1100 Wayne Avenue, Suite 1100
Silver Spring, Maryland 20910
301/587-8202

A resolution test chart featuring various patterns of vertical and horizontal lines. Each pattern is accompanied by a numerical value representing its resolution. The values are arranged in a grid-like fashion, with larger values (1.0, 1.1, 1.25, 1.4, 1.6, 1.8) on the left and smaller values (2.0, 2.2, 2.5, 2.8, 3.2, 3.6, 4.0, 4.5, 5.0, 5.6, 6.3, 7.1, 8.0) on the right. The patterns consist of groups of three lines, with the number of lines increasing as the resolution value increases.

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Second Year Report on Grant DE-FG06-88ER13890 (1990)

D-Erythroascorbic acid: Its Preparations, Chemistry, and Metabolism (Fungi and Plants).

**Frank A. Loewus, Institute of Biological Chemistry, Washington State University, Pullman,
Washington 99164-6340**

and

**Paul A. Seib, Department of Grain Science and Industry, Kansas State University,
Manhattan, Kansas 66506**

Chemical Synthesis of D-Erythroascorbic acid: Second Method

In the 1st year of this grant, D-erythroascorbic acid (EAA), trivial name for *D-glycero-2-pentenono-1,4-lactone*, was synthesized from D-glucose in four steps (Y.T.S. Liang, X.S. Liu, P.A. Seib 1990 J. Carbohydr. Chem., 9: 75-84). The overall yield, 10%, provided sufficient material for comparison with L-ascorbic acid (AA), in studies on stability, characterization by HPLC with electrochemical detection, and oxidation with Cu(II) or Fe(II) as well as material for peroxygenation studies on C2/C3 cleavage of EAA to oxalic acid (OA) and a C₃ fragment.

The poor recovery experienced in that first synthetic approach prompted a fresh effort that would provide a better yield and a more expeditious route. This was achieved with commercially available 3,4-*O*-benzylidene-D-ribo-1,4-lactone which could be oxidized with activated manganese dioxide to its 2-keto derivative in 76% yield. Oxidation of the latter with ruthenium tetroxide or methyl sulfoxide/acetic anhydride followed by methanolysis with ion-exchange resin as the acid catalyst gave a mixture of products, most of which tautomerized to EAA. The final product was recrystallized from acetonitrile with overall yield of 59% (L-X Gan, P.A. Seib 1991 Carbohydr. Res. 220: 117-125). Since the starting compound can be prepared from D-glucose, yield from the latter would be a theoretical 28%. This new synthesis provides an attractive route for the preparation of isotopically labeled EAA from the many forms of labeled D-glucose available commercially.

Biosynthesis of EAA and OA in Raft-supported *Sclerotinia sclerotiorum*

Procedures developed in the 1st year of this study in which *S. sclerotiorum* was grown on porous polypropylene rafts to create conditions comparable to those encountered in an infected host were used to explore aspects of EAA biosynthesis and its breakdown to OA. When *S.*

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sclerotiorum was grown on 0.1% D-glucose for 4 days, the mycelial extract contained very little EAA (<50 µg/g dry weight) but an unknown electrochemical product (presumably the open-chain form of EAA) accumulated (>800 µg/g dry weight) and OA was over 1.4 mg/g dry weight. When D-arabinose, a purported pentose precursor of EAA, replaced D-glucose as the carbon source, EAA accumulated as well as the unknown. D-Arabinose was less than 50% as efficient as D-glucose as a carbon source, that is, fungal growth was slower. Combining the two carbon sources (0.05% of each) gave intermediate results. There was no synergism. When growth was continued for 10 days, D-arabinose produced more EAA and the unknown acid but the OA present in mycelial extracts diminished, probably due to secretion into the medium. These results are shown in Figure 1.

The experiments just described provided important information regarding distribution of OA (Figure 2). After 10 days of growth in D-glucose or D-arabinose, very little OA remained in the mycelia, either as soluble OA or as acid-extractible OA (insoluble salts such as Ca oxalate). Sclerotia which are fully developed at 10 days were devoid of OA. Virtually all of the OA had been secreted into the medium.

The effective role of D-arabinose in EAA biosynthesis in fungi is hardly surprising. This pentose is present in fungi and readily participates in pentose metabolism in these organisms. It is important to emphasize that it is the enantiomer of L-arabinose which is found in higher plants where it is a common constituent of cell wall polysaccharides. The latter is a product of UDP-D-glucuronate metabolism. To further examine D-arabinose as a precursor of EAA, an experiment was designed to test the next oxidation state of D-arabinose, D-arabinono-1,4-lactone. The experimental conditions followed those used in the previous comparative study of D-glucose and D-arabinose. As seen in Figure 3, D-arabinonolactone was almost as effective as D-arabinose but when both the sugar and its acid-lactone were combined in a single experiment, results were no better than when each was supplied separately.

Results clearly demonstrate the role of D-arabinose as a precursor of EAA and its unknown acidic partner as well as EAA's intermediary role in OA production. [It should be added

here that since these studies were completed, new experiments with forms of D-arabinose specifically labeled in carbons that are destined for OA have confirmed these earlier results. Unpublished work of K. Saito and F. A. Loewus, 1992.]

Metabolism of D-[5-³H]arabinose in *S. sclerotiorum*

An experiment with raft-grown *S. sclerotiorum* that had been supplied with a trace amount of D-[5-³H]arabinose in 0.1% D-glucose provided important information on the fate of the C₃ fragment. In addition to producing ³H-labeled EAA and the accompanying unknown acid, ³H-labeled D-glyceric acid was also isolated from the mycelial extract as would be expected if EAA was cleaved at C2/C3 to give OA and the expected C₃ fragment.

Oxidation of AA and EAA by AA Oxidase

EAA was oxidized by AA oxidase (Sigma Chem. Co.) at about 1/3 the rate of AA. Assays were made by both HPLC and uv detection.

In Summary

S. sclerotiorum contains EAA and a closely related reducing acid, possibly the open-chain form of EAA. The organism cleaves one of these products or possibly both to yield OA and D-glyceric acid. The OA is rapidly secreted into the medium. An analogy can be made between AA-linked OA biosynthesis in higher plants and EAA-linked OA biosynthesis in fungi as exemplified by *S. sclerotiorum*. For details regarding studies reported here, please refer to the Final Report (copy attached).

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Products from Glc and/or Ara *Sclerotinia sclerotiorum*, 4 days growth

Figure 1A

Products from Glucose and/or Arabinose *Sclerotinia sclerotiorum*, 10 days growth

Figure 1B

Oxalate Production from Glc and/or Ara *Sclerotinia sclerotiorum*, 10 days growth

Figure 2

Products from Selected Carbon Sources *Sclerotinia sclerotiorum*, 4 days growth

Figure 3

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