min. In dry systems, the ϵ-pyrrole-lysine level represented the extent of nonenzymatic Maillard reactions well. Thus, the method not only is suited for shelf life quality control purposes but also is valuable for elucidating Maillard reaction mechanisms.

Registry No. ϵ-Pyrrole-lysine, 74509-14-1.

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Terpenoid Aldehydes in Upland Cottons: Analysis by Aniline and HPLC Methods

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Lysigenous pigment glands in Upland cotton contain a diverse mixture of terpenoid aldehydes, including gossypol, hemigossypolone, and heliocides H_1, H_2, H_3, and H_4. These terpenoids are involved in plant resistance to some phytophagous insects. A high-performance liquid chromatographic (HPLC) method has been developed for quantitating each of these terpenoids in seed, leaves, and flower buds using a data base from 14 cultivars and experimental lines grown in five diverse field environments with four replicates per environment. Hemigossypolone and heliocides H_1, H_2, H_3, and H_4 were the major terpenoid aldehydes in leaves, and gossypol was the major tepenoid aldehyde in flower buds and essentially the only one in seed. Results from these analyses have been compared with those obtained on the same tissue by the aniline method of analysis; aniline analysis detected an average of about 50% of the terpenoid aldehydes in leaves and flower buds compared to the HPLC method. Seed analysis by the two methods gave nearly identical amounts of gossypol. HPLC analysis provides the concentration of each individual terpenoid aldehyde, while the aniline method measures only the total terpenoid aldehydes. The former method gives a more accurate measure of total terpenoid aldehydes, yet because the aniline method is fast, relatively inexpensive, and highly correlated with terpenoid content in plant tissues as determined by the HPLC method (r = 0.80–0.99, $P < 0.01$), it remains a useful procedure.

Cotton (*Gossypium spp.*) and other members of the Gossypieae tribe contain lysigenous glands in vegetative and reproductive plant parts (Lukefahr and Fryxell, 1967). Cottonseed is a rich source of protein, but its use for human consumption or animal feed has been limited by a toxic substance, gossypol, in the seed glands. The structure of gossypol (G) was determined by Adams and co-workers (review 1960) as shown in Figure 1. On the basis of the assumption that G is the only terpenoid aldehyde in pigment glands, Smith developed a spectrophotometric method for determining G in seed (1958) and in leaves and flower buds (1967). Smith's method involved reaction of G with aniline to form a yellow Schiff base. With this method, both free and so-called "bound gossypol" could be determined (Smith, 1967). Bound refers to G that has the aldehyde group condensed with free amino groups on proteins to form a Schiff base. The formation of a Schiff base is a reversible reaction; when heated with excess aniline, bound gossypol is liberated to form the soluble aniline Schiff base. Free and bound gossypol taken together are called total gossypol. A modification of Smith's method is widely used by breeders and entomologists (Dilday, 1983). Pons et al. (1958) developed an alternative method for determining total G in cottonseed based on the aniline reaction using 3-amino-1-propanol as a complexing agent.

Studies on insect resistance to *Heliothis*, *Spodoptera*, and other insect pests assumed G was the terpenoid aldehyde conferring resistance [reviews by Bell and Stipa-

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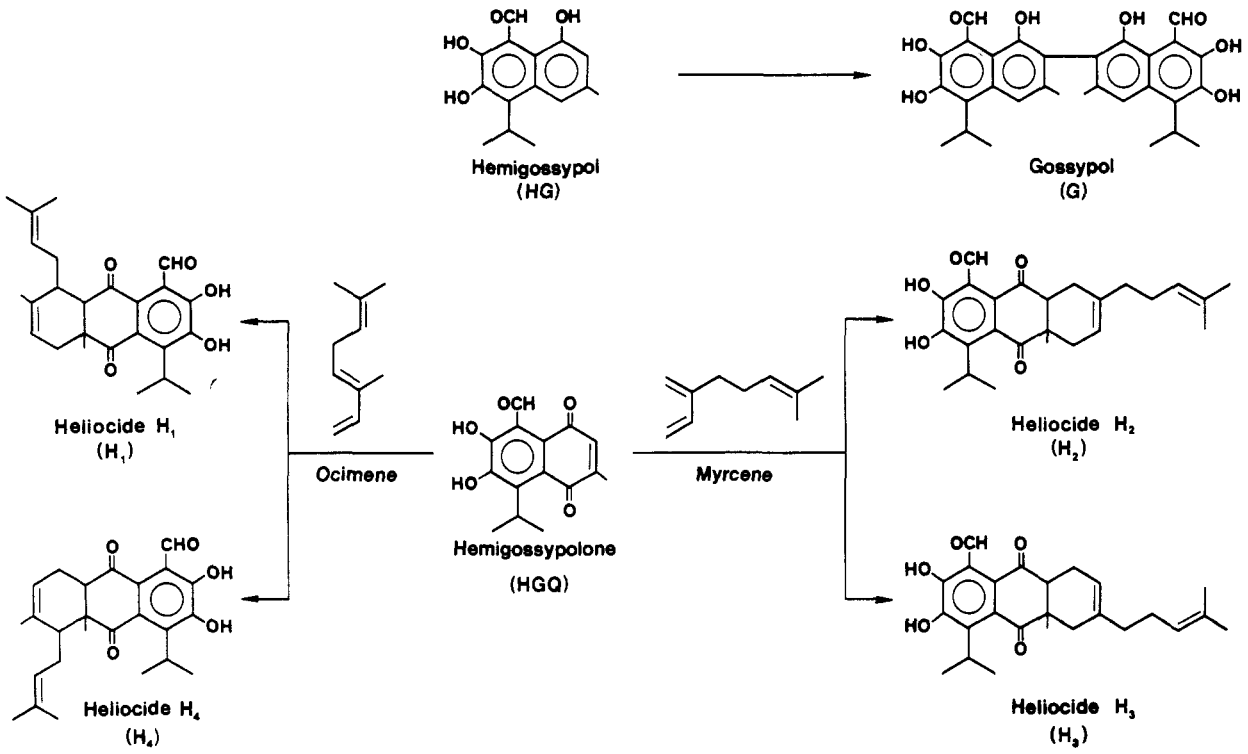

Figure 1. Terpenoid aldehydes in vegetative and reproductive plant parts of *G. hirsutum*.

novic (1977) and Hedin et al. (1981)]. The discovery of other terpenoids related to G in the root and stem [review by Bell and Stipanovic (1977)] and in the glands of foliar plant parts (Gray et al., 1976; Stipanovic et al., 1977b, 1978a,b; Bell et al., 1978) showed the importance of glands in this resistance. Thus, glandless varieties devoid of "gossypol glands" were extremely susceptible to these pests (Bottger et al., 1964; Jenkins et al., 1966). G, the heliocides H_1–H_4 (H_1, H_2, H_3, and H_4, respectively), and hemigossypolone (HGQ) (Figure 1) have been bioassayed against *Heliothis virescens* and other insect pests in artificial diets (Stipanovic et al., 1977a; Chan et al., 1978; Hedin et al., 1981). H_1–H_4 and G retard growth of *H. virescens* about equally, while HGQ is slightly less effective.

The role of the heliocides in *Heliothis* resistance has not been fully appreciated because they were purported to be present in relatively small amounts (Elliger et al., 1978; Waiss et al., 1981). In these studies, G ranged from 45 to 60% of the total terpenoids in flower buds. The authors did not point out that, in their bud samples, the total concentration of the other terpenoid aldehydes (e.g., H_1–H_4 and HGQ) was nearly equal that of G. Also, insect feeding sites other than flower buds were not considered. An NMR study showed that the heliocides H_1–H_4 and HGQ comprise the majority of the terpenoid aldehydes in 2- to 3-day-old bolls (Stipanovic et al., 1977a). This finding has been confirmed in 20- to 35-day-old bolls (Chan and Waiss, 1981). Mahoney and Chan (1985) used an HPLC method to show that the heliocides and HGQ are the predominant terpenoid aldehydes in floral bracts of *Gossypium hirsutum* cv. ST-7A (G, 5%; H_1–H_4, 90%; HGQ, 5%) and are slightly less than half of the terpenoid aldehydes in flower buds in *G. hirsutum* cv. VHG (G, 54%; H_1–H_4, 31%; HGQ, 15%).

A study of 130 lines of wild cotton demonstrated the importance of the heliocides to *Heliothis* resistance (Seaman et al., 1977). An increase in resistance correlated with the simultaneous increase of all the major terpenoids (G, H_1–H_4, HGQ) in flower buds. Using thin-layer chromatography, Seaman calculated that G represented 44% of the total active terpenoid fraction, with H_1–H_4 representing an equal amount.

Specific methods have been developed for analyzing individual terpenoid aldehydes in cotton. These include NMR (Stipanovic et al., 1977a; Waiss et al., 1978), GC (Chan et al., 1983), and HPLC (Greenblatt and Stipanovic, 1984; Mahoney and Chan, 1985). Mahoney and Chan (1985) have compared the HPLC and NMR methods.

We report a comparison of the standard aniline method of analysis for total terpenoid aldehydes and an HPLC analysis for individual extractable terpenoid aldehydes. These methods have been applied to seed, leaves, and flower buds from 14 cultivars or experimental lines of *G. hirsutum* grown in diverse field environments at five locations.

EXPERIMENTAL SECTION

Statistical Design. Eleven experimental lines and three cultivars of Upland cotton, *G. hirsutum*, were planted in a randomized complete block design with four replicates at each of five locations in 1984. Preliminary data for three years at one location indicated that the concentration of total terpenoids in flower buds ranged from low (0.4%) to high (2.0%) in these cottons. Analyses of variance were computed for all chemical measurements on an individual location basis. Following appropriate tests for error heterogeneity, means were computed for each of the 14 experimental lines and cultivars. Correlation coefficients were then calculated for these 14 entry means between all chemical measurements. Standard curves for all individual terpenoids were computed with regression analysis for both the aniline and HPLC methods. For the aniline methods, a standard curve was constructed as suggested by Smith (1967). Standard curves were similarly constructed for HGQ, H_1, and H_2. The response was linear for these compounds between 0.03 and 1.0 absorbance unit. Regression was also used to develop prediction equations for individual terpenoid aldehydes, with total terpenoid aldehydes by the aniline method being the independent variable and the respective terpenoid being the dependent

variable. (These prediction parameters are listed in the supplementary material.)

Plant Material Sampling. The five locations, soil types, and average monthly temperatures at the time of sample collection were as follows: (1) USDA Worksite, Brownsville, TX, Rio Grande silt loam, high 31.0 °C, low 21.5 °C; (2) Rio Farms, Inc., Monte Alto, TX, Hidalgo sandy loam, high 32.4 °C, 21.1 °C; (3) Texas A&M University Agricultural Research and Extension Center, Corpus Christi, TX, Orleia fine sandy loam, high 32.6 °C, 22.4 °C; (4) Texas A&M University Research Farm, College Station, TX, Ships clay, high 32.3 °C, 21.9 °C; (5) Texas A&M University Agricultural Research and Extension Center, Halfway, TX, Pullman clay loam, high 32.7 °C, 19.1 °C. All locations except Corpus Christi were irrigated. Leaf and flower bud samples were taken 3 weeks after first bloom; sample age was standardized by collecting only the first nonglossy leaf on terminals and flower buds at the third-grown square stage of development. All tissue samples were immediately placed on dry ice, later freeze-dried, and ground with a Wiley mill using a 20-mesh screen. Mature seed cotton was sampled from each plot at harvest and ginned. The seed was dehulled and ground as above for one replicate from each of the five locations.

Preparation of Standards. G was obtained from Dr. Walter Pons (deceased) of USDA, Southern Regional Research Laboratory, New Orleans; HGQ, H_1, and H_2 were isolated from plant sources as previously reported (Stipanovic et al., 1977b, 1978a,b). For HPLC analysis, a standard curve was obtained for G, HGQ, H_1, and H_2, with concentrations in the range of 25–6000 ng in 19 increments. Standard curves for H_1 and H_2 were also used for H_4 and H_3, respectively.

Aniline Method. Cottonseed was analyzed for G according to the method of Pons et al. (1958). Flower buds were analyzed as described by Dilday (1983), except 100 mg of sample was used. This is a modified method of Smith (1967) in which bound and free gossypol are analyzed simultaneously. This is the method extensively used in cotton-breeding programs. Leaves were analyzed by the method of Smith (1967). The 14 cultivars were sampled for all four replicates at each of the five locations for leaves and flower buds, but only one replicate from each location was sampled for seed. Free gossypol and bound gossypol in flower buds (i.e., total gossypol) were determined simultaneously at 440 nm with hexane as the solvent and as the reference in order to expedite the analysis (Dilday, 1983). Analyses of flower buds from 30 different *G. hirsutum* experimental lines showed that the use of hexane as the reference gave readings that were higher than those using a duplicate extract without aniline as reference, but the data sets were linearly correlated ($r = 0.97$, $P < 0.01$). For ease of analysis, the results reported here for flower buds were obtained with hexane as a reference blank, and the actual values have been calculated by multiplying the spectrophotometer readings by 0.85. Samples were diluted if necessary to give values below 1.0 absorbance unit. UV spectra for the aniline derivatives [λ_{max}, nm (ϵ)]: H_1, 376 (10700), 458 (7700); H_2, 376 (11300), 456 (8600); HGQ, 418 (9900), 440 (8300).

HPLC Method. Extracting solvents were ACS grade; chromatography solvents were either HPLC grade or ACS grade filtered through a 0.45-μm filter. Extractions were conducted in subdued light. Samples were analyzed on a Hewlett-Packard 1090 liquid chromatograph equipped with a diode array detector, multichannel integrator, and autoinjector using a 4.6 mm × 25 cm Scientific Glass Engineering LC-18 (5-μm) column at 55 °C. A mobile phase of ethanol–methanol–isopropyl alcohol (IPA)–acetonitrile (ACN)–water–ethyl acetate (EtOAc)–dimethylformamide–phosphoric acid (16.7:4.6:12.1:20.2:37.4:3.8:5.1:0.1) was monitored at 272 nm at a flow of 1.25 mL/min. All samples were checked by duplicate injections of 20 μL.

Samples of flower buds or leaves (100 mg) were shaken (350 rpm) in a capped Erlenmeyer flask (125 mL) with 15 mL of glass beads, 10 mL of hexane–EtOAc (3:1; solvent 1), and 100 μL of 10% acetic acid (HOAc) (for flower buds) or 200 μL of 10% HCl (for leaves) for 30 min. The solution was filtered (fritted filter funnel) into a 50-mL pear-shaped flask and the residue rinsed with solvent 1 (3×). The solvent was evaporated and the flask washed with solvent 1 (4 × 150 μL) and transferred to a silica Sep-Pak. The Sep-Pak was dried with N_2 gas and washed with 5 mL of IPA–ACN–H_2O–EtOAc (35:21:39:5), and 1 mL of the eluent was transferred to a crimp-top vial.

One replicate of seed from each location was sampled by HPLC analysis. Samples of seed (500 mg) were shaken as above with 20 mL of EtOH–H_2O–ether–HOAc (59:24:17:0.2, solvent 2) for 30 min (Smith, 1968). The residue was quantitatively filtered through a Buckner funnel (Whatman No. 1) into a 50-mL volumetric flask with solvent 2 and diluted to volume. One milliliter of this sample was filtered (0.45 μm) into a crimp-top vial for analysis.

Recovery of Terpenoid Aldehydes by HPLC Analysis. Standard solutions of G, H_1, H_2, and HGQ were prepared in solvent 1 to simulate the ratios of these compounds in a highly glanded line. Three aliquots of the standard solution were prepared to approximate the concentrations in medium, low, and very low glanded lines. A second standard solution was prepared to simulate the concentration of the terpenoid aldehydes in a highly glanded flower bud sample. Aliquots were taken as above. Glandless lyophylized *G. hirsutum* leaf (cv. Rogers GL-6) and flower bud (cv. ESP) samples were ground to a powder. Solvent 1 (10 mL) was added to 100-mg samples of leaf and flower bud powder. The terpenoid aldehydes were added to flasks to duplicate ranges of concentrations in the appropriate plant tissue. Glass beads (15 mL) were added and the samples analyzed as indicated above. Samples of leaf and flower bud powder containing no additional terpenoids were extracted and analyzed as a control. Average recoveries from leaf: G, 77%; H_1, 97%; H_2, 97%; HGQ, 94%. Average recoveries from bud: G, 87%; H_1, 98%; H_2, 95%; HGQ, 92%.

RESULTS AND DISCUSSION

Statistical Analysis. The analyses of variance calculated for HPLC terpenoid measurements by location for leaves and flower buds showed relatively large experimental errors and error heterogeneity as indicated by Bartlett's test (Snedecor and Cochran, 1967). Examination of means plotted against variances or standard deviations as well as nonparametric rank analyses indicated relationships that warranted data transformation. Subsequent logarithmic transformations for HPLC and aniline analyses of leaves and flower buds yielded acceptable experimental error values (Table I). The relationship between means and variances could be an artifact of area under the curve integration by the HPLC-dedicated computer. Error heterogeneity was not eliminated for every measurement across locations by data transformation, but the magnitude of differences was reduced enough to allow pooling raw entry means for correlation analyses (Gates, 1986).

In general, the HPLC method for individual compounds gives less experimental error than the aniline method for both leaves and buds at all locations (Table I). The same

Table I. Coefficients of Variation (%) for Terpenoid Aldehydes (TA) in Leaves and Flower Buds for 14 Varieties of *G. hirsutum* (HGQ = Hemigossypolone, G = Gossypol, H_1–H_4 = Heliocides H_1, H_2, H_3, and H_4) Grown at Five Locations As Determined by HPLC and Aniline Methods of Analysis

compd[a]	location[b] Bv	MA	CC	CS	Hw
	HPLC Method for Leaves				
HGQ	7.0	5.8	4.5	7.0	4.0
G	5.4	6.8	5.7	6.9	6.5
H_1	2.7	3.2	3.0	3.9	3.4
H_2	2.1	2.2	2.0	2.6	1.7
H_3	2.6	3.0	2.5	2.9	2.0
H_4	7.9	7.1	5.9	10.4	9.4
	Aniline Method for Leaves				
total TA	9.4	8.0	12.6	14.6	4.2
	HPLC Method for Flower Buds				
HGQ	5.2	2.1	4.1	3.3	3.8
G	2.6	1.1	2.2	2.1	1.2
H_1	5.5	2.3	4.2	2.4	2.4
H_2	4.0	1.9	3.3	3.2	2.7
H_3	5.1	2.3	4.2	3.9	3.2
H_4	8.6	2.8	6.3	4.4	5.1
	Aniline Method for Flower Buds				
total TA	9.0	4.2	6.7	5.9	4.1

[a] Key: HGQ = hemigossypolone; G = gossypol; H_1–H_4 = heliocides H_1, H_2, H_3, and H_4; TA = terpenoid aldehydes. [b] Key: Bv = Brownsville, TX; MA = Monte Alto, TX; CC = Corpus Christi, TX; CS = College Station, TX; Hw = Halfway, TX.

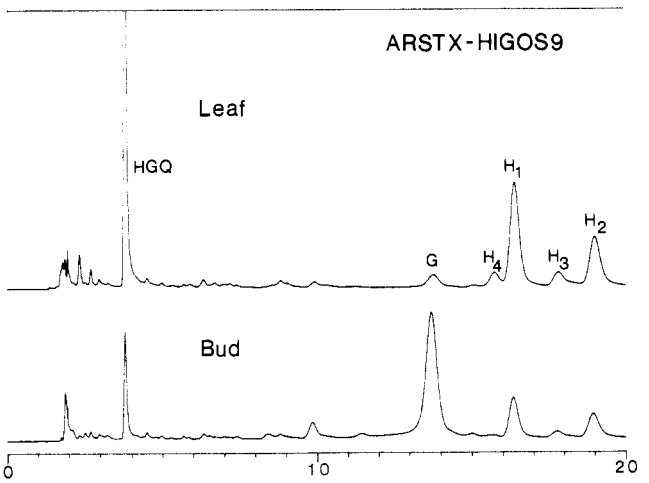

Figure 2. Chromatogram comparing the concentrations of gossypol (G), hemigossypolone (HGQ), and heliocides H_1, H_2, H_3, and H_4 (H_1, H_2, H_3, H_4) in leaves and flower buds of a *G. hirsutum* experimental line ARSTX-HIGOS9.

is true for the analysis pooled across the five locations. In leaves, the most precise measurements were for H_1, H_2, and H_3; H_1 and H_2 are present in the highest concentration, and H_3 is least variable and best resolved by the HPLC column. In flower buds, G is present in the highest concentration and gives the smallest error.

Seed. HPLC analysis showed that G is essentially the only terpenoid aldehyde in seed (Table II). Agreement between the two methods is excellent. The HPLC method gives values that vary between 93 and 98% of those obtained by the aniline method.

Leaves and Flower Buds. A chromatogram illustrating the analysis of leaves and buds from one sample of a cotton line is shown in Figure 2. In leaves, the heliocides are the major terpenoid aldehydes present; G is a minor constituent (Table II). However, on an individual basis, HGQ is usually the largest single component present.

Table II. Mean Concentration of Terpenoid Aldehydes (ppm) in Leaves, Flower Buds, and Seeds for 14 Varieties of *G. hirsutum* Grown at Five Locations As Determined by HPLC and Aniline Methods of Analysis

compd[a]	location[b] Bv	MA	CC	CS	Hw
	HPLC Method for Leaves				
HGQ	2364	5230	2900	1921	856
G	735	1039	785	670	413
H_4	302	447	549	335	238
H_1	2188	4178	3016	2146	1242
H_3	984	1088	984	771	698
H_2	2583	2316	2556	1887	1710
sum	9156	14298	10790	7730	5157
	Aniline Method for Leaves				
total TA	4263	6309	6237	4185	4521
	HPLC Method for Flower Buds				
HGQ	874	930	1010	885	692
G	7071	8170	10173	9279	10381
H_4	208	256	298	301	192
H_1	926	1249	1174	1140	873
H_3	425	432	475	450	421
H_2	1044	1012	1122	1060	1067
sum	10548	12049	14252	13115	13626
	Aniline Method for Flower Buds				
total TA	5344	5817	6242	5987	5850
	HPLC Method for Seed				
G	10616	11287	8879	8537	9813
	Aniline Method for Seed				
G	11375	11574	9444	9086	10224

[a,b] See footnotes, Table I.

In flower buds, G is the major constituent, representing 60–70% of the total terpenoid aldehydes. These data for 14 cultivars show a slightly higher proportion of G than previously reported by Mahoney and Chan (1985) where G was 54% of the terpenoid aldehydes in the flower buds of a single cultivar. G is the primary terpenoid aldehyde in the glands on the anthers and flower petal (Bell, 1986). Because the glands on these organs compose a major portion of the glands in the flower bud, the preponderance of G is understandable.

The HPLC analytical method gives a total terpenoid aldehyde content higher than that obtained by the aniline method (Table II). The HPLC method measures only the extractable free terpenoid aldehydes; the aniline method also measures only free terpenoid aldehydes in leaves, but both free and bound terpenoid aldehydes in flower buds. In part, the low readings with the aniline method result from using dianilinogossypol for constructing the standard curve from which the total terpenoid aldehydes are calculated even though other terpenoid aldehydes are present. Dianilinogossypol has a λ_{max} at 440 nm (ϵ 41 450). The ϵ values for the aniline derivatives of the heliocides and HGQ at 440 nm are considerably lower than for dianilinogossypol. These ϵ values for the aniline derivatives for HGQ, H_1, and H_2 at 440 nm are 8300, 7000, and 7800, respectively, which would indicate only about 20% of their actual concentration based on the standard curve for dianilinogossypol.

The total terpenoid aldehyde concentration reported for the aniline method in leaves (Table II) is considerably larger than what would be obtained by adding the G concentration as determined by the HPLC method to 20% of the concentration of the other terpenoid aldehydes as measured by the aniline method. In leaves, both methods are based on only free terpenoid aldehydes. Two explanations are possible for these results. The solvents used

in the aniline method may more efficiently extract the terpenoid aldehydes. Artifacts may also make a considerable contribution to the absorbance at 440 nm in the aniline method. Heating the extracts in the presence of aniline may produce colored products of extraneous compounds, leading to erroneous readings that are higher than those predicted based on the ϵ values. High readings have been noted in the aniline analysis of seed and attributed in part to oxygenated lipid (Stipanovic et al., 1984).

In flower buds the results are quite different. At all locations, the concentration of G obtained by the HPLC method is higher than that obtained by the aniline method, which includes both free and bound G. These results are surprising when one considers the excellent agreement between the two methods in the seed analysis. The reasons for these discrepancies cannot be explained without additional experiments.

The HPLC method shows variation in concentrations of specific compounds in leaves and flower buds among locations (environments). For example, terpenoid concentrations in leaves at Halfway deviated substantially from the values for the other locations with greatly reduced concentrations of HGQ and H_1 (Table II). Total terpenoid aldehydes also were approximately half that at the other locations. Among the individual compounds, HGQ fluctuated most across environments. Leaf samples from Monte Alto had 5.6 times the concentration of those grown at Halfway, and samples from Brownsville had 44% more HGQ than at Monte Alto, yet these locations are less than 100 km apart.

Heliocide Ratios. Stipanovic et al. (1978a) earlier proposed that the heliocides are formed by a Diels–Alder reaction between HGQ and either myrcene to give H_2 and H_3 or β-ocimene to give H_1 and H_4. The Diels–Alder reactions between HGQ and myrcene and ocimene in vitro in a nonpolar solvent gave ratios of 0.66 for H_3 to H_2 and 0.50 for H_4 to H_1. However, these ratios were at variances with the ratios in vivo in preliminary studies on race stocks (Stipanovic et al., 1978a). The present study confirms these in vivo observations. We found significant variations in the ratios among the 14 varieties at the five locations. The ratio of H_4 to H_1 in buds varied from 0.14 (±0.01) to 0.33 (±0.02) and in leaves from 0.09 (±0.01) to 0.24 (±0.04). The ratio of H_3 to H_2 was more consistent varying from 0.32 (±0.01) to 0.40 (±0.01) and 0.34 (±0.01) to 0.47 (±0.01) in buds and leaves, respectively. These variations indicate other factors are influencing the Diels–Alder reaction between HGQ and the dienes. For example, interaction of HGQ with a protein, or variations in the polarity of the medium within the gland, could influence the product ratio.

Association of Chemical Analyses. Correlation coefficients between measurements for leaves, flower buds, and seed show a moderate to high relationship for all methods and even different plant tissues (Table III). As expected, those compounds derived via the same biosynthetic pathway correlate well within the same plant tissue (e.g., H_4 with H_1 and H_3 with H_2 in leaves or buds). Seed G, as determined by either the aniline or HPLC method, is not as strongly correlated as the HPLC method for leaf and flower buds with the other compounds from diferent plant tissue. This moderate association could result from calculating means from only one replication per location for seed samples. Total terpenoid aldehydes, as determined by the aniline method, correlate best with H_1, H_2, H_3, and H_4 in leaves and with G in buds. Total terpenoid aldehydes as determined by the aniline method in buds were highly correlated with individual terpenoid aldehydes

Table III. Correlation Coefficients[a] for Individual Terpenoid Aldehydes As Determined by High-Performance Liquid Chromatography and Total Terpenoid Aldehydes As Determined by the Aniline Method for the Leaf, Flower Bud, and Seed of 14 Varieties Grown at Five Locations with Four Replicates/Location[b]

plant tissue–compd[c]	HPLC							aniline			HPLC					
	L–HGQ	L–G	L–H_4	L–H_1	L–H_3	L–H_2	L–TTA	L–TTA	S–G	B–TTA	B–HGQ	B–G	B–H_4	B–H_1	B–H_3	B–H_2	B–TTA
L–G	0.88**																
L–H_4	0.88**	0.85**															
L–H_1	0.81**	0.78**	0.97**														
L–H_3	0.91**	0.81**	0.96**	0.91**													
L–H_2	0.90**	0.79**	0.93**	0.86**	0.99**												
L–TTA	0.95**	0.88**	0.98**	0.94**	0.98**	0.96**											
L–TTA	0.87**	0.77**	0.95**	0.91**	0.97**	0.95**	0.95**										
S–G	0.78**	0.81**	0.78**	0.78**	0.74**	0.72**	0.81**	0.68**									
B–TTA	0.87**	0.80**	0.90**	0.87**	0.92**	0.91**	0.93**	0.86**	0.73**								
B–HGQ	0.67**	0.55*	0.54*	0.44	0.66**	0.70**	0.61*	0.61*	0.34	0.80**							
B–G	0.83**	0.75**	0.89**	0.86**	0.91**	0.90**	0.90**	0.85**	0.67**	0.99**	0.79**						
B–H_4	0.78**	0.69**	0.84**	0.79**	0.89**	0.88**	0.85**	0.86**	0.57*	0.91**	0.80**	0.91**					
B–H_1	0.81**	0.72**	0.88**	0.87**	0.89**	0.86**	0.89**	0.85**	0.62*	0.92**	0.71**	0.92**	0.96**				
B–H_3	0.78**	0.67**	0.72**	0.63*	0.82**	0.83**	0.77**	0.75**	0.51	0.90**	0.94**	0.89**	0.93**	0.88**			
B–H_2	0.77**	0.65*	0.67**	0.58*	0.79**	0.81**	0.74**	0.71**	0.49	0.89**	0.96**	0.87**	0.89**	0.82**	0.99**		
B–TTA	0.83**	0.74**	0.86**	0.81**	0.90**	0.89**	0.88**	0.84**	0.63*	0.98**	0.86**	0.99**	0.94**	0.94**	0.94**	0.93**	
S–G	0.64*	0.61*	0.59*	0.54*	0.59*	0.60*	0.62*	0.53	0.86**	0.56*	0.33	0.49	0.50	0.47	0.44	0.42	0.48

[a] ** and * indicate $P < 0.01$ and $P < 0.05$, respectively. [b] Only one replication per location for each of the 14 varieties for all seed analysis. [c] Key: L–HGQ = leaf hemigossypolone; L–G = leaf gossypol; L–H_1 to L–H_4 = leaf heliocides H_1, H_2, H_3, and H_4; L–TTA = leaf total terpenoid aldehydes; S–G = seed gossypol; B–TTA = bud total terpenoid aldehydes; B–HGQ = bud hemigossypolone; B–G = bud gossypol; B–H_1 to B–H_4 = bud heliocides H_1, H_2, H_3, and H_4.

as determined by the HPLC method in leaves. A similar but moderately high correlation was found between total terpenoid aldehydes in leaf and individual terpenoid aldehydes in buds. Total terpenoid aldehyde values using HPLC analysis had a very high correlation with aniline values for both leaves and flower buds. These data justify the derivation of prediction equations given in the Supplementary Material.

CONCLUSIONS

The HPLC method provides a detailed analysis of variations of individual terpenoid aldehydes among *G. hirsutum* while the aniline method gives only total terpenoid aldehydes. Furthermore, the HPLC method gives a more accurate measure of total terpenoid aldehydes. However, the aniline method has adequate reliability and is faster and less expensive to run. G is the major terpenoid aldehyde in *G. hirsutum* seed, and the agreement between the HPLC and the aniline method for this tissue is good. Thus, the aniline method is the preferred method of analysis for seed.

For host plant resistance studies, the HPLC method provides a means of correlating resistance with specific terpenoid aldehydes, while the aniline method only allows inference to total terpenoid aldehydes. Correlation with specific terpenoid aldehydes could be a useful guide to cotton breeders, entomologists, and other scientists concerned with understanding the action of these allelochemicals. For example, it might be possible to increase resistance to *Heliothis* by increasing selected terpenoid aldehydes and yet hold constant the total terpenoid aldehyde content. It is important to note that there is no clear understanding of the interaction of these compounds nor do we know how these various terpenoids relate to actual field resistance. HPLC analysis thus represents an expansion of the aniline method by providing previously unavailable information. In general, the correlation values support the use of Dilday's and Smith's aniline methods in many areas of research where a quick and inexpensive evaluation of relative concentrations is required, such as in genetic screening of a large number of experimental genotypes.

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Registry No. G, 303-45-7; HGQ, 35688-47-2; H_1, 65024-84-2; H_2, 63525-06-4; H_3, 64960-69-6; H_4, 64960-68-5.

Supplementary Material Available: Prediction parameters from regression for flower bud and leaf terpenoid aldehydes (1 page). Ordering information is given on any current masthead page.

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Fluorescence Detection and Measurement of Ferulic Acid in Wheat Milling Fractions by Microscopy and HPLC

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Ferulic (4-hydroxy-3-methoxycinnamic) acid is known to occur in high concentrations in the aleurone cell walls of wheat kernels, to a lesser extent in the seed coat and embryo, and in only trace amounts in the starchy endosperm. High-performance liquid chromatography (HPLC) is used to quantitatively examine the distribution of ferulic acid and, thus, its morphological host among milling fractions. Fluorescence photomicrographs corroborate the HPLC data and show that ferulic acid is a meaningful indicator of the nonendosperm tissues in the milling process. The application of this specific and sensitive method allows inferences regarding the efficiency of physical separation at selected steps of the milling process. A high correlation exists between HPLC and microspectrofluorometry techniques for determining ferulic acid in a reasonable range. The comparative results establish the potential for rapid ferulic acid determination of bran carryover in flour during milling.

The aim of dry milling of wheat is to separate the bran and germ from the starchy endosperm. Recently, we developed a sensitive analytical liquid chromatographic method chemically specific for bran in order to allow assessment of the efficiency of separation by milling (Pussayanawin and Wetzel, 1987). The rationale for this approach and its application are presented in this report. The various tissues of wheat are comprised of different structures and chemical constituents that ultimately determine the nutritional value and functional properties of the milling end products. Several chemical compounds in wheat can be used as precise indicators of selected botanical parts of the seed: pericarp, testa, aleurone, embryo, endosperm. Besides proteins, carbohydrates, and lipids, cereal grains also contain lesser quantities of noteworthy organic compounds such as vitamins, phenolics, aromatic amines, and amino acids. All of these components are synthesized and stored in specific tissues, making three major grain fractions (bran, germ, endosperm) chemically and morphologically distinct from each other.

Ferulic (4-hydroxy-3-methoxycinnamic) acid autofluoresces in the blue region of the spectrum, and prior to this work, fluorescence microscopy had been used to localize ferulic acid in cereal kernels (Fulcher et al., 1972; Fulcher and Wong, 1979; Fulcher, 1982; Smart and O'Brien, 1979). Ferulic acid was found in high concentration in the aleurone cell walls and also in the seed coat and embryo of wheat, but not in significant quantities in the starchy endosperm of the mature grains (Fulcher, 1982). The measurement of botanical parts by using the fluorescence characteristics of pericarp, aleurone, and endosperm was previously done on wheat fractions where data were evaluated by a statistical model (Jensen et al., 1982; Jensen and Martens, 1982). The model was initially calibrated against fluorescence data for manually dissected botanical parts and synthesized mixtures with known compositions. The resulting profile of 10 portions successively removed from the outside to the inside of the kernel suggested that the determination of ferulic acid by fluorescence measurements might be a desirable way of establishing the purity of endosperm separated during milling.

In the milling process, the efficiency of separation also needs to be determined by measuring the quality of the

Table I. Pilot Milling (Kansas State University) Fractions in Order of Mill Flow[a]

break system	residue system	reduction system
prebreak	tailings (purifier)	fine-sizing redn (top)
1st break	2nd quality stock	fine-sizing redn (bottom)
2nd break	suction recovery	coarse-sizing redn (top)
3rd break		coarse-sizing redn (bottom)
4th break		1st middlings (top)
5th break		1st middlings (bottom)
bran duster		2nd middlings (top)
		2nd middlings (bottom)
		3rd middlings
		4th middlings
		5th middlings
		6th middlings

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