BIOSYNTHESIS OF SUGARS FOUND IN BACTERIAL POLYSACCHARIDES

PART I. BIOSYNTHESIS OF L-RHAMNOSE

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Received November 30, 1961

ABSTRACT

Azotobacter indicum was grown on media which contained D-glucose-1-C^{14}, D-glucose-2-C^{14}, D-glucose-6-C^{14}, D-mannose-1-C^{14}, or D-galactose-1-C^{14}. The radioactive polysaccharides produced were hydrolyzed and the specific activities of the isolated D-glucose and L-rhamnose were measured. The sugars were degraded and the distribution of activity in the individual carbon atoms was determined.

The results suggest that some L-rhamnose is formed from D-glucose by a pathway which involves the breakdown of the glucose carbon skeleton.

INTRODUCTION

The conversion of D-glucose to deoxy sugars of the L-series has received considerable attention. For example, using isotopic tracer techniques the biosynthesis of L-fucose has been studied in bacteria (1–3) and in man (4). The results favor a direct conversion of D-glucose to L-fucose. A similar conclusion (5) has been reached in the case of colitose (3-deoxy-L-fucose). The biosynthesis of L-rhamnose has been investigated in bacteria (6–9) and in plants (10), and again a direct conversion of D-glucose to L-rhamnose is indicated.

Bacterial extracts can transform guanosine diphosphate D-mannose to guanosine diphosphate L-fucose (11) and thymidine diphosphate D-glucose to thymidine diphosphate L-rhamnose (12–14). Intermediates in these interconversions have recently been characterized (15, 16) and they provide strong evidence for the existence of direct pathways, although they do not preclude the existence of alternative pathways in vivo.

Azotobacter indicum produces an extracellular polysaccharide when grown in suitable media containing sugar substrates (17, 18). Studies in this department (19) have shown that, when a peptone medium is used, the polysaccharide contains D-*glycero*-D-*manno*-heptose, D-glucose, D-mannose, D-glucuronic acid, L-rhamnose, and arabinose.

In the present paper the results of growing the organism in a peptone medium, containing a variety of C^{14}-labelled hexoses, are reported. The polysaccharides were hydrolyzed and the distribution of activity in the carbon atoms of D-glucose and L-rhamnose was determined. The possible origin of the heptose sugar will be discussed in a future communication.

EXPERIMENTAL

Materials

The radioactive sugars were purchased from Merck and Co., Montreal, Que. *A. indicum*-446 was kindly supplied from the Culture Collection of the National Research Laboratories, Ottawa, Ontario.

Determination of Radioactivity

Carbon dioxide was precipitated as barium carbonate. Hydrazones, dimedon derivatives, iodoform, and barium carbonate were plated in triplicate on sintered-glass planchets (6–12 mg per plate). The samples were counted in a proportional methane gas flow counter. The barium carbonate self-absorption curve was used to correct all solid samples to their activity at "infinite thickness" (20).

Liquid samples containing radioactive sugars were evaporated on aluminum plates and counted at "infinite thinness". The activity was converted to microcuries (μc), assuming 50% counting efficiency. The values are not absolute and should not be directly compared with the activities of solid samples.

Growth of A. indicum and Isolation of Polysaccharide

The culture medium contained dipotassium hydrogen phosphate (12 mg), magnesium sulphate (5 mg), calcium carbonate (500 mg), peptone (25 mg), sugar (1.00 g with 100 μc activity), and distilled water (25 ml). The suspension was autoclaved and inoculated with *A. indicum*, growing in a similar liquid medium. Sterile, carbon dioxide free air was slowly drawn through the medium; the evolved carbon dioxide was collected in a trap containing 2 *N* sodium hydroxide (200 ml). The specific activity of the carbon dioxide was determined.

Generally, the medium became quite viscous after 7–10 days. After about 2 weeks the medium was diluted with water (25 ml) and shaken to dissolve the slime which accumulated on the flask wall. The suspension was centrifuged (12,000 r.p.m. for 90 minutes) and the clear supernate was poured into ethanol (2 vol.). The gel-like precipitate was collected by centrifugation, washed with 60% ethanol (4×), absolute ethanol (2×), acetone, and ether, and dried in a vacuum desiccator containing silica gel. The supernate and ethanol washings were combined and evaporated to a syrup, which was dried.

In a control experiment radioactive glucose was added to a non-radioactive solution of polysaccharide. When the polysaccharide was isolated in the above manner it contained no appreciable activity.

The yields of extracellular polysaccharide and carbon dioxide produced, when *A. indicum* was grown in media containing C^{14}-labelled sugars, are shown in Table I.

TABLE I

Yields of extracellular polysaccharide and evolved carbon dioxide

Sugar substrate	Weight of sugar (g)	Time of growth (days)	Weight of polymer isolated (g)	Weight of substrate recovered (g)	Yield of polymer (%)	Yield of CO_2 (%)
D-Glucose-U-C^{14}	1.00	12	0.149	0.585	36	65
D-Glucose-1-C^{14}	1.00	22	0.150	0.480	29	58
D-Glucose-2-C^{14}	1.00	14	0.095	0.591	23	85
D-Glucose-6-C^{14}	1.00	18	0.174	0.461	32	74
D-Mannose-1-C^{14}	1.00	14	0.224	0.415	38	65
D-Galactose-1-C^{14}	1.00	14	0.156	0.507	32	73

The specific activities of the sugar substrates and evolved carbon dioxide are shown in Table II.

TABLE II

Specific activities of evolved carbon dioxide, hydrolyzates, and isolated sugars

Sugar substrate	Specific activity of sugar (μc/mmole C)	Specific* activity of CO_2 (μc/mmole C)	Specific activity of hydrolyzate (μc/mmole C)	Specific activities of sugars (μc/mmole C)	
				Glucose	Rhamnose
D-Glucose-U-C^{14}	3.00	1.73	2.36	—	—
D-Glucose-1-C^{14}	3.00	1.46	1.80	3.12	2.73
D-Glucose-2-C^{14}	3.00	1.42	1.88	3.09	2.80
D-Glucose-6-C^{14}	3.00	1.08	1.94	2.89	2.05
D-Mannose-1-C^{14}	3.00	1.60	2.05	3.03	2.93
D-Galactose-1-C^{14}	3.00	1.62	2.09	3.04	2.86

*The average molecular weight of the sugars in the hydrolyzate was taken to be 180.

Hydrolysis of Polysaccharide

The polysaccharide was hydrolyzed with 90% formic acid for 7.5 hours at 100° C in a sealed tube. After evaporation, *N* sulphuric acid was added to the syrup. The solution was heated on a boiling-water bath for 5 hours, neutralized (barium carbonate), filtered, and passed through Amberlite IR 120 (H^+) ion-exchange resin. The eluate was evaporated to a syrup, which was dried in a vacuum desiccator containing silica gel. Usually 50–60% by weight of the polysaccharide was recovered as monosaccharides. The specific activity of the hydrolyzate was measured and is shown in Table II.

Isolation of Radioactive Sugars

The No. 3 MM Whatman paper on which the hydrolyzates were fractionated was prewashed with the developing solvent—butan-1-ol–pyridine–water (10:3:3, v/v). Sugars were fractionated by multiple development (21) using developing times of 16, 12, 12, and 17 hours. The chromatograms were placed in contact with X-ray films for 8 days and the areas containing radioactive sugars were eluted with water. The eluates

which contained glucose and rhamnose were diluted to known volumes. The concentration of these sugars was estimated on triplicate samples by the phenol – sulphuric acid method (22). The specific activities of these sugars are recorded in Table II.

"Carrier" D-glucose and L-rhamnose were added to their respective solutions, which were then evaporated to syrups. Glucose was crystallized from aqueous ethanol, and rhamnose from moist acetone.

Degradation of L-*Rhamnose*

L-Rhamnose was degraded by the reactions outlined in Fig. 1.

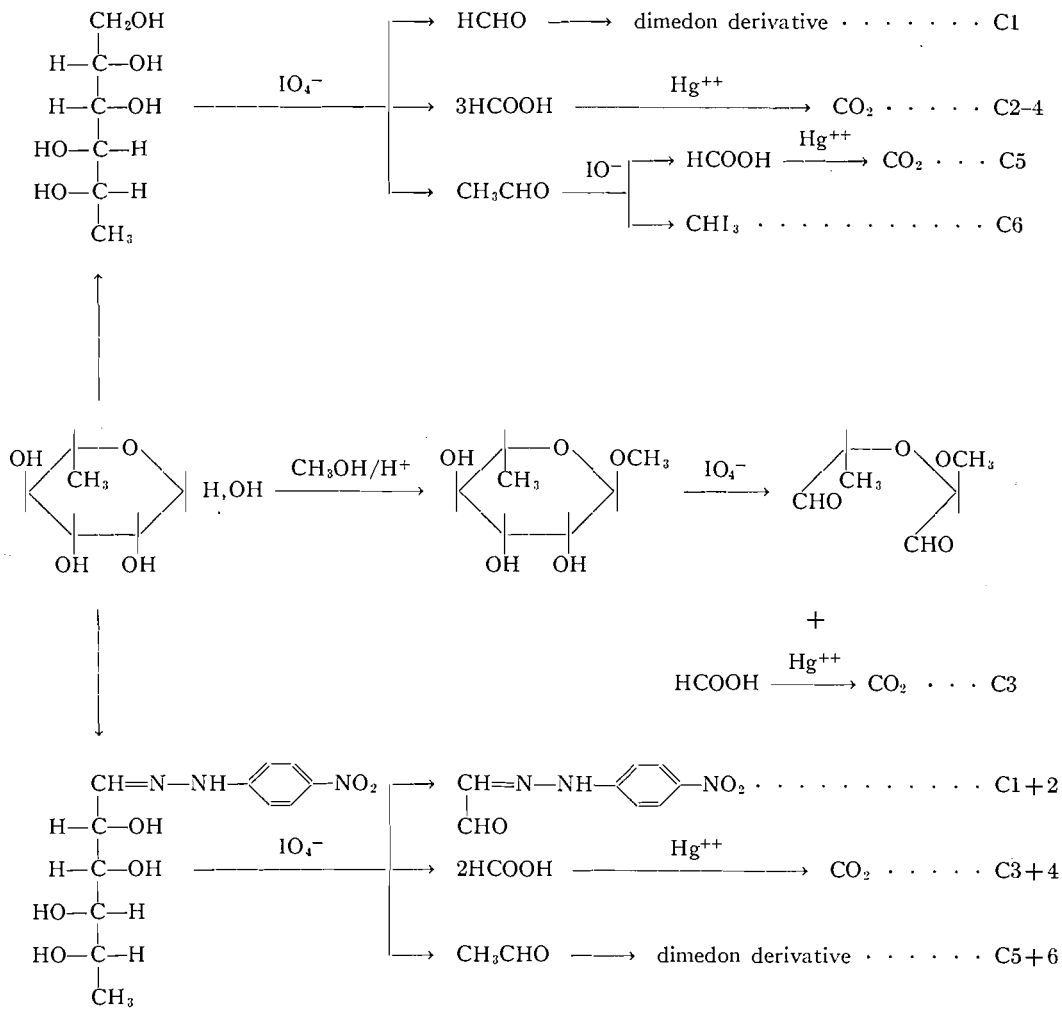

FIG. 1. Degradation of L-rhamnose-C^{14}.

Determination of C^{14} *in C1, C2–4, C5, and C6*

L-Rhamnitol was prepared *in situ* by reducing L-rhamnose (20 mg) with 1% sodium borohydride (2 ml). Excess borohydride was destroyed after 12 hours by the addition of 2 *N* sulphuric acid. The rhamnitol was oxidized by the procedure developed by Nicolet and Shinn (23). Phosphate buffer at pH 8 (10 ml) was added to the solution together with glycine (200 mg) and 0.3 *M* sodium metaperiodate (1.5 ml). Nitrogen was bubbled through the solution to remove acetaldehyde (C5+6), which was collected in 2% sodium metabisulphite. The bisulphite solution was concentrated *in vacuo* to a small volume and potassium carbonate (1.0 g) was added (24) followed by 9 *N* potassium hydroxide (0.05 ml) and 0.1 *M* iodine in 20% potassium iodide (2 ml). The iodoform (C6) was collected by filtration after 15 minutes, washed well with water,

and plated. The plates were dried *in vacuo* over silica gel and their specific activities determined. The material, m.p. 117–118° C, was obtained in 30–35% yield. The filtrate and washings were acidified with 2 *N* sulphuric acid and iodine was destroyed by the addition of dilute sodium thiosulphate. The solution was neutralized and the formic acid (C5) was oxidized to carbon dioxide with mercuric acetate (25) in about 30% yield.

The solution obtained after removal of acetaldehyde contained formaldehyde (C1) and formic acid (C2–4); it was acidified with acetic acid, and dimedon was added. The formaldehyde dimedon was collected after 20 hours and was obtained in about 70% yield, after recrystallization from aqueous ethanol; m.p. 188° C. The derivative was dried in a vacuum desiccator containing silica gel and its specific activity determined. The formic acid in the filtrate was oxidized to carbon dioxide (25) in about 70% yield, and its specific activity was determined.

Determination of C^{14} in C1–6

L-Rhamnose *p*-nitrophenylhydrazone was prepared in the usual manner in yields of 80–85%; m.p. 190–192° C. Portions of the hydrazone (ca. 8 mg) were ground to a fine powder in ether and the suspensions were plated. The plates were dried in an oven at 100° C for 15 minutes and their specific activities were determined.

Determination of C^{14} in C1+2 and C5+6

The *p*-nitrophenylhydrazone (50 mg) was dissolved in pH 8 phosphate buffer (8 ml), and 0.3 *M* sodium metaperiodate (1.5 ml) was added to the chilled solution. Glyoxal *p*-nitrophenylhydrazone (C1+2), which precipitated within a few seconds, was removed by centrifugation and recrystallized from aqueous acetone. The material was obtained in 50–60% yield; m.p. 198–200° C; it was plated and dried in a vacuum desiccator prior to the determination of its specific activity.

Nitrogen was bubbled through the supernate and the acetaldehyde was collected in an ice-cold solution of dimedon at pH 4.3 (26). The precipitated derivative was collected after 20 hours by filtration, and, after recrystallization from aqueous ethanol, was obtained in 30–40% yield; m.p. 139–140° C. The specific activity of C5+6 was determined.

Determination of C^{14} in C3

L-Rhamnose (50–100 mg) was dissolved in methanolic hydrogen chloride (2 ml concentrated hydrochloric acid in 100 ml methanol) and the solution refluxed for 10 hours. The solution was diluted with water and passed through Duolite A4 (OH^-) resin. The eluate was concentrated to a syrup and fractionated chromatographically on Whatman No. 3 MM paper using a neutral solvent—butan-1-ol–ethanol–water (3:1:1, v/v). End strips of the chromatogram were sprayed with silver nitrate (27) and the area of paper containing methyl α-L-rhamnopyranoside was eluted with water. The solution was concentrated, and the syrup allowed to crystallize. When a sufficient quantity of glycoside was available it was recrystallized from ethyl acetate; m.p. 100–101° C.

The glycoside (30–40 mg) was dissolved in water (1 ml) and oxidized with 0.3 *M* periodic acid (1 ml). After 2.5 hours the solution was passed through Duolite A4 (OH^-) resin, which was then washed with water (100 ml). The column was washed with 0.2 *M* barium hydroxide (15 ml) and water (30 ml). The eluate was neutralized with 3 *N* sulphuric acid and the formic acid (C3) oxidized to carbon dioxide in the usual manner (25).

Degradation of D-*Glucose*

Determination of C^{14} in C1+6 and C2–5

D-Glucitol was prepared *in situ* by reducing D-glucose (15 mg) with 1% sodium borohydride (2 ml). Excess borohydride was destroyed after 12 hours by the addition of 2 *N* sulphuric acid. Phosphate buffer at pH 8 was added to the solution together with 0.3 *M* sodium metaperiodate (1.5 ml). Barium acetate was added after 2 hours and the insoluble barium salts were removed by filtration. The solution was acidified with acetic acid and dimedon was added. The formaldehyde dimedon was collected after 20 hours and recrystallized from aqueous ethanol; m.p. 188° C. The material was obtained in 70% yield and was used to determine the specific activity of C1+6. The specific activity of C2–5 was measured by oxidizing the formic acid in the filtrate to carbon dioxide (25).

Determination of C^{14} in C1–6

Glucose (4–6 mg) was oxidized to carbon dioxide by Van Slyke's procedure (28) and its specific activity determined. Glucose *p*-nitrophenylhydrazone was prepared in 70–75% yield; m.p. 188–189° C. The specific activity of C1–6 was determined by directly counting the hydrazone.

Determination of C^{14} in C1+2 and C6

Glucose *p*-nitrophenylhydrazone was oxidized in a manner similar to that described for rhamnose *p*-nitrophenylhydrazone. Glyoxal *p*-nitrophenylhydrazone (C1+2) was recrystallized from aqueous acetone and its specific activity was determined. Dimedon was added to the filtrate and the formaldehyde dimedon was collected after 20 hours. The derivative, after recrystallization from aqueous ethanol, was obtained in about 50% yield; it was used to determine the specific activity of C6.

The specific activities of the fragments obtained during the degradation of rhamnose are recorded in Table III.

TABLE III

Specific activities of rhamnose fragments (c.p.m./mmole C)

Rhamnose carbons	Sugar substrate				
	G-1-C^{14}	G-2-C^{14}	G-6-C^{14}	M-1-C^{14}	Gal-1-C^{14}
C1–6	10,170	20,010	19,380	22,060	18,940
C1+2	23,780	46,900	5,250	45,670	39,080
C5+6	4,350	6,560	46,170	12,460	5,490
C1	44,610	13,780	13,610	91,840	—
C2–4	1,310	31,540	0	1,860	2,570
C5	2,300	—	0	2,300	3,120
C6	6,230	0	97,250	24,270	8,690
C3	1,970	7,870	—	2,460	3,940

NOTE: G = glucose, M = mannose, Gal = galactose.

The specific activities of the fragments obtained during the degradation of glucose are shown in Table IV.

TABLE IV

Specific activities of glucose fragments (c.p.m./mmole C)

Glucose carbons	Sugar substrate				
	G-1-C^{14}	G-2-C^{14}	G-6-C^{14}	M-1-C^{14}	Gal-1-C^{14}
C1–6*	8,730	18,600	25,440	31,590	13,320
C1–6†	—	19,140	26,100	29,250	11,970
C1+2	21,960	50,760	6,390	80,100	34,020
C6	4,140	2,340	159,840	32,000	13,500
C1+6	21,690	7,200	79,020	84,060	36,720
C2–5	1,000	25,250	590	2,570	2,610

NOTE: G = glucose, M = mannose, Gal = galactose.
*Determined by Van Slyke oxidation (28) of glucose.
†Determined by directly counting glucose *p*-nitrophenylhydrazone.

The percentage distribution of activity in the carbon atoms of L-rhamnose and D-glucose is shown in Table V. The specific activities shown in Tables III and IV were multiplied by the number of carbon atoms they represented to give total activities. The total activity of the rhamnose molecule was calculated by summing the activities of C1, C2–4, C5, and C6. Generally, this value corresponded (±7%) with the activity of C1–6, determined by an independent method. The total activity of the glucose molecule was calculated by summing the activities of C1+6 and C2–5. The value corresponded (±7%) with the activity of C1–6, determined by two independent methods. When D-glucose-6-C^{14} was the substrate the value obtained for C6 was slightly greater than that of C1+6. In this case the total activity was compounded from the sum of the activities of C1+2, C2–5, and C6.

TABLE V

Distribution of C^{14} in individual carbon atoms of isolated sugars

Sugar substrate	Sugar isolated	C^{14} as % total in monosaccharide					
		C1	C2	C3	C4	C5	C6
G-1-C^{14}	Rhamnose	78	←——	7	—→	4	11
	Glucose	83	←————	8	———→		9
G-2-C^{14}	Rhamnose	11	66	7	5	11	0
	Glucose	10	77	←———	11	——→	2
G-6-C^{14}	Rhamnose	12	←————	0	———→		88
	Glucose	7	←————	1	———→		91
M-1-C^{14}	Rhamnose	74	←——	4	—→	2	20
	Glucose	76	←————	6	———→		18
Gal-1-C^{14}	Rhamnose	80	←——	8	—→	3	9
	Glucose	72	←————	12	———→		16

NOTE: G = glucose, M = mannose, Gal = galactose.

DISCUSSION

A. indicum was grown in aerated, buffered media which contained C^{14}-labelled sugar substrates. Generally about 30% of the sugar utilized was converted to extracellular polysaccharide and most of the remainder was oxidized to carbon dioxide.

The specific activity of the evolved carbon dioxide was substantially lower than that of the sugar substrate, even when D-glucose-U-C^{14} was the substrate. This isotope dilution may be due to peptone in the medium serving as an additional carbon source. Also, acidic materials may be liberated into the medium by the bacteria, displacing inactive carbon dioxide from calcium carbonate. Furthermore an exchange reaction between metabolic carbon dioxide and inactive carbonate may occur. Nevertheless, the specific activity of carbon dioxide evolved during the D-glucose-6-C^{14} growth was noticeably lower than in the other cases, indicating that the hexose monophosphate shunt is probably operating in the organism.

The isolated polysaccharides were hydrolyzed and usually 50–60% of their weight was recovered. However, although the specific activities of the hydrolyzates were quite similar, they were lower than those of the original substrates, indicating that they contained inactive materials.

The specific activities of the isolated glucose and rhamnose were determined. They were usually quite similar to each other and to the specific activities of the original substrates. However, the specific activity of L-rhamnose isolated from the D-glucose-6-C^{14} growth was approximately 30% lower than in the other cases. This result is believed to be significant and indicates that C6 of D-glucose contributes only about 70% as much to the L-rhamnose skeleton as does C1 or C2 of D-glucose or C1 of D-mannose and D-galactose.

A number of degradations of L-rhamnose have been reported in the literature. In this work rhamnose of low specific activity was occasionally isolated and it was necessary to devise a procedure which required less than 1 mmole of sugar. Quite often the majority of the activity is found in the terminal carbon atoms and it is then unnecessary and time consuming to use a procedure which gives the activity of each carbon atom. It is an advantage, therefore, to use a procedure which initially gives the activity of the terminal carbon atoms.

Hauser and Karnovsky (6) oxidized potassium rhamnonate to carbon dioxide (C1), formic acid (C2–4), and acetaldehyde (C5+6). However, about 0.5 mmole of rhamnose is required for this degradation, and in one case (8) poor recoveries of activity were reported. The same information can be readily obtained by oxidation of rhamnitol, using the procedure of Nicolet and Shinn (23), and only about 0.12 mmole of sugar is required.

Rhamnose phenylosazone has been used (6) to determine the activity of C1–3, C4, and C5+6. However, on a 0.5-mmole scale the present authors were unable to obtain better than 25% yield of recrystallized osazone. Rhamnose *p*-nitrophenylhydrazone could be prepared in over 80% yield and was therefore used to determine the specific activities of C1+2 and C5+6.

The isotope content of C3 was determined by periodate oxidation of methyl α-L-rhamnopyranoside. Watkin and Neish (10) have recently described a method for degrading rhamnose which involves the periodate oxidation of its glycopyranosides. The specific activities of C2, C3, C4, C5, and C6 were determined directly while that of C1 was calculated by difference, about 1 mmole of rhamnose being required for the degradation.

The degradative studies revealed that about 77±6% of the activity was retained in C1 of the isolated monosaccharides, when D-glucose-1-C^{14}, D-mannose-1-C^{14}, or D-galactose-1-C^{14} were substrates. The majority of the remaining radioactivity was located in C6 of the hexoses.

When D-glucose-6-C^{14} served as substrate most of the activity was recovered in C6 of the hexoses (90±2%). The remaining activity was found in C1 of these sugars.

Approximately 72±6% of the activity was recovered in C2 and 10% in C5 of D-glucose and L-rhamnose, when D-glucose-2-C^{14} was substrate. In addition about 10% of the activity was found in C1 of these sugars. The latter result further indicates that C1 decarboxylation is occurring and suggests that some of the pentose formed by the hexose monophosphate shunt is reutilized for hexose synthesis. Apparently about 10% of hexose is resynthesized by this route and it can presumably equilibrate with substrate hexose.

About 10% of activity was detected in C6, C5, or C1 of D-glucose and L-rhamnose, when D-glucose-1-C^{14}, D-glucose-2-C^{14}, or D-glucose-6-C^{14} were the respective substrates.

These results suggest that there is a hexose pool which at equilibrium contains about 20% of hexose resynthesized from equilibrated trioses (formed from D-fructose 1,6-diphosphate via the Embden–Meyerhof pathway) and about 10% of hexose resynthesized from pentose (produced by the hexose monophosphate shunt) by the action of transketolase.

The specific activity of L-rhamnose derived from D-glucose-6-C^{14} was about 30% lower than that derived from 1-C^{14}-labelled precusors, and so it will be assumed that 70% of L-rhamnose is synthesized directly from D-glucose. This transformation probably proceeds via the thymidine diphosphate derivatives (12–14) and requires three epimerizations and a reduction of $-CH_2OH$ to $-CH_3$. An intermediate in this interconversion, the thymidine diphosphate derivative of 6-deoxy-4-oxo-D-glucose, has recently been isolated from a cell-free enzyme system of *Pseudomonas aeruginosa* (16). Presumably a second pathway, involving scission of the glucose carbon skeleton, contributes to rhamnose biosynthesis in *A. indicum*. A possible route, which has not been demonstrated experimentally, may involve the transfer of a dihydroxy acetone unit from D-fructose 6-phosphate to L-pyruvaldehyde (produced by glycolysis) by the action of transaldolase. The resultant 6-deoxy-L-sorbose might then be converted to L-rhamnulose.

It is interesting to note that the same sugar can form different nucleotides. For example, glucose can form uridine diphosphate glucose and thymidine diphosphate glucose, which presumably results in a channelling of the sugar to different metabolic pathways. If glucose 1-phosphate and the glucose nucleotides can act as glycosyl donors in polysaccharide synthesis, then different linkages may be produced in the polysaccharide chain.

There are a number of other studies on the biosynthesis of L-rhamnose using isotopic tracer techniques. Hauser and Karnovsky (6, 7) have studied the biosynthesis of the rhamnolipide produced by *P. aeruginosa*. They found that glycerol and propanediol could serve equally well for L-rhamnose synthesis. Degradation of L-rhamnose indicated that it was formed by the combination of two triose units. When D-glucose-6-C^{14} in the presence of inactive glycerol was the substrate, the rhamnose contained 35% of its activity in C1 and 48% in C6. On the other hand, with D-fructose-6-C^{14} as substrate, 83% of the activity was recovered in C6 of L-rhamnose. These results presumably reflect fundamental metabolic differences of the two sugars. Although the results indicate that L-rhamnose is formed from two triose units, it is possible that a hexose precursor is first formed which is then directly transformed to L-rhamnose.

Southard and co-workers (8) have investigated the conversion of D-glucose-1-C^{14} and D-glucose-6-C^{14} to L-rhamnose of streptococcal cell walls. Although only 60–80% of the total activity was recovered in their degradations, the majority of activity was retained in the terminal carbon atoms. However, the specific activity of the L-rhamnose isolated

from D-glucose-6-C^{14} was about 30% higher than that derived from D-glucose-1-C^{14}. It would be necessary to isolate and degrade bacterial glucose to determine the significance of this result.

Taylor and Juni (9) have studied the biosynthesis of D-glucose and L-rhamnose in a bacterial capsular polysaccharide. Unlabelled polysaccharide was isolated when D-glucose-1-C^{14} and D-glucose-2-C^{14} were the substrates. However, with D-glucose-6-C^{14}, the isolated D-glucose and L-rhamnose contained twice the initial specific activity. The bacterial D-glucose was not degraded but the L-rhamnose contained 50% of its activity in C1 and 43% in C6. The results indicated that the polysaccharide was derived only from C4, C5, and C6 of D-glucose. It was not possible to decide whether L-rhamnose arose directly from trioses or from a six-carbon sugar which was synthesized from the trioses.

Recently, Watkin and Neish have studied the biosynthesis of L-rhamnose from D-glucose in buckwheat (10). These authors showed that the isolated D-glucose and L-rhamnose possessed similar specific activities and distributions of activity, suggesting a direct conversion of D-glucose to L-rhamnose.

Whatever interpretation is placed upon the results presented in this paper, there appears to be a pathway for L-rhamnose biosynthesis which involves the breakdown of the glucose carbon skeleton.

ACKNOWLEDGMENTS

The authors are grateful to Dr. G. B. Krotkov and Dr. C. D. Nelson of the Biology Department of Queen's University for making available the counting apparatus. They are indebted to the National Research Council for grants (T-39 and N.R.C. 706) and for the award of a Studentship (to R. J. S.).

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