A solution of 0.5 g. of the alkaloid in 5 cc. of dilute nitric acid (2 cc. concd. nitric acid diluted to 10 cc. with water) was heated for twenty minutes on the steam-bath. The cooled solution was treated with pellets of sodium hydroxide while cooling until alkaline when hydrastinine separated in colorless crystals. When this was recrystallized from ether the hydrate of hydrastinine was obtained melting at 115–115°. This and all of the following derivatives were compared with authentic specimens by mixed melting point determinations. The hydrastinine was converted into a mixture of oxyhydrastine (m. p. 96–97°) and hydrohydrastinine (picrate, m. p. 178°) by heating with alcoholic potash on the steam-bath for five hours.[6]

The alkaline filtrate from which the hydrastinine had separated was acidified with hydrochloric acid and exhausted with ether. The combined ether extract was evaporated to a small volume and heated with an excess of sodium hydroxide on the steam-bath overnight, a few granules of aluminum being added to convert the nitrate to ammonia. The acidified solution was again extracted with ether and the residue from the ether washed with cold water to remove the 3,4-methylenedioxyphthalic acid. (This was recovered from the aqueous solution, sublimed *in vacuo*, and the resulting anhydride recrystallized from acetone–ether, m. p. 136°.) The water insoluble portion was recrystallized first from boiling water and then from very dilute ethanol and obtained in colorless plates melting sharply at 232° (3,4-methylenedioxyphthalide).

***dl*-Adlumidine or Capnoidine.**—A mixture of 0.1 g. of each of adlumidine and of capnoidine was dissolved in boiling chloroform. The solvent was largely evaporated and the resinous residue treated with *ca.* 5 cc. of hot methanol and momentarily heated to yield a homogeneous solution. Almost immediately the *dl*-base separated in stout prisms which melted sharply at 205°. One recrystallization from chloroform–methanol did not alter the melting point.

## Summary

*Corydalis incisa* (Thunb.) Pers. has yielded three known alkaloids (protopine, adlumidine, and *l*-corypalmine) as well as one (F62) which has not been identified. The structures of adlumidine and of capnoidine have been determined as the same as that of bicuculline. They are optical antipodes and on admixture yield a racemic compound.

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[CONTRIBUTION FROM THE GEORGE M. MOFFETT LABORATORIES OF THE CORN PRODUCTS REFINING COMPANY]

# Amylose Complexes

BY WALTER J. KATZBECK AND RALPH W. KERR

Rundle and co-workers,[1-9] Bear,[10] and others, have developed the theory that when complexes are formed between amylose and agents such as iodine, alcohols and fatty acids, the long, linear starch molecules form helices around the complexing agents. X-Ray diffraction patterns of these powdered complexes were best explained in all cases by a crystalline structure consisting of closely packed helices. Since the complexing agent can be removed from or introduced into the crystalline structure without materially altering the diagram, it was believed that the complexing agent is placed in the holes in the crystal structure. At least in the case of the fatty acid complexes and possibly also the iodine complex, the amount of complexing agent held by the crystal structure can be explained satisfactorily with reference to the amount of space within the helices, in contrast to the insufficient amount of interstitial space between helices. These workers have pointed out, furthermore, that all of these complexing agents have one obvious property in common: possession of a permanent electric moment, or, in the case of iodine, a tendency to assume an induced moment. They have proposed that the stability of the complex is the result of dipolar action.

Although this explanation appears to be satisfying and is entirely consistent with all the facts observed, one of these experimental approaches which has been used in the past to develop this hypothesis has of necessity been limited, and this limitation, among others, has given rise to alternate explanations from time to time to account for complex formation by amylose. The alternate explanation most commonly advanced in the past has been that complex formation is an adsorption phenomenon between the agent and the hydrophilic groups along the amylose chain.[11-18]

One experimental approach to the problem used by Rundle and co-workers has been to compare the relatively large amounts of iodine and fatty acids taken up by "V" form amylose in the solid state with insignificant amounts taken up by other solid forms of amylose which give "A" or "B" type, X-ray diffraction patterns. The "A" and "B" forms of amylose are exemplified by the retrograded (spontaneously crystallized) and

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[1] R. E. Rundle and D. French, This Journal, **65**, 558 (1943).

[2] R. E. Rundle and D. French, *ibid.*, **65**, 1707 (1943).

[3] R. E. Rundle and F. C. Edwards, *ibid.*, **65**, 2200 (1943).

[4] R. E. Rundle and R. R. Baldwin, *ibid.*, **65**, 554 (1943).

[5] F. Bates, D. French and R. E. Rundle, *ibid.*, **65**, 142 (1943).

[6] R. E. Rundle, J. F. Foster and R. R. Baldwin, *ibid.*, **66**, 111 (1944).

[7] R. R. Baldwin, R. S. Bear and R. E. Rundle, *ibid.*, **66**, 2116 (1944).

[8] F. F. Mikus, R. M. Hixon and R. E. Rundle, *ibid.*, **68**, 1115 (1946).

[9] R. S. Stein and R. E. Rundle, *J. Chem. Physics*, **16**, 195 (1947).

[10] R. S. Bear, This Journal, **66**, 2122 (1944).

[11] C. S. Hanes, *New Phytologist*, **34**, 101 and 189 (1937).

[12] C. S. Hanes and M. Cattle, *Proc. Roy. Soc.* (*London*), **125B**, 387 and 414 (1939).

[13] L. Lehrman, This Journal, **64**, 2144 (1942).

[14] T. J. Schoch, *ibid.*, **64**, 2954 (1942).

[15] T. J. Schoch and C. B. Williams, *ibid.*, **66**, 1232 (1944).

[16] R. Whistler and G. E. Hilbert, *ibid.*, **66**, 1721 (1944).

[17] T. J. Schoch, *Advances in Carbohydrate Chem.*, **1**, 247 (1945).

[18] T. J. Schoch, *Bakers' Digest*, **21**, No. 1, 1 (1947).

granular states and these pattern types are believed to indicate a linear and parallelwise orientation.

However, it seems reasonable to assume that in the "A" and "B" configurations, the linearly oriented amylose molecules are associated largely through their hydroxyl groups, and these associations no doubt account for the water insolubility of amylose in the retrograded and granular states. That is, in these forms the very groups which many adsorption proponents believe are active in complex formation are blocked because of associations which already exist between these groups, whereas, in the "V" configuration there is obviously very little intermolecular association, as evidenced by the rapid and complete water solubility of the amylose.

The purpose of this paper is to describe the preparation of a new solid form of amylose in which the molecules are not oriented and in which there is substantially no intermolecular association through hydrophilic groups, since the product is both amorphous and water soluble, and to show that this form does not enter into complex formation in the solid state to the very pronounced degree to which amylose in the "V" form does.

## Experimental

**Preparation of Amylose Samples.**—Corn amylose in amorphous, water-soluble form was prepared by the use of special drying procedures for the Pentasol precipitated fraction obtained from a corn starch sol according to the general method outlined by Schoch.[19] The moist amylose–Pentasol complex, obtained from the centrifuge, contained 84.5% of volatile substance, 70.0 being water and the balance Pentasol. This semi-fluid mass was fed continuously to the valley between two heated rolls, held close together and rotating in opposite directions toward each other at 5 r. p. m. The rolls were of polished steel, 24 inches in diameter and 24 inches wide and were heated to a surface temperature of 160° by means of steam under pressure. The product emerged from the rolls as a white, very thin, gauze-like sheet having about 4% moisture content and containing substantially no Pentasol. It was ground to a fluffy powder: iodine affinity 16.6.

The product was inspected by Dr. G. E. Hilbert and his associates at the Northern Regional Research Laboratories and was found to be amorphous by examination under polarized light and by X-ray diffraction. One gram of the product was found to dissolve readily to form a reasonably clear solution when vigorously stirred into 100 ml. of water at 90°.

Amyloses of corn, potato and tapioca starches were prepared in the "V" form by precipitation of dilute starch sols with Pentasol according to the general method of Schoch.[19] The corn starch sol was prepared by autoclaving a 2% defatted starch slurry at 17 lb. steam pressure for thirty minutes at *p*H 6.2. The potato and tapioca starch sols were prepared by heating 1% starch slurries to a boil at *p*H 6.0. In all cases Pentasol was stirred into the sol as soon as the temperature had been lowered to 90°. These amyloses (A-fractions, according to Schoch) were twice crystallized from aqueous butanol.

The retrograded form was prepared by dispersing corn amylose in boiling water, using 1.5 g. per 100 ml., and cooling. After forty-eight hours the mass was centrifuged and dried in a current of warm, dry air with frequent stirring. When dry, the mass was pulverized into a gritty powder.

The frozen gel form was prepared by dispersing 3 g. of corn amylose in 100 ml. of water in a boiling water-bath, with vigorous stirring. The amylose was first mixed with a small amount of aqueous butanol and the moistened mass added to the hot water. Heating and stirring were continued to volatilize the alcohol. The dispersion was cooled without stirring and then frozen at about −5° for forty-eight hours. The mass was warmed to room temperature, then dehydrated as far as possible on a suction filter and washed with two liters of cold water. The mass was dehydrated in methanol, ground and freed of methanol in a vacuum desiccator over sulfuric acid.

**Iodine Absorption.**—One-gram samples of the various amylose preparations, in glass dishes provided with covers, were placed in a desiccator over phosphorus pentoxide. An additional sample of corn A-fraction was also prepared on which moisture determinations were made at frequent intervals. After a period of about two weeks the moisture content of the corn A-fraction had been reduced to 2.98%. All samples were weighed and placed in a desiccator which contained a petri dish holding about 15 g. of resublimed iodine, excepting the additional sample of corn A-fraction, which was placed in a similar but otherwise empty desiccator. This latter sample was used as a moisture control and was manipulated in all the many weighing operations in a manner identical to the samples exposed to iodine vapor. The control sample varied in moisture content as shown at the bottom of Table I, finally increasing to 5.80% and it was assumed that all other test samples had the same moisture contents as the control. The samples exposed to iodine vapor were removed periodically and weighed over a period of twenty-six days, at which time increase in weight due to iodine absorption had substantially ceased. All A-fractions rapidly became very dark in color whereas the others remained very light.

**Butanol Absorption.**—In the first series of tests with butanol vapor the amylose samples were desiccated over phosphorus pentoxide until the control had a moisture content of 2.10%. Then the samples were placed in a desiccator which contained dishes of both phosphorus pentoxide and butanol and it was assumed that any increase in weight of the samples was due to absorption of the alcohol. In the second series of tests the samples were equilibrated at a moisture vapor pressure sufficient to yield a moisture content 6.13%. These were placed over dry butanol and no phosphorus pentoxide was placed in this desiccator. The gains in weights of the samples in the two series of tests are shown in Tables II and III.

**Fatty Acid Absorption.**—Two series of experiments were run, one in which the moisture content of the samples had been reduced to 1.62% and one in which the samples had approximately 7% moisture as shown in Table IV. The fatty acid absorption procedure was the same in all cases. One gram of the amylose sample was heated under reflux in a solution of 10 g. of fatty acid in 25 ml. of absolute methanol, at the boiling point for five hours. They were filtered into a Soxhlet thimble and washed with 25 ml. of cold methanol. After air-drying overnight, the samples were treated in a Soxhlet extraction apparatus for three hours with carbon tetrachloride and again air-dried.

Absorbed fatty acid was determined by five extractions in boiling 85% aqueous methanol. The combined extracts were quantitatively filtered into weighed dishes, evaporated to dryness on a steam-bath and oven-dried at 80° to constant weight. The extracted samples were analyzed for possible additional fatty acid by transferring to a flask with the aid of 50 ml. of water and adding an equal volume of hydrochloric acid sufficient to make the acidity 5 *N*. The flask was heated on a steam-bath for two hours. The liquors were cooled, transferred to a separatory funnel and shaken with three 100-ml. portions of petroleum ether. The extract was filtered into an evaporating dish and evaporated on a steam-bath, followed by oven-drying at 80°. No residue was found from the petroleum ether extract, within the experimental error for weighing the dish.

## Discussion

It will be observed from the data presented

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[19] T. J. Schoch, THIS JOURNAL, **64**, 2957 (1942).

TABLE I

PER CENT. BY WEIGHT ABSORPTION OF IODINE VAPOR AND MOISTURE CONTENT OF SOLID AMYLOSE SAMPLES

| Amylose | % Absorption at time in days 1 | 4 | 8 | 12 | 19 | 26 |
|---|---|---|---|---|---|---|
| Corn A | 7.74 | 14.74 | 18.61 | 19.88 | 20.24 | 20.70 |
| Potato A | 3.62 | 8.74 | 15.73 | 19.31 | 19.71 | 20.22 |
| Tapioca A | 5.53 | 12.69 | 19.73 | 21.52 | 21.81 | 22.13 |
| Frozen Gel | 1.27 | 3.08 | 4.80 | 5.98 | 5.75 | 6.32 |
| Retrograded | 0.54 | 1.67 | 3.16 | 4.34 | 4.06 | 4.38 |
| Amorphous | 0.24 | 0.68 | 2.05 | 3.69 | 3.42 | 4.03 |
| Moisture, % | 3.80 | 4.04 | 4.88 | 5.27 | 5.65 | 5.80 |

in Table I and Fig. 1, that amyloses in the "V" form, the corn, potato and tapioca A-fractions, took up 19–20% of iodine, by weight, within the first twelve days at room temperature, following which there was less than 1% gain in weight during the next fourteen days. In contrast, the amorphous form of corn amylose absorbed less iodine than the retrograded form, which was about 4%, whereas amylose prepared from a frozen gel state took up slightly more. It was estimated that the amylose samples had increased in moisture content to 5.80% during the interval of the test and corrections were applied to the gains in weight, accordingly. Actually, the gain in weight of the "V" forms was 24-25%, approaching the value reported by Rundle and co-workers.[2]

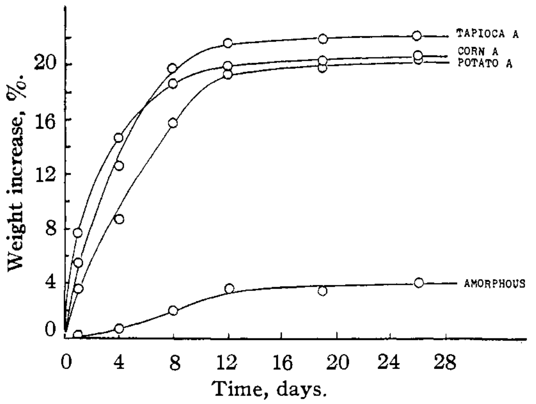

Fig. 1.—Complex formation between dry amylose samples and iodine vapor at room temperature.

It was likewise found that amyloses in the "V" form, at 6% moisture content, absorbed about 20% by weight of butanol vapor at room temperature whereas both the retrograded and amorphous forms absorbed only insignificant quantities, actually less than 0.5%, as is shown in Table II.

Table IV shows that the "V" forms of corn and tapioca amyloses at 7% moisture, absorbed nearly 2% by weight of oleic acid whereas the amorphous form, presenting a much larger surface area per unit weight than any of the other samples, absorbed an insignificant amount which was less than 0.1%.

Inasmuch as the amorphous corn amylose sample was found to be readily soluble in water, it may be presumed that the hydroxyl groups in this material were as free to associate with groups on other molecules capable of forming hydrogen bonds, such as alcohols and acids, as were the hydroxyl groups on the water soluble amyloses in the "V" form. That the amorphous form did not absorb significant quantities of these materials under conditions where the water soluble, "V" form did, is fairly convincing reason for believing that amylose molecules adsorb these polar materials by means other than through hydrogen bonds. Indeed, considering the comparatively large surface area comprised of unoriented molecules in the amorphous sample and the minor amounts of alcohol, fatty acid and iodine taken up compared to the very much larger amounts taken up by well oriented amyloses in the "V" form, precludes any adsorption mechanism as a satisfying explanation for the effect. Rather, it appears for complex formation to take place a definite configuration of the linear molecules is required, which is indicated from a "V" type diffraction pattern, and this applies not only for the iodine complex but also for the fatty acid and alcohol complexes of amylose as well.

It is of interest to note that the data in Table

TABLE II

PER CENT. BY WEIGHT ABSORPTION OF BUTANOL VAPOR BY SOLID AMYLOSE SAMPLES AT 5–6% MOISTURE CONTENT

| Amylose | % Absorption at time in days 1 | 2 | 6 | 18 | 20 | 27 | 34 | 41 | 62 |
|---|---|---|---|---|---|---|---|---|---|
| Corn A | 5.62 | 8.05 | 12.49 | 12.95 | 16.0 | 18.2 | 19.8 | 21.4 | 21.4 |
| Tapioca A | 5.74 | 8.93 | 13.50 | 13.85 | 18.1 | 18.2 | 20.5 | 18.1 | 17.1 |
| Retrograded | 0.20 | 0.10 | 0.20 | 0.20 | 0.2 | 0.2 | 0.2 | 0.2 | 0.4 |
| Amorphous | 0.20 | 0.10 | 0.20 | 0.30 | 0.25 | 0.35 | 0.25 | 0.30 | 0.25 |
| Moisture, % |  | 5.18 | 4.80 | 4.96 | 5.30 | 5.10 | 5.45 | 5.00 | 5.26 |

TABLE III

PER CENT. BY WEIGHT ABSORPTION OF BUTANOL VAPOR BY SOLID AMYLOSE SAMPLES AT 2% MOISTURE CONTENT

| Amylose | % Absorption at time in days 1 | 4 | 5 | 6 | 12 | 18 | 25 | 32 | 39 |
|---|---|---|---|---|---|---|---|---|---|
| Corn A | 4.44 | 5.39 | 5.49 | 5.99 | 6.80 | 7.90 | 9.23 | 9.02 | 7.86 |
| Tapioca A | 3.74 | 5.10 | 5.71 | 6.56 | 8.02 | 8.84 | 10.31 | 10.39 | 9.44 |
| Retrograded | 0.34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Amorphous | 0.41 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

TABLE IV

PER CENT. BY WEIGHT ABSORPTION OF FATTY ACIDS BY SOLID AMYLOSE SAMPLES AT DIFFERENT MOISTURE LEVELS

| Amylose | Moisture content | Oleic acid absorbed | Moisture content | Oleic acid absorbed | Palmitic acid absorbed |
|---|---|---|---|---|---|
| Corn A | 1.62 | 0.1 | 7.26 | 1.90 | 3.40 |
| Tapioca A | 1.62 | 0.1 | 7.11 | 1.91 | |
| Retrograded | 1.62 | 0 | 6.81 | 0.35 | |
| Amorphous | 1.62 | 0 | 6.81 | 0.10 | 0.06 |

TABLE V

PERCENTAGE GAIN IN WEIGHT OF CORN A-FRACTION DRIED TO CONSTANT WEIGHT AT 110°

| Time in days | Over iodine and calcium chloride | Over calcium chloride |
|---|---|---|
| 3 | 7.47 | 0.07 |
| 7 | 9.06 | .10 |
| 15 | 10.35 | .13 |
| 36 | 11.21 | .16 |
| 180 | 13.05 | .22 |

II indicate a maximal absorption of 22% by weight of butanol at completion of complex formation by "V" form amylose. This is very nearly equivalent to a ratio of one butanol molecule for every two glucose residues in the amylose molecule, an unusual ratio to be accounted for on the basis of adsorption through carbohydrate hydroxyls.

It is also of interest to note that a percentage of moisture of the order of 5% greatly facilitates complex formation with all of the agents tried. In the case of iodine, one is tempted to speculate on the function of water on the basis that it promotes an orthodox chemical reaction between amylose and iodine. Thus, for example, in the presence of moisture the iodine might conceivably be reduced by some part of the amylose to hydrogen iodide which could be a necessary intermediate in forming the complex. However, explanations of this nature are difficult to expand so as to include the effect of moisture on fatty acids and alcohols. Nevertheless, in order to throw further light on the iodine–amylose interaction, another sample of corn A-fraction was dried *in vacuo* for four hours at 110°. At this time the sample had come substantially to constant weight and was assumed to be moisture free. A one-gram portion was placed in a desiccator at room temperature over trays containing iodine and calcium chloride. Another gram portion was placed in a similar desiccator over calcium chloride only. The percentage gains in weight with time are shown for both samples in Table V. It is apparent that moisture, at least above trace amounts, is not essential for complex formation between "V" form amylose and iodine although it is obvious, when these results are compared to those in Table I, that water, even as little as 5%, greatly facilitates the interaction.

Therefore, in view of these results, and of the present information concerning the generality of the effect with complexing agents it seems reasonable to conclude that the function of water is a physical one and that its action is directed primarily to the amylose. Rundle and Edwards[3] studied the X-ray diffraction patterns of wet and dried butanol precipitates of amylose and found evidence that the wet precipitate had a shorter period along the unit cell axis than the dried precipitate. On the basis of a helical configuration with six glucose residues per turn, then a shorter period along the helical axis could be taken to indicate an enlargement in helical diameter. It is conceivable, therefore, that moisture may induce a slight enlargement of the helices of very dry amylose, thus facilitating, possibly, the entry of the complexing agent within the helices.

## Summary

1. Preparation of an amorphous form of amylose has been described.

2. Amorphous amylose, in the solid form, adsorbs only trace amounts of butyl alcohol vapor, fatty acid and about 4% of iodine, the latter being less than adsorbed by amylose in the frozen gel and retrograded states.

3. The negative results obtained with amorphous amylose was taken as proof that amylose-complex formation is not an adsorption phenomenon.

4. Only the "V" form of amylose was found to take up significant quantities of iodine vapor, butyl alcohol and fatty acid, confirming the conclusion that a particular molecular configuration of amylose is a necessary requisite for complex formation.

5. Moisture assists complex formation between "V" form amylose and iodine vapor, butyl alcohol and fatty acid and its effect, accordingly, appears to be physical rather than chemical.

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