Pyrogenesis of Aromatic Hydrocarbons Present in Cigarette Smoke II

Pyrolytic Products of some Representative Constituents of the Hexane Soluble Fraction of Tobacco*

by W. S. Schlotzhauer and I. Schmeltz

INTRODUCTION

In the foregoing report (1), we noted the efficacy of the hexane soluble fraction of flue-cured tobacco to produce, on pyrolysis (860° C, N_2), a relatively high yield of aromatic hydrocarbons. Comprising but 6 % of dry leaf weight, this fraction was shown to account for as much as 60 % of the benzo(a)pyrene (BaP) produced by pyrolysis of tobacco at the approximate burn temperature of a cigarette (860° C). This observation attested to the importance of the hexane soluble fraction as a whole in influencing the chemical and biological properties of cigarette smoke. However, we felt that further insight into precursor-product relationships would be gained by evaluating the role played by individual components of this complex fraction in the pyrolytic process, and by assessing their corresponding contributions to the levels of certain products (i. e. BaP) present in pyrolysates. To this end, we pyrolyzed a number of compounds structurally similar, if not identical, to those present in the hexane soluble fraction of tobacco, and examined the products thus formed. Compounds pyrolyzed were selected on the basis of previous reports (2—5) which have described the hexane soluble fraction of flue-cured tobacco as consisting largely of aliphatic and cyclic paraffins, fatty acids, polyenes, phyto-sterols, and steryl esters. Our objective was to correlate the pyrolytic products obtained to the structural characteristics of the materials pyrolyzed, if possible, and ultimately to extrapolate the findings to the pyrolytic processes occurring in a burning cigarette.

EXPERIMENTAL

Materials Pyrolyzed

n-Hexane was obtained by distillating commercial "Skellysolve"** and recovering the fraction boiling at 67—69° C. Squalene and dotriacontane, both reagent grade, were obtained from the Eastman Chemical Company, and phytol, distilled grade, from K&K Laboratories. Stearic acid, purity > 99 %, was obtained from Humko Products Co. and recrystallized from acetone before use. Linolenic acid (all *cis*, purity > 99 %) and its methyl ester were obtained from the Hormel Institute, University of Minnesota, and β-sitosterol, chemically pure grade, from the Mann Chemical Corporation.

Pyrolysis

Pyrolyses were performed in quartz tubes, constantly flushed by a stream of dry nitrogen (30 ml/min), at a temperature generally of 860 ± 5° C; apparatus and product collection techniques have been described previously (6).

Fractionation of Pyrolysate

Pyrolysates were recovered from the collection traps by extraction with diethyl ether, and 0.5 % NaOH (aqueous) where applicable (1, 6). In the case of non-oxygenated starting materials pyrolyzed under nitrogen, the ether extracts were assumed to contain all condensable products formed. The pyrolysates of phytol, β-sitosterol, and the fatty acids were additionally examined for acidic products, including phenols (6), although none of the latter was actually found in significant amounts. Representative fractions of the various pyrolysates were ultimately obtained in dry ethereal solutions concentrated (0° C, *in vacuo*) to convenient volumes for gas chromatographic analyses (1, 6).

Product Analysis

Neutral products were examined by gas-liquid chromatography on a Loenco Model 70 "Hi-flex" gas chromatograph equipped with dual columns in conjunction with dual thermal conductivity and flame ionization detectors. Separations were achieved on columns (5′ × 0.25″ o.d., stainless steel) containing 20 % SE-30 on 60/80 mesh Chromosorb W, programmed from 70—250° C at a rate of 6° C/minute. Detector and injector temperatures were maintained at 260° C; the helium flow rate was 70 ml/min (1).

The method used proved applicable to the quantitative

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** Mention of a specific commercial item does not imply endorsement by the Department over similar products not amed.

Table 1 Relative yields of pyrolytic products**

Products*	a	b	c	d	e	f	g	h	i
Benzene	99	190	261	358	352	166	267	404	82
Toluene	121	20	32	44	200	213	197	322	97
Ethylbenzene	26	—	—	—	26	25	28	75	17
Styrene	39	29	50	64	160	172	72	74	37
Xylenes	15	—	—	—	15	30	20	26	11
Indene	25	14	19	30	59	77	38	45	31
Naphthalene	201	201	186	164	317	377	192	411	299
Alkyl-naphthalenes	37	10	14	17	69	73	53	81	89
Biphenyl	11	16	15	10	24	26	16	21	9
Acenaphthylene	35	39	36	37	46	46	38	55	42
Fluorene	18	9	13	13	23	30	15	32	26
Anthracene Phenanthrene	68	61	51	41	65	70	45	101	171***
Alkyl-anthracenes Alkyl-phenanthrenes	19	8	6	4	23	27	14	41	50
Fluoranthene	9	19	15	9	11	19	5	17	19
Pyrene	16	27	28	16	14	21	5	23	46
Total	739	633	726	807	1404	1372	1005	1728	1026

Key
a: hexane-extract (flue-cured tobacco)
b: n-hexane
c: dotriacontane
d: stearic acid
e: linolenic acid
f: methyl linolenate
g: phytol
h: squalene
i: β-sitosterol

* In order of elution from gas chromatographic column. For BaP see Figure 1.
** Figures represent areas adjusted to an attenuation of 1 and are based on 1 g of starting material.
*** Predominately phenathrene

estimation of products eluting up to and including pyrene which had an elution time of 40 minutes. Effluents corresponding to various peaks were collected and characterized by suitable spectral techniques, in addition to retention data, and cochromatography with authentic samples. Relative yields of products in the various pyrolysates were determined by measuring corresponding gas chromatographic peak areas, adjusted to an attenuation of 1. For purposes of comparisons, equal aliquots of equivalent solutions of pyrolysates were analyzed.
Levels of benzo(a)pyrene in pyrolysates were estimated by a thin layer chromatographic procedure described previously in detail (1, 7).

RESULTS AND DISCUSSION

The relative yields, based on gas chromatographic peak areas, of neutrals obtained from the pyrolysis of the eight model compounds (860° C, N_2) are presented in Table 1. The same information is presented as percent of neutral pyrolysate in Table 2. For comparison, the previously reported data from the pyrolysis studies of the hexane soluble fraction of flue-cured tobacco are included (1). In general, pyrolysates from all the model compounds are qualitatively similar. This is in agreement with previous observations (8). In terms of total yields and yields of the various individual components identified, however, dissimilarities occur which require some discussion. Pyrolysis of three straight chain saturated compounds, *n*-hexane (C_6), stearic acid (C_{18}), and dotriacontane (C_{32}), produced pyrolysates markedly similar both qualitatively and quantitatively. These compounds produced the lowest yields of neutral condensables among pyrolysates examined, the product distributions being distinguished by the relatively high preponderance of benzene and naphthalene, the low percentage of toluene, and the absence or presence of trace amounts of other alkyl substituted aromatic rings. The observation that in the case of saturated compounds in-

Table 2 Product distribution (% neutral products)

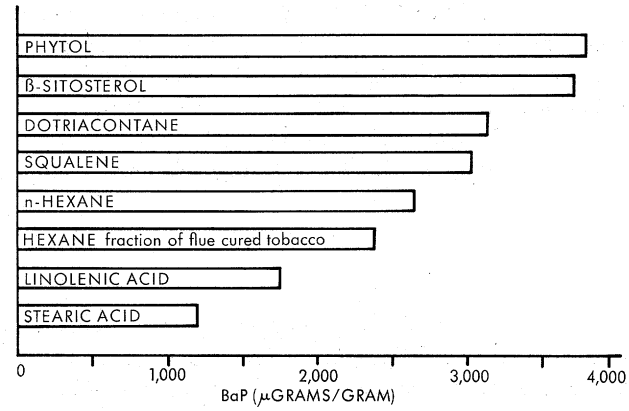
Products	a	b	c	d	e	f	g	h	i
Benzene	13.40	29.55	35.95	44.36	25.07	12.10	26.57	23.38	7.99
Toluene	16.37	3.11	4.41	5.45	14.25	15.52	19.60	18.63	9.45
Ethylbenzene	3.52	—	—	—	1.85	1.82	2.79	4.34	1.66
Styrene	5.28	4.51	6.89	7.93	11.40	12.54	7.16	4.28	3.61
Xylenes	2.03	—	—	—	1.07	2.19	1.99	1.50	1.07
Indene	3.38	2.18	2.62	3.72	4.20	5.61	3.78	2.60	3.02
Naphthalene	27.20	31.26	25.62	20.32	22.58	27.48	19.10	23.78	29.14
Alkyl-naphthalenes	5.01	1.56	1.93	2.11	4.91	5.32	5.27	4.69	8.67
Biphenyl	1.49	2.49	2.07	1.24	1.71	1.90	1.59	1.22	0.88
Acenaphthylene	4.74	6.07	4.96	4.58	3.28	3.35	3.78	3.18	4.09
Fluorene	2.44	1.40	1.79	1.61	1.64	2.19	1.49	1.85	2.53
Anthracene Phenanthrene	9.20	9.49	7.02	5.08	4.63	5.10	4.48	5.84	16.67
Alkyl-anthracenes Alkyl-phenanthrenes	2.57	1.24	0.83	0.50	1.64	1.97	1.39	2.37	4.87
Fluoranthene	1.22	2.95	2.07	1.12	0.78	1.38	0.50	0.98	1.85
Pyrene	2.17	4.20	3.86	1.98	1.00	1.53	0.50	1.33	4.48

creasing chain lengths have little effect on the amount of aromatic hydrocarbons produced at high temperatures supports the postulations of *Badger* et al. (9). Pyrolysis of a molecule displaying branching of the chain, e.g. phytol, resulted in two noticeable effects: 1) a marked increase in the total yield of neutrals examined, and 2) a substantial increase in the amounts of toluene and other alkyl-substituted aromatic products. These same effects were evident in the pyrolysis of the unsaturated fatty acid, linolenic acid (9, 12, 15 octadecatrienoic acid), and its methyl ester which differ from stearic acid (octadecanoic acid) by the presence of 3 double bonds in the chain. In addition, the unsaturated acid produced a significantly higher yield of styrene (11 % of the neutrals examined). Corroborating the observation that unsaturation or branching in the carbon chain is favorable for production of aromatic hydrocarbons at high temperatures are the data obtained from pyrolysis of squalene ($C_{30}H_{50}$), a polyene possessing both unsaturation and methyl side chains. The yield of products from squalene pyrolysis is considerably higher than that of the saturated straight chain compounds examined (nearly threefold that of *n*-hexane), and includes substantial quantities of toluene, ethylbenzene, and other alkyl substituted aromatic compounds. Pyrolysis of the common plant sterol, β-sitosterol, gave results which must be interpreted in light of the fact that sterols possess an internal phenanthrene nucleus. Pyrolysis of sitosterol resulted in formation of substantial amounts of phenanthrene (approx. 16 %) which was, of course, not unexpected, and naphthalene (29 %). *Badger* et al. (10). report similar high yields of phenanthrene from the closely related sterol, stigmasterol. To help rationalize the formation of naphthalene, we pyrolyzed the sterol under nitrogen at a lower temperature (650° C). The neutral fraction of the pyrolysate thus obtained displayed, on gas chromatography, two peaks of equal proportions in the "naphthalene region" of the chromatogram. One peak was definitely identified by cochromatography and ultraviolet absorption spectroscopy as naphthalene, whereas the other component cochromatographed with and possessed mass spectral characteristics of tetralin. This data would strongly indicate that a tetralin-type intermediate precedes naphthalene in the pyrolytic pathway from "sterol" to "products".

Yields of benzo(a)pyrene from the model compounds investigated are presented in Figure 1. β-Sitosterol, and phytol were observed to be the most effective precursors of BaP. The sterol's self-contained polycyclic nucleus might easily undergo further condensation and aromatization to more highly condensed ring systems. Our results with respect to phytol are in accordance with the previous observation of *Lam* (11) that branching of the chain is related to increased yields of BaP. For the same reason, squalene should have perhaps given rise to greater quantities of BaP than we observed. However, the magnitude of the difference between yields of BaP from phytol and squalene may not be great enough to be considered significant.

In light of the data obtained in the present study, we find it difficult to correlate the nature and quantity of the pyrolytic products with the structural characteristics of the materials pyrolyzed and arrive at meaningful generalizations. Some of the data do seem to indicate that branching of and unsaturation in the hydrocarbon chain of pyrolysands do enhance pyrolytic production of aromatic hydrocarbons (Table 1), although this correlation is not apparent in the data (Figure 1) obtained for BaP formation. In addition, two hydrocarbons of very different chain length (C_6 vs. C_{32}) were shown to produce very similar qualitative and quantitative patterns in their respective pyrolysates (Table 1, Figure 1). Apparent in the data, as well, is the lack of correlation between BaP levels and total neutrals in pyrolysates. For example, although phytol was the most efficient precursor of BaP among the materials pyrolyzed, it ranks fifth in total yield of neutrals listed in Table 1 (or fourth if the data for methyl linolenate, for which we have no BaP values, is ignored).

Figure 1 **Yields of BaP (in μg per g pyrolysand)**



In comparing the pyrolytic behavior of the model compounds and the hexane-soluble fraction of tobacco, we note that the levels of products from the latter (Table 1, Figure 1) are, in general, intermediate to the levels of products obtained from the model compounds. This finding is to be expected, perhaps, if the model compounds are truly representative of the hexane-soluble fraction; on the other hand, the hexane-soluble fraction behaved like an "average" model compound.

Finally, on the basis of the data obtained, we must arrive at the usual conclusion with respect to pyrogenesis. That is, at high temperatures, "anything is bound to occur", or is at least energetically possible, and at the present time, a simple mechanistic explanation for the conversion of starting material to the complex mixture of products generally obtained still eludes us.

SUMMARY

In continued studies to reevaluate the contribution of the hexane-soluble fraction of flue-cured tobacco to the formation of aromatic compounds, especially polynuclear aromatic hydrocarbons, present in cigarette smoke, some compounds representative of that fraction have been pyrolyzed (860° C, N_2). The straight chain saturated compounds, *n*-hexane, stearic acid, and dotriacontane,

produced pyrolysates distinguished by relatively low yields of total neutrals and the absence of any significant quantities of aromatic products with alkyl side chains. Compounds containing double bonds, such as the unsaturated fatty acid, linolenic acid and its methyl ester, and the polyene squalene, which like phytol is also characterized by methyl groups on the chain, produced relatively higher yields of neutral products, including alkyl-substituted aromatic hydrocarbons. Phytol, of course, also exhibited this tendency. Pyrolysis of the plant sterol, β-sitosterol, produced the greatest amounts of phenanthrene and BaP. Estimates of BaP levels in pyrolysates indicate that phytol, in addition to the above mentioned sterol, is a relatively effective high temperature precursor of this important carcinogen. The hexane solubles of flue-cured tobacco were shown to produce BaP in levels intermediate to the least effective (stearic acid) and most effective (β-sitosterol) precursor examined.

RÉSUMÉ

Dans le cadre des études destinées à une réévaluation de la contribution de la fraction soluble dans l'hexane, du tabac flue-cured à la formation des composés aromatiques, en particulier des hydrocarbures aromatiques polynucléaires, présents dans la fumée de cigarette, on a pyrolysé (860° C, N_2), quelques constituants représentatifs de cette fraction. Les composés saturés à chaîne droite, *n*-hexane, acide stéarique et dotriacontane, ont produit des pyrolysats se distinguant par des rendements relativement bas en substances neutres totales et par l'absence de quantités significatives de produits aromatiques à chaînes latérales alcoylées. Des composés possédant des doubles liaisons, tels que l'acide linolénique, acide gras non saturé, et son ester méthylé, et le squalène, polyène qui comme le phytol est aussi caractérisé par des groupes méthyl sur la chaîne, produisaient des quantités relativement importantes de produits neutres, comprenant des hydrocarbures aromatiques alkyl-substitués. Le phytol, naturellement, présentait aussi cette tendance. La pyrolyse d'un stérol des végétaux, le β-sitostérol, produisait les plus grandes quantités de phénanthrène et de benzo(a)pyrène (BaP). L'estimation des taux de BaP dans les pyrolysats indique que le phytol, outre le stérol sus-mentionné, est, à haute température, un précurseur relativement efficace de cet important cancérogène. Il est apparu que les constituants du tabac flue-cured solubles dans l'hexane produisent des taux de BaP se situant entre ceux du moins efficace (acide stéarique) et de ceux du plus efficace (β-sitostérol) des précurseurs examinés.

ZUSAMMENFASSUNG

In weiteren Untersuchungen über den Beitrag, den die hexanlösliche Fraktion des Blattabaks (nach flue-curing) zur Bildung aromatischer Verbindungen, insbesondere der im Cigarettenrauch vorkommenden polycyclischen aromatischen Kohlenwasserstoffe liefert, wurden einige typische Verbindungen dieser Fraktion bei 860° C unter Stickstoff pyrolysiert. Aus den langkettigen gesättigten Verbindungen, n-Hexan, Stearinsäure und Dotriacontan entstanden Pyrolysate, in denen die gesamten Neutralanteile relativ gering vertreten waren und in denen nennenswerte Mengen an Aromaten mit Alkyl-Seitenketten nicht vorlagen. Eine relativ höhere Ausbeute an Neutralanteilen (einschließlich Alkyl-substituierte aromatische Kohlenwasserstoffe) lieferten die Pyrolyse von Verbindungen mit Doppelbindungen. Dazu gehören die ungesättigten Fettsäuren, die Linolensäure und ihre Methylester sowie das Polyen Squalen, das ebenso wie Phytol durch Methylgruppen in der Kette gekennzeichnet ist. Für Phytol gilt dieses Verhalten natürlich auch.
Die Pyrolyse des Pflanzensterols β-Sitosterin führte zu den größten Ausbeuten an Phenantren und 3,4 Benzpyren. Aus den Benzpyrengehalten in den Pyrolysaten läßt sich schließen, daß das Phytol zusammen mit dem o.g. Sterin bei hohen Temperaturen ein relativ bedeutsamer Vorläufer dieses wichtigen Cancerogens ist. In unseren Untersuchungen lag die hexanlösliche Fraktion des Tabaks (flue cured) in bezug auf die Ausbeute an Benzpyren im Pyrolysat in der Mitte zwischen den Vorläufern mit der geringsten (Stearinsäure) und der höchsten Ausbeute (β-Sitosterin).

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Author's address:

Eastern Utilization Research and Development Division,
Agricultural Research Service,
United States Department of Agriculture,
Philadelphia, Pa., 19118, USA