to the procedures of Chen and McGinnis (1981). The results (Table VI) show that there is significantly more xylose; 20.5% (and thus xylan; hence, hemicellulose) in resistant whorl tissue than in susceptible whorl tissue; 13.9%, while the glucose (hence cellulose) content was slightly higher in resistant tissue but probably not biologically important. The presence of the sugars arabinose and mannose is presumptive evidence of two other hemicelluloses, the arabans and the mannans. Thus, the total hemicellulose content was 17.6% for the susceptible line and 24.0% for the resistant line. The difference in the sum of the sugars from 100% can be attributed to proteins, salts, and other non-sugar constituents. The higher analysis for xylose in MpSWCB-1 × Mp496 suggests that xylans may be a source of resistance.

Comparable results for sugars in cob, husk, stalk, and leaf were determined by Krull and Inglett (1978) and are also given in Table VI. From this analysis, it can be deduced that cellulose, starch, and xylan polymers are the major sources of residue in corn leaf. Lignin (1.52–1.63%) and salts (analyzed as ash, 4.4–6.4%) evidently contribute only to a limited degree.

In summary, the fiber and residue contents are significantly higher in a number of resistant lines. The cellulose and hemicelluloses comprise an important portion of the fiber and can be expected to contribute to leaf toughness, indigestibility, and intractibility to metabolism by the insect. Thus, they evidently explain at least in part a basis of resistance in the cultivars studied.

ACKNOWLEDGMENT

We thank Tom Davis and Thomas Oswalt of this laboratory for expert technical service.

Registry No. 6-Methoxybenzoxazolinone, 532-91-2; cellulose, 9004-34-6; hemicellulose, 9034-32-6.

LITERATURE CITED

Batra, G. K.; Kuhn, C. W. *Phys. Plant Pathol.* **1975**, *5*, 239.
Chen, C. C.; McGinnis, G. D. *Carbohydr. Res.* **1981**, *90*, 127.
Chippendale, G. M. *Environ. Entomol.* **1974**, *3*, 233.
Davis, F. M.; Henderson, C. A.; Scott, G. E. *J. Econ. Entomol.* **1972**, *65*, 519.
Davis, F. M.; Williams, W. P. *J. Econ. Entomol.* **1980**, *73*, 704.
Guthrie, W. D.; Russell, W. A.; Jennings, C. W., Proceedings of the 26th Annual Corn and Sorghum Research Conference, Chicago, IL, 1971, p 165.
Hedin, P. A.; Davis, F. M., USDA, Mississippi State, MS, unpublished data, 1983.
Henderson, C. A.; Bennett, S. E.; McQueen, H. F. *J. Econ. Entomol.* **1966**, *59*, 360.
Hyodo, H.; Uritani, I. *Arch. Biochem. Biophys.* **1967**, *122*, 299.
Klun, J. A.; Brindley, J. A. *J. Econ. Entomol.* **1966**, *59*, 711.
Krull, L. H.; Inglett, G., Northern Regional Research Center, USDA, Peoria, IL, personal communication, 1978.
Matsumoto, I.; Okazawa, M. Japanese Patent 7275593, 28 (CA); *Chem. Abstr.* **1974**, *81*, 63612.
Nicollier, G. F.; Hedin, P. A.; Davis, F. M. *J. Agric. Food Chem.* **1982**, *30*, 1133.
Robinson, J. F.; Klun, J. A.; Brindley, T. A. *J. Econ. Entomol.* **1978**, *71*, 461.
Scott, G. E.; Davis, F. M. *Agron. J.* **1974**, *66*, 773.
Sullivan, S. L.; Grocen, V. E.; Ortega, A. *Environ. Entomol.* **1974**, *3*, 718.
Venis, M. A.; Watson, P. J. *Planta* **1978**, *142*, 103.

Received for review June 17, 1983. Accepted October 28, 1983. 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 or Mississippi State University and does not imply its approval to the exclusion of other products or vendors that may also be suitable. Mississippi Agricultural and Forestry Experiment Station Journal Article 5537.

Roles of Tobacco Cellulose, Sugars, and Chlorogenic Acid as Precursors to Catechol in Cigarette Smoke

Steven G. Carmella,* Stephen S. Hecht, T. C. Tso, and Dietrich Hoffmann

Tobacco was extracted sequentially with hexane and methanol–H_2O, and the extracts were pyrolyzed at 650 °C in order to identify likely leaf precursors to the tobacco smoke cocarcinogen catechol. The results demonstrated that the methanol–H_2O extract and the extracted tobacco residue were good pyrolytic precursors to catechol. Subfractions of the methanol–H_2O extract were isolated by HPLC and pyrolyzed. Fructose, glucose, sucrose, and chlorogenic acid were thus identified as important pyrolytic precursors to catechol. Cellulose, a component of the extracted tobacco residue, was also found to be a good precursor to catechol in pyrolysis experiments. To determine the role of these substances as precursors to catechol under the conditions prevailing in a burning cigarette, either [^{14}C(U)]cellulose, [^{14}C(U)]fructose, or various levels of the unlabeled polyphenols chlorogenic acid or rutin were added to cigarettes and the mainstream smoke was analyzed for [^{14}C]catechol and catechol. On the basis of these experiments, we estimated the minimum contributions of these compounds to mainstream smoke catechol levels as follows: cellulose, 7–12%; total of fructose, glucose, and sucrose, 4%; chlorogenic acid, 13%; rutin, <1%. It is suggested that a significant portion of the remaining catechol in mainstream cigarette smoke is formed from pectin, starch, and hemicellulose.

The cocarcinogens of tobacco smoke are likely to be among the most important constituents responsible for its carcinogenic activities in experimental animals and man (Hoffmann et al., 1978; U.S. Department of Health and Human Services, 1982). These compounds, while not carcinogenic themselves, significantly enhance the tumorigenic activities of the polynuclear aromatic hydrocarbon carcinogens. In the absence of cocarcinogens and tumor promoters, the levels of polynuclear aromatic hydrocarbons present in tobacco smoke are not sufficient to induce tumors on mouse skin (Hoffmann et al., 1978). Catechol

Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York 10595, and Tobacco Laboratory, Beltsville Agricultural Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.

0021-8561/84/1432-0267$01.50/0

(1,2-dihydroxybenzene) is a major cocarcinogen in tobacco smoke. It occurs in mainstream cigarette smoke typically in the range of 136–328 μg/cigarette (Brunnemann et al., 1976). Pure catechol as well as subfractions of cigarette smoke condensate containing catechol significantly increases the carcinogenicity of benzo[a]pyrene on mouse skin (Van Duuren and Goldschmidt, 1976; Hecht et al., 1981). These results indicated that reduction of the levels of catechol in cigarette smoke would result in products with less carcinogenic activity. Since significant quantities of catechol have not been detected in tobacco leaf, it apparently is formed during smoking. The purpose of the present study was to identify possible precursors to mainstream smoke catechol. In preliminary experiments, tobacco extracts, their subfractions, and selected tobacco components were pyrolyzed and the pyrolysates were analyzed for catechol. This information was then used to design experiments in which potential precursors were added to cigarettes, and the effects on catechol levels in mainstream smoke were evaluated.

EXPERIMENTAL SECTION

Apparatus. GC analyses were performed on a Hewlett-Packard Model 5830A gas chromatograph equipped with a flame ionization detector and a 6 ft × $^1/_8$ in. i.d. column packed with 10% UCW 98 on Chromosorb WHP. The oven temperature was held at 90 °C for 8 min and then programmed at 4 °C/min to 230 °C with a flow rate of 50 mL/min He. GC–MS was carried out on a Hewlett-Packard Model 5982A instrument. HPLC was performed on a system consisting of two Model 6000A solvent delivery systems, a Model 660 solvent programmer, a Model U6K injector, and a Model 440 UV–visible detector or a Model R-401 differential refractometer (Waters Associates, Milford, MA). Scintillation counting was performed with a Nuclear Chicago Isocap 300 system. Chlorogenic acid and rutin were sprayed on cigarette tobacco with a Chromamist spray unit, Gelman Instrument Co., Ann Arbor, MI. Cigarettes were prepared by using a Laredo cigarette maker, Brown and Williamson Tobacco Corp., Lexington, KY. Draw resistance was measured with a Filtrona Instruments Model PDALP flow meter, Cigarette Components, Ltd., London, England. Cigarettes were smoked on a Heinrich Borgwaldt automatic smoking machine, RM-20/68.

Chemicals. Phenol, catechol, and hydroquinone were obtained from Fisher Scientific Co., Springfield, NJ, and *o*-, *m*-, and *p*-cresol, chlorogenic acid [3-(3,4-dihydroxycinnamoyl)quinic acid], rutin (3,3′,4′,5,7-pentahydroxyflavone 3-rutinoside), and 3- and 4-methylcatechol were obtained from Aldrich Chemical Co., Inc., Milwaukee, WI. Regisil RC-2 [bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane] was obtained from Regis Chemical Co., Morton Grove, IL. Sucrose, fructose, glucose, and α-cellulose were procured from Sigma Chemical Co., St. Louis, MO. [^{14}C(U)]Cellulose from tobacco (10 μCi/mg), prepared by a modification of methods described for isolation of α-cellulose (Green, 1963), was obtained from New England Nuclear, Boston, MA, as were D-[^{14}C(U)]fructose (1.9 mCi/mg) and [^{14}C(U)]catechol (2.5 mCi/mmol).

Extraction of Tobacco. A preliminary series of experiments was performed with Type 16 low-nicotine Bright tobacco provided by the U.S. Department of Agriculture (Beltsville, MD). Ten-gram aliquots of tobacco were sonically dispersed with 30 mL of either *n*-hexane, benzene, chloroform, or methanol for 15 min. The solvent was removed and the sonication repeated twice with two additional 30-mL portions of solvent. The extracts were combined and concentrated.

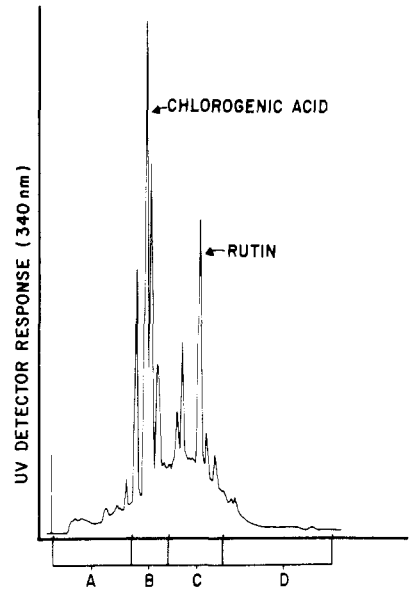

Figure 1. High-pressure liquid chromatogram of the methanol–H_2O extract of tobacco. Fractions A–D were collected for pyrolysis studies.

In a second series of experiments, 34 g of either Type 16 low-nicotine Bright tobacco or University of Kentucky 1R1 cigarette tobacco was extracted for 3 h with 750 mL of *n*-hexane in a Soxhlet extractor. Then the *n*-hexane was removed and the tobacco was extracted with 90% methanol in H_2O for 4 h. The extracts were concentrated to dryness under reduced pressure prior to pyrolysis. Aliquots equivalent to 3.5 g of tobacco were pyrolyzed.

Fractionation of Methanol–H_2O Extract to Fractions A–D. The 90% methanol extract (1.1 g) of low-nicotine Bright tobacco was suspended in 10 mL of methanol at 40 °C. Insoluble material was removed by passing the mixture through a C-18 Sep-PAK (Waters Associates, Milford, MA). The eluant was concentrated to 3 mL, and aliquots were injected on a 22.1 mm i.d. × 50 cm Whatman Magnum 20 ODS-2 column eluted with a program as follows: 100% 0.1 N KH_2PO_4 linear to 100% of 90% methanol–10% H_2O in 70 min at 7.5 mL/min. Fractions A–D were collected as indicated in Figure 1. Fractions were concentrated by rotary evaporation to remove most of the methanol. The remaining aqueous portion was lyophilized and the residue sonically dispersed with methanol to separate the tobacco constituents from the buffer. The methanolic solutions were then concentrated to dryness for pyrolysis.

Subfractions of Methanol–H_2O Fraction A. An aliquot of fraction A (197 mg) in 2 mL of H_2O was injected on a Partisil 10 ODS 3 Magnum 9 column (Whatman, Ann Arbor, MI), cooled in an ice bath, and eluted with H_2O at 1 mL/min. Subfractions A-1–A-5 were collected according to refractive index detection as indicated in Figure 2. The subfractions were lyophilized and the residues prepared for pyrolysis. To confirm the presence of sugars, aliquots of subfractions A-1–A-5 were reinjected on a 3.9 mm × 30 cm Waters Associates carbohydrate analysis column as previously described (Bell, 1975).

Pyrolysis Experiments. The pyrolysis apparatus consisted of a Lindberg Type 55035A electric furnace (Lindberg Co., Watertown, WI.) equipped with a 0.5 m × 2.2 cm (i.d.) Vycor combustion tube. The exit of the tube was connected to two 100-mL cold traps in series, cooled with dry ice–acetone, and finally connected to a 250-mL gas wash bottle containing 100 mL of 0.1 N HCl. The second cold trap was two-thirds filled with 7-mm glass

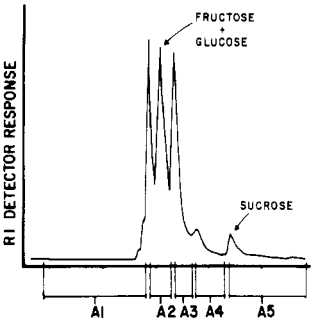

Figure 2. High-pressure liquid chromatogram of fraction A. Subfractions A-1–A-5 were collected for pyrolysis studies.

beads. Nitrogen (600 mL/min) was swept through the entire apparatus during pyrolysis. In experiments on cellulose pyrolysis, N_2–air mixtures were also used. Samples were introduced into the hot zone by means of a 14 cm × 2 cm (i.d.) quartz tube. Samples were pyrolyzed for 10 min.

Analysis of Pyrolysates for Catechol. The pyrolysis products in the two cold traps and in the pyrolysis tube were collected by rinsing with 200 mL of ether and 100 mL of 1 N HCl. The resulting mixture was combined with the 0.1 N HCl from the gas wash bottle, and [^{14}C(U)]catechol (1×10^5 dpm) was added. The layers were separated and the aqueous phase was washed once with an equal volume of ether. The ether layers were combined, dried (Na_2SO_4), and concentrated in vacuo to dryness. This residue was brought to a known volume (usually 2.0 mL) with acetonitrile. A 100-μL aliquot was removed, transferred to a 1-mL Reacti-Vial (Pierce, Rockville, IL), treated at 70 °C for 60 min with 400 μL of Regisil RC-2, and analyzed by GC. A second aliquot was counted to determine recovery.

Addition of Precursors to Cigarettes. [^{14}*C(U)*]*Cellulose.* Univeristy of Kentucky 1R1 cigarette tobacco (17.7 g) was spread in a 125 mm diameter recrystallizing dish and moistened with 2–3 mL of methanol. Three grams of the tobacco was removed and mixed throughly with [^{14}C-(U)]cellulose (125 mg), which had been ground to a fine powder with a glass tissue homogenizer. The resulting mixture was then thoroughly mixed with the remaining tobacco. The tobacco was allowed to dry at room temperature and then equilibrated at 60% relative humidity. An aliquot of the tobacco was sent to New England Nuclear, Boston, MA, for combustion analysis. Cigarettes were prepared and 10 were selected by weight and draw resistance (see Table IV).

[^{14}*C(U)*]*Fructose.* Cigarette tobacco (17.7 g) was spread in a recrystallizing dish and moistened by spraying with 2 mL of H_2O. A solution of 10 mg of [^{14}C(U)]fructose in 4 mL of H_2O was added to the tobacco, 100 μL at a time, with a syringe. During the addition, the tobacco was continually mixed manually. After the addition was complete, the tobacco was equilibrated to 60% relative humidity for 24 h. An aliquot of the tobacco was sent to New England Nuclear, Boston, MA, for combustion analysis. Ten cigarettes were prepared and selected for analysis according to weight and draw resistance (Table IV).

Chlorogenic Acid and Rutin. Levels of chlorogenic acid and rutin in 1R1 cigarettes were determined as described (Court, 1977; Snook and Chortyk, (1982). Tobacco from 30 1R1 cigarettes was placed in a 17.8 × 25.4 cm polyethylene tray and sprayed with 3.4 mL of MeOH containing varying amounts of either chlorogenic acid (210, 420, or 630 mg) or rutin (60, 120, or 210 mg). The tobacco was allowed to dry at room temperature and then equilibrated to 60% relative humidity. Cigarettes were prepared, and for each analysis, 10 cigarettes were selected according to weight and draw resistance as summarized in Table IV.

Smoking of Cigarettes. For each analysis, 10 weight and draw resistance selected cigarettes were smoked under standard conditions (one puff/min, puff duration 2 s, puff volume 35 mL, butt length 23 mm) in a laboratory maintained at a relative humidity of 60 ± 5% and at 22 ± 2 °C. The cigarettes to which ^{14}C-labeled precursors had been added were smoked on a machine in a fume hood. The mainstream smoke was led through a 250-mL cold trap cooled to −60 °C in a dry ice–ethylene glycol monomethyl ether bath. A filter holder with a 44-mm Cambridge CM-113 glass fiber filter pad was placed between the gas wash bottle and the vacuum pump.

Analysis of Cigarette Smoke. The cold trap, tubing, and Cambridge filter were washed thoroughly with a total of 150 mL of 0.1 N HCl and 150 mL of ether. For the chlorogenic acid and rutin experiments, [^{14}C(U)]catechol (1×10^5 dpm) was added to the washings as an internal standard. The two washings were shaken together in a separatory funnel. The layers were separated and the aqueous phase was washed with an additional 150 mL of ether. The ether layers were combined, dried (Na_2SO_4), and concentrated at reduced pressure. The residue was redissolved in CH_3CN (5.0 mL for chlorogenic acid and rutin experiments, 1.0 mL for fructose and cellulose experiments). An aliquot (100 μL for chlorogenic acid and rutin experiments; 200 μL for fructose and cellulose experiments) was treated with Regisil RC-2 (400 μL for chlorogenic acid and rutin experiments; 200 μL for fructose and cellulose experiments) at 70 °C for 60 min. For the chlorogenic acid and rutin experiments, 3-μL aliquots were analyzed for catechol by GC and another aliquot was used to determine recovery of [^{14}C(U)]catechol, which ranged from 80 to 90%. For the fructose and cellulose experiments, 3-μL aliquots were analyzed for catechol by GC, and recoveries were based on the average recoveries in the chlorogenic acid and rutin experiments.

To determine the specific activity of the [^{14}C]catechol formed from either [^{14}C(U)]fructose or [^{14}C(U)]cellulose, 20-μL aliquots of the silylated mixture were separated by GC and the peak corresponding in retention time to catechol was collected in a glass capillary tube cooled with dry ice by using a split ratio of 1/5 detector/heated collection port. The efficiency of collection was determined under the conditions of each experiment by injecting and collecting a known volume and amount of [^{14}C(U)]catechol. Efficiencies ranged from 50 to 80%. The glass capillary collection tube was rinsed alternately with five 10-μL aliquots of CH_3CN and 1% acetic acid in H_2O. Under these conditions, the bis(trimethylsilyl) derivative was rapidly hydrolyzed to catechol. The rinsings were collected in a 1-mL Reacti-Vial (Pierce Chemical Co.) for further purification by HPLC with a program as follows: 1% aqueous acetic acid to 100% methanol in 50 min with a flow rate of 1 mL/min. One-milliliter fractions were collected and analyzed by scintillation counting. Catechol was quantified by UV detection at 254 and 280 nm. The specific activity of the catechol was calculated. For the cigarettes treated with [^{14}C(U)cellulose], the reported values are the average of two determinations.

RESULTS

In preliminary experiments catechol formation during the pyrolysis of tobacco was found to be temperature dependent. At a pyrolysis temperature of 900 °C, 100 μg of catechol was formed/g of tobacco pyrolyzed. The corresponding levels of catechol per gram of tobacco pyrolyzed

Table I. Catechol in Pyrolysates of Tobacco and Its Extracts at 650 °C[a,b]

material pyrolyzed	weight of tobacco or extract pyrolyzed: weight, g		weight of tobacco or extract pyrolyzed: % of total[c]		μg of catechol in pyrolysate		% conversion to catechol[d]	
	Bright[a]	1R1	Bright[a]	1R1	Bright[a]	1R1	Bright	1R1
whole tobacco	3.5	3.5	100	100	2230	1590	0.064	0.045
hexane extract	0.15	0.16	4	5	5	n.d.[e]	0.003	
methanol-H_2O extract	1.1	1.1	31	31	2090	1900	0.19	0.18
extracted residue	2.5	2.2	71	63	2680	1000	0.11	0.046

[a] Mean of two experiments. [b] Low nicotine Bright tobacco or Kentucky 1R1 cigarette tobacco was extracted sequentially with hexane and methanol-H_2O and the extracts and residue were pyrolyzed. See Experimental Section for details. [c] Traces of solvent remained in the methanol-H_2O extract and in the extracted residue. [d] Percent conversion based on weight. [e] n.d. = not detected.

Table II. Catechol in Pyrolysates of Fractions A-D of the Methanol-H_2O Extract of Tobacco[a]

methanol-H_2O fraction pyrolyzed	weight, mg	% of total[b]	μg of catechol in pyrolysate	% conversion to catechol[c]
A	466	65	364	0.078
B	87	12	90	0.10
C	78	11	12	0.015
D	55	8	11	0.020

[a] The methanol-H_2O extract (1.1 g) of low-nicotine Bright tobacco (3.5 g) was fractionated by HPLC as illustrated in Figure 1. In addition to fractions A-D, 28 mg of material that was insoluble in the eluting solvent was removed prior to fractionation. Fractions A-D were pyrolyzed at 650 °C. [b] Corrected for 65% recovery in the fractionation. [c] Percent conversion based on weight.

were 560 and 720 μg at 750 and 650 °C, respectively. Pyrolysis of *n*-hexane, benzene, chloroform, or methanol extracts of tobacco revealed that significant amounts of catechol were formed only from the methanol extracts. Therefore, in subsequent experiments tobacco was extracted sequentially with *n*-hexane and methanol-H_2O and the extracts and residue were pyrolyzed at 650 °C. Catechol was identified by GC–MS, as were several other major phenolic constituents including phenol and *o*-, *m*-, and *p*-cresol. The levels of catechol in pyrolysates of tobacco, its hexane and methanol-H_2O extracts, and the extracted residue are summarized in Table I. These data indicate that precursors to catechol were present in the methanol-H_2O extract and in the extracted tobacco. Extracted Bright tobacco was a better pyrolytic precursor to catechol than was extracted 1R1 tobacco. In all other respects, the two tobacco types gave similar results in this experiment.

To investigate the precursors in the methanol-H_2O extract of Bright tobacco, it was fractionated by HPLC using a system that separates two of the major polyphenols of tobacco, chlorogenic acid, and rutin, as illustrated in Figure 1 (Court, 1977; Snook and Chortyk, 1982). The four fractions A–D were collected and pyrolyzed. The results are summarized in Table II. Although the sum of the catechol levels in fractions A–D did not equal that in the methanol-H_2O extract, it was evident that fraction A, which comprised 65% of the extract, contained important precursors. Therefore, fraction A was investigated in more detail.

We suspected that fraction A contained sugars because of its early retention time on reverse-phase HPLC, its relatively high weight contribution to the extract, and its minor UV absorbance. Fraction A was subfractionated by using a system similar to one designed for separation of carbohydrates (Heyrand and Rinaudo, 1980). Five subfractions A-1–A-5 were collected according to refractive index detection, as illustrated in Figure 2, and were pyrolyzed. The results are summarized in Table III. Subfraction A-2, which contained primarily fructose and glucose, comprised 53% of fraction A and was converted to catechol in 0.064% yield based on weight. Pyrolysis of a mixture of 140 mg of fructose and 140 mg of glucose gave 0.14 mg of catechol (0.05% conversion based on weight, 0.08% yield).

Table III. Catechol in Pyrolysates of Subfractions A-1–A-5 of Fraction A[a]

subfraction	weight, mg	% of total[b]	μg of catechol in pyrolysate	% conversion to catechol[c]
A-1	13	8	7.3	0.056
A-2	86	53	55.4	0.064
A-3	40	25	5.6	0.014
A-4	8	5	6.0	0.075
A-5	15	9	6.8	0.054

[a] Fraction A (197 mg) of the methanol-H_2O extract of tobacco was subfractionated by HPLC as illustrated in Figure 2 and the subfractions were pyrolyzed at 650 °C. [b] Corrected for 82% recovery in fractionation. [c] Percent conversion based on weight.

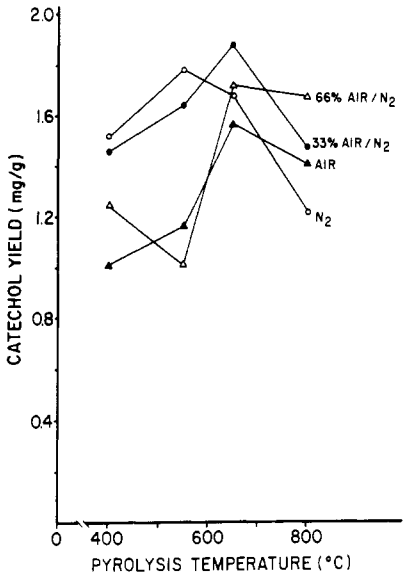

Figure 3. Formation of catechol upon pyrolysis of 1 g of α-cellulose under various conditions.

Since the extracted residue of tobacco was a major precursor to catechol, based on its mass and percent conversion to catechol, we pyrolyzed one of its constituents, cellulose, at various temperatures and in different N_2–air mixtures. The results are illustrated in Figure 3. The conversion of cellulose to catechol at 650 °C ranged from 0.16% to 0.19%, based on weight (0.26–0.31% yield per unit of glucose). Yields of catechol were not markedly

Table IV. Draw Resistance and Weights of Experimental Cigarettes Selected for Analysis[a]

cigarette type	draw resistance, mm	weight, g, ±0.02
[^{14}C(U)]cellulose	84 ± 13	1.18
[^{14}C(U)]fructose	71 ± 14	1.18
chlorogenic acid 0[b]	96 ± 10	1.13
chlorogenic acid 7	97 ± 10	1.20
chlorogenic acid 14	96 ± 6	1.25
chlorogenic acid 21	97 ± 9	1.25
rutin 0[c]	96 ± 10	1.13
rutin 2	94 ± 8	1.18
rutin 4	98 ± 6	1.18
rutin 7	95 ± 9	1.20

[a] Mean ± SD for 10 cigarettes. [b] 0 = amount of chlorogenic acid added (mg). [c] 0 = amount of rutin added (mg).

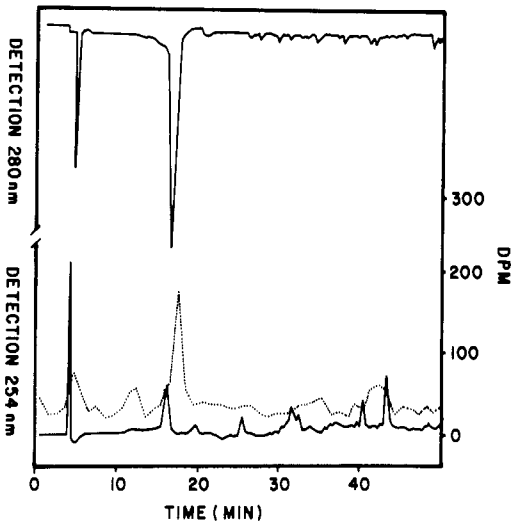

Figure 4. High-pressure liquid chromatogram of catechol isolated from the mainstream smoke of cigarettes enriched with [^{14}C-(U)]cellulose: (—) UV detection at 280 nm (upper trace) or 254 nm (lower trace); (···) detection by disintegrations per minute (dpm). Catechol eluted at 15.9 min; the radioactivity was detected 1.3 min later than UV absorption.

affected by the air concentration.

To determine the role of fructose as a precursor to catechol in mainstream smoke, [^{14}C(U)]fructose was added to 1R1 tobacco, and cigarettes were prepared and smoked. The draw resistance and weights of the cigarettes selected for this study as well as for the other smoking studies are summarized in Table IV. Bis(trimethylsilyl)catechol was isolated by collection from GC, hydrolyzed, and analyzed by HPLC. The specific activity of the catechol isolated by HPLC was 21 dpm/μg of catechol. The mainstream smoke contained 138 μg (2900 dpm) of catechol. According to combustion analysis, the tobacco used to prepare the cigarettes contained 4.4×10^7 dpm of [^{14}C(U)]fructose/g of tobacco. The cigarettes weighed 1.18 g (Table IV), but only 62 mm of the 85-mm cigarette was smoked. Thus, the amount of [^{14}C(U)]fructose in the tobacco column smoked was $4.4 \times 10^7 \times 1.18 \times (62/85)$ or 3.8×10^7 dpm. The yield of [^{14}C]catechol was, therefore, $(2.9 \times 10^3)/(3.8 \times 10^7) \times 100 = 0.008\%$.

The same procedure was used to determine the efficiency of conversion of [^{14}C(U)]cellulose in tobacco to [^{14}C]catechol in mainstream cigarette smoke. Figure 4 illustrates a chromatogram obtained by HPLC analysis of the [^{14}C]catechol isolated from these cigarettes. The average specific activity, based on two HPLC analyses, was 53 ± 8 dpm/μg of catechol. Since the mainstream smoke contained 121 μg of catechol/cigarette, the average level of radioactive catechol per cigarette was 6410 ± 970 dpm. The tobacco contained 3.70×10^7 dpm of [^{14}C(U)]cellulose/g or 3.2×10^7 dpm in the tobacco column smoked. Thus, the yield of [^{14}C]catechol was $(6.4 \times 10^3)/(3.2 \times 10^7) \times 100 = 0.02\%$.

Table V. Effects on Mainstream Smoke Catechol of Adding Chlorogenic Acid or Rutin to Cigarettes[a]

chlorogenic acid added, mg	Part A total chlorogenic acid per cigarette, mg	% increase in smoke catechol
0	7.8	b
7.0	14.9	13
14.0	21.8	26
21.0	28.8	40
rutin added, mg	**Part B total rutin per cigarette, mg**	**% increase in smoke catechol**
0	3.2	b
2.0	5.2	4
4.0	7.2	0
7.0	10.2	9

[a] 1R1 tobacco was sprayed with solutions of chlorogenic acid or rutin in methanol and cigarettes were prepared. [b] Mainstream smoke contained 230 μg of catechol/cigarette.

The effects of adding chlorogenic acid to cigarettes are summarized in Table V (A). A linear increase in mainstream smoke catechol was observed for each 7-mg increase in chlorogenic acid. The effects of adding rutin to cigarettes are summarized in Table V (B). No significant increase in the levels of mainstream smoke catechol was observed.

DISCUSSION

In this study, we used pyrolysis as an exploratory method to identify likely precursors to catechol. Despite its limitations, some of which are discussed below, pyrolysis can provide qualitative guidelines for potentially more reliable, but expensive, experiments in which radiolabeled precursors are added to cigarette tobacco. We focused only on those fractions that gave relatively high yields of catechol and were abundant in leaf. By these criteria, the methanol–H_2O extract and the extracted residue of tobacco can be considered as major pyrolytic precursors to catechol. These results are in agreement with previous studies in which ethanol extracts or extracted residues of Bright tobacco varieties have been shown to contain major pyrolytic precursors to catechol and several other phenols (Bell et al., 1966; Schlotzhauer et al., 1972, 1982; Schlotzhauer and Chortyk, 1981).

Qualitatively, the best precursors to catechol among the fractions of the methanol–H_2O extract were fractions A and B. Fraction A contained the tobacco leaf sugars—fructose, glucose, and sucrose—and fraction B the leaf polyphenol, chlorogenic acid. Another polyphenol, rutin, was concentrated in fraction C but the conversion of this fraction to catechol was only about one-sixth as efficient as in the pyrolysis of fraction B. The results of the pyrolyses of subfraction A-2, containing mainly fructose and glucose, and of subfraction A-5, containing sucrose, support the role of these sugars as pyrolytic precursors to catechol. Because of its relative abundance in leaf, the sugar fraction would appear to be an important extractable pyrolytic precursor to catechol, at least under our conditions.

In agreement with the results of the present study, Schlotzhauer and co-workers have emphasized the role of chlorogenic acid as a pyrolytic precursor to catechol (Schlotzhauer and Chortyk, 1981; Schlotzhauer et al., 1982). For example, the levels of chlorogenic acid were higher in several Bright tobacco types (1.6–2.1%) than in Burley types (0.2–1.0%), as were the levels of catechol higher in the Bright tobacco pyrolysates (Schlotzhauer et al., 1982). However, it should be noted that Bright tobacco varieties generally also have higher levels of reducing sugars (6–8%) than do Burley tobaccos (3–5%) (Gori, 1976).

The extracted tobacco residue, or marc, was also found to be an important pyrolytic precursor to catechol. Schlotzhauer and Chortyk attributed this partially to lignin by comparison of their results to those obtained upon pyrolysis of softwood lignin (Schlotzhauer and Chortyk, 1981). While we did not investigate the residue thoroughly in this study, we did observe that pyrolysis of cellulose, which typically comprises 5–12% of whole leaf (Bowman et al., 1973; Bokelman et al., 1983) and is a major component of its extracted residue, yielded relatively high levels of catechol. Several other components of the extracted tobacco residue that contain six carbon sugars or the corresponding uronic acids would also be expected to be good pyrolytic precursors to catechol. These include pectin (10.7% of Bright tobacco lamina), starch (3.2%), and hemicellulose (3.6–13.0%) (Bokelman et al., 1983).

The quantitative aspects of the pyrolysis experiments require further investigation. The total amounts of catechol obtained in the pyrolyses of the methanol–H_2O extracts and the extracted tobacco residues were approximately twice as great as those obtained in the pyrolyses of whole tobacco. Schlotzhauer and Chortyk observed a similar phenomenon although their recombined level of catechol was only 22% greater than that observed in the whole tobacco (Schlotzhauer and Chortyk, 1981). These observations may be due to the physical differences between whole tobacco and its extracts. In contrast to these results, the total amounts of catechol obtained in the pyrolyses of fractions A–D or subfractions A-1–A-5 were less than those obtained in the pyrolyses of the corresponding parent fractions, even after correction for losses in fractionation. This may have resulted from the use of successively smaller quantities in the pyrolyses. In addition, there could have been synergistic effects during pyrolysis that may have increased or decreased yields of particular components. Nevertheless, the results of the pyrolysis experiments did suggest that chlorogenic acid, sugars, cellulose, and possibly some related components of the extracted tobacco residue are likely leaf precursors to catechol in cigarette smoke.

The results of the smoking experiments support the use of pyrolysis as a qualitative indicator for leaf precursors to catechol. As in the pyrolysis studies, cellulose was found to be a relatively good precursor to smoke catechol. The lower percent conversion of cellulose to catechol in the smoking studies compared to that in the pyrolysis studies is in agreement with previous work, in which the levels of various other components generated from cellulose under smoking and pyrolysis conditions were compared (Sakuma et al., 1981).

The [^{14}C(U)]cellulose employed in this study was isolated by a procedure which, when applied to 1R1 tobacco, gave a cellulose value of 8.2%. Therefore, the tobacco column smoked contained 0.082 × 1.18 g × (62/85) = 0.071 g of cellulose or 71 mg/180 mg = 0.39 mmol of glucose units. Since 0.02% of [^{14}C(U)]cellulose was converted to [^{14}C]catechol, the amount of catechol formed from cellulose was 0.0002 × 0.39 mmol = 7.9×10^{-5} mmol or 9 μg. Since the mainstream smoke contained 121 μg of catechol, 7% was formed from cellulose. This calculation assumes that the [^{14}C(U)]cellulose used in this study contained only glucose units and that 1 mol of [^{14}C]catechol is theoretically formed/mol of glucose. When calculated on a weight basis, the amount of catechol formed from cellulose is 0.071 g × 0.0002 = 14 μg or 12% of the catechol in mainstream smoke.

Holocellulose accounts for 32% of 1R1 cigarette tobacco (Gori, 1977). Holocellulose is comprised of cellulose, hemicellulose, and probably significant amounts of related constituents that are obtained in the chlorite process used for its isolation (Wise et al., 1946; Green, 1963). Thus it is not appropriate to use the 32% value to calculate the contribution of cellulose to smoke catechol. However, we strongly suspect that hemicellulose, starch, and pectin would be good precursors to catechol and suggest that the appropriate studies with labeled precursors be carried out.

1R1 tobacco contains 4.6% of fructose, 2.4% of glucose, and 1.0% of sucrose (Oakley, 1973). The 62-mm tobacco column smoked in this study therefore contained 40 mg (0.22 mmol) of fructose. Since the conversion of fructose to catechol was 0.008%, 1.8×10^{-5} mmol, or about 2 μg, of the catechol in mainstream smoke originated from fructose. This accounts for approximately 1.4% of the catechol in smoke. In a previous series of experiments, we added [^{14}C(U)]fructose or [^{14}C(U)]glucose or [^{14}C(U)]sucrose to Type 16 low-nicotine Bright tobacco by the syringe injection technique (Carmella et al., 1980). By using methods similar to those described in this study, we determined that the percent conversions to catechol of the various sugars were as follows: fructose, 0.005; glucose, 0.006; sucrose, 0.004. These results are in good agreement with that obtained for [^{14}C(U)]fructose added to 1R1 tobacco and indicate that the conversions of the three sugars to catechol were similar. We estimate that the overall contribution of glucose, sucrose, and fructose to smoke catechol is approximately 4%. The lower contribution to smoke catechol of sugars as compared to that of cellulose parallels the results of the pyrolysis studies.

The role of chlorogenic acid as a precursor to catechol is more difficult to assess. Since [^{14}C]chlorogenic acid was not available, we enriched cigarettes with unlabeled chlorogenic acid. This is less desirable than using a labeled precursor because addition of milligram amounts of a compound could affect the burning characteristics of the cigarette. Nevertheless, in the cigarettes sprayed with chlorogenic acid, a linear increase in levels of smoke catechol was observed: approximately 13% for each 7 mg of chlorogenic acid added. By extrapolation to 0 mg of chlorogenic acid added, it can be calculated that approximately 30 μg of the 230 μg of catechol in the mainstream smoke of these cigarettes, or 13%, originated from chlorogenic acid. This could represent a minimum value because of the presence in tobacco of neochlorogenic acid and 4-*O*-caffeoylquinic acid, which also contain the catechol moiety (Snook and Chortyk, 1982).

In contrast to the results obtained when chlorogenic acid was added to tobacco, we did not observe a consistent increase in smoke catechol in cigarettes that had been enriched with rutin. From these results we conclude that leaf rutin is not an important precursor to smoke catechol.

A possible limitation of the present study was the relatively low mainstream smoke catechol levels of 121 and 138 μg/cigarette, respectively, in the cigarettes prepared from tobacco to which [^{14}C(U)]cellulose or [^{14}C(U)]fructose had been added. The mainstream smoke of the cigarettes

prepared for the chlorogenic acid and rutin studies contained 230 μg of catechol/cigarette, which was in good agreement with the value of 210 μg/cigarette that we obtained upon analysis of catechol in the mainstream smoke of machine-made 1R1 cigarettes. The lower catechol levels in the mainstream smoke of the ^{14}C-labeled cigarettes does not appear to have been caused by their somewhat lower draw resistance because in separate experiments we observed that a 20% difference in draw resistance had little effect on catechol levels. In order to obtain maximum specific activity in the tobacco to which we added [^{14}C-(U)]cellulose or [^{14}C(U)]fructose, we used less tobacco than for the preparation of the cigarettes sprayed with chlorogenic acid or rutin. Therefore, when we prepared cigarettes from the ^{14}C-labeled tobacco, we had to incorporate a greater percentage of the smaller particles generated during manipulation of the tobacco. This may have affected the mainstream smoke catechol levels. The results of this study might also have been influenced by differing distributions in the tobacco of the naturally occurring precursors compared to those which we added.

CONCLUSIONS

Pyrolysis studies showed that the methanol–H_2O extract of tobacco and the extracted tobacco residue were good precursors to catechol. Individual components of these fractions that are good pyrolytic precursors to catechol are fructose, glucose, chlorogenic acid, and cellulose. Smoking studies agreed qualitatively with the pyrolysis experiments. Estimated minimum contributions to mainstream smoke catechol based on our results can be summarized as follows: total of fructose, glucose, and sucrose, 4%; cellulose, 7–12%; chlorogenic acid, 13%. We suggest that a significant portion of the remaining catechol in mainstream smoke is formed from pectin, starch, and hemicellulose.

Registry No. Catechol, 120-80-9; fructose, 57-48-7; glucose, 50-99-7; sucrose, 57-50-1; chlorogenic acid, 327-97-9; cellulose, 9004-34-6; rutin, 153-18-4.

LITERATURE CITED

Bell, J. H. *Recent Adv. Tob. Sci.* **1975**, *1*, 51.
Bell, J.; Saunders, A. O.; Spears, A. W. *Tob. Sci.* **1966**, *10*, 138.
Bokelman, G. H.; Ryan, W. S., Jr.; Oakley, E. T. *J. Agric. Food Chem.* **1983**, *31*, 897.
Bowman, D. R.; Tso, T. C.; Chaplin, J. F. *Tob. Sci.* **1973**, *17*, 8.
Brunnemann, K. D.; Lee, H. C.; Hoffmann, D. *Anal. Lett.* **1976**, *9*, 939.
Carmella, S. G.; Hecht, S. S.; Hoffmann, D., Abstracts of the 34th Tobacco Chemists Research Conference, 1980, p 16.
Court, W. A. *J. Chromatogr.* **1977**, *130*, 287.
Gori, G. B., Ed. *DHEW Publ. (NIH) (U.S.)* **1976**, *No. NIH 76-111*, 24.
Gori, G. B., Ed. *DHEW Publ. (NIH) (U.S.)* **1977**, *No. NIH 77-1280*, 38.
Green, J. W. *Methods Carbohydr. Chem.* **1963**, *3*, 9.
Hecht, S. S.; Carmella, S.; Mori, H.; Hoffmann, D. *JNCI, J. Natl. Cancer Inst.* **1981**, *66*, 163.
Heyrand, A.; Rinaudo, M. *J. Liq. Chromatogr.* **1980**, *3*, 721.
Hoffmann, D.; Schmeltz, I.; Hecht, S. S.; Wynder, E. L. In "Polycyclic Hydrocarbons and Cancer"; Gelboin, H. V.; Ts'o, P. O. P., Eds.; Academic Press: New York, 1978; p 85.
Oakley, E. T. *Tob. Sci.* **1973**, *17*, 99.
Sakuma, H.; Munakata, S.; Sugawara, S. *Agric. Biol. Chem.* **1981**, *45*, 443.
Schlotzhauer, W. S.; Chortyk, O. T. *Tob. Sci.* **1981**, *25*, 6.
Schlotzhauer, W. S.; Higman, E. B.; Schmeltz, I. In "The Chemistry of Tobacco and Tobacco Smoke"; Schmeltz, I., Ed.; Plenum Press: New York, 1972; p 65.
Schlotzhauer, W. S.; Martin, R. M.; Snook, M. E.; Williamson, R. E. *J. Agric. Food Chem.* **1982**, *30*, 372.
Snook, M. E.; Chortyk, O. T. *Tob. Sci.* **1982**, *25*, 6.
U.S. Department of Health and Human Services *DHHS Publ. (PHS)* **1982**, *No. PHS 82-50179*.
Van Duuren, B. L.; Goldschmidt, B. M. *J. Natl. Cancer Inst. (U.S.)* **1976**, *56*, 1237.
Wise, L. E.; Murphy, M.; D'Addicco, A. A. *Pap. Trade J.* **1946**, *122*, 35.

Received for review March 28, 1983. Revised manuscript received November 28, 1983. Accepted December 9, 1983. This study was supported by Contract 53-32U4-9-186 from the U.S. Department of Agriculture. Mention of a trademark or proprietary product does not consistitute 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 that may also be suitable. These data were presented in part at the 34th, 35th, and 36th Tobacco Chemists Research Conferences, Richmond, VA, Oct 1980, Winston-Salem, NC, Oct 1981, and Raleigh, NC, Oct 1982. This paper is no. LXXVII in the series "Chemical Studies on Tobacco Smoke".