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Preliminary communication

Degradation of nonreducing carbohydrates by alkaline hydrogen peroxide and a ferrous salt*

HORACE S. ISBELL, HARRIET L. FRUSH, and EDWIN W. PARKS
Department of Chemistry, The American University, Washington, D.C. 20016 (U. S. A.)
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Eighty years ago, Fenton reported that a combination of hydrogen peroxide and a ferrous salt effectively oxidizes a wide variety of organic compounds[1]. The reagent attacks primary and secondary hydroxyl groups, with production of carbonyl groups, by a free-radical mechanism[2]. Notwithstanding many meritorious investigations, the degradation of carbohydrates by Fenton's reagent has not been clearly understood[3]. We have shown previously that, *under alkaline conditions*, hydrogen peroxide combines rapidly with carbohydrates having a free, or potentially free, carbonyl group, and that the resulting adduct, a hydroperoxide hydrin, decomposes in a manner characteristic of the structure involved. Adducts from *alpha*-hydroxy carbonyl compounds, in general, decompose with formation of lower aldehydes which, by repetition of the process, are degraded further. By this mechanism, aldoses[4], ketoses[5], uronic acids[6], and related compounds[7] are degraded stepwise, to formic acid, oxalic acid, and carbon dioxide.

In the absence of a free-radical catalyst, carbohydrates lacking a carbonyl, or potential carbonyl, group are inert to alkaline hydrogen peroxide, but with Fenton's reagent they are oxidized, with formation of carbonyl groups. Our results indicate that, with alkaline hydrogen peroxide in large excess, the carbonyl groups generated by Fenton's reaction initiate stepwise degradation, presumably by reactions described in prior papers of this series. Thus, the measurements given in Table I show that, in a reaction mixture containing a ferrous salt and an excess of alkaline hydrogen peroxide, alditols are converted almost entirely into formic acid, and aldonic acids into formic acid, oxalic acid, and carbon dioxide. Scheme 1 depicts the degradation of a nonreducing carbohydrate, starting with the well-known Fenton's reaction, followed by an iron-catalyzed, hydroperoxide cleavage reaction.

Stepwise degradation of the oxidation product formed by Fenton's reaction from an alditol having n carbon atoms, and cleavage of the adducts, produces n moles of formic acid, with continuous regeneration of ferrous ion. The results indicate that Fenton's re-

*Reactions of Carbohydrates with Hydroperoxides, IX. For Part VIII, see ref. 9.

TABLE I

DEGRADATION OF NONREDUCING CARBOHYDRATES BY ALKALINE HYDROGEN PEROXIDE AND A FERROUS SALT[a]

Substrate	*Millimoles of product per millimole of substrate*		
	Formic acid	*Carbon dioxide*	*Oxalic acid*
D-Mannitol	5.9	0.00	0.00
D-Glucitol	5.8	0.00	0.00
Inositol	5.5	0.03	0.00
Lactitol	11.2	0.00	0.00
D-Xylonic acid	3.7	0.91	0.12
L-Arabinonic acid	3.6	0.81	0.10
D-Gluconic acid	4.5	0.76	0.04
Lactobionic acid	10.4	1.2	0.15
Maltobionic acid	10.2	1.2	0.15

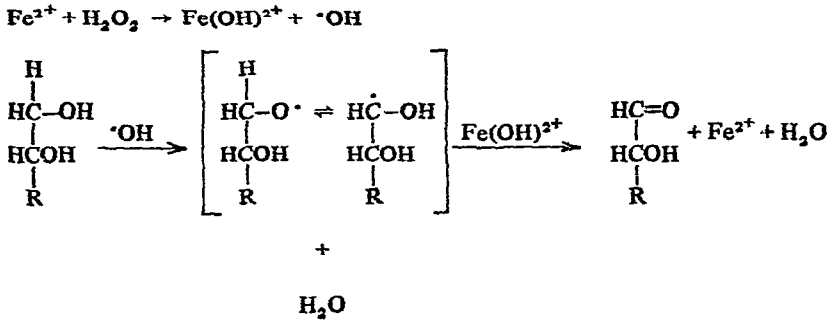
[a]To 1 ml of substrate, dissolved in 10 ml of ice-cold, 1.5M KOH, was added 2.5 ml of 30% H_2O_2, followed by 2.5 ml of 1mM $FeSO_4$, the latter added in 0.5-ml portions at 30-min intervals. After the mixture had been kept for 24 h at 4°, the addition of H_2O_2 and $FeSO_4$ was repeated. After an additional 24 h, formic acid, carbon dioxide, and oxalic acid were determined by methods previously described[6].

agent, under alkaline conditions, preferentially attacks the primary hydroxyl groups of an alditol. Oxidation of a secondary hydroxyl group, with formation of a ketose, and degradation of this by hydroperoxide cleavage, would give an intermediate carboxylic acid, and, finally, carbon dioxide as well as formic acid. The absence of an appreciable amount of carbon dioxide in the products from alditols indicates that the degradation begins, in large measure, with oxidation of primary hydroxyl groups.

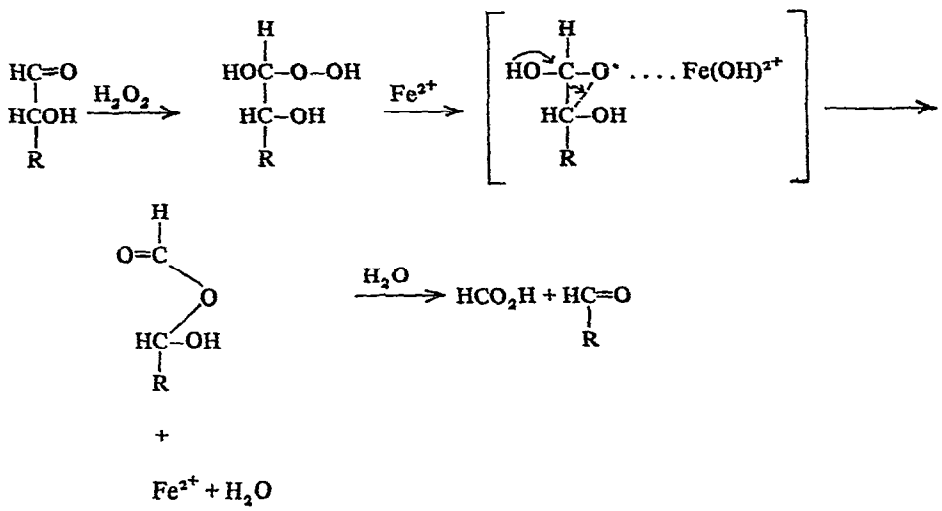
The products obtained from aldonic acids likewise suggest that the degradation is initiated, principally, by oxidation of the primary hydroxyl group, with formation, in this case, of a uronic acid. By hydroperoxide cleavage, this presumably yields formic acid, oxalic acid, and carbon dioxide by the reactions described in ref. 6. By these mechanisms, the sum of the carbon dioxide and oxalic acid should be one mole per mole of substrate. Intermediate formation of a keto acid by the initial oxidation of a secondary hydroxyl group would give a sum greater than one mole. With lactobionic and maltobionic acids, this sum is actually 1.35 moles each. The high value may indicate that Fenton's reagent, in alkaline solution, attacks the substrate, to a minor degree, at a secondary hydroxyl group.

Complete degradation of lactitol, lactobionic acid, and similar compounds is noteworthy, because it shows, as with disaccharides[5], that the glycosidic bond of the substrate is readily split under the conditions used. Starting with carbon atom 1 of the D-glucitol residue of lactitol, the reagent presumably initiates stepwise degradation to 2-*O*-D-galactosyl-D-erythrose (see Scheme 2). Alternatively, starting with carbon atom 6, the process affords 2-*O*-D-galactosyl-L-xylose. The glycosidic bond of either intermediate may be split by addition of hydrogen peroxide to the aldehyde group, followed by either a heterolytic, intra-

Fenton's reaction



Hydroperoxide cleavage reaction (iron-catalyzed)



Scheme 1

molecular cleavage of the adduct, or an iron-catalyzed, homolytic cleavage, with subsequent hydrolysis. The resulting D-glyceraldehyde (or L-threose) and D-galactose would be degraded to formic acid by further reaction with hydrogen peroxide. Concurrent oxidation of carbon atoms 1 and 6 of the D-glucitol residue would lead to the same final products.

Degradation of sucrose and other nonreducing saccharides by alkaline hydrogen peroxide in the presence of a ferrous salt is slower and less definitive than that of the open-chain alditols and aldonic acids.

Gal = D-galactosyl group

Scheme 2

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