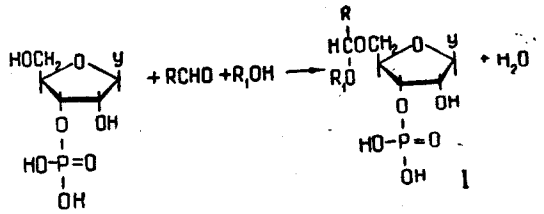
In order to show the applicability of this reaction to the case under consideration, when one of the alcohols is the carbohydrate part of a nucleotide, we have synthesized 5'-O-(α-butoxyethyl) uridine 3'-phosphate (Ia) by treating uridine 3'-phosphate with an excess of acetic anhydride and butan-1-ol in the presence of anhydrous alumina at 15° C for 6–7 hr. Under these conditions about 28% of 5'-O-(α-butoxyethyl) uridine 3'-phosphate is formed together with traces of 2',5'-O,O-di-(α-butoxyethyl) uridine 3'-phosphate. The 5'-O-(α-butoxyethyl)-uridine 3'-phosphate was isolated from the reaction mixture by means of chromatography on cellulose powder; the solvent was a mixture of isopropanol, concentrated ammonia, and water (7:1:2). This substance proved to be identical with the 5'-O-(α-butoxyethyl) uridine 3'-phosphate prepared by our previous method [1] by its partition coefficient in paper chromatography with the solvent isopropanol – conc. ammonia – water (7:1:2), with respect to its mobility on paper electrophoresis in a 0.05 M solution of triethylammonium hydrogen carbonate, by its behavior in 2 N acetic acid, and by its IR spectrum. 5-O-(α-Alkoxyalkyl) derivatives of uridine 3'-phosphate with other aldehydes and alcohols – (Ib)-(Ih) have also been obtained.



1a CH_3- $CH_3CH_2CH_2CH_2-$
1b CH_3- CH_3CHCH_2- (CH_3)
1c CH_3- $CH_3CH_2CHCH_3$ (CH_3)
1d CH_3- CH_3-C- (CH_3)
1e CH_3- $C_6H_5CH_2$
1f CH_3CHCH_2 (CH_3) $CH_3CH_2CH_2CH_2-$
1g C_6H_5 $CH_3CH_2CH_2CH_2-$
1h CH_3CHCH_2 (CH_3) CH_3CHCH_2- (CH_3)

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SPECIFIC ALKYLATION OF CHYMOTRIPSIN WITH THE AZIDE OF THE S-CARBOXYMETHYLMERCAPTIDE OF p-MERCURIBENZOIC ACID

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We have studied the acylation of chymotripsin with the azide of the S-carboxymethylmercaptide of p-mercuribenzoic acid [1]. Chymotripsin was treated in an acetate buffer (pH 5.0) with a large excess of the azide at 2° C for 36 hr, after which the enzyme had lost its esterase activity as determined by the hydrolysis of p-nitrophenyl acetate. The low-molecular-weight components of the mixture were eliminated by gel filtration on Sephadex G-25 equlibrated with 0.002 N hydrochloric acid; then the acylated enzyme was freeze-dried.

A determination by the dithizone method showed that the molecule of the acyl-enzyme contained one atom of mercury. The treatment of the acyl-enzyme with hydroxylamine at pH 6.0 led to the cleavage of 1 mole of hydroxamate from 1 mole of acyl-enzyme. The acylated chymotripsin was completely reactived after incubation for 20 hr at pH 5.0 or 2 hr at pH 6.0.

The selective acylation and the high reactivity of the bond formed permit the assumption that it is the active hydroxyl of serine located in the catalytic center of chymotripsin that reacts with the azide of the S-carboxymethyl-

mercaptide of p-mercuribenzoic acid. This is confirmed by the absence of a reaction between the azide and diisopropylphosphorylchymotripsin.

The S-carboxymethylmercaptide of p-mercuribenzoylchymotripsin can be used for X-ray studies.

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