USE OF L-[^{14}C]GLYCEROTETRULOSE FOR THE ENZYMATIC PREPARATION OF ^{14}C-LABELLED INTERMEDIATES OF THE PENTOSE PHOSPHATE PATHWAY.

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SUMMARY

L-/^{14}C/glycerotetrulose (L-/^{14}C/erythrulose) was prepared by a two step process. Step one involved the formation of L-/^{14}C/glycerotetrulose 1-phosphate by condensation of specifically ^{14}C-labelled 1,3-dihydroxydimethyl ketone 1-phosphate (dihydroxyacetone phosphate) with formaldehyde as catalysed by crystalline rabbit muscle aldolase. Step two involved the enzymatic dephosphorylation of L-/^{14}C/erythrulose 1-phosphate. Based on the criteria of the constancy of the specific activity of L-erythrulose and its derivatives, namely L-erythrulose phenylosazone and L-erythrulose o-nitrophenylhydrazone, the compound was concluded to be pure.

Using /1-^{14}C/- and /2-^{14}C/dihydroxyacetone phosphate, L-/1-^{14}C/- and L-/2-^{14}C/erythrulose were prepared and used for the enzymatic preparation of D-/1-^{14}C/- and /2-^{14}C/xylulose 5-phosphate, D-/1-^{14}C/- and /2-^{14}C/fructose 6-phosphate, D-/1-^{14}C/- and /2-^{14}C/altroheptulose 7-phosphate, D-/1-^{14}C/- and /2-^{14}C/mannoheptulose 7-phosphate and D-/1-^{14}C/- and /2-^{14}C/glycero D-ido octulose 8-phosphate. The authenticity of each of the prepared ^{14}C-labelled sugar phosphates was verified by chromatographic and specific degradative procedures.

Abbreviations

ATP, adenosine 5'-triphosphate; NAD^+, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide, reduced form.

INTRODUCTION

Transketolase, a group transferring enzyme of the anaerobic segment of the pentose phosphate pathway, has a remarkably broad specificity for its ketol donor and aldo acceptor substrates[1,2]. Of the known ketol donors L-erythrulose affords three advantages for the enzymatic synthesis of C-1 and/or C-2 ^{14}C-labelled keto sugar phosphates. Firstly, L-erythrulose reacts with transketolase and the aldehyde acceptor (reaction I) to give a product aldehyde (glycolaldehyde) which can be removed by coupling with a second enzyme, alcohol dehydrogenase (reaction II).

$$\text{L-Erythrulose} + \underset{\text{(phosphorylated)}}{\text{acceptor aldehyde}} \rightleftharpoons \text{glycolaldehyde} + \underset{\text{(phosphorylated)}}{\text{product ketol}} \qquad (I)$$

$$\text{Glycolaldehyde} + \text{NADH} \longrightarrow \text{glycol} + NAD^+ \qquad (II)$$

Secondly, as the equilibrium of reaction (II) lies far to the right at pH 7.4[3], the formation of the keto sugar phosphate can be monitored spectrophotometrically by observing the decrease in absorbance at 340 nm due to the oxidation of NADH to NAD^+. Thirdly, as only one product and one substrate are phosphorylated and these differ by two carbon atoms, the isolation of the product keto sugar phosphate may be readily achieved by paper chromatography. In this paper use is made of the above properties of L-erythrulose ([1-^{14}C] and [2-^{14}C]) for the preparation of D-[1-^{14}C]- and [2-^{14}C]xylulose 5-phosphate, D-[1-^{14}C]- and [2-^{14}C]fructose 6-phosphate, D-[1-^{14}C]- and

[2-^{14}C] altroheptulose 7-phosphate, D-[1-^{14}C]- and [2-^{14}C] mannoheptulose 7-phosphate and D-[1-^{14}C]- and [2-^{14}C] glycero D-ido octulose 8-phosphate.

The previous enzymatic methods for the preparation of D-xylulose 5-phosphate(4) and D-[1-^{14}C] xylulose 5-phosphate(5) always gave rise to preparations contaminated with D-ribulose 5-phosphate and D-ribose 5-phosphate. The method described in this paper allows the preparation of high specific activity and high purity D-xylulose 5-phosphate where this may be labelled at C-1, C-2, or C-1 and C-2 as derived from the correspondingly ^{14}C-labelled L-erythrulose. The method described in this paper for the preparation of D-[1-^{14}C]- and [2-^{14}C] fructose 6-phosphate is an alternative method for the preparation of this substance free of D-glucose 6-phosphate.

Recent studies on the reaction scheme of the non-oxidative pentose phosphate pathway(6,7) have led to the proposal of a new pathway of carbohydroate metabolism(8) involving the new intermediates D-arabinose 5-phosphate, D-glycero D-ido octulose 1,8-diphosphate, D-glycero D-ido octulose 8-phosphate and D-altroheptulose 1,7-diphosphate. Mandatory to the studies on the reaction scheme of this new pathway have been methods for the preparation of specifically ^{14}C-labelled heptulose and octulose phosphates. To the authors' knowledge no previous methods have been described for the preparation of specifically ^{14}C-labelled D-altroheptulose 7-phosphate, D-mannoheptulose 7-phosphate and D-glycero D-ido octulose 8-phosphate.

EXPERIMENTAL SECTION

Enzymatic synthesis of [^{14}C] dihydroxyacetone phosphate

L-[3-^{14}C] Glycerol(9) and [2-^{14}C] glycerol (The Radiochemical Centre, Amersham, Bucks, England) were used for the preparation of L-[1-^{14}C]- and [2-^{14}C]-erythrulose 1-phosphate respectively by the following method. The reaction mixture was composed of 1 mmole of hydrazine, 0.2 mmole of glycine, 2 μmoles $MgCl_2$, 3.0 μmoles of ATP, 3.0 μmoles of NAD^+, 25 μg of L-α-glycerol

phosphate dehydrogenase (1.2 units*) and 20 μCi of [^{14}C]glycerol (the solution of L-[3-^{14}C]glycerol and [2-^{14}C]glycerol had initial specific radioactivities of 27.5 μCi/μmole and 8 μCi/μmole and were adjusted to a final radioactivity of 10 μCi/ml and 3 μCi/ml, respectively) in a total volume not exceeding 8 ml and adjusted to pH 9.8. The reaction was commenced by the addition of 100 μg of glycerokinase (8.5 units) and the progress of the rate and extent of production of [^{14}C]dihydroxyacetone phosphate was monitored spectrophotometrically by recording the increase in absorbance at 340 nm due to the reduction of NAD^+ in a suitably diluted aliquot of the reaction mixture. When no further change in optical density at 340 nm was evident (the reaction was usually found to be quantitative after 180 minutes) the reaction mixture was adjusted to pH 3.5 with 10 M HCl and passed through a column (0.5 x 10 cm) of cationic resin (BioRad, AG 50W-X4 200-400 mesh in the hydrogen form) to cleave the hydrazone and to remove NAD^+, NADH and protein. The acid eluate was assayed for dihydroxyacetone phosphate[10] and radioactivity. Because of the instability of this compound[11] it was used the same day or stored as the acid solution at -20°C; a decomposition of 15-20% was detected when stored under these conditions for 6 months.

Enzymatic synthesis of L-[^{14}C]erythrulose.

L-[1-^{14}C]- and L-[2-^{14}C]erythrulose were prepared from the corresponding ^{14}C-labelled dihydroxyacetone phosphate by a two step procedure. The first step involved the preparation and isolation of L-[^{14}C]erythrulose 1-phosphate by a modification of the procedure of Charalampous and Mueller[12]. The reaction mixture was composed of 0.5 mmole of borate buffer, pH 7.3, 0.75 mmole

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* 1 unit of enzyme activity is defined as the amount of enzyme that converts a micromole of substrate to product in 1 minute under optimal conditions of assay at 30°C.

of $MgCl_2$, 30μmoles of [^{14}C]-dihydroxyacetone phosphate (equivalent to approximately 20μCi), 15μmoles of formaldehyde and 2 mg of aldolase (18 units). The reaction was allowed to proceed at 38°C for 3 hours under a nitrogen atmosphere, at which time the entire reaction mixture was passed through a column (0.5 x 10 cm) of AG 50W-X4 resin as described above. The eluted material including the deionized water washings (4 bed volumes) was concentrated by evaporation under vacuum at 40°C to 0.2 ml and applied to Whatman 3 MM paper as a band 20 cm wide. With authentic L-erythrulose 1-phosphate as a reference the L-[^{14}C] erythrulose 1-phosphate was isolated from possible contaminants by ascending chromatography using the GW_3 solvent system described by Wood(13). L-[^{14}C]Erythrulose 1-phosphate was eluted from the paper *, assayed and the specific radioactivity determined (Table 1). The average yield of L-[^{14}C]erythrulose 1-phosphate from four preparations was 7 ± 2μmoles. The second step involved conversion of L-[1-^{14}C]- and [2-^{14}C]erythrulose 1-phosphate to the corresponding ^{14}C-labelled erythrulose by enzymatic dephosphorylation. The reaction mixture contained 1 ml of sample, 3 mg of plant acid phosphatase (type I, Sigma Chemical Co., St. Louis, Mo.), 30μmoles of $MgCl_2$ and 0.3 mmole of sodium acetate-acetic acid buffer, pH 4.8 in a total volume of 4.0 ml. Dephosphorylation was allowed to proceed at 37°C for 3 hours after which time the reaction mixture was passed through a mixed-bed resin (2 g) composed of equal parts of Dowex 50W (H^+ form) and Dowex 1 (HCO_3^- form). The resin was washed with 10 ml H_2O and the combined eluate (pH 3.5) was evaporated to dryness under vacuum at a temperature not exceeding 40°C. The semicrystalline residue was extracted with cold methanol. Upon removal of the methanol in a stream of cold air the residue of L-[^{14}C]-

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* L-erythrulose is unstable and was not stored as the free compound but as L-erythrulose 1-phosphate on paper chromatograms which had been irrigated with the GW_3 solvent(13).

TABLE 1. Properties of synthesized ^{14}C-labelled sugar phosphates.

Synthesized [^{14}C] sugar phosphate	Yield[a] (%)	R_f^b	R_{PO_4}	Specific Radioactivity (d.p.m./μmole)
L- [1-^{14}C] erythrulose 1-phosphate	23.5	0.29	0.57	1.42×10^6
L- [2-^{14}C] erythrulose 1-phosphate	22.9			1.39×10^6
D- [1-^{14}C] xylulose 5-phosphate	98.7	0.29	0.57	1.48×10^6
D- [2-^{14}C] xylulose 5-phosphate	99.2			1.47×10^6
D- [1-^{14}C] fructose 6-phosphate	95.7	0.21	0.41	1.35×10^6
D- [2-^{14}C] fructose 6-phosphate	96.9			1.40×10^6
D- [1-^{14}C] altroheptulose 7-phosphate	98.9	0.19	0.38	1.42×10^6
D- [2-^{14}C] altroheptulose 7-phosphate	99.4			1.35×10^6
D- [1-^{14}C] mannoheptulose 7-phosphate	78.2	0.19	0.38	1.39×10^6
D- [2-^{14}C] mannoheptulose 7-phosphate	76.4			1.48×10^6
D- [1-^{14}C] glycero D-ido octulose 8-phosphate	82.1	0.14	0.27	1.46×10^6
D- [2-^{14}C] glycero D-ido octulose 8-phosphate	81.6			1.40×10^6

Methods for the preparation of each of the ^{14}C-labelled sugar phosphates are given in the text. [a]The yield of L- [^{14}C] erythrulose 1-phosphate was calculated relative to the quantity of [^{14}C] dihydroxyacetone phosphate, and the yield of other ^{14}C sugar phosphates relative to the quantity of L- [^{14}C] erythrulose, employed in the respective syntheses. [b]R_f and R_{PO_4} values are given for GW_3 solvent, run twice in the same direction(13).

erythrulose was dissolved in 1 ml of ice-cold 0.01 M acetic acid, assayed enzymatically[14] and the specific radioactivity determined (average yield for 5 preparations was 19.2% from [^{14}C]glycerol).

Exactly 0.5 μmole of the prepared L-[^{14}C]erythrulose was added to 500 μmoles of unlabelled L-erythrulose, the phenylosazone prepared[12], and recrystallized from alcohol (m.p. 166.5 - 167°C, uncorrected; reported 168°C[15].) Identical amounts of the prepared radioactive L-erythrulose and unlabelled L-erythrulose were used for the preparation of the o-nitrophenyl hydrazone derivative[16] (m.p. 152-153°C, uncorrected; reported 153°C[16].) The specific radioactivity of each derivative was determined by collecting approximately 20 mg onto a pre-weighed filter disc and counting in a Gas Flow Counter. All counts were corrected to infinite thinness. The specific radioactivities of the [^{14}C] erythrulose phenylosazone and o-nitrophenyl hydrazone were 6.1 x 10^2 and 5.9 x 10^2 d.p.m./μmole, respectively.

Partial degradation only of the prepared ^{14}C-labelled L-erythruloses was attempted. Each L-[^{14}C]erythrulose (1 μmole) was diluted with 250 μmoles of unlabelled material and oxidized with periodic acid according to the method of Reeves[17]. Oxidation of L-[1-^{14}C]erythrulose by this method gave rise to the formation of radioactive formaldehyde, derived from C-1 and C-4, which accounted for 97.2% of the total ^{14}C contained by the molecule. Although discrimination between formaldehyde derived from either C-1 or C-4 was not possible the radioactive formaldehyde was concluded to have derived from C-1 as indicated by the distribution of ^{14}C in D-[^{14}C]fructose 6-phosphate formed from L-[1-^{14}C] erythrulose and D-erythrose 4-phosphate (reaction I) and shown in Table 2. L-[2-^{14}C]Erythrulose, when similarly degraded gave rise to unlabelled formaldehyde (Table 2). From the distribution of ^{14}C in D-[^{14}C]fructose 6-phosphate formed from L-[2-^{14}C]erythrulose and D-erythrose 4-phosphate (Table 2), C-2 only of L-[2-^{14}C]erythrulose was concluded to be labelled.

Enzymatic preparation of D-[^{14}C]xylulose 5-phosphate

D-[1-^{14}C]- and [2-^{14}C]xylulose 5-phosphate were prepared from the corresponding ^{14}C-labelled L-erythrulose by the following method which involved reactions (I) and (II), where the acceptor aldehyde and product ketol of reaction (I) were D-glyceraldehyde 3-phosphate and D-[^{14}C]xylulose 5-phosphate respectively. The reaction mixture contained 5 ml of 0.25 M glycylglycine-KOH buffer pH 7.4, 0.05 ml of 1.05 mM thiamine pyrophosphate, 0.05 ml of 0.3 M $MgCl_2$, 0.25 ml of 10 mM NADH, 2.0 μmoles of D-glyceraldehyde 3-phosphate (or 4 μmoles of DL-glyceraldehyde 3-phosphate), 4.0 μmoles (approximately 2.8 μCi) of L-[^{14}C]erythrulose and 0.1 mg of alcohol dehydrogenase (25 units). The reaction was commenced by the addition of 0.26 mg of transketolase (5 units of type IV, Sigma Chemical Co., St. Louis, Mo.) and the production of D-[^{14}C]xylulose 5-phosphate was monitored spectrophotometrically by recording the oxidation of NADH (reaction II) in a suitably diluted aliquot of the reaction mixture. The reaction reached equilibrium after approximately 30 minutes at 30°C when the yield of D-[^{14}C]xylulose 5-phosphate and glycol was 95-100% of theoretical. The reaction mixture was passed through a column (0.5 x 10 cm) of Dowex 50W (H^+ form) and the eluate plus 5 ml of washings concentrated to 0.2 ml by evaporation under vacuum at 40°C. D-[^{14}C]Xylulose 5-phosphate was isolated by paper chromatography using the GW_3 solvent system described by Wood[13]. With authentic L-erythrulose, D-xylulose 5-phosphate and D-glyceraldehyde 3-phosphate as reference markers and by scanning for radioactivity (Actigraph III, Nuclear Chicago, Ill.) the D-[^{14}C]xylulose 5-phosphate band was detected on the paper, cut out and eluted. The eluate of D-[^{14}C]xylulose 5-phosphate was acidified (pH 3.5) with dilute acetic acid to prevent decomposition[18], assayed enzymatically[19] and the specific radioactivity determined (Table 1). The specific radioactivity of the xylulose moiety obtained by enzymatic dephos-

phorylation and paper chromatography in two separate solvent systems was also determined (Table 2). The radiochemical purity and authenticity of each of the prepared D-[1-^{14}C]- and [2-^{14}C]xylulose 5-phosphates were established by conversion (of approximately 1 μmole) to the corresponding ^{14}C-labelled species of D-ribose 5-phosphate using the enzymes ribose 5-phosphate 3-epimerase (2 units) and ribose 5-phosphate isomerase (10 units) in 1 ml of 0.25 M glycylglycine-KOH buffer, pH 7.4. After 30 minutes at 25°C the reaction mixture (composed of D-ribose 5-phosphate, D-ribulose 5-phosphate and D-xylulose 5-phosphate in the ratio, 1.25:1:3) was added to 1 ml of 0.6 M $HClO_4$. The denatured protein was removed by centrifugation and the supernatant fluid adjusted to pH 6.5 with saturated $KHCO_3$ solution. Subsequent dilution of the radioactive D-ribose 5-phosphate with unlabelled material (500 μmoles) followed by the isolation of D-ribose 5-phosphate[7] and degradation of the purified ribose moiety[20] was used to verify the position of the label (Table 2).

Enzymatic preparation of D-[^{14}C]fructose 6-phosphate

D-[1-^{14}C]- and [2-^{14}C]fructose 6-phosphate were prepared from the corresponding ^{14}C-labelled L-erythrulose and D-erythrose 4-phosphate in an identical manner to that described above for the preparation of D-[^{14}C]xylulose 5-phosphate. D-Erythrose 4-phosphate replaced D-glyceraldehyde 3-phosphate as the acceptor aldehyde of reaction (I). The radiochemical purity and authenticity of the D-[1-^{14}C]- and [2-^{14}C]fructose 6-phosphate (Table 1) were established by conversion to D-glucose and the degradation by methods previously described[7]. The specific radioactivity of the D-fructose moiety obtained by enzymatic dephosphorylation and paper chromatography in two solvent systems was also determined (Table 2).

TABLE 2. PROPERTIES OF THE SUGAR MOIETIES OF THE SYNTHESIZED ^{14}C-LABELLED SUGAR PHOSPHATES.

$[^{14}C]$ Sugar	Solvent System 1		Solvent System 2		Distribution of radioactivity							
	R_f	Spec. radioact.	R_f	Spec. radioact.	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
L-$[1-^{14}C]$erythrulose	0.62	1.50×10^6	0.44	1.37×10^6	48.6	2.8		48.6[c]				
L-$[2-^{14}C]$erythrulose		1.47×10^6		1.48×10^6	1.0	98.0		1.0[c]				
D-$[1-^{14}C]$xylulose	0.55	1.39×10^6	0.41	1.31×10^6	92.4	2.0		5.6				
D-$[2-^{14}C]$xylulose		1.42×10^6		1.29×10^6	1.6	98.4		0				
D-$[1-^{14}C]$fructose	0.31	1.43×10^6	0.32	1.40×10^6	96.2	1.2	2.0	0.1	0.3	0.2		
D-$[2-^{14}C]$fructose		1.38×10^6		1.36×10^6	0.9	97.2	0	1.5	0.1	0.3		
D-$[1-^{14}C]$altroheptulose	0.29	1.51×10^6	0.29	1.49×10^6	97.9	0.9			1.2			
D-$[2-^{14}C]$altroheptulose		1.48×10^6		1.31×10^6	2.2	94.2			3.6			
D-$[1-^{14}C]$mannoheptulose	0.27	1.35×10^6	0.28	1.42×10^6	96.2	2.8			1.0			
D-$[2-^{14}C]$mannoheptulose		1.40×10^6		1.40×10^6	1.2	97.2			1.6			
D-$[1-^{14}C]$glycero D-ido octulose	{0.41[d] / 0.17	1.39×10^6	0.21	1.51×10^6	90.1	4.3	1.0	0.4	1.9	0.1	2.1	0.1
D-$[2-^{14}C]$glycero D-ido octulose		1.42×10^6		1.48×10^6	1.1	89.4	1.1	1.8	1.1	1.4	2.9	1.2

Methods for the dephosphorylation of each synthesized ^{14}C-labelled sugar phosphates are given in the text, as are the methods for the degradation of the sugar moieties.

The specific radioactivity of each sugar was determined on the material eluted from the chromatogram. Radioactivity measurements on the aqueous sugar solutions were determined using the scintillant of Patterson and Greene(23). Colorimetric methods were used for the determination of xylulose(24), fructose(25), total heptulose(26) (E max. 580 nm) and octulose(27).

Solvent system 1: ethyl acetate: pyridine: H_2O, 12:5:4 by vol., ascending.

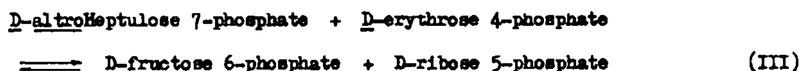
Solvent system 2: 1-butanol: acetic acid: water, 100:21:50 by vol., descending.

[c] L-[^{14}C]erythrulose was partially degraded(17); the values given are composite values for C-1 plus C-4, and C-2 plus C-3 and are discussed in the text.

[d] The faster-moving compound (R_f 0.41) was concluded to be due to the presence of an anhydro-octulose.

Enzymatic preparation of D-[^{14}C]altroheptulose 7-phosphate.

D-[1-^{14}C]- and [2-^{14}C]altroheptulose 7-phosphate were prepared from the corresponding ^{14}C-labelled L-erythrulose and D-ribose 5-phosphate in an identical manner to that described above for the preparation of D-[^{14}C]-xylulose 5-phosphate. D-Ribose 5-phosphate replaced D-glyceraldehyde 3-phosphate as the acceptor aldehyde in reaction (I). The radiochemical purity and authenticity of the D-[1-^{14}C]- and [2-^{14}C]altroheptulose 7-phosphate (Table 1) were established by the degradation of D-fructose 6-phosphate from unlabelled erythrose 4-phosphate and the D-[^{14}C]heptulose 7-phosphate in a transketolase catalysed reaction (reaction III).



D-altroHeptulose 7-phosphate + D-erythrose 4-phosphate

$\rightleftharpoons$ D-fructose 6-phosphate + D-ribose 5-phosphate (III)

The reaction mixture for the formation of D-fructose 6-phosphate contained 1 μmole of D-altroheptulose 7-phosphate, 0.25 mmole of glycylglycine-KOH buffer, pH 7.4, 0.01 ml of 1.05 mM thiamine pyrophosphate, 0.01 ml of 0.3 M $MgCl_2$, 2.0 μmoles of D-erythrose 4-phosphate and 26 μg of transketolase (0.5 units). The equilibrium of reaction (III) favoured the formation of D-fructose 6-phosphate and D-ribose 5-phosphate (K_{eq} was found to be approximately 2.1) and under the conditions specified above was found to reach equilibrium after approximately 40 minutes at 30°C. D-[^{14}C]Fructose 6-phosphate was isolated from the reaction mixture and degraded by methods described previously[7]. The specific radioactivity of the D-altroheptulose moiety obtained by enzymatic dephosphorylation and paper chromatography in two separate solvent systems was also determined (Table 2).

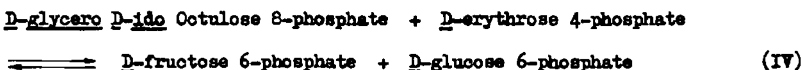
Enzymatic preparation of D-[^{14}C]mannoheptulose 7-phosphate

D-[1-^{14}C]- and [2-^{14}C]mannoheptulose 7-phosphate were prepared from

the corresponding ^{14}C-labelled L-erythrulose and D-arabinose 5-phosphate (prepared by the method of Volk[21]) in an identical manner to that described above for the preparation of D-[^{14}C]xylulose 5-phosphate. D-Arabinose 5-phosphate replaced D-glyceraldehyde 3-phosphate as the acceptor aldehyde in reaction (I). The radiochemical purity and authenticity of the D-[1-^{14}C]- and [2-^{14}C]mannoheptulose 7-phosphate were established by the same method as that described above for D-altroheptulose 7-phosphate (Table 1). The specific radioactivity of the D-mannoheptulose moiety obtained by enzymatic dephosphorylation and paper chromatography in two separate solvent systems was also determined (Table 2).

Enzymatic preparation of D-[^{14}C]glycero D-ido octulose 8-phosphate

D-[1-^{14}C]- and [2-^{14}C]glycero D-ido octulose 8-phosphate were prepared from the corresponding ^{14}C-labelled L-erythrulose and D-glucose 6-phosphate in an identical manner to that described above for the preparation of D-[^{14}C]-xylulose 5-phosphate. D-Glucose 6-phosphate replaced D-glyceraldehyde 3-phosphate as the acceptor aldehyde in reaction (I). The radiochemical purity and authenticity of the D-[1-^{14}C]- and [2-^{14}C]glycero D-ido octulose 8-phosphate were established by the degradation of D-fructose 6-phosphate[7] formed from unlabelled D-erythrose 4-phosphate and the D-[^{14}C]-glycero D-ido octulose 8-phosphate in a transketolase catalysed reaction (reaction IV).



D-glycero D-ido Octulose 8-phosphate + D-erythrose 4-phosphate
$\rightleftharpoons$ D-fructose 6-phosphate + D-glucose 6-phosphate (IV)

At pH 7.4 and 30°C the equilibrium of reaction (IV) favoured the formation of D-fructose 6-phosphate and D-glucose 6-phosphate (K_{eq} was found to be approximately 9). D-[^{14}C]Fructose 6-phosphate was isolated and degraded as described above. The specific radioactivity of D-glycero D-ido octulose moiety obtained by enzymatic dephosphorylation and paper chromatography in the two solvent systems was also determined (Table 2).

DISCUSSION

Since only C-1 and C-2 are transferred to the aldehyde acceptor in the transketolase catalysed reaction (I) DL-[1-^{14}C]glycerol may also be used as the starting material for the preparation of C-1 labelled keto sugar phosphates. DL-[1-^{14}C]Glycerol, however gives rise to L-[1,3-^{14}C]erythrulose which cannot be distinguished from L-[2,4-^{14}C]erythrulose by the degradative procedures employed and therefore does not allow the verification of the [^{14}C] substrate for reaction (I). L-[3-^{14}C]Glycerol[9] was thus the preferred starting material.

As shown in Table 1 relatively lower yields of the product keto sugar phosphates, D-mannoheptulose 7-phosphate and D-glycero D-ido octulose 8-phosphate were obtained when D-arabinose 5-phosphate and D-glucose 6-phosphate were the respective acceptor aldehydes for reaction (I). This observation was in agreement with previous reports[1,22] that the substrates D-arabinose 5-phosphate and D-glucose 6-phosphate are less active than either D-glyceraldehyde 3-phosphate or D-erythrose 4-phosphate as acceptor aldehydes for transketolase.

The identification of each of the synthesized ^{14}C-labelled compounds, apart from L-[^{14}C]erythrulose, was determined by (i) the formation of identifiable products from an enzymatic reaction (for example, in the transketolase catalysed reactions III & IV each synthesized ^{14}C-labelled compound gave rise to products whose identities were totally consistent with theoretical predictions based on the established mechanism of the enzyme[1]); and (ii) the chromatographic characteristics of the sugar moiety when compared with published values.

All ^{14}C-labelled compounds synthesized were stored as aqueous preparations (approximately 1 mM) pH 3.5 and at -20°C. Under these conditions only L-[^{14}C]-erythrulose 1-phosphate and D-[^{14}C]xylulose 5-phosphate showed any decom-

position (approximately 10%) after 3 months. Both of these compounds were found to be particularly unstable in neutral or slightly alkaline solutions even at 0°C.

The method described in this communication permitted the preparation of keto sugar phosphates labelled with ^{14}C in carbon atoms one and two. Although the preparation of these compounds with other carbon atoms labelled is not discussed in this paper, the method has successfully been used for the preparation of D-[8-^{14}C] glycero D-ido octulose 8-phosphate from unlabelled L-erythrulose and D-[6-^{14}C] glucose 6-phosphate.

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REFERENCES

1. Racker, E. - In The Enzymes, vol. 5, p.397. Ed. by Boyer, P.D., Lardy, H. and Myrbäck, K. New York : Academic Press Inc. (1961).
2. Davies, D.D. - Intermediary Metabolism in Plants, p.19. Cambridge : University Press (1961).
3. Holzer, H. and Goedde, H.W. - In Methods of Enzymatic Analysis, p.297 Ed. by Bergmeyer, H.U. New York : Academic Press Inc. (1965).
4. Horecker, B.L. and Smyrniotis, P.Z. - In Methods in Enzymology, vol. 1, p.371. Ed. by Colowick, S.P. and Kaplan, N.O. New York : Academic Press Inc. (1955).
5. Clark, M.G., Williams, J.F. and Blackmore, P.F. - Biochem. J., 125 : 381 (1971).
6. Williams, J.F., Rienits, K.G. and Clark, M.G. - Biochem. J., 123 : 915 (1971).

7. Williams, J.F., Rienits, K.G., Schofield, P.J. and Clark, M.G. - Biochem. J., 123 : 923 (1971).
8. Williams, J.F. and Clark, M.G. - Search, 2 : 80 (1971).
9. Rose, I.A., Kellermeyer, R., Stjernholm, R. and Wood, H.G. - J. Biol. Chem., 237 : 3325 (1962).
10. Bücher, T. and Hohorst, H. - In Methods of Enzymatic Analysis, p.246. Ed. by Bergmeyer, H.U. New York : Academic Press Inc. (1965).
11. Dawson, R.M.C., Elliott, D.C., Elliott, W.H. and Jones, K.M. - In Data for Biochemical Research, p.106. Oxford : Clarendon Press (1969).
12. Charalampous, F.C. and Meuller, G.C. - J. Biol. Chem., 201 : 161 (1953).
13. Wood. T. - J. Chromatog., 35 : 352 (1968).
14. Holzer, H. and Goedde, H.W. - In Methods of Enzymatic Analysis, p.208. Ed. by Bergmeyer, H.U. New York : Academic Press Inc. (1965).
15. Iwadare, K. - Bull. Chem. Soc. Japan, 14 : 131 (1939).
16. Müller, H., Montigel, C. and Reichstein, T. - Helv. Chem. Acta., 20 : 1468 (1937).
17. Reeves, R.E. - J. Am. Chem. Soc., 63 : 1476 (1941).
18. Novello, F. and McLean. P, - Biochem. J., 107 : 775 (1968).
19. Racker, E. - In Methods of Enzymatic Analysis, p.201. Ed. by Bergmeyer H.U. New York : Academic Press Inc. (1965).
20. Bernstein, I.A. - J. biol. Chem. 205 : 309 (1953).
21. Volk, W.A. - In Methods in Enzymology, vol. 9, p.974. Ed. by Colowick, S.P. and Kaplan, N.O. New York : Academic Press Inc. (1955).
22. Datta, A.G. and Racker, E. - J. biol. Chem. 236 : 617 (1961).
23. Patterson, M.S. and Greene, R.C. - Analyt. Chem., 37 : 854 (1965).
24. Dische, Z. and Borenfreund, E. - J. biol. Chem., 192 : 583 (1951).
25. Graham, D. and Smydzuk, J. - Analyt. Biochem., 11 : 246 (1965).
26. Mejbaum, W. - Z. Physiol. Chem., 258 : 117 (1938).
27. Bartlett, G.R. and Bucolo, G. - Biochim. Biophys. Acta., 156 : 240(1968).