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ANTIMICROBIAL TERPENOIDS OF *GOSSYPIUM*: HEMIGOSSYPOL, 6-METHOXYHEMIGOSSYPOL AND 6-DEOXYHEMIGOSSYPOL

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Key Word Index—*Gossypium*; *Cienfuegosia*; *Hampea*; *Thespesia*; *Gossypioides*; *Hibiscus*; Malvaceae; cotton; disease resistance; phytoalexins; sesquiterpenoid aldehydes; hemigossypol; 6-methoxyhemigossypol; 6-deoxyhemigossypol.

Abstract—The sesquiterpenoid aldehydes, hemigossypol (**1a**), 6-methoxyhemigossypol (**1b**), and 6-deoxyhemigossypol (**1c**), were isolated and identified from *Verticillium*-infected stele tissue of *Gossypium barbadense*. Structures were established by spectral (UV, IR, NMR, MS) evidence and chemical transformations. This is the first report of (**1b**)* and (**1c**) in nature, and of NMR and m.p. data for crystalline pure (**1a**). Compound (**1a**) occurred in diseased stele tissues of all 21 *Gossypium* species examined and in the genera, *Cienfuegosia*, *Gossypioides*, *Hampea*, and *Thespesia*; it was absent in three *Hibiscus* spp. Compound (**1b**) occurred in the same taxa as (**1a**), except that it was absent in species of two cytogenetic groups (A and B genome) of *Gossypium*. Compound (**1c**) occurred in trace quantities, or was not detected, in most species; however, its distribution appeared to be similar to that of (**1a**).

INTRODUCTION

Tissues of cotton (*Gossypium* spp.), when stressed by disease or chemical injury, biosynthesize gossypol [1] and at least five related aromatic aldehydes [2]. All these compounds formed characteristic pink to magenta derivatives with phloroglucinol [2] and were fungitoxic [3]. Their probable importance in the disease resistance of cotton has been reviewed [4, 5]. Our preliminary data [3, 4] indicated that the major fungitoxin in diseased stele tissues of *G. hirsutum* was the sesquiterpenoid aldehyde, hemigossypol (1,6,7-trihydroxy-5-isopropyl-3-methyl-8-naphthaldehyde, **1a**). *Gossypium barbadense* also contained (**1a**), and a second compound that appeared identical except for one less free hydroxyl group [4]. Zaki *et al.* [6, 7] more recently reported the isolation of two major antifungal compounds from *G. barbadense* plants infected with *Verticillium albo-atrum*. They proposed the structures (**1a**) and 7-methoxy-5-isopropyl-3-methyl-8-naphthaldehyde (trivial name, vergosin) [6]. They obtained (**1a**) as a yellow oil with UV absorption at λ_{max}^{MeOH} 228, 255, 288, 294 and 370 nm and with a carbonyl-absorption band at 1710 cm^{-1} in the IR. They did not report a crystalline (**1a**) or its NMR spectrum. We report here the isolation and more complete characterization of pure crystalline (**1a**) and the isolation of the closely related sesquiterpenoid aldehydes, 6-methoxyhemigossypol (**1b**) and 6-deoxyhemigossypol (**1c**). Also, a partial survey of the occurrence of (**1a**), (**1b**) and (**1c**) in the Malvaceae was made.

RESULTS

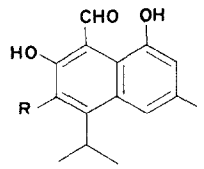
Identification of hemigossypol (**1a**)

Our isolation provided (**1a**) as bright yellow crystals with m.p. 159–163° from $CHCl_3$. A color change from yellow to yellow–green was noted on heating, or prolonged exposure to air, with a rapid change to dark green in the temp. range 111–115°. Hemigossypol (**1a**) formed a magenta derivative [λ_{max}^{EtOH} 553 (21 500) nm] with phloroglucinol, a red chelate with antimony, and a burnt orange 2,4-dinitrophenylhydrazone. Compound (**1a**) readily

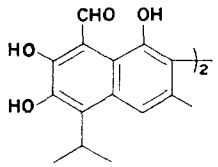
* Since this manuscript was submitted, the authors became aware of a recent communication (Seshadri, V., Batta, A. K. and Rangaswami, S. (1973) *Indian J. Chem.* **11**, 825) which suggests structure **1b** for a compound isolated from root bark of *Bombax malabaricum*. There are slight discrepancies between the m.p. UV and NMR data of this compound and **1b** reported in this paper.

formed a bright yellow complex with $B_4O_7^{2-}$, indicating an aromatic *cis*-dihydroxyl group.

The NMR spectrum of (**1a**) showed an aromatic isopropyl group with a six-proton doublet at 1·48 and a methine septet at 3·81. An aromatic methyl group resonated at 2·39 (3 H, *s*). Two phenolic hydroxyl protons appeared as a broad peak centered at 6·00. Aromatic protons appeared at 6·60 (1 H, *s*), and 7·45 (1 H, *s*). The presence of an aldehyde proton was indicated by a peak at 11·11, which was shifted downfield due to a hydrogen bonding of the aldehyde with a phenolic proton at 14·75. The overall NMR spectrum of (**1a**) generally agreed with that of gossypol (**2**).



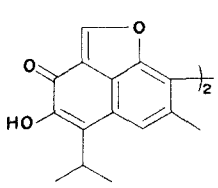
(1a) R = OH
(1b) R = OMe
(1c) R = H



(2)

The UV absorbance of hemigossypol (**1a**), λ_{max}^{EtOH} 229 (38300), 279 (10600), sh 288, sh 298, 374 (6800) nm, was also very similar to that of gossypol (**2**), λ_{max}^{EtOH} 236 (76100), sh 283, 289 (28800), 376 (15500) nm, but with A values about half those of gossypol. The two naphthyl rings of gossypol are not coplanar and have restricted rotation about the binaphthyl bond [8–10]. This allows a minimum of delocalized electronic interaction between the two rings. Thus, the absorbance of gossypol (**2**) is expected to be twice that of hemigossypol (**1a**). In gossypol (**2**) there is some hydrogen bonding between the C_1, C_1-hydroxyl groups [11]. This probably accounts for the small variations in A maxima between (**1a**) and (**2**).

The high-resolution MS of (**1a**) indicated a molecular composition of $C_{15}H_{16}O_4$ (Found: 260·104691, 100%. Required: 260·104840). Major ionic fragments were noted at *m/e* 245 (M^+-Me, 32%), 242 (M^+-H_2O, 24%), and 227 (M^+-H_2O–Me, 48%). These fragments were in agreement with the proposed structure. The loss of methyl from the isopropyl group was expected to be a favored process. The formation of anhydrogossypol (**3**) by the elimination of 2 mol H_2O from gossypol is well documented [12, 13]. Furthermore, the MS of gossypol (M^+ 518, 5%) is characterized by the loss of 1 and 2 mol H_2O [*m/e* 500, 85% (M^+-H_2O) and 482, 100% (M^+-$2H_2O$)], and the loss of one and two water molecules and a methyl group [*m/e* 485, 17% (M^+-H_2O–Me), and 467, 50% (M^+-$2H_2O$–Me)]. Thus, the formation of fragment *m/e* 242 from (**1a**) was most easily accounted for by the anhydrohemigossypol radical ion (**4**). Since the MS of hemigossypol (**1a**) was determined by direct probe insertion at 20°, most of the anhydrohemigossypol ions (**4**) must have been formed by electron impact, and not by ionization of thermally dehydrated hemigossypol.



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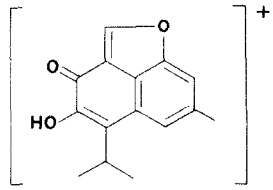


(4)

Identification of 6-*methoxyhemigossypol* (**1b**)

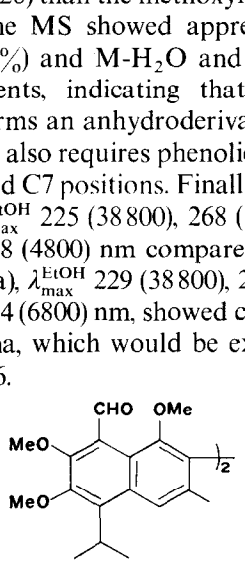
Compound (**1b**) was obtained as yellow crystals with m.p. 156–160° from benzene. On heating or prolonged exposure to air, (**1b**) developed first a burnt orange and later an orange–brown color. It formed a deep rose-colored derivative [λ_{max}^{EtOH} 548 (20300) nm] with phloroglucinol, a burnt orange 2,4-dinitrophenylhydrazone, and a yellow–orange chelate with $SbCl_3$. No complex was formed with $B_4O_7^{2-}$. Under UV light (365 nm), (**1b**) showed yellow fluorescence, which was intensified by increasing the pH.

Comparison of the NMR spectrum of (**1b**) with hemigossypol (**1a**) indicated very strong similarities between the compounds. Aromatic isopropyl (1·47, 6 H, *d* and 3·86, 1 H, *sept*) and methyl (2·39, 3 H, *s*) groups were evident in (**1b**), and aromatic protons appeared at 6·68 (1 H, *s*) and 7·49 (1 H, *s*). As with hemigossypol, the aldehyde proton was again shifted downfield and resonated at 11·09 (1 H, *s*), due to hydrogen bonding of the aldehyde with the C_7 phenolic hydroxyl (14·05, 1 H, *s*). An additional phenolic hydroxyl gave a broad singlet at 6·23 (1 H). The only major difference in the NMR spectrum was the appearance of a three-proton singlet at 3·89, which is characteristic of an aromatic methoxyl group.

High-resolution MS of (**1b**) indicated a molecular formula of $C_{16}H_{18}O_4$ (Found: 274·119543,

100%. Required: 274·120490). Diagnostically important peaks also occurred at *m/e* 259 (24%, M^+-Me), 256 (43%, M^+-H_2O), and 241 (95%, M^+-H_2O–Me).

Compound (**1b**) is apparently 1,7-dihydroxy-5-isopropyl-6-methoxy-3-methyl-8-naphthaldehyde (trivial name: 6-methoxyhemigossypol). The methoxyl group is assigned to the C6-position for several reasons. The downfield shift of the aldehyde and the one hydroxyl proton clearly indicate strong hydrogen bonding, which would result only from a C7-hydroxyl group. The methoxyl group is not at C1, since compound (**1b**) did not complex with $B_4O_7^{2-}$ (a complex would result from *cis*-dihydroxyl groups at C6, C7). Also, Datta *et al.* [14] have shown that the C1-OMe in (+)-gossypol hexamethylether (**5**) resonates at a higher field (3·26) than the methoxyls at C6 and C7 (3·95–4·00). The MS showed appreciable M-H_2O (*m/e* 256, 43%) and M-H_2O and Me (*m/e* 241, 95%) fragments, indicating that compound (**1b**) readily forms an anhydroderivative (**6**). The formation of (**6**) also requires phenolic hydroxyls at both the C1 and C7 positions. Finally, the UV spectrum of (**1b**), λ_{max}^{EtOH} 225 (38800), 268 (10600), sh 281, 352 (5100), 388 (4800) nm compared to that of hemigossypol (**1a**), λ_{max}^{EtOH} 229 (38800), 279 (10600), sh 288, sh 298, 374 (6800) nm, showed characteristic shifts in maxima, which would be expected for a methoxyl at C6.



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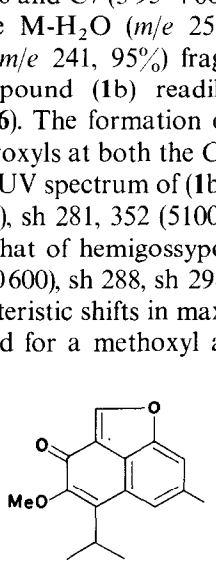

(6)

Identification of 6-*deoxyhemigossypol*

A third minor compound (**1c**) was also obtained as yellow crystals from benzene (m.p. 174–178·5°). This compound showed behavior similar to that of 6-methoxyhemigossypol (**1b**) during chromatographic separations and in chemical reactions. It formed a rose colored derivative [λ_{max}^{EtOH} 540 (12000) nm] with phloroglucinol, a burnt orange 2,4-dinitrophenylhydrazone, and a yellow–orange antimony chelate. It did not complex with $B_4O_7^{2-}$, and under UV light (365 nm) it showed yellow–green fluorescence, which was intensified by increasing the pH.

The NMR spectrum of (**1c**) indicated aromatic isopropyl (1·36, 6 H, *d*, and 3·58, 1 H, *sept*) and methyl (2·42, 3 H, *s*) groups, and a highly deshielded aldehyde proton (11·10, 1 H, *s*). Compound (**1c**) showed three aromatic protons, all of which appeared as singlets (6·72, 1 H, *s*; 6·97, 1 H, *s*; 7·39, 1 H, *s*). Because of the small amount of material available, the hydroxyl groups could not be clearly distinguished in the NMR spectrum, but hydroxyl absorption was observed in the IR (3260 cm^{-1}).

High-resolution MS of (**1c**) indicated a molecular formula of $C_{15}H_{16}O_3$ (Found: 244·111090, 100%. Required: 244·109930). The M^+ readily lost water (*m/e* 226, 65%) and water plus a methyl group (*m/e* 211, 24%). The UV spectrum of (**1c**), λ_{max}^{EtOH} 222 (24300), 259 (10600), sh 281, 336 (2310), 389 (2900) nm, was very similar to that of 6-methoxyhemigossypol (**1b**).

Compound (**1c**) is apparently 1,7-dihydroxy-5-isopropyl-3-methyl-8-naphthaldehyde (trivial name: 6-deoxyhemigossypol). The following evidence indicates that the hydroxyl groups are located at the C1 and C7 positions. The appreciable loss of water in the MS indicates the formation of the anhydro ion, which requires both a C1 and C7 hydroxyl. The deshielding of the aldehyde proton and lack of coupling of the three aromatic protons (i.e. aromatic protons are not on adjacent carbon atoms) also indicates that the hydroxyls must occur at C1 and C7. The similarity of UV spectra indicates that the hydroxyls of **1b** and **1c** are in the same positions. Finally, the failure to complex with $B_4O_7^{2-}$ indicates the absence of *cis*-dihydroxyl groups in the C6 and C7 positions.

Distribution of (**1a**), (**1b**), *and* (**1c**) *in the Malvaceae*

The occurrence of the sesquiterpenoid aldehydes in *Verticillium*-infected stele tissues of various *Gossypium* spp. and genera of the Malvaceae is shown in Table 1. Hemigossypol (**1a**) occurred in all subdivisions of the genus *Gossypium* [15] and in other genera of the tribe Gossypieae [16]. Deoxyhemigossypol (**1c**), the probable biosynthetic precursor of (**1a**), also appeared to occur generally in the Gossypieae. However, its concentrations were so small that definite confirmation of its presence was not always possible with the quantities of materials used in these studies. Compounds (**1a**), (**1b**) or (**1c**) were not found in the three *Hibiscus* spp. from the tribe Hibisceae.

Table 1. Occurrence of hemigossypol (HG), 6-methoxyhemigossypol (6-MHG), and 6-deoxyhemigossypol (6-DHG) in *Verticillium*-infected stele tissues of various *Gossypium* spp. and other genera of the Malvaceae

Cytogenetic Group	Genus and species	Relative occurrence* HG	6-MHG	6-DHG
A genome (Africa, Middle East, Far East)				
	Gossypium arboreum L. (10)†	+ + +	−	+
	G. herbaceum L. (3)	+ + +	−	+
B genome (Centered in Africa)				
	G. anomalum Waw. & Pey.	+	−	−
C genome (Australia)				
	G. australe Muell.	+ +	+	−
	G. bickii Prokh.	+ +	+	−
	G. sturtianum J. H. Willis	+ + +	−	−
D genome (Centered in Mexico)				
	G. aridum (Rose and Standl.) Skov.	+	+	−
	G. armourianum Kearn.	+	+	−
	G. gossypioides (Ulbr.) Standl.	+	+ + +	+
	G. harknessii Brandeg.	+ + +	+ + +	+
	G. klotzschianum Anderss.			
	var. *klotzschianum*	+	+	−
	var. *davidsonii* (Kell.) Hutch.	+ +	+	−
	G. lobatum Gentry	+	+	+ +
	G. raimondii Ulbr.	+ + +	+	+
	G. thurberi Tod. (2)	+ + +	+	+
	G. trilobum (DC.) Skov.	+ +	+	+
E genome (Arabian peninsula)				
	G. areysianum Deflers	+	+	−
	G. somalense (Gürke) Hutch.	+ +	+	−
	G. stocksii Mast.	+	−	−
F genome (East Africa)				
	G. longicalyx Hutch. & Lee	+	+	−
AD genome (centered in Central and South America)				
	G. barbadense L. (4)	+ + +	+ +	+
	G. hirsutum L. (7)	+ + +	+	+
	G. hirsutum var. *marie-galante* (Watt) Hutch. (4)	+ + +	+ +	+
Genera closely related to *Gossypium*				
	Cienfuegosia heterophylla (Vent.) Garcke	+ + +	+ + +	+
	C. hildebrandtii Garcke	+	+	−
	C. yucatanensis Millspaugh	+	+	−
	Gossypioides kirkii (Mast.) Skov. ex Hutch.	+ +	+ +	−
	Hampea rovirosae Standl.	+	+	−
	Thespesia populnea (L.) Sol. ex Corr.	+ + +	+ +	−
Other genera of the Malvaceae				
	Hibiscus esculentus L.	−	−	−
	H. rosa-sinensis L.	−	−	−
	H. syriacus L.	−	−	−

* Estimated (− = not detectable; + = clearly detectable; + + + = most intense concentration) from intensity of rose color developed 20 min after spraying chromatograms with 2% phloroglucinol in a mixture of EtOH–conc HCl (1 : 1). Concentrated extracts, each from 0·5 g fresh stele inoculated for 72 hr with *Verticillium dahliae*, were spotted on 0·5 mm layers of polyamide and developed with $CHCl_3$–Me_2CO–HCO_2H (95 : 4 : 1) to separate the sesquiterpenoid aldehydes.

† Number in parentheses represents the number of genetic strains of the species examined.

The unique occurrence of 6-methoxyhemigossypol (**1b**) might represent advanced evolution of terpenoid structure in *Gossypium*. None of the Old World cultigens (10 strains of *G. arboreum* and 3 strains of *G. herbaceum*; A genome) produced the methoxylated compound (**1b**). Likewise, *G. anomalum* (the only species of the B genome examined) did not produce (**1b**). The absence of methoxylation in these groups has been confirmed by using terpenoid fractions from as much as 10 g fresh tis-

sue per spot for chromatography. Compound (**1b**) was also absent in preparations from younger plants.

DISCUSSION

Hemigossypol (**1a**), 6-methoxyhemigossypol (**1b**), and 6-deoxyhemigossypol (**1c**) are apparently the major sesquiterpenoid aldehydes of *Gossypium*. Triterpenoid aldehydes, formed by random dimerization of (**1a**), (**1b**), and (**1c**), also occur in diseased tissue of *Gossypium* and are particularly abundant in seedling roots [17, 18]. However, the triterpenoid aldehydes (R_f 0·70–0·75) are readily separated from the sesquiterpenoid aldehydes (R_f 0·30–0·50) by TLC on polyamide powder, using $CHCl_3$–Me_2CO–H_2CO_2H (95 : 4 : 1) as a solvent.

The identification of vergosin [6] as a sesquiterpenoid aldehyde is apparently incorrect. Zaki *et al.* assigned a proton at 7·3 as the aldehyde proton of vergosin; this is outside the normal range for aldehyde protons [19]. Also, the aldehyde protons of (**1a**), (**1b**), (**1c**), and the three triterpenoid aldehydes reported elsewhere [17] occur at 11·09–11·17. The C4 aromatic proton of (**1a**), (**1b**), and (**1c**) occurred at 7·39–7·45 and corresponded closely to the proton that Zaki *et al.* assigned to the aldehyde. Further, they reported that vergosin reacted slowly with phloroglucinol to give only a pink color. The six terpenoid aldehydes that we have studied react rapidly with phloroglucinol reagent to form intense rose to magenta derivatives [λ_{max}^{EtOH} 540–550 (12000 to 46000)]. The failure of vergosin to form a colored derivative readily with phloroglucinol is further evidence that it contains no aldehyde group.

Other compounds, which reacted with phloroglucinol and were found sporadically on chromatograms of *Gossypium* terpenoids, apparently represented anhydro derivatives of the aldehydes or hybrid triterpenoid molecules that had an aldehyde group in only one of the two naphthyl rings. On drying TLC plates or in alcoholic solutions, small amounts of organic or inorganic acids catalyze dehydration of the terpenoid aldehydes (unpublished). The use of activated silica gel or alumina also causes some dehydration of the aldehydes. The anhydro derivatives react with aldehyde reagents to give the same product as the parent aldehyde. We have not determined if the anhydro derivatives occur naturally.

The extensive occurrence of hemigossypol (**1a**) in the Gossypieae was expected. Compound (**1a**) is the logical biosynthetic precursor to gossypol, which previously was reported to occur in seeds, roots, and foliar subepidermal glands of numerous *Gossypium* spp. [20, 21] and of several genera of the tribe Gossypieae [16, 22]. Methoxylated terpenoids have not been reported previously from cotton. Their absence in the A and B genome of *Gossypium* and wide variation in concentration in the D and AD genomes suggest that they may be important in the ecological adaptation of certain species.

EXPERIMENTAL

Gossypium barbadense L. cv. Seabrook Sea Island 12B2 and the various species listed in Table 1 were grown in the field at College Station, Texas. Stems were harvested from 5-month-old plants. Stele tissues were prepared for inoculation by cutting the main stem into 10 cm sections and peeling off the cortical tissue. These were immediately dipped into conidial suspensions of the fungus and incubated over moist towels in loosely folded plastic bags in the dark for 72 hr.

Preparation of fungal inoculum. Shaken cultures of *Verticillium dahliae* strain 277 were grown in a sterile medium containing 900 ml potato broth (prepared by heating 200 g fresh peeled and sliced Russet potatoes in 1 l. H_2O at 1 kg/cm^2 for 20 min), 100 ml V-8 juice, and 20 g sucrose. After 4–5 days of incubation, cultures were filtered (4 layers of Kimwipes, type 900–S) to remove mycelia and culture debris. Conidia were then centrifuged and washed (H_2O 2 ×). Conidia were finally suspended in a buffer containing 0·01 M each of KH_2PO_4 and K_2HPO_4. Concentrations were adjusted to an A of 0·5 at 600 nm (*ca* 10^7 conidia/ml).

Preparation of crude terpenoid fraction. The diseased stele tissues were cut into 2 cm sections and shaken with 95% EtOH (2 vol./g fresh stele) for 1 hr before filtering. The EtOH extract was mixed thoroughly with equal vol. of H_2O, satd NaCl soln, and EtOAc in a separatory funnel, and the H_2O phase was discarded. The EtOAc extract was washed 2× with equal vol. of 50% satd NaCl soln and finally with satd NaCl soln before drying over Na_2SO_4. The EtOAc was removed in a rotary evaporator at 30°, and the residue was dissolved in a minimum vol. of EtOAc–hexane (1 : 3) and filtered through a silica gel column (3 × 5 cm). The column was eluted with an additional 200 ml of the EtOAc–hexane, and the combined eluates were dried *in vacuo* at 30°. This residue was dissolved in EtOAc (1 ml/100 g fresh stele) for chromatography or in Et_2O (1 ml/10 g) for $B_4O_7^{2-}$ complexing.

Chromatography. All procedures were conducted in minimum light or in the dark. Layers (0·5 mm) of absorbent were spread on TLC plates (20 × 20 cm) and allowed to air-dry overnight; no further activation was used. Polyamide layers were developed with $CHCl_3$–Me_2CO–HCO_2H (95:4:1), System 1; or MeOH–HCO_2H (49 : 1), System 2. Silica gel G_{254} was developed with $CHCl_3$–Me_2CO–HCO_2H (95 : 4 : 1), System 3; naphtha solvent–Et_2O–HCO_2H (70 : 30 : 1), System 4; $CHCl_3$, System 5; EtOAc–hexane (1 : 3), System 6; or, C_6H_6–MeOH (19 : 1), System 7. Each compound was purified until it appeared as a single quenched spot under UV (254 nm) or a fluorescent one at 365 nm in each system. Initially, zones containing terpenoid

aldehydes were detected on TLC plates by spraying with : (a) 2% phloroglucinol in EtOH–conc HCl (1 : 1); (b) 2,4-dinitrophenylhydrazine (satd) in aq 2 N HCl; or (c) 2% $SbCl_3$ in $CHCl_3$. During preparative stages, the compounds were located by their visible yellow color and UV quenching or fluorescence. Zones containing the terpenoids were scraped from plates with a razor blade immediately after development. Terpenoids were eluted from polyamide powder with MeOH–HCO_2H (49:1) which was immediately mixed with 3 vol. of 2% $NaHCO_3$ in 50% satd NaCl soln and 1 vol. of Et_2O. The Et_2O was washed 2 × with 50% satd NaCl soln, dried, filtered, and taken to dryness *in vacuo* at 30°. The residue was again dissolved in EtOAc for further purification. Terpenoids were eluted from silica gel with Et_2O and were immediately taken to dryness, unless HCO_2H was used in the TLC solvent. In the latter case, the Et_2O soln was treated with $NaHCO_3$ soln and washed as with the eluate from polyamide. Careful attention to the removal of trace amounts of HCO_2H was necessary, because it quickly catalyzed dehydration of the terpenoid aldehydes, either on TLC or during removal of solvents *in vacuo*.

Spectral data. UV spectra were determined in 95% EtOH or 95% EtOH plus 0·03 M NaOH (EtONa) and are reported as $\lambda_{max}(\epsilon)$. IR spectra were determined in KBr. 100 MHz NMR spectra were determined in $CDCl_3$ soln at 23°. Hydroxyl protons were detected by exchange with D_2O. All NMR data are reported in δ units. The probe temperature for the MS of (**1a**), (**1b**), and (**1c**) was 20° and for (**2**) was 180°; source temp. was 200° for all compounds.

Isolation of hemigossypol (**1a**). Compound (**1a**) was extracted from Et_2O soln into 2% $Na_2B_4O_7$ soln, leaving (**1b**) and (**1c**) in the Et_2O phase. The borate soln was washed repeatedly with Et_2O and acidified to pH 3. Compound (**1a**) was then re-extracted into Et_2O and washed with H_2O to remove residual H_3BO_3. This extract was dried *in vacuo* at 30°, and the residue was dissolved in EtOAc. Compound (**1a**) was purified by TLC using, sequentially, systems 1 (R_f 0·31), 3 (R_f 0·64), 2 (R_f 0·45) and 6 (R_f 0·47). Residue from the final TLC system was dissolved in a minimum vol. of warm $CHCl_3$ and mixed with an equal vol. of hexane to give crude crystals. Recrystallization from $CHCl_3$ yielded pure (**1a**) as yellow crystals. M.p. 159–163° (decomp). UV: $\lambda_{max}^{EtOH}(\epsilon)$ 229 (38300), 279 (10600), 288 (sh), 298 (sh), 374 (6800) nm; $\lambda_{max}^{EtONa}(\epsilon)$ 237 (34100), 263 (sh), 296 (sh), 383 (6000) nm. IR: ν_{max}^{KBr} 3530, 3350, 1615 cm^{-1}. MS (*m/e*): 260·104691 (M^+, 100%), 245 (M^+-Me, 32%), 242 (M^+-H_2O, 24%), 227 (M^+-Me-H_2O, 48%), 199 (12%), 115 (15%). NMR ($CDCl_3$): 1·48 (6 H, *d*), 2·39 (3 H, *s*), 3·81 (1 H, *sept*), 6·0 (2 H, *bs*), 6·60 (1 H, *s*), 7·45 (1 H, *s*), 11·11 (1 H, *s*), 14·75 (1 H, *s*).

Isolation of 6-methoxyhemigossypol (**1b**) *and 6-deoxyhemigossypol* (**1c**). The Et_2O soln remaining after aqueous $Na_2B_4O_7$ extraction was washed with H_2O and dried. The Et_2O was removed *in vacuo* at 30°, and the residue was dissolved in EtOAc. Compounds **1b** and **1c** together were purified by TLC chromatography in systems 5 (R_f 0·43) and 4 (R_f 0·42). They were then separated by repeated chromatography in system 1 (R_f **1b** 0·47, **1c** 0·39), and were finally chromatographed in system 6 (R_f 0·47). Each was then dissolved in a minimum vol. of C_6H_6, and an equal vol. of hexane was added to give crude crystals. Recrystallization from C_6H_6 yielded pure (**1b**) as yellow crystals. M.p. 156–160° (decomp). UV: $\lambda_{max}^{EtOH}(\epsilon)$ 225 (38800), 268 (10600), 281 (sh), 352 (5100), 388 (4800) nm; $\lambda_{max}^{EtONa}(\epsilon)$ 230 (sh), 269 (10400), 296 (sh), 341 (5000), 420 (5700) nm. IR: ν_{max}^{KBr} 3300, 1610, 1260 cm^{-1}. MS (*m/e*): 274·119543 (M^+, 100%), 259 (M^+-Me, 24%), 256 (M^+-H_2O, 43%), 242 (17%), 241 (M^+-Me-H_2O, 95%), 225 (12%), 223 (11%), 213 (13%), 211 (15%), 169 (10%), 141 (17%), 139 (10%), 128 (15%), 115 (26%). NMR ($CDCl_3$): 1·47 (6 H, *d*), 2·39 (3 H, *s*), 3·86 (1 H, *sept*), 3·89 (3 H, *s*), 6·23 (1 H, *bs*), 6·68 (1 H, *s*), 7·49 (1 H, *s*), 11·09 (1 H, *s*), 14·05 (1 H, *s*). When recrystallized from C_6H_6, (**1c**) was also obtained as yellow crystals. M.p. 174–178·5° (decomp.). UV: $\lambda_{max}^{EtOH}(\epsilon)$ 222 (24300), 259 (10600), 281 (sh), 336 (2310), 389 (2900) nm; $\lambda_{max}^{EtONa}(\epsilon)$ 230 (sh), 265 (9700), 290 (sh), 337 (2600), 419 (3000) nm. IR: ν_{max}^{KBr} 3260, 1615 cm^{-1}. MS (*m/e*): 244·111090 (M^+, 100%), 243 (M^+-H, 23%), 229 (M^+-Me, 12%), 226 (M^+-H_2O, 65%), 211 (M^+-H_2O-Me, 24%), 198 (18%), 185 (15%), 183 (23%), 155 (14%), 153 (10%), 152 (12%), 131 (15%), 128 (17%), 127 (12%), 119 (12%), and 115 (18%). NMR ($CDCl_3$): 1·36 (6 H, *d*), 2·42 (3 H, *s*), 3·58 (1 H, *sept*), 6·72 (1 H, *s*), 6·97 (1 H, *s*), 7·39 (1 H, *s*), 11·10 (1 H, *s*).

Survey of Malvaceae for (**1a**), (**1b**) *and* (**1c**). Equal amounts of the crude terpenoid fraction from each species or cv. (5 μl, equivalent of 0·5 g fr. wt.) were spotted side by side and chromatographed in system 1. Plates were immediately sprayed with phloroglucinol reagent. When present, **1a** (R_f 0·31), **1b** (R_f 0·47), and **1c** (R_f 0·39) developed magenta, deep rose, and rose colors, respectively. The concentration of each compound was arbitrarily rated from + = clearly developed color to + + + = most intense color.

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