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Volatile and Nonvolatile Constituents of the Cotton Bud. The Fatty Acid Composition[1]

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VANDERZANT et al. reported that insects of the genera *Pectinophora* and *Ephestia* (17) have a dietary requirement for fat which can be satisfied by linoleic or linolenic acid. Previously no other insects were found to require fats in the diet although a beneficial effect on growth was observed when fatty acids were present. Recently Vanderzant and Davich (18) demonstrated increased egg production by the boll weevil, *Anthonomus grandis* Boheman, on an artificial diet to which corn oil and fat soluble vitamins had been added. This result suggested that these fatty acids may exert some effect on the boll weevil also.

Since cotton is the preferred host plant of the boll weevil, a study of the fatty acid content of the square (flower bud)—the site of ovipositioning and larval development—was undertaken to determine the number and quantity of fatty acids present.

Information on fatty acid content in cotton (*Gossypium hirsutum* L.) plants has been limited mostly to that in seeds (1). The literature does not appear to contain reports on the fatty acid content in cotton leaves or squares other than the report by Lambremont and Blum (10), who showed that about two-thirds of the fatty acids in cotton squares were unsaturated and 95% were of a long chain nature (C_{14} or above). Lambremont et al. (personal communication) also showed that these characteristics were approximately the same for the anther, ovary, and pistil fatty acids.

MATERIALS AND METHODS

Cotton squares containing the sepal, calyx, and petal were ground in a blender and lyophilized. Total lipids were extracted from the lyophilized powder by the method of Folch et al. (3) with chloroform-methanol (2:1 v/v). After removal of the chloroform-methanol in vacuo in a nitrogen atmosphere, a portion of the resulting lipid extract was further fractionated on a silicic acid column to separate the neutral lipids (11). Total and neutral lipids were analyzed separately for their fatty acid content by the following method.

Lipid extracts were saponified in 10% methanolic potassium hydroxide by the method of James (6) under nitrogen. After saponification was complete, the methanol was distilled in vacuo and the resulting basic residue extracted with ether until no visible color remained in the ether. Salts were converted to the acids with 10% sulfuric acid and the acids extracted with ether. Acids were converted to their methyl esters by refluxing for 2 hours with a 10-fold (w/v) excess of methanol containing 1% sulfuric acid and 1% 2,2-dimethoxypropane in the sealed tube at 65° (12).

Qualitative and quantitative analyses were performed in a gas-liquid chromatographic system equipped with a flame ionization detector. Separation of the methyl esters was achieved on a 10-foot (spiral) column of 20% diethylene glycol succinate as the stationary phase and Chromosorb-W[R] (Johns Manville, New York, N. Y.) as the support. The separations were carried out with both isothermal (180° C.) and temperature programmed runs (50–180° C. at a rate of 8° C./min.). Helium was the carrier gas, with a flow rate of 100 ml. per minute.

Identification of unknown methyl esters from the total lipids was achieved by: *(1)* Comparing the relative retention volumes with those of known standards; *(2)* employing the method of James (7) in which the logarithm of the adjusted retention volumes of the saturated fatty methyl esters are plotted relative to the number of carbon atoms in the chain; *(3)* plotting the logarithms of the adjusted retention volumes of the unsaturated fatty esters according to the method outlined by Orr and Callen (15); and, *(4)* calculating increased peak areas of the saturated components upon catalytic hydrogenation of the unknown fatty ester mixture with 5% palladium-on-carbon.

RESULTS AND DISCUSSION

Table 1 shows the retention data of methyl esters of fatty acids derived from lipids of cotton bud samples.

Quantitative analysis of several samples of squares obtained from the same field grown cotton indicated little variation in the fatty acid content except in the unsaturated fatty acids. The average for five analyses is given in Table 2. Qualitatively the same fatty acids were found to be present in each sample. Thus, cotton square lipids contain at least 17 fatty acids (1 component unidentified), 5 of which accounted for about 85% of the total.

Table 1. Retention data of the fatty acid methyl ester fraction from total lipids of cotton squares.*

Component number	Common name	Chain length	Retention time	
			Minutes	Relative
1	Caproic	C_6	3.5	0.21
2	Caprylic	C_8	5.6	0.34
3	Capric	C_{10}	7.7	0.46
4	Lauric	C_{12}	9.8	0.59
5	Myristic	C_{14}	12.5	0.76
6		$C_?$	12.9	0.78
7	Pentadecanoic	C_{15}	13.2	0.80
8	Pentadecenoic	$C_{15:1}$	14.9	0.90
9	Palmitic†	C_{16}	16.5	1.00
10	Palmitoleic	$C_{16:1}$	17.8	1.08
11	Heptadecanoic	C_{17}	20.7	1.25
12	Stearic	C_{18}	22.2	1.34
13	Oleic	$C_{18:1}$	24.0	1.45
14	Linoleic	$C_{18:2}$	26.1	1.58
15	Linolenic	$C_{18:3}$	30.1	1.82
16	Octadecatetraenoic	$C_{18:4}$	33.4	2.02
17	Arachidic	C_{20}	36.0	2.18

* Gas chromatograph condition: Sample: Fatty acid methyl esters. Detector: Hydrogen flame. Substrate: Diethylene glycol succinate. Temperatures: Temperature programmed 50-180 °C at a rate of 8 °C/min, flame detector temp. 230 °C, inlet temp. 205 °C. Carrier gas helium, flow rate 100 ml/min. Column size: 10 ft × 1/4 in Stainless Steel. † Methyl ester of palmitic acid was used as the internal standard.

Table 2. Quantitative analysis of the fatty acid methyl ester fraction from total lipids of cotton squares, from neutral cotton square lipids, and from cotton square lipids after catalytic hydrogenation.

Fatty acid chain length	Common name	Percent of fatty acids from		
		Total lipids	Neutral lipid	After hydrog'n
C_6	Caproic	0.47		
C_8	Caprylic	0.65		
C_{10}	Capric	1.11	1.44	
C_{12}	Lauric	0.61	0.38	0.40
C_{14}	Myristic	0.99	1.16	1.11
$C_?$		0.43		
C_{15}	Pentadeeanoic	0.07		
$C_{15:1}$	Pentadecenoic	0.51	0.18	
C_{16}	Palmitic	24.58	24.02	25.84
$C_{16:1}$	Palmitoleic	1.73		
C_{17}	Heptadecanoic	1.91		
C_{18}	Stearic	2.56	3.00	60.90
$C_{18:1}$	Oleic	16.13	14.92	
$C_{18:2}$	Linoleic	17.60	16.40	
$C_{18:3}$	Linolenic	10.84	7.12	
$C_{18:4}$	Octadecatetraenoic	15.63		
C_{20}	Arachidic	5.84	5.60	5.16

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The percentage of fatty acids derived from the neutral lipids, (Table 2) on gas-liquid chromatography are qualitatively consistent with those from total lipids, except for the absence of the $C_{18:4}$ acid attributed to component 16 (Table 1). Smaller amounts of octadecaenoic ($C_{18:1}$), octadecadienoic ($C_{18:2}$), and octadecatrienoic ($C_{18:3}$) acids were present.

Sixty-three percent of the total fatty acids were unsaturated, with the C_{18} components accounting for the largest amounts. Of the saturated fatty acids, C_{16} (palmitic) was the largest component, accounting for 24%.

The assignment of component 16 to octadecatetraenoic acid ($C_{18:4}$) was indicated by the following evidence. The experimentally observed retention volume of this component (3312 ml) divided by the calculated retention volume (3346 ml) gave a relative retention of 0.99. More significant evidence that this component was not an oxygenated or hydroxylated fatty acid was obtained by catalytic hydrogenation of the mixture of cotton square fatty esters. The resulting saturated fatty acids were analyzed quantitatively and the results for C_{12} — C_{20} are summarized in Table 2.

Hydrogenation of the fatty ester sample resulted in the loss of components attributed to the C_{18} unsaturated acids (No. 13, 14, 15, and 16, Table 1) with a subsequent 60% increase in the C_{18} (stearic) acid component, Table 2. This increase is essentially equivalent to the combined percentages of the unsaturated C_{18} fatty esters lost upon hydrogenation as shown in Table 2.

James and Martin (8) pointed out that when a mixture of unsaturated fatty acid esters containing varying amounts of unsaturation was incompletely brominated, the degree of bromination increased by the order: 1 double bond < 2 double bonds < 3 double bonds. When their procedure was applied to the fatty esters from cotton squares, a complete loss of the component attributed to octadecatetraenoic ($C_{18:4}$) acid occurred. There was, at the same time, a decrease in the amounts of the other C_{18} unsaturated acids in the order described above.

Infrared analysis of a sample of component 16 gave absorptions similar to those for the standard C_{18} unsaturated acids. The absence of absorption above 3000 $cm.^{-1}$ eliminated the possibility of this component being a hydroxy acid. Attempts to gain information as to the length of the carbon chain by the method of Jones et al. (9), in which the appearance of a number of bands in the region of 1350–1180 $cm.^{-1}$ is characteristic of chain length, failed due to the unsaturated nature of this component. Ultraviolet analysis of the fatty acid fraction did not reveal the presence of a conjugated unsaturated system.

The mode of formation of fatty acids in plants is not completely understood. Mead and Howton (13) suggested that linoleic acid is a parent compound of a series of polyunsaturated acids with the terminal structure $CH_3(CH_2)_4CH=$, that linolenic acid is a parent compound of a series of polyunsaturates with a terminal structure of $CH_3CH_2CH=$ (14), and that oleic and palmitoleic acids can give rise to polyunsaturated acids with terminal structures of $CH_3(CH_2)_7CH=$ and $CH_3(CH_2)_5CH=$ (5), respectively. Mead's studies suggest that the octadecatetraenoic acid reported in this paper probably was formed from linoleic acid with gamma-linolenic acid as a likely intermediate. The evidence of other workers (5, 14) suggested that arachidonic acid (eicosa-5,8,11,14-tetraenoic acid) also may be an intermediate. The C_{18} tetraenoic acid resulting from arachidonic acid through beta-oxidation would be the 3,6,9,12-tetraenoic acid. If instead, the octadecatetraenoic acid was formed directly from gamma-linolenic acid, it probably would possess the cis-6,9,12,15-structure shown to exist in small quantities in some plant seed oils (2, 16) and fish oil (4). Efforts are being made in this laboratory to collect sufficient quantities of the pure component, $C_{18:4}$, acid to fully characterize the positions of unsaturation.

The high percentage (60%) of unsaturated fatty acids present in the cotton bud substantiates the dietary requirements for insects as reported by Vanderzant et al. (17). The presence of octadecatetraenoic acid could account for the preference of the cotton bud over the present artificial diets for the boll weevil.

Moreover, studies now in progress in this laboratory to identify components in the bud which elicit a feeding stimulus to the boll weevil implicate the lipid fraction in part. While the contribution of specific fatty acids to the total response can only be a subject for speculation at this time, it may eventually prove desirable to select cotton lines which are less attractive because of their particular lipid content.

SUMMARY

Cotton squares grown under field conditions were studied qualitatively and quantitatively for their fatty acid content. There were at least 17 fatty acids present in the total lipids of the cotton square of which 57% were the C_{18} unsaturated fatty acids. There was only slight variation in the concentrations of saturated fatty acids derived from the total and neutral lipids. Octadecatetraenoic ($C_{18:4}$) was found to be present in the acids derived from total lipids but absent from the acids from neutral lipids. Octadecatrienoic ($C_{18:3}$) acid showed a decrease of about 3% in the neutral lipids from that of the total lipids.

Octadecatetraenoic acid has not previously been reported as being present in green plant material. The assignment of the $C_{18:4}$ fatty acid structure to this component was made on the following evidence:

1. The retention volume of this component, when compared with that actually obtained with gas-liquid chromatographic analysis, was equal to 1.
2. Catalytic hydrogenation of the fatty acid methyl esters derived from the total lipids resulted in the loss of the $C_{18:4}$ component as well as the other assigned C_{18} unsaturated acids with the subsequent increase in the C_{18} saturated component, equal in percentage to the total C_{18} unsaturates lost. The hydrogenation did not result in the appearance of any new components.
3. When the total fatty acid esters were incompletely brominated in ether at 0° the percentage of the unsaturated components removed was in the order 1 double bond < 2 double bonds < 3 double bonds < 4 double bonds. This is indicative of the degree of unsaturation.
4. Infrared analysis of a trace amount of the sample, collected from the GC-column and assigned the $C_{18:4}$ structure, contained the same absorption pattern of the other C_{18} unsaturated methyl esters.
5. Ultraviolet analysis of the impure $C_{18:4}$ component did not show the presence of a conjugated unsaturated system.

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