sulfate. After filtering, the solution was taken to dryness on a steam-bath under reduced pressure and the residue was taken up in anhydrous acetone, filtered, and again reduced to dryness. The tan friable solid which weighed 57.4 g. (93%) was finally dried in an Abderhalden pistol at 57° over phosphorus pentoxide and paraffin at 1 mm. pressure for five days, m. p. 72° (sintered). The acetyl compound was tasteless and odorless. It gave no color with ferric alum solution and was insoluble in water. *Anal.* Calcd. for $C_{70}H_{62}O_{40}$: C, 54.5; H, 4.05. Found: C, 54.4; H, 4.08.

d-Arabitol Pentagallate.—30.8 g. of penta-(triacetyl-galloyl)-*d*-arabitol was taken up in 60 cc. of acetone and an oxygen-free stream of nitrogen passed into the solution while 330 cc. of 1 N sodium hydroxide solution was added with mechanical stirring at such a rate that the reaction temperature was maintained at 0° (ice–salt-bath). The gummy mass which separated was brought into solution by the addition of 100 cc. of acetone. Stirring was continued under a nitrogen atmosphere for two and one-half hours at 0°, 330 cc. of cold 1 N sulfuric acid was added, and the acetone was removed from the solution under reduced pressure. The residual solution was extracted with neutral ethyl acetate and the solvent layer dried over sodium sulfate, filtered, and concentrated to dryness under reduced pressure. The golden colored, friable hygroscopic glass produced was suspended in chloroform and then filtered. Repetition of this suspension gave 20 g. of a water soluble product. After drying at 57° over phosphorus pentoxide and paraffin at 1 mm. pressure for five days the color was a very pale tan, m. p. 83° (sintered). This product gave a blue-black color with dilute ferric alum solution. *Anal.* Calcd. for $C_{40}H_{32}O_{26}$: C, 52.6; H, 3.53. Found: C, 52.5; H, 3.52.

Hexa-(triacetylgalloyl)-sorbitol.—The method employed by Fischer[1] for the corresponding mannitol derivative was applied to sorbitol. The white, odorless tasteless product gave no coloration with ferric alum, m. p. 106° (sintered). After drying in an Abderhalden pistol as described for the derivative of *d*-arabitol it was converted to the gallic ester.

Sorbitol Hexagallate.—18.5 g. of hexa-(triacetyl-galloyl)-sorbitol was treated by the method employed by Fischer[1] for the production of mannitol hexagallate to give 8.6 g. of gritty, white solid which was dried in a pistol as indicated above (m. p. 76° sint.). It has an astringent taste, was soluble in water and in neutral ethyl acetate, but was insoluble in petroleum ether. It gave a blue color with ferric alum. *Anal.* Calcd. for $C_{48}H_{38}O_{30}$: C, 52.5; H, 3.49. Found: C, 52.6; H, 3.51.

The experimental tannages were carried out on pickled calf-skin using the following standard procedure. A piece of selected pickled calfskin (8″ × 4″, wt. 25–35 g.), covered with three times its weight of 4% aqueous sodium chloride was treated with 25% of its weight of the material to be tested and the whole was rotated slowly in a bottle for from twenty-four to forty-eight hours. The piece of skin was now washed with water to a *p*H of 4.5, dried and compared as to color, feel, texture, flexibility and fullness with a similar piece tanned with gallotannin.

Summary

A number of simple esters and polyesters of gallic acid have been prepared and tested for leather forming properties. None of the simple esters showed any such properties. However, leather forming properties were shown by the polyesters of gallic acid with various polyhydric alcohols. The leather forming properties of the members of the latter series were poor but definite, and seemed to improve on ascending the series.

The Catalytic Properties of Charcoal. IV. Factors Influencing the Indophenol Reaction[1,2]

BY CLAUDE SCHWOB, WITH JOHN E. BIEGNER, KENNETH J. CARSON AND GEORGE V. SCOTT

The indophenol reaction has been used in this Laboratory to investigate the oxidase and peroxidase action of charcoal.[3,4] In this reaction indophenol is formed by the oxidation of a mixture of *p*-phenylenediamine and α-naphthol by oxygen or hydrogen peroxide in the presence of charcoal. It was found that when equimolar amounts of *p*-phenylenediamine and α-naphthol are used, the yield of indophenol diminishes after the first

minute. Moreover, in later work, considerable difficulty was encountered in duplicating results.

In an attempt to clear up these points, and in order to gain further knowledge of the mechanism of the reaction, four modes of attack were employed: (a) variation in the concentrations of the individual reagents, (b) study of the *p*H during the course of the reaction, (c) effect of catalyst poisons and (d) study of the factors affecting the recovery of indophenol.

1. Reagent Concentrations.—Previous evidence[4] indicated that excess reagents are responsible for the drop in the yield of indophenol after

(1) Paper III, THIS JOURNAL, **60**, 2483 (1938).
(2) Presented in part at the 102nd meeting of the American Chemical Society, Atlantic City, N. J., September, 1941.
(3) Schwob, THIS JOURNAL, **58**, 1115 (1936).
(4) O'Brien, Tkac and Schwob, *ibid.*, **60**, 2480 (1938).

the first minute. Figure 1 summarizes the results obtained when the amounts of the reagents are varied individually. The concentrations are expressed as multiples of the "normal" amount, the reaction mixture normally containing equimolar amounts of p-phenylenediamine and α-naphthol, with a 20-fold excess of hydrogen peroxide, in the presence of sucrose charcoal, the whole being buffered to pH 4.5.[4]

Charcoal (curve 1): As expected, the ten-minute yield of indophenol is proportional to the amount of charcoal used, from one-half to twice the normal amount.

α-Naphthol (curve 2): Increasing concentrations of α-naphthol diminish the yield in a very marked manner, indicating that this substance is the chief culprit in the observed destruction of the indophenol. Due to the low over-all yield of indophenol (less than 6% in all cases) only a small amount of α-naphthol is needed for the actual formation of the dye. The excess "destroys" the indophenol in a manner not yet investigated in this Laboratory.

p-Phenylenediamine (curve 3): Excess amine does not affect the yield of indophenol very much. The yield diminishes in the expected manner when the concentration is below "normal."

Hydrogen Peroxide: Experiments involving variation in peroxide concentration were inconclusive. This is very probably due to the fact that charcoal has definite oxidase properties.[4] As the concentration of peroxide is lowered, the oxidase action predominates and obscures the role of the peroxide. However, at high concentrations of peroxide the yield of indophenol is somewhat lower (0.32 mg. of indophenol for 2.5 times normal concentration of hydrogen peroxide). Attempts to investigate the role of the peroxide anaerobically are futile due to the unavoidable decomposition of peroxide by the charcoal, providing an ample supply of oxygen.

2. **Hydrogen-ion Concentration.**—In addition to the change in the shape of the time–yield curve previously observed with pH variation, there is a definite increase in yield as the hydrogen-ion concentration decreases.[4] In the present investigation it was observed that during the course of the indophenol reaction relatively large quantities of an acid substance are formed, leading to a decrease in pH in spite of the presence of the buffer. For the "normal" reaction mixture the pH drop, measured by means of a glass elec-

trode, was from 4.5 to 2.8 in seven minutes. A large increase in conductivity was observed also as the reaction progressed. It is likely that these effects are due to the liberation of hydrochloric acid as the p-phenylenediamine dihydrochloride is consumed, either in the formation of indophenol, or by being independently oxidized to unknown products by the peroxide under the influence of the charcoal.[5]
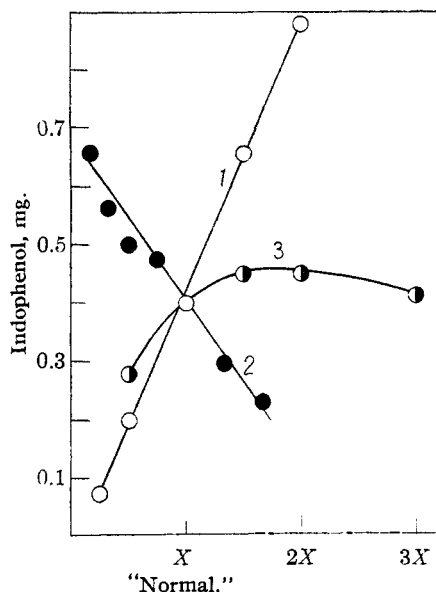

Fig. 1.—Ten-minute yield of indophenol in the presence of varying amounts of: charcoal (curve 1), α-naphthol (curve 2), and p-phenylenediamine (curve 3).

3. **Catalyst Poisons.**—The effects of potassium cyanide and amyl alcohol on the indophenol reaction have already been described.[4] The cyanide inhibits the formation as well as the destruction of the dye, but permits the uncatalyzed formation of indophenol to proceed at the same rate as in the absence of charcoal. The amyl alcohol stops all processes, so that the yield does not change with time after its addition. These different and characteristic effects were reinvestigated by means of conductivity measurements. Using a Fisher Titrimeter, the resistance of the normal reaction mixture, in the absence of poisons, drops with time, reaching a constant value of about one-half the original resistance after thirty minutes. This points to the liberation of hydrochloric acid for thirty minutes. Since indophenol is not being formed throughout

(5) Preliminary observations point to the feasibility of using a conductance method, involving this catalyzed oxidation of p-phenylenediamine dihydrochloride, for the rapid estimation of certain enzymes.

this period, the p-phenylenediamine must be losing some of its basic character by being oxidized independently.

In the presence of potassium cyanide (0.005 N) no drop in resistance can be observed. This indicates that not only does the cyanide poison the charcoal surfaces responsible for the formation and destruction of indophenol, but it also inhibits the independent oxidation of p-phenylenediamine.

On the contrary, in the presence of amyl alcohol the usual drop in resistance takes place, showing that the charcoal-catalyzed oxidation of the p-phenylenediamine is not affected by the amyl alcohol. Hence it is probable that there are at least two distinct surfaces of charcoal active in the indophenol reaction: (a) the portion of the surface which catalyzes the formation and destruction of indophenol, inhibited by both cyanide and amyl alcohol, and (b) the portion which catalyzes the oxidation of p-phenylenediamine, inhibited by cyanide but not by amyl alcohol.

4. Recovery of Indophenol.—It has been observed throughout this investigation that the indophenol-bearing charcoal must not be dried at too elevated a temperature or the indophenol will be destroyed. Another recurring source of annoyance in determining yield has been the varying shades of red which toluene solutions of indophenol exhibit, making colorimetric comparisons difficult and sometimes impossible.

The solid phase destruction of indophenol has been investigated by preparing indophenol-bearing charcoal, analyzing a portion and submitting other portions of the same batch to various treatments before analysis. The charcoal was prepared by using it to catalyze the formation of indophenol, using the "normal" concentrations. After filtering, the charcoal was washed with water and with 70% alcohol in an endeavor to remove most of the reagents and by-products. Alternately pure indophenol was adsorbed on charcoal from alcoholic solution.

The effect of heat was investigated first. Heating the charcoal in the dry state for progressively longer periods at the same temperature, or for the same time at increasing temperatures, causes a gradual destruction of the indophenol. For example, heating at 80° for sixty minutes decreased the indophenol content of a sample from 0.9 mg. per g. of charcoal to 0.5 mg. per g. In an attempt to find the lowest temperature at which indophenol is still destroyed at a significant rate,

it was observed that a good deal of destruction takes place at room temperature. A sample of charcoal decreased in indophenol content from 0.6 mg. per g. to 0.3 mg. on exposure to air at room temperature for seventy-two hours.

The effect of excess reagents on this dry destruction of indophenol was studied by allowing the dye-bearing charcoal to adsorb one or another of the reagents from suitable solutions. As expected, the results are generally consistent with the findings described in the first part of this paper. Excess α-naphthol considerably increases the rate of destruction, while excess p-phenylenediamine actually increases the indophenol content of the charcoal after heating for one hour at 80°. This seems to be due to α-naphthol on the charcoal carried over from the reaction mixture and reacting with the added diamine. Further support for this view is furnished by the fact that if charcoal is supplied with indophenol by a reaction mixture containing only one-half the usual amount of α-naphthol, the increase in indophenol content on heating in the presence of added p-phenylenediamine is much less.

A study of the effect of reagents on indophenol adsorbed on charcoal from solutions of the pure dye in alcohol should be enlightening in these respects, since then none of the reaction mixture would be present to obscure the results. Unfortunately, as soon as charcoal so prepared was dried thoroughly, toluene no longer extracted the indophenol, and these experiments were perforce abandoned.

The second disturbing effect in estimating indophenol yields is the color variation exhibited by different toluene solutions of the dye. It was found that the presence of a trace of ethyl alcohol shifts the color toward the blue. Acid fumes make the solutions more yellow.

5. Conclusions.—A consideration of the present findings in the light of the previous work on the charcoal catalysis of the indophenol reaction enables us to list some of the processes taking place simultaneously or concurrently: A, charcoal catalyzed (1) formation of indophenol, (2) destruction of indophenol when its concentration reaches a definite level dependent on the charcoal, the temperature, and on the pH, (3) oxidation of the p-phenylenediamine, (4) decomposition of hydrogen peroxide due to the catalase action of the charcoal; B, uncatalyzed (1) slow formation of indophenol.

Summary

The oxidation of α-naphthol and p-phenylenediamine in the presence of activated charcoal appears to involve several simultaneous and successive processes. The measurement of the indophenol production is not an adequate test of the charcoal activity.

Cocarboxylase and Related Esters

BY JOHN WEIJLARD

In previous publications concerning synthetic cocarboxylase, a rather indefinite acid mixture prepared by dehydrating orthophosphoric acid has been employed for the phosphorylation.[1,2,3] A different method was employed by H. Weil-Malherbe,[4] who prepared cocarboxylase by interaction of bromo-thiamine with silver pyrophosphate, a reaction that does not fall within the scope of this paper.

The obvious method to prepare cocarboxylase would be the interaction of pure pyrophosphoric acid and thiamine hydrochloride (I). It was found, however, that pyrophosphoric acid itself did not form any thiamine ester whatever, but with anhydrous sodium pyrophosphate as catalyst produced thiamine orthophosphoric acid ester (IV) in fair yields instead of the expected pyrophosphoric acid ester, as shown by the fact that mild hydrolysis produced no phosphoric acid.[2] Similarly, 4-methyl-5-hydroxyethylthiazole (II) formed 4-methyl-5-hydroxyethyl thiazole orthophosphoric acid ester (VI) with pyrophosphoric acid and pyrophosphates. Concentrated sulfuric acid with anhydrous sodium pyrophosphate produced thiamine sulfuric acid ester (III) from thiamine hydrochloride in good yields, but no thiamine pyrophosphoric acid ester could be detected in the reaction mixture.

Phosphorus pentoxide was hydrated to give metaphosphoric acid,[5] and with the acid thus produced it was actually possible to esterify thiamine hydrochloride to thiamine pyrophosphoric acid ester chloride (cocarboxylase) (V). Yields similar to those previously reported[3] of thiamine pyrophosphoric acid ester were obtained when thiamine hydrochloride was esterified with a mixture of phosphorus pentoxide dissolved in

pyrophosphoric acid with sodium pyrophosphate as catalyst. Such a solution should be a mixture of meta and pyro acids, with perhaps some unchanged phosphorus pentoxide, similar to the mixture obtained upon dehydrating orthophosphoric acid.[6,7] The mixture from dehydrated orthophosphoric acid gave with 4-methyl-5-hydroxyethylthiazole the compound, 4-methyl-5-hydroxyethylthiazole pyrophosphoric acid ester (VII), isolated as the silver salt,[2,8] which varies in composition according to varying pH and salt concentration.

2-Methyl-4-amino-5-bromomethylpyrimidine hydrobromide (VIII) was condensed with 4-methyl-5-hydroxyethylthiazole pyrophosphoric acid ester (VII) on the one hand, and with 5,β-chloroethyl-4-methylthiazole (IX) in the presence of silver pyrophosphate on the other to form cocarboxylase. Cocarboxylase was obtained in these reactions as ascertained by biological tests, but the yields were no higher than those obtained by the more simple direct phosphorylation of thiamine hydrochloride.

Experimental

Thiamine Orthophosphoric Acid Ester.—Five grams of pyrophosphoric acid was heated to faint fuming to remove any excess water, 2 g. of anhydrous sodium pyrophosphate was dissolved in the hot acid, 2 g. of thiamine hydrochloride was added and the mixture held at 150–155° for twenty minutes while stirring continuously. The cooled mass was dissolved in 100 cc. of water, and a slight excess of concentrated barium hydroxide solution was added. The precipitate was removed by centrifugation and discarded, the excess barium removed by adding a slight excess of 10% sulfuric acid and centrifuging. The solution was then concentrated to 60 cc. *in vacuo*. Sufficient one normal silver nitrate solution was added to precipitate the chloride, and the precipitate was removed by centrifuging and was discarded. The solution was neutralized with ammonia and an excess of 1 N silver nitrate solution added. The

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