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OXIDATION OF 2,3,4,6-TETRA-*O*-METHYL-D-GLUCOSE WITH ALKALINE HYDROGEN PEROXIDE*†

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ABSTRACT

Treatment of 2,3,4,6-tetra-*O*-methyl-D-glucose with 10 molar equivalents of 30% aqueous hydrogen peroxide and 2 molar equivalents of potassium hydroxide afforded, after chromatographic separation, 2,3,4,6-tetra-*O*-methyl-D-gluconolactone, 1-*O*-formyl-2,3,5-tri-*O*-methyl-D-arabinose methyl hemiacetal (**7**), 2,3,5-tri-*O*-methyl-D-arabinonolactone, methyl 2,3,5-tri-*O*-methyl-D-arabinoside, *O*-(2,4-di-*O*-methyl-D-erythrose)-(1′→3)-2,4-di-*O*-methyl-D-erythronic acid, and *O*-(2,4-di-*O*-methyl-D-erythrose)-(1′→2)-3-*O*-methyl-D-glyceraldehyde. The proportions of the products depended on the reaction conditions, especially the time, temperature, and the presence or absence of magnesium hydroxide. Formation of the products is explained by a series of reactions beginning with the addition of hydrogen peroxide to the carbonyl form of the methylated sugar. The adduct, with the help of superoxide radical and a molecule of hydrogen peroxide, breaks up in two ways, giving 2,3,4,6-tetra-*O*-methyl-D-gluconic acid and **7**. The formic ester, on hydrolysis, gives 2,3,5-tri-*O*-methyl-D-arabinose, which undergoes a similar series of reactions, affording 2,3,5-tri-*O*-methyl-D-arabinonic acid, and presumably, 1-*O*-formyl-2,4-di-*O*-methyl-D-erythrose methyl hemiacetal. Apparently, the latter compound, on hydrolysis, forms a dimer, which, with alkaline hydrogen peroxide, undergoes a similar series of reactions, ultimately affording *O*-(2,4-di-*O*-methyl-D-erythrose)-(1→3)-2,4-di-*O*-methyl-D-erythronic acid and *O*-(2,4-di-*O*-methyl-D-erythrose)-(1→2)-3-*O*-methyl-D-glyceraldehyde. With a larger amount of alkali, under more-severe conditions, oxidation of 2,3,4,6-tetra-*O*-methyl-D-glucose proceeds further, with production of up to 3 moles of formic acid per mole of methylated sugar.

INTRODUCTION

The reaction between tetra-*O*-methyl-D-glucopyranose (**1**) and alkaline hydrogen peroxide was first investigated by Gustus and Lewis[2] in 1926. By oxidation of the

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sugar with alkaline hydrogen peroxide in large excess for many days at 45°, they obtained, from 100 g of sugar, 13.42 g of carbon dioxide, 33.78 g of formic acid, and 51.33 g of non-volatile material, consisting of a dimethyl-D-arabinonolactone, methyl esters of tetronic acids, and demethylated hexonic and pentonic acids. The authors suggested that the products isolated were formed by "oxidation of the dissociated 1,2-enol of the sugar", a concept, originated by Nef[3], that has since been discredited.

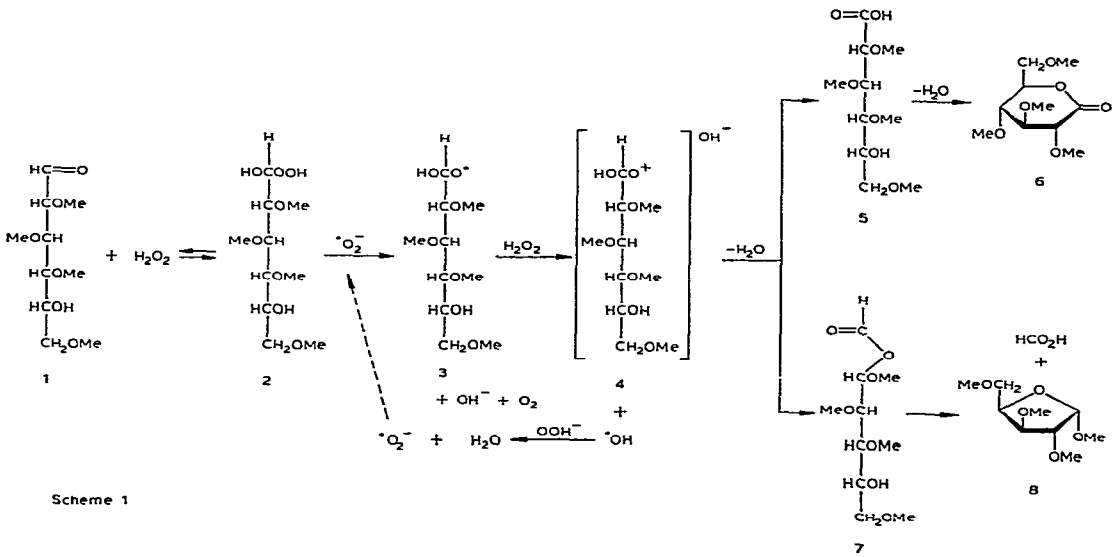
Later, Fraser-Reid and colleagues[4] reported that tetra-*O*-methyl-D-glucopyranoside is demethylated and oxidized by hydrogen peroxide and ferrous ion under acid conditions. In neutral solution, demethylation and oxidation proceeded only to a limited extent, and in the absence of the ferrous salt, no demethylation occurred. They rationalized the demethylation reaction by oxidation of the methyl groups by hydroperoxide radical, but did not consider the reactions under alkaline conditions.

The reactions of 2,3,4,6-tetra-*O*-methyl-D-glucose seem to be particularly suitable for testing some of the mechanisms proposed by Isbell and co-workers[1,5–10] for oxidation of carbohydrates by alkaline hydrogen peroxide. According to the ideas promulgated, degradation of reducing sugars and related compounds by alkaline hydrogen peroxide begins with addition of hydrogen peroxide to the carbonyl form of the sugar: the adduct so formed decomposes in a predictable manner. In alkaline solution, addition of hydrogen peroxide to the carbonyl group of an aldose is rapid and prevails over enolization and related reactions. The adduct decomposes rapidly by the so-called α-hydroxy-hydroperoxide cleavage-reaction, with formation of formic acid and the next-lower aldose. This type of cleavage is not possible for a substance lacking an α-hydroxyl, or other electron-releasing, group. Hence, such a compound must react by another mechanism.

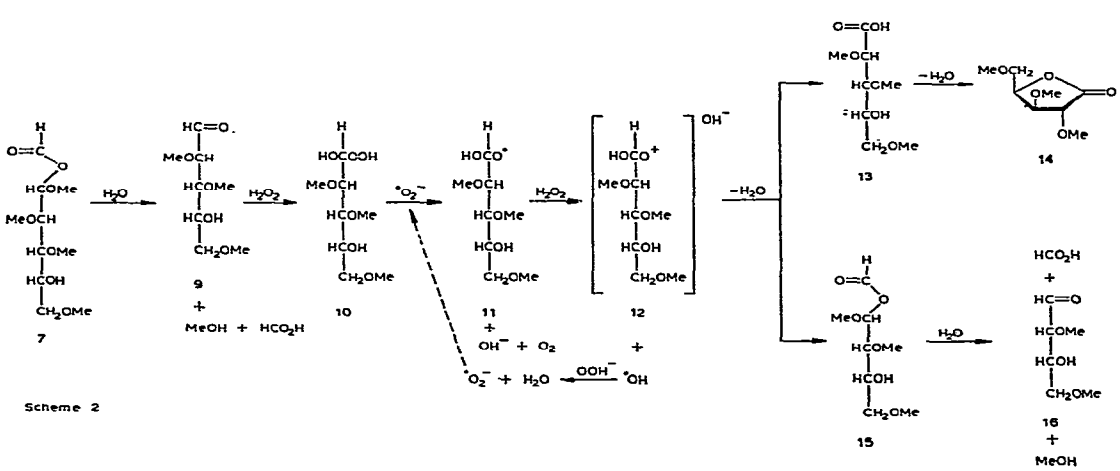
In a prior study[1], it was shown that 2-deoxy-D-*arabino*-hexose, a sugar that lacks an α-hydroxyl group, undergoes, on treatment with alkaline hydrogen peroxide, two separate oxidation-processes. The primary process involves addition of hydrogen peroxide to the carbonyl form of the sugar, reduction of the adduct, and oxidation of the resulting oxy radical. This sequence produces an electron-deficient intermediate, which collapses, with elimination of a hydrogen ion, to form 2-deoxy-D-*arabino*-hexonic acid, or the formic ester of D-arabinitol. The other process begins with attack by hydroxyl radical on one of the hydroxyl groups of the substrate. This route results in a carbonyl derivative that is subsequently degraded, in large measure, to formic acid, by the α-hydroxy–hydroperoxide cleavage-reaction.

It has now been found that 2,3,4,6-tetra-*O*-methyl-D-glucose (**1**) gives a series of reactions (Schemes 1–3) similar in most respects to those of 2-deoxy-D-*arabino*-hexose. Thus (Scheme 1), treatment of **1** with alkaline hydrogen peroxide yielded, in addition to formic acid, 2,3,4,6-tetra-*O*-methyl-D-gluconic acid (**5**) and 1-*O*-formyl-2,3,5-tri-*O*-methyl-D-arabinose methyl hemiacetal (**7**). Ester **7**, by elimination of formic acid, afforded methyl 2,3,5-tri-*O*-methyl-D-arabinoside (**8**). Hydrolysis of ester **7** (Scheme 2) afforded 2,3,5-tri-*O*-methyl-D-arabinose (**9**), which underwent a series of reactions similar to those in Scheme 1. These produced 2,3,5-tri-*O*-methyl-

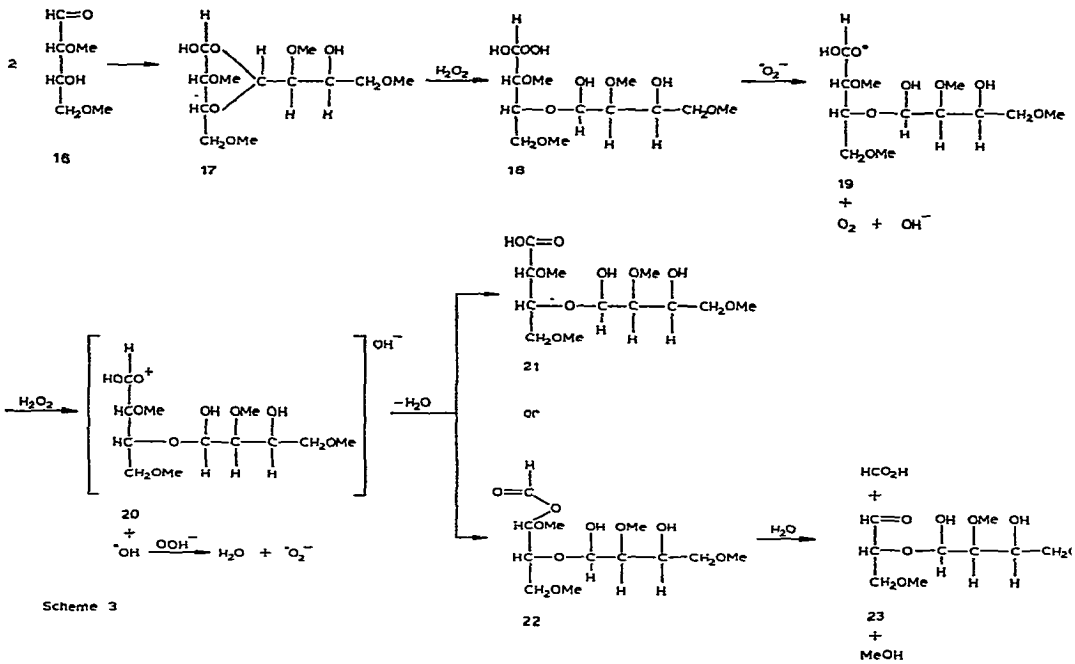
D-arabinonic acid (**13**) and, presumably, 1-*O*-formyl-2,4-di-*O*-methyl-D-erythrose methyl hemiacetal (**15**). Surprisingly, this hypothetical ester, on hydrolysis, formed a dimer (Scheme 3), which was oxidized to *O*-(2,4-di-*O*-methyl-D-erythrose)-(1→3)-2,4-di-*O*-methyl-D-erythronic acid (**21**), and to *O*-(2,4-di-*O*-methyl-D-erythrose)-(1→2)-3-*O*-methyl-D-glyceraldehyde (**23**). The procedures used for separation and identification of the products are described in the following section.



Scheme 1



Scheme 2



Scheme 3

DISCUSSION

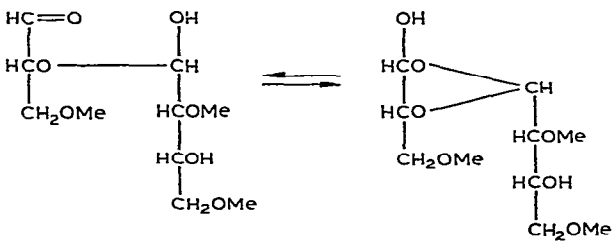
Samples of **1** were treated with hydrogen peroxide and potassium hydroxide under various conditions. Each mixture was separated by ion-exchange resins into a neutral fraction and an acid fraction. Chromatographic separation of the constituents afforded three compounds from each fraction. Mass spectra and ^{13}C-n.m.r. spectra were recorded for the pure compounds. In each case, the mass spectrum was interpreted by splitting-patterns reported to be characteristic of the methyl sugars[11]. The ^{13}C-n.m.r. spectra were rationalized by characteristic resonances previously found for carbohydrates[12,13].

The slowest-moving constituent of the neutral fraction was isolated by preparative-layer chromatography (p.l.c.) as a chromatographically pure syrup. Spectroscopic evidence indicated it to be 1-*O*-formyl-2,3,5-tri-*O*-methyl-D-arabinose methyl hemiacetal (**7**). Its mass spectrum showed a distinct molecular ion at *m/e* 252. Its i.r. spectrum exhibited the characteristic carbonyl absorption-band at 1770 cm^{-1}. The ^{13}C-n.m.r. spectrum showed resonance at 176 p.p.m., characteristic of a carbonyl carbon atom, and resonance at 82 p.p.m. characteristic of the arabinose entity. Other resonances were also in agreement with structure **7**.

The second, slow-moving component of the neutral fraction was isolated as a pure syrup and characterized as methyl 2,3,5-tri-*O*-methyl-α-D-arabinoside (**8**). It showed the same specific rotation ($[\alpha]_D^{25}$ +80.7°) and i.r. spectrum as the previously known[14] methyl 2,3,5-tri-*O*-methyl-α-D-arabinoside, prepared, for comparison, from tri-*O*-methyl-D-arabinose.

The fastest-moving component separated from the neutral fraction was also isolated as a pure syrup. Spectroscopic evidence indicated it to be *O*-(2,4-di-*O*-methyl-D-erythrose)-(1→2)-3-*O*-methyl-D-glyceraldehyde (**23**). Its mass spectrum showed an intense ion at *m/e* 149, presumably arising from cleavage of the di-*O*-methyl-D-erythrose group. The spectrum also showed ions indicative of cleavage between C-1 and C-2 (*m/e* 119), C-2 and C-3 (*m/e* 75), and C-3 and C-4 (*m/e* 45) of the erythrose component. An ion at *m/e* 223 corresponded to cleavage of the C-1–C-2 bond of the glyceraldehyde entity. An ion at *m/e* 234 corresponded to the molecular weight of 252 minus water.

The ^{13}C-n.m.r. spectrum of compound **23** was complex; it showed 10 resonance bands, whereas the compound contains only 7 carbon atoms. The complex spectrum suggests that the compound may exist in more than one form, as for example, in ring–chain tautomerism of the type depicted.



The fastest-moving constituent of the acid fraction was isolated by column chromatography as a syrup, and was shown to be 2,3,4,6-tetra-*O*-methyl-D-gluconolactone (**6**). Its identity was established by comparison of its i.r. spectrum and optical rotation with the properties of the known[15] 2,3,4,6-tetra-*O*-methyl-D-gluconolactone.

The second-fastest-moving constituent of the acid fraction was separated crystalline, and identified as 2,3,5-tri-*O*-methyl-D-arabinonolactone (**14**). Its mass spectrum showed an intense molecular ion at *m/e* 190, and its i.r. spectrum and m.p. agreed with those of the previously known lactone[14], prepared, for comparison, by Weerman degradation of **1**.

The third consituent of the acid fraction, presumably *O*-(2,4-di-*O*-methyl-D-erythrose)-(1→3)-2,4-di-*O*-methyl-D-erythronic acid (**21**), was separated by chromatography as a syrup. Its mass spectrum showed *m/e* peaks at 149, 119, 89, 75, and 45, in agreement with the splitting-pattern depicted in Fig. 1. The ^{13}C-n.m.r. spectrum of the compound showed resonances at 176.92, 79.98, 74.38, and 72.23 p.p.m., corresponding to the erythronic acid portion of the molecule, and at 83.13, 79.6, 71.9, and 69.53 p.p.m., corresponding to the erythrosyl group. The i.r. spectrum showed absorption bands at 1820 and 3550 cm^{-1}, indicative, respectively, of the carbonyl and hydroxyl groups. The neutralization equivalent (285) was in substantial accord with the proposed structure (**21**).

Of the compounds separated and identified, **6**, **7**, **8**, and **14** were expected, from the reactions of Schemes 1 and 2; however, compounds **21** and **23** were not

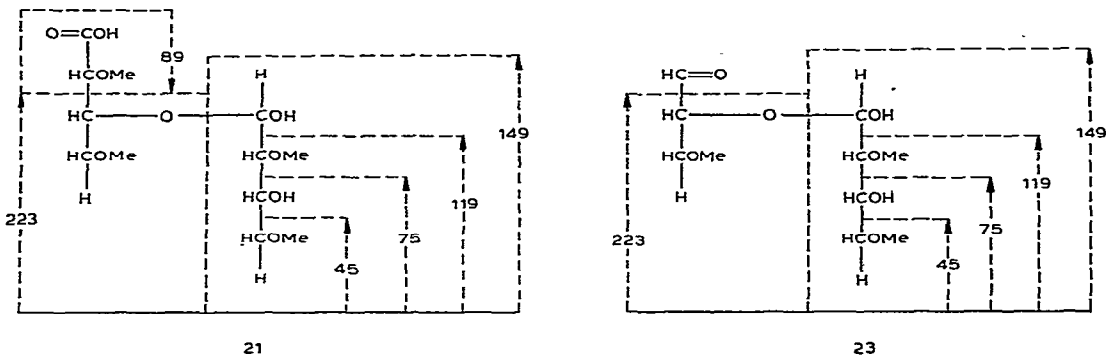


Fig. 1. Splitting-patterns of mass spectra for compounds **21** and **23**.

expected. They appear to have been derived from the dimeric form of 2,4-di-*O*-methyl-D-erythrose, a β-hydroxyaldehyde, by the process of Scheme 3. Reaction of the hypothetical dimer (**17**) with alkaline hydrogen peroxide gives an adduct that decomposes, with the help of superoxide radical and hydrogen peroxide, affording *O*-(2,4-di-*O*-methyl-D-erythrose)-(1→3)-2,4-di-*O*-methyl-D-erythronic acid (**21**) or *O*-(2,4-di-*O*-methyl-D-erythrose)-(1→2)-3-*O*-methyl-D-glyceraldehyde (**23**). Formation of the dimers is in accord with the properties of β-hydroxyaldehydes in general[16]. Supposedly, in continuation of the degradation process, compounds **21** and **23** would be hydrolyzed, with formation of 2,4-di-*O*-methyl-D-erythronic acid, 2,4-di-*O*-methyl-D-erythrose, and 3-*O*-methyl-D-glyceraldehyde. The latter compound would be degraded by reactions of the type already described, affording 3-*O*-methyl-D-glyceric acid, 2-*O*-methylglycolic acid, and formic acid. The first two of these compounds were not found in the present investigation.

The six products isolated and identified indicate that the reaction follows the course depicted in Schemes 1–3. The methylated sugar is degraded in steps, each involving formation and decomposition of a hydroperoxide adduct, with production of either the corresponding *O*-methylaldonic acid or the formic ester of the next-lower methylated sugar, which, on hydrolysis, is degraded further by repetition of the process. Formic acid is formed by hydrolysis of the esters and by further oxidation of the substrate and intermediate products. In the experiments used for identification of intermediate products, reactions were restricted by the amount of alkali added. However, in experiments employing a large excess of alkaline hydrogen peroxide at 40°, only 3 mol of formic acid were produced per mol of **1**.

EXPERIMENTAL

General methods. — Evaporations were conducted under diminished pressure below 45°. Optical rotations were recorded with a Perkin–Elmer Model 141 polarimeter and use of 1-dm tubes. Melting points were determined on a Kofler hot-stage and are uncorrected. Silica Gel 7731 (Merck) was used for p.l.c., detection being effected by

charring with sulfuric acid. Preparative chromatography (p.l.c.) was performed on silica gel plates (fluorescent, 20 × 20 cm); the constituent zones were located by u.v. light, excised, and eluted. Wet-column chromatography[12] was performed on Silica Gel 7734 (Merck). I.r. spectra were recorded with a Perkin–Elmer spectrophotometer. The spectra were interpreted by splitting-patterns presumably characteristic of methyl sugars[11]. The ^{13}C-n.m.r. spectra were rationalized by characteristic resonances previously reported for similar structures[11,13]. The reagents used were of the highest commercial grade. The hydrogen peroxide was represented as containing <0.005% Fe, and the potassium hydroxide, <0.001% Fe. Compound yields are expressed as mole-percent, unless otherwise indicated.

Reference compounds. — 2,3,4,6-Tetra-*O*-methyl-D-glucose, $[\alpha]_D^{25}$ +83° (*c* 4, water), was prepared by the method described in ref. 17. 2,3,4,6-Tetra-*O*-methyl-D-gluconolactone, $[\alpha]_D^{25}$ +102.3° (*c* 0.5, water), was prepared by oxidation[18] of 2,3,4,6-tetra-*O*-methyl-D-glucose with iodine. 2,3,5-Tri-*O*-methyl-D-arabinonolactone, m.p. 35°, $[\alpha]_D^{25}$ +45.4° (*c* 0.5, water), was prepared by Weerman degradation of 2,3,4,6-tetra-*O*-methyl-D-gluconolactone[14]. Methyl 2,3,5-tri-*O*-methyl-D-arabinoside, $[\alpha]_D^{25}$ +80.7° (*c* 0.8, water), was prepared by the action of 2% methanolic hydrogen chloride on 2,3,5-tri-*O*-methyl-D-arabinose[14].

*Reaction of 2,3,4,6-tetra-*O*-methyl-D-glucose with alkaline hydrogen peroxide at 25°.* — Hydrogen peroxide (10 mL, 30%) was added, dropwise, to an ice-cold solution of tetra-*O*-methyl-D-glucose (**1**; 0.590 g, 2.5 mmol) in a mixture of water (10 mL) and 2M potassium hydroxide (30 mL). After 5 min at 0°, the mixture was allowed to warm to 25° and kept for 43 h at 25°. It was then digested with activated carbon (1 g) in a boiling-water bath until evolution of oxygen had ceased. The mixture was filtered, and the filtrate was passed, successively, through columns of cation-exchange resin (H^+, Rexyn) and anion-exchange resin (Duolite A-4). The resulting solution was evaporated under diminished pressure to dryness. The residue, hereafter called the "neutral fraction", weighed 0.106 g (18% of weight of original substrate); $[\alpha]_D^{25}$ +12° (*c* 1, water).

The Duolite column containing the acid constituents was eluted with 10% aqueous ammonia, and the eluate and washings were evaporated under diminished pressure to remove ammonia. The ammonia-free solution was passed through a column containing cation-exchange resin (Amberlite 120-H^+). The resulting solution was evaporated to dryness, and formic acid was removed by successive addition and evaporation of three 10-mL portions of toluene. The resulting "acid fraction" weighed 0.186 g (31.5% of weight of substrate); $[\alpha]_D^{25}$ +16.1° (*c* 0.5, water).

By t.l.c. (1:4 ethanol–hexane) the neutral fraction showed five constituents, two of which were minor; the other three were separated by p.l.c. The fastest-moving constituent was obtained as a syrup (0.043 g, 12.3%), $[\alpha]_D^{25}$ +25.4° (*c* 0.5, methanol). The syrup was characterized by its mass spectra as *O*-(2,4-di-*O*-methyl-D-erythrose)-(1→2)-3-*O*-methyl-D-glyceraldehyde (**23**). The second-fastest constituent from the neutral fraction was obtained as a syrup, methyl tri-*O*-methyl-D-arabinoside (**8**; 0.018 g, 3.5%), $[\alpha]_D^{25}$ +80.4° (*c* 0.5, water). The third-eluted constituent was obtained

as a syrup and identified as 1-*O*-formyl-2,3,5-tri-*O*-methyl-D-arabinose methyl hemiacetal (**7**; 0.030 g, 4.7%), $[\alpha]_D^{25}$ +52° (*c* 0.2, water).

The acid fraction of the product was passed through a column of silica gel, by use of 1:6 ethanol–hexane as eluant. The first-eluted constituent was obtained as a syrup and identified as 2,3,4,6-tetra-*O*-methyl-D-gluconolactone (**6**; 0.022 g, 4.0%), $[\alpha]_D^{25}$ +102.5° (*c* 0.5, water). Its i.r. spectrum was the same as that of authentic **6**. The second-eluted constituent of the acid fraction was crystallized from ethanol and identified as 2,3,5-tri-*O*-methyl-D-arabinonolactone (**14**); yield 0.036 g (7.4%), m.p. and mixed m.p. 35°, $[\alpha]_D^{25}$ +45.4° (*c* 0.5, water). Its i.r. spectrum was identical with that of authentic **14**. The third-eluted constituent was obtained as a syrup and identified as *O*-(2,4-di-*O*-methyl-D-erythrose)-(1→3)-2,4-di-*O*-methyl-D-erythronic acid (**21**); yield 0.060 g (14.4%), $[\alpha]_D^{25}$ +57° (*c* 0.2, water).

Repetition of the preceding experiment, with a reaction period of **14** days at 4° instead of 43 h at 25°, gave a mixture of constituents **6**, **7**, **14**, **21**, and **23**, which were isolated in yields of 2, 1, 4, 2.5, and 2.6%, respectively.

Reaction of **1** *with alkaline hydrogen peroxide in the presence of magnesium hydroxide.* — The process reported in the previous section was repeated with substitution of magnesium sulfate (10 mL, 0.01M) for water, and a reaction time of 14 days instead of 43 h, at 25°. The neutral fraction weighed 0.208 g (35.3% of weight of substrate). By p.l.c., it afforded the formic ester **7** (0.017 g, 2.8%), arabinoside **8** (0.029 g, 5.8%), and glyceraldehyde **23** (0.013 g, 3.9%). The acid fraction weighed 0.257 g (40.2% of weight of substrate). It afforded, by wet chromatography, gluconolactone **6** (0.021 g, 3.5%), arabinonolactone **14** (0.036 g, 7.4%), and erythronic acid **21** (0.019 g, 4.6%). Determination of formic acid in a separate experiment afforded 1.3 mmol per mmol of **1**. The molecular yields of identified products amounted to 28% of the substrate.

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