Powdered Glass.—Pyrex and Kimble glass tubing was ground in an iron mortar and pestle, the product which passed a 150-mesh screen collected on a 200-mesh screen, digested with concentrated hydrochloric acid for 48 hours at 25–30°, thoroughly washed with distilled water and dried at 145° for 2 hours. In the calculation of surface areas an average diameter of 0.0088 cm. was assumed. The density of Pyrex glass was taken to be 2.25 g./cm.[3] and that of the Kimble glass 2.5 g./cm.[3].

Enzyme and Specific Substrate.—The α-chymotrypsin was an Armour preparation lot No. 10705. The acetyl-L-tyrosinhydroxamide, m.p. *ca.* 140° dec., $[\alpha]^{25}D$ 37.0° (*c* 5% in water), was prepared essentially as described by Hogness and Niemann.[6]

Enzyme Experiments.—All reactions were conducted as described by Hogness and Niemann[6] except that the improved analytical procedure described by Foster, Jennings and Niemann[10] was substituted for the one used earlier.[6] In every case plots of both $([S]_0 - [S]_t)$ *vs.* *t* and $\ln [S]_0/[S]_t$ *vs.* *t* were made and then corrected as described by Jennings and Niemann[5] using a value of $K_S = 43 \times 10^{-3}$ *M*. The values of v_0 given in Table I are the averages of those obtained from the corrected zero and first order plots which in no case differed by more than $\pm 0.005 \times 10^{-3}$ *M*/min.

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Epimerization and Fragmentation of Glucose by Quaternary Ammonium Base Type Anion Exchange Resins[1]

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The interconversion and degradation of mono- and disaccharides through the action of anion exchange resin (quaternary ammonium base type) in its hydroxyl form has been reported recently by several workers.[2-4] By analogy one would expect the mechanism of these reactions to be similar to that of the interaction of saccharides and strong alkali; however, failure to find mannose in the interconversion products has been cited by Rebenfeld and Pacsu[4] as evidence that the ene-diol mechanism for the Lobry du Bruyn transformation is not involved. In the case of the degradation products, five acidic compounds have been detected, but only two have been identified, these being lactic and glycolic acids.[2,3] The mechanism of the formation of these acids has not been investigated thoroughly.

In the present study, mechanism of the interconversion and degradation of glucose through the action of Amberlite IRA-400, a quaternary ammonium base type resin, was probed by the use of radioactive glucose-2-C^{14}. The interconversion products were examined by means of chromatography and radioautography. Lactic and glycolic acids were isolated from the degradation products and their respective isotopic distribution patterns is determined by degradation studies.

Experimental

Isolation of Fractions.—A solution containing 1.1 g. of glucose-2-C^{14} with a total activity of 1.66×10^5 c.p.m. was passed through a column 1.6 cm. diameter and 32 cm. long charged with 39 g. of IRA-400 (hydroxyl form). The absence of radioactivity in the effluent indicated that there was complete retention of the sugar on the column.

After standing 24 hours at room temperature the stoppered column was eluted with 1 *N* $(NH_4)_2CO_3$. After the removal of $(NH_4)_2CO_3$ by partial evaporation, the eluate subsequently was passed through an IR-112 cation exchange column to remove any remaining ammonium ions, and through an IR-4B weakly basic exchange column to remove any acidic constituents. The effluent from the latter thus contained only the sugars and other neutral components and gave a total activity of 1.07×10^5 c.p.m. or 64% of the original activity.

The original column was eluted with 2 *N* H_2SO_4 to recover the acidic products retained on the IRA-400. This was subsequently combined with the 2 *N* H_2SO_4 eluate of the IR-4B column. Organic acids then were recovered from the combined eluates by evaporation and exhaustive ether extraction. The mixed acid fraction so obtained had an activity of 2.48×10^4 c.p.m. or 15% of the original activity.

Identification of Interconversion and Degradation Products.—Paper chromatography of the mixed sugar fraction in the 80% phenol–water system revealed three components when sprayed with the normal carbohydrate reagents such as aniline phthalate and 3,5-dinitrosalicylic acid (R_f 0.33, 0.41 and 0.50). However, when ammoniacal silver nitrate was used, a fourth component of low R_f value (R_f 0.16) also slowly appeared. Radioautographs of these chromatograms showed all four spots to be radioactive. Three of these components (R_f 0.33, 0.41 and 0.50) were identified by co-chromatography as glucose, fructose and mannose, respectively. The fourth component is as yet unidentified.

Paper chromatography of the mixed acid fraction using a pentanol–formic acid–water system and a multiple-spray developing technique[5] revealed six components (R_f 0.08, 0.15, 0.23, 0.35, 0.56 and 0.74). Radioautography, however, indicated only three of these were radioactive (R_f 0.35, 0.56 and 0.74). These were identified, by color reactions with the various spray reagents and co-chromatography with authentic samples, to be: lactic acid (major constituent), glycolic acid and lactyllactic acid. The latter compound presumably results from the autoesterification of lactic acid during concentration of the samples for paper chromatography.[5,6] The other three non-radioactive components have not yet been identified.

Isolation of Acids.—Half of the mixed acid fraction was subjected to silica gel column chromatography according to the procedure of Bulen, Varner and Burell.[7] Three peaks were observed: a large radioactive lactic acid band containing essentially all of the starting activity, a very small radioactive glycolic acid band and a small non-radioactive band. The purity and identity of all fractions were established by paper chromatography. The unknown non-radioactive acid component could not be detected readily on paper chromatograms and it is as yet unidentified.

The glycolic acid content of the combined glycolic acid fractions was assayed colorimetrically to be 0.95 mg. according to the procedure of Newburgh and Burris.[8] After addition of the carrier, the diluted glycolic acid was isolated as its calcium salt. The lactic acid was isolated from the corresponding fractions as the zinc salt without dilution; yield 41.5 mg. Zn lactate trihydrate.

Degradation of Glycolic and Lactic Acids.—The glycolic acid was oxidized with lead tetraacetate according to a modification of the procedure of Schou, *et al.*[9] To a frozen mixture of calcium glycolate in glacial acetic acid was added

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the lead tetraacetate, and the mixture was heated at 90° on a water-bath for 30 minutes. Carbon dioxide from the oxidation was swept out of the system with a slow stream of nitrogen gas and trapped in carbon dioxide-free sodium hydroxide. The residue in the reaction flask was cooled and neutralized with sodium hydroxide, and the formaldehyde, derived from the carbinol carbon, was distilled into an ice-cooled receiver. The formaldehyde was then oxidized to formic acid with sodium hypoiodite according to the procedure of Sakami[10] and the resulting formic acid steam distilled from the reaction mixture. This was then oxidized to carbon dioxide by mercuric chloride in glacial acetic acid as described by Pirie.[11]

The lactic acid was oxidized with acid permanganate according to the method of Wood, Lifson and Lorber.[12] The acetaldehyde was trapped in bisulfite solution and the carbon dioxide from the carboxyl carbon trapped in CO_2-free sodium hydroxide. A portion of the acetaldehyde–bisulfite complex was taken for formation of acetaldehyde 2,4-dinitrophenylhydrazone and the remainder was treated with sodium hypoiodite to give iodoform and formic acid. The formic acid was oxidized to carbon dioxide as described previously.[11]

Radioactivity Measurements.—The specific activities of the sugar and acid fractions were determined as negligible thickness plates by direct plating. All other compounds were determined by appropriate wet or dry combustion to carbon dioxide and counted as barium carbonate with correction for background and self-absorption in the conventional manner.

Results and Discussion

The identification of fructose and mannose, as well as glucose, in the interconversion products by means of chromatography and radioautography strongly suggested that the interaction of quaternary ammonium base type resin with glucose is similar to the base-catalyzed Lobry du Bruyn transformation and can be explained readily by the well established ene-diol mechanism.

The isotopic distribution patterns of glycolic and lactic acids isolated from the glucose degradation mixture are given in Table I.

TABLE I

DISTRIBUTION OF C^{14} IN GLYCOLIC AND LACTIC ACIDS FROM GLUCOSE-2-C^{14}

Compound	Specific activity, c.p.m. × 10^4 per mM.	Total, %
Original glucose-2-C^{14}	2.71	100
Glycolic acid whole molecule	2.78	100
(1) COOH	1.45	52
(2) CH_2–OH	1.20	42
	1.33[a]	
Lactic acid whole molecule	1.22	100
(1) COOH	0.01	1
(2) CHOH	1.25	103
(3) CH_3	0	0

[a] By difference.

Since the specific activity of lactic acid is approximately one-half that of glucose, it is evident that glucose probably has undergone a C_3–C_3 split followed by the conversion of both C_3 units to lactic acid. This is further supported by the degradation studies which reveal that the labeling of lactic acid occurs exclusively in carbon atom 2. The finding is in line with the mechanism originally proposed by Evans[13] and subsequently modified by Gibbs[14] concerning the degradation of glucose by strong alkalies.

Although a relatively small amount of glycolic acid was formed in the glucose degradation process, the labeling pattern of this acid has revealed a rather interesting situation. The specific activity of this acid is practically the same as that of glucose, indicating the following possibilities: (1) that a C_2–C_4 cleavage of glucose has taken place with the C_2-unit, corresponding to glucose carbon atoms 1 and 2, in turn converted to glycolic acid; or (2) that a C_3–C_3 split of glucose has given rise to two unequivalent units with only one of these units degraded to the C_2-acid. Yet it seems probable that the latter mechanism is not involved, as suggested by the lactic acid data which demonstrate the practically complete equivalent of the C_3 units in the glucose degradation. Consequently, it appears that a C_2–C_4 cleavage of glucose was the initial step in the glycolic acid formation. The finding that both the carbon atoms were approximately equally labeled defined the nature of the C_2 compounds as symmetrical in structure and suggested that glycolic aldehyde may have been the intermediate compound which was randomized by way of the ene-diol mechanism and oxidized to glycolic acid by means of autoöxidation.

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Two-stage Polymerizations. III. Infrared Evidence for the Course of Polymerization of Vinyl Ethers of Unsaturated Phenols[1]

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Previous work[2,3] has shown that compounds containing two or more double carbon-to-carbon linkages which vary considerably in their relative tendencies to form induced ions or free radicals can be made to undergo two-stage polymerizations. Allyl ethers of phenols containing unsaturated side chains were found to undergo polymerization through a cationic mechanism in which only the double bond of the allyl ether group was involved. The unsaturated side chains of these linear polymers could be made to enter into polymerization reactions only by thermal means. Vinyl ethers of unsaturated alcohols were found to produce linear polymers through the catalytic influence of boron trifluoride at relatively low temperatures. These linear polymers were subject to cross-linking through the catalytic effect of peroxide catalysts, as well as to copolymerization with other monomers to produce cross-linked materials. Due to the known reactivity of vinyl ethers toward polymerization by a cationic mechanism[4] it was assumed that

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