

Spectral Techniques for Structural Analysis of the Cotton Terpenoid Aldehydes Gossypol and Gossypolone[†]

Valeria A. Phillips and Paul A. Hedin*

Crop Science Research Laboratory, USDA—ARS, Mississippi State, Mississippi 39762-5367

Positive-ion EI mass and infrared spectra were obtained from gossypol (G) and gossypolone (GQ), two allelochemic terpenoid aldehydes (TA's) present in cotton (*Gossypium spp.*) plants. MS and IR spectra were also obtained from their methoxyamine (oxime) and trimethylsilyl derivatives. These reagents appear to have considerable applicability for structural elucidation of microgram quantities of the TA's.

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 compounds also contribute to the expressed resistance (Waiss et al., 1981; Bell and Stipanovic, 1983; Hedin et al., 1983a,b). The need to analyze for the terpenoid aldehydes present in thousands of experimental lines has led to the development of a number of analytical procedures including colorimetry and spectrometry, high-pressure liquid chromatography (HPLC), and infrared, nuclear magnetic resonance, and mass spectrometries. Perhaps the most facile and best initial approach to the identification of microquantities of compounds such as would be obtained from HPLC eluates is mass spectrometry. Infrared and nuclear magnetic resonance spectrometries may be feasible when sufficient quantities are available. MS, NMR, and IR data have been collected in several instrument modes for many terpenoid aldehydes and have been published and therefore can be used for comparisons (Haas and Shirley, 1965; Gray and Mabry, 1976; Stipanovic et al., 1977, 1978; Matlin and Zhou, 1984).

Positive-ion EI-MS via solid probe is normally the procedure of choice for preliminary analysis of chromatographic isolates. However, extensive fragmentation often occurs, and the molecular ion may not be present or apparent, so the use of other instrument modes, the preparation of derivatives, and the use of other spectrometric techniques may be necessary to obtain definitive data.

This report developed from efforts to identify a HPLC peak from a partially purified cotton plant extract that eluted from a C₁₈ reversed-phase column slightly earlier than gossypol. The peak was subsequently identified as gossypolone on the basis of its synthesis from gossypol and the preparation of derivatives of gossypol and gossypolone that were analyzed by EI-MS and IR. While various spectral data from gossypolone and gossypol have been published (Haas and Shirley, 1965; Matlin and Zhou, 1984), MS and IR data for the compounds and their methoxyamine and trimethylsilyl derivatives have not been reported. The use of this integrated approach appears to have considerable applicability for structural elucidation of terpenoid aldehydes.

Table I. Infrared Data [Wavenumbers (cm⁻¹) with Intensities (s, m, w)] for Gossypol (G), Gossypolone (GQ), Their Methoxyamine Derivatives, and Methoxyamine (MA)

G	GQ	GMA	GQMA	MA
3440 m		3450 m		
	3424 m		3420 m	3424 s
2970 m	2970 m	2960 m	2960 m	2980 m
2940 m	2930 m	2930 m	2930 m	2940 m
				2712 w
	1715 w		1720 w	
	1640 s		1645 s	1655 s
1620 s		1620 w		1620 m
1590 s		1580 w		
		1550 w	1550 w	
1440 s		1449 w	1440 m	1440 w
1425 m	1425 m	1420 s		
1390 m	1390 m			
1340 s		1330 s		
	1320 s			
1290 s	1290 s	1290 w	1295 s	
1250 m		1240 w		
1170 m		1170 m		
		1070 m	1070 m	
1050 m	1040 w	1050 m	1040 m	1040 m
		940 m	955 w	
				880 m
840 m	850 w	830 w	845 w	
750 m	750 s	770 m	750 m	

MATERIALS AND METHODS

Synthesis of Gossypolone. Gossypolone (GQ), 1,1',4,4'-tetrahydro-6,6',7,7'-tetrahydroxy-3,3'-dimethyl-5,5'-bis(1-methylethyl)-1,1',4,4'-tetraoxo[2,2'-binaphthalene]-8,8'-dicarboxaldehyde, was synthesized by oxidation of gossypol (G), 1,1',6,6',7,7'-hexahydroxy-3,3'-dimethyl-5,5'-bis(1-methylethyl)[2,2'-binaphthalene]-8,8'-dicarboxaldehyde with ferric chloride in hydrochloric acid Haas and Shirley, 1965).

Preparation of Methoxyamine Derivatives. G or GQ (30 mg) was dissolved in 3 mL of ethanol to which 30 mg of methoxyamine hydrochloride and one drop of concentrated HCl were added. After 2 h at room temperature, the reaction appeared complete based on TLC, and 10-mL portions each of H₂O and CHCl₃ were added. The organic layer was collected, washed with H₂O, and dried with Na₂SO₄. Upon concentration, the product crystallized.

Preparation of Trimethylsilyl Derivatives. A slight excess of BSA [*N,O*-bis(trimethylsilyl)acetamide], Analabs Inc., North Haven, CT, in CS₂ was reacted with the compounds (0.1-1.0 mg) by heating in capped vials near the boiling point (Pierce, 1968). Mass spectra could be taken on the reaction mixture without further processing, although anhydrous sodium sulfate was added to deter hydrolysis if a delay in analysis was anticipated.

Mass and Infrared Spectra. Mass spectra were taken at 70 eV in the positive-ion EI mode with a HP-5985B quadru-

[†] Taken in part from the M.S. Thesis of V.A.P., Mississippi State University, Mississippi State, MS, Dec 1988.

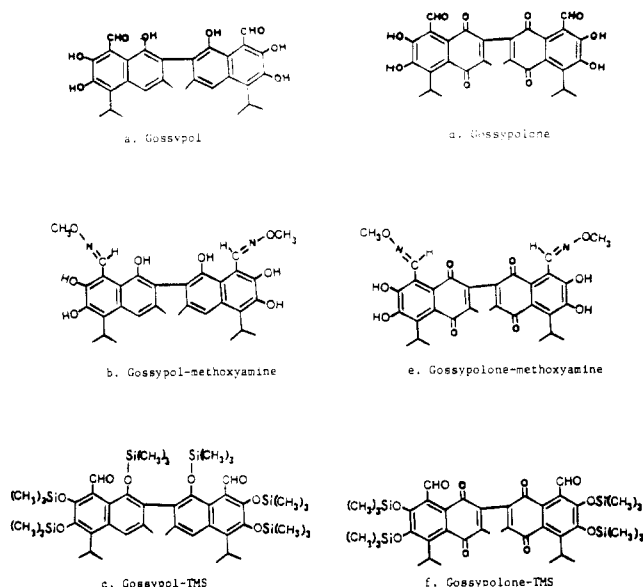


Figure 1. Structures of gossypol (G) and gossypolone (GQ) and their methoxyamine (MA) and trimethylsilyl (TMS) derivatives.

pole mass spectrometer. The sample was introduced into the source of the instrument via a direct-insertion probe. The source was maintained at a temperature of 200 °C, and the probe was ballistically heated from ambient temperature to 350 °C. IR

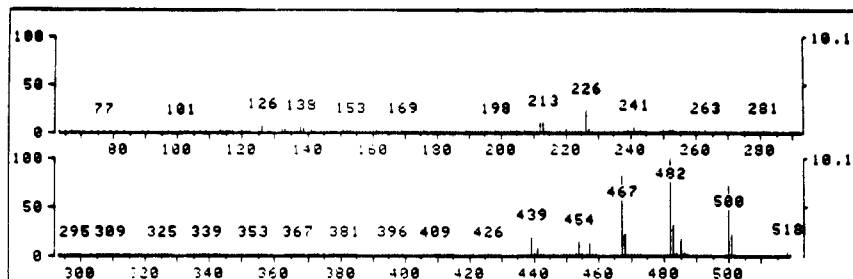
spectra were taken as cast films with a Nicolet 7000 instrument. Mass and infrared spectra were taken on 5–10- μ g quantities.

Isolation of Gossypolone from Cotton Buds. Buds (*Gossypium hirsutum*, var. ST213) were extracted at room temperature with cyclohexane/ethyl acetate/glacial acetic acid (500/500/1). The extract was back-extracted with methanol/water (3/1) so that the chlorophyll and less polar lipids remained in the upper, less polar layer, while the terpenoid aldehydes including gossypolone were located in the lower, more polar layer. The concentrate could then be analyzed by reversed-phase C_{18} high-pressure liquid chromatography (HPLC) according to the procedure of Stipanovic et al. (1988). A peak coincident with authentic gossypolone with a relative retention volume to gossypol of 0.80 accounting for 5.0% of the total terpenoids (HPLC chromatogram) gave mass spectra identical with that for the synthetic compound.

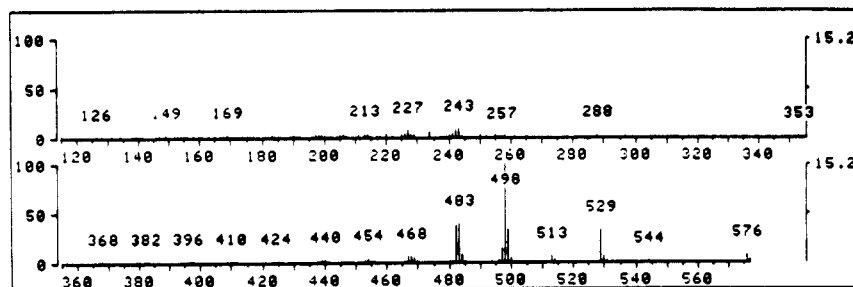
RESULTS AND DISCUSSION

The structures of gossypol and gossypolone and their MA and TMS derivatives are given in Figure 1. The mass spectra for these compounds are given in Figures 2 and 3. Table I is a summary of the IR data for G, GQ, their oximes, and methoxyamine hydrochloride.

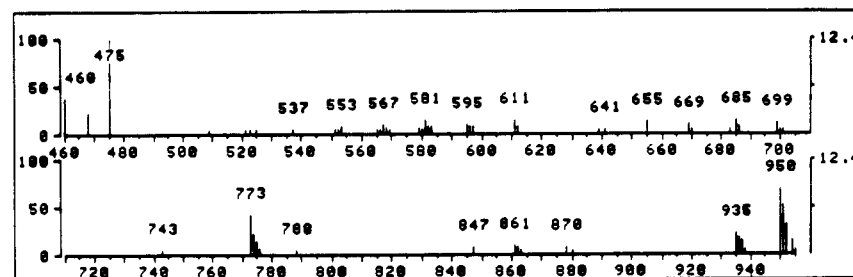
The EI mass spectrum for gossypol is essentially identical with that reported by Matlin and Zhou (1984) with a weak M^+ (m/e 518) and large fragments at m/e 500, 482, and 467 resulting from the loss of two H_2O and a CH_3^+ ion. The losses of two H_2O may be attributed to



a. Gossypol - MW = 518 amu

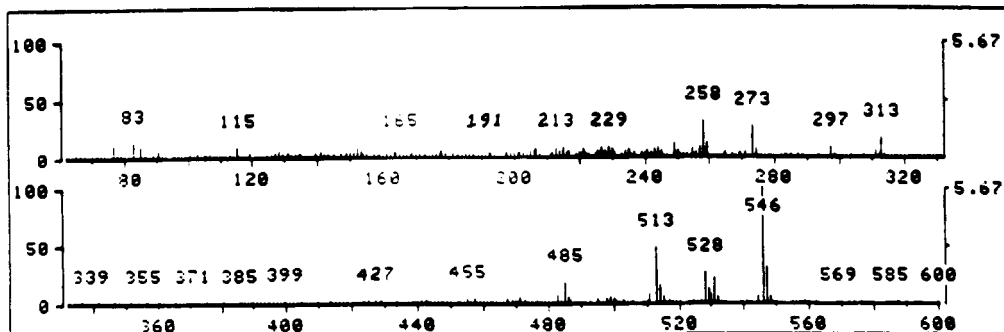


b. Gossypol-methoxyamine derivative - MW = 576 amu

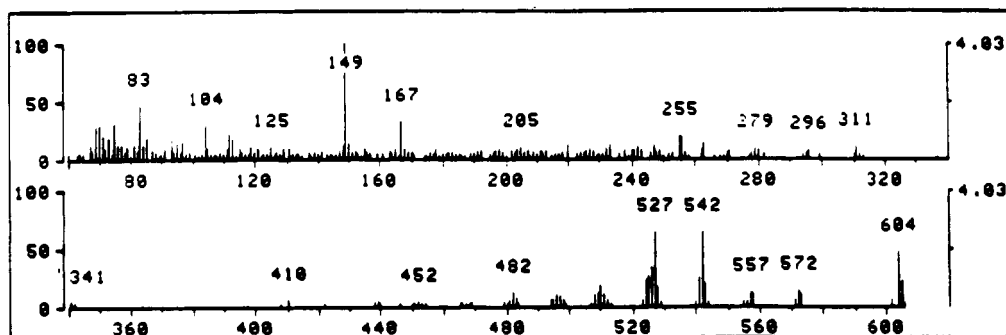


c. Gossypol-TMS derivative - MW = 950 amu

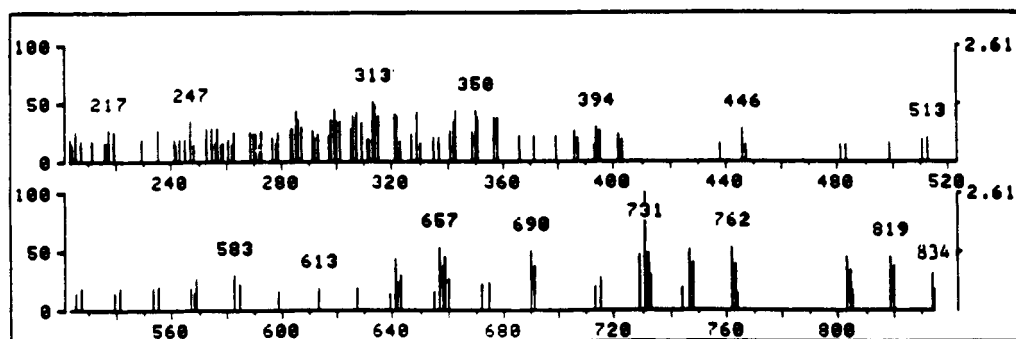
Figure 2. EI-MS of gossypol and its MA and TMS derivatives.



a. Gossypolone - MW = 546 amu



b. Gossypolone-methoxyamine derivative - MW = 604 amu



c. Gossypolone-TMS derivative - MW = 834 amu

Figure 3. EI-MS of gossypolone and its MA and TMS derivatives.

a partial thermal dehydration or ionization or both. Cleavage of the rings is indicated by fragments at m/e 226 and 213 (Figure 2).

Although gossypolone was synthesized by Haas and Shirley (1965), they did not provide mass spectral data, and only a CI-MS (NH_3) spectrum was reported by Matlin and Zhou (1984). In this work using the EI mode, the M^+ (m/e 546) was the base peak. Fragments at m/e 531, 528, and 513 result from losses of water and CH_3^+ ions. Further fragmentation, which gave m/e 485, was attributed to the loss of CO. Cleavage of the rings is indicated by fragments at m/e 273 and 258 (Figure 3).

No report could be found about the recording of mass spectra of gossypol methoxyamine (GMA) derivatives although Matlin and Zhou (1984) performed MS studies on another Schiff's base. With the gossypol oxime, the M^+ (m/e 576) was very weak. The most prominent fragments were at m/e 529 ($-\text{NH}_2\text{OCH}_3$), m/e 498, and m/e 483. Minor fragments at m/e 257, 243, and 227 suggested some cleavage of the rings.

The gossypolone methoxyamine (GQMA) derivative, by contrast, gave a much stronger M^+ (m/e 604) and fragments at m/e 573, 572, 557, 542, and 527. These fragments were generated by cleavage and/or rearrangements involving $-\text{CH}_3$, $-\text{OCH}_3$, and NH_2OCH_3 . Additional fragments at m/e 311, 296, 279, and 255 suggested some cleavage of the rings. Fragments at m/e 167 and 149 are attributed to system impurities.

The EI mass spectra for the trimethylsilyl derivatives of gossypol (G-TMS) and gossypolone (GQ-TMS) consisted of ions generated mostly by the losses of CH_3^+ , H_2O , and TMS fragments. G-TMS gave a strong M^+ (m/e 950) with significant $\text{M}^+ + 1$ and $\text{M}^+ + 2$ ions. Significant ions were present at m/e 935 ($-\text{CH}_3^+$), 878 ($-\text{1 TMS}$), 861 ($-\text{1 TMS}, -\text{1 H}_2\text{O}$), 847 ($-\text{2 CH}_3^+, -\text{1 TMS}$), 788 ($-\text{1 CH}_3^+, -\text{2 TMS}$), 773 ($-\text{2 CH}_3^+, -\text{2 TMS}$), 699 ($-\text{2 CH}_3^+, -\text{3 TMS}$), 685 ($-\text{3 CH}_3^+, -\text{3 TMS}$), 655 ($-\text{4 TMS}$), 611 ($-\text{3 CH}_3^+, -\text{4 TMS}$), 553 ($-\text{1 CH}_3^+, -\text{5 TMS}$), 475 (ring cleavage), 468 ($-\text{3 CH}_3^+, -\text{6 TMS}$), and 460 (ring cleavage, $-\text{2 CH}_3^+$). Previously, Abou-Donia et al. (1970)

reported that the major fragments were m/e 475 and 476, obtained from ring cleavage.

The mass spectrum of GQ-TMS evidently has not previously been reported. It had a significant M^+ (m/e 834) with a base peak at m/e 731 (-2 CH_3^+ , -1 TMS). Other significant ions were present at m/e 819 (-1 CH_3^+), 804 (-2 CH_3^+), 762 (-1 TMS), 747 (-1 CH_3^+ , -1 TMS), 690 (-2 TMS), 657, 659 (-2 CH_3^+ , -2 TMS), 641 (-3 CH_3^+ , -2 TMS), 613 (-3 TMS), 583 (-2 CH_3^+ , -3 TMS), and 513 (-2 CH_3^+ , -4 TMS). Cleavage fragments were not prominent, but ions at m/e 446 and 402 ($417 - \text{CH}_3^+$) were present.

The major reason for obtaining mass spectra of trimethylsilyl derivatives of polyphenolic compounds is that volatility of the molecule is enhanced, increasing the likelihood that the molecular ion will be present. While it was possible to obtain a count of the number of phenolic groups in these two instances (6 and 4), accompanying losses of CH_3^+ complicate the spectra so that the number in an unknown may not be deduced with certainty.

Table I lists the IR absorptions obtained from gossypol, gossypolone, their oximes, and methoxyamine hydrochloride. Procedures to obtain IR spectra of the trimethylsilyl derivatives were briefly explored but not pursued. The most helpful differences appear to be the absorptions in the region from 1720 to 1550 cm^{-1} that relate to the unsaturated aldehyde, quinone, and oxime functions that are present. The α,β -unsaturated aldehyde absorptions of gossypol are found as a doublet at 1620 and 1595 cm^{-1} and also appear in spectra of the related oxime (GMA). GQ and GQMA give a weak absorbance at 1715 – 1720 cm^{-1} and a strong singlet at 1640 – 1645 cm^{-1} . An oxime-related absorption at 1550 cm^{-1} appears in both GMA and GQMA. Although methoxyamine has an absorption at 1655 cm^{-1} with a shoulder at 1620 cm^{-1} , it lacks several of the absorptions in the fingerprint region that are present in the other compounds so that the overall features are clearly different.

The quinone absorptions of GQ and GQMA are found at a slightly lower wavelengths (1640 – 1655 cm^{-1}) than that reported by Silverstein et al. (1981) for quinones having carbonyl groups in the same ring (1690 – 1655 cm^{-1}). This may be attributed to the unsaturated aldehyde function in the adjoining ring.

There are a number of other evident diagnostic differences at lower wavelengths, notably 1390 , 1330 – 1340 , 1240 – 1250 , 1170 , 1070 , and 940 – 955 cm^{-1} , that relate to formation of either the oxime or the quinone function.

In summary, oxime and trimethylsilyl derivatives can easily be prepared from microquantities of G and GQ. Mass and infrared spectra obtained from these derivatives have characteristic features that are helpful for structural analysis. This approach may aid in the identification of other compounds that are found in association with gossypol.

ACKNOWLEDGMENT

We thank Harshad Anjaria at Mississippi State University for the infrared spectroscopy work.

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Received for review April 25, 1989. Accepted September 13, 1989.

Registry No. G, 303-45-7; GMA, 112725-54-9; G-TMS, 27864-30-8; GQ, 4547-72-2; GQMA, 124155-80-2; GQ-TMS, 124155-81-3.