

THE CONTENT OF COUMARIN ANALOGUES IN RED LEAVES OF HIGHER PLANTS

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Abstract—A remarkable amount of bound *o*-coumaric acid accompanied by a small amount of coumarin was isolated from red leaves of 20 plant species. It is noteworthy that the leaves of deeper red colour contain a higher amount of the bound *o*-coumaric acid. The amount of *o*-coumaric acid in one species reaches a maximum at the beginning of the red colouring, and decreases somewhat as the red colour deepens. This phenomena is interesting from biosynthetical point of view, since it has been shown that both coumarin and anthocyanins are synthesized through shikimic acid pathway.¹

INTRODUCTION

COUMARIN is widely distributed in various plant families, but there has been no report concerning its quantitative seasonal variation. Recently, *o*-coumaric acid glucoside (melilotoside) was found to increase in autumn and reach the maximum at the beginning of the red colouration in the leaves of *Prunus yedoensis* Matsum. (cherry tree) and three related species. The amount of this glucoside was then found to decrease somewhat as the red colour deepens as shown in Fig. 1.² A trace of coumarin was detected in the leaves throughout the investigation. The amount of *o*-coumaric acid glucoside in the cherry tree was greatest in

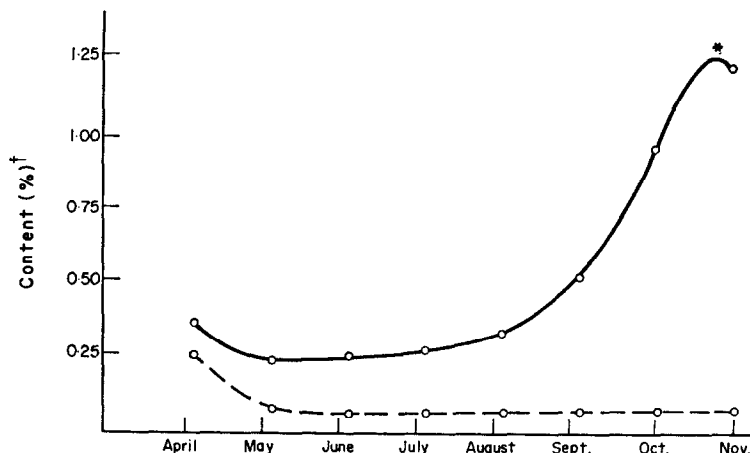


FIG. 1. CONTENTS OF COUMARIN AND *o*-COUMARIC ACID GLUCOSIDE IN THE LEAVES OF CHERRY TREE.
(—○—) *o*-Coumaric acid glucoside; (---○---) coumarin.

* The beginning of the red colouring.

† On a fresh weight basis.

¹ J. B. HARBORNE, *Biochemistry of Phenolic Compounds*, pp. 268, 340, Academic Press, London (1964).

² K. TAKAISHI, *J. Pharm. Soc. Japan* **88**, 1467 (1968).

the leaves of deeper red colour. Further, it has been often experienced that red leaf plants when growing in a group outdoors give out a sweet coumarin odour. To find out whether these phenomena occur in various plant species whose leaves turn red in autumn, the coumarin analogues in the leaves of 20 plant species were investigated.

RESULTS

The red and yellowish red leaves of 20 species of plants collected in October contain fairly high amounts of bound *o*-coumaric acid as shown in Table 1. Larger amounts of *o*-coumaric acid were observed in the deep red leaves than in yellowish red leaves. The bound *o*-coumaric acid content of red leaves of one plant was compared with the green leaves of another species belonging to the same genus. It was found that the red leaves contained much more *o*-coumaric acid than the green leaves of the closely related species (Table

TABLE 1. CONTENTS OF *o*-COUMARIC ACID IN RED LEAVES

Name of plant	Family	Content (%)*	Colour of leaf
<i>Cornus controversa</i> Hemsl.	Cornaceae	1.70	deep red
<i>Sorbus commixta</i> Held.	Rosaceae	1.63	deep red
<i>Prunus yedoensis</i> Matsum.	Rosaceae	1.34	deep red
<i>Tripetaleia paniculata</i> Sieb., et Zucc.	Ericaceae	1.05	deep red
<i>Euonymus oxyphylla</i> Miq.	Celastraceae	0.89	deep red
<i>Acer sieboldianum</i> Miq.	Aceraceae	0.75	red
<i>Prunus jamasakura</i> Sieb., et Zucc.	Rosaceae	0.72	red
<i>Hypericum patulum</i> Thunb.	Guttiferae	0.70	red
<i>Hamamelis japonica</i> Sieb., et Zucc.	Hamamelidaceae	0.60	red
<i>Acer palmatum</i> Thunb.	Aceraceae	0.57	red
<i>Rhus silvestris</i> Sieb., et Zucc.	Anacardiaceae	0.42	red
<i>Viburnum urceolatum</i> Sieb., et Zucc.	Caprifoliaceae	0.38	red
<i>Quercus serrata</i> Thunb.	Fagaceae	0.38	red
<i>Quercus aliena</i> Blume	Fagaceae	0.35	yellowish red
<i>Corylus sieboldiana</i> Blume	Betulaceae	0.26	yellowish red
<i>Acer rufinerve</i> Thunb.	Aceraceae	0.20	yellowish red
<i>Stachyurus praecox</i> Sieb., et Zucc.	Stachyuraceae	0.20	yellowish red
<i>Hydrangea paniculata</i> Sieb., et Zucc.	Saxifragaceae	0.18	yellowish red
<i>Cornus kousa</i> Buerger.	Cornaceae	0.18	yellowish red
<i>Viburnum furcatum</i> Blume	Caprifoliaceae	0.13	yellowish red

* On a fresh weight basis.

TABLE 2. CONTENTS OF *o*-COUMARIC ACID IN RED AND GREEN LEAVES

Name of plant	Family	Content (%)*	
		Red leaf	Green leaf
<i>Viburnum fureatum</i> Blume	Caprifoliaceae	0.13	—
<i>Viburnum awabuki</i> K. Koch	Caprifoliaceae	—	0.00
<i>Hydrangea paniculata</i> Thunb.	Saxifragaceae	0.18	—
<i>Hydrangea Macrophylla</i> Seringe	Saxifragaceae	—	0.02
<i>Quercus serrata</i> Thunb.	Fagaceae	0.38	—
<i>Quercus phillyraeoides</i> A. Gray	Fagaceae	—	0.02
<i>Sorbus commixta</i> Held.	Rosaceae	1.63	—
<i>Spiraea cantoniensis</i> Lour.	Rosaceae	0.20	0.00

* On a fresh weight basis.

TABLE 3. CONTENTS OF *o*-COUMARIC ACID IN RED AND GREEN LEAVES

Name of plant	Family	*Content (%)	
		May	November
<i>Sorbus commixta</i> Helund	Rosaceae	0.21	1.40
<i>Cornus controversa</i> Hemsl.	Cornaceae	0.20	1.35
<i>Prunus yedoensis</i> Matsum.	Rosaceae	0.25	1.34
<i>Quercus serrata</i> Thunb.	Fagaceae	0.15	0.60
<i>Quercus aliena</i> Blume	Fagaceae	0.12	0.40

* On a fresh weight basis.

2). The intensity of the red colour of leaves of a single species was correlated with a higher content of *o*-coumaric acid when the leaves are collected at the same time. The bound *o*-coumaric acid content of plants which showed red colour in autumn were tested again in the spring of next year found to contain a very small amount of *o*-coumaric acid as shown in Table 3.

DISCUSSION

o-Coumaric acid was isolated together with reducing sugars in large quantities when ethanolic extracts of leaves were hydrolysed with acid, while only small amounts of the acid could be isolated from direct ethyl ether and ethanolic extracts. Therefore the *o*-coumaric acid is present in a bound form, perhaps as a glycoside in the leaves.

It has long been known that leaves of *Melilotus* spp. and *Coumarona odorata* contain large amounts of *o*-coumaric acid and its glucoside. However, this is the first demonstration that the above compounds are present in large quantities in the leaves of plants which turn red in autumn.

There are some plants which turn red in early spring such as *Acer planatum* *Mallotus japonica* and *Photinia glabra*. No *o*-coumaric acid has been detected in these plants and, therefore, the existence of the acid in red autumn leaves in large quantities may be due to the special metabolism in the latter season. Thus there is a close correlation between the formation of anthocyan and *o*-coumaric acid. This is interesting from the biosynthetic viewpoint since both anthocyan and *o*-coumaric acid are thought to be synthesized through the shikimic acid pathway.

EXPERIMENTAL

Plant materials were collected from mountains in Kyoto, Osaka, Hyogo, and Nagano Pref., Japan.

Isolation of Coumarin from the Red leaves

A known weight (5–50 g) of fresh red leaves was heated with steam for 30 sec to kill enzymes and extracted 3 × by heating for 1 hr with Et₂O, using 10 ml/g of fresh red leaves. The Et₂O extract was cooled, dried, filtered and evaporated to dryness. From the residue coumarin was isolated. The coumarin was sublimed *in vacuo* and was recrystallized from ethanol, m.p. 68–70°. It was identified with authentic coumarin by mixed melting point and TLC (silica gel G–propanol-NH₄OH, 7:3).

Isolation of o-Coumaric Acid from the Red Leaves

After isolation of the coumarin, the residual leaves were extracted 3 × by heating for 3 hr each with EtOH, using 10 ml/g of fresh red leaves. The extract was evaporated to a small volume, and the residue was hydrolysed by heating for 6 hr under reflux with 0.3 N H₂SO₄, using 10 ml/g of fresh red leaves, and then steam

distilled. The steam distillate was extracted with Et₂O and the extract evaporated. From the residue *o*-coumaric acid was obtained and recrystallized from EtOH, m.p. 206–207°. It was identified with authentic *o*-coumaric acid by m.m.p. and TLC (silica gel G–propanol-NH₄OH, 7:3). The residue after isolation of *o*-coumaric acid gave a fragrant coumarin odour and its solution in aq. NaOH gave yellow fluorescence³ u.v. light on heating. It also showed on TLC (silica gel G–propanol-NH₄OH, 7:3 colouration by iodine vapour, u.v. yellow fluorescence³ in hot alkali) a major spot corresponding to coumarin and a faint spot of *o*-coumaric acid. No other spot was detected. The formation of a small amount of coumarin from synthetic *o*-coumaric acid was also observed on the acid treatment followed by steam distillation carried out as above. The syrupy residue from the filtration of *o*-coumaric acid crystal, after washing with NaHCO₃, showed the u.v. yellow fluorescence³ in hot alkali, and authentic coumarin was done by TLC and also by u.v. and i.r. spectra. After isolation of the *o*-coumaric acid, reducing sugar was obtained from the hydrolysates. The sugar was detected by PPC (BuOH–AcOH–H₂O, 4:1:5).

Quantative Assay of o-Coumaric Acid

o-Coumaric acid was determined by measuring the u.v. absorption (278 nm) of the steam distillate (2 l.) from the hydrolysate of the extract of the red leaves (5 g) from which free coumarin had been removed, using a suitable standard as control. The steam distillates from the hydrolysates of both the leaf extracts and the synthetic *o*-coumaric acid were chromatographed on silica gel G plates in several solvents (propanol–NH₄OH, 7:3, ethanol–NH₄OH–H₂O, 80:5:15, butanol–AcOH–H₂O, 4:1:5) and no other spot than those of *o*-coumaric acid and coumarin was detected.

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³ F. FEIGL, *Spot tests in Organic Analysis*, p. 424, Elsevier, New York (1960).