

average for five experiments repeated. This figure is lower than that obtained by Freudenberg (20–25%). It was found, however, that vanillin once produced was decomposed under the condition of oxidation of lignin, and only about 55% of vanillin was actually recovered from reaction mixture. Therefore, the total yield of vanillin from alkali lignin might be accounted to be about 20%.

On the other hand, the thioglignin which was obtained from meal of red pine by cooking with NaSH has resulted in giving a smaller yield of vanillin amounting in average to 6.61%.

The sulfur content of the thioglignin prepared by us is 5.71%. If the elementary composition of lignin is assumed to be C 65%, H 6%, and OCH_3 15%, the sulphur content of the thioglignin as mentioned corresponds to the atomic ratio of 1 S to 30 C. In other words, it results that one sulfur atom is combined with every three building units of lignin ( c.c.c). If the sulfur atom or its radical hinders the formation of vanillin from lignin by any means, the yield of vanillin from the thioglignin should be $\frac{2}{3}$ the yield from alkali lignin, namely about 7%. The actual yield obtained by us, that is 6.61% agrees fairly good with this.

We are now investigating the chemical nature of the combined sulfur atom in the lignin molecule.

67. Studies on Biocatalyses. (XI)

On the Absorption of Carbon Dioxide by Plant.

Kinsuke Kondo, Shigeki Mori and Fumio Kawai.

In the previous paper (This Report 18, 33 (1949); 19, 68, 69 (1949)) we reported the presence and distribution of carbonic anhydrase in various plants. In this paper we mention on the nature of the enzyme relating to carbon dioxide absorption by plant. The enzyme was prepared from pepper plant leaves, the material was ground and the juice was pressed out, after separating chloroplasts by centrifuge, the supernatant liquor was purified by adsorption with kieselguhr and elution was repeated by the cake with sodium phosphate buffer, pH 7.2.

The enzyme solution thus prepared has the activity of carbonic anhydrase causing decomposition of substrate, bicarbonate, but no potency of absorption of carbon dioxide, while on adding minute amount of histidine or other $-\text{NH}$ compound it catalysed the absorption of carbon dioxide strongly. In the second experiment we have ascertained that carbon dioxide absorption is catalysed by the enzyme on adding chlorophyll b. The added chlorophyll b was isolated from the same plant material.

From these results we assume that carbonic anhydrase catalyses the absorption of carbon dioxide in the presence of chlorophyll b in the plant body. The combination of carbon dioxide with chlorophyll b seems to occur in the two -NH radicals of tetra-pyrrole forming carbamate.

68. Studies on Biocatalyses. (XII)

On the Trace Metals in Chloroplasts.

Kinsuke Kondo, Shigeki Mori and Morikazu Kajima.

The green leaves (Plant, Pepper, *Capsicum anuum* var *grossum*), previously soaked thoroughly in ice-cold water for one hour, were ground, pressed and filtered through thick cloth.

The filtered juice were kept for 10 hours in a refrigerator at 0~2°C. By centrifuge, the chloroplastic matter was separated at the bottom and cytoplasmic matter as well as water soluble substances on the upper side.

On the two fractions, the trace metals, that is, iron, copper, zinc and manganese were analysed, and as the result we found that the greater part of iron (60% of total Iron) was accumulated in chloroplastic matter and 11.7% Cu, 15.1% Zn and 12.6% Mn, each of their total amount, respectively were localized in this part. But it is uncertain, whether they are actually the essential constituents of chloroplasts or not.

69. Polarographic Studies of Serum Protein. (I)

On the Brdička Denaturation Test.

Tokio Sasai and Masao Egawa.

We have studied the digest reaction of polarographic cancer tests (Brdicka's), regarding its fundamental conditions of serum denaturation. Measuring the heights of protein double-wave obtained in cobaltous ammonium buffer solution, we called them H_1 and H_2 respectively. Since the H_1 and H_2 changed always correlatively and the change of H_2 was greater than that of H_1 , we adopted the change of H_2 as an indicator, and called the higher one "activated" and the lower one "inactivated". Although the change of the height was almost negligible when serum was left at room temperature for a week, the height in 60°C water-bath increased about 50% in 2 minutes and then decreased. In case of the alkaline denaturation ($\frac{1}{2}$ N. KOH) at room temperature, the height was at its maximum