

Mass Spectrometry of Some Gossypol Ethers

The present work represents a study of the mass spectra of gossypol hexamethyl ether and the trimethylsilyl ether of gossypol. The spectra are in full agreement with the structure of gossypol, as has

been proposed by Adams and coworkers. These reference spectra can be utilized for identification of gossypol and its metabolites in studies of gossypol metabolism in plant and animal tissues.

Gossypol is a yellow pigment occurring in cottonseed. Adams and coworkers proposed in 1938 that gossypol was a symmetrically substituted 2,2'-di-(1-naphthol) derivative. This proposed structure was verified and the compound was synthesized and showed to be 1,1',6,6',7,7'-hexahydroxy-3-3'-dimethyl-5,5'-diisopropyl-2,2'-binaphthyl-8,8'-dialdehyde (Edwards, 1958). The ultraviolet spectrum of gossypol, which supported the presence of the binaphthyl group was obtained (Frampton *et al.*, 1948). The infrared spectra of chloroform solutions of gossypol and of its 13 derivatives and degradation products strongly support the chemical evidence that gossypol occurs in tautomeric forms (O'Connor *et al.*, 1954). Recent work using nuclear magnetic resonance spectroscopy indicated that acetic acid in the crystalline gossypol acetic acid complex may be present as a clathrate since no interaction was indicated (Shirley, 1966).

Originally the purpose of this work was to present the mass spectra of gossypol and its derivatives for identification of gossypol and the metabolites in studies of gossypol metabolism in animals. Several experiments which were carried out to obtain the mass spectra of free gossypol, gossypol hexaacetate, methyl gossypol, and dianilinogossypol had failed. This may be attributed to their instability toward thermal decomposition. Gossypol hexamethyl ether and the trimethylsilyl ether derivative of gossypol were the only thermally stable gossypol derivatives tested and their mass spectra were obtained.

EXPERIMENTAL

Gossypol hexamethyl ether (m.p. 175° C) was prepared by the reaction of gossypol acetic acid and dimethyl sulfate according to the method of Adams *et al.* (1938). Trimethyl-

silyl ether derivative of gossypol was easily formed by reaction of gossypol acetic acid and N,O-bis(trimethylsilyl) acetamide in carbon disulfide according to the method of Raju and Cater (1967).

The spectra were recorded in triplicate at 70 e.v. on a CEC 21-110B high resolution mass spectrometer. Gossypol hexamethyl ether was run with an all-glass probe at a temperature of 180° C and a source temperature of 200° C. The trimethylsilyl ether derivative of gossypol was run at a probe temperature of 160° C and a source temperature of 190° C.

RESULTS AND DISCUSSION

The mass spectrum of gossypol hexamethyl ether, Figure 1,

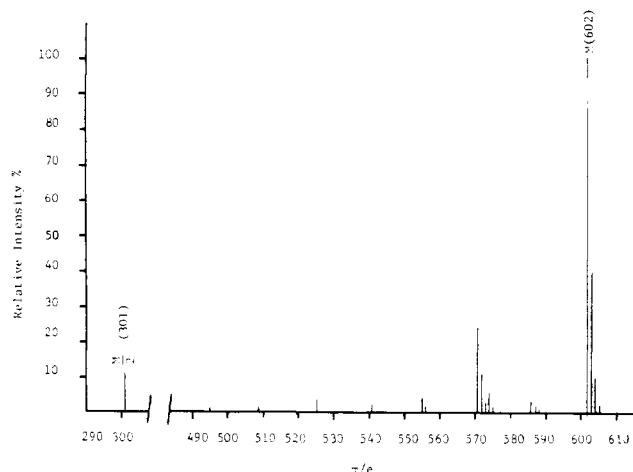
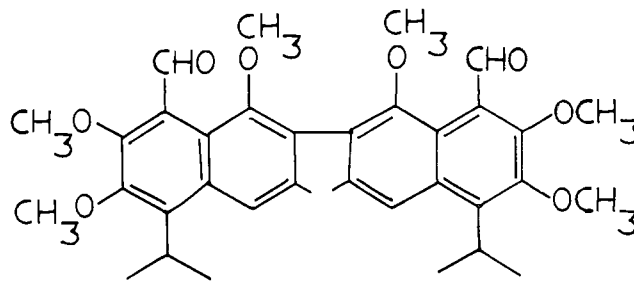


Figure 1. The mass spectrum of gossypol hexamethyl ether

is relatively simple. Table I lists the major relative mass intensities *vs.* mass/charge. An intense molecular ion peak ($m/e = 602$) reflects the chemical stability of the molecule and is probably due to charge stabilization through resonance in the polycyclic structure of the molecule. A medium intensity peak M-31 at m/e 571 and a less intense peak at 301 were recorded. Most other peaks were of low intensity. The m/e 301 peak indicates that a dimer is present, *i.e.*, the m/e 301 fragment is one-half the mass of the molecular ion. Also the ion at m/e 301 may be a doubly charged molecular

Table I. Relative Mass Intensities *vs.* Mass/Charge for Gossypol Hexamethyl Ether^a

m/e	Relative Intensity
31	3.6
262	4.5
301	10.6
571	23.4
572	10.9
574	5.3
602	100.0
603	39.4

^a This table includes only those m/e in which at least one compound exhibits a relative intensity greater than 3%.

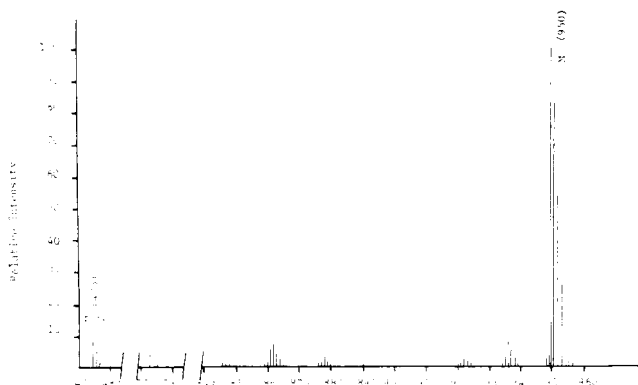
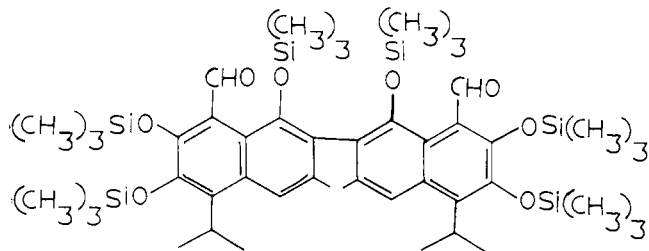


Figure 2. The mass spectrum of the trimethylsilyl ether of gossypol

ion, a species common in large aromatic systems. A check on this point is whether there is a sufficiently intense peak at m/e 301.5 corresponding to the double charged ^{13}C isotope, *i.e.*, m/e 603. The relative intensity of the 301.5 to 301 peaks should be similar to that of the 603 to 602 peaks, if the 602 mass compound gives a double charged 301 ion. This hypothesis is not valid since the spectrum shows a very weak peak at 301.5. This supports the argument constructed on the dimeric nature of the compound. The M-31 peak (m/e 571) indicates the presence of one or more CH_3O groups. M/e 602 was measured at high resolution and was found to be 602.2836. Possible elemental compositions were taken from Lederberg. On the assumption that nitrogen is absent, two CHO combinations must be considered. These are; $\text{C}_{43}\text{H}_{38}\text{O}_3$ calc. 602.2821 and $\text{C}_{38}\text{H}_{42}\text{O}_3$ calc. 602.2880. The dimeric nature of the molecule rules out the possibility of an odd number of oxygens; therefore, the elemental composition of the molecule must be $\text{C}_{38}\text{H}_{42}\text{O}_3$. The structural information is in complete agreement with structural formula (I). The ion at m/e 571—the second most abundant fragment-ion in the spectrum—was formed by loss of a methoxyl radical from the molecular ion. It is noted that it is not possible to determine which methoxyl group was cleaved. Alternatively, the molecular ion could expel methoxyl ion with m/e 31. The peak at m/e 587 was formed from the molecular ion by losing a methyl radical. Also, the molecular ion could lose an isopropylene group to form the peak observed at m/e 41.

The mass spectrum of trimethylsilyl ether of gossypol, Figure 2, is recognized from the "isotopic clusters" produced



II

by the presence of six silicon atoms. Table II lists the major relative mass intensities *vs.* mass/charge. The molecular ion recorded the most intense peak at m/e 950, which reflects the stability of this gossypol derivative. The ion at m/e 73—the second most intense peak in the spectrum—and the ion at m/e 147 arise from the trimethylsilyl group and do not contain structural information. The observed peak of m/e 475,

Table II. Relative Mass Intensities *vs.* Mass/Charge for Trimethylsilyl Ether of Gossypol^a

m/e	Relative Intensity
29	3.6
45	3.4
73	62.2
74	5.6
75	17.1
77	3.0
147	14.6
475	11.6
476	5.7
861	5.6
862	7.3
863	4.6
878	3.2
935	9.8
936	9.1
937	6.1
938	3.2
949	3.7
950	100.0
951	82.9
952	53.7
953	25.6
954	6.1
955	3.7

^a This table includes only those m/e in which at least one compound exhibits a relative intensity greater than 3%.

which is one-half the mass of the molecular ion, is in harmony with the dimeric nature of the parent compound. The ion at m/e 935 was formed by loss of a methyl radical from the molecular ion. The peak at m/e 862 was formed by a subsequent loss of trimethylsilyl group from the ion 935. In this case it is not possible to determine which trimethylsilyl group was lost. The remaining fragment peaks were of very low intensity. The spectrum is characteristic of a polycyclic structure and is in agreement with the structural formula (II), with a molecular weight of 950, and elemental composition of $\text{C}_{38}\text{H}_{78}\text{O}_3\text{Si}_6$, although groups cannot be located. Because of the absence of mass markers in the 950 region, an elemental composition was not obtained.

ACKNOWLEDGMENT

Acknowledgment is made to Dr. R. D. Grigsby for running the mass spectra. This study was supported by USDA-ARS Contract No. 12-14-100-9497(72).

LITERATURE CITED

- Adams, R., Geissman, T. A., Morris, R. G., *J. Amer. Chem. Soc.* **60**, 2967 (1938).
 Adams, R., Morris, R. C., Geissman, T. A., Butterbaugh, D. J., Kirkpatrick, E. C., *J. Amer. Chem. Soc.* **60**, 2193 (1938).
 Edwards, J. D., Jr., *J. Amer. Chem. Soc.* **80**, 3798 (1958).
 Frampton, B. L., Edwards, J. D., Jr., Henze, H. R., *J. Amer. Chem. Soc.* **70**, 3944 (1948).
 Lederberg, J., "Computation of Molecular Formulas from Mass Spectrometry," Holden-Day, San Francisco, Calif. (1964).
 O'Connor, R. T., Haar, P. V., Dupre, E. F., Brown, L. E., Pominiski, C. H., *J. Amer. Chem. Soc.* **76**, 2368 (1954).
 Raju, P. K., Cater, C. M., *J. Amer. Oil Chem. Soc.* **44**, 465 (1967).
 Shirley, D. A., Proc. of Conf. on Inactivation of Gossypol with Mineral Salts, p. 11, New Orleans, La., April 4-5, 1966.

Mohamed B. Abou-Donia
 Julius W. Dieckert
 Carl M. Lyman¹

Department of Biochemistry and Biophysics
 Texas A&M University
 College Station, Texas 77843

¹ Deceased.

Received for review September 22, 1969. Accepted February 12, 1970.