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PMR INVESTIGATION OF 3-O-(β-D-GLUCOSYL)MALVIDIN STRUCTURAL TRANSFORMATIONS IN AQUEOUS SOLUTIONS

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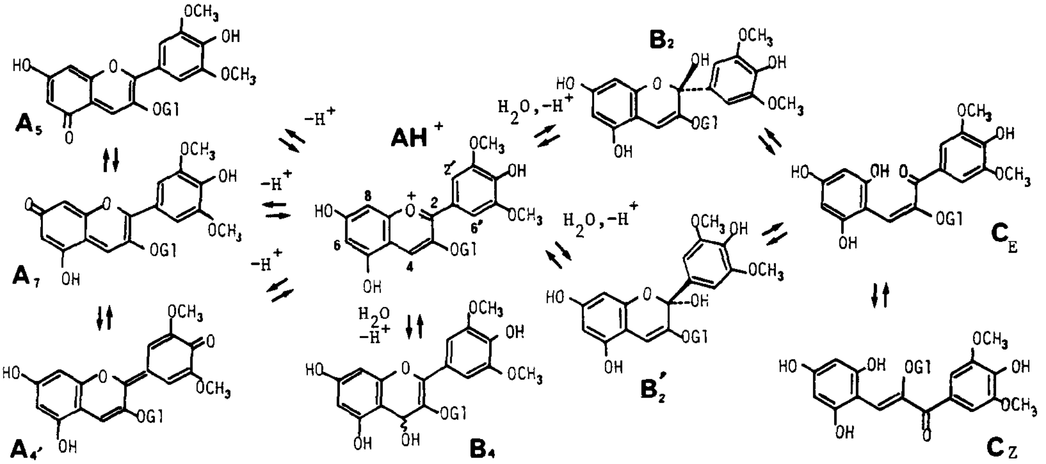
Summary : In slightly acidic aqueous solutions, 3-O-(β-D-glucosyl)malvidin was shown to exist in seven different structures by means of PMR spectrometry.

With the exception of polyacylated pigments, it is well established that in slightly acidic aqueous solutions, most of the natural anthocyanins are rapidly and almost completely converted into colourless forms[1,2]. It has been demonstrated that under such conditions, four anthocyanin structures exist in equilibrium : the flavylium cation AH^+, the quinoidal base A, the carbinol pseudobase B and the chalcone pseudobase C (Scheme 1). The flavylium cation dominates at low pH but it quickly disappears as the pH increases to give the unstable neutral quinoidal base, which then slowly evolves to the much more stable mixture of the neutral pseudobases. Reactions $A \rightarrow AH^+ \rightarrow B \rightarrow C$ are all endothermic[1]; thus any temperature rise will strongly favor the open chalcone form at the expense of the other species.

These compounds have been extensively studied by UV-visible spectrometry[3]. NMR has been used to elucidate the structure of anthocyanins only recently[4] ; in most cases, however, the pigment was in the cationic flavylium form. In this paper, we report PMR spectra of seven different structures of 3-O-(β-D-glucosyl) malvidin in slightly acidic aqueous solutions.

3-O-(β-D-glucosyl)malvidin was dissolved, at room temperature, in a CD_3CN - D_2O mixture (1/4). CD_3COONa was added to the solution at a concentration close to the anthocyanin concentration ($\simeq 1.4 \times 10^{-2}$ M). Under such conditions the hydronium ion concentration ranges approximately from 10^{-4} to 10^{-5} M. Addition of CD_3CN resulted in a greater solubility and a better resolution of the PMR spectra. One should notice that the H-6 and H-8 protons of the aglycone are slowly exchanging with deuterium atoms ; this is clearly indicated by a decrease in the intensities of the corresponding peaks. PMR measurements were made on a BRUKER AM 400 spectrometer. The anthocyanin sample was obtained from *Vitis vinifera* according to the procedure previously described[1].

Spectrum 1a, obtained within 15 minutes after dissolution, shows signals assignable to the quinoidal base A (loss of proton from the cation) and to three other species B_2, B'_2 and B_4 arising from the nucleophilic attack of the water molecule on the cation 2 and 4 carbon atoms (Scheme 1). In fact, three prototropic tautomers A_5, A_7 and $A_{4'}$ can exist ; they are in extremely fast equilibrium and only a single signal can be observed for each type of proton[5] .



Scheme 1 : Different 3-O-(β-D-glucosyl) malvidin structures in equilibrium in aqueous solution.

Although the formation in large quantity of a water 2-adduct is generally accepted[1], it has not been possible until now to establish the existence of the two epimers B_2 and B'_2 which possess inverse configurations at C-2. At 296 K, B_2 and B'_2 PMR signals are well resolved (fig. 1a and 1c). With temperature increase, these signals become broader, then coalesce as the interconversion rate of the two species through the flavylium cation (present in trace amount) increases (fig. 1b). Coalescence was found to take place at about 313 K. The existence of B_4 was previously considered[5], but its presence has never been detected in the case of natural anthocyanins. In the temperature range investigated, no evidence could be given for the existence of two epimers for the water 4-adduct.

Spectrum 1b was recorded within 15 minutes after heating the solution to 333 K, whereas spectrum 1c was taken about 30 minutes later, after cooling this solution back to room temperature. A temperature increase favors the open chalcone pseudobase. Indeed, we observe two new species C_E and C_Z formed from the carbinol as shown in table 1 ; C_E and C_Z, the formation of which has been previously discussed[6], are identified as chalcone E and Z respectively.

Figure 1d shows the spectrum observed within a few minutes after addition of concentrated DCl to the solution characterized by spectrum 1c. It is clearly seen that the hydrated forms B_2, B'_2 and B_4 reverse immediatly and quantitatively to the flavylium cation, whose PMR aglycone spectrum is shown in figure 1f. The chalcone C_E, still present on figure 1d, is slowly converted to the cation by cyclisation, whereas the chalcone C_Z, the structure of which does not allow cyclisation to take place rapidly, is still present four hours later (Fig. 1e).

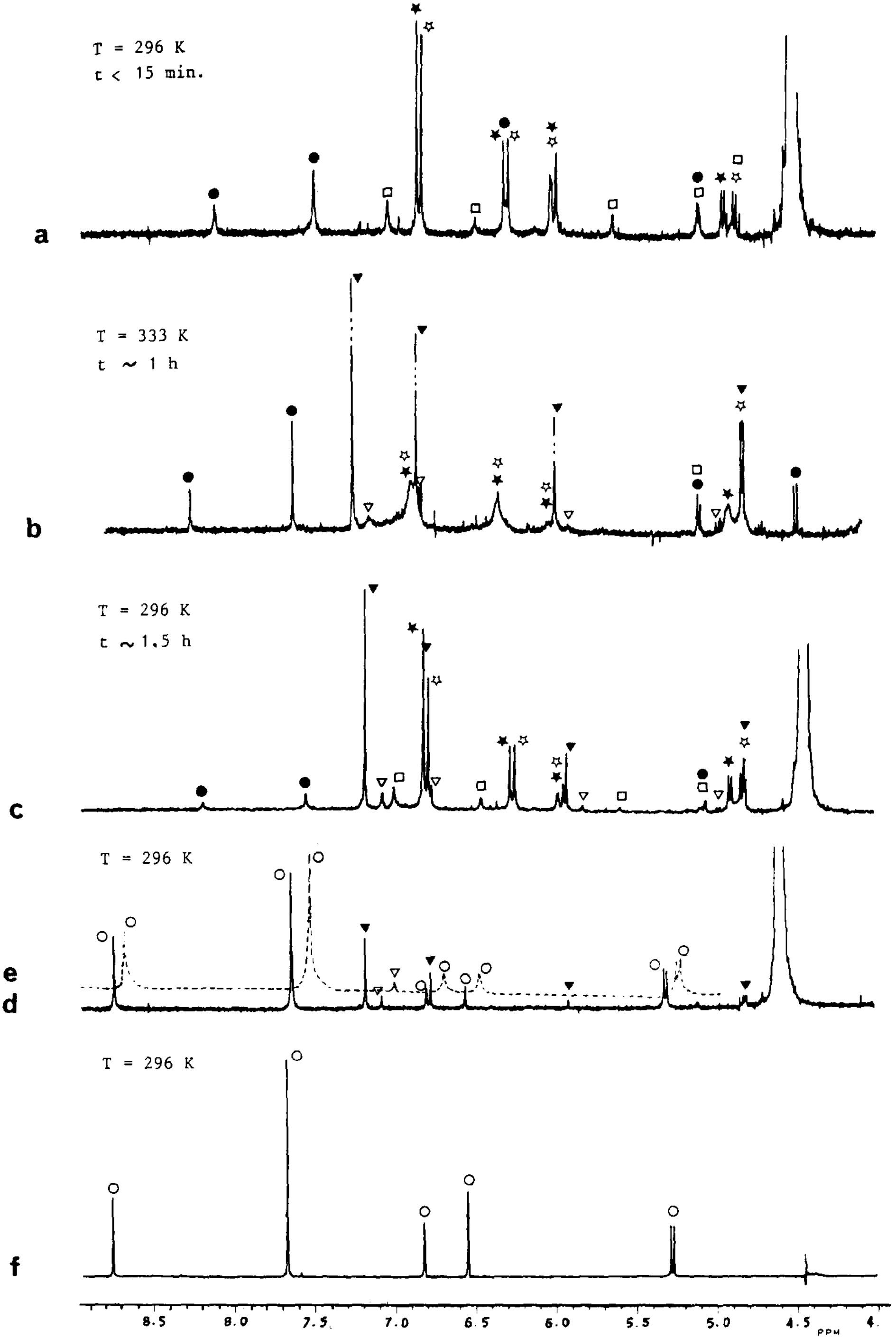


Figure 1 : PMR spectra of 3-O-(β-D-glucosyl)malvidin in aqueous solutions[7]
a - c : in slightly acidic conditions
d : after addition of concentrated DCl
e : d, 4 hours later
f : AH$^+$ reference spectrum.(○) AH$^+$; (●) A; (✶ , ✫) B_2, B'_2 or B'_2 , B_2;(□) B_4; (▼) C_E; (▽) C_Z.

Spectrum	AH^+	A	B_2 & B'_2	B_4	C_E	C_Z
t < 15 min[a], 296 K : 1a	-	31	63	6	-	-
t ≃ 30 min, 296 K (not shown)	-	13	76	11	traces	-
t ≃ 1 h, 333 K : 1b	-	11	38	coalescence	51 (C_E & C_Z)	
t ≃ 1.5 h, 296 K : 1c	-	6	47	10	33	4
1c with DCl, 296 K : 1d	72	-	-	-	24	4
1d after 4 h, 296 K : 1e	97	-	-	-	-	3

Table 1 : Percentages[b] of the different malvidin 3-glucoside structures in slightly acidic aqueous solution as a function of time and temperature.

a. t = 0 refers to the anthocyanin dissolution

b. average values over the time of accumulation of the signal (100-200 scans, 3.7 s per scan); these values do not correspond to equilibrium conditions.

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7. PMR (400 MHz, CD_3CN/D_2O 1/4, CD_3COONa, 1.2 x 10^{-2} M), 296 K. **Cation AH^+** (o) : 8.80 (1H, d, J = 0.6 Hz, H-4) ; 7.71 (2H, s, H-2' & H-6') ; 6.86 (1H, dd, J = 2 & 0.6 Hz, H-8) ; 6.59 (1H, d, J = 2 Hz, H-6) ; 3.87 (6H, s, $-OCH_3$) ; 5.32 (1H, d, J = 7.7 Hz, H-1Gl). **Quinoidal base A** (●) : 8.13 (1H, s, H-4) ; 7.5 (2H, s, H-2' & H-6') ; 6.3 (1H, H-6 or 8) ; 5.11 (1H, d, J = 4 Hz, H-8 or 6) ; 3.79 (6H, s, $-OCH_3$) ; 4.50 (1H, d, J = 7.8 Hz, H-1Gl). **Carbinols B_2 and B'_2** (✶☆ or ✡★) : 6.86 (s) & 6.83 (s) (both 2H, H-2' & H-6') ; 6.32 (s) & 6.29 (s) (both 1H, H-4); 6.02 (complex) & 5.97 (br.) (both 2H, H-6 & H-8) ; 3.76 (s) & 3.75 (s) (both 6H, $-OCH_3$) ; 4.94 (d, J = 8 Hz) & 4.87 (d, J = 8 Hz) (both 1H, H-1Gl). **Carbinol B_4** (□) : 7.05 (2H, br.s, H-2' & H-6') ; 6.5 (1H, br.s, H-4) ; 5.63 (1H, br.s, H-6 or 8) ; 5.1 (1H, br.s, H-8 or 6) ; 3.73 (6H, s, $-OCH_3$) ; 4.9-4.8 (1H, d, H-1Gl). **Chalcone C_E** (▼) : 7.24 (2H, s, H-2' & H-6') ; 6.85 (1H, s, H-4) ; 5.99 (2H, s, H-6 & H-8) ; 3.85 (6H, s, $-OCH_3$) ; 4.83 (1H, d, J = 7.6 Hz, H-1Gl). **Chalcone** C_Z (▽) : 7.13 (2H, s, H-2' & H-6') : 6.82 (1H, s, H-4) ; 5.87 (H-6 & H-8 ?) ; 3.79 (6H, s, $-OCH_3$); 5.02 (1H, d, J = 8 Hz, H-1Gl). The proton signals for the sugar moiety, in the cation spectrum are characteristic of a (β -D-glucosyl) group.

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