

Base-Catalyzed Fructose Degradation and Its Relation to Nonenzymic Browning

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Aqueous fructose solutions were degraded with alkali either by adding additional base as needed to maintain alkalinity or by allowing the initially alkaline mixture to become acidic as the reaction progressed. Fifteen ether-soluble products were identified in each case, and 12 of these were found in both reactions. The products included two furfuryl alcohols, two furanones, hydroxyacetone,

three hydroxybutanones, three carboxylic acids, four cyclopentanediones, one cyclohexanedione, γ -butyrolactone, and 2,5-hexanedione. A mechanism for formation of the cyclic diketones from three- and four-carbon hydroxyketones is presented. The relationship of these results to nonenzymic browning of foodstuffs such as coffee, pineapple juice, maple sirup, and dehydrated citrus powders is discussed.

Increasing interest in processed food products has led to a greater need for fundamental information about nonenzymic browning in foods (Talbert, 1961). One approach to obtaining this information has been to study model systems containing a few of the constituents of a particular foodstuff. In addition to end-product brown polymers which form, intermediate products of lower molecular weight are also produced (Reynolds, 1963). Because of their volatility and greater reactivity these intermediates are more likely to influence flavors and odors of the product.

Chemical changes in dehydrated citrus powders during storage at ambient temperatures are under investigation at our laboratory (Berry and Tatum, 1965). From a fructose-acid model system a previous report described some low molecular weight degradation products and their relationship to nonenzymic browning products of dehydrated orange powder (Shaw *et al.*, 1967; Tatum *et al.*, 1967a). From that study there remained some browning products from stored orange powders, which had not been present in the model system. Thus, the model studies were extended to include base-catalyzed fructose degradation in an effort to identify some of those remaining compounds. Some of the products formed by base-catalyzed fructose degradation, the mechanism of their formation, and their role in nonenzymic browning are the subject of the current report.

PROCEDURE

Ultraviolet spectra were recorded in 95% ethanol on a Cary 14 spectrophotometer, infrared spectra were obtained on a Perkin-Elmer 137 Infracord, mass spectra were determined with a Bendix Model 12-100 time-of-flight mass spectrometer, and pH measurements were made with a Beckman Zeromatic pH meter.

Chromatography. Gas-liquid chromatography (GLC) was performed on an F & M Model instrument using $\frac{1}{4}$ -inch stainless steel columns packed with 20% Carbowax 20M on 60- to 80-mesh Gas-Chrom P under two sets of conditions. For the 9-foot column, helium flow was 150 ml. per minute and the temperature program was: initial temperature 80° C. for 6 minutes, 130° at 6 minutes at a rate of 60° per minute, then at 16 minutes increase was 2° per minute to 216°. For the 18-foot column, helium flow was 180 ml. per minute. All temperature increases were at 60° per minute and the temperature program was: initial temperature 80° C. for 6 minutes, 130° at 6 minutes, 135° at 14 minutes, 140° at 18 minutes, 155° at 22 minutes, 165° at 36 minutes, 180° at 54 minutes, 190° at 64 minutes, 200° at 68 minutes. The instrument was equipped with a dual-flame dual-column and a 5-to-1 effluent splitter. GLC samples were collected and thin-layer chromatography (TLC) was carried out as described by Tatum *et al.* (1967a).

Reagents. Authentic samples of the following compounds as listed in Table I were obtained from the source indicated and were purified by GLC prior to their use: compounds 1, 7, 8, and 9 from Eastman Organic Chemi-

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cals, Rochester, N. Y.; compounds 2, 5, and 13 from K and K Laboratories, Inc., Plainview, N. Y.; compounds 4, 6, and 18 from Fisher Scientific Co., Fair Lawn, N. J.

The comparison sample of compound 17 had been isolated in earlier fructose-acid degradation studies (Shaw *et al.*, 1967).

Authentic samples of the following two compounds were prepared according to procedures in the references cited: 3,4-dimethyl-2-hydroxy-2-cyclopenten-1-one (Gianturco and Friedel, 1963); 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Henry and Silverstein, 1966).

5-Methyl-2-furfuryl alcohol was prepared by the reduction of 5-methylfurfural with sodium borohydride and purified by GLC.

1-Hydroxy-2-butanone. To 0.1 gram of sodium hydroxide in 0.5 ml. of water was added 0.5 ml. of 1-hydroxy-2-butanone acetate and the resulting mixture stirred at 50° until a clear solution resulted (3 minutes). After an additional 25 minutes at room temperature, a portion of the reaction mixture was subjected to GLC to give 1-hydroxy-2-butanone; infrared (CS₂), 3440 m, 1720 s, 1400 m, 1345 m, 1270 m, 1090 s, 1030 s, 980 s, 743 cm.⁻¹ w; *m/e* (%), 88 (16), 57 (88), 56 (10), 31 (65), 29 (100), 27 (51).

Fructose-Base Degradation. KEPT ALKALINE. A solution of 240 grams of D-fructose in 440 ml. of water was adjusted to pH 11.5 with 50% sodium hydroxide solution. The solution was boiled for 3 hours and the pH checked every 15 minutes. Sodium hydroxide solution was added as required to keep the pH above 8 at all times. The reaction mixture was cooled (the pH was about 8 at that point), 50 grams of sodium chloride were added, and the mixture was extracted with three 250-ml. portions of ether. The combined extracts were dried over sodium sulfate and the ether was removed under reduced pressure. The residue (0.89 gram) was connected to a liquid nitrogen trap and distilled at 60° and 1 mm. for 1 hour. This distillation step was found necessary to prevent deposition of non-volatile materials on the GLC column (Langner and Tobias, 1967). The material which condensed in the trap (0.15 gram) was dissolved in ether and concentrated to a small volume. This solution was used for GLC separation. There remained 0.71 gram of undistilled pot residue which could be shown by GLC to contain the compounds present in the distillate as well as nonvolatile material. All products except 10, 12, and 15 (Table I) were identified by GLC retention time and infrared and mass spectral comparison with authentic samples. These three compounds were identified by infrared comparison with published spectra (Gianturco *et al.*, 1963).

NOT KEPT ALKALINE. The above fructose degradation reaction rapidly became acidic if additional base was not added to maintain alkalinity. Thus, the pH dropped to about 8 after 10 minutes of heating, to about 6 after 30 minutes, and was 5.75 after the 3-hour heating period. The reaction mixture was worked up and distilled as described above and the products were separated by GLC and identified.

Condensation of Hydroxyketone Mixture. 1-Hydroxy-2-butanone was prepared as described above from 3 ml. of 1-hydroxy-2-butanone acetate, 0.6 gram of sodium hydroxide, and 3 ml. of water. The crude reaction mixture was used in the following reaction.

The 1-hydroxy-2-butanone solution, thus prepared, was diluted with 440 ml. of water and 1 ml. of 3-hydroxy-2-butanone, 1 ml. of 1-hydroxy-3-butanone, and 12 ml. of 50% aqueous hydroxyacetone were added. The pH was 6 at that point and was raised to 10 by adding 50% sodium hydroxide solution dropwise. The solution was heated on a steam bath for 3³/₄ hours and sodium hydroxide solution was added as required to maintain pH 9 or higher. The reaction mixture was cooled, adjusted to pH 7.4 with 1*N* hydrochloric acid, and extracted with ether. The ether extract was concentrated and the dark-brown oily residue was separated on both the 9- and 18-foot GLC columns.

Both 2-hydroxy-3-methyl-2-cyclopenten-1-one (13) and 3,4-dimethyl-2-hydroxy-2-cyclopenten-1-one (14) were obtained as single peaks from the GLC column and were identical to authentic samples as shown by retention time, infrared and mass spectra, and TLC comparison. 3,5-Dimethyl-2-hydroxy-2-cyclopenten-1-one (12) was the major component in a peak collected at the proper GLC retention time as shown by infrared, mass spectral, and TLC comparison with a sample isolated from fructose-base degradation. The main impurity had a molecular weight of 140 as shown by the mass spectrum. Similarly, 3-ethyl-2-hydroxy-2-cyclopenten-1-one (15) was obtained from the GLC column as the major component in a peak at the proper retention time as shown by infrared, mass spectral, and TLC comparison with a sample isolated from fructose-base degradation. From TLC two impurities were observed, and the mass spectrum showed impurities of mass 140 and 152 to be present. Other GLC conditions were tried to improve separation but without success.

RESULTS AND DISCUSSION

Base-catalyzed degradation of D-fructose was carried out under two sets of conditions; the products identified in both cases are listed in Table I. In the first case, alkalinity was maintained during the reaction by adding sodium hydroxide solution periodically. In the second case, when additional base was not added, the reaction mixture rapidly became acidic. This was probably due to the formation of metasaccharinic acids which are the expected major products of such a base-catalyzed sugar degradation (Anet, 1964). Ether extraction in both cases separated the ether-soluble minor products from the saccharinic acids and from any unreacted fructose. Individual components of the ether extract were isolated by GLC separation.

From the fructose-base degradation where alkalinity was maintained, the 15 products identified are listed in Table I in order of their GLC retention times. In contrast to previous fructose-acid model system studies where eight furan derivatives were identified (Shaw *et al.*, 1967), only three were found in this fructose-base study, and none of the three had been formed by acid catalysis. These were furfuryl alcohol, 5-methyl-2-furfuryl alcohol, and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone. The latter compound has previously been reported as a rhamnose degradation product (Hodge *et al.*, 1963) and as a component of pineapple flavor concentrate (Rodin *et al.*, 1965).

Degradation products 10, 12, 13, 14, and 15 in Table I are the five cyclic diketones previously found in the "aroma complex" of roasted coffee (Gianturco *et al.*, 1963). All but compound 10 were major ether-soluble degradation

Table I. Base-Catalyzed Fructose Degradation Products

Compound	GLC, R_t , Min.		R_f	TLC Color with spray
	Kept alkaline ^a	Not kept alkaline ^b		
1 3-Hydroxy-2-butanone ^c	19.8	10.8	0.35	Yellow
2 Hydroxyacetone	20.6	11.2	0.47	Green-brown
3 1-Hydroxy-2-butanone	24.3		0.31	Purple
4 Acetic acid	26.0	12.0		
5 2,5-Hexanedione	32.0		0.67	Dark brown
6 Propionic acid		17.8		
7 4-Hydroxy-2-butanone	34.2	20.0	0.40	Rust
8 Furfuryl alcohol ^c	42.2	25.7	0.66	Purple
9 γ -Butyrolactone ^{c,d}	43.2	25.7		
10 2-Hydroxy-3-methyl-2-cyclohexen-1-one ^{c,d}	48.0	28.2	0.96	Blue
11 5-Methyl-2-furfuryl alcohol ^c	48.0	29.4	0.78	Brown
12 3,5-Dimethyl-2-hydroxy-2-cyclopenten-1-one ^c	57.0	33.4	0.90	Rust
13 2-Hydroxy-3-methyl-2-cyclopenten-1-one	62.0	35.3	0.70	Yellow
14 3,4-Dimethyl-2-hydroxy-2-cyclopenten-1-one ^c	62.3		0.75	Rust
15 3-Ethyl-2-hydroxy-2-cyclopenten-1-one ^c	68.0	38.7	0.83	Purple, then green-brown
16 2,5-Dimethyl-4-hydroxy-3(2H)-furanone	78.5	46.0	0.62	Brown
17 4-Hydroxy-2-hydroxymethyl-5-methyl-3(2H)-furanone		57.0	0.33	Yellow
18 Benzoic acid		65.3	0.73	White

^a 18-foot column.

^b 9-foot column.

^c Minor component when not kept alkaline.

^d Minor component when kept alkaline.

products when alkalinity was maintained. A plausible mechanism for their formation from fructose, discussed below, is based on the observation that four hydroxyketones were among the ether-soluble degradation products isolated: the three possible hydroxybutanones (1, 3, and 7) and hydroxyacetone (2).

Combinations of the four hydroxyketones through base-catalyzed condensation can lead to all five cyclic diketones, as illustrated in Figure 1. However, in the formation of the cyclohexanedione (10), either dehydration of 4-hydroxy-2-butanone to methyl vinyl ketone followed by Michael addition, or prior activation of the hydroxyl group followed by S_N2 displacement would be required. As noted, this was the only one of the five cyclic diketones which was not formed in appreciable quantity by alkaline degradation of fructose.

When a mixture of the four hydroxyketones was heated with alkali in dilute aqueous solution, diketones 12, 13, 14, and 15 were isolated by GLC but none of 10 was found. Other workers had suggested that this type of condensation is responsible for formation of these diones in coffee and in alkali treatment of carbohydrates. Thus, Gian-turco (1967) found 14 and 15 in the base-catalyzed condensation of glyceraldehyde and butanone. Fray (1961) isolated compound 13 from the base-catalyzed condensation of glycidaldehyde with acetone and from the degradation of glucose with base, but only in the presence of acetone. However, these cyclopentanediones had not previously been isolated by combining the three- and four-carbon hydroxyketones used here.

If alkalinity was not maintained during fructose degradation, the reaction quickly became slightly acidic and remained so during most of the heating period (see Procedure). The product mixture in this case differed from that found when alkalinity was maintained, in that compounds 3, 5, and 14 were absent, while three additional

products (6, 17, and 18) were identified. Furanone (17) had been isolated from earlier acid-catalyzed degradation studies (Shaw *et al.*, 1967). Neither 6 nor 18 had been found under either strictly acidic or alkaline conditions.

The relationship of these fructose-base studies to storage decomposition in dehydrated citrus powders was not as straightforward as previous fructose-acid studies had been. Of the 18 compounds identified from base degradation, only six were also present in stored orange powder (compounds 4, 8, 9, 13, 17, and 18) (Tatum *et al.*, 1967a). Two of these six (4 and 17) had previously been found in acid-

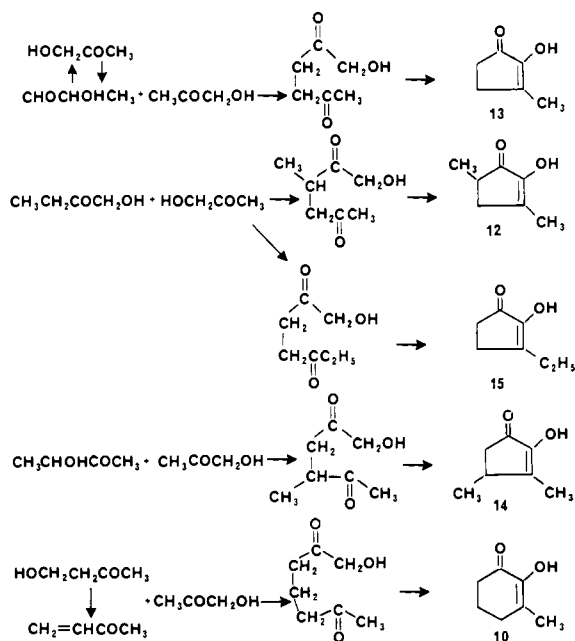


Figure 1. Base-catalyzed formation of the five cyclic diketones

catalyzed fructose degradation (Shaw *et al.*, 1967). Even though orange juice is acidic, localized base-catalyzed degradations contributing to storage decomposition might occur in the solid state such as encountered with orange powder. Such base-catalysis could be due to the proximity of a carbonyl to an amino group, for example. Recently, another model study has shown that ascorbic acid can be a precursor for some of these fructose-base products plus others known to be present in stored orange powder (Tatum *et al.*, 1967b).

With respect to other foods, the fructose-base model system which was not kept alkaline may help to explain the finding of methyl cyclopentenolone (13) as a flavor component of maple sirup (Filipic *et al.*, 1965). The characteristic color and flavor of maple sirup develop during the boiling process when maple sap is concentrated, and the amount of invert sugar present affects the color produced (Underwood *et al.*, 1961). Also, the pH of maple sap is between 6 and 7. As boiling progresses, the pH rises to above 8 and then drops to below the original value (Bois and Dugal, 1940). This model study suggests that fructose is a precursor for methyl cyclopentenolone in maple sirup. Of the five cyclic diketones (10, 12, 13, 14, and 15), only methylcyclopentenolone was isolated in appreciable quantity in this model system where alkalinity was not maintained, and it was the only one reported in maple sirup.

Interesting odors were noted for several of the compounds isolated in these studies. All five cyclic diketones had strong caramel-like odors, the 3,4-dimethyl compound (14) being the most powerful in this respect. Furanone (16) possessed a sweet, cotton-candy odor, while furanone

(17) had the unpleasant odor of charred paper. Thus, these diketones, in whatever foods they might occur, would undoubtedly influence flavor and aroma.

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Received for review May 10, 1968. Accepted August 19, 1968. References to brand names are for identification and do not imply endorsement. Work done in a laboratory of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.