Formula	Analyses, % Calcd.	Found
$C_{10}H_{17}ON_5$	N 31.35	31.32
$C_7H_{13}N_5$	N 41.87	41.96
$C_{11}H_{21}N_5 \cdot 3HCl$	Cl 32.14	32.10
$C_{13}H_{26}N_6 \cdot 2C_6H_3O_7N_3$	N 21.70	21.68
$C_{17}H_{33}N_5 \cdot 3C_6H_3O_7N_3$	N 19.72	19.99
$C_{12}H_{21}N_5 \cdot 3HCl$	Cl 30.91	30.83
$C_{11}H_{19}ON_5 \cdot 3HCl$	Cl 30.73	30.67
$C_{12}H_{23}N_5 \cdot 2C_6H_3O_7N_3$	N 22.14	22.14
$C_{13}H_{25}N_5 \cdot 2C_6H_3O_7N_3$	N 21.70	21.42
$C_{13}H_{23}N_5 \cdot 2C_6H_3O_7N_3$	N 21.79	21.75
$C_{12}H_{21}ON_5 \cdot 3HCl$	Cl 29.53	29.51
$C_9H_{17}N_5 \cdot 2C_6H_3O_7N_3$	N 23.56	23.19
$C_{10}H_{19}N_5 \cdot 2C_6H_3O_7N_3$	N 23.17	23.06
$C_8H_{16}N_6 \cdot 4C_6H_3O_7N_3$	N 22.65	22.83
$C_{14}H_{28}N_6 \cdot 3HCl$	Cl 27.34	27.37
$C_{13}H_{25}ON_5 \cdot 3C_6H_3O_7N_3$	N 20.53	20.43
$C_{15}H_{29}ON_5 \cdot 3C_6H_3O_7N_3$	N 19.67	19.75
$C_{18}H_{36}N_6 \cdot 4HCl$	Cl 29.46	29.46
$C_{19}H_{36}N_6 \cdot 4HCl$	Cl 28.06	28.00
$C_{19}H_{34}O_2N_6 \cdot 4C_6H_3O_7N_3$	N 19.68	19.20
$C_{22}H_{40}N_6 \cdot 4C_6H_3O_7N_3$	N 19.46	19.32
$C_{21}H_{36}O_2N_6 \cdot 4C_6H_3O_7N_3$	N 19.40	19.48
$C_{22}H_{44}O_2N_6 \cdot 3C_6H_3O_7N_3$	N 18.88	18.81
$C_{26}H_{52}O_2N_6$	N 16.49	16.93

hol solution of the base. The salt after recrystallization from *n*-butanol–ether mixture melted at 209–211°.

2-Amino-4-(ω-aminohexylamino)-pyrimidine.—A paste consisting of 40 g. (0.31 mole) of 2-amino-4-chloropyrimidine and 126 g. (1.09 moles) of hexamethylenediamine[7] was placed in a flask equipped with a reflux condenser. The mixture was heated in an oil-bath at 155° for five hours, cooled and 65 g. of flake sodium hydroxide added. The mixture was warmed overnight by steam, the liquid was decanted, and the residue washed with a little pyridine. After distillation of the pyridine, the residue was distilled from a modified Claisen flask and the fraction boiling at 218–221° (3 mm.) was collected; yield 43 g. (66.3%). The product was a light yellow, viscous oil which crystallized on cooling and after recrystallization from petroleum ether melted at 93°. The picrate was prepared in ethanol and after recrystallization melted at 208–209°.

Summary

2-Amino-4-chloropyrimidine reacts with primary or secondary basically-substituted aliphatic amines to yield the corresponding 2-amino-4-basically-substituted pyrimidines. Twenty-four compounds of this type have been prepared.

(7) Furnished by the courtesy of E. I. du Pont de Nemours & Co., Inc.

STATE COLLEGE, PENNSYLVANIA

RECEIVED APRIL 12, 1945

[CONTRIBUTION FROM STARCH AND DEXTROSE DIVISION, NORTHERN REGIONAL RESEARCH LABORATORY,[1] PEORIA, ILLINOIS]

Separation of Amylose and Amylopectin by Certain Nitroparaffins[2]

BY ROY L. WHISTLER AND G. E. HILBERT

Schoch's[3] fundamental discovery that butanol (or isoamyl alcohol) separates starch into two fractions—amylose and amylopectin[4]—having widely different properties and molecular configurations[5] is of importance from two standpoints:

1. A preparative method for the fractions, which appears to be applicable to starches in general, has been provided.

2. The procedure used for effecting the fractionation is an unusual one involving the formation of a complex between amylose and butanol which is insoluble in the system water–butanol while amylopectin is soluble under the same conditions. Practically nothing is known regarding the mechanism of formation of the butanol–amylose complex. Schoch,[3b] for example, states: "The reason for the selective precipitating action of normal butyl and isoamyl alcohols is obscure, possibly depending on some undefined optimum of molecular volume or 'hydrophil balance'." Information on the mechanism of formation or on the nature of the butanol–amylose complex obviously would be of value as a guide in developing new procedures for separating the components of starch and possibly other mixtures of high polymers.

Very few data are available on the composition or structure of the butanol–amylose complex. From Schoch's[3a] work, it is apparent that the complex contains butanol, but the ratio of butanol to amylose is not known. Rundle and Edwards,[6] on the basis of X-ray diffraction data, have concluded that the complex is composed of helically shaped amylose molecules[7] with butanol occupying the core of the helix.

The wide occurrence of hydrogen bonding[8] suggests that the association of butanol and starch

(1) This is one of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Original manuscript received August 14, 1944.

(3) (a) Schoch, *Cereal Chem.*, **18**, 121 (1941); (b) Schoch, THIS JOURNAL, **64**, 2957 (1942); (c) Wilson, Schoch and Hudson, *ibid.*, **65**, 1380 (1943); see also Wiegel, *Koll. Zeit.*, **102**, 145 (1943).

(4) Schoch did not name the two different fractions separated by means of butanol. The conventional terms now in use for designating the starch fractions are amylose and amylopectin. The amylose fraction, consisting essentially of linear molecules, is prepared by butanol precipitation or elution from swollen granules. Amylopectin is the fraction remaining after the removal of the amylose, and is composed mainly of branched or tangled molecules.

(5) Meyer, "Advances in Colloid Sciences," Interscience Publishers, Inc., New York, N. Y., 1942, pp. 143–165.

(6) Rundle and Edwards, THIS JOURNAL, **65**, 2200 (1943).

(7) Freudenberg, *Naturwissenschaften*, **27**, 841 (1939).

(8) Hilbert, Wulf, Hendricks and Liddel, THIS JOURNAL, **58**, 548 (1936); Gordy and Sanford, *J. Chem. Phys.*, **8**, 170 (1940).

involves this type of binding. Butanol, and alcohols in general, can act either as donors or acceptors of electrons ($\overset{\uparrow}{-O}-H$, $-O-H\leftarrow$). Starch also possesses both donor and acceptor groups ($\overset{\uparrow}{-O}-H$, $-O-H\leftarrow$, $-\overset{\uparrow}{O}-$). If the intermolecular attraction between amylose and butanol does involve hydrogen bonding it is reasonable to expect starch to be fractionated, not only by alcohols as found by Schoch, but also by compounds belonging to entirely different classes, having donor, acceptor, or both, groups. This has now been found to be the case. Compounds possessing nitro, ester, ketone, mercapto, and carboxyl groups, and cyclic nitrogen (=N—), have been found to be excellent agents for fractionating starch. Specific examples are nitropropane, nitrobenzene, amyl acetate, methyl ethyl ketone, butyric acid, butyl mercaptan, and pyridine.[9] Of particular interest is the fact that some of these compounds have only donor groups. Amylose, therefore, can form complexes with a wide variety of compounds many of which can serve as agents for fractionating starch. This article, however, is restricted to the investigation of the fractionation of starch by nitroethane, 1-nitropropane, and 2-nitropropane.

Treatment of a defatted starch sol with nitroethane, 1-nitropropane, or 2-nitropropane under conditions similar to those recommended by Schoch for forming the amylose–butanol complex results in the "crystallization" of a complex between amylose and the nitro compound. The quantity of amylose separating varies with the type of nitro compound and kind of starch used. From corn starch the average yields of crude amylose, using 1-nitropropane, 2-nitropropane, and nitroethane as the fractionating agents, are 26–28, 29–31, and 22–23%, respectively. Nitromethane, under similar conditions, does not produce a precipitate. On fractionation with 1-nitropropane, the yield of crude amylose from potato starch is 26–27%, from wheat starch 31–32%, and from tapioca 20–21%. Thus, approximately the same yield of amylose is obtained through precipitation with these nitroparaffins as with butanol. Much less of the nitroparaffin than butanol, however, is required to effect satisfactory fractionation. Amylose fractions were also obtained through nitroparaffin precipitation of the aqueous extract of swollen corn starch granules by a modification[10] of Kerr and Severson's[11] procedure. 1-Nitropropane, like butanol, precipitates about 85% of the starch in the extracted fraction, while 2-nitropropane precipitates almost all of it. The average yields of amylose from corn starch obtained through the crude and "recrystallized" nitroparaffin–amylose complexes are shown in Table I. Amylose obtained by 1-nitro- or 2-nitropropane precipitation contains 0.02% nitrogen and from 0.003 to 0.009% phosphorus.

TABLE I

YIELDS OF AMYLOSE FROM CORN STARCH

Precipitating agent	Character of precipitate	Number of prepns.	Yield, %
Nitroethane	Repptd.	4	22–23
1-Nitropropane	Crude	10	26–28
1-Nitropropane	Repptd.	4	26–28
2-Nitropropane	Crude	11	29–31
2-Nitropropane	Repptd.	4	26–29

The properties of the amylose–nitroparaffin solvates are closely similar to those of the amylose–butanol solvate. The amylose–nitroparaffin complexes precipitate from dispersions of starch in water saturated with nitroparaffin in the form of dumbbell- or needle-shaped "crystals" frequently as long as 0.02 mm. On reprecipitation, the complexes separate so rapidly that they are usually obtained in a "microcrystalline" condition; at times sheaf-like bundles of needles separate. Sometimes the "crystals" are organized like disks or spheres. Due to their fineness, the crystals are only faintly birefringent. The complexes are stable in water saturated with nitroparaffin and show a solution point between 70 and 80°. In water, the complex disperses rapidly to give a clear solution. On collection in a centrifuge, the complex is obtained as a white creamy gelatinous paste which, on dehydration, forms a translucent horny mass. By this operation, as well as by suspension in alcohol, the complex is destroyed and the amylose freed. Because of the unstability of the complexes and their intractable nature, the ratio of nitroparaffin to amylose in the complex was not determined.

X-Ray diffraction patterns of the nitroethane–, 1-nitropropane– and 2-nitropropane–amylose complexes appear to be identical. The spacings and the relative order of intensity of the rings are shown in Table II. A halo, apparently due

TABLE II

OBSERVED SPACING AND RELATIVE ORDER OF INTENSITY OF DIFFRACTION RINGS

Spacing, Å.	Order of intensity
12.9	4 W
10.3	5 W
9.1	9 VW
7.0	6 W
5.8	8 VW
5.0	1 S
4.5	2 MS
4.1	7 VW
3.6	3 M
3.1	10 VW

(9) It is of historical interest to note that perhaps the first crystalline amylose complex reported in the literature was the amylose–pyridine complex (Reschke and Hartmann, *Naturwissenschaften*, **22**, 451 (1934)), although the amylose was not characterized as such. Somewhat earlier Hess, Pfleger and Trogus, *Ber.*, **66**, 1505 (1933), reported the X-ray diffraction pattern of a starch–pyridine paste to be different than the well-known A, B, C, and V patterns of starch. The new pattern was believed to be due to a pyridine–starch complex.

(10) Jeanes, Whistler and Hilbert, unpublished results.

(11) Kerr and Severson, THIS JOURNAL, **65**, 193 (1943); Meyer, Bretano and Bernfeld, *Helv. Chim. Acta*, **23**, 845 (1940).

to the presence of some gel in the complex, is also present in the pattern. The X-ray diagrams of the nitroparaffin–amylose complexes are different than those of the butanol–amylose complex.[4]

The amylose fractions obtained through precipitation with nitroparaffins, as well as the original complexes, are colored bright blue by iodine. Quantitative data on the amount of iodine adsorbed by these amylose fractions, as determined by potentiometric iodine titration[3c,12] are shown in Table III. The amount of iodine adsorbed by the nitroparaffin-precipitated amyloses is generally less than that taken up by the amylose fraction prepared by butanol precipitation. Larger quantities of iodine are taken up by amylose fractions separated by means of the nitroparaffins from aqueous extracts of swollen granules (Table III). The amount of iodine adsorbed by these amylose fractions is about the same as that taken up by amylose separated from aqueous extracts of swollen granules through butanol precipitation.

TABLE III

AMOUNT OF IODINE BOUND BY REPRECIPITATED AMYLOSE PREPARATIONS

Source of amylose	Precipitating agent	Iodine bound in complex mg./g. of amylose (±1.5%)
Corn starch	1-Nitropropane	149
Corn starch	2-Nitropropane	142
Corn starch	Nitroethane	161
Corn starch	Butanol	178
Wheat starch	1-Nitropropane	167
Potato starch	1-Nitropropane	182
Tapioca starch	1-Nitropropane	173
Corn starch[a]	1-Nitropropane	199
Corn starch[a]	2-Nitropropane	195
Corn starch[a]	Nitroethane	182

[a] From aqueous extract of swollen granules.

Characteristic properties of amylose, in addition to iodine adsorption, are its behavior on retrogradation and the strength of films[13] prepared from its triacetate derivative. Amylose prepared by fractionation of whole corn starch with nitroethane, 1-nitropropane, or 2-nitropropane retrogrades rapidly from 1% aqueous solutions at 3° and to the extent of 84, 93, or 71%, respectively, in twenty-four days. Films prepared from the triacetates of the amylose fractions are clear, tough, and pliable, and have tensile strengths of 6.7 to 8.1 kg./mm.^{2}.

These properties clearly show that the fractions precipitated by nitroparaffins consist mainly of amylose. The extent of conversion to maltose by β-amylase has been reported to be another useful method for characterizing amylose.[14] In our hands, however, the procedure as described in the literature was found to be difficult to carry out and unreliable from the quantitative standpoint (for details see the experimental section).

The fraction remaining after removal of the amylose–nitroparaffin complex has properties typical of amylopectin. The fraction retrogrades extremely slowly from water at 3°. Films prepared from its triacetate, although self-supporting, are so brittle that the tensile strength could not be determined. Corn and potato amylopectin fractions obtained in this work respond somewhat differently toward iodine; the former is colored violet and adsorbs 11.6 mg. I_2/g. and the latter is colored magenta and adsorbs 0.1 mg. I_2/g.

Experimental

Materials.—The corn starch and wheat starch used were pilot-plant preparations produced by the wet-milling process, the sulfur dioxide content of the steeping liquor being held below 0.25 g./100 ml. The starches were dried in a forced draft at or below 74°. The potato starch used was the "Eupenco" brand from the New England Starch Company and the tapioca starch was the "Sando" brand from the Corn Products Refining Company. Before use the major portion of the fatty material in the corn starch and wheat starch was removed by methanol extraction.[15] Since the other starches have low fat contents they were used directly.

The nitroethane, 1-nitropropane, and 2-nitropropane were commercial samples which, before use, were washed once with a small amount of sodium bicarbonate solution and three times with distilled water to remove acidic impurities.

Preparation of Amylose–Nitroparaffin Complexes.—The fractionation procedure used was quite similar to that described by Schoch. Two hundred and forty grams of starch (moisture content, 10%) was stirred with water to form a thick slurry which was poured slowly into 6 liters of hot water (85 to 90°) with rapid stirring. The resulting uniform paste was diluted to 8 liters and heated in an autoclave at 120° under steam pressure for three hours. Clarification of the hot solution was effected by passage through a Sharples supercentrifuge fitted with a clarifier bowl and operating at 50,000 r. p. m. Material representing about 1.0 to 2.0% of the starch was deposited in the bowl and discarded; this product consisted of some incompletely dispersed granular fragments, but, for the most part, was partially solidified starch from the upper inner surface of the container.

The centrifugate (which had cooled to about 45 to 50° during passage through the centrifuge) was immediately heated to 85°; nitroparaffin was added (200 ml. of 1-nitropropane, 200 ml. of 2-nitropropane, or 500 ml. of nitroethane); and the solution was vigorously stirred. The *p*H of the solution was usually about 5.5 to 6.0. When more acidic, the *p*H of the solution was adjusted to this range by addition of a small quantity of sodium bicarbonate. Variation of the *p*H over the range 5.5 to 7, however, did not noticeably affect the yield of the amylose–nitroparaffin complex. Vaporization of the nitroparaffin was retarded by a rubber gasket fitted over the mouth of the container and around the shaft of the stirrer. The container was insulated with cloth to decrease the rate of cooling. After standing for twenty-four hours, the solution had cooled to room temperature. Precipitation was practically complete at this point as indicated by the fact that very little additional precipitate formed in the supernatant liquor when allowed to stand a week. After stirring was stopped, most of the precipitate settled to the bottom of the container as a flocculent white mass.

(12) Bates, French and Rundle, THIS JOURNAL, **65**, 142 (1943).

(13) Whistler and Hilbert, *Ind. Eng. Chem.*, **36**, 796–798 (1944).

(14) Meyer, Bernfeld and Press, *Helv. Chim. Acta*, **23**, 1465 (1940); Meyer and Heinrich, *ibid.*, **25**, 1038 (1942); Kerr and Severson, THIS JOURNAL, **65**, 193 (1943); Ling and Nanji, *J. Chem. Soc.*, 2677 (1926); McCready and Hassid, THIS JOURNAL, **65**, 1154 (1943); Freeman and Hopkins, *Biochem. J.*, **30**, 446 (1936); Samec, *Z. physiol. Chem.*, **236**, 103 (1935).

(15) Schoch, THIS JOURNAL, **64**, 2954 (1942).

Good recovery of the precipitate was obtained by centrifuging the solution in cups in an ordinary laboratory centrifuge. In order to obtain more accurate yield measurements, the precipitate was collected in a Sharples supercentrifuge. To convert the pasty precipitate to a dry powdery condition, it was vigorously stirred into ethanol (approximately 1 part by volume of precipitate to 5 parts by volume of ethanol); the mixture was filtered; and the amylose was twice more treated with ethanol. The product, on drying *in vacuo* over calcium chloride, was a colorless, fine powder.

The crude complex from the centrifuge bowl was purified; ordinarily, by dissolving in sufficient hot water and nitroparaffin to make a 1 to 2% solution of amylose, the amount of nitroparaffin present being more than enough to saturate the water at room temperature. After the solution had been slowly cooled to room temperature, the precipitate was separated, washed, and dried as already described. Purification of the crude precipitate also was effected by suspending the wet product from the centrifuge bowl in water saturated with nitroparaffin, stirring the mixture vigorously for several minutes, and again removing the precipitate by centrifugation. Yields obtained in this way were roughly the same (within 1 to 3%) as those obtained by dissolving and precipitating the amylose complex.

The quantity of nitroparaffin used to precipitate the amylose was approximately one-third more than that required to saturate 8 liters of water. This excess of precipitant was used only as a precautionary method to ensure complete precipitation of the amylose fraction. Addition of just sufficient 2-nitropropane to saturate the solution caused no decrease in the amount of precipitate formed. Addition of only half the amount of precipitant required to saturate the solution, on the other hand, resulted in a noticeable decrease in the quantity of precipitate formed.

Amylose fractions were prepared from aqueous extracts of swollen starch granules in the following manner. A 3% corn starch paste was gently stirred at 85° for sixteen hours. The swollen granules were separated by centrifuging the paste in cups. By analysis, 12% of the starch was found to be present in the water extract. This solution was heated to 85° and precipitated by 1-nitropropane, 2-nitropropane, or nitroethane. In all cases, beautiful, fine, needle-like "crystals" were formed when the solution was cooled slowly. The product was collected by centrifuging.

Potentiometric Iodine Titration.—The iodine-sorbing capacity of the fractions was determined by the method of Bates, French and Rundle,[12] as modified by Schoch, Wilson and Hudson,[3c] except that the starch samples were dissolved in 1 *N* potassium hydroxide at 0°. The use of low temperature greatly facilitated the solution of starch fractions. Solution was usually complete after one to two hours at 0° whereas at room temperature many hours or several days were required.

X-Ray Diffraction Patterns.—All patterns were obtained by exposing for one hour a 1 mm.-thick portion of the fresh moist complex contained in a thin-walled glass cell to unfiltered Cu radiations at 37 K. V. P., 15 ma. and a specimen-to-film distance of 5 cm. Identical patterns were obtained by the use of filtered radiation. On drying, to a transparent horny film, the specimens exhibit a "B" X-ray diagram.

Extent of Retrogradation.—For retrogradation experiments, 5 g. of amylose was dissolved in 100 ml. of 3% sodium hydroxide by allowing the mixture to stand at 0° under nitrogen for eighteen hours. The solution was diluted to 400 ml., neutralized with sulfuric acid, and brought to a volume of 500 ml. After holding the solution at 3° for twenty-four days, aliquots were centrifuged in cups, at 2800 times gravity, and the supernatant liquids were analyzed for starch content by a modification of the dichromic acid oxidation method.[16] The amount of amylose that retrograded was obtained by difference.

Amylose Triacetate Films.—Amylose triacetate was prepared by acetylating 1 part of amylose in 3.2 parts of acetic anhydride and 3.7 parts of pyridine at 100° for three hours. Films were prepared by casting 8% solutions of the amylose triacetates in chloroform on a glass plate using a casting knife with 0.02" clearance. The tensile strength of the films was measured by a method previously described.[13]

β-Amylase Hydrolysis.—Several investigators[14] have reported that amylose is completely or almost completely converted to maltose by β-amylase. We have been unable to obtain trustworthy values of amylose conversion by β-amylase hydrolysis. The rapidity with which amylose associates or retrogrades and is thus removed from the sphere of action of the enzyme appeared to be the main interfering factor.

In attempts to develop a satisfactory method, various modifications of procedures described in the literature were investigated. The β-amylase used in this work was prepared according to the procedure of Kneen, Sandstedt and Hollenbeck.[17] An aqueous extract of ground, ungerminated wheat (1943 Trumbull) was dialyzed three days against running distilled water and then treated at *p*H 3.0 for two hours at 30° to destroy α-amylase. The enzyme was precipitated from the aqueous extract by means of ethanol, the fraction which separated when the concentration of ethanol in the extract was raised from 50 to 80% being used in this work. The purified enzyme was free from maltase. The amount of reducing sugar present in the β-amylase hydrolysis mixtures was determined by the ferricyanide method[18] and calculated as maltose after correcting for the reducing power of the enzyme.

The following modification of Meyer and Heinrich's method[19] gave the highest conversion, but even by this procedure hydrolysis of amylose fractions was not complete. After dissolving amylose in 1 *N* sodium hydroxide at 0°, acetic acid was added to bring the *p*H to 4.7. This solution, containing about 0.3% amylose, was treated with β-amylase, and the conversion carried on at 30° for twenty-four hours. A precipitate consisting at least in part of amylose was always formed during the hydrolysis. To increase the extent of conversion, the precipitate was dissolved by treating the hydrolysis mixture with alkali and heating to 50°. The solution was then adjusted to *p*H 4.7 with acetic acid and a second large portion of enzyme added at 30°. After twenty-four hours, the solution was again treated with alkali, neutralized to *p*H 4.7, and submitted to the action of β-amylase.

After two enzyme treatments, the amylose fraction obtained through nitroparaffin precipitation was usually hydrolyzed to the extent of 70 to 80% and after three treatments to about 90%. That this procedure was severe and leaves much to be desired is shown by the fact that amylopectin was hydrolyzed to the extent of 45 to 55, 60, and 68%, by one, two, and three enzyme treatments, respectively. The end reaction mixture in all the experiments carried out in this work gave a deep blue coloration with iodine.

Data on the extent of conversion of amylose by β-amylase are of doubtful value from a quantitative or characterization standpoint unless (1) the β-amylase has been shown to be free from maltase and α-amylase, (2) the amylose is completely dispersed and remains dispersed, and (3) the response of the end product of hydrolysis is negative toward iodine. In addition, Haworth, Kitchen and Peat[20] have pointed out that conversion methods based on reducing sugar methods may be in error due to the presence of reducing substances other than maltose. According to these investigators, accurate analyses must be based on the isolation of crystalline maltose.

Separation of Amylopectin.—After removal of the precipitate formed by treating dispersions of corn starch or potato starch with 1-nitropropane or 2-nitropropane, the

(16) Launer, *Bur. Standards J. Res.*, **18**, 333 (1937).

(17) Kneen, Sandstedt and Hollenbeck, *Cereal Chem.*, **20**, 399 (1943).

(18) Sandstedt, *ibid.*, **14**, 603 (1937); Kneen and Sandstedt, *ibid.*, **18**, 237 (1941).

(19) Meyer and Heinrich, *Helv. Chim. Acta*, **25**, 1038 (1942).

(20) Haworth, Kitchen and Peat, *J. Chem. Soc.*, 619 (1943).

resulting amylopectin solution was concentrated under reduced pressure to a thick paste which was precipitated by pouring slowly with vigorous stirring into ethanol (1 part of paste to 5 parts of ethanol). The precipitate was filtered, thrice vigorously stirred with fresh portions of ethanol, and dried in a vacuum desiccator over calcium chloride.

Acknowledgments.—We are indebted to Dr. N. Cyril Schieltz for the X-ray data and to Mrs. Jane Rehn Von Korff and Messrs. Stanley A. Watson and William D. Johnson for carrying out parts of the experimental work.

Summary

In addition to alcohols, as found by Schoch, representatives of many different classes of organic compounds such as esters, ketones, mercaptans, carboxylic acids, nitroparaffins, and pyridine, which are capable of forming hydrogen bonds with amylose, have been found to form complexes with this carbohydrate and can serve as agents for fractionating starch.

"Crystalline" amylose–nitroparaffin complexes are formed when starches are fractionated with nitroethane, 1-nitropropane, and 2-nitropropane. The yields of amylose, using 1-nitropropane and 2-nitropropane as fractionating agents, from corn starch, potato starch, tapioca starch, and wheat starch are about the same as those obtained using butanol. Some of the properties of amylose–nitroparaffin complexes are described. The X-ray diffraction patterns of the three nitroparaffin complexes are identical. The amylose fractions have been characterized by potentiometric iodine titration, retrogradation, and the film-forming properties of the triacetate derivative.

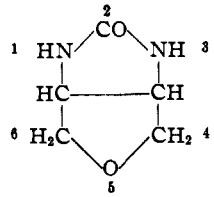
PEORIA, ILLINOIS RECEIVED APRIL 16, 1945

[CONTRIBUTION NO. 564 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

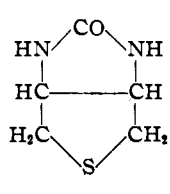
Furan and Tetrahydrofuran Derivatives. V. The Synthesis of a Racemic Hexahydro-2-oxo-4-methyl-1-furo[3,4]imidazole[1,2]

BY KLAUS HOFMANN AND ANNA BRIDGWATER

The ring system of hexahydro-2-oxo-1-furo-[3,4]imidazole (I)[3] has so far not been studied, and as it is closely related to hexahydro-2-oxo-1-thieno[3,4]imidazole (II),[3] the nucleus of biotin, the preparation of certain representatives of this class of compounds was desirable.



(I)



(II)

This report describes a new procedure for the synthesis of a hexahydro-2-oxo-4-methyl-1-furo-[3,4]imidazole isomer (VII), which is applicable to the preparation of a variety of 4-substituted hexahydro-2-oxo-1-furo[3,4]imidazoles, and thus makes these compounds easily available.

Recently, a number of 3,4-diaminocarbethoxy-2-substituted furans have been prepared[4] with the expectation that the introduction of aminocarbethoxy groups would labilize the furan nucleus and make it susceptible to low pressure catalytic hydrogenation.

This has now been found to be the case, and it was observed that 3,4-diaminocarbethoxy-2-methylfuran (III) absorbed two moles of hydrogen with remarkable ease and was transformed into a mixture of hydrogenation products from which *cis* - 3,4 - diaminocarbethoxy - 2 - methyltetrahydrofuran (IV) could be isolated. Attempts to remove the carbethoxy groups from this compound by mild hydrolysis with dilute barium hydroxide did not afford the expected 3,4-diamino-2-methyltetrahydrofuran, but gave a substance which analyzed for $C_6H_{10}O_2N_2$. The structure of this new material was established as hexahydro-2-oxo-4-methyl-1-furo[3,4]imidazole (VII) by the following reactions. Drastic hydrolysis with strong barium hydroxide opened the urea ring with the formation of *cis*-3,4-diamino-2-methyltetrahydrofuran, which was characterized as the crystalline sulfate (VIII). Treatment of a sodium bicarbonate solution of (VIII) with phosgene reintroduced the CO group into the molecule and the resulting bicyclic urea derivative was identical with (VII). Thus, treatment of the urethan (IV) with dilute barium hydroxide effected a ring closure and resulted in the formation of the desired bicyclic urea derivative (VII). The great tendency of (IV) to undergo this ring closure provides direct chemical proof for the *cis* position of the aminocarbethoxy groups in compound (IV) and consequently establishes the *cis* configuration of the rings in compound (VII). Treatment of compound (IV) with strong barium hydroxide at 140° for six hours gave a small amount of (VIII), most of the material being transformed into (VII). Only prolonged hydrolysis at 140° resulted in a complete transformation of (IV) into (VIII), indicating that (IV) is even under these

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(3) These ring systems are named and numbered according to the rules followed by Chemical Abstracts and the Ring Index (American Chemical Society Monograph No. 84).

(4) Hofmann and Bridgwater, THIS JOURNAL, **67**, 738 (1945).