

# Composition Studies on Tobacco. XVII. Steam-Volatile Acidic Fraction of Cigarette Smoke

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Past studies have described differences in the steam-volatile neutral (Burdick *et al.*, 1963) and acidic (Schmeltz *et al.*, 1963) components of tobacco types and grades and in the neutral components of blended and unblended cigarette smoke (Burdick and Stedman, 1963). Characteristic patterns of volatile acids were found in the leaves of the four cigarette tobacco types, including distinctive distributions of isomeric acids in the C<sub>4</sub>-C<sub>7</sub> range.

The principal objective of the present work was to study the isomeric distribution of the above acids in blended and unblended cigarette smoke. Buyske *et al.* (1957) have described a method for determining volatile acids in cigarette smoke and presented quantitative values for unblended bright, burley, and Turkish cigarettes. Although the method insures against losses due to volatility, separation of the isomeric acids in the C<sub>4</sub>-C<sub>7</sub> range is not obtained. Since isomeric separation is obviously essential, a gas chromatographic method was devised to study the smoke of blended and unblended cigarettes.

Since the adopted method permitted concurrent determination of phenols, data on these components are also included herein. Although great interest exists in smoke phenols (Hoffman and Wynder, 1963; Spears, 1963a, b), only Rayburn *et al.* (1953)

have studied the phenols in smoke from unblended bright, burley, Maryland, and Turkish cigarettes. A colorimetric method was employed by these workers. The present report describes correlative data on the smoke phenols of cigarette tobaccos before and after blending.

## Experimental

*Cigarettes and Fractionating Procedure:*—The cigarettes, their physical characteristics, smoking conditions, and the initial fractionation of the condensates have been previously described (Burdick and Stedman, 1963). In general, the smoke condensate from the cooled traps was dissolved by successive washings with ether and 5% aqueous NaOH. Bases and acids were removed from the pooled ether extract by successive extraction with 1N aqueous HCl and 5% aqueous NaOH. The latter was combined with the alkaline extracts used to wash the traps. The pH of the combined alkaline extract was adjusted to 1.0 with H<sub>2</sub>SO<sub>4</sub>. After saturating with NaCl, the acidified solution was repeatedly extracted with ether (total vol, 250 ml) and all ether extracts were combined.

The combined ether extract containing acidic substances was steam distilled, and both ether and distillate (500 ml) were collected in a cooled receiver. NaCl was added to the ether-distillate mixture until the aqueous layer was saturated and, after vigorous shaking, the layers were separated. The aqueous layer was extracted repeatedly with ether

and all ether extracts were combined (total vol, 400 ml). The combined ether extract was dried over MgSO<sub>4</sub> and evaporated to a volume of 0.5 ml on the Stedman and spinning band columns (Burdick *et al.*, 1963). This concentrate was analyzed for volatile acids. For the phenols, the concentrate was subsequently diluted to 20 ml with ether and extracted with saturated NaHCO<sub>3</sub> solution (3 times, 15 ml total). The ether solution was then dried over MgSO<sub>4</sub> and the volume was reduced to 0.5 ml for analysis of phenols.

*Gas Chromatography:*—Volatile acids were studied by the methods previously outlined (Schmeltz *et al.*, 1963). Qualitative studies were made on the Tween<sup>2</sup>-H<sub>3</sub>PO<sub>4</sub> and diethylene glycol adipate polyester-H<sub>3</sub>PO<sub>4</sub> (DEGA) columns; in most cases quantitative measurements were made on the DEGA column with flame ionization detection, using peak height as a measure of quantity. Various volumes of the 0.5 ml concentrates were injected to yield peaks which did not exceed the width of the chart paper at 1 mv full scale deflection. If necessary, dilutions of the concentrate were made with ether. Standard curves of concentration versus peak height for authentic acids were used for determining concentrations of unknowns.

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For the determination of phenols, a 0.25 in. dia. 8 ft column of 25% di-n-octyl sebacate on Gas Chrom P (60-80 mesh) was employed at 180°C and a helium flow rate of 60 ml per minute with thermal conductivity detection. Quantitative measurements were made in the same manner as the acids.

**Calculation of Data:**—All values are reported as either mg acid per 105 cigarettes or  $\mu\text{g}$  phenol per cigarette based on smoking 55% of the length of each cigarette using calculations discussed earlier (Burdick and Stedman, 1963).

**Identifications:**—Acids were identified by gas cochromatography with knowns on the above two columns, ultraviolet and infrared spectra, and paper chromatographic separations with several solvents in a manner similar to the leaf acids (Schmeltz *et al*, 1963). Phenols were identified by cochromatography on two columns (Osman *et al*, 1963) and by infrared and ultraviolet spectral comparisons with known compounds.

## Results and Discussion

Figure 1 is a chromatogram of the volatile acidic fraction from Turkish cigarette smoke. Although very similar to the corresponding fraction of leaf (Schmeltz *et al*, 1963), certain differences are noted. Peak 16 in Figure 1 contains mostly phenol and o-cresol, which are essentially absent in the leaf, and small amounts of n-caprylic acid. Peak 17 is isomeric

xyleneols or m- or p-cresol, all of which are also absent in the leaf, and pelargonic acid. Peaks 5a and 6a are present in very small amounts in the leaf compared to smoke. A small peak eluting after peak 17 and containing 3, 5-xyleneol or m- or p-ethylphenol is not shown in Figure 1.

Cochromatography of the acidic fractions from the smoke of the unblended bright, burley, Maryland, and Turkish cigarettes showed that the peaks chromatographed in an identical manner. Thus, the volatile, strongly acidic fraction of smoke differs from the neutral fraction in that the former does not contain large numbers of components formed during burning and not present in the leaf.

Quantitative differences among the smoke condensates were apparent. Recovery experiments with known acids indicated that recoveries were low (10-30%) with the  $\text{C}_1\text{-C}_8$  acids but were satisfactory (70-100%) for quantitative comparisons of the higher acids in the range in which distribution of isomers ( $\text{C}_4\text{-C}_7$ ) is important. The lack of sensitivity of formic acid in flame ionization detection and the difficulties of resolving formic and acetic or propionic (Schmeltz *et al*, 1963) with thermal conductivity detection did not permit determination of this acid. Buyske *et al* (1957) have reported quantitative values for the  $\text{C}_1\text{-C}_8$  acids in smoke condensates.

Table 1 shows the values obtained with the condensates from blended

and unblended cigarettes. The data are given as ratios of acid levels using the value for the unblended bright cigarettes as a reference. The decreasing order of total acids in the  $\text{C}_4\text{-C}_7$  range was Turkish, bright, Maryland, and burley for the unblended cigarettes. Table 2 shows pertinent isomeric distributions of acids among the samples. The tendency for higher proportions of branched-chain to normal acids in the  $\text{C}_4\text{-C}_7$  range of the Turkish smoke condensate compared to bright parallels previous findings of the acids of leaf. The relative patterns of distributions in the blended cigarettes reflect the blending of types having different proportions of isomers. This is especially evident in the case of Turkish tobacco, which contributes a large amount of  $\beta$ -methylvaleric acid to the blend.

Table 3 shows a comparison of observed and "calculated" ratios of levels of identified acids for condensates of the blended cigarettes. The calculated ratios are essentially the ratios expected if each individual tobacco in a blend contributed its acids additively to the total. For most acids, the observed ratios are higher than the calculated ratios for the blend containing Turkish. The reverse is true for the blend without Turkish, although the differences between observed and calculated ratios are relatively small. In general, this alteration of an additive effect through blending parallels previous findings on the volatile neutral

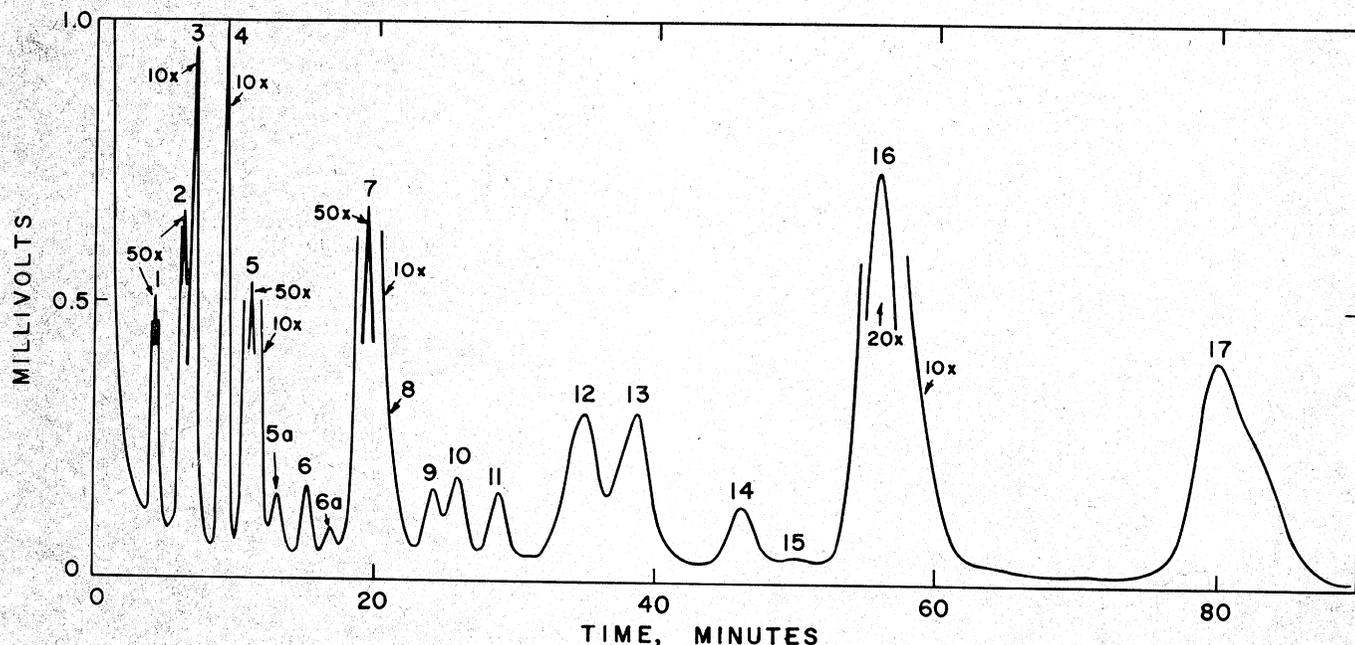


Figure 1. Chromatogram of volatile acid fraction of Turkish cigarette smoke (diethylene glycol adipate polyester- $\text{H}_3\text{PO}_4$  column, 125°C, 40 ml helium per min). Peak identities: peak (1) acetic; (2) propionic; (3) isobutyric; (4) n-butyric; (5) isovaleric; (6) n-valeric; (7)  $\beta$ -methylvaleric; (9) n-caproic; (13) n-heptylic; (16) phenol, o-cresol, and n-caprylic acid; (17) m-, p-cresol isomeric xyleneols and n-pelargonic acid. Peaks 5a, 6a, 10-12, and 14-15 are unknowns. Peak 8 is isocaproic which is resolved from  $\beta$ -methylvaleric in all smoke condensates except Turkish.

**Table 1. Quantitative differences in certain volatile acids of smoke condensates from various blended or unblended cigarettes.**

Acid or unknown peak no.	Mg per 105 cigarettes** (bright)	Ratios of amounts*					
		F	B	M	T	FBM	FBMT
Isobutyric	2.4	1.0	.78	.71	1.03	.74	1.17
n-Butyric	4.6	1.0	.36	.53	.58	.46	.87
Isovaleric	5.9	1.0	.84	.88	1.81	.77	1.63
Peak 5a	0.6	1.0	.38	.77	.63	.50	.94
n-Valeric	1.0	1.0	.38	.46	.50	.61	.68
Peak 6a	0.2	1.0	.37	.57	.62	.89	1.11
$\beta$ -Methylvaleric	2.6	1.0	.70	.76	8.7	.70	3.93
Isocaproic	1.7	1.0	.64	.90	—	.80	—
n-Caproic	0.6	1.0	.58	1.07	.97	.75	1.23
Peak 10	0.9	1.0	.48	.97	1.00	.73	.96
Peak 11	0.9	1.0	.17	.91	.78	.68	.77
Peak 12	3.0	1.0	.35	.83	.95	.62	.90
n-Heptylic	5.0	1.0	.46	.68	.60	.65	1.23
Peak 14	1.2	1.0	.12	1.32	.72	.74	1.02

\* Values for  $\frac{\text{mg per 105 cigarettes of indicated composition}}{\text{mg per 105 unblended bright cigarettes}}$ . Weights were based on smoking 55% of cigarette length. F=bright, B=Burley, M=Maryland, T=Turkish, FBM=blend (60% bright, 35% burley, 5% Maryland), FBMT=blend (40% bright, 35% burley, 5% Maryland, 20% Turkish).  
 \*\* Total weights (mg per 105 cigarettes) for indicated acids were as follows: Turkish, 48.3; bright, 30.6; Maryland, 23.7; burley, 16.5; FBM blend, 20.5; FBMT blend, 40.5. Unidentified acids were calculated as n-caproic acid.

**Table 2. Distribution of C<sub>4</sub>-C<sub>6</sub> isomers in various smoke condensates.**

Cigarette composition	Relative distribution of isomeric acids in smoke*		
	i-C <sub>4</sub> n-C <sub>4</sub>	i-C <sub>5</sub> n-C <sub>5</sub>	BMV n-C <sub>6</sub>
Bright	1.0	1.0	1.0
Burley	2.2	2.2	1.2
Turkish	1.8	3.6	9.0
Blend with Turkish**	1.3	2.4	3.2
Blend without Turkish**	1.6	1.3	.93

\* i-C<sub>4</sub> = Ratio of amount of isobutyric acid (from Table 1)

n-C<sub>4</sub> = Ratio of amount of n-butyric acid (from Table 1)

C<sub>5</sub> = valeric, C<sub>6</sub> = caproic, BMV =  $\beta$ -methylvaleric.

\*\* See footnote, Table 1, for blend compositions.

**Table 3. Percentage difference between calculated and observed ratios of volatile acid levels in condensates of blended cigarettes.**

Acid	Percentage difference*	
	FBM	FBMT
Isobutyric	-19	+27
n-Butyric	-39	+30
Isovaleric	-17	+48
n-Valeric	-19	+3
$\beta$ -Methylvaleric	-21	+62
Isocaproic	-8	—
n-Caproic	-12	+45
n-Heptylic	-18	+71

\* Percentage difference =  $(100 \times \frac{\text{observed ratio}}{\text{calculated ratio}}) - 100$ . Calculated ratios obtained from known percentage composition of blends (see Table 1) and ratios for bright, burley, Maryland, and Turkish cigarettes in Table 1. Observed ratios are in Table 1. Isocaproic acid is not resolved in blended or unblended Turkish cigarette smoke condensates (see Figure 1).

components of smoke (Burdick and Stedman, 1963). However, both blends showed an antagonistic effect in the latter case.

Since the four tobacco types possess distinctly different pH, the possibility was considered that the different responses of the two blends in respect to volatile acids was a reflection of pH alteration on blending. Determination of the pH of aqueous suspensions of the blends showed the two to be identical. However, *in situ* differences in pH may exist which are not detectable by such measurements and which may contribute to the observed effects.

Values for the major phenols are presented in Table 4. The levels are generally within the range reported by other workers, including Rayburn *et al* who studied unblended cigarettes. No consistent pattern of suppression or enhancement of the phenols on blending the types is evident. The blend without Turkish contained slightly less phenol than the other blend.

### Summary

The steam-volatile strongly and weakly acidic fractions of smoke from unblended and blended cigarettes were studied. Qualitatively, the strongly acidic fraction was generally similar to the corresponding fraction of tobacco leaf. Important differences in the distribution of C<sub>4</sub>-C<sub>6</sub> isomeric acids were noted in the various types of smoke. The con-

tribution of  $\beta$ -methylvaleric acid from the Turkish tobacco in blended cigarettes was especially significant. The inclusion of Turkish tobacco in the blend caused an enhancement of the expected  $C_4$ - $C_7$  acid levels on burning. Data are presented on the major volatile phenols of smoke from unblended and blended cigarettes.

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**Table 4. Levels of phenols in smoke of blended and unblended cigarettes.**

Compound	Micrograms per cigarette						Blending effect**	
	F*	B	M	T	FBM	FBMT	FBM	FBMT
Phenol	179	79	141	98	100	121	-30	-5
o-Cresol	28	14	18	20	21	20	-9	-5
m- and/or p-Cresol	47	30	51	48	42	52	0	+21
m-, p-Ethylphenol or 3,5-xyleneol	27	15	18	25	31	25	+24	+14

\* See footnote, Table 1, for designations. Di-n-octyl sebacate column operated as described by Osman et al (1963).

\*\*  $(100 \times \frac{\text{observed level}}{\text{calculated level}}) - 100$ . Calculated level for blends obtained from known percentage composition of blends (see footnote, Table 1) and observed levels for unblended types. Small amounts of 2,4-(and/or 2,5-) xyleneol (7-15 micrograms) were also found in the smoke condensates.

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