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Presence of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, a precursor of a mutagenic nitroso compound, in soy sauce

(nitrosable precursor of mutagen)

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ABSTRACT **After treatment with nitrite, Japanese soy sauce was strongly mutagenic to *Salmonella typhimurium* TA100 without S9 mixture. Two precursors of the mutagen were isolated from Japanese soy sauce, and these were identified as (−)-(1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid [(−)-(1*S*,3*S*)-MTCA] and its stereoisomer (−)-(1*R*,3*S*)-MTCA. After treatment with nitrite, 1-mg samples of these compounds induced 17,400 and 13,000 revertants of TA100, respectively, without S9 mixture. Quantitative analysis of various kinds of soy sauces produced in Japan showed the presence of 82–678 μg of MTCA per ml. The mutagenicities of these compounds with nitrite accounted for 16–61% of the total mutagenicity of soy sauce with nitrite. Most soy sauces produced in the United States were less mutagenic than those produced in Japan and little, if any, of these two precursors of the mutagen was found in them. A major reaction product of (−)-(1*S*,3*S*)-MTCA and nitrite was a compound having a nitroso substitution at position N-2, but this compound was not mutagenic. Thus, the mutagen(s) formed from (−)-(1*S*,3*S*)-MTCA and nitrite was a minor product(s), and its specific mutagenic activity must be very high.**

Epidemiological studies have indicated the importance of environmental factors, especially dietary factors, as causes of human cancer (1). There are many reports of naturally occurring carcinogens in foods, such as mycotoxins (2), pyrrolizidine alkaloids (3), polycyclic hydrocarbons (4), heterocyclic amines (5), and *N*-nitrosamines (6).

N-Nitroso compounds are known to be potent carcinogens (7). They are found in various foods, cigarette smoke, and air samples from rubber factories (6, 8–10). Linxian County in China is a high-risk area for esophageal cancer, and *N*-nitroso compounds in foods are likely candidates as causes of cancer in this area (11). It has been proposed that nitroso compounds, nitrate, and nitrite are formed endogenously (12, 13).

The mortality from gastric cancer is much higher in Japan than in Europe or the United States (14). Recently, Fine *et al.* (15) showed that there is a good correlation between nitrate ingestion per capita and mortality from gastric cancer in various countries, and a similar correlation was also shown in Narino in the Andes of southern Columbia (16). The average total intake of nitrate by Japanese has been estimated to be about three times that of Americans (8). Conversion of nitrate to nitrite *in vivo* is thought to be a microbial process in the oral cavity (17) and intestinal tract (18), and nitrite and secondary amines produce *N*-nitroso compounds in the acidic conditions in the stomach (19). Therefore, attention has been directed to the presence of secondary amines at high concentrations in Japanese foods, such as raw and processed fish (20). Recently, treatment of fava beans with nitrite was found to result in formation of a mutagen, but the structures of this compound and its precursor have not yet been determined (21).

We found that, after treatment with nitrite, soy sauce shows a strong direct-acting mutagenicity on *Salmonella typhimurium* TA100. This paper reports the isolation and structures of two precursors of mutagenic compounds present in Japanese soy sauce. Quantitative analyses of these precursors in various soy sauces are also reported, and the structure of the mutagenic nitroso compound is discussed.

MATERIALS AND METHODS

Foods. Soy sauces A–G and various foods were obtained at local markets in Japan. Soy sauces I–K were purchased at markets in Honolulu, and soy sauces H, L, and M, in Madison, WI. Soy sauces A–H are produced in Japan, and I–K, in Hawaii. Soy sauce L is produced in Ohio and M, in Wisconsin. Fish sauces of the Philippines and Thailand were obtained at markets in Manila and Bangkok, respectively.

Mutation Test. The mutation assay was carried out by the preincubation method (22). *S. typhimurium* TA98 and TA100 were used.

Revertants induced by samples without nitrite treatment were subtracted from those produced by samples with nitrite treatment, and the resulting values were defined as the mutagenic activities of substances produced by nitrite treatment. The numbers of spontaneous revertants of TA98 were between 15 and 38 and those of TA100 were between 106 and 160. All assays were carried out in duplicate.

Nitrite Treatment. *Foods.* Samples of 1 ml of liquid, such as soy sauce, were mixed with 1 ml of 0.1 M sodium nitrite and adjusted to pH 3.0 with 6 M HCl. The mixtures were incubated in the dark for 1 hr at 37°C. Nitrosation was stopped by addition of 1 ml of 0.1 M ammonium sulfamate. The mixtures were sterilized by passage through a Millex-HA filter (0.45 μm, Millipore), and 20-, 50-, 100-, and 200-μl portions of the filtrates were tested for mutagenicity. Samples of 10 g of solid food were homogenized with 10 ml of water in a Polytron homogenizer for 3 min and then centrifuged at 13,000 rpm for 20 min. The precipitates were reextracted with 10 ml of water and 2-ml samples of pooled supernatant were mixed with 0.5 ml of 0.2 M sodium nitrite containing 0.48 mmol of sodium chloride, as reported by Piacek-Llanes and Tannenbaum (21). The amount of ammonium sulfamate used was the same as that of sodium nitrite.

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Abbreviations: MTCA, 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid; MNTCA, 1-methyl-2-nitroso-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid.

Preparation for isolation of precursors and synthetic precursors. Appropriately diluted preparations at various isolation steps in 1 ml of water or 2-mg samples of synthetic compounds in 1 ml of water were mixed with 0.6 ml of 80 mM citrate/Na_2HPO_4, pH 3.0, and 0.2 ml of 0.5 M sodium nitrite. When necessary, the solutions were adjusted to pH 3.0 with 6 M HCl. Nitrosation was stopped by addition of 0.2 ml of 0.5 M ammonium sulfamate. The incubation was carried out as described above.

Isolation of Precursors of Mutagenic Nitroso Compounds from Soy Sauce. A volume of 80 ml of soy sauce A was diluted 1:17 with water and applied to a column (4.0 × 16.0 cm) of charcoal (Wako Pure Chemical Industries, Osaka). The column was washed with 2 liters of water and then material was eluted successively with 2 liters each of methanol, 80% methanol/1 M acetic acid, methanol, and 80% methanol/1 M NH_4OH. The fractions eluted with methanol, 80% methanol/1 M acetic acid, and methanol again were pooled and evaporated to dryness, and the dry material (3.364 g) was dissolved in 90 ml of water and loaded on an Amberlite XAD-7 column (4.0 × 19.3 cm). The column was washed with 1.25 liters of water and then material was eluted with 1.25 liters of methanol. The eluate was evaporated to dryness and the residue (266 mg) was separated by HPLC in a Toyo Soda HLC-803A apparatus. The residue applied was dissolved in 8.4 ml of 50% methanol and one-quarter of this solution was injected into a reversed-phase LS-110 styrene column (21.5 × 600 mm, Toyo Soda, Tokyo). Material was eluted with 50% methanol at a flow rate of 6 ml/min at ambient temperature, and the absorbance of the eluate at 270 nm was monitored. This procedure was repeated four times. Fractions with elution volumes of 246–282 ml were pooled and evaporated. The residue (68 mg) was dissolved in 13.6 ml of 20% methanol and purified further by HPLC on a reversed-phase LS-410 octadecyl silyl column (21.5 × 300 mm, Toyo Soda) with 20% methanol as eluant. The volume of material injected into the HPLC column was 1 ml, and other HPLC conditions were as for the LS-110 column. At this step, two white crystalline compounds were isolated having elution volumes of 450–480 ml (peak I) and 540–576 ml (peak II) as nitrosable precursors of mutagens.

Synthesis of (−)-(1*S*,3*S*)-MTCA and (−)-(1*R*,3*S*)-MTCA. (−)-(1*S*,3*S*)-MTCA was synthesized by the method of Brossi *et al.* (23) and crystallized twice from 30% methanol. (−)-(1*R*,3*S*)-MTCA was obtained as follows. A mixture of (−)-(1*S*,3*S*)- and (−)-(1*R*,3*S*)-MTCA was synthesized by the method of Jacobs and Craig (24) and then the (−)-(1*R*,3*S*) compound was purified by HPLC on an LS-410 column. (−)-(1*R*,3*S*)-MTCA was identified by comparison of its physical properties with those reported by Brossi *et al.* (23).

Separation of Mutagen Produced from (−)-(1*S*,3*S*)-MTCA by Nitrite Treatment. A 2-mg sample of (−)-(1*S*,3*S*)-MTCA was treated with nitrite. After destruction of nitrite with ammonium sulfamate, the reaction mixture was extracted with two 2-ml portions of ethyl acetate and the extracts were combined and evaporated at 20°C. The residue was dissolved in 1 ml of methanol and subjected to HPLC on an LS-310 silica gel column (21.5 × 300 mm; Toyo Soda). Material was eluted with methanol at a flow rate of 6 ml/min at ambient temperature, and the absorbance of the eluate at 270 nm was monitored. The eluate was evaporated at 20°C, and the residue was subjected to the mutation test on TA100 without S9 mixture.

Synthesis of (−)-(1*S*,3*S*)-1-Methyl-2-nitroso-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid. A 100-mg sample of (−)-(1*S*,3*S*)-MTCA was treated with nitrite as described for isolation of precursors and synthetic precursors, and the reaction mixture was extracted with two 100-ml portions of ethyl acetate. The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated. The residue (95 mg) was crystallized from 50% methanol to yield 77 mg of yellow plates. The mass spectra of this compound and its methyl ester derivative, obtained by treatment with diazomethane prepared from *p*-toluenesulfonyl-*N*-methyl-*N*-nitrosamide (Tokyo Kasei Kogyo, Tokyo), showed molecular ions at m/z 259 (M^+) and 273 (M^+), respectively. The UV spectrum of the crystallized material was as follows: λ_{max}^{MeOH} nm (log ε) 224 (4.67), 273 (4.06) (sh), 282 (3.99) (sh), 290 (3.89). The ^1H NMR spectrum indicated that this compound is present in two conformations with respect to the orientation of the nitroso group in the solution. The major/minor conformer ratio was about 4 at room temperature. The chemical shifts were δ($C^2H_3O^2H$) CH_3, 1.58 and 1.89 (minor); CH_2CH, 2.98 (minor) and 3.20; $CHCOOH$, 3.50 (minor) and 3.76; CH_3CH, 5.81 (minor) and 6.03; aromatic (4H), 6.99–7.48. These data suggest that this compound was (−)-(1*S*,3*S*)-1-methyl-2-nitroso-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid [(−)-(1*S*,3*S*)-MNTCA]. The structure was confirmed by x-ray crystallography.

Spectral Measurements. ^1H NMR spectra were measured with a Bruker CXP-300 spectrometer operated at 300 MHz. High- and low-resolution mass spectra were measured with JEOL O1SG-2 and Hitachi RMU-6M spectrometers, respectively. UV spectra were obtained with a Hitachi 320 spectrophotometer. Optical rotations were recorded on a JASCO DIP-181 digital polarimeter.

Quantitative Analyses of (−)-(1*S*,3*S*)- and (−)-(1*R*,3*S*)-MTCA in Various Kinds of Soy Sauce by HPLC. Samples (50 μl) of soy sauce were loaded on a silica gel $60F_{254}$ plate (5 × 10 cm; Merck, Darmstadt) and the plate was developed with chloroform/methanol, 2:8 (vol/vol). The region (R_f 0.25–0.75) including (−)-(1*S*,3*S*)- and (−)-(1*R*,3*S*)-MTCA was scraped off and extracted with 5 ml of ethyl acetate/methanol, 1:1 (vol/vol) by mixing in a Vortex for 1 min. The mixture was centrifuged at 2,000 rpm for 10 min, and the precipitate was extracted twice more. The supernatants were pooled and evaporated to dryness, and the residue was dissolved in 1.5 ml of 20% methanol and passed through a Millipore FH filter (0.50 μm) to remove insoluble material. The filtrate was evaporated, and the residue was dissolved in 200 μl of 20% methanol. This solution was analyzed quantitatively for (−)-(1*S*,3*S*)-MTCA and (−)-(1*R*,3*S*)-MTCA by HPLC. When a 50-μl sample of soy sauce was supplemented with 22 or 11 μg of (−)-(1*S*,3*S*)-MTCA, the average recovery of this compound was 97%. The recovery of exogenous (−)-(1*R*,3*S*) isomer was 83% when 5 or 2.5 μg of this compound was added to 50 μl of soy sauce. The minimum detectable amounts of (−)-(1*S*,3*S*)-MTCA and (−)-(1*R*,3*S*)-MTCA were both 0.1 μg/50 μl of soy sauce. Conditions of HPLC were as follows: column, LS-410 (4 × 300 mm); flow rate, 1 ml/min; eluant, 20% methanol; detection, 270 nm; temperature, room temperature.

RESULTS

Mutagenicities of Various Foods after Nitrite Treatment. The mutagenicities of various foods after nitrite treatment are shown in Table 1. Of those tested, soy sauce was especially mutagenic: 1 ml of soy sauce A produced in Japan induced 25,200 revertants of TA100 without S9 mixture. Bean paste was mutagenic but fermented soybeans (*natto*) were not, although both are soybean fermentation products. Bean curd, bean milk, and boiled green soybeans, which are also soybean products, were not mutagenic. Fermented rice products, such as Japanese sake and rice paste, were not mutagenic. Three kinds of fish sauce produced by fermentation of fish were mutagenic, but their mutagenicities were less than that of soy sauce. Worcestershire

Table 1. Appearance of mutagenicity in food products after nitrite treatment

Food	Revertants
Soybean product	
Soy sauce*	25,200
Bean paste	6,200
Fish sauce	
Japanese	2,300
Philippine	3,000
Thai	9,400
Beans	
Fava	2,100

Mutagenicity was assayed on TA100 without S9 mixture. Results are per ml or g of food.
* Soy sauce A in Table 2.

sauce and processed, brie, and blue cheeses were not mutagenic. Soybeans were not mutagenic, although fava beans were, as reported by Piacek-Llanes and Tannenbaum (21). Red beans and mottled kidney beans also were not mutagenic.

Since soy sauce A had a marked direct-acting mutagenic effect, various kinds of soy sauce were tested for mutagenicity after treatment with nitrite. The results are given in Table 2. All soy sauces produced in Japan, except soy sauce G, were strongly mutagenic, while all those produced in the United States, except M, were only slightly mutagenic. It is noteworthy that soy sauce M was as mutagenic as the Japanese products. Soy sauce J was not mutagenic.

Isolation and Identification of Precursors of Mutagenic Compounds in Soy Sauce. Precursors of mutagens that became mutagenic after treatment with nitrite were separated from soy sauce A. The results of the purification are summarized in Table 3.

At the step of HPLC on an LS-410 column, the relative intensities of UV absorption and relative mutagenic activities of the fractions in the two major peaks coincided well, as shown in Fig. 1. White crystalline compounds **I** and **II** were obtained as precursors of mutagens from peaks I and II, respectively. Nineteen and 2% of the mutagenic activity of the original soy sauce A after treatment with nitrite were recovered as compounds **I** and **II**, respectively (Table 3).

Table 2. Mutagenicity after treatment with nitrite and total amounts of (−)-(1*S*,3*S*)- and (−)-(1*R*,3*S*)-MTCA in various kinds of soy sauce

Soy sauce	Country and state of production	Revertants, no./ml	MTCA,* μg/ml	% mutagenicity explained by MTCA
A	Japan, Chiba	25,200	668	44
B	Japan, Chiba	18,300	678	61
C	Japan, Chiba	24,300	378	26
D	Japan, Mie	9,600	95	16
E	Japan, Chiba	22,200	604	45
F	Japan, Chiba	24,900	521	34
G	Japan, Aichi	2,700	82	50
H	Japan, Mie	17,300	275	26
I	USA, Hawaii	4,100	55	22
J	USA, Hawaii	0	<4†	
K	USA, Hawaii	2,300	34	24
L	USA, Ohio	6,100	<4†	
M	USA, Wisconsin	26,600	711	44

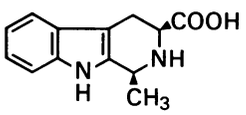
Mutagenicity was assayed on TA100 without S9 mixture.
* The ratio of (−)-(1*S*,3*S*)-MTCA to (−)-(1*R*,3*S*)-MTCA was about 4 in every case.
† Minimum amount detectable by HPLC.

Table 3. Purification of nitrosable precursors of mutagens in soy sauce A

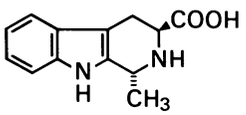
	Weight, mg	Specific activity, revertants per mg	Recovery of mutagenicity, %
Soy sauce (80 ml)	32,800	58.7	100
Charcoal column			
H_2O fraction	29,095	3	4
MeOH fraction	2,463	147	19
MeOH/CH_3COOH fraction	855	913	41
MeOH fraction	46	4,435	11
MeOH/NH_4OH fraction	793	179	7
XAD-7 column			
H_2O fraction	3,426	80	14
MeOH fraction	266	2,270	31
LS-110 column	68	8,220	29
LS-410 column			
Fraction I	19.4	18,300	19
Fraction II	4.2	10,400	2

Mutagenicity was assayed on TA100 without S9 mixture.

The mass spectrum of compound **I** showed a molecular ion at m/z 230. The elemental composition of compound **I** was found to be $C_{13}H_{14}N_2O_2$ (mass:calculated; 230.1055; found; 230.1045). The UV spectrum of this compound in methanol showed absorption maxima at 221, 271, 278, and 288 nm. The ^1H NMR spectrum of compound **I** indicated the presence of one methyl, one methylene, and two methine groups and four aromatic protons. The optical rotation of the compound was $[\alpha]_D^{25}$ −104° (*c* 0.045, 50% pyridine). From these observations, compound **I** was deduced to be (−)-(1*S*,3*S*)-MTCA. This deduction was confirmed by comparing its mass, UV, and ^1H NMR spectra and its optical rotation with those of the synthetic compound. The structure of compound **I** is shown below.



I



II

The mass and UV spectra of compound **II** were superimposable on those of compound **I**, but the optical rotation was $[\alpha]_D^{25}$ −68° (*c* 0.050, 50% pyridine). From these data, compound **II** was concluded to be a stereoisomer of compound **I**. The physical properties of compound **II** were identical with those of synthetic (−)-(1*R*,3*S*)-MTCA.

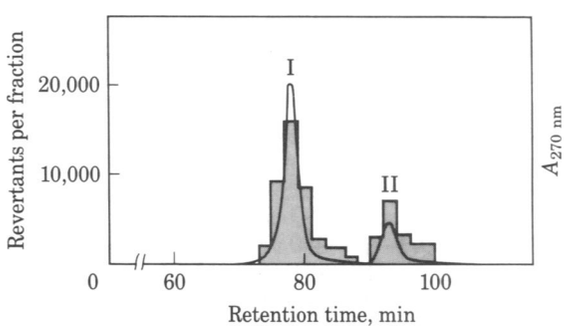


FIG. 1. HPLC patterns of nitrosable precursors of mutagens on a column of LS-410.

The mutagenicities of synthetic (−)-(1S,3S)- and (−)-(1R,3S)-MTCA after treatment with nitrite were tested. (−)-(1S,3S)-MTCA induced 1,740 and 140 revertants of TA100 and TA98, respectively, per 100 μg without S9 mixture, while (−)-(1R,3S)-MTCA induced 1,300 and 240, respectively. The mutagenic effects of these two compounds were reduced by the addition of S9 mixture. (−)-(1S,3S)- and (−)-(1R,3S)-MTCA were themselves not mutagenic to TA100 and TA98 either with or without S9 mixture. The maximum mutagenicities of (−)-(1S,3S)- and (−)-(1R,3S)-MTCA on TA100 without S9 mixture at 37°C were obtained after 20 min and 10 min treatment with nitrite, respectively, and remained at plateaus on treatment for up to 120 min.

Mutagen(s) Formed from (−)-(1S,3S)-MTCA with Nitrite. (−)-(1S,3S)-MTCA was treated with nitrite, and then the reaction mixture was extracted with ethyl acetate. More than 90% of the mutagenic activity produced was recovered in the ethyl acetate layer. This ethyl acetate fraction was separated by HPLC. The mutagenic activity was observed in fractions eluted before most of the UV absorbing material, which was not mutagenic. The recovery of the mutagenic activity was 95% of the total applied. The activity of a methanol solution of the mutagenic material decreased by half within 20 hr at room temperature.

The structure of the main UV-absorbing compound was also examined. The UV and mass spectra of its methyl derivative (produced by treatment with diazomethane) were identical with those of authentic (−)-(1S,3S)-MNTCA.

Amounts of (−)-(1S,3S)-MTCA and (−)-(1R,3S)-MTCA in Various Kinds of Soy Sauce. The amounts of (−)-(1S,3S)- and (−)-(1R,3S)-MTCA in various kinds of soy sauce were examined by HPLC, and the values corrected for the recovery are shown in Table 2. Soy sauces A–F and H, which were strongly mutagenic after nitrite treatment, contained large amounts of (−)-(1S,3S)- and (−)-(1R,3S)-MTCA. The proportions of the mutagenic effect of soy sauce attributable to the mutagenicities of these two precursors were calculated from the mutagenicities of the synthetic compounds after treatment with nitrite (Table 2). Of the Japanese products, soy sauces A, B, E, and G contained sufficient (−)-(1S,3S)- and (−)-(1R,3S)-MTCA to explain about 50% of their mutagenicities.

(−)-(1S,3S)- and (−)-(1R,3S)-MTCA were present in only small amounts in soy sauces I and K, which were very weakly mutagenic, and were not detected in soy sauce J, which was not mutagenic after treatment with nitrite. Interestingly, MTCA isomers were also not found in soy sauce L, although this soy sauce was mutagenic after nitrite treatment. American soy sauce M, which was highly mutagenic, contained as much (−)-(1S,3S)- and (−)-(1R,3S)-MTCA as did Japanese products.

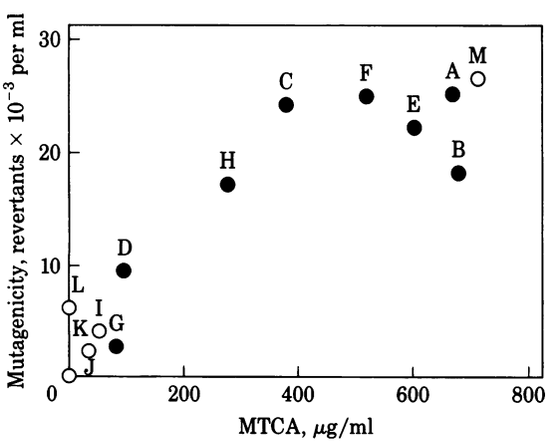


FIG. 2. Relationship between amounts of (−)-(1S,3S)- and (−)-(1R,3S)-MTCA and mutagenicities after nitrite treatment of various kinds of soy sauce produced in Japan (●) and the United States (○).

DISCUSSION

Soy sauce is taken with most Japanese foods. In this work, we found that it had a marked direct-acting mutagenic effect toward TA100 after nitrite treatment. (−)-(1S,3S)- and (−)-(1R,3S)-MTCA were isolated from soy sauce as precursors of the mutagen formed on nitrite treatment. These precursors were probably formed from L-tryptophan and acetaldehyde during fermentation and brewing of soy sauce. Soy sauce J, produced in Hawaii, is made by acid hydrolysis of soybeans, a procedure in which tryptophan should be degraded. This might be why soy sauce J does not contain (−)-(1S,3S)- and (−)-(1R,3S)-MTCA. The relationship between the amounts of the two isomers and the mutagenicities after nitrite treatment of various soy sauces is shown in Fig. 2. A good correlation was observed with all soy sauces except soy sauce L, which is a Chinese style sauce made in Ohio. The differences in the amounts of (−)-(1S,3S)- and (−)-(1R,3S)-MTCA in various soy sauces is presumably related to differences in the production processes. (−)-(1S,3S)- and (−)-(1R,3S)-MTCA can explain 44% of the mutagenicity of soy sauce A after nitrite treatment. L-Tryptophan, which must be a precursor of both isomers, has also been reported to be weakly mutagenic after nitrite treatment (25). However, the amount of L-tryptophan in soy sauce A can explain less than 1% of the total mutagenicity.

About 25 μg and 5 μg of (−)-(1S,3S)- and (−)-(1R,3S)-MTCA were detected in 1 g of bean paste produced in Japan and 1 ml of fish sauce produced in Thailand, respectively. However, these compounds were not found in all foods that were mutagenic after nitrite treatment: they were not found in soy sauce L (Chinese style), fish sauces produced in Japan and the Philippines, and fava beans.

Previously, Lin *et al.* (26) reported that soy sauces produced in Taiwan were mutagenic after nitrite treatment. However, their mutagenicities were higher with S9 mixture than without, contrary to the mutagenicities of nitrite-treated soy sauce A and nitrite-treated MTCA isomers.

It is important to isolate the direct-acting mutagen(s) produced by reaction of (−)-(1S,3S)-MTCA with nitrite. A major product, which is a N-2 nitroso-substituted compound, was found not to be mutagenic. Thus, we suspect that most of the mutagenicity is due to a minor product(s). Since, after treatment with nitrite, about 80% of (−)-(1S,3S)-MTCA was recovered as the nonmutagenic N-2 nitroso-substituted compound, the specific activity of the actual mutagenic product(s) must be very high; its specific activity seems to be similar to or more than that of *N*-nitrosomethylurea, which is 47 revertants per μg.

According to the data of the Food Agency of Japan in 1978, the average annual consumption of soy sauce is about 10 liters per person in Japan (27). Therefore, the total daily ingestion of (−)-(1S,3S)- and (−)-(1R,3S)-MTCA in soy sauce is calculated to be 2–18 mg per person, based on the contents of these compounds in soy sauces A–G.

It has been reported that various aldehydes are produced by peroxidation of unsaturated fatty acids and that they may react with tryptophan, tyrosine, cysteine, and histidine (28). In fact, β-carboline-3-carboxylates have been isolated from human urine and brain tissues (29). These observations suggest that (−)-(1S,3S)- and (−)-(1R,3S)-MTCA could be formed from L-tryptophan and acetaldehyde in the body.

A good correlation between nitrate intake per capita and mortality from gastric cancer in various countries has been reported (15). Although nitrate and its reduced product, nitrite, formed by microbes, do not induce gastric cancer (30), it is gen-

erally accepted that nitrosamine or nitrosamide formed *in vivo* from amines or amides could be a cause of human gastric cancer. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine, which is a direct-acting mutagen, induces gastric cancer in experimental animals (31). An extract of nitrite-treated fish, which shows direct-acting mutagenicity, induced tumors in the glandular stomachs of rats when given repeatedly by gastric intubation (32). We suspect that direct-acting mutagens produced from (−)-(1*S*,3*S*)- and (−)-(1*R*,3*S*)-MTCA and nitrite could be causes of human gastric cancer. Soy sauce is reported to be noncarcinogenic to rats (33), but the carcinogenicity of soy sauce with nitrite has not yet been tested. It is a very important and urgent problem to clarify the carcinogenicity of (−)-(1*S*,3*S*)- and (−)-(1*R*,3*S*)-MTCA and also of soy sauce in the presence of nitrite by long-term animal experiments.

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