THE CHEMICAL CONSTITUENTS OF TOBACCO AND TOBACCO SMOKE

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CONTENTS

I. INTRODUCTION

A vast amount of literature has appeared on tobacco and its combustion products, and over the years many books and reviews have appeared (27, 59, 75, 76, 85, 136, 140, 168, 293, 306, 318, 389, 390), an authoritative guide to the earlier literature of tobacco chemistry being Brückner's *Biochemie des Tabaks* (27), whilst an extremely useful and comprehensive bibliography of the literature on smoke has recently been published (10). There is an increasing interest in the chemical constituents of tobacco and the transformations occurring during its processing, the seed and the immature plant receiving less but a not inconsiderable amount of attention. There has recently been a revival of interest in the composition of tobacco smoke, particularly on account of its supposed

connection with some forms of lung cancer, but tobacco manufacturers, themselves, are aware of the value of such information in relation to the quality of tobacco products. The purpose of this review is to collate and discuss the chemical constituents of tobacco from the standpoint of the organic chemist; hence closely related aspects in botany, plant physiology, and biosynthesis have been introduced only where strictly necessary. Many substances have been reported in tobacco and smoke, but their identification does not always satisfy the criteria of classical organic chemistry; this applies particularly to tobacco smoke. "Isolations and identifications" based mainly on R_f values, color tests, and ultraviolet spectra are not compatible with the classical meaning of these terms, but in view of the minute quantities of some of these substances, such information has often given the only indication of their chemical nature. The aim of this review also has been to draw the distinction between such reports and those in which the identity of a compound has been more fully established. The terminology applied to tobacco in the literature is often loose or vague (see Section II), whilst the conditions used to obtain tobacco smoke have varied from straightforward incineration in a continuous draft to the more desirable, mechanically simulated, human smoking conditions. Where possible, the history of the tobacco sample examined is indicated. The review is not exhaustive with regard to the literature, preference having been given to the latest works in which most of the earlier references can be found. Copies of some journals are difficult to obtain, but the original literature has been consulted wherever possible; the exceptions are indicated by references to abstracts.

II. Terminology in Tobacco Processing

An authoritative account of the methods and terminology of tobacco processing has been given (85). The processing of tobacco from harvested leaf to a satisfactory smoking product is a skilled and complex technique divided into stages which depend on the type of commercial product required. The terminology is loose and often vaguely applied in the literature: "curing," for example, may denote the entire processing from harvest to smoking material but is more conveniently used to describe the first phase of processing, i.e., drying, which can be either rapid, as in flue curing, or much slower, as in air, fire, or sun curing. The temperature of fire curing is very little above that of air curing, but the flavor of the leaf is altered by the smoke from the open fires, whilst sun curing, a modification of air curing, is a drying process carried out in the open; sun- and fire-cured materials comprise only a very small proportion of commercial tobaccos.

The type of processing to which leaf is subjected depends on the use to which the end-product will be put: namely, its use as cigaret, pipe, or cigar tobacco. The bulk of manufactured British cigarets usually contain a high proportion of flue-cured tobacco, whereas American cigarets often incorporate more air-cured material. The leaves for flue curing are picked from the plant individually as they reach maturity and are hung in barns where they are artificially heated for a short time at fairly low temperatures, which are gradually increased until

the process is completed at temperatures of about 80°C. The whole curing process lasts only a short period; the rapid drying produces a material which is low in moisture content (10–15 per cent) and in which enzymic action has been halted at an early stage.[1] Since whole leaves are cured, no translocation of mineral salts from leaf to stem can occur. The rapid halting of enzymic activity means that although most of the starch is used up, there is still a substantial quantity of free reducing sugars. There is relatively little change in contents of polyphenols, alkaloids, and proteins. After flue curing, the leaf is "aged" (at a moisture content of about 10 per cent) by storage in barrels for a few years before manufacture into cigarets. The mainstream smoke of such cigarets has an acid reaction.

Pipe tobaccos are prepared by air curing, for which the whole plant is cut down so that leaves of several biological ages are cured and considerable translocation of mineral salts takes place at the same time. In air curing, the tobacco is hung in barns at ambient temperatures and humidities permitting enzymic action to continue so that most of the starch and sugars are used up and protein and alkaloid contents are reduced, whilst the polyphenol content is much less than in flue-cured leaf. The air-cured leaf is then simply aged at a moisture content of about 10 per cent for use as pipe tobacco, the mainstream smoke of which is usually just on the acid side.

Cigar tobaccos are air-cured like the pipe tobaccos, but only individual mature leaves are used as in flue curing. Again, all the starch and sugars are used up, but there is no translocation of mineral salts or protein and the alkaloid content is much the same as in air-cured pipe tobacco. At this stage, some cigar tobaccos are given a short aging. The leaf is then subjected to "fermentation," the aim of which is the reduction of the quantity of nitrogenous compounds which impart an undesirable flavor to the smoke and also to enhance the smoking quality of the tobacco. The air-cured leaves are packed into tight staples and left to stand, when some form of anaerobic process occurs, as evidenced by an increase in temperature of the staples which are broken apart and rebuilt several times to complete the fermentation. The term "fermentation" does not imply a similarity to alcoholic fermentation, since it is not yet clear whether the cause is enzymic or is due to the mediation of bacteria and fungi, or both together. An excellent review, completed in 1950 (75, 76), dealt with the chemical conversions in the harvested tobacco leaf, changes in chemical content, and likely transition mechanisms, and discussed hypotheses regarding the possible causative agents of tobacco fermentation. Fermentation is often regarded as synonymous with "sweating" (85). The fermentation takes place at the expense of proteins, alkaloids, and possibly pectic substances, so that there is a great reduction in dry weight and the mineral content becomes proportionately greater. The mainstream smoke from cigars is alkaline, and the large mineral content gives to cigar tobacco its even-burning property, so that whole leaf can be used as a smoking product.

[1] Some enzymic activity may still remain in flue-cured leaf (see Section IV,H).

Nicotiana tabacum is the main cultivated variety in the West, but in Eastern Europe and Russia *Nicotiana rustica* (*Markhorka*) is also cultivated and used in commercial tobaccos. The leaf is normally air-cured and then given a mild fermentation, although much less than cigar tobacco.

The so-called "black," fermented tobaccos are first air-cured and then packed into piles at high temperatures and humidities. The piles are not stripped and fermentation is allowed to proceed to a very advanced stage before the tobacco is used for manufacture.

Whilst it is desirable in the literature that the history of a tobacco sample should be known, the manner in which the preceding terms have sometimes been applied, if at all, can make it exceedingly difficult to decide the exact nature of the tobacco described and to correlate the results so obtained.

III. General Remarks

Investigations into the chemical composition of tobacco smoke are usually carried out on the "tar" obtained by condensing the smoke at low temperatures or by passing it through a number of absorption vessels containing solvents. The complex and unstable nature of tobacco tar, which contains many free radicals, has made handling and investigation difficult. Research on smoke has centered mainly on the organic compounds, a number of them having been identified and many more indicated by analytical methods, particularly column, paper, and vapor-phase chromatography supplemented by ultraviolet and infrared spectra. The smoke is usually and preferably obtained by a machine designed to puff the cigarets (or cigars or pipes) intermittently in much the same way as a human smoker does. The machines may be designed to draw air through a cigaret at either constant pressure or constant volume (24, 207). A close control is necessary so that each cigaret is smoked under standard conditions requisite for quality control analysis and the production of material for biological tests (322). There seems to be no general agreement on the number of puffs to be taken each minute, but the accepted puff volume for cigarets is usually about 35 cc. in the United States and 25 cc. in Great Britain. The temperature of the burning zone of cigarets has been measured (346) and is normally in the range $884 \pm 30°C$.

IV. Chemical Constituents of Tobacco and Tobacco Smoke

The chemical constituents of tobacco and smoke have been treated under the headings shown in this section, although in a few instances these classifications have not been strictly adhered to. The tables list the compounds that have been reported in tobaccos (of all types) and in tobacco smoke.

A. Hydrocarbons

A small number of hydrocarbons have been definitely identified in tobacco or tobacco smoke, but evidence exists for the presence of a further large number in smoke alone. The hydrocarbons may conveniently be classified as aliphatic and aromatic.

TABLE 1

Aliphatic hydrocarbons found in tobacco and tobacco smoke

Hydrocarbon	Tobacco	Smoke	Hydrocarbon	Tobacco	Smoke
Acetylene		+	Isoprene		+
Butadiene		+	Isosqualene		+
Butane		+	1,8-*p*-Menthadiene		+
C_{25}-C_{33} paraffins	+	+	Methane		+
β-Carotene	+		Methylacetylene		+
Neo-β-carotene	+		Phytadienes	+	+
Dipentene		+	Propane		+
Ethane		+	Propylene		+
Ethylene		+	Squalene		+
Isobutane		+	Xanthophylls	+	
Isobutylene		+			

1. Aliphatic hydrocarbons (see table 1)

(a) Paraffins

The literature contains many early references to the presence of waxlike hydrocarbons in tobacco and smoke. The waxes from processed leaf were separated by fractional crystallization into two parts, which exhibited no unsaturation and had elementary analyses and melting points corresponding to those of hentriacontane, $C_{31}H_{64}$, and heptacosane, $C_{27}H_{56}$ (152, 338). Later work showed that these fractions were normal paraffins but impure, since the hentriacontane contained C_{33} and C_{29} homologs whilst the heptacosane was probably a mixture of three or four hydrocarbons—the C_{29}, C_{27}, and C_{25} paraffins (42). Numerous reports have appeared on the presence of hentriacontane in tobacco smoke (43, 135, 388, 412), but most of the identifications have rested on elementary analyses and melting points alone, criteria of purity which are insufficient in this class of compounds. There is little doubt that the paraffin identified as hentriacontane is mainly such, although recent x-ray evidence has indicated that when isolated from smoke it probably contains a little tritriacontane (357), and a brief report stated that mass spectrography showed it to contain about 10 per cent of some homologs and isomers from C_{27} to C_{36} (412). Paraffins of green leaf were separated into two components, again presumably mixtures, but mainly hentriacontane and heptacosane (114).

Hydrocarbons obtained from extracts of green leaf (*N. tabacum*), processed tobacco, and tobacco smoke by chromatography on alumina (36) were treated with urea in the usual way, the complex was decomposed, and the hydrocarbons were crystallized once from ethanol/benzene. Ultraviolet and infrared spectroscopy indicated no unsaturation, whilst mass spectrometry of the three samples showed them to have very similar compositions, the main constituents being normal hentriacontane and isohentriacontane. Considerable proportions of isoparaffins were present in addition to the normal ones. Vapor-phase chromatography gave very similar results, and it is interesting that in addition to the odd-numbered paraffins, small quantities of even-numbered ones appeared to be present as well. The results of the mass-spectrum analysis are briefly set out in

TABLE 2

Paraffin hydrocarbons found in tobacco and tobacco smoke

Number of Carbon Atoms	Green Leaf		Processed Leaf		Tobacco Smoke	
	Normal	Iso	Normal	Iso	Normal	Iso
25	+		+			
26	+		+		+	
27	+	+	+	+	+	+
28	+		+		+	
29	+	+	+	+	+	+
30	+	+	+	+	+	+
31	+	+	+	+	+	+
32	+	+	+	+	+	+
33	+	+	+	+	+	+

table 2. The percentages of the major hydrocarbons of processed tobacco are as follows: *n*-C_{29}, 6.3; iso-C_{29}, 11.0; *n*-C_{31}, 26.6; iso-C_{31}, 20.4; *n*-C_{33}, 13.1; iso-C_{33}, 3.9; the *n*-alkane/isoalkane ratio is based on an accepted sensitivity ratio of 1/10.

(b) Terpenes

Tobacco and its combustion products elaborate a whole range of terpene hydrocarbons from isoprene to the carotenoids, although so far no sesquiterpene has been isolated. The presence of terpenoid compounds in an extract of cigarets and in tobacco smoke condensate was briefly reported (412).

(*1*) *Isoprene*, C_5H_8, has been identified in tobacco smoke by fractionation of the gas phase followed by infrared analysis (225, 238), and its presence has been indicated by vapor-phase chromatography (40, 92, 119, 120). It possibly arises through the destruction of the naturally occurring terpenoids upon the combustion of tobacco.

(*2*) *Dipentene*, $C_{10}H_{16}$, in tobacco smoke condensate was isolated from a methanol-volatile fraction (43) which has been shown by vapor-phase chromatography to be a mixture of at least eleven substances (36). The isolated dipentene had the expected elementary analysis and was further characterized through its tetrabromide. A second closely similar monoterpene, 1,8-*p*-menthadiene, obtained from cigaret smoke condensate by fractional distillation, was characterized through its infrared spectrum and the preparation of a tetrabromide (270).

(*3*) *Phytadienes*, $C_{20}H_{38}$, have been isolated and identified in flue-cured and air-cured tobaccos (94, 213, 271), a "black," fermented tobacco (36), green leaf (39), and tobacco smoke (36, 94). A number of derivatives (adducts) have been prepared, and the substance itself degraded to formaldehyde and a saturated long-chain acid. Neophytadiene might arise from phytol, since the latter can be chemically dehydrated to a number of phytadienes, one of which was similar to the naturally occurring isomer (271); phytol, itself, has not been reported in tobacco, although it is, of course, a component of chlorophyll. Physical constants of the isolated phytadienes are shown in table 3, along with those of some synthetic phytadienes.

TABLE 3

Physical constants of some phytadienes

Phytadiene	n_D	α_D	Maximum	log ϵ_{max}	Reference
			$m\mu$		
Neophytadiene	1.4604	0.0°	224.5	4.4	(271)
γ-Phytadiene	1.4628	+0.08°	224	4.19	(213)
A phytadiene	1.4720	+8.5°	226	—	(39)
A phytadiene	1.4634	+7.5°	227	4.3	(36)
Phytadiene A	1.4636	+2.1°	227	4.3	(271)
Phytadiene B	1.4604	0.0°	225	4.23	(271)
Phytadiene C	1.4704	−9.58°	235	4.38	(271)

(*4*) *Squalene*, $C_{30}H_{50}$, was isolated and rigorously identified in tobacco smoke simultaneously by two groups of workers (141, 359). It is interesting to note that although squalene has been isolated from smoke, its presence in fresh or processed tobacco has not yet been reported. Traces of an isomer, isosqualene, were found in the squalene from tobacco smoke (141).

(*5*) *Carotenes*, $C_{40}H_{56}$, occur in fresh tobacco but none has been isolated and identified as crystalline material. Column chromatography and ultraviolet spectroscopy suggest the presence of β-carotene (128, 401) and neo-β-carotene (401). The oxygenated carotenes lutein, neoxanthin (226, 332, 401), violaxanthin (401), and flavoxanthin (226) have been reported.

(c) Gaseous hydrocarbons

The gas phase of tobacco smoke has been shown to contain a number of volatile hydrocarbons by techniques involving low-temperature fractionation, infrared spectroscopy, and vapor-phase chromatography, although none of the compounds was characterized, where possible, through a derivative except possibly acetylene. Acetylene (40, 73, 225, 230, 238), butane (40, 230, 238), butadiene (40, 225, 238), ethane (40, 225, 230, 238), ethylene (40, 225, 230), methane (40, 225, 230), propane (40, 225, 230, 238), and propylene (40, 230, 238) have been identified by several groups of workers, whilst isobutane (230), isobutylene (40, 238), *trans*- and *cis*-2-butenes (40), and methylacetylene (21) have each been reported once.

(d) Miscellaneous hydrocarbons

A few volatile hydrocarbons have been reported to occur in the essential oils of tobacco leaf. Early work on the steam-volatile portion of an extract of Kentucky leaf indicated the presence of a compound having the formula $C_{10}H_{16}$ (101); later, two more hydrocarbons were isolated, $C_{11}H_{20}$ and possibly $C_{10}H_{18}$ (104), from fresh Hungarian tobacco. No confirmation or further evidence for these reports has appeared, although a hydrocarbon having an odor of pinene was found in green leaf (277). Recently an unsaturated hydrocarbon, stated to have an infrared spectrum resembling that of myrcene but with one or two notable differences (214), has been shown to be a phytadiene (213).

2. Aromatic hydrocarbons

A large amount of research has been concentrated on the aromatic hydrocarbon content of tobacco smoke, whilst fresh and processed tobaccos have attracted specifically less attention.

After chromatography on alumina of an extract of fresh and processed leaves, the polycyclic hydrocarbons anthracene, pyrene, fluoranthene, 1,2-benzanthracene, and 3,4-benzopyrene were identified by ultraviolet spectroscopy; they were present in too small a quantity to account for their concentration in tobacco smoke and may have arisen on the leaf through deposition of soot (32, 33). A small amount of 3,4-benzopyrene was also detected on normally cured tobacco leaf (11). Recently, aged Japanese Burley tobacco was extracted and the hydrocarbons examined by chromatography on silicic acid followed by alumina and fractional crystallization of the solids so obtained. In this way anthracene, phenanthrene, pyrene, and fluoranthene were isolated and identified by comparison of melting points (mixed), ultraviolet spectra, and infrared spectra with authentic materials (212). Moreover, these compounds were present in much larger quantities than has previously been reported, and since polycyclic aromatic hydrocarbons of this sort are very unusual constituents of plants, it would be desirable to know more of the history of the tobacco used in the investigation.

The emphasis on the polycyclic aromatics in smoke has arisen because of its supposed connection with lung cancer and the known carcinogenic activity of some of the higher polycyclic hydrocarbons. Nevertheless, because of the low concentration of these compounds in tobacco smoke it has not been possible to isolate and identify them in the usual way, except in two cases (116, 369),[2] and the main evidence of their nature arises from paper and column chromatography coupled with ultraviolet and fluorescence spectroscopy. The interpretation of individual spectra of fractions from such complex mixtures is difficult, particularly in the presence of "background absorption," and although some of the published work reaches a high standard, much must be regarded with caution. In many cases no spectra have been recorded. No attempt has been made here to comment on the identifications of the individual aromatic hydrocarbons which are recorded in table 4. It would be desirable to have further proof of the existence in tobacco smoke of many of these compounds. The highly potent carcinogen, 3,4-benzopyrene, has received a large proportion of the attention paid to polycyclic hydrocarbons in smoke and proof of its presence, or that of a derivative, seems fairly conclusive with regard to chromatographic and spectroscopic evidence (11), although the amount is extremely small.

Azulene has been obtained in crystalline form from tobacco smoke and its trinitrobenzene complex has been prepared (116), whilst a hydrocarbon which melted at 94–95°C. (carbon, 92.3 per cent; hydrogen, 7.7 per cent) and which formed a picrate was isolated from the smoke of cigars burned in pipes (369).

Tentative suggestions have been advanced as to the origin and mode of formation of aromatic polycyclic hydrocarbons during smoking (71, 154, 155, 412).

[2] At an informal symposium held in London on June 6, 1959 Professor E. L. Wynder announced the isolation of a crystalline specimen of 3,4-benzopyrene from tobacco smoke.

TABLE 4

Aromatic hydrocarbons of tobacco smoke

Hydrocarbon	References	Hydrocarbon	References
Acenaphthene	(21, 88)	3,4-Dihydro-3,4-benzopyrene	(1)
Acenaphthylene	(21, 49, 50, 88, 89)	9,10-Dimethyl-1,2-benzanthracene	(245)
Alkylchrysene	(356)	Dimethylchrysene	(1)
Anthanthrene	(49, 50, 88, 89, 167)	Dimethylfluoranthene	(358)
Anthracene	(21, 46, 49, 158, 412)	1,8-Dimethylnaphthalene	(21)
Anthraceno-2,3-9,10-phenanthrene	(167)	2,5-Dimethylphenanthrene	(1)
Azulene	(88, 89, 164)	Fluoranthene	(49, 88, 89, 161, 355, 412)
1,2-Benzanthracene	(1, 21, 88, 89, 158, 164)	Fluorene	(1, 21, 88, 89)
Benzene	(238)	2-Methylanthracene	(49)
3,4-Benzofluoranthene	(412)	3-Methyl-1,2-benzanthracene	(21)
7,8-Benzofluoranthene	(356)	9-Methyl-1,2-benzofluorene	(1, 21)
8,9-Benzofluoranthene	(356)	1-Methylchrysene	(1, 358)
11,12-Benzofluoranthene	(167)	8-Methylfluorene	(355)
1,1*a*-1*a*,10*a*,10-Benzofluoranthene (benzo[*m*, *n*, *o*]fluoranthene)	(356)	9-Methylfluorene	(203)
1,2-Benzofluorene	(1, 21, 356)	2-Methylnaphthalene	(49, 88)
2,3-Benzofluorene	(1)	9-Methylphenanthrene	(1, 21)
1,2-Benzonaphthacene	(412)	1-Methylpyrene	(21, 358)
1,12-Benzoperylene	(49, 355)	3-Methylpyrene	(21, 49, 88, 89)
3,4-Benzophenanthrene	(356)	4-Methylpyrene	(355)
1,2-Benzopyrene	(21, 158, 167, 224)	Naphthalene	(21, 88, 89)
3,4-Benzopyrene	(11)	2,1-Naphtho-1,2-fluorene	(1)
Chrysene	(1, 167, 358)	2′,3′-Naphtho-3,4-pyrene	(412)
Coronene	(88, 89)	1,8,9-Perinaphthoxanthene	(356)*
5,6-Cyclopentenobenzanthracene	(21, 167, 203)	Perylene	(88, 167, 358)
6,7-Cyclopentenobenzanthracene	(1, 21, 167, 203)	Phenanthrene	(1, 21, 49)
1,2-5,6-Dibenzanthracene	(245, 356)	Phenylacetylene	(21)
1,2-7,8-Dibenzofluorene	(167)	Pyrene	(1, 21, 46, 49, 89, 158, 167, 224, 412)
1,2-7,8-Dibenzonaphthacene	(412)		
1,2-3,4-Dibenzopyrene	(165, 412)	Toluene	(238)
3,4-8,9-Dibenzopyrene	(167, 412)	1,2-3,4-5,6-Tribenzanthracene	(167)
3,4-9,10-Dibenzopyrene	(21, 203)	1,2,4-Trimethylbenzene	(21)
		1,3,5-Trimethylbenzene	(21)

* Heterocyclic.

B. ALCOHOLS AND ESTERS (SEE TABLE 5)

Methanol in tobacco and its products may arise from pectins, and the methoxyl content of tobacco during processing has been investigated (197). Ethanol has been reported to be present (197). Studies of Japanese flue-cured tobacco resulted in the identification of methanol, β-phenethyl alcohol, and benzyl alcohol by conversion to the 3-nitrophthalates followed by fractional crystallization; the same alcohols were found esterified with acetic acid. The separation of the esters was effected by chromatography, and the acid component was identified as acetic acid by paper chromatography of the hydroxamic acid derivative (215). Lauric, myristic, and palmitic acids present in the same source as their methyl esters were separated chromatographically, and their identity was confirmed by reversed-phase chromatography of the 2,4-dinitrophenylhydrazides. The same group of workers have isolated methanol, ethyl acetate, and furfuryl alcohol from flue-cured Japanese and Virginia tobaccos (214, 216). Furfuryl alcohol had previously been isolated from Japanese yellow leaf and North American tobacco

TABLE 5

Alcohols and esters found in tobacco and tobacco smoke

Alcohol	Tobacco	Smoke	Ester	Tobacco	Smoke
Benzyl alcohol	+	+	Benzyl acetate	+	
Borneol	+		$C_{30}H_{61}COOC_{31}H_{63}$		+
$C_{16}H_{24}O$ or $C_{18}H_{26}O$		+	Ethyl acetate	+	+
Diethylene glycol		+	Ethyl *n*-butyrate		+
Ethanol	+	+	Ethyl *n*-caproate		+
Ethylene glycol		+	Ethyl isovalerate		+
Furfuryl alcohol	+		Ethyl β-methylvalerate		+
Glycerol	+	+	Ethyl propionate		+
l-Linaloöl	+		Glycerides	+	
Methanol	+	+	Methyl acetate		+
β-Phenethyl alcohol	+	+	Methyl laurate	+	
Solanesol	+	+	Methyl myristate	+	
Triethylene glycol		+	Methyl nitrite		+
			Methyl palmitate	+	
			Methyl salicylate	+	
			β-Phenethyl acetate	+	
			Solanesol esters	+	

when it was converted to the α-naphthylurethan. Similar derivatives of two unidentified polyhydric alcohols were reported in the same investigation (114).

Partially fermented Moroccan tobacco leaf was found to contain borneol and *l*-linaloöl; the compounds were isolated by steam distillation of a petroleum ether extract, borneol being characterized as its acid phthalate and *l*-linaloöl as its diphenylurethan (277, 278).

The glycerides of tobacco seed oil are obtainable in quantity and have been investigated by several groups of workers in view of their possible commercial interest (265, 279, 360, 361).

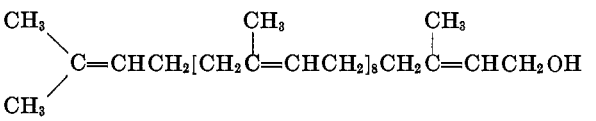
The addition of glycerol, related polyhydric alcohols, and their derivatives to tobacco as "humectants" or moistening agents is a practice of long standing, and the determination of such agents in tobacco and tobacco smoke has formed the subject of several investigations. Glycerol has been identified and estimated in tobacco samples (41, 74). The estimation involved conversion to isopropyl iodide, which was allowed to react with silver nitrate (41). Diethylene glycol was identified similarly by conversion to the urethan. Such humectants are carried into the mainstream smoke, from which they have been isolated and identified (74). Ethylene glycol in smoke has been detected by a color reaction (261), glycerol has been estimated by oxidation with periodic acid (34), and triethylene glycol has been determined by infrared absorption spectroscopy (19).

The alcohols and esters of tobacco smoke have been investigated by a variety of methods. Methanol and methyl acetate have been shown to be present in the smoke of American cigarets by vapor-phase chromatography (119), and methanol and methyl nitrite have been identified by infrared analysis (237, 238). Methanol has been isolated from the smoke of East Indian and North American pipe tobaccos (198). The steam distillate of the neutral extract from the smoke of cigarets composed mainly of Japanese flue-cured tobacco was found to contain β-phenethyl alcohol, benzyl alcohol, and ethanol. The alcohols were converted

to 3,5-dinitrobenzoates and separated by a "chromatostrip" technique. The esters contained ethanol as the only alcoholic component. The acids were identified by the R_f values of their hydroxamic acid derivatives on paper chromatography. Thus the presence of ethyl acetate and ethyl propionate was established, whilst the probable presence of ethyl *n*-butyrate, ethyl isovalerate, and either ethyl *n*-caproate, ethyl β-methylvalerate, or both, was inferred. As the derivatives of the acids and their branched-chain isomers possessed the same R_f values, the postulated structures were based on those of the free acids, which had been previously found in smoke by the same workers (122, 123).

An alcohol, $C_{16}H_{20}O$ or $C_{18}H_{28}O$, boiling at 90°C./2 mm., has been isolated from the smoke of British cigarets (43), and an ester, $C_{30}H_{61}COOC_{31}H_{63}$, from American cigarets (412).

The isolation of a pentaterpenoid alcohol, solanesol (I), from aged, flue-cured tobacco leaf, Dixie bright leaf, green leaf, and cigaret smoke is of great interest (184, 274). Solanesol also occurs in flue-cured tobacco esterified with palmitic, linolenic, linoleic, oleic, myristic, and other fatty acids (273).



I

Solanesol

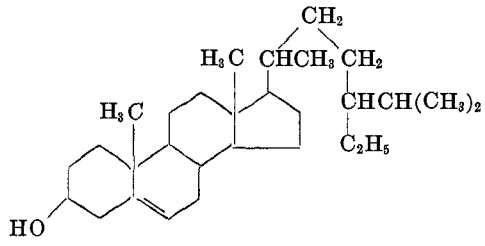
The alcohol is present in flue-cured tobacco in relatively large amounts and constitutes about 0.4 per cent of the dry weight of the leaf. The structure is supported by analytical data for the compound and its derivatives and was suggested by the similarity of the infrared spectrum to that of farnesol. Further evidence was obtained by catalytic hydrogenation to the saturated alcohol (usually accompanied by the hydrocarbon $C_{50}H_{102}$, a hydrogenolysis product; 93 per cent in one case), by treatment with ozone followed by oxidative or reductive fission of the product, and by oxidation of the terminal primary alcoholic group to a carboxyl group. Solanesol isolated from smoke is said to be accompanied by closely related compounds (184).

Recently, phthalates were isolated from flue-cured leaf (324) and hydrolyzed to phthalic acid and the free alcohols, which by vapor-phase chromatography were shown to be 1-propanol, 1-butanol, and an unidentified alcohol.

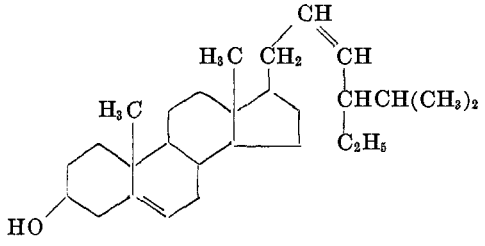
C. STEROLS

The sterols of plant origin have been studied for many years. They present a difficult and complex problem for analysis, as purification is often achieved only by tedious fractional crystallization which must usually be carried out with small quantities of material. The methods of identification have been summarized authoritatively (12), and in recent years the sterols of tobacco have been investigated according to the criteria suggested therein as far as has been possible with the small quantities of material isolated.

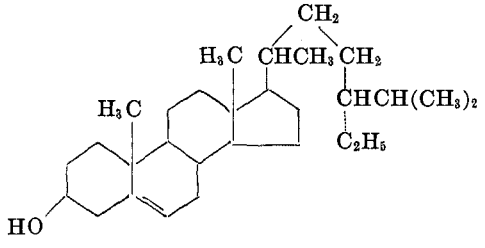
The most important of the sterols of tobacco (see table 6) are stigmasterol (II), β-sitosterol (I), and γ-sitosterol (III), with ergosterol (IV) occurring as a minor component.



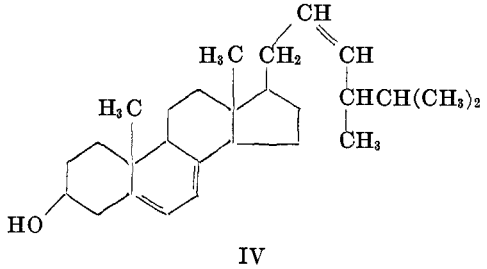
I
β-Sitosterol



II
Stigmasterol



III
γ-Sitosterol



IV
Ergosterol

Stigmasterol and both the sitosterols are 3-β-hydroxysterols possessing $\Delta^{5,6}$-unsaturation. Stigmasterol also possesses trans $\Delta^{22,23}$-unsaturation, which gives rise to a characteristic infrared absorption band at 972 cm.$^{-1}$, useful for

TABLE 6

Sterols found in tobacco and tobacco smoke

Sterol	Tobacco	Smoke	Sterol	Tobacco	Smoke
Chalinasterol	+		γ-Sitosterol	+	+
Ergosterol	+		Stigmasterol	+	+
β-Sitosterol	+	+			

the determination of the sterol in mixtures of other sterols carrying a saturated side chain (130). Selective hydrogenation of the C 22,23 double bond of stigmasterol gives β-sitosterol, thus establishing the relationship between these two compounds. The configuration of stigmasterol at C 24 is arbitrarily designated as *b*, for the absolute configuration of this center has not yet been determined (72). The C 24 epimer of stigmasterol is poriferasterol (24*a*-ethylcholesterol) and that of β-sitosterol is clionasterol. It is not certain whether γ-sitosterol is identical with the C 24 epimer of β-sitosterol (clionasterol) or whether it possesses, in addition, the opposite configuration at C 20 (15). The point remains to be settled when larger quantities of pure γ-sitosterol become available.

Stigmasterol, β-sitosterol, and γ-sitosterol give similar colors in the Liebermann–Burchardt reaction (addition of a drop of concentrated sulfuric acid to a solution of the sterol in chloroform containing acetic anhydride). Ergosterol is indicated by its ultraviolet spectrum. Many mixtures of plant sterols possess a similar absorption spectrum, indicating the presence of diene sterols.

Earlier workers reported the presence of sterols in tobacco leaf (300, 349). There have been several investigations of tobacco seed oil; from the data given, what is almost certainly β-sitosterol has been isolated and converted to derivatives (269, 279). A sterol of slightly higher melting point has been isolated from the same source (360).

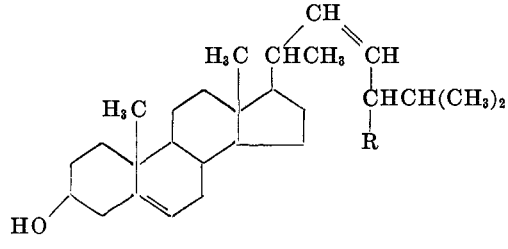
An Indian chewing tobacco afforded a glucoside of γ-sitosterol (134); acid hydrolysis of the glucoside gave glucose and the sterol, which was identified by conversion to derivatives for which analytical data and specific optical rotations were obtained.

Chromatographic techniques were applied quite early to the examination of tobacco tar and the isolation of what was possibly β-sitosterol, m.p. 135°C., was described (289). Recent studies on tobacco smoke have confirmed the presence of sterols, the identities of which have been well established by measurement of the infrared spectra, the melting points, and the specific optical rotations of the sterols and their derivatives. In this manner, the presence of stigmasterol in the smoke of American cigarets has been demonstrated (142), as has also the presence of stigmasterol, β-sitosterol, and γ-sitosterol in the smoke of British cigarets (35).

A neutral extract of green leaf which had not been hydrolyzed yielded free stigmasterol and an ester of β-sitosterol, probably the oleate (38). The sterols found in American flue-cured leaf have been thoroughly investigated (65, 66, 99, 325, 327). By extraction of the leaf with ethanol, β-sitosterol-D-glucoside was

obtained, whereas extraction with Skellysolve afforded free stigmasterol. Exhaustive extraction of the above flue-cured leaf, followed by acid and alkaline hydrolysis and precipitation of the sterols as digitonides, showed that sterols comprise 0.15 per cent by weight of the moisture-free tobacco. A semimicro method for the determination of 3-β-hydroxysterols has been developed and was used to show that the total sterol levels of tobacco samples studied lay between 0.1 and 0.5 per cent depending on the variety. Percentages of free sterols, esterified sterols, and sterol glycosides were determined also. Flue-cured Maryland and Burley tobaccos have a greater sterol content than Turkish, fire-cured, and cigar leaf tobaccos, and in the last three there is a progressive decrease in the amount of sterols. The processes of flue curing and aging tobacco do not seem to affect the sterol content to any great extent.

Ergosterol and γ-sitosterol were isolated from flue-cured tobacco, but the former is a minor component (66). The ultraviolet spectrum of a sterol from "black," fermented Argentinian cigarets suggested the presence of a cyclic diene (35). Another unknown steroidal glucoside has been obtained (66), which on hydrolysis gave glucose and a sterol resembling stigmasterol in some respects. The infrared spectum of the sterol acetate was similar to that of stigmasterol acetate and possessed the characteristic absorption band at 972 cm.$^{-1}$ but of somewhat lower intensity. Catalytic hydrogenation yielded campestanol, thus establishing the stereochemistry of the compound and suggesting structure V, identical with that originally postulated for chalinasterol.



V

Chalinasterol: R = 24α-methyl

The physical constants were in most respects in accord with those given for chalinasterol (14, 16), but the original authors later modified the structure proposed for chalinasterol (13) on the grounds that the work had originally been carried out on impure material, and the unsaturation was placed at $\Delta^{24(28)}$ rather than at Δ^{22}. The infrared spectrum of 24-methylenecholesterol was shown to be quite different from that of the tobacco sterol and the two are thus not identical.

D. ALDEHYDES AND KETONES (SEE TABLE 7)

Until recently, there was little information concerning the carbonyl compounds of tobacco leaf. Furfural may occur in the steam distillate of Dewbek tobacco (a Russian cigaret tobacco) or it may arise as an artefact formed in the distillation process (299). Acetaldehyde was reported in fresh leaf (197), and methylglyoxal in cigar and cigaret tobaccos (138).

TABLE 7

Aldehydes and ketones found in tobacco and tobacco smoke

Aldehyde	Tobacco	Smoke	Ketone	Tobacco	Smoke
Acetaldehyde	+	+	Acetone	+	+
Acrolein		+	2,3-Butanedione		+
p-Anisaldehyde	+		Diethyl ketone		+
Benzaldehyde	+	+	Dipropyl ketone		+
Butyraldehyde		+	Hydroxypyruvic acid	+	
Crotonaldehyde	+	+	Methyl ethyl ketone		+
Formaldehyde		+	Palmitone		+
Furfural	+	+	2,3-Pentanedione		+
Glycolaldehyde	+		α-Pyrryl methyl ketone	+	
Glyoxal	+		Reductic acid		+
5-Hydroxymethylfurfural	+				
Isobutyraldehyde	+	+			
Mesoxaldialdehyde	+				
5-Methylfurfural	+				
Methylglyoxal	+	+			
Propionaldehyde		+			
Reductone	+				
Salicylaldehyde	+				
m-Tolualdehyde	+				

In a series of Japanese papers on the steam-volatile essential oils of flue-cured tobacco the carbonyl compounds were isolated and separated by chromatography of the 2,4-dinitrophenylhydrazones on silica. The melting points and infrared spectra of the compounds thus obtained were compared with those of authentic specimens, and in this way acetaldehyde, isobutyraldehyde, benzaldehyde, 5-hydroxymethylfurfural, and what was possibly *m*-tolualdehyde were identified (208, 209, 217). Salicylaldehyde was identified in the phenolic fraction and α-pyrryl methyl ketone was found in the neutral and alkali-soluble fractions, as was crotonaldehyde. The authors commented that the latter compound may arise by degradation during the steam distillation; indeed, they later demonstrated that a greater part of the furfural derivatives were produced by degradation of carbohydrates during the steam distillation process (209, 214, 216, 218). It would be expected, however, that condensation reactions might occur in aldehydes subjected to basic conditions, and crotonaldehyde may arise in such a manner. Comparisons of Virginia tobacco before and after redrying and aging showed a decrease in furfural and an increase in 5-methylfurfural and 5-hydroxymethylfurfural, whilst acetone occurred only after aging (210). The carbonyl compounds of flue-cured leaf increased on aging (218), and a comparison of flue-cured and Burley tobaccos showed that the significant differences were the predominance of benzaldehyde amongst the carbonyl compounds of Burley, coupled with a lower furfural level and complete absence of 5-methylfurfural (211).

Possible products of carbohydrate degradation in Virginia tobacco have been identified by preparation of their phenylhydrazones. Reductone [CH(OH)=C(OH)CHO] and its oxidation products, hydroxypyruvic acid and mesoxaldialdehyde, were found and also glycolaldehyde, possibly arising through the decarboxylation of hydroxypyruvic acid (376, 379).

Tobacco smoke has been extensively studied. Aldehydes and ketones from the smoke of pipe tobacco were separated by silver oxide oxidation of the aldehydes to the corresponding acids, which were then distilled and analyzed. Formic, acetic, butyric, and benzoic acids were formed from the aldehydes, the formation of benzoic acid being attributed to the decomposition of complex aromatic compounds. Diethyl and dipropyl ketones were identified by conversion to 2,4-dinitrophenylhydrazones and semicarbazones (196). Higher ketones were also reported.

Conversion of carbonyl compounds to 2,4-dinitrophenylhydrazones, followed by paper chromatography of the mixed derivatives, has been used by several groups of workers as a method of examination of tobacco smoke (29, 105, 169, 185). Comparisons of ultraviolet spectra and R_f values with those of authentic materials have served to demonstrate the presence of the following: formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, furfural, acrolein, crotonaldehyde, glyoxal, methylglyoxal, 2,3-butanedione (biacetyl), and 2,3-pentanedione. Sufficient material was obtained for the further purification of some of these, so that melting points and infrared spectra of the 2,4-dinitrophenylhydrazones of acetaldehyde, acetone, and methyl ethyl ketone could be compared with those of authentic specimens (185).

Vapor-phase chromatography indicated in smoke the presence of acetaldehyde, acrolein, glyoxal, isobutyraldehyde, propionaldehyde, acetone, and methyl ethyl ketone (119, 120). The gaseous phase of smoke has been condensed and fractionated, and the fractions have been shown to contain acetaldehyde, methyl ethyl ketone, acetone, and biacetyl by an infrared compensation technique (225, 238).

Biacetyl, which is said to play an important role in the flavor of tobacco smoke (284, 285), has been separated from and estimated in tobacco smoke by conversion to the dioxime, which was then precipitated as the nickel complex. Other, more recently reported α-diketones in smoke (105, 169) might be expected to cause some interference with the estimation of biacetyl, if present in comparable amounts.

What may be dipalmityl ketone, m.p. 79°C., has been isolated (289), and a mixture of long-chain ketones, m.p. 65–75°C., with infrared spectra virtually identical to that of palmitone has been obtained from the smoke of American cigarets (357).

Reductic acid has been shown to occur in cigaret smoke by paper chromatography of the free acid and its osazone. In the same study the presence of methylglyoxal was demonstrated by conversion to the osazone, followed by light-absorption measurements in alkaline solution (379).

E. ACIDS (SEE TABLE 8)

The tobacco plant contains a high percentage of acidic compounds other than polyphenols, amino acids, and uronic acids. A large proportion of the carboxylic acid fraction is composed of fatty and nonvolatile components, although in keeping with an earlier suggestion (76), an increasing number of volatile acids has

TABLE 8

Acids found in tobacco and tobacco smoke

Acid	Tobacco	Smoke	Acid	Tobacco	Smoke
Acetic	+	+	Lactic	+	+
Adipic		+	Lauric	+	+
Arachidic	+	+	Levulinic		+
Arachidonic	+		Linoleic	+	+
Benzoic	+	+	Linolenic	+	+
Butyric		+	Maleic	+	
C_{10}-C_{23} saturated	+	+	Malic	+	+
$C_{10}H_{12}O_2$		+	Malonic	+	+
$C_{12}H_{12}O_5$		+	Methylethylacetic	+	
Caproic	+	+	β-Methylvaleric	+	+
Caprylic		+	Myristic	+	+
Cerotic		+	Nicotinic	+	+
Citric	+		Nonylic		+
trans-Crotonic	+		Oleic	+	+
Formic	+	+	Oxalic	+	+
A fluorenecarboxylic acid		+	Palmitic	+	+
Fumaric	+		Palmitoleic		+
Furoic	+	+	Phenylacetic	+	
Glutaric		+	Phthalic		+
Glutamic	+	+	Propionic	+	+
D-Glyceric	+		Pyruvic	+	+
Glycolic		+	Reductic		+
Glyoxylic	+	+	Resin	+	+
Heptylic		+	Stearic	+	+
Isobutyric	+	+	Succinic	+	+
Isovaleric	+		Valeric	+	+
α-Ketoglutaric	+	+			

been discovered. The nonvolatile, water-soluble acids are composed mainly of citric, malic, and oxalic acids, the latter possibly being an oxidation product of plant metabolism. Citric and malic acids bear some kind of relationship to each other, the sum total usually remaining fairly constant but the actual proportions of the two acids varying a great deal. The possible role of citric and malic acids in the degradation of sugars has been discussed (76), as has the metabolism of the organic acids in tobacco leaf (329). Citric, malic, and oxalic acids were early isolated and identified in the fresh leaf of *N. glauca* (305) and *N. tabacum* (362) and in processed tobacco (296, 302), although the absence of oxalic acid has been reported in fresh leaf (413). Chromatography on paper and silicic acid has proved invaluable for the rapid determination of these acids, and the use of such techniques has enabled their presence to be demonstrated in Bulgarian leaf (176), flue-cured Canadian tobacco (125), green leaf (90), green and cured Japanese tobaccos (371), and a cured tobacco (264). It has been shown that malic acid occurs exclusively in *N. tabacum* leaf as its levorotatory form (363), whilst a similar form was isolated from *N. glauca* (305).

Tobacco yields a number of other dibasic acids, some of which, like maleic and fumaric acids, bear an obvious relationship to those already mentioned. Fumaric acid was isolated from processed leaf but not from fresh tobaccos (296), although further reports have appeared of its presence in cured leaf (371) and in fresh leaf (362). Maleic acid, another possible degradation product of malic

acid, was indicated in paper chromatographic studies which also revealed the presence of lactic and malonic acids (90). Malonic acid has been isolated from fresh and cured American and Oriental tobaccos, the substance being identified through its melting point, the melting point of its amide, and its infrared spectrum (8). Terephthalic acid has been reported (75).

Succinic acid has been obtained from tobacco (296) and found in a number of chromatographic investigations (90, 125, 371).

Analytical determinations of acidity in tobacco always reveal a substantial difference between the total acidity as found and the total calculated from the sum of the individual component acids (malic, citric, oxalic, etc.), a fact indicating the existence of other unidentified acids, whilst the above-mentioned chromatographic studies on the nonvolatile tobacco acids also usually revealed the presence of acids which could not be accounted for. A recent investigation of the acids in fresh Virginia leaf by chromatography on silica gel and ion-exchange resin has thrown further light on this "unknown acids" fraction (62). Two new acids, glyoxylic and α-ketoglutaric, were isolated and identified by preparation of derivatives; shortly thereafter these acids, together with pyruvic acid, were discovered in uncured and cured American Burley and Bright tobaccos (95). Since glyoxylic and α-ketoglutaric acids are present in substantial quantity, they may make up a considerable part of the discrepancy between the observed and the calculated total acidities in the tobacco plant. D-Glyceric acid has been identified in mature leaves of *N. tabacum* (227).

The flowers of *Nicotiana* species have yielded caprylic (octanoic) acid (identified as its amide), acetic acid, and also possibly formic acid (275).

The leaf of fresh and processed tobaccos contains a number of volatile acids which may arise through oxidative deamination of amino acids or oxidation of saccharides.

Formic and acetic acids have been detected in green (277), uncured, and cured American, Dewbek, and Trapesund tobaccos (301).

An acid extract of a smoking tobacco was esterified with ethyl alcohol and the resulting esters fractionally distilled to give *n*-valeric and β-methylvaleric acids. *n*-Valeric acid was characterized through its *p*-bromophenacyl ester, whilst the dextrorotatory β-methylvaleric acid was identified through its amide, its *p*-toluidide, and its *p*-bromophenacyl ester (276). A valeric acid was isolated from fresh Hungarian tobacco leaf, although the evidence presented for its being the iso compound is somewhat slender (104). Recent investigations of Japanese Virginia tobaccos have confirmed the presence of formic, acetic, and β-methylvaleric acids and demonstrated the existence of several more (220, 221, 222). The acids were first chromatographed on silicic acid and then converted to *p*-bromophenacyl esters which were compared with samples of authentic specimens; in this way *trans*-crotonic, propionic, methylethylacetic, *n*-caproic, phenylacetic acids, and, possibly, isobutyric acid were identified. The propionic and methylethylacetic acids were reported to arise on aging the cured leaf (221). Benzoic and 2-furoic acids were isolated and gave no melting-point depression with authentic specimens.

In addition to these low-molecular-weight volatile and nonvolatile acids, the

tobacco plant elaborates a number of fatty acids predominantly of the C_{16} and C_{18} type. Tobacco seed and the oil which can be expressed from it are particularly abundant sources of long-chain saturated and unsaturated acids and their glycerides. The possible commercial exploitation of this oil has been explored.

Fractional distillation and crystallization of the esterified fatty acids of tobacco seed oil were used to isolate palmitic, stearic, oleic, and linoleic acids, the latter being further characterized by bromination to tetrabromostearic acid (265). Saponification of tobacco seed oil, followed by fractional crystallization of the lead and barium salts, enabled stearic, palmitic, myristic, oleic, and linoleic acids to be separated, and alkaline oxidation with potassium permanganate afforded dihydroxy- and tetrahydroxystearic acids, thereby confirming the presence of oleic and linoleic acids (360). Bromination of the unsaturated acids gave tetrabromostearic acid; no hexabromostearic acid was detected, indicating the absence of linolenic acid in any quantity. Other investigations of tobacco seed oil have qualitatively shown much the same composition of fatty acids (26, 241, 269), although arachidic acid has been reported in Philippine tobacco seed oil (53).

Tobacco leaf, like the seed, was found to contain palmitic, oleic, and linoleic acids, but in addition the more unsaturated linolenic acid was isolated as its hexahydroxy derivative and a noncrystalline acid, $C_{30}H_{55}O_4$, was reported (292). A more recent investigation of Japanese flue-cured Virginia leaf by paper chromatography of the 2,4-dinitrophenylhydrazides of the fatty acids indicated the presence of palmitic, myristic, and lauric acids (218), the same acids being further identified by their melting points and by analysis of their *p*-bromophenacyl esters (21b). The acids of a flue-cured leaf were methylated and distilled, and a fraction (b.p. 195–200°C. (air bath)/0.5 mm.) was taken for mass-spectrometric analysis. The fraction was complex, the predominant acids being stearic, palmitic, linolenic, and linoleic, together with small quantities of all the other saturated long-chain acids from C_{10} to C_{23} (except C_{13}) and the unsaturated arachidonic and oleic acids (36).

Tobacco leaf resin yields a group of nonvolatile, noncrystalline, and indeterminate "resin acids." Early work (101) on Kentucky leaf reported the isolation of kentuckinic acid ($C_{22}H_{30}O_7$), kentuckinilic acid ($C_{23}H_{40}O_5$), and kentuckinolic acid ($C_{22}H_{34}O_6$), whilst similar work on a fermented tobacco gave α-, β-, and γ-tobacco acids, the α-tobacco acid more or less corresponding to kentuckinic acid (57). A benzene extract of tobacco yielded a resin acid ($C_{24}H_{41}O_5$) similar in its properties to kentuckinilic acid (293), and extraction of *Makhorka* resin gave acid fractions (α-, β-, and γ-tabacenic acids) similar to the α-, β- and γ-tobacco acids (303).

An early investigation of cigar smoke referred to the identification of formic, acetic, propionic, butyric, and valeric acids as their silver salts (369); later, the dry distillation of tobacco was reported to yield formic, acetic, and propionic acids as well as an unsaturated one, $C_{10}H_{12}O_2$ (84). Formic, acetic, butyric, valeric, caproic, C_7 acids, and C_8 acids were isolated from tobacco smoke by fractional distillation and precipitation as silver salts (196).

More recently, formic and acetic acids were identified in cigaret smoke (25)

and these two, together with propionic, butyric, isobutyric, benzoic, valeric, isovaleric, caproic, heptylic, caprylic, nonylic, and higher acids, were obtained by chromatography on silica and paper (30). The normal and iso acids were not separated by chromatography, but their presence was indicated by infrared spectroscopy of the mixtures.

Acids of cigaret smoke have been isolated on a Dowex resin and converted to methyl esters before examination by vapor-phase chromatographic techniques which indicated the presence of lactic, glycolic, succinic, malonic, oxalic, furoic, levulinic, glutaric, adipic, malic, and phthalic acids, together with five unidentified ones (255).

No derivatives of the previously mentioned acids were prepared however, but chromatography on silica and paper and the preparation of *p*-bromophenacyl esters enabled Japanese investigators to identify acetic, propionic, isovaleric, and *n*-butyric acids by comparison with authentic specimens, whilst β-methylvaleric and *n*-caproic acids were possibly obtained (122). Also, benzoic acid, which previously had been detected by its ultraviolet spectrum (23, 30, 122), was isolated and identified by comparison with authentic material.

Vapor-phase chromatography and mass spectrometry of the higher-boiling acids in cigaret smoke served to identify lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic, and cerotic acids and to demonstrate the presence of several unidentified ones (43).

An acid fraction of cigaret smoke was methylated and chromatographed on alumina yielding a crystalline material (m.p. 33–34°C.), methyl oleate, and methyl linoleate, the last two being characterized through the preparation of derivatives (357). The crystalline compound, resembling methyl palmitate in having a superimposable infrared spectrum and the correct elementary analysis, when subjected to mass spectrometry was shown to be a mixture of methyl esters of acids from C_{12} to C_{20}.

Chromatography on alumina of an acid fraction of cigaret smoke eluted a material which had an ultraviolet spectrum similar to that of a fluorenecarboxylic acid and which, after heating with calcium oxide, yielded a residue with an ultraviolet spectrum like that of fluorene (23).

α-Ketoglutaric, glyoxylic, and pyruvic acids were demonstrated in smoke by paper chromatography of the 2,4-dinitrophenylhydrazones (95).

Preliminary communications briefly summarized the presence in tobacco smoke of palmitic, oleic, further C_{18} unsaturated, aromatic, long-chain C_{20}, and phthienoic acids and also one having the formula $C_{12}H_{12}O_5$ (411, 412). Glutamic, nicotinic (28), and reductic acids (377), reported in tobacco smoke, have been dealt with in other sections.

A number of poorly characterized "resin acids" have been described (393, 394).

F. PHENOLS AND POLYPHENOLS (SEE TABLE 9)

An underlying complexity is suggested by the number of phenols indicated in tobacco; however, many of these are present in limited amounts and are of

TABLE 9

Phenols and polyphenols found in tobacco and tobacco smoke

Phenol	Tobacco	Smoke	Phenol	Tobacco	Smoke
p-Allylcatechol	+		Kaempferol glycosides	+	
p-Anisaldehyde	+		Melilotic acid	+	
Caffeic acid	+		Mesitol		+
Catechol	+	+	1-Naphthol		+
Chlorogenic acids	+		2-Naphthol		+
p-Coumarylquinic acid	+		Phenol	+	+
o-Cresol		+	Quinic acid	+	
m-Cresol	+	+	Resorcinol		+
p-Cresol		+	Rutin	+	
Eugenol	+		Salicylaldehyde	+	
Guaiacol	+	+	Scopoletin	+	+
Hydroquinone		+	Scopoletin glycosides	+	
o-Hydroxyacetophenone	+		Scopolin	+	
m-Hydroxyacetophenone		+	Shikimic acid	+	
p-Hydroxyacetophenone		+	2,4-Xylenol		+
Isoeugenol	+		3,5-Xylenol		+
Isoquercitrin	+				

doubtful significance. The phenols of processed tobacco and smoke may not originate wholly from the phenols of green leaf.

In green tobacco the phenols, amongst which rutin and chlorogenic acid predominate, form part of an oxidation-reduction system, and this system is greatly affected by leaf-processing conditions. It is difficult to ascertain the structures of the end-products, apart from unchanged polyphenols, in processed tobacco. The nature of phenols isolated from the latter source suggests that they may have some different origin such as lignin or carbohydrates. The problem of deciding whether the phenols of tobacco smoke originate by decomposition of polyphenols, by pyrolysis of lignins and carbohydrates, or by other routes would form an interesting topic for research. Some studies along these lines have been reported (188, 392).

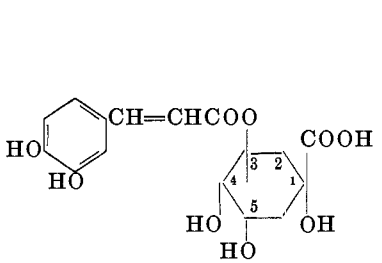
It is believed that the polyphenols of tobacco can be correlated in some manner with the "smoking quality" of a cigaret; whilst this is a subjective factor to a large extent, many studies have been made of the relationship between grade of tobacco and polyphenol content. The polyphenols of tobacco have recently been reviewed (323). Although a direct relationship between phenols and quality may not be proved, their role in the formation of the dark brown pigments of tobacco during air curing is more certain, and chlorogenic acid plays an important part in these reactions. The disappearance of polyphenols during the air-curing and fermentation processes is accompanied by a deepening of the tobacco color, sometimes almost black in comparison with flue-cured leaf, which is bright yellow.

A number of papers have been published recently on the variation of polyphenol content and its decrease in processing, a topic in which paper chromatographic techniques are extremely useful (56, 235, 380). The earlier classical studies have been described (27). The principal polyphenols have been isolated and studied, but many of the minor components have been indicated only by

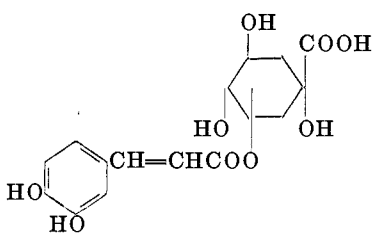
measurements of R_f values, and the small quantities present make further confirmation of identity extremely difficult.

1. Chlorogenic acid

The major polyphenols of tobacco are rutin and the depside chlorogenic acid (3-caffeoylquinic acid).



Chlorogenic acid



Isochlorogenic acid

Chlorogenic acid possesses several isomers. Isochlorogenic acid has the depside link at position 5 in the quinic acid residue and trans to the carboxyl group; it has been suggested that lactonization would account for the anomalous neutralization equivalent shown by this isomer (4). Neochlorogenic acid was isolated as a crystalline compound from an extract of peaches by counter-current distribution, but the position of the depside link is unknown (51).

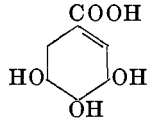
Paper chromatography of chlorogenic acid in aqueous acid affords two spots due to cis and trans isomerism about the cinnamic double bond (408), and further investigation showed that neochlorogenic acid also gives two spots, whereas isochlorogenic acid gives three, the third spot being attributed to a lactone form (266).

An early report of the occurrence of what was probably chlorogenic acid (294) described the isolation from unfermented Tyk-Kulak tobacco of a compound thought to be a glycoside of chlorogenic acid, containing rhamnose. The isolation technique was later improved (139) and chlorogenic acid, m.p. 206°C., and quinic acid were isolated as crystalline compounds from German tobacco, whilst chlorogenic acid was further obtained from extracts of American flue-cured tobacco by precipitation with lead acetate (239) and column chromatography on cellulose (380).

The variable behavior of chlorogenic acid and its isomers on paper chromatography would account partly for the complexity of the results obtained by this method (178, 232, 268, 326). Dried and green Pennsylvania seed filler tobacco showed on paper chromatography chlorogenic acid and two other spots resembling the latter in giving positive Hoepfner reactions (109, 268). Chlorogenic and neochlorogenic acids have been reported from flue-cured American and South African tobaccos (234, 380), and *cis*- and *trans*-chlorogenic acids from Bulgarian fermented leaf (178). Eight spots showing chlorogenic-like reactions could be demonstrated in a lead salt precipitate of an aqueous extract of flue-cured South Carolina leaf (260).

Reports on the presence of caffeic acid in tobacco conflict, but its presence now seems well established. It was synthesized from labelled phenylalanine in tobacco seedlings, and its presence in the aqueous extract was demonstrated by the method of isotope dilution (87). Paper chromatography indicated the presence of caffeic acid in flue-cured leaf but not in fresh tobacco (235, 259, 268). It was isolated from Japanese Bright Yellow leaf, and in the same investigation a compound of nicotine with a polyphenol, closely related to chlorogenic acid, was also reported (2).

The presence of quinic acid in tobacco leaf has been rarely reported, and several workers have stated its absence in leaf extracts examined by paper chromatography. However, it has been identified by this technique in Japanese flue-cured tobacco, where shikimic acid was also found (193), and in American mature cigar tobacco, where it is said to be a normal component of leaf and not derived from chlorogenic acid (229). It is lost by drying at 80°C.



Shikimic acid

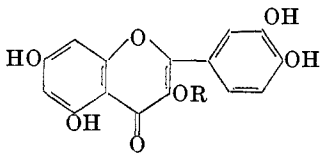
2. Rutin

Rutin (quercitrin-3-rhamnosidoglucoside) is one of the principal polyphenolic constituents of most types of tobacco, in which it occurs in appreciable quantity, and there are several accounts of its isolation and characterization (2, 200, 380). In view of the pharmaceutical value of rutin in the treatment of capillary fragility, an examination of *Nicotiana* species for rutin content was made, but better yielding plants were later found (323).

There is a decrease in rutin content during the curing process, more notably in air curing than in flue curing, which would suggest that an enzymic process may be responsible. As rutin is not directly oxidized by polyphenoloxidase, it may be oxidized by the quinonoid form of chlorogenic acid produced by this enzyme; thus the constancy of chlorogenic acid and the decrease in rutin would be explained. It has been shown that the presence of chlorogenic or caffeic acid is necessary for the oxidation of rutin by a tobacco polyphenolase (380). In flue curing the enzyme would be rapidly inactivated by dehydration and the high temperature, but in air curing the lower temperature would permit activity to continue until all the rutin had been used up and further reactions of the resulting quinones would give the dark brown pigments characteristic of air-cured tobacco (199).

Rutin has been isolated from flue-cured American tobacco (380) and fresh leaf (200), and paper chromatographic studies have confirmed its presence in green tobacco (268), Bulgarian fermented tobacco (178), and many varieties of American tobacco (56, 235). When comparative studies were made of several tobacco types and the variation of rutin content with cultural and manufacturing practices (235), it was found that there was a similarity in polyphenol content if dif-

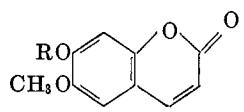
ferent types were cultivated and cured alike. The reported absence of rutin in greenhouse-grown tobacco (235) has been disputed, however (381).



Rutin: R = rutinose (L-rhamnosido-D-glucose)
Isoquercitrin: R = D-glucose

3. Isoquercitrin

Isoquercitrin was isolated from the leaves of unfermented Tyk-Kulak tobacco, where it is said to occur to the extent of 1.7 per cent, but the quantity is generally about 0.25 per cent in most smoking tobaccos (151, 153). Its structure was established by hydrolysis, followed by isolation and analysis of the sugar and aglycone. Methylation of the glycoside and hydrolysis to 5,7,3′,4′-tetramethylquercitrin established the position of the 3-glycoside linkage. Isoquercitrin was later obtained in low yield from air-cured Kentucky Burley tobacco (112). Recent paper chromatographic studies on tobacco have shown that the presence and quantity of isoquercitrin vary greatly in tobacco types, whilst the same technique was used to demonstrate its presence in fermented Bulgarian tobacco (178). There was no evidence for the compound in more than trace quantities in Pennsylvania seed filler tobacco (268), and a trace only was detected in flue-cured American tobacco. Flue-cured S. Carolina leaf yielded isoquercitrin as a minor component (260).



Scopolin: R = D-glucose
Scopoletin: R = H

4. Scopoletin

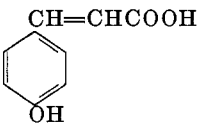
Scopoletin (7-hydroxy-6-methoxycoumarin) is well established as a constituent of tobacco. It has been isolated from an ethanolic extract of Japanese Bright Yellow leaf (0.15 g. from 2 kg.) (2), and identified by analysis, ultraviolet spectrum, and comparison with authentic material. It has also been isolated from roots of tobacco (17, 180).

To determine the quantities present in cigaret, cigar, pipe tobaccos and other commercial tobacco products, paper chromatographic techniques have been used followed by elution of the spots and estimation by ultraviolet absorption at 344 mμ (416, 418). The characteristic blue fluorescence of coumarins in the ultraviolet renders their location on paper relatively simple, and many chromatographic studies have shown the presence of scopoletin in a wide variety of tobacco types (129, 178, 235, 259, 354, 380). Scopolin, the 7-glucoside, has been identified similarly on paper by R_f values and by hydrolysis of the eluted material to glucose

and scopoletin (380). The scopolin content of tobacco decreases during the curing process (380), and there is an increase of scopoletin during air curing (235). Scopoletin glycosides and unknown scopoletin-like substances have been reported in flue-cured South Carolina leaf (259, 260).

5. Simpler phenols of tobacco leaf

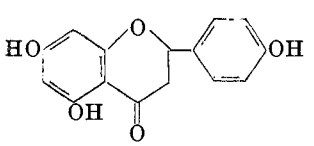
For a long time simple phenolic compounds in tobacco leaf received little attention from research workers.



Melilotic acid

Melilotic acid (4-hydroxycoumaric acid) has been mentioned as occurring in leaf (86), and the occurrence of caffeic acid is discussed in Section IV,F,1. Eugenol was isolated from the flowers of *N. tabacum* and identified as the benzoate (275). More recently a group of Japanese workers have made a full study of the steam-volatile components of various types of tobacco, obtaining in most cases crystalline derivatives of the compounds and comparing infrared spectra. The steam distillate from flue-cured Japanese Virginian tobacco was fractionated, and phenol and guaiacol were identified as their aryloxyacetic acid derivatives (216). Salicylaldehyde was also found in this fraction and was identified as its 2,4-dinitrophenylhydrazone, as were methyl salicylate (216), *p*-anisaldehyde, and *o*-hydroxyacetophenone (217). Eugenol was obtained and converted to its 2,4-dinitrophenyl ether. *m*-Cresol was found to be present before aging the tobacco but not afterwards, and there was a general decrease in the quantities of those phenols not possessing a carbonyl function (218).

In Japanese Burley tobacco leaf the same group of workers identified guaiacol, phenol, eugenol, *p*-allylcatechol, and *m*-cresol by column chromatography of the 3,5-dinitrobenzoates, which were then compared with authentic specimens by infrared spectra and melting points. Guaiacol is the predominant phenolic constituent of Japanese Burley, which has a much higher phenol content than Japanese flue-cured tobacco leaf (219). Isoeugenol has been identified in flue-cured tobacco (66a).



Kaempferol

6. Minor phenolic constituents

The major polyphenols of tobacco have been characterized and studied, but the complexity of the polyphenolic pattern and the presence of many minor components have only been revealed by sensitive separative techniques such as paper

chromatography; unfortunately, the quantities of material available make it impossible to identify the compounds in a manner which would satisfy the criteria of classical organic chemistry.

Paper chromatography shows that kaempferol is a component of Bulgarian tobacco, originally present as a glucoside (179); from S. Carolina flue-cured tobacco a kaempferol glycoside was extracted which yielded rhamnose and glucose on hydrolysis (260). Evidence has been given for the presence of quercitrin in air-cured Burley tobacco (112). Column chromatography on alumina of an extract from Burley lugs gave three yellow pigments which from chemical reactions and ultraviolet spectra appeared to be flavones.

A chromatographic study of the products of hydrolysis of a compound obtained from an American flue-cured tobacco extract indicated that it was possibly a *p*-coumarylquinic acid (380).

It has been reported that at least sixty phenolic compounds were apparent on paper chromatography of an extract of S. Carolina flue-cured leaf (259). In a study of fermented Bulgarian tobacco by this method the presence of four unidentified coumarin derivatives and some unidentified flavonol glycosides was reported in addition to rutin, isoquercitrin, and *cis*- and *trans*-chlorogenic acids (178). Many chromatographic studies record the presence of such unidentified compounds, and it is probable that some light will be shed on the nature of these by enzymic studies on the oxidation of polyphenols.

7. Phenols of tobacco smoke

The presence of phenol and "creosote" in tobacco smoke was established as early as 1871, the compounds being identified by isolation of the silver salts (369). Catechol was isolated as a lead salt (187).

More recently, the phenols have been identified by chromatographic techniques supplemented by ultraviolet spectra (23), and in this way phenol, catechol, resorcinol, and mesitol have been found in smoke. Hydroquinone, identified in the smoke of cigarets smoked in a reducing atmosphere containing sulfur dioxide, was considered to be present normally as *p*-benzoquinone. Paper chromatographic techniques have been used to separate and determine the *p*-nitrobenzene-azo derivatives of phenol, guaiacol, *m*- and *o*-cresols, and the steam-volatile phenols from American cigarets (257).

Investigators of the smoke from English cigarets, using for identification the ultraviolet spectra of the methyl ethers which had been separated by chromatography on alumina, revealed more of the complexity of this fraction and indicated the presence of phenol, *o*-, *m*-, and *p*-cresols, 1- and 2-naphthols, resorcinol, and hydroquinone (47, 48). A preliminary report has also mentioned the presence of 2,4-xylenol (412).

Vapor-phase chromatography of the methyl ethers (37) and of the phenols themselves, confirmed by mass spectrometry (43), indicated in addition the occurrence of 3,5-xylenol, several other substituted phenols, and some of hydroaromatic nature. These conclusions have been borne out in a further investigation in which phenol, *o*-, *m*-, and *p*-cresols, catechol, and *m*- and *p*-hydroxyacetophenones were separated by vapor-phase chromatography and counter-current

distribution followed by conversion of the pure compounds to crystalline derivatives which were identical with those of authentic specimens in melting point and in ultraviolet and infrared spectra (36).

The method of estimation of scopoletin which was used for its determination in tobacco products (418) has been applied to its estimation in cigaret smoke (416).

Phenolic esters have been reported in cigaret smoke (123).

G. ALKALOIDS AND OTHER BASES (SEE TABLE 10)

Since the first isolation of nicotine (249) a considerable amount of research has centered on the alkaloid fraction of tobacco as regards both the isolation and the identification of the constituents and their biosynthesis and transformations. A number of excellent reviews have appeared on the alkaloids of tobacco (68, 81, 124, 149, 168, 190, 318).

The various species and strains of *Nicotiana* may be broadly classified according to the major alkaloid elaborated in the plant. Usually the principal alkaloid constituent is nicotine or nornicotine and sometimes anabasine (313); along with these bases a number of so-called secondary alkaloids are formed. The alkaloid content of commercial tobacco also depends on the processing to which it has been subjected; consequently the pattern of alkaloids in the smoking material and the smoke can vary a great deal. Earlier work on the tobacco and smoke bases often failed to describe the type and history of tobaccos used in the investigations, thus losing some of its value, since it is not now clear whether the sub-

TABLE 10

Alkaloids and other bases found in tobacco and tobacco smoke

Substance	Tobacco	Smoke	Substance	Tobacco	Smoke
Ammonia	+	+	Nicotimine	+	
Anabasine	+	+	Nicotinamide	+	+
Anatabine	+	+	Nicotine	+	+
Anodmine		+	Nicotine oxide	+	
Collidine		+	Nicotinic acid	+	+
Cotinine	+	+	Nicotoin	+	
Dimethylamine		+	Nicotyrine	+	+
2,6-Dimethylpyridine		+	Nornicotine	+	+
2,3′-Dipyridyl	+		Nornicotyrine		+
2,4-Di(3-pyridyl)pyridine	+		Obeline		+
Ethylamine		+	Picoline		+
Gudham		+	Piperidine	+	
Isonicotein	+		Poikiline		+
Lathrein		+	Pyridine	+	+
Lohitam		+	Pyridine-3-aldehyde		+
Lutidine		+	3-Pyridyl ethyl ketone		+
Methylamine		+	3-Pyridyl methyl ketone	+	+
N-Methylanabasine	+		3-Pyridyl propyl ketone	+	+
N-Methylanatabine	+		Pyrrole		+
N-Methylmyosmine		+	Pyrrolidine	+	
2-Methylpyridine		+	Quinoline		+
3-Methylpyridine		+	α-Socratine		+
N-Methylpyrrolidine	+		β-Socratine		+
Myosmine	+	+	γ-Socratine		+
Nicotein	+		Trimethylamine	+	+
Nicotelline	+				

stances isolated were present in the leaf or arose on processing, or even through laboratory treatment. Recently, systematic investigations have been carried out on tobacco leaf at the different stages of processing with an aim to elucidating the fate of the alkaloids (81), whilst their biogenesis has been widely studied (54, 55, 68, 117, 157, 190, 330). The application of modern analytical techniques such as paper chromatography (143, 147, 150, 156, 248, 336, 353, 383, 387, 400, 407), column chromatography (150, 223), vapor-phase chromatography (253), and electrophoretic chromatography (174) has facilitated research on tobacco and smoke, as has the concomitant use made of ultraviolet and infrared spectra.

Several alkaloids—nicotimine, nicotein (242, 244), nicotelline, isonicotein, and nicotoin (205)—were described and later nicotein was separated into two components (69), but since then the tobacco alkaloids have been separated and identified so that it is now possible to comment on these earlier isolated bases. It is thought that nicotimine must have been an impure specimen, since of all the alkaloids so far isolated, none has properties resembling those of this substance. One of the bases into which nicotein had been separated was similar to anatabine (3′,4′-dehydroanabasine), also isolated later from an extract of tobacco (321), and it was shown that nicotein was, in fact, a mixture of nornicotine and anatabine (315). A specimen of nicotelline (205) was shown by semimicro oxidation and paper chromatography to be 2,4-di(3-pyridyl)pyridine (146), the investigators being of the opinion that the original was not a laboratory artefact, since 4,3′ linkages are absent in the other tobacco alkaloids. The structure of nicotelline was confirmed by synthesis (337). Nicotoin, which was not amply described, has never been found again, and isonicotein proved to be identical with 2,3′-dipyridyl (314, 321). Further alkaloids separated in earlier work include nornicotine (320), anabasine (316) [the chief alkaloid of *N. glauca* (309)], nicotyrine (317), *N*-methylanabasine (317), and *N*-methylanatabine (317), whilst several simpler bases isolated were ammonia (321), trimethylamine (321), pyrrolidine (243), *N*-methylpyrrolidine (314), *N*-methylpyrroline (37), piperidine (321), and traces of pyridine (20). A recent paper chromatographic investigation of the volatile bases of *N. tabacum* and *N. rustica* showed the presence of ammonia, methylamine, and isoamylamine, but not trimethylamine, in fresh leaves (331).

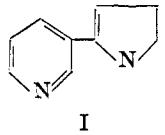
During air curing of cigar tobaccos there is a loss of about 10 per cent of alkaloids, a decrease which could not entirely be accounted for by volatilization or transformations, and it was suggested that bacterial action might be responsible (81). Several investigations of the bacterial degradation of nicotine have shown that the pyridine ring itself can readily be split, the end-products being dibasic acids such as oxalic acid and volatile bases such as ammonia (81, 370, 373). There appeared to be little change in alkaloid content during aging but fermentation proceeded at the expense of the alkaloids, which were transformed into other products, some having been identified: namely, cotinine, oxynicotine, 3-pyridyl propyl ketone, 3-pyridyl methyl ketone, nicotinamide, *N*-methylnicotinamide, and nicotinic acid (81). Myosmine (78, 126) and 2,3′-dipyridyl (321) have been found in tobacco, and a base resembling metanicotine was isolated from a nicotine-free variety (375).

Nicotine glycosides obtained by the dialysis of tobacco extracts have been re-

ported but not confirmed. One glycoside, tabacilin, on hydrolysis gave glucose, nicotine, and other products (414), whilst the glycoside tabacin yielded nicotine on hydrolysis (7).

Early research on the smoke of cigars burned in pipes reported the isolation and identification of several pyridine homologs. Pyridine, C_5H_5N, and the homologs $C_nH_{2n-5}N$ (where n = 2–7) were obtained and analyzed in the free state and as platinichlorides, but nicotine was said to be absent (369). Pyridine, itself, has been found often in tobacco smoke (10) and a few publications refer to its homologs. 2- and 3-Methylpyridines and 2,6-dimethylpyridine were identified by ultraviolet spectroscopy after chromatography on alumina of a basic fraction of cigaret smoke (22). Recent studies on the pyrolysis of nicotine have shown the formation of pyridine-3-aldehyde, *N*-methylmyosmine, nornicotyrine, and nicotinamide (352), the latter having been previously reported (28) in cigaret smoke. Pyrrole has been estimated by its color reaction with isatin (83).

Two ketones, 3-pyridyl methyl ketone and 3-pyridyl ethyl ketone, have been isolated (254, 287). Quinoline was reported in a dry distillate of tobacco along with ammonia, methylamine, dimethylamine, trimethylamine, and a picoline (84). Paper chromatographic studies on the volatile bases of cigaret smoke showed the presence of ammonia, methylamine, and ethylamine. In this investigation a base was isolated which formed a picrate melting at 250°C. (analysis: carbon, 29.4 per cent; hydrogen, 1.93 per cent; nitrogen, 22.4 per cent) (121); this base may have been ammonia (analysis for ammonium picrate: carbon, 29.2 per cent; hydrogen, 2.4 per cent; nitrogen, 22.7 per cent). The picrate of obeline, a substance obtained from cigar smoke (399), was later shown to be ammonium picrate (148). The same series of studies in which obeline was isolated yielded several more bases of unknown constitution: α-, β-, and γ-socratines, poikiline, anodmine, gudham, lathrein, and lohitam, all isolated as picrates or picrolonates (395, 396, 398, 399). The constitutions of these bases remained unknown until recently, when investigators having access to original specimens were able to elucidate the identities of some of them by application of modern analytical techniques. γ-Socratine was found to be identical with *l*-nornicotine, and a crude mixture of α- and β-socratines (the only sample available) was shown to consist mainly of nicotyrine and 2,3′-dipyridyl with small quantities of nicotinic acid, nornicotine, and possibly anatabine (148). The bases anodmine, gudham, lathrein, and lohitam were originally isolated from a non-steam-volatile fraction of cigar smoke and a similar fraction has been shown to contain a large number of bases, amongst which were myosmine, 2,3′-dipyridyl, anabasine, anatabine, nornicotine, and nicotinic acid (148). The last-named bases, together with cotinine, have also been identified in cigaret smoke (254). The constitution of myosmine, originally isolated from cigar smoke (395), was largely elucidated by experiments on the base (319) and a synthesis of the supposed structure (I).



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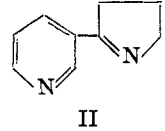
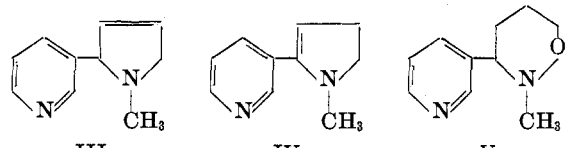


II

It was shown later that myosmine has no N—H frequencies in the infrared (67) and this, coupled with the ultraviolet spectrum which indicates a double bond in conjugation with the pyridine ring (333), finally establishes structure II for the base. Myosmine dissolves in water with scission of the ring to give 3-pyridyl ω-aminopropyl ketone (102), possibly identical with the cigar smoke base poikiline, a compound which slowly yielded myosmine picrate on treatment with an aqueous solution of picric acid (397).

There would seem to be some confusion over "*N*-methylmyosmine." The product of partial hydrogenation of nicotyrine has an ultraviolet spectrum resembling that of nicotine rather than that of myosmine (333), indicating structure III for dihydronicotyrine and not structure IV, which is that of a compound described as *N*-methylmyosmine.



III IV V

It is clear from structure II that no *N*-methylmyosmine can exist except possibly as a quaternary base, and to avoid confusion it was suggested that this name should no longer be used (410). "*N*-Methylmyosmine" (or better, 2′,3′-dehydronicotine) has been obtained from pseudo-oxynicotine (103) and has the double bond in the 2′,3′-position, whilst the dihydronicotyrine obtained by partial hydrogenation of nicotyrine has the double bond in the 3′,4′-position (333).

Nicotine oxide yields 2-methyl-6-(3-pyridyl)tetrahydro-1,2-oxazine (V) on distillation *in vacuo* (258).

H. PROTEINS AND AMINO ACIDS (SEE TABLE 11)

Although the tobacco chemist has long possessed detailed quantitative information regarding the distribution and fate of the nitrogen of the leaf during processing, a fresh approach to the chemistry of the amino acids and proteins has been made possible by newer techniques.

Primarily, these have been applied to fresh tobacco, and it must be borne in mind that, in view of the complexity of the degradation in tobacco processing, paper chromatographic studies in this context may be especially misleading and the possibility of obtaining products similar in chromatographic behavior to the naturally occurring amino acids cannot be overlooked. Identification based solely on R_f values can be misleading. Breakdown and change of nitrogen is extensive; in the case of a fermented cigar leaf 90 per cent of the amino acid nitrogen originally present was found to be converted into other products (75, 76).

The high-molecular-weight nitrogenous compounds of fermented tobacco have been fractionated by solution in different solvents followed by selective reprecipitation and enzymic hydrolyses, using trypsin to study the protein fraction (384, 385, 386). Accounts of analytical studies on curing the cigar leaf have been given with full details of the methods used (79, 80, 127). In addition to protein break-

TABLE 11

A. Amino acids of tobacco

α-Alanine	Betaine	Glutamic acid	Leucine	Serine
β-Alanine	Choline	Glutamine	Lysine	Threonine
α-Aminobutyric acid	Citrulline	Glycine	Methionine	Tryptophan
γ-Aminobutyric acid	Cysteine	Histidine	Phenylalanine	Tyrosine
Asparagine	Cystine	Isoleucine	Proline	Valine
Aspartic acid				

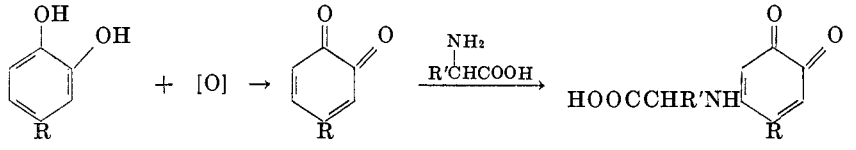
B. Amino acids of tobacco smoke

Glutamic acid	Glutamine	Nicotinic acid	Nicotinamide

down, there is an increase of water-soluble nitrogen, including amides, ammonia, thiamine, and pantothenic acid. However, proteolysis is accompanied by little increase of peptide nitrogen.

The degradation of leaf proteins is an extremely important aim of tobacco processing, as it is believed that these compounds are associated with poor smoking quality. The paucity of methods available for investigation of some of the end-products (water-insoluble, polymeric, nitrogen-containing compounds of high molecular weight) has meant that their chemical nature has remained obscure, but possible schemes for their origin have been suggested.

Oxidative deamination is an important mode of amino acid breakdown. Amino acids condense with *o*-quinones, arising in the leaf by oxidation of polyphenols, to give complexes which catalyze the oxidative deamination of amino acids to α-keto acids with the liberation of ammonia, and indeed a dark colored substance, obtained from the enzymic oxidation of a mixture of caffeic acid and L-proline, is known to catalyze the oxidation of glycine (108). It is of interest that brown substances obtained from processed tobacco showed an ultraviolet absorption curve similar to that of *p*-benzoquinone (100).



Complex which further catalyzes oxidative deamination

It has also been demonstrated that breakdown products of carbohydrates may, by combination with amino acids, give deep brown substances of the Schiff base type. Methylglyoxal, when warmed with amino acids, underwent deamination and decarboxylation to a lower aldehyde, and similar deamination may be effected by other degradation products of sugars such as mesoxaldialdehyde (195, 378).

In recent years, extensive studies of the proteins of green leaf have been undertaken, and the soluble protein of the cytoplasm has been obtained from the young leaf of *N. tabacum* (Havana and Samsun varieties) (405). This protein, termed

"Fraction I" protein, possesses auxin and phosphatase activities (403). It is a nucleoprotein, containing 5–15 per cent nucleic acid. The nature of the bound phosphorus was investigated by digestion with trichloroacetic acid, followed by hydrochloric acid, the phosphorus content being estimated at each stage (404). Electrophoresis and ultracentrifuge studies showed that this protein comprises 23–50 per cent of the total cytoplasmic protein and displays a high degree of homogeneity. The molecular weight is of the order of 600,000, and the sedimentation constant is about 18 Svedberg units (304). The suggestion has been made that Fraction I protein may play some role in the photosynthetic process, as it is found only in the leaves of plants containing chlorophyll and protochlorophyll (61).

A minor protein component, termed "Fraction II" protein, was also studied and found to be heterogeneous in composition. It has greater electrophoretic mobility than the Fraction I protein, and the sedimentation constant is about 4 Svedberg units. Considerable enzymatic activity is associated with this fraction (403).

Ultracentrifuge techniques were used to examine the changes in protein fractions on curing tobacco. It was found that Fraction I protein was rapidly degraded, whereas the remainder of the proteins, possessing much enzymic activity, was relatively more stable. Pectin methylesterase activity of this fraction in air-cured Burley leaf was assayed and shown to reside in Fraction II protein. It is remarkable that pectin methylesterase activity remained in a sample of flue-cured Bright tobacco leaf which had been subjected to a temperature of 80°C. for 18 to 24 hr., as is usual in flue-curing practice (246).

It has been stated that there is evidence for the presence in leaf, even after flue curing, of protease, lipase, emulsin, amylase, invertase, phosphatase, glycolase, pectase, ketone-aldehyde mutase, oxidase, peroxidase, catalase, and reductase activities (85).

Investigations of green tobacco leaf grown in South Africa, France (Paraguay variety), Italy (Round tip, Sumatra, and Scafati 7 varieties), and elsewhere, using paper chromatography, have indicated the presence of the following as the principal free amino acid constituents: alanine, α-aminobutyric acid, asparagine, aspartic acid, glutamine, lysine, phenylalanine, proline, serine, tryptophan, and tyrosine. In addition, β-alanine, methionine, valine, citrulline, histidine, and cysteine, as well as unidentified amino acids, have been occasionally reported (91, 233, 267, 339). The overall picture seems to vary with the source of the tobacco, but glutamic acid, aspartic acid, γ-aminobutyric acid, and serine have been reported as relatively more abundant.

It has been suggested that green leaf may actually contain few amino acids but that many may arise by rapid proteolysis.

The change in the amino acid composition of leaf immediately following picking (256), during forced and natural fermentation (339), and the amino acids of the processed leaf (91, 172, 177, 233, 267) have been studied by paper chromatography. Investigation, by this technique, of free and bound amino acids of four classes of fermented Bulgarian tobacco from both the Gorna-Dzhumaya and the

Melnick districts showed that the amino acid composition of the tobacco differed in the two provinces; although the protein amino acid composition was constant throughout the four categories from each province, the free amino acid composition varied (177). Processed Burley, Xanthi, Virginia, and Havana tobaccos were examined by concentrating the amino acids on Dowex 50 resin and subjecting the resultant acids to paper chromatography and paper electrophoresis. Twenty-four amino acids were identified; α-alanine, γ-aminobutyric acid, glutamine, glutamic acid, asparagine, aspartic acid, phenylalanine, valine, and proline occurred in all types, whilst the remainder of the amino acid content was variable. Havana tobacco lacked asparagine and glutamine, and Burley differed from the other types in containing substances that were probably citrulline and α-aminobutyric acid (172).

Aspartic acid, glutamic acid, glycine, lysine, and leucine comprise 65 per cent of the amino acids of leaf protein. In curing tobacco, protein breakdown occurs with the liberation of amino acids, accompanied by an increase in amide nitrogen as glutamine and asparagine (77, 127). When Kentucky tobacco shoots were incubated in water for a period, proteolysis took place affording amino acids of which the following were isolated and identified: arginine, lysine, glutamine, histidine, and probably leucine (350).

Asparagine has been isolated from Burley tobacco, where it was found in appreciable quantity even after long storage, 4.4 g. being obtained from 200 g. of tobacco (113). Glutamine and asparagine content varies with the variety, and the relatively large amounts found in shade-grown tobacco were not paralleled in Pennsylvania Seed leaf, where little was found at the corresponding stage of processing (79).

Studies throughout the processing of Italian varieties indicated that arginine disappeared during air curing whilst there was an increase of citrulline, and the appearance of methionine was linked with the disappearance of cysteine (91). The first of these findings may result from the possible reaction during processing:

$$\text{Arginine} + \text{water} \rightarrow \text{citrulline} + \text{ammonia}$$

During normal fermentation many amino acids disappeared but dicarboxylic acids seemed more resistant to decomposition (339).

Betaine, $N^+(CH_3)_3CH_2COO^-$, has been isolated from dried tobacco leaf and identified by conversion to derivatives; melting points were recorded and the gold salt analyzed (58), whilst the corresponding alcohol, choline, was isolated from shoots of *N. tabacum* after proteolysis (350).

Variations in the glutathione content of tobacco during the vegetative cycle and curing have been studied, but there is little information concerning other peptides (340, 341). Although, in processing, breakdown of protein occurs with liberation of amino acids, there is little increase in peptide nitrogen and few investigations of free peptides in processed tobacco have been reported. Burley tobacco contained several peptides but none were found in U. S. Bright, Virginia, Xanthi, and Havana tobaccos (processed). The peptides were separated from other nitrogenous fractions by chromatography on ion-exchange resins and then

isolated by paper chromatography. The amino acid sequences were studied in the usual way and the peptides found to have the following structures:

A asp. acid (glu. acid, ser, ala) glyc
B asp. acid (glu. acid, ser, gly) ala
C, D asp. acid, glu. acid, gly or asp. acid, glu. acid, glu. acid, gly

The occurrence of aspartic acid as an end group in every case is worthy of note (100).

The preparation of crystalline tobacco seed globulin has been described. The substance has been shown to possess a molecular weight of 350,000 ± 20,000 by measurement of unit cell dimensions and density of the dry crystals. Solubility studies indicated the presence of two or possibly three components, although on electrophoresis the material migrated in a homogeneous manner (52, 63). Investigations of the amino acid content of the globulin have been reported (311), and microbiological assay was used to determine the following percentages of amino acids: arginine, 16.4; cystine, 1.11; histidine, 2.22; leucine, 10.5; isoleucine, 5.3; lysine, 1.58; methionine, 2.18; phenylalanine, 5.70; threonine, 4.17; valine, 6.72 (310, 312, 364). Color tests suggested the presence of tryptophan and tyrosine (311). The content of cystine and methionine does not account for the total amount of sulfur present (310).

The free amino acids of the seeds of *N. rustica* were studied using paper chromatographic techniques. The variation in amino acid content with age of seed suggests that loss of germinative capacity was accompanied by a decrease in tryptophan and an increase in valine (93).

The presence of glutamine and glutamic acid has been demonstrated in tobacco smoke (28).

I. CARBOHYDRATES (SEE TABLE 12)

Lignins, celluloses, and hemicelluloses, common constituents of the cell wall of plants, are present in tobacco along with starch, a reserve carbohydrate, and simpler saccharides.

Although forming a moderately high percentage of stem tissue (191), the lignins of tobacco have not attracted a great deal of attention. The lignins, separated from other material by solution in strong acid, were fully methylated and decomposed in the usual way to yield at least three acids: dehydrodiveratric, isohemipinic, and veratric. Culture studies of plants fed with methionine isotopically labelled on the methyl group have shown that the latter is incorporated

TABLE 12

Carbohydrates of tobacco

Ascorbic acid	Glucose	Maltose	Raffinose	Sorbitol
Cellulose	Hemicellulose	Pectins	Rhamnose	Stachyose
Deoxyribose	Inositol	Pentoses	Ribose	Starch
Fructose	Lignin	Planteose	Rutinose	Sucrose

Tobacco smoke contains 1,6-anhydro-D-glucose.

as a whole into the methoxyl group of the lignins, which are probable end-products of plant metabolism (31). The transmethylation from sulfur to oxygen, a reaction in which methionine was much more effective than formate, may be a step in the biosynthesis of the lignins; later work has shown that isotopically labelled glycine, serine, and formaldehyde were incorporated into the lignin of plants fed with these substances (106).

Curing, essentially a plant starvation process, is accompanied by the usually complete loss of starch, the reserve carbohydrate. The two components of starch, amylose and amylopectin, occur in the ratio 23:77 in tobacco (419). The amylose was estimated to have a chain length of forty to forty-seven anhydroglucose units, whilst by periodate studies the amylopectin was shown to contain twenty-six glucose residues per nonreducing end group (228). Experiments with $^{14}CO_2$ showed that the carbon was first incorporated in the polysaccharide, only appearing later in sucrose and the simple sugars (366). Similar studies had previously demonstrated that whilst $^{14}CO_2$ is assimilated and incorporated into starch, glucose, and fructose, little appeared in the proteins, celluloses, hemicelluloses, and polyuronides (247). It has also been found that dissimilation of starch did not proceed as far as maltose, or if it did, the maltose must have been used up before it could be generally released (145). A simple method for the isolation of plant starches, including those from tobacco leaf, was recently described (131).

Investigations of Japanese Bright Yellow leaf, stem, and root have enabled the polysaccharides to be roughly separated into celluloses, hemicelluloses, pectins, and lower-molecular-weight polysaccharides (181, 182, 183). A cellulose fraction insoluble in 20 per cent sodium hydroxide solution was hydrolyzed to give only glucose, as shown by paper chromatography; on acetylation octaacetylcellobiose was obtained. Another leaf cellulose, soluble in 20 per cent sodium hydroxide and precipitated with ethanol after neutralization, gave mainly galactose with small quantities of arabinose and ribose on hydrolysis, and so may be considered as a galactan. The corresponding cellulose from stem on acid hydrolysis yielded arabinose and glucose, whilst that from root gave arabinose, ribose, glucose, and galactose.

The polysaccharides of the hemicellulose group in tobacco have been widely investigated. The hemicelluloses from the stalk of cured Havana seed tobacco were fractionated and purified by re-solution, reprecipitation, and treatment with Fehling's solution. The product exhibited no optical activity and complete hydrolysis gave a sugar (probably xylose), melting at 148°C. with decomposition, having a specific rotation, $[\alpha]_D^{22}$, of +19.42°, and giving a positive phloroglucinol test (9). The polysaccharide also contained uronic acids and was possibly a xylan. It is reported that the pentosan content of tobacco does not change during normal curing (127). The above-mentioned Japanese workers separated the hemicelluloses into a fraction soluble in 5 per cent sodium hydroxide and precipitated with ethanol after neutralization and a second fraction soluble in hot 5 per cent sodium hydroxide (181, 183). The principal constituent sugars obtained by hydrolysis of these hemicelluloses are shown in table 13, traces of other sugars being omitted.

TABLE 13

Sugars obtained by hydrolysis of hemicellulose fractions

	Rhamnose	Arabinose	Xylose	Mannose	Glucose	Galactose	Galacturonic acid
First hemicellulose fraction:							
Leaf		+	±		+	+	±
Stem		+					+
Root		+	±		±	+	+
Second hemicellulose fraction:							
Leaf		+	±		±	+	
Stem	+	+		±	±	+	+
Root		+			+	+	+

+ = large amount; ± = small amount.

Pectic substances now known to consist of a mixture of polysaccharides (a galactan, an araban, and the methyl ester of a galacturonan) have been estimated in tobacco by measurement of the carbon dioxide evolved on boiling with strong acids (6, 374). Russian workers investigating *N. tabacum*, *N. glauca*, and *N. rustica* isolated free pectic acid and also calcium magnesium pectate (144). Hydrolysis yielded 'a' and 'b' tetragalacturonic acids, whilst on oxidation mucic, oxalic, and trihydroxyglutaric acids were isolated; pentoses and hexoses were reported to be present also. Calcium magnesium pectate, $[\alpha]_D = +131.3°$, isolated earlier (202), gave on hydrolysis the tetragalacturonic acids and galacturonic acid. The latter has also been obtained from a hydrolysate of tobacco polysaccharide (45), and its presence in a cured tobacco has been demonstrated by paper chromatography (264). A furfural determination of the isolated calcium magnesium pectate gave a value in excess of the calculated one, a result which was interpreted as being due to the presence of pentoses (202)—in particular arabinose, which had previously been detected in a pectin extract of tobacco (5). The recent Japanese work on the polysaccharides of leaf extracted with ammonium oxalate has shown that acid hydrolysis gives predominantly galacturonic acid, galactose, and arabinose (183), a result in harmony with the present knowledge of the pectins. The pectic substances of stem were very similar to those of leaf, but smaller percentages of galactose and arabinose were present. In addition to these sugars, the pectin from roots yielded considerable quantities of rhamnose, mannose, fructose, xylose, and ribose (181).

A polysaccharide fraction of leaf, soluble in ethanol/water (1:1), was considered to be an arabogalactan of relatively low molecular weight (183).

Complete acid hydrolysis of a trisaccharide isolated from tobacco seed gave D-glucose, D-fructose, and D-galactose as the constituent sugars, whilst partial hydrolysis yielded D-glucose and a ketose disaccharide (372). The trisaccharide could be split by an α-galactosidase, giving sucrose and D-galactose, and these observations suggested its identity with planteose [*O*-α-D-galactopyranosyl-(1→6)-*O*-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside] (82). The trisaccharide, isolated from tobacco seed as crystalline material, had an optical rotation and X-ray diffraction picture identical with those of planteose and, like the latter, gave an acetate melting at 137°C.

A hot aqueous extract of Japanese Bright Yellow leaf was purified by treat-

ment with lead acetate and subjected to paper chromatography; the oligosaccharides thus isolated included stachyose [*O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside] and raffinose [*O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside], which were further hydrolyzed to their constituent sugars (182).

The di- and monosaccharides of tobacco have been investigated at all stages of the vegetative cycle from seed to fermented leaf. Paper chromatographic studies on the seeds of Xanthi, Herzogavinia, Virginia, and Maryland tobaccos showed that raffinose, sucrose, glucose, and fructose occurred in the seed, together with an unidentified sugar possibly having three to four carbon atoms (280). After germination raffinose disappeared, maltose and ribose appeared, and at the stage of transplanting all the sugars decreased to some extent.

Fructose and aldoses were found in fresh leaf and a phenylosazone corresponding to that of glucose, fructose, or mannose was prepared, although it was doubted whether the latter was present, since no characteristically insoluble phenylhydrazone could be obtained (250). Only fructose was isolated from fresh leaves of Tyk-Kulak tobacco, whereas the stems contained glucose, fructose, and a small quantity of "pentose" thought to be like quinovose (291). Glucose, fructose, sucrose, and maltose were demonstrated in fresh leaf by paper chromatography (182), results which have been confirmed by similar techniques during investigations of Chinese and Oriental tobacco (251, 252), and sucrose was crystallized from the sugars of stems (181).

Glucose, fructose, and possibly sucrose were found in a hot ethanolic extract of flue-cured tobacco (204), observations which were affirmed by paper chromatography (382). During air curing the sucrose disappears, leaving a little fructose and glucose, but on fermentation the fructose and most of the glucose are used up (90).

Early experiments on Russian cured and fermented American tobacco and fresh Tyk-Kulak leaf indicated that fructose was the main constituent monosaccharide with very little of the other sugars (295). Fructose has been isolated from freshly dried Kentucky tobacco (347). D-Fructose and sucrose were obtained from a carefully purified aqueous extract of tobacco by paper chromatography, followed by elution of the sugars from the paper and measurement of their infrared spectra (263).

Studies with isotopically labelled sugars have shown that when fed to young plants, glucose was incorporated into sucrose and the fructose half of the latter was also derived from glucose (365). Uniformly, labelled maltose appeared mainly in the plant as sucrose, although a little of the radioactivity was evident in the starch (145).

Sorbitol or D-glucitol has been obtained from aqueous extracts of fresh cigaret and pipe tobaccos by treatment of the extracted material with benzaldehyde, when di-and tribenzalsorbitols crystallized out. The benzalsorbitols were decomposed and the free sorbitol recrystallized, analyzed, and converted to its hexaacetyl derivative (m.p. 101–102°C.; no depression with authentic material (201)). Another report of the presence of sorbitol has appeared (107).

myo-Inositol, a polyalcohol closely related to the sugars, has been isolated and

identified a number of times and has also been found during paper chromatographic studies (182), whilst a report of its presence in cigaret smoke has appeared (412). Inositol, precipitated as its lead complex from an aqueous extract of Russian cigaret tobaccos (*N. tabacum*) and regenerated by decomposition of the complex with hydrogen sulfide, was recrystallized from aqueous alcohol and characterized by analysis, color reactions, and the preparation of derivatives (297). It was later extracted from both *N. tabacum* and *N. rustica* (307, 308), and more recently from American flue-cured leaf (240). Phytin, the calcium magnesium salt of inositol hexaphosphate, has been found in tobacco seed (298). Quinic and shikimic acids, closely related to inositol and occurring in tobacco, have been discussed in Section IV,F,1.

The phytosterols and polyphenols of tobacco exist in part as glycosides, and some of these sugar residues have been identified. Hence the well-known rutinose (6-*O*-β-L-rhamnosyl-D-glucose) occurs in rutin, rhamnose in kaempferol, and D-glucose combined with β-sitosterol. An extract of fresh leaf has been treated with lead acetate, the precipitated complex decomposed, and the residue hydrolyzed with acid. The sugars so produced were investigated by paper chromatography; mannose, glucose, galactose, and rhamnose were obtained together with faint traces of xylose, fructose, and desoxyribose (182). Two glycosides of nicotine, tabacin (7) and tabacilin (414), have been reported, the former yielding a reducing sugar on hydrolysis and the latter yielding glucose. Ribose and deoxyribose are the sugar components of the nucleoproteins.

Ascorbic acid was identified in tobacco by the Indophenol Blue reduction method (340, 341), and a further report of its presence has appeared (160).

The existence of "tabakose," a sugar peculiar to tobacco (3), has never been substantiated and the report of its discovery is now regarded as an error.

The increase in reducing capacity of mainstream cigaret and cigar smoke before and after hydrolysis was ascribed to the presence of anhydrosugars (189). Levoglucosan (1,6-anhydro-β-D-glucopyranose), a product of the pyrolysis of cellulose, starch, and glucosides, has been obtained from the aqueous portion of smoke condensate (391).

J. INORGANIC COMPONENTS (SEE TABLE 14)

The inorganic content of any tobacco sample will be affected by such factors as the nature of the soil in which cultivation took place, possible translocation during some types of curing, and additives which may be used as fertilizers or insecticidal sprays.

References to the literature on the minor elements are available in collected form (18). The presence of arsenic, a known carcinogen, in tobacco and smoke has been the subject of many papers and reviews, and recently a critical summary with bibliography has been published (10). Lead arsenate sprays were used as insecticides, and it was pointed out that arsenic might enter the human body from this source (262), but the replacement of these arsenical sprays and other sources of arsenic has reduced the amount present in mainstream smoke to exceedingly low quantities, being 0.3 to 1.4 μg. in the smoke of an American cigaret.

The inorganic elements that have been detected and estimated in tobacco

TABLE 14

Inorganic elements in tobacco ash and smoke

Element	Ash	Smoke	Element	Ash	Smoke
Aluminum	+	+	Magnesium	+	+
Arsenic	+	+	Manganese	+	+
Barium	+		Nickel		+
Boron	+		Potassium	+	+
Calcium	+	+	Rubidium	+	
Cesium	+		Silicon	+	
Chromium	+	+	Sodium	+	+
Copper	+	+	Strontium	+	+
Iron	+	+	Titanium	+	+
Lead	+	+	Zinc	+	+
Lithium	+				

ash by spectrometric and colorimetric methods are aluminum, arsenic, barium, boron, calcium, cesium, chromium, copper, iron, lead, lithium, magnesium, manganese, potassium, rubidium, silicon, sodium, strontium, titanium, and zinc (44, 171, 262, 334, 348, 415).

Quantitatively, sodium, potassium, and calcium are more important, and the variations in the less important metals indicate that the origin and processing of the tobacco govern the detectable quantities of these elements. Analysis of a large number of samples suggested that it might be possible to determine the area of origin of a tobacco sample by estimation of the ratios of some elements present.

A recent study of the transfer of the metallic constituents of tobacco to the mainstream smoke possesses implications which are of interest in connection not only with the inorganic but also with the organic constituents of tobacco smoke (334). It was found that about 150 μg. of the metallic constituents was transferred to the mainstream smoke under conditions simulating human smoking and calculations were made of percentage transfers.

The mainstream smoke comprised mainly potassium (90 per cent), sodium (5 per cent), and traces (total 5 per cent) of aluminum, arsenic, calcium, copper, lead, manganese, magnesium, strontium, titanium, and zinc (44). Nickel has also been reported (409). Transfer took place by vaporization of volatile compounds of the metals or of the metals themselves and not by mechanical means. The unburned portion of a cigaret acted as an effective filter for any solid particles entrained in the smoke. In accord with the transfer data the compounds most likely to appear in the smoke are the volatile chlorides. The important role of volatility in transfer would also apply to organic substances, and those constituents of tobacco which possess even a low vapor pressure below their decomposition temperature might be expected to occur in tobacco smoke, which exists in part as an aerosol, so that a low tendency to vaporize does not necessarily mean that a substance will rapidly condense again.

K. MISCELLANEOUS COMPONENTS (SEE TABLE 15)

Considerable attention has been paid to the analysis of the gaseous phase of smoke from all types of tobacco with particular reference to the more toxic com-

TABLE 15

Miscellaneous components of tobacco and tobacco smoke

Component	Tobacco	Smoke	Component	Tobacco	Smoke
Auxin and indoleacetic acid	+		Hydrogen thiocyanide		+
$C_{10}H_{14}O$		+	Methyl chloride		+
Carbon dioxide		+	2-Methylfuran		+
Carbon monoxide		+	Nucleic acids	+	
Carbon oxysulfide		+	Phosphatides	+	
Chlorophyll	+		Purines	+	
Cyanogen		+	Saponins	+	
Furan		+	Solanochromene	+	
Hydrogen cyanide		+	Thiocyanogen		+
Hydrogen sulfide		+	α-Tocopherol	+	

ponents and carbon dioxide. A good source of references is available (10), and a study of the carbon monoxide and carbon dioxide contents of smoke includes a critical discussion of earlier work (73). Results of reduction methods, such as the iodine pentoxide estimation of carbon monoxide, were considered to be very prone to error owing to the presence of other reducing substances.

The hydrogen cyanide, hydrogen sulfide, and hydrogen thiocyanide contents of the gaseous and particulate phases of smoke from acid and alkaline tobaccos have been investigated by absorption and gravimetric procedures (286, 288). Cigar smoke contained greater quantities of these gases, weight for weight of tobacco smoked, than did cigaret smoke. Cyanogen and thiocyanogen have been reported (342, 343, 344, 345).

Nitrous oxide and methyl nitrite were identified and semiquantitatively estimated by infrared absorption spectroscopy (19).

An analysis, said to be reasonably complete, of the gas phase of smoke from Turkish and American cigarets was carried out by fractionation followed by infrared compensation spectroscopy. The presence of the following was shown: carbon dioxide, carbon monoxide, acetaldehyde, acetone, methyl ethyl ketone, methanol, hydrogen cyanide, biacetyl, carbon oxysulfide, methyl chloride, furan, 2-methylfuran, and hydrocarbons. Confirmatory evidence was obtained by mass spectrometry (225, 238). Vapor-phase chromatography of the condensed vapor phase of cigaret smoke has also indicated the presence of furan and 2-methylfuran in addition to other substances mentioned elsewhere in this review (119).

A review of the biochemical and physiological aspects of the tobacco plant has appeared (328). Only brief mention will be made here of a few topics which have been studied with these aspects in mind.

Interest in the tobacco mosaic virus has stimulated research into the nucleic acids of the healthy and the virus-infected plant, methods having been described for the extraction and separation of nucleoprotein and nucleic acid from *N. tabacum* and *N. rustica* by differential ultracentrifugation and electrophoresis (111, 159). Electrophoresis experiments indicated a principal nucleoprotein identical with "Fraction I" protein (see Section IV,H) and a more mobile nucleic

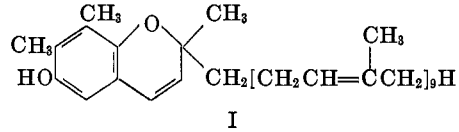
acid fraction (159). Hydrolysis of the free nucleic acids afforded the common aglycones adenine, guanine, cytosine, and uracil. Nucleic acids in leaf homogenates have been determined by investigation of the amounts of purines, pyrimidines, sugars, and phosphorus (170), and it was reported that 30 per cent of the total phosphorus in young leaf is combined in ribonucleic acid and 7 per cent in deoxyribonucleic acid (110). Paper chromatography and electrophoresis were used to determine the free purines of cured tobacco after concentration by precipitation with silver and absorption on an ion-exchange resin. Adenine, guanine, and possibly a little xanthine were found in Burley, Virginia, Xanthi, and Havana tobaccos (173).

The synthesis and control of auxin production in green tobacco have been studied (162, 163, 406). "Fraction I" protein on hydrolysis with alkali or proteolytic enzymes is reported to yield an auxin resembling indoleacetic acid (403). A new, naturally occurring indole hormone found in Maryland Mammoth tobacco was not identical with any of the common ones in its behavior on paper chromatography (368); however, similar but conflicting experiments on Virginia Bright and Maryland Mammoth tobaccos reported the presence of indoleacetic acid (133, 231, 367).

The chlorophyll content of green tobacco has been estimated by ultraviolet absorption measurements on ether extracts (98, 401). The same technique was used to follow the changes in chlorophyll content at various stages of maturity in Burley tobacco, a decrease in total chlorophyll being found on maturity of the leaf, whilst the proportion of chlorophyll a increased (97). Similar investigations and results on *Makhorka* have appeared (236). During growth, chlorophyll a seems to be formed more rapidly than chlorophyll b (194). The chlorophylls of detached immature leaves are more resistant to degradation during induced senescence than those of more mature and senile leaves, and the stability of the chlorophyll appears to be related to the age of the pigment systems (402).

Phosphatides occur in tobacco (206, 279), and saponins have been reported (137). The constitution of the lipide fraction of tobacco has been described (107a).

Solanochromene (I), interesting because of its relationship to solanesol (see



I

Section IV,B) and the tocopherols, was isolated from flue-cured American tobacco by chromatography of the phenolic fraction on silicic acid. The ultraviolet spectrum of solanochromene suggested a relationship to the tocopherols; after hydrogenation, its infrared spectrum was similar to that of γ-tocopherol (272). α-Tocopherol was isolated in the same investigation, tocopherols having already been reported in tobacco seed oil (265).

Changes in the amounts of vitamin B complex on curing cigar tobacco have been studied (60).

The finally processed tobacco leaf may contain a number of additives in the form of flavorings or blendors, humectants, and the residues of insecticidal sprays and fumigants. The subject of tobacco additives is extensive and not easy to review, as the actual materials used by manufacturers are not often disclosed. Nevertheless, the topic has been reviewed (351), a number of short articles have appeared (161, 290, 335), and patent specifications provide examples. Essential oils of plants and not pure substances are often used as additives and this, in itself, can complicate the investigation of tobacco smoke. Also, the customs regulations concerning tobacco additives vary from country to country; for example, in Great Britain none is allowed in cigaret tobacco after removal from bond.

Humectants, generally of the di- or trihydroxy alcohol type, are added to most tobaccos; some of them have been discussed in Section IV,B.

Less desirable in tobacco are insecticidal spray residues (70), which may be transferred to the smoke. The use of lead arsenate, now discontinued in the United States, has been referred to, but organic insecticides also suffer from the disadvantage of volatility. The isolation of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (TDE) has been reported in an investigation of American cigaret smoke, where it occurred in a concentration of 1.6 mg. per 1000 cigarets (186).

Fresh solutions of tobacco smoke condensate exhibit considerable light-sensitive fluorescence, 50 per cent of which is extractable with water (281, 282). These observations were substantiated in experiments in which other substances besides tobacco were burned and the smoke shown to have a similar fluorescence behavior (132). On exposure to artificial and ordinary light it was found that 90 per cent of the fluorescence was irreversibly lost by an apparent first-order process which had two time constants, corresponding to fluorescence contributed by stable and unstable substances (64). The quenching of the fluorescence does not seem to be due to oxidation, since it proceeded unchanged under an atmosphere of nitrogen or in the presence of oxidizing agents. The absolute rate at which the fluorescence decreased was proportional to the intensity of the exciting radiation (283).

The light-sensitive fluorescent content of smoke may be related to free radicals, of which a high proportion has been found in the same source. A suggestion that smoking conditions are favorable to the production of free radicals (96) was substantiated by electron resonance spectrometry, which showed a concentration of about 10^{15} free radicals per gram of smoke condensate at $-60°C$. (118, 166); although warming this tar to room temperature reduced the concentration of free radicals somewhat, a considerable number of relatively stable free radicals persisted. It was found that 20 per cent of the free radicals could be extracted with water and 72 per cent by treatment with alkali and acid (166) in contrast to the fluorescent substances, 50 per cent of which could be extracted with water. It would be interesting to determine whether there is any relationship between the concentration of free radicals and the intensity of the unstable fluorescence. Substances which are highly fluorescent and have been reported in tobacco smoke include coumarin (412), scopoletin (417), and some polycyclic hydrocarbons.

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