formation which AChE or ronnel oxon might have to distort to in order to react. However, such a rotation *would* cause the phosphorus to be in a slightly different position relative to the other ring substituents. This may be partially responsible, then, for changes in I_{50} and/or LD_{50} values as a result of subtle steric influences of the insecticide AChE. In this regard it would be quite interesting to compare the toxicities of the pure *d* and *l* forms of ronnel oxon; a 1:1 racemic mixture is present in a centrosymmetric space group. But, in order to make better comparisons and predictions, many heretofore unreported insect and mammalian I_{50} and LD_{50} values will need to be investigated and tabulated. In addition CNDO calculations will need to be performed to obtain a better idea of the charge distribution and potential energy barriers which may be necessary to overcome in aduct formation.

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Supplementary Material Available: A listing of the observed and calculated structure factor amplitudes (9 pages). Ordering information is given on any current masthead page.

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Lipid Distribution in Flue-Cured Tobacco Plants

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The increasing use of reconstituted tobacco sheet in cigarette manufacture and the reported relationship between tumorigenicity of smoke and lipid content of cigarettes has generated interest in lipid analysis of tobacco plant parts used in the manufacture of sheet. We analyzed various plant parts of three varieties of flue-cured tobacco and reported data for hexane extractables, total solanesol, neophytadiene, hydrocarbon waxes, total major fatty acids, and total sterols. Total lipid content was highest in strip, followed by whole leaf, stem, and stalk in decreasing order. Solanesol, the major lipid, was present only in whole leaf and strip. Concentrations of the other lipids generally followed the same decreasing order as seen for the total lipids.

The amount of reconstituted tobacco sheet used in the manufacture of commercial cigarettes has increased from about 15% in 1964 to the present level of 30% in some blends (Wynder and Hoffmann, 1967; DeJong et al., 1975). The increase has been due to the monetary savings made possible by the manufacture of sheet from the normally discarded stems and fines. Alterations in smoke composition related to the increased stem content of cigarettes

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made from a blend of reconstituted sheet and leaf have been investigated. Wynder and Hoffmann (1965) reported reduced tumorigenicity in smoke condensates from cigarettes made with reconstituted sheet. Halter and Ito (1972) confirmed this initial report and noted in some instances a 60% reduction in tumorigenicity of tar from sheet cigarettes in comparison with control cigarettes made from a natural tobacco blend. Chouroulinkov et al. (1969) and Hoffmann and Wynder (1972) reported significantly lower biological activity of condensates from cigarettes consisting solely of leaf stem and main veins than from cigarettes made from leaf lamina.

Reports of the composition of smoke condensates of cigarettes made from sheet, stem, and blended mixtures have generally included data for total particulate matter (TPM) and components such as nicotine, hydrocarbons, phenols, and benzo[*a*]pyrene (B*a*P). Thus, Klimisch (1970) reported a reduction in aliphatic and aromatic hydrocarbons, nicotine, and phenols in sheet smoke condensate compared with control cigarette condensate. Hoffmann and Wynder (1972) reported lower levels of tar, total polynuclear aromatic hydrocarbons (PAH), and volatiles in the condensate of stem cigarettes compared with blended control cigarettes. Ishiguro et al. (1976) compared smoke condensates of cigarettes made from lamina and from stem (midribs). The stem condensate was 45% lower in TPM, 40% lower in the neutral fraction (containing aliphatic and aromatic hydrocarbons), and 53% lower in basic compounds, while the acidic and phenolic fractions were 6 and 10% higher, respectively.

Schlotzhauer and Chortyk (1975) pyrolyzed whole leaf, stems, and a sheet tobacco made essentially from stems and midribs. Stem and sheet pyrolyzates yielded significantly lower amounts of bases, neutrals, and phenolics, but only slightly less acids than the whole leaf pyrolyzate. The neutral fractions of each condensate were divided into five subfractions, two of which were biologically active. One of these fractions contained PAH. Sheet and stem pyrolyzates yielded significantly lower amounts of the active subfractions than whole leaf pyrolyzate. As suggested by pyrolytic studies, lower levels of PAH reflect a correspondingly low level of lipid components in stem utilized in sheet manufacture (Schlotzhauer et al., 1969). A recent experimental method for tobacco production involves close growing of tobacco plants, harvesting of the whole plant, and manufacture of the cured product into sheet. Cigarettes made from close-grown, stalk-cured tobacco sheet yielded condensate with reduced biological activity as compared with condensates from commercial tobacco blend cigarettes (Zilkey, 1975).

Darkis et al. (1952) reported the levels of 18 components in the lamina, total vein tissue, midribs, lateral veins, veinules, whole leaf, and stalk of a flue-cured tobacco. The ratio of petroleum ether extractables in lamina to those in stem usually ranged from 6 or 7 to 1.

Since the petroleum ether- or hexane-extractable fraction (lipid fraction) of tobacco is the major source of PAH precursors (Schlotzhauer et al., 1969, 1976), we examined the lipid composition of the various plant parts being used increasingly in the manufacture of reconstituted sheet, and therefore in cigarettes. We analyzed the plant parts of three flue-cured tobaccos using a method developed recently for the quantitation of solanesol, total major fatty acids, total sterols, neophytadiene, and the hydrocarbon waxes in tobacco (Ellington et al., 1977; Severson et al., 1977).

EXPERIMENTAL SECTION

Tobacco samples were obtained from the Tobacco Research Laboratory, ARS, USDA, Oxford, N.C. The Coker 139 and Speight G-28 were close-grown tobaccos of the 1975 crop (close-grown, 35 000 plants/acre; normal, 6000 plants/acre). (Coker 139 is used for experimental purposes and is not grown commercially.) Whole plants were harvested and cured intact. They were then separated into whole leaf, stalk, stem, strip, and top (flowers). (Whole leaf consists of stem plus strip.) The NC2326 (1976 crop) sample was grown and cured in the manner normal for flue-cured varieties. The NC2326 leaves were likewise separated into whole leaf, stem, and strip fractions. (Stalks and flowers were not available.) Coker and Speight flower samples were analyzed for informational purposes only, since flowers are not used in sheet manufacture. Stalk analyses were of interest, since the close-grown, whole-plant sheet contains a high percentage of stalk. All samples were ground to 32 mesh and refrigerated until used.

Table I. Hexane Extractables

		% dry weight	
		Soxhlet	Hydrolyzate
Coker	Whole leaf	5.78	5.26
	Stem	1.25	0.88
	Strip	6.24	5.02
	Stalk	0.32	0.11
	Flower	ND	ND
Speight	Whole leaf	6.15	3.94
	Stem	1.27	0.77
	Strip	6.61	5.01
	Stalk	0.29	0.11
	Flower	ND	ND
NC2326	Whole leaf	8.62	6.59
	Stem	1.06	0.67
	Strip	9.91	7.75

Table II. Total Solanesol

	% dry weight		
	Coker	Speight	NC2326
Leaf	0.67	1.28	3.12
Stem	0	0	0
Strip	0.99	1.33	3.96
Stalk	0	0	ND
Flowers	0	0	ND

The amount of hexane extractables was determined by a 24-h Soxhlet extraction of the ground samples (Ellington et al., 1977). Our quantitative scheme (Ellington et al., 1977) for solanesol, fatty acids, sterols, neophytadiene, and the hydrocarbons was used with a slight modification in the posthydrolysis workup. This modification improved recoveries of labeled cholesterol and cholesteryl palmitate from 90 and 97% to 96 and 98%, respectively. The hydrolysis of cholesteryl palmitate was 98% complete.

In the modified procedure, 1 g of tobacco of known moisture content was refluxed 1 h with 40 mL of 2 N KOH in 95% ethanol under a nitrogen atmosphere. The tobacco was removed from the hydrolyzate by suction filtration with a sintered glass filter and washed with three 10-mL portions of hot benzene–ethanol (1:1). The filtrate was diluted with 50 mL of water, adjusted to pH 2 with concentrated HCl and transferred to a separatory funnel. The filter flask was rinsed with 10 mL of benzene–ethanol and the rinsing added to the hydrolyzate. Solid KCl was added with intermittent shaking until saturated. The filter flask was further rinsed with three 50-mL portions and one 25-mL portion of hexane; each of the rinsings was used to extract the lipid material in the separatory funnel. The hexane extracts were combined in a 200-mL volumetric flask and adjusted to volume with hexane. This sample solution was transferred to a brown bottle and aliquots were removed for the various analyses.

Table III. Neophytadiene and C_{27}-C_{34} Hydrocarbons

	% dry weight					
	Coker		Speight		NC2326	
	Neophytadiene	C_{27}-C_{34} hydrocarbons	Neophytadiene	C_{27}-C_{34} hydrocarbons	Neophytadiene	C_{27}-C_{34} hydrocarbons
Leaf	0.017	0.181	0.023	0.258	0.118	0.153
Stem	0.002	0.043	0.004	0.053	0.011	0.059
Strip	0.021	0.340	0.020	0.252	0.248	0.339
Stalk	0	0.020	0	0.024	ND	ND
Flowers	ND	ND	ND	ND	ND	ND

Table IV. Total Major Fatty Acids

		% dry weight		
		C_{16}	$C_{18}A$[a]	$C_{18}B$[b]
Coker	Leaf	0.36 (41)[c]	0.40 (46)	0.11 (13)
	Stem	0.12 (32)	0.22 (58)	0.04 (10)
	Strip	0.27 (39)	0.30 (43)	0.13 (18)
	Stalk	0.10 (35)	0.16 (55)	0.03 (10)
	Flower	0.14 (15)	0.73 (78)	0.07 (7)
Speight	Leaf	0.25 (33)	0.38 (50)	0.13 (17)
	Stem	0.13 (29)	0.26 (59)	0.05 (12)
	Strip	0.27 (39)	0.30 (43)	0.13 (18)
	Stalk	0.09 (33)	0.15 (56)	0.03 (11)
	Flower	0.74 (11)	5.56 (83)	0.44 (6)
NC2326	Leaf	0.21 (22)	0.52 (55)	0.21 (22)
	Stem	0.09 (22)	0.24 (60)	0.07 (18)
	Strip	0.22 (23)	0.54 (56)	0.21 (21)

[a] 18A = oleic, linoleic, linolenic. [b] 18B = stearic. [c] Numbers in parentheses are relative percentages.

Each sample was hydrolyzed in duplicate and the values in the tables are the averages. The lipid analyses are described in greater detail in Ellington et al. (1977) and Severson et al. (1977). The total fatty acids and total solanesol trimethylsilyl (Me_3Si) derivatives were quantitated by gas chromatography (GC) on a 2 ft × 0.125 in. stainless steel column containing 5% Dexsil 300 GC. The Me_3Si derivatives of undecanoic and dimyristin were used as internal standards for the fatty acid and solanesol analyses, respectively. Overlapping GC elution times for the Me_3Si sterols and hydrocarbon waxes necessitated their fractionation by silicic acid column chromatography before quantitation. The sterols were quantitated, after Me_3Si derivitization, on a 6 ft × 0.125 in. stainless steel column containing 3% SP-2250 with hexacosanol as the internal standard. Neophytadiene and the hydrocarbon waxes in the Soxhlet extracts were chromatographed on a silicic acid column and eluted with hexane and quantitated by GC on the 2 ft × 0.125 in. 5% Dexsil column with docosane as internal standard.

RESULTS AND DISCUSSION

On the basis of previous reports (Tso, 1972; Schlotzhauer et al., 1976), we used the amount of hexane extractables as a measure of the neutral lipid content of tobacco. Table I gives the yields of hexane extractables obtained by direct Soxhlet extraction and by extraction of the hydrolyzates of tobacco plant parts. The lipid content of strip was approximately 20 times that of stalk and 5 times that of stem. The lipid distribution was very similar to that reported by Darkis et al. (1952). The relative lipid levels of strip, stem, and stalk were the same for hydrolyzates and Soxhlet extracts. The lower extraction of lipids by the hydrolyzate method as compared to the Soxhlet method could have been due to several factors, such as (1) some water-soluble material was also extracted by cyclic siphoning during the Soxhlet extraction, (2) water-soluble compounds were generated by breakdown of water-insoluble lipids during the alkaline hydrolysis, or (3) alkaloids, including nicotine, were rendered water soluble by the acidification with hydrochloric acid.

The low lipid levels in stem agree with reported low PAH levels in smoke condensate of cigarettes made from stem (Schlotzhauer and Chortyk, 1975). Schlotzhauer and Schmeltz (1968) found that approximately 60% of the B*a*P in tobacco pyrolyzates were derived from the hexane extract.

Table II indicates that solanesol is present in strip only. This is of interest because solanesol is the major component of the hexane extract and because of the known translocation of volatile oils, resins, waxes, and carbohydrates from leaf during stalk curing (Wynder and Hoffmann, 1967). The levels of solanesol were lower in the Speight and Coker samples than any other flue-cured samples we have analyzed by this method. The reason(s) for the lower levels is not known, but might be related to the growing regime or the curing method, or both. Speight and Coker samples grown and cured in the normal manner were not available for analysis.

Table III shows that the neophytadiene and hydrocarbon wax levels were considerably higher in strip than in stem. Stalk contained approximately half as much of the hydrocarbons as stem and no neophytadiene.

The relative percentage of unsaturated fatty acids was

Table V. Total Sterols

		% dry weight			
		Cholesterol	Campesterol	Stigmasterol	β-Sitosterol
Coker	Leaf	0.019 (12)[a]	0.030 (20)	0.061 (40)	0.043 (28)
	Stem	0.008 (13)	0.009 (15)	0.028 (47)	0.015 (25)
	Strip	0.028 (14)	0.045 (23)	0.069 (36)	0.052 (27)
	Stalk	0.004 (11)	0.010 (29)	0.012 (34)	0.009 (26)
	Flower	0.008 (10)	0.024 (30)	0.030 (37)	0.018 (23)
Speight	Leaf	0.022 (13)	0.037 (22)	0.064 (40)	0.042 (25)
	Stem	0.009 (14)	0.011 (17)	0.033 (52)	0.011 (17)
	Strip	0.026 (13)	0.043 (21)	0.078 (38)	0.057 (28)
	Stalk	0.003 (9)	0.007 (21)	0.014 (43)	0.009 (27)
	Flower	0.007 (8)	0.022 (26)	0.027 (32)	0.028 (34)
NC2326	Leaf	0.024 (10)	0.067 (28)	0.081 (34)	0.066 (28)
	Stem	0.009 (12)	0.012 (16)	0.036 (49)	0.017 (23)

[a] Numbers in parentheses are relative percentages.

highest in the stem and stalk samples (Table IV). In Speight stalk the unsaturated C_{18} fatty acids comprised 56% of the quantitated acids. The high value (5.56%) in Speight flowers as compared to Coker flowers might be attributed to differences in plant maturity.

The plant parts varied slightly in the relative percentage for cholesterol and slightly more in the percentages of campesterol, stigmasterol, and β-sitosterol (Table V). Grunwald (1975) reported increased relative percentages of stigmasterol with increasing leaf maturity. This increase was probably due to an increased concentration of stigmasterol in the stem. As noted in Table V, stigmasterol was the major sterol in all plant parts analyzed.

The data presented here indicate sterols, fatty acids, and hydrocarbons were present in all plant parts although at reduced levels in fibrous tissues. Absence of solanesol in the stem and stalk might indicate that solanesol is localized in the laminal tissue of the leaf. Neophytadiene was found in strip and stem tissue only. If sheet made from close-grown, chopped, whole-plant tobaccos becomes commercially important because of economic considerations, we can expect cigarettes of the future, which contain such sheet material, to have correspondingly lower amounts of the PAH precursors, solanesol, and neophytadiene.

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Gas Chromatographic Analysis of Tebuthiuron and Its Metabolites in Grass, Sugarcane, and Sugarcane By-Products

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Analytical procedures are described for determining residues of the herbicide tebuthiuron, *N*-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-*N*,*N*'-dimethylurea, and its major metabolites in forage grass, sugarcane, and sugarcane by-products. Extracts of the plant tissue and sugarcane by-products are purified by liquid–liquid partitioning and alumina column chromatography. Measurement is accomplished by gas chromatography using flame photometric detection (GC–FPD) and by gas chromatography–mass spectrometry (GC–MS) using single ion detection. The methods are capable of determining 0.01–0.3 ppm of tebuthiuron and metabolites.

Tebuthiuron (I), *N*-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-*N*,*N*'-dimethylurea, is the active ingredient in the herbicides SPIKE and PERFLAN (Elanco Products Company, Indianapolis, Ind.). Tebuthiuron is a preemergence and postemergence herbicide for total vegetation control on rights-of-way and industrial sites (Walker et al., 1973). It has exhibited potential for rangeland brush control (Bovey et al., 1975) and for broad spectrum weed control in sugarcane (Pafford and Hobbs, 1974).

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Several metabolites of tebuthiuron have been identified in plant tissue (Table I). The major metabolites in grass and sugarcane have been identified as compounds II and III. Compound IV is a major metabolite in grass but is only a minor metabolite in sugarcane (Eaton et al., 1976). The presence of these metabolites requires that analytical procedures for determining tebuthiuron residues in forage and food crops be capable of determining the major metabolites.

Saunders and Vanatta (1974) have reported the derivatization of tebuthiuron with trifluoroacetic anhydride for electron-capture detection by gas chromatography. This derivative exhibits adequate sensitivity for some residue determinations. However, it has not been generally

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