RESULTS AND DISCUSSION

Research has been published which disclosed that the proportions of palmitate decreased and oleate increased considerably in the PE and PC remaining after storage of orange juice at 85°F (Nagy and Nordby, 1970). They reported a loss of approximately 90% of the PE and PC during 85°F storage of orange juice. This means that the changes in palmitate and oleate were measured from fatty acid determinations on only the remaining 10% of PE and PC. Also, Nagy and Nordby showed in their Table III that the four major fatty acids in the free fatty acid fraction increased by 85–90% during storage at 85°F. These data do not indicate any selective hydrolysis of the 90% of PE and PC reported as destroyed, but only that the 10% of unhydrolyzed PE and PC had less palmitate and more oleate than in the initial concentrations of these two phospholipids. These authors postulated that the changes in these two fatty acids were due to a specific action of phospholipases in the juice, resulting in complete hydrolysis of certain phospholipids, as no lyso-phospholipids were detected.

The findings presented in Table I show that practically all of the palmitate is at the 1 position of both PE and PC and that the predominant acid at the 2 position is linoleate. It could be reasoned that since no lyso compounds were present, the complete enzymatic hydrolysis of both the 1 and 2 positions of PE and PC should also result in significant decreases of linoleic acid. The number of phospholipids containing two palmitate molecules would also be small, because only about 2–3% of this acid is esterified at the 2 position.

Since the data of Nagy and Nordby (1970) do not indicate any significant decrease during storage of any fatty acid other than palmitate in the remaining PE and PC, it appears that the changes reported were not entirely due to lipase or phospholipase hydrolysis. Knowing that orange juice is an acidic substrate, is subjected to heat treatment during processing, and contains many degradable compounds which may interact with phospholipids causing losses, it seems that phospholipid changes during storage are primarily due to random nonenzymatic degradation, not specific hydrolysis by phospholipases. A careful study of the activity of lipolytic enzymes in citrus juices is needed in order to more fully characterize changes in the lipids which may lead to quality changes in the juice.

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Occurrence of Chlorophyll in Dried Bract of Cotton Plant

Dried bracts from field-grown frost-killed cotton plants but not from hothouse grown plants were shown by visible spectroscopy and thin-layer chromatography to contain chlorophyll a and chlorophyll b. The ratio of chlorophyll a to b was 54:46 in dried bract but 65:35 in green living bract. The total chlorophyll content of dried bract was about 0.3%. An investigation as to whether chlorophyll and pheophytin can be released from bract by lung tissue and the effect of chlorophyll and pheophytin on lung tissue to determine if these compounds can contribute to the acute response of byssinosis may be worthwhile.

Bract, a leaf-like part under the boll of the cotton plant and a reportedly prominent trash component of cotton dust that arises during textile processing, is thought to contain the chemical causative of byssinosis (Bouhuys, 1966; Bouhuys and Nicholls, 1966; Stoll, 1971; Taylor *et al.*, 1971). Plant pigments, in addition to many other compounds, have been suggested (Taylor *et al.*, 1971) as a cause of this industrial pulmonary disease. We, therefore, have undertaken a study of the chemical composition of the dried bract of the mature cotton plant, with special emphasis on the pigments present in this friable material. In this study, we report the identification of chlorophyll (Chl) in dried bract.

EXPERIMENTAL SECTION

Bracts from frost-killed field-grown cotton (High Plains, Lubbock, Texas area; variety, Paymaster 111) were collected (in November of 1971) by hand with special care being taken that the sample contained only dried bracts from under mature bolls (FDB). Samples of dried bract (HHDB), green leaf, and green bract (1 day prior to anthesis) were collected from glanded varieties of cotton grown in a hothouse. The dried bract samples were ground in a Wiley mill (room temperature; 20 mesh) and the green materials were cut into small pieces with scissors. A portion (2–3 g) of each was separately dispersed in methanol (50 ml) or acetone, each containing $MgCO_3$ (100 mg). After stirring at 0° for 1 hr, the dispersion was filtered (Whatman No. 41 paper), and the methanolic solution was partitioned with CCl_4 and the acetone was partitioned with ether. To obtain the green (red fluorescing) pigments, water saturated with $MgCO_3$ was added to the solvent mixture. The CCl_4 or ether phase, containing the green pigments, was evaporated to dryness in a stream of N_2 gas. For thin-layer chromatographic analysis, the samples of the extracts and of authentic Chl a and b were chromatographed on silica gel G (Merck) thin layers at room temperature in a lined tank using the following sol-

Table I. R_f Values of Chl a and Chl b

Compound	Eluent[a] I	Eluent[a] II	Eluent[a] III	Detection: Ultraviolet	Detection: Visible
Chl a[b]	0.70	0.20	0.52	Red	Green
Chl b	0.60	0.03	0.27	Red	Yellow-green

[a] Eluents I and II on silica; eluent III on sucrose. [b] On silica this may be pheophytin a instead of Chl a (see text).

vent systems, acetone–benzene (1:4, w/w) (eluent I) and petroleum ether (bp 30–60°)–acetone–*n*-propyl alcohol (90:10:0.5, v/v/v) (eluent II), as separate one-dimensional eluents and also as a two-dimensional eluent pair, eluent I being used for the first development and eluent II for the second. In addition, samples of the extracts and of authentic Chl a and b were chromatographed on sucrose (confectioner's sugar that contains 3% cornstarch; Imperial Sugar Co.) layers in lined tanks in the dark using petroleum ether (bp 30–60°)–chloroform (3:1, v/v) (eluent III) as the eluent. The separated chlorophylls were detected under ultraviolet light by their red fluorescence. For spectral analysis, the extract residue was dissolved in ether. The visible spectra were measured and used to investigate for the presence of Chl and to calculate the relative amounts of the chlorophylls if they were present (Comar and Zscheile, 1942).

To estimate the content of total chlorophyll by magnesium determination, accurately weighted samples of plant material (1 g) were extracted with methanol (30 ml; no $MgCO_3$ added), with constant stirring at 0° for 1.5 hr, filtered, and further extracted with methanol for 30 min. The combined extracts were filtered quantitatively into a volumetric flask (50.0 ml) and diluted with methanol. Aliquot parts (5.0 ml) were transferred into volumetric flasks (25.0 ml), standard magnesium solution was added, and samples were diluted to volume with water. The blank and 100% absorption reference solution contained methanol (20% by vol) and magnesium (1 ppm). The magnesium content was measured by atomic absorption spectrophotometry. This method for determining total Chl content assumes that Chl is the only source of magnesium in the plant tissue that is extractable with methanol, which may not be true. Small amounts of magnesium from other sources, if present, could cause a large error in this estimation.

RESULTS AND DISCUSSION

Examination by thin-layer chromatography (tlc) indicated that the extracts, except those from HHDB, contained compounds that had the same mobility as authentic samples of Chl a and b. In one-dimensional chromatography on silica, the separation of Chl a from Chl b and of these compounds from other pigments was not always complete. The two-dimensional chromatographic separation on silica of the compounds was excellent, Chl a and b being well separated from each other and from the other pigments. On sucrose, Chl a and b were well separated from interfering pigments into discrete spots and are not destroyed on this substrate (Sestak, 1967). Many other tlc systems for the separation of the chlorophylls from each other and from other pigments have been reported (Sestak, 1967). Chl is usually chromatographed on cellulose because Chl does not decompose as readily on this substrate as it does on silica (Sestak, 1967). However, tlc on cellulose does not usually give a good separation of Chl a and b and of these two compounds from other pigments; the spots become very large and tail badly as they are eluted up the cellulose layer. Tlc on sucrose has been described (Colman and Vishniac, 1964; Nutting *et al.*, 1965; Smith *et al.*, 1965), although these systems need improvement by elution selection. Our systems do give a very good separation.

To determine which Chl has the higher R_f value in the described systems, the upper and lower green pigments from the extract of FDB and from the authentic Chl sample were isolated by preparative tlc (silica, eluent II; sucrose, eluent III), being eluted from the support with ether. The spectra of the green pigment with the lower R_f value showed absorption characteristics of Chl b with slight pheophytin b contamination (λ_{max} 434 and 655 nm) if it had been obtained by silica tlc, but the lower green pigment contained no pheophytin b contamination when sucrose tlc was used. Pheophytin contamination might be expected with silica, since it is not an inert chromatographic medium for these pigments (Sestak, 1967). The green pigment with the higher R_f value showed only pheophytin a (λ_{max} 408 and 667 nm) when tlc on silica was used, but showed only Chl a when tlc on sucrose was used. Spectral results, similar to those for the pigments obtained by tlc on silica, were observed when the green pigments had been obtained by eluting a sucrose column under vacuum with petroleum ether. It follows that the upper green pigment was Chl a and the lower was Chl b. In addition, on silica, Chl a is decomposed to pheophytin at a faster rate than Chl b. Table I contains the thin-layer chromatographic R_f values obtained for (authentic) Chl a and Chl b in our systems.

Acetone only removes trace amounts of Chl from the bracts. It has been reported that methanol preferentially removes Chl b (Comar and Zscheile, 1942). This may not be true with our materials, since a higher amount of Chl a than Chl b was found in green bracts (65:35) and green leaves (71:29) from the cotton plant. By using a standard method (Comar and Zscheile, 1942) for determining Chl in plant tissue, a method which gives an acceptable value for spinach of about 70% Chl a (Comar and Zscheile, 1942; Leowenschuss and Wakelyn, 1972a), green bract was found to be 65% Chl a and green leaf to be 71% Chl a. Methanol, therefore, was used as the extracting solvent. By tlc, the methanol extract contained three components [as yet unidentified; none of which were gossypol [gossypol was identified separately, under different conditions, as a component of dried bract (Loewenschuss and Wakelyn, 1972b)]] in addition to Chl a and b; after partitioning with CCl_4, no Chl, only these three compounds, remain in the methanol. Traces of one of the additional components were found by tlc along with Chl a and Chl b in the CCl_4 layer. Thus, the chlorophylls from these extracts could be completely transferred from the methanol into an equal volume of CCl_4 by the addition of water containing $MgCO_3$.

From the visible spectra of the extracts (CCl_4 phase), the relative amounts of Chl a and b in the different plant parts were estimated. It was seen that FDB contained Chl, of which about 46% was Chl b, but not pheophytin or other contaminating pigments. Green bracts contain Chl a and b, with 35% being Chl b, but bracts that had been grown and dried on the plant in a hothouse (HHDB) contained no Chl.

The total Chl content of FDB as determined by atomic absorption spectrophotometry was 0.32%. Also for comparison, the extract of dried bract used for this determination was chromatographed on sucrose thin layers in a manner similar to that described by Loewenschuss and Wakelyn (1972a). The portions of the chromatogram containing Chl a and b were used to estimate the Mg content (in this methanol extract) that originated only from Chl a and b. This estimation of Mg due to dissolved Chl was approximately the same as the value for Mg that was obtained when the Mg content was determined in the original extract. Such an observation suggests that our estimation of total chlorophyll may be reasonable.

By visible spectroscopy and tlc, FDB has been shown to

contain Chl a and b. That HHDB does not may be due to FDB probably being killed rapidly by frost before translocation or chemical alteration of the Chl, whereas in a hothouse, under humid conditions, these processes would have taken place by the time the bract becomes dried material. The relatively high content in Chl b of FDB is different from the ratio usually found in Chl-containing tissues (Allen, 1966; Comar and Zscheile, 1942; Robinson, 1967; Shlyk, 1971; Strain and Svec, 1966) and in green bract; this may have implications for the function of cotton bract and of Chl b, which is presently thought to be biosynthesized from Chl a (Shlyk, 1971).

The waste obtained from ginning cotton is often made into pellets which are used as feed for animals. This waste material contains bract, and bract has been shown to contain chlorophyll, which can be readily converted to pheophytin. It has been suggested that pheophytin may bind Cu, making it unavailable for utilization by cattle, etc., and therefore accounting for the observations that the Cu status of cattle falls during the summer grazing period and improves during winter, even in diets that contain less Cu (Mills, 1964). Copper pheophytin has been shown to be readily formed (Mills, 1964) after treatment of aqueous acid suspensions of grass with ionic Cu. The isolated product is stable even in the presence of sulfide and chelating agents and decomposes appreciably to ionic Cu only below pH 3.2. It is not known whether chlorophyll and magnesium can be released from dried bract by the surface of lung tissue as it can from plant tissue under the physiological conditions of the rumen. In addition, the effect of pheophytin, magnesium, or chlorophyll on lung tissue is not presently known nor has any connection between these materials and byssinosis been investigated. On the other hand, magnesium poisoning in humans has been reported (Wacker and Parisi, 1968). Since FDB contains chlorophyll and pheophytin, which are highly reactive substances, it is suggested that it may be worthwhile to investigate whether these materials can be released from bract by lung surface tissue and if so to investigate the effect of these compounds on lung surface lining to determine if these materials can contribute to the acute response of byssinosis.

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Rapid Field Method for Evaluating Hydrocyanic Toxicity of Cassava Root Tubers

A simple and rapid method for field evaluating the hydrocyanic toxicity of cassava roots is described. The method uses the blue copper-benzidine reaction, is reliable, and can be performed in a few minutes in the field.

An important part of the crop diversification program at the Cocoa Research Centre (CEPEC) is the selection of new cultivars for cassava (*Manihot esculenta* Crantz). However, the considerable variation in the HCN toxicity levels of cassava root tubers has led to the development of a quick field method for evaluating this toxicity, especially in view of the large number of selections being studied. The toxicity level is often further complicated by the prevailing ecological conditions and the management system used for the cultivation of cassava.

The conventional method of using sodium picrate paper strips is tedious and requires some laboratory equipment which is difficult to take to the field. In addition, the 3 hr needed for the full chemical reaction to become apparent does not lend itself to field conditions, where a quick yet accurate response is preferred.

An adaption of the benzidine blue test (Feigl, 1954), which is accurate down to 0.25 μg of HCN, gave good results when employed for field work with cassava root tubers. No interfering substances are present. The results are reported in the present paper.

MATERIALS

For the experimental work, squares (3 cm × 3 cm) of Whatman No. 1 filter paper, a dropper flask (all glass eyedropper), and a sharp knife were used.

REAGENTS

The reagent used is made up of two parts: solution A consists of 1.43 g of cupric acetate·H_2O dissolved in 500 ml of distilled water, while solution B consists of 275 ml of saturated benzidine acetate solution mixed with 225 ml of