HEAVENLY BLUE ANTHOCYANIN III. STRUCTURE OF BIS-DEACYL HEAVENLY BLUE ANTHOCYANIN, A CONTROLLED ALKALINE HYDROLYSIS PRODUCT OF HEAVENLY BLUE ANTHOCYANIN[1]

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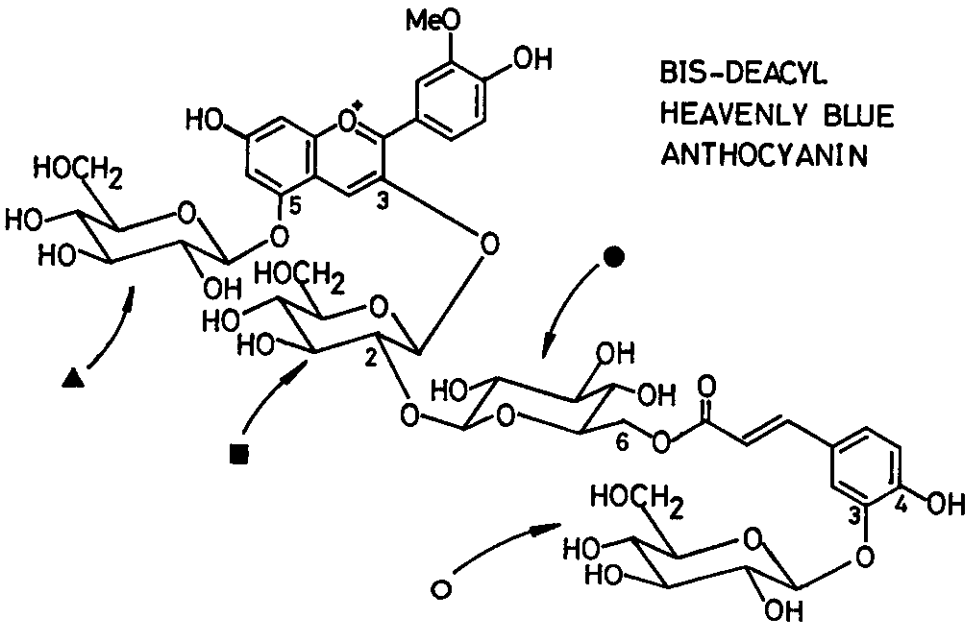
Abstract — Structure of bis-deacyl heavenly blue anthocyanin was determined to be 3-O-(2-O-(6-O-(trans-3-O-(β-D-glucopyranosyl)caffeyl)β-D-glucopyranosyl)β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl)peonidine.

Heavenly blue anthocyanin (HBA) is a constituent of blue flowers of a morning glory, Ipomoea "Heavenly Blue".[2] We have reported the components of HBA (1) to be peonidine 3-β-sophoroside-5-β-glucoside (tris-deacyl HBA) (2),[3] trans-4-O-(6-O-(trans-3-O-(β-D-glucopyranosyl)caffeyl)-β-D-glucopyranosyl)caffeic acid (3), and trans-3-O-(β-D-glucopyranosyl)caffeic acid (4).[1]

Alkaline hydrolysis of HBA using a variety of basic reagents such as aq NaOH, triethylamine, or dicyclohexylamine at higher or lower temperatures afforded only two anthocyanin products; bis-deacyl HBA (5) and tris-deacyl HBA (2). The best condition to obtain bis-deacyl HBA (5) is that HBA (1) chloride is treated with 60% methanol containing 1% NaOH at 25 °C for 10 min under nitrogen atmosphere. After the usual work-up, the products were separated by Avicel tlc using AcOH:HCl:H_2O (5:1:40) as eluent. Bis-deacyl HBA (5) was eluted from the tlc plates with 1/100 N HCl and, after the usual work-up, obtained as its chloride (red powder, yield 28%); $\lambda_{max}^{0.01\%HCl\text{-}MeOH}$ nm (log ε): 282 (1.48), 295 (1.39), 321 (1.22), 524 (2.11). Its fd-mass [m/z 1112 (M+1)] and pmr spectrum[4] (Fig. 1) show that 5 consists of peonidine + 4 x glucose + 1 x caffeic acid.

Bis-deacyl HBA (5) was hydrolyzed with 4% NaOH in 50% methanol at 25 °C for 10 min to afford tris-deacyl HBA (2) and an aromatic acid, which, after methylation with diazomethane, was identified as methyl trans-3-O-(β-D-glucopyranosyl)caffeate by tlc comparisons with the authentic sample.[1] Thus, bis-deacyl HBA (5) is composed from tris-deacyl HBA (2) acylated with trans-3-O-(β-D-glucopyranosyl)caffeic acid.



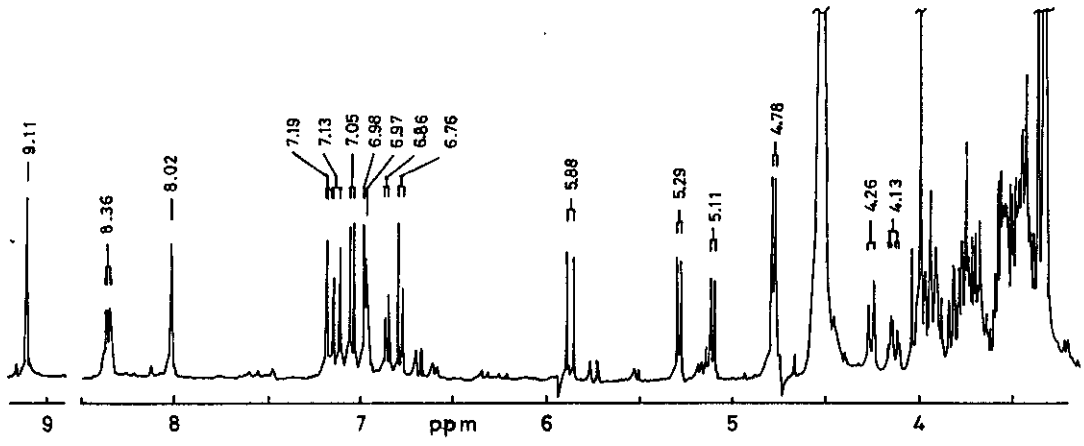


Fig. 1. Pmr spectrum (400 MHz) of bis-deacyl HBA (5) in CD_3OD containing 0.1% DCl at 60 °C

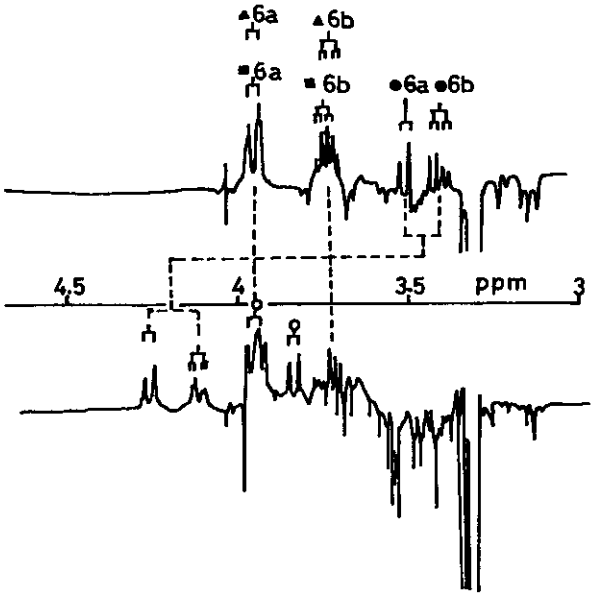


Fig. 2. PRFT pmr spectra (400 MHz) of tris-deacyl HBA (2) (upper) and bis-deacyl HBA (5) (lower) in CD_3OD containing 0.1% DCl at room temp.

Position of the acylation on the sugar moiety of tris-deacyl HBA (2) was deduced to be one of the three CH_2OH groups of glucose moieties since the signals of one of $C\underline{H}_2O$- groups appeared at δ 4.13 (1H, dd, J = 2.5 and 11 Hz) and 4.26 (1H, d, J = 11 Hz), while pmr spectrum of 2 showed the signals of all of the three $C\underline{H}_2OH$ above 4 ppm. That the signals at δ 4.13 and 4.26 are indeed for $C\underline{H}_2O$- grouping was confirmed by the partially relaxed Fourier transform method (PRFT) in which four $C\underline{H}_2O$- groups were differentiated from other signals as shown in Fig. 2.

In the PRFT spectrum of 5 the signals at δ 3.44 and 3.56 corresponding to the $C\underline{H}_2OH$ of ● glucose in tris-deacyl HBA (2) disappeared. It can be best interpreted by assuming that those signals were shifted to δ 4.13 and 4.26 in bis-deacyl HBA (5). Thus, it is evident that the acyl moiety is attached to the CH_2OH group of ● glucose. Incidentally the $C\underline{H}_2OH$ signals of methyl 3-O-(β-D- glucopyranosyl)caffeate appeared at δ 3.97 (1H, d, J = 12 Hz) and 3.81 (1H, dd, J = 7 and 12 Hz). Thus, the structure of bis-deacyl HBA (5) was determined to be 3-O-(2-O-(6-O-(trans-3-O-(β-D-glucopyranosyl)caffeyl)β-D-glucopyranosyl)β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl)peonidine.

The above results show that HBA (1) consists of bis-deacyl HBA (5) acylated with the glucosyl-

caffeylglucosylcaffeic acid 3. Our preliminary experiments applying the PRFT method (not shown) strongly suggests that the acid 3 is attached to the CH_2OH group of O glucose moiety; thus HBA (1) may have a long side chain of the glucosylcaffeylglucosylcaffeylglucosylcaffeyl group on peonidine 3-sophoroside-5-glucoside (tris-deacyl HBA) (2).

Acknowledgements — We thank Mr Y. Ohnishi and Mr T. Okada, the University Farm, for cultivating the morning glory.

REFERENCES AND NOTES

1. Preceding paper: Heavenly Blue Anthocyanin II, T. Goto, T. Kondo, H. Imagawa, and I. Miura, Tetrahedron Letters, 1981, 22, 3213.
2. N. Ishikura and M. Shimizu [Kumamoto J. Sci. Biol., 1975, 12, 41] 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., 1977, 16, 1118]. 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. T. Goto, T. Kondo, H. Imagawa, S. Takase, M. Atobe, and I. Miura, Chem. Letters, 1981, 883.
4. Pmr spec. of 5: (0.1% DCl-CD_3OD at 60 °C; 400 MHz) δ 4.00 (3H, s), 4.13 (1H, dd, J = 5 and 12 Hz), 4.26 (1H, d, J = 12 Hz), 4.78 (2H, d, J = 7.5 Hz), 5.11 (1H, d, J = 7.5 Hz), 5.29 (1H, d, J = 7.5 Hz), 5.88 (1H, d, J = 16 Hz), 6.76 (1H, d, J = 8.5 Hz), 6.86 (1H, dd, J = 2 and 8.5 Hz), 6.97 (1H, s), 6.98 (1H, s), 7.05 (1H, d, J = 9 Hz), 7.13 (1H, d, J = 16 Hz), 7.19 (1H, d, J = 2 Hz), 8.02 (1H, d, J = 2 Hz), 8.36 (1H, dd, J = 2 and 9 Hz), 9.11 (1H, s).

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