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Determination of Origin of Ephedrine Used as Precursor for Illicit Methamphetamine by Carbon and Nitrogen Stable Isotope Ratio Analysis

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The sale of ephedrine, one of the precursors of methamphetamine, is strictly controlled and monitored in various countries to prevent the production of illicit methamphetamine. There are three kinds of production scheme for ephedrine manufacture, and it is very useful for precursor control to investigate the origin of ephedrine used for the synthesis of illicit methamphetamine. By means of stable isotope ratio mass spectrometry (IR-MS), we investigated the origin of ephedrine based on the δ^{13}C and δ^{15}N values. The various origins of ephedrine (biosynthetic, semisynthetic, or synthetic) could be discriminated clearly by using these values. The δ^{15}N values of synthetic ephedrine were more negative than those of ephedrine from other sources. By the repeated distillation of methylamine in our laboratory, we confirmed that this could be due to isotope separation during distillation for the purification of methylamine used for ephedrine synthesis. The values for ephedrine used as the precursor were well-correlated with those for methamphetamine synthesized from it. This drug characterization analysis should be useful to illuminate the origin of the precursors used for clandestine methamphetamine and to trace the diversion of medicinal ephedrine for illicit manufacture of methamphetamine.

Isotope ratio analyses at natural abundance levels have been used to establish the environmental source or the geographic origin of various biological and nonbiological materials.[1−4] Isotope ratio analyses have also been used for examination of legitimate items of commerce.[5,6] The natural isotopic variation of organic products in plants is fixed during biochemical synthesis, and the observed isotopic variations reflect differences in metabolic processes or environmental conditions during growth. For example, the carbon stable isotope ratio (δ^{13}C) values of plants grown under low-humidity or water-stressed conditions are more positive than those of plants grown under conditions of high humidity or high soil water.[7] Variations in plant nitrogen stable isotope ratio (δ^{15}N) values of 10‰ or more occur and represent a record of soil and microbial N_2 status.[8] Several reports have shown that δ^{13}C and δ^{15}N values for cocaine and heroin are valuable to trace their geographical origin.[9−11] Ephedrine, one of the precursors of methamphetamine, is produced in substantial amounts for medical purposes. Commercial ephedrine is produced by (a) extraction from ephedra plant, namely biosynthesis; (b) fully chemical synthesis; and (c) semisynthesis, as shown in Figure 1. Originally, ephedrine was commonly produced from ephedra plant, but recently, large amounts have been produced by synthesis or semisynthesis. On the other hand, illicit ephedrine is still generally thought to be produced from ephedra plants.

The abuse of amphetamine-type stimulants (ATS) is an increasing problem. In Asia, including Japan, the abuse of methamphetamine itself is the most serious problem. Trade in precursors of methamphetamine is strictly controlled and monitored in various countries, since methamphetamine can easily be clandestinely manufactured from its precursors. To prevent the production of illicit methamphetamine, it is important not only to control and monitor the trade in ephedrine, but also to evaluate the origin of

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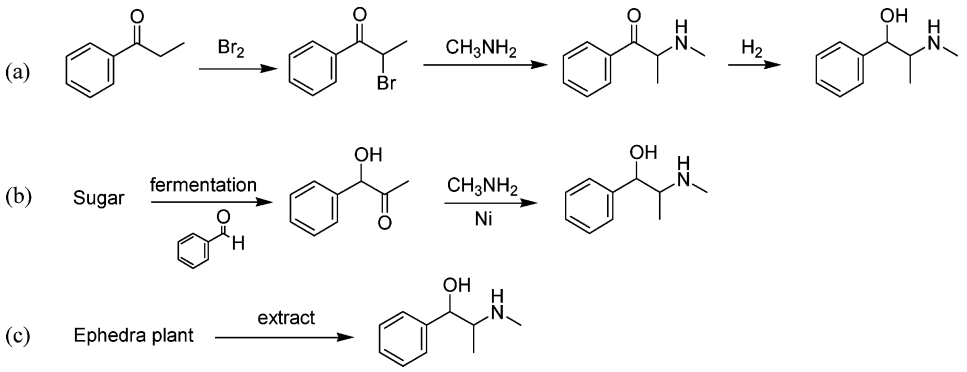

Figure 1. Production schemes of ephedrine: (a) bromination of propiophenone followed by amination, (b) fermentation of sugar followed by amination, and (c) extraction from ephedra plant.

ephedrine used as a precursor of seized methamphetamine by scientific analyses. It is possible to identify the precursor or the method of synthesis of methamphetamine by analyzing impurities in methamphetamine.[12−16] Analyses of impurities by HPLC have enabled identification of the kind of ephedrine used for the synthesis of seized methamphetamine,[17] but these methods are not sufficient to determine the origin of the ephedrine.

Chemically, no differences are observed among ephedrine samples produced by different methods. Nevertheless, the δ^{13}C and δ^{15}N values differ. Furthermore, the isotope ratio of biosynthetic ephedrine will be different depending upon the environmental conditions during growth of the ephedra plants. In this paper, we report the discrimination of the source of ephedrine on the basis of the δ^{13}C and δ^{15}N values using IR-MS, and we describe the relationship of the δ^{13}C and δ^{15}N values of the precursor ephedrine and those of methamphetamine synthesized from it.

EXPERIMENTAL SECTION

Materials and Chemicals. Thirteen ephedrine hydrochloride samples were used. Four of them, purchased from Fujiyakuhin (Saitama, Japan), Maruishi Pharmaceutical (Osaka, Japan) and Dainippon Pharmaceutical (Osaka, Japan), were medicines produced by bromination of propiophenone followed by amination[18] or by extraction from ephedra plants. Three other samples were produced by extraction from ephedra plants, and the rest were produced by fermentation of sugar followed by amination, namely semisynthesis.[19] A sample of medical methamphetamine hydrochloride was purchased from Dainippon Pharmaceutical (Osaka, Japan), and three methamphetamine hydrochloride samples were synthesized from ephedrine by the Nagai method or the Emde method, as shown in Figure 2, in our laboratory. Twenty-one illicit methamphetamine samples seized in Japan were also used. Methylamine hydrochloride was purchased from Sigma-Aldrich (MO).

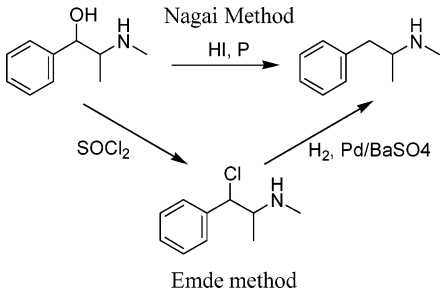

Figure 2. Synthetic pathways of methamphetamine from ephedrine in this study.

Instruments and IR-Mass Analysis. A stable isotope ratio mass spectrometer Delta^{Plus} (ThermoFinnigan, U.S.A.) equipped with an elemental analyzer Flash EA1112 (ThermoFinnigan, U.S.A.) was used for the measurements of carbon and nitrogen isotope ratios of samples. Individual samples (250 μg) wrapped in tin foil were flash-combusted on an elemental analyzer to afford CO_2, NO_x, and H_2O in an O_2 atmosphere in a quartz reactor packed with Cr_2O_3 on alumina and Co_3O_4/Ag. The gases were passed through a copper reactor to reduce NO_x to N_2. H_2O was trapped with $Mg(ClO_4)_2$. Then CO_2 and N_2 were separated on a GC column (Porapaq QS), and subjected to IR-MS to obtain the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios. The stable isotope ratios are expressed relative to the conventional standards, that is, Peedee Belemite for carbon and atmospheric N_2 for nitrogen. The δ value is defined according to the following equation:

$$\delta\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000$$

where $R = {}^{13}C/{}^{12}C$ or $^{15}N/^{14}N$. Each sample was measured five times. The precisions routinely obtained were 0.1‰ or less for δ^{13}C and 0.2‰ or less for δ^{15}N.

Distillation of Methylamine. Methylamine hydrochloride (10 g) was dissolved in 200 mL of 1 mol/L NaOH aq. The resulting free base was distilled by heating. The distillate was conducted into 1 mol/L HCl aq, where it was neutralized. The volume of distilled methylamine was monitored in terms of the pH of the HCl solution. The early distillate was collected, concentrated, and recrystallized from acetone. These procedures were repeated three times.

RESULTS AND DISCUSSION

δ^{13}C and δ^{15}N Values for Ephedrine. We investigated the origin of ephedrine on the basis of the δ^{13}C and δ^{15}N values

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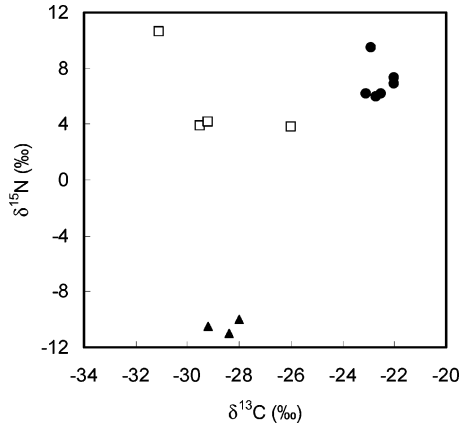

Figure 3. Carbon and nitrogen isotope ratios of ephedrine samples: natural (□), synthesized (▲), and semisynthetic (●).

obtained by using IR-MS. δ^{13}C and δ^{15}N values for ephedrine samples are given in Figure 3. The δ^{13}C values for synthetic medical ephedrine derived from propiophenone were −29.2 to −28.0‰, and those of biosynthetic ephedrine extracted from ephedra plants, known as C_3-photosynthetic plants, were −31.1 to −26.0‰. Typical values for natural organic compounds from C_3-photosynthetic plants are −28.1 ± 2.5,[20] and this range is essentially the same as that observed for biosynthetic ephedrine. It is thought that the variations of δ^{13}C values reflect the differences in humidity conditions of the areas where the source plants were grown. The δ^{13}C values for ephedrine produced by fermentation of sugar followed by amination, namely semisynthesis, were −23.1 to −22.0‰. The starting material for semisynthetic ephedrine is sugar extracted from C_4-photosynthetic plants such as sugarcane, in which typical δ^{13}C values are −13.5±1.5.[20] Acetaldehyde generated by sugar fermentation is condensed with benzaldehyde to form phenylacetyl carbinol. Phenylacetyl carbinol reacts with methylamine over a Ni catalyst to produce ephedrine. The δ^{13}C values of acetaldehyde from sugar are more positive than those of C_3-photosynthetic plants or products derived from petroleum.[21] It is supposed that acetaldehyde contributes 2 of the 10 carbon atoms in ephedrine. Thus, the value of δ^{13}C can be used to differentiate semisynthetic ephedrine from biosynthetic or synthetic ephedrine (Figure 3), but it does not discriminate biosynthetic ephedrine from synthetic ephedrine.

On the other hand, δ^{15}N showed remarkable differences: δ^{15}N values for semisynthetic or biosynthetic ephedrine were 3.8 to 10.6‰, and those for synthetic ephedrine were −10.5 to −10.0‰. This is because the origins of the ephedrine nitrogen are different. The source of nitrogen in both synthetic and semisynthetic ephedrine is methylamine, as indicated in Figure 1, but the source of nitrogen in the biosynthetic material is nitrate or ammonia in soil. In the case of synthetic ephedrine, it is considered that isotope separation occurs during distillation for purification of methylamine used for ephedrine synthesis. Figure 4 shows the variation of the δ^{15}N values of methylamine during successive distillations in our laboratory. The δ^{15}N value changed from −5.4 to −7.6‰ after the first distillation, from −7.6 to −9.4‰ after the second distillation, and from −9.4 to −11.9‰ after the third distillation. This supports the above hypothesis.

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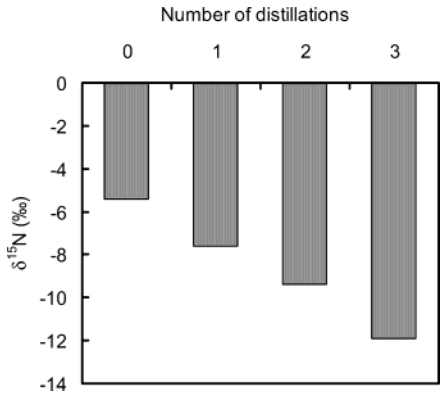

Figure 4. The variation of δ^{15}N values of methylamine with increasing number of distillations.

Table 1. The δ^{13}C and δ^{15}N Values of Ephedrines Used as Precursors of Methamphetamine Synthesis and Methamphetamine Synthesized from the Ephedrine

compound	synthetic pathway	δ^{13}C (‰)	δ^{15}N (‰)
methamphetamine	Nagai	−29.2	−11.1
ephedrine (synthetic)		−29.2	−10.5
methamphetamine	Nagai	−23.0	9.5
ephedrine (semisynthetic)		−22.9	9.5
methamphetamine	Nagai	−23.1	5.8
ephedrine (semisynthetic)		−23.1	6.2
methamphetamine	Emde	−26.1	4.4
ephedrine (natural)		−26.0	3.8
methamphetamine	Nagai	−29.8	3.7
ephedrine (natural)		−29.5	3.9
methamphetamine	Nagai	−31.4	10.9
ephedrine (natural)		−31.1	10.6
methamphetamine	Nagai	−29.5	3.9
ephedrine (natural)		−29.2	4.2

Thus, as shown in Figure 3, ephedrine samples prepared in the three ways could be clearly discriminated by using both δ^{13}C and δ^{15}N values.

Relationship of δ^{13}C and δ^{15}N Values for Ephedrine and Methamphetamine. Table 1 summarizes the δ^{13}C and δ^{15}N values for ephedrine precursors and for methamphetamine synthesized from them. The δ^{13}C and δ^{15}N values for ephedrine were well-correlated with those for methamphetamine; therefore, it is possible to know what kind of ephedrine was used as a precursor by measuring the values of δ^{13}C and δ^{15}N of methamphetamine.

***δ*^{13}C and *δ*^{15}N Values for Methamphetamine.** Figure 5 shows δ^{13}C and δ^{15}N values of seized methamphetamine samples together with those of medical methamphetamine and those of methamphetamine synthesized from ephedrine in our laboratory, as indicated in Table 1. Values of δ^{13}C and δ^{15}N of seized methamphetamine were in the ranges of −33.1 to −25.5‰ and +3.3 to +7.4‰, respectively. Although δ^{13}C and δ^{15}N each showed a distribution, the values of illicit samples seized on a given occasion were all similar. These results indicate that analysis of δ^{13}C and δ^{15}N can provide a means to distinguish apparently similar samples seized on different occasions. The δ^{13}C and δ^{15}N values of a medical methamphetamine sample were −25.5 and +3.4‰. It can be inferred that the medical methamphetamine and all seized methamphetamine samples examined in this study were synthesized from ephedrine extracted from

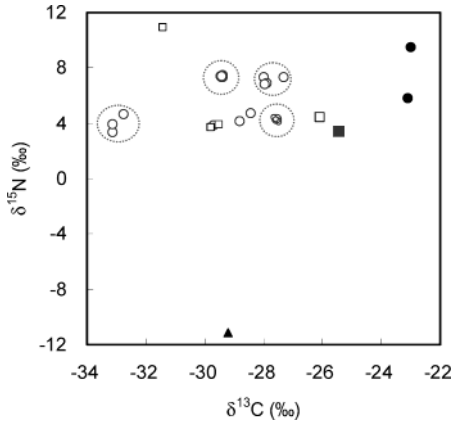

Figure 5. Carbon and nitrogen isotope ratios of methamphetamine samples: seized (○), synthesized from natural ephedrine (□), synthesized from synthetic ephedrine (▲), synthesized from semisynthetic ephedrine (●), and commercial methamphetamine (■). Dotted circles indicate samples that were seized on the same occasion.

ephedra plants, not from synthetic ephedrine. Further, as the isotope ratios of plants differ depending on their growing location and conditions, the variations of $\delta^{13}C$ and $\delta^{15}N$ among the seized samples must reflect differences in the source of the ephedra plants. Thus, by using this IR-MS technique, we can further distinguish for the first time among samples that do not show marked differences in their impurity profiles. Therefore, it is possible to obtain a great deal of information about illicit methamphetamine by measuring the values of $\delta^{13}C$ and $\delta^{15}N$.

CONCLUSIONS

It has been shown that the origins of ephedrine can be discriminated by $\delta^{13}C$ and $\delta^{15}N$ analysis and that the values of the precursor ephedrine are reflected in those of methamphetamine obtained from it. The more negative $\delta^{15}N$ values of synthetic ephedrine appear to be due to isotope separation during distillation for purification of methylamine used in ephedrine synthesis. Thus, $\delta^{13}C$ and $\delta^{15}N$ analysis of methamphetamine can tell us the origin of the precursor ephedrine. In addition, this analysis makes it possible to distinguish methamphetamine samples seized on different occasions, even if they are difficult to discriminate on the basis of their impurity profiles. It is impossible to get such information by other techniques; therefore, this technique will be very useful for profiling of methamphetamine and monitoring the sources of ephedrine used for clandestine manufacture of methamphetamine. In the future, it may be possible to establish a relationship between the sites where ephedra plants are grown and the $\delta^{13}C$ and $\delta^{15}N$ values of ephedrine.

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