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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 year's 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 occurring on thermolysis of tobacco which affect the composition (and ultimately the flavor) of tobacco smoke.
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\textsubscript{3} - C\textsubscript{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 nitrogenous 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 occurring 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

<table>
<thead>
<tr>
<th>CHEESY, SWEATY TYPE NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>STE &amp; GTE FLAVOR PRECURSORS</td>
</tr>
<tr>
<td>o 3-METHYLVALERIC ACID</td>
</tr>
<tr>
<td>o ISOVALERIC ACID</td>
</tr>
<tr>
<td>o 2-METHYLBUTYRIC ACID</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CEDAR, AMBER TYPE NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>LABDANOID DERIVED</td>
</tr>
<tr>
<td>o NORAMBREINOLIDE</td>
</tr>
<tr>
<td>o DEHYDRONORAMBREINOLIDE</td>
</tr>
<tr>
<td>o SCLARAL</td>
</tr>
<tr>
<td>o 1-BICYCLOHOMOFARNESAL</td>
</tr>
<tr>
<td>o AMBROX</td>
</tr>
</tbody>
</table>
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-O-acetyl-2,3,4-tri-O-[(+)-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)

<table>
<thead>
<tr>
<th>Acid</th>
<th>Tobacco (μG/G)</th>
<th>Smoke (μG/Cig)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionic</td>
<td>17.0</td>
<td>66.8</td>
<td>+293</td>
</tr>
<tr>
<td>Butyric</td>
<td>2.5</td>
<td>9.7</td>
<td>+288</td>
</tr>
<tr>
<td>Valeric</td>
<td>4.3</td>
<td>3.6</td>
<td>-16</td>
</tr>
<tr>
<td>2-Methylbutyric</td>
<td>2.1</td>
<td>12.4</td>
<td>+490</td>
</tr>
<tr>
<td>Isovaleric</td>
<td>5.1</td>
<td>19.7</td>
<td>+286</td>
</tr>
<tr>
<td>3-Methylvaleric</td>
<td>12.0</td>
<td>62.0</td>
<td>+417</td>
</tr>
<tr>
<td>Hexanoic</td>
<td>7.5</td>
<td>3.9</td>
<td>-48</td>
</tr>
<tr>
<td>Octanoic</td>
<td>1.7</td>
<td>6.2</td>
<td>+265</td>
</tr>
<tr>
<td>Phenylacetic</td>
<td>48.9</td>
<td>19.9</td>
<td>-59</td>
</tr>
</tbody>
</table>
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 occurring at full maturity. These workers also demonstrated that the tetraesters of Oriental STE tended to have higher amounts of the more aromatic C₅ and C₆ 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 thermolysis 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).
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)

NORAMBREINOLIDE (SCLAREOLIDE)

SCLAREOL

CrO₃

SCLAREOL OXIDE

KMnO₄

NORAMBREINOLIDE (SCLAREOLIDE)

LIAŁH₄/H⁺

AMBROX

LIAŁH₄/H⁺

CHO

Pyrolysis

β-BICYCLOHOMOFARINESAL
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 chromosome A controls production of the C\textsubscript{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 transferring 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 major 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). Biomimetic 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 biomimetic 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
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)

\[ C_{13} \]
\[
\begin{align*}
(\text{A, B}) & \quad (\text{A, B}) & \quad (\text{A, B}) & \quad (\text{A, B}) \\
(\text{b}) & \quad (\text{b}) & \quad (\text{b}) & \quad (\text{b}) \\
(\text{b}) & \quad (\text{b}) & \quad (\text{b}) & \quad (\text{A, B}) \\
(\text{A, B}) & \quad & & \\
\end{align*}
\]

\[ C_{11} \]
\[
\begin{align*}
(\text{A}) & \quad (\text{A, B}) & \quad (\text{A, B}) & \quad (\text{b}) \\
\end{align*}
\]

\[ C_{10} \]
\[
\begin{align*}
(\text{b}) & \quad (\text{A, B}) & \quad (\text{A, B}) & \quad (\text{A, B}) \\
(\text{A, B}) & \quad & & \\
\end{align*}
\]

\[ C_{9} \]
\[
\begin{align*}
(\text{A, B}) & \quad (\text{A, B}) & \quad (\text{A, B}) & \quad (\text{A, B}) \\
(\text{A, B}) & \quad & & \\
\end{align*}
\]

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)
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-trimethylcyclohex-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])

<table>
<thead>
<tr>
<th>Structure</th>
<th>Virginia %</th>
<th>Burley %</th>
<th>Oriental %</th>
<th>Tobacco Flavor (39, 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>0.62</td>
<td>0.65</td>
<td>0.49</td>
<td>EARTHY, HARSH, Burley note</td>
</tr>
<tr>
<td>(A, B, C)</td>
<td>0.26</td>
<td>0.38</td>
<td>0.54</td>
<td>TOBACCO, HAY, STRAW, SWEET FLUE-CURED</td>
</tr>
<tr>
<td>(C)</td>
<td>0.18</td>
<td>0.10</td>
<td>0.55</td>
<td>WEAK, SOUR</td>
</tr>
<tr>
<td>(A, B)</td>
<td>0.43</td>
<td>0.24</td>
<td>0.23</td>
<td>SWEET, GREEN-HAY, SAFFRON, ENHANCED TOBACCO AROMA</td>
</tr>
<tr>
<td>(B, D, E, H, I)</td>
<td>0.83</td>
<td>0.60</td>
<td>0.93</td>
<td>WEAK, SLIGHT COOLING</td>
</tr>
<tr>
<td>(A)</td>
<td>0.77</td>
<td>0.57</td>
<td>0.49</td>
<td>ORIENTAL, WOODY, IONONE</td>
</tr>
<tr>
<td>(A)</td>
<td>0.26</td>
<td>0.61</td>
<td>0.94</td>
<td>SWEET, FLORAL Burley like, CAMPHORACEOUS</td>
</tr>
<tr>
<td>(A, B, J, K, L)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>SWEET, FLORAL, WOODY, SMOOTHING</td>
</tr>
<tr>
<td>(A, B, J, K, M, N)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>SWEET, FRUITY, FLORAL, WOODY, SMOOTHING</td>
</tr>
<tr>
<td>Structure</td>
<td>Virginia %</td>
<td>Burley %</td>
<td>Oriental %</td>
<td>Tobacco Flavor (39,73)</td>
</tr>
<tr>
<td>-----------</td>
<td>------------</td>
<td>----------</td>
<td>------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>(A)</td>
<td>+</td>
<td>NR</td>
<td>NR</td>
<td>Sweet, Floral</td>
</tr>
<tr>
<td>(B)</td>
<td>+</td>
<td>0.09</td>
<td>+</td>
<td>Sweet, Flue-cured</td>
</tr>
<tr>
<td>(C)</td>
<td>0.28</td>
<td>0.70</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>(D)</td>
<td>0.93</td>
<td>0.95</td>
<td>0.50</td>
<td>Floral</td>
</tr>
<tr>
<td>(E)</td>
<td>2.92</td>
<td>1.70</td>
<td>3.26</td>
<td>Fruity, Enhanced Burley notes</td>
</tr>
<tr>
<td>(F)</td>
<td>0.13</td>
<td>0.25</td>
<td>+</td>
<td>Rose-floral, Sweet, Green, Burley note</td>
</tr>
<tr>
<td>(G)</td>
<td>7.03</td>
<td>5.64</td>
<td>5.32</td>
<td>Spicy Burley notes, adds strength</td>
</tr>
<tr>
<td>(H)</td>
<td>NR</td>
<td>+</td>
<td>NR</td>
<td>Sweet, rich Flue-cured</td>
</tr>
</tbody>
</table>

Also found in:
(A) Osmanthus (B) Black Tea (C) Saffron (D) Tomato (E) Cassia (F) Apples (G) Grapes (H) Cassie (I) Ambergris (J) Blackberries (K) Passion Fruit (L) Whiskey (M) Raspberries (N) Carrots (O) Coffee (P) Beer (Q) Red Fox (R) Roman (S) Chamomille (T) Bulgarian (U) Rose
higher polyrenols, decaprenol and isodecaprenol, (62) which are homologs of the major polyrenoid, 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)

µmoles/gram dwt

Days From Harvest

Leaf #2
Leaf #17
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 usable. 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)

μmoles/gram

0 5 10 15 20 25

Days From Harvest
FIGURE 11.

Ammonia Increase
Burley Air-curing (7)

μmoles/gram

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 standpoint 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)

Air-cured
Virginia

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 anomaly 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-PROLINE)-D-FRUCTOSE

\[ \text{DDMP} \]

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 occurring 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>
<thead>
<tr>
<th>Acyloin</th>
<th>Time / Temp</th>
<th>Products (Yield %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR : C</td>
<td>DMP*</td>
</tr>
<tr>
<td>Acetol</td>
<td>247 : 22</td>
<td>2.3</td>
</tr>
<tr>
<td>Acetoin</td>
<td>17.5 : 22</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>15.3 : 60</td>
<td>--</td>
</tr>
<tr>
<td>Acetol +</td>
<td>247 : 22</td>
<td>--</td>
</tr>
<tr>
<td>Acetoin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 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 occurring 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)

<table>
<thead>
<tr>
<th>AMMONIUM ACETATE</th>
<th>GLYCINE</th>
<th>GLUTAMATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-METHYLPYRAZINE</td>
<td>14.39</td>
<td>0.78</td>
</tr>
<tr>
<td>2,5-DIMETHYLPAZINE</td>
<td>3.75</td>
<td>6.92</td>
</tr>
<tr>
<td>2,6-DIMETHYLPAZINE</td>
<td>13.96</td>
<td>3.05</td>
</tr>
<tr>
<td>TRIMETHYLPAZINE</td>
<td>1.47</td>
<td>28.59</td>
</tr>
<tr>
<td>TETRAMETHYLPAZINE</td>
<td>0.04</td>
<td>7.14</td>
</tr>
<tr>
<td>2-ETHYL-3,5-DIMETHYL-PYRAZINE</td>
<td>--</td>
<td>4.12</td>
</tr>
<tr>
<td>2-METHYLIMADAZOLE</td>
<td>1.58</td>
<td>19.65</td>
</tr>
<tr>
<td>2-ACETYLPRROLE</td>
<td>1.39</td>
<td>0.23</td>
</tr>
<tr>
<td>5-METHYL-6,7-DIHYDRO-[SH]-CYCLOPENTA-PYRAZINE</td>
<td>0.25</td>
<td>0.03</td>
</tr>
<tr>
<td>ACETOL</td>
<td>--</td>
<td>3.37</td>
</tr>
<tr>
<td>ACETOIN</td>
<td>--</td>
<td>2.66</td>
</tr>
</tbody>
</table>
and tetramethylpyrazine formation is enhanced when both acetoin and acetol are present appears to hold true, even though 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 dependence 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-methylpropanal, 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 occurring in tobacco.
FIGURE 14.

2-Methylpropanal
From Valine at 80° C

Arnoldi et al. (1)
Roasting At +20° C/min to a Final Temp. of 225° C

FIGURE 15.
Aroma Classes From Threonine and Serine (2)
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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