The Biosynthesis of Polysaccharides

INCORPORATION OF D-[1-¹⁴C]GLUCOSE AND D-[6-¹⁴C]GLUCOSE INTO PLUM-LEAF POLYSACCHARIDES

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1. The utilization of specifically labelled D-glucose in the biosynthesis of plum-leaf polysaccharides has been studied. After these precursors had been metabolized in plum leaves, the polysaccharides were isolated from the leaves, and their monosaccharide constituents isolated and purified. 2. Both the specific activities and the distribution of ¹⁴C along the carbon chains of the monosaccharides were determined. Significant ¹⁴C activity was found in units of D-galactose, D-glucose, D-xylose and L-arabinose, but their specific activities varied widely. The labelling patterns suggest that in the leaves the other monosaccharides all arise directly from D-glucose without any skeletal change in the carbon chain, other than the loss of a terminal carbon atom in the synthesis of pentoses. 3. The results indicated that within the leaf there are various precursor pools for polysaccharide synthesis and that these pools are not in equilibrium with one another.

Starch, pectin and hemicellulose fractions were isolated from plum leaves in admixture with each other by successive extractions with water and aqueous alkalis, whereas cellulose remained in the insoluble residue (Andrews & Hough, 1958a). The heterogeneity in properties of the individual soluble polysaccharides, indicated by their appearance together in fractions extracted with different solvents, was further demonstrated by experiments in which plum leaves were allowed to photosynthesize in the presence of ¹⁴CO₂ and then metabolize the assimilated ¹⁴C for 48 hr. (Andrews & Hough, 1958b). Monosaccharides isolated from each polysaccharide fraction differed from one another in specific activity, but in addition most monosaccharides differed in specific activity as between one polysaccharide fraction and another.

The incorporation of ¹⁴C from D-[1-¹⁴C]- and D-[6-¹⁴C]-glucose into plum-leaf polysaccharides has now been investigated to obtain more information on the metabolic activity of these polysaccharides and the biosynthesis of their monosaccharide components. Both the specific activities and the labelling at some individual carbon atoms of the main constituent monosaccharides have been determined.

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MATERIALS AND METHODS

Radioactivity measurements. The ¹⁴C-labelled compounds were burnt in a stream of oxygen and the ¹⁴CO₂ so produced was converted into Ba¹⁴CO₃ disks of infinite thickness for counting (Anderson, Andrews & Hough, 1961). The ¹⁴CO₂ liberated in degradation experiments was likewise converted into Ba¹⁴CO₃ for counting. Radioactivity was determined with a thin-end-window Geiger counter, the performance of which was frequently checked by using a disk of poly([¹⁴C]-methyl methacrylate) as a standard reference source.

Labelled compounds. These were purchased from The Radiochemical Centre, Amersham, Bucks.

Procedure for incorporation experiments. The labelled monosaccharide was fed to five non-fruited spurs, each bearing seven to nine leaves, that had been cut from plum trees (*Prunus domestica* var. Giant Prune) and kept with the cut ends of the stems in water until required (1–2 hr. after cutting).

The labelled compounds (Table 1) were dissolved in water (1.5 ml.), sufficient to provide 0.3 ml. of solution for each spur, which imbibed the solution from a small glass cup through the cut end of the stem in about 10 min. Two further 0.3 ml. portions of water were taken up by each spur from the same cups, then water was supplied freely from larger containers. A gentle current of air was maintained over the leaves during experiments and negligible amounts of radioactivity were found in the water remaining in the containers at the end of the experiments. The illumination received by the leaves was about 400 ft.-candles.

Metabolism of the labelled compounds was terminated after 5 hr. by rapidly breaking up the leaves and immediately plunging the laminae into boiling ethanol. After 2–3 min. the pieces were transferred to a Soxhlet apparatus and

Table 1. Incorporation of ^{14}C -labelled glucoses into plum leaves.

Experimental details are given in the text.

Compound supplied			Wt. of alcohol-insoluble material from leaves (g.)	^{14}C in leaf fractions after 5 hr. metabolism (% of ^{14}C supplied)	
Name	Wt. (mg.)	Activity (μC)		Alcohol-soluble material	Alcohol-insoluble material
D-[1- ^{14}C]Glucose	3.8	100	6.75	37	17
D-[6- ^{14}C]Glucose	1.2	50	8.99	44	18

extracted for several hours with methanol, then with ether. The insoluble leaf residue was dried at 50° , powdered, dried to constant weight over phosphoric oxide and assayed for ^{14}C . The carbon content of this material was 43.3%.

Fractionation of the leaf polysaccharides. The dry alcohol-insoluble material was extracted successively with water at 80° , N -sodium hydroxide at room temperature and 2.5 N -sodium hydroxide at 80° . In each case extraction with solvent (200 ml. portions) was continued until the extract, after clarification by centrifugation, contained only very small amounts of carbohydrate material as shown by a Molisch test. The clarified extracts obtained with each solvent were combined and the resultant solutions (approx. 1.5 l. in each case) worked up separately. The alkaline extracts were carefully neutralized by the slow addition of 2*N*-acetic acid and dialysed against tap water for 5 days. The three extracts from each batch of leaves were then evaporated under reduced pressure to approx. 100 ml., octan-2-ol being added as an antifoaming agent as necessary, the solutions were clarified by centrifugation and ethanol (3 vol.) was added to precipitate polysaccharides. The precipitates were isolated, washed with ethanol and ether on the centrifuge and dried under reduced pressure over silica gel.

The fraction of the leaves insoluble in hot 2.5 N -alkali was, in each case, washed with water until the washings were neutral, dehydrated with ethanol, washed with ether and dried as above.

Isolation of ^{14}C -labelled monosaccharides from the polysaccharide fractions extracted with water or alkali. A 2.5% (w/v) solution or suspension of each polysaccharide fraction in N - H_2SO_4 was heated at 100° for 8 hr. The small amounts of brown material remaining undissolved at the end of this time were discarded. The cold hydrolysates were neutralized by adding with stirring a slurry of BaCO_3 in water, and then the precipitated BaSO_4 and excess of BaCO_3 were removed on the centrifuge and washed three times with water. The combined supernatant and washings were concentrated to approx. 10 ml., a fine suspension of BaSO_4 and BaCO_3 was removed on the centrifuge and the clear solution was finally concentrated to a syrup that was dried to constant weight over silica gel.

The constituent monosaccharides of each syrup were separated on paper chromatograms. The syrup was dissolved in water, and the solution was applied to sheets of Whatman no. 3MM chromatography paper to give a loading of approx. 1.5 mg. of syrup/cm. width of paper. Chromatography was by the descending method for 48 hr., with butan-1-ol-pyridine-water (10:3:3, by vol.) as mobile phase, the solvent being allowed to drip from the serrated bottom edge of the paper. The chromatograms were dried at room temperature and the monosaccharide bands located

by spraying guide strips with *p*-anisidine hydrochloride reagent (Hough, Jones & Wadman, 1950).

Glucose and galactose were not completely separated from each other, nor were arabinose and xylose, so in each case the band of mixed sugars was divided horizontally into halves. Soxhlet extraction of the paper strips with methanol then afforded syrups greatly enriched in one of the four monosaccharides. Further purification was effected by repeating the paper chromatography, but with a loading of 0.5–1 mg./cm. width of paper and with the multiple development technique to improve separation. Maximum separation of glucose and galactose was obtained after four excursions of the solvent as given by the relationship $1/R_F - 1$ (Jeanes, Wise & Dimler, 1951). To remove a little mannose as well as xylose from the arabinose samples, ethyl acetate-acetic acid-water (9:2:2, by vol.) was used as chromatographic solvent, otherwise the butan-1-ol-ethanol-water mixture was used as above. The purified monosaccharides were eluted from the paper with methanol, and after evaporation of the methanol the syrups were dissolved in water and the solutions deionized by passage through columns of Amberlite IR-120 (H^+ form) and IRA-400 (CO_3^{2-} form) ion-exchange resins. The solutions were then evaporated to dryness and the residual monosaccharides dried to constant weight over phosphoric oxide.

To each of the purified radioactive monosaccharides was added sufficient carrier monosaccharide to yield, after recrystallization, enough material (approx. 0.5 m-mole) for an assay for specific activity, and for degradation to determine the pattern of labelling. D-Glucose, D-galactose and L-arabinose were recrystallized from methanol-ethanol, and D-xylose from methanol-acetone, in 65–80% yield.

Isolation of D-glucose from cellulose. Polysaccharides other than cellulose were removed from the insoluble material, remaining after extraction of each batch of leaves with alkali, by heating it as a 2.5% (w/v) suspension in N - H_2SO_4 at 100° for 8 hr.; the acid-resistant residue was washed with water until the washings were free from acid, and then with ethanol and ether, and dried over silica gel and assayed for ^{14}C . Further heating in acid, followed by washing and drying, effected no change in its specific activity. The cellulose contained in the resultant brown amorphous product was then hydrolysed to glucose by the method of Monier-Williams (1921). To 200 mg. of the material was added 1 ml. of 72% (w/w) H_2SO_4 . After standing for 1 week at room temperature and dilution to 50 ml., the suspension was heated to 100° for 8 hr. This hydrolysate was treated in the same way as the hydrolysates of the other polysaccharide fractions, giving a crystalline sample of D-glucose.

Degradation of ^{14}C -labelled monosaccharides. (a) Prepara-

tion of potassium D-gluconate, D-galactonate and L-arabinonate. These aldones were prepared in about 90% yield from the aldoses by modification of the method of Moore & Link (1940). The aldose (0.48 m-mole) was dissolved in the minimum quantity of water and transferred with the aid of methanol (2 ml.) to a solution of I_2 (280 mg.) in methanol (6 ml.) at 40° . A 5% (w/v) solution of KOH in methanol (6 ml.) was then added dropwise with stirring. After 15 min. the reaction mixture was cooled to room temperature, and the precipitated aldinate was filtered off, washed with methanol and ether and dried over silica gel.

(b) Preparation of potassium D-xyloate. D-Xylose (0.48 m-mole) was oxidized in methanolic solution as described above, but, since potassium xyloate is soluble in methanol, $BaI_2 \cdot 2H_2O$ (280 mg.) was also included in the reaction mixture to effect the precipitation of the product as barium xyloate. The material so isolated contained some $BaCO_3$, suggesting that the presence of $BaCO_3$ in preparations of barium salts of aldonic acids may well account for the low recovery of ^{14}C sometimes encountered when degrading these salts with sodium metaperiodate. Accordingly, the crude barium xyloate, after being dried and weighed, was extracted with water (3 ml.) three times, and the undissolved residue was dried and weighed. The difference in weight was assumed to be soluble barium xyloate, from which the potassium salt was prepared by adding the calculated amount of K_2SO_4 and discarding the precipitated $BaSO_4$.

(c) Oxidation of the $[^{14}C]$ aldonates. The method of Bernstein (1953) and Eisenberg (1954) was adapted for oxidation of 0.4 m-mole quantities of aldones, and further modified in detail. The reactions are outlined in Scheme 1. A solution of the aldinate in 0.2 M-sodium phosphate buffer, pH 5.9 (10 ml.), was placed in a reaction vessel covered with black tape, to exclude light, and flushed with N_2 (CO_2 -free) for 15 min. The reaction vessel was then connected to an absorption vessel containing $Ba(OH)_2$ solution (previously saturated at 20°), maintained at 80° to encourage the formation of a granular precipitate of $BaCO_3$, and sodium metaperiodate (Hopkin & Williams Ltd.; special grade 'for glycerol determinations') (480 mg.) was added to the aldinate solution. The flow of N_2 was continued for 2 hr. and the $BaCO_3$ in the absorption vessel collected for ^{14}C assay.

After dissolution of KI (300 mg.) in the oxidation mixture, the excess of periodate was destroyed by the gradual addition of 0.2 M-NaAsO₂ until the precipitate of I_2 appeared to have reached a maximum. This precipitate was filtered off and the addition of arsenite continued until disappearance

of the iodine coloration in the solution. Removal of the precipitated I_2 by reaction with arsenite, instead of filtration, resulted in the appearance of free I_2 during steam-distillation at a later stage.

The colourless solution of oxidation products was made alkaline to phenolphthalein by the addition of N-NaOH and distilled at atmospheric pressure to near-dryness several times, with the intermediate addition of water. The distillate (about 200 ml.), containing formaldehyde, was collected in a receiver cooled in ice.

The solution remaining in the still was acidified to pH 4 with N- H_2SO_4 (final vol. 15–20 ml.) and steam-distilled in a Markham (1942) apparatus until the distillate was no longer acid. To this solution, which contained formic acid, were added N-acetic acid (6 ml.) and a boiling-stone, the solution then being heated under reflux for 10 min. in a continuous flow of N_2 to remove dissolved CO_2 . When the solution had cooled, 0.3 M-mercuric acetate in CO_2 -free water (20 ml.) was added, and an absorption vessel containing $Ba(OH)_2$ solution was attached to the top of the reflux condenser. During a further 30 min. heating under reflux, the formic acid was oxidized to CO_2 and this was swept into the baryta solution, from which the precipitated $BaCO_3$ was collected for ^{14}C assay.

The distillate containing formaldehyde (see above) was cooled until partly frozen. Then, to oxidize the formaldehyde to formic acid, N-NaOH (20 ml.) and 0.5 M- I_2 in KI solution (2 ml.) were added, and the mixture was allowed to warm to room temperature. After 1 hr. the solution was acidified to pH 2 and 0.3 M-NaAsO₂ added to discharge the iodine colour, and then after the addition of N-NaOH to make the solution alkaline to phenolphthalein it was concentrated to about 15 ml. Formic acid was isolated from the acidified solution by steam-distillation, oxidized to CO_2 with mercuric acetate, and the CO_2 collected as $BaCO_3$, as described above.

RESULTS AND DISCUSSION

Degradation of ^{14}C -labelled monosaccharides. The pattern of labelling in each of the four labelled monosaccharides (D-glucose, D-galactose, L-arabinose, and D-xylose) isolated from plum-leaf polysaccharides was determined by a method (Scheme 1) that gave the specific activity of each terminal carbon atom, and a mean value for the other carbon

Scheme 1. Degradation scheme for aldoses (for hexose, $n = 4$; for pentose, $n = 3$). Experimental details are given in the text.

atoms. The percentage distribution of ^{14}C along the carbon chain was calculated from these values. Application of the method to samples of D-[1- ^{14}C]-, D-[2- ^{14}C]- and D-[6- ^{14}C]-glucose, kindly supplied by Dr J. R. Catch of The Radiochemical Centre, gave satisfactory results (Table 2). The conclusion from these results is that significant errors are unlikely to arise from the reactions and manipulations involved in the preparation of carbon dioxide from the various carbon atoms of labelled monosaccharides by the method employed. The labelling in many of the monosaccharides isolated from plum-leaf poly-

saccharides was largely at one position (Table 3), and ^{14}C at these positions was estimated with a possible error of $\pm 2\%$ (determined by repetitive counting of assay samples), whereas the possible error in estimations for positions of intermediate specific activity was $\pm 5\%$. Although uncertainty in the estimates of ^{14}C at the most lightly labelled positions was greater (10–20%), such errors have little overall effect on the values for relative amounts of ^{14}C at the different locations. In general, the values in Table 3 for percentage distribution of ^{14}C along the carbon chain of monosaccharides are considered to have a possible error of 2 units. Uncertainty due to counting errors in the estimates of specific activity of sugar carbon is $\pm 2\%$ for the high-activity glucose and $\pm 5\%$ for the other monosaccharides.

^{14}C -incorporation experiments. More than half the ^{14}C supplied to the plum spurs in labelled glucose was found in the leaves 5 hr. after its absorption through the cut ends of the stems (Table 1). Some ^{14}C was also detected in the stems, but not assayed, and presumably more was lost from the leaves as $^{14}\text{CO}_2$ during metabolism. About a quarter of the activity in the leaves was isolated in the alcohol-insoluble fraction that contained the polysac-

Table 2. *Chemical degradation of specifically labelled D-[^{14}C]glucose*

Degradation was effected by the method outlined in Scheme 1. Radioactivity at each location is expressed as a percentage of the total radioactivity found in the degradation products.

Substance	Location of ^{14}C		
	C-1	C-2 + C-3 + C-4 + C-5	C-6
D-[1- ^{14}C]Glucose	99.8	0.1	0.1
D-[2- ^{14}C]Glucose	0.5	99.5	0
D-[6- ^{14}C]Glucose	0	0.9	99.1

Table 3. *Labelling in monosaccharide constituents of plum-leaf polysaccharides after the incorporation of ^{14}C -labelled glucose*

Experimental details are given in the text. To facilitate comparison between the two experiments, results for D-[6- ^{14}C]glucose incorporation have been corrected for the use of only 50 μC of ^{14}C in this experiment (compared with 100 μC of D-[1- ^{14}C]glucose), and the distribution of ^{14}C amongst polysaccharides in a greater weight of material (Table 1). Results of monosaccharide degradations are expressed as in Table 2.

Compound supplied	Period of metabolism (hr.)	Polysaccharide fraction	Monosaccharide constituent	Specific activity of sugar carbon ($\mu\text{C}/\text{mg. atom of C}$)	Location of ^{14}C					
					C-1	C-2	C-3	C-4	C-5	C-6
D-[1- ^{14}C]Glucose	5	Hot-water-soluble	D-Glucose	0.53	64	← 13 →				23
			D-Galactose	0.031	77	← 7 →				16
			L-Arabinose	0.027	91	← 6 →			3	
			D-Xylose	0.020	65	← 29 →			6	
		Hot-alkali-soluble	D-Glucose	0.82	60	← 13 →				27
			D-Galactose	0.035	68	← 13 →				19
			L-Arabinose	0.017	84	← 9 →			7	
			D-Xylose	0.016	89	← 7 →			4	
		Cellulose	D-Glucose	0.009	72	← 7 →				21
D-[6- ^{14}C]Glucose	5	Hot-water-soluble	D-Glucose	0.56	29	← 2 →				69
			D-Galactose	0.064	22	← 3 →				75
			L-Arabinose	0.008	67	← 25 →			8	
			D-Xylose	0.007	68	← 32 →			0	
		Hot-alkali-soluble	D-Glucose	0.45	27	← 11 →				62
			D-Galactose	0.040	19	← 15 →				66
			L-Arabinose	0.006	51	← 26 →			23	
			D-Xylose	0.004	40	← 44 →			16	
		Cellulose	D-Glucose	0.009	23	← 2 →				75

charides. Three polysaccharide fractions were extracted from each batch of alcohol-insoluble material, but only the first and third of these (extracted with hot water and hot 2.5 N-sodium hydroxide respectively) were examined further, since investigation of the labelling in plum-leaf polysaccharides after $^{14}\text{CO}_2$ incorporation (Andrews & Hough, 1958b) had indicated that labelling in the second fraction (isolated by extraction with N-sodium hydroxide) was intermediate between that in the other two. The water-soluble polysaccharide from 6 g. of alcohol-insoluble leaf residue yielded 10–25 mg. each of purified D-glucose, D-galactose and L-arabinose, and 5 mg. of D-xylose, whereas the corresponding alkali-soluble polysaccharides gave 25–50 mg. each of all four monosaccharides. These yields were roughly the same as those obtained from similar polysaccharide fractions by Andrews & Hough (1958b), who also used paper chromatography extensively to effect purification.

The material remaining after final extraction of soluble polysaccharides from the leaves with hot alkali contained cellulose (Andrews & Hough, 1958a) together with small amounts of hemicelluloses. The removal of glucans other than cellulose from this preparation was considered important in case their ^{14}C activity was high in relation to that of cellulose, for small amounts of contaminating glucans would then give an erroneous picture of the metabolic activity of cellulose. Accordingly, the material was heated with dilute mineral acid and thoroughly washed, before the cellulose was hydrolysed with stronger acid. The yield of pure D-glucose from 200 mg. of α -cellulose was 20 mg., but a trace of xylose was detected in the cellulose hydrolysates.

The specific activity of D-glucose isolated from water- and alkali-soluble polysaccharides after the incorporation of either D-[1- ^{14}C]- or D-[6- ^{14}C]-glucose was at least 50-fold greater than that of D-glucose from cellulose of the same leaves, and showed somewhat greater randomization of ^{14}C between the terminal carbon atoms (Table 3), suggesting that D-glucose derivatives for the synthesis of soluble glucans are perhaps drawn from a pool different from that used for the synthesis of cellulose. On the assumption that the glucose of high specific activity is combined in starch, the differences in specific activity of the glucose samples reflect the dynamic role of starch in leaf metabolism and the relatively inert character of cellulose. However, some metabolic activity for cellulose is indicated by the fact that, in wheat roots, the incorporation of ^{14}C into cellulose was reversible (Margerie & Péaud-Lenôel, 1961). Heterogeneity of the leaf starch is indicated by the fact that the water-soluble polysaccharides yielded glucose differing in specific activity from the glucose of the alkali-soluble polysaccharides. A possible reason is

that amylose and amylopectin did not attain the same specific activity, as observed in wheat grain (McConnell, Mitra & Perlin, 1958; Whistler & Young, 1960), and the relative amounts of these polysaccharides extracted in the two fractions were not the same. Incorporation of ^{14}C from $^{14}\text{CO}_2$ into glucose of the soluble polysaccharides similarly indicated heterogeneity in starch metabolism (Andrews & Hough, 1958b).

A major portion of the ^{14}C in glucose from both starch and cellulose of plum leaves was located at the same terminal position that labelling occupied in the [^{14}C]glucose administered to the leaves, whereas much of the redistributed ^{14}C was at the other terminal position. Such metabolic redistribution of isotope between the terminal carbon atoms of D-glucose is now well known, for among plants the glucose used in cellulose synthesis by cotton bolls (Shafizadeh & Wolfrom, 1955), wheat plants (Brown & Neish, 1954; Neish, 1955, 1958; Altermatt & Neish, 1956), wheat seedlings (Edelman, Ginsburg & Hassid, 1955) and barley seedlings (Shibko & Edelman, 1957) is labelled in this way, and so also is the glucose used in starch synthesis by wheat plants (McConnell *et al.* 1958), after the incorporation of D-[1- ^{14}C]- or D-[6- ^{14}C]-glucose by the plants. This pattern of labelling probably arises from aldolase breakdown of the specifically labelled glucose molecules by competing enzymes of the Embden-Meyerhof glycolytic pathway to dihydroxyacetone 1-phosphate and D-glyceraldehyde 3-phosphate, followed by isomerization of these compounds by triose phosphate isomerase, and subsequent resynthesis of glucose by a reversal of the pathway. If these reactions are the main cause of the randomization of ^{14}C in glucose of plum-leaf polysaccharides, then, by the argument of Shibko & Edelman (1957), at least 50% of this glucose had been resynthesized in the leaves from labelled precursors.

The expectation was that incorporation of ^{14}C into pectic polysaccharides and xylan, which are associated with plants cell walls and are regarded as structural materials, would be much less than that into starch, but comparable with that into cellulose. The specific activities of D-galactose, L-arabinose and D-xylose of plum-leaf polysaccharides after the incorporation of D-[1- ^{14}C]glucose were in accordance with this view. A marked deviation in relative specific activities from the results of $^{14}\text{CO}_2$ incorporation (Andrews & Hough, 1958b) occurred in the labelling of xylose of the water-soluble polysaccharides. Whereas, in the $^{14}\text{CO}_2$ experiment this xylose had only one-eighth the specific activity of arabinose of the same fraction, in the [^{14}C]glucose experiment both monosaccharides had about the same specific activity. The reason for this difference is not understood.

The pattern of labelling in galactose bore more resemblance to that in cellulose glucose than to that in starch glucose, which suggests that in plum leaves the synthesis of D-galactose from D-glucose, which doubtless proceeds via UDP-glycosyl compounds (Hassid, Neufeld & Feingold, 1959) utilizes glucose from a pool concerned with the synthesis of structural polysaccharides rather than from one concerned with starch synthesis. These conclusions are consistent with the recent observations that ADP-D-glucose is the preferred precursor of starch (Leloir, 1964; Recondo & Leloir, 1961; Espada, 1962; Kauss & Kandler, 1962; Recondo, Dankert & Leloir, 1963) and GDP-D-glucose of cellulose (Elbein, Barber & Hassid, 1963). The breakdown of cell walls in growing plant tissue (MacLachlan & Young, 1962) might well contribute to one pool but not to the other. The orderly and closely linked working of enzymes in cell-wall synthesis is indicated by the observations (Neish, 1955; Altermatt & Neish, 1956) that xylose is apparently not readily used for xylan synthesis in wheat plants without intermediate conversion into hexose. The presence in plum leaves of the route for pentose synthesis that involves loss of C-6 from hexose (Altermatt & Neish, 1956; Seegmiller, Axelrod & McCready, 1955; Slater & Beevers, 1958; Hassid, 1962) was indicated by the fact that the specific activities of arabinose and xylose, relative to that of galactose, were much less after the incorporation of D-[6-¹⁴C]-glucose than after the incorporation of D-[1-¹⁴C]-glucose. Further evidence is provided by a comparison of labelling patterns in the monosaccharides. In pentoses isolated from polysaccharide fractions after the incorporation of D-[1-¹⁴C]glucose, ¹⁴C was concentrated mainly at C-1 and very little was at C-5. The ratios of amounts of ¹⁴C at C-1 and at C-2-C-5 of the pentoses were similar to the ratios of amounts of ¹⁴C at C-1 and at C-2-C-5 of hexoses from the same leaves. After the incorporation of D-[6-¹⁴C]glucose, the pentoses again had more ¹⁴C at C-1 than at any other position, and, so far as the degradations can show, the labelling patterns in the pentoses were similar to those at C-1-C-5 of the hexoses of the alkali-soluble polysaccharides of the same leaves. On the other hand, labelling in hexoses from the other polysaccharide fractions did not correspond in the same way with that of the pentoses. If interconversion of arabinose and xylose via their UDP derivatives (Hassid *et al.* 1959) occurs in plum leaves, the differences in labelling patterns of the two pentoses indicate incomplete equilibration between pools involved in their metabolism.

Overall, the results suggest the existence of differences in the metabolic behaviour and relationship with precursors of the various plum-leaf polysaccharides that are additional to the already recognized difference in metabolic activity between

starch and structural polysaccharides. Although the labelling patterns found in the monosaccharide units are generally in accordance with known pathways of monosaccharide biosynthesis, the precursors for polysaccharide synthesis in plum leaves seem to exist in pools that are not all in equilibrium with each other. A picture is emerging of specific interrelationships between leaf polysaccharides and precursor pools, rather than one of polysaccharide synthesis drawing on a common supply of monosaccharide precursors.

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