Base (III) with mp 222-225°C, identified as reserpinine [5], was isolated by treating the fraction from the buffer with pH 2.2 with methanol. An exhaustive chloroform extract was chromatographed on a column of alumina (neutral, activity grade II, 1:30). Elution was performed with ethyl ether, ethyl ether–chloroform, chloroform, chloroform–methanol, and methanol.

Fractions 6-11 eluted by ethyl ether yielded a crystalline base (IV) with mp 185-186°C (methanol). Base (IV) was identified as majdine [6].

The alkaloids vincamajoreine, vincamajine, reserpine, and majdine have been isolated previously from the herbage of Vinca major L. introduced into Georgia [1, 2].

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INFLUENCE OF MAGNESIUM IONS ON COTTON PLANT PYROPHOSPHATASE

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UDC 633.511:577.15

Pyrophosphatase reactions take place at a considerable rate if ions of bivalent metals are present in the system [1, 2]. We have previously reported the detection and separation of two forms of cotton plant pyrophosphatase [3]. We have now studied the influence of Mg^{2+} ions on the hydrolytic capacity of the alkaline pyrophosphatase.

The results have shown that magnesium ions are activators of the enzyme in all the concentrations considered and the activation effect is proportional to the amount of $MgCl_2$ in the medium (Fig. 1a). The optimum concentration of $MgCl_2$ was 5 mM, regardless of the concentration of the substrate. At higher concentrations of the metal in the incubation mixture the activity of the enzyme fell and the curve of the dependence of the rate of the reaction assumed a linear nature.

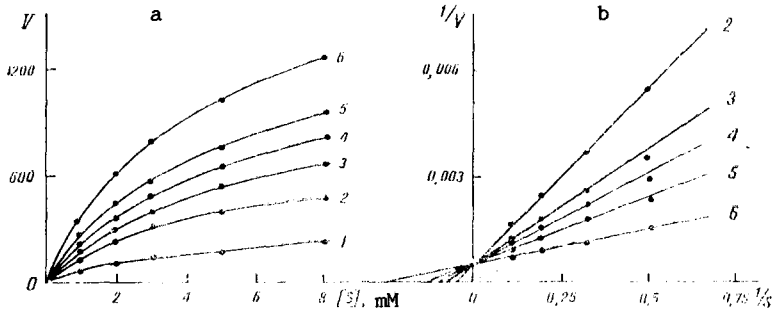

Fig. 1. Action of Mg^{2+} ions on pyrophosphatase activity: a) dependence of the rate of the reaction on the concentrations of substrate and metal; b) the same in the Lineweaver–Burk coordinates; 1) without Me; 2) 0.5 mM; 3) 1 mM; 4) 2 mM; 5) 3 mM; 6) 5 mM.

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The results for the dependence of the rate of the reaction on the concentration of sulfate at fixed concentration of Mg^{2+} in the range of all the concentrations of the ion are linearized satisfactorily in the Lineweaver–Burk coordinates (Fig. 1b). The maximum rate of the reaction, $V = K_{cat} \cdot E_0$ (where E_0 is the concentration of the enzyme and K_{cat} is the constant of the catalytic stage of the reaction) did not change with a variation in the amount of activator. Conversely, the value of the Michaelis constant (the intercept on the axis of abscissa, Fig. 1b) decreased with a rise in the concentration of magnesium ions, which, in its turn, indicates an increase in the role of the metal in the affinity of the enzyme and the substrate, since the smaller is the Michaelis constant the higher is the efficiency of an enzymatic reaction.

Thus, the facts that we have observed indicate that magnesium ions are an activator of cotton plant alkaline pyrophosphatase and there is no effect of inhibition by an excess of the metal.

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DETECTION OF CYSTINE AND ITS PEPTIDES ON TLC

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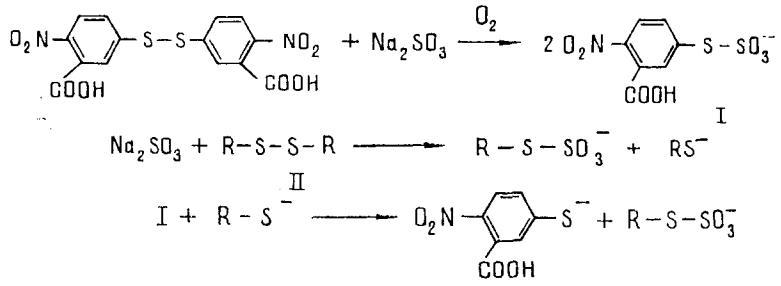
UDC 543.544:547.964

Cystine and its derivatives are detected on chromatograms by the use of the nitroprusside reagent. The plates are sprayed with sodium cyanide and dried and are then sprayed with a solution of sodium nitroprusside [1]. This method is inconvenient for two reasons: in the first place it is fairly lengthy and, in the second place, it requires the use of the highly poisonous sodium cyanide. In view of this, the search for safer reagents and for convenient procedures for revealing cystine and its derivatives is necessary. A quantitative spectrophotometric method of determining disulfide bonds in proteins and peptides in solution has recently been proposed [2]. The Ellman reagent [dithiobis(nitrobenzoic acid)] is converted into the S-sulfo derivative (I), the protein or peptide (II) is treated with sodium sulfite and then reagent (I) is added and the absorption at 412 nm is measured.



We have established that a similar method can be used for detecting cystine and its peptides on TLC. The spraying of the plate with a 1 M solution of sodium sulfite, drying, and spraying with reagent (I) permits cysteine and cystine to be detected on a chromatogram, but the sensitivity of this method is no better than 100 μg. This is probably connected with the rapid oxidation in air of the mercapto groups formed on the action of sulfite on a disulfide bond. However, since reagent (I) is obtained in an excess of sodium sulfite, the stage of the preliminary spraying with a solution of sulfite can be omitted.

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