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IDENTIFICATION OF AROMATIC ALDEHYDES IN CIGARETTE SMOKE* AND IN TOBACCO

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Abstract—The following free phenolic aldehydes have been identified in the particulate phase of smoke from cigarettes: *p*-hydroxybenzaldehyde, *m*-hydroxybenzaldehyde, vanillin, and syringaldehyde. These aldehydes together with protocatechualdehyde and 5-hydroxymethylfurfural have also been identified in the smoke from cigarettes prepared without the usual flavorings or other additives, and in the ether and ethyl acetate extracts of tobacco from cigarettes.

INTRODUCTION

RECENTLY, Yang and Wender[1] have reported finding 5-hydroxymethylfurfural and protocatechualdehyde in smoke from cigarettes and have indicated the presence of four other aldehydes in the particulate phase of the mainstream smoke of these cigarettes. This paper describes the isolation and identification of these remaining four aldehydes.

RESULTS AND DISCUSSION

An "aldehyde fraction" was isolated from the mainstream smoke of cigarettes by extracting with 2 M sodium bisulfite solution the ether and ethyl acetate solubles of the particulate phase collected on a "Cambridge" filter. After separation and purification by paper chromatography, *p*-hydroxybenzaldehyde, *m*-hydroxybenzaldehyde, vanillin, and syringaldehyde have been identified in this fraction. Similar studies on the "aldehyde fraction" from the smoke of 1000 cigarettes especially prepared by the manufacturer so as to contain no flavorings or other common additives, revealed the presence of all six aldehydes found in the smoke from regular cigarettes.

The "aldehyde fraction" from the extracts of tobacco was found to be slightly different in chromatographic pattern than that from the smoke. The compounds, other than those identified in this paper, which persisted in the "aldehyde fraction" from tobacco itself were also different from those in the smoke. In this study, approximately 1150 g of tobacco powder obtained from 1200 cigarettes of the five brands of cigarettes (240 cigarettes of each brand) were used. The combined ether and ethyl acetate solution (Soxhlet extractor) of the tobacco powder was shaken with 2 M sodium bisulfite solution for separation of the "aldehyde fraction". The aldehydes which have been identified in the extracts of tobacco thus far include *p*-hydroxybenzaldehyde, vanillin, syringaldehyde, protocatechualdehyde, and 5-hydroxymethylfurfural. In contrast with its predominant occurrence in smoke,

* A part of the results described for the aldehydes of the smoke from cigarettes was presented at the Second Annual Meeting of the Plant Phenolic Group of North America held in Oregon State University, Corvallis, Oregon, August 24–25, 1962.

[1] C.-H. YANG and S. H. WENDER, *Tobacco Science*, **6**, 156 (1962).

5-hydroxymethylfurfural was isolated only in poor yield from the tobacco. Experimental evidence based on chromatographic behavior and color tests indicates that extracts of tobacco contain an extremely small amount of *m*-hydroxybenzaldehyde. No quantitative studies, however, have been undertaken.

EXPERIMENTAL

Materials and General Procedures

Five brands of non-filter cigarettes (Camel, Lucky Strike, Philip Morris, Chesterfield, and Pall Mall) were purchased on the open, retail market. Raleigh cigarettes especially prepared to contain no flavorings or other common additives were kindly supplied by the Brown and Williamson Corp., Louisville, Kentucky.

Details of the smoking of the cigarettes, collection of the particulate phase of the mainstream smoke, and the procedure for isolation of an "aldehyde fraction" have been reported previously.[1]

Separation and Purification of the "Aldehyde Fraction" from the Smoke of Regular Cigarettes

By descending chromatography on Whatman No. 1 paper using the BzAW system (see Table 2), the concentrate of the "aldehyde fraction" was first separated into five zones (Table 1). Tentative identification of compounds present in these zones is included in Table 1.

TABLE 1. PRELIMINARY SEPARATION OF THE "ALDEHYDE FRACTION"

Separation in BzAW		
Zone	R_f values	Tentative identification
1	0·00–0·05	Protocatechualdehyde
2	0·05–0·20	Protocatechualdehyde
3	0·20–0·46	5-Hydroxymethylfurfural and *p*-hydroxybenzaldehyde
4	0·46–0·79	Vanillin, syringaldehyde, *p*- and *m*-hydroxybenzaldehydes, and 5-hydroxymethylfurfural
5	0·79–0·94	Syringaldehyde and vanillin

The methyl alcohol eluates of zones 1 and 2 were subjected to two-dimensional studies in the solvent system combinations, BzAW-2% HOAc and BAW-2% HOAc (see Table 2). Similar eluates of zones 3, 4, and 5 were separated using BzAW-2% HOAc and BAmW-2% HOAc, respectively (see Table 2).

Purification of p-*hydroxybenzaldehyde.* The eluate of zone 3 (Table 1) was streaked on thick paper which was then developed in BAmW to separate *p*-hydroxybenzaldehyde from 5-hydroxymethylfurfural. The separated components were eluted and the combined eluates containing *p*-hydroxybenzaldehyde were next developed in BzAW, followed by cutting, sewing and development in BAmW. Additional development in the CAW system (see Table 2) removed other major impurities from the *p*-hydroxybenzaldehyde zone. Eluates of the latter were further subjected to paper chromatography, in order, with 2% HOAc, BAW and BAmW, using the previously described cutting and sewing techniques. After this purification, each zone of *p*-hydroxybenzaldehyde exhibited a strong characteristic color change with ammonia vapor as seen under long wavelength (366 mμ) ultraviolet light (Table 2). The same procedure described above was also applied to purification of *p*-hydroxybenzaldehyde contained in the

eluate of zone 4. Final purification of the combined *p*-hydroxybenzaldehyde for identification studies was carried out successivley in 2% HOAc, BzAW and BAmW on chromatography paper prewashed with BAW.

Purification of m-*hydroxybenzaldehyde, vanillin, and syringaldehyde.* Preliminary separation of the aldehydes of zone 4 was obtained by development in BAmW. With the 2,4-dinitrophenylhydrazine spray reagent (Table 3), *m*-hydroxybenzaldehyde was located near the solvent front, poorly separated from the fastest moving dark purple zone (R_f 0·87). This spray also revealed the location of the other three zones containing *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde with R_f values of 0·50, 0·38, and 0·30, respectively.

TABLE 2. R_f VALUES AND COLORS OF THE AROMATIC ALDEHYDES UNDER ULTRAVIOLET LIGHT

Compound	R_f					UV			
	BzAW	CAW	BAW	2% HOAc	BAmW	luv	luv+NH_3	suv	suv+NH_3
p-Hydroxybenzaldehyde	0·53	0·72	0·91	0·73	0·54	—	dP	dkP	dkP
m-Hydroxybenzaldehyde	0·62	0·78	0·91	0·74	0·86	—	dY	dkP	lY
Vanillin	0·81	0·89	0·89	0·73	0·42	P	dP	dkP	dP
Syringaldehyde	0·80	0·91	0·86	0·69	0·31	P	dP	dkP	dP

R_f: Average value by descending chromatography with Whatman No. 1 paper; distance traveled by solvents: 40–45 cm.

BzAW = benzene–acetic acid–water (6:7:3, v/v/v); CAW = chloroform–acetic acid–water (2:1:1, v/v/v); BAW = n-butyl alcohol–acetic acid–water (6:1:2, v/v/v); 2% HOAc = 2% aqueous acetic acid solution; BAmW = n-butyl alcohol saturated with 2% aqueous ammonia solution.

luv: long wavelength (366 mμ) ultraviolet light (Spotlight, Blak-Ray Model B-100); suv = short wavelength (253·7 mμ) ultraviolet light (Mineralight Model R-51).

l = light; d = deep; dk = dark; P = purple; Y = yellow.

Characteristic color changes of *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde with ammonia vapor, as observed under long wavelength ultraviolet light (Table 2), also provided a convenient means of location of each aldehyde on the chromatogram. Thus, by cutting at this stage, vanillin was separated satisfactorily from *p*-hydroxybenzaldehyde, but poorly from syringaldehyde. Separation and recovery of crude syringaldehyde and vanillin from the eluate of zone 5 was likewise carried out by paper chromatography.

Two developments in BzAW, followed by 2% HOAc and BAmW with cutting and sewing, removed several zones of impurities from the eluate containing-*m*-hydroxybenzaldehyde. Final purification was completed on the chromatography paper (prewashed with BAW) by using the 2% HOAc, BzAW, and then BAW. The purified *m*-hydroxybenzaldehyde zone of each final chromatogram showed a characteristic change to a deep yellow color on exposure to ammonia vapor, as seen under long wavelength ultraviolet light.

Further separation of vanillin and syringaldehyde was carried out separately by repeating the chromatographic development in BzAW, 2% HOAc, and BAmW in the order described. Final purification was completed by chromatography using the CAW, 2% HOAc, and BAmW.

Identification Studies

(1) *Chromatographic behavior and color reaction.* The chromatographically pure eluates of *p*- and *m*-hydroxybenzaldehydes, vanillin, and syringaldehyde were each checked against

authentic samples of each respective aldehyde on one- and two-dimensional paper chromatograms, using solvent systems listed in Table 2, alone and in combination. In every case, the isolated aldehyde corresponded to the respective reference aldehyde on the same chromatogram. Color reactions of isolated aldehydes (Tables 2 and 3) also checked with those obtained with the corresponding authentic aldehydes.

TABLE 3. COLORS (IN VISIBLE LIGHT) PRODUCED BY SPRAY REAGENTS PLUS THE AROMATIC ALDEHYDES

Compound	DNPH	PDA	Benzidine	DzSA + 10% Na_2CO_3	FRSGG + 10% Na_2CO_3	$FeCl_3$ + $K_3Fe(CN)_6$
p-Hydroxybenzaldehyde	Br	dY	Y	lY	lR	lBl
m-Hydroxybenzaldehyde	Y	lY	lY	—	lY	lBl
Vanillin	O	OY	Y	lOY	P	dBl
Syringaldehyde	BrO	dO	OY	lBrY	lBl	dBl

l = light; d = deep; Bl = blue; Br = brown; O = orange; P = purple; R = red; Y = yellow.
DNPH = 2,4-dinitrophenylhydrazine.
PDA = 1% aqueous *p*-phenylenediamine.
Benzidine = 0·1 M alcoholic benzidine solution–1 N hydrochloric acid (1:1, v/v).
DzSA = diazotized sulfanilic acid.
FRSGG = stabilized diazo salt of *p*-nitroaniline.
$FeCl_3 + K_3Fe(CN)_6$ = 1% aqueous ferric chloride–1% aqueous potassium ferricyanide.

(2) *Ultraviolet absorption spectra.* By the procedure similar to that previously described,[1] the absorption spectrum in water of each aldehyde isolated from smoke was compared with the spectrum of the corresponding reference aldehyde. In each case, the reference and the corresponding unknown compound had identical spectra (Table 4).

TABLE 4. ULTRAVIOLET ABSORPTION SPECTRA OF PHENOLIC ALDEHYDES

Compound	H_2O* λ_{max} (mμ)	log ε
p-Hydroxybenzaldehyde	221	3·99
	283	4·10
m-Hydroxybenzaldehyde	216	4·17
	254	3·95
	314	3·37
Vanillin	230	4·03
	279	3·91
	307	3·86
Syringaldehyde	214	4·31
	303	3·98

* Almost identical values were obtained, when measured in 0·1 N HCl.

TABLE 5. 2,4-DINITROPHENYLHYDRAZONES OF THE PHENOLIC ALDEHYDES; R_f VALUES AND COLORS (IN VISIBLE LIGHT) PRODUCED BY SPRAY REAGENTS

Compound	R_f				10% Na_2CO_3	5% NaOH	$FeCl_3$+ $K_3Fe(CN)_6$
	BzAW	BAmW	MeOH–C_7H_{16}	C_6H_{12}–EtOAc			
p-Hydroxybenzaldehyde–DNPHO	0·84	0·83	0·62	0·73	YBr	OBr	*
m-Hydroxybenzaldehyde–DNPHO	0·88	0·92	0·67	0·76	Y	YBr	*
Vanillin–DNPHO	0·92	0·72	0·48	0·69	OBr	OBr	*
Syringaldehyde–DNPHO	0·89	0·60	0·40	0·52	PBr	PBr	*
DNPHI	0·96	0·95	0·73	0·93	Y	YBr	*

R_f: average value by descending chromatography with Whatman No. 1 paper; distance traveled by solvents: 40–45 cm.
DNPHO = 2,4-dinitrophenylhydrazone; DNPHI = 2,4-dinitrophenylhydrazine.
BzAW = benzene–acetic acid–water (6:7:3, v/v/v).
BAmW = n-butyl alcohol saturated with 2% aqueous ammonia solution.
MeOH–C_7H_{16} = methyl alcohol saturated with n-heptane.
C_6H_{12}–EtOAc = cyclohexane–ethyl acetate (1:1, v/v).
Br = brown; O = orange; P = purple; Y = yellow.
* = All of these compounds give a pale green blue spot which turns dark blue on standing, except for the syringaldehyde–DNPHO which fades to white on standing.

(3) *2,4-Dinitrophenylhydrazones.* The 2,4-dinitrophenylhydrazone[1] of each isolated aldehyde was checked by paper chromatography against the corresponding derivative of the reference aldehyde using the solvent systems listed in Table 5. In every test, the derivative from the isolated aldehyde solution and from the corresponding reference had the same R_f value. The 2,4-dinitrophenylhydrazones of the isolated and corresponding reference aldehydes behaved similarly toward all the spray reagents used (Table 5).

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