1206 Sesqui- and Sesterterpenoids of Cotton: Biosynthesis and Function

Robert D. Stipanovic*, Alois A. Bell, Jinggao Liu and Lorraine S. Puckhaber
United States Department of Agriculture
Agricultural Research Service
Southern Plains Agricultural Research Center
College Station, Texas 77845

Key Words: Gossypol, heliocides, hemigossypolone, raimondal, biosynthesis, desoxyhemigossypol, hemigossypol.

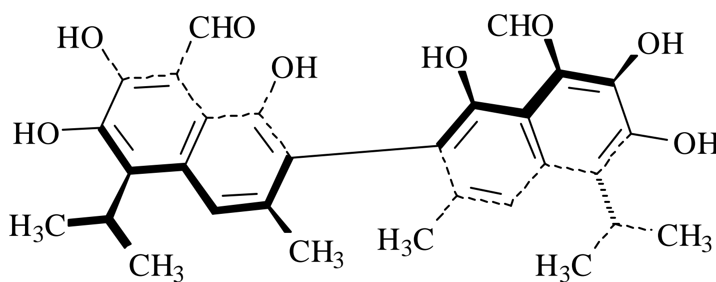
* Corresponding author: rdstip@cpru.usda.gov

ABSTRACT

Cotton produces a diverse mixture of sesquiterpenoids and sesterterpenoids that protect the plant from pathogens and insects. These compounds may be preformed and/or induced by pathogen attack or insect herbivory. The preformed compounds occur in subepidermal lysigenous glands, while the pathogen-induced compounds are synthesized by specialized cells such as paravascular cells appressed to xylem vessels infected by fungal pathogens or in palisade or mesophyll cells adjacent to infection sites on leaves. Some of the genes involved in the biosynthesis of these compounds have been identified and the structures of several biosynthetic intermediates have been elucidated. The structures, occurrence, biological activity toward fungal pathogens and insects, and biosynthesis of these compounds are the subjects of this paper.

Structures and Occurrence

Sesquiterpenoids – Sesquiterpenoids are a class of terpenes that consist of three isoprene units. Gossypol (top row, Fig. 1), which is the most widely studied cotton terpenoid, is produced in the plant by the dimerization of two molecules of hemigossypol and, as such, it is best classified as a dimeric-sesquiterpenoid. Due to hindered rotation around the binaphthyl bond, gossypol exists as two enantiomers (i.e., mirror images of one another and not super imposable). There are significant differences in the biological activity of these enantiomers.



(S)-(+)-Gossypol

Sesquiterpenoids that are structurally related to gossypol, such as, desoxyhemigossypol and hemigossypol and their methylated derivatives (second row, Fig. 1), are phytoalexins. Phytoalexins are antibiotics produced by a plant that is under microbial attack and are toxic to the attacking organism. These phytoalexins were first identified in pathogen-infected stele tissue of *Gossypium barbadense* (Bell et al., 1975; Stipanovic et al., 1975a). Desoxyhemigossypol and hemigossypol may occur in cottonseed but only in very low concentrations. The predominant terpenoid in seed is gossypol which may occur at levels as high as 3.6% of the total embryo weight (*G. davidsonii*; Stipanovic et al., 2005). In some species, gossypol is accompanied by relatively moderate concentrations of gossypol-6-methyl ether and gossypol-6,6-dimethyl ether (top row, Fig. 1).

In the D genome cotton species *G. aridum, G. davidsonii, G. klotzchianum*, *G. lobatum*, *G. thurberi*, *G. trilobum* and *G. turneri*, gossypol is the only sesquiterpene found in cotton leaves (Stipanovic et al., 1977a; Altman et al., 1990); however, in other species in the D genome (eg., *G.*

gossypioides and *G. raimondii*), very little gossypol occurs in the leaves. In *G. raimondii*, the predominant terpenoid aldehyde is raimondal (**2**) (Fig. 2) (Stipanovic et al., 1980). A related terpenoid, raimondalone (**3**) was isolated from the leaves of a plant derived from a *G. hirsutum* x *G. raimondii* hybrid (Stipanovic et al., 1994). In *G. hirsutum*, an AADD allotetraploid species, hemigossypolone (left center, Fig. 1) is the primary sesquiterpene found in the foliar-glands (Gray et al., 1976). Hemigossypolone-6-methyl ether (right center, Fig. 1) occurs with this compound in other *Gossypium* species (Stipanovic et al., 1977a; Bell et al., 1978; Altman et al., 1990).

Stipanovic et al. (1975b, 1981) identified in aged cotton bracts 2,7-dihydroxycadalene (**17a**) (Fig. 3) and lacinilene C (**18a**) plus their methyl ether derivatives **17b** and **18b**. Davila-Huerta et al. (1995) reported 7-hydroxycalamenene (**13**) in cotton leaves that were inoculated with *Xanthomonas campestris* pv. *malvacearum* (*Xcm*). Beckmann and Heitefuss (1998) reported that *Xcm* elicits the synthesis of desoxyhemigossypol and hemigossypol (second row, Fig. 1) in cotyledons. Davila-Huerta et al. (1995) also reported the isolation of *trans*-7-hydroxycalamenen-2-one (**15**) (Fig. 3) and *cis*-7-hydroxycalamenen-2-one (**16**) from *Xcm*-infected leaves. Isomerization between **15** and **16** can not be excluded; therefore, only one of these compounds may actually be present in the plant. Stipanovic et al. (1998) identified 3-hydroxy-α-calacorene (**14**) in cold shocked *G. hirsutum* seedlings. Lastly, Zhang et al. (1998) reported the identification of two new sesquiterpenoid glycosides **1a** and **1b** (Fig. 2) from cotton oil cake.

Sesterterpenoids – Terpenoids resulting from the combination of five isopropenyl units are referred to as sesterterpenoids. Analysis of leaves and squares from *G. hirsutum* showed the presence of a group of sesterterpenoids that are referred to as heliocides (bottom rows and middle, Fig. 1) (Stipanovic et al., 1977b; 1978a; 1978b; Bell et al., 1978). Fourteen genotypes of *G. hirsutum*, with terpenoid contents that varied from normal to very high, were grown at five diverse locations in

Texas over 2 years (Altman et al., 1989). Squares and terminal leaves were analyzed for gossypol, hemigossypolone, and heliocides H_1, H_2, H_3 and H_4. Mean concentrations of terpenoids in tissues from different locations varied from less than twofold for flower bud heliocide H_2 to as high as eightfold for leaf hemigossypolone. Genetic x environment interactions gave smaller variance components than genetic variance components in all instances. The authors concluded that selection for higher terpenoid levels is an attainable goal.

Biological Activity

Insects and pathogens - In glanded plants, terpenes such as hemigossypolone and the heliocide H_1, H_2, H_3, and H_4 (bottom half, Fig. 1) together with gossypol (top row, Fig. 1) are found in the subepidermal pigment glands (Elzen et al., 1985). These compounds are toxic to herbivorous insects (Stipanovic et al., 1977a). Gossypol shows greater toxicity than other glandular terpenoids in the laboratory, but field studies show that the levels of heliocides H_1 and H_2, and hemigossypolone correlate with resistance better than gossypol (Hedin et al., 1992). Herbivorous insects, especially early instars, avoid feeding on the glands (Parrott et al., 1983). Cotton terpenoids affect the rate of insect development. For example, gossypol at very low concentrations stimulates growth and feeding while, at higher concentrations, it is an antifeedant/toxin (Williams et al., 1987; Gunasena et al., 1988). Volatile terpenes also occur in foliar glands and some volatile terpenoids enhance the toxicity of gossypol to the tobacco budworm (*Heliothis virescens*) larvae (Gunasena et al., 1988).

Insect feeding damage can affect the whole plant inducing biosynthesis of acyclic and homo-terpenoids that may be absent or present in only minute quantities in undamaged plants. These acyclic and homo-terpenoids are not found in the subepidermal glands (Donath and Boland, 1995; Paré and Tumlinson, 1998). In a feeding choice test, *Spodoptera littoralis* larvae fed

predominately on young leaves from an undamaged plant compared to undamaged leaves from a previously damaged plant (Alborn et al., 1996). In a no-choice test, this same study found that third instar *S. littoralis* larvae that were fed undamaged young leaves from a damaged plant died by the seventh day while larvae fed young leaves from an undamaged plant survived and pupated in 11 days. McAusland and Alborn (2000) reported that when *S. littoralis* larvae were placed on the terminal foliage of cotton, they moved down the plant to feed and they fed more extensively on foliage at the bottom of plants that were previously damaged compared to undamaged plants. Larvae feeding sites were evenly distributed on glandless plants and no differences were noted in preferred feeding sites between damaged and undamaged glandless plants. These same authors (McAuslane and Alborn, 1998) reported that larvae also preferred (33:1) to feed on leaves from undamaged plants compared to leaves from plants that had been damaged by *S. littoralis* larvae seven days prior to the trial. When the same test was conducted on glandless plants the undamaged leaves were preferred but only by a margin of 2.6:1. Seven days after herbivory, the concentration of heliocides H_1 increased 3.5 times over the levels in control plants, and heliocide H_4 increased almost 4 times that found in the controls. Hemigossypolone and gossypol showed a smaller but significant increase (1.5 times and 1.2 times, respectively). Bezemer et al. (2004) findings were somewhat different. They found that seven days after feeding by *S. littoralis* larvae on mature cotton (*G. herbacium*) leaves, the synthesis of hemigossypolone and heliocides H_1, H_2, H_3, and H_4 in young leaves was induced but not in the damaged leaves. Gossypol levels, in general, were not enhanced. Root herbivory by wireworms (*Agriotes lineatus*) also induced synthesis in both mature and young leaves, but the levels were lower than found after foliage herbivory. After root herbivory, the concentration of hemigossypol in root tissue increased 4.0 times compared to control

plants, and methoxyhemigossypol increased 3.3 times compared to the controls; gossypol levels increased slightly (1.4 to 1.9 times).

Bell (1967) showed that xylem and boll endocarp tissues, which are normally devoid of terpenoids, rapidly synthesize gossypol and related terpenoid aldehydes in response to infection by fungal pathogens. After inoculation with *V. dahliae*, accumulation of hemigossypol, desoxyhemigossypol and their 6-methyl ethers (second row, Fig. 1) can be detected in stele tissue 24 hours sooner in resistant *G. barbadense* cultivars than in susceptible *G. hirsutum* (Bianchini et al., 1999). The terpenoid aldehydes are synthesized by paravascular cells appressed to the xylem vessels and are exuded first into the vessels and then into the surrounding intercellular spaces (Mace, 1978; Mace et al., 1976; 1989; Daayf et al., 1997). The use of histochemical methods allowed Mace et al. (1976) to show that one of the induced phytoalexins, hemigossypol, was deposited on fungal hyphae in xylem vessels *in situ*. Hemigossypol is the major compound formed in *G. hirsutum*, but its more toxic naphthofuran precursor, desoxyhemigossypol, is also present. The latter occurs at about 1/3 the concentration of hemigossypol by 48-72 hours after inoculation with fungal spores; however, 20 μg/ml of desoxyhemigossypol will kill all mycelia of the defoliating *V. dahliae* isolate V-76 while 35 μg/ml of hemigossypol is required to achieve the same effect. The solubility of desoxyhemigossypol at pH 6.3, the pH of infected cotton stele tissue, is 50 μg/ml (Mace et al. 1985) which is significantly higher than the concentration required to kill mycelia or conidia of *V. dahliae* and *Fusarium oxysporum* f. sp. *vasinfectum* (*F.o.v.*) (Zhang et al., 1993). Howell et al. (2000) showed that the biocontrol agent *Trichoderma virens* induces the biosynthesis of hemigossypol and desoxyhemigossypol in cotton roots and that this induction is an essential mechanism in the biocontrol of *Rhizoctonia solani*-incited seedling disease.

The mechanism of action of desoxyhemigossypol (second row, Fig. 1) has been investigated. Stipanovic et al. (1992), and Mace and Stipanovic (1995) found that the toxicity of desoxyhemigossypol to *V. dahliae* was correlated with its ability to form free radicals and hydrogen peroxide. These reactive species may oxidize critical fungal wall components causing membrane disruption and cell death.

Essenberg et al. (1982; 1990) found that inoculation of cotton leaves or cotyledons with *Xcm* elicits the synthesis of 2,7-dihydroxycadalene (**17a**) (Fig. 3) and lacinilene C (**18a**) as well as 2-hydroxy-7-methoxycadalene (**17b**) and lacinilene C 7-methyl ether (**18b**). All of these compounds are toxic to *Xcm* except **17b**; of the other three, 2,7-dihydroxycadalene was the most toxic to *Xcm*. Lacinilene C has one asymmetric center and, thus, exists as two enantiomers. Both enantiomers have been identified in different cotton cultivars. Of the two enantiomers, the (-)-enantiomer is more toxic to *Xcm* (Essenberg et al., 1982), and this enantiomer was identified in the cultivar that was more resistant to *Xcm*. The (-)-enantiomer has the *S* configuration at C-1 (Stipanovic et al., 1986a). *Xcm* also elicits the biosynthesis of desoxyhemigossypol and hemigossypol in cotyledons (Abraham et al., 1999).

Biosynthesis

Terpenoids are compounds derived from isopentenyl diphosphate (IDP). Terpenoids with two isoprene units (C-10 compounds) are designated as monoterpenes. The monoterpenoids β-ocimene and myrcene (center, Fig. 1) are incorporated into the final structure of the heliocides as discussed below.

Sesquiterpenes – A combination of three isoprene units leads to the production of sesquiterpenes (C_{15}). Early work based on ^{14}C incorporation studies seemed to indicate that *Z,Z*-farnesyl diphosphate (**4**) (Fig. 2) was the biosynthetic precursor of gossypol (Heinstein et al., 1970). Later

however, Essenberg et al. (1985) using (1,2-$^{13}C_2$) acetate found that 2,7-dihydroxycadalene (**17a**) (Fig. 3) is formed in *Xanthomonas*-infected cotton by a folding pattern consistent with *Z,E*-farnesyl diphosphate (*ZE*-FDP) (**5**) (Fig. 2) or an intermediate equivalent to the nerolidol cation (**7a, 7b**) (Fig. 3). This finding led to a reevaluation of *ZZ*-FDP as the precursor to gossypol using ^{14}C-labelled mevalonate (Masciadri et al., 1985) or (1,2-$^{13}C_2$)-acetate (Stipanovic et al., 1986b). Both studies demonstrated that gossypol is derived from *E,E*-FDP or its equivalent (**6a**, **6b**), and not the ZZ configuration. Thus, it was established that the biosynthesis of the foliar phytoalexins and gossypol agree with the early steps established for cadinene sesquiterpenoids in other species. Benedict et al. (1995) used (1-*R,S*)-[1-^{2}H]-(*E,E*)-farnesyl diphosphate (**6a**, **6b**) to study the biosynthesis of δ-cadinene (**11a**, **11b**). Cane et al. (1980) had previously established an ion pair intermediate in the conversion of farnesyl to nerolidyl diphosphate during the biosynthesis of cyclonerodiol in which the integrity of the protons on carbon-1 is retained. In the experiment by Benedict et al. (1995), the same scenario would produce the ion pair **7a** and **7b** from **6a** and **6b**. Electrophilic attack on the cation in **7a** and **7b** then gives the germacradienyl cation (**8**). The mass spectrum of deuterated δ-cadinene produced in the Benedict study clearly established the 1,3-hydride shift that occurs to give the allylic carbocation (**9)** during the cyclization as previously observed by Cane et al. (1993) in the biosynthesis of epicubenol. Thus, the cyclization of (1-*S*)-[1-^{2}H]-farnesyl diphosphate (**6a**) gives [11-^{2}H]-δ-cadinene (**11a**) while that of (1-*R*)-[1-^{2}H]-farnesyl diphosphate (**6b**) gives [5-^{2}H]-δ-cadinene (**11b**). Subsequently, Davis and Essenberg (1995) working with *Xcm*-infected plants showed that it is the δ-cadinene produced in cotton is the (+)-enantiomer. They also showed that δ-cadinene synthase is the first enzyme unique to the biosynthesis of the terpenoid aldehydes including gossypol (Fig. 1). Other work by Benedict's group (Alchanati et al., 1998) showed that cotton stele tissue infected with *V. dahliae* converts

[4,4,13,13,13-^{2}H]-farnesyl diphosphate into [8,8,15,15,15-^{2}H]-δ-cadinene. These data support the concept that *E,E*-farnesyl diphosphate undergoes isomerization in the enzyme pocket to form a stereochemically appropriate nerolidyl diphosphate cation (**7**) (Fig. 3) that undergoes cyclization (Benedict et al., 2001).

Utilizing a *G. arboretum* cell suspension culture, Chen et al. (1995) isolated two cDNA clones that contain open reading frames coding for proteins of 554 amino acids with M_r 64,096 and 64,118. The encoded protein from the XpC1 cDNA was expressed in *Escherichia coli* and purified. The protein synthesized (+)-δ-cadinene from *E,E*-farnesyl diphosphate. Purification of this enzyme also has been reported by Davis et al. (1996). In addition, a very similar (+)-δ-cadinene synthase gene has been identified and cloned from *G. arboreum* L. cv. Nanking (Chen et al., 1996). On the basis of sequence similarities, these three genes were grouped into two subfamilies, the *cad*1-C that includes C1 and C14, and the *cad*1-A gene. Davis et al. (1998) isolated a cDNA from *G. hirsutum* (*cdn*1) that was over 95% identical to the *G. arboreum cad*1-C1 and *cad*1-C14. Meng et al. (1999) isolated a new member of the (+)-δ-cadinene synthase from *G. arboreum.* The cDNA clone encodes a protein that is 80% identical to the CADl-Cl and C14 clones of (+)-δ-cadinene synthase from *G. arboreum.* Heterologous expression of this cDNA produced a 64 kD protein that catalyzed the cyclization of farnesyl diphosphate to (+)-δ-cadinene.

Luo et al. (2001) identified a cytochrome P_{450} enzyme in cotton. When expressed in *Saccharomyces cerevisiae*, it catalyzed the hydroxylation of (+)-δ-cadinene to give 8-hydroxy-(+)-δ-cadinene (**12**) (Fig. 3). They proposed this to be an early step in hemigossypol biosynthesis.

Davis and Essenberg (1995) found that 7-hydroxycalamenene (**13**) (Fig. 3) in *G. hirsutum* is derived from (+)-δ-cadinene and, in this (+)-δ-cadinene, the carbon to which the isopropyl group is attached has the *S* configuration. Davis, Davila-Huerta, and Essenberg (personal communication)

also found that 3-hydroxy-α-calacorene (**14**) is derived from (+)-δ-cadinene. Stipanovic et al. (2006b) showed that the 3-hydroxy-α-calacorene in cotton is the (+)-enantiomer and determined that carbon-8, to which the isopropyl group is attached, also has the *S* configuration as shown (**14**). It appears likely that the sesquiterpenoid glycosides **1a** and **1b** (Fig. 1), identified by Zhang et al. (1998), also are derived from 3-hydroxy-α-calacorene.

Since the carbon atom to which the isopropyl group is attached has the *S* configuration in both (+)-δ-cadinene and in (+)-3-hydroxy-α-calacorene in *G. hirsutum*, it seems likely that 7-hydroxycalamenene in *G. hirsutum* has the 1*R*, 4*S* configuration as shown in **13** (Fig. 3)**,** and that compounds **1a** and **1b** (Fig. 2) have the 1*S*, 2*S*, 4*S*, configuration. These latter assignments are conjectural and have not been confirmed.

The proposed steps involved in the biosynthesis of hemigossypolone (middle of Fig. 1) involve hydroxylation at C-4 of desoxyhemigossypol. The resulting product (4-hydroxydesoxyhemigossypol) is proposed to undergo subsequent oxidations to give hemigossypolone. These steps have not been substantiated.

G. raimondii produces raimondal (**2**) (Fig.2), but not hemigossypolone (left center, Fig. 1), while the interspecific hybrid *G. hirsutum* x *G. raimondii* produces raimondalone (**3**) (Fig. 2) (Stipanovic et al., 1994). Genetic analysis for raimondal and raimondalone has been investigated *via* F_1, F_2 and backcross progeny (Bell et al., 1994; Kohel and Bell, 1999). The study showed that segregation for raimondal was consistent with this character being controlled by two dominant genes with epistasis (i.e., hydroxylation at C-2 and methylation) and that raimondalone is formed by genes from both *G. raimondii* (C-2 hydroxylation and methylation) and *G. hirsutum* (C-4 hydroxylation).

The variation in the enantiomeric distribution of (+)- and (-)-gossypol observed in cottonseed (Stipanovic et al., 2005), and in root and foliar tissues (Stipanovic et al., 2006a) implies that a protein must control this dimerization. Studies on the protein that controls this ratio are presented elsewhere in these Proceedings (see Liu et al.).

Sesterterpenoids -- Heliocides H_1 and H_4, and H_2 and H_3 (bottom, Fig. 1) are the reaction products of hemigossypolone and either β-ocimene or myrcene, respectively. This biosynthetic process is a Diels-Alder reaction and it can be reproduced in the laboratory by dissolving hemigossypolone and either β-ocimene or myrcene in a nonpolar organic solvent and allowing them to stand for a few days (Stipanovic et al., 1977b; 1978a) to yield a mixture of the appropriate heliocides. In cotton, the glands appear to offer a similar reaction "vessel" in that hemigossypolone, and both β-ocimene and myrcene (Elzen et al., 1985) are dissolved in a lipophilic matrix in the glands. In the laboratory preparation, the ratio between heliocides H_4 and H_1, and between H_3 and H_2 are about 0.50 and 0.66, respectively. In contrast, in cotton tissue, these ratios vary greatly. In a study of fourteen cotton varieties at five different geographical locations, the H_4/H_1 ratio varied from 0.14 (± 0.01) to 0.33 (± 0.02) in flower buds and from 0.09 (± 0.01) to 0.24 (± 0.04) in leaves while the H_3/H_2 ratio varied from 0.32 (± 0.01) to 0.40 (± 0.01) in flower buds and from 0.34 (± 0.01) to 0.47 (± 0.01) in leaves (Stipanovic et al., 1988). These variations imply the involvement of a protein (Stipanovic, 1992). Evidence to support this proposal has been provided by research with other organisms, including the work by Oikawa et al. (1995) wherein they report the isolation of a Diels-Alderase enzyme from *Alternaria solani*.

***O*-Methylation of Terpenoids --** Addition of a methyl group to the phenolic group at the C-6 position of any of the sesquiterpenoids or sesterterpenoids (Fig. 1) reduces the biological activity of that terpenoid in cotton. For example, field or bioassay studies have shown that the methylation

causes the heliocides to be less toxic to the tobacco budworm (Stipanovic et al., 1977a), desoxyhemigossypol and hemigossypol to be less toxic to *Verticillium dahliae* and *Fusarium* (Mace et al., 1985, 1995), and gossypol to be a less effective spermatocide (Hoffer et al., 1987). Liu et al. (1999) purified, characterized and cloned the enzyme responsible for methylation of desoxyhemigossypol and showed that desoxyhemigossypol is the unique substrate for this enzyme (i.e., hemigossypol did not act as substrate). The cDNA that codes for a 365-residue polypeptide with a calculated molecular weight of 40.6 kDa was cloned (Liu et al., 2002). The cloned gene was successfully expressed in *Escherichia coli*. This enzyme controls the production of several terpenoids in cotton including hemigossypol-6-methyl ether, hemigossypolone-6-methyl ether, the B heliocides, gossypol-6-methyl ether and gossypol-6,6-dimethyl ether.

Conclusion

The cotton industry has seen a significant increase in the understanding of the cotton plant's secondary product chemistry and biochemistry. Developments in molecular biology are providing new tools for agricultural scientists to utilize the secondary plant metabolites, such as the terpenoids, to provide unprecedented technologies that enhance pest control, increase productivity, yield and utilization. The launch pad for many of these enhancements will continue to be our fundamental chemical and biochemical knowledge-based studies of plant secondary products — what they are, their mechanism of action, how they are synthesized in nature, and their role in pest resistance and biocontrol activity.

REFERENCES

Abraham, K. J., Pierce, M. L., and Essenberg, M. 1999. The phytoalexins desoxyhemigossypol and hemigossypol are elicited by *Xanthomonas* in *Gossypium* cotyledons. *Phytochemistry*

52:829-836.

Alborn, H. T., Rose, U. S. R., and McAuslane, H. J. 1996. Systemic induction of feeding deterrents in cotton plants by feeding *Spodoptera* spp. larvae. *J Chem Ecol* 22:919-932.

Alchanati, I., Acreman Patel, J. A., Liu, J., Benedict, C. R., Stipanovic, R. D., Bell, A. A., Cui, Y., and Magill, C. W. 1998. The enzymatic cyclization of nerolidyl diphosphate by δ-cadinene synthase from cotton stele tissue infected with *Verticillium dahliae*. *Phytochemistry* 47:961-967.

Altman, D. W., Stipanovic, R. D., and Bell, A. A. 1990. Terpenoids in foliar pigment glands of A, D, and AD genome cottons: introgression potential for pest resistance. *J Hered* 81:447-454.

Altman, D. W., Stipanovic, R. D., and Benedict, J. H. 1989. Terpenoid aldehydes in Upland cottons. II. Genotype-environment interactions. *Crop Sci* 29:1451-1456.

Beckmann, B., and Heitefuss, R. 1998. Formation of gossypol and related substances in glandless cotton after inoculation with *Xanthomonas campestris* pv. m*alvacearum*. J. *Phytopathology* 146:459-463.

Bell, A. A. 1967. Formation of gossypol in infected or chemically irritated tissues of *Gossypium* spp. *Phytopathology* 57:759-764.

Bell, A. A., Stipanovic, R. D., Howell, C. R., and Fryxell, P. A. 1975. Antimicrobial terpenoids of *Gossypium*: Hemigossypol, 6-methoxyhemigossypol and 6-desoxyhemigossypol. *Phytochemistry* 14:225-231.

Bell, A. A., Stipanovic, R. D., Mace, M. E., and Kohel, R. J. 1994. Genetic manipulation of terpenoid phytoalexins in *Gossypium*: effects on disease resistance, pp. 231-249. *In* B. E. Ellis, G. W. Kuroki, and H. A. Stafford (eds.) Genetic Engineering of Plant Secondary Metabolism, Recent Advances in Phytochemistry. Vol. 28. Plenum Press, New York.

Bell, A. A., Stipanovic, R. D., O'Brien, D. H., and Fryxell, P. A. 1978. Sesquiterpenoid aldehyde quinones and derivatives in pigment glands of *Gossypium*. *Phytochemistry* 17:1297-1305.

Benedict, C. R., Alchanati, I., Harvey, P. S., Liu, J., Stipanovic, R. D., and Bell, A. A. 1995. The enzymatic formation of δ-cadinene from farnesyl diphosphate in extracts of cotton. *Phytochemistry* 39:327-331.

Benedict, C. R., Lu, J., Pettigrew, D. W., Liu, J., Stipanovic, R. D., and Williams, H. J. 2001. The cyclization of farnesyl diphosphate and nerolidyl diphosphate by a purified recombinant δ-cadinene synthase. *Plant Physiol* 125:1754-1765.

Bezemer, T. M., Wagenaar, R., van Dam, N. M., van Der Putten, W. H., and Wäckers, F. L. 2004. Above- and Below-Ground Terpenoid Aldehyde Induction in Cotton, *Gossypiup herbaceum*, Following Root and Leaf Injury. *J Chem Ecol* 30:53-67.

Bianchini, G. M., Stipanovic, R. D., and Bell, A. A. 1999. Induction of δ-cadinene synthase and sesquiterpenoid phytoalexins in cotton by *Verticillium dahliae*. *J Agric Food Chem* 47:4403-4406.

Cane, D. E., Tandon, M., and Prabhakaran, P. C. 1993. Epicubenol synthase and the enzymatic cyclization of farnesyl diphosphate. *J Amer Chem Soc* 115:8103-8106.

Cane, D. E., Iyengar, R., and Shiao, M.-S. 1980. Cyclonerodiol biosynthesis and the enzymatic conversion of farnesyl to nerolidyl pyrophosphate. *J Amer Chem Soc* 103:914-931.

Chen, X., Chen, Y., Heinstein, P., and Davisson, V. J. 1995. Cloning, expression, and characterization of (+)-d-cadinene synthase: a catalyst for cotton phytoalexin biosynthesis. *Arch Biochem Biophys* 324:255-266.

Chen, X., Wang, M., Chen, Y., Jo Davisson, V., and Heinstein, P. 1996. Cloning and heterologous expression of a second (+)-delta-cadinene synthase from *Gossypium arboreum*. *J Nat Prod* 59:944-951.

Daayf, F., Nicole, M., Boher, B., Pando, A., and Geiger, J. P. 1997. Early vascular defense reactions of cotton roots infected with a defoliating mutant strain of *Verticillium dahliae. Europ J Plant Pathol* 103:125-136.

Davila-Huerta, G., Hamada, H., Davis, G. D., Stipanovic, R. D., Adams, C. M., and Essenberg, M. 1995. Cadinane-type sesquiterpenes induced in *Gossypium* cotyledons by bacterial inoculation. *Phytochemistry* 39:531-536.

Davis, E.M., Chen, Y.-S., Essenberg, M., and Pierce, M. L. 1998. cDNA sequence of a (+)-delta-cadinene synthase gene (Accession No. U883818) induced in *Gossypium hirsutum* L. by bacterial infection. *Plant Gene Register PGR 98-040 Physiol* 116:1191.

Davis, E. M., Tsuji, J., Davis, G. D., Pierce, M. L., and Essenberg, M. 1996. Purification of (+)-δ-cadinene synthase, a sesquiterpene cyclase from bacteria-inoculated cotton foliar tissue. *Phytochemistry* 41:1047-1055.

Davis, G.D., and Essenberg, M. 1995. (+)-δ-Cadinene is a product of sesquiterpene cyclase activity in cotton. *Phytochemistry* 39:553-567.

Donath, J., and Boland, W. 1995. Biosynthesis of acyclic homoterpenes: enzyme selectivity and absolute configuration of the nerolidol precursor. *Phytochemistry* 39:785-790.

Elzen, G. W., Williams, H. J., Bell, A. A., Stipanovic, R. D., and Vinson, S. B. 1985. Quantification of volatile terpenes of glanded and glandless *Gossypium hirsutum* L. cultivars and lines by gas chromatography. *J Agric Food Chem* 33:1079-1082.

Essenberg, M., Doherty, M. d'A., Hamilton, B.K., Henning, V. T., Cover, E. C., Mcfaul, S. J., and Johnson, W. M. 1982. Identification and effects on *Xanthomonas campestris* pv. *malvacearum* to two phytoalexins from leaves and cotyledons of resistant cotton. *Phytopathology* 72:1349-1356.

Essenberg, M., Grover, P. B., Jr., and Cover, E. C. 1990. Accumulation of antibacterial sesquiterpenoids in bacterially inoculated *Gossypium* leaves and cotyledons. *Phytochemistry* 29:3107-3113.

Essenberg, M., Stoessl, A., and Stothers, J. B. 1985. The biosynthesis of 2,7-dihydroxycadalene in infected cotton cotyledons: the folding pattern of the farnesol precursor and possible implications for gossypol biosynthesis. *J Chem Soc (London), Chem Commun* 1985:556-557.

Gray, J. R., Mabry, T. J., Bell, A. A., and Stipanovic, R. D. 1976. *para*-Hemigossypolone: a sesquiterpenoid aldehyde quinone from *Gossypium hirsutum*. *J Chem Soc (London), Chem Comm* 1976:109-110.

Gunasena, G. H., Vinson, S. B., Williams, H. J., and Stipanovic, R. D. 1988. Effects of caryophyllene, caryophyllene oxide, and their interaction with gossypol on the growth and development of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae). *J Econ Entomol* 81:93-97.

Hedin, P. A., Parrott, W. L., and Jenkins, J. W. 1992. Relationships of glands, cotton terpenoid square aldehydes and other allelochemicals to larval growth of *Heliothis virescens* (Lepidoptera: Noctuidae). *J Econ Entomol* 85:359-364.

Heinstein, P. F., Herman, D. L., Tove, S. B., and Smith, F. H. 1970. Biosynthesis of gossypol. *J Biol Chem* 245:4658-4665.

Hoffer, A. P., Agarwal, A., Meltzer, P., Herlihy, P., Naqvi, R. H., Lindberg, M. C., and Matlin, S. A. 1987. Ultrastructural, fertility, and spermicidal studies with isomers and derivatives of gossypol in male hamsters. *Biol Reprod* 37:909-924.

Howell, C. R., Hanson, L. E., Stipanovic, R. D., and Puckhaber, L. S. 2000. Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. *Phytopathology* 90:248-252.

Kohel, R. J., and Bell, A. A. 1999. Genetic analysis of two terpenoid variants in cotton *Gossypium hirsutum* L. *J Hered* 90:249-251.

Liu, J., Benedict, C. R., Stipanovic, R. D., and Bell, A. A. 1999. Purification and characterization of *S*-adenylosyl-L-methionine: desoxyhemigossypol-6-methyltransferase from cotton plants. An enzyme capable of methylating the defense terpenoids of cotton. *Plant Physiol* 121:1017-1024.

Liu, J., Benedict, C. R., Stipanovic, R. D., C. W. Magill, and Bell, A. A. 2002. Cloning and expression of desoxyhemigossypol-6-methyltransferase from cotton (*Gossypium barbadense*). *J Agric Food Chem* 50:3165-3172.

Luo, P., Wang, Y.-H., Wang, G.-D., Essenberg, M., and Chen, X.-Y. 2001. Molecular cloning and functional identification of (+)-δ-cadinene-8-hydroxylase, a cytochrome P450 mono-oxygenase (CYP706B1) of cotton sesquiterpene biosynthesis. *The Plant J* 28:95-104.

Mace, M. E. 1978. Contributions of tyloses and terpenoid aldehyde phytoalexins to Verticillium wilt resistance in cotton. *Physiol Plant Pathol* 12:1-11.

Mace, M. E., Bell, A. A., and Beckman, C. H. 1976. Histochemistry and identification of disease-induced terpenoid aldehydes in Verticillium wilt-resistant and -susceptible cottons. *Can J Bot* 54:2095-2099.

Mace, M. E., and Stipanovic, R. D. 1995. Mode of action of the phytoalexin desoxyhemigossypol against the wilt pathogen, *Verticillium dahliae*. *Pestic Biochem Physiol* 53:205-209.

Mace, M. E., Stipanovic, R. D., and Bell, A. A. 1985. Toxicity and role of terpenoid phytoalexins in Verticillium resistant cotton. Physiol. *Plant Pathol* 26:209-218.

Mace, M. E., Stipanovic, R. D., and Bell, A. A. 1989. Histochemical localization of desoxyhemigossypol, a phytoalexin in *Verticillium dahliae*-infected cotton stems. *New Phytologist* 111:229-232.

Masciadri, R., Angst, W., and Arigoni, D. 1985. A revised scheme for the biosynthesis of gossypol. *J Chem Soc (London), Chem Commun* 1985:1573-1574.

McAuslane, H. J., and Alborn H. T. 1998. Systemic Induction of Allelochemicals in Glanded and Glandless Isogenic Cotton by *Spodoptera exigua* Feeding. *J Chem Ecol* 24:399-416.

McAuslane, H. J., and Alborn, H. T. 2000. Influence of previous herbivory on behavior and development of *Spodoptera exigua* larvae or glanded and glandless cotton. *Entomol Exp Appl* 97:283-291.

Meng, Y.-L., Jia, J.-W, Liu, C.-J., Liang, W.-Q., Heinstein, P., and Chen, X.-Y. 1999. Coordinated accumulation of (+)-δ-cadinene synthase mRNAs and gossypol in developing seeds of *Gossypium hirsutum* and a new member of the *cad*1 family from *G. arboreum*. *J Nat Prod* 62:248-252.

Oikawa, H., Katayama, K., Suzuki, Y., and Ichihara, A. 1995. Enzymatic activity catalyzing exo-selective Diels-Alder reactions in solanapyrone biosynthesis. *J Chem Soc (London), Chem. Commun* 13:1321-1322.

Paré, P. W., and Tumlinson, J. H. 1998. Cotton volatiles synthesized and released distal to the site of insect damage. *Phytochemistry* 47:521-526.

Parrott, W. L., Jenkins, J. N., and McCarty, J. C. Jr. 1983. Feeding behavior of first-stage tobacco budworm (Lepidoptera: Noctuidae) on three cotton cultivars. *Ann Entomol Soc Am* 76:167-170.

Stipanovic, R. D. 1992. Natural product biosynthesis via the Diels-Alder reaction, pp. 319-328. *In* R. J. Petroski and S. P. McCormick (eds.) Secondary-Metabolite Biosynthesis and Metabolism. Plenum Press, New York.

Stipanovic, R. D., Altman, D. W., Begin, D. L., Greenblatt, G. A., and Benedict, J. H. 1988. Terpenoid aldehydes in upland cottons: analysis by aniline and HPLC methods. *J Agric Food Chem* 36:509-515.

Stipanovic, R. D., Bell, A. A., and Howell, C. R. 1975a. Naphthofuran precursors of sesquiterpenoid aldehydes in diseased *Gossypium*. *Phytochemistry* 14:1809-1811.

Stipanovic, R. D., Bell, A. A., and Lukefahr, M. J. 1977a. Natural insecticides from cotton (*Gossypium*). *In* P. A. Hedin (ed.) Host Plant Resistance to Pests. ACS Symposium Series, No. 62. American Chemical Society, Washington, D.C., pp. 197-214.

Stipanovic, R. D., Bell, A. A., and O'Brien, D. H. 1980. Raimondal, a new sesquiterpenoid from pigment glands of *Gossypium Raimondii*. *Phytochemistry* 19:1735-1738.

Stipanovic, R. D., Bell, A. A., O'Brien, D. H., and Lukefahr, M. J. 1977b. Heliocide H_2: an insecticidal sesterterpenoid from cotton (*Gossypium*). *Tetrahedron Lett* 6:567-570.

Stipanovic, R. D., Bell, A. A., O'Brien, D. H., and Lukefahr, M. J. 1978a. Heliocide H_1. A new insecticidal C_{25} terpenoid from cotton (*Gossypium hirsutum). J Agric Food Chem* 26:115-118.

Stipanovic, R. D., Bell, A. A., O'Brien, D. H., and Lukefahr, M. J. 1978b. Heliocide H_3 an insecticidal terpenoid from *Gossypium hirsutum*. *Phytochemistry* 17:151-152.

Stipanovic, R. D., Greenblatt, G. A., Beier, R. C., and Bell, A. A. 1981. 2-Hydroxy-7-methoxycadalene. The precursor of lacinilene C 7-methyl ether in *Gossypium*. *Phytochemistry* 20:729-730.

Stipanovic, R. D., Kim, H. L., Altman, D. W., Bell, A. A., and Kohel, R. J. 1994. Raimondalone, a sesquiterpene from a cotton interspecific hybrid. *Phytochemistry* 36:953-956.

Stipanovic, R. D., Mace, M. E., Bell, A. A., and Beier, R. C. 1992. The role of free radicals in the decomposition of the phytoalexin desoxyhemigossypol. *J Chem Soc, Perkin Trans I* 1992: 3189-3192.

Stipanovic, R. D., McCormick, J. P., Schlemper, E. O., Hamper, B. C., Shinmyozu, T., and Pirkle, W. N. 1986a. Corroboration of techniques for assigning absolute configuration: lacinilene C methyl ether as an exemplary study. *J Org Chem* 51:2500-2504.

Stipanovic, R. D., Puckhaber, L. S., and Bell, A. A. 1998. Biological activity and synthesis of a kenaf phytoalexin highly active against fungal wilt pathogens, pp. 318-324. *In* D. R. Baker, J. G. Fenyes, G. S. Basarab, and D. A. Hunt (eds.) Synthesis and Chemistry of Agrochemicals V, American Chemical Society, Washington, D. C. 339 pp.

Stipanovic, R. D., Puckhaber, L. S., and Bell, A. A. 2006a. Ratios of (+)- and (-)-gossypol in leaf, stem and roots of selected accessions of *Gossypium hirsutum* var. *marie galante* (Watt) Hutchinson. *J Agric Food Chem* 54:1633-1637.

Stipanovic, R. D., Puckhaber, L. S., Bell, A. A., and Percival, A. E. 2005. Occurrence of (+)- and (-)-gossypol in seed of wild species of cotton and in *Gossypium hirsutum* var. *marie-galante* (Watt) Hutchinson. *J Agric Food Chem* 53:6266-6271.

Stipanovic, R. D., Puckhaber, L. S., Reibenspies, J. H., and Williams, H. J. 2006b.

Determination of the absolute configuration of 3-hydroxy-α-calacorene. *Phytochemistry* 67:1304-1308.

Stipanovic, R. D., Stoessl, A., Stothers, J. B., Altman, D. W., Bell, A. A., and Heinstein, P. 1986b. The stereochemistry of the biosynthetic precursor of gossypol. *J Chem Soc (London), Chem Commun* 1986:100-102.

Stipanovic, R. D., Wakelyn, P. J., and Bell, A. A. 1975b. Lacinilene C, a revised structure, and lacinilene C 7-methyl ether from *Gossypium* bracts. *Phytochemistry* 14:1041-1043

Williams, H. J., Stipanovic, R. D., Smith, L. A., Vinson, S. B., Darnell, P. O., Montandon, R., Begin, D. L., Elzen, G. W., Gunasena, H., and Bell, A. A. 1987. Effects of gossypol and other cotton terpenoids on *Heliothis virescens* development. *Rev Latinoamer Quim* 18:119-131.

Zhang, H. L., Nagatsu, A., Okuyama, H., Mizukami, H., and Sakakibara, J. 1998. Sesquiterpene glycosides from cotton cake oil. *Phytochemistry* 48:665-668.

Zhang, J., Mace, M. E., Stipanovic, R. D., and Bell, A. A. 1993. Production and fungitoxicity of the terpenoid phytoalexins in cotton inoculated with *Fusarium oxysporum* f. sp. *vasinfectum*. J. *Phytopathology* 139:247-252.

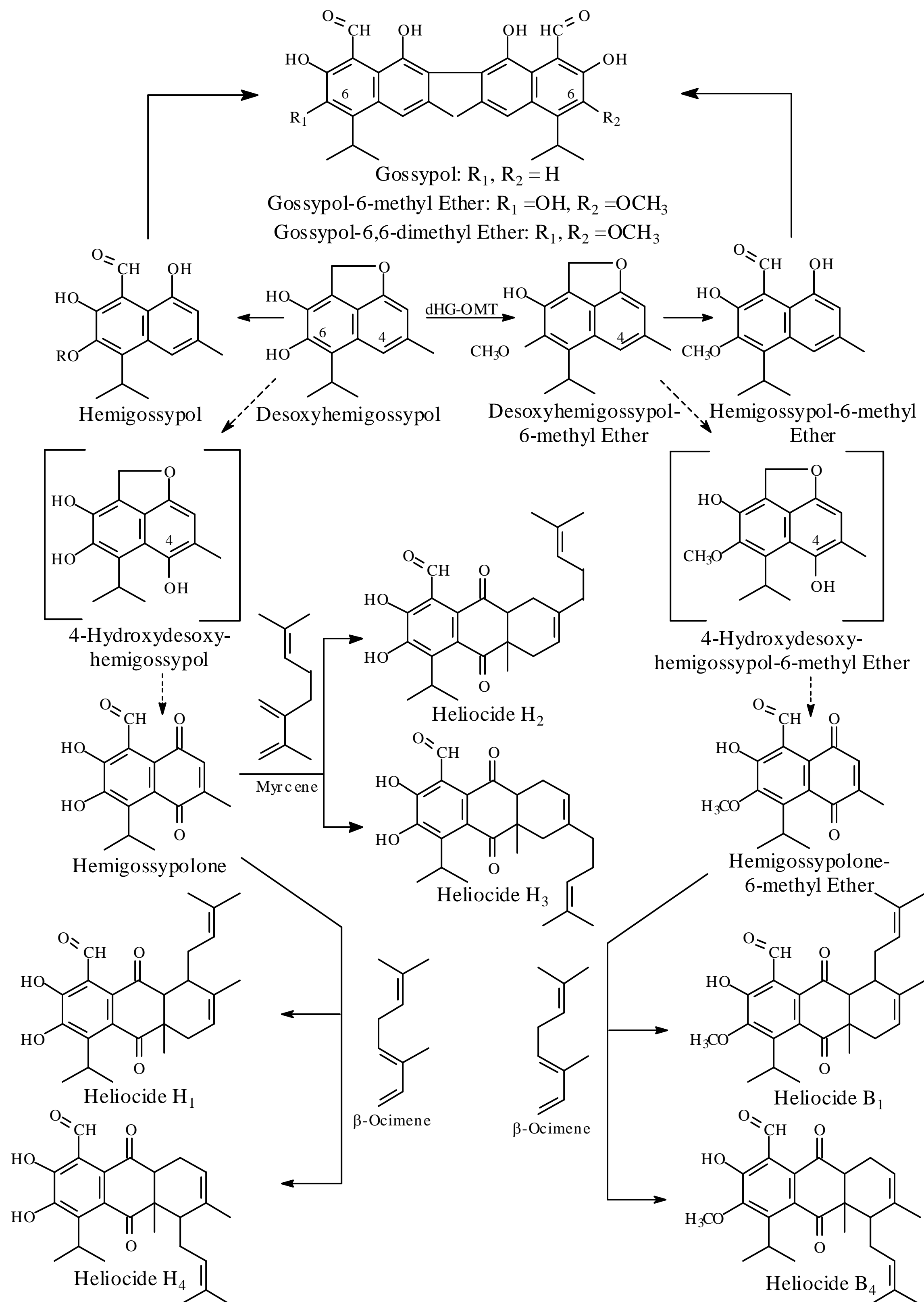


Figure 1. Proposed biosynthesis of foliar terpenoids in cotton.

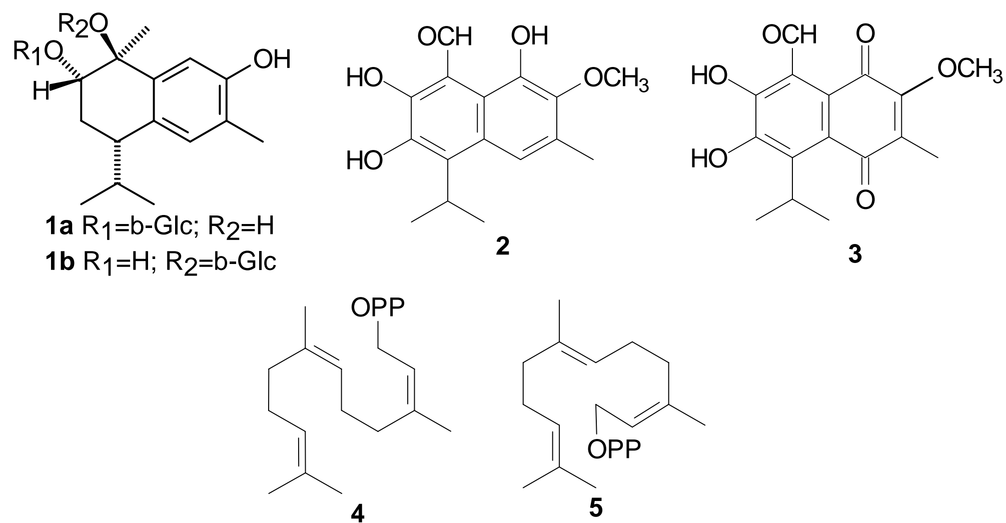


Figure 2. Terpenoids purported to occur or identified in various cotton tissues.

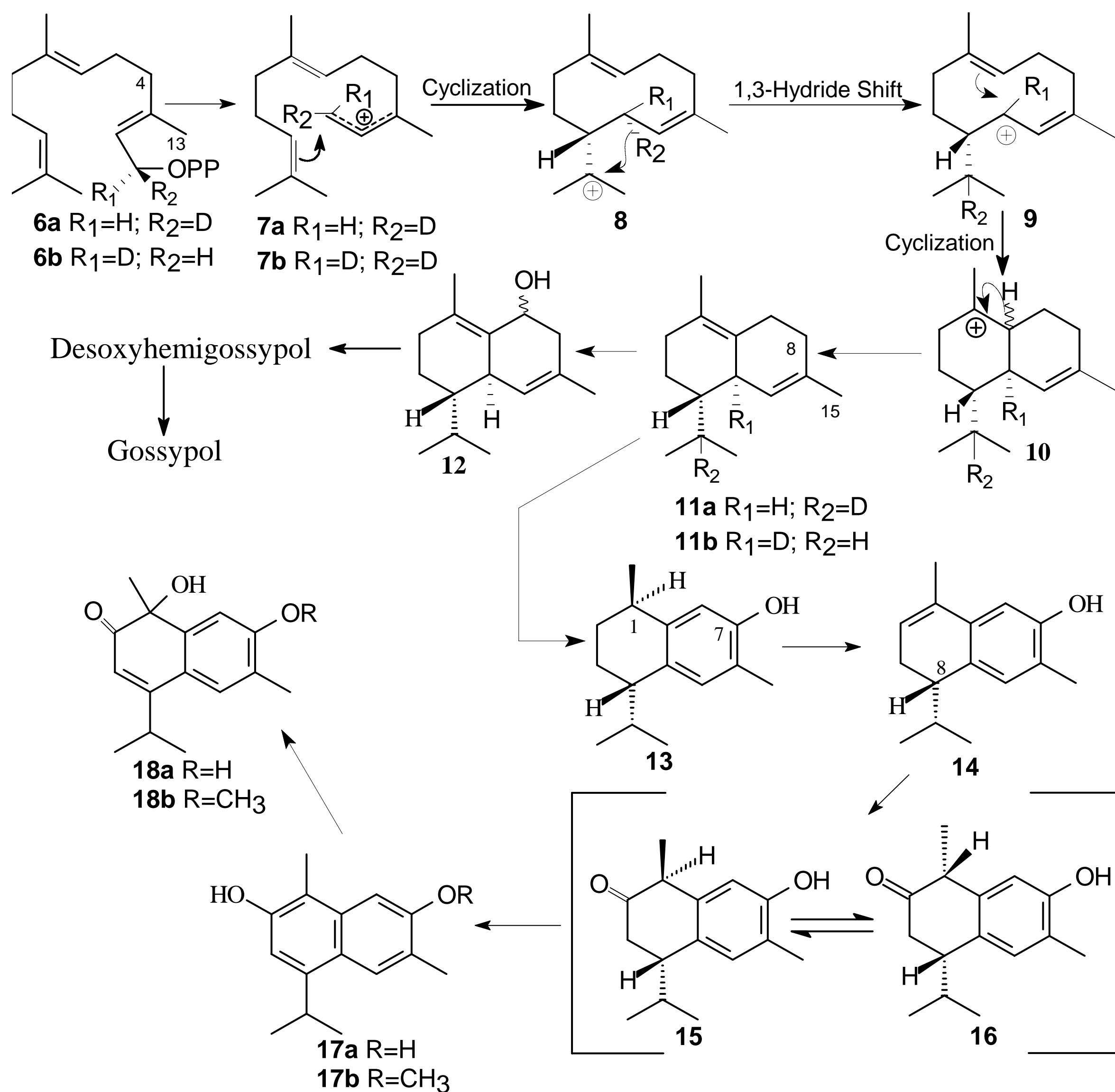


Figure 3. Proposed biosynthesis of 2,7-dihydroxycadalene, 2-hydroxy-7-methoxycadalene, lacinilene C and lacinilene C methyl ether.