[CONTRIBUTION FROM THE MULTIPLE FELLOWSHIP OF THE COTTON RESEARCH FOUNDATION, MELLON INSTITUTE]

Composition of Cottonseeds. II. Catalase

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During an investigation of cottonseed proteins, hydrogen peroxide was added to an aqueous extract of cottonseeds in an attempt to bleach the colored extractives. The extensive foaming which occurred suggested that a catalase was present in appreciable amounts. The results of further experiments indicated that cottonseeds contained more catalase than any of several other seeds examined.

The following routine method of assay for catalase activity was used.[1] To 5 cc. of approximately 0.02 *N* hydrogen peroxide solution were added 1 cc. of 0.067 *N* phosphate buffer (*p*H 6.85) and 1 cc. of catalase extract suitably diluted so that 10 to 20% of the peroxide was decomposed in two minutes at room temperature (25–28°). At the end of this period 10 cc. of 10% sulfuric acid was added. The peroxide remaining was determined iodimetrically; 10 cc. of 10% potassium iodide and three drops of 1 *N* ammonium molybdate solution were added, and the liberated iodine was titrated with 0.005 *N* sodium thiosulfate with starch for an indicator.

In most cases parallel determinations were run at four- and six-minute intervals. The computed monomolecular reaction constant diminished with increased time of reaction. Activity indices were calculated from the reaction constant at two minutes rather than from that obtained by extrapolation to 0 time in order to obtain minimum values. The index of catalase activity ("Kat. f." of von Euler and Josephson[2]) was obtained by dividing the reaction constant by the weight of solids added in the catalase extract. Approximate indices were obtained experimentally with crude aqueous extracts of a number of ground whole seeds (dehulled in the case of cottonseed, hempseed, and soybean), and rat and beef liver (Table I). Zeile[3] reported that an extract of pumpkin seeds was one-fifteenth as effective as one prepared from horse liver by the same method. According to Sumner and Dounce[4] the "Kat. f." (0°) of crystalline beef liver catalase is 35,000.

TABLE I

CATALASE CONTENT OF AQUEOUS EXTRACTS FROM VARIOUS SOURCES

Source of extract	Kat. f.
Cottonseed[a]	600
Corn	90
Hempseed[a]	60
Wheat	40
Flaxseed[a]	15
Soybean[b]	15
Rapeseed[a]	1
Beef liver	5,000
Rat liver	3,000

[a] Ether extracted. [b] Petroleum ether extracted.

Ether-extracted cottonseed meats[5] were leached with water to obtain the active enzyme solutions. Preserved with chloroform, the extracts retained their activity for several days at 0°. A number of experiments designed to concentrate the catalase fraction were only partially successful.

In the extraction of catalase from cottonseed meal by dilute acids and alkalies, the most active preparations were obtained with neutral or slightly alkaline extractants. Acids destroyed the catalase activity. Aqueous extracts were most effective at a *p*H of 6.8 to 7.2 (phosphate and borate buffers). However, there was no sharp peak in the *p*H–activity curve; the preparations were almost equally active in the *p*H range 6 to 9.

The addition of ammonium sulfate to 0.6 saturation did not precipitate the enzyme, but at 0.75 saturation most of the activity could be accounted for in the precipitate. During the dialysis procedure used to remove the ammonium sulfate, the catalase content spontaneously decreased so that no marked concentration was effected by this method.

Several attempts to concentrate the enzyme by adsorption on moist alumina[6] were unsuccessful, as was the dioxane precipitation method described by Sumner and Dounce.[4]

The catalase was partially precipitated from its aqueous solution by the addition of an equal part of anhydrous alcohol. With 60% alcohol precipitation was complete. The total activity rapidly decreased in the presence of this solvent.

(1) Adapted from K. G. Stern, *Z. physiol. Chem.*, **204**, 259 (1932), and others.

(2) H. V. von Euler and K. Josephson, *Ann.*, **452**, 158 (1927).

(3) K. Zeile, *Ergebnisse Enzymforschung*, **3**, 265 (1934).

(4) J. B. Sumner and A. L. Dounce, *J. Biol. Chem.*, **121**, 417 (1937); *ibid.*, **127**, 439 (1939).

(5) H. S. Olcott and T. D. Fontaine, THIS JOURNAL, **61**, 2037 (1939).

(6) K. G. Stern, *J. Biol. Chem.*, **114**, 473 (1938).

Acetone precipitation was most satisfactory for the preparation of concentrates. The fraction soluble at 50% but insoluble in 55% aqueous acetone was redissolved in water. This solution had a "Kat. f." of 1800, representing a threefold concentration of the original activity. Stern has used acetone in the preparation of liver catalase concentrates.[7]

A cottonseed extract (*p*H 6.4) was only slightly affected by heating at 50° for thirty minutes. At 60° the catalase was destroyed completely in fifteen minutes, and at 70° no activity remained after five minutes. However, dry ether-extracted cottonseed meats could be heated at 105° for several hours without destruction of the enzyme. Commercial cottonseed meal contained no catalase; this material is generally subjected to a steaming process for twenty to forty minutes prior to the pressing operation.

The activity of concentrates was almost entirely repressed in 0.02 *N* sodium cyanide solution.

During the first forty-eight hours of germination, the catalase concentration of whole seeds increased from 120 to 140% of the original content.

The characteristics of this enzyme are not essentially different from those of other plant catalases.[3] The availability of a plant source of somewhat greater original catalase content should facilitate its further investigation.

(7) K. J. Stern, *J. Biol. Chem.*, **112**, 661 (1935–36).

Summary

Cottonseeds contain appreciably more catalase than do a number of other seeds examined. Some properties of the enzyme have been described.

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Reduction of Diazonium Salts to Hydrocarbons with Alkaline Formaldehyde

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It is well known that the classical method[1] for the reduction of a diazonium salt such as phenyldiazonium chloride or sulfate to a hydrocarbon by reaction with an alcohol produces an ether as well as the hydrocarbon. In most cases the ether is the major product. An experiment by Hantzsch and Jochem,[2] which is typical of many recorded in the literature, produced 1.6 g. of benzene and 34.1 g. of phenetole from the reaction of ethyl alcohol upon 64 g. of phenyldiazonium chloride. Similar results with varying proportions in the yield of hydrocarbon (hydrogen reaction, I) and ether (alkoxy reaction, II) have been obtained by a large number of investigators.[3] The extent to which the reaction between any given diazonium salt and an alcohol will follow one course or the other depends upon the character of the radical (R) and upon the nature of the alcohol. In general the yield of hydrocarbon increases with the substitution of a carboxyl, halogen or nitro group ortho to the diazonium radical, and the hydrogen reaction becomes quite predominant in the case of 2,4,6-tribromophenyldiazonium sulfate where the yield of tribromobenzene[4] is 75–78% of the theoretical quantity. The alcohols used most commonly are methyl and ethyl, of which the latter usually gives the better yield of hydrocarbon. Some experiments using other alcohols are to be found in the literature and they are being extended in this Laboratory.

$$RNNCl + C_2H_5OH \longrightarrow RH + CH_3CHO + HCl + N_2 \quad (I)$$

$$RNNCl + C_2H_5OH \longrightarrow ROC_2H_5 + HCl + N_2 \quad (II)$$

In the deamination of certain aminodiphenyl ether derivatives in which we were interested, the alkoxy reaction occurred almost exclusively and it was necessary to devise some other method for replacement of the amino group by hydrogen. Inasmuch as the hydrogen reaction involves a reduction of the carbon atom to which the diazonium salt is joined, it would seem that a reducing agent considerably stronger than an alcohol should be used. Such reducing agents as sodium alcoholate,[5] stannous chloride[6] and hypophosphorous acid[7] in certain cases have increased somewhat the yield of the hydrocarbon but it would seem that the more effective reducing action of an

(1) Griess, *Ann.*, **137**, 69 (1866).
(2) Hantzsch and Jochem, *Ber.*, **34**, 3340 (1901).
(3) A. W. Hofmann, *Ber.*, **17**, 1919 (1884); Remsen and Orndorff, *Am. Chem. J.*, **9**, 387 (1887); Cameron, *ibid.*, **20**, 229 (1898); Franklin, *ibid.*, **20**, 455 (1898), and many others.
(4) *Org. Syntheses*, **13**, 96 (1933).
(5) Beeson, *Am. Chem. J.*, **16**, 250 (1894).
(6) Friedländer, *Ber.*, **22**, 587 (1889).
(7) Mai, *ibid.*, **35**, 163 (1902).