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DIVERSITY IN CYCLIC SESQUITERPENE PRODUCTION BY *GOSSYPIUM HIRSUTUM*

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Key Word Index—*Gossypium*; malvaceae; aromadendrene; cyclase; selinene; sesquiterpene.

Abstract—Major sesquiterpene components of oil of Texas Race Stock 810 of *Gossypium hirsutum* were α- and β-selinene. This is the seventh cyclic terpene type found to date in this genus. Both α- and β-selinene, along with aromadendrene, were found but only as minor components of extracts of several domestic cultivars of *G. hirsutum*.

INTRODUCTION

Domestic cottons (*Gossypium hirsutum* L.), which are allotetraploid derivatives of wild diploid cotton species, have been developed over a period of several thousand years on at least three continents. Because of the economic importance of cotton, the secondary metabolites have been studied extensively. A variety of monoterpenes and sesquiterpenes, sesquiterpene dimers, and Diels–Alder adducts of monoterpines with sesquiterpenes are stored in subepidermal glands found in leaves and buds of most varieties [1–6]. Individual chemicals and mixtures from the sesquiterpene group have been shown to possess insecticidal, fungicidal, and bacteriocidal properties. Presence or absence of given chemicals is also a useful taxonomic marker in breeding programmes. The goal of our studies is to obtain a better understanding of specificity and genetic variation of sesquiterpene cyclases in cotton species and of biosynthetic pathways leading to functionalized terpene allelochemicals in cotton. Analysis of major previously unidentified volatile sesquiterpenes in Texas Race Stock 810 [7] of *G. hirsutum* has led to identification of three minor components of volatile oil of domestic cultivars, indicating that a unique metabolic pathway exists for this cultivar.

RESULTS AND DISCUSSION

Domestic cotton cultivars produce cyclic sesquiterpenes of the copaane, caryophyllane, aromadendrane, humulane, cadinane, and bisabolane families, and relative amounts of the individual components in extracts of given plants can be an important indicator of breeding history. The compounds found in the locally grown *G. hirsutum* variety TAMCOT CAMD-E with 'average' sesquiterpene profile, in order of elution on BP-1 capillary gas chromatography, are copaene (**1**), caryophyllene (**2**), an unknown compound, humulene (**4**), three unknown compounds, bisabolene (**7**), cadinene (**8**), caryophyllene oxide (**9**), spathulenol (**10**), gossonorol (**11**), and bisabolol (**12**). In the course of a screening programme to determine the volatile terpene profiles of Texas Race Stocks of cotton, Stock 810 was found to be unique in that two of the previously unidentified components, which occur only in trace quantities in domestic cultivars, made up a major portion of the sesquiterpene fraction. These components were purified by packed column and capillary gas chromatography and analysed by NMR and mass spectroscopy. Spectral analysis and comparison of data with literature values [8, 9] and with spectra obtained on samples acquired as a gift or prepared from commercial celery oil allowed identification of these components as β- and α-selinene (**5** and **6**). ^{13}C NMR data on α-selinene was available for comparison [8] and mass spectra was available from the National Bureau of Standards (NBS) data bank. In the literature β-selinene is referred to as being known but no useful NMR data could be found other than ^{1}H data for the olefinic protons and the two methyl groups [9]. Complete spectral data of both compounds with complete assignments are presented here to aid future identification. Aromadendrene (**3**), another minor constituent of cotton volatile oil eluting between caryophyllene and humulene, was also identified. Aromadendrene is

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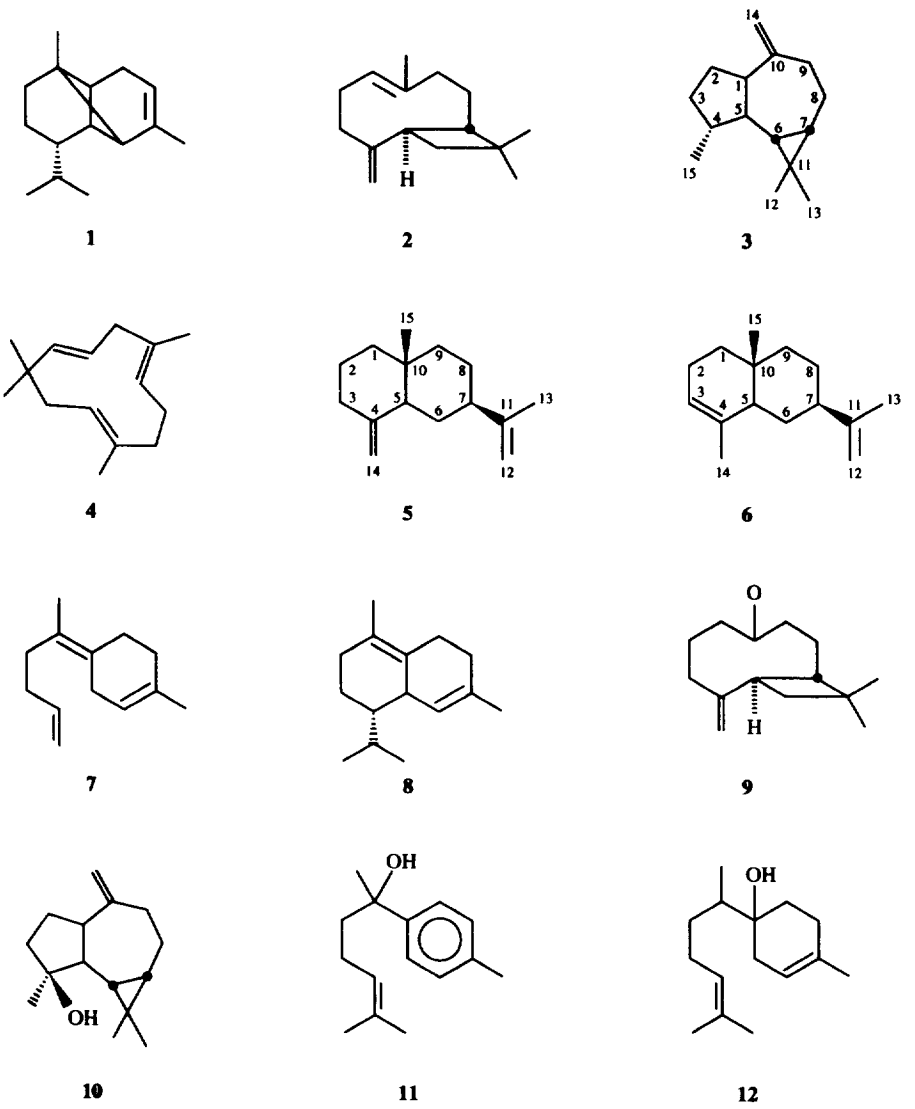

Scheme 1. Cyclic sesquiterpenes found in cotton plants.

presumed to be the precursor to the sesquiterpene alcohol spathulenol (**10**) [5]; its spectral data are also presented (Tables 1 and 2).

The eudesmane components α- and β-selinene make up a major portion of the sesquiterpene fraction of volatile oil produced by Texas Race Stock 810, which indicates that a cyclase with altered function is present in this species. It is unknown whether these are accumulated end-products or are intermediates on the pathway to other metabolites. Of the sesquiterpene skeletal types found in cotton, only cadinane derivatives are used in production of the highly functionalized aromatic compounds that are related to gossypol and thought to be responsible for most of cotton's resistance to herbivory and pathogens; however, volatile sesquiterpenes have also been shown to influence resistance [10]. Ability to biosynthesize other cyclic structures is inherited in a predictable manner, indicating that more than one sesquiterpene cyclase is present [11]. Cadinene itself is also present but only as a minor component, presumably because most of it is further processed to more oxidized derivatives. Production of minor terpene components, in many cases, may be the result of action of nonspecific cyclases. In these cases, major and minor components would segregate together on breeding, as has been shown in cotton for certain monoterpenes [11]. Fairly small changes in enzyme active site structure in a cyclase may alter specificity and could lead to a minor component becoming the major product.

EXPERIMENTAL

Cotton extracts were prepared by soaking fresh whole green leaves in ethyl ether for 1 hr. Extracts were rotary evaporated at 20°, dissolved in hexane:ethyl acetate (1:1), and filtered through a column of alternate layers of

Table 1. NMR data of identified compounds

	α-Selinene (6)*†			β-Selinene (5)*†			Aromadendrene (3)*		
C		δ_C	δ_H		δ_C	δ_H		δ_C	δ_H
1	CH_2	37.9	1.31	CH_2	41.9	1.43 1.26	CH	53.7	2.20 (*ddd*,5.8, 10.7, 11.6)
2	CH_2	22.9	2.07 1.94	CH_2	23.5	1.60	CH_2	29.4	1.66 (*dddd*, 2.1, 5.8, 5.8, 11.7)α 1.54 (*dddd*, 6.2, 11.6, 11.6, 11.7)β
3	CH	120.9	5.29	CH_2	36.9	2.31 2.01	CH_2	35.0	1.17 (*dddd*, 5.8, 11.6, 11.6, 12.0)α 1.84 (*dddd*, 2.1, 6.2, 6.3, 12.0)β
4	C	135.1		C	150.9		CH	35.2	2.08 (*dddq*, 6.3, 7.3, 10.7, 11.6)
5	CH‡	46.8	1.89	CH	49.9	1.81	CH	43.6	1.36 (*ddd*, 10.7, 10.7, 10.8)
6	CH_2	28.9	1.74 1.13	CH_2	29.5	1.55 1.29	CH	29.1	0.59 (*dd*, 9.5, 10.8)
7	CH‡	46.7	1.95	CH	45.8	1.96	CH	27.3	0.66 (*ddd*, 6.0, 9.5, 11.0)
8	CH_2	26.8	1.55	CH_2	26.8	1.55	CH_2	24.7	1.02 (*dddd*, 1.0, 11.0, 13.0, 14.1)α 1.94 (*dddd*, 1.7, 6.0, 6.2, 14.1)β
9	CH_2	40.2	1.41 1.16	CH_2	41.2	1.49 1.27	CH_2	38.9	2.39 (*ddd*, 1.0, 6.2, 13.5)α 2.04 (*ddd*, 1.7, 13.0, 13.5)β
10	C	32.3		C	35.9		C	154.6	
11	C	151.0		C	151.0		C	19.9	
12	CH_2	108.2	4.71	CH_2	108.1	4.71	CH_3‡	15.7	0.96 (*s*)
13	CH_3	20.9	1.75 (*s*)	CH_3	21.0	1.75 (*s*)	CH_3‡	28.6	1.02 (*s*)
14	CH_3	21.2	1.58 (*s*)	CH_2	105.3	4.69 4.42	CH_2	105.2	4.60 (*s br*)
15	CH_3	15.6	0.77 (*s*)	CH_3	16.3	0.71 (*s*)	CH_3	17.1	0.95 (*d*, *7.3*)

*^{13}C NMR: 125 MHz, $CDCl_3$, *^{1}H NMR 500 MHz, $CDCl_3$; †multiplicity of δ_H: if not stated otherwise signals appeared as complex multiplet or broad singlets, that could not be interpreted; ‡pairwise interchangeable.

Table 2. Mass spectra of identified compounds (*m/z*(%))*

Aromadendrene	204 (21.4), 189 (16.6), 162 (10.2), 161 (50.5), 148 (13.8),147 (20.0), 135 (15.3), 134 (10.8), 133 (36.4), 122 (10.8), 121 (31.7), 120 (14.0), 119 (39.6), 109 (11.7), 108 (14.2), 107 (48.5), 106 (16.6), 105 (54.9), 95 (19.7), 94 (20.1), 93 (56.2), 92 (16.3), 91 (72.5), 82 (10.5), 81 (37.6), 80 (10.9), 79 (54.6), 78 (10.8), 77 (37.9), 69 (36.4), 67 (41.6), 65 (17.7), 55 (42.3), 53 (28.7), 43 (20.1), 42 (10.7), 41 (100.0).
β-Seleniene	204 (56.4), 189 (47.6), 175 (25.0), 162 (18.5), 161 (55.6), 149 (10.4), 148 (26.6), 147 (45.7), 135 (25.8), 134 (19.2), 133 (51.5), 123 (16.1), 122 (22.8), 121 (60.4), 120 (17.7), 119 (47.4), 109 (21.3), 108 (50.0), 107 (88.5), 106 (26.1), 105 (100.0), 95 (40.5), 94 (31.7), 93 (97.8), 92 (17.9), 91 (80.7), 82(22.3), 81 (64.8), 80 (29.6), 79 (90.3), 78 (13.2), 77 (49.4), 69 (17.9), 68 (22.8), 67 (70.2), 65 (21.2), 55 (46.6), 53 (39.3), 43 (11.9), 41 (76.8).
α-Seleniene	204 (55.9), 190 (14.5), 189 (100.0), 175 (30.5), 162 (22.8), 161 (38.9), 148 (10.8), 147 (34.4), 134 (15.8),133 (62.0), 123 (14.1), 122 (20.5), 121 (30.5), 120 (10.5), 119 (27.9), 109 (33.4), 108 (19.3), 107 (69.2),106 (11.2), 105 (58.9), 95 (28.8), 94 (12.8), 93 (75.8), 92 (10.7), 91 (66.7), 81 (54.9), 79 (54.8), 77 (36.6), 69 (14.7), 68 (10.5), 67 (37.4), 65 (14.5), 55 (36.3), 53 (27.2), 43 (10.3), 41 (52.2).

*Intensities greater than 10%.

silica gel and polyamide. The final volume was adjusted to give a 1 ml 10 g^{-1} fresh leaves. Analytical gas chromatography was performed using a 25 m × 0.33 mm i.d. BP-1 column, He carrier gas at 20 psig [pounds per sq. inch guage, psi + 1 atm (15 lb)], temperature-programmed at 60° for 1 min, then to 250° at 15° min^{-1}. Retention times and normalized amounts of sesquiterpene hydrocarbons were caryophyllene (100, 9.30 min), aromadendrene (2, 9.51 min), humulene (23, 9.73 min), β-selinene (35, 10.14 min), and α-selinene (34, 10.27 min). Samples of purified chemicals for analysis were prepared by preparative gas chromatography using a 1.83 m × 4 mm i.d. glass column packed with 3% OV-101 on Chromosorb® 750 (100–120 mesh) with nitrogen carrier gas at 60 ml min^{-1}. Selinenes were separated from each other using a 12.5 m × 0.53 mm i.d. BP-1 column

with helium carrier gas at 1.8 ml min^{-1} operated isothermally. Mass spectra were obtained using a Hewlett–Packard 5971 instrument using a 30 m × 0.33 mm i.d. DB-5 column. NMR spectra were acquired on a Bruker ARX-500 500 MHz spectrometer in $CDCl_3$. Assignments relied on DEPT, C-H correlation, COSY, TOCSY, and HMBC interactions as necessary.

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