Physical Stability of the Blue Pigments Formed from Geniposide of Gardenia Fruits: Effects of pH, Temperature, and Light

Young-Sook Paik,*,† Chang-Min Lee,† Man-Ho Cho,‡ and Tae-Ryong Hahn‡

Department of Chemistry and Genetic Engineering, and Plant Metabolism Research Center, Kyung Hee University, Suwon 449-701, Korea

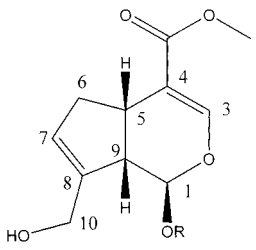
Fruits of *Gardenia jasminoides* contain geniposide which can be transformed to blue pigments by a simple modification. Colorless geniposide obtained from gardenia fruits by charcoal and silica gel column chromatographies was hydrolyzed with β-glucosidase to yield genipin. The resulting genipin was transformed to blue pigments by reaction with amino acids (glycine, lysine, or phenylalanine). The stability of the blue pigments against heat, light, and pH was studied to examine the blue dye for possible use as a value-added food colorant. Thermal degradation reactions at temperatures of 60−90 °C were carried out at different pH levels within the range 5.0−9.0 (pH 5.0, acetate buffer; pH 7.0, phosphate buffer; and pH 9.0, CHES buffer). The blue pigments remained stable after 10 h at temperatures of 60−90 °C, and in some cases, more new pigments formed. The pigments were more stable at alkaline pH than neutral and acidic pH. Similarly, the pigments were stable under light irradiance of 5000−20 000 lux. In this case, pH effect was not significant.

Keywords: *Gardenia jasminoides; geniposide; genipin; glycine; lysine; phenylalanine; physical stability; gardenia blue pigments*

INTRODUCTION

Fruits of *Gardenia jasminoides*, easily obtainable in southern parts of the Korea peninsula, have long been used as folklore medicine for antiphlogistic, diuretic, laxative, choleretic, and hemostatic purposes in the treatment of trauma by external application (*1*). Gardenia fruits also have been used as a yellow dye for staining foods and fabrics. The yellow color components of gardenia fruits are composed of carotenoids and related compounds (*1*, *2*). Colorless components of gardenia fruits can also produce blue colorants by a simple modification of an enzyme reaction followed by the treatment of primary amines (*2*). With growing concern over the safety of synthetic dyes, the importance of natural colorants suitable for use in foods has gained increasing attention. The only natural blue colorants commercially feasible today are those derived from gardenia fruits and algae (*3*). The protein dye phycocyanin, derived from blue green algae, has stability problems as a colorant in alcoholic beverages or in aqueous solutions of different pH levels (*3*). The blue pigments produced by gardenia fruits have been widely used in east Asia, including Korea and Japan. Although the effect of pH on gardenia blue pigments was reported to be relatively stable (*3*), a detailed description of the physical stability of the pigments has yet to be reported.

In this research we isolated geniposide **1** (Figure 1), a colorless iridoid glycoside, from gardenia fruits by charcoal and silica gel column chromatographies. Geniposide was hydrolyzed with β-glucosidase to produce aglycon genipin **2** (Figure 1). The resulting genipin was transformed to blue pigments by reactions with amino acids (glycine, lysine, or phenylalanine). Thermal and light stabilities of the blue pigments in aqueous solutions were tested at pHs within the range of 5.0−9.0.



R = Glucose 1 (geniposide)

H 2 (genipin)

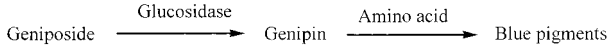


Figure 1. Chemical structures of geniposide **1** and genipin **2** and the procedure of blue pigments formation from geniposide of gardenia fruits.

EXPERIMENTAL PROCEDURES

Materials. Dried fruits of *Gardenia jasminoides* were obtained from the local region of Kyungnam province and stored in the refrigerator. The silica gel for column chromatography (Kiesel gel 230−400 mesh) and TLC plate (Kiesel gel 60 F_{254}) were purchased from Merck. β-Glucosidase, glycine, lysine, phenylalanine, and reagents for buffer preparation, including CHES (2-[*N*-cyclohexylamino]ethanesulfonic acid) and sodium phosphate, were purchased from Sigma Chemical Co. Other chemicals including chloroform, ethanol, and methanol were obtained from Hayman Ltd. and Tedia Company, Inc.

* To whom correspondence should be addressed. Tel: 82-31-201-2422. Fax: 82-31-202-7337. E-mail: paikys@khu.ac.kr.
† Department of Chemistry.
‡ Department of Genetic Engineering.

10.1021/jf000978f CCC: $20.00
Published on Web 12/20/2000

Spectral Analysis. The UV−vis spectra were recorded on a Milton Roy Spectronics 3000 spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were measured on a 400 MHz FT-NMR(JEOL) at 400 and 100 MHz, respectively. Melting points were taken in open capillaries in a Mel-Temp apparatus and are uncorrected. EIMS spectra were obtained with a JEOL JMS-AS505 WA mass spectrometer.

Isolation of Geniposide 1 and Genipin 2. Geniposide of *G. jasminoides* was isolated by the methods of Endo and Taguchi (*4*) and Lee et al. (*5*) with minor modifications. Gardenia fruits (100 g) were ground and extracted with 0.5 L of $CHCl_3$ three times to remove lipid components. The dried residue was extracted with 0.5 L of CH_3OH three times at room temperature for 3 h with stirring. The combined extracts were concentrated to a small volume, dissolved into water, and applied to charcoal. The concentrates in charcoal were eluted with water, followed by 10% aqueous ethanol, and finally with methanol. The methanol fractions were applied to a silica gel column and eluted with a $CHCl_3$/CH_3OH mixture (7:3) and then crystallized in acetone to give geniposide (1.95 g). Mp, 162−164 °C (163−164 °C, (*4*)), UV (CH_3OH) λ_{max} 237 nm, EIMS, *m*/*z* 388. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.45 (s, H-3), 5.67 (br. s, H-7), 5.11 (d, J = 6.8 Hz, H-1), 5.02 (d, J = 5.4 Hz, G2-OH), 4.96 (d, J = 5.1 Hz, G3-OH), 4.92 (d, J = 5.1 Hz, G4-OH), 4.72 (t, J = 5.4 Hz, 10-OH), 4.52 (d, J = 7.8 Hz, H-G1), 4.45 (t, J = 5.8 Hz, G6-OH), 4.12 (br. d, J = 15.0 Hz, H-10), 3.96 (br. d, J = 15.0 Hz, H-10), 3.64 (m, H-G6), 3.63 (s, $-OCH_3$), 3.41 (m, H-G6), 3.16 (m, H-G3), 3.11 (m, H-G5), 3.05 (m, H-G4), 3.05 (m, H-5), 2.97 (m, H-G2), 2.67 (m, H-6), 2.63 (m, H-9), 2.03 (m, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.9 ($-CO_2-$), 151.6 (C-3), 144.1 (C-8), 125.5 (C-7), 110.9 (C-4), 98.6 (C-G1), 95.7 (C-1), 77.2 (C-G5), 76.6 (C-G3), 73.3 (C-G2), 70.0 (C-G4), 61.0 (C-G6), 59.3 (C-10), 51.0 ($-OCH_3$), 45.9 (C-9), 38.0 (C-6), 34.4 (C-5).

Geniposide (1.0 g) and β-glucosidase (5 mg) were added into 50 mL of acetate buffer (pH 5.0) at 37 °C and stirred for 5 h. The resulting aglycon was extracted with ether three times. The combined extracts were treated with sodium sulfate followed by filtration and concentration in vacuo. The concentrates were crystallized in ether to yield 0.5 g of genipin (*4*−*7*). Mp, 118−120 °C (120−121 °C, (*7*)), UV (CH_3OH) λ_{max} 240 nm, EIMS, *m*/*z* 226. ^{1}H NMR ($CDCl_3$) δ: 7.53 (s, H-3), 5.88 (s, H-7), 4.82 (d, J = 8.5 Hz, H-1), 4.35 (d, J = 13.2 Hz, H-10), 4.29 (d, J = 13.2 Hz, H-10), 3.74 (s, $-OCH_3$), 3.22 (ddd, J = 9.5, 8.5, 8.5 Hz, H-5), 2.89 (ddt, J = 16.8, 8.5, 1.4 Hz, H-6), 2.54 (ddd, J = 8.5, 8.5, 1.5 Hz, H-9), 2.07 (ddt, J = 16.8, 9.5, 1.8 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.9 ($-CO_2-$), 152.4 (C-3), 142.1 (C-8), 130.9 (C-7), 110.8 (C-4), 96.3 (C-1), 61.3 (C-10), 51.3 ($-OCH_3$), 48.2 (C-9), 39.0 (C-6), 36.7 (C-5).

Formation of Genipin to Gardenia Blue Pigments. Genipin (10 mg) and amino acids (glycine, lysine, or phenylalanine, 0.44 mmol) were added respectively into 2 mL of 100 mM phosphate buffer (pH 7.0) at 70 °C and stirred for 5 h. The three reaction vessels containing blue pigments were wrapped with aluminum foil and stored in the refrigerator for further use.

Effects of pH, Temperature, and Light on Gardenia Blue Pigments. Aliquots of the refrigerated blue pigments (10 μl) were added into 0.99 mL of buffer solutions (pH 5.0, 100 mM acetate buffer; pH 7.0, 100 mM phosphate buffer; pH 9.0, 100 mM CHES buffer). Thermal stabilities of the blue pigments in the pH range of 5.0−9.0 were measured with scanning intervals of 30 min at temperatures of 60−90 °C. Light stabilities of the blue pigments at pHs within the range of 5.0−9.0 were also tested at light intensities of 5000−20 000 lux (light source, metal halide lamp).

Sample Preparation and Reaction Condition. Blue pigments transformed from genipin and an amino acid were dissolved in buffer solution. The pigment concentration was adjusted to give an initial absorbance between 0.7 and 0.8 at $\lambda_{vis,max}$. Degradation reactions were carried out in 1.0-mL quartz spectrophotometer cuvettes. The prepared samples were degraded at 60, 70, 80, and 90 °C. Duplicate tests of thermal degradation reactions were performed. Photodegradation reactions of the blue pigments at 4 °C were carried out in a phytotron (Vision Co.) with different light intensities of 5000−20 000 lux. Light intensities were adjusted with a lux meter and sample cuvettes were placed on it. Thermal degradation and photodegradation of the blue pigments were monitored spectrophotometrically at 580 nm.

RESULTS AND DISCUSSION

Geniposide **1** obtained from gardenia fruits was hydrolyzed with β-glucosidase and the resulting genipin **2** was transformed to the blue pigments by the reactions with amino acids. Our previous work (*5*) with blue pigments formation indicated that the optimum pH for pigment formation was 7.0 and the best choice of the amino acids was glycine, lysine, or phenylalanine. UV/vis spectra for the formation of the blue pigments in phosphate buffer (pH 7.0) with scanning intervals of 5 min showed that λ_{max} at 240 nm of genipin disappeared rapidly when genipin was treated with amino acids, and λ_{max} at about 290 nm of intermediates peak started to appear, and finally λ_{max} at about 570−600 nm produced blue pigment polymers (*5*). Blue pigment polymers contained many blue components (*3*). Absorption maxima of the blue pigments formed from genipin with glycine, lysine, and phenylalanine were 580, 583, and 589 nm, respectively.

The stability of pigments is affected by many factors such as heat, light, and pH (*8*). Thermal degradation reactions of gardenia blue pigments were carried out at pH of 5.0 (100 mM acetate buffer), 7.0 (100 mM phosphate buffer), and 9.0 (100 mM CHES buffer) at different temperatures within the range of 60−90 °C. The degree of degradation was determined by measuring absorbance changes at 580 nm. When the degree of degradation was plotted on a semilogarithmic scale, the plots showed a curved line, indicating that the reaction did not follow simple first-order kinetics. Table 1 summarizes thermal stabilities of the blue pigments formed from genipin with glycine, lysine, and phenylalanine at pH levels of 5.0, 7.0, and 9.0 and at temperatures of 60, 70, 80, and 90 °C. As shown in Table 1, the stability of the blue pigments formed from genipin with glycine was more stable in the alkaline condition than in acidic or neutral conditions. For example, the percents of remaining blue pigments after 10 h at 60 °C under the conditions of pH 5.0, 7.0, and 9.0 were 97%, 99%, and 105%, respectively. It seems likely that more blue pigments could be formed from monomer or dimer intermediates at alkaline pH. Similar, but more dramatic, results occurred in the case of the blue pigments formed from genipin and lysine. The percent of remaining blue pigments after 10 h at 60 °C under the conditions of pH 5.0, 7.0, and 9.0 were 104%, 102%, and 110%, respectively. It is likely that the extra primary amino group of lysine plays a crucial role for the formation of the blue pigments. The percent of remaining blue pigments formed from genipin and phenylalanine after 10 h at 60 °C under the conditions of pH 5.0, 7.0, and 9.0 were 95%, 99%, and 100%, respectively, indicating that these blue pigments were also heat stable components.

Fujikawa et al. (*3*) reported that the ratios of the remaining absorbances of the gardenia blue pigments after two weeks at 40 °C in the dark under different pHs (pH 3−8) were 65−80%. These values are similar to the present data even though reaction conditions are different. Blue color of phycocyanin (*3*), obtained from blue green algae, disappeared completely after two weeks at 40 °C in the dark at pH 3, 4, and 8 in aqueous

Table 1. Percent Remaining Blue Pigments (A_{580}) after Different Times at Various pH Levels and Temperatures

		pH 5.0				pH 7.0				pH 9.0			
		60	70	80	90 °C	60	70	80	90 °C	60	70	80	90°C
Gly	0 h	100	100	100	100	100	100	100	100	100	100	100	100
	2 h	99	94	90	89	101	99	98	96	102	102	102	101
	4 h	98	92	87	85	101	98	97	93	103	103	103	101
	6 h	97	92	84	82	101	97	94	90	104	104	102	98
	8 h	97	92	83	80	101	96	94	88	104	103	99	94
	10 h	97	90	81	78	99	94	94	85	105	103	99	93
Lys	0 h	100	100	100	100	100	100	100	100	100	100	100	100
	2 h	101	99	100	87	102	101	99	98	103	103	105	105
	4 h	102	99	98	83	101	101	98	94	105	106	105	105
	6 h	102	99	98	81	101	102	97	91	107	108	105	103
	8 h	103	100	95	79	101	102	95	89	109	109	103	101
	10 h	104	100	95	79	102	101	94	85	110	108	102	102
Phe	0 h	100	100	100	100	100	100	100	100	100	100	100	100
	2 h	98	94	91	89	100	99	97	96	100	99	99	98
	4 h	97	92	88	85	100	99	96	95	100	98	100	100
	6 h	96	92	86	83	99	98	95	91	99	98	102	98
	8 h	95	92	83	79	99	98	92	90	99	99	99	98
	10 h	95	88	83	79	99	96	92	87	100	97	99	96

Table 2. Percent Remaining Blue Pigments (A_{580}) after Different Times at Various pH Levels and Light Intensities

		pH 5.0				pH 7.0				pH 9.0			
		0	5000	10 000	20 000 lux	0	5000	10 000	20 000 lux	0	5000	10 000	20 000 lux
Gly	0 h	100	100	100	100	100	100	100	100	100	100	100	100
	1 h	100	96	96	94	100	97	97	95	100	98	97	95
	2 h	99	95	95	91	99	97	95	92	100	97	95	92
	5 h	99	93	91	84	99	94	92	85	100	95	90	82
	10 h	98	90	86	76	98	91	86	76	100	90	83	70
Lys	0 h	100	100	100	100	100	100	100	100	100	100	100	100
	1 h	99	96	96	95	100	97	96	96	99	95	95	93
	2 h	98	95	95	92	99	95	94	93	98	94	92	89
	5 h	97	93	91	86	99	94	91	87	97	90	87	80
	10 h	96	91	87	78	97	91	87	80	96	87	79	67
Phe	0 h	100	100	100	100	100	100	100	100	100	100	100	100
	1 h	99	95	91	90	99	98	96	91	99	96	94	91
	2 h	98	93	87	84	99	96	92	85	99	95	91	85
	5 h	97	91	79	69	98	95	86	71	98	89	83	69
	10 h	96	83	69	53	97	85	74	56	96	79	70	51

solutions, showing that the gardenia blue pigments have much better stabilities than phycocyanin for use as value-added food colorants.

The photodegradation reactions of blue pigments were carried out under different light intensities of 5000−20 000 lux at pH 5.0, 7.0, and 9.0. These reactions were carried out at 4 °C to study the effect of light for usage in cold beverages and ices. Table 2 summarizes light stability of the blue pigments (formed from genipin with glycine, lysine, or phenylalanine) with and without light for 10 h in solutions of pH 5.0, 7.0, and 9.0. As summarized in Table 2, pH effect was not significant. For example, the percent remaining absorbances of the blue pigments from genipin with glycine after 10 h with light intensity of 5000 lux under the conditions of pH 5.0, 7.0, and 9.0 were 90%, 91%, and 90%, respectively. The percent remaining absorbances of the blue pigments from genipin with glycine after 10 h with light intensity of 20 000 lux under the conditions of pH 5.0, 7.0, and 9.0 were 76%, 76%, 70%, respectively. The blue pigments formed from phenylalanine with the light intensity of 20 000 lux at pH 9.0 after 10 h showed 51% of remaining absorbance, indicating that this blue dye was the least stable pigment when exposed to intense light. In conclusion, gardenia blue pigments were very stable with regard to pH, temperature, and light conditions and may have potential for use as value-added colorants for foods.

LITERATURE CITED

(1) Tang, W.; Eisenbrand, G. Chinese Drugs of Plant Origin; Springer-Verlag: Berlin, 1992; pp 539−543.

(2) Hendry, G. A. F.; Houghton, J. D. Natural Food Colorants, 2nd ed.; Chapman & Hall: London, 1996; pp 318−321.

(3) Fujikawa, S.; Fukui, Y.; Koga, K.; Kumada, J. Brilliant Skyblue Pigment Formation from Gardenia Fruits. *J. Ferment. Technol.* **1987**, *65*, 419−424.

(4) Endo, T.; Taguchi, H. The Constituents of *Gardenia jasminoides*: Geniposide and Genipin-gentiobioside. *Chem. Pharm. Bull.* **1973**, *21*, 2684−2688.

(5) Lee, J.-Y.; Hahn, T.-R.; Paik, Y.-S. Physicochemical Characteristics for the Transformation of Blue Pigments from Genipin of *Gardenia jasminoides* with Amino Acids. *Agric. Chem. Biotechnol.* **1998**, *41*, 399−404.

(6) Drewes, S. E.; Kayonga, L. Iridoid Molluscicidal Compounds from *Apodytes dimidiata*. *J. Nat. Prod.* **1996**, *59*, 1169−1170.

(7) Djerassi, C.; Nakano, T.; James, A. N.; Zalkow, L. H.; Eisenbraun, E. J.; Shoolery, J. N. Terpenoids XLVII. The Structure of Genipin. *J. Org. Chem.* **1961**, *26*, 1192−1206.

(8) Attoe, E. L.; von Elbe, J. H. Photochemical Degradation of Betanine and Selected Anthocyanines. *J. Food Sci.* **1981**, *46*, 1934−1937.

Received for review August 8, 2000. Revised manuscript received October 23, 2000. Accepted October 30, 2000. This work was supported by a grant from the National Science and Engineering Foundation (2000) through the Plant Metabolism Research Center.

JF000978F