Histochemistry and Cell Wall Specialization of Oil Cells related to the Essential Oil Accumulation in the Bark of *Cinnamomum cassia* Presl. (Lauraceae)

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Abstract: Chinese cinnamon (*Cinnamomum cassia*) has economic value as an aromatic and medicinal plant, and its bark oil has a very high trans-cinnamaldehyde content. To gain insight into the accumulation of bark oil and the biological mechanisms which permit the accumulation of a high level of aldehydes in *C. cassia* bark, the morphology and histochemistry of oil cells and the specialization in its walls were assessed by light and fluorescence microscopy. The histochemical tests localized in situ the main chemical classes of metabolites in oil cells, which included aldehydes, lipids and terpenoids. In oil cells the aldehydes distributed in the area surrounding the oil sac were compartmentalized from lipid compounds in the center; the oil sac in an oil cell was attached by multiple cupules rather than one cupule. The autofluorescence of oil-cell walls was attributed to the presence of suberin and lignin, and was confirmed by different methods. The lignified and suberized walls probably serve as protective barriers against the cytotoxicity of high contents of trans-cinnamaldehyde to the surrounding active cells. These results contribute to our knowledge of the structure of oil cells and accumulation of essential oil in Chinese cinnamon bark.

Key words: Bark, *Cinnamomum cassia*, Essential oil, Histochemistry, Lignified wall, Oil cells. Suberized wall.

*Cinnamomum cassia* Presl. (Lauraceae), also known as Chinese cinnamon, is an indigenous evergreen tree which is widely distributed and cultivated in Southeast Asia, and was introduced to Indonesia, South America and Hawaii (Wang et al., 2009). Cinnamon is one of the earliest known spices (Lee and Balick, 2005). The bark of *C. cassia* is an important Chinese medicine material (China Pharmacopoeia Commission, 2005). Bioassays and component analyses of cinnamon bark oil in naturally occurring samples have been reported. The bark oil exhibits antioxidant (Lin et al., 2005), antifungal (Giordani et al., 2006; Ooi et al., 2006; Lee et al., 2007) and antibacterial activities (Chang et al., 2001; Ooi et al., 2006) and has a potential as a repellent and antitumor agent (Chang et al., 2006; Shin et al., 2006). This plant is commonly cultivated in the southern provinces of China such as Guangdong, Guangxi, Hainan and Yunnan. There are more than 270,000 ha of *C. cassia* trees in China (Wei et al., 2006). Because of its characteristic cinnamon aroma, the bark oil of *C. cassia* is of considerable importance in the food, pharmaceutical, perfumery, cosmetics and flavor industries. The bark of *C. cassia* contains 1–2% (v/w) volatile oil, 60–80% (w/w) of which is the cinnamaldehyde that provides its bioactivity (Ooi et al., 2006; Geng et al., 2011). In contrast, in many other aromatic plants, the lipophilic materials secreted by oil cells mainly contain volatile low-molecular-weight terpenes (Fahn, 1988). Such a high content of aldehydes is toxic to surrounding metabolically active cells, and Choi et al. (2001) reported that cinnamaldehyde in *C. cassia* oil has potent cytotoxicity. In general, plant volatiles are produced in specialized secretory structures which can minimize the risk of autotoxicity and simultaneously allow the presence of a high level of metabolic components at sites where their defensive and/or attractive role may be vital (Figueiredo et al., 2008).

In the Lauraceae family, oil cells containing oil drops are known to be primary sites of essential oil biosynthesis, secretion and storage (Fahn, 1988). Oil cells are commonly present in roots, stems and fruit, as well as leaves of the Lauraceae (Metalff and Chalk, 1983; Baas and Gregory, 1987).
1985; Chu and Hu, 1998, 1999), and the morphology of secretory idioblasts in Lauraceae have been well studied (Maron and Fahn, 1979; Bakker and Gerritsen, 1989; Bakker and Gerritsen, 1996; Chu et al., 1999; Chu and Hu, 1999, 2001a, 2001b). The main structural events in oil cell differentiation are the deposition of a suberized layer inside the outer cellulosic wall and the formation of the cupule, a localized cell wall protuberance, to which the oil sac is attached (Mariani et al., 1989; Chu and Hu, 2001a). Although the cupule and the suberized cell wall in the oil cell have been studied, many problems concerning the structure and function of idioblastic cells remain unsolved (Maron and Fahn, 1979; Metcalfe and Chalk, 1983). Little is known about the biological and compartmentalization mechanisms that permit the accumulation of cinnamaldehyde in cinnamon bark tissues. Due to its economic potential, it is important to study the function of idioblastic cells and the accumulation of essential oil in C. cassia bark.

In this paper, we report the morphology and structure of oil cells in C. cassia bark using light and fluorescence microscopy, and discuss the compartmentalization of aldehyde compounds and lipid compounds in oil cells based on the results of histochemistry and GC-MS analysis. The suberized and lignified cell wall in oil cells of C. cassia bark was confirmed, and considered to be an important mechanism of compartmentalizing oil cells from the surrounding cells.

Materials and Methods

1. Plant material

Fresh bark of C. cassia was collected in March 2009 from the Tree Garden of the South China Agricultural University (Guangzhou, Guangdong Province, China). The fresh samples were used for both morphological and histochemical investigations. C. cassia trees at different stages of growth (4–11 years old, 4–8 m tall) were harvested in April 2009 from Zhaoqing (Guangdong province, China) for the plant anatomic structure and the chemical analysis. The age of each stem and branch was determined by counting the annual rings of the lower section. The stem bark and the branch bark of each tree were collected separately. The materials used for paraffin sections were fixed in formalin-acetic acid-alcohol immediately after being cut down. The materials used for extraction of essential oil were placed in open containers in a dust free environment for three weeks to dry naturally. Ambient temperatures ranged from 24– to 27°C and the relative humidity from 30 to 50%. Voucher specimens were deposited in the Herbarium of South China Agricultural University.

2. Morphological studies

We investigated the special morphology of oil cells and their distribution by means of light microscopy (LM). Both transmitted light and epi-fluorescence (UV or blue-light excitation) were employed to observe the difference between oil cells and their surrounding cells.

(1) Paraffin sections: Fixed plant material was dehydrated in an alcohol series, and then embedded in paraffin. Serial sections were cut at a 12–15 μm thickness using a microtome (Leica RM 2135) and doubly stained with Safranin O and Fast Green.

(2) Fresh sections: Fresh material was frozen and sectioned at a 60–80 μm thickness using a cryostat microtome (Thermo Scientific, Microm HM525), and the sections were mounted in glycerine on glass slides with cover slips. Observations were made with a Zeiss Axioskop 40 light microscope under transmitted and fluorescence light in which blue light was excited at 450–480 nm using a Zeiss LP 515 barrier filter, and UV-light was excited at 330–385 nm using a Zeiss LP 420 barrier filter.

3. Histochemical investigations

Identifying the main groups of metabolites in oil cells: Fresh material was frozen, sectioned and stained with the following reagents: Schiff’s reagent (Lewinsohn et al., 1998) and 2, 4-dinitrophenylhydrazine (Liu and Deng, 1979; Ni et al., 2007) for aldehydes; Sudan III (Ruzin, 1999; Rodrigues et al., 2008) and Neutral Red under UV (Ascensao et al., 1999) for total lipids; Nadi reagent for terpenes (Moura et al., 2005); and concentrated H2SO4 for sesquiterpenes (Cappelletti et al., 1986; Nikolakaki and Christodoulakis, 2007). In addition, starch in parenchyma cells and stone cells was identified using I2-KI reagent (Ruzin, 1999). Standard control procedures were conducted simultaneously to assess the specificity of labeling in several control tests by (1) direct observation without staining, (2) staining with the same reagent after being extracted in an alcohol series.

Testing for the presence of suberin and lignin in oil-cell walls: Fresh material was frozen, sectioned and stained with the following reagents: phosphate buffer (pH 9.1) under UV and Sudan Black B reagent for oil cells and suberin of cell walls (Ruzin, 1999); berberine–aniline-blue stain (Brundrett et al., 1988), phloroglucinol-HCl (Ruzin, 1999; Patten et al., 2005) and Mäule reagent (Patten et al., 2005) for lignin of cell walls, respectively. Standard control procedures were conducted simultaneously as mentioned above.

4. Extraction and analysis of essential oil

The essential oil in the bark of 2-year-old and 9-year-old trees was extracted by hydrodistillation in accordance with the method in the Pharmacopoeia of the People’s Republic of China 2005 Volume 1 (China Pharmacopoeia Commission, 2005). Forty grams of cinnamon bark powder precisely weighed was mixed with 800 mL distilled water. The mixture was then heated at 100°C for 4 hr. The
Fig. 1. Morphology and distribution of oil cells in *Cinnamomum cassia* bark. (A-D) Details of cross-sections of fresh bark: (A) oil cell with a large sac, in which the surrounding area appears black with the center area bright under transmitted-light; (B) the same section with (A), showing an intense blue–white autofluorescence emitted from the oil-cell wall under UV-light excitation; (C) the section stained with berberine–aniline blue, showing the morphology of an oil sac in an oil cell under transmitted-light; (D) the section stained with berberine, showing the morphology of an oil sac and cupule in an oil cell under transmitted-light. (E and F) Cross-sections of fresh bark observed under UV-light excitation: exhibiting the distribution of oil cells in young bark of two years’ growth (E) and older bark of nine years’ growth (F). Arrowheads, oil cell; CA, center area of oil sac; CU, cupule; DOC, destroyed oil cell; SA, area surrounding the oil sac; OS, oil sac. Bars = 100 μm.

Fig. 2. Histochemistry of oil cells in *Cinnamomum cassia* bark. (A) Schiff’s reagent, the same section observed under transmitted light (A1) and UV (A2), respectively, showing the difference in color between the surrounding and center areas of the oil sac; (B) 2,4-dinitrophenylhydrazine, the same section observed under transmitted light (B1) and UV (B2), showing the difference in color between the surrounding and center areas of the oil sac; (C) Sudan III; (D) Neutral Red under UV, showing the difference of color between the surrounding and center area of oil sac; (E) Nadi reagent; (F) concentrated H2SO4. Arrowheads, oil cell; CA, center area of oil sac; DOC, destroyed oil cell; SA, area surrounding the oil sac; OS, oil sac. Bars = 100 μm.
Fig. 3. The developing oil sacs and multiple cupules in oil cells of Cinnamomum cassia bark. (A-D) Cross-sections of fresh bark following staining with Sudan III: (A) an oil cell at early-developing stage, a small oil sac is attached to a short cupule and surrounded by a lot of lipophilic substances in the cell lumen; (B) an oil cell at near-mature stage, the oil sac has become larger, and is attached to a longer cupule and surrounded by a litter of lipophilic substances in the cell lumen; (C-D) a mature oil cell, the oil sac is larger and attached to one cupule (C) or multiple cupules (D), and all lipophilic substances in the cell lumen have been secreted into the oil sac. (E-F) The same section stained with berberine-aniline blue but observed at different foci under transmitted-light, showing two cupules in one oil cell. Arrowheads, oil cell; CL, cell lumen; CU, cupule; OS, oil sac; Bars=50 μm.

Fig. 4. The suberized and lignified walls of oil cells in Cinnamomum cassia bark. (A-B) Details of suberized walls of oil cells: (A) mounted in 0.02 M phosphate buffer (pH 9.1), showing the suberized oil-cell walls fluorescing yellow-white from the median layer under UV; and (B) stained with Sudan Black B, showing the suberized oil-cell walls stained blue at the median layer. (C-F) Details of lignified walls of oil cells: (C) stained with berberine and observed under UV, showing the intense yellow-green fluorescence from the lignified walls of oil and fiber cells; (D) stained with berberine-aniline blue and observed under UV, showing the lignified walls of oil and fiber cells fluorescing yellow-green, while the cellulose walls of the parenchymatous cells surrounding the oil cells are blue; (E-F) respectively stained with phloroglucinol-HCl (E) and Mäule reagents (F), showing the lignified walls of oil and fiber cells stained red. Arrowheads, oil cell; FC, fiber cell; ML, the median layer of oil-cell wall; OL, the outer layer of oil-cell wall; Bars=50 μm.
In addition, the oil-cell wall had an intense blue–white autofluorescence under UV-light excitation (Fig. 1B). Fluorescence of this nature, clearly different from that emitted by other tissues, is evidence of the specialized function of the cell (Gersbach, 2002). The special autofluorescence of the cell wall enabled the oil cell to be clearly distinguished from the surrounding parenchyma cells.

The distribution pattern of the oil cells in the younger bark was different from that in the older bark. In the young bark of 1- to 3-year-old trees, the oil cells were scattered in the cortex and phloem (Fig. 1E); however, they were mainly situated in the secondary phloem in the older bark of 4- to 11-year-old trees, and most of them were distributed in the area near the vascular cambium, which was produced later (Fig. 1F). There was a greater density of oil cells in the young compared to the older bark.

2. Histochemistry of oil cells

The histochemical test localized in situ the main chemical classes of metabolites present in oil cells of Chinese cinnamon bark, including aldehydes, lipids and terpenoids. Some of the test reactions are presented in Table 1 and Figure 2. The content of the oil cells was stained purple–red with Schiff’s reagent (Fig. 2A1), revealing its aldehyde nature; confirmed by brown–red staining with 2, 4-dinitrophenylhydrazine (Fig. 2B1). The lipid nature of the oil cell contents was identified by orange-red staining with Sudan III (Fig. 2C) and by orange fluorescence with Neutral Red reagent under UV excitation (Fig. 2D), respectively. The purple color with Nadi reaction indicated the presence of terpenoids in oil cells (Fig. 2E). The concentrated H2SO4 reaction to sesquiterpenes, which appeared orange, indicated the presence of sesquiterpenes (Fig. 2F). In addition, the obtained volatile extract was kept at 4°C for further analysis. GC-MS analysis was made using a gas chromatograph (Finnigan Trace GC-2000)-Mass Spectrometer (Thermo Finnigan, American). A 0.25 mmID ×30 m HP-1 bonded-phase fused-silica capillary column with a film thickness of 1μm was used. The injector temperature was 200°C. The oven temperature was programmed from 45°C (3 min isothermal) to 80°C at 5°C min\(^{-1}\) and then to 170°C (held for 10 min at final temperature) at 8°C min\(^{-1}\). The interface was kept at 200°C, and mass spectra were obtained at 70 eV. The effluent of the capillary column was introduced directly into the ion source of the mass spectrometer. The sector mass analyzer was set to scan from 35 to 335 amu. The linear velocity of the helium carrier gas was 1.0 mL min\(^{-1}\) at a split ratio of 1:60. Components of essential oil were identified by comparing mass spectra of each peak with those of authentic samples in a mass spectra library (The NIST Registry of Mass Spectral Data). Especially, identification of cinnamaldehyde was conducted by comparing both the retention times and mass spectra with the commercially available authentic samples.

Results

1. Morphology and distribution of oil cells

The morphology and distribution of oil cells in the Chinese cinnamon bark are shown in Figure 1. The oil cells were single, isolated and larger than the surrounding cells (Fig. 1A). In the cross-section, the length of oil cells was generally 50–100 μm, and their shapes were spherical to ellipsoidal (Fig. 1). A mature oil cell at the secretory stage had, in vivo, a characteristic faint-yellow color under transmitted light (Fig. 1A), and its cytoplasm and nucleus had disintegrated, leaving a large oil sac occupying almost the entire cell lumen (Fig. 1C, D). The area surrounding the oil sac appeared black, while the center bright (Fig. 1A). In addition, the oil-cell wall had an intense blue–white autofluorescence under UV-light excitation (Fig. 1B).

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numerous particles in parenchyma cells and stone cells of phloem were confirmed to be starch granules by the black–blue coloring when stained with I\textsubscript{2}–KI (data not shown).

The different staining methods revealed a color pattern in the area surrounding the oil sac different from that in the center of the oil sac in mature oil cells, implying that the substances in the area surrounding the oil sac were different from those in the center. For example, fresh sections stained with Schiff’s reagent and 2, 4-dinitrophenylhydrazine showed the characteristic colors of aldehydes distributed in the area surrounding the oil sac in the oil cell, while the center had a different color (Fig. 2A1 and B1). This phenomenon was clear under UV excitation: the surrounding area emitted a red fluorescence, while the center was dim when stained with Schiff’s reagent (Fig. 2A2); or the surrounding area emitted a brown fluorescence, and the center a yellow fluorescence, when stained with 2, 4-dinitrophenylhydrazine (Fig. 2B2). These results indicated that aldehydes were mainly distributed in the area surrounding the oil sac. In contrast, when stained with Neutral Red and observed under UV light excitation, the center of the oil sac showed a characteristic orange–yellow fluorescence of lipids, while the surrounding area emitted an orange–red fluorescence (Fig. 2D), indicating that lipids were mainly distributed in the center of the oil sac.

3. **Multiple cupules in oil cells**

Cupules were clearly observed in a number of oil cells of *C. cassia* bark (Fig. 3), especially in sections stained with Sudan III and observed with an oil-immersion objective lens (Fig. 3A-D).

| No. | Compound | Area percentage (%) |
|-----|----------|---------------------|
|     |          | 2 years old | 9 years old |
| 1   | Benzaldehyde        | 0.82        | 0.18        |
| 2   | Benzenepropanal     | 0.20        | –           |
| 3   | cis-Cinnamaldehyde  | 1.15        | 0.64        |
| 4   | 2-Methoxy-benzaldehyde | 0.07        | –           |
| 5   | trans-Cinnamaldehyde| 73.47       | 81.32       |
| 6   | para-Methoxy cinnamic aldehyde | 1.89 | 0.57 |
| 1   | Cinnamyl acetate    | 7.08        | 11.15       |
| 1   | trans-Caryophyllene | 0.50        | 0.44        |
| 2   | α-Bergamotene       | 0.42        | –           |
| 3   | α-Farnesene         | 0.33        | –           |
| 4   | Juniperene          | 0.29        | 0.20        |
| 5   | α-Isobolene         | 1.02        | –           |
| 6   | \(\delta\)-Cadinene | 0.67        | 0.26        |
| 7   | α-Muaroene          | 0.33        | –           |
| 8   | \(\delta\)-Cadinol  | 0.27        | –           |
| 9   | Spathulenol         | 0.14        | –           |
| 10  | α-Isobolol          | 0.72        | –           |
| 1   | Isopropylbenzene    | 1.26        | 0.42        |
| 2   | Propyl benzene      | 2.09        | 0.71        |
| 3   | 1-Ethyl-3-methyl-benzene | 1.52 | – |
| 4   | 1-Ethyl-4-methyl-benzene | 0.49 | 0.53 |
| 5   | Cinnamyl alcohol    | 0.08        | 0.19        |
| 6   | 1-(1,5-Dimethyl-4-hexenyl)-4-methyl-benzene | 0.49 | 0.31 |
| 7   | 2,4,5-Trimethyltriphenylmethane | 0.30 | 0.54 |
|     | **Total**            | **96.20**   | **97.75**   |
cupules were shorter and attached to a small oil sac surrounded by a lot of lipophilic substances, and lipophilic substances filled the cell lumen (Fig. 3A, B); while in mature oil cells, cupules were longer and oil sacs larger, and lipophilic substances in the cell lumen were gradually transferred into the oil sac (Fig. 3C, D). The cupules were stained the same color as the oil sacs when stained with Sudan III. In addition, there were 2–4 cupules attached to each oil sac in each oil cell and the cupules were located on any side of the cell (Fig. 3C, D). This phenomenon was observed more clearly when stained with berberine–aniline blue, which gave a better contrast of photographs, and the different cupules could be clearly identified at different foci in an oil cell (Fig. 3E, F).

4. Lignification and suberization of oil-cell walls

Both under UV and blue light excitation, cell wall autofluorescence was observed in oil cells. Especially there was an intense bright blue fluorescence at the median wall layer of the oil cell under UV light excitation (Fig. 1B). Such autofluorescence should be due to a suberized layer (Brundrett et al., 1988; Bakker and Gerritsen, 1990; Surový et al., 2009) – this supposition was then tested by two techniques as described by Ruzin (1999) (Fig. 4). When fresh sections were mounted in 0.02 M phosphate buffer (pH 9.1), the cell walls in the median layer fluoresced yellow–white, indicating the presence of suberin (Fig. 4A). When the sections were stained with Sudan Black B, the median wall layer was stained blue showing a positive reaction for suberin (Fig. 4B). These results confirmed the presence of suberin in the oil-cell walls. In addition, the blue fluorescence emitted from the outer layer of oil walls showed the same pattern as that emitted from the fiber-cell walls, which are known as being lignified (Fig. 4A), thus implying the presence of lignin in the oil-cell wall. This supposition was then tested using three different staining methods: berberine–aniline blue, phloroglucinol–HCl and Mäule reagent. After berberine fluorescence staining the walls of oil and fiber cells showed an intense yellow–green fluorescence under UV light excitation (Fig. 4C). Background fluorescence and nonspecific berberine staining was efficiently quenched by providing a fluorochrome for callow when using aniline blue counterstaining (Brundrett et al., 1988); however, yellow–green fluorescence from the oil-cell and fiber-cell walls was also observed (Fig. 4D). In general, such a fluorescence pattern from the oil-cell walls has been attributed to lignified walls (Brundrett et al., 1988). When phloroglucinol–HCl and Mäule reagents were used, the walls of both oil and fiber cells stained red (Fig. 4E, F) – further confirming the presence of lignin in the oil-cell wall.

5. Composition of essential oil

The essential oil of C. cassia bark was bright yellow and viscous. After extraction of the essential oils by hydrodistillation, GC-MS was used to identify the volatile compounds in it. As shown in Table 2, 24 and 14 components, including aldehydes, esters, terpenoids and others could be unambiguously identified in the bark of 2- and 9-year-old trees, respectively. Trans-cinnamaldehyde was confirmed to be the major component in the oil in the bark of both 2- and 9-year-old trees, with the highest area percentage of 73.47% and 81.52%, respectively, and cinnamyl acetate was the second main component with an area percentage of 7.68% and 11.15%, respectively. Thus, aldehydes and lipids were the dominating components in the bark oil of C. cassia.

Discussion

The histochemical assays revealed that oil cells are the main sites of accumulation of the essential oils in C. cassia bark, while the results of GC-MS analysis showed that aldehydes and lipids were the main compounds of the bark oil. Interestingly, the two main chemical classes of essential oil, aldehydes and lipids, were distributed in different areas of the same oil sac in the present study (Fig. 3). In fact, the area surrounding the oil sac was black, while the center area of the oil sac was bright under transmitted light in the non-stained sections (Fig. 1A). These phenomena indicate compartmentalization of aldehydes and lipids in oil cells. Although secondary metabolites in plants have been suggested to be compartmented at tissue and subcellular levels (Santiago et al., 2000; Bosabalidis, 2010), there are no reports concerning the compartmentalization of aldehydes and lipids in oil cells. Such a phenomenon may be related to the accumulation of essential oil in oil cells, as aldehyde substances would be produced or transported into oil sac later than lipid substances during development of the oil cell. Studies on the compartmentalization of different substances are needed to elucidate the accumulation mechanism of essential oil in oil cells.

The cupule in an oil cell is too small to observe at either microscopic or ultramicroscopic levels, and thus the function of cupules in the development of oil cells remains obscure. The cupule has been suggested to be a localized cell wall protrubance always located on the longer side of the cell (Mariani et al., 1989; Bakker and Gerritsen, 1990; Chu and Hu, 2001a); however, the present results are not in full agreement with this assumption. We observed 2–4 cupules attached to an oil sac, which was located on any side of the cell (Fig. 3C-F). This finding suggests that the cupule may play an important role in supporting or fixing the oil sac at a special location in the cell lumen. To our knowledge, this is the first report that an oil cell contains multiple cupules rather than a single cupule.

Oil has been reported to be synthesized in the plastids, released into the cytoplasm and secreted through the surrounding plasmalemma into the oil cavity (i.e. oil sac)
In our observations of the development of oil cells, lipids presented a similar transportation pattern from the cytoplasm to the oil sac with the development of the oil cell (Fig. 3A-C). Interestingly, both the cupule and the oil sac were stained the same color with Sudan III, indicating lipids in both structures. In fact, the presence of lipids in the cupule is not unique in the Lauraceae family. Oil produced inside the protoplast was secreted to the outside of the plasmalemma, and accumulated as a drop at the place predetermined by the cupule of oil cells in *Laurus nobilis* L., a closely related plant species (Maron and Fahn, 1979). This result suggests that the cupule may be associated with the accumulation of lipids in the oil sac, and may also play an important role in transporting oil from the cell lumen to the oil sac.

The biosynthesis and accumulation of volatile compounds are associated with the presence of superficial or subcutaneous specialized structures that compartmentalize these often toxic components from metabolically active cells (Salgueiro et al., 2010). Our previous study showed that trans-cinnamaldehyde, the main component of Chinese cinnamon bark oil, represented up to 53.7–76.4% of the bark oil of trees of different ages (Geng et al., 2011). The presence of high concentrations of aldehydes is not unique in oil cells of the Lauraceae; cinnamaldehyde accounted for 60.17 and 89.8% of the essential oil of *C. burmannii* (Wang et al., 2009) and *C. panceiflorum* leaves (Nath et al., 2006), respectively. Such a high content of cinnamaldehyde is toxic to the surrounding metabolically active cells, so oil-cell walls would be expected to be an effective protection mechanism.

The suberized layer between the outer and inner cellulose wall layers is typical of oil cells (Metcalfe and Chalk, 1983; Baas and Gregory, 1985; Mariani et al., 1989; Bakker and Gerritsen, 1990). The suberized layers in secretory idioblasts have been hypothesized to compartmentalize potential toxic substances by preventing apoplastic transport of the cell contents (Bakker and Baas, 1993), and to act as barriers to prevent leakage to the surrounding cells (Bakker and Gerritsen, 1989). Our study confirmed the presence of the suberized layer of oil-cell walls in Chinese cinnamon barks using phosphate-buffer fluorescence and Sudan Black B staining methods. However, the presence of lignin as well as suberin in the oil-cell walls was also confirmed in this study. In general, lignified cell walls are commonly thought to strengthen plant tissues, increasing protection against invasion by phytopathogens (Hahlbrock and Scheel, 1989; Donaldson et al., 1999) and increasing waterproofing of the cell wall (Xu et al., 2006). Interestingly, the lignified cell walls in lemongrass oil cells were thought to serve as an impermeable barrier keeping citral compartmentalized from neighboring tissues; the citral, a natural mixture of two isomeric acrylic monoterpenic aldehydes, represents up to 75–85% of the essential oil (Levinsohn et al., 1998). However, there are no previous reports of such lignified cell walls of secretory idioblasts in Lauraceae species. As a consequence, we tentatively suggest that the combination of suberized and lignified walls acts as a barrier to compartmentalize the highly concentrated cinnamaldehyde in oil cells of *C. cassia* bark from the surrounding cells.

**Conclusions**

In this study, the morphology of oil cells in Chinese cinnamon bark was determined using histochemical and fluorescence microscopy. Aldehydes were found to be compartmentalized from lipids in the oil sac. The oil sac in an oil cell was attached by 2–4 cupules rather than one cupule. The presence of lignin and suberin in oil-cell walls was confirmed. The lignified and suberized walls likely serve as protective barriers against the cytotoxicity of high contents of trans-cinnamaldehyde to the surrounding active cells. These results contribute to our knowledge of the mechanism of accumulation of essential oil in Chinese cinnamon bark. However, further studies on the structure and function of oil cells using confocal laser scanning microscopy and transmission electron microscopy (Chu and Hu, 2001b, Xu et al., 2006) are needed.

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