acid, 2 ml. of aniline and 61 g. (0.5 mole) of recrystallized *p*-hydroxybenzaldehyde, there was obtained after three recrystallizations from water, 57 g. (70%), m. p. 210.5–211 (206°, 52%[8]), (207°, 33%[12a]), (62%, no m. p.[12b]), (206°, 70%[12c]), (206°, 80%[12d]), (no m. p., 80% crude yield[12e]).

The acid chlorides were prepared according to the procedure described for the preparation of *n*-butyryl chloride (thionyl chloride) by Helfrich and Schaefer[13] (R = *n*-C_5H_7, *n*-C_6H_{11}, *n*-C_7H_{15}) or by the procedure described by Hickenbottom[14] using phosphorus trichloride (R = C_2H_5, *n*-$C_{11}H_{23}$, *n*-$C_{15}H_{31}$) and were purified by distillation.

The *p*-acyloxycinnamic acids were prepared by esterification of *p*-hydroxycinnamic acid by the method described by Ullman and Nadai.[15]

The reaction mixture was added to a dilute sulfuric acid solution and either a solid or a heavy oil precipitated. The heavy oils soon solidified on standing. This product was then purified by recrystallization, the first recrystallization being carried out with the addition of Norite. For example, from 41.6 ml. (0.3 mole) of caproyl chloride and 25.5 g. (0.155 mole) of *p*-hydroxycinnamic acid in 175 ml. of dry pyridine, there was obtained 66.8 g. (85%) of a white crystalline solid, m. p. 149.7–150.7°, 175.6–177.6°. These *p*-acyloxycinnamic acids are described in Table I.

p-Acetoxycinnamic acid was prepared by the Perkin procedure according to the method described by v. Konek and Pacsu (38%).[8]

Isolation of By-Products in the Preparation of *p*-Acyloxycinnamic Acids.—On effecting solution of the crude *p*-butyroxycinnamic acid in absolute ethanol, there was obtained a small quantity of a white crystalline material, more insoluble than the main product, m. p. 210.5–211.5°. This may be the product resulting from the esterification of *p*-butyroxycinnamic acid by *p*-hydroxycinnamic acid.

Anal. Calcd. for $C_{22}H_{20}O_6$: C, 69.46; H, 5.31. Found: C, 69.45; H, 5.29.

In a similar way a white crystalline by-product was obtained from *p*-caproxycinnamic acid, m. p. 193.5–194.5°.

Anal. Calcd. for $C_{24}H_{24}O_6$: C, 70.57; H, 5.92. Found: C, 70.54; H, 5.85.

***p*-Acyloxystyrenes.**—The decarboxylation procedure was similar to that described by Walling and Wolfstirn[7] with the following modifications. This procedure was used to prepare all the styrenes except R = $C_{15}H_{31}$. *p-t*-Butylcatechol was used as the inhibitor. The period of decarboxylation as determined by carbon dioxide evolution (gas buret and lime water) required about forty minutes. Decarboxylation usually started between 140 and 180° (maximum rate of decarboxylation was reached about 205–210°) and continued as the temperature was raised to the boiling point of quinoline. The colorless liquid styrenes were purified by several distillations from modified Claisen flasks. A typical experiment employed 10 g. of a *p*-acyloxycinnamic acid, 30 g. of quinoline, 1 g. of copper powder and a small amount of *p-t*-butylcatechol. When R = $C_{11}H_{23}$, the initial distillation gave a fraction, b. p. 144–157° (1 mm.), which solidified. Four recrystallizations from 95% methanol gave the desired product. This procedure gave a lower yield for R = $C_{11}H_{23}$ than that reported in Table II.

An alternate procedure was used in the preparation of the solid styrenes, R = $C_{11}H_{23}$, $C_{15}H_{31}$ (Table II). The reaction mixture after decarboxylation was separated from the copper powder by filtration. Ether was added to the filtrate and the ether solution treated as described in reference 7, except that additional acidic washes were used. After removal of the ether, the styrenes were recrystallized from 95% methanol. Four or five recrystallizations were necessary in order to obtain an analytical sample.

The use of copper powder in nitrobenzene or aqueous mineral acid to effect the decarboxylation was unsuccessful.

Summary

The preparation and properties of six new *p*-acyloxycinnamic acids and six new *p*-acyloxystyrenes are described.

BROOKLYN, NEW YORK RECEIVED SEPTEMBER 2, 1949

(12) (a) Kurien, Pandya and Surange, *J. Indian Chem. Soc.*, **11**, 823 (1934); (b) Posner, *J. prakt. Chem.*, (2) **82**, 425 (1910); (c) Eigel, *ibid.*, **20**, 2527 (1887); (d) Zincke and Leisse, *Ann.*, **322**, 220 (1902); (e) Sonn, *Ber.*, **46**, 4050 (1913).

(13) Helfrich and Schaefer, "Organic Syntheses," Vol. 9, John Wiley and Sons, Inc., New York, N. Y., 1929, p. 32.

(14) Hickenbottom, "Reactions of Organic Compounds," Longmans, Green and Co., 1946, p. 195.

(15) Ullman and Nadai, *Ber.*, **41**, 1870 (1908); see also Einhorn, *Ann.*, **301**, 95 (1898).

[CONTRIBUTION FROM THE IOWA AGRICULTURAL EXPERIMENT STATION]

Studies on the Schardinger Dextrins. III. Redistribution Reactions of *Macerans* Amylase[1]

BY ETHELDA NORBERG[2] AND DEXTER FRENCH

The formation of Schardinger dextrins from starch and linear glucosidic dextrins has been attributed to a type of "glucosidic exchange" action.[3] Here, *macerans* amylase is apparently capable of bringing about the exchange of the energy from one α-1,4-glucosidic bond in a linear chain to another new α-1,4-glucosidic bond in a cyclic dextrin. This action has been found to be reversible.[4] *Macerans* amylase has been shown to be capable of transforming purified cyclic dextrins into longer linear molecules in the presence of suitable cosubstrates, such as glucose, maltose, α-methyl glucoside, sucrose, cellobiose, and maltobionic acid.

The studies reported here have shown that the glucosidic exchange action of *macerans* amylase is not limited to reactions involving cyclic dextrin molecules. In the presence of the *macerans* enzyme, linear dextrins are also capable of entering into reaction with each other, which effects redistribution of the available glucose residues among

(1) Journal Paper No. J-1674 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1116. Supported in part by a grant from the Corn Industries Research Foundation.

(2) A portion of the thesis submitted to the Graduate School, Iowa State College, by Ethelda Norberg in partial fulfilment of the requirements for the degree of Doctor of Philosophy, June, 1949. Present address: Biochemical Division, Medical School, University of California, Berkeley 4, California.

(3) Cori, *Federation Proc.*, **4**, 226 (1945).

(4) French, Pazur, Levine and Norberg, THIS JOURNAL, **70**, 3145 (1948).

dextrins of longer and shorter chain lengths. These redistribution reactions among linear reducing oligosaccharides and dextrins we have called "homologizing" reactions.

Electrophoretic techniques have been developed for analysis of the Schardinger dextrins and the reducing oligosaccharide fractions of enzyme digests. Thus, the action of *macerans* amylase on amyloheptaose[5] has been studied by analysis of digest aliquots inactivated at varying time intervals up to 500 conversion periods.[6] The Schardinger dextrins were removed from each digest aliquot by precipitation with trichloroethylene and analyzed separately. The remaining reducing oligosaccharides were oxidized with alkaline hypoiodite, and the potassium salts of the corresponding acids analyzed electrophoretically.

The electrophoretic patterns of some Schardinger dextrin fractions from aliquots of a 10% amyloheptaose digest are shown in Fig. 1. These analyses confirm the findings of McClenahan, Tilden and Hudson,[7] that the alpha dextrin is formed first and disappears as the beta dextrin is formed. The gamma dextrin is formed even later. No alpha dextrin could be detected in the digest before one conversion period by microscopic identification of the characteristic alpha-dextrin–triiodide complex crystals.

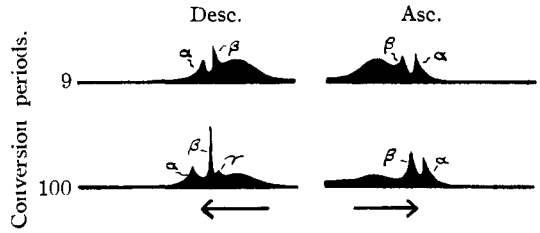


Fig. 1.—Electrophoretic patterns of Schardinger dextrin fractions from aliquots of a *macerans* amylase digest of amyloheptaose.

Figure 2 shows the electrophoretic patterns of the oxidized linear oligosaccharide fractions of aliquots of the heptasaccharide digest. At zero time, there is present only the amyloheptaonate anion, corresponding to the unaltered amyloheptaose substrate. Within one-fourth to one conversion period, approximately one-half the amyloheptaose substrate has been converted primarily to one shorter and one longer chain dextrin. The mobilities calculated for these peaks indicate that the components present at one conversion period correspond to the acid anions of amylotetraose, amyloheptaose, and amylodecaose. After 100 conversion periods, there is evidence for the presence of linear oligosaccharides including maltose, amylotriose, amylotetraose, and unresolved higher saccharides. At 500 conversion periods, glucose is also present.

The results of these electrophoretic analyses

(5) French, Levine and Pazur, THIS JOURNAL, **71**, 356 (1949).
(6) French, Levine, Pazur and Norberg, *ibid.*, **71**, 353 (1949).
(7) McClenahan, Tilden and Hudson, *ibid.*, **64**, 2139 (1942).

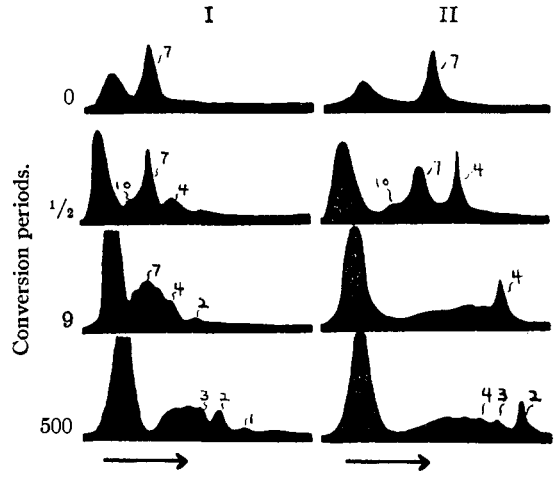


Fig. 2.—Ascending electrophoretic patterns of oxidized linear oligosaccharide fractions of a *macerans* amylase digest of amyloheptaose: column I includes patterns taken at time intervals from 60 to 100 minutes; column II includes from 125 to 160 minutes; numbers refer to the number of glucose residues in peak components.

seem to indicate that the initial reactions in the action of *macerans* amylase on amyloheptaose are those of equations 1 and 2 (where α is alpha dextrin, G_7 is amyloheptaose, G_4 is amylotetraose, etc.).

$$2G_7 \longrightarrow G_4 + G_{10} \quad (1)$$

$$G_{10} \longrightarrow G_4 + \alpha \quad (2)$$

The initial predominance of these reactions in the presence of excess substrate would account for the presence of only three major linear components in the earlier stages of the digestion up to three conversion periods.

Successive stages of *macerans* amylase action on amyloheptaose, as shown by electrophoretic analysis, may then be represented by the general equations 3.

$$G_7 \longrightarrow G_4 + G_7 + G_{10} + \alpha \longrightarrow G_1 + G_2 + G_3 + G_4 + G_5 + \text{higher saccharides} + \beta + \gamma \quad (3)$$

The action of *macerans* amylase on a maltose substrate has also been studied. Here, there has been no detectable amount of Schardinger dextrins formed, but homologizing reactions have occurred.

The initial redistribution reaction of *macerans* amylase on maltose must be that of equation 4.

$$2G_2 \longrightarrow G_1 + G_3 \quad (4)$$

Therefore, *macerans* action on a maltose substrate can be followed by determination of the glucose produced with time. Results of such a study are given in Fig. 3. On the basis of an unpublished mathematical treatment of the reactions catalyzed by *macerans* amylase, one would expect the amount of glucose formed to approach an equilibrium value of approximately 25% of the total original maltose. The slightly higher conversion

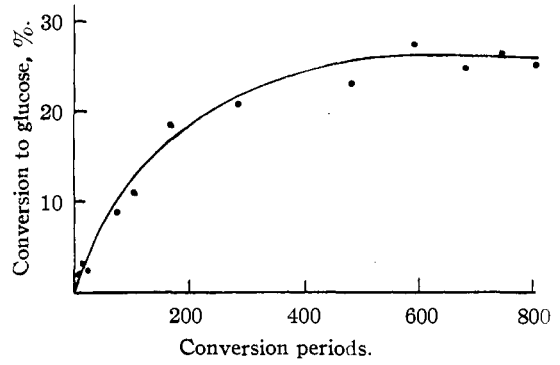


Fig. 3.—Glucose formation during *macerans* amylase action on maltose.

to glucose in Fig. 3 may be accounted for by the hydrolytic activity of the crude bacterial filtrate.

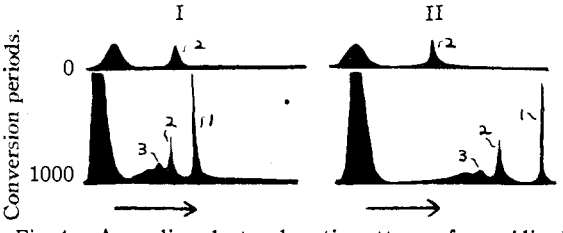


Fig. 4.—Ascending electrophoretic patterns of an oxidized *macerans* amylase digest of maltose: numbers refer to the number of glucose residues in peak components.

Figure 4 shows the electrophoretic patterns of an oxidized digest of maltose plus purified *macerans* amylase at zero time and after 1000 conversion periods. Mobility calculations indicate that the over-all reaction may be represented by equation 5.

$$G_2 \longrightarrow G_1 + G_2 + G_3 + \text{higher saccharides} \quad (5)$$

The mixture of saccharides from a maltose digest has been subjected to alcohol precipitation to effect a separation of the lower sugars (glucose, maltose, etc.) from the longer chain oligosaccharides. The isolated longer chain fraction was shown to contain normal amylooligosaccharides by its hydrolysis with salivary amylase and its conversion to Schardinger dextrins with *macerans* amylase.

These reactions serve to correlate the different redistribution reactions of *macerans* amylase so far observed: cyclic dextrin formation, coupling reactions and homologizing reactions can occur simultaneously.

Macerans amylase is apparently capable of acting on amylaceous substrates ranging from starch to maltose. However, the slow rate of *macerans* action on maltose, compared to amyloheptaose, and the different rates of formation of the alpha, beta and gamma cyclic dextrins show that individual reactions proceed at different relative rates. *Macerans* amylase action may then be pictured as an approach to a state of dynamic equilibrium through a series of concurrent redistribution reactions.

Experimental

Enzyme Preparation.—*Macerans* amylase in 2 l. of *Bacillus macerans* filtrate was precipitated with an equal volume of 95% ethanol (precooled so that precipitation temperature did not rise above 0°), centrifuged, extracted with one-tenth volume cold water, adsorbed on acetone-extracted potato starch (1 g. per 300 units activity) from 25% acetone solution,[8] eluted with 0.2% solution of Schardinger beta-dextrin, evaporated in a viscose tubing bag in a cold air stream, and dialyzed. All steps were carried out in a cold room at 2°. Solutions used in sterile digests were filtered aseptically through a bacterial fritted glass filter.

The solutions so prepared were clear and colorless. Total activity recovery was about 60%. R_{Cu} determinations on *macerans* digested starch substrates showed 2.4% apparent hydrolysis after 100 conversion periods.

Amyloheptaose Digest.—To 50 ml. of a sterile 10% solution of amyloheptaose was added 10 ml. of sterile *macerans* enzyme solution at 15 units per ml. (Tilden assay[9]). After thorough mixing, 6-ml. aliquots (0.5 g. substrate) were transferred to sterile cotton-plugged tubes and incubated at 33°. These conditions allowed a conversion period of one hour for the digest. Aliquots were removed and inactivated by heating at intervals of 1/4, 1/2, 1, 3, 9, 60, 100, 250 and 500 conversion periods.

Each inactivated aliquot was made up to 10 ml. volume and the Schardinger dextrins precipitated with trichloroethylene. The complex precipitate was filtered off, dissolved in water and boiled to remove the trichloroethylene, dried, and redissolved in 12 ml. of 0.087 *M* potassium iodide for electrophoretic analysis.

The filtrates from the Schardinger dextrin precipitations contained the soluble reducing sugars and dextrins. These were oxidized to the corresponding monocarboxylic acids by an adaptation of the alkaline hypoiodite procedure of Goebel.[10] To each filtrate (0.44 millimolar in reducing groups) was added 5 ml. of 0.3 *N* iodine in 0.15 *M* calcium iodide. To this was added 0.13 g. of calcium hydroxide over a three-minute period, and the mixture was allowed to react at room temperature for fifteen minutes. To this was added 0.33 g. of oxalic acid, and the excess neutralized with calcium carbonate. The solution was then filtered, evaporated to 10 ml. volume at 60° under reduced pressure, and twice thrown out of solution with 300 ml. of acetone.

The calcium salts of the sugar acids were converted to the potassium salts by solution in 5 ml. of 0.1 *M* potassium acetate, passing through a column of potassium-saturated IR-100 Amberlite resin,[11] and elution with 0.1 *M* potassium acetate to a total volume of 12 ml., which was suitable for electrophoretic analysis.

Oxidation of synthetic mixtures of glucose, maltose, and heptasaccharide showed the total recovery of oxidized oligosaccharides to be less than 100%, but recoveries of different constituents were approximately proportional to their concentration in the original solution.

Maltose Digests.—A 5% solution of maltose (purified by dialysis through viscose tubing and recrystallization) was incubated at 40° with sufficient purified *macerans* amylase to effect a six-minute conversion period in an all-glass polarimeter tube under toluene. The angular rotation fell at a steady rate, gradually levelling off; the readings at various conversion periods were as follows: 6.57°, initial; 6.51°, 18 conversion periods; 6.37°, 71 conversion periods; 6.11°, 180 conversion periods; 6.08°, 500 conversion periods. After 1000 conversion periods, the

(8) (a) Holmbergh, *Biochem. Z.*, **258**, 134 (1933); (b) Schwimmer and Balls, *J. Biol. Chem.*, **176**, 465–466 (1948).

(9) Tilden and Hudson, *J. Bact.*, **43**, 527 (1942).

(10) Goebel, *J. Biol. Chem.*, **72**, 809 (1927).

(11) Product of Resinous Products and Chemical Co., distributed by Fischer Scientific Co., Pittsburgh, Penna.

enzyme was inactivated and the digest prepared for electrophoretic analysis as with the amyloheptaose digest.

The rate of glucose formation on digestion of maltose with *macerans* amylase was determined by analysis of aliquots removed at varying time intervals from a digest of a 3% maltose substrate with the filtrate from a pure culture of *Bacillus macerans*. Incubation was at 37 to 40° for intervals up to 800 fifteen-minute conversion periods. Glucose was determined by the micro colorimetric method of Tauber and Kleiner.[12,13]

Fractionation of the products of a similar digest was effected by precipitating with 90% ethanol and seeding with crystalline maltose hydrate. The precipitate was not crystalline and showed an average DP of approximately 4, based on reducing value. About 0.2 g. of this precipitate was dissolved in 25 ml. of water and the solution was divided into two portions. One fraction of the solution was pretreated with salivary amylase and exhibited a doubling of reducing value, and a drop in observed rotation from +1.27 to +1.16° (sodium light, 2-dm. tube). The remaining fraction was not hydrolyzed. Aliquots of both fractions were then treated with *macerans* amylase for five conversion periods. The untreated solution showed pronounced Schardinger dextrin formation, while the predigested fraction showed no conversion to Schardinger dextrins.

Electrophoretic Analyses.—The Schardinger dextrin mixtures were resolved in solution in 0.087 *M* potassium iodide against 0.087 *M* potassium iodide electrolyte, at 190 volts and 32 m. a. for four to five hours.

Oligosaccharide acid mixtures were resolved in 1 to 2% solution in 0.1 *M* potassium acetate against 0.1 *M* potassium acetate electrolyte, at 190 volts for one to three hours.

Ascending and descending mobilities calculated under the above conditions for a synthetic mixture of alpha, beta and gamma Schardinger dextrins were α = 3.9, 3.1; β = 2.8, 2.4; and γ = 2.3, 2.2 cm.^{2} sec.$^{-1}$ v.$^{-1}$ × 10^5. Ascending mobilities calculated under the above conditions for an oxidized synthetic mixture of reducing oligosaccharides were glucose = 11.4; maltose = 8.6; amyloheptaose = 4.8 cm.^{2} sec.$^{-1}$ v.$^{-1}$ × 10^5.

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

(13) The experimental work of this paragraph was carried out by Mrs. Doris W. Knapp.

Summary

1. Electrophoretic procedures have been developed for qualitative analysis of mixtures of Schardinger dextrins and mixtures of reducing oligosaccharides.

2. These electrophoretic procedures have been used to follow the course of action of *macerans* amylase on the homogeneous substrates, amyloheptaose and maltose.

3. *Macerans* amylase has been shown to be capable of effecting redistribution reactions among linear amylooligosaccharides concurrently with reactions involving the cyclic Schardinger dextrins.

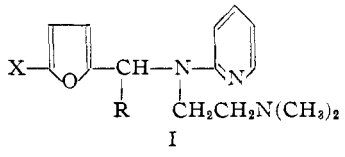
AMES, IOWA RECEIVED AUGUST 8, 1949

[A CONTRIBUTION FROM EATON LABORATORIES, INC., NORWICH, N. Y.]

Some Furan Antihistaminic Agents

BY KENYON HAYES, GABRIEL GEVER AND JAMES ORCUTT

Viaud[1] reported the high antihistaminic activity of N,N-dimethyl-N′-(2-furyl)-methyl-N′-(2-pyridyl)-ethylenediamine. This was of much interest to us because of our continuing study of the pharmaceutical applications of furan derivatives. We have prepared and tested a limited series of compounds of general structure I.



I

After this work was completed a number of the compounds presented were described by other workers in the field.[2,2a,2b]

Since the synthetic route employed differs from that of the previous workers and some of the intermediates and furylalkylethylenediamines are new, we wish to describe the work.

The general method of preparation involved the condensation of furfural or 5-halo-2-furaldehydes, with 2-aminopyridine or 2-aminothiazole to yield the azomethines. Of these, N-furfurylidene-2-aminopyridine has been reported by Ridi[3] and Ziering and Buck.[3a] In all instances investigated this condensation proceeded *via* an intermediate N,N′-furfurylidenebisamino compound, as observed by Kirpal and Reiter[4] in the case of benzaldehyde and 2-aminopyridine.

The azomethines were catalytically hydrogenated by a modification of the method of Adkins and Winans[5] to yield the N-furylmethyl-2-aminopyridines. One member of this group, N-(2-furyl)-methyl-2-aminopyridine, has been reported by Ziering and Buck[3a] without experimental directions.

In some cases the Schiff bases were reductively alkylated with a Grignard reagent by the procedure of Moffett and Hoehn.[6] The physical properties of the furylalkylamines are shown in Table I.

Some of the secondary furylalkylamines thus obtained were lithiated with lithium amide in benzene and treated with 2-dimethylaminoethyl

(1) Viaud, *Produits Pharmaceutiques*, **2**, 53 (1947).

(2) Vaughan and Anderson, THIS JOURNAL, **70**, 2607 (1948).

(2a) Kyrides and Zienty, *ibid.*, **71**, 1122 (1949).

(2b) Biel, *ibid.*, **71**, 1306 (1949).

(3) Ridi, *Gazz. chim. ital.*, **71**, 462 (1941).

(3a) Ziering and Buck, "Jubilee Vol. Emil Barell," 378 (1946). We are indebted to the Referee for pointing out Ridi's prior work on this substance. The melting point of this azomethine, as reported by these authors, is not in agreement with our findings. See Experimental.

(4) Kirpal and Reiter, *Ber.*, **60**, 664 (1927).

(5) Adkins and Winans, U. S. Patent 2,175,585 (1939).

(6) Moffett and Hoehn, THIS JOURNAL, **69**, 1792 (1947).