Plant Physiol. (1997) 113: 649–655

*Rapid Communication*

# Isolation and Characterization of Soluble Boron Complexes in Higher Plants[1]

## The Mechanism of Phloem Mobility of Boron

**Hening Hu, Sharron G. Penn, Carlito B. Lebrilla, and Patrick H. Brown***

Department of Pomology (H.H., P.H.B.), Department of Chemistry (S.G.P., C.B.L.), University of California, Davis, California 95616

**Boron (B) polyol complexes have been isolated and characterized from the phloem sap of celery (*Apium graveolens* L.) and the extrafloral nectar of peach (*Prunus persica* L.). In celery the direct analysis of untreated phloem sap by matrix-assisted laser desorption-Fourier transform mass spectrometry, with verification by high-performance liquid chromagraphy and gas chromatography-mass spectrometry, revealed that B is present in the phloem as the mannitol-B-mannitol complex. Molecular modeling further predicted that this complex is present in the 3,4 3′,4′ bis-mannitol configuration. In the extrafloral nectar of peach, B was present as a mixture of sorbitol-B-sorbitol, fructose-B-fructose, or sorbitol-B-fructose. To our knowledge, these findings represent the first successful isolation and characterization of soluble B complexes from higher plants and provide a mechanistic explanation for the observed phloem B mobility in these species.**

B is an essential micronutrient for higher plants and its deficiency results in the rapid inhibition of plant growth. The uptake, transport, and function of B in plants appears to be dependent on the formation of B complexes. B uptake, for example, is a passive, nonmetabolic process determined in part by the formation of nonexchangeable B complexes within the cytoplasm and cell wall (Brown and Hu, 1994). When B is present at low to adequate concentrations, the majority of cellular B (>95%) is associated with cell wall pectins where it may be critical for normal cell wall expansion (Hu and Brown, 1994). Recently, B was found to be present as a B-rhamnogalacturonan complex within plant cell walls (Ishii and Matsunaga, 1996; Kobayashi et al., 1996; O'Neill et al., 1996), and the B requirement of a particular plant species has been shown to correlate with the pectin content of the cell wall (Matoh et al., 1993; Hu et al., 1996).

Historically, B has been considered a phloem-immobile element in plants (Oertli and Richardson, 1970). The occurrence of B-deficiency symptoms in young, growing tissue also indicates that B is not readily retranslocated within the plant. Recently, however, it has been demonstrated that B is phloem-mobile in species that translocate significant amounts of sorbitol in the phloem (Brown and Hu, 1996). Based upon these results and in vitro evidence it was proposed that the mobility of B in these species is mediated by the formation of B-sorbitol complexes (Brown and Hu, 1996). Subsequently, we have found that B is also phloem-mobile in species that translocate significant amounts of mannitol or dulcitol, further suggesting that B complexes with sorbitol, mannitol, or dulcitol may mediate the phloem mobility of B.

The identification of B complexes is central to an improved understanding of B physiology. In this report we describe the isolation and characterization of soluble B complexes from phloem sap or of nectar from phloem-fed extrafloral nectaries. The physiological significance of these results is discussed.

## MATERIALS AND METHODS

Phloem mobility of foliar-applied isotopic B was demonstrated in celery (*Apium graveolens* L.) according to the methodology of Brown and Hu (1996). Celery was grown in 11-L pots filled with a perlite:super soil mixture (2:1, v/v). Each pot contained 2 plants and there were 16 replicate plants. The plants were grown in a greenhouse with day/night temperatures of 27/17°C. Plants were fed weekly with one-half-strength complete Hoagland solution (Hoagland and Arnon, 1950). After 2 months of growth three leaflets from leaf number 4 (counting from bottom) on eight replicate plants were immersed for 10 s in 50 mM [$^{10}$B]-enriched (95.91% $^{10}$B) boric acid solution with 0.05% (v/v) surfactant L-77 (Loveland Industries, Inc., Greeley, CO). The leaves were then gently shaken and blotted to

[1] This work was supported by grants from the National Institute of General Medical Sciences, National Institutes of Health (no. GM49077-01), the National Science Foundation (CHE-9531236), and the University of California to C.B.L. and S.G.P., and by grants from the U.S. Department of Agriculture (no. 9601359) and U.S. Borax.

* Corresponding author; e-mail phbrown@ucdavis.edu; fax 1-916-752-8502.

Abbreviations: DHB, 2,5-dihydroxybenzoic acid; MALDI-FTMS, matrix-assisted laser desorption ionization-Fourier transform mass spectrometer.

remove the excess solution. For each replicate, one leaflet from each plant was harvested at 1, 5, and 15 d after treatment. The same number of untreated leaves were collected as the controls. Leaves were washed for 1 min in deionized water, dry-ashed at 500°C, and analyzed for B using an inductively coupled plasma-mass spectrometer (Elan 500, Perkin-Elmer Cetus).

Phloem sap was collected from 6-month-old plants and cultivated as above, with one plant per pot. Five fully mature leaflets on each stem were submerged briefly in a 50 mM boric acid solution that contained 0.05% (v/v) L-77. The leaflets were gently shaken and blotted to remove the excess solution. Care was taken to prevent the contamination of any neighboring leaves or stems with the boric acid solution. Phloem sap was collected 1 d after foliar B application as follows: celery stems were cut about 4 cm from the soil surface and sap was collected from the basipetal cut end (with leaves attached) using a fine-tip borosilicate glass microcapillary (approximately 200 μm in diameter). We found no evidence of B contamination from these microcapillaries within the time frame used. The glass microcapillary was placed directly in contact with the droplet that formed at the cut end of the vascular bundles. The vascular bundles in celery were clearly identifiable and no exudate was observed from any other stem material. Each cut surface yielded about 0.05 to 0.2 μL. The entire process was repeated with sequential cuts, each approximately 5 cm apart on each of the eight stems, until 2 to 3 μL of sap was collected. Between each sap collection, the microcapillary was rinsed with double-deionized water and blotted dry. The sap was kept in plastic tubes in an ice bath, diluted 50-fold with deionized water, and kept frozen prior to analysis.

Droplets of extrafloral nectar totaling 50 μL were collected from the nectary that was present at the base of the leaves of 1-year-old Nemared peach (*Prunus persica* L.) seedlings grown in vermiculite. The nectar was diluted 10-fold with double-deionized water prior to analysis. Because of the small amount of phloem sap and extrafloral nectar collected, the B concentration was estimated from the diluted solution by an inductively coupled plasma-mass spectrometer using a peak height routine.

Aliquots of each solution (celery phloem sap and peach extrafloral sap) were then analyzed by MALDI-FTMS, HPLC, and GC-MS. The MALDI-FTMS (IonSpec Corp., Irvine, CA) was equipped with a 4.7-tesla superconducting magnet and a nitrogen laser at 337 nm (Li et al., 1994; Carroll et al., 1996). All spectra were obtained in the negative ion mode using 3-aminoquinoline (Aldrich) as the matrix. A more common matrix for the analysis of sugars by MALDI is DHB (Harvey, 1993; Stahl et al., 1994). However, when DHB was used as a matrix for the B-containing samples, only peaks corresponding to a complex of B with DHB (DHB-B-DHB) were observed. We found no evidence of a complex formation between B and 3-aminoquinoline; this was predicted given the absence of suitable B complexing sites in this molecule. The matrix was prepared at a concentration of 0.4 M in ethanol. Spectra were obtained by placing 2 μL of the sample on a stainless steel probe tip and adding 1 μL of matrix. The probe tip was held in a cool air stream until crystals formed.

Additional aliquots of celery phloem sap and peach extrafloral sap were analyzed for polyol content by HPLC without purification (Moing et al., 1992). The results were further verified by GC-MS (Greve and Labavitch, 1991; Tao et al., 1995).

MALDI-FTMS is well suited to determine the nature of biological complexes because of its high-mass sensitivity and gentle ionization technique. In FTMS the ion cyclotron resonance of an ion in a magnetic field is measured. The ion cyclotron resonance frequency can be measured very accurately and is proportional to the mass and charge of the ion. Therefore, with FTMS, a high mass resolution ($m/\Delta m$ = 1 million) and accurate mass determination can be obtained (Wu et al., 1995). The chemical composition and stoichiometry of the analyte are obtained from the mass and isotopic pattern. Within the mass range considered here (100–600 $m/z$), each peak can be unambiguously assigned to a specific, unique combination of atoms; isomers, however, cannot be distinguished. Additional information on the identity of the analyte can be obtained by HPLC with further verification by GC-MS. The combination of these techniques was used to unambiguously identify B-polyol complexes that are present in celery phloem and peach nectaries. Standards containing various concentrations of mannitol (Sigma), sorbitol (Fisher Scientific), and/or B were used throughout. The pH of celery sap and peach extrafloral nectar were both around 7.0. All standard solutions were adjusted to pH 7.0 with diluted KOH prior to analysis.

## RESULTS

Figure 1 shows the changes in the B isotopic concentrations following [$^{10}$B] labeling in celery. An 8-fold increase in the [$^{10}$B] concentration was observed 1 d after labeling. Because leaves were washed before analysis, this increase represents B that had been absorbed by the leaf tissue. The

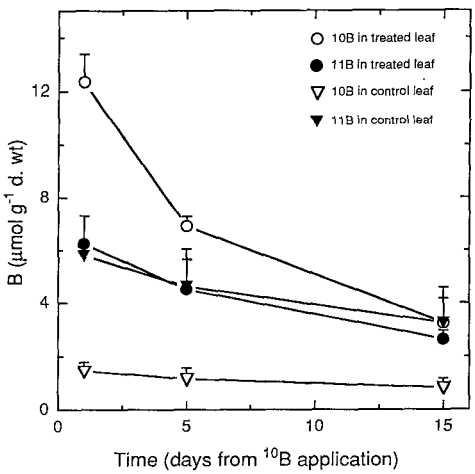



**Figure 1.** Changes in leaf B concentration following application of 50 mM $^{10}$B-enriched boric acid at time 0. Each point is a mean of two replicates ± SE.

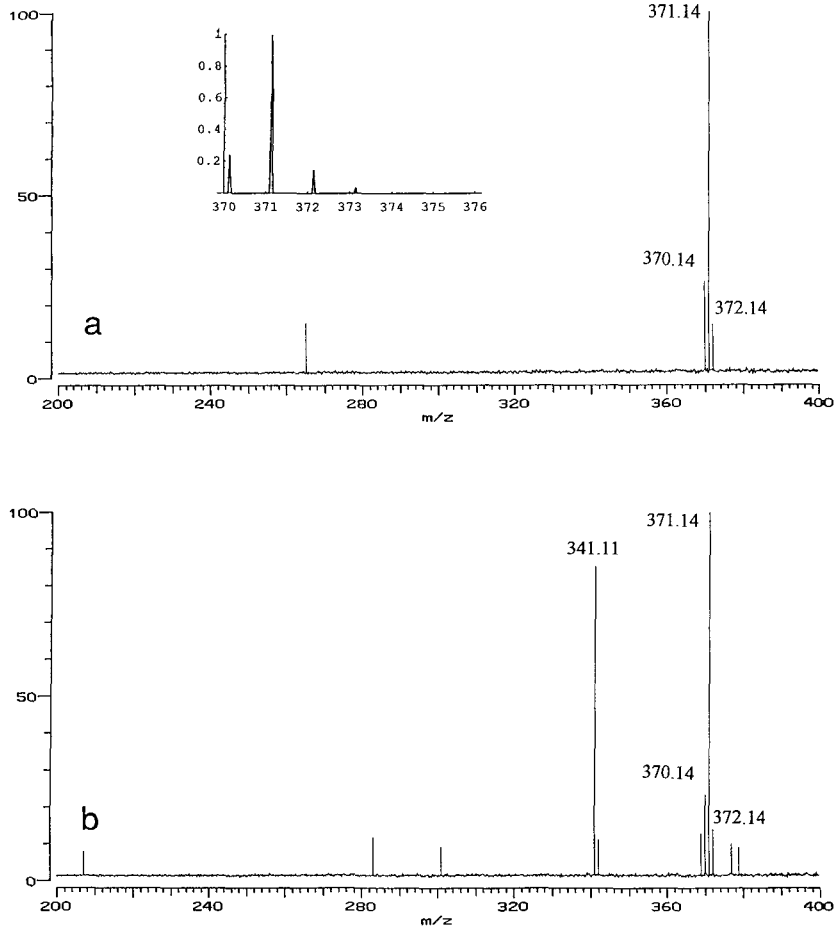



**Figure 2.** Spectra of MALDI-FTMS. a, 10 mM mannitol 1 $mM^{-1}$ boric acid; b, celery phloem sap. Inset, Predicted peak height of mannitol-B-mannitol complex based upon relative isotopic abundance (peaks are identified in Table I).

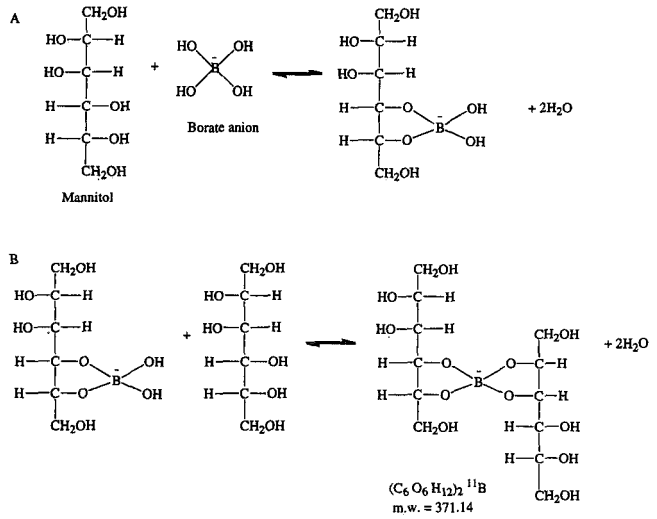
high concentration of [$^{10}$B] decreased rapidly, and after 5 d only 56% of the original [$^{10}$B] remained in the treated leaf. By d 15 there was a 75% reduction in the labeled [$^{10}$B] in the treated leaves. A small increase in [$^{11}$B] was also detected in the treated leaves following [$^{10}$B] application as a result of the 4.09% (atom %) [$^{11}$B] content of the applied [$^{10}$B]-enriched boric acid. During this period no B accumulation was found in the nontreated plants. At the completion of this experiment the distribution of B within the celery plant was determined. The youngest growing tissue (apical meristem plus emerging leaflets) had a B concentration of 104 $\mu g\ g^{-1}$ B; new, fully expanded leaflets had a concentration of 49 $\mu g\ g^{-1}$ B; and the oldest leaves had a concentration of 32 $\mu g\ g^{-1}$ B. This B accumulation pattern is characteristic of a phloem-mobile element (van Goor and van Lune, 1980). Together, these results clearly indicate that B is phloem-mobile in celery.

Figure 2a shows a typical MALDI-FTMS mass analysis of a 10 mM mannitol 1 $mM^{-1}$ boric acid standard (pH 7.0). Two distinct peaks of mass/charge ($m/z$), 370.14 and 371.14, are shown, which coincide exactly with the mass of mannitol-[$^{10}$B]-mannitol and mannitol-[$^{11}$B]-mannitol. The $m/z$ 372.14 is mannitol-$^{11}$B-mannitol with one $^{13}$C atom (Table I). As the $m/z$ of the mannitol-B-mannitol complex and the relative isotopic abundance of [$^{10}$B]:[$^{11}$B] (20%:80%) and $^{12}$C:$^{13}$C (99%:1%) are known, it is possible to predict the relative peak height and exact $m/z$ of the spectra (Fig. 2a, inset). The predicted and measured peaks coincide closely and unambiguously demonstrate the presence of B in these complexes. No other B peak was observed in the mass range that we

**Table I.** *Mass to charge ratio of B complexes determined by MALDI/FTMS*

| Formula (all-1 charge) | Molecular Weight | Description |
|---|---|---|
| $(C_6O_6H_{10})_2{}^{10}B$ | 366.11 | Borate complex with 2 Glc (Fru) |
| $(C_6O_6H_{10})_2{}^{11}B$ | 367.11 | Borate complex with 2 Glc (Fru) |
| $C_6O_6H_{12} + C_6O_6H_{10}{}^{10}B$ | 368.12 | Borate complex with Glc (Fru) and sorbitol |
| $C_6O_6H_{12} + C_6O_6H_{10}{}^{11}B$ | 369.12 | Borate complex with Glc (Fru) and sorbitol |
| $(C_6O_6H_{12})_2{}^{10}B$ | 370.14 | Borate complex with 2 sorbitol (mannitol) |
| $(C_6O_6H_{12})_2{}^{11}B$ | 371.14 | Borate complex with 2 sorbitol (mannitol) |
| $(^{13}C_6O_6H_{12})_2{}^{11}B$ | 372.14 | Borate complex with 2 sorbitol (mannitol) with a [$^{13}$C] atom |

studied (100–600 *m/z*; results not shown). These results agree with the NMR studies that predict a full complexation of B and mannitol as the mannitol-B-mannitol complex when present at the pH and at the relative concentrations used here (Makkee et al., 1985; van der Berg et al., 1994). The proposed reaction is as follows:




**Scheme 1.**

These reactions result in *m/z* of 370.14 for mannitol-$^{10}$B-mannitol (0.5) and 371.14 for mannitol-$^{11}$B-mannitol (0.5), both with one negative charge (Table I).

The MALDI-FTMS spectrum of the celery phloem sap collected from plants that received foliar B application is shown in Figure 2b. As in Figure 2a, two distinct peaks (370.14 and 371.14) are present. These peaks occur in the exact ratio predicted from the isotopic abundance of [$^{10}$B]:[$^{11}$B]. The occurrence of mannitol in the phloem of celery was further verified by HPLC and GC-MS analysis (data not shown). Thus, it is very likely that B is present in celery phloem as the mannitol-B-mannitol complex. From the exact mass the peak at *m/z* 341.11 is assigned as a deprotonated disaccharide (Suc).

MALDI-FTMS analysis of standard solutions of B with Fru as well as B with sorbitol are shown in Figure 3, a and b, respectively. In Figure 3a the *m/z* 366.11 corresponds to Fru-[$^{10}$B]-Fru, whereas the larger peak, 367.11, is Fru-[$^{11}$B]-Fru (Table I). In Figure 3b two distinct peaks of *m/z*, 370.14 and 371.14, are present, coinciding with the mass of sorbitol-[$^{10}$B]-sorbitol and sorbitol-[$^{11}$B]-sorbitol (Table I). These two complexes are present in the ratios expected of the B complexes.

Figure 3c shows the MALDI-FTMS spectrum of peach nectar. Analysis of nectar from the peach leaf reveals a greater complexity than what we observed in standard solutions. Two peaks at *m/z* 366.11 and *m/z* 367.11 correspond to Fru-[$^{10}$B]-Fru complex (or Glc-[$^{10}$B]-Glc) and Fru-[$^{11}$B]-Fru (or Glc-[$^{11}$B]-Glc), respectively. Fru-[$^{10}$B]-Glc and Fru-[$^{11}$B]-Glc complex (*m/z* 366.11 and *m/z* 367.11, respectively) may also occur. A *m/z* of 368.12 corresponds to Fru-[$^{10}$B]-sorbitol, whereas a *m/z* of 369.12 corresponds to Fru-[$^{11}$B]-sorbitol and a *m/z* of 371.14 corresponds to the sorbitol-[$^{11}$B]-sorbitol complex. The *m/z* 370.14, corresponding to sorbitol-[$^{10}$B]-sorbitol, is not detectable at these concentrations. Because the overall borate ester stability of D-Fru is about two orders of magnitude higher than the borate ester stability of D-Glc (Makkee et al., 1985), the observed *m/z* 366.11 and 367.11 must be dominated by Fru-B complex and not Glc-B complex, because Fru and Glc are present at about same concentration in *Prunus* nectar (Bieleski and Redgwell, 1980; Caldwell and Gerhardt, 1986). These results and the abundance of sorbitol in peach (Moing et al., 1992) suggest strongly that Fru and sorbitol are the primary B complexing compounds in peach nectar.

## DISCUSSION

It has been proposed that B (which occurs as $H_3BO_3$ at a physiological pH) cannot be translocated out of the leaf, because the inherent, high-membrane permeability of $H_3BO_3$ would lead to a leakage of B from the phloem to the adjacent (and less concentrated) xylem (Oertli and Richardson, 1970). This "leakage" of B back into the xylem would effectively prevent B loss from the leaf, resulting in B immobility. Although this mechanism is valid for many plants, it is contrary to the findings in the *Malus*, *Prunus*, and *Pyrus* species, where it has been demonstrated that B is highly mobile (van Goor and van Lune, 1980; Hanson, 1991; Picchioni et al., 1995; Brown and Hu, 1996). To explain B mobility in those species, Brown and Hu (1996) proposed that the mobility of B is due to the formation and transport of complexes between B and polyols. Furthermore, they suggest that B would be phloem-mobile in other species that transport B-binding polyols in their phloem. Among the polyols known to form complexes with B, sorbitol, mannitol, and dulcitol occur as the major photosynthates in members of the Rosaceae (sorbitol), Oleaceae, Rubiaceae, and Umbelliferae (mannitol), and Celestraceae (dulcitol) families and may occur in lesser amounts in other plant species (Bieleski, 1982).

The observed mobility of B in celery described here further supports our contention that phloem-B transport is mediated by the formation of polyol-B complexes, because in celery mannitol is the primary photosynthetic product and the major form of translocated carbon (Davis et al., 1988). MALDI-FTMS analysis of celery phloem sap strongly suggests that B was present as the mannitol-B-mannitol complex. These results are in agreement with in vitro kinetic NMR analysis, which suggests that when mannitol:B ratios are in excess of 10:1, at pH 7.0, almost all B will be present as mannitol-B-mannitol (Makkee et al., 1985). In the present study phloem mannitol concentrations were estimated between 150 and 300 mM by HPLC, whereas the B concentration was about 1 to 1.5 mM 1 d following foliar B application. The mannitol:B molar ratio in these samples was in excess of 100:1. This, in combination with the relative isotopic abundance, the unique *m/z* ratio of the peaks, and HPLC and GC-MS verification of mannitol concentrations, strongly suggests that B is transported primarily as the mannitol-B-mannitol complex in the phloem of celery.

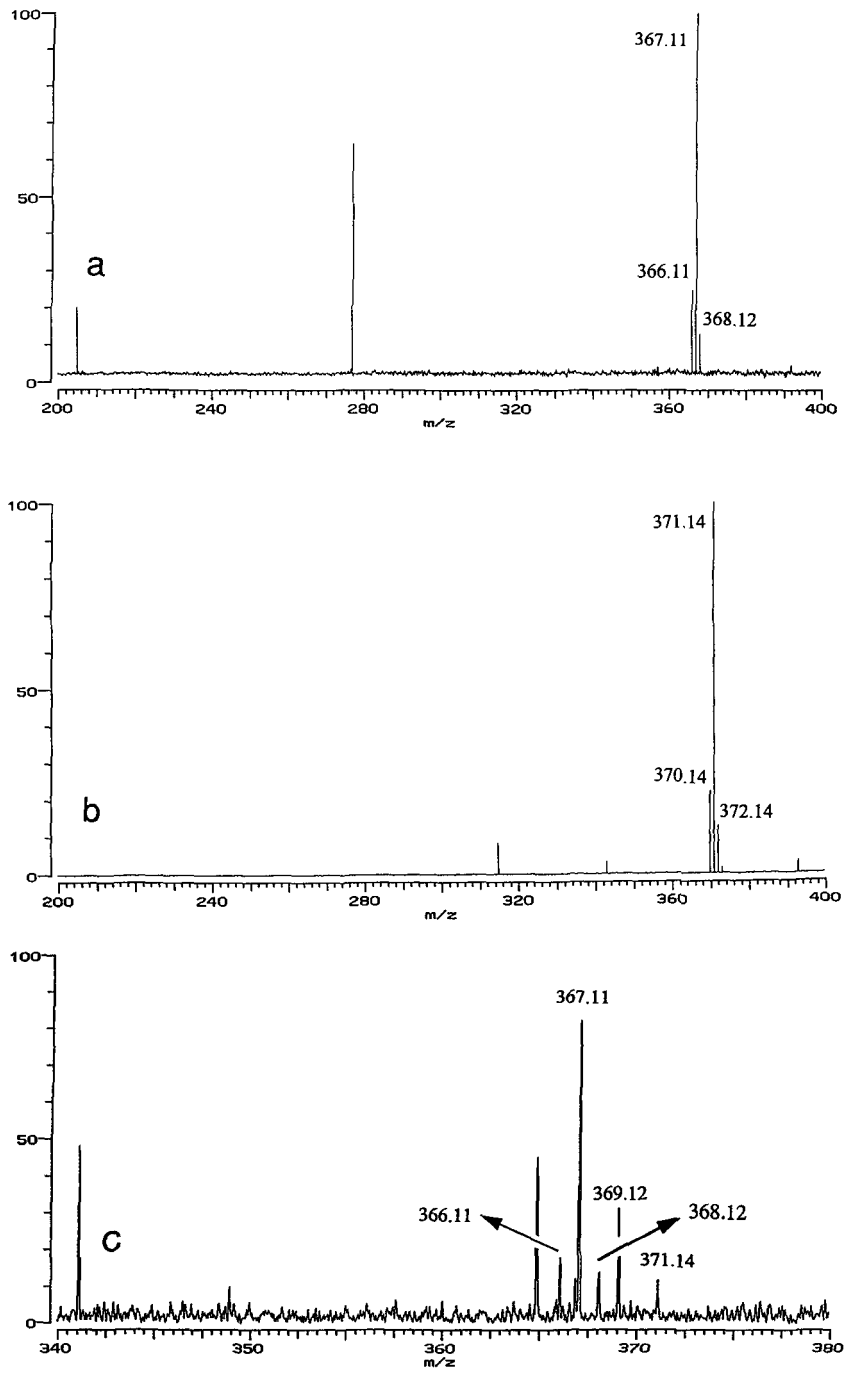



**Figure 3.** Spectra of MALDI-FTMS. a, 10 mM Fru 1 $mM^{-1}$ boric acid; b, 10 mM sorbitol 1 $mM^{-1}$ boric acid; and c, peach nectar.

MALDI-FTMS is ideally suited for analyzing phloem sap because the method does not require any sample pretreatment and can be used on small sample sizes. The high mass resolution of FTMS coupled with the ability to determine isotope ratios allows the unambiguous determination of both elemental composition and molecular identity.

Molecular modeling calculations (SPARTAN Wavefunction Inc., Irvine, CA) were carried out on the mannitol-B-mannitol complex to gain information on the most favorable conformation. Makkee et al. (1985) found from [$^{11}$B] and [$^{13}$C] NMR that the 3- and 4-hydroxyl groups of mannitol bound preferentially with borate. From preliminary semiempirical calculations (AM1, Dewar et al., 1985) the lowest energy conformer that was found for the 1,2 1,2 mannitol borate complex had $\Delta H_f$ = −737.7 kcal/mol, whereas the 3,4 3,4 mannitol borate complex had ΔHf = −741.9 kcal/mol. This result confirms that the 3,4 3,4 borate ester is energetically more favorable (Table II; Fig. 4). Additional information on this configuration and other B-diol complexes is provided elsewhere (S.G. Penn, H. Hu, P.H. Brown, and C.B. Lebrilla, unpublished data).

**Table II.** *Calculated heat of formation of various Bis-mannitol borate configurations*

| Configuration of Borate Ester | Δ Hf[a] |
|---|---|
| | *kcal/mol* |
| 1,2 1',2' Bis-mannitol borate | −737.7 |
| 3,4 3',4' Bis-mannitol borate | −741.9 |
| 1,2 3',4' Bis-mannitol borate | −738.2 |

[a] Δ Hf, Heat formation.

Sorbitol is the primary photosynthetic product and the major form of translocated carbon in *Prunus* (Loescher, 1987), and B has been shown to be phloem-mobile in members of this species (Brown and Hu, 1996). Given the technical difficulty of obtaining phloem sap directly from vascular tissue of *Prunus* species, we chose to use the readily available extrafloral nectar collected from the nectaries at the base of almost fully expanded leaves. Nectar produced from these glands is supplied primarily from phloem and is subject to the metabolism in the nectaries prior to exudation (Bentley, 1977). Here we observed that B in the nectary exudate was present as a complex with either Fru-B-Fru, sorbitol-B-sorbitol, or Fru-B-sorbitol. In peach, as in celery, the relative concentrations of B to ligand is such that the 2 ligand-B conformation is favored.

The occurrence of the Fru-B complexes in the nectary exudate is likely the result of post-phloem metabolism and does not necessarily indicate the occurrence of Fru in the phloem. Moing et al. (1992) found no evidence of Glc or Fru in the phloem sap of peach, and the nectaries of peach are known to metabolize sorbitol via the enzyme sorbitol dehydrogenase with Fru as the primary product (Bieleski and Redgwell, 1980). Given the high concentrations of sorbitol found in the phloem of *Prunus* species (Moing et al., 1992) and the observed phloem mobility of B in *Prunus* (Brown and Hu, 1996), it is reasonable to assume that B is transported in the phloem as the sorbitol-B-sorbitol complex and, subsequently, metabolized in the nectary and exuded as a mixture of the sorbitol- and Fru-B complexes.

The isolation and characterization of the mannitol-B-mannitol complexes from the phloem of celery and the presence of the sorbitol- and Fru-B complexes in peach provide a mechanistic explanation for the observed phloem mobility of B in these species. The complexes we have described, to our knowledge, represent the first soluble B complexes isolated from higher plants.

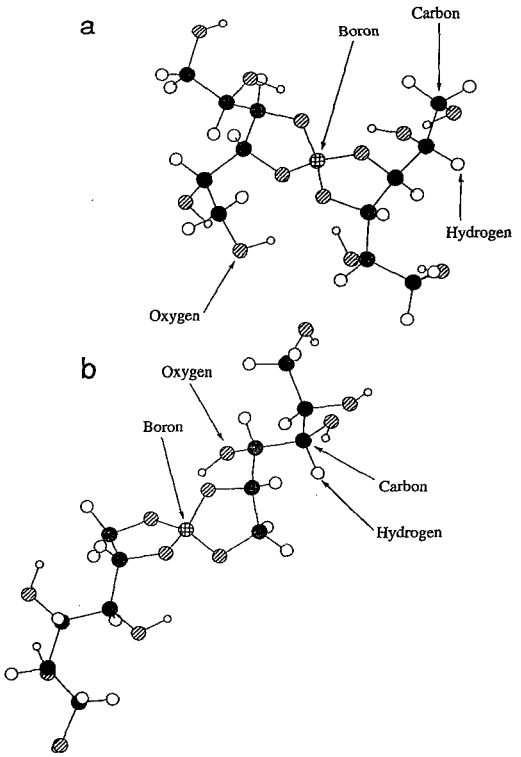



**Figure 4.** Predicted three-dimensional configuration of 3,4 3,4 (a) and 1,2 1,2 bis-mannitol borate (b).

## ACKNOWLEDGMENTS

H.H. and P.H.B. are grateful to Nacer Bellaloui, Carl Greve, James Stangoulis, and Betty Hess-Pierce for their help with GC-MS and HPLC analysis. We would also like to thank M. Kirk Green for generation of the predicted isotopic pattern for the bis-mannitol borate ester.

Received August 28, 1996; accepted November 4, 1996.


## LITERATURE CITED

**Bentley BL** (1977) Extrafloral nectaries and protection by pugnacious bodyguards. Annu Rev Ecol Sys **8:** 407–427

**Bieleski RL** (1982) Sugar alcohols. *In* FA Loewus, W Tanner, eds, Encyclopedia of Plant Physiology. New Series V13A Plant Carbohydrates. I Intercellular Carbohydrates. Springer-Verlag, New York, pp 158–192

**Bieleski RL, Redgwell RJ** (1980) Sorbitol metabolism in nectaries from flowers of *Rosaceae*. Aust J Plant Physiol **7:** 15–25

**Brown PH, Hu H** (1994) Boron uptake by sunflower, squash and cultured tobacco cells. Physiol Plant **91:** 435–441

**Brown PH, Hu H** (1996) Phloem mobility of boron is species dependent: evidence for phloem mobility in sorbitol-rich species. Ann Bot **77:** 497–505

**Caldwell DL, Gerhardt KO** (1986) Chemical analysis of peach extrafloral nectary exudate. Phytochemistry **25:** 411–413

**Carroll JA, Penn SG, Fannin ST, Wu J, Cancilla MT, Green MK, Lebrilla CB** (1996) A dual vacuum chamber Fourier transform mass spectrometer with rapidly interchangeable LSIMS, MALDI, and ESI sources-initial results with LSIMS and MALDI. Anal Chem **68:** 1798–1804

**Davis JM, Fellman JK, Loescher WH** (1988) Biosynthesis of sucrose and mannitol as a function of leaf age in celery (*Apium graveolens* L.) Plant Physiol **86:** 129–133

**Dewar MJS, Zoebisch EG, Healy EF, Stewart JP** (1985) AM1: a new general purpose quantum mechanical molecular model. J Am Chem Soc **107:** 3902–3909

**Greve C, Labavitch JM** (1991) Cell wall metabolism in ripening fruit. V. Analysis of cell wall synthesis in ripening tomato tissue using D-[U-$^{13}$C]glucose tracer and gas chromatography-mass spectrometry. Plant Physiol **97:** 1456–1461

**Hanson EJ** (1991) Movement of boron out of tree fruit leaves. Hortic Sci **26:** 271–273

**Harvey DJ** (1993) Quantitative aspects of the matrix-assisted laser desorption mass spectrometry of complex oligosaccharides. Mass Spectrom Rev **7:** 614–619

**Hoagland DR, Arnon DI** (1950) The water-culture method for growing plants without soil. California Experiment Station Circular 347. The College of Agriculture, University of California, Berkeley, CA

**Hu H, Brown PH** (1994) Localization of boron in cell walls of squash and tobacco and its association with pectin. Evidence for a structural role of boron in the cell wall. Plant Physiol **105:** 681–689

**Hu H, Brown PH, Labavitch JM** (1996) Species variability in boron requirement is correlated with cell wall pectin. J Exp Bot **47:** 227–232

**Ishii T, Matsunaga T** (1996) Isolation and characterization of a boron-rhamnogalacturonan-II complex from cell walls of sugar beet pulp. Carbohydr Res **284:** 1–9

**Kobayashi M, Matoh T, Azuma J-I** (1996) Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. Plant Physiol **110:** 1017–1020

**Li YZ, Mclver R.T. Jr, Hunter RL** (1994) High-accuracy molecular mass determination for peptides and proteins by Fourier transform mass spectrometry. Anal Chem **66:** 2077–2083

**Loescher WH** (1987) Physiology and metabolism of sugar alcohols in higher plants. Physiol Plant **70:** 553–557

**Makkee M, Kieboom APG, van Bekkum H** (1985) Studies on borate esters III. Borate esters of D-mannitol, D-glucitol, D-fructose and D-glucose in water. Recl Trav Chim Pays-Bas Belg **104:** 230–235

**Matoh T, Ishigaki K-I, Kaori O, Azuma J-I** (1993) Isolation and characterization of a boron-polysaccharide complex from radish roots. Plant Cell Physiol **34:** 639–642

**Moing A, Carbonne F, Rashad MH, Gaudilere JP** (1992) Carbon fluxes in mature peach leaves. Plant Physiol **100:** 1878–1884

**O'Neill MA, Warrenfeltz D, Kates K, Pellerin P, Doco T, Darvill AG, Albersheim P** (1996) Rhamnogalacturonan-II, a pectic polysaccharide in the walls of growing plant cells, forms a dimer that is covently cross-linked by a borate ester. J Biol Chem **271:** 22923–22930

**Oertli JJ, Richardson WF** (1970) The mechanism of boron immobility in plants. Physiol Plant **23:** 108–116

**Picchioni GA, Weinbaum SA, Brown PH** (1995) Retention and the kinetics of uptake and export of foliage-applied, labeled boron by apple, pear, prune, and sweet cherry leaves. J Amer Soc Hortic Sci **120:** 28–35

**Stahl B, Thurl S, Zeng J, Karas M, Hlllenkamp F, Steup M, Sawatzki G** (1994) Oligosaccharides from human milk as revealed by matrix-assisted laser desorption ionization mass spectrometry. Anal Biochem **223:** 218–226

**Tao R, Uratsu SL, Dandekar AM** (1995) Sorbitol synthesis in transgenic tobacco with apple cDNA encoding NADP-dependent sorbitol-6-phosphate dehydrogenase. Plant Cell Physiol **36:** 525–532

**van der Berg R, Peter JA, van Bekkum H** (1994) The structure and (local) stability constants of borate esters of mono- and disaccharides as studied by $^{11}B$ and $^{13}C$ NMR spectroscopy. Carbohydr Res **253:** 1–12

**van Goor BJ and van Lune P** (1980) Redistribution of potassium, boron, magnesium and calcium in apple trees determined by an indirect method. Physiol Plant **48:** 21–26

**Wu JY, Fannin ST, Molinski TF, Lebrilla CB** (1995) Exact mass determination for elemental analysis of ions produced by matrix-assisted laser desorption. Anal Chem **67:** 3788–3792