

## A. CHEMICAL STUDY OF THE PHENOL OXIDASE OF THE COTTON PLANT

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We have previously reported that the phenol oxidase of the cotton plant contains sugars [1].

The protein (2 mg) was dissolved in 0.5 ml of 3 N HCl and was hydrolyzed at 105°C for 3 h. The hydrolyzate was analyzed in a thin layer of cellulose in the pyridine-isoamyl alcohol-water (1:1:0.8) system. The spots were revealed with aniline phthalate solution. Arabinose, xylose, glucose, and galactose were detected. The amounts of the sugars were determined by the GLC method on an LKM-7A chromatograph. Their total amount was 26%. The arabinose, xylose, galactose, and glucose were present in a ratio of 4:2:6:1, respectively.

When the native protein was chromatographed on paper in the butanol-acetic acid-pyridine-water (15:3:10:12) system we found three protein bands. These bands were eluted separately and were hydrolyzed with 3 N HCl at 105°C for 3 h. Qualitatively identical sugars were found in each of them. When the native protein was treated with such agents as formic acid (70%), a 10% solution of caustic soda in 50% ethanol, and a 1 N solution of acetic acid, only one protein band, containing the same sugars, was obtained. The changes are apparently due to the presence of a quaternary structure of the enzyme.

In order to elucidate the nature of the bond of the carbohydrate moiety with the protein, we hydrolyzed it with 14% Ba(OH)<sub>2</sub> at 105°C for 20 h. Carbon dioxide was passed into the hydrolyzate, and the BaCO<sub>3</sub> was eliminated by centrifuging. The supernatant liquid was evaporated, and the residue was dansylated. From the mixture obtained an oligosaccharide was isolated by chromatography in a thin layer of silica gel in the acetone-isopropanol-25% ammonia (9:6:2) system. After the acid hydrolysis of this carbohydrate, sugars and also DNS-serine were found. The stability of the bond between the serine and the oligosaccharide on alkaline hydrolysis shows that the carbohydrate moiety is attached to the protein through the hydroxyl of the serine.

Thus, the phenol oxidase of the cotton plant contains an oligosaccharide which is attached to the protein moiety of the molecule through a series hydroxyl. The multiplet forms contain identical sugars.

### LITERATURE CITED

1. T. S. Yunusov and P. Kh. Yuldashev, *Khim. Prirodn. Soedin.*, 112 (1975) [in this issue].

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