Isolation of Secretory Cells from Plant Glandular Trichomes and Their Use in Biosynthetic Studies of Monoterpenes and Other Gland Products

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The natural products that accumulate in or exude from plant glandular trichomes are biosynthesized by secretory cells located at the apex of the trichome. To investigate the formation of glandular trichome constituents in several species of mints (Lamiaceae), a new procedure was developed for isolating large numbers of highly purified secretory cells. In this method, the leaf surface is gently abraded with glass beads in a way that fragments the glandular trichomes and yields clusters of intact secretory cells. The isolated, intact secretory cells and cell-free preparations derived from them are very active in monoterpene biosynthesis and provide useful starting materials for the purification of several key enzymes of monoterpene metabolism. The procedure described is adaptable to a broad range of plant species and should find wide application in the preparation of whole cell and cell-free systems for biosynthetic studies of plant natural products found in glandular trichomes.

Plants secrete a diverse array of natural products within modified epidermal hairs called glandular trichomes. Among these substances are terpenoids, phenolics, sucrose esters, and organic acids (1-5). Found in numerous plant families, glandular trichomes vary widely in shape and structure (6,7). In the mint family (Lamiaceae), two structural types of glandular trichomes are common (8-12) (Fig. 1). Peltate (subsessile) glandular trichomes, with a short, unicellular stalk and a large, multicellular head, accumulate secretory products in a cavity that forms between the cuticle and underlying cells, whereas capitate (clavate) glandular trichomes, with a one- or two-celled head atop a single- or multicellular stalk, extrude secretion products outside of the gland.

Several lines of biochemical evidence (13-15) suggest that the natural products secreted by glandular trichomes are biosynthesized in situ. For example, in spearmint (Mentha spicata), which secretes an essential oil containing principally monoterpenes in its glandular trichomes, the three enzymes specific to monoterpene biosynthesis were shown to be located exclusively in the glandular trichomes and not in the remainder of the leaf (15). Ultrastructural studies also implicate glandular trichomes as the site of synthesis of secreted gland products. The specialized cells of the trichome head, called secretory cells, display many features indicative of active metabolism and secretion of lipophilic material (6,7). Thus, studies of the biosynthesis of natural products found in glandular trichomes require the use of preparations containing glandular trichome secretory cells or extracts derived from such cells.

A number of methods have been devised for obtaining extracts enriched in glandular trichome secretory cells or their contents, including gentle brushing of leaf surfaces with a toothbrush (16), wiping of leaf surfaces with a microscope cover glass (13), mechanical abrasion of leaf surfaces with glass beads (17), use of epidermal peels (18), and homogenization of leaf tissue followed by separation of the isolated glandular trichomes on a Percoll density gradient (19). When applied to studies of monoterpene and sesquiterpene biosynthesis in members of the Lamiaceae, none of these methods gave
a preparation of sufficient quality or quantity to permit purification of key biosynthetic enzymes. Therefore, new techniques for the selective isolation of glandular trichome secretory cells were sought.

Here we report a method for isolating clusters of biosynthetically active secretory cells in high purity and excellent yield. Secretory cells isolated by this method from peppermint (M. × piperita) and spearmint (M. spicata) glandular trichomes were able to efficiently incorporate radiolabeled precursors into monoterpenes, and therefore constituted very useful preparations for investigations of monoterpene biosynthesis. In addition, cell-free extracts of these isolated secretory cells contained much higher levels of monoterpene biosynthetic activities than have been obtained using other types of extraction techniques, and so provided excellent starting materials for the purification of several enzymes of monoterpene metabolism. This paper describes procedures for both the isolation of intact, metabolically competent secretory cells, and the preparation of very active cell-free extracts from the isolated cells. The methods were developed with peppermint and spearmint, two species that synthesize and accumulate large quantities of monoterpenes in glandular trichomes; however, the techniques discussed appear to be adaptable to a wide range of plants that produce a variety of different types of natural products in glandular trichomes.

MATERIALS AND METHODS

Plant materials, substrates, and reagents. Peppermint (M. × piperita L.) and spearmint (M. spicata L.) plants were propagated from rhizomes or stem cuttings in peat moss:pumice:sand (55:35:10, v/v/v) and grown in a greenhouse with supplemental lighting (16 h, 21,000 lux minimum) and a 30°/15°C (day/night) temperature cycle. Plants were watered as needed and fertilized daily with a complete fertilizer (N:P:K, 20:20:20) plus iron chelate and micronutrients. Apical buds and newly emerged leaves (5-15 mm long) of vegetative stems (3-7 weeks old) were used as the source of glandular trichomes. Nylon mesh was obtained from Small Parts Inc. (Miami, FL). Polystyrene resin beads (Amberlite XAD-4, Rohm and Haas, Philadelphia, PA) were prepared for use by washing in sequence with 95% ethanol, 3 N HCl, and distilled water, according to standard procedures (20). [U-14C]Sucrose (24.8 GBq/mmol) and [2-14C]mevalonic acid (130 MBq/mmol) were purchased from duPont. [1-3H]Isopentenyl pyrophosphate (835 MBq/mmol) (21) and [8-3H]geranyl pyrophosphate (2.30 GBq/mmol) (22) were synthesized as previously described. All other reagents were purchased from Research Organics, Aldrich, or Sigma Chemical Co.

Secretory cell isolation. The procedure was modified from a previously described mechanized technique for leaf surface abrasion (17). Apical buds and leaves were harvested, soaked in ice-cold, distilled water for 1 h, and then abraded using a cell disrupter (Bead Beater, Bio-spec Products, Bartlesville, OK). The finned 300-ml polycarbonate chamber was filled with 15-20 g of plant material, 100-130 g of glass beads (0.5 mm diameter), XAD-4 resin (1 g/g plant material), and isolation buffer to full volume. The isolation buffer consisted of 25 mM Hepes, adjusted to pH 7.3 with KOH, containing 200 mM sorbitol, 10 mM sucrose, 5 mM dithiothreitol, 10 mM KCl, 5 mM MgCl₂, 0.5 mM potassium phosphate, 0.1 mM PP₃ (Fluka), 0.6% (w/v) methyl cellulose, and 1% (w/v) polyvinylpyrrolidone (PVP₃. M. 40,000). The filled chamber was fitted with the Teflon rotor supplied with the unit, sealed, and attached to the motor. Leaves and buds were abraded by 2-4 pulses of operation of 1 min each. This procedure was carried out at 4°C, and after each pulse the chamber was allowed to cool on ice for at least 30 s. The rotor speed was controlled with a rheostat set between 75-90 V. Larger batches of plant material (40-50 g) were abraded in 600-ml chambers of our own manufacture with relative proportions similar to those of the 300-ml chamber.

Following abrasion, the contents of the chamber were filtered through 350-μm nylon mesh to remove the plant material, glass beads, and XAD-4 resin. The residual plant material and beads were scraped from the mesh and rinsed twice with additional isolation buffer that was also passed through the 350-μm mesh. The isolation buffer used in these additional rinses and all subsequent steps lacked polyvinylpyrrolidone and methyl cellulose in order to lower the viscosity and reduce the time required for filtration. The 350-μm filtrate was then passed through a 105-μm nylon mesh to remove small tissue fragments. Finally, clusters of secretory cells (which were approximately 60 μm in diameter) were collected by passing the 105-μm mesh filtrate through a 20-μm mesh. Collected cell clusters were then resuspended in additional isolation buffer and refiltered through 20-μm mesh several times until the filtrate was free of detectable mint monoterpene odor. Isolated glandular trichome secretory cell clusters were quantified by counting with a hemacytometer.

Biochemical studies with intact secretory cells. Clusters of glandular trichome secretory cells, isolated as described above, were suspended in isolation buffer (without polyvinylpyrrolidone or methyl cellulose) and incubated with various radiolabeled precursors, including [U-14C]sucrose (2 μM), [2-14C]mevalonic acid (120 μM), [1-3H]isopentenyl pyrophosphate (40 μM), and [8-3H]geranyl pyrophosphate (6 μM). Preliminary trials showed evidence for efficient incorporation of prenyl pyrophosphate precursors and uptake of exogenous co-
FIG. 1. (A) Scanning electron micrograph of a peppermint leaf showing peltate (P) and capitate (C) glandular trichomes and nonglandular trichomes (NG). Both peltate and capitate glandular trichomes produce secretions containing monoterpenes. Glandular trichomes of these two basic types are found in many other species of the Lamiaceae. Samples were prepared for microscopy by fixation in 3% (v/v) glutaraldehyde in 100 mM sodium phosphate, pH 7.3; postfixation in 2% (w/v) aqueous osmium tetroxide; dehydration in an ethanol series; critical point drying; and sputter coating with gold. Specimens were viewed in a Hitachi S-570 microscope at 20 kV. (B) Drawings of cross-sections of peppermint peltate and capitate glandular trichomes showing the three kinds of cells present in these secretory structures. Peltate glandular trichomes have eight secretory cells, one stalk cell, and one basal cell, whereas capitate glandular trichomes have one secretory cell, one stalk cell, and one basal cell. In peltate glandular trichomes, the secretion is stored in an enlarged subcuticular cavity and is not released unless the cuticle is damaged, whereas in capitate glandular trichomes, the secretion apparently volatilizes to the atmosphere through pores in the cuticle.
factors, such as NADPH, suggesting that the isolated secretory cells were permeable to low molecular weight, water-soluble substances and, as a consequence, might have been depleted in essential cofactors. Thus, the incubation buffer was supplemented with 0.5 mM MnCl₂, 1 mM NADPH, 1 mM NAD⁺, 1 mM CoA. A typical incubation contained 1–2 × 10⁶ clusters suspended in a volume of 3 ml buffer and was allowed to proceed for 3 h at room temperature. Air was bubbled vigorously through the suspension of clusters during incubation, and a 2-ml pentane overlay was used to trap volatile monoterpene products.

After incubation, the pentane overlay was removed and passed through a short column of anhydrous MgSO₄. The aqueous suspension of cell clusters was then extracted twice with 1-ml portions of diethyl ether, and the resulting ether extracts were also passed through the MgSO₄ column. Radioactivity in the combined organic extract was determined by removing an aliquot for liquid scintillation spectrometry. Radioactivity measurements were performed in a solution of 30% (v/v) ethanol in toluene, containing 0.4% (w/v) Omnifluor (duPont), using a Packard TriCarb 460D liquid scintillation counter (efficiency for ³H 41%, efficiency for ¹⁴C 89%). After addition of authentic carriers, the remaining organic extract was concentrated under N₂ for radio-GLC analysis.

Radio-GLC was performed on a Gow-Mac 550P gas chromatograph (He carrier gas at 36 ml/min, injector at 170°C, thermal conductivity detector at 200°C and 160 mA) attached to a Nuclear Chicago 7357 gas proportional counter. The column (0.085 in id × 12 ft stainless steel) was 15% AT-1000 (polyethylene glycol ester) on 100/120 mesh Gas-Chrom Q II (Alltech) programmed from 100°C (5 min hold) to 220°C (5°C/min). Thermal conductivity and radioactivity output channels were monitored with a SICA 7000A Chromatogram Processor, and radioactivity measurements were externally calibrated with [³H]- and [¹⁴C]toluene.

Preparation of cell-free extracts from isolated secretory cells and enzyme assays. Suspensions of secretory cell clusters (5–10 × 10⁶ clusters/ml) were sonicated using a microprobe (Braun-Sonic 2000) operated at maximum power for four to six 30-s bursts. For measurement of limonene synthase (cyclase) activity, sonication was carried out in a buffer of 25 mM potassium phosphate, pH 6.0, containing 10% (w/v) glycerol, 10 mM Na₂SO₃, 10 mM sodium ascorbate, 1 mM EDTA, 1 mM sodium ascorbate, and 1% (w/v) polyvinylpyrrolidone, to which was added 0.6% (w/v) XAD-4 resin. Cell suspensions were allowed to cool on ice for at least 1 min after each burst of sonication. The resulting cell-free extract was filtered through 20-µm nylon mesh and the filtrate centrifuged at 27,000g for 20 min and then at 195,000g for 90 min. The final supernatant was exchanged into a buffer of 20 mM Mopso, pH 7.0, containing 10% (w/v) glycerol, 5 mM potassium phosphate, 1 mM sodium ascorbate, and 1 mM dithiothreitol, via dialysis or passage through a desalting column (Econo-Pac 10DG, Bio-Rad). Assays were performed with [l-³H]geranyl pyrophosphate as previously described (23).

For measurement of limonene hydroxylase activity, sonication was carried out in a buffer of 25 mM potassium phosphate, pH 7.4, containing 10% (w/v) glycerol, 1 mM EDTA, 1 mM dithiothreitol, 5 µM FAD, 5 µM FMN, and 0.6% (w/v) XAD-4 resin. After filtration through 20-µm nylon mesh, the sonicate was centrifuged sequentially at 3000g (10 min), 18,000g (30 min), and 195,000g (90 min), and the resuspended 195,000g pellet was assayed as previously described (24).

Assays for geranyl pyrophosphate phosphohydrolase activity were performed according to a previously published procedure (25). Protein concentrations were estimated by the dye-binding method of Bradford (26) using the Bio-Rad protein assay kit with bovine serum albumin as standard. To compare the enzyme activities of secretory cell sonicates with those of other types of cell-free extracts, batches of peppermint tissue har-
FIG. 2. Light micrograph of a preparation of secretory cell clusters (SCC) isolated from the peltate glandular trichomes of young peppermint leaves by procedures described in the text. Secretory cells were isolated as disc-like clusters of eight cells each, approximately 60 μm in diameter. Each cluster was derived from the fragmentation of a single glandular trichome. In this figure, some of the clusters are oriented so that the depression on one side of the disk (the former site of stalk cell attachment) can be seen (*). Also present are other cell and tissue fragments found in these preparations at low levels: ruptured cuticular sheaths that formerly covered the subcuticular cavity of the peltate glandular trichomes (CS), cells of capitate glandular trichomes (C), and large nonglandular trichomes (NG).

RESULTS AND DISCUSSION

Isolation of glandular trichome secretory cells. Gentle abrasion of the leaf surfaces of peppermint and spearmint with glass beads fragmented the glandular trichomes and afforded disc-like clusters of intact secretory cells in high yield. Microscopic examination indicated that each cluster was approximately 60 μm in diameter and contained eight cells (Fig. 2). Since the peltate glandular trichomes of both peppermint and spearmint possess eight secretory cells, each cluster was apparently derived from the fragmentation of an individual peltate glandular trichome with the eight secretory cells being released as a unit.

The isolation procedure was developed by modifying a previously described method for preparing plant surface extracts using glass beads and a commercial cell disrupter (17). The published method was altered to maximize the yield of intact secretory cell clusters which could then be separated from other constituents of the extract by multiple filtration steps. The relative proportion of beads to plant material in the abrasion chamber, the time of abrasion, and the viscosity of the isolation buffer were all changed from the original protocol in order to fragment as many of the glandular trichomes as possible while avoiding disruption of the secretory cell clusters once isolated. The shear forces generated by the single-speed motor were found to be too great for isolating large quantities of intact glandular trichome cell clusters and, therefore, the rotor speed was reduced with a rheostat set between 75–90 V. Soaking the plant material in water for 1 h prior to abrasion significantly increased turgidity of the tissue, resulting in higher recoveries of secretory cell clusters. Since isolated secretory cells were found to be nonspecifically permeable to low molecular weight, water-soluble com-
pounds (McCaskill, D., Gershenzon, J., and Croteau, R., unpublished results), the buffer used was formulated to mimic the pH, osmotic strength, and ionic composition of aqueous plant cytoplasm in order to maximize the retention of metabolic activity. Methylcellulose was included to increase the viscosity of the buffer (thus improving the recovery of intact secretory cell clusters) without significantly altering osmotic strength. The polymeric adsorbents polyvinylpyrrolidone and polystyrene (XAD-4) resin were added to adsorb the terpenoids and phenolics released during abrasion. The isolated secretory cell clusters were separated from other cells and cell fragments in the abraded mixture by sieving through a series of nylon meshes. The clusters, being approximately 60 μm in diameter, readily passed through meshes of 350 and 105 μm, and were collected on 20-μm mesh.

Following these procedures, it was possible to obtain preparations containing large numbers of peltate glandular trichome secretory cells from young peppermint and spearmint leaves on a routine basis. Over 90% of the peltate gland secretory cells originally present on these leaves were recovered as intact cell clusters. The remainder either were not removed from the leaf (3-5%) or were disrupted during the abrasion process, based on microscopic observations and comparison of the enzyme activities of intact secretory cells with those of the cell-free extract. Typically, 3–5 × 10^4 secretory cell clusters were obtained per gram of plant material. The purity of these preparations was also quite high. Secretory cells of peltate glandular trichomes, along with the ruptured cuticular sheath that formerly covered the subcuticular cavity (Fig. 2), generally accounted for over 90% of the isolated material. The chief contaminants were fragments of capitate glandular trichomes and nonglandular trichomes. Although these procedures were developed for peppermint and spearmint, the methodology appears to be adaptable to many other plant species bearing glandular trichomes. In preliminary trials, secretory cells have been isolated in high yield from garden sage (Salvia officinalis), American wormseed (Chenopodium ambrosioides), tansy (Tanacetum vulgare), and wild tomato (Lycopersicon hirsutum).

The facile detachment of intact secretory cells from leaf glandular trichomes and the tight adhesion of the cells in clusters after detachment (Fig. 2) are critical to the success of this procedure. However, the reasons for such fortuitous phenomena are at present unclear. The stalk cell, which is situated immediately adjacent to the secretory cells (Fig. 1B), has a rigid, heavily cutinized cell wall (9) that may render the glandular trichome very susceptible to cleavage between the stalk and secretory cells during abrasion of the leaf surface. Abrasion also causes rupture of the segment of cuticle surrounding the subcuticular space and the loss of the monoterpenoid secretion droplet stored in this compartment, thus releasing the secretory cells as an intact, disc-shaped cluster. After fragmentation of the glandular trichome, the secretory cells may remain tightly bound to each other in clusters as a result of the thickened cell walls between them (9).

Researchers in several other laboratories have described methods for obtaining preparations of intact glandular trichome cells. Slone and Kelsey (19) isolated clusters of secretory cells from sagebrush (Artemisia tridentata ssp. vaseyana) by homogenizing leaf and floral tissue in a Waring blender, filtering and centrifuging the extract to recover intact cells, and separating the gland heads (which contain the secretory cells) on a Percoll density gradient. Keene and Wagner (13) reported a procedure for removing gland heads from the leaves of tobacco simply by touching a microscope cover glass to the leaf surface. Gland heads adhere to the cover glass, apparently because of the sticky exudate coating the surfaces of these structures.

**Biosynthetic studies with intact secretory cells.** The clusters of intact secretory cells isolated from peppermint and spearmint peltate glandular trichomes seemed likely to be very suitable experimental systems for the investigation of monoterpenoid biosynthesis, since secretory cells are specialized for monoterpenoid production and secretion (15). Under the light microscope, nearly all of the isolated secretory cells appeared cytologically normal (Fig. 2), having remained turgid with little evidence of plasmolysis.

To determine the utility of peppermint secretory cell clusters for monoterpenoid biosynthetic studies, incubations were carried out with various radiolabeled precursors in the presence of required cofactors. [U-14C]-Sucrose, [2-14C]mevalonic acid, [1-3H]isopentenyl pyrophosphate, and [8-3H]geranyl pyrophosphate were all incorporated into monoterpenes at easily measurable rates (Table 1). Precursors that were farther along the biosynthetic sequence (Figure 3) gave higher levels of percentage incorporation. The radiolabeled monoterpenoid products formed during these incubations consisted primarily of limonene and menthone (identified by radio-GLC), both typical monoterpenes of young peppermint leaves (23).

**TABLE 1**

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Incorporation (% of total administered)</th>
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<tbody>
<tr>
<td>[U-14C]Sucrose</td>
<td>0.6</td>
</tr>
<tr>
<td>[2-14C]Mevalonic acid</td>
<td>1.1</td>
</tr>
<tr>
<td>[1-3H]Isopentenyl pyrophosphate</td>
<td>1.9</td>
</tr>
<tr>
<td>[8-3H]Geranyl pyrophosphate</td>
<td>34</td>
</tr>
</tbody>
</table>

**Figure 3**

Incorporation of Various Biosynthetic Precursors into Monoterpenes in Intact Secretory Cells Isolated from the Peltate Glandular Trichomes of Peppermint.
The levels of precursor incorporation observed in these experiments with secretory cell clusters were much higher than those obtained in almost all previous monoterpene biosynthetic studies using intact cells (28,29). For example, in previous work with detached stems, leaves, and leaf discs, the efficiency of mevalonic acid incorporation has usually been less than 0.01%. However, with isolated secretory cell clusters over 1% of the administered [2-14C]mevalonic acid was incorporated into monoterpene (Table 1). This success can be attributed, at least in part, to the fact that secretory cells are specialized for monoterpene biosynthesis, and thus there should be less diversion of precursor to other pathways. In addition, the plasmodesmata of the secretory cells apparently remain open after isolation (McCaskill, D., Gershenzon, J., and Croteau, R., unpublished results), a feature also observed in the isolated bundle sheath cells of several species of C₄ plants (30). This circumstance undoubtedly enhances the uptake of small, water-soluble precursors, such as mevalonic acid, isopentenyl pyrophosphate, and geranyl pyrophosphate.

Cell-free preparations from isolated secretory cells. Most recent advances in monoterpene biosynthesis have resulted from the use of cell-free extracts. However, progress in this area has been hindered by the instability and relatively low activity levels of monoterpene synthesizing enzymes in typical cell-free extracts, and by the presence of competing activities (27). Therefore, various techniques were evaluated for preparing cell-free extracts from the isolated clusters of peppermint and spearmint secretory cells, including sonication, treatment with a vibrating glass bead mill, mechanized homogenization with a high-speed rotor (Tekmar Tissumizer), blending at high speed with glass beads (Bead-Beater), and repeated freeze–thaw cycles. The most efficient extraction was achieved by sonication. When a concentrated suspension of secretory cell clusters was sonicated as described under Materials and Methods, most of the cells were broken open, based on microscopic examination, and excellent yields of monoterpene biosynthetic enzymes were obtained. Freezing secretory cell clusters in liquid nitrogen and then grinding them with a mortar and pestle also afforded high yields of monoterpene enzyme activity, but this technique was less suitable for studying monoterpene cyclase activity since it gave higher yields of competing phosphohydrolase activities (see below). Sonication was carried out at pH 6.0 in the presence of reducing agents, polyvinylpyrrolidone, and polystyrene (XAD-4) resin beads in order to maximize enzyme activity by adsorbing phenolics and lipophilic materials and by mitigating the action of polyphenol oxidases (27).

For the investigation of monoterpene biosynthesis, sonicated secretory cell extracts are the most active cell-free preparations thus far obtained. Table 2 illustrates the activities of three key enzymes of monoterpene biosynthesis in several different types of cell-free preparations derived from peppermint and spearmint leaves. 4S-Limonene synthase is a soluble enzyme that catalyzes the cyclization of the ubiquitous isoprenoid intermediate, geranyl pyrophosphate, to the olefin, (−)-4S-limonene (Fig. 3). 4S-Limonene-3-hydroxylase and 4S-limonene-6-hydroxylase are microsomal, cytochrome P450-dependent monooxygenases which convert 4S-limonene to (−)-3S,4R-trans-isopiperitenol and (−)-4R,6S-trans-carveol, respectively. In sonicated extracts of secretory cells, the activities of these enzymes were much greater than in extracts prepared by homogenization of whole leaves or by mechanized abrasion of leaf surfaces according to previously published methods (17,27).

The high activity of monoterpene synthesizing enzymes in cell-free preparations derived from intact se-
TABLE 2

Comparison of Enzyme Activities in Different Types of Cell-Free Extracts

<table>
<thead>
<tr>
<th>Enzyme (Species)</th>
<th>Activity (Specific activity)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Whole leaf homogenate</td>
</tr>
<tr>
<td>Monoterpenes biosynthetic enzymes</td>
<td></td>
</tr>
<tr>
<td>4S-Limonene synthase [1]</td>
<td>0.82 (0.11)</td>
</tr>
<tr>
<td>(peppermint)</td>
<td></td>
</tr>
<tr>
<td>4S-Limonene-3-hydroxylase [2]</td>
<td></td>
</tr>
<tr>
<td>(peppermint)</td>
<td>Not determined</td>
</tr>
<tr>
<td>4S-Limonene-6-hydroxylase [3]</td>
<td></td>
</tr>
<tr>
<td>(spearmint)</td>
<td>Not determined</td>
</tr>
<tr>
<td>Competing activities</td>
<td></td>
</tr>
<tr>
<td>Geranyl pyrophosphate phosphohydrolase</td>
<td>20.4 (2.7)</td>
</tr>
<tr>
<td>(peppermint)</td>
<td></td>
</tr>
</tbody>
</table>

* Extracts were prepared as described in Materials and Methods.

Data are from Ref. (24).

cretory cells is at least partly due to the relatively low levels of interfering substances present. In making these extracts, the secretory cells are disrupted only after they have been separated from most of the other cells of the leaf, so much lower levels of phenolic substances and bulk protein are present than in the other types of cell-free extracts. Another potential source of interference is that due to phosphohydrolases. These enzymes, which are ubiquitous in higher plants, compete effectively for the monoterpene cyclase substrate geranyl pyrophosphate in cell-free preparations, converting this precursor to geranyl phosphate and geraniol, neither of which is a substrate for monoterpene cyclases (25,27). However, sonicates of peppermint secretory cells have less than 10% of the phosphohydrolase activity of corresponding leaf surface extracts and less than 1% of that of whole-leaf homogenates (Table 2). Therefore, secretory cell extracts provide much more suitable experimental systems for investigation of monoterpene cyclization reactions than do other types of cell-free preparations.

In summary, new procedures have been devised for isolating plant glandular trichome cells that are engaged in the synthesis and secretion of gland products. These methods were developed using peppermint and spearmint glandular trichome secretory cells and have provided excellent starting materials for enzyme purification. The specific activities of cyclases and hydroxylases in these preparations are much higher than in any other type of cell-free extract (Table 2). In addition, the fact that secretory cell clusters can be collected on a nylon mesh and then resuspended and sonicated in a small volume of buffer allows the volume of the extracts to be kept to a minimum, which increases enzyme stability and is more convenient for subsequent chromatographic steps.

In summary, new procedures have been devised for isolating plant glandular trichome cells that are engaged in the synthesis and secretion of gland products. These methods were developed using peppermint and spearmint, but are adaptable to a wide range of other species. Both whole cell preparations and cell-free extracts were active in the biosynthesis of gland products and therefore provide excellent experimental systems for investigating the formation of glandular secretions.

The cell-free preparations can serve as very valuable first steps in the purification of the enzymes involved in the biosynthesis of glandular natural products.

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