Tetrahedron Letters,Vol.24,No.21,pp 2181-2184,1983 0040-4039/83/212181-04$03.00/0
Printed in Great Britain

STRUCTURE OF PLATYCONIN, A DIACYLATED ANTHOCYANIN ISOLATED FROM THE CHINESE BELL-FLOWER PLATYCODON GRANDIFLORUM

Toshio Goto*, Tadao Kondo†, Hirotoshi Tamura, and Kiyoko Kawahori
Laboratory of Organic Chemistry, Faculty of Agriculture, Nagoya University and
†Chemical Instruments Center, Nagoya University; Chikusa, Nagoya 464, Japan

Hiroyuki Hattori
National Institute for Basic Biology, Okazaki 444, Japan

Structure of platyconin is determined to be 3-O-(6-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl)-7-O-(6-O-(trans-4-O-(6-O-(trans-4-O-(β-D-glucopyranosyl)-caffeoyl)-β-D-glucopyranosyl)caffeoyl)-β-D-glucopyranosyl)delphinidin.

Acylated anthocyanins having more than one acyl group have unusually good stability[1,2] in neutral and weakly acidic aqueous solutions, in which usual anthocyanins are mostly hydrated to form the corresponding colorless pseudobase. An intramolecular interaction between the anthocyanin and the organic acids has been suggested.[1,2] Platyconin, one of such stable anthocyanins, was isolated by Saito et al. from the flower petals of Chinese bell-flower Platycodon grandiflorum (Japanese name: kikyo) as its crystalline chloride[3] and anhydrobase.[1] They suggested its structure to be delphinidin 3-dicaffeoylrutinosido-5-glucoside from the chemical evidence.[1,3] To clarify the cause of unusual stability of this anthocyanin, it must be necessary first to determine its complete structure and stereochemistry, which we report herewith.

Platyconin was extracted from deep-freezed flower petals of P. grandiflorum with 0.5% HCl-MeOH. The extract was concentrated and washed with ether. The remaining aqueous solution was evaporated to dryness, dissolved in water, and chromatographed on an Amberlite XAD-7 column using aqueous methanol as eluant to give a crude pigment. It was purified by ODS (Nomura Develosil) HPLC [solvent: CF_3COOH-CH_3COOH-CH_3CN-H_2O (1.0:6:7.5:85.5) to give pure platyconin (1) as its chloride (reprecipitated from its HCl-MeOH solution with ether) [red powder blackened over 112 °C without melting until 260 °C (lit.[3] mp 193-195 °C, dec); FAB-mass m/z = 1422 (M+1); Calcd for $C_{63}H_{73}O_{37}^+$ = 1421; UV-VIS and PMR[4]].

Platyconin (1) was hydrolyzed with 1% NaOH in 50% MeOH under Ar atmosphere at 0 °C for 15 min. The reaction mixture was acidified with HCl and evaporated to dryness. The residue was dissolved in 1% HCl-EtOH, centrifuged to remove NaCl, and evaporated to dryness. The residue was dissolved in a 1:5/32 mixture of 1% aq CF_3COOH (solvent A) and CH_3CN-AcOH-H_2O (25:20:55) containing 1% CF_3COOH (solvent B) and fractionated by gradient ODS HPLC using the solvents from A:B = 1:5/32 to 1:1. Monitoring was made at 295 and 520 nm absorptions.

The pigment fractions were evaporated to dryness to give, after reprecipitation from its HCl-MeOH solution with ether, bisdeacylplatyconin (2) chloride [dark red powder melted at 136-146 °C with blackening; FAB-mass m/z 774 (M+1); Calcd for $C_{33}H_{41}O_{21}^{+}$ = 773; UV-VIS and PMR[5]].

The fractions having UV absorption at 295 nm were combined and purified by gradient ODS HPLC using the solvents from A:B = 1:0.5 to 1:1 to afford methyl 4-O-(β-D-glucopyranosyl)-caffeate (3), whose PMR spectrum was completely identical with that of the authentic sample;[6] the 3-O-glucosyl isomer or free methyl caffeate was not detected.

Saito et al.[3] also reported that bisdeacylplatyconin (2) was identical with the deacylation product of violanin[7] and hence must be delphinidin 3-rutinosido-5-glucoside. However, our HPLC analysis disclosed that they are not identical; 2 was clearly distinguished from deacyl-violanin by gradient ODS HPLC from A:B = 1:1/8 to 1:1 [A = 1.5% aq H_3PO_4; B = CH_3CN-AcOH-H_2O (25:20: 55) containing 1.5% H_3PO_4; detected at 295 nm].

Structure of bisdeacylplatyconin (2) was determined to be delphinidin 3-rutinosido-7-glucoside as follows: 1) Rutinose [6-O-(α-L-rhamnopyranosyl)glucose] must be attached at 3 position, for it was isolated from platyconin (1) by treatment with H_2O_2 followed by ammonolysis[8] and identified by comparison of its CMR spectrum with that of authentic rutinose. The position of attachment was further confirmed by NOE (Fig. 2). 2) PMR and FAB-mass spectra of 2 indicated that it consists of delphinidin, one molecule each of rutinose and a hexose. 3) The hexose was determined to be glucose in the β-pyranoside form by a spin-spin decoupling analysis of the sugar part signals in the PMR spectra of pertrifluoroacetate of bisdeacylplatyconin (2) (Fig. 1); all of the $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ of the hexose moiety are 8-10 Hz. 4) That the glucose is attached at 7 position was deduced from NOE (Fig. 2). Incidentally, the assignment of the H-8 signal of delphinidin nucleus was done by the observation of a spin-spin coupling (0.5 Hz) between H-4 and H-8 signals.

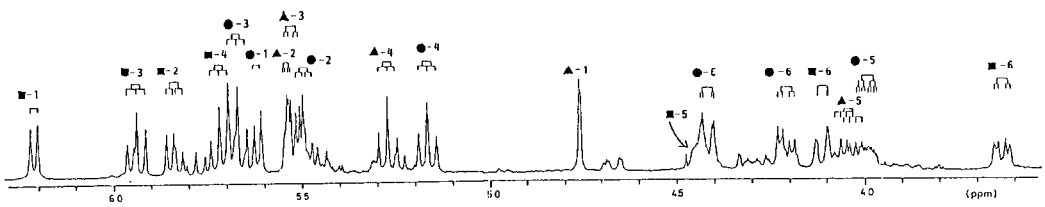

Fig. 1. PMR spectrum (400 MHz) of pertrifluoroacetate of bisdeacylplatyconin (2) (2 was dissolved in a mixture of $(CF_3CO)_2O$ and C_6D_6 (3:2))

Platyconin (1) consists of bisdeacylplatyconin (2) and two molecules of trans-4-O-(β-D-glucopyranosyl)caffeic acid as evident from the FAB-mass and PMR spectra[4] (Fig. 3). Analysis of the PMR spectrum by the spin-spin decoupling and partially relaxed Foruier transform methods[9] showed that the signals corresponding to two $-CH_2O-$ groups in glucose moieties

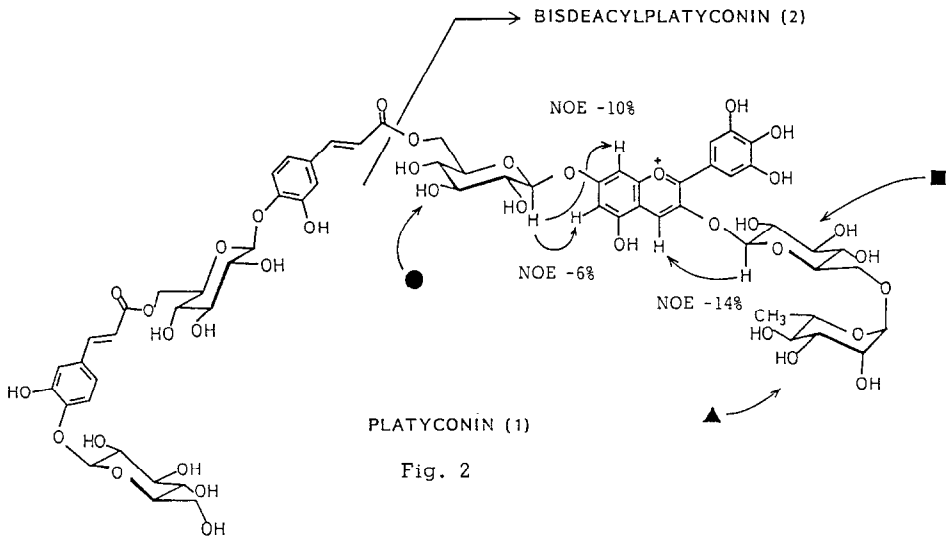

Fig. 2

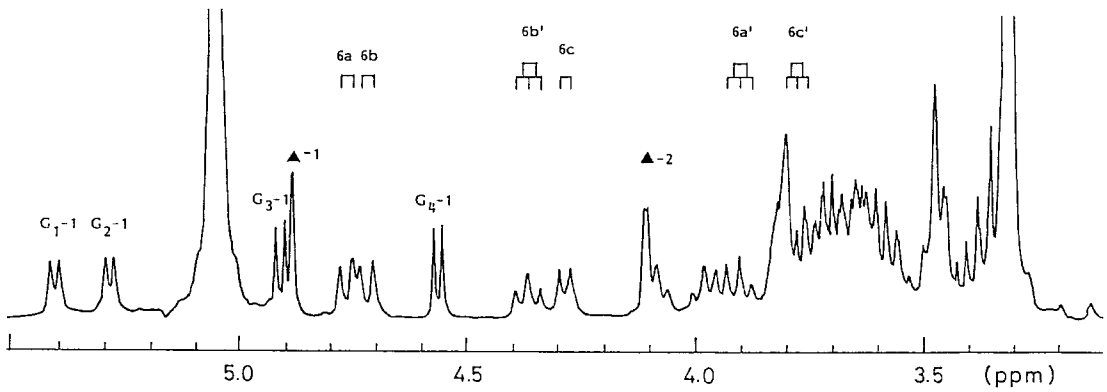

Fig. 3. PMR spectrum (400 MHz) of platyconin (1)
(in 1% DCl-CD_3OD)

are shifted more than 0.5 ppm toward lower fields than those of $-CH_2O-$ groups in 2, indicating that two caffeic acid moieties in 1 are attached at the 6 position of two glucose moieties. All of four glucose moieties in 1 exist in the β-pyranosyl form, for the coupling constants of their anomeric proton signals are 8-10 Hz. Thus the structure of platyconin (1) is determined to be 3-O-(6-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl)-7-O-(6-O-(trans-4-O-(6-O-(trans-4-O-(β-D-glucopyranosyl)caffeoyl)-β-D-glucopyranosyl)caffeoyl)-β-D-glucopyranosyl)delphinidin.

Platyconin (1) (quinonoidal base form) is quite stable in weakly acidic aqueous solutions (Fig. 4), whereas bisdeacylplatyconin (2) is easily hydrated to the corresponding pseudobase.[1]

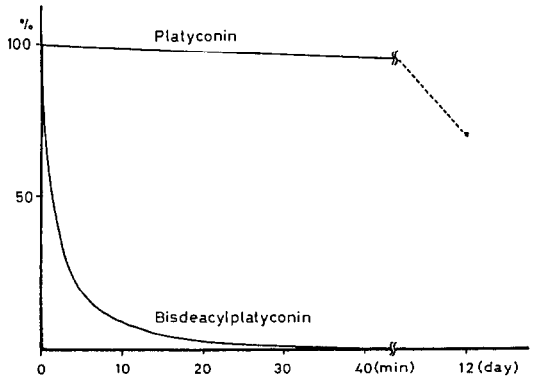

Fig. 4. Stability of platyconin (1) and bisdeacylplatyconin (2) in aq. solution at pH 5.0 (conc. 4×10^{-5}M in acetate buffer; observed at λ of the visible absorption maximum)

Two acyl groups in 1 prevent the anthocyanidin chromophore from the attack of water molecule similar to the case of gentiodelphin as discussed in the previous paper.[10] Indeed, construction of a CPK model of 1 having the anthocyanidin part sandwiched between the acyl groups is possible.

Acknowledgements —— We thank Mr Y. Ohnishi and Mr T. Okada, the University Farm, for cultivating P. grandiflorum. We also thank JEOL Co. Ltd. for measurements of 400 MHz PMR spectra.

REFERENCES AND FOOTNOTES

1. N. Saito, Y. Osawa and K. Hayashi, Bot. Mag. Tokyo, 85, 105 (1972).
2. K. Yoshitama and K. Hayashi, Bot. Mag. Tokyo, 87, 33 (1977); S. Asen, R. N. Stewart and K. H. Norris, Phytochem., 11, 1139 (1972); K. Yoshitama, Bot. Mag. Tokyo, 91, 207 (1978).
3. N. Saito, Y. Osawa and K. Hayashi, Phytochem., 10, 445 (1971).
4. Platyconin (1) chloride: UV-VIS (0.01% HCl-MeOH) nm (log ε) 549 (4.54), 323 (sh 4.43), 303 (sh 4.51), 286 (4.63), 237 (4.60); E_{320}/E_{549} = 0.779, E_{440}/E_{549} = 0.175; PMR (400 MHz, 1% DCl-CD_3OD) ppm 8.54 (1H, s), 7.76 (2H, s), 7.55 (1H, d, J=16 Hz), 6.97 (1H, br.s), 6.90 (1H, d, J=16 Hz), 6.88 (1H, d, J=1.5 Hz), 6.83 (1H, dd, J=1.5 & 7.5 Hz), 6.77 (1H, d, J=1.5 Hz), 6.71 (1H, d, J=1.5 Hz), 6.67 (1H, d, J=7.5 Hz), 6.57 (1H, d, J=7.5 Hz), 6.53 (1H, d, J=16 Hz), 6.35 (1H, dd, J=1.5 & 7.5 Hz), 6.11 (1H, d, J=16 Hz), 1.25 (3H, d, J=6.5 Hz), for other signals, see Fig. 3.
5. Bisdeacylplatyconin (2) chloride: UV-VIS (0.01% HCl-MeOH) nm (log ε) 540 (4.59), 350 (3.71), 300 (sh 3.98), 282 (4.32); E_{440}/E_{540} = 0.174; PMR (400 MHz, 1% DCl-CD_3OD) ppm 8.85 (1H, s), 7.83 (2H, s), 7.27 (1H, br.s), 6.92 (1H, d, J=1.5 Hz), 5.42 (1H, d, J=8 Hz), 5.28 (1H, d, J=8 Hz), 4.68 (1H, d, J=1.0 Hz), 1.17 (3H, d, J=6.5 Hz), no signal between 4.2 and 5.0 ppm except the signal at 4.68 ppm.
6. T. Goto, T. Kondo, H. Imagawa and I. Miura, Tetrahedron Lett., 22, 3213 (1981).
7. T. Goto, S. Takase and T. Kondo, Tetrahedron Lett., 2413 (1978).
8. P. Karrer and G. de Meuron, Helv. Chim. Acta, 15, 507 (1932); K. Hayashi ed., "Shokubutsu Shikiso", Yokendo, Tokyo, 1980, p 168.
9. T. Goto, H. Imagawa, T. Kondo and I. Miura, Heterocycles, 17, 355 (1982).
10. T. Goto, T. Kondo, H. Tamura, H. Imagawa, A. Iino and K. Takeda, Tetrahedron Lett., 23, 3695 (1982).

(Received in Japan 21 February 1983)