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Chemical and Sensory Aspects of Tobacco Flavor

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INTRODUCTION TO SYMPOSIUM

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The advances in knowledge about tobacco composition in the last quarter century have been substantial. The following chart approximates the total number known of tobacco and smoke constituents reported from 1956 to date and is a rough measure of the scientific progress made towards understanding the product of this industry. As the complexity of tobacco and smoke composition has been unraveled, tobacco chemists have gained insights into the chemical and sensory factors which comprise tobacco flavor.

It is appropriate that this years symposium, entitled "Chemical and Sensory Aspects of Tobacco Flavor" will address the current state of knowledge in this area. Our speakers this morning will provide an interesting series of papers on important flavor constituents of tobacco, sensory methodology for measuring the acceptance of tobacco products and aspects of chemical transformations occuring on thermolysis of tobacco which affect the composition (and ultimately the flavor) of tobacco smoke.

FIGURE

Components Reported in
Tobacco & Tobacco Smoke
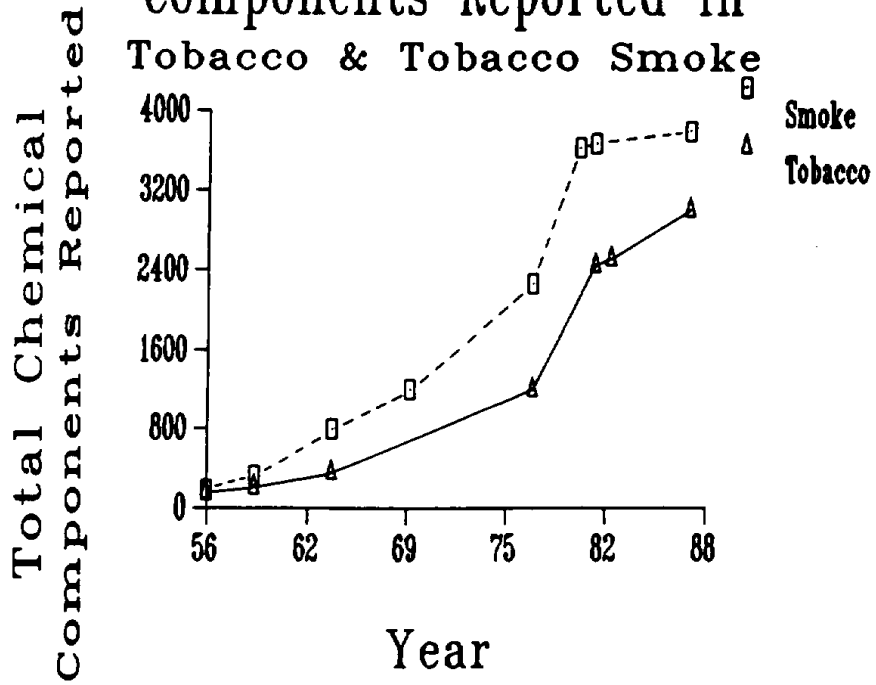
TCRC SYMPOSIUM SUMMARY: CHEMICAL & SENSORY
ASPECTS OF TOBACCO FLAVOR - AN OVERVIEW.

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ABSTRACT

An overview of selected topics related to
tobacco aroma and flavor is presented.

Sugar esters represent a unique example of
thermally labile precursors for generation of
$C_3 - C_8$ carboxylic acid flavorants in tobacco
smoke. The importance of norlabdanoids as the
cedar-amber notes in Oriental and cigar tobacco is
discussed along with the commercial importance of
such materials for the fragrance industry.
Carotenoids, which comprise as much as 2000 ppm in
green tobacco, degrade to a series of flavorants
important to the flavor and aroma of not only
tobacco, but also tea, osmanthus and rose. Flavor
properties of the major carotenoid degradation
products in tobacco are given along with
techniques for generating such compounds through
the application of biotechnology. Changes in the
composition of nitrogeneous components in tobacco
during curing and aging is presented in
relationship to smoke pH and flavorant formation.
Special emphasis is placed on recent findings
relative to the formation of pyrazines and related
materials in model systems under conditions which
simulate those in tobacco leaf.

PREFACE

Our speakers this morning have provided us with considerable insight into the complexities of tobacco - in the areas of product sensory assessment, thermolysis of tobacco types, and aroma chemicals which are important to the flavor of tobacco (as well as the aroma and flavor of many other natural products).

My portion of this symposium will focus on selected topics related to tobacco flavor and aroma, some of which expand on subjects mentioned by several of our earlier speakers, and some of which attempt to broaden the understanding of how specific chemical changes occuring in tobacco affect flavor.

SUGAR ESTERS AND NORLABDANOIDS

Of all tobacco types, the aromatic smoking character of Oriental (or "Turkish") is the most pronounced - and the best understood from a flavor standpoint.

The smoke of Oriental tobacco is primarily identified with two well defined aromatic notes (Table 1) which we will describe as -

> o A cheesy-sweaty-buttery odor reminiscent of isovaleric acid and known to be due primarily to (+)-3-methylvaleric acid, isovaleric acid and 2-methylbutyric acid.

TABLE 1.

ORIENTAL TOBACCO SMOKE FLAVOR & AROMA

CHEESY, SWEATY TYPE NOTES

- STE & GTE FLAVOR PRECURSORS

    o 3-METHYLVALERIC ACID

    o ISOVALERIC ACID

    o 2-METHYLBUTYRIC ACID

CEDAR, AMBER TYPE NOTES

- LABDANOID DERIVED

    o NORAMBREINOLIDE

    o DEHYDRONORAMBREINOLIDE

    o SCLARAL

    o 𝜸 - BICYCLOHOMOFARNESAL

    o AMBROX

o A cedary-amber aromatic character due
to compounds of labdanoid origin.

For many years, the origin and identity of
the specific flavor chemicals responsible for the
aroma of Oriental smoke was a mystery, because the
leaf itself is only slightly aromatic while its
smoke is powerful in these characteristics. The
ratio of the cheesy:cedar aroma may vary
considerably by source - the smoke of Greek and
Macedonian tobaccos possessing more cedary
character while smoke of Smyrna tobacco possesses
more of the cheesy type notes.

The relative increase of low molecular weight
acids in Oriental tobacco smoke as compared to
levels of the free acids in tobacco has been
quantified by Heckman et. al. (Table 2) (20),
presumably for a typical Oriental blend used
commercially.

The isolation, structure and synthesis of 6-0-
acetyl-2,3,4-tri-0-[(+)-3-methylvaleryl]-beta-D-
glucopyranose (a glucose tetraester) from Oriental
tobacco was published in 1970 (56); but 11 years
passed before Spears et. al., of Lorillard and
Heckman et. al., of RJR clearly stated in 1981
that this sugar tetraester was (one of) the
thermally labile precursor(s) of the lower
carboxylic acid aroma components responsible for
Oriental flavor (20,63). However, patent
literature including the potential use of sugar
esters as non-volatile precursors for generation
of such tobacco aroma had appeared as early as
1956 by Jones (25).

TABLE 2.

MAJOR AROMATIC ACIDS IN ORIENTAL SMOKE (20)

Acid	Tobacco (ug/g)	Smoke (ug/cig)	% Change
Propionic	17.0	66.8	+293
Butyric	2.5	9.7	+288
Valeric	4.3	3.6	-16
2-Methylbutyric	2.1	12.4	+490
Isovaleric	5.1	19.7	+286
3-Methylvaleric	12.0	62.0	+417
Hexanoic	7.5	3.9	-48
Octanoic	1.7	6.2	+265
Phenylacetic	48.9	19.9	-59

In 1981, Severson et. al. (59-61) found
that the cuticular waxes of a tobacco budworm
resistant tobacco contained a series of sucrose
tetraesters (STE) which are the probable
precursors of 6-O-acetyltriacylglucopyranosides
[Glucose Tetraesters (GTE)] isolated from the
hexane soluble fraction of Oriental tobacco
(56,51) (see Figure 1).

Einolf and Chan have quantified the
accumulation of STE for Coker 319 (Virginia),
Kentucky 14 (burley) and Smyrna (Oriental)
tobaccos utilizing flue-curing, air-curing and
sun-curing respectively to mimic the field curing
practices for each tobacco type (10). The graphic
representation (Figure 2) shows that accumulation
of STE for Oriental and burley is greater than for
Virginia tobacco, with the Oriental and burley
maximums occuring at full maturity. These workers
also demonstrated that the tetraesters of Oriental
STE tended to have higher amounts of the more
aromatic C_5 and C_6 carboxylic acids (see also
reference No. 59 for details on the isolation and
characterization of sucrose esters).

From a flavor standpoint, it is interesting
that GTE and STE readily release free carboxylic
acids on thermolysis while totally esterified
sucrose and glucose esters (such as glucose
pentaisovalerate and the sucrose octaesters) do
not (easily) release their acid moieties (25,36),
suggesting that the tetraesters "unzip" on
thermolysis via a neighboring group participation
similar to that observed in cyclohexane systems
(37).

FIGURE 1.

SUGAR TETRAESTERS PRESENT IN TOBACCO
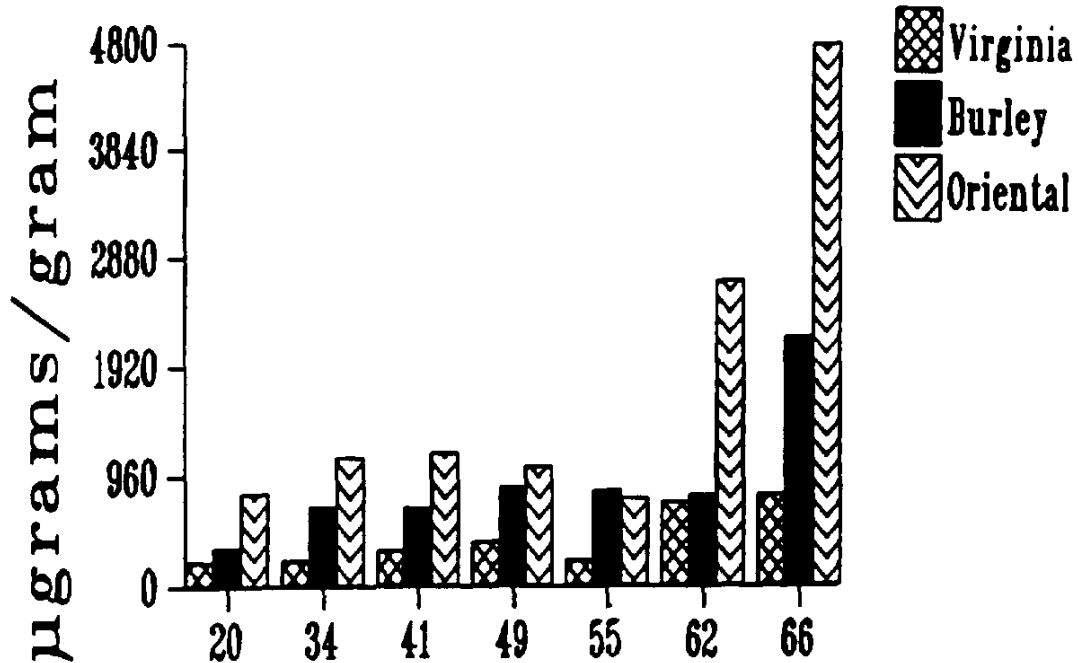[ACID SMOKE AROMA PRECURSORS]
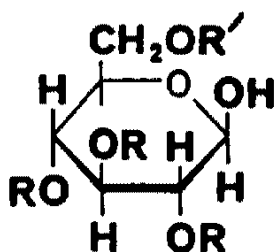
GLUCOSE TETRAESTERS
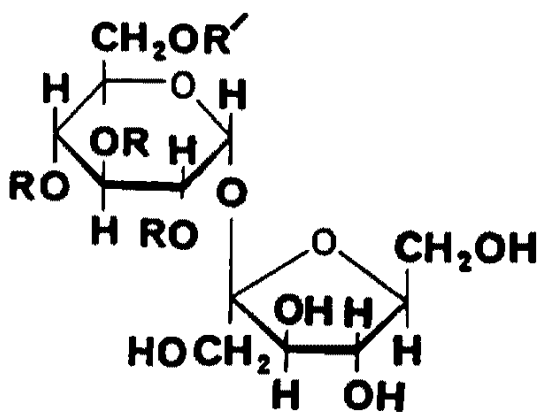
SUCROSE TETRAESTERS

$R = C_3-C_8$ CARBOXYLATE
$R' = $ ACETATE

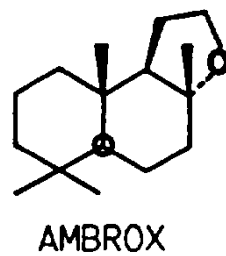FIGURE 2.
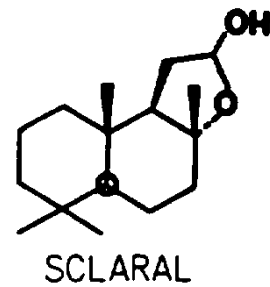
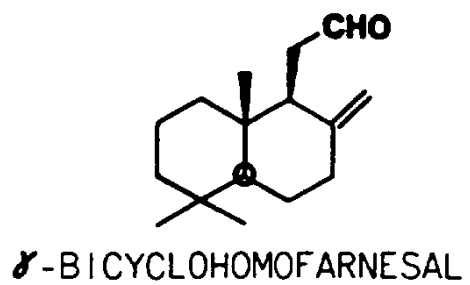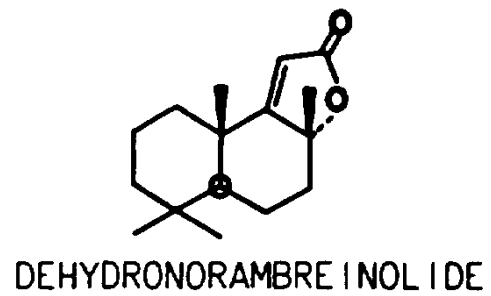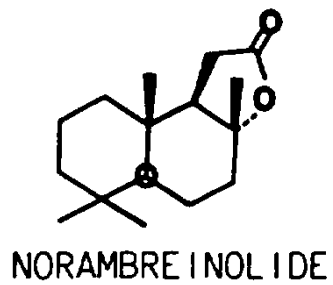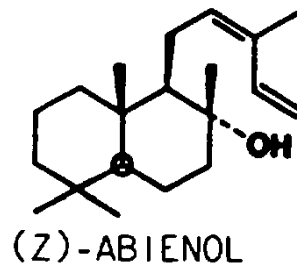# Total Sucrose Tetraester
Levels in Tobacco Types

Days From Transplant
Einolf & Chan (10)

Since the aromatic character of Oriental tobacco smoke due to thermolyis of GTE and STE is of great importance to the flavor of American blend type cigarettes, the work of Gwynn et. al., demonstrating that this trait is genetically determined by a single gene is a significant finding (16). Genetic selection (as monitored by analysis of STE and GTE from cuticular waxes) may now allow the rapid development of improved tobaccos exhibiting Oriental characteristics for production in geographic regions where trade restraints prohibit or restrict importation of Oriental tobaccos. While genetic selection work has been in progress for many years based on subjective smoking qualities, we now have the scientific tools to speed improvements in the development of Oriental flavor characteristics in tobacco leaf.

The cedar-amber flavor components of Oriental tobaccos are due to a series of labdanoid derived compounds. The first C_{20} labdanoids reported in tobacco were the alpha & beta-levantenolides by Giles and Schumacher in the early 1960's (15).

Several C_{20} labdanoid compounds formed in tobacco are potential precursors of the five C_{16} norlabdanoids shown which are reported to exhibit the cedary odor properties of Oriental (39,58). (Z)-Abienol is a major labdanoid in green Oriental leaf (and a regulator of plant growth) which undergoes drastic reduction in concentration when the leaf is cured; it is the likely precursor of many oxygenated C_{20} labdanoids and the cedar-amber norlabdanoids (67,50a) (Figure 3).

FIGURE 3.

(Z)-ABIENOL

NORAMBREINOLIDE

DEHYDRONORAMBREINOLIDE

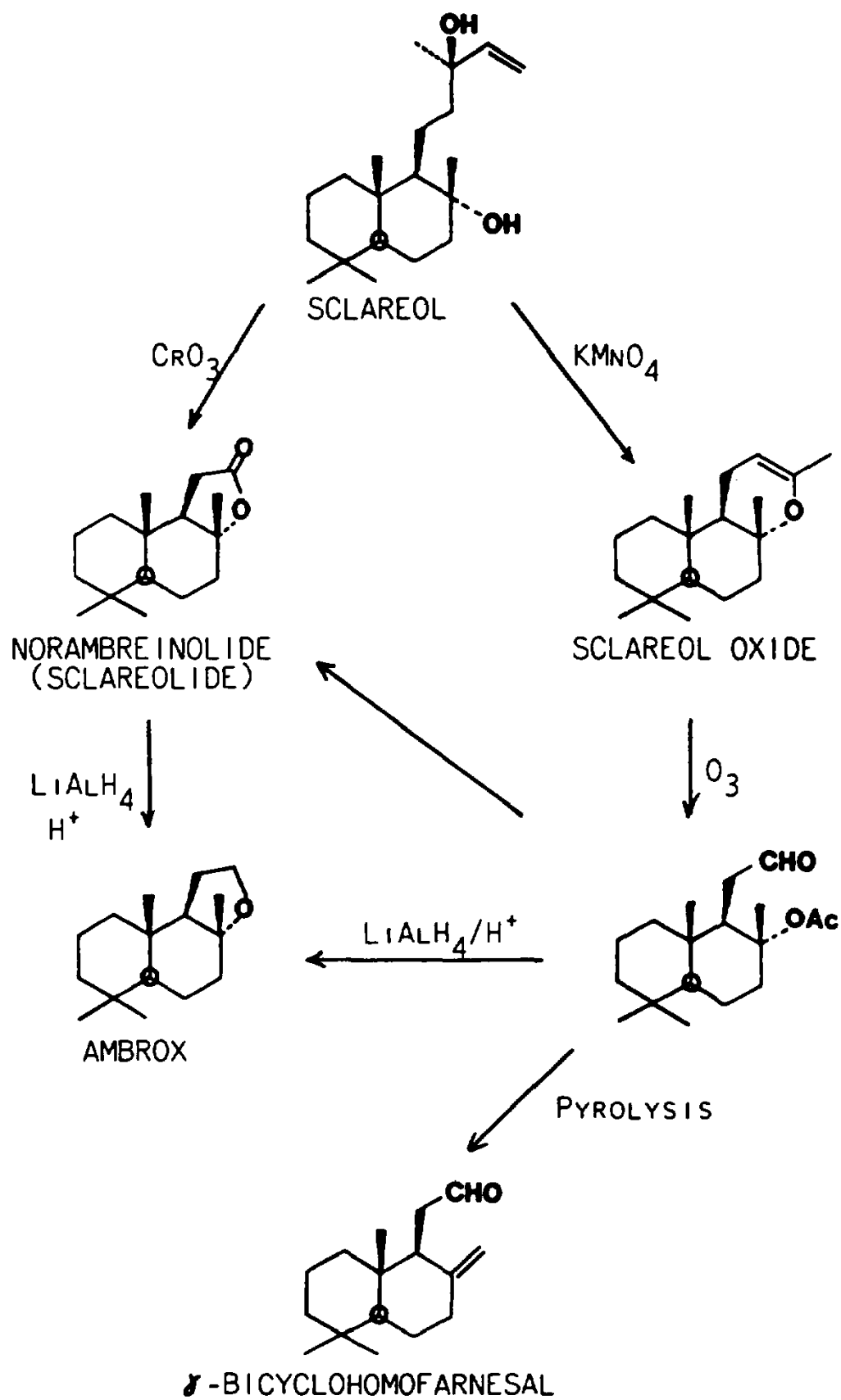ɣ-BICYCLOHOMOFARNESAL

SCLARAL

AMBROX

In 1959, norambreinolide (sclareolide), an important constituent of Oriental tobacco and cigar tobacco was patented (57) as possessing a cedar character on smoking although isolation from tobacco (cigar leaf) was not reported until the early 1970's by Kaneko (28).

Possible mechanistic pathways for oxidation of C_{20} labdanoids to the C_{16} norlabdanoids in tobacco have been recently reviewed by Wahlberg and Enzell (67), who also reviewed the presence in tobacco of many other labdanoid derivatives. A possible alternative route to norlabdanoids via a stereospecific enzymatic cyclization of the tobacco isolate farnesyl acetone to the tobacco isolate sclareol oxide [a known intermediate in the commercial production of norambreinolide from sclareol via permanganate oxidation (54)] can not yet be ruled out.

As discussed earlier in this symposium by Mookherjee, the tobacco constituents sclareol (12,50) and norambreinolide (28) are commercially important to the fragrance industry for the production of the Ambergris odor substitute, Ambrox (4). Sclareol is a major constituent of Salvia sclarea (Clary Sage) and this crop is now grown in North Carolina yielding both Clary Sage oil, which is used extensively in perfumery, and sclareol which is oxidized to norambreinolide as an intermediate for the production of Ambrox (38,43) (Figure 4). The demand for Ambrox in perfumery now exceeds the world's supply, with nearly every fragrance manufacturer being on allocation. In recent years, the chronic shortage

FIGURE 4. (46)

SCLAREOL

CrO₃

KMnO₄

NORAMBREINOLIDE
(SCLAREOLIDE)

SCLAREOL OXIDE

LiAlH₄
H⁺

O₃

LiAlH₄/H⁺

AMBROX

CHO

OAc

PYROLYSIS

CHO

γ-BICYCLOHOMOFARNESAL

of sclareol (and norambreinolide) production
needed for production of this important fragrance
material has fueled extensive research both in
Japan and Europe for alternative routes to the
production of Ambrox and its intermediate,
norambreinolide (18,30,44,24,72,6,29,31).

Until today, Ambrox had not been reported as
a constituent of either tobacco or tobacco smoke.
Roberts has now reported (at this symposium) that
Ambrox was isolated by J. N. Schumacher at RJR
from Oriental tobacco in 1978; it was also
isolated in 1979 from tobacco smoke by A.
McMurtrie and E. D. Alford of B & W (76).

The chemistry of ambergris odorants has been
reviewed by Ohloff and further information can be
obtained in these references (47,48).

Interestingly, norambreinolide is repeatedly
(but erroneously) reported in the literature as
being odorless (65); in fact, a strong cedar-amber
character is apparent when this material is
aerosoled (36).

Suffice it to say that the chemistry of the
norlabdanoids is still, after 40 years, an area of
intense research.

Eventhough Oriental tobacco contains many of
the same volatile constituents as Virginia and
burley (air-cured) tobaccos, most of these
constituents are only background flavor notes.
The predominant flavor characteristics of Oriental
are derived from 1.) sugar tetraesters due to
their thermally labile character which releases
lower molecular weight $C_3 - C_8$ carboxylic acids
into the smoke and 2.) labdanoid derived

flavorants (e.g., norambreinolide, sclaral) possessing cedar-amber notes.

From the genetic view toward imparting the cedar-amber characteristic of Oriental tobacco, it has been found by Tomita et. al. that a single gene positioned on chromosone A controls production of the C_{20} norlabdanoid precursor, (Z)-Abienol (66). Methods for measuring this compound and the related cedary odor material, sclaral, have been reported (23).

The importance of these findings may lead to the genetic design of commercial tobacco varieties other than classic Orientals which have this desired character.

In fact, Earl Wernsman of North Carolina State University has recently succeeded in transfering the genetic characteristics which impart Oriental taste (genetic factors for producing Z-abienol and sugar esters) into both flue-cured and burley tobaccos (77).

In the case of normal Virginia and burley tobaccos, the class of labdanoid derived chemicals are virtually absent; but in cigar tobaccos, the norlabdanoids are extremely important for the cedary note characteristic of fine quality leaf (e.g., Havana wrapper).

CAROTENOIDS

It is known that carotenoid degradation products such as beta-ionone, damascenone, beta-damascone, 2,2,6-trimethylcyclohex-2-ene-1,4-dione and the megastigmatrienones are important to the

aroma of flue-cured and burley tobacco, as well as to many other natural products (27). It is less clear as to which of the originating tobacco carotenoids is most important to the generation of flavor. In green tobacco four <u>major</u> carotenoids are present : lutein, beta-carotene, violaxanthin and neoxanthin (28) (Figure 5). In addition, seven minor and a host of trace other carotenoids are present. In green leaf, total carotenoids may comprise 2000 ppm (0.2% by weight of dry leaf) which drops (Figure 6) to about 100 - 400 ppm after maturation, senescence, curing and aging (an 80-95% decrease!). It has been observed that the degree of degradation of the major tobacco carotenoid, lutein, is greater for flue-cured tobacco than burley on curing (8). Biomimitic studies by Burton and co-workers (7) indicate that carotenoid pigments are only partially degraded into volatile (flavor) components (7), (e.g., 15% conversion to volatiles via oxidative photolysis in 48 hours).

Our knowledge of carotenoid degradation pathways are limited to studies of enzymatic degradation in tomatoes (64), the degradation of beta-Carotene by a tea enzyme preparation in the presence of tea flavanols (55), the conversion of violaxanthin into 3-hydroxy-beta-ionone and beta-carotene into beta-ionone and beta-ionone epoxide by soybean lipoxygenase (13), and biomimitic studies (i.e., photooxidation of beta-carotene and ionone related materials (12,67). An inverse relationship between tobacco flavor and carotenoid content has been reported by
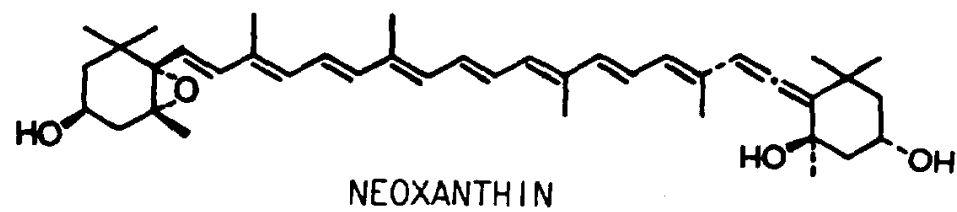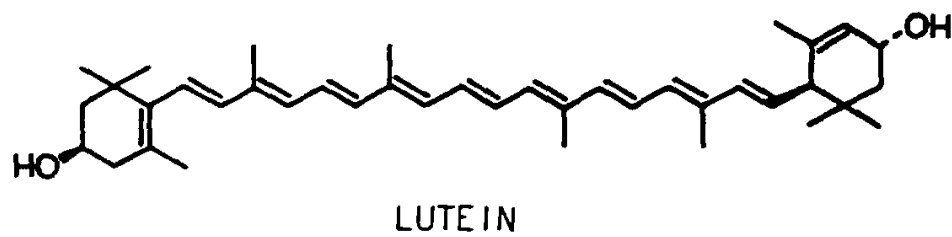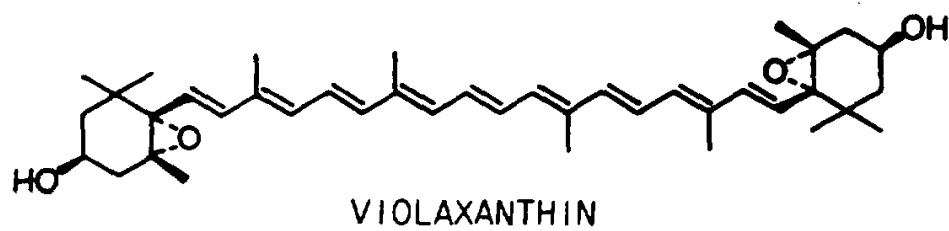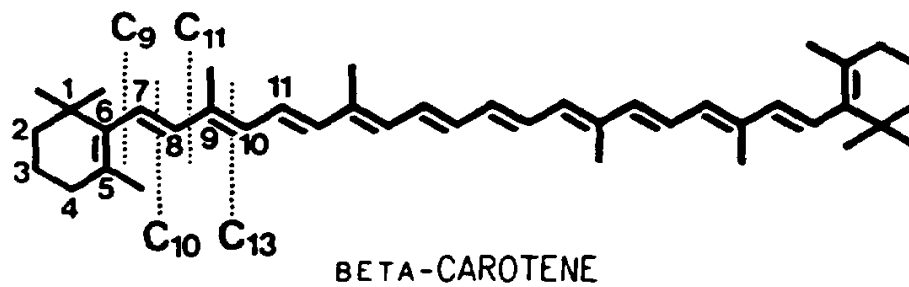
FIGURE 5.

BETA-CAROTENE

VIOLAXANTHIN

LUTEIN
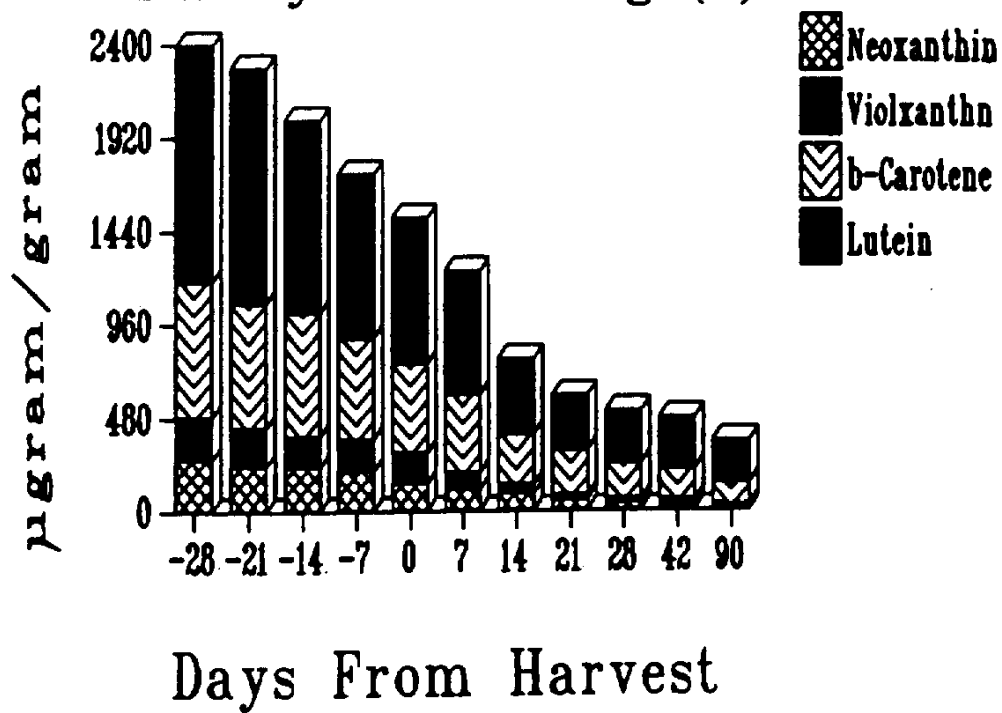

NEOXANTHIN

FIGURE 6.

Carotenoid Degradation
Burley Air-curing (8)

Days From Harvest

Roberts (53).

The complexity of carotenoid derived products is exhibited in Figure 7. showing some of the degradation products common to tea, osmanthus and tobacco (27).
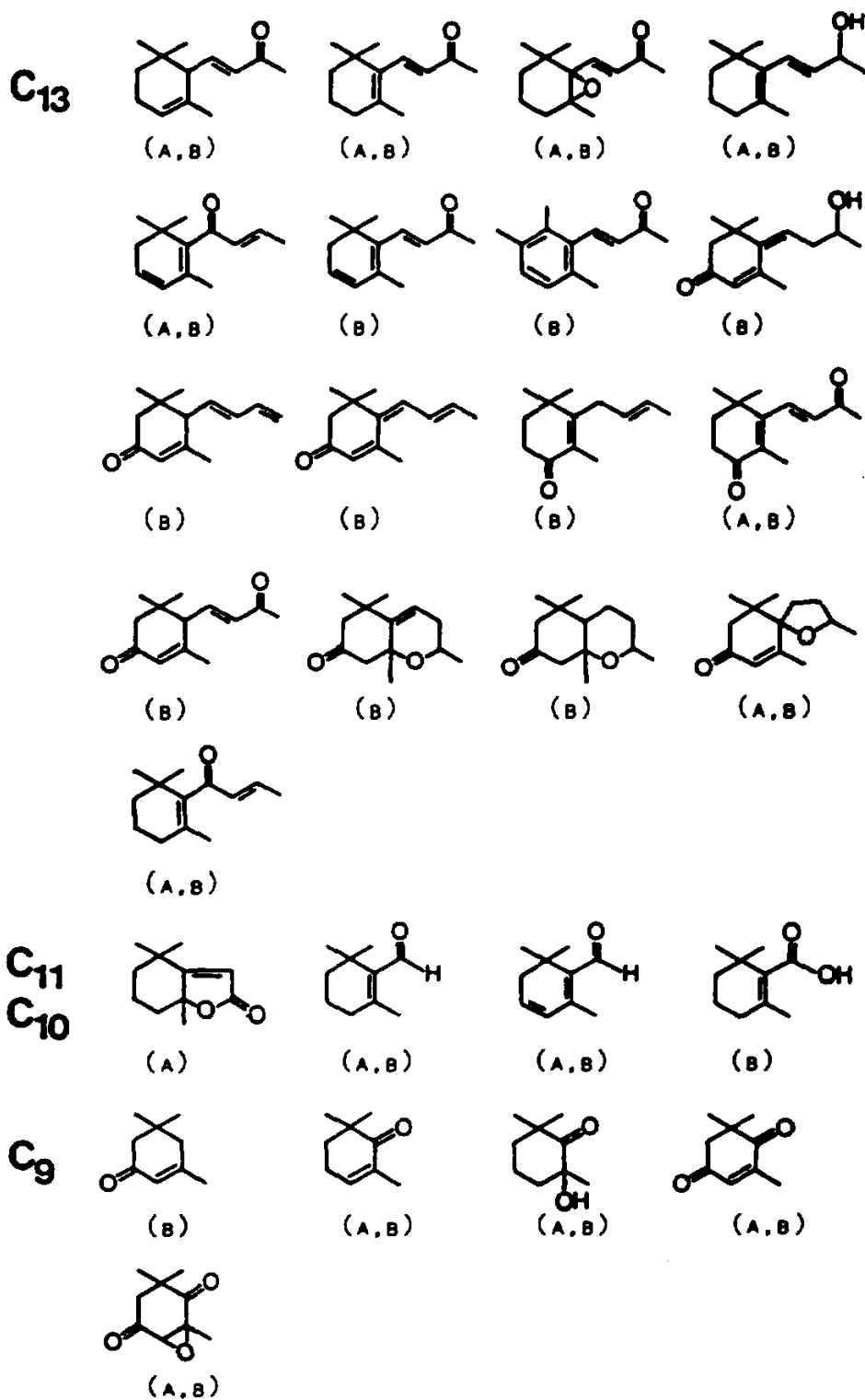
Kaiser et. al., at Givaudan, have demonstrated that enzymatic processes based on (fermenting) the fungi of Lasiodiplodia theobromae with the ionones (Figure 8) or damascone can generate materials with tobacco like aroma and containing many known tobacco isolates (22,26,32,33). Similar work has been patented by Barnes and Bevan (3). This is of particular interest in that it represents a practical application of biotechnology for the formation of complex aromas with distinct tobacco notes.

Undoubtedly the role of curing methodology and tobacco specific enzymatic and microbial processes are important in formation of carotenoid derived flavor compounds in tobacco. The possibility of enzymatic treatment of tobacco prior to curing (just as beef is sometimes treated in the meat industry to improve flavor) is an area of potential interest.

Beatson et. al. (5) have been able to genetically manipulate increases in carotenoid pigment in flue-cured tobacco, but correlation of improved smoking quality could not be attributed unequivocally to increased carotenoid levels since other chemical variables also changed (e.g., nicotine levels tended to increase along with carotenoids). Weeks (70) has demonstrated that recessive cultivars with lower nicotine (and

FIGURE 7.

CAROTENE DEGRADATION PRODUCTS
COMMON TO TOBACCO, TEA & OSMANTHUS (26,27)

- -

C₁₃

(A,B) (A,B) (A,B) (A,B)

(A,B) (B) (B) (B)

(B) (B) (B) (A,B)

(B) (B) (B) (A,B)

(A,B)

C₁₁
C₁₀

(A) (A,B) (A,B) (B)

C₉

(B) (A,B) (A,B) (A,B)

(A,B)

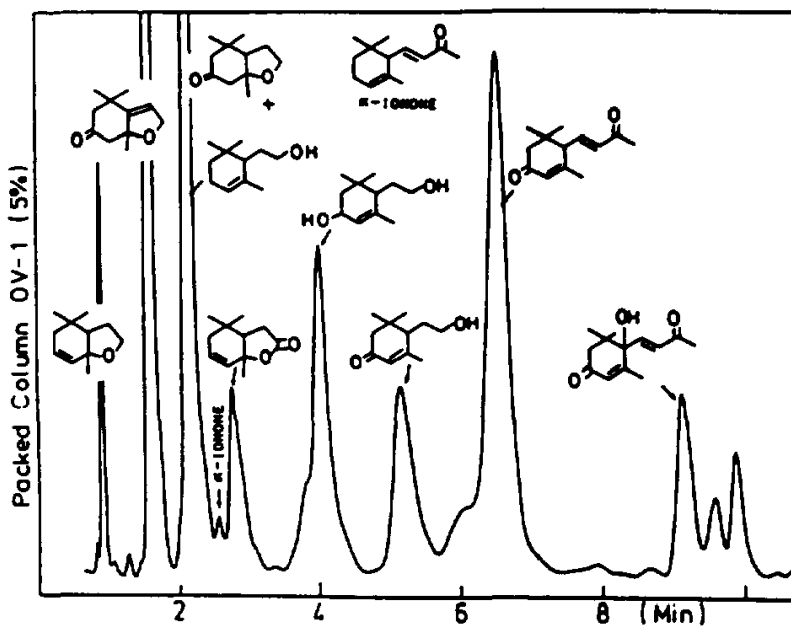- -

ALL PRODUCTS ARE FOUND IN TOBACCO
(A) ALSO FOUND IN BLACK TEA
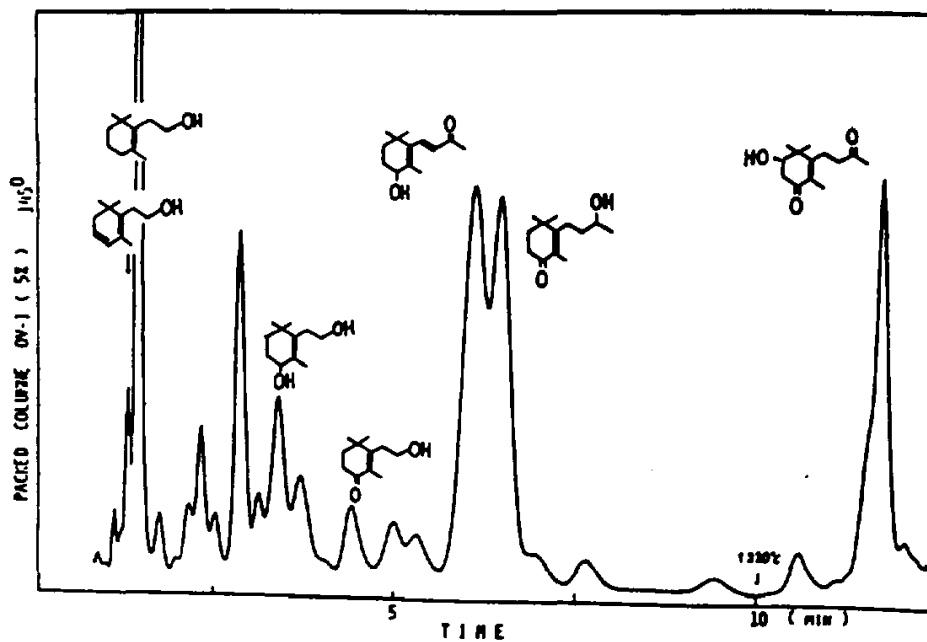(B) ALSO FOUND IN OSMANTHUS

FIGURE 8.

IONONE FERMENTATIONS WITH LASIODIPLODIA THEOBROMAE (26)

ALPHA-IONONE FERMENTATION PRODUCTS

BETA-IONONE FERMENTATION PRODUCTS (EARLY STAGE)

presumably lower carotenoid pigment levels) also produced less volatile carotenoid degradation products.

Carotenoid derived products present in tobacco (Table 3) such as beta-ionone, beta-damascone, damascenone, etc. find extensive utility in fragrances and food flavors (e.g., beta-ionone and damascenone possess delightful fruity notes). One tobacco isolate is known to possess a straw (hay-like) tobacco aroma on dilution (9,45); this material, 2,6,6-trimethyl-cyclohex-2-en-1,4-dione, has been claimed in recent years as one of the most important new tobacco notes in perfumery (9). Other carotenoid derivatives, such as the megastigmatrienones have been reported as being very important to tobacco aroma/taste (45,70).

In summary, carotenoid degradation products are important to tobacco aroma and flavor; while no one chemical so derived appears to be responsible for tobacco flavor, in total, they contribute significantly to both aroma and taste.

POLYPRENOIDS

Before leaving the subject of terpenoid related flavor materials one rarely mentioned class of materials deserves comment. The tobacco isolate neophytadiene [an acyclic non-carotenoid C_{20} terpenoid] is a virtually odorless material which exhibits flavor enhancing properties in tobacco smoke (39b). Similar effects have recently been observed by Shibata et. al. for the

TABLE 3.

MAJOR TOBACCO CAROTENOID DEGRADATION PRODUCTS
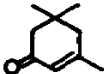### (PERCENT OF TOBACCO VOLATILE OILS [73])
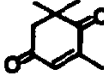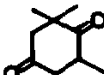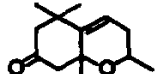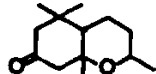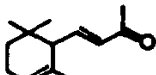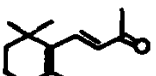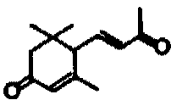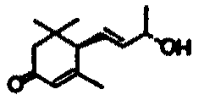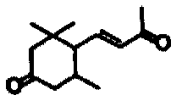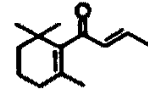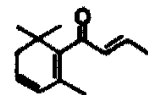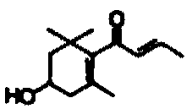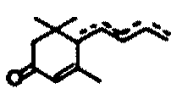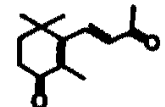
STRUCTURE	VIRGINIA %	BURLEY %	ORIENTAL %	TOBACCO FLAVOR(39,73)
(A)	0.62	0.65	0.49	EARTHY, HARSH, BURLEY NOTE
(A,B,C)	0.26	0.38	0.54	TOBACCO, HAY, STRAW, SWEET FLUE-CURED
(C)	0.18	0.10	0.55	WEAK, SOUR
(A,B)	0.43	0.24	0.23	SWEET,GREEN-HAY, SAFFRON, ENHANCED TOBACCO AROMA
(B,D,E,H,I)	0.83	0.60	0.93	WEAK, SLIGHT COOLING
(A)	0.77	0.57	0.49	ORIENTAL, WOODY, IONONE
(A)	0.26	0.61	0.94	SWEET, FLORAL BURLEY LIKE, CAMPHORACEOUS
(A,B,J,K,L)	+	+	+	SWEET, FLORAL, WOODY, SMOOTH-ING
(A,B,J,K,M,N)	+	+	+	SWEET, FRUITY, FLORAL, WOODY, SMOOTHING

TABLE 3. (CONTINUED)

MAJOR TOBACCO CAROTENOID DEGRADATION PRODUCTS
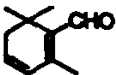(PERCENT OF TOBACCO VOLATILE OILS [73])

STRUCTURE	VIRGINIA %	BURLEY %	ORIENTAL %	TOBACCO FLAVOR(39,73)
(A)	+	NR	NR	SWEET, FLORAL
	+	0.09	+	SWEET, FLUE-CURED
	0.28	0.70	0.30	----
(A,B,S)	0.93	0.95	0.50	FLORAL
(A,B,F,G,M,O,P,R,S)	2.92	1.70	3.26	FRUITY, ENHANCED BURLEY NOTES
	0.13	0.25	+	ROSE-FLORAL, SWEET, GREEN, BURLEY NOTE
(A,G)	7.03	5.64	5.32	SPICY BURLEY NOTES, ADDS STRENGTH
(A,B,Q)	NR	+	NR	SWEET, RICH FLUE-CURED

ALSO FOUND IN:
(A) OSMANTHUS   (H) CASSIE   (O) COFFEE
(B) BLACK TEA   (I) AMBERGRIS   (P) BEER
(C) SAFFRON   (J) BLACKBERRIES   (Q) RED FOX
(D) TOMATO   (K) PASSION FRUIT   (R) ROMAN
(E) CASSIA   (L) WHISKEY   CHAMOMILLE
(F) APPLES   (M) RASPBERRIES   (S) BULGARIAN
(G) GRAPES   (N) CARROTS   ROSE

higher polyprenols, decaprenol and isodecaprenol,
(62) which are homologs of the major polyprenoid,
solanesol. While solanesol is present at levels of
up to 4% by weight in tobacco dry leaf lamina,
Davis has suggested that solanesol may only
influence smoke aroma indirectly (75). It has been
proposed that such higher molecular weight
materials, which possess no flavor character
themselves, enhance tobacco smoke flavor by
entrapment of lower molecular weight flavor
volatiles into the aerosol droplets of smoke, in
effect acting as a flavor transfer carrier in the
mainstream aerosol (19).

NITROGENOUS CONSTITUENTS

Flavor quality for both flue-cured and burley
tobaccos is dependent on a variety of complex
interacting factors related to genetics,
agricultural practices, soil types, nutrients,
weather conditions, plant disease, and stalk
position as well as harvesting and curing
practices.

In general, elevated levels of proteins and
amino acids appear to contribute negatively to
smoke flavor. This is particularly true of burley
tobaccos grown in cool moist climates with
insufficient sunshine, which are over fertilized,
harvested and air-cured prematurely. In such
cases the normal catabolic changes during
senescence and curing which result in the
enzymatic hydrolysis of Fraction 1 protein to
amino acids (which then can undergo further

FIGURE 9.

Burley Protein Decrease
Air-curing (40)
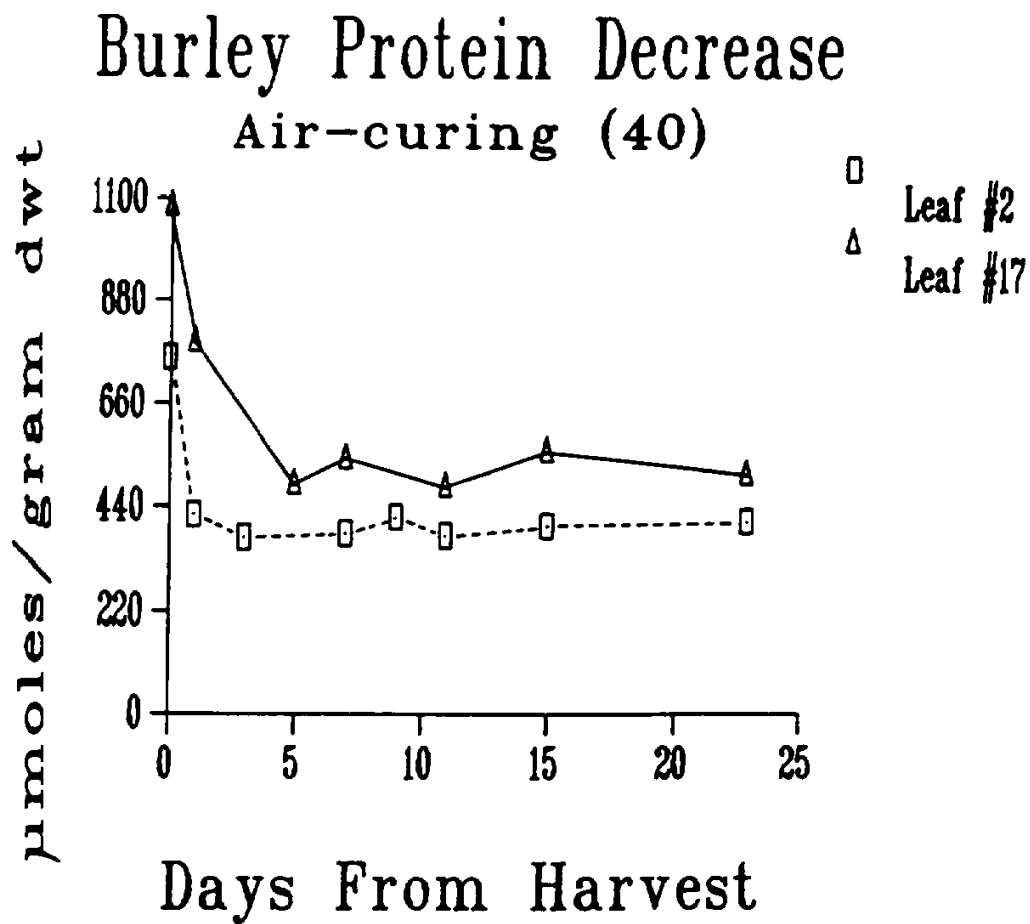
catabolic changes) are at least partially
arrested. Such immature tobaccos possess a
proteinaceous aroma on smoking which is
unpleasant. Indirect evidence exists indicating
that a balance between carotenoids and
proteinaceous constituents is required for a
balanced smoke (i.e., carotenoids mask
proteinaceous off notes) (69). Even secondary
fermentation of such poor tobaccos often cannot
provide sufficient improvement to make them
useable. By comparison to normal burley, in which
approximately 50% of total protein undergoes
hydrolysis to amino acids, such poor tobaccos may
exhibit less than 15% protein hydrolysis during
air-curing (21).

Normal air-curing of fully mature burley
shows a rapid drop in protein (Figure 9) with a
concomitant initial increase in free amino acids
for the first few days in cure (Figure 10).
Thereafter, there is a decrease and finally a
leveling off in total free amino acid
concentration. The decrease in free amino acid
content after the 5th day of air-curing is due to
both metabolic deamination and decarboxylation as
well as nitrogen translocation. Even though a
slight decrease from a maximum in total free amino
acids occurs during the cure, some individual
amino acids show a net decrease while others have
a net increase (7,40).

It should be noted that the ammonia level of
air-cured tobacco rapidly increases during this
period (Figure 11) (7).

The increase in ammonia levels of air-cured

FIGURE 10.

Total Free Amino Acids
Burley Air-curing (7)

Days From Harvest
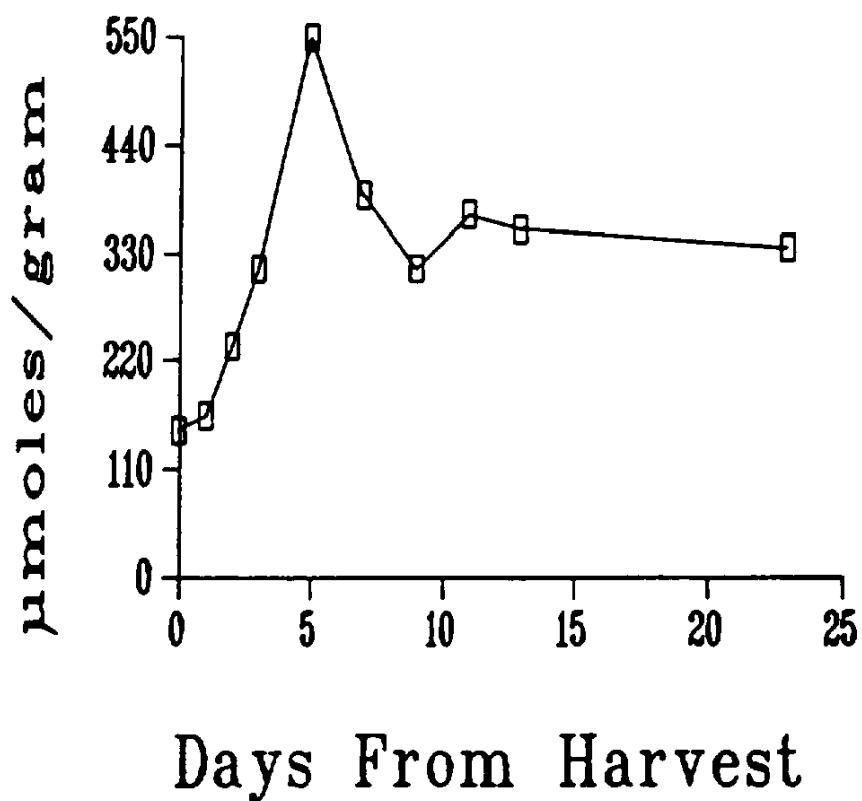
FIGURE 11.

Ammonia Increase
Burley Air-curing (7)

Days From Harvest
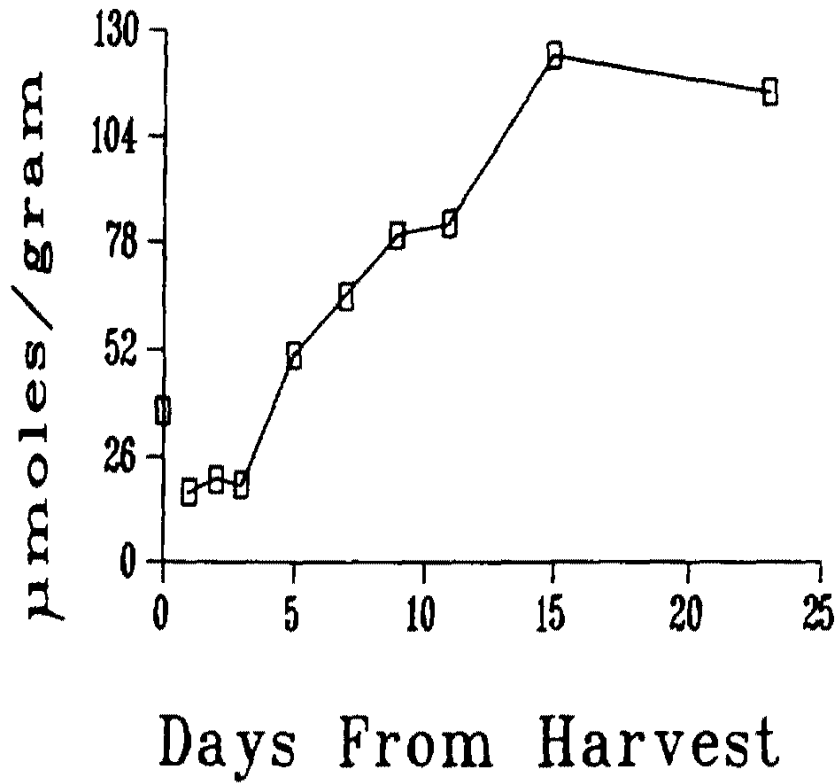
tobacco would presumably have two major flavor
effects. First, it has an effect on the ratio of
nicotine/nicotine salts delivered on smoking
relative to high sugar and/or more acidic tobacco
types wherein the nicotine is complexed (more) as
a salt in the smoke. Since pH of smoke in air-
cured tobacco is considerably more alkaline than
flue-cured or Oriental, the ratio of nicotine base
to nicotine salts increases (11). This causes the
sensory perception of increased nicotine strength
(and harshness). It should also be mentioned that
the pH of air-cured tobacco smoke increases with
succeeding puffs from such cigarettes (Figure 12)
(11). Accordingly, the increased alkalinity of
straight air-cured cigarettes renders them
virtually unacceptable to nearly all smokers as
the high smoke pH imparts an alkaloid harshness.
"...Nicotine alone does not determine smoking
flavor..." (35). As has been shown by R. A.
Fenner (this symposium), air-cured burley
generates significantly more ammonia on
thermolysis than Virginia, a fact that must
influence increased smoke pH.

It is probable that smoke pH also plays a
synergistic role in the perception of flavors in
tobacco smoke - in analogy to other oropharyngeal
stimuli (e.g., foodstuffs).

Second, but more important from the stand-
point of flavor formation, is the role that
ammonia plays in the formation of aroma and taste
compounds.

Before addressing this subject, however, let
us discuss briefly the role of certain amino acids

FIGURE 12.

Cigarette Smoke pH
(Cumulative Puffs)

Cumulative Smoke pH
Vatskerok Apparatus

Cig. Butt Length (mm)
Adapted From Elson (11)
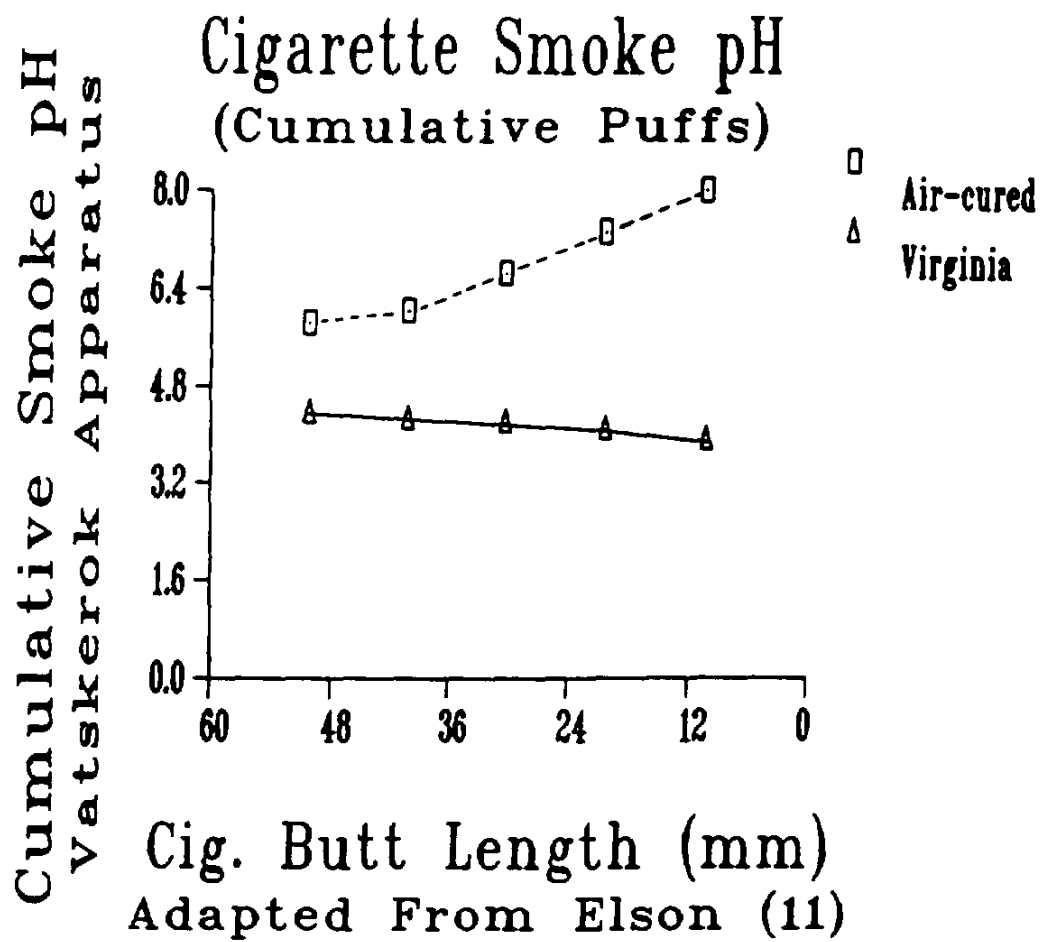
in flue-cured tobacco.

Protein hydrolysis with formation of free
amino acids occurs also at the yellowing and early
stages of drying during flue-curing, although
flue-cured tobacco contains only about 20-25% of
the total amount of protein/amino acids as burley.

The major amino acid in flue-cured tobacco,
proline, seems to be an anomoly in that as much as
a 25 fold increase has been observed during the
curing schedule for both flue-cured and air-cured
(Maryland) tobaccos (71); this appears to be
greater than can be accounted for by proteolysis
of Fraction 1 protein - a hypothesis (17) being
that proline is partially formed by a metabolic
conversion of the pyrrolic portion of chlorophyll
(which is rapidly decreasing during this stage).

In flue-cured tobaccos, proline reacts with
the reducing sugar, glucose, to form 1-deoxy-1-(L-
prolino)-D-fructose, an Amadori compound which
comprises as much as 1-1.5% of dry aged tobacco
weight (42). This compound is significant in that
it has been shown (41) to undergo low temperature
degradation primarily to the probable precursor
(2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
[DDMP]) of the important volatile flavorant,
maltol, found in flue-cured (Figure 13). It has
also been shown that the smoking properties of
DDMP are identical to that of maltol (35).

The role of amino compounds both alone and in
conjunction with sugars to form flavor precursors
(Amadori compounds, fructosazines and
deoxyfructosazines) has been reviewed in previous
TCRC symposia (70,20,35,14) and elsewhere (34).

FIGURE 13.

1-DEOXY-1-(L-PROLINO)-D-FRUCTOSE

DDMP

MALTOL
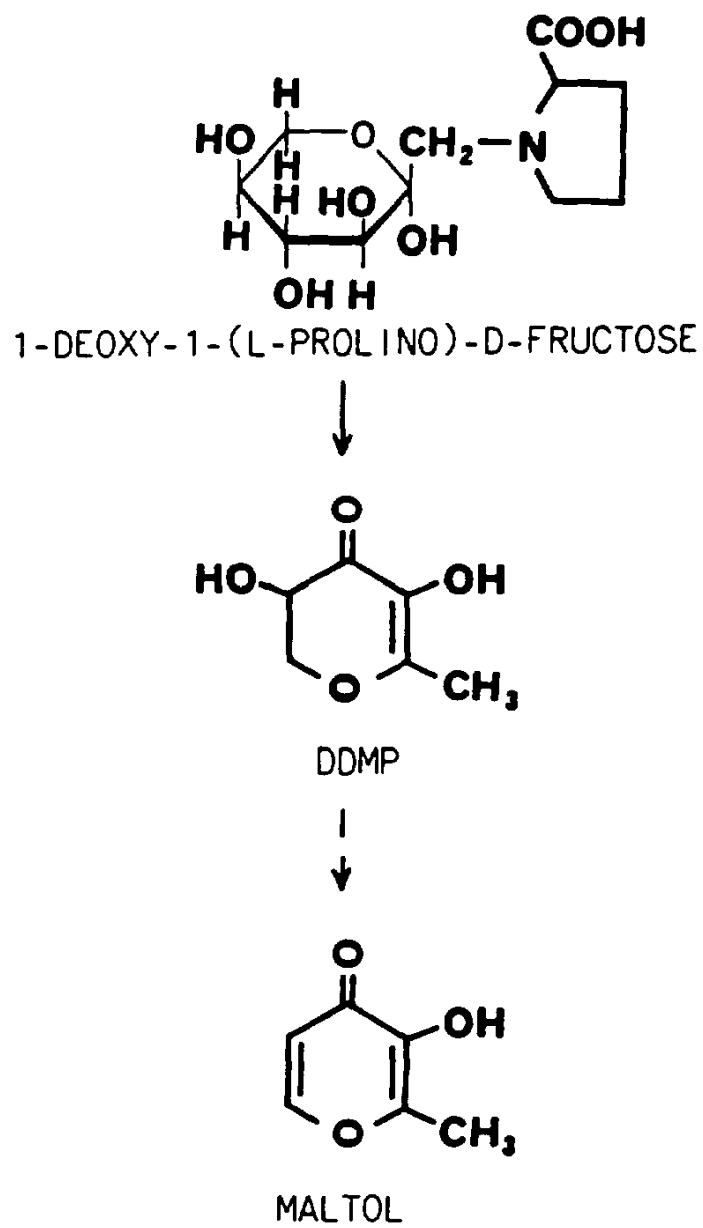
Suffice it to say that in this area of natural products chemistry, as well as the chemistry of cembranoid, carotenoid and labdanoid derived aroma materials, that research on tobacco and tobacco smoke constituents has contributed significantly to the present scientific knowledge of naturally occuring aroma constituents.

Of particular interest to tobacco scientists are the improvements in tobacco smoking quality that occur with aging. In the case of flue-cured tobacco, the reduction in undesirable "green" notes with aging can be associated with decreasing amounts of such flavorants as cis-3-hexenol, hexanal and 2-hexenal (68). Improvements in the sweetness of flue-cured can also be associated with increases in Amadori compounds (42) and certain carotenoid derived compounds (68). Development of the "nutty" character of flue-cured on aging may be associated with increases in formation of pyrazines (12). I will focus here on the latter subject relative to ammonia in tobacco.

Recent work has shed considerable light on the formation of pyrazines using model systems. The sugar derived products, acetol (1-hydroxy-2-propanone) and acetoin (3-hydroxy-2-butanone), present in flue-cured tobacco, are now known to form pyrazines (Table 4) at room temperature in the presence of ammonium salts under weakly acidic conditions at relatively low moisture levels (similar to the aging conditions in tobacco) (52).

In flue-cured tobacco, the levels of pyrazines (and other nitrogenous flavor constituents) increase substantially in storage

TABLE 4.

PYRAZINE FORMATION FROM ACYLOINS AND AMMONIUM
ACETATE UNDER MILD CONDITIONS AT 15% MOISTURE (52)

ACYLOIN	TIME / TEMP HR : C	PRODUCTS (YIELD %)		
		DMP*	Tri-MP	TMP
ACETOL	247 : 22	2.3	0.001	0.001
ACETOIN	17.5 : 22	--	--	13.0
	15.3 : 60	--	--	47.0
ACETOL + ACETOIN	247 : 22	--	20.5	29.9

* Equimolar amounts of 2,5- & 2,6-DMP

DMP = Dimethylpyrazine
Tri-MP = Trimethylpyrazine
TMP = Tetramethylpyrazine

with age (12). The primary pyrazines that
increase during aging are:

> Tetramethylpyrazine
> Trimethylpyrazine
> 2,6-Dimethylpyrazine
> 2,5-Dimethylpyrazine
> 2,3-Dimethylpyrazine

as measured by headspace analysis. Rizzi has
shown that four of these pyrazines can be
accounted directly for from acetol and acetoin
(52).

This does not preclude the possible formation
of pyrazines on tobacco aging via degradation of
Amadori compounds or deoxyfructosazines, but
neither would it require such mechanisms. In all
probability, several processes are occuring
simultaneously.

Also of interest is the recent work by Wong
and Bernhard (74) on the generation of pyrazines
and related compounds in model systems utilizing
various nitrogen sources with glucose in aqueous
systems (Table 5). Of the thirty isolates
identified, all of which have been identified in
cooked foods, 26 have been positively identified
as components of tobacco or tobacco smoke.
Although absolute yields are not provided, the
relative weight percentage of the various
pyrazines formed is dramatically influenced by the
nitrogen source (74). Additionally, the
relationships shown by Rizzi that dimethylpyrazine
formation is related to acetol and that trimethyl-

TABLE 5.

GLUCOSE - NITROGEN SOURCE REACTIONS AT 110 C (74)
(RELATIVE WEIGHT PERCENTS)

	AMMONIUM ACETATE	GLYCINE	GLUTAMATE
2-METHYLPYRAZINE	14.39	0.78	1.75
2,5-DIMETHYLPYRAZINE	3.75	6.92	18.03
2,6-DIMETHYLPYRAZINE	13.96	3.05	0.42
TRIMETHYLPYRAZINE	1.47	28.59	2.74
TETRAMETHYLPYRAZINE	0.04	7.14	0.10
2-ETHYL-3,5-DIMETHYL-PYRAZINE	--	4.12	0.29
2-METHYLIMADAZOLE	1.58	19.65	43.18
2-ACETYLPYRROLE	1.39	0.23	0.51
5-METHYL-6,7-DIHYDRO-[5H]-CYCLOPENTA-PYRAZINE	0.25	0.03	3.29
ACETOL	--	3.37	21.06
ACETOIN	--	2.66	0.72

and tetramethylpyrazine formation is enhanced when both acetoin and acetol are present appears to hold true, eventhough the reaction conditions were quite different. Such reactions are highly pH dependent and pH was not constant in these reactions; accordingly it is difficult to assess the dependance on reactants alone.

Several studies have been carried out on model roasting reactions with amino acids in the presence and absence of carbohydrates under relatively low moisture conditions. It has been found that the Strecker degradation of leucine and valine to produce 3-methylbutanal and 2-methyl-propanal, respectively, occurs more efficiently and at lower temperatures in lipid systems with carbohydrates than in aqueous carbohydrate systems (Figure 14). In fact, the reaction occurs even without carbohydrates in lipid systems at low moisture (1).

Studies on serine and threonine (Figure 15) indicate that these amino acids more efficiently produce pyrazines on roasting when carbohydrates are absent at low initial moisture; and that the presence of carbohydrates generates a significantly different profile of aroma classes (2).

These studies of model food systems have considerable potential significance because they not only shed light on the mechanisms involved in formation of food flavorants, but also simulate processes occuring in tobacco.

FIGURE 14.

2-Methylpropanal
From Valine at 80°C

Legend:
□ COCOA BUTTER/H₂O FRUCTOSE
△ COCOA BUTTER/H₂O
X H₂O/FRUCTOSE

Y-axis: 200 mg of Valine mg from

X-axis: Minutes

Arnoldi et. al. (1)

FIGURE 15.

Aroma Classes From
Threonine and Serine (2)

Legend:
- ALKYLFURANS
- FURAN ALDEHYDES
- FURFURYL ALCOHOLS
- ALKYLPYRROLES
- N-PYRROLYL-ALKANOLS
- ALKYLPYRAZINES

Y-axis: % of Volatiles from MS ion current

X-axis categories: SER:THREO:SUCROSE, SER:THREO

Roasting At +20° C/min to a Final Temp. of 225° C

SUMMARY:

This symposium has provided us with a look at chemical and sensory aspects of Tobacco from a diversity of viewpoints.

R. A. Fenner has provided an approach to looking at the thermolysis products of tobacco. He has shown us that evolution of constituents which affect pH of smoke (such as ammonia and formic acid) differ considerably for Virginia and burley tobaccos, both in amount and thermolysis temperatures required.

Don Roberts has enlightened us with an overview of natural tobacco constituents with a view as to classes and importance to tobacco flavor.

Lance Reynolds has given us an appraisal of the vagaries, difficulties and advances in methodology for the elusive task of quantifying sensory assessment of tobacco products.

Braja Mookherjee provided us with an assessment of the importance of chemicals, which are also tobacco constituents, to the flavor and fragrance industry.

And finally, I have provided a few comments on a variety of subjects related to tobacco flavor.

I would like to thank all of the speakers at this 15th annual TCRC symposia on behalf of the editorial committee and our audience.

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