BIOGENESIS OF CARBOHYDRATES IN WOOD

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It is believed that the primitive earth had on its surface only simple carbon compounds. It is generally agreed that the atmosphere was a reducing one and that most of the carbon was in the form of methane and carbon dioxide, with methane predominating. There was much hydrogen and water. Calvin has shown that irradiation of a mixture of water and carbon dioxide in a cyclotron yielded formic acid, acetic acid, succinic acid and other derivatives[1]. If carbon dioxide was replaced by methane and ammonia was added the more rapid formation of these acids was observed and, in addition, glycine and other amino-acids were produced[2, 3].

Formic acid on decomposition yields carbon monoxide and this with hydrogen could yield methanol and formaldehyde. Many years ago it was shown that formaldehyde could be polymerized by basic solutions, such as lime water, lead carbonate or an aqueous suspension of calcium carbonate, to yield a mixture of sugars called *formose*[4]. This mixture has been analysed and the mechanism of its formation has been investigated extensively. It contains mainly pentulose and hexulose sugars together with traces of heptuloses and substances with fewer carbon atoms[5]. The mixture of sugars has been reduced and the acetylated materials were examined in the gas chromatographic apparatus. The major products were identified as

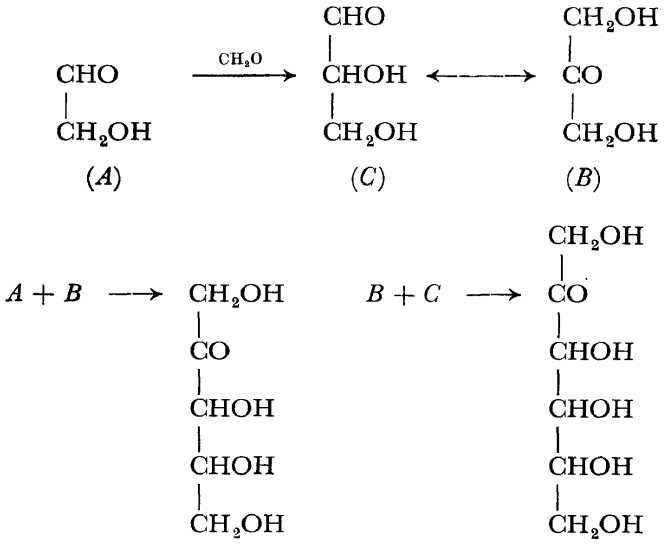


Figure 1. Formose synthesis

penitol and hexitol acetates[6]. These sugars are produced by mechanisms shown in *Figure 1.*

The key compound is glycollic aldehyde (*A*) which, it has been suggested, catalyzes the reaction according to the following scheme: It reacts with formaldehyde to yield DL-glycerose (*C*) which isomerizes to dihydroxypropanone (*B*), which in turn condenses with a molecule of formaldehyde to yield DL-erythrulose (*D*), which isomerizes to a tetrose (*E*), the tetrose then splits into two molecules of glycollic aldehyde (*A*) and the reaction continues[7] (*Figure 2*).

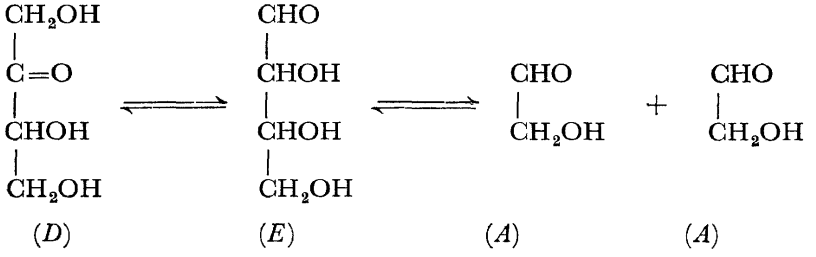


Figure 2

The ketoses which are first produced are converted in part to aldoses under the influence of basic catalysts. It is very interesting that the major substances produced in the formose reaction are those which are commonly encountered in natural products, *e.g.* ribulose, xylulose, fructose and heptulose. None of the products produced in the formose reaction is optically active. Optical activity may have developed as a result of irradiation by polarized light, from the moon for example—or resolution may have occurred by chromatography over optically active materials such as quartz or even by zone melting or by some other mechanism. Why the D-form of glucose rather than the L-form should be favoured is unknown at present.

The development of plants took place some 400,000,000 years ago. The consequent conversion of carbon dioxide to complex polysaccharides takes place on a very large scale, an example is illustrated in *Table 1.*

Table 1. Comparison of the amount of food produced by photosynthesis and the amount of wood produced by stands of several tree species

Species	*Annual net photosynthesis* (kg carbon per hectare)	*Annual net production of wood* (kg carbon per hectare)
Douglas Fir	18,500	7,000
Beech	13,500	3,700
Spruce	11,100	4,100
Larch	10,700	3,100
Birch	8,900	2,300
Oak	6,300	2,800
Scots Pine	4,700	2,900

The mechanism of conversion of carbon dioxide to sugars and to polymers has been studied by Hassid[8], a pioneer in this work, and by Krotkov[9], Calvin[1, 2], Neish[10] and by many others. ^{14}C-labelled compounds have generally been used to follow the interconversion of the sugars. While much is known of the origin of sugars in plants, relatively little information is available on carbohydrate metabolism in trees. Thus, many of the suggested metabolic pathways result from analogy with known sources in plants and fungi.

Some sugars occur in the free form in plants and trees, *e.g.* D-glucose, L-arabinose, L-fructose, L-rhamnose, vitamin C, hexitols, heptitols, octitols and sucrose and its derivatives. The following are some sugars which are found combined in polysaccharides:

D-Glucose
D-Galactose (and its 3-*O*-methyl ether)
D-Mannose
D-Fructose
L-Arabinose
D-Xylose (and its 2-*O*-methyl ether)
L-Rhamnose
L-Fucose (and its 2-*O*-methyl ether)
D-Glucuronic acid (and its 4-*O*-methyl ether)
D-Galacturonic acid

It appears that all these sugars arise from carbon dioxide *via* triose fragments (*H*) and D-fructose-1,6-diphosphate (*I*) (*Figure 3*). It has been shown

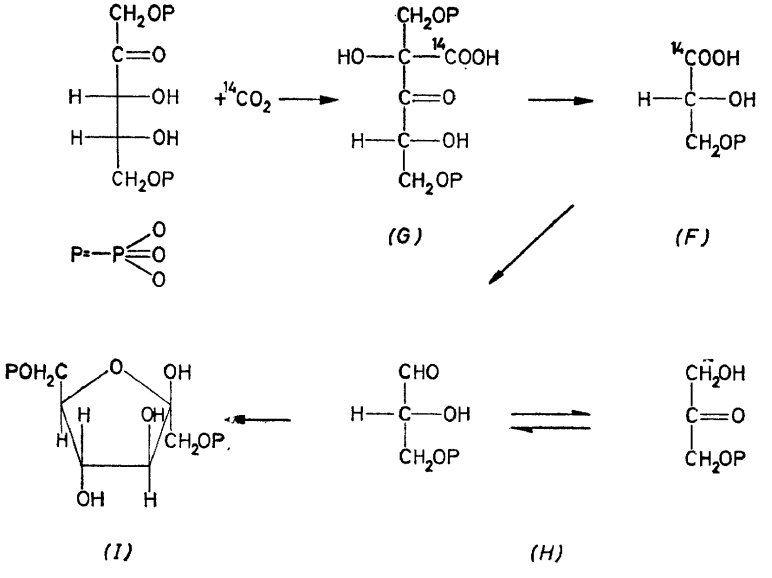


Figure 3. Origin of hexoses

that carbohydrates may be phosphorylated preferentially on the primary alcohol groupings by heating them with phosphoric acid or with its salts[12]. Perhaps this is how the vital sugar phosphates were first produced long ago.

Calvin and his co-workers[1] have shown that the first product of photosynthesis is 3-D-phosphoglyceronic acid (*F*) which arises from a D-erythro-pentulose diphosphate (*G*) derivative.

Krotkov, Nelson, and Shiroya[13] of Queen's University have kindly allowed me to use some of their results to illustrate the photosynthetic activities of pine trees. When a seedling of *Pinus strobus* is allowed to photosynthesize for eight hours in light and in an atmosphere of $^{14}CO_2$, sucrose and raffinose are the major primary water-soluble products.

The photosynthesized material is translocated mainly as non-reducing saccharides, as is illustrated in *Table 2*.

Table 2. 2500 FT-c photosynthesis

	Shoot (%)	*Stem* (%)	*Root* (%)
Pentose	—	—	—
Fructose	11·2	1·5	1·8
Glucose	10·0	1·4	2·3
Sucrose	74·3	94·6	94·2
Disaccharide	0·9	0·8	—
Raffinose	1·2	1·7	1·7
Trisaccharide	0·1	—	—
Neutral substance	1·8	—	—
Organic acid	0·5	—	—
Total	100·0	100·0	100·0

The small amounts of acids which are produced are of particular interest as they contain shikimic and quinic acids which may be utilized to make lignin—an aspect of carbohydrate chemistry which was discussed earlier by Freudenberg[14] (see *Table 3*).

Table 3. Distribution of ^{14}C in organic acid fractions as percentage of total activity spotted on chromatogram

Organic acid	*Expt. 11A* (%)	*Expt. 11B* (%)
Quinic acid (overlapped with oxalic acid)	39·5	23·9
Shikimic acid	7·5	5·4
Citric acid	9·8	16·3
Malic acid	19·4	28·5
Succinic acid	2·5	3·1
Fumaric acid	0·8	1·3
Unknown I (Hexose phosphate?)	12·8	14·5
Unknown II (Phosphoglyceronic acid?)	5·7	4·4
Unknown III	2·0	2·6
Total	100·0	100·0

Sucrose is a common major component of the fluid in the sieve tube but higher molecular weight sugars are also found[15] (*Table 4*).

Table 4. Sugars in sieve-tube exudate of some American trees

Family	*Name of tree*	*Sucrose* (%)	*Raffinose* (%)	*Stachyose* (%)	*Verbascose* (%)
Salicaceae	Aspen	>10	Trace	Trace	—
Fagaceae	Beech	>10	Trace	—	—
	Chestnut	>10	Trace	—	—
	White Oak	>10	—	—	—
	Chestnut Oak	>10	—	—	—
	Red Oak	>10	—	—	—
Ulmaceae	Elm	2–10	2–10	2–10	—
Magnoliaceae	Tulip Tree	>10	Trace	Trace	—
Rosaceae	Black Cherry	>10	Trace	—	—
Leguminosae	Black Locust	>10	—	—	—
Aceraceae	Striped Maple	>10	Trace	—	—
	Sugar Maple	>10	—	—	—
Rhamnaceae	Buckthorn	>10	Trace	Trace	—
Tiliaceae	Basswood	>10	0·5–2	0·5–2	—
Nyssaceae	Black Gum	>10	Trace	—	—
Oleaceae	White Ash	2–10	2–10	10	Trace

These are derivatives of sucrose and contain α-linked D-galactopyranose residues (*Figure 4*). Free D-galactose does not appear in the sieve tube fluid. It is of interest that α-linked D-galactopyranose residues are found in some wood polysaccharides, (*e.g.* glucomannans)[16].

Maple sap besides containing sucrose also contains fructosylsucrose and a polysaccharide which is composed of L-arabinose, D-galactose and L-rhamnose[17]. The problem is to explain the origin of these and other sugars from carbon dioxide.

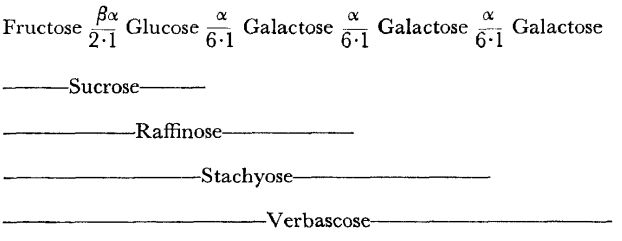


Figure 4. D-Galactose containing oligosaccharides

Mannitol, a common constituent, is believed to arise from D-mannose by reduction, the hexose sugar itself arising from D-fructose-6-phosphate by isomerization. Sorbitol is formed by the reduction of D-fructose, this is a reversible reaction.

As we saw earlier, the D-fructose, 1,6-diphosphate (*I*) originates from the condensation of two triose fragments under the influence of the enzyme aldolase. D-Fructose-6-phosphate is split by the enzymes *transaldolase* and *transketolase* in different ways as is illustrated in *Figure 5* [19].

Transketolase and transaldolase require thiamine pyrophosphate as a *co-factor*. These two enzymes between them can cause the formation of pentose residues with no great change of energy and no formation of carbon dioxide as was suggested by Neish a few years ago. The net result is that

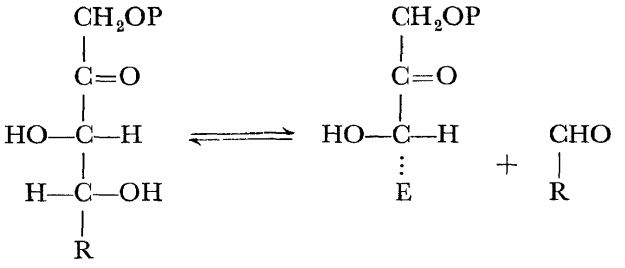


Figure 5(a). Transaldolase reaction

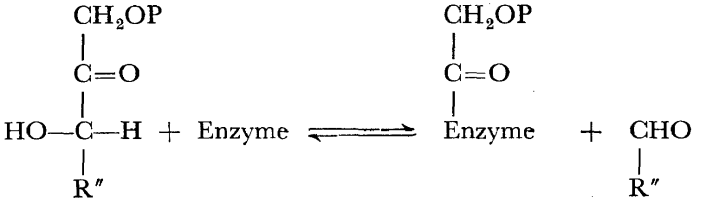
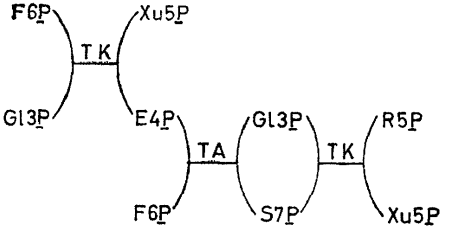


Figure 5(b). Transketolase reaction

two moles of D-fructose-6-phosphate and one mole of D-glycerose-3-phosphate are converted to 3 moles of pentose-5-phosphate[10] (*Figure 6*).



F6P = D-Fructose-6-phosphate
Gl3P = D-Glycerose-3-phosphate
Xu5P = D-Xylulose-5-phosphate
E4P = D-Erythrose-4-phosphate
S7P = D-Sedoheptulose-7-phosphate
R5P = D-Ribose-5-phosphate

TK = Transketolase
TA = Transaldolase

Figure 6. Origin of pentose sugars

Thus a possible route to pentoses from hexoses can be advanced.

A second method (see *Figure 7*) of pentose formation is *via* the aldolase reaction. For example, D-fructose-1,6-diphosphate is split by aldolase to give dihydroxypropanone phosphate and D-glycerose-3-phosphate (*H*). The enzyme is specific for the ketose only and the triose-aldehyde can be replaced by a diose *e.g.* glycollic aldehyde when D-xyulose-1,5-diphosphate results or with D-erythrose when D-sedoheptulose-1,7-diphosphate is obtained[18].

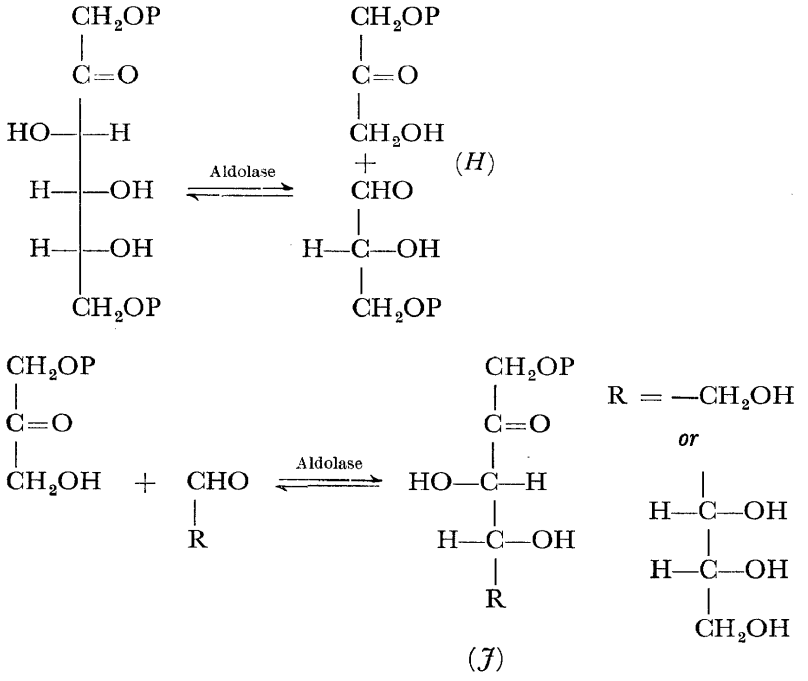


Figure 7. Origin of pentose and heptose sugars

The hydroxyl groups on C–3 and C–4 possess the D-*threo* configuration, *i.e.* they are *trans* to one another. However, if the product (*J*) of enzyme action existed initially in the zig-zag conformation, the two hydroxyl groups would be *cis* to one another as might be expected for a reaction on an enzyme surface (*Figure 8*).

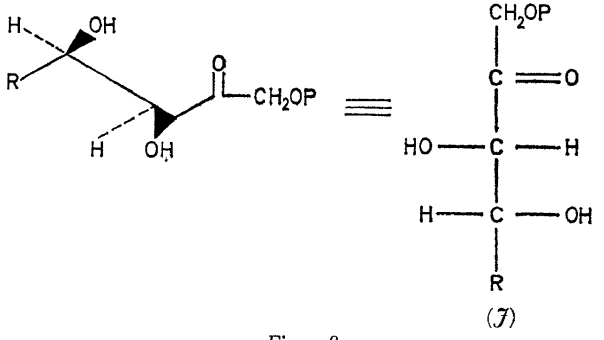
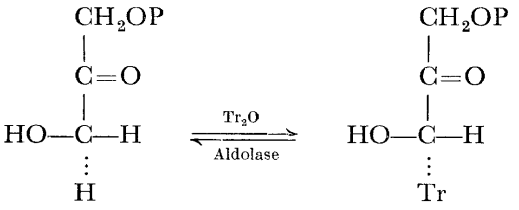
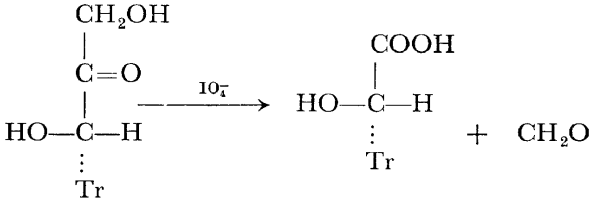


Figure 8

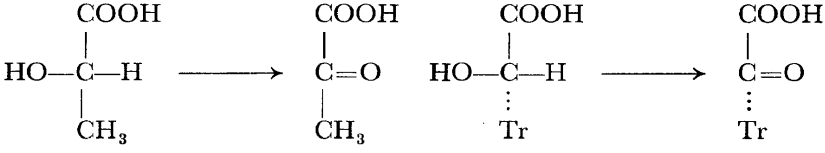
It has been proved conclusively by Rose that only one hydrogen of the dihydroxypropanone phosphate is involved in this reaction in the following way. The substance was incubated in tritiated water with aldolase when one hydrogen was substituted by tritium:



The tritiated product was dephosphorylated and oxidized with periodate:



The glycollic acid was then dehydrogenated with L-lactic acid dehydrogenase. Hydrogen only was removed[20]:



Yet a third method of obtaining the pentose residues which are so commonly found in trees is the decarboxylation process first suggested by the French chemist Maquenne and later amplified and discussed by Hirst[21]. As a result of much work mainly by Hirst[22], Hassid[8] and Neish[10], it has been shown that a derivative of D-glucose can be oxidized to D-glucuronic acid and then decarboxylated to a D-xylose derivative. This transformation occurs at the monosaccharide level.

It has been shown recently that the uridine diphosphate derivatives are the key compounds in these transformations. When D-mannose or L-galactose is the sugar transformed, guanosine or thymidine may be the heterocyclic system involved[23a, b].

The mechanism of formation of uridine diphosphate glucose (UDPG) is pictured in *Figure 9*.

Uridine diphosphate glucose is also involved in the formation of sucrose from D-glucose-1-phosphate and very probably it is responsible for the synthesis of starch from D-glucose-1-phosphate. The rarer sugars, L-fucose and L-rhamnose, probably arise from purine-diphosphate-glucose derivatives without fragmentation of the sugar molecule, but the exact sequence of events is unknown. However, it is known that in L-rhamnose synthesis the hydroxyl group at C–6 is probably first converted to CH_3, perhaps, first by elimination of the elements of water followed by reduction and then the

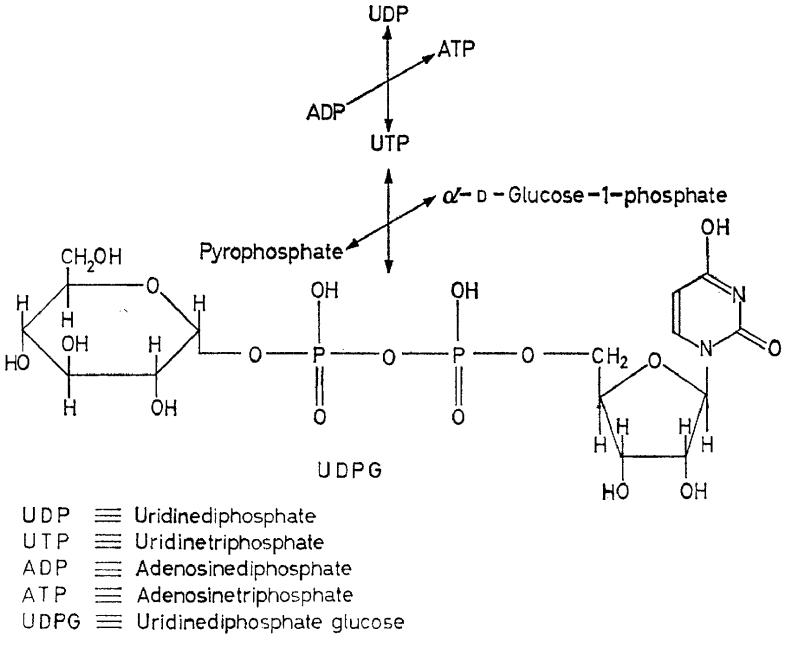


Figure 9. Formation of uridine diphosphate glucose

hydroxyl group at C–4 is oxidized to a keto group[24] (*Figure 10*). (The last two steps may occur simultaneously.)

D-Glucose (100 per cent 1–^{14}C) ⟶ L-Rhamnose (89 per cent 1–^{14}C)

as *as*

Thymidine diphosphate D-glucose Thymidine diphosphate L-rhamnose

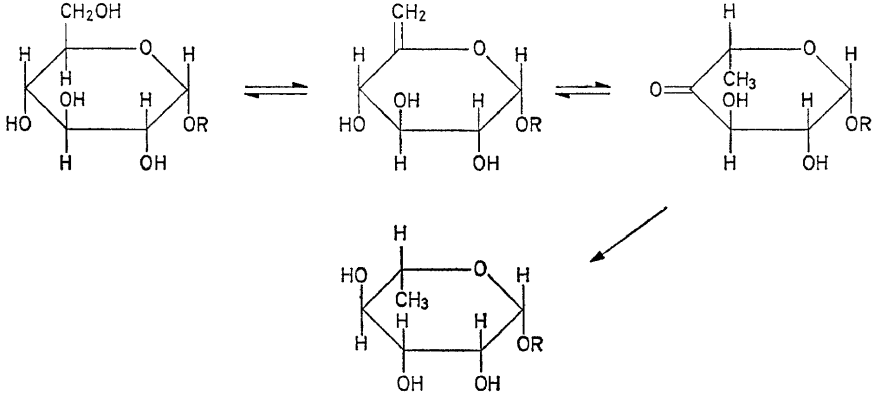
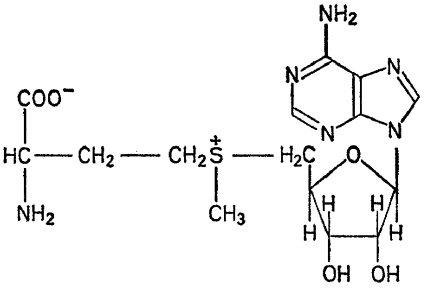


Figure 10. Formation of L-rhamnose

The methoxyl grouping which is found on D-galactose at C–3, on D-glucuronic acid at C–4, on C–2 of D-xylose and L-fucose, on the carbomethoxy group in pectin and which is present as an ether in lignin probably

originate from methionine ($CH_3SCH_2CH_2CHNH_2COOH$) *via* an adenine derivative.



Vitamin C which is found in the needles of spruce and pines may originate from D-glucose* by the following series of reactions as it does in cress seedlings[25].

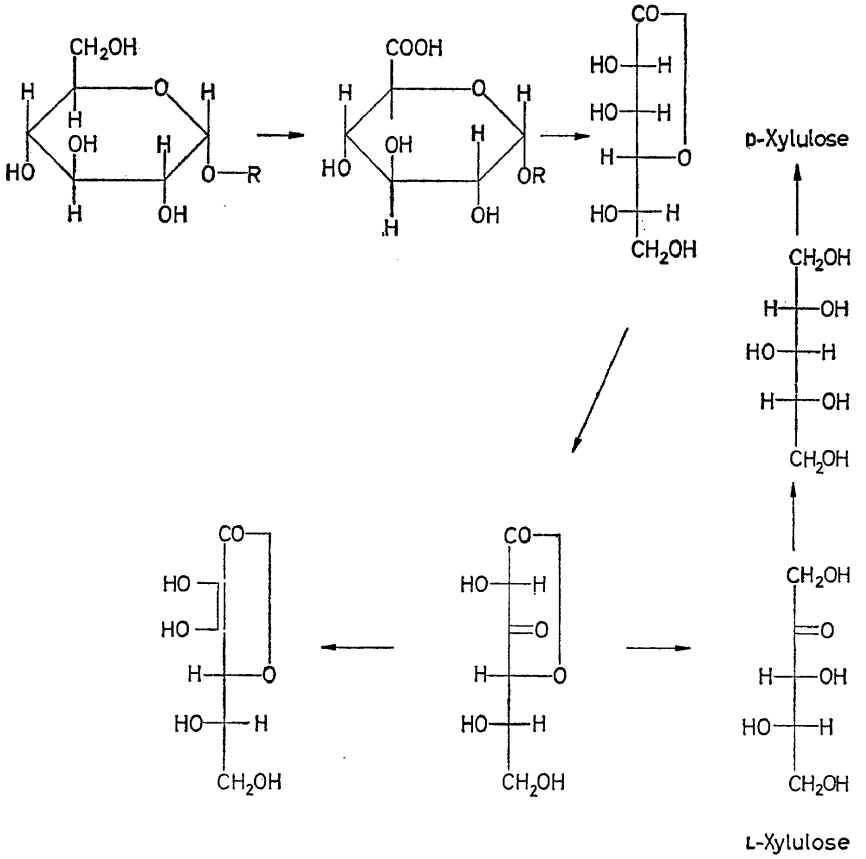


Figure 11. Vitamin C biosynthesis

* Vitamin C may also originate from D-glucose without inversion of the molecule[26] (Loewus; personal communication from Dr A. C. Neish).

The synthesis of some of these sugars from carbon dioxide may be illustrated by showing some of the results of Andrews and Hough[27] who have examined the photosynthesis of sugar in plum leaves. I wish to thank these authors for their kind permission to reproduce these results. They allowed leaves, which had been kept in the dark for 24 hours to photosynthesize in the presence of $^{14}CO_2$. After 5 hours, when all the $^{14}CO_2$ had been absorbed, photosynthesis was allowed to continue in the presence of $^{12}CO_2$. Samples of leaves were removed at intervals and

Table 5. Leaf fractions as percentage of ^{14}C supplied in $^{14}CO_2$

Period of photosynthesis (h)	1	1·5	3	6	15·5	24	48
Activity:							
in leaf extract	18·4	24·5	53·8	58·4	56·3	52·3	49·0
in insoluble leaf residue	6·2	9·5	25·0	39·9	40·7	38·7	32·3
Total	24·6	34·0	78·8	98·3	97·0	91·0	81·3

Table 6. Relative distribution of ^{14}C between leaf extracts

Leaf extract	75	72	68	59·5	58	57·5	60
Insoluble leaf residue	25	28	32	40·5	42	42·5	40

Table 7. Polysaccharide sugar–carbon (%) originating from $^{14}CO_2$ after 48 hours photosynthesis

	Water soluble	*Soluble in cold* N NaOH	*Soluble in hot* 2·5N NaOH
D-Glucose	—	21·6	18·5
D-Galactose	1·66	—	1·72
L-Rhamnose	0·94	—	1·12
L-Arabinose	1·58	0·90	0·74
D-Xylose	0·21	0·30	0·49
D-Galacturonic acid	0·08	—	—

examined. *Tables 5* and *6* show the uptake of $^{14}CO_2$ at various time intervals. The total activity in leaf residues did not exceed that of the leaf extracts. It is evident that the rate of incorporation of $^{14}CO_2$ into metabolically inert polysaccharides is slow under these experimental conditions. Whistler has shown that hemicelluloses (xylan) once formed are not broken down.

The percentage of sugars of the three fractions which are labelled is given in *Table 7.*

Each monosaccharide was crystallized, sometimes with the addition of a carrier, and converted to a crystalline derivative before combustion and assay as ^{14}C-barium carbonate. D-Galactose was converted to mucic acid, L-arabinose to its benzhydrazide, D-xylose to the tosylhydrazone, and D-galacturonic acid was assayed as $NaCa(C_6H_9O_3)_3 \cdot 6H_2O$.

Table 8. 48 h Plum leaves[27]

Monosaccharides from polysaccharides which are:	*Monosaccharide constituent*	*Weight isolated* (mg)	*Specific activity of undiluted sugar* (μc/milli-atom-equiv. of C)
Water soluble	D-Galactose	16·0	0·338
	D-Galacturonic Acid	9·3	0·017
	L-Arabinose	14·0	0·320
	D-Xylose	2·6	0·043
	L-Rhamnose	5·5	0·190
Soluble in cold N NaOH	L-Arabinose	5·4	0·182
	D-Glucose	41·0	4·40
	D-Xylose	8·0	0·062
Soluble in hot 2·5N NaOH	D-Galactose	19·7	0·350
	L-Arabinose	31·0	0·149
	D-Glucose	45·3	3·76
	D-Xylose	26·5	0·100
	L-Rhamnose	8·1	0·227

Table 9. Incorporation of 1-(^{14}C)-D-glucose into plum leaf polysaccharides

Period of incorporation (h)	*Polysaccharide fraction*	*Monosaccharide constituent*	*Weight isolated* (mg)	*Specific activity of sugar carbon* (μc/mA)	*Distribution of* ^{14}C *along carbon chain of monosaccharide* (%) C_1 C_2 C_3 C_4 C_5 C_6
5	Hot water soluble	D-Glucose	9·6	0·53	64·6 ← 13·7 → 21·7
		D-Galactose	11·6	0·031	77·3 ← 6·7 → 16·5
		L-Arabinose	15·4	0·027	91·4 ← 6·2 → 2·4
		D-Xylose	2·9	0·020	65·0 ← 28·8 → 6·2
		L-Rhamnose	3·7	0·022	56·4 ← 7·8 → 1·9 33·9
5	Hot alkali soluble	D-Glucose	40·3	0·815	59·9 ← 12·8 → 27·3
		D-Galactose	26·0	0·035	68·5 ← 12·9 → 18·7
		L-Arabinose	95·3	0·017	85·0 ← 9·1 → 7·1
		D-Xylose	42·1	0·016	89·0 ← 7·0 → 4·0
		L-Rhamnose	19·0	0·022	78·5 ← 3·0 → 1·1 17·4
5	Cellulose	D-Glucose	138·6	0·0094	71·8 ← 6·6 → 21·6
20	Cellulose	D-Glucose	115·5	0·0072	77·8 ← 1·8 → 20·4

The activities of the sugars varied from fraction to fraction as is illustrated in *Table 8*. This effect has been noted in starch metabolism by Perlin[29], Porter and others. The very small activity in D-galacturonic acid suggests that pectic acid is not a very active metabolite and that once D-galacturonic acid is produced it is decarboxylated to L-arabinose. Note also that the rate of conversion of D-glucose to D-xylose is not very high because starch is a major product which is metabolized. It suggests that xylan is not an active metabolite[30].

It is interesting that no sucrose was detected—it was probably present during the early stages of photosynthesis.

Table 10. Incorporation of 6-(^{14}C)-D-glucose into plum leaf polysaccharides

Period of incorporation (h)	*Polysaccharide fraction*	*Monosaccharide constituent*	*Weight isolated* (mg)	*Specific activity of sugar carbon* (μc/mA)	*Distribution of* ^{14}C *along carbon chain of monosaccharide* (%) C_1 C_2 C_3 C_4 C_5 C_6
5	Hot water soluble	D-Glucose	24·1	0·21	28·6←1·8→69·5
		D-Galactose	19·9	0·024	21·6←3·2→75·2
		L-Arabinose	16·4	0·0029[a]	66·6←24·9→8·3
		D-Xylose	4·9	0·0058[a]	78·0←32·0→0·0
		L-Rhamnose	7·2	0·017	20·0←4·0→2·9 73
5	Hot alkali soluble	D-Glucose	65·1	0·17	27·1←10·7→62·2
		D-Galactose	10·7	0·015	19·5←15·0→65·5
		L-Arabinose	21·3	0·0023[b]	51·0←26·5→22·5
		D-Xylose	20·0	0·0014[b]	40·0←44·0→16·0
		L-Rhamnose	4·1	0·0062	10·8←13·0→ [c] 76·3
5	Cellulose	D-Glucose	46·8	0·0034	23·1←1·9→75·0
20	Cellulose	D-Glucose	116·6	0·0065	21·5←6·0→72·5

[a] Activities determined to standard deviation of 4%.
[b] Activities determined to standard deviation of 10%.
[c] C_5 lost during preparation of sample.

Tables 9 and *10* illustrate the changes in radioactive polysaccharides during photosynthesis. Cellulose metabolism is not reversible and the intermediates are slowly converted to cellulose.

In conclusion, the conversion of sugars to aromatic compounds may be illustrated. These pathways are known to occur in fungi and are very probably present in plants and trees according to the following scheme.

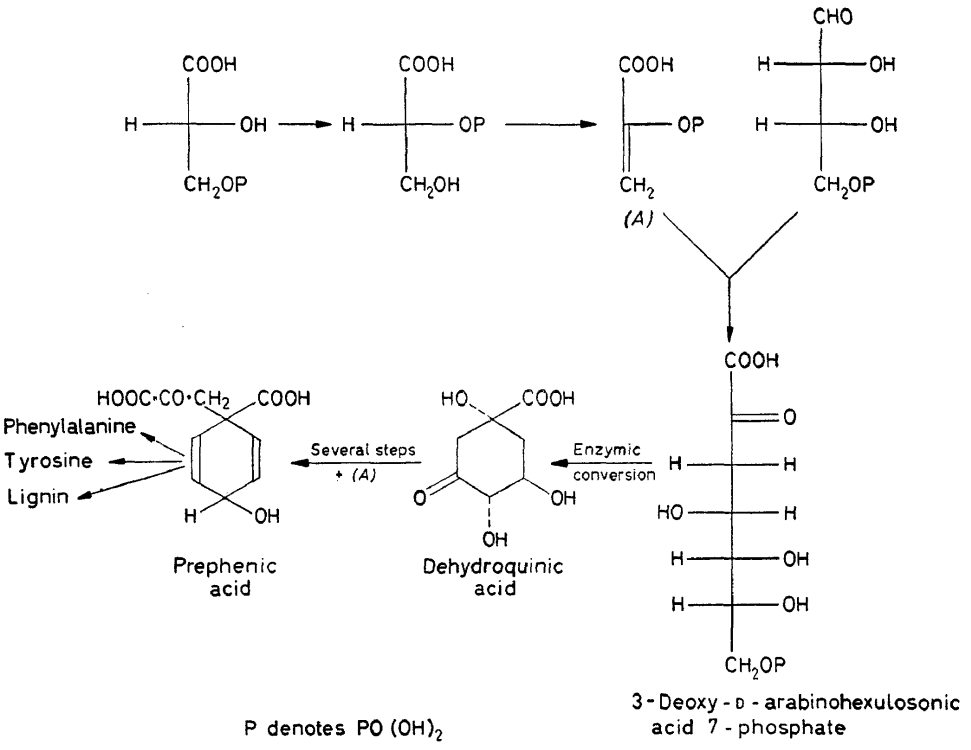


Figure 12. Biosynthesis of phenylalanine, tyrosine and (?)lignin

It will be noted that D-glycerose-3-phosphate and D-erythrose-4-phosphate are intermediate in an aldol-like condensation which results in the formation of a 3-deoxy-2-keto-D-arabinohexulosonic acid which then undergoes conversion to an intermediate cyclohexane derivative[31].

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