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Carbon Exchange in Hot Alkaline Degradation of Glucose

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Received May 7, 2002

The decomposition of 1-^{13}C−D-glucose, 6-^{13}C−D-glucose, and 1-^{13}C-sodium lactate has been studied in hot (145 ± 3 °C) alkaline (3.5 M) sodium hydroxide solution in order to understand the mechanisms of carbon exchange in the alkaline degradation of glucose. The results show that in the formation of lactate from glucose the carboxylate (COO^-) carbon is formed preferentially from C1 carbons but methyl (CH_3) carbon is formed preferentially from C6 carbons. However, on further decomposition of lactate to ethanol and carbonate, ^{13}C-labeled carboxylate (COO^-) is scrambled equally among carbonate and both carbons in product ethanol molecules. In the production of glycolate, the labeled C1 carbon mainly ends up as carboxylate (COO^-) carbon, while for C6-labeled glucose the labeled carbon mainly ends up as alcoholic (CH_2OH) carbon. In the production of acetate and formate there is also discrimination between C1 and C6 label.

Introduction

Although the decomposition mechanism of glucose in alkaline solution has been known for a long time,[1−11] little is known of the pathway by which products are formed in highly alkaline solution (3.5 M) at high temperatures (140−150 °C), yet knowledge of this process is important in industrial processing of plant derived material, for example, in the Bayer process for separating alumina in bauxite. In this process organic matter in the bauxite is known to play a significant role in affecting crystal growth and quality. Glucose from carbohydrates in plant detritus plays an important role.[12]

The major products from hot alkaline degradation of glucose are lactic acid, formate, glycolic acid, and acetate.[1−5,11,12] However, the mechanism of formation of these and other compounds has not been established. Thus in this work we use ^{13}C-labeled glucose to follow decomposition pathways using ^{13}C NMR spectroscopy. Harris[11] used the same technique to establish preliminary mechanistic results at different temperatures and concentrations not related to Bayer processing. The results described here are more detailed and quite different and demonstrate a change in mechanism under different conditions.

Results and Discussion

Isotope Exchange. It is well-known[13] that the concentration of the noncyclic open form of glucose and its furanoic forms in water are negligible, and that equilibrium values are 64% β and 36% α for the pyranose forms, which is confirmed here, ±2% for the labeled isomers. Table 1 shows the ^{13}C distributions of all the products identified from digestion of unlabeled and labeled 1-^{13}C-D-glucose and 6-^{13}C-D-glucose after 1 h digestion at 145 °C in 3.5 M NaOH. Table 1 also shows enhanced yields of ^{13}C in these compounds relative to that in the original unlabeled mixture of products. It is clear that these values are greater than one, and that label from 1-^{13}C-D-glucose and 6-^{13}C-D-glucose ends up in all carbons in the products. It is shown in Table 1 that labeled lactate is the predominant compound formed from both 1-^{13}C-D-glucose and 6-^{13}C-D-glucose under Bayer simulated laboratory conditions. The labeling occurs at all three lactate carbons but in different proportions.

The percentage distributions of the label in a given compound are readily calculated. For 1-^{13}C-D-glucose the production of C1-labeled lactate (49.5/(49.5 + 1.0 + 37.8)

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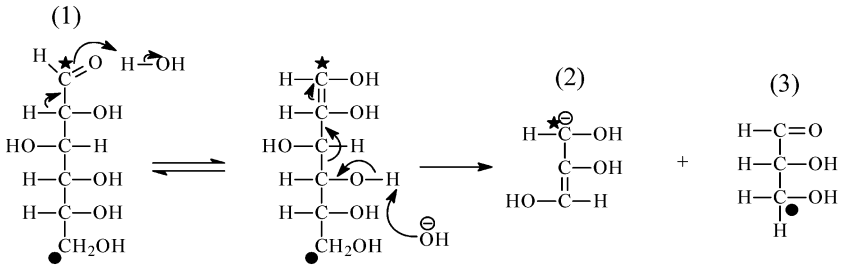
10.1021/jo025912t CCC: $22.00
Published on Web 11/02/2002

TABLE 1. ^{13}C Distribution (%) and Enhanced Yield in Products from Unlabeled Digested α-D-(+)-Glucose and Labeled 1-^{13}C-D-Glucose and 6-^{13}C-D-Glucose Digestions in 3.5 M NaOH for 1 h at 145 °C

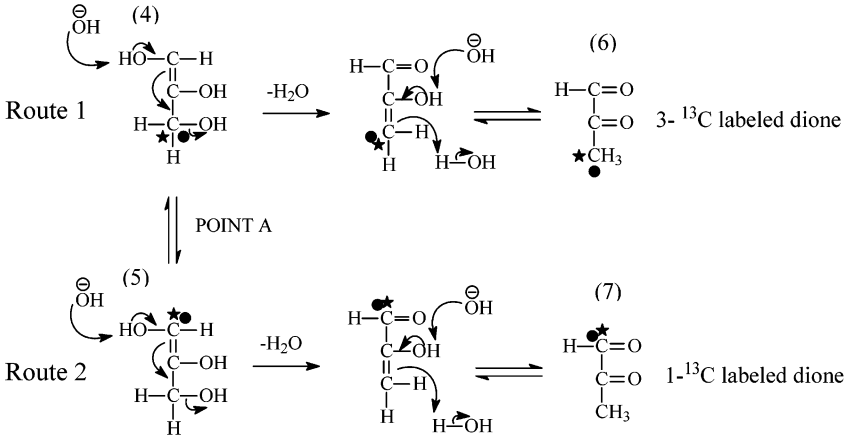
product	chemical shift δ (ppm)	unlabeled α-D-(+)-glucose digestion (1 h)	1-^{13}C-D-glucose digestion (1 h)	6-^{13}C-D-glucose digestion (1 h)	enhanced yield[a] 1-^{13}C-D-glucose digestion (1 h)	enhanced yield[a] 6-^{13}C-D-glucose digestion (1 h)
lactate	185.6	28.7	49.5	24.2	155.2	76.2
COO^-	|	|	|	|	|	|
|	71.1	24.8	1.0	1.1	3.7	3.7
CHOH	|	|	|	|	|	|
|	23.2	21.6	37.8	33.7	157.9	140.5
CH_3						
glycolate	184.6	2.7	3.5	2.9	116.6	96.4
COO^-	|	|	|	|	|	|
|	66.5	2.8	0.1	31.7	3.7	1019.2
CH_2OH						
acetate	184.1	0.1	0.1	0.1	90.0	90.0
COO^-	|	|	|	|	|	|
|	26.3	0.1	1.3	0.4	1169.8	359.9
CH_3						
ethanol	57.0	3.0	0.1	0.1	2.8	2.8
CH_2OH	|	|	|	|	|	|
|	17.6	3.2	0.3	0.1	8.3	2.8
CH_3						
formate $HCOO^-$	173.8	0.1	1.3	0.5	1169.8	449.9
carbonate CO_3^{2-}	170.9	9.7	0.4	0.4	3.7	3.7
other products		3.2	4.6	4.8	129.5	135.0

[a] Enhanced yield is defined as the yield of the functional group in ^{13}C-labeled experiment (g/100 g)/yield in unlabeled experiment (g/100 g) = [(% distribution in labeled experiment (g/100 g) × fraction of label enhancement in glucose, 0.99)/(% distribution in unlabeled experiment (g/100 g) × fraction of natural label enhancement (0.011 for ^{13}C in natural glucose))].

SCHEME 1. Mechanism for Glucose Cleavage To Form a Carbanion and an Aldehyde



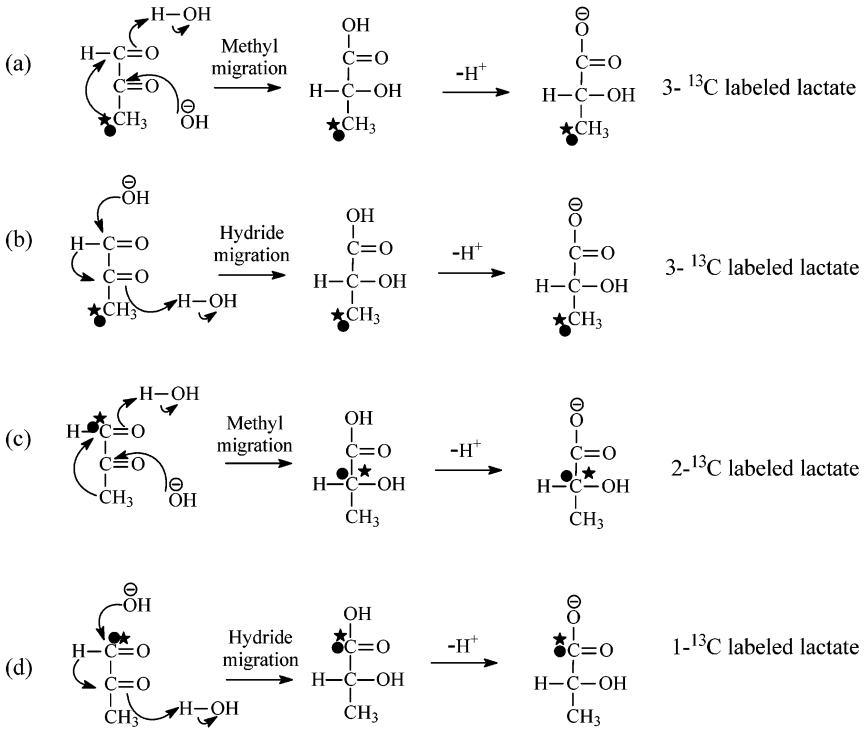
SCHEME 2. Triose−Enediol β-Elimination To Form Diones



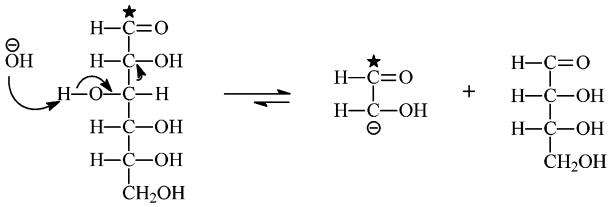
= 56.1%) is favored over other lactate carbons while from 6-^{13}C-D-glucose the production of C3-labeled lactate (57.1%) is favored. The differences between labeled C1 and labeled C3 lactate formation can be explained in terms of isomerization as shown in Schemes 1 and 2. For C1-labeled glucose the label is marked with the symbol ★ and for C6-labeled glucose the label is marked with the symbol ●.

Initial cleavage of the glucose molecule (**1**) gives rise to a 3-carbon carbanion (**2**) and an aldehyde (**3**). The aldehyde **3** can further undergo enolization to **4** (Scheme 2), followed by isomerization. The carbanion **2** may pick up a proton from water to also give **4** where the labels at C1 and C6 then become synonymous. Species **4** may isomerize to form **5** by picking up a proton β to the original labeled carbon.

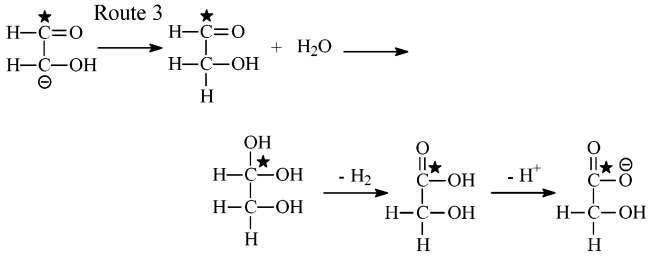
SCHEME 3. Formation of Labeled Lactate from Diones



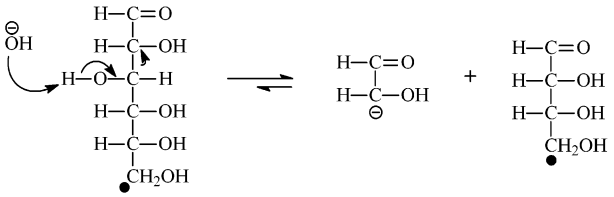
SCHEME 4. Retroaldolization of 1-^{13}C-D-Glucose into a Labeled 2-Carbon Carbanion and a Tetrose



SCHEME 5. Formation of C2-Labeled Glycolate from C1-Labeled Glucose



SCHEME 6. Retroaldolization of 6-^{13}C-D-Glucose into a 2-Carbon Carbanion and a Labeled Tetrose



In Scheme 2 the formation of 1,2-diones **6** and **7** from **4** and **5** involves β-elimination of water. The C3-labeled intermediate dione **6** rearranges to give only C3- labeled lactate (Scheme 3, reactions a and b). On the other hand the C1-labeled dione **7** can rearrange to give either C2- or C1-labeled lactate through either methyl (Scheme 3, reaction c) or hydride transfer (Scheme 3, reaction d), respectively. However, reaction 3c rarely occurs. The completeness of the isomerization between the trioses **4** and **5** (see Scheme 2, Point A) is reflected in the ratio of the sum of C1 and C2 to that of C3 label. For example, for 1-^{13}C-D-glucose this ratio is 1.34 = [((56.1 + 1.1)/42.8) or from Table 1, (49.5 + 1.0)/37.8].

For the 6-^{13}C-D-glucose the labeled glycolate produced is predominately at the C2 (CH_2OH, 91.6%) with only 8.4% at the C1 position (COO^-). ^{13}C-labeled C2 units can be formed from C2 carbon retroaldolization of C1-labeled glucose (Scheme 4). The carbanion is then protonated and rearrangement forms glycolate labeled at the C2 position (Route 3, Scheme 5) by hydroxide attack at OH. Table 1 shows that from C1-labeled glucose the predominant form of the label is at the carboxyl unit in the glycolate. That is, Route 3 is important.

For C6-labeled glucose the label would be on the tetrose as shown in Scheme 6. This then undergoes further reaction to give glycolate shown in Scheme 7. If the carbanion, Route 4, is protonated then a diol is formed. If the labeled aldehyde is formed by Route 5, then on addition of water to the carbonyl group and loss of hydrogen (Scheme 8, Route 6), the label appears on the CH_2OH group. This is clearly the predominant case for the C6-labeled glucose and must be an important mechanism. Hydrogen has been demonstrated to be formed readily in strong basic solution.[14]

Interestingly, the acetate produced from 1-^{13}C-D-glucose and 6-^{13}C-D-glucose has the ^{13}C distribution almost entirely on the CH_3 group, 92.9% and 80.0%, respectively. Depicted in Scheme 9 (Routes 7 and 8) is

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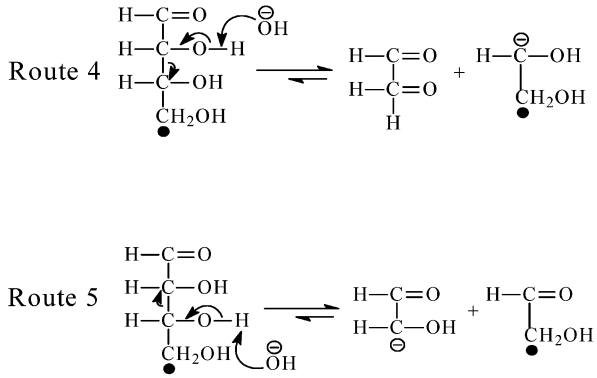
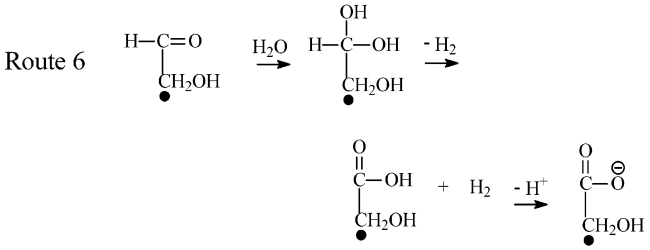
SCHEME 7. Decomposition of the Tetrose To Give Two 2-Carbon Fragments



SCHEME 8. ^{13}C Incorporation at Hydroxyl Carbon in Glycolate



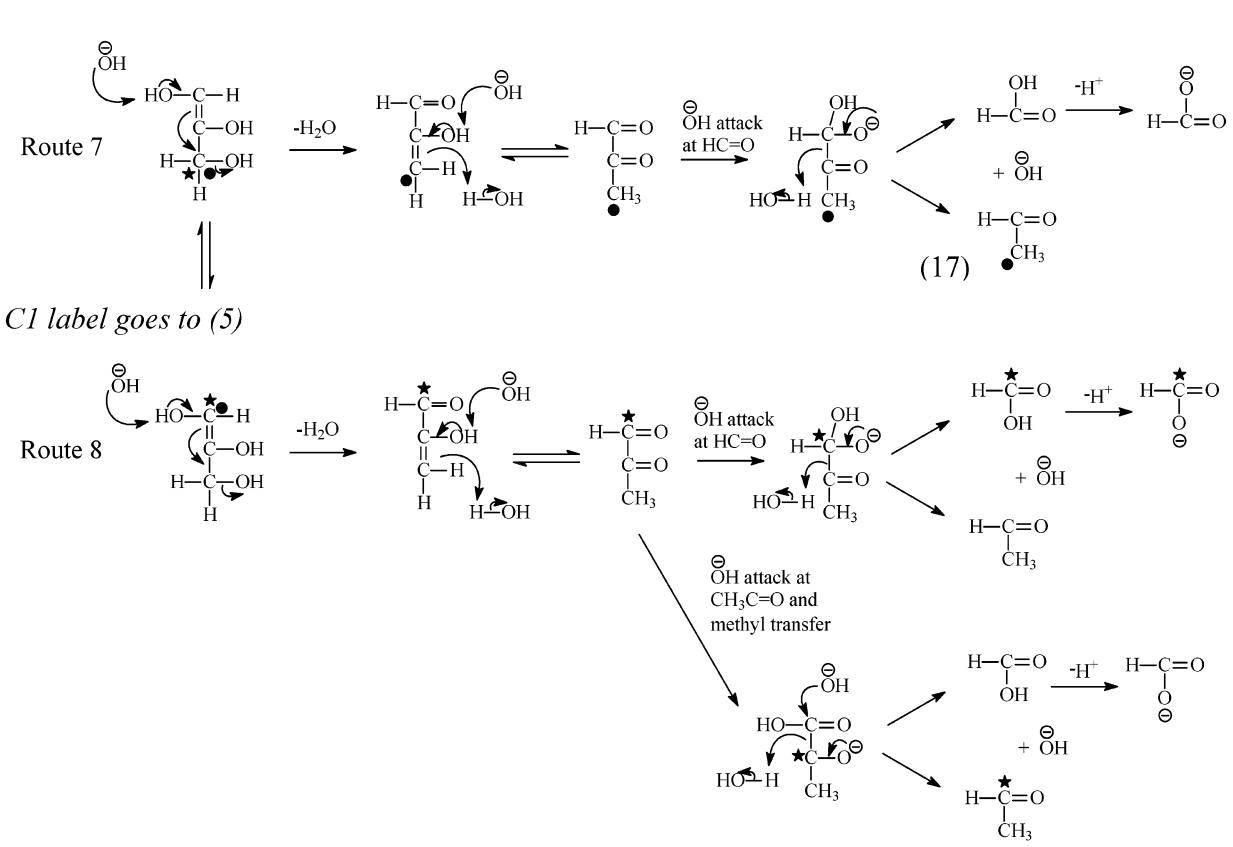
the formation of CH_3-labeled ethanal (**17**) from two different labeled propdienones (**4** and **5**) formed as in Scheme 2. The ethanal would readily oxidize to acetic acid via addition of water and loss of hydrogen. However, these are probably not important routes to acetate. The intermediate **4** yields only labeled CH_3 (Route 7). The intermediate **5** primarily forms labeled formate but not acetate. It can produce labeled acetate but only if hydroxide attack is at the unlabeled C=O carbon and there is methyl migration. The results therefore testify to the importance of methyl migration by Route 8, if acetate can form this way. However, the scheme offers no explanation as to why acetate labeled at COO^- differs in amount from C6- and C1-labeled glucose. It must be true that reactions from **4** can occur which transfer hydride and OH so that the labeled carbon becomes carboxyl and then cleavage occurs. One possibility is given in Scheme 10.

Route 8 yields labeled formate over Route 7 in Scheme 9, and this is reflected in the product distributions since formate preferentially forms from C1-labeled glucose. Labeled formate arising from C6-labeled glucose is also found in significant amounts, although less than from C1-labeled glucose. A mechanism for this is proposed in Scheme 11.

Labeled studies were carried out on 1-^{13}C-sodium-L-lactate to establish the mechanism of formation of carbonate and ethanol from lactate. Table 2 shows the ^{13}C distribution both before and after digestion in 3.5 M NaOH at 145 °C.

Neither ethanol nor carbonate produced from 1-^{13}C-D-

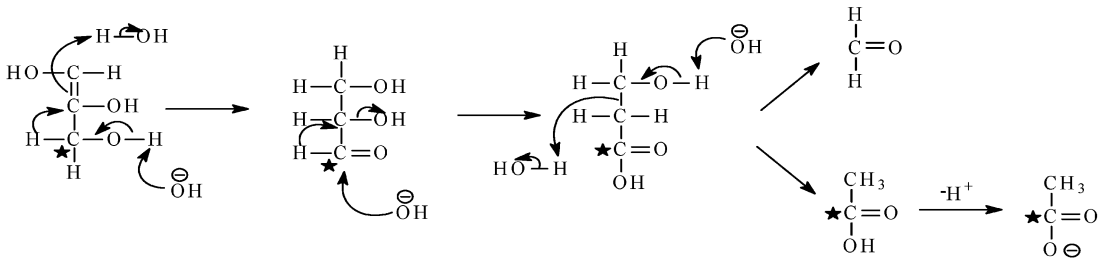
SCHEME 9. Proposed Mechanism for Formate Formation via 1,2-Dicarbonyl Cleavage of Trioses[a]



[a] The dual label symbol is dropped for convenience after depiction of **4** and **5** from Scheme 2.

SCHEME 10. Formation of Carboxyl-Labeled Acetate by Hydride and OH Migration

(4)



SCHEME 11. Cleavage of Labeled Formate from an End-Labeled CH_2OH Unit

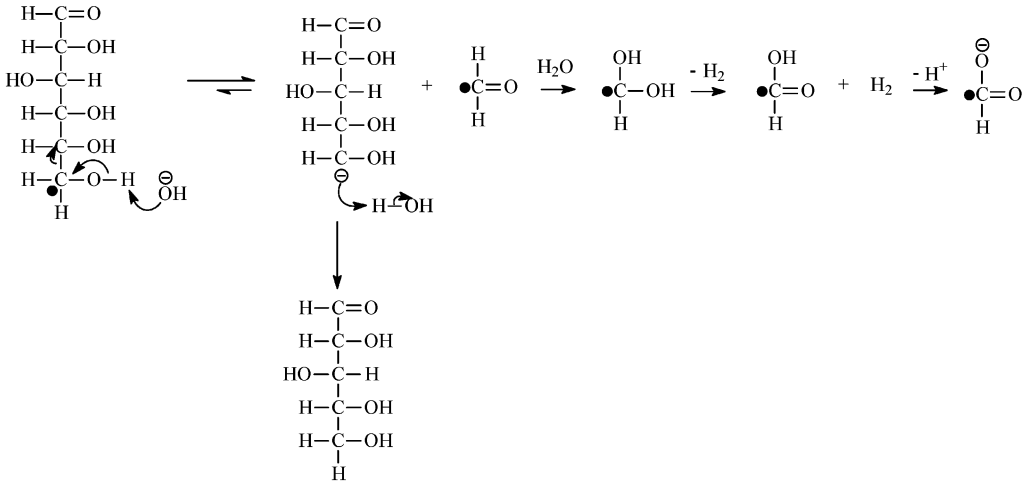
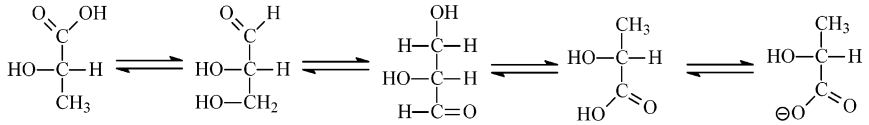


TABLE 2. ^{13}C Distribution in Unlabeled Digested Sodium-L-(+)-lactate and 1-^{13}C-Sodium-L-lactate before and after 1 h Digestion in 3.5 M NaOH at 145 °C

	chemical shift δ (ppm)	% ^{13}C distribution			enhanced yield[a]
		original sodium-L-(+)-lactate digestion (1 h)	1-^{13}C-sodium-L-lactate before digestion	1-^{13}C-sodium-L-lactate digestion (1 h)	1-^{13}C-sodium-L-lactate digestion (1 h)
lactate	185.6	31.7	96.9	96.8	274.5
COO^-	|	|	|	|	|
|	71.1	30.9	1.5	1.2	3.7
CHOH	|	|	|	|	|
|	23.2	32.1	1.6	1.2	3.7
CH_3					
ethanol	57.0	1.5		0.3	18.4
CH_2OH	|	|	not observed	|	|
|	17.6	1.6		0.3	16.5
CH_3					
carbonate CO_3^{2-}	170.9	2.2[b]	not observed	0.2[b]	8.3

[a] See legend to Table 1. [b] Yield of carbonate is not equal to yields of ethanol carbons as some carbon dioxide is in the gas phase.

SCHEME 12. Hydride and Hydroxyl Shifts for C1 and C3 Equivalence in Lactate



glucose or 6-^{13}C-D-glucose is selectively ^{13}C enhanced. Thus when lactate decomposes carbon is scrambled among all positions.

This must be through hydride and OH shifts such as that shown in Scheme 12 for C1 and C3 equivalence). Similarly for C1 and C2 equivalence hydride, hydroxyl, and methyl migration gives Scheme 13.

Both these include a rather improbable first step, and a 1,2,3-cyclopropanetriol intermediate would appear to be the mechanism for such exchange. Decomposition of lactate can be influenced by a number of factors. Agents that inhibit hydride, hydroxy, or methyl transfer may be effective since the stability of transition states to the species shown will be sensitive to a number of reaction conditions. It was thought that labeled carbonate would arise from the decarboxylation of the C1-labeled carboxyl group on the lactate. This is the case but the carbon is equally spread through the ethanol formed as well

SCHEME 13. Hydride and Hydroxyl Shifts for C1 and C2 Equivalence in Lactate

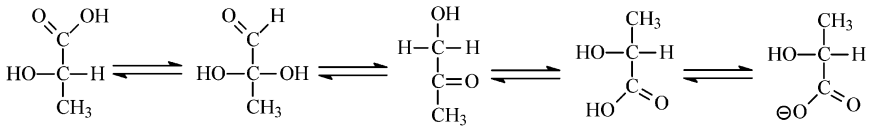


TABLE 3. Chemical Shift (ppm) Data and $^{13}C-^{13}C$ Coupling Constants (J (Hz)) for Labeled and Unlabeled D-Glucose (0.416 M) in Distilled Water

	chemical shift δ^a		6-^{13}C-α-D-glucose		6-^{13}C-β-D-glucose		1-^{13}C-α-D-glucose		1-^{13}C-β-D-glucose	
carbon	α-D-(+)-glucose	β-D-(+)-glucose	chemical shift δ^a	J (Hz)	chemical shift δ^a	J (Hz)	chemical shift δ^a	J (Hz)	chemical shift δ^a	J (Hz)
C1	94.70	98.52	94.70	$^{13}C_6-^{13}C_1$ $J = 2.75$	98.51	$^{13}C_6-^{13}C_1$ $J = 3.75$	94.80		98.51	
C2	74.16	76.85	74.16	$^{13}C_6-^{13}C_2$ $J = 0$	76.84	$^{13}C_6-^{13}C_2$ $J = 0$	74.14	$^{13}C_1-^{13}C_2$ $J = 46.1$	76.83	$^{13}C_1-^{13}C_2$ $J = 45.8$
C3	75.45	78.49	75.45	$^{13}C_6-^{13}C_3$ $J = 3.13$	78.44	$^{13}C_6-^{13}C_3$ $J = 3.96$	75.45	$^{13}C_1-^{13}C_3$ $J = 0$	78.47	$^{13}C_1-^{13}C_3$ $J = 0$
C4	72.25	72.13	72.29	$^{13}C_6-^{13}C_4$ $J = 0$	72.25	$^{13}C_6-^{13}C_4$ $J = 0$	72.29	$^{13}C_1-^{13}C_4$ $J = 0$	72.25	$^{13}C_1-^{13}C_4$ $J = 0$
C5	73.99	78.40	73.99	$^{13}C_6-^{13}C_5$ $J = 43.1$	78.47	$^{13}C_6-^{13}C_5$ $J = 43.1$	73.99	$^{13}C_1-^{13}C_5$ $J = 0$	78.41	$^{13}C_1-^{13}C_5$ $J = 0$
C6	63.27	63.42	63.26		63.41		63.28	$^{13}C_1-^{13}C_6$ $J = 2.71$	63.43	$^{13}C_1-^{13}C_6$ $J = 3.71$

[a] δ in ppm.

because of the rapid scrambling process, so that no preference is found for carbonate or ethanol.

Mechanism. Rearrangements in aqueous alkaline media have been demonstrated and show that alkaline degradation reactions are a dynamic interconversion of monosaccharides in which carboxylic acids such as lactate, acetate, and formate form. The results show that in the formation of lactate from glucose the carboxylate (COO^-) carbon is formed preferentially from C1 carbons but methyl (CH_3) carbon is formed preferentially from C6 carbons. The process involves some exchange through enolysis, hydride, and methyl migration. Once formed label then becomes indiscriminately scrambled as lactate decomposes. 1-^{13}C-sodium-L-lactate studies showed that ^{13}C-labeled carboxylate (COO^-) is scrambled equally among carbonate and both carbons in product ethanol molecules.

The production of glycolate generally results in a high degree of selectivity for carbon. From C1-labeled glucose, labeled carbon ends up as carboxylate (COO^-) carbon, but from C6-labeled glucose the labeled carbon ends up as alcoholic (CH_2OH) carbon. A possible explanation is the position of attack of hydroxy ion at carbonyl rather than OH functionality.

Formate must arise through a range of end chain carbon cleavage reactions and especially that from the 6 carbon of glucose. Dienone intermediate routes may be possible but are not significant. The production of acetate and formate also discriminates between 1-C- and 6-C-labeled carbon.

Experimental Section

Alkaline Digestion of ^{13}C-Labeled 1-^{13}C-D-Glucose, 6-^{13}C-D-Glucose, and 1-^{13}C-Sodium Lactate. Alkaline digestions were carried out in a Parr reactor (25 mL) adjusted to 2.5 mL with a Teflon plug. Individual digestions were carried out on 0.416 M solutions (1.0 mL) of unlabeled α-D-(+)-glucose, unlabeled sodium-L-lactate, 1-^{13}C-D-glucose, 6-^{13}C-D-glucose, and l-^{13}C-sodium lactate in 3.5 M NaOH (previously purged with nitrogen). All labeled compounds contained 99% ^{13}C. Each 1.0-mL solution was placed in the 2.5-mL Parr reactor and sealed under 5.2×10^5 Pa of nitrogen then heated in a silicon oil bath at 145(±3) °C. The temperature dropped to 120 °C and then rose to 145 °C in 30 min. The reaction was then carried out for 1 h from this time. After each digestion the bomb was cooled for 5 min under a cold tap water.

Analysis. Quantitative ^{13}C NMR spectra were obtained prior to digestion for 0.416 M solutions of unlabeled α-D-(+)-glucose, unlabeled sodium-L-lactate, 1-^{13}C-D-glucose, 6-^{13}C-D-glucose, and l-^{13}C-sodium lactate in distilled water to check purity and ^{13}C distributions (refer to Table 3 for chemical shifts (ppm) and $^{13}C-^{13}C$ coupling constants (J (Hz)). It should be noted that α-D-(+)-glucose undergoes mutarotation and therefore there are 12 separate carbon resonances, 6 from the α anomer and 6 from the β anomer in the pure solutions.

Quantitative inverse gated ^{13}C NMR spectra were obtained on a Bruker DRX300 instrument, operating at 75.4 MHz for ^{13}C. A sample (400 μL) of each solution was added to a 5 mm NMR tube and 0.3 M 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt in D_2O (100 μL) was placed in a coaxial tube as an internal reference (δ 0 ppm). A 45° pulse was used (pulse width 2.50 μs). The experiment required 1536 transients with a recycle delay of 30 s to ensure complete relaxation. A short (0.14 s) acquisition time was used to minimize nuclear Overhauser effects (NOE). All data was collected in 5 k data points and Fourier transformed using a line-broadening factor of 3 Hz. Quantitative ^{13}C NMR spectra were obtained for solutions both before and after alkaline digestion in 3.5 M NaOH. Assignments of major product peak chemical shifts (ppm) are formate 173.8, acetate 26.3, 184.1, lactate 23.2, 71.1, 185.6, glycolate, 66.5, 184.6, carbonate 170.9, and ethanol 17.6, 57.0.

Acknowledgment. We thank Alcoa Australia and the Australian Research Council for financial support.

Note Added after ASAP Posting. In Table 3, two coupling constants were reported incorrectly in the version posted November 2, 2002; the corrected version was posted November 5, 2002.

JO025912T