Comparison of the Steam-Volatile Components of Commercial Cigarette, Pipe, and Chewing Tobaccos

Edmond J. LaVoie,* Akemi Shigematsu, Patricia L. Tucciarone, John D. Adams, and Dietrich Hoffmann

The steam-volatile components of cigarette, pipe, and chewing tobaccos were analyzed by capillary gas chromatography–mass spectrometry. In comparison with tobacco from the 1R1 reference cigarette, several compounds were found to be unique to specific commercial tobacco blends or present in significantly greater quantity. Maltol, benzoic acid, anethole, piperonal, triacetin, coumarin, myristicin, *p*-cresol, 4-ethoxy-3-hydroxybenzaldehyde, and butyl 4-hydroxybenzoate were among the tobacco flavorants detected in commercial tobaccos.

The flavor and aroma of tobacco and tobacco smoke are important qualities to the users of tobacco (Leffingwell, 1976). The organoleptic nature of tobacco products can be altered by selecting aromatic varieties, adding tobacco extracts, and by the addition to the blend of natural flavors and extracts or synthetic flavor components. The use of additives in the blending of pipe, snuff, and chewing tobaccos has been a common practice. The increased popularity of low-yield cigarettes (<10 mg tar/cigarette) in recent years has also stimulated increased emphasis on the addition of flavorants to cigarette tobacco.

The organoleptic qualities of tobacco and tobacco flavors have been extensively studied (Enzell et al., 1977; Enzell and Wahlberg, 1980; Fujimori and Kaneko, 1979; Heckman et al., 1981; Leffingwell, 1976; Lloyd et al., 1976). Reviews have also outlined the types of additives which have been investigated as tobacco flavorants (Gutcho, 1972; Leffingwell et al., 1972; Second Report of the Independent Committee on Smoking and Health, 1979). In the interest of characterizing the types of components which may be present in commercial tobaccos as influenced by the selection of certain cultivars or by addition of tobacco flavorants, we analyzed the steam distillate of various tobacco products.

This study reports the identification of 21 steam-volatile compounds which were found to be either unique to specific commercial tobacco blends or present in significantly greater quantity than found in the tobacco of the 1R1 reference cigarette (the University of Kentucky's research reference cigarette). In this study, the steam-volatiles of three commercial brands of low-yield cigarettes, three commercial pipe tobaccos, and two commercial chewing tobaccos were compared.

EXPERIMENTAL SECTION

Apparatus. Capillary gas chromatographic profiles of the steam distillable components of tobacco were obtained from a Hewlett Packard Model 5790 gas chromatograph equipped with a split-splitless injector, flame ionization detector, and a Model 3390A calculating integrator. The GC columns used in this study were a 50 m × 0.25 mm i.d. OV-101 fused silica capillary column or a 50 m × 0.25 mm i.d. Dexsil 300 GC fused silica capillary column (Quadrex Corp.). Mass spectral analyses were performed at 70 eV on a Model 5982A dual-source GC/MS interfaced with a Model 5933A data system (Hewlett Packard). High performance liquid chromatography was performed with a Waters Associates Model ALC/GPC-204 chromatograph equipped with a Model 6000A solvent delivery system, a Model 660 solvent programmer, a Model U6K septumless injector, a Model 480 UV spectrophotometer and a μBondapak C-18 10 μm column, 300 mm × 4 mm (Waters Associates).

Reagents. Commercial cigarettes, pipe tobacco, and chewing tobacco were purchased on the open market in Westchester County, NY during 1984. (Commercial cigarettes selected were low-yield brands, tar <10 mg/cigarette). The 1R1 Reference Cigarette was obtained from the University of Kentucky. All cigarette tobaccos were conditioned for 24 h at 60% relative humidity and 20 °C. Pipe and chewing tobaccos were analyzed as packaged. [^{14}C]Benzyl alcohol (specific activity, 2.6 mCi/mmole) was obtained from California Bionuclear, Inc.

Procedures. For the analysis of its steam-distillable components, 50 g of tobacco was mixed with 150 mL of 0.5 N sulfuric acid in a round-bottom flask. A separate aliquot of tobacco was analyzed for moisture content by Dean-Stark distillation with benzene as the azeotropic solvent. To the tobacco in the round-bottom flask was added 1.8 μg (95,000 dpm) of [^{14}C]benzyl alcohol as a quantitative internal standard and the mixture was subjected to steam distillation (produced from tap water with a Büchi steam generator) until 1000 mL of distillate was collected. The steam distillate obtained from the acidified aqueous suspension of tobacco does not contain basic steam-volatile components, such as nicotine and related tobacco alkaloids. The abundance of these alkaloids in tobacco would overshadow the analysis of other components in the steam distillate. Extraction of this distillate was performed 3 times with equal volumes of methylene chloride and this extract was carefully concentrated by using rotary evaporation at room temperature and 380 mmHg vacuum. Samples were then concentrated under N_2 to 1.0 mL to provide suitable samples for capillary GC analysis. Concentration of methylene chloride in this manner minimizes losses of volatile components as we have shown with our studies on volatile *N*-nitrosamines.

For the GC analyses, a 50 m OV-101 or 50 m Dexsil 300 GC fused silica capillary column was employed. Both columns were operated under split conditions (20:1) with a helium flow of 1 mL/min, injector temperature of 200 °C, detector temperature of 250 °C, and a temperature gradient from 60 to 230 °C at 2 °C/min. Helium was used as a makeup gas with a flow of 35 mL/min. Confirmations of the 21 steam-volatile components were made by GC/MS analyses. An aliquot of each distillate was taken for liquid scintillation counting in order to determine the recovery rate. Typical recoveries of >70% were obtained based upon [^{14}C]benzyl alcohol.

The quantitative analyses of coumarin in pipe tobaccos were accomplished by high performance liquid chromatography. The steam distillate of the various pipe tobaccos was obtained from a mixture of 15 g of pipe tobacco in 50 mL of 0.5 N sulfuric acid as previously outlined. Ap-

Naylor Dana Institute, Division of Environmental Carcinogenesis, Dana Road, Valhalia, New York 10595.

0021-8561/85/1433-0876$01.50/0

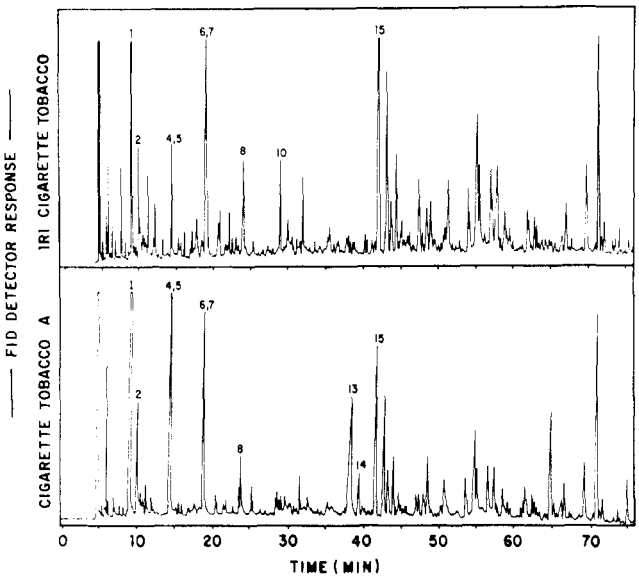

Figure 1. GC profiles of the steam-volatile components in 1R1 cigarette tobacco and cigarette tobacco A on a 50 m *OV*-101 fused silica capillary column. Numbered peaks are identified in Table I.

proximately 450 mL of distillate was obtained for each tobacco studied. This distillate was then transferred to a 500-mL volumetric flask. The volume was brought up to 500 mL with distilled water. Analyses on HPLC were performed by injection of 100-μL aliquots of the distillates onto a reverse-phase column maintained at 56 °C by means of 30-cm Allteck water jacket and a constant temperature circulating water bath. The variable wavelength ultraviolet detector was operated at 280 nm. The solvent system employed for these analyses was 25% methanol in 0.005 M ammonium acetate (adjusted to pH 4 with phosphoric acid). Under these chromatographic conditions, coumarin eluted at 10.2 min with a flow rate of 1.0 mL per min.

RESULTS AND DISCUSSION

Comparison of the nonbasic steam-volatile components in the tobacco obtained from three commercial brands of low-yield cigarettes with the tobacco of the 1R1 reference cigarette did reveal major differences in composition. Triacetin (peak 14, Figure 1), which is present in filter tips of commercial cigarettes at a concentration of 14.9 mg/20 mm tip (Mathis, 1983), was detected in the commercial cigarette tobaccos. This finding is consistent with the fact that all three brands were filter-tipped. No triacetin (peak 14) was detected in tobacco from the nonfilter 1R1 reference cigarette. In cigarette tobaccos A and B there were significantly higher levels of furfural than in the 1R1 tobacco. However, the presence of furfural might very well be an artifact which can form from pectin during steam distillation under acidic conditions (Green et al., 1980). In the steam distillate of tobacco A there were also elevated levels of both benzaldehyde and 5-methylfurfural (peaks 4 and 5, Figure 1). Furfural, furfuryl alcohol, and 5-methylfurfural were previously identified among the neutral steam volatiles of several different tobacco types. The levels of these compounds varied with the tobacco type and grade (Burdick et al., 1986a; Burdick and Stedman, 1963b: Kim et al., 1982). While trace levels of menthol were found in the tobacco of 1R1 cigarettes and commercial cigarettes B and C, no menthol was detected in commercial cigarette A. Piperonal, which has been reported to be a component of Burley tobacco (Fujimori and Kaneko, 1979), was detected in the distillate of cigarette tobacco A (peak 13, Figure 1). Piperonal has been characterized as imparting a "sweet, floral, cherry–vanilla undertone" to smoke taste (Leffingwell et al., 1972). Anethole was detected only in the distillate of cigarette tobacco C. This is a known tobacco flavorant and has been previously identified as a component of Burley tobacco (Demole and Berthet, 1972). The structural identification of each of the components of these steam distillates was based upon the mass spectral data obtained by capillary GC–MS and by co-injection of reference standards on either the capillary OV-101 and/or Dexsil 300 columns.

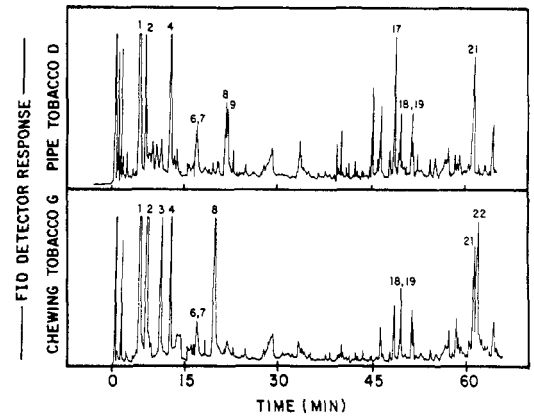

Figure 2. GC profiles of the steam-volatile components of pipe tobacco D and chewing tobacco G on 50 m OV-101 fused silica capillary columns. Numbered peaks are identified in Table I.

The steam-volatile components from three commercial pipe tobaccos were also compared with those obtained from the 1R1 cigarette tobacco. As anticipated, several qualitative and quantitative differences were observed in the steam distillates (Table I and Figure 2). Pipe tobaccos D and E had increased levels of furfuryl alcohol and 5-methylfurfural. Maltol, a known tobacco flavorant, was confirmed as a unique constituent of pipe tobacco D (peak 9, Figure 2). Pipe tobacco D also contained myristicin (peak 17, Figure 2). Both pipe tobaccos D and F contained *p*-cresol and 2,6-di-*tert*-butyl-*p*-cresol which were not detected in the steam distillate from the 1R1 cigarette tobacco. Coumarin was a major flavorant detected in pipe tobaccos E and F. Coumarin has been previously detected as an additive of tobacco and in tobacco smoke (Grob, 1969; Von Nesemann and Seehofer, 1971; Viart, 1970). We observed that the level of coumarin was highest in pipe tobacco F, namely 820 μg per g of tobacco as determined by HPLC. Since this tobacco contained 16% water, the additive constituted >0.1% of the dry weight of this tobacco. Tobacco F also contained 4-ethoxy-3-hydroxybenzaldehyde which was not detected in any of the other tobaccos. While vanillin and ethylvanillin have been reported as additives identified in tobacco, 4-ethoxy-3-hydroxybenzaldehyde has not been previously identified as a flavorant in commercial tobaccos. Butyl 4-hydroxybenzoate is reported to add a "sweet and weakly fruity" aroma to smoke (Leffingwell et al., 1972). In all three commercial pipe tobaccos, it was detected as a component of the distillate. Butyl 4-hydroxybenzoate was not found in 1R1 cigarette tobacco. Benzoic acid was also detected as a major component in the steam distillate of pipe tobacco E. These data reflect the extent to which additives have been used in the formulation of pipe tobacco blends. While there may be an increased interest in the use of tobacco flavorants in the formulation of tobacco blends for use in low-yield cigarettes, our data suggest that the levels of additives being used are not comparable to those presently employed in pipe tobacco blends.

Table I. Steam-Volatile Compounds in Various Tobaccos[a]

			cigarette				pipe			chewing	
peak no.	compound	retention time[b]	1R1[c]	A	B	C	D	E	F	G	H
1	furfural[d]	8.1	+	+[e]	+[e]	+	+[e]	+[e]	+[e]	+[e]	+[e]
2	furfuryl alcohol	9.0	+	+	+	+	+[e]	+[e]	+[e]		+[e]
3	unknown	11.6								+	+
4	benzaldehyde	13.0 (16.7)[f]	+	+[e]	+	+		+			+
5	5-methylfurfural	13.0 (17.4)[f]	+	+	+	+	+[e]	+	+[e]	+[e]	+[e]
6	benzyl alcohol	18.1 (22.0)[f]	+	+	+	+	+	+[e]	+	+[g]	+[g]
7	phenylacetaldehyde	18.1 (22.7)[f]	+	+	+	+	+		+	+	+
8	2-phenylethanol	22.7	+	+	+	+	+	+[e]		+	+
9	maltol	23.1					+				
10	menthol	27.8	+		+	+					
11	benzoic acid	33.02						+			+
12	anethole	36.3				+					
13	piperonal	37.3		+				+			
14	triacetin	39.5		+[h]	+[h]	+[h]					
15	solanone	41.2	+	+	+	+		+	+		
16	coumarin	43.9						+	+		
17	myristicin	48.5					+				
18	*p*-cresol	50.1					+		+	+	+
19	2,6-di-*tert*-butyl-*p*-cresol	50.4					+		+	+	+
20	4-ethoxy-3-hydroxybenzaldehyde	50.6							+		
21	butyl 4-hydroxybenzoate	62.6					+	+[i]	+	+	+
22	isomer of butyl hydroxybenzoate	63.1								+	

[a] The plus symbol (+) refers to confirmation of the presence of listed compounds as determined by GC–MS. [b] Retention on 50 m OV-101 column unless otherwise noted. [c] 1R1 is a University of Kentucky Research cigarette which was employed as a reference standard. [d] Furfural may be formed as an artifact. [e] Levels present were substantially greater than in the steam volatiles present in 1R1 cigarette tobacco. [f] Retention on 50 m Dexsil-300 GC column. [g] Levels present were substantially lower than in the steam volatiles present in 1R1 cigarette tobacco. [h] Tobacco obtained from a filter-tipped cigarette. [i] Levels present were substantially higher than in other commercial pipe tobaccos.

The steam distillate of two brands of commercial chewing tobacco was also compared with that obtained from 1R1 tobacco. While several components such as furfural, furfuryl alcohol, and 5-methylfurfural were present in quantities greater than those detected in the steam distillate of 1R1 tobacco, several components present in the 1R1 tobacco distillate were not detected in these chewing tobaccos or were present in reduced quantity (Figure 2, Table I). Since these furfural derivatives are common breakdown products of reducing sugars, their increased presence in chewing tobacco may be related to the level of sugars added to these tobacco blends. Certain tobacco flavorants which were detected in commercial pipe tobaccos such as *p*-cresol, 2,6-di-*tert*-butyl-*p*-cresol, and butyl 4-hydroxybenzoate were also detected in the distillate of these chewing tobaccos.

Several of the steam-volatile compounds listed in Table I have been evaluated as mutagens in *S. typhimurium* (Buchanan et al., 1982; Florin et al., 1980; Sekizawa and Shibamoto, 1982; To et al., 1982). While most of these compounds did not exhibit mutagenic activity in these assay systems, maltol is mutagenic in *S. typhimurium* TA100 (Bjeldanes and Chew, 1979). The significance of coumarin as a health risk to man has not been conclusively established. There exist several conflicting reports on the potential health effects of coumarin. Its genetic effects and potential toxic effects to humans have been reviewed (Cohen, 1978; Grigg, 1977). Anethole and myristicin are structurally related to safrole in that both of these compounds have a propenyl group attached to the phenyl ring. While the hepatocarcinogenic activity of safrole is well documented, anethole and myristicin are not carcinogenic in mice or rats (Miller et al., 1983). Further studies are in progress in our laboratory to assess the potential health effects of several of the components detected in the steam-volatile portion of these specific commercial tobacco blends.

Analysis of additives in tobacco products is important for understanding the type of exposure an individual may have to the flavorants themselves by their transfer rate in smoke or by direct exposure in smokeless tobaccos. It is also important to monitor the pyrolytic products which may result from a particular additive. Further studies are in progress to monitor the types and levels of flavorants presently in use in commercial tobacco products to provide some insight into their potential to contribute to the toxicity of tobacco products and tobacco smoke.

Registry No. Furfural, 98-01-1; furfuryl alcohol, 98-00-0; benzaldehyde, 100-52-7; 5-methylfurfural, 620-02-0; benzyl alcohol, 100-51-6; phenylacetaldehyde, 122-78-1; 2-phenylethanol, 60-12-8; maltol, 118-71-8; menthol, 89-78-1; benzoic acid, 65-85-0; anethole, 104-46-1; piperonal, 120-57-0; triacetin, 102-76-1; solanone, 1937-54-8; coumarin, 91-64-5; myristicin, 607-91-0; *p*-cresol, 106-44-5; 2,6-di-*tert*-butyl-*p*-cresol, 128-37-0; 4-ethoxy-3-hydroxybenzaldehyde, 2539-53-9; butyl 4-hydroxybenzoate, 94-26-8; butyl hydroxybenzoate, 1322-01-6.

LITERATURE CITED

Bjeldanes, L. F.; Chew, H. *Mutat. Res.* **1977**, *67*, 367.

Buchanan, R. L.; Goldstein, S.; Budroe, J. D. *J. Food Sci.* **1982**, *47*, 330.

Burdick, D.; Schmeltz, I.; Miller, R. L.; Stedman, R. L. *Tob. Sci.* **1963**, *7*, 97.

Burdick, D.; Stedman, R. L. *Tob. Sci.* **1963**, *7*, 113.

Cohen, A. J. *Fd. Cosmet. Toxicol.* **1979**, *17*, 277.

Demole, E.; Berthet, D. *Helv. Chem. Acta* **1972**, *55*, 1866.

Enzell, C. R.; Wahlberg, I. *Recent Adv. Tob. Sci.* **1980**, *6*, 64.

Enzell, C. R.; Wahlberg, I.; Aasen, A. J. In "Progress in the Chemistry of Organic Natural Products"; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer-Verlag: New York, 1977; Vol. 34, p 1.

Florin, I.; Rutberg, L.; Curvall, M.; Enzell, C. R. *Toxicology* **1980**, *15*, 219.

Fujimori, T.; Kaneko, H. *Nippon Nogeikagaku Kaishi* **1979**, *54*, 95.

Green, C. R.; Colby, D. A.; Copper, P. J.; Heckman, R. A.; Lyerly, L. A.; Thome, F. A. *Recent Adv. Tob. Sci.* **1980**, *6*, 123.

Grigg, G. W. *Mutat. Res.* **19778** *47*, 161.

Grob, K; Völlmin, J. A. *Beitr. Tabakforsch.* **1969**, *5*, 52.

Gutcho, S. "Tobacco Flavoring Substances and Methods"; Noyes Data Corporation, NJ, 1972.

Heckman, R. A.; Dube, M. F.; Lynn, D.; Rivers, J. M. *Recent Adv. Tob. Sci.* **1981**, *7*, 107.

Kim, K. R.; Zlatkis, A.; Park, J.-W.; Lee, U.-C. *Chromatographia* **1982**, *15*, 559.
Leffingwell, J. C. *Recent Adv. Tob. Sci.* **1976**, *2*, 1.
Leffingwell, J. C.; Young, H. J.; Bernasch, E. "Tobacco Flavoring for Smoking Products"; R. J. Reynolds Tobacco Co.: Winston-Salem, NC, 1972.
Lloyd, R. A.; Miller, C. W.; Roberts, D. L.; Giles, J. A.; Dickerson, J. P.; Nelson, N. H.; Rix, C. E.; Ayers, P. H. *Tob. Sci.* **1976**, *20*, 43.
Mathis, D. E. *Beitr. Tabakforsch.* **1983**, *12*, 1.
Miller, E. C.; Swanson, A. B.; Phillips, D. H.; Fletcher, T. L.; Liem, A.; Miller, J. A. *Cancer Res.* **1983**, *43*, 1124.
Second Report of the Independent Scientific Committee on Smoking and Health. "Developments in Tobacco Products and the Possibility of "Lower-Risk" Cigarettes"; Her Majesty's Stationery Office: London, 1979.
Sekizawa, J.; Shibamoto, T. *Mutat. Res.* **1982**, *101*, 127.
To, L. P.; Hunt, T. P.; Andersen, M. E. *Bull. Environ. Contam. Toxicol.* **1982**, *28*, 647.
Viart, P. *Serv. Exploit. Ind. Tob. Allumettes, Ann.* **1970**, *8*, 151.
Von Nesemann, E.; Seehofer, E. *Beitr. Tabakforsch.* **1971**, *5*, 290.

Received for review January 14, 1985. Accepted April 29, 1985. This study was supported by Grant CA-29580 from the National Cancer Institute.

Changes in Chemical Composition of Tobacco Lamina during Senescence and Curing. 1. Plastid Pigments

Harold R. Burton* and M. J. Kasperbauer

Chlorophyll a, chlorophyll b, neoxanthin (I), violaxanthin (II), lutein (III), and β-carotene (IV) were quantified in burley tobacco (*Nicotiana tabacum* L.) during senescence and air-curing. During senescence the decrease of pigment concentrations varied from 70% reduction of chlorophyll b to a 30% decrease of violaxanthin. During air-curing 99% of the chlorophylls were catabolized, whereas, approximately 70% of the lutein (III) and carotene were degraded. The majority of pigment degradation occurs during the first two weeks of curing. Curing environments also influenced the rates of degradation of the pigments. Presence of light during curing retarded destruction of the carotenoids during early stages, whereas at final cure there were few differences in the concentrations of the pigments for different treatments.

INTRODUCTION

In recent years there has been increased interest in changes in chemical composition during plant senescence. This is particularly true for tobacco since the final consumable product has gone through growth, senescence, and curing. Chemical changes that occur during curing have a dramatic impact on the cured product. A recent review (Long and Weybrew, 1981) summarized many of the major chemical changes which occur during senescence and curing of tobacco. Also, a review (Burton et al., 1983) reported on many of the chemical changes occurring during air-curing of burley tobacco. One major change during the cell death is the destruction of chrlorophyll a, chlorophyll b, and the four major carotenoids: neoxanthin (I), violaxanthin (II), lutein (III), and carotene (IV). The degradation of the chlorophylls is almost complete with only low levels detected in the cured lamina; whereas, there is approximately 80% destruction of the carotenoids (Forest and Vilicens, 1979). The degradation of the carotenoids can lead to the formation of many components which enhance the aroma of tobacco. Recent reviews (Enzell and Wahlberg, 1980; Demole and Dietrich, 1977) have indicated approximately 80 aroma constituents in tobacco can be derived from the oxidative degradation of the carotenoids. Because of the importance of carotenoid degradation to the formation of flavor components, a study was initiated to quantify the decrease of carotenoids in tobacco lamina during air-curing. This allowed for identifying the stage of curing when the majority of the carotenoids were being metabolized.

Department of Agronomy and ARS, USDA, University of Kentucky, Lexington, Kentucky 40546.

EXPERIMENTAL SECTION

Plant Materials and Procedures. Burley tobacco (*Nicotiana tabacum* L. cv. Ky 14) plants were grown at the Kentucky Agricultural Experimental Farm near Lexington. Recommended fertilization and cultural practices were followed during the growing season (Atkinson et al., 1976). Plants designated for all curing environments were harvested on the same date. At harvest, plants were stalk cut and six stalks speared on each stick. One third of the tobacco was cured in a conventional curing barn at ambient conditions. Another third was placed in a controlled environmental chamber and cured at 24 °C and 70% RH in darkness. The remainder was also cured at the same conditions but under continuous illumination from cool white fluorescent lamps. The lamps were arranged for uniform illumination of the tobacco to be sampled with light intensity of 80 ft-c.

Four randomized replicate samples were taken at harvest and 1, 2, 3, 4, 7, 9, 11, 14, 16, 18, 21, 24, 27, 35, 42, and 90 days to determine the concentration changes that occurred during curing. Five leaves—excepting the top two—were taken from the upper one-third of each plant sampled. Midveins were removed from the leaf, the lamina weighed, and the leaf areas were determined with a leaf area meter. The lamina was freeze-dried and reweighed to determine moisture content. Samples were ground to pass a 40-mesh screen and stored at −40 °C until analyzed.

Chemical Analyses of Plant Pigments. The procedure for the analyses of chlorophyll a, chlorophyll b, neoxanthin, violaxanthin, lutein, and β-carotene was a modification of a procedure described by Eskins and Dutton (1979). A 250-mg sample of the dried tissue was placed in a 10 × 40 mm extraction thimble and extracted with acetone (20 mL) in a microsoxhlet apparatus for 1 h.

0021-8561/85/1433-0879$01.50/0