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HEAVENLY BLUE ANTHOCYANIN II[1]

TRANS-4-O-(6-O-(TRANS-3-O-(β-D-GLUCOPYRANOSYL)CAFFEYL)-β-D-GLUCOPYRANOSYL)-CAFFEIC ACID, A NOVEL COMPONENT IN HEAVENLY BLUE ANTHOCYANIN

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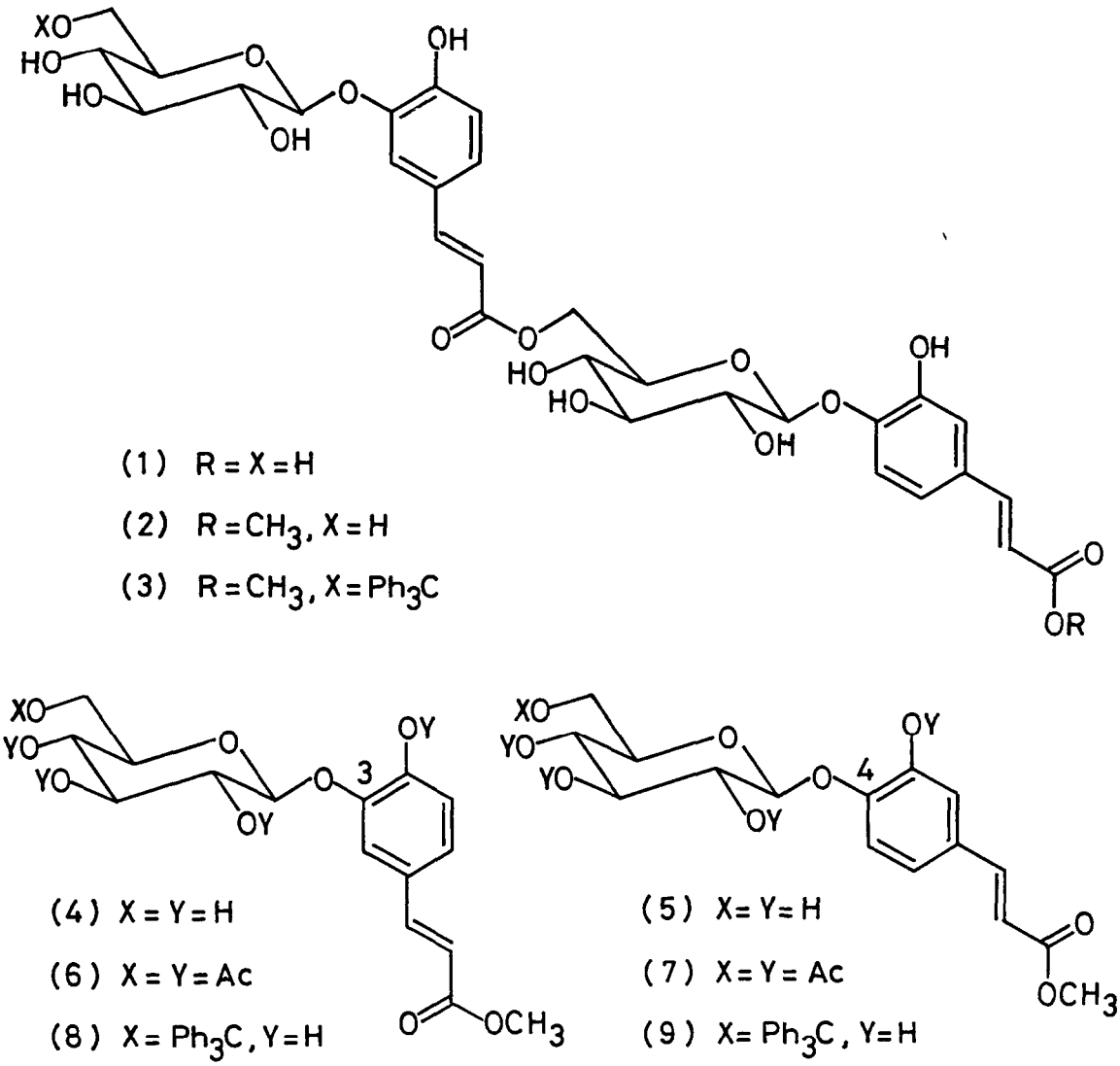
Summary: Structure of an alkaline hydrolysis product of heavenly blue anthocyanin was determined to be trans-4-O-(6-O-(trans-3-O-(β-D-glucopyranosyl)caffeyl)-β-D-glucopyranosyl)caffeic acid (1).

Blue flower of a morning glory, Ipomoea "Heavenly Blue",[2] contains heavenly blue anthocyanin (HBA), that consists of peonidin with six molecules of glucose and three molecules of caffeic acid;[3] it is one of the most complex natural anthocyanins.[4]

Hydrolysis of HBA with a solution of 8% NaOH in 60% aq methanol at 25 °C for 10 min (and then treatment with methanolic HCl) afforded, besides tris-deacyl HBA [3-O-(β-D-sophorosyl)-5-O-(β-D-glucosyl)peonidin],[1] a product that absorbs uv light on silica gel tlc. That it was a mixture of methyl trans-3-O-(β-D-glucopyranosyl)caffeate (4)[5] and methyl trans-4-O-(β-D-glucopyranosyl)caffeate (5)[6] was deduced as follows: Separation of the mixture by tlc was only effective after acetylation with acetic anhydride and pyridine to give the pentaacetate (6),[7] mp 79-81 °C, and its isomer (7),[8] mp 168.5-169.5 °C. These pentaacetates gave easily analyzable pmr spectra in hexadeuteriobenzene (100 MHz); the sugar part of these compounds could be assigned as β-glucopyranose by proton decoupling experiments ($J_{1',2'}$ = 8 Hz, $J_{3',4'} = J_{4',5'}$ = 10 Hz) and the trans configuration of caffeic acid was evident from the coupling constant (16 Hz) of the olefin.[9] The position of attachment of glucose on caffeic acid was determined by nuclear Overhauser effect (NOE) measurements between the anomeric proton and one of the aromatic ring protons; thus, the 3-O-glucoside (6) gave 15% NOE between H-2 of the phenyl group and the anomeric proton of the glucose moiety, whereas 5% NOE was observed between the anomeric proton



and H-5 in the case of the 4-O-isomer (7).

Controlled alkaline hydrolysis of HBA at -20 °C with 0.5% KOH in 75% aq methanol for 4 hours followed by methylation with diazomethane afforded the methyl ester (2)[10] of the title acid (1), white powder, mp 149.5-151 °C, FD-mass m/z 703 (M + Na), in 17% yield. The structure of (2) was determined as follows: treatment of (2) with triphenylmethyl (trityl) chloride and pyridine afforded in 76% yield the monotrityl derivative (3),[11] mp 168-172°C, which was methanolyzed with 1N sodium methoxide at room temp overnight to give the tritylated m-O-glucosylcaffeate (8) (36%) and the p-O-glucosylcaffeate (5)[6] (22%). Structure of the trityl derivative (8) was

determined by comparisons of uv and nmr spectra with those of the authentic samples, (8),[12] mp 109-111 °C, and (9),[13] mp 115.5-117.5 °C, prepared from (4) and (5), respectively, by tritylation. Thus, the structure of (1) was established as shown in the title.

Quite a few natural anthocyanins contain acyl group(s) such as coumaric, caffeic, ferulic acids, etc.,[4] but the HBA is the first case to have glycosylated aromatic acids as its components.

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REFERENCES AND NOTES

1. Preceding paper: Heavenly Blue Anthocyanin I., T. Goto, T. Kondo, H. Imagawa, S. Takase, M. Atobe, and I. Miura, Chem. Lett., submit for publication.
2. N. Ishikura and M. Shimizu [Kumamoto J. Sci. Biol., 12, 41 (1975)] reported that the morning glory "Heavenly Blue" is Ipomea rubro-caerulea Hook, whereas Ipomoea tricolor Cav was assigned for "Heavenly Blue" by S. Asen, R. N. Stewart and K. H. Norris [Phytochem., 16, 1118 (1977)]. Our species has not been identified either. Seeds of "Heavenly Blue" were purchased from Takii Seeds Co., Kyoto, and cultivated at our Univeristy Farm.
3. T. Goto, T. Kondo, H. Imagawa and I. Miura, to be published. Ishikura and Shimizu,[2] and Asen et al.[2] reported that the anthocyanin of "Heavenly Blue" is peonidin 3-dicaffeylsophoroside-5-glucoside.
4. C. F. Timberlake and P. Bridle, in The Flavonoids, Ed., J. B. Harborne, T. J. Mabry and H. Mabry, 1975, pp 214-266. Chapman and Hall, London; C. F. Timberlake and P. Bridle, in Developments in Food Colours-1, Ed., J. Walford, 1980, pp 115-149. Applied Sci. Publ., Essex, England.
5. (4) was obtained by deacetylation of (6) with sodium ethoxide in ethanol: mp 108-110 °C; FD m/z 379 (M + Na), 356 (M); uv (MeOH) nm (log ε) 233.5 (4.11), 316 (4.25); (MeOH-NaOH) 248 (3.92), 310 (3.77), 365 (4.39).
6. (5) was obtained by deacetylation of (7) with sodium methoxide in methanol: mp 200-205 °C; FD m/z 356 (M), 357 (M + H); uv (MeOH) nm (log ε) 239 (4.04), 290 (4.21), 319 (4.12); (MeOH-NaOH) 263 (4.22), 302 (4.17), 360 (3.89). Ratio of yields (4)/(5) is ca 2:1. This ratio indicates that the third caffeic acid that was not freed from the anthocyanin moiety by the controlled alkaline hydrolysis exists in the form of ester of 3-O-(β-D-glucopyranosyl)-caffeic acid.
7. (6): FD m/z 566 (M); uv (MeOH) nm (log ε) 223 (4.15), 275 (4.23), 278 (4.23); (MeOH-NaOH) 249 (3.93), 311 (3.77), 366 (4.36); pmr (C_6D_6) δ 1.65, 1.67, 1.81, 1.92 and 2.03 (each 3H,s, 5 x Ac); 3.08 (1H, ddd, J = 4.0, 4.5 & 10 Hz, H-5); 3.45 (3H, s, OMe); 3.9-4.0 (2H, m, H_2-6'); 4.64 (1H, d, J = 8.0 Hz, H-1'); 5.00 (1H, t, J = 10 Hz, H-4'); 5.2-5.5 (2H, m, H-2' & 3'); 6.40 (1H, d, J = 16 Hz, H-α); 6.69 (1H, s, H-2); 7.0-7.15 (2H, m, H-5 & 6); 7.70 (1H, d, J = 16 Hz, H-β).

8. (7): FD m/z 566 (M); uv (MeOH) nm (log ε) 223 (4.20), 287 (4.39); (MeOH-NaOH) 264 (4.28), 302 (4.23), 361 (3.93); pmr (C_6D_6) δ 1.66 (9H, s), 1.93 (3H, s), and 2.06 (3H, s) (5 x Ac); 3.11 (1H, ddd, J = 2.5, 5 & 10 Hz), H-5'); 3.47 (3H, s, OMe); 3.91 (1H, dd, J = 2.5 & 12 Hz, H-6a'); 4.15 (1H, dd, J = 5 & 12 Hz, H-6b'); 4.68 (1H, d, J = 8 Hz, H-1'); 5.12 (1H, t, J = 10 Hz, H-4'); 5.2-5.5 (2H, m, H-2' & 3'); 6.22 (1H, d, J = 16 Hz, H-α); 6.64 (1H, d, J = 9 Hz H-5); 6.7-6.95 (2H, m, H-2 & 6); 7.61 (1H, d, J = 16 Hz, H-β).
9. That all of three caffeic acid moieties in HBA have trans configuration is evident from analysis of pmr spectrum of HBA.[3] The possibility of isomerization during the isolation is, therefore, excluded.
10. (2): uv (MeOH) nm (log ε) 235.5 (4.44), 293 (4.55), 316 (4.54); (MeOH-NaOH) 260 (4.46), 304 (4.39), 364 (4.58); pmr (CD_3OD) δ 3.3-4.1 (10H, m), 3.74 (3H, s), 4.2-4.6 (2H, m), 4.74 (1H, d, J = 7.5 Hz), 4.80 (1H, d, J = 7.5 Hz), 6.07 (1H, d, J = 16 Hz), 6.29 (1H, d, J = 16 Hz), 6.79 (1H, dd, J = 1.5 & 8.0 Hz), 6.80 (1H, d, J = 8.0 Hz), 6.96 (1H, d, J = 1.5 Hz), 7.04 (1H, d, J = 8.0 Hz), 7.12 (1H, dd, J = 1.5 & 8.0 Hz), 7.35 (1H, d, J = 16 Hz), 7.46 (1H, d, J = 1.5 Hz), 7.53 (1H, d, J = 16 Hz).
11. (3): FD m/z 946 (M + Na + H); uv (MeOH) nm (log ε) 294 (4.16), 315 (4.14); (MeOH-NaOH) 260 (4.19), 303 (3.99), 364 (4.17).
12. (8): FD 621 (M + Na); uv (MeOH) nm (log ε) 316 (4.11); (MeOH-NaOH) 364 (4.25); pmr (CD_3OD) δ 3.4-3.7 (6H, m), 3.64 (3H, s), 4.90 (1H, d, J = 8 Hz), 6.22 (1H, d, J = 16 Hz), 6.92 (1H, d, J = 9 Hz), 7.0-7.65 (18H, m).
13. (9): FD m/z 621 (M + Na); uv (MeOH) nm (log ε) 292 (4.13), 318 (4.09); (MeOH-NaOH) 263 (4.10), 303 (4.05), 362 (3.97); pmr (CD_3OD) δ 3.4-3.8 (6H, m), 3.76 (3H, s), 4.90 (1H, d, J = 8 Hz), 6.32 (1H, d, J = 16 Hz), 6.93 (1H, dd, J = 2 & 9 Hz), 7.0-7.7 (18H, m).

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