Note

Identification of L-Bornesitol and Changes in Its Content during Flower Bud Development in Sweet Pea (Lathyrus odoratus L.)

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An unidentified carbohydrate was isolated from sweet pea (Lathyrus odoratus L. cv. Diana) petals using HPLC. The isolated compound was identified as L-1-O-methylmyo-inositol, called L-bornesitol, using 1H-NMR, 13C-NMR, and CI-MS. L-Bornesitol was distributed in all organs at high concentrations. L-Bornesitol concentration of petals gradually decreased during flower bud development, but the L-bornesitol content increased by about 5 times.

Key words: L-bornesitol; cut flower; soluble carbohydrate; sweet pea

Sweet peas are suitable for cut flowers because of their wide range of colors and exceptional fragrance. The flowers of sweet pea are sensitive to ethylene, and the longevity of the cut flower is very short.1-3)

Since the amount of sugars contained in cut flowers is limited, addition of carbohydrates such as sucrose to vise water is effective in improving the vase life of cut flowers.4) Ichimura and Hiraya5 recently reported that the vase life of cut sweet peas was markedly improved by treatment with sucrose. This suggests that sugars are important in extending the vase life of cut sweet peas. However, there have not yet been investigations on sugar metabolism of sweet peas. The purpose of this study is to identify soluble carbohydrates and examine their distribution in sweet peas.

Sweet peas (Lathyrus odoratus L. cv. Diana) were grown in a greenhouse under frost-free conditions. The petals (1 g) were taken from fully opened flowers and extracted with 10 volumes of 80% 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 a minimum of water and passed through a Sep-Pak C-18 (Millipore, Milford) with water. The eluate was separated using HPLC with a refractive index detector on a Pb-loaded cation exchange column of Shodex Sugar SP0810 (8×300 mm, Showa Denko, Tokyo). 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 petals of sweet peas. Besides glucose, fructose, sucrose, and myo-inositol, two unidentified major peaks, B and C, and one minor peak, A were detected. Since the retention time of peak A was coincident with that of pinitol, which is a main carbohydrate in many leguminous plants such as soybeans,6-8) peak A would be pinitol. However, peak A was very small in all the samples tested, and thus we did not investigate peak A further.

To isolate an unidentified compound B, we obtained a water-soluble fraction from 20.5 g of petals. The sample was first purified by a Shodex C18-5E (10×300 mm, Showa Denko), which was eluted with water at a flow rate of 2 ml/min. Fractions containing peak B were further purified on a Shodex NH2 column (5E, 10×300 mm, Showa Denko), which was eluted with 65% acetonitrile at a flow rate of 3 ml/min. Fractions containing peak B were finally purified on the Shodex Sugar

Fig. 1. HPLC Elution Profile on Pb-Loaded Cation Exchange Column of Carbohydrate Sample Extracted from Sweet Pea cv. Diana Petals.

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. Standard carbohydrates except for pinitol were obtained from Wako Pure Chemical Industries. Pinitol was obtained from carnation leaves as described in ref. 15. Left; standard, Right; sample. 1, sucrose (9.55 min); 2, glucose (11.45 min); 3, pinitol (12.17 min); 4, fructose (14.85 min); 5, myo-inositol (23.75 min); A, unknown compound A (12.25 min); B, L-bornesitol (18.25 min); C, unknown compound C (21.75 min).

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SP0810 column, which was kept at 80°C and eluted with water at a flow rate of 0.8 ml/min to yield 17 mg. The chemical structure of the purified compound was analyzed by CI-MS using a Jeol JMS-SX 102A instrument and 1H-NMR (270 MHz, D2O), 13C-NMR (68 MHz, D2O), 1H-1H COSY and 1H-13C COSY using a Jeol JNM-EX270 instrument. Acetone was used as an internal standard in NMR analyses. CI-MS gave a molecular ion peak at m/z 195 [M+H]+. In the 1H-NMR spectrum (Fig. 2), a singlet signal of three protons at δ 3.42 indicated the presence of one methyl group. Furthermore, six protons besides the three protons of the methyl group were observed. The 13C-chemical shifts were assigned as follows: 80.52 (C-1), 71.09 (C-3), 72.28 (C-4), 74.45 (C-5), 71.65 (C-6), 56.72 (OCH3). One of them at δ 56.72 was assigned to a methyl group by 1H-13C COSY. From these assignments, the compound was found to be a methyl derivative of an isositol isomer. Since a carbon attached to the alkoxy group (-OR) is known to be shifted downfield compared with that attached to the free hydroxy group (-OH),9 the peak shifted most downfield in the 13C-NMR (δ 80.52) is assigned to the carbon attached to the methoxy group. In the 1H-NMR spectrum, three triplet signals at δ 3.26, 3.61, and 3.63 gave large coupling constants, 9.41, 9.74, and 9.57 Hz, respectively, suggesting the presence of three coupling systems of Hax-Hax-Hax. Two doublet signals at δ 3.19 and 3.48 indicate the presence of two coupling systems of Hax-Hax-Hex. A triplet signal at δ 4.29 gave a small coupling constant (J=2.81 Hz), suggesting the presence of a coupling system of Hax-Heq-Hax. It was proved by 1H-13C COSY that the proton resonating at δ 3.19 was correlated with the carbon attached to the methoxy group. When this proton and carbon are named H-1 and C-1, the signals of protons H-2 to H-6 and carbons C-2 to C-6 could be assigned by 1H-1H and 1H-13C COSY without contradiction, as shown in Fig. 2 and assignments of 13C-NMR spectrum. Furthermore, the specific rotation of the purified compound was [α]D90°=–26.4° (C=0.5 in water) while that of D-bornesitol has been reported to be [α]D =+30.6° (C=0.8 in water).10 From these structural analyses, this compound was identified as L-1-O-methyl-myo-inositol, called L-bornesitol.

Next, we isolated an unidentified compound C from petals (25 g) using the HPLC system on the Shodex C18 column under the same conditions as above to yield 17 mg and investigated its chemical structure. The 1H-NMR spectrum suggested that this compound was not derived from carbohydrates (data not shown). This view is strengthened by the CI-MS spectrum, which gave a molecular ion peak at m/z 139 [M+H]+. However, we have not yet identified the chemical structure of compound C.

We measured the soluble carbohydrate concentrations in the petal, the remaining part of the flower, leaf, and stem. Flowering stems were harvested at normal harvest maturity. Each organ (1 g) obtained from 3 plants was extracted with 80% ethanol and purified using the Sep-Pak C18 as above. Soluble carbohydrate concentrations were measured using HPLC on the Shodex Sugar SP0810 column. L-Bornesitol was distributed in all the organs with high concentrations (Table 1). L-Bornesitol has been detected in only 5 plant families.11 Although Plouvier11 reported that L-bornesitol was detected in sweet pea, he did not measure its concentration. To our knowledge, this is the first report that L-bornesitol is a major soluble carbohydrate in sweet peas.

The physiological role of L-bornesitol have not yet been investigated. To explore its possible role, we examined changes in sugar contents of petals during flower bud development. Flowers (buds) were harvested at 5 different stages: Stage 1; bud length was about 1.5 cm. Stage 2; bud length was about 2 cm. Stage 3; bud length was about 2.5 cm. Stage 4; bud length was about 3 cm. Stage 5; flower within 1 day of opening. L-Bornesitol was the most abundant soluble carbohydrate at stage I. Glucose, fructose, and sucrose concentrations increased during flower bud development. On the contrary, L-bornesitol concentration gradually decreased (Fig. 3). Since the fresh weight of petals increased, the carbohydrate contents of petals increased, except for myo-inositol.

The L-bornesitol concentration of petals was high, particularly in stage I. This suggests that L-bornesitol contributes to maintaining an osmotic balance. Large quantities of sugars are required for flower bud opening as substrates for respiration and cell walls as well as osmolytes.46 Pinitol, an isomer of bornesitol, is mainly dis-

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**Table 1. Carbohydrate Concentrations of Various Organs**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Glucose (mg/gFW)</th>
<th>Fructose (mg/gFW)</th>
<th>Sucrose (mg/gFW)</th>
<th>myo-Inositol (mg/gFW)</th>
<th>L-Bornesitol (mg/gFW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petal</td>
<td>11.9±1.7</td>
<td>2.4±0.5</td>
<td>10.8±1.5</td>
<td>0.3±0.0</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>Flower</td>
<td>10.1±1.4</td>
<td>2.6±0.1</td>
<td>17.9±1.9</td>
<td>0.2±0.0</td>
<td>5.0±0.2</td>
</tr>
<tr>
<td>Stem</td>
<td>12.5±0.8</td>
<td>2.2±0.2</td>
<td>8.1±0.5</td>
<td>0.1±0.0</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>Leaf</td>
<td>1.3±0.5</td>
<td>1.1±0.4</td>
<td>5.1±0.6</td>
<td>0.3±0.0</td>
<td>3.7±0.4</td>
</tr>
</tbody>
</table>

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

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**Fig. 2.** 1H-NMR Spectrum and Structure of L-Bornesitol (Compound B).
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Fig. 3. Changes in Fresh Weight (A), Carbohydrate Concentrations (B) and Contents (C) of Petals during Flower Bud Development. Values are means (n=6 for fresh weight and n=3 for carbohydrate concentrations and contents)±standard errors.

Contributed in the cytoplasm of Mesembryanthemum leaves. Cyclitols and their methyl ethers including pinitol are found to act as an osmolyte or compatible solute. Therefore, in sweet pea petals, l-bornesitol may act as an osmoregulator, particularly in cytoplasm.

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References