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# Structure of Monoacylated Anthocyanins Isolated from Red Cabbage, Brassica oleracea#

Eiichi IDAKA, Kaneyoshi SUZUKI, Hiroshi YAMAKITA, Toshihiko OGAWA,
Tadao KONDO,*+ and Toshio GOTO *++
Department of Chemistry, Faculty of Engineering, Gifu University,
Yanagido, Gifu 501-11
+Chemical Instrument Center, Nagoya University, Chikusa, Nagoya 464
++Laboratory of Organic Chemistry, Faculty of Agriculture,
Nagoya University, Chikusa, Nagoya 464

Five monoacylated anthocyanins from Brassica oleracea were isolated and their structures determined to be 3-O-(6-O-acyl-2-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl)cyanidins, in which the acyl group is p-coumaryl, feruly1, sinapyl, 4-O-(β-D-glucopyranosyl)-E-p-coumaryl, or 4-O-(β-D-glucopyranosyl)-E-feruly1.

The anthocyanin pigments extracted from red cabbage have wide usefulness for food industries as safe natural coloring materials. During the past five decades,[1-7] several anthocyanins have been isolated from red cabbage and charaterized by hydrolysis to be acylated anthocyanins consisting of a common skeleton, cyanidin 3-sophoroside-5-glucoside, acylated by one or two molecules of the organic acid such as malonic, sinapic, ferulic, or p-coumaric acid on the sophorose moiety,[7] but the acylating position has not rigorously been determined because of the lack of authentic samples corresponding to the hydrolysis products.

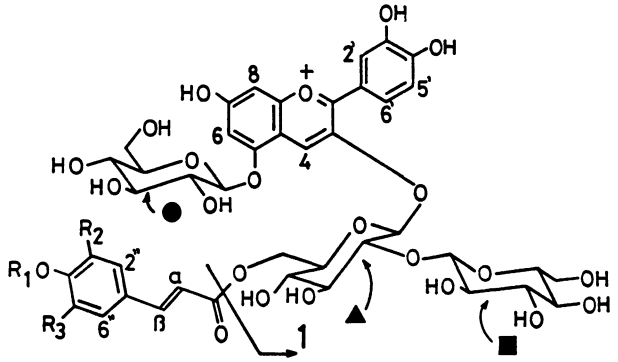
In this paper we wish to report determination of the complete structures and stereochemistry of three known and two new monoacylated anthocyanins isolated from Brassica oleracea.

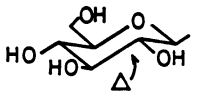
Deep reddish purple coloring leaves of red cabbages (10 Kg) were deep-freezed with liq. nitrogen, powdered by a blender, and extracted with MeOH containing 1% trifluoroacetic acid (TFA). The extract was chromatographed on an Amberlite XAD-7 column by stepwise elution from $H_2O$ to MeOH containing 1% TFA. The water fraction (Fr.) mainly contained an anthocyanin (**1**), 25% MeOH Fr. a mixture of two (**2** and **3**; a ratio of 1:1), and 30% MeOH Fr. a mixture of three (**4**, **5**, and **6**). Each fraction was further separated by preparative ODS-HPLC using a solvent system consisting of a suitable proportion of AcOH-$CH_3CN$-$H_2O$ containing 1% $H_3PO_4$, and purified by precipitation with MeOH-$Et_2O$ containing 1% HCl. The chloride **1** (160 mg), **2** (3 mg), **3** (5 mg) and **4** (272 mg) were obtained as a dark-red amorphous powder, respectively. A mixture of the chloride **5** and **6** (1:1, 67 mg) thus obtained was further separated by paper chromatography using HCl-BuOH-$H_2O$ (3 : 50 : 50) to give dark-red amorphous solids (yields; **5**, 1.9 mg and **6**, 1 mg).[8]

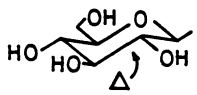
# Dedicated to Professor Teruaki Mukaiyama on the occasion of his 60th birthday.

On hydrolysis with 2% NaOH in aq. MeOH followed by treatment with 3% TFA in MeOH, **4**, **5**, and **6** afforded, besides **1** as the anthocyanin fraction, methyl p-coumarate, methyl ferulate, and methyl sinapate, respectively. The aromatic acids were identified by HPLC comparison with authentic samples. Assignments of all signals in $^1$H NMR spectra of these anthocyanins, **1-6**, were carried out by means of the 2D COSY, homo-spin-decouplings and low temperature difference NOE experiments.[8)]

Fast atom bombardment mass spectrum (FABMS) of **1** indicates the molecular ion peak at m/z 773. The $^1$H NMR of **1** shows the presence of cyanidin and three hexosides, all of which are deduced to be β-D-glucopyranosides[9)] from their coupling constants ($J_{1,2}$=7.5 Hz, and $J_{2,3}$=$J_{3,4}$=$J_{4,5}$=9.0 Hz). H-4 in the flavylium skeleton appears at the lowest field (9.01 ppm).[10)] A proton at 7.11 ppm having a long-range coupling to H-4 (J=0.5 Hz) can be assigned to H-8, which is further spin-coupled with H-6 (7.07 ppm; J=2 Hz). The positions of the glycosidic linkages (each glucose is differentiated by geometric symbols) were determined as follows; irradiation at each of ▲-1 (5.51 ppm) and ●-1 (5.18 ppm) caused a negative NOE to H-4 (-12%, at -10 °C) and to H-6 (-8%), but no effect to H-8. $H_2O_2$ oxidation of **1** gave sophorose. Thus, the structure of **1** is determined to be 3-O-(2-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl)cyanidin.



$R_1$ =  $R_2$ = $R_3$ = H **2**

$R_1$ =  $R_2$ = $OCH_3$ $R_3$ = H **3**

$R_1$ = $R_2$ = $R_3$ = H **4**

$R_1$ = $R_3$ = H $R_2$ = $OCH_3$ **5**

$R_1$ = H $R_2$ = $R_3$ = $OCH_3$ **6**

$^1$H NMR and FABMS (m/z 919) of **4** show the presence of E-p-coumaric acid ($J_{\alpha,\beta}$= 16 Hz) and **1**. In comparison with $^1$H NMR spectrum of **1**, the signals[11)] of ▲-$CH_2$O- group (4.44 and 4.50 ppm) are shifted to lower fields about 0.8 ppm, indicating that this group is acylated. Hence, the structure of **4** is determined to be 3-O-(6-O-(E-p-coumaryl)-2-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl)cyanidin. Similarly, the structures of **5** and **6** (FABMS: m/z 949 and 979, respectively) are elucidated to be **1** esterified with E-ferulic and E-sinapic acid, respectively, at the 6 position of ▲-glucose.

Analysis of the 2D COSY spectrum of **2** (FABMS: m/z 1081) indicates the presence of one molecule each of cyanidin and p-coumaric acid, and four molecules of hexoses. Since all vicinal J values of ▲-, ●-, and ■-hexoses except △ are 7.5-9.0 Hz, the hexoses must be β-D-glucopyranosides. Irradiation of anomeric protons, ▲-1, ●-1, ■-1, and △-1, produces strong negative NOE on H-4 (-32%), H-6 (-21%), ▲-2 (-17%), and H-3" and H-5" (-17%), respectively, indicating the structure of **2** to be **1** acylated with 4-O-hexosyl-p-coumaric acid. The chemical shifts of △-2 and △-3 are very close, so that the multiplicities are too complicated to be analyzed. In order to simplify the spectrum, $^1$H NMR of the pertrifluoroacetate of **2** was recorded according to our procedure.[12)] The J values from H-1 to H-5 of the

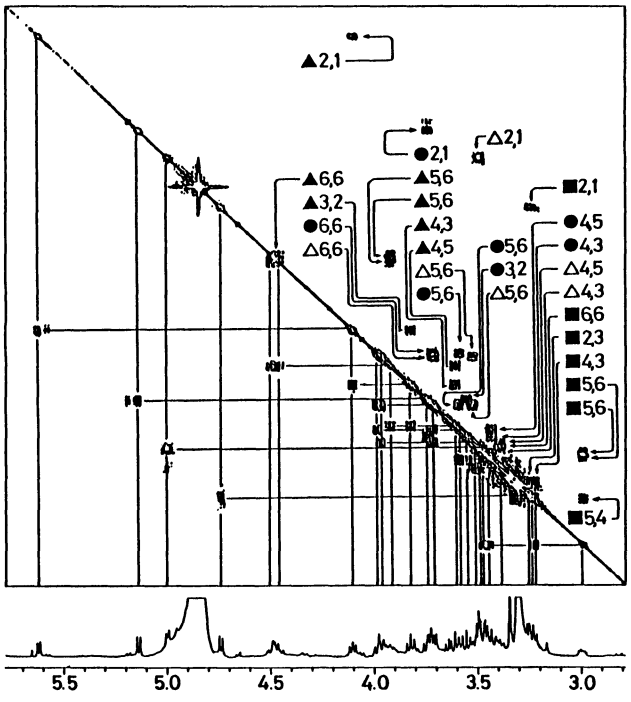



Fig. 1. $^{1}H$-$^{1}H$ COSY spectrum of the sugar region of **2** at 500 MHz in 8% $CF_3COOD$-$CD_3OD$.

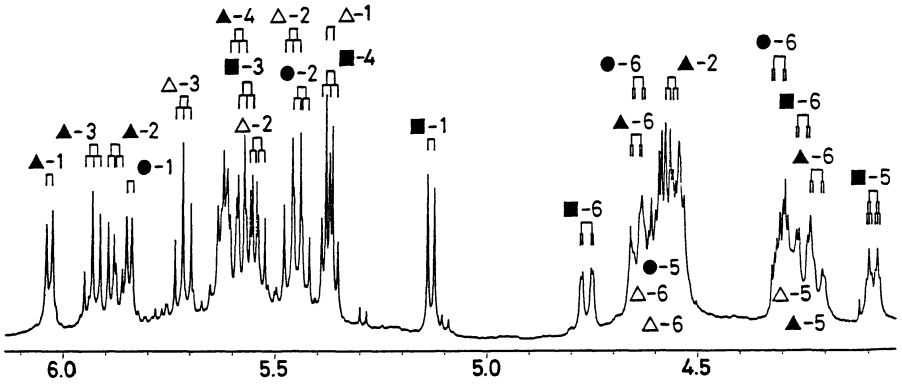



Fig. 2. $^{1}H$ NMR of the sugar region of pertrifluoroacetate of **2** in $(CF_3CO)_2O$-$CDCl_3$ (1:2).

△-hexose moiety are 7.0-9.5 Hz (Fig. 2), indicating that △ is also β-D-glucopyranoside. Thus **2** must be 3-O-(2-O-(β-D-glucopyranosyl)-6-O-(4-O-(β-D-glucopyranosyl)-E-p-coumaryl)-β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl)cyanidin. The $^{1}H$ NMR of **3** (FABMS m/z 1111) is similar to that of **2**, but the spin system in the aromatic region indicated the presence of E-ferulic acid in place of p-coumaric acid. Thus **3** is the derivative of **1** esterified at the ▲-6 position with β-D-glucopyranosyl-E-ferulic acid.

Red cabbage contains, besides these monoacylated anthocyanins, several diacylated anthocyanins, and their structural elucidation is now in progress.

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References

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8) Spectral data of the monoacylated anthocyanins ( **1** - **6** ).

| | **1** | **2** [a] | **3** [a] | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| UV (0.01% HCl) $\lambda_{max}$ | 511 | 521 | 523 | 520 | 523 | 523 |
| | – | 305 | 315 | 314 | 328 | 330 |
| | 275 | 279 | 279 | 279 | 280 | 280 |
| $E_{UV\ max}/E_{Vis\ max}$ | – | 0.64 | 0.58 | 0.58 | 0.62 | 0.56 |
| $^1$H NMR (8% $CF_3COOD$-$CD_3OD$, at 25 °C, δ ) | | | | | | |
| H-4 | 9.10 | 8.91 | 8.93 | 8.94 | 8.97 | 8.97 |
| H-6 | 7.07 | 6.97 | 6.96 | 6.99 | 6.99 | 6.99 |
| H-8 [b] | 7.11 | 6.89 | 6.89 | 6.92 | 6.92 | 6.88 |
| H-2' | 8.08 | 8.01 | 8.01 | 8.01 | 8.02 | 8.01 |
| H-5' | 7.09 | 7.06 | 7.06 | 7.05 | 7.08 | 7.07 |
| H-6' [c] | 8.03 | 8.25 | 8.27 | 8.25 | 8.27 | 8.29 |
| H-2" | – | 7.34 | 6.96 | 7.25 | 6.90 | 6.64 |
| H-3" | – | 7.07 | – | 6.76 | – | – |
| H-5" | – | 7.07 | 7.13 | 6.76 | 6.79 | – |
| H-6" [d] | – | 7.34 | 6.97 | 7.25 | 6.89 | 6.64 |
| H-α | – | 6.24 | 6.24 | 6.15 | 6.16 | 6.16 |
| H-β | – | 7.34 | 7.32 | 7.33 | 7.33 | 7.31 |
| $CH_3O$ | – | – | 3.84 | – | 3.85 | 3.82 [e] |
| ▲-1 | 5.51 | 5.63 | 5.63 | 5.61 | 5.61 | 5.61 |
| ▲-2 | 4.08 | 4.11 | 4.11 | 4.10 | 4.11 | 4.11 |
| ▲-3 | 3.78 | 3.83 | 3.83 | 3.82 | 3.84 | 3.85 |
| ▲-4 | 3.50 | 3.61 | 3.64 | 3.62 | 3.67 | 3.71 |
| ▲-5 | 3.62 | 3.93 | 3.92 | 3.91 | 3.91 | 3.90 |
| ▲-6 | 3.93 | 4.46 | 4.44 | 4.44 | 4.42 | 4.40 |
| ▲-6 | 3.95 | 4.51 | 4.54 | 4.50 | 4.56 | 4.61 |
| ●-1 | 5.18 | 5.14 | 5.14 | 5.16 | 5.16 | 5.16 |
| ●-2 | 3.69 | 3.75 | 3.74 | 3.74 | 3.75 | 3.76 |
| ●-3 | 3.55 | 3.56 | 3.55 | 3.56 | 3.56 | 3.56 |
| ●-4 | 3.55 | 3.42 | 3.42 | 3.45 | 3.45 | 3.46 |
| ●-5 | 3.58 | 3.59 | 3.58 | 3.59 | 3.58 | 3.57 |
| ●-6 | 3.73 | 3.72 | 3.69 | 3.62 | 3.72 | 3.70 |
| ●-6 | 3.75 | 3.99 | 3.97 | 3.97 | 3.96 | 3.95 |
| ■-1 | 4.79 | 4.74 | 4.74 | 4.75 | 4.75 | 4.74 |
| ■-2 | 3.19 | 3.25 | 3.25 | 3.25 | 3.26 | 3.23 |
| ■-3 | 3.29 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 |
| ■-4 | 3.22 | 3.24 | 3.23 | 3.45 | 3.23 | 3.2 |
| ■-5 | 2.95 | 3.01 | 3.01 | 3.01 | 3.03 | 3.04 |
| ■-6 | 3.44 | 3.4 | 3.4 | 3.46 | 3.47 | 3.47 |
| ■-6 | 3.48 | 3.5 | 3.5 | 3.50 | 3.52 | 3.54 |
| △-1 | – | 5.00 | 5.01 | – | – | – |
| △-2 | – | 3.5 | 3.55 | – | – | – |
| △-3 | – | 3.5 | 3.51 | – | – | – |
| △-4 | – | 3.44 | 3.40 | – | – | – |
| △-5 | – | 3.53 | 3.5 | – | – | – |
| △-6 | – | 3.72 | 3.70 | – | – | – |
| △-6 | – | 3.97 | 3.96 | – | – | – |

b; J=0.5 & 2.0 Hz.
c; J=2.0 & 9 Hz.
d; J=16 Hz.
e; two $CH_3O$-.

a; the molecular formulae of **2** and **3** are $C_{48}H_{57}O_{28}$ and $C_{49}H_{59}O_{29}$ respectively.

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