Table 1. Distribution of Fe^{59} in *S. marcescens* cells and pigments. Total Fe^{59} counts in 70 ml of sample was 3.4×10^6 count/min.

Fraction	Weight (mg)	Total count/min in fraction	Specific activity (count/min mg)
Dry cells	750	1.2×10^6	1.6×10^3
Entire pigment	1.00	10.4×10^3	10.4×10^3
Blue component	0.51	8.3×10^3	16.3×10^3
Combined red components	0.49	1.3×10^3	2.7×10^3

Table 2. Distribution of Ca^{45} in *S. marcescens* cells and pigments. Total Ca^{45} counts in 70 ml of sample was 11.9×10^6 count/min.

Fraction	Weight (mg)	Total count/min in fraction	Specific activity (count/min mg)
Dry cells	760	2.8×10^6	3.7×10^3
Entire pigment	1.00	1.2×10^3	1.2×10^3
Blue component	0.50	0.65×10^3	1.3×10^3
Combined red components	0.50	0.51×10^3	1.0×10^3

that he could find no evidence for the presence of iron in the pigment itself. Bortels stated that Samkow (*3*) had found iron to be a constant component of the ash produced after combustion of the pigment.

During the course of chemical investigations carried out on the pigment components, which had been separated by paper chromatography (*4*), we found that the pigment fractions contain a large amount of ash. Most of the ash could be removed by treatment with disodium ethylenediaminetetraacetate (Versene). A qualitative thiocyanate test established that the ash contained iron. Spectrochemical analysis (*5*) demonstrated that other metal contaminates also were present in the ash, the principal ones being, in addition to iron, calcium and magnesium. We have investigated the presence of iron and calcium in the pigment fractions by use of the radioactive isotopes of the metals (*6*).

Serratia marcescens, strain Nima, was grown in liter flasks containing a thin layer of a liquid casein hydrolyzate-yeast extract medium to which isotopic Fe^{59} or Ca^{45} had been added. The cultures were incubated for 7 days at 30°C, and then the cells were harvested. The pigment was extracted and fractionated into its components by paper chromatography according to methods described elsewhere (*4*). The amount of isotopic iron in the various components was determined by means of a scintillation counter; the amount of isotopic calcium, by means of a thin-window Geiger-Müller tube.

The results of the experiments carried out with Fe^{59} and Ca^{45} are presented in Tables 1 and 2. Owing to the fact that the various red components (*4*) were present in quantities too small for accurate isotopic counts to be made, the three red fractions were combined to form the "combined red components." Examination of the tables reveals that both iron and calcium are incorporated into the cellular components of *S. marcescens*. However, the uptake of iron is greater. Approximately 34 percent of the added isotopic iron is found in the cells, whereas only 24 percent of the isotopic calcium was incorporated. Distribution of the isotopes in the various fractions according to their specific activity demonstrates that iron is specifically incorporated into the pigment. Calcium, however, is present to a lesser degree in the pigment than in the intact cells. The specific-activity calculations also emphasize the fact that iron is incorporated into the blue fraction of the pigment about 6 times as much as the metal is incorporated into the combined red fractions. Calcium is distributed more or less equally between both pigment fractions.

These results establish the fact that *S. marcescens* pigments will incorporate metals. However, the exact interpretation of the data must await the elucidation of the chemical structure of the pigment fractions. It is interesting to note that Wrede and Rothhaas (*7*) stated that the pigment was a tripyrrole compound. This structure would provide three nitrogen atoms as potential electron donors whose electrons could be shared with polyvalent metals in a chelate association. The increased affinity of the pigments for iron in lieu of calcium could be explained by the fact that iron can exist as a trivalent ion, but calcium is always divalent. Spectral and chemical data indicate that the blue component is a possible dimer of the red components (*8*). Such a structure could explain the greater binding of iron in the blue fraction, since more combining sites would be available than in the red fractions of the pigment.

ROBERT P. WILLIAMS
JAMES A. GREEN*
DONALD A. RAPPOPORT
Departments of Microbiology, Biochemistry, and *Radiology, Baylor University College of Medicine, Houston, Texas*

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* Candidate for the degree of doctor of philosophy, biology department, Rice Institute, Houston, Tex.

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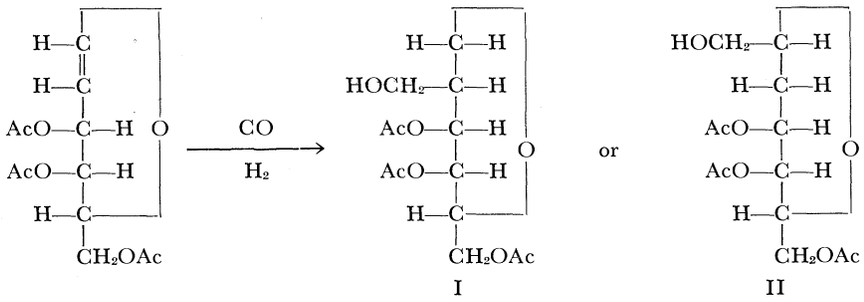
Lengthening the Carbon Chain of Sugars

Sugars that have more carbon atoms than the starting compounds have been prepared by several methods involving the addition of hydrocyanic acid, nitromethane, diazomethane, and the Grignard reagent to the appropriate sugar. A critical evaluation of these methods has been presented by Pigman and Goepp (*1*).

We have now found (*2*) that the carbon chain of carbohydrates can be lengthened by the addition of carbon monoxide and hydrogen to unsaturated sugars (glycals, *1, 3*). To the best of our knowledge, this is the first application of the oxo reaction (*4*) to the field of carbohydrates.

To a solution of 8.5 g of 3,4,6-tri-O-acetyl-D-galactal (*5*), 5.8 ml of ethyl orthoformate, and 0.4 g of dicobalt octacarbonyl (*6, 7*) in 20 ml of purified benzene contained by the liner of a reaction vessel having a void of 280 ml, there was added carbon monoxide (200 lb/in.^{2}) followed by hydrogen (1670 lb/in.^{2}) After the reactants had been heated at 130° to 140°C for 3 hours (pressure drop of 170 lb/in.^{2} at 22°C), the inorganic material was removed, and evaporation under vacuum yielded 9.3 g of a sirup. The products were chromatographically fractionated (*8*) using acid-washed aluminum oxide as absorbent and benzene-ethanol as developer. Two fractions were obtained. The lower (A) fraction (yield, 8.0 g) was deacetylated using 0.05*N* sodium methylate, and the product was then deionized using Amberlite 1R-120. The product was recrystallized several times from a solution of methyl alcohol in ethyl acetate (paper chromatograms showed one zone). Determined specifications of the product were as follows: mp = 158.5° to 159.5°C



(corrected), $[\alpha]_D^{21} = +37.6°$ (0.013 g/cm³ water).

The percentage composition of compound A that was calculated for $C_7H_{14}O_5$ $[C_7H_{10}O(OH)_4]$ consisted of the following values: C, 47.23; H, 7.86; OH, 38.18. Values found on microanalysis (*9*) were: C, 47.43; H, 8.16; OH, 37.4 (±1 in duplicate).

Compound A exhibited a strong hydroxy band in the infrared spectrum. It was undoubtedly a hydroformylated (*6*) product that was subsequently reduced in the presence of the large excess of hydrogen. The formation of A from 3,4,6-tri-0-acetyl-D-galactal may be illustrated by the reaction that appears at the top of this page, where the structure of A is represented by either (I) or (II) (*10*).

In a slightly modified experiment, a solution of 12.8 g of 3,4,6-tri-0-acetyl-D-glucal (*5*) in 45 ml of benzene and 3 ml of ethyl orthoformate was heated with 0.75 g of cobalt acetate tetrahydrate (*6*) under a 1 to 1 mixture of carbon monoxide and hydrogen (combined gas pressure at room temperature was 1550 lb/in.²) at 110° to 150°C for 5 hours. When the crude product was fractionated by chromatography (*8*), using acid-washed Magnesol-Celite as adsorbent and benzene as developer, a crystalline product (B) was obtained from the upper zone (yield, 20 percent). Compound B was recrystallized twice from ethyl acetate in petroleum ether at 30° to 60°C. Determined specifications of the product were as follows: mp = 76.5° to 78°C (corrected), $[\alpha]_D^{24} = +100°$ (0.00179 g/cm³ 95-percent ethanol). After hydrolysis, B reduced Fehling's solution in 15 seconds.

The percentage composition of B that was calculated for $C_{17}O_{28}O_9$ $[C_7H_9O_4(CH_3CO)_3(OC_2H_5)_2]$ consisted of the following values: C, 54.23; H, 7.51; CH_3CO, 34.3. The calculated molecular weight was 376. Values found on microanalysis (*11*) were: C, 55.56; H, 7.07; CH_3CO, 34.0; molecular weight, 350 (cryoscopic).

The analysis of compound B agrees most closely with the theoretical analyses of the diethylacetal derivative that might be expected to be produced by the subsequent reaction of the hydroformylation product with ethyl orthoformate. The lower zone of the chromatogram yielded a mixture consisting mainly of dihydro-3,4,6-tri-0-acetyl-D-glucal (*12*). The proof of structure of the compounds is in progress.

A. ROSENTHAL
D. READ
C. CAMERON
Department of Chemistry, University of British Columbia, Vancouver, Canada

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10. Configurations at carbon atoms No. 2 (I) and 1 (II) are not known.
11. Performed by W. Manser, Nerdstrasse 140, Zurish 37, Switzerland, and by G. Weiler and F. B. Strauss, 164 Banbury Road, Oxford, England.
12. Details of the work are being incorporated in a report that is in preparation.

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Increased Oxidative Effects on Irradiation in Glass

During a study on the radiation-induced flavor changes of meat fractions, we noticed a marked lack of analytic consistency in peroxide determinations in our irradiated lipid samples. Those portions of the sample that were adjacent to the walls of the glass container gave a much higher peroxide value than the portions in the center. These samples had been irradiated to 3×10^6 rep, with gamma radiation of a mixed energy spectra, in the canal of the Materials Testing Reactor, Phillips Petroleum Company, Arco, Idaho. A second series of samples irradiated in metal cans showed no such increases in the layers adjacent to the walls. We therefore ran two experiments to determine the extent of the effect of the glass during the irradiation of lipid materials (*1*).

A large sample of ether-extracted beef fat was irradiated in a 3-in. section of 3-cm Pyrex-glass tubing. The irradiation level and conditions were the same as those described in the preceding paragraph. Upon return, the sample was frozen solid with Dry Ice and pushed out of the tubing. The cylinder of lipid was sectioned to give an outer, a middle, and a core fraction. The peroxide data obtained on these fractions are given in Table 1. The peroxide determination was that of Hartman (*2*).

A second set of samples was irradiated in which ground glass was incorporated directly into the sample of lipid. The lipid used was ether-extracted pork fat. These mixtures were irradiated in cans to a level of 3×10^6 rep. The control samples were stored and handled in the same way as the experimental samples but received no irradiation. The peroxide values obtained on these samples are shown in Table 2.

It can be seen from both sets of data that there is a definite increase in the peroxide value of lipid samples irradiated in the presence of glass. Although the total effect may be slight when a large sample has been irradiated, it is of importance to be aware of the possibility of these effects. We have seen further evidence of this phenomena upon the irradi-

Table 1. Irradiation of a lipid sample in glass and metal containers.

Fraction	Milligrams of benzoyl peroxide per gram of fat	
	Glass	Metal
Outside	0.6	0.25
Middle	0.2	0.27
Core	0.05	Not sampled

Table 2. Irradiation of lipid in presence of glass.

Sample	Milligrams peroxide per gram of fat	
	Irradiated	Control
Pork fat	0.355	0.186
Pork fat + 10% w/w fine glass	0.420	0.175
Pork fat + 10% w/w coarse glass	0.500	0.188