First characterization of anthocyanin-Ge and anthocyanin-B complex formation through UV-vis spectroscopy and DFT quantum chemical calculations

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Supporting Information

Table S1. Hyperchromic and bathochromic shift in red basil anthocyanins at different pH (5, 6, 7) and anthocyanin:metal(loid) stoichiometric ratio (1:1; 1:10; 1:100; 1:500). Values are the mean of five independent replicates. Different lower case letters indicate statistical significance (p = 0.05) of each parameter within the same element, independently of pH and anthocyanin:metal(loid) stoichiometric ratio. Different capital letters denote statistical significance (p = 0.05) of each parameters among different elements [1:500 anthocyanin:metalloid stoichiometric ratio at pH 5].

		1:1		**1:10**		**1:100**		**1:500**	
		Hypercromic shift (OD)	Bathochromic shift (nm)	Hypercromic shift (OD)	Bathochromic shift (nm)	Hypercromic shift (OD)	Bathochromic shift (nm)	Hypercromic shift (OD)	Bathochromic shift (nm)
Ge	pH 5	0.158 f	33.0 c	0.531 c	36.0 c	0.589 b	35.5 c	0.663 aA	35.5 cA
	pH6	0.167 f	40.5 b	0.353 d	43.0 ab	0.373 d	43.5 ab		
	pH7	0.051 g	49.5 a	0.309 e	55.5 a	0.313 e	55.0 a		
B	pH 5	0.017 d	2.5 c	0.085 b	14.5 b	0.072 b	13.0 b	0.376 aB	30.5 aB

pH equilibria of the uncomplexed cyanidin-3-OMe.

The pH equilibria of the uncomplexed cyanidin-3-OMe have been explored. The cation, neutral and anion forms were optimized at the B3LYP/6-31++G(d,p)/PCM (solvent = water) level of theory. Theoretical absorption spectra were obtained by means of time-dependent (TD) DFT (TD/ B3LYP/6-31++G(d,p)/PCM (solvent = water)) calculations.

The acid dissociation equilibria were analyzed as the pH increases where two deprotonation steps were included for the study: from the cation to the neutral and from the neutral to the anion form. Hydroxyl groups at 4', 7 and 5 were considered as potential acid sites. The relative Gibbs energies for the first and second deprotonations are shown in Table S2.

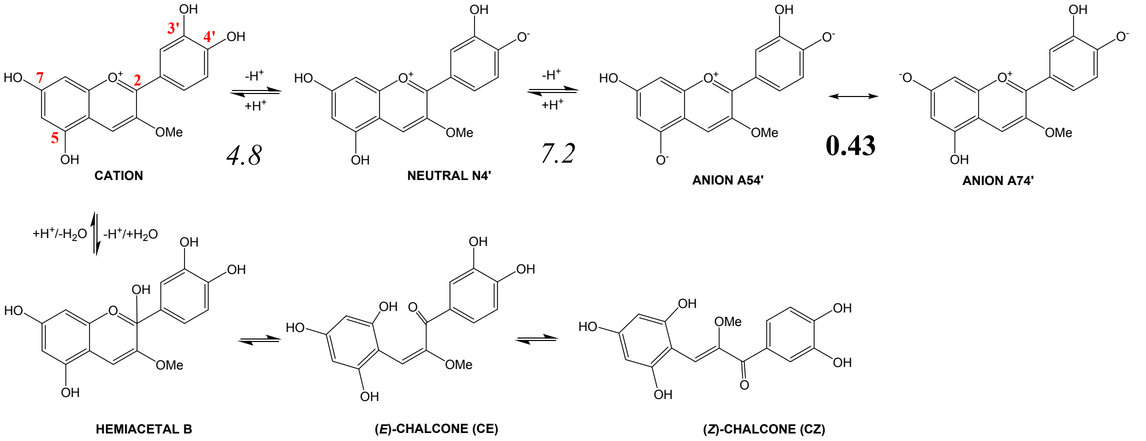
Table S2. Relative Gibbs energies (ΔG, in kcal·mol^{-1}) among possible tautomer of the neutral and anionic forms.

NEUTRAL tautomers			**ANION tautomers**	
N4'	**N5**	**N7**	**A74'**	**A54'**
0.0	4.11	3.86	0.50	0.0

DFT calculations indicate (Table 2) that tautomer **N4'** is the most favorable neutral form. Energy difference among them (**N5** or **N7**, resulting from deprotonation at H5 or H7, respectively) ($\Delta G_{(\mathbf{N4'}\text{-}\mathbf{N5/N7})}$ ~ 4.11/3.86 kcal·mol^{-1}) suggest that only **N4'** would exist in the pH equilibria while raising pH. The energy difference would come from intramolecular hydrogen bonding between H3' and O4' (comparison between conformers of **N4'**; one with dihedral angle C4'-C3'-O3'-H3' of 180º and the other with dihedral angle C4'-C3'-O3'-H3' of 0º gives rise to an energy difference between conformers of 4.95 kcal/mol).

Concerning second deprotonation leading to both anions **A54’** and **A74’**, DFT calculations indicate they are nearly isoenergetic (ΔG = 0.50 kcal·mol^{-1}; ΔE =0.43 kcal·mol^{-1}) and would be both present in the mixture at similar ratios.

Colorless forms have been also computed (Scheme 1). Hemiacetal form has been suggested to be the most abundant form of the colorless species in slightly acidic to neutral media. Our calculations indicate hemiacetal is more stable than *E*-chalcone and *Z*-chalcone, by 2.4 and 3.5 kcal·mol^{-1}, respectively, in good agreement with experimental studies.



Scheme 1. pH equilibria for the cyanidin-3-OMe model system. Estimated (see text) pK_a values (p$K_{a1(\mathrm{cation}\rightarrow \mathbf{N4'})}$: 4.8 and p$K_{a2(\mathbf{N4'}\rightarrow \mathbf{A54'})}$: 7.2) and energy difference between tautomers of the anion form, $\Delta E_{\mathbf{A54'}\rightarrow\mathbf{A74'}}$ (in bold, in kcal·mol^{-1}), are shown.

TD-DFT calculations were performed to simulate the UV-vis spectra of all possible forms of Cy. Wavelength of maximum absorbance, λ_{max}, corresponding to the HOMO -> LUMO transition together with oscillator strength values were obtained and they are collected in Table S3.

Table S3. Absorbance wavelength, λ, in nm, together with the associated oscillator strength value, f, (f > 0.1) for the different species of Cy-3-OMe that could exist in the pH equibria.

Cy species	**λ (nm)**	**f**
CATION	524.13/400.25/279.30	0.8284/0.1636/0.2485
NEUTRAL N4’	544.19/307.01/262.95	1.2722/0.2027/0.1439
NEUTRAL N5	629.47/442.22/308.24/264.49	0.2975/0.6173/0.1837/0.2104
NEUTRAL N7	567.15/404.87/291.45	0.6907/0.2410/0.5097
ANION A57	599.73/501.35/352.35/291.41	0.1414/0.6426/0.1334/0.4224
ANION A54’	624.08/483.20/314.69/294.36	0.7905/0.4427/0.2313/0.2123
ANION A74’	621.07/432.44/312.38	1.1172/0.1527/0.3299
ANION A3’4’	716.93/446.29	0.6476/0.6612
HEMI-BASE	292.80/285.81	0.4212/0.2069
Chalcone CE	429.44/328.10/285.96/271.95/266.24	0.1197/0.2180/0.2562/0.2012/0.2993
Chalcone CZ	396.99/335.08/292.43/263.30	/0.4501/0.1658/0.2132/0.3044

Prediction of the first and second pK_a of Cy.

In order to find out which species are largely present in the control solution at the different pH values explored, it is necessary to obtain the relative population of each of the color forms at the different pH values explored (pH 5-7). This requires to know the corresponding pK_a values. It should be considered as well, how shifted is the equilibria towards colorless forms. This latter has been accounted for by taking into account the hydration constant, pK_h = 3.01, reported in literature (see main text).

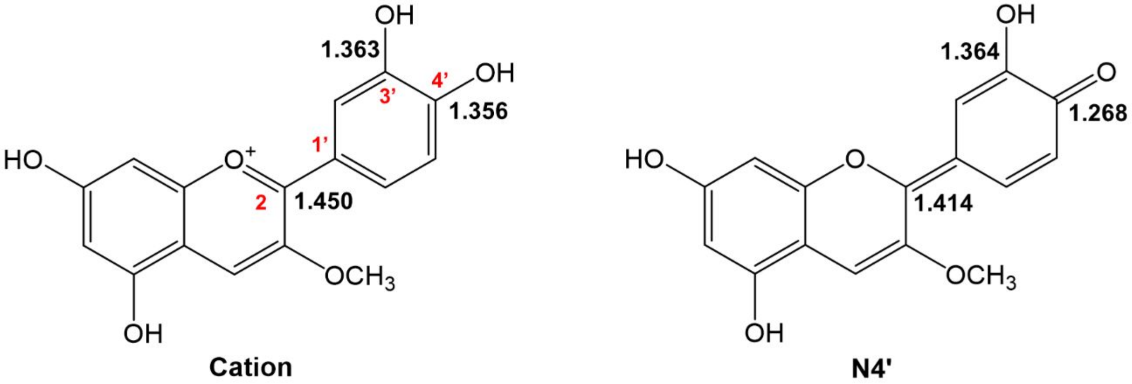
pK_a values have been obtained by following a strategy previously reported (see reference 47 in the main text). Shortly, this strategy is referred to as the parameters fitting method. It consists of using the experimental pK_a values of a set of small reference molecules (Table S4) to obtain two empirical parameters (k and C_0) by fitting the following linear equation:

$$\text{p}K_{\text{a(exp)}} = \text{k}*\Delta G_{\text{BA}} + C_0$$

where ΔG_{BA} is the difference in Gibbs energy between the conjugated base and the corresponding acid ($G_{\text{calc}}(\text{A}^-)$-$G_{\text{calc}}(\text{HA})$), calculated at the same level of theory than the investigated molecules, here B3LYP/6-31++G(d,p) and SMD (solvent = water). The same reference set, used previously, was chosen based on the kind of acid chemical group involved, in our case phenols (Table S4). After obtaining parameters k and C_0, they are used to calculate the pK_a of the molecule of interest. Their values in this work are k = 0.226 mol/kcal and C_0 = 56.383, with $R^2 = 0.935$. With these values, we obtain a pK_{a1} =4.8 and pK_{a2} = 7.2.

Table S4. Experimental pK_a values and difference in Gibbs energy, $\Delta G_{\mathrm{BA},}$ (in kcal·mol^{-1}) between the conjugated base, B, and the corresponding acid, A, of the selected set of phenols.

	E_A (Hartress)	E_B (Hartress)	ΔG_{AB}	pK_a
phenol	-307.425871	-306.95867	293.2	9.98
***o*-Clphenol**	-767.030763	-766.57061	288.8	8.56
***m*-Clphenol**	-767.032098	-766.57076	289.5	9.02
***p*-Clphenol**	-767.031726	-766.56837	290.8	9.38
***p*-Mephenol**	-346.722066	-346.25266	294.6	10.14
***m*-Mephenol**	-346.723399	-346.25418	294.4	10.08
***o*-Mephenol**	-346.721213	-346.25247	294.1	10.29
***m*-OHphenol**	-382.652947	-382.18711	292.3	9.65
***p*-OHphenol**	-382.650534	-382.18055	294.9	9.96
***m*-OMephenol**	-421.928218	-421.46159	292.8	9.65
***p*-OMephenol**	-421.925377	-421.45521	295.0	10.21
***p*-NO_2phenol**	-511.945615	-511.49948	279.9	7.15
***m*-NO_2phenol**	-511.941787	-511.48481	286.8	8.4
***o*-NO_2phenol**	-511.944524	-511.48987	285.3	7.23
***p*-NH_2phenol**	-362.774600	-362.30242	296.3	10.3
***m*-NH_2phenol**	-362.778212	-362.30924	294.3	9.87



Scheme S2. Molecular structure of the cation and neutral form (N4') of Cy where C2-C1', C3'-O and C4'- O distances (in Å) are shown for comparison.

QTAIM analysis of the metalloid-Cy bonding situation.

Table S5. QTAIM electron density properties of **a1** and **b1** complexes of B/Ge-Cy.

		M-O3'			M-O4'		
		ρ_b	$\nabla^2\rho_b$	H_b	ρ_b	$\nabla^2\rho_b$	H_b
M=Ge	**a1**	0.1322	0.6324	-0.0421	0.1288	0.6054	-0.0406
	b1	0.1316	0.6451	-0.0404	0.1175	0.5203	-0.0359
M=B	**a1**	0.1954	0.8494	-0.1389	0.1900	0.8234	-0.1329
	b1	0.1434	0.4408	-0.1100	0.1232	0.3132	-0.0878

Complexation energies.

Table S6. Complexation energies, ΔE_{sol}, in kcal·mol^{-1} for the reaction between the corresponding color (**N4'**, **A54'** and **A74'**) and colorless (hemiacetal and chalcone Z) form of Cy and the metalloid (B or Ge).

	N4'	**A54'**	**A74'**	Hemiacetal	Chalcone Z
B	1.28	-2.12	-2.01	9.82	10.47
Ge	-21.32	-24.85	-24.83	0.23	1.63