Pergamon

0031-9422(94)00958-9

Phytochemistry, Vol. 39, No. 3, pp. 531–536, 1995

0031–9422/95 $9.50 + 0.00

CADINANE-TYPE SESQUITERPENES INDUCED IN *GOSSYPIUM* COTYLEDONS BY BACTERIAL INOCULATION

GUADALUPE DAVILA-HUERTA, HIROKI HAMADA,* GORDON D. DAVIS, ROBERT D. STIPANOVIC,† CHRISTOPHER M. ADAMS‡ and MARGARET ESSENBERG

Department of Biochemistry and Molecular Biology and Department of Chemistry (‡), Oklahoma State University, Stillwater, OK 74078-0454, U.S.A.; †Southern Crops Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Route 5, Box 805, College Station, TX 77845, U.S.A.

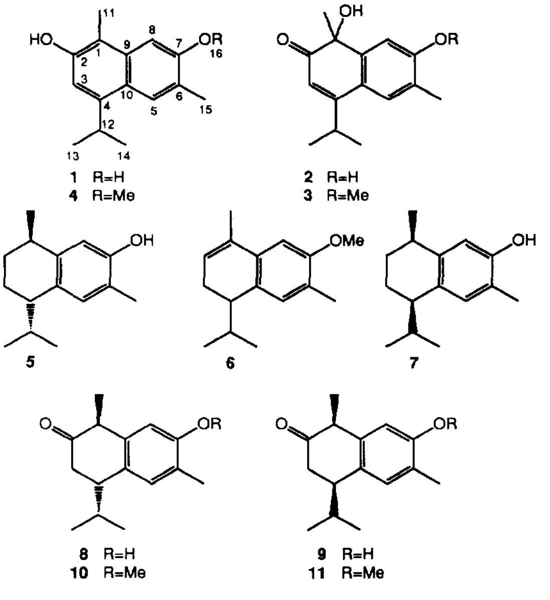
(*Received in revised form* 4 *October* 1994)

Key Word Index—*Gossypium hirsutum*; Malvaceae; cotton; *Xanthomonas campestris* pv. *malvacearum*; bacterial blight of cotton; sesquiterpenes; 7-hydroxycalamenene; 7-hydroxycalamenen-2-one.

Abstract—Two new sesquiterpenes, the *cis*- and *trans*-diastereomers of 7-hydroxycalamenen-2-one, as well as *trans*-7-hydroxycalamenene, have been identified in extracts from cotyledons of bacterial blight-resistant *Gossypium hirsutum* harvested during the period of sesquiterpenoid phytoalexin biosynthesis following inoculation with the bacterial pathogen *Xanthomonas campestris* pv. *malvacearum*. The *cis*- and *trans*-diastereomers were distinguished by NOE correlations predicted from molecular modelling calculations.

INTRODUCTION

When leaves or cotyledons of bacterial blight-resistant cotton are inoculated with strains of *Xanthomonas campestris* to which they are resistant, the sesquiterpenoid phytoalexins 2,7-dihydroxycadalene (**1**), lacinilene C (**2**), and lacinilene C 7-methyl ether (**3**), as well as the structurally related sesquiterpenoid 2-hydroxy-7-methoxycadalene (**4**) are biosynthesized [1, 2]. The phytoalexins accumulate in the hypersensitively necrotic cells at infection centres [3]. The high local concentrations attained by the phytoalexins strongly suggest that they play a significant role in resistance of cotton to bacterial blight [4]. As the first stage in a study of how this resistance response is regulated, an investigation of the biosynthetic pathway to the phytoalexins was undertaken. We report here the identification of three additional sesquiterpenes identified in extracts made from cotton cotyledons harvested during the period of phytoalexin biosynthesis, whose structures suggest that they may be biosynthetic precursors to the phytoalexins.



Structures of bacterial infection-induced cadinane-type sesquiterpenoids (**1**–**5**, **8** and **9**) from *Gossypium* and some synthetic analogues (**6**, **7**, **10**, **11**). The carbon numbers shown for **1** apply to all 11 compounds. Natural products **2** and **3** have been isolated with varying enantiomeric excess of *R* or *S* configuration [1]. Absolute configurations of **5**, **8** and **9** are not known. Synthetic **6**, **7**, **10** and **11** were racemic mixtures.

RESULTS AND DISCUSSION

A preliminary survey was made for infection-induced compounds which had characteristics expected of cadinane-type sesquiterpenes. Extracts were made of highly resistant cotyledons harvested during the period of phytoalexin accumulation (40–60 hr after inoculation) and of mock-inoculated control cotyledons. The extracts were analysed by GC–MS. The chromatographic patterns from inoculated cotyledons exhibited greater quantities and diversity of sesquiterpenes than from control cotyle-

---

*Present address: Department of Applied Science, Okayama University of Science, 1-1 Ridai-cho Okayama 700, Japan.

Table 1. ^{1}H NMR spectral data of compounds **5**, **7**–**11** (400 MHz, $CDCl_3$, TMS as internal reference)

H	**5** *trans*	**7** *cis*	**8** *trans*	**9** *cis*	**10** *trans*	**11** *cis*
1	2.71 *m*	2.80 *m*	3.42 *q* (7.3)	3.41 *q* (7.3)	3.46 *q* (7.3)	3.45 *q* (7.3)
2	1.93 *m* 1.81 *m*	1.79 *m* 1.62 *m*				
3	1.93 *m*	1.71 *m*	2.60 *dd* (16*, 4*)	2.67 *m* (16*, 5*)	2.64 *dd* (6.2)	2.69 *m*
	1.81 *m*	1.62 *m*	2.72 *dd* (16*, 2*)	2.69 *m* (16*, 2*)	2.71 *dd* (3.3)	2.72 *m*
4	2.63 *m*	2.52 *dd*	2.74 *m* (12*, 4*, 2*)	2.71 *m* (12*, 5*, 2*)	2.78 *m* (3.2)	2.71 *m*
5	6.95 *s*	6.95 *s*	6.93 *s*	6.93 *s*	6.95 *s*	6.94 *s*
8	6.65 *s*	6.57 *s*	6.67 *s*	6.65 *s*	6.67 *s*	6.64 *s*
11	1.24 *d* (6.8)	1.23 *d* (7.1)	1.46 *d* (7.3)	1.45 *d* (7.3)	1.50 *d* (7.3)	1.50 *d* (7.3)
12	2.19 *m* (6.8)	2.22 *m* (6.8)	1.79 *m* (6.7)	1.64 *m* (6.6)	1.81 *m* (6.8)	1.61 *m*
13	0.70^a *d* (6.8)	0.76^a *d* (6.8)	0.88^a *d* (6.7)	0.92^a *d* (6.6)	0.89 *d* (6.8)	0.91^a *d* (6.7)
14	0.99^a *d* (6.8)	1.02^a *d* (6.8)	0.91^a *d* (6.7)	0.93^a *d* (6.6)	0.89 *d* (6.8)	0.93^a *d* (6.7)
15	2.21 *s*	2.21 *s*	2.25 *s*	2.25 *s*	2.21 *s*	2.22 *s*
16					3.84 *s*	3.83 *s*
OH-7	4.48 *s*	4.44 *br s*				

Spectra of **5** and **7** were recorded at 12°, and of **8**–**11** at 8°.

*These coupling constants were predicted by the MOPAC molecular modeling program, and when used with the NMR spin simulation program, generated patterns similar to the experimental split signals.

^{a}Values within the same column may be interchanged.

Coupling constants (in Hz) in parentheses.

dons. Compounds whose mass spectra exhibited the expected losses of 15 and 43 atomic mass units (methyl and isopropyl groups, respectively) were observed with apparent molecular ions of *m/z* 204, 216, 218, 232, 234, 242 and 258. Identification of the M_r 204, 218 and 232 compounds was undertaken, because their probable molecular formulae of $C_{15}H_{24}$, $C_{15}H_{22}O$ and $C_{15}H_{20}O_2$ made them reasonable candidates for biosynthetic precursors to the phytoalexins. Identification of M_r 204 compound will be reported separately in this journal.

The ^{1}H NMR (Table 1) and mass spectra of the M_r 218 compound closely matched published data for 7-hydroxycalamenene with *trans* orientation of the methyl and isopropyl substituents on the cyclohexene ring (**5**) [5, 6]. Since the amount of the compound obtained from inoculated cotton tissues was small, a reference sample of 7-hydroxycalamenene was prepared from 7-methoxy-α-calacorene (**6**) which had been synthesized by McCormick *et al.* [7]. The calacorene was catalytically reduced to 7-methoxycalamenene, followed by demethylation with BBr_3. The resulting synthetic 7-hydroxycalamenene exhibited an exact mass corresponding to the expected $C_{15}H_{22}O$ composition; however, its ^{1}H NMR spectrum (Table 1) did not match that of our M_r 218 compound, but rather matched the published data for *cis*-7-hydroxycalamenene (**7**) [5, 6, 8]. Uemura *et al.* observed a similar stereoselectivity upon catalytic reduction of 7-methoxy-α-calacorenal to *cis*-7-methoxycalamenene without contamination by the *trans* isomer [5]. The stereochemical assignment of the *cis* and *trans* diastereomers of 7-hydroxycalamenene is based upon the X-ray crystallographic identification of the *p*-bromobenzoate of the *cis* isomer isolated from *Eremophila drummondii* by Croft *et al.* [8], and upon the stereochemically specific synthesis of both diastereomers by Uemura *et al.* [5]. Bohlmann and colleagues have both synthesized *cis*- and *trans*-7-hydroxycalamenene [6] and isolated them from *Osteospermum* species [9]. Their detailed ^{1}H NMR analysis agrees with those of Uemura [5] and Croft [8]. However, the 7-hydroxycalamenene isolated from *Heritiera ornithocephala*, which was putatively assigned as the *cis*-isomer on the basis of NOE data [10], differs in its ^{1}H NMR spectrum from the *cis*-isomer; rather it agrees more closely with that of the *trans*-isomer. Based on the general agreement with the ^{1}H NMR data of the *trans*-isomer in Uemura's and Bohlmann's work, we have assigned this geometry (**5**) to the 7-hydroxycalamenene isolated from cotton.

Further NMR studies were performed on synthetic **7** (Tables 2 and 3). ^{1}H–^{1}H COSY connectivities (Table 2)

Table 2. ^{1}H–^{1}H COSY observed connectivities.

H	7 *cis*	8 *trans*	9 *cis*	10 *trans*	11 *cis*
1	H-2				
	H-11	H-11	H-11	H-11	H-11
2	H-1				
	H-3				
3	H-2				
	H-4	H-4	H-4	H-4	H-4
4	H-3	H-3	H-3	H-3	H-3
	H-12	H-12	H-12	H-12	H-12
11	H-1	H-1	H-1	H-1	H-1
12	H-4	H-4	H-4	H-4	H-4
	H-13	H-13	H-13	H-13	H-13
	H-14	H-14	H-14	H-14	H-14
13	H-12	H-12	H-12	H-12	H-12
	H-14	H-14	H-14	H-14	H-14
14	H-12	H-12	H-12	H-12	H-12
	H-13	H-13	H-13	H-13	H-13

Spectra of **5** and **7** were recorded at 12°, and of **8**–**11** at 8°.

Table 3. ^{13}C NMR spectral data (400 MHz, $CDCl_3$, TMS as internal reference)

C	7 *cis*	10 *trans*	11 *cis*
1	32.6	46.0	46.1
2	28.6	162.5*	162.6*
3	19.7	40.2*	42.5
4	42.6	45.8	46.0
5	130.5	131.1*	131.0
6	120.7	124.3*	124.3*
7	151.3	156.5*	156.5*
8	114.3	108.2*	109.3
9	141.9^{a}	141.7^{a}*	137.4^{a}*
10	132.0^{a}	130.4^{a}*	130.5^{a}*
11	23.2	17.8	18.2
12	31.1	32.2	33.0
13	17.5^{b}	21.5^{b}	19.5^{b}
14	21.4^{b}	19.5^{b}	21.6^{b}
15	15.6	16.0	16.0
16		55.3	55.3

Spectra of **5** and **7** were recorded at 12°, and of **8**–**11** at 8°.

[a,b] Values within the same column may be interchanged.

*These carbon number assignments were made by comparison with assignments for related compounds [13].

supported the proton assignments of Table 1. HETCOR ^{1}H–^{13}C correlations led to the ^{13}C assignments of Table 3, and a DEPT experiment revealed all predicted multiplicities. ^{13}C chemical shifts were in good agreement with those reported by Bunko *et al.* [11] for *cis*-7-hydroxycalamenene synthesized from dihydroxyserrulatic acid of known absolute configuration (no differences greater than 0.5 ppm). However, our HETCOR correlations indicate that their assignments (which were based on 1D ^{13}C NMR) of the signals for C-1 and C-12 should be interchanged, as should those for C-5 and the more shielded of the quaternary C-9 and C-10. The ^{13}C chemical shifts obtained do not agree with those reported for the 7-hydroxycalamenene from *H. ornithocephala* [10] (differences of $\geqslant$ 1 ppm for C-2, C-3, C-8 and C-11). These differences suggest that further studies are warranted on the compound isolated from *H. ornithocephala.*

The two M_r 232 compounds (**8** and **9**) were resolved from one another by HPLC on a porous graphitized carbon column [12]. The EI-mass spectra of **8** and **9** were indistinguishable. Direct probe high-resolution EI-mass spectrum of an approximately 3:2 mixture of **8** and **9** established for both compounds $[M]^+$ of $C_{15}H_{20}O_2$ (calc: 232.1463; found: 232.1443) with major fragment ions resulting from loss of isopropyl (calc for $C_{12}H_{13}O_2$: 189.0916; found: 189.0972) and subsequent loss of carbonyl (calc for $C_{11}H_{13}O$: 161.0966; found: 161.0967).

The ^{1}H NMR spectrum provided considerable structural information (Table 1). Compound **8** presented a pair of 3H doublets (J = 6.7 Hz) at δ0.88 and 0.91, which are coupled (Table 2) to a 1H multiplet at δ1.79, indicating the presence of an isopropyl moiety. Methyl resonances occurred as a 3H singlet of δ2.25 for the aromatic Me and as a 3H doublet of δ1.46 for the non-aromatic Me, the latter coupled to a 1H quartet (Table 2). There were 1H singlets at δ6.67 and 6.93 indicating two aromatic hydrogens in a *para* relationship. These chemical shifts and splitting patterns of **8** exhibit the expected similarities and differences to those reported for a 3-keto analogue [13] and a 2-hydroxy analog [14]. The ^{1}H NMR spectrum for **9** presented similar splitting patterns and chemical shifts (Table 1).

To confirm the structural properties of **8** and **9**, other spectral analyses were performed on **8** (more abundant and more stable in $CDCl_3$ soln than **9**). The occurrence of IR absorption bands at 3385 and 1700 cm^{-1} confirmed the presence of hydroxyl and carbonyl functions. Structural identification of **8** and **9** was supported by analysis of the corresponding synthetic 7-methoxycalamenen-2-ones [7]. As expected, the synthetic material was resolved into two compounds (**10** and **11**) by HPLC on the porous graphitized carbon column. Their mass spectral fragmentation patterns strongly suggested that the synthetic compounds are the methyl ethers of the M_r 232 natural product sesquiterpenes of this study. This conclusion that the natural products are the two diastereomers of 7-hydroxycalamenen-2-one (**8** and **9**) was supported by ^{1}H NMR and COSY experiments (Tables 1 and 2). We have found no previous report of these compounds in the literature.

The ^{1}H NMR, COSY and HETCOR spectra did not, however, indicate which of the ketones had *trans* orientation of the aliphatic substituents on the cyclohexene ring (**8** and **10**) and which had *cis* orientation (**9** and **11**). Semi-empirical energy minimization calculations for **8**–**11** were carried out using the MOPAC 6.0 molecular orbital program [15]. The predicted most stable conformations of **10** and **11** are shown in Fig. 1; those for **8** and **9** were very similar to **10** and **11**, respectively. The nonaromatic ring in **10** appeared with what has been termed the half-chair conformation of cyclohexene [16], whereas in **11** the predicted conformation is a slightly twisted boat. The methyl and isopropyl groups were pseudoaxial in both predicted conformations, as has been observed for alkyl substituents of 9,10-dihydroanthracenes [17]. The predicted coaxial orientation of these groups in the *cis* isomers **9** and **11** achieves a 1.7 Å proximity between H-12 (the methine proton of the isopropyl group) and the nearest proton of Me-11 (Fig. 1). In the *trans* isomers **8** and **10**, the calculated interatomic distance between the axial H-3 and Me-11 is 2.5 Å, and between H-1 and H-12 it is 3.5 Å. Evidence for these distinguishing proximities through space was sought by nuclear Overhauser effect (NOE) difference spectrometry. When Me-11 was irradiated, H-8 exhibited strong NOE enhancement in both **10** and **11**. Unfortunately, irradiation of Me-11 ($\delta = 1.50$ ppm) of **11** had a direct effect on the neighboring signal for H-12 ($\delta = 1.61$ ppm), obscuring any NOE enhancement. Chemical shifts for Me-11 and H-12 of **10** (1.50 and 1.81 ppm, respectively) were sufficiently different that partial saturation did not occur and NOE could have been detected, had it occurred. However, irradiation of Me-11 of **10** resulted in no detectable enhancement at H-12, consistent with the *trans* configuration. Irradiation of Me-11 resulted in no enhancement of H-3 resonance in either compound. However, this is not surprising, since H-3 is a methylene proton, and its relaxation is expected to be dominated by its geminal partner, so that more distant protons will contribute little to its relaxation and will induce only small NOEs ([18] and pp. 77–81 in [19]). Irradiation of H-12 of **10** and **11** resulted in the expected medium-intensity enhancements of H-5. Irradiation of H-12 yielded a medium-intensity enhancement of H-1 in **10**, but not in **11**, consistent with the predicted 3.5 Å proximity of the *trans* compound **10**. On the basis of the NOE results, we propose the assignments of *trans* (**10**) and *cis* (**11**) configurations to the two synthetic ketones as listed in Table 1.

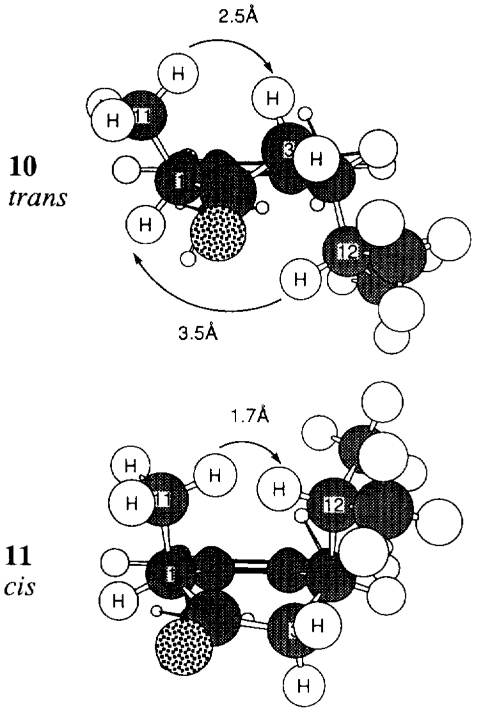


Fig. 1. Conformations of *trans* (**10**) and *cis* (**11**) isomers of 7-methoxycalamenen-2-one predicted by energy minimization calculations using MOPAC with semi-empirical Hamiltonian PM3.

The ^{1}H NMR chemical shifts (Table 1) for H-4, H-8, and H-12 of *trans* isomer **10** were downfield to those for *cis* isomer **11**, whereas those for H-13 and H-14 were relatively upfield. Corresponding differences in ^{1}H chemical shifts of the natural product phenols enable us to distinguish the *trans* **8** and *cis* **9** isomers (Table 1).

The GC–MS analysis of the natural products **8** and **9** revealed that during gas chromatography of either isomer, a mixture of the two was generated. New GC columns caused less isomerization than older ones, suggesting that interaction with residues of previous samples promoted the isomerization. This interconversion, presumably via enolization at positions 1 and 2, suggests that nonenzymatic isomerization may occur *in planta* or during isolation. Thus it is possible that only one of these diastereomers is enzymatically biosynthesized in cotton cotyledons.

This report has presented evidence that the new sesquiterpenes **8** and **9**, as well as **5**, are present in extracts of inoculated, bacterial blight-resistant *Gossypium* cotyledons harvested during production of phytoalexins **1**–**3** and the structurally related sesquiterpenoid **4**. The structures of **5**, **8**, and **9** suggest that they may be biosynthetic precursors to the phytoalexins. Alternatively, they may be precursors or products of side pathways. We are planning isotope incorporation studies to test these alternatives.

EXPERIMENTAL

Plant tissue inoculation and extraction. OK 1.2, a bacterial blight-resistant, glanded line of *Gossypium hirsutum* L., which is a high producer of sesquiterpenoids **1**–**4** [20], was used for all experiments. Plants were grown in a controlled environment chamber [21] and were inoculated 11 or 12 days after planting. The bacterial pathogen was *X. campestris* pv. *malvacearum* strain 3631 [21]. Inocula (*ca* 5×10^6 cfu ml^{-1}) were prepared in sterile, saturated $CaCO_3$ soln and used to infiltrate the intercellular spaces of cotyledons of 11- or 12-day-old plants as previously described [20]. Mock-inoculated control cotyledons were infiltrated with sterile, saturated $CaCO_3$ solution. Cotyledons were harvested 40–60 hr after inoculation or mock-inoculation, quick-frozen in liquid nitrogen, and stored at $-70°$ until extraction. Sesquiterpenes **5**, **8**, and **9** were isolated from bacteria-inoculated cotyledons.

Since the sesquiterpenoid end products **1**–**4** are unstable to light and oxygen [22, 23], samples were isolated in reduced light and stored under argon in the dark at − 70°. Frozen cotyledons were processed by repetition of the following procedure: 100 g were homogenized in 400 ml of chilled HPLC-grade MeOH–H_2O (4:1) in an ice bath with a Sorvall Omni-mixer at maximum speed, 30 sec on, and 30 sec off, repeated 4 times. The homogenate was centrifuged, and the residue was extracted one more time with half the original volume of MeOH–H_2O. The pooled hydro-organic phase was subsequently concd to 140 ml on a rotary evaporator at room temperature. The crude extract was treated with 40 g of preconditioned HPLC-grade C_{18} silica gel. After decantation, the retained sesquiterpenoids were eluted with MeOH–H_2O (17:3), using 1 ml per g fr. wt of tissue. The methanol content was adjusted to 30% by addition of water, followed by $CHCl_3$ back-extraction. The combined extracts were concentrated by rotary evapn and dried with a stream of argon, then stored at − 70°.

Synthesis of **7**, **10**, *and* **11**. 7-Methoxy-α-calacorene (**6**) (200 mg), prepared as previously reported [7], was dissolved in EtOAc (5 ml). Palladium on carbon (10%, 50 mg) was added, and **6** was reduced with H_2 at atmospheric pressure. The catalyst was removed by filtration, and the solvent was evapd. The product was distilled bulb-to-bulb (135°/1.0 mm Hg) providing 81 mg of *cis*-7-methoxycalamenene. Demethylation with BBr_3 [24] provided *cis*-7-hydroxycalamenene (**7**). The 7-methyl ethers **10** and **11** were prepared from the acid chloride of 2-(3′-methoxy-4′-methylphenyl)propionic acid [25] by reaction with 3-methyl-1-butene and aluminum chloride [26].

Isolations. The 7-hydroxycalamenenes **5** and **7** were isolated by chromatography of crude plant extract from infected cotton tissue (containing natural product **5**) or demethylation products (containing synthetic **7**) on a radially compressed silica HPLC cartridge (10 μm, 100 × 8 mm). The elution gradient was with 1% EtOAc in hexane (v/v) for 25 min, followed by an increase of 1% ethyl acetate min^{-1} to a final concentration of 18%, with a flow rate of 1.5 ml min^{-1}. Repeated injections of crude tissue extract, equivalent to 25 g fr. wt tissue, were performed under these conditions. Sesquiterpenoids were detected with a fixed-wavelength UV detector, using an interference filter of 280 nm. Solvent was evapd with argon. Purification was achieved by rechromatography in the same system. Final yields were *ca* 40 μg of natural product **5** from *ca* 100 g of inoculated tissues and 4.3 mg of synthetic **7**.

The natural product ketones **8** and **9** were isolated by reversed phase chromatography of crude plant extract on a semiprep. C_{18} column (5 μm, 250 × 10 mm). Elution was with a linear gradient of MeOH–H_2O increasing from 60 to 80% MeOH (v/v) in 38 min delivered at a flow rate of 3.5 ml min^{-1}, with detection at 280 nm. A fraction with a retention time of 23–24 min (*ca* 1 min after DHC) was collected and recovered by partition with $CDCl_3$. The organic layer was evapd to a few μl in a rotary evaporator at room temp. The sample was transferred to a conical tube (on ice) and dried under a gentle argon stream. Capillary GC-FID analysis of the HPLC-collected fraction indicated the presence of two closely related compounds. This was confirmed by GC–EI–MS (70 eV).

A more selective HPLC column was employed to achieve separation of the closely related compounds. The partially purified fraction was further chromatographed by consecutive injections on a porous graphitized carbon HPLC column (Hypercarb by Alltech, 7 μm, 50 × 4.6 mm) with a linear gradient of MeOH–H_2O increasing from 77 to 100% MeOH (v/v) in 8 min, at a flow rate of 1 ml min^{-1}. The organic phase was evapd to dryness, and purity of the resulting material was monitored by GC-FID. The final yields were 150 μg of **8** and *ca* 90 μg of **9** from 400 g of inoculated tissue. These compounds were stable to storage only when perfectly dry under argon at − 70°. The synthetic ketones **10** and **11** were similarly resolved by chromatography on the porous graphitized carbon column; yields were *ca* 7.8 mg and *ca* 4.4 mg, respectively.

GC analysis. Purity of the isolated compounds was determined by GC-FID with on-column injection onto a WCOT SE 54 capillary column 30 m × 0.25 mm i.d. (0.25 μm film thickness), using He at 33 cm sec^{-1}. Detector and injector temperatures were 300° and 55°, respectively. The temperature gradient was programmed to maintain the initial temperature of 85° for 1 min, then increase at 21.7° min^{-1} to 150°, maintain 150° for 1 min, then increase to 250° at 2° min^{-1}.

Spectral analysis. Electron impact, 70 eV mass spectrometry was performed on a VG ZAB 2-SE by direct probe or by GC-MS with a GC separation similar to that described above, interfaced to a VG TS-250 mass spectrometer. The ^1H NMR, ^1H-^1H COSY, NOE, ^{13}C NMR, ^{13}C DEPT, ^1H–^{13}C HETCOR and spin simulation spectra were acquired on a Varian XL 400A NMR spectrometer. All NMR experiments were referenced to TMS with $CDCl_3$ as solvent. With the exception of the NOE studies, NMR sample solutions were at 8° or 12°. NOE difference experiments were performed on ambient temperature $CDCl_3$ solutions of **10** and **11** which had been degassed by the 'freeze–pump–thaw' method (p. 216 in [19]). NOE measurements were made with spectral width of *ca* 5636 Hz and 32 K data points, resulting in a digital resolution of 0.32 Hz per point. Numbers of transients were 112 (**10**) and 320 (**11**). Protons Me-11 and H-12 were irradiated for 5 sec at power sufficient for 95–100% saturation. NOE difference spectra were recorded for qualitative display of the NOE enhancements. Quantitative NOE enhancement values were calculated from intensities in the irradiated and control spectra (p. 231 in [19]) and were characterized as weak (1–4%), medium (5–9%) or strong (10–20%).

Molecular modelling. All semi-empirical calculations were carried out using the semi-empirical molecular orbital program MOPAC 6.0 developed by Stewart [15], which is parameterized for the semi-empirical Hamiltonian PM3 [27, 28]. Initial Z-matrix data for each compound were obtained after a molecular mechanics (MM2) calculation using PCModel by Serena Software. Energy minimizations in MOPAC followed the eigenvector minimization routine (EF). The final Z-matrix output was then read by PCModel on a Macintosh computer. Vicinal coupling constants (H-C-C-H) were calculated using the internal programs of Haasnoot [29] and Garbisch [30] in

PCModel. Dihedral angles and interatomic distances were determined using PCModel and Chem3D. MOPAC-predicted coupling constants for **8** and **9** (Table 1) were used with the Varian NMR spin simulation program to generate patterns similar to the experimental ones and facilitated estimation of the coupling constants of complex multiplet signals.

trans-7-*Hydroxycalamenene* (**5**). MS: *m/z* (rel. int.): 218 $[M]^+$ (10), 175 $[M - \text{iso-Pr}]^+$ (100), 160 $[M - \text{iso-Pr}{-}\text{Me}]^+$ (7), 145 $[M - \text{iso-Pr}{-}2\text{Me}]^+$ (7).

cis-7-*Hydroxycalamenene* (**7**). MS: *m/z* (rel. int.): 218.1666 (calc. for $C_{15}H_{22}O$: 218.1671) $[M]^+$ (8), 175.1136 (calc. for $C_{12}H_{15}O$: 175.1123) $[M - \text{iso-Pr}]^+$ (100), 160 $[M - \text{iso-Pr} - \text{Me}]^+$ (7), 145 $[M - \text{iso-Pr} - 2\text{Me}]^+$ (7).

trans-7-*Hydroxycalamenen-2-one* (**8**). CAS registry no. 155662-81-0. MS: *m/z* (rel. int.): 232 $[M]^+$ (19), 189 $[M - \text{iso-Pr}]^+$ (57), 161 $[M - \text{iso-Pr} - \text{CO}]^+$ (100). UV: $\lambda_{\max}^{\text{EtOH}}$ nm (log ε): 207.4, (4.325); 284.6 (3.511). CD: $[\theta]_{293.2}$ + 2260 (EtOH; *c* 0.0073) IR: $\nu_{\max}^{\text{CHCl}_3}$ cm^{-1}: 3385, 2959, 2853, 1700, 1618.

cis-7-*Hydroxycalamenen-2-one* (**9**). CAS registry no. 155662-80-9. MS: *m/z* (rel. int.): 232 $[M]^+$ (20), 189 $[M - \text{iso-Pr}]^+$ (57), 161 $[M - \text{iso-Pr} - \text{CO}]^+$ (100).

trans-7-*Methoxycalamenen-2-one* (**10**). MS: *m/z* (rel. int.): 246 $[M]^+$ (19), 203 $[M - \text{iso-Pr}]^+$ (71), 175 $[M - \text{iso-Pr} - \text{CO}]^+$ (100).

cis-7-*Methoxycalamenen-2-one* (**11**). MS: *m/z* (rel. int.): 246 $[M]^+$ (18), 203 $[M - \text{iso-Pr}]^+$ (72), 175 $[M - \text{iso-Pr} - \text{CO}]^+$ (100).

Acknowledgements—We thank Roushan A. Samad for technical assistance with extraction and purification of the natural products and for culture and inoculation of the cotton plants, S. O. Sigle for the NMR analyses, T. E. Vickstrom for the mass spectrometric analyses, J. P. Devlin for the I.R. analysis, A. R. Engles for the CD analysis, H. J. Williams for helpful comments on the NOE spectra, and S. P. White for discussion of the manuscript. The work was supported by the National Science Foundation (Grant Nos. DMB-8616650 and EHR-9108771), by the U.S. Department of Agriculture (Agreement No. 91-37303-6652) and by the Oklahoma Agricultural Experiment Station. We acknowledge support of the National Science Foundation for the Varian XL-400A NMR spectrometer (Grant No. CHE-8718150) and the VG TS-250 and ZAB MS systems (Grant No. BBS-8704089) and further support for the same instruments from the Oklahoma Center for the Advancement of Science and Technology (OCAST-MOST), Grant Nos. FGM-3478 and RE-B1-003, respectively).

REFERENCES

1. Essenberg, M., Grover, P. B. Jr. and Cover, E. C. (1990) *Phytochemistry* **29**, 3107.
2. Davis, G. D., Eisenbraun, E. J. and Essenberg, M. (1991) *Phytochemistry* **30**, 197.
3. Essenberg, M., Pierce, M. L., Hamilton, B., Cover, E. C., Scholes, V. E. and Richardson, P. E. (1992) *Physiol. Mol. Plant Pathol.* **41**, 85.
4. Essenberg, M., Pierce, M. L., Cover, E. C., Hamilton, B., Richardson, P. E. and Scholes, V. E. (1992) *Physiol. Mol. Plant Pathol.* **41**, 101.
5. Uemura, M., Isobe, K., Take, K. and Hayashi, Y. (1983) *J. Org. Chem.* **48**, 3855.
6. Krause, W. and Bohlmann, F. (1987) *Tetrahedron Letters* **28**, 2575.
7. McCormick, J. P., Shinmyozu, T., Pachlatko, J. P., Schafer, T.R., Gardner, J. W. and Stipanovic, R. D. (1984) *J. Org. Chem.* **49**, 34.
8. Croft, K. D., Ghisalberti, E. L., Hocart, C. H., Jefferies, P. R., Raston, C. L. and White, A. H. (1978) *J. Chem. Soc., Perkin I* 1267.
9. Bohlmann, F., Wallmeyer, M., Jakupovic, J. and Ziesche, J. (1983) *Phytochemistry* **22**, 1645.
10. Cambie, R. C., Lal, A. R. and Ahmad, F. (1990) *Phytochemistry* **29**, 2329.
11. Bunko, J. D., Ghisalberti, E. L. and Jefferies, P. R. (1981) *Aust. J. Chem.* **34**, 2237.
12. Bassler, B. J. and Hartwick, R. A. (1989) *J. Chromatogr. Sci.* **27**, 162.
13. Bowden, B. F., Coll, J. C., Engelhardt, L. M., Tapiolas, D. M. and White, A. H. (1986) *Aust. J. Chem.* **39**, 103.
14. Bohlmann, F. and Zdero, C. (1979) *Phytochemistry* **18**, 1185.
15. Stewart, J. J. P. (1991) *QCPE Catalog* **21**, 455.
16. Anet, F. A. L. (1989) in *The Conformational Analysis of Cyclohexenes, Cyclohexadienes, and Related Hydroaromatic Compounds* (Rabideau, P. W., ed.), p. 1. VCH, New York.
17. Rabideau, P. W. (1989) in *The Conformational Analysis of Cyclohexenes, Cyclohexadienes, and Related Hydroaromatic Compounds* (Rabideau, P. W., ed.), p. 89. VCH, New York.
18. Hall, L. D. and Sanders, J. K. M. (1980) *J. Am. Chem. Soc.* **102**, 5703.
19. Neuhaus, D. and Williamson, M. (1989) *The Nuclear Overhauser Effect in Structural and Conformational Analysis.* VCH, New York.
20. Pierce, M. and Essenberg, M. (1987) *Physiol. Mol. Plant Pathol.* **31**, 273.
21. Pierce, M. L., Essenberg, M. and Mort, A. J. (1993) *Phytopathology* **83**, 344.
22. Sun, T. J., Essenberg, M. and Melcher, U. (1989) *Mol. Plant–Microbe Interact.* **2**, 139.
23. Stipanovic, R. D., Mace, M. E., Bell, A. A. and Beier, R. C. (1992) *J. Chem. Soc. Perkin Trans. I*, 3189
24. Vickery, E. H., Pahler, L. F. and Eisenbraun, E. J. (1979) *J. Org. Chem.* **44**, 4444.
25. Stipanovic, R. D. and Steidl, J. (1986) *Synth. Commun.* **16**, 1809.
26. Jeffs, P. W. and Lynn, D. G., (1978) *Tetrahedron Letters* **19**, 1617.
27. Stewart, J. J. P. (1989) *J. Comput. Chem.* **10**, 209.
28. Stewart, J. J. P. (1989) *J. Comput. Chem.* **10**, 221.
29. Haasnoot, C. A. G., De Leeuw, F. A. A. M. and Altona, C. (1980) *Tetrahedron* **36**, 2783.
30. Garbisch, E. W. (1964) *J. Am. Chem. Soc.* **86**, 5561.