

# Determination of Stable Carbon Isotopic Compositions of Low Molecular Weight Dicarboxylic Acids and Ketocarboxylic Acids in Atmospheric Aerosol and Snow Samples

Kimitaka Kawamura\* and Tomomi Watanabe

Institute of Low Temperature Science, Hokkaido University, N19 W8, Kita-ku, Sapporo 060-0819, Japan

**We report a new method developed for the determination of stable carbon isotopic composition of homologous  $\alpha,\omega$ -dicarboxylic acids and phthalic acid isolated from environmental samples such as atmospheric aerosols and snow. Dicarboxylic acids are derivatized with  $\text{BF}_3/1$ -butanol to dibutyl esters, which are analyzed for the stable carbon isotopic composition using a capillary GC interfaced to on-line combustion isotope ratio mass spectrometer. The  $\delta^{13}\text{C}$  values for individual dicarboxylic acid are then calculated from  $\delta^{13}\text{C}$  of 1-butanol and butyl ester derivative using a mass balance equation. The accuracy of the  $\delta^{13}\text{C}$  measurement for  $\text{C}_2$ – $\text{C}_{10}$  diacids is within 0.8‰. We report a few examples of the  $\delta^{13}\text{C}$  ratios of saturated  $\text{C}_2$ – $\text{C}_9$   $\alpha,\omega$ -dicarboxylic acids, unsaturated (maleic, phthalic) diacids, and oxocarboxylic acids in the aerosol and snow samples.**

Low molecular weight (LMW) dicarboxylic acids are one of the most abundant organic compound classes present in the atmospheric aerosols.<sup>1–3</sup> Because of the hygroscopic properties, diacids have been considered to act as cloud condensation nuclei and to play an important role in altering the chemical and physical properties of atmospheric particles.<sup>4</sup> LMW dicarboxylic acids, thus, have a potential impact on the radiative forcing of the Earth's climate. LMW diacids including oxalic acid are emitted into the atmosphere through incomplete combustion of fossil fuels<sup>5</sup> and biomass burning.<sup>6</sup> However, a major portion of the atmospheric dicarboxylic acids is produced in situ by the atmospheric photochemical oxidation of anthropogenic hydrocarbons as well as biogenic organic matter.<sup>2</sup>

Recently, the stable carbon isotopic composition of individual organic molecules in geochemical samples has been studied using gas chromatography/isotope ratio mass spectrometry (GC/

irMS).<sup>7</sup> This technique has been used for *n*-alkanes, alcohols, and fatty acids in the atmospheric aerosols, and their isotopic ratios provided source information on the contribution of  $\text{C}_3$  and  $\text{C}_4$  plants to organic aerosols.<sup>8–10</sup> The GC/irMS technique has also been applied to gaseous hydrocarbons in the air.<sup>11–13</sup> These studies demonstrated that isotopic ratios of the light hydrocarbons are primarily dependent on the intrinsic isotopic ratios of sources but are secondarily controlled by atmospheric OH oxidation. In fact, stable carbon isotopic ratios of ethane and other hydrocarbons are enriched in  $^{13}\text{C}$  as a result of photochemical oxidation in the atmosphere.<sup>11–13</sup>

Carbon isotopic ratios of LMW polar organic compounds have rarely been studied. Monocarboxylic acids ( $\text{C}_2$ – $\text{C}_5$ ) isolated from carbonaceous chondrite were determined for carbon isotopic composition,<sup>14</sup> where the organic acids are characterized by heavier isotopic ratios up to +22‰. Formic and acetic acids in rainwater from Los Angeles were determined for the carbon isotope ratios by employing an ion chromatographic isolation followed by the conventional oxidation to  $\text{CO}_2$  and mass spectral analysis.<sup>15</sup> More recently, GC/irMS technique has been utilized to determine the carbon isotopic ratios of carboxyl carbon and alkyl carbon in volatile organic acids such as formic and acetic acids in the environmental samples.<sup>16–18</sup> However, there is no report on the carbon isotopic ratios of homologous  $\alpha,\omega$ -dicarboxylic acids and aromatic diacids in the environmental samples, although carbon isotopic composition of oxalic acid has been reported in marine aerosols.<sup>19</sup>

\* Corresponding author. Fax: 81-11-706-7142. E-mail: kawamura@lowtem.hokudai.ac.jp.

- (1) Kawamura, K.; Ikushima, K. *Environ. Sci. Technol.* **1993**, *27*, 2227–2235.
- (2) Kawamura, K.; Kasukabe, H.; Barrie, L. A. *Atmos. Environ.* **1996**, *30*, 1709–1722.
- (3) Kawamura, K.; Sakaguchi, F. *J. Geophys. Res.* **1999**, *104*, 3501–3509.
- (4) Saxena, P.; Hildemann, L. M.; McMurry, P. H.; Seinfeld, J. H. *J. Geophys. Res.* **1995**, *100*, 18755–18770.
- (5) Kawamura, K.; Kaplan, I. R. *Environ. Sci. Technol.* **1986**, *21*, 105–110.
- (6) Narukawa, M.; Kawamura, K.; Takeuchi, N.; Nakajima, T. *Geophys. Res. Lett.* **1999**, *26*, 3101–3104.

- (7) Hayes, J. M.; Freeman, K. H.; Popp, B. N.; Hoham, C. H. *Org. Geochem.* **1990**, *16*, 1115–1128.
- (8) Simoneit, B. R. T. *Atmos. Environ.* **1997**, *31*, 2225–2233.
- (9) Conte, M. H.; Weber, J. C. *Nature* **2002**, *417*, 639–641.
- (10) Fang, J.; K. Kawamura, Ishimura, Y.; Matsumoto, K. *Environ. Sci. Technol.* **2002**, *36*, 2598–2604.
- (11) Rudolph, J. E.; Lowe, D. C.; Martin, R. J.; Clarkson, T. S. *Geophys. Res. Lett.* **1997**, *24*, 659–662.
- (12) Tsunogai, U.; Yoshida, N.; Gamo, T. J. *J. Geophys. Res.* **1999**, *104*, 16, 033–16, 039.
- (13) Saito, T.; Tsunogai, U.; Kawamura, K.; Nakatsuka, T.; Yoshida, N. *J. Geophys. Res.* **2002**, *107*, D4, ACH 2–1 to 2–9.
- (14) Yuen, G.; Blair, N.; Des Marais, D. J.; Change, S. *Nature* **1984**, *307*, 252–254.
- (15) Sakugawa, H.; Kaplan, I. R. *Geophys. Res. Lett.* **1995**, *22*, 1509–1512.
- (16) Dias, R. F.; Freeman, K. H. *Anal. Chem.* **1997**, *69*, 944–950.
- (17) Dias, R. F.; Freeman, K. H.; Franks, S. G. *Org. Geochem.* **2002**, *33*, 161–168.
- (18) Yamada, K.; Tanaka, M.; Nakagawa, F.; Yoshida, N. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1059–1064.

In this study, we developed a new analytical technique to measure stable carbon isotopic composition of homologous series of dicarboxylic acids and related compounds and applied to the environmental samples including atmospheric aerosols and snow. Here, we report a novel method to determine stable carbon isotopic composition of homologous C<sub>2</sub>–C<sub>10</sub> dicarboxylic acids using a capillary GC/isotope ratio monitoring mass spectrometry employing dibutyl ester derivatization technique.

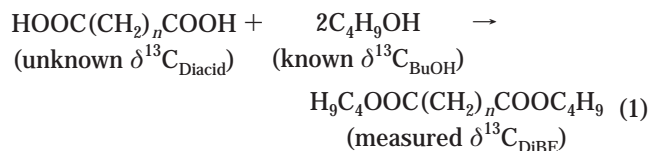
## EXPERIMENTAL SECTION

**Principle of  $\delta^{13}\text{C}$  Measurement for Dicarboxylic Acids Using GC/irMS.** Dicarboxylic acids either from atmospheric samples or from authentic standards were reacted with 14% BF<sub>3</sub> in 1-butanol at 100 °C for 30 min to derive dibutyl esters. Prior to the stable carbon isotopic analyses, the esters were determined using a capillary GC-FID to obtain their concentrations. After an appropriate amount of internal standard (*n*-C<sub>13</sub> alkane) was spiked to the ester fraction, the esters were injected to a gas chromatograph interfaced to isotope ratio mass spectrometer (Finnigan-MAT Delta plus) to determine their stable carbon isotopic composition. A Hewlett-Packard GC (HP 6890) installed with a HP manual on-column injector and a capillary column (CIP-Sil 8CB, 60 m × 0.32 mm × 0.25 μm) was used with a column oven temperature programmed from 50 to 120 °C at 30 °C/min and then to 300 °C at 6 °C/min. Flow rate of carrier gas (He) was maintained at 1.7 mL/min.

The esters and internal standard separated on GC column were transferred to a combustion furnace (0.3 mm i.d. × 30 cm long) and oxidized on-line to CO<sub>2</sub> at 850 °C. The resulting CO<sub>2</sub> gas was transferred to the mass spectrometer to monitor the stable isotopic composition of CO<sub>2</sub> at *m/z* 44 (<sup>12</sup>C<sup>16</sup>O<sub>2</sub>), 45 (<sup>13</sup>C<sup>16</sup>O<sub>2</sub> and <sup>12</sup>C<sup>16</sup>O<sup>17</sup>O), and 46 (<sup>12</sup>C<sup>16</sup>O<sup>18</sup>O, <sup>12</sup>C<sup>17</sup>O<sup>17</sup>O, and <sup>13</sup>C<sup>16</sup>O<sup>17</sup>O). The signals were amplified and corrected for the presence of <sup>17</sup>O at mass 45 using the Craig correction.<sup>20</sup> The isotopic analytical precision was ≤0.5‰ for a peak height (*m/z* 44) of 0.2–8 V. The isotopic composition of diacids and their derivatives is reported in the  $\delta$  notation relative to the Pee Dee Belemnite (PDB) standard<sup>20</sup> as follows:

$$\delta^{13}\text{C} (\text{‰}) = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{sample}}/({}^{13}\text{C}/{}^{12}\text{C})_{\text{PDB}} - 1] \times 10^3$$

After the measurement of isotopic compositions of butyl esters,  $\delta^{13}\text{C}$  of individual diacids are calculated by the following mass balance equation.



$$\delta^{13}\text{C}_{\text{DiBE}} = f_{\text{Diacid}}\delta^{13}\text{C}_{\text{Diacid}} + f_{\text{BuOH}}\delta^{13}\text{C}_{\text{BuOH}} \quad (2)$$

where  $\delta^{13}\text{C}_{\text{Diacid}}$ ,  $\delta^{13}\text{C}_{\text{BuOH}}$ , and  $\delta^{13}\text{C}_{\text{DiBE}}$  are the carbon isotopic composition of diacid, 1-butanol, and diacid dibutyl ester, respectively, whereas  $f_{\text{Diacid}}$  and  $f_{\text{BuOH}}$  are fractions of carbon in the ester

Table 1. Molar Fractions of Original Compound Carbon (Dicarboxylic Acids, Ketocarboxylic Acids,  $\alpha$ -Dicarbonyls) and 1-Butanol-Derived Carbon in the Derivatives (Dibutyl Esters or Dibutoxy Acetals)

diacids, abbr	carbon nos		fraction	
	original compds	derivs	$f_{\text{original}}$	$f_{\text{BuOH}}$
oxalic acid, C <sub>2</sub>	2	10	0.200	0.800
malonic acid, C <sub>3</sub>	3	11	0.273	0.727
succinic acid, C <sub>4</sub>	4	12	0.333	0.667
glutaric acid, C <sub>5</sub>	5	13	0.385	0.615
adipic acid, C <sub>6</sub>	6	14	0.429	0.571
pimelic acid, C <sub>7</sub>	7	15	0.467	0.533
suberic acid, C <sub>8</sub>	8	16	0.500	0.500
azelaic acid, C <sub>9</sub>	9	17	0.529	0.471
sebacic acid, C <sub>10</sub>	10	18	0.556	0.444
methylmalonic acid, iC <sub>4</sub>	4	12	0.333	0.667
methylsuccinic acid, iC <sub>5</sub>	5	13	0.385	0.615
2-methylglutaric acid, iC <sub>6</sub>	6	14	0.429	0.571
maleic acid, M	4	12	0.333	0.667
fumaric acid, F	4	12	0.333	0.667
methylmaleic acid, mM	5	13	0.385	0.615
phthalic acid, Ph	8	16	0.500	0.500
ketomalonic acid, kC <sub>3</sub>	3	11	0.273	0.727
4-ketopimelic acid, kC <sub>7</sub>	7	15	0.467	0.533
malic acid, hC <sub>4</sub>	4	12	0.333	0.667
pyruvic acid, Pyr	3	15	0.200	0.800
2-oxoethanoic (glyoxylic) acid, $\omega\text{C}_2$	2	14	0.143	0.857
3-oxopropanoic acid, $\omega\text{C}_3$	3	15	0.200	0.800
4-oxobutanoic acid, $\omega\text{C}_4$	4	16	0.250	0.750
glyoxal, Gly	2	18	0.111	0.889
methylglyoxal, MeGly	3	11	0.273	0.727

derived from diacid and butanol, respectively. In the case of oxalic acid,  $f_{\text{Diacid}}$  and  $f_{\text{BuOH}}$  are 0.2 and 0.8, respectively. Table 1 gives molar fractions of original compound carbon and butanol-derived carbon in the derivatives of various organic compounds studied.

To check the GC/irMS conditions, a mixture of normal C<sub>15</sub>–C<sub>34</sub> alkanes having known stable carbon isotopic compositions was injected into the GC/irMS and their  $\delta^{13}\text{C}$  values were calculated using *n*-C<sub>22</sub> alkane as an internal standard. The calculated isotopic ratios were compared with their own  $\delta^{13}\text{C}$  values, which have been determined individually using a conventional method (off-line oxidation followed by irMS measurement<sup>21</sup>). This standard mixture was used as a working standard and injected everyday into a GC/irMS system to monitor the instrumental conditions. Prior to the run of actual samples, we confirmed that the  $\delta^{13}\text{C}$  values of the working standards were equivalent to the theoretical values within an analytical error of 0.2‰.

**Determination of  $\delta^{13}\text{C}$  of 1-Butanol and Dicarboxylic Acids.** The  $\delta^{13}\text{C}$  value of 1-butanol, a derivatizing reagent, was determined using a Carlo Erba NA 1500 elemental analyzer (EA) interfaced to Finnigan MAT Delta plus isotope ratios mass spectrometer (EA/irMS), as follows. About 0.3–0.5 μL of 14% BF<sub>3</sub>/1-butanol (Alltech Associates) was transferred onto ~200 mg of Chromosorb (ThermoQuest, 80–100 mesh) that was placed within a tin cup (ThermoQuest; 8 mm diameter × 8 mm high). The tin cup was pre-extracted with acetone to remove potential organic contaminants, dried in an oven, and stored in a glass vial with a Teflon-lined cap. The 1-butanol adsorbed on the Chromosorb in the tin cup was combusted in the combustion furnace (set

(19) Turekian, V. C.; Macko, S. A.; Keene, W. C. *J. Geophys. Res.* **2003**, *108*, NO. D5, 4157.

(20) Craig, H. *Geochim. Cosmochim. Acta* **1957**, *12*, 133–149.

(21) Ishiwatari, R.; Uzaki, M.; Yamada, K. *Org. Geochem.* **1994**, *21*, 801–808.

temperature of 850 °C, but actual temperature jumps up to 1400 °C as a result of the reaction between tin and oxygen). The resulting CO<sub>2</sub> was purified on a packed GC column installed in the EA system and then determined with a thermal conductivity detector. An aliquot of purified CO<sub>2</sub> was transferred to a mass spectrometer through a Conflow II interface (Thermoquest).

The reagent (BF<sub>3</sub>/1-butanol) was repeatedly (*n* = 5) measured for the stable carbon isotopic composition. The isotopic values were corrected for the procedural blank of CO<sub>2</sub> (tin cup + Chromosorb + EA/irMS system blank), which is typically 1% or less than that of the 1-butanol reagent. The  $\delta^{13}\text{C}$  value of 1-butanol was then used to calculate the stable carbon isotopic ratio of diacids from the  $\delta^{13}\text{C}$  values of esters using a mass balance equation (eq 2).

As a candidate for the internal standard to be coinjected to GC/irMS with dibutyl esters, several normal alkanes (C<sub>13</sub>, C<sub>15</sub>, C<sub>18</sub>, C<sub>25</sub>) and 5 $\alpha$ -cholestane were measured for their stable carbon isotopic ratios using EA/irMS by the method stated above. By comparing the GC retention times of *n*-alkanes with those of diacid dibutyl esters from an authentic standard as well as real environmental samples, we decided to use *n*-C<sub>13</sub> alkane as an internal standard because this compound does not coelute with any diacid dibutyl esters or any other peaks on the GC chromatogram of real samples. The alkane (*n*-C<sub>13</sub>) elutes ~60 s before the peak of oxalic acid dibutyl ester on the GC chromatogram.

Authentic  $\alpha,\omega$ -dicarboxylic acids (C<sub>2</sub>–C<sub>10</sub>) including branched-chain C<sub>4</sub>–C<sub>6</sub> species as well as aromatic (phthalic) acid were purchased from either Wako Pure Chemicals (Doshomachi 3-chome, chuo-ku, Osaka, Japan) or Tokyo Kasei (Nihonbashi-honcho, chuo-ku, Tokyo, Japan). The stable carbon isotopic ratios of diacids were individually determined as follows. A total of 300–600  $\mu\text{g}$  of each standard was taken into a small tin cup (5-mm diameter, 5-mm height) and analyzed in triplicate for  $\delta^{13}\text{C}$  values by EA/irMS as stated above. To check for isotopic fractionation during esterification, an authentic dicarboxylic acid (C<sub>2</sub>–C<sub>10</sub>) standard solution was prepared with pure water and derivatized with BF<sub>3</sub>/1-butanol as stated above. The esters were repeatedly analyzed for their  $\delta^{13}\text{C}$  ratios. Further, authentic diacid (C<sub>2</sub>–C<sub>6</sub>; ~500 ng each) standard solution was spiked onto a precombusted quartz filter. The spiked filter was extracted three times with pure water, and the extracts were derivatized to butyl esters.<sup>1,22</sup> The esters were analyzed with GC/FID for the recovery and with GC/irMS for the  $\delta^{13}\text{C}$  of diacids.

**Application to Environmental Samples.** Aerosol samples were collected in Sapporo using a high-volume air sampler (Kimoto AS-800) and precombusted quartz fiber filter (Pallflex) on the roof of Institute of Low-Temperature Science, Hokkaido University. Filter samples were stored in a clean glass jar (150 mL) with a Teflon-lined cap in a freezer (–20 °C) room prior to analysis. Fresh snow samples were also collected on the rooftop of the Institute building and stored in a clean glass bottle with mercuric chloride after the melting. Melted snow samples were stored at 4 °C prior to analysis.

An aliquot of filter sample was extracted with organic-free pure water to separate low molecular weight dicarboxylic acids and related compounds.<sup>1,22</sup> The extracts were concentrated with a rotary evaporator under a vacuum and passed through a Pasteur

Table 2. EA/irMS Measurements of Stable Carbon Isotopic Composition ( $\delta^{13}\text{C}$ ) of Authentic *n*-Alkanes, 1-Butanol, and Dicarboxylic Acids<sup>a</sup>

authentic compounds	$\delta^{13}\text{C}$ , ‰
<i>n</i> -tridecane, <i>n</i> -C <sub>13</sub>	–27.24 ± 0.04
<i>n</i> -pentadecane, <i>n</i> -C <sub>15</sub>	–30.05 ± 0.06
<i>n</i> -octadecane, <i>n</i> -C <sub>18</sub>	–34.06 ± 0.20
<i>n</i> -pentadodecane, <i>n</i> -C <sub>25</sub>	–30.48 ± 0.05
1-butanol, C <sub>4</sub>	–29.70 ± 0.19
oxalic acid, C <sub>2</sub>	–22.60 ± 0.18
malonic acid, C <sub>3</sub>	–22.96 ± 0.11
succinic acid, C <sub>4</sub>	–26.12 ± 0.06
glutaric acid, C <sub>5</sub>	–23.62 ± 0.14
adipic acid, C <sub>6</sub>	–25.25 ± 0.04
azelaic acid, C <sub>9</sub>	–16.03 ± 0.03
sebacic acid, C <sub>10</sub>	–27.91 ± 0.07
methylmalonic acid, iC <sub>4</sub>	–9.30 ± 0.34
methylsuccinic acid, iC <sub>5</sub>	–10.21 ± 0.14
2-methylglutaric acid, iC <sub>6</sub>	–27.00 ± 0.22
phthalic acid, C <sub>8</sub>	–27.81 ± 0.15

<sup>a</sup> For 1-butanol, carbon isotopic analyses were repeated five times. Other compounds were measured in triplicate.

pipet packed with quartz wool to remove filter debris and particles. Dicarboxylic acids were dried in a pear-shaped flask using a rotary evaporator and then a nitrogen blow-down system and reacted with 14% BF<sub>3</sub>/1-butanol to derive diacid dibutyl esters at 100 °C for 30 min.<sup>1,22</sup>  $\omega$ -Oxocarboxylic acids were derivatized to  $\omega,\omega$ -dibutoxy butyl esters whereas glyoxal was derivatized to 1,1,2,2-tetrabutoxyethane. Liquid samples were directly concentrated using rotary evaporation under a vacuum, passed through a quartz wool column, and then derivatized with BF<sub>3</sub>/1-butanol. The butyl esters were washed with pure water and then dissolved in 50 or 100  $\mu\text{L}$  of *n*-hexane. A 2- $\mu\text{L}$  sample of the ester solution was injected into the GC-FID to determine the concentrations of series of C<sub>2</sub>–C<sub>10</sub> dicarboxylic acids and related compounds (C<sub>2</sub>–C<sub>9</sub> ketocarboxylic acids, and C<sub>2</sub>–C<sub>3</sub> dicarbonyls).

Before the determination of the stable carbon isotopic composition for dicarboxylic acid butyl esters, an aliquot of ester fraction (typically 10  $\mu\text{L}$ ) was taken in a 1.5-mL glass vial and diluted with *n*-hexane (e.g., 6 times) and an internal standard (IS: *n*-C<sub>13</sub> alkane) was added to the ester fraction to adjust the *n*-C<sub>13</sub> concentration to be roughly equal to major compounds (generally, oxalic, malonic, and succinic acids). The amount of IS was adjusted to obtain a *m/z* 44 signal of between 3 and 4 V. To measure the  $\delta^{13}\text{C}$  ratios of minor species, a second aliquot of the ester fraction was analyzed using a smaller IS addition. The GC/irMS measurements of the derivatives were conducted in duplicate. Procedural blanks were run together with the samples. Although blanks showed a small peak of dibutyl ester of oxalic acid (<1%) and phthalic acid (<5%) on the GC-FID chromatogram, they are too small to be detected with GC/irMS.

## RESULTS AND DISCUSSION

**GC/irMS Measurements of Dicarboxylic Acid Dibutyl Esters.** Table 2 presents averaged  $\delta^{13}\text{C}$  values of normal alkanes and 1-butanol obtained by EA/irMS measurement based on replicate analyses. The  $\delta^{13}\text{C}$  value of the internal standard (*n*-C<sub>13</sub> alkane) was determined to be –27.24 ± 0.04‰ whereas that of BF<sub>3</sub>/1-butanol (Alltech Associate, Lot No. 11440) was determined

(22) Kawamura, K. *Anal. Chem.* **1993**, *65*, 3505–3511.

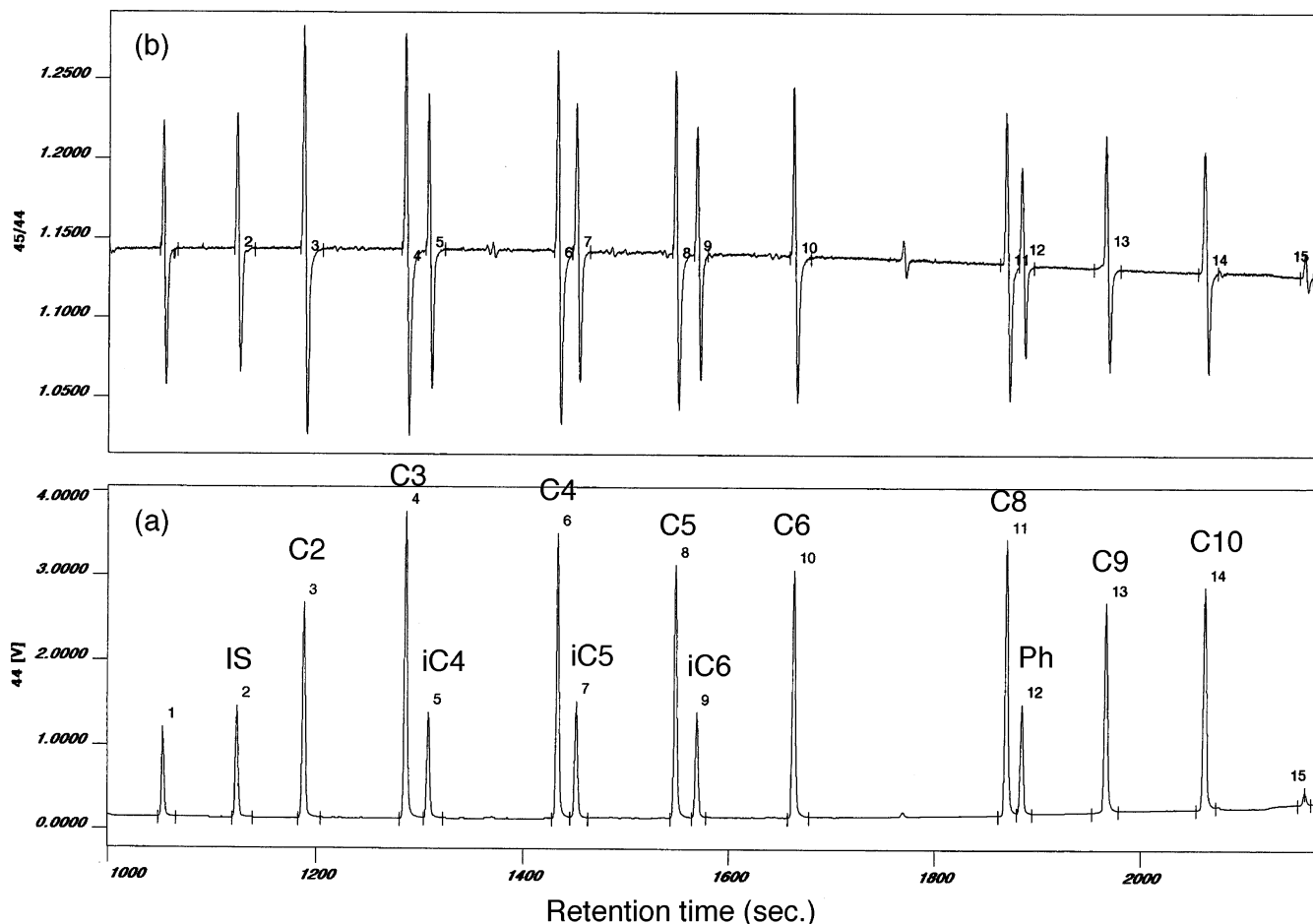


Figure 1. GC/irMS traces of  $m/z$  44 and ratios of  $m/z$  45 to 44 for dibutyl esters of homologous series of authentic aliphatic  $C_2$ – $C_{10}$  dicarboxylic acids, branched (iso) chain  $C_4$ – $C_6$  diacids, and phthalic acid. For the abbreviations, see Table 3. IS, internal standard ( $n$ - $C_{13}$  alkane). The signal of  $m/z$  45 is multiplied by 100 times.

Table 3. Replicate Measurements of  $\delta^{13}C$  of Diacid Butyl Esters and Calculated  $\delta^{13}C$  of Authentic Diacids<sup>a</sup>

diacids	measd $\delta^{13}C$ of DiBE by GC/irMS					calcd $\delta^{13}C$ for diacid					measd $\delta^{13}C$ by EA/irMS (b)	difference (a – b)
	first	second	third	av	SD	first	second	third	av (a)	SD		
C <sub>2</sub>	–28.30	–28.11	–28.22	–28.21	0.10	–22.70	–21.75	–22.30	–22.25	0.48	–22.60	0.35
C <sub>3</sub>	–27.67	–27.64	–27.85	–27.72	0.11	–22.26	–22.15	–22.92	–22.44	0.42	–22.96	0.52
C <sub>4</sub>	–28.43	–28.42	–28.49	–28.45	0.04	–25.89	–25.86	–26.07	–25.94	0.11	–26.12	0.18
C <sub>5</sub>	–27.33	–27.26	–27.30	–27.30	0.04	–23.54	–23.36	–23.46	–23.45	0.09	–23.62	0.17
C <sub>6</sub>	–27.56	–27.58	–27.76	–27.63	0.11	–24.71	–24.75	–25.17	–24.88	0.26	–25.25	0.37
C <sub>8</sub>	–31.26	–31.27	–31.35	–31.29	0.05	–32.82	–32.84	–33.00	–32.89	0.10	no data	no data
C <sub>9</sub>	–22.26	–22.48	nd <sup>b</sup>	–22.37	0.16	–15.65	–16.06	nd	–15.85	0.29	–16.03	0.17
C <sub>10</sub>	–28.84	–29.07	–28.88	–28.93	0.12	–28.15	–28.57	–28.22	–28.31	0.22	–27.91	–0.40
iC <sub>4</sub>	–23.02	–23.18	–23.05	–23.08	0.09	–9.66	–10.14	–9.75	–9.85	0.26	–9.30	–0.55
iC <sub>5</sub>	–22.17	–22.64	–22.22	–22.34	0.26	–10.12	–11.34	–10.25	–10.57	0.67	–10.21	–0.37
iC <sub>6</sub>	–29.03	–28.79	–28.72	–28.85	0.16	–28.14	–27.58	–27.41	–27.71	0.38	–27.00	–0.71
Ph	–28.78	–28.44	nd	–28.61	0.24	–27.86	–27.18	nd	–27.52	0.48	–27.81	0.29

<sup>a</sup> Dibutyl esters (10–20 ng each) were injected to GC/irMS system. <sup>b</sup> nd, not determined.

as  $-29.70 \pm 0.19\%$ . Isotopic compositions of homologous series of dicarboxylic acids ( $C_2$ – $C_{10}$  dicarboxylic acids) measured by EA/irMS, are shown in Table 2. Their  $\delta^{13}C$  values range from  $-9.30$  to  $-27.91\%$ . These values were used to evaluate a potential isotopic fractionation during the analytical procedure including a derivatization of dicarboxylic acids to their dibutyl esters.

Figure 1 presents a GC/irMS trace at  $m/z$  44 and isotope ratios of 45/44 for dibutyl esters derived from authentic dicarboxylic acids ( $C_2$ – $C_{10}$ ). The shape of the 45/44 plots demonstrates that

the esters enriched with  $^{13}C$  elute from the GC column a little earlier than those depleted with  $^{13}C$ . Table 3 presents the results of replicate GC/irMS measurements for the  $\delta^{13}C$  values of dibutyl esters. Standard deviations for the measurements of  $\delta^{13}C$  values for dibutyl esters are less than  $0.26\%$ . Using the carbon isotopic ratios of dibutyl esters derived from  $BF_3$  and 1-butanol ( $-29.70\%$ ), we calculated the stable carbon isotopic composition of dicarboxylic acids based on a mass balance equation (eq 2). Table 3 also presents calculated  $\delta^{13}C$  values of diacids from GC/irMS and



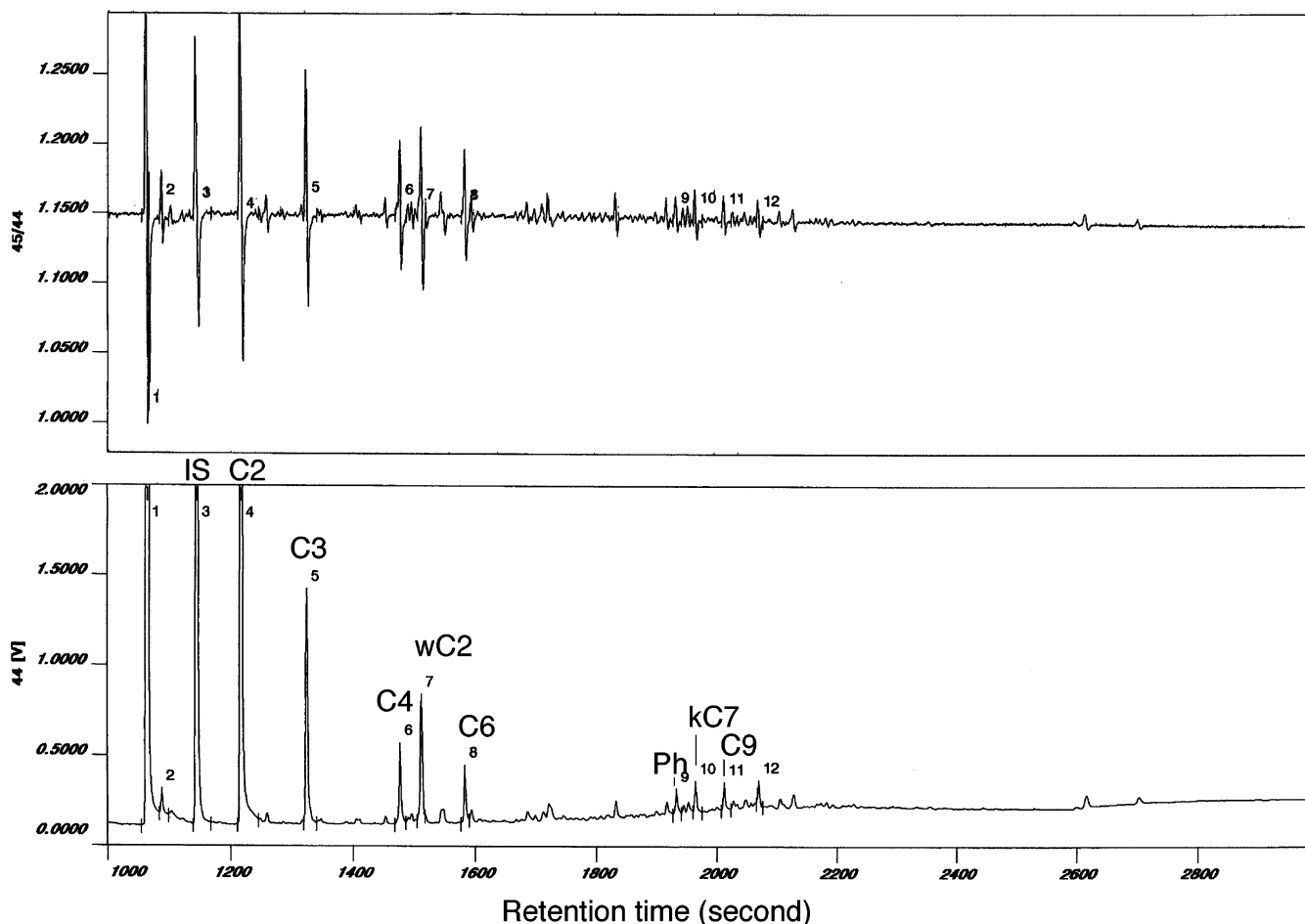


Figure 2. GC/irMS traces of  $m/z$  44 and ratios of  $m/z$  45 to 44 for dibutyl esters of dicarboxylic acids isolated from aerosol sample (QFF 2566) collected in Sapporo, Japan. For abbreviations, see Table 3.

theoretical  $\delta^{13}\text{C}$  values for authentic diacids determined by EA/irMS. Comparisons show that the differences between theoretical and calculated  $\delta^{13}\text{C}$  values are less than 0.71‰ during the dibutyl ester derivatization. Uncertainties of  $\delta^{13}\text{C}$  measurements depend on the unstable condition of GC column (i.e., bleeding of liquid phase and overlapping of unknown peak on GC).

The standard deviation of replicate analyses ( $n = 8$ ) of  $\text{C}_2$ – $\text{C}_{10}$  diacid dibutyl esters ranged from 0.10 to 0.21‰ depending on the diacid species. The calculated  $\delta^{13}\text{C}$  values of diacids were also found to be equal to the theoretical  $\delta^{13}\text{C}$  values of authentic dicarboxylic acids with an accuracy of 0.05–0.64‰. These results indicate that the isotopic fractionation during the butyl ester derivatization step is small.

**Examination of Possible Isotopic Fractionation during Analytical Procedure.** To evaluate possible isotopic fractionation that may occur during potential evaporation loss of dicarboxylic acid butyl esters, dibutyl esters of authentic normal saturated  $\text{C}_2$ – $\text{C}_6$  diacids, unsaturated  $\text{C}_4$  diacids (maleic and fumaric acids), and phthalic acid were dissolved in *n*-hexane, dried in a 1.5-mL glass vial, and exposed to a nitrogen blow-down stream for up to 30 min. at room temperature. Although the recoveries of oxalic and malonic acid butyl esters were relatively low (46–52%), no shift in the  $\delta^{13}\text{C}$  values was observed. A negligible isotopic fractionation was also confirmed for  $\text{C}_4$ – $\text{C}_6$  diacids and phthalic acid. Further, dibutyl esters of  $\text{C}_2$ – $\text{C}_6$  diacids were stored in a 1.5-mL vial with a loosened cap and at  $-15^\circ\text{C}$  for 4 months to partially evaporate

Table 4. Comparison of Calculated  $\delta^{13}\text{C}$  Values (‰) of Dicarboxylic Acids Recovered in the Spiked Experiments 1 and 2 from the Theoretical  $\delta^{13}\text{C}$  Values of Diacids<sup>a</sup>

diacids	experiment 1		experiment 2	
	calcd $\delta^{13}\text{C}$	difference <sup>b</sup>	calcd $\delta^{13}\text{C}$	difference <sup>b</sup>
$\text{C}_2$	$-22.48 \pm 0.46$	0.12	$-22.42 \pm 2.09$	0.18
$\text{C}_3$	$-22.17 \pm 0.18$	0.80	$-22.24 \pm 0.15$	0.72
$\text{C}_4$	$-25.31 \pm 0.32$	0.81	$-26.21 \pm 0.27$	-0.09
$\text{C}_5$	$-23.76 \pm 0.46$	-0.14	$-24.09 \pm 0.55$	-0.47
$\text{C}_6$	$-25.61 \pm 0.21$	-0.35	$-26.51 \pm 0.56$	-1.26

<sup>a</sup> GC/irMS measurement was conducted in duplicate. <sup>b</sup> Difference means a deviation from theoretical  $\delta^{13}\text{C}$  values of diacids (Table 2).

the esters. There was no isotopic fractionation for  $\text{C}_2$ – $\text{C}_6$  diacids within our analytical accuracy of  $\leq 0.8\text{‰}$  when the organic solvent (*n*-hexane) was completely evaporated (the recoveries of the esters were better than 86%).

Table 4 presents changes in the  $\delta^{13}\text{C}$  ratios of authentic diacids ( $\text{C}_2$ – $\text{C}_6$ ) that were spiked to a quartz filter during the whole analytical procedure from the water extraction to GC analysis. The recovery of oxalic acid was 77% and that of other diacids  $>85\%$ . The  $\delta^{13}\text{C}$  ratios of diacids recovered in the spiked experiments show that there is no serious change (less than 0.8‰, except for the result of  $\text{C}_6$  in experiment 2) in the  $\delta^{13}\text{C}$  values of diacids

Table 5. Concentrations and Stable Carbon Isotopic Composition of Dicarboxylic Acids, Ketocarboxylic Acids, and Dicarbonyls in the Aerosol and Snow Samples Collected in Sapporo<sup>a</sup>

comps	aerosol (July 23–29, 2002)				aerosol (May 26–27, 2003) QFF 2631		snow (Feb 19–20, 2003)		snow Feb 26, 2003	
	QFF2566, first analysis		QFF2566, second analysis							
	concn, ng m <sup>-3</sup>	$\delta^{13}\text{C}$ , ‰	concn, ng m <sup>-3</sup>	$\delta^{13}\text{C}$ , ‰	concn, ng m <sup>-3</sup>	$\delta^{13}\text{C}$ , ‰	concn, $\mu\text{g L}^{-1}$	$\delta^{13}\text{C}$ , ‰	concn, $\mu\text{g L}^{-1}$	$\delta^{13}\text{C}$ , ‰
C <sub>2</sub>	204.0	-19.2 ± 0.4	184.5	-19.8 ± 0.2	261.3	-17.0 ± 0.0	73.2	-27.8 ± 0.8	18.16	-28.4 ± 1.3
C <sub>3</sub>	46.5	-23.0 ± 0.2	43.1	-23.5 ± 1.4	76.8	-19.9 ± 1.5	19.0	-36.3 ± 1.1	6.12	-32.8 ± 2.3
C <sub>4</sub>	16.1	-23.1 ± 1.0	15.3	-23.9 ± 1.1	67.0	-22.3 ± 1.9	30.7	-26.1	6.48	
C <sub>5</sub>	7.1	-21.3 ± 0.3	6.4	-22.1 ± 0.4	11.2	-18.4 ± 1.2	10.4	-25.3 ± 1.9	1.96	-27.3 ± 3.9
C <sub>6</sub>	2.2		2.1		4.0	-15.3 ± 0.5	5.6	-22.8	1.39	-21.8 ± 0.7
C <sub>7</sub>					1.6					
C <sub>8</sub>	2.7		2.9		2.3	-15.8	2.2	-25.4 ± 2.4	0.35	-27.2
C <sub>9</sub>	9.6	-25.8 ± 0.5	9.3	-25.2 ± 1.3	5.5	-23.1 ± 0.2	8.8	-26.1 ± 2.1	2.21	-25.9 ± 0.1
C <sub>10</sub>					0.3					
iC <sub>4</sub>	0.8		0.7		3.8 <sup>b</sup>		0.9		0.4	
iC <sub>5</sub>	1.1		1.2		5.9		6.1		1.5	
iC <sub>6</sub>	3.3		3.3		1.6		2.9		0.8	
M	2.0		1.8		2.8	-28.0	11.7	-19.3 ± 1.3	2.6	-29.9 ± 0.7
F	2.1		2.1		2.5		2.0		0.5	
mM					2.4					
Ph	4.7	-26.0 ± 1.2	4.8	-25.2 ± 1.0	8.9	-26.8 ± 0.2	18.5	-27.0 ± 0.2	8.3	-27.4 ± 0.1
kC <sub>3</sub>										
kC <sub>7</sub>	6.1	-25.9 ± 5.2	6.7	-32.9 ± 0.2	5.7	-15.3 ± 2.5	6.1	-16.6	1.5	
malic	4.3		4.6				7.3	-17.8 ± 2.9	1.7	-24.2
$\omega\text{C}_2$	16.3	-11.2 ± 0.8	12.2	-12.0 ± 1.6	31.3	-10.1 ± 4.2	44.0	-29.8 ± 0.7	12.9	-20.3 ± 5.9
$\omega\text{C}_3$					0.5					
$\omega\text{C}_4$	2.6		2.1		0.2		2.2	-30.4 ± 6.0	3.2	-26.4 ± 3.0
Gly					1.3	19.0	7.3	3.9 ± 7.0	4.1	-16.5 ± 3.7
MeGly					1.8					

<sup>a</sup> Averaged  $\delta^{13}\text{C}$  ratios are given with a standard deviation. The data without standard deviation mean that the data were based on single measurement. <sup>b</sup> This value includes kC<sub>3</sub> as well. GC/irMS analyses were conducted in duplicate and the averaged values are presented with standard deviation.

throughout the analytical procedure. These  $\delta^{13}\text{C}$  differences in the spiked experiments are similar to that obtained during the derivatization procedure (see Table 3). In conclusion, these comparisons indicate that the  $\delta^{13}\text{C}$  ratios of dicarboxylic acids in aerosol samples can be determined with an analytical precision of 0.8‰.

#### Application of the Method to Aerosol and Snow Samples.

Figure 2 shows an example GC/irMS trace for dicarboxylic acid dibutyl esters isolated from an aerosol sample collected in Sapporo, Japan, in July 2002. An internal standard was added so that the peak height was similar to that of the major diacid dibutyl ester peaks. Butanol derivatives of C<sub>2</sub>–C<sub>4</sub> and C<sub>9</sub> diacids, phthalic acid, 4-ketopimelic acid, and glyoxylic acid were detected in the *m/z* 44 chromatogram. Their  $\delta^{13}\text{C}$  values were used to calculate the stable isotopic composition of the organic acids. Some ester fractions were injected to GC/irMS without dilution.

Table 5 presents stable carbon isotopic compositions calculated for C<sub>2</sub>–C<sub>9</sub> dicarboxylic acids and some ketocarboxylic acids (2-oxoethanoic acid and 4-oxobutanoic acid) and dicarbonyl (glyoxal) in the aerosol and snow samples. Standard deviations for major diacid species in aerosols are generally less than 1‰, but those of minor species are sometimes larger (>2‰). Reproducibility of  $\delta^{13}\text{C}$  ratios for snow samples is more variable for some species when concentrations are relatively low (Table 5). One July 2002 aerosol sample was analyzed in duplicate in order to evaluate the reproducibility of the concentration measurements. Analytical errors for the concentrations are within 10% for both major and minor species. The first and second analyses of the same July

2002 aerosol sample also gave good agreement in the  $\delta^{13}\text{C}$  values of C<sub>2</sub>–C<sub>9</sub> diacids, phthalic acid and glyoxylic acid (Table 5).

The  $\delta^{13}\text{C}$  values of oxalic acid in aerosol samples range from -17 to -20‰. The  $\delta^{13}\text{C}$  value of oxalic acid in the July sample is similar to that reported for Bermuda aerosols (-21‰<sup>19</sup>) and is similar to that of bulk marine organic matter.<sup>24,25</sup> With the exception of the C<sub>5</sub> acid, the  $\delta^{13}\text{C}$  values for longer chain aliphatic diacids ( $\geq\text{C}_3$ ) tend to have lighter isotopic ratios (-23 to -26‰). The light  $\delta^{13}\text{C}$  ratio (-25‰) of azelaic (C<sub>9</sub>) acid is consistent with the hypothesis that its primary source is unsaturated fatty acids (a double bond exists at C-9 position) of terrestrial higher plant origin via the photochemical oxidation in the atmosphere.<sup>10,23</sup> On the other hand, phthalic acid in the aerosols showed relatively constant  $\delta^{13}\text{C}$  values at  $-26 \pm 1\%$ , suggesting that this aromatic acid is derived from anthropogenic sources either directly or indirectly via photochemical oxidation of aromatic hydrocarbons.<sup>1</sup>

The May 2003 aerosol sample has a heavier  $\delta^{13}\text{C}$  value (-17‰) for oxalic and other acids with the exception of phthalic acid whose  $\delta^{13}\text{C}$  value (-25‰) was similar to that of the July sample. The heavier isotope ratios of aliphatic diacids may be associated with atmospheric reactions of diacids because this aerosol sample was collected during an event of strong inflow of biomass burning plume from Siberia over the Northern Japan including Hokkaido

(23) Kawamura, K.; Gagosian R. B. *Nature* **1987**, 325, 330–332.

(24) Sackett, W. M. In *Handbook of Environmental Isotope Geochemistry*; Fritz, P., Fontes, J. Ch., Eds.; Elsevier Scientific: New York, 1987; Vol. 3, pp 139–169.

(25) Fry, B.; Hopkinson Jr., C. S.; Nolin, A.; Wainright, S. C. *J. Geophys. Res.* **2003**, 108, NO. D5, 4157.

Island.<sup>26</sup> During a long-range atmospheric transport of aerosols, dicarboxylic acids may have been subjected to photochemical production and decomposition, which may cause isotopic fractionation of diacids. Alternatively, the difference in the stable carbon isotopic composition could have arisen from differences in the sources. It is of interest to note that we observed heavier carbon isotopic ratios of oxalic and malonic acids (+4 to +15‰ and -3 to +11‰, respectively) in the remote marine aerosols collected in the Central Pacific (Kawamura, unpublished results, 2003).

Snow samples collected at Sapporo in February 2003 were characterized by a lighter isotopic composition for oxalic and malonic acids (-28 to -36‰). These small diacids in snow samples may have sources different from those of aerosols collected near ground level, as snow crystals scavenge atmospheric gases and particles through the air column of several kilometers above the ground. The organics in snow may be derived from photochemical oxidation of volatile and semivolatile organic compounds emitted from terrestrial vegetation because  $\delta^{13}\text{C}$  ratios of terrestrial  $\text{C}_3$  plants are roughly 10‰ lighter than that of marine organic matter.<sup>24</sup> However,  $\text{C}_6$  diacid showed an isotopically heavier value at  $\sim -22\text{‰}$ . Because adipic acid in aerosols has been proposed as an oxidation product of anthropogenic cyclohexene,<sup>27,28</sup> this acid may have an isotopic composition different from other aliphatic diacids. On the contrary, phthalic acid in snow showed  $\delta^{13}\text{C}$  ratios similar to those of aerosols. This

aromatic diacid may be derived from phthalates that are used as plasticizer or produced in the atmosphere by photochemical oxidation of naphthalene.<sup>1</sup>

## CONCLUSION

We developed a new technique to measure stable carbon isotopic composition of homologous dicarboxylic acids ( $\text{C}_2\text{--C}_9$ ) in the environmental samples using a capillary GC/irMS system employing dibutyl esters. Accuracy of the determination of low molecular weight diacids is 0.8‰. We applied the new method to aerosol and snow samples and report stable carbon isotopic ratios of oxalic acid (-17 to -28‰), malonic acid (-20 to -36‰), succinic acid (-22 to -26‰), glutaric acid (-18 to -27‰), adipic acid (-15 to -22‰), azelaic acid (-23 to -26‰), and phthalic acid (-25 to -27‰). A wide range of  $\delta^{13}\text{C}$  values of aliphatic diacids suggests that their stable carbon isotopic compositions are controlled by several factors such as source strength and formation and transformation processes in the atmosphere. In contrast, phthalic acid showed a narrow range of  $\delta^{13}\text{C}$  values, suggesting a more specific source including anthropogenic emission or photochemical production from anthropogenic aromatic hydrocarbons.

## ACKNOWLEDGMENT

We thank R. Ishiwatari for providing *n*-alkane standards. This study was partly supported by the Japanese Ministry of Education, Culture, Sports, Science and Technology through grant-in-aid 14204055.

Received for review April 1, 2004. Accepted July 13, 2004.

AC049491M

(26) <http://info.nies.go.jp:8094/dust/> [http://www.jma.go.jp/JMA\\_HP/jma/press/0305/22b/sunshine.pdf](http://www.jma.go.jp/JMA_HP/jma/press/0305/22b/sunshine.pdf).

(27) Grosjean, D.; Van Cauwenberghe, K.; Schmid, J. P.; Kelley, P. E.; Pitts, J. N. Jr. *Environ. Sci. Technol.* **1978**, *12*, 313–317.

(28) Hatakeyama, S.; Ohno, M.; Weng, J.; Takagi, H.; Akimoto, H. *Environ. Sci. Technol.* **1987**, *21*, 52–57.