Note

Anthocyanins in The Dark Purple Anthers of Tulipa gesneriana: Identification of Two Novel Delphinidin 3-O-(6-O-(Acetyl-α-Rhamnopyranosyl)-β-Glucopyranosides)

Masayoshi Nakayama,1 Masa-atsu Yamaguchi,2 Osamu Urashima,3 Yukiko Kan,4 Yuko Fukui,5 Yuichi Yamaguchi,6 and Masaji Koshioka1,†

1Department of Floriculture, National Research Institute of Vegetables, Ornamental Plants and Tea (NIVOT), Ministry of Agriculture, Forestry and Fisheries, 360 Kusawa, Ano, Mie 514-2392, Japan
2Minami-Kyushu University, Takanabe, Miyazaki 884-0003, Japan
3Toyama Agricultural Research Center, 288 Gorohmaru, Tonami, Toyama 939-1327, Japan
4Suntory Institute for Bioorganic Research (SUNBOR), 1-1-1 Wakayamadai, Shimamoto, Osaka 618-8503, Japan
5Institute for Fundamental Research, Suntory Ltd., 1-1-1 Wakayamadai, Shimamoto, Osaka 618-8503, Japan
6Department of Tea Processing Technology, NIVOT, 2769 Kanaya, Kanaya, Shizuoka 428-8501, Japan

Received April 13, 1999; Accepted May 12, 1999

Two novel anthocyanins, delphinidin 3-O-(6-O-(2-O-acetyl-α-rhamnopyranosyl)-β-glucopyranoside) and delphinidin 3-O-(6-O-(3-O-acetyl-α-rhamnopyranosyl)-β-glucopyranoside), were identified from the anthers of Tulipa gesneriana. These and delphinidin 3-O-(6-O-(α-rhamnopyranosyl)-β-glucopyranoside) made up over 80% of the anthocyanin content in the dark purple anthers and could be responsible for the intense color of the anthers.

Key words: Tulipa gesneriana; dark purple anther; delphinidin 3-O-(6-O-(α-rhamnopyranosyl)-β-glucopyranoside); delphinidin 3-O-(6-O-(2-O-acetyl-α-rhamnopyranosyl)-β-glucopyranoside); delphinidin 3-O-(6-O-(3-O-acetyl-α-rhamnopyranosyl)-β-glucopyranoside)

A number of angiosperms have a system to attract nectar-seeking insects to their central flower parts for pollination. The prominent color of the central flower parts of Tulipa gesneriana (tulip) is thought to be one such system. Some cultivars have red petals as well as dark purple basal petals and anthers to give appeal to the central flower parts. We have been investigating the anthocyanin distribution to identify whether a qualitative difference would cause the color changes in organs of a T. gesneriana flower. The major anthocyanins in T. gesneriana flowers have been reported to be the 3-O-(6-O-(α-rhamnopyranosyl)-β-glucopyranosides) of pelargonidin, cyanidin, and delphinidin.1,2 In the course of our study, we detected the occurrence of two novel anthocyanins in the dark purple anthers and determined their structures.

Dark purple anthers of T. gesneriana cvs. ‘Ben van Zanten’ and ‘Ile de France’ were extracted with 5% HCO3H. The extract was analyzed by ODS-HPLC in an Inertsil-2 column (ϕ 4.6 × 250 mm, GL Science) with a linear-gradient solvent system of 20–100% of solvent A (25% MeCN, 20% HCO3H, and 1.5% phosphoric acid in water) in solvent B (1.5% phosphoric acid) for 40 min at 40°C at a flow rate of 0.6 ml/min. Three major anthocyanins (1–3) were detected in both the extracts. The tR and UV-Vis (240–580 nm) spectra recorded during on-line HPLC by a photodiode array detector were compared with those of the authentic anthocyanins of T. gesneriana.3,4 The spectra of 1 were identical to those of authentic delphinidin 3-O-(6-O-(α-rhamnopyranosyl)-β-glucopyranoside), but neither 2 nor 3 corresponded to the authentic samples (Table 1).

Anthocyanins 1–3 were extracted from dark purple anthers of cvs. ‘Ben van Zanten,’ ‘China Pink,’ ‘Ile de France,’ ‘Jan Reus,’ and ‘Murasaki Suishou’ (total 150 g fresh weight) with 5% HCO3H. The extract was applied to an Amberlite XAD-7 column and eluted with 5% EtOH. The eluate was partitioned in n-BuOH–HCO3H–H2O (4:1:5), and the aqueous phase was loaded into an ODS column. Anthocyanins 1 and 2 were eluted in the 7.5% EtOH (5% HCO3H) fraction, and 3 was eluted in the 15.0% EtOH (5% HCO3H) fraction. These anthocyanins were successively purified by cellulose TLC with n-BuOH–HCO3H–H2O (1 and 2: 4:1:2; 3: 6:1:2) and with 10% HCO3H. Each anthocyanin was subjected to Sephadex LH-20 chromatography, eluting with EtOH–HCO3H–water (10:1:9). The eluate was further purified by ODS-HPLC in a Senshu Pak ODS-4253-D column (ϕ 10 × 250 mm), eluting with 25% of solvent C (MeCN–HCO3H–H2O, 4:1:5) in solvent D (10% HCO3H) at 40°C at a flow rate of 2 ml/min (1 and 2) or with 30% of solvent C in solvent D and 40% of solvent E (EtOH–HCO3H–H2O, 4:1:5) in solvent D at 35°C at a flow rate of 2 ml/min (3). Finally, 1 (20 mg), 2 (8 mg) and 3 (15 mg) were obtained.

Anthocyanins 1–3 were analyzed by FABMS (JEOL JMS SX-102A) and NMR (Bruker DMX-500). The FABMS data for 1 showed (M+) at m/z 611, and the 1H-NMR spectrum of 1 was assigned by DQF-COSY and TOCSY to delphinidin 3-O-(6-O-α-rhamnopyranosyl-β-glucopyranoside), resulting in its identification (Table 2).

† To whom correspondence should be addressed. Fax: 81-59-268-1339; E-mail: masaji@nivot.afric.go.jp
The FABMS data for both 2 and 3 showed (M+*) at m/z 653 corresponding to delphinidin 3-O-(6-O-α-rhamnopyranosyl-β-glucopyranoside) with an acetyl. The detailed structures of 2 and 3 were established from 1H- and 13C-NMR measurements consisting of DQF-COSY, TOCSY, NOESY, HSQC-editing, and HMBC methods. Anthocyanin 2 was found to contain the same chromophore (delphinidin) and two sugars (β-glucopyranose and α-rhamnopyranose) as 1, based on information regarding coupling constants and integration data in the 1H-NMR spectrum, as well as DQF-COSY and TOCSY data. Furthermore, the presence of an acetyl was indicated by the proton signal of the H3 singlet at δ 2.06, and the carbon signals at δ 21.6 and δ 173.3. A proton signal of the H-3 rhamnose of 2 was shifted to a lower field at δ 4.81 than that of 1 at δ 3.64, indicating acylation at the
C-3 rhamnose of 2. NOE was found between the H-1 glucose and H-4 delphinidin, and the HMBC spectrum showed cross-peaks between the H-1 glucose and C-3 delphinidin, H-1 rhamnose and C-6 glucose, and H-3 rhamnose and carbonyl carbon of the acetyl. Therefore, the structure of 2 was determined to be delphinidin 3-O-(6-O-(3-O-acetyl-α-rhamnopyranosyl)-β-glucopyranosyl). In a similar fashion, the structure of 3 was determined to be delphinidin 3-O-(6-O-(2-O-acetyl-α-rhamnopyranosyl)-β-glucopyranoside).

A variety of acylated anthocyanins have been reported as flower pigments. General aliphatic acids attached to anthocyanins are of dicarboxylic type such as malonic acid. Acetyl anthocyanins are restricted to the 3-O-β-galactoside and the 3-O-(2-O-galloyl-β-galactoside) types from Nymphaea. Pelargonium, Verbena hybrida and Zinnia elegans, and the 3-O-(6-O-rhamnosyl-β-glucoside) type from Eurya japonica and T. gesneriana (2 and 3).

While 1-3 showed the visible maxima at 525 nm, the visible maxima of the 3-O-(6-O-α-rhamnopyranosyl-β-glucopyranosides) of pelargonidin and cyanidin, which are the other known anthocyanins of T. gesneriana, were at 505 nm and 520 nm, respectively (Table 1). It is conceivable that 1-3 could express more purple color than the other T. gesneriana anthocyanins. Anthocyanins 1-3 were the major ones and made up over 80% of total anthocyanins in the anthers of both 'Ben van Zanten' and 'Ile de France.' Anthocyanins 1-3 could be responsible for the intense color of the dark purple anthers of Tulipa gesneriana.

Acknowledgments
We are grateful to Dr. Y. Tanaka (Institute for Fundamental Research, Suntory Ltd.), Dr. H. Naoki (SUNBOR) and Dr. N. Oyama (NIVOT) for analytical assistance, to Ms. M. Taya (Toyama Agricultural Research Center) for preparing the samples, and to Dr. R. W. King (CSIRO) for valuable advice. This work was supported by grant-aid from the Ministry of Agriculture, Forestry and Fisheries of Japan.

References