that these extraordinarily high values of BOD and consequent anaerobic conditions in the water were attributable to the *Gonyaulax* and were important contributing factors in the mass mortality of fish in Offatt Bayou.

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The Synthesis of L-Ascorbic Acid Uniformly Labeled with C^{14} [1]

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The synthesis of L-ascorbic acids isotopically labeled in different positions is of great interest in biochemical research. Recently Burns and King (*1*) have described a synthesis of 1-C^{14}-L-ascorbic acid.[2] We wish to report the synthesis of uniformly enriched L-ascorbic acid.[2] Our synthesis was patterned after that of Reichstein and Grüssner (*4*) and adapted to working on a semimicro scale.

A mixture of uniformly enriched sucrose, glucose, and fructose was prepared by allowing bean leaves to photosynthesize for 24 hr in an atmosphere of $C^{14}O_2$ (*2*).

The sucrose was hydrolyzed, and a mixture consisting mainly of glucose was precipitated by the addition of absolute ethanol. The mixture, weighing 392 mg and having a specific activity of 1.4 μc/mg, was hydrogenated in an alkaline aqueous medium using Raney nickel. The sorbitol produced was oxidized to sorbose with *Acetobacter suboxydans* by the method of Wells, Stubbs, Lockwood, and Roe (*6*). After the fermentation the organism was removed by centrifuging, and the supernatant liquid was passed through Amberlite IR-100-H and Duolite A-4 ion exchange columns to remove ionic impurities. Crystallization and decolorization with Darco G-60 gave 344 mg of pure sorbose, mp 163°–165° C.

The sorbose was diluted with 135 mg of carrier, and acetonated, using freshly distilled dry acetone, and sulfuric acid as a catalyst. The diacetonesorbose was extracted from unreacted material and monoacetone derivative with ether. Oxidation of the diacetonesorbose with 6% potassium permanganate was carried out in alkaline medium (*3*), the unattacked material from the first oxidation being removed by ether extraction and reoxidized. The two batches were carried through separately to the last step. The reaction mixture was filtered and acidified to pH 2, after which the diacetone-2-ketogulonic acid was extracted with ethyl acetate and refluxed with water for 40 min to effect deacetonation. Lyophilization gave white, powdery 2-ketogulonic acid, mp 170°–171° C. The methyl ester was prepared by passing a large excess of diazomethane into a methanolic solution of the acid at −15° C. By crystallization from methanol-acetone, 68 mg of white crystals, mp 145°–150° C, was obtained. There was obtained in addition 220 mg of residual oil, consisting partly of methyl 2-ketogulonate, which could not be induced to crystallize and was therefore diluted with 205 mg of carrier and carried through the last step. Treatment under nitrogen of the methanolic solutions of the ester with stoichiometric amounts of 4.65*N* sodium methoxide in methanol and 2.60*N* hydrogen chloride in methanol, gave, after purification according to the method of Szent-Györgyi (*5*), 30 mg of L-ascorbic acid having specific activity of 0.80 μc/mg, and 70 mg having specific activity 0.16 μc/mg. The products melted at 189°–190° C, and gave a single spot of proper R_f value on a paper chromatogram.

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[2] According to suggestions for the naming of isotopically labeled compounds proposed at the Chemistry Conference, Brookhaven National Laboratory, and shortly to be published, these acids would be known as L-ascorbic acid-1-C^{14}_1, and L-ascorbic acid-ue-C^{14}_6, respectively.