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FLORAL PIGMENTS OF THE YELLOW *CAMELLIA*,  
*CAMELLIA CHRYSANTHA* (THEACEAE)

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ABSTRACT

Floral pigments of *Camellia chrysantha* consist of three flavonoids (quercimeritrin, rutin, and isoquercitrin) and two xanthophylls (neoxanthin and an unidentified xanthophyll). The transfer of yellow petal coloration from *C. chrysantha* to other *Camellia* species may be hampered by interspecific genetic incompatibilities and by the complexities of genetic regulation of biosynthesis of the pigments involved.

Key words: *Camellia chrysantha*, Theaceae, floral pigments, flavonoids, carotenoids, plant breeding.

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INTRODUCTION

A *Camellia* with deeply golden-yellow petals was first described by Hu (1965) as *Theopsis chrysantha* Hu. This taxon was subsequently transferred to the genus *Camellia* by Tuyama (1975) as *C. chrysantha* (Hu) Tuyama. The discovery and increasing availability of a long-sought-after yellow *Camellia* has generated great excitement among *Camellia* growers and breeders as a potential source for a new range of *Camellia* cultivar floral colors, to be obtained through interspecific hybridization and artificial selection activities (Brown 1985). A knowledge of the chemical nature of floral pigments and their inheritance patterns is of paramount importance as a guide for the design of effective and efficient flower-color breeding experiments. An important contributor to the yellow petal color of *C. chrysantha* was reported by Miyajima, Uemoto, Sakata, Arisumi, and Toki (1985) to be quercetin 7-O-glucoside. The present report identifies additional flavonoid and carotenoid constituents of the petals of *C. chrysantha* and considers aspects of the production of these pigments relevant to flower-color breeding programs.

MATERIALS AND METHODS

Fresh petal material of *Camellia chrysantha* was provided by the staff of the Huntington Botanic Garden, San Marino, California, from plants in cultivation.

Petals were exhaustively extracted with 100% methanol, into which all yellow pigmentation dissolved. The concentrated methanolic extract was applied to a small (1 × 8 cm) column of silica gel (type 1, 60-200 mesh). The column was washed with benzene and a yellow-colored, benzene-elutable fraction was collected. The column was then washed with 100% methanol to yield a second, yellow-colored pigment fraction which was methanol elutable.

Preparative and analytical thin-layer chromatography (tlc) was performed on the benzene-elutable fraction using silica gel G (activated) as adsorbent and benzene : ethyl acetate : methanol (75:20:5, v:v:v) as a solvent.

The methanol-elutable pigment fraction was examined initially by 2-D paper chromatography (pc) using tert-butyl alcohol : acetic acid : water (3:1:1, v:v:v) and

15% acetic acid as solvents. Subsequently, flavonoids were purified by 1-D pc using 15% acetic acid as solvent. Paper chromatography, R<sub>f</sub> determinations, acid hydrolysis, and UV spectroscopy were performed using standard methods (Mabry, Markham, and Thomas 1970).

## RESULTS

### *Carotenoids*

Analytical tlc of the benzene-elutable pigment fraction revealed two carotenoid constituents, a major carotenoid and a trace constituent with lower chromatographic mobility. On the basis of their yellow color, methanol solubility, and absorption spectra, these pigments are shown to be xanthophylls. The two xanthophylls were separated and purified by preparative tlc and the major component was examined spectroscopically in a variety of solvents. The spectroscopic properties of the major xanthophyll are shown in Table 1. This xanthophyll is provisionally identified on the basis of spectroscopic properties as neoxanthin (Davies 1976). Identification must remain provisional at present because the very limited samples available precluded further analysis. Co-chromatography (tlc) of benzene extracts of *C. chrysantha* petals and anthers revealed the same major xanthophyll constituent in both tissues. Several additional yellow-colored carotenoids (presumed xanthophylls) also occur in anther tissue in much lesser amounts.

### *Flavonoids*

Two-dimensional pc of the methanol-elutable pigment fraction revealed one major and two minor flavonoid constituents. The results of Miyajima et al. (1985) were confirmed in that the major flavonoid constituent is quercetin 7-O-glucoside. The two additional flavonoid constituents are quercetin 3-O-rutinoside (rutin) and quercetin 3-O-glucoside (isoquercitrin). The three flavonoid constituents were characterized with respect to hydrolysis product (quercetin in all cases), R<sub>f</sub> values in four solvents (TBA, BAW, water, and 15% HOAc), and UV-spectroscopic properties. Chromatographic and spectroscopic properties of the three *Camellia* pigments agreed in all respects with published values for these compounds (Anyos and Steelink 1960; Harborne and Hall 1964; Mabry et al. 1970).

## DISCUSSION

### *Floral Coloration in Camellia chrysantha*

Flavonol 7-glycosides are among the few classes of flavonoid pigments which impart to flowers a coloration (usually yellow) which is visible to humans (Harborne 1976). As noted by Miyajima et al. (1985), quercetin 7-glucoside is a possible source of the yellow petal coloration in *C. chrysantha*. The two additional flavonoids (rutin and isoquercitrin) may function to impart "body" to the appearance of the petal, but provide no coloration to which the human eye is sensitive.

Carotenoids frequently impart yellow coloration to flowers (Scogin 1983) and the xanthophylls in *C. chrysantha* petals certainly contribute to the yellow petal color. The relative importance of quercetin 7-glucoside and xanthophylls in providing petal coloration in this taxon is difficult to assess at present. Additional research will be required for this assessment. One possible investigation is the

Table 1. Spectroscopic properties of the major xanthophyll from *Camellia chrysantha* petals and neoxanthin (from Davies 1976).

Solvent	Absorption maxima (nm)	
	<i>Camellia</i> xanthophyll	Neoxanthin
Ethanol	417, 439, 467	415, 438, 467
Benzene	427, 453, 479	426, 453, 483
Heptane	418, 441, 468	418, 442, 476
Chloroform	423, 448, 477	423, 448, 476
Carbon disulfide	465, 494	463, 493
Pyridine	429, 452, 481	unavailable

examination of artificial selections or natural mutants among cultivars of *C. chrysantha* exhibiting flowers which lack one or the other pigment class. These "deficient" flowers can be analyzed for petal color and chemical constitution. A second possibility is the phytochemical characterization of additional *Camellia* species of the section *Chrysantha*, which are reported by Chang and Bartholomew (1984) to exhibit varying hues and intensities of yellow petal coloration.

#### *Implications of Floral Pigments for Camellia Breeding*

When two plant taxa which exhibit different chemical constitutions are hybridized, the resultant product frequently produces constituents characteristic of both parents (Harborne and Turner 1984). Thus, *Camellia* breeders might well expect hybrids between *C. chrysantha* and other species to produce yellow pigments characteristic of the former. However, the transfer of yellow coloration to other *Camellia* species may be hampered by several considerations: 1) the presence of high anthocyanin concentrations in many *Camellia* cultivars; 2) generic incompatibility barriers between *C. chrysantha* and the *Camellia* species from which the most popular cultivars have been selected; and 3) genetic regulatory systems which control the combinations and relative amounts of floral pigments produced.

The first two barriers have been commented upon by Nagao (1985) in connection with his attempts to hybridize *C. chrysantha* with other species. Crosses with *C. reticulata* Lindl. cultivars yielded viable hybrids, but with no apparent yellow petal coloration, due (at least in part) to masking by high concentrations of petal anthocyanins. Nagao's attempts to hybridize *C. chrysantha* with *C. japonica* L. failed to yield viable seed, probably due to genetic incompatibilities. Similar results have been described by Lifang (1984) for the *C. chrysantha* breeding program at the Kuming Botanical Institute.

The third difficulty in transferring yellow pigmentation to hybrid camellias results from the necessity to transfer production of a flavonol 7-glycoside to plants in which a different flavonoid group (namely, flavonol 3-glycosides) is the predominant pigment and the complexity of the genetic system regulating the production of different flavonoid groups. Since the production of yellow camellias appears to depend, at least in part, on the production of substantial quantities of quercetin 7-O-glucoside in the petals, the process of 7-glycosylation, its inheritance, and its regulation must be considered in some detail.

*Distribution of quercetin 7-glucoside.*—Among *Camellia* species examined to date, *C. chrysantha* is unique in producing a flavonol 7-glycoside. In all other *Camellia* species examined, only 3-glycosides of flavonols were found in leaf materials (Roberts, Wight, and Wood 1958) and in floral materials (Parks and Kondo 1974; C. R. Parks, pers. comm.). The distinct possibility exists that quercetin 7-glucoside occurs commonly as a floral constituent among *Camellia* species of section *Chrysantha*. Yellow flower color typifies this section (Chang and Bartholomew 1984) and most of the 10 species exhibit the golden-yellow flower color found in *C. chrysantha*. The occurrence of quercetin 7-glucoside in the leaves of *C. chrysantha* is unknown at present, but is under current investigation. The leaves of three species [*C. sinensis* Kuntze, *C. irrawadiensis* P. K. Barua, *C. taliensis* (W. W. Sm.) Melchior] of the same subgenus (*Thea*), but a different section (*Thea*), were reported by Roberts et al. (1958) to contain only flavonol 3-glycosides. If flavonol 7-glycosides are found in leaves of *C. chrysantha*, they may serve as a unique floral and foliar phytochemical marker among camellias within section *Chrysantha*. Alternatively, 7-glycosides may be present in the flower, but absent from the leaves, a situation which has been reported for *Baptisia sphaerocarpa* Nutt. (Alston, Rosler, Naifeh, and Mabry 1965) and attributed to tissue-specific genetic regulation.

*Enzymology of 7-glycosylation.*—Glycosylation of flavonols solely at the seven position is relatively uncommon among angiosperms, with 3-glycosylation and 3,7-diglycosylation occurring much more frequently. The uncommon occurrence of 7-glycosylation has resulted in the accumulation of relatively little data regarding the enzymology, regulation, and inheritance of sugar attachment at this position. A 7-glycosylating enzyme (UDP-glucose flavone/flavonol 7-O-glucosyltransferase) has been purified from parsley [*Petroselinum crispum* (Mill.) Mansf.] cell suspension cultures (Sutter, Ortmann, and Grisebach 1972). This enzyme exhibits strict positional specificity (glycosylating only at the 7-position) and strict substrate specificity (it will accept only aglycones as substrate, albeit of either flavone or flavonol classes). The 7-glycosylating enzyme will not accept 3-glucosides as a substrate to generate 3,7-diglucosides, so that in the presence of large intracellular amounts of the commonly occurring flavonol 3-glycosylating enzyme (and hence, high concentrations of flavonol 3-glucosides), the 7-glycosylating enzyme would have almost no available substrate. Most *Camellia* species examined probably produce large amounts of flavonol 3-glycosyltransferase. There is no direct enzymological evidence for this, but the observation that all *Camellia* species examined by Roberts et al. (1958) produce flavonol 3-glycosides in substantial quantities is indirect evidence. Therefore, in *Camellia* hybrids involving *C. chrysantha*, there may be present in the petal little suitable substrate (i.e., quercetin) for a 7-glycosylating enzyme, even if such an enzyme is produced.

*Inheritance of 7-glycosylation activity.*—The relatively uncommon occurrence of solely 7-glycosylation has yielded relatively few studies in which a flavonol 7-glycosylating taxon was hybridized with a flavonol 3-glycosylating taxon and the resulting hybrid examined phytochemically. One such study is that of Parks (1965) on *Gossypium*, the results of which suggest that, whereas 3-glycosylation is not genetically dominant over 7-glycosylation, the production of 7-glycosides

is reduced in the hybrid and the regulation of production of glycosylating enzymes in hybrid plants is under complex and unpredictable (at present) genetic control.

Parks (1965) reported crosses between *Gossypium* species in which one parental type produced quercetin 3-glucoside (Q3glu) and the other produced both Q3glu and quercetin 7-glucoside (Q7glu) in their flowers. In both of the hybridizing combinations [*G. raimondii* Ulb. (Q3glu) × *G.* (near white) *hirsutum* L. (Q3glu + Q7glu) and *G. thurberi* Tod. (Q3glu) × *G.* (near white) *hirsutum* (Q3glu + Q7glu)], the resultant hybrids produced both Q3glu and Q7glu, but Q7glu was often reduced in quantity relative to the parental type. If a similar phenomenon occurs in hybrids involving *C. chrysantha*, then reduced Q7glu production and, hence, reduced yellow coloration may be expected in the hybrid.

*Genetic regulation of glycosyltransferase production.* — Alston and Simmons (1962) explained their observation that flavonol 7-glycosides were present in the leaves of the hybrid *Baptisia sphaerocarpa* × *B. leucantha* Torr. & Gray, but were absent from the leaves of both of the parental taxa (being present only in the flowers of *B. sphaerocarpa*), as occurring due to the disruption of a complex genetic regulation mechanism controlling tissue-specific production of the 7-glycosylating enzyme. A complex regulatory mechanism was also invoked by Alston and Turner (1963) to explain the observed variability in the particular suite of chemical constituents expressed among different hybrid individuals from the same parental taxa. More details on the complexity of the regulation of glycosyltransferase production are coming to light from studies of the *bronze* mutant in maize. The phenotypic expression of this mutant derives from differential production of UDP-glucose flavonol 3-O-glucosyltransferase (Dooner and Nelson 1979). The activity of the gene producing this enzyme is regulated by a transposable element controlling an adjacent activator. Clearly, glycosylation enzyme activity is under complex genetic regulation and this finely tuned regulation may be disrupted in crosses involving *C. chrysantha* with distantly related *Camellia* species.

The transfer of flavonol 7-glycosylating ability from *C. chrysantha* to an intersubgeneric hybrid may be thwarted by the absence of a suitable substrate in the hybrid (i.e., by the occurrence only of flavonol 3-glycosides in the hybrid), by the depression of flavonol 7-O-glucosyltransferase activity (as in *Gossypium*), or disruption of finely tuned genetic regulatory controls which result in low (or absent) production of 7-glycosylating enzyme. Many attempts with different hybridization combinations may be required before a serendipitous combination permits circumvention of these barriers to yield the full expression of quercetin 7-glucoside production in the petals of a hybrid plant.

*Genetics of xanthophyll inheritance.* — The biosynthetic pathways and genetic regulation of biosynthesis enzymes of carotenoids is more poorly understood than those of flavonoids. Little can be said at this time regarding the effects of hybridization on xanthophyll constitution or regulation.

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