treatments for both lactalbumin and casein.

Four rat bioassays were used to examine the effect of heat on casein and lactalbumin. The absolute values of the four assays are listed for the unheated and 90-min autoclaved samples of lactalbumin and casein (Table III). Also listed are relative protein quality (r.p.q.) values (unheated lactalbumin = 100). Table III demonstrates that casein is significantly ($P < 0.01$) less heat-labile than lactalbumin regardless of the assay method. The extent of protein quality loss in lactalbumin varies depending on the bioassay used. The differing r.p.q. values of lactalbumin:A-90 correlate with values obtained with low-quality proteins by McLaughlan (1976). Yanez and McLaughlan (1970) believe that PER underestimates protein quality of lower quality proteins because it has no measure of maintenance protein needs. The NPR and NPU overestimate it due to protein conservation in the rats fed a nonprotein diet. The modified PER values are likely to be the most accurate measure of protein quality (McLaughlan and Keith, 1975); they usually compare well with the more complex and accurate slope–ratio assay of Hegsted and Chang (1965). This is caused by the realistic estimate of the maintenance protein requirement found within the calculations of the modified PER.

In summary, it has been determined that the protein quality of lactalbumin is more heat-labile than that of casein. These observations are important in deciding if lactalbumin or casein is to be used as the reference protein in studies of protein quality of foods. The degree of destruction of protein quality can be reduced by washing the lactalbumin, which results in the removal of the lactose. The method of evaluation of changes of protein quality should be a bioassay, specifically the modified PER, since the amino acid profile technique does not reflect the loss of bioavailability of some essential amino acids.

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Production of Nonglandular Terpenoid Aldehydes within Diseased Seeds and Cotyledons of *Gossypium hirsutum* L.

Cottonseed and cotyledons of germinating seedlings were examined for the presence of fungal-induced, nonglandular, toxic terpenoids. The terpenoid aldehyde hemigossypol was produced in cottonseed during exposure to 9 days of nearly continuous rainfall in the field. However, no productions of terpenoid aldehydes was observed in mature seeds weathered under normal rainfall or deteriorated by *Aspergillus niger* at constant 20% seed moisture. Hemigossypol and gossypol were produced in isolated lesions and vascular tissues of cotyledons of glandless seedlings inoculated with conidia of *Verticillium dahliae* and *Colletotrichum dematium*. Because terpenoids are formed in deteriorating seeds only under extremely moist conditions conducive to germination or rapid deterioration, they should not be a problem in the use of glandless cottonseed for food.

Gossypol, the major terpenoid aldehyde found in pigment glands of cottonseed (*Gossypium hirsutum* L.), is formed by peroxidative dimerization of the sesquiterpenoid aldehyde hemigossypol (Veech et al., 1976). Only trace amounts of hemigossypol occur in glands of cottonseed (Stipanovic et al., 1975), but this terpenoid aldehyde may be formed in major amounts in cells outside glands in response to fungal infection. Considerable amounts of hemigossypol and lesser amounts of gossypol, for example, form in vascular and boll tissue infected by fungi (Bell and Stipanovic, 1978; Mace et al., 1976). Both terpenoids also are formed in the epidermis of healthy roots (Mace et al.,

1974). Production of gossypol and hemigossypol is restricted to members of the plant tribe Gossypieae and has never been reported to occur in fungi (Bell and Stipanovic, 1978). The possible presence of nonglandular terpenoids in cottonseed or cotyledons of either glanded or glandless cotton has not been studied.

Gossypol is thought to be a major contributor to the occurrence of off-colors in baked products prepared from cottonseed flour. Low concentrations of gossypol which occasionally are found in samples of glandless cottonseed are presumed to be due to contamination by glanded seed (Wilcke, 1978); however, disease-induced nonglandular terpenoids might also be involved. Whereas contamination of cottonseed protein preparations by glandular gossypol could be reduced or eliminated by use of the liquid cyclone process (Gastrock et al., 1969), nonglandular terpenoids would not be removed by this process and could present a continuing problem for the use of cottonseed as food.

Cottonseed infected by fungi commonly exhibit a delay between the time of seed infection and the time of embryo infection (Halloin, 1975). This delay occurs in both glanded and glandless seeds (Halloin et al., 1978), and the induced production of fungitoxic terpenoid aldehydes (phytoalexins) could be an explanation for the delay. Thus, we studied the production of induced terpenoid aldehydes in cottonseed and seedlings to determine if these compounds might account for the occurrence of terpenoid aldehydes in some samples of glandless cottonseed and be involved in the apparent resistance of embryos to infection.

MATERIALS AND METHODS

Seed Production and Germination. Seeds of "TAMCOT SP-37" (glanded) and "Rogers" GL-6′ (glandless) cotton were produced by self pollination in field plantings at College Station, TX. Prime seeds were harvested immediately following complete boll opening; seeds were ginned and delinted with concentrated H_2SO_4. Seeds were germinated in rolls of moistened filter paper (Whatman No. 3) held in darkness at 30 °C for 4 days.

Weathered seeds usually were those subjected to at least 2 months of field exposure between the times of boll opening and harvesting. One sample of weathered, glanded seeds was collected following a 9-day period of nearly continuous rain. Many seeds in this sample had germinated in the bolls, but only nongerminated seeds were selected for these experiments.

Seed Deterioration and Seedling Inoculation. The fungi *Aspergillus niger* VanTieghem and *Colletotrichum dematium* (Pers. ex Fr.) Grove were grown for 7 days at 22 °C on plates of potato dextrose agar. Cultures were then flooded with a 0.1% solution of Tween-20 and scraped with a glass stirring rod. Conidia were rinsed from the culture with sterile distilled water and filtered through two layers of facial tissue. The conidial suspensions were adjusted to 10^7 conidia/mL with sterile distilled water. The fungus *Verticillium dahliae* Kleb. was grown for 3 days at 22 °C in shake cultures of potato broth containing 2% sucrose and 10% V-8 juice. The cultures were filtered through two layers of facial tissue, and centrifuged for 3 min at 1000*g*, and the resulting conidial pellets were resuspended in sterile distilled water and adjusted to 10^7 conidia/mL.

Seeds to be deteriorated were inoculated with *A. niger*. Sufficient conidial suspension was added to raise the moisture of the seed mass to 20%. Following complete imbibition of this liquid, seeds were incubated in sealed jars at 25 °C for periods of 4–22 days.

Filter paper rolls containing glandless seedlings were opened after 4 days of germination and the seedlings were repositioned so that their hypocotyls and cotyledons protruded above the top edges of the papers. The papers were rerolled and cotyledons were briefly immersed either in distilled water (checks) or in conidial suspensions of either *C. dematium* or *V. dahliae*. Seedlings then were placed in darkness at 22 °C and approximately 100% relative humidity for 4 days.

Table I. Terpenoid Aldehydes Isolated from Cottonseed and Seedling Cotyledons

sample	gossypol	hemigossypol
seeds		
prime seeds		
glanded	+	−
glandless	−	−
weathered seeds		
9 days of continuous rain		
glanded	+	+
2 months of field exposure		
glanded	+	−
glandless	−	−
deteriorated seeds		
(*Aspergillus niger*, 20% moisture, 25 °C)		
glanded	+	−
glandless	−	−
cotyledons (glandless)		
noninoculated check	Tr[a]	Tr
inoculated with *Verticillium dahliae*	+	+
inoculated with *Colletotrichum dematium*	+	+

[a] Tr = trace (faint staining on thin-layer chromatogram).

Extraction and Detection of Terpenoids. Embryos (cotyledons and embryonic axes) were excised from seeds, and cotyledons were excised from seedlings. These tissues were freeze-dried and ground to a fine powder with a mortar and pestle under liquid nitrogen. Crude terpenoid fractions were prepared from these powders as described by Stipanovic et al. (1975). The fractions were spotted adjacent to terpenoid standards on TLC plates coated with 0.5 mm of polyamide. Chromatograms were developed with benzene/chloroform/methanol/acetic acid (150:50:3:2), air-dried, and sprayed with ethanolic 5% phloroglucinol/concentrated hydrochloric acid (1:1) as described by Stipanovic et al. (1975). Terpenoid aldehydes were identified by their chromatographic mobilities and the color of derivatives formed with phloroglucinol. Terpenoid aldehydes were detected histochemically in situ in the seedling cotyledons with saturated antimony trichloride in concentrated perchloric acid as described by Mace et al. (1974).

RESULTS AND DISCUSSION

The occurrences of terpenoid aldehydes in various tissues are summarized in Table I. Hemigossypol was not detected in mature, prime cottonseed. Therefore, the presence of hemigossypol in seeds was taken as evidence of induced synthesis of terpenoid aldehydes. No hemigossypol was detected in either glanded or glandless seeds incubated with *A. niger* for periods of 4–22 days. All seeds in this experiment failed to germinate after 15 days of incubation. Seeds subjected to 2 months of field exposure had low germination (less than 50%), but showed no evidence of induced synthesis of terpenoids. The sample of glanded seeds exposed to 9 days of continuous rain, however, contained hemigossypol; all of these seeds failed to germinate. Thus, it appears that induced synthesis of terpenoid aldehyde phytoalexins can occur in cottonseeds, but only under extreme conditions of sustained high moisture, that favor either germination or rapid deterio-

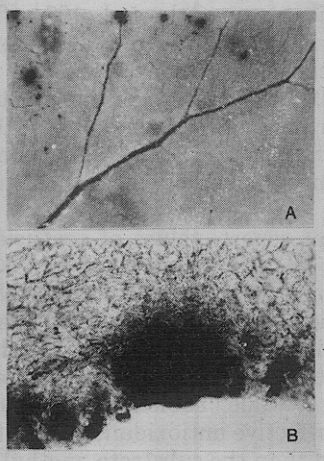

Figure 1. Localization of terpenoid aldehydes (black areas) in cotyledons of germinating glandless cotton seedlings. (A) Terpenoids in lesions and vascular tissue of cotyledons inoculated with *Verticillium dahliae* (×50). (B) Terpenoids in lesion on abaxial surface of cotyledon inoculated with *Colletotrichum dematium* (×300).

ration. Pfleger and Harman (1975) reported failure of deteriorating pea seeds to synthesize phytoalexins, but moisture levels in their seeds never exceeded 20%.

Cotyledons of glandless cotton have the capacity to synthesize terpenoid aldehydes (Table I, Figure 1) when inoculated with either the cotton wilt pathogen *V. dahliae* or a nonpathogenic isolate of *C. dematium*. Induced synthesis of terpenoid aldehydes occurred in localized lesions and in vascular tissues. Histochemical staining of noninoculated cotyledons revealed terpenoids in their vascular tissues as well, thereby accounting for the traces of terpenoid aldehydes extracted from them.

In summary, cottonseed and seedling cotyledons have the capacity to synthesize nonglandular terpenoid aldehydes, but do so only at moisture levels conducive to germination or rapid deterioration. Thus, synthesis of phytoalexins is not a determining factor in resistance of seed embryo tissues to infection by fungi, but may contribute to disease resistance in seedlings. Additionally, gossypol found in preparations of glandless seed is more likely due to contamination by glanded seed than to production of nonglandular terpenoids. Microbial-induced terpenoids should present no problem in production of gossypol-free or low-gossypol food products from glandless and mechanically deglanded seed preparations.

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Antioxidative and Quantum Chemical Properties of Some Hydroxy N-Heterocyclic Compounds

The inhibiting effects of some N-heterocyclic compounds on autoxidation of methyl linoleate increased with decreasing the highest molecular orbital energies (E_{ho}) of these compounds. 5-Hydroxy-2,3-dimethylindole ($E_{ho} = \alpha + 0.3\beta$) was found to have a marked antioxidant activity.

Amino acids are well known to affect the course of lipid oxidation (Kawashima et al., 1977a,b). Their antioxidative effects have been generally recognized to be of synergistic nature. Certain amino acids such as tryptophan and histidine may, however, occasionally function as primary antioxidants themselves. Mitsuda et al. (1965) reported that tryptophan and tryptamine were effective for preventing the development of oxidized flavor in raw and dried milk preparations. The antioxidant activities of tryptophan and the related indole compounds were shown to correlate to some extent with the highest occupied molecular orbital (HOMO) energies of the molecules (Mitsuda et al., 1966, 1967).

In the present communication, HOMO energies of some heterocyclic compounds such as indole and quinoline derivatives were calculated by the simple Hückel molecular

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