How-To-Do-It

The Influence of pH on the Color of Anthocyanins and Betalains

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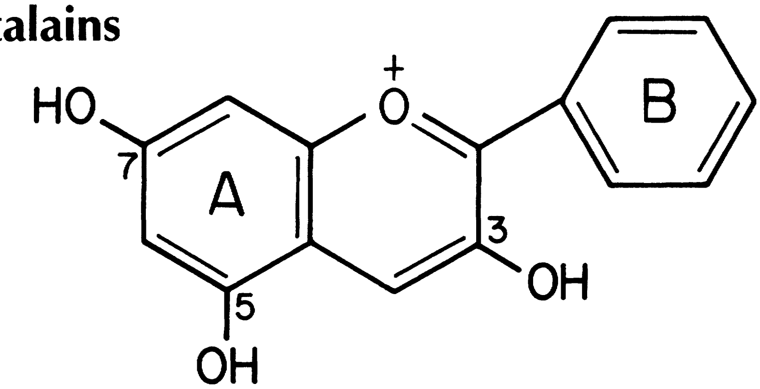

Figure 1. Structure of anthocyanidin. Position 3 of ring A is always glycosylated with glucose galactose, rhamnose, xylose-glucose, rhamnose-glucose, or glucose-glucose. Position 5 is sometimes glycosylated—if so, it is glycosylated with glucose. Position 7 is almost never glycosylated—if so, it is glycosylated with glucose.

Anthocyanins are a water-soluble group of pigments called flavonoids that are widespread among angiosperms, usually absent in liverworts and algae, and rare in mosses and gymnosperms. More than one anthocyanin often occurs in the same flower or organ, and these pigments can impart violet, blue, purple, red and scarlet colors to flowers, stems, fruits and leaves.

Anthocyanins are typically present as glycosides of one or two sugar molecules attached to anthocyanidin (Figure 1), which is also colored. The presence of sugars on the molecule increases its solubility, and allows anthocyanins to accumulate in the aqueous sap of the central vacuole of plant cells. Hydroxylation or methylation of ring B of anthocyanidin (Figure 1) produces anthocyanins of different colors (Figure 2).

Anthocyanins probably function in attracting pollinators to flowers, and also contribute to the spectacular colors of autumn leaves. Yellow and orange carotenoids (e.g., in tomatoes) responsible for similar colors are unrelated to anthocyanins.

The color of anthocyanins depends on several factors:

Associations with other compounds. For example, an association with phenolic compounds usually produces a blue color.

Substituent groups on the B ring of anthocyanidin. For example, the presence of methyl groups on this ring reddens the pigment.

pH of the cellular sap. Most anthocyanins are purple or blue at high pH, and become reddish as the pH decreases. This pH-dependent change in color results from ionization of hydroxyl groups on the B ring, followed by electron shifts on the ring.

Betalains include red and yellow pigments called betacyanins and betaxanthins. Despite their similar colors, betalains are unrelated to anthocyanins, and are found in only 10 families of plants, all of which are members of the order Caryophyllales (Centrospermae). Indeed, betalains and anthocyanins do not occur in the same plant—individual plants cannot synthesize both of these pigments.

Betalains, like anthocyanins, are glycosides of sugars and colored groups. For example, betanin (the betalain in roots of red beets) is a glycoside of glucose and betanidin. Betanidin is red and has the structure shown in Figure 3. Like anthocyanins, betalains are water soluble and accumulate in the vacuole of plant cells.

Anthocyanins and betalains can be distinguished by their differing responses to changes in pH—betalains typically do not undergo extensive changes of color with pH as anthocyanins do. The following experiment is a "sure-fire," rapid and inexpensive means of demonstrating the influence of pH on the color of anthocyanins and betalains.

Objectives

The objectives of this experiment are to demonstrate 1) the effect of pH on the color of anthocyanins and betalains, and 2) how a simple chemical test (i.e., changing pH) can be used to distinguish between betalains and anthocyanins.

Materials

- 10 test tubes, 10-15 ml capacity
- red cabbage, 20 g
- red beet roots, 20 g
- balance to weigh tissue
- blender
- buchner funnel
- filter paper
- sidearm flask, 500 ml capacity
- water aspirator
- 2 beakers, 500 ml capacity, with labels
- 0.1 N HCl, 20 ml
- 0.01 N KOH, 20 ml

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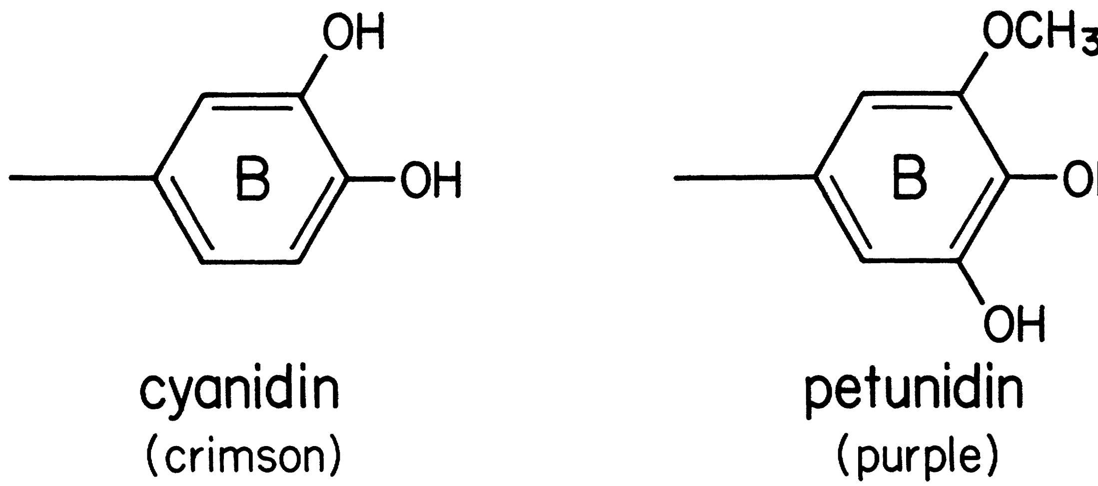

Figure 2. Cyanidin (first isolated from blue cornflower) is a hydroxylated anthocyanidin, whereas petunidin (isolated from petunias) is a methylated anthocyanidin.

0.1 N KOH, 20 ml
crystalline KOH or NaOH, 20 pellets
1 N HCl, 30 ml
test tube rack

Procedure

1. Separately homogenize 20 g of red cabbage and red beet roots in 400 ml of water in a blender.
2. Filter each homogenate through filter paper. This filtration is facilitated by using a buchner funnel in a sidearm flask attached to an aspirator.
3. Discard the filter and solid material.
4. Place the two extracts into separate labeled flasks.
5. Add 5 ml of cabbage extract containing anthocyanin to each of 5 test tubes numbered 1-5.
6. Add 5 ml of beet extract containing betalains to each of 5 test tubes numbered 6-10.
7. Perform the following treatments:
   Tubes 1 & 6: None (untreated controls)
   Tubes 2 & 7: Add 1.0 ml of 0.1 N HCl, mix, and note any color change
   Tubes 3 & 8: Add 0.5 ml of 0.01 N KOH, mix, and note any color change (the cabbage extract will become violet or, if too basic, blue)
   Tubes 4 & 9: Add 1.0 ml of 0.1 N KOH, mix, and note any color change
   Tubes 5 & 10: Add one pellet of KOH or NaOH, mix, and note any color change (the anthocyanin will become yellow)
8. These pH-dependent changes in pigment color are fully reversible. This can be demonstrated by adding drops of 0.1 N HCl to tubes 3, 4, 8 and 9, or 1 N HCl for tubes 5 & 10. Observe the changes in color.

Questions for Students:

Do betalains and anthocyanins show the same changes in color in response to differing pH values? Why or why not?

Can differences in pH account for all blue or red colors of flowers containing anthocyanins? Why or why not?

Further Reading

Goodwin, T.W. (Ed.) (1976). *Chemistry and biochemistry of plant pigments.* (2nd ed.). Vol. 1. London: Academic Press.

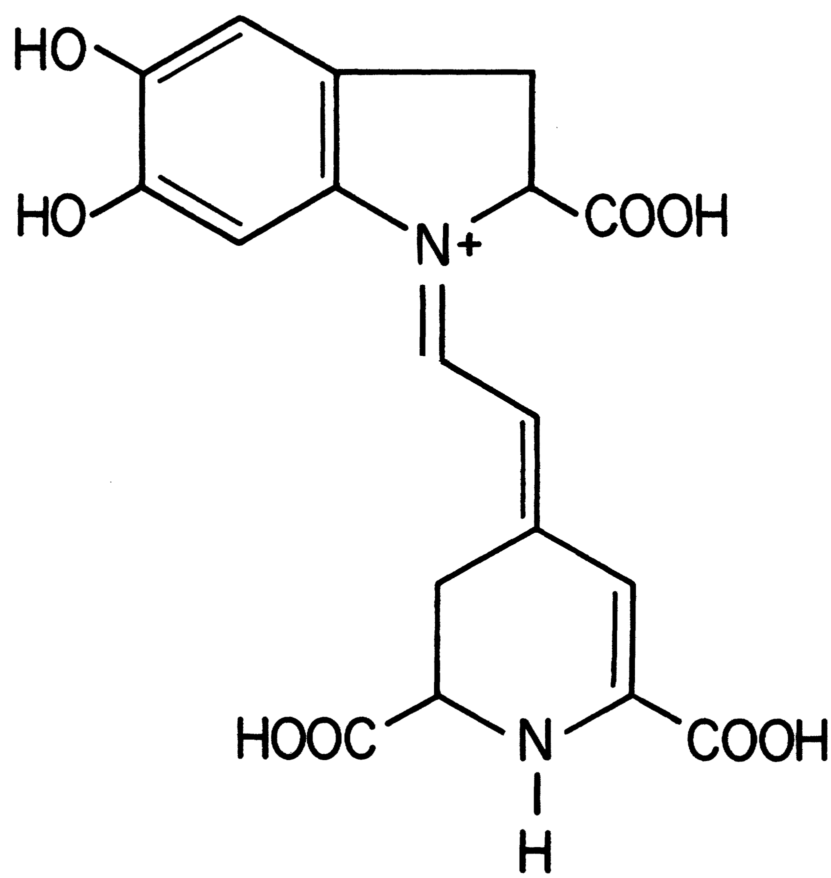

Figure 3. Structure of betanidin.