and 3-nitroacrylonitrile (48.2%) as well as 2-chloro-3-nitropropionitrile. The products from reaction in ethyl ether are very unstable and violent decomposition may occur when they are distilled. 2-Chloro-3-nitropropionitrile prepared from nitryl chloride and acrylonitrile must be distilled carefully several times at reduced pressure before it reaches maximum stability. The chloronitronitrile is very corrosive and blisters skin rapidly. It forms dark red solutions rapidly in dilute alkaline media.

Reaction of 2-Chloro-3-nitropropionitrile and Sodium Acetate; 3-Nitroacrylonitrile.—2-Chloro-3-nitropropionitrile (402 g., 3 moles) was added dropwise in one hour to a stirred suspension of anhydrous sodium acetate (297 g., 3.3 moles) in absolute ethyl ether (500 ml.) at 0°. Immediately after addition was completed, the red suspension was filtered free of sodium acetate and sodium chloride. Ethyl ether and acetic acid were removed at reduced pressure; the residue was distilled under nitrogen to yield: (1) volatile product (265.5 g., 93%), b.p. 59–87° (3–4 mm.) and (2) residue (31.9 g.). Refractionation of the volatile product under nitrogen in a glass-packed column (24 × 2 cm.) gave: **3-nitroacrylonitrile**, a yellow oil (231 g., 2.35 moles, 78.9%), b.p. 53–54° (3.3–3.4 mm.), $n^{20}D$ 1.4929, d^{20}_4 1.268, and residue (26 g.). The residue is composed principally of unreacted 2-chloro-3-nitropropionitrile.

Anal. Calcd. for $C_3H_2N_2O_2$: C, 36.73; H, 2.04; N, 28.57. Found: C, 36.43; H, 2.04; N, 28.12.

3-Nitroacrylonitrile is a powerful vesicant and lachrymator. It is oxidized by permanganate but absorbs bromine slowly. The nitronitrile can be stored for long periods at 0–5° without serious decomposition.

Reaction of 3-Nitroacrylonitrile and Sulfuric Acid; 3-Nitroacrylamide.—A mixture of 3-nitroacrylonitrile (3.0 g., 0.030 mole) and 85% sulfuric acid (25 ml.) was heated at 50–55° for 7 hours and then poured on ice. A yellow solid precipitated (1.23 g.), m.p. 165° (dec.); continuous extraction of the aqueous filtrate yielded additional solid (0.64 g.), m.p. 155–160°, m.p. 165° after recrystallization from benzene, and an oil (1.1 g., unidentified) that did not crystallize. An infrared spectrogram of the solid contained definite absorption bands for α-nitrounsaturated (6.45–6.50 microns), carbonyl (5.85–5.90 microns), amide (2.85–3.05 microns), and olefinic groups (6.0–6.05 microns); no bands characteristic of hydroxyl or nitrile groups were present. (A possible product of this reaction is 2-hydroxy-3-nitropropionitrile.) On the basis of the spectrographic and analytical data, the product is assigned the structure, **3-nitroacrylamide** (54% yield).

Anal. Calcd. for $C_3H_4N_2O_3$: C, 31.04; H, 3.45; N, 24.14. Found: C, 31.25; H, 3.46; N, 24.53.

Conversion of 3-Nitroacrylonitrile to 3-Nitroacrylic Acid by Hydrolysis and Deamination.—A mixture of 3-nitroacrylonitrile (2.5 g., 0.025 mole) and 85% sulfuric acid (20 ml.) was heated at 70–80° for 2.5 hours. The mixture was cooled, and a concentrated solution of sodium nitrite (3.5 g., 0.05 mole) in water was added slowly. After the mixture stopped foaming, it was heated to 80° for 10 minutes. The solution was cooled, diluted with water, and continuously extracted with ethyl ether. After the ether had been evaporated, a yellow solid remained which, after recrystallization from chloroform, was identified as 3-nitroacrylic acid (2.0 g., 0.0171 mole, 69.4%); m.p. 134.5°, no depression by an authentic sample; neut. equiv. (calcd.) 117, neut. equiv. (found) 116.7.

COLUMBUS 10, OHIO

Microbiological Synthesis of C^{14}-Labeled Streptomycin

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Radioactive streptomycin was prepared by microbiological synthesis using *S. griseus* as the active microörganism. Glucose and starch, uniformly labeled with C^{14}, were added to the medium as precursors in separate experiments. Glucose was the superior substrate since it appeared to be preferentially utilized for streptomycin formation. Radiostreptomycin was isolated as the crystalline helianthate. The specific activity of the product was ≃0.01–0.025 μc./mg. streptomycin base.

Streptomycin is a metabolism product of *Streptomyces griseus*.[1] The problem of labeling streptomycin with carbon-14 by microbiological synthesis can be solved, in principle, by adding to the fermentation medium a C^{14}-labeled compound utilized by the organism. This presupposes, of course, a knowledge of streptomycin precursors, and of the extent of incorporation of these substrates into the desired product.

One potential difficulty encountered in this approach is the rather large quantity of carbon present initially in the usual medium in the form of broth ingredients. Unless the radioactive precursor is of such a nature as to be used by the organism in preference to the normal broth components, the net effect will be a considerable diminution in specific activity of the carbon in the precursor with resultant formation of low specific activity product.

A logical substrate for labeling is glucose which is a common component of many fermentation media. Glucose uniformly labeled with C^{14} was employed in two of the experiments to be reported. Experiments were also performed with uniformly labeled starch and with a crude starch hydrolysate. Nutrient medium in shake flasks was inoculated with a *S. griseus* culture and the microörganism allowed to grow for about 24 hr., at which time the C^{14} substrate was added. The broth was harvested 1–3 days later. After filtration of the mycelium, the streptomycin in the filtrate was removed by adsorption on IRC 50 resin column and subsequent elution with 1 *N* HCl.

In all cases, evidence for the synthesis of radioactive streptomycin was obtained from concomitant measurements of the radioactivity and microbial activity of the column eluates, as shown in Table I. The carbon content of the broth, necessary for computing the item "broth carbon c.p.m./mg.," was determined by microanalysis of broth components. Allowance was also made for the carbon content of precursors, which was determined in the case of the starch hydrolysate. The quantity of carbon lost from the broth as carbon dioxide during the 28-hr. period prior to addition of precursor was found from blank experiments to amount to ≃10% of the initial total carbon content (≃1.2 g.) of the medium. This correction was made so that the specific activity of broth carbon recorded in the table refers to that prevailing at the time of addition of the substrate. Succeeding items show the total radioactivity (column eluate c.p.m.)

(1) A. Schatz, E. Bugie and S. A. Waksman, *Proc. Soc. Exp. Biol. & Med.*, **55**, 66, (1944).

and the streptomycin content (streptomycin mg.) of the column eluate determined microbiologically. The next two rows of the table contain the calculated specific activities of the streptomycin and of the carbon in the streptomycin. Finally is shown the C^{14} enrichment in the streptomycin over that in the broth, *i.e.*, the ratio of the "strep. carbon" specific activity to the "broth carbon" specific activity. The radioactivity of the eluate is assumed to be associated only with streptomycin. Specific activities (c.p.m./mg.) computed therefrom may be high if other radioactive products are present in the eluate.

TABLE I

ISOLATION OF RADIOACTIVE STREPTOMYCIN

Fermentation	I	II	III	IV
Precursor	Starch	Glucose	Glucose	Starch hydrolysate
Precursor, mg.	2.8	78	52	..
Precursor, c.p.m.[a]	11.3×10^6	9.38×10^6	6.15×10^6	3.36×10^8
Broth carbon c.p.m./mg.	10,400	8,470	5,600	2.23×10^5
Duration, days	3	1	3	3
Column eluate, c.p.m.	208,000	51,000	226,000	4.28×10^6
Streptomycin,[b] mg.	45	10.5	33.4	23.8
Streptomycin, c.p.m./mg.	4630	4860	6770	1.80×10^5
Strep. carbon c.p.m./mg.	10,700	11,200	15,600	4.16×10^5
C^{14} enrichment	1.14	1.48	3.10	2.02

[a] Q-gas counter. [b] Microbiological assay; mg. free base.

In one of the glucose runs (fermentation III) and in the starch hydrolysate experiment (fermentation IV), streptomycin was successfully isolated as the crystalline helianthate,[2] and converted to the trihydrochloride by metathesis with calcium chloride.[3] The successful production of radioactive streptomycin was evidenced by the constancy of specific activity of helianthates obtained after successive crystallizations. Table II shows the results pertaining to the product obtained from fermentation III.

TABLE II

SPECIFIC ACTIVITY OF FERMENTATION III PRODUCT

Crystals, c.p.m.[a]/mg.	I	III	IV
Strep. helianthate	1600	1670	1600
Strep. in helianthate (microbial assay)	5400	5750	6290
Carbon in strep.	13,500	14,400	15,800
Streptomycin (glucosamine assay)	....	5130	5060

[a] I represents the crystalline helianthate obtained initially; III and IV represent the same product after two and three recrystallizations from 30–50% methanol, respectively.

The first set of figures shows the successive specific activities of the helianthate after repeated recrystallization. The specific activity of the helianthate based on weight of the crystals after drying in vacuum at room temperature for two hours is constant within experimental error. Corresponding values for the streptomycin proper based on microbial assay of these helianthates are shown in the next series of figures. Specific activities are constant within the error of the microbial assays. The streptomycin carbon specific activities based on these microbial assays are in fair agreement with that observed for the column eluate (15,600 c.p.m./mg.) similarly determined. In view of the uncertainty of the microbial assay, and possible variations in composition of the helianthate, portions of III and IV were analyzed[4] colorimetrically for streptomycin after conversion to the streptomycin trihydrochloride. The agreement between specific activities based on these assays is excellent.

Experimental

Fermentation.—The organism used for the production of radioactive streptomycin was a streptomycin producing strain of *Streptomyces griseus*. One loopful of a well-sporulated culture grown on agar slants was used to inoculate a 50-ml. portion of a nutrient broth medium containing beef extract, N-Z amine (Type E[5]), NaCl and dextrose.

After good growth (about 5 to 6 mg. per ml. dry wt.) was obtained, a number of 250-ml. erlenmeyer flasks containing 50-ml. portions of a production medium were inoculated with 2 ml. of the culture and placed on the shaker (220 r.p.m., 1.5" throw) in a 28° incubator. This medium contained 24.0 mg. total carbon per ml. of which 8.0 mg. was supplied by dextrose, and 16.0 mg. by complex nitrogenous substances. The flasks were incubated until good growth had occurred (about 28 hours), at which time the sterile radioactive carbohydrate (starch or glucose) was added under aseptic conditions. Immediately after the addition of the radioactive substance was made, sterile aerating devices were inserted into the flasks and the flasks returned to the shaker and hooked up to an aerating train consisting, in the order named, of a pressure regulator, humidifier, cotton air filter, fermentation flasks, a second cotton air filter and three scrubbing bottles containing 10% caustic solution. Air was allowed to flow through the aerating train at a slow rate throughout the remainder of the fermentation. The flasks were harvested at about 24 or 72 hours after the addition of the radioactive material.

Isolation from Broth.—The streptomycin was recovered from the harvested broth by adsorption on Rohm and Haas IRC 50 resin.[6a,b] The column was eluted with aqueous acid and washed with water as described elsewhere.[6a] The total volume of eluate collected was 40–50 ml. Further treatment of column eluates will be illustrated with data from fermentation III.

Radioactive Materials.—The radioactive starch[7] was kindly provided by Dr. Martin Gibbs of the Brookhaven National Laboratories. It was uniformly labeled[8] with C^{14} and had a stated activity of $\simeq 7$ μc. per mg.

Uniformly labeled glucose[9] was obtained from the Oak Ridge National Laboratories. It had a specific activity of $\simeq 0.12$ μc./mg.

Dr. G. R. Noggle of the Oak Ridge Institute for Nuclear Studies supplied the crude starch hydrolysate[9] which was prepared by acid hydrolysis of plant extracts partly treated for recovery of C^{14} labeled sugars.[10]

Concentration of Radioactive (C^{14}) Streptomycin from Resin Column Eluate (Fermentation III).—The biologically active column eluate, volume 39 ml. (after removal of 4 ml. for assay purposes), was adjusted to *p*H 6.8, frozen, and dried from the frozen state. The dry residue was extracted with 10 ml. of absolute methanol. The methanol extract was poured into 200 ml. of acetone to give a white, amorphous precipitate. Evaporation of the mother liquors to dryness yielded a small amount of amorphous residue. The

(2) F. A. Kuehl, R. L. Peck, C. E. Hoffhine, R. P. Graber and K. Folkers, THIS JOURNAL, **68**, 1460 (1946).

(3) R. L. Peck, N. G. Brink, F. A. Kuehl, E. H. Flynn, A. Walti and K. Folkers, *ibid.*, **67**, 1866 (1945).

(4) Courtesy of Dr. G. E. Boxer of this Laboratory.

(5) Obtained from Sheffield Farms, Co., Inc., New York City.

(6) (a) U. S. Patent 2,541,420, Feb. 13, 1951; (b) H. M. Doery, E. C. Mason and D. E. Weiss, *Anal. Chem.*, **22**, 1038 (1950).

(7) Obtained upon allocation by the Isotopes Division of the Atomic Energy Commission.

(8) M. Gibbs, R. Dumrose and F. Archer, A.E.C. Report AECU-329.

(9) Obtained upon allocation by the Isotopes Division of the Atomic Energy Commission.

(10) G. R. Noggle and R. A. Bolomey, *Plant Physiol.*, **26**, 174 (1951).

yields, *i.e.*, biological activity and radioactivity data, are summarized below in Table III.

TABLE III

CONCENTRATION OF RADIOACTIVE STREPTOMYCIN

Fraction	Amount	Microbial activity Units/mg.	Total units[a]	Total counts per min.
Column eluate	39 ml.	..	30,300	205,000
Freeze-dried eluate	1.2953 g.	..	....	.....
Methanol-insoluble	1.0296 g.	..	1,925	17,400
Methanol-soluble acetone precipitated	0.2037 g.	119	24,200	189,000
Acetone mother liquor fraction	.0636 g.	..	100	2,880

[a] 1000 units correspond to 1 mg. of free base.

These data indicate that the bulk of the radioactivity ($\simeq$ 92%) and the microbial activity (80%) is recoverable in the amorphous precipitate obtained by precipitation of the methanol extract of the freeze-dried eluate with acetone. Furthermore the procedure served to concentrate the streptomycin from 2.3% of total solids in the column eluate to 12% in the acetone precipitate.

Crystalline Helianthate of C^{14}-Streptomycin (Fermentation III).—Further purification was effected by conversion to the crystalline helianthate. A 198-mg. sample of the methanol-soluble acetone-precipitated radioactive streptomycin concentrate described above was stirred on a small sintered filter with 1 ml. of absolute methanol for a few minutes. The methanol extract was then removed by filtration. The insoluble portion, after drying, weighed 128.6 mg. The methanol solution was evaporated to an amorphous residue in a stream of dry air, redissolved in 1 ml. of methanol, and mixed with a hot solution of 50 mg. of methyl orange dissolved in 3 ml. of water. On cooling, the originally clear solution deposited typical coppery crystals of streptomycin helianthate. The crystals were collected on a filter, washed with water, and dried; yield 50 mg. (I). A 47.2-mg. portion of this product was recrystallized once from 4 ml. of 50% methanol (II) and a second time from 2 ml. of 50% methanol to give 20.2 mg. of crystals (III) (m.p. 206–214° dec.). Ten milligrams of the recrystallized material was recrystallized once more from 1.5 ml. of 30% methanol to give 7.4 mg. of crystals (IV) (m.p. 207–214° dec.). Data obtained on the first, third and fourth crystallization products are summarized below in Table IV. The net recovery of streptomycin in helianthate IV crystals is 14% based on microbial activity and 13% based on radioactivity.

TABLE IV

FORMATION OF CRYSTALLINE STREPTOMYCIN HELIANTHATE

Fraction	Amount	Microbial activity Units/mg. or units/ml.	Total units	Radioactivity Total counts per min.
Methanol-insoluble fraction	128.6 mg.	32	4100	35,500
Crystalline helianthate (I)	50 mg.	296	14,800	80,000
Helianthate (I) mother liquor	8.7 ml.	512	4,450	53,500
Helianthate (II) mother liquor	5.0 ml.	955	4,775	29,900
Helianthate (II)	20.2 mg.	290	5,850	33,600
Helianthate (III) mother liquor	4.2 ml.	550	2,310	....
Helianthate (IV)	7.4 mg	254	1,880	11,800
Authentic streptomycin helianthate		280	....	....

In the case of fermentation IV product, the column eluate was enriched with 166 mg. of ordinary streptomycin as carrier to facilitate recovery. The yield[11] at the helianthate IV stage was 39% of the streptomycin present in the column eluate.

(11) The authors are grateful to Mr. G. P. Condon of this Laboratory for performing these crystallizations.

Regeneration of Radioactive (C^{14}) Streptomycin Trihydrochloride or Calcium Chloride Double Salt (Fermentation III).—A 6.873-mg. sample of final recrystallized radioactive (C^{14}) streptomycin helianthate IV was dissolved in 3 ml. of methanol. To the solution was added 0.1 ml. of methanol containing 5 mg. of calcium chloride. Since no precipitate formed, 0.4 ml. of water was added and the solution evaporated to about 0.5 ml. and diluted to a volume of 1.5 ml. with water. A precipitate of calcium helianthate separated at once. This precipitate was removed by filtering through a small pad of Darco G-60. The colorless filtrate of streptomycin trihydrochloride, containing excess calcium chloride, was then used for counting and assayed colorimetrically for streptomycin by the glucosamine method. Crystals of double salt could be obtained by the usual procedure,[6a] if a solid crystalline product was desired. A portion (6.674 mg.) of crystalline helianthate III was similarly converted and assayed for radioactivity and streptomycin.

Radioactivity Measurements.—Carbon 14 measurements were made in a Q-Gas counter. Solutions of materials under investigation were evaporated in aluminum planchets $\simeq$ 3 cm. in diameter and 2 mm. deep, and residue activities determined. Appropriate corrections, including those for self-absorption, were made.

Streptomycin Assays.—The microbial determination of streptomycin in the several fractions cited above was performed by a cup assay method[12] employing *Bacillus subtilis* as the test organism. The N-methylglucosamine assay of Elson and Morgan,[13] as modified by Scudi, Boxer and Jelinek,[14] was employed to determine the streptomycin content of the streptomycin trihydrochloride regenerated from helianthates III and IV.

Discussion

Results compiled in Table I indicate that, inasmuch as all column eluates were radioactive, radiocarbon from all substrates employed was incorporated in the streptomycin isolated by the resin process. As seen for fermentation III, the specific activity of the purified streptomycin was quite close to that in the column eluate. Accordingly, comparison of values for "strep. carbon c.p.m./mg." with corresponding figures for "broth carbon c.p.m./mg.," which is reported in the table as a ratio under the heading "C^{14} enrichment," would reveal how well the precursor carbon is utilized as compared to other broth ingredients.

In the case of radiostarch (ferm. I), but slight enrichment (ratio 1.14) of C^{14} in the streptomycin was noted. Radioglucose was most favorable for the labeling of streptomycin, ratios of 1.48 and 3.10 being obtained after 1 and 3 days of fermentation, respectively. The lag in achieving greater enrichment was probably due to utilization of the sugar for fostering growth of the microörganism during the early stages (1–2 days) of the fermentation, rather than for streptomycin production. This supposition is supported by the considerably greater rate of $C^{14}O_2$ evolution in the early stages. Thus 21% of the total radioactivity was liberated as CO_2 (trapped in alkali and precipitated as $BaCO_3$ for measurement) in 1 day (ferm. II), whereas 29% was liberated as CO_2 in 3 days (ferm. III).

The starch hydrolysate produced a twofold enrichment of C^{14} in the product, thereby occupying a place intermediate between starch and glucose. Its effect is probably due principally to the presence of glucose (and fructose) in the hydrolysate.

(12) Federal Register, Title 21, Part 1, Section 141 (June 3, 1948).

(13) L. A. Elson and W. T. Morgan, *J. Biochem.*, **27**, 1825 (1933).

(14) J. V. Scudi, G. E. Boxer and V. C. Jelinek, *Science*, **104**, 486 (1946).

The relative constancy of specific activity of the streptomycin produced in fermentation III, in proceeding from the column eluate, through the helianthate crystals I–IV, to the regenerated trihydrochloride, is strong proof of the identity of the radioactivity with the streptomycin, *i.e.*, the radioactivity is associated with the streptomycin and not with incidental impurities.

The exact specific activity of the preparation is somewhat uncertain due to discrepancies between the various methods of determining streptomycin. Thus microbial assays of fermentation III helianthates III and IV, and of an authentic helianthate prepared from ordinary streptomycin, indicates 29.0, 25.4 and 28.0%, respectively, whereas the colorimetric (glucosamine assay) procedure showed more reasonable values such as 32.5, 31.5 and 33.5% streptomycin, respectively.

On the basis of the latter assay, the specific activity of the product from fermentation III is 5100 c.p.m./mg. streptomycin base, measured with a counting efficiency of ≃30%, or ≃0.008 μc. per mg. Fermentation IV product, after dilution with carrier, had a specific activity of ≃0.025 μc./mg., so that the original product must have had an activity of 0.2 μc./mg. It is obvious that the quantity of precursor C^{14} actually converted to streptomycin is ≃1% of that added. Higher specific activities would require precursors more efficiently utilized than glucose, or larger quantities of radioglucose.

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, PURDUE UNIVERSITY]

Crystalline Derivatives of Xylobiose[1,2]

BY ROY L. WHISTLER, JOSEPH BACHRACH AND CHEN-CHUAN TU

Partial hydrolysis of xylan leads to the isolation of a new disaccharide for which the name xylobiose is suggested. Four crystalline derivatives of xylobiose are described. Xylobiose is shown to consist of two xylopyranose units linked by a β-D-1,4′ bond.

By partial hydrolysis of xylan there has been obtained a disaccharide which is shown to be composed of two D-xylose units connected with a β-D-1,4′ linkage. Consequently the disaccharide is termed xylobiose to correspond with the disaccharide cellobiose similarly obtained from cellulose. By proper reactions xylobiose has been converted to four crystalline derivatives. This is the first time that crystalline products have been obtained from a disaccharide composed only of pentose units.

By acetolytic degradation of dimethylxylan of esparto grass Haworth and Percival[3] were able to recognize among the products a partly methylated, non-crystalline disaccharide which they showed to be composed of two xylose units linked by a β-D-1,4′ linkage.

In the present investigation xylan from corn cobs which is readily soluble in fuming hydrochloric acid was subjected to controlled partial hydrolysis. From the mixture of hydrolysis products the disaccharide was conveniently separated by the chromatographic technique of Whistler and Durso.[4] The disaccharide on acetylation yielded crystalline xylobiose hexaacetate. Deacetylation of xylobiose hexaacetate followed by hydrolytic cleavage of the amorphous disaccharide and reacetylation of the product yielded β-D-xylose tetraacetate in 85% yield.

The structure of xylobiose has been established by the procedure used by Haworth and Percival.[3] Crystalline xylobiose hexaacetate was deacetylated and oxidized to xylobionic acid which after repeated methylation with dimethyl sulfate and sodium hydroxide followed by methyl iodide and silver oxide yielded the methyl ester of hexamethylxylobionic acid. Hydrolytic cleavage of this product yielded crystalline 2,3,4-trimethyl-D-xylose in 86% of theoretical yield which was shown to be identical with an authentic specimen and a second component 2,3,5-trimethyl-γ-D-xylonolactone which distilled as a liquid in a yield of 80% of theoretical. This component was converted to the crystalline amide of the corresponding 2,3,5-trimethylxylonic acid and was shown to be identical with an authentic specimen.

Since xylobiose and its derivatives are strongly levorotatory and since this rotation decreases on hydrolysis, it is suggested that its two components are linked by a β-D-1,4′ bond.

Experimental

Hydrolysis of Xylan.—Xylan (1 g.) prepared as previously described[5,6] was dissolved in 100 ml. of fuming hydrochloric acid (sp. gr. 1.21) at −15°. This mixture was then placed in an ice–water-bath and 10-ml. samples were withdrawn at regular intervals. The samples were analyzed for reducing values.[7] When the reducing value data were plotted on a semi-logarithmic scale, a straight line was obtained. The rate constant during the initial 3–4 hours is approximately 3×10^{-4} sec.$^{-1}$; $[\alpha]^{25}D$ +40° at the time the hydrolysis is two-thirds complete.

Preparation of Xylobiose.—Xylan (30 g.) ground to pass a 60-mesh sieve was placed in a 2-l. flask and 1.5 l. of fuming hydrochloric acid (sp. gr. 1.21) previously cooled to −15° was added. Solution was complete within 30 minutes. The flask was then placed in an ice–water-bath. Hydroly-

(1) Journal Paper No. 526 of the Purdue University Agricultural Experiment Station.

(2) Paper presented before the Division of Sugar Chemistry and Technology at the 116th Meeting of the American Chemical Society, Atlantic City, N. J., 1949.

(3) W. N. Haworth and E. G. V. Percival, *J. Chem. Soc.*, 2850 (1931).

(4) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

(5) R. L. Whistler, R. Bowman and J. Bachrach, *Arch. Biochem.*, **19**, 25 (1948).

(6) R. L. Whistler, E. Heyne and J. Bachrach, THIS JOURNAL, **71**, 1476 (1949).

(7) Method of P. A. Shaffer and A. F. Hartman, *J. Biol. Chem.*, **45**, 365 (1920).