Identification of L-Inositol and Scyllitol and Their Distribution in Various Organs in Chrysanthemum

Kazuo Ichimura, Katsunori Kohata, Yuichi Yamaguchi, Mitsuru Douzono, Hiroshi Ikeda, and Mamoru Koketsu

National Research Institute of Vegetables, Ornamental Plants and Tea, Ano, Mie 514-2392, Japan
Faculty of Engineering, Gifu University, Gifu 501-1193, Japan

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Two unidentified soluble carbohydrates were isolated from chrysanthemum (Dendranthema × grandiflorum (Ramat.) Kitamura) leaves using HPLC. The compounds were identified as 1-L-chiro-inositol, called L-inositol (1) and scyllo-inositol, called scyllitol (2) from the results of 1H-NMR, 13C-NMR, and CI-MS spectra. L-Inositol and scyllitol were distributed in four cultivars tested. L-Inositol concentration of petals gradually decreased during the flower bud development, but the L-inositol content increased by about 7 times. Scyllitol was detected only at an early stage of flower bud.

Key words: chrysanthemum; cut flower; L-inositol; scyllitol; soluble carbohydrate

Addition of sugars to the vase water has been shown to extend the vase life of cut flowers because it supplies their carbon source.1,2) Furthermore, when flowering stems are cut at an early stage of the flower bud, sugar is indispensable to open the flower because large amounts of energy are required for flower opening.3,5) Chrysanthemum is one of the most popular ornamental plants, and widely used for cut flowers. In chrysanthemum, glucose, fructose and sucrose are known to be the main sugar constituents.3-8) Trusty and Miller9) reported that an unknown sugar-like substance is present in large amounts in chrysanthemum leaves. Similarly, Rajapakse and Kelly7) found the presence of an unknown substance in the stem. In a preliminary experiment, we found the occurrence of two unidentified carbohydrates in chrysanthemum leaves. The purpose of this study is to identify the two carbohydrates and examine their distribution in chrysanthemums.

Chrysanthemums (Dendranthema × grandiflorum (Ramat.) Kitamura cv. Gem, Kibo-no-hikari, Ki-shuho-no-chikara, and Viking) were grown in a greenhouse. The leaves (1 g) were taken from the plant and extracted with 10% volumes of ethanol at 75°C for 30 min, then homogenized. The homogenate was centrifuged at 3,000 × g for 10 min. The resulting supernatant was evaporated in vacuo below 50°C. The concentrate was dissolved in 1.0 ml of water and passed through a Sep-Pak C-18 (Millipore, Milford) with water. The eluate was separated using HPLC on a Pb-loaded cation exchange column of Shodex Sugar SP0810 (8 × 300 mm, Showa denko, Tokyo) with a refractive index detector. The column was kept at 80°C and eluted with water at a flow rate of 0.8 ml/min. Figure 1 shows the elution profile of the ethanol extract from leaves of chrysanthemum cv. Ki-shuho-no-chikara. Besides glucose, fructose, sucrose, and myo-inositol, two unidentified peaks, A and B, were detected.

To isolate the compound 1 corresponding to peak A, the vapor of the eluate was introduced into the HPLC system and the eluate was collected at peak 1. The collected sample was evaporated in vacuo and redissolved in water. HPLC was used to confirm whether the sample was compositionally identical to authentic L-inositol (Tokyo Kasei Industry). The sample was subjected to HPLC analysis and the results were compared with those of authentic L-inositol. The results showed that the sample was compositionally similar to authentic L-inositol.

Fig. 1. HPLC Elution Profile on Pb-loaded Cation Exchange Column of Carbohydrate Sample Extracted from Chrysanthemum cv. Ki-shuho-no-chikara Leaves. The separation was done on a Shodex Sugar SP0810 column kept at 80°C and eluted with water at 0.8 ml/min. Sample size was 50 μl. Detection was by a refractive index detector. Authentic carbohydrates were obtained from Wako Pure Chemical Industries. Left; authentic carbohydrates, Right; sample. 1, sucrose (9.75 min); 2, glucose (11.46 min); 3, fructose (14.42 min); 4, myo-inositol (21.98 min); A, L-inositol (17.34 min); B, scyllitol (18.50 min).
A, we obtained a water-soluble fraction from 35 g of leaves of chrysanthemum cv. Ki-shuho-no-chikara. The sample was first purified on a column of Shodex C18-SE (10 × 250 mm, Showa Denko), which was eluted with water at a flow rate of 1.0 ml/min. Fractions containing 1 were combined and further purified by HPLC on a Shodex NH2-SE (10 × 250 mm, Showa Denko) column, which was eluted with 65% acetonitrile at a flow rate of 2.0 ml/min. Fractions containing 1 were finally purified on the Shodex NH2-SE under the same conditions except that 75% acetonitrile was used as the solvent to yield 1 as a colorless powder, 12 mg. A compound 2 corresponding to peak B was also purified from 120 g of leaves of the same cultivar by the same procedure as above to yield 9 mg of 2 as a colorless powder.

The chemical structure of the purified compound was analyzed by CI-MS (Jeol JMS-SX 102A) using isobutane as a reagent gas and 1H-NMR (500 MHz, D2O) and 13C-NMR (126 MHz, D2O) using a Jeol JNM-A500 instrument. Acetonitrile was used as an internal standard in NMR analyses. The retention times of peaks A and B in the HPLC chromatogram were between fructose and myo-inositol (Fig. 1). Furthermore, CI-MS of both compounds had a molecular ion peak at m/z 181 [M + H]+, suggesting that they might be monosaccharides.

1H-NMR and 13C-NMR spectra of 1 showed the presence of three protons at δ 3.58 (double doublet), δ 3.75 (multiplet), and δ 4.02 (doublet) and three carbon signals at δ 70.48, 71.70, and 72.79. These results suggested that this compound might be an inositol isomer with a higher symmetrical structure. The 1H-NMR of 1 coincided with that of authentic L-d-chiro-inositol. Furthermore, the purified compound was strongly levorotatory ([α]D = −52° (c = 1.15 in water)). According to the reference, L-d-chiro-inositol is dextrorotatory ([α]D = +65°). Therefore, 1 was identified as L-d-chiro-inositol, called l-inositol (Fig. 2).

1H-NMR and 13C-NMR spectra of 2 showed a singlet signal and a methine signal at δ 3.34 and δ 73.64, respectively, indicating the presence of an inositol isomer of which all protons and carbons were under exactly the same circumstances. The 1H-NMR and 13C-NMR spectra of 2 coincided with those of authentic scyllo-inositol. Therefore, 2 was identified as scyllo-inositol, called scyllitol (Fig. 2).

Next, we measured the soluble carbohydrate concentrations in various organs such as petal, the remaining part of flower, stem, and leaf of cv. Ki-shuho-no-chikara, Gem, Kibo-no-Hikari, and Viking. Flowering stems were cut at a normal harvest stage. Each organ (1 g) obtained from 3 plants was extracted with 80% ethanol and purified using the Sep-Pak C18 as described above. Carbohydrate concentrations were measured using HPLC on the Shodex Sugar SP0810 column. L-Inositol was distributed

\[ \text{Fig. 2. Structures of L-Inositol (1) and Scyllitol (2).} \]

\[ \text{Fig. 3. Changes in Fresh Weight (A), Carbohydrate Concentrations (B) and Contents (C) in Petals during Flower Bud Development in Chrysanthemum cv. Kibo-no-Hikari.} \]

Values are means (n = 6 for fresh weight and n = 3 for sugar concentration and content) ± standard errors. The concentration and content of carbohydrates were obtained from 3 independent experiments.
in all the organs of all the cultivars tested at fairly high concentrations. Scyllitol was also distributed in all the organs except for petals of all the cultivars tested and the remaining part of the flower of cv. Ki-shuho-no-chikara (Table 1). Distribution of L-inositol and scyllitol in higher plants is limited. L-Inositol and scyllitol are found in only 2 and 7 families, respectively. To our knowledge, this is the first report that L-inositol and scyllitol are soluble carbohydrate constituents in chrysanthemums. In chrysanthemum leaves and stem, the presence of unidentified sugar like compounds in large amounts have been reported. The compounds may be L-inositol or scyllitol. The concentrations of L-inositol and scyllitol are high in leaves and stem. Cyclitols including myo-inositol and their methyl ethers including pinitol are found to act as an osmolyte or compatible solute. These findings suggest that L-inositol and scyllitol may be osmolytes in these organs.

Soluble carbohydrates play important roles in flower opening as substrates for respiration and cell walls as well as osmolytes. The concentrations of metabolic sugars such as glucose and fructose increase during flower opening in many plants including carnation and sweet pea. To explore possible roles of L-inositol, scyllitol, and myo-inositol, we examined changes in soluble carbohydrate contents of petals during flower opening. Flowers (buds) cv. Kibo-no-hikari were harvested at 5 different stages: Stage 1; petal length was about 1.4 cm. Stage 2; petal length was about 1.8 cm. Stage 3; petal length was about 2.6 cm. Stage 4; petal length was about 3.6 cm. Stage 5; petal length was about 4.5 cm and petals were completely unfolded within 1 day of opening. The concentrations of soluble carbohydrates were measured as above. Glucose and fructose concentration increased during flower bud development. On the contrary, the concentration of L-inositol as well as myo-inositol and sucrose almost constant (Fig. 3). Scyllitol was only detected at stages 1 and 2. Since the fresh weight of petals increased, the carbohydrate contents of petals increased, except for scyllitol. During flower bud development, the contents of L-inositol and myo-inositol increased about 7-fold on an organ basis. Petal growth associated with flower opening is the result of cell expansion, which requires the influx of water and osmolytes into the vacuoles. Metabolic sugars such as glucose and fructose accumulate in vacuoles of the petals in *Hippeastrum* and *Tulip*. On the other hand, pinitol, a methylated inositol, is mainly accumulated in cytoplasm. Similarly, in rose petals and *Delphinium* sepals, glucose and fructose accumulate in vacuoles but sucrose and myo-inositol are mainly present in cytoplasm. From these findings, we assume that L-inositol and myo-inositol may act as an osmolyte in cytoplasm to counteract the osmotic potential caused by metabolic sugars accumulated in vacuoles in chrysanthemum. On the other hand, scyllitol was only detected at stage 1 and 2 with relatively low concentrations, suggesting that scyllitol appears not to contribute to flower opening.

**myo-Inositol acts as a reserve compound in higher plants.** Thus, it can be speculated that L-inositol and scyllitol act not only as osmolytes but also as reserve carbohydrates in chrysanthemum. Further studies are needed to clarify whether L-inositol and scyllitol also act as reserve carbohydrates in chrysanthemum.

### Table 1. Carbohydrate Concentrations of Various Organs in Chrysanthemum Cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Organ</th>
<th>Glucose (mg/gFW)</th>
<th>Fructose (mg/gFW)</th>
<th>Sucrose (mg/gFW)</th>
<th><em>myo</em>-Inositol (mg/gFW)</th>
<th>L-Inositol (mg/gFW)</th>
<th>Scyllitol (mg/gFW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gem</td>
<td>Petal</td>
<td>13.6 ± 0.3</td>
<td>14.9 ± 0.5</td>
<td>2.8 ± 0.1</td>
<td>1.2 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Flower</td>
<td>1.7 ± 0.3</td>
<td>1.9 ± 0.2</td>
<td>4.0 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>0.1 ± 0.0</td>
<td>1.5 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>0.6 ± 0.0</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>0.4 ± 0.4</td>
<td>1.0 ± 0.0</td>
<td>2.7 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>Kibo-no-hikari</td>
<td>Petal</td>
<td>12.8 ± 0.5</td>
<td>17.0 ± 0.4</td>
<td>3.0 ± 0.1</td>
<td>0.5 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Flower</td>
<td>0.9 ± 0.1</td>
<td>4.1 ± 0.2</td>
<td>2.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.0</td>
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<td>4.4 ± 0.1</td>
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<td>0.2 ± 0.0</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>0.9 ± 0.0</td>
<td>2.3 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>0.5 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.5 ± 0.0</td>
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<tr>
<td>Ki-shuho-no-chikara</td>
<td>Petal</td>
<td>6.3 ± 1.8</td>
<td>7.3 ± 2.0</td>
<td>2.0 ± 0.3</td>
<td>0.6 ± 0.0</td>
<td>0.5 ± 0.0</td>
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</tr>
<tr>
<td></td>
<td>Flower</td>
<td>0.1 ± 0.0</td>
<td>1.1 ± 0.3</td>
<td>2.8 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
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<tr>
<td></td>
<td>Stem</td>
<td>0.2 ± 0.0</td>
<td>3.5 ± 0.6</td>
<td>3.0 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.1</td>
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</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>0.3 ± 0.1</td>
<td>1.0 ± 0.4</td>
<td>2.7 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>Viking</td>
<td>Petal</td>
<td>15.1 ± 0.9</td>
<td>18.4 ± 1.0</td>
<td>3.7 ± 0.2</td>
<td>0.9 ± 0.0</td>
<td>0.6 ± 0.0</td>
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</tr>
<tr>
<td></td>
<td>Flower</td>
<td>1.0 ± 0.0</td>
<td>2.4 ± 0.1</td>
<td>5.5 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>0.1 ± 0.1</td>
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<tr>
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<td>2.5 ± 0.5</td>
<td>3.8 ± 0.4</td>
<td>0.9 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>0.6 ± 0.1</td>
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<tr>
<td></td>
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<td>1.0 ± 0.1</td>
<td>3.0 ± 0.3</td>
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<td>1.3 ± 0.1</td>
<td>0.5 ± 0.0</td>
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</tbody>
</table>

* Carbohydrates were measured by HPLC as described in the text.
* Values are means of 3 independent experiments ± standard errors.
Acknowledgments

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References