Dyeing/Cross-Linking Property of Natural Iridoids to Protein Fibers**

Part II. Color-forming rules and mechanism of methylamine and protein fibers dyed by four natural iridoids

by

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Abstract

Color-forming rules and mechanism of methylamine and protein fibers dyed by four iridoids, genipin, loganin aglycone, oleuropein aglycone and E-6-*O*-methoxycinnamoyl scandoside methyl ester aglycone were researched. The results showed that, before the formation of final pigments, intermediates were formed. A continuous color change following classical color matching principles was observed when the pigments formed by genipin and oleuropein aglycone reacted individually with methylamine were mixed. However, when genipin and oleuropein aglycone reacted simultaneously with methylamine, the situation was different. The results of single dyeing and combination dyeing of protein fibers showed that color of the dyed protein was the same as that formed by each iridoid with methylamine. This means that the structure of iridoid is the dominant factor for the color-forming process. The classical color-matching principle would be observed when two iridoids were applied to dye protein fibers separately. However, during simultaneous combination dyeing, the classical color-matching principle was not observed.

Resumen

Los mecanismos así como las reglas que rigen los colores producidos por tintura de fibras proteínicas y metil amina, teñidas por cuatro irridoides: genipín, loganin aglicon, oleuropin aglicon y E-6-O-metoxicinamoil scandocide metil ester aglicona fueron investigados. Los resultados indicaron, que antes de la formación de los pigmentos finales, productos intermedios fueron formados. Un cambio continuo del color de acuerdo con los principios convencionales, se observaron cuando los pigmentos formados por genipin y oleuropin aglicon reaccionaron individualmente con metil amina, y luego fueron mezclados. La situación, sin embargo, es diferente cuando genipin y oleuropin aglicon [juntos] reaccionaron simultáneamente con metil amina. Los resultados del teñido único y los combinados de fibras proteínicas, demuestran que el color del teñido proteínico fue el mismo al formado por cada irridoide con metil amina. Esto significa que la estructura del irridoide es el factor dominante en el proceso de la formación del color. El caso clásico del principio de obtención del tono requerido se daría cuando se aplicaran separadamente cada irridoide a la fibra colagénica. Es decir que en el caso de teñidos combinados múltiples, las reglas de los principios clásicos para obtener el deseado tono no son aplicables.

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INTRODUCTION

The reaction of genipin (GP), an iridoid aglycone prepared from *Gardenia jasminoides Ellis,* with primary amines, such as amino acids, can produce edible blue pigments. This principle has been applied by the food industry of East Asia, including Korea and Japan.[1] Previous research also reported that, in addition to GP, three other novel iridoids could dye protein fibers to black or yellow.[2-3] These results suggested the possibility of iridoid compounds as potential "natural reactive dyes" for materials containing primary amino-groups, such as leather, wool, silk, etc. More than 1400 iridoid compounds have been isolated from nature at present and their contents in some plants are fairly abundant.[4] In the present study, four iridoid compounds, genipin (GP), loganin aglucon (LA), oleuropein aglucon (OA) and E-6-*O*-methoxycinnamoyl scandoside methyl ester aglycone (EA), which were prepared from *Gardenia jasminoides Ellis, Lonicera japonica* Thunb, *Olea europaea Linn* and *Hedyotis diffusa* (Willd) Roxb respectively, were applied to dye methylamine (MA), the simplest primary amine, and to protein fibers such as hide powder, silk and white hair. By single dyeing reactions and combination dyeing reactions, the changes in color during different dyeing periods were detected via UV-vis spectrophotometery. Mechanisms for color-forming reactions of iridoids with methylamine (MA) and protein fibers are proposed.

EXPERIMENTAL

Materials

The four iridoid aglycones (Figure 1), GP, LA, OA and EA were prepared from *Gardenia jasminoides Ellis, Lonicera japonica* Thunb, *Olea europaea Linn* and *Hedyotis diffusa* (Willd) Roxb respectively in our laboratory. Their structures were identified by ESI-MS, ^{1}H NMR, ^{13}C NMR and compared with chemical evidence in figures from related reference. Bovine hide powder was provided by the National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University. Individual silk fibers and white hair were purchased from the market. β- glucosidase was the product of Sigma and provided by Shanghai Bio-chemical Co. Ltd. Other chemicals are analytical grade from Chengdu Chemical agents Co. Ltd. All experiments were performed at least three times to evaluate reproducibility.

Reaction of MA with Iridoid Aglycones[5]

Reaction of individual iridoid aglycones with MA. Each iridoid aglycone was dissolved in 50% EtOH-H_2O (v/v) to obtain 20mg/mL solution. One mL of this solution was added to 3 mL of PBS (pH=7.5) and stirred in a 20 mL tube at 35°C for 15 min, then 20 mg CH_3NH_2.HCl was added, the temperature was increased to 50 °C and reacted for 6 h. UV-vis spectra of the reaction solutions (colorant) were detected every 10 min.

UV-vis spectra of the colorant mixture from GP and OA. The final colorants produced by the reactions of GP and OA with MA as described above, were mixed at different ratios (v/v), and the UV-vis spectrum of each mixture was detected.

Simultaneous reaction of GP and OA with MA. Different ratios (w/w) of GP and OA were dissolved in 50% (v/v) EtOH-H_2O, keeping the total concentration of iridoids at 20mg/mL. One mL of these solutions was added to 3 mL of PBS (pH=7.5) and stirred in a 20 ml tube at 35°C for 15 min, then 20 mg CH_3NH_2.HCl was added and the temperature increased to 50°C. UV-vis spectra of the reaction solutions (colorants) were detected after 6 h.

Dyeing Reaction of Four Iridoids with Protein Fibers

Reaction of individual iridoids with protein fibers. *S*ilk and white hair were soaked in 10% Na_2CO_3 at 40°C for 30 min, then washed with common shampoo, rinsed with water, and dried at ambient temperature. Each protein fiber (hide powder, silk and white hair) was first hydrated with 1500% (w/w) distilled water in a flask overnight at ambient temperature, filtered and resuspended in phosphate buffered saline (PBS) at pH =7.5-8.0. The dyeing processes were evaluated using 0.5 g protein fiber in 7.5 mL buffer in a thermostated shaking bath. Reaction parameters were the optimized conditions described in the previous work [1]: The dosage for each iridoid was 5% (w/w) (based on the dry weight of protein fiber), T=35°C, t = 6h and the pH was maintained at 7.5-8.0 during the process. At the completion of dyeing, the resulting mixture was filtered and washed with distilled water until the effluent was clear. The dyed protein fiber was then dried at ambient temperature.

Dyeing of hide powder first with GP and then with OA. Hide powder, as an example of protein fibers, was dyed with 2.5%, 1.6%, 1.3%, 1.0% of GP (w/w, based on the dry weight of the hide powder) first, to produce a blue color, then the blue hide powder was redyed with 2.5%, 3.4%, 3.7%, 4.0% of OA. The dyeing process for each step was the same as described above, and the total amount of iridoid aglycones was kept at 5% of the dry weight of the hide powder.

Dyeing of hide powder with GP and OA simultaneously. Hide powder, as an example of protein fibers, was dyed with GP and OA in the same solution. The ratios of GP/OA were 1/1, 1/2, 1/3, 1/4, 1/5 (w/w); and the total dosage of iridoids was kept at 5% (w/w, based on the dry weight of hide powder). The dyeing processes were as the same as described above.

RESULTS AND DISCUSSIONS

Iridoid treatment of MA

Reaction of individual iridoids with MA. The previous research in this laboratory has confirmed that, the four iridoid aglycones were stable and did not polymerize in PBS, or only

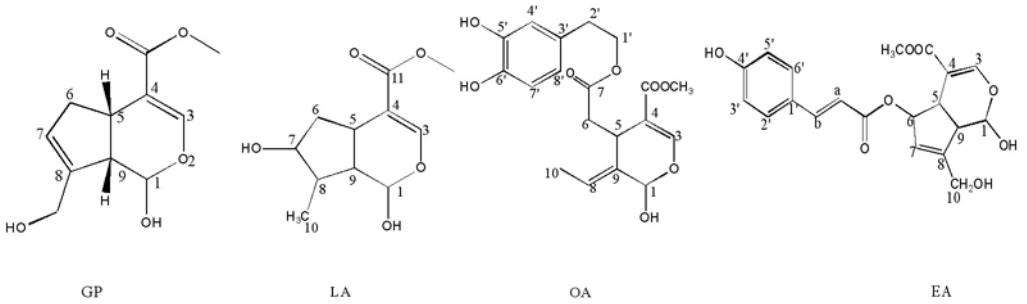

Figure 1. Chemical structures of the four iridoid aglycones

limited polymerization occurred, i. e., iridoid aglycones were reacted with protein fibers in the form of monomer.[6] To review the previous work by J. E. Park et al [1] and R. Touyama et al,[7,8] the mechanism for the formation of blue pigments when GP is reacted with MA could be summarized as follows, whether under an inert atmosphere or under an oxygen atmosphere, before the formation of the final blue pigment, intermediates (1 through 4, as illustrated in figure 2) would be formed. While intermediate 1 was colorless, intermediates 2 through 4 were brownish-red. The final blue pigment is considered to be a mixture of polymers consisting on average of 40-44 monomer units such as intermediate 1 (Figure 2).

In addition to GP, our experiments with the other three iridoids confirmed the previous work described by R. Touyama and J. E. Park et al. For example, during the reaction of EA with MA (Figure 3), the characteristic EA peak at 239 nm disappeared within 10 min, after which the longer wavelength absorbance peaks at 291 nm and 377 nm appeared. At 70 min, the peak at 377 nm disappeared, the peak at 291 nm became stronger and a new peak at 580 nm appeared. After that, the peaks at 291nm and 580 nm increased continuously until the final mauve pigment formed. Similar phenomena occurred during the processes of the other three iridoids reacted with MA, and indicated that complicated intermediates would be formed also. The final colors of pigments formed by each iridoid compound with MA are shown in Table 1.

UV-vis spectral analysis of the mixture colorants from GP and OA reacted with MA. From the results shown in Table 2, we could see that, with increasing dosage of yellow pigment, the mixture's color changed from blue-green, to light-green, to lemon yellow. From the UV spectra for the mixture (Figure 4), we could see that, the peaks at 290 nm and 580 nm belonged to the blue pigment of GP and the peak at 341 nm belonged to the yellow pigment of OA. These results indicated that the classical color-matching principles were observed when mixing the pigments formed by GP and OA reacted with MA respectively. The UV-vis spectrum of the mixture was the superposition of those of the two pigments.

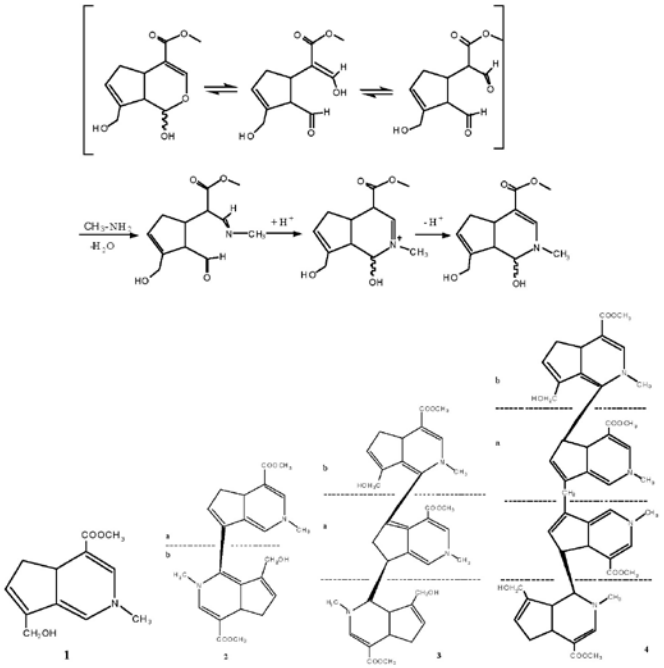

Figure 2. Intermediates during the formation of blue pigments by GP reacting with MA

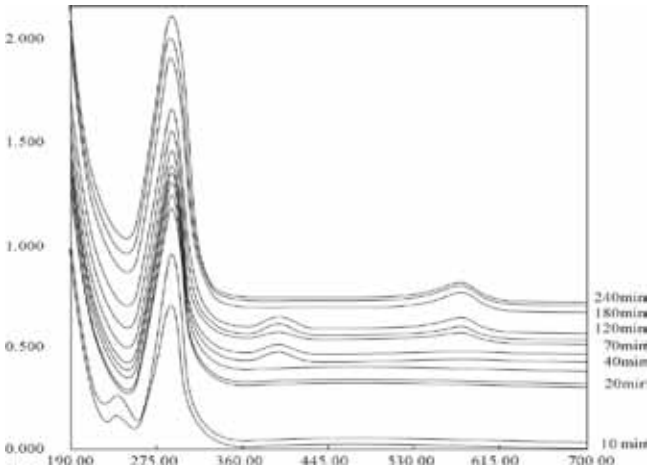

Figure 3. UV spectra for EA reacted with methylamine at different time (T=35°C, pH=7.5)

TABLE 1
Color of the pigments from iridoids reacted with MA

Iridoid	GP	LA	OA	EA
Color	dark blue	Yellow-brown	yellow	mauve

TABLE 2
Colors of the mixtures of blue pigment formed by GP and yellow pigment formed by OA

No.	Blue pigment (μL)	Yellow pigment (μL)	Color of mixture
J-1	20	20	Blue-green
J-2	20	35	Light-green
J-3	20	50	Lemon-yellow

TABLE 3
Color of pigments formed by GP and OA reacting with MA simultaneously

No.	GP (mg)	OA (mg)	Color
L-1	10.0	10.0	Light-yellow
L-2	6.6	13.4	Light-yellow
L-3	5.0	15.0	yellow

UV-vis spectral analysis for GP and OA reacted simultaneously with MA. Color and UV-vis spectra of the pigments from GP and OA reacted with MA in one solution are illustrated in Table 3 and Figure 5(a). With the decreasing GP/OA ratio (1/1, 1/2, 1/3), the color of the pigments changed from light yellow to yellow, and two new characteristic peaks at 280nm and 360nm appeared. These peaks differed from those produced when GP and OA were reacted with MA separately. These results indicated that, new chromogen characteristic of polymerized intermediates formed by GP and OA reacting with MA, might be produced (Figure 5 (b)). During this process, classical color-matching principles would not be observed.

Dyeing reaction of four iridoids with protein fibers

Dyeing of protein fibers with individual iridoids. In Table 4 we could see that the color formed by each iridoid reacting with each protein fiber was the same as that formed by the same iridoid reacting with MA (Table 1). These results indicated that, the chemical structure of the iridoid was the dominant factor for the resultant color. The structure of the compounds containing primary amines, such as MA or protein fibers had little or no - effect on the color. The presumed mechanism for the cross-linking and polymerization by GP reacted with hide powder is illustrated in Figure 6.

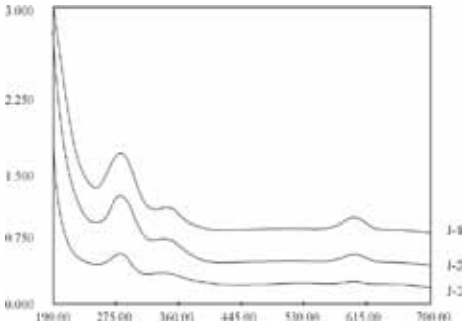

Figure 4. UV spectra for the mixture of blue pigment and yellow pigment

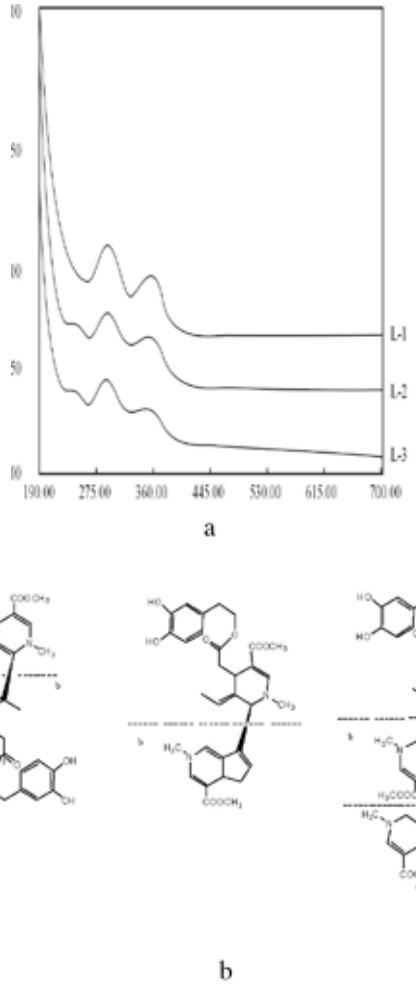

Figure 5. UV spectra and presumed mechanism for colorant of GP and OA reacting with MA in one solution

TABLE 4
Colors formed by dyeing process of iridoids to protein fibers

	Hide powder	silk	White hair
GP	dark blue	blue	blue
LA	yellow	yellow	yellow
OA	light yellow	light yellow	light yellow
EA	mauve	mauve	mauve

TABLE 5
Color of hide powder dyeing by GP and OA separately

No.	GP (%)	OA (%)	Color
W-1	2.5	2.5	blue
W-2	1.6	3.4	light blue
W-3	1.3	3.7	green
W-4	1.0	4.0	lemon yellow

TABLE 6
Color of hide powder dyed by GP and OA simultaneously

No.	GP (%)	OA (%)	Color
P-1	2.5	2.5	dark blue
P-2	1.6	3.4	light blue
P-3	1.3	3.7	green
P-4	1.0	4.0	lemon yellow
P-5	0.8	4.2	deep yellow

Combination dyeing of hide powder with GP and OA. The colors formed when GP and OA reacted with hide powder, step-by-step, are shown in Table 5. The results indicated that, with decreasing GP/OA ratio (1/1, 1/2, 1/3, 1/4), color of the dyed hide powder changed from blue, to green, to lemon yellow, therefore, the classical color-matching principle was observed. However, during combination dyeing of hide powder by GP and OA simultaneously, the classical color-matching principle was not observed (Table 6). The reason might be that, new chromophores with different iridoid backbones coupled in the same or different protein molecular chains, would be formed (Figure 7). This is in contrast with the step-by-step dyeing process using each iridoid compound separately (Figure 6).

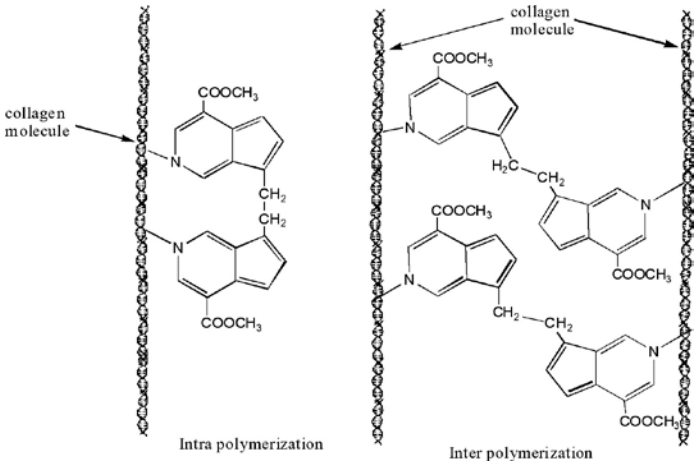

Figure 6. Presumed mechanism for the crosslinking and polymerization by GP reacted with hide powder

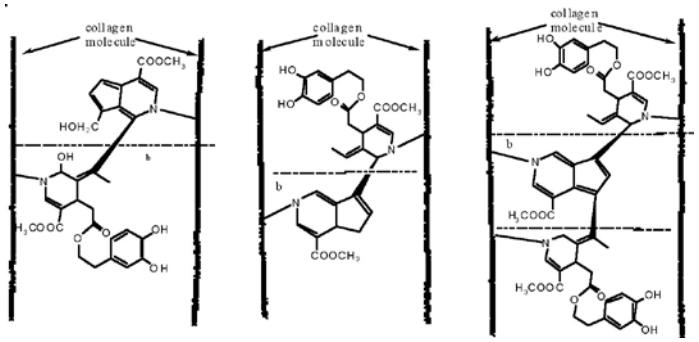

Figure 7. Presumed mechanism for the crosslinking and polymerization by GP and OA dyeing hide powder simultaneously

CONCLUSIONS

The following are the conclusions derived from this present work;

- The pigment-forming reaction of iridiods with MA is a considerably complicated process. Before producing the final pigments, several intermediates would be formed and accompanied by a continuous color change; different iridoids would form pigments with different colors. The reason might be that, chromogens with complicated molecular structures were formed during the process, and the side groups combining with the backbones of different iridoids acted as auxochromes. Different auxochromes would form pigments with different colors.

- The classical color-matching principles would be observed when mixing the pigments formed by GP and OA reacted with MA respectively. The UV-vis spectrum of the mixture was the superposition of those of the two pigments; however, when GP, OA and MA reacted in one solution, new chromophores coupled by the intermediates formed by the two iridoids reacted with MA, would be

produced. During this process, the classical color-matching principle would not be observed.

- The resultant's color formed by each iridoid reacting with each protein fiber was the same as that formed by each iridoid reacting with MA. This result confirmed that, the chemical structure of the iridoid was the dominant factor for the resultant color, and there was little or no relationship with the structures of the compounds containing primary amines, such as MA and protein fibers.

- During the combination dyeing process of hide powder by GP and OA, the classical color-matching principle would be observed when they were applied step-by-step. However, classical color-matching principle was not observed when they were applied to hide powder simultaneously. The presumed mechanism might be that, new chromogens with different iridoid backbones coupled in the same or different protein molecular chains, would be formed.

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