Pergamon

0031-9422(95)00140-9

Phytochemistry, Vol. 39, No. 6, pp. 1437–1441, 1995

0031-9422/95 $9.50 + 0.00

ACYLATED ANTHOCYANINS FROM RED *HYACINTHUS ORIENTALIS*

KEIZO HOSOKAWA,*† YUKIO FUKUNAGA,‡ ERI FUKUSHI§ and JUN KAWABATA§

*Iwate Biotechnology Research Center, 22-174-4 Narita, Kitakami, Iwate 024, Japan; ‡Gakken Institute of Plant Technology, 1-2-14 Honson, Chigasaki, Kanagawa 253, Japan; §Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan

(*Received in revised form* 11 *January* 1995)

Key Word Index—*Hyacinthus orientalis*; Liliaceae; red flowers; acylated anthocyanin; pelargonidin; cyanidin; *p*-coumaric acid; malonic acid.

Abstract—Two novel anthocyanins, pelargonidin 3-*O*-β-D-glucoside-5-*O*-(6-*O*-malonyl-β-D-glucoside) and pelargonidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(4-*O*-malonyl-β-D-glucoside) have been isolated from red flowers of *Hyacinthus orientalis* cv Holly Hock, along with seven known anthocyanins, two of which include *cis*-*p*-coumaric acid as the acyl moiety. Their complete structures were unambiguously elucidated by 1D and 2D NMR techniques and other spectral evidence.

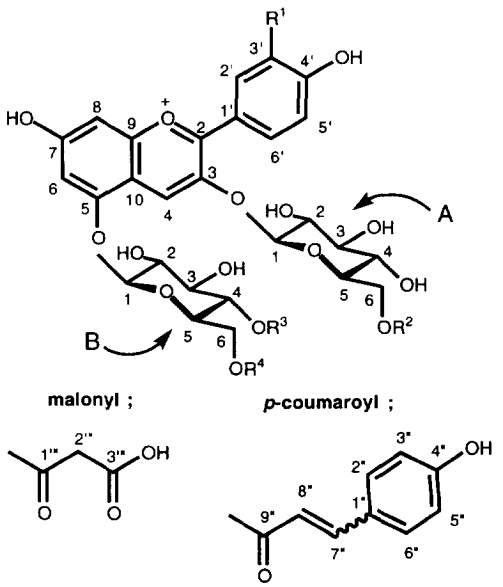
INTRODUCTION

Hyacinthus orientalis L. is a popular ornamental plant with blue, red, pink, yellow or white flowers. The anthocyanins of the blue flowers of *H. orientalis* L. cv Delft Blue have been reported to be seven acylated anthocyanins [1]. Although cyanidin 3,5-diglucosides have been reported in red flowers of *H. orientalis* L. cv Jan Bos [2], the structures of the other anthocyanins have not been elucidated. We now report two novel acylated anthocyanins as well as seven known anthocyanins in red double flowers of *H. orientalis* L. cv Holly Hock.

RESULTS AND DISCUSSION

Anthocyanins (**1**–**9**) of the red double flowers of *H. orientalis* were isolated by an Amberlite XAD-7 column chromatography, followed by preparative HPLC. UV-vis and FAB-mass spectra of the nine anthocyanins are shown in Table 1.

The FAB-mass spectrum of **8** showed a molecular ion at *m/z* 827, in good agreement with the mass calculated for $C_{39}H_{39}O_{20}$. Fragment peaks were also observed at *m/z* 579 [M − 248 (malonylhexose)]$^+$, 519 [M − 308 (coumaroylhexose)]$^+$ and 271 [aglycone]$^+$, indicating **8** to be comprised of pelargonidin, malonylhexose and coumaroylhexose. In the UV-vis spectra, $E_{440}/E_{vis,\,max}$ was 0.17, indicating the presence of pelargonidin 3,5-diglycosides [3]. Analysis of the ^{1}H NMR spectrum indicated the presence of pelargonidin, two glucoses, *trans*-*p*-coumaric acid ($J_{7'',8''}$ = 16 Hz) and malonic acid (Table 2). Signals from the sugar moiety were observed in the region of δ3.52–5.36 and all vicinal coupling constants of two glucose moieties were at 7.5–9.6 Hz. The chemical shifts and the large coupling constants of two anomeric protons appeared at δ5.36 (*d*, *J* = 7.8 Hz, glucose A) and δ5.21 (*d*, *J* = 7.5 Hz, glucose B), showing



	R^1	R^2	R^3	R^4	7''/8''
2	H	H	H	H	--
3	H	*p*-coumaroyl	H	H	*cis*
4	H	*p*-coumaroyl	H	H	*trans*
5	H	H	H	malonyl	--
6	H	*p*-coumaroyl	H	malonyl	*cis*
7	H	*p*-coumaroyl	H	malonyl	*trans*
8	H	*p*-coumaroyl	malonyl	H	*trans*
9	OH	*p*-coumaroyl	H	malonyl	*trans*

†Author to whom correspondence should be addressed.

Table 1. Spectral properties of anthocyanins from the red flower of *H. orientalis* L. cv Holly Hock

Anthocyanin	UV-vis (0.1% HCl–MeOH)					FAB-mass spectra
	$\lambda_{vis.\,max}$ (nm)	$\lambda_{acyl.\,max}$ (nm)	$E_{acyl}/E_{vis.\,max}$	$E_{440}/E_{vis.\,max}$	$AlCl_3$ shift (nm)	$[M]^+$ & fragment ions
1	511			0.39	0	443, 271
2	508			0.16	0	595, 433, 271
3	512	313	0.44	0.18	0	741, 579, 433, 271
4	508	316	0.58	0.19	0	741, 579, 433, 271
5	506			0.18	0	681, 519, 433, 271
6	513	310	0.38	0.20	0	827, 579, 519, 271
7	509	316	0.55	0.19	0	827, 579, 519, 271
8	508	316	0.56	0.17	0	827, 579, 519, 271
9	528	314	0.56	0.11	+ 7	843, 595, 535, 287

both glucose units to be β- D-glucopyranoside. By analysis of the proton network of the glucose moieties, the anomeric protons of glucose A and B were finally correlated to non-equivalent methylene protons of C-6 at δ4.45 and 4.51, and δ3.63 and 3.79–3.83, respectively. The downfield shift of the methylene of glucose A and H-4 (δ4.95) of glucose B indicated the coumaroyl/ malonyl moieties to be attached to OH-6 of glucose A and to OH-4 of glucose B.

To confirm the position of the ester linkage, the heteronuclear multiple-bond correlation (HMBC) spectrum was determined. The correlation peak between H-6 (δ4.45 and 4.51) of glucose A and carbonyl carbon of *p*-coumaric acid (δ169.2) was observed, indicating *p*-coumaric acid to be attached to OH-6 of glucose A through an ester bond. Similarly, malonic acid is attached to the OH-4 of glucose B, shown by the presence of the correlation peak between H-4 (δ4.95) of glucose B and one of the carbonyl carbons (δ168.1) of malonic acid.

The position of the glucosidic linkage was determined by HMBC and nuclear Overhauser effect (NOE) difference spectra. In the HMBC spectum, correlation between the anomeric proton (δ5.36) of glucose A and C-3 (δ145.7) of pelargonidin was observed, indicating glucose A to be attached to OH-3 of pelargonidin. Similarly, glucose B was attached to OH-5 of pelargonidin, shown by the presence of the correlation between the anomeric proton (δ5.21) of glucose B and C-5 (δ156.7) of pelargonidin. These connectivities were also confirmed by NOE experiments, in which negative NOEs of H-4 (δ9.00) and H-6 (δ7.01) of pelargonidin to the anomeric protons of glucoses A and B were observed, respectively. Anthocyanin **8** is thus pelargonidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(4-*O*-malonyl-β-D-glucoside).

Anthocyanin **5** was found to have a molecular ion at *m/z* 681 by FAB-mass spectrometry, in good agreement with mass calculated for $C_{30}H_{33}O_{18}$. Fragment peaks were observed at *m/z* 519 $[M - 162\ (hexose)]^+$, *m/z* 433 $[M - 248\ (malonylhexose)]^+$ and 271 $[pelargonidin]^+$ which indicated **5** to be comprised of pelargonidin, malonylhexose and hexose. The ^1H NMR spectrum was similar to that of **8**, except for the signals of H-6 of glucose A and H-4 and H-6 of glucose B. The rather high-field shift (δ3.70 and 3.97) of H-6 of glucose A compared to **8** indicated that OH-6 of glucose A was unsubstituted. In the glucose B, the low-field shift of H-6 (δ4.34 and 4.54) was observed in place of that of H-4 in **8**, which indicated the malonic acid to be attached to OH-6 in **5**. The total connectivities were confirmed by HMBC. Anthocyanin **5** is thus pelargonidin 3-*O*-β-D-glucoside-5-*O*-(6-*O*-malonyl-β-D-gucoside).

Anthocyanins **1**–**4**, **6**, **7** and **9** were also obtained and these structures were elucidated by the complete assignments of the ^1H and ^{13}C NMR signals deduced with 1D and 2D techniques (Tables 2 and 3). These structures are pelargonidin 3-*O*-β-D-glucoside (**1**), pelargonidin 3-*O*-, 5-*O*-di-β-D-glucoside (**2**), pelargonidin 3-*O*-(6-*O*-*cis*-*p*-coumaroyl-β-D-glucoside)-5-*O*-β-D-glucoside (**3**), pelargonidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-β-D-glucoside (**4**), pelargonidin 3-*O*-(6-*O*-*cis*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside (**6**), pelargonidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside (**7**) and cyanidin 3-*O*-(6-*O*-*trans*-*p*-coumaroyl-β-D-glucoside)-5-*O*-(6-*O*-malonyl-β-D-glucoside) (**9**).

Pelargonidin 3,5-diglucoside acylated with malonic acid at the 5-*O*-glucose as in **5** has not been reported before. Anthocyanin **8** is also a novel anthocyanin although anthocyanins which have 4-malonylglucose have been reported in *Monarda didyma* [4], *Salvia splendens* [4–6] and *S. farinancea* [6]. While *cis–trans* isomerization of cinnamate in acylated anthocyanins is known to occur spontaneously [7], the anthocyanins **3** and **6** containing *cis*-*p*-coumarate as acyl moiety of anthocyanins were first found in flowers of *H. orientalis* L. cv Holly Hock.

EXPERIMENTAL

Plant material. Bulbs (*ca* 6 cm in diameter) of *H. orientalis* L. cv Holly Hock were obtained from Heiwaen Co.

Table 2. ^{1}H NMR spectral data of anthocyanins from *Hyacinthus* (in MeOH-d_4 containing 10% TFA-*d*)

	1	2	3	4	5	6	7	8	9
Aglycone									
4	9.07 *s*	9.21 *s*	8.72 *s*	8.90 *s*	9.19 *s*	8.58 *s*	8.92 *s*	9.00 *s*	8.84 *s*
6	6.67 *d* (2.0)	7.08 *br s*	6.96 *d* (1.5)	6.95 *d* (1.8)	7.03 *d* (1.8)	6.84 *d* (1.5)	6.94 *d* (2.0)	7.01 *d* (1.7)	6.94 *d* (1.4)
8	6.92 *d* (2.0)	7.12 *br s*	6.89 *d* (1.5)	6.87 *d* (1.8)	7.12 *d* (1.8)	6.78 *d* (1.5)	6.97 *d* (2.0)	6.97 *d* (1.7)	6.86 *d* (1.4)
2′	8.59 *d* (9.1)	8.66 *d* (9.0)	8.52 *d* (9.2)	8.47 *d* (9.1)	8.65 *d* (9.1)	8.46 *d* (9.0)	8.52 *d* (9.1)	8.56 *d* (9.2)	7.92 *d* (2.3)
3′	7.05 *d* (9.1)	7.06 *d* (9.0)	7.06 *d* (9.2)	6.98 *d* (9.1)	7.06 *d* (9.1)	7.05 *d* (9.0)	7.01 *d* (9.1)	7.03 *d* (9.2)	
5′	7.05 *d* (9.1)	7.06 *d* (9.0)	7.06 *d* (9.2)	6.98 *d* (9.1)	7.06 *d* (9.1)	7.05 *d* (9.0)	7.01 *d* (9.1)	7.03 *d* (9.2)	6.95 *d* (8.7)
6′	8.59 *d* (9.1)	8.66 *d* (9.0)	8.52 *d* (9.2)	8.47 *d* (9.1)	8.65 *d* (9.1)	8.46 *d* (9.0)	8.52 *d* (9.1)	8.56 *d* (9.2)	8.15 *dd* (8.7, 2.3)
Glucose A									
1	5.27 *d* (7.8)	5.27 *d* (7.7)	5.40 *d* (7.8)	5.41 *d* (7.7)	5.28 *d* (7.9)	5.44 *d* (7.9)	5.43 *d* (7.7)	5.36 *d* (7.8)	5.48 *d* (7.8)
2	3.64 *dd* (9.1, 7.8)	3.67 *dd* (8.6, 7.7)	3.71 *dd* (9.2, 7.8)	3.71 *dd* (8.9. 7.7)	3.67 *dd* (9.2, 7.9)	3.72 *dd* (9.2, 7.9)	3.72 *dd* (8.9, 7.7)	3.71 *dd* (8.8, 7.8)	3.76 *dd* (8.9, 7.8)
3	3.53 *dd* (9.2, 9.1)	3.55 *dd* (9.4, 8.6)	3.57 *dd* (9.2, 9.1)	3.61 *dd* (9.2, 8.9)	3.53 *dd* (9.2, 9.1)	3.60 *dd* (9.2, 9.2)	3.61 *dd* (8.9, 8.9)	3.59 *dd* (9.0, 8.8)	3.64 *dd* (9.1, 8.9)
4	3.43 *dd* (9.6, 9.2)	3.39 *dd* (9.4, 9.1)	3.43 *dd* (9.5, 9.1)	3.55 *dd* (9.4, 9.2)	3.39 *dd* (9.5, 9.1)	3.43 *dd* (9.6, 9.2)	3.49 *dd* (9.6, 8.9)	3.52 *dd* (9.4, 9.0)	3.49 *dd* (9.5, 9.1)
5	3.55 *ddd* (9.6, 6.1, 2.1)	3.62 *br dd* (9.1, 7.0)	3.93 *ddd* (9.5, 9.1, 2.4)	3.88 *ddd* (9.4, 6.5, 2.9)	3.62 *ddd* (9.5. 7.1, 2.0)	3.95 *ddd* (9.6, 9.6, 2.5)	3.93 *ddd* (9.6, 7.8, 2.8)	3.87 *ddd* (9.4, 6.8, 2.8)	3.95 *ddd* (9.5, 8.0, 2.7)
6	4.70 *dd* (12, 6.1)	3.71 *dd* (12, 7.0)	4.55 *dd* (12, 9.1)	4.45 *dd* (12, 6.5)	3.70 *dd* (12, 7.1)	4.65 *dd* (12, 9.6)	4.42 *dd* (12, 7.8)	4.45 *dd* (12, 6.8)	4.41 *dd* (12, 8.0)
	4.91 *dd* (12, 2.1)	3.97 *br d* (12)	4.40 *dd* (12, 2.4)	4.50 *dd* (12, 2.9)	3.97 *dd* (12, 2.0)	4.35 *dd* (12, 2.5)	4.51 *dd* (12, 2.8)	4.51 *dd* (12, 2.8)	4.51 *dd* (12, 2.7)
Glucose B									
1		5.16 *d* (7.6)	5.17 *d* (7.8)	5.16 *d* (7.7)	5.17 *d* (7.7)	5.21 *d* (7.8)	5.19 *d* (7.8)	5.21 *d* (7.5)	5.19 *d* (7.8)
2		3.68 *dd* (8.7, 7.6)	3.76 *dd* (9.2, 7.8)	3.75 *dd* (9.2. 7.7)	3.69 *dd* (9.2, 7.7)	3.78 *dd* (9.3, 7.8)	3.78 *dd* (9.2, 7.8)	3.85 *dd* (9.5, 7.5)	3.78 *dd* (9.1, 7.8)
3		3.57 *dd* (9.5, 8.7)	3.57 *dd* (9.2, 9.2)	3.60 *dd* (9.4, 9.2)	3.57 *dd* (9.2, 9.1)	3.62 *dd* (9.3, 9.2)	3.59 *dd* (9.1, 9.2)	3.80 *dd* (9.5, 9.2)	3.59 *dd* (9.2, 9.1)
4		3.46 *dd* (9.5, 9.2)	3.47 *dd* (9.7, 9.2)	3.46 *dd* (9.5, 9.4)	3.46 *dd* (9.6, 9.1)	3.51 *dd* (9.5, 9.2)	3.46 *dd* (9.7, 9.1)	4.95 *dd* (9.6, 9.2)	3.46 *dd* (9.7, 9.2)
5		3.52 *br dd* (9.2, 9.0)	3.61 *ddd* (9.7, 6.1, 2.3)	3.61 *m*	3.81 *ddd* (9.6, 6.8, 2.2)	3.86 *ddd* (9.5, 5.7, 3.1)	3.80 *ddd* (9.7, 6.2, 2.1)	3.79–3.83 *m*	3.80 *ddd* (9.7, 6.2, 2.0)
6		3.74 *dd* (12, 5.4)	3.81 *dd* (12, 6.1)	3.74 *dd* (12, 5.0)	4.34 *dd* (12, 6.8)	4.53 *dd* (12, 5.7)	4.22 *dd* (12, 6.2)	6.63 *dd* (12, 6.6)	4.22 *dd* (12, 6.2)
		3.95 *br d* (12)	4.03 *dd* (12, 2.3)	3.97 *dd* (12, 2.3)	4.54 *dd* (12, 2.2)	4.55 *dd* (12, 3.1)	4.52 *dd* (12, 2.1)	3.79–3.83 *m*	4.52 *dd* (12, 2.0)
Coumaric acid moiety									
2″			7.23 *d* (8.7)	7.20 *d* (8.6)		7.07 *d* (8.7)	7.19 *d* (8.6)	7.26 *d* (8.6)	7.16 *d* (8.6)
3″			6.38 *d* (8.7)	6.69 *d* (8.6)		6.28 *d* (8.7)	6.69 *d* (8.6)	6.75 *d* (8.6)	6.68 *d* (8.6)
5″			6.38 *d* (8.7)	6.69 *d* (8.6)		6.28 *d* (8.7)	6.69 *d* (8.6)	6.75 *d* (8.6)	6.68 *d* (8.6)
6″			7.23 *d* (8.7)	7.20 *d* (8.6)		7.07 *d* (8.7)	7.19 *d* (8.6)	7.26 *d* (8.6)	7.16 *d* (8.6)
7″			6.57 *d* (13)	7.31 *d* (16)		6.36 *d* (13)	7.33 *d* (16)	7.37 *d* (16)	7.31 *d* (16)
8″			5.75 *d* (13)	6.17 *d* (16)		5.71 *d* (13)	6.21 *d* (16)	6.20 *d* (16)	6.20 *d* (16)
Malonic acid moiety									
2‴					nd*	3.84 *s*	3.38 *s*	nd*	3.41 *s*

*nd: Not detected because of the intensity reduction by H/D exchange.
Coupling constants *J* (in Hz) in parentheses.

Table 3. ^{13}C NMR spectral data of anthocyanins from *Hyacinthus* (in MeOH-d_4 containing 10% TFA-*d*)

	1	2	3	4	5	6	7	8	9
Aglycone									
2	164.7	165.5	164.6	164.9	165.5	163.9	165.1	165.6	164.3
3	154.5	146.5	145.9	145.4	146.6	145.8	145.7	145.7	145.7
4	137.8	137.1	134.5	136.2	136.9	133.5	135.7	136.7	134.7
5	159.5	157.0	156.7	156.7	156.7	156.1	156.6	156.7	156.5
6	103.5	105.9	105.1	106.0	106.1	104.9	106.2	106.3	106.1
7	170.8	169.9	169.7	170.1	169.8	169.4	169.9	170.0	169.6
8	95.2	97.5	97.2	97.6	97.4	96.9	97.5	97.7	97.4
9	158.0	157.5	157.0	157.0	157.4	156.6	157.1	157.3	156.8
10	113.7	113.7	113.3	113.3	115.5	113.1	113.3	113.4	113.1
1′	120.9	120.7	120.7	120.4	120.7	120.5	120.5	120.6	120.9
2′	135.7	136.3	136.1	136.1	136.3	136.0	136.1	136.2	118.5
3′	117.9	118.1	118.1	118.1	118.1	118.1	118.1	118.1	147.5
4′	166.6	167.3	167.2	167.3	167.3	167.2	167.4	167.4	156.7
5′	117.9	118.1	118.1	118.1	118.1	118.1	118.1	118.1	117.6
6′	135.7	136.3	136.1	136.1	136.3	136.0	136.1	136.2	128.8
Glucose A									
1	103.9	104.2	102.1	102.9	104.2	101.5	102.6	103.1	102.2
2	74.8	74.7	74.3	74.5	74.7	74.6	74.4	74.6	74.4
3	78.1	78.4	78.2	78.0	78.4	78.3	78.1	78.0	78.2
4	71.1	71.4	72.1	71.7	71.4	72.3	72.2	71.8	72.3
5	78.8	79.0	75.9	75.7	79.0	75.9	75.6	75.8	75.5
6	62.4	62.7	64.4	64.1	62.7	64.4	64.5	64.2	64.5
Glucose B									
1		102.8	102.3	102.9	102.3	101.5	102.8	102.9	102.8
2		74.5	74.7	74.8	74.4	74.2	74.7	74.7	74.8
3		77.7	77.8	77.8	77.5	77.7	77.7	76.5	77.7
4		71.1	71.3	71.2	71.4	71.3	71.0	72.9	71.0
5		78.7	78.7	78.8	75.9	75.9	75.9	75.4	76.0
6		62.4	62.6	62.5	65.2	65.1	65.1	62.0	65.2
Coumaric acid moiety									
1″			127.2	126.7		127.0	126.7	126.8	126.7
2″			133.4	131.4		133.3	131.4	131.4	131.4
3″			115.6	117.2		115.5	116.7	116.9	116.7
4″			159.6	161.2		159.5	161.2	161.3	161.2
5″			115.6	117.2		115.5	116.7	116.9	116.7
6″			133.4	131.4		133.3	131.4	131.4	131.4
7″			144.2	147.0		143.6	147.0	147.0	147.0
8″			115.9	114.6		115.8	114.7	114.6	114.7
9″			168.9	169.1		169.0	169.2	169.2	169.2
Malonic acid moiety									
1‴					168.6	168.6	168.5	168.1	168.6
2‴					nd.*	42.0	41.9	42.2	41.9
3‴					170.4	170.3	170.5	170.4	170.5

*nd: Not detected.

(Tenri, Japan), in September 1991, and then grown in an experimental field. Fresh red flowers were collected in March to April 1991 and freeze-dried.

Isolation of the anthocyanins. Freeze-dried flowers (25 g) were extracted with EtOH–HOAc–H_2O (10:1:9) at 4°. The concd extract was adsorbed on an Amberlite XAD-7 column and washed with 5% HOAc. The anthocyanins were eluted by 50% MeOH containing 5% HOAc. For further purification, the crude anthocyanins were purified by prep. HPLC using Chromatorex-ODS (Fuji Silysia Chemical Ltd.) column with flow rate of 5–8 ml min^{-1} monitoring at 510 nm for anthocyanins. The solvent systems used were as follows; 15–60% MeCN–HOAc–H_2O containing 0.5% TFA, and then 10–25% MeOH containing 15% HOAc and 0.5% TFA. To replace the counter anion of anthocyanins with trifluoroacetate, the concentrated fractions were adsorbed on activated Sep-Pak tC 18 (Waters Associates, Milford, MA, U.S.A.). The cartridge was washed with 0.5% aq. TFA and then eluted with MeOH containing 0.5% TFA. The purified anthocyanins were concentrated to dryness under N_2 stream. The residues were dissolved in small amount of 1% TFA and then freeze-dried to give nine anthocyanins as powders (**1**; 1.7 mg, **2**; 3.6 mg, **3**; 2.5 mg, **4**; 26.2 mg, **5**; 10.0 mg, **6**; 13.1 mg, **7**; 388.5 mg, **8**; 6.4 mg, **9**; 5.6 mg).

Acknowledgement—The authors thank Prof. Masahiro Mii (Faculty of Horticulture, Chiba University) for helpful discussions and for a critical reading of the manuscript.

REFERENCES

1. Hosokawa, K., Fukushi, E., Kawabata, J. and Fukunaga, Y. (1994) *Phytochemistry* **38**, 1293.
2. Shibata, M., Morita, A., Hirai, S. and Ishikura, N. (1967) *Kumamoto J. Sci., Ser. B* **8**, 21.
3. Harborne, J. B. (1958) *Biochem. J.* **70**, 22.
4. Kondo, T., Nakane, Y., Tamura, H. and Goto, T. (1985) *Tetrahedron Letters* **26**, 5879.
5. Tomas-Barberan, F., Harborne, J. B. and Self, R. (1987) *Phytochemistry* **26**, 2759.
6. Kondo, T., Yoshikane, M., Yoshida, K. and Goto, T. (1989) *Tetrahedron Letters* **30**, 6729.
7. Yoshida, K., Kondo, T., Kameda, K. and Goto, T. (1990) *Agric. Biol. Chem.* **54**, 1745.