

Biologically Active Aliphatic Acetogenins from Specialized Idioblast Oil Cells

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Abstract: More than three decades ago, Kashman and coworkers [1,2] reported for the first time a class of closely related compounds from avocados (Lauraceae). This class of compounds contains several highly oxygenated long-chain acetylenic and olefinic natural products derived from fatty acids (e.g. possibly C-18 oleic, linoleic, linolenic, or stearic acid as precursors). These products form part of a group of compounds known as the "biologically active aliphatic acetogenins", the distribution of which is thought to be restricted to the Annonaceae and Lauraceae. Current studies indicate that the Lauraceous acetogenins are synthesized during early plant development from specialized idioblast oil cells and transported from the oil cells to other parts of the plant.

While idioblast oil cells are found throughout the plant kingdom, their function in many plant families has been the focus of considerable speculation because little is known about the chemistry or biological activity of the oil in the cells. Until recently, the idioblast cells were generally viewed simply as unusual storage organs. In avocados, increasing evidence indicates that the cells, and the oil they contain, may play an important defensive role against pathogens and insects. Several of the compounds produced in avocado oil cells, reported to occur almost exclusively in the genus *Persea*, are now known to have antibacterial, antifungal, and insecticidal activity. In the present paper we review the distribution, synthesis, biological activity, and mode of action of the most studied Lauraceous acetogenins: the diene persin, (12Z, 5Z)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene, and the persin-derived furans known as avocadofurans.

I. INTRODUCTION

In the past few decades, the search for new natural substances to control insects has accelerated, mainly due to an increasing perception of potential environmental risks associated with the use of petroleum-based pesticides and the much stricter regulatory conditions for agrochemicals. Problems such as development of pesticide resistance and an increase in secondary pests that reach damaging status due to elimination of their natural enemies are also driving the search for new pesticidal materials [3]. Thus, because

plants have evolved a wide range of pesticidal products [4], it is reasonable to expect that plants may constitute a source for potentially useful new materials for anthropogenic use. However, natural plant products are likely to provide suitable alternatives to synthetic pesticides only if they are safer and more selective than current pesticides, in addition to more biodegradable, environmentally acceptable, economically viable, and compatible with other integrated pest management techniques [5].

The goal of this review is to investigate the oil in the unusual idioblast oil cells as alternative sources for new unidentified natural plant chemistries with potential biological activity. Although oil cells can be found in many plant species, relatively few plants have been studied in detailed for the distribution, chemistry, biological

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activity, and function of oil cells. Therefore we will focus on one of the better known cases: the secretory idioblast oil cells from avocados, *Persea americana* Mill. (Lauraceae). Avocado oil cells contain a novel group of compounds known as the "biologically active aliphatic acetogenins" [6]. In this chapter we review the current knowledge on the unusual chemistry of idioblast oils, the biosynthesis of the active constituents, their biological activity, and presumed mode of action against herbivores.

II. SECRETORY IDIOBLAST OIL CELLS

Plants produce a wide diversity of chemicals that mediate important interactions among organisms in their natural environment. For instance, many phytochemicals are toxic to a variety of pathogens and insects (among others) [7] and may serve to protect plants from herbivory [8,9,10,11]. These substances are often believed to be by-products of the plant's primary metabolism and are commonly referred to as secondary plant metabolites. This terminology may be misleading in that the possible alternative roles of many of these metabolites have not been investigated. Regardless, many plant secondary metabolites are isolated from the neighboring cells in specialized structures or removed entirely from the plant body due to potential autotoxicity [12]. The secretory structures, which are responsible for the synthesis and/or storage of secondary metabolites, vary widely in degree of specialization and location in the plant [13]. They may consist of a single cell or small to large group of cells. Some are external, such as trichomes, epidermal glands, and nectaries; while others are internal such as secretory cells, secretory spaces, and laticifers.

The functions and chemistries of the internal secretory cells are in most instances unknown. Some of the secreted substances may serve to attract pollinators or vectors involved in seed dispersal, while others may deter phytophagous animals [14]. Furthermore, the secretory cells tend to be well differentiated from parenchymal cells and contain a variety of substances generally classified as balsams, resins, oils, tannins,

mucilages, gums, and crystals [13]. Among the oils secreted by these cells, the most common constituents are terpenes, fats, waxes, and flavonoid aglycones [14]. Secretory cells occur in all parts of a plant and are commonly known as secretory idioblasts when they differ conspicuously from neighboring cells among which they are embedded. Secretory idioblasts are commonly classified based on their contents. However, this classification is somewhat nebulous considering that many of the cell contents remain to be identified.

One of the common types of secretory idioblasts is an oil cell [13]. Baas and Gregory [15] define the characteristics of idioblast oil cells and provide a review of their distribution in the dicotyledons. Idioblast oil cells are common. They have been reported in the leaves and other organs of species from several plant families, including the Araceae, Aristolochiaceae, Calucanthaceae, Lauraceae, Magnoliaceae, Piperaceae, and Saururaceae, among others [14,15,16]. The cells differ in size from neighboring cells and possess an oil drop termed an "oil sac" [14]. Fahn [14] indicated that the oil drop is attached to a wall protuberance, called cupule, which occurs in fixed places on the wall. The wall of a mature oil cell consists of three layers, in contrast to the typical parenchymal cell wall which consists of only two layers. An oil cell wall contains an outer cellulotic layer, an intermediate suberin layer, and an inner cellulotic layer [15], but some variations may be observed (see Postek and Tucker [17]).

The chemical constituents of idioblast oil are commonly summarized as oils or lipids, or as combinations of terpenes, fats, and flavonoid aglycones [15]. Baas and Gregory [15] indicated that the terpenes from oil cells are of lower molecular weight than in resin cells. Mariani *et al.* [18], in one of the few studies that included histochemistry of the oil, found evidence for sesquiterpene lactones in oil cells of *Liriodendron tulipifera* L.

Platt and Thomson [19] indicate that the reason for the lack of biochemical information on the oil is due to the fact that oil cells make up a small

percentage of the total tissue volume, often less than one or two percent. Any biochemical analyses require development of an extraction method to permit collection of a large quantity of oil cells, and ultimately oil, in reasonably pure form. Fortunately, an efficient method has recently been developed to extract fairly large amounts of the cells, and the cell oil, from ripe avocado fruit [19,20].

III. AVOCADO OIL CELLS

Cummings and Schroeder [21] were the first to report the occurrence of oil cells in avocado fruit. The presence of these cells has been subsequently verified for avocados and other species in the genus *Persea* [22,23]. Avocado oil cells are distributed uniformly throughout the fruit mesocarp, and in volume make up about 2% of the total edible portion of the fruit [21]. The oil cells in the fruit are typically larger than the surrounding parenchymal cells. A typical idioblast avocado oil cell has a diameter of 80 μm , whereas a parenchyma cell measures about 40-60 μm in diameter. In addition, the walls of these unusual cells are 4 μm in thickness in contrast to 2.5 μm of parenchyma walls due to the presence, in addition to cellulose, of suberin in the cell wall of oil cells [23]. Platt-Aloia *et al.* [24] indicated that a possible function of the extra layer containing suberin is to isolate the toxic cell contents from the adjacent cells. In the mature fruit, the oil cells are very large, enucleate, and possess a vestigial cytoplasm.

Idioblast oil cells also can be found in other plant tissues besides the fruit. They have been reported from the peduncle and pedicel, leaf blades and root tissue [22,25]. There are structural similarities among the oil cells throughout the plant tissues, e.g. the oil cells in the avocado leaf is similar to that of the oil cells in the mature fruit [22]. The oil in the idioblasts of avocado occurs as a single, large drop which fills the cell, while the other oils in surrounding cells occur as many small droplets [19]. The structure of oil cells in avocado appears to be strongly conserved. Platt-Aloia *et al.* [24] found that avocado oil cells from fruit mesocarp are structurally similar to oil cells of

Laurus leaves [26]. Armstrong [22] reported strong morphological similarities among oil cells across genera in the family Lauraceae, suggesting close taxonomic relationships.

The chemistry of the oil present in the avocado idioblasts is more complex than that of parenchymal cells, as demonstrated by tests involving microchemical reagents and pH indicators [21,23]. The oil in parenchymal cells consists primarily of triacylglycerides [27,28]. Platt and Thomson [19] reported preliminary evidence that a variety of chemicals, including alkaloids and sesquiterpene hydroperoxides, are likely present in avocado oil cells. Moreover, studies have shown that the oil from avocado idioblasts accumulates early in tissue development. Studies on the oil cell development in avocado fruit showed that the oil cells reach mature structural stage in very small fruit (< 1 cm long) [24]. Similarly, Armstrong [22] had previously indicated the presence of oil cells in avocado fruit 2 to 3 days after pollination. The presence of a fully formed oil droplet in early tissue growth implies a function of oil cells other than the passive storage of lipids.

IV. BIOLOGICAL ACTIVITY OF AVOCADO OIL CELLS

Rodriguez-Saona and Trumble [29] provided the first evidence of a potential defensive role of the avocado oil cells against insect herbivores. They found that the avocado oil cells are biologically active against larvae of the non-adapted generalist *Spodoptera exigua* (Hübner). The oil cells reduced growth, increased mortality, and acted as an antifeedant. Subsequent studies [30] demonstrated that intact avocado oil cells are less toxic to two adapted avocado feeding insects, *Sabuloides aegrotata* (Guenée) and *Pseudoplusia includens* (Walker). A post-ingestive mechanism of adaptation allows the avocado-feeding insects to feed on diets containing oil cells [30].

The toxic effects of intact oil cells in plants appear to be selective to certain herbivorous organisms depending on the type of attack. In contrast to chewing herbivores, avocado oil cells

are not effective against a common avocado-infecting fungus, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sack [31]. However, the extracted oil proved toxic. The authors concluded that the thick cell wall of the avocado oil cells may prevent hyphal contact with the toxic cell content, thus minimizing potential antifungal benefit.

In the past three decades, several biologically-active compounds have been isolated and identified from avocado idioblast oil cells. The avocado compounds were first described by Kashman *et al.* [1,2] as "highly oxygenated long chain acetylenic and olefinic compounds". Interestingly, these same compounds were later found to be the main constituents responsible for the unpleasant bitter-type flavor in avocados [32]. This new class of compounds from the family Lauraceae, originally named avocatinins [33], was recently included in a class of natural products known as the "biologically active aliphatic acetogenins" [6]. Aliphatic acetogenins are typically found in the Annonaceae and Lauraceae. However, although Annonaceous acetogenins have been well studied and their biological activity extensively documented (e.g. Mikolajczak *et al.* [34]), few researchers have reported the toxicity of acetogenins from the family Lauraceae.

V. A NEW CLASS OF BIOLOGICALLY ACTIVE COMPOUNDS: THE LAURACEOUS ACETOGENINS

V.1 Persin and Related Compounds

V.1.1 Biosynthesis

Magalhaes Alves *et al.* [33] speculated on the possible biosynthetic pathways leading to the new

class of avocado acetogenins. They indicated that the compounds were derived from biosynthetic variants in the normal pathways involved in the biosynthesis of long chain fatty acids and natural acetylenes. Saturated and unsaturated C-18 fatty acids, possibly stearic, oleic, linoleic, or linolenic acids, were suggested as potential precursors. Most of these C-18 fatty acids, especially oleic and linoleic acid, are abundant in avocado tissues [27,28]. Some of the precursors are now believed to be stored in the oil cells as triacylglycerides. For example, Rodriguez-Saona *et al.* [35] identified triolein, the triglyceride of oleic acid, in the oil of avocado oil cells.

The first compound identified from avocado oil cells was the diene persin, (12Z, 5Z)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene I, "Fig. (1)" [31,36]. Persin is the deoxy-derivative of glyceride and is closely related structurally to the monoglyceride of linoleic acid II, "Fig. (1)", even to the position and the (Z,Z)-stereochemistry of the two double bonds [37,38]. Bull and Carman [37], and Carman and Duffield [38] indicated that the reaction from persin to glyceride, with the insertion of an oxygen atom, could be carried out by a Baeyer-Villager type oxidation. However, no enzymes or chemical procedures are currently known to carry out the reverse process from glyceride to persin with extrusion of an oxygen atom. Thus the final step in the biosynthesis of persin in avocado tissues remains unknown.

The concentration of persin in avocado tissues is regulated by lipoxygenase activity. These enzymes are responsible for the oxydation of the diene. An increase in lipoxygenase activity causes a decrease in persin levels during fruit ripening [39]. Lipoxygenases are themselves regulated by

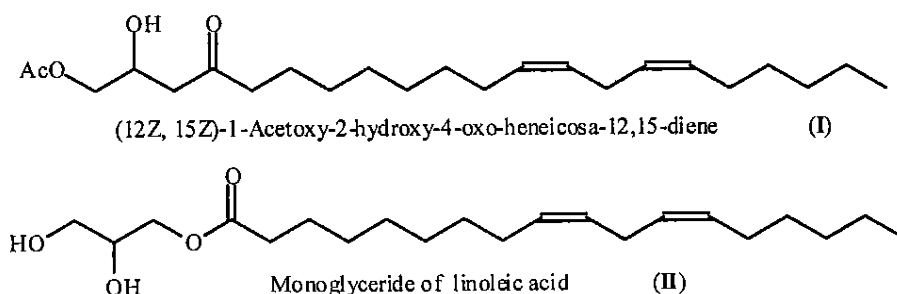


Fig. (1). Structural similarities between persin (I) from avocado idioblast oil cells and the monoglyceride of linoleic acid (II) (after Rodriguez-Saona *et al.* [36]).

the enzyme's endogenous inhibitor, epicatechin. For instance, the levels of epicatechin decrease during ripening [40], and coincide with the increase of lipoxygenases and the decrease of persin. Not surprisingly, epicatechin concentrations in a pathogen-susceptible cultivar decrease to 60-130 $\mu\text{g/g}$ fruit weight when the fruit softens, while in resistant cultivars epicatechin concentrations ranged from 636-1740 $\mu\text{g/g}$ at the time of softening. Thus, higher levels of epicatechin inhibited the lipoxygenases, preventing the decrease in persin levels and providing higher resistance to potential invaders.

Most of persin in the fruit mesocarp is sequestered in isolated oil cells, however no significant lipoxygenase activity was detected in the oil cells [31]. The lack of enzyme activity in the oil cells indicated that the levels of persin remain high in the oil cells both of unripe and ripe mesocarp, while concentrations outside the oil cells were variable. Persin is synthesized in the avocado oil cells and then transported to other tissues (e.g. fruit pericarp) where concentrations are regulated enzymatically. Recent studies conducted with radiolabeled malonyl-CoA or acetate confirmed that persin was synthesized in the idioblast oil cells and subsequently exported from the cells to the pericarp of the fruit [41]. Furthermore, compared to the levels of epicatechin in the fruit peel, the levels of epicatechin in the mesocarp were very low (about 1.2 $\mu\text{g/g}$ fresh weight, while the concentrations present in the peel were 1000 times higher) [42].

V.1.2 Distribution

Persin has been reported only in the genus *Persea*. This compound has been extracted from the leaves [38,43,44,45] and fruit [46] of avocados. Concentrations vary with cultivar. Prusky *et al.* [40] reported levels of persin in fresh peel as high as 1420 $\mu\text{g/g}$ fresh weight in a 'Fuerte' cultivar and 620 $\mu\text{g/g}$ in the more fungal resistant 'Hass' cultivar. As suggested earlier, the concentration of persin in the fruit peel varies as fruit ripens. Levels decreased to 120 $\mu\text{g/g}$ after 6 days of harvest in the susceptible 'Fuerte' cultivar but required 14 days to decline to 159 $\mu\text{g/g}$ in the 'Hass' cultivar. Most

of the diene in the mesocarp is found in the oil cells (1486 $\mu\text{g/g}$ fresh weight compared to 230 $\mu\text{g/g}$ outside the oil cells [31]). In contrast to the levels of persin outside the oil cells, the concentrations of persin in the oil cells of the mesocarp remained high even during fruit ripening (700-1000 $\mu\text{g/g}$ fresh weight [31]).

The accretion of persin in avocado leaves varied depending on the variety. For example, Carman and Duffield [38] reported 2000 $\mu\text{g/g}$ of persin from fresh 'Reed' leaf and Chang *et al.* [43] extracted 600 $\mu\text{g/g}$ of persin from fresh avocado leaves from an unspecified variety. Levels of persin as high as 9000 $\mu\text{g/g}$ have been recorded [45]. Carman and Handley [47] compared the concentration of persin in leaves from different avocado varieties. They documented that concentrations ranged from 4500 $\mu\text{g/g}$ of fresh leaf in the Guatemalan 'Hass' cultivar to undetectable amounts in the Guatemalan natural hybrids G775b and G775c. They also found no seasonal variation in the levels of persin in the leaf of any particular cultivar. In addition, no significant differences were found in the concentrations of persin between young and mature leaves [47].

V.1.3 Biological Activity

V.1.3.1 Antifungal Activity

Most of our knowledge of avocado idioblast oil comes from studies conducted to determine the antifungal activity of the constituents. Two antifungal compounds have been isolated and identified from the avocado oil cells [31]. Persin, isolated from the peel of unripe avocado fruit, inhibits growth of the fungus *C. gloeosporioides* [46]. The fungus causes a disease known as avocado anthracnose which has symptoms that are observed only during fruit ripening. Although the fungus can attack avocado fruit throughout the fruit development, the damage typically remains superficial and the pathogen nearly quiescent until the fruit is harvested. However, when the fruit ripens, the fungus reinitiates growth, resulting in significant decay losses during storage and marketing. Prusky *et al.* [46] demonstrated that the antifungal diene confers resistance to *C. gloeosporioides*. They found that the high

concentrations of persin in the peel of unripe fruit (1600 $\mu\text{g/ml}$) prevented fungal growth (persin inhibited spore germination completely at 790 $\mu\text{g/ml}$). Thus, the decline in concentration of persin to about 160 $\mu\text{g/ml}$ in the peel during ripening appears to be a critical in allowing renewed growth of the fungus.

Other antifungal compounds were subsequently extracted from avocado fruit. Sivanathan and Adikaram [48] reported four related compounds from immature fruit that had activity against *C. gloeosporioides*. Although a detailed chemical identity for all the compounds was not provided in the original paper, one of the compounds isolated matched the spectral data of persin. In a later paper the identities of the three remaining compounds were reported [49]. These compounds were identified as 1,2,4-trihydroxyheptadeca-16-yne, 1,2,4-trihydroxyheptadeca-16-ene, and the monoyne 1-acetoxy-2,4-dihydroxyheptadeca-16-yne. All of the compounds belong to the same class of natural compounds as persin and had been previously reported in extracts from avocado seeds and fruit [1,2]. Another antifungal compound, present in the oil cells of avocado fruit [31], was identified by Prusky *et al.* [50] as the monoene 1-acetoxy-2,4-dihydroxyheptadeca-16-ene. The maximal concentration of the antifungal monene in unripe fruit was 800 $\mu\text{g/g}$ fresh weight. As in the case of persin, the monoene decreased to 40 $\mu\text{g/g}$ fresh weight during ripening which corresponded with the appearance of disease symptoms [50].

In comparative bioassays, the diene persin was reportedly more fungitoxic than both the monoyne [48] and the monoene [50]. In addition, the diene and the monoyne were more toxic to *C. gloeosporioides* than 1,2,4-trihydroxyheptadeca-16-yne and 1,2,4-trihydroxyheptadeca-16-ene [48], although the latter had strong antibacterial activity [50]. Thus, the compound persin for many years was considered the main component in avocado fruit contributing to resistance against the fungus *C. gloeosporioides*.

The two major fungicidal compounds, the diene persin and the monoene, are largely compartmentalized in the specialized oil cells of

the avocado mesocarp [31]. The oil cells in avocado fruit are only present in the mesocarp and completely absent in the pericarp. However, despite the presence of antifungal compounds in the mesocarp, unripe unharvested and harvested avocado fruit are susceptible to *C. gloeosporioides* [31]. This probably occurs because 70 to 90% of the diene and up to 50% of the monoene in the fruit mesocarp are localized in the oil cells and only subfungitoxic concentrations are found outside the oil cells [31]. The compartmentalization of antifungal compounds in oil cells accounts for the susceptibility of the mesocarp to fungal attack because the thick idioblast oil cell walls prevent hyphal contact with the contents.

V.1.3.2 Insecticidal Activity

Persin was the first compound isolated from the oil cells of avocado shown to have insecticidal activity. This compound was reported to inhibit larval feeding of the host specific (specialized) silkworm, *Bombyx mori* L. at concentrations in artificial diet of 200 $\mu\text{g/g}$ or higher [43,44]. The reported concentration of persin in the avocado leaves (600 $\mu\text{g/g}$) was substantially above the inhibitory inhibition levels for *B. mori* [44].

More recently, Rodriguez-Saona *et al.* [36] demonstrated the effects of persin on another lepidopteran, *S. exigua*. Inhibitory effects were observed for both larval growth and feeding at concentrations of 200 $\mu\text{g/g}$ and 400 $\mu\text{g/g}$ of diet, respectively. Persin showed insecticidal activity against *S. exigua* at levels well below those found in the oil cells of ripe mesocarp fruit tissue (700-1000 $\mu\text{g/g}$; [31]).

V.1.3.3 Activity Against Other Organisms

Avocados have long been known for their potential toxicity to mammals [52,53,54,55]. The reports indicate a toxic action of avocados on mammary glands and also strong evidence for cardiotoxicity. Until recently, the cause of these toxicities remained unknown. Oelrichs *et al.* [45] identified persin as the main cause for necrosis of the epithelium of the lactating mammary gland and the myocardium. Persin at a dose of 60-100 mg/kg

had the same effect on mammary glands in lactating mice as did avocado leaves. However, the mechanism(s) of action on the mammary gland and the myocardium still remain to be determined. Interestingly, a patent has been filled reporting the potential of persin as treatment for cancer in mammals, in particular breast cancer [56].

Although various methods for the synthesis of persin have been published [37,38,57], evidence that persin loses activity due to double bond oxidation [40] has led the search for more stable compounds. A more stable stearic acid analogue, a tetrahydro derivative, has been found in the leaves of avocados [47]. The tetrahydro derivative is present at about 2% the concentration of persin (Carman, R., personal comm.). The tetrahydro derivative was found as active as persin against *B. mori* [44] and in anti-cancer and anti-lactation screens [56]. A synthesis for this tetrahydro analogue has also been described [58].

V.1.4 Mode of Action

Little is known of the mode of action of persin. Most of the speculation on its potential mode of action has focused on the close similarity between persin and the monoglyceride of linoleic acid "Fig. (1)". Persin may mimic (possibly irreversibly) the monoglyceride of linoleic acid in glyceride synthesis [37,56]. Thus, persin appears to affect tissue cells by interfering with normal lipid biosynthesis [38].

V.2 Avocadofurans

V.2.1 Biosynthesis

A persin-derived group of furans have also been isolated from avocado oil cells "Fig. (2)" [35]. These compounds, commonly referred to as avocadofurans [59,60,61], are readily formed when persin is exposed to mild acidic conditions [1,2,33,43]. Laboratory studies have shown that avocadofurans can be prepared from persin by addition of traces of p-toluenesulfonic acid in ether or by refluxing persin in hexane in the presence of anhydrous CuSO_4 as a mild Lewis acid [2,43]. In plant tissues, one possible pathway in the synthesis of avocadofurans from persin is by elimination of water, followed by the departure of the acetate group, and subsequent formation of the furan moiety by ring closure and proton abstraction [2].

Because of the low stability of persin, and a propensity for forming a furan ring in acid environments, scientists have questioned whether avocadofurans are an artifact of the method employed during extraction of avocado oils (e.g. Kashman *et al.* [1]). However, Kashman *et al.* [2] provided evidence of the existence in nature of avocadofurans. They were able to obtain the avocadofurans by a rapid acid-free chromatographic extraction of the crude avocado oil. In addition, the pH of the avocado is slightly acidic (about 5) indicating that the conditions in

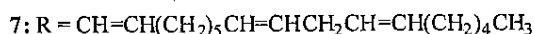
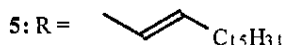
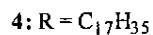
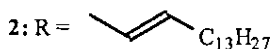
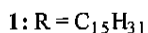
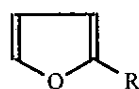


Fig. (2). Structures of 2-alkyl furans extracted from avocado idioblast oil cells (from Rodriguez-Saona *et al.* [35]).

the tissues are suitable for the synthesis of avocadofurans [1].

V.2.2 Distribution

The occurrence of 2-alkyl furans appears to be widespread in the genus *Persea* (Table 1). Many avocadofurans, with various side-chain lengths and numbers of unsaturated sites, have been isolated from at least three species of *Persea*. Although avocadofurans are found almost exclusively in the genus *Persea*, two avocadofurans named avocadynofuran and avocadenynofuran [59], were isolated from aquatic plant *Elodea canadensis* Michaux (Hydrocharitaceae) [62]. The potential biological role in the aquatic plant was not discussed.

The avocadofurans are distributed throughout the plant. They are located in the leaves, seed, and fruit of *P. americana*, leaves of *Persea tolimanensis* Schieber, and leaves and fruit of *Persea indica* (L.) K. Spreng. (Table 1). In the avocado fruit, and most likely in oil cells of other plant tissues, the avocadofurans are sequestered in idioblast oil cells [35]. Therefore, the oil cells might constitute the place of storage and synthesis of avocadofurans from precursors such as persin.

V.2.3 Biological Activity

V.2.3.1 Insecticidal Activity

The 2-alkyl furans have insecticidal activity [35]. However, to date only few studies have

tested the insecticidal potential of avocadofurans. In a search for new compounds from avocado leaves with insecticidal activity, Murakoshi *et al.* [44] tested the avocadofuran 2-(8Z, 11Z-heptadecadienyl)furan. In contrast to persin, where activity was found at concentrations as low as 200 $\mu\text{g/g}$ in diet [43,44], no activity of the avocadofuran was obtained at concentrations below 300 $\mu\text{g/g}$.

Rodriguez-Saona *et al.* [35] showed low to moderate insecticidal activity of a group of avocadofurans from the idioblast oil cells of avocado fruit. The two most toxic avocadofurans against *S. exigua* larvae were 2-(pentadecyl)furan and 2-(heptadecyl)furan. Both avocadofurans had similar toxicities against young and old *S. exigua* larvae [35,63]. In no-choice bioassays initiated with neonates, the $\text{LC}_{50\text{s}}$ were 612 and 581 $\mu\text{g/g}$ of diet for 2-(pentadecyl)furan and 2-(heptadecyl)furan, respectively. The $\text{LC}_{50\text{s}}$ for bioassays initiated with third instars were 842 and 1040 $\mu\text{g/g}$, respectively. Choice tests indicated that the avocadofurans act as feeding deterrents at concentrations equivalent to the LC_{10} or higher [63].

Not all avocadofuran chemistries are equally toxic to *S. exigua* [35]. The unsaturated 2-(1E-pentadecenyl)furan and 2-(8Z, 11Z heptadecadienyl)furan are less toxic than the saturated analogs 2-(pentadecyl)furan and 2-(heptadecyl)furan. In addition to saturation, the length of the alkyl side-chain may also affect the

Table 1. Occurrence and Reported Activity of Avocadofurans Extracted from *Persea* Plants

| Species | Location | Activity | Reference |
|------------------------|---|--|-----------|
| <i>P. americana</i> | Seed and fruit | None to moderate | [1,2,50] |
| | | antibacterial effects | |
| | Seed | Not tested | [32] |
| | | Toxic to hepatic tissue in rats | [67,68] |
| | Leaves | No effect on <i>B. mori</i> at < 300 $\mu\text{g/g}$ | [42,43] |
| Fruit (oil cells) | Effect on <i>S. exigua</i> at > 600 $\mu\text{g/g}$ | [34,62,63] | |
| <i>P. tolimanensis</i> | Leaves | Not tested | [59] |
| <i>P. indica</i> | Leaves | Not tested | [58] |
| | Fruit | Not tested | [60] |

toxicity of 2-alkyl furans [64]. The naturally occurring 2-(pentadecyl)furan and 2-(heptadecyl)furan, with long odd-numbered side chains, proved more toxic than the synthesized 2-(tetradecyl)furan that has a shorter and even-numbered side chain. Rodriguez-Saona *et al.* [64] speculated that avocados may produce those avocadofurans with an odd-numbered carbon side chain to provide optimal protection against insect herbivory. Alternatively, constraints in the biosynthetic pathways of avocadofurans may limit the number and types of structures produced. For instance, Magalhaes Alves [33] suggested that avocadofurans can be derived from long-chain fatty acids; avocados produce almost exclusively fatty acids of 16 and 18 carbons [27,28]. Moreover, the presence of the aromatic furan substructure appears to be more critical than the length of the side chain in relation to the insecticidal toxicity of the 2-alkylfurans. The 2-alkyl tetrahydrofuran analogs were significantly less toxic to *S. exigua* larvae than the corresponding avocadofurans [65].

V.2.3.2 Activity Against Other Organisms

Avocadofurans also have been examined for antibacterial activity [51]. Two avocadofurans, 2-(trideca-12-yne)furan and 2-(trideca-12-ene)furan, were bioassayed against 13 species of bacteria and a yeast. 2-(Trideca-12-yne)furan had no activity. A moderate inhibitory activity was reported for 2-(trideca-12-ene)furan against *Bacillus subtilis* (Ehrenberg) Cohn and *Staphylococcus aureus* Rosenbach [51]. However, the antibacterial activity of the two avocadofurans was lower than the related 1,2,4-trihydroxyheptadeca-16-ene.

Recent evidence indicates that avocadofurans may adversely affect mammals [66]. Oils from avocados inhibit growth in rats, increase hepatic lipids, and decrease levels of triacylglycerides in the blood. Subsequently, the same research group showed that rats fed diet with avocado seed oils exhibited a decrease in skin lysyl oxidase activity and an increase in skin soluble collagen [67]. Recently, Rosenblat *et al.* [68,69] attributed these toxic effects on the rat liver to several avocadofurans identified from an avocado oil. The avocadofurans were the active factor for the

inhibition of the enzyme lysyl oxidase, which catalyzed the reaction that initiated covalent cross-linkages of collagenic tissues. The enzyme deaminates lysine and hydroxylysine residues in collagen, converting them into peptidyl aldehydes, which are involved in covalent cross-links. Inhibition of lysyl oxidase activity causes a reduction of cross-linkages. Rosenblat *et al.* [69] concluded that furan-containing lipids from avocado seeds may prove useful as an antifibrotic drug in the treatment of diseases associated with excess collagen deposition.

V.2.4 Mode of Action

Naturally-occurring furans have long been shown to cause hepatic and pulmonary toxicity to several animal species [70,71,72]. Similarly, the toxicity of avocadofurans has been associated with effects on the hepatic tissue (Table 1) [68,69]. The importance of the furan moiety with respect to this activity has been emphasized [65,72,73]. Experiments demonstrating that catalytic hydrogenation of the furan moiety in furan-containing products eliminates their toxic effects support the contention that the furan moiety is vital to their biological activity [74]. The double bonds in the furan ring of avocadofurans appear required for at least some biological activity, because the toxicity of these compounds against an insect herbivore is significantly reduced in their absence [65].

The toxicity of furans has mainly been attributed to the metabolic activation of the furan moiety to form a furan epoxide [73]. This reaction is catalyzed by microsomal P-450 oxygenase enzymes [71,75]. The resulting epoxide, the activated form of the toxin, may potentially bind to tissue macromolecules such as proteins and nucleic acids [71,75]. For instance, Neal and Wu [76] found that furanocoumarins, a group of natural photoactivated furan-containing molecules that react to DNA, RNA, proteins, and lipids (see Diawara and Trumble [77] for review), are oxidized by cytochrome P-450 at the double bond of the furan ring. They also indicated that the resulting unstable epoxide may inhibit the insect's cytochrome P-450.

Currently, we know of only one case where avocadofurans are reported to cause enzymatic inhibition (lysyl oxidase; [68,69]). However, it has not been proven that the toxicity of avocadofurans to insects occurs through a metabolic epoxidation of the double bond into an activated form after ingestion, possibly involving cytochrome P-450. In addition, the target organ(s) of the activated form, and the exact molecule(s) it binds to, also remain unidentified.

VI. CONCLUSION

Plant secondary metabolites have attracted attention from entomologists for many years because of their importance in ecological processes and probable evolution as plant defenses against herbivores. Indeed, compounds of plant origin have been used for insect control for thousands of years because of their availability and effectiveness. Insecticides from natural sources currently are in vogue due to the questionable belief that they are less toxic to humans than most synthetic insecticides because of their occurrence in nature. Whereas this contention is arguable, it has renewed interest in plant-derived compounds with new chemistries for use in agriculture.

Idioblast oil cells in plants serve as reservoirs for natural products with biological activity. These cells are clearly involved in the synthesis and storage of secondary products useful in defense against herbivores. The fact that avocado oil cells occur in internal tissues suggests that the cells are particularly effective against chewing herbivores. However, at least some of the oil constituents, e.g. persin, are transported peripherally to other plant tissues where they are involved in plant resistance against pathogenic organisms such as fungi. Whether the compounds in the oil cells serve other functions for the plant in addition to protection against potential attackers remains unknown.

The oil produced from the avocado oil cells is toxic to various organisms ranging from fungi to insects, and most likely also to mammals. Some of the chemicals in avocados, such as avocadofurans (Table 1) and their related analogs, may have potential for use in insect suppression. Their

relatively simple syntheses have been described [35], and would make them attractive for large scale production. However, considerable evidence remains to be collected to clarify their potential as lead compounds for a new class of insecticides. In particular, there is little information available as to their non-target (especially mammalian) toxicity, chemical- and photo-stability, and phytotoxicity.

Other chemicals found in the idioblast oil, such as persin, have limited potential for development due to their more complex structure, and particularly, their limited stability. However, it may be possible to find or develop more stable analogs [38,58]. For instance, Carman and Handley [47] identified a saturated analog (tetrahydropersin) in avocado leaves. This compound presumably is more stable because it lacks the easily oxidized 1,4 diene of persin (Carman R., personal comm.). Furthermore, although two syntheses of persin have been reported [37,57], neither is really amenable to the inexpensive and large-scale production required for an agrochemical.

Substantial additional information will be required on field rates, mammalian toxicity, photostability, and phytotoxicity before avocado idioblast oil cell components can be considered for commercialization. However, avocados have not been reported as a human health concern, humans have ingested fresh and processed avocados for centuries, and avocado oils are widely used in cosmetics [78]. One possible explanation for this apparent lack of toxicity following ingestion relates to the nature of the idioblast cells; these cells have a tough cell wall that may not be degraded by human digestion, so that the cells may pass through the digestive system intact. Additional research will be required to clarify the role of the cell wall in prevention of mammalian toxicity.

VII. ACKNOWLEDGEMENTS

The authors thank the support by the California Tomato Commission and the California Celery Research Advisory Board.

VIII. REFERENCES

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