3-Methyl-2(1*H*)-pyrazinones, the Asparagine-Specific Maillard Products Formed from Asparagine and Monosaccharides

Chi-Kuen Shu* and Brian M. Lawrence

R. J. Reynolds Tobacco Company, Bowman Gray Technical Center, 950 Reynolds Boulevard, Winston-Salem, North Carolina 27105

Four unknown compounds from the reactions of monosaccharides and asparagine have been positively identified by spectroscopic elucidation and synthesis as 3,5-dimethyl-6-ethyl-2(1*H*)-pyrazinone, 3,6-dimethyl-5-ethyl-2(1*H*)-pyrazinone, 3,5,6-trimethyl-2(1*H*)-pyrazinone, and 3-methyl-5,6-diethyl-2(1*H*)-pyrazinone, which are novel to model reaction studies. This group of compounds can be recognized as asparagine-specific Maillard products. The formation mechanism of them has been proposed. Basically, 3-aminosuccinimide, asparagine, and isoasparagine may be at an equilibrium state. Condensation of isoasparagine and α-dicarbonyls (the degradation products of the monosaccharide) leads to the formation of 3-(carboxymethyl)-5,6-dialkyl-2(1*H*)-pyrazinones, decarboxylation of which generates 3-methyl-5,6-dialkyl-2(1*H*)-pyrazinones. Identification of this group of compounds from Maillard reaction was herein reported for the first time in literature.

Keywords: *Asparagine; fructose; glucose; rhamnose; alkylpyrazines; Maillard reaction; 3,6-dimethyl-5-ethyl-2(1H)-pyrazinone; 3,5-dimethyl-6-ethyl-2(1H)-pyrazinone; 3,5,6-trimethyl-2(1H)-pyrazinone; 3-methyl-5,6-diethyl-2(1H)-pyrazinone; 3,6-dimethyl-5-propyl-2(1H)-pyrazinone; 3,5-dimethyl-6-propyl-2(1H)-pyrazinone*

INTRODUCTION

It is well known that Maillard reaction products possess a wide range of interesting aromas. In the literature, a large number of model reactions between amino acids and sugars have been studied and many components identified, of which alkylpyrazines were always readily produced (Vernin and Parkanyi, 1982). It is not surprising because any amino acid can react with an α-dicarbonyl compound arising from sugar degradation to form an α-amino carbonyl via the Strecker degradation. Condensation of these α-amino carbonyls yields alkylpyrazines; however, these alkylpyrazines are nonspecific amino acid Maillard products.

A survey of the literature reveals that only a few publications have described the identification and chemistry of the specific Maillard products that are generated from specific amino acids. Pyrroles and tetrahydroindolizin-6-ones were determined as hydroxyproline-specific Maillard products (Tressl et al., 1985a, 1986). The proline-specific Maillard products determined are 2,3-dihydro-1*H*-pyrrolizines (Tressl et al., 1985b), 7*H*-cyclopenta[*b*]pyridin-7-ones (Helak et al., 1989), 2-(1-pyrrolidinyl)-2-cyclopentenones, and cyclopent[*b*]azepin-8(1*H*)-ones (Tressl et al., 1985c).

The objective of this study was to determine whether asparagine could thermally generate specific reaction products during the course of the Maillard reaction. Specifically, we attempted to identify such products and to propose the formation mechanism. Initially, fructose was chosen to react with asparagine for the identification work, and then other monosaccharides were used to replace fructose for the rest of the study.

EXPERIMENTAL PROCEDURES

Preparation of the Reaction Mixtures. A solution of asparagine (0.05–0.1 mol), monosaccharide (fructose, glucose, rhamnose; 0.05 mol), and water (100 mL) was heated at 200 °C for 1 h in an enclosed reaction vessel (Parr Instrument Co.; Model 4563). The reaction mixture obtained was cooled to room temperature and extracted with methylene chloride (3 × 30 mL), after which the extracts were combined, dried over anhydrous Na_2SO_4, and concentrated to about 1 mL by a rotary evaporator prior to the GC/MS analysis.

GC/MS Analysis. The concentrated extract was analyzed by GC/MS on a DBWAX fused silica column (30 m × 0.32 mm, 0.15 μm film thickness). The oven temperature was programmed from 50 to 190 °C at 6 °C/min; a mass selective detector (EI; 70 eV) was used. Retention indices (I_k) were calculated on the basis of the literature method (Majlat et al., 1974) on the same column.

Preparative GC. The peaks of interest were collected from a preparative glass capillary column (Supelcowax 10; 30 m × 0.75 mm, 1.0 μm film thickness) using a thermal conductivity detector under the same chromatographic conditions.

IR Analysis. The peaks of interest collected from GC were analyzed on a Mattson Polaris FT-IR microscope.

NMR Analysis. Proton NMR spectra were obtained from a 360 MHz spectrometer using $CDCl_3$ as solvent. All chemical shifts are reported in ppm downfield of internal TMS.

Synthesis of 3,5-Dimethyl-6-ethyl-2(1*H*)-pyrazinone and 3,6-Dimethyl-5-ethyl-2(1*H*)-pyrazinone. The procedure was adapted from Karmas and Spoerri (1952) with slight modifications in that α-bromopropionamide instead of ethyl α-bromopropionate was used as the starting material to prepare alaninamide hydrochloride, which was, in turn, reacted with 2,3-pentanedione in the presence of NaOH to form a mixture of both 2(1*H*)-pyrazinones. Each pyrazinone was isolated by GC.

Preparation of 3-Methyl-2(1*H*)-pyrazinones. In an enclosed reaction vessel, 0.05 mol of asparagine, 0.01 mol of α-dicarbonyl compound (diacetyl, 2,3-pentanedione, 2,3-hexanedione, 3,4-hexanedione), and 50 mL of water were placed. The workup procedure was the same as that for the preparation of the reaction mixtures above.

Chemicals. Chemicals were purchased from standard chemical suppliers as follows: α-bromopropionamide, diacetyl, 2,3-pentanedione, 2,3-hexanedione, and 3,4-hexanedione.

RESULTS AND DISCUSSION

At the early stage of the study, identification of the components from the reaction between fructose and

* Author to whom correspondence should be addressed.

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Table 1. Proton NMR Data

2(1*H*)-pyrazinone	chemical shift, ppm
3,6-dimethyl-5-ethyl-	R_3: 2.42 (s, CH_3). R_5: 2.51 (q, CH_2), 1.23 (t, CH_3), both J = 7.5 Hz. R_6: 2.26 (s, CH_3).
3,5-dimethyl-6-ethyl-	R_3: 2.42 (s, CH_3). R_6: 2.54 (q, CH_2), 1.18 (t, CH_3), both J = 7.5 Hz. R_5: 2.24 (s, CH_3).
3,5,6-trimethyl-	R_3: 2.42 (s, CH_3). R_5: 2.22 (s, CH_3). R_6: 2.25 (s, CH_3).
3-methyl-5,6-diethyl-	R_3: 2.43 (s, CH_3). R_5: 2.52 (q, CH_2), 1.19 (t, CH_3). R_6: 2.54 (q, CH_2), 1.24 (t, CH_3), all J = 7.5 Hz.
3,6-dimethyl-5-propyl-	R_3: 2.42 (s, CH_3). R_6: 2.26 (s, CH_3). R_5: 1.15–2.50 (7H, propyl).
3,5-dimethyl-6-propyl-	R_3: 2.42 (s, CH_3). R_5: 2.24 (s, CH_3). R_6: 1.15–2.55 (7H, propyl).

asparagine by GC/MS revealed that alkylpyrazines, alkylpyridines, and alkylimides (such as 3,4-dimethylmaleimide) were predominant. Alkylpyrazines and alkylpyridines are commonly found in most of thermal reactions between reducing sugar and amino acid (Vernin and Parkanyi, 1982). Alkylimides have also been found from the roasting of aspartic acid or asparagine alone without sugars (Wilken and Baltes, 1990). Therefore, all of these three groups of compounds are not asparagine-specific Maillard products.

In addition to those compounds identified, the components possessing retention times of 29 min or greater were unable to be identified because no reference mass spectra were available. When the quantitative information was considered, these unknown peaks totaled over 18%, which implied that they could play an important role in the chemistry of this reaction. Consequently, an attempt was made to identify them.

Examination of the mass spectra of the unknown peaks 1, 2, and 3 indicated that they were homologs with molecular weights of 152 and 166. The IR spectrum of peak 1 collected from GC showed strong absorptions at 1641 and 1605 cm^{-1}, which suggested an amide moiety in the molecule. The proton NMR spectrum of this peak showed two methyl groups (2.26 ppm, s; 2.42 ppm, s) and one ethyl group (1.24 ppm, t, J = 7.5 Hz; 2.51 ppm, q, J = 7.5 Hz). Combination of all the spectral data above strongly suggested that the unknown peak 1 might be a kind of pyrazinone. In order to achieve positive identification of the homolog unknown compounds, synthesis work was then performed.

Using the modified literature method, two pyrazinone isomers were initially synthesized from alaninamide hydrochloride and 2,3-pentanedione as 3,6-dimethyl-5-ethyl-2(1*H*)-pyrazinone and 3,5-dimethyl-6-ethyl-2(1*H*)-pyrazinone. The mass spectra of both isomers were identical to those obtained from unknown peaks 1 and 3. It was apparent that the unknown peaks 1 and 3 were certainly these two isomers; however, no information was available to determine which unknown peak was which isomer. Therefore, both unknown compounds were submitted for proton NMR analyses. The proton NMR data of these isomers (Table 1) are similar to each other. The significant difference between them is that the chemical shift of the methyl proton at C-5 (2.24 ppm) is slightly lower than that at C-6 (2.26 ppm) (MacDonald et al., 1975). As a result, the unknown peak 1 has been assigned as 3,6-dimethyl-5-ethyl-2(1*H*)-pyrazinone and the unknown peak 3 as 3,5-dimethyl-6-ethyl-2(1*H*)-pyrazinone.

On the basis of the identification of these isomers, it was postulated that these pyrazinones were derived from 2,3-pentanedione, which was supposed to be generated from fructose. If this hypothesis was correct, the same pyrazinones should be formed from asparagine and 2,3-pentanedione. In order to test it, the reaction of asparagine and 2,3-pentanedione was performed under the same conditions as those for the reaction of asparagine and fructose. As expected, the same two pyrazinones were formed. The mass spectra, NMR spectra, and GC retention times of these two compounds are the same as those obtained above.

Table 2. Mass Spectra and Retention Indices Data

2(1*H*)-pyrazinone	mass fragment, *m/e* (%)
3,6-dimethyl-5-ethyl-	152 (M^+, 37), 151 (6), 109 (100), 68 (11), 54 (7), 53, (8), 42 (23), 41(11). I_k = 2385.
3,5-dimethyl-6-ethyl-	152 (M^+, 61), 123 (18), 109 (100), 83 (53), 68 (34), 42 (48), 41 (28), 39 (20). I_k = 2445.
3,5,6-trimethyl-	138 (M^+, 66), 110 (34), 109 (100), 95 (34), 68 (22), 54 (26), 42 (66), 41 (26). I_k = 2410.
3-methyl-5,6-diethyl-	166 (M^+, 79), 165 (22), 151 (22), 137 (17), 123 (100), 97 (22), 82 (65), 56 (21), 42 (31), 41 (27), 39 (23). I_k = 2413.
3,6-dimethyl-5-propyl-	166 (M^+, 30), 151 (13), 138 (100), 109 (76), 68 (14), 42 (38), 41 (21), 39 (13). I_k = 2457.
3,5-dimethyl-6-propyl-	166 (M^+, 32), 151 (20), 138 (100), 109 (63),69 (89), 68 (25), 42 (59), 41 (35). I_k = 2537.

Four other homologs were also prepared from asparagine and α-dicarbonyls in order to further prove the formation pathway and the general synthesis procedure. 3,5,6-Trimethyl-2(1*H*)-pyrazinone, 3-methyl-5,6-diethyl-2(1*H*)-pyrazinone, and isomers of 3,6-dimethyl-5-propyl-2(1*H*)-pyrazinone and 3,5-dimethyl-6-propyl-2(1*H*)-pyrazinone were prepared from diacetyl, 3,4-hexanedione, and 2,3-hexanedione, respectively. The NMR data and the mass spectral data along with the retention indices of all the pyrazinones prepared from this study have been compiled in Tables 1 and 2, respectively.

It is interesting to note that the retention times of 3,5,6-trimethyl-2(1*H*)-pyrazinone, 3-methyl-5,6-diethyl-2(1*H*)-pyrazinone, and the unknown peak 2 are very close to each other. Comparison of their mass spectra and retention indices revealed that the unknown peak 2 is composed of 3,5,6-trimethyl-2(1*H*)-pyrazinone and 3-methyl-5,6-diethyl-2(1*H*)-pyrazinone.

Two other monosaccharides, glucose and rhamnose, were also chosen to react with asparagine under the same conditions as those for the reaction of fructose and asparagine. It was found that all of these four 2(1*H*)-pyrazinones were formed from both reactions. Categorically, these compounds are 3-methyl-5,6-dialkyl-2(1*H*)-pyrazinones. Recently, a publication on the reaction of glucose and asparagine did not report these pyrazinones (Bohnenstengel and Baltes, 1992).

The formation mechanism of these compounds from asparagine and monosaccharide is proposed in Figure 1. Basically, 3-aminosuccinimide, asparagine, and isoasparagine may be at an equilibrium state. Condensation of isoasparagine and α-dicarbonyls (the degradation products of the monosaccharide) leads to the formation of 3-(carboxymethyl)-5,6-dialkyl-2(1*H*)-pyrazinones, decarboxylation of which generates 3-methyl-5,6-dialkyl-2(1*H*)-pyrazinones.

CONCLUSION

Four unknown compounds from the reactions between monosaccharides and asparagine have been positively

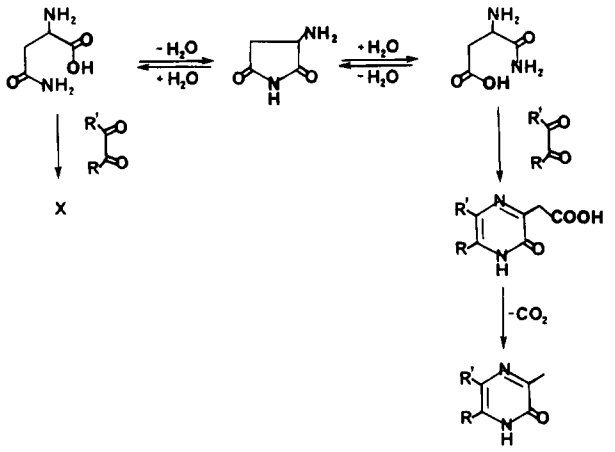

Figure 1. Proposed formation mechanism.

identified by spectroscopic elucidation and synthesis as 3-methyl-5,6-dialkyl-2(1*H*)-pyrazinones, which are novel to model reaction studies. This group of compounds can be recognized as asparagine-specific Maillard products. A formation mechanism for them is proposed. Basically, 3-aminosuccinimide, asparagine, and isoasparagine may be at an equilibrium state. Condensation of isoasparagine and α-dicarbonyls (the degradation products of the monosaccharide) leads to the formation of 3-(carboxymethyl)-5,6-dialkyl-2(1*H*)-pyrazinones, decarboxylation of which generates 3-methyl-5,6-dialkyl-2(1*H*)-pyrazinones. Identification of this group of compounds from the Maillard reaction is herein reported for the first time.

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