Malvidin 3-Rutinoside as the Pigment Responsible for Bract Color in Curcuma alismatifolia

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Malvidin 3-rutinoside was the only anthocyanin identified from pink bracts of Curcuma alismatifolia cultivars. The concentration of malvidin 3-rutinoside in three cultivars increased as the intensity of the pink color in the bracts increased.

Key words: Curcuma alismatifolia; Zingiberaceae; pink bract; anthocyanin; malvidin 3-rutinoside

The Curcuma genus belongs to the Zingiberaceae family and is native to tropical Asia. Due to the large, hooded pink bracts which are the most visible feature of C. alismatifolia, this species is now grown as a cut flower. Since only a few cultivars are being commercially grown, it is important to breed new cultivars with different bract colors. Understanding the chemical composition of the bract pigments will aid plant breeders to expand the range of colors in breeding and selecting new color forms. The anthocyanins in pink bracts of C. alismatifolia cultivars were analyzed qualitatively and quantitatively.

Three cultivars of C. alismatifolia, 'Pink Pearl', 'Shalom', and 'Red Shalom', were grown in the field at Kochi Prefectural Agriculture Research Center in Japan. After excising the green tip of the bracts, the anthocyanins of the pink bracts of each cultivar were extracted with HCO3H–H2O (10:90). The presence of other pigments could not be visually detected after the anthocyanin had been extracted. The crude extracts were analyzed by ODS-HPLC with a Hewlett-Packard HP 1100 system. The following conditions were used: column, Inertsil ODS-2 (4.6 μm × 250 mm, GL Science); solvent, linear gradient of 20–100% of solvent A (HCO3H–H2O–MeCN-phosphoric acid, 40:107:50:3) in solvent B (H2O-phosphoric acid, 197:3) for 40 min; flow rate, 0.6 ml/min; column temperature, 40°C; monitoring, UV-Vis 240–600 nm by a photodiode array detector. An anthocyanin which was eluted at tR 22.7 min made up over 77% of the absorbance peak area at 530 nm in each of these three cultivars' bract extract.

The anthocyanins were extracted again with HCO3H–H2O (9:5) from the bracts of C. alismatifolia (100 g dry weight) that had been grown in a greenhouse at USDA in U.S.A. Again, only one anthocyanin was detected in the crude extract. The extract was passed through an Amberlite XAD-7 column and eluted with EtOH–HCO3H (95:5). The eluate was loaded into an ODS column, and the anthocyanin was eluted with EtOH–HCO3H–H2O (19:10:171). The fraction was developed by cellulose TLC with n-BuOH–HCO3H–H2O (6:1:2), and then the anthocyanin was subjected to Sephadex LH-20 chromatography, eluting with EtOH–HCO3H–H2O (10:1:9). The anthocyanin was further purified by ODS-HPLC under the following conditions: column, Senshu Pak ODS-4235-D (10 μm × 250 mm); solvent, 25% of solvent C (EtOH–HCO3H–H2O, 40:10:50) in solvent D (HCO3H–H2O–H2O, 10:90); flow rate, 2 ml/min; column temperature, 40°C. The fraction of tR 10.7 min was collected, dissolved in a small amount of CF3CO2H in MeOH, and 10 mg of the anthocyanin CF3CO2H salt was finally obtained.

The anthocyanin was analyzed by 1H- and 13C-NMR in CD3OD–CF3CO2D (9:1) by a JEOL EX-400 spectrometer. The proton signals in the region of δ 6.63–9.80 and at δ 3.95 were assigned to those of

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Abbreviations: Glc, glucose; Mv, malvidin; Rhm, rhamnose
malvidin.\(^1\) The other proton signals were assigned to two hexose moieties, based on information on the coupling constants and integration data in the \(^1\)H-NMR spectrum, as well as on the \(^1\)H-\(^1\)H-COSY and the HOHAHA spectral data. The proton signals of one sugar ring associated with the anomic proton (\(\delta 5.28, J=8 \text{ Hz}\)) had coupling constants in the range \(J=8-10 \text{ Hz}\), indicating that the hexose was \(\beta\)-glucopyranose. The proton signals of the other sugar ring associated with the anomic proton (\(\delta 4.60, J=2 \text{ Hz}\)) had \(J=3 \text{ Hz}\) between H-2 and H-3, and \(J=10 \text{ Hz}\) between H-3 and H-4 and between H-4 and H-5. The methyl proton signal at \(\delta 1.10 (J=6 \text{ Hz})\) was also present at H-6 of the hexose. The second hexose was thus determined to be \(\alpha\)-rhamnopyranose.

The HMBC spectrum showed cross-peaks between H-1 of glucose and C-3 of malvidin and between H-6 of glucose and C-1 of rhamnose. This indicates binding of glucose to OH-3 of malvidin and binding of rhamnose to OH-6 of glucose through glycosidic bonds. This binding of rhamnose to OH-6 of glucose was also suggested by the lower-field shift of the proton signals of H2-6 of glucose. Therefore, the anthocyanin was determined to be malvidin 3-O-(6-O-(\(\alpha\)-rhamnopyanosyl)-\(\beta\)-glucopyranoside), that is, malvidin 3-rutinoside (1). \[^1\]H-NMR \(\delta_6:\) 1.10 (3H, d, \(J=6\), Rhm H-6), 3.27 (1H, t, \(J=10\), Rhm H-4), 3.35 (1H, dd, \(J=9, 10\), Glc H-4), 3.48 (1H, qd, \(J=6, 10\), Rhm H-5), 3.51 (1H, t, \(J=9\), Glc H-3), 3.54 (1H, dd, \(J=7, 11\), Glc H-6a), 3.56 (1H, dd, \(J=3, 10\), Rhm H-3), 3.60 (1H, dd, \(J=8, 9\), Glc H-2), 3.68 (1H, ddd, \(J=2, 7, 10\), Glc H-5), 3.74 (1H, dd, \(J=2, 3\), Rhm H-2), 3.95 (6H, s, Mv OCH\(_3\)), 4.11 (1H, dd, \(J=2, 11\), Glc H-6b), 4.60 (1H, d, \(J=2\), Rhm H-1), 5.28 (1H, d, \(J=8\), Glc H-1), 6.63 (1H, d, \(J=2\), Mv H-6), 6.88 (1H, brd, \(J=2\), Mv H-8), 7.92 (2H, s, Mv H-2', 6'), 9.80 (1H, brs, Mv H-4). \[^13\]C-NMR \(\delta_C:\) 19.7 (Rhm C-6), 59.1 (Mv OCH\(_3\)), 69.7 (Glc C-6), 71.6 (Rhm C-5), 73.2 (Glc C-4), 73.7 (Rhm C-2), 74.3 (Rhm C-3), 75.7 (Rhm C-4), 76.8 (Glc C-2), 79.4 (Glc C-5), 80.0 (Glc C-3), 97.3 (Mv C-8), 104.1 (Rhm C-1), 105.4 (Mv C-6), 105.6 (Glc C-1), 112.6 (Mv C-2', 6'), 115.4 (Mv C-10), 121.6 (Mv C-1'), 138.5 (Mv C-4), 147.5 (Mv C-3), 148.2 (Mv C-4'), 151.6 (Mv C-3', 5'), 159.7 (Mv C-9), 161.0 (Mv C-5), 165.6 (Mv C-2), 172.7 (Mv C-7). FABMS (JEOL JMS SX-102A system): \(m/z\) 369 (M\(^+\)) corresponding to C\(_{26}\)H\(_{37}\)O\(_{16}\)^{+}. UV-Vis \(\lambda_{max}\) (on-line HPLC): 280 nm and 530 nm.\]

The malvidin type of anthocyanins in most plants are generally malvidin 3-glucoside or 3,5-diglucoside, and the occurrence of malvidin 3-rutinoside is not common.\(^{2,3}\) At present, malvidin 3-rutinoside has been reported in flowers of *Sinningia speciosa*\(^6\) and *Gladiolus*\(^5\) and in fruits of *Liriope platyphylla*, *Parthenocissus tricuspidata*, *Berberis fortueta*,\(^{6}\) and *Buxus*\(^7\).

The contents of malvidin 3-rutinoside in the bracts of each cultivar were calculated on the basis of the absorbance peak area at 530 nm as analyzed by the first HPLC. The intensity of the bract color and the malvidin 3-rutinoside content in each *C. alismatifolia* cultivar were as follows: 'Pink Pearl', light pink, 330 nmol/g fresh weight; 'Shalom', pink, 550 nmol/g fresh weight; 'Red Shalom', deep pink, 920 nmol/g fresh weight. The concentration of malvidin 3-rutinoside corresponds with the intensity of the pink color of the bracts of these *C. alismatifolia* cultivars. Malvidin 3-rutinoside, which is the major anthocyanin in *C. alismatifolia* bracts, could therefore account for the pink bract color.

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