any effect on the spontaneous activity of mice. In *in vitro* studies hydrotrichlorothiazide did not exhibit any antihistaminic, anticholinergic, or antispasmodic activity. Hydrotrichlorothiazide had one-half the carbonic anhydrase inhibiting activity of chlorothiazide and five times that of hydrochlorothiazide (SHEPPARD).

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Research Department, CIBA, Summit (New Jersey), February 9, 1960.

Zusammenfassung

In der Reihe der Dihydro-benzothiadiazine wurde mit Hydrotrichlorthiazid (III) ein neues, ausserordentlich stark wirksames Diuretikum gefunden. Herstellung und pharmakologische Evaluation werden kurz beschrieben. Im Vergleich zu Hydrochlorthiazid (I) ist III beim Hund *per os* bis zu 20mal stärker diuretisch wirksam.

Effect of Methanol and Dioxan on the Action of Chymotrypsin on L-Phenylalanine Methyl Ester

Many studies on the kinetics and specificity of chymotrypsin have been carried out in methanol-water mixtures because of the limited solubility in water of the synthetic substrates employed in these studies[1–4]. Thus, SNOKE and NEURATH[4] investigated the action of chymotrypsin on benzoyl-L-phenylalanine methyl ester in a system containing 30 vol.% of methanol. The present paper deals with the effect of methanol and dioxan on the action of chymotrypsin on non-benzoylated L-phenylalanine methyl ester which, like the corresponding ethyl ester[5], is readily hydrolyzed by chymotrypsin in aqueous solution.

Worthington crystalline, salt-free chymotrypsin was used. Its action was followed by measuring the disappearance of the ester, using Hestrin's hydroxamic acid method[6]. Each reaction mixture contained, in 5 ml 0·04 *M* phosphate buffer: chymotrypsin, 0·2 mg; substrate, approximately 50 μmoles (L-phenylalanine methyl ester) or 25 μmoles (benzoyl-L-phenylalanine methyl ester). The temperature was 30°C. One ml samples were tested by Hestrin's method (15 min treatment with hydroxylamine prior to the addition of HCl and $FeCl_3$). The results of the experiments are summarized in the Table. The figures in the Table represent the Klett-Summerson colorimeter readings (Filter 54). Blanks run simultaneously without addition of enzyme gave, at the end of the experiments, values which were equal or very close to the initial values.

As shown in the Table, the action of chymotrypsin on L-phenylalanine methyl ester was completely inhibited in the presence of 30 vol.% of either methanol or dioxan. Under the same conditions, benzoyl-L-phenylalanine methyl ester was readily hydrolyzed. The Table also shows that 15 vol.% of methanol very strongly inhibited the action of chymotrypsin on L-phenylalanine methyl ester, and that even 7·5 vol.% caused a strong inhibition.

Effect of methanol and dioxan on the action of chymotrypsin

Substrate	Medium	pH	Time, min		
			0	10	20
PME*	Water	7·5	370	180	50
PME*	Methanol 7·5%	7·5	370	325	260
PME*	Methanol 15 %	7·5	380	365	340
PME*	Methanol 30 %	7·5	385	385	375
PME*	Dioxan 30 %	7·5	375	375	365
BPME**	Methanol 30 %	7·5	365	20	15
BPME**	Methanol 30 %	7·5	360***	120***	30***
BPME**	Dioxan 30 %	7·5	365	25	15
BPME**	Dioxan 30 %	7·5	375***	120***	30***
PME*	Water	6·5	390	175	50
PME*	Methanol 30 %	6·5	420	415	415
BPME**	Methanol 30 %	6·5	395	160	90

* L-Phenylalanine methyl ester hydrochloride.
** Benzoyl-L-phenylalanine methyl ester.
*** With half the amount of enzyme.

The findings here reported on the complete inhibition of the action of chymotrypsin on phenylalanine methyl ester by methanol or dioxan under conditions where the corresponding benzoyl derivative was readily hydrolyzed, may be of interest and deserve a closer investigation. In a previous communication[7] it was suggested that chymotrypsin does not hydrolyze the phenylalanine ester directly, but first converts it, by a transfer reaction, to a dipeptide ester (or to an ester of a higher peptide), and that this compound, bearing a 'secondary peptide bond' is then rapidly hydrolyzed by the enzyme. Since methanol or dioxan did not, in our experiments, prevent the hydrolysis of the benzoyl derivative of the ester, this assumption, if correct, would mean that both organic solvents inhibit the primary transfer reaction.

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Zusammenfassung

Die Spaltung von L-Phenylalanin-methylester durch Chymotrypsin ist in Gegenwart von 30 Vol.% Methanol oder Dioxan vollständig gehemmt, während unter denselben Bedingungen Benzoyl-L-phenylalanin-methylester intensiv hydrolisiert wird.

Incorporation of S^{35}-Methionine in the Microsomes and Soluble Proteins During the Early Development of the Sea Urchin Egg

Previous work from this Laboratory has shown that S^{35}-methionine given to unfertilized eggs of *Paracentrotus lividus* is stored entirely in the so-called nonprotein fraction (fraction soluble in cold 10% trichloroacetic acid) and largely converted into glutathione[1,2].

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