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STRUCTURE CONFIRMATION OF TRIS-DEACYL HEAVENLY BLUE ANTHOCYANIN, AN ALKALINE HYDROLYSIS PRODUCT OF HEAVENLY BLUE ANTHOCYANIN OBTAINED FROM FLOWER OF MORNING GLORY "HEAVENLY BLUE"[1]

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Structure of tris-deacyl heavenly blue anthocyanin was determined to be 3-O-(β-sophorosyl)-5-O-(β-glucosyl)peonidin by PMR analysis.

Heavenly blue anthocyanin (HBA) is the anthocyanin isolated from blue petals of a morning glory, Ipomoea[2] "Heavenly Blue". In aqueous solution at pH 7.5, it gives beautiful blue color similar to that of the flower petals,[3,4] and differs from usual anthocyanins by its stability.[3] These characteristics prompted us to investigate its structure by means of NMR.[5]

Ishikura and Shimizu[6] reported HBA to be peonidin 3-diglucoside-5-glucoside with two molecules of caffeic acid, which was further modified by Asen et al.[3] to peonidin 3-(dicaffeylsophoroside)-5-glucoside. This structure has been assumed, however, only from analysis by ppc and UV, and not firmly confirmed. In contradiction to these results, we have decided the composition of HBA to be peonidin with six molecules of glucose and three molecules of caffeic acid.[7] Thus, it is the largest monomeric anthocyanin ever known, and hence for determination of its complete structure and stereochemistry the structure of the much simpler component, tris-deacyl HBA, must be confirmed first.[8]

Alkaline hydrolysis of HBA gave tris-deacyl HBA [$\lambda_{max}^{0.01\%\ HCl\text{-}MeOH}$ 279 nm (ε 11,700), 523 (24,100)], whose molecular weight was determined as 787 (flavylium cation) by FD-mass spectral analysis, suggesting its molecular composition to be peonidin[9] and three molecules of glucose.[10] Its 400 MHz PMR spectrum is shown in Fig. 1 [δ 9.17 (br.s, H-4), 8.37 (J = 2.0 & 8.8 Hz, H-6'), 8.13 (J = 2.0 Hz, H-2'), 7.20 (br. s, H-8), 7.12 (J = 8.8 Hz, H-5'), 7.10 (br.s, H-6), 4.05 (s, OMe); see Fig. 1 for other signals]. The PMR signals of the sugar part (δ 2.9 ∿ 5.8 ppm) were analyzed as follows: Signals of the three anomeric protons appeared at 5.18 (J=8.0Hz, ▲-1), 5.56 (J=8.0Hz, ■-1), and 4.77 ppm (J=8.0Hz, ●-1), and those of three $\underline{C}H_2OH$ groups were differentiated from other signals by partially relaxed FT (PRFT) NMR method as shown in Fig. 2. Assignment of ▲-2 signal was done by

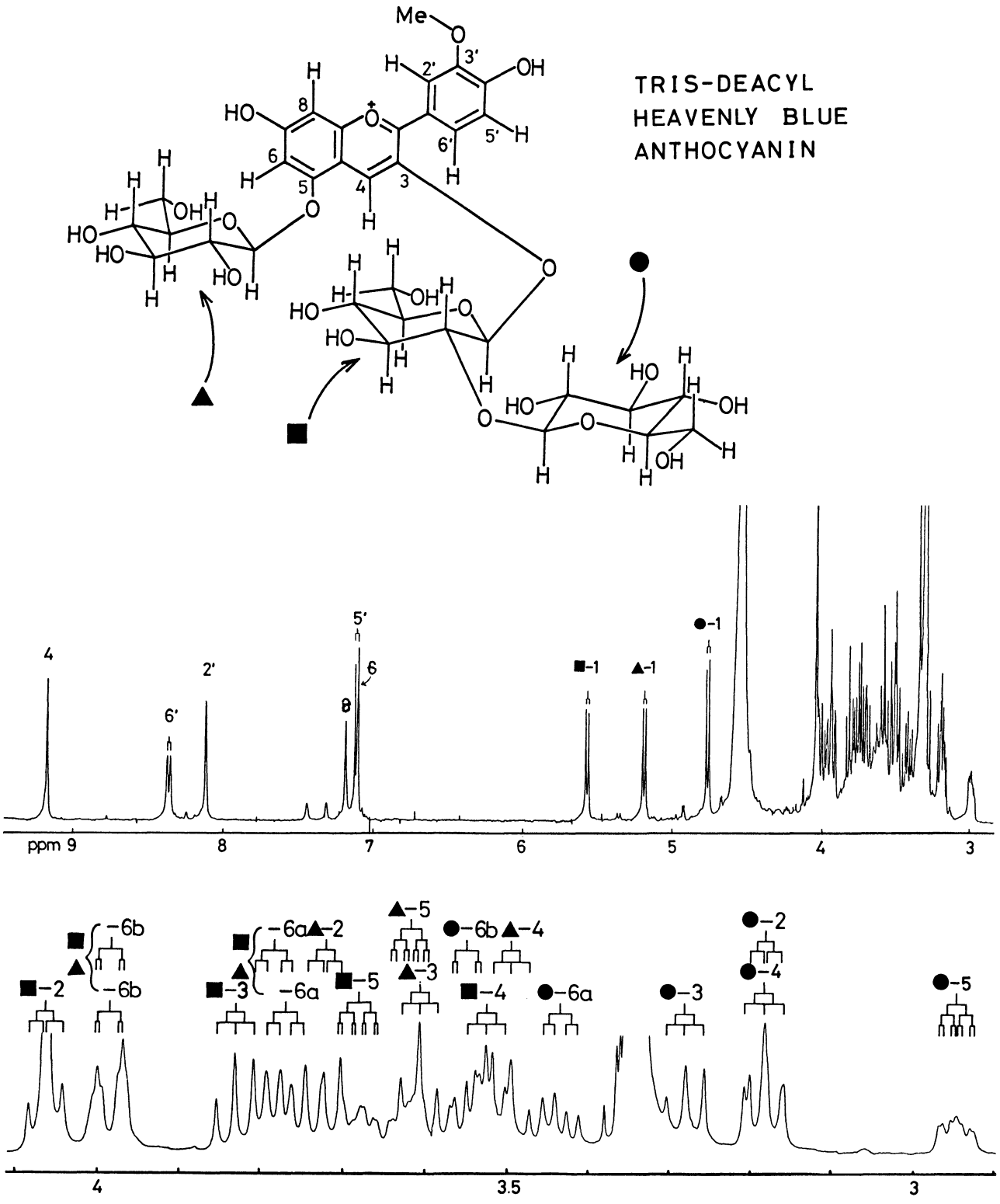

Fig. 1. PMR spectrum of tris-deacyl heavenly blue anthocyanin at 400 MHz in CD_3OD (0.1 % DCl) at room temp.

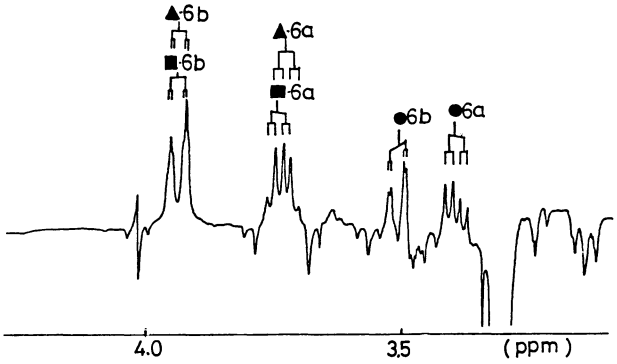

Fig. 2. Assignment of H-6 signals of three glucose moieties by PRFT method

PRFT/NMDR (decoupling by irradiation at ▲-1 signal under PRFT condition erasing all of H-6 signals of the sugar part). Other signals were correlated to each other by proton decoupling experiments. Since all of $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ of three glucose moieties are 9.2 Hz, and all of the $J_{1,2}$ are 8 Hz, three sugar moieties must be all β-glucosides with chair conformation. Positions of attachment of the sugars to peonidin nucleus were determined by NOE measurements; between the anomeric proton of ▲glucose and H-6 of peonidin, and between that of ■ glucose and the aromatic H-4 were observed NOE in 15 % and 19 %, respectively, indicating that ▲glucose and ■glucose are attached to the hydroxyl groups at 5 and 3 positions of peonidin, respectively. NOE (21 %) was also observed between 3'-OMe and 2'-H of peonidin nucleus.

The third glucose moiety (●) was determined in the following way to be attached to 2 position of ■glucose: Tris-deacyl HBA was dissolved in trifluoroacetic anhydride at room temp. and then diluted with $CDCl_3$ (4:1).[11] This solution gave a well-defined PMR spectrum of pertrifluoroacetate of tris-deacyl HBA (Fig. 3). The assignments shown in Fig. 3 were done by NOE and proton decoupling experiments similar to the case of HBA; the H-2 signal of ■glucose was shifted down field only slightly, whereas large downfield shifts were observed on other signals except H-5 ones, thus indicating the attachment of ●glucose at 2-OH of ■glucose.[12]

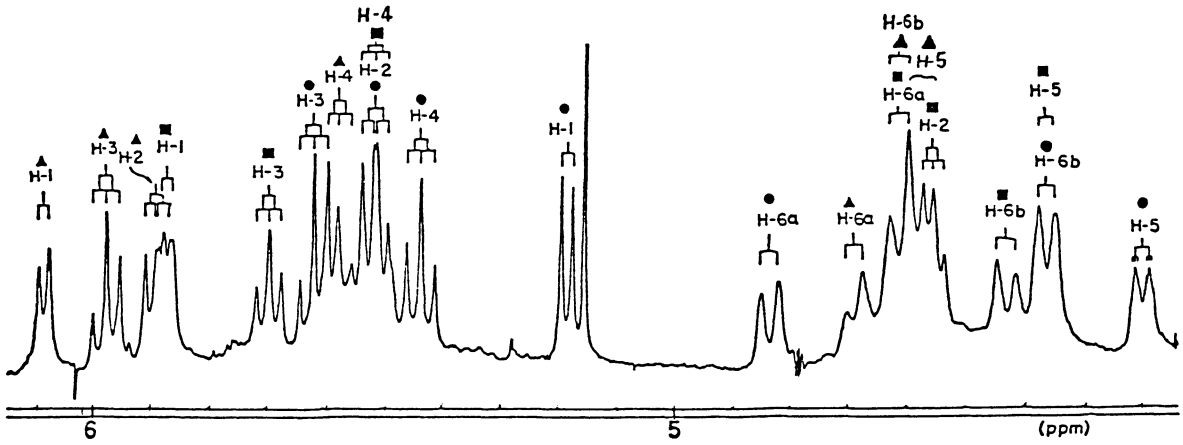

Fig. 3. PMR spectrum of pertrifluoroacetate of HBA

The above results confirmed the structure of tris-deacyl HBA to be 3-O-(β-sophorosyl)-5-O-(β-glucosyl)peonidin.

Acknowledgement: We thank Mr. Y. Ohnishi and Mr. T. Okada, the University Farm, for cultivation of the morning glory.

REFERENCES AND NOTES

1. Heavenly Blue Anthocyanin I.
2. Ishikura and Shimizu[6] reported that the morning glory "Heavenly Blue" is Ipomea rubro-caerulea Hook, whereas Ipomoea tricolor Cav was assigned for "Heavenly Blue" by Asen et al.[3] Our species has not been identified either. Seeds of "Heavenly Blue" were purchased from Takii Seeds Co., Kyoto, and cultivated at our University farm.
3. S. Asen, R. N. Stewart and K. H. Norris, Phytochem., 16, 1118 (1977); see also M. P. Imbert, C. E. Seaforth and D. B. Williams, J. Am. Soc. Hortic. Sci., 88, 481 (1966); A. B. Pomilio and J. F. Sproviero, Phytochem., 11, 2323 (1972).
4. Asen et al.[3] reported that pH of epidermal tissue of petals of Ipomoea tricolor is 7.5.
5. We have established the method of obtaining well-defined PMR spectra of natural anthocyanins [T. Goto, S. Takase and T. Kondo, Tetrahedron Lett., 1978, 2413].
6. N. Ishikura and M. Shimizu, Kumamoto J. Sci. Biol., 12, 41 (1975); N. Ishikura and U. Takahama, Kumamoto J. Sci. Biol., 11, 13 (1972).
7. T. Goto, T. Kondo, H. Imagawa and I. Miura, to be published.
8. Structure of the deacylated HBA was suggested by Asen et al.[3] as peonidin 3-sophoroside-5-glucoside by ppc and UV analyses of acid hydrolysis products (peonidin 3,5-diglucoside, 3-glucoside, 5-glucoside, and 3-sophoroside); anomeric configurations have remained undetermined.
9. Peonidin nucleus was confirmed by degradation with 10% $Ba(OH)_2$ to vanilic acid.
10. ^{13}C NMR of deacyl HBA [25.05 MHz, CD_3OD + 0.1% DCl, r.t.]:δ(ppm) 169.4 (s, 8a); 164.9 (s, 2); 157.0 (s x 2), 156.6 (s), 149.7 (s), and 146.1 (s) (3, 7, 3', and 4'); 136.3 (d, 4); 130.3 (d, 6'); 121.1 (s, 4a); 117.6 (d, 5'); 114.7 (d, 2'); 113.4 (s, 1'); 105.6 (d, 8); 97.5 (d, 6); 56.9 (q, OMe); (sugar parts): 104.0 (d) and 102.4 (d x 2) (3 x anomeric); 81.4 (d), 78.4 (d), 77.5 (d), 75.5 (d), 74.7 (d), 71.3 (d), and 70.9 (d) (12 x CHOH); 62.2 (t x 3, CH_2OH).
11. M. Ranganathan and P. Balarm, Org. Mag. Res., 13, 220 (1980).
12. That this disaccharide is sophorose was reported in the literatures[3] and further confirmed by us by its isolation and comparison with authentic sample by means of ^{13}C NMR [25.05 MHz in D_2O at room temp. (ppm from MeOH = 0 ppm): 55.1, 53.8, 45.7, 42.9, 32.5, 31.9, 26.8, 24.4, 22.8, 22.3, 20.6, 11.7] and FD-mass spectra [m/z 365 $(M + Na)^+$]. We thank professor K. Matsuda, Tohoku University, for generous gift of authentic sophorose.

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