

THE VOLATILE BASES OF CIGAR SMOKE 2119

Abstract—The following pyridine bases were isolated from cigar smoke condensate using temperature programmed gas chromatography: pyridine; α -, β -, and γ -picoline; 2,3-, 2,4-, 2,5-, and 2,6-lutidine; 3-ethylpyridine; 3-vinylpyridine; nicotine; nornicotine; myosmine, and 2,3'-dipyridyl. Tentative identifications were also obtained for 3,5-lutidine and 3-acetylpyridine. Semiquantitative measurements on the amounts of these compounds present in the smoke condensate were obtained.

INTRODUCTION

RECENT publications from this laboratory have been concerned with the volatile acidic and neutral fractions¹⁻³ as well as the non-volatile acids⁴ of cigar smoke. In a continuation of this work the volatile bases[§] have now been studied and are the subject of this report. Whereas the alkaloids present in tobacco smoke have been studied quite extensively, e.g. the work of Kuffner *et al.*,⁵ relatively little has been reported on the other bases therein. Although a number of bases of smoke have been isolated by a variety of procedures, none of these methods was considered satisfactory for the qualitative and semiquantitative comparisons desired in the present study. These previously used methods were generally based on paper chromatography (usually of the derivatives) and permitted the analysis of only a relatively few compounds at a time. In 1959 Quin⁶ reported the separation of a number of alkaloids and alkyl pyridyl ketones by gas chromatography. We have now extended the use of gas chromatographic analysis to include the more volatile bases through the technique of temperature programming. Using this method a number of compounds in the basic fraction of the smoke condensate were isolated and identified including some that, to our knowledge, have not previously been reported. In addition, semiquantitative data on the amounts of many of these compounds in the smoke condensate are presented for the first time.

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§ Arbitrarily defined as those bases which are eluted in less than 2 hr under the chromatographic conditions outlined in the Experimental.

¹ A. I. SCHEPARTZ, *Tobacco Sci.* **4**, 12 (1960).

² I. SCHMELTZ and W. S. SCHLOTZHAUER, *Tobacco Sci.* **5**, 92 (1961).

³ S. OSMAN, I. SCHMELTZ, H. C. HIGMAN and R. L. STEDMAN, *Tobacco Sci.* **7**, 141 (1963).

⁴ I. SCHMELTZ and W. S. SCHLOTZHAUER, *Tobacco Sci.* **6**, 88 (1962).

⁵ F. KUFFNER, K. SCHICK and H. BÜHN, *Monatsh.* **87**, 749 (1956).

⁶ L. D. QUIN, *J. Org. Chem.* **24**, 911 (1959).

RESULTS AND DISCUSSION

The isolation method for the bases (see Experimental) was designed to reduce losses due to volatility and unfavorable partitioning effects. Control experiments indicated that, in general, recoveries were in the order of 60–70% for the identified compounds. It should be noted that known tobacco bases of high volatility, e.g., methylamine, or of relatively lower solubility in ether, e.g., nicotinamide, will not be isolated by this procedure.

A chromatogram of the volatile basic fraction for which the conditions are given in the Experimental section, is shown in Fig. 1. The first 20 min of the chromatogram which contains three solvent and two unidentified peaks has been deleted. The compounds identified are as follows: 6, pyridine; 7, α -picoline; 9, 2,6-lutidine; 10, β - and γ -picoline; 11, 2,4- and 2,5-lutidine; 12, 2,3-lutidine; 13, 3-ethylpyridine; 15, 3-vinylpyridine; 21, nicotine; 26, nornicotine; 27, myosmine; and 29, 2,3'-dipyridyl. Resolution of nornicotine and myosmine was not observed when either alkaloid was present in large amounts compared to the other, which

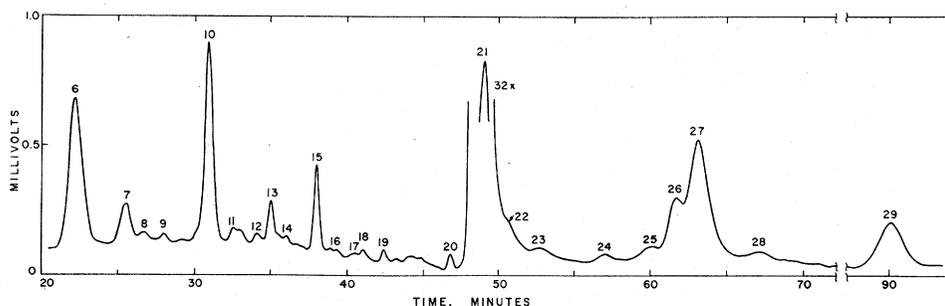


FIG. 1. VOLATILE BASES OF CIGAR SMOKE.

Except for peak 26, the numbered peaks were common to all chromatograms.

Identification as follows: 6, pyridine; 7, α -picoline; 9, 2,6-lutidine; 10, β - and γ -picoline; 11 (a doublet), 2,4- and 2,5-lutidine; 12, 2,3-lutidine; 13, 3-ethylpyridine; 15, 3-vinylpyridine; 21, nicotine; 26, nornicotine; 27, myosmine; 29, 2,3'-dipyridyl. Tentatively, 14 is 3,5-lutidine, and 20, 3-acetylpyridine. Remaining peaks unknown.

occurred in most instances. Most of the above identifications were established by co-chromatography, paper co-chromatography and i.r. and u.v. spectral comparisons with known compounds. Lack of authentic 2,3'-dipyridyl prevented corroboration of the peak identity by gas and paper chromatography; however, the i.r. and u.v. spectra were comparable to those reported by Onishi.⁷ Peaks 14 and 20 have tentatively been identified as 3,5-lutidine and 3-acetylpyridine, respectively, by gas co-chromatography. The remaining peaks in the chromatogram have yet to be identified; a number of these exhibit carbonyl absorption in their i.r. spectra and lack characteristic amino or pyridyl absorption. Onishi⁷ has also reported the presence of carbonyl contaminants in the basic fraction.

This is the first report, to our knowledge, of the presence of 2,4-, 2,5-, 2,6-, and 3,5-lutidines in tobacco smoke condensate. The presence of 3-vinylpyridine and 3-ethylpyridine in cigarette smoke condensate has been mentioned by Glock and Wright,⁸ however, experimental details have not been published to date. The other compounds have previously been reported in smoke condensate although quantitative data are lacking for most of them.

Table 1 contains the levels of these bases found in the smoke condensate for two types of

⁷ I. ONISHI and K. YAMASCKI, *Bull. Agr. Chem. Soc. Japan* **21**, 177 (1957).

⁸ E. GLOCK and M. P. WRIGHT, Abstracts, 16th Tobacco Chemists' Research Conference, Richmond, Virginia, September 1962.

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commercial cigar. These values were obtained using area-concentration curves of authentic samples in the usual way. One value for both myosmine and nornicotine is given in the table as two distinct peaks were not apparent in either case, presumably due to relatively small amounts of nornicotine as compared to myosmine. The composition of the β - and γ -picoline peak was estimated to be about 90% β -picoline by i.r. analysis, using the bands at 1580 cm^{-1}

TABLE 1. LEVELS OF BASES PRESENT IN CIGAR SMOKE CONDENSATE*

Compound	Cigar A†		Cigar B†	
	($\mu\text{g}/\text{cigar}$)	($\mu\text{g}/\text{g tob. smoked}$)	($\mu\text{g}/\text{cigar}$)	($\mu\text{g}/\text{g tob. smoked}$)
Pyridine	218	43.6	170	40.2
α -Picoline	51	10.2	38	8.9
β - and γ -Picoline	92	18.4	69	16.1
2,6-Lutidine	10	2.0	7	1.6
2,5-Lutidine	4	0.8	4	0.9
2,4-Lutidine	13	2.6	10	2.3
2,3-Lutidine	trace	—	trace	—
3-Ethylpyridine	32	6.4	8	1.8
3-Vinylpyridine	21	4.2	18	4.2
Nicotine	2150	430	1780	420
Nornicotine and myosmine	28	5.6	20	4.7
2,3'-Dipyridyl	40	8.0	37	8.6

* Uncorrected for losses during isolation (see text).

† Both types of cigar were of perfecto shape and contained domestic fillers. The cigars (15 in all of each) were all 13 cm in length.

and 1600 cm^{-1} for β - and γ -picoline, respectively. It should be emphasized that the values cited herein should be considered semiquantitative in nature and are presented to illustrate the ranges for the volatile bases found in cigar smoke condensate when the above conditions are employed.

EXPERIMENTAL

The smoking machine and smoking conditions used in obtaining the smoke condensate have been described previously.^{2, 3, 9} The condensate obtained from smoking approximately 400 cigars was used for identification purposes but analytical data were obtained on the condensate of fifteen cigars.

Isolation of the Basic Fraction from the Total Smoke Condensate

The condensate was removed from the traps by successive washings with ether (150 ml total per 15 cigars) and 0.1 N HCl saturated with NaCl (50 ml total); the ether solution was further extracted with 50 ml of 0.1 N HCl. Both acidic solutions were combined and continuously extracted with ether to remove non-basic contaminants; the purified aqueous solution was adjusted to pH 11.0 with 0.1 N NaOH. The free bases were removed from the aqueous solution by continuous ether extraction under good reflux. The ether extract was dried over anhydrous sodium sulfate and then concentrated to 1.0 ml by distillation of the

⁹ A. I. SCHEPARTZ, *Tobacco Sci.* 3, 144 (1959).

solvent through a spinning band column. Aliquots of this sample were analyzed by gas chromatography.

Gas Chromatographic Conditions

An Aerograph* Model A-350 equipped with dual thermal conductivity cells was used. The columns (10 ft \times 0.25 in.) were packed with Chromosorb W (60–80 mesh) containing 20% Carbowax 20M. The chromatograph was operated isothermally at 100° column temperature for 20 min, programmed at 4°/min to 240° and then maintained at 240° for the remaining portion of the chromatogram. The detector and injector temperatures were maintained at 260° throughout the analysis. The same conditions were employed for obtaining qualitative and quantitative data.

Identification of Chromatographic Peaks

Preliminary identifications were made by co-chromatography of authentic samples with the basic fraction from the smoke condensate. Eluates corresponding to the peaks in the chromatogram were then collected in U-shaped tubes submerged in a solid CO₂–acetone bath. Infrared spectra were obtained on a Perkin–Elmer 237 Infracord by pressing an aliquot (ca. 1.0 μ l) between sodium chloride plates. In all cases the sample was either liquid or semi-solid at room temperature. Ultra-violet spectra were determined on eluates collected in 0.1 N HCl solutions using a Perkin–Elmer Model 202 Spectrophotometer. Paper chromatography was run according to the procedure described by Brandsch.¹⁰ The bases were chromatographed as the corresponding hydrochlorides and were developed on Whatman Paper No. 1 with butanol saturated with 2 N HCl.

Quantitative Analysis

When possible, area concentration curves were derived for the identified compounds in the chromatogram using the corresponding authentic sample. Although the supply of authentic 3-vinylpyridine was sufficient for identification work using chromatographic and spectrophotometric methods, there was insufficient material to weigh accurately for preparation of a solution of known concentration; therefore, the amounts of 3-vinylpyridine were calculated from the area-concentration curve for 3-ethylpyridine. Similarly, the levels of 2,3'-dipyridyl were calculated from the curve for myosmine. Areas were measured by triangulating the chromatographic peaks with suitable corrections for background elution.

Recovery Experiments

A solution of authentic compounds was subjected to the isolation procedure described above. The recoveries were as follows: pyridine, 72%; α -picoline, 78%; β -picoline, 85%; 2,4-lutidine, 82%; nicotine, 70%; and myosmine, 63%.

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* Mention of a specific commercial product does not constitute an endorsement by the USDA over similar items not mentioned.

¹⁰ J. BRANDSCH, *Rev. Chim. (Bucharest)* **9**, 334 (1958).