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Formaldehyde-derived tetrahydroisoquinolines and tetrahydro-β-carbolines in human urine

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Abstract

Human urine samples were examined for the occurrence of formaldehyde-derived tetrahydroisoquinolines and tetrahydro-β-carbolines generated by condensation of the methanol oxidation product with biogenic amines. Positive results were obtained for the tryptamine condensation product 1,2,3,4-tetrahydro-β-carboline and the serotonine condensation product 6-hydroxy-1,2,3,4-tetrahydro-β-carboline as well as for the condensation products with tyramine, dopamine, adrenaline and noradrenaline 1,2,3,4-tetrahydroisoquinoline, 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, N-methyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline, 4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline, and the metabolite 6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline. Negative results were obtained for N-methyl-1,2,3,4-tetrahydroisoquinoline and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, N-methyl-1,2,3,4-tetrahydro-β-carboline, 6-methyl-1,2,3,4-tetrahydro-β-carboline, and 6-methoxy-1,2,3,4-tetrahydro-β-carboline in samples of chronic alcoholics as well as in the urine of healthy volunteers. No correlation between alcohol ingestion or state of alcoholization could be demonstrated.

Keywords: Tetrahydroisoquinolines; Tetrahydro-β-carbolines

1. Introduction

During the last two decades investigations on the molecular basis of alcoholism and its aetiology per se were concentrated on the generation and signification of endogenous neurochemical factors. Condensation products between acetaldehyde, the oxidation product of ethanol, and biogenic amines of the nervous system received increasing interest. Alkaloids produced via the Pictet–Spengler [1] reaction of phenylethylamines or indole ethylamines with aldehydes or α-keto acids (Fig. 1) – tetrahydroisoquinolines (TIQ) and tetrahydro-β-carbolines (THBC) as shown in Fig. 2 – were seen as connecting links between alcoholism and opioid mania [2,3]. The formation occurs readily under physiological conditions [4–6] and results in substances that can function as neurotransmitters or neuromodulators. Their pharmacological, neuroanatomical and physiological attributes in specific regions of the brain were investigated. Endogenous formation of condensation products and a connection to chronic alcoholism were discussed controversially [7–17].

Investigations during the last two decades demonstrated that TIQ and THBC compounds occurred not only in plants but also in liquids and organs of

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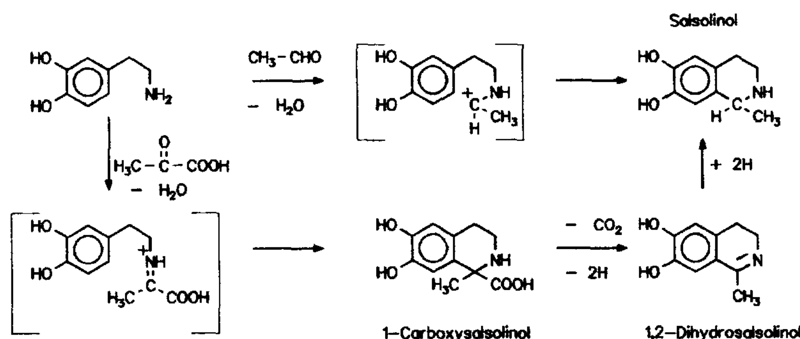

Fig. 1. Biosynthesis of salsolinol from dopamine in mammals. Pictet–Spengler reaction of phenylethylamines with aldehydes to form tetrahydroisoquinolines by mechanism of the Mannich reaction: primarily a carbinole amine is formed, which substitutes the benzene ring at position o as mesomeric carbenium immonium ion after proton-catalysed OH separation. Besides condensation with acetaldehyde, a condensation with pyruvate is described to form 1-carboxysalsolinol. From this intermediate salsolinol is generated by oxidative decarboxylation to 1,2-dihydosalsolinol followed by hydration. Pictet–Spengler reaction between indole ethylamines and aldehydes formed tricyclic indole derivatives, tetrahydro-β-carbolines.

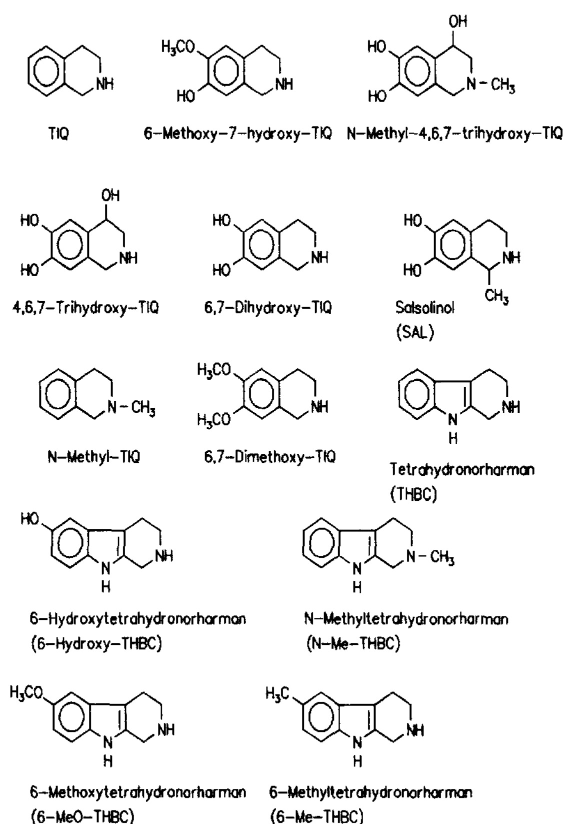

Fig. 2. Structures of investigated tetrahydroisoquinolines and tetrahydro-β-carbolines.

humans and animals (Table 1 and Table 2). First a demonstration in vivo took place under experimental conditions, when concentration of one of the reaction partners was increased by gearing in the biological system. Subsequently such alkaloids could be demonstrated as endogenous, physiological substances. In particular a connection with preceding alcohol consumption was discussed and interest focused on the hypothesis that endogenously formed TIQ and THBC compounds could contribute to psychotic effects in alcoholism or Parkinson's disease [18–24]. Acute and chronic administration of selected TIQ and THBC compounds to rats have been reported to alter alcohol consumption significantly [25–30]. Normally the ethanol oxidation product acetaldehyde has been regarded as the reaction partner to form TIQ and THBC compounds. Recent investigations proved that alcoholic beverages contain not only ethanol but also congener alcohols, especially methanol [31]. This alcohol is used as an alcoholism marker and is discussed as a decisive factor in the research on the aetiology of chronic alcoholism. It is regarded as an exogenous formaldehyde source [32–34]. This oxidation product is a more potent reaction partner for TIQ and THBC formation than acetaldehyde [35]. There are some known metabolic pathways of formaldehyde generation, which may account for in vivo TIQ and THBC formation in tissues [36–39], but up to now nothing is known

Table 1
Tetrahydroisoquinolines identified in mammals

Substance	Occurrence	Ref.
Tetrahydropapaveroline		
Experimental:	Rat (brain and liver; incubation with dopamine by AlDH, inhibition with acetaldehyde)	[40]
	Guinea-pig (liver mitochondria; incubation with dopamine without NAD^+)	[41,42]
	Rat (brain; animals treated with L-dopa+dopa decarboxylase, inhibitor (2–15 μg THP/g) or L-dopa+ethanol (10–25 μg THP/g)	[43]
	Rat (brain; animals treated with L-dopa (0.42 pmol THP/g) or L-dopa+ethanol (4.02–482 pmol THP/g)	[44]
4-Hydroxysalsolinol		
Experimental:	Cow (adrenal medulla; perfusion with acetaldehyde)	[45,46]
2-Methyl-4-hydroxysalsolinol		
Experimental:	Cow (adrenal gland; perfusion with acetaldehyde)	[45,46]
Salsolinol (SAL)		
Experimental:	Human (urine; excretion by parkinsonian patients after L-dopa therapy; basal excretion about 400–1000 μg SAL/d increased by ethanol treatment to 1200–3800 μg SAL/d))	[47]
	Rat (brain; simultaneous giving of ethanol+aldehyde dehydrogenase inhibitor pyrogallol)	[48,49]
	Rat (brain; no demonstration after acute administration of ethanol only, but after chronical treatment)	[50–54]
	Human (cerebrospinal fluid (CSF); parkinsonian patients after implantation of dopamine producing cells; after operation drastic increase of SAL concentration; forced after treatment with L-dopa	[55,56]
Physiological:	Human (urine, plasma, cerebrospinal fluid (CSF), brain; SAL and SALN in different investigations of physiological substances, also in untreated collectives)	[57–61] and others
After alcohol ingestion:	Human (urine; SAL and SALN as excretion products of alcoholics (28.8 μg SAL/d; 111.4 μg SALN/d) significant increase versus healthy volunteers (1.1 μg SAL/d; 20.6 μg SALN/d))	[62]
	Human (urine, CSF; in alcoholics significantly higher concentrations of SAL and SALN versus healthy volunteers; single ethanol ingestion of 80 g resulted in no effects in healthy volunteers)	[59,63]
	Human (brain; alcoholics with higher concentrations of SAL (0.18–0.30 nmol SAL/g) and SALN than in a healthy collective (0.007–0.05 nmol SAL/g))	[60,61]
	Human (urine, only minimal differences in excretion rates of alcoholics versus controls)	[64,65]
	Human (urine; 26.2 pmol SAL/mg creatinine in moderate drinkers versus 6.6 pmol SAL/mg in non-drinkers)	[66]
	Human (urine; connection between acetaldehyde concentration in blood after alcohol ingestion and SAL excretion, demonstrated by orientals with AlDH I-deficiency)	[67]

Table 1
Tetrahydroisoquinolines identified in mammals

Substance	Occurrence	Ref.
	Human (urine; great interindividual differences in SAL excretion of alcoholics)	[68]
	Human (plasma; SAL concentration of alcoholics increased (50–1331 pg SAL/ml) versus healthy volunteers (0–232 pg SAL/ml); correlation with parallel measured dopamine concentrations)	[57]
	Human (alcoholics with lower SAL excretion (10.2–17.2 μg/d) than healthy volunteers (approximately 40 μg/d); also children with SAL excretion (approximately 10 μg/d))	[58]
1-Carboxysalsolinol (1-CA-SAL)		
Experimental:	Rat (plasma, brain; after chronic ingestion of ethanol)	[69]
After alcohol ingestion:	Human (brain, urine; alcoholics with ethanol in their blood at autopsy)	[6,70]
6,7-Dihydroxy-TIQ		
Physiological:	Rats (brain)	[71]
After alcohol ingestion:	Human (urine; demonstrated by investigations of catechol fractions, but interpreted as an artefact)	[9]
Tetrahydroisoquinoline		
Physiological:	Rat (brain)	[72]
	Human (brain of parkinsonian patients)	[73–75]
N-Methyl-TIQ		
Physiological:	Rat (brain)	[72]
	Human (brain of parkinsonian patients)	[73–75]
2-Methyl-6,7-dihydroxy-TIQ		
Physiological:	Human (brain of parkinsonian patients and healthy people)	[76]
N-Methylsalsolinol		
Physiological:	Human (brain of parkinsonian patients and healthy people)	[76]

about how formation in humans depends on the oxidation product of ingested methanol.

In our opinion chronic consumption of alcoholic beverages coupled with methanol accumulation may cause an induction of TIQ and THBC forming mechanisms. Such formaldehyde derived compounds could be involved in the aetiology of alcoholism.

We recently reported on the qualitative gas chromatographic–mass spectrometric (GC–MS) screening procedures for the measurement of formaldehyde-derived TIQ and THBC compounds [99,100]. Now we investigated the occurrence of the following compounds in human urine: 1,2,3,4-tetrahydroisoquinoline (TIQ), N-methyl-1,2,3,4-tetrahydroisoquinoline (N-Me-TIQ), 6-methoxy-7-hydroxy-1,2,3,4-tetrahydoisoquinoline (6-MeO-7-OH-TIQ), 4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline (4,6,-7-triOH-TIQ), N-methyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline (N-Me-4,6,7-triOH-TIQ), 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (6,7-diOH-TIQ), salsolinol, and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (6,7-diMeOH-TIQ), 1,2,3,4-tetrahydro-β-carboline (THBC), N-methyl-1,2,3,4-tetrahydro-β-carboline (N-Me-THBC), 6-methoxy-1,2,3,4-tetrahydo-β-carboline (6-MeO-THBC), 6-methyl-1,2,3,4-tetrahydro-β-carboline (6-Me-THBC), and 6-hydroxy-1,2,3,4-tetrahydro-β-carboline (6-OH-THBC).

2. Experimental

2.1. Materials

Methanol, ethyl acetate (Uvasol, Merck, Darmstadt, Germany), acetonitrile (gradient grade,

Table 2
β-Carbolines identified in mammals

Substance	Occurrence	Ref.
6-Methoxytetrahydroharmane (1-methyl-6-methoxy-THBC)		
Experimental:	Rat (urine; demonstration after treatment of animals with 5-methoxytryptamine (MAO-inhibitor), Disulfiram (AlDH-inhibitor) and ethanol	[77]
6-Hydroxytetrahydroharmane (1-methyl-6-hydroxy-THBC)		
Experimental:	Rat (homogenated brain; incubation with 5-hydroxytryptamine (5-HT, serotonin) and acetaldehyde	[78]
Physiological:	Human, cat (urine)	[79]
Tetrahydronorharmane (THBC)		
Physiological:	Human (urine, platelets, plasma)	[80–84]
	Rat (brain)	[85–87]
2-Methyltetrahydronorharmane (N-Me-THBC)		
Physiological:	Rat (brain)	[86]
6-Methoxytetrahydronorharmane (6-MeO-THBC)		
Physiological:	Rat (brain, adrenal gland)	[86,87]
6-Hydroxytetrahydronorharmane (6-hydroxy-THBC)		
Experimental:	Human (urine); rat (brain, platelets); after ingestion of 5-hydroxytryptophan	[88]
Tetrahydroharmane (1-methyl-THBC)		
Physiological:	Human (platelets); rat (brain)	[84,89]
After alcohol ingestion:	Human (urine, platelets, plasma); rat (urine) after alcohol addiction	[90,91]
Harmane (1-methyl-β-carboline)		
Physiological:	Human (platelets)	[92–94]
After alcohol ingestion:	Human, rat (urine; excretion product after addition of ethanol); rat (urine, brain; after chronic addiction)	[91,95]
	Human (urine, plasma; increased concentrations for alcoholics (41.6 pg/ml) in comparison to healthy volunteers (20.8 pg/ml))	[16,96,97]
Norharmane (β-carboline)		
After alcohol ingestion:	Human (plasma; increased concentrations for alcoholics (99.5 pg/ml) in comparison with healthy volunteers (26.9 pg/ml))	[16,97]
1-Carboxytetrahydronorharmane		
Experimental:	Rat (brain; i.v. application of tryptamine and pyruvate)	[98]

Merck), 1-chlorobutane (for chromatography, Merck), water (HPLC grade, Baker, Gross-Gerau, Germany), THBC, TIQ hydrochloride, 5-hydroxytryptamine creatinine sulphate, β-glucuronidase type H-1 (all from Sigma, Deisenhofen, Germany), methyl chloroformate, 5-methoxytryptamine hydrochloride, 5-methyltryptamine hydrochloride, N-methyltryptamine, 3,4-dimethoxyphenylethylamine, N-methylphenylethylamine and 3-O-methyldopamine hydrochloride (all from Aldrich,

Steinheim, Germany) were obtained from the respective suppliers.

Samples of 4,6,7-triOH-TIQ and N-Me-4,6,7-triOH-TIQ were purchased from Dr. Oebels (Bayer AG, Dormagen, Germany).

The inorganic chemicals used were boric acid, sodium tetraborate, potassium dihydrogenephosphate, dipotassium hydrogenephosphate, potassium hydroxide, orthophosphoric acid, sodium carbonate, sodium hydrogenecarbonate, sodium hydroxide, hydrobromic acid, hydrochloric acid, acetic acid, paraformaldehyde, glyoxylic acid and semicarbazide (all p.a., Merck).

Chem-Elut extraction columns (20-ml volume, diatomaceous earth as sorbent) were purchased from Analytichem International (ICT-Handelsgeselschaft, Frankfurt, Germany). Worldwide Monitoring Clean Up C_{18} end-capped extraction columns (100 mg, 1 ml) were purchased from Amchro (Sulzbach/Taunus, Germany).

Borate buffer (pH 9.0) consisted of 835 ml of solution A (12.37 g of boric acid+100 ml of 1 *M* sodium hydroxide with 0.05 *M* sodium tetraborate made up to 1 l) and 165 ml solution B (0.1 *M* hydrochloric acid).

2.2. Organic syntheses

Samples of TIQ compounds were prepared according to the method of Buck [101] with a modification according to the procedure of Barker et al. [71].

Characterization of prepared compounds was performed by elemental analysis, melting-point determination, mass spectrometry and ^{1}H NMR spectroscopy (^{1}H NMR (80 MHz) Bruker WP 80; all measurements in DMSO-d_6/TMS).

2.3. 6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride

A 2-g ($1.1 \cdot 10^{-3}$ mol) amount of 3,4-dimethoxyphenylethylamine was dissolved by stirring in 10 ml of distilled water containing 0.4 g ($1.3 \cdot 10^{-3}$ mol) of paraformaldehyde. After addition of 1 ml of concentrated hydrochloric acid the solution was refluxed for 3 h. After cooling to room temperature the white crystalline precipitate was collected on a filter and washed with 50 ml of acetone and the residue was dried in a vacuum desiccator over phosphorus pentoxide.

Yield: 1.71 g ($7.45 \cdot 10^{-3}$ mol), 68%; m.p. 250–254°C. Calc. for $C_{11}H_{15}NO_2$–HCl: C, 57.6; H, 7.0; N, 6.1. Anal.: C, 56.5; H, 7.1; N, 6.0. ^{1}H NMR: δ=3.0 ["t", J=6 Hz, 2 H, H−4], 3.4 ["t", J=6 Hz, 2 H, H−3], 3.75 [s, 6 H, OCH_3] 4.2 [bs, 2 H, H−1], 6.7−6.95 [2s, 2 H, H−5, H−8], 9.9 [bs, 1 H, NH]. EI–MS: (m/z) [ion assignment] (rel. int.): 193 $[M]^+$ (53), 192 $[M-H]^+$ (55), 164 $[M-29\ (-C_2H_5)]^+$ (100), 149 $[164-(-CH_3)]$ (21).

2.4. 6-Methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride

A 1-g ($4.9 \cdot 10^{-3}$ mol) amount of 3-O-methyldopamine hydrochloride was dissolved in 5 ml of distilled water containing 0.2 g ($1.3 \cdot 10^{-2}$ mol) of paraformaldehyde. After addition of 0.5 ml of concentrated hydrochloric acid the mixture was refluxed for 3 h. After cooling to room temperature the white crystalline sediment was filtered under vacuum and washed with 50 ml of acetone and the residue was dried in a vacuum desiccator over phosphorus pentoxide.

Yield: 0.52 g, 49%; m.p. 254–258°C. Calc. for $C_{10}H_{13}NO_2$–HCl: C, 55.7; H, 6.5; N, 6.5. Anal.: C, 54.7; H, 6.5; N, 6.2. ^{1}H NMR: δ=3.75 [s, 3 H, OCH_3] 6.8 [s, 1 H, H−5], 6.95 [s, 1 H, H−8], 9.7 [bs, 1 H, NH]. EI–MS: (m/z) [ion assignment] (rel. int.): 179 $[M]^+$ (50), 178 $[M-H]^+$ (47), 150 $[M-29\ (-C_2H_5)]^+$ (100), 135 $[150-15\ (-CH_3)]$ (37).

2.5. 6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrobromide

A 1-g ($4.4 \cdot 10^{-3}$ mol) amount of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride was dissolved in 20 ml of bromic acid (48%) and refluxed for 2 h. Then the mixture was evaporated under vacuum to a volume of 10 ml and incubated over night at 5°C. White needles as a sediment were filtered under vacuum and washed with 20 ml of acetone and dried in a vacuum desiccator over phosphorus pentoxide.

Yield: 0.93 g, 86%; m.p. 265–268°C. Calc. for

$C_9H_{11}NO_2$–HBr: C, 43.9; H, 4.9; N, 5.7. Anal.: C, 43.7; H, 5.0; N, 5.6. ^{1}H NMR: δ=2.9 [t, J=6 Hz, 2 H, H−4], 3.35 [t, J=6 Hz, 2 H, H−3], 4.2 [bs, 2 H, H−1], 6.75 [1s, 2 H, H−5, H−8], 9.1 ["bs", >2 H, NH, OH]. EI–MS: (m/z) [ion assignment] (rel. int.): 165 $[M]^+$ (39), 164 $[M-H]^+$ (66), 136 $[M-29\ (-C_2H_5)]^+$ (100), 104 $[(C_8H_8)]$ (32).

Samples of THBC compounds were prepared according to the method of Ho and Walker [102]. This method is generally applicable to the preparation of 1-unsubstituted tetrahydro-β-carbolines providing the 1-carboxylic acid precursor is soluble in the hot acid used to effect decarboxylation. A slight modification of this procedure yielded N-Me-THBC according to Elliott [103].

2.6. 6-Hydroxy-1,2,3,4-tetrahydro-β-carboline hydrochloride

A 1.5-g ($3.7\cdot10^{-3}$ mol) amount of 5-hydroxytryptamine creatinine sulphate was dissolved by stirring in 30 ml of distilled water at 45°C (pH 4). Cooling to room temperature and addition of 750 mg of glyoxylic acid dissolved in 1.5 ml distilled water was followed by slow addition (about 3 min) of 1.5 ml of a cooled solution of potassium hydroxide (20%). After stirring at ambient temperature for 1 h the white solid was collected on a filter and washed thoroughly with 10 ml distilled water. The residue was resuspended in 15 ml of distilled water and a 1.5-ml volume of concentrated hydrochloric acid was slowly added and the mixture was boiled for 30 min before an additional volume of 1.5 ml concentrated hydrochloric acid was added. Heating was continued for another 15 min and the resulting solution was allowed to cool to room temperature. The precipitated hydrochloride salt was collected on a filter and washed with 10 ml of distilled water and dried in a vacuum desiccator over phosphorus pentoxide.

Yield: 0.72 g, 86%; m.p. 275–278°C. Calc. for $C_{11}H_{12}N_2O$–HCl: C, 58.8; H, 5.8; N, 12.5. Anal.: C, 58.5; H, 5.7; N, 12.1. ^{1}H NMR: δ=2.9 ["t", J=6 Hz, 2 H, H−4], 3.45 ["t", J=6 Hz, 2 H, H−3], 4.4 ["s", 2 H, H−1], 6.7−6.95 [m, 2 H, H−5, H−7], 7.3 [d, J=9 Hz, 1 H, H−8], 8.9 [s, 1 H, NH], 9.9 [s, 1 H, OH], 10.9 [s, 1 H, NH]. EI–MS: (m/z) (rel. int.): 188 $[M]^+$ (37), 159 $[M-29\ (-C_2H_5)]^+$ (100).

2.7. 6-Methoxy-1,2,3,4-tetrahydro-β-carboline hydrochloride

A 1-g ($4.4\cdot10^{-3}$ mol) amount of 5-methoxytryptamine hydrochloride was dissolved by stirring in 20 ml of distilled water at 45°C (pH 4). Further preparation was carried out following the procedure described above.

Yield: 0.84 g, 82%; m.p. 262–266°C. Calc. for $C_{12}H_{14}N_2O$–HCl: C, 60.4; H, 6.3; N, 11.7. Anal.: C, 63.8; H, 6.4; N, 11.9. ^{1}H NMR: δ=2.95 [t, J=6 Hz, 2 H, H−4], 3.45 [t, J=6 Hz, 2 H, H−3], 3.9 [s, 3 H, OCH_3], 4.4 [bs, 2 H, H−1], 6.8−7.5 [m, 4 H, C_6H_4], 11.35 [s, 1 H, NH]. EI–MS: (m/z) [ion assignment] (rel. int.): 202 $[M]^+$ (34), 199 $[M-3\ H]^+$ (13), 173 $[M-29\ (-C_2H_5)]^+$ (100), 158 $[173-15\ (-CH_3)]^+$ (71).

2.8. 6-Methyl-1,2,3,4-tetrahydro-β-carboline hydrochloride

A 1-g (4.7×10^{-3} mol) amount of 5-methyltryptamine hydrochloride was dissolved by stirring in 20 ml of distilled water at 45°C (pH 4). Further preparation was carried out following the procedure described above.

Yield: 0.81 g, 76%; m.p. 257–261°C. Calc. for $C_{12}H_{14}N_2$–HCl: C, 64.7; H, 6.8; N, 12.6. Anal.: C, 60.9; H, 6.4; N, 11.8. ^{1}H NMR: δ=2.35 [s, 3 H, CH_3], 2.95 [t, J=6 Hz, 2 H, H−4], 3.4 [s, 1 H, NH], 3.45 [t, J=6 Hz, 2 H, H−3], 4.4 [bs, 2 H, H−1], 6.9−7.45 [m, 4 H, C_6H_4], 11.1 [s, 1 H, NH]. EI–MS: (m/z) [ion assignment] (rel. int.): 186 $[M]^+$ (32), 183 $[M-3\ H]^+$ (32), 157 $[M-29\ (-C_2H_5)]^+$ (100), 142 $[157-15\ (-CH_3)]^+$ (1).

2.9. N-Methyl-1,2,3,4-tetrahydro-β-carboline

A 0.5-g (2.9×10^{-3} mol) amount of N-methyltryptamine hydrochloride was dissolved in 4 ml of distilled water by slow addition of concentrated hydrochloric acid and by cooling on an ice bath. After addition of 250 mg of glyoxylic acid dissolved in 0.5 ml of distilled water the solution was set at pH 1 with concentrated hydrochloric acid and boiled for 1 h. After cooling on ice the slow addition of a solution of sodium hydroxide (10 M) followed until precipitation of a white-brown solid

occurred. This was collected on a filter and washed with acetone and dried in a vacuum desiccator over phosphorus pentoxide.

Yield: 0.32 g, 59%; m.p. 235–240°C. Calc. for $C_{12}H_{14}N_2$: C, 64.7; H, 6.3; N, 12.6. Anal.: C, 63.4; H, 6.6; N, 11.5. ^{1}H NMR: δ=2.4 [s, 3 H, CH_3], 2.8 s (integration ca. 4 H, (H−3, H−4)) 3.65 [bs, 2 H, H−1], 6.9−7.55 [m, 4 H, C_6H_4]. EI–MS: (*m/z*) [ion assignment] (rel. int.): 186 $[M]^+$ (16), 143 [M−43 (−C_2H_5N)] (100).

2.10. Instrumentation

A Model 5890A gas chromatograph with a 5970A mass selective detector (MSD) (Hewlett-Packard) and a OV1 fused-silica capillary column (12 m×0.2 mm I.D.; film thickness 0.33 μm) were used.

2.11. Sample preparation and data analysis

The sample preparation and data analysis procedure for the identification of TIQ compounds was outlined previously [100]: The sample consisted of 10 ml of urine with 0.1 ml of a solution of semicarbazide (1 *M*) adjusted to pH 4.5 with acetic acid, and to which 2500 U β-glucuronidase were added. For enzymatic hydrolysis the sample was incubated for 2 h at 55°C. Then the mixture was saturated with sodium hydrogencarbonate to adjust the pH to 9.0. Finally 20 ml of 5% propionic anhydride in ethyl acetate were added. The sample was shaken for 30 min and centrifuged (5 min, 1000 *g*), and the organic layer was transferred to a glass tube. The procedure was repeated, and combined organic layers were evaporated to dryness. To derivatize the rest of free TIQ compounds, the residue was reconstituted in 50 μl of propionic anhydride–pyridine (1:1) and incubated for 30 min at 80°C. After cooling to room temperature, 2 ml of water were added for solid-phase extraction.

Before application of a sample, the C_{18} extraction columns were conditioned with 2 ml of methanol followed by 2 ml of water. Prepared samples were applied to the columns under vacuum at a flow-rate of approximately 1 ml/min. The columns were washed with 1 ml of water followed by 1 ml of 30% acetonitrile in water and dried by centrifugation (5 min, 1000 *g*). The TIQ compounds were eluted with two 0.75-ml volumes of acetonitrile and collected in a vial. The eluate was evaporated to dryness under a stream of nitrogen at 50°C. The residue was dissolved in 20 μl of ethyl acetate and a 2-μl aliquot was subjected to GC–MS.

The temperature of the GC–MS system was programmed from an initial value of 60°C, held for 2 min, followed by a linear increase to 210°C with 30°C/min and then by a linear increase to 300°C at 10°C/min. The final temperature was held for 5 min. The split–splitless injector (2 min split off) was maintained at 270°C.

The sample preparation and data analysis procedure of THBC compounds was also outlined previously [99]: The sample consisted of 10 ml of urine with 0.1 ml of a solution of semicarbazide (1 *M*) adjusted to pH 4.5 with acetic acid, to which 2500 U β-glucuronidase had been added. For enzymatic hydrolysis the sample was incubated for 2 h at 55°C. Then 7 ml of dipotassium hydrogenphosphate were added to adjust the pH to 7.2, followed by 0.1 ml methyl chloroformate. The sample was vortex-mixed and allowed to stand for 10 min. The pH was increased to 9.5 by addition of 2.5 ml of saturated sodium carbonate. Then another 0.1 ml of methyl chloroformate was added and the sample was allowed to stand for another 15 min.

For extraction the whole sample volume was applied to a Chem-Elut column and eluted twice with 20 ml of 1-chlorobutane. The eluate was evaporated to dryness under vacuum and the residue was prepared for solid-phase extraction by reconstitution in 2 ml of borate buffer.

Before application, the C_{18} extraction columns were conditioned with 2 ml of methanol followed by 2 ml of water and 1 ml of borate buffer. Prepared samples were applied to the columns under vacuum at a flow-rate of approximately 1 ml/min. The columns were washed with 1 ml of water followed by 1 ml of 50% methanol in water and dried by centrifugation (5 min, 1000 *g*). The THBC compounds were eluted with two 0.75-ml volumes of methanol and collected in a vial. The eluate was evaporated to dryness under a stream of nitrogen at 50°C. The residue was dissolved in 20 μl of ethyl acetate and a 2-μl aliquot was subjected to GC–MS.

The temperature of the GC–MS system was

Table 3
Characteristic mass fragments of propionyl derivatives of investigated TIQ compounds together with retention times, recoveries determined with external standard (1 ng substance per ml) and coefficients of correlation (r) (linearity was obtained over the range 0.1–50 ng/ml) (n=5)

Propionyl derivative	Characteristic mass fragments (m/z)	Retention time (min)	Recovery (mean±S.D.) (%)	Coefficient of correlation (r)
N-Me-TIQ	42, 104, 146	5.52	93.7±8.7	0.993
TIQ	104, 117, 132, 189	7.94	93.5±8.0	0.994
6,7-diMeO-TIQ	164, 192, 249	10.79	89.5±6.3	0.998
6-MeO-7-OH-TIQ	150, 162, 235, 291	12.43	80.9±7.1	0.998
N-Me-4,6,7-triOH-TIQ	152, 176, 233, 289	12.61	74.3±6.2	0.996
SAL	164, 220, 276, 332, 347	13.88	80.6±7.5	0.999
6,7-diOH-TIQ	57, 221, 333	14.02	76.3±6.8	0.999
4,6,7-triOH-TIQ	162, 219, 275, 331	15.51	69.9±7.1	0.997

programmed from an initial value of 100°C, held for 2 min, followed by a linear increase to 300°C with 40°C/min. The final temperature was held for 5 min. The split–splitless injector (2 min split off) was maintained at 270°C.

Retention times were recorded and diagnostic mass fragments of the derivatives were chosen for an acquisition in the selected-ion monitoring (SIM) mode. The following mass fragments were chosen to monitor for the presence of TIQ compounds: m/z 42, 104 and 146 for N-Me-TIQ; m/z 104, 117, 132 and 189 for TIQ; m/z 164, 192 and 249 for 6,7-DiMeO-TIQ; m/z 150, 162, 235 and 291 for 6-MeO-7-OH-TIQ; m/z 152, 176, 233 and 289 for N-Me-4,6,7-triOH-TIQ; m/z 164, 220, 276, 332 and 347 for SAL; m/z 57, 221 and 333 for 6,7-diOH-TIQ; m/z 162, 219, 275 and 331 for 4,6,7-triOH-TIQ. The mass fragments m/z 102, 142, 174 and 244 were chosen to monitor for the presence of N-Me-THBC, m/z 115, 143 and 230 were used for THBC, m/z 157, 229, and 244 were used for monitoring of 6-Me-THBC, m/z 158, 173 and 260 for 6-MeO-THBC, and the mass fragments selected for 6-OH-THBC were m/z 158, 217, 289 and 304. Experiments with spiked urine samples revealed that linearity was obtained over the range of 0.1 to 50 ng TIQ or THBC compound per ml, with coefficients of correlation from 0.993 to 0.999 (Table 3 and Table 4). Recoveries determined by spiked samples (1 ng/ml) ranged from 74.3±6.2 to 93.7±8.7% (n=5). Using the routine method described above, the minimum detectable concentrations of the TIQ and THBC compounds were 50–100 pg/ml (signal-to-noise ratio 3:1). A sample was regarded as positive when retention time together with diagnostic mass fragments and specific ion ratios corresponded to a

Table 4
Characteristic mass fragments of carbonic acid derivatives of investigated THBC compounds together with retention times, recoveries determined with external standard (1 ng substance per ml) and coefficients of correlation (r) (linearity was obtained over the range 0.1–50 ng/ml) (n=5)

Carbonic acid derivative	Characteristic mass fragments (m/z)	Retention time (min)	Recovery (mean±S.D.) (%)	Coefficient of correlation (r)
N-Me-THBC	102, 142, 174, 244	9.60	79.9±8.3	0.995
THBC	115, 143, 230	9.75	85.4±7.5	0.994
6-Me-THBC	157, 229, 244	10.56	83.2±7.3	0.998
6-MeO-THBC	158, 173, 260	11.74	80.3±5.9	0.998
6-OH-THBC	158, 217, 289, 304	13.77	84.7±6.4	0.999

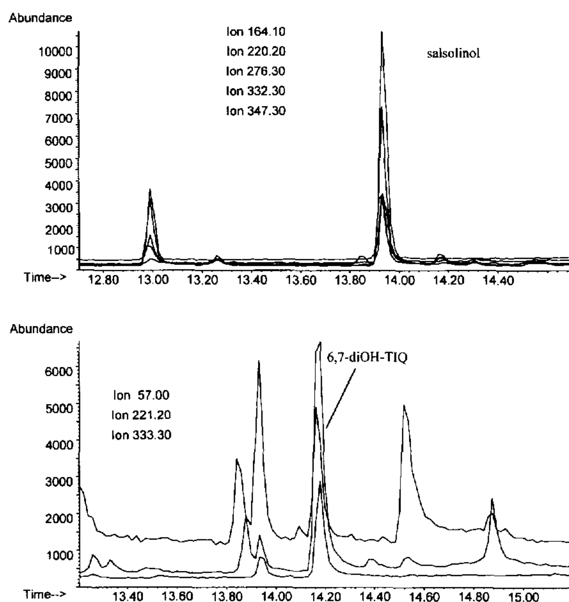

Fig. 3. Identification of SAL and 6,7-diOH-TIQ in an authentic urine sample of a chronic alcoholic.

comparison. In Fig. 3 the identification of SAL and 6,7-DiOH-TIQ in an authentic urine sample of a chronic alcoholic is demonstrated.

2.12. Sample material

Urine samples of alcoholics were taken from patients, who had been admitted in an alcoholized state for alcohol withdrawal. Samples were collected in three different states of alcoholization, with breath alcohol concentration (BAC) higher than 0.5 g/kg, lower than 0.5 g/kg and on the next morning. A second collection of samples was taken from healthy volunteers with no alcohol consumption for the previous 24 h.

3. Results and discussion

Screening investigations for the identification of formaldehyde-derived TIQ and THBC compounds in human urine samples revealed positive results for TIQ, 6-MeO-7-OH-TIQ, N-Me-4,6,7-OH-TIQ, SAL, 6,7-diOH-TIQ, 4,6,7-tri-OH-TIQ, THBC and 6-OH-THBC and negative results for N-Me-TIQ, 6,7-di-MeO-TIQ, N-Me-THBC, 6-Me-THBC and 6-MeO-THBC.

Positive results for the TIQ compounds could be obtained for alcoholics in all states of alcoholization. TIQ was identified in 6 of 64 samples in the higher state (state 1: >0.5 g ethanol/kg), in 3 of 59 samples in the lower state (state 2: <0.5 g/kg) and in 5 of 65 samples in a sober state on the next morning (state 3). For 6-MeO-7-OH-TIQ 1 sample in state 1, 2 samples in state 2 and 5 samples in state 3 were positive. N-Me-4,6,7-triOH-TIQ was detected 22 times in state 1, 23 times in state 2 and 21 times in state 3. The highest frequencies of detection were obtained for SAL and 6,7-diOH-TIQ with 52 and 55 positive samples respectively in state 1, 49 and 51 positive samples in state 2, and 56 and 54 positive samples respectively in the sober state. 4,6,7-triOH-TIQ was found in 37 samples in state 1, 31 samples in state 2 and 29 samples in state 3.

In urine samples of chronic alcoholics THBC was detected in 86 of 188 samples. The identification of 6-OH-THBC, the condensation product of serotonine and formaldehyde, was obtained in 81 samples. In higher states of alcoholization 32 samples revealed positive results for THBC and 25 for 6-OH-THBC, in lower states 26 samples were positive for THBC and 6-OH-THBC. In the sober state on the next morning THBC was demonstrated in 28 samples and 6-OH-THBC was found in 30 urine samples.

No significant correlation between the states of alcoholization and the occurrences of TIQ or THBC compounds was observed. However a decreasing frequency of THBC identification with an increasing frequency of 6-OH-THBC could be observed (Fig. 4).

Investigations of urine samples of healthy volunteers proved that also TIQ (2 of 50 samples positive), 6-MeO-7-OH-TIQ (4 samples positive), N-Me-4,6,7-triOH-TIQ (9 samples positive), SAL (44 samples positive), 6,7-diOH-TIQ (38 samples positive) 4,6,7-triOH-TIQ (22 samples positive), THBC (20 samples positive) and 6-OH-THBC (18 samples positive) could be detected, but no sample was positive for N-Me-TIQ, 6,7-diMeO-TIQ, N-Me-THBC, 6-Me-THBC or 6-MeO-THBC (Fig. 5).

The identification of TIQ, 6-MeO-7-OH-TIQ, N-Me-4,6,7-triOH-TIQ and 4,6,7-triOH-TIQ as excretion products in human urine samples is described

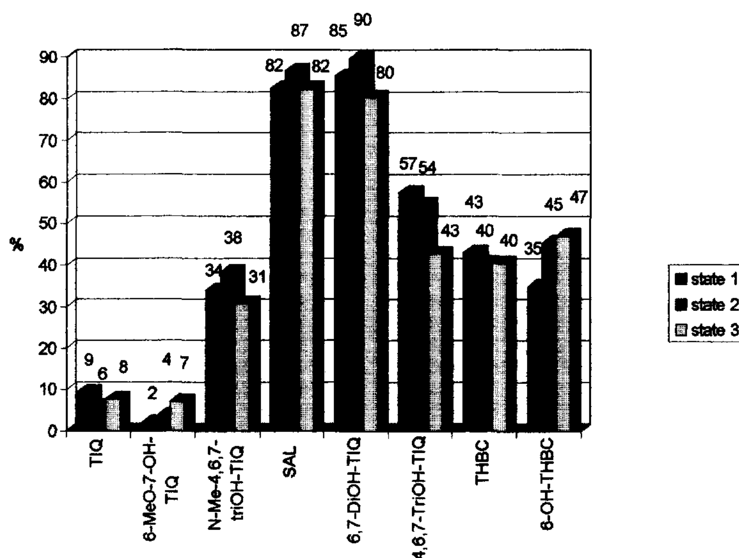

Fig. 4. Percentage frequencies of TIQ and THBC compounds identified in human urine samples in different states of alcoholization (state 1: >0.5 g ethanol/kg; state 2: <0.5 g/kg; state 3: sober on the next morning).

for the first time in this study. TIQ was previously found only in brain samples and formation was discussed in connection with psychic deficiency of suffering persons [73–75,104]. 6-MeO-7-OH-TIQ is a metabolite of 6,7-diOH-TIQ formed by 6-O-methylation. Beside the known SAL, positive results for the analogous formaldehyde–dopamine condensation product 6,7-diOH-TIQ were obtained together with positive results for the adrenaline and noradrenaline condensation products N-Me-4,6,7-triOH-TIQ and

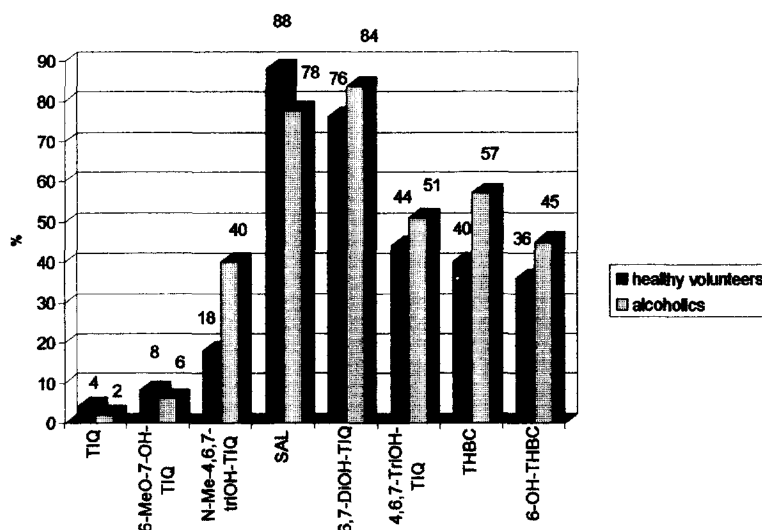

Fig. 5. Percentage frequencies of TIQ and THBC compounds identified in human urine samples of alcoholics ($n=188$) and healthy volunteers ($n=50$).

4,6,7-triOH-TIQ. The latter were found only in lower frequencies, because concentrations of the educts are not so high. It was generally noticed that in most of urine samples peak height ratios for SAL and 6,7-OH-TIQ varied. In samples of chronic alcoholics the peak height relations of 6,7-OH-TIQ and SAL seemed to be higher than in samples of healthy volunteers.

The identification of unchanged THBC could not be expected, because fast metabolization to aromatic β-carbolines by hydroxylation at positions 6 and 7 with following conjugation and transformation is described [105]. Although the formation of 6-MeO-THBC is described, the missing proof in our study could be explained by O-demethylation with following conjugation before excretion [106,107].

Rommelspacher et al. described a correlation between ethanol ingestion and the occurrence of the aromatic β-carboline harmane resulting from the tryptamine–acetaldehyde condensation product [108]. Furthermore a correlation between ethanol ingestion and harmane excretion in the urine of alcoholic patients could be observed [96]. Harmane concentrations in erythrocytes of alcoholics were increased after ethanol ingestion [95]. Comparing alcoholic patients and healthy volunteers only differences in plasma norharmane concentrations were found [16,97]. Norharmane is the C_1-condensation product generated by oxidation of THBC. Concentrations of norharmane were increased in patients who had delirium or hallucinations in the phase of detoxication, but they were never increased in persons with vegetative withdrawal symptoms. So a symptomatic and pathobiological appearances exists similar to schizophrenic patients with psychedelic psychoses and with disarrangements in C_1 metabolism and generation of formaldehyde–neuroamine condensation products [104].

Our study shows no correlation between the occurrence of formaldehyde-derived TIQ or THBC compounds and the state of alcoholization or alcohol elimination. Even in a sober state and in samples of healthy volunteers TIQ and THBC compounds could be demonstrated. These findings give rise to the assumption that formaldehyde is formed continuously. Further investigations are necessary. Dietary sources of tested persons have to be taken into account. Drinking habits and the kind of alcoholic beverages preferred by tested people could be important, too.

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