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SESQUITERPENOID ALDEHYDE QUINONES AND DERIVATIVES IN PIGMENT GLANDS OF *GOSSYPIUM*

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Key Word Index—*Gossypium*; Malvaceae, Gossypieae; cotton; host plant resistance; chemotaxonomy; sesquiterpenoids; heliocides B_1 and B_4; hemigossypolone-7-methyl ether; gossypol.

Abstract—The resistance of *Gossypium* species to insects is enhanced by compounds in their lysigenous pigment glands. In cultivated cottons, glands in achlorophyllous plant parts contained predominately the terpenoid aldehyde gossypol in *G. hirsutum*, and gossypol and its methyl and dimethyl ethers in *G. barbadense*. Glands in young green tissues, however, contained hemigossypolone as the predominant terpenoid aldehyde in *G. hirsutum*, and a new quinone, hemigossypolone-7-methyl ether, in *G. barbadense*. As glands aged in green tissues, the sesquiterpenoid quinones were replaced by several C_{25}-terpenoids formed by the Diels–Alder reaction of the quinones with myrcene or *trans*-β-ocimene. Two C_{25}-terpenoids isolated from *G. barbadense*, but not *G. hirsutum*, were the methyl ethers of heliocides H_1 and H_4 and were designated heliocides B_1 and B_4, respectively. A dark red pigment, gossyrubilone, from glands of young leaves of both species is the isopentylimine of hemigossypolone. Similar red imines, formed from sesquiterpenoid quinones and amino acids, resembled the red coloration of the envelope cells surrounding the gland sac. The terpenoid quinones of *Gossypium* had physical characteristics different from quinones in *Bombax* which apparently were incorrectly identified as being the same. A survey of the terpenoid quinones and their heliocide derivates in wild *Gossypium* species and related genera in the Gossypieae showed considerable diversity which may be used for establishing biochemical and phylogenetic relationships.

INTRODUCTION

Pigment glands are one of two major characters used to define the tribe Gossypieae (family Malvaceae), which includes *Gossypium* (cotton) and seven other genera [1]. The gland is composed of an ovoid or spherical sac, 100–400 μm in diameter, and an envelope of one or more layers of flattened cells [2, 3]. The sac is formed by a lysigenous process involving several cells originally arranged in a spherical pattern. Glands generally are located below hypodermal cells of stems and palisade cells of leaves, but they also occur throughout the secondary phloem tissue in bark. While glands occur in most organs and tissues, they are particularly dense in seed embryos, young carpel walls (bolls), young leaves, and old root and stem bark near the soil line. Although the oil in the sac is golden or orange, the glands in green tissue are dark in appearance because of a red or purple pigment in the envelope cells.

Cook [4] first suggested that the pigment glands of cotton are important for host plant resistance to boll weevils and bollworms. Subsequently, Withers and Carruth [5] demonstrated that the pigment gossypol (**2a**) was associated with the glands and was primarily responsible for the toxicity of cottonseed to swine, poultry, and rodents. McMichael [6] reported two mutant recessive genes gl_2 and gl_3 which completely remove pigment glands from cotton plants and thus give nontoxic cottonseed free of gossypol (**2a**). However, when glandless cottons are grown in the field, they are sometimes attacked by various insect species that do not normally feed on glanded cotton, and many normal insect pests of cotton cause more damage on the glandless plants [7]. Conversely, wild races or hybrids of cotton with abnormally high gland density show less damage from major cotton insects than do normally glanded cottons [7]. Thus, gland contents apparently are toxic or repulsive to many insects.

Bottger *et al.* [8] first showed the toxicity of gossypol (**2a**) to insects, and postulated that it may be the insecticidal compound in glands. Other studies [7] have since correlated the insecticidal activity of glanded cotyledons and flower buds with gossypol (**2a**) content as determined by aniline or anisidine reagents. Lukefahr *et al.* [9], however, reported that fresh flower buds from certain wild or primitive lines of *G. hirsutum* showed more antibiosis to the tobacco budworm than would be expected from their gossypol (**2a**) content as determined by the aniline test. Thus, we undertook several studies to examine the terpenoid aldehyde content of pigment glands in various plant tissues.

In leaves [10], flower buds [11], and young bolls [13] of glanded *G. hirsutum* we found several terpenoid aldehydes, besides gossypol (**2a**), occurring in appreciable concentration. These terpenoids, like gossypol (**2a**), were missing from glandless cottons. The major terpenoid aldehyde in glands of young leaves was identified as the sesquiterpenoid quinone *para*-hemigossypolone (**3a**) [10]. This quinone (**3a**) also was isolated from flower buds [12] and was a major terpenoid in glands of 2- to 3-day-old bolls [13]. Four other aldehydes were identified as C_{25}-terpenoids formed by a Diels–Alder reaction. Thus,

heliocides H_2 (**5b**) and H_3 (**5c**) are the structural isomers formed by the reaction of hemigossypolone (**3a**) with myrcene [13, 14], while heliocides H_1 (**5a**) and H_4 (**5d**) are formed from hemigossypolone (**3a**) and *trans*-β-ocimene (**6**) [15]. Seaman *et al.* [16] found good correlations between the toxicity of flower bud extracts from 130 races of *G. hirsutum* and the content of individual heliocides, gossypol (**2a**), or total terpenoid aldehydes in the extracts. In controlled feeding experiments, however, heliocide H_1 (**5a**) was more active against budworms than heliocides H_2 (**5b**) or H_3 (**5c**) [17, 18]. Thus, the resistance of cotton to insects might be improved by breeding for specific heliocide contents in the pigment glands.

In the present study, we compared the terpenoids in pigment glands of different tissues and organs of *G. hirsutum* and *G. barbadense*. Three terpenoids unique to pigment glands in green tissues of *G. barbadense*, and a dark red pigment from glands in young leaves of both species, are identified as terpenoid quinones and derivatives. A survey was made of the occurrence of terpenoid quinones and their heliocide derivatives in *Gossypium* species and related genera to determine their usefulness in chemotaxonomy.

RESULTS

Identification of hemigossypolone-7-methyl ether (3b)

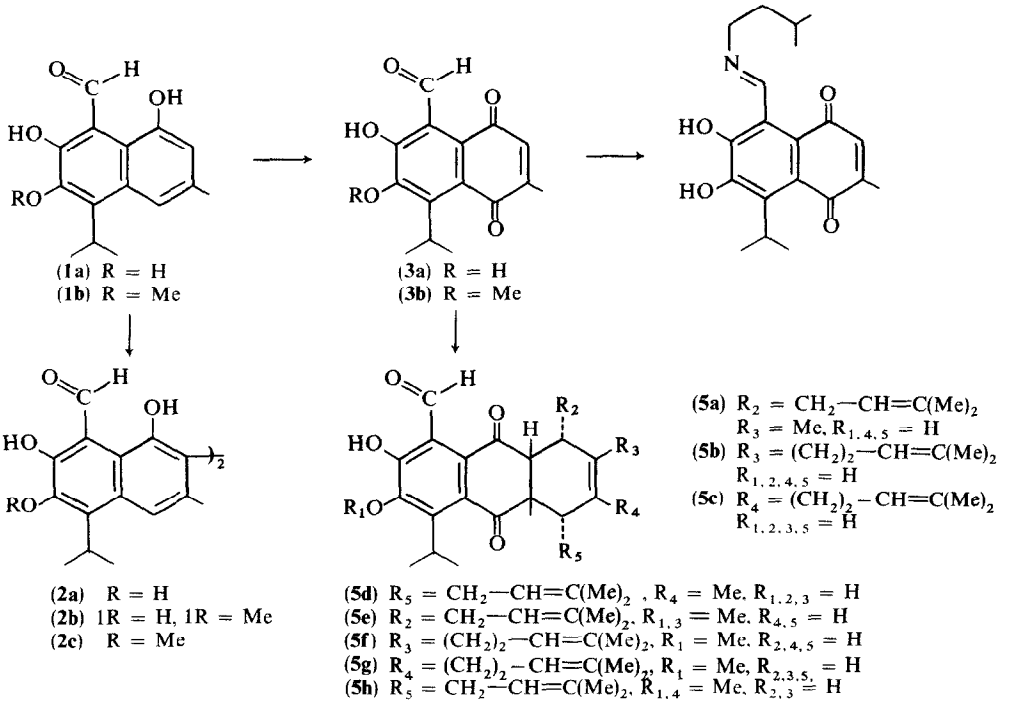
Compound **3b** (yellow crystals, mp 57–59°) was the major terpenoid isolated from young leaves and bolls of glanded *G. barbadense* plants but it was absent from glandless leaves. The occurrence of **3b** among tissues and among *Gossypium* sp. was consistently associated with that of hemigossypolone (**3a**). Compound **3b**, like **3a**, formed a magenta derivative with acidic phloroglucinol

Table 1. ^{13}C-NMR chemical shifts (δ) of hemigossypolone-7-methyl ether (**3b**), heliocides B_1 (**5e**) and B_4 (**5h**)*

Carbon No.	Compound (δ) 3b	5e	5h
C-1	186.8	201·7	200·9
C-2	149.3	49.0	51.6
C-3	133.4	56.9	51.2
C-4	185.9	198.7	199.3
C-5	117.0	115.3	115.0
C-6	158.4	158.8	159.1
C-7	152.4	151.9	152.2
C-8	150·4	149.7	150.7
C-9	126.7	129.0	129.9
C-10	130.5	135.9	135.4
C-11	198.0	197.1	197.7
C-12	28.7	29.1	29.7
C-13	20.8	20.9	20.7
C-14	20.8	21.0	21.3
C-15	16.3	23.0	23.8
C-16		32.4	49.0
C-17		118.4	136.3†
C-18		134.8†	117.6
C-19		38.9	26 4
C-20		21.3	21.5
C-21		27.4	30.5
C-22		123.3	124.2
C-23		133.3†	131.6†
C-24		25.6	25.5
C-25		17.6	17.6
$-OCH_3$	60.5	60.4	60.6

*^{13}C-NMR shifts are expressed in ppm downfield from TMS using the central resonance of $CDCl_3$ as reference (δ 76.9 ppm). Shift assignments of structurally similar types of carbon atoms were distinguished through differences in 2, 3, of 4-bond carbon–hydrogen coupling [32].

† Shift assignments of carbon atoms may be interchanged.



Scheme 1 Structures and probable biosynthetic relationships of terpenoid aldehydes and imines found in pigment glands of *Gossypium*.

and an orange derivative with 2,4-dinitrophenylhydrazine, indicating the presence of an aldehyde group.

Comparisons of spectral data of **3a** and **3b** showed that **3b** is the 7-methyl ether of hemigossypolone. The high resolution MS of **3b** indicated a molecular composition of $C_{16}H_{16}O_5$ (Found: 288.098270, 87%. Required: 288.099750). The ^{1}H-NMR spectrum of **3b** was very similar to that of **3a** [13]. However, the spectrum of **3b** had an additional three proton singlet at δ 3.98 and only one hydroxyl proton (δ 12.65). The δ 3.98 peak was similar to the methyl ether proton resonance peaks in spectra of hemigossypol-6-methyl ether (**1b**) [17] and gossypol-6,6′-dimethyl ether (**2c**) [18]; thus the methyl of (**3b**) was probably at the equivalent position (C-7). Further, the chemical shift of the hydroxyl group indicated that it was strongly hydrogen bonded to the aldehyde and therefore at C-6. The ^{13}C-NMR spectrum of hemigossypolone-7-methyl ether (**3b**) (Table 1) was very similar to that of hemigossypolone (**3a**) [13]. A major difference was the presence of a methyl ether peak at δ 60.5 in **3b**, but not in **3a**.

The structure of hemigossypolone-7-methyl ether (**3b**) was proved by its synthesis from hemigossypol methyl ether (**1b**). Compound **1b** was previously isolated and identified from *G. barbadense* [19]. When **1b** was oxidized with $FeCl_3$, several products were obtained. One was identical (mp, mmp, IR, UV, ^{1}H-NMR and MS) to the hemigossypolone-7-methyl ether isolated from *G. barbadense*.

Identification of heliocides B_1 *and* B_4

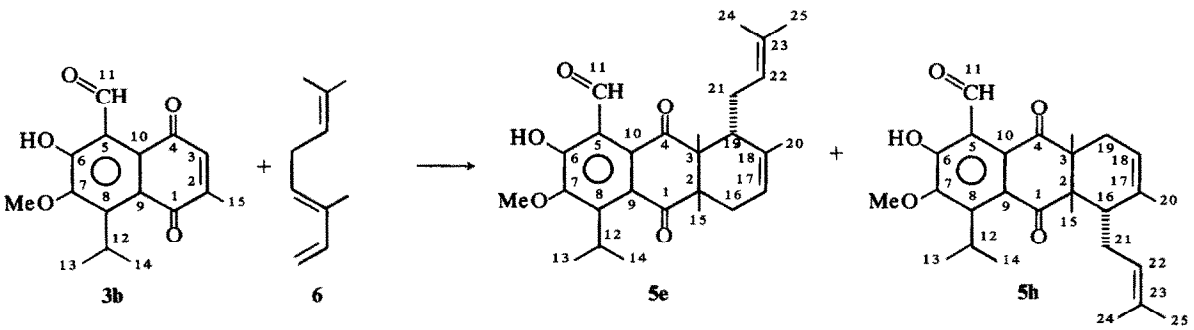
Extracts from young leaves or bolls of *G. barbadense* contained four compounds that formed yellow-orange derivatives with acidic phloroglucinol and yellow derivatives with 2,4-dinitrophenylhydrazine. The two compounds present in minor amounts were identified as heliocides H_1 (**5a**) and H_4 (**5d**) by comparing their chromatographic behaviour and MS and ^{1}H-NMR spectra with those of heliocide H_1 (**5a**) and H_4 (**5d**) synthesized from hemigossypolone (**3a**) and *trans*-β-ocimene (**6**) [15]. The remaining two compounds were different from heliocides H_{1-4} (**5a–d**) previously isolated from *G. hirsutum* [13–15]. We designated them as heliocides B_1 (**5e**) and B_4 (**5h**) to indicate their discovery in *G. barbadense* and structural relationships to heliocides H_1 (**5a**) and H_4 (**5d**), respectively.

* The molecular composition of ions were determined by high resolution MS.

High resolution MS of heliocides B_1 (**5e**) and B_4 (**5h**) indicated a molecular composition of $C_{26}H_{32}O_5$ [Found: for B_1 (**5e**) 424.223823, 8%, for B_4 (**5h**) 424.225076, 8%. Required: 424.224950]. Both compounds readily underwent reverse Diels–Alder fragmentations; B_1 gave major fragments of $C_{16}H_{17}O_5$* (*m/e* 289, 57%) and $C_{10}H_{15}$* (*m/e* 135, 100%), while B_4 gave fragments of $C_{16}H_{18}O_5$* (*m/e* 290, 53%) and $C_{10}H_{15}$* (*m/e* 135, 100%). Similar fragmentations were noted in the MS of heliocides H_1 (**5a**) and H_4 (**5d**) [15]. The alkyl side chain of heliocides B_1 (**5e**) and B_4 (**5h**) was indicated by fragments of $C_{21}H_{23}O_5$*, (*m/e* 355; 4% for B_1, 15% for B_4) and C_5H_9* (*m/e* 69, 24% for B_1, 44% for B_4).

The ^{13}C-NMR (Table 1) and ^{1}H-NMR spectra indicated that heliocides B_1 (**5e**) and B_4 (**5h**) were identical to heliocides H_1 (**5a**) and H_4 (**5d**), respectively, except for the presence of a methyl ether at one of the hydroxyl groups. The methyl ether groups of heliocides B_1 (**5e**) and B_4 (**5h**) appeared at δ 3.99 and 4.00 in the ^{1}H-NMR spectra and at δ 60.4 and 60.6 in the ^{13}C-NMR spectra, respectively. The chemical shifts of the hydroxyl protons (δ 12.71 and 12.98) and the aldehyde protons (δ 10.13 and 10.41) of heliocides B_1 (**5e**) and B_4 (**5h**), respectively, indicated that the hydroxyl protons were strongly chelated with the aldehyde oxygen. Such strong chelation could only result from a free hydroxyl at C-6. Thus, the methyl group was located at C-7.

Identification of heliocides B_1 (**5e**) and B_4 (**5h**) as Diels–Alder products of hemigossypolone-7-methyl ether (**3b**) and *trans*-β-ocimene (**6**) was confirmed by synthesis (Scheme 2). The reaction gave heliocides B_1 (**5e**) and B_4 (**5h**) in an approximate ratio of 3:1, which is the same ratio of B_1 (**5e**) and B_4 (**5h**) extracted from pigment glands. We previously presented arguments that formation of the Diels–Alder adduct heliocide H_1 (**5a**) with alkyl groups at C-18 and C-19 should be kinetically favoured over that of the adduct heliocide H_4 (**5d**) with alkyl groups at C-17 and C-16 because formation of the latter (**5d**) involves greater steric interference from the ocimene alkyl groups with the aromatic isopropyl group during adduct formation [15]. By similar reasoning, the isopentenyl group of the major product, heliocide B_1 (**5e**), and minor product, heliocide B_4(**5h**), would be at C-19 and C-16, respectively. Location of the isopentenyl groups at these positions was confirmed by comparisons of ^{13}C-NMR resonances of carbons in the cyclohexenyl rings (Table 2). When heliocides H_1 (**5a**) and B_1 (**5e**), or H_4 (**5d**) and B_4 (**5h**), were compared with each other, the alkyl carbons each showed $\Delta\delta$ differences less than 1.0,



Scheme 2

Table 2. Comparisons of the ^{13}C-NMR chemical shifts (δ) of carbons in the cyclohexenyl ring of heliocides H_1 (**5a**) and B_1 (**5e**), and H_4 (**5d**) and B_4 (**5h**)

Carbon	5a	5e	$\Delta\delta$	5d	5h	$\Delta\delta$
C-2	49.0	49.0	0.0	51.6	51 6	0.0
C-3	57.3	56.9	0.4	51.3	51.2	0.1
C-16	32.3	32.4	0.1	49.0	49 0	0.0
C-17	118.6	118.4	0.2	136 2	136.3	0.1
C-18	135.0	134.8	0.2	117.7	117.6	0.1
C-19	39.5	38.9	0 6	26 1	26.4	0.3

indicating the same alkyl substitutions within each pair of heliocides. Thus, heliocides B_1 and B_4 are the 7-methyl ether derivatives of heliocides H_1 and H_4, and have structure **5e** and **5h**, respectively.

Identification of gossyrubilone (**4**)

Extracts from terminal leaves contained a dark red pigment that we named gossyrubilone (**4**). From 200 g of dried terminal leaves, we obtained 12 mg of pure **4**, mp 140–142°. The high resolution MS of **4** indicated a molecular formula of $C_{20}H_{25}NO_4$ (Found: 343.179843. Calculated: 343.178335). Prominent peaks at *m/e* 258* ($C_{15}H_{14}O_4$, 63%) and 256* ($C_{15}H_{12}O_4$, 49%) were due to loss of $C_5H_{11}N$ and $C_5H_{13}N$ ions, respectively, while loss of these ions and a methyl group gave peaks at *m/e* 243* ($C_{14}H_{11}O_4$, 53%) and 241* ($C_{14}H_9O_4$, 51%), respectively.

The ^{1}H-NMR spectrum also indicated the imine structure **4**. The aldehyde proton (δ 10.7) of hemigossypolone (**3a**) was replaced by an imine proton (δ 9.65) in gossyrubilone (**4**). The spectrum also confirmed that the aromatic part of **4** was the same as in hemigossypolone (**3a**) [13]. The ^{1}H-NMR data also indicated that the alkyl group attached to the imine nitrogen was an isopentyl group. This was confirmed by synthesis. Isopentylamine reacted quickly with hemigossypolone in benzene to give high yields of **4**. Amino acids (alanine, isoleucine and phenylalanine) also reacted with hemigossypolone overnight at room temp. in methanol to give appreciable yields of red coloured imines. Thus, the aldehyde of the terpenoid quinones (**3a**, **3b**) is very reactive with amino groups.

Terpenoid content of pigment glands in different tissues

The major terpenoid aldehydes found in pigment glands in various tissues of flowering plants of *G. hirsutum* and *G. barbadense* are summarized in Table 3. In different tissues of *G. barbadense*, 30 to 70% of the terpenoids were methylated at the hydroxyl at C-7. Less than 5% of the terpenoids in any tissue of *G. hirsutum* were methylated. Pigment glands shielded from light (seed embryo, stem phloem, and root bark) or located in achlorophyllous tissues (petals and stamens) contained 90–95% gossypol (**2a**) and its methyl ethers (**2b**, **2c**). Glands in green tissues (leaves, petioles, stems, bracts, bolls), however, contained predominantly the terpenoid quinones (**3a**, **3b**) and their heliocide derivatives (**5a–h**). Gossypol (**2a**) and its methyl ethers (**2b**, **2c**) made up only 10 to 30% of the terpenoid aldehyde content in green tissue. Hemigossypol (**1a**) and its methyl ether (**1b**, in *G. barbadense* only) were present in all pigment glands but never made up more than 5% of the total aldehydes.

Changes in terpenoid composition of glands during growth of the plant were observed in cotyledons, leaves,

Table 3 Major terpenoid aldehyde components in pigment glands in different tissues of cultivated cotton

Tissue	Upland cotton (*G. hirsutum*)	Egyptian cotton (*G. barbadense*)
Seed:		
Embryo	G*	G,MG, DMG
Coat	no glands	no glands
Stems:		
Cortex	H_2, H_3, (H_1, H_4)†	B_1, B_4, H_1, H_4
Secondary Phloem	G	G, MG, DMG
Xylem	no glands	no glands
Leaves:		
Cotyledonary	G	G, MG, DMG
True	HGQ, H_2, H_3 (H_1, H_4)	HGQ, MHGQ, B_1, B_4, H_1, H_4
Petiole	H_2, H_3, (H_1, H_4)	B_1, B_4, H_1, H_4
Flowers:		
Bracts and calyx	H_2, H_3, (H_1, H_4)	B_1, B_4, H_1, H_4
Petals and stamens	G	G, MG, DMG
Pollen	no glands	no glands
Ovary and stigma	HGQ, H_2, H_3, (H_1, H_4)	HGQ, MHGQ, B_1, B_4, H_1, H_4
Roots:		
Cortex‡	G	G, MG, DMG
Secondary Phloem	G	G, MG, DMG
Xylem	no glands	no glands

* Abbreviations: G = gossypol (**2a**); MG = 6-methyl ether of gossypol (**2b**); DMG = 6,6'-dimethyl ether of gossypol (**2c**); H_1, H_2, H_3, H_4, B_1, and B_4 = heliocides H_1 (**5a**), H_2 (**5b**), H_3 (**5c**), H_4 (**5d**), B_1 (**5e**), and B_4 (**5h**); HGQ = hemigossypolone (**3a**); and MHGQ = hemigossypolone-7-methyl ether (**3b**).

† Parentheses indicate that these components were prominent in most wild strains but only in a few cultivated varieties.

‡ Considerable amounts of terpenoid in this tissue were in the epidermis and periderm of the root as well as in glands.

and bolls. Cotyledons in the seed contained only gossypol (**2a**) and its methyl ethers (**2b**, **2c**). After the cotyledon emerged from the soil following seed germination, the quinones (**3a**, **3b**) and heliocides (**5a–h**) appeared and increased progressively during growth of the cotyledon. Very young leaves and bolls contained terpenoid quinones (**3a**, **3b**) as major components. As these tissues aged, however, the quinones (**3a**, **3b**) were replaced by the heliocides (**5a–h**). In *G. hirsutum*, the free quinone (**3a**) was largely replaced by heliocides (**5a–d**) by the time bolls or leaves were 2–3 weeks old. Conversely, *G. barbadense* leaves and bolls contained appreciable free quinones (**3a**, **3b**) even when 1 month old.

Terpenoid content of glands in the tribe Gossypieae

Young leaves from 30 *Gossypium* species and five other genera in the Gossypieae were examined for their terpenoid content in glands. Results for the *Gossypium* species are shown in Table 4. Among the genera of the Gossypieae, *Cienfuegosia heterophylla*, *C. hildebrandtii*, *C. yucatanensis*. *Thespesia grandiflora*, *T. garckeana*, *T. populnea* and *Hampea nutricia* accumulated mostly gossypol (**2a**) along with a trace of hemigossypol (**1a**) but not the terpenoid quinones (**3a**, **3b**) or their derivatives (**4**, **5a–h**). *Lebronnecia kokioides* contained prominent amounts of hemigosspyol (**1a**) and gossypol (**2a**) and small amounts of hemigossypol methyl ether (**1b**) and gossypol methyl ethers (**2b**, **2c**); it also contained small amounts of hemigossypolone (**3a**) and hemigossypolone ether (**3b**) and traces of heliocides H_1 (**5a**) and B_1 (**5e**). *Gossypioides kirkii* was the only genus in the Gossypieae other than *Gossypium*, that contained heliocides (H_1, **5a** and H_4, **5d**) as the major terpenoids in leaf glands. *G. kirkii* also contained appreciable gossypol (**2a**), a small amount of hemigossypolone (**3a**), and a trace of hemigossypol (**1a**).

DISCUSSION

Dissimilarity of terpenoid aldehyde quinones in Gossypium *and* Bombax

Several lines of evidence firmly establish the structures of the two major terpenoid quinones of *Gossypium* as hemigossypolone (**3a**) and hemigossypolone-7-methyl ether (**3b**). These include the synthetic conversion of hemigossypol (**1a**) to both gossypol (**2a**) [20] and hemigossypolone (**3a**) [10, 12]. X-ray crystallographic analyses of heliocide H_2 (**5b**) which was synthesized from hemigossypolone (**3a**) further establish the location of the functional groups in **3a** [13]. Likewise, hemigossypolone-7-methyl ether (**3b**) from *Gossypium* was identical to (**3b**) synthesized from hemigossypol-6-methyl ether (**1b**). The position of the methyl ether group in **1b** was previously

Table 4. Relative content* of binaphthyl and naphthoquinone derivatives formed from hemigossypol (**1a**) and hemigossypol-7-methyl ether (**1b**) in pigment glands of young leaves of *Gossypium* sp.

Gossypium sp.	Cytogenetic group and designation	Naphthols		Binaphthols		Naphthoquinones		Naphthoquinone adducts With ocimene		With myrcene†	
		1a	**1b**	**2a**	**2b,2c**	**3a**	**3b**	**5a**	**5e**	**5b, 5c**	**5f, 5g**
G. herbaceum	A_1	tr	—	+	—	++	—	+++	—	+	—
G. arboreum	A_2	tr	—	+	—	+	—	++	—	++	—
G. anomalum	B_1	tr	tr	+	tr	++	+	+	tr	+++	++
G. triphyllum	B_2	+	+	+	++	++	+++	tr	+	—	—
G. barbosanum	B_3	tr	tr	+	+	++	++	+	+	++	++
G. sturtianum	C_1	++	++	—	—	+++	+++	tr	+	tr	tr
G. australe	C_3	tr	—	—	—	+	tr	+	tr	—	—
G. bickii	C_4	+	tr	—	—	+++	+	++	++	tr	tr
G. nelsonii	C	tr	—	+	—	++	tr	+++	+	++	+
G. thurberi	D_1	tr	—	+++	—	—	—	—	—	—	—
G. harknessi	D_2	tr	—	++	—	—	—	—	—	—	—
G. armourianum	D_{2-1}	tr	—	++	—	—	—	—	—	—	—
G. davidsonii	D_{3-d}	tr	—	++	—	—	—	—	—	—	—
G. klotzschianum	D_{3-k}	+	—	+++	—	—	—	—	—	—	—
G. aridum	D_4	tr	—	+++	—	—	—	—	—	—	—
G. raimondii	D_5	tr	—	+	tr	—	—	—	—	—	—
G. gossypioides	D_6	tr	—	tr	—	+	—	+++	—	+++	—
G. lobatum	D_7	tr	+	++	++	—	—	—	—	—	—
G. trilobum	D_8	+	—	+++	—	—	—	—	—	—	—
G. laxum	D_9	+	—	+++	—	—	—	—	—	—	—
G. stocksii	E_1	+	tr	++	tr	++	tr	++	—	+	—
G. somalense	E_2	+	tr	++	+	—	—	—	—	—	—
G. areysianum	E_3	tr	tr	++	+	+	tr	+	—	+	—
G. incanum	E_4	+	tr	++	+	—	—	—	—	—	—
G. longicalyx	F_1	tr	+	—	—	+++	++	++	++	tr	tr
G. hirsutum	AD_1	tr	—	+	—	++	—	+	—	++	—
G. barbadense	AD_2	tr	tr	+	+	++	++	++	++	tr	tr
G. tomentosum	AD_3	+	+	—	—	++	++	+	+	tr	tr
G. mustelinum	AD	+	—	++	—	++	—	+++	—	+	—
G. darwinii	AD	+	++	tr	+	++	+++	—	tr	—	—

* Terpenoids were separated by TLC, reacted with phloroglucinol reagent, and estimated from spot size and intensity: +++ = large intense spot, + = small distinct spot, tr = trace, and — = none detected.

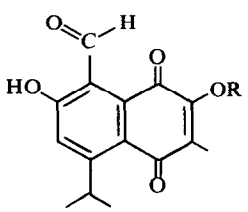
† The minor adducts from ocimene, **5d** and **5h**, occurred at the same R_f values as **5b**, **5c** and **5f**, **5g**, respectively, and made slight contributions to the intensity of these spots when appreciable **5a** or **5e**, respectively, were present.

established when its dimeric product (**2c**) from *Gossypium* was shown to be identical with **2c** synthesized from gossypol [21].

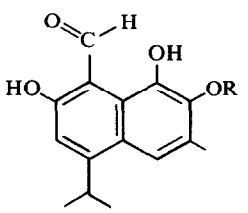
Seshadri *et al.* [22] identified terpenoids isolated from root bark of *Bombax malabaricum* as hemigossypolone (**3a**) and hemigossypolone-7-methyl ether (**3b**). In addition they tentatively identified a naphthol as hemigossypol-6-methyl ether (**1b**) [23]. Comparisons of physical and spectral characteristics of the terpenoids from *Bombax* with those of the comparable terpenoids from *Gossypium*, however, show that the compounds are different. Compounds **1b**, **3a** and **3b** from *Gossypium* had mp of 156–160° [19], 167–169° [12], and 57–59°, respectively, whereas putative **1b**, **3a**, and **3b** from *Bombax* had mp of 144°, 167–168°, and 87–88° [22, 23], respectively. UV spectra of the *Gossypium* terpenoids were different from those of the *Bombax* terpenoids: λ_{max} (ε) were 225 (388000), 268 (10600), 281 (*sh*), 352 (5100), and 388 (4800) for **1b** from *Gossypium* [19], but 235 (1260), 273 (6460), and 353 (10000) for putative **1b** from *Bombax* [23]; 270 (23500), 313 (9900), and 417 (2300) for **3a** from *Gossypium*, but 234 (10480), 252 (9770), and 294 (7080) for putative **3a** from *Bombax* [22]; and 266 (22900), 302 (*sh*) and 362 (2500) for **3b** from *Gossypium*, but 248 (15140), 272 (13180), and 338 (3980) for putative **3b** from *Bombax* [22].

There is also a considerable difference in the NMR spectra between the protons of the naphthoquinone ring system of the *Gossypium* terpenoids and those assigned to the naphthoquinone rings of the *Bombax* terpenoids. The quinoid protons at C-3 in hemigossypolone (**3a**) and hemigossypolone-7-methyl ethers (**3b**) have chemical shifts of δ 6.62 and 6.60, respectively. In contrast, Seshadri *et al.* [22] assigned a proton at δ 7.32 as the quinoid proton of putative **3b** from *Bombax*, and indicated that a similar proton occurred in putative **3a** from *Bombax*. Because of the above conflicts, we recently extracted terpenoids from bark and diseased tissue of an American cultivar of *Bombax malabaricum*. On TLC the terpenoids from *Bombax* gave R_f values and chromogenic reactions with acidic phloroglucinol different from those of the *Gossypium* terpenoids.

The terpenoid aldehyde quinones of *Bombax* probably are isohemigossypolone (**7a**) and isohemigossypolone-3-methyl ether (**7b**). The proton at δ 7.32 in putative **3b** from *Bombax* is better assigned as the aromatic proton at C-7 of **7b**. In 6-deoxyhemigossypol, an aromatic proton comparable to that at C-7 in **7b** occurs at δ 6.97 [19], whereas the quinoid protons of **3a** and **3b** occurs at δ 6.62 and 6.60, respectively. Likewise the UV spectra of the terpenoid quinones from *Bombax* show Band 1 absorption for a naphthoquinone at longer wavelengths (294 and 272 nm) than **3a** and **3b** (270 and 266 nm, respectively). The shift to the longer wavelengths would be expected from a hydroxyl and methoxyl substitution on the quinone ring as occurs in **7a** and **7b**, respectively [24]. By similar reasoning, putative **1b** from *Bombax* [23] probably is isohemigossypol-2-methyl ether (**8b**). The terpenoids of *Bombax* need further study before their structures can be definitely ascertained.



(**7a**) R = H
(**7b**) R = Me



(**8a**) R = H
(**8b**) R = Me

Biosynthesis and localization of terpenoid aldehydes

Previous studies [25] indicated that methylation of cotton terpenoids occurs early in biosynthesis before formation of the aldehyde group. Thus, high concentrations of hemigossypolone methyl ether (**3b**) and methylated heliocides (**5e–h**) were expected in *G. barbadense*, because hemigossypol methyl ether (**1b**) occurs in about the same concentration as hemigossypol (**1a**) in this species [21, 26]. The discovery of more than 50% methylation of the quinone and the heliocide derivatives in *G. barbadense* (Table 3) further shows that methylation probably precedes formation of hemigossypol methyl ether (**1b**).

Hemigossypol (**1a**) and its ether (**1b**) apparently have alternate routes of biosynthetic conversion that are under both genetic and physiological control. As shown in Scheme 1, the naphthol aldehydes may be either dimerized to gossypol (**2a**) and its ethers (**2b**, **2c**) or oxidized to naphthoquinones (**3a**, **3b**). The dimerization reaction is catalyzed by the enzyme peroxidase [20], but the enzyme catalyzing quinone formation is unknown. Ten of the 11 species of the American wild diploid cottons (D cytogenetic group, Table 4) apparently lack the enzyme and structural gene necessary for quinone formation. Consequently, these cottons accumulate only gossypol (**2a**), or **2a** and its methyl ethers (**2b**, **2c**) in pigment glands in all tissues. The complete inability to form terpenoid quinones, but marked ability to accumulate gossypol (**2a**), also occurred in pigment glands of the genera *Cienfuegosia*, *Thespesia*, and *Hampea* that, like *Gossypium*, are in the tribe Gossypieae [1].

Species or genera that have the genetic ability to form terpenoid quinones do so only in certain tissues. The enzyme that catalyzes quinone formation apparently is stimulated by light, either in its synthesis or activity, and it occurs only in tissues containing chlorophyll (Table 3). Consequently, pigment glands in seeds, root bark, flower petals, or stamens contain predominantly gossypol (**2a**) and its ethers (**2b**, **2c**), while glands in other tissues of the same plant contain mostly quinones (**3a**, **3b**) and their heliocide derivatives (**5a–h**) (Table 3). Glands formed in cotyledons during seed development contained only gossypols (**2a–c**), but after seedling emergence and exposure to light, quinones (**3a**, **3b**) and heliocides (**5a–h**) increased progressively in concentration during growth of the cotyledon. This indicates that within the cotyledon, glands formed in the dark, make only gossypols (**2a–c**), while those formed in light make mostly quinones (**3a**, **3b**) and heliodides (**5a–h**).

The principal products formed from the terpenoid quinones in *Gossypium* are the heliocides (Scheme 1). The heliocides apparently are formed by a spontaneous Diels–Alder reaction of the monoterpene dienes and the terpenoid quinones within the pigment glands. Thus, hemigossypolone (**3a**) reacts with *trans*-β-ocimene (**6**) to form heliocides H_1 (**5a**) and H_4 (**5d**) [15] (Scheme 2) or with myrcene to form heliocides H_2 (**5b**) and H_3 (**5c**) [13, 14]. Likewise, hemigossypolone methyl ether (**3b**) reacts with *trans*-β-ocimene to form heliocides B_1 (**5e**)

and B_4 (**5h**) or with myrcene to form heliocides B_2 (**5f**) and B_3 (**5g**). These conclusions are supported by the findings that *G. hirsutum*, which makes heliocides H_{1-4} (**5a–d**), contains both myrcene and *trans*-β-ocimene in its essential oils [27]; but *G. barbadense*, which makes only heliocides H_1, H_4, B_1, and B_4 (Table 3), contains *trans*-β-ocimene but not myrcene in its essential oil [28]. While we have synthesized heliocides other than H_{1-4} (**5a–d**) and B_{1-4} (**5e–h**) by reacting the quinones (**3a**, **3b**) with conjugated dienes such as allocimene and phellandrene [15], the only natural heliocides found to date are formed from *trans*-β-ocimene or myrcene.

Imines such as gossyrubilone (**4**) apparently are a minor group of compounds formed from the terpenoid quinones. For example, only 12 mg of gossyrubilone (**4**) compared to nearly 500 mg of heliocides (**5a–d**) was obtained from 200 g of dry terminal leaves of *G. hirsutum* cultivar 'TM-1' [10]. Gossyrubilone, and other imines that form spontaneously when terpenoid quinones (**3a**, **3b**) are mixed with amines and amino acids at room temp, are all bright red pigments. Thus, the pigments in the flattened envelope cells surrounding the pigment gland sac in green tissues most likely are imines formed by reaction of diffused quinones (**3a**, **3b**) with proteins or amino acids in the envelope cells. This concluson contrasts with earlier reports that incorrectly considered gossypol as the only terpenoid in glands and the red pigments of the gland as anthocyanins [2, 3]. The few *Gossypium* species that contain gossypol (**2a**) but no quinones (**3a**, **3b**) in glands of the leaves (Table 4), in fact, have no red pigmentation around the gland sac.

Phylogeny of terpenoid biosynthesis and its regulation

The presence or absence of terpenoid quinones (**3a**, **3b**) and structural variations in the quinones and their derivatives (**4**, **5a–h**) may be useful in establishing phylogenetic relationships among *Gossypium* species and related genera. *Gossypium* species have been divided, based on cytogenetic data, into the groups A, B, C, D, E, F, and AD as shown in Table 4 [29]. Species in each group, except AD, are diploids with 13 pairs of chromosomes. The AD species are amphidiploids (26 pairs of chromosomes) formed by the hybridization of an A and D species followed by doubling of chromosome number. The distributions of terpenoid quinones (**3a**, **3b**) and their heliocide derivatives (**5a–h**) among species (Table 4) generally support the cytogenetic groupings [29]. The distributions also give further evidence and amplification of phylogenetic relationships previously established by phenetic analysis [30] and show a possible biochemical advantage for formation of the AD amphidiploids.

With respect to quinone formation, four stages of phylogenetic advancement appear in the tribe Gossypieae. The most primitive chemical state is the inability to make quinones and is found in the D cytogenetic group of *Gossypium* and in the genera *Cienfuegosia*, *Thespesia*, and *Hampea*. The ability to form quinones (**3a**, **3b**) but not appreciable amounts of heliocides is probably the next stage of advancement. This character is shown by *G. triphyllum* of the B group, *G. sturtianum* of the C group, and *G. tomentosum* and *G. darwinii* of the AD group. The ability to form heliocides from only ocimene, followed by the ability to form heliocides from ocimene and myrcene, probably represents further biochemical advancement. Species that make heliocides predominantly from ocimene include *G. herbaceum* of the A group, *G. sturtianum* and *G. bickii* of the C group, *G. longicalyx* of the F group, and *G. barbadense* and *G. mustelinum* of the AD group. Species that make heliocides from both ocimene and myrcene include *G. arboreum* of the A group, *G. anomalum* and *G. barbosanum* of the B group, *G. nelsonii* of the C group, *G. gossypioides* in the D group, *G. stocksii* in the E group, and *G. hirsutum* of the AD group; most of these species are also considered to be the most phylogenetically advanced in their groups based on cytogenetic or phenetic characters [29, 30].

Other phylogenetic changes in terpenoid structure and its regulation have occurred in the substituents on the aromatic carbon positioned *meta to* the aldehyde group. This carbon is hydroxylated in all *Gossypium* species and related genera of the Gossypieae but apparently is not substituted in the sesquiterpenoid aldehydes of *Bombax* which is in a distinct family, the Bombacaceae. Methylation of the *meta* hydroxyl represents a more phylogenetically advanced state than the free hydroxyl group and occurs in all *Gossypium* species except the Old World (Asiatic) species of the A cytogenetic group (Table 4) [19]. Even though most species in the D cytogenetic group, and *G. hirsutum* and *G. mustelinum* of the AD group, have the structural gene for methylation, as can be shown from terpenoids synthesized in infected cambial tissues [19] or young roots [21], these species do not contain appreciable amounts of methylated terpenoids in the pigment glands. The diminution of methylation in glands probably is due to a regulator gene, similar to that in *G. hirsutum* [7], that inhibits methylation. Thus, inhibition of methylation in glands (less than 5%) coupled with appreciable methylation (10–50%) in juvenile tissues (seedling roots or diseased cambian) is due to both a structural and regulatory gene for methylation, and is a more phylogenetically advanced character than unregulated methylation. The latter is due to only the structural gene and occurs in similar percentages in juvenile tissues and glands.

Formation of the amphidiploid cotton species (AD group), because of enzymatic complementation, allows the formation of several antibiotic terpenoid aldehydes that can not be made by the diploid species in either the A or D groups. Species in the A group readily form terpenoid quinones and heliocides (Table 4) but apparently lack the enzyme and structural gene for methylation of terpenoids (Table 4) [19]. Species of the D group, on the other hand, apparently lack the enzyme and structural gene for quinone formation but readily form hemigossypol methyl ether [19] and, except for *G. lobatum* and *G. raimondii*, also contain a regulatory gene that suppresses methylation in glands (Table 4). Consequently, formation of the AD amphidiploid brings together the methylating-control genes of the D species with the quinone gene of the A species and allows formation of hemigossypolone methyl ether (**3b**) and heliocides B_{1-4} (**5e–h**) uniquely in the AD species. In addition, amphidiploid (AD) species can have high levels of methylated hemigossypol (**1b**) and gossypol (**2b**, **2c**) in young roots and infected tissue coupled with predominantly unmethylated heliodices (H_{1-4}; **5a–d**) in pigment glands of the foliage. This unique combination is not possible in the diploid A or D species (Table 4).

Detailed knowledge of the biochemical and genetical variation of terpenoid structure in *Gossypium* species allows the possible development of cotton cultivars with new chemical composition of their pigment glands.

Crosses between *G. hirsutum* and *G. barbadense*, for example, gave F_2 progeny which fell into six classes based on the chemical compositions of their pigment glands [7]. Three of these classes did not occur naturally in the parent species and therefore were created by the interspecies hybridization. Because the different gossypols (**2a–c**) and heliocides (**5a–h**) do vary considerably in their toxicity to tobacco budworm [17, 18], and probably other insects, it is desirable to breed into cotton pigment glands those chemicals that will give maximum protection. Detailed understanding of terpenoid biochemistry and genetics in the Malvaceae should facilitate the accomplishment of this objective.

EXPERIMENTAL

Isolation of hemigossypolone-7-methyl ether (**3b**) *and heliocide* B_1 (**5e**) and B_4 (**5h**). Young bolls (2–3 days old) of *Gossypium barbadense* L. cv 'Seabrook Sea Island 12B2' were collected in the field at College Station, Texas, and terpenoids were extracted and concentrated as previously described [14, 15]. Crude terpenoids were applied to a Si gel column and eluted with EtOAc–hexane–HOAc (10:90:0.25) in the following sequential order heliocides B_1 (**5e**), B_4 (**5h**), H_1 (**5a**), hemigossypolone-7-methyl ether (**3b**), heliocide H_4 (**5d**), hemigossypolone (**3a**), and finally gossypol (**2a**) mixed with its ethers (**2b**, **2c**). Fractions containing **3b**, or **5e** and **5h**, were combined, conc., and chromatographed on a Si gel column eluted with $CHCl_3$–HCO_2H (200:1). Fractions containing **3b** were then chromatographed on a Si gel column eluted with Et_2O–hexane–HCO_2H (5:95:0.25, solvent 1) and finally on Si gel TLC developed with hexane–EtOAc–HCO_2H; (90:10:0.25, solvent 2). Hemigossypolone-7-methyl ether (**3b**) crystallized from hexane in two forms (mp 57–59° and 72–75°), but usually with mp 57–59°. Heliocides B_1 (**5e**) and B_4 (**5h**) were further purified by CC (solvent 1) followed by TLC (solvent 2), and were crystallized from hexane heliocides B_1 (**5e**), mp 83–87°, and B_4 (**5h**), mp 87–88.5°.

Synthesis of hemigossypolone-7-methyl ether (**3b**) *and heliocides* B_1 (**5e**) *and* B_4 (**5h**). Hemigossypolone-7-methyl ether (**3b**) was synthesized from hemigossypol-6-methyl ether (**1b**) by oxidation with $FeCl_3$ in the presence of HOAc [31]. For the synthesis of heliocides, hemigossypolone-7-methyl ether (**3b**) (75 mg) was dissolved in C_6H_6 (0.5 ml), and *trans*-β-ocimene (**6**) (10 ml) was added. The reaction mixture, under N_2, was heated at 90° for 8 hr and stirred overnight at room temp. *Ca* the same product ratio and yield was achieved by stirring the reaction mixture under N_2 for 8 days at room temp. Excess ocimene (**6**) was removed as an azeotrope with MeOH (4 × 350 ml) on a rotoevaporator. The crude product was separated by Si gel TLC developed with hexane–Et_2O–HCO_2H (90:10:0.5). The upper band (R_f 0.5) was heliocide B_1 (**5e**) (44.8 mg), and the lower band was heliocide B_4 (**5h**) (21.0 mg). Residual ocimene (**6**) moved with the solvent front. Heliocides B_1 (**5e**) and B_4 (**5h**) each were then chromatographed on Si gel plates developed 4 × in hexane–Et_2O–HCO_2H (95:5:0.5) before the compounds were removed. Heliocide B_1 (**5e**) gave crystals with mp 83–86° (hexane) and mmp with naturally occurring heliocide B_1 of 83–87°. Heliocide B_4 (**5h**) gave crystals with mp 85–89° (hexane) and mmp with naturally occurring heliocide B_4 of 86–89°. Heliocide B_4 (**5h**) was less stable than heliocide B_1 (**5e**) during purification and in soln, but it was stable in its crystalline state.

Spectra of hemigossypolone-7-methyl ether (**3b**). MS (Probe 30°): *m/e* (rel. int.) 288* ($C_{16}H_{16}O_5$, 87), 274 (19), 273* ($C_{15}H_{13}O_5$, 100), 271 (11), 258 (13), 245* ($C_{14}H_{13}O_4$, 16), 231 (10), 230* ($C_{13}H_{10}O_4$, 33), 229 (15), 227 (10), 217 (15), 128 (10), 115 (14), 91 (10), 77 (10). UV $\lambda_{max}^{CHCl_3}$ nm (ε): 378 (2300), 305 (11100), 268 (25700), 261 (*sh*); $\lambda_{max}^{EtOH/HCl}$ nm (ε): 362 (2500), 302 (*sh*), 266 (22900); $\lambda_{max}^{EtOH/NaOH}$ nm (ε) 528 (3000), 323 (11600), 239 (23700). ^{1}H-NMR ($CDCl_3$): δ 1.36 (6H, *d*), 2.14 (3H, *bs*), 3.98 (3H, *s*), 4.03 (1H, *sept*), 6.60 (1H, *bs*), 10.49 (1H, *s*), 12.65 (1H, *s*, exchanged with D_2O). IR ν_{max}^{KBr} cm^{-1}: 1665, 1645.

Spectra of heliocide B_1 (**5e**). MS (Probe 70°) *m/e* (rel. int.) 424 (8), 328 (15), 327 (31), 290 (17), 289 (57), 249 (10), 136 (12), 135 (100), 107 (13), 93 (40), 91 (15), 69 (24). UV $\lambda_{max}^{CHCl_3}$ nm (ε): 350 (3800), 267 (31000); $\lambda_{max}^{EtOH/HCl}$ nm (ε): 348 (3500), 265 (29800); $\lambda_{max}^{EtOH/NaOH}$ nm (ε): 385 (6300), 339 (9700), 276 (20500). ^{1}H-NMR ($CDCl_3$): δ 1.34 (6H, *d*), 1.35 (3H, *s*), 1.44 (3H, *bs*), 1.57 (3H, *bs*), 1.79 (3H, *bs*), 2.0–2.8 (5H, *m*), 3.24 (1H, *d*), 3.72 (1H, *sept*), 3.99 (3H, *s*), 4.95 (1H, *m*), 5.35 (1H, *m*), 10.13 (1H, *s*), 12.71 (1H, *s*, exchanged with D_2O). IR ν_{max}^{KBr} cm^{-1}: 1690, 1650.

Spectra of heliocide B_4 (**5h**). MS (Probe 100°) *m/e* (rel. int.): 424 (8), 355 (15), 337 (10), 328 (17), 327 (12), 290 (53), 259 (24), 249 (12), 241 (16), 149 (28), 147 (38), 136 (16), 135 (100), 129 (90), 113 (21), 112 (38), 111 (16), 107 (11), 93 (36), 91 (14), 84 (11), 83 (16), 81 (11), 71 (29), 70 (26), 69 (44). UV $\lambda_{max}^{CHCl_3}$ nm (ε): 352 (3600), 268 (32700); $\lambda_{max}^{EtOH/HCl}$ nm (ε): 364 (2800), 298 (*sh*), 266 (35200); $\lambda_{max}^{EtOH/NaOH}$ nm (ε): 377 (*sh*), 339 (17200), 291 (22800), 266 (22800), 255 (*sh*). ^{1}H-NMR ($CDCl_3$): δ 1.38 (6H, *d*), 1.39 (3H, *s*), 1.46 (3H, *bs*), 1.56 (3H, *bs*), 1.74 (3H, *bs*), 1.7–2.1 (5H, *m*), 3.14 (1H, *m*), 3.84 (1H, *sept*), 4.00 (3H, *s*), 4.95 (1H, *m*), 5.43 (1H, *m*), 10.41 (1H, *s*), 12.98 (1H, *s*, exchanged with D_2O). IR ν_{max}^{KBr} cm^{-1}: 1629, 1655.

Isolation of gossyrubilone (**4**). Terminal leaves of *Gossypium hirsutum* cv 'TM-1' were collected from the field and freeze dried. Dried leaves (200 g) were ground to 60 mesh, and crude terpenoids were extracted and prepared as described previously [21]. Gossyrubilone was purified by PLC using cyclohexane–Me_2CO (9:1), $CHCl_3$–HCO_2H (200:1) and Et_2O–hexane–HCO_2H (25:75:1) in successive stages of purification. In each system gossyrubilone was the only red pigment with an R_f greater than 0.1. Crystallization from cyclohexane yielded 12 mg of **4**.

Synthesis of gossyrubilone (**4**). Hemigossypolone (**3a**) (5 mg) was dissolved in deuterobenzene (0.5 ml) and a catalytic amount of deuteroacetic acid in a 5 mm NMR tube. Isomylamine (30 µl) was added, and a red colour developed on mixing. In the NMR spectrum of the reaction mixture, the aldehyde proton of hemigossypolone (δ 10.59) was completely replaced in a few min by an imine proton at δ 9.65. The crude soln was added to Et_2O, extracted successively with dilute HCl, H_2O, and NaCl soln. The NMR spectrum ($CDCl_3$) indicated a complex series of peaks in the region of δ 1.3, and the mp of the crystals was broad (128–140°). After 24 hr, with no additional purification, the NMR spectrum was much cleaner and the mp had narrowed to 136–142°. The initial product was probably a mixture of *syn*- and *anti*-isomers that equilibrates to the *anti*-isomer upon standing.

Spectra of gossyrubilone (**4**). IR λ_{max}^{KBr} cm^{-1}: 1655, 1629, 1543, 1500, 1431, 1320, 1282 and 1258. UV λ_{max}^{EtOH} nm (ε): 513.5 (2700), 400 (*sh*), 351.5 (7900), 269.5 (21800), 236 (18500), 216.5 (21000) [illegible] nm (ε) 511 (2200), 400 (*sh*), 351.5 (6700), 269.5 (22700), 215 (22000) [illegible] nm (ε) 577 (2200), 358 (*sh*), 254.5 (21000); MS (Probe, 90°): *m/e* (rel. int.) 343 (100, $C_{20}H_{25}NO_4$), 329 (12), 328 (50), 259 (18), 258 (65, $C_{15}H_{14}O_4$), 257 (22), 256 (57, $C_{15}H_{12}O_4$), 255 (14), 244 (14), 243 (60), 242 (15), 241 (51), 230 (13), 229 (10), 128 (11), 115 (10), 88 (13), 86 (21), 69 (10), 57 (10). NMR ($CDCl_3$): δ 0.98 (6H, *d*), 1.40 (6H, *d*), 1.70 (3H, *m*), 2.10 (3H, *bs*), 3.66 (2H, *t*), 3.86 (1H, *sept*), 6.43 (1H, *bs*), 9.65 (1H, *s*).

Survey of terpenoids in tissues and species. Seeds of *Gossypium* spp. and related genera in the tribe Gossypieae were from the species collection maintained by P. A. Fryxell at Texas A&M University, College Station, Texas, U.S.A. Seeds of *Bombax malabaricum* were obtained from John Brudy's Rare Plant House†, P.O.B. 1348, Cocoa Beach, Florida, U.S.A. Plants of all wild species were grown in the greenhouse at College Station, Texas and were sampled when 2 and 4 months old. Comparisons of terpenoids in plant parts (Table 4) were made from *Gossypium hirsutum* L. cvs 'Acala SJ-1', 'TAMCOT SP-37', and 'Stoneville 213', and *Gossypium barbadense* cvs 'Seabrook Sea Island 12B2', 'Tanguis 66-1' and 'Pima S-2'

† Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

grown in the field. Tissues were harvested about 2 weeks after flowering began. Similar results were obtained for each cultivar within a species; therefore, only data for the species is given.

Crude terpenoids were extracted from whole tissues by two methods. (a) Fresh tissue (2 g) was extracted with Et_2O for 1 min and then 10 min followed with EtOAc for 50 min The extracts were combined, reduced to dryness *in vacuo* at 30° and taken up in a minimal volume of EtOAc for TLC. (b) Fresh tissue (2 g) was immersed in 2.5 ml of a soln containing 90% EtOH and 10% of an aq. 1% soln of Na_2HSO_3. After sitting overnight, 4.5 ml 9% NaCl soln containing 1% HCl was added. The organic layer, which floated to the top of the tube, was applied directly to Si gel TLC. Best results were obtained with unactivated TLC plates that were air dried overnight at 40–60% r.h. Plates were developed two-dimentionally with Et_2O–hexane–HCO_2H (15:85:0.5) and cyclohexane–Me_2CO (95:5) to estimate quinones (**3a**, **3b**) and heliocides (**5a–h**), and with cyclohexane–Me_2CO–HCO_2H (80:20:0.25) and Et_2O–hexane–HCO_2H (15:85:0.5), developed twice with intermittent drying, to estimate hemigossypol (**1a**), gossypol (**2a**), and their ethers (**1b**, **2b**, **2c**). Plates were sprayed with a fresh mixture of equal amounts of 5% phloroglucinol in EtOH and conc. HCl. Hemigossypol (**1a**), gossypol (**2a**), and their ethers (**1b**, **2b**, **2c**) formed rose to magenta colours; quinones (**3a**, **3b**) gave magenta to maroon colours; and heliocides (**5a–h**) formed yellow to orange colours. Quantities of compounds were estimated from spot size and colour intensity. The localization of terpenoid aldehydes to glands was ascertained by histochemical observations [26].

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