Some Compounds Formed during Nonenzymic Browning of Orange Powder

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An analytical study was made of nonenzymic browning products formed in stored dehydrated orange powder. The isolation of eight furan-type compounds, three pyrroles, three acids, one lactone, and methylcyclopentenolone is reported. All the compounds were separated by GLC and identified by spectroscopic methods in comparison with authentic samples.

Because of increasing expansion of the dehydrated food industry, the effects of storage on the chemical composition of dehydrated food products are of increasing interest (*18*). Many foods are susceptible to nonenzymic browning, especially orange powder (*5*) which undergoes undesirable changes in odor and flavor during storage at ambient temperatures (*4*). In many dehydrated materials of high carbohydrate content, these changes are directly related to the degradation of sugars, as described by Anet (*1*).

Because orange powder is such a complex mixture, a preliminary study of model systems is helpful for developing methods of analysis and suitable techniques. A recent report from this laboratory (*16*) described the identification of 11 compounds that were products of nonenzymic browning in a fructose-acid model system.

Analytical methods developed in the model system study were applied to orange powder and the isolated compounds were compared to those previously found in the model study. The results are the subject of this report. More specifically, this work was carried out to determine what degradation products were formed in stored and heated orange powder and which of these might be responsible for objectionable odors and flavors. Besides clarifying or explaining the mechanism by which such changes in composition may occur, the results of such studies may provide leads for inhibiting or blocking the responsible reactions.

EXPERIMENTAL

Chromatographic Methods. GLC. Gas-liquid chromatography using stainless steel columns was carried out with the following conditions: A 9-foot × 0.25-inch Carbowax 20M packed column (20% on 60- to 80-mesh Gas Chrom P) was operated at a helium flow rate of 180 ml. per minute. The temperature program was as follows: Initial temperature was 80° C. for 6 minutes, the temperature was increased to 130° C. at 6 minutes, 135° C. at 14 minutes, 140° C. at 24 minutes, 155° C. at 30 minutes, 180° C. at 46 minutes, 190° C. at 56 minutes, 200° C. at 64 minutes, and 215° C. at 76 minutes. All temperature increases were at the rate of 60° C. per minute, and all times were from the moment the sample was injected on the column.

The instrument employed was an F & M Model 810, equipped with a dual-flame dual column and a 5 to 1 effluent splitter. Samples were collected at the exit port which has a male luer lock with a hypodermic needle attached. Capillary tubes were placed over the needle and cooled with liquid nitrogen from below to condense the sample.

TLC. Thin-layer chromatography was carried out as described by Berry and Tatum (*4*). The only change was in the solvent system. Plates were developed in an equilibrated mixture of benzene, ethanol, water, and ammonium hydroxide (200:47:15:1, by volume), using the top organic phase.

Spectrophotometric Methods. The infrared spectra were run neat or in CS_2 on a Perkin Elmer 137 spectrophotometer. The ultraviolet spectra were run on a Cary-14 recording spectrophotometer. The mass spectra were determined on a Bendix Time-of-Flight 12-100 instrument.

Extraction and Solvent Separation. Orange powder prepared on a crater-type foam-mat dryer (*3*) was sealed in No. 2 cans ($3^7/_{16} \times 4^9/_{16}$ inches) containing 168 grams per can and placed in 37.5° C. storage. After two months, the samples were treated in the following manner: To 168 grams of orange powder were added 300 ml. of 50% acetone–water. This was mixed for 10 minutes with a Brookfield counterrotating mixer, Model L998, and then allowed to settle for 20 minutes at room temperature. This mixture was extracted with one 300-ml. portion of diethyl ether and then with three successive 200-ml. portions in an open beaker, mixing for 4 minutes for each extraction. The ether layer was decanted each time. To the combined ether extracts were added 100 ml. of water. This mixture was evaporated under vacuum using only tap water as a heat source until most of the ether and acetone was removed. The remaining water fraction was filtered through Hyflo Super-Cel to remove the carotenoids and other water-insoluble material. Fifty grams of NaCl were added to the water fraction which was extracted after 10 minutes with four 150-ml. portions of diethyl ether. The ether extract was dried and the ether removed under vacuum using tap water as a heat source. Twelve 168-gram samples of powder were extracted by this procedure and the residues combined. This combined residue (about 2.5 grams) was then connected to a liquid nitrogen trap and distilled at 60° C. and 1 mm. of Hg. The liquid nitrogen trap was washed with ether and the washings were transferred to a flask. The ether was removed under vacuum using tap water as a heat source. When the ether had evaporated, the flask was immediately removed and stored in a freezer. The GLC analysis was run on this fraction.

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Synthesis of Compounds. *N*-ETHYLPYRROLE-2-CARBOXALDEHYDE. To 0.10 gram (2 mmoles) of 50% sodium hydride dispersion (Metal Hydrides, Inc., Beverly, Mass.) in 2 ml. of dimethylformamide was added 0.19 gram (2 mmoles) of pyrrole-2-carboxaldehyde (K&K Laboratories, Inc., Plainview, N.Y.) in 2 ml. of dimethylformamide. After 1 hour at room temperature, a solution of 0.31 gram of ethyl iodide in 1 ml. of dimethylformamide was added to the mixture. The reaction was complete after 1.25 hours as shown by thin-layer chromatography. The product was isolated by GLC of the crude reaction mixture and was identical in retention time to compound 9 of Table I (*15*); *J* (oil film) 1660(s), 1520(w), 1475(m), 1400(m), 1365(m), 1320(m), 1220(w), 1070(m), 765(s), 745 cm.$^{-1}$(s); *m/e* 123, 94, 39, 108, 122, 106 (listed in order of decreasing intensity).

5-METHYLPYRROLE-2-CARBOXALDEHYDE (*9*, *17*). Dimethylformamide (1.6 grams) was cooled in an ice bath as 3.4 grams of phosphorus oxychloride was added dropwise. The solution was stirred for 15 minutes at room temperature and then cooled in ice as 5 ml. of methylene dichloride was added. When the solution had cooled to 5°, 1.6 grams of 2-methylpyrrole (*6*) in 15 ml. of methylene dichloride was added dropwise and the resulting solution was refluxed for 15 minutes, cooled, and treated with 15 grams of sodium acetate in 20 ml. of water, and the layers were separated. The aqueous layer was made alkaline, and extracted with methylene dichloride, and the extract was dried (Na_2SO_4) and concentrated to a small volume. Separation by GLC yielded 5-methylpyrrole-2-carboxaldehyde as the main product. The bulk of the crude product crystallized on standing. Two recrystallizations from acetone gave massive prisms, m.p. 67–68.5° C. (0.11 gram); *J* (CS_2) 3170(s), 2740(m), 1640(s), 1190(s), 1045(s), 805(s), 775 cm.$^{-1}$(s); *m/e* 109, 108, 80, 53, 52.

2-ACETYLPYRROLE was prepared by the method of Berlin (*2*), m.p. 88–88.5° C.: *J* (CS_2), 3200(s), 1630(s), 1130(s), 1040(s), 928(s), 745 cm.$^{-1}$(s); *m/e* 94, 109, 66, 39, 43.

2-HYDROXYACETYLFURAN was synthesized by the procedure of Kipnis, Soloway, and Ornfelt (*12*) and Miller and Cantor (*14*), m.p. 78–9° C.

RESULTS AND DISCUSSION

Nineteen compounds have been identified from an extract of stored orange powder which had previously given relatively large amounts of hydroxymethylfurfural (*4*). These constituents were separated from the hydroxymethylfurfural by vacuum distillation and then separated from each other by gas chromatography. Table I lists these compounds along with their chromatographic data and the character of their odors.

A comparison of the results of a fructose-acid model system (*16*) with the current study shows the compounds in the orange powder apparently may be formed in part by acid degradation of sugars. Eight of the compounds found in the model system study also were found in the orange powder (Table I, footnote *a*). There are probably compounds other than sugars that can degrade to give the same products. Eight compounds were found in the stored powder that were not present in the model system or in the fresh powder. There were three compounds found in the powder which were probably artifacts.

The identity of most compounds was confirmed by comparing their infrared and ultraviolet spectra, mass spectral cracking patterns, R_f on thin-layer chromatography, and retention times on Carbowax 20M with those of commercially available samples. When authentic samples

Table I. Compounds Found in the Analysis of Stored Orange Powder

		TLC		GLC	
		R_f 15 cm.	Color with spray	R_t[a], min.	Odor
1	Furfural[b]	0.94	Blue	17	Sharp
2	2-Acetylfuran	0.94	Red-brown	19.5	
3	5-Methyl-2-furfural[b]	0.90	Blue	23.5	Sharp grape
4	Furfuryl alcohol	0.66	Purple	29	Coconut
5	2-Hydroxyacetylfuran[b]	0.53	Blue	55.2	
6	4-Hydroxy-2-hydroxymethyl-5-methyl-3(2H)-furanone[b]	0.33	Yellow	71	Charred sugar
7	Hydroxymethylfurfural[b]	0.38	Green	87	
8	Acetylformoin[b]			19.5	Burnt sugar
9	*N*-ethylpyrrole-2-carboxaldehyde	0.99	Yellow	26.6	
10	2-Acetylpyrrole	0.83	Yellow	52.8	
11	5-Methylpyrrole-2-carboxaldehyde	0.79	Yellow	60	Sharp
12	Acetic acid[b]			15	
13	Levulinic acid[b]	0.44	Green	75.2	
14	Benzoic acid	0.53		83	
15	α-Angelica lactone[b,c]	0.92	Blue, green	16.2	
16	β-Angelica lactone[b,c]	0.80	Blue	31.2	
17	γ-Butyrolactone			28	Rancid oil
18	Valencene[c]			35.2	
19	Methylcyclopentenolone	0.66	Yellow	41.6	Maple sirup
20	Diacetone alcohol[c]			12	

[a] Carbowax 20M.
[b] Also found in fructose-acid model system (*16*).
[c] Not degradation products.

were not available they were synthesized in our laboratory. For final identification, synthesis was required of compounds 5, 8, 9, and 11 from Table I (see Experimental).

The following furans were identified (see Table I): furfural, 2-acetylfuran, 5-methyl-2-furfural, furfuryl alcohol, hydroxyacetylfuran, and hydroxymethylfurfural. The first four have been reported in coffee oil by Gianturco (*9*). 4-Hydroxy-2-hydroxymethyl-5-methyl-3(2*H*)-furanone was also found. This compound has not been previously reported from any source except as a product from acid degradation of fructose (*16*). Acetylformoin (4-hydroxy-2,3,5-hexanetrione) also was present, and for its identification the reference sample was synthesized by the method of Goto (*10*).

Three pyrroles (*N*-ethylpyrrole-2-carboxaldehyde, 2-acetylpyrrole, and 5-methylpyrrole-2-carboxaldehyde), three carboxylic acids (acetic, levulinic, and benzoic), and three lactones (α- and β-angelica lactone and γ-butyrolactone) were also identified. The α- and β-angelica lactones were probably artifacts formed from levulinic acid during distillation (*13*). 2-Acetylpyrrole and 5-methylpyrrole-2-carboxaldehyde have been reported as constituents of coffee oil (*9*).

Diacetone alcohol, valencene, and methylcyclopentenolone were the remaining identified compounds. Methylcyclopentenolone had been reported previously as a flavor component of maple sirup (*7*) and also was found in coffee oil (*8*) but not in orange juice.

A control sample of orange powder which had been kept at $-20°$ C. and which had not undergone browning was extracted by the procedure used on stored powder. Of the compounds reported in this study, only diacetone alcohol and valencene were identified from this extract. Valencene is a sesquiterpene found in orange oil (*11*) and its presence in control powder indicates incomplete removal during the dehydration process. Diacetone alcohol may have formed during the extraction procedure, since a large volume of acetone is required.

In the extract from the stored orange powder, there remained 17 unidentified peaks on the GLC chromatogram. Most of these were present in trace amounts and only two had disagreeable odors. Of the 19 compounds identified in this study, 13 have not been previously reported as constituents of orange powder or as products of nonenzymic browning of orange juice.

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