Constituents of the Cotton Bud

Mass Spectrometric Identification of the Major High-Molecular-Weight Hydrocarbons in Buds and Flowers

R. F. Struck, J. L. Frye, and Y. F. Shealy

Hydrocarbon fractions of cotton buds and flowers (*Gossypium hirsutum* var. Deltapine Smoothleaf) were obtained by petroleum ether extraction, separated from nonhydrocarbon components by thin-layer chromatography, and examined by gas chromatographic and mass spectral techniques. The major high-molecular-weight hydrocarbons were shown unequivocally to be C_{23}, C_{25}, C_{27}, C_{29}, and C_{31} on the basis of a combination of retention time data and high-resolution mass measurements. Even-numbered hydrocarbons (C_{24} to C_{32}), although present, constituted a relatively minor portion of the total hydrocarbon fraction.

During our investigation of the components of buds and flowers of *Gossypium hirsutum* for feeding-stimulant activity for the boll weevil, *Anthonomus grandis* Boheman, we examined petroleum ether extracts of these plant materials and noted significant activity in such extracts (Struck *et al.*, 1968). Thin-layer chromatographic separation of the extracts gave fractions whose infrared and proton magnetic resonance spectra showed only typical hydrocarbon bands, and elemental analysis gave carbon and hydrogen analyses totaling 98 to 100%. Although the hydrocarbon fraction lacked feeding-stimulant activity for the boll weevil (Temple *et al.*, 1968), we were interested in verifying the only previous reports (Power and Chesnut, 1925, 1926; Sadykov, 1965; Sadykov *et al.*, 1964) in the literature of the hydrocarbons from comparable plant parts. This paper describes the identification of the major high-molecular-weight hydrocarbons from cotton buds and flowers using gas chromatographic and mass spectrometric techniques. Such a combination of techniques seemed advisable in view of recent reports (Farnsworth *et al.*, 1967b) which emphasize the need for use of proper identification methods before unequivocal identification of high-molecular-weight hydrocarbons can be claimed.

Two hydrocarbons, which were reported (Sadykov *et al.*, 1964) as $C_{30}H_{62}$ and $C_{35}H_{72}$ on the basis of infrared, melting point, and elemental analytical data alone, had previously been isolated from cotton flowers. Sadykov (1965) also reported that tetracosane, hexacosane, octacosane, triacontane, dotriacontane, and hexatriacontane were isolated from the leaves, bolls, and flowers of *Gossypium* species but gave no details on the method of identification. Power and Chesnut (1925, 1926) reported the isolation of triacontane and pentatriacontane from cotton foliage, squares, and flowers and based their identification on melting point and elemental analytical data; however, Chibnall *et al.* (1934) later showed that both solids were mixtures of paraffins. Using gas chromatography, Kuksis (1964) examined the hydrocarbon fraction of refined cottonseed oil and identified many components by cochromatography with known standards, relative retention times, or certain other demonstrated characteristics of hydrocarbons in gas chromatographic systems. Fargher and Probert (1923) reported the isolation of crystalline triacontane and hentriacontane from cotton fiber.

Southern Research Institute, Birmingham, Ala. 35205

EXPERIMENTAL

Apparatus. Gas chromatography (GLC) was performed with an F & M Model 5756A research chromatograph. Mass spectral measurements were obtained with a Hitachi high resolution double-focusing mass spectrometer, RMU-6D-3, equipped with peak-matching device and mass marker. Thin-layer chromatographic (TLC) purification of extracts was performed on E. Merck silica gel precoated preparative layer glass plates obtained from Brinkmann Instruments, Inc.

Isolation of Hydrocarbon Fractions. Deltapine Smoothleaf cotton buds or flowers (whole or ground) were extracted with petroleum ether (b.p. 30° to 60° C.) by stirring overnight at room temperature. After filtration, the petroleum ether extracts were evaporated to dryness in vacuo, and the waxy residues were applied to silica gel plates (100 mg. per plate). Development in petroleum ether (b.p. 30° to 60° C.) produced a band at the solvent front which was collected, stirred 15 minutes with petroleum ether, and filtered. Evaporation of the filtrate gave a colorless, waxy semisolid, which served as the starting material for the GLC and mass spectral examination. Such a procedure produced approximately 10 mg. of purified hydrocarbons per plate.

Gas Chromatographic Operating Conditions. Purified hydrocarbon fractions from cotton buds and flowers were separated on a $^1/_8$-inch × 6-foot stainless steel column containing 10% (w./w.) Union Carbide W-98 (methyl vinyl silicone gum rubber) on F and M Diaport S. Carrier gas was helium at a flow rate of 22 ml. per minute and inlet pressure of 40 p.s.i.g. Detection was by thermal conductivity cell at 300° C. and cell current of 150 ma. Injection

port temperature was 330° C., and column temperature was maintained at 310° C. Two of the major hydrocarbon bands were collected in capillary tubes during GLC operation for mass spectral examination as purified, individual hydrocarbons.

RESULTS AND DISCUSSION

Gas Chromatographic Analysis. The total hydrocarbon fractions were analyzed by gas chromatography. Table I lists retention times and percentages of the components present in the mixtures. Peak identities are also included as adjudged from cochromatography with known standards (n-C_{24}, n-C_{28}, n-C_{32}, and n-C_{36}). On the basis of retention time data and cochromatography, it appeared that, although the even-numbered hydrocarbons reported by Sadykov (1965) are present, they are by far in the minority as compared with the odd-numbered hydrocarbons. Table I shows also that the buds are slightly richer in the higher molecular weight hydrocarbons than the flowers.

The total hydrocarbon fractions could be conveniently crystallized from absolute ethanol and yielded white solids with melting points of approximately 50° to 60° C. Such treatment eliminated most of the lower molecular weight hydrocarbons (approximately C_{13} to C_{21}) which were present in the total fractions and were observable as minor components in the chromatograms of the total fractions; the method of preparation of the various fractions, which included drying in vacuo, also removed some of the lower hydrocarbons. GLC separation of the crystallized hydrocarbon fractions gave the results shown in Table I. Figure 1 is typical of chromatograms obtained from the crystalline samples. As expected, the ethanol filtrates contained a higher concentration of the lower molecular weight hydrocarbons, as illustrated by the filtrate from the bud hydrocarbon crystallization (Table I).

Mass Spectrometric Analysis. To confirm the identities of the major hydrocarbons found in cotton buds and flowers, two of the most abundant hydrocarbons (C_{27} and C_{29}) were collected during the GLC examination for subsequent mass spectral examination. Low resolution mass spectra showed that the two hydrocarbons produced

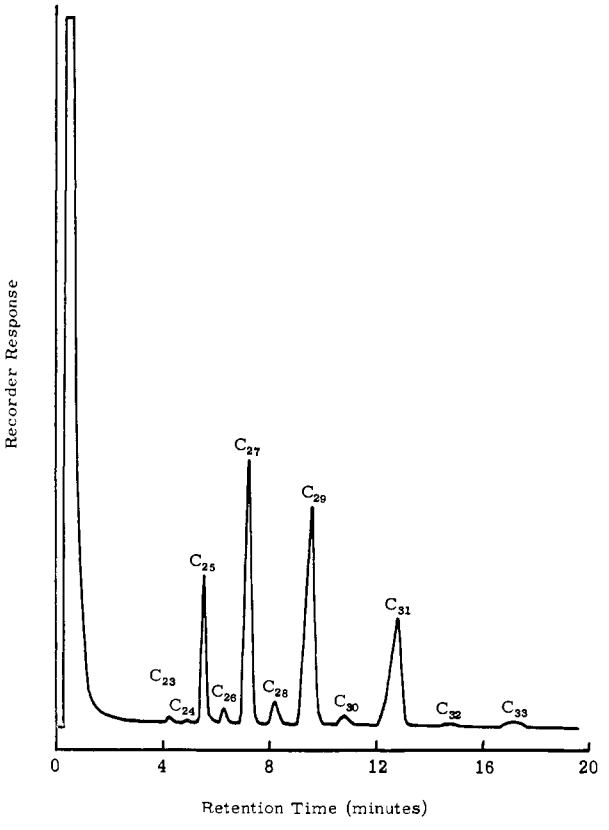

Figure 1. GLC of crystallized bud hydrocarbon fraction

molecular ions of 380 and 408, respectively, in accord with molecular formulas of $C_{27}H_{56}$ and $C_{29}H_{60}$. In addition, the mass fragmentation patterns were identical to typical straight-chain hydrocarbon fragmentation patterns—for example, Bieman (1962)—as shown in Figure 2 for $C_{29}H_{60}$, and indicated that the major hydrocarbons are normal isomers.

Unequivocal identification of the major hydrocarbons (n-C_{23}, n-C_{25}, n-C_{27}, n-C_{29}, and n-C_{31}) in cotton buds and flowers was obtained by resorting to high resolution mass

Table I. GLC of Hydrocarbon Fractions from Cotton Buds and Flowers

	Total Hydrocarbon Fractions			Crystallized Hydrocarbon Fractions			
	% Composition			% Composition			
				Buds			
Identity	Buds	Flowers	T_R, min.[a]	Solid	Filtrate	Flowers (solid)	T_R, min.[a]
C_{23}	5.6	9.2	1.8	1.4	17.5	<1	4.2
C_{24}	<3	<3	2.2	1.4	3.9	<1	4.7
C_{25}	16.3	27.0	2.6	9.9	32.0	2.5	5.7
C_{26}	<3	<3	3.2	2.8	2.9	1.9	6.3
C_{27}	30.0	42.2	3.8	28.2	22.3	30.2	7.2
C_{28}	<3	<3	4.6	4.2	1.9	4.4	8.1
C_{29}	28.6	16.5	5.4	31.0	12.6	37.7	9.5
C_{30}	<3	<3	6.6	2.8	<1	1.9	10.8
C_{31}	8.6	3.5	8.0	16.9	4.9	16.4	12.5
C_{32}	<3	<3	9.6	<1	<1	<1	15.0
C_{33}	...	...	...	1.4	<1	3.1	17.0

[a] Differences in T_R for same component in total and crystallized hydrocarbon fractions—e.g., 1.8 *vs.* 4.2 for C_{23}—arose as a result of extensive use of the column and a time lapse of several months between GLC measurements; significant conditioning of the column and bleeding of the stationary phase are not unexpected for a column maintained at 310° for extended periods. Since known standards were used in both instances, identity of the components is valid. It becomes obvious from comparison of the two sets of data that the crystallized fraction was examined first.

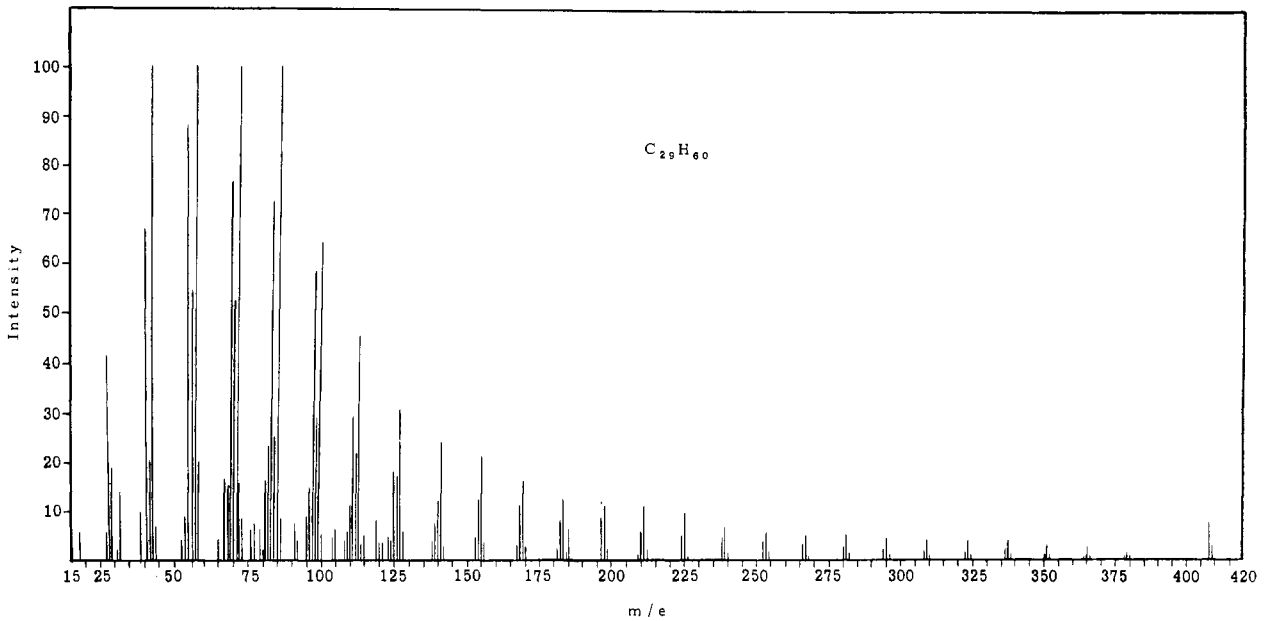

Figure 2. Mass spectrum of $C_{29}H_{60}$ isolated from a cotton flower hydrocarbon fraction

measurements. Certain oxygenated cyclic and unsaturated structures are permissible from whole mass unit measurements in this molecular weight range and would not be excluded by low resolution mass spectrometry; high resolution mass spectrometry showed these structures to be untenable from observed *m/e* values, even apart from retention time, infrared, and elemental analytical data. Mass measurements of the major hydrocarbons, in isolated form or in the total hydrocarbon mixtures, gave the results shown in Table II. High resolution mass spectrometry, then, can serve as a means of establishing molecular formulas of high molecular weight hydrocarbons even in total natural product extracts.

Refined cottonseed oil is also rich in some (C_{19}, C_{21}, and C_{29}) of the odd-numbered hydrocarbons (Kuksis, 1964) but differs significantly from buds and flowers in that the total amounts of C_{23}, C_{25}, and C_{27} (including normal, iso, ante-iso, and cyclohexyl types) are less than 1%; another significant difference is the presence of relatively large amounts of the even-numbered hydrocarbons C_{20} and C_{30}. Many workers (Del Castillo *et al.*, 1967; Eglinton *et al.*, 1962a, b; Farnsworth *et al.*, 1967a; Hargreaves, 1966; Manni and Sinsheimer, 1965; Suzuki *et al.*, 1966) have likewise found that the odd carbon atom compounds are predominant in other plants.

Table II. High-Resolution Mass Measurements of Molecular Ions of Major Hydrocarbons in Cotton Buds and Flowers

Peak Measured, M_0^+	High Resolution Mass, *m/e*		Identity
	Found	Calcd.	
324	324.377 ± 0.003	324.376	$C_{23}H_{48}$
352	352.409 ± 0.002	352.407	$C_{25}H_{52}$
380	380.441 ± 0.005	380.438	$C_{27}H_{56}$
408	408.470 ± 0.002	408.470	$C_{29}H_{60}$
436	436.499 ± 0.002	436.501	$C_{31}H_{64}$

ACKNOWLEDGMENT

The authors thank W. C. Coburn, Jr., and M. C. Kirk for the mass spectral measurements and C. H. Gwin, R. P. Robinson, and W. R. Laseter for the gas chromatographic analysis.

LITERATURE CITED

Bieman, K., "Mass Spectrometry. Organic Chemical Applications," p. 79, McGraw-Hill, New York, 1962.
Chibnall, A. C., Piper, S. H., Pollard, A., Williams, E. F., *Biochem. J.* **28**, 2189 (1934).
Del Castillo, J. B., Brooks, J. W., Cambia, R. C., Eglinton, G., Hamilton, R. J., Pellitt, P., *Phytochemistry* **6**, 391 (1967).
Eglinton, G., Gonzalez, A. G., Hamilton, R. J., Raphael, R. A., *Phytochemistry* **1**, 89 (1962a).
Eglinton, G., Hamilton, R. J., Martin-Smith, M., *Phytochemistry* **1**, 137 (1962b).
Fargher, R. G., Probert, M. E., *J. Textile Inst.* **14**, 49 (1923).
Farnsworth, N. R., Wagner, H., Hörhammer, L., Hörhammer, H.-P., *J. Pharm. Sci.* **56**, 1369 (1967a).
Farnsworth, N. R., Wagner, H., Hörhammer, L., Hörhammer, H.-P., Constantine, G. H., Jr., *J. Pharm. Sci.* **56**, 1534 (1967b).
Hargreaves, K. R., *Nature* **211**, 417 (1966).
Kuksis, A., *Biochemistry* **3**, 1086 (1964).
Manni, P. E., Sinsheimer, J. E., *J. Pharm. Sci.* **54**, 1541 (1965).
Power, F. B., Chesnut, V. K., *J. Am. Chem. Soc.* **47**, 1751 (1925); **48**, 2721 (1926).
Sadykov, A. S., *J. Sci. Ind. Res. India* **24**, 77 (1965).
Sadykov, A. S., Padkudina, Z. P., *Nauchn. Tr., Tashkentsk. Gos. Univ.* No. **263**, 88 (1964); *Chem. Abstr.* **62**, 16553d (1965).
Struck, R. F., Frye, J. L., Shealy, Y. F., Hedin, P. A., Thompson, A. C., Minyard, J. P., *J. Econ. Entomol.* **61**, 270 (1968).
Suzuki, M., Ikekawa, N., Akaike, E., Tsuda, K., *Chem. Pharm. Bull. (Tokyo)* **14**, 837 (1966).
Temple, C. G., Roberts, E. C., Frye, J. L., Struck, R. F., Shealy, Y. F., Thompson, A. C., Minyard, J. P., Hedin, P. A., *J. Econ. Entomol.* **61** (1968), in press.

Received for review May 13, 1968. Accepted August 14, 1968. Part XIV in a series entitled "Constituents of the Cotton Bud." Part XIII is "Further Studies on a Non-polar Boll Weevil Feeding Stimulant," C. G. Temple, E. C. Roberts, J. L. Frye, R. F. Struck, Y. F. Shealy, A. C. Thompson, J. P. Minyard, and P. A. Hedin, J. Econ. Entomol. **61** *(1968), in press. Investigation performed in cooperation with the Boll Weevil Research Laboratory, Entomology Research Division, U. S. Department of Agriculture, State College, Miss., supported by the Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture; the National Cotton Council of America; and the institute-sponsored research program of Southern Research Institute.*