ALKALINE DECOMPOSITION OF D-XYLOSE-*1*-^{14}C, D-GLUCOSE-*1*-^{14}C, AND D-GLUCOSE-*6*-^{14}C*

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ABSTRACT

The distribution of radioactivity in the three- and four-carbon saccharinic acids, lactic acid and 2,4-dihydroxybutyric acid, obtained from D-xylose-*1*-^{14}C, D-glucose-*1*-^{14}C, and D-glucose-*6*-^{14}C, was measured. The relative importance of the various mechanisms for forming 2,4-dihydroxybutyric acid was determined. Recombination of two-carbon fragments was found to be an important mechanism at the high alkalinity and temperature employed.

INTRODUCTION

The formation of 2,4-dihydroxybutyric acid (**1**) from D-glucose has been satisfactorily explained by assuming a C-2–C-3 cleavage followed by the β-elimination of a hydroxyl group and a benzilic acid type of rearrangement [1-3]. However, the occurrence of significant proportions of this acid among the alkaline degradation products of 2-deoxy-D-*arabino*-hexose[4], D-xylose[5], and L-rhamnose[4] as well as D-glucose[5] and D-fructose[5], indicates that other mechanisms must exist. The most plausible possible mechanisms are apparently (*1*) initial C-2–C-3 or a C-4–C-5 cleavage followed by elimination and rearrangement, or (*2*) an aldol condensation of two-carbon fragments with the resulting tetrose undergoing elimination and rearrangement, or a combination of mechanisms *1* and *2*. This work was undertaken to determine the relative importance of these mechanisms.

D-Xylose-*1*-^{14}C, D-glucose-*1*-^{14}C, and D-glucose-*6*-^{14}C were each subjected to alkaline degradation by refluxing in ~4.0M sodium hydroxide. The resulting mixture of acids was converted into the anilides[6], and the major components, lactic anilide, 2,4-dihydroxybutyric anilide, and D-α,β-glucometasaccharinic anilide (or its five-carbon homolog), were separated by column chromatography. The anilides of lactic acid and **1** were then converted into their corresponding benzimidazoles, which were systematically degraded[7] to determine the distribution of radioactivity (Table I).

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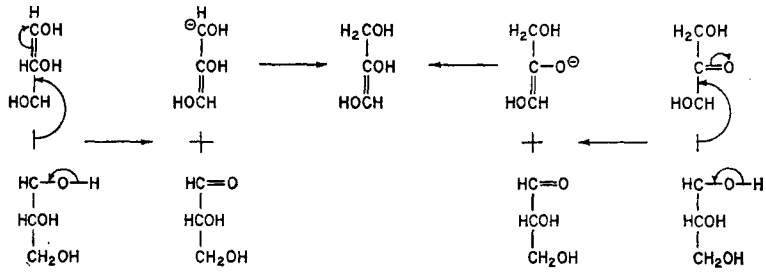
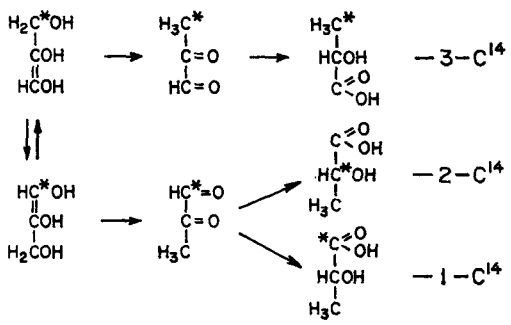
DISCUSSION

All of the lactic acid samples were found labeled predominantly in the methyl group; all had a significant amount of activity at the central carbon atom (C-2).



Scheme 1

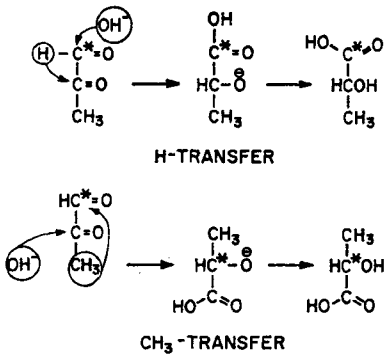
The preponderance of activity at the methyl carbon is in accord with the currently accepted mechanism[3] illustrated in Scheme 1. By this mechanism, the aldehyde carbon of the triose-enediol released from the reducing end originates from C-3 of the aldose. In the absence of isomerization (Scheme *2*), lactic acid-*3*-^{14}C



Scheme 2

would be formed from a C-1 labeled sugar. In contrast, the carbonyl group of the D-glyceraldehyde formed from the nonreducing end of a hexose originates from C-4. It follows that hexoses labeled at either C-1 or C-6 would yield lactic acid labeled predominantly at C-3. This conclusion agrees with the data of Table I, but a considerable quantity of lactic acid-*1*-^{14}C, which results from the isomerization shown in Scheme 2, is also formed.

The C-1 labeled triosulose shown in Scheme 3 rearranges, via a benzilic acid rearrangement, by addition of a hydroxyl ion to either C-1 or C-2. In the reaction at C-1, the rearrangement involves a hydride-ion transfer resulting in lactic acid-*1*-^{14}C. Hydroxyl ion attack at C-2 results in the transfer of the methyl group and a change in the order of the carbon atoms, with the active carbon becoming C-2. The hydride-ion



Scheme 3

shift predominates, but under the conditions used here, appreciable proportions of C-2 labeled lactic acid were also formed. The proportion of methyl migration is equal to the ratio of the activity at C-2 to the sum of that at C-1 and C-2. From the data of Table I the ratios were calculated to be 0.241, 0.184, and 0.113, for D-xylose-*1*-^{14}C, D-glucose-*1*-^{14}C, and D-glucose-*6*-^{14}C, respectively. The difference in values obtained for D-glucose-*1*-^{14}C and D-glucose-*6*-^{14}C is probably not significant, as the amount of radioactivity at C-2 is small and is calculated by difference. Under conditions milder than used here, this reaction is much less important. Sowden and Pohlen [8] found no methyl-group transfer in 1.68M NaOH at 25°, whereas Gibbs[9] recorded a ratio of 0.06 in 3.0M KOH at 50°.

Since the C-3 labeled intermediate HCO–CO–C*H_3 can rearrange only to lactic acid-*3*-^{14}C, even if methyl-group transfer occurs, the completeness of the isomerization $H_2C^*OH–COH=CHOH \rightleftharpoons HC^*OH=COH–CH_2OH$ is reflected in the ratio of the sum of radioactivity at C-1 and C-2 to that at C-3. From the data of Table I, it is apparent that equilibrium was not attained prior to dehydration. Calculated as the ratio of actual sum of lactic acid-*1*-^{14}C and lactic acid-*2*-^{14}C to what would be present had complete equilibrium been reached (one-half of the total activity of the lactic acid), the amount of isomerization is 81.5, 71.0, and 81.6%, an average of 76%.

Gibbs[9] found the lactic acid-^{14}C obtained from glucose-*1*-^{14}C equally labeled at C-1 and C-3. From this, it may be concluded that the nascent, radioactive triose-enediol, liberated from the reducing end of the molecule, reached complete equilibrium prior to dehydration. He also found that, under the same reaction conditions, D-glucose-*3,4*-^{14}C produced lactic acid-^{14}C labeled principally at C-1; the ratio of activity at C-1 to that at C-3 was 2.6. For this sugar, the isomerization was incomplete, even though the same experimental conditions had been used. This fact could be explained by assuming that the D-glyceraldehyde originating from the nonreducing end of the D-glucose molecule is incompletely isomerized when dehydration occurs. If this were true, a significant difference in the proportion of radioactivity at C-3 of the lactic

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TABLE I

SPECIFIC RADIOACTIVITY (SpAc) AND DISTRIBUTION IN ACIDIC PRODUCTS

Aldose		*Product*								
	SpAc (μCi/mmole)	*Lactic acid*				*2,4-Dihydroxybutyric acid*				D-α,β-*Glucometasaccharinic acid*
		SpAc (μCi/mmole)	*Distribution (%)*			*SpAc (μCi/mmole)*	*Distribution (%)*			*SpAc (μCi/mmole)*
			C-1	*C-2*	*C-3*		*C-1*	*C-2*	*C-3+C-4*	
D-Xylose-*1*-^{14}C	0.186	0.102	30.9	9.8	59.3	0.157	26.0	24.1	49.9	
D-Glucose-*1*-^{14}C	0.230	0.115	29.0	6.5	64.5	0.118	22.9	17.9	59.2	0.236
D-Glucose-*6*-^{14}C	0.198	0.0938	36.2	4.6	59.2	0.135	17.5	10.5	72.0	0.202

acid-^{14}C from D-glucose-*1*-^{14}C and D-glucose-*6*-^{14}C might be expected. However, as seen in Table I, the distribution is about the same for both hexoses. This indicates that, under these reaction conditions, there is little difference between the three-carbon fragments liberated from each end of the hexose.

In agreement with previous investigations[9,10], each of the terminal carbon fragments of D-glucose is shown to contribute approximately equally as the source of lactic acid. This is precisely true for the D-glucose-*1*-^{14}C sample, whereas with D-glucose-*6*-^{14}C, 47.5% of the lactic acid originates from the terminal end of the D-glucose molecule. The activity of the lactic-^{14}C acid derived from D-xylose-*1*-^{14}C reflects the relative lability of the C-2–C-3 and the C-3–C-4 bonds; 45.2% originates from C-2–C-3 cleavage, the remainder from C-3–C-4 cleavage. Thus, the C-3–C-4 bond is somewhat more labile than the C-2–C-3 bond.

The almost uniform distribution of radioactivity among the carbon atoms of **1** derived from D-xylose-*1*^{14}C indicates that the source is almost entirely from the recombination of completely isomerized, two-carbon fragments, with only minor amounts originating from C-1–C-2 or C-4–C-5 cleavage. Ignoring the small amount of C-2 label that could have arisen from $-CH_2-CH_2-OH$ migration (equivalent to methyl-group migration), calculation shows the distribution of sources for the recovered acid to be 90.1% from recombination, 6.8% from C-1–C-2 cleavage, and 3.1% from C-4–C-5 cleavage. As, under these conditions, the molar yield of **1** from D-xylose is approximately 18%, the extent of two-carbon fragment recombination and conversion into **1** must be in excess of 40%.

Fragmentation of D-xylose at these experimental conditions is primarily at one of the central bonds. An estimate of the lability of the various linkages shows that 1% occurs at C-4–C-5, 2% at C-1–C-2, 44% at C-2–C-3, and 53% at C-3–C-4.

Interpreting the data of radiochemical distribution for **1** derived from D-glucose is considerably more involved than that for D-xylose because of the large extent of secondary fragmentation that occurs. Four-carbon fragments from primary cleavage undergo further cleavage and produce two-carbon fragments, some of which recombine. However, the participation of each mechanism for forming **1** may be calculated by combining the data for D-glucose-*1*-^{14}C and D-glucose-*6*-^{14}C and imposing the requirement that the ratio of C-2–C-3 cleavage to C-4–C-5 cleavage (the $-CH_2-CH_2OH$ transfer) and the amount of secondary fragmentation, be the same for both sugars. The result of this calculation indicates that the approximate distribution of sources for **1** is 46% from C-2–C-3 cleavage, 40% from recombination of two-carbon fragments, and 14% by C-4–C-5 cleavage. There is about 10% transfer of $-CH_2-CH_2OH$ groups and 30% of secondary fragmentation of four-carbon fragments. Approximately 58% of the two-carbon fragments generated recombined to give four-carbon units.

The extent of isomerization of the tetrose is approximately 34% compared with 76% for the triose and 100% for the two-carbon fragment. The yield of **1** from D-glucose was 20.6 mol %. Because, under these conditions, the yields of lactic and glucometasaccharinic acids are approximately 50% and 20%, respectively, the

stability of the C-2–C-3 bond relative to the C-3–C-4 bond must be considerably greater than that for xylose.

It is concluded that the recombination of two-carbon fragments contributes significantly to the formation of **1**. The acid would be expected to be found in any alkaline reaction medium where this type of two-carbon fragment is formed, the presence of four contiguous oxygenated carbons in the original carbohydrate being unnecessary.

EXPERIMENTAL

General. — Radioactive sugars (Calatomic*, Los Angeles) were diluted with an aqueous solution of the corresponding inactive sugars. Crystals, obtained by concentration and addition of ethanol, were washed with ethanol and dried (vacuum, overnight, 70°). T.l.c. and C, H analyses indicated no impurities.

Specific activities were determined by scintillation counting (Packard Tricarb 3003). Triplicate or duplicate samples were dissolved in a suitable solvent (0.2 ml) and scintillation liquid in *p*-dioxane added (15 ml). The effect of the solvent was negligible.

T.l.c. was performed on silica gel HF thin-layer plates. Anilides were chromatographed in 1:1 benzene–acetone, benzimidazoles of the saccharinic acids in 9:1 chloroform–methanol, and benzimidazole 2-carboxylic acid in 9:1 chloroform–acetic acid. All were visible in u.v. light. Free acids and lactones were chromatographed in 4:1:1 ethyl acetate–acetic acid–water and visualized with ammoniacal silver nitrate.

All evaporations were under diminished pressure below 50°.

Preparation of anilides. — The aldose (30 g) in water (50 ml) was added to 50 ml of refluxing 8M NaOH over a 1-min period. Refluxing was continued for an additional 2 min. The cooled solution was applied to a column (475 × 5 cm) of Dowex 50- × 8 (H^+), and the acids eluted with water. The eluate was concentrated (26–28 g), ethanol (200 ml) added, and the solution re-evaporated. Ethanol (25 ml), aniline (25 ml), and glacial acetic acid (3 ml) were added to the resulting syrup and the mixture heated on a steam bath (1.5 h). Evaporation of the reaction mixture yielded a crude, dark mixture of anilides that was partially purified by adding 400 ml of water and by heating with trituration on a steam bath (30 min). This solution was refrigerated overnight, filtered, and evaporated. The mixture of water-soluble anilides was dissolved in 25:1 chloroform–ethanol, charged to a silica gel column of (475 × 5 cm, Mallinckrodt, SilicAR CC4, 100–200 mesh), and eluted with a 2 liters of the same solvent to elute the anilides of lactic acid and of 2,4-dihydroxybutyric acid. The fractions were pooled and evaporated. The anilides of the acids of higher molecular weight were eluted with 800 ml of acetone. None of the lactic acid anilides thus recovered was crystalline, but all of the anilides of **1** crystallized spontaneously from the concentrated syrups. These were crystallized twice from acetone, and dried, m.p. 115–116° (lit.[5]: 115–116°). The mixture of D-α,β-glucometasaccharinic acid anilides from both

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hexoses crystallized spontaneously. These crystals were recrystallized from acetone. Attempts to crystallize the corresponding fraction from D-xylose-*1*-^{14}C were unsuccessful.

The specific activity of all of the crystalline anilides was measured by dissolving them in water (0.2 ml) before adding the scintillation liquid. The results were the following:

Anilide of 2,4-dihydroxybutyric acid (μCi/mmole): from D-glucose-*1*-^{14}C, 0.119; from D-glucose-*6*-^{14}C, 0.135; from D-xylose-*1*-^{14}C, 0.157.

Anilide of D-α,β-glucometasaccharinic acid (μCi/mmole): from D-glucose-*1*-^{14}C, 0.236; from D-glucose-*6*-^{14}C, 0.202.

Conversion of lactic anilide into 2-(1-hydroxyethyl)benzimidazole. — Lactic anilide (2.0 g) in 4M NaOH (2 ml) was refluxed for 2 h. The cation was removed by exchange on Dowex 50×8 (16 ml). *o*-Phenylenediamine dihydrochloride (3.0 g) was added and the solution evaporated to dryness. The residue was dissolved in water (4–6 ml) and heated (2 h, 125°) until it became a thick syrup or a solid. The resulting material was dissolved in water (10 ml), carbon was added, and the suspension centrifuged. The decanted liquid was filtered, and concentrated to 25 ml. The pH was adjusted to 7.5–7.8 with ammonium hydroxide (25%), and precipitation allowed to occur overnight in a refrigerator. The light-brown precipitate was filtered and air-dried (average yield 2.1 g). It was dissolved in hydrochloric acid (5%), and precipitated with ammonium hydroxide (25%), filtered, washed with a small amount of cold water, and air-dried (average yield, 1.5 g). The air-dried solid was then sublimed (0.3 torr, 145°) to give a white, crystalline solid, m.p. 179—180° (lit.[11]: 179–181°). Samples were dissolved in 0.2 ml of methanol for scintillation counting.

Conversion of 2,4-dihydroxybutyric anilide into 2-(1,3-dihydroxypropyl)benzimidazole. — 2,4-Dihydroxybutyric anilide (2.0 g) in M NaOH (25 ml) was refluxed for 0.5 h. Sodium ion was removed (IR-120, 50 ml), and the solution evaporated to dryness. An identical procedure as that for lactic anilide was used to prepare the benzimidazole. The product was recrystallized twice by dissolving in hydrochloric acid (5%) and by precipitating with ammonium hydroxide (25%). The product was washed with cold water, and dried (vacuum, overnight, 30°), m.p. 189–190°.

Anal. Calc. for $C_{10}H_{12}N_2O_2$: C, 62.50; H, 6.25; N, 14.58. Found: C, 62.03; H, 6.18; N, 14.32.

Preparation of benzimidazole-2-carboxylic acid and benzimidazole. — The procedure of Roseman[7], slightly modified, was used to oxidize the benzimidazoles. A hot 3% solution of potassium permanganate (250 ml) was added to a boiling, alkaline solution (375 ml water, 0.5 g sodium carbonate) of the benzimidazole (1.25 g). Additional oxidant was introduced to maintain an excess by adding crystals of permanganate. After refluxing for 30 min, the solution was cooled, and excess permanganate removed by adding ethanol. The liquid was decolorized (carbon) and evaporated to dryness. The dry residue was dissolved in water (25 ml), and filtered. The filtrate was brought to pH 5.0 with glacial acetic acid, and placed in the refrigerator overnight. The crystals of dehydrate were washed with water, dried under

vacuum overnight at ambient temperature, and then dried under vacuum with phosphorus pentoxide for 3 days. Care had to be taken to ensure complete drying while avoiding decarboxylation; the temperature did not have to exceed 35°. Purity of all samples was checked by C, H assay and t.l.c. For scintillation counting, samples were dissolved in 0.2 ml of 0.1M NaOH.

Activity of benzimidazole-2-carboxylic acid-^{14}C: from 2-(1-hydroxyethyl)-benzimidazole-^{14}C (μCi/mmole): from D-glucose-*1*-^{14}C, 0.0408; from D-glucose-*6*-^{14}C, 0.0383; from D-xylose-*1*-^{14}C, 0.0415.

From 2-(1,3-dihydroxypropyl)-benzimidazole-^{14}C (μCi/mmole): from D-glucose *1*-^{14}C, 0.0481; from D-glucose-*6*-^{14}C, 0.0378; from D-xylose-*1*-^{14}C, 0.0787.

The benzimidazole-2-carboxylic acid-^{14}C samples (200 mg) were decarboxylated in a sublimator under conditions (180°, 0.3 torr) suitable for sublimating the resulting benzimidazole. The dry, white crystalline products obtained melted in the range 169–171° (lit: 170°). Samples for scintillation counting were dissolved in 0.2 ml of ethanol. The following results were obtained:

Activity of benzimidazole-^{14}C: from 2-(1-hydroxyethyl)benzimidazole-^{14}C (μCi/mmole): from D-glucose-*1*-^{14}C, 0.0334; from D-glucose-*6*-^{14}C, 0.0340; from D-xylose-*1*-^{14}C, 0.0315.

From 2-(1,3-dihydroxypropyl)benzimidazole-^{14}C (μCi/mmole): from D-glucose-*1*-^{14}C, 0.0270; from D-glucose-*6*-^{14}C, 0.0236; from D-xylose-*1*-^{14}C, 0.0409,

Yield of 2,4-dihydroxybutyric acid (**1**). — To circumvent the problem of preparing **1** in high purity, the easily purified anilide of **1** was used.

A mixture of free acids was prepared from D-glucose-*1*-^{14}C (29.90 g) by the procedure previously described. An aliquot corresponding to 0.932 g of glucose-*1*-^{14}C was then diluted with inert **1**. This inert material was prepared by alkaline hydrolysis (M NaOH, 0.5 h) of a purified sample of the anilide of **1** (102.0 mg). Twice crystallized anilides of **1** were prepared from both the diluted and the undiluted solutions. The yield of **1** (18.9 mol%) was calculated by comparing the specific activities of the diluted (0.119 μCi/mmole) and the undiluted material (0.0772 μCi/mmole). Because some of the labeled **1** from the anilide may have been decomposed during hydrolysis or because hydrolysis may have been incomplete, this figure (18.9 mol%) represents the minimum yield. The maximum yield of **1** (22.3 mol %) was obtained by the same procedure, but inert D-glucose (0.857 g) was used, and the acid mixture diluted with labeled **1** prepared from the crystalline labeled anilide of **1** (100.0 mg, 0.119 μCi/mmole). The activity of the anilide prepared from this diluted mixture was 0.0391 μCi/mmole. The minimum yield of **1** from D-xylose-*1*-^{14}C, determined in the same manner was found to be 16.4%.

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REFERENCES

1 O. SCHMIDT, *Chem. Revs.*, 17 (1935) 137.
2 H. S. ISBELL, *J. Res. Nat. Bur. Stand.*, 32 (1944) 45.
3 J. C. SOWDEN, *Advan. Carbohyd. Chem.*, 12 (1957) 35.
4 J. F. HARRIS, unpublished data.
5 A. ISHIZU, B. LINDBERG, AND O. THEANDER, *Acta Chem. Scand.*, 21 (1967) 424.
6 J. W. GREEN, *J. Amer. Chem. Soc.*, 78 (1956) 1894.
7 S. ROSEMAN, *J. Amer. Chem. Soc.*, 75 (1953) 3854.
8 J. C. SOWDEN AND E. K. POHLEN, *J. Amer. Chem. Soc.*, 80 (1958) 242.
9 M. GIBBS, *J. Amer. Chem. Soc.*, 72 (1950) 3964.
10 J. C. SOWDEN, M. G. BLAIR, AND D. J. KUENNE, *J. Amer. Chem. Soc.*, 79 (1957) 6450.
11 R. J. DIMLER AND K. P. LINK, *J. Biol. Chem.*, 143 (1942) 557.