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OXIDATION OF SODIUM SALTS OF ALDURONIC AND GLYCULOSONIC ACIDS BY SODIUM PEROXIDE*

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ABSTRACT

Because, in the presence of a large excess of alkaline hydroperoxides, aldoses are oxidized stepwise to formic acid, it was expected that alduronic acids would be degraded to formic acid and carbon dioxide by the mechanism previously proposed. However, in addition to the products expected, substantial yields of oxalic acid were found, indicating a second mechanism, proposed here. With alkali-metal hexuronates, one mechanism yields five moles of formic acid and one mole of carbon dioxide per mole of substrate, whereas the second mechanism yields four moles of formic acid and one mole of oxalic acid. The results show that, under highly alkaline conditions, the two mechanisms are of approximately equal importance. Oxidation of a D-*xylo*-5-hexulosonate begins with the cleavage of glycolic acid, and this is followed by degradation of the resulting tetruronic acid by the two mechanisms described for the degradation of hexuronic acids. With 2-hexulosonates, also, two reaction-mechanisms appear to be necessary to account for the products formed under highly alkaline conditions; the main reaction (80%) yields one mole each of carbon dioxide and pentonic acid per mole, whereas the other yields one mole of oxalic acid and four moles of formic acid.

Under moderately alkaline conditions, both the alduronic acids and the 2-keto acids react almost entirely by the mechanism that yields carbon dioxide; no detectable amount of oxalic acid was found. In all cases, small amounts of unknown products, not further investigated, are formed.

The following compounds were studied: sodium D-galacturonate, sodium D-glucuronate, potassium D-mannuronate, sodium D-*lyxo*-2-hexulosonate, sodium D-*arabino*-2-hexulosonate, calcium D-*xylo*-5-hexulosonate, and glyoxylic acid.

DISCUSSION

In prior publications of this series[1,2], we advanced the hypothesis that the oxidative degradation of reducing sugars by alkaline hydrogen peroxide takes place

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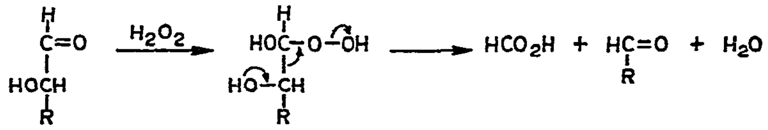
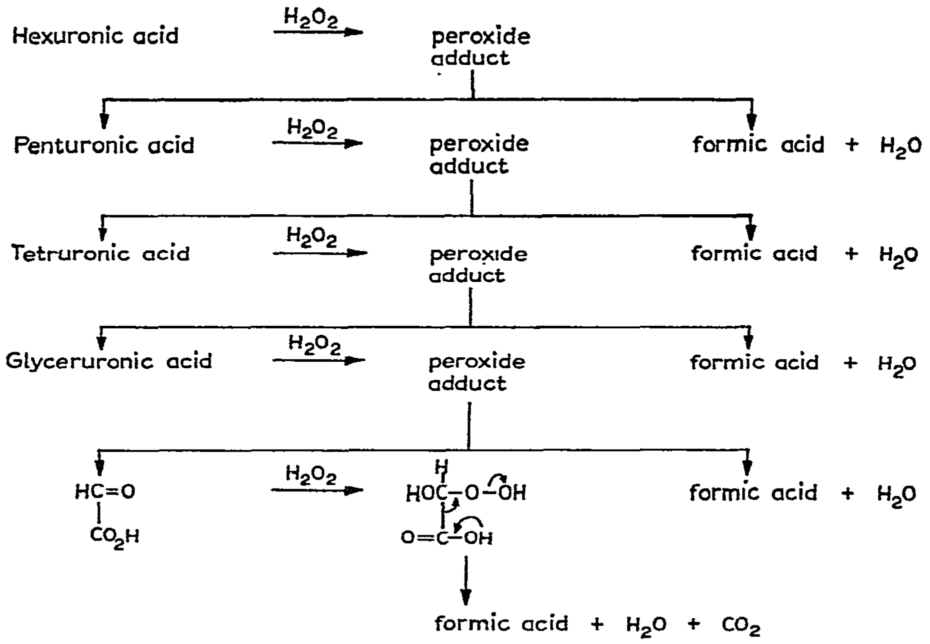
by a nucleophilic addition of the hydroperoxide anion to the carbonyl form of the sugar, followed by oxidative cleavage of the adduct (see Scheme 1). By this process, under suitable conditions, an aldose is degraded stepwise to formic acid, and a 2-ketose to glycolic acid and formic acid. We have now studied the oxidation of certain hexuronic acids and glycosulosonic acids.



Scheme 1.

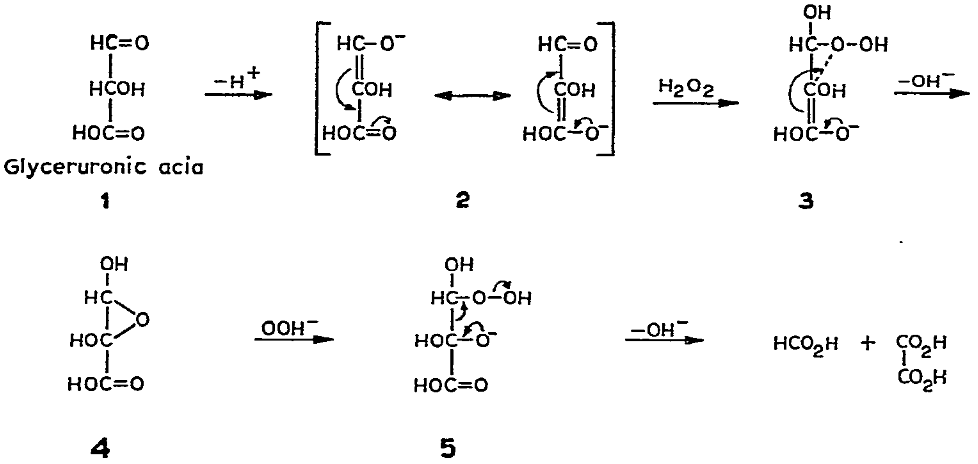
Oxidation of hexuronic acids by sodium peroxide in excess. — From our previous work, we expected that alkali-metal salts of hexuronic acids would be degraded stepwise to lower alduronic acids, producing formic acid from C-1 to C-4, and intermediate glyoxylic acid from C-5 and C-6 (see Scheme 2). It is known[3] that glyoxylic acid reacts rapidly with aqueous sodium peroxide to yield formic acid and carbon dioxide. Experimentally, we found that the reaction of hexuronic acids with sodium peroxide in large excess yields not only formic acid and carbon dioxide, but also



Scheme 2.

oxalic acid. The process of Scheme 2 accounts for the first two products, but not for oxalic acid.

However, Scheme 3 presents a suggested mechanism for the formation of oxalic acid from the adduct of the intermediate glyceruronic acid (2-hydroxy-3-oxopropionic acid) (**1**), a compound that would be expected to yield the resonance-stabilized, enolic form (**2**). Addition of the hydroperoxide anion to **2** would yield the adduct **3** and the epoxide **4**. Attack of the hydroperoxide anion on **4**, and decomposition of **5** would yield formic acid and oxalic acid. The reaction may well proceed through an alternative mechanism starting with **5** and leading to a mixed anhydride by way of an epoxide. In alkaline medium, the anhydride is hydrolyzed to the corresponding acids.



Scheme 3.

Degradation of a hexuronic acid by Scheme 2 would produce five moles of formic acid and one mole of carbon dioxide per mole of substrate, whereas degradation by Scheme 2 as modified by Scheme 3 would afford four moles of formic acid and one mole of oxalic acid. Thus, the amount of carbon dioxide formed is a measure of the occurrence of Scheme 2, and the amount of oxalic acid, a measure of that of Scheme 3.

The results reported in Table I show that, under highly alkaline conditions, the reactions postulated in Schemes 2 and 3 are of approximately equal importance. In the absence of side reactions, the sum of the moles of carbon dioxide and oxalic acid per mole of substrate should equal unity. The summed values found are somewhat less than 1. Small amounts of undetermined products may be formed, but the constituents determined account for over 90% of the carbon atoms in the substrate. The amount of formic acid found is in accord with the occurrence of the reactions shown in Schemes 2 and 3, which yield, respectively, 5 and 4 moles of formic acid per mole of substrate. The almost quantitative conversion of sodium glyoxylate into carbon

TABLE I

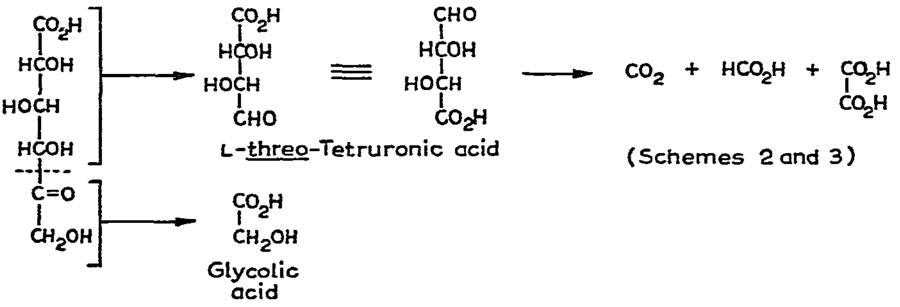
OXIDATION OF ALKALI HEXURONATES AND GLYCULOSONATES AT 4° BY SODIUM PEROXIDE IN EXCESS[a]

Substrate	*Millimoles formed per milliequivalent of substrate*					*Milliatoms of carbon recovered, per milliequivalent of substrate*
	Formic acid	*Carbon dioxide*	*Oxalic acid*	*Residual acid*[b]	CO_2 + *oxalic acid*	
7	4.42	0.41	0.53		0.94	5.89
8	4.44	0.47	0.43		0.90	5.77
9	4.49	0.47	0.44		0.91	5.84
10	0.83	0.62	0.20	0.75[c]	0.82	5.60
11	0.92	0.74	0.19	0.77[d]	0.93	5.89
12	2.19	0.62	0.51	0.93[e]	1.13	5.69
13	1.00	0.96	trace		0.96	1.96

[a]Reaction mixtures and analytical methods given in Experimental section. [b]Milliequivalents by titration. [c]Largely D-lyxonic acid. [d]Largely D-arabinonic acid. [e]Glycolic acid.

dioxide and formic acid shows that the oxalic acid found does not arise from oxidation of intermediate glyoxylic acid.

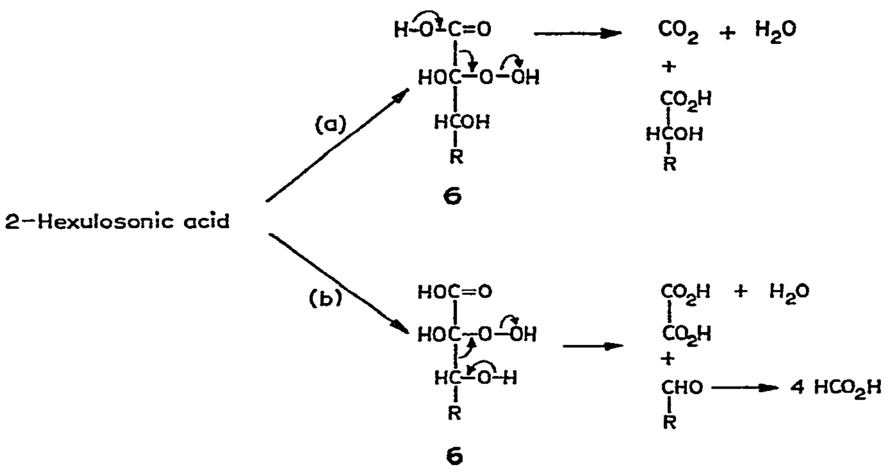
Oxidation of glyculosonic acids by sodium peroxide in excess. — Reaction of calcium D-*xylo*-5-hexulosonate with sodium peroxide, depicted in Scheme 4, involves processes characteristic of the degradation of a 2-ketose and a tetruronic acid. The first step yields one mole each of glycolic acid and L-*threo*-tetruronic acid per mole. The subsequent degradation of the alduronic acid should be the same as that of the enantiomorphic D-*threo*-tetruronic acid, presumably formed from D-galacturonic acid after elimination of two carbon atoms as formic acid. In both cases, stepwise degradation of the tetruronic acid by the reactions depicted in Schemes 2 and 3 should give formic acid, carbon dioxide, and oxalic acid. In accordance with the reaction mechanisms postulated, calcium D-*xylo*-5-hexulosonate gave approximately one mole of glycolic acid per mole of substrate, and amounts of carbon dioxide and oxalic acid comparable to those found for the alkali hexuronates (see Table I). As



Scheme 4.

expected, the yield of formic acid was two moles less than that found for the hexuronates, because of the elimination of C-5 and C-6 as glycolic acid.

Oxidation of 2-hexulosonic acids by sodium peroxide presumably occurs by the reactions depicted in Scheme 5. Decomposition of the hydrogen peroxide adduct 6 by rupture of the C-1–C-2 bond gives carbon dioxide and the next lower (pentonic) acid (reaction 5a); this acid does not react further. Alternatively, decomposition of 6 by rupture of the C-2–C-3 bond yields oxalic acid and an aldotetrose (reaction 5b). One mole of the latter is degraded to three moles of formic acid and one mole of formaldehyde, which is subsequently oxidized to formic acid. Under the conditions indicated in Table I, degradation of 1 mole of the 2-hexulosonic acids gave approximately 0.8 mole of carbon dioxide and 0.2 mole of oxalic acid. Thus, about 80% of the degradation occurred by rupture of the C-1–C-2 bond, and 20% by rupture of the C-2–C-3 bond. According to process 5b, the yield of formic acid should be four times that of the oxalic acid. The experimental ratios (4.1 and 4.8) are somewhat high, but are of the expected magnitude.



Scheme 5.

Oxidation of sodium D-*glucuronate and sodium* D-arabino-*2-hexulosonate by hydrogen peroxide under moderately alkaline conditions.* — As shown in the preceding sections, degradation of alduronic acids by sodium peroxide in excess at pH 13 occurs in large measure by two mechanisms, one of which leads to the formation of carbon dioxide, and the other, of oxalic acid. Formation of oxalic acid (see Scheme 3) involves an enolization step that occurs only under highly alkaline conditions. As shown in Table II, it was found that, with hydrogen peroxide in excess, little or no oxalic acid is formed from sodium D-glucuronate under moderately alkaline conditions. Hence, under these conditions, degradation proceeds largely by the process depicted in Scheme 2.

TABLE II

OXIDATION OF SODIUM D-GLUCURONATE AND SODIUM D-*arabino*-2-HEXULOSONATE AT 25° BY HYDROGEN PEROXIDE UNDER MODERATELY ALKALINE CONDITIONS[a]

Substrate	*Millimoles formed per millimole of substrate*				*Milliatoms of carbon recovered per millimole of substrate*
	Formic acid	*Carbon dioxide*	*Oxalic acid*	*Residual acid*	
8	4.93	0.96	none		5.89
11	0.01	0.93	none	1.00[b]	5.94

[a]Reaction mixtures and analytical methods given in Experimental section. [b]D-Arabinonic acid.

In the oxidation of sodium D-*arabino*-2-hexulosonate, cleavage of the C-2–C-3 bond and formation of oxalic acid involve ionization of an alcoholic hydroxyl group, a process favored by high alkalinity. The results given in Table II show that little or no oxalic acid was formed under moderately alkaline conditions, and, hence, the reaction proceeds almost entirely by the mechanism given in Scheme 5a.

The mechanism given here for the degradation of sugars by alkaline hydrogen peroxide differs from certain mechanisms suggested by prior workers. Küchlin[4] suggested that the noncatalytic degradation of D-glucose occurs by stepwise formation of lower alduronic acids, with repetitive stripping of formic acid units. By this mechanism, an aldose and the corresponding alduronic acid should yield the same products, but our present work has shown that this is not the case. Others[5] suggested that the degradation of an aldose occurs by scission of formaldehyde, with stepwise formation of lower aldoses, and oxidation of the intermediate formaldehyde to formic acid. However, no convincing mechanism has been presented for the stepwise cleavage of formaldehyde from an aldose by hydrogen peroxide. We now postulate direct cleavage of a peroxide adduct without the intermediate formation of formaldehyde, which is produced only in the terminal cleavage; this formaldehyde is then oxidized to formic acid.

The oxidation of formaldehyde with hydrogen peroxide has been extensively investigated. In 1898, Blank and Finkenbeiner[6] reported that the reaction of formaldehyde with hydrogen peroxide and sodium hydroxide gives sodium formate and hydrogen. Lyford[7] postulated the following reactions.

$$HCHO + H_2O_2 \rightarrow HCO_2H + H_2O \qquad (1)$$

$$2\,HCHO + H_2O_2 \rightarrow 2\,HCO_2H + H_2 \qquad (2)$$

$$HCO_2H + H_2O_2 \rightarrow CO_2 + 2\,H_2O \qquad (3)$$

Reaction *3* is a consecutive reaction whereby the formic acid originally formed is slowly decomposed; in highly alkaline solutions, this reaction is of little importance. Reactions *1* and *2* yield formic acid from formaldehyde in the ratio of 1:1, and hence the yield is not characteristic of either process. To account for the formation of hydrogen, Wieland and Wingler[8] postulated the intermediate formation of bis-(hydroxymethyl) peroxide, a compound previously crystallized by Fenton[9]. In accordance with this mechanism, Wirtz and Bonhoeffer[10] demonstrated that the hydrogen of reaction *2* arises from the aldehyde, and not from the peroxide or the solvent. The mechanism is in accord with the data of Fry and Payne[11] showing that the yield of hydrogen from formaldehyde is highest when the ratio of aldehyde to peroxide is 2:1.

Base-catalyzed decomposition of the bis(hydroxymethyl) peroxide presumably proceeds by the following mechanism[8]. The mechanism has been confirmed by considerable experimental work. However, under our experimental conditions, the concentration of bis(hydroxymethyl) peroxide must be insignificant, because the concentration of the hydroperoxide anion is high compared to that of the carbonyl substrate.

$$\mathrm{HO{-}CH_2{-}OOH} + \mathrm{H_2C{=}O} \longrightarrow \mathrm{HO{-}CH_2{-}O{-}O{-}CH_2{-}OH}$$

EXPERIMENTAL

General. — Sodium D-galacturonate (7) (ref. 12), sodium D-glucuronate·H_2O (8) (ref. 13), and potassium D-mannuronate·H_2O (9) (ref. 14) were prepared by the methods cited. Sodium D-*lyxo*-2-hexulosonate (**10**) was prepared from D-galactonic acid[15]. Sodium D-*arabino*-2-hexulosonate (**11**) and calcium D-*xylo*-5-hexulosonate·2.5 H_2O (**12**) were commercial products. Sodium glyoxylate (**13**) was prepared from commercial glyoxylic acid. Sodium peroxide and 30% hydrogen peroxide were commercial, A.C.S.-certified products.

Oxidation measurements of Table I. — Reaction mixtures were prepared by dissolving each substrate (2 milliequivalents) in ice-cold, 1M sodium peroxide solution (50 ml). The mixtures were kept for 24 h at 4°, and then diluted to 100 ml with carbon dioxide-free water. Aliquots were treated as follows.

a. Carbon dioxide. An aliquot (25 ml) was transferred to a 100-ml flask connected to a conventional apparatus for the titrimetric determination of carbon dioxide. Sulfuric acid (5 ml of 2.5M) was added, and the resulting carbon dioxide was absorbed in 0.05M barium hydroxide, the excess of which was titrated with 0.1M hydrochloric acid (phenolphthalein). Correction was made for a blank determined on a sample of the peroxide solution.

b. Formic acid. An aliquot (20 ml), combined with glacial acetic acid (5 ml), sodium acetate trihydrate (4 g), and mercuric chloride solution (40 ml of 5%), was heated for 2 h on a boiling-water bath. The resulting mercurous chloride was separated, dried, and weighed. Formic acid = HgCl × 0.0975.

c. Oxalic acid. An aliquot (20 ml) was acidified with hydrochloric acid, and warmed, to decompose the excess of peroxide and remove carbon dioxide. The solution was made neutral with ammonium hydroxide, and oxalic acid was determined in the conventional way by precipitation as calcium oxalate, separation by filtration, and titration with 0.5N potassium permanganate.

d. Residual acids. An aliquot (25 ml) was warmed with activated carbon (0.3 g) and Celite (0.3 g) until evolution of oxygen ceased. The solids were filtered off, the filtrate was passed through Dowex-50W X-4 ion-exchange resin (50 ml), and the eluate evaporated under diminished pressure. Volatile acids were removed by three successive additions and evaporations of water and toluene. The residue was dissolved in water, calcium acetate (0.05 g) was added, and the resulting calcium oxalate was filtered off and discarded. The filtrate was again passed through Dowex-50W X-4 resin (5 ml), and acetic acid was removed from the eluate by repeated addition and evaporation of water and toluene. The residual acid was dried to constant weight, and titrated with 0.1M potassium hydroxide (phenolphthalein).

Identification of residual acids. — Samples of each substrate (4 milliequivalents) were oxidized under the conditions described for the oxidations given in Table I. The residual acids were separated and identified as follows.

The residual acid from **10** crystallized spontaneously as an impure lactone mixture. After the product had been recrystallized from ethanol, its m.p. (113–114°) and infrared (i.r.) spectrum were identical with that of authentic D-lyxono-1,4-lactone.

The residual acid from **11** was neutralized with potassium hydroxide, and the resulting salt was recrystallized. The i.r. spectrum of the salt was identical with that of authentic potassium D-arabinonate.

The residual acid from **12** was neutralized with calcium carbonate, and the excess of the carbonate was filtered off. The filtrate was concentrated to a thin syrup, and the resulting crystals of calcium glycolate were separated, and recrystallized from hot water. The salt showed the same i.r. spectrum as calcium glycolate prepared from authentic glycolic acid.

Oxidation of sodium D-*glucuronate* (**8**) *and sodium* D-arabino-2-*hexulosonate* (**11**) *under moderately alkaline conditions.* — Reaction mixtures were prepared from 2 millimoles of **8** or **11**, ice-water (10 ml), and 30% hydrogen peroxide (2 ml). After 30 min, 0.25M sodium hydroxide (60 ml for **8**; 20 ml for **11**) was added dropwise during 2 h. After 18 h at 25°, each mixture was diluted to 100 ml, and aliquots were taken for determination of formic acid, oxalic acid, carbon dioxide, and residual acids by the methods already described.

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