predictable way, by chemical modification.

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Hexose–Amino Acid Degradation Studies Involving Formation of Pyrroles, Furans, and Other Low Molecular Weight Products

Philip E. Shaw* and Robert E. Berry

Degradation reactions involving fructose and alanine, fructose and γ-aminobutyric acid, and rhamnose and alanine were carried out at pH 3.5; and the volatile, ether-soluble products, which included pyrrole and furan derivatives, were identified. The only derivatives formed in appreciable quantities by the rhamnose–alanine reaction were 5-methylfurfural and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone. The fructose–amino acid reactions produced several furans plus 2-acetylpyrrole and 5-methylpyrrole-2-carboxaldehyde, both of which have been reported as browning products in stored instant orange juice. A critical step in formation of the pyrroles by the fructose–alanine reaction is believed to be a Strecker degradation of alanine with a 1,2-hexosulose intermediate. By this reaction the hexosulose is reduced and incorporates the nitrogen atom.

Pyrroles and furans have been isolated from many foods whose flavors had been improved or impaired by nonenzymic browning (Shigematsu et al., 1972, and references therein). They are believed to be formed during nonenzymic browning, which results from interaction between sugars and amino acids present (Hodge, 1953). Since many pyrroles and furans have powerful flavors and aromas, more knowledge of their origin might enable food scientists to control their formation to either enhance desirable flavors or minimize off-flavors. Pyrroles and furans were shown to form in dehydrated instant orange juice (IOJ) during storage at ambient temperature (Tatum et al., 1967) and to contribute to its off-flavor which developed during storage (Shaw et al., 1970). In the latter study, *N*-ethylpyrrole-2-carboxaldehyde had the lowest flavor threshold of any browning product found in stored IOJ.

Model studies have shown that pyrroles can be produced by a Maillard-type reaction between hexoses and either amines or amino acids. Thus, Langner and Tobias (1967) identified 2-acetylpyrrole as a degradation product from heated model systems containing dextrose and lysine or lactose and lysine. Ferretti and Flanagan (1971, 1973) identified two 2-acetylpyrroles and three pyrrole-2-carboxyaldehydes from lactose–amino acid model systems. Kato and Fujimaki (1970) heated aqueous, glucose–alkylamine solutions and obtained several pyrrole carboxyaldehydes as degradation products. Shigematsu et al. (1972) roasted a glucose–alanine mixture in the dry state and obtained pyrazine and pyrrole derivatives. Kato (1967) treated pentoses and hexoses with alkylamines and proposed a mechanism for formation of the pyrrole-2-carboxyaldehydes that he found as degradation products of these reactions. The formation of furans in sugar–amino acid model systems has been reviewed by Anet (1964) and by Reynolds (1965). Acid-catalyzed degradations of sugars to furan derivatives have also been studied and related to nonenzymic browning in foods (Reynolds, 1965; Shaw et al., 1967). Many furans are formed from sugars in acid or amino acid catalyzed reactions that involve enolization and dehydration but not reduction, oxidation, or fragmentation (Anet, 1964; Hodge, 1953).

We report the degradation reactions of aqueous fructose–amino acid and rhamnose–amino acid solutions and propose a mechanism for formation of the pyrrole derivatives from hexose–amino acid degradation.

Citrus and Subtropical Products Laboratory, one of the laboratories of the Southern Region, Agricultural Research Service, U.S. Department of Agriculture, Winter Haven, Florida 33880.

EXPERIMENTAL SECTION

General Conditions for Hexose–Amino Acid Degradation Reactions. A solution of 0.81 mol of hexose (D-fructose or α-L-rhamnose) and 0.34 mol of amino acid (DL-α-alanine or γ-aminobutyric acid) in 325 mL of water

Table I. Products from Hexose-Amino Acid Degradation at pH 3.5

Product	GLC peak area percent of ether-soluble products		
	Fructose-alanine	Fructose-GAB[a]	Rhamnose-alanine
Acetic acid		0.81	Tr
Acetylfuran	0.71	0.13	
2-Acetylpyrrole	0.07	0.13	
β-Angelica lactone			0.23
Diacetone alcohol[b]	0.22	3.23	
2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone			11.10
Furfural	1.45	4.42	
Furfuryl alcohol	0.24	0.11	
Hydroxyacetylfuran	3.49	1.76	
Hydroxymethylfurfural	91.45	82.71	
Methylcyclopentenolone	0.13		0.44
5-Methylfurfural	0.39	1.49	87.28
5-Methylpyrrole-2-carboxaldehyde	0.62		
Mol wt 100	0.44		
Total unidentified	0.79	5.23	0.95

[a] GAB = γ-aminobutyric acid. [b] Artifact from acetone solvent.

was adjusted to pH 3.5 by the addition of 6 N HCl, and the resulting solution was refluxed for 5 h. Portions (10 mL) were taken initially and at 1-h intervals during the reaction period for visible absorption measurements at 490 nm. The reaction mixture (pH 3.15) was cooled, 3 N NaOH was added to a pH of 7.0, and the reaction mixture was extracted with three 150-mL portions of ether. The extracts were combined, washed with saturated sodium chloride solution, dried, and concentrated to a small volume to afford a dark-yellow, viscous oil that was separated by chromatographic methods described below. The yield of this oil was as follows: 0.36% from fructose–alanine, 0.33% from fructose–γ-aminobutyric acid, and 5.8% from rhamnose–alanine, based on the amount of sugar employed and the major reaction product.

Chromatographic Methods. Studies of gas chromatography–mass spectrometry were carried out by use of a Varian Model 1400 gas chromatograph coupled to a du Pont Model 21-490 mass spectrometer. A 0.10-in. i.d. × 9 ft stainless steel column packed with 20% Carbowax 20M on 60/80 mesh Gas Chrom P was used at a helium flow rate of 30 mL/min; injection port temperature of 225 °C; and a flame ionization detector with a 1:1 splitter was employed. For quantitative measurements, samples were injected directly onto a similar column in a Hewlett Packard Model 7620 gas chromatograph equipped with a thermal conductivity detector and a Hewlett Packard Model 3380 digital integrator. Helium flow rate was the same as above. Large samples of individual compounds for infrared analysis were isolated by use of a 0.20-in. i.d. × 9 ft stainless steel column packed with 20% Carbowax 20M on 60/80 mesh Gas Chrom P at a helium flow rate of 100 mL/min. The temperature for all runs was programmed from 70 to 210 °C at 4 °C/min. Quantitative data reported are averages of three consecutive runs for each sample.

Thin-layer chromatography (TLC) was carried out on commercially prepared 2 × 8-in. silica gel G plates with development in the organic phase of an equilibrated mixture of benzene, ethanol, water, and ammonium hydroxide (200:47:15:1, by volume) (Tatum et al., 1967). Plates were sprayed with a mixture of sulfuric acid, anisaldehyde, and ethanol (1:1:18, by volume) and were heated in an oven at 120 °C for 4 min for color development.

Spectroscopic Measurements. Infrared (IR) spectra were determined with the samples as either carbon disulfide or chloroform solutions or as oil films on a Perkin-Elmer Model 137 Infracord spectrophotometer.

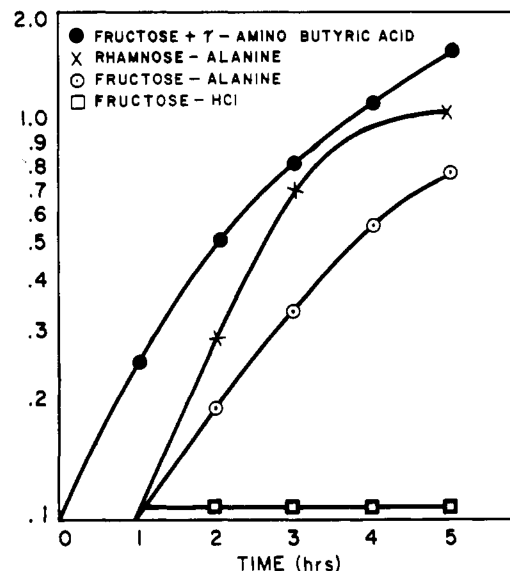


Figure 1. Rate of color formation in hexose–amino acid degradation reactions (log of absorbance vs. time).

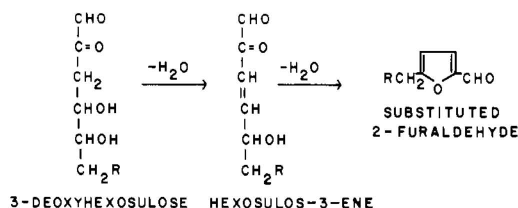
Absorptions of the browning reaction mixtures, appropriately diluted were measured with a Beckman Model DU spectrometer at 490 nm. All compounds were identified by comparison of IR, mass spectra, and GLC retention times with those for authentic samples. Authentic samples were obtained as described previously (Tatum et al., 1967). For the pyrrole derivatives, R_f values and color development by TLC were compared with those reported previously for these compounds (Tatum et al., 1967).

RESULTS AND DISCUSSION

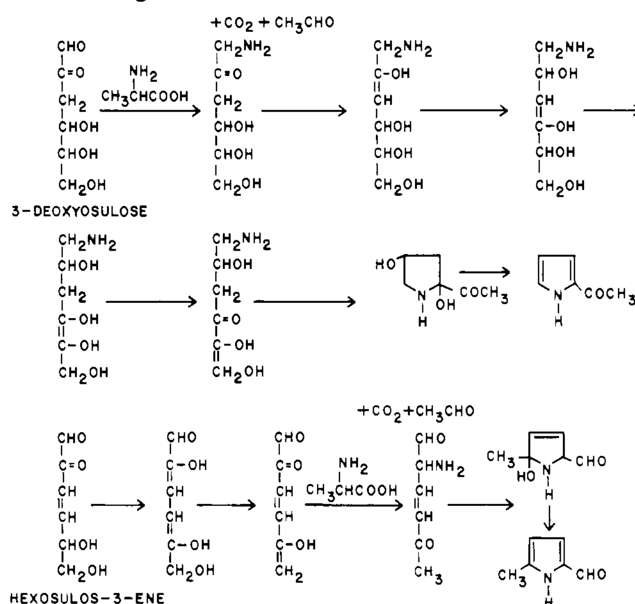
The volatile, ether-soluble components that were formed in the degradation reactions are listed in Table I along with the relative percentages, based on GLC peak areas, in the ether extracts. Although these values are not molar percentages (response factors were not determined), they do indicate approximate relative percentages of the components. These compounds of relatively small molecular weight represent only a fraction of the total products, since the major products of such sugar–amino acid degradation reactions are higher molecular weight melanoidins (Hodge, 1953).

The melanoidins are believed largely responsible for the characteristic brown color that develops in this type of reaction. In Figure 1 the rate of color formation at 490

Scheme I. Degradation of Aldoses and 2-Ketoses to 2-Furaldehydes



Scheme II. Synthesis of the Two Pyrroles by Strecker Degradation



nm was measured for the three degradation reactions studied, as well as for a fructose–HCl degradation reaction (Shaw et al., 1967) which contained no amino acid to form melanoidins. The rate of color formation for the fructose–γ-aminobutyric acid reaction was higher than that for the fructose–alanine reaction, as had been found by Wolfrom et al. (1974) under similar conditions. The rate of color formation for the rhamnose reaction was intermediate between those for the fructose–amino acid reactions after a 1-h induction period. Color formation for the fructose–HCl reaction was very low even though browning did develop, and furan derivatives were isolated (Shaw et al., 1967).

Color development in the three hexose–amino acid reactions did not parallel formation of the volatile, ether-soluble products. Thus, the total yield of ether-soluble material was more than ten times as high in the rhamnose reaction as in either of the fructose reactions. In the rhamnose reaction, 5-methyl-2-furfural was the major ether-soluble product (Table I). This result agrees with the findings of Anet (1964) that hexoses are degraded under acidic conditions through the 3-deoxyosulose by a series of enolizations and dehydrations to the corresponding furan derivatives with no oxidation or reduction steps necessarily involved (Scheme I, R = H). Similarly, fructose afforded 5-hydroxymethylfurfural as the main furan derivative (Table I and Scheme I, R = OH). Both an aldose (rhamnose) and a 2-ketose (fructose) could form the common intermediate, 3-deoxyhexosulose, shown in Scheme I.

The reactions involving fructose produced other furan derivatives besides 5-hydroxymethylfurfural. Their formation, as well as that of acetic acid and methylcyclopentenolone (2-hydroxy-3-methyl-2-cyclopenten-1-one), by acid catalysis, has been discussed previously (Shaw et al., 1967). In the rhamnose–alanine reaction, no furans other than the major component, 5-methylfurfural, were identified. The only other component found in appreciable quantity was 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, a compound first isolated by Hodge et al. (1963) from the reaction of rhamnose with piperidine acetate. The other compounds identified, acetic acid, β-angelica lactone, and methylcyclopentenolone, are expected acid-catalyzed degradation products from hexoses (Shaw et al., 1967).

No pyrrole derivatives were isolated from the reaction between rhamnose and alanine. By analogy with furan formation in Scheme I, and the mechanism proposed by Kato (1967) for formation of pyrrole derivatives from the reaction of 3-deoxyhexosuloses with amino acids, both 2-acetylpyrrole and 5-methylpyrrole-2-carboxaldehyde would be expected products of the reaction between rhamnose and an amino acid (Shigematsu et al., 1972). Such products are expected in a mechanism involving only addition of ammonia, followed by dehydration and enolization without oxidation or reduction.

2-Acetylpyrrole was identified in both fructose reactions. The production of this compound and 5-methylpyrrole-2-carboxaldehyde from fructose and an α-amino acid (α-alanine) could be explained on the basis of currently accepted mechanisms for nonenzymic browning (Scheme II). Thus, the 3-deoxyhexosulose derivative of fructose could undergo Strecker degradation with the α-amino acid, α-alanine, to afford a 1-amino-3-deoxy-2-ketose (Hodge, 1953). In this step, the sugar would be reduced. Then, after a series of enolization and dehydration steps, 2-acetylpyrrole would be formed, as outlined in Scheme II. Similarly, the hexosulos-3-ene from fructose could undergo enolization, Strecker degradation, cyclization, and dehydration to afford 5-methylpyrrole-2-carboxaldehyde, as shown in Scheme II. Strecker degradation would provide the necessary reduction step to enable the production of this derivative from fructose.

2-Acetylpyrrole was the only pyrrole derivative isolated from the reaction between fructose and γ-aminobutyric acid. Since an α-amino acid is required for Strecker degradation, the mechanism for formation of 2-acetylpyrrole in this reaction is not as straightforward. Addition of ammonia (from the amino acid) to the 3-deoxyhexosulose, as suggested by Kato (1967), would require reduction at some other point in the degradation for the formation of 2-acetylpyrrole from fructose.

One other pyrrole derivative that we hoped to find in this study was *N*-ethylpyrrole-2-carboxaldehyde, which has been found in stored IOJ (Tatum et al., 1967). Theoretically, alanine could provide the *N*-ethyl group needed through decarboxylation. No *N*-ethylpyrrole-2-carboxaldehyde could be seen in any of these reactions as determined by GLC and use of an authentic sample for comparison of retention times. Shigematsu et al. (1972) found this pyrrole derivative by roasting glucose with alanine at a much higher temperature (250 °C) than that used in our study.

Thus, the formation of two pyrrole derivatives found in stored IOJ could be explained by the model studies described. Of the pyrroles associated with browning in stored IOJ, the most potent in flavor, *N*-ethylpyrrole-2-carboxaldehyde, probably is formed by some mechanism other than that involving a Strecker degradation between the hexoses and amino acids present in IOJ.

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On the Occurrence of *N*-Methyl-*N*-formylhydrazones in Fresh and Processed False Morel, *Gyromitra esculenta*

Heikki Pyysalo* and Aimo Niskanen

Nine volatile *N*-methyl-*N*-formylhydrazones were identified from fresh false morel mushrooms, *Gyromitra esculenta*, at an average combined level of 57 mg/kg. Using high-resolution glass capillary GLC, a method was developed for the control of hyrazone residues in false morel products. Investigation was made of the decrease of levels of hydrazones during boiling and drying processes and of the effect of process conditions on the level of toxin residues. After prolonged drying, the level of hydyrazone residues fell below 3 mg/kg of dried mushroom, and a boiling time of 10 min was required to reduce the level below 1 mg/kg. The results were compared with measured values of the toxicity of the main component of the residues, acetaldehyde *N*-methyl-*N*-formylhydrazone, and with an estimation of the suitability of false morel as an edible mushroom.

False morel, *Gyromitra esculenta* (Pers.) Fr., is a widely distributed spring mushroom eaten dried or boiled in many countries as a choice edible mushroom. Commercial products are also prepared from this mushroom. Fresh false morel is highly poisonous, and raw or incompletely processed mushrooms have caused many fatal cases of food poisoning (Mlodecki et al., 1962; Franke et al., 1967). The toxicity of false morel has been reported to result not from the so-called helvella acid (Boehm and Kuelz, 1885), but from a compound named gyromitrin. In its structure, gyromitrin is a hydrazone, i.e., acetaldehyde *N*-methyl-*N*-formylhydrazone (acetaldehyde MFH) (List and Luft, 1968a). In addition to acetaldehyde MFH, three of its higher homologues have also been isolated from the steam distillate of *Gyromitra esculenta*. These homologues have been shown to be compounds **4**, **5**, and **6** in Table I, (Pyysalo, 1976a).

The toxicity of acetaldehyde MFH has recently been shown to be for rabbits about LD = 70 mg/kg, for rats about 320 mg/kg, and for chickens over 400 mg/kg (Mäkinen et al., 1977). In a 90-day "short-term" test, peroral administration of acetaldehyde MFH was employed and "no-effect" values obtained were 0.05 mg kg^{-1} day^{-1} for chickens and 0.5 mg kg^{-1} day^{-1} for rabbits (Niskanen et al., 1976). In preliminary animal tests the known higher MFH homologues **4**, **5**, and **6** were shown to have lower values of acute toxicity for rabbits than did acetaldehyde MFH itself (Pyysalo, 1975).

As considerable residues of acetaldehyde MFH have been detected in dried and boiled false morel, the suitability of the mushroom as a commercial food product has been doubted (Gray, 1972; List and Sundermann, 1974; Schmidlin-Meszaros, 1974, 1975; Pyysalo, 1976b). On the other hand, the toxic properties of acetaldehyde MFH and the amount of MFH homologues in false morel have not been known until recently, and the reported results of quantitative estimations of acetaldehyde MFH differ considerably from the results obtained in the present work.

This report is part of a program of research dealing with the toxicity of false morel. The aim has been to develop a sensitive method for estimation of *N*-methyl-*N*-formylhydrazone (MFH) which would be suitable for process control and to clarify the processing conditions necessary to eliminate hydrazone residues or reduce their amount to acceptable levels on the basis of results from animal experiments.

Technical Research Centre of Finland, Food Research Laboratory, Biologinkuja 1, SF-02150 Espoo 15, Finland.

EXPERIMENTAL SECTION

Materials and Processing Conditions. Fresh, fully grown false morel mushrooms, pileus diameter approximately 5 cm, were used. The mushrooms were collected in early June in southern Finland. Drying tests were carried out in dry outdoor air (15–20 °C), in a heat-controlled chamber supplied with through-flow draught and in a commercial fruit and vegetable drier, in which a strong controlled-temperature air current was passed through the mushrooms. In boiling tests a 50-g sample was placed in boiling water, after which boiling resumed in 30 s. The moment of resumption of boiling was taken as zero. After boiling, the mushrooms were filtered and rapidly rinsed once with running tap water.

Quantitative Estimation of *N*-Methyl-*N*-formylhydrazones Using GLC. A known amount of compound **5** was added to the mushrooms as an internal standard and was allowed to adsorb into the mushroom material in a Soxhlet apparatus. MFH compounds were extracted for