The analytical reaction described above was performed at room temperature. When the N-methylated porphyrin was treated with methylmagnesium iodide at 100°, more methane was liberated and part of the porphyrin was converted back to etioporphyrin II, as shown by identification of the porphyrin after its liberation from the magnesium salt by acid. Acid treatment of the magnesium salt of N-methyletioporphyrin II obtained at room temperature, in contrast, yielded unchanged N-methyletioporphyrin II.

Summary

1. N-Methyletioporphyrin II has been prepared and its properties and analysis recorded.

2. The absorption spectrum of this compound shows a shift of all the characteristic porphyrin bands toward the red.

3. Oxidation of the N-methylporphyrin yielded N-methylethylmethylmaleic imide.

4. Thermal decomposition of N-methyletioporphyrin II at its melting point or treatment of it with methylmagnesium iodide at 100° followed by acidification regenerated etioporphyrin II.

5. N-Ethyletioporphyrin II has also been prepared and analyzed.

6. Attempts to prepare N-propyl- and N-butylporphyrins by direct alkylation met with failure.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF IOWA STATE COLLEGE]

The Complexes of Fatty Acids with Amylose[1]

BY F. F. MIKUS, R. M. HIXON AND R. E. RUNDLE

Recent observations by Schoch and Williams[2] that fatty acids interfere with formation of the amylose–iodine complex and selectively precipitate the amylose (unbranched) component of starch have led us to study the interaction between fatty acids and the components of starch. Our interest in this problem arose because it appeared likely that amylose and the fatty acids formed molecular compounds or complexes similar to those which amylose forms with iodine[3] and the alcohols.[3,4] The influence of the tenaciously held fatty materials commonly associated with starch on the physical properties of starch suspensions, pastes and gels is of practical as well as academic interest.

The type of linkage between starch and its "fat-by-hydrolysis" (*i.e.*, that part of the fatty material which cannot be extracted from starch by ether, carbon tetrachloride and the other common fat solvents) has been the subject of several investigations. Early workers were inclined to believe that "fat-by-hydrolysis" was bound to starch by a chemical bond, such as the ester linkage. More recently Schoch[5] has found that "fat-by-hydrolysis" can be extracted from starch almost completely by methanol, 80% dioxane and other hydrophilic fat solvents. By use of solutions of fatty acids in these solvents he was able to reintroduce fatty acids into defatted starches. The reintroduced fatty acids were bound as tightly as "fat-by-hydrolysis", *i.e.*, they were not removed by prolonged carbon tetrachloride extraction, but were removed by methanol. Schoch's work has generally been acknowledged as adequate evidence that the "fat-by-hydrolysis" is not combined by the ester linkage or similar primary chemical bond.

Following the publication of Schoch's work, adsorption has been gaining favor as an explanation of the interaction between starch and "fat-by-hydrolysis."[6,7] The evidence presented below precludes surface phenomena and indicates quite conclusively that the mechanism of starch–fatty acid interaction is similar to that previously advanced for the complexes which amylose forms with iodine and the alcohols.[3,4]

X-Ray Study of the Fatty Acid Complexes

Schoch and Williams[2] have reported that amylose is precipitated by fatty acids in a microcrystalline condition. The crystalline form is unlike that of amylose precipitating from water solution spontaneously, as shown by both optical and X-ray examination. The birefringence of the material is very pronounced. All evidence points to the fact that the fatty acid is contained within the crystalline regions and is responsible for this particular crystalline form of the amylose.

Microscopic observation of the birefringent product of this precipitation indicated that the precipitate was similar to the butanol precipitate of amylose. Since the butanol precipitate has been examined by X-rays,[3d] the most direct way of comparing the fatty acid precipitate with the butanol precipitate appeared to be through X-ray diffraction.

X-Ray Samples and Diagrams.—The method of Schoch and Williams[2] was used to obtain amylose–fatty acid precipitates. Interest in the vari-

(1) Journal Paper No. J-1292 of the Iowa Agricultural Experiment Station, Project 639. Supported in part by a grant from the Corn Industries Research Foundation. The major portion of this paper was presented before the Division of Sugar Chemistry and Technology of the American Chemical Society at the Cleveland Convention, April 6, 1944.

(2) T. Schoch and C. Williams, THIS JOURNAL, **66**, 1232 (1944).

(3) (a) R. Rundle and R. Baldwin, *ibid.*, **65**, 554 (1943); (b) R. Rundle and D. French, *ibid.*, **65**, 558 (1943); (c) *ibid.*, **65**, 1707 (1943); (d) R. Rundle and F. Edwards, *ibid.*, **65**, 2200 (1943); (e) R. Rundle, J. Foster and R. Baldwin, *ibid.*, **66**, 2116 (1944).

(4) R. Bear, *ibid.*, **66**, 2122 (1944).

(5) T. Schoch, *ibid.*, **60**, 2824 (1938).

(6) L. Lehrman, *ibid.*, **64**, 2144 (1942).

(7) R. Whistler and G. Hilbert, *ibid.*, **66**, 1721 (1944).

TABLE I

AMYLOSE–FATTY ACID PRECIPITATES

Indices	Intensity[b] I	Intensity[b] II	$\sin^2\theta$ (observed)[a] Dried butanol ppt.	Oleic amylose (dry)[c]	Palmitic amylose (dry)[c]	Palmitic amylose (wet)	Lauric amylose (wet)
(110) (020)	M	M	0.0042	0.0043	0.0042	0.0042	0.0040
(011)	VW	VW	.0103	.0106	.0103	....	....
(200) (130)	S	VS	.0126	.0126	.0126	.0124	.0124
(210)	MW	M	.0137	Not resolved	.0135	....	.0134
(040) (220)	VVW	VW	.0166	.166	.0164	.0162	.0159
(131) (201)	VW	W	.0216	.0216	.0219	.0215	....
(221) (041)	W	W	.0262	.0258	.0261	.0256	.0255
(310) (150)	VS	VS	.0295	.0295	.0297	.0290	.0289
(320)	W	S	.0326	....	.0320	....	....
(330) (060)	M	MS	.0379	.0380	.0378	.0370	.0374
(321)	MW	VW	.0420	....	.0433	....	....
(331) (061)	W	W	.0469	.0467	.0469	....	.0460
(410) (070)	...	VW	.0515	....	....	....	....
(420) (170)	VW	W	.0548	...	.0547	....	.0537

[a] $\sin^2\theta$ values for CuKα radiation. [b] Intensity notation: S, strong; M, medium; W, weak; V, very. [c] Dry in this table refers to samples dried over phosphorus pentoxide at 70° in an Abderhalden evacuated with an aspirator. I = Fatty acid complexes, II = dried butanol precipitate.

ation of the precipitates with variation in the chain length and unsaturation of the fatty acids led us to use lauric, palmitic, stearic and oleic acids as precipitating agents. In all cases only that fatty acid which could not be removed by Soxhlet extraction with carbon tetrachloride was counted as bound fatty acid, and, for all our X-ray samples, the bound fatty acid determined as "fat-by-hydrolysis" amounted to about 7% of the precipitate.

X-Ray diagrams were made of samples wet, as obtained from the centrifuge, of samples dried over phosphorus pentoxide in an Abderhalden drying tube at 70° and evacuated by an aspirator, and of samples dried at 100° over phosphorus pentoxide in an Abderhalden at pressures below 1 mm. The two methods of drying were given careful study when it was discovered that a significant and interesting difference in the X-ray diagrams was caused by the more thorough drying.

All maxima recorded in the tables below are from X-ray diagrams made with CuKα radiation, nickel filtered, and a cylindrical powder camera of 10-cm. radius. The powder samples were sealed in thin-walled, glass capillaries of about 0.3-mm. diameter. Exposure times were from three to five days using a gas-filled tube at 15 m.a. and 40 KVP.

In order to reduce the time necessary for preparing X-ray diagrams, not all samples dried in the three different ways were examined in the 10-cm. camera. Identification diagrams were made of all samples using fairly thick X-ray samples, a flat film and a sample-to-film distance of 5 cm. These diagrams could be made in a few hours and proved sufficient to reveal all but subtle changes in the X-ray samples.

The identification diagrams revealed no differences in precipitates made with different fatty acids and dried in a similar manner. Diagrams of samples dried in a relatively poor vacuum were scarcely distinguishable from diagrams of samples taken directly from the centrifuge, while those given more thorough drying produced diagrams shifted to larger scattering angles, with some maxima considerably altered in intensity.

Several palmitic acid precipitates were studied in the 10 cm. camera. The $\sin^2\theta$ values reported in the tables below are averages. In general the variations in $\sin^2\theta$ values in similarly treated palmitic acid precipitates were as great as the variation from fatty acid to fatty acid.

The X-Ray Structures.—In Table I are listed the maxima from wet fatty-acid amyloses, and of samples dried at aspirator pressure. Included for comparison are maxima of the dried butanol-precipitated amylose.[3d] The variations in positions of the maxima of the dried fatty acid precipitates are thought to be within the reproducibility of the samples and measurements. The slight differences in the positions of the maxima of the wet samples are probably real. The relative intensities of maxima of wet and dried samples appeared to be identical.

The chief difference between the diagrams of the fatty-acid amyloses dried at 70° and the dried butanol–precipitated amylose appears to be in the relative intensities of certain of the weak maxima, particularly those with indices (hkl) with $l \neq 0$. The fatty acid complex produces more diffuse maxima of this form. In this respect it is more like the amylose–iodine complex where maxima with $l \neq 0$ are barely visible. We attribute this to a random arrangement of the fatty acids or iodine molecules along c (*vide infra*).

As previously reported,[3d] the dried butanol–precipitated amylose is at least pseudo-hexagonal with a unit $a_0 = 27.4$ Å., $c_0 = 8.05$ Å. For reasons previously discussed we believe the true unit to

be orthorhombic with a unit a_0 = 13.7 Å., b_0 = 23.8 Å.,[8] c_0 = 8.05 Å.[3d]

Within the experimental errors, the unit for the fatty acid complex, dried at 70° in a partial vacuum, is identical. In Table II are listed maxima from three different samples of more thoroughly dried fatty acid–amylore. These contained the same percentage of fatty acid as the unextracted samples, but the X-ray lines have clearly shifted to larger angles. The X-ray diagram is now very similar to that of the amylose iodine complex shown in the same table (Column 6).[3c] Though the maxima can no longer be indexed on a hexagonal basis (the axial ratio b_0/a_0 = 1.77 instead of 1.73 as required for an orthohexagonal unit), the positions of the lines are such as to make easy correlation with the maxima from the iodine complex, and the intensities and positions are still related to the complexes listed in Table I. On this basis a very similar orthorhombic unit has been chosen for the dry fatty acid–amyloses with dimensions a_0 = 13.0 Å., b_0 = 23.0 Å., c_0 = 8.05 Å. (Note that corresponding lines have the same indices for all these complexes.[3c,d])

TABLE II

AMYLOSE–FATTY ACID PRECIPITATES (DRY)[a]

		Sin²θ (Observed)[b]				
Indices	Intensity[c]	Lauric amylose	Palmitic amylose	Stearic amylose	Amylose iodine complex	Sin²θ (calculated)[d]
(110)	M	0.0046	0.0045	0.0046	0.0047	0.0046
(011)	VVW	....	.0109	....	....	.0102
(200)	S	.0137	.0137	.0138	.0140	.0138
(040)	VVW	.0177	....	.0179	.0188	.0179
(131)	VW	.0234	.0229	.0226	.0236	.0226
(221)	W	.0275	.0275	....	.0274	.0274
(310)	VS	.0324	.0323	.0323	.0328	.0323
(330)	VW, diffuse	....	.0404	.0412	.0422	.0412
(331)	W	....	.0503	.0503	.0519	.0503

[a] Dried at 100° over phosphorus pentoxide in an Abderhalden at a pressure below 1 mm. [b] Sin²θ values are for CuKα radiation. [c] Intensity notation: S, strong; M, medium; W, weak; V, very. [d] Calculated sin²θ values are for the fatty acid precipitates, based on an orthorhombic unit, a_0 = 13.0 Å., b_0 = 23.0 Å., c_0 = 8.05 Å.

In view of the optical and x-ray evidence previously presented,[3] we consider that there is no satisfactory alternative to a helical model for the amylose chain in the amylose–iodine complex and butanol–precipitated amylose.[9] The X-ray diagrams clearly indicate a very similar structure for fatty acid–amylose.

The amylose helices must be packed in the unit cells of the fatty acid–amyloses in a manner quite like that previously reported for the iodine complex and butanol–precipitated amylose.[3c,d] In the pseudo-hexagonal cell of the dried, fatty acid–amyloses there are four helices, two in the orthorhombic unit. The nearly circular tubes are close-packed. For the reasons previously outlined,[3d] the space group of the structures is probably $P2_12_12_1$ and alternate helices are directed oppositely. In the wet and partially dried samples the amylose helix has a diameter of 13.7 Å. In the more thoroughly dried complex the helix has a diameter of 13.0 Å., equal to that found for the helix in the amylose–iodine complex.[3c]

Densities of the two forms of the fatty acid complexes have been determined by flotation in organic solvents. The fatty acids are not removed by solvents and these complexes are not particularly hygroscopic. Consequently the densities should be more significant than those reported for the dried butanol precipitate[3d] and the amylose iodine complex.[3c] The samples dried at 70° had densities ranging from 1.45 to 1.465 g./cc. After more thorough drying the densities of various samples were from 1.46 to 1.475 g./cc. The decrease in unit cell volume is about 8% upon drying at 100°. The fact that the density is scarcely changed by this large decrease in volume requires that material be lost from the unit cell upon drying. From the method of treatment it seems reasonable to suppose that there has been a loss in water. If there were one molecule of water of crystallization per glucose unit, a 10% loss of weight on drying would be expected.

For a helix of six glucose residues per turn the observed density of the fatty acid complex dried at 70° under partial vacuum seems to require twelve water molecules per orthorhombic unit, or one water of hydration per glucose unit. The more thoroughly dried complex on the other hand appears to be essentially anhydrous. Tentatively we suggest that the amylose helix has a 13.0 Å. diameter when anhydrous and 13.7 Å. when monohydrated. These two helix diameters have also been encountered in the case of the iodine complex (13.0 A.) and the dried butanol complex (13.7 Å.). The observed density of the former can be explained in terms of anhydrous amylose, and the latter in terms of a monohydrate.

It is not possible to determine the positions of the fatty acids in these complexes in a direct way from the X-ray diagrams. The X-ray results are, however, suggestive of the position of the fatty acids since the packing of the amylose helices is not affected in any important manner. (It is to be noted that the butanol has been removed from the dried butanol precipitate, so that fatty acid is not replacing butanol.) This requires that the fatty acid be placed in holes in the structure. For reasons given in the discussion we conclude that it is more probable that the fatty acids lie within the helices than that they lie within the interstices between the helices.

It is probably significant that in diagrams from both the iodine complex and the fatty acid complex the reflections (hkl) with $l \neq 0$ are relatively less intense and not so sharp as are these reflections in diagrams from the dried butanol–precipi-

(8) This dimension was erroneously reported as 24.8 Å.[3d]

(9) It should be noted that the amylose chain in granular and retrograded starch is linearly extended, not helical. See Rundle, Daasch and French, THIS JOURNAL, 66, 130 (1944).

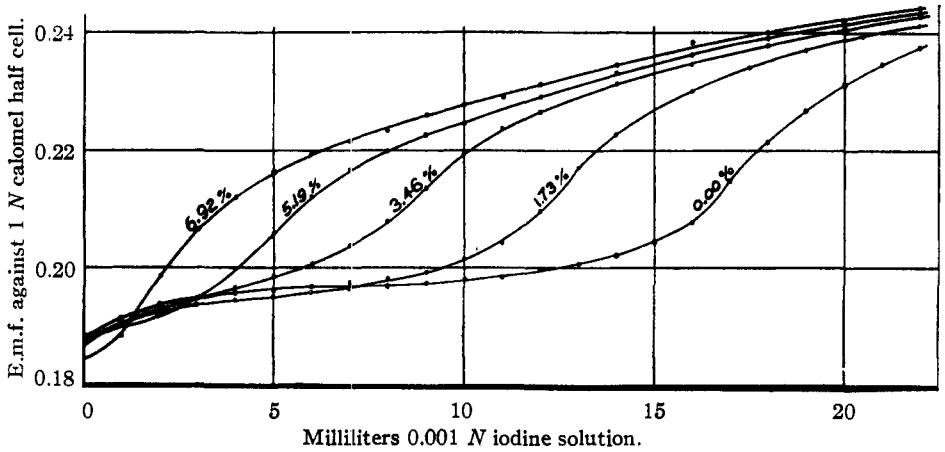

Fig. 1.—Potentiometric titration of amylose dispersion to which the indicated percentages of oleic acid have been added.

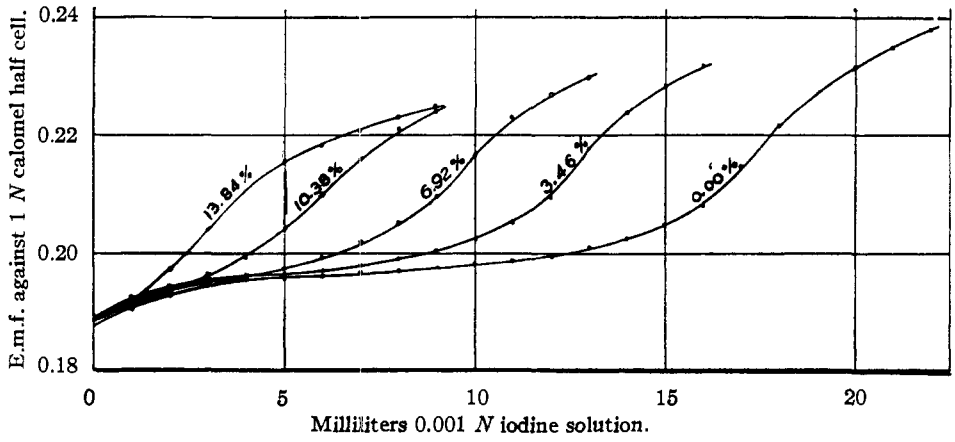

Fig. 2.—Potentiometric titration of amylose dispersion to which the indicated percentages of palmitic acid have been added.

tated amylose.[10] In the latter material the complexing agent, butanol, has been removed while in the iodine and fatty acid complexes it remains. It is probable that the periodicity along the axis of the amylose helix itself is good, but that the complexing agents are arranged at random along the helix axis.

The Influence of Fatty Acids on Amylose–Iodine Complex Formation.—Schoch and Williams[2] have already noted that small amounts of fatty acids repress the iodine binding power of corn starch as determined by the starch–iodine titration.[11] The repression was shown to increase as the fatty acid content of the starch increased, and a 10% fatty acid content was sufficient to repress totally the iodine binding of amylose.

We have examined the influence of fatty acids on amylose–iodine complex formation in more detail. In Fig. 1, the titration curves of amylose with various amounts of oleic acid added to the amylose solution are reproduced. The amylose was a butanol precipitate of lily bulb starch, prepared by the method of Schoch.[12] The iodine titration of the pure amylose (Fig. 1, Curve 0.00%) indicates that the amylose used was about 90% pure.[13] In Fig. 3 the iodine required to reach the end-point of the titration is plotted as a function of added oleic acid. The relation is linear up to the addition of 6–7% oleic acid, at which point the amylose no longer binds iodine as determined by the iodine titration. A very similar situation resulted from the addition of lauric acid to an amylose solution (Fig. 3).

When palmitic (Fig. 2) and stearic acids were added to amylose solutions the repression of iodine binding was not nearly so marked. These fatty acids are less soluble in water and it appears likely that simple addition of these acids to a cold, water solution was not sufficient to cause complete

(10) It is not obvious from the intensities reported in Table I that this is true. There is quite a difference in intensity between the weaker set of maxima, comprised chiefly of the (*hkl*) reflections, and the more intense set, comprising the (*hk*0) reflections. Hence visual, relative estimates of intensities for each diagram separate the weak from the strong reflections within the set, but do not permit comparisons of weak reflections against each other from diagram to diagram.

(11) F. Bates, D. French and R. Rundle, THIS JOURNAL, **65**, 142 (1943).

(12) T. Schoch, *ibid.*, **64**, 2957 (1942).

(13) Purity was based on Kerr's crystalline amylose as outlined in ref. 11.

reaction of the amylose and fatty acid. Consequently palmitic acid was introduced into butanol precipitated amylose from a methanol solution by Schoch's method, and the excess acid was removed with carbon tetrachloride. In Fig. 3 the iodide bound by the amylose as determined potentiometrically is plotted as a function of the palmitic acid content, determined as "fat-by-hydrolysis." Under these circumstances the influence of palmitic acid in repressing the iodine binding of amylose is quite comparable with the influence of oleic and lauric acids.

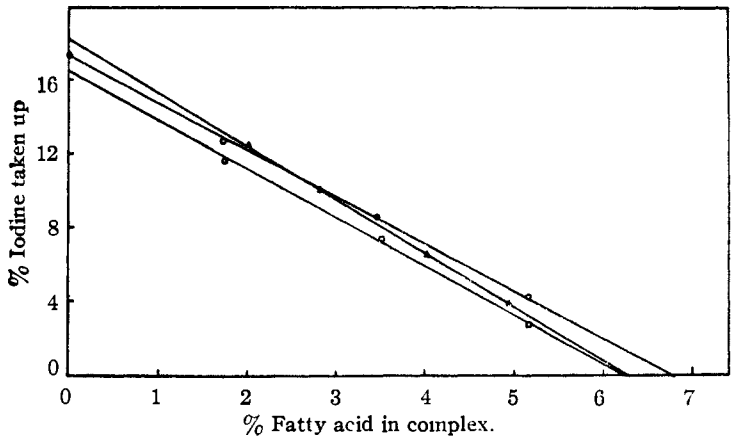

Fig. 3.—Binding of iodine by amylose-fatty acid complexes containing varying amounts of fatty acid: △, palmitic acid; ○, oleic acid; □, lauric acid.

The stoichiometry of the reaction between fatty acids and amylose appears rather complicated from the above results. In the complexes there are 17.6 glucose residues per lauric acid, 22.6 per palmitic acid, and 25.0 per oleic acid molecule, respectively; *i.e.*, the longer the fatty acid the more amylose it binds. Pursuing this observation further we note that the lengths of the fully extended fatty acids, including van der Waals distances, are about 19 Å. for lauric acid, 24 Å. for palmitic acid and 27 Å. for oleic acid.[14] The apparent length of helix associated with each acid can be found by multiplying the number of glucose residues per acid by the length of a turn in the helix (8 Å.) and dividing by the number of glucose residues per turn (six). The distances are then 23.5 Å for lauric acid, 30 Å. for palmitic acid, and 33 Å. for oleic acid, in each case about 1.2 times the length of the fatty acid. If correction were made for the 10% amylopectin which binds no fatty acid,[2] the agreement between the length of the fatty acid would be even better. (The ratio would become about 1.1:1 in each case.)

It is, therefore, attractive to explain the fatty acid content of the complexes in terms of the capacity of the helices. The diameter of the hole in the helix should be approximately the correct size to accommodate the fully extended fatty acid aligned along the helix axis. The theoretical fatty acid capacity of the helices, making these assumptions, is only about ten per cent. greater (correcting for amylopectin) than the experimental binding power.

Vapor Phase Reaction of Iodine with Fatty Acid–Amylose Complex.—It has been shown that amylose, precipitated with alcohols and dried and having a "V" X-ray diagram characteristic of the helical configuration, takes up iodine vapor readily and in quantity (up to 26% of the dry weight of the amylose), while amylose producing an "A" or "B" type diffraction diagram will not take up significant amounts of iodine vapor.[3c] The fatty acid–amylose complex produces a "V" type X-ray diagram, but the space within the helix is here presumed to be occupied and hence not available for iodine. However, upon treating the fatty-acid amylose complex with iodine vapor, iodine is taken up in quantity, although more slowly than in the case of the dried butanol precipitate.

A palmitic acid–amylose complex containing 8.3% fatty acid was heated for eight days at 78° in the presence of iodine vapor in an evacuated Abderhalden. The weight gained due to the iodine was 20.3% of the weight of the amylose or about 78% of the amount the amylose could take up. After this treatment the amylose was rinsed with carbon tetrachloride, resulting in the removal of 75% of the palmitic acid. The remainder was held tightly and was determined as "fat-by-hydrolysis." It amounted to 1.82% of the original weight of amylose, or 21.8% of the palmitic acid originally present in the amylose complex.

It is to be noted that carbon tetrachloride will not extract fatty acids from the amylose complexes even upon prolonged treatment. The above experiment demonstrates that approximately 80% of the fatty acid in the complex was displaced by iodine, and that the amount of iodine required for this displacement was about 80% of the total capacity of amylose for iodine. This proportionate displacement of fatty acid by iodine we interpret as additional evidence that iodine and fatty acid occupy the same position in their amylose complexes. Apparently the reaction is reversible.

$$\text{Amylose}\cdot I_2 + \text{Fatty acid} \rightleftarrows \text{Amylose}\cdot\text{Fatty acid} + I_2$$

In water solution equilibrium lies far to the right, but with dry solids and iodine in the vapor phase it is possible to reverse the reaction. The partial pressure of iodine necessary to reverse the reaction has not as yet been given study. The reaction is apparently slow, since after eight days the amylose was still gaining weight.

Surface *vs.* Crystalline Configuration in Influencing Complex Formation.—Schoch[5] has

(14) Distances and angles were taken from Pauling, "Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1939.

shown that fatty acids can be reintroduced into defatted starches by suspending the defatted starch granules in solutions of fatty acids in methanol. This procedure has been given further study by Lehrman,[7] who found that defatted rice starch takes up more fatty acid than defatted corn starch and both take up more than potato starch. The original fat content of rice starch is greater than that of corn starch and the fat content of potato starch is negligible. Lehrman believed the binding of fat was adsorption and that previous fat content might influence the surface. It has been pointed out by Whistler and Hilbert[8] that the fatty acid reintroduced is roughly proportional to the surface area of the granules. Lehrman also pointed out that with the starches he examined the uptake of fatty acid followed a typical Freundlich adsorption isotherm.

To investigate the possible effect of surface we have used Lehrman's method to study the uptake of fatty acids by amylose and amylopectin individually. With amylose it is necessary to examine at least two crystalline modifications, *i.e.*, the "V" modification and the "A" or "B" modification in which the chains are linearly extended. Accordingly we have suspended dried butanol-precipitated amylose ("V") and retrograded amylose ("B") as well as alcohol-precipitated amylopectin in solutions of methanol containing various concentrations of fatty acid. Table III shows the relationship between the amount of palmitic acid present and the amount of acid taken up by these substances. Amylose in the "B" configuration and amylopectin were found not to take up significant amounts of fatty acids by Lehrman's treatment. (Fatty acid extracted by carbon tetrachloride was not counted as bound.) On the other hand, amylose in the "V" configuration bound acid in amounts far exceeding those which Lehrman found for granular starch. The palmitic acid so bound is not extracted by carbon tetrachloride, but is extracted by Soxhlet extraction with methanol. The palmitic acid analyses were made as "fat-by-hydrolysis."

TABLE III

BINDING OF PALMITIC ACID BY AMYLOSE AND AMYLOPECTIN

Grams of palmitic acid[a]	Palmitic acid bound[b]				
	Amylose in "V" configuration		Amylose in "B" configuration	Amylopectin	
	Trial 1	Trial 2		Trial 1	Trial 2
0.2	0.84	1.09	0.23	0.10	..
0.5	1.53	1.84	.23	.15	0.07
1.0	1.94	2.14	.29	.07	.07
1.5	2.95	3.26	.22	.21	..
2.0	2.90	3.55	.19	.05	.12
3.0	4.05	3.80	.18	.15	..
4.0	4.02	4.03	.18	.21	.21

[a] One-gram samples of starch material were suspended in 10-ml. portions of methanol containing the indicated weight of acid. [b] Expressed as % by weight of amylose.

The results reported in Table III show rather large experimental errors in the amounts of palmitic acid bound. The variation is probably due largely to differences in degrees of crystallinity. Two samples of this same amylose, crystallized in a form about twice as voluminous, bound 3.81 and 4.35% palmitic acid after refluxing 12 hours under similar conditions. These values increased to 4.00 and 4.50%, respectively, after ninety hours treatment. In spite of these experimental variations, the values are undoubtedly of the right magnitude for equilibrium conditions.

Both retrograded amylose in the "B" configuration and amylopectin were precipitated in forms possessing relatively great surface area. Even examined microscopically and for birefringence both appeared amorphous. By contrast, the microscopic appearance of "V" or helical amylose was obviously crystalline and quite birefringent. X-ray maxima of the retrograded "B" material and the helical or "V" material used are also remarkably different in sharpness of diffraction line. The maxima of the "B" material are very broad, while those of the helical material are very much sharper. All these observations indicate that the retrograded "B" material has a smaller particle size and, as a consequence, a greater surface area than the helical amylose. Certainly the amorphous amylopectin must have a larger surface area. Both retrograded amylose and amylopectin can be safely assumed to have greater surface areas than any of the granular starches examined by Lehrman.

By comparison of the values in Table III, it is seen that the binding of fatty acids by amylopectin and by amylose in the "B" configuration is insignificant when compared with the binding of fatty acids by amylose in the "V" or helical configuration. Moreover, from Lehrman's work it is clear that defatted corn and rice starches in the granule form bind appreciably more fatty acids than does retrograded "B" amylose. The evidence indicates that helical configuration is more important than surface in the binding of fatty acids by starch. If surface enters into the problem it does so for that portion of the fatty acid bound more loosely, and extractable with carbon tetrachloride.

Effect of Solvent

Lehrman's procedure for reintroducing fatty acids has been applied to helical amylose using carbon tetrachloride instead of methanol as a solvent for the fatty acids. The use of carbon tetrachoride results in the introduction of much larger amounts of fatty acid into helical amylose at given concentrations of fatty acid. Thus when one gram of amylose was refluxed with 0.04 g. of palmitic acid in 20 ml. of carbon tetrachloride for two weeks, 0.012 g. of palmitic acid (1.2%) was introduced into the amylose (and even this is almost certainly below the equilibrium amount for these conditions). For a similar binding of palmitic acid dissolved in methanol, a concentration

of between 0.4 and 1.0 g. of acid in 20 ml. of methanol would be required (Table III).

On the other hand, equilibrium is very slowly attained if carbon tetrachloride is the solvent. Attempts to reach equilibrium in the introduction of palmitic acid into helical amylose from carbon tetrachloride were apparently unsuccessful after two weeks. Approaching equilibrium from the other side, one can only note that prolonged Soxhlet extraction of the fatty acid complex by carbon tetrachloride removes no detectable amount of fatty acid. One can only guess that the equilibrium activity of fatty acid over the complex is very low, and that in the experiment described above, nearly all the fatty acid would have been removed from the carbon tetrachloride by the amylose in time. The reaction is probably slow because of poor wetting and slow penetration of amylose by carbon tetrachloride.

Discussion

Position of Complexing Agents in the Complexes.—It has been shown by X-ray diffraction that amylose helices pack together in nearly identical manner whether they are complexed by iodine, fatty acids, or are free of complexing agent (as in dried butanol precipitate). The distances between helices are not altered measurably by the complexing agents. Consequently, the complexing agents must be placed in holes in the structure.

The obvious holes in the structure are of two sorts: the hole in the center of the helix, and the interstitial spaces between helices. Where molecules fill interstitial spaces, lattice parameters are generally enlarged. Since this is not the case here we feel that this is evidence that the complexing agents are not in a position to influence materially the forces between helices. The fact that two forms of the packing (with 13.0 and 13.7 Å. diameters of the helices) exist, both with and without complexing agents, serves to emphasize the fact that complexing agents have almost nothing to do with the packing of the amylose. The hole within the helix seems, on this basis, to be the more probable position for the complexing agents.

Moreover, for the fatty acid complexes and the iodine complex, the amount of complexing agent bound by helical amylose can be explained satisfactorily in terms of the capacity of the helices. It is to be noted that for the close packing of circular tubes, which the amylose helices closely approximate, there are two interstitial holes per helix as contrasted to one in the center of the helix. These interstitial spaces would have to be very large to accommodate fatty acid without change of X-ray spacing. For fully extended fatty acids such interstitial spaces would be only about half filled by the amounts of fatty acids found in the complexes. (If the fatty acids could fold in these interstices, they would be less than half full.) Hence, if the interstices between helices bind fatty acids, the amounts are unexplained.

Finally, the hypothesis that the complexing agents are contained within the helices permits an understanding of Lehrman's experiments and those described above. Either adsorption or placing the complexing agents in the interstices between helices is far less successful in this respect, as shown below.

Nature of Binding of Complexing Agents by Amylose.—Many organic molecules are now known to form precipitates with amylose similar to the complexes formed with iodine, the fatty acids and alcohols.[4,8] These molecules have in common only a fairly linear structure and a sizeable dipole (or in the case of iodine, a high polarizability). The mechanism of complex formation can be understood in terms of the recently proposed mechanism of iodine-complex formation[3e] according to which the amylose helix is presumed to possess a considerable dipole moment parallel to the helix axis. Linear polar molecules should be held within the helix with their dipoles directed antiparallel to the dipole on the helix. This interpretation has also been used by Bear.[4] The suggestion that amylose forms these complexes with molecules which can form hydrogen bonds ignores the fact that iodine forms with amylose a complex having the same structural form as the fatty acids, alcohols, etc. Certainly iodine cannot form hydrogen bonds of significant strength.

Attempts to explain the lack of ability of amylopectin to form complexes cannot be explained in terms of hydrogen bond formation, although this has been suggested.[8] Certainly amylopectin, like amylose, has —OH groups to form hydrogen bonds if this were all that were necessary for complex formation. Moreover, it has ample surface to make —OH groups available in large numbers. In contrast, it seems quite reasonable that the many branch-points in amylopectin would interrupt helix formation sufficiently often to make for a low helix dipole and poor attraction between complexing agent and amylopectin. In this respect its failure to form complexes with fatty acids, alcohols, etc., parallels its failure to form a stable iodine complex. It is to be noted, too, that if the complexing agents were held by hydrogen bonding to the outside of the amylose helices the forces between helices would certainly be influenced. Consequently, one would expect the packing of the helices to be materially altered, which, as we have noted, is not the case.

Finally, since the complexes of amylose with fatty acids and iodine are alike structurally, it seems reasonable to suppose that the inability of amylopectin to form complexes with iodine and with fatty acids derives from the same reason. Hydrogen bonding, as previously mentioned, can have little influence in the iodine complex.

Interpretation of the Binding of Fatty Acids by Defatted Starches.—Lehrman's experiments, coupled with those reported above, show that defatted corn and rice starch granules bind more

fatty acid than does granular potato starch, or even more than retrograded "B" amylose containing substantially more surface than any of the granular starches. The amounts bound correlate well with previous fat content.

The mechanism proposed here for the binding of fatty acid by amylose explains why previous fat content should condition defatted starch granules to bind fatty acid as reported by Lehrman. The amylose in the granule originally binding fatty material would have a helical configuration in contrast to the bulk of the starch granule which is known to have an extended "A" or "B" type configuration. The helical configuration of amylose chains is not altered by alcohol extraction of the fatty material, leaving the helices capable of recombining with fatty acid.

In granular starch, where the number of helical chains is very small in comparison with extended chains, the helical chains can hardly be packed together closely in a crystalline array as in "V" starch. This contributes valuable evidence in regard to the nature of the binding, since regular intersticial holes between helices, such as exist in crystalline "V" amylose, probably never would exist in this case. The fact that the binding of fatty acid by granular starches seems to be conditioned by previous fat content is confirmatory evidence both that the binding is by the helical chains and that the fatty acid enters the interior of the helices and not intersticial spaces between helices.

It is true that the fatty acid taken up by defatted starches in Lehrman's experiments is not exactly equal to fatty material previously removed, and even potato starch, which contains essentially no fat originally, will bind a small amount of fatty acid when treated by Lehrman's technique. Probably under the influence of alcohol, heat and fatty acids used in extraction and reintroduction of fatty acids, a few nearly free chains can be induced to alter their configurations. Under more drastic conditions nearly all the chains can be led to form a helical configuration in the presence of complexing agents. This undoubtedly accounts for Schoch's introduction of large quantities of fatty acids by heating starch to dryness with alcohol solutions of fatty acids.

Interpretation of the Differences in Solvents.—It has been shown that there is a pronounced difference in solvents both in the extraction and in the reintroduction of fatty acids. Methanol is an effective extractant, and likewise relatively large concentrations of fatty acids are necessary for the reintroduction of fatty acids into helical starch from methanol solution. Carbon tetrachloride is ineffective in the extraction of fatty acids, and even small fatty acid concentrations in this solvent permit the introduction of fatty acids into helical amylose.

On the basis of the theory presented here, methanol should be capable of entering the amylose helix and of competing with fatty acids for space in the helix. Its effectiveness as an extractant may be attributed to this property. The ineffectiveness of carbon tetrachloride, ether, etc., may be attributed to their inability to enter the helix or their inability to compete for space because of a low dipole and consequent lack of binding force.

It is interesting to note that with methanol the amount of reintroduced fatty acid is a function of fatty acid concentration. For the reaction

$$\text{Amylose–CH}_3\text{OH} + \text{Fatty acid} \rightleftharpoons \text{Amylose–Fatty acid} + \text{CH}_3\text{OH}$$

where the methanol complex and fatty acid complex are independent phases, the equilibrium constant would be $K_{eq} = 1/a$ (fatty acid), independent of concentration of fatty acid in dilute methanol solution. This is not the case, and it seems probable that fatty acid bound is increasing the concentration of fatty acid in the methanol complex, so that there is but one complex phase over a wide range of fatty acid and methanol contents of the amylose. This property would produce the type of concentration dependence found by Lehrman[7] without requiring adsorption. On the other hand, carbon tetrachloride cannot enter the helix, so that in carbon tetrachloride the reaction is

$$\text{Amylose} + \text{Fatty acid} \overset{CC_{14}}{\rightleftharpoons} \text{Amylose–fatty acid}$$

By analogy with iodine complex formation, as demonstrated by the iodine titration,[11] we presume that the amylose–fatty acid complex represents one phase and amylose another, so that the fatty acid activity in an amylose–fatty acid complex should be independent of fatty acid content until saturation of the amylose is reached. Extraction experiments indicate that the activity of fatty acid in an amylose–fatty acid complex is very low indeed, even when the amylose is saturated with fatty acid.

It seems likely that where different molecules can each enter the helix the relative amounts within the helix will be determined by the activities of each molecule and a constant characteristic of the binding power of amylose for each. On the basis of the present theory the binding power should be determined largely by dipole moment and/or polarizability of the complexing agent. Activity studies of different complexing agents with helical amylose would obviously be of interest in this connection.

Schoch[5] has noted that 80% dioxane is a good fat extractant while anhydrous dioxane is not. The effect of water is probably to loosen chains. It is known that water causes helical amylose to change slowly to extended chain amylose when complexing agents are removed. It is likely that the mechanism of fat extraction by dioxane-water is more complex than a simple replacement of complexing agent by penetration of the helix by solvent.

The Fatty Acid Content of Waxy Maize Starch.—It is interesting to note that waxy maize starch, containing essentially pure amylopectin, contains about one-seventh as much "fat-by-hydrolysis" as ordinary corn starch.[15] Since this difference is not due to the amount of fat available in the respective types of corn kernels, it probably can be attributed to the inability of amylopectin to combine readily with fatty acids.

Summary

X-Ray and optical investigation of the complex between amylose and fatty acid reveal it to have its own particular crystalline form. Amylose with a helical chain configuration will bind fatty acid, while amylose with an extended chain configuration will not, independent of relative particle size of the two materials. These points are interpreted to mean that a molecular complex, rather than surface adsorption, is involved.

Structurally, the fatty acid complex resembles closely the iodine complex, consisting of the close-packing of helical amylose tubes. The packing of the amylose is not materially influenced by the complexing agents, indicating that the complexing agents are contained in holes in the structure. Chemical evidence is presented that the holes occupied by iodine and fatty acid are the same. A number of reasons are presented for believing the holes in the center of the helices, rather than the holes between helices, are involved.

The above structure of the complex explains quantitatively the fatty acid content of the complex, the effect of previous fat content on the binding power of defatted granules, the low fat content of waxy starches, the differences between many solvents in fat extraction, the inability of amylopectin to bind fatty acid, etc. It is shown that surface adsorption and hydrogen bond formation and the placing of amylose between helices leave most of these points unexplained.

The force between amylose and complexing agent is explained in terms of dipolar interactions, adapting the mechanism suggested for the iodine complex. It is pointed out that hydrogen bonding as a force will not explain the great similarity between fatty acid and iodine complexes.

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An Equation for the Line of Saturation of Liquids and Vapors

BY JOHN E. HAGGENMACHER

No single mathematical relationship comprising all four variables of saturated fluids has been published. In the following a simple expression between vapor–volume, liquid–volume, pressure and temperature is established.

Consider the behavior of a system of saturated liquid and vapor reported on rectangular p, v,T-coördinates. It is a particularity of this state that corresponding volumes of liquid and vapor lie on parallels to the v-axis. The system is determined by one variable. The loci of all possible values of p,v,T, constitute the line of saturation. Geometrically the line is a skew curve, quadratic with respect to v, and situated on a ruled surface parallel to the v-axis.

The equation of such a three-dimensional curve may be put in the form

$$v = \frac{v_g + v_L}{2} \pm \frac{v_g - v_L}{2} \qquad (1)$$

where v_g and v_L are the roots of a quadratic p,v,T-function. Therefore, if a rectangular equation of reasonable accuracy can be found for the line of saturation, it is, by purely mathematical means, possible to obtain expressions for $v_g + v_l$ and $v_g - v_l$, which may be simpler of construction than the original function. Both expressions are useful for thermodynamic considerations of saturated fluids.

The following relationship between pressure, volumes and temperature of saturated fluids was chosen for its good all around accuracy and simplicity.

TABLE I

COMPARISON BETWEEN EQUATION (2) AND VAN DER WAALS' FOR WATER[3] (SATURATED CONDITIONS)

$$p = \frac{CT}{v + B} - \frac{A}{T(v + B)^2} \qquad (2)$$

$C = 0.0045548 \quad A = 6.4469 \quad B = 0.0035593$

$$p = \frac{CT}{v - b} - \frac{a}{v^2}$$

$C = 0.0045548 \quad a = 0.016808 \quad b = 0.0016892$

Temp., °C.	van der Waals	p atm. Eq. (2)	Lit.	% difference from lit. van der Waals	Eq. (2)
0	0.0060316	0.0060303	0.0060273	+ 0.07	+0.05
40	0.072951	0.072820	0.072748	+ 0.28	+0.10
80	0.47092	0.46977	0.46740	+ 0.75	+0.51
120	1.9911	1.9799	1.9595	+ 1.61	+1.04
160	6.2887	6.2032	6.1032	+ 3.04	+1.64
200	16.135	15.687	15.332	+ 5.22	+2.32
240	35.585	33.818	33.044	+ 7.69	+2.34
280	70.090	64.533	63.343	+10.6	+1.88
320	125.99	112.11	111.40	+13.1	+0.64
360	200.24	182.30	184.26	+ 8.67	−1.07
374.11	310.7	(218.167)	218.167	+42.4	0.0

The general behavior of equation (2) in comparison with van der Waals' may be judged from the summary in Table II. The saturation pressures were calculated for the two equations and compared with experimental values.