crystallization from 60% methanol. The average chain length from reducing value was 14.4 glucose units and the material was completely converted to maltose by β-amylase. Measured amounts of standardized solutions of the dextrins were allowed to react with measured quantities of periodate under various conditions. Analyses for the concentration of unreacted periodate were carried out by titration of the iodine liberated on addition of excess potassium iodide and standard arsenite to the bicarbonate buffered solution. Tests for formic acid were made by destroying the periodate with an excess of propylene glycol and titrating with 0.1 *N* sodium hydroxide to the phenolphthalein end-point. The alkali titrations of 15-ml. aliquots of the oxidation mixtures were equivalent to those obtained on periodate blanks, indicating that no formic acid was produced. The dimedon test[12] did not reveal the presence of formaldehyde in any of the digests.

Total Consumption of Periodate and Rotational Changes.—Conditions necessary to avoid over-oxidation were ascertained by treating 0.01 *M* β-dextrin with varying amounts of sodium periodate at room temperature. The reaction was followed by measuring the periodate consumption (Fig. 1) as well as the change in optical rotation; when an excess of oxidant was present the apparent rotations dropped to about −11°, then very gradually rose 3 to 4°.

The addition of buffers to the oxidation mixtures caused wide differences in the optical rotations observed. With either sodium or potassium periodate, no added buffer, and *p*H about 4, the rotation dropped to −11°. In the presence of a *p*H 6.5 potassium phosphate buffer the final rotation was 75–78°, and with sodium periodate buffered with acetic acid–sodium acetate to *p*H 4.1 or 5.5 the final rotation was 50°. Since no appreciable amount of acid was produced in the reaction, there was very little shift in the *p*H of reaction mixtures without additional buffer. This effect of buffers on rotation was not investigated further.

Following the preliminary experiments with β-dextrin, solutions of α-, β- and γ-dextrins were oxidized under identical conditions with 1.1 moles of sodium metaperiodate/glucose residue. The oxidations were carried out at 28° without added buffer. The rotational changes and periodate consumption are plotted in Figs. 2 and 3. After four days, the consumption of periodate was not greater than 1.03 moles/glucose residue.

Kinetic Analysis.—The order of the initial part of the reaction at 4° was determined in 25-minute oxidations with beta dextrin:

S_0	P_0	$-\Delta P/25$ min.	
0.00254	0.01660	0.00037	
.00254	.04975	.00100	Order in periodate = 0.90
.00506	.01660	.00070	Order in Schardinger dextrin = 0.92

Oxidation of dextrin–periodate mixtures (dextrins *ca.* 0.04 *M* with respect to glucose residues) was then followed at 4° over 2–3 days, the amount of periodate consumed determined by titrating aliquots, and the data used for the determination of the rate constants as explained above. The values of the second order rate constants k_1 and k_2 so obtained are: α, $k_1 = 9.0$, $k_2 = 5.4$; β, $k_1 = 33$, $k_2 = 8.3$; γ, $k_1 = 105$, $k_2 = 21.0$; all expressed as liters × mole^{-1} × sec.$^{-1}$ × 10^{-5} at 4°, with an uncertainty of about 5% of the stated values for each constant. The curves calculated for these constants together with the experimental points appear in Fig. 4. Comparable data for a Nägeli-type amylodextrin of average chain length approximately 14.4 glucose residues are compared (Fig. 4) with a curve calculated on the assumption that all the glucose residues are oxidized at the same rate; $k_{av.} = 65 \times 10^{-5}$ liters × moles^{-1} × sec.$^{-1}$.

Effect of Iodate on Reaction Velocity.—To each of five flasks was added sufficient sodium periodate and β-dextrin so that the initial concentration after dilution was 0.0133 *M* and 0.00161 *M*, respectively, together with sodium iodate to the extent of 0, 0.0056, 0.0140, 0.0280 and 0.0420 *M*. After 61 min. at 4° the remaining periodate was determined to be the same in each flask: 0.0126 ± 0.0001 *M*.

Summary

Oxidation of the α-, β- and γ-dextrins with sodium periodate has been characterized as producing no formic acid or formaldehyde and consuming one mole of periodate per glucose residue. The kinetics of the reaction have been analyzed by an approximate method and up to about 40% total oxidation agree with curves calculated for an initially hindered reaction followed by a more rapid oxidation. The rate of the initial oxidation increases in the order: alpha, beta, gamma. The initial oxidation of the γ-dextrin is still subject to an inhibition not characteristic of a low molecular weight amylodextrin.

AMES, IOWA RECEIVED APRIL 28, 1950

(12) Vorlander, *Z. anal. Chem.*, **77**, 241 (1929).

[CONTRIBUTION FROM THE IOWA AGRICULTURAL EXPERIMENT STATION]

Studies on the Schardinger Dextrins. VI. The Molecular Size and Structure of the γ-Dextrin[1]

BY DEXTER FRENCH, DORIS W. KNAPP AND J. H. PAZUR

The application of crystallographic procedures for the determination of molecular size to the α- and β-dextrins indicated that these are composed of six and seven glucose residues, respectively.[2] A similar study has now been directed toward the establishment of the size of the γ-dextrin,[3] previously regarded by Freudenberg[4] as a cyclic heptasaccharide. In the present study it was not found possible to determine unambiguously the number of glucose units per molecule of γ-dextrin by crystallographic procedures alone, but in addition it was necessary to examine

(1) Journal Paper No. J-1782 of the Iowa Agricultural Experiment Station, Ames, Iowa. Proj. 1116; supported in part by a grant from the Corn Industries Research Foundation.

(2) French and Rundle, THIS JOURNAL, **64**, 1651 (1942).

(3) French, Levine, Pazur and Norberg, *ibid.*, **71**, 353 (1949).

(4) (a) Freudenberg and Jacobi, *Ann.*, **518**, 102 (1935); (b) Freudenberg, Plankenhorn and Knauber, *Chem. and Ind.*, 731 (1947); (c) *Ann.*, **558**, 1 (1947); (d) FIAT Report No. 1096 (duplicate publications). (e) A late publication, Borchert, *Z. Naturforsch*, **3b**, 464 (1948), presents X-ray evidence that the γ-dextrin is either a tetrasaccharide or an octasaccharide. (f) A tetrasaccharide structure is not favored by Freudenberg and Cramer, *ibid.*, **3b**, 464 (1948), as being sterically unlikely as well as being out of line with the trend in optical rotations established by the α- and β-dextrins. Freudenberg and Cramer now accept the hexasaccharide character of the α-dextrin and the heptasaccharide character of the β-dextrin.

other evidence, especially the character of the initial product of acid hydrolysis. The findings reported here, taken together with previously available information on the regularity of the γ-dextrin structure[4,5] indicate that the gamma dextrin is composed of eight glucose residues symmetrically arranged in a ring and joined mutually by α-1,4-glycosidic bonds. The name *cycloöctaamylose* is now suggested as appropriate for the γ-dextrin, in that it symbolizes the essential structural characteristics and is in harmony with the names *cyclohexaamylose* and *cycloheptaamylose* previously used for the α- and β-dextrins, respectively.

Just as the initial hydrolytic product from cycloheptaamylose is amyloheptaose,[6] similarly the hydrolysis of cycloöctaamylose would be expected to yield amyloöctaose. Indeed, the product obtained by the mildest acid treatment of γ-dextrin is amyloöctaose, as judged by (a) its optical rotation and reducing value, (b) its complete conversion to maltose by β-amylase (c) its location on paper chromatograms in relation to the series of amyloöligosaccharides obtained from linear starch by acid hydrolysis, and (d) the conversion by more extensive acid hydrolysis into a mixture of oligosaccharides including all members from glucose to amyloöctaose, similarly resolved and identified on paper chromatograms.

The crystallographic space group of the γ-dextrin–propanol complex has been found to be $P42_1$ (tetragonal), with 48 glucose residues per unit cell. With this symmetry, there must be 8 molecules per cell if the molecules are completely lacking in symmetry, 4 molecules with a twofold axis of symmetry, or 2 molecules with a fourfold axis of symmetry. The only possibility consistent with the arguments of the preceding paragraph is that the gamma dextrin crystallizes with propanol in such a way that there are two molecules displaying the complete symmetry allowed, *i.e.*, a fourfold axis, while four other molecules lie on twofold axes, probably normal to the fourfold-axis. This high degree of demonstrable symmetry of cycloöctaamylose is of interest, not only in that it is the most symmetrical known carbohydrate (excepting the carbohydrate alcohols and cyclitols), but that it indicates that any structural feature or irregularity in the γ-dextrin would appear four times per molecule. Together with the suggestive results of periodate oxidation,[5] these findings constitute convincing evidence that the γ-dextrin is a regular, symmetrical cyclic octasaccharide, cycloöctaamylose, homologous with cyclohexaamylose (α-dextrin) and cycloheptaamylose (β-dextrin).

Acknowledgment.—We wish to thank Dr. N. K. Richtmyer for making available to us ref. 4f. The chromatographic procedures herein described are in part adaptations of techniques shown us by the staff members of the Northern Regional Research Laboratory, Starch and Dextrose Division, Peoria, Illinois.

(5) French and McIntire, THIS JOURNAL, **72**, 5148 (1950).

(6) French, Levine and Pazur, *ibid.*, **71**, 356 (1949).

Experimental

γ-Dextrin.—The air-dry propanol complex was prepared as previously described.[3] The carbohydrate content of the beautifully crystalline material was determined by carrying a weighed sample repeatedly through the sequence: solution in water, boiling, and evaporation practically to dryness; until the propanol was removed. The aqueous sirup was then allowed to crystallize, air-dried, and dried to constant weight at 70° *in vacuo;* nonvolatile, 76.6%. The density of well-formed crystals of the air-dry propanol complex was determined to be 1.351 by flotation in a mixture of toluene and carbon tetrachloride.

X-Ray Diffraction Measurements.—The unit cell and space-group data were obtained as previously described[2] using oscillation and goniometer patterns with nickel-filtered copper radiation: $a_0 = 23.7$ Å.; $c_0 = 22.2$ Å.; space-group $P42_1$ (no systematic extinctions except odd orders of $(h00)$); glucose residues per unit cell

$$\frac{(1.351)\ (0.766)\ (23.7)^2\ (22.2)\ (0.606)}{(162.1)} = 48.2 \simeq 48$$

Acid Hydrolysis.—The pure propanol complex of γ-dextrin, 25 g., was freed from propanol as above and refluxed with 0.001 *N* hydrochloric acid four hours. The acid was exactly neutralized with lithium carbonate and the unchanged γ-dextrin precipitated with toluene[3,6]; yield *ca.* 80%. The filtrate from the toluene complex was then concentrated *in vacuo* to a sirup and precipitated with absolute alcohol. The product was rubbed under fresh alcohol to a coarse powder, filtered off and washed with a little dry butanol to prevent gumming: and dried *in vacuo* at 70° to constant weight; $[\alpha]^{27}D$ 180.0° (*c* 1, water), calcd. for amyloöctaose,[7] 185°; molecular weight by alkaline copper oxidation,[8] 1350, calcd., 1314. A more extensively hydrolyzed product was prepared by refluxing for twenty-four hours instead of four hours. Similar hydrolyses were carried out with the α- and β-dextrins.

Paper Chromatography.—Droplets of *ca.* 0.01 ml. of 1–10% solutions of the substances being tested were placed at intervals of 1 inch along a line ruled 0.5 inch from one edge of a rectangle, generally 8 in. by 10 in., of filter paper.[9] After drying, the paper was rolled into a cylinder and held in this form by a wire staple at each end, thus giving a cylinder capable of supporting itself even when wet with solvent. The cylinder, with the sample spots near the bottom, was then placed in a shallow layer of solvent (3 parts water, 4 parts pyridine, 6 parts butanol, by volume)[10] such that the sample spots were above the solvent level. The solvent vessel was kept away from marked thermal disturbances, out of direct light, and tightly closed by a glass plate. With smaller paper cylinders, screw-cap bottles have been found most convenient. After the solvent had climbed by capillary attraction to the top of the cylinder, it was removed from the vessel, air-dried and oven-dried, then returned to the solvent for one or more additional climbs. For the resolution of oligosaccharides in the octasaccharide range from six to twelve climbs may be needed. Finally the dried cylinder was unrolled and sprayed lightly with an alkaline copper reagent,[11] heated in an oven at 105° for about five minutes, then sprayed with a phosphomolybdic acid reagent[12] to locate the areas in

(7) Levine, Foster and Hixon, *ibid.*, **64**, 2331 (1942).

(8) (a) Swanson and Cori, *J. Biol. Chem.*, **172**, 797 (1948); (b) Shaffer and Somogyi, *ibid.*, **100**, 695 (1933).

(9) Eaton and Dikeman 613, Allied filter paper, distributed by Geo. T. Walker and Co., Minneapolis. This paper has given us better resolution than Whatman No. 1.

(10) Chargaff, Levine and Green, *J. Biol. Chem.*, **175**, 67 (1948).

(11) Reagent 60 (ref. 8b) made up without potassium iodate or potassium iodide.

(12) Tauber and Kleiner, *J. Biol. Chem.*, **99**, 249 (1932).

which reduction of the copper reagent has taken place. The reducing carbohydrates show up as blue spots against a white background, which gradually becomes somewhat blue.

When examined by this technique, a linear starch degradation product of average size about 3 glucose units, showed clearly resolved spots at least through the octasaccharide, while beyond the octasaccharide partially resolved components through the dodecasaccharide appeared. Beyond this a continuum of components extended to those which failed to move under the chromatographic conditions used. The initial hydrolytic products from α-, β- and γ-dextrins corresponded very exactly in position with the hexasaccharide, heptasaccharide and octasaccharide components of the linear starch hydrolysate. In the 24-hour hydrolysates, it was easily possible to see the spots corresponding to each oligosaccharide from glucose through heptasaccharide (β-dextrin) and from glucose through octasaccharide (γ-dextrin). There was no evidence for components higher than the heptasaccharide or octasaccharide, although as mentioned previously these components were clearly indicated in the linear starch degradation product.

β-Amylase Digestion.—An excess of soybean β-amylase[13] was added to a sample of the 4-hour hydrolysate and the amount of maltose formed determined by alkaline copper reduction[8]: 101, 104, 100%; calcd. for $(C_6H_{10}O_5)_7$-$(C_6H_{12}O_6)$: 104.0. No components other than maltose were detected by paper chromatography of the digest.

Summary

X-Ray crystallographic measurements, acid hydrolysis, paper chromatography and enzyme digestion together with previous periodate oxidation studies indicate that the γ-dextrin is the cyclic octasaccharide of the amylose series, cyclooctaamylose.

(13) Newton and Naylor, *Cereal Chem.*, **16**, 71 (1939).

AMES, IOWA RECEIVED MAY 8, 1950

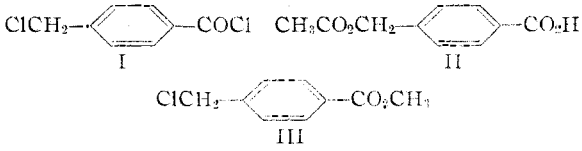
[CONTRIBUTION FROM THE CENTRAL RESEARCH DEPARTMENT, MONSANTO CHEMICAL COMPANY]

Some Esters Based on *p*-Chloromethylbenzoyl Chloride

BY WILLIAM S. EMERSON AND ROBERT A. HEIMSCH

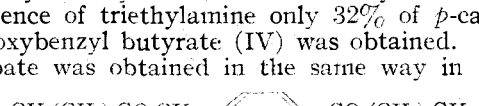
p-Chloromethylbenzoyl chloride (I), readily preparable in 91–95% yields by the chlorination of *p*-toluyl chloride, constitutes a very versatile intermediate for the preparation of *p*-toluic acid derivatives. In this paper are described certain fundamental reactions of this compound, which involve both the alcohol and acid functions of the molecule.

Treatment of *p*-chloromethylbenzoyl chloride with sodium acetate in acetic acid followed by hydrolysis yielded 72% of *p*-carboxybenzyl acetate (II), which was further hydrolyzed with aqueous alkali to the known *p*-carboxybenzyl alcohol.

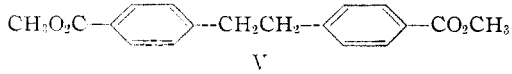


p-Chloromethylbenzoyl chloride reacted with cold aqueous alkali to give 98% of *p*-chloromethylbenzoic acid. The *n*-butyl and methyl esters (III) were prepared in 87% and 93% yields, respectively, by using the appropriate alcohol[1] in place of water.

n-Butyl *p*-chloromethylbenzoate did not esterify readily. With sodium butyrate in the presence of triethylamine only 32% of *p*-carbobutoxybenzyl butyrate (IV) was obtained. The adipate was obtained in the same way in 12% yield along with 6% of monocarbobutoxybenzyl adipate.



Methyl *p*-chloromethylbenzoate (III) reacted with alcoholic potassium thiocyanate to give 58% of methyl *p*-isothiocyanomethylbenzoate. The thiocyanate rearranged probably during its isolation. With Raney alloy in boiling water[2] methyl *p*-chloromethylbenzoate yielded principally methyl *p*-toluate (53%) together with 17% of 1,2-bis-(*p*-carbomethoxyphenyl)-ethane (V).



Methyl *p*-chloromethylbenzoate reacted with sodium ethyl malonate to give 66% of ethyl *p*-carbomethoxybenzylmalonate.[3] This compound was hydrolyzed, decarboxylated and reesterified to give a 46% over-all yield of methyl β-(*p*-carbomethoxyphenyl)-propionate. The 2-ethylhexyl ester also was prepared.

Experimental

p-Chloromethylbenzoyl Chloride.—*p*-Toluyl chloride was chlorinated at 75–90°. At 42–50% conversions yields ranged from 91% to 95%. A sample, b. p. 140–156° (20 mm.) (150–155° (22 mm.)),[4] solidified on standing. It was crystallized from hexane, m. p. 30–31°,[5] (33–35°).[6]

Anal.[7] Calcd. for $C_8H_8OCl_2$: C, 50.8; H, 3.17. Found: C, 50.7; H, 3.12.

(1) Blicke and Lilienfeld, THIS JOURNAL, **65**, 2281 (1943), prepared the ethyl ester in 90% yield from the acid chloride and ethanol in the presence of a pyridine catalyst.

(2) Bun-Hoi and Hoán, *J. Org. Chem.*, **14**, 1023 (1949).

(3) Titley, *J. Chem. Soc.*, 2571 (1928), condensed ethyl *p*-bromomethylbenzoate with sodium ethyl chloromalonate, and ethyl *m*-chloromethylbenzoate with sodium ethyl methylmalonate.

(4) Badische Anilin-Soda Fabrik, German Patent 239,311; *Frdl.*, **10**, 118 (1910).

(5) All of the melting points are uncorrected.

(6) P. B. Report No. 82040; Frames 2986-2995.

(7) All of the analyses are microanalyses performed by Mr. P. J. Adams and Mr. Donald Stolz of this laboratory and by the Micro Tech Laboratories, 8000 Lincoln Ave., Skokie, Ill.