Plant Physiology, January 1999, Vol. 119, pp. 191–197, www.plantphysiol.org © 1999 American Society of Plant Physiologists

Metabolism of D-*Glycero*-D-*Manno*-Heptitol, Volemitol, in Polyanthus. Discovery of a Novel Ketose Reductase[1]

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Volemitol (D-*glycero*-D-*manno*-heptitol, α-sedoheptitol) is an unusual seven-carbon sugar alcohol that fulfills several important physiological functions in certain species of the genus Primula. Using the horticultural hybrid polyanthus (*Primula* × *polyantha*) as our model plant, we found that volemitol is the major nonstructural carbohydrate in leaves of all stages of development, with concentrations of up to 50 mg/g fresh weight in source leaves (about 25% of the dry weight), followed by sedoheptulose (D-*altro*-2-heptulose, 36 mg/g fresh weight), and sucrose (4 mg/g fresh weight). Volemitol was shown by the ethylenediaminetetraacetate-exudation technique to be a prominent phloem-mobile carbohydrate. It accounted for about 24% (mol/mol) of the phloem sap carbohydrates, surpassed only by sucrose (63%). Preliminary $^{14}CO_2$ pulse-chase radiolabeling experiments showed that volemitol was a major photosynthetic product, preceded by the structurally related ketose sedoheptulose. Finally, we present evidence for a novel NADPH-dependent ketose reductase, tentatively called sedoheptulose reductase, in volemitol-containing *Primula* species, and propose it as responsible for the biosynthesis of volemitol in planta. Using enzyme extracts from polyanthus leaves, we determined that sedoheptulose reductase has a pH optimum between 7.0 and 8.0, a very high substrate specificity, and displays saturable concentration dependence for both sedoheptulose (apparent K_m = 21 mM) and NADPH (apparent K_m = 0.4 mM). Our results suggest that volemitol is important in certain *Primula* species as a photosynthetic product, phloem translocate, and storage carbohydrate.

Alditols (sugar alcohols or acyclic polyols) may be chemically described as reduction products of aldose or ketose sugars. The most prevalent plant alditols are the hexitols sorbitol, mannitol, and galactitol. However, as many as 17 different alditols occur naturally in higher plants (for review, see Bieleski, 1982; Lewis, 1984; Loescher and Everard, 1996). The lesser-known alditols are often restricted in their occurrence but still fulfill important functions in those plants where they do occur. Volemitol (Fig. 1) is a good example of a less common but important alditol. This seven-carbon sugar alcohol seems to be confined to certain sections of the genus *Primula*, so much so that it has been suggested as a useful chemotaxonomical marker (Kremer, 1978). Very little is known about the physiology and metabolism of volemitol in primulas, except that it was an early photosynthetic product in cowslip (*Primula veris*) and oxslip (*Primula elatior*) (Kremer, 1978).

The physiological roles of alditols are manifold and largely resemble those of disaccharides and oligosaccharides. They include photosynthetic assimilation, translocation and storage of carbon, and reducing power, as well as protection against different types of stresses (for review, see Bieleski, 1982; Lewis, 1984; Loescher and Everard, 1996; Stoop et al., 1996). The biosynthetic pathways of the hexitols sorbitol (glucitol), mannitol, galactitol (dulcitol), and the pentitol ribitol have been established in higher plants. They generally use NADPH as a hydrogen donor and aldose phosphate as a hydrogen acceptor, in concert with the corresponding phosphatases. One exception might be galactitol, which was suggested to be formed directly from unphosphorylated Gal (and NADPH) (Negm, 1986). Although all foliar alditols are thought to be phloem-mobile (Lewis, 1984), this has only been demonstrated for sorbitol, mannitol, and galactitol (Zimmermann and Ziegler, 1975; Davis and Loescher, 1990; Moing et al., 1992; Flora and Madore, 1993).

To expand our knowledge of alditol metabolism in higher plants beyond that of hexitols, we studied the carbohydrate metabolism of polyanthus (*Primula* × *polyantha*). This popular horticultural hybrid of primrose (*Primula vulgaris*), oxlip, and cowslip (Mabberley, 1997) was chosen because preliminary experiments showed that its volemitol content is very high, similar to that of the wild-type species, and because it may be easily grown both outdoors and indoors.

We give a general overview on volemitol metabolism in polyanthus with special emphasis on the role of volemitol in plant development and phloem transport. We also report on a novel enzyme, a NADPH-dependent ketose reductase, which forms volemitol by the reduction of sedoheptulose.

MATERIALS AND METHODS

Chemicals

Biochemicals were obtained from Sigma or Fluka unless stated otherwise in the text. The following rare carbohydrates, which are not commercially available, were generous gifts: volemitol from R. Honegger (University of Zurich, Switzerland), P. Köll (University of Oldenburg, Germany), S.J. Angyal (University of New South Wales,

[1] This work was supported by the Swiss National Foundation.

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Abbreviations: PAD, pulsed amperometric detection; sedoheptulose, D-*altro*-2-heptulose; volemitol, D-*glycero*-D-*manno*-heptitol, α-sedoheptitol.

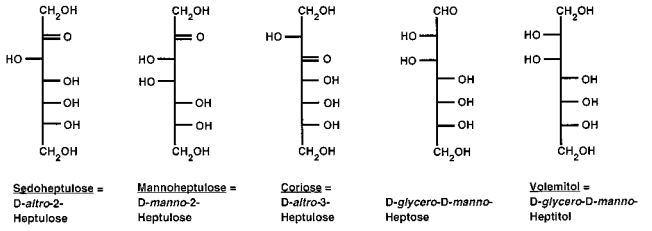

Figure 1. Fischer projections of volemitol and its four structurally related seven-carbon sugars. Nomenclature follows that of Collins (1987); trivial names are underlined.

Sydney, Australia), and T. Okuda (Okayama University, Japan); sedoheptulose from P. Köll, coriose from T. Okuda, and D-*glycero*-D-*manno*-heptose from S.J. Angyal. Sedoheptulose was also isolated from desalted cryo sap of *Sedum album* leaves and coriose from *Coriaria japonica* leaves harvested from the University of Zurich Botanical Garden by HPLC purification on a Ca column (see below).

Plant Material and Growth

Polyanthus (*Primula* × *polyantha* Hort.) plants were grown from seed (Kraft, Nesslau, Switzerland) in a mixture of commercial standard soil and sand (10:1; v/v) in a greenhouse. After about 40 d the plantlets had two to four leaves and were transferred to plastic pots. The average day and night temperatures in the greenhouse were 25°C ± 3°C and 12°C ± 2°C, respectively, and the RH was 55% ± 5%/78% ± 6% (day/night). In winter illumination was supplemented with incandescent lamps (200 μmol m^{-2} s^{-1}). For some experiments, we used polyanthus plants grown outdoors at the University of Zurich Botanical Garden.

Enzyme Extraction and Assays

Up to 1 g of freshly harvested plant material was finely chopped with a razor blade and extracted on ice in a glass homogenizer containing 5 volumes of extraction buffer (20 mM Hepes/KOH, pH 7.5, 5 mM DTT, 5 mM $MgCl_2$, 2% [w/v] PEG-20,000, and 2% [w/v] PVP K30). The homogenate was centrifuged at 20,000*g* at 4°C for 20 min. The supernatant was immediately centrifuge-desalted through Sephadex G-25 preequilibrated with the appropriate assay buffer (Helmerhorst and Stokes, 1980). This fraction was designated as desalted crude enzyme extract. In preliminary experiments the addition of 0.1% (w/v) Triton X-100 in the extraction buffer and/or freezing leaves in liquid N_2 prior to extraction did not have any demonstrable effect on the sedoheptulose reductase activity.

Sedoheptulose reductase activity was measured either spectrophotometrically by monitoring the continuous oxidation of NADPH at 340 nm or chromatographically using a fixed-time assay based on the quantification of the end product, volemitol, by HPLC-PAD. The spectrophotometric assay mixture (1 mL) contained 25 mM sedoheptulose, 1 mM NADPH, extraction buffer, and 500 μL of desalted crude enzyme extract. The chromatographic assay mixture (100 μL) was composed similarly but contained only 50 μL of enzyme extract. Boiling in a water bath for 5 min stopped the assay. After centrifugation at 14,000*g* at 4°C for 3 min, the reaction mixture was desalted and volemitol was determined by HPLC-PAD as described below. One unit of sedoheptulose reductase activity corresponds to the amount of enzyme that catalyzed the formation of 1 μmol of product (NADP or volemitol) per minute under the assay conditions chosen.

Carbohydrate Extraction and Analysis

Water-soluble carbohydrates were extracted by the cryo-sap method and immediately desalted and liberated from phenols by the mixed-bed microfiltration method described previously (Bachmann et al., 1994). Carbohydrates were routinely determined by HPLC-PAD using cation-moderated partitioning chromatography on a Ca-carbohydrate column (300 × 7.8 mm; model SS-100, Benson Polymeric, Reno, NV) with 50 mg L^{-1} Ca-EDTA as the eluant (at 0.6 mL min^{-1} and 90°C). For additional identification of the peaks obtained by cation chromatography, anion-exchange chromatography using a CarboPak MA1 column (250 × 4 mm) fitted with a CarboPak MA1 guard column (50 × 4 mm), both from Dionex (Sunnyvale, CA), with 0.6 M NaOH as the eluant (at 0.4 mL min^{-1}, at room temperature) and PAD was used. The HPLC systems used have been described in detail previously (Keller and Ludlow, 1993; Bachmann et al., 1994). Quantification of the water-soluble carbohydrates was by the external standard method using authentic standards. Because sedoheptulose cannot be obtained in crystalline form, it was quantified using the response factor of mannoheptulose. For the study on the ontogenetic pattern of nonstructural leaf carbohydrates (Fig. 3), we used lyophilized leaf tissue and followed exactly the procedure described for pigeon pea (Keller and Ludlow, 1993).

Phloem Exudation

Phloem exudates were obtained by the EDTA method described previously (Bachmann et al., 1994). The three youngest fully expanded leaves of 3-month-old plants were excised and their petioles were recut under a collection solution of 5 mM phosphate buffer and 5 mM EDTA, pH 7.5. The petioles were quickly placed into small plastic dishes (3.2 × 2.6 × 1.6 cm) containing 5 mL of fresh collection solution per leaf. About 0.6 mm of the petiole was immersed in the solution. The samples were kept in an airtight, translucent plexiglass (light incubation) or black, plastic (dark incubation) chamber lined with moist filter paper to maintain high humidity. At the times indicated (usually in 3-h intervals), 4.5 mL of the exudation solution was withdrawn and immediately replaced by the same volume of new collection solution. Exudation solutions were desalted and analyzed by HPLC-PAD as described above. The amounts of exuded carbohydrates are expressed as micromoles per gram fresh weight of the leaves at the beginning of the experiment.

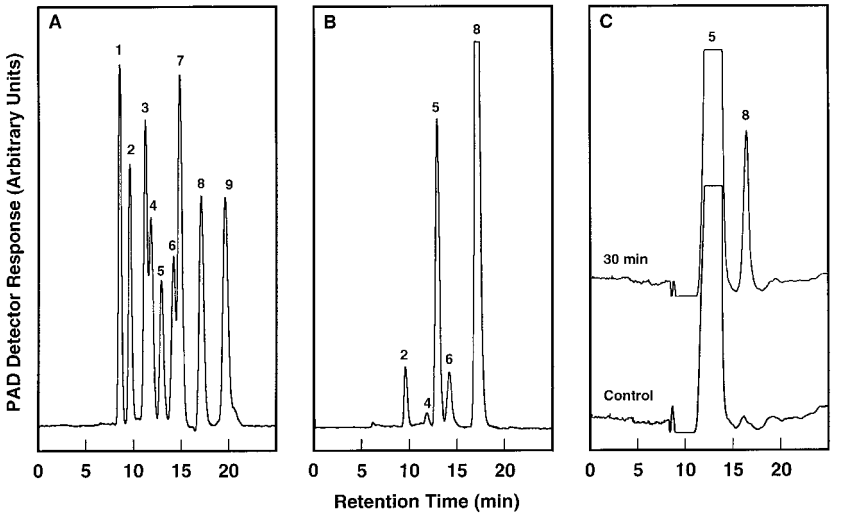

Figure 2. HPLC chromatograms of a carbohydrate standard (A), water-soluble carbohydrates (B), and sedoheptulose reductase reaction products (C) of fully mature polyanthus leaves. Carbohydrates were extracted by the cryo-sap method and determined by HPLC-PAD. The sedoheptulose reductase assay was performed as described in "Materials and Methods." The control in C was a boiled enzyme blank incubated for 30 min. 1, Raffinose; 2, Suc; 3, galactinol; 4, Glc; 5, sedoheptulose; 6, Fru; 7, *myo*-inositol; 8, volemitol; 9, perseitol.

In preliminary experiments the optimum composition of the collection solution was determined; EDTA concentration was varied between 0 and 20 mM, and pH was varied between 6.0 and 8.0. Virtually no carbohydrates exuded when tap water was used as the collection solution. When the EDTA-containing collection solution was replaced with tap water after 4 h of EDTA exudation, exudation slowed and came to a complete halt within the next 19 h.

RESULTS

Volemitol Occurrence

Water-soluble carbohydrates were analyzed by HPLC-PAD (Fig. 2, A and B). Table I shows that the two main soluble carbohydrates detected in leaves of polyanthus, oxslip, and *Primula juliae* (all members of the section *Primula*) were volemitol and sedoheptulose; Suc, Glc, and Fru were also present but in much smaller concentrations. In leaves of *Primula denticulata* (section *Denticulata*), Suc, Glc, and Fru but not volemitol (or any other known alditol) and only traces of sedoheptulose were found. In a small survey, the presence of volemitol was further confirmed for leaves of an additional two members of the section *Primula*, cowslip and primrose; its absence was observed in leaves of 13 *Primula* species belonging to the sections *Auriculastrum*, *Aleuritia*, *Proliferae*, *Sikkimensis*, *Oreophlomis*, and *Muscarioides* (data not shown). Our results on volemitol occurrence are identical to those published by Kremer (1978), and confirm the importance of volemitol as a chemotaxonomic marker of the genus *Primula*.

Leaf volemitol concentrations of plants harvested in spring were high, ranging from 23 to 50 mg g^{-1} fresh weight (13%–25% of the dry weight) (Table I). Sedoheptulose concentrations were also quite high, but generally a bit lower than volemitol concentrations, ranging from 14 to 36 mg g^{-1} fresh weight (7%–18% of the dry weight). The volemitol concentrations of mature leaves of wild-grown polyanthus plants were about twice as high in March (flowering) as in June (postflowering), indicating possible seasonal variations (data not shown); and volemitol concentrations of leaves of warm-grown greenhouse plants were generally lower (by a factor of 2–3) than those of cold-grown greenhouse or wild-grown spring plants, also indicative of seasonal variation (Fig. 3; F. Keller, unpublished observations).

Changes of Leaf Nonstructural Carbohydrate Concentrations during Development

To study the ontogenetic pattern of leaf carbohydrate concentration, we analyzed a 4-month-old greenhouse-grown (25°C/12°C day/night temperatures) plant that had

Table I. *Main soluble carbohydrates and sedoheptulose reductase activities of leaves of some Primula species*

Youngest fully mature leaves were harvested in March from plants grown under natural conditions in the University of Zurich Botanical Garden. Data are means ± SE from three plants.

Species	Soluble Carbohydrate					Sedoheptulose Reductase Activity
	Volemitol	Sedoheptulose	Suc	Glc	Fru	
	mg g^{-1} fresh wt					*milliunits g^{-1} fresh wt*
P. × polyantha	50.1 ± 10.0	35.7 ± 11.2	4.1 ± 0.3	1.0 ± 0.1	6.2 ± 1.7	42.1 ± 6.8
P. elatior	23.4 ± 2.0	14.4 ± 1.9	6.7 ± 1.2	3.9 ± 0.3	3.5 ± 0.8	25.5 ± 11.3
P. juliae	24.5 ± 3.1	27.7 ± 3.5	14.1 ± 0.8	2.2 ± 0.9	5.8 ± 0.4	21.0 ± 5.3
P. denticulata	nd[a]	0.4 ± 0.1	4.1 ± 0.6	3.4 ± 0.2	7.2 ± 0.8	nd

[a] nd, Not detected.

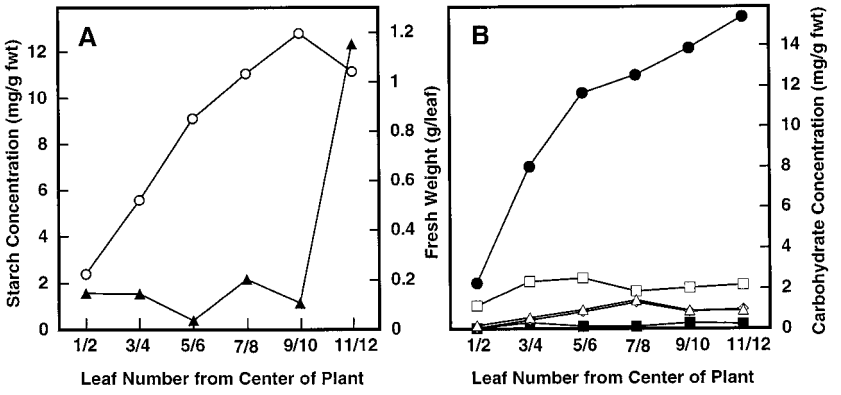

Figure 3. Changes of fresh weight and nonstructural carbohydrate concentrations in polyanthus leaves during development. The plant analyzed was greenhouse-grown (25°C/15°C; day/night), 4 months old (postflowering), and had reached its 12-leaf stage. The leaves were harvested about 3 h into the light period. The carbohydrates were determined by the HPLC-PAD method described in "Materials and Methods." Two successive leaves were combined for analysis. A, Leaf fresh weight (fwt) (○) and starch (▲). B, Volemitol (●), sedoheptulose (□), Suc (■), Glc (◇), and Fru (△).

reached its 12-leaf stage (postflowering). The youngest leaves analyzed (leaves 1 and 2) were in the center of the rosette, still partly unfolded and pale green. Leaves 7 and 8 were fully expanded and totally green, whereas the outer leaves (11 and 12) were slightly smaller and starting to senesce. Fig. 3A shows the gradual increase of the leaf biomass with age, which reached a maximum in leaves 9 and 10.

Volemitol was the dominating carbohydrate in leaves at all stages of development, gradually increasing from 2.3 mg g^{-1} fresh weight in the youngest leaves (1 and 2) to 15.4 mg g^{-1} fresh weight in the oldest leaves (11 and 12), and contributing between 43% and 73% to the totality of the nonstructural carbohydrates (Fig. 3B). The second most prominent soluble carbohydrate was sedoheptulose. Unlike volemitol, its concentration remained constant with development (between 1.1 and 2.5 mg g^{-1} fresh weight). The common sugars Suc, Glc, and Fru were minor components. Starch was also a minor carbohydrate in young to mature leaves but became prominent in the oldest two leaves (12.3 mg g^{-1} fresh weight; Fig. 3A).

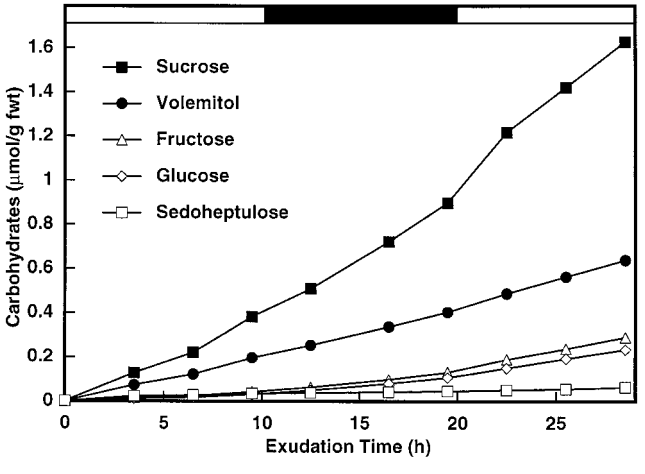

Figure 4. Diurnal pattern of soluble carbohydrates found in phloem exudates of mature leaves of 3-month-old polyanthus plants. Exudation was performed with excised leaves placed into a 5 mM EDTA solution at pH 7.5. The carbohydrates were determined by the HPLC-PAD method described in "Materials and Methods." Black bar indicates nighttime. fwt, Fresh weight.

Similar patterns of nonstructural carbohydrate distributions within polyanthus rosettes were observed in plants of a variety of ages (from the 2-leaf to the 15-leaf stage) and growing conditions (cold/warm). Volemitol concentrations were always low in sink leaves and high in source leaves, whereas no distinct concentration gradients were apparent for sedoheptulose. The absolute values of the carbohydrate concentrations, however, varied greatly with the history of the plants (F. Keller, unpublished results).

Phloem Mobility of Volemitol

The diurnal course of carbohydrate exudation from mature polyanthus leaves is shown in Figure 4. The two main phloem-mobile carbohydrates were Suc and volemitol. No clear diurnal fluctuations in the levels of these two carbohydrates were seen, with the possible exception of a transient increase in the Suc level during the first hours of the light period. Minor components found in the phloem exudates were Fru and Glc, with traces of sedoheptulose also seen. Because the two hexoses were present in about equal molar amounts and they are generally not phloem mobile, we attribute their origin to invertase products of translocated Suc rather than the phloem. Comparing average exudation rates calculated from the data shown in Figure 4, of the 106 nmol (31.5 μg) of carbohydrates that were exuded per gram of leaf fresh weight per hour, about 58% were found in Suc and 21% in volemitol.

In a similar experiment in which the phloem exudates were collected over a 6-h period, the average exudation rate was 143 nmol (41.5 μg) carbohydrate g^{-1} fresh weight, and Suc and volemitol made up 67% and 27%, respectively, of the total carbohydrates exuded (also calculated on a molar basis). The carbohydrate composition of the phloem is thus clearly distinct from that of the whole-leaf tissue, where volemitol was the main component (71% of the total soluble leaf carbohydrate on a molar basis), followed by sedoheptulose (14%), and Suc was a minor component (2%) (data not shown, see Table I).

Sedoheptulose Reductase, a Novel Enzyme

HPLC revealed that when a desalted crude enzyme extract of mature polyanthus leaves was incubated in the

presence of sedoheptulose and NADPH, volemitol was formed (Fig. 2C). Under the assay conditions chosen, the reaction was linear for at least 30 min, and doubling the amount of enzyme also doubled the sedoheptulose reductase activity (data not shown). Similar reaction rates were obtained when the oxidation of NADPH to NADP was monitored spectrophotometrically at 340 nm. When the pH of the reaction mixture was varied between 4.0 and 9.0 using the four buffer systems Mes-KOH (pH 5.5–6.5), Hepes-KOH (pH 6.5–7.5), Tricine-KOH (pH 7.5–9.0), and McIlvaine (pH 4.0–7.0), a broad pH optimum between 7.0 and 8.0 was determined (data not shown). The temperature dependence of sedoheptulose reductase showed a maximum at 45°C (data not shown). Sedoheptulose reductase activity decreased slowly with decreasing temperature and retained 44% of its maximum activity, even at 14°C. Sedoheptulose reductase showed saturable concentration dependence for both the substrate, sedoheptulose (Fig. 5A) with an apparent K_m value of 20.8 mM, and the cosubstrate, NADPH (Fig. 5B) with an apparent K_m value of 0.395 mM.

The specificity of sedoheptulose reductase was very high for both the cosubstrate and the substrate. Enzymatic sedoheptulose reduction was observed only with NADPH, not with NADH (at 1 mM) as the reductant (data not shown). Of the 14 different substrates tested, only 2 showed sedoheptulose reductase activity, sedoheptulose and D-xylulose. The survey was performed in triplicate under standard assay conditions, with different polyanthus enzyme samples containing sedoheptulose reductase activities between 30 and 100 milliunits g^{-1} fresh weight (as measured with sedoheptulose). No products were detected by the sensitive HPLC-PAD assay from the seven-carbon sugars, D-*glycero*-D-*manno*-heptose, mannoheptulose (D-*manno*-2-heptulose), coriose (D-*altro*-3-heptulose) (all potential precursors structurally related to volemitol [Fig. 1]), D-mannoheptose (D-*glycero*-D-*galacto*-heptose), and D-glucoheptose (D-*glycero*-D-*gulo*-heptose), the seven-carbon sugar phosphate, D-sedoheptulose-7-phosphate, and the six-carbon sugars, D-Glc, D-Fru, L-sorbose, D-psicose, D-tagatose, the pentulose D-ribulose, as well as the ubiquitous disaccharide, Suc. D-Mannoheptose, mannoheptulose, coriose, Glc, and Fru were also tested with the spectrophotometric assay for their ability to oxidize NADPH, and did not show any detectable activity. The sedoheptulose reductase activity with the pentulose D-xylulose was between 7% and 10% of the rate with sedoheptulose and was observed with both the chromatographic and spectrophotometric assay. On the Ca column, the product coeluted with arabinitol, which is one of the two expected xylulose reduction products (the other is xylitol).

To further test whether sedoheptulose is the precursor of volemitol synthesis in planta, preliminary photosynthetic $^{14}CO_2$ pulse-chase, radiolabeling experiments were performed with polyanthus leaf discs. After a 4-min pulse, most of the label of the neutral soluble fraction was in sedoheptulose, followed by Glc, Suc, and Fru; volemitol was still unlabeled. Some label started to appear in volemitol after a 10-min pulse. A more comprehensive study of the kinetics of sedoheptulose and volemitol production is under way.

Volemitol does not seem to be exclusively synthesized in green tissue. When different parts of 6-month-old polyanthus plants were analyzed for sedoheptulose reductase activity, leaf laminae, fleshy nongreen petioles, and roots all displayed comparable sedoheptulose reductase activities in the range of 50 to 100 milliunits g^{-1} fresh weight (data not shown).

Sedoheptulose reductase activity was only found in volemitol-containing *Primula* species (Table I). Leaf enzyme extracts of *P. denticulata*, which lack volemitol, did not show any measurable activity. Recombination experiments mixing extracts of leaves of polyanthus and *P. denticulata* gave no indication of inhibitors. Enzyme extracts of perseitol-containing and volemitol-lacking avocado leaves also did not show any sedoheptulose reductase activity (data not shown), supporting the conclusion that sedoheptulose reductase is typical for volemitol-containing tissue.

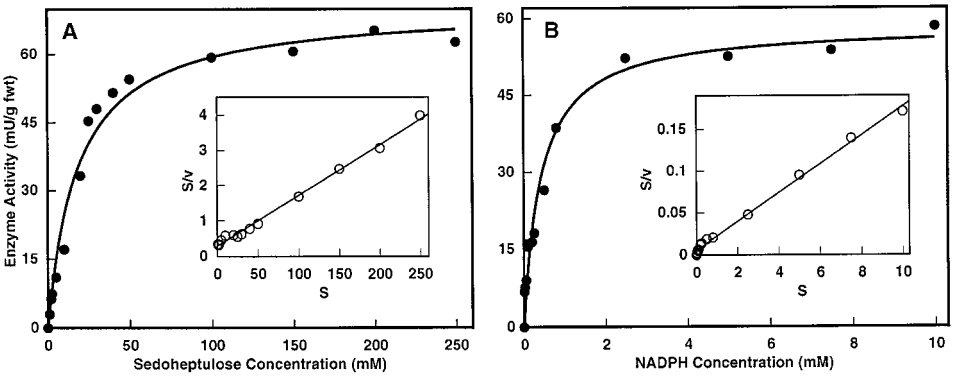

Figure 5. Effect of sedoheptulose (A) and NADPH (B) concentration on polyanthus leaf sedoheptulose reductase activity. Enzyme activities were determined by the HPLC-PAD method described in "Materials and Methods." The concentrations of NADPH and sedoheptulose were 1 and 25 mM in A and B, respectively. Insets show the Hanes-Woolf replots of the data. fwt, Fresh weight.

DISCUSSION

At the onset of our study, quantitative data on the occurrence of soluble carbohydrates in *Primula* species were scarce and based on somewhat outdated methods. At best, they had been obtained using one- or two-dimensional paper chromatography or TLC (e.g. Kremer, 1978; Beck and Hopf, 1990). In an attempt to upgrade to HPLC as the main analytical tool, we compared the performance of two HPLC columns to separate the carbohydrates expected to be present in primulas: a Ca-loaded ion-moderated partition column and an anion-exchange column at high pH. The Ca column proved to be suitable, reliable, and rapid for routine work (Fig. 1). It must be emphasized, however, that this column was not totally selective; for example, it was unable to separate sedoheptulose from Gal. Fortunately, by using the more selective (but slower and more delicate) MA1 anion-exchange column, we found that Gal is present only in trace amounts (more than 2 orders of magnitude lower than sedoheptulose) in *Primula* leaves. The identity of volemitol and sedoheptulose isolated from polyanthus leaves was confirmed by GC-MS (performed by Dr. A. Richter of the University of Vienna) and that of volemitol by ^{1}H-NMR spectroscopy (performed by Dr. P. Rüedi of the University of Zurich).

The volemitol concentration in *Primula* leaves (Table I) was similar to that of many other alditol plants (up to 25% of the dry weight; Dietz and Keller, 1997). Such a high concentration is clear evidence for the role of volemitol as a storage carbohydrate. As with other alditols, this implies that volemitol ideally combines storage of carbon and reducing power, because alditols are more reduced than sugars.

Of special interest was the occurrence of high concentrations of free sedoheptulose (up to 18% of the dry weight, Table I) and the invariable co-occurrence of sedoheptulose and volemitol in *Primula* leaves. Although sedoheptulose is universal in the plant kingdom, it is normally only found in the form of its mono- and bisphosphate esters, in the pentose phosphate cycles. Free sedoheptulose has rarely been described and seems to be confined to selected vascular plants, mostly of the Crassulaceae (Hegnauer, 1964; Okuda and Mori, 1974). Only speculations exist about the physiological roles of sedoheptulose; they include carbon storage and cryoprotection (Kull, 1967). In this paper we present evidence for a third role of sedoheptulose: as an important metabolic precursor. Therefore, the coexistence of volemitol and sedoheptulose in primula is not surprising.

The occurrence of volemitol in phloem exudates of polyanthus leaves is a new discovery. Although expected because hexitols such as mannitol (Davis and Loescher, 1990; Flora and Madore, 1993; Flora and Madore, 1996) and sorbitol (Moing et al., 1992, 1997) have been shown to be important phloem-mobile carbohydrates, no heptitols have actually been demonstrated to play the same role.

The molar Suc-to-volemitol ratio in the phloem sap of polyanthus was about 2.5, which is similar to the Suc-to-mannitol ratio in parsley phloem (Flora and Madore, 1996), but higher than the Suc-to-mannitol ratios reported for celery phloem (about 0.5; Daie, 1987). In the woody Rosaceae species peach, the molar Suc-to-alditol ratio in the phloem is even more in favor of the alditol sorbitol with values up to 0.25 (Moing et al., 1997). The implications of the high phloem Suc-to-volemitol ratio are not totally clear, but may be an indication that in polyanthus, the primary role of volemitol is storage and that of Suc is translocation.

A further physiological role of volemitol might be cryoprotection. In temperate regions, wild-growing polyanthus is found mainly in open grassy habitats, where it flowers in early spring (March/April), undergoes a phase of leaf expansion in early summer (June/July), and overwinters with young green leaves. The observed winterhardiness implies freezing tolerance of both the aboveground and the underground organs. Volemitol is a possible candidate as a cryoprotectant because it is present in both types of organs at considerable concentrations, and because nonreducing carbohydrates such as Suc, raffinose oligosaccharides, and various polyols have been suggested to be involved in the acquisition of freezing tolerance (for review, see Popp and Smirnoff, 1995; Keller and Pharr, 1996; Loescher and Everard, 1996). However, because no data in support of the role of volemitol as a cryoprotectant are available, it can only be considered an interesting possibility.

The finding that volemitol is synthesized by the action of a NADPH-dependent ketose reductase is somewhat surprising because the best-studied reductases involved in vascular plant alditol biosynthesis use aldoses (not ketoses) and phosphate esters (not free sugars) as their substrates; i.e. Glc-6-P is used for sorbitol formation, Man-6-P for mannitol formation, and Rib-5-P for ribitol formation (for review, see Loescher and Everard, 1996). Exceptions have been described; galactitol is thought to be synthesized by a NADPH-dependent aldose reductase (albeit with an unusually high K_m value of 227 mM for Gal; Negm, 1986) and an alternative pathway for sorbitol synthesis has been proposed, which involves a free ketose (Fru) as the substrate and NADH as the cosubstrate (Doehlert, 1987). The discovery of sedoheptulose reductase described here, which involves a free ketose as the substrate, is an exciting expansion of our knowledge of plant alditol biosynthesis. Whether such a nonsugar-phosphate/ketose biosynthetic pathway is typical for plant heptitols (e.g. perseitol synthesis from mannoheptulose) is an intriguing question that will have to be answered in the future.

The involvement of sugar phosphates in volemitol biosynthesis cannot be totally ruled out, because only one potential candidate, sedoheptulose-7-phosphate, was tested and it did not show any activity with either NADH or NADPH as cosubstrates. Other seven-carbon sugar phosphates such as mannoheptulose-phosphate, coriose-phosphate, and D-*glycero*-D-*manno*-heptose-phosphate are theoretically possible (Fig. 1), but could not be tested because they are not commercially available. It is noteworthy that in the benthic marine brown alga *Pelvetia canaliculata*, volemitol was shown to be synthesized from a sugar phosphate, sedoheptulose-7-phosphate, by the action of a NADH-dependent reductase via volemitol-1-phosphate (Kremer, 1977). Whether this type of reductase is limited to certain brown algae is not known. It would be interesting to do a comparative study of the volemitol biosynthetic

pathways expected to occur in the variety of known volemitol-containing organisms ranging from basidiomycetes and yeasts to algae, lichens, liverworts, and vascular plants (for review, see Bieleski, 1982; Lewis, 1984).

In conclusion, we have shown that: (a) volemitol and sedoheptulose are the two main nonstructural carbohydrates, (b) volemitol and sedoheptulose are photosynthetic products, (c) volemitol is phloem-mobile (like Suc), and (d) volemitol biosynthesis proceeds by a novel, NADPH-dependent, ketose reductase, tentatively called sedoheptulose reductase because of its high substrate specificity. The stage is now set to further study and define the physiological roles of volemitol and the subcellular compartmentation of its biosynthetic pathway, as well as to characterize sedoheptulose reductase in depth at both the biochemical and molecular levels.

ACKNOWLEDGMENTS

We thank Mason Pharr for critical comments on the manuscript and Helen Greutert for expert technical assistance.

Received July 13, 1998; accepted October 2, 1998.

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