

## Characterization of anthocyanins in wild *Lycium ruthenicum* Murray

by HPLC-DAD/QTOF-MS/MS

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## Supplementary material

### ■ Fragmentation pathway of pure anthocyanins

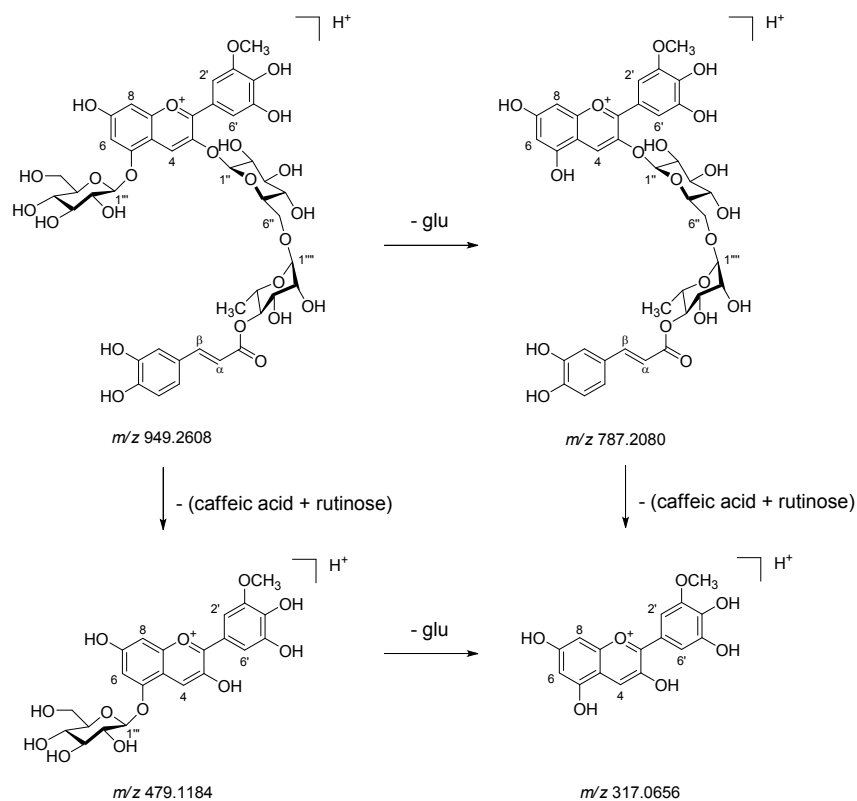


Fig. S1. Proposed fragmentation pathway of A1

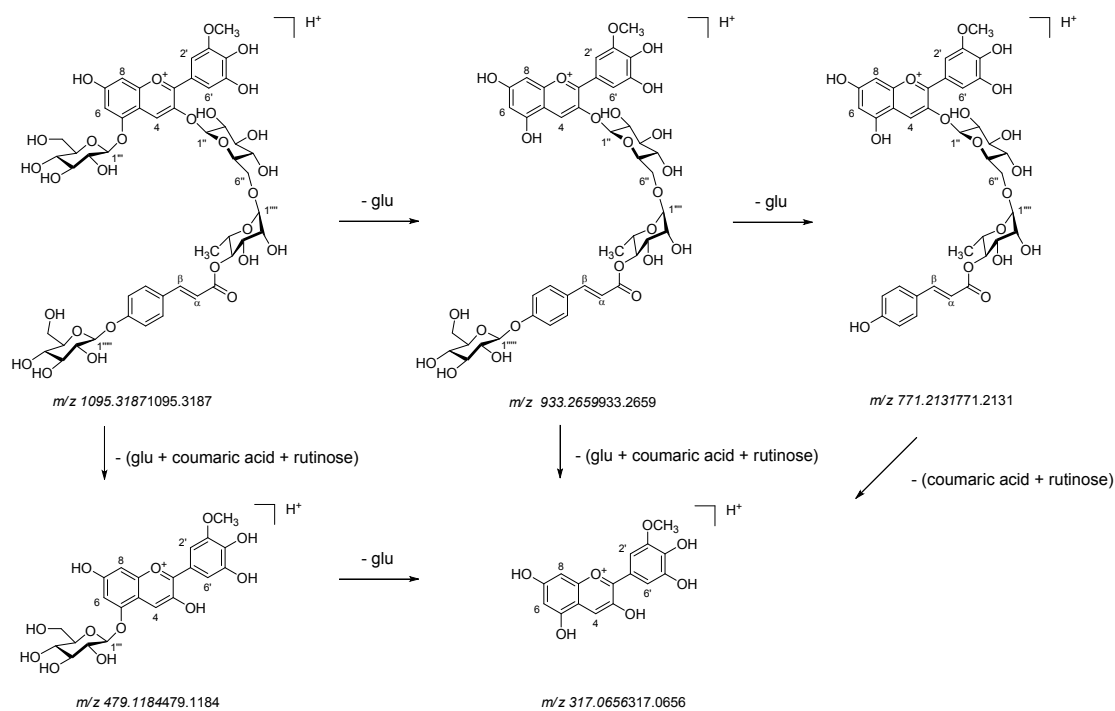


Fig. S2. Proposed fragmentation pathway of A5

■ Preparation of anthocyanin from *Lycium ruthenicum* Murray

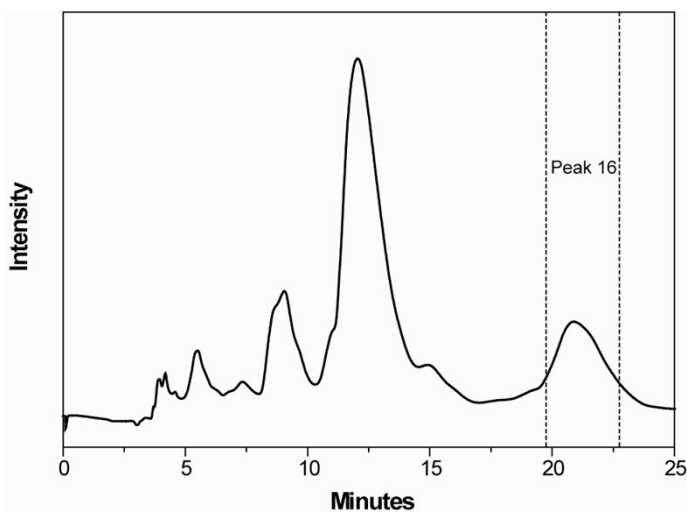


Fig. S3. Preparative chromatogram of the peak 16 from Fr. 12

■ Structure identification

Peak 16 in the extract with high purity was obtained from fraction 12, and identified by mass spectrum,  $^1\text{H}$  NMR and 2D NMR. It was identified as Malvidin 3-*O*-[6-*O*-(4-*O*-(4-*O*-*trans*-( $\beta$ -D-glucopyranoside)-*p*-coumaroyl)- $\alpha$ -L-rhamnopyranosyl)- $\beta$ -D-glucopyranoside]-5-*O*-[ $\beta$ -D-glucopyranoside], was isolated from *L. ruthenicum* for the first time. The specific structural identification results were as follows:

**Peak 16**, dark purple powder,  $[\text{M}+\text{H}]^+$ : 947.2798, calculated for  $\text{C}_{44}\text{H}_{53}\text{O}_{23}$ , 947.2798 with error 2.14 ppm;  $^1\text{H}$  NMR see Table S1. Compared with the literature [1], **Peak 16** was identified as Malvidin 3-*O*-[6-*O*-(4-*O*-(4-*O*-*trans*-( $\beta$ -D-glucopyranoside)-*p*-coumaroyl)- $\alpha$ -L-rhamnopyranosyl)- $\beta$ -D-glucopyranoside]-5-*O*-[ $\beta$ -D-glucopyranoside]. The chemical structure was showed in Figure S1.

Table S1 <sup>1</sup>H NMR data for prepared anthocyanin in CD<sub>3</sub>OD/TFA-*d* (95:5, v/v)

| H                            | Peak 16       |
|------------------------------|---------------|
| <i>Anthocyanidin</i>         |               |
| 4-H                          | 9.05 s        |
| 6-H                          | 7.07 s        |
| 8-H                          | 7.16 s        |
| 2'-H                         | 8.06 s        |
| 5'-H                         |               |
| 6'-H                         | 8.06 s        |
| 3'-OCH <sub>3</sub>          | 4.04 s        |
| 5'-OCH <sub>3</sub>          | 4.04 s        |
| <i>3-O-Glucopyranoside</i>   |               |
| 1''                          | 5.53 d (7.7)  |
| 2''                          | 3.84          |
| 3''                          | 3.75          |
| 4''                          | 3.63          |
| 5''                          | 3.71          |
| 6a                           | 3.98          |
| 6b                           | 4.05          |
| <i>5-O-Glucopyranoside</i>   |               |
| 1'''                         | 5.22 d (7.8)  |
| 2'''                         | 3.82          |
| 3'''                         | 3.73          |
| 4'''                         | 3.61          |
| 5'''                         | 3.72          |
| 6a                           | 3.85          |
| 6b                           | 3.86          |
| <i>6''-O-Rhamnopyranosyl</i> |               |
| 1''''                        | 4.74          |
| 2''''                        | 3.58          |
| 3''''                        | 3.56          |
| 4''''                        | 4.93 d (9.7)  |
| 5''''                        | 3.50          |
| -CH <sub>3</sub>             | 1.01 d (6.2)  |
| <i>Hydroxycinnamic acid</i>  |               |
| 2                            | 7.45 d (8.5)  |
| 3                            | 6.83 d (8.4)  |
| 5                            | 6.83 d (8.4)  |
| 6                            | 7.45 d (8.5)  |
| $\alpha$                     | 6.29 d (15.9) |
| $\beta$                      | 7.60 d (15.9) |

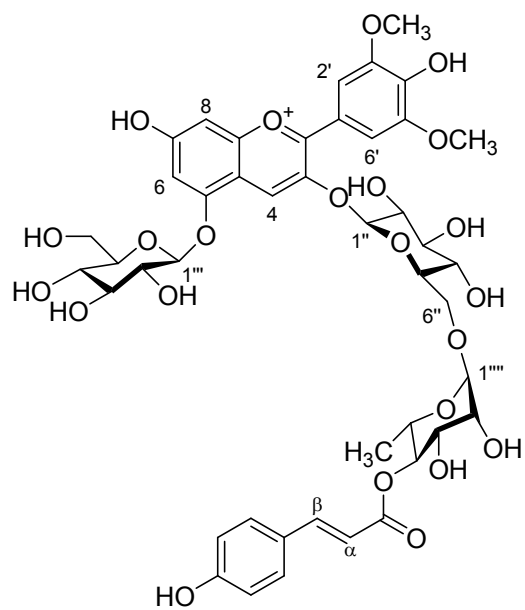


Fig. S5. The chemical structure of the prepared anthocyanin

## Reference

- [1] T. Ando, N. Saito, F. Tatsuzawa, T. Kakefuda, K. Yamakage, E. Ohtani, M. Koshi-ishi, Y. Matsusake, H. Kokubun, H. Watanabe, T. Tsukamoto, Y. Ueda, G. Hashimoto, E. Marchesi, K. Asakura, R. Hara, H. Seki, *Biochem. Syst. Ecol.* 27 (1999) 623.