THE DETECTION OF MAUSAMINE IN THE BRAIN OF MOUSE

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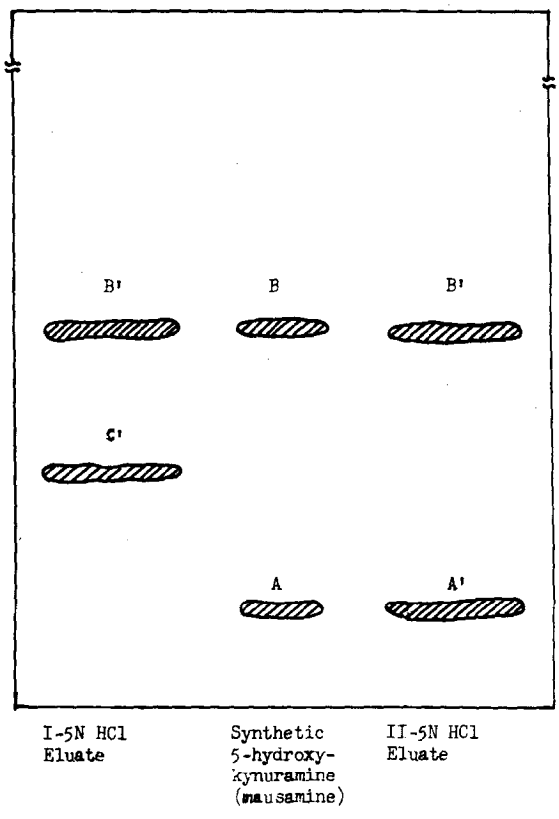
It was reported recently by one of the authors (K.M.) that mausamine (5-hydroxykynuramine) was excreted in the urine of mouse.[1] In this paper we report the detection of mausamine in mouse brain tissues: 130 g of fresh mice brain (300 normal mice of DD-strain) were homogenized under cooling with ice, and, after addition of 560 ml of 0.2N HCl and then 100 ml of 6N HCl, were deproteinized by heating in a boiling water bath for ten minutes, and centrifuged. The supernatant was adjusted by the addition of sodium hydroxide solution to an acidity corresponding to 0.18N HCl (confirmed by titration), and passed through a Dowex 50 (H^+ form)-column (2.2 x 26cm). The column was washed with 1.2 l of 0.2N HCl, then with 200 ml each of 2N and 3.5N HCl, and finally eluted three times with 200 ml each of 5N HCl. The three eluates were concentrated under reduced pressure in the atmosphere of nitrogen. After desalting by treating the resulting syrups with alcohol repeatedly, the residual thick syrups were chromatographed with ascending technique using Toyo-Roshi filter paper No. 53 and a solvent system-isobutanol, acetic acid and water (4:1:5) (Fig. 1). It was sprayed with Pauli-Monda's reagent.[1] At Rf 0.07 (A^t-band) and Rf 0.21 (C^t-band) dark purple to violet-colored bands, and at Rf 0.31 a cherry-red band (B^t-band) were seen.

Compared with the synthetic 5-hydroxykynuramine (HK), which was chromatographed simultaneously with the above eluate, the Rf of A^t and

Fig 1.



B' nearly coincided with those of A and B* of the synthetic 5-hydroxy-kynuramine giving the same color with Pauli-Monda's reagent respectively.

As there were few diazo-positive compounds in its neighborhood, B' was especially easily detectable on the paper chromatogram of mice brain eluate, and its identity with B was confirmed as follows: B' was cut out, inlaid in the starting line of a new wide filter paper and purified by rechromatography with another solvent system. The identity of the purified B'-band with the purified B from the synthetic sample was confirmed by paper chromatography (Table I) using four different solvent systems as shown in the preceding paper, and by comparing its

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* mausamine B: autoxidation product of A[1], whose structure is tentatively formulated in Fig. 4 as a β-indoxyl derivative, because it showed no coloration with Ehrlich's reagent.

Table I

Identification of band B' from mice brain with HK(band B)

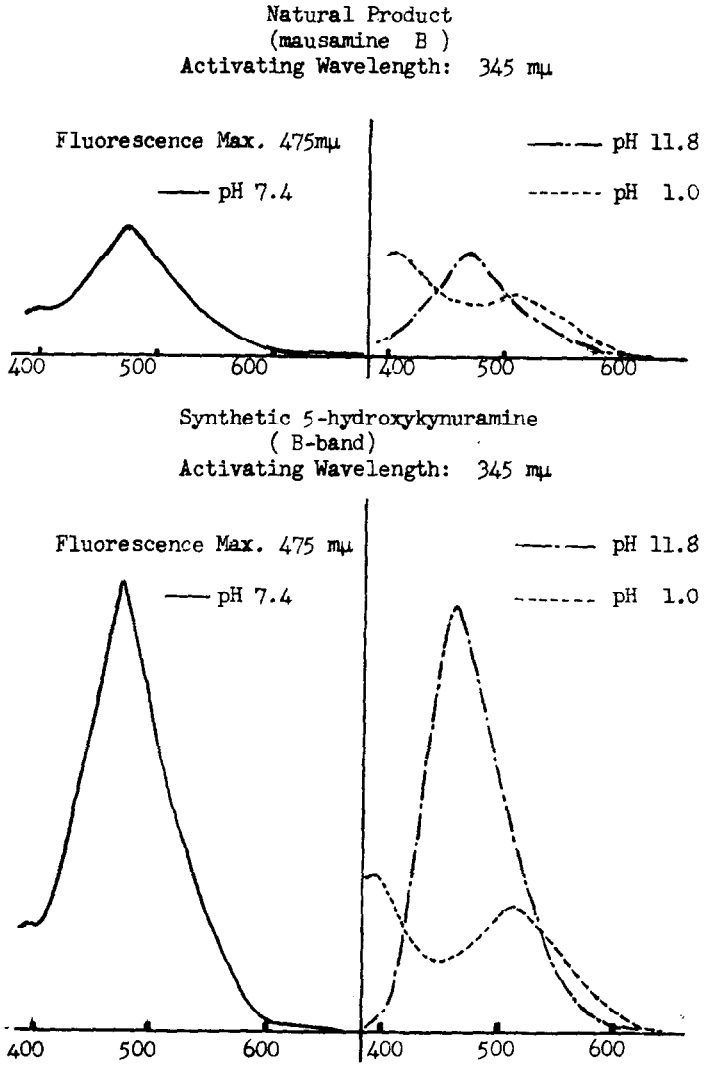
solvent systems	Rf band B	Rf band B'
a	0.28	0.29
b	0.13	0.13
c	0.23	0.23
d	0.59	0.60

a, isobutanol, acetic acid and water (4:1:5)
b, n-butanol saturated with water
c, isopropanol, concentrated ammonia and water (100: 8:8)
d, methanol, n-butanol, benzene and water (2:1:1:1)

fluorescence spectrum with that of B. The wave length of their maxima and the shift at three different pH were coincident with each other, as can be seen from Fig. 2 (at pH 7.4 fluorescence max. was 475 mμ, while at pH 1.0 it separated into two peaks and the strength of the fluorescence decreased strikingly). The precursor of mausamine is perhaps 5-hydroxykynurenine because the latter is easily converted to HK, 4,6-dihydroxyquinoline and 6-hydroxykynurenic acid in mice brain homogenate (Fig. 3 and 4). 4,6-Dihydroxyquinoline has been found by us in mice urine in a small amount, and 6-hydroxykynurenic acid detected by Roy and Price in pig urine. Serotonine could not be converted to HK in mice brain and liver homogenate. Aminco spectrophoto-fluorometer is suitable not only for the identification of HK (B-band) as shown above, but also presumably for its quantitively analysis.

5-Hydroxykynuramine showed a blood pressure effect: various amounts of the newly prepared sample of this compound dissolved in saline solution were injected intravenously into the urethan-anesthetized rabbits and the blood pressure change in the common carotid artery were recorded.

Fig. 2

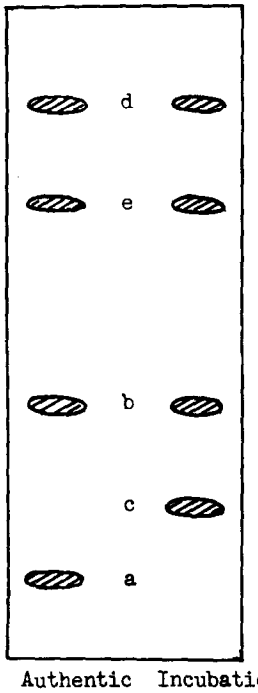


5 μg of this compound per kilogram of body weight showed 15 mm Hg-pressure drop, 10 μg of the amine per kilogram body weight 22 mm Hg-pressure drop, and 50 μg per kilogram body weight 50 mm Hg-pressure drop, respectively. The duration of this blood pressure lowering activity lasted about 4 to 5 minutes with the amount of 100 μg per kilogram body weight.

Fig. 3

Incubation of 5-hydroxykynurenine with mice brain

0.5g of mice brain was homogenized with 0.5mg of 5-hydroxykynurenine in 1.5ml of 0.05M phosphate buffer (pH 7.4), incubated at 37° C for one hour, and an aliquot was taken for paper chromatographic analysis, authentic samples being developed simultaneously.
Solvent system: isobutanol, acetic acid and water=4:1:5.
Ascending technique. Spots were detected with Mineralight.



d: 4,6-dihydroxyquinoline

e: 6-hydroxykynurenic acid

b: 5-hydroxykynuramine (B)

c: unchanged DL-5-hydroxykynurenine

a: 5-hydroxykynuramine (A)

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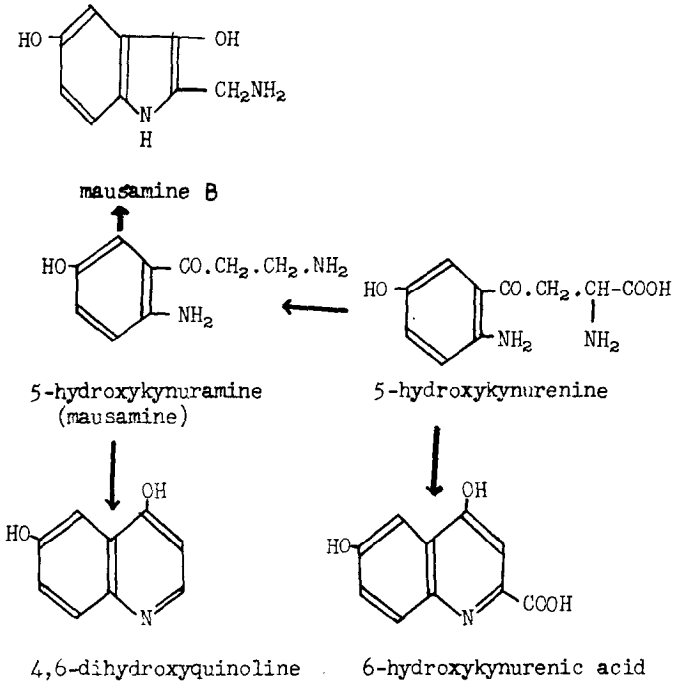
Fig. 4



References

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2. Roy, J. K. and Price, J. M., J. Biol. Chem., 234, 2759(1959)