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I. LITERATURE REVIEW

General

Dulcitol (galactitol) is a hexitol belonging to the class of carbohydrates known as polyols, sugar alcohols, or glycitols. In recent years the knowledge of polyol metabolism in microorganisms has increased considerably although little is yet known about the biosynthesis and intermediary metabolism of these compounds in plants. Evidence has also accumulated showing that polyols may be normal intermediates in the intermediary metabolism of mammals (1). Polyols have also been implicated in pathological conditions in mammals (2,3).

Table I lists some polyols, in order of increasing chain length, which occur in many varied organisms (4).

TABLE I

Natural Occurrence of the Acyclic Polyols

<u>Polyol</u>	<u>Source</u>
Erythritol	Algae Grasses Lichens Fungi Residue from fermented molasses Urine of normal humans
D-Arabitol	Lichens Fungi Uredospores of wheat-germ rust Residue from fermented molasses
L-Arabitol	Urine of pentosuric humans
DL-Arabitol	Urine of normal humans
Ribitol	In riboflavin and its derivatives throughout nature Bacteria (in cell walls and in cytidine diphosphate ribitol of gram-positive bacteria, and in pneumococcal polysaccharide) Plants Honeydews of wax scale insects (Ceroplastes)*
Xylitol	Rat lens (after D-xylose feeding)
Sorbitol	Algae (seaweed) Fungi (yeast) Higher plants of many types, especially fruit trees Silkworm (diapause egg of <u>Bombyx</u> silkworm) Animals (fetal blood, seminal vesicles and plasma; the lens of the alloxan-diabetic or D-xylose-fed rat)

2(a)

TABLE I

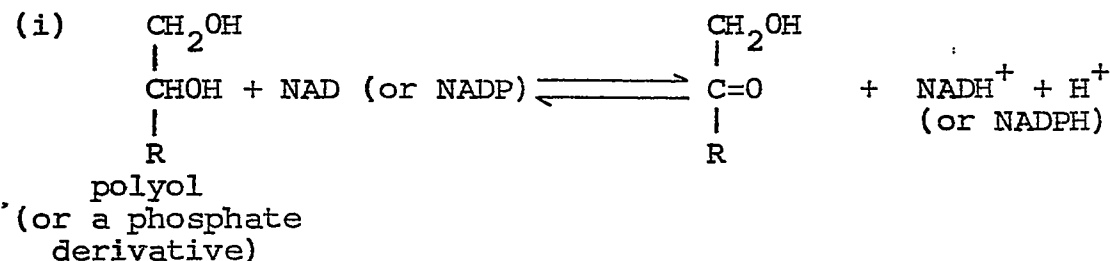
Natural Occurrence of the Acyclic Polyols (continued)

<u>Polyol</u>	<u>Source</u>
D-mannitol	Bacteria (<u>L. arabinosus</u>) Algae (eg. all brown seaweeds) Grasses Lichens Higher plants of many types
L-Iditol	Berry of mountain ash (<u>Sorbus aucuparia</u>)
Dulcitol	Algae (seaweed) Fungi (yeast) Higher plants Honeydew of <u>Aphis euonymi</u> Fabr.*
Perseitol	Avocado Algae
Volemitol	Lichens Fungi (mushrooms) Plants
B-Sedohepitol	Plant (<u>Sedum spectabile</u>)
D-Erythro-D-galacto-octitol	Avocado

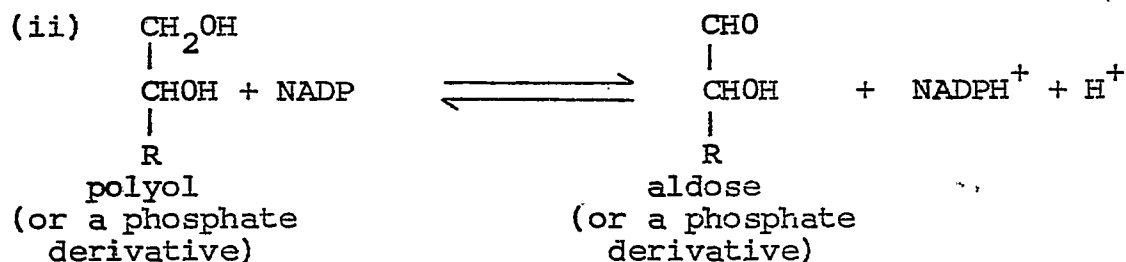
* Polyol may be of dietary origin

It has been generally believed that polyols originate from sugars by reduction, and the first step in the utilization of these compounds is via their oxidation. In many plants which contain a polyol, the corresponding ketose is also found.

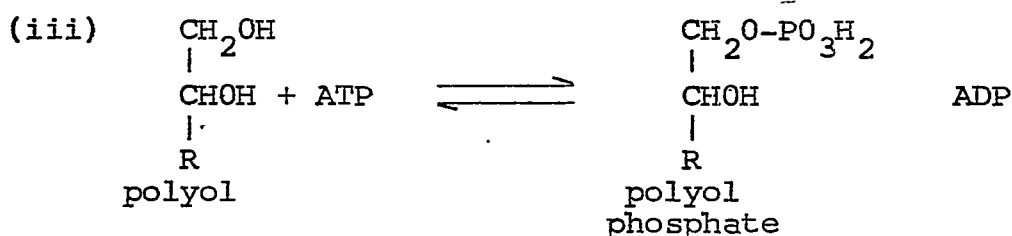
Enzymes which utilize polyols as substrates have been found to be widely distributed. The reactions catalyzed by these enzymes are of three known types:



An example of reaction (i) is the conversion of allitol to allulose in Itea ilicifolia under respiratory conditions (5) and the conversion of L-arabitol to L-ribulose in several species of plants (6).



Although the formation of polyols by the reduction of an aldose is very common (4), very little information is available which shows the reverse reaction of the metabolism of a polyol by direct conversion to an aldose as illustrated by reaction (ii).



The metabolism of mannitol to fructose in celery has been shown to take place via phosphorylated intermediates (7,8) as shown by reaction (iii).

These three different reactions may not be the only reactions by which polyols are transformed. Indeed, the fate of dulcitol in *E. japonica*, as will be shown later, appears to be different than any of the three reactions mentioned above.

The fate of the polyol can be shown to be dependant upon the specificity of the enzymes (dehydrogenases or reductases) from individual organisms, whether a coenzyme is needed in the transformation of the polyol, and if so, the specific coenzyme required and whether the polyol has been phosphorylated or not. These factors can be illustrated by Table II (4).

TABLE II

Enzyme Catalyzed Interconversions of Polyols and Sugars

<u>Polyol</u>	<u>Sugar</u>	<u>Coenzyme and Source of Enzyme</u>
<u>Polyol Ketose</u>		
D-Arabitol	D-Xylulose	Nil, <u>A. suboxydans</u> ; NAD, <u>A. aerogenes</u> NADP, <u>A. suboxydans</u>
L-Arabitol	L-Ribulose	NAD, <u>P. chrysogenum</u>
L-Arabitol	L-Xylulose	NAD, <u>P. chrysogenum</u>
Ribitol	D-Ribulose	NAD, mammals; <u>A. aerogenes</u>
Ribitol	L-Ribulose	Nil, <u>A. suboxydans</u>
Xylitol	D-Xylulose	NAD, mammals; <u>P. chrysogenum</u> ; <u>C. albicans</u>
Xylitol	L-Xylulose	NAD, <u>Ps. fluorescens</u> ; <u>A. aerogenes</u> NADP, guinea-pig mitochondria; <u>P. chrysogenum</u>
Allitol	D-Allulose	NAD, liver
Allitol	L-Allulose	Nil, <u>A. suboxydans</u>
Dulcitol	D-Tagatose	NAD, <u>Ps. fluorescens</u>
Sorbitol	D-Fructose	NAD, mammals; <u>Ps. fluorescens</u> ; <u>C. utilis</u> ; <u>A. suboxydans</u>
Sorbitol	L-Sorbose	Nil, <u>A. suboxydans</u> ; NAD, <u>C. utilis</u> ; NADP, <u>A. suboxydan</u>
L-Glucitol	L-Fructose	NAD, <u>Ps. fluorescens</u>
D-Iditol	D-Sorbose	NAD, <u>Ps. fluorescens</u>
L-Iditol	L-Sorbose	NAD, mammals; <u>Ps. fluorescens</u>
D-Mannitol	D-Fructose	Nil, <u>A. suboxydans</u> ; NAD, <u>A. suboxydans</u> , <u>L. brevis</u> , <u>A. aerogenes</u> , <u>C. utilis</u> , brewer's yeast; NADP, <u>A. suboxydans</u> , <u>A. aceti</u>
D-Talitol	D-Allulose	NAD, <u>Ps. fluorescens</u>
D-Talitol	D-Tagatose	Nil, <u>A. suboxydans</u>

5(a)

TABLE II

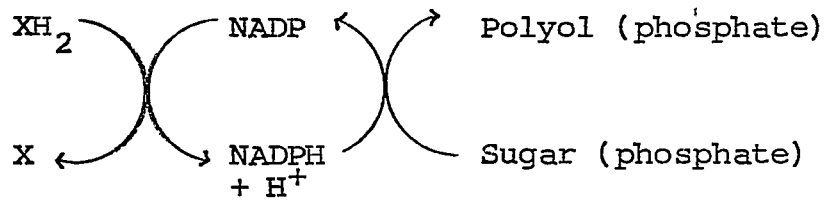
Enzyme Catalyzed Interconversions of Polyols and Sugars (continued)

<u>Polyol</u>	<u>Sugar</u>	<u>Coenzyme and Source of Enzyme</u>
<u>Polyol-aldose</u>		
L-Arabitol	L-Arabitol	NADP, <u>P. chrysogenum</u>
Xylitol	D-Xylose	NADP, mammals, <u>P. chrysogenum</u> , <u>C. albicans</u>
Sorbitol	D-Glucose	NADP, mammals
<u>Polyol phosphate-ketose phosphate</u>		
D-Galactitol 6-phosphate	D-Tagatose 6-phosphate	NAD, <u>E. coli</u>
Sorbitol 6-phosphate	D-Fructose 6-phosphate	NAD, <u>E. coli</u> , <u>L. casei</u>
D-Mannitol 6-phosphate	D-Fructose 6-phosphate	NAD, <u>Diplococcus pneumoniae</u> ; <u>E. coli</u> , <u>Piricularia oryzae</u>
<u>Polyol phosphate-aldose phosphate</u>		
D-Ribitol-5- phosphate	D-Ribose-5- phosphate	NADP, silkworm
D-Sorbitol-6- phosphate	D-Glucose-6- phosphate	NADP, silkworm

The function of polyols also varies from one organism to another. The roles of polyols which have been demonstrated to date can be summarized as follows (9):

TABLE III
Roles of Polyols

- (1) Structural; eg. ribitol teichoic acids of bacteria (10)
- (2) Interconversion of sugars; eg. transformation of glucose-fructose via sorbitol in seminal vesicles (11, 12)
- (3) Coenzyme regulation
- (4) Storage of reducing power:



- (5) Storage of carbohydrates
- (6) Translocation of carbohydrates
- (7) Osmoregulation

The information given in Tables II and III demonstrates that the group of compounds classed as polyols, although similar chemically, show great diversity in synthesis, metabolism and function.

Botanical Occurrence of Dulcitol

Dulcitol has been shown to be present in higher plants, algae and fungi but has not been demonstrable in lichens (9). Thirteen acyclic polyols have been found in higher plants, but unlike some polyols (eg. mannitol), which have been found in over fifty plant families, dulcitol is mainly characteristic of two families, Celastraceae and Hippocrateaceae (13,14).

Plouvier (15,16) in 1949 showed the presence of dulcitol in the branches, barks and leaves of numerous species belonging to the Celastraceae. Included in the list of species belonging to the genus Euonymus are: E. atropurpureus, E. europaeus, E. alatus, E. wilsonii, E. bungeanus, E. americanus, E. latifolius, E. radicans and E. yedoensis. Among the species of Celastrus which contained dulcitol were the following: Celastrus acuminatus, C. gemmata, C. paniculatus, C. scandens, C. buxifolius, C. angulatus, C. orbiculatus, C. punctatus, C. rosthornianus and C. strigillosus. The presence of dulcitol is also indicated in two other members of Celastraceae, namely, Catha edulis and Maytenus boaria. More recently, Plouvier (14) has reported finding dulcitol in

several species of Hippocrateaceae including Loeseneriella ritschardii and Hippocratea indica. This author has also reported the presence of dulcitol in Cassytha filiformis of the Lauraceae, Cuscuta reflexa of the Convolvulaceae, and in some species of Melampyrum in the Scrophulariaceae.

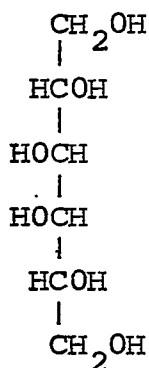
The concentration of dulcitol varies from one species to another and from one plant part to another (eg. stems, roots, barks, leaves). Schradie (17) reported that the leaves of E. japonica contained 2.68 per cent of dulcitol based on the dry weight of the leaves. E. atropurpurea was reported by Bliss and Ramstad (18) to contain 2 per cent of the dry weight in the root barks. The dulcitol concentration of the powdered stem of S. chinensis (19) and in the stems of E. pellucidifolius (20) has been reported to comprise 0.06 per cent of the stem bulk.

Evidence (9) has been obtained which indicates that the differences in concentrations of polyols in different parts of the same plant may be due to the translocation of the polyol in the phloem from the point at which synthesis occurred.

Physical and Chemical Properties of Dulcitol

Physical

Using the Fischer representation, the structure of dulcitol can be depicted by the following structure:



The representation shows that dulcitol has a meso structure and, therefore, has no specific rotation. This property can be used to distinguish dulcitol from other polyols with similar chemical properties such as sorbitol or mannitol.

Dulcitol is a white crystalline solid with a melting point of 188-189° (21). It can easily be crystallized from a mixture of methanol and water (4:1) (18). Dulcitol is soluble to the extent of one gram in 30 ml distilled water at 25° and one gram in two ml boiling water. Dulcitol is somewhat soluble in alcohols, but this solubility decreases rapidly with increasing chain length. Like most carbohydrates, dulcitol is soluble in pyridine. This solubility plus the basic properties of pyridine make this solvent an excellent media in which the trimethylsilyl ethers of carbohydrates can be prepared for separation by gas chromatography (22).

Chemical

The only functional group present in dulcitol and other polyols is the hydroxy moiety. The chemical properties

of dulcitol can therefore be separated into two classes. In the first class are those reactions in which the hydroxyl groups react separately, while the reactions of the second class are those in which two or more hydroxyl groups are involved in a reaction. The reactions of the first group include esterification, etherification and certain oxidations. A reaction such as the periodate oxidation serves as an example of the reactions of the latter type.

Etherification was an important reaction in the study of dulcitol because of the ability to separate trimethylsilyl ethers of carbohydrates by gas chromatography. Paper chromatography and thin layer chromatography have limited application in the separation of hexitols from hexaaldoses and hexaketoses because the R_f values are very similar.

II. INTRODUCTION TO THE PROBLEM

The leaves of E. japonica were found to contain 2.68 per cent dulcitol on a dry weight basis (17). The presence of dulcitol has been demonstrated in several families and in several species belonging to the genus Euonymus (14,15). Therefore, in the biological development of these plants, some change must have occurred resulting in the formation of a pool of this polyol.

Initially, a pathway for the biosynthesis of dulcitol was postulated. The primary step was thought to involve the synthesis of glucose-1-P from CO_2 . Ginsburg (23) and Neufeld et al. (24) have shown the presence of UDP-D-glucopyrophosphorylase, UDP-D-galactose-4-epimerase, and UDP-D-galactosepyrophosphorylase in plant tissues which synthesize galactose. The synthesis of dulcitol was therefore proposed to proceed via the following pathway as shown in Figure 1 (25).

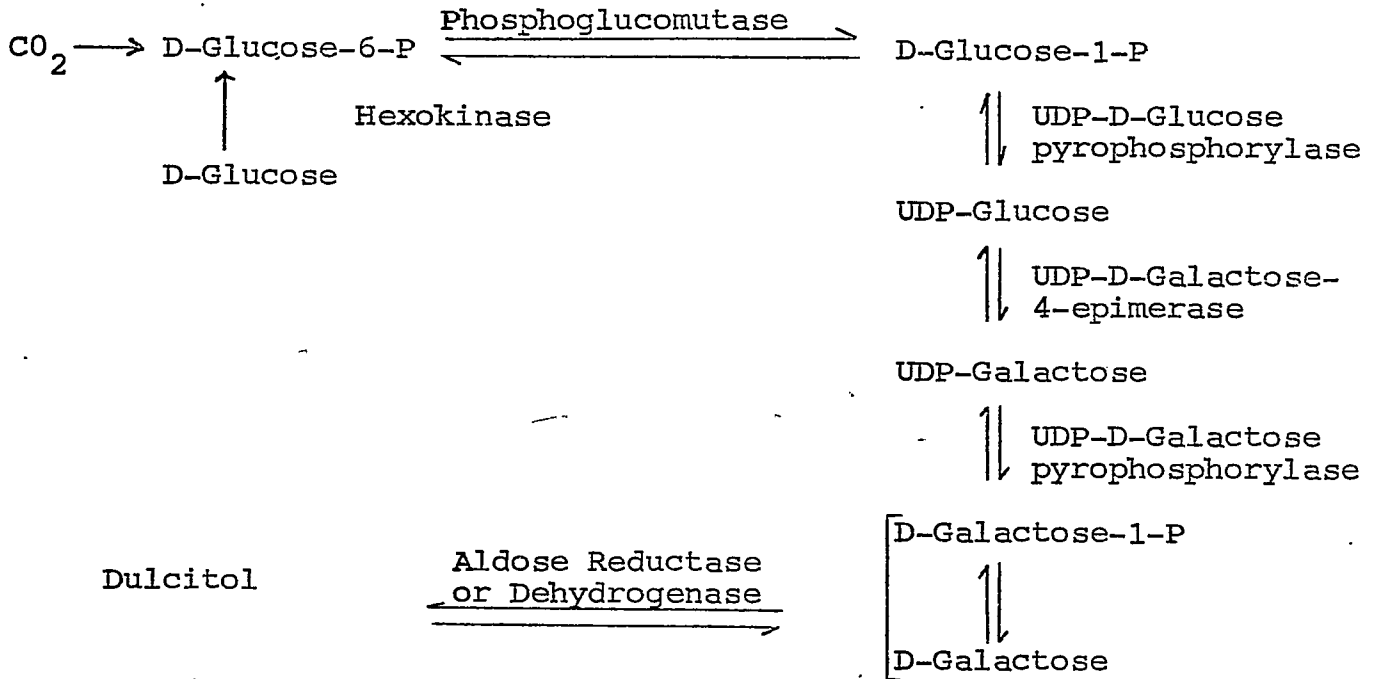


Figure 1: Hypothetical Pathway for Dulcitol Biosynthesis

