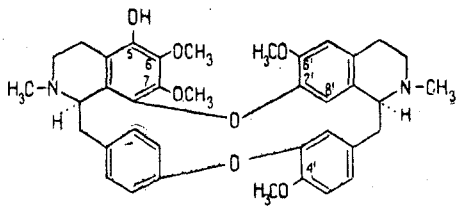
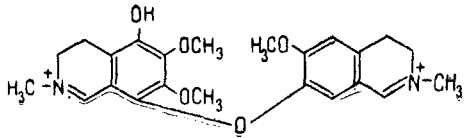
As was shown earlier [1], thalisopine $C_{38}H_{42}N_2O_7$ (II) belongs to the bisbenzylisoquinoline bases. Its IR spectrum has a band at 3500–3400 cm^{-1} (hydroxy group); in the spectrum of O-acetyl-(II) (III) there is the band of a phenol ester at 1770 cm^{-1}. When (II) was methylated with diazomethane, amorphous O-methyl-(II) with mp 163–166° C (decomp.); $[\alpha]_D^{19}$ −54° (c 1.0; chloroform), was obtained. The NMR spectrum of (II) has the signals of the protons of two N-methyl groups at 7.57 and 7.52. The signals of the protons of OCH_3 groups appear at 7.00 (C-7), 6.71 (C-6'), 6.30 (C-6), and 6.14 (C-4'). The one-proton signal at 4.90 is due to the proton of the hydroxy group. In the weak-field region there are the signals of nine aromatic protons at 3.69; 3.62; 3.43; 3.23; 3.15; 2.94. The signal at 3.69 relates to the C-8' proton. In the spectrum of (III) the signal of the three protons of a CH_3—COO group appear at 7.75.

Since the methoxy group in position 6' resonates in the strong field, the two asymmetric atoms possess the same configuration [2]. When thalisopine was decomposed with sodium in liquid ammonia, L-6-methoxy-1-(4'-methoxybenzyl)-2-methyltetrahydroisoquinoline was obtained [1]. Consequently, thalisopine has the L,L-configuration.

In the mass spectrum of thalisopine (II), the peak of the molecular ion with m/e 638 makes up 11% of the main peak. The peak with m/e 206 (100%) corresponds to the doubly-charged ion *a*. The splitting off of dimethyl ester from the ion *a* leads to a fragment with m/e 183 (17). Fragment *a* corresponds to a singly-charged ion radical with m/e 412 (89). The latter, losing a methyl radical, gives a fragment with m/e 397 (38). In addition, the peaks of ions with m/e 221 (18), 174 (18), 173 (29), 172 (89), 90 (9), 89 (20) are recorded.



II



a

On the basis of what has been said above, we proposed for thalisopine structure (II) as the most probable.

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INVESTIGATION OF GLOBULIN A

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From the seeds of the type 108-F cotton plant we have isolated by chromatography on DEAE-cellulose a homogeneous globulin component which we have called globulin A. The same component was obtained from Acala glandless [1] as acalin A, and its homogeneity was shown. We have confirmed the homogeneity of globulin A by electrophoresis in polyacrylamide gel and by chromatography on DEAE cellulose.

The molecular weight was determined by gel filtration through Sephadex G-200 (column 2.5 × 50 cm) [2]. The protein was dissolved and eluted with 1M sodium chloride solution (pH 7.6). To construct the calibration curve, we used the proteins γ-globulin, blood serum albumin, hemoglobin, and ribonuclease. The elution curves were constructed after the determination of the amount of protein on an SF-4a instrument. The molecular weight of globulin A proved to be ≈170 000.

The N-terminal amino acids were determined by the fluorodinitrobenzene method [3]. A solution of 10 mg of globulin A in 5 ml of 0.1 M ammonium acetate (pH 8.7) was treated with 0.2 g of FDNB and the mixture was stirred at 40° C for 2 hr. The excess of reagent was eliminated with peroxide-free ether. To eliminate the dinitrophenol, the reaction mixture was dialyzed against water and freeze-dried. The dry FDN-protein was hydrolyzed in tubes with 5.7 N

hydrochloric acid at 100° C for 16 hr. The N-terminal amino acids were isolated by thin-layer chromatography on silica gel [4]. Two spots were found with R_f 0.70 and 0.38 in the propanol—34% ammonia system, corresponding to histidine and arginine.

The C-terminal amino acids were determined by the hydrazine and carboxypeptidase methods. A mixture of 50 mg of protein, 0.125 g of hydrazine sulfate, and 0.5 ml of anhydrous hydrazine was sealed in a tube which was heated in a water bath at 60° C for 16 hr. The excess of hydrazine was eliminated in a vacuum desiccator over sulfuric acid. The hydrazides of the free amino acids were separated on B3 polyacrolein resin [5]. The latter was filtered off and the filtrate was investigated for its content of amino acids by thin-layer chromatography on silica gel and in a mixed layer of cellulose and silica gel [6]. In the ethanol—34% ammonia system on silica gel we identified valine with R_f 0.45 and aniline with R_f 0.55; in the mixed layer in the phenol—water system we identified the same amino acids with R_f 0.55 and 0.66. The revealing agent was 0.5% ninhydrin in ethanol.

In the carboxypeptidase method, 0.02 ml of a suspension of carboxypeptidase in water (corresponding to ≈1 mg of enzyme) was added to 10 ml of a 0.5% solution of globulin A in a 1% solution of sodium hydrogen carbonate (ph 7.8), and the mixture was incubated at 27° C for 4 hr. The enzyme was inactivated by boiling in the water bath for 5 min, and after the addition of 2 ml of 20% TCA the mixture was centrifuged. The supernatant liquid was separated off and evaporated to dryness in vacuum at 40° C. The residue was dissolved in 0.02 ml of water. The amino acids were determined by thin-layer chromatography on silica gel in the ethanol—34% ammonia system. The revealing agent was 0.5% ninhydrin in ethanol. The two spots with R_f 0.45 and 0.55 were identified as valine and alanine.

Thus, the C-terminal amino acids of globulin A are valine and alanine, and the N-terminal acids histidine and arginine.

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ALANINE-OXOGLUTARATE AMINOTRANSFERASE FROM THE SEEDS OF THE COTTON PLANT

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Continuing an investigation of the albumin fraction of the proteins of cottonseed [1], we have isolated a fraction with alanine-oxoglutarate aminotransferase activity.

The acetone powder obtained by B. Mondovi's method [2] was suspended in five volumes of a 10^{-3} M solution of glutathione pyruvate, the pH of the mixture was brought to 6, and it was stirred at 4° C [3]. The suspension was centrifuged at 9000 × g for 15 min. The residue was discarded and the supernatant was centrifuged at 45 000 × g for 90 min. The liquid present in the intermediate layer was collected, filtered through glass wool, concentrated, and dialyzed in the cold against 3 *l* of 0.02 M phosphate (potassium) buffer (pH 7.6) for 12 hr. The contents of the dialysis bag were centrifuged at 100 000 × g for 30 min and transferred to a column of DEAE-cellulose (2.5 × 35 cm) equilibrated with the same buffer. The rate of elution was 24 ml/hr. The column was washed with 500 ml of the same buffer and eluted with 0.1 M phosphate (potassium) buffer (pH 7.4) until the density of the fraction at 280 mμ had become less than 0.3. Then, it was eluted with 0.15 M phosphate (potassium) buffer (pH 7.4) and elution was continued until the density at 280 mμ had fallen to 0.1.

The experimental results enabled a graph of the elution of the protein from the column to be plotted. All the fractions eluted were investigated for alanine-oxoglutarate aminotransferase activity [4]. The fractions were collected,