the units of the main chain are split once, and that the other half of the units of the main chain, or those at the points of branching, are not split at all.

Experimental

Periodate Oxidation.—Following the method of Hirst, a 400-mg. sample of guaran was dissolved in 100 ml. of potassium chloride solution (5 g. of potassium chloride per 100 ml. of water) in a 500-ml. glass-stoppered bottle. Then 10 ml. of 0.3 *M* sodium periodate solution and 10 ml. of water were added and the mixture shaken at 25° for about one hundred hours. At this point the reaction was complete and ethylene glycol was added to consume the excess periodate. The formic acid present was titrated with 0.01 *N* barium hydroxide solution. One mole of formic acid was produced for 2.7 anhydrohexoside units. Samples of 200 mg. and 100 mg. gave similar values.

The presence of formic acid was confirmed by oxidation with mercuric chloride by the method of Auerbach and Zeglin.[6] Formic acid (*ca.* 40 mg.) formed from a 400-mg. sample of guaran was removed from the final reaction mixture by extraction with ether in a liquid–liquid extractor for ten days. A slight excess of sodium hydroxide was added to the ether extract and the mixture concentrated to about 5 ml. to remove ether and then was diluted to 60 ml. with water. After neutralization with 1 *N* hydrochloric acid, 1 ml. excess of acid and 3 g. of sodium acetate were added. The solution was filtered into an Erlenmeyer flask and 20 ml. of 5% mercuric chloride solution was added. The flask was covered with an inverted beaker and the mixture heated on a steam-bath for two hours. Precipitated mercurous chloride was filtered on a medium porosity sintered glass crucible, washed with hot water and ethanol, dried at 100°, and weighed. The weight of the precipitate corresponded to 103% of the formic acid determined by the above method of direct titration.

(6) F. Auerbach and H. Zeglin, *Z. physik. Chem.*, **103**, 161 (1922).

DEPARTMENT OF AGRICULTURAL CHEMISTRY
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RECEIVED FEBRUARY 28, 1948

COMMUNICATIONS TO THE EDITOR

REVERSIBLE ACTION OF *macerans* AMYLASE[1]

Sir:

The action of *Bacillus macerans* amylase[2] on starch has been interpreted by Cori[3] as the exchange of a glycosidic bond in starch for a corresponding bond in a cyclic Schardinger dextrin (cycloamylose) molecule. In view of the small ΔF which would be expected for such an exchange, it might be expected that the reaction should be readily reversible. The reverse type reaction

$$\text{Cyclohexaamylose} + \text{Maltose} \xrightarrow{\textit{macerans}\ \text{amylase}} \text{Higher saccharides}$$

has been tested with crystalline substrates and verified; *macerans* amylase thus has a synthetic as well as degradative action.

Pure cyclohexaamylose,[4] 2.0 g., and C. P. maltose, 0.7 g., were dissolved in water, heated to complete mutarotation of the maltose, and treated with four units[2] of *macerans* amylase. The solution was made up to 100 ml. and the increase in rotation[5] was followed in the polarimeter: initial rotation, 7.87°; after two hours, 8.10°. At this point the enzyme was inactivated by boiling and the reaction products separated by fractional precipitation. The least soluble fraction, 0.14 g., sirupy, had $[\alpha]_D$ +163°; average chain length by alkaline ferricyanide,[6] 8.9 glucose units. These values indicate that the sample is probably a mixture of saccharides containing some non-carbohydrate impurities. It gave a slight deepening of the color of I_2–KI solution and no unchanged cyclohexaamylose could be detected by the Tilden micro test.[2] On treatment with *macerans* amylase the fraction was rapidly reconverted in part into cyclohexaamylose as indicated by the formation of the characteristic I_2–KI complex.

Results indicating a similar synthetic action of *macerans* amylase have been obtained from cyclohexaamylose with glucose, α-methylglucoside, sucrose, cellobiose or maltobionic acid as co-substrates; also from cycloheptaamylose[4] with maltose or glucose as co-substrates. These studies are being continued and will be reported in full at a later date.

(1) Journal Paper No. J-1581 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 964.

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

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

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

(5) McClenahan, Tilden and Hudson, *ibid.*, **64**, 2139 (1942).

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

CHEMISTRY SECTION
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RECEIVED AUGUST 10, 1948

FORMATION OF FLUORESCING SUBSTANCES FROM AMINO ACIDS

Sir:

Tauber[1] has reported recently on the formation of a fluorescing compound formed by the reaction of tryptophan with perchloric acid at room tem-

(1) Tauber, THIS JOURNAL, **70**, 2615 (1948).

perature. In the course of some preparative work we have noted the formation, also at room temperature, of striking blue fluorescing substances after treatment of tyrosine, phenylalanine and tryptophan with Denigès reagent.[2]

A fairly stable reagent was prepared by dissolving one part of paraformaldehyde in five parts of concentrated sulfuric acid. The reaction was carried out by dissolving a small sample in 2 ml. of sulfuric acid, adding 1 ml. of reagent and diluting with water after three minutes at room temperature. Fluorescence could be measured with the same filter combinations as are used for thiochrome determinations.

Under these conditions equal weights of the amino acids phenylalanine, tyrosine and tryptophan give intensities in the ratio of 100:4:1. If the acid solution containing tyrosine stood ten minutes before addition of reagent, no fluorescence was observed; this treatment did not affect the reaction with phenylalanine or tryptophan.

The fluorescing solutions are quite stable in the dark, less so in diffuse light, and lose about half their intensity after twenty minutes of ultraviolet irradiation.

Fluorescence is quenched by addition of bromine water, picric acid, hydrogen peroxide, or sodium sulfite, hydrosulfite or nitrite. Cysteine, but not cystine or methionine, inhibits the formation of the fluorescing compounds.

The reaction is also given by N-acetyltyrosine and ephedrine; not by alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glycine, histidine, isoleucine, lysine, methionine, proline, serine, threonine, valine, thiamine, riboflavin, niacinamide, pteroylglutamic acid, calcium pantothenate, rutin, dextrose, sucrose and maltose.

Crude substances have been isolated from reaction mixtures following treatment of phenylalanine or tyrosine, respectively. These substances react with ninhydrin (Van Slyke method) to yield carbon dioxide virtually equivalent to their total nitrogen content. This is contrary to what might be expected from the tetrahydroisoquinoline or carboline compounds formed with formalin by these amino acids under other conditions.[3] Possibly a reversible condensation product of the triformal glycine ester type[4] is formed. This would fit fairly well with the presently available analytical data on the tyrosine and phenylalanine compounds.

The relation between intensity of fluorescence and concentration of phenylalanine is practically linear over the range from 2–8γ per ml. of diluted reaction mixture.

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RECEIVED AUGUST 25, 1948

(2) Denigès, *Compt. rend.*, **136**, 583 (1900).

(3) French and Edsall, "Advances in Protein Chemistry," Vol. II, Academic Press, New York, N. Y., 1945.

(4) Bergmann, *et al.*, *Z. physiol. Chem.*, **131**, 18 (1923).

REACTIONS OF IONS IN AQUEOUS SOLUTION WITH GLASS AND METAL SURFACES

Sir:

Radioactive tracers afford a more direct, sensitive and rapid means than any previously[1] used for studying the sorption of ions in solution on solid surfaces. Using them we have carried out exploratory investigations on some of the variables controlling such reactions. The method consists of immersing small flat samples in a solution of the radioactively tagged ion,[2] removing, rinsing and drying, and determining the intensity of the radioactivity on each with the aid of a Geiger–Mueller counter.

Soft glass samples immersed in 0.05 *M* sodium carbonate solution show a rapid sorption of sodium ions which approaches an apparent equilibrium value of about 0.5 "monolayers"[3] at 25° and 5 monolayers at 90° after two to three hours immersion. The initial rate of sorption shows an approximately five-fold linear decrease with decrease in *p*H from 12 to 5.

Samples flamed prior to immersion show a much greater rate of sorption than those cleaned only by washing with water and vapor degreasing with carbon tetrachloride. The apparent activation energy[4] was the same, however, *i. e.*, about 10,000 cal. mole^{-1} in neutral solution. This suggests that the flame does not produce new types of reactive centers but uncovers more of the kind already available by cleaning the surface more completely than water and carbon tetrachloride alone. Presoaking of samples in 9 *N* HCl increased the activation energy for sorption in neutral solution to 13,500 cal. mole^{-1} suggesting that the exchange of sodium ion with an —Si—OH bond may be more difficult than with an —Si—ONa bond.

Sodium ions sorbed on soft glass are removed only very slowly by rinsing in water at room temperature up to ten hours, but are removed somewhat more readily at higher temperature.

The sorpion of silver and cesium ions is qualitatively similar to that of sodium ions. Presoaking of glass in stannous chloride solution, such as used in preparing glass for silvering, caused an increase in the sorption of silver ions.

From 0.2 to 5 monolayers of sodium ion are sorbed by cleaned aluminum, steel, silver and platinum during a few hours immersion at room temperature.

The sorption of carbonate ion on glass or steel is slight, of the order of 0.001 monolayer.

Radioautographs have been useful in determining the homogeneity of distribution of sorbed

(1) W. A. Weyl, "Some Practical Aspects of the Surface Chemistry of Glass," Glass Technology Institute, State College, Pa.

(2) U. S. Atomic Energy Commission Radioisotope Catalog No. 2.

(3) A "monolayer" is arbitrarily defined here as the number of ions required to cover the macro surface area of the sample if each ion covers an area equal to the square of its ionic crystal diameter.

(4) Calculated from the graph of the logarithm of the initial rate of sorption *vs.* $1/T$.