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Note

3-Deoxy-L-*glycero*-pentos-2-ulose (3-deoxy-L-xylosone) and L-*threo*-pentos-2-ulose (L-xylosone) as intermediates in the degradation of L-ascorbic acid*

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In acidic solution, L-ascorbic acid undergoes decarboxylation with the formation of 2-furancarbaldehyde, suggesting that a five-carbon fragment is probably produced as an intermediate in the reaction. Isbell[1] proposed 3-deoxy-L-*glycero*-pentos-2-ulose (3-deoxy-L-xylosone, **1**) as a reaction intermediate. In subsequent investigations, Kurata and Sakurai[2,3] isolated a number of dicarbonyl and carbonyl compounds from acid-catalyzed partial degradation reactions, and reported the detection of **1**, as well as L-*threo*-pentos-2-ulose (L-xylosone, **2**) as evidenced by t.l.c. analysis of 2,4-dinitrophenylhydrazone derivatives. In neutral and alkaline solutions, L-ascorbate readily undergoes an oxygen-dependent, free-radical mediated oxidation to dehydro-L-ascorbic acid[4], which also exists in equilibrium with 2,3-diketo-L-gulonic acid, its open-chain form. In neutral and basic solutions, however, the degradation is more complex than in acidic solution, with carbon-chain fragmentation a predominant feature of the reaction[5]. 2-Furancarbaldehyde is produced under acidic conditions, but it is not a reaction product under neutral and basic conditions. We recently found[6] that considerable decarboxylation occurs during the degradation reaction, even at pH 7.0 and 37°, with as much as 35% of the ascorbate undergoing decarboxylation within a few days, suggesting that five-carbon fragments may well be involved in the degradations over the entire pH range. This paper reports an investigation of the degradation of L-ascorbic acid over the range of pH 2.0–8.0 with respect to the presence of neutral five-carbon compounds that are present and which may serve as reaction intermediates.

EXPERIMENTAL

Materials and Methods. — For routine detection of compounds, a Shimadzu model GC-9A gas-liquid chromatograph, equipped with a 15-m Supelco bonded capillary column (SPB-5) using helium as the carrier gas, was employed. Mass spectra were collected using a double-focusing Kratos MS-25 instrument equipped with a

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DS-55 data system and interfaced with a Hewlett-Packard g.l.c. instrument equipped with a bonded SE-30 capillary column.

*Preparation of 3-deoxy-*L-glycero-*pentos-2-ulose (3-deoxy-*L-*xylosone)* (**1**), *and* L-threo-*pentos-2-ulose (*L-*xylosone)* (**2**). — Compound **1** was prepared using the same methodology as has been described for the preparation of the 3-deoxyhexosulose derivative by Madson and Feather[7]. The crystalline bisbenzoylhydrazone had m.p. 230° and was chromatographically pure by t.l.c., having R_F 0.45 in 4:1 (v/v) benzene–methanol. The ^{13}C-n.m.r. spectrum was also consistent with the structure, with signals at δ 148.6 (C-1), 154.4 (C-2), 29.7 (C-3), 65.2 (C-4), and 7.10 (C-5). The aromatic carbon resonances were found as multiplets at δ 127–129. The **1** obtained from the bisbenzoylhydrazone was obtained as a syrup, which, on reduction and conversion to the alditol acetate derivative and examination by g.l.c. gave, as expected, two peaks. The mass spectra of the peaks were identical and gave a peak at *m/z* 231 (M − 73). Conversion of **1** to the oxime–trimethylsilyl derivative[8] also gave two peaks by g.l.c., with mass spectral peaks at *m/z* 435 (M − 15) and 345 (M − 15 − 90).

Compound **2** was prepared as described by Salomon *et al.*[9] The mass spectra of the alditol acetate derivatives showed peaks at *m/z* 289 (M − 73), 217 (M − 73 − 72), and 145 (M − 73 − 72 − 72). The mass spectrum of the trimethylsilyl-dioxime derivative[8] gave peaks at *m/z* = 538 (M^+), 523 (M − 15), and 435 (M − 103).

Isolation of dehydration reaction products. — L-Ascorbic acid (0.396 g, 2.25 mmol), in 10 mL of solution, was heated for 2 h in a boiling water bath in aq. buffer solutions at pH 2.0, 4.0, 6.0, and 8.0. The reaction solution was then cooled quickly to 25° in an ice bath, the pH adjusted to 7.0, and an excess of sodium borohydride (25 mg) was added to the solution. After standing in a refrigerator overnight, the solution was treated with Dowex-50 [H^+] and then passed through a column of Dowex-1 [CO_3^{2-}]. The eluate was evaporated to dryness, and the products were converted to the alditol acetate derivatives and examined by g.l.c.–m.s.

RESULTS AND DISCUSSION

The basic strategy used in the experiments involved treating buffered solutions of L-ascorbic acid for 2 h at 100°, quickly cooling the reaction to below 25°, neutralizing to pH 7.0, and immediately treating the mixture with sodium borohydride (or borodeuteride) to reduce any carbonyl-containing compounds to the less reactive alcohols.

3-Deoxy-D-*glycero*-pentos-2-ulose (**1**) was prepared using the procedure described previously for the hexos-2-ulose[7]. The n.m.r. and m.s. data for this compound (see Experimental) are consistent with the structure and are analagous to those obtained previously for the hexos-2-ulose derivative[7]. When **1** was reduced with borohydride, acetylated and examined by g.l.c.–m.s., the characteristic *m/z* 231 fragment was observed corresponding to M − 73 (cleavage between C-1 and C-2 or between C-4 and C-5). This assignment was confirmed by repeating the reduction with borodeuteride, which gave peaks at *m/z* 232 and 233.

The structural authenticity of L-xylosone (**2**) was confirmed by the g.l.c.–m.s. of the alditol acetate derivatives. Peaks identical to authentic xylitol and L-lyxitol acetates were observed, and m.s. showed the characteristic peak at m/z 289, ($M^+ - 73$), corresponding to cleavage between C-4 and C-5 (or between C-1 and C-2), shifted to m/z 290 and 291 when the experiment was repeated using borodeuteride as the reducing agent.

For the L-ascorbic acid-derived samples, peaks corresponding to both reduced **1** and **2** were clearly visible, along with L-threose (identified as L-threitol tetraacetate). Compounds **1**, **2**, and L-threitol were identified by comparison with the mass spectral data collected for the standard compounds described above. Confirmation that the compounds were **1** and **2** was evidenced by borodeuteride reduction, which gave mass spectra identical with the borodeuteride-reduced standards. This result confirms that the samples were, in fact diuloses, since two carbon-bound deuterons were found on the molecules in the expected positions. No traces of any other pentoses were detected, based on comparison with authentic standards (ribitol and L-arabinitol).

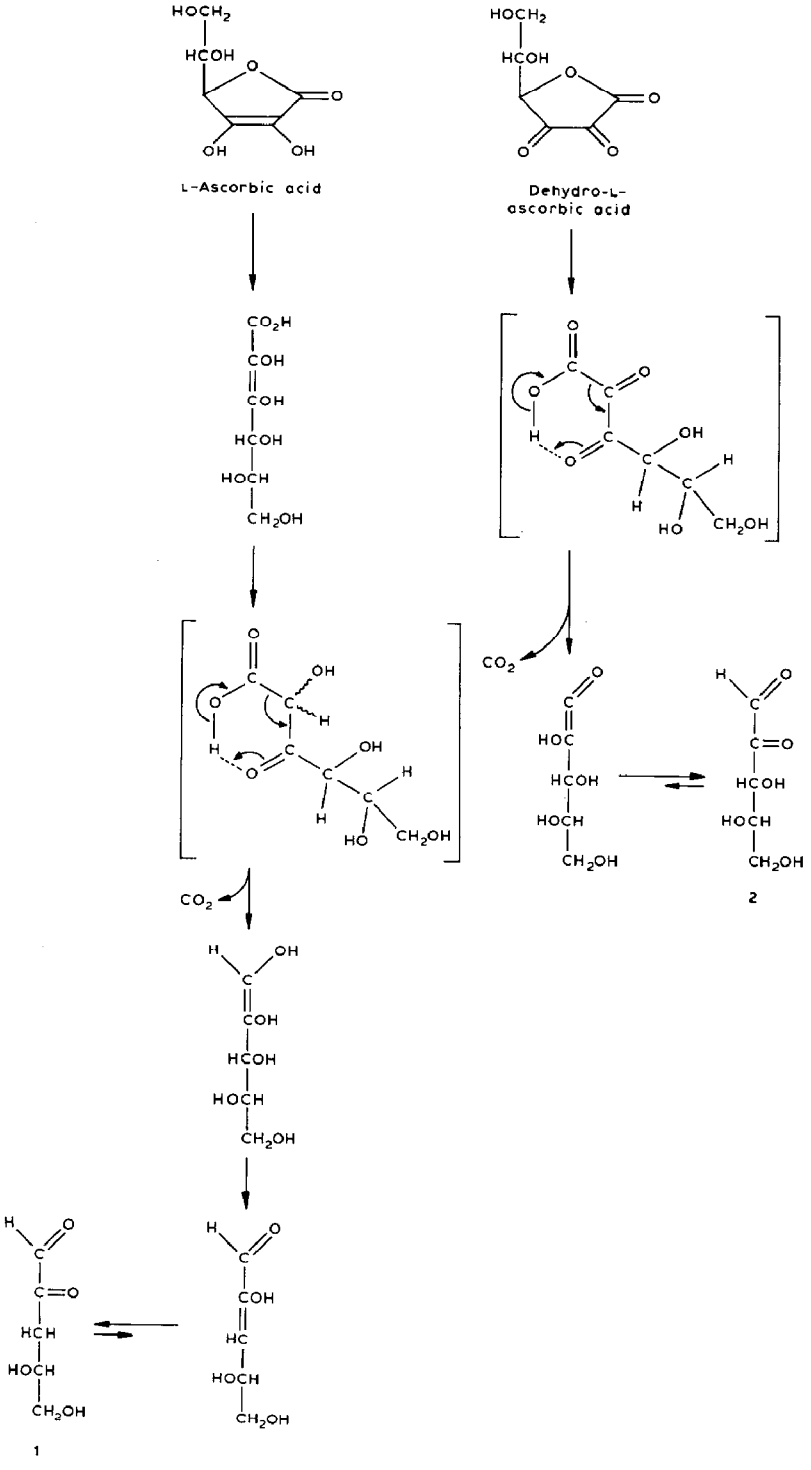
Although **1**, **2** and L-threose were found in all degradation reaction mixtures, the amounts were not constant. Yield data are given in Table I. The presence of both **1** and **2** is consistent with a β-keto acid decarboxylation of L-ascorbic (after ring opening and formation of the 3-keto form) and dehydro-L-ascorbic acid, respectively as is shown in Sheme 1. The ratios of **1** and **2** probably represent the ratios of the reduced and oxidized forms, respectively, of ascorbic acid in the reaction mixture. (No attempt was made to exclude oxygen from the reactions.) The fact that the borodeuteride-reduced samples from the degradation reaction mixture were identical to standards with respect to ratios of isotope suggests that pentoses are not intermediates in this reaction. The first-formed product of the decarboxylation of L-ascorbic acid is the 1,2-enediol of L-xylose (or L-lyxose). Presumably this immediately reacts further to give **1**, without equilibration with the keto form, to undergo further degradation. It is likely that the enolic form of **1**, which is predicted to form initially in the decarboxylation reaction, reacts immediately on formation. The evidence for this stems from conversions of ascorbic acid to 2-furaldehyde in acidified, tritiated water. The 2-furancarbaldehyde formed in this reac-

TABLE I

Yields of **1**, **2** and L-threose obtained from L-ascorbic acid at various pH

	pH of Solution			
Compound	*2.0*	*4.0*	*6.0*	*8.0*
1	2.4	3.3	0.5	trace
2	0.6	15.2	8.1	6.2
L-Threose	1.3	2.4	2.8	3.2

[a] Expressed as μg per 100 mg of starting L-ascorbic acid, measured by g.l.c. using sucrose octaacetate as the internal standard.


L-Ascorbic acid
Dehydro-L-ascorbic acid
CO2
CO2
1
2

tion contains no carbon-bound tritium at H-3 of the furan ring[10], suggesting that the keto form of **1** is not present in appreciable concentrations during the degradations reaction.

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