Changes in Chemical Composition of Homogenized Leaf-Cured and Air-Cured Burley Tobacco Stored in Controlled Environments

Roger A. Andersen,* Michael J. Kasperbauer, Harold R. Burton, James L. Hamilton, and Elmon E. Yoder

Effects of controlled-environment storage (aging) on chemical components in tobacco (*Nicotiana tabacum* L.) were determined. Homogenized leaf-cured (HLC) Ky 14 burley tobacco was prepared with and without removal of protein. Conventionally air-cured and HLC tobaccos were stored at 8, 12, and 16% moisture and at 20 and 30 °C. Tobaccos kept in the six environments for 0, 20, 40, and 52 weeks were analyzed. After 52 weeks, concentrations of HLC and low-protein HLC tobacco components were greatly reduced (17–83%) in the case of total alkaloids, nitrate N, nitrite N, ammonia N, total volatile nitrogenous base N, amino acids, and petroleum ether extractables; only nitrate N decreased significantly (22%) in air-cured tobacco. In contrast, *N*-nitrosonornicotine (NNN) increased in all tobacco samples during storage. After 52 weeks, NNN concentrations were 200–300× higher in HLC than in air-cured tobacco. Prior to the tobacco being aged, NNN was also much higher in HLC tobacco. Net changes in component concentrations were increased by storage at higher temperatures and moistures.

Although relatively little published information is available about the chemical reactions that occur during the aging of conventionally air-cured burley tobacco, it is generally agreed that the quality of the leaf is greatly improved by storage under specific conditions albeit at ambient temperatures for 1–3 years. The major benefits cited for the process include development of desirable aroma, elimination or reduction of harshness or bitterness, attainment of a more uniform shade of color with disappearance of any greenness, decrease in sticky properties, and improved combustibility (Garner, 1947; Bates et al., 1975).

An experimental homogenized leaf-curing (HLC) procedure for the rapid curing of burley tobacco is currently under investigation. The process involves homogenization of ripe green leaves, incubation of the homogenate, and dehydration of the material (Tso et al., 1975; Yoder et al., 1976). The process could provide a means to reduce concentrations of chemical components that are associated with the smoking–health problem. No investigations have been conducted on the chemical changes that occur during the storage (aging) of HLC tobacco. However, we have observed that the appearance and aroma of HLC tobacco were improved after prolonged storage.

The present investigation was undertaken to determine some of the chemical compositional changes that take place during the prolonged storage of conventionally air-cured and HLC burley tobacco under controlled-environment conditions that approximate some of the conditions encountered in the conventional aging of burley tobacco. The effects of aging on probable precursors of health-related components in leaf that may form undesirable or toxic components in smoke were emphasized in this study.

MATERIALS AND METHODS

Plant Materials and Curing Procedures. Burley tobacco (*Nicotiana tabacum* L. cv. Ky 14) plants were grown at the Kentucky Agricultural Experiment Station Spindletop Farm near Lexington. Recommended fertilization and cultural practices were followed during the growing season (Atkinson et al., 1976). Plants designated for each curing method were harvested on the same date, i.e., when the tobacco was ready for conventional harvest. Plants to be air-cured were hung on sticks and cured in a conventional burley barn with ambient temperatures. Tobacco leaves for the HLC process were removed (primed) from the stalks in the field and taken directly to the HLC facilities.

Leaves were mechanically chopped and then homogenized in the manner similar to that described for homogenized leaf-cured bright tobacco (DeJong et al., 1975), except that no antioxidant was added. The grinding equipment used was described by Yoder et al. (1976). Part of the homogenized material was reduced in protein content prior to incubation in a manner similar to that described by DeJong and Lam (1979). The liquid phase of the homogenate was separated from the solids, and the soluble protein was denatured by injection of steam into the liquid held at 80 °C. The resultant insoluble protein was removed by flotation (skimmed off), and the remaining liquid was recombined with the solids of the original homogenate. The homogenized and low-protein homogenized materials were incubated for 20 h at 40 °C with constant mixing and aeration and then air-dried to 3–4% moisture according to the methods of Yoder et al. (1976). The dry HLC materials were stored in plastic bags in darkness at room temperature until the air-cured samples were ready (after about 6 weeks). Air-cured leaves (corresponding to the same stalk positions used for the HLC process) were removed from the stalks and then cut into strips about 3 mm wide in order to facilitate handling and sampling. After completion of the respective cures, samples of each of the materials were stored in sealed containers at –50 °C prior to their use as "zero-time" controls. Moisture contents of portions of the remaining materials were adjusted in humidity chambers as described under Storage Conditions.

Storage Conditions. Six samples were prepared for each of the three cures. Every sample contained 1 kg of dry tobacco plus enough moisture to give 8, 12, or 16% moisture on a "wet weight" basis. Each set of six samples included the three moisture contents and two storage temperatures (20 and 30 °C).

Samples were packed into 9-L glass desiccators, which served as storage chambers during the aging experiment. Desiccators were selected because they were of uniform

Agricultural Research Service, U.S. Department of Agriculture (R.A.A., M.J.K., J.L.H., and E.E.Y.), and Departments of Agronomy (R.A.A., M.J.K., H.R.B., and J.L.H.) and Agricultural Engineering (E.E.Y.), University of Kentucky, Lexington, Kentucky 40546-0091.

0021-8561/82/1430-0663$01.25/0 © 1982 American Chemical Society

size, could be sealed to maintain the moisture content of the tobacco, and could be opened easily for sampling. Thus, all chambers had the same dry weight of tobacco in the same volume of space. The containers were sealed, but gases were not evacuated. The seals were broken briefly at weekly intervals to allow gaseous exchange without appreciable change in moisture content of the tobacco. Storage chambers were kept in darkness during the aging period (except during sampling), and temperatures were maintained at ±0.5 °C of the indicated temperatures in controlled environments.

Sampling Procedures. Samples were taken from each of the treatments at weekly intervals for the first 4 weeks and at 4-week intervals for the remainder of a year. At each sampling, the tobacco within each chamber was thoroughly mixed and 20 g of sample collected. After each sample was removed, the remaining tobacco was repacked and the container resealed and returned to darkness at its respective temperature. Samples were placed in plastic bags and stored at -50 °C prior to chemical analyses. The present study utilized samples collected after 0, 20, 40, and 52 weeks of storage under the various environments. Samples were equilibrated to ambient moisture overnight in darkness on a laboratory bench before grinding to pass a 40-mesh screen in preparation for chemical analyses. The ground samples were refrigerated in sealed containers and kept in darkness prior to analyses.

Reflectance Measurements. Analyses were performed on finely ground tobacco samples by using a Shimadzu MPS-50L variable-wavelength recording spectrophotometer with a reflectance attachment. Scans were made from 400–800 nm.

Chemical Analyses. Total alkaloids were determined spectrophotometrically at 460 nm after reaction with cyanogen bromide and aniline (Harvey et al., 1969). Results were expressed as nicotine equivalent.

Nornicotine and nicotine were determined by the following unpublished gas chromatographic method. A half gram of tobacco, 0.3 g of $Ba(OH)_2$, 3 mL of saturated $Ba(OH)_2$ solution, 20 mL of diethyl ether, and 5 mg of the internal standard (7-methylquinoline) were placed in a serum vial. The mixture was shaken for 30 min. After the solids in the mixture were allowed to settle, an aliquot (ca. 3.8 μL) was injected into the Varian Model 3700 GC system equipped with a thermionic-specific detector and a 1.83 m × 6.3 mm glass column packed with 10% Carbowax 20M–2% KOH on 80/100 Chromosorb WAW (Supelco, Inc., Bellfonte, PA). The column was maintained at 180 °C and helium was the carrier gas. Quantification was by internal standardization after calibration with authentic nicotine and nornicotine.

N-Nitrosonornicotine was determined by the high-performance liquid chromatography (HPLC)–thermal energy analysis (TEA) procedure (Hecht et al., 1975) except that the preliminary basic alumina column chromatography step was omitted and two Ultrasil-NH_2 columns were used in series (10-μm particle size; 250 × 4.6 mm) with an isooctane–dichloromethane–methanol (89:9:2) solvent system and thermal energy detection (TEC Model 502 analyzer, Thermo Electron Corp., Waltham, MA 02154). Extractions and solvent partitioning were performed in our laboratories and the HPLC–TEA was completed by the Analytical Services Laboratory of the Thermo Electron Corp.

Nitrate and nitrite N analyses were carried out by a spectrophotometric procedure. After reduction of nitrate to nitrite with the nitrate reductase preparation isolated from *Escherichia coli*, combined nitrite (reduced nitrate plus endogenous nitrite) was measured with a color-developing reagent (Lowe and Gillespie, 1975). Endogenous nitrite was determined directly in the absence of reductase enzyzme, while nitrate was calculated as the difference between endogenous nitrite and combined nitrite.

Total N was determined by spectrophotometry with the aid of a Technicon autoanalyzer after Kjeldahl digestion of the sample followed by reaction of the digest with phenol and hypochlorite (Russell, 1944). Protein N was assayed by the procedure for total N except that the sample was extracted with 0.5% acetic acid and then the Kjeldahl method was employed as given above. Ammonia N was also determined by a modification of the total N procedure that omitted the concentrated sulfuric acid digestion steps. A 0.5-g tobacco sample was extracted with 25 mL of pH 6.4 citrate buffer. The solution was filtered and the filtrate was diluted to 50 mL prior to spectrophotometric measurement.

Total volatile nitrogenous base N was determined by titration with sulfamic acid after the following pretreatment sequence: initial steam distillation of tobacco from an alkaline solution into dilute hydrochloric acid, concentration of the acid solution and normal Kjeldahl digestion of the residue with sulfuric acid, solution again made basic, and final steam distillation of bases as ammonia (Milner and Zahner, 1960; Neurath et al., 1966).

In the determination of total free amino acids, a 0.2-g sample was mixed with 19.8 mL of 0.1 N HCl in a 30-mL centrifuge tube that contained about 0.4 g of glass beads (5-μm diameter). The mixture was first cooled in an ice bath for 15 min and then sonified vigorously for 20 s. The cooling and sonification procedures were repeated once. After addition of 5 mL of 0.1 N HCl, the sample was centrifuged at 8000 rpm for 8 min. After dilution of the supernatant with water, the total free amino acids were estimated with the aid of a Technicon autoanalyzer by the procedure described by Piez and Morris (1960) with modifications described in the "Technicon Instruction Manual AAA-1" (1967) and "Research Bulletin No. 20" (1963). Quantification was achieved by comparison with a standard mixture of amino acids (mean molecular weight of 120).

The total protein (bound amino acids) assay was carried out with a 0.2-g sample. After addition of 9.6 mL of 6 N HCl, the mixture was autoclaved for 6 h at 121 °C. After the mixture was cooled and diluted to 25 mL with water, a 1-mL aliquot was diluted further to 200 mL with water and assayed as described above for total free amino acids. Total protein-bound amino acids were calculated as the difference between the amounts of amino acids in the hydrolysate and free amino acids.

Petroleum ether extractables (PEE) were determined by a modification of an AOAC procedure (Wilkham and Blackmore, 1963). A 0.5-g sample was extracted in a micro-Soxhlet extractor with 20 mL of petroleum ether (bp 35–60 °C) for 1 h in a tared flask. After evaporation of the solvent on a steam bath, the flask contents were dried in an oven at 100 °C for 1 h. The residue weight was calculated as PEE.

Total soluble phenols were estimated by spectrophotometric measurement of the reduction of Folins phenol reagent and determination of the difference in absorbance before and after the addition of insoluble poly(vinylpyrrolidone) (Andersen and Todd, 1968).

Calcium was determined by atomic absorption using a Varian AA6 spectrometer. A 0.2-g sample of tobacco was placed in a flask, covered, and then digested in 10 mL of a mixture of HNO_3–$HClO_4$ (9:1 v/v) overnight at 80 °C.

Table I. Chemical Composition of HLC and Air-Cured Burley Tobacco Aged for One Year Compared to That of Unaged (Zero-Time) Controls[a]

component	HLC			low-protein HLC			air-cured		
	unaged, mg/g	aged, mg/g	change, %	unaged, mg/g	aged, mg/g	change, %	unaged, mg/g	aged, mg/g	change, %
total alkaloids, nicotine equiv	11.8	3.6	−70	10.3	1.7	−83	21.6	21.5	−0.50
nornicotine	0.2	<0.05	−75	<0.05	<0.05	0	0.20	0.11	−55
N-nitrosonornicotine	0.36	0.81	+125	0.50	0.98	+96	0.0017	0.0051	+241
nitrate N	15.4	8.1	−47	12.7	4.1	−68	16.9	13.2	−22
nitrite N	2.6	1.0	−61	2.4	0.8	−67	0.02	trace	
ammonium N	0.9	0.6	−33	0.8	0.5	−38	3.8	4.4	+16
total volatile nitrogenous base N	5.2	4.0	−23	3.9	3.2	−18	9.0	9.1	+1
protein N	26.0	26.5	+2	18.7	17.2	−8	15.4	15.9	+3
total N	41.5	38.2	−8	29.0	28.0	−3	44.0	43.3	−2
free amino acids	22.9	12.6	−45	19.2	8.2	−57	41.5	41.6	0
protein-bound amino acids	162.4	165.9	+2	118.4	120.9	+2	85.0	91.8	+8
petroleum ether extractables	22.5	5.4	−76	30.8	6.4	−79	29.5	27.4	−7
total phenols, chlorogenic acid equiv	1.8	2.1	+17	6.0	4.2	−30	1.9	1.1	−42

[a] Each entry is the mean value of three to five laboratory analyses per sample. Entries for aged tobacco are averaged over all six treatments of storage temperature and moisture parameters and have been corrected for change in dry weight by a factor obtained from calcium analyses performed on aged and zero-time control samples.

The flask and contents were then placed on a hot plate, and the mixture was heated with fuming until evaporation was complete. After the residue was cooled, 10 mL of 1 N HCl was added to dissolve the salts. The solution was diluted with 200 parts of water, and the absorption was measured in an acetylene–nitrous oxide flame.

RESULTS AND DISCUSSION

During the early stages of storage (up to about 20 weeks) the HLC and low-protein HLC tobaccos became less green in color as aging time lengthened. The relative reflectances of HLC and air-cured tobacco samples that were stored 20 weeks were determined from measurements at the minima which occurred at 660–690 nm. This wavelength region corresponds to green colorations that are associated with improperly or incompletely cured burley tobacco. Improved tobacco quality is, therefore, likely to be associated with increased relative reflectance values in this wavelength region. The order of increasing reflectance values for cured tobacco samples that were either unaged or aged for 20 weeks was HLC < low-protein HLC < air-cured. Higher relative reflectance values were a function of storage at each successively higher temperature and moisture content for HLC and low-protein HLC tobacco. Air-cured tobacco reflectance changes were relatively small during storage, and the observed differences may not be significant. While the color of HLC tobaccos continued to improve during the remainder of the controlled aging treatments, the rates of these changes were slower after the 20-week storage period.

The net quantitative changes in the chemical composition of tobacco after 1 year of storage are summarized in Table I for nitrogenous compounds, petroleum ether extractables, and total phenols. The values for concentrations of the components were corrected for changes in dry weights of samples by factors obtained from Ca contents of aged relative to zero-time control samples. Calcium was assumed to undergo no losses during aging of the tobaccos, and Ca contents ranged from 2.93 to 4.21% on a dry weight basis among all the samples.

In general, more components in HLC and low-protein HLC tobaccos showed greater concentration changes than in air-cured tobacco. The magnitudes of some chemical changes in HLC tobaccos were high enough to indicate that curing was incomplete or fermentation processes occurred during storage (Table I). The moisture contents of various tobaccos during fermentation were reported to range from 14 to 40% (Garner, 1947), and our samples stored at 16% moisture were within this range. During storage, HLC and low-protein HLC tobaccos underwent large concentration decreases of total alkaloids as nicotine (70 and 83%, respectively), nitrate N (47 and 68%), nitrite N (61 and 67%), ammonia N (33 and 38%), total volatile nitrogenous base N (23 and 18%), free amino acids (45 and 57%), and petroleum ether extractables (76 and 79%). Smaller concentration changes in stored HLC and low-protein HLC tobaccos occurred for protein N, total N, and protein-bound amino acids. However, there was a 28% reduction of the initial protein content during the extraction phase of the low-protein HLC procedure. The protein N and protein-bound amino acid determinations provided alternate analyses for the same components. Relatively small changes in concentrations were noted for the following components in air-cured tobacco samples: total alkaloids, nitrite N (low zero-time concentrations were also observed), total volatile nitrogenous base N, protein N, total N, free amino acids, and petroleum ether extractables. Palmer (1963) reported larger reductions in some nitrogenous components than we observed for aging air-cured burley tobacco, namely, reductions of nicotine N, ammonia N, α-amino N, and total N.

In contrast to the reductions or relative constancy noted for the foregoing constituents, *N*-nitrosonornicotine levels increased markedly in HLC (125%) and low-protein HLC tobaccos (96%) (Table I). Although large increases in *N*-nitrosonornicotine were also noted for stored air-cured tobacco, the final concentrations of this compound after 1 year of aging were about 200 times greater in HLC and low-protein HLC samples than in air-cured samples. The apparent biosynthesis of NNN in our samples during aging may have resulted from storage conditions that promoted specific reactions among known endogenous precursors of NNN, namely, nitrate, nitrite, and nicotine (Hecht et al., 1978; Digenis and Issidorides, 1979).

Decreased levels of nornicotine occurred in aged HLC and air-cured samples, while nitrate N and PEE concentrations decreased in air-cured tobacco during aging. Concentrations of total phenols decreased in low-protein HLC and air-cured samples, but increased in HLC samples. Although the reasons for this anomaly are unknown, the analytical method may lack sufficient precision at the relatively low phenolic concentrations found in the burley materials.

In nonaged samples the concentrations of total alkaloids (as nicotine) in HLC and low-protein HLC tobacco were only about half that of the air-cured tobacco (Figure 1).

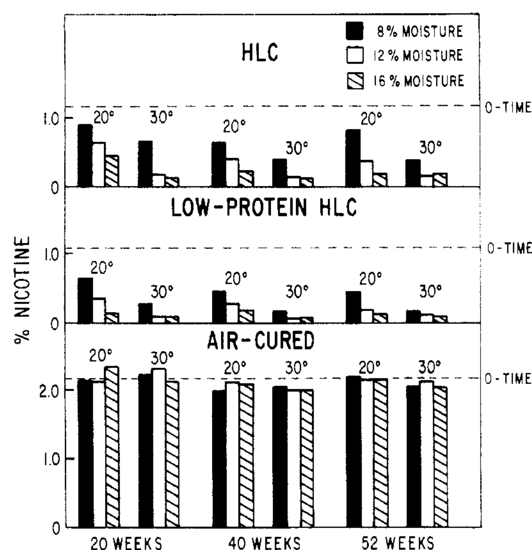


Figure 1. Effects of storage temperature, moisture, and duration on total alkaloids (as nicotine) in HLC, low-protein HLC, and air-cured tobacco. LSD for total alkaloids among entries is 0.08% for HLC, 0.10% for low-protein HLC, and 0.40% for air-cured tobacco at $P < 0.05$.

Continued losses in the total alkaloid fraction occurred during storage of both HLC tobaccos; these losses were directly related to increased moisture content, temperature, and duration of the aging treatments. In contrast to HLC tobaccos, air-cured samples had only small decreases in total alkaloid concentrations at the 40- and 52-week storage times relative to those at zero time. The chemical changes that occurred in the aging HLC tobaccos were evidenced by the total alkaloid losses. In addition, these losses were accompanied by the evolution of variable quantities (less than 200 mg) of an orange-red substance (sublimate) that collected on the upper walls of the storage chambers housing the 1-kg lots of HLC and low-protein HLC tobacco. More sublimate appeared on storage chambers housing HLC materials at higher temperature or moisture levels than at lower temperature or moisture levels. Also, the amounts of sublimate appeared to be about the same from HLC and low-protein HLC tobaccos stored under the same environments. This sublimation (or sublimation–condensation) reaction was not observable in the air-cured tobacco. Although only partial characterization of the sublimate has been accomplished, major components of the material were identified as nicotinic acid and volatile bases. On the basis of the known transformation reactions of nicotine in cigar leaf tobacco during aging (Frankenburg and Gottscho, 1952) and the nicotine losses we observed during the storage of HLC tobaccos, it seems probable that nicotinic acid in the HLC sublimate derived from the leaf nicotine present in the HLC tobacco. The substantial concentration changes in aging HLC and low-protein HLC tobaccos observed for nicotine, nitrate N, nitrite N, free amino acids, petroleum ether extractables, and total phenols resemble the transformation (fermentation) reactions in cigar tobacco (Frankenburg and Gottscho, 1952; Garner, 1947).

In nonaged samples the concentrations of nitrate N were not remarkably different among the HLC and air-cured tobacco samples (Figure 2). In contrast, nitrite N levels in HLC and low-protein HLC tobaccos were about 10 times higher than those in air-cured samples. Although decreases in both nitrate N and nitrite N occurred as a consequence of aging in both HLC and air-cured tobaccos, the magnitudes of these changes were at least 2–3 times greater for the HLC tobaccos. Nitrate and nitrite are precursors

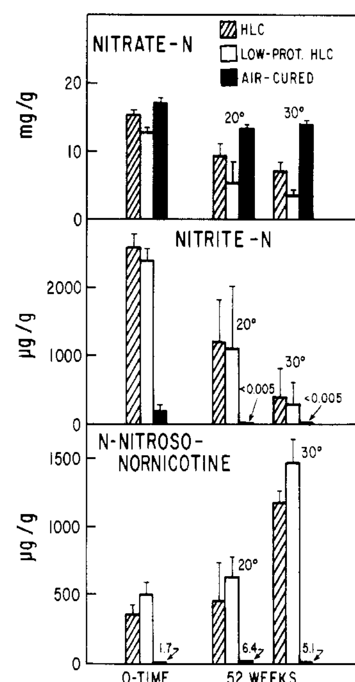


Figure 2. Effect of storage duration and temperature on N-nitrosonornicotine and N-nitrosonornicotine precursor concentrations averaged over storage moisture variables for aged tobaccos. Unaged controls are included for comparison. Bars are standard errors.

of N-nitroso compounds (Digenis and Issidorides, 1979) including nicotine and the carcinogenic tobacco-specific N-nitroso compound N-nitrosonornicotine (NNN) (Hecht et al., 1978). Therefore, it was considered important from the tobacco–health viewpoint to determine the effects of storage and curing variables on levels and relationships among nitrate N, nitrite N, nicotine, and NNN. As in the case of nitrite N, levels of NNN were much higher in HLC and low-protein HLC tobacco samples (200–300×) than in the air-cured counterparts (Figure 2). It was also evident that increased duration of storage and the higher temperature (30 °C) during aging increased the levels of NNN in all of the tobacco samples. Nicotine levels in HLC and low-protein HLC tobacco decreased, however, under these same conditions (Figure 1). Thus, each known precursor of NNN that we measured, i.e., nitrate N, nitrite N, and nicotine, underwent concentration changes (decreases) that were consistent with the biosynthesis of NNN from these endogenous precursors during storage of the three tobacco materials. The origin of high levels of nitrite N in the HLC and low-protein HLC tobacco remains unknown, but it seems probable that increased activities of nitrate reductase acting upon nitrate N are favored by the homogenization and incubation processes. The nitrite formed is believed to react with nicotine or nornicotine to yield NNN under conditions which favor the nitrosation reaction (Hecht et al., 1978). The concentrations we report for NNN in the experimental HLC and low-protein HLC burley tobaccos (up to about 1500 μg/g) are higher than those reported for this compound in marketable tobacco products [cf. Hecht et al. (1978)] that, heretofore, have not exceeded 100 μg/g. High NNN levels in HLC tobaccos are not desirable from the standpoint of quality improvement through the use of new tobacco materials, and future efforts need to be made toward finding the means of eliminating or decreasing the formation of this component. It seems probable that utilization of tobacco varieties grown under conventional cultural practices in the flue-cured regions for experimental HLC tobacco production by

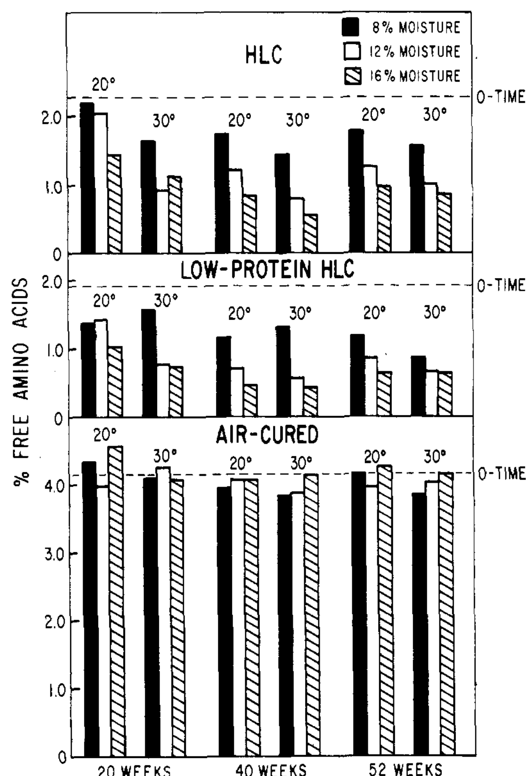


Figure 3. Effects of storage temperature, moisture, and duration on free amino acid levels in HLC, low-protein HLC, and air-cured tobacco. LSD for free amino acids is 0.32% for HLC, 0.28% for low-protein HLC, and 0.38% for air-cured tobacco at $P < 0.05$.

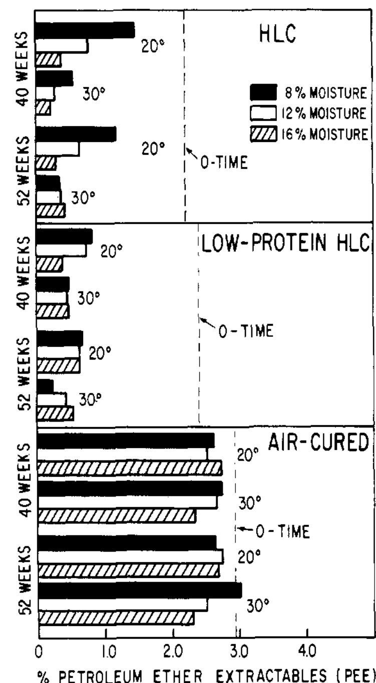


Figure 4. Effects of storage temperature, moisture, and duration on levels of petroleum ether extractables (PEE) in HLC, low-protein HLC, and air-cured tobacco. LSD for PEE is 0.36% for HLC, 0.37% for low-protein HLC, and 0.27% for air-cured tobacco at $P < 0.05$.

methods similar to those described herein for burley may result in HLC materials with lower NNN concentrations, because of lower nitrate contents in flue-cured tobaccos compared to those of burley (Broaddus et al., 1965).

As was the case for nicotine, nonaged HLC and low-protein HLC tobaccos had concentrations of total free amino acids and petroleum ether extractables that were only about 50% or less of the air-cured tobacco (Figures 3 and 4, respectively). Continued reductions in free amino acid levels in the HLC tobaccos occurred during aging (at least up to 40 weeks), and the magnitudes of these losses were also related to increased moisture content and temperature during storage (Figure 3). Similarly, there were further decreases in PEE concentrations in the HLC and low-protein HLC samples, and these reductions were, likewise, proportional to increased moisture content and temperature (Figure 4). In contrast to HLC tobaccos, air-cured samples had mostly nonsignificant changes in free amino acid concentrations at the 20-, 40-, and 52-week aging durations compared to corresponding free amino acid levels at zero time (Figure 3). The PEE concentrations in air-cured tobacco also changed very little with duration of aging, although there was a tendency for samples aged 40–52 weeks to have some reductions ranging up to 22% after 52 weeks aging at 16% moisture and 30 °C (Figure 4). Free amino acids (as well as protein) are generally considered to be negative factors in chemical indices that influence either the quality or usability of tobacco leaves (Tso and Gori, 1975). The lower concentrations at zero time of free amino acids in HLC and low-protein HLC tobacco compared to those of air-cured samples and the corresponding reductions in concentrations during aging seem to have moved in the direction of meeting the goals of improving tobacco quality through postharvest processing. The concomitant decrease of soluble proteins by the low-protein HLC processing prior to aging is also believed to favor improvement of the quality and usability of the HLC tobacco. Petroleum ether extractables contain some components that are desirable as well as some that are undesirable for tobacco usability (Tso and Gori, 1975). The reductions of PEE levels in HLC and low-protein HLC tobacco during aging may be beneficial to the potential use of these experimentally cured tobaccos.

Although concentrations of total soluble phenolics changed in the tobacco, the final levels of this component fraction after 52 weeks of storage (Table I) were all in a low concentration range compared to those of flue-cured tobacco varieties (Andersen et al., 1970). Statistical evidence based on correlations of the chemical composition of experimental cigarettes and the biological activity of their derived smoke indicated that high levels of soluble phenols in leaf may be undesirable [*DHEW Publ. (NIH) (U.S.)*, 1976, 1977; *U.S., Dep. Agric., Tech. Bull.*, 1977].

ACKNOWLEDGMENT

We thank Dr. R. H. Lowe for his advice during the initiation of this work and Dr. Stephen Hecht of the American Health Foundation for sending us authentic N-nitrosonornicotine. We gratefully acknowledge the assistance of T. Sutton, J. Crutchfield, L. Staples, T. H. Vaughn, and members of the Analytical Services Laboratory and Tobacco and Health Research Institute, University of Kentucky.

LITERATURE CITED

Andersen, R. A.; Chaplin, J. F.; Currin, R. E.; Ford, Z. T. *Agron. J.* **1970**, *62*, 415.
Andersen, R. A.; Todd, J. R. *Tob. Sci.* **1968**, *12*, 107.
Atkinson, W. O.; Sims, J. R.; Calvert, J. R. *Tob. Sci.* **1976**, *20*, 32.
Bates, W. W., Jr.; Mitchem, A. R.; Rogers, J. D. *Tob. Int.* **1975**, *177*, 48.
DeJong, D. W.; Lam, J J., Jr. *Tob. Res.* **1979**, *5*, 1.
DeJong, D. W.; Lam, J.; Lowe, R.; Yoder, E.; Tso, T. C. *Beit. Tabakforsch.* **1975**, *8*, 93.
DHEW Publ. (NIH) (U.S.) **1976**, *No. NIH 76-1111.*
DHEW Publ. (NIH) (U.S.) **1977**, *No. NIH 77-1280.*
Digenis, G. A.; Issidorides, C. H. *Bioorg. Chem.* **1979**, *8*, 97.

Frankenburg, W. G.; Gottscho, A. M. *Ind. Eng. Chem.* **1952**, *44*, 301.
Garner, W. M. "The Production of Tobacco"; The Blakiston Co.: Philadelphia, 1947; Chapter 20.
Harvey, W. R.; Stahr, H. M.; Smith, W. C. *Tob. Sci.* **1969**, *13*, 13.
Hecht, S. S.; Chen, C. B.; Hirota, N.; Ornaf, R. M.; Tso, T. C.; Hoffmann, D. *J. Natl. Cancer Inst. (U.S.)* **1978**, *60*, 819.
Hecht, S. S.; Ornaf, R. M.; Hoffmann, D. *Anal. Chem.* **1975**, *47*, 2046.
Lowe, R. H.; Gillespie, M. C. *J. Agric. Food Chem.* **1975**, *23*, 783.
Milner, O. I.; Zahner, R. J. *Anal. Chem.* **1960**, *32*, 294.
Neurath, G.; Krull, A.; Pirmann, B.; Wandrey, K. *Proc. Int. Tob. Sci. Congr., 4th* **1966**, 774.
Palmer, J. K. *Tob. Sci.* **1963**, *7*, 93.
Piez, K. A.; Morris, L. *Anal. Biochem.* **1960**, *1*, 187.
"Research Bulletin No. 20"; Technicon Corp.: Chauncy, NY, 1963.
Russell, J. *J. Biol. Chem.* **1944**, *156*, 457.
"Technicon Instruction Manual AAA-1"; Technicon Corp.: Chauncy, NY, 1967.
Tso, T. C.; Gori, G. B. *Beit. Tobakforsch.* **1975**, *8*, 167.
Tso, T. C.; Lowe, R.; DeJong, D. W. *Beit. Tabakforsch.* **1975**, *8*, 44.
U.S., Dep. Agric., Tech. Bull. **1977**, *No. 1551*.
Wilkham, J. E.; Blackmore, R. H. *J. Assoc. Off. Agric. Chem.* **1963**, *46*, 425.
Yoder, E. E.; Lowe, R. H.; Lam, J. J., Jr.; DeJong, D. W.; Tso, T. C. *Trans. ASAE* **1976**, *19*, 359.

Received for review November 20, 1981. Accepted April 12, 1982. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may be suitable. Presented in part at the 34th Tobacco Chemists Research Conference, Richmond, VA, Oct 1980. This investigation is in connection with a project at the Kentucky Agricultural Experiment Station, and the results are published as KAES Journal Article 81-3-2-231.

Formation of Alkyl Heteroaromatics in the Pyrolysis of Pyrazylethanol and Pyridylethanol Derivatives

Richard A. Kornfeld* and Yoram Houminer*

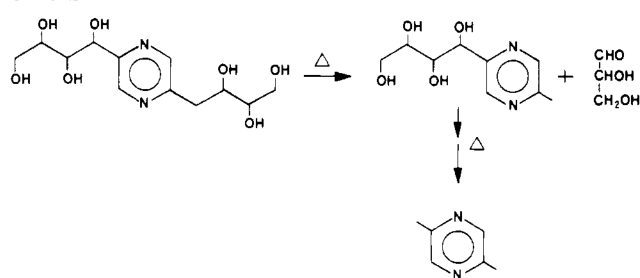
Several substituted pyrazylethanols (1–15) and pyridylethanols (16–18) have been prepared and their pyrolysis at 450 °C has been studied. Each of these compounds decomposes to yield the parent methyl heteroaromatic and the corresponding aldehyde or ketone by way of a retro-ene type mechanism. Characteristic also to the pyrolysis of the pyrazylethanols is the formation of small amounts of 1,2-dipyrazylethanes (19–23), as well as small amounts of dehydration products. The isomeric 2-, 3- and 4-pyridylethanols differ strongly in their thermal reactivities.

Alkyl heteroaromatics such as alkylpyridines and, in particular, alkylpyrazines have desirable flavor properties. These compounds have been detected in a wide variety of food products, especially ones that have undergone cooking or roasting (Ohloff and Flament, 1979). The mechanisms by which these important flavors are formed are still under extensive study. Several pathways have been proposed for the formation of alkylpyrazines in the systems mentioned above. One mechanism involves the reaction of 1,2-dicarbonyl compounds, formed from sugars, with ammonia. A second proposed pathway is the dimerization of α-amino ketones, the latter being formed from the reaction of sugars with amino acids (Garnero, 1980).

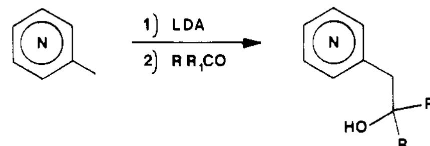
A number of possible intermediates in these reaction schemes have been isolated. One of these compounds, 2,5-deoxyfructosazine has been isolated from natural products such as tobacco leaves (Green et al., 1980; Shigematsu and Kitami, 1978). This compound is also known to be formed in the dehydration of 2-amino-2-deoxy-D-glucose (Eitelman and Feather, 1979). The deoxyfructosazine is proposed to undergo thermal decomposition to form 2,5-dimethylpyrazine in a multistep reaction. One part of this decomposition may involve a retro-ene type reaction as shown in Scheme I.

The role of this retro-ene reaction in the formation of alkyl heteroaromatics has been demonstrated in a few systems (Houminer, 1980; Ohsawa et al., 1979). All of these studies were performed in solution and at relatively low temperatures (<200 °C). The investigations reported here focus on the formation of alkylpyrazines from a large variety of 2-pyrazylethanols as model compounds. The reactions were carried out in the molten state and at a relatively high temperature (450 °C). These studies have also been extended to isomeric pyridylethanols in an attempt to obtain basic pyrolysis data and to correlate structure and reactivity.

Scheme I



Scheme II



Philip Morris U.S.A., Research Center, Richmond, Virginia 23261.

RESULTS AND DISCUSSION

The preparations of the pyrazyl- and pyridylethanols were carried out by reacting the alkyl heteroaromatic anion

 © 1982 American Chemical Society