# FLUE-CURED TOBACCO FLAVOR. II. CONSTITUENTS ARISING FROM AMINO ACID—SUGAR REACTIONS

By J. P. DICKERSON, D. L. ROBERTS, C. W. MILLER, R. A. LLOYD and C. E. RIX
Research Dept., R. J. Reynolds Tobacco Co.
Winston-Salem, N. C. 27102

Twenty-one heterocyclic compounds of the type produced in the Maillard reaction were isolated from flue-cured leaf. Included were seven pyrrole-2-carboxaldehydes, five pyrazines, eight oxygen heterocycles and 2-acetylpyrrole. Sixteen of these compounds were new flue-cured tobacco constituents and eight had not been previously isolated from any type of tobacco. A novel series of N-substituted-2-formylpyrroles, not previously found in natural products, was isolated from flue-cured leaf. A procedure for synthesizing 2-(2-formylpyrrol-l-yl) alkyl acids from 2-formypyrrole and $\alpha$-bromoesters is disclosed.

## INTRODUCTION

The non-enzymic browning or Maillard reaction between amino acids and sugars plays an important role in the development of characteristic flue-cured tobacco flavors and aromas (3). This reaction produces complex mixtures which include volatile compounds and polymeric brown pigments (26). While the exact mechanism of the Maillard reaction and the identity of the reaction products have not been totally elucidated, the initial step in the reaction appears to be the formation of sugar-amino acid compounds *via* an Amadori Rearrangement (9). The labile Amadori products decompose during curing and aging as well as by pyrolysis to form a variety of compounds.

The presence of Amadori compounds in flue-cured tobacco is well documented. Noguchi *et al.* have isolated a number of fructosyl amino acids which arise from the reaction of glucose with an amino acid (24,35,36). These amino acid-sugar compounds account for as much as two percent of the total weight of flue-cured tobacco leaf (35) and are reported to be important contributors to the characteristic aromas of tobacco and cigarette smoke (3).

The nature of the contribution of Amadori compounds to the flavor and aroma of flue-cured tobacco has received little attention. However, studies of model amino acid-sugar reactions have been performed in an effort to determine the mechanism of the browning reaction in food products. As a result, a number of volatile compounds which originate from the Maillard reaction have been identified. In the course of our continuing investigation of tobacco flavor (20,28), many of these same compounds were found in the essential oil of flue-cured tobacco. This report describes the isolation and characterization of heterocyclic flue-cured constituents which are believed to arise from the decomposition of Amadori compounds.

*Contribution received September 5, 1975; Tob. Sci. XX: 59–63, 1976.*

## EXPERIMENTAL

Infrared spectra were run neat on Perkin-Elmer 221 and 267 infrared spectrophotometers. NMR spectra were run with a Varian A60 spectrometer with deuterochloroform as the solvent and tetramethylsilane as the internal standard. Mass spectral data were obtained using a Varian MAT-CH5 mass spectrometer coupled with a Varian Aerograph 1740 gas chromatograph. The column used was a $\frac{1}{8}'' \times 10'$ column packed with 3% OV-101 coated on 100–120 mesh Chromosorb W-AWDMCS (Applied Science Laboratories).

### *Isolation Procedures*

The detailed description of the isolation and characterization of compounds discussed in this paper is presented by Lloyd *et al.* (20). All compounds were isolated from a chloroform extract of flue-cured tobacco. The molecular distillate of the alkaloid-free extract was separated into acidic, basic and neutral fractions. Individual components were isolated by liquid chromatography over silicic acid and gas chromatography. The isolates were identified by comparison of their IR, NMR and mass spectra and GC retention times with those of authentic samples. The syntheses of compounds not previously described in the literature are given below.

### *2-(2-Formyl-5-hydroxymethylpyrrol-1-yl)-3-phenylpropionic acid lactone* 2

Compound 2 was prepared and isolated in *ca.* 1% yield from phenylalanine and D-glucose according to the procedure described by Shigematsu *et al.* (32) for the preparation of pyrrole-lactone 1. Spectral data for 2-(2-formyl-5-hydroxymethylpyrrol-1-yl)-3-phenylpropionic acid lactone: IR 2800, 2730, 1755, 1667, 1500, 1240, 1195, 1045, 960, 815, 750, 720, 700 $cm^{-1}$; MS (relative intensity) $M^+$255(18); 91(100), 255(18), 65(14), 108(10); NMR $\delta$ 9.19 (–C*H*O), 7.38–6.81 (phenyl), 7.10 (–C*H*=C), 6.04 (–C*H*=C), 6.03 (–C*H*–), 4.20 (–C$H_2$–O–), 3.47 (–CH–C$H_2$–Ph).

### *Methyl 2-(2-Formylpyrrol-1-yl) propionate*

Commercial sodium hydride (8.4 g of 57% oil dispersion, 0.2 moles) was washed twice with toluene and then suspended in 350 ml of toluene. To this suspension was added 19 g (0.2 moles) of pyrrole-2-carboxaldehyde. The mixture was refluxed under nitrogen overnight. A solution of 32 g (0.2 moles) of methyl 2-bromopropionate in 200 ml of toluene was added to the sodium salt of pyrrole-2-carboxaldehyde in toluene and the reaction mixture was kept at 65° for four days. At the end of this time 250 ml of water was added and the toluene layer was separated. The toluene solution was washed with an additional 250 ml of water and the combined

aqueous layers were back extracted with ether. The combined ether and toluene solutions were then dried and concentrated. Twenty grams of oil were obtained which by GC and MS analyses were shown to be 89% methyl 2-(2-formylpyrrol-1-yl)propionate. Chromatography of 10 g of the oil on silicic acid gave 7 g of the desired ester. This ester was not identical with the esters of the natural products isolated. Spectral data: IR 2770, 2720, 1745, 1650, 1532, 1210, 1088, 1777, 752 $cm^{-1}$; MS (relative intensity) $M^+$181(21); 122(100), 94(84), 39(36), 41(30), 67(29); NMR δ 9.35 (–C*H*O), 7.2 (–C*H*=C), 6.95 (–C*H*=C), 6.22 (–C*H*=C), 5.77 (–C*H*–), 3.60 ($CH_3$0–), 1.63 ($CH_3$–CH–).

*2-(2-Formylpyrrol-1-yl)propionic acid*

To a solution of methyl 2-(2-formylpyrrol-1-yl)propionate (3.0g) in 25 ml ethanol was added 25 ml of 10% aqueous potassium hydroxide. After stirring for 3 hrs, water was added and the solution was extracted with ether. The aqueous solution was then acidified to pH 5 with 6N hydrochloric acid and extracted thoroughly with chloroform. Concentration of the chloroform extract gave 1.7 g of crude 2-(2-formylpyrrol-1-yl)propionic acid. Spectral data: IR 2770, 2720, 2580 (broad), 1721, 1654, 1370, 1220, 1060, 777, 750 $cm^{-1}$. Attempts to purify the material by decolorization and crystallization resulted in polymerization.

*Methyl 2-(2-Formylpyrrol-1-yl)-3-methylbutyrate*

Pyrrole-2-carboxaldehyde (9.5 g, 0.1 mole) was converted to the sodium salt by the procedure described previously. To this was added 19 g of methyl 2-bromo-3-methylbutyrate and the mixture was heated at 65° for three days and worked up as before. Gas chromatographic analysis showed primarily starting materials, but liquid chromatography on neutral silicic acid allowed the isolation of 74 mg of methyl 2-(2-formylpyrrol-1-yl)-3-methylbutyrate. This material was identical with the methyl ester of the tobacco isolate. Spectral data: IR 2720, 1745, 1667, 1527, 1368, 1206, 1075, 758, 750 $cm^{-1}$; MS (relative intensity) $M^+$ 209(81); 95(100), 209(81), 94(73), 150(64), 55(62); NMR δ 9.57 (–C*H*O), 7.38 (–C*H*=C), 6.93 (–C*H*=C), 6.30 (–C*H*=C), 5.98 (–CH–C*H*–$CO_2$), 3.72 (–$CO_2CH_3$), 2.35 ($CH_3CH$–$CH_3$), 1.0 ($CH_3$–CH–$CH_3$).

The reaction was repeated; a mixture of 1.5 g of pyrrole-2-carboxaldehyde and 3 g of bromoester was heated at 70–90° for seven days. Work-up gave 1.17 g of oil containing about 10% methyl 2-(2-formylpyrrol-1-yl)-3-methylbutyrate. Acidification of the aqueous layer from the extraction and subsequent chloroform extraction gave 0.71 g of acidic material which was shown by NMR to be 37% 2-(2-formylpyrrol-1-yl)-3-methylbutanoic acid (3) and 63% 3-methyl-2-butenoic acid; these were separated by liquid chromatography on silicic acid. Spectral data for 3: IR 2780, 2720, 2570 (broad), 1710, 1655, 1523, 1370, 1252, 1070, 750 $cm^{-1}$; NMR δ 10.8 (–$CO_2H$), 9.53 (–C*H*O), 7.38 (–C*H*=C), 6.95 (–C*H*=C), 6.32 (–C*H*=C), 6.00 (–C*H*–$CO_2$H), 2.4 (C*H*–CH–), 1.05 ($CH_3$–CH–$CH_3$).

*Methyl 2-(2-Formylpyrrol-1-yl)-4-methylvalerate*

Isobutylmalonic acid (34) was brominated (22) to give 2-bromo-4-methylvaleric acid. Methylation of the bromo acid was accomplished by refluxing the acid with 10% boron trifluoride in methanol. Methyl 2-bromo-4-methylvalerate (bp 83°/15 mm) was obtained in 59% overall yield from the malonic acid. Spectral data: IR 1741, 1284, 1200, 1155, 1130 $cm^{-1}$; NMR δ 4.27 (–C*H*Br–), 3.73 ($CH_3$O–), 1.9 (–C*H*– and –$CH_2$–), 0.95 ($CH_3CH$–$CH_3$).

Pyrrole-2-carboxaldehyde (19 g, 0.2 mole) was converted to the sodium salt as described previously; to this was added dropwise 41 g (0.2 mole) of methyl 2-bromo-4-methylvalerate in 200 ml of toluene. The reaction mixture was kept at 70–80° for seven days before being worked up. The neutral material recovered weighed 31.5 g and the acidic material 12.2 g. Liquid chromatography of the neutral material on silicic acid gave 6.7 g of methyl 2-(2-formylpyrrol-1-yl) 4-methylvalerate. This compound was identical with one of the esterified tobacco isolates. Spectral data: IR 2777, 2720, 1741, 1657, 1528, 1370, 1204, 1080, 760 $cm^{-1}$; MS (relative intensity) $M^+$ 223(16.5); 108(100), 41(54), 194(43), 94 (43), 80(37); NMR δ 9.53 (–C*H*O), 7.22 (–C*H*=C), 6.9 (–C*H*–C), 6.25 (–C*H*=C), 6.05 ($CH_2$–C*H*–$CO_2$–), 3.65 (–$CO_2CH_3$), 2.0 (–$CH_2$–), 1.37 ($CH_3$–C*H*–$CH_3$), 0.90 ($CH_3$–CH–$CH_3$).

The acidic fraction was primarily 2-(2-formylpyrrol-1-yl)-4-methylvaleric acid (4) according to NMR and IR. Spectral data: IR 2780, 2720, 2520 (broad), 1720, 1654, 1528, 1370, 1216, 756 $cm^{-1}$; NMR δ 10.88 (–$CO_2H$), 9.38 (–C*H*O), 7.25 (–C*H*=C), 6.95 (–C*H*=C), 6.28 (–C*H*=C), 6.0 ($CH_2$–C*H*–$CO_2$H), 2.0 (–$CH_2$–), 1.40 ($CH_3CH$–$CH_3$), 0.90 ($CH_3$–CH–$CH_3$).

*Methyl 2-(2-Formylpyrrol-1-yl)-3-methylvalerate*

Methyl 2-bromo-3-methylvalerate (bp 74°/7 mm) was prepared from *sec*-butyl malonic acid (34) by the procedures described for the preparation of methyl 2-bromo-4-methylvalerate. Spectral data: IR 1740, 1267, 1200, 1150, 1000 $cm^{-1}$; NMR δ 4.2 (–C*H*Br–), 3.73 ($CH_3$O–), 1.2–2.2 (–$CH_2$–C*H*–$CH_3$), 0.95 ($CH_3$–$CH_2$– and $CH_3$–CH–).

Methyl 2-bromo-3-methylvalerate (41 g, 0.2 mole) in 200 ml of toluene and the sodium salt of 19 g (0.2 mole) of pyrrole-2-carboxaldehyde were allowed to react for five days at 60–70°. Following the usual work-up, the neutral and acidic products were chromatographed on silicic acid to give 99 mg of methyl 2-(2-formylpyrrol-1-yl)-3-methylvalerate and 318 mg of 2-(2-formylpyrrol-1-yl)-3-methylvaleric acid (5), respectively. The ester was shown to be identical with an esterified component of flue-cured tobacco by comparison of the mass spectra. Spectral data for methyl 2-(2-formylpyrrol-1-yl)-3-methylvalerate: IR 2780, 2720, 1742, 1665, 1528, 1341, 1206, 760 $cm^{-1}$; MS (relative intensity) $M^+$ 223(4.7); 41(100), 39(42), 95(32), 108(27), 69(26); NMR δ 9.48 (–C*H*O), 7.32 (–C*H*=C), 6.85 (–C*H*=C), 6.22 (–C*H*=C), 6.0 (CH–C*H*–$CO_2$–), 3.67 (–$CO_2CH_3$), 2.2 (–$CH_2$–C*H*–CH–), 1.3 (–$CH_2$–), 0.9 ($CH_3CH_2$– and $CH_3CH$–). Spectral data for 5: IR 2780, 2735, 2590 (broad), 1725, 1658, 1532, 1376, 1225, 1080, 760 $cm^{-1}$; NMR δ 11.5 (–$CO_2H$), 9.36 (–C*H*O), 7.20 (–C*H*=C), 7.05 (–C*H*=C), 6.3 (–C*H*=C), 6.1 (–CH–C*H*–$CO_2$H), 2.2 (–$CH_2$–C*H*–$CH_3$), 1.3 (–$CH_2$–), 1.0 ($CH_3$–$CH_2$– and $CH_3$–CH–).

## RESULTS AND DISCUSSION

Eight pyrrole derivatives, shown in **Table 1**, of the type produced in the Maillard reaction were isolated from flue-cured tobacco. These included five pyrrole-2-carboxaldehydes 1–5 which had not been previously found in tobacco or any other natural product. The remaining pyrrole-2-carboxaldehydes 6–7 and 2-acetylpyrrole (8) had been isolated from one or more types of tobacco. However, only 2-acetylpyrrole was reported specifically as a flue-cured constituent (10).

The isolation of pyrrole-aldehydes **1** and **4** was particularly significant since these compounds had been isolated from model browning reactions but had not been found in natural systems. Shigematsu *et al.* (32) isolated pyrrole-lactone **1** by pyrolysis of a glucose-alanine mixture. In a similar manner, Kato and Fujimaki (12) isolated pyrrole-acid **4** by pyrolysis of a xylose-leucine mixture. In both cases, the pyrrole derivatives were proposed as important contributors to the characteristic flavors and aromas produced by the Maillard reaction.

The structures of pyrrole-lactones **1** and **2** were confirmed by comparison with authentic samples. Pyrrole **1** gave IR, mass and NMR spectra identical with those reported by Shigematsu *et al.* (32). Pyrrole-lactone **2**, which had not been reported in the literature, gave IR, mass and NMR spectra consistent with the proposed structure. The structure was confirmed by synthesis through pyrolysis of a mixture of L-phenylalanine and D-glucose in the manner described (32) for the preparation of compound **1**. A low yield of pyrrole-lactone **2**, which was identical with the flue-cured isolate, was obtained.

Pyrrole-acids **3–5** were isolated as methyl esters from a mixture of flue-cured tobacco acids which had been treated with diazomethane. Two gas chromatography fractions containing these esters were isolated. The compound in the first fraction was assigned structure **3** on the basis of IR and mass spectra. This structure, which could arise from the reaction of valine with a pentose or a 5-carbon atom hexose degradation product, was confirmed by synthesis. The mass and IR spectra of a second chromatography fraction were similar to those of pyrrole-acid **3**. The molecular weight of the compound from this fraction was consistent with two structures which could be produced by an amino acid-sugar reaction. Neither structure **4**, which is derived from leucine, nor structure **5**, which is derived from isoleucine, could be excluded on the basis of spectral data. Comparison of mass spectra of authentic samples with those of the flue-cured acid fraction indicated that the second chromatography fraction was a mixture of compounds **4** and **5**.

Authentic samples of pyrrole-acids **3–5** were prepared by reaction of the sodium salt of pyrrole-2-carboxaldehyde with the appropriate α-bromoesters. A mixture of the 2-(2-formylpyrrol-1-yl) alkyl acids and methyl esters was obtained from the reaction due to partial saponification of the esters during the reaction and its work up. The 2-(2-formylpyrrol-1-yl)propionic acid was found not to be an isolate in our work although there were indications of other pyrrole-carboxaldehyde compounds being present in flue-cured leaf.

The 3-methylbutyrate (**3**) and the 3-methylvalerate (**5**) derivatives were formed in very low yields due to the steric hindrance of the methyl groups alpha to the reaction site. The hindrance is demonstrated in the mass spectra by the increased intensity of the m/e 95 peak compared with the m/e 94. In the hindered compounds the m/e 95 fragment is the major peak and is larger than the m/e 94. The instability of the pyrrole-acids was another factor which contributed to the low yields of these compounds. On standing at room temperature, the free acids readily formed dark brown pigments. However, the methyl ester derivatives appeared to be stable.

Kato (11, 12) proposed a mechanism for the formation of pyrrole-2-carboxaldehydes from sugar-amine reactions and suggested that the key reaction intermediate was a 3-deoxyosulose (**1**). According to this mechanism, the formation of pyrroles **1–7** is due to the reaction of an amine with a sugar to form an Amadori compound (**9**) which is converted to a 3-deoxyosulose (**10**), as shown in **Figure 1**. Reaction of the amine with the 2-carbonyl of the osulose followed by cyclization and loss of water produces a substituted pyrrole derivative. The substituents on the pyrrole ring are determined by the structure of the sugar and amine precursors. Compounds **1** and **2** are derived from hexoses while compounds **3–7** are derived from pentoses. The type of substituent on the pyrrole nitrogen is determined by the amine precursor. Pyrrole-2-carboxaldehydes **1–5** are obviously derived from amino acids whereas 1-methyl-2-formylpyrrole (**6**) and 2-formylpyrrole (**7**) may be derived from methylamine and ammonia, respectively. However, the possibility that amino acids contribute to the formation of pyrroles **6** and **7** cannot be excluded. The decarboxylation of amino acids in model Maillard reactions has been observed. Thus 1-methyl-2-formylpyrrole (**6**) could arise from the reaction of glycine with a 3-deoxyosulose, followed by decarboxylation. On the other hand, ammonia formed by the deamination of amino acids may contribute to the formation of 2-formylpyrrole.

The formation of significant amounts of 2-acetylpyrrole (**8**) in model glucose-amino acid browning reactions (7, 17, 30) suggests that amino acids serve as a source of ammonia in the Maillard reaction. Although a mechanism for the formation of the acetylpyrrole in amino acid-sugar reactions has not been established, the carbon skeleton of this compound is apparently derived from the sugar precursor. The fact that several different amino acids react with glucose to form 2-acetylpyrrole (7, 17, 30) indicates that the amino acid acts as an ammonia donor in this reaction.

Pyrrole-aldehydes have been proposed as important contributors to the characteristic flavors and aromas of food products (11–13, 32), but the effect of these compounds on the flavor of tobacco smoke has not been previously reported. Pyrrole derivatives isolated from flue-cured tobacco were evaluated as tobacco flavor-

**Table 1. Heterocyclic compounds isolated from flue-cured tobacco**

| Compound | Smoke Flavor | Previous Leaf Isolation Reference |
|---|---|---|
| 2-(5-Hydroxymethyl-2-formyl-pyrrol-1-yl) propionic acid lactone (1)[1] | | |
| 2-(5-Hydroxymethyl-2-formyl-pyrrol-1-yl)-3-phenyl propionic acid lactone (2)[1] | Hot, peppery | |
| 2-(2-Formylpyrrol-1-yl)-3-methylbutanoic acid (3)[1] | | |
| 2-(2-Formylpyrrol-1-yl)-4-methylvaleric acid (4)[1] | Smooth, sweet, nutty | |
| 2-(2-Formylpyrrol-1-yl)-3-methylvaleric acid (5)[1] | Soapy, sweet, slightly floral and fruity | |
| 1-Methyl-2-formylpyrrole (6) | Sweet, cherry, adds body (19) | 4 |
| 2-Formylpyrrole (7) | Sweet, smoothing (19) | 4,29 |
| 2-Acetylpyrrole (8) | Floral, green, winey, adds body (19) | 4,10,29 |
| Methylpyrazine[1] | Dully sweet, aromatic (19) | 4 |
| 2,6-Dimethylpyrazine[1] | Dull herbal sweetness (19) | |
| Trimethylpyrazine[1] | Burley character, sweet, adds body (19) | |
| Tetramethylpyrazine[1] | Burley note, smoothing, mellowing (19) | 4 |
| 2,3-Dimethyl-5-ethylpyrazine[1] | Green, woody note (18) | |
| 5-(Hydroxymethyl)-2-furfural | Sweet, floral, adds body, flue-cured note (19) | 10,33 |
| 2-Furoic acid | Weak, sweet, nutty (19) | 10,15,33 |
| Furfuryl alcohol | Cereal, bran, oily, adds body (19) | 4,33 |
| 5-Methyl-2-furfural | Sweet, adds body (19) | 4,33 |
| 2-Acetylfuran[1] | Green, herbaceous, adds harshness (19) | 4,10,33 |
| 2-Acetyl-5-methylfuran[1] | Sweet, aromatic spicy, enhanced burley note (19) | 4,14,29 |
| Furfural[1] | Sweet, yeasty-bread, buttery (19) | 4,33 |
| Maltol[1] | Sweet (19) | 25,30 |

[1] *Not reported previously as flue-cured constituents.*

Figure 1. Formation of formyl pyrroles from Amadori compounds

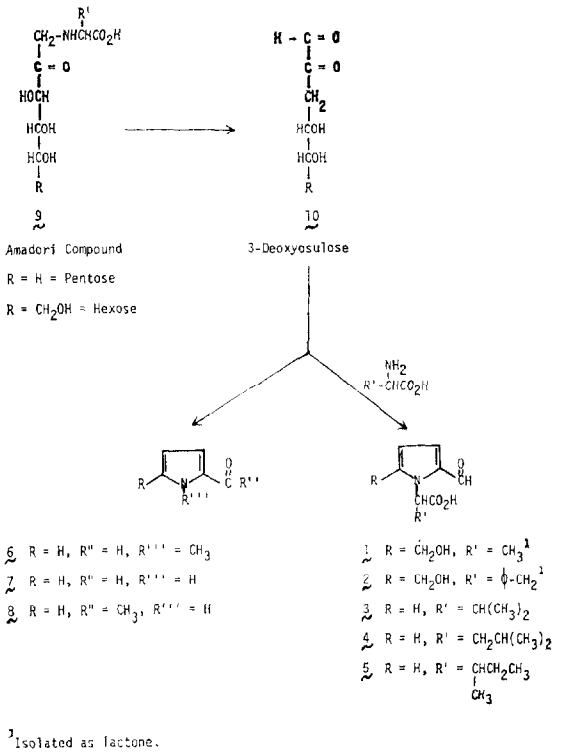


**Figure 1. Formation of formyl pyrroles from Amadori compounds**

ants according to the procedure of Leffingwell *et al.* (19). The contribution of these pyrrole derivatives to the characteristic flavor of flue-cured tobacco smoke is illustrated in **Table 1.**

Five pyrazines of the type produced by amino acid-sugar reactions were isolated from flue-cured tobacco. None of these pyrazines, shown in **Table 1**, has been previously reported as a flue-cured leaf constituent. However, methylpyrazine and tetramethylpyrazine have been isolated from burley leaf (4). Model studies (6, 16) indicate that the reaction of amino acids with aldoses is a major source of pyrazines in roasted food and that the formation of these compounds contributes to characteristic flavors and aromas. Thus, the presence of pyrazines in flue-cured tobacco leaf suggests that part of the contribution of the Maillard reaction to flue-cured flavor and aroma (3) is due to pyrazine formation. The effect of the pyrazines on the smoke flavor of tobacco is described in **Table 1.**

The eight oxygen heterocycles in **Table 1** were isolated from the essential oil of flue-cured tobacco and were evaluated as tobacco flavorants. Four of these compounds—maltol, 2-acetyl-5-methylfuran, 2-acetylfuran and furfural—have not been previously reported as flue-cured constituents. However, each of these compounds has been isolated from other types of tobacco (10, 33). The remaining furan derivatives—5-(hydroxylmethyl)-2-furfural, 2-furoic acid, furfuryl alcohol and 5-methyl-2-furfural—have been reported as flue-cured tobacco constituents (10).

The oxygen heterocycles isolated from flue-cured tobacco are typical products of Maillard type nonenzymic browning reactions (26). All of the isolates of this type in **Table 1** were isolated by Ferretti *et al.* (5) in model studies of a casein-lactose browning system. Although the furan derivatives and maltol may arise from sugar decompositions which do not involve the Maillard reaction (2), the addition of amino acids to sugars enhances the formation of these oxygen heterocycles (8, 26).

## SUMMARY

Twenty-one heterocyclic compounds of the type produced by amino acid-sugar reactions were isolated from flue-cured tobacco. These included eight pyrrole derivatives, five pyrazines and eight oxygen heterocycles. Of these heterocycles, seventeen have not been previously reported as flue-cured constituents and eight have not been found in any type of tobacco.

A novel series of *N*-substituted-2-formylpyrroles, not previously isolated from natural products, was isolated from flue-cured leaf. Two of the pyrrole derivatives, 2-(5-hydroxymethyl-2-formylpyrrol-1-yl) propionic acid lactone and 2-(2-formylpyrrol-1-yl)-4-methylvaleric acid, were reported as products of model amino acid-sugar reaction. The remaining three pyrrole-aldehydes, 2-(5-hydroxymethyl-2-formylpyrrol-1-yl) phenylpropionic acid lactone, 2-(2-formylpyrrol-1-yl)-3-methylbutyric acid and 2-(2-formylpyrrol-1-yl)-3-methylvaleric acid, have not been reported in the literature. A procedure for synthesizing the 2-(2-formylpyrrol-1-yl) alkyl acids from pyrrole-2-carboxaldehyde and α-bromoesters was developed.

The heterocyclic compounds isolated in this study are believed to arise from the decomposition of Amadori compounds during the curing and aging of flue-cured tobacco. We propose that part of the contribution of amino acid-sugar reactions to desirable flue-cured flavor in tobacco smoke is due to the formation of these oxygen and nitrogen heterocycles.

## ACKNOWLEDGMENTS

The authors express their gratitude to J. A. Giles for encouragement and guidance in this work. We thank G. W. Young, F. A. Thome, J. S. White and K. L. Rush for providing necessary spectra and P. H. Ayers for technical assistance. We gratefully acknowledge Mrs. J. H. Dickerson, Mrs. B. P. Hege and F. N. Wendelboe for their assistance in synthesizing some om the compounds described in this paper.

## LITERATURE CITED

1. Anet, E. F. L. J., Degradation of Carbohydrates. III. Unsaturated Hexosones. **Austral. J. Chem.** 15:503–509. 1962.
2. Anet, E. F. L. J., Formation of Furan Compounds from Sugars. **Chem. and Ind.:** 262. 1962.
3. Constantinescu, T., The Possible Contribution of Sugars, Amino Acids and Polyphenols to Formation of Tobacco Aroma and Color. **Ind. Alimentara** (Bucharest) 24:136–139. 1973.
4. Demole, E. and D. Berthet, A Chemical Study of *Burley* Tobacco Flavor (*Nicotiana tabacum* L.) I. Volatile to Medium-volatile Constituents (b.p. ≦ 84°/0.001 torr). **Helv. Chim. Acta** 55:1866–1882. 1972.
5. Ferretti, A., V. P. Flannagan and J. M. Ruth, Nonenzymatic Browning in a Lactose-Casein Model System. **Jour. Agr. Food Chem.** 18:13–18. 1970.
6. Fujimaki, M., M. Tajima and H. Kato, Volatile Basic Compounds Identified from a Heated D-Glucose/L-Alanine Mixture. **Agr. Biol. Chem.** 36:663–668. 1972.
7. Heyns, K., H. Röper and H. Koch, Zur Frage der Entstehung von Nitrosaminen bei der Reaktion von Monosacchariden mit Aminosäuren (Maillard-Reaktion) in Gegenwart von Natriumnitrit 2. Mitteilung. **Z. Lebensm. Unters. Forsch.** 154:193–200. 1974.
8. Hodge, J. E., B. E. Fisher and E. C. Nelson, Dicarbonyls, Reductone and Heterocyclics Produced by Reactions of Reducing Sugars with Secondary Amine Salts. **Am. Soc. Brewing Chemists Proc.:** 84–92. 1963.
9. Hodge, J. E. and C. E. Rist, The Amadori Rearrangement Under New Conditions and Its Significance for Nonenzymatic

Browning Reactions. **J. Am. Chem. Soc.** 75:316–322. 1953.

10. Johnstone, R. A. and J. R. Plimmer, The Chemical Composition of Tobacco and Tobacco Smoke. **Chem. Rev.** 59:885–936. 1959.

11. Kato, H., Chemical Studies on Amino-Carbonyl Reaction III. Formation of Substituted Pyrrole-2-aldehydes by Reaction of Aldoses with Alkylamines. **Agr. Biol. Chem.** 31:1086–1096. 1967.

12. Kato, H. and M. Fujimaki, Formation of *N*-substituted Pyrrole-2-aldehydes in the Browning Reaction between D-xylose and Amino Compounds. **J. Food Sci.** 33:445–449. 1968.

13. Kato, H. and M. Fujimaki, Formation of 1-Alkyl-5-(hydroxymethyl) pyrrole-2-aldehydes in the Browning Reaction between Hexoses and Alkylamines. **Agr. Biol. Chem.** 34:1071–1077. 1970.

14. Kimland, B., A. J. Aasen and Curt R. Enzell, Tobacco Chemistry 10. Volatile Neutral Constituents of Greek Tobacco. **Acta. Chem. Scand.** 26:2177–2184. 1972.

15. Kimland, B., A. J. Aasen, S. O. Almqvist, P. Arpino and C. R. Enzell, Volatile Acids of Sun-Cured Greek Nicotinia Tabacum. **Phytochemistry** 12:835–847. 1973.

16. Koehler, P. E., M. E. Mason and J. A. Newell, Formation of Pyrazine Compounds in Sugar-Amino Acid Model Systems. **Jour. Agr. Food Chem.** 17:393–396. 1969.

17. Langner, E. H. and J. Tobias, Isolation and Characterization of Ether Soluble Sugar-Amino Acid Interaction Products. **Jour. Food Sci.** 32:495–502. 1967.

18. Leffingwell, J. C., Tobacco Flavoring for Smoking Products. II. **Tob. Sci.** 18:55–58. 1974.

19. Leffingwell, J. C., H. J. Young and E. Bernasek, Tobacco Flavoring for Smoking Products. R. J. Reynolds Tobacco Co., Pub. 1972.

20. Lloyd, R. A., Jr., C. W. Miller, D. L. Roberts, J. A. Giles, J. P. Dickerson, N. H. Nelson, C. E. Rix and P. H. Ayers, Flue-cured Tobacco Flavor. I. Essence and Essential Oil Components. **Tob. Sci.** XX: 43–51, 1976.

21. Maga, A. J. and C. E. Sizer, Pyrazines in Food. **CRC Crit. Rev. Food Technol.** 4:39–115. 1973.

22. Marvel, C. S. and V. Du Vigneaud, Organic Syntheses, Collective Vol. 2, Wiley, New York, N. Y.: 93–95. 1943.

23. Neurath, G. and M. Dunger, Isolation of Weakly Basic Heteroaromatic Compounds from Tobacco Smoke. **Beitr. Tabakforsch.** 5:1–4. 1969.

24. Noguchi, M., Y. Satoh, K. Nishida, S. Andoh and E. Tamaki, Studies on Storage and Ageing of Leaf Tobacco. Part IX. Changes in the Content of Amino Acid-Sugar Compounds During Ageing. **Agr. Biol. Chem.** 35:65–70. 1971.

25. Novotny, M. and K. Tesarik, Surface Treatment of Glass Capillaries for Gas Chromatography. **Chromatographia:** 332–333. 1968.

26. Reynolds, T. M. Chemistry of Nonenzymic Browning. 1. The Reaction Between Aldoses and Amines. **Advances in Food Research** 12:1–52. 1963.

27. Rizzi, G. P., Formation of *N*-Alkyl-2-acylpyrroles and Aliphatic Aldimines in Model Nonenzymic Browning Reactions. **J. Agr. Food Chem.** 22:279–282. 1974.

28. Roberts, D. L., C. W. Miller and R. A. Lloyd, Jr., Tobacco Carotenoids. Presented at the 27th Tobacco Chemists' Research Conference, Winston-Salem, N. C. 1973.

29. Roberts, D. L. and W. A. Rhode, Isolation and Identification of Flavor Components of Burley Tobacco. **Tob. Sci.** 16: 107–112. 1972.

30. Scanlan, R. A. and L. M. Libbey, *N*-Nitrosamines not Identified from Heat-Induced D-glucose/L-alanine Reactions. **J. Agr. Food Chem.** 19:570–571. 1971.

31. Schumacher, J. N. and L. Vestal, Isolation and Identification of Some Components of Turkish Tobacco. **Tob. Sci.** 18:43–48. 1974.

32. Shigematsu, H., T. Kurata, H. Kato and M. Fujimaki, Formation of 2-(5-Hydroxymethyl-2-formylpyrrol-1-yl) alkyl Acid Lactones on Roasting Alkyl-α-amino Acid with D-Glucose. **Agr. Biol. Chem.** 35:2097–2105. 1971.

33. Stedman, R. L., The Chemical Composition of Tobacco and Tobacco Smoke. **Chem. Rev.** 68:153–207. 1968.

34. Teague, C. E., Jr., Process of Preparing Sodium Salts of Substituted Malonic Acids. U. S. Patent 2,899,464. 1959.

35. Tomita, H., M. Noguchi and E. Tamaki, Chemical Studies on Ninhydrin-Positive Compounds in Cured Tobacco Leaves III. **Agr. Biol. Chem.** 29:959–961. 1965.

36. Yamamoto, K. and M. Noguchi, Isolation of Sugar Compounds of Asparagine, Phenylalanine, Tyrosine and Valine from Cured Tobacco Leaves. Ibid. 37:2185–2187. 1973.