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Quantitative variation of flavonoids and related compounds in *Cosmos bipinnatus*

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Abstract

Quantitative variation of nine flavonoid compounds and two related phenolic acids in several parts of three garden varieties of *Cosmos bipinnatus* Cav. were examined by means of paper chromatography followed by a spectrophotometric procedure.

INTRODUCTION

Some kinds of flavonoids and related polyphenolics have been known to coexist in floral parts of higher plants (Lawrence, Scott-Moncrieff, 1935; Nakaoki, 1938; Hattori et al., 1952; Shimokoriyama, Hattori, 1953; Hattori, Shimokoriyama, 1956) and, in some cases, the distribution of these compounds has been observed to be restricted regularly to a tissue or an organ of a plant (Hattori et al., 1956; Murphy 1957; Watanabe, Wender, 1965; Saito 1974; Saito 1976). Numerous views concerning the role of polyphenolics in plants and their interactions with the plant organism have been proposed in numerous papers in the past decades (Blank, 1947; Towers, 1964; Siegelman, 1964; Cruickshank, Dawn, 1964). The plant polyphenolics have been until recently considered by many workers to be inactive secondary metabolic products which plants store or excrete by various mechanisms. However, latest studies have revealed the fact that these substances exist in state of dynamic equilibrium and are not static end products of plant metabolism (Zenk, 1967; Jaffe, Galston, 1967; Wittaker, 1970; Levin, 1971; Levin, 1972; Crevoisier et al., 1974; Mc Clure, 1975; Barz, Hösel, 1975). For studying the dynamic state

of these chemicals quantitative analysis in each organ or tissue of the plant material seems to be indispensable.

In this communication the results obtained from quantitative studies on nine flavonoid pigments and two related phenolic acids in the vegetative parts of *Cosmos bipinnatus* Cav. are reported.

EXPERIMENTAL

Material

Three varieties of cosmos plants, the ray flowers of which when fully opened, were collected at intervals during August-September at Murasakino, Kitakami-shi, Iwate-ken, Japan. Fresh plant material of the pink-coloured form was free-hand dissected into the following pars

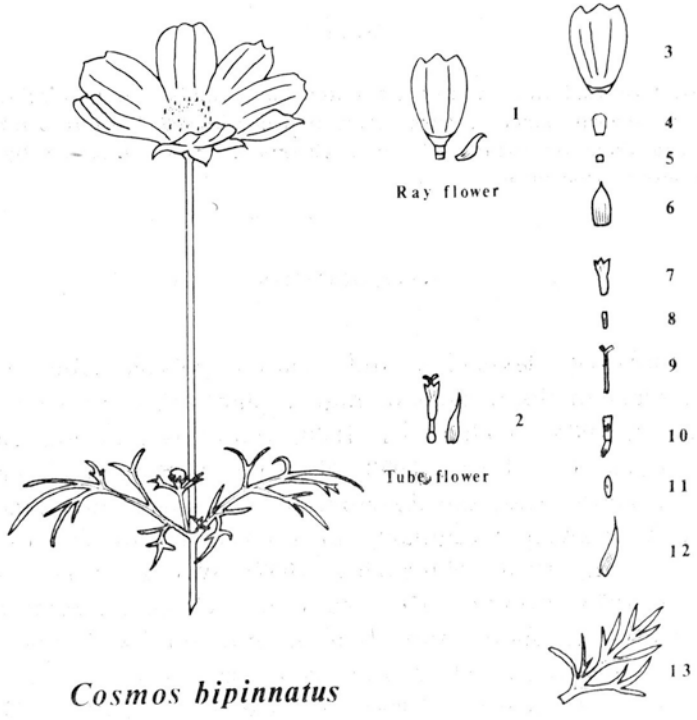

Fig. 1. Vegetative parts of *Cosmos bipinnatus*

1 — Ray flower, 2 — Tube flower, 3 — Coloured part of corolla, 4 — Colourless part of corolla, 5 — Ovary, 6 — Bract, 7 — Coloured part of corolla, 8 — Colourless part of corolla, 9 — Stigma plus style, 10 — Stamen, 11 — Ovary, 12 — Bract, 13 — Leaf

and used in this study: ray flower (bract — 5.04 g, coloured part of corolla — 12.81 g, colourless part of corolla — 3.03 g, ovary — 0.15g), tube flower (bract — 1.46 g, coloured part of corolla — 1.17 g, colourless part of corolla — 1.04 g, stigma plus style — 2.40 g, stamen —

0.93 g, ovary — 3.07 g), leaf — 19.05 g (Fig. 1). Ray flowers of white and crimson varieties were separated into four parts (bract — 1.48 g; 1.53 g, coloured part of corolla — 5.35 g; 5.80 g, colourless part of corolla — 0.94 g; 1.41 g, ovary — 0.14 g; 0.27 g). Besides the above parts, the buds of the pink-coloured form collected at growing stage were also used in the present study for examining quantitative changes in poliphenolics during bud swelling.

Methods

1. Extraction procedure

The freshly collected corolla or bract of ray flowers from pink and crimson varieties was soaked in 80% methanol (3—5 vol./g fresh wt.) containing 1% HCl, and kept for about 10 hours at room temperature, then followed by an alcoholic solution and filtered. The extraction was repeated two times more under the same conditions. The combined methanol extracts were evaporated to a small volume in a rotary evaporator at 35—37°C. The resulting concentrates were filtered and stored at 2—4°C for analysis of cosmocyanin content.

All the dissected parts were separately extracted with hot 80% methanol (3—4 vol./g fresh wt.) each time for 20—30 minutes until the fresh portion of solvent was no longer coloured. Methanol extracts were combined and concentrated to an appropriate volume. The residual aqueous concentrate was filtered, washed with petroleum ether to remove ether-soluble compounds. The aqueous phase was reconcentrated *in vacuo* to a small volume and used as stock solution for quantitative analysis of each polyphenolic substance.

2. Separation and purification of flavonoids and related compounds

A given volume of stock solution was applied to Whatman No. 3 MM paper and separated chromatographically with the use of an ascending one-dimensional technique in n-butanol/acetic acid/water (4 : 1 : 5, v/v/v upper phase). The fractions from chromatograms were extracted with methanol and rechromatographed separately in 30% acetic acid and then in n-butanol/ethanol/water (5 : 1 : 4, v/v/v, upper phase). Each poliphenolic band on the air-dried paper chromatograms was cut out, eluted with 80% methanol and used for polyphenol content determination.

3. Estimation of polyphenol content in various parts of cosmos plants

The concentration of each polyphenol separated was determined spectrophotometrically with a spectrophotometer (Hitachi Perkin-Elmer, type 139). The readings of the amounts of the chemical constituents under investigation were taken from the standard curves.

RESULTS

Methanol extracts from freshly collected cosmos plants revealed the presence of nine flavonoids and two related phenolic acids, all of which had already been characterized chromatographically (Saito,

Table 1

Characteristics of polyphenolic compounds from various parts of *Cosmos bipinnatus*

Compound	R_f value in solvent system			Absorption * maximum in methanol (nm)
	A	B	C	
Butein	0.86	0.15	0.79	381
Coreopsin	0.49	0.33	0.51	384
Cosmosiin	0.71	0.48	0.45	336
Luteolin-7-glucuronide	0.48	0.43	0.34	349
Chrysoeriol-7-glucuronide	0.56	0.34	0.19	347
Trifolin	0.76	0.58	0.70	367
Isoquercitrin	0.67	0.50	0.59	364
Nelumboside	0.51	0.54	0.46	364
Cosmocyanin	—	—	—	538 **
Caffeic acid	0.85	0.59	0.56	326
Chlorogenic acid	0.64	0.73	0.42	324

* absorption maximum (Band I) in 80% methanol
** absorption maximum (Band I) in 80% methanol containing 1% HCl
A: *n*-butanol/acetic acid/water (4:1:5, v/v/v, upper phase)
B: 30% acetic acid
C: *n*-butanol/ethanol/water (5:1:4, v/v/v, upper phase).

Table 2

Quantitative variation of flavonoids and related compounds in buds during their swelling stage

Compound \ Diameter*	2	3	4	5	6	7	8	9	10
Butein	0.02	0.03	0.1	0.6	1.0	4.1	5.3	5.6	6.8
Coreopsin	0.3	1.4	6.4	18	39	72	77	82	86
Cosmosiin	4.9	14	24	49	122	421	632	663	1378
Luteolin-7-glucuronide	0.8	4.6	13	16	19	22	23	28	34
Chrysoeriol-7-glucuronide	1.3	2.9	10	16	20	35	45	72	86
Isoquercitrin	1.9	2.3	4.4	5.9	14	16	18	19	21
Nelumboside	3.5	4.0	7.7	9.4	10	13	17	18	26
Cosmocyanin	—	—	—	0.04	0.1	0.6	2.0	10	27
Caffeic acid	3.8	7.9	16	19	20	22	29	30	40
Chlorogenic acid	10	17	19	22	34	50	69	82	92
Weight of bud **	21	47	79	137	229	311	448	535	548

* millimeter
** mg/bud
All values are expressed in μg/g dry weight.
Under the conditions of the present study, flower-bud swelling by one millimeter in diameter, lasted 48 hours.

1974; Saito 1976). Table 1 shows the R_f values and absorption maxima (Band I) (Mabry et al., 1970) of the phenolic substances isolated from various parts of *Cosmos bipinnatus*. Flavonoids and related phenolic acids accumulate gradually during flower-bud swelling (Table 2). Cosmocyanin (cyanidin-gluco rhamnoside) content which is characteristic for the coloured part of the corolla of ray flowers in the crimson or pink variety, accumulates in an about 15 times higher amount in crimson-coloured form than in the pink-coloured one (Table 3) Co-

Table 3

Quantitative variation of flavonoids and related compounds in ray flowers of three varieties of *Cosmos bipinnatus*

Part		Compound	Cosmosiin	Luteolin-7-glucuronide	Chrysoeriol-7-glucuronide	Isoquercitrin	Nelumboside	Cosmocyanin	Caffeic acid	Chlorogenic acid
Ray flower	Bract	A	—*	—	—	96	171	tr.**	33	148
		B	—	—	—	66	129	tr.	41	163
		C	—	—	—	76	183	tr.	64	145
	Coloured part of corolla	A	1739	144	221	—	—	—	18	36
		B	1874	129	261	—	—	97	21	34
		C	1835	182	118	—	—	1457	38	68
	Colourless part of corolla	A	—	—	—	—	—	—	51	34
		B	—	—	—	—	—	—	75	46
		C	—	—	—	—	—	—	80	54
	Ovary	A	—	—	—	—	—	—	104	120
		B	—	—	—	—	—	—	138	130
		C	—	—	—	—	—	—	343	351

A: White-coloured form, B: Pink-coloured form, C: Crimson-coloured form.
* compound not detected
** trace
All values are expressed in μg/g dry weight.

mosiin (apigenin-7-glucoside) is commonly detected in highest amounts in the corolla of ray flowers and the content was calculated to be about ten times higher than that of luteolin-7-glucuronide and chrysoeriol-7-glucuronide (Table 3). Caffeic acid and chlorogenic acid which are found in all parts of ray flowers of the three varieties show a higher level in the ovary of the crimson-coloured form (Table 4). The leaves have been found to contain three types of flavonoid glyco-

sides, trifolin (kaempferol-3-galactoside), isoquercitrin (quercetin-3--glucoside) and nelumboside (quercetin-3-glucoglucuronide) and two types of phenolic acids, caffeic acid (3, 4-dihydroxycinnamic acid) and chlorogenic acid (3-caffeylquinic acid) (Saito, 1974). In these substances nelumboside and chlorogenic acid predominate in those parts (Table 4). In addition to isoquercitrin, nelumboside, caffeic acid and

Table 4

Quantitative variation of flavonoids and related compounds in tube flower and leaf of pink variety of *Cosmos bipinnatus*

Part \ Compound		Butein	Coreopsin	Trifolin	Isoquercitrin	Nelumboside	Caffeic acid	Chlorogenic acid
Tube flower	Bract	0.1	43	—	196	124	110	130
	Coloured part of corolla	47	283	—	85	51	127	152
	Colourless part of corolla	—	—	—	—	—	47	41
	Stigma+Style	4.0	85	—	85	52	18	29
	Stamen	159	88	—	52	—	175	246
	Ovary	—	—	—	—	—	36	42
Leaf		—	—	48	98	532	49	35

All values are expressed in μg/g dry weight.

chlorogenic acid, other types of flavonoid pigments — butein and coreopsin — occur in the tube flower (Saito, 1974). Butein (2′, 4′ 3, 4-tetrahydroxychalcone) was found mainly in the stamen but with little in the bract, stigma plus style and coloured part of corolla. On the contrary, coreopsin (butein-4′-glucoside) was detected in large quantities in the bract, stigma plus style and coloured part of corolla, but was much less abundant in the stamen. Caffeic and chlorogenic acids are present at a relatively high level in the bract, coloured part of corolla and stamen (see Table 4).

DISCUSSION

The results obtained in the present study show that the content of flavonoids and related phenolic acids varies with the growth stage and the vegetative part of the cosmos plant. The data may be considered in reference to the well-established biogenetic mechanisms

(Neish, 1960; Schmidt, 1963; Neish, 1964; Hanson, 1966; Grisebach, 1966; Grisebach, Barz, 1969).

In the studies on quantitative variation of polyphenolic substances, both flavonoid pigments and phenolic acids were shown to accumulate gradually during the stage of flower-bud swelling. This may reflect the fact that the synthetic activity of these compounds is controlled by an internal or external regulatory mechanism of plant polyphenolic biosynthesis.

Caffeic acid and chlorogenic acid are distributed uniformly in all parts examined. This fact shows that the synthesising system of the acids exists in all above-ground parts. Isoquercitrin and nelumboside are also found in vegetative parts exept the corolla of the ray flower. Though each flavonol glycoside is found to occur in different amounts in the parts investigated, the synthetic route of these two compounds may also be operative as extensively as those of caffeic and chlorogenic acids in this plant.

The most conspicuous difference between the coloured forms is the amount of cosmocyanin. The synthetic pathway leading to the red pigment in the corolla of the crimson-coloured form may be operating more actively than that of the pink coloured one. The route is genetically blocked in the white variety.

Another interesting fact is that conspicuous quantitative difference in butein and coreopsin are found between the bract, corolla, stigma plus style and stamen. This suggests that the pathway for the synthesis of glycoside in the bract, corolla and stigma plus style may predominate as compared with that in the stamen. On the contrary, as regards the pathway for the synthesis of aglycone, a reverse relation is observed. The foregoing data clearly indicate the possibility that an alternate route is operative between butein and coreopsin in tube flowers. The co-occurrence of glycoside and its aglycone in one vegetative part of a plant sets many interesting problems from the enzymic and histochemical point of view.

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Zmienność ilościowa flawonoidów i związków pokrewnych u Cosmos bipinnatus Cav.

Streszczenie

Wyizolowano dziewięć związków flawonoidowych i dwa pokrewne kwasy fenolowe z części kwiatowych trzech ogrodowych odmian gatunku *Cosmos bipinnatus* Cav. Zawartość ich określano używając metody spektrofotometrycznej. Przeprowadzając badania ilościowe związków polifenolowych w pąkach kwiatowych wykazano, że wszystkie przebadane polifenole akumulują się podczas fazy nabrzmiewania pąków. Pod względem występowania ilościowego charakterystyczny był wysoki poziom kosmosyny w kwiatach języczkowych i znaczna zawartość nelumbozydu w liściach. Szkarłatnie zabarwione formy kwiatów wyróżniały się wyższą koncentracją kosmocyaniny, kwasu kawowego, kwasu chlorogenowego w określonych częściach kwiatu języczkowego. Koreopsyna i jej aglikon, buteina, które zlokalizowane były w żółtych częściach kwiatu rurkowatego wykazywały odwrotną relację w ich zawartości.