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# Molecular and carbon and hydrogen isotopic composition of *n*-alkanes in plant leaf waxes

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**Abstract**

Molecular composition and compound-specific carbon and hydrogen stable isotope ratios of leaf wax *n*-alkanes are presented for 26 plant species operating $C_3$, $C_4$ and CAM photosynthetic pathways. In contrast to $\delta^{13}C$ values, $\delta D$ values are not diagnostic discriminators for $C_3$, $C_4$ and CAM plants. $\delta D$ and $\delta^{13}C$ values of *n*-alkanes from $C_4$ plants seem to express an inverse linear relationship while some $C_3$ plants showed a positive relationship. The carbon and hydrocarbon isotopic correlation is dependent on plant types as well as photosynthetic pathways. However, our data imply that the combined use of carbon and hydrogen isotopic characterization may have superior diagnostic potential for source apportionment when compared with more limited isotopic approaches.


## 1. Introduction

Normal alkanes occur almost ubiquitously in soil, water, sediment, atmosphere, plants and fossil fuels (Simoneit et al., 1977; Fu et al., 1990; Pio et al., 2001). Sources of environmental and atmospheric *n*-alkanes are incomplete combustion of fossil fuels and biomass, fossil fuel seepages and leaks, and epicuticular waxes produced by vascular plants. Although the chemical distribution of *n*-alkanes in atmospheric aerosols has been widely used for source identification, several factors exist that limit the usefulness of the chemical composition as a source indicator. First, many natural and anthropogenic sources produce similar *n*-alkane profiles (Lichtfouse et al., 1994; Orous and Simoneit, 2001). There are no diagnostic features distinguishing *n*-alkanes from plant waxes versus those from anthropogenic sources (e.g., cigarette smoke; Kavouras et al., 1998). Second, the *n*-alkane composition in aerosol can change due to mixing of various sources, or via partial *n*-alkane loss to photochemical reactions, especially affecting lighter hydrocarbons during atmospheric transport.

Stable isotope ratios offer independent and complementary information about sources and sinks of environmental hydrocarbons. For example, compound-specific carbon isotope analysis (CSIA) of individual hydrocarbons has been widely used recently as a technique for source elucidation (Sun et al., 2000; Filley et al., 2001; Schimmelmann et al., 2004). No significant carbon isotope fractionation of hydrocarbons has been reported for volatilization, photo-oxidation, thermal oxidation and microbial degradation processes (O'Malley et al., 1994, 1997; Mazeas et al., 2002). Furthermore,

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the different sources do have distinct characteristics for the $\delta^{13}C$ values (Collister et al., 1994; McRae et al., 1999). The *n*-alkanes from plants with $C_3$ and $C_4$, and CAM photosynthetic pathways differ widely in their isotopic signatures (Collister et al., 1994). $C_3$ plants are relatively $^{13}C$-depleted (*n*-$C_{24}$ to *n*-$C_{35}$ typically range from $\delta^{13}C = -31‰$ to $-39‰$) and $C_4$ plants are $^{13}C$-enriched ($\delta^{13}C = -18‰$ to $-25‰$). Because crassulacean acid metabolism (CAM) plants can utilize both $C_3$ and $C_4$ carbon fixation pathways they have an intermediate $\delta^{13}C$ range (e.g., *n*-$C_{21}$ to *n*-$C_{35}$ typically range from $-23‰$ to $-29‰$) (Collister et al., 1994; Simoneit, 1997). Ballentine et al. (1998) demonstrated that carbon isotopic signatures of fatty acids in plants are also diagnostic with a $C_3$ range from $-32.4‰$ to $-38.5‰$ and a $C_4$ range of $-21.1‰$ to $-28.2‰$.

The typical range of $\delta^{13}C$ variation for *n*-alkanes in a single plant is less than 10‰ (Collister et al., 1994; Chikaraishi and Naraoka, 2003; Conte et al., 2003). The relatively narrow range of carbon isotope values limited the application of the carbon isotope techniques. However, hydrogen isotopes are commonly fractionated to a much greater extent by different processes than carbon isotopes. For example, the $\delta D$ variation for individual *n*-alkanes in crude oils obtained from the Western Canada Sedimentary Basin is up to 160‰ (Li et al., 2001). Ward et al. (2000) demonstrated that hydrogen isotopes of toluene fractionated approximately 60‰ during in vitro anaerobic biodegradation. Generally, the hydrogen isotope ratios are strongly influenced by environmental and biochemical variables (Hassan and Spalding, 2001; Li et al., 2001). Sauer et al. (2001) have shown that the average apparent fractionation between sterols and environmental water was $-201 \pm 10‰$. Fractionation between *n*-alkyl lipids and growth water observed by Sessions et al. (1999) in living organisms was 113–262‰. The advantage of hydrogen isotopes is mainly that they better respond to the hydrological cycle, e.g., meteoric water availability and isotopic differences. Therefore, such strong fractionation of hydrogen isotopes in combination with carbon isotopes will provide a very powerful isotopic approach.

Recently, Huang and his colleagues demonstrated the hydrogen isotopic variability of individual alkanes in crude oils with gas chromatography-thermal conversion-isotope ratio mass spectrometry (GC-TC-IRMS) (Li et al., 2001; Wang and Huang, 2001; Pond et al., 2002). There have been some studies on D/H ratios of individual lipid compounds as a proxy for environmental and biological studies (Sternberg, 1988; Sessions et al., 1999; Xie et al., 2000). However, few published works (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004) have confirmed the hydrogen isotopic application in source apportionment of individual alkanes in plants waxes, which are especially dominant compared to the anthropogenic components in rural and remote areas (Simoneit, 1997; Pio et al., 2001; Gogou et al., 1996; Conte and Weber, 2002a,b). The purpose of this study was to examine the molecular composition and the range of hydrogen isotopic fractionation for leaf waxes between $C_3$, $C_4$ and CAM plants. In addition, the carbon isotopic compositions for plant waxes were also measured in order to compare with the literature values. The relationship between $\delta D$ and $\delta^{13}C$ values of *n*-alkanes from different plants was also discussed in this study.

## 2. Experimental

### 2.1. Sample collection and purification

All plants were obtained from the South China Botanical Garden, the Chinese Academy of Sciences, Guangzhou (Lat. N: 23.1°; Long. E: 113.3°, 7 m above sea level). It was one of the three largest gardens in China with an area of ca. 3 million $m^2$. Guangzhou is situated in sub-tropical zone. The climate is under the strong influence of the Asian monsoon system. Winter seasons are characterized by a strong monsoon wind and dry weather whereas summer seasons are hot and humid. Mean annual temperature was 20–22 °C. The mean annual precipitation was 1600 mm. More significant rainfall was received between March and September.

A total of 26 specimens representative of $C_3$, $C_4$ and CAM plants were collected during summer 2002 and spring 2003. All of them are the major species in South China. The plants chosen for this study and their growth conditions are shown in Table 1. Three indoor plants were grown in a greenhouse with an area of 150 $m^2$, the other outdoor plants were grown in natural conditions. The normal indoor temperature is 2–3 °C higher than outdoors. For bulk leaf isotopic analyses, some matured leaves of each species were dried at 40 °C for 24 h, and then were ground with a pestle (40–90 mesh). About 1–1.5 mg aliquot was used for isotopic analysis. The remaining leaves were dried overnight at 25 °C. The leaf waxes were extracted by immersion in hexane.

Aliphatic fractions containing *n*-alkane homologues were then purified using silica–alumina column chromatography eluting with hexane (Bi et al., 2002). This was followed by rotary evaporative concentration to about 1 ml. Before GC analysis the volume was further reduced using a gentle stream of nitrogen. The aliphatic fractions of plant samples were characterized by relatively small concentrations of compounds other than *n*-alkanes. When necessary, a silica–$AgNO_3$ column was used to remove interfering alkenes. Optimum sample injection volumes, identification and retention times of the compounds of interest were determined for all samples using

Table 1
Plants sampling date, species, type, carbon fixation modes, growth conditions, and *n*-alkane parameters

| Code letter | Sampling date | Plant genera and species | Plant family | Plant type | Carbon fixation pathway | Growth environment | Chain length range | $C_{max}$ | CPI | ACL |
|---|---|---|---|---|---|---|---|---|---|---|
| A | August 28, 2002 | *Hylocereus undatus* (Ham.) Britt. et Rose | Cactaceae | Succulent | CAM | Greenhouse | 18–37 | 33 | 5.1 | 32.2 |
| B | August 28, 2002 | *Euphorbia trigona* How. | Euphorbiaceae | Shrub-like | CAM | Greenhouse | 24–37 | 33 | 13.5 | 31.5 |
| C | August 28, 2002 | *Opuntia dillenii* (Ker-Gawl) Haw. | Cactaceae | Succulent, shrub-like | CAM | Greenhouse | 23–36 | 29 | 3.7 | 29.8 |
| D | August 28, 2002 | *Euphorbia pulcherrima* Willd. | Euphorbiaceae | Shrub | $C_3$ | Open air | 22–33 | 29 | 4.2 | 28.7 |
| E | August 28, 2002 | *Codiaeum variegatum* (L.) Bl. var. *pictum* M.-A. forma *crispum* Pax | Euphorbiaceae | Shrub | $C_3$ | Open air | 22–35 | 33 | 4.0 | 32.2 |
| F | July 29, 2002 | *Ficus altissima* Bl. | Moraceae | Tree | $C_3$ | Open air | 27–34 | 31 | 12.7 | 31.2 |
| G | July 29, 2002 | *Ficus microcarpa* Linn. f. | Moraceae | Tree | $C_3$ | Open air | 25–34 | 31 | 7.5 | 31.1 |
| H | July 29, 2002 | *Osmanthus fragrans* Lour. | Oleaceae | Small tree | $C_3$ | Open air | 24–35 | 31 | 4.9 | 31.2 |
| I | July 29, 2002 | *Kigelia africana* (am.) Benth. | Bignoniaceae | Tree | $C_3$ | Open air | 24–35 | 31 | 5.6 | 31.3 |
| J | July 29, 2002 | *Syzygium cumini* (L.) Skeels | Myrtaceae | Tree | $C_3$ | Open air | 24–33 | 31 | 11.1 | 30.2 |
| K | August 28, 2002 | *Swietenia mahagoni* (L.) Jacq. | Meliaceae | Tree | $C_3$ | Open air | 24–35 | 31 | 9.8 | 30.9 |
| L | August 28, 2002 | *Pistia stratiotes* | Araceae | Floating herb | $C_3$ | Open air | 24–37 | 31 | 54.3 | 31.0 |
| M | August 28, 2002 | *Caryota mitis* Lour. | Arecaceae | Small tree | $C_3$ | Open air | 20–35 | 31 | 5.4 | 31.4 |
| N | July 29, 2002 | *Cinnamomum burmanni* (Nees) Bl. | Lauraceae | Tree | $C_3$ | Open air | 22–35 | 31 | 3.9 | 30.1 |
| O | August 28, 2002 | *Araucaria cunninghamii* Sweet | Araucariaceae | Tree | $C_3$ | Open air | 22–35 | 31 | 2.3 | 30.5 |
| P | August 28, 2002 | *Alternanthera dentata* ' *Rubiginosa* ' | Amaranthaceae | Herb | $C_3$ | Open air | 22–35 | 29 | 3.2 | 29.9 |
| Q | August 28, 2002 | *Alternanthera versicolor Regel* | Amaranthaceae | Herb | $C_3$ | Open air | 18–35 | 31 | 3.7 | 30.2 |
| R | August 28, 2002 | *Alternanthera bettzickiana* (Regel) Nichols. | Amaranthaceae | Herb | $C_3$ | Open air | 24–33 | 31 | 4.3 | 29.9 |
| S | August 28, 2002 | *Holmskioldia sanguinea* Retz. | Verbenaceae | Shrub | $C_3$ | Open air | 22–37 | 35 | 4.3 | 31.6 |
| T | April 1, 2003 | *Zea mays* L. | Gramineae | Herb | $C_4$ | Open air | 14–33 | 29 | 7.3 | 28.1 |
| U | April 1, 2003 | *Amaranthus tricolor* L. | Amaranthaceae | Herb | $C_4$ | Open air | 14–33 | 31 | 5.1 | 28.4 |
| V | April 1, 2003 | *Amaranthus paniculatus* L. | Amaranthaceae | Herb | $C_4$ | Open air | 14–33 | 31 | 7.6 | 28.7 |
| W | April 1, 2003 | *Imperata cylindrica* var. *major* (Ness) C. E. Hubb. | Gramineae | Herb | $C_4$ | Open air | 14–35 | 31 | 8.9 | 31.4 |
| X | April 1, 2003 | *Bothriochloa ischaemum* Keng | Gramineae | Herb | $C_4$ | Open air | 14–35 | 31 | 15.0 | 29.4 |
| Y | April 1, 2003 | *Zoysia japonica* Stued | Gramineae | Herb | $C_4$ | Open air | 14–35 | 33 | 12.3 | 31.8 |
| Z | April 1, 2003 | *Saccharum sinense* Roxb. | Gramineae | Herb | $C_4$ | Open air | 14–33 | 27 | 12.9 | 27.7 |

$C_{max}$: *n*-alkane with maximum abundance; carbon preference index (CPI) = $\sum$odd $C_{15}$–$C_{37}$/$\sum$even $C_{14}$–$C_{36}$; average chain length (ACL) = $(\sum[c_i] \times i)/[c_i]$ for $i = 24$–35, where $i$ is the concentration of the *n*-alkane containing $i$ carbon atoms.

gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). GC-IRMS analyses were then performed on the *n*-alkane fractions, which had been checked for interfering compounds.

### 2.2. GC and GC-MS analysis

The GC analysis was conducted using a Hewlett–Packard 5890 gas chromatograph with flame ionization detector (FID). The injector temperature was maintained at 290 °C, with a detector temperature of 300 °C. The GC temperature program featured a 50 °C hold for 1 min, a step of 4 °C/min to 290 °C, followed by a hold for 30 min. I μl solution was injected in splitless mode. A 50 m × 0.25 mm i.d. DB-5 (film thickness 0.25 μm) capillary column was used to separate compounds. Components were identified using a Platform II mass spectrometer operated in electron impact mode (70 eV) with a scan range of m/z 50 to 500. The carrier gas was helium at a constant flow of 1 ml/min. *n*-Alkanes were identified by comparison of mass spectra with those obtained from the NBS 75 mass spectra library and by matching of retention times and fragmentation profiles against corresponding standards.

### 2.3. Carbon isotopic analysis

Bulk leaf tissue samples were combusted quantitatively using an elemental analyzer (Flash EA 1112 series, CE Instruments) coupled on-line to a Finnigan MAT Delta Plus XL mass spectrometer (ThermoFinnigan MAT, Bremen, Germany). The stable carbon isotopic ratios of individual *n*-alkanes were determined using an HP 6890 gas chromatograph interfaced on-line via a CuO furnace (940 °C) to a Delta Plus XL isotope ratio mass spectrometer. A 30 m × 0.32 mm i.d. HP-5 (film thickness 0.25 μm) capillary column was used. The GC temperature program for *n*-alkane separation ramped from 50 to 240 °C (1 min) at 20 °C/min, then to 290 °C (20 min) at 4 °C/min. Ultra-high purity helium was used as carrier gas with a constant flow rate of 1.5 ml/min. Each sample was injected in the on-column mode. For isotopic standardization, $CO_2$ reference gas was automatically introduced into the isotopic ratio mass spectrometer in a series of pulses at the beginning and the end of each analysis.

Prior to carbon isotope analyses, the $CO_2$ reference gas was calibrated relative to Vienna Pee Dee Belemnite (VPDB). Instrument performance was routinely checked using *n*-alkane standard mixes with known $\delta^{13}C$ values ($nC_{12}$–$nC_{32}$ provided by Malvin Bjorϕy) (Xiong and Geng, 2000). Precision for replicate measurements of the standard *n*-alkanes ranged between 0.02‰ and 0.39‰. Isotopic compositions are reported as $\delta^{13}C$ values relative to VPDB averaging at least four replicate measurements.

### 2.4. Hydrogen isotopic analysis

Hydrogen isotopic analyses of individual alkanes via GC-TC-IRMS utilized an HP-6890 GC and a high-temperature pyrolysis unit that was connected on-line via a GCC III interface to a Finnigan MAT Delta Plus XL isotope ratio mass spectrometer. Individual compounds separated by GC were pyrolysed to $H_2$ and C at 1450 °C. The $H_2$ was then introduced into the mass spectrometer. The temperature program and capillary column were identical to those used for $\delta^{13}C$ analysis.

The $H_3$ factor for the mass spectrometer was determined every 4–6 injections by observing changes in the (mass-3)/(mass-2) ion-current ratio as the pressure of $H_2$ in the ion source varied. The instrument was tuned to ensure that the $H_3$ factor was always around 10. The reproducibility and accuracy of the hydrogen isotopic analyses were evaluated routinely using GC-IRMS reference materials provided by Indiana University and laboratory isotopic standards with known $\delta D$ values. There are two types of laboratory standards. One contains nine *n*-alkane homologues ($C_{12}$, $C_{14}$, $C_{16}$, $C_{18}$, $C_{20}$, $C_{25}$, $C_{28}$, $C_{30}$, $C_{32}$), and the other one contains three *n*-alkane homologues ($C_{18}$, $C_{20}$, $C_{24}$). Typically, during the analyses of unknown samples, laboratory standards were injected periodically (typically one standard injection per six sample analyses) to ensure that the mass spectrometer and $H_3$ factor were stable. The $\delta D$ values of standard compounds injected did not vary by more than 3‰. $\delta D$ values of sample compounds were calculated relative to pulses of $H_2$ gas and were calibrated against the VSMOW scale. Reported $\delta D$ values represent averages of four to six repeat analyses.

## 3. Results and discussion

Table 1 provides the growth conditions and some parameters describing *n*-alkane distributions for the plants examined. *n*-Alkanes from $C_{14}$–$C_{37}$ were identified in the plants. *n*-$C_{25}$–$C_{35}$ congeners were the major compounds with carbon number maximum ($C_{max}$) between $C_{27}$–$C_{35}$. All species showed a strong odd-numbered carbon dominance with carbon preference index (CPI) values ranging from 2.3 to 54.3. Most of these CPI values were significantly higher than those observed in aerosol samples, even though they were thought to have a higher plant input (Gogou et al., 1996; Pio et al., 2001; Simoneit et al., 1990; Conte and Weber, 2002b). Average chain length (ACL) values varied from 27.7 to 32.2. The relative abundance of the surface wax *n*-alkanes is presented in Table 2. The *n*-alkane chain length distributions overlapped among plants with $C_3$, $C_4$ and CAM biochemistry and indicated that this cannot be used as a tool which allows $C_3$, $C_4$ or CAM plant sources to be separated.

Table 2
Molecular distributions as percentage of total $n$-$C_{21}$–$C_{35}$ alkanes

| $n$-Alkanes | A | B | T | U | V | W | X | Y | Z |
|---|---|---|---|---|---|---|---|---|---|
| 21 | 0.28 | n.d. | 8.87 | 2.12 | 0.62 | 0.63 | 2.68 | 14.64 | 0.26 |
| 23 | 1.06 | 0.96 | 4.18 | 4.44 | 2.06 | 1.20 | 8.02 | 9.99 | 0.45 |
| 25 | 1.33 | 1.66 | 9.52 | 12.66 | 8.81 | 1.91 | 6.40 | 1.55 | 5.23 |
| 27 | 1.53 | 6.88 | 24.26 | 21.82 | 26.57 | 3.32 | 15.92 | 3.09 | 60.81 |
| 29 | 2.93 | 33.36 | 32.41 | 17.45 | 17.40 | 8.62 | 24.74 | 7.85 | 18.11 |
| 31 | 23.84 | 29.92 | 8.17 | 29.24 | 33.51 | 39.17 | 26.48 | 16.38 | 5.82 |
| 33 | 44.59 | 4.95 | 1.53 | 0.72 | 0.87 | 26.93 | 8.33 | 32.09 | 3.34 |
| 35 | 8.85 | 1.20 | n.d. | n.d. | n.d. | 8.43 | 1.52 | 7.85 | n.d. |

| | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 21 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 0.02 | n.d. | n.d. | n.d. | 0.53 | n.d. | n.d. |
| 23 | n.d. | 0.56 | 0.15 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 0.05 | 0.64 | 1.38 | 0.69 | 0.99 | n.d. | 0.13 |
| 25 | 9.11 | 4.40 | 0.64 | n.d. | 0.22 | 0.42 | 0.23 | 0.41 | 0.03 | 0.04 | 0.11 | 1.52 | 2.55 | 1.79 | 1.10 | 1.10 | 0.60 |
| 27 | 4.19 | 17.63 | 1.16 | 2.40 | 0.77 | 1.19 | 1.75 | 3.26 | 0.30 | 0.36 | 0.75 | 10.36 | 5.22 | 8.03 | 4.29 | 5.55 | 7.54 |
| 29 | 6.65 | 42.95 | 2.09 | 14.14 | 11.81 | 10.28 | 13.78 | 27.40 | 22.87 | 23.26 | 6.51 | 21.04 | 15.48 | 26.99 | 20.56 | 26.20 | 15.99 |
| 31 | 16.42 | 13.43 | 17.78 | 45.91 | 54.55 | 50.48 | 39.60 | 59.29 | 47.29 | 51.16 | 49.95 | 30.30 | 23.79 | 26.96 | 44.10 | 44.08 | 17.45 |
| 33 | 44.52 | 1.80 | 56.11 | 30.23 | 20.93 | 18.46 | 24.25 | 1.41 | 20.13 | 21.58 | 26.20 | 14.75 | 18.30 | 10.57 | 6.56 | 4.33 | 14.85 |
| 35 | 12.24 | n.d. | 2.21 | n.d. | n.d. | 2.11 | 5.13 | n.d. | 0.15 | 1.89 | 0.86 | 0.85 | 3.14 | 1.19 | 0.77 | n.d. | 25.40 |

### 3.1. Carbon isotopic composition

Plant carbon isotopic compositions are presented in Tables 3 and 4. $\delta^{13}C$ measurements were not performed on some plant $n$-alkanes due to the relatively low concentrations extracted from plant surface wax. It can be seen that $n$-alkane molecular isotopic compositions were measured with good reproducibility in most of the cases (mean standard deviation of 0.17‰ for all of the samples). The $C_4$ plants examined had relatively heavy $\delta^{13}C$ values compared to $C_3$ plants. The bulk leaf $\delta^{13}C$ values for $C_3$ and $C_4$ plants were in the range of −30.1‰ to −27.1‰ and −14.0‰ to −10.8‰, respectively, which were consistent with the literature values (Collister et al., 1994; O'Malley et al., 1997; Conte et al., 2003).

For individual compounds, the $\delta^{13}C$ values of $n$-alkanes ranged between −14.1‰ and −38.9‰. Distinct isotopic patterns were evident for the plants with differing carbon dioxide metabolisms. The $C_4$ plants were the most enriched in $^{13}C$. The $\delta^{13}C$ values for $C_4$-derived $n$-alkanes were all within the range of −26.4‰ to −14.1‰, and $C_3$ plants were the most depleted in $^{13}C$ with an overall $\delta^{13}C$ range of −38.9‰ to −29.1‰. CAM plants had an intermediate $\delta^{13}C$ range (−29.5‰ to −21.5‰), which were consistent with the utilization of both $C_3$ and $C_4$ carbon fixation pathways. Such depletion of $^{13}C$ was explained by the biosynthesis of lipid from $^{13}C$-depleted acetate precursors, and with an additional fractionation occurred at biosynthetic branch points (Hayes, 1993). Similar $\delta^{13}C$ values for individual $n$-alkanes from $C_3$, CAM and $C_4$ plants were reported in the literature (Collister et al., 1994; O'Malley et al., 1997; Lockheart et al., 1997). In addition, Tables 3 and 4 show that the range of $\delta^{13}C$ values for individual $n$-alkanes within each species was 0.2‰ to 6.2‰. There was no clear relationship between the molecular weight and $\delta^{13}C$ values, although the even carbon-number compounds tended to be more depleted in $^{13}C$ than the odd carbon-containing compounds (Fig. 1). This trend has also been reported by other researchers (Collister et al., 1994; O'Malley et al., 1997; Chikaraishi and Naraoka, 2003).

Isotopic fractionation during wax biosynthesis was observed among individual species. In general, the individual $n$-alkanes from leaf surface waxes were depleted in $^{13}C$ by 6.7–11.0‰ relative to bulk leaf tissue. These results were consistent with data from previous studies (Collister et al., 1994; Lockheart et al., 1997; Chikaraishi and Naraoka, 2003; Conte et al., 2003). In this study, the $C_4$ plants showed weighted mean carbon isotopic signatures of $n$-alkanes ranging from −24.4‰ to −14.6‰, which were depleted in $^{13}C$ relative to total leaf tissue by 6.7–11.0‰. $C_3$ plants weighted mean $\delta^{13}C$ values varied from −37.9‰ to −30.0‰ and were 6.8–9.7‰ more depleted in $^{13}C$ than leaf tissue (Tables 3 and 4). The results also indicated that isotopic fractionation was independent of the photosynthetic pathway. The isotopic changes are more likely when environmental conditions vary between the time when the structural carbon was laid down and the period when the waxes on the sampled leaf were synthesized. Conte et al. (2003) suggested that wax biosynthetic isotopic fractionation, i.e. the offset in wax $\delta^{13}C$ relative to bulk carbon, is not significantly affected by environmental conditions.

### 3.2. Hydrogen isotopic composition

The hydrogen isotopic composition of individual leaf wax $n$-alkanes in $C_3$, $C_4$ and CAM plants are

Table 3
$\delta^{13}C$ values of individual *n*-alkanes[a] and mean weighted $\delta^{13}C$ values of all *n*-alkanes[b] for CAM and $C_4$ plants

| *n*-Alkanes | A[a] | B | C | T | U | V | W | X | Y | Z |
|---|---|---|---|---|---|---|---|---|---|---|
| Bulk carbon (TT) | n.d. | n.d. | n.d. | −10.8 | −13.7 | −14.0 | −13.1 | −12.8 | −13.6 | n.d. |
| Isotopic fractionation $\Delta\delta$ (TT-WMA) | | | | 11.0 | 10.7 | 10.3 | 6.7 | 8.4 | 9.3 | |
| 21 | n.d. | n.d. | n.d. | −18.8 ± 0.2[b] | n.d. | n.d. | n.d. | −19.6 ± 0.2 | −21.3 ± 0.2 | n.d. |
| 23 | n.d. | n.d. | n.d. | −22.1 ± 0.1 | −26.1 ± 0.1 | −25.3 ± 0.1 | n.d. | −19.2 ± 0.3 | −22.0 ± 0.2 | n.d. |
| 25 | n.d. | n.d. | n.d. | −23.4 ± 0.2 | −24.1 ± 0.1 | −24.2 ± 0.1 | −22.0 ± 0.3 | −20.3 ± 0.2 | n.d. | −14.7 ± 0.2 |
| 26 | n.d. | n.d. | n.d. | −25.0 ± 0.2 | n.d. | −25.5 ± 0.3 | n.d. | n.d. | n.d. | n.d. |
| 27 | n.d. | −24.6 ± 0.3 | −22.7 ± 0.1 | −21.7 ± 0.1 | −24.3 ± 0.1 | −25.1 ± 0.2 | −22.1 ± 0.1 | −20.7 ± 0.1 | −23.5 ± 0.2 | −14.1 ± 0.1 |
| 28 | n.d. | n.d. | n.d. | −24.4 ± 0.2 | n.d. | −26.4 ± 0.2 | n.d. | −22.2 ± 0.2 | n.d. | n.d. |
| 29 | n.d. | −28.7 ± 0.3 | −24.2 ± 0.2 | −21.3 ± 0.3 | −25.8 ± 0.2 | −25.4 ± 0.1 | −21.0 ± 0.1 | −21.0 ± 0.3 | −24.1 ± 0.2 | −15.3 ± 0.3 |
| 30 | n.d. | n.d. | −24.8 ± 0.4 | −24.7 ± 0. 1 | n.d. | −25.3 ± 0.1 | −22.1 ± 0.1 | −23.6 ± 0.2 | n.d. | n.d. |
| 31 | −21.8 ± 0.1 | −29.4 ± 0.1 | −25.1 ± 0.1 | −22.1 ± 0.1 | −23.4 ± 0.1 | −22.9 ± 0.1 | −19.2 ± 0.1 | −22.1 ± 0.3 | −23.4 ± 0.1 | −16.7 ± 0.2 |
| 32 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | −24.3 ± 0.3 | n.d. |
| 33 | −21.5 ± 0.1 | −29.5 ± 0.4 | n.d. | −23.5 ± 0.2 | n.d. | n.d. | −19. 6 ± 0.4 | −22.4 ± 0.3 | −23.1 ± 0.1 | n.d. |
| 34 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | −23.9 ± 0.2 | n.d. |
| 35 | −21.5 ± 0.3 | n.d. | n.d. | n.d. | n.d. | n.d. | −19.9 ± 0.2 | −23.1 ± 0.3 | −24.2 ± 0.2 | n.d. |
| Weighted mean alkanes (WMA)[c] | −21.6 | −29.1 | −24.5 | −21.8 | −24.4 | −24.3 | −19.8 | −21.2 | −22.9 | −14.6 |
| $\Delta\delta$[d] | 0.3 | 4.9 | 2.4 | 6.2 | 2.7 | 3.5 | 2.9 | 4.4 | 3.0 | 2.6 |

Code letters refer to plants listed in Table 1.
n.d. = not determined.
[a] A to C are CAM plants; T to Z are C4 plants.
[b] Indicated uncertainties are standard deviations of replicate analyses.
[c] Weighted mean alkanes = $(\sum[c_i]\times\delta_i)/\sum[c_i]$; for $i = 21$–35, where $c_i$ is the concentration of the *n*-alkane containing *i* carbon atoms.
[d] $\Delta\delta = \delta^{13}C$ range for individual *n*-alkanes.

Table 4
$\delta^{13}C$ values of individual *n*-alkanes[a] and mean weighted $\delta^{13}C$ values of all *n*-alkanes[b] for $C_3$ plants

| *n*-Alkanes | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|
| 27 | −37.7 ± 0.3 | n.d. | n.d. | n.d. | n.d. | n.d. | −34.2 | n.d. |
| 28 | −38.9 ± 0.1 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 29 | −38.0 ± 0.3 | n.d. | −33. 9 ± 0.1 | −31.0 ± 0.1 | −35.7 ± 0.1 | −33.1 ± 0.3 | −37.2 ± 0.1 | −34.1 ± 0.2 |
| 30 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 31 | −37.4 ± 0.2 | −35.1 ± 0.1 | −36.1 ± 0.1 | −33.1 ± 0.3 | −37.0 ± 0.1 | −33.3 ± 0.3 | −35.5 ± 0.2 | −35.9 ± 0.1 |
| 32 | n.d. | −36.2 ± 0.2 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 33 | n.d. | −37.2 ± 0.1 | −36.7 ± 0.1 | −36.5 ± 0.4 | −35.2 ± 0.1 | −33.2 ± 0.2 | n.d. | −38.3 ± 0.1 |
| 34 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 35 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Weighted mean alkanes (WMA) | −37.9 | −36.6 | −35.9 | −33.4 | −36.4 | −33.3 | −36.0 | −36.0 |
| $\Delta\delta$[c] | 1.5 | 2.1 | 2.8 | 5. 5 | 1.8 | 0.2 | 3.0 | 4.2 |
| | L | M | N | O | P | Q | R | S |
| Bulk carbon (TT) | | | | | −30.1 | −28.4 | −27.1 | −28.2 |
| Isotopic fractionation $\Delta\delta$ (TT-WMA) | | | | | 6.8 | 8.9 | 9.8 | 7.0 |
| 27 | −37.1 ± 0.3 | n.d. | −33.0 ± 0.1 | n.d. | −36.3 ± 0.2 | n.d. | −35.7 ± 0.2 | −34.5 ± 0.2 |
| 28 | n.d. | n.d. | n.d. | n.d. | −36. 8 ± 0.1 | n.d. | −36.3 ± 0.3 | n.d. |
| 29 | −36.6 ± 0.2 | n.d. | −33.3 ± 0.1 | −30.1 ± 0.2 | −36.6 ± 0.2 | −36.7 ± 0.2 | −36.5 ± 0.1 | −35.3 ± 0.3 |
| 30 | n.d. | n.d. | n.d. | −30.1 ± 0.2 | −37.5 ± 0.2 | −37.6 ± 0.1 | −36.9 ± 0.1 | n.d. |
| 31 | −37.1 ± 0.1 | −35.5 ± 0.1 | −37.2 ± 0.2 | −30.5 ± 0.1 | −37.2 ± 0.1 | −37.5 ± 0.1 | −37.2 ± 0.1 | −33.8 ± 0.1 |
| 32 | n.d. | −34.9 ± 0.3 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 33 | −36.9 ± 0.1 | −35.7 ± 0.2 | −37.9 ± 0.2 | −29.1 ± 0.6 | −36.9 ± 0.3 | −38.3 ± 0.1 | −37.4 ± 0. 1 | −34.4 ± 0.1 |
| 34 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 35 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | −36.9 ± 0.1 |
| Weighted mean alkanes (WMA) | −36.9 | −35.5 | −35.7 | −30.0 | −36.9 | −37.3 | −36.9 | −35.2 |
| $\Delta\delta$[c] | 0.5 | 0.8 | 4.9 | 1.4 | 1.3 | 1.6 | 1.7 | 3.1 |

Code letters refer to plants listed in Table 1.
n.d. = not determined.
[a] Indicated uncertainties are standard deviations of replicate analyses.
[b] Weighted mean alkanes = $(\sum[c_i]\times\delta_i)/\sum[c_i]$; for $i = 27$–35, where $c_i$ is the concentration of the *n*-alkane containing *i* carbon atoms.
[c] $\Delta\delta = \delta^{13}C$ range for individual *n*-alkanes.

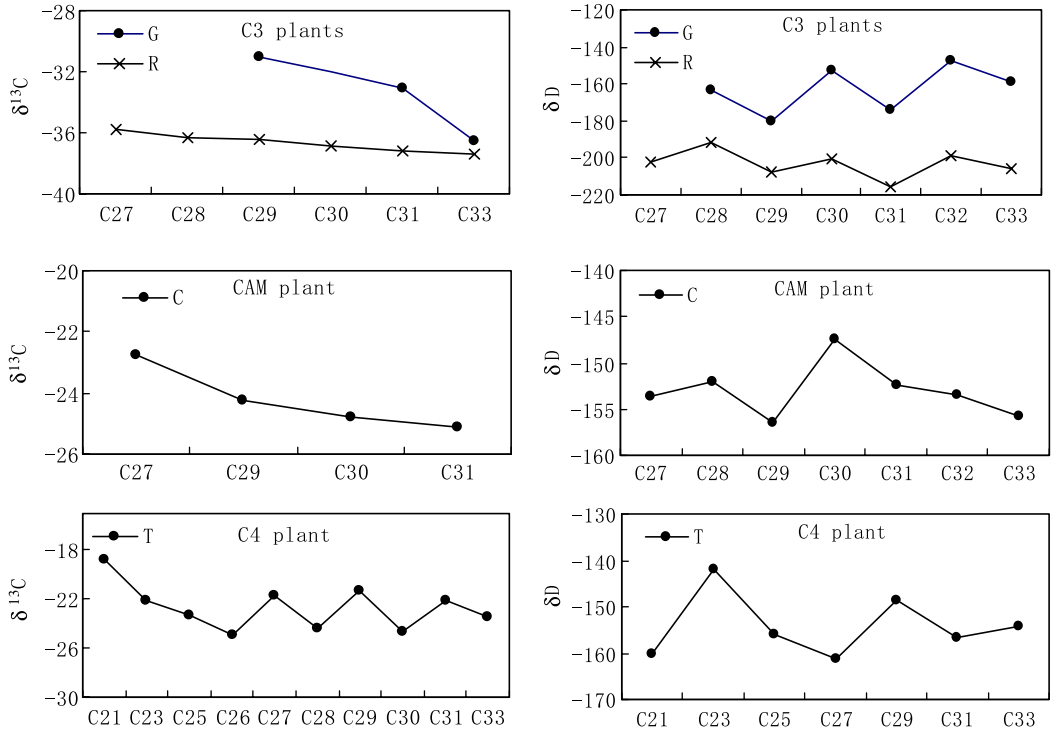


Fig. 1. Compound-specific plant wax *n*-alkane $\delta^{13}C$ (left) and $\delta D$ values (right) vs *n*-alkane carbon number. (For code letters, refer Table 1.)

presented in Tables 5 and 6. Hydrogen isotope results displayed a large compositional variation. Weighted mean $\delta D$ values of *n*-alkanes for $C_3$, $C_4$ and CAM plants were $-175.7 \pm 29.5‰$, $-150.4 \pm 42.6‰$ and $-170.4 \pm 14.8‰$, respectively. The range of weighted mean $\delta D$ variation of different plant types was more than 100‰, which is significantly higher than for carbon isotopes (23.3‰). Estep and Hoering (1980) also found that plants from several natural populations also fractionated hydrogen isotopes during photosynthesis by an average of −90‰ to −110‰. Other results from a variety of plants representing a wide geographical range and plant classes reveal that water source and uptake are important factors in determining the $\delta D$ value of cellulose hydrogen (Dawson and Ehleringer, 1993; Sternberg, 1988; Yapp and Epstein, 1982). Moreover, biogeochemistry pathway of the stable carbon and hydrogen isotopes may be different in different regions. However, for a given sampling place with the same water source and biogeochemistry status in South China Botanical Garden, plant classes and some environmental factors may play an important role in hydrogen isotope fractionation. Therefore, in comparison with $C_3$ and CAM plants, the slightly larger but not significant D value of $C_4$ plants could mainly ascribe to its higher water use efficiency (as indicated by lower $^{13}C$ value in bulk leaf) and isotope discrimination during *n*-alkanes biosynthesis.

The hydrogen isotopic compositions of individual *n*-alkanes were widely variable in each plant. $\delta D$ values of individual *n*-alkanes ranged from −233.1‰ to −80.7‰. $\delta D$ variations among *n*-alkane homologues for individual plants ranged from 6.3‰ to 41.2‰ and averaged 21.8‰ (Tables 5 and 6) with no difference among the $C_3$, $C_4$ and CAM plants examined. The reason for the large isotopic variations of *n*-alkane $\delta D$ values in each plant may be due to production of different homologues in differing proportions during the growth cycle of the leaves, with the plants being exposed to diverse conditions of temperature, watering and nutrient availability (Yapp and Epstein, 1982; Guelz et al., 1991; Maffei and Scannerini, 1992; Collister et al., 1994). Like carbon isotopic composition, most species also exhibited a zigzag pattern in $\delta D$ value dependent on carbon number. However, in the case of $\delta D$, the even carbon-numbered compounds were more enriched in D than the odd carbon-numbered compounds (Fig. 1). While most plants showed a trend towards increasing D enrichment with increasing carbon number (e.g., plants F, G, I). Some others exhibited an opposite trend (e.g., plants M, R, W). The remainder of the plants exhibited isotope distributions independent of carbon number.

Table 5
$\delta$D values of individual *n*-alkanes[a] and mean weighted$\delta$D values of all *n*-alkanes[b] for CAM and $C_4$ plants

| *n*-Alkanes | A | B | C | T | U | V | W | X | Y | Z |
|---|---|---|---|---|---|---|---|---|---|---|
| 21 | n.d. | n.d. | n.d. | −160.2 ± 0.3 | n.d. | n.d. | n.d. | n.d. | −146.5 ± 1.3 | n.d. |
| 23 | n.d. | n.d. | n.d. | −142.0 ± 0. 7 | n.d. | n.d. | n.d. | −138.3 ± 1.4 | −127.3 ± 1.7 | n.d. |
| 25 | n.d. | n.d. | n.d. | −156.0 ± 4.2 | −125.6 ± 1.0 | −110.1 ± 1.7 | n.d. | −129.5 ± 2. 9 | n.d. | −226.8 ± 1.6 |
| 27 | n.d. | −201.6 ± 0.8 | −153.6 ± 2.0 | −161.1 ± 3. 8 | −123.42 ± 0.9 | −94.5 ± 0.5 | −149.5 ± 2.0 | −130. 7 ± 2.1 | n.d. | −233.1 ± 1.5 |
| 28 | n.d. | n.d. | −152.0 ± 2.3 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 29 | n.d. | −177.6 ± 3.3 | −156.4 ± 0.9 | −148.7 ± 2.4 | −123.1 ± 2.2 | −94. 5 ± 1.6 | −174.2 ± 3.3 | −131.8 ± 1.8 | −120.1 ± 1.0 | −218.4 ± 3.2 |
| 30 | n.d. | n.d. | −147.5 ± 1.8 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 31 | −170.8 ± 1.4 | −181.6 ± 2.3 | −152.4 ± 1.6 | −156.8 ± 2.8 | −119.3 ± 1.6 | −107.8 ± 0.9 | −184.9 ± 0.5 | −142.9 ± 1.0 | −129.6 ± 1.0 | −207.0 ± 2. 7 |
| 32 | −166.6 ± 1.6 | −167.8 ± 2.2 | −153.4 ± 2.6 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 33 | −181.3 ± 2.8 | −180.2 ± 1.1 | −155.8 ± 2.8 | −154.2 ± 2.5 | n.d. | n.d. | −182.3 ± 2.8 | −148.8 ± 1.7 | −124.3 ± 0.4 | n.d. |
| 34 | −165.3 ± 1.0 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 35 | −181.2 ± 1.3 | n.d. | n.d. | n.d. | n.d. | n.d. | −183.1 ± 2.7 | n.d. | −116.6 ± 1.8 | n.d. |
| Weighted mean alkanes (WMA)[c] | −176.4 | −181.3 | −153.6 | −154.5 | −122.2 | −101.2 | −181.4 | −136.9 | −128.2 | −228.1 |
| $\Delta\delta$[d] | 16.0 | 33.8 | 8.9 | 19.0 | 6.3 | 15.6 | 35.4 | 19.3 | 29.9 | 26.1 |

Code letters refer to plants listed in Table 1.
n.d. = not determined.
[a] A to C are CAM plants; T to Z are C4 plants.
[b] Indicated uncertainties are standard deviations of replicate analyses.
[c] Weighted mean alkanes = $(\sum[c_i]\times\delta_i)/\sum[c_i]$; for $i$ = 21-35, where $c_i$ is the concentration of the *n*-alkane containing $i$ carbon atoms.
[d] $\Delta\delta = \delta$D range for individual *n*-alkanes.

Table 6
$\delta$D values of individual *n*-alkanes[a] and mean weighted$\delta$D values of all *n*-alkanes[b] for $C_3$ plants

| *n*-Alkanes | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|
| 27 | −195.9 ± 3.1 | n.d. | −174.9 ± 0.9 | n.d. | −160.9 ± 0.2 | −156.8 ± 2.6 | −145.6 ± 0.6 | n.d. |
| 28 | −193.4 ± 2.2 | n.d. | n.d. | −163.0 ± 5.0 | −163.7 ± 4.1 | −148.4 ± 0.8 | −143.8 ± 2.7 | n.d. |
| 29 | −198.5 ± 2.9 | −155.7 ± 1.7 | −165.7 ± 2.6 | −180.0 ± 2.6 | −174.9 ± 3.7 | −158.4 ± 1.4 | −161.2 ± 3.8 | −171.0 ± 2.1 |
| 30 | −188.6 ± 1.6 | −155.0 ± 0.2 | −152.4 ± 2.3 | −152.9 ± 2.6 | −169.1 ± 2.1 | −145.4 ± 2.8 | −151.1 ± 0.9 | −167.2 ± 0.1 |
| 31 | −199.4 ± 1.9 | −168.1 ± 2.1 | −159.4 ± 0.7 | −173.8 ± 1.1 | −171.3 ± 1.3 | −158.3 ± 0.9 | −162.4 ± 1.6 | −170.8 ± 3.3 |
| 32 | n.d. | −162.9 ± 3.5 | −144.8 ± 5.2 | −147.3 ± 5.6 | −166.9 ± 5.5 | −142.7 ± 1.5 | −137.0 ± 3.1 | −164.2 ± 1.2 |
| 33 | n.d. | −172.6 ± 1.6 | −155.1 ± 1.1 | −158.6 ± 3.7 | −174.4 ± 4.6 | −147.7 ± 1.9 | −148.0 ± 5.3 | −171.7 ± 1.4 |
| 34 | n.d. | −155.1 ± 1.6 | n.d. | n.d. | −166.3 ± 2.7 | n.d. | n.d. | n.d. |
| 35 | n.d. | n.d. | n.d. | n.d. | −177.1 ± 0.8 | −136.1 ± 0.8 | n.d. | n.d. |
| Weighted mean alkanes (WMA) | −190.6 | −168.8 | −158.6 | −168.8 | −171.8 | −152.6 | −160.2 | −170.5 |
| $\Delta\delta$[c] | 10.8 | 17.6 | 30.1 | 32.7 | 16.2 | 22.3 | 25.4 | 7.5 |
| | L | M | N | O | P | Q | R | S |
| 27 | −168.5 ± 0.9 | n.d. | −208.9 ± 3.8 | −121.8 ± 1.5 | −199.8 ± 2.0 | −198.7 ± 0.5 | −202.4 ± 1.5 | −201.6 ± 3.8 |
| 28 | −160.2 ± 1.0 | n.d. | −204.4 ± 1.3 | −105.5 ± 1.6 | −196.7 ± 1.3 | −185.6 ± 2.7 | −191.5 ± 0.1 | −199.2 ± 0.7 |
| 29 | −161.2 ± 0.9 | −196.4 ± 2.1 | −206.7 ± 2.4 | −109.6 ± 2.2 | −203.8 ± 0.9 | −208.9 ± 1.8 | −207.5 ± 1.9 | −204.1 ± 1.6 |
| 30 | −142.7 ± 1.4 | −188.7 ± 0.5 | −184.1 ± 1.9 | −96.6 ± 0.9 | −194.8 ± 1.1 | −204.7 ± 0.3 | −200.1 ± 1.5 | −209.0 ± 1.8 |
| 31 | −153.5 ± 1.8 | −205.0 ± 0.7 | −191.4 ± 2.2 | −95.2 ± 1.9 | −200.6 ± 3.4 | −213.1 ± 1.3 | −215.8 ± 1.0 | −210.4 ± 2.0 |
| 32 | −142.8 ± 1.5 | −190.9 ± 1.4 | −177.2 ± 1.4 | −80.7 ± 2.3 | −186.5 ± 2.0 | −199.0 ± 4.0 | −198.4 ± 2.0 | −204.4 ± 2.3 |
| 33 | −144.0 ± 1.1 | −199.2 ± 2.9 | −190.6 ± 1.6 | −80.7 ± 2.1 | −198.2 ± 1.0 | −207.5 ± 2.1 | −206.0 ± 1.0 | −211.2 ± 2.8 |
| 34 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | −204.3 ± 1.9 |
| 35 | −139.7 ± 2.1 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | −206.4 ± 3.0 |
| 36 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | −203.2 ± 1.6 |
| 37 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | −203.1 ± 3.6 |
| Weighted mean alkanes (WMA) | −152.9 | −200.8 | −196.4 | −95.9 | −199.8 | −208.4 | −209.1 | −206.7 |
| $\Delta\delta$[c] | 28.9 | 16.2 | 31.7 | 41.2 | 17.3 | 27.5 | 24.2 | 12.0 |

Code letters refer to plants listed in Table 1.
n.d. = not determined.
[a] Indicated uncertainties are standard deviations of replicate analyses.
[b] Weighted mean alkanes = $(\sum[c_i]\times\delta_i)/\sum[c_i]$; for $i = 27$–35, where $c_i$ is the concentration of the*n*-alkane containing $i$ carbon atoms.
[c] $\Delta\delta = \delta$D range for individual *n*-alkanes.

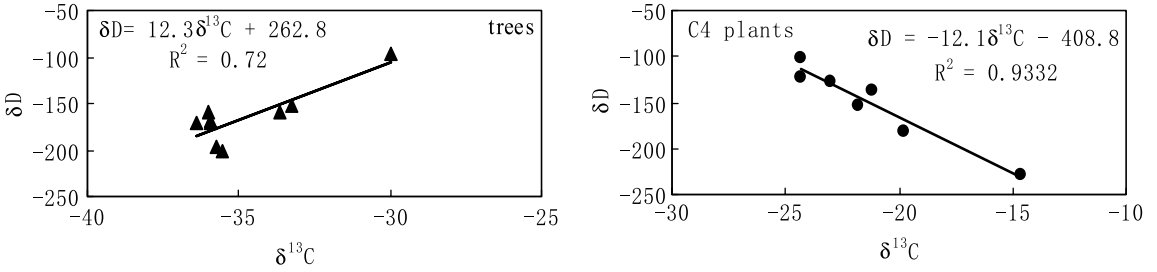


Fig. 2. Relationships between weighted mean $\delta$D and $\delta^{13}$C values for *n*-alkanes from plants with different photosynthetic pathways (▲: $C_3$ plants (trees); ●: $C_4$ plants).

Carbon and hydrogen isotopic compositions of *n*-alkanes are expected to be dependent on biosynthetic processes and environmental conditions (Sternberg, 1988; Hayes, 1993; Chikaraishi and Naraoka, 2003; Otieno et al., 2005). However, the relationship between carbon and hydrogen isotopic compositions has not been clarified for each plant. From Fig. 2, it can be seen that there was a strong negative relationship between weighted mean $\delta^{13}$C and $\delta$D in $C_4$ plants ($R^2 = 0.93$). The slope was found to be negative, indicating a decrease in $\delta$D with increasing $\delta^{13}$C. In the case of $C_3$ plants, however, the relationship is not obvious ($R^2 = 0.55$). It is worth noting that all the tested $C_4$ plants are herb type. If limited to the data from nine tree species, the correlation coefficient was positive and increased to $R^2 = 0.72$. Hence, the reason for no obvious relationship between $\delta$D and $\delta^{13}$C in $C_3$ plants might be partly due to the different life types (tree, shrub and herb). Because of the limited number of samples, the correlation for CAM plants is still unclear.

## 4. Conclusions

Plants with different photosynthetic pathways can be clearly distinguished using carbon isotopes, whereas hydrogen isotopes were not diagnostic discriminators for $C_3$, $C_4$ and CAM plants. *n*-Alkanes in $C_4$ plants were most $^{13}$C-enriched with $\delta^{13}$C values ranging from $-26.4‰$ to $-14.1‰$. In contrast, $C_3$ plants had the most negative $\delta^{13}$C values ranging from $-29.1‰$ to $-38.9‰$. CAM plants expressed an intermediate $\delta^{13}$C range. Compound-specific $\delta^{13}$C values from $C_3$ and $C_4$ *n*-alkane leaf surface waxes were more depleted in $^{13}$C relative to total leaf tissue by 6.7–11.0‰. Hydrogen isotopes displayed a greater compositional variation than carbon isotopes. $\delta$D values of individual *n*-alkanes ranged between $-233.1‰$ and $-80.7‰$. There was a strong negative relationship between weighted mean $\delta^{13}$C and $\delta$D in $C_4$ plants while plant type tree expressed a positive relationship in $C_3$ plants. These results suggest that the carbon and hydrocarbon isotopic correlation was dependent on plant types as well as photosynthetic pathways. Thus combined analyses of carbon and hydrogen isotopes may have superior diagnostic potential for tracing sources of organic compounds in the environment than analytical approaches that rely on only carbon or hydrogen alone.

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