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STRUCTURE OF LOBELININ A AND B, NOVEL ANTHOCYANINS ACYLATED WITH THREE AND FOUR DIFFERENT ORGANIC ACIDS, RESPECTIVELY

Tadao Kondo,[†*] Junko Yamashiki, Kiyoko Kawahori and Toshio Goto[*]
[†]Chemical Instrument Center, Nagoya University
Laboratory of Organic Chemistry, Faculty of Agriculture, Nagoya University
Chikusa, Nagoya 464, Japan

Structures of lobelinin A and B obtained from bluish violet petals of Lobelia erinus have been determined to be 3-coumarylrutinosyl-5-malonyl-glucosyl-3',5'-di(caffeylglucosyl)delphinidin and 3-coumarylrutinosyl-5-malonylglucosyl-3'-caffeylglucosyl-5'-ferulylglucosyldelphinidin. These pigments are stable in neutral aqueous solutions possibly by intra-molecular sandwich type stacking.

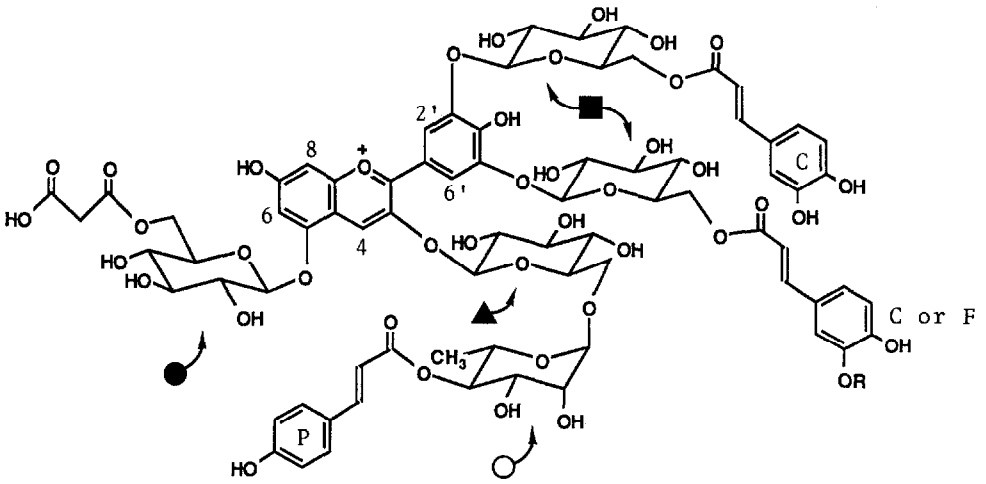
In 1977 Yoshitama[1)] isolated from bluish violet petals of Lobelia erinus a complex anthocyanin pigment and reported its structure to be caffeylferulyl-p-coumaryl delphinidin 3-rutinoside-5,3',5'-triglucoside. They also reported that this anthocyanin is unusually stable in neutral aqueous solution. We have isolated from the flower two anthocyanin pigments, lobelinin A (1) and lobelinin B (2), whose structures are reported herewith.

Lobelia petals (180 g) stored at -40 °C were extracted with 3% CF_3COOH(TFA)-methanol at room temp. The extract was concentrated, washed with ether, and chromatographed on an Amberlite XAD-7 column using stepwise elution from 1% aq TFA to 80% MeOH containing 1% TFA. The 50% methanol fraction containing pigments was dried up (1.45 g) and purified by preparative ODS-HPLC using a mixture of 3% H_3PO_4-solvent A (2:1) (solvent A = a mixture containing 20% acetic acid, 25% acetonitrile, and 55% water). To remove phosphoric acid from the eluates, the eluates were diluted with 1% TFA and passed through an ODS column to adsorb the pigment on the column, which was eluted with solvent A containing 1% TFA, yielding lobelinin A (1) TFA salt (75 mg) and lobelinin B (2) TFA salt (20 mg) as a dark red powder.

Lobelinin A (1) TFA salt[2)] gave a molecular ion peak at m/z 1653 (flavylium ion) in its FABMS. ^{1}H NMR spectrum[2)] showed a typical H-4 signal of anthocyanidin nucleus at 8.57 (br.s) ppm. A signal at 6.73 ppm was assigned to H-8 by observing a long range spin coupling between H-4 and H-8 in HOMOSD spectrum. A doublet (J = 2 Hz, H-6) at 6.92 ppm was spin-coupled with H-8. A very broad 2H signal appeared around 8.0 ppm at room temp became singlet above 30 °C, which is assignable to H-2' and 6'.[3)] Analysis of other signals appeared around the aromatic proton region in the spectrum measured at 50 °C

suggested the presence of a molecule of p-coumaric acid and two molecules of caffeic acid. The coupling constants (ca 16 Hz) between a and b proton signals indicated that all of the double bonds in these cinnamic acid derivatives have a trans configuration.

Five anomeric proton signals[4] present in the spectrum indicated the presence of five sugar moieties. To elucidate the structure of sugar portions, difference NOE, HOHAHA, and COSY spectra were measured and all signals corresponding to the protons in the five sugar moieties were assigned.[2] The signals corresponding to two sugar moieties (■ x 2) were completely overlapped. Four sugar moieties (■ x 2, ●, ▲) showed $J_{1,2}$ = 8 Hz and $J_{2,3} = J_{3,4} = J_{4,5}$ = 9.5 Hz, indicating that these are all β-glucopyranosyl. A remaining sugar (○) showed a 3H doublet at 1.14 ppm, and $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 3.5 Hz, $J_{3,4} = J_{4,5}$ = 9.5 Hz, and $J_{5,6}$ = 7 Hz, thus attributable to an α-rhamnopyranosyl structure.



Lobelinin A (1) R = H

Lobelinin B (2) R = CH_3

(One of two ■ is replaced by □)

Since the difference NOE spectrum obtained by irradiation at the ■-1 (5.39 ppm) showed negative NOE's on the H-2' and 6', two ■-glucose moieties are attached to 3'- and 5'-OH. Similarly, irradiation of ●-1 (5.22 ppm) or ▲-1 (4.96 ppm) showed NOE on H-6 or H-4, respectively, indicating that ●-glucose and ▲-glucose are attached to 5-OH and 3-OH, respectively. Two ■-glucose moieties attached at 3' and 5' are acylated at 6 position as evident from the chemical shifts of the ■-6 (4.34 and 4.72 ppm). All the signals belonging to two caffeic acid moieties are completely duplicated as well as the signals of

two ■-glucose moieties, suggesting that each caffeic acid is attached to the 6-position of each ■-glucose. Irradiation on the anomeric proton signal of rhamnose moiety (○) revealed NOE on ▲-6 (4.21 ppm), indicating that rhamnose is attached to ▲-6. Chemical shifts of ▲-6 and 6' (4.21 and 3.96 ppm) also verified this assignment (not acylated).

There are two remaining acid moieties, a p-coumaric acid and a malonic acid (deduced from M.W. of 1). ^{1}H NMR showed that ●-6 (4.60 & 4.46 ppm) and ○-4 (4.99 ppm) must be acylated. To determine the exact position of attachment of these acids on the sugars, lobelinin A (1) was hydrolyzed with 1M HCl-MeOH at 45 °C for 4.5 h followed by chromatography with an ODS column to give demalonyllobelinin A (3).[5] In ^{1}H NMR spectrum of 3, ●-6,6' signals were shifted upfield (4.04 and 3.78) about 0.6 ppm, thus concluding that malonic acid is attached to ●-6-OH and p-coumalic acid to ○-4-OH. Thus, structure of lobelinin A (1) is determined to be 3-O-(6-O-(4-O-(E)-p-coumaryl-α-L-rhamnopyranosyl)-β-D-glucopyranosyl)-5-O-(6-O-malonyl-β-D-glucopyranosyl)-3',5'-di-O-(6-O-(E)-caffeyl-β-D-glucopyranosyl)delphinidin.

Lobelinin B (2),[6] which is a pigment very similar to lobelinin A (1), showed a molecular ion peak at 1667 (flavylium ion); only difference is that 2 contains a ferulic acid moiety instead of a caffeic acid in 1. ^{1}H NMR signals corresponding to two substituents on 3' and 5' positions were not overlapped in contrast to the case of 1. Careful comparisons of NMR spectra of 2 with those of 1 deduced the structure of lobelinin B (2) to be 3-O-(6-O-(4-O-(E)-p-coumaryl-α-L-rhamnopyranosyl)-β-D-glucopyranosyl)-5-O-(6-O-malonyl-β-D-glucopyranosyl)-3'-O-(6-O-(E)-caffeyl-β-D-glucopyranosyl)-5'-O-(6-O-(E)-feruloyl-β-D-glucopyranosyl)delphinidin, whose composition is corresponding to that of the pigment reported by Yoshitama.[1]

These anthocyanins are very stable in neutral and weakly acidic aqueous solutions possibly by intramolecular sandwich type stacking as reported previously.[7]

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References and Notes

1) Yoshitama, K. Phytochem. 1977, 16, 1857.

2) Lobelinin A (1) TFA salt: ^{1}H NMR (500 MHz, 3% CF_3COOD-CD_3OD, 25 °C) δ ppm (J in Hz) 8.57 (1H, s, H-4), 8.07-7.82 (2H, br., H-2' & 6'), 7.36 (1H, d, 16, P-β), 7.14 (2H, d, 8.5, P-2 & 6), 6.97 (2H, d, 15.5, C-β), 6.92 (1H, d, 2, H-6), 6.73 (1H, d, 2, H-8), 6.70 (2H, d, 8.5, P-3 & 5), 6.46-6.25 (6H, br., C-2, 5 & 6), 5.98 (1H, d, 16, P-α), 5.92 (2H, br., C-α), 5.39 (2H, br., ■-1), 5.22 (1H, d, 8, ●-1), 4.99 (1H, t, 9.5, ○-4), 4.96 (1H, d, 8, ▲-1), 4.90 (at 50 °C)[4] (1H, br.d, 1, ○-1), 4.72 (2H, dd, 2 & 11.5, ■-6'), 4.60 (1H, dd, 2 & 12, ●-6'), 4.46 (1H, dd, 7 & 12, ●-6), 4.34 (2H, dd, 10 & 11.5, ■-6), 4.21 (1H, d, 11, ▲-6'), 4.04 (1H, dd, 3.5 & 9.5, ○-3), 4.01 (1H, dd, 1.5 & 3.5, ○-2). 3.96 (1H, dd, 2.5 & 11, ▲-6), 3.96 (1H, dd, 7 & 9.5. ○-5), 3.91-3.86 (2H, m, ■-5), 3.90-3.85 (3H, m, ○-5, ▲-4 & 5), 3.80(1H, dd, 8 & 9.5, ▲-2), 3.76 (1H, dd, 8 & 9.5, ●-2), 3.70-

3.66 (4H, m, ■-2 & 3), 3.66 (1H, t, 9.5, ▲-3), 3.65 (1H, t, 9.5, ●-3), 3.57 (1H, t, 9.5, ●-4), 3.41 (2H, t, 9, ■-4), 1.14 (3H, d, 7, O-6); NOE ▲-1 ⌒ H-4 (-14%), ●-1 ⌒ H-6 (-10%), ■-1 ⌒ H-2' & 6' (-15%), O-1 ⌒ ▲-6 (-2%).

3) The very broad signal around 8.0 ppm at room temp became a sharp singlet above 50 °C and two singlets below -20 °C. These phenomena can be explained by assuming a restricted rotation of B ring below room temp possibly by an intramolecular stacking of aromatic acids[7] in the two chains attached to 3' and 5' positions with the anthocyanidin nucleus.

4) One of five anomeric proton signals (O) lay under HOD at room temp, but it appeared at 50 °C.

5) Demalonyllobelinin A (3) TFA salt: ^{1}H NMR (500 MHz, 3% CF_3COOD-CD_3OD, 26 °C) δ ppm (J in Hz) 8.50 (1H, s, H-4), 8.01-7.55 (2H, br., H-2' & 5'), 7.35 (1H, d, 16, P-β), 7.17 (2H, d, 8.5, P-2 & 5), 6.97 (2H, d, 15.5, C-β), 6.90 (1H, d, 2, H-6), 6.71 (1H, d, 2, H-8), 6.71 (2H, d, 8.5, P-3 & 5), 6.51-6.41 (2H, br., C-6), 6.38-6.23 (4H, br., C-2 & 5), 5.97 (1H, d, 16, P-α), 5.96-5.83 (2H, br., C-α), 5.36 (2H, br.d, 5, ■-1), 5.25 (1H, d, 7.5, ●-1), 4.97 (1H, t, 9.5, O-4), 4.92 (1H, d, 7.5, ▲-1), 4.89 (1H, br.s, O-1), 4.68 (2H, dd, 1.5 & 11, ■-6'), 4.37 (2H, dd, 9.5 & 11, ■-6), 4.18 (1H, d, 11, ▲-6), 4.04 (1H, dd, 2.5 & 12.5, ●-6'), 3.99 (1H, dd, 4 & 11, ▲-6'), 3.95 (1H, O-2), 3.88 (2H, dt, 2 & 11, ■-5), 3.80 (1H, dd, 7.5 & 9, ▲-2) 3.78 (1H, dd, 7.5 & 12.5, ●-6), 3.72 (1H, dd, 7.5 & 9, ●-2), 3.69-3.64 (4H, m, ■-2 & 3), 3.66 (1H, t, 9, ●-3), 3.52 (1H, t, 9, ●-4), 3.39 (2H, t, 9.5, ■-4), 1.15 (3H, d, 5, O-6).

6) Lobelinin B (2) TFA salt: ^{1}H NMR (500 MHz, 5%CF_3COOD-CD_3OD, 25 °C) δ ppm (J in Hz) 8.52 (1H, s, H-4), 7.94-7.77 (2H, br., H-2' & 6'), 7.28 (1H, d, 16, P-β), 7.06 (2H, d, 9, P-2 & 6), 7.03 (1H, d, 16, F or C-β), 6.90 (1H, d, 16, F or C-β), 6.83 (1H, d, 2, H-6), 6.61 (2H, d, 9, P-3 & 5), 6.60 (1H, d, 2, H-8), 6.47-6.41 (1H, br., F or C-5), 6.38 (1H, d, 1.5, F or C-2), 6.36-6.29 (2H, br., F or C-5 & 6), 6.27-6.20 (2H, br., F or C-2 & 6), 5.92 (1H, br., F or C-α), 5.91 (1H, d, 16, P-α), 5.86-5.78 (1H, br., d, 16, F or C-α), 5.45-5.41 (1H, br.s, ■-1), 5.37-5.32 (1H, br.s, □-1), 5.25 (1H, d, 8, ●-1), 5.02 (1H, t, 10, O-4), 4.98 (1H, d, 7.5, ▲-1), 4.88 (at 50 °C)[4] (1H, br.d, 1, O-1), 4.80 (1H, dd, 2 & 11.5, ■-6'), 4.76 (1H, dd, 2 & 11.5, □-6'), 4.65 (1H, dd, 2 & 12, ●-6'), 4.47 (1H, dd, 7 & 12, ●-6), 4.37 (2H, dd, 10 & 11.5, ■ & □-6), 4.21 (1H, d, 11, ▲-6'), 4.08 (1H, dd, 3 & 10, O-3), 4.04 (1H, dd, 2 & 3, O-2), 4.00 (1H, dd, 2.5 & 11, ▲-6), 3.97 (1H, dd, 6 & 10, O-5), 3.96-3.92 (1H, m, ■-5), 3.93-3.88 (1H, m, ●-5), 3.89 (1H, dd, 8.5 & 10, O-5), 3.90-3.81 (2H, m, ■-3 & 5), 3.83 (1H, dd, 8 & 9.5, ▲-2), 3.79 (1H, dd, 8 & 9.5, ●-2), 3.75-3.69 (4H, m, ■-2 & 3 and □-2 & 3), 3.69 (3H, s, CH_3O), 3.71-3.67 (1H, t, 9.5, ●-3), 3.67 (1H, t, 8, ▲-4), 3.59 (1H, t, 9.5, ●-4), 3.46 (1H, t, 10, ■-4), 3.44 (1H, t, 10, □-4), 1.04 (3H, d, 6, O-6).

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