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CYANIDIN 3-*p*-COUMAROYLGLUCOSIDE IN *CAMELLIA* SPECIES AND CULTIVARS

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Key Word Index—*Camellia japonica*; *C. hiemalis*; *C. sasanqua*; Theaceae; *Hyacinthus orientalis*; Liliaceae; acylated anthocyanin; cyanidin 3-*O*-β-(6-*O*-*p*-coumaroyl-D-glucoside); cyanidin 3-glucoside; delphinidin 3-glucoside; flower colour.

Abstract—The major anthocyanin of red flowers of *Camellia hiemalis*, *C. japonica* and *C. sasanqua* was determined to be cyanidin 3-*O*-β-D-(6-*O*-*p*-coumaroylglucoside) by fast atom bombardment mass spectrometry and NMR spectroscopy. It was identical with the pigment, hyacinthin, of the bulb scales of *Hyacinthus orientalis*. This pigment and cyanidin 3-glucoside are widely distributed in the flowers of *Camellia japonica* and many *Camellia* cultivars.

INTRODUCTION

The anthocyanin pigments of *Camellia* cultivars were first reported to be the aglycone and 3-glucoside of cyanidin in ref. [1]. However, the presence of free cyanidin was not confirmed by Harborne [2]. Recently, Yokoi [3] found a delphinidin glycoside in *Camellia hiemalis* and *C. sasanqua*. Also, the presence of several 3-glucosides of cyanidin was reported in *C. japonica*, *C. saluenensis*, *C. reticulata*, and their hybrids [3–6]. However, these anthocyanins have not been studied in detail although the 3-glucoside and 3-galactoside of cyanidin were thought to be [1, 6].

This report describes the isolation and determination of the major anthocyanins of *Camellia hiemalis*, *C. sasanqua*, *C. japonica*, and 20 cultivars of *C. japonica*, together with the structure determination of hyacinthin, cyanidin 3-*p*-coumaroylglucoside, isolated from the mauve bulb scales of *Hyacinthus orintalis*.

RESULTS AND DISCUSSION

During a survey of 20 cultivars of *Camellia japonica*, two anthocyanins, cyanidin 3-glucoside and its acylated derivative, were observed as major pigments. The acylated anthocyanin was isolated from red flowers of *Camellia hiemalis* 'Kanjiro' with 0.1% HCl–MeOH, and purified using Sephadex LH-20, PC and TLC (solvent, BAW). The anthocyanin was obtained likewise from *C. sasanqua* and *C. japonica* using a similar procedure. Hyacinthin, cyanidin 3-*p*-coumaroylglucoside [7], was isolated from mauve bulb scale of *Hyacinthus orientalis* cultivars for comparison with the acylated *Camellia* anthocyanin.

To investigate the structure of these acylated anthocyanins, three types of degradation were carried out as follows: (i) acid hydrolysis produced cyanidin, glucose and *p*-coumaric acid, (ii) partial acid hydrolysis gave rise to cyanidin 3-glucoside, and (iii) *p*-coumaroylglucose was obtained by the hydrogen peroxide degradation [8].

The acylated *Camellia* anthocyanin and hyacinthin gave ^{1}H NMR signals in the region δ3.1–4.0 due to four glucose protons (Table 1). Acylated sugars normally show signals in the region δ4.0–5.0 attributed to the proton(s) geminal to the acyloxy group [9, 10, 12]. Both anthocyanins gave two characteristic signals at δ4.50 (1H, *d*, J = 11 Hz) and 4.17 (1H, *dd*, J = 7 and 9 Hz) in hyacinthin, and 4.48 and 4.15 in the acylated *Camellia* anthocyanin, indicating that the acyl group was attached to C-6 glucose [4, 10]. Also, both these signals showed the characteristic geminal coupling (J = 11 Hz) usually observed for the magnetically non-equivalent C-6 methylene protons, giving further evidence that acyl groups were attached to C-6 glucose carbon. The anomeric proton (δ5.46 and 5.42) in both anthocyanins was observed to be coupled with H-2 (glucose) (J = 7.5 Hz), indicating that the compound is β-D-glucopyranoside [9, 10, 12].

The fast atom bombardment mass spectrometry of each acylated anthocyanin gave its molecular ion at 595 *m/z* $[M]^+$, in good agreement with the mass calculated for $C_{30}H_{27}O_{13}$ (*m/z* 595), establishing its composition. Thus, the chemical properties and the ^{1}H NMR evidence together with the FAB-MS data indicate that the acylated *Camellia* anthocyanin and hyacinthin are cyanidin 3-*O*-β-(6-*O*-*p*-coumaroyl-D-glucopyranoside). This paper reports for the first time that the *p*-coumaroyl residue in the hyacinth pigment is located at the 6-position of the glucose residue.

Another major anthocyanin isolated from *Camellia* cultivars was identified as cyanidin 3-glucoside according to standard procedures [11], and as reported by Hayashi and Abe [1]. Delphinidin 3-glucoside was confirmed as a minor component of *Camellia hiemalis* and *C. sasanqua* [3]. Finally, an electrophoretic analysis was carried out according to the procedure of ref. [13]. The *Camellia* and *Hyacinthus* pigments extracted with the methanol–acetic acid–water solvent did not move to the anode. Consequently the native anthocyanins in both plants are identical with the anthocyanins extracted with methanolic hydrochloric acid.

Table 1. ^{1}H NMR analyses of cyanidin 3-*p*-coumaroylglucoside using DMSO-d_6 with DCl (chemical shifts in ppm from TMS)

	Cyanidin 3-*p*-coumaroylglucoside		
	Acylated *Camellia* anthocyanin	Hyacinthin	*Malvidin* 3-*p*-coumaroylglucoside*
H-4	8.84	8.92	8.96
H-6	6.72 (J = 2)	6.78 (J = 2)	6.56 (J = 2.0)
H-8	6.83 (J = 2)	6.90 (J = 2)	6.91 (J = 2.0, 0.7)
H-2′	8.00 (J = 2)	8.02 (J = 2)	7.96†
H-5′	6.80 (J = 8)	6.82 (J = 8)	
H-6′	8.20 (J = 2, 9)	8.20 (J = 2, 9)	
Anomeric H	5.42 (J = 7.5)	5.46 (J = 7.5)	5.38 (J = 7.6)
Glu CH_2	4.15–4.48	4.17–4.50	4.2–4.6
Glucosyl	3.10–3.95	3.09–3.96	3.4–3.9
C<u>H</u> = CH–COOR	7.44 (J = 16)	7.46 (J = 16)	7.42 (J = 16.1)
CH = C<u>H</u>–COOR	6.28 (J = 16)	6.30 (J = 16)	6.20 (J = 16.1)
H-2″, 6″‡	7.37 (J = 8)	7.40 (J = 8)	7.30 (J = 8.8)
H-3″, 5″‡	7.03 (J = 8)	7.05 (J = 8)	6.78 (J = 8.8)

*Ref. [12].
†H-2′ and 6′.
‡Protons of *p*-coumaric acid.

EXPERIMENTAL

Extraction and purification of anthocyanins. The colour petals of *C. hiemalis* (1.5 kg), *C. sasanqua* (500 g), *C. japonica* (500 g) and 20 cultivars of *C. japonica* collected in the campus-garden of Chiba University and also mauve bulb scales of *Hyacinthus orientales* cultivars were extracted with 0.1% HCl–MeOH. After each red residue of concd extracts was passed through a column of Sephadex LH-20 with 0.1% HCl–MeOH in order to separate flavonols and other phenolic components, the extracts were separated and purified by PC and TLC (cellulose: *n*-BuOH–AcOH–H_2O, 4:1:5). The approximate yields of cyanidin 3-*p*-coumaroylglucoside and cyanidin 3-glucoside were 50 mg and 35 mg in *C. hiemalis*. 15 mg and 10 mg in *C. sasanqua*, 2 mg and 5 mg in *C. japonica*. Delphinidin 3-glucoside (5 mg) was obtained from the extracts of *C. hiemalis*, with a further small quantity from *C. sasanqua*.

Pigment identifications were carried out by standard procedures, involving H_2O_2 oxidation, deacylation with alkali, hydrolysis with acid and electrophoresis at pH 4.4 [2, 11, 13].

R_f values and spectral properties of anthocyanins cyanidin 3-p-coumaroylglucoside. The acylated *Camellia* anthocyanin and hyacinthin displayed bathochromic shifts on the addition of $AlCl_3$ showing the presence of a catechol system on the B-ring. These anthocyanins showed identical λ_{max} values at 528 and 282 nm, and also shoulder at 310 nm in 0.1% HCl–MeOH. The values of $E_{440}/E_{vis,max}$ of both anthocyanins were 23%, and $E_{310}/E_{vis,max}$ were 77%. R_f values of TLC were 0.42 in BAW (*n*-butanol–acetic acid–H_2O, 4:1:5), 0.48 BuHCl (*n*-butanol–2N-HCl, 1:1), 0.03 1% HCl, 0.22 AcOHCl (acetic acid–HCl–H_2O, 15:3:82).

Cyanidin 3-glucoside. R_f values (TLC) were 0.33 BAW, 0.24 BuHCl, 0.07 1% HCl and 0.22 AcOHCl. λ_{max} 282, 528 nm in 0.1% HCl–MeOH and $E_{440}/E_{vis,max}$ 25%.

Delphinidin 3-glucoside. R_f values of TLC, 0.14 BAW, 0.08 BuHCl, 0.01 1% HCl, 0.08 AcOHCl; λ_{max} 277, 540 nm in 0.1% HCl–MeOH and $E_{440}E_{vis,max}$ 21%.

^{1}H NMR and fast atom bombardment mass spectrometry. ^{1}H NMR of anthocyanins was obtained with JEOL FX-100 spectrometer and samples were measured in 10% TFA-90% DMSO-d_6 and also DMSO-d_6 adding one drop of DCl [10]. Mass spectra were taken with JEOL JMS D-300 spectrometer.

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