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STRUCTURE DETERMINATION OF HEAVENLY BLUE ANTHOCYANIN, A COMPLEX MONOMERIC ANTHOCYANIN FROM THE MORNING GLORY IPOMOEA TRICOLOR, BY MEANS OF THE NEGATIVE NOE METHOD[1]

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The structure of heavenly blue anthocyanin was elucidated to be 3-O-(2-O-(6-O-(trans-3-O-(β-D-glucopyranosyl)caffeyl)-β-D-glucopyranosyl)-6-O-(trans-4-O-(6-O-(trans-3-O-(β-D-glucopyranosyl)caffeyl)-β-D-gluco-pyranosyl)caffeyl)-β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl)peonidin by application of the negative NOE difference spectroscopy.

Heavenly blue anthocyanin (HBA)[2] isolated from blue petals of the morning glory, Ipomoea tricolor, is the largest monomeric anthocyanin known[3] and its structure could not have been unambiguously determined by the methods employed previously for structure elucidation of complex anthocyanins,[4] since heavily overlapped signals of sugar moieties prevented complete assignment of its ^{1}H-NMR spectrum. We wish to report the structure elucidation of HBA by application of the negative nuclear Overhauser effect (NOE) difference spectroscopy.[5]

Heavenly blue anthocyanin (1)[6] consists of a molecule of peonidin, six molecules of glucose and three molecules of caffeic acid. Its molecular weight is 1759 (flavylium cation) as determined by FABMS. Controlled alkaline hydrolysis of HBA afforded bis-deacyl HBA, whose structure was already determined to be 2,[7] and trans-4-O-(6-O-(trans-3-O-(β-D-glucopyranosyl)caffeyl)-β-D-glucopyranosyl)caffeic acid (mGCpGC) (3).[8] Thus, HBA (1) was composed of bis-deacyl HBA (2) and mGCpGC (3). Extensive analysis of the COSY spectrum (not shown) made possible to correlate most of the protons in the molecule, but unambiguous assignment of protons of all glucose units were unsuccessful due to heavily overlapped signals. The application of the negative NOE method[9] made possible to assign all of the proton signals and to determine the position of attachment of mGCpGC (3) on the sugar moieties unambiguously.

The proton NOE (500 MHz; steady state) of HBA was measured in 8% TFA-d:CD_3OD at 5 °C. Irradiation of the C(1)-5 [H-5 on caffeic acid-(1)] proton signal gave the NOE difference spectrum (Figure 1d) in which 2, 6, α, and β proton signals of the C(1) as well as the anomeric proton (△-1) of a glucose moiety were relieved.[10] Thus, it was concluded that △-glucose is attached to the 4-OH of C(1). Similarly, irradiation of C(2)-2 and C(3)-2 protons disclosed that □- and ○-glucose units are attached to the 3-OH of C(2) and C(3), respectively (Figures 1b and 1c). The two glucose units, ▲- and ●-glucose, were attached to the 3 and 5 positions, respectively, of the peonidin nucleus, as deduced from the observation of NOE

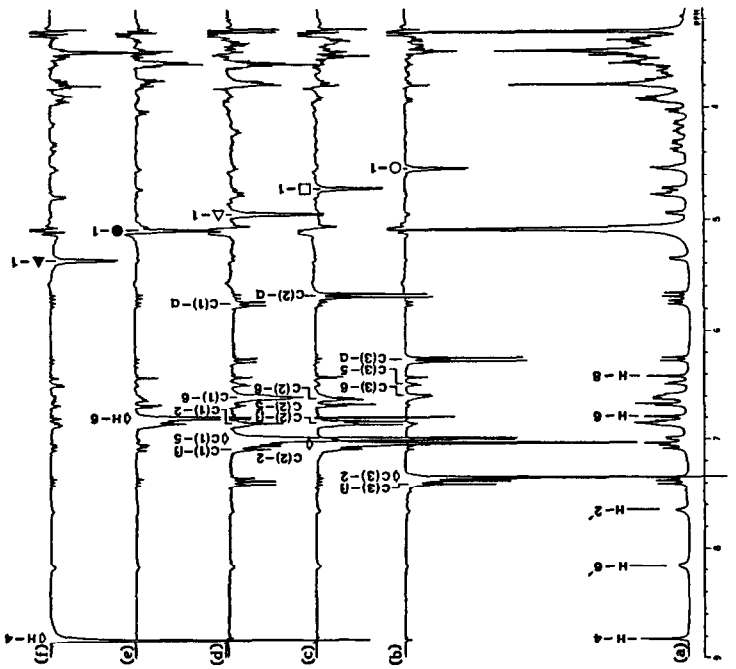


Figure 1. ^{1}H-NMR NOE difference spectra of HBA (1) by irradiation of the aromatic protons (8% TFA-d:CD_3OD at 5 °C, 500 MHz). (a) Normal spectrum; (b)-(f) NOE difference spectra by irradiation at the position indicated by the arrow. (Some long-distance NOEs are observed but their intensities are low so as not to interfere with the assignments.)

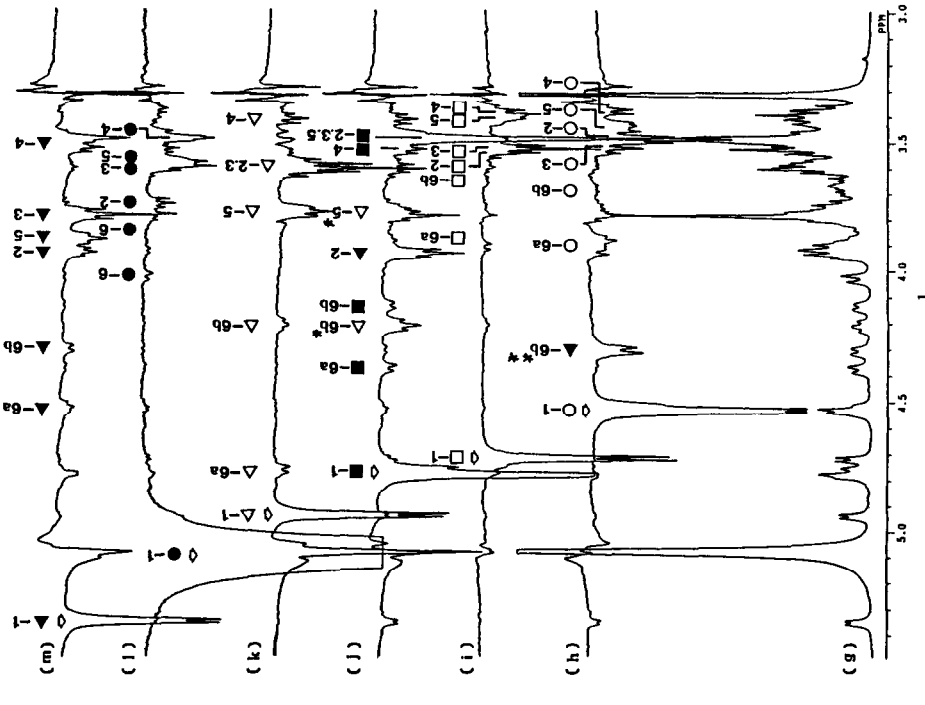
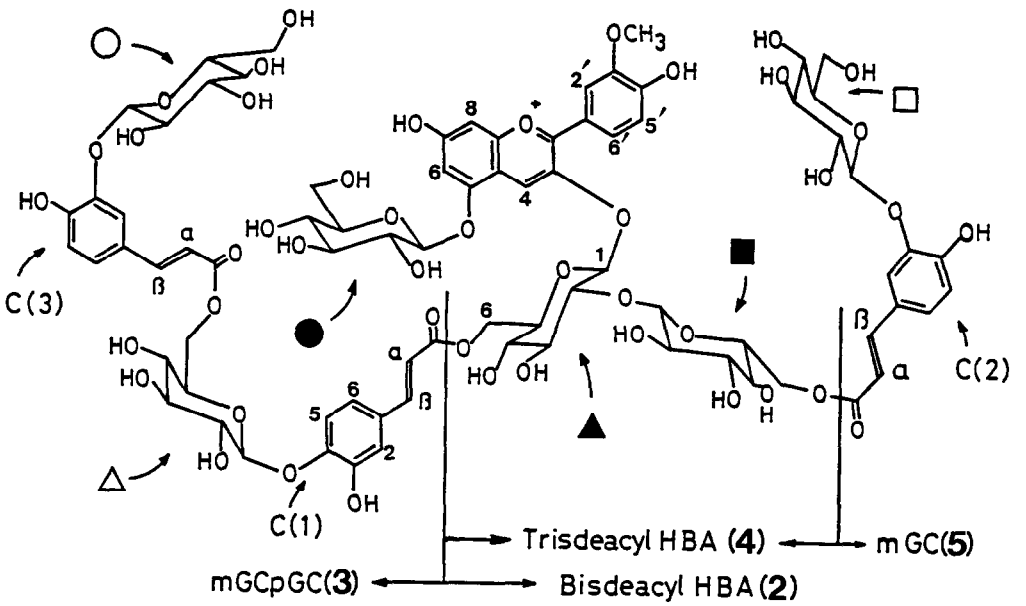


Figure 2. High field region of ^{1}H-NOE difference spectra of HBA (1) by irradiation of the anomeric protons of the glucose moieties (8% TFA-d:CD_3OD at 5 °C, 500 MHz). (g) Normal spectrum; (h)-(m) NOE difference spectra by irradiation at the position indicated by the arrow. * Since Δ-6_a is also irradiated, the NOE signals for Δ-6_a and Δ-5_a appear in this spectrum. ** Since ▲-6_a is also irradiated, the NOE signals for ▲-6_b appears in this spectrum.

between ▲-1 and H-4 and between ●-1 and H-6 (Figures 1f and 1e). Thus, all of the six anomeric protons of the glucose moieties were correlated with peonidin and three caffeic acid moieties.

Next, each anomeric proton of the six glucose moieties was irradiated as shown in Figure 2h-m. Irradiation of the ▲-1 proton relieved all protons attached to the carbon atoms of ▲-glucose, in which two ▲-6 protons were at δ 4.30 and δ 4.52, indicating that ▲-CH_2OH is acylated. Since bis-deacyl HBA (2) has no acyl group on the ▲-glucose unit,[7] the ▲-6 position in HBA (1) must be esterified with mGCpGC (3). This conclusion was further confirmed by assigning all of other signals (Fig. 1 and 2).



Heavenly Blue Anthocyanin (1)

The temperature for production of a maximum negative NOE depends on mol. wt.;[11] in 8% TFA-d:CD_3OD at 500 MHz, 5 °C for HBA (1)(mol. wt. 1759), -20 °C for bis-deacyl HBA (2)(mol. wt. 1111), and -40 °C for tris-deacyl HBA (4)(mol. wt. 787). Using a viscous solvent such as DMSO makes the temperature of maximum negative NOE higher;[11,12] for example, bis-deacyl HBA in TFA-d:DMSO-d_6 (1:2) gives a maximum negative NOE at room temperature.

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REFERENCES AND NOTES

1. Heavenly Blue Anthocyanin IV. Preceding paper: reference 7.

2. N. Ishikura and M. Shimizu, Kumamoto J. Biol. 1975, 12, 41; S. Asen, R. N. Stewart, and K. H. Norris, Phytochem. 1977, 16, 1118.

3. T. Goto, T. Kondo, H. Imagawa, S. Takase, M. Atobe, and I. Miura, Chem. Lett. 1981, 883.

4. T. Goto, T. Kondo, H. Tamura, H. Imagawa, A. Iino, and K. Takeda, Tetrahedron Lett. 1982, 23, 3695; T. Goto, T. Kondo, H. Tamura, K. Kawahori, and H. Hattori, Tetrahedron Lett. 1983, 24, 2181; T. Goto, T. Kondo, T. Kawai, and H. Tamura, Tetrahedron Lett. 1984, 25, 6021.

5. L. D. Hall and J. K. M. Sanders, J. Am. Chem. Soc. 1980, 102, 5703, and references therein.

6. HBA trifluoroacetate: dark red powder, m.p. 140-145 °C (dec.); λ_{max} (0.01% HCl-MeOH) nm (ε) 532 (31,600), 318 (42,000), 294 (45,700), 234 (45,700); E_{318nm}/E_{538nm} = 1.33; ^{1}H-NMR (8% TFA-d:CD_3OD, 5 °C, 500 MHz) [Assignments were done by ^{1}H-^{1}H COSY and NOE difference spectroscopy (steady state)] δ ppm (J in Hz): 8.82 (1H, s, H-4), 8.15 (1H, br.s, H-6'), 7.64 (1H, br.s, H-2'), 6.86 (1H, d, 7.5, H-5'), 6.79 (1H, d, 1, H-6), 6.41 (1H, br.s, H-8), 3.78 (3H, s, CH_3O); for other signals see figures 1 and 2.

7. T. Goto, H. Imagawa, T. Kondo, and I. Miura, Heterocycles 1982, 17, 355.

8. T. Goto, T. Kondo, H. Imagawa, and I. Miura, Tetrahedron Lett. 1981, 22, 3213.

9. Since the maximum value of a negative NOE[11] is 100%, the negative NOE can transmit over a long-distance. In the extreme case spin diffusion occurs.[13] Such a phenomenon has been observed in the case of high molecular substances such as proteins.[14] For a regular case, however, where the protons are grouped in several domains, the spin diffuses only into restricted domain(s), if the domains are separated from each other in space and/or the connecting parts among the domains are mobile.[14,15] In the case of substances with molecular weights of 600-2000 and using NOE difference spectroscopy, an appropriate enhancement of the negative NOE by lowering the temperature[11,12] or by using a viscous solvent results in relief of the all proton signals corresponding to only restricted domain(s) such as the glucosylcaffeic acid moiety in HBA molecule as shown in Figures 1 and 2.

10. Actually, signals of protons other than the anomeric proton on the carbon atoms of the glucose unit are also relieved, indicating that the glucosylcaffeic acid moiety forms a domain, but irradiation of the anomeric proton signal rather than the aromatic proton signal gave a better NOE difference spectrum for analysis of the sugar moiety.

11. J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect", Academic Press, New York and London, 1971.

12. E. M. Krauss and S. I. Chan, J. Am. Chem. Soc. 1982, 104, 6953; M. Ishitsuka, T. Kusumi, H. Kakisawa, Y. Kawakami, Y. Nagai, and T. Sato, Tetrahedron Lett. 1986, 27, 2639.

13. A. Kalk and H. J. C. Berendsen, J. Magnetic Resonance 1976, 24, 343.

14. K. Akasaka, M. Konrad, and R. S. Goody, FEBS Lett. 1978, 96, 287; K. Akasaka, J. Mag. Resonance 1983, 51, 14.

15. T. Endo, In "*NMR-Sosetsu to Jikkengaido [II]*". T. Miyazawa and Y. Arata Ed., Nankodo, Tokyo, 1983; pp 95-118; F. M. Paulsen, J. C. Hoch, and C. M. Dobson, Biochemistry 1980, 19, 2597.

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