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Origin of the Color of Cv. Rhapsody in Blue Rose and Some Other So-called "Blue" Roses

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Flowers of the rose cultivar Rhapsody in Blue display unusual colors, changing as they age, from a vivid red-purple to a lighter and duller purple, which are based on tonalities corresponding to hue angles between 340 and 320° in the CIELAB scale. Unexpectedly, the chemical basis of these colors is among the simplest, featuring cyanin (cyanidin 3,5-di-*O*-glucoside), the most frequent anthocyanin in flowers, as the sole pigment and quercetin kaempferol glycosides as copigments at a relatively low copigment/pigment ratio (about 3/1), which usually produces magenta or red shades in roses. This color shift to bluer shades is coupled with the progressive accumulation of cyanin into vacuolar anthocyanic inclusions (AVIs), the occurrence of which increases as the petals grow older. In addition to the normal λ_{max} of cyanin at ∼545 nm, the transmission spectra of live petals and of epidermal cells exhibit a second λ_{max} in the 620−625 nm range, the relative importance increasing with the presence of AVIs. In petals of fully opened flowers, the only pigmented structures in the vacuoles of epidermal cells are AVIs; their intense and massive absorption in the 520−640 nm area produces a much darker and bluer color than measured for the vacuolar solution present at the very first opening stage. Cyanin is probably "trapped" into AVIs at higher concentrations than would be possible in a vacuolar solution and in quinonoidal form, appearing purple-blue because of additional absorption in the 580−630 nm area. Quite similar pigmentation features were found in very ancient rose cultivars (cv. L'Evêque or Bleu Magenta), also displaying this type of so-called "blue" color.

KEYWORDS: Blue rose; cyanin; anthocyanic vacuolar inclusions (AVI); flower color

INTRODUCTION

For many years, the quest for the "holy grail" in horticulture, the blue rose, never attained its ultimate goal. However, many breeders have anticipated this achievement because the commercial names of more than 100 cultivars refer to the term of blue, bleu (in French), or blaü (in German).

In fact, on the basis of Royal Horticultural Society (RHS) color codes (*1*), and colorimetric measurements for some, the color palette of these "blue" varieties covers the red-purple (RHS sheets 70−74), purple (sheets 75−79), purple-violet (sheets 80−82), and violet (sheets 83−88) groups and, exceptionally, the violet-blue group (sheets 89−98). The roses corresponding to the first two color groups display a wide range of color "intensities", from light to dark coupled to dull to vivid colors, that is, from purplish-pink to purplish-red, whereas those in the second group are generally restricted to light and dull colors, such as lavander or mauve. On the basis of objective color measurement, their basic tonalities correspond to hue angles on the CIELAB color wheel (*2*) approximately between 355 and 345° for the first group and between 345 and 325° for the second one. The color gap between these tonalities and really blue ones is impressive because blue colors cover the ∼275−240° portion of the color wheel. In the RHS color chart, the blue group is represented by sheets 99−110, and there is no rose cultivar having a color code in this area.

Recently, a newly introduced shrub rose cultivar was reported to represent "an important color break", and its color was described as a striking "iridescent purple" that in overcast conditions looks remarkably blue (*3*). Its original name, cv. Frantasia, was finally changed to cv. Rhapsody in Blue when the rose was introduced into the commercial market. It also has won several awards. The availability of this plant material in quantities represented an interesting opportunity to investigate the physical and chemical basis of this apparently unusual color in roses. "Cyanic" colors (red to purple and up to blue shades) are currently based on the presence of water-soluble anthocyanin pigments, the color expression of which depends on multiple factors in the vacuolar compartment: the nature of the basic pigment itself; vacuolar pH effects; and the presence and relative concentration of other phenolic molecules named copigments (*4*−*7*). This study was undertaken to determine the factors responsible for the "blue" color of the cv. Rhapsody in Blue rose variety.

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MATERIALS AND METHODS

Plant Source. Cv. Rhapsody in Blue (Frank Colishaw, breeder) plants were cultivated in fields at the GAEC Orard (Feyzin, France). Fresh flowers were harvested and stored at −18 °C. Some other cut flowers were also placed in a culture room (OSRAM Biolux 965 fluorescent lamps, color temperature = 6500 K; illuminance level = 3000 lx; and daytime = 14 h; T = 22 °C) for spectroscopic and colorimetric measurements of petals at five successive blooming steps ranging from (A) just-opening bud, (B) half-opened flower, and (C) just fully opened flower to (D, E) largely spread petals at ∼24 h interval.

Additional flower material employed for comparisons was cultivated by the author: cv. Lancôme (Delbard, breeder); and a few flowers of ancient varieties found at the Jardin Botanique du Parc de la Tête d'Or (Lyon, France), cv. L'Evêque (unknown breeder, before 1790), and cv. Bleu Magenta (Van Houtte, breeder, ∼1900).

Color Specifications of Petals. The spectrocolorimetric procedure employed for the measurement of petal colors has been previously described (*8*, *9*). Spectral reflectance curves (between 380 and 780 nm; specular component included) were recorded on two or three circular areas (diameter = 6 mm) on each of the five petals measured at each blooming stage by a Datacolor International CS5 spectrocolorimeter. Each color is numerically specified in the CIELAB scale (for the CIE D_{65}/10° illuminant/observer condition) by the psychometric correlates of its attributes of color perception by the human visual system: hue angle (h_{ab}, correlate of chromatic tonality or hue), lightness (L^*), and metric chroma (C^*, saturation) (*2*). RHS codes were visually and instrumentally assessed using the conditions and system previously reported (*9*, *10*); however, the system of numerical assessment of RHS codes was updated with the introduction of the $CIEDE_{2000}$ color difference calculations (*11*).

Spectroscopic Measurements of Live Petals. Transmission curves between 380 and 780 nm were recorded using a Kontron Uvikon 943 spectrophotometer on petals fixed on a glass microscope slide (1 mm thickness). The reference was recorded on another microscope glass slide, covered by several layers of a diffusing opalescent adhesive tape; the correction for baseline flatness was run by the calculation utilities of the Uvikon 943 software.

In Vivo Microscopical Observation and Spectroscopic/Colorimetric Measurements of Epidermal Cell Content. Upper epidermal tissue was peeled off the petals and mounted into a drop of an isotonic 0.1 M aqueous sucrose solution. Brightfield and difference interferential contrast (DIC) observations were performed with an Olympus BHA microscope at magnifications between 100× and 1000×.

Spectroscopic curves of portions of individual epidermal cells were recorded using a Leitz MPV single-beam microphotometer. Readings were performed between 400 and 700 nm at a 2.5 nm interval (bandwidth ∼10 nm) using a continuous interferential filter; the wavelength correspondence with the filter numerical scale was checked using an Agilent 8453 diode array spectrophotometer. The reference transmission baseline was recorded on the vacuole of unpigmented cells in the adjacent mesophyll. The transmission spectra of vacuolar sap of epidermal cells were recorded on ∼15 μm circular areas (using 40× objective and 25× eyepiece), wheras for anthocyanic vacuolar inclusions (AVIs) the measurement zone was reduced to ∼3−4 μm only in diameter (90× objective and 25× eyepiece), to provide some color heterogeneity. Subsequent absorbance and colorimetric calculations (CIELAB scale, LCH notation for the D_{65}/10° CIE illuminant/observer condition) were applied to those transmission curves with the help of specially developed software using the spectral and colorimetric data adopted by the CIE (*2*, *12*).

Extraction of the Pigment Mixture and Isolation of the Anthocyanin. Anthocyanin and flavonol copigments were extracted twice for 20 min from frozen (liquid N_2) fresh petals (50 g, of a mixture of petals harvested at different stages) with a boiling mixture of MeOH/EtOH (1:1, v/v, 500 mL). Lipophilic surface material was removed by centrifugation of the refrigerated (−18 °C, overnight) methanolic crude solution obtained after concentration of the initial alcoholic extract. The sole anthocyanin present in the petals of cv. Rhapsody in Blue rose was isolated as a pure compound by following the same procedure employed for the isolation of the anthocyanins of the cv. RedRox carnation (*9*) applied to an aliquot of the methanolic solution. Additional extractions of a few petals harvested at specific stages B and D (∼1.5 g of each) were performed for comparison of their pigment contents.

Chromatographic Analysis. TLC of anthocyanin(s) was performed on cellulose plates (Merck, 5577) with the solvents HAW (HCl/HOAc/H_2O, 3:15:82, v/v) and BAW (1-butanol/HOAc/H_2O, 4:1:5, v/v, upper phase) in the presence of authentic samples of cyanidin 3-*O*- and 3,5-di-*O*-glucosides. TLC of anthocyanidin(s) obtained after hydrolysis with 2 N HCl for 40 min of a few drops of the solution and extraction with 1-butanol was performed on the same plates with the Forestal solvent (HCl/HOAc/H_2O, 3:30:10, v/v) in the presence of authentic samples of cyanidin and pelargonidin. TLC of flavonol aglycons after hydrolysis and extraction by Et_2O was performed on DC11 polyamide (Merck, 5555) with the solvent toluene/methyl ethyl ketone/MeOH (4:3:3, v/v), in the presence of authentic samples of quercetin and kaempferol. ExtraSynthèse (Genay, France) supplied all reference samples.

HPLC analyses were performed using an Agilent 1100 series chromatograph controlled by Agilent ChemStation software. The methanolic pigment extract diluted with MAW (MeOH/HOAc/H_2O, 25:4:21, v/v) was injected (20 μL) on a 150 mm × 4.6 mm i.d., 3.5 μm reversed-phase C_{18} X-Terra MS (Waters Associates) column. The elution was run at 21 °C and at a 0.5 mL/min flow rate using two successive linear gradients of A (2% aqueous TFA) and B (H_2O/acetonitrile/TFA, 70:30:1, v/v): 20−45% B in 20 min, then 45−90% B in 22 min followed by a short isocratic step (90% B, 3 min), and finally back to initial conditions (20% B, 12 min). On-line chromatographic and spectroscopic readings (220−640 nm interval) were recorded with a diode array spectrophotometric detector (DAD, Agilent G1315B); simultaneous detection of anthocyanin and flavonol glycoside copigments was achieved by signal monitoring at 520, 280, and 350 nm, respectively. Nine flavonol glycosides were found in the HPLC profile and their aglycons assigned by on-line UV spectrophotometry: t_R = 33.75 min [quercetin (Q), λ_{max} 257, (278) 357 nm, relative amount = 1.5%], t_R = 34.78 min (Q, 32.2%), t_R = 36.30 min (Q, 14.4%), t_R = 38.19 min (Q, 2.8%), t_R = 39.10 min (Q, 12.1%), t_R = 39.61 min [kaempferol (K), λ_{max} 270, 355 nm, 23.7%], t_R = 41.89 min (K, 5.9%), t_R = 43.26 min (K, 1.1%), and t_R = 44.56 min (K, 6.4%).

Anthocyanin and Flavonol Glycoside Quantification. Anthocyanin concentration in flowers was quantified in triplicate by direct spectrophotometry (Agilent 8453) of the methanolic solutions diluted by MeOH/HCl (100:0.1, v/v) using $E^{1‰}$ = 49.4 for cyanin. Flavonol glycosides were also quantified (expressed as rutin equivalents) by differential photometric measurement of the extracts using EtOH/$AlCl_3$ with the extract diluted by EtOH/$AlCl_3$ versus extract diluted by EtOH only, at 405−410 nm and a reference $E^{1‰}$ = 32. Copigment/pigment ratio (concentration/concentration) in the extract was then calculated from these data.

RESULTS AND DISCUSSION

Color and Spectroscopic Features of Cv. Rhapsody in Blue Flowers. This cultivar exhibits very important variation of petal color during blooming, which takes about 3 days in summer. It was reported as having "dark plum-colored flowers (RHS color chart 77A), which fade to a striking shade of slate-blue (83B)" (*3*). **Table 1** lists the CIELAB L^*, C^*, and h_{ab} colorimetric coordinates of petals measured at blooming stages A−E and their RHS codes numerically assessed for the corresponding illumination/observer condition (D_{65}/10°).

The most intensely colored petals were those at the initial stage (the lowest lightness L^* and highest saturation C^* levels), based on a red-purple shade (h_{ab} = 340°). Then, their color continuously faded as the flower grew older, by the combined effects of loss of saturation (the most influential parameter in stages B and C) and increased lightness (especially in stages D and E). Simultaneously, the hue continuously shifted to bluer tonalities, down to h_{ab} = 323°. However, this final hue remained far from truly blue, and all of the RHS codes based on these

Table 1. CIELAB Coordinates and RHS Color Codes (for the CIE $D_{65}/10°$ Illuminant/Observer Condition) of Petals of Flowers of Cv. Rhapsody in Blue Rose at Blooming Stages A–E

flower blooming stage	L^*	C^*	h_{ab}	RHS code[a]	$CIEDE_{2000}$ color difference
A	27.60	49.29	340.53	71A	9.97
B	27.38	33.40	335.09	79B	8.85
C	29.72	27.62	328.33	79B	5.31
D	31.65	27.75	326.32	79B	4.02
E	41.84	25.25	322.75	79C	2.11

[a] Determination of RHS codes is based on the search of smallest color differences ($CIEDE_{2000}$) between the CIELAB coordinates of the petals and of all the chips in the RHS chart. Petals at stages B–D have the same code (79B), but the color of the petals at stage D is the closest to this reference.

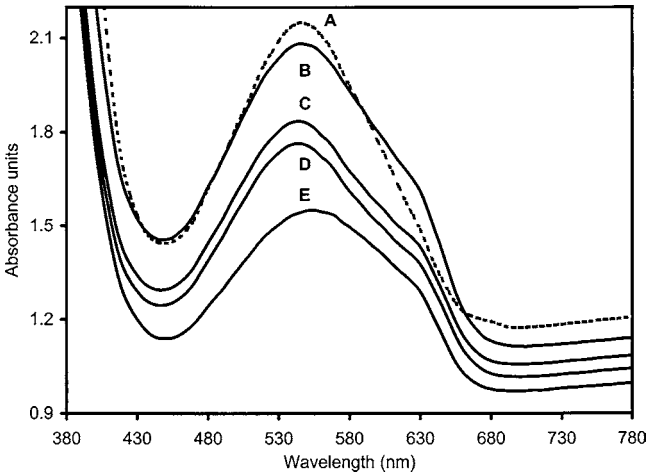

Figure 1. Transmission spectra of live petals of flowers of cv. Rhapsody in Blue rose at blooming stages A–E: (A) just-opening bud; (B) half-opened flower; (C) just fully opened flower; (D, E) largely spread petals at ~24 h interval.

measurements (code 79B and 79C) were in the purple and violet groups, except for the red-purple one for stage A (code 71A).

Transmission spectra of live petals (**Figure 1**) revealed the physical origin of these color variations. At stage A, the spectrum of the petal tissue featured a relatively broad absorption band with λ_{max} at 545 nm and an additional discrete shoulder at ~625 nm (ratio $A_{\lambda max}/A_{625} = 2.7$). As the petals were opening, their transmission λ_{max} values progressively shifted to higher wavelengths (up to 554 nm, stage E), whereas absorbance decreased; simultaneously, the absorption at 625 nm gained in relative importance (ratio $A_{\lambda max}/A_{625} = 1.8$ at stage C and =1.6 at stage E). Decreasing absorbance and a broadened peak caused higher lightness and lower saturation of petal color; the emergence of the second absorption at ~625 nm, coupled with the bathochromic shift at the λ_{max}, was mainly responsible for the hue becoming bluer.

Pigment Analysis. TLC of the methanolic extract showed the presence of only one magenta-red anthocyanin. HPLC of the same extract confirmed the presence of a single pigment ($t_R = 23.32$ min) with on-line UV−visible spectrum λ_{max} 278 and 514 nm ($A_{440nm}/A\lambda_{max,vis} = 0.17$, $A\lambda_{max,UV}/A\lambda_{max,vis} = 0.46$). This molecule was identical by HPLC and TLC cochromatography to the reference cyanin (cyanidin 3,5-di-*O*-glucoside), confirmed by its spectroscopic properties in 0.1% MeOH/HCl (λ_{max} 278 and 529 nm; $A_{440nm}/A\lambda_{max,vis} = 0.16$, $A\lambda_{max,UV}/A\lambda_{max,vis} = 0.54$) and hydrolytic methods. The average cyanin concentration in petals was 19.59 ± 0.91 mg/g of dry weight. Cyanin concentrations measured at stages B and D varied from 24.3 to 19.01 mg/g, respectively.

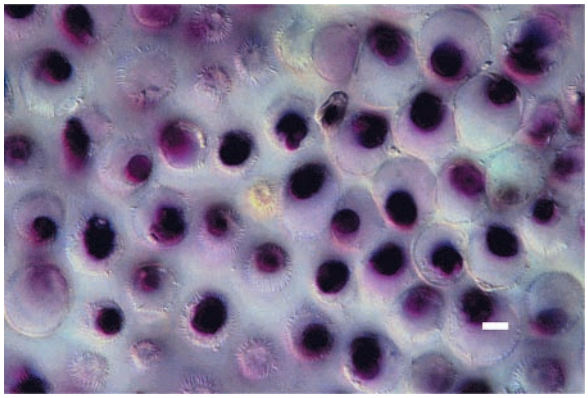

Figure 2. Polar view of inner epidermal cells of petals of cv. Rhapsody in Blue rose at blooming stage B [difference interferencial contrast (DIC) microscopy]. Bar = 10 μm.

Two flavonols—quercetin (major compound) and kaempferol (minor compound)—were detected by polyamide TLC of the copigment aglycons after acid hydrolysis of the extract. The quercetin/kaempferol ratio of the nine flavonol glycosides detected was 63/37. Finally, on the basis of spectrophotometric measurements, the copigment (flavonols) to pigment (cyanin) concentration ratio gave an average value of 3.04 ± 0.15. Comparable values were obtained for the petals harvested at stages B and D (2.86 and 3.03, respectively).

The variations measured for the pigment concentrations and copigment/pigment ratios between petals at blooming stages from B to D are in accordance with the colorimetric changes observed: higher lightness coupled with lower chroma.

This global pigment/copigment pattern is remarkably simple and very close to the one previously reported (*13*) and newly reinvestigated for cv. Lancôme. Cyanin again was the sole pigment with an average concentration ~18 mg/g of dry weight, the constitution of the HPLC pattern of flavonol glycosides copigments having a 60/40 quercetin/kaempferol ratio and a copigment-to-pigment ratio of 3.6/1. However, in flowers of cv. Lancôme, as in many other "cyclamen-red" roses, the in vivo spectroscopic and colored expressions of this pigment pattern are strikingly different from the ones observed in cv. Rhapsody in Blue. This cultivar had a symmetrical transmission spectrum ($\lambda_{max} = 536$ nm) and a vivid magenta-red shade of petals ($L^* = 47$, $C^* = 60$, and $h_{ab} = 360°$, RHS code 66A) typical of most "basic" cyanin/quercetin−kaempferol simple glycoside mixtures at these concentration levels. Consequently, the unusual, markedly bluer, color displayed by the comparable vacuolar pigment content in flowers of cv. Rhapsody in Blue suggested that another predominant factor was involved.

Microscopy and Microspectrophotometry—Colorimetry of Petal Epidermis. Polar and lateral observations by DIC and brightfield microscopy of peeled inner and outer epidermal cells of petals revealed in both a particular accumulation mode of pigments in the vacuolar compartment. At the initial blooming stage (A), vacuoles of most cells displayed a uniform and light, vivid, purplish-pink coloration, but, in the others, there were one—or two—dark purplish-red globular bodies ~9−12 μm in diameter (polar view), localized at the center of a poorly colored vacuole and corresponding to structures described in *Lisianthus* and carnations, and recently renamed AVIs (*14*). At the next development stage (B, **Figure 2**), nearly all vacuoles contained anthocyanic globules on a very pale magenta vacuolar surround. At stage C, no color at all was perceptible in the vacuolar

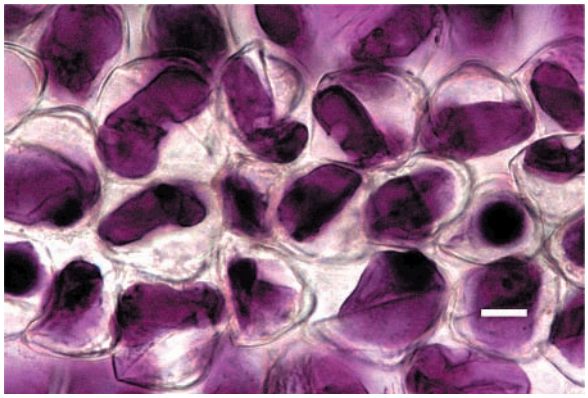

Figure 3. Slighly squashed epidermal cells of petals of cv. Rhapsody in Blue rose at blooming stage D (brightfield observation). When viewed sideways, AVIs appear as having irregular cylindrical shapes. Bar = 10 μm.

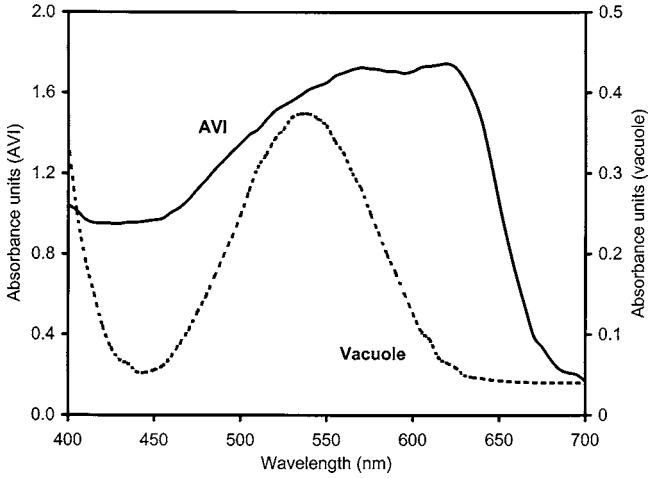

Figure 4. Absorption spectra of a vacuolar solution (stage A) and of an AVI (stage E) in epidermal cells of petals of cv. Rhapsody in Blue rose (microspectrophotometry).

compartment, the only colored portion being the dark AVI. At the last two stages, D and E (**Figure 3**), the vacuolar bodies had grown in size (between 10 and 14 × 20−32 μm, "diameter" × length) and became more irregularly shaped, and their color had turned dark purple.

The visible absorption spectrum of the vacuolar sap of an epidermal cell (optical path length ∼20−25 μm) at the young stage, A (**Figure 4**), showed a unique peak at λ_{max} = 537−540 nm; accordingly, the CIELAB coordinates calculated from the corresponding transmission curve of this in vivo pigment mixture are those of a light medium purplish-pink, L^* = 80.8, C^* = 32.1, and h_{ab} = 324.4°. In contrast, a typical spectrum of an AVI (**Figure 4**) showed a massive absorption zone in the center of the visible spectrum with λ_{max} ∼ 568 and 621 nm. Readings of different bodies displayed very similar shapes, the variations affecting the absorbance scale only. The AVI measured in **Figure 4** displayed a dark and relatively dull color (L^* = 21.4 and C^* = 32.8), based on a typically violet-blue hue (301.4°).

The simple cyanin-based pigment/copigment pattern of the flowers of cv. Rhapsody in Blue is quite comparable to those reported for many rose cultivars displaying "classic" cyanic colors in different "intensities" (pink, red, dark red, purplish-red, etc.), according to the absolute pigment accumulation level (*13*, *15*), but its unusual color expression is coupled with the abundant presence of AVIs, which seem to be the most influential parameter of the color shift observed. Comparable structures, originally described as blue "spherules", were observed in roses more than 30 years ago in the red cv. Crimson Glory and their bluing effect was reported in aging petals; it was hypothesized that the blueness of the spherule resulted from the synergistic effects of a colorless tannin-like substance, cyanin, and iron (*16*), the latter two probably being connected individually with the surface of the spherule (*17*). Spectroscopic data of live tissues (*17*) were very similar to the ones measured in the present work. More recent reports stated that such anthocyanic globular inclusions, also called anthocyanoplasts, might be protein matrices without a membrane or an internal structure (*18*). Finally, Markham et al. (*14*) demonstrated the nature of anthocyanoplasts in flowers of *Lisianthus* and carnations, renaming them AVIs, and showed their influence on the color of anthocyanins. AVIs appear to be "membrane-less proteinaceous matrices with a high specificity for certain anthocyanin-3,5-diglucosides", the association with metals being excluded. In carnations, the shape of the transmission spectra of petals without and with AVIs, especially a noticeable shoulder at ∼625 nm, is quite comparable to those of transmission spectra recorded at stages A (AVI-free) and C−E (AVI-rich), respectively, during the blooming of cv. Rhapsody in Blue. The CIELAB calculations (D_{65}/10°) performed by the present author on transmission curves of "normal" and AVI-containing carnation petals recorded by Markham show a hue angle shift of about −12 to −15° on the color circle to bluer tonalities when AVIs are present.

The darker color of AVIs is assumed to result from the attachment, through hydrogen bonding, of the anthocyanins (specifically diglycosides) to the protein matrix, leading to the accumulation of the "trapped" pigment at higher levels than is possible in the vacuolar sap (*14*). According to the authors, this bonding of the pigment, and/or the self-association of anthocyanins resulting from their high concentration, could also be responsible for the bluing effect by favoring the quinonoidal base forms of the pigment. An additional peak at ∼625 nm in the spectroscopic records of live petals of carnations and cv. Rhapsody in Blue and the massive absorption of the AVIs themselves in the 560−630 nm area are quite similar to the spectroscopic features reported for anthocyanin quinonoidal bases (*6*).

It was reported in the Introduction that the color of cv. Rhapsody in Blue represented an "important color break" in roses (*3*). In reality, it seems that this color type was introduced many decades ago in the subgenus *Rosa*. In the Jardin Botanique du Parc de la Tête d'Or in Lyon (France), several ancient varieties exhibit intense purple shades, visually close to the ones of cv. Rhapsody in Blue, and present a comparable evolution of their color with aging, including cvs. L'Evêque, Tuscany Superb, Hippolyte, Cardinal de Richelieu, and Bleu Magenta. In the Helpmefind rose database (*19*), all of these are reported as having mauve and mauve blend colors. All but cv. Bleu Magenta, a *Multiflora* hybrid of unknown parentage, also belong to the *Gallicanae* section of the subgenus *Rosa*.

Only a few petals each of cvs. Bleu Magenta and L'Evêque were available for a rapid comparative investigation. In opened flowers of both varieties, microscopy again revealed that pigmentation was exclusively accumulated in dark red-purple AVIs. Cyanin was also the sole anthocyanin present, and eight of the nine flavonol copigments detected in the HPLC profile of cv. Rhapsody in Blue were also found, the main difference being that kaempferol glycosides were dominant in cv. Bleu Magenta (Q/K = 33/67 vs 86/14 in L'Evêque), copigment/

pigment ratios being 2.6/1 for cv. Bleu Magenta and 3.8/1 for cv. L'Evêque. CIELAB coordinates of petals of cvs. L'Evêque and Bleu Magenta at both blooming stages corresponding to B and D closely matched those of petals of cv. Rhapsody in Blue. In addition, in cv. Cardinal de Richelieu, cyanin was also reported to represent >97% of the anthocyanin content, accompanied by traces of its *p*-coumaroyl derivative (1%) and kuromanin (cyanidin 3-*O*-glucoside, 1%) (*20*). Consequently, it appears that the newly obtained cv. Rhapsody in Blue and at least two ancient cultivars share very close global color and pigment features, probably as the result of some common history or lineage; it is also noteworthy that a "Gallica" cultivar (*R. gallica* Violacea) figures in the parentage of cv. Rhapsody in Blue (*3*).

Although cv. Rhapsody in Blue does not have really blue flowers, the chemical basis of their purple color represents an interesting example of an unusual colored expression of a very classical and simple cyanin−flavonols pattern; it mainly results from accumulation in AVIs, causing an important shift of its basic magenta-red tonality to bluer ones. One cannot expect to generate blue roses by hybridization of this new cultivar—or more ancient ones—exhibiting this original pigment feature, but its particular color seems to be missing in modern roses (tea hybrids) and using cv. Rhapsody in Blue as a source for hybridization can be considered.

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LITERATURE CITED

(1) RHS. *The Royal Horticultural Society Colour Chart*, 1st−3rd eds.; London, U.K., 1966, 1986, 1995.

(2) CIE. *Colorimetry. Recommendations on Uniform Colour Spaces, Colour Difference Equations. Psychometric Terms*, 2nd ed.; Publication 15-2; Central Bureau of the CIE: Vienna, Austria, 1986.

(3) Page, M.; Garbutt, S. Blue rose success for an amateur. *Garden* **2002**, *127*, 584.

(4) Asen, S.; Stewart, R. N.; Norris, K. H. Anthocyanin-flavonol co-pigments and pH responsible for larkspur flower color. *Phytochemistry* **1975**, *14*, 2677−2682.

(5) Brouillard, R. The in vivo expression of anthocyanins colour in plants. *Phytochemistry* **1983**, *22*, 1311−1323.

(6) Brouillard, R.; Dangles, O. Flavonoids and flower colour. In *The Flavonoids—Advances in Research since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, U.K., 1994; pp 565−588.

(7) Goto, T. Structure, stability and color variation of natural anthocyanins. *Prog. Chem. Org. Nat. Prod.* **1987**, *52*, 113−158.

(8) Gonnet, J.-F. CIELAB measurement, a precise communication in flower colour: An example with carnation (*Dianthus caryophyllus*) cultivars. *J. Hortic. Sci.* **1993**, *68*, 499−510.

(9) Gonnet, J.-F.; Fenet, B. "Cyclamen" red colors based on a macrocyclic anthocyanin in carnation flowers. *J. Agric. Food Chem.* **2000**, *48*, 22−26.

(10) Gonnet, J.-F. A colorimetric look at the RHS Chart—Perspectives for an instrumental determination of codes. *J. Hortic. Sci.* **1995**, *70*, 191−206.

(11) CIE. *Improvement to Industrial Colour-Difference Evaluation*; CIE Publication 142; Central Bureau of the CIE: Vienna, Austria, 2001.

(12) ISO. *Norme ISO/CIE 10526: Illuminants Colorimétriques Normalisés. Norme ISO/CIE 10527: Observateurs Colorimétriques Normalisés*; CIE: Paris, France, 1991.

(13) Biolley, J. P.; Jay, M. Anthocyanins in modern roses: Chemical and colorimetric features in relation to the colour range. *J. Exp. Bot.* **1994**, *44*, 1725−1734.

(14) Markham, K. R.; Gould, K. S.; Winefiled, C. S.; Mitchell, K. A.; Bloor, S. J.; Boase, M. R. Anthocyanic vacuolar inclusions—their nature and significance in flower colouration. *Phytochemistry* **2000**, *55*, 327−336.

(15) Biolley, J. P.; Jay, M.; Viricel, M. R. Flavonoid diversity and metabolism in 100 *Rosa* × *hybrida* cultivars. *Phytochemistry* **1995**, *35*, 413−419.

(16) Yasuda, H. Studies on "bluing effect" in the petals of red rose. I. Some cytochemical observations on epidermal cells having a blue tinge. *Bot. Mag., Tokyo* **1970**, *83*, 233−236.

(17) Yasuda, H. Studies on "bluing effect" in the petals of red rose. VI. Further observations on the development of blue color of the spherule. *Cytologia* **1982**, *47*, 717−23.

(18) Nozue, M.; Yamada, K.; Nakamura, T.; Kubo, H.; Kondo, M.; Nishimura, M. Expression of vacuolar protein (VP24) in anthocyanin-producing cells of sweet potato in suspension culture. *Plant Physiol.* **1997**, *115*, 1065−1072.

(19) Helpmefind rose database: http://www.helpmefind.com/Roses; last accessed June 12, 2003.

(20) Mikanagi, Y.; Saito, N.; Yokoi, M.; Tatsuzawa, F. Anthocyanins in flowers of genus *Rosa*, sections *Cinnamomeae* (= *Rosa*), *Chinenses*, *Gallicanae* and some modern roses. *Biochem. Syst. Ecol.* **2000**, *28*, 887−902.

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