Degradation Products from Ascorbic Acid

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An analytical study was made of degradation products formed when ascorbic acid was heated in aqueous solution. The isolation of 10 furan-type compounds, two lactones, three acids, and 3-hydroxy-2-pyrone is reported. Eight of these compounds were identified as nonenzymic browning products found in dehydrated orange and grapefruit powders. All the compounds were separated by GLC and identified by spectroscopic methods in comparison with authentic samples.

Dehydrated orange and grapefruit powders undergo nonenzymic browning during prolonged storage at ambient temperatures (Berry and Tatum, 1965). Tatum *et al.* (1967) reported the identification of 16 nonenzymic browning products from stored orange juice powder (IOJ). Two of the constituents of these citrus powders which are likely to be precursors of these browning products are ascorbic acid (Lamden and Harris, 1950) and fructose.

Shaw *et al.* (1967a,b) reported on both fructose-acid and fructose-base model studies as related to browning in citrus powders. Those studies showed that fructose can be a precursor of many of the 16 compounds previously isolated from dehydrated orange powder.

An ascorbic acid model system was studied to determine if this substance could be a precursor for some of the known browning products present in stored orange powder. This model system was analyzed using methods developed in the fructose degradation studies. The products identified from ascorbic acid degradation and the relationship of these findings to nonenzymic browning in dehydrated citrus powders are the subject of this report.

EXPERIMENTAL

Chromatographic Methods. Gas-liquid chromatography (GLC) using stainless steel columns was carried out under the following conditions: A 4-foot × 0.25-inch Carbowax 20M–packed column (40% 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 40° C. for 4 minutes, and the temperature was increased to 110° at 4 minutes, 130° at 8 minutes, 140° at 16 minutes, 150° at 20 minutes, 170° at 30 minutes, 180° at 46 minutes, 190° at 52 minutes, and 215° at 58 minutes. All temperature increases were at the rate of 60° C. per minute and all times were from the moment of injection. 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.

Spectrophotometric Methods. Infrared spectra were obtained neat or in CS_2 on a Perkin-Elmer 137 spectrophotometer. Ultraviolet spectra were obtained on a Cary-14 recording spectrophotometer. Mass spectra were determined on a Bendix Time-of-Flight 12-100 instrument. The nuclear magnetic resonance (NMR) spectrum was determined on a Varian A-60 instrument equipped with a time-averaging computer using tetramethylsilane as an internal standard.

Degradation and Extraction Procedures. L-Ascorbic acid (140 grams) was dissolved in 400 ml. of water and the solution was heated in a beaker over an open flame for 5 hours. Water was added as needed to maintain about

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constant volume. In an alternate procedure the L-ascorbic acid was placed in a 1000-ml. round-bottomed flask, 400 ml. of water were added, and a water-cooled reflux condenser was attached. The ascorbic acid solution was heated on a steam bath for 5 hours.

In both procedures, ascorbic acid solutions were extracted three times with 200-ml. portions of diethyl ether in an open beaker, mixed 4 minutes for each extraction with a Brookfield counter-rotating mixer, Model L998. The ether layer was decanted each time. The ether extracts were combined and dried with anhydrous sodium sulfate, then evaporated to about 5 ml. under vacuum at tap water temperature. The GLC analysis was carried out on these concentrated fractions. The only observed difference in the compounds identified from the two systems was an increase in the amount of furfural in the closed system.

Synthesis of Compounds. γ-CROTONOLACTONE. Fifty microliters of α-bromo-γ-butyrolactone were injected into the GLC; injection port temperature was 190°. About 10% of the injected material was converted to γ-crotonolactone.

3-Hydroxy-2-pyrone was synthesized by a modification of the procedure of Wiley and Jarboe (1956), which was followed through collection of the distillate. At that point, 20 grams of NaCl were added to the distillate, which was then extracted three times with 150-ml. portions of ether. The combined ether extract was dried with anhydrous sodium sulfate and concentrated. The concentrate was examined by GLC. Four compounds were identified: acetic acid, γ-crotonolactone, 3-hydroxy-2-pyrone, and 2-furoic acid. The relative ratios present by peak area were 0.5/0.3/0.8/1.

2-Hydroxyacetylfuran was synthesized by the published procedure (Kipnis *et al.*, 1948; Miller and Cantor, 1952) (m.p. 78–9° C.).

Benzoin-Type Condensation. One milliliter of furfural was added to 1 ml. of ethanol and 0.1 gram of NaCN. After 30 minutes the reaction mixture was filtered and the filtrate separated by GLC. Four major reaction products were formed: 2-furoic acid ethyl ester, deoxyfuroin, furoin, and furil. After 2 hours, traces of 2-hydroxyacetylfuran and 2-furoic acid were present.

2-Furoic acid ethyl ester was made by mixing 2-furoic acid, ethanol, and a trace of sulfuric acid. The ester was isolated by GLC separation of the crude reaction mixture.

Deoxyfuroin was prepared by mixing 0.5 gram of furil thoroughly with 25 grams of zinc dust, and then adding 10 ml. of acetic acid. The mixture was stirred thoroughly; it became hot and in about 20 minutes appeared dry. Water (100 ml.) was added after the major portion of acetic acid was removed under vacuum. Solid $NaHCO_3$ was added to neutralize the remaining acetic acid. Thirty grams of NaCl were added and then the solution was extracted twice with 75-ml. portions of diethyl ether. The combined ether extract was dried and concentrated. GLC of this ether fraction gave four major components. The first compound to come off was the reduced furil, or 2,2′-ethylidenedifuran. The third compound was deoxyfuroin: infrared, $cm.^{-1}$ (oil film) 1665(s), 1590(w), 1560(m), 1500(w), 1480(s), 1380(w), 1300(w), 1240(m), 1220(w), 1210(w), 1150(w), 1145(m), 1080(w), 1075(w), 1040(w), 1010(s), 940(w), 915(w), 900(w), 885(s), 800(w), 765(w), 735(s); *m/e* 176, 95, 81, 53, 44, 40, 39; NMR (acetone-d_6) CH_2 at 4.27 p.p.m., vinyl protons at 6.35 (2*H*), 6.67 (1*H*), 7.40 (2*H*), and 7.81 p.p.m. (1*H*). The remaining two components were not identified.

RESULTS AND DISCUSSION

Fifteen compounds have been identified as degradation products from ascorbic acid. Table I lists these compounds along with their chromatographic data. It also indicates those which have been reported previously from instant orange juice (IOJ) or from model systems of fructose in acid or base.

Eight of the compounds from ascorbic acid degradation had been previously reported from stored IOJ. This indicates that, in IOJ, ascorbic acid can serve as a source for the formation of these substances during storage. Three of these compounds have also been previously reported from acid degradation of fructose and four from basic degradation of fructose. As shown in Table I, two of the compounds previously found to be formed during storage of IOJ (2-acetylfuran and 3-hydroxy-2-pyrone) were not found in the previous studies of fructose degradation products (Shaw, 1967a,b), but were found as ascorbic acid degradation products. This suggests that in the degradation of IOJ during storage, ascorbic acid serves as a precursor for these two compounds.

The identity of most of the compounds reported was confirmed by comparing their infrared and ultraviolet spectra, mass spectral cracking patterns, and retention times on Carbowax 20M with those of commercially available samples. When authentic samples were not available, they were synthesized in our laboratory. For

Table I. Compounds Isolated from Ascorbic Acid Degradation

	Compounds	Rt,[a] Min.	Previously Reported in: Stored IOJ	Fructose-acid	Fructose-base
1.	Acetic acid	12.1	X	X	X
2.	Furfural	13.3	X	X	
3.	2-Acetylfuran	14.6	X		
4.	2,2′-Difuryl-methane	18			
5.	Furfuryl alcohol	19.2[b]	X		X
6.	γ-Butyro-lactone	19.2[b]	X		X
7.	γ-Croto-no-lactone	24			
8.	Methylcyclo-pentenolone	26.4	X		X
9.	3-Hydroxy-2-pyrone	34.5	X		
10.	2-Hydroxy-acetylfuran	35.6	X	X	
11.	2,5-Dihydro-furoic acid	48			
12.	Deoxyfuroin	54			
13.	2-Furoic acid	60			
14.	Furoin	72			
15.	Furil	81			

[a] Retention time in minutes on Carbowax 20M.
[b] Resolved on a 9-foot column.

final identification synthesis was required of compounds 7, 9, 10, and 12 from Table I. The one compound not identified by comparison to an authentic sample was number 4, which was identified as 2,2′-difurylmethane by comparison of its mass spectral cracking pattern with that published by Gautschi *et al.* (1967) for this substance. The infrared spectrum was in accord with the proposed structure.

From heat degradation of ascorbic acid, the following furans were identified (Table I): furfural, 2-acetylfuran, 2,2′-difurylmethane, furfuryl alcohol, 2-hydroxyacetyl furan, 2,5-dihydrofuroic acid, deoxyfuroin, 2-furoic acid, furoin, and furil. One of these, 2,5-dihydro-2-furoic acid, has been reported as a degradation product from ascorbic acid by Coggiola (1963). In that work, the mass spectrum of this compound had a base peak of 69 but no mass peak. The mass spectrum of the same compound obtained in the current study on a Time-of-Flight Bendix Model 12-100 mass spectrometer run at 70 e.v. gave a base peak of 69 and two small peaks of about equal intensity at 112 and 114. To determine if these small peaks may be due to impurities, a sample of the acid was rerun through the GLC to purify it. The two peaks were still present in the same ratio. Thus, it appears that a small percentage of this compound is being converted to 2-furoic acid in the ionization chamber of the mass spectrometer.

In addition to the furans mentioned above, two lactones were identified: γ-butyrolactone and γ-crotonolactone. 3-Hydroxy-2-pyrone was isolated and identified by comparison to a sample synthesized by the procedure of Wiley *et al.* (1956). Methylcyclopentenolone and acetic acid were also found.

Of the 15 compounds reported, seven were present in concentrations above trace amounts: furfural, 3-hydroxy-2-pyrone, 2-hydroxyacetylfuran, 2,5-dihydro-2-furoic acid, 2-furoic acid, furoin, and furil.

Five peaks remain unidentified on the GLC chromatogram of the extract from heat-degraded ascorbic acid. Four of these compounds were present in trace amounts only.

In an effort to determine a possible mechanism for formation of some of these compounds, a benzoin-type condensation was carried out on furfural. This yielded five of the compounds that had been found from ascorbic acid degradation. Since furfural is the major ascorbic acid degradation product, self-condensation probably led to formation of these products from furfural.

CONCLUSIONS

Fifteen compounds have been reported as degradation products from ascorbic acid. Only furfural, 2,5-dihydro-2-furoic acid, and 2-furoic acid (Kamiya, 1959) have been previously reported from ascorbic acid degradation. Condensation of furfural is probably responsible for the formation of two of the other major components. In stored IOJ the formation of 2-acetylfuran and 3-hydroxy-2-pyrone probably derives from ascorbic acid.

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Received for review October 11, 1968. Accepted November 20, 1968. References to brand names are for identification and do not imply endorsement. The Fruit and Vegetable Products Laboratory is a laboratory of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.