# 3-DEOXY-D-GLYCERO-2,5-HEXODIULOSONIC ACID, A NEW INTERMEDIATE IN THE METABOLISM OF POLYGALACTURONATE

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Following the original identification of an unsaturated disaccharide resulting from the enzymatic catabolism of hyaluronic acid, described by Linker et al. (1), numerous studies have reported the formation of unsaturated oligosaccharides among the degradation products of various polygalacturonate polymers.

The purpose of the present communication is to present evidence for the identification of a new intermediate, 3-deoxy-D-glycero-2,5-hexodiulosinic acid, II, in the complete metabolism of polygalacturonate as outlined in Fig. 1. It should be noted that I is the same compound reported earlier (2) to arise from D-glucuronic- and L-iduronic acid containing polymers.

A pseudomonad isolated from soil was grown with polygalacturonate as sole carbon source and cultured under conditions previously described for alginic acid (3). Cell free extracts, prepared by sonic disintegration and purified about 14-fold, readily catalyzed the breakdown of polygalacturonate with the formation of a variety of unsaturated oligosaccharides which slowly disappeared upon prolonged incubation. Two monosaccharide end-products, isolated by column chromatography on Dowex-1-formate, were identified as 4-deoxy-L-threo-5-hexosulose uronic acid, compound I (30% of the initial substrate) and D-galacturonic acid, (70% of the initial substrate).

Characteristic spectra for a 2-keto acid were obtained when I was assayed by O-phenylenediamine (4) and semicarbizide (5). Treatment with ceric sulfate resulted in the formation of equimolar amounts of $CO_2$. Upon

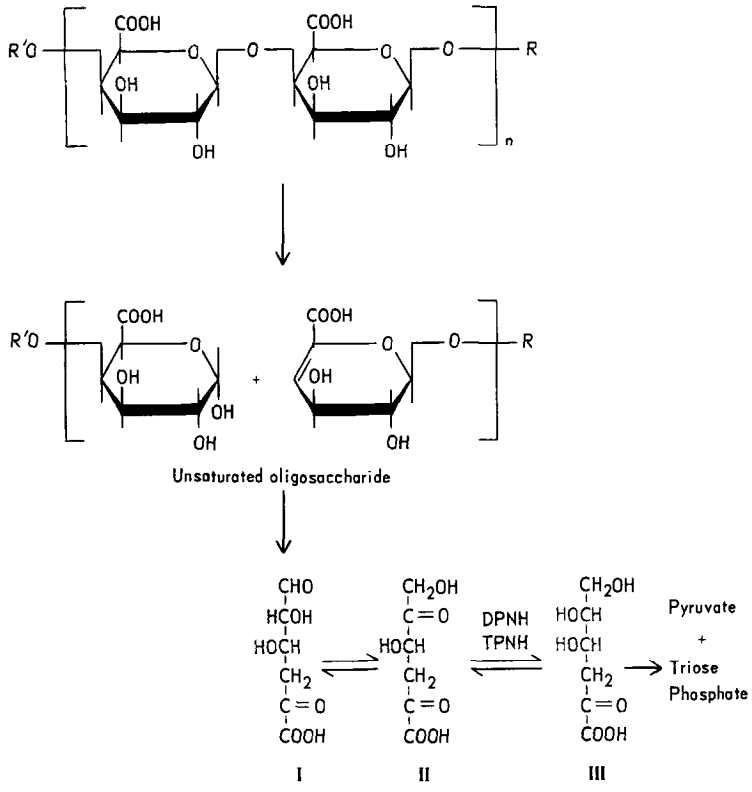


Figure 1

periodate oxidation, 2 moles of periodate were consumed and 1 mole of β-formyl pyruvate was produced as determined by the thiobarbiturate assay (6); no formaldehyde was detected. Reduction of I with $KBH_4$ prior to periodate treatment resulted in the liberation of 1 mole of formaldehyde per mole of I (Table I).

In order to determine the specific configuration of the two hydroxyl groups about carbon atoms 2 and 3, compound I was reduced with borohydride and the resulting metasaccharinic acids converted to the 2-deoxypentose derivative by methods previously described (3). The derivative was characterized as 2-deoxy-L-xylose by the following criteria: (a) The product reacted quantitatively as a deoxypentose in the diphenylamine (7), cysteine-sulfuric acid (8) and thiobarbiturate assay (9). (b) It co-chromatographed with 2-deoxy-xylose in three paper chromatographic systems and in borate electrophoresis. In each case, the product was readily resolved from

TABLE I

Analytical data of compounds from polygalacturonate digestion

| Sample | Periodic acid degradation | | | | |
|---|---|---|---|---|---|
| | β-Formyl-pyruvate | Formaldehyde [a] | | Glycolic acid [b] | Periodate consumption |
| | | Before reduction | After reduction | | |
| | μmoles/ml | μmoles/ml | μmoles/ml | μmoles/ml | μmoles/ml |
| Compound I | 15.6 | 0.0 | 16.1 | 0.0 | 30.7 |
| Compound II | 4.0 | 0.28 | -- | 3.68 | 4.2 |
| Compound III | 6.0 | 6.2 | -- | -- | 11.1 |

a Reference (13)
b Reference (14)

2-deoxyribose. (c) Optical rotation studies, in dry pyridine, revealed the derivative to be strongly levorotatory, $[\alpha]_D^{20}$ -38°. Under similar conditions, authentic 2-deoxy-D-xylose exhibited a positive rotation $[\alpha]_D^{20}$ + 47° (10). In each case, the concentration was determined by the diphenylamine assay.

Incubation of I with a 0.45-0.90 ammonium sulfate fraction of the cell free extract resulted in the formation of a second compound which was easily separated from I by paper chromatography as well as by column chromatography on Dowex-1-formate. In contradistinction to I, compound II consumed only 1 mole of periodate and liberated 1 mole each of glycolic acid and β-formyl pyruvate. The identification of glycolic acid in stoichiometric amounts strongly supports the proposed keto-configuration for II. It was noted that the rate of periodate oxidation of II was unusually rapid and, under the conditions of the thiobarbiturate assay, was essentially complete within 1 minute.

Upon incubation in the presence of either DPNH or TPNH, II was rapidly reduced to 2-keto-3-deoxy-D-gluconic acid, compound III. Under these conditions, I reacted more slowly and only after a lag period. Purification

of the above dehydrogenase activity (130-fold) yielded a preparation which was highly specific for II.

The reduction product III was identified by co-chromatography with an authentic sample in several solvent systems and distinguished from its 5-epimer, 2-keto-3-deoxy-galactonic acid by differential rate of periodate oxidation (11). More definitively, III was shown to serve as a substrate for ketodeoxygluconic kinase and the product of this reaction to be cleaved by phosphodeoxygluconic aldolase. The latter two enzymes were shown to be present in the Pseudomonas cell free extract in high titer.

The second monosaccharide end product of polygalacturonate degradation by Pseudomonas, identified as D-galacturonic acid, was shown to be metabolized by means of the tagaturonic acid pathway as previously demonstrated in Escherichia coli (12).

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