Extraction of Lipids from Cottonseed Tissue: III. Cyclopropenoid Fatty Acids from Glanded and Glandless Seed[1]

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ABSTRACT

Principal storage sites of cyclopropenoid fatty acids in glanded and glandless cottonseed tissues were investigated by measuring the content of cyclopropenoid fatty acids in lipids obtained by a cytoplasmic disruptive solvent (hexane-acetone-water) and a nondisruptive solvent (hexane-acetone). The content of cyclopropenoid fatty acids in lipid obtained by either solvent did not differ quantitatively, indicating that cyclopropenoid fatty acids are not stored preferentially in extraspherosomal cytoplasm. Since this observation was also made with glanded tissue, whose glands are thoroughly disrupted by hexane-acetone-water but not by hexane-acetone, the lipoidal material of glands are also not rich in cyclopropenoid fatty acids. These observations indicate that oil-rich spherosomes are the principal sites of cyclopropenoid fatty acids, as well as the reserve oil of cottonseed, and suggest that the greater content of cyclopropenoid fatty acids in lipid prepared by increased periods of solvent-extraction is from release of binding to tissue components, rather than a thorough extraction of somewhat inaccessible (extraspherosomal) areas of tissue.

INTRODUCTION

Storage oils of oilseeds are localized principally in intracellular organelles named "spherosomes" (1). Depletion of spherosomal contents results from extraction of lipids from cottonseed tissue with hexane or with hexane-acetone (2). In addition to spherosomal depletion, disruption of intracellular structures occurs upon extraction of lipids with hexane-acetone-water (2). These cytological observations and the finding that lipids extracted by the two types of solvents differ in quantitative composition (3) indicate that disruptive solvents, e.g., hexane-acetone-water, extract cytoplasmic materials in addition to spherosomal lipids.

The finding that concentrations of cyclopropenoid fatty acids in oils extracted by hexane increase with increased periods of contact between tissue and solvent has indicated that cyclopropenoid fatty acids might be contained within areas of cottonseed somewhat inaccessible to hexane (4). If the principal intracellular storage site of cyclopropenoid fatty acid were cytoplasmic rather than spherosomal, disruptive solvents would produce a significant increase in the concentration of cyclopropenoid fatty acids in extracted lipids with respect to the concentration of cyclopropenoid fatty acids obtained by nondisruptive solvents. Therefore it was of interest to identify the storage sites of cyclopropenoid fatty acids, by determining whether thorough extraction of cyclopropenoid fatty acids is obtained by disruptive or nondisruptive solvents. In addition, lipids were extracted from both glanded and glandless tissues to ascertain the contribution of glands to the concentration of cyclopropenoid fatty acids in oil.

[1]Previous paper in this series is given in Reference 2.

[2]ARS, USDA.

TABLE I

Lipid and Cyclopropenoid Fatty Acids from Glanded and Glandless Cottonseed[a]

Seed	Solvent	Lipid, g	Cyclopropenoid,[b] mg
Glanded	HA[c]	1.42 ± 0.01	8.1 ± 0.1
Glanded	HAW[d]	1.49 ± 0.01	7.6 ± 0.4
Glandless	HA	1.61 ± 0.01	12.2 ± 0.5
Glandless	HAW	1.65 ± 0.01	13.4 ± 1.3

[a]Materials in each miscella are from 4 g of flakes. Values represent means ± standard deviations from the means.

[b]As methyl malvalate.

[c]HA = anhydrous hexane-acetone 31:20 v/v.

[d]HAW = HA plus 1.8% water by volume.

MATERIALS AND METHODS

Extracting media were: a nondisruptive solvent, HA, which is anhydrous hexane-acetone 31:20 v/v and a disruptive solvent, HAW, which is HA containing 1.8% water by volume (2,3). Hexane was Skellysolve B.

Dehulled glanded and glandless (Watson GSL-16) cottonseeds were flaked to a thickness averaging 0.3 mm. Occasional hull fragments were culled from both samples by hand, and cleaned flakes were stored over P_2O_5 in vacuo.

Dried flakes were stirred continuously with extracting media in fritted glass Buchner funnels of medium porosity (12 ml solvent per 4 g tissue; 5 min contact), and resultant miscellae were collected by filtration with reduced pressure for 2 min (3). Each marc was extracted with fresh solvent three more times, and the four miscellae obtained from each sample were combined. Weights of the extracted lipids were determined after solvents were removed in vacuo.

Concentrations of cyclopropenoids in the lipids were determined by the procedure of Brown (5). In brief, duplicate samples of lipid from each of three separate miscellae for every tissue-solvent combination were (a) converted to methyl esters, (b) passed through alumina columns with diethyl ether-methanol 39:1 v/v, (c) passed through alumina columns with petroleum ether, and (d) titrated with HBr in glacial acetic acid.

The abilities of solvents to disrupt pigment glands were estimated from amounts of pigments in miscellae obtained from isolated glands. Glands were prepared from disintegrated flakes by flotation (6) and were rinsed with hexane before storage over P_2O_5 in vacuo. Dried glands were mixed with solvents (5 ml solvent per 10 mg glands; 5 min contact), and absorbancies of miscellae at 360 nm, due to gossypol pigments (6), were determined spectrophotometrically. Hexane in the solvents was spectrograde quality.

RESULTS AND DISCUSSION

To determine the effects of solvents on the pigment glands of glanded tissue, isolated glands were treated with hexane, HA and HAW. Absorbancies at 360 nm of miscellae obtained with hexane, HA and HAW were 0.01, 0.58 and 20.4 units, respectively. These results show that HAW

readily disrupted pigment glands and indicate that the miscella obtained from glanded tissue with HAW contained both spherosomal and glandular oils. Unfortunately, since a large portion of glandular weight is extractable pigment (6), lipids in miscellae from isolated glands could not be practicably assayed for content of cyclopropenoid fatty acids.

Amounts of lipid and concentrations of cyclopropenoid fatty acids in oils were determined in miscellae produced from glanded and glandless tissues with HA and HAW. Results (Table I) show that amounts of lipid extracted from glandless tissues were greater than amounts extracted from glanded tissues. This difference probably reflects differences in genotypes and environments during seed development. As observed previously (3), more lipid was extracted by HAW than by HA, because HAW extracts extraspherosomal lipids and, in the case of glanded tissue, glandular lipids.

If cyclopropenoid fatty acids were located in intracellular, extraspherosomal cytoplasm, concentrations of cyclopropenoid fatty acids would significantly increase in lipids from both glanded and glandless tissues when HAW rather than HA was the medium of extraction. Since the concentrations did not significantly change (Table I), apparently extraspherosomal cytoplasm was not a principal site of storage of cyclopropenoid fatty acids. Instead, the results indicate that the bulk of cyclopropenoid fatty acids in cottonseed is principally located in spherosomes, which are also the principal sites of reserve oil.

In addition to disruption of intracellular, cytoplasmic structures, HAW also extracts a large amount of pigment from pigment glands—two effects not obtained by HA. With the assumption that the presence of pigment glands comprised the only principal difference between glanded and glandless tissues, and since HAW, but not HA and hexane, readily extracted grandular contents, the results also indicate that glandular contents were not the source of the increase in concentration of cyclopropenoid fatty acids in hexane-extracted oil as observed by Bailey et al. (4).

In summary, these results indicate that pigment glands and extraspherosomal cytoplasm are not significant storage sites for cyclopropenoid fatty acids. Instead, the principal sites appear to be spherosomes, which are the repository sites for reserve oil (1) and which are readily emptied by lipid solvents (2). Perhaps a characteristic binding of cyclopropenoids to other tissue components (7) might account for the increased concentrations of cyclopropenoid fatty acids in lipid that occurs as the period of contact between solvent and tissue increases (4).

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[Received October 18, 1972]