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https://doi.org/10.5109/23808

出版情報：九州大学大学院農学研究院紀要. 29 (4), pp.257-266, 1985-06. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 29 (4), 257-266 (1985)

Yellow Pigment of *Camellia chrysantha* Flowers

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(Received February 22, 1985)

The principal yellow pigment which contributes to the petal colour of yellow-flowered ***Camellia chrysantha*** was isolated and identified chromatographically as quercetin-7-glucoside (quercimeritrin).

INTRODUCTION

Since the discovery of pure yellow-flowered camellias in Kwangsi Province in the southwestern part of the People's Republic of China, they have been classified into more than ten species (Chang, 1981). One of these species, Camellia ***chrysantha,*** has engaged the attention of plant breeders as a cross-parent for various entirely new colours in garden ***Camellia.***

Although flower pigmentation of Camellia has been investigated by many researchers and more than ten components have been identified as anthocyanin glycosides (Parks ***et al.,*** 1968 ; Yokoi, 1975 ; Sakata ***et al.,*** 1980, 1981), a yellow pigment has never been reported in ***Camellia*** species. In this study, the main agent of yellow-flowered C. ***chrysantha*** was isolated and identified chromatographically.

MATERIALS AND METHODS

Fresh petals were collected from fully opened flowers and some of them were dried through lyophilization at -40°C. These fresh and dried petals were used as the samples.

The absorption spectrum of fresh petals was measured by a Shimadzu multi-purpose spectrophotometer MPS-5000 in reflected light, and that in transmitted light by a Shimadzu DB spectrophotometer UV-300. In the latter measurement, small pieces of petals (2 cm×5 mm) were placed against the wall of cuvettes filled with distilled water, and the spectral absorption curves were measured against a blank of a white camellia cultivar, "Kumasaka". To check the distribution of the relevant yellow pigment within the petal

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tissue, fresh petals were thinly cross sectioned with a razor blade and observed microscopically.

Chemical analysis of pigments was done by (1) thin-layer chromatography (TLC) of crude extract and (2) purification of the relevant pigment and its chromatographic characterization.

(1) TLC of crude extract

Dried and powdered samples of flower petals were allowed to stand overnight in MeOH at room temperature and were filtered. The filtrate was evaporated almost to dryness under reduced pressure at 35°C and washed repeatedly with petroleum ether to remove the ether-soluble impurities.

The concentrated residual extract was applied to a corner of a *20 x20* cm glass plate coated with micro-crystalline cellulose powder and developed two-dimensionally, using the following solvent systems : n-BuOH-HOAc-H_2O (4 : 1: 5 v/v, upper phase) for the first dimension, and 15 % HOAc for the second. By this procedure the position of the relevant yellow pigment on the chromatogram and some of its properties were clarified.

(2) Purification of the relevant pigment and its chromatographic characterization

To further characterize the relevant pigment, it was purified by successive preparative paper chromatographies, first with n-BuOH-HOAc-H_2O (4: 1: 5 v/v, upper phase) and second with 15% HOAc. After each development, the pigment band was marked under UV light and cut out, and then eluted with MeOH. The eluate obtained from the chromatogram of second development was concentrated under reduced pressure and allocated to either controlled or complete acid hydrolysis, the former for the investigation of the number of bound sugars and the latter for the identification of aglycone and sugar.

For controlled acid hydrolysis, a small portion of the residue was hydrolyzed at 100°C with 2 ml of 2 N HCI, and samples taken at appropriate intervals from 0 to 120 min. hydrolysis were used for TLC with solvent system n-BuOH-HOAc-H_2O (4: 1: 5 v/v, upper phase).

For the identification of the aglycone, another portion was hydrolyzed for 90 min. at 100°C with 2 ml of 2N HCl. The hydrolysate obtained was divided into two portions. One was used for co-chromatography with some authentic flavonol aglycones. Another portion was analyzed by high performance liquid chromatography (HPLC), in which chromatograms were run on a TRIROTER III using a 25 cmx4 mm Nucleosil 7-C_{18} column with a UVIDEC 100-111 detector set at 370 nm. A flow rate of 1.0 ml/min. was maintained employing a mixture of H_2O-$HClO_4$-MeOH (30: 0.05 : 70 v/v) as eluent.

For the identification of the sugar, the acid hydrolysate was neutralized with IN NaOH, and the NaCl produced was eliminated through absorption to the ion-exchange resin. The residual sugar solution was evaporated to dryness, again dissolved in MeOH, and used for co-chromatography with some authentic sugars using the solvent system EtOAc-pyridine-HOAc-H_2O (5: 5: 1: 2 v/v). The development of sugars on the chromatogram was based on the method of Bryson and Mitchell (1951).

Another purified sample, which was prepared in the manner mentioned before, was used for characterization at the level of glycosides. The sample was co-chromatographed with some authentic flavonol glycosides. The same sample was also used for analysis by HPLC. The running conditions of HPLC were somewhat different from those used for identification of the aglycone. Namely, chromatograms were run on a BIP-I using a 25 cm $\times$4.6 mm Nucleosil 7-C_{18} column with a UVIDEC 100-IV detector set at 390 nm. The flow rate was 0.8 ml/min., and two kinds of eluent mixtures were employed, H_2O-$HClO_4$-MeOH (45: 0.05: 55 v/v) and 0.1% HOAc-CH_3CN (75: 25 v/v).

RESULTS AND DISCUSSION

(1) *In vivo* absorption spectra and pigment distribution in fresh petals

As shown in Fig. 1, the *in vivo* absorption spectra showed a pronounced maximum at 380nm and slight shoulders at 450 and 490nm. From their wavelengths these shoulders might suggest a co-occurrence of carotenoid.

In the microscopic observation on cross sections of fresh petals, the most intensely yellow-coloured portions were the vacuoles of epidermal and subepidermal cells (Fig. 2), although in the cells of the mesophyll some yellow

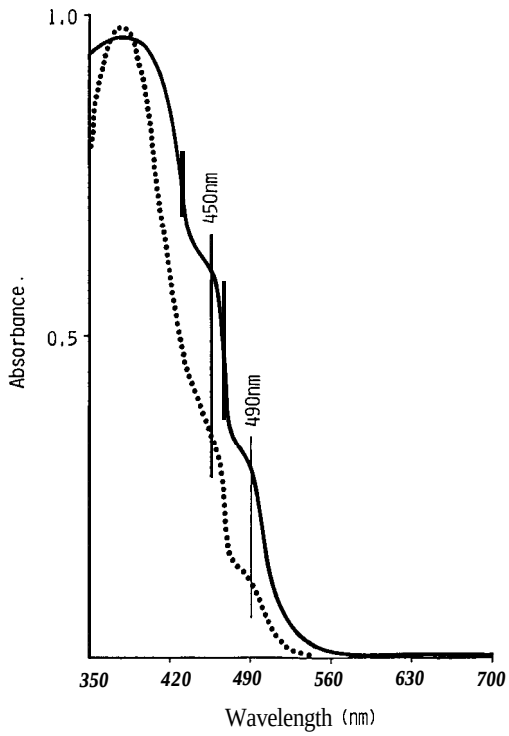

Fig. 1. *In vivo* absorption spectra of ***Camellia chrysantha*** petals in reflected light (solid line) and transmitted light (dotted line).

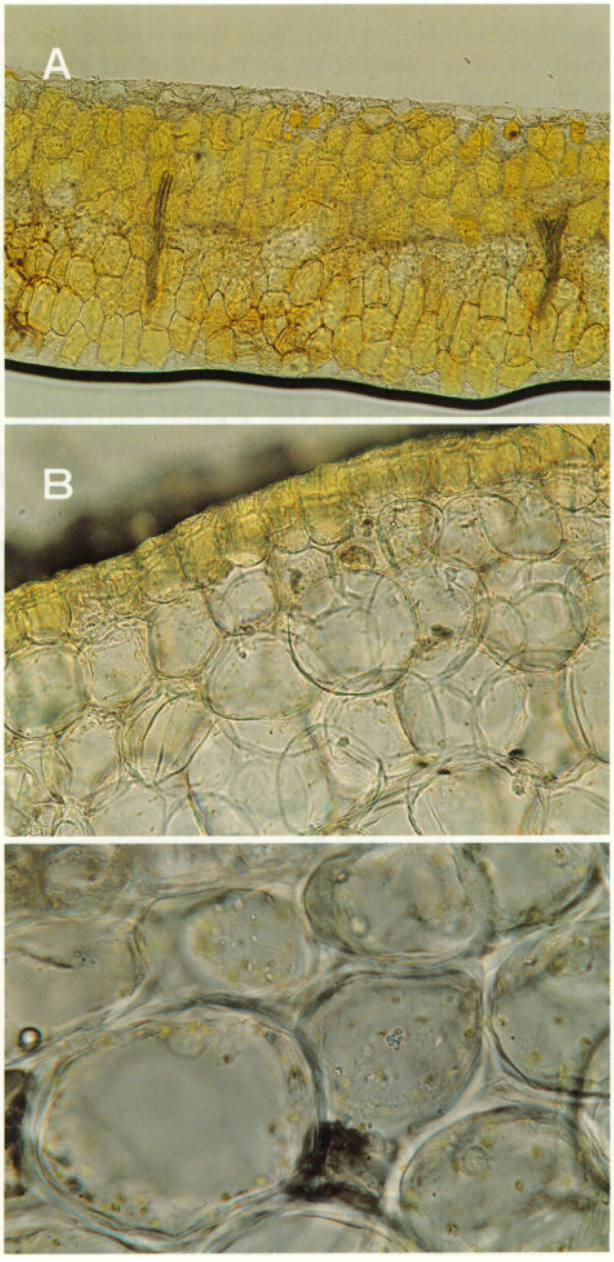

Fig. 2. Photomicrograph of ***cross*** section of fresh petal of C. *chrysantha*. A, apical portion; B, median portion.

Fig 3. Photomicrograph of chromoplasts in the mesophyll of C. ***chrysantha.***

chromoplasts also occurred, which might be evidence of a small contribution of carotenoid to the yellow colour. The frequency distribution of these chromoplasts in cells of the mesophyll in C. *chrysantha* was very low (Fig. 3), as compared with other yellow-flowered plants, such as ***Rosa hybrida***, in which carotenoids play the major role in the development of their yellow colour.

From these findings it was concluded that the major agency for yellow colour in C. ***chrysantha*** was the unknown water-soluble yellow pigment, probably one of the flavonoids, dissolved in the vacuoles of epidermal and subepidermal cells, and not the carotenoid contained in the chromoplasts.

(2) **Chromatographic identification of yellow pigment**

Based on the foregoing results, chromatographic analyses of flavonoid pigments in C. ***chrysantha*** were conducted. Figure 4 shows a two-dimensional thin-layer chromatogram of the crude extract, which exhibited 7 distinct spots. Some chemical properties of these spots are shown in Table 1. From

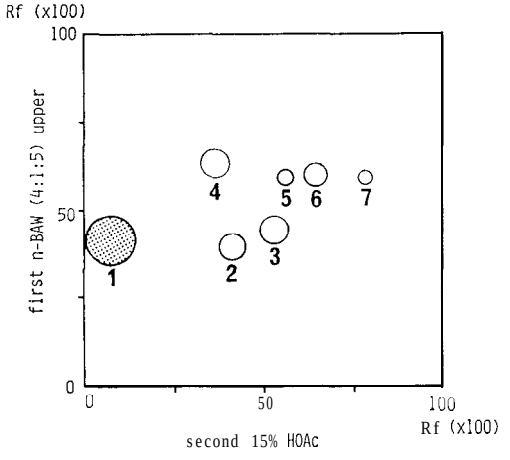

Fig. 4. Schematic representation of C. ***chrysantha*** pigments appearing on a two-dimensional thin-layer chromatogram.

these properties, especially their consistent colour change to yellow when fumed with NH_3, all of these spots were presumed to be flavonols. Although spots 2 to 7 were clearly detectable under UV light, the only yellow pigment in visible light in intact condition (without NH_3) was spot 1, and further characterization of this spot was done after purification by successive preparative paper chromatographies.

Upon acid hydrolysis the aglycone yielded was identified as quercetin (Fig. 5). As shown in Fig. 6 (A) and (B), the data of HPLC substantiated this identification. Moreover, the sugar residue attached was identified as glucose (Fig. 7) and no intermediate was found under controlled acid hydrolysis (Fig. S), indicating that this glycoside should be a monoglucoside of quercetin.

Table 1. Properties of C. *chrysantha* pigments appearing on a two-dimensional thin-layer chromatogram.

Spot' number	Rf (X100) in		Colour in			
	n-BAW[2]	15% HOAc	Visible	u v	Visible (+NH_3)	UV (+NH_3)
1	41	10	yellow	bright yellow	deep yellow	bright yellow
2	41	43	none	dull brown	yellow	dull yellow
3	45	47	none	dull brown	yellow	dull yellow
4	67	40	none	dull brown	yellow	bright yellow
5	62	55	none	dull brown	yellow	bright yellow
6	62	63	none	dull brown	yellow	bright yellow
7	59	80	none	dull brown	yellow	bright yellow

1 Numbers correspond to the spot numbers represented in Fig. 4
2 n-BuOH-HOAc-H_2O (4: 1: 5 v/v, upper phase)

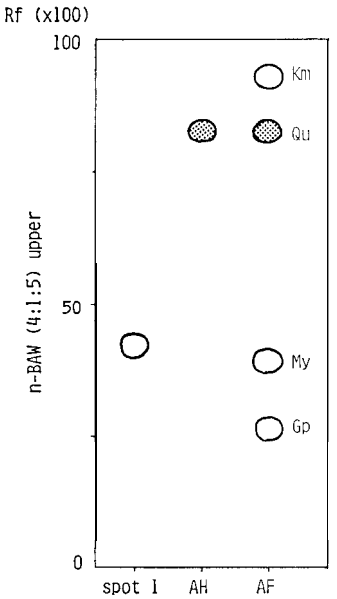

Fig. 5. Thin-layer chromatogram of acid hydrolysate of spot 1 in parallel with authentic flavonol aglycones.
AH, acid hydrolysate of spot 1; AF, authentic flavonol aglycones; Gp, gossypetin ; My, myricetin ; Qu, quercetin ; Km, kaempferol.

Therefore, using four solvent systems, co-chromatography with some authentic quercetin glycosides was conducted, including quercetin-4'-glucoside and quercetin-7-glucoside, both of which were reported to contribute to the yellow colours of some plant species (Harborne, 1976). The results obtained are summarized in Table 2. As seen in this table, the Rf values and the colours of spot 1 completely agreed with those of quercetin-7-glucoside. The

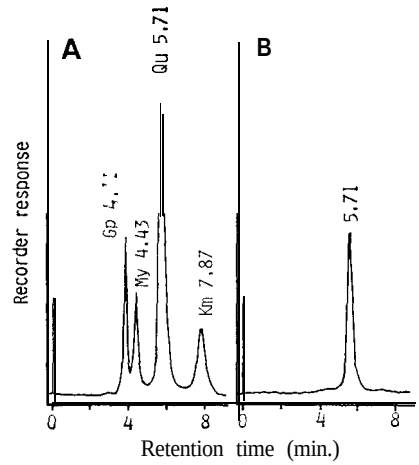

Fig. 6. High performance liquid chromatographic separations of authentic flavonol aglycones (A) and the aglycone of C. *chrysantha* spot 1 (B).

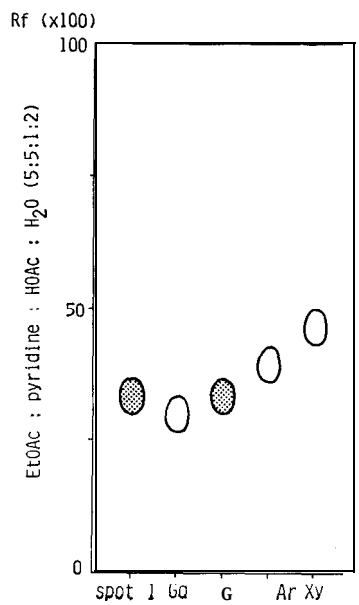

Fig. **7.** Thin-layer chromatogram of component sugar of C. *chrysantha* spot 1 in parallel with authentic sugars.
Ga, galactose; G, glucose; Ar, arabinose; Xy, xylose.

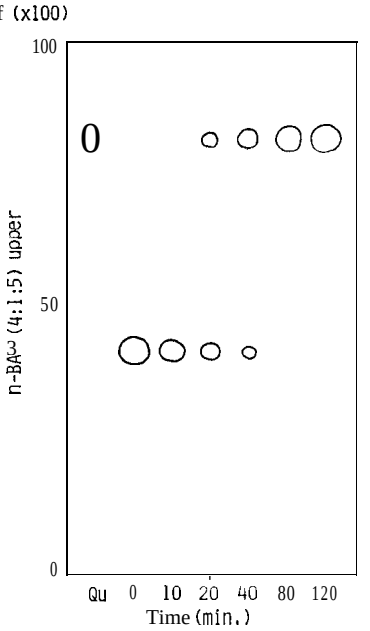

Fig. 8. Thin-layer chromatogram of the controlled acid hydrolysates of C. *chrysantha* spot 1 in parallel with an authentic sample of quercetin (Qu).

Table 2. Properties of Spot 1 occurring in petals of C. *chrysantha*, and those of authentic flavonols.

	Rf (×100) in				Colour in	
	n-BAW[1]	15% HOAc	H_2O	PhOH[2]	UV	UV ($+NH_3$)
spot 1	41	10	01	43	bright yellow	bright yellow
Authentic flavonols[3]						
Qu 3G	67	40	08	64	dull brown	bright yellow
Qu 3GR	62	63	22	56	dull brown	bright yellow
Qu 4'G	52	15	01	48	dull yellow	bright yellow
Qu 7G	41	10	01	43	bright yellow	bright yellow

1 n-BuOH-HOAc-H_2O (4: 1: 5 v/v, upper phase)

2 water-saturated PhOH

3 Qu 3 G (quercetin-3-monoglucoside) and Qu 3 GR (quercetin-3-monorutinoside) were provided by a commercial source. Qu 4'G (quercetin-4'-monoglucoside) was provided by Dr. N. Saito, Meiji-gakuin University and Qu 7 G (quercetin-3-monoglucoside) was prepared from petals of Gossypium *arboreum* (Parks, 1965).

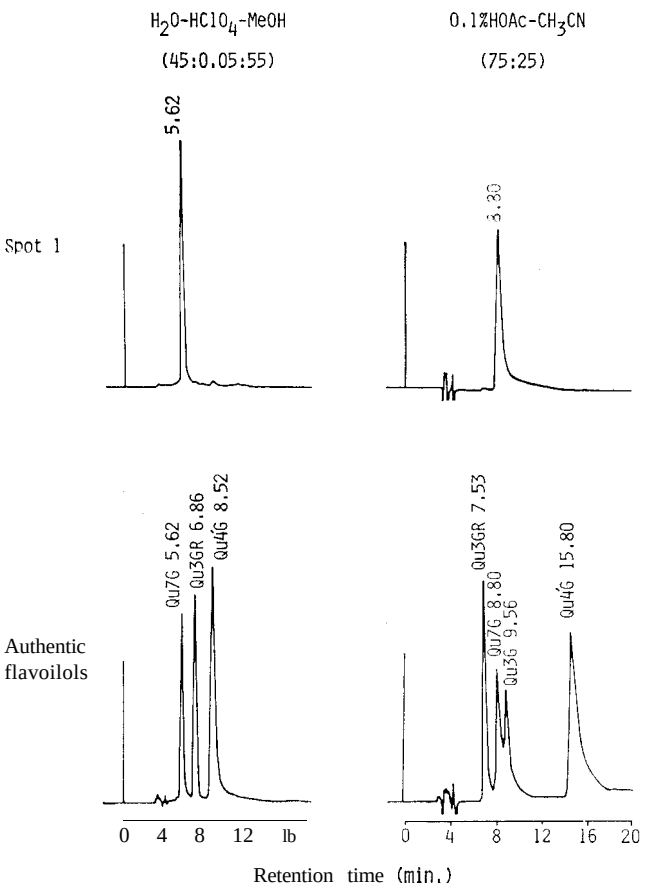

Fig. 9. High performance liquid chromatographic separations of C. ***chrysantha*** spot 1 and authentic flavonol glycosides.
Qu 3 G, quercetin-3-monoglucoside ; Qu 3 GR, quercetin-3-monorutinoside ; Qu 7 G, quercetin-7-monoglucoside ; Qu 4′G, quercetin-4'-monoglucoside

data of HPLC also completely coincided with quercetin-7-glucoside (Fig. 9).

From the findings mentioned above, it may be concluded that the yellow flower colour of C. ***chrysantha,*** which in the near future will offer various entirely new colours in garden camellias, ***is*** based on the presence of a flavonol, quercetin-7-glucoside (quercimeritrin).

ACKNOWLEDGEMENTS

The authors wish to thank Professor Y. Osajima, Laboratory of Food Analysis, and Dr. K. Shinohara, Laboratory of Food Chemistry, Faculty of Agriculture, Kyushu University, for their technical guidance concerning the photometric measurement. Thanks are due to Mr. M. Nakashima, Laboratory

of Food Analysis, for his technical advice during part of this work. We also wish to express appreciation to Dr. N. Saito, Meiji-gakuin University, for his donation of a valuable authentic specimen of quercetin-4′-glucoside.

REFERENCES

Bryson, J. L. and T. J. Mitchell. 1951 Improved spraying reagent for the detection of sugars on paper chromatograms. ***Nature,*** 167: 864

Chang, H. T. 1981 A taxonomy of the genus Camellia. *J.* Sun *Yatsen* Univ. (Natural Science), 1: 101-107

Harborne, J. B. 1967 ***Comparative Biochemistry of the Flavonoids.*** Academic Press, London

Parks, C. R. 1965 Floral pigmentation studies in the genus *Gossypium.* I. Species specific pigmentation patterns. *Amer. Jour. Bot., 52 (3):* 309-316

Parks, C. R., A. Griffiths, Jr. and K. R. Montgomery. 1968 A possible origin of anthocyanin (red) pigmentation in the flowers of Camellia ***sasanqua. Amer. Camellia Yearbook, 1968: 229-242***

Sakata, Y., S. Nagayoshi and K. Arisumi. 1980 Studies on the flower colours in *Camellia.* I. On the anthocyanin constitution in C. juponica, C. ***sasanqua*** and C. *vernalis* surrounding Hirado. ***Bull.*** *Fac.* ***Agr. Kagoshima Univ., 30: 35-40*** (in Japanese with English summary)

Sakata, Y., S. Nagayoshi and K. Arisumi. 1981 Studies on the flower colours in ***Camellia.*** II. On the anthocyanin constitution in the cultivars of C. *japonica,* C. ***juponica*** subsp. ***rusticana, C. sasanqua, C. hiemalis, C.*** *vernalis* and C. ***wabisuke. Mem.*** *Fac.* ***Agr. Kagoshima Univ., 17: 79-94***

Yokoi, M. 1975 Colour and pigment distribution in the cultivars of selected ornamental plants, with special reference to their contribution to the ornamental value of plants. Transactions *Fac. Hort.* ***Chiba Univ., 14: 1-65***