

EXCRETION OF TETRAHYDROHARMANE AND HARMANE INTO THE URINE OF MAN AND RAT AFTER A LOAD WITH ETHANOL

Hans ROMMELSPACHER, Sabine STRAUSS and Jürgen LINDEMANN[†]

Institut für Neuropsychopharmakologie der Freien Universität Berlin, Ulmenallee 30, D-1000 Berlin 19 and [†]Institut für Pharmazie, Abteilung für Pharmazeutische Chemie, der Freien Universität Berlin, Königin-Luise-Str. 2 D-1000 Berlin 33, Germany

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1. Introduction

It is well known that ethanol is oxidized to acetaldehyde in the mammalian organism. Whether the symptoms of acute or chronic ethanol ingestion are caused exclusively by ethanol and acetaldehyde remains to be elucidated. Further pharmacodynamically active substances may be formed in the organism by other metabolic reactions, such as tetrahydroharmane (THH), also known as eleagnin, which is the condensation product of acetaldehyde and tryptamine. In principle the reaction occurs *in vivo*, since it was demonstrated in man and rat for tryptamine and 5-hydroxytryptamine with formaldehyde yielding tetrahydronorharmane and 6-hydroxytetrahydronorharmane, respectively.

Here, the existence of the β -carbolines THH and harmane in the urine of man and rat is demonstrated.

2. Materials and methods

Female Wistar rats (200–220 g) received 8 ml 0.9% NaCl orally in the evening and were kept individually in a metabolic cage. The collection vessels contained 4 ml 1 N HCl and 200 μ l of a solution of 268.2 mg semicarbazide in 10 ml distilled water. The urine was collected over 12 h. Thereafter, the urine specimen was transferred to an Erlenmeyer flask saturated with dry NaCl, and adjusted to pH 10.2. The solution was shaken with 3-fold vol. diethylether. The ether had been saturated with water containing semicarbazide. The procedure was repeated with the same amount of ether. The two organic phases were combined and evaporated. The dry residue was dis-

solved in methanol (Merck, Darmstadt, p.a. grade). The compounds of the solution were separated by gel filtration on Sephadex G-25 (fine grade, Pharmacia, Freiburg). The elution medium consisted of 11.4 mmol/l triethylamine (Merck) and 8 mmol/l acetic acid (Merck) in distilled water containing 10% methanol (v:v). The fractions were collected by a fraction collector (Ultrorac, LKB, Lochham).

The retention times of THH and harmane had been measured by adding authentic substances (purchased from Ferak, Berlin and Sigma Chemie, München) to rat urine. In the experiments performed to investigate the physiological occurrence of the β -carbolines in the urine, the fractions with retention times similar to those of authentic THH and harmane, respectively, were collected and then lyophilized. The dry residue was dissolved in methanol (Uvasol-grade, Merck, Darmstadt) further separated by HPLC (Hewlett Packard, model 1084B) and analyzed by mass spectrometry.

In a second experiment 8 rats were loaded with 100 mg/kg tryptamine (Sigma Chemie) injected *i.p.* and 2 g/kg ethanol in 4 ml 0.9% NaCl/os 90 min later. The urine samples were collected, separated, and combined before the analysis by mass-spectrometry as above.

In a third experiment 5 mg authentic THH (i.e., $>10^3$ -times the amount detectable by mass-spectrometry, 1–3 μ g) were added to the collection vial of the metabolic cage at the beginning of the collection period. The urine sample was treated as above and analyzed by mass-spectrometry.

Human urine was investigated in the following way: Two male and two female volunteers which were not on diet, ingested either no ethanol or 30 g, or 100 g

ethanol mixed with orange juice in the evening hours. The urine was collected over 12 h into a glass cylinder containing 5 ml 5 N HCl and 50 mg semicarbazide-HCl. The specimen (~750 ml) was lyophilized. The residue was dissolved in 100 ml distilled water. The solution was saturated with dry NaCl and adjusted to pH 10.2. Then, twice the amount of diethylether containing semicarbazide was added to the beaker. After shaking and centrifuging the organic phase was removed. The procedure was repeated once. The organic phases were combined and separated as described above for the rat urine. The sample was analyzed by mass-spectrometry.

3. Results

The urine of rats treated with saline was first analyzed by mass-spectrometry. No characteristic fragments of tetrahydronorharmine (THH) and harmine were found under the conditions of mass-spectrometry. Therefore, the experiments were repeated with rats loaded with 100 mg/kg tryptamine and 2 g ethanol. Under these conditions both, THH and harmine, could be detected. The spectra were similar as those depicted in fig.2,3 for human urine.

To exclude that the harmine detected in the urine sample is formed *in vitro* during the collection period, a urine specimen was assayed as usual but containing 5 mg THH. No harmine could be detected by mass-spectrometry.

Human urine was analyzed by ultraviolet spectrometry as well as mass-spectrometry. Compounds of the urine sample of a subject loaded with 100 g ethanol were separated by gel-filtration and HPLC. The ultraviolet spectra of the authentic and extracted substances are in good agreement (fig.1).

In the upper part of fig.2, a mass-spectrum is depicted of authentic THH, in the lower part that of the extracted compound. The spectra show the characteristic peaks which can be expected from the fragmentation of the substance under the conditions of mass-spectrometry [1-3]. The peak of the molecule ion appears at m/z 186 with a medium relative intensity. Furthermore, a smaller peak (M-1) was found and the base-peak (M-15). Presumably both fragment ions are formed by elimination of a hydrogen- and methyl-radical from the 1-position in the course of an onium fission. The further fragmentation of the (M-15)-ion m/z 171 under the elimination of HCN is proven by a meta-stable peak.

Caused by the substituent in position 1 the retro-Diels-Alder fragmentation is less pronounced compared with the fragmentation of THN (see [11], fig.1,2). Instead of the base peak a signal at m/z 157 can be detected of medium intensity followed by the loss of HCN and/or H^+ . For m/z 186→157→130→103 and m/z 156→129 the respective peaks are found in the mass-spectrum.

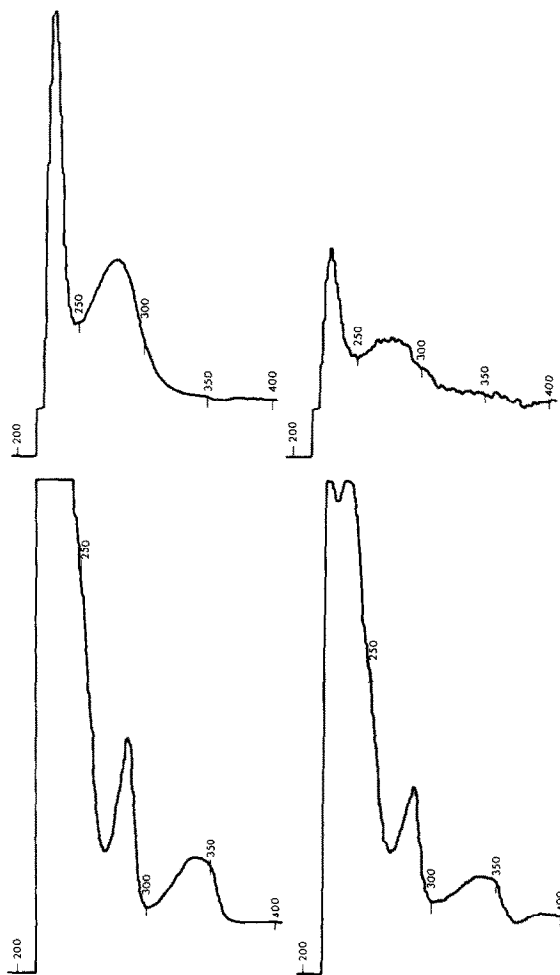


Fig.1. Ultraviolet spectrum of authentic THH (upper left) and the ultraviolet spectrum of a compound extracted from human urine separated by gel-filtration and HPLC (RP-8.7 μ m; elution conditions: isocratic, 30% acetonitrile, 70% acetic acid (8 mmol/l) and triethylamine (11 mmol/l, pH 8) upper right. The spectrum was recorded by the variable wavelength detector of the HPLC apparatus. Ultraviolet spectrum of authentic harmine (lower left) and the ultraviolet spectrum of a second compound extracted from human urine separated and recorded as above (lower right).

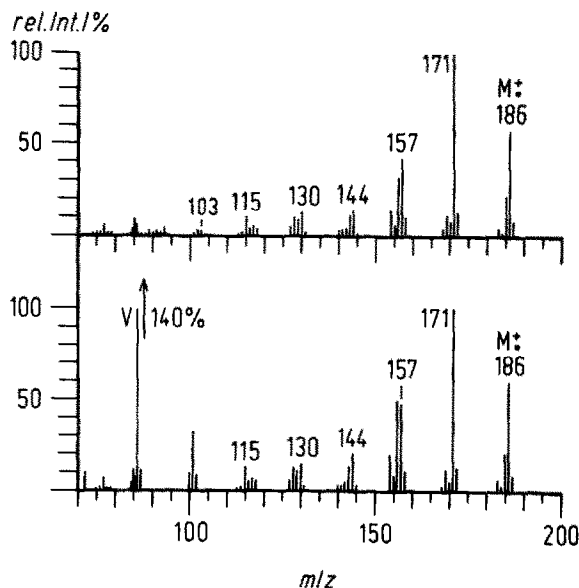
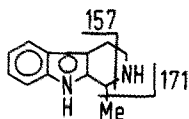


Fig.2. Mass-spectrum of authentic THH (upper part) and the spectrum of the compound extracted from human urine (lower part). 'V': contamination from the eluent. Conditions: Varian, MAT CH 7A, mass-spectrograph; 70 eV, 100°C.

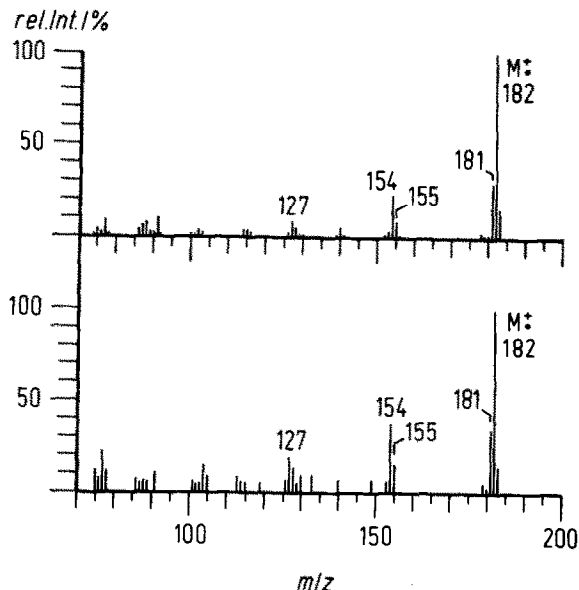
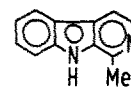


Fig.3. Mass-spectrum of authentic harmaline (upper part) and the spectrum of the compound extracted from human urine (lower part).

The mass-spectra of authentic harmaline (upper part) and the compound extracted from the urine (lower part) are shown in fig.3.

In contrast to THH the chemical structure of harmaline exhibits the maximal number of double bonds. Thus, the onium fission or the retro-Diels-Alder processes cannot further dominate the fragmentation of the substance. Besides the molecule ion peak (m/z 182) a relative pronounced peak at 181 can be demonstrated as a result of a tropylium-fission. The ions decay by elimination of H^+ or Me^+ or HCN and $MeCN$. In addition meta stable peaks are registered for m/z 182→155→128. Thus, the spectra of authentic harmaline and the extracted compound are in good agreement.

However, in samples from individuals who had ingested no ethanol or 30 g ethanol none of the carbolines was found. Thus, only a load with a high amount of ethanol leads to an excretion of condensation products in a concentration range large enough to be detected by mass-spectrometry ($\sim 3 \mu g$).

4. Discussion

Acetaldehyde constitutes a physiologically occurring substance in the mammalian organism. After ethanol ingestion its concentration increases [4–7]. Furthermore, tryptamine is found in the mammalian organism, too [8]. Therefore, it was of interest whether acetaldehyde and indoles react *in vivo* by a Mannich reaction yielding β -carbolines. Such a reaction was described to occur between tryptamine and formaldehyde in man and rat [9–12] and between 5-hydroxytryptamine and formaldehyde [13,14]. Here evidence is presented that the condensation product between tryptamine and acetaldehyde is excreted into the urine of man and rat after ethanol ingestion.

The role of *in vivo* β -carbolines and other condensation products in alcoholism remains to be elucidated. Condensation between acetaldehyde and the catecholamines adrenaline and noradrenaline *in vitro* was first described in [15]. After ingestion, this quinoline elicited excitation, drowsiness, hallucinations, seizures, and changes of the blood pressure

[16,17]. There is not yet a pharmacological study of THH.

However, there are several reports dealing with harmine and with congeners of the two identified β -carbolines. Harmine was tentatively found together with still other unidentified β -carbolines in the nucleus arcuatus of rat brain [23]. Tetrahydronorharmine (THN), 6-OH-THN, 6-MeO-THN, and carbonyl-methyl-THN exert serotonergic activities and antagonize the effects of dopamine-receptor stimulants [14,18–21]. In combination with tetrahydropapaveroline THN increases the voluntary ethanol ingestion in rats [22]. Harmaline and harmine elicit excitation and euphoria in humans [24–26]. The effects of harmaline in 30 volunteers were reported [27]. He observed autism and colorful hallucinations. In conclusion, despite the fact that only little is known about the significance of the two identified β -carbolines, the available pharmacological data support the view that the compounds may participate in the effects of ethanol.

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