its R_f values and mass and NMR spectra. From the fourth fraction we obtained magnoflorine and berberine iodides.

Thus, from *Th. strictum*, which has not previously been studied chemically, we have isolated ten bases: thalicminine, O-methylcassyfiline [1], thalicmine, argemonine, 2,3,7-trimethoxy-N-methyl-8,9-methylenedeoxypavinan, thalicthuberine, preocoteine, thalicsimidine, magnoflorine, and berberine. Thalicminine has been isolated from the roots of *Th. minus*, *Th. simplex*, and *Th. isopyroides* [5] but in the plant under investigation thalicminine was found in the seeds, leaves, and epigeal part but it was not detected even chromatographically in the roots. 2,3,7-Trimethoxy-N-methyl-8,9-methylenedioxypavinan is a new base which we first isolated from the epigeal part of *Th. strictum* [1, 2].

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ISOLATION OF AMINOACYL-tRNA SYNTHETASES AND THEIR USE IN THE AMINOACYLATION OF tRNA

A. A. Kolmakova, B. F. Abdullaev, and R. N. Nuriddinov

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In the present communication we give the results of the isolation of aminoacyl-tRNA synthetases (ARSases) from the seeds of the cotton plant of variety 108-F and their use in the aminoacylation of the tRNA from the same source.

The ARSases were obtained by the method of Merrick and Dure [1] with some modifications. A dry defatted powder of cotton seeds was extracted with homogenization in the cold in 0.1 M tris-HCl buffer, pH 8.0, containing 0.01 M $MgCl_2$, 0.005 M 2-mercaptoethanol, and 0.001 M EDTA. The ratio of seeds to buffer was 1:6. The homogenate was centrifuged at 27,000g, The precipitate was discarded, and the supernatant was treated with 2% protamine sulfate to eliminate nucleic acids. After centrifuging, the supernatant liquid was fractionated with $(NH_4)_2SO_4$, and the fraction between 30% and 60% saturation was collected. It was dissolved in the minimum volume of the extraction buffer and stored at −20°C. The protein was determined by Lowry's method [2].

In aminoacylation, we used the tRNA isolated from cotton seeds by the method of Holley et al. [3] with some modifications. The incubation mixture with a volume of 0.25 ml contained (μmole): tris-HCl buffer pH 7.0-9.0, 25; EDTA, 0.25; 2-mercaptoethanol, 0.25; Mg^{2+}, 2.5; ATP, 0.25-2.5; NH_4Cl, 2.5; [^{14}C]-(amino acid)s, 2 μCi; tRNA, 2-3 o.u.; enzyme, 3 mg of protein. The time of incubation was varied.

Samples with a volume of 50 μl each were deposited on paper disks, these were washed several times with 5% TCA, ethanol, and ether, and were dried in the air. The radioactivities of the disks were counted in a LS-100C liquid scintillation counter with an efficiency of 65% for ^{14}C.

In a study of the influence of ATP and Mg^{2+} on the catalytic activity of the enzyme it was found that the largest amount of valyl-tRNA was synthesized when 2.5 μmole of Mg^{2+} and

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent.

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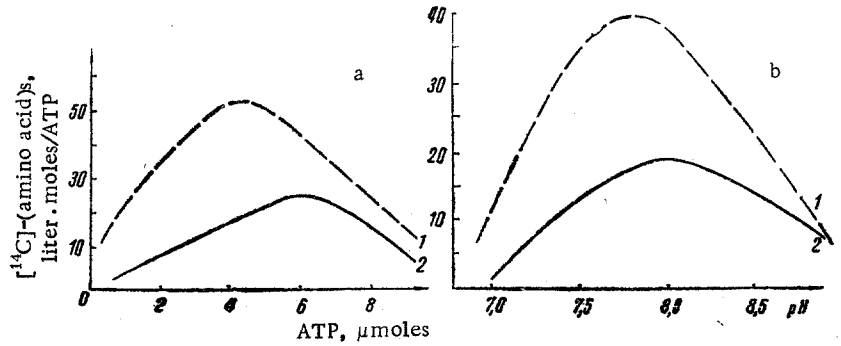

Fig. 1. Dependence of the degree of acylation of tRNA on the concentration of ATP (A) and on the pH of the incubation medium (B): 1) leucine; 2) valine.

1.5 μmole of ATP (Mg^{2+}:ATP = 1.66) were present in the mixture, and for the formation of the maximum amount of leucyl-tRNA 2.5 μmole of Mg^{2+} and 1 μmole of ATP (Mg^{2+}:ATP = 2.5) were required. The results of the experiments are shown in Fig. 1a.

The optimum time of aminoacylation at this composition of the incubation mixture for valine and leucine was 40 min.

Taking literature information on the various optimum pH values for several ARSases [4, 5] into account, we tried the pH range from 7.0 to 9.0. The maximum activity of the valyl-tRNA synthetase was observed at pH 8.0, which agrees with the findings of Jakubowski and Paweikiewicz [4] for the valyl-tRNA synthetase from European yellow lupin seeds. The optimum pH for the leucyl-tRNA synthetase was in the region of pH 7.8 (Fig. 1b).

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