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Hippurylcholine

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Hippurylcholine, the hippuric acid ester of choline, has been synthesized and its chemical, biochemical and pharmacological properties described.

Hippurylcholine is not identical with the "F component" present in ox spleen. This component may be a choline ester of an aromatic acid derivative containing glycine.

During investigations in this laboratory concerning the natural occurrence of choline esters other than acetylcholine in animal organisms, *hippurylcholine* or a derivative thereof was thought to be present in ox spleen and identical with the unidentified ester ("F component") reported by Banister, Whittaker and Wijesundera [1]. Hippurylcholine was therefore synthesized and tested in the chromatographic analysis of the isolated bases from the spleen. It was demonstrated, however, that the new ester is neither identical with the "F component", nor present in the material studied.

Hippurylcholine, the hippuric acid ester of choline, has not hitherto been described in the literature. Its synthesis together with some of its chemical, biochemical and pharmacological properties are reported briefly in the present paper.

Chemistry. Hippurylcholine chloride (HiCh), $C_6H_5CONHCH_2CO\text{-}OCH_2CH_2N^+(CH_3)_3Cl^-$, was prepared by methylating β-chloroethyl hippurate, $C_6H_5CONHCH_2CO\text{-}OCH_2CH_2Cl$. The latter compound was synthesized from hippuric acid and 2-chloro-ethanol. The methods used were similar to those described by von Euler *et al.* [2].

HiCh crystallizes with one molecule of water in white non-hygroscopic prisms, m. p. 91°, very soluble in water and ethanol, insoluble in ether, ethylacetate, chloroform, benzene, acetone. The rate of spontaneous hydrolysis in distilled water is similar to that of acetylcholine chloride. HiCh can be estimated quantitatively using the hydroxylamine-ferric chloride test [3], and isolated as reineckate or chloroaurate.

Paper chromatography; comparison with the "F component" of oxspleen. HiCh was chromatographed on paper (Fig. 1, A) using the technique recently described [4]. The R_F value was 0.50 compared with acetylcholine (ACh) 0.37, pro-

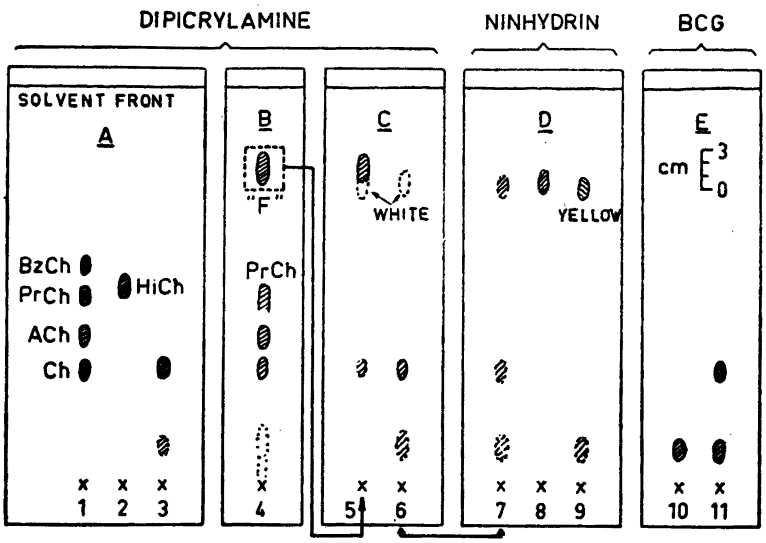

Fig. 1. Ascending chromatograms of hippurylcholine (HiCh) and other choline esters (ACh, acetyl-; PrCh, propionyl-; BzCh, benzoyl-choline), compared with the chromatographic results obtained with the "F component" isolated from ox spleen. Solvent: n-butanol-ethanol-acetic acid-water (8:2:1:3). Development of chromatograms as indicated; BCG, bromocresol green. 1. Choline (Ch) and esters. 2. HiCh. 3. HiCh after alkaline hydrolysis. 4. Isolated bases from spleen. 5. Rechromatogram of "F component". 6 and 7. "F" after alkaline hydrolysis. 8. Hippuric acid. 9. p-aminobenzoic acid. 10. Glycine. 11. Hippuric acid after hydrolysis + choline + glycine.

pionylcholine (PrCh) 0.48, and benzoylcholine (BzCh) 0.55. After alkaline hydrolysis of HiCh, choline (R_F 0.30) was traced on the paper developed with dipicrylamine together with a faintly coloured area (R_F 0.10) which was identified as glycine.

As was mentioned in the introductory section of the present paper, HiCh was assumed to be identical with the "F component" present in ox spleen. The basis for this hypothesis was the following. On the chromatogram of the isolated bases of ox spleen (Fig. 1, B) the "F component" was identified as a yellow spot (dipicrylamine) with the approximative R_F 0.80. The biological activity of this area was verified by testing on the isolated frog rectus abdominis muscle. The "F" area was extracted and rechromatographed (Fig. 1, C). In addition to the "F component" the new chromatogram showed after development with dipicrylamine a white area (R_F 0.77) close to the "F" area and a yellow spot (R_F 0.30) which was identified as choline. After alkaline hydrolysis of the "F component" the original area disappeared and the new areas (R_F 0.77 and 0.30) were more clearly visible; in addition, a third faintly coloured spot (R_F 0.10) appeared. The white area (R_F 0.77) was probably due to an acid compound. By comparing the R_F value of this compound with a great many acid compounds we found *p*-aminobenzoic acid (0.76), *o*-aminobenzoic acid (0.80), *p*-hydroxy benzoic acid (0.84), and hippuric acid (0.77) to come close

Table 1. Effect of hippurylcholine chloride (HiCh) on cholinesterase activity. Corrections made for non-enzymatic hydrolysis.

HiCh *M*	Electric tissue acetylcholinesterase		Human serum cholinesterase	
	b_{30}	% inhibition	b_{30}	% inhibition
Control	68	—	118.5	—
10^{-3}	67	0	13	90
10^{-4}	69	0	57	52
10^{-5}	67.5	0	109.5	8

to the acid compound obtained from the "F component" (Fig. 1, D and E). The slow running compound (R_F 0.10) came close to glycine. The "F component" was therefore assumed to be composed of choline, glycine, and an aromatic acid, presumably an aminobenzoic acid. The working hypothesis was put forward that the new component is a choline ester of a hippuric acid derivative (*e. g.*, *p*-aminohippurylcholine). Choline esters of hippuric acid or its derivatives have not hitherto been described and experiments were therefore performed to synthesize such compounds. Hippurylcholine chloride (HiCh) is the only ester so far synthesized; *p*-aminohippurylcholine has not been obtained in a pure form.

The results obtained by paper chromatography with the "F component" (Fig. 1) demonstrate that hippurylcholine is not identical with this component. The problem to be investigated is whether some derivative of hippuric acid is the acid moiety of the "F component" in ox spleen.

Biochemistry. HiCh is not split enzymatically by the acetylcholinesterase of electric tissue or by a purified preparation of human serum cholinesterase. It has no inhibiting effect in a 10^{-3} *M* solution on the acetylcholinesterase, but inhibits in this concentration the human serum cholinesterase to 90 %. HiCh is regarded as a useful selective inhibitor of the latter enzyme (Table 1).

Pharmacology. HiCh has practically no pharmacological effects when tested on general test objects. No effects were obtained with 1 mg HiCh on isolated guinea-pig ileum, sensitive to 0.1 μg acetylcholine, and on frog rectus abdominis muscle. The compound has no anti-acetylcholine effect.

The blood pressure of the cat is not influenced by 1, 10 and 100 μg HiCh; after 1 mg intravenously a small decrease of the blood pressure was observed, but this effect was probably not specific.

There was no effect on the nictitating membrane, neither stimulating nor inhibiting. The transmission in the superior cervical ganglion of the cat was not influenced after 1 mg HiCh.

HiCh has no antihistaminic effect.

EXPERIMENTAL

β-Chloroethyl hippurate. Hippuric acid (5 g) was dissolved in 2-chloroethanol (10 ml), and hydrochloric acid gas bubbled through the solution for 3 hours. Water was added to the product and when the separated oily phase was mixed with more water a white precipitate was formed. The precipitate was filtered off, washed with water, and dried in a

desiccator. The product was dissolved in hot benzene and precipitated with petrol ether. The pure compound, recrystallized from benzene and dried over P_2O_5, had m. p. 59° C (uncorr.). Yield 74 %. (Found: C 54.88; H 4.97; N 5.78. Calc. for $C_{11}H_{12}O_3NCl$ (241.68): C 54.54; H 5.00; N 5.79.)

Hippurylcholine chloride (HiCh). *a*-Chloroethyl hippurate (1 g) was mixed with a solution (5 ml) containing anhydrous trimethylamine (1 ml) and benzene (4 ml). The mixture was heated in a sealed tube for about 20 hours at 60° C. The oily residue was removed and washed with benzene. An excess of acetone was added to the product which dissolved on heating and crystallized in prisms on cooling. Recrystallization from ethanol (by adding ethyl acetate to the solution) gave white prisms, m. p. 91° C (uncorr.). Yield 25 %. (Found: C 52.94; H 7.27; N 8.77; Cl 10.60. Calc. for $C_{14}H_{21}O_3N_2Cl.H_2O$ (318.80): C 52.75; H 7.27; N 8.79; Cl 11.12.)

Hippurylcholine reineckate, $C_{14}H_{21}O_3N.Cr(NH_3)_2(SCN)_4$. An aqueous solution of HiCh was precipitated with Reinecke salt (aqueous solution). After two hours in the refrigerator the precipitate was filtered off, washed and recrystallized from acetone. The red crystalls had m. p. 135° C (uncorr.).

Hippurylcholine chloroaurate, $C_{14}H_{21}O_3N_2Cl.AuCl_4$, was prepared by a method similar to that used for the reineckate. Recrystallization of the water-insoluble product from acetone gave yellow neddles, m. p. 173° C (uncorr.).

Paper chromatography was carried out according to the method described recently [4,5]. The solvent used was a *n*-butanol-ethanol-acetic acid-water mixture (8:2:1:3). The ascending technique was employed in most cases.

Enzyme and pharmacological studies. Cholinesterase activity was determined by the technique developed in this laboratory [6]. The pharmacological experiments were carried out with test objects in general use.

Isolation of tissue bases was carried out according to the method described recently for the central nervous system of the honey bee [5].

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