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Identification of 4-Methylspinaceamine, a Pictet−Spengler Condensation Reaction Product of Histamine with Acetaldehyde, in Fermented Foods and Its Metabolite in Human Urine

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Previous study demonstrated that 4-methylspinaceamine (4-methyl-4,5,6,7-tetrahydro-1*H*-imidazo-[4,5-*c*]pyridine), a Pictet−Spengler condensation reaction product of histamine with acetaldehyde, is present in human urine. The current study sought to determine whether 4-methylspinaceamine is present in fermented foods; its presence might be expected since both histamine and acetaldehyde are often present in these foods. Soy sauce, fish sauce, cheese, and shao hsing wine (Chinese wine) were found to contain 4-methylspinaceamine. The concentration of 4-methylspinaceamine excreted in human urine was greatly elevated after ingestion of a meal containing soy sauce as a dietary source of 4-methylspinaceamine, demonstrating that the level of 4-methylspinaceamine in human urine was affected by dietary foods. In addition, a metabolite of 4-methylspinaceamine in human urine was investigated. An enhanced peak in the HPLC chromatogram of human urine samples after ingestion of 4-methylspinaceamine-containing foods was observed. A peak at the same retention time was also observed from a human urine sample after administration of 4-methylspinaceamine, suggesting that the peak was due to a metabolite. By comparison with the newly synthesized authentic compound, the metabolite was identified as 1,4-dimethylspinaceamine.

KEYWORDS: 4-methylspinaceamine; 1,4-dimethylspinaceamine; 4-methyl-4,5,6,7-tetrahydro-1*H*-imidazo-[4,5-*c*]pyridine; 1,4-dimethyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine; acetaldehyde; histamine; Pictet−Spengler reaction; fermented foods; metabolite

INTRODUCTION

1-Methyl-1,2,3,4-tetrahydro-β-carbolines and salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) are produced by a Pictet−Spengler condensation reaction of acetaldehyde with indoleamines and dopamine, respectively (*1*, *2*). These compounds are present in mammalian tissue and fluid samples (*3*−*5*). They are also distributed in various foods and beverages (*6*−*16*). 1-Methyl-1,2,3,4-tetrahydro-β-carbolines and salsolinol are known to possess various neuropharmacological properties (*3*−*5*). Several reports suggest that their presence in mammalian tissue and fluid samples is caused by the reaction of acetaldehyde with indoleamines and dopamine *in vivo* (*17*−*26*).

Along with indoleamines and dopamine, histamine is an important biogenic amine of the central nervous system. Although it has been reported that the chemical reaction of histamine with acetaldehyde produces 4-methylspinaceamine via the Pictet−Spengler reaction under physiological conditions (*27*, *28*), little attention has been paid to this interaction in biological systems or to the identification of 4-methylspinaceamine in biological samples. Previously, it was reported that 4-methylspinaceamine was present in human urine (*29*).

1-Methyl-1,2,3,4-tetrahydro-β-carbolines are known to be present in various fermented foods (*7*−*9*, *13*) and are believed to be produced by the reaction of acetaldehyde with indoleamines during the processes of fermentation, ripening, and storage (*13*, *30*). It has also been well documented that both histamine and acetaldehyde are often present in various fermented foods (*31*−*39*). This suggests that the reaction of histamine with acetaldehyde can occur during fermentation, ripening, and storage.

Recent research has demonstrated that spinaceamine derivatives including 4-methylspinaceamine can inhibit semicarbazide-sensitive amine oxidase (SSAO) (*40*). In this work, the occurrence of 4-methylspinaceamine was studied. In addition, a metabolite of 4-methylspinaceamine in human urine was investigated.

MATERIALS AND METHODS

^{1}H NMR spectra were recorded on a JEOL JNM-A500 (500 MHz) spectrometer with trimethylsilyl[2,2,3,3-$^2H_4$]propionate (TSP) as an internal standard. FAB-MS spectra were measured on a JEOL JMS-

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700 mass spectrometer. UV absorption spectra were obtained on a JASCO V-560 UV/vis spectrophotometer.

Materials. Histamine dihydrochloride, 1-naphthyl isothiocyanate, and *N*-methylpiperazine were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Acetaldehyde, 1-methylhistamine, and 3-methylhistamine were purchased from Sigma Chemical Co. (St. Louis, MO). Acetaldehyde-d_4 (99 atom % D) was purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI). Bond Elut CBA cartridges (100 mg, 1 mL) were provided by Varian International (Harbor City, CA). 4-Methylspinaceamine, 4-methylspinaceamine-d_4 (4-[$^2H_3$]methyl-4-[^{2}H]5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine), and the naphthylthiourea derivative of 4-methylspinaceamine (**1**) were prepared as reported previously (*29*).

Preparation of 1,4-Dimethylspinaceamine (1,4-Dimethyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine). A mixture of 3-methylhistamine dihydrochloride (100 mg) and acetaldehyde (0.1 mL) in 60 mL of 0.2 M phosphate buffer solution (pH 7.5) was maintained at 70 °C for 8 h and kept at room temperature overnight. After the solution was brought to pH 12 with 4 M NaOH, it was evaporated to dryness. EtOH (50 mL) was added to the residue, and the solution was filtered to separate sodium phosphate. The filtrate was evaporated to dryness and the residue was subjected to column chromatography on silica gel (80 mL) with $CHCl_3$/28% aq NH_4OH/MeOH (5:0.1:1). The eluate was evaporated to dryness. The residue was rechromatographed using the same conditions as above, and the eluate was evaporated to dryness. The residue was dissolved with EtOH (20 mL), and HCl gas was bubbled into the solution. The solution was evaporated to dryness, and 1,4-dimethylspinaceamine dihydrochloride (45 mg) was obtained as a white solid upon reprecipitation from MeOH−EtOAc. HR-FAB-MS *m/z*: 152.1178 $[M + H]^+$. Calcd for $C_8H_{14}N_3$: 152.1188. Anal. calcd for $C_8H_{15}N_3Cl_2 \cdot 0.25H_2O$: C, 42.03; H, 6.83; N, 18.38. Found: C, 42.33; H, 6.76; N, 18.36. ^{1}H NMR (D_2O): δ 1.67 (d, 3H, CH_3-4, $J = 6.7$ Hz), 3.04−3.08 (m, 2H, H-7), 3.51−3.57 (m, 1H, H-6), 3.77−3.83 (m, 1H, H-6′), 3.77 (s, 3H, CH_3-1), 4.73 (q, 1H, H-4, $J = 6.7$ Hz), 8.44 (s, 1H, H-2).

Preparation of 3,4-Dimethylspinaceamine (3,4-Dimethyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-*c*]pyridine). 3,4-Dimethylspinaceamine (42 mg) was obtained from the reaction of 1-methylhistamine dihydrochloride (100 mg) and acetaldehyde (0.1 mL) as described above. HR-FAB-MS *m/z*: 152.1191 $[M + H]^+$. Calcd for $C_8H_{14}N_3$: 152.1188. Anal. calcd for $C_8H_{15}N_3Cl_2 \cdot 0.25H_2O$: C, 42.03; H, 6.83; N, 18.38. Found: C, 42.09; H, 6.84; N, 18.28. ^{1}H NMR (D_2O): δ 1.71 (d, 3H, CH_3-4, $J = 6.7$ Hz), 3.00−3.13 (m, 2H, H-7), 3.63−3.67 (m, 2H, H-6), 3.86 (s, 3H, CH_3-3), 4.96 (q, 1H, H-4, $J = 6.7$ Hz), 8.63 (s, 1H, H-2).

Preparation of Naphthylthiourea Derivative of 1,4-Dimethylspinaceamine (2). To a solution of 1,4-dimethylspinaceamine dihydrochloride (34 mg) in 0.2 M phosphate buffer (pH 8.1, 20 mL) was added a solution of 1-naphthyl isothiocyanate (45 mg) in CH_3CN (20 mL), and the mixture was heated at 55 °C with stirring in a screw-capped flask for 30 min. After cooling, the solution was extracted with $CHCl_3$ (2 × 20 mL). The combined $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The resulting solid was subjected to column chromatography on silica gel (70 mL) with $CHCl_3$/MeOH (10:1, v/v). The eluate was evaporated until solid began to precipitate. Compound **2** (36 mg) was obtained as a white solid upon precipitation from *n*-hexane. HR-FAB-MS *m/z*: 337.1491 $[M + H]^+$. Calcd for $C_{19}H_{21}N_4S$: 337.1487.

HPLC Conditions. The HPLC system consisted of a NP-KX-100 pump (Nihon Seimitsu, Tokyo, Japan), a UV-2075 spectrophotometer (Jasco, Tokyo, Japan), a model 7725 injector (Rheodyne, Cotati, CA), and a Chromatocorder 21 integrator (System Instruments, Tokyo, Japan). HPLC separation was achieved on a 150 mm × 6 mm i.d. Wakosil-II 3C18 HG ODS column (Wako Pure Chemical Industries, Osaka, Japan) with a mobile phase of 0.01 M phosphate buffer (pH 7.4)/CH_3CN (7:3, v/v) at a flow rate of 1.0 mL/min at ambient temperature. The peaks were detected at 220 nm.

Ion Spray Mass Spectrometric (IS-MS) Conditions. An API 300 mass spectrometer (PE-Sciex, Thornhill, Canada) was used with an ion spray ionization interface. Sample injection was conducted with a Rheodyne bulb set between an ion spray probe and a syringe drive that pumped out CH_3CN/0.05% HOAc (7:3, v/v) at a constant flow rate (5 μL/min). The orifice potential and ring potential were set at 25 and 250 V, respectively.

Urine Samples. Urine was collected from healthy volunteers (age range 26−55 years) in plastic tubes and stored immediately at a temperature below −30 °C.

Food and Beverage Samples. All food and beverage samples were purchased in local supermarkets.

Solid samples (0.5−2 g) were homogenized with 10 mL of 0.1 M HCl containing semicarbazide (1 mg/mL) and centrifuged. To an aliquot of supernatant (1 mL), 50 μL of *N*-methylpiperazine (200 nmol/mL) was added as an internal standard, and the solution was used for HPLC analysis as described below.

Liquid samples (0.1−1 mL) were made up to 1 mL with water. To the solution were added 50 μL of semicarbazide (20 mg/mL) and 50 μL of *N*-methylpiperazine (200 nmol/mL) as an internal standard. The entire solution was used for HPLC analysis as described below.

HPLC Analysis of Food Samples. The analytical procedure was performed according to the method described previously (*29*) with slight modifications. To the solution obtained as described above were added NaCl (400 mg) and Na_2CO_3 (100 mg). After vortex mixing, the solution was extracted with *n*-BuOH (3 × 1.5 mL). The combined organic layer was washed with 0.1 M NaOH saturated with NaCl (1 mL) and transferred to a screw-capped tube containing 5 mL of *n*-heptane. The mixture was extracted with 0.03 M hydrochloric acid (1.5 mL). The aqueous layer was transferred to a screw-capped tube and brought to pH 8 by the addition of 0.6 M disodium hydrogen phosphate solution (0.5 mL). To the resulting solution was added 0.7 mL of 1-naphthyl isothiocyanate solution in CH_3CN (8 mg/mL). The solution was heated to 55 °C for 30 min with occasional vortex mixing. After the solution was cooled in an ice bath, 140 μL of 4 M HCl was added, and the excess 1-naphthyl isothiocyanate was removed by extracting with Et_2O (3 × 2 mL). To the aqueous layer was added 170 μL of 4 M NaOH. This was then extracted with $CHCl_3$/MeOH (5:1, v/v, 3 × 2 mL). The combined organic layer was evaporated to dryness under a stream of nitrogen, and the resulting residue was dissolved in MeOH (100 μL). An aliquot (10 μL) of the solution was analyzed by HPLC.

HPLC Analysis of Human Urine. The analysis was performed according to the method described previously (*29*).

IS-MS Analysis of Food Samples. The entire MeOH solution (100 μL) obtained from the above procedure for HPLC analysis was evaporated to about 20 μL under a stream of nitrogen. Water (50 μL) was added to the solution, and the entire solution was injected onto the HPLC. The HPLC conditions were the same as described above. The peak fractions corresponding to 4-methylspinaceamine were collected, and the UV spectrum of the fraction was measured before continuing on to the next procedure. After the pH was adjusted to >8.0 with 1 M NaOH, the fraction was extracted twice with the same volume of $CHCl_3$/MeOH (10:2, v/v). The organic layer was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 50 μL of MeOH, and an aliquot (5 μL) was analyzed by IS-MS.

IS-MS Analysis of Human Urine. To 10 mL of urine were added semicarbazide hydrochloride (10 mg), NaCl (4 g), and Na_2CO_3 (700 mg). After vortex mixing, the solution was extracted with *n*-BuOH (2 × 10 mL). The combined organic layer was washed with 0.1 M NaOH saturated with NaCl (4 mL) and evaporated to dryness. The residue was dissolved in 10 mM phosphate buffer (pH 7.0, 1 mL). The resulting solution was then subjected to a Bond Elut CBA column (100 mg, 1 mL), which had been preconditioned with MeOH (2 mL), H_2O (3 mL), and 10 mM phosphate buffer (pH 7.0, 2 mL). The column was washed with H_2O (3 mL) and eluted with 20 mM HCl (3 mL). The eluate was evaporated to dryness. The residue was dissolved in 0.2 M phosphate buffer (pH 8.0, 0.6 mL), and the resulting solution was transferred to a screw-capped tube. To the solution was added 0.3 mL of 1-naphthyl isothiocyanate solution in CH_3CN (6 mg/mL). The solution was then heated at 55 °C for 30 min with occasional vortex mixing. After the solutions was cooled in an ice bath, 70 μL of 4 M HCl was added, and the excess 1-naphthyl isothiocyanate was removed by extracting with Et_2O (3 × 1 mL). To the aqueous layer was added 90 μL of 4 M NaOH. This was then extracted with $CHCl_3$/MeOH (5:1, v/v, 3 × 1 mL). The combined organic layer was evaporated to dryness under a stream of nitrogen. The resulting residue was dissolved in MeOH (30 μL). Water

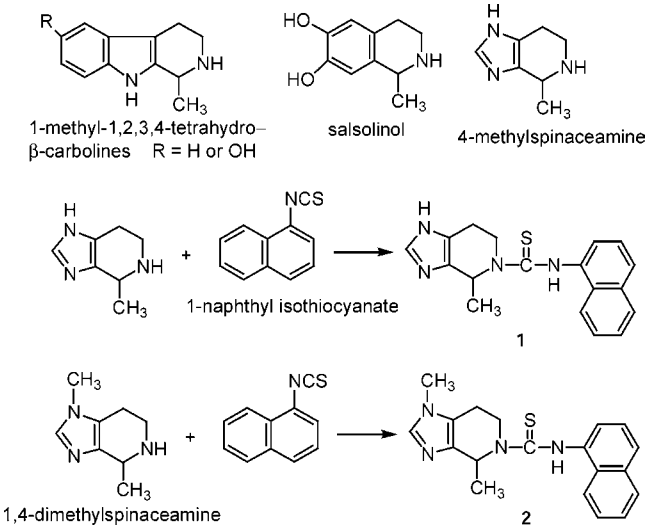

Figure 1. Pictet–Spengler reaction products of biogenic amines with acetaldehyde and derivatization reactions of 4-methylspinaceamine and 1,4-dimethylspinaceamine with 1-naphthyl isothiocyanate.

(50 μL) was added to the solution, and the entire solution was injected onto the HPLC. The HPLC conditions were the same as described above. Peak fractions corresponding to 4-methylspinaceamine and 1,4-dimethylspinaceamine were collected. The UV spectrum of the fraction corresponding to 1,4-dimethylspinaceamine was measured before continuing on to the next procedure. After the pH was adjusted to >8.0 with 1 M NaOH, the fraction was extracted with $CHCl_3$/MeOH (5:1, v/v, 3 × 1 mL). The organic layer was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 30 μL of MeOH, and an aliquot (5 μL) was analyzed by IS-MS.

Creatinine Assay. Urinary creatinine was determined by a creatinine assay kit (Wako Pure Chemical Industries).

Determination of Histamine. Histamine in foods was determined according to the procedure described by Moret et al. (*41*).

RESULTS AND DISCUSSION

Identification of 4-Methylspinaceamine in Fermented Foods. In a previous study, the identification and quantitative analysis of 4-methylspinaceamine in human urine was reported (*29*). The analytical method was as follows: 4-methylspinaceamine was partially purified by solvent extraction from a sample spiked with *N*-methylpiperazine as an internal standard, derivatized with 1-naphthyl isothiocyanate to a naphthylthiourea compound (**1**, **Figure 1**), and analyzed by HPLC. In this study, the same method was used in the analysis of 4-methylspinaceamine in fermented foods. The reliability of the method was examined by adding various amounts of standard 4-methylspinaceamine to soy sauce sample. Precision (coefficients of variation) and accuracy (relative error) ranged from 1.60% to 2.43% and from −0.50% to 1.67%, respectively. Among fermented foods tested in this study, soy sauce, fish sauce, cheese, and shao hsing wine (Chinese wine) were found to contain 4-methylspinaceamine (**Table 1**). **Figure 2** shows typical chromatograms of 4-methylspinaceamine (10 nmol) spiked with *N*-methylpiperazine (10 nmol) as an internal standard and food samples spiked with the internal standard (10 nmol/mL or g in analytical samples). A prominent peak in these chromatograms corresponding to 4-methylspinaceamine was observed. To identify this peak, the corresponding HPLC fraction was collected and analyzed by UV absorption spectrometry and IS-MS. All of the HPLC fractions exhibited the same spectra as authentic compound **1**. The results verify that these peaks were identical with that of **1**.

Table 1. Concentrations of 4-Methylspinaceamine in Fermented Foods

sample	4-methylspinaceamine (nmol/mL or g)
tamari soy sauce A	22.9
tamari soy sauce B	165.1
soy sauce A	29.9
soy sauce B	14.8
soy sauce C	1.8
soy sauce D	*a*
soy sauce E	17.4
soy sauce F	16.4
fish sauce A	3.3
fish sauce B	37.8
fish sauce C	6.7
fish sauce D	14.9
Appenzeller extra cheese	127.1
Roquefort cheese	1.7
Gorgonzola Pi cheese A	2.1
Gorgonzola Pi cheese B	14.7
Parmigiano Reggiano cheese	7.5
Conte extra cheese	1.0
Roaring 40's cheese	21.8
Gruyere cheese	2.1
Ridder cheese	*a*
Edam cheese	*a*
Gouda cheese	*a*
Stilton cheese	*a*
Pecorino cheese	*a*
Emmental cheese	*a*
Cheddar cheese	*a*
shao hsing wine A	5.2
shao hsing wine B	11.6
shao hsing wine C	8.8
shao hsing wine D	4.0
sake (Japanese wine)	*a,b*
wine	*a,c*
miso paste	*a,c*
anchovy fillet	*a,d*
anchovy paste	*a,b*
salami sausage	*a,b*
beer	*a,c*

[a] Not detected. [b] Five samples were tested. [c] Six samples were tested. [d] Four samples were tested.

It has been reported that 1-methyl-1,2,3,4-tetrahydro-β-carboline was formed as an artifact during the analytical procedure from the precursors tryptamine and acetaldehyde (*42*). To test whether the formation of 4-methylspinaceamine might occur during the analytical procedure, acetaldehyde-d_4 (2 μmol) was added to an analytical sample of soy sauce A (**Table 1**) that contained 0.32 μmol of histamine. The IS-MS spectrum of the HPLC peak fraction corresponding to 4-methylspinaceamine showed no formation of tetradeuterated 4-methylspinaceamine, indicating that artifact formation was not an issue in this analytical procedure.

Table 1 shows the concentrations of 4-methylspinaceamine in fermented foods. The content of the compound varied both between different types of food and within samples of the same type of food and ranged from an undetectable amount to a high of 165 nmol/mL. It has been reported that 1-methyl-1,2,3,4-tetrahydro-β-carbolines are present in a wide variety of foods (*6*−*13*). However, only four of the foods tested in this study contained 4-methylspinaceamine. This may be due to the low reactivity of acetaldehyde toward histamine at acidic pH (*28*). Fermented foods usually become acidic due to the production of organic acids by microorganisms during the fermentation process. In contrast, acetaldehyde has much higher reactivity toward indoleamines under acidic conditions (*28*, *30*). Therefore, 4-methylspinaceamine-containing foods could be much less

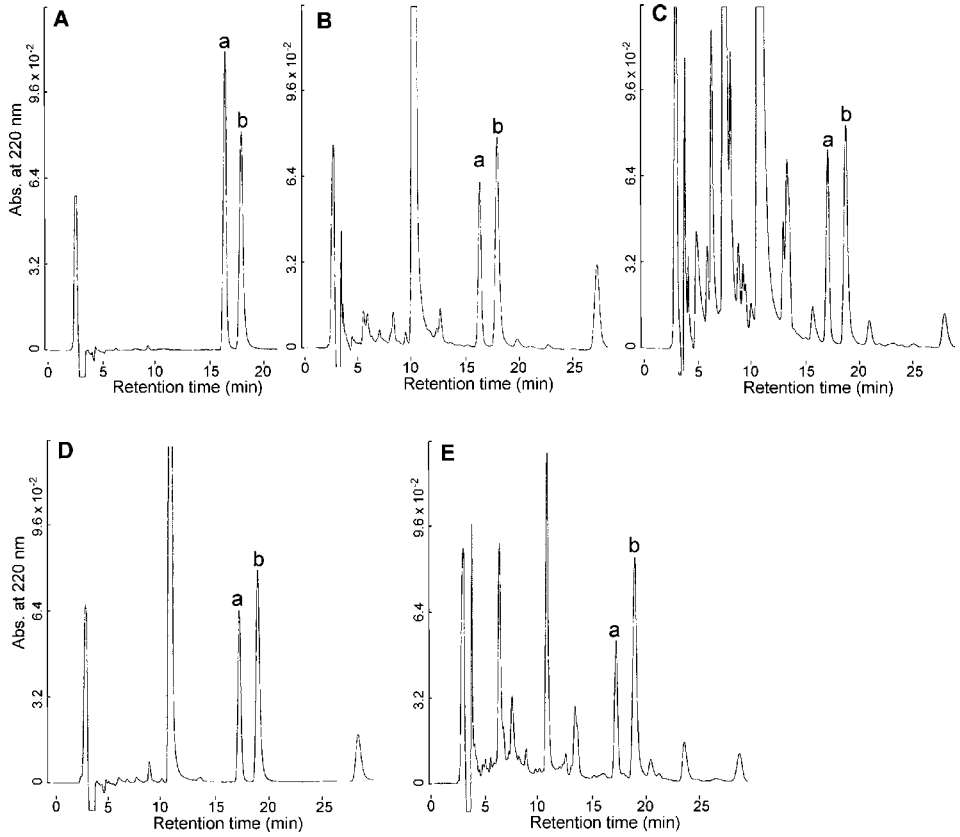

Figure 2. HPLC analysis of 4-methylspinaceamine in fermented foods: (**A**) standard 4-methylspinaceamine (10 nmol); (**B**) soy sauce (0.2 mL); (**C**) fish sauce (0.2 mL); (**D**) cheese (0.05 g); (**E**) shao hsing wine (1 mL). Each sample was spiked with *N*-methylpiperazine (10 nmol) as an internal standard. Peak assignments: **a**, 4-methylspinaceamine; **b**, internal standard.

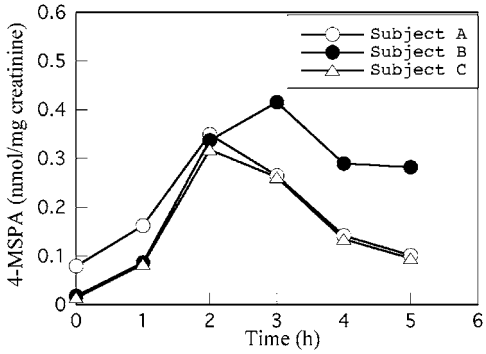

Figure 3. Urinary excretion of 4-methylspinaceamine supplied exogenously. Subjects ate a meal that contained 9 mL of soy sauce (sample A, equivalent to 269 nmol of 4-methylspinaceamine).

common than 1-methyl-1,2,3,4-tetrahydro-β-carboline-containing foods.

In this study, several foods were found to contain 4-methylspinaceamine. Therefore, it can be postulated that the intake of foods that contain 4-methylspinaceamine can affect the urinary level of 4-methylspinaceamine. In Japan, the daily consumption of soy sauce is estimated to be 23−30 mL per person. Three subjects ate a meal that contained 9 mL of soy sauce (sample A, equivalent to 269 nmol of 4-methylspinaceamine), and urinary 4-methylspinaceamine was subsequently measured. As shown in **Figure 3**, urinary excretion of 4-methylspinaceamine was greatly elevated by the food ingestion, reaching a maximum after 2−3 h. Therefore, exogenous intake was found to contribute to the presence of 4-methylspinaceamine in human urine. Several reports have suggested that the presence of 1-methyl-1,2,3,4-tetrahydro-β-carbolines and salsolinol in mammalian tissues and fluid samples was due to the *in vivo* condensation of acetaldehyde with indoleamines or dopamine (*17*, *22*, *24*, *25*). On the other hand, Tsuchiya et al. (*7*, *8*) proposed that most fractions of 1-methyl-1,2,3,4-tetrahydro-β-carbolines found in human urine were exogenously supplied via dietary sources. In the previous study (*29*), urine samples were collected from human subjects who were not under dietary control. Thus, it was not clear whether 4-methylspinaceamine identified in human urine was exogenously supplied or derived from the reaction of acetaldehyde with histamine *in vivo*. Since acetaldehyde has much higher reactivity toward histamine, resulting in the formation of 4-methylspinaceamine, than toward indoleamines under physiological conditions (pH 7.4, 37 °C) (*28*), the possibility of endogenous formation of 4-methylspinaceamine cannot be eliminated. Further investigation is required.

Identification of a Metabolite of 4-Methylspinaceamine in Human Urine. An enhanced peak was observed with a retention time of 25.5 min in the HPLC chromatograms of urine samples collected from subjects after consumption of 4-methylspinaceamine-containing soy sauce in the above trial or cheese. The peak was not a constituent of these food samples (**Figure 2**). **Figure 4B,C** shows representative HPLC chromatograms of urine samples before and after consumption of cheese (Appenzeller extra cheese, 10 g, equivalent to 1.27 μmol of 4-methylspinaceamine). The HPLC peak fraction at the retention time 25.5 min was collected and analyzed by IS-MS. A molecular ion at *m*/*z* 337 suggested that the peak was due to the methylated metabolite of 4-methylspinaceamine. To identify this peak, methylated 4-methylspinaceamines (1,4-dimethylspinaceamine and 3,4-dimethylspinaceamine) were synthesized. **Figure 4A** shows the HPLC chromatogram of authentic

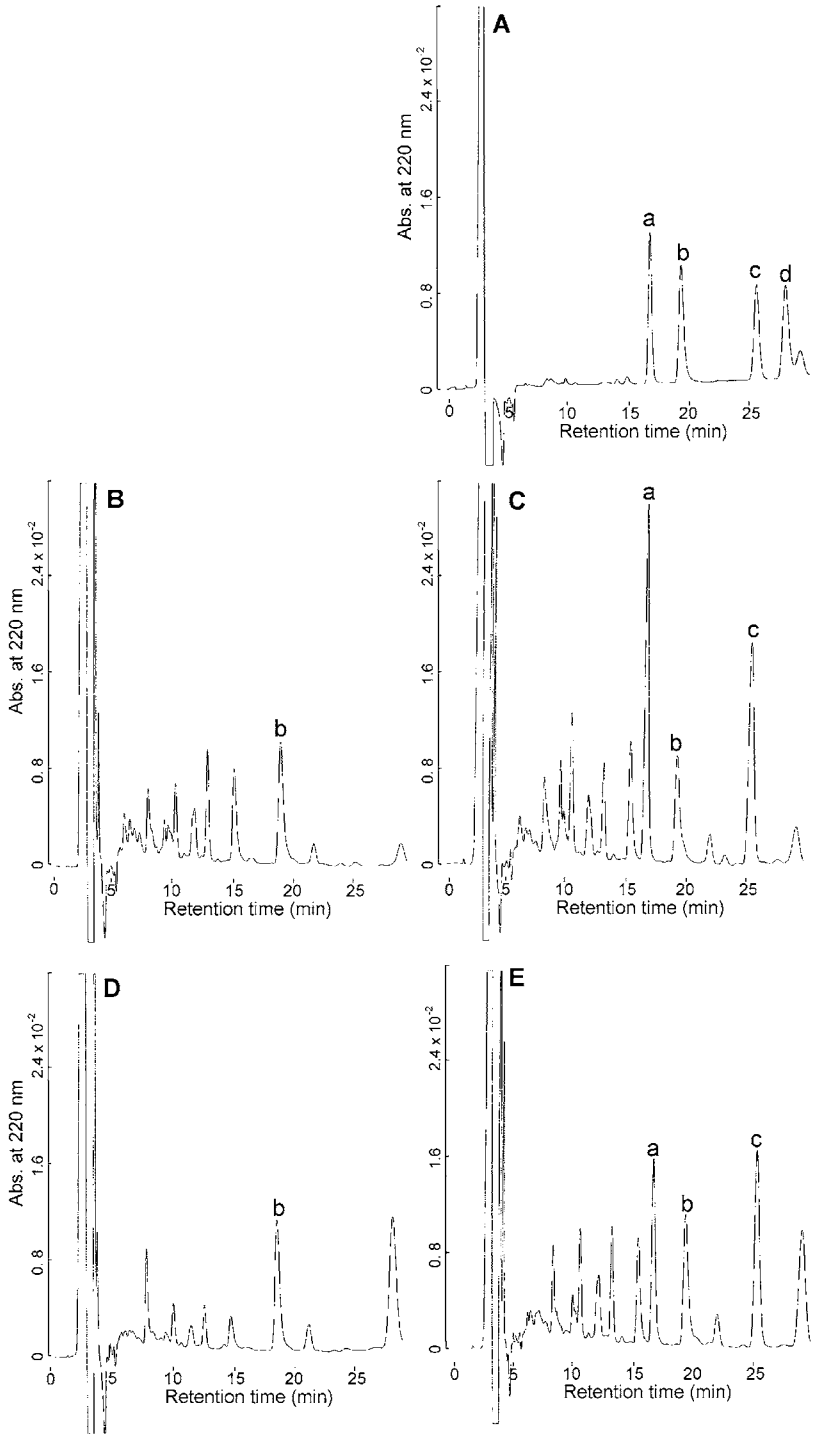

Figure 4. HPLC analysis of 1,4-dimethylspinaceamine in human urine: (**A**) a mixture of standard 4-methylspinaceamine (1 nmol), 1,4-dimethylspinaceamine (1 nmol), and 3,4-dimethylspinaceamine (1 nmol); urine samples (**B**) before and (**C**) 2 h after ingestion of cheese (Appenzeller extra cheese, 10 g); urine samples (**D**) before and (**E**) 4 h after administration of 4-methylspinaceamine (1 μmol). Each sample was spiked with *N*-methylpiperazine (1 nmol) as an internal standard. Peak assignments: **a**, 4-methylspinaceamine; **b**, internal standard; **c**, 1,4-dimethylspinaceamine; **d**, 3,4-dimethylspinaceamine.

4-methylspinaceamine, 1,4-dimethylspinaceamine, and 3,4-dimethylspinaceamine (1 nmol each), spiked with *N*-methylpiperazine (1 nmol) as an internal standard. A peak corresponding to 1,4-dimethylspinaceamine appeared at the retention time of 25.5 min. In addition, the authentic naphthylthiourea derivative of 1,4-dimethylspinaceamine (**2**, **Figure 1**) exhibited the same IS-MS and UV absorption spectra as the HPLC peak fraction at the retention time of 25.5 min. From these results, the structure of the compound was identified as 1,4-dimethylspinaceamine.

To confirm that 1,4-dimethylspinaceamine excreted in human urine was produced by the methylation pathway of 4-methylspinaceamine in the human body, a subject (the author) took 4-methylspinaceamine (1 μmol), and the urine was analyzed by HPLC. As shown in **Figure 4E**, the enhanced peak at the retention time of 25.5 min was observed. In addition, the subject took tetradeuterated 4-methylspinaceamine (1 μmol), and the HPLC peak fraction corresponding to 1,4-dimethylspinaceamine was analyzed by IS-MS. A protonated molecular ion was obtained at *m*/*z* 341, indicating that 1,4-dimethylspinaceamine-

Table 2. Urinary Excretion of 4-Methylspinaceamine and 1,4-Dimethylspinaceamine Two Hours after Ingestion of 4-Methylspinaceamine-Containing Foods

		4-methyl-spinaceamine (nmol/mg of creatinine)	1,4-dimethyl-spinaceamine (nmol/mg of creatinine)
subject A[a] (trial 1)	before ingestion	*c*	*c*
	after ingestion	2.16	1.93
subject A[a] (trial 2)	before ingestion	0.16	0.27
	after ingestion	1.82	1.46
subject B[a]	before ingestion	*d*	*d*
	after ingestion	0.95	0.86
subject C[a]	before ingestion	0.13	0.21
	after ingestion	1.25	1.02
subject A[b]	before ingestion	*d*	*d*
	after ingestion	0.34	0.23
subject B[b]	before ingestion	0.08	0.12
	after ingestion	0.35	0.36
subject D[b]	before ingestion	*d*	*d*
	after ingestion	0.32	0.2

[a] Subjects ingested cheese (Appenzeller extra cheese, 10 g, equivalent to 1.27 μmol of 4-methylspinaceamine). [b] Subjects ate a meal that contained 9 mL of soy sauce (sample A, equivalent to 269 nmol of 4-methylspinaceamine). [c] Trace. [d] Not detected.

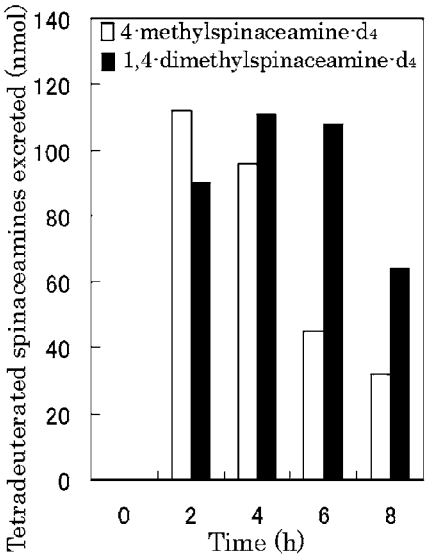

Figure 5. Urinary excretion of 4-methylspinaceamine-d_4 and 1,4-dimethylspinaceamine-d_4 after administration of 4-methylspinaceamine-d_4 (1 μmol).

d_4 was excreted in the urine sample. These results clearly show that 1,4-dimethylspinaceamine identified in the urine samples after ingestion of 4-methylspinaceamine-containing foods was the methylated metabolite of 4-methylspinaceamine. The concentrations of 4-methylspinaceamine and 1,4-dimethylspinaceamine excreted in human urine before and after ingestion of 4-methylspinaceamine-containing foods from healthy volunteers are listed in **Table 2**. 4-Methylspinaceamine and 1,4-dimethylspinaceamine were determined by the HPLC method using *N*-methylpiperazine as an internal standard. The reliability of the determination of 1,4-dimethylspinaceamine was examined by adding various amounts of standard 1,4-dimethylspinaceamine to urine sample. Precision (coefficients of variation) and accuracy (relative error) ranged from 2.01% to 7.35% and from 0.82% to 5.68%, respectively. In all cases, urinary excretion of both 4-methylspinaceamine and 1,4-dimethylspinaceamine was elevated by food ingestion. **Figure 5** shows the amounts of 4-methylspinaceamine-d_4 and 1,4-dimethylspinaceamine-d_4 excreted in urine samples collected at 2 h intervals after administration of 4-methylspinaceamine-d_4 (1 μmol). The total amount of 4-methylspinaceamine-d_4 and 1,4-dimethylspinaceamine-d_4 excreted after 8 h was 658 nmol (285 nmol of 4-methylspinaceamine-d_4 and 373 nmol of 1,4-dimethylspinaceamine-d_4). Although only one metabolite could be identified in this analytical procedure, the above result suggests that 1,4-dimethylspinaceamine may be the major metabolite of 4-methylspinaceamine.

A recent study has demonstrated that spinaceamine derivatives including 4-methylspinaceamine possess an inhibitory effect against semicarbazide-sensitive amine oxidase (SSAO); thus, these compounds are expected to be effective for the treatment or prophylaxis of SSAO-mediated complications (*40*). Since 1,4-dimethylspinaceamine is a newly identified compound, the biological effect is unknown. Further investigations are required to elucidate the effect of ingesting foods that contain 4-methylspinaceamine.

ABBREVIATIONS USED

IS-MS, ion spray mass spectrometry; SSAO, semicarbazide-sensitive amine oxidase.

Supporting Information Available: Precision and accuracy data for the determinations of 4-methylspinaceamine and 1,4-dimethylspinaceamine and UV and IS-MS spectra of HPLC peak fractions corresponding to 4-methylspinaceamine collected from food samples and corresponding to 1,4-dimethylspinaceamine collected from human urine sample after consumption of 4-methylspinaceamine-containing food. This material is available free of charge via the Internet at http://pubs.acs.org.

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