Pyrolytic Studies on the Contribution of Tobacco Leaf Constituents to the Formation of Smoke Catechols

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Pyrolyses of different tobacco leaf varieties, under conditions designed to simulate cigarette smoke formation, demonstrated a correlation between leaf polyphenol levels and pyrolyzate catechol. Bright tobacco varieties contained significantly higher levels of polyphenols as compared to identically cured Burley tobacco varieties and produced correspondingly higher pyrolyzate catechol yields. Leaf constituents, including polyphenols, lignin, cellulose, and sugars, were pyrolyzed to determine potential conversion to catechol and alkylcatechols. Chlorogenic acid, the polyphenol generally present in highest amounts in tobacco leaf, produced the highest pyrolytic conversions to catechol and 4-ethylcatechol. Flavanoids (rutin and quercetin) produced lesser amounts of catechol and 4-alkylcatechols. The polymeric phenolic leaf constituent, lignin, produced significant yields of catechol.

Catechol (1,2-dihydroxybenzene), considered an active cocarcinogen (Van Duuren et al., 1973) and the most abundant phenol in cigarette smoke, has been identified (Schlotzhauer et al., 1978; Hecht et al., 1981) as the principal component of the weakly acidic fraction of cigarette smoke condensate which has been shown to possess tumor-promoting activity (Wynder and Hoffmann, 1964; Bock et al., 1969, 1971). A number of compounds, known to be present in tobacco leaf, have been shown to produce catechol under various pyrolytic conditions. Zane and Wender (1963) produced catechol and alkylcatechols by thermal degradation of the leaf tannin, chlorogenic acid, and of the flavanoids, rutin and quercetin, at reported melt temperatures of 600 °C. Caffeic acid, a structural component of chlorogenic acid, was reported (Jones and Schmeltz, 1968) to produce catechol (31.60 mol % yield) by hot tube (700 °C) pyrolysis under nitrogen. Since neither of these studies attempted to closely simulate actual conditions occurring in a burning cigarette, no realistic quantitative relationships between these leaf constituents and smoke catechol levels were determined. Recently, Carmella et al. (1980) extracted tobacco leaf with hexane, chloroform, benzene, and methanol and concluded, through pyrolysis studies, that the catechol precursors resided mainly in the methanol extract, which contained chlorogenic acid and sugars. Using a method designed to simulate the formation of cigarette smoke (Schlotzhauer et al., 1979), Schlotzhauer and Chortyk (1981), by pyrolysis of extracts obtained with solvents of increasing polarity, estimated that half of the catechol content of tobacco smoke is attributable to the ethanol extractable polyphenols of tobacco leaf, with the remainder of smoke catechol precursors residing in the lignin–cellulose-containing leaf fibers. Since type, cultivation, and curing of the tobacco plant have been shown to result in considerable variation in levels of chlorogenic acid and total polyphenols (Tso, 1972), we felt it of importance to determine the relationship between leaf polyphenol content and smoke catechol levels from different tobacco varieties. In this study, a series of Bright and Burley varieties, with known polyphenol contents [as determined by colorimetric

(Williamson, 1975) and high-pressure liquid chromatographic (Snook and Chortyk, 1981) analyses] were pyrolyzed and catechol yields were determined. Representative leaf polyphenols and additional compounds reported to produce phenolic degradation products, including lignin (Clark, 1968) and carbohydrates (Bell et al., 1966), were pyrolyzed under identical conditions to determine their contributions to the yield of smoke catechol.

EXPERIMENTAL SECTION

Tobacco Leaf Samples. Fourteen tobacco leaf varieties (nine Bright and five Burley) grown at the Tobacco Research Laboratory, Oxford, NC, in three replicate plots, were selected for these studies. For preservation of uniformity, all plants were cultured, harvested, and cured as for conventional flue-cured tobacco. After being cured, all harvests from a plot were bulked, the midribs were removed, and a portion of the resulting leaf material was converted to standard cigarette shred. Prior to pyrolysis, the shredded samples were equilibrated to 10% moisture content by placing them in a chamber containing a tray of saturated aqueous sodium bromide solution for 48 h. A second portion of leaf material was ground to pass a 40-mesh (1-mm) screen to produce samples for high-pressure liquid chromatographic determination of leaf polyphenol content.

Standard Leaf Compounds. Chlorogenic acid, mp 207–209 °C, and caffeic acid, 99%+, predominantly the trans compounds, were obtained from Aldrich Chemical, Inc. Rutin (crystalline trihydrate), cellulose (α-cellulose fiber), β-D-(–)-fructose (crystalline), and sucrose (Grade 1, crystalline) were obtained from Sigma Chemical Co. Quercetin (dihydrate) was obtained from Fluka A.G. As the best representative plant lignin, a Kraft softwood lignin was obtained from the Southeastern Forest Experiment Station, U.S. Department of Agriculture Forest Service, Macon, GA.

Pyrolyses. Samples (25 g) were pyrolyzed by using apparatus (Smith et al., 1975, Higman et al., 1977) and experimental parameters (Schlotzhauer et al., 1979) previously described. Pyrolysis products were collected in traps containing chloroform–methanol (9:1 v/v, distilled-in-glass grade). The pyrolyzate solutions were concentrated under vacuum on rotary evaporators (30 °C) to an accurate volume, generally 25 mL, sufficiently concentrated for subsequent gas chromatographic analyses.

Glass Capillary Gas Chromatography (GC-2). Aliquots of the pyrolyzate solution were combined with a known quantity of the internal standard, *p*-(*sec*-butyl)-

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Table I. Pyrolyzate Catechol Yields and Leaf Polyphenol Contents in Bright and Burley Tobacco Varieties

variety[a]	type	catechol, mg/g pyr	% total polyphenol colorimetric[b]	HPLC[c]	% chlorogenic acid isomers, HPLC[c]
White Mammoth	Bright	1.17	3.89	2.47	1.66
Speight G-10	Bright	1.04	3.93	2.05	1.46
North Carolina 95	Bright	1.03	4.26	2.83	2.09
Canadel	Bright	1.02	3.81	2.65	1.78
North Carolina 2326	Bright	1.01	4.17	2.41	1.68
Virginia 115	Bright	1.01	4.01	2.47	1.72
Speight G-28	Bright	0.98	3.68	2.79	1.83
401 Cherry Red Free	Bright	0.91	4.17	2.28	1.60
Coker 319	Bright	0.88	3.72	2.45	1.60
Kentucky 34	Burley	0.82	2.61	1.47	0.97
Gr 46	Burley	0.68	1.88	0.97	0.48
Warner	Burley	0.60	1.22	0.48	0.20
Kentucky 57	Burley	0.53	1.61	0.59	0.23
H 47	Burley	0.46	1.74	0.89	0.38
average for Bright		1.00	3.96	2.49	1.71
average for Burley		0.62	1.81	0.81	0.46

[a] All values obtained for mean of three replicate plot samples. [b] Determined by colorimetric method of Williamson (1975). [c] Determined by high-pressure liquid chromatography (HPLC) method of Snook and Chortyk (1981). HPLC method more specific for polyphenolic components than traditional colorimetric methods.

Table II. Major Pyrolysis Products of Selected Tobacco Leaf Components

compd pyrolyzed	M_r	product	yield (mg/g of compd pyrolyzed)	mol % converted
chlorogenic acid	354	catechol	43.3	13.9
		4-ethylcatechol	40.2	10.3
		phenol	13.9	5.2
		5-(hydroxymethyl)furfural	13.2	3.7
quercetin (dihydrate)	338	catechol	19.6	6.0
		4-methylcatechol	4.6	1.2
rutin (trihydrate)	601	catechol	7.8	4.3
		4-methylcatechol	5.5	2.7
		4-ethylcatechol	4.6	2.0
		4-propylcatechol	1.2	0.5
caffeic acid	180	catechol	3.6	0.6
lignin	undefined	catechol	9.5	a
		guaiacol	5.2	a
		4-methylcatechol	4.3	a
		phenol	2.3	a
		isoeugenol	1.1	a
cellulose	80–500 000	furfural	5.4	a
		levoglucosan	2.9	a
sucrose	342	furfural	27.7	9.8
		5-(hydroxymethyl)furfural	19.5	5.3
fructose	180	furfural	38.6	6.9
		5-(hydroxymethyl)furfural	19.5	3.5

[a] Indeterminate.

phenol, in a reaction vial. Excess BSTFA reagent [*N,O*-bis(trimethylsilyl)trifluoroacetamide] (Pierce Chemical Co.) was added, and the vial was sealed and placed in a heating block at 75 °C for 30 min. The silylated samples were directly analyzed by GC-2 on a Hewlett-Packard 5720A gas chromatograph equipped with flame ionization detectors and converted to utilize a 25-m SE-54 capillalry column which had been dynamically coated (Arrendale et al., 1980). The detector temperature was 300 °C, the injection port was 280 °C, and the oven temperature was programmed from 50 to 260 °C at 2 °C/min. The carrier gas (helium) linear flow velocity was 25 cm/s, the split flow was 100 ml/min, and the helium makeup was 30 mL/min. Under these conditions, the trimethylsilyl derivative of catechol eluted at 15.9 (±2%) min. Quantitative data, obtained with a Hewlett-Packard 3351B automation system, were based on response and retention values of authentic catechol.

High-Pressure Liquid Chromatography (HPLC). Quantitation of leaf chlorogenic acid contents was obtained by the method of Snook and Chortyk (1981). Tobacco samples were subjected to an ultrasonification extraction with water and were directly analyzed by reverse-phase HPLC. This method has been shown to be reproducible with a relative standard deviation of less than 10%.

RESULTS AND DISCUSSION

Pyrolytic yields of catechol (Table I) showed that flue-cured Bright tobacco varieties produced significantly higher levels of this compound than for flue-cured Burley varieties. The Bright varieties exhibited a higher range of total polyphenolic leaf constituents as determined both colorimetrically and by HPLC. Chlorogenic acid data, obtained by HPLC, indicated that chlorogenic acid isomers (chlorogenic, neochlorogenic, and 4-*O*-caffeoylquinic acids) constitute a greater portion of the total polyphenols in "high" polyphenol varieties than in "low" polyphenol tobaccos. The average catechol yield for the Bright varieties was 61% greater than that determined for the Burley varieties. Average total polyphenol and total chlorogenic acid levels ranged from 118 to 271% greater for Bright tobaccos. These data indicate that although polyphenol

Table III. Effect of Agronomic Variables on Pyrolyzate Catechol Levels[a]

field nitrogen application	pyrolyzate catechol, % change from recommended	
	Pale Yellow-10	Kentucky 34
recommended nitrogen		
low nitrogen	+51	+137
high nitrogen	+6	-50

plant stalk position	pyrolyzate catechol, % change from bottom position		
	Pale Yellow-10	Special Yellow-A	Coker 139
bottom			
middle	+16	-2	+7
top	+90	+23	+74

[a] As determined by relative response vs. the p-(sec-butyl)phenol internal standard.

content of tobacco leaf correlates to a high statistical probability with catechol levels (probability = 0.0002 for total polyphenol vs. catechol, with 0.01 highly significant), additional precursors (e.g., lignin) as previously suggested (Schlotzhauer and Chortyk, 1981) contribute to total catechol content of smoke.

Previous data (Schlotzhauer and Chortyk, 1981) had shown that 50% of the catechol content of the pyrolyzate was attributable to the polyphenol-containing ethanol-soluble leaf extract and the remainder to the sugars (methanol and water extracts) and the lignin–cellulose residue. Accordingly, individual leaf constituents were pyrolyzed under smoke simulation conditions (Table II). Chlorogenic acid was shown to produce the highest yields of catechol and 4-ethylcatechol. Significant amounts of 5-(hydroxymethyl)furfural, a thermal degradation product of the quinic acid moiety of this caffetannin and previously reported in pyrolyzates of the ethanol-soluble leaf extract, were produced. Quercetin and rutin, generally present in tobacco leaf in lesser quantities than those reported for chlorogenic acid, also produced significant amounts of catechol. Interestingly, rutin produced a series of 4-alkylcatechols, which have also been reported in cigarette smoke (Brunnemann et al., 1976; Schlotzhauer et al., 1978). In the latter study, the presence of vinylcatechols were reported in a biologically active fraction of cigarette smoke condensate. 4-Vinylcatechol has been reported to be a major cigarette smoke compound by Ishiguro et al., (1976). The source of this catechol derivative is easily rationalized from the thermal breakdown of the caffeic acid moiety of chlorogenic acid. Under our smoke simulation conditions, only catechol was found as a major pyrolysis product. When we performed a destructive distillation of caffeic acid, at 190 °C (oil bath) and under nitrogen, and directly reacted the distillate with BSTFA, we obtained as the major product a compound with mass spectral characteristics of trimethylsilylvinylcatechol. If formed under our pyrolysis conditions, apparently this compound was not stable under the standard workup conditions in our analyses.

Of the sugar and leaf fiber constituents, only lignin produced significant amounts of phenolic products, including catechol and guaiacols. These data suggest that lignin is a significant contributor to catechol levels in tobacco smoke. The polysaccharide cellulose, the disaccharide sucrose, and the monosaccharide fructose produced furfural and substituted furfurals as principal pyrolytic products. However, recent smoke studies (Carmella et al., 1981) performed with ^{14}C-labeled cellulose added to cigarettes suggest that cellulose may be a more important precursor of smoke catechol than might be deduced from pyrolytic data alone.

Although lignin content shows relatively slight variation among tobacco varieties, polyphenol levels vary considerably amount type, cultivation, stalk position, and curing parameters (Tso, 1972). Effects of some agronomic variables on pyrolyzate catechol levels for selected tobacco varieties are shown in Table III. The apparent inverse relationship of pyrolyzate catechol with nitrogen application closely correlates with the observations of Andersen et al. (1970) that leaf polyphenol content is lowered with increased nitrogen availability. The trend in pyrolyzate catechol yields for leaf samples obtained from the upper, middle, and lower stalk positions is reasonable, since leaf tannins increase dramatically from lower to middle stalk positions and reach maximum concentrations in the upper stalk positions (Tso, 1972). These data reinforce the previous conclusions as to the importance of polyphenol content of leaf in determining catechol content of tobacco smoke.

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