Bei intraperitoneal infizierten Mäusen wird somit durch die LPS-Vorbehandlung ein völlig geänderter Verlauf der lokalen Leukozytenreaktion auf die Infektion hervorgerufen. Während bei nicht vorbehandelten Tieren im Bereich der hohen Bakteriendosen eine Aufhebung der Leukozytenreaktion erfolgt, ist nach LPS-Vorbehandlung auch bei den höchsten Infektionsdosen eine starke intraperitoneale Leukozytenansammlung vorhanden. Da die Leukozytenreaktion in quantitativer Relation zur Schutzwirkung der LPS-Vorbehandlung steht, stellt sie offenbar einen besonders wesentlichen Faktor für die Erhöhung der Resistenz durch LPS dar.

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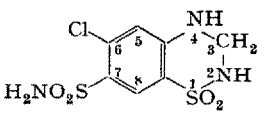
Forschungslaboratorien der CIBA Aktiengesellschaft, Pharmazeutische Abteilung, Basel, 4. Januar 1960.

Summary

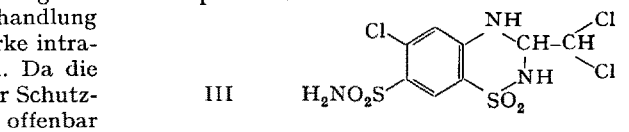
Intraperitoneal aggregation of leucocytes is produced in an identical manner by intraperitoneal application of different amounts of living and dead bacteria and bacterial products. The maximum accumulation occurs with medium dosages of bacteria or their products, with high dosages no leucocytic accumulation is produced. Pretreatment with selected LPS of bacteria enhances the intraperitoneal accumulation of leucocytes also with the high amounts of bacteria, whereas the reaction in not pretreated with LPS is suppressed. Parallel to the increase of the intraperitoneal leucocytic accumulation the animals are protected against the infection with high amounts of bacteria, to which they succumb if not pretreated with LPS.

The Chemistry and Pharmacology of Hydrotrichlorothiazide

Since the discovery of the potent orally effective nonmercurial diuretic drug, 6-chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxide (I), (hydrochlorothiazide) in our Laboratories[1], the ground work was laid for preparation of a considerable number of analogs of this heterocyclic system. As described in our first communication, compounds of this type are readily prepared by condensing an aldehyde or acetal with 4-amino-6-chloro-m-benzenedisulfonamide (II). In this way a large variety of 3-substituted derivatives of I have been synthesized. One of the several hundred compounds[2] we have prepared in this series is the subject of this report.

I 

The condensation of the disulfonamide II with dichloroacetaldehyde or diethyldichloroacetal was carried out in anhydrous diethyleneglycol dimethyl ether containing a catalytic amount of hydrogen chloride. The condensation could also be run in 15% hydrochloric acid solution. The resulting substance, 6-chloro-3-dichloromethyl-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxide (III)[3] was recrystallized from methanol:acetone:water (1:1:1) to yield a white crystalline powder, m. p., 248–250°C with decomposition.

III 

Anal. Calculated for $C_8H_8Cl_3N_3O_4S_2$; C, 25·25; H, 2·08; N, 11·05. Found: C, 25·40; H, 2·33; N, 11·01.

Pharmacology

General Pharmacological Properties. Hydrotrichlorothiazide (III) has shown marked diuretic activity in the unanesthetized dog after oral administration. The diuretic, saluretic, kaluretic, and chloruretic activities of this compound were compared to those of hydrochlorothiazide (I). Both drugs were tested in the unanesthetized dog after the oral administration of the drugs. The exact method of the assay employed has been previously described by Barrett *et al.*[4]. The relative potency of III compared to that of hydrochlorothiazide was computed by using a 2 × 2 factorial assay. The results obtained for each of the four parameters measured are summarized in the following Table along with the 95% confidence limits. Hydrotrichlorothiazide at doses of 1·25 and 20 μg/kg was compared with hydrochlorothiazide at doses of 20 and 310 μg/kg. Thus, in terms of water excretion, this substance is 22 times more potent than I and in terms of sodium excretion 20 times more potent than I. Since it was not possible to obtain a dose response curve for urinary potassium excretion after the administration of hydrotrichlorothiazide, one could not calculate a relative potency value for this parameter.

Parameter	Relative Potency (Hydrochlorothiazide = 1)	95% Confidence Limits
Water	22	9·0–51
Sodium	20	9·0–46
Chloride	11	7·0–18·0

The relative diuretic potency of hydrotrichlorothiazide and hydrochlorothiazide was determined for the rat utilizing four separate assays. The method of assay was similar to that previously reported[5]. Compound III was tested at doses of 0·08 and 0·02 mg/kg and hydrochlorothiazide at 0·32 and 0·08 mg/kg for each assay. The calculation of relative potencies of the two compounds showed that hydrotrichlorothiazide was 6·3 times as potent in its effect on water excretion, 3·8 times as potent in terms of sodium excretion, 4·6 times in terms of potassium excretion, and 4·9 times as potent with respect to chloride excretion. This difference in potency is less for the rat than for the dog.

Hydrotrichlorothiazide produced no effect on the blood pressure of the anesthetized dog at doses up to 9·0 mg/kg (i. v.). In the anesthetized dog it did not exhibit any anticholinergic activity nor adrenolytic activity. It had no effect on the behaviour of the unanesthetized dog in the doses employed in the diuretic studies, neither did it have

[1] G. deStevens, L. H. Werner, A. Halamandaris, and S. Ricca, Jr., Exper. *15*, 463 (1958).

[2] In a recent paper we have described in detail the preparation of compounds substituted at the 2, 4, 5, 6, and 7 positions. See L. H. Werner, A. Halamandaris, S. Ricca, Jr., L. Dorfman, and G. de Stevens, J. Amer. Chem. Soc. *82*, in press (1960).

[3] Generic name: Hydrotrichlorothiazide.

[4] W. Barrett *et al.* Toxicol. appl. Pharmacol. *1*, 333 (1959).

[5] A. H. Renzi, J. Chart, and R. Gaunt, Toxicol. appl. Pharmacol. *1*, 406 (1959).

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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Department of Biological Chemistry, The Hebrew University, Jerusalem (Israel), November 3, 1959.

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 non-protein fraction (fraction soluble in cold 10% trichloroacetic acid) and largely converted into glutathione[1,2].

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[2] J. E. SNOKE and H. NEURATH, Arch. Biochem. *21*, 351 (1949).
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[4] J. E. SNOKE and H. NEURATH, J. biol. Chem. *182*, 577 (1950).
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[6] S. HESTRIN, J. biol. Chem. *180*, 249 (1949).
[7] S. KUK-MEIRI and N. LICHTENSTEIN, Biochim. biophys. Acta *25*, 182 (1957).

[1] E. NAKANO and A. MONROY, Exp. Cell Res. *14*, 236 (1958).
[2] E. NAKANO and A. MONROY, Exper. *14*, 367 (1958).