High-performance liquid chromatography of magnolia flower pigments

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Background. Hybrid yellow-flowering magnolias were bred in the 1950s at the Brooklyn Botanic Garden, resulting in introduction of the M. × 'Elizabeth' and M. × 'Yellow Bird' hybrids (Koerting, et al., 1979; Koerting, 1981). These hybrids were the results of crossings of *M. acuminata* with *M. heptapeta* and of *M. acuminata* with *M.* × *brooklynensis* 'Evamaria,' respectively. In addition to breeding and selecting superior magnolia hybrid cultivars, we are interested in determining the biochemical basis for flower color and pigment inheritance in magnolia through the use of chromatography.

Thin-layer chromatography has been employed to determine the anthocyanin and carotenoid composition in magnolia (Santamour, 1965; Demuth and Santamour, 1978). The two anthocyanin aglycones identified for several species and cultivars of magnolia were cyanidin and peonidin (Santamour, 1965). Lutein-5,6-epoxide and alpha-carotene-5,6-epoxide were the major carotenoids found in magnolia (Demuth and Santamour, 1978). Other pigments may be present in trace quantities. Previous results are not sufficiently quantitative for determining the inheritance of flower color in magnolia and for using this information in taxonomic identification.

Chromatography achieves separation of chemicals in a sample on the basis of their differential adsorption to various substrates in the presence of migrating solvents. Unlike traditional column liquid chromatography, which relies on gravity or capillary action to generate a flow of solvent at little or no pressure, high-performance liquid chromatography (HPLC) involves the use of a densely packed column of adsorbent (usually silica gel) through which solvent is pumped. A photometric detector can be used to quickly analyze a complex sample quantitatively and qualitatively with excellent reproducibility.

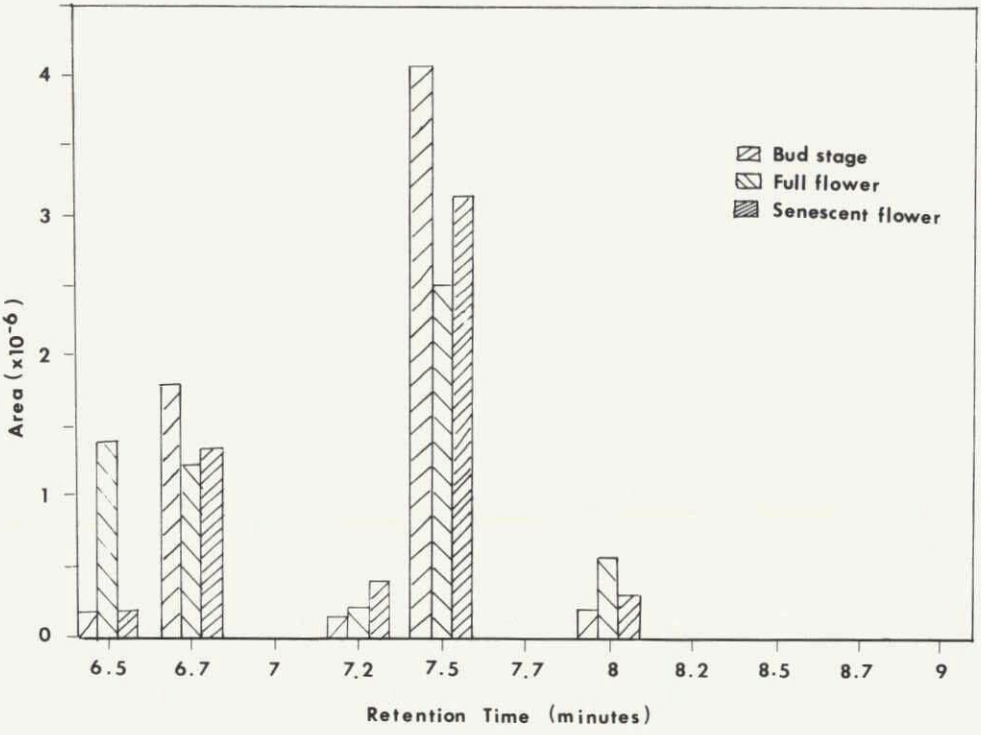
The advent of HPLC has greatly improved the capability to detect and quantify complex mixtures of pigments. Through use of HPLC, the carotenoid and flavonoid composition or makeup in the flowers of plants in the magnolia collection at the Brooklyn Botanic Garden is being analyzed to establish a data base from which the inheritance of flower color in magnolia can be determined. The ability to chemically identify magnolia species and cultivars is a secondary goal of this research. The following report

Table 1. Comparison of total carotenoids and the relative percentage of lutein epoxide.

Cultivar Identity	Flower Color	Total Carotenoids[a]	Lutein Epoxide[b] percent
M. heptapeta	white	2.77	37.3
Merrill	white	3.70	37.6
Evamaria	purple/yellow	15.59	68.6
Woodsman	purple/yellow	18.20	56.5
#251	purple/yellow	11.50	61.4
#204	purple/yellow	4.22	46.0
Susan	purple	0.89	48.7
#149	purple	3.52	19.9
M. acuminata	yellow/green	57.52	75.6
Elizabeth	yellow	6.41	63.5

[a] Total chromatographic peak area/10^6.
[b] Relative percentage of total carotenoids.

Figure 1. Effect of flower stage on carotenoids of M. × *'Elizabeth.'*



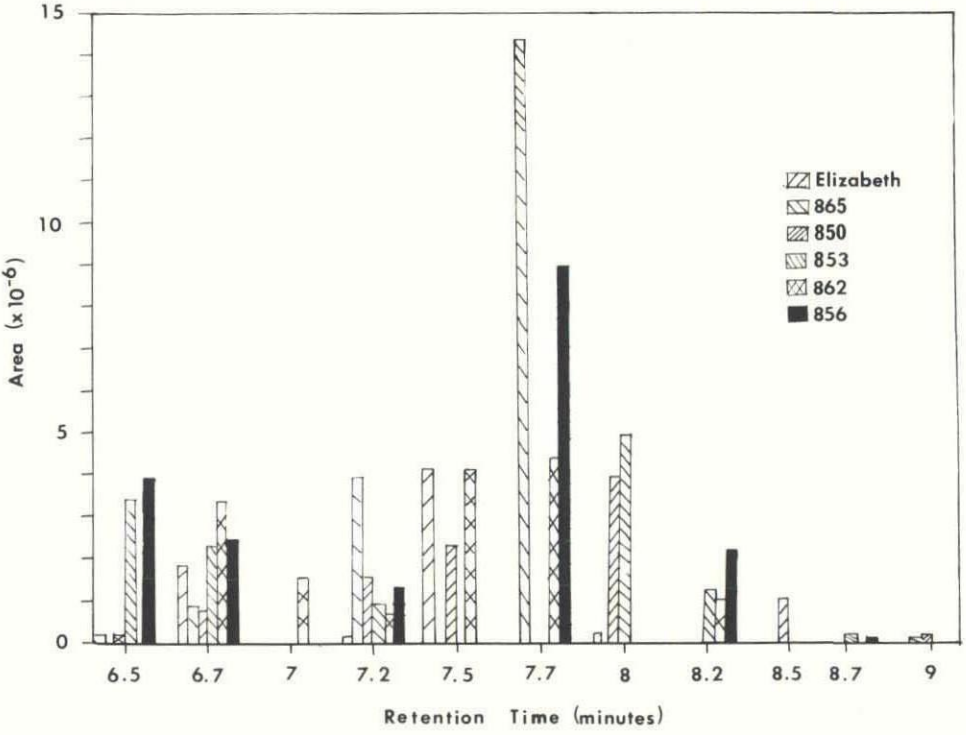
describes recent progress.

Methodology. Samples to be used with the HPLC method were prepared as described by Eskins and Dutton (1979). Magnolia tepals were collected in spring 1983, 1984 and 1985. The tepals were stored at -20° C until lyphilization of the tissue could be performed by use of a freeze-drying apparatus (Virtis model 10-010). The lyophilized tepals were ground to a fine powder and passed through a fine mesh screen prior to extraction. Fifty milligrams (mg) of this powder were extracted with 10 milliliters (ml) of 70 percent methanol. The resulting homogenate, with 80 mg of Celite added, was forced through a 0.45 micron filter into a Waters Associates Sep-Pak cartridge (reverse-phase, C18-Bondapak) using a syringe. The filtrate of 70 percent methanol was saved for flavonoid analysis while the carotenoids remained bound to the adsorbent within the Sep-Pak cartridge. The carotenoids were eluted with 5 ml of acetone and prepared for HPLC analysis by evaporating the acetone solvent under nitrogen and dissolving the residue in 1 ml of 10 percent ethyl-acetate in 90 percent methanol.

An ISCO model 2300 pump was used to deliver a 1 ml/minute flow of solvent (10 percent ethylacetate in 90 percent methanol) through a 15 centimeter (cm) Supelcosil LC-18 (5 micron) column. The 20 microliter sample loop of the Rheodyne 7120 injector was loaded, using a 50 microliter syringe. Detection of separated compounds was achieved by using an ISCO model UA-5 Absorbance Monitor with a type 6 optical unit (436 nanometers, or nm, billionths of a meter). Peak detection and quantitation was performed using a Varian 4270 integrator.

Analysis. *Effect of flower stage.* Flowers from M. × 'Elizabeth' were

Figure 2. Comparison of carotenoid compositions of six yellow flowering magnolias.



collected and tested at three different stages of maturity: in bud, in full bloom and in senescent or post-bloom. Five carotenoids were detected in all 3 stages of flower development (figure 1), the predominant carotenoid being lutein-5,6-epoxide. The peak area for this component ranged between 2.5 and 4.0 ($\times 10^6$). At a retention time of 6.7 minutes, a second, unidentified carotenoid with a peak area of 1.25 to 1.75 ($\times 10^6$) exists in all samples. This carotenoid and the other three unidentified carotenoids were undetectable previously when thin-layer chromatography methods were used. Since the carotenoid composition of all three stages of flower development were similar, we concluded that the stage of flower development does not affect the results significantly.

Hybrid comparisons. The yellow-flowering hybrids had unique carotenoid compositions (Figure 2). BBG hybrid #865 had only 4 carotenoids, while BBG #862 apparently had 6. The relative composition and total amounts of carotenoids for the 6 hybrids depicted are also distinctly different. Remarkably, these 6 hybrids all are siblings of M. × 'Elizabeth' and, on the basis of carotenoid composition alone, it would be easy to distinguish these 6 hybrids one from the other.

In contrast, the white-flowering magnolias, M. heptapeta (denudata) and *M.* × *loebneri* 'Merrill,' had much less total carotenoids (Table 1), though a trace of lutein-5,6-epoxide was evident in each, along with traces of other carotenoids.

Purple-flowering magnolias such as *M.* × *brooklynensis* 'Evamaria,' 'Woodsman,' BBG #251, and BBG #204, which have some yellow-green suffusion in their tepals due to their lineage from *M. acuminata*, all have significant amounts of carotenoids, the predominant one being lutein-5,6-