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PRODUCTS OF ALKALOID OXIDATION BY BLOOD PLASMA HEMOPROTEIN

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A heme-containing protein with mol. wt. of about 75,000 daltons, inactivating alkaloids of the atropine group, was isolated previously from blood. The abbreviation HP_{aa} (antialkaloid hemoprotein) is suggested for this protein.

In the present investigation the mechanism of the reaction of HP_{aa} with alkaloids was studied by identifying inactivation products of atropine and also of the alkaloid arecoline, which differs from it in its pharmacologic action and structure.

EXPERIMENTAL METHOD

The method of isolating the enzyme from rat blood plasma, including fractionation of the proteins by precipitation with ammonium sulfate, ion-exchange chromatography, and gel-chromatography, was described previously [1]. The concentration of the enzyme was 1/12,000 of the total blood plasma proteins.

To study inactivation of atropine and arecoline the reaction mixture contained 0.3-7.7 μmoles of substrate and 20 μg of enzyme in 0.3 ml of 0.025 M K-phosphate buffer, pH 7.4. Samples were incubated for 60 min at 37°C, 0.3 ml of ethanol was added, and the mixture was dried in vacuo. The material was extracted with 0.2-4.8 ml of ethanol and aliquots, each of 0.025-0.05 ml, were applied to Silufol chromatographic plates (length of run 7 cm). In the case of atropine fractionation was carried out in a system of 96% ethanol—chloroform—25% ammonia (15:10:4), and in the case of arecoline, 80% ethanol—25% ammonia (97:3). One pole of the chromatogram was developed with Dragendorf's reagent [3] or with iodine vapor. Zones corresponding to the reaction products found were cut out and the material eluted with 7 ml of 0.05 M Na,K-phosphate buffer, pH 7.4. Atropine, arecoline, and their conversion products were estimated quantitatively by the reaction with bromthymol blue [2]. For preparative isolation of the reaction products they were eluted from the sorbent with 0.5 ml of ethanol. The eluate was centrifuged and dried in vacuo. The residue was dissolved in a small volume of ethanol, the solution was applied to KRS-5 glass, and dried in vacuo at 45°C. IR spectra were recorded on the UR-20 and Specord 75-UR spectrophotometers.

EXPERIMENTAL RESULTS

After incubation of atropine and arecoline with the enzyme, besides the original substances (R_f 0.70 and 0.58, respectively), single products with R_f values of 0.58 and 0.27, respectively, also were found in the reaction mixture (Fig. 1).

IR spectra of atropine and its enzymic conversion product are shown in Fig. 2. The only difference in principle is the appearance of a strong and narrow absorption band for the lat-

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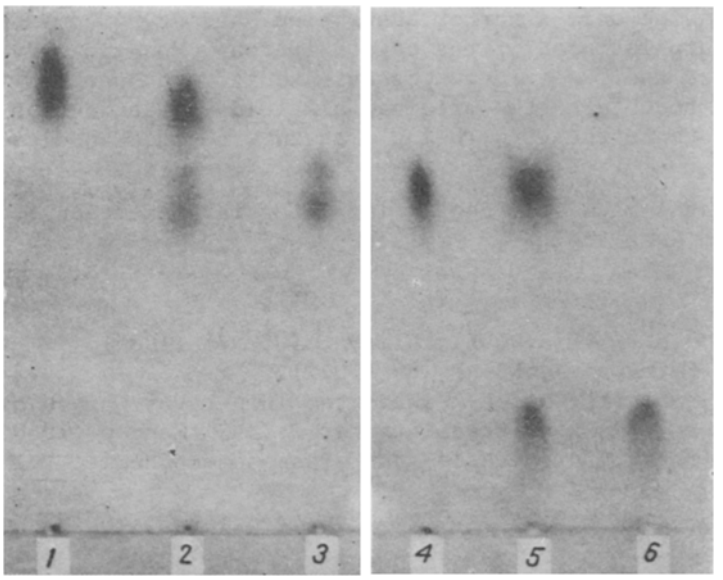

Fig. 1. Chromatographic fraction of products of enzymic and chemical conversion of atropine and arecoline. 1, 4) Original atropine and arecoline, respectively; 2, 5) products of their enzymic conversion; 3, 6) products of their oxidation by hydrogen peroxide.

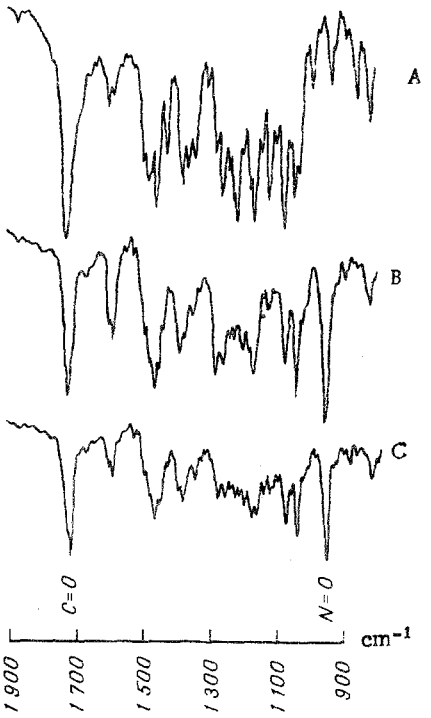

Fig. 2. IR spectra of atropine (A) and products of its enzymic (B) and chemical (C) conversion.

ter at 950 cm^{-1}, which is characteristic of valency oscillations of the N=O bond. The enzymic reaction product was identified with the known N-oxide of atropine, obtained by oxidizing atropine with hydrogen peroxide in ethanol [5].

The N-oxide was isolated from the reaction mixture by preparative thin-layer chromatography in the same system as the product of the enzymic reaction. Products of enzymic and chemical conversion of atropine had the same R_f value (Fig.1) and similar IR spectra (Fig. 2). Products of enzymic conversion of arecoline and its oxidation with hydrogen peroxide also had the same R_f value (Fig. 1), and their IR spectra were identical, and differed from the spec-

trum of arecoline in having only a narrow absorption band at 940 cm^{-1} ν, N=O, comparable in intensity with the absorption band of the carbonyl group. Enzymic conversion products of atropine and arecoline, like their N-oxides, possess oxidative properties (oxidation of the iodide ion) and ability to be reduced to the original alkaloids by the action of zinc in an acid medium. This last fact prevents them from being classed with the N,N-disubstituted hydroxylamines [4].

HP_{aa} thus inactivates alkaloids containing a tertiary nitrogen atom in the aliphatic ring, by converting them into N-oxides.

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