

linear relation between the equivalents of titer added and the resistance offered to the conductivity cell had not been established.

Summary

1. Electrometric and conductometric curves for the titration of very dilute solutions of phosphoric acid with sodium hydroxide have been presented.

2. The first stoichiometric point of the reaction is analytically indicated by a maximum in the conductometric curve and by an inflection in the electrometric curve.

3. Within the limits of concentration studied, the second stoichiometric point cannot be ascertained from the second inflection point by either of the methods which were used.

CLEVELAND, OHIO

RECEIVED JUNE 5, 1941

[CONTRIBUTION FROM THE IOWA AGRICULTURAL EXPERIMENT STATION]

The Significance of X-Ray Diffraction Patterns Obtained from Starch Granules¹

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In contrast to the situation with cellulose, little information regarding the structure of starch has been gained from application of X-ray diffraction methods.² However, Katz and his co-workers³ have shown that starches offer a variety of types of diffraction pattern, and once these are interpreted much can be expected from study of the diffraction data.

X-Ray investigation of starches has been limited to powder patterns which, while indicating in many instances a surprising degree of crystalline organization, are relatively weak and diffuse and have offered no great wealth of information. It is impossible, even microscopically, to observe external crystal symmetries, and no one has yet succeeded in obtaining oriented fiber patterns.⁴ In the absence of knowledge concerning geometrical relationships between visible structure and diffraction pattern, and between separate diffractions, the problem is a difficult one indeed.

While it may be unnecessary to abandon hope of securing oriented diffractions eventually, in the present paper this limitation is accepted. In attempting to determine what progress can be made from powder patterns alone, one soon discovers that with starch the variety of patterns exhibited compensates somewhat for the limitations of individual ones. By obtaining accurate information for a number of related patterns, and by comparing line positions and intensities

in these, it is possible to draw conclusions regarding the structures responsible for them.

Experimental

Choice of Materials.—Native granular starches exhibit the most satisfactory degree of crystalline perfection while offering a variety of similar, yet recognizably different, patterns. Náray-Szabó⁵ and Katz and van Itallie⁶ indicate that granular starches yield patterns which are of two chief types, designated by the latter investigators as "A" (cereal starches) and "B" (tuber or potato starches). A "C" class represents intermediates which are usually fairly close to one or other of the extremes. It is of interest to make a detailed comparison of representatives of the A and B types, because of a feeling that similar molecular structures must underlie the observed essential similarities in gross structure and optical properties existing in all types of granule.

For this purpose the present paper describes experiments employing cornstarch as a source of A patterns and potato starch for the B type. Intermediates, chosen to bridge the gap between types partially, are waxy maize starch which yields almost an A pattern, and banana starch whose diffractions depart from the B variety.

Diffraction Methods.—Data required for interpretation of powder photographs must be accurate. In the case of starch, accuracy is difficult to achieve because of a certain amount of lack of definition in the patterns themselves, the low scattering ability of the atoms involved and possibly also the presence of amorphous material diluting the crystalline component. The points of experimental procedure listed below were found essential, and failures to observe one or more of them are responsible for the fact that all previously published data are inadequate for the present purpose.

(1).—Starches must be kept moistened during the photographing of their diffraction patterns. Katz and Derksen⁷ have shown that water is essential to the observation of a crystalline starch pattern. It is equally true that the pres-

(1) Journal Paper No. J-880 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 639. Supported in part by a grant from the Corn Industries Research Foundation.

(2) See H. Mark, *Chem. Rev.*, **26**, 184 (1940).

(3) J. R. Katz, *et al.*, series of papers in *Z. physik. Chem.*, Ser. A, 1930-1939. See particularly those cited below.

(4) See, for example, K. H. Meyer, H. Hopff and H. Mark, *Ber.*, **A62**, 1111 (1929).

(5) St. v. Náray-Szabó, *Ann.*, **465**, 299 (1928).

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

(7) J. R. Katz and J. C. Derksen, *ibid.*, **A150**, 100 (1930).

ence of as much water as the starch will absorb at ordinary temperatures improves the sharpness of the diffractions. Potato starch patterns are very noticeably improved by moisture, cornstarch ones less so. Changes in relative diffraction ring intensities, as well as minor shifts of positions, also result from alteration of water content. To provide a standard condition for the photography of the diffraction patterns, a small excess of water was ordinarily placed along with the starch in thin-walled glass capillaries, whose ends were then sealed to prevent evaporation.

(2).—Fine collimating pinholes or slits must be used to ensure resolution of diffractions reflected at very nearly identical angles. In using a 10-cm. cylindrical camera a slit combination of the following description was found satisfactory: two parallel slits, each 0.25×1.5 mm. and with a separation between them of 10 cm., were interposed between specimen and X-ray tube window. The use of slits aided materially in shortening exposure time.

(3).—Monochromatic radiation is essential to avoid certain false rings that arise from $K\beta$ radiation and confuse regions in which strong diffractions occur. Crystal monochromatization was impractical because of long exposure times required, so that with Cu radiation a nickel foil was employed. The X-ray tube was of the gas type and general radiation was not excessive.

(4).—Calibration of the camera is desirable. In the case of starch investigations a crystalline powder, to be mixed intimately with the moistened starch, would need to have the following characteristics: it should be capable of being finely powdered; it should be chemically stable and inert, insoluble and unable to swell in water; its lattice should be known with fair accuracy; it should show actual diffraction rings up to about 16 \AA .; and it is desirable that it have fair scattering power without too much absorption for Cu $K\alpha$ radiation. All of these requirements are difficult to satisfy in one material, and furthermore superposition of an additional powder pattern upon that of starch would further confuse an already difficult situation. It has been assumed adequate for the starch investigations to calibrate most of the diffraction region with sodium chloride and potassium alum powders, photographed separately but under conditions which were as nearly identical as possible with those used for the starch.

With all of the above experimental requirements satisfied as much as a week of continuous exposure may be required for a single photograph. However, there is no substitute for proper procedure in the case of starch investigations.

Results.—In each of the photographs obtained in the manner described above about two dozen diffraction rings, or approximately twice the number ever before reported in the literature, can be observed. Of these, the inner dozen are most distinct and intense. Only these will be considered here, since to investigate others satisfactorily will require even greater perfection of technique. Limitation to the inner rings is undesirable since as many diffractions as possible must be investigated eventually. However, the diffractions excluded represent the finer details,

up to interplanar spacings of about 3.3 \AA ., and the rest, from 3.3 to 16 \AA ., can be expected to furnish the most important information.

Reproductions of the photographs in the region of interest are given in Fig. 1, while Table I lists the measured values of ring position in terms of the convenient $(2 \sin \theta/\lambda)^2$, which is related to the interplanar spacing, d_{hkl} , according to: $2 \sin \theta/\lambda = 1/d_{hkl}$. θ is half the angle of diffraction observed with radiation of wave length λ . For purposes of orientation Table I also indicates relative intensities for the corn and potato starch patterns, and d_{hkl} for the latter.

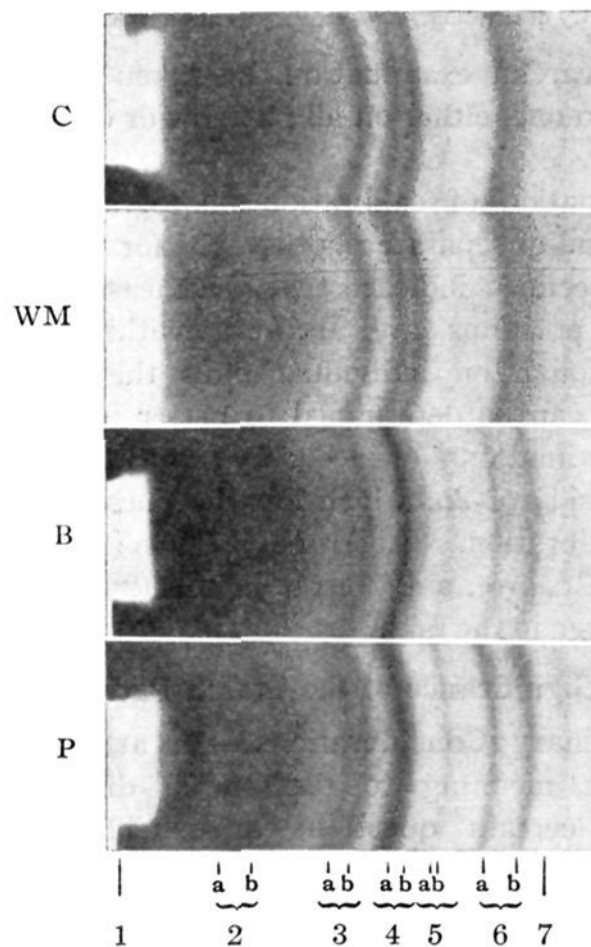


Fig. 1.—Powder patterns of moistened starch granules: C, corn; WM, waxy maize; B, banana; P, potato. The figures and letters at the bottom refer to the ring designations used in the first column of Table I. A patterns are at the top, B at the bottom.

In the interest of brevity, comparison of the results given here is not made with those published by other investigators. In many instances close doublets, previously noted only as diffuse or probably double, have been clearly resolved. There is some advantage in retaining the nomenclature of Katz and van Itallie,⁶ as is done in the first column of Table I with modification to allow for several new lines. In this designation numbering starts with inner rings and progresses outward, the letters indicating rings which might,

TABLE I
 PROMINENT RINGS IN THE DIFFRACTION PATTERNS OF GRANULAR STARCHES

No.	d_{hkl} , Å.	<i>I</i>	Potato			Banana			<i>E</i> , %	Indices (<i>hkl</i>)	Waxy maize			<i>E</i> , %	<i>I</i>	Corn		
			$\left(\frac{2 \sin \theta}{\lambda}\right)^2 \times 10^4$ Obsd.	$\left(\frac{2 \sin \theta}{\lambda}\right)^2 \times 10^4$ Caled.	<i>E</i> , %	$\left(\frac{2 \sin \theta}{\lambda}\right)^2 \times 10^4$ Obsd.	$\left(\frac{2 \sin \theta}{\lambda}\right)^2 \times 10^4$ Caled.	<i>E</i> , %			$\left(\frac{2 \sin \theta}{\lambda}\right)^2 \times 10^4$ Obsd.	$\left(\frac{2 \sin \theta}{\lambda}\right)^2 \times 10^4$ Caled.	<i>E</i> , %			$\left(\frac{2 \sin \theta}{\lambda}\right)^2 \times 10^4$ Obsd.	$\left(\frac{2 \sin \theta}{\lambda}\right)^2 \times 10^4$ Caled.	<i>E</i> , %
1	15.8	s	40.2	38.3	5.0	40.9	38.7	5.7	(100)	38.8	41.6	-6.8	o		
2a	8.9	f	126	120	5.0	125	121	3.3	(010)	127	126	0.8	vf	128	127	0.8		
2b	7.88	vf	161	159	1.3	161	160	0.6	($\bar{1}$ 10)	161	161	...	vf	160	160	...		
3a	6.36	m	247	247	...	251	251	...	(001)	o		
3b	5.92	ms	285	285	0	286	290	-1.4	(101)	290	290	...	s	290	290	...		
4a	5.22	s	367	367	0	369	372	-0.8	(011)	366	366	...	s	367	367	...		
4b	...	o	412	411	0.2	($\bar{1}$ $\bar{1}$ 1)	425	430	-1.2	s	425	428	-0.7		
5a	4.56	f	481	481	...	483	483	...	(020)	504	504	...	f	506	506	...		
5b	4.40	vf	517	519	-0.4	525	522	0.6	(120)	o		
6a	4.04	ms	614	614	...	615	615	...	(400)	666	666	...	s	668	668	...		
6b	3.70	ms	732	728	0.5	737	734	0.4	(021)	726	723	0.4	m	730	727	0.4		
7	3.42	m	855	861	-0.7	861	866	-0.6	(401)	876	876	...	m	882	882	...		

I = intensity description; *E* = error; s = strong; ms = moderately strong; m = moderate; f = faint; vf = very faint; o = absent.

without careful examination, be taken as combined into one, either on all patterns or on one of them.

Examination of calibration photographs and comparison of separate photographs for the same starch specimen indicate that the measured diffraction positions are accurate within about 0.5%, though on individual films the relative positions can be determined to better than this. These estimations of accuracy apply only to rings outside of 2b, which are the ones covered by the calibration. An error of 0.5% in $(2 \sin \theta / \lambda)$ means, of course, a 1% error in the $(2 \sin \theta / \lambda)^2$ values given in the table.

The Significance of the Starch Patterns

Preliminary Considerations.—In attempting to assign meaning to the starch diffraction patterns certain questions arise. These are stated below and answers are indicated.

Since starch granules are not in the ordinary sense pure crystalline materials, is it necessary to assume that all of the diffraction rings are caused by a single crystalline starch component? The answer seems to be in the affirmative. Especially purified samples, such as those which have been electro-dialyzed or subjected to prolonged treatment to remove fatty materials, show patterns which are identical with those prepared without special precautions. Retrogradation, a sort of recrystallization process, allows one to precipitate from a starch solution insoluble material which gives diffractions typical of granular starches ordinarily exhibiting a B pattern.⁶ During the present investigations the observation of Katz and Derksen⁸ that A patterns can be

obtained with starches recrystallized from concentrated solutions at higher temperatures has been confirmed. Finally the demonstrations that synthetic potato⁹ and muscle¹⁰ polysaccharides yield typical starch patterns would seem to decide the matter beyond all doubt.

What is the relation between the A and B types of diffraction? Do they represent very different modifications or are they indicative of but slight variations in starch chain organization? An assumption made below is that in going from an A to a B modification relatively slight alterations in unit cell dimensions and angles are necessary. As a consequence, rings in corresponding locations on the two patterns are taken to have arisen from corresponding atomic planes of the similar structures. The correctness of this view is particularly apparent in Fig. 2, in which are shown photographs of materials prepared from the same commercial soluble starch sample at various temperatures according to the method of Katz and Derksen mentioned above. In each instance equal quantities of starch and water were subject to autoclaving (120°), let stand for twenty-four hours at the temperature indicated in the figure, and finally allowed to dry in air at the same temperature. Small pieces of the opalescent glassy solids thus obtained gave surprisingly good "crystal" patterns, those prepared below 50° yielding patterns resembling the B type, those at 50° and above manifesting chiefly the A characteristics. While in the present paper the granule series has been used because of superiority in pattern distinctness, the temperature series is important because it shows that all intermediate stages

(8) J. R. Katz and J. C. Derksen, *Z. physik. Chem.*, **A165**, 228 (1933).

(9) W. T. Astbury, F. O. Bell and C. S. Hanes, *Nature*, **146**, 558 (1940).

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

between the pure A and B extremes can be prepared.

With the above criterion of temperature behavior used to place corresponding individual diffractions side by side in Table I no crossing over or coalescence of diffractions has been necessary. Of the various differences between the patterns, most striking are the following: In the pure B pattern of potato starch the ring 4*b* is missing, while this same ring is very strong in cornstarch patterns. However, 4*b* is faintly observed with banana starch, which is otherwise typically B. On the other hand, the pure A or cornstarch pattern is lacking in the 1, 3*a* and 5*b* rings, the absence of the 1 ring being the most noticeable. This ring is, however, present in waxy maize, which is otherwise typically A.

Starch Unit Cells.—It is theoretically possible to determine unit cells of crystalline materials from powder patterns alone provided sufficiently accurate measurements of diffraction positions are available and certain important diffractions closest to the central undiffracted beam are not missing or too faint to be observed. Runge¹¹ has described how this may be done for any crystal system. Unfortunately, his method cannot often be carried out successfully, largely because of the habit of most crystalline materials of halving one or more of the spacings along axis directions. With starch one is compelled at least to try Runge's procedure, and one expects that success is most likely to be attained by using it with the B patterns, for these possess the fullest complement of observable diffractions, particularly showing clearly the innermost 1 ring.

Fortunately, with potato and banana starch diffractions Runge's method appears to work out in a very straightforward manner. A probable reason for its success in these cases will be given below. As a result of its application the indices, apart from sign, were determined as given in the central column of Table I. Although the indices were thus chosen, in the interest of brevity they can be assumed. It can then be determined how accurately they account for the starch diffractions.

Potato and banana patterns are so closely alike that for present purposes they may be taken as essentially the same. Therefore, the fact that one set of indices can be used to explain both cannot be taken as very good proof of the cor-

(11) C. Runge, *Physik. Z.*, **18**, 509 (1917).

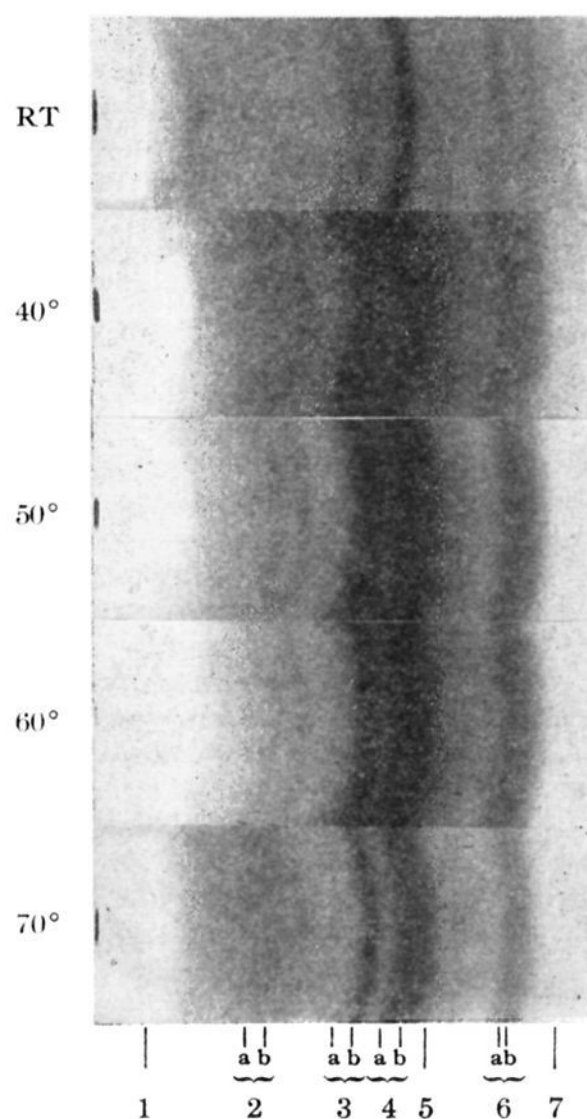


Fig. 2.—Powder patterns of soluble starch dried in air from concentrated solutions at various temperatures: RT, at room temperature; others at the centigrade temperature indicated. The figures and letters at the bottom refer to the ring designations used in the first column of Table I. A patterns are at the bottom, B at the top.

rectness of the assignments. In a certain sense the chief value of oriented or single crystal patterns in the case of the usual X-ray crystallographic investigations is that of checking index assignments by determining whether the diffractions have appropriate geometrical relationships. In the absence of the possibility of applying this method of confirming indices for starch one may ask whether another is available.

Frevel¹² has suggested using temperature variations in unit cell dimensions, hence the resulting diffraction displacements, to confirm the indexing of powder patterns. In the case of the starches much the same possibility arises when it is admitted, as developed in the preceding section, that A and B patterns arise from similar structures whose unit cells may be expected to be slightly but measurably different. A test for

(12) L. K. Frevel, *Phys. Rev.*, **51**, 997 (1937); *J. Appl. Phys.*, **8**, 553 (1937).

the correctness of the assignments can be made by determining with what success they can be applied to the A patterns of waxy maize and corn.

In the most general case the relation between the halved diffraction angle θ , wave length λ and the planar indices can be shown to be

$$(2 \sin \theta / \lambda)^2 = Ah^2 + Bk^2 + Cl^2 + Dhk + Ekl + Fhl \quad (1)$$

in which A, B, C, D, E and F are constants for any one crystalline material. These constants are related to unit cell dimensions a_0, b_0 and c_0 and angles α, β and γ according to¹³

$$\begin{aligned} A &= \sin^2 \alpha / a_0^2 \Delta \\ B &= \sin^2 \beta / b_0^2 \Delta \\ C &= \sin^2 \gamma / c_0^2 \Delta \\ D &= 2(\cos \alpha \cos \beta - \cos \gamma) / a_0 b_0 \Delta \\ E &= 2(\cos \beta \cos \gamma - \cos \alpha) / b_0 c_0 \Delta \\ F &= 2(\cos \alpha \cos \gamma - \cos \beta) / a_0 c_0 \Delta \end{aligned}$$

and

$$\Delta = 1 + 2 \cos \alpha \cos \beta \cos \gamma - \cos^2 \alpha - \cos^2 \beta - \cos^2 \gamma$$

Given six pairs of values for $(2 \sin \theta / \lambda)^2$ and associated indices, it is possible to determine from these equations the six lattice constants.

In attempting this with the figures of Table I it is soon found that for the B patterns within experimental error D, E and F are zero, hence that $\alpha = \beta = \gamma = 90^\circ$ and the axes are orthogonal. For the calculated values of Table I the three rings with indices (001), (020) and (400) have been used to determine A, B and C for substitution in equation 1.

For the A patterns relatively small but definitely non-zero values of D, E and F are required, while A, B and C are not altered greatly. Unfortunately, to determine these constants most of the diffractions must be used (these indicated by the dashes in the error columns), although several are left over for confirmation.

The constants indicated for the various types of starch granule are given in Table II. The ex-

TABLE II

CONSTANTS DESCRIBING STARCH DIFFRACTIONS						
	Av. B modifica- tion $\times 10^4$	Potato $\times 10^4$	Banana $\times 10^4$	Waxy maize $\times 10^4$	Corn $\times 10^4$	Av. A modifica- tion $\times 10^4$
A	38.5	38.3	38.7	41.6	41.8	41.7
B	120.6	120.3	120.8	126.0	126.5	126.3
C	249	247	251	261	260	260.5
D	0	0	0	6.6	8.3	7.5
E	0	0	0	- 21.0	- 19.5	- 20.3
F	0	0	0	- 12.7	- 11.3	- 12.0

(13) See "Internationale Tabellen zur Bestimmung von Kristallstrukturen," Bd. I, Gebrüder Borntraeger, Berlin, 1935, p. 69.

cellence of fit thus obtained is indicated in the error columns of Table I, which give differences between measured and calculated values of $(2 \sin \theta / \lambda)^2$ in percentages. The agreement is remarkable except for the innermost two rings, where errors are apt to be higher. It must be remembered, however, that errors in actual measurement of the first power of $\sin \theta$ are only half those given.

Table III lists the results of conversion of the constants to the unit cell dimensions and angles.

TABLE III
DIMENSIONS OF STARCH UNIT CELLS

	a_0 , Å.	b_0 , Å.	c_0 , Å.	α , °	β , °	γ , °	Vol., Å. ³
Av. B mod.	16.1	9.11	6.34	90.0	90.0	90.0	930
Av. A mod.	15.4	8.87	6.18	87.0	86.9	92.8	843

It is apparent that the cells for A and B modifications are indeed quite closely similar. While B cells are referable to orthogonal axes, it seems improbable that they can be truly orthorhombic. The line-for-line correspondence between A and B patterns would seem to indicate this, for if the difference were actually one of symmetry, the (110) diffraction in the B pattern, for example, should break up into two, representing (110) and ($\bar{1}$ 10) planes, in the definitely triclinic A pattern. From one point of view the non-orthogonality of the A axes is a fortunate circumstance, for it allows an assignment of indices which is complete even as to signs, except in the case of the $5b$ or (120) ring, which is present only in the B patterns. It is a curious fact that all of the angles for the A cell depart from 90° by about the same amount, namely, 3° .

The Number of Glucose or Maltose Residues per Cell.—The determination of the number of glucose residues in a unit cell of starch cannot be done with as great certainty as is usual in crystallographic investigations. The granules have undoubtedly a colloidal organization, and densities and compositions of whole granules may not be those of the actual starch crystallites responsible for the X-ray patterns. The closeness with which the whole-granule figures may be taken to approach those for the crystallites depends on what is assumed to be the proportion of the granules made up by the crystalline component.

It already has been shown that no other crystalline component is very important. Is there any evidence in the diffraction patterns for components which may be amorphous in character?

Though there is always background blackening of films, at least in the times necessary to secure good development of the crystalline interferences, one does not observe any broad but localized bands characteristic of amorphous materials. It therefore seems probable that a large fraction of the starch of the granule is in the form whose crystal pattern is evident. Also, starch which may be more poorly organized can perhaps be expected to have nearly the same hydration and density as the crystalline material, hence the assumption seems justified that whole granule and crystallite values may not be too different, at least for air-dried granules in which "pore-swelling" is not too significant.

At any rate an estimate of the number of residues per cell can be attempted from the figures 1.50 for density and 18% for water content, taken as representative of air-dried potato starch from data found in Samec's book.¹⁴ These values, along with the unit cell given in Table II, which does not change significantly from the air-dried to the fully hydrated state, suggest a number of glucose residues per cell equal to four within a few per cent. Considerable deviations in density and water content could be allowed before this number would be changed to the next higher or lower integral value. Furthermore, there probably should be an even number of glucose residues, hence an integral number of maltose residues, per cell. Apparently there are two maltose residues per cell, probably in all granular starches since their unit cells, densities and water contents are similar.

Space Group.—If starches belong to the triclinic system it becomes a simple matter to determine the proper space group. Of the two triclinic space groups, C_1^1 and C_i^1 , only the former permits optical activity. While optical activity of starch crystallites, or even of granules, cannot be demonstrated directly, the fact that hydrolysis leads to a single optical isomer permits one to conclude that the original material must also have been able to rotate polarized light.

According to this reasoning the starch space group is therefore C_1^1 , which has lowest possible symmetry. No systematic absences of diffractions are required, which may account for the success with which the Runge method of powder pattern analysis could be applied above.

(14) M. Samec, "Kolloidchemie der Stärke," Verlag von Theodor Steinkopff, Leipzig, 1927, pp. 97 and 106.

Discussion

Criticism of Earlier Suggestions Regarding Starch Unit Cells and Symmetry.—Four attempts have been made by other investigators to determine unit cells, axial ratios or otherwise come to conclusions as to crystal symmetry and molecular periodicities in starch from X-ray investigations. These are briefly criticized in the light of present knowledge in the following paragraphs:

1. Herzog and Jancke¹⁵ believed that cereal starch crystallites have axial ratios 0.7252 : 1 : 0.5509, with orthogonal axes. These ratios appear to have no simple relation to the axial lengths suggested above, nor are the cereal starch angles truly orthogonal. Herzog and Jancke gave no experimental data, so it is impossible to see precisely wherein they differ from present conclusions.

2. Sponser¹⁶ concluded that the unit cells for various starches are tetragonal, with $a_0 = b_0 = 5.94 \text{ \AA.}$, $c_0 = 5.05 \text{ \AA.}$, and that there is one glucose residue per cell. Apart from the fact that such a high degree of symmetry is not to be expected of a cell containing only one asymmetric residue, this cell is unable to account for several of the innermost rings, which Sponser did not observe.

3. Basing his conclusions on rough calculations using the density of starch and the positions of the innermost diffraction ring observed, Ott¹⁷ decided that the maximum possible number of glucose residues per cell of starch is two. From his published photographs and the fact that his innermost ring had an interplanar spacing of 5.21 \AA. it is clear that his data were at fault.

4. Náray-Szabó⁵ made the best of these attempts and arrived at a tetragonal cell $a_0 = b_0 = 16 \text{ \AA.}$, $c_0 = 9.8 \text{ \AA.}$, with 16 glucose residues per cell. This description was thought to hold for all types of starch, though he recognized that there are two chief varieties of starch pattern. Judging from experience with other carbohydrates it is scarcely to be expected that starch show as much symmetry, but in any event many of the measurements with which Náray-Szabó worked were those of unresolved rings.

Validity of the Unit Cells Suggested.—The following arguments can be advanced in support of a belief that the unit cells proposed in this paper may be the true unit cells of starches:

(15) R. O. Herzog and W. Jancke, *Ber.*, **B53**, 2162 (1920).

(16) O. L. Sponser, *J. Gen. Physiol.*, **5**, 757 (1920).

(17) E. Ott, *Physik. Z.*, **27**, 174 (1926).

1. They account accurately and simply for diffraction positions, for the hitherto unexplained similarities between A and B patterns, and for the observed continuous transition from one type to the other.

2. Their volumes are the smallest necessary for this.

3. It is reassuring to note that assignment of indices for diffraction according to them has not been forced, as witness the sample indices of Table I.

4. As nearly as can be ascertained at present, they contain integral numbers of glucose or maltose residues.

5. While not proof of their validity, it is gratifying to note that the number of residues per cell is the same as has been suggested for the two varieties of cellulose.¹⁸ The number of residues per cell is sufficient to build structures which are at least as complex as those which have been described for the celluloses.

While the proposed unit cells are open to the criticism of having been (necessarily) derived from the relatively few powder diffractions exhibited by starches, the data of the present paper do establish clearly the close relationship between the A and B types of pattern, whether these be observed with native granules or produced by variation of temperature of crystallization.

Meaning of the Cells for Starch-Granule Organization.—The uniaxial character of starch granule double refraction of light would seem to demand that the triclinic crystallites be arranged with random orientation about one of their axes, this one axis in each crystallite remaining more or less in coincidence with the granule radius through its vicinity. Whether starch chains or spirals terminate with crystallite boundaries; what axis it is that runs radially; whether the diffuseness of starch diffractions is caused by small crystallites or by imperfect ones—these are all questions which cannot be answered as yet. It is clear that a colloidal organization rather than that of a perfect crystal is demanded. Several comments regarding the relation of the present diffraction results to certain much discussed viewpoints of the structure of starch may be offered:

1. The fact that granular starches, like cellulose, contain two disaccharide units (maltose,

(18) K. H. Meyer and L. Misch, *Helv. Chim. Acta*, **20**, 232 (1937); K. R. Andress, *Z. physik. Chem.*, **B4**, 190 (1929).

cellobiose) per cell, and comparisons of the starch cells with those of simple carbohydrates related to starch, to be published subsequently, suggest that a straight unbranched chain structure may suffice to account for the diffraction data under consideration as has been found sufficient with cellulose. However, this may not deny the possibility of branching chains,¹⁹ since diffractions from a structure whose unit cell contains only four glucose residues cannot be expected to tell much regarding a situation which is supposed to occur once approximately every 24–30 residues. The fact that glycogen, which is said to represent an α -glucose polymer with considerable branching, cannot be made to yield any of the crystalline starch diffraction patterns²⁰ suggests also that these originate from configurations assumed by unbranched chains.

2. The number of glucose residues per cell of starch, four, is not very suggestive of spiral models for starch chains,²¹ since those which have been proposed call for periodicities of about six. This does not exclude the possibility that spiral formations are present in starch under other conditions. Frey-Wyssling²² has noted the difficulty of accounting for the sign of the double refraction of starch granules on the basis of spiral chains arranged radially.

The above experimental facts and conclusions apply only to the configurations existing in starch granules and in retrograded starches, *i. e.*, in general to cold-water-insoluble material. Another crystalline starch spectrum, termed "V" by Katz, *et al.*,²³ is exhibited by starches precipitated from aqueous solution or paste in various ways, *e. g.*, with alcohol. In this form, the starch is often readily redispersible by cold water. The significance of its diffraction pattern is under investigation.

Summary

1. By careful investigation of representative starch granule diffraction patterns, using moistened samples and a well collimated beam of

(19) K. H. Meyer, W. Brentano and P. Bernfeld, *Helv. Chim. Acta*, **23**, 845 (1940); K. H. Meyer and P. Bernfeld, *ibid.*, **23**, 890 (1940); E. L. Hirst and G. T. Young, *J. Chem. Soc.*, 951, 1471 (1939); H. Staudinger and E. Husemann, *Ber.*, **B71**, 1057 (1938).

(20) M. Samec and J. R. Katz, *Z. physik. Chem.*, **A158**, 321 (1932).

(21) C. S. Hanes, *New Phytologist*, **36**, 101, 189 (1937); K. Freudenberg, E. Schaff, G. Dumpert and T. Ploetz, *Naturwissenschaften*, **27**, 850 (1939).

(22) A. Frey-Wyssling, *ibid.*, **28**, 78 (1940).

(23) J. R. Katz and L. M. Rientsma, *Z. physik. Chem.*, **A150**, 60 (1930); J. R. Katz and J. C. Derksen, *ibid.*, **A167**, 129 (1933).

monochromatic X-radiation, accurate measurements of resolved diffraction rings have been obtained.

2. Evidence is cited which indicates that the diffractions are those of a single major component of the starch granules, and that there is ring-for-ring correspondence between the extreme (A and B) types of pattern.

3. From analysis of potato (B) patterns, it is possible to set up a unit cell and arrive at indices of planes responsible for the individual diffractions. These assignments can be carried over

to the corn (A) starch patterns with comparatively slight changes in unit cell dimensions and angles, thus providing confirmation. The number of maltose residues per cell seems to be two. Starch granule crystallites are apparently built on triclinic lattices whose axes are, however, very nearly orthogonal.

4. The validity of the chosen unit cells and the relation of the results to straight, branched and spiral chain models for starch are discussed briefly.

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RECEIVED APRIL 28, 1941

[CONTRIBUTION FROM THE GEORGE HERBERT JONES LABORATORY, THE UNIVERSITY OF CHICAGO]

Factors Determining the Course and Mechanisms of Grignard Reactions. I. Preliminary Study: The Effects of Metallic Compounds on Some Grignard-Carbonyl Interactions

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In an earlier study in this Laboratory¹ it was found that when pure, magnesium-free, ethereal Grignard reagents are used, the nature of the Grignard-carbonyl interaction (whether reduction or addition) is determined (for some carbonyl compounds, at least) by the nature of the organic radical of the Grignard reagent. As a preliminary and a supplement to more extensive investigation in this field the catalytic effects of several metallic compounds have been examined in the present study.

It is well-known that various metals (including magnesium) and metallic compounds significantly affect the yields, the nature of the products obtained, and the rates of reaction in various Grignard reactions, and, further, that some interactions that do not otherwise take place may be effected in the presence of metallic catalysts.² Accordingly, the purest supply of reagent magnesium available was sought for these studies. A sample of sublimed magnesium, kindly donated by the Dow Chemical Company, was found to be spectrographically free of manganese, copper, tin, iron, and silver.³ The only heavy metal

detected, lead, was estimated to be present to the extent of approximately 0.01%.

Initial studies were made on the benzophenone-isobutylmagnesium bromide interaction, which normally gives high yields of a single product (benzohydrol). Cuprous chloride in concentrations up to 1.0 mole % was substantially without effect on the course of the reaction and the yields obtained. In the presence of manganous chloride, however, the addition of the first few drops of ketone solution to the Grignard solution gives rise to the deep cherry-red coloration characteristic of the magnesium ketyls, and the reaction product upon hydrolysis yields benzopinacol as well as benzohydrol. The proportion of benzopinacol in the product varies directly with the concentration of manganous chloride up to 2.0 mole %. The effect of chromic chloride is similar to that of manganous chloride, but less pronounced. A single experiment with 2 mole % of ferric chloride indicated activity similar in kind and degree to that of chromic chloride (71% benzopinacol). Experimental data are summarized in Figs. 1 and 2. The total yields of the hydrol and pinacol can be computed easily from the two curves.

The observations recorded show that benzophenone may undergo either a two-electron or a one-electron Grignard reduction, the former leading to benzohydrol, and the latter to benzopinacol.

(1) Kharasch and Weinhouse, *J. Org. Chem.*, **1**, 209 (1936).

(2) See, e. g., Gilman, *et al.*, (a) *Rec. trav. chim.*, **48**, 155 (1929); (b) **50**, 578 (1936); (c) *THIS JOURNAL*, **53**, 1581 (1931); (d) Young, Prater and Winstein, *ibid.*, **55**, 4908 (1933); Johnson and Adkins, (e) *ibid.*, **53**, 1520 (1931); (f) **54**, 1943 (1932); Job, *et al.*, (g) *Bull. soc. chim.*, [4] **41**, 814 (1927); (h) [4] **47**, 279 (1930).

(3) The writers are indebted to Dr. W. C. Pierce and Mr. W. W. Marshall for spectrographic analyses of several magnesium samples.