Table V. Odor Properties of Cola Fractions

fraction no.	character
2	weak
3	sweet, vanillin
4	vanillin
5	phenolic, smokey, coumarin-like
6	tonka bean, sweet
7	tonka bean
8	cinnamic aldehyde, resinoid
9	clean cinnamon
10	cassia-like, cinnamic aldehyde
11	cola lime, cinnamon
12	limey, cinnamic, piney
13	piney, musty, oily
14	citral, lemon
15	fresh lime, citrus, nootkatone-like
16	harsh, floral, petitgrain-like
17	citrus, orange
18	orange, aldehydic
19	cola-citrus, orange, woody
20	ginger, lime
21	carrot seed oil
22	carrot note
23	tomato, vegetable
24	green tomato, metallic, rosemary
25	vegetative, green
26	herbaceous
27	weak

icals can then be fractionated by a solvent gradient. (3) Overall recoveries of aroma chemicals are generally over 80%. (4) The fractions can be evaluated on sniff strips as well as by standard (GC, GC–MS, and HPLC) methods. (5) The presence of significant levels of soluble solids does not cause interference. (6) The procedure is inherently nondestructive, since the catalytic activity of silica gel and the heat associated with gas chromatography are eliminated.

The value of this procedure has been shown with a methyl ester model system, a synthetic flavor mixture containing a variety of functional groups, a peppermint oil, and a carbonated beverage.

LITERATURE CITED

Bemelmans, J. M. "Progress in Flavor Research"; Land, D. G.; Nursten, H. E., Eds.; Applied Science Publishers, Inc.: London, 1979; pp 79–98.
Bock, R. M.; Ling, N. *Anal. Chem.* **1954**, *26*, 1543.
Charalambous, G. "Analysis of Foods and Beverages", Headspace Techniques; Academic Press: New York, 1978.
Kirkland, J. *Analyst (London)* **1974**, *99*, 859.
Likens, S. T.; Nickerson, G. B. *J. Chromatogr.* **1966**, *21*, 1.
May, W. E.; Chesler, S.; Cram, S.; Gump, B.; Hertz, H.; Enagonio, D.; Dysezel, S. *J. Chromatogr. Sci.* **1975**, *13*, 535.
Merritt, C. "Preparative Gas Chromatography"; Zlatkis, A.; Pretorius, V., Eds.; Wiley-Interscience: New York, 1971; pp 235–276.
Ogan, K.; Katz, E.; Slavin, W. *J. Chromatogr. Sci.* **1978**, *16*, 517.
Schultz, T. H.; Flath, R. A.; Mon, T. R.; Eggling, S. B.; Teranishi, R. *J. Agric. Food Chem.* **1977**, *25*, 466.
Weurman, C. *J. Agric. Food Chem.* **1969**, *17*, 370.

Received for review August 11, 1980. Revised December 18, 1980. Accepted March 6, 1981.

Effects of Plant Growth and Air Curing on Amounts of *trans*-Coniferyl and *trans*-Sinapyl Alcohols in Midveins of Burley Tobacco

Roger A. Andersen,* Michael J. Kasperbauer, Thomas H. Vaughn, and Harold R. Burton

A quantitative gas chromatographic method was developed for the estimation of *trans*-coniferyl and *trans*-sinapyl alcohols in leaf midveins of field-grown Ky 14 burley tobacco. It was confirmed by gas chromatography–mass spectrometry that both alcohols were major phenylpropanoids in hot-water extracts of the leaf midvein samples. Concentrations of both alcohols increased as the maturity of plants increased. Greater quantities of alcohols were obtained after air curing than at harvest. Leaf position on the stalk also affected the amounts of the alcohols recovered, but the magnitude and direction of this effect depended on plant maturity and air curing parameters. Levels of *trans*-sinapyl alcohol among all the samples were only about one-third those of *trans*-coniferyl alcohol; the amounts found ranged from 7.3 to 70.5 mg of coniferyl alcohol and 0 to 24.6 mg of sinapyl alcohol per kg dry weight of sample.

Coniferyl alcohol [3-(4-hydroxy-3-methoxyphenyl)-2-propen-1-ol] and sinapyl alcohol [3-(-hydroxy-3,5-dimethoxyphenyl)-2-propen-1-ol] are considered to be precursors of the monolignol building blocks of plant lignin (Freudenberg, 1966), and they were recently identified as phenolic phenylpropanoids in hot-water extracts of burley tobacco stalk (Andersen et al., 1980). These alcohols were thought to be weakly bound to non-cross-lined regions of lignin or polysaccharide and capable of release by hydrolysis with hot water. Coniferyl alcohol has been identified as a constituent in a phenolic fraction of cigarette tobacco smoke condensate; its concentrations were twice as high in midvein condensate as in leaf lamina condensate (Ishiguro et al., 1976). Since tobacco leaf midveins are used in smoking tobacco (Moshy, 1967), it is important to learn whether contents of these phenolic phenylpropanoids in tobacco leaf midveins are influenced by plant growth environment, stage of plant development, and curing.

In this report we identify *trans*-coniferyl alcohol and *trans*-sinapyl alcohol in superheated water extracts of burley tobacco leaf midveins by gas chromatography–mass spectrometry (GC–MS). The effects of plant population

Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, and Department of Agronomy, University of Kentucky, Lexington, Kentucky 40546.

density, maturity, and air curing on the amounts of these alcohols were estimated by quantitative gas chromatography (GC).

MATERIALS AND METHODS

Plant Materials. Burley tobacco plants (*Nicotiana tabacum* L. cv. Ky 14) were grown in three plant population densities at the Kentucky Agricultural Experiment Station South Farm near Lexington. Plant spacings were "close" (30 × 30 cm), "conventional" (45 × 100 cm), and "wide" (125 × 125 cm). Field cultural practices were those recommended for burley tobacco in Kentucky (Atkinson et al., 1976). Midveins were taken from green leaves during rapid plant growth (6 weeks after transplant), at the time of first flowering (10 weeks after transplant), and at the time of harvest (~3 weeks after "topping", i.e., removal of the inflorescences and small upper leaves when ~25% of the plants had one or more open flowers). Midveins were also taken from leaves after conventional air curing of the leaves on the stalks.

In all cases except for the 6-week sample, midveins were from leaves grown on the top, middle, and bottom positions of stalks from all three plant population densities. The top stalk position included leaves 1, 2, and 3; the middle included leaves 9, 10, and 11; the bottom included leaves 18, 19, and 20. Leaf 1 was the uppermost after topping. Each stalk position sample consisted of the 3 leaves from each of 10 stalks. Only middle positions were sampled in the 6-week sample. Midveins were largest from plants grown in the wide spacing and smallest from those grown in close spacing.

Midveins were separted immediately from lamina of green-harvested leaves. They were freeze-dried, ground to 40-mesh size, and stored in darkness until chemical analyses were performed. Midveins from cured samples were separated from lamina after the cure was completed; they were then ground and stored in the same manner as the green samples.

Reference Compounds and Internal Standard for Gas Chromatography (GC). All the foregoing phenylpropanoid compounds were trans isomers. *p*-Coumaric acid, ferulic acid, sinapic acid, and caffeic acid were obtained from Aldrich Chemical Co., Milwaukee, WI. *p*-Coumaraldehyde, coniferaldehyde, sinapaldehyde, and their alcohol congeners were synthesized by reduction of their acetylated acid chlorides with metal hydrides (Andersen et al., 1980). Caffeic acid *n*-propyl ester was used as the internal standard for most GC analyses and was prepared by the reaction of caffeic acid and *n*-propyl alcohol saturated with HCl gas (Andersen and Vaughn, 1970). Trimethylsilyl (Me_3Si) derivatives of up to 200-μg amounts of the reference compounds or internal standard in 50 μL of ethyl acetate were prepared by their reaction with 30 μL of bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) at 65 °C in a sealed vial for 15 min. One- to five-microliter amounts were injected into the GC.

Gas Chromatography and Gas Chromatography–Mass Spectrometry (GC and GC–MS). A microprocessor-controlled gas chromatograph with a flame ionization detector (FID) was used for analyses of the Me_3Si derivatives of the reference compounds and tobacco midvein extracts. Inlet and detector temperatures were 200 and 250 °C, respectively. The column temperature was programmed from 150 to 200 °C at 10 °C/min. N_2 was the carrier gas at a flow rate of 30 mL/min. A 1.83 m × 2 mm glass column with 3% (w/w) OV-101 silicone stationary phase on 80–100-mesh Chromosorb WHP was used. Relative retention times (t_R) of the 10 Me_3Si-phenylpropanoid reference compounds were determined and are tabulated in Table I for the temperature-programmed column conditions. For isothermal analyses of phenylpropanoid alcohols in midvein extracts, the operating temperatures for inlet and FID were 200 and 250 °C, respectively. Argon was used as the carrier gas at a flow rate of 60 mL/min. A 1.83 m × 4 mm coiled glass column with 5% (w/w) OV-101 silicone stationary phase on 80–90-mesh Anakrom AS at 170 °C was used.

Table I. GC Relative Retentions (t_R) of Me_3Si-phenylpropanoid Reference Compounds on a 3% OV-101 Column Programmed from 150 to 200 °C at 10 °C/min

Me_3Si-phenylpropanoid ref compd	t_R [a]
p-coumaraldehyde	0.25
coniferaldehyde	0.38
p-coumaryl alcohol	0.42
coniferyl alcohol	0.49
p-coumaric acid	0.50
sinapaldehyde	0.55
scopoletin	0.62
ferulic acid	0.74
sinapyl alcohol	0.76
caffeic acid *n*-propyl ester (ISTD)	1.00
sinapic acid	1.17

[a] Relative retention time calculated by considering caffeic acid *n*-propyl ester as 1.00. Mean value of three determinations.

The GC–MS analyses were performed in either the electron impact or chemical ionization mode in the same manner described for tobacco and alfalfa extracts (Andersen et al., 1980).

Extraction and Analysis of Tobacco. The following procedures were used for the identifications and preliminary quantitative analyses. A 0.5–2.0-g sample of freeze-dried midvein was refluxed with *n*-hexane (50 mL) for 30 min. The mixture was filtered with suction through a 0.45-μm Millipore (fluoropore) filter, and the precipitate was air-dried on the filter. The filter pad and its contents were extracted with 50 mL of chloroform, acetone, or methanol for a specified period of 5–30 min and filtered as before; these solvents were used either as principal extractants or, more commonly, one was used as a preextractant prior to extraction with hot water. When a final extraction with hot water was omitted, the nonchloroform filtrates were evaporated to dryness, taken up in water (50 mL), and partitioned with chloroform as described below for the posttreatment of hot-water extracts for GC or GC–MS analyses.

For final hot-water extractions, the filter pad containing preextracted midveins was transferred to an oversize (500-mL) Erlenmeyer flask containing water (50 mL). The flask was covered with an inverted beaker and autoclaved at 121 °C with a pressure of 154 cmHg for a specific time (15–180 min). The mixture was then filtered as before. The flask and residue were washed with chloroform (10 mL). The combined water–chloroform filtrate washing was transferred to a separatory funnel and partitioned 3 times with chloroform (total chloroform volume = 120 mL). The chloroform phases were combined and taken to near dryness on a rotary vacuum evaporator. The remaining solvent was evaporated with a stream of nitrogen. The residue was dissolved in 50 μL of ethyl acetate containing 180 μg of *trans*-caffeic acid *n*-propyl ester and 30 μL of BSTFA containing 1% TMCS for silylation before analysis. The silylation solution was heated at 65 °C in a sealed vial for 15 min before injection of 1–5 μL into the gas chromatograph.

For quantitative estimations of *trans*-coniferyl alcohol and *trans*-sinapyl alcohols in field-grown burley tobacco midveins, 0.5-g samples were extracted and prepared for GC analysis as described above except for the following details. The samples were preextracted with *n*-hexane for 30 min, chloroform for 30 min, and methanol for 5 min. Residues were then extracted with hot water for 45 min, and the extracts were filtered, partitioned with chlorofom, and silylated. Isothermal GC column conditions were used. Quantitation was accomplished by an internal standardization method calibrated from ratios of retention times and detector responses for known amounts of the reference phenylpropanoid alcohols and the internal standard caffeic acid *n*-propyl ester. The mean phenylpropanoid alcohol content of a sample in a given subgroup was compared with those for other samples in the subgroup by means of the Student's *t* test. Phenylpropanoid alcohol concentrations determined by this method were comparable among samples, but individual results were not corrected for incomplete recoveries of the alcohols that occurred during the extraction and solvent-partitioning steps.

Lignin Determination. A gravimetric method was used for the determination of lignin (Van Soest, 1963). A calculation was performed to determine the sum of the weight percentages of *trans*-coniferyl and *trans*-sinapyl alcohols that were equivalent to the total lignin content in a sample.

RESULTS AND DISCUSSION

GC and GC–MS Identification Experiments. Two major peaks were obtained for each midvein extract with retentions that closely approximated those for the trans isomers of reference coniferyl alcohol and *p*-coumaric acid (the first peak) and sinapyl alcohol and ferulic acid (the second peak). GC–MS analyses confirmed the presence of only *trans*-coniferyl alcohol in the first peak and *trans*-sinapyl alcohol in the second peak. Figure 1 shows an isothermal GC chromatogram of a silylated residue from the chloroform-partitioned hot-water extract of tobacco midvein (preextracted successively with chloroform and methanol). Peaks 1 and 2 had t_R values of 0.37 and 0.68, respectively; *trans*-caffeic acid *n*-propyl ester (ISTD) was peak 3. These t_R values closely approximated those of the trans isomers of reference Me_3Si-coniferyl alcohol (0.38) and Me_3Si-sinapyl alcohol (0.67) analyzed with the isothermal column conditions.

trans-Coniferyl alcohol and *trans*-sinapyl alcohol were not detected in chloroform or acetone extracts of midvein samples after 30-min extraction periods, and *trans*-coniferyl alcohol was barely detectable in methanol extracts after 30-min extraction periods. These solvent effects on yields of the phenylpropanoid alcohols were in contrast to that observed when hot water was used as the extraction solvent. Chloroform, acetone, or methanol readily dissolved reference coniferyl alcohol and sinapyl alcohol, however. The differential solvent effects were interpreted to mean that hot water hydrolyzed coniferyl alcohol and sinapyl alcohol bound to tobacco midvein lignin or lignin–carbohydrate complexes in the same manner as that hypothesized for tobacco stalk (Andersen et al., 1980) and for coniferyl alcohol in spruce wood (Freudenberg, 1966). Another possible cause for the observed solvent extraction effects on yields of the phenylpropanoid alcohols may be related to the "compartmentation phenomenon" observed for plant phenolics in angiosperm leaves (Monties and Rambourg, 1978). The description of the result of this phenomenon cited that the same phenolic compound in leaf was extracted with two different solvents of increasing polarity used successively.

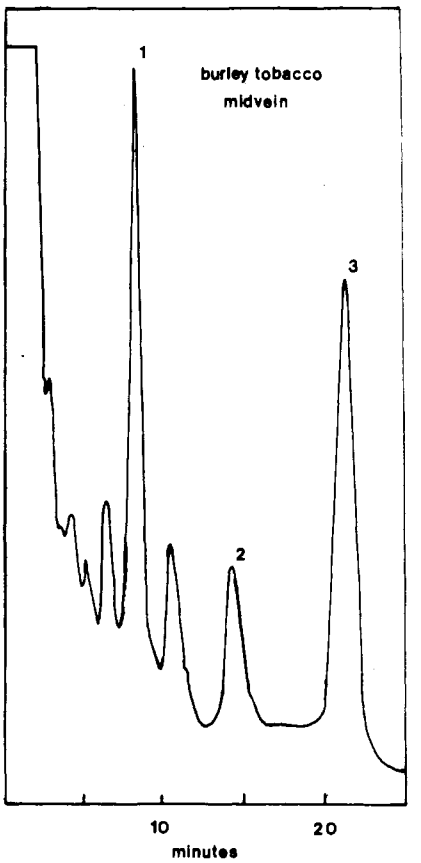

Figure 1. GC chromatogram obtained on an 5% OV-101 column at 170 °C with an FID detector for a silylated hot-water extract of burley tobacco midvein (31.2 mg equiv of tobacco midvein from upper leaves of wide-spaced tobacco at the floral bud stage). Peaks 1, 2, and 3 correspond to Me_3Si-*trans*-coniferyl alcohol, Me_3Si-*trans*-sinapyl alcohol, and the internal standard Me_3Si-*trans*-caffeic acid *n*-propyl ester, respectively.

Preliminary experiments showed that preextractions for 30 min with chloroform, followed by 5 min with methanol, reduced the elevated base lines and permitted the resolution of peaks in the chromatograms of the hot-water extracts used for GC–MS and GC analyses. The same preextractions did not noticeably affect the yields of the two phenylpropanoid alcohols in the hot-water extracts.

Structural identifications of the alcohols were based in part upon the electron impact and chemical ionization mass spectra of the GC-separated Me_3Si derivatives, the relative intensities of the MS charged ions, and the comparison of specific spectra with those of the available reference compounds. Mass spectra of phenylpropanoids extracted from midveins were compared with spectra of phenypropanoids in tobacco stalk obtained in a previous study (Andersen et al., 1980) and with other literature values when available; fragmentation patterns produced by electron impact have been tabulated for three underivatized *p*-hydroxycinnamyl aldehydes (Nakamura et al., 1974) and for coniferyl alcohol and coniferaldehyde (Nakamura and Higuchi, 1976). Chemical ionization mass spectra were interpreted by comparison of the product ions obtained with those predicted as formed with methane according to the pinciples of chemical ionization mass spectrometry (Munson, 1971). Our MS data pertaining to confirmations of chemical structure for Me_3Si derivatives of coniferyl alcohol and sinapyl alcohol along with probable fragment assignments and relative intensity (percent) follow. *Electron impact*: Me_3Si-*trans*-coniferyl alcohol *m/e* 324 (M^+, 39), 309 ($M^+ - CH_3$, 7), 294 (15), 293 ($M^+ - CH_3O$, 25), 235 (18), 204 (29), 131 (17), 103 (12), 89

Table II. Plant Maturity and Air Curing Effects on Amounts of Phenylpropanoid Alcohols Extracted from Tobacco Midveins

weeks after transplant	growth stage	phenylpropanoid alcohols released by hot-water extraction, mg/kg dry wt of midveins[a]		sum of phenylpropanoid alcohols released	
		trans-coniferyl alcohol	*trans*-sinapyl alcohol	mg/kg dry wt of midveins	as % of total lignin
	Closely Spaced Tobacco (111 000 Plants/Hectare)				
6	rapid	8.8 a	3.4 a	12.2	0.04
10	first flowering	29.6 b	7.5 ab	37.1	0.25
13	mature—at harvest	31.6 bc	12.3 b	43.9	0.30
13 + 7 weeks of air curing	air cured	66.6 d	22.7 c	89.3	0.35
	Conventionally Spaced Tobacco (22 000 Plants/Hectare)				
6	rapid	7.3 a	2.7 a	10.0	0.03
10	first flowering	30.8 b	5.7 ab	36.5	0.21
13	mature—at harvest	39.6 c	16.4 c	56.0	0.37
13 + 7 weeks of air curing	air cured	62.6 d	17.4 c	80.0	0.22
	Widely Spaced Tobacco (6400 Plants/Hectare)				
6	rapid	7.8 a	8.1 ab	15.9	0.05
10	first flowering	34.0 c	5.1 a	39.1	0.23
13	mature—at harvest	30.4 bc	9.3 ab	39.7	0.26
13 + 7 weeks of air curing	air cured	70.5 d	24.6 c	95.1	0.35

[a] Mean values are from three to five laboratory analyses per sample of midveins from the middle leaf position on the stalk. Values in each column of a tobacco plant population subgroup not followed by same letter are significantly different at the 0.05 level of probability. LSD for coniferyl alcohol and sinapyl alcohol among all entries is 7.6 and 3.3 mg/kg dry weight, respectively, at $P < 0.05$.

(5), 73 [$(CH_3)_3Si$, base ion]; Me_3Si-*trans*-sinapyl alcohol *m/e* 354 (M^+, 37), 339 ($M^+ - CH_3$, 7), 324 (23), 323 ($M^+ - CH_3O$, 21), 293 (7), 265 ($M^+ - C_7H_5$, 13), 234 (26), 204 (18), 149 (12), 134 (11), 73 [$(CH_3)_3Si$, base ion]. *Chemical ionization*: Me_3Si-*trans*-coniferyl alcohol *m/e* 237 (base ion), 309 ($M^+ - CH_3$), 325 ($M^+ + H$), 353 ($M^+ + C_2H_5$); Me_3Si-*trans*-sinapyl alcohol *m/e* 264, 339 ($M^+ - CH_3$, base ion), 355 ($M^+ + H$), 383 ($M^+ + C_2H_5$).

Optimization of Extraction Conditions. In preliminary experiments, midvein samples were extracted with water heated on a hot plate. Foaming and temperature fluctuations of the extract solution occurred. These difficulties were avoided, and the yields of phenylpropanoids released were improved by extraction with superheated water at 121 °C and 154 cmHg in an autoclave. Experiments were carried out to determine the hot-water extraction time needed for maximum yields of *trans*-coniferyl and *trans*-sinapyl alcohols from midvein samples preextracted with *n*-hexane for 30 min, chloroform for 30 min, and methanol for 5 min. Midveins were extracted with superheated water for 15-, 30-, 45-, 60-, 120-, and 180-min periods. The extracts were quantitatively analyzed for coniferyl and sinapyl alcohols by GC (Figure 2). Yields of the two alcohols became greater as the extraction time increased from 15 to 30 min. At extraction times between 30 and 120 min, the yields were maximized and were not significantly different. Extraction for 180 min caused a loss in relative yield. Several antioxidants were added to the extraction solution to determine whether their presence would improve the yields of the alcohols. No changes in yields were observed (even following a 180-min extraction period) with additions of 1 mM mercaptoethanol, ascorbic acid, or thiourea. A 30% recovery of reference coniferyl alcohol was obtained when 1 mg of this alcohol was carried throughout the GC quantitative estimation procedure but omitting the preextraction steps.

Effects of Plant Growth Conditions and Air Curing. Field-grown burley tobacco midvein samples were quantitatively analyzed for *trans*-coniferyl alcohol, *trans*-sinapyl alcohol, and total lignin. The amounts of

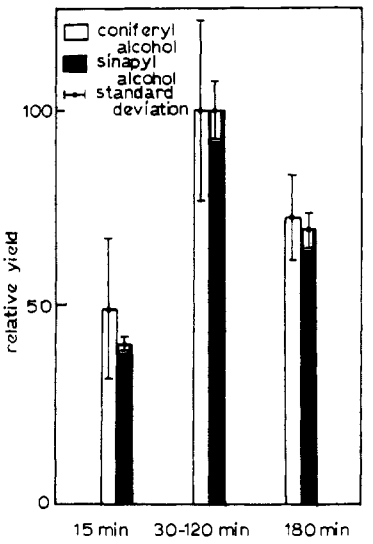

Figure 2. Effect of superheated water (120 °C; 154 cmHg) extraction time on the yield of phenylpropanoid alcohols released from Ky 14 burley tobacco midveins.

the alcohols extracted from tobacco leaf midveins from plants grown at high-, conventional- and low-population densities as a function of plant maturity and air curing are given in Table II. Results are expressed as means of individual analyses on samples from middle leaf positions on the stalk. For each population density, increased concentrations of both alcohols were found in midveins of the successively more mature growth stages, and the postcure concentrations were greater than those obtained from samples taken at harvest. Although air curing of burley tobacco caused dry weight losses of 15–20% in the leaf (Palmer, 1963), similar weight losses in the presently studied air-cured leaf midveins would not account for the approximate doubling of phenylpropanoid alcohol concentrations that we observed after air curing.

The effects of stalk position on the amounts of *trans*-coniferyl and *trans*-sinapyl alcohols in midvein samples

Table III. Stalk Position Effect on the Amounts of Phenylpropanoid Alcohols Extracted from Tobacco Midveins

leaf position on stalk	*trans*-coniferyl alcohol released by hot-water extraction, mg/kg dry wt of midveins[a]			*trans*-sinapyl alcohol released by hot-water extraction, mg/kg dry wt of midveins[a]		
	10 weeks after transplant	13 weeks after transplant	after air curing	10 weeks after transplant	13 weeks after transplant	after air curing
	Closely Spaced Tobacco (111 000 Plants/Hectare)					
upper	22.4 a	38.9 c	67.1 b	0.0 a	9.5 a	20.3 a
middle	29.6 b	31.6 ab	66.6 b	7.5 b	12.3 ab	22.7 b
lower	34.7 c	30.1 a	54.1 a	11.9 c	13.9 b	18.5 a
	Conventionally Spaced Tobacco (22 000 Plants/Hectare)					
upper	19.2 a	41.7 b	42.6 a	0.0 a	13.6 ab	10.9 a
middle	30.8 b	39.6 a	62.6 c	5.7 b	16.4 b	17.4 b
lower	32.7 b	38.1 a	49.2 b	7.4 bc	11.5 a	20.1 bc
	Widely Spaced Tobacco (6400 Plants/Hectare)					
upper	19.5 a	41.3 b	56.3 a	0.0 a	18.8 b	20.1 a
middle	34.0 b	30.4 a	70.5 b	5.1 b	9.3 a	24.6 b
lower	57.1 c	30.4 a	54.1 a	11.3 c	16.0 b	20.7 a

[a] Mean values are from four laboratory analyses per sample. Values in each column of a tobacco plant population subgroup not followed by same letter are significantly different at the 0.05 level of probability. LSD for coniferyl alcohol and sinapyl alcohol among all entries is 3.8 and 3.7 mg/kg dry weight, respectively, at $P < 0.05$.

of different maturity stages are given in Table III. At the time of first flowering, midvein concentrations of both phenylpropanoid alcohols in samples representing all population densities increased in the order of descending stalk position. At the time of harvest, there was a stalk position effect for coniferyl alcohol levels in samples of all population densities that generally was in reverse order to that at the time of first flowering. The direction of stalk position effects on sinapyl alcohol concentrations at harvest varied among population densities, however. Phenylpropanoid alcohol concentrations were usually maximal in cured midveins from middle leaf positions on the stalk.

It was noted that *trans*-sinapyl alcohol levels were only about one-third those of *trans*-coniferyl alcohol in the midvein samples from plants grown at the three spacings (Tables II and III). This relationship of 3 parts of coniferyl alcohol to 1 part of sinapyl alcohol in tobacco leaf midveins contrasted with nearly equimolar concentratons of these two alcohols that were determined in tobacco stalk (Andersen et al., 1980).

The amounts of extractable coniferyl and sinapyl alcohols were greater in midvein samples from tobacco leaves that had higher lignin contents. The calculated values for the sum of the trans isomers of coniferyl alcohol and sinapyl alcohol expressed as percentage of the total lignin content (Tables II and III) may be representive of the amounts of these phenylpropanoid alcohols which were weakly bound to the non-cross-linked regions of lignin and released by hot-water extraction or hydrolysis. It was estimated that up to 0.4% of the total lignin was accounted for by the recovered alcohols, although the phenylpropanoid alcohols determined by the GC procedure were probably underestimated because of their incomplete recoveries during extraction steps. Only 0.05 or less of the lignin in midveins of 6-week samples was equivalent to extractable coniferyl and sinapyl alcohols, but percentages increased 4–11-fold in the more mature and air-cured samples of midveins. Approximately 90 mg of the combined alcohols were determined per kg of the air-cured midvein samples, representing $\sim^1/_{300}$ of the total lignin content of these samples (total lignin content was ~30 mg/g of air-cured midvein). Our data for the amounts of hot water extractable phenylpropanoid alcohols do not account for the composition of the major fraction of lignin in tobacco midveins. The major fraction of lignin is believed to consist of nonhydrolyzable cross-linked residues of monolignols derived from *trans*-coniferyl alcohol, *trans*-sinapyl alcohol, and *trans*-*p*-coumaryl alcohol (Freudenberg, 1966). It is possible that the coniferyl alcohol identified in cigarette smoke condensate (Ishiguro et al., 1976) originated either directly from the water-extractable coniferyl alcohol or from coniferyl alcohol released from the thermolytic cracking of lignin in cured tobacco during the smoking process.

ACKNOWLEDGMENT

We thank R. Laine (Biochemistry Department) and C. Hughes (Tobacco and Health Research Institute) for the mass spectral analyses.

LITERATURE CITED

Andersen, R. A.; Vaughn, T. H. *J. Chromatogr.* **1970**, *52*, 385.
Andersen, R. A.; Vaughn, T. H.; Kasperbauer, M. J. *J. Agric. Food Chem.* **1980**, *28*, 427.
Atkinson, W. O.; Sims, J. L.; Calvert, J. R. *Tob. Sci.* **1976**, *20*, 32.
Freudenberg, K. In "Symposium on Lignin Structure and Reactions"; Gould, R., Ed.; American Chemical Society: Washington, DC, 1966; pp 1–21.
Ishiguro, S.; Sato, S.; Sugawara, S.; Kaburaki, Y. *Agric. Biol. Chem.* **1976**, *40*, 977.
Monties, B.; Rambourg, J. C. *Ann. Technol. Agric.* **1978**, *27*, 629.
Moshy, R. J. In "Tobacco and Tobacco Smoke"; Wynder, E. L.; Hoffmann, D., Eds.; Academic Press: New York, 1967; pp 47–85.
Munson, B. *Anal. Chem.* **1971**, *43*, 28.
Nakamura, Y.; Higuchi, T. *Wood Res.* **1976**, *59*, 101.
Nakamura, Y.; Nakatsubo, F.; Higuchi, T. *Wood Res.* **1974**, *56*, 1.
Palmer, J. K. *Tob. Sci.* **1963**, *7*, 93.
Van Soest, P. J. *J. Assoc. Off. Agric. Chem.* **1963**, *46*, 829.

Received for review November 18, 1980. Revised March 23, 1981. Accepted April 16, 1981. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may be suitable. Presented in part at the 33rd Tobacco Chemists Research Conference, Lexington, KY, Oct 1979. This investigation is in connection with a project at the Kentucky Agricultural Experiment Station (KAES), and the results are published as KAES Journal Article 80-3-164.