that are characteristic for the protein and the dinitroaniline group of the inhibitor. At a concentration of the inhibited enzymes of 1 mg/ml, the optical densities at 280 and 360 mμ were, respectively: for human pepsin, 1.3 and 0.465; for gastricsin, 1.3 and 0.480; and for porcine pepsin, 1.3 and 0.410. This showed that in human pepsin and gastricsin, and also in procine pepsin, there is one inhibitor residue per molecule of the enzyme. A comparison of the content of basic amino acids in the two enzymes and the absorption at 360 mμ gives a similar result. Thus, DDE inhibits human pepsin and gastricsin, porcine pepsin [1], hen pepsin [2], and calf rennin [5]. The data given in the papers mentioned permits the conclusion that DDE is apparently a general inhibitor for proteases of the pepsin group.

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N-ACETYL-3-HYDROXY-4-METHYLANTHRANILIC ACID FROM AN ACTINOMYCETE—THE PRODUCING AGENT OF THE ACTINOMYCIN ANTIBIOTIC AURANTIN

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In a study of the mechanism of the actinomycin antibiotics it was shown that the precursor of the phenoxazine part of the molecule is 3-hydroxy-4-methylanthranilic acid, detected in the culture liquid of the producing agent in trace amounts [1].

We have established that the producing agent of the Russian actinomycin antibiotic aurantin liberates into the medium an N-acyl derivative of 3-hydroxy-4-methylanthranilic acid (HMAA) which accumulates in considerable amounts [2]. The substance has been isolated and characterized, and its structure has been shown, apart from the organic acid acylating the amine group. The N-acyl-HMAA has the following elementary composition (%): C 58.23, 57.61; H 5.65, 5.68; N 6.51, 6.56. This agrees with the elementary composition of N-acetyl-HMAA (C 57.41; H 5.30; N 6.70) and differs little from the composition of N-propionyl-HMAA (C 59.19; H 5.82; N 6.27).

The choice between these two compounds was made on the basis of the chromatography of hydrolysates of the substance under investigation. The volatile organic acid formed as a result of hydrolysis was converted into the hydroxamate by a published method [3], as modified by us, and was chromatographed with reference materials. The N-acyl-HMAA was hydrolyzed with 2 N sulfuric acid at 105° C for 4 hr, the volatile acid was distilled off with steam, the distillate was neutralized and evaporated to dryness, and the residue was dissolved in 2 ml of ethanol and treated with 0.1 ml of thionyl chloride. After 1 hr, the mixture was neutralized with 12.5% alkali in methanol, and 1 ml of hydroxylamine solution was added (5% hydroxylamine hydrochloride in methanol was mixed with an equal volume of 12.5% caustic soda in methanol). After 18 hr, the precipitate was filtered off. The filtrate was evaporated in vacuum, and the residue was chromatographed in the solvent system amyl alcohol—acetic acid—water (4:1:5). The hydroxamates were revealed on the chromatogram with a 15% aqueous solution of ferric chloride. In the solvent used, the chromatograms showed the presence of acetohydroxamic acid. This means that the hydrolysate of the N-acyl-HMAA contained acetic acid. Consequently, the producing agent of the actinomycin antibiotic aurantin synthesizes and liberates into the medium N-acetyl-3-hydroxy-4-methylanthranilic acid.

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