Constituents of the Cotton Bud. The Carbonyl Compounds

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Carbonyl compounds isolated from the buds (squares) of Deltapine Smoothleaf cotton by steam distillation, column chromatography, and Girard T procedure were identified by reaction gas chromatographic techniques, by thin layer chromatography of derivatives, and by ultraviolet and infrared spectroscopy. The 14 components identified of the 40 found present accounted for 88% of the carbonyl fraction, which itself constitutes 1.4% of the square oil. A qualitative survey of the total oil composition is presented. Carbonyl compounds identified and their quantity (parts per million) in the oil were: acetone (98), isobutyraldehyde (14), butyraldehyde (56), isovaleraldehyde (70), hexanal (6900), heptanal (1050), *trans*-2-hexenal (2690), nonanal (252), 2-octenal (56), benzaldehyde (238), 2-nonenal (406), *trans*-2-*cis*-6-nonadienal (364), *p*-tolualdehyde (28), and myrtenal (56).

Carbonyl compounds are important contributors to the flavor and aroma of foods and plants, frequently to a degree well out of proportion to the relative amounts present (*1, 7, 19*). As in the present case, the quantities of individual aldehydes and ketones in the essential oils are often so small that trapping them from a gas chromatograph for identification by their infrared or NMR spectra is very difficult. Mass spectrographic examination of the gas chromatographic effluent is probably the method of choice for such identification, but the required instrumentation is not generally available. When this technique cannot be used, other sensitive but less specific methods of analysis such as gas liquid chromatography (GLC) and thin layer chromatography (TLC) must be employed. A variety of such methods has been used in this work.

Ample laboratory (*9, 11*) and field (*17*) evidence exists for the presence of a volatile attractant in the cotton plant, *Gossypium* sp., for the boll weevil, *Anthonomus grandis* Boheman. This laboratory is investigating cotton plant constituents to elucidate the nature of those which cause such attraction or other specific host plant–insect behavior. In a continuing study of the aroma profile of the cotton plant (*15, 16*), the authors have isolated and identified a number of carbonyl compounds from the cotton square (flower bud). In addition, this report describes the preliminary fractionation and gives a quantitative survey of the total cotton volatile oil. The entire carbonyl fraction from this oil was unattractive to the boll weevil (*9*).

Experimental

Apparatus. The gas chromatographic and carbon skeleton chromatographic equipment employed (*15, 16*) was modified to allow trapping and derivative formation at the exhaust. Gas chromatographic operating conditions are listed in Table I.

Spectra were obtained with a Beckman DK-2A ratio recording spectrophotometer in 1.0-cm. matched silica cells, a Beckman IR-5A infrared spectrophotometer, and a Varian A-60 analytial NMR spectrometer. TLC plates were prepared with Brinkmann apparatus on 20 × 20 cm. glass, 250-micron bed depth, and developed in the ascending fashion in solvent vapor saturated chambers to a height of 10 cm. from the start.

Isolation of the Cotton Square Oil. An enlarged version of the still described previously (*15*) was constructed from two 55-gallon drums. The horizontal steam boiler passed steam into the vertical distillation drum through a manifold and four $^1/_2$-inch pipes which extended to the bottom of the container. The top of the distillation drum was removable and was fitted with a snap-lock ring clamp and a gum rubber gasket to ensure a steam-tight enclosure. Distillate passed through a 1.25-cm. o.d. copper tube in a 7-meter long water-jacketed condenser to an ice chilled 22-liter distilling flask receiver, which was connected in turn to a 4-liter vacuum flask in an acetone–dry ice bath. Gas burners (ca. 38,000 kcal. per hour) heated the two vessels. The entire apparaus was thoroughly cleaned with several solvents and by distilling water through it before use.

Deltapine Smoothleaf cotton buds (50 kg.) ground to pass a 1-cm. mesh screen in a tractor-driven hammermill were steam-distilled for 1.5 hours. The 20 liters of distillate were extracted with dichloromethane and worked up as before (*17*). A total of 2975 kg. (6550 pound) of squares gave 569 grams of crude extract. Vacuum distillation showed that 19.1% of the crude extract was solvent, indicating a true yield of 460 grams (0.0155%) of essential oil, d_{25} 0.952.

Initial GLC surveys of the qualitative composition of the first crude square oil obtained (*4*) were conducted on a temperature-programmed SE-30 column in an Aerograph A-700 instrument (Table I). Kováts' indices, I_K, (*12*) of peaks and odors of materials emerging at the exhaust gave several clues to component identities. Reaction gas chromatographic techniques (*5, 23*) used to detect specific compound classes among the GLC peaks supported these and showed that a large number of carbonyls were present in small concentrations.

Volatile Carbonyl Separations and Derivative Formation. Two distinct approaches to the problem of isolation and identification of the carbonyl compounds were followed. The volatile carbonyls were isolated effectively as less volatile derivatives, whereas the less volatile materials were isolated in the free state with little loss, and as such permitted a greater variety of

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Table I. Gas Chromatographic Operating Conditions

	Carbowax 4000	Apiezon L	DNPH SE-30	Survey SE-30	Carbon Skeleton, Apiezon L
Detector	H_2FID[a]	H_2FID	EC,[a] H_2FID	TC[a]	H_2FID
Column length, ft.	10	10	5	20	20
Column diam., in.	1/4	1/4	1/8	3/8	1/8
Column material	Al	Al	S.S.	Al	Cu
Per cent stationary phase (w./w.)	28.5	20.0	5.0	10.0	20.0
Solid support	A[b]	A	B[b]	B	A
Carrier gas; flow rate, ml./min.	N_2; 65	N_2; 65	N_2; 32	He; 200	H_2; 20
Inlet pressure,[c] p.s.i.g.	12	12	30	50	18
Temperatures, ° C.					
Column	(a) 105°; (b) 140°; (c) 100–175° at 2°/ min., 175° iso.		230	150°/10 min.; 150–200° at 15°/ min.; 200°/16 min.; 200–240° at 10°/min.	68, 120
Injector	265		270	270	290
Detector	220		230	252	68, 120

[a] FID, flame ionization detector; EC, electron capture; TC, thermal conductivity.
[b] A, 60 -to 80-mesh Gas Chrom P, hexamethyldisilazane treated.
B, 60- to 80-mesh Chromasorb W, hexamethyldisilazane treated.
[c] Outlet pressure, 0 p.s.i.g.

characterization tests, including derivative formation.

Crude square oil (10 ml.) was chromatographed in 250-μl. portions on SE-30, and 29 2,4-dinitrophenylhydrazone (DNPH) derivative fractions were prepared by bubbling the column effluent into separate vials containing reagent in phosphoric acid (*10*). Each derivative sample was filtered, washed with a minimum of water, and put into a small quantity of methanol. Several of the more volatile carbonyls gave derivatives in sufficient quantity to allow recrystallization from methanol and melting point determinations on a Fisher-Johns block.

The DNPH's were spotted and developed ont wo TLC systems: Silica gel G, benzene–petroleum ether (38°–50° C.), 4 to 1 v./v., and polyamide, methanol–water, 95 to 5 v./v. Standard derivatives and dye test mixture (Stahl) were spotted adjacent to unknowns of suspected identity. All TLC migration data were expressed as R_y values relative to butter yellow.

Each of the first six DNPH fractions in methanol was streaked on silica gel G TLC plates and developed in benzene–petroleum ether, 4 to 1. The bands on the resulting chromatograms were scraped off individually, and equivalent bands from a fraction were composited. These subfractions were eluted from the adsorbent with a minimum of anhydrous methanol and examined by GLC on the 5% SE-30 column (Table I), splitting the exhaust between electron capture and flame ionization detectors (*21*). Standards were run in the same way and GLC retention data were recorded as Kováts' indices. Fractions above number 6 were not examined in this manner because the carbonyls in fraction 6 were as large as nonanal and GLC retention times of the DNPHs were nearly 30 minutes.

Though this technique proved to be quite useful for the low molecular weight compounds, Girard T isolation of the free carbonyls appeared to be a more profitable route for identification of compounds of higher mass. This second phase of the study was undertaken as follows.

Column Fractionation of Square Oil. As part of the total analytical program, the crude square extract was fractionated as described by Kugler and Kováts (*13*) on Baker Analyzed silica gel coated with Carbowax 20M (0.75%, w./w.). The coated adsorbent was washed exhaustively with methanol, filtered, and dried at 125° C. A 4-cm. i.d. × 50-cm. Kontes Chromaflex extender column fitted with an adapter, frit, O-ring, and clamp was filled with adsorbent (387 grams) to within 0.5 cm. of the top. A 4-cm. circle of filter paper was placed over the smoothed adsorbent surface, after which a pad of glass wool, a second glass frit, O-ring, adapter, and clamp were added. This column was used as described (*13*) with a 1-liter separatory funnel solvent reservoir and a 2-way stopcock outlet.

Crude square oil (100 ml.) loaded onto the evacuated column from the bottom was eluted successively by 1500 ml. of pentane (α fraction) and 1900 ml. of 2-chloropropane (β fraction). The column then was inverted, and elution was continued with 1750 ml. of methanol (γ fraction). A total of 460 ml. of crude square oil was separated in this manner, and equivalent fractions were composited. Table II indicates the expected qualitative composition of these fractions. Solvent was removed

Table II. Quantitative Composition of Oil from Deltapine Smoothleaf Cotton Buds[a]

Fraction	Expected Chemical Classes	Per Cent of True Square Oil[a]	Amount in Plant, P.P.M.
α	Hydrocarbons; terpenes, sesquiterpenes	48.8	76
β_1	Carbonyl compounds	1.4	2
β_2	Larger esters, ethers, epoxides, etc.	6.7	10
γ	Small esters, alcohols, polyfunctional compounds, very high polarity compounds	43.1	67
		Total	155

[a] Based on "true square oil"–i.e., crude dichloromethane extract of steam distillate corrected for residual solvent contents.

from the α and β fractions at 35° C. under vacuum, and all fractions were stored at 0° C. until further use.

Isolation of Carbonyl Compounds from the β Fraction. To the composited fraction, expected to contain the carbonyl compounds, were added anhydrous methanol (100 ml.), (carboxymethyl)trimethylammonium chloride, hydrazide (Girard's reagent T, 70 grams), and Amberlite IRC-50 ion exchange resin (2.0 grams) (*22*). After refluxing the mixture for 1.5 hours, the clear yellow solution was decanted into 400 ml. of distilled water and extracted four times with 200-ml. portions of dichloromethane. The aqueous phase was added to 1.6 liters of formalin, allowed to stand overnight, and extracted six times with a total of 1800 ml. of pentane. The pentane was washed five times each with 400 ml. of water and removed under vacuum at 15° C. to give 30 ml. of solution containing 5.2 grams of the carbonyls (fraction β_1). The solvent from the dichloromethane extract was removed similarly to give 50 ml. of fraction β_2, which was stored at −27° C. for future studies. The β_1 fraction was stored at −27° C. under nitrogen in a flask wrapped with aluminum foil and equipped with a serum bottle septum for sample removal. A reduced scale blank preparation on all reagents and solvents was run in a similar manner.

In a preliminary study, 20.1 grams of crude square oil extract was carried through the Kováts column and Girard T. separations with reagent ratios as before. The resulting fractions were weighed after complete solvent removal and the gross analytical composition of the true cotton square oil calculated (Table II).

Separation and Derivative Formation on β_1 Carbonyls. Thirty peaks appeared in the temperature-programmed gas chromatogram of fraction β_1 on Carbowax 4000 (Table I, Figure 1). Five of these were also present in the reagent blank. Each peak was trapped at dry ice–acetone temperature in a separate trap-injector constructed essentially as described by Beroza (*4*), bracketing paraffins were added, and reinjected on an Apiezon L column. Similarly, each temperature-programmed Carbowax fraction was trapped and rechromatographed isothermally on Carbowax. I_k values were calculated for peaks produced on both columns, which were maintained at 140° C. for components through number 11 (nonanal) and at 175° C. for the others. When a single Carbowax maximum gave multiple peaks on Apiezon L, each component was retrapped as needed and checked on both columns to determine that it was not a degradation artifact. Separation in this manner indicated that β_1 contained at least 40 components. These components, purified as needed on both columns, were finally trapped for carbon skeleton determination (*3*).

DNPH's of the β_1 components were prepared directly on silica gel G TLC plates at the column exhaust. A drop of the phosphoric acid reagent was spotted at the starting line, and the emerging component was directed at the drop center at an exhaust to plate distance of 1

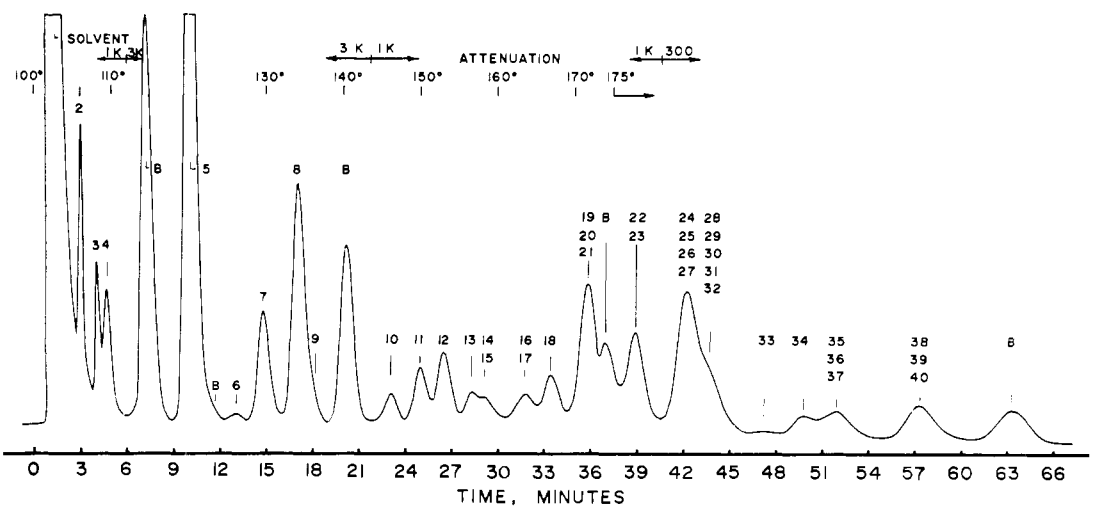

Figure 1. Temperature-programmed chromatogram of fraction β_1 of cotton square oil on Carbowax 4000

Conditions, Table I; peak identifications, Table IV

mm. Immediate formation of the derivative was usually apparent. After development of the plates in benzene–petroleum ether, 4 to 1, the derivative runs on Carbowax and Apiezon L with intermediate trapping and reinjection as necessary gave DNPH's of nearly all components from 8 ml. of β_1. Standard DNPH's were prepared and purified for comparison, some by the GLC-TLC method. Visible absorption spectra of DNPH's eluted from the TLC adsorbent with methylal were determined in methylal and compared with standards determined in the same way.

The DNPH's in methylal were spotted and developed on three separate TLC systems, along with dye test mixture and standards. Two of these systems have been described; the third was silica gel G coated with Carbowax 600 (*1*), developed with heptane–benzene, 4 to 1 (v./v.). Freshly activated plates were immersed in 20% Carbowax 600 in acetone, withdrawn, drained, and air-dried 2 hours before use. R_y values for all unknowns and standards in all systems were calculated.

Carbon Skeleton Chromatography. Pure components injected from the trap-injectors onto a skeleton unit (*3, 17*) (Table I) gave hydrocarbon reduction products whose I_k values were calculated and compared with standard paraffins. Occasionally similar quantities of standards suspected to be identical with the unknowns were injected in the same manner.

Synthesis and Isolation of Standards. 2-Heptenal, 2-octenal, and 2-decenal were prepared from the saturated aldehydes through the enol acetates and methyl acetals (*2*). Distillation of the α-bromodimethyl acetals was omitted since GLC showed them to be relatively pure. The aldehydes regenerated from the acetals were extracted with pentane and purified on an Apiezon L GLC column at 175° C.

Myrtenal (2-carboxyaldehyde-6,6-dimethylbicyclo-[3.1.1]hept-2-ene) was prepared by selenium dioxide oxidation of α-pinene in 95% ethanol (*6*). Vacuum distillation of the filtered reaction mixture gave a major fraction (b.p. 102–104° C./13 mm.) which was chromatographed on Apiezon L at 175° C. The major component (ca. 90% of total) was the only DNPH-positive substance in the mixture, and gave a semicarbazide, m.p. 206–7° C. (lit. 206° C.) (*6*). The PMR spectrum of this material was consistent with the structure of myrtenal, having an unsplit aldehyde proton signal a δ 9.40, an allylic methylene triplet at δ 2.54, and a lower field vinyl proton signal (δ 6.60) than α-pinene (δ 5.18), though it lacked the vinyl methyl signal at δ 1.12 p.p.m. in α-pinene.

Forss and coworkers (*7*) showed that *trans*-2-*cis*-6-nonadienal is the major carbonyl component in cucumber volatiles, comprising 30% of the total carbonyls. Since this compound was suspected in cotton, minced cucumbers (18 kg.) were steam distilled and the essential oil worked up as with the cotton square oil. The carbonyls were isolated by the Girard T procedure and gas chromatographed on Carbowax 4000 under standard programmed-temperature conditions (Table I). The major component in the resulting chromatogram was characterized as *trans*-2-*cis*-6-nonadienal on the basis of its characteristic cucumber odor, GLC retention time relative to 2-nonenal on Apiezon L and Carbowax 4000, and its DNPH (m.p., visible spectrum) (*7*).

Quantitation. The area of each carbonyl GLC peak in the temperature-programmed Carbowax separation was triangulated, and its percentage of the total carbonyl area was calculated. These values were further subdivided according to the relative areas of peaks appearing in the corresponding Apiezon L chromatograms. The internal percentages were converted to percentages of the square oil by multiplying by 0.014.

Results and Discussion

Qualitative Survey of Square Oil. A chromatogram of the cotton square oil obtained early in this study (Figure 2) aided in establishing a perspective on the cotton volatiles identification. Table III lists the peak numbers then assigned and the I_k values, odor descriptions, and identifications made to date. A large amount of component overlap obviously occurs in the chromatogram, and many of the large peaks contain more than one compound.

The α and β_1 fractions (Table II) have been accounted for in this and previous work (*16, 17*); studies of the β_2 and γ fractions are under way. For brevity, Figure 2 is terminated at peak 44, I_k = 1764, though the SE-30

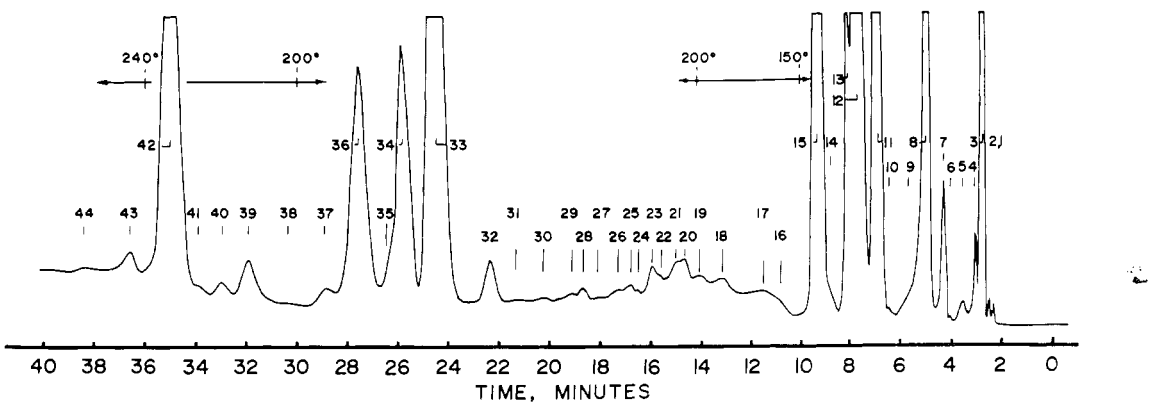

Figure 2. Temperature-programmed survey chromatogram of cotton square oil on SE-30

Conditions, Table I; peak identifications, Table III

Table III. Cotton Square Oil Component Survey by GLC on SE-30

Peak No.	I_k	Odor	Identification
1	...	.....	Air
2	400	.....	.....
3	510	Rancid, sour	Acetone, dichloromethane, isobutyraldehyde
4	605	Sour butter	Butyraldehyde
5	690	Amine-like	.....
6	773	.....	.....
7	796	Grassy, leafy	Hexanal
8	849	Stink bug, lachrymatory	*trans*-2-Hexenal
9	904	Fatty	Heptanal
10	937	Nutty	.....
11	959	Minty, piney	α-Pinene, camphene, benzaldehyde
12	987	Peppermint	β-Pinene, myrcene
13	1000	Nutty, minty	α-Phellandrene, α-terpinene
14	1025	.....	.....
15	1043	Minty, terpene	*trans*-β-Ocimene, limonene
16	1082	Fruity	Terpinolene, 2-octenal
17	1093	Lemony	Nonanal, *p*-tolualdehyde
18	1115	Citrus	.....
19	1130	.....	.....
20	1170	Cucumber	*trans*-2-*cis*-6-Nonadienal
21	1176	Citrus, cucumber	2-Nonenal
22	1187	.....	.....
23	1194	Spicy, cinnamon	.....
24	1219	.....	.....
25	1238	Aromatic aldehyde?	.....
26	1246	Sweet, sharp, pleasant	Myrtenal
27	1273	Soapy	.....
28	1292	Soapy	.....
29	1300	Soapy	.....
30	1346	Soapy	.....
31	1380	.....	.....
32	1400	Tree sap, wet cotton lint	Copaene
33	1460	Citrus, wet cotton lint	*trans*-α-Bergamotene, caryophyllene
34	1487	Pine sap	α-Humulene
35	1494	Ionone like, violets	.....
36	1525	Floral, musty	γ-Bisabolene, δ-gauiene
37	1556	Floral, sharp, sweet	δ-Cadinene
38	1580	.....	.....
39	1617	Oak sap, piney	.....
40	1649	Pine, sweet	.....
41	1665	Piney	.....
42	1699	Floral, roses	.....
43	1740	Sweet, citrus, piney	.....
44	1764	Rosin	.....

GLC survey showed small maxima out to 106 minutes (peak 57, I_k ca. 2800). The high boiling or high polarity materials in the later peaks may account for a moderate amount of the γ fractions, though a number of smaller alcohols and esters are also present.

Evaluations of the odors of components emerging from the GLC exhaust were of particular aid in establishing group and compound types present. Because of the odors and I_k values, the terpenes (peaks 11–16), sesquiterpenes (peaks 32–36), and several specific components such as 2-hexenal (peak 8), hexanal (peak 7), nonanal (peak 17), and 2-nonenal (peak 20) were early suspects. Although I_k values (Table III) obtained in such an irregularly temperature-programmed run were subject to differences of as much as ± 15 units from isothermal values, they still were useful in specifying peak position and indicating relative molecular weights of components.

Relative quantities of the compounds present were altered somewhat in oil isolated at a later date than that

chromatographed in Figure 2. Much lower quantities of 2-hexenal (peak 8) were found in oil isolated from freshly picked and processed squares compared with oil from frozen squares several months old (Figure 2). This effect also has been noted in tea leaves (*18*) and may indicate that postharvest enzymatic processes give rise to the larger quantities of hexenal (Figure 2), though it is present in oil from buds processed immediately after harvest. The complexity of the β_1 fraction may account for the inability of Power and Chesnut (*20*) to detect this compound in their early study of cotton volatiles, but the authors' data indicate that hexenal concentration may be as high as 5% of the whole plant oil.

The infrared spectrum of peak 8 (Figure 2) matched that of authentic *trans*-2-hexenal, the trans assignment being made on the basis of the strong 970-cm.$^{-1}$ absorption. The characteristic "stink bug" aroma of this compound led to its identification.

Fraction β_1. Table IV lists the component identifications, GLC retention index, odor, and quantitative data on the carbonyls in fraction β_1, whose chromatogram on Carbowax 4000 is given in Figure 1. Of the 40 components present, 14 carbonyl compounds were identified and several more tentatively identified. These 14 compounds account for 88% by weight of the β_1 fraction. The presence of a number of medium and small branched and straight chain aldehydes, as well as a few α,β-unsaturated aldehydes was expected. Many of the later peaks, from 23 on, appear to have highly unsaturated branched chain, cyclic, or aromatic structures, possibly arising from degradation of carotenoids or similar plant precursors.

The peaks marked *B* in Figure 1 are noncarbonyl impurities introduced from the reagents used in the Girard T isolation technique. No special purification of these reagents was attempted, though Gadbois, Mendelsohn, and Ronsivalli (*8*) recently reported that such purification gave a very clean reagent blank chromatogram. The reagent blank peaks did not obscure any major carbonyl components; but because of differential recovery of components by the Girard T procedure, the quantitative data reported in Table IV should be considered as only semiquantitative. For this reason, no attempt was made to refine the GLC quantitation by including response factors in the calculations, particularly since they are nearly equal for all the compounds in such a homogeneous group.

Component 9 was tentatively identified as 5-heptenal. Its watermelon rind–fatty odor is reminiscent of heptanal (component 7) and its boiling point (Apiezon L index, I_k^A = 879) is only slightly lower than heptanal. Carbon skeleton chromatography indicates a straight chain 7-carbon aldehyde or 6-carbon ketone. The I_k^A value definitely supports the 7-carbon aldehyde; the I_k^C rules out a 6-carbon ketone unless it has an α,β-unsaturated structure. The absorption spectrum of its DNPH corresponds to a saturated aldehyde or an unconjugated, unsaturated aldehyde, which rules out the possibility of a 6-carbon ketone. Thus, a 7-carbon aldehyde with an isolated unsaturation is implicated, since the ΔI_k value of 402 ($I_k^C - I_k^A$) agrees with that obtained from the sum of the partial ΔI_k values (*14*) of 343 for an aldehyde function and 58 for an isolated double bond.

Component 9 was isolated from 1000 μl. of β_1 by trapping it into pentane from the Carbowax column at 105° C. and rechromatographing on Apiezon L at 100° C. Residual amounts of component 8 (2-hexenal) were separated from component 9 on this second column, from which both were trapped separately into 0.6 ml. of 2% neutral aqueous potassium permanganate. Sodium carbonate (4 mg.) was added, and each sample was brought just to a boil on a hot plate, whereupon all the permanganate decolorized with precipitation of MnO_2. Each sample was acidified with 3 drops of concentrated HCl, extracted twice with 5 ml. of Spectrograde diethyl ether, and the ether solution was evaporated to a volume of 0.3 ml. Butyric acid was identified from component 8 by GLC on a diethylene glycol adipate–phosphoric acid column (*14*). Acetic acid found in component 9 implied a Δ^5 unsaturation. The column employed degraded glutaric acid standards, so that none was observed as a second fragment of component 9. To the authors' knowledge, 5-heptenal has never been reported in volatile oils. This analytical procedure would seem to be quite useful in micro structure analysis and can be applied successfully to 1 mg. or slightly less of unsaturate.

Component 10 has the appropriate I_k values, carbon skeleton behavior, and R_y values for 2-heptenal except for a small deviation in R_y on Carbowax-coated silica gel G. However, the ΔI_k value and λ_{max} on the DNPH were both low for an α,β-unsaturated aldehyde, so the identification of component 10 as 2-heptenal is questionable. I_k values also check for methyl heptenone, but R_y values on polyamide and Carbowax-silica definitely rule out this possibility.

Component 29 has I_k values that agree within two units with those of acetophenone. Further, the carbon skeleton maxima I_k values indicate ethylcyclohexane, methylcyclohexane, and cyclohexane as reduction products; however, there was an insufficient amount of this component for DNPH formation for TLC and spectrophotometric confirmation. Components 28, 30, 33, 34, 56, and 38 also appear to be aromatic, based on DNPH λ_{max}, ΔI_k, and TLC data, though identifications with all available aromatic standards were excluded for various reasons.

The finding that component 31 is myrtenal is of interest because of its relationship to α-pinene, a major terpene constituent of cotton square oil (*16*). Identification was based on its odor, GLC, and TLC behavior compared with that of synthetic myrtenal, and the absorption spectrum of its DNPH. Of greatest value, however, was its behavior upon carbon skeleton chromatography, where it gave a complex pattern of nine maxima. An equal quantity of the standard produced this same pattern when it was chromatographed immediately before or after component 31. The relative sizes of maxima in both chromatograms were dependent on the catalyst condition, temperature, and sample size. Apparently, thermal or catalytic decomposition of myrtenal occurs in the carbon skeleton unit, and the strong dependency of results upon conditions empha-

Table IV. Identity, GLC Retention, Odor, and Quantitative Data of Carbonyl Compounds in Fraction β_1 ofCotton Square Oil

Sample Peak No.[a]	Identity		I_k		Odor	Quantity in Square Oil[d], P.P.M.
	Compound	Methods[b]	C4M[c]	ApL[c]		
1	Acetone	A–E, G, I	842	470	.....	98
2	Isobutyraldehyde	A–C, G, H	842	500	Rancid butter	14
3	Butyraldehyde	A–C, G, H	906	594	Rancid butter	56
4	Isovaleraldehyde	A–C, G, H	938	655	Buttery	70
5	Hexanal	A–I	1122	779	"Green," grassy	6900
6	.....	....	1185	938	.....	98
7	Heptanal	A–F, H	1222	879	Watermelon rind, fatty	1050
8	*trans*-2-Hexenal	A–J	1274	838	Stink bug	2690
9	5-Heptenal?	E, F, H	1276	874	Fatty, watermelon	322
10	2-Heptenal?	A, B, C, F	1364	948	Like methylheptenone	98
11	2,6-Dimethyl-octanal?	....	1394	1038	Citrus, myrcene-like	182
12	Nonanal	A–H	1418	1081	Fatty, "green"	252
13	.....	....	1474	1000	Cucumber	98
14	2-Octenal	A–F, H	1496	1055	Cucumber, "green"	56
15	.....	....	1496	1090	Cucumber	28
16	.....	....	1527	1046	.....	28
17	.....	....	1527	1109	.....	84
18	.....	....	1566	1149	.....	154
19	Benzaldehyde	A–F, H	1602	1016	.....	238
20	.....	....	1602	1131	.....	70
21	2-Nonenal	A–F, H	1622	1155	Cucumber-like	406
22	*trans*-2-*cis*-6-Nonadienal	A–F, H	1653	1144	Cucumber	364
23	.....	....	1653	1175	.....	56
24	*p*-Tolualdehyde	A–F, H	1706	1122	Like aromatic aldehyde	28
25	.....	....	1706	1222	.....	Trace
26	.....	....	1706	1247	.....	196
27	.....	....	1706	1276	.....	28
28	.....	....	1732	1069	.....	14
29	Acetophenone?	A, F	1732	1119	.....	14
30	.....	....	1732	1140	.....	14
31	Myrtenal	A–F, H	1732	1223	Honeybee, piney	56
32	.....	....	1732	1244	.....	14
33	.....	....	1753	1219	Like aromatic aldehyde	Trace
34	.....	....	1778	1206	.....	42
35	.....	....	1801	1203	.....	14
36	.....	....	1801	1228	Soapy	28
37	.....	....	1801	1327	Soapy	28
38	.....	....	1839	1228	Spicy, like aromatic aldehyde	98
39	.....	....	1839	1280	.....	14
40	.....	....	1839	1318	.....	Trace
					Total	14,000

[a] Reagent blank maxima not assigned numbers.
[b] A, GLC retention on Carbowax 4000 and Apiezon L. B, DNPH–TLC, R_y value on silica gel G, benzene/pet. ether, 4/1. C, DNPH–TLC, R_y value on polyamide, methanol/water, 95/5. D, DNPH–TLC, R_y value on Carbowax 600/silica gel G, heptane/benzene, 4/1. E, DNPH visible absorption spectrum. F, Carbon skeleton chromatography, expected maxima or identity with standard. G, GLC retention of DNPH on SE-30. H, Odor. I, DNPH m.p. J, Infrared spectrum of carbonyl compound.
[c] Components 1–4, 105° C.; 5–12, 140° C; 13–40, 175° C., isothermal.
[d] Footnote *a*, Table II.

sizes the need for careful comparison of standard and unknown when labile substances are examined by this technique.

Since myrcene is another important terpene component in square oil, myrcenal (2-methyl-6-methylene-2,7-octadienal) was prepared by selenium dioxide oxidation of myrcene and isolated in a manner similar to that used with myrtenal. No components in β_1 were identical to synthetic myrcenal though GLC and TLC behavior indicate component 11 may be the reduced form (2,6-dimethyloctanal). Carbon skeleton chromatography of component 11 gives a hydrocarbon with an I_k equal to that of 3-methyloctane, as expected from 2,6-dimethyloctanal. Several attempts to reduce the crude oxidation mixture catalytically failed because of selenium catalyst poisoning, and further attempts to purify the aldehyde caused decomposition. No report of a preparation of 2,7-dimethyloctanal was found in the literature.

A number of components having odors reminiscent of *Cucurbitaceae* are present in cotton. Most striking is the identification of component 22 as *trans*-2-*cis*-6-nonadienal, the characteristic aroma contributor in cucumbers (*Cucurbitaceae*) and in violet leaves (*Violaceae*). The presence of this aldehyde in cotton (*Malvaceae*) as well as in two other phylogenetically dissimilar families may indicate a more widespread occurrence of the substance than has been recognized heretofore.

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