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# NAPHTHOFURAN PRECURSORS OF SESQUITERPENOID ALDEHYDES IN DISEASED *GOSSYPIUM**†

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**Key Word Index**—*Gossympium barbadense*; Malvaceae; cotton; phytoalexins; 3,4-dihydroxy-5-isopropyl-7-methyl-2*H*-naphtho[1,8-*bc*]furan; 3-hydroxy-5-isopropyl-4-methoxy-7-methyl-2*H*-naphtho[1,8-*bc*]furan; vergosin; hemigossypol; 6-methoxyhemigossypol; *Verticillium dahliae*.

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**Abstract**—Crude terpenoid fractions from stele tissues of *Gossypium barbadense* infected with *Verticillium dahliae* contained two prominent compounds that were nonaldehydic, but readily autooxidized to the terpenoid aldehydes hemigossypol and 6-methoxyhemigossypol. Spectral analyses, chemical studies, and comparisons with model compounds showed that these compounds were the sesquiterpenoids 3,4-dihydroxy-5-isopropyl-7-methyl-2*H*-naphtho[1,8-*bc*]furan (**1a**) and 3-hydroxy-5-isopropyl-4-methoxy-7-methyl-2*H*-naphtho[1,8-*bc*]furan (**1b**). The trivial names desoxyhemigossypol and desoxy-6-methoxyhemigossypol are proposed for (**1a**) and (**1b**). The sesquiterpenoid, previously isolated and named vergosin, is apparently desoxy-6-methoxyhemigossypol.

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## INTRODUCTION

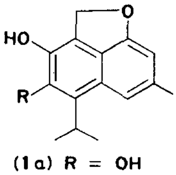
The occurrence of antifungal substances in cotton plants (*Gossypium hirsutum* L. and *G. barbadense* L.) infected with *Verticillium dahliae* Kleb. is well documented [1–5]. These substances have generally been referred to as the gossypol-related phytoalexins. Zaki *et al.* [4,5] extensively analyzed two of these compounds. One was identified as hemigossypol (1,6,7-trihydroxy-5-isopropyl-3-methyl-8-naphthaldehyde), and the second was purported to be 1-hydroxy-5-isopropyl-7-methoxy-3-methyl-8-naphthaldehyde (vergosin). We confirmed the structure of hemigossypol and isolated the related aldehydes 6-methoxy hemigossypol (1,7-dihydroxy-5-isopropyl-6-methoxy-3-methyl-8-naphthaldehyde) and 6-deoxy hemigossypol (1,7-dihydroxy-5-isopropyl-3-methyl-8-naphthaldehyde) but failed to find an aldehyde corresponding with vergosin [3].

In our previous studies [3] we isolated two sesquiterpenoids that did not contain aldehyde groups. Our present data indicate that these compounds are the 2*H*-naphtho[1,8-*bc*]furans (**1a** and **1b**). Compound (**1b**) is identical to the compound previously reported, but incorrectly identified as vergosin [4,5].

## RESULTS

### *Identification of desoxy-6-methoxyhemigossypol*

Desoxy-6-methoxyhemigossypol (**1b**) was identified as 3-hydroxy-5-isopropyl-4-methoxy-7-methyl-2*H*-naphtho[1,8-*bc*]furan. The high-resolution MS of (**1b**) showed the molecular formula $C_{16}H_{18}O_3$ (found: *m/e* 258·126003, 100%; required: 258·125580). Major fragment ions were also noted at *m/e* 243 (80%, $M^+$–Me) and 228 (26%, $M^+$–2Me).



(**1a**) R = OH
(**1b**) R = OMe

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* A preliminary report of this work was presented at the 29th Southwest Regional Meeting of the American Chemical Society, San Antonio, Texas, 5–7 December, 1973.

† For the previous paper in this series see Ref. [3].

Three fragment ions observed in the MS by Zaki *et al.* [4] were important in their analyses. In our analysis of (**1b**), the elemental composition of these three ions was determined by high resolution mass measurements. The ion at *m/e* 229 ($M^+$–29, 8%) had the formula $C_{14}H_{13}O_3$ (found: 229·085792; required: 229·086455) formed by the loss of $C_2H_5$, not CHO as previously suggested [4]. The ions at *m/e* 213 (4%) and 212 (4%) had the formulae $C_{13}H_9O_3$ (found: 213·055830; required: 213·055155) and $C_{14}H_{12}O_2$ (found: 212·083142; required: 212·083720), respectively. Thus, *m/e* 213 was formed by the loss of three Me groups, and *m/e* 212 resulted from the loss of a Me group and a OMe group. These ions are unrelated to any type of tautomerization between a *peri* hydroxyl and aldehyde as previously suggested [4]. Our IR of (**1b**) showed hydroxyl absorption at 3425 $cm^{-1}$, but there was no carbonyl peak at 1680 $cm^{-1}$.

The PMR of (**1b**) indicated the presence of an isopropyl group in which the chemical shift of both the methine proton ($\delta$ 3·70, 1H, sept) and the methyl protons ($\delta$ 1·50, 6H, d) were shifted down field, due to deshielding by the aromatic ring current. An aromatic Me group and a OMe group were indicated by three proton singlets at $\delta$ 2·40 and 3·73; two aromatic protons appeared as singlets at $\delta$ 6·35 and 7·10. A phenolic proton appeared as a broad peak center at $\delta$ 5·89, which disappeared on $D_2O$ exchange and a two-proton singlet was observed at $\delta$ 5·61.

Assignment of the two-proton singlet at $\delta$ 5·61 to the aromatic methylene group was based on comparison with two model compounds. This singlet appears at $\delta$ 5·70 in 2*H*-naphtho[1,8-*bc*]furan [6], and at $\delta$ 5·79 in desoxygossypol tetramethyl ether [7]. 2*H*-Naphtho[1,8-*bc*]furan and the homocyclic analogue phenalene readily form stable delocalized radicals [6,8], which account for the rapid oxidation of (**1b**) in air, especially in the presence of UV light. The position of methoxylation in (**1b**) was confirmed by its photooxidation to give predominantly 6-methoxyhemigossypol.

### *Identification of desoxyhemigossypol*

Desoxyhemigossypol (**1a**) was identified as 3,4-dihydroxy-5-isopropyl-7-methyl-2*H*-naphtho[1,8-*bc*]furan. The molecular formula $C_{15}H_{16}O_3$ was confirmed by the high resolution MS (found: *m/e* 244·109651, 74%; required: 244·109930). The PMR spectrum showed signals for aromatic isopropyl ($\delta$ 1·47, 6H, *d* and $\delta$ 3·59, 1H, sept) and Me ($\delta$ 2·40, 3H, *s*) groups. Resonance signals for two phenolic protons appeared as a broad peak centered at $\delta$ 5·55. Two aromatic protons absorbed at $\delta$ 6·29 (*s*) and 6·97 (*s*). The methylene protons appeared as a sharp two-proton singlet at $\delta$ 5·57, superimposed on the broad phenol proton resonance.

The MS of (**1a**), like that of (**1b**), showed loss of Me (*m/e* 229, 100%), $C_2H_5$ (*m/e* 215, 12%), 2Me (*m/e* 214, 16%), and 3Me (*m/e* 199, 9%). The structure of (**1a**) was confirmed by its photooxidation to hemigossypol.

## DISCUSSION

Desoxy-6-methoxyhemigossypol (**1b**) isolated in our studies appears to be identical to the compound previously isolated and identified as vergosin by Zaki *et al.* [4]. Both their data and ours show the same empirical formulae and similar UV, PMR, MS and chromatographic behavior. Compound (**1b**), like their compound, slowly turned pink when treated in light with acidic phloroglucinol, a reagent for aldehydes. This reaction was apparently preceded by autooxidation of (**1b**) to the aldehyde 6-methoxyhemigossypol.

Zaki *et al.* [4] concluded that their compound contained an aldehyde group because of three observations: (a) an IR absorption at 1680 $cm^{-1}$; (b) fragments in the MS at *m/e* 229, 213 and 212, that they attributed to loss of CHO, $CHO_2$ and $CH_2O_2$, respectively, from the $M^+$; and (c) an NMR peak at $\delta$ 7·3 that they concluded was due to an aldehyde proton.

We found no evidence for an aldehyde group in (**1b**). No carbonyl absorption occurred at 1680 $cm^{-1}$ in freshly purified (**1b**), although some carbonyl absorption occurred in the autooxidized compound because of formation of the corresponding aldehyde. High resolution MS of the ionic fragments *m/e* 229, 213 and 212 from (**1b**) showed that these were due to the loss of $-C_2H_5$, –3Me and –Me–OMe, respectively, and therefore were not derived from an aldehyde structure. In the six terpenoid aldehydes that we had previously identified from cotton [9], the C-4 protons occurred at $\delta$ 7·39–7·70, and the aldehyde protons occurred at $\delta$ 10·96–11·17. Aldehyde protons generally appear downfield beginning at $\delta$ 8·9 [10]. Thus, the proton that others observed at $\delta$ 7·3 is the $C_4$-aromatic proton.

The correct structure of both (**1b**) and the compound isolated by Zaki *et al.* [4] appears to be 3-hydroxy-5-isopropyl-4-methoxy-7-methyl-2*H*-naphtho[1,8-*bc*]furan. The appropriate trivial name for this compound is desoxy-6-methoxyhemigossypol, because of its relationship to hemigossypol [3], and to desoxygossypol tetramethyl ether that was synthesized by Adams and Dial [11]. The structure of (**1b**) was confirmed by its photooxidation to 6-methoxyhemigossypol. The structure of (**1b**) is further supported by its co-occurrence with desoxyhemigossypol (**1a**) that readily photooxidizes to hemigossypol.

Antimicrobial activity was reported for vergosin [4,5] but the bioassay consisted of direct layering of nutrient agar on TLC plates followed by seeding with fungal spores and incubation for several days. Under these conditions, desoxy-6-methoxyhemigossypol is readily oxidized to 6-methoxyhemigossypol, a known antimicrobial substance [3]. Thus, more careful bioassays are required to evaluate the biological activities of the 2*H*-naphtho[1,8-*bc*]furans.

Compounds (**1a**) and (**1b**) accumulate rapidly in cotton tissues 12–96 hr after infection with several fungi (unpublished data). The ready conversion of these compounds to the phytoalexins, hemigossypol and 6-methoxyhemigossypol, by UV-catalyzed oxidation, suggests that they are the natural biological precursors of these phytoalexins. Veech and Bell (unpublished) have recently shown that a peroxidase-type of enzyme converts hemigossypol to gossypol in cotton. Since such conversion normally involves free radical formation, the same or a similar enzyme might convert (**1a**) and (**1b**) to their corresponding aldehydes.

## EXPERIMENTAL

Crude terpenoid fractions were prepared from stele tissues of *Gossypium barbadense* L. "Sea Brook Sea Island 12B2" 48 hr after inoculation with *Verticillium dahliae* (strain 117). Extraction procedures have been described [3,12] and all were conducted in subdued light or in darkness. Chromatography was on polyamide (System 1) and Si gel (Syst. 3–6) as in [3], or Si gel with $C_6H_6$, (Syst. 8), and $Et_2O$–naphtha (1:1), (Syst. 9). TLC plates were irradiated with UV (254 nm) for 1 hr to convert the desoxy terpenoids to their aldehydes which were then detected with 2% phloroglucinol in EtOH–conc HCl (1:1).

*Spectra.* UV spectra were determined in 95% EtOH or 95% EtOH containing 0·03 M NaOH (EtONa) and are reported as $\lambda_{max}$ ($\epsilon$). IR spectra were determined in KBr. The 100 MHz NMR spectra were determined in $CDCl_3$ at 23° and are reported in $\delta$ units. Hydroxyl protons were detected by exchange with $D_2O$. MS were obtained by direct probe at 25° with a source temp. of 200°.

*Isolation of desoxyhemigossypol* (**1a**) *and desoxy-6-methoxyhemigossypol* (**1b**). Compound (**1a**) was purified from crude terpenoid extracts by TLC with sequential use of systems 4 ($R_f$ 0·22), 3 ($R_f$ 0·39), 1 ($R_f$ 0·33), and 6 ($R_f$ 0·47) given in Ref. [3]. Compound (**1b**) was purified by use of systems 8 ($R_f$ 0·31), 3 ($R_f$ 0·77), and 4 ($R_f$ 0·41) in sequence as described in Ref. [3]. Compound (**1a**) crystallized from $C_6H_6$–hexane as pale yellow crystals. Mp 134–138°. UV $\lambda_{max}^{EtOH}$ ($\epsilon$) 224 (31000), 246 (38800), 251 (sh), 294 (7900), 303 (7800), 331 (sh) nm; $\lambda_{max}^{EtONa}$ ($\epsilon$) 254 (44700), 260 (sh), 299 (10800), 310 (10900), 325 (8800), 338 (8400) nm. IR: $\nu_{max}^{KBr}$ 3400–3300, 1655, 1633, 1505, 1291 $cm^{-1}$. Compound (**1b**) also crystallized from $C_6H_6$–hexane as pale yellow crystals. Mp 121·5–123°. UV $\lambda_{max}^{EtOH}$ ($\epsilon$) 224 (39000), 247 (62500), 284 (sh), 295 (10000), 306 (10700), 325 (sh), 339 (5300) nm; $\lambda_{max}^{EtONa}$ ($\epsilon$) 254 (65700), 290 (sh), 299 (15000), 311 (16500), 341 (10000) nm. IR: $\nu_{max}^{KBr}$ 3430–3340, 1655, 1642, 1622, 1508 $cm^{-1}$.

*Photooxidation.* Pure (**1a**) and (**1b**) were developed in TLC system 5 [3], and plates were immediately exposed to UV radiation (both 254 and 366 nm) in air for 1 hr. Oxidation products were eluted from the bands with $Et_2O$ and purified by TLC. The major oxidation product of (**1a**) was hemigossypol. It was purified by sequential TLC in systems 1 ($R_f$ 0·31), 3 ($R_f$ 0·64), and 4 ($R_f$ 0·41). The major oxidation product of (**1b**) was 6-methoxygossypol. It was purified in systems 5 ($R_f$ 0·43) and 9 ($R_f$ 0·59). The aldehydes formed by photooxidation were identified by comparing their chromatographic behavior and MS with those of authentic samples.

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