5-HYDROXYKYNURAMINE(MAUSAMINE) IN THE URINE OF MOUSE

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Received July 17, 1961

In the course of our study on the neutral and basic metabolites which were excreted in the urine of mice injected with serotonine, we have found on the paper chromatogram of the urine extract a compound having similar properties to those of synthetic HK* (I).

The investigation was performed as follows: fifteen mice of DD strain weighing 20 to 25 g each and fed on a synthetic diet** were injected with 10 mg of serotonine creatinine sulfate daily for three days, and their urine was collected for three days in 0.2 N HCl, which was then passed through Dowex 50 (H^+ form)-column(2 cm x 26 cm), washed with 1.2 L of 0.2 N HCl and then eluted with 0.4 L of 5 N HCl.

This eluate was found to contain the greater part of HK-like substances but only a very small amount of serotonine. It was concentrated under reduced pressure of nitrogen below 45°, and the residue was applied in a band form to a filter paper No. 53 (40 cm x 40 cm) of the Toyoh Roshi Co., Nihonbashi, Tokyo, with ascending technique at the room temperature. The solvent system used was the supernatant of the mixture of isobutanol-acetic acid-water (4:1:5)

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* 5-Hydroxykynuramine (we intend to call this mausamine)

** Purified casein 20%, lard 15%, starch 60%, Mc Collum salt 5%; moreover, for 100 g of the above diet, thiamine 2.0 mg, riboflavin 0.4 mg, pyridoxine 0.1 mg, calcium pantothenate 3.0 mg, biotin 0.01 mg and inositol 10 mg; one drop of cod liver oil for each animal daily.

The detection was performed by PMR* . The chromatogram of the above eluate showed two or three dark violet bands near Rf 0.3 or below it. According to our experience, except for 5-hydroxykynurenine or HK, all other substances likely to be encountered in urine gave colors of yellow, brown or reddish tones. Therefore, the chromatogram of the urine eluate was compared with that of HK, which was prepared from the analytical pure precursor and which gave an analytical pure picrate. When run with the above solvent system and sprayed with PMR, the eluate immediately showed three bands (A', B' and C') corresponding to the three spots, A, B and C obtained by paper chromatography of synthetic HK, respectively. All these spots and bands gave colors of dark purple or bluish purple tones as shown in Fig. I, a. Almost simultaneously or soon after that, band G appeared showing a bluish color, which turned within a short time to green. In about ten minutes another pink band (F) appeared and the highest band (B') was enclosed with an orange-brown color (Fig. I, b). The following day spot A and A' became sooty.

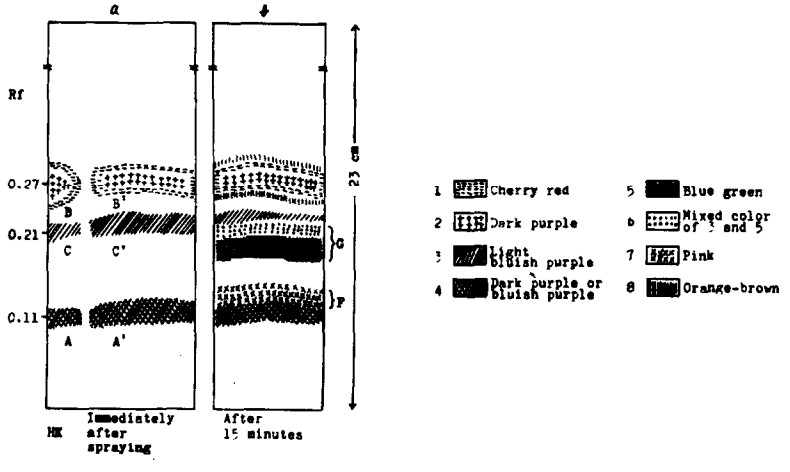

Fig. 1

* Pauli-Monda's reagent, prepared from 3 ml of 0.9% sulfanilic acid in 10% HCl, 3 ml of 5% $NaNO_2$ and 20 ml of N NaOH according to the ordinary percaution before use.

The purple tone of spot B and B' rapidly faded and a cherry-red color became distinguished in turn. Owing to these circumstances the chromatographic pattern of the urine eluate became obscure, but the residual dark purple color of HK was visible on holding up the chromatogram to the light. Spot A gave not only Pauli-Monda reaction but also an orange color with ER[x]. Therefore, A was thought to be HK in the original sense having a p-aminophenolic structure. B was confirmed to be a converted compound from A presumably by autoxidation and gave no more Ehrlich reaction: a newly prepared chromatogram of HK was placed for some time in a dark room and the sharply separated A-band was cut out, which was inserted in the starting line of a new filter paper. On running with the above solvent system, it showed besides A another strong band which was indistinguishable in its properties from B-band originating from the starting material (HK sample). C might be also a converted product from A; the HK sample prepared 6 months ago, when treated with Dowex 50 (H^+ form) showed with the above solvent system two main bands, one having the same RF as that of B and the other the same Rf as that of C. The spot corresponding to A became very scanty in amount.

As B or B' seemed to be rather stable, for further identification, the strongly fluorescent B'-band of the urine eluate chromatogram, obtained by running with the above solvent, was cut out and inlaid in the starting line of new filter papers simultaneously with spot B, and run with different solvent systems. The Rf's were determined by observing the white fluorescence under ultraviolet ray and a purple or pink color caused by spraying PMR. As shown in table I, the Rf's coincided in both cases, showing the identity of the two substances. Respect to the ultraviolet absorption

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[x] Ehrlich's reagent: 400 mg of dimethyl p-aminobenzaldehyde in 20 ml of HCl (one part of concentrated HCl diluted with 6 parts of water)

TABLE I

solvent system	ascending distance of solvent	Rf B	Rf B'
a	26.7	0.37	0.37
b	23.2	0.28	0.28
c	25.3	0.57	0.56
d	25.4	0.55	0.55
e	25.0	0.70	0.71
fluorescence		white	white

a, N-butanol, acetic acid and water (4:1:5); b, isobutanol, acetic acid and water (4:1:5); c, methanol, N-butanol, benzene and water (2:1:1:1); d, same as c with the exception of containing 1% HCl in it; e, 50% ethanol

spectrum, both B and B' had no characteristic peak though they showed broad absorptions. So far was the result with the urine of the serotonine-treated mice.

In the case of the urine eluate of non-treated mice, we have obtained chromatograms nearly similar to those of serotonine-treated mice urine, though the quantity of excreted HK-like substances seemed smaller than in the case of serotonine-treated. Therefore, whether HK and its converted products were derived from

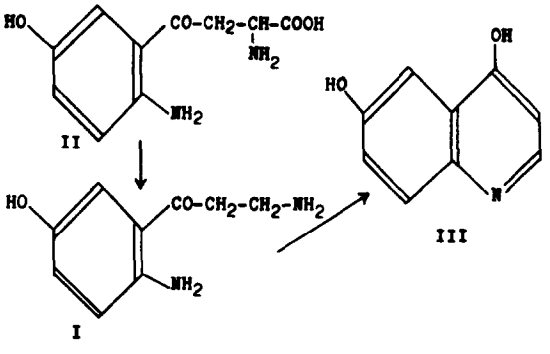

Fig. 2

serotonine or not, is still undecided. Our previous paper (Makino, K. and Takahashi, H., J. Am. Chem. Soc., 76, 6193(1954)) showed that 5-hydroxykynurenine (11) was easily converted to 4,6-dihydroxy-quinoline (111) by incubating with liver homogenate.

The investigation was conducted by using paper chromatography. The unknown spot in the previous paper which had been thought to be the intermediate of this reaction, was recently identified as HK.

On the other hand, such a minute quantity as 5 μg of HK injected intravenously into an urethane-anesthetized rabbit lowered the blood pressure in the common carotid artery, indicating that HK had a stronger action than dimethyl-kynuramine or N-dimethyl-5-hydroxykynuramine. The details of these latter subjects and the synthesis of HK will be reported by Dr. Hitoshi Takahashi and the author elsewhere. The author thanks Miss Atsuko Watanabe and Dr. Yoshinori Joh for their assistance.