formed during the isolation of rosemary antioxidant. Triterpene alcohols and acids (erythrodiol, betulin, ursolic and oleanolic acids) could be associated as synergists with the antioxidant activity of the rosemary complex.

Antioxidant Activity

Table III shows reports the induction period (hr) to record a decrease in the oxygen pressure on the surface of the sample (chicken fat) showing an oxygen absorption on the substrate, according to the Astell method (11).

From these data, it appears that rosemary and sage prolong the induction period in chicken fat and show antioxidant activity comparable with BHA and BHT. Moreover, they are more effective than cocoa shell distillate as antioxidants. The antioxidant properties of rosemary distillate were also evaluated in potato flakes according to the methods already described. Figure 4 gives the antioxidant activity measured as residual oxygen (%) and pentane (IU 10^4) in the headspace of the potato flakes packaging, the amount of residual carotenoids (mg/kg) in the product and the organoleptic scores.

From these data, it is clear that rosemary antioxidant retards linoleic degradation (pentane), carotenoid loss and protects lipids and lipid-like materials from oxygen attack. This protection has been confirmed by taste testings which show satisfactory correlation with the analytical figures.

We conclude that natural antioxidants, obtained as described here and added to oxygen-sensitive foods, improve their shelf-life and avoid deterioration of the organoleptic properties.

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Absence of the Cotton Dust/Bract Antigen in House Dust, Clean Cotton Fibers and Cottonseed Hulls and Proteins

A.A. SEKUL and **R.L. ORY**, Southern Regional Research Center[1], New Orleans, LA 70179

ABSTRACT

Aqueous extracts of cotton dust and cotton bract induced the formation of antibodies in rabbits. The antisera cross-reacted with both extracts as well as with extracts of stem, leaf, baled cotton, and gin trash. No reaction was obtained with extracts of cottonseed hulls, cottonseed proteins, noncontaminated cotton fibers, or house dust. None of the anitgens reacted with normal rabbit serum.

INTRODUCTION

Byssinosis, a respiratory disease, has been shown to affect the respiratory function of susceptible textile workers (1-3) as well as workers who handle flax, soft hemp and sisal (4,5). Neither the causative agent(s) nor the mode of action by which byssinosis is produced is known. The symptoms are generally ascribed to the prolonged inhalation of dust in textile mills that process raw fibers. Cotton dust is a heterogeneous material which varies in composition among work areas (6). Cotton bract has been identified as one of the major contaminants (32-52%) of the cotton plant trash which is associated with the fiber (7). Botanically, cotton dust at the textile mill is composed mainly of micronized plant parts such as bract, stem, leaf and cotton fiber. In the cottonseed oil mill, the botanical nature of the dust in the linter handling and processing consists largely of seed-coat fragments and linter trash microparticulates (8).

In the only study done in the U.S. oil mills, Jones et al. (9) reported a low prevalence of chronic airways diseases (2.3% byssinosis and 4% chronic bronchitis) among workers in several oil mills. This byssinosis prevalence level is about the level of false positives one would expect to find. Studies by Noweir et al. (10) showed that extremely high dust levels (73-590 mg/m^3) generated in cottonseed-handling operations (loading and unloading) in oil mills led to some respiratory complaints, but that dust from other operations (grinding and oil extraction) was inert.

Opinions regarding the etiology of byssinosis are conflicting. The symptoms are presumed to be caused by airway constriction brought about by induction of histamine release by cotton and hemp dust (11,12). Recently, reports have appeared (13) as well as our own studies (14), showing that cotton dust activates the complement system of proteins. Previous studies in this laboratory (15,16) showed that antibodies to water extracts of cotton card-room dust, as well as cotton bract and a purified fraction of dust, gave positive immunological responses in rabbits. In view of the low prevalence of byssinosis in oil mills, the purpose of this investigation was to test water extracts of clean cotton, cottonseed kernels (proteins plus aller-

[1] One of the facilities of the Southern Region, Science and Education Administration, U.S. Department of Agriculture.

gens), cottonseed hulls and house dust for cross-reactions with the cotton dust/bract antigen.

EXPERIMENTAL PROCEDURE

Materials

The materials extracted for testing as possible antigens were: cotton cardroom dust (collected from V-cell air filters in a commercial textile mill), cotton plant tissues (including bract, leaf and stem), gin trash, baled cotton, cottonseed hulls, cottonseed proteins (fat-extracted flour), clean cotton lint (greenhouse-grown) and house dust.

Preparation of Samples (Antigens)

A typical extraction consisted of wetting the material for 15 min with three changes of deionized water (1:10, w/v) at 25 C and pH 7. The residue was discarded. Extracts were pooled and centrifuged at 9,000 × g for 30 min and the supernatant was filtered by gravity through Whatman No. 1 filter paper. This residue was also discarded, and the filtrate was freeze-dried. The material was extracted repeatedly with 85% methanol at ambient temperature until the extracting solution showed only slight yellow coloration. The residue, methanol-insoluble (f-3) was air-dried and used as antigenic material in subsequent experiments. The methanol-soluble fraction was found to be immunologically inactive.

A sample of f-3 was purified by dialysis through membrane tubing of MW cut-off 50,000 at 4 C and neutral pH, against deionized water for 5 days, with daily changes of water and daily collection of the diffusate. Both the diffusate and the residue were freeze-dried. Only the residue was active immunologically.

Preparation of Rabbit Serum and Antiserum (Antibodies)

Extracts of cotton dust (f-3) and cotton bract (b-Ag) were used to produce antibodies. White New Zealand rabbits (2-3 kg) were first bled to obtain normal serum (NS), then were injected subcutaneously in the footpad with a total of 2.5 mg of antigenic material in Freund's complete adjuvant. After 10 days, booster injections of the same amount in incomplete adjuvant were given subcutaneously every 2 weeks for a total of 7 injections. Antisera to dust (AD) and to bract (AB) were pooled and concentrated ca. 10-fold by 40% saturation with ammonium sulfate at 4 C. The precipitated antibodies were resuspended in a minimal amount of 0.01 M phosphate-buffered saline (PBS) at pH 7.2 and dialyzed exhaustively against several changes of PBS. NS was concentrated in a similar manner. AD, AB and NS were used in double diffusion experiments to detect the antibodies.

Detection of Antibodies

Double diffusion in agarose plates was the most satisfactory and simplest method for detecting antibodies. Glass plates (8.5 × 9.5 cm) were prepared by pouring 10 ml of a 1% molten solution of agarose in barbital buffer of 0.02 ionic strength and pH 8.6. After the gel had set, circular wells of 50 µl were punched out with a cork borer and filled once with appropriate serum, antiserum, or antigen. The antigen was applied as 100 mg/ml solution in deionized water. Diffusion proceeded for 24-30 hr at 25 C. Plates were washed with 0.2 M NaCl for 24 hr before staining with 0.5% solution of Amido Black 10B.

RESULTS AND DISCUSSION

Figure 1 shows a typical example of cross-reaction between

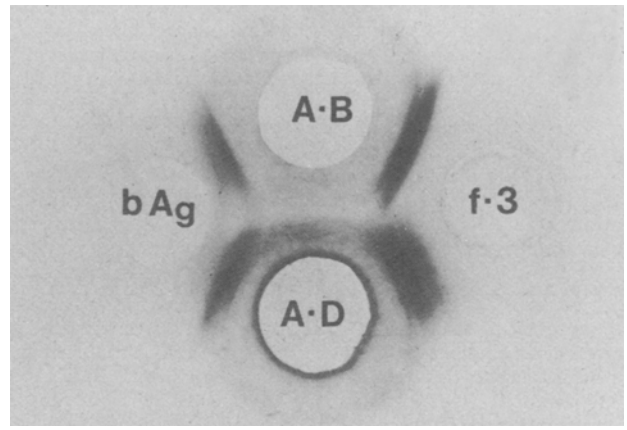

FIG. 1. Double diffusion of purified cotton dust (f-3) and bract extract (b-Ag) against rabbit antisera to dust (AD) and bract (AB) in 1% agarose.

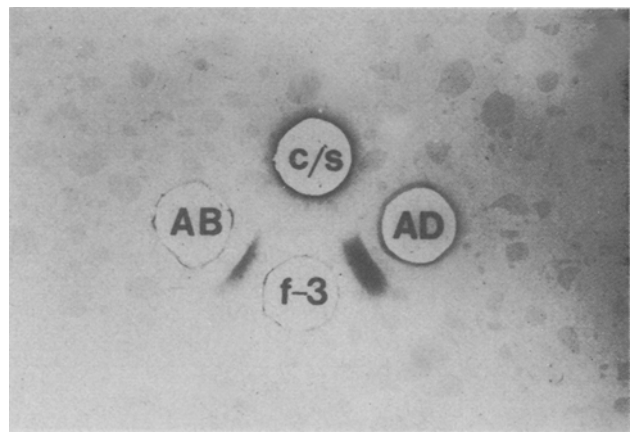

FIG. 2. Double diffusion of cotton dust (AD) and cotton bract (AB) antisera against purified dust antigen (f-3) and cottonseed proteins (C/S).

rabbit antisera raised against dust and bract and their antigens. Figure 2 illustrates reactions between a purified fraction of cotton dust and AD and AB. No reaction was obtained with cottonseed proteins (CS). Similar negative reactions were obtained with cottonseed hulls, clean cotton fibers and house dust. Precipitin lines resulting from reactions between antigens and cotton plant tissue parts (Fig. 1 and Table I) confirm previous reports that antigenic material in the dust originates from vegetable trash. Immunodiffusion reactions between all active material and AD produced precipitin lines that fuse in a reaction of identity, indicating common antigenic determinants. No reaction was obtained with NS. Thus, the precipitation effect obtained between AD and antigens are specific immuno-precipitates. Table I summarizes reactions between AD, AB, NS, and active and inactive antigens. No reaction was obtained with extracts of hulls, proteins, or cotton fibers (cotton grown in the greenhouse). Thus, antigens in cotton dust are not derived from cottonseed tissue, but rather from cotton plant parts other than the fiber. House dust also did not react with preformed antibodies to cotton dust. House dust, therefore, does not possess the same antigenic material found in cotton dust, as had previously been reported (17).

Several mechanisms have been suggested to explain the symptoms of byssinosis. One popular view is that cotton dust induces the release of endogenous histamine to produce bronchoconstriction (18). Studies with oil mill dust

TABLE I

Reactivity between Rabbit Antisera to Cotton Dust and Bract, Normal Rabbit Serum and Extracts of Cotton Dust, Cotton Tissues and House Dust

Antigen	AD[a]	AB	NS
Cotton dust	+	+	–
Cotton bract	+	+	–
Cotton stem	+	+	–
Cotton leaf	+	+	–
Baled cotton	+	+	–
Gin trash	+	+	–
Cottonseed hulls	–	–	–
Cottonseed proteins	–	–	–
Cotton lint (clean)	–	–	–
House dust	–	–	–

[a](AD), antisera to dust; (AB), antisera to bract; (NS), normal serum; (+), positive reaction; (–), no reaction.

indicate that this dust possesses relatively little physiological activity, as measured by the capacity to induce in vitro release of histamine from pig lung tissue (19).

Cotton dust very effectively activates in vitro the human complement cascade (13,14). Extracts from cotton plant tissues are active immunologically with antisera to cotton dust, and extracts from cottonseed tissues are inactive. If this cotton dust antigen is proven to be associated with the symptoms of byssinosis, the results of these tests could provide a basis for the reported low prevalence of the disease in oil mills. Although the cottonseed kernels contain proteins, these data suggest that the seed proteins are not associated with antigens found in cotton dust from textile mills, dust that has been implicated in the symptoms/causes of byssinosis.

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Separation of Methyl Malvalate from Methyl Sterculate

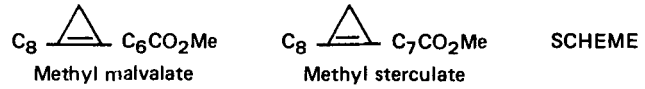
N.E. PAWLOWSKI, P.M. LOVELAND and **R.O. SINNHUBER**, Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331

ABSTRACT

Methyl malvalate and sterculate, labile and differing by a single methylene, are difficult to separate completely. They have been separated in our laboratory by high vacuum spinning band distillation. Each of these fatty esters has been prepared completely free of the other.

The unique fatty acids, malvalic and sterculic, contain a cyclopropene ring in the center of their carbon chains. Cottonseed and kapok oils, which contain these acids, are consumed in large amounts by the world's population and cottonseed flour or meal is finding increasing use as a source of protein for human consumption. These cyclopropenoid fatty acids are responsible for several physiological disorders in farm and laboratory animals (1-10), including cocarcinogenic (11-13) and carcinogenic activity in rainbow trout (14). Their mechanisms of action remain for the most part a mystery. Previous work in these laboratories (12,15) has indicated that the biological activity of malvalic acid may differ from that of sterculic acid, and a complete separation of these two fatty acids, never before achieved, would be desirable.

Research with cyclopropenoid fatty acids is complicated by their high ground-state energy which renders them unstable, or quite reactive. Methyl malvalate and sterculate differ by a single methylene group (Scheme I) and have nearly identical chemical and physical properties. Both instability and similarity create a major task for their separation.



SCHEME I

Nunn (16) and Kircher (17) purified sterculic acid from *Sterculia foetida* oil via crystallization of the urea inclusion complexes. This technique provides a reasonably pure sterculic acid, but not one that is totally free of malvalic acid. Shenstone and Vickery, and Fogerty and coworkers (18,19) have separated the cyclopropenoid fatty acids by reverse-phase column chromatography. This method is quite laborious and in our hands did not clearly separate the two acids.

Surprisingly, cyclopropenoid fatty esters can be distilled under vacuum. Methyl malavate has a slightly lower boiling point and faster distillation rate than sterculate which is the basis of our separation. The low pressure of oxygen during distillation abates free radical initiated polymerizations and while higher temperatures enhance decomposition