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^{13}C NMR SPECTRA OF CYANIDIN AND CHRYSANTHEMIN[1]

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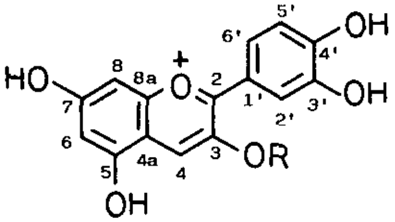
Abstruct ——— On the ^{13}C nmr spectrum of cyanidin (**1**), the signal assignments of all the carbon atoms of **1** were carried out by means of selective decoupling and long-range selective ^{1}H decoupling (LSPD) techniques. The ^{13}C nmr spectrum of chrysanthemin (**2**) isolated from the fruits of Morus alba L. was analyzed with the aid of insensitive nuclear enhanced by population transfer (INEPT) technique as well as of comparison with the ^{13}C nmr spectrum of **1**.

Goto, et al.[2] reported the signal assignments in the ^{13}C nmr spectrum to the carbon atoms of tris-deacyl heavenly blue anthocyanin. In the report, however, no proof was described. Further, no paper has been presented on the signal assignments to the carbon atoms of anthocyanidin moiety.[3] In the course of our studies on the constituents of the mulberry tree,[4] the signal assignments of the carbon atoms were carried on cyanidin (1)[5] and chrysanthemin (cyanidin-3-monoglucoside, 2)[5] obtained from the fruits of Morus alba L.[6]

On the ^{13}C nmr spectrum (0.04N DCl-CD_3OD, 25 MHz) of cyanidin (1) derived from quercetin,[7] the signals were assigned to the carbon atoms at C-4, 6, 8, 2', 5', and 6' positions by selective decoupling technique (Fig. 1). The signal assignments of the carbon atoms having no hydrogen atoms were achieved by long-range selective ^{1}H decoupling (LSPD)[8] and selective $^{13}C-\{^{1}H\}$ nuclear Overhauser effects (NOE)[8b] technique. As an example of the application of these analytical tools the analysis of the C-3 is described. When the signal at δ 8.59 (C-4-H) was weakly irradiated, the signal at δ 146.3 was appeared as singlet and increased the area (77%). The results are shown in Fig. 2 and Table 1.

On the ^{13}C nmr spectrum (0.04N DCl-CD_3OD, 25 MHz) of chrysanthemin (2), the signal assignments of the carbon atoms of cyanidin moiety were carried out by



1: R = H

2: R = glucosyl

insensitive nuclear enhanced by population transfer (INEPT)[9] technique as well as by comparing the spectrum with the ^{13}C nmr spectrum of 1.[10] The results are shown in *Table 2*.

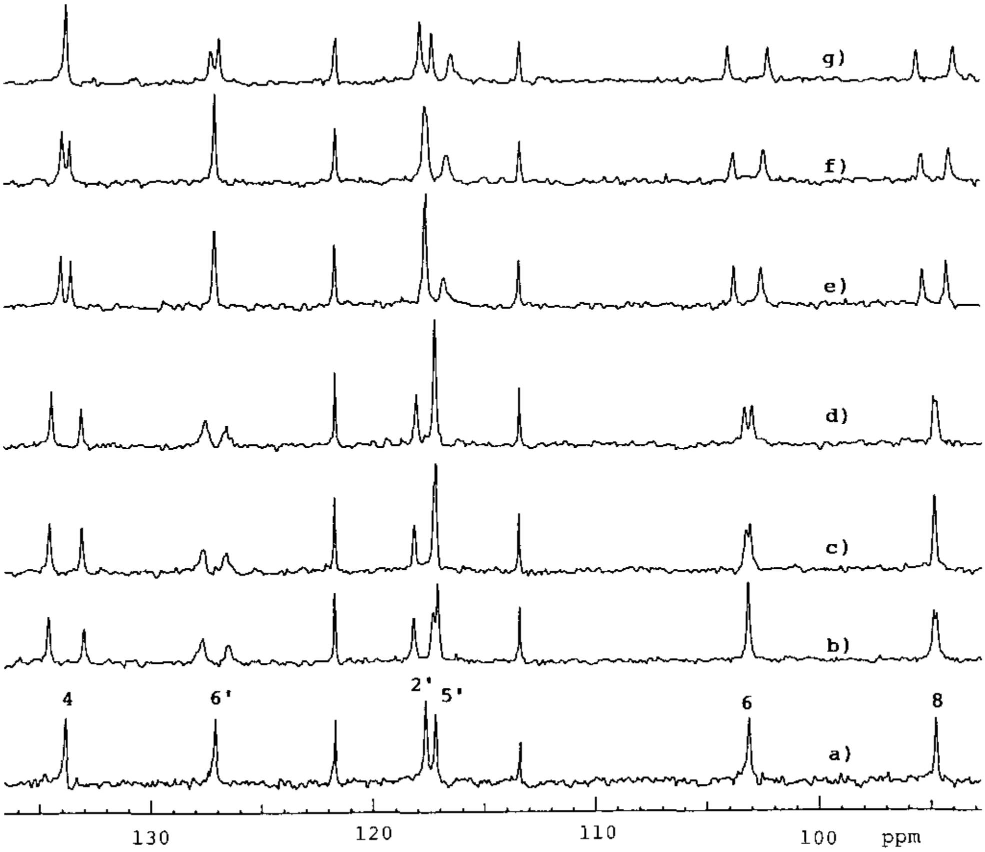


Fig. 1 ^{13}C selective decoupling spectra of cyanidin (1)

a): complete decoupling; b): irradiated at 6-H; c): irradiated at 8-H;

d): irradiated at 5'-H; e): irradiated at 2'-H; f): irradiated at 6'-H;

g): irradiated at 4-H.

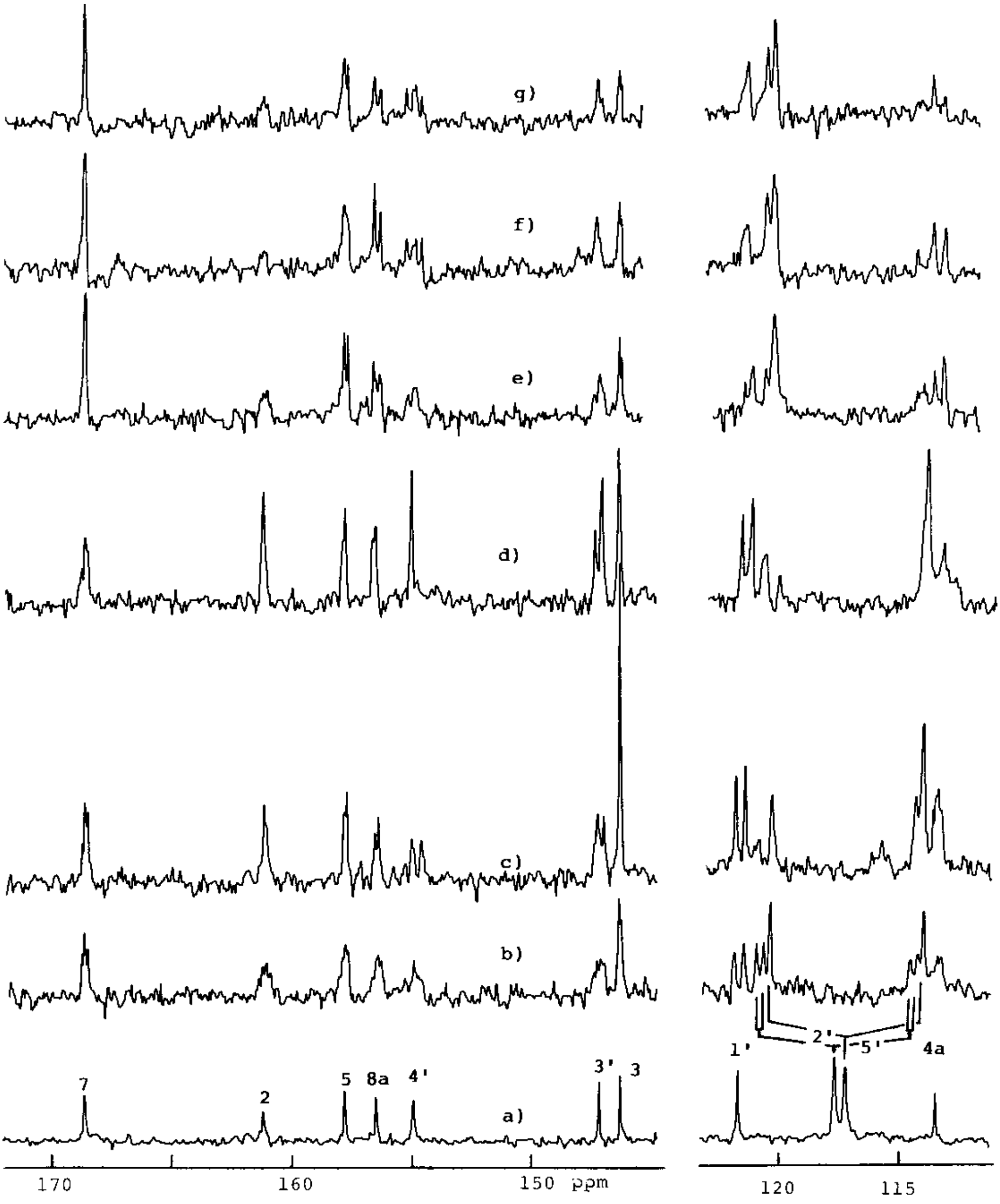


Fig. 2 LSPD and selective ^{13}C-$\{^1$H$\}$ NOE spectra of cyanidin (1)

a): complete decoupling; b): reference (irradiated at 9.18 ppm);

c): irradiated at 4-H; d): irradiated at 2'-H; e): irradiated at 6-H;

f): irradiated at 8-H; g): irradiated at 5'-H.

Table 1 LSPD and selective $^{13}\mathrm{C}$-$\{^{1}\mathrm{H}\}$ NOE data of cyanidin (1)

irrad. at	C-2 (161.3 ppm) m ($\nu_{\frac{1}{2}}$=12 Hz)	C-3 (146.3) d (J=2.5, $\nu_{\frac{1}{2}}$=5)	C-4a (113.4) br ($\nu_{\frac{1}{2}}$=20)	C-5 (157.9) m ($\nu_{\frac{1}{2}}$=10)	C-7 (168.8) dd (J=2.5, 4)	C-8a (156.6) m ($\nu_{\frac{1}{2}}$=14)	C-1' (121.7) br d (J=10, $\nu_{\frac{1}{2}}$=16)	C-3' (147.2) m ($\nu_{\frac{1}{2}}$=16)	C-4' (155.0) m ($\nu_{\frac{1}{2}}$=22)
4-H (8.59 ppm)	d (J=2 Hz)	s ($\nu_{\frac{1}{2}}$=2.5)	br ($\nu_{\frac{1}{2}}$=14)	br d (J=3)		d (J=4)		sharp 11) ($\nu_{\frac{1}{2}}$=13)	br t 11) (J=10)
NOE		**77%**	**40%**						
6-H (6.63 ppm)			br s ($\nu_{\frac{1}{2}}$=6)	br d (J=4)	br s 12) ($\nu_{\frac{1}{2}}$=2)	dd 12) (J=2.5,6)			m 13) ($\nu_{\frac{1}{2}}$=22)
NOE				**65%**	**100%**	**57%**			**24%**
8-H (6.89 ppm)			sharp ($\nu_{\frac{1}{2}}$=6)	m ($\nu_{\frac{1}{2}}$=9)	br s 14) ($\nu_{\frac{1}{2}}$=2)	d (J=6)	br s 15) ($\nu_{\frac{1}{2}}$=11)	br s 15) ($\nu_{\frac{1}{2}}$=8)	sharp 15)
NOE					**100%**	**36%**			
2'-H (8.13 ppm)	br s ($\nu_{\frac{1}{2}}$=5)	s 16) ($\nu_{\frac{1}{2}}$=4.5)		br d 16) (J=3)		br d 16) (J=4)	d (J=10, $\nu_{\frac{1}{2}}$=14)	d (J=7)	s 17) ($\nu_{\frac{1}{2}}$=4)
NOE	**33%**	**28%**					**35%**	**37%**	
5'-H (7.02 ppm)				sharp 18) ($\nu_{\frac{1}{2}}$=2)	s 18) ($\nu_{\frac{1}{2}}$=2)	d 18) (J=7)	s ($\nu_{\frac{1}{2}}$=10)	br d (J=7)	dd (J=6, 10)

$\nu_{\frac{1}{2}}$: The widths at half signal height.

11) - 18): see notes 11 - 18.

Table 2 ^{13}C nmr chemical shifts of cyanidin (1) and chrysanthemin (2)

C	1	2	C	1	2	C	1	2
2	161.3	164.2	8	94.8	95.1	6'	127.1	128.3
3	146.3	145.6	8a	156.6	157.6	sugar		103.7*
4	133.9	136.8	1'	121.7	121.2			74.8
4a	113.4	113.3	2'	117.7	118.4			78.1
5	157.9	159.2	3'	147.2	147.4			71.1
6	103.1	103.3*	4'	155.0	155.8			78.8
7	168.8	170.4	5'	117.2	117.4			62.4

*: Assignments may be reversed.

EXPERIMENTAL

All the melting points are uncorrected. ^{1}H and ^{13}C nmr spectra were measured with tetramethylsilane (TMS) as the internal reference. Chemical shifts were expressed in ppm down field from TMS, and coupling constants (J) in Hz. Abbreviations : s = singlet, d = doublet, m = multiplet, br = broad, sh = shoulder, infl. = inflection. The following instruments were used: melting points; Mitamura's micromelting point apparatus (hot stage type), uv spectra; Hitachi 340 UV Spectrometer, ir spectra; Hitachi 295 Spectrometer, ^{1}H nmr spectra; JEOL FX-100 and GX-400 NMR Spectrometers. ^{13}C nmr spectra; JEOL FX-100 NMR Spectrometer. The conditions of the LSPD and selective $^{13}C-\{^{1}H\}$ NOE were as follows: spectral width, 5 KHz; data points, 8 K; repetition time, 0.9 sec; pulse width, 10 μsec (40 °); power level for LSPD ($\gamma H_2/2\pi$), 21 Hz; no. of pulses, 16000. For column chromatography, Polyamide C-200 and cellulose powder (AVICEL) were used.

Isolation of chrysanthemin (2)^{5} from the fruits of morus alba L.

Fresh fruits (25 Kg) of M. alba L., collected in neighbourhood of Sakato, Saitama Prefecture, Japan, in June 1983, was extracted with 0.012N HCl-EtOH (17 l x 2), and the extracts were concentrated to residue (1.5 l) under reduced pressure. The residue (200 ml) was chromatographed successively on cellulose powder (solvent : AcOH:HCl:H_2O = 3:1:8), polyamide (solvent : 0.012N HCl-MeOH), cellulose powder (0.012N HCl-MeOH), cellulose powder (0.012N HCl-MeOH; AcOH:HCl:H_2O = 15:3:82), and polyamide (0.012N HCl-MeOH), to give chrysanthemin (**2**, 7 mg) as its chloride, reddish brown powder, mp 235 °C (decomp.), reprecipitated from 0.1% HCl-MeOH, FD-MS m/z: 449 (M^+), 287; uv $\lambda_{max}^{HCl+MeOH}$ nm (log ε): 210 (4.72), 281 (4.53), 330 (sh 3.67), 380 (sh 3.95), 440 (infl. 4.10), 525 (4.84); $\lambda_{max}^{HCl+MeOH+AlCl_3}$: 210 (4.73), 238 (4.59), 275 (sh 4.37), 290 (sh 4.37), 313 (4.14), 400 (sh 3.95), 575 (4.91); ^{1}H nmr (1% DCl-CD_3OD, 100 MHz): 3.40-4.00 (6H, m, protons of sugar moiety), 5.36 (1H, d, J = 8, anomeric - H), 6.86 (1H, d, J = 9, C - 5' - H), 7.86 (1H, d, J = 2.5, C - 2' - H), 8.08 (1H, dd, J = 2.5 and 9, C - 6' - H), 8.81 (1H, br s, C - 4 - H).

Cyanidin (1)^{5}

Dry ether (200 ml) and lithium aluminum hydride (1 g) were placed in the flask of Soxhlet extractor and quercetin (290 mg) was placed in the Soxhlet cup. The mixture was refluxed for 30 h, and poured into ice water, acidified with HCl, and extracted with ether. The acidic solution was absorbed on a polyamide column, which was eluted with 0.012 N HCl-MeOH solution to give crude pigment. It was chromatographed successively on cellulose powder (AcOH:HCl:H_2O = 3:1:8), and on polyamide (0.012N HCl-MeOH) to give cyanidin (**1**, 47 mg), brown powder, without melting until 300 °C (reprecipitated from 0.1N HCl-MeOH); FD-MS m/z: 287 (M^+); uv $\lambda_{max}^{HCl+MeOH}$ nm (log ε): 277 (3.53), 400 (2.96), 440 (3.10), 536 (3.76); $\lambda_{max}^{HCl+MeOH+AlCl_3}$: 260 (sh 3.44), 310 (3.17), 400 (2.76), 565 (3.76); ^{1}H nmr (0.1% DCl-CD_3OD, 400 MHz): 6.63 (1H, d, J = 2.1, C - 6 - H), 6.89 (1H, dd, J = 0.9 and 2.1, C - 8 - H), 7.02 (1H, d, J = 8.6, C - 5' - H), 8.13 (1H, d, J = 2.4, C - 2' - H), 8.24 (1H, dd, J = 2.4 and 8.6, C - 6' - H), 8.59 (1H, d, J = 0.9, C - 4 - H).

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10. As the solubility of **2** in the solution (DCl + CD_3OD) was poor, further studies could not be performed.
11. The 2'- and 6'-protons were irradiated with lower power because of the close chemical shifts of H-4, H-2' and H-6'.
12. The 8-proton was irradiated with lower power because of the close chemical shifts of H-6 and H-8.
13. The 5'-proton was irradiated with lower power because of the close chemical shifts of H-6 and H-5'.
14. The 6-proton was irradiated with lower power because of the close chemical shifts of H-6 and H-8.
15. The 5'-proton was irradiated with lower power because of the close chemical shifts of H-8 and H-5'.
16. The 4-proton was irradiated with lower power because of the close chemical shifts of H-4 and H-2'.
17. The 6'-proton was irradiated with lower power because of the closed chemical shifts of H-2' and H-6'.
18. The 6- and 8-protons were irradiated with lower power because of the closed chemical shifts of H-6, H-8 and H-5'.

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