THE CHEMICAL COMPOSITION OF TOBACCO AND TOBACCO SMOKE

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CONTENTS

I. INTRODUCTION

The last comprehensive review of the constituents of tobacco and tobacco smoke appeared in 1959 (263). Since then, numerous reports linking smoking and health (527) have contributed greatly to increased effort in this field, and several books and reviews have been published dealing with selected chemical aspects of smoking and health relationships. A comprehensive review with more than 6000 literature citations is available dealing mostly with medical aspects but containing some information on chemical carcinogens, ciliostatic agents, and irritants (307). Two other books have appeared on the biological effects of tobacco and smoke, one having two short chapters on the chemistry of smoke (254) and the other including information on alkaloids (595). A tabulation of known components of cigarette smoke has been published which is supplemented periodically (38); however, no critical evaluation of the cited reports is given therein. An extensive Russian work on the chemistry of tobacco has been

translated into English, but much of the data must be regarded as historical (513). Two reviews dealing with the general composition of tobacco and smoke have been published, but coverage of the subject is not complete in both cases (437, 554). However, an exhaustive survey has appeared on the relationships between the chemical composition and experimental carcinogenic effects of tobacco (644); an expanded version of this work will be published as a book in late 1967 (648). Several surveys with varying degrees of age and thoroughness are available covering selected groups of leaf or smoke constituents: polynuclear aromatic hydrocarbons (152), terpenes (47), phenols (223, 628), and alkaloids (399, 492).

None of these works can be considered an adequate sequel to the comprehensive survey on composition published in 1959. The present work is intended to fill this role. The procedure followed herein is to provide information which supplements the 1959 review or other surveys for which coverage is considered adequate. In listing the known constituents of tobacco leaf and smoke, literature citations are given for every component; for some compounds, the citations are to the 1959 survey or to other reviews which, in turn, list the original references. However, original publications were consulted in making the lists. The inclusion of references to components was deemed necessary to facilitate the location of original reports which was difficult in the 1959 review since compounds were listed merely as being absent or present. Except for certain trivial names which are widely used in the field, the nomenclature of *Chemical Abstracts* is employed herein.

Many of the newly reported constituents of leaf and smoke occur in trace amounts; in some instances, the isolated quantities have been much less than 1 mg. Application of classical methods to identify the isolates has not been possible in such cases, and identification has been based on chromatographic and spectrometric comparisons with authentic compounds. Under the conditions, these procedures appear to be adequate for identification provided the criteria are not limited excessively. The present survey lists those compounds for which claims of identity appear to be reasonably justified.

II. Tobacco Processing and Physicochemical Properties of Tobacco Smoke

Most tobacco products use processed leaves of *Nicotiana tabacum* as their major ingredient. In Eastern Europe, the Soviet Union, and perhaps India, *Nicotiana rustica* is used to some extent. Traditionally, tobacco is classified into types which differ in the conditions of growth, processing, and eventual use; the major cigarette types used in the United States and Europe are flue-cured ("bright," "Virginia"), burley, Maryland, and Turkish ("Oriental"). After harvesting, all tobacco is subjected to a curing process which is basically a dehydration accompanied by certain chemical changes resulting in the development of desired color and other properties. The major curing processes are flue-curing, in which heat is provided to effect the drying, and air-curing, in which ambient temperatures are employed. Following curing, tobacco is treated in a series of industrial processes, including moisture adjustment, aging or fermentation, blending, and addition of humectants and flavors (117, 164, 165, 213). The exact treatment will vary with the ultimate form of the smoking product and the particular manufacturer's practice. Fermentation is a vigorous process involving storage at elevated temperatures with relatively high moisture levels in the tobacco and is employed with cigar and snuff tobaccos. Aging is a relatively mild prolonged (up to 3 years) storage at ambient temperature and relatively low moisture levels and is used for cigarette tobaccos. On the basis of sparse information, the chemical changes occurring during aging are more subtle than those of fermentation (134, 165, 166).

For research and development purposes, cigarettes, cigars, and pipes are smoked mechanically in machines which superficially simulate the act of smoking by humans. Most smoking machines operate on a constant-volume or constant-pressure principle, but other designs have been reported including a "pressure procedure" which is claimed to reduce the aging effects of smoke (371). Many different machines have been proposed for research purposes (151, 332, 490, 644), and fully automatic models are commercially available with daily smoking capacities as high as 1000 cigarettes (151). Whole smoke is collected in a variety of ways depending on the end use, but, for large-scale compositional work, condensation at low temperature is usually employed. Other methods include collection of particulate matter on Cambridge filters or condensation of smoke by electrostatic precipitation, both of which give essentially the same yields as low-temperature condensation (41) although significant compositional differences may exist, *e.g.*, free-radical contents (644). Artifacts may be introduced by many systems of collection and storage since smoke components in the trapped material may react to form products not in the fresh smoke, *e.g.*, nitrophenols (274) and cyanohydrins (361). To reduce the inherent variability in these systems, standard methods of collecting and handling smoke condensate have been developed for analytical (41) and biological (118) use. Although such standardizations are desirable, they provide little assistance in one of the current major problems involving smoke composition: the determination of the constituents actually present in the particulate matter of fresh mainstream smoke emitting from a cigarette or other smoking product. Little progress has been made in this area, mainly because of the inadequacies of current methodological

procedures. The solution of many pragmatic problems involving the organoleptic properties and biological effects of tobacco smoke may lie in this direction.

Cigarette smoke is an aerosol having a discontinuous phase (about 8% of the total weight) and a continuous phase composed of vapor constituents (19%), excess nitrogen (15%), and air (58%) (283). The vapor constituents of unfiltered tobacco smoke include a variety of hydrocarbons, oxygenated compounds, and related constituents (411), some of which have physiological activity including the ability to inhibit the motion of the cilia lining the respiratory tract, *i.e.*, ciliostasis. The discontinuous or particulate phase contains a large number of identified and unidentified compounds. The physical characteristics of smoke change rapidly on emission from the cigarette tip (239) with the particles rapidly agglomerating (281). Fresh mainstream smoke contains about 1×10^9 to 5×10^9 particles per ml (135, 281, 385), and each particle contains 0–10 charges. Studies on the charge distributions of American cigarettes (385) have shown the following percentages of particles with the indicated numbers of charges: more than 2, 1%; 2, 7%; 1, 47%; and 0, 45%. Approximately equal numbers of positively and negatively charged particles are present, and the proportion of charged particles increases with particle diameter. The primary mechanism of charge origin may be chemionization in the reaction zone of the cigarette coal rather than thermionic emission or collision with atmospheric ions (385). Chemionization results when molecules, atoms, and radicals combine in exothermic reactions and the products collide with excited species producing positive ions.

Significant differences in the magnitude and sign of the charges have been shown for cigarettes containing various tobaccos. British cigarettes containing all flue-cured tobaccos yield (9) a smoke which is strongly positive ($+2.2 \times 10^{-11}$ to $+2.4 \times 10^{-11}$ coulomb), but American cigarettes, made of a blend of the four major cigarette tobacco types, may be either electrically neutral (239) or have a net negative or positive charge of lower magnitude ($+1.3 \times 10^{-11}$ to -1.3×10^{-11} coulomb) than British cigarettes (9). Alteration and neutralization of the net charge on smoke particles by the blending of the cigarette tobaccos has been demonstrated experimentally (9). Filter tips show no effect in altering the charge on the individual smoke particles (9).

Various ranges of particle sizes have been reported for tobacco smoke, depending on the age of the aerosol and the measuring technique employed. Mean diameters of about 0.16–1.1 μ have been reported for fresh smoke (216, 239, 281, 385). The diameters of almost all smoke particles are in the range of about 0.1–1.0 μ (216, 281, 385), and smoke from cigarettes having filters show a slightly less medial diameter than unfiltered smoke (239). No significant differences have been observed in the particle diameters and numbers of particles in smoke from the major cigarette tobacco types (281). Whether or not the composition of smoke particles varies with particle size or charge is not known. The infrared spectra of negatively and positively charge particles show no distinct differences (159), but other indirect measurements show some degree of heterogeneity (45, 112). Of course, the questions of particle-size distribution and of homogeneity of smoke particles are of great importance in work on the development of filters for cigarette smoke.

Cigarette smoke contains large numbers of free radicals and certain ions which are discussed in section IV.

III. Chemical Composition of Tobacco Leaf and Smoke

Since 1959, the list of known components in tobacco and smoke has risen from about 400 to more than 1200, not including the individual components in complex substances such as the brown pigments and resins, which have not been resolved. Since most of the major components of leaf and smoke are apparently known, recent effort has concentrated on investigating minor and trace components. Many of these compounds may contribute significantly to the organoleptic and physiological properties of leaf and smoke, and brief comments on these points will be given when appropriate.

A. Alkanes (Table I)

In addition to the ubiquitous normal and isoparaffins, tobacco leaf contains significant amounts of other branched-chain alkanes, including anteiso (3-methyl) homologs. Treatment of the paraffinic fraction of tobacco leaf wax with molecular sieve permits separation of normal and branched isomers, each of which can be separated by gas chromatography. Resolved iso and anteiso compounds can then be distinguished by differences in mass spectral fragmentation since scission occurs at the bonds adjacent to the tertiary carbon atoms, yielding ions of variable intensities for the two classes of isomers (349). Both isomers cleave pre-

Table I
Alkanes in Tobacco Leaf and Smoke

	Leaf		Smoke	
Confign	Carbon no.	Ref	Carbon no.	Ref
Normal	8–35	84, 87, 90, 133, 188, 263, 349	1–9, 12–36	78, 79, 84, 85, 205, 263, 296, 378, 397, 415, 531
Iso	27–34	84, 263, 349	4–6, 27–33	79, 84, 205, 263, 378, 415
Anteiso	28, 30, 32, 34	349	6	79, 378, 415
Cyclic	...	...	5, 6	205, 206, 378, 413

dominately at the C_2–C_3 linkage, giving major peaks at P − 43 and P − 29 for the iso and anteiso isomers, respectively, and this difference may be used in conjunction with gas chromatographic data to identify and determine quantitatively the levels of the isomers. Analysis of leaf paraffins in this way has shown that the branched-chain compounds comprise about 24–45% of the total and consist of approximately equal parts of iso and anteiso compounds in the major cigarette tobaccos. In the normal and iso series, the odd-numbered homologs predominate, and the C_{31} compounds are present in the largest amounts. In the anteiso series, even-numbered compounds are exclusively found, and the C_{32} hydrocarbon predominates.

Although the major paraffins in leaf are the C_{25}–C_{35} components, small amounts of normal and iso homologs in the C_8–C_{24} range occur therein (84, 349). Also, analysis of the headspace vapors of Turkish tobacco has shown the possible presence of pentane, hexane, and heptane based on gas chromatographic comparisons of Kovats retention indices with known compounds and the failure of the eluted peaks to react with functional group reagents (558).

Mixtures of paraffins of indeterminate structure have been isolated from flue-cured tobacco, but resolution into single components has not been accomplished despite intensive effort (552, 553). Based on elementary analyses, hydrogenation, reactivity toward selected reagents, and infrared and mass spectral data, the components appear to be cyclic in nature with molecular weights of about 500–900; in general, they resemble the naphthenes of petroleum, but distinctive differences are noted. Although the possibility that these components are artifacts arising from the deposition of flue gases on leaves during curing has been largely discounted, confirmation of their presence as natural constituents of tobacco is required.

The lower molecular weight saturated hydrocarbons of cigarette smoke are present in the gaseous phase and are easily separated and identified by a combination of gas chromatography and mass spectrometry (205). The cycloalkanes in smoke are cyclopentane (378), methylcyclopentane (378, 413), and cyclohexane (206, 413). Branched-chain, aliphatic paraffins having C_{12} to C_{33} and C_6 skeletons occur in smoke, but the positions of substitution have not been determined (78, 85, 296, 531).

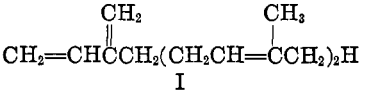
Analytical methods are available for the determination of paraffins in tobacco leaf (429, 545). The levels vary somewhat with tobacco type and are not related to leaf quality (546). As expected from the inert character of these constituents, there is little or no change in the paraffins during tobacco fermentation (429). The levels of C_{12} to C_{33} paraffins in cigarette smoke are about 0.5–1.4 mg per cigarette, and 9–18% of the mixture is composed of branched-chain constituents (531).

The paraffinic hydrocarbons of leaf have been suggested as precursors of polynuclear aromatic hydrocarbons in smoke (see section V). The higher alkanes show a tendency to retard the tumorigenic effect of benzo[*a*]pyrene in animals (644), but their contribution, if any, to the neoplastic effect of smoke condensate in animals is not known.

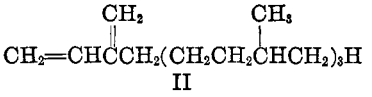
B. ALKENES AND ALKYNES

1. Isoprenoid Hydrocarbons

The presence of the sesquiterpene farnesene (I) and the diterpene neophytadiene (II) in tobacco leaf and/or smoke has been established. Farnesene in smoke may arise by pyrolysis of solanesol (see section III.D.-



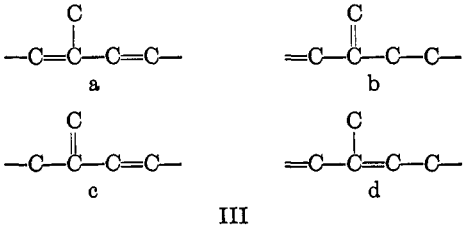
I



II

2), neophytadiene, or unidentified C_{15} isoprenoid alcohols (457) in leaf. Farnesiferols have also been suggested as possible leaf precursors of I, but no evidence for the occurrence of these compounds in tobacco exists (52).

Smoke from American cigarettes contains a series of neophytadiene isomers which have a single set of conjugated double bonds in different terminal and internal positions. Evidence has been obtained (452) for at least four basic combinations of olefinic linkages within a given isoprene unit or between adjacent units (IIIa–d).

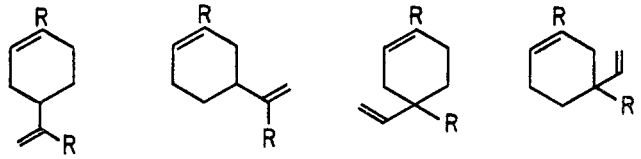


III

As many as 12 of these phytadienes may occur in smoke exclusive of neophytadiene and geometric isomers. Also, a 1,2,4-trialkyl-1,3-butadiene may be present. In the reported isolation (452), the phytadienes were separated into groups by column chromatography on alumina, and the mixtures were allowed to react with 1,4-naphthoquinone, giving the Diels–Alder adducts. The resulting alkylanthraquinones were oxidized to the corresponding carboxylic acids, which were separated and found to vary in the number and position of carboxyl groups, depending on the location of methyl substituents relative to the double bonds in the original terpene. These acids and methyl derivatives thereof were identified by mixture melting point determinations and infrared spectral analyses. The possibility of the

isomers being artifacts was considered negligible and some experimental evidence on this point was provided.

Other hydrocarbons related to neophytadiene in smoke are norphytene (2,6,10,14-tetramethyl-1-pentadecene) and a mixture of neophytadiene dimers (IV,



IV

R = 4,8,12-trimethyltridecyl). These dimers are identical with the major products resulting when neophytadiene is heated at 190–200° and are not believed to be artifacts resulting from experimental manipulation of cigarette smoke extracts (265). In a study of British cigarettes neophytadiene was found to comprise more than 99% of the acyclic phytadienes of smoke (265). Neophytadiene isomers may have been present in the remainder of the fraction, but no evidence of this was presented. In the above study describing the presence of neophytadiene isomers with moieties IIIa–d in American cigarettes, no quantitative yields were given so that comparisons of the two investigations cannot be made; however, it appears that yields of these isomers were much greater than the 1% unaccounted for above. Some of the discrepancy may have been due to compositional differences in the cigarettes.

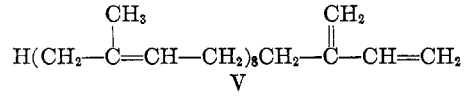
Since phytadiene C is a mixture of isomers (265), the claim of its presence in leaf is questionable (245); instead, an artifact with a moiety similar to IIIa may have been isolated as a result of isomerization of neophytadiene on acid-washed alumina (248). This absorbent will isomerize squalene, neophytadiene, and possibly other terpenes during chromatographic separation (550).

An analytical method for neophytadiene in leaf has been developed (49). The levels of the terpene in the different tobacco types show differences, but no relationship with leaf quality can be demonstrated. Biologically, the phytadienes do not produce hyperplasia or sebaceous gland destruction when applied to mouse epithelium (644).

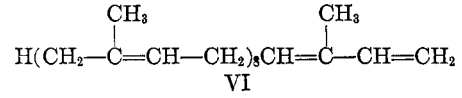
The presence of the triterpene squalene in smoke has been established and its occurrence in leaf has been cited (168). The reported presence of isosqualene in smoke (295) may have been a result of isomerization of squalene by acid-washed alumina used in the separation; however, other studies have described the isolation of "regenerated" squalene (590) and other isomeric squalenes (463) in smoke when acid-washed alumina was not employed in the separation.

The tetraterpenoid hydrocarbons of tobacco leaf comprise the familiar colorless polyenes and carotenoid pigments which were isolated in an intensive study of aged burley tobacco (631, 638, 639). Identification was made by standard procedures for such plant components: chromatographic behavior on magnesium oxide and calcium hydroxide columns, selected color and isomerization tests, and ultraviolet spectral examinations. In addition to those listed in Table II, three partially identified unsaturated hydrocarbons were isolated, including one compound that resembled isodemethylaxerophthene and two constituents that were similar to the lycopenes.

The solanesenes in smoke are a mixture of trisesquiterpenes structurally related to solanesol and formed from dehydration of solanesol or pyrolysis of its acetate (463). In tobacco smoke, the major components in the solanesene mixture are V and VI. The structures of



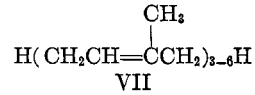
V



VI

these compounds have been revealed by sodium–alcohol reduction of the isolated mixture which yielded a hydrocarbon, dihydrosolanesene, with an infrared spectrum having diminished 6.25 and 11.22 μ absorption indicative of double-bond migration from a terminal to an internal position (463). A quantitative study of the ozonization products of the solanesenes and dihydrosolanesene established the basic structures of V and VI. Confirmatory evidence for V was obtained by Diels–Alder reaction of the isolated mixture with 1,4-naphthoquinone, yielding an alkylanthraquinone which was oxidized to anthraquinone-2-carboxylic acid in the same general manner as the phytadienes. However, the presence of the expected product from VI, anthraquinone-1,2-dicarboxylic acid, could not be demonstrated in the reaction mixture, indicating that VI was relatively inert. A similar pattern of reactivity was observed earlier (452) with the phytadienes (IIIa–d) in Diels–Alder reactions. The sequence of elution from alumina is neophytadiene isomers, neophytadiene, isomeric squalenes, and solanesenes (463).

The isoprenoid polyolefins listed in Table II are a series of compounds (VII) found in smoke which are



VII

probably formed by pyrolysis of leaf constituents such as solanesol. These olefins have an all-*trans* configuration and occur in levels of about 2–5 μg per cigarette.

Conflicting reports have appeared concerning the presence of the acyclic monoterpene, alloocimene, in cigarette smoke. Initially (647) the terpene was claimed to be a smoke component having cocarcinogenic

TABLE II

ALKENES AND ALKYNES IN TOBACCO LEAF AND SMOKE

Hydrocarbon	Reference: Leaf	Reference: Smoke
	Isoprenoid	
α-Carotene	631	...
β-Carotene	133, 263, 631	...
2,4-Dimethyl-4-vinylcyclohexene	...	100, 264
Dipentene	...	100, 204, 205, 263, 264, 395, 644
Farnesene	...	52
Isomeric squalenes	...	263, 296, 463
Isoprene	...	79, 202–206, 263, 378, 415, 621
Isoprenoid polyolefins	...	116
1-Methyl-4-isopropyl-1-cyclohexene	...	205
Neo-β-carotene	263, 631	...
Neophytadiene	46, 187, 466	85, 263, 461
Neophytadiene dimers	...	265
Norphytene	...	265
Phytadienes	...	452, 461
Phytoene	631	...
Phytofluene	631	...
β-Pinene	...	206
Solanesenes	...	461, 463, 644
Squalene	...	85, 263, 296, 461, 644
	Others	
Acetylene	...	79, 263, 378, 415
Allene	...	79, 378, 415
1,2-Butadiene	...	378, 415
1,3-Butadiene	...	79, 263, 378
1-Butene	...	79, 378, 415
2-Butene	...	79, 263, 378, 415
3-Buten-1-yne	...	415
1-Butyne	...	415
Cyclohexene	...	205
1,3-Cyclopentadiene	...	415
Cyclopentene	...	378, 415
1-Decene	...	205
2,3-Dimethyl-1-butene	...	413
3,3-Dimethyl-1-butene	...	205, 413, 415
Ethylene	...	79, 263, 378, 415
1-Hexene	...	413
2-Hexene	...	413
Methylacetylene	...	78, 79, 263, 415
2-Methyl-1-butene	...	78, 79, 378, 415
2-Methyl-2-butene	...	78, 378, 415
3-Methyl-1-butene	...	79, 378, 415
1-Methyl-1-cyclopentene	...	413
3-Methyl-1-cyclopentene	...	413
4-Methyl-1-cyclopentene	...	413
2-Methyl-1-pentene	...	413
2-Methyl-2-pentene	...	79, 413
3-Methyl-1-pentene	...	413
4-Methyl-1-pentene	...	378, 413
4-Methyl-2-pentene	...	378, 413
2-Methylpropene	...	79, 263, 415
Monoolefins (C_{10}–C_{32})	...	155
1,2-Pentadiene	...	79
1,3-Pentadiene	...	79, 205, 378, 415
1,4-Pentadiene	...	378, 415
1-Pentene	...	78, 79, 205, 378, 415
2-Pentene	...	78, 79, 378, 415
Propene	...	78, 79, 378, 415

activity and occurring in relatively large amounts (0.5%) in condensate. These findings were disputed in a later report (352) showing that alloocimene, if present at all in condensate, occurs in levels of less than 0.006%.

The presence of α-pinene and myrcene in cigarette smoke has been indicated (644), but details of the isolation and identification have not been published.

2. *Other Alkenes and Alkynes*

The lower molecular weight alkenes and alkynes occur chiefly, if not exclusively, in the gaseous phase of smoke. The list of known constituents in this group has grown considerably since 1959. Many of the newly reported constituents have been separated and identified by a combination of gas chromatography and mass spectrometric analysis. Although some of the constituents in the gaseous phase of tobacco smoke show important physiological activity, *e.g.*, ciliostasis, the lighter hydrocarbons therein are believed to be relatively nontoxic at low dosage (78).

The C_{10}–C_{32} monoolefins listed in Table II are a series of ethylenic hydrocarbons isolated from smoke by several procedures including thin layer chromatography on silicic acid containing silver nitrate. Six homologous series (VIII) of compounds have been found, including all of the possible *cis* and *trans* isomers. The structures were determined by identification of the alkanes after

(a) $CH_3(CH_2)_nCH{=}CH_2$	n = 7–25
(b) $CH_3(CH_2)_nCH{=}CHCH_3$	n = 9–28
(c) $(CH_3)_2CH(CH_2)_nCH{=}CHCH_3$	n = 7–26
(d) $C_2H_5(CH_3)CH(CH_2)_nCH{=}CHCH_3$	n = 6–25
(e) $(CH_3)_2C{=}CH(CH_2)_nCH_3$	n = 8–27
(f) $CH_2{=}C(CH_3)(CH_2)_nCH_3$	n = 9–28

VIII

hydrogenation and the methyl esters of the acids obtained by oxidation of the olefins. The total yield of olefins was about 3 mg per 1000 cigarettes, and the compounds are not present in the leaf.

C. AROMATIC HYDROCARBONS (TABLE III)

With the possible exceptions of benzene (558), toluene (558), isomeric xylenes (558), and a few polynuclear aromatic hydrocarbons (PAH) (75, 391), this class of compounds is found exclusively in tobacco smoke. The trace amounts of benzo[*a*]pyrene (BAP) and related PAH in leaf originate from atmospheric pollution during growth (74) or contamination with steam or air during curing and industrial processing (39, 74).

In addition to the usual chromatographic and spectrometric methods, precipitation with 1,3,5-trinitrobenzene has been used to obtain concentrates of low molecular

TABLE III

AROMATIC HYDROCARBONS IN TOBACCO SMOKE

Compound	Ref	Compound	Ref
Acenaphthene	86, 152, 263	2,6-Dimethylnaphthalene	100, 152, 266
Acenaphthylene	152, 263	2,7-Dimethylnaphthalene	100, 152, 266
Alkylbenzo[*a*]pyrene	152	2,5-Dimethylphenanthrene	152, 263
Alkylchrysene	152	Ethylbenzene	100, 152, 204–206, 267, 395
Alkylfluoranthene	152		
Alkylpyrene	152	Ethyltoluenes (*o*-, *m*-, *p*-)	100, 204–206, 395
Anthanthrene	86, 152, 263	Fluoranthene	16, 86, 100, 263, 449
Anthracene	16, 86, 152, 263, 449	Fluorene	152, 263, 449
Azulene	152, 263	Indene	100
Benz[*a*]anthracene	16, 86, 152, 263	Indeno[1,2,3-*cd*]fluoranthene	152
Benzene	79, 100, 152, 202–204, 206, 263, 378, 621	Indeno[1,2,3-*cd*]pyrene	16, 152
		Ionene	148
Benzo[*b*]fluoranthene	152, 263, 591	4-Isopropenyltoluene	100, 152, 267
Benzo[*g,h,i*]fluoranthene	86, 152	Isopropylbenzene	152, 267
Benzo[*j*]fluoranthene	152, 263	4-Isopropyltoluene	100, 205
Benzo[*k*]fluoranthene	152, 263	2-Methylanthracene	86, 152, 263
Benzo[*m,n,o*]fluoranthene	152, 263	9-Methylanthracene	449
5H-Benzo[*a*]fluorene	152	3-Methylbenz[*a*]anthracene	152, 263
11H-Benzo[*a*]fluorene	16, 86, 152, 263	5-Methylbenz[*a*]anthracene	152
Benzo[*b*]fluorene	152, 263, 449	11-Methyl-11H-benzo[*a*]fluorene	16, 152
7H-Benzo[*c*]fluorene	152	Methylbenzo[*a*]pyrene	587
11H-Benzo[*b*]fluorene	152	Methylchrysene	16, 152, 263
Benzo[*a*]naphthacene	152	8-Methylfluoranthene	152
Benzo[*g,h,i*]perylene	152, 449	1-Methylfluorene	86, 152, 263
Benzo[*c*]phenanthrene	152, 263	9-Methylfluorene	152, 263
Benzo[*a*]pyrene	16, 86, 152, 263, 449	1-Methylnaphthalene	100, 152, 266
Benzo[*e*]pyrene	86, 152, 263, 449	2-Methylnaphthalene	100, 152, 263, 266
Biphenyl	86, 449	1-Methylphenanthrene	86
Chrysene	16, 152, 263	9-Methylphenanthrene	86, 152
Coronene	152, 263	1-Methylpyrene	86, 152, 263, 449
Dibenz[*a,h*]anthracene	152, 263, 449	2-Methylpyrene	152, 263
Dibenzo[*a,i*]fluorene	152, 263	4-Methylpyrene	86, 152, 263
Dibenzo[*a,c*]naphthacene	152, 263	Methylstyrenes (*o*-, *m*-)	206
Dibenzo[*a,j*]naphthacene	152	Naphthacene	86, 100, 152, 263, 449
Dibenzo[*b,h*]phenanthrene	152	Naphthalene	86, 152, 263, 266
Dibenzo[*a,h*]pyrene	86, 152, 263	11H-Naphtho[2,1-*a*]fluorene	152, 263
Dibenzo[*a,i*]pyrene	86, 152, 263	Naphtho[2,3-*a*]pyrene	152, 263
Dibenzo[*a,l*]pyrene	86, 152, 263	Perylene	86, 152, 263, 449
Dibenzo[*cd,jk*]pyrene	152	Phenanthrene	16, 100, 152, 263, 449
9,10-Dihydroanthracene	152	Phenylacetylene	152, 263
5,6-Dihydro-8H-benzo[*a*]cyclopent[*h*]-anthracene	152, 263	Pyrene	16, 86, 152, 263, 449
		Styrene	100, 152, 204–206, 267, 395
10,11-Dihydro-9H-benzo[*a*]cyclopent-[*i*]anthracene	152, 263	Toluene	79, 100, 202–206, 267, 395, 621
3,4-Dihydrobenzo[*a*]pyrene	152, 263	Tribenz[*a,c,h*]anthracene	152, 263
16,17-Dihydro-15H-cyclopent[*a*]-phenanthrene	152	1,2,3-Trimethylbenzene	204, 206
		1,2,4-Trimethylbenzene	100, 152, 204–206, 395
9,10-Dimethylbenz[*a*]anthracene	152, 263	1,3,5-Trimethylbenzene	100, 152, 204–206
Dimethylchrysene	16, 152, 263	1,3,6-Trimethylnaphthalene	100, 152, 266
Dimethylfluoranthene	152, 263	Xylenes (*o*-, *m*-, *p*-)	100, 152, 204, 205, 267, 395
1,6-Dimethylnaphthalene	100, 152, 266, 267		
1,8-Dimethylnaphthalene	263, 266		

weight aromatic hydrocarbons which were subsequently identified by classical methods (100, 266). Levels of benzene, naphthalene, and alkyl derivatives thereof vary from 0.17 to 46 μg per cigarette (100).

Great interest has been shown in the PAH of tobacco smoke since many of these compounds are carcinogenic. More than 60 PAH compounds having three or more rings have been isolated thus far, not including the heterocyclic polynuclear aromatic compounds (see section III.J) and alkyl derivatives of PAH for which structures are not given. Details of the isolation, identification, and biological importance of PAH in smoke have been published elsewhere (644), and the present discussion will be limited to certain salient points and more recent supplementary information.

The major hydrocarbon of biological significance is BAP which occurs in smoke condensate in levels of about 1–2 ppm; other biologically active PAH are

generally present in lower concentrations with the possible exception of chrysene. The trace amounts of these compounds in smoke have necessitated extensive use of paper, thin layer and gas chromatography, and fluorescence spectrometry for isolation and identification (484–486, 644). The recent application of gas chromatographic capillary columns with electron-capture detection has aided greatly in the identification and analytical determination of these compounds in smoke (86, 449). Separation of the isomeric benzopyrenes has been achieved on capillary columns (77) and by gas–solid chromatography on columns of larger diameter containing lithium chloride (92).

PAH forms complexes with nitro aromatic compounds and purines such as caffeine and 1,3,7,9-tetramethyluric acid (TMU) (351, 588). TMU can be used to partition complex mixtures of PAH on a relatively large scale by countercurrent distribution between cyclohexane and 90% methanol (351) or to obtain concentrates of PAH by elution from silicic acid with a highly polar solution of the complexing agent and applied voltage (465). Small-scale separations can be achieved by paper electrophoretic methods using the same polar solution of complexing agent (465).

The fluorescence spectra of many of the PAH in tobacco smoke are available (586).

Conclusive identification of the minor PAH in tobacco smoke is difficult for several reasons. The small quantities of isolated material are readily susceptible to photodecomposition, especially on thin layer plates. Isolation of a single PAH free of closely related analogs and "background" spectral absorption is difficult to obtain with complete assurance. The isolated quantities frequently require spectral analysis by fluorescent methods, which may have higher absorptivities for PAH but may be less specific than ultraviolet measurements. In many reported cases, identifications of PAH must be considered as tentative if not questionable.

In addition to the listed compounds, the possible presence of several other PAH has been cited, including picene, benzo[*a*]fluoranthene, and dibenz[*a,c*]anthracene (86). On capillary columns, these compounds elute with benzo[*g,h,i*]perylene, benzo[*b*]fluoranthene, and dibenz[*a,h*]anthracene, respectively, all of which are established smoke constituents. The presence of these new PAH requires confirmation.

Published analytical methods to determine BAP and related PAH in smoke have been listed (120, 484–486, 644), and the obtained values for cigarette, cigar, and pipe smoke have been discussed (644). Since appreciable losses of BAP occur during isolation, the use of a radioactive marker is necessary to obtain accurate results, and most recent studies have employed the C^{14}-labeled hydrocarbon (16, 120, 449, 644) although perylene may be used as a substitute for radioactive BAP (120).

With the exception of arsenious oxide (312), certain radionuclides (see section IV), and the controversial nitrosamines (see section III.J), the only known tumor initiators in smoke are PAH and heterocyclic analogs (see section III.J). By following the distribution of tumorigenic activity of smoke condensate during chemical fractionation, it has been shown that the bulk of the tumor-initiating material occurs in the neutral subfractions eluted from silicic acid columns by hexane (644) or carbon tetrachloride (649). These subfractions contain many of the carcinogenic PAH of smoke, *e.g.*, benzo[*a*]pyrene, but in amounts too small to explain the total tumorigenic activity of smoke condensate in animals (138, 527, 641, 649). The difference between the PAH contribution and the over-all activity is usually explained by the cocarcinogenic activities of components in the acidic fractions, *e.g.*, phenols, which act to supplement the tumor-initiating properties of PAH without possessing activity themselves. However, data have appeared which show that levels of benzo[*a*]pyrene are not directly related to the tumor-initiating properties (311, 464). On the other hand, reduction in benzo[*a*]-pyrene has been employed as one of several criteria to determine the relative tumorigenicity of different smoke samples (646), and biological data confirming the use of such criteria have been obtained (233, 641). Thus, the question of what chemical compounds are responsible for the tumorigenic activity of smoke cannot be answered categorically. Assuming that the interaction of phenols and PAH are not exclusively responsible for the effect, two possibilities are evident: either unidentified tumor initiators and tumor promoters are present in the smoke, or, the various known biologically active compounds therein interact in a complex manner involving synergism, antagonism, and additive effects. Data to show that PAH may act competitively in reducing the tumorigenic activity of pure compounds have appeared recently (160). The biological activities of mixtures of PAH have not been studied extensively, and further information in this area is required. In relation to this, chrysene, a weak or "borderline" carcinogen, has been shown to act as a potent tumor initiator in the presence of croton resin, a strong promoter (591). The synthesis and biological activity of certain dibenzopyrenes, naphthopyrenes, and indenopyrenes have also been studied only recently (235).

Lists of carcinogenic polynuclear compounds found in tobacco smoke have appeared (232, 312, 587, 644) which contain from 7 to 17 compounds, including the polynuclear nitrogen-containing heterocyclics. Differences in the numbers of compounds listed may be reflections of the authors' interpretations of the relative importance of compounds having borderline tumorigenic activity since a paucity of biological data exists for many PAH.

Conspicuous by its absence from all lists is 2-methylcholanthrene, a highly potent carcinogen found in many pyrolytic products but unreported in cigarette smoke.

D. STEROLS AND OXYGENATED ISOPRENOID COMPOUNDS (TABLE IV)

Significant progress has been made recently in elucidating the nature of these leaf and smoke constituents, and several compounds not found previously in any plant species have been isolated and identified.

1. Sterols

The presence of free and bound stigmasterol, campesterol, and β-sitosterol in both leaf and smoke is well established. All of these compounds are 3-β-hydroxysterols having endocyclic unsaturation at C_5 and side chains at C_{17} which vary in carbon number and the presence of unsaturation (XXVI). Campesterol was initially found in the free form in leaf (146) and later as the glucoside in smoke (275) and as esters in leaf (104). Although the isolation of free ergosterol in leaf has been described in only one report (144), a $\Delta^{5,7}$ sterol having an ultraviolet spectrum similar to ergosterol has been found in the smoke of Argentinian cigarettes (80). A new unique tobacco sterol was reported some time ago in tobacco leaf (144) and then erroneously cited by others as chalinasterol (263). At the time, identification of this sterol was based on melting points and infrared spectral characteristics of the sterol and derivatives thereof. Later, the availability of gas chromatography permitted a reevaluation of these findings (275), and the sterol was finally shown to be a mixture of stigmasterol, β-sitosterol, and campesterol. The possible presence of β-sitostanol in leaf has been indicated (83), but no conclusive evidence was presented. In the earlier literature, free γ-sitosterol was reported in both leaf (263) and smoke (80, 263, 275, 296). However, more recent work (571) has shown that γ-sitosterol is probably a mixture of β-sitosterol and campesterol.

Recently, the presence of esterified cholesterol in flue-cured tobacco has been reported (104). This occurrence is somewhat unexpected since evidence for the presence of this sterol in plants has only been obtained in the last few years (37). Initial separation of esterified cholesterol and other sterols (stigmasterol, β-sitosterol, and campesterol) from a crude mixture was effected using countercurrent distribution and gel permeation chromatography. Cholesterol was identified mainly by mass spectrometric characteristics after further separation of the sterol mixture by preparative scale gas chromatography of the silylated and acetylated derivatives.

The presence of a ketosterol in leaf has been indicated, but no structural information was provided (133).

TABLE IV
STEROLS, OXYGENATED TERPENES, AND OTHER ISOPRENOIDS IN TOBACCO LEAF AND SMOKE

Sterols	Reference: Leaf	Reference: Smoke
Sterols		
Campesterol	104, 146, 263, 275	275
Cholesterol	104	...
Ergosterol	144	...
β-Sitosterol	83, 104, 133	80, 263, 275, 296, 462, 480
Stigmasterol	83, 104, 145, 162, 263, 275, 634	80, 263, 275, 296, 462, 480
Monoterpenes		
Borneol	263	...
l-Linalool	263	...
Diterpenes		
3,8,13-Duvatriene-1,5-diol (α-, β-)	470	...
4,8,13-Duvatriene-1,3-diol (α-, β-)	451	...
12α-Hydroxy-13-epimanoyl oxide	182	455
α_2-Levantanolide	183	...
Levantenolide (α-, β-)	181	101
α-5,8-Oxido-3,9,13-duvatrien-1-ol	471	457
α-5,8-Oxido-3,9(17),13-duvatrien-1-ol	471	457
β-5,8-Oxido-3,9(17),13-duvatrien-1-ol	471	...
Phytol	...	392, 457
Triterpenes		
β-Amyrin	167	167
Tetraterpenes		
Cryptoxanthin	631, 638	...
Flavoxanthin	263	...
Lutein	263, 631, 638	...
Neoxanthin	263, 631, 638	...
Violaxanthin	263, 631, 638	...
Zeaxanthin	631	...
Trisesquiterpene		
Solanesol	48, 247	263, 453, 461, 462
Related Isoprenoids		
6,8-Dihydroxy-11-isopropyl-4,8-dimethyl-14-oxo-4,9-pentadecadienoic acid	288	...
Farnesylacetone	...	396
Hexahydrofarnesylacetone	505	102
Solanochromene	467	...
Solanone	171, 262	102, 392
Tocopherols	467, 551	454, 461
Vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone)	468	...

In addition to campesterol, β-sitosterol, and stigmasterol, tobacco grown in tissue culture contains cycloartenol, 24-methylenecycloartenol, citrostadienol, and 28-norcitrostadienol (42). The identification of these sterols was made by mixture melting point determinations of steryl derivatives and mass and nmr spectrometric characteristics. In addition, a sterol was found having a molecular weight corresponding to cholesterol, but conclusive identification was not made. Evidence has also been obtained for the presence of cycloartenol in young tobacco leaves metabolizing in C^{14}-labeled sodium acetate solution (150).

The sterolins in leaf and smoke are glucosidated stigmasterol, β-sitosterol, and campesterol (145, 275, 634); although these compounds are heat-labile, demonstration of their distillation at 250° and atmospheric pressure (275) has confirmed that the glucosides can pass into the smoke unchanged. Details of the composition of the steryl esters of smoke leaf are given in section III.E.

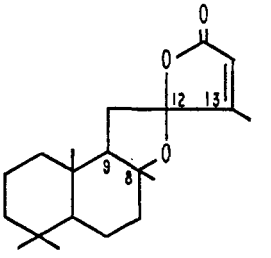
Because of the chemurgic value of sterols as starting materials in the synthesis of certain hormones, the levels of these compounds in leaf have been studied extensively. Total 3-β-hydroxysterols were determined by a gravimetric method based on precipitation with digitonin (544), a steroidal saponin which precipitates 3-β-hydroxysteroids regardless of the confirmation of the hydroxyl or presence of unsaturation. Using this reagent, the concentrations of total free and bound sterols in leaf were found to vary with tobacco type, but the maximum levels (about 0.45% of leaf weight) were too low to consider tobacco as an economic source of phytosterols. Also, no relationship between levels of total phytosterols and leaf quality was observed (546).

The sterols of tobacco may play some role in the biological effects of smoke either through *in situ* autoxidation to hydroperoxides and related compounds during curing and aging or through pyrolysis to polynuclear aromatic hydrocarbons during burning of the cigarette. In this regard, a hydroperoxide of cholesterol has been shown to be carcinogenic (162), and stigmasterol has been pyrolyzed to benzo[*a*]pyrene at 750°. Also, the pyrolysis products of a crude mixture of tobacco sterols have been shown to be carcinogenic in animals (651). The pyrolytic mechanisms of sterol degradation are discussed in section V.

2. *Oxygenated Isoprenoid Constituents*

The monoterpenes borneol and l-linalool were reported in leaf almost 25 years ago (263), but no confirmation of their presence has appeared since that time. Geraniol was initially claimed to be a constituent of smoke condensate (649), but later work indicated the isolate was probably solanesol (627).

Several new diterpenes have been isolated recently from tobacco and smoke. The levantenolides are epimeric lactones related to labdanolic acid which were obtained from a hexane extract of 1000 pounds of Turkish tobacco. α-Levantenolide (IX) is more stable

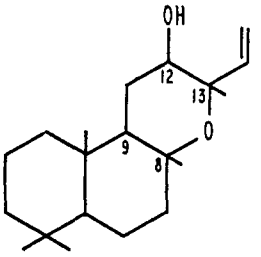


IX

and occurs in larger amounts in leaf than the β epimer, but both compounds are minor leaf components (about 0.0034% of leaf weight). In the determination of the basic structure of the epimers, hydrogenation and spectral analysis indicated the presence of a monounsaturated compound having a probable β-methylvinyl lactone group (181). Reduction with $LiAlH_4$, reaction with hydrazine, or saponification followed by lactonization yielded the same triol, pyridazone or lactone, respectively, from the two epimers and indicated the carbon (C_{12}) to which the lactone ring is attached. The ease of conversion in these reactions showed that the third oxygen is also linked to the C_{12} carbon and indicated the presence of a hemiketal or hemiacetal moiety in the structure. The basic skeleton was confirmed when treatment of the dihydropyridazone with KOH under Wolff–Kishner conditions yielded epimeric labdanolic acids. Further evidence of the structures was obtained by ozonization followed by either reductive or oxidative hydrolysis to yield either polyols or a tricyclic lactone of known composition in which the original C_{12} had been oxidized.

α_2-Levantanolide is an epimeric dihydro derivative of α-levantenolide found in Turkish tobacco. The structure of this terpene was assigned after a comparative study of the reduction products of α-levantenolide. Catalytic hydrogenation yielded two epimers (α_1 and α_2) having greatly different ratios of P to P − 15 ions in their respective mass spectra. Since the epimer with less steric hindrance of the methyl groups at C_{13} would cleave more readily, the compound having the higher ratio was assigned the 13-(*S*) configuration and was designated α_2. Confirmation of this designation was obtained by a sequence of reactions yielding methyl 13-epilabdanolate which is known to have the above configuration.

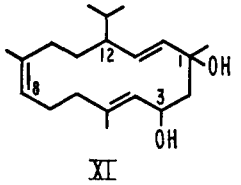
12α-Hydroxy-13-epimanoyl oxide (X) has been isolated from Turkish tobacco leaf and smoke. Hydrogenation, elemental and spectral analyses, and active hydrogen determination indicated the presence of a monounsaturated alcohol having two oxygens, one hydroxyl, one probable ether, and a terminal vinyl



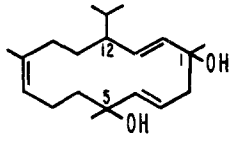
X

group. The nmr spectra of the isolated compound and the known 13-epimanoyl oxide were similar except for differences attributable to an α-C_{12} hydroxyl. Similarities in mass spectra between the isolated compound and certain known diterpenes (sclareol, manoyl oxide, and manool) confirmed the basic skeleton of the isolated compound. Ozonization followed by oxidative hydrolysis of the ozonide gave a lactone and an acetoxy acid of 16 and 18 carbon atoms, respectively, which were of known constitution and were consistent with the postulated structure. Oxidation of the isolated compound yielded a 12-keto derivative which, on Wolff–Kishner reduction, formed the known dihydro-13-epimanoyl oxide. The configuration of the hydroxyl group was established by the nmr spectrum and stereospecific reduction of dihydro-12-keto-13-epimanoyl oxide to give a saturated equatorial alcohol, which was not similar to the product obtained from the isolated compound, indicating an axial or α configuration for the tobacco isolate.

The diterpenoid duvatrienols and related ethers are a series of substituted macrocyclic olefins, which, with one exception (cembrene), have not been found previously in natural products. The proposed structures for the diols in this group are represented by XI and XII, and



XI

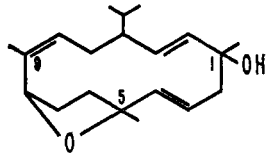


XII

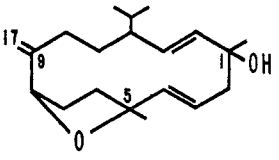
the C_1 epimers of both diols have been isolated from cigarette tobaccos. In the case of the 1,3-diols (XI), elemental, spectrometric, and related analyses showed a C_{20} compound with two hydroxyls (one secondary and one tertiary), three nonconjugated substituted double bonds, and an isopropyl group in both epimers (451). Oxidation of the perhydro derivative of α-XI yielded a keto alcohol which, on alkaline cleavage of the C_1–C_2 bond, gave a diketone. Hypoiodite oxidation of this diketone showed the presence of two methyl keto groups and established the partial structure C_1 to C_3. Oxidation of the C_3 hydroxyl of α-XI with manganese dioxide yielded a keto alcohol in which the carbonyl group was conjugated with a trisubstituted ethylenic bond having a single methyl group and provided further information on fragment C_2 to C_4. Retroaldol reaction of this keto alcohol gave ring scission between C_1 and C_2, and ultraviolet and nmr spectra showed the product to be a diketone with one carbonyl conjugated with a *trans* double bond which in turn was adjacent to a methine carbon. Thus, most of partial structure C_1–C_5 and C_{12}–C_{14} was established. Refluxing the diketone in sodium hydroxide split off acetone and gave a new diketone having one unconjugated, methyl-substituted carbonyl; in this case, cleavage occurred at the C_3–C_4 linkage and the reaction established the position of the C_5 methyl group. The nmr spectrum of α-XI showed the presence of methyls in an isopropyl group attached to a methine carbon and substitution at C_{12} was assigned, thus establishing all details of the partial structure C_{12}–C_{14} and C_1–C_5. The position of the remaining trisubstituted olefinic bond was determined by the isolation of the oxidation products, levulinic acid and 5-keto-2-isopropylhexanoic acid, resulting from the oxidative cleavage of the C_8 and C_{13} double bonds. Similar reactions were performed with β-XI, and the isolation of a common product from both the α and β compounds in certain cases indicated the compounds were C_1 epimers although epimerism at C_3 also remained a possibility. The geometrical orientations of the double bonds were established by spectrometric analysis or by analogy with acyclic systems, except for Δ^8 which was assigned *cis* without supporting data.

The epimeric 1,5-diols (XII) gave the same saturated hydrocarbon as the 1,3-diols on dehydration and hydrogenation, indicating a common ring system (470). Various analyses showed the presence of an allylic tertiary hydroxyl, a *trans*-disubstituted olefinic linkage, one $C(CH_3)=C$ group, two CH_3COH groups, and a hindered isopropyl group. Chromic acid oxidation of β-XI and β-XII yielded β-4,8,13-duvatrien-1-ol-3-one, which confirmed the general similarity of the diols and indicated an allylic rearrangement of β-XII presumably to β-XI during oxidation. In addition, levulinic and 5-keto-2-isopropylhexanoic acids were isolated, confirming the general similarity of XI and XII in respect to the structural fragment from C_9 to C_{13}. Although both isomers of XI rearranged to the isomers of XII on column chromatography using alumina, it was felt that the 1,5-diols were not artifacts produced from 1,3-diols by experimental manipulation since suitable precautions were taken throughout the work. β-XII was isolated in amounts corresponding to 0.0015% of tobacco leaf.

The duvatrienol ethers in Table IV have been isolated initially from flue-cured, burley, and Turkish tobaccos and later from cigarette smoke. The presence of an ether linkage in these compounds was established indirectly since an active hydrogen analysis accounted for only one of the two oxygens found by elemental analysis and molecular weight determination. In the case of XIII, the position of the ether linkage was obtained by perbenzoic acid oxidation of α-XI which gave an epoxide shown to be the 8,9 derivative by nmr analysis. Conversion of the 8,9-epoxide to α-XIII was then accomplished under a variety of conditions. The orientation of the C_1 hydroxyl was established by analogy with α-XI. The structure of the other oxidoduvatrienol (XIV) was determined by similarities in the elemental analyses and dehydration and hydrogenation products of α-XIII and α-XIV. The presence of exocyclic un-



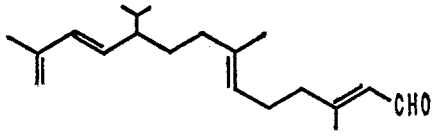
XIII



XIV

saturation at C_9 was deduced from nmr spectral data.

Recently, further evidence of the orientation of the C_8 olefinic bond in the duvatrienols has been obtained (106). In a study on the composition of tobacco flowers, an all-*trans* C_{20} aldehyde (XV) was isolated

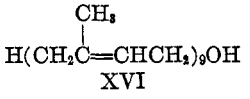


XV

which was identical with the product obtained by reaction of the β isomer of 4,8,13-duvatriene-1,3-diol (XI) with *p*-toluenesulfonic acid. On the basis of this evidence, it was claimed that the C_8 double bond in α- and β-XI is *trans* instead of the *cis* originally proposed but not actually determined.

Several esters containing either the diterpenoid alcohol, phytol, or the triterpenoid alcohol, β-amyrin, have been found recently in cigarette tobacco or smoke and are discussed in section III.E.

Among the higher terpenes, solanesol (XVI) has been



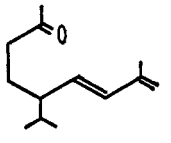
XVI

of interest to workers in fields other than tobacco chemistry. The alcohol was isolated initially from flue-cured tobacco (469) and is now believed to be a relatively ubiquitous plant constituent. Its occurrence in rat and human liver tissue (194) has been attributed to the ingestion of solanesol-containing foods. The alcohol was characterized originally as a pentaterpene, but later work established the presence of only nine isoprenoid units (157, 468, 515). Solanesol has gained the attention of biochemists because of its value as a source of isoprene units for the laboratory synthesis of metabolically active quinones, *e.g.*, ubiquinones and vitamin K analogs (294, 353, 383, 473, 515). A total synthesis of solanesol has been reported (473, 475) in which *cis* and *trans* forms of the alcohol are obtained. The *trans* compound, which is the natural isomer, occurs in two forms (α and β) having different melting points and spectral characteristics in the solid state. X-Ray diffraction studies have shown that the α form is planar, and the β form has successive isoprene units bending against one another (474). In tobacco leaf, solanesol is accompanied by olefinic analogs or isomers (81) and oxidation products (345, 447, 559), and an analytical method for "solanesol-like substances" (SLS) has been developed (48). The levels of SLS are relatively high, ranging from 1.9 to 2.5% for the different tobacco types, and most of this material is believed to be solanesol itself. Although no conclusive relationship between the levels of SLS and leaf "quality" exists (48), pyrolytic studies have shown that solanesol may be the source, at least in part, of dipentene in cigarette smoke (207), and thereby influence smoke aroma indirectly (see section V). Also, solanesol and other leaf terpenes have been postulated as primary leaf precursors of polynuclear aromatic hydrocarbons in smoke (179). Solanesol does not produce hyperplasia or destroy sebaceous glands when applied to mouse skin (644).

Solanesol occurs in both free and esterified forms; the esters are described in section III.E.

Since the structure of the isoprenoid benzopyran, solanochromene, was determined in part by analogy to solanesol (467), revision of the former structure to include one less isoprene unit has also been made (294, 468). Solanochromene may be formed by isomerization of Kofler quinone (2,3-dimethyl-7-solanesyl-1,4-benzoquinone) during chromatographic separation on acid-washed alumina (222).

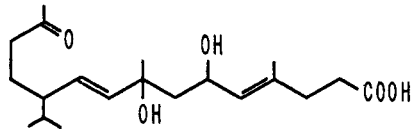
Several oxidative or other degradative products of terpenes are present in tobacco leaf and/or smoke. L-(+)-Solanone (XVII) was isolated initially from



XVII

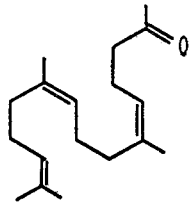
cigarette tobaccos (262) and later from cigarette (102) and cigar (392) smoke. The compound was characterized by elemental analysis, functional group tests, oxidative degradation, nmr spectral analysis, and syn-

thesis. The L configuration was established by synthesis of the D-(+) enantiomer of perhydrosolanone from D-3-isopropyl-6-ketoheptanal, giving a product which was identical with the hydrogenated tobacco isolate except for reversed optical rotation. In a later study (288) two isomers of an isoprenoid precursor (XVIII) of



XVIII

solanone were isolated from tobacco leaf and characterized by the general methods employed for solanone. XVIII may arise (288) by oxidative fission of the Δ^8 bond of the duvatriene-1,3-diols (XI) during growth, curing, or processing of tobacco. Since α-XI is quite labile at room temperature and is sensitive to acids (451), the possibility also exists that XVIII may be produced at least in part from α-XI during experimental manipulation. Farnesylacetone (XIX) was isolated in



XIX

cigar smoke condensate and identified by gas chromatographic and spectrometric comparison of the isolated compound and its hydrogenated product with synthetic XIX and hexahydro XIX. Hexahydrofarnesylacetone has also been found in cigarette smoke (102) and characterized by gas and spectrometric characteristics including a similarity with the C_{18} ketone obtained by ozonolysis of phytol or dihydroneophytadiene (46).

A few partially characterized isopenoids have been described in burley leaves including a monohydroxy α-carotene, β-carotene aldehyde, and possibly α-ionone (631). In a study of crude fractions of cigarette smoke containing phytol (457), several unsaturated alcohols of probable isoprenoid structure have also been isolated. On the basis of gas chromatographic behavior and spectral characteristics of the alcohols and oxidation products thereof, compounds having the properties of farnesol, dihydrofarnesol, and analogous C_{20}, C_{25}, and C_{30} alcohols were found.

Approximations have been made of the levels of several isoprenoid compounds in smoke from different tobacco types. The amounts of neophytadiene, phytadienes, squalene, solanesenes, α-tocopherol, and free and esterified solanesol and phytosterols show significant but not marked differences in the types (461).

E. ALCOHOLS (TABLE V) AND ESTERS (TABLE VI)

Extensive compositional studies on the vapor phase of cigarette smoke have shown the presence of a large number of organic compounds therein, including low molecular weight alcohols (202, 203, 206). In the most recent report in this series (205), a combination of gas chromatographic separation on capillary columns followed by mass spectrometric determination of effluents was employed to identify 84 components with certainty, including hydrocarbons, alcohols, aldehydes, ketones, nitriles, heterocyclic compounds, and a mercaptan. Probable identifications, empirical formulas, and molecular weights were also obtained on an additional 84 constituents. Estimates of the amounts of more than 35 of these constituents are available (203, 206). Knowledge of the vapor-phase constituents of smoke is important in studies on cigarette smoke filtration (see section VI).

The higher fatty alcohols occur in tobacco "sand," a mixture of small tobacco particles and soil covered with tobacco exudate ("gum") which is separated from the whole leaf during industrial processing. These alcohols have been characterized by melting points and infrared and mass spectral characteristics of the acetylated

TABLE V
ALCOHOLS IN TOBACCO LEAF AND SMOKE

Aliphatic	Reference: Leaf	Reference: Smoke
Aliphatic		
Butyl alcohol	...	263
sec-Butyl alcohol	...	263
1-Docosanol	81, 335	102
1-Eicosanol	335	103
Ethyl alcohol	263, 390, 558	206, 263
1-Heneicosanol	335	103
1-Heptadecanol	335	103
Isobutyl alcohol	...	263
Methanol	263, 558	203, 204, 206, 263, 621
3-Methyl-1-pentanol	390	...
1-Nonadecanol	335	103
1-Octadecanol	335	103
Propyl alcohol	...	263
1-Tetracosanol	...	103
1-Tricosanol	335	103
Aromatic		
Benzyl alcohol	263, 390	263
β-Phenethyl alcohol	263, 390	263
Polyols		
Diethylene glycol	170, 305	263
Ethylene glycol	...	263
Glycerol	109, 170, 263, 406	263, 308, 321
Propylene glycol	95, 109, 170, 305	263, 308, 321, 623
Triethylene glycol	629	50, 263
Cyclic		
Furfuryl alcohol	68, 263	69, 210
Inositol	1, 263	...
Menthol	319, 336, 390	321, 336

TABLE VI
ESTERS IN TOBACCO LEAF AND SMOKE

Compound	Reference: Leaf	Reference: Smoke
β-Amyrenyl esters	167	167
Benzyl acetate	68, 263	69, 263
Benzyl benzoate	...	495, 503
Benzyl cinnamate	...	495, 503
Butyl acetate	...	206
Dibutyl phthalate	541, 551	...
Di(2-ethylhexyl) phthalate	87, 644	...
Dipropyl phthalate	541, 551	...
Esters of higher fatty alcohols	...	460, 644
Ethyl acetate	263	204, 206, 263, 378
Ethyl butyrate	263	263
Ethyl caproate	263	206, 263
Ethyl formate	...	263, 378
Ethyl isovalerate	263	263
Ethyl β-methylvalerate	263	263
Ethyl propionate	263	263
Ethyl valerate	263	...
Glycerides	104, 219	...
Glyceryl triacetate	...	321, 623
Hentriacontanyl hentriacontanoate	...	263
Isopropyl formate	...	378
Methyl acetate	...	79, 204–206, 378, 621
Methyl acrylate	...	206
Methyl and ethyl esters of higher fatty acids	263, 549	...
Methyl formate	...	202–204, 206, 378, 621
Methyl isocyanate	...	412
Methyl nitrite	...	263, 410, 520
Methyl propionate	...	79
Methyl salicylate	263	...
Methyl thionitrite	...	414
β-Phenethyl acetate	263	...
Phytyl esters	...	459
Solanesyl esters	87, 247, 468	453, 461, 462, 644
Steryl esters	104, 551, 559	461, 462
Undecyl acetate	87	...
Vinyl acetate	...	378

derivatives. The major component in both smoke and "sand" is 1-heptadecanol. A similar series of higher fatty alcohols was probably isolated from Maryland tobacco (107, 594), but conclusive identifications were not made.

The humectants and flavoring additives used in domestic cigarettes are frequently transferred into the smoke. The glycols, glycerol, and menthol listed in Table V arise mostly from this source, although significant amounts of naturally occurring glycerol are found in the leaf. The levels of total glycerol in processed cigarette tobacco are about 3–40 times the naturally occurring amounts (109). One report has appeared on the use of 1,3-butylene glycol as a humectant in foreign cigarettes (592). Although this compound may act as a fungicide, the more commonly used diethylene glycol and glycerine are inactive (592). Several methods for the determination of glycerol and the listed glycols have been published (95, 109, 170, 305, 321, 333, 406, 629, 640).

Phytin, a calcium magnesium salt of inositol, is present in tobacco seeds (263) but has not been reported in leaf.

More than 300 esters have been identified in tobacco leaf and smoke (Table VI), and the bulk of these compounds consist of sterols, terpenes, and fatty alcohols esterified with higher fatty acids. In most reports identifications have been by gas cochromatography of known compounds with alcohols and acids released from the isolated esters after saponification. In a few instances, mass spectral data have been obtained to support the gas chromatographic findings. Stigmasterol and β-sitosterol esterified with lauric, myristic, palmitic, stearic, oleic, linoleic, and linolenic acids have been found in cigarette smoke (462). More recently, cholesterol and campesterol esterified with an incompletely defined mixture of C_{14}–C_{18} fatty acids have been reported in flue-cured leaf (104). Studies on the esterified terpenoid alcohols have generally shown a wider range of fatty acids therein compared to the reported steryl esters. The mixture of phytyl esters found in smoke contains a complex spectrum of acids: fourteen normal (C_{11}–C_{24}), two monounsaturated (C_{15} and C_{18}), one diunsaturated (C_{18}), four triunsaturated (C_{13}, C_{14}, C_{16}, C_{18}), and seven uncharacterized branched-chain, unsaturated acids. β-Amyrin has been found in both leaf and smoke esterified with octacosanoic and/or hentriacontanoic acids. Solanesol occurs as the acetate (453, 468) and in combination with the same fatty acids as stigmasterol and β-sitosterol in both leaf and smoke. The presence of solanesyl octanoate and decanoate in Oriental tobacco has also been claimed (247).

The most thorough investigation of the higher molecular weight esters of leaf or smoke has been performed on the waxes. These compounds were isolated as complex mixtures from smoke condensate and were characterized by two experimental approaches. In the first, the mixture was saponified, and the acidic fraction was methylated and separated by gas chromatography. The alcoholic fraction was then oxidized to the corresponding acids and similarly separated. Gas chromatographic and mass spectral analyses of the separated components were used to identify the acidic and alcoholic constituents. In the second approach, the crude ester mixture was pyrolyzed at 475°, splitting the ester and dehydrating the alcoholic moieties to the corresponding alkenes. The alkenes were oxidized with permanganate to the acids, and all acids were methylated and characterized as above. In this manner, 16 normal fatty alcohols (C_{12}–C_{27}) were found to be combined with 17 known (C_{14}–C_{28}, oleic, and linolenic) and several unidentified acids. Thus, more than 272 individual esters were probably present in the original mixture. Based on the demonstrated hyperplastic

effect of lower fatty alcohols on animal skin, the possibility that such esters may be tumor-promoting has been suggested (644).

The presence of methyl and ethyl esters of higher fatty acids (palmitic, stearic, oleic, linoleic, and linolenic) in flue-cured tobacco has been reported. In one case (549), both methanol and ethanol were found after saponification of the isolated esters and were identified by mixture melting point determinations of the phenylurethan derivatives.

Several aromatic esters have been found in leaf and smoke including at least three phthalates which are true leaf constituents and not contaminants obtained from plastic tubing or other extraneous sources. Several esters of benzoic and cinnamic acids have been isolated from the nitromethane-soluble neutrals of smoke condensate, including benzyl benzoate and benzyl cinnamate which were identified by spectral (infrared and mass) and gas chromatographic characteristics. Evidence was also obtained for cinnamyl cinnamate and styryl cinnamate (495), but conclusive identification could not be claimed. Styryl cinnamate is of particular interest since this compound has not been synthesized or isolated from a natural source. The primary source of the benzoate and cinnamate esters in smoke may be the flavoring agents used in cigarette tobaccos (495).

The relatively low molecular weight esters occur mainly in the vapor phase of smoke. In addition to the listed components, evidence has been obtained for the presence of methyl propionate in Turkish tobacco (558). Whether or not these esters contribute to ciliostatic activity or organoleptic properties has not been shown.

Glycerides have been found in tobacco seeds (263) as well as in leaf and smoke. Saponification of the leaf constituents yields the common higher fatty acids (lauric, palmitic, stearic, oleic, linoleic, and linolenic) and glycerol, which has been identified tentatively by conversion to acrolein (104). Glyceryl triacetate is a plasticizer used as an additive in cigarette filters; apparently the compound volatilizes sufficiently during smoking to yield detectable amounts in smoke condensate (623).

The occurrence of methyl 2-furoate in tobacco leaf has been claimed (200), but no details of the identification were presented. The compound is believed to contribute to the aroma and flavor of cigarette smoke.

F. ALDEHYDES, KETONES, AND QUINONES (TABLE VII)

Aldehydes and ketones may contribute to the organoleptic properties of leaf and smoke, and extensive qualitative and quantitative studies have been conducted on the carbonyl compounds in different tobacco types, grades, and varieties (616, 617). In this work, the aldehydes and ketones were obtained by steam distillation, and the distilled carbonyls were allowed to react with 2,4-dinitrophenylhydrazine, giving the corresponding substituted hydrazones from which the free carbonyls were released by an exchange reaction with α-ketoglutaric acid and analyzed directly by gas chromatography. Correlations between leaf aroma and carbonyl levels were found to be positive with acetone and 2-butanone and negative with isobutyraldehyde and isovaleraldehyde. The total carbonyl levels in tobaccos were shown to be related indirectly to the moisture content. In respect to curing, a direct relation between carbonyl level and oxygen content of the atmosphere was demonstrated (616).

A detailed study of the composition of leaf trichomes has been made since these structures may contain organoleptically important components (87). Using an ingenious procedure, 20,000 green tobacco leaves were individually brushed to remove the trichomes, and about 7 g of ether-soluble oil was obtained. Several previously unidentified compounds were isolated from this extract and identified by the methods discussed above, including 2-pentanone and 4-methyl-2-pentanone. Representatives of several other chemical classes were also found in the trichome extract and are cited elsewhere in this report. Two special procedures have been proposed for the collection of the vapors from tobacco leaf without reducing the moisture content of tobacco (268, 558). Using one of these methods, butyraldehyde, valeraldehyde, and caproaldehyde were identified tentatively in Turkish tobacco (588).

The presence of several relatively high-boiling ketones in the high-vacuum distillate of tobacco leaf has been claimed (200): 4-methylacetophenone, 2-methyl-5-isopropylacetophenone, 6-methyl-2-hepten-2-one, and 2,6-dimethyl-2,6-undecadien-10-one. However, details of the characterizations were not presented. These ketones were claimed to enhance the flavor and aroma of cigarette smoke when added to cigarette tobacco.

Most of the low molecular weight aldehydes and ketones in cigatette smoke have been isolated from the vapor phase and identified by mass spectral characteristics (205) and/or gas cochromatography with authentic compounds (79, 202, 378). Classical identification by the preparation of 2,4-dinitrophenylhydrazones or related derivatives has usually been limited to the lower boiling constituents which occur in amounts sufficient to use semimicro methods. The levels of low-boiling aldehydes and ketones in smoke are influenced markedly by the moisture content of the tobacco: fivefold increases of acetaldehyde in smoke may occur on reducing the moisture content by one-half (403). However, some reported analytical values for aldehydes and ketones may be low due to the interreaction of HCN and carbonyls during smoke collection (361). The compounds formed in these reactions have not been identified, but acetaldehyde cyanohydrin may be present. On the basis of limited data (621), it appears

TABLE VII

ALDEHYDES, KETONES, AND QUINONES IN TOBACCO LEAF AND SMOKE

Compound	Reference: Leaf	Reference: Smoke
Aldehydes		
Acetaldehyde	245, 263, 511, 617	202–205, 263, 309, 340, 378, 403, 621
Acrolein	263	204–206, 309, 378, 621
p-Anisaldehyde	263	...
Benzaldehyde	263	263
Butyraldehyde	245	202–206, 263, 378, 403
Caproaldehyde	...	204–206, 378
Crotonaldehyde	263	79, 203, 205, 206, 378, 621
Formaldehyde	511	378
Furfural	263, 511	204, 205, 340, 448
Glycolaldehyde	263	...
Glyoxal	263	328
5-Hydroxymethylfurfural	263, 657	51, 657, 658
Isobutyraldehyde	263, 511, 617	79, 202, 204–206, 378
Isovaleraldehyde	87, 511, 617	79, 203–206, 340, 378
Mesoxaldialdehyde	263	...
Methacrolein	...	79, 203–206, 378
2-Methylbutyraldehyde	...	205
5-Methylfurfural	263	196
Methylglyoxal	263	263
2-Methyl-4-pentenal	...	205
Methylreductone	600	...
2-Methylvaleraldehyde	...	378
Pivaldehyde	...	204–206, 378
Propionaldehyde	245, 511, 617	79, 202–206, 263 340, 378
Reductone	263	...
m-Tolualdehyde	68, 263	...
Valeraldehyde	245, 617	79, 205, 206, 340, 378
Ketones		
Acetone	87, 263, 511, 617	79, 203–206, 263, 309, 340, 378, 403
2-Acetylfuran	...	205
2,3-Butadione	...	79, 204, 205, 263, 378, 621
2-Butanone	87, 263, 511, 617	79, 204–206, 621
Butenone	...	203, 205, 378
Cyclopentanone	...	204, 205
2,4-Dimethyl-3-pentanone	...	204, 205
4-Heptanone	...	204, 205, 263
2-Hexanone	...	204, 205
3-Hexanone	...	204, 205
3-Methyl-2-butanone	...	79, 202, 204–206, 378
3-Methyl-3-buten-2-one	...	204–206
Methyl naphthyl ketone	...	86
2-Methyl-3-pentanone	...	204, 205
3-Methyl-2-pentanone	...	205
4-Methyl-2-pentanone	87	204, 205
Methyl α-pyrryl ketone	263	...
Palmitone	...	263
2,3-Pentadione	...	204, 205, 263
2-Pentanone	87	202, 203, 205, 206, 263, 378
3-Pentanone	...	79, 204, 205, 378, 621
4-Penten-2-one	...	205
4-Penten-3-one	...	204, 205
Reductic acid	...	263
Quinones		
9,10-Anthraquinone	390	...
2,3,6-Trimethyl-1,4-naphthoquinone	...	89

that many of the low-boiling aldehydes and ketones occur mostly in the vapor phase of cigarette smoke.

Formaldehyde, acrolein, and crotonaldehyde have marked ciliostatic activity when tested in aqueous solution against the cilia of the water mussel (642). However, anomalous results have been obtained by another technique in which smoke is separated by gas chromatography and the peak containing acrolein is tested directly for ciliostasis (604).

Only one quinone has been found in cigarette smoke. An early citation (38) of the presence of 1,4-benzoquinone in cigarette smoke is erroneous; actually, hydroquinone was isolated (58, 569), and the suggestion was offered (58) that the compound may be present in smoke as the quinone. 2,3,6-Trimethyl-1,4-naphthoquinone has been isolated from cigarette smoke in small quantities (about 1.5 mg per 50,000 cigarettes) and identified by spectrometric methods. The infrared spectrum of the isolate showed a highly conjugated carbonyl group (6.05 μ) and aromatic absorption which was the reverse of the usual pattern: a strong characteristic doublet appeared at 6.20–6.28 μ but a relatively weak band occurred at 11.80 μ. The mass spectrum gave a fragment (*m/e* 82) indicative of –COC (CH_3)=C(CH_3)– and characteristic of the 1,3 cleavage known to occur with quinones. Since tetrasubstituted double bonds absorb in the 6.25-μ region (due to C=C stretching) but not at the higher wavelengths (due to the absence of C–H vibrations), the enhanced absorption of the unknown in the 6.20–6.28-μ range resulted from a combination of aromaticity and tetrasubstituted olefinic unsaturation. The nmr spectrum indicated three aromatic hydrogens, six methyl hydrogens adjacent to an olefinic linkage, and three methyl hydrogens connected to an aromatic ring. The position of the aromatic substituent was established through synthesis of the two possible isomers by chromic acid oxidation of the corresponding trimethylnaphthalenes and comparison of the spectral and chromatographic characteristics of the synthetic compounds with the isolate.

The leaf precursor of the quinone in smoke was postulated to be 2-methyl-3-phytyl-1,4-naphthoquinone (vitamin K_1).

The presence of probable analogs of plastoquinone (2,3-dimethyl-5-solanesyl-1,4-benzoquinone) in senescent tobacco leaves has been cited (572) and an unidentified quinone amine has been reported in tobacco leaf (593). Phenolic aldehydes and ketones are discussed in section III.I.

G. NITRILES, CYCLIC ETHERS, AND SULFUR COMPOUNDS (TABLE VIII)

The known aliphatic nitriles and cyclic ethers occur in the vapor phase of smoke and have been separated on capillary columns and identified by mass spectral comparisons and cochromatography with authentic compounds (205). Cinnamonitrile and 3-phenylpropionitrile have also been found in cigar (392) and/or cigarette smoke (89) condensates.

Methyl thionitrite (CH_3SNO) and carbon disulfide have been isolated from cigarette smoke and identified in an interesting infrared and mass spectrometric study (414). Strong bands appear at 5.22, 6.52, 7.69, and 15.27 μ for methyl thionitrite, and major fragments are obtained at mass 77 (parent peak) and 94, which is dimethyl disulfide, a product of recombination. Approximations of the levels of methyl thionitrite and carbon disulfide in the gaseous phase of smoke have been made on the basis of spectral absorption at 6.52 and 6.57 μ, respectively, and the obtained values were about 0.2 and 8–10 ppm, respectively.

Recently, dimethyl sulfide was isolated from cigarette smoke and identified in a similar fashion (409). The mass spectrum of this compound shows a strong fragment at m/e 62, a base peak at P−15, and numerous other ions attributable to $(CH_2S)^+$, $(CHS)^+$, $(H_3S)^+$, etc. In another study (343) dimethyl disulfide was identified in tobacco smoke during a study of sulfur compounds therein (343). Cigarette smoke was passed through mercuric chloride solution and the precipitated sulfur compounds were regenerated with acid or alkali, yielding eight components of which dimethyl disulfide and methanethiol were identified mainly by mass spectral characteristics. Dimethyl disulfide may be derived from methyl thionitrite or methanethiol in the smoke.

Although generally associated with undesirable organoleptic properties, low molecular weight sulfur compounds contribute to the characteristic flavor of several foods, such as garlic, onion, and horseradish. Synergisms may occur in mixing individual components which are undetectable individually, resulting in unusual organoleptic effects (362). Although present in very low concentrations, the sulfur compounds in cigarette smoke may act in a similar fashion to influence flavor and aroma.

TABLE VIII
NITRILES, CYCLIC ETHERS, AND SULFUR COMPOUNDS IN TOBACCO SMOKE

Compound	Reference
Nitriles	
Acetonitrile	79, 202, 203, 205, 206, 378
Acrylonitrile	202, 205, 206, 378
Butyronitrile	202, 204–206
Capronitrile	202, 204–206
Cinnamonitrile	495
Crotononitrile	205, 378
Cyanogen	263
Hydrogen cyanide	205, 378
Isobutyronitrile	202, 204, 206
Isocapronitrile	204, 205
Isovaleronitrile	204, 205
Methacrylonitrile	79, 204, 205, 378
3-Phenylpropionitrile	89, 392
Propionitrile	202–206, 378
Valeronitrile	202, 204–206
Ethers	
2,5-Dimethylfuran	79, 100, 202, 203, 205, 206, 267, 378, 621
Furan	79, 202–206, 378, 621
Methylfuran	79, 202–205, 378, 621
Tetrahydrofuran	206, 378
Tetrahydropyran	204, 206
Sulfur Compounds	
Carbon disulfide	414
Carbonyl sulfide	263
Dimethyl disulfide	343
Dimethyl sulfide	388, 409
Hydrogen sulfide	205, 378, 263
Methanethiol	205, 343
Methyl thionitrite	414
Thiocyanic acid	263
Thiocyanogen	263
Thiophene	206, 378

Sulfur-containing amino acids and oxygenated derivatives of furan are discussed in sections III.M and F.

H. ACIDS (TABLE IX)

Both domestic (497, 504) and foreign (128, 246, 271) tobaccos contain significant amounts of the C_1–C_{10} fatty acids including branched-chain isomers. Turkish and flue-cured leaves contain more of the C_3–C_{10} acids than burley and Maryland (504). Characteristically, Turkish tobacco and smoke have a high proportion of β-methylvaleric acid and high ratios of branched-chain to normal isomers of the C_5 and C_6 fatty acids (539). These compositional differences apparently contribute to the distinct organoleptic properties of Turkish tobacco smoke since a mixture of isovaleric and β-methylvaleric acid can be substituted for Turkish tobacco in blended cigarettes (547, 548). The hydroxylated derivatives of valeric, β-methylvaleric, and isocaproic acids are also believed to influence the aroma of Turkish tobacco smoke. The industrial practice of blending the four tobacco types in making American

TABLE IX

ACIDS FOUND IN TOBACCO LEAF AND SMOKE

Acid	Reference: Leaf	Reference: Smoke
Acetic	87, 246, 255, 263, 271, 272, 389, 497, 504	128, 220, 263, 498, 538
Adipic	...	263, 499
Arachidic	263	263
Arachidonic	263	...
Auxin and indoleacetic acid	263, 280, 407	...
Azelaic	174	...
Benzoic	174, 263, 271, 272	263, 394, 499
Butyric	87, 271, 497, 504	128, 263, 538, 539
C_{10}–C_{23} (saturated)	99, 219, 263, 624	263
C_{10}–C_{34} (normal)	347	...
C_{15}–C_{26} (iso, anteiso)	347	...
C_{16} + C_{18} (hydroxy)	347	...
C_{22}–C_{25} (cyclohexyl)	347	...
$C_{10}H_{12}O_2$	...	263
$C_{12}H_{12}O_5$	...	263
Caproic	246, 263, 271, 497, 504	128, 263, 538, 539
Cerotic	...	263
Citric	255, 263	...
Crotonic	263, 271	...
Decanoic	246	73
A fluorenecarboxylic acid	...	263
Formic	246, 255, 263, 271, 272, 497	128, 263, 498
Fumaric	255, 263	...
Furoic	263, 271	263, 438, 499
Glutaric	...	263, 499
D-Glyceric	263	...
Glycolic	14	263, 438, 499
Glyoxylic	13, 263	263
Heptanoic	497, 504	263, 538, 539
α-Hydroxyisocaproic	173	...
β-Hydroxyisocaproic	173	...
α-Hydroxy-β-methylvaleric	172	...
β-Hydroxy-β-methylvaleric	172	...
Hydroxypyruvic	263	...
α-Hydroxyvaleric	173	...
Isobutyric	263, 271, 504	128, 263, 538, 539
Isocaproic	504	538, 539
2-Isopropylmalic	175	...
Isovaleric	263, 271, 497, 504	128, 538, 539
α-Ketoglutaric	13, 263	263
Lactic	14, 263	263, 438, 499
Lauric	246, 263	263
Levulinic	...	263, 438, 499
Linoleic	263, 560	263
Linolenic	263, 560	263
Maleic	255, 263	...
Malic	255, 263	263, 499
Malonic	255, 263	263, 438
α-Methylbutyric	263, 271	...
β-Methylvaleric	263, 271, 389, 497, 504	263, 538, 539
Myristic	87, 248, 263, 560	263
Nonanoic	504	73, 263
Octanoic	246, 497, 504	263
Oleic	248, 263, 560	263
Oxalacetic	13, 189	...
Oxalic	255, 263	263, 438, 499
Palmitic	87, 248, 263, 560	263, 499
Palmitoleic	...	263
Phenylacetic	263, 271, 389	394
α-Phenyllactic	174	...
α-Phenylpropionic	...	394
Phenylpyruvic	13	...
Phthalic	...	263, 499
Propionic	87, 255, 263, 271, 497, 504	128, 263, 538
Pyruvic	255, 263	263
Sorbic	...	394
Stearic	263, 560	263
Succinic	255, 263	263, 438, 499
Terephthalic	263	...
Toluic acids (*m*-, *p*-)	...	394
Valeric	248, 263, 271, 497, 504	538, 539

cigarettes produces unexpected quantitative changes in the volatile acid content of the smoke. Apparently, the inclusion of Turkish tobacco synergizes the release of the C_4–C_7 acids into the smoke possibly through alteration of the burn rate of the cigarette (539). As a group, the volatile fatty acids are believed to contribute to the over-all leaf aroma and smoke flavor. Indications of a relationship between the levels of C_1–C_7 acids and organoleptic properties have been obtained (504, 536, 538), but the relationship is influenced by the amounts of simple pyridine components in the case of cigarette smoke (502, 538). The carbohydrates of tobacco are apparently not a primary source of the volatile acids of tobacco smoke (438, 538).

Much of the above data were obtained by gas chromatographic separations and estimations of the volatile fatty acids and methyl esters thereof. Although such methods are satisfactory for comparing samples with large differences, significant losses of compounds may occur during manipulation (543), and other procedures must be used to determine volatile fatty acids quantitatively, *e.g.*, separation of the sodium salts by partition chromatography (73) or *in situ* methylation on ion-exchange resins (387). Using such methods, formic and acetic acids are found to comprise about 75% of the volatile acids in cigarette (73) or cigar (498) smoke and are the predominant components in the volatile acid fraction of tobacco leaf (270).

Although the major higher fatty acids of tobacco are the common C_{16}–C_{18} saturated or unsaturated compounds (560), about 15–25 minor components are found in this fraction. The skeletal structures of some of these acids have been determined recently (347). The free and bound acids extracted from tobacco were saponified and converted to hydrocarbons by a reaction sequence involving methylation, reduction of the

methyl esters to the alcohols by $LiAlH_4$, halogenation of the alcohols, and reductive dehalogenation of the resulting halides to paraffins. The normal, iso and anteiso hydrocarbons were separated by molecular sieve and urea occlusion, and gas chromatographic and mass spectral analyses were used to identify these and other branched-chain components. Cyclohexyl compounds were detected by characteristic peaks at *m/e* 82 and 83, but cyclopentyl derivatives were absent. Approximately 90% of the total acids had 10–34 carbon atoms, 4.1% were methyl- (C_{15}–C_{26}) or cyclohexyl- (C_{22}–C_{25}) substituted compounds, and 5.9% were more complex in structure. The polar acids were mainly hydroxylated derivatives of palmitic and stearic acids. Although this method provides a means of determining carbon skeletons, differentiation of saturated and unsaturated acids is not possible.

Superficial studies on differences in the free higher fatty acids contents of various tobacco types show that air-cured and fire-cured tobaccos contain less than flue-cured and Turkish (560). In flue-cured tobacco, a slight tendency toward lower levels of linolenic acid in lower quality grades is observed, but this trend may be insignificant. A quantitative method for such determinations in cigarette smoke has been announced recently (626).

The nonvolatile acids of tobacco leaf can be isolated and determined accurately in tobacco leaf using low-temperature, liquid–liquid partition chromatography on silicic acid (255). The major acids in flue-, air-, and fire-cured tobaccos are citric, malic, oxalic, and malonic, and the proportions vary widely with tobacco type. The minor nonvolatile acids are glycolic, succinic, maleic, fumaric, and pyruvic. In general, the differences in the proportions of major and minor acids are a reflection of widely variable cultural and curing practices. The keto acids of tobacco leaf have been analyzed satisfactorily by paper chromatographic separation of the 2,4-dinitrophenylhydrazones and ultraviolet spectral determination of the eluted spots (189).

The partition chromatographic method used for leaf acids has not been applied to cigarette smoke. A favorite procedure for determining nonvolatile strong acids in smoke condensate involves the following steps: extraction of condensate with aqueous alkali; acidification of the aqueous solution followed by discontinuous or continuous ether extraction; methylation of the partitioned acids with diazomethane or boron trifluoride; and gas chromatographic separation of the methyl esters. A column chromatographic step may be inserted prior to gas chromatographic analysis (394). The major objections to this experimental approach are the poor yields obtained in the methylation and the inefficiency of the ether extraction due to unfavorable partition coefficients. Using these methods, recoveries of lactic, glycolic, oxalic, malonic, furoic, levulinic, and succinic acids as low as 58% have been reported (394, 438). The total isolated levels of these acids are about 20–200 μg per cigarette, and succinic, lactic, and glycolic acids are the major components. In cigar smoke, succinic, furoic, lactic, and oxalic acids are the predominate constituents (499), and the amounts of total nonvolatile acids are lower than those of cigarette smoke on the basis of yield per gram of tobacco smoked. In general, keto acids in smoke are better estimated by the 2,4-dinitrophenylhydrazone method used for leaf acids, which gives values for pyruvic, α-ketoglutaric, and glyoxylic acids of 188, 64, and 23 μg per cigarette, respectively (189). Qualitatively, the method has revealed the presence of oxalacetic acid in leaf and the possible occurrence of oxalacetic, 2-ketoadipic, and 2-ketobutyric acids in cigarette smoke (189).

The sorbic acid reported in cigar smoke is derived from the microbial inhibitor added to cigars during manufacturing (394). No sorbic acid has been found in cigarette smoke.

The presence of phthienoic acid in cigarette smoke has been cited (649), but details of the identification were not given.

Formic, acetic, propionic, and butyric acids are ciliostatic when tested in fresh water mussels (642), and the strongly acidic fraction of cigarette smoke shows slight cocarcinogenic activity when painted on the backs of mice (645). The higher fatty acids may serve as tumor promoters in the over-all activity of tobacco smoke condensate in animals (644).

Amino, phenolic, and terpenoid acids are discussed in sections III.M.I and D.2, respectively.

I. PHENOLS AND PHENOLIC ETHERS (TABLE X)

Within this group are many components believed to influence tobacco quality or contribute to the physiological effects of smoke. Since 1962 three reviews on phenols in leaf and smoke have appeared, but none gives comprehensive coverage of the chemical constituents. One survey lists the components of leaf but not of smoke (628). Another work is concerned primarily with smoke constituents, including quantitative data, and gives some details on biosynthesis of polyphenols (223); in this review, the cited occurrences of the methyl ethers of catechol, cresols, and hydroxyacetophenones in smoke (223) are probably erroneous since the isolated compounds were extracted initially by aqueous alkali and then methylated for subsequent gas chromatographic analysis (82). The third survey emphasizes the physiological effects of phenols in smoke, including cocarcinogenesis and ciliostasis (644).

The present report supplements these reviews and includes details of earlier work omitted therein. Although original reports were consulted in developing Table X, the cited references in some instances are to the above reviews which list the earlier works. In-

TABLE X

PHENOLS AND RELATED COMPOUNDS IN TOBACCO LEAF AND SMOKE

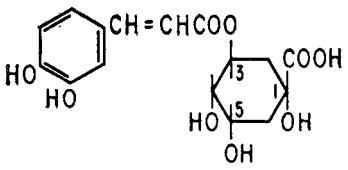
Compound	Reference: Leaf	Reference: Smoke
4-Allylcatechol	263	...
p-Anisaldehyde	263	...
Anisole	...	202, 206
Caffeic acid	223, 263, 656	174, 223, 655
1-O-Caffeoylglucose	665	...
4-Caffeoylquinic acid	665	...
Catechol	263	82, 223, 263, 525, 569
Chlorogenic acid	223, 255, 259, 263, 665	534
p-Coumaric acid	656	174, 223
p-Coumarylquinic acid	223, 263, 665	...
m-Cresol	263	82, 108, 223, 263, 398, 525, 539
Cresols (*o*-, *p*-)	...	82, 108, 223, 263, 398, 525, 539
2,6-Dimethoxyphenol	...	277
Esculetin	132	52, 132
Esculetin 7-glucoside	477	...
2-Ethylphenol	...	398, 525
3-Ethylphenol	...	223, 398, 525
4-Ethylphenol	...	223, 525
Eugenol	223, 263	458
Ferulic acid	174, 223, 656	223
1-O-Feruloylglucose	665	...
3-Feruloylquinic acid	665	...
Guaiacol	263	223, 263, 526
Hydrocaffeic acid	186	...
Hydroquinone	...	223, 263, 525, 569
Hydroxyacetophenone (*o*-, *m*-, *p*-)	263	263
Hydroxybenzaldehyde (*m*-, *p*-)	657	657
3-Hydrobenzoic acid	656	223
4-Hydroxybenzoic acid	656	174, 223
2-Hydroxyphenylacetic acid	656	223
3-Hydroxyphenylacetic acid	656	223
4-Hydroxyphenylacetic acid	174, 656	174, 223
3-Hydroxyphenylpropionic acid	656	223
4-Hydroxyphenylpropionic acid	656	223
Isoeugenol	223	458
Isoquercetrin	223, 263, 358	...
Isovanillic acid	...	535
Kaempferol 3-rhamnoglucoside	223, 358	...
Melilotic acid	223	...
3-Methoxyphenol	...	223
4-Methoxyphenol	...	223
Methyl salicylate	263	...
1-Naphthol	...	223, 263
2-Naphthol	...	223, 263
Naringenin	186	...
Naringin	186	...
Neochlorogenic acid	259, 263, 665	534
Phenol	263	82, 108, 223, 263, 398, 525, 539
Protocatechuic acid	656	174, 223
Protocatechuic aldehyde	657	223, 657, 658
Quercetin methyl ethers	358, 654	...
Quercimeritrin	186	...
Quinic acid	263, 380	...
Quinic acid γ-lactone	...	360
Resorcinol	...	223, 263, 525
Rutin	223, 263, 358	...
Salicylaldehyde	263	223
Salicylic acid	...	535
Scopoletin	131, 223, 263, 272	52, 223, 263, 569, 608
Scopoletin 7-glucoside	129, 131, 223, 263	...
Scopoletin rhamnoglucoside	653	...
Shikimic acid	263, 380	...
Sinapic acid	...	223, 656
Syringaldehyde	657	657
Syringic acid	656	223
Thymol	...	342, 343
1,2,3-Trimethoxybenzene	390	...
2,3,5-Trimethylphenol	...	223, 525
2,4,6-Trimethylphenol	...	223
Vanillic acid	656	174, 223
Vanillin	657	657
2,3-Xylenol	...	525
2,4-Xylenol	...	108, 223, 263, 398, 525
2,5-Xylenol	...	223, 398, 525
2,6-Xylenol	...	108, 223, 525
3,4-Xylenol	...	223, 398, 525
3,5-Xylenol	...	263, 392, 398, 525

cluded in the table are certain polyfunctional phenols and components which are not true phenols but are intimately related to this group, *e.g.*, quinic and shikimic acids. The polyphenolic pigments are discussed in section III.K.

Much of the recent work on phenols has concerned the identification of minor constituents of leaf and smoke. Because of the small quantities isolated, identifications have been made mainly by spectrometric and paper and gas chromatographic methods. Since techniques for the gas chromatographic separation of flavonoids, aromatic aldehydes and ketones, and depsides of quinic acid have not been available until very recently, identifications of these compounds have been made mostly by spectrometric and paper chromatographic methods, including color reactions of separated spots. Studies on the simple phenols have used mostly gas chromatographic methods, although colorimetric techniques have been employed in quantitative procedures.

1. Chlorogenic Acids

The major polyphenols in tobacco leaf are chlorogenic acid and rutin. Tobacco and other natural products also contain isomers of chlorogenic acid, and the structures of these isomers have now been established. Four possible monodepsides of quinic and caffeic acids are possible, including the common 3 isomer, chlorogenic



XX

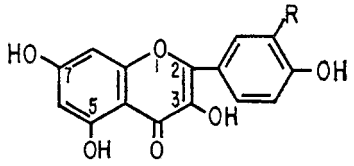
acid (XX). In earlier investigations, isochlorogenic acid was believed to be 5-caffeoylquinic acid (263), but recently the substance has been shown to be a complex mixture of dicaffeoylquinic acid isomers, with linkages at the 3,4, 3,5, and 4,5 positions, and methyl ethers thereof. The positions of substitution were established by a sequence of reactions culminating in periodate oxidation to establish the positions of hydroxyl groups (489) and by nmr data in which the presence of four olefinic protons and expected chemical shifts and spin–spin couplings of other hydrogens were observed (105). Similar approaches were used to establish the structure of neochlorogenic acid as 5-caffeoylquinic acid (488, 602). Prior to this information, the isolation and identification of isochlorogenic and neochlorogenic acids from tobacco leaf had been described, and the properties of the latter compound had been claimed to be similar to synthetic 1-caffeoylquinic acid (260). Obviously, neither identity was correct, although it is possible that one of the dicaffeoylquinic acid isomers in the isochlorogenic acid mixture was isolated. 1-Caffeoylquinic acid is not believed to occur naturally (602), and its presence in leaf is questionable.

Early studies have described the isolation of a rhamnoside (512) of chlorogenic acid and a nicotine–chlorogenic acid complex from leaf (599, 601), but no confirmation of these reports has ever appeared. However, compounds of this type could be related biosynthetically to the recently isolated high molecular weight pigments containing chlorogenic acid, rutin, amino acids, silicone, and alkaloids (see Section III. K).

The possible occurrence of lactones of chlorogenic acid in tobacco leaf has been cited (259, 446), but identifications of such compounds have not been made.

2. *Other Polyphenols*

The principal flavonols of tobacco leaf, quercetin and kaempferol (XXI), occur mostly as glycosides, but

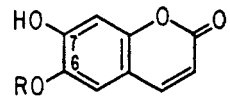


R = OH = QUERCETIN
R = H = KAEMPFEROL

XXI

small amounts of the aglycones may also be present (358, 628). In addition to rutin (quercetin 3-rhamnoglucoside), quercetin linked with glucose at the 3 position (isoquercetin) and the 7 position (quercimeritrin) have been found in leaves. Quercetin-3,3′-dimethyl ether has also been reported (654), and evidence for the presence of the 3-methyl and 3,7-dimethyl ethers in leaves has appeared (358). Kaempferol has been isolated from tobacco as the 3-rhamnoglucoside in several studies, and the possible occurrence of the 3-methyl ether of kaempferol has been cited (654). Only one report of the presence of flavanones has been published: naringenin (4′,5,7-trihydroxyflavanone) and naringin (the 5-rhamnoglucoside of naringenin) were isolated from leaves.

Scopoletin and esculetin (XXII) are the two couma-



R = H = ESCULETIN
R = CH_3 = SCOPOLETIN

XXII

rins occurring in both leaf and smoke. The presence of scopoletin 7-glucoside (scopolin) in leaf is well established, but only one report of a scopoletin rhamnoglucoside (653) has appeared. Two glycosides of scopoletin have been found in tobacco stems and roots but not in leaves: a xyloglucoside (primveroside) and a gentiobioside (482). The possible occurrence of esculetin 6-glucoside (esculin) in leaf or leaf suckers has also been cited (273, 591).

Tobacco flowers contain a variety of polyphenolic ethers and glycosides, some of which have not been reported in leaves: astragalin (kaempferol 3-glucoside), the 7-glucoside of nictoflorin (kaempferol 3-rhamnoglucoside), rutin 7-glucoside, and other glucosides of quercetin, kaempferol, ferulic acid, and *p*-coumaric acid (609, 610).

Tissue cultures of tobacco cells and metabolizing leaf disks also contain compounds not found in the growing plant or cured tobacco, such as glucosides, glucosamines, and glucose esters of *p*-coumaric, ferulic, and caffeic acids (44, 479).

The levels of rutin and chlorogenic acid in air-cured and flue-cured tobaccos differ markedly. In flue-curing a maximum temperature of about 80° is reached which inactivates the leaf enzyme responsible for oxidizing polyphenols, polyphenoloxidase. In air-curing, ambient temperatures are employed, and the enzyme is not inactivated significantly so that lower levels of chlorogenic acid and rutin are found in these tobacco types. The *in vitro* or *in vivo* oxidation of

polyphenols produces a variety of products which may condense with alkaloids or amino acids in "browning" or analogous reactions; experimental data and postulated mechanisms of these reactions in tobacco and other plants have been discussed elsewhere (169, 223, 224, 263, 329, 408, 445, 517, 597, 611, 628). The brown, polymeric pigments of tobacco leaf may be produced, at least in part, through such pathways and may be responsible for the deep brown color of air-cured tobaccos although there is evidence (*vide infra*) that these concepts are oversimplifications.

Polyphenols have attracted industrial attention in quality control and, to a lesser degree, in chemurgy. A direct relationship exists between leaf quality and levels of chlorogenic acid and rutin (628). Rutin has been used therapeutically in the treatment of capillary fragility and associated cardiovascular disease (199). Although tobacco leaf was the initial source of rutin for therapeutic testing, *Eucalyptus* and *Sophora* species have been used recently.

The polyphenols of tobacco leaf may serve as precursors of smoke phenols during burning of tobacco (see section V). Although chlorogenic acid (54) and eugenol (591) are weak cocarcinogens, their role in the tumorigenic activity of tobacco leaf extracts (53, 55, 591) or smoke condensates in laboratory animals is still unresolved. Rutin does not show tumorigenic activity in animals (644).

3. *Other Phenols and Phenolic Ethers*

Tobacco leaf contains small amounts of simple phenols and phenolic aldehydes, ketones, and acids. During burning of a cigarette many of these components enter the smoke through distillation and other physical mechanisms. However, the bulk of the phenols in smoke are formed by pyrolysis of cellular constituents (see section V). Since phenol, eugenol, and related compounds in cigarette smoke may act as ciliostats, cocarcinogens, etc., considerable attention has been devoted to the qualitative and quantitative composition of these smoke constituents.

Distillation of the steam-volatile, ether-soluble weakly acidic fraction of cigarette smoke condensate yields two fractions, bp 50–55° (2 mm) and 55–90° (2 mm), representing about 80 and 20% of the total, respectively (96). The first fraction contains almost all the phenol, cresols, and related phenols. The second fraction has many uncharacterized constituents, including possibly tetrahydro-2-naphthol. The fraction which is not steam-volatile contains complex, unidentified phenols, including possibly dihydroxypyrenes. Bioassays of fractions from distillations performed under slightly different conditions show that both the volatile and nonvolatile fractions have cocarcinogenic activity in animals (644); in this case, oleic and lauric acids, which are tumor promoters, are present in the nonvolatile residue. Gas chromatographic separations of the volatile phenols obtained by *in vacuo* or steam distillation is easily accomplished on many stationary phases, such as polyethylene glycols, silicones (398), and di-*n*-octyl sebacate (231); however, the sebacate loses resolution rapidly on aging (528). As expected, capillary columns are more effective for separation of certain isomers than larger diameter columns and can resolve the acetates of *m*- and *p*-cresols (525).

Intensive studies on the filtration of phenols from cigarette smoke (see section VI) have required the development of precise analytical methods. Colorimetric techniques for phenols have been employed using 4-aminoantipyrine (317), diazotized *p*-nitroaniline (523, 524, 526), and phenylazobenzenesulfonic acid (278) as reagents with coefficients of variation of less than 6% in some cases. Gas chromatographic methods (108, 231, 528) also have acceptable reproducibilities and may be preferable to colorimetric methods which frequently respond to interfering substances. A correlation can be shown between values obtained by gas chromatography and a colorimetric (4-aminoantipyrine) method (386). Analyses of commercial cigarettes by gas chromatographic techniques (229, 528) show the proportions of phenol:cresols (*m*-, *p*-):*o*-cresol:xylenols (2,4- and 2,5-) to be 10:5:2:2; however, larger proportions of phenol have been reported using a colorimetric method (526). The total levels of phenol, cresols, and related compounds in smoke from cigarette, cigar, and pipes may vary widely (229), and an indirect relationship exists between the moisture content of tobacco and the quantity of phenols in the mainstream smoke (153, 370).

Methods for the quantitative determination of catechol in cigarette smoke have been developed (348, 607), and values ranging from 40 to 500 μg per cigarette have been reported. In burley tobacco smoke, the catechol levels in smoke condensate are inversely proportional to the nitrate contents of the leaf (274) owing to reactions between catechol and oxides of nitrogen forming nitrocatechols as artifacts in the smoke collection system.

The pharmacologically active aromatic compound, myristicin (5-allyl-2,3-methylenedioxyphenyl methyl ether), has been identified in cigarette smoke condensate by infrared and mass spectral characteristics (501). This ether is a component of several natural products (276) and is responsible for the well-known physiological effects of nutmeg oil, *i.e.*, nausea, tachycardia, cyanosis, etc. (514, 612). The level of myristicin in smoke is at least 0.64 μg per cigarette and its contribution, if any, to the physiological effects of tobacco smoke is unknown (501). Myristicin occurs in the nitromethane-soluble neutral fraction of smoke and is accompanied by benzyl benzoate and benzyl cinnamate (503) which are common

components of natural oils and resins (275). Since domestic cigarettes contain flavoring additives (195, 213), it is possible that the myristicin in smoke is derived from this source rather than the tobacco leaf. Another possible source is through pyrolytic fission of elemicin (5-allyl-2,3-dimethoxyphenyl methyl ether), which may be present in cigarette leaf although strong evidence is lacking (390).

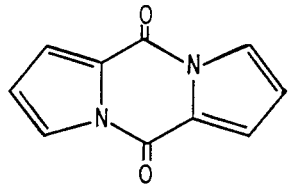
J. ALKALOIDS AND OTHER BASES (TABLE XI)

Two reviews on the alkaloids of tobacco and its smoke have appeared recently (492, 595). Although both works are concerned mainly with pharmacological and metabolic properties of alkaloids, some information on structure (399), occurrence (299, 492), biogenesis (399), and pyrolysis (299) was presented by various authors therein.

Since 1959, more than 70 new alkaloids and bases have been found in leaf and smoke. Alkaloids listed in older studies and subsequently shown to be mixtures, *e.g.*, obeline, sokratine, etc. (263), are not included in Table XI, although occasional reports on the presence of such components in tobacco still appear, *e.g.*, nicotimine (516). For the sake of convenience, Table XI includes some nitrogen-containing components which are more neutral than basic, such as aromatic secondary amines. The purines and pyrimidines listed therein occur in the free state; the presence of free xanthine in leaf is doubtful and free hypoxanthine is absent (331). Oxynicotine (nicotine N-oxide) has been claimed to occur in cigarette smoke (244); however, this alkaloid is thermolabile and cannot be recovered in the smoke from cigarettes containing added oxynicotine (428). Levels of metanicotine in leaf have appeared in an agronomic study but proof of identity was not provided (405).

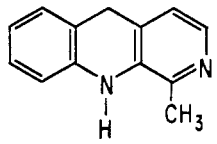
Relatively few studies have appeared recently on the basic chemistry of the alkaloids known prior to 1959. Syntheses have been reported for nicotine (220, 221, 320), nicotine analogs (156, 613), myosmine (220), N-methylmyosmine (320), and anatabine (434). The autooxidation of nicotine apparently occurs through a free-radical mechanism (313); although hydroperoxides have not been isolated from the oxidative products, myosmine, nicotyrine, cotinine, nicotine N-oxide, nicotinic acid, and possibly high molecular weight components occur therein (176, 598). The reaction between nicotine and ethylene oxide (an industrial fumigant and fermentation accelerator) is mainly an alkylation yielding N-2-hydroxyethylnicotine as the major product (17); the tartrate of this product has also been isolated from tobacco treated with the gaseous oxide (17). The mass spectral fragmentation of nicotine and other tobacco alkaloids has been studied using the deuterated bases (140). The principal peak in the fragmentation of nicotine occurs at *m/e* 84 corresponding to the N-methylpyrrolidinyl ion. A major peak occurs at *m/e* 133 (P − 29) which may be due to an ion formed by expulsion of C_3 and C_4 in the saturated ring possibly accompanied by hydrogen transfer from the 2 or 4 position in the pyridyl moiety. Similar patterns of fragmentation occur with nornicotine, anabasine, and cotinine, although the individual mechanisms of cleavage may differ from that of nicotine. Myosmine splits to give a base peak at *m/e* 118 (M − 28), and loss of the pyridyl moiety does not occur to any significant extent.

Pyrocoll, harmane, and norharmane have been isolated from leaf and/or smoke and identified conclusively. Isolation of pyrocoll (XXIII) was accomplished by



XXIII

solvent partition of cigarette smoke condensate followed by chromatography of the aqueous methanol solubles on alumina and distillation of the alkaloid from the benzene eluate (346). The isolated compound was characterized by elemental analysis, ultraviolet and infrared spectral characteristics, and/or mixture melting point determinations of the free base and its alkaline hydrolytic product, pyrrole-2-carboxylic acid, with authentic compounds. The level of pyrocoll in smoke is 1.4 μg per cigarette. Harmane



XXIV

(XXIV) and norharmane were isolated from cigarette tobaccos and smoke condensates by chromatography of the basic fraction on cellulose columns (421, 422). Identifications of the bases were made by paper chromatographic and ultraviolet, infrared, and fluorescent spectral characteristics. Although the alkaloids are present in both leaf and smoke, the levels in smoke are about 40–100-fold greater than those in leaf, indicating that pyrosynthesis of the bases occurs during burning (see section V). The levels of harmane and norharmane in smoke are about 10–20 μg per cigarette. The occurrence of pyrazine, alkylpyrazines, quinoxaline, benzimidazole, pyrrolo[2,3-*b*]pyridine, and pyrido[2,3-*b*]indole was reported recently in fractions of smoke condensate containing the harmanes. Analogs of some of these bases may also be present, *e.g.*, methylbenzimidazole (565). The presence of the benzacri-

TABLE XI: ALKALOIDS AND OTHER BASES IN TOBACCO LEAF AND SMOKE

Base	Leaf ref	Smoke ref
Adenine	331	...
Alkylcarbazoles	...	495, 503
Allylamine	...	400
Ammonia	263, 372	263, 368
Amylamine	...	341, 400, 401
sec-Amylamine	...	400
Anabasine	244, 263	244, 263, 269, 368, 436, 502
Anatabine	263, 516	263, 368, 436
Aniline	...	368, 400, 401
m-Anisidine	...	400
Benzimidazole	...	565
Butylamine	...	341, 368, 400, 401
sec-Butylamine	...	400
Carbazole	...	449, 456, 495, 567, 568, 503
Collidine	...	263
Cotinine	263	263, 436
3,4-Dehydropiperidine	...	368, 400
Dibenz[*a*,*h*]acridine	...	589
Dibenz[*a*,*j*]acridine	...	76, 589
7H-Dibenzo[*c*,*g*]carbazole	...	589
Diethylamine	...	368, 400
Dihydrometanicotine	...	368
9,9-Dimethylacridan	...	334
Dimethylamine	...	263, 368, 400
2,3-Dimethylaniline	...	400
2,4-Dimethylaniline	...	400
2,5-Dimethylaniline	...	400
2,6-Dimethylaniline	...	400
3,5-Dimethylaniline	...	400
Dimethylindoles	...	230, 456, 495, 503
2,6-Dimethylpyrazine	...	111, 565
2,3-Dimethylpyridine	...	393
2,4-Dimethylpyridine	...	393
2,5-Dimethylpyridine	...	393, 502
2,6-Dimethylpyridine	...	263, 393, 502
3,4-Dimethylpyridine	...	502
3,5-Dimethylpyridine	...	502
Diphenylamine	...	334
Dipropylamine	...	400
Ethylamine	372	263, 341, 368, 400
2-Ethylaniline	...	400
4-Ethylaniline	...	400
3-Ethylindole	...	230
3-Ethylpyridine	...	192, 393, 502, 652
Guanine	331	...
Hexylamine	...	400, 401
Harmane	421, 422	111, 421, 422, 565
Indole	...	111, 230, 456, 503, 566–568
Isoamylamine	372, 659	368, 400, 401
Isobutylamine	372, 659	368, 400, 401
N-Isobutylbutylamine	...	400
Isonicotein (2,3′-bipyridyl)	263	393, 502
Isopropylamine	...	400
N-Isopropylpropylamine	...	400
Isoquinoline	...	111, 269, 565
Metanicotine	...	368, 502
Methylamine	372, 659	263, 341, 368, 400, 401
3-Methylaminopyridine	...	368
N-Methylanabasine	263	...
N-Methylanatabine	263	...
N-Methylaniline	...	400
2-Methylbutylamine	...	400
N-Methylbutylamine	...	368, 400
Methylcarbazole	...	456, 495
Methylethylamine	...	368, 400
3-Methylindole	...	230, 456, 503, 566–568
N-Methylmyosmine	...	192, 263
N-Methylnicotinamide	263	...
N-Methyl-2-phenylethylamine	372	368
2-Methylpyrazine	...	111, 565
2-Methylpyridine	...	111, 263, 393, 502, 652
3-Methylpyridine	...	263, 393, 502, 565, 652
4-Methylpyridine	...	393, 502
N-Methylpyrrole	...	205
2-Methylpyrrolidine	659	368, 373, 400
N-Methylpyrrolidine	263, 659	...
N-Methyl-3-pyrroline	419	...
Myosmine	263	192, 263, 269, 368, 393, 436, 502
1-Naphthylamine	...	400
Nicotelline	263	263
Nicotinamide	263	263
Nicotine	244, 263, 335	192, 244, 263, 269, 393, 502
Nicotine N-oxide	263, 405	...
Nicotinic acid	244, 263	244, 263
Nicotinonitrile	...	502
Nicotyrine	244, 263	2, 244, 263
Norharmane	421, 422	111, 421, 422, 565
Nornicotine	244, 263, 335	192, 244, 263, 269, 368, 393, 436
Nornicotyrine	...	263
1,8,9-Perinaphthoxanthene	...	589
2-Phenylethylamine	372	368, 400
N-Phenyl-4-isopropyl-phenylamine	...	334
N-Phenyl-2-naphthylamine	...	334
Piperidine	263, 372	368, 373, 400
Propylamine	...	341, 368, 400
Pyrazine	...	111, 565
Pyridine	263	111, 192, 263, 269, 393, 502, 565, 652
Pyridine-3-aldehyde	...	263
3-Pyridinol	...	500
Pyrido[2,3-*b*]indole	...	111, 565
3-Pyridyl ethyl ketone	...	263, 439
3-Pyridyl methyl ketone	263	263, 439, 502, 652
3-Pyridyl propyl ketone	263	263
Pyrocoll	...	346, 456, 566, 567
Pyrrole	...	205, 263, 502, 566, 567
Pyrrolidine	263, 372	368, 373, 400
3-Pyrroline	...	400
Pyrrolo[2,3-*b*]pyridine	...	565
Quinoline	...	111, 263, 269, 565
Quinoxaline	...	111, 565
1,2,5,6-Tetrahydropyridine	372	...
Thymine	630	...
Toluidines (*o*-, *m*-, *p*-)	...	400, 401
Trimethylamine	263	263
2,4,6-Trimethylaniline	...	400
Trimethylindoles	...	456, 495
3-Vinylpyridine	...	192, 393, 502, 652

dines and 7H-dibenzo[*c,g*]carbazole in cigarette smoke (589) is of special interest since these compounds are carcinogenic and may contribute to the weak tumorigenic activity of the basic fraction in laboratory animals (644).

Alkylindoles and alkylcarbazoles occur in smoke condensate, but the positions of substitution have not been determined conclusively with the exception of skatole and 3-ethylindole (230). The latter compound was characterized by ultraviolet, infrared, and mass spectral characteristics. The dimethylindoles in smoke are not the 2,3, 2,5, 2,7, 3,5, or 3,7 derivatives (456); possibly one or more of the isolated compounds could be ethylindoles since the mass spectral molecular peaks of dimethyl- and ethylindoles are identical (503). Propylindoles may also occur in smoke condensate (230).

Although the presence of phenylamines and naphthylamines as pyrolytic products in cigarette smoke has been suspected for many years, the isolation of these components has been reported only recently. The four aromatic secondary amines listed in Table XI are essentially neutral in character and are found in the nitromethane-soluble neutral fraction of smoke condensate. Identification of these amines was made by cochromatography and mass and infrared spectral comparisons with authentic compounds. The amines occur in low levels (0.013–0.1 μg per cigarette) and, with the exception of diphenylamine, have not been tested for tumorigenic activity in animals. A report of papilloma formation by diphenylamine may not be reliable because of the presence of 4-aminobiphenyl in the tested material (334). The known aromatic primary amines in cigarette smoke have been reported within the past 2 years (368, 400, 401). In two studies, the compounds were isolated by passage of the basic fraction of smoke condensate through an ion-exchange column and reaction of the bases *in situ* with trifluoracetic anhydride (400, 401). The resulting substituted acetamides were separated on gas chromatographic capillary columns and identifications were made by comparisons of retention times and mass spectra of eluted peaks with known compounds. In addition to the listed components, evidence for the following types of amines in cigarette smoke was also obtained (400): alkyl derivatives of piperidine, dehydropiperidine, pyrroline, and pyrrolidine; aminofluorenes; aliphatic and aromatic diamines; and higher molecular weight, aliphatic monoamines.

The potent bladder carcinogen, 2-naphthylamine, cannot be detected in cigarette smoke with available methods (400) and, if present at all, occurs in levels of no more than 0.04 μg per cigarette (334a).

The relatively low-boiling bases of cigarette smoke consist of the aliphatic amines, saturated heterocyclic bases, and simple pyridine derivatives, and these compounds have been studied extensively. Direct separation of the basic fraction of cigarette smoke condensate by gas chromatography (435, 436, 502) shows the presence of at least 35 components (502) of which at least 14 can be identified conclusively by comparisons with known compounds. The identified components are pyridine, alkylpyridines, acylpyridines, 3-vinylpyridine, pyrrole, nicotinonitrile, and alkaloids, including metanicotine. The isolation of the low-boiling aliphatic amines by this procedure is difficult owing to losses during solvent evaporation; however, such compounds can be separated from the crude smoke bases by reaction with 4′-nitroazobenzene-4-carboxylic acid chloride, and the resulting carboxamides can be resolved by column and thin layer chromatography (368, 372, 373). Using this method, 27 bases in cigarette smoke have been detected of which 24 have been identified, including several compounds not reported previously, *e.g.*, 1,2,5,6-tetrahydropyridine and dihydrometanicotine (368). A comparable study on leaf has shown that a marked qualitative similarity exists between volatile bases in leaf and smoke (372).

The presence of 3-pyridinol in cigar smoke (500) is of interest since this compound is amphoteric. Identification has been made chiefly by infrared spectral interpretation and confirmed by gas and thin layer chromatography. Spectral differentiation of the three isomers is simple since the 2- and 4-pyridinols exhibit tautomerism and the predominating pyridone forms show amido bands at 5.88 to 6.25 μ characteristic of lactams in the solid state; in 3-pyridinol, tautomerism does not occur but extensive intermolecular bonding is present, giving a very broad hydroxyl and C–H stretching absorption centered at about 4.11 μ and no absorption in the amido region.

The occurrence of putrescine in foreign grown tobacco has been cited (561), but no confirmation of the report has appeared.

The presence or absence of nitrosamines in tobacco smoke is currently a controversial question. Interest in this problem has arisen since many nitrosamines are known to be potent carcinogens. Based on the known presence of secondary amines and oxides of nitrogen in tobacco smoke, the suggestion was made in 1962 that these components could react and produce nitrosamines, which might explain the tumorigenic activity of tobacco smoke in animals (136). This suggestion led to the development of methods for the isolation and detection of trace amounts of these compounds in smoke (367, 425) and to the synthesis of N-nitroso derivatives of anabasine, dihydrometanicotine, metanicotine, and nornicotine (64, 367). The nitroso derivatives of the smoke components, anabasine (64), piperidine (64,137), methylaniline (64), pyrrolidine (136), and nornicotine (63), were shown to be carcinogenic to laboratory animals, producing pulmonary

tumors in some cases (63). However, on addition of nitrosoanabasine to cigarettes, no nitrosamine could be isolated from the mainstream smoke.

The first evidence for the presence of nitrosamines in cigarette smoke was reported in 1964 (375). In this study, cigarette smoke condensate and smoke trapped in pentane were extracted with aqueous HCl to remove bases. The fraction containing neutrals and acids was then reacted with $LiAlH_4$, which reduced nitrosamines to hydrazines. The fraction was extracted again with hydrochloric acid which removed the newly formed hydrazines without contamination from neutral and acidic components. The fraction containing the hydrazines was allowed to react with 5-nitro-2-hydroxybenzaldehyde, forming the substituted hydrazones which were separated and identified by thin layer chromatographic and ultraviolet spectral comparisons with known compounds. In this way, N-nitrosomethylbutylamine and two unidentified nitrosamines were found in the smoke of cigarette tobaccos containing high levels of nitrates and volatile bases but not in the smoke from cigarettes with normal amounts of these leaf constituents. A later study confirmed these general findings and reported the probable presence of the nitrosamines of dimethylamine, diethylamine, and piperidine in cigarette smoke (508). However, further work indicated that all of the isolated nitrosamines may be artifacts formed in the smoke collection train and may not actually exist in the mainstream smoke (374). Kinetic studies showed that the vapor-phase reaction between dimethylamine and nitrogen trioxide or nitric oxide was extremely rapid, giving yields of nitrosamine up to 36% with a contact time of 6 sec. Also, high yields of the nitrosamine could be obtained by treating nitrogen trioxide or nitrogen dioxide with the free base in pentane at $-80°$, one of the conditions used in smoke collection. A reexamination of the nitrosamines found in collection trains showed that the proportion of different nitrosamines varied in different parts of the train and that the levels increased on standing. From all of these findings it was concluded that the nitric oxide present in cigarette smoke may be oxidized progressively to nitrogen dioxide in the traps and the combination of oxides may react with the amines to form the nitrosamines as artifacts. However, a subsequent report appeared giving evidence of the presence of nitrosamines in tobacco leaf (509). Large-scale extraction of burley tobacco and subsequent fractionation gave on gas chromatography a peak which cochromatographed with authentic N-nitrosopiperidine. Other components were found which gave color reactions for nitrosamines on thin layer and column chromatographic separation of the hydrazones prepared as described above; however, N-nitrosopiperidine could not be detected in this way.

No valid conclusion can be reached at this time regarding the presence or absence of nitrosamines in smoke immediately leaving the butt end of a cigarette. From the pragmatic viewpoint, inhaled smoke is not completely expelled during the first exhalation. Components in the smoke retained in the lungs may react and produce products not found in smoke immediately leaving the cigarette. Whether or not this extended reaction time could result in a vapor-phase synthesis of nitrosamines is obviously unknown. Resolution of the entire nitrosamine question requires further work.

Interest in the role of nicotine and other bases in the physiological effects and the organoleptic properties of smoke has prompted the development of many quantitative methods. Nornicotine is believed to be an undesirable leaf and smoke component which produces myosmine and pyridine in large amounts on pyrolysis and gives smoke an undesirable taste (192). New or improved paper chromatographic (191), colorimetric (190, 193), gas chromatographic (440), and nonaqueous titrimetric (110) methods for determining nornicotine, nicotine, myosmine, pyridines, and related bases have appeared within the past few years. Also, a chromatographic method for indoles has been reported (230); studies on smoke condensates with this method show that aging of smoke condensates causes up to a 10-fold increase in the proportion of skatole to indole. The levels of certain volatile bases and alkaloids can be related to the relative flavor of cigarette smoke from flue-cured tobaccos (502); however, a balance between the levels of these bases and the C_2–C_8 aliphatic acids is apparently necessary for desirable smoke flavor.

K. BROWN PIGMENTS

The presence of brown, acidic substances in tobacco leaf was reported 100 years ago, and subfractions of these substances were given names such as "Tabakensäure (α, β, γ)" and "Kentuckinsäure" (143). In later work, constituents with similar properties were isolated from cigar smoke (614). Although no reports of the isolation or structure of these components have appeared in the modern literature, recent work has shown the presence in leaf and smoke of certain high molecular weight pigments which may be related to these "brown acids."

The leaf pigments (LP) can be extracted from cured or aged cigarette tobaccos with water (270, 632, 637), aqueous alkali (93, 252, 253), and neutral buffer (252) in amounts up to about 6.5% of the leaf weight. The infrared spectra of these pigments and fractions thereof show no special features with broad absorption at about 2.9 and 6.0 μ and nonspecific absorption at other wavelengths. LP may be fractionated by solubility, dialysis, and gel filtration to yield many subfractions, none of which is probably a pure compound. By ultracentrifugal analysis, the two major subfractions of water-

soluble LP have molecular weights of about 4000 and 20,000 to 30,000, respectively (632, 637). Subfractions of the alkaline-extractable LP range in molecular weight from <3000 to more than 100,000 with major components in the 5000–60,000 range (91).

Prior to 1966, studies on the hydrolytic and alkaline fusion products of LP showed slight qualitative and quantitative compositional differences in the subfractions with all pigments containing chlorogenic acid and amino acids and some fractions having rutin and iron. However, a recent demonstration of the presence of alkaloids and a silicone in the closely related smoke pigment (*vide infra*) prompted a reexamination of LP and confirmed the presence of these additional components therein, at least in the alkaline-extractable material (142). Although classical "browning reactions" between polyphenols (or their quinones) and amino acids may be involved in biosynthesis of these pigments, the presence of alkaloidal and silicone moieties in some, if not all, of the LP subfractions shows that other reactions are involved. The nature of the linkages within the molecules is unknown although chelation between iron and the phenolic hydroxyl groups of chlorogenic acid (637) or a salt linkage (493) has been suggested. The presence of a conventional salt bond as the only linkage involving nicotine is unlikely since steam distillation from an alkaline solution of the leaf pigment yields no nicotine (141).

Cigarette smoke condensate contains a group of brown acidic pigments which are superficially similar to the leaf pigments. These smoke pigments (SP) occur in relatively high concentrations (up to 4% of the smoke condensate), and chlorogenic acid, amino acids, iron, silicone, and alkaloids and other bases are linked within the molecule (143, 540). The major subfraction of SP has a higher molecular weight ($\geqslant$100,000) than the comparable subfraction of LP, and the percentage of chlorogenic acid resistant to conventional hydrolysis is higher in SP (143, 540). The relative strengths of the linkages are well illustrated by the recoveries of partially degraded pigments after high-temperature (260–280°), prolonged (1.5 hr) alkaline fusion (142). Eighty per cent of the SP is recovered with molecular weights of 30,000–100,000, but only 20% of LP can be isolated with molecular weights of <4000. Although both LP and SP contain alkaloids and related bases, important qualitative and quantitative differences are noted. In comparing the nondialyzable subfraction of the alkaline-extractable SP and LP, fewer alkaloids and lower proportions of volatile bases to nicotine are found in LP. Table XII presents some representative results on this point; in addition to the listed components, eight other volatile bases with similar relative proportions have been isolated from both pigments (142).

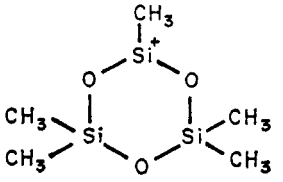
Based on these findings a postulation of the origin of the smoke pigment has been suggested (142, 143, 540). As a result of the sharp thermal gradient behind the cigarette coal (214), cellular eruption may occur adjacent to the burning zone, expelling the cell contents, including LP, into the aerosol stream. These cellular particles may serve as nuclei for further aerosol formation or be adsorbed on preformed nuclei. In this transition, the leaf pigments undergo structural alteration and react with the volatile bases and alkaloids known to be present in smoke, thus increasing in molecular weight and acquiring higher proportions of volatile bases:nicotine and other alkaloids.

TABLE XII
VOLATILE BASES AND ALKALOIDS IN THE BROWN PIGMENTS

Compound	Relative amounts: Leaf pigment	Relative amounts: Smoke pigment
Cotinine	0.23	5.7
Dihydrometanicotine	...	0.7
Metanicotine	...	4.1
2-Methylpyridine	0.10	1.4
3-Methylpyridine	0.42	3.9
N-Methylpyrrolidine	0.02	2.2
Nicotine	1.0	1.0
Nornicotine	...	3.2
Pyridine	0.50	2.0
Pyrrole	0.23	5.7
3-Vinylpyridine	0.03	2.6

One anomaly in the findings to date is the isolation of pyridines substituted in the 2 position and 3-vinylpyridine in LP. Except for isonicotein (2,3′-bipyridyl) and nicotelline [2,4-di(3′-pyridyl)pyridine], 2-pyridyl constituents are not found in leaf. Also, 3-vinylpyridine occurs only in smoke. However, on the basis of recent work (579), it appears that biochemical transformations of piperidine derivatives into 2-pyridyl compounds may occur in tobacco leaf. Although the products listed in Table XII were obtained by alkaline fusion of the leaf pigments, the possibility that they are artifacts formed by degradation of nicotine is excluded since fusion of nicotine under identical conditions results in recovery of more than 95% of the alkaloid (142).

The silicone present in the pigment is a minor moiety and is similar to the component in cigar smoke (491) postulated to be a polymeric methylsiloxane in the range of $-[(CH_3)_2SiO]_{10-50}-$. This similarity was determined by infrared spectral comparisons and by the appearance of a major fragmentation peak at *m/e* 207 (XXV) in the mass spectrum (143), indicative of the pentamethyl-substituted cyclic structure of the



XXV

polymeric siloxanes. The silicone is actually linked within SP and is not a contaminant from experimental manipulation since the isolation procedure involves several steps which would remove contaminating silicones: exhaustive extraction of SP with ether followed by dissolution of the ether-insoluble pigment in aqueous buffer and prolonged dialysis of the solution (143). The occurrence of organic silicon compounds in bacteria and higher plants has been reported previously (217, 218).

Pigments are found in the smoke condensates from unblended cigarettes of the four major tobacco types (88), and the distributions of molecular weight in the different condensates vary with the type. In contrast, the distributions of molecular weight in LP from these four tobaccos show a high degree of uniformity (91). Thus, the differences in SP from different tobacco types appear to be more related to the burning characteristics of the tobaccos than to variations in the composition of LP precursors.

A relatively low molecular weight brown pigment also occurs in flue-cured tobacco (476). The pigment is dialyzable and contains equimolecular amounts of rutin, chlorogenic acid, and scopolin. Based on the stability in the presence of chelating agents and the response to hydrolysis, the linkages of the polyphenols in the pigment may not be covalent in nature.

Much, if not all, of the published work on these pigments has been done on probable mixtures since available methods for the separation of closely related high molecular substances and criteria for purity are inadequate. However, some valid information can be gained by such studies although complete structural elucidation may not be accomplished, *e.g.*, recent studies on animal and plant melanins (418). Further progress in this field must await the development of new or improved methods for isolation and identification.

The brown leaf pigments may play some role in leaf quality since they are believed to be responsible for the color of air-cured tobaccos (637), and color is one industrial criterion for judging acceptability. The smoke pigments resemble the acidic brown polymeric substances of smoke which have been reported to act as cocarcinogens (57, 59, 365).

In addition to the brown pigments, some tobaccos with a high proportion of nornicotine to nicotine contain a "cherry red" pigment which has not been isolated or characterized but is believed to be formed by reactions between the quinone form of chlorogenic acid and nornicotine (408).

L. CARBOHYDRATES (TABLE XIII)

Carbohydrates constitute a large part of the tobacco leaf, and considerable recent work on these components has been done since 1959 including effort on pyrolytic degradations which is discussed in section V.

TABLE XIII

CARBOHYDRATES IN TOBACCO LEAF

Carbohydrate	Ref	Carbohydrate	Ref
Arabinose[a]	98	Salt of gum-like polysaccharide	636
Arabogalactan	263	Lignin	263
Cellulose	94, 263, 481	Maltose	263
1-Deoxy-1-L-alanino-D-fructose	562, 576	Mannose	338
		Pectins	60, 251, 263, 430, 431
1-Deoxy-1-(N-γ-aminobutyric acid)-D-fructose	562, 576	Pentoses	263
1-Deoxy-1-L-prolino-D-fructose	562, 575	Planteose	263
		Raffinose	263
		Rhamnose	263, 338
Deoxyribose	263	Ribose	98, 263
Erythrose	98	Rutinose	263
Fructose[a]	12, 263, 338	Sorbitol	263
Galactan	263	Stachyose	263
Galactosamine	618	Starch	263, 329a, 337, 420
Galactose	98, 338		
Galacturonic acid	98	Sucrose	12, 263
		Xylan	263
Glucosamine	338	Xylose[a]	338
Glucose[a]	12, 263		

[a] These compounds (292) and 1,6-anhydro-β-pyranose (263) are also present in smoke.

Three fructosamines have been identified among the ninhydrin-positive substances which accompany pipecolic acid and pyrrolidine-2-acetic acid in cured tobacco (see section III.M), and several other substances are present, giving on hydrolysis aspartic acid, threonine, valine, and components with positive responses to the phthalic acid–aniline reagent for sugars (576). The total content of amino acid–sugar compounds in flue- or sun-cured tobaccos may be more than 2% of the dried leaf weight, and these constituents may be important contributors to smoke flavor and aroma (576).

The reported pentoses and hexoses in cigarette smoke have been isolated by ion-exchange chromatography of aqueous extracts and identified by paper chromatographic techniques including the use of color reagents (292). These components may enter the smoke by thermal cellular eruption in a manner similar to the brown pigments. The separation and identification of erythrose, galactose, ribose, arabinose, and galacturonic acid in "tobacco products" by thin layer chromatography and fluorescence spectral determination have been described, but it is not clear whether these compounds were found in the free or combined state (98). Free and glycosidated mannose, galactose, xylose, and fructose have been isolated from green Japanese tobacco (338). Plantebiose has been reported in tobacco seeds (339).

A few investigations have appeared recently on the polysaccharides of tobacco leaf. The level of starch extracted from Japanese green tobacco by calcium

chloride solution is about 2.4% of fresh leaf weight (337). The amylose:amylopectin ratio shows diurnal variation with values of 1:3.7 for daylight hours and 1:4.3 for darkness. The former ratio is in agreement with a previous approximation of 23:77 in starch from American tobacco (263). Various methods of isolating starch have been studied to determine the resulting degree of structural alteration (329a). Chloral hydrate, perchloric acid, and sodium hydroxide were used as extracting solvents, and the extracted materials were precipitated with iodine. After splitting of the starch–iodine complex with alkali, the polysaccharide fraction was purified by dialysis or electrodialysis and analyzed for viscosity, approximate chain length, and hydrolytic products, including components not present in starch, *i.e.*, arabinose and galactose, to determine the specificity of the method. These data were compared with comparative findings on starch granules obtained by mechanical disruption of leaf cells. Starch obtained by any of the solvent extractions was degraded, but perchloric acid appeared to be the most efficient solvent. A quantitative method has appeared in which 4 *N* perchloric acid is used to extract leaf starch after prior removal of sugars with ethanol (420). Spectrometric readings of the starch–iodine complex are made at 400 mμ and compared with an absorption–concentration curve obtained with potato starch.

The pectins of tobacco leaf have received some attention. Both green and flue-cured leaves contain pectins which yield D-galacturonic acid, D-galactose, L-rhamnose, and L-arabinose as major products and D-glucose, D-xylose, fucose, and 2-O-methylxylose as minor constituents on acidic hydrolysis (60). In green leaves, the yields of these major sugars and the methoxyl values of the pectin are higher, but total uronic acid is lower than in cured leaves. However, equivalent levels of rhamnose can be obtained from pectin of cured leaves by diborane reduction of the pectin prior to acidic hydrolysis. Partial enzymatic hydrolysis of the pectin from cured leaves yields three α-1,4-linked D-galacturonic acids with degrees of polymerization of 2, 3, and 4. Comparable hydrolysis of green leaves gives several oligosaccharides including the three found in cured leaf pectin and another three which contain galacturonic acid and galactose with or without rhamnose. One of the latter compounds appears to be 2-O-(D-galactopyranosyl uronic acid)-L-rhamnose (aldobiouronic acid). These hydrolytic patterns indicate a fundamental difference in the position of rhamose within the structures of pectins from green and cured leaves. It is known that rhamnose may be linked within the uronic acid chain or may exist in side chains (60) which may be more accessible to acidic and enzymatic hydrolysis. In cured leaves the increased yields of rhamnose after diborane reduction and the failure to isolate rhamnose-containing oligosaccharides may be reflections of the less accessible positions of rhamnose in the chain. Thus, the curing of tobacco results in major alterations in the structure of the pectins.

Significant differences are found in the amounts and kinds of pectic substances in cigarette and cigar tobacco stems (251). Galacturonic acid, methoxyl, and acetyl determinations (431) show that pectin degradation is directly related to the severity of curing and fermentation (430). Some positive correlation may exist between the uronic acid content of flue-cured tobacco and the body and tensile strength of the leaf (417). However, a trend toward a negative correlation has been claimed in other work (430).

The gum-like substance listed in Table XIII is a calcium–magnesium salt of a polysaccharide which contains glucuronic acid, galactose, arabinose, and rhamnose. The salt can be isolated from aged burley leaf during separation of the brown pigments. Acidic hydrolysis yields the above sugars and glucuronolactone, which is known to be formed from glucuronic acid under these conditions. These hydrolytic products are qualitatively identical with the compounds obtained by similar hydrolysis of gum arabic. Eleven amino acids are also detectable among the hydrolytic products of the tobacco isolate although these components may be contaminants accompanying the polysaccharide. The acid form of the polysaccharide has an equivalent weight of 924 which would indicate a repeating unit having molar proportions of 2:2:1:1 for galactose, arabinose, rhamnose, and glucuronic acid. The isolated salt comprises about 0.9% of dry leaf weight.

The inclusion of the arabogalactan, galactan, and xylan in Table XIII is based on a previous evaluation of the claimed identities (263).

Cellulose can be isolated from tobacco leaf or midribs by a sequence of steps consisting of extraction with water and organic solvents, digestion with hot aqueous alkali, and washing with acetic acid, water, and organic solvents (94). The isolated cellulose is obtained with a minimum of degradation or contamination with lignin and other substances. Using a viscosimetric method, the degree of polymerization (DP) of cellulose from leaf lamina varies with the tobacco type and grade (481) but is generally low (about 1100–1650) compared to wood (~3000) and fiber (6000–8000) celluloses. A slight tendency for lower DP values in flue-cured tobaccos compared to air-cured types is observed. As expected, leaf midribs contain higher levels of cellulose and a larger DP (about 1600–1800) than lamina. No conclusive relation between quality and cellulose characteristics can be demonstrated, but some tendency for higher cellulose levels and lower DP may be found in the lower grades (481). The cellulose in cigarette tobaccos may contribute significantly to the taste of smoke. The addition of various concentrations and physical forms of cellulose to cigarettes produces a wide variation

in harshness (603). The presence of noncellulosic components in cigarette tobaccos appears to modify the burning of cellulose and ameliorate the harshness associated with the polysaccharide.

M. AMINO ACIDS, PROTEINS, AND RELATED COMPOUNDS (TABLE XIV)

Since 1959, the list of known amino acids and related compounds in tobacco leaf and smoke has grown considerably. Although most of the newly reported acids are familiar cellular constituents, two compounds are of special interest. In a study of unidentified ninhydrin-positive substances in tobacco leaf, pyrrolidine-2-acetic acid was isolated from a natural source for the first time (562, 574). The compound was extracted from flue-cured leaves with 70% methanol and separated on cationic exchange resin and cellulose columns. Crystallization of a column eluate yielded 60 mg of acid from 16 kg of leaves. Elementary analyses and paper chromatographic and spectrometric characteristics of the isolate were identical with synthetic pyrrolidine-2-acetic acid. The compound is either absent or present in only trace amounts in green tobacco. The second new amino acid is pipecolic acid (piperidine-2-carboxylic acid), which was isolated in the above separation and crystallized from a chromatographic fraction eluting before pyrrolidine-2-acetic acid. The isolated compound gave no depression of melting point and an infrared spectrum identical with synthetic L-pipecolic acid, a well-known constituent of natural products. This amino acid is present in easily detectable amounts in green tobacco and is accompanied by other ninhydrin-positive substances (574), three of which have been shown recently to be condensation products of amino acids and sugars (see section III.L).

Since 1959, the number of known amino acids in cigarette smoke has grown from 2 to 14. Isolation of the newly reported amino acids was accomplished by trapping of smoke in water and separation of the water-soluble fraction on ion-exchange resins after removal of ether-soluble components (249, 250). Identifications were established by paper cochromatographic separations with authentic amino acids. α-Alanine was the major acid in the smoke from seven types of tobacco and was present in relatively high levels, 11–268 μg per cigarette (250).

Much of the recent work on the amino acids of tobacco leaf has concerned the qualitative and quantitative differences in tobacco types and the effects of various cultural and curing conditions on acid contents. The major free amino acid in Rhodesian flue-cured leaf is proline, occurring in levels of 0.4–1.3% (258). Among American tobaccos, some qualitative differences are noted in the free amino acids in flue-cured and air-cured types, *e.g.*, the absence in burley and presence in flue-cured leaf of homocystine and hydroxyproline

TABLE XIV
AMINO ACIDS AND RELATED COMPOUNDS IN TOBACCO LEAF AND SMOKE

Compound	Reference: Leaf	Reference: Smoke
α-Alanine	32, 258, 263, 359, 382, 580, 618	249, 250
β-Alanine	263, 359, 382, 580, 618	249, 250
α-Aminoadipic acid	618	...
α-Aminobutyric acid	263, 580	...
γ-Aminobutyric acid	126, 258, 263, 359, 382, 580, 618	249, 250
Arginine	32, 243, 359, 382, 580, 618	...
Asparagine	126, 258, 263, 359, 382, 580	...
Aspartic acid	32, 126, 243, 258, 263, 359, 580, 618	249, 250
Betaine	263	...
Choline	263	...
Citrulline	263, 580	...
Cysteic acid	580, 618	...
Cysteine	263, 580	...
Cystine	263, 580, 618	...
Glutamic acid	32, 126, 243, 258, 263, 359, 382, 580, 618	249, 250, 263
Glutamine	243, 258, 263, 359, 580	249, 250, 263
Glutathione	263	...
Glycine	32, 126, 243, 263, 352, 382, 580, 618	249, 250
Histidine	126, 258, 263, 359, 382, 580, 618	...
Homocystine	618	...
Homoserine	633	...
Hydroxyproline	184, 185, 618	...
Isoleucine	126, 243, 258, 263, 382, 580, 618	...
Leucine	126, 243, 258, 263, 382, 580, 618	249, 250
Lysine	126, 243, 263, 359, 382, 580, 618	...
Methionine	32, 126, 243, 263, 382, 580, 618	...
Methionine sulfone	185, 618	...
1-Methylhistidine	382, 662	...
Norleucine	580	...
Ornithine	382, 580	249, 250
Phenylalanine	126, 243, 258, 263, 359, 382, 580, 618	250
Pipecolic acid	562, 574	...
Proline	126, 258, 263, 359, 382, 580, 618	249, 250
Pyrrolidine-2-acetic acid	562, 574	...
Serine	32, 126, 243, 258, 263, 359, 382, 580	249, 250
Taurine	618	...
Threonine	32, 126, 243, 258, 263, 359, 382, 580	250
Tryptophan	32, 258, 263, 359, 580, 635	...
Tyramine	580	...
Tyrosine	32, 126, 243, 258, 263, 382, 580, 618	...
Valine	126, 243, 258, 263, 359, 382, 580, 618	249, 250

(618). Although rapid hydrolysis of protein is known to occur during curing, 7 out of 27 amino acids decrease in the process, showing that other reactions are proceeding which affect the acid levels markedly. These reactions may include condensation with sugars, producing compounds such as 1-deoxy-1-L-prolino-D-fructose (562, 575) and decarboxylations and oxidative deaminations, yielding such products as acetaldehyde, isobutyraldehyde, glutamine, and asparagine, all of which accumulate during curing (616, 618). In this regard the apparent relationship between leaf aroma and some aliphatic carbonyls (617) may be actually a reflection of changes in amino acid composition during curing. The observed decreases in amino acids during fermentation of tobacco have been attributed to reactions with polyphenols yielding melanin-like compounds (243). Qualitative and quantitative differences are found in the amino acid contents of leaf midribs and lamina in different tobacco types (633). Homoserine occurs in the midribs but not in the lamina of flue-cured tobacco, and burley midribs contain asparagine, glycine, and tryptophan, which are absent in the flue-cured type. Interest in the composition of midribs has been prompted by the current commercial use of these previously discarded leaf parts in homogenized tobacco sheet or in cigarette blends after pulverization (see section VI).

Several investigations have been reported on the changes in amino acid composition of tobacco leaf in different stalk positions (126) and under various cultural (381, 382, 580, 662) and curing (663) conditions.

Few significant studies on the protein composition of tobacco leaf have appeared in the recent literature. A method for isolating "fraction I" and "fraction II" proteins (263) from green leaf has been described utilizing ultracentrifugation through a density boundary followed by dialysis (404). The isolated "fraction II" contains about 60% protein and has a sedimentation constant of about 3 Svedberg units, which is within the range of an earlier reported value (263). The proteins of tobacco leaf grown under shade or in direct sunlight show differences in electrophoretic mobilities on starch (666). One major fraction is obtained from shade-grown leaf protein, but two fractions are present in the sun-grown material; however, none of the substances is believed to be homogenous. A protein, "phytomyosin," has been isolated from tobacco leaf by ammonium sulfate precipitation and gel filtration (661). The substance was claimed to be homogenous and to have marked adenosine triphosphatase activity. A study has been made of the effectiveness of conventional precipitants in removing proteins from tobacco leaf extracts (432). Trichloracetic acid and acetic acid were found to be preferable to cupric hydroxide, but all precipitants contained large amounts of nonproteinaceous material. Using these techniques superficial differences were determined in the proteins from tobacco subjected to different methods of curing and fermentation (433).

N. MISCELLANEOUS COMPONENTS

1. *Inorganic Constituents* (*Table XV*)

The occurrence and agronomic influence of trace elements in tobacco leaf have been reviewed recently (578).

The continued trend toward replacement of arsenical sprays with other pesticides has been reflected in progressively lower arsenic contents in leaf (522) and cigarette smoke (237). The suggestion has been made that triphenylarsine may be present in cigarette smoke and may contribute to the tumorigenic effect of smoke condensate in laboratory animals (237). A new analytical method using silver diethyldithiocarbamate has been claimed to have equivalent precision and greater

TABLE XV
INORGANIC ELEMENTS IN TOBACCO LEAF AND SMOKE

Element	Reference: Leaf	Reference: Smoke
Aluminum	263, 578, 596	263
Arsenic	3, 227, 237, 263, 522, 578	263
Barium	263, 578, 596	...
Beryllium	620	...
Boron	263, 578	...
Calcium	263, 596	263
Cesium	263, 578	...
Chlorine	578	...
Chromium	263, 578	263
Cobalt	578, 596	...
Copper	263, 578, 596	263
Fluorine	533	...
Iodine	578	...
Iron	263, 578, 596	143, 263
Lead	263, 578, 596	263
Lithium	263, 578, 596	...
Magnesium	263, 578, 596	263
Manganese	263, 578, 596	263
Mercury	578	...
Molybdenum	578, 596	...
Nickel	578, 596	121, 263, 402, 557
Polonium	426, 578	161, 284, 330, 426, 441, 442, 660
Potassium	263	263
Radium	161, 327, 578, 581, 583	...
Rubidium	263, 578	...
Selenium	578	...
Silicon	142, 263, 578	143, 491
Silver	578	...
Sodium	263, 578, 596	263
Strontium	263, 578, 596	263
Thallium	177, 178, 578	...
Tin	578, 596	...
Titanium	263, 286, 596	263
Uranium	578	...
Vanadium	578, 596	...
Zinc	263, 578, 596	263

ease of performance than the Gutzeit procedure in determining arsenic in tobacco (227).

The levels of thallium in cigars and cigarettes are relatively similar and are in the range of 24–100 ppb (177, 178). Experiments on the fate of thallium during smoking suggest that one-half of the quantity in cigars and cigarettes may be transferred into the smoke (177), but thallium has not been detected therein.

The amounts of beryllium in the cigarette tobacco types vary from 0.075 ppm for Maryland to 0.015 ppm for flue-cured (620). Beryllium does not transfer into the smoke and is distributed between the ash (63%) and the butt (37%).

In a preliminary report on selenium, levels of 2–4 ppm in tobacco and about 10 ppm in cigarette paper have been described (7), but no mention was made of the transference of selenium into cigarette smoke.

The possible presence of nickel carbonyl in tobacco smoke has been suggested (557), but neither this compound nor related volatile carbonyls, *e.g.*, cobalt carbonyl, has been isolated from smoke. Analysis of the nickel content of smoke from domestic and foreign cigarettes has given different results, but all reported levels are less than 1 μg per cigarette (402, 557). Estimates of the percentage transference of nickel from leaf to smoke have varied from less than 0.1 (121) to 20% (557), and part of this variation may be due to differences in smoking conditions (402). Although nickel carbonyl has not been found in smoke and is known to decompose at a temperature (200°) far below the coal temperature, a patent has appeared listing a series of chemical additives for cigarette filters capable of reacting with nickel carbonyl (70).

Gold and platinum may effect the biosynthesis of alkaloids, but no information on the levels of these elements in tobacco leaf is available (578).

Tobacco leaf contains significant amounts of nitrates, and analytical methods for determining this anion have been published recently (97, 369, 519). Values of 0.1–5% have been obtained for cigarette and cigar tobaccos (65, 369, 519). The nitrates of tobacco may be of importance as precursors of oxides of nitrogen in smoke.

Polonium and other radionuclides of tobacco leaf and smoke are discussed in section IV.

2. *Agricultural Chemicals (Table XVI)*

Since residues of agricultural chemicals on tobacco are a source of off-flavor in smoke (154) and have been suggested as a possible factor in smoking-health relationships (527), interest in these components has been stimulated markedly in the last few years. A comprehensive review of agricultural chemicals used on tobacco and residues thereon has appeared recently (209), and the present discussion will be limited to a brief summary of the points covered in this review and to certain other published information (62, 310). The full chemical names for all cited chemicals are given in the review.

Most of the published work has concerned residues obtained on tobacco grown under experimental conditions. Detection of agricultural chemicals in leaf and smoke from such samples is no assurance that the same results will be obtained in commercial products. Marked differences in the quantitative transfer of residues from leaf to smoke have been noted between experimental and commercial samples; however, some basic characteristics of thermostability and volatility can be determined by using experimental tobaccos.

The residues of agricultural chemicals found in tobacco and smoke are derived from insecticides, fungicides, and sucker-inhibiting agents. Leaf and soil fumigants probably do not leave residues, although they may react with leaf constituents, *e.g.*, alkylation by the fumigant methyl bromide. In the United States, insecticides are used extensively, but fungicides are employed rarely (209).

All of the pesticides listed in Table XVI have been found in both green and cured leaves except Malathion, which was present only in green leaves.

Substantial losses of applied insecticide are observed after application to the growing plant and during curing. Reductions of about 50–80% of chlorinated compounds (TDE and Endrin) and 90% or greater of carbamate (Carbaryl) and thiophosphate (Guthion) insecticides may occur prior to harvest, and flue-curing may result in a further decrease of about 40 and 80%, respectively.

TABLE XVI
AGRICULTURAL CHEMICALS AND DECOMPOSITION PRODUCTS THEREOF IN TOBACCO LEAF AND SMOKE

		Leaf		
Chemical	Source[a]	Green or cured	Cigarettes	Smoke
Carbaryl	C, E	+	+	+
2-Chloraniline	E	−	−	+
DDT	C	+	+	−
Dieldrin	C	+	−	−
Dimethoate	U	+	−	−
Dyrene	E	+	−	+
Endosulfan	E	+	−	+
Endrin	C, E	+	+	+
Guthion	E	+	−	+
Malathion	E	+	−	−
Maleic hydrazide	E	+	+	+
Maneb	E	+	−	−
Oxyguthion	E	+	−	−
Sevin	E	+	−	+
TDE	C, E	+	+	+
TDEE	C, E	−	−	+
Telodrin	E	+	−	+
Thiodan	E	+	−	+
Toxaphene	C	+	−	−
Trichlorfon	E	+	−	−
Zineb	C	+	+	−

[a] C = commercial, E = experimental, U = unknown.

Losses during air-curing are much less. Aging and subsequent manufacturing processes do not appear to reduce the amounts of residues. The levels of Endrin and TDE vary widely in commercial tobaccos, but commercial blending of various crops and types in making cigarettes apparently reduces this variation. Values of 11–22 ppm of TDE and 0.2–1.3 ppm of Endrin have been reported from 1956 to 1966 (61). The following percentages of transference from leaf to mainstream smoke were obtained on smoking experimental cigarettes (62, 209): TDE, 19–22; Endrin, 18–30; Telodrin, 4–5; Endosulfan, 3; Thiodan, 3; Sevin, 1; Carbaryl, 1; and Guthion, <1. In addition, a known pyrolytic product of TDE, 1-chloro-2,2-bis-(4′-chlorophenyl)ethylene (TDEE) (61, 350, 495), has been found in smoke in amounts approximately equivalent to the TDE levels (209). Some variation in these percentages were found in measuring transference of TDE and Endrin in smoke from commercial cigarettes. Levels of 0.06 (Endrin), 1.6 (TDE), and 1.1 (TDEE) μg per cigarette have been reported (209), but such values may vary significantly (61).

Little work has been done on the loss of fungicidal residues during growth, curing, processing, and smoking (209). Levels of Dyrene (a chlorinated anilinotriazine) on leaves are apparently not reduced greatly on storage. In experimental cigars 1% of Dyrene was transferred to the mainstream smoke. Losses of residual Zineb and Maneb, two salts of thiocarbamic acid, were 43–78% during curing and industrial processing of bright and burley tobaccos; apparently, Zineb is more stable than Maneb. Diclone (2,3-dichloro-1,4-naphthoquinone) is a fungicide which has been used on Canadian tobacco in conjunction with maleic hydrazide. On experimental tobaccos grown in the field, residues of Diclone up to 2 ppm were found in leaves (236).

Maleic hydrazide (MH-30) is a commonly used sucker-inhibiting agent which gives increased crop yields of leaf (215) but controversial effects on quality (8, 215, 354). Variable levels of residual MH-30 are found in experimental flue- and air-cured tobaccos (6, 8), but information on the fate of MH-30 during burning is sparse. Experimental cigarettes containing 10 and 30 ppm MH-30 gave 0 and $\leqslant 2$ ppm of unchanged MH-30 in the smoke, respectively (556). Using C^{14}-labeled MH-30, 23.4% of the added radioactivity was found in CO_2, CO, and "tars" of the smoke, and 31% was calculated or found in the butt and ash. The remainder was assumed to have been lost in the sidestream smoke.

With a few exceptions, the identities of the decomposition products of agricultural chemical residues on tobacco or in the smoke are unknown. In addition to TDEE, 2-chloroaniline has been isolated from the smoke of cigars containing Dyrene and oxyguthion, an oxidation product of Guthion, appears during curing (209). The presence of a Δ-keto derivative of Endrin in cigarette smoke is suspected (209). Although present in trace amounts, the use of modern chromatographic and spectrometric techniques should expedite the isolation and identification of pyrolytic products of pesticidal residues in the next few years.

Regarding biological activity, tumorigenic activity has been reported for MH-30 when the hydrazide was administered parenterally in rats (130). Comparisons of carcinogenic activity of smoke from cigarettes with or without Dimethoate (a thiophosphate) have shown no statistical difference in tumor rates (379).

3. *Other Constituents (Table XVII)*

Recent studies on the major gases of cigarette smoke have concerned mainly the changes in concentration which occur during smoking. In the region of the cigarette cone, large amounts of carbon monoxide and carbon dioxide are formed in an atmosphere which is deficient in oxygen (377). As the smoke passes through the cigarette the oxygen concentration is increased due to dilution with air drawn through the porous cigarette paper and around the burning cone. An increase in the airflow through the cigarette produces a decrease in oxygen and increases in oxides of carbon (257). In the mainstream smoke, about 60% of the carbon dioxide and 47% of the carbon monoxide are derived from atmospheric oxidation of carbon monoxide or carbon (34). Carbon monoxide comprises 3–5% of the smoke from commercial cigarettes (357), and the level is not influenced significantly by the moisture content of the tobacco. The carbon monoxide in smoke causes small increases in the carboxyhemoglobin levels in smokers but may not be a hazard to normal individuals (285).

Since nitrogen dioxide is more toxic and ciliostatic than nitric oxide, the proportion of these compounds in

TABLE XVII
MISCELLANEOUS COMPONENTS IN TOBACCO LEAF AND SMOKE

Component	Reference	
	Leaf	Smoke
$C_{10}H_{14}O$	263	...
Carbon dioxide	...	56, 205, 257, 263, 377
Carbon monoxide	...	56, 205, 257, 263, 348, 377
Chlorophyll	263	...
Methyl chloride	...	79, 205, 263
Methyl isocyanate	...	412
Nitric oxide	...	56, 205, 212, 263, 378, 384
Nitrous oxide	...	410
Nucleic acids	263	...
Phosphatides	263	...
Resins	87, 219, 447, 537, 559	447
Saponins	263	...
Silicones	142	143, 491

cigarette smoke has been investigated (56, 384). Levels of 24–54 μg of nitric oxide per puff have been obtained for commercial cigarettes, and nitrogen dioxide is either absent or present only in trace amounts. In fact, nitrogen dioxide can only be detected if a delay occurs between smoke collection and analysis, thus permitting oxidation of nitric oxide (378). The nitric oxide content in the smoke from different tobacco types varies from 145 to 665 ppm (212).

The possible presence of ethyl chloride in the gaseous phase of cigarette smoke has been cited (205).

The resins are a large group of acidic and neutral substances which comprise at least 3% of the dry weight of flue-cured leaves (537) and have defied intensive effort to be identified. Although variable in properties, the resins are usually viscous, tacky substances having odor, color, high molecular weight, and large carbon to hydrogen ratios. The infrared spectra are generally nondescript and show short unbroken methylene chains, variable amounts and types of oxygenated functions, and little or no unsaturation (559). These substances can be separated into "hard" and "soft" resins which differ in solubility and paper chromatographic mobility (219, 447). The over-all characteristics of the resins suggest they are oxidative and/or polymeric products produced from terpenes, sterols, etc. The presence of apparent oxidation products of solanesol in aged tobacco has been noted (48, 345). During smoking, leaf resins may undergo structural changes during transference into the smoke (447).

A silicone has been isolated from the ether-soluble neutral fraction of cigar smoke condensate (491). Based on the infrared spectrum and X-ray diffraction pattern, the substance appears to be a polymeric methylsiloxane which is generally similar to the silicone isolated from the alkaline fusion products of the brown pigments from cigarette leaf and smoke (see section III.K). The silicone from cigar smoke is not a contaminant derived from stopcock grease or laboratory chemicals during the isolation. Although it is possible that the substance originates from deposition of lubricants or other industrial chemicals on cigars during manufacturing, the presence of bound silicone in the brown pigments would indicate that these compounds occur naturally.

Except for a report on the degradation of chlorophyll to phaeophytin (423), no new findings have appeared on the other constituents listed in Table XVII. The purines and pyrimidines isolated from the free nucleic acids of leaf are adenine, guanine, cytosine, and uracil (263). No confirmation of a report on the occurrence of coumarin in leaf has appeared (649). No further information has been published on the occurrence of vitamin B complex in tobacco (263); the presence of nicotinic acid and nicotinamide in leaf was cited in section III.J.

The total reducing substances in cigarette smoke from different tobacco types and quality grades have been determined (619) and are believed to include compounds other than reducing sugars (292).

Microscopic examination of commercial tobaccos has shown that large numbers of fungal spores may be present in some samples (163). By growing organisms isolated therefrom on timothy hay and exposing animals to heavy doses of the smoke from such hay, respiratory changes suggestive of emphysema have been demonstrated. A parallel experiment with tobacco could not be done since the organisms did not grow consistently on leaf. Although many questions remain unanswered in this study, the findings indicate that the presence of mycotoxins in leaf and smoke is a possibility. Thus far, isolation of such substances from leaf or smoke has not been reported. Organisms in tobacco are not transferred into the smoke (521).

IV. Special Properties of Tobacco and Smoke

A. Radioactivity

Within the past 10 years and probably as a result of increased emphasis on the physiological aspects of tobacco smoking, there has been a developing interest in the radioactivity of tobacco and its smoke. Earlier reports were concerned mainly with the β activity of leaf and smoke. On smoking cigarette and cigar tobaccos, the bulk of such activity is found in the ash (10, 11, 355, 478, 532) and is presumed to be due mainly to the naturally occurring K^{40} although isotopes of rubidium (10, 11, 355), strontium (10, 11, 197), and cesium (197) are also present. The amounts of K^{40} transferred to the smoke of cigarettes (less than 1% of total leaf activity) were calculated to be about 10,000-fold less than the dose required for tumor formation (532) and only slightly more than the α activity of Rn^{222} and Rn^{220} normally inhaled from the atmosphere (478). Also, the inhaled K^{40} may be in a "soluble" form which is readily eliminated from the lung tissue (478). A process of steaming tobacco has been claimed to reduce the radioactivity of tobacco by more than threefold thus producing a relatively nonradioactive smoke (10, 11), but details are unavailable. The Sr^{90} content of Canadian tobacco has been measured (66) and is believed to be a negligible factor in the tumorigenic activity of cigarette smoke since the nuclide is not volatile at the temperature of a burning cigarette.

Recently, the α activity of tobacco has received considerable attention. Although the absence of radioelements in the radium and thorium series in leaf was reported in early work (11), later studies have shown variable levels of α activity in green leaf and tobacco products. Total α activity amounting to 10–1100 pcuries per 100 g of tobacco has been reported

in green tobacco, cigarettes, and cigars (327, 584), and about 20–25% of this activity (584) is due to Ra^{226} and Ra^{228}. Other reports (161, 581) give values of 9.9–47 pcuries per 100 g for Ra^{226}. Variations in α activity of leaf are found depending on geographic origin and growth and processing conditions (198, 325, 581, 583). Pb^{210} and Po^{210} with half-lives of 19.4 years and 138.4 days, respectively, are daughter elements of Ra^{226} which also occur in tobacco leaf (158, 198, 581–583). In addition, bismuth-210 (half-life, 5.9 days) may be present but cannot be detected (581). The measured levels of these daughter elements are higher than calculated values based on the Ra^{226} content of the growing leaf showing that a source other than decay of Ra^{226} contributes to the Pb^{210} and Po^{210} contents. The source may be direct absorption of the nuclides from the soil rather than foliar intake of atmospheric Rn^{222} (half-life, 3.83 days), a gaseous precursor of the lead and polonium nuclides (582). An earlier report which attributed the Po^{210} content of leaf to the curing process (43) has not been confirmed (582). In general, contamination with radioactive fallout is not believed to be a major contributor to the activity of tobacco leaf (327, 582).

The presence of α activity in leaf has led to a search for such activity in smoke. In earlier work, almost all the long-lived α activity in leaf was believed to be retained in the ash, and the only source in smoke could be the gaseous Rn^{222} arising from decay of Ra^{226} in leaf (584). Calculations based on such reasoning showed that the intake of Rn^{222} by smokers was negligible. However, these findings were ultimately disputed (327) and interpreted in another way to show that significant loss of other α emitters, such as Po^{210}, may occur during burning of a cigarette. The controversy was finally resolved by the demonstration of Po^{210} in cigarette smoke (441) although the biological significance of these findings still remains questionable.

Po^{210} is volatile at 500° and is easily transferred from leaf to smoke during burning. The nuclide has been isolated from mainstream smoke using a method for separating radium isotopes and identified by determination of the decay pattern (441). Recovery experiments on cigarette tobaccos and smoke have accounted for 80–100% of the Po^{210} in leaf (161, 426, 441) with approximate distribution percentages as follows: ash, 10; butt, 35; mainstream smoke, 25; sidestream smoke, 30. The reported levels in mainstream smoke are 0.029–0.139 pcuries per cigarette (161, 284, 441, 660) and are apparently dependent on the conditions of mechanical smoking (284). The nuclide occurs mostly in the particulate phase of smoke, and the ratio of particulate matter to Po^{210} in smoke varies over a relatively narrow range (284). Filter cigarettes contain smaller amounts of Po^{210} in mainstream smoke than nonfilters, but this effect is apparently due to nonspecific removal of particulate matter (284, 330, 441, 442).

Various interpretations of the biological significance of Po^{210} in cigarette smoke have appeared. Initial estimates of the excess α activity in lung or other body tissue due to smoking (441) were disputed (226, 240, 326, 518) and then rebutted (315, 443). Most recently, analyses of tissues for Po^{210} and its precursor, the long-lived Pb^{210}, have provided data on retention and distribution within the body, and detailed discussions of the physiological significance of these nuclides have appeared (241, 316).

B. FREE RADICALS AND IONS

Since free radicals may play some role in the induction of tumorigenicity, attention has been directed to the free-radical content of tobacco smoke. The levels of radicals in cigarette smoke are about 10^{14}–10^{16} free radicals per g of smoke (242, 322, 323), which is in the range found for soots and atmospheric smoke (322, 323). The stability of these radicals is variable (242, 322, 327); although the number of free radicals is drastically reduced when smoke condensate is warmed from very low temperatures to room temperature or higher (242, 322, 323), free radicals can still be detected in flue-cured cigarette smoke even after 300 hr by reaction with the radical scavenger, α,α'-diphenyl-β-picrylhydrazyl (DPPH) (563). The retention of free radicals in rabbit lungs after exposure to cigarette smoke has been demonstrated recently by esr techniques (472).

The chemical structures of the free radicals in smoke may be quite diverse. Gross differences in the free radicals of smoke condensate are readily apparent based on solubility (322) and chromatographic behavior on alumina (323). A linear relationship exists between loss of fluorescence and decreased reactivity with DPPH when smoke solutions are irradiated (323). Thus, experimental handling of smoke may influence the quantity and structure of the free radicals with concurrent effects on the biological activity.

The stability of free radicals in smoke are actually reflections of structural differences. In general, stability is determined by the potentialities for migration of the free electron throughout the strucutre, *i.e.*, movement in a delocalized orbital. Structures containing large numbers of fused aromatic rings afford a high potential for extensive orbital delocalization, and such structures may be major contributors to the stable free-radical complement of cigarette smoke. Comparisons of free-radical concentrations in pyrolytic products of organic matter and the percentage of carbon in these products tend to confirm this concept (242). The "semiconductor" theory of carcinogenesis by such hydrocarbons is based on the migration of an electron from an excited protein molecule to pair with the mobile

electron in the hydrocarbon molecule at the proper energy level, thus resulting in what is essentially a "free-radical" protein (242).

The free radicals in smoke are derived primarily from the pyrolytic reactions which occur during burning. A possible secondary source is distillation of intracellular free radicals produced by *in situ* α irradiation or by autooxidative processes (327). As discussed in section III.N.3, leaf contains relatively large amounts of unfractionated resins which possess structural features suggestive of terpenoid or steroidal oxidation products. Since peroxides are formed in autooxidations, the peroxides detectable in smoke (see section IV.C) could conceivably originate by distillation from tobacco during burning of a cigarette. In general, however, the contribution of a secondary source of free radicals to the total content of smoke must be relatively minor and probably insignificant.

Free radicals are produced by burning all types of organic matter, and a relationship exists between temperature and the yield of free radicals. One published example (242) shows that the rate of free-radical generation increases markedly at about 400°, peaks at about 500–600°, and declines at about 650–1000°. However, heating tobacco or cellulose isothermally at different temperatures in this range results in a progressive increase in free-radical-mediated pyrosynthesis of benzo[*a*]pyrene (BAP) (see section V). Considering the complex burning pattern of a cigarette, it is obviously not possible to predict the temperature in a cigarette at which free-radical generation would proceed maximally. Nevertheless, alteration of the burning characteristics of a cigarette to change the yields of free radicals and polynuclear aromatic hydrocarbons remains an attractive hypothesis, and some effort in this area has been reported (see section VI).

Most of the published work on free radicals in smoke has involved the relatively stable entities therein. The patterns of pyrolytic generation and the general properties of very short-lived radicals are relatively unknown because of obvious experimental difficulties. Also, other phenomena associated with the generation of smoke and subsequent biological effects of such smoke are difficult to correlate, *e.g.*, the possibilities of free radicals acting as "scavengers" for each other or of detoxifying effects by adsorption on carbon blacks (242).

In addition to free radicals, cigarette smoke contains large amounts of structurally unidentified gaseous ions (287, 318, 615) which possess energies in the range of 6 eV. Small ions of this nature are known to be bactericidal in much lower doses than ultraviolet and X-ray irradiation, but large ions are not believed to have a biological effect attributable to charge alone. Although sidestream and mainstream smoke contain large amounts of large ions, mainstream smoke shows relatively low levels of small ions, indicating a loss during passage through the tobacco column by a mechanism other than filtration (287).

The ionic current in sidestream smoke increases greatly after puffing and on removal of the accumulated ash. Based on spatial and kinetic considerations, it has been estimated (287) that the concentration of small ions in a spheroidal volume having a radius of 25 cm from the coal of a cigarette would be 2.8×10^5 ions per ml which is about 1000 times the ionic level in a room not containing smoke. The ionic behavior of sidestream smoke from cigarettes is much different from smoke from cigars and pipes; cigarette smoke shows a persistent retention of ionic concentration up to 6 min after a puff, but the ionic levels of cigar or pipe smoke decay rapidly up to 2.5 min after puffing.

In general, the contribution of radioactive substances, free radicals, and ions to the biological effects of smoke in animals has been relatively ignored. Such agents may act in conjunction with other chemical carcinogens and promoters in smoke, which have been studied more extensively and, perhaps, overemphasized. A fundamental link exists between chemically and physically induced carcinogenesis in that both can act by disorientation of the normal electronic patterns in molecules. Much work is required to determine the relative contribution of charged particles in smoke to the tumorigenic effect in animals.

C. ALKYLATION AND ENZYMATIC INHIBITION

Biological alkylating agents act by alkylation of essential cellular metabolites in producing toxic, mutagenic, and/or tumorigenic effects. Some aspects of biological alkylation relating to tobacco and its use have been reviewed earlier (4).

Although the presence of alkylating activity in cigarette smoke has been known for several years (424, 487), details of this property have been studied only recently (542). Alkylations are primarily nucleophilic reactions and a colorimetric method employing the nucleophile 4-(4′-nitrobenzyl)pyridine has been used to determine the level and distribution of activity in cigarette smoke condensate. Unfractionated smoke condensate from domestic, commercial cigarettes had the alkylating equivalent of 20 μg of 2-iodobutane per cigarette. On separation of the smoke condensate into twelve fractions of different solubilities, alkylating activity was found in every fraction. With the possible exception of the water-soluble acidic substances, the bulk of the activity was found in the cyclohexane-soluble neutrals (26% of total). The crude, weakly acidic brown pigments comprised 6.8% of the condensate weight and had 8.5% of the total activity. The largest specific activity was found in a precipitate which formed on addition of hydrochloric acid to an ether solution of the bases and neutrals. An accurate approximation of the alkylating activity of the water-soluble acidic fraction could not be

made because of artifact formation. The final step in the isolation of these substances required evaporation of a 0.1 *N* hydrochloric acid solution containing these acids. Apparently, some hydrohalogenation of the unsaturated acids occurred during solvent removal, yielding halogenated compounds with high alkylating activity and fallaciously high values for this fraction. However, when calculated by difference, these substances had the highest activity of any fraction (33% of total).

The smoke constituents responsible for this alkylation are not known. Methyl chloride is an obvious candidate, but this compound should not be present in the fractions because of its volatility. Other possibilities are γ-lactones (*e.g.*, levantenolides, IX), nitrosamines, and pesticides, such as halogenated hydrocarbons or phosphate esters which may be present in trace amounts in leaf and smoke (see section III.N.2). In fact, the presence of any alkylating agent in smoke condensate known to contain nucleophiles, *e.g.*, pyridines, would seem to be anomalous. One possible explanation is that alkylating reactions have already occurred in smoke during collection, and the activity being measured is a small residual one. Another possibility is that the pattern is a reflection of differences in the rates of nucleophilic reactions. In the SN1 mechanism, rates are related to the dielectric constant of the solvent, and cooled (−79°) smoke collection traps may not be favorable in this respect. In the SN2 mechanism, alkylation is a function of the nucleophilicity of the nucleophile, and the analytical reagent may be more active than the bases occurring naturally in smoke.

Tobacco smoke inhibits the activity of several enzymes including urease (483), succinic dehydrogenase, (483), glyceraldehyde 3-phosphate dehydrogenase (GAPD) (306), yeast alcohol dehydrogenase (306), and monamine oxidase (72). The inhibition of urease and succinic dehydrogenase is reversed by cysteine or glutathione (483). With GAPD, two inhibitory factors are present, one of which is reversed by cysteine and is not removed by inhalation (306). Addition of catalase to smoke prior to reacting with the GAPD results in complete disappearance of the inhibitory effect. Fresh smoke accelerates the autooxidation of cysteine markedly but inhaled smoke has a negligible effect (577). Thus, at least part of the inhibitory action of smoke is probably due to oxidation of sulfhydryl groups in enzymes. Since small amounts of peroxides can be detected in smoke (564), these compounds may be responsible for some of the inhibition. In addition, the free radicals in smoke may contribute to the effect. Transfer of electrons from free radicals to sulfhydryl groups is possible, yielding thio radicals which could react with oxygen to give thioperoxy radicals (577) and eventually disufides or sulfinic and sulfonic acids as oxidative products.

The effect of tobacco smoke on monoamine oxidase has been studied superficially (72). This enzyme acts by oxidative deamination of a number of substrates, including serotinin, a pharmacologically active compound which occurs in many animal tissues and has an effect on cilia. Using spermine as a substrate, the inhibitory activity of smoke is found in both the gaseous and particulate phases, but the effect is not due to nicotine.

Elucidation of a specific mechanism of inhibition by tobacco smoke in a given enzyme system is difficult. Tobacco smoke contains several components which are known to react noncompetitively with many enzymes, *e.g.*, carbon monoxide, hydrogen sulfide, and simple phenols. Depending on the enzyme, several inactivating reactions may occur simultaneously, and separation of these effects can be a formidable task. Considering the potential physiological effects of enzyme inhibition, such studies comprise an important area of work for future investigation.

V. THERMAL ALTERATION OF TOBACCO CONSTITUENTS

Since tobacco is consumed mainly by smoking, numerous investigations have been reported on the chemical changes during heating and burning of tobacco. Most of the studies have concerned the pyrolytic products of tobacco constituents with particular emphasis on precursors of smoke components having tumorigenic or organoleptic properties. The present discussion will be limited to studies related to tobacco chemistry and is not intended as a general review of thermal and pyrolytic effects on organic compounds.

When stored in closed containers at room temperature and atmospheric pressure, tobacco emits vapors which can be trapped and examined compositionally (558) using subtle collection and analytical systems. These vapors consist generally of low-boiling constituents which have been discussed in section III. On warming tobacco progressively, moisture and volatile organic compounds are lost, and changes in the carbohydrates take place. On prolonged heating up to 60°, glucose is converted into unidentified high molecular weight constituents (123), hydroxymethylfurfural (124), formic acid (125), tetrahydroxyvaleric acid (125), and other oxidative products (123). Differential thermal analysis of the heating pattern shows a marked endothermic reaction occurring in the 60–80° range attributable to loss of water and low-boiling volatiles (147). As the temperature is increased, a second endotherm is noted at 160–170° which has been attributed to loss of water of hydration (71) and decomposition or melting of leaf constituents such as carbohydrates and pectins. Significant losses of alkaloids by volatilization also occur in this temperature range (293). Further heating of tobacco causes substantial weight losses (147) which are somewhat different for the different tobacco types

(71, 416). Large weight losses are encountered at temperatures approaching the ignition point which is about 400–450° (71, 573) although a value of 230° has been reported (416). At temperatures above the ignition point, classical free-radical reactions of pyrolysis are initiated and drastic changes occur in the components of heated tobacco. From 400 to 1000°, the yields of condensable pyrolysate decrease progressively but the levels of polynuclear aromatic hydrocarbons (PAH) in the condensates increase rapidly (201). The condensates contain many constituents found in cigarette smoke as well as compounds not occurring in smoke, *e.g.*, 20-methylcholanthrene and anthraquinone (297).

In a burning cigarette, cigar, or pipe, a sequential pattern of thermal changes superficially similar to the above events occurs. The maximum temperature in the cigarette cone is about 880° and variable temperatures in cigars and pipes have been reported (644). In cigarettes, a sharp thermal gradient is found in a narrow region behind the burning cone (214), and this gradient may vary with the length of the remaining butt (149). Volatilization, distillation, sublimation, pyrolysis, chemical interaction, and, possibly, mechanical cellular eruption (540) occur chiefly within this region, and oxygen plays a minor role in the reactions (228). The formed aerosol proceeds rapidly down the cigarette during the "draw" and is mixed with air which permeates the cigarette paper. Partial deposition of the aerosol occurs during this passage and the condensed material is again subjected to heat and revolatilized as the cone moves down the cigarette. The compositional nature of the aerosol thus changes from puff to puff, giving higher concentrations of aerosol and components therein as smoking proceeds (376).

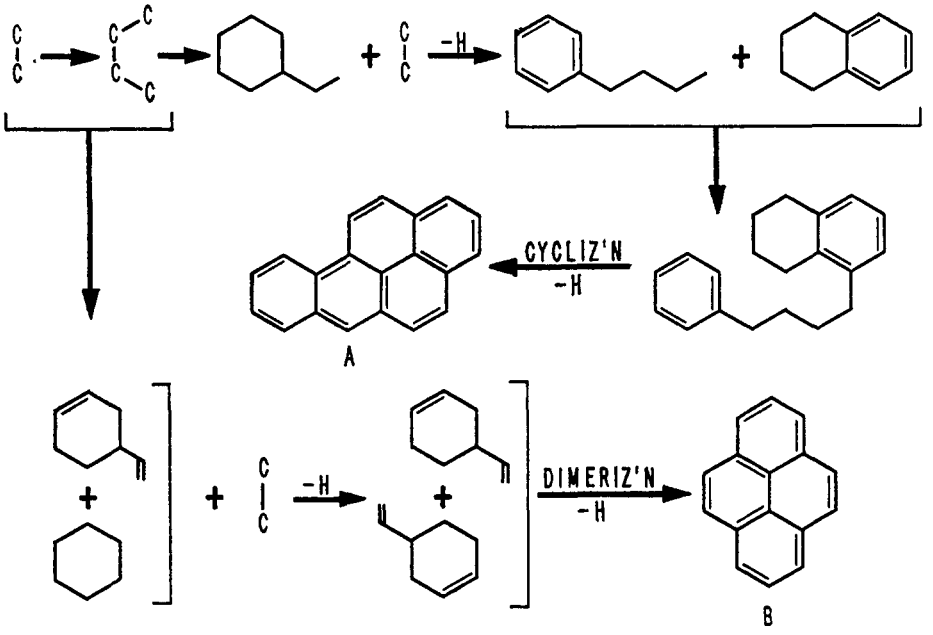
Earlier investigations on the precursors of the PAH were tacitly based on the concept that one or, at most, a few leaf components act as such precursors. Since most, if not all, organic compounds produce PAH at the temperatures of a burning cigarette, *e.g.*, oxalic acid (180), the concept of a single precursor is obviously an oversimplification. However, leaf components vary widely in their yields of PAH on heating (93, 180) and may contribute disproportionately to the over-all PAH in mainstream smoke. A major obstacle in studies on leaf precursors has been the mechanics of designing pyrolytic experiments to simulate the thermal environment of a burning cigarette. For example, pyrolysis of cellulose at 880° gives a yield of benzo[*a*]pyrene (BAP) which is 1000-fold greater than the amount of BAP produced by smoking a cigarette (450). Extrapolations of such pyrolytic data to the events which occur during the burning of a cigarette are tenuous although some progress has been reported recently: by varying experimental conditions carefully, indirect correlations of phenol yields from pyrolytic and smoking experiments can be made (15, 530). However, most published studies have involved pyrolysis of tobacco constituents and extracts in heated tubes in the conventional manner.

A. PARAFFINIC HYDROCARBONS

When pyrolyzed in air or nitrogen at temperatures at or near that of a burning cigarette, dotriacontane or a mixture of aliphatic paraffins from tobacco leaf produces at least 30 PAH having three or more rings (300–303) and the yield of BAP is almost 100-fold greater than that obtained from glucose (201). Maximum levels of BAP are found at 850°, and the quantities of PAH formed at 700° are about one-third to one-tenth the amounts obtained (301, 302, 304) at 800°. At 700°, significant amounts of methane, ethylene, propylene, acetylene, benzene, toluene, naphthalene, and styrene are also produced (20, 24). No aromatic compounds are formed at or below 600° (302).

The mechanisms of these pyrosyntheses have been studied in detail. In general, fragmentation of the paraffinic hydrocarbon chain to C_2 and C_4 units occurs, and the units may polymerize by several routes to yield aromatic hydrocarbons of one to six rings. The pyrosyntheses of BAP (Scheme I, A) (18, 29) and pyrene (Scheme I, B) (18) illustrate the generalities of two representative cases and are given here without regard to the positions of unpaired electrons which are variable and transitory. For BAP, the sequence involves combination of C_2 and C_4 units to form ethylcyclohexyl, butylphenyl, and tetralinyl radicals, the last two of which cyclize and dehydrogenate to BAP. In the pyrene synthesis, the C_2 and C_4 fragments combine in several steps to form a pair of 4-vinylcyclohexenyl radicals which then dimerize and produce pyrene after dehydrogenation. However, cleavage of the higher aliphatic hydrocarbons to C_2 and C_4 fragments is not a necessity since synthesis may begin when fragmentation to C_6, C_8, or larger units has occurred (27). The effect of temperature on the generation of PAH from a C_{10} compound, butylbenzene, is generally similar to the pattern noted above for tobacco paraffins and little or no PAH synthesis occurs below 450° (26). Evidence for these general mechanisms has been obtained by extensive studies on the yields of PAH from such precursors as acetylene, butadiene, styrene, ethylbenzene, indene, benzene, toluene (18), and naphthalene (30), including some C^{14}-labeled compounds. Mechanisms superficially similar to Scheme I have been suggested for the pyrosynthesis of anthracene (28), phenanthrene (19, 23, 28), chrysene (22–25), benzofluorenes (22, 25), benzofluoranthenes (24, 25, 30), benzanthracene (28), perylene (19, 25, 30), benzoperylene (27), benzopyrenes (19, 21, 25), dibenzopyrenes (19, 27), anthanthrene (27), and coronene (27). Similar mechanistic schemes can also be used to explain the presence of the mono-

SCHEME I



and dicyclic aromatic compounds found in cigarette smoke.

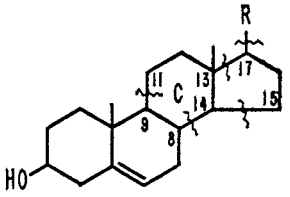
The initial demonstration of PAH generation by pyrolysis of tobacco leaf paraffins inspired related studies on the hexane and cyclohexane extracts of leaf. Such extracts are extremely complex in composition and contain many groups of components other than aliphatic paraffins, *e.g.*, sterols, terpenes, esters, fatty acids, etc. (537). Pyrolysis of extracts at temperatures from 560 to 880° and bioassay of the pyrolysates have shown a direct relationship between tumorigenic activity and temperature (650), and no tumors are observed with the 560° pyrolysate. However, attempts to reduce the BAP content of smoke by extracting tobacco have shown conflicting results (see section VI).

The concept that paraffin hydrocarbons of tobacco are major contributors to the PAH of smoke has been challenged (444). No significant reduction in substances absorbing at or near 385 mμ (attributable to PAH) were observed in the smoke from hexane-extracted cigarette tobaccos, and no significant increase was found in smoke from extracted cigarettes containing added C^{14}-tagged paraffins or hexane extract. However, other authors (644) have criticized these findings because of the nonspecificity of the substances absorbing at 385 mμ and other methodological factors. A more general objection can be made concerning any experiment in which leaf substances are extracted and subsequently added to cigarettes to note the changes in smoke composition. Such additions result in components occupying a position relative to the leaf cells which may be completely unlike their natural *in situ* location. The same substance located within a cell or on a cell surface may undergo different patterns of thermal alteration. Also, the addition of significant amounts of substances to cigarettes to alter the smoke composition may modify the basic burn characteristics and produce compositional changes which are unrelated to the chemical nature of the additive. However, experimental limitations frequently make the objectionable approach the only choice.

Higher paraffinic hydrocarbons yield phenol on heating in air at temperatures greater than 500°. The yields are greater than those obtained from cellulose and pectin and are equivalent to the levels obtained from heating tobacco (35).

B. STEROLS

Sterols of tobacco have also been implicated as precursors of PAH in smoke. Since these compounds contain the elements of a polynuclear system, they can be easily dehydrogenated to phenanthrene-like compounds using a palladium–charcoal catalyst (303) and, on pyrolysis at 700–840° in nitrogen, can form more than 50 hydrocarbons, including 30 PAH (20, 651). High yields of phenanthrene and chrysene are formed in contrast to the products from dotriacontane which contain toluene and styrene as major constituents (20). Thus, a different mechanism of formation of aromatic hydrocarbons is indicated for the sterols, a concept that would be theoretically predictable. The bond dissociation energies of all the carbon–carbon bonds of dotriacontane are approximately similar (about 80 kcal/mole), but the presence of tertiary carbons and the relatively strained five-membered ring in the sterol skeleton (XXVI) give the structure a versatility of lower bond energies. Scission of the C_{13}–C_{17} and C_{14}–C_{15} bonds with subsequent dehydration and dehydrogenation would yield phenanthrene. Splitting off of the C_{17} aliphatic chain, scission of the C_{14}–C_{15} bond,



XXVI

and migration of the angular methyl on C_{13} to the C_{14} position would yield chrysene after dehydration, dehydrogenation, and cyclization; such migrations of methyl groups in sterols occur frequently (20). BAP would result from scission of the C_9–C_{14} bonds in ring C, giving tetralin (after dehydration and dehydrogenation) which could split to butylbenzene and yield BAP *via* the reactions in Scheme I. The formation of binaphthyl, benzofluoranthenes, pyrene, perylene, and dibenzopyrenes can be illustrated similarly (20).

Significantly larger amounts of BAP are formed from phytosterols at 850° compared to 720° (650), and the pyrolytic products at 800° (in nitrogen) show tumorigenic activity of the same order of magnitude as the pyrolysate of a hexane extract of tobacco from which the paraffinic hydrocarbons are removed (651).

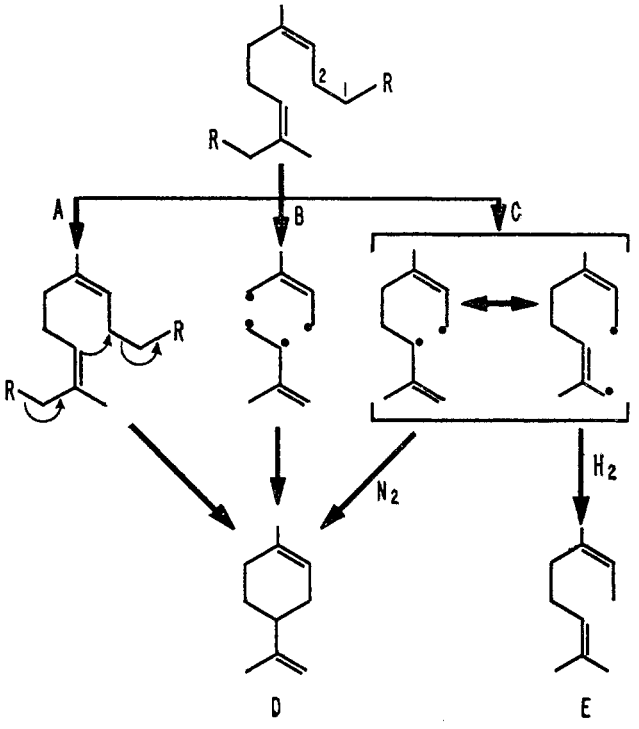
C. TERPENES

The effect of heat on some terpenes of tobacco leaf has been studied in detail. Experimental evidence and postulations have implicated neophytadiene, carotene, solanesol, and cyclic diterpenes as possible precursors of 2,4-dimethyl-4-vinylcyclohexene, dipentene, phytadiene dimers, naphthalenes, PAH, and aldehydes and ketones of smoke.

On heating to 180°, the conjugated system of neophytadiene was initially reported (452) to migrate toward the center of the chain producing isomers similar to the phytadienes isolated from smoke (see section III.B.1). Later work (265) showed that bond migration occurs to a limited extent and the major products are the previously discussed series of dimers (IV) which are formed through Diels–Alder condensation and are smoke constituents.

An early postulation of the formation of dipentene in smoke included cleavage of leaf isoprenoid compounds to isoprene which subsequently dimerized to *p*-mentha-1,8-diene (dipentene) (179). However, since dimerization of isoprene was know to yield *m*- and *p*-mentha-1,8-dienes and a mixture of dimethylvinylcyclohexenes but tobacco smoke itself contained only *p*-mentha-1,8-diene and one cyclohexene (see section III.B.1), it was apparent that a route other than simple dimerization of isoprene must be operative (264). Later experimental evidence showed that terpenes may still act as precursors for dipentene without necessarily involving isoprene. The proposed

SCHEME II



mechanisms of dipentene formation from the trisesquiterpenoid alcohol, solanesol (XVI), are shown in Scheme II. At 550°, an internal monoterpenoid unit may split in three ways. In route A, cyclization accompanied by scission of the remainder of the chain yields dipentene (D) directly (207). Cleavage between C_1 and C_2 is expected since this bond has the lowest dissociation energy of the carbon to carbon bonds in the diisoprenoid moiety (264). In route B, the C_{10} unit is fragmented into two isoprene diradicals, which may exist in different hybrid forms, two of which are illustrated. Dimerization of the diradicals gives D (207). In route C, a C_{10} diradical is formed which, in one hybrid form, may cyclize to form the hydrocarbon in an inert gas atmosphere. Evidence for this C_{10} diradical is indicated by the isolation of a dimethyloctadiene (E) when pyrolysis is conducted in a hydrogen atmosphere (208). In addition to dipentene, the pyrolysis also yields mixed dimethylvinylcyclohexenes, but these compounds have not been resolved so that the actual presence of the smoke constituent, 2,4-dimethyl-4-cyclohexene, among the pyrolytic products cannot be claimed. Dipentene formed in these reactions may further undergo aromatization, alkylation, and dealkylation yielding such compounds as ethyltoluenes, trimethylbenzenes, naphthalenes, *p*-cymene, and PAH (179). However, the contribution of these routes to the PAH complement of tobacco smoke is considered minor since isoprene and dipentene probably volatilize before being exposed to a temperature sufficiently high to produce PAH (100). Pyrolysis of solanesol at

650° yields unidentified aromatic compounds without concurrent formation of isoprenoid substances (208).

Tetraterpenes and, possibly, the cyclic diterpenes may be important precursors of aromatic hydrocarbons in smoke. Heating β-carotene at 188–300° produces ionene (1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene) as a major volatile product (122, 148, 324) and toluene, xylenes, and 2,6-dimethylnaphthalene in small amounts (148, 324). Two basic mechanisms may be operative in the formation of these compounds from carotene: one pathway involves cyclization of a terminal C_{13} moiety to yield ionene (148, 324), and the other mechanism is based on cyclization of the polyene chain to give the alkylbenzenes and alkylnaphthalenes (324). However, the principal thermal product of β-carotene degradation at 240° *in vacuo* is a series of nonvolatile, uncharacterized substances with molecular weights up to 983 (324). The duvatrienols (XI–XIV), levantenolides (IX), levantanolide, and 12α-hydroxy-13-epimanoyl oxide (X) of tobacco leaf may act as precursors for alkylnaphthalenes and PAH, but no evidence of such relationships is available.

D. CARBOHYDRATES, LIGNIN, AND ACIDS

Tobacco contains about 65% carbohydrates, lignin, and related substances, and much interest has been shown recently in the effects of heat on these components.

At temperatures under 450°, a variety of products are formed from oligosaccharides and polysaccharides. The compounds formed from glucose on heating tobacco at 60° have been discussed above. At 300°, glucose is fragmented mainly to 1,4:3,6-dianhydro-D-glucopyranose, furans, aldehydes, and ketones; the furans may arise from cleavage of polymers which form initially in the pyrolysis (225). Heating of sucrose yields mainly furfural and 5-hydroxymethylfurfural and the following minor products: 2-acetylfuran, β-angelicalactone, γ-butyrolactone, and two hydroxymethylcyclopentenones (261). At 375–420°, cellulose is split to low molecular weight aldehydes, ketones, aliphatic acids, and levoglucosan (1,6-anhydro-β-D-glucopyranose) (238, 507).

Recent studies on the effect of heat on carbohydrates have been concerned mostly with the formation of phenols and PAH at temperatures above 400°. Lignin, pectin, cellobiose, glucose, and glucuronic and polygalacturonic acids give higher levels of phenol and cresols than cellulose when heated isothermally at 700° in nitrogen (494). Isoeugenol and 2-propylphenol, compounds related to lignin, give very high yields of these compounds, as expected. Under isothermal conditions, the optimum temperature range for phenol formation from lignin is 500–600° and the yields in nitrogen are higher than in air. Other products identified in the thermal decomposition products of tobacco lignin are guaiacol, pyrogallol 1,3-dimethyl ether, and *p*-creosol (4-methyl-2-methoxyphenol) (279). When pyrolyzed in a system permitting programmed heating to a maximum temperature of about 700°, glucose, sucrose, starch, cellulose, and pectin generally give lower percentage yields of phenol than tobacco itself (35). Analysis of the smoke from cigarettes containing C^{14}-tagged glucose have shown that the conversion of the sugar to phenol during burning is higher than the expected yield based on pyrolysis of the pure carbohydrate (35). Based on this conversion, it was estimated that about 40% of the total phenol of smoke is contributed by the carbohydrates of the leaf. The remaining phenol may be formed by pyrolysis of chlorogenic acid, brown pigments, and other substances.

The formation of PAH from the following carbohydrates and related leaf components has been demonstrated: xylose, glucose, sucrose, maltose, fructose, starch, cellulose, xylan, pectin, and lignin (180, 201, 450). Suggestions of a relationship between the cellulose content of leaf and the PAH level of smoke (363, 427) have led to detailed studies on the pyrolysis of this polysaccharide. Two mechanisms are evident for the formation of BAP from cellulose depending on whether heat is applied isothermally in a series of steps from 450 to 880° or is programmed over this range (450). The isothermal mechanism is inhibited by iron, cobalt, and nickel, and a direct relationship between BAP yield and temperature is observed. The mechanism operative in programmed heating is insensitive to metals and gives lower amounts of BAP that vary indirectly with temperature. Comparisons of BAP yields from monosaccharides (glucose and xylose), disaccharides (cellobiose, maltose, and sucrose), and polysaccharides (amylose, soluble starch, and xylan) related to cellulose show that the nature of the C_1–C_4 steric configuration is not a major factor in the BAP produced from the monomers and dimers but is important in the polymers (450). However, oxidation of the C_6-hydroxymethylene groups in both glucose and cellulose markedly reduces the BAP yields.

Malic, citric, and oxalic acids are products of carbohydrate metabolism that occur in substantial amounts in tobacco. Pyrolysis of these compounds at 650° yields PAH although the level obtained with oxalic acid is quite small (180). Malic, citric, *cis*-aconitic, and fumaric acids give phenol and cresols on pyrolysis at 700° in yields smaller than the common carbohydrates, but sodium lactate produces more phenols than glucose and cellobiose (496).

E. ALKALOIDS

Nicotine, 3-(2′-N-methylpyrrolidinyl)pyridine, is probably the most unique component of tobacco and a major precursor of the volatile bases of smoke. On heating nicotine at temperatures greater than 400°,

various patterns of fragmentation occur depending on the gaseous atmosphere and other experimental conditions; for example, the use of quartz chips, activated alumina, and metallic oxides in reactors gives a wide range of yields of myosmine (625) and 3-cyanopyridine (624) from nicotine at 500–780°. The major identified pyrolytic products of nicotine and two other tobacco alkaloids at 400–900° in a nitrogen or helium atmosphere are shown in Table XVIII. In addition to the listed components, ammonia, methylamine, nicotyrine, N-methylmyosmine, and pyridine-3-aldehyde have been found in pyrolysates of nicotine (577a, 625). The qualitative and quantitative data in Table XVIII

TABLE XVIII
EFFECT OF TEMPERATURE ON THE COMPOSITION OF THE PYROLYTIC PRODUCTS OF MAJOR TOBACCO ALKALOIDS

	Nicotine[a]				Nornicotine			Myosmine	
Products	600°	700°	800°	900°	400°	500°	600°	500°	550°
Pyridine	1	2	3	2	–	–	–	+	+
3-Methylpyridine	2	3	2	1	3	2	1	1	2
3-Ethylpyridine	3	2	1	1	2	3	1	1	2
3-Vinylpyridine	3	3	2	1	+	+	1	1	+
Metanicotine	1	1	1	–	–	–	–	–	–
Benzonitrile	–	–	1	2	–	–	–	–	–
3-Cyanopyridine	2	3	3	1	2	1	2	1	2
Naphthalene	–	–	1	2	–	–	–	–	–
3-(Buta-1,3-dienyl)-pyridine	2	1	–	–	–	–	–	–	–
2-Cyanopyridine	–	–	1	1	–	–	–	–	–
Quinoline	1	3	3	2	1	2	3	1	2
Isoquinoline	1	2	3	2	1	2	3	1	2
1,7-Diazaindene	–	–	1	1	–	–	–	–	–
Nicotine	3	–	–	–	–	–	–	–	–
Nornicotine	–	–	–	–	–	–	–	–	–
Nornicotyrine	1	1	1	–	–	–	–	+	–
Myosmine	3	–	–	–	3	1	+	3	+

[a] Relative amounts: 1 = smallest. + = in trace or undescribed amounts.

are based on several studies (31, 256, 289–291) and give some insight into the mechanism of fragmentation. The arbitrary values in the table are for use only in comparing relative yields of a given product from a given alkaloid at the indicated temperatures and do not reflect the absolute yields of products at one temperature.

Nicotine is not fragmented significantly at <600° in reactors without packing. At 600°, and in an inert atmosphere, about two-thirds of the nicotine is split mainly into myosmine (1′,2′-dehydronornicotine) and 3-vinylpyridine. At 700°, nicotine is completely decomposed, and the major products are 3-vinylpyridine, 3-methylpyridine, and pyridine. At 800 and 900°, extensive cleavage and combination of fragments occur yielding such products as quinoline, naphthalene, and benzonitrile. From this over-all pattern, it is evident that dehydrogenation, demethylation, and scission of the pyrrolidine ring are initial steps in the pyrolysis of nicotine. In a C_1–C_2 fragmentation of this ring, dehydrogenation of the resulting N-methylaminoalkyl chain would give metanicotine (256) which, on further elimination, would form the observed 3-(1,3-butadienyl)pyridine and subsequently 3-vinylpyridine by appropriate cleavage. Fragmentation of the pyrrolidine ring in the 2,3 or 1,5 positions might ultimately give 3-cyanopyridine after dealkylation of the side chain.

The mechanism of thermal degradation of nornicotine is generally similar to nicotine although differences in thermostability exist (31, 293). Nornicotine is less stable than nicotine and the pyrrolidine ring fragments at <400°. At 400° and in an inert atmosphere, myosmine and 3-methylpyridine are major pyrolytic products, but at 500° only a small amount of myosmine is formed. Since myosmine is relatively stable (44% unchanged) on pyrolysis at 500°, this compound may not be an intermediate of nornicotine pyrolysis at higher temperatures (31). An alternative explanation is that myosmine formed from nornicotine may react with other pyrolytic products which are not produced when myosmine is pyrolyzed alone.

Although the available evidence is sparse, it appears that the pyrolytic mechanism of myosmine is different from that of nicotine and nornicotine (31). At 500° and in an inert atmosphere, myosmine gives lower yields of 3-methylpyridine and 3-ethylpyridine and higher yields of 3-cyanopyridine than nornicotine. This pattern may indicate that a C_3–C_4 split is favored in the five-membered ring; scission of this bond might be preferred over a C_2–C_3 fragmentation adjacent to the relatively stable C=N bond.

One superficial study has appeared on the pyrolytic products of 3-(2′-piperidinyl)pyridine (anabasine) heated at 580–650° in a charcoal-filled reactor (139). Exclusive of gases, the major products were reported to be pyridine, 2-methylpyridine, 2-ethylpyridine, 5-methylisoquinoline, and 2,3′-bipyridyl. The preponderance of the 2-alkylpyridines might indicate preferential cleavage of the pyridine ring which would be unexpected; evaluation of these findings is difficult since the use of charcoal in the reactor may have produced a catalytic effect and altered the pyrolytic reactions markedly.

Many of the pyrolytic reactions of the alkaloids at high temperatures are undoubtedly free radical although ion-controlled mechanisms may be operative to some extent (256). The appearance of such compounds as benzonitrile, naphthalene, and 1,7-diazaindene at these temperatures indicates that a high degree of molecular destruction and combination of fragments is occurring. Under such conditions, it is not surprising that acridine (314) and derivatives thereof, *e.g.*, dibenz[*a*,*j*]acridine and dibenz[*a*,*h*]acridine, occur in the products of nicotine and/or pyridine on pyrolysis at 750–850° (589). Another possible pyrosynthetic route for the acridines in smoke might involve cyclization of aromatic secondary amines

therein, *e.g.*, N-phenyl-4-isopropylphenylamine (see section III.J). If present in smoke, the *ortho* isomer of this compound could cyclize to form 9,9-dimethylacridan, a known smoke component. Demethylation and dehydrogenation of the substituted acridan would yield acridine which may further condense to form benzacridines or related compounds.

Amino acids of leaf have been implicated as precursors of three alkaloids in smoke. The amounts of harmane and norharmane in leaf account for only 1% of the levels found in smoke and further quantities of these alkaloids are probably formed from tryptophan during burning. The addition of C^{14}-labeled tryptophan to cigarettes results in recovery of radioactive harmane and norharmane in yields of about 80% of the predicted values (421, 422). Pyrocoll may also be derived from amino acids in leaf. Pyrolysis of gelatin at about 260–290° is known to yield this alkaloid, and the precursor is believed to be proline or hydroxyproline. The same pattern may exist in tobacco leaf and smoke, although experimental evidence on this point is lacking.

Decarboxylation of amino acids in leaf has been suggested as one of several reactions which could produce the low-boiling aliphatic bases in smoke (368). Other processes might involve pyrosynthesis from alkaloids or condensations of low molecular weight fragments from a variety of molecular scissions.

F. MISCELLANEOUS COMPONENTS

Thermal effects on the polyphenolic constituents of tobacco have not been studied extensively. Dry distillation of rutin, quercetin, and chlorogenic acid in air at temperatures up to 600° produces (664) several phenolic compounds (Table XIX). The furan derivatives

TABLE XIX
PYROLYTIC PRODUCTS OF THE MAJOR POLYPHENOLS OF TOBACCO

	Polyphenol		
Product	Rutin	Quercetin	Chlorogenic acid
Benzoic acid	−	−	+
Catechol	+	+	+
4-Ethylcatechol	−	−	+
Furfural	+	−	−
5-Hydroxymethylfurfural	+	−	−
4-Methylcatechol	+	+	+
5-Methylfurfural	+	−	−
Phloroglucinol	−	+	−
Quinic acid γ-lactone	−	−	+
Resorcinol	+	+	−

in the pyrolytic products of rutin may arise from the disaccharide side chain. The phloroglucinol in the products from quercetin is probably derived from scission of the benzopyrone moiety. Apparently, glycosidation of quercetin in the 3 position affects the bond energy of the ethereal linkage so that phloroglucinol is not formed from rutin. Catechols and alkylcatechols probably originate from the caffeic acid moiety of chlorogenic acid and the phenyl ring of quercetin and rutin. Benzoic acid is apparently formed from more complex reactions of fragments from the polyphenolic structures. In comparative studies, phenol is formed in smaller quantities from rutin than from glucose, sucrose, pectin, and cellulose (35). However, chlorogenic acid is a potent phenol precursor, giving yields 13-fold greater than those from carbohydrates and lignin (15). It is claimed that the quinic acid group of chlorogenic acid is primarily responsible for these high yields (15), which may be unexpected since the caffeic acid moiety contains a more thermostable aromatic structure.

The complex brown pigments (see section III.K) of leaf produce polynuclear aromatic hydrocarbons (PAH), phenols, volatile bases, and alkaloids on pyrolysis. At 850° in an atmosphere of nitrogen, 27 PAH are formed including many known smoke constituents (93). The addition of the pigment to cigarettes produces a significant increase in the level of benzo[*a*]pyrene in the smoke (93). The pigment is also a more potent precursor of phenols and cresols than tobacco cellulose, lignin, and pectin (494). On heating at 857° in nitrogen, quinoline, isoquinoline, 3-cyanopyridine, pyridine, and seven alkyl- or alkenylpyridines are formed from the pigment in a quantitative pattern which is similar to that produced when tobacco is heated under identical conditions (493). At 700°, nicotine and nornicotine appear in the pyrolytic products and the level of nicotine increases progressively as the temperature is lowered to 300° for both pigment and tobacco. Apparently, at higher pyrolytic temperatures, the nicotine, nornicotine, and other alkaloids linked in the pigment structure are pyrolytically released and tend to fragment into the products characteristic of alkaloidal pyrolysis.

With one exception, the thermal effects on simple phenols have not been studied. Vanillin produces phenol and *o*-cresol on heating and increases slightly the levels of these compounds in mainstream smoke when added to cigarettes (277).

Pyrolysis of neutral "tobacco resins" at 600° results in the formation of large numbers of hydrocarbons, acids, phenols, esters, and carbonyl compounds (344). Many of these components are known tobacco constituents, and their occurrence in such pyrolysates would be anticipated.

VI. TECHNOLOGY

Although not a primary objective in the present report, no comprehensive review of the chemical composition of tobacco and its smoke would be complete without some mention of technological studies. Since surveys on some phases of this subject are available

(555, 644, 648), the present report is simply a general summary of current knowledge.

Most technological investigations have concerned ways of altering the physiological effects of tobacco smoke with special emphasis on cigarette smoke. As indicated previously, unfiltered cigarette smoke has carcinogenic, cocarcinogenic, and ciliostatic activities when tested in animals, and the major known carcinogenic components are the polynuclear aromatic hydrocarbons (PAH). The major cocarcinogens in smoke are believed to the phenols, but terpenes, long-chain alcohols, higher fatty acids, certain esters, and other unidentified substances may contribute to the effect (365, 644); in addition, many recently isolated components having unknown biological activity may play some role in tumorigenesis. The important ciliostatic agents are mainly volatile gases or liquids such as hydrogen cyanide, formic acid, formaldehyde, and acrolein, but phenols (115) and other components may also be involved. In general, smoke components may occur in the vapor phase (*e.g.*, hydrogen cyanide and isoprene), the particulate phase (*e.g.*, PAH), or both phases (*e.g.*, phenol), depending primarily on volatility (206, 366, 621). Substances in both phases can be removed by filtration, and the removal of vapor-phase constituents may be selective in some cases. At present, nonvolatile compounds in the particulate phase cannot be removed preferentially by filtration but can be reduced or eliminated by altering the fundamental burning process, at least from a theoretical viewpoint. Attempts to modify the composition of smoke have involved a variety of approaches utilizing all of these facts.

One approach involves the extraction of tobacco or tobacco substitutes (510) with solvents prior to incorporation into cigarettes. Such treatment has been claimed to reduce the PAH of tobacco smoke in some studies (74) but not in others (606). One investigation on hexane-extracted tobacco has shown that benzo[*a*]pyrene (BAP) concentrations in smoke are not reduced, but a lower yield of smoke condensate is obtained (651). A process has been developed to diminish the PAH in smoke by extraction of tobacco with halogenated hydrocarbons and impregnation of the extracted tobacco with aluminum diethyl malonate or other organometallic compounds (364). Attempts to confirm the chemical claims of this process have failed (114), and the statistical inferences supporting the biological claims have been disputed (585). In general, selective solvent extraction of tobacco is regarded as an approach of little promise in reducing the tumorigenic activity of smoke in animals.

Another avenue of modifying smoke is to incorporate additives into cigarettes which alter the burning process. As noted above, the tumorigenic activity of pyrolytic products produced on heating tobacco sterols and hexane extracts of tobacco is a function of the pyrolytic temperature (650). This pattern is generally similar to the relationship between free-radical generation and temperature discussed in section IV. Therefore, the concept has been developed to lower the PAH yield by altering drastically the coal temperature of the cigarette. Many additives have been claimed to have this ability to a large extent, including aluminum foil, alumina trihydrate, and metallic silicates (555), but most of these claims cannot be confirmed. A comprehensive study of a large number of potential depressants has shown that significant alteration of coal temperature is difficult (33). One reason is that large quantities of additives are required (mostly 20–50% of cigarette weight) to produce any detectable change, and the additive cannot be uniformly distributed throughout the cigarette, resulting in large variations in temperature and difficulties in statistical evaluations of the results. However, moderate alterations of temperature ($\leqslant 200°$) can apparently be achieved with a few additives in high concentrations, *e.g.*, basic magnesium carbonate (33).

The incorporation of certain additives into cigarettes can reduce the amounts of BAP and phenol in smoke and the levels of tumorigenic activity of smoke condensate (233, 644) in animals. Copper nitrate and sodium nitrate have been especially effective in this respect. In the low levels employed (less than 10% of cigarette weight), it is doubtful that these additives influence significantly the burn temperature of the cigarettes. In the case of sodium nitrate, the mechanism is believed to involve thermal decomposition of nitrate to yield oxides of nitrogen which act as electron scavengers and inhibit the generation of free radicals. However, tobaccos with low and high nitrate contents give BAP levels in smoke which are not significantly different (36). Many other types of cigarette additives have been tested for the ability to reduce the PAH content of smoke; nitrites, glycerol, and ethylene glycol were found to be successful to some degree (40), but the effect of glycerol could not be confirmed (127). In general, the use of additives to alter smoke composition is a worthwhile area for future work.

The contribution of the cigarette paper to the PAH content of smoke has been accorded an unwarranted degree of attention. The paper represents about 5% of the weight of a cigarette and, although it may contribute disproportionately to the PAH content of smoke (5), the tobacco is still the major source of these compounds. Treatment of cigarette paper with ammonium sulfamate and other salts has been claimed to reduce the PAH in smoke (5), but these findings could not be confirmed (113).

By far, the modification of mainstream cigarette smoke by the use of filters has attracted the most attention. In general, filters may act selectively in removing

certain components from smoke, but this effect is presently limited to constituents in the vapor phase. Claimed reductions in total particulate matter (TPM) or "tar" of smoke are mostly a reflection of nonselective mechanical filtration of the aerosol and a function of filter compactness or the degree of porosity of the cigarette paper (506), both of which may be easily changed. However, cigarette tobaccos vary significantly in their yields of TPM, and the blending of the proper tobaccos can also contribute to reductions in the TPM of smoke (605). The basic filter material in cigarettes is composed of cellulose or acetylated cellulose although many polymeric and other substances may be employed (555). The selective removal of simple phenols has been studied extensively. Cellulose diacetate or triacetate filter tows show some selectivity for phenol (234, 529) and pyridine (298). The addition of glyceryl triacetate and other plasticizers to filters increases this selectivity and impregnated filters can remove much of the phenol in the vapor phase (119, 234, 529). To a lesser extent, nicotine may also be removed selectively by filters containing polyols (356). Another common plasticizer, di(2-methoxyethyl) phthalate, and other filter additives show significant removal of isoprene, acetaldehyde, methanol, acetone, toluene, and related constituents from the vapor phase (282).

Activated carbon filters remove selectively many gaseous components from smoke (378) and significantly reduce ciliostatic (643) and irritative (211) effects. Multiple carbon–cellulose acetate filters are superior to cellulose acetate filters in removing factors which inhibit growth and interfere with protein synthesis in human cells grown in tissue culture (570). However, one of the shortcomings of presently used carbon filters is limited adsorptive capacity for vapor-phase constituents. During the last few puffs of a cigarette, desorption may occur, resulting in higher concentrations of acetaldehyde and related constituents compared to earlier puffs (622). The type of carbon and method of making the carbon filter influence significantly the degree of ciliostatic efficiency (643).

Recently, an experimental filter containing an ion-exchange resin has been described which reduces markedly the Po^{210} content of mainstream smoke (67).

By mixing finely pulverized tobacco, ground midribs, and various chemical agents, a slurry is obtained which can be spread into thin sheets and dried, thus forming the familiar homogenized tobacco "sheet" or "reconstituted tobacco." This product is very uniform in character and is used with ground midribs as a substitute for cut tobacco leaf in many tobacco products. The use of reconstituted tobacco and ground midribs as a partial replacement for shredded tobacco reduces the yield of smoke condensate obtained from cigarettes (646).

The cut of cigarette tobacco is related to the yield of BAP obtained in the smoke (644). Increases in cigarette paper porosity accelerate the burning rate and reduce the yields of total particulate matter per cigarette, but the concentration of BAP in the smoke is not altered (646).

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VII. ADDENDUM

Between May 1, 1967 and September 27, 1967, several pertinent papers have come to the attention of the author. The designations in parentheses below refer to the related section in the present publication.

The occurrence of 9-fluorenone and 1-indanone in the smoke condensate of cigarettes containing dark tobaccos has been reported (III.F) (564a). The isolated levels were 0.25 (9-fluorenone) and 2.3 μg (1-indanone) per cigarette. The occurrence of free β-amyrin and phytol and free and esterified cycloartenol and 24-methylenecycloartenol has been observed in *N. tabacum* seedlings and tissue slices fed C^{14}-mevalonic acid (III.D.1 and 2) (44a). 2′-Deoxymaltose and an oligosaccharide described as 2,2-dideoxyglucobiose have been isolated from tobacco plants fed 2-deoxy-D-glucose (III.L) (162a), and the extraction of cytochrome f from tobacco has been reported (III.N.3) (241a). A correlation between nitrate levels in cigarette tobacco and concentrations of oxides of nitrogen in cigarette smoke has been observed, and removal of up to 44% of oxides of nitrogen has been claimed using a cellulose acetate filter with or without impregnation with activated carbon (III.N.3) (562a). This claim requires confirmation since filtration of nitric oxide from smoke by such filters has been reported previously to be unsuccessful (378).

Details have appeared on the method of separating polynuclear aromatic hydrocarbons (PAH) from smoke condensates (see section III.C) by migration of the tetramethyluric acid–PAH complexes on chromatographic columns under the influence of high electrical potential (III.C) (465a).

Additional comments on the nitrosamine problem have appeared (III.J) (378a).

Further data on the levels of Po^{210} in cigarette tobaccos, cigarette smoke, and selected tissues and areas of the respiratory tract of smokers have been published (IV.A) (443a). The ranges of concentration in different cigarettes and smoke therefrom are slightly higher than previously reported values.

A study (IV.B) (104a) of the behavior of the free radicals of tobacco smoke condensate in acidic media has shown that a limited number of radical types exists therein and the predominating cationic species is derived from PAH. Also, PAH may dimerize at high temperatures or be structurally altered by the presence of other smoke constituents (IV.B) (104a), *e.g.*, quinones of PAH, resulting in increased stability of the free-radical species (IV.B) (162b).

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