Mass Spectral Techniques for the Identification of Cotton Terpenoid Aldehydes[†]

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Mass spectra of four cotton terpenoid aldehydes (TA's), gossypol, hemigossypolone, heliocide H_1, and heliocide H_2, have been obtained in positive EI and CI modes and the negative CI mode. The use of these complimentary modes resulted in the production of molecular ions for each compound in at least one mode. To obtain confirmatory information, several derivatives of these TA's were prepared and examined by MS. PFA (2,3,4,5,6-pentafluoroaniline) derivatives gave satisfactory molecular ions with the negative ion CI in methane mode. Trimethylsilyl and methoxyamine derivatives gave the expected molecular ions in the positive ion EI mode. Hemigossyplone (HGQ) was found to be capable of enolization, because approximately 10% of the tris(trimethylsilyl) species was produced. Although mass spectra for these compounds have previously been reported, mostly in the positive EI mode, this present integrated approach should facilitate identification of microquantities of these and other TA's as obtained from high-pressure liquid chromatography (HPLC) of plant extracts.

Efforts have continued at this laboratory and others over the past 30 years to identify cotton *Gossypium spp.* lines with resistance to *Heliothis spp.* and other cotton insects and to determine the chemical factors associated with the resistance. A consensus has developed that gossypol and related terpenoid aldehydes are the most important allelochemicals although some other cotton compounds also contribute to the expressed resistance (Bell and Stipanovic, 1983; Waiss et al., 1981; Hedin et al., 1983a,b; Stipanovic et al., 1988).

The need to analyze for the terpenoid aldehydes present in thousands of experimental lines of cotton has led to the development of a number of analytical procedures including colorimetry and spectrometry, high-pressure liquid chromatography (HPLC), and infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry (MS). Perhaps the most facile and best initial approach to identification of microquanties of isolates such as these terpenoid aldehydes would be obtained from HPLC eluates is mass spectrometry. MS data have been collected in several instrumental modes from a host of cotton terpenoid aldehydes and have been published and therefore can be used for comparison (Matlin and Zhou, 1954; Gray et al., 1976; Stipanovic et al., 1977, 1978).

Positive EI-MS via solid probe is normally the procedure of choice for general MS analysis of large numbers of samples. However, with extensive fragmentation often occurring, the molecular ion (M^+) may not be present or apparent, so CI-MS may be used to locate or confirm the M^+. Paradoxically, we found that gossypol gives a small M^+ (RA = 10.7%) with EI-MS but no M^+ with CI-MS (methane). It occurred to us that the methane-generated molecular ion might be a negative ion. This hypothesis led to the collection of spectra of four terpenoid aldehydes by several EI and CI instrumental techniques. Additionally, because mass spectra alone are not always conclusive, the use of derivatives to generate confirming MS data was explored.

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MATERIALS AND METHODS

EI and CI Techniques. A HP-5985-B quadrupole mass spectrometer was used to obtain positive EI spectra and positive CI spectra with methane and isobutane as reagent gases. A Finnigan 4500 unit was used to obtain positive and negative CI data.

In the EI experiments, the source was maintained at 200 °C, the pressure in the source was approximately 2×10^{-7} Torr, and the electron energy was 70 eV. The direct-insertion probe was programmed to heat ballistically from ambient temperature to 350 °C. The CI experimental conditions were as follows: the source pressure was 2×10^{-4} Torr, the electron energy was 235 eV, and the source temperature was 150 °C.

Terpenoid Aldehydes. Gossypol was obtained as a gift from the Southern Regional Research Center, USDA—ARS, New Orleans, LA. (It was received as the acetate, converted to the free state by shaking with water and ethyl ether, recovered in the ether phase.) Hemigossypolone, heliocide H_1, and heliocide H_2 were obtained as gifts from Dr. Robert Stipanovic, USDA—ARS, College Station, TX. Key: gossypol (G), 1,1',6,6',7,7'-hexahydroxy-3,3'-dimethyl-5,5'-bis(1-methylethyl)[2,2'-binaphthalene]-8,8'-dicarboxaldehyde; hemigossypolone (HGQ), 5,8-dihydro-2,3-dihydroxy-6-methyl-4-(1-methylethyl)-5,8-dioxo-1-naphthalenecarboxaldehyde; heliocide H_1 (H_1), 5,8,8a,9,10,10a-hexahydro-2,3-dihydroxy-7,10a-dimethyl-8-(3-methyl-2-butenyl)-4-(1-methylethyl)-9,10-dioxo-(8α,8aβ,10aβ)-1-anthracenecarboxaldehyde; heliocide H_2 (H_2), 5,8,8a,9,10,10a-hexahydro-2,3-dihydroxy-10a-methyl-4-(1-methylethyl)-7-(4-methyl-3-pentenyl)-9,10-dioxo-*cis*-1-anthracenecarboxaldehyde (Figure 1).

Trimethylsilyl Derivatives. A slight excess of BSA (*N*,*O*-bis(trimethylsilyl)acetamide; Analabs, North Haven, CT) in CS_2 was reacted with the compounds and heated in capped vials near the boiling point (Pierce, 1968). Mass spectra were taken on an estimated 5 µg of the product of the reaction mixture. Anhydrous sodium sulfate was added to deter hydrolysis if a delay in analysis was anticipated.

PFA Derivatives. Samples were dissolved in chloroform, and 10 µL of solution containing 100 µg was mixed with 2 L of 10% aqueous thiourea, 1 µL of 1 N HCl, 10 µL of 1-butanol, and 10-20 µL of PFA (2,3,4,5,6-pentafluoroaniline; Fluka Chemical Corp., Ronkonkoma, NY) (Horwitz, 1975). The procedure was modified by the use of 1-butanol in place of chloroform to achieve the higher boiling point required to drive the reaction and the use of variable amounts of PFA dependent on the number of reaction sites of the test compound.

Methoxyamine Derivatives. Methoxyamine hydrochloride (30 mg; Sigma Chemical Co., St. Louis, MO) was dissolved in 3 mL of absolute ethanol with a drop of concentrated HCl
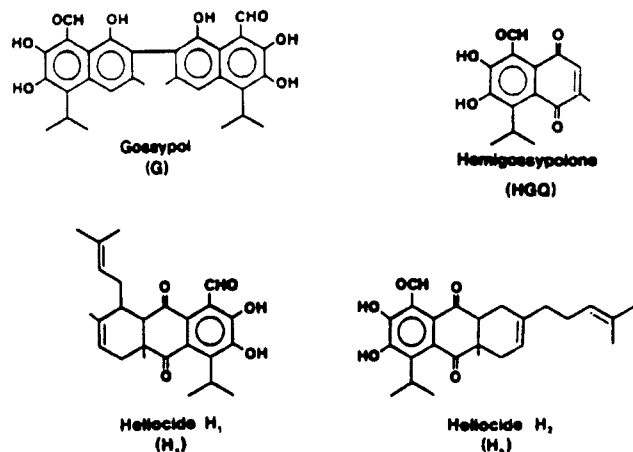
Figure 1. Structures of four terpenoid aldehydes; gossypol, hemigossypolone, heliocide H_1, and heliocide H_2.

and the mixture allowed to stand for ca. 2 h, at which time the reaction was shown to be completed by TLC; then, 10 mL of chloroform and 10 mL of water were added. The organic layer containing the product was collected, washed with water, and dried with anhydrous sodium sulfate. The product was a solid that could be recrystallized (Fried and Nutile, 1962).

RESULTS AND DISCUSSION

EI and CI Techniques. With positive ion EI spectra of aromatic aldehydes, the molecular ion $[M]^+$, the $[M - 1]^+$, and the $[M - CHO]^+$ ions are major peaks (Hill, 1966). With CI spectra with methane as reagent gas, ions of $[M + 1]^+$, $[M + 16]^+$, $[M + 29]^+$, and $[M + 41]^+$ are often seen. These ions are due to the attachment of reagent gas ions to the molecules. The $[M + 1]^+$ ion can be the result of self-protonation, which also sometimes gives an $[M - 1]^+$ ion. It may also result from proton transfer from CH_5^+. The $[M + 16]^+$ ion results from the addition of the CH_4^+ ion, $[M + 29]$ from the $C_2H_5^+$ ion, and $[M + 41]^+$ from the $C_3H_5^+$ ion (Harrison, 1983).

In a preliminary test, positive ion CI/MS (methane) did not produce a "quasi-molecular ion" (hereafter referred to as "molecular") for gossypol even though positive ion EI does produce a molecular ion. It was hypothesized that the molecular ion of gossypol produced in CI with methane as the reagent gas might be a negative ion. This is conceivable because gossypol is a large conjugated system bearing electron-attracting substituents (Harrison, 1983).

The spectral data obtained for gossypol, hemigossypolone, and heliocides H_1 and H_2 are given in Table I. The molecular ion for gossypol (m/e 518) is seen with positive EI; its intensity was 11% of the base peak. Positive EI gave a fragment (m/e 500) showing the loss of a water molecule and the base peak (m/e 482) showing the loss of a second water molecule. No molecular ion is seen in positive CI (methane), but the $[M]^-$ m/e 518 ion is one of the major peaks in negative CI. This seems to support the hypothesis that the molecular ion formed in CI is a negative ion. The PFA derivative, although formed and detected, did not appear to enhance the production of the negative molecular ion since the molecular ion peak of m/e 848 had a lower total ion abundance than the total molecular ion abundance observed in negative CI of pure gossypol.

The spectrum for hemigossypolone was fairly simple; a relatively large ion at m/e 274 indicated a stable molecular ion. The major fragmentation was the loss of a methyl group to form the ion at m/e 259. With positive ion CI (methane), the most abundant ion produced was the [M

+ 1]$^+$ ion (m/e 275). Also present were the $[M + 29]^+$ ion (m/e 303) and the $[M + 41]^+$ ion (m/e 315), which resulted from the addition of reagent gas ions. Very little fragmentation was observed. Negative ion CI produced the $[M - 1]^-$ ion (m/e 273), the $[M + 16]^-$ ion (m/e 290), and the $[M - 15 - 1]^-$ ion (m/e 258). A precise mass determination could be made from both the positive and negative CI spectra. The PFA derivative ($[M]^-$, m/e 439) did not seem to enhance the formation of a negative molecular ion. An ion also was present at m/e 499. While a conclusive explanation is not available, the presence of this ion would be consistent with a species resulting from the reaction of the PFA–HGQ with thiourea (which was present in the acidic reaction mixture) with the loss of ammonia.

With heliocide H_1, a very small molecular ion (m/e 410) was observed in the positive EI spectrum. However, in the positive CI (methane) spectrum, the most abundant ion observed was the $[M + 1]^+$ ion (m/e 411). Also observed was the $[M + 29]^+$ ion (m/e 439). Negative ion CI produced the molecular ion (m/e 410). A precise mass determination could be made from both the positive and negative CI spectra. The PFA derivatization evidently was not 100% complete since unreacted starting material was observed in the spectrum. The molecular ion of the PFA derivative (m/e 575) was observed.

With heliocide H_2, positive ion EI produced a larger molecular ion peak (m/e 410) for H_2 than for H_1, indicating that H_2 produced a more stable ion than H_1. The ion of mass m/e 382 indicated the loss of carbon monoxide $[M - 28]^+$. The m/e 274 peak could arise from a reverse Diels–Alder reaction of H_2 giving the m/e 274 peak (HGQ). Positive CI (methane) produced the $[M + 1]^+$ (m/e 411), and it was the most abundant ion in the spectrum. The $[M + 29]^+$ ion (m/e 439) was also observed. In the negative ion CI spectrum, the molecular ion (m/e 410) was observed. Also, the $[M + 16]^-$ ion (m/e 426) was produced. As with HGQ and H_1, an exact-mass determination could be made from both the positive and negative CI spectra. The molecular ion of the PFA derivative (m/e 575) was observed.

EI and CI are complementary techniques, EI giving more fragmentation and positive ion CI giving mostly molecular ions, except in the case of gossypol. Because CI with methane did not produce a molecular ion for gossypol, the use of isobutane as reagent gas was considered because it has been reported to be a more specific protonating agent (Harrison, 1983). However, the only major ion produced was m/e 483 $[M - 36 + 1]^+$.

The negative ion CI methane spectra for G, HGQ, H_1, and H_2 were very similar, all giving molecular ions. Ions resulting from the addition of reagents gas were seen as expected.

The negative ion CI (methane) of PFA derivatives (Table I) did not give an enhanced formation of a negative molecular ion as was expected, although the PFA derivatives of all four were present.

Terpenoid Aldehyde (TA) Methoxyamine–Trimethylsilyl Derivatives. The reaction of gossypol with aniline (Horwitz, 1975) occurred as demonstrated by TLC but gave an apparent molecular ion (positive ion EI) of m/e 482, the fragment expected from gossypol less two molecules of H_2O. The pentafluroaniline (PFA) derivatives of the four TA's all gave the expected molecular ions in the negative CI in methane mode (Table I).

However, for wider applicability, a derivative was required that would give strong molecular ions in the pos-

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Table I. Mass Spectral Fragmentation Patterns for Four Terpenoid Aldehydes

compd[a]	fragmentation pattern[b]
	Positive EI
G	518 [M]$^+$ (11), 500 (77), 482 (100), 467 (72), 454 (13), 226 (20)
HGQ	275 (7), 247 [M]$^+$ (48), 259 (100), 241 (5), 231 (10), 217 (7)
H$_1$	410 [M]$^+$ (5), 393 (3), 313 (40), 275 (47), 135 (100)
H$_2$	411 (31), 410 [M]$^+$ (30), 392 (3), 383 (23), 382 (100), 313 (57), 274 (53)
	Positive CI (Methane)
G	511 (10), 501 (12), 484 (30), 483 (100), 469 (5), 447 (7)
HGQ	315 (4), 303 (15), 276 (15), 275 [M + 1]$^+$ (100), 259 (2)
H$_1$	439 (12), 412 (25), 411 [M + 1]$^+$ (100), 393 (18), 343 (30)
H$_2$	439 (8), 412 (24), 411 [M + 1]$^+$ (100), 409 (6), 393 (2)
	Negative CI (Methane)
G	532 (15), 519 (45), 518 [M]$^-$ (96), 501 (57), 500 (100), 484 (19)
HGQ	290 (11), 276 (82), 274 [M]$^-$ (86), 273 (100), 260 (23), 258 (25)
H$_1$	426 (7), 412 (24), 411 (35), 410 [M]$^-$ (100), 395 (9), 384 (5), 342 (7)
H$_2$	426 (7), 413 (16), 412 (49), 411 (42), 410 [M]$^-$ (100), 409 (20), 408 (11)
	Negative CI (Methane), PFA
G	850 (18), 849 (57), 848 [M]$^-$ (100), 830 (2), 829 (2)
HGQ	500 (29), 499 (86), 441 (26), 439 [M]$^-$ (37), 423 (100), 348 (54)
H$_1$	576 (81), 575 [M]$^-$ (100), 486 (4), 411 (27), 410 (65)
H$_2$	587 (3), 576 (12), 575 [M]$^-$ (100), 557 (4)

[a] Key: G = gossypol, HGQ = hemigossypolone, H$_1$ and H$_2$ = heliocides H$_1$ and H$_2$. [b] m/e values with relative abundance in parentheses.

Table II. Mass Spectral Fragmentation Patterns for Four Terpenoid Aldehyde Trimethylsilyl Derivatives

ion mass	m/e	rel abund	explanation
gossypol	950	69	gossypol (518) = 6 TMS gps
	935	22	950 − 15
	878	8	518 = 5 TMS gps
	773	42	878 − 90 − 15
	743	5	773 − 15 − 15
	475	100	950/2
	460	38	475 − 15
hemigossypolone	490	7	HGQ (274) = 3 TMS gps
	475	7	490 − 15
	418	28	274 = 2 TMS gps
	386	55	490 − 89 − 15
	299	100	
heliocide H$_1$	554	2	H$_1$ (410) + 2 TMS gps
	539	6	554 − 15
	483	23	410 + 1 TMS gp
	419	100	554 − (90 + 45)
	347	40	
heliocide H$_2$	554	4	H$_2$ (410) + 2 TMS gps
	539	33	554 − 15
	449	7	554 − 90 − 15
	418	17	554 − (90 + 45) − 1
	403	27	418 − 15

itive EI mode. Methylations with diazomethane, methyl iodide, and dimethyl sulfate were evaluated. TLC indicated that reactions occurred, but none of these derivatives gave the expected molecular ions in the positive EI mode. However, trimethylsilyl derivatives were found to give strong positive EI molecular ions.

Table II gives the mass spectral results of the TMS derivatives of G, HGQ, H$_1$ and H$_2$. Gossypol–TMS gave the expected molecular ion (positive ion EI) of m/e 950 with a characteristic [M − 15]$^+$ ion at m/e 935. A species was also observed at m/e 878 in which five of the six TMS groups were added, but at a low relative abundance indicating that very little of this species was formed. Intra- or intermolecular hydrogen bonding of adjacent phenolic and carbonyl groups may prevent total silylation, thus accounting for the m/e 878 ion.

The hemigossypolone–TMS (HGQ–TMS) derivative (Table II) gave some rather unexpected results. HGQ has two phenolic groups and was expected to give a molecular ion of m/e 418 on reaction with BSA. While this was a major ion, a smaller ion peak of m/e 490 was also observed indicating that three TMS groups were added.

Scheme I
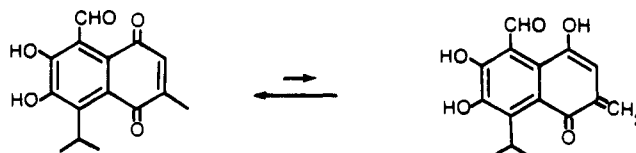
To determine the site of the third TMS group, additional mass and NMR spectra were acquired.

The ^{1}H NMR spectrum (deuterated chloroform as solvent) exhibited signals at δ 10.76 (aldehyde), 6.52 (OH, not hydrogen bonded), 13.03 (OH, hydrogen bonded to the aldehyde), 1.42 (6 H, methyls of isopropyl), septet at 4.13 (1 H, isopropyl), 2.16 (3 H, methyl), and 6.66 (1 H, vinyl). These data are consistent with those reported by Gray et al. (1976). Also observed was a small signal at δ 5.7, which is within the region for methylene hydrogens, and a small signal (0.1 proton) at δ 6.42, which suggests that a phenolic hydrogen atom was generated from the enolization in Scheme I.

Additional positive ion EI-MS data were obtained to determine the site of the third TMS group. First, the aldehyde carbonyl of the HGQ was reacted with methoxyamine to form a Schiff's base (an oxime). Positive ion EI-MS gave the expected molecular ion of m/e 303. Subsequent reaction of HGQ–MA with BSA gave a species of m/e 519 (relative ion abundance 0.8), the result of three TMS groups added to the molecule, and a species with a larger abundance of m/e 447 (relative ion abundance 6.7), the result of two TMS groups added. The ratio of m/e 519 to m/e 447 was 1:8.4, which is consistent with the similar ratio obtained from the ^{1}H NMR to demonstrate that the enol–quinone equilibrium exists.

Heliocide H$_1$ (Table II) gave an expected molecular ion at m/e 554, indicating an addition of two TMS groups. Enolization involving the methinyl hydrogen, thus generating a third TMS function, appears possible but evidently did not occur. The peak at m/e 539 is due to the loss of a methyl group, [M − 15]$^+$. Other prominent ion species and their possible structures are listed. Heliocide H$_2$ (Table II) gives a spectrum very similar to that of H$_1$, with an ion of m/e 554 indicating the addition of two TMS groups.

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Table III. MS Fragmentation Patterns for TA–Methoxy-amine (MA) Derivatives

ion mass	m/e	rel abund	explanation
gossypol	576	10	gossypol + 2 MA gps
	529	36	$576 - NH_2OCH_3$
	498	100	$529 - OCH_3$
hemigossypolone	303	52	HGQ + 1 MA gp
	292	93	$303 - OCH_3$
	256	100	$303 - NH_2OCH_3$
heliocide H_1	439	15	H_1 + 1 MA gp
	408	38	$439 - OCH_3$
heliocide H_2	439	9	H_2 + 1 MA gp
	411	59	$439 - CO$
	408	39	$439 - OCH_3$
	380	100	$439 - CH_2{=}NOCH_3$

The four terpenoid aldehydes were also reacted with methoxyamine to produce oximes (note spectral data on HGQ–MA in previous section). The mass spectral data (Table III) show that the expected molecular ions were obtained with each compound; G, m/e 576 (2 carbonyls); HGQ, m/e 303 (1 carbonyl); H_1, m/e 439 (1 carbonyl); H_2, m/e 439 (1 carbonyl). Other prominent fragments were the $[M - 31]^+$ ion and the $[M - 47]^+$ ion resulting from the loss of $-OCH_3$ and NH_2OCH_3, respectively. Methoxyamine reacted with each available aldehyde group.

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