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Highly sensitive method for the determination of 1-methyl-1,2,3,4-tetrahydro-β-carboline using combined capillary gas chromatography and negative-ion chemical ionization mass spectrometry

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SUMMARY

A sensitive method was developed for the determination of deuterated and non-deuterated 1-methyl-1,2,3,4-tetrahydro-β-carboline by combined capillary gas cheomatography and negative-ion chemical ionization mass spectrometry. 1-Methyl-1,2,3,4-tetrahydro-β-carboline was converted into a trifluoroacetyl derivative after pretreatment with fluorescamine and extraction with ethyl acetate. The derivative was separated by capillary gas chromatography and determined by selected-ion monitoring. In the determination, $[3,3,4,4-^2H_4]$-1-methyl-1,2,3,4-tetrahydro-β-carboline was used as an internal standard. The method developed in this work was used for the determination of deuterated and non-deuterated 1-methyl-1,2,3,4-tetrahydro-β-carboline in human urine samples collected before and after administration of $[3,3-^2H_2]$-L-tryptophan.

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INTRODUCTION

1,2,3,4-Tetrahydro-β-carbolines are formed from indoleamines and aldehydes through Pictet–Spengler condensation. They have recently attracted special interest because of speculation over their occurrence in mammalian tissues [1–4] and reports regarding their neuropharmacological effects, such as the inhibition of monoamine oxidase [5], the inhibition of the uptake of 5-hydroxytryptamine [6,7] and binding to benzodiazepine receptors [8,9]. However, in vivo formation of 1,2,3,4-tetrahydro-β-carbolines has not been confirmed clearly.

Recently many kinds of analytical method have been reported for the quantification of 1,2,3,4-tetrahydro-β-carbolines in biological samples, including thin-layer chromatography [10,11], high-performance liquid chromatography [12] and gas chromatography–mass spectrometry [4,13–18]. For the determination of 1,2,3,4-tetrahydro-β-carbolines, it may be potentially important to suppress artifactual formation besides achieving high sensitivity [18]. In most methods reported, semicarbazide hydrochloride was used as an aldehyde-trapping agent for this purpose. However, Bosin et al. [18,19] suggest that the removal of aldehyde might be incomplete.

This paper describes a highly sensitive method for the determination of 1-methyl-1,2,3,4-tetrahydro-β-carboline (MTHC) using combined capillary gas chromatography– negative-ion chemical ionization mass spectrometry (GC–NICI-MS). The method, coupled with fluorescamine pretreatment to suppress artifactual formation during various kinds of analytical treatment is not only highly sensitive and reliable for urinary MTHC but also involves only simple procedures. This method was also applied to the investigation of the in vivo formation of MTHC using [3,3-^{2}H$_2$]-L-tryptophan (Trp-d$_2$) as a precursor of [^{2}H$_2$]tryptamine.

EXPERIMENTAL

Reagents

MTHC was synthesized from tryptamine (TA) hydrochloride and acetaldehyde according to the method reported by Akabori and Saito [20]. It was then purified by silica gel chromatography and recrystallized from an aqueous ethanol solution. [3,3,4,4-^{2}H$_4$]-1-Methyl-1,2,3,4-tetrahydro-β-carboline (MTHC-d$_4$) was prepared from [$\alpha,\alpha,\beta,\beta$-^{2}H$_4$]tryptamine hydrochloride and acetaldehyde in the same manner. The isotopic purity was as follows: ^{2}H$_0$ = 0.124%, ^{2}H$_1$ = 0.091%, ^{2}H$_2$ = 0.285%, ^{2}H$_3$ = 5.146%, ^{2}H$_4$ = 94.291% and ^{2}H$_5$ = 0.063%, calculated from the relative intensities of peaks at m/z 378–388 measured for MTHC and MTHC-d$_4$, assuming that the relative peak heights of the isotopic ions M$^-$, [M+1]$^-$, [M+2]$^-$, and [M+3]$^-$ of each species are analogous. Standard stock solutions (400 μg/ml) were prepared by dissolving

the substances in ethanol and stored at $-20°C$. The solutions were daily diluted with ethanol in appropriate concentrations before use. Trp-d_2 was synthesized according to the method reported in a previous paper [21]. [$\alpha,\alpha,\beta,\beta$-$^2H_4$]Tryptamine (TA-$d_4$) was obtained from Merck Frosst Canada (Montreal, Canada). Fluorescamine was purchased from Wako (Osaka, Japan). Water was used after redistillation in an all-glass apparatus. The other reagents and solvents were all reagent grade.

Administration of TRP-d_2

Trp-d_2 (10 mg/kg body weight), which was thoroughly suspended in 200 ml of orange juice by sonicating in an ultrasonic bath, was administered orally to a healthy male, aged 44 years, at 10:00 a.m. The urine samples were collected before and after dosing at specific intervals, and aliquots were stored at $-20°C$ after measurement of the volume until analysis.

Procedure for the determination of urinary MTHC

A 200-μl volume of urine sample was pipetted into a 15-ml polypropylene tube, and 50 μl of the internal standard solution (100 ng MTHC-d_4 per ml ethanol) and 0.5 ml of 0.1 M phophate buffer (pH 8.50) were added. A 0.5-ml volume of a fluorescamine solution (1.6 mg/ml of acetone) was added under vortex-mixing. MTHC was then extracted with 5 ml of ethyl acetate by shaking for 5 min after the addition of 2 ml of 0.2 M Na$_3$PO$_4$. MTHC was then extracted from the organic layer with 1 ml of 0.1 M HC1 by shaking for 5 min. After removal of the organic layer, MTHC was again extracted from the aqueous layer with 5 ml of ethyl acetate after the addition of 2 ml of 0.2 M Na$_3$PO$_4$. The ethyl acetate was transferred to a 25-ml glass centrifuge tube and evaporated to dryness under reduced pressure after being dried over sodium sulphate. The residue was redissolved in 0.5 ml of trifluoroacetic anhydride–acetonitrile (1:1) and transferred to a siliconized glass ampoule. The solution was evaporated under a stream of nitrogen, and the residue was redissolved in 50 μl of ethyl acetate prior to analysis by GC–NICI-MS.

Gas chromatography–mass spectrometry

A Finnigan 4000 gas chromatograph–mass spectrometer equipped with a pulsed positive-ion negative-ion chemical ionization accessory (Finnigan, CA, U.S.A.) was used for the recording of mass spectra and for selected-ion monitoring. The GC separations were achieved with a solvent-cut injector (Gasukuro Kogyo, Tokyo, Japan) connected to an OV-1701 bonded fused-silica capillary column (25 m×0.25 mm I.D., film thickness 0.3 μm, Gasukuro Kogyo) using methane as a carrier gas. The end of the column was inserted directly into the ion source of the mass spectrometer. The capillary column head-pressure was held at 0.6 kg/cm^2, which gave a methane flow-rate of 1 ml/min. The

injector port and transfer line temperatures were maintained at 285 and 280°C, respectively. The GC oven was kept at a temperature of 230°C. The mass spectrometer was operated in the NICI mode. The reagent gas, methane, was delivered to the ion-source, which was maintained at a pressure of 0.15 Torr. The temperatures of the source and analyser section of instrument were maintained at 250 and 80°C, respectively. The emission current and the electron energy were maintained at 300 μA and 90 eV, respectively.

RESULTS AND DISCUSSION

In a previous paper [22], we discussed effective derivatization for the highly sensitive detection of many indole compounds for GC–NICI-MS. From the data, the derivative shown in Fig. 1 was selected for use in the highly sensitive determination of MTHC. A methane NICI mass spectrum of the TFA derivative consists of a single fragment anion, which we assigned to M$^-$. The TFA derivative showed not only good ionization efficiency in the methane NICI mode, but also good GC properties. Fig. 2 shows a selected-ion current profile, which was generated by the derivative related to 10 fg of MTHC.

One of the most important requirements in the analysis of the 1,2,3,4-tetrahydrocarbolines is the suppression of their formation during the analytical procedure. In most reported methods semicarbazide and the other aldehyde-trapping reagents were used to remove aldehydes, which react with the precursor indoleamines to produce 1,2,3,4-tetrahydroβ-carbolines. However, Bosin et al. [18,19] indicated that it was difficult to remove aldehydes using conven-
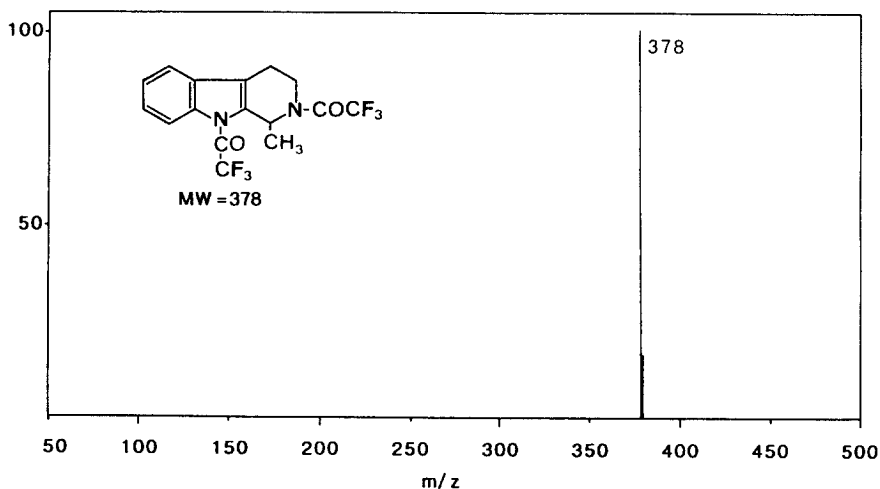

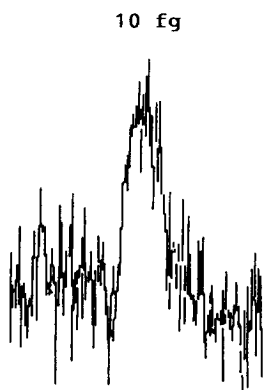Fig. 1. Methane NICI mass spectrum of the TFA derivative of MTHC.

10 fg

Fig. 2. Selected-ion current profile obtained by monitoring the ion at m/z 378 for MTHC : 100 ng of MTHC were derivatized as described in Experimental, the product was diluted with ethyl acetate, and the sample solution equivalent to 10 fg of MTHC was analysed.
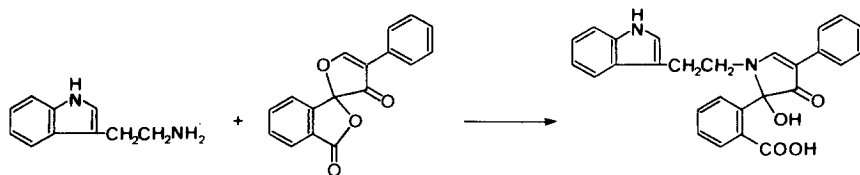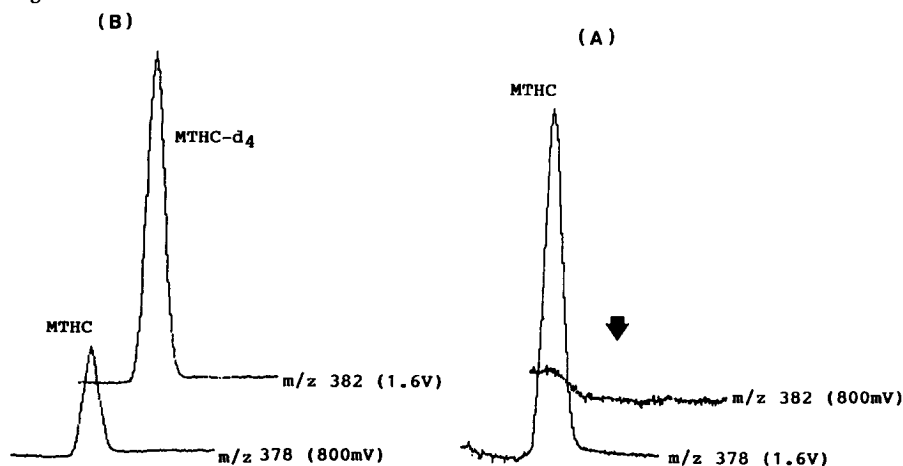

Fig. 3. Reaction of fluorescamine with TA.

Fig. 4. SIM chromatograms obtained from human urine samples spiked with [$\alpha,\alpha,\beta,\beta$-$^2H_4$]tryptamine (5 μg/ml urine) with (A) and without (B) fluorescamine treatment.

tional aldehyde-trapping agents. Also it seems troublesome to remove aldehydes from the reagents and solvents used for the determination of 1,2,3,4-tetrahydro-β-carbolines. An attempt was therefore made to suppress the artifactual formation of 1,2,3,4-tetrahydro-β-carbolines through the removal of the precursor indoleamine. Fluorescamine was reported as a fluorescent label-
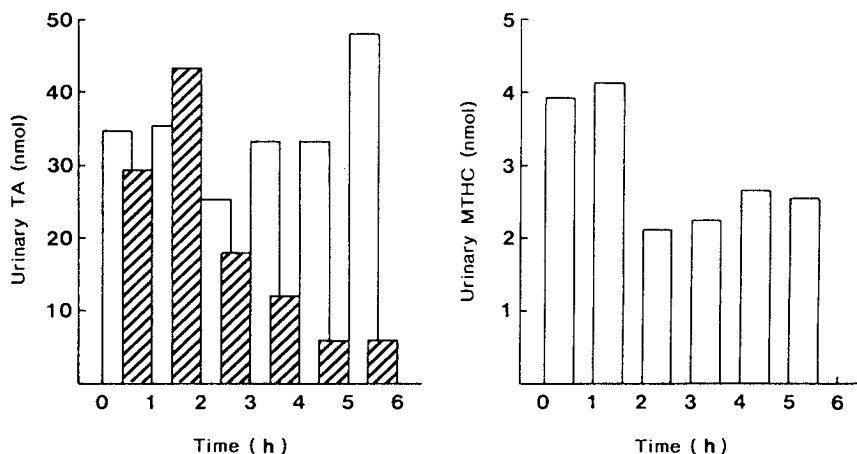
Fig. 5. Urinary excretion profiles of tryptamine and MTHC (□), and deuterated tryptamine and MTHC (▨) after oral administration of $[3,3\text{-}^2\text{H}_2]$-L-tryptophan (10 mg/kg body weight) to a healthy male subject aged 44 years.

ing reagent for the compounds with a primary amino group [23,24]. It shows high reactivity to the primary amino group, but not to the secondary amino group. The precusor indoleamine, TA, was converted into a compound that contains a carboxy group, as shown in Fig. 3. The use of this reaction with the solvent extraction method described in detail in Experimental readily made it possible to remove the precursor indoleamine. Fig. 4 shows the results obtained from a urine sample spiked with 1 μg of TA-d_4, with or without the fluorescamine treatment. Although the peak related to MTHC-d_4 produced from TA-d_4 is clearly observed on the chromatogram obtained from the sample not treated with fluorescamine, it is not observed on the trace from the sample treated with fluorescamine. Furthermore, the treatment was also ascertained by GC with flame ionization detection to be effective for the prepurification of the urine sample.

A calibration graph for the determination of MTHC was prepared by plotting the peak-height ratio of MTHC to MTHC-d_4 on the ordinate after correction for the signal due to the isotopic peak, and the amounts of MTHC on the abscissa. The graph was linear in a concentration range of at least 40 pg to 100 ng of MTHC per sample and passed through the origin. Analytical reproducibility was estimated using human urine samples. In eight different determinations, the coefficient of variation was 3.5%, and the mean value of the urinary MTHC was 20.8 pmol/ml.

The possibility of the in vivo production of tetrahydro-β-carboline is of great interest in consideration of the etiology of alcohol dependence. Metabolic studies using the stable labelled compound Trp-d_2 were therefore carried out to investigate whether or not MTHC was produced in vivo.

Fig. 5 represents the time course of amounts of tryptamine, MTHC and their deuterated analogues in urine after the oral administration of Trp-d$_2$ (10 mg/kg). The determination of deuterated and non-deuterated TA was performed according to the method in the previous paper. The excretion of TA-d$_2$ began to increase immediately after dosing, and maximum excretion was observed in urine collected 2 h later. The maximum ratio of excreted TA-d$_2$ to TA was 1.25. After maximum excretion, TA-d$_2$ excretion decreased slowly. On the other hand, no measurable amount of MTHC-d$_2$ observed. These observations do not suggest in vivo production of MTHC.

In conclusion, this paper describes a GC–NICI-MS method for the determination of urinary MTHC. This method is highly sensitive and reliable. The derivatization and pretreatment employed for the determination of MTHC should be applicable to the other biological important tetrahydro-β-carbolines. The metabolic studies using Trp-d$_2$ did not suggest in vivo formation of MTHC. Detailed data about in vivo formation will be reported in a further paper.

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