Reeves and Woodham (1974), while attempting to show chromatographic tracings of sediment and water nonfortified and fortified with methomyl, obtained badly resolved double peaks for methomyl in the fortified chromatograms. No satisfactory explanations or remedies were given. However, in the method described here, better resolution was obtained in the chromatograms of green tobacco by operating the gas chromatograph with temperature programming as shown in Figure 3. Similar chromatograms were obtained for cured tobacco.

The oxime derivative of methomyl was analyzed instead of methomyl because the derivative was found to be three times as sensitive as the parent compound under identical conditions of the GLC.

Hence, by combining the two methods, a rapid, sensitive, reliable method of analysis resulting in a high percent recovery of methomyl is developed. The methomyl extracted was also successfully identified as such by GC–MS and TLC.

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LITERATURE CITED

Pease, H. L., Kirkland, J. J., *J. Agric. Food Chem.* **16**, 554 (1968).
Reeves, R. G., Woodham, D. W., *J. Agric. Food Chem.* **22**, 76 (1974).
Williams, I. H., *Pestic. Sci.* **3**, 179 (1972).

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A Survey of the Volatile Constituents of Cotton Lint and Waste with Regard to Byssinosis

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The volatile constituents of lint cotton and gin waste were compared with those of growing buds, bolls, bracts, and hulls by GLC–MS as part of a study on byssinosis, the respiratory disease of cotton mill workers. Of 158 compounds identified from the 5 samples, the growing tissues contained mostly terpenoids, while the lint and waste which have been implicated in byssinosis contained aromatic and alicyclic carbonyl compounds, aromatic alcohols, phenols, esters, lactones, pyrans, epoxides, and pyrazines in addition to the terpenoids. These latter components are believed to be produced in part by microbiological action, oxidation, and heat.

Byssinosis is an occupational respiratory disease which may be caused by inhalation of the dusts of fiber crops such as flax, soft hemp, and cotton. It is characterized by Monday symptoms of chest tightness, cough, and dyspnea accompanied by a marked decrease in expiratory flow rate, and has been associated with an increased prevalence of chronic bronchitis and diminished ventilatory capacity. The prevalence of byssinosis in selected United States textile mills, as determined in five recent independent surveys involving more than 4000 workers, has varied from 20 to 40% in the high-risk preparation areas, and as high as 25% in lower risk yarn processing weaving areas (Merchant et al., 1973).

Byssinosis was first described in Italy in 1705 by Ramazzini, who observed that flax workers developed characteristic respiratory symptoms. Subsequently, investigators such as Prausnitz (1936) and Schilling (1955) described similar respiratory symptoms in cotton-mill workers. At present, the specific causative agents of this respiratory disease are unknown, although Taylor et al. (1971) implicated plant pigments of the flavonoid type, and Tuma et al. (1973) speculated that a bacterial enzyme present in cotton dust might be the causative agent. Model compounds such as methyl piperonylate (Hitchcock et al., 1973) and polymers of quercetin and quercitrin (Kilburn et al., 1973; Hamilton et al., 1973) have been reported to evoke some of the byssinotic symptoms. Steinfeld (1972) reported that phenols and aldehydes were implicated in lung disease.

Similarly, the mechanisms of production of byssinotic symptoms, of decreased flow rates, and of subsequent chronic bronchitis are unknown. Bouhuys et al. (1960) and Bouhuys and Lindell (1961) have suggested that a histamine release in mast cells of the lung occurs. Merchant and associates (1973) described byssinosis as a dose–toxicity–response phenomenon. Although clinical investigators have used human subjects to evaluate samples and fractions, efforts are in progress to develop small-animal bioassays. Kilburn (1972) has exposed hamsters and guinea pigs to respirable dust and subsequently observed recruitment of polymorphonuclear leukocytes through the epithelium of the airways. Changes were also observed in the alveoli, with an increase in macrophages, which had phagocytized the dust particles.

Recently, Merchant and associates (1973) reported that steaming cotton effects a decrease in the byssinotic response by susceptible workers. Almost no drop occurred in the forced expiratory (FEV_1) volume, and only 12% of these workers showed byssinotic symptoms. Although several approaches to a commercially feasible procedure for steaming cotton are being investigated, Merchant and associates suggest that this could best be accomplished at the cotton gin. Later work by Merchant et al. (1974) suggests that steaming reduces dust levels, but does not remove the causative agent.

In 1971, a study was initiated at the Boll Weevil Research Laboratory to survey the chemical constituents of

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the cotton plant, various parts, and its lint in an effort to identify constituents which could be involved in the initiation of byssinosis. In the initial phase, a chemical survey of the essential oil of cotton mill dust resulted in the identification of 67 compounds which included a number of aromatic hydrocarbons, terpene hydrocarbons, aromatic and terpenoid carbonyl compounds, aromatic and terpenoid alcohols, furans, pyrans, epoxides, and nitrogen-containing compounds (Hedin et al., 1974). This work has been hindered by the lack of opportunity to obtain biological evaluations of prepared fractions. Recently, Dr. Ragnar Rylander, Institute of Social and Preventative Medicine, University of Geneva, Switzerland, who has been studying pulmonary cell reactions after exposure to different preparations of the cotton plant, evaluated several of the fractions prepared here and found that some elevated the leucocyte and macrophage levels in the airways of guinea pigs (Rylander, 1974). He also reported commercial lint samples with high counts of several bacteria, including *Bacillus subtilus, Escherichia coli,* and *Enterobacter sp.,* increased the recruitment of leucocytes and macrophages. He also found that an extract could be prepared by steeping cotton lint in boiling water that would greatly increase these cell levels. In all instances particulate material was removed before testing. It is the further intent of his work to link the leucocyte reaction in guinea pigs and other species with effects which are encountered in human exposures. The work reported here is with cotton fractions which were either prepared similarily to those of Rylander or evaluated by his guinea pig bioassay.

MATERIALS AND METHODS

Isolation and Fractionation. The bale lint cleaner waste (BLCW) derived from mixed upland cottons that were collected by the Palins Ginning Research Laboratory, Lubbock, Tex. (15 kg), was steam distilled in an all-glass system, and the distillate was extracted with ethyl ether. The oil after removal of the solvent was chromatographed on a 2 × 25 cm cold-water-jacketed Florisil column. The hydrocarbons were eluted with 100 ml of pentane, and the polar compounds were eluted successively with 100 ml each of 5, 10, 20 and 50% Et_2O in pentane, and finally with 100% Et_2O. Progress of the elution and recombination of all fractions into seven reconstructed fractions of gradually increasing polarity was monitored by silica gel TLC.

In similar fashion, fresh buds, sterile cotton lints obtained by removing the green hulls, and green hulls and bracts from *Gossypium hirsutum* L. var. Deltapine Smoothleaf cotton plants grown locally were steam distilled and fractionated by column chromatography for subsequent analysis by gas–liquid chromatography/mass spectrometry (GLC–MS). The oil yields varied from 30 ppm for the sterile lint to 330 ppm for the BLCW.

To obtain a preparation similar to the boiling water extract of Rylander which elevated the leucocyte and macrophage levels of guinea pigs, 2 kg of a commercial lint sample was refluxed in boiling water in a flask equipped with a cold water condenser for 2 hr, and the contents were filtered by gravity through coarse filter paper to yield a clear extract. Extraction of the aqueous extract with ethyl ether or methylene chloride and subsequent solvent removal yielded a yellow oil with a noxious, choking odor. This oil also elicited a feeling of chest tightness in informal sensory tests by laboratory personnel. The aqueous phase, which had initially possessed a musty odor, was rendered essentially odorless by the extraction with organic solvents.

The lint oil was fractionated on a Florisil column in essentially a similar fashion to that employed with the BLCW except that mixtures of ether and methanol were required to elute some of the components because of their higher polarity. The fractionation was monitored by TLC, and all fractions were ultimately recombined into five reconstructed fractions, the third of which possessed essentially all of the odorous and physiological properties of the original oil. This fraction was eluted with 2% methanol in ethyl ether after fraction 2 was eluted with 30% ether in pentane.

Analytical GLC–MS. Fractions were introduced into a Hewlett Packard 5930 quadrupole mass spectrometer from either (1) a 250 ft × 0.03 in. capillary column coated with OV 17 which was programmed from 90 to 180° at 2°/min (gas flow, 8 ml/min of He), (2) a 20 in. × ⅛ in. column packed with 10% UCW-98 on Chromosorb Q which was programmed from 120 to 230° at 4°/min (gas flow, 40 ml/min of He), or (3) a solid probe. Mass spectra were obtained at 70 eV. Peak identity was confirmed by comparison with standards where possible. Solid probe analysis showed that a number of other components in the mass range M^+ 250–400 were also present, but they are not included except for β-sitosterol which was unequivocally identified.

RESULTS AND DISCUSSION

From the fractions and by comparison with previous work in this laboratory (Hedin et al., 1973), mass spectral data from a commercial lint water extract, the bale lint cleaner waste oil, fresh buds, sterile lint, and green hulls and bracts were interpreted and tabulated. Table I lists 30 alkyl hydrocarbons, 18 aromatic hydrocarbons, 25 aliphatic carbonyl compounds, 14 aromatic carbonyl compounds, 35 alcohols and phenols, 3 esters, 5 lactones, 10 furans, pyrans, or epoxides, 13 fatty acids, and 5 miscellaneous compounds, a total of 158 compounds that were present in one or more of the samples. Since isolation, spectral analysis, and other studies were not performed, the identifications should be considered tentative. Also, it is likely that some of the higher boiling compounds were not eluted from the GLC column; thus, not all steam distillable compounds were subjected to GLC–MS analysis.

Table II lists 10 carbonyl compounds, 7 alcohols and phenols, 5 lactones, 3 furans and pyrans, and 2 nitrogen containing compounds. These compounds were identified either in the BLCW oil fraction that was reported by Rylander (personal communication) to elicit an appreciable increased leucocyte and macrophage recruitment in the airways of guinea pigs, or in the commercial lint water extract fraction that was found by us to possess the previously described odor and physiological properties.

The components of the sterile cotton lints (Table I) were almost entirely limited to the mono- and sesquiterpene hydrocarbons, β-caryophyllene oxide, and β-bisabolol, all found in fresh buds. There were, additionally, a number of minor peaks that could not be identified, some of which were probably carbonyl compounds and alcohols. The total yield of volatiles from the lints was very low (30 ppm).

The components of the fresh buds and the green hulls and bracts were similar to each other and to that previously reported by us (Hedin et al., 1973). Those present included a series of mono- and sesquiterpene hydrocarbons comprising about half the oil, a number of low molecular weight aldehydes, ketones, and alcohols, and several oxygenated mono- and sesquiterpenoids, mostly alcohols.

By comparison, the BLCW oil and the commercial lint oil contained chiefly oxygenated compounds such as aromatic and alicylic carbonyl compounds and alcohols, several phenols, esters, lactones, furans, pyrans, epoxides, fatty acids, chlorinated hydrocarbons, indole, and tetramethylpyrazine. They also contained a number of components present in the fresh plant, which is to be expected.

These components are among the 10 carbonyl compounds, 7 alcohols and phenols, 5 lactones, 3 furans and pyrans, and 2 nitrogen-containing compounds listed in Table II that were found in fractions that were biologically active or possessed noxious odor properties.

Table I. Volatile Components from Growing Cotton, Aged Bracts, and Commercial Lint

Compound	M+	I_k[a]	Ref. or fragmentation	Comm. lint water ext.	BLCW[b] steam distillate	Fresh buds	Sterile cotton lints	Green hulls + bracts
			Alkyl Hydrocarbons					
α-Pinene	136	1080	c		X	X	X	X
Camphene	136	1095	c			X		
β-Pinene	136	1105	c		X	X	X	X
Myrcene	136	1115	c			X	X	X
α-Phellandrene	136	1140	c			X		
α-Terpinene	136	1155	c			X		
l-Limonene	136	1168	c			X		
β-Phellandrene	136	1185	c			X		
trans-β-Ocimene	136	1210	c			X	X	X
γ-Terpinene	136	1222	c			X		
Terpinolene	136	1240	c			X		
n-( )-Decene	140	1020	c	X				
n-Decane	142	1000	c	X				
( )-Dodecane	170	1200	c	X				
α-Curcumene	202	1620	c		X			
Copaene	204	1520	c		X	X		
l-trans-α-Bergamotene	204	1540	c			X		
l-Caryophyllene	204	1582	c	X	X	X	X	X
Farnesene	204	1595	c			X		
α-Humulene	204	1605	c	X	X	X	X	X
α-Bisabolene	204	1610	c		X			
α_2-Bisabolene	204	1615	c		X			
cis-γ-Bisabolene	204	1618	c			X		
l-δ-Guaiene	204	1622	c			X		
l-δ-Cadinene	204	1675	c			X		
α-Santalene	204	1630	c		X			
β-Santalene	204	1633	c		X			
epi-β-Santalene	204	1636	c		X			
$C_{15}H_{26}$	206	1580	69, 70, 83, 97, 111		X			
3-Methyltetradecane	212	1490	43, 41, 57, 197, 212		X			
			Aromatic Hydrocarbons					
Toluene	92	860	c	X	X			
Ethylbenzene	106	1015	c	X	X			
o-Xylene	106	1005	c		X			
Propylbenzene	120	1130	c		X			
o-Ethyltoluene	120	1135	c	X	X			
m-Ethyltoluene	120	1142	c	X				
p-Ethyltoluene	120	1148	c		X			
1,3,5-Trimethylbenzene	120	1145	c	X				
Naphthalene	128	386	c	X				
1-Methyl-2-propylbenzene	134	1175	c	X				
( )-Diethylbenzene[d]	134	1165	c	X				
tert-Butylbenzene	134	1175	c	X				
1-Methylnaphthalene	142	1497	c	X				
2-Methylnaphthalene	142	1526	c	X				
Acenaphthene	154	1585	c	X				
( )-Methyl-( )-ethylnaphthalene	170	1587	c	X	X			
2-Ethylbiphenyl	182	1675	c	X				
Diethylnaphthalene	184	1680	c	X				
			Aliphatic Carbonyl Compounds					
Acetone	58	625	c			X		
Isobutyraldehyde	72	740	c			X		
Butyraldehyde	72	750	c			X		
Isovaleraldehyde	86	835	c			X		
2,4-Hexadienal	96	1020	c		X			
Cyclohexanone	98	920	c	X				
trans-2-Hexenal	98	965	c			X		
1-Hexanal	100	945	c			X		

Table I *(Continued)*

Compound	M+	I_k[a]	Ref. or fragmentation	Comm. lint water ext.	BLCW[b] steam distil- late	Fresh buds	Sterile cotton lints	Green hulls + bracts
5-Methyl-2-furfural	110	1265	*c*	X				
Cycloheptanone	112	1015	*c*	X				
Cyclohexane-carboxaldehyde	112	1035	*c*	X				
Heptanal	114	1040	*c*			X		
2-Octenal	126	1170	*c*			X		
trans-2,*cis*-6-Nonadienal	138	1330	*c*			X		
2-Nonenal	140	1265	*c*			X		
1-Nonanal	142	1225	*c*			X		X
Myrtenal	150	1340	*c*		X	X		X
Perillaldehyde	150	1360	*c*	X				
Verbenone	150	1365	*c*		X			
Isopulegone	152	1315	*c*		X			
4-Isopropyl-1-cyclohexene-1-carboxaldehyde	152	1380	*c*		X			
Isopinocamphene	152	1385	*c*		X			
α-Campholene aldehyde	152	1370	*c*		X			
Citronellal	154	1355	*c*		X			
β-Ionone	192	1480	*c*			X		X
			Aromatic Carbonyl Compounds					
Benzaldehyde	106	1172	*c*	X	X	X		X
p-Tolualdehyde	120	1230	*c*	X	X	X		X
( )-Tolualdehyde	120	1240	*c*		X			
Phenylacetaldehyde	120	1248	*c*		X			
Acetophenone	120	1270	*c*	X				
2′-Methylacetophenone	134	1360	*c*		X			
( )-Phenyl propionaldehyde	134	1415	92, 91, 105, 119, 134		X			
Benzyl methyl ketone	134	1385	92, 91, 70, 55, 134		X			
Cumic aldehyde	148	1490	*c*		X			
4′-Ethylacetophenone	148	1490	133, 105, 148, 91, 119		X			
Vanillin	152	1620	*c*	X				
2,4-Dimethylacetophenone	180	1580	*c*		X			
5-*tert*-Butyl-3,3-dimethyl-( )-indanone	216	1620	*c*		X			
Germacron	218	1640	*c*		X			
			Alcohols and Phenols					
1-Penten-3-ol	86	910	*c*			X		X
Isopentyl alcohol	88	870	*c*			X		X
2-Methyl-1-butanol	88	875	*c*			X		X
3-Methyl-1-butanol	88	875	*c*			X		X
1-Pentanol	88	880	*c*	X		X		X
Phenol	94	1230	*c*	X				
trans-2-Hexen-1-ol	100	995	*c*			X		X
1-Hexanol	102	980	*c*			X		X
cis-3-Hexen-1-ol	100	990	*c*			X		X
4-Hexen-1-ol	100	985	*c*			X		
Cyclohexanol	100	940	*c*			X		
Benzyl alcohol	108	1260	*c*	X	X	X		
4-Hydroxy-4-methyl-2-pentanone	116	1105	*c*	X				
2-Phenylethanol	122	1350	*c*	X	X	X		
6-Octen-4-ol	128	1195	43, 55, 45, 41, 99			X		
m-Tolyl ethyl ether	136		*c*	X				
Cuminyl alcohol	150	1525	*c*		X			
Thymol	150	1532	*c*		X			
Carvacrol	150	1537	*c*		X			
Myrtenol	152	1490	*c*		X			

Table I *(Continued)*

Compound	M+	I_k[a]	Ref. or fragmentation	Comm. lint water ext.	BLCW[b] steam distillate	Fresh buds	Sterile cotton lints	Green hulls + bracts
Carveol	152	1385	*c*			X		
α-Terpineol	154	1340	*c*			X		X
Isoborneol	154	1315	*c*			X		X
Nerol	154	1410	*c*			X		X
Geraniol	154	1425	*c*			X		X
Thujyl alcohol	154	1415	*c*		X			
Citronellol	156	1326	*c*			X		
2-(Cyclohexadienyl)-4-methyl-1-pentanol	194	1665	69, 95, 136, 151, 176		X			
α-Caryophyllene alcohol	222	1740	*c*		X			
α-Bisabolol	222	1765	*e*		X	X		
β-Bisabolol	222	1795	*f*	X	X	X	X	X
Nerolidol	222	1705	*c*			X		
o-Phenylphenol	170	1760	*c*	X				
( )-Di-*sec*-butylphenol	206	1680	*c*	X				
β-Sitosterol	414	S.P.	*c*	X		X		X
			Esters					
Ethyl acetate	88	980	*c*	X				
Methyl linoleate	294	2130	*c*		X			
Methyl oleate	296	2105	*c*		X			
			Lactones					
γ-Butyrolactone	86	895	*c*	X				
γ-Caprolactone	114	1090	*c*		X			
δ-Octalactone	142	1290	*c*	X				
γ-Nonalactone	156	1380	*c*	X				
γ-Undecalactone	184	1585	*c*	X				
			Furans, Pyrans, and Epoxides					
Dihydropyran	84	910	*c*	X	X			
2,5-Dimethylfuran	96	990	*c*		X			
5-Methyl-2-furfural	110	1265	*c*	X				
2,3-Dihydropyran-( )-carboxaldehyde	112	1180	*c*		X			
2-Acetyl-4-methylfuran	124	1215	*c*		X			
2-Furyl isopropyl ketone	138	1280	*c*		X			
2-Butyl-4-methylfuran	138	1280	67, 95, 123, 138, 109		X			
2-Butyl-4-vinylfuran	150	1410	53, 81, 108, 107, 135		X			
Bisabolene oxide	220	1710	*g*		X	X		X
β-Caryophyllene oxide	220	1725	*h*, 79, 93, 55, 91, 121		X	X	X	X
			Fatty Acids					
Acetic acid	60	782	*c*	X				
Nonanoic acid	158	1480	*c*	X				
Decanoic acid	172	1585	*c*	X				
Undecanoic acid	184	1678	*c*	X				
Pentadecanoic acid	242	2085	*c*	X				
Hexadecanoic acid	256	2190	*c*	X				
Heptadecanoic acid	270	SP	*c*	X				
( )-Heptadecenoic acid	268	SP	*c*	X				
( )-Heptadecadienoic acid	266	SP	*c*	X				
Stearic acid	284	SP	*c*	X				
Linoleic acid	280	SP	*c*	X				
Linolenic acid	278	SP	*c*	X				
			Miscellaneous					
Indole	117	1585	*c*	X	X			
Benzothiazole	135	1480	*c*	X				
( )-Dichlorobenzene	146	1175	*c*	X				
Tetramethylpyrazine	136	1180	*c*	X	X			
Tetrachloroethylene	164	920	*c*	X				

[a] Kováts (1961) indices: e.g., *n*-pentadecane = 1500; *n*-hexadecane = 1600. [b] Hedin et al., 1974. [c] Stenhagen et al., 1969. [d] Parentheses on diethylbenzene and all subsequent compounds indicate the precise isomer could not be assigned. [e] Hedin et al., 1971. [f] Minyard et al., 1968. [g] Hedin et al., 1972. [h] Minyard et al., 1969.

Table II. Compounds Present in Fractions from the Commercial Lint Water Extract and Bale Lint Cleaner Waste Essential Oil Which May Induce Byssinotic Symptoms

Compound	Leucocyte–macrophage bioassay	Human sensory bioassay
	Carbonyls	
Cyclohexanone		X
Cycloheptanone		X
Phenylacetaldehyde	X	
Cyclohexanecarboxaldehyde		X
Perillaldehyde		X
Benzaldehyde	X	
p-Tolualdehyde	X	
2,3-Dihydropyran-( )-aldehyde	X	
5-Methyl-2-furfural		X
Vanillin		X
	Alcohols	
2-Phenylethanol	X	X
4-Hydroxy-4-methyl-2-pentanone		X
Benzyl alcohol	X	X
Cuminyl alcohol	X	
Thymol	X	
Carvacrol	X	
Myrtenol	X	
	Lactones	
γ-Butyrolactone		X
γ-Caprolactone	X	
δ-Octalactone		X
γ-Nonalactone		X
γ-Undecalactone		X
	Furans and Pyrans	
Dihydropyran	X	X
2,5-Dimethylfuran	X	
2-Acetyl-5-methylfuran	X	
	Nitrogen Compounds	
Indole	X	X
Tetramethylpyrazine	X	X

The presence of these oxygenated compounds may be attributed in part to microbiological contamination on the basis of several model system studies. Morgan et al. (1966) found ethanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methylpropanal, and 3-methylbutanal in the headspace vapor of milk cultures of *Streptococcus lactis*. Sheldon et al. (1971) also found phenylacetaldehyde, phenylethanol, ethyl butyrate, ethyl hexanoate, and ethyl decanoate in this same culture. Kaminski et al. (1974) found 3-methyl-1-butanol, 3-octanone, 3-octanol, 1-octen-3-ol, 1-octanol, and 2-octen-1-ol in culture distillates of *Aspergillus, Penicillium,* and *Fungi imperfecti*. Compounds identified tentatively were butyl and isobutyl alcohol, butyl, pentyl, and octyl acetate, pyridine, hexanol, nonanone, dimethyl- and tetramethylpyrazine, benzaldehyde, propylbenzene, and phenylethanol. *Pseudomonas fragi* produced several esters including ethyl acetate, ethyl propionate, ethyl butyrate, ethyl isovalerate, and ethyl hexanoate (Reddy et al., 1968). Several of these components are also present in the cotton fractions. Rylander, as previously described (personal communication), found elevated counts of *E. coli, Bacillus subtilus,* and *Enterobacter sp.* in commercial cotton lints that elicited leucocyte and macrophage recruitment in guinea pigs. Another compound which may be of interest although it has not been biologically evaluated in this context is scopoletin which was found in dried bracts of the cotton plant by Wakelyn et al. (1974). They cited other data that scopoletin occurs in cigarette smoke and tobacco, and, while it is naturally occurring in plants, it has been reported to increase greatly in fruits and vegetables infected by microorganisms. Cotton plants affected with *Verticillium* wilt also apparently contain scopoletin, and infection greatly enhances the production of scopoletin in the cotton plant.

It is possible that one or several components chemically related and diverse, produced by processes such as microbiological involvement, oxidation, and heating, are responsible for initiation of byssinotic effects. It should be noted that most of the components identified are volatile to some degree, and thus are more likely to be transported to their site of action. By comparison, the components in fresh tissue exist in a much less oxidized state, and few are known to possess any physiological activity.

LITERATURE CITED

Bouhuys, A., Lindell, S. E., *Experientia* **17,** 211 (1961).
Bouhuys, A., Lindell, S. E., Lundin, G., *Br. Med. J.* **1,** 324 (1960).
Hamilton, J. D., Halprin, G. M., Kilburn, K. H., Merchant, J. A., Ujda, J. R., *Arch. Environ. Health* **26,** 120 (1973).
Hedin, P. A., Thompson, A. C., Gueldner, R. C., *Toxicol. Environ. Chem. Rev.* **1,** 291 (1973).
Hedin, P. A., Thompson, A. C., Gueldner, R. C., *Ann. N.Y. Acad. Sci.* **221,** 174 (1974).
Hedin, P. A., Thompson, A. C., Gueldner, R. C., Minyard, J. P., *Phytochemistry* **10,** 1693 (1971).
Hedin, P. A., Thompson, A. C., Gueldner, R. C., Ruth, J. M., *Phytochemistry* **11,** 2118 (1972).
Hitchcock, M., Piscitelli, D. M., Bouhuys, A., *Arch. Environ. Health* **26,** 177 (1973).
Kaminski, E., Stawicki, S., Wasowiez, E., *Appl. Microbiol.* **27,** 1001 (1974).
Kilburn, K. H., Lynn, W. S., Tres, L. L., *Lab. Invest.* **28,** 55 (1973).
Kováts, E. sz., *Z. Anal. Chem.* **181,** 351 (1961).
Merchant, J. A., Lumsden, J. C., Kilburn, K. H., Germino, V. H., Hamilton, J. D., Lynn, W. S., Byrd, H., Baucom, D., *Br. J. Ind. Med.* **30,** 237 (1973).
Merchant, J. A., Lumsden, J. C., Kilburn, K. H., O'Fallon, W. M., Copeland, K., Germino, V. H., McKenzie, W. N., Baucom, D., Curren, P., Stilman, J., *Br. J. Ind. Med.* **31,** 261–274 (1974).
Minyard, J. P., Hardee, D. D., Gueldner, R. C., Thompson, A. C., Hedin, P. A., *J. Agric. Food Chem.* **17,** 1093 (1969).
Minyard, J. P., Thompson, A. C., Hedin, P. A., *J. Org. Chem.* **33,** 909 (1968).
Morgan, M. E., Lindsay, R. C., Libbey, L. M., Pereira, R. L., *J. Dairy Sci.* **49,** 15 (1966).
Prausnitz, H., "Investigations on Respiratory Dust Disease in Operatives in the Cotton Industry", His Majesty's Stationary Office, London, 1936.
Reddy, M. C., Bills, D. D., Lindsay, R. C., Libbey, L. M., Miller, A., Morgan, M. E., *J. Dairy Sci.* **51,** 656 (1968).
Rylander, R., Symposium on Cotton Dust, American Conference of Governmental Industrial Hygienists, Atlanta, Ga., Nov 1974.
Schilling, R. S. F., *Br. J. Ind. Med.* **12,** 217 (1955).
Sheldon, R. M., Lindsay, R. C., Libbey, L. M., Morgan, M. E., *Appl. Microbiol.* **22,** 263 (1971).
Steinfeld, J. L., Ed., "The Health Consequences of Smoking", A Report of the Surgeon General, 1972, p 139.
Stenhagen, E., Abrahamsson, S., McLafferty, F. W., "Atlas of Mass Spectral Data", Vol. 1, 2, and 3. Interscience, New York, N.Y., 1969.
Taylor, G., Massoud, A. A., Lucas, F., *Br. J. Ind. Med.* **28,** 143 (1971).
Tuma, J., Parker, G., Braun, D. C., *J. Occup. Med.* **15,** 409 (1973).
Wakelyn, P. J., Stipanovic, R. D., Bell, A. A., *J. Agric. Food Chem.* **22,** 567 (1974).

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