(+)-*trans*-Bromethrin has been resolved from the racemic mixture by use of D(−)-threo-2-(dimethylamino)-1-(*p*-nitrophenyl)-1,3-propanediol (Muller *et al.*, 1968). Preliminary toxicity studies of the resolved compound have shown that the (+) form is approximately twice as active as the (±) form ((+)-*trans*-resmethrin is also approximately twice as toxic to insects as the racemic form) (Casida, 1973). It is probable that the resolution of (±)-*trans*-fluorethrin to the (+) enantiomer will also result in a significant increase in insecticidal activity.

EXPERIMENTAL SECTION

All melting and boiling points were uncorrected. Ethyl chrysanthemate was purchased from Pfaltz and Bauer, Inc., Flushing, N.Y. Nmr spectra were taken employing a Jeolco JNM-PS-100 or a JNM-MH60-II.

(±)-*trans*-3-(2,2-Difluorovinyl)-2,2-dimethylcyclopropanecarboxylic Acid. *tert*-Butyl (±)-*trans*-3-formyl-2,2-dimethylcyclopropanecarboxylate, 22.0 g, and triphenylphosphine, 33.5 g, were dissolved in 100 ml of dimethylformamide and placed in a three-necked flask. The flask was fitted with a nitrogen inlet tube, reflux condenser, and powder inlet port, and was heated in an oil bath to 165–170° (bath temperature). Sodium chlorodifluoroacetate, 25.5 g, was added over 1 hr (carbon dioxide was evolved). The reaction flask was cooled and the contents were washed with water and diethyl ether. The ether was dried over anhydrous magnesium sulfate and removed *in vacuo*. Distillation of the residue afforded 9.0 g of colorless oil: bp 80–90° (17 mm); nmr ($CDCl_3$) δ 3.98 (ddd, 1, J = 3, 8, 24 Hz, =CH), 1.89 (m, 1), 1.52 (d, 1, J = 5 Hz, $CHCO_2$), 1.44 (s, 9), 1.22 (s, 3), and 1.12 ppm (s, 3). Hydrolysis of the *tert*-butyl ester, 9.0 g, was carried out with *p*-toluenesulfonic acid in refluxing toluene to give 5.0 g of crude acid. Recrystallization from cold hexane gave 4.5 g of white solid which melted below room temperature: nmr ($CDCl_3$) δ 11.98 (s, 1), 4.06 (ddd, 1, J = 3, 8, 24 Hz, =CH), 2.00 (m, 1), 1.47 (d, 1, J = 6 Hz, $-CHCO_2$), 1.28 (s, 3), and 1.12 ppm (s, 3). An amine salt of the acid was prepared from D(−)-*threo*-2-dimethylamino-1-(*p*-nitrophenyl)-1,3-propanediol, mp 132–134°. *Anal.* Calcd for $C_{19}H_{26}F_2N_2O_5$: C, 56.99; H, 6.55; N, 7.00. Found: C, 56.84; H, 6.78; N, 7.15.

(5-Benzyl-3-furyl)methyl (±)-*trans*-3-(2,2-Difluorovinyl)-2,2-dimethylcyclopropanecarboxylate. 3-(2,2-Difluorovinyl)-2,2-dimethylcyclopropanecarboxylic acid, 4.5 g, was added to 50 ml of hexane containing 2.7 ml of thionyl chloride. After standing 24 hr, the hexane was removed and excess $SOCl_2$ removed *in vacuo*. The crude acid halide was added to a solution of 3.0 ml of pyridine and 3.7 g of (5-benzyl-3-furyl)methyl alcohol in 250 ml of dry benzene. After standing overnight in the dark, the pyridine hydrochloride was filtered off and the benzene removed *in vacuo*. The ester was purified employing silica gel chromatography and eluting with hexane–ethyl acetate (9:1). Removal of the solvents *in vacuo* gave a colorless oil: nmr ($CDCl_3$) δ 7.28 (s, 1, =CHO), 7.18 (s, 5, C_6H_5), 6.00 (s, 1), 4.88 (s, 2), 4.00 (ddd, 1, J = 3, 8, 24 Hz), 3.88 (s, 2), 2.00 (m, 1), 1.44 (d, 1, J = 6 Hz, $-CHCO_2$), 1.20 (s, 3), and 1.05 ppm (s, 3). *Anal.* Calcd for $C_{20}H_{20}F_2O_3$: C, 69.35; H, 5.82. Found: C, 69.54; H, 6.11.

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COMMUNICATIONS

Effects of Freeze-Drying on Residues of TDE, DDT, and Endosulfan in Tobacco

This study was conducted to examine the effect of freeze drying of cured tobacco shreds, with or without extraction, on removal of TDE, DDT, and endosulfan residues. Tobaccos were utilized having initially low and high levels of pesticide. Freeze drying of cured tobacco shreds significantly reduced residue levels, with up to 42% reduction for total TDE, 41% for total DDT, and 43% for total endosulfan. Because of the expansive effect of freeze drying, residue levels on a volumetric basis (micrograms/milliliter) were reduced up to 74%. With the exception of the group of tobacco having low initial levels of TDE and DDT, the extraction step prior to freeze drying did not contribute significantly to pesticide reduction in comparison with standard freeze drying. Analyses of water extracts substantiated this conclusion.

Recognition of pesticide residues on tobacco as a potential health hazard has led to increased emphasis on measures for reducing pesticide levels. The problem of pesticide residues on tobacco was recently discussed (Guthrie and Sheets, 1970; Guthrie, 1973) in the context of changes necessitated by legislation in the Federal Republic of Germany which extended pesticide tolerances to tobacco (Bundesgestzblatt, 1972). This legislation, providing regulations to become effective January 1, 1978, may preclude sale of tobacco to the German manufacturer if residues on

the leaf product exceed tolerances. While restrictions to the sale or use of persistent insecticides, including DDT and TDE, are producing dramatic reductions in residue levels of U.S. auction market tobacco (Domanski and Sheets, 1973; Domanski and Sheets, 1975; Gibson *et al.*, 1974), it may be difficult to reduce residues to such low levels.

Reduction in pesticide residues may be achieved through various means including restricting the use of persistent pesticides, utilizing nonpersistent, readily degraded pesticides, minimal use practices in pest control, etc. In addition, there are opportunities for reducing residue levels in tobacco manufacturing processes.

Zabik and Dugan (1971) reported significant reduction in chlorinated hydrocarbon insecticides from whole eggs by freeze drying, which suggests that similar reductions may be found for tobacco. Their research showed reductions of 79% for lindane, 37% for dieldrin, and 44% for total DDT. Factors which may relate to ease of removal included pesticide vapor pressure, concentration of pesticide, and density of the product containing the pesticide.

The freeze-drying process for cured tobacco shreds (Johnson, 1970, 1973) provides turgoring and sublimation conditions which may effectively contribute to reduced residue levels on cured tobacco. The turgoring, or vacuum impregnation, phase permits the addition of water to defined levels. Process variables of water temperature, water/solids ratio, soak time prior to freezing, and sublimation conditions can be controlled over a wide range. Extraction following impregnation is also possible by removal of a portion of the initial water added.

This investigation was undertaken to examine the potential of freeze drying of cured tobacco shreds, with and without extraction, for removal of TDE, DDT, and endosulfan from tobaccos having initially high and low levels of residues.

EXPERIMENTAL PROCEDURE

Experimental Design. Four groups of flue-cured tobacco were selected from the stocks of the Pesticide Residue Research Laboratory at North Carolina State University as follows: (A) low concentrations of TDE and DDT; (B) low concentrations of endosulfan; (C) high concentrations of TDE and DDT; (D) high concentrations of endosulfan. Tobacco samples were selected from each group to provide three replications for each of three treatments identified as (I) control, (II) standard freeze drying, and (III) extraction plus freeze drying.

Procedure. All tobacco samples were shredded at approximately 12–16 cuts/cm. To assure uniformity of moisture levels prior to treatment and to permit handling with minimum shred breakage, all samples were placed into an Aminco-Aire humidity-temperature control chamber for 24 hr of conditioning at approximately 20° and 80% relative humidity. Subsamples oven dried at 110° for 3.25 hr provided dry weight estimates for treatment samples. Moisture levels after conditioning were calculated to be in the range of 21.5–22.9% (dry basis). At this time the 12 control samples were placed into sealed glass jars and stored at −18°.

Samples for the standard freeze-drying treatment (II) were processed as follows. Approximately 350 g of conditioned tobacco was placed into a 29.2 cm × 62.2 cm stainless steel tray to form a mat of approximately 1.3 cm thickness. A predetermined amount of water at 25° was added by a vacuum turgoring technique described by Johnson (1973) to establish a mat moisture content of 450%. Vacuum turgoring was accomplished within 60 sec. After a 10-min rest time which allowed essentially complete imbibition of water, the samples were transferred to a Vir-Tis Model FFD-40-WS freeze dryer and vacuum frozen within 5 min. The frozen samples were stored at −70° prior to freeze drying.

Samples for the extraction plus freeze drying treatment (III) were processed in a similar manner. However, the samples were vacuum conditioned to a moisture content of 600% with 25° water, allowed a 3-min rest time, and then vacuum drained for about 60 sec. Moisture contents of samples after extraction were in the range of 390–420%. The water extracts were collected, measured volumetrically, and stored at 3° in sealed glass containers for analysis later. After a rest time of 10 min from vacuum conditioning, the samples were vacuum frozen and placed into the freezer at −70°.

After the 24 samples for treatments II and III were vacuum conditioned and frozen, the samples were transferred to a Vir-Tis Model FFD-100 freeze dryer with shelves precooled to −18°. Vacuum was established and samples were allowed to equilibrate for 2 hr without applied heat. Shelf temperatures were increased to 25° for overnight operation and then further increased to 80° for final drying. Chamber pressure during freeze drying was generally less than 1.0 Torr. After completion of drying, the samples were weighed, removed from the trays, and stored in glass jars for subsequent analyses.

Analytical Methods for Determining TDE, DDT, and Endosulfan. Prior to extraction, the tobacco was ground in a Wiley mill; then, the pesticides were extracted and subsequently the extracts were analyzed by gas chromatographic procedures. Details of these methods were previously described (Domanski *et al.*, 1972; Domanski and Sheets, 1973). Residue values have been corrected to a 13% moisture content of the tobacco.

A well-stirred 100-ml portion of the water extract from the extraction plus freeze-drying treatment was extracted three times with 100 ml of diethyl ether in pentane (1:3, v/v). The diethyl ether–pentane extract was washed with 100 ml of an aqueous 2% NaCl solution. The aqueous layer was discarded and the diethyl ether–pentane layer was filtered through anhydrous Na_2SO_4. The dried extraction was reduced in volume for subsequent gas chromatographic analysis.

RESULTS AND DISCUSSION

In all cases, treatments of standard freeze drying and extraction plus freeze drying reduced the level of TDE and DDT residues below that of the control (Table I) and there were greater reductions for some isomers than for others.

With group A tobacco (low level of TDE and DDT), extraction plus freeze drying appeared more effective than standard freeze drying in reducing the pesticide level, with all values significantly different from their respective controls. Extraction plus freeze drying reduced total TDE and total DDT by 28 and 21%, respectively, whereas only about 10 and 11% reductions were noted for standard freeze drying. Since a definite trend of reduction was obtained for each constituent, the extraction step was apparently beneficial.

A different response was obtained with the group C tobacco (high level of TDE and DDT). The standard freeze drying reduced total TDE and total DDT by 42 and 41%, respectively, but extraction plus freeze drying reduced values by 26 and 20%. It is difficult to explain this reversal of trend for the group C tobacco from that of group A. The extraction step, nevertheless, apparently did not contribute to significant pesticide removal for group C tobacco. The higher residue levels for the extraction plus freeze drying treatment than for standard freeze drying can be partially explained on the basis of dry matter losses during extraction. Weight data indicated that the extraction step removed from 8.60 to 9.85% of initial dry matter, which, in the absence of pesticide removal, would serve to increase the residue level on a parts per million basis. It was initially believed that a lower percentage moisture was obtained for the samples after extraction; however,

Table I. Effect of Two Freeze-Drying Treatments on TDE and DDT Residues in Shredded Tobacco (Values Represent Averages over Three Replications)[a,b]

Initial pesticide level	Freeze-drying treatment	ppm								
		p, p'-TDEE	*o, p*-TDE	*p, p'*-TDE	Total TDE	*p, p'*-DDE	*o, p*-DDT	*p, p'*-DDT	Total DDT	TDE + DDT
Low (Group A)	Control	0.13	0.52	2.42	3.07	0.15	0.35	1.59	2.09	5.16
	Std freeze drying	0.10 (23%)	0.34* (35%)	2.32 (4%)	2.75 (10%)	0.11+ (27%)	0.25* (29%)	1.51 (5%)	1.87 (11%)	4.62 (10%)
	Extr. + freeze drying	0.07* (46%)	0.29* (44%)	1.85* (24%)	2.21* (28%)	0.10* (33%)	0.23* (34%)	1.32+ (17%)	1.65+ (21%)	3.87* (25%)
High (Group C)	Control	0.87	2.65	11.85	15.37	0.69	1.44	7.04	9.17	24.53
	Std. freeze drying	0.44* (49%)	1.16* (56%)	7.25** (39%)	8.86** (42%)	0.34* (51%)	0.77* (47%)	4.28* (39%)	5.38** (41%)	14.24** (42%)
	Extr. + freeze drying	0.57+ (34%)	1.41* (47%)	9.46* (20%)	11.43** (26%)	0.39* (43%)	0.81* (44%)	6.14 (13%)	7.34+ (20%)	18.77* (23%)

[a] Comparisons are limited to within the level of pesticide comparisons and within a single residue. A plus (+) symbol indicates a significant difference from the control at the 10% level. Similarly, a single asterisk (*) and double asterisk (**) indicate significance at the 5 and 1% levels, respectively. [b] Values within parentheses refer to percentage pesticide reduction in comparison with the control.

Table II. Effect of Two Freeze-Drying Treatments on Endosulfan Residues in Shredded Tobacco (Values Represent Averages over Three Replications)[a,b]

Initial pesticide level	Freeze-drying treatment	ppm			
		Endosulfan I	Endosulfan II	Endosulfan sulfate	Total endosulfan
Low (Group B)	Control	0.12	0.98	2.43	3.53
	Std freeze drying	0.05** (58%)	0.56** (43%)	1.59** (35%)	2.20** (37%)
	Extr. + freeze drying	0.05** (58%)	0.59** (40%)	1.68** (31%)	2.32** (34%)
High (Group D)	Control	0.25	2.35	7.65	10.24
	Std freeze drying	0.11** (56%)	1.27** (46%)	4.45** (42%)	5.83** (43%)
	Extr. + freeze drying	0.10** (60%)	1.29** (45%)	4.94* (35%)	6.33** (38%)

[a] Comparisons are limited to within the level of pesticide comparisons and within a single residue. A single asterisk (*) and a double asterisk (**) indicate significance at the 5 and 1% levels, respectively, with respect to the control. [b] Values within parentheses refer to percentage pesticide reduction in comparison with the control.

Table III. Residues of TDE, DDT, and Endosulfan in Tobacco Expressed on a Weight per Volume Basis (Microgram/Milliliter)[a]

Initial pesticide level	Freeze-drying treatment	Total TDE	Total DDT	Total endosulfan
Low	Control	1.07 ± 0.18	0.73 ± 0.10	1.26 ± 0.12
	Std freeze drying	0.43 ± 0.08 (60%)	0.30 ± 0.05 (59%)	0.36 ± 0.05 (71%)
	Extr. + freeze drying	0.32 ± 0.07 (70%)	0.24 ± 0.04 (67%)	0.33 ± 0.04 (74%)
High	Control	4.66 ± 0.43	2.78 ± 0.42	3.47 ± 0.52
	Std freeze drying	1.41 ± 0.22 (70%)	0.85 ± 0.22 (70%)	0.93 ± 0.25 (73%)
	Extr. + freeze drying	1.63 ± 0.20 (65%)	1.04 ± 0.20 (63%)	0.92 ± 0.22 (73%)

[a] Values were obtained by dividing concentrations (micrograms/gram) by specific volumes for corresponding samples. Confidence intervals shown are at the 95% level.

correction of the moisture data to account for dry matter losses showed moisture contents in the range of 440–460% with a mean of 451%, which is practically the same as for the standard freeze-drying samples.

The freeze-drying treatments significantly decreased endosulfan residues for tobaccos having both low and high initial pesticide levels (Table II). Standard freeze drying decreased total endosulfan by 38 and 43% for the low and high levels, respectively, whereas extraction plus freeze drying gave reductions of 34 and 38%. Differences between freeze-drying treatments with and without extraction were not significant. A distinct trend of reduction was also noted among the three isomers, with percentage reduction greatest for endosulfan I.

Analysis of the water extract from the extraction plus freeze-drying procedure showed that for DDT, TDE, and endosulfan less than 0.1% of the total pesticide present on the tobacco was removed as a result of the water extraction.

Since the freeze-drying process substantially increases the specific volume (and hence filling capacity) of tobacco (Johnson, 1970), it was of interest to examine the pesticide residue data on a volumetric basis (micrograms/milliliter). For this purpose, specific volumes of the control and freeze-dried samples were determined on a dry basis. Known quantities of tobacco were ground through a 20-mesh screen, poured into a graduated cylinder, and tamped to equilibrium. Standard freeze drying increased the specific volume from 90 to 120%, whereas extraction plus freeze drying gave increases in the range of 114–145%. The latter values are higher, at least partially due to dry matter losses associated with the extraction step. Table III presents the residue data for total TDE, total DDT, and total endosulfan on a volumetric basis. These values were obtained by dividing the appropriate residue data from Tables I and II by the corresponding specific volumes. On this basis, significant reductions in pesticide residues were obtained by the freeze-drying treatments for all components and for both levels of initial pesticide. Per

cent reduction ranged from 60 to 70% for total TDE, 59 to 70% for total DDT, and 71 to 74% for total endosulfan.

While the percentage reduction of pesticides is significant, further investigation of process parameters might establish optimal conditions for even further reductions. For example, factors such as water temperature, water/solids ratio, rest time prior to freezing and/or extraction, and product temperature during sublimation may relate to ease of pesticide removal.

It is also likely that, in addition to freeze drying, other manufacturing processes might contribute to pesticide reduction. This could be expected, particularly for new or existing processes which involve moistening tobacco to moderately high levels followed by drying at elevated temperatures. Results of this study should be important to future approaches aimed at reducing pesticide residues in tobacco.

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Quantitative Determination of 4-Methylimidazole as 1-Acetyl Derivative in Caramel Color by Gas–Liquid Chromatography

A gas–liquid chromatographic (glc) method has been developed for the quantitative determination of 4-methylimidazole (4-MeI) in caramel color. Prior to glc the 4-MeI was converted to a 1-acetyl derivative. The identity of 4-MeI in caramel color extracts has been confirmed by thin-layer chromatography, retention time data on two stationary phases of differing polarity, and mass spectrometry. The quantitation has been achieved by using 2-methylimidazole as internal standard. The accuracy and precision of the method were also tested, by recovery experiments and replicate analyses on four different commercial caramel colors. The acylation step has also been studied, by nuclear magnetic resonance.

Caramel color is a frequently used food additive in various food commodities, *e.g.*, carbonated beverages, baked products, and dry mixes, as a coloring and flavoring agent. Caramel color is the amorphous dark brown material resulting from the controlled heat treatment of various food grade carbohydrates such as dextrose, invert sugar, lactose, malt syrup, molasses, or starch hydrolysates. There are various acids, alkalis, and salts which may be used to assist caramelization. If ammonium hydroxide or ammonium salts are used as a process aid, the color is specified "prepared by the ammonia process." During this procedure small quantities of substituted imidazoles can be formed, *e.g.*, 4-methylimidazole (4-MeI) from pyruvaldehyde and ammonia in aqueous solution, according to Grimmett and Richards (1965). Commercial caramel colors made by the ammonia process contain up to several hundred milligrams of 4-MeI/kg.

The presence of 4-MeI in the caramel color is undesirable because of its toxicity. The toxic effect of various imidazoles on mice has been investigated by Nishie *et al.* (1969).

In view of these facts, further information about the levels of 4-MeI in caramel colors is needed as is also a suitable method for the determination of this compound. Such a method for the isolation and determination of 4-MeI in caramel color has been developed recently by Wilks *et al.* (1973). Their method is based on extraction and direct chromatography of the 4-MeI in free form and subsequent quantitation by an external standard technique. The gas chromatographic step was carried out on an alkali-treated polar stationary phase. It was necessary to prepare a new standard curve for each day's runs. In the present study another technique has been tried, involving derivatization and application of a suitable internal standard.

It has been recently noted that Begg and Grimmett (1972) have successfully separated several imidazoles as their 1-acetyl derivatives by glc. It seemed feasible that such a derivative could facilitate the determination of 4-MeI in caramel color extracts, too. For the quantitative assessment, 2-methylimidazole (2-MeI) was chosen as internal standard. It was found that this compound was absent in the caramel colors examined in this work and also that 2-MeI acetylated simultaneously with 4-MeI and the derivatives separated well on a common, polar stationary phase (STAP).