}_1 , for tin, calculated from these data by the usual methods, are given in

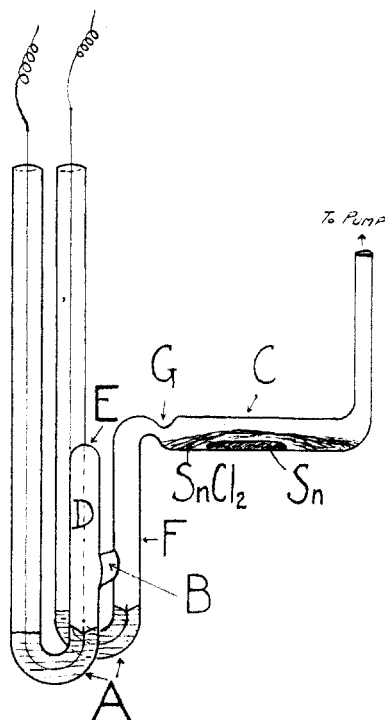


Fig. 1.

(1) Summarized by Seltz, *Trans. Electrochem. Soc.*, **LXXVII**, 233 (1940).

(2) Strichler and Seltz, *THIS JOURNAL*, **58**, 2084 (1936).