Dyeing Property of Four Natural Iridoids to Methylamine and Protein Fibers

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Abstract: Dyeing reactions of four iridoids, genipin(GP), loganin aglucon(LA), oleuropein aglucon(OA) and E-6-*O*-methoxycinnamoyl scandoside methyl este aglycone(EA) with methylamine (MA) and protein fibers such as hide powder, silk and white hair, were researched in this paper. The color changing tendency in different dyeing period were detected by UV-vis, the color-forming mechanism were deduced. The results showed that: (1) The pigment-forming reaction of iridiods with MA was a considerably complicated process. Before the formation of the final pigment, a lot of intermediates would be formed and accompanied with a continuous color changing, and different iridoids would form pigments with different color. (2) The result of combination dyeing experiments indicated that the classical color-matching principles would be observed when mixing the pigments formed by GP or OA reacted with MA respectively; however, when GP, OA and MA reacted in one solution, the situation would be different. (3) The results of single dyeing and combined dyeing experiments to protein fibers showed that the resultant's color formed by each iridoid was the same as that formed by each iridoid reacting with MA. This result indicated that the chemical structure of iridoids was the dominate factor for the resultant's color. The classical color-matching principle would be observed when two kinds of iridoids were applied to dye protein fibers step by step separately. However, during the combination dyeing process by two iridoids in one step, classical color-matching principle was not observed.

Key words: iridoid; methylamine; protein fibers; natural reactive dye

1 Introduction

The reaction of genipin (GP), an iridoid aglycone prepared from *Gardenia jasminoides Ellis,* with primary amines, such as amino acids, could produce edible blue pigments. This principle has been applied by the food industry of East Asia, including Korea and Japan [1]. The previous research work also reported that，besides GP, three other novel iridoids could dye protein fibers to black or yellow[2-3]. These results have suggested the possibility of iridoid compounds as potential "natural reactive dyes" special for materials containing primary aminol-group, such as leather, wool, silk, etc. More than 1400 of 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 este aglycone(EA), which were prepared from *Gardenia jasminoides Ellis, Lonicera japonica* Thunb, *Olea europare Linn* and *Hedyotis diffusa* (Willd) Roxb respectively, were applied to react with methylamine(MA), the simplest primary amines, and protein fibers such as hide powder, silk and white hair. By single dyeing reaction and combination dyeing reaction, the color changing tendency in different dyeing period were detected via UV-vis, the color-forming mechanism of iridoids with methylamine (MA) or protein fibers were deduced.

2 Experimental

2.1 Materials

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The four iridoid aglycones (Figure 1), GP, LA, OA and EA were prepared from *Gardenia jasminoides Ellis, Lonicera japonica* Thunb, *Olea europare Linn* and *Hedyotis diffusa* (Willd) Roxb respectively in our laboratory, their chemical structures were identified by ESI-MS, ^{1}H NMR, ^{13}C NMR and comparing their chemical evidence with figures from related reference[5]. Bovine hide powder was provided by the Key Laboratory for Leather Chemistry & Engineering of Education Ministry, Sichuan University. Silk and white hair were common samples. β- glucosidase was the product of Sima and provided by Shanghai Bio-chemical Co. Ltd. Other chemicals are analytical class from Chengdu Chemical agents Co. Ltd. All experiments were performed at least twice to evaluate reproducibility.

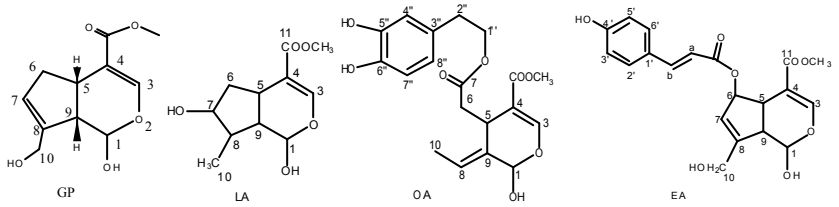


Fig. 1 Chemical structures of the four iridoid aglycones

2.2 Reaction of MA with iridoid aglycones[5]

2.2.1 Each iridoid aglycone reacts alone 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 put in 3 ml of PBS (pH=7.5) and stirred in a 20 ml tube at 35℃ for 15 min, then 20 mg CH_3NH_2.HCl were added and increased temperature to 50℃ and reacted for 6 h. UV-vis spectra of the reaction solution (colorant) was detected every 10 min.

2.2.2 UV-vis spectra of the mixture colorant from GP and OA

Mix the final colorants produced by GP and OA with MA in 2.2.1 at different ratios, UV-vis spectra of each mixture was detected.

2.2.3 GP and OA reacted with MA in one solution

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

2.3 Dyeing reaction of four iridoids with protein fibers

2.3.1 Dyeing reaction of each iridoid alone with protein fibers

Pretreatment of samples: Silk and white hair were soaked in 10% Na_2CO_3 at 40°C for 30 min, then they were washed with common shampoo and rinsed with water, dried in ambient temperature.

Each protein fiber (hide powder, silk and white hair) was first hydrated with 1500% 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 in a thermostated shaking bath. Reacting parameters were adopted the optimized conditions 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 maintained the pH at 7.5-8.0 during the whole 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.

2.3.2 Dyeing of GP and OA to hide powder in two steps

Taking hide powder as an example of protein fibers, dyed it with 2.5%, 1.6%, 1.3%, 1.0% of GP (w/w, based on the dry weight of hide powder) first, then dyed the light blue hide powder with 2.5%, 3.4%, 3.7%, 4.0% of OA. The dyeing process for each step were as the same as 2.3.1.

2.3.3 Dyeing reaction of GP and OA with hide powder in one step

Taking hide powder as an example of protein fibers, dyed it 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 kept the total dosage of iridoids at 5% (w/w, based on the dry weight of hide powder). The dyeing process were as the same as 2.3.1.

3 Results and discussion

3.1 Iridoids react with MA

3.1.1 Each iridoid reacts alone with MA

To review the previous work by J. E. Park et al [1] and R. Touyama et al [6, 7], the forming mechanism of blue pigments from GP reacted with MA could be summarized as that, whether under an inert atmosphere or under an oxygen atmosphere, before the formation of the final blue pigment, intermediates such as 1, 2, 3, 4,……, would be formed, while 1 was colorless but 2, 3, 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 1.

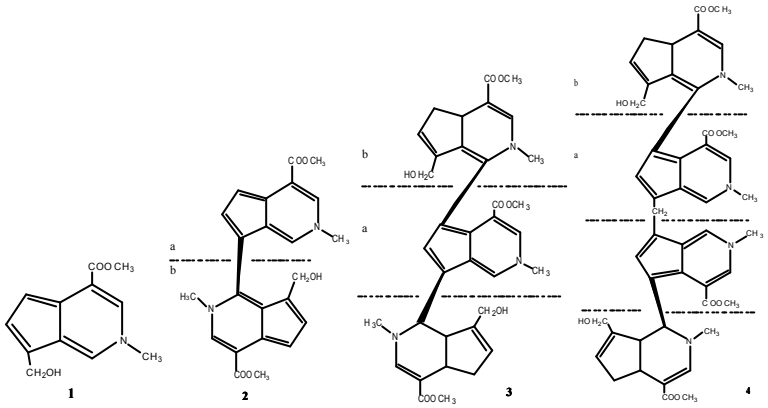


Fig. 2 Intermediates during the formation of blue pigments from GP reacting with MA

Besides GP, our experiments with the other three iridoids confirmed the previous work by R. Touyama and J. E. Park et al. For example, during the reaction of EA with MA (Figure 3, (d)), the characteristic peak of EA at 239 nm disappeared within 10 min, while the absorb peaks in long-wave region, 291nm and 377 nm would appear. 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. The color and UV-vis spectra for the other three iridoids reacted with MA at different time were illustrated in Figure 3.

Table 1 Colors of the pigments from iridoids reacted with MA

Iridoid	GP	LA	OA	EA
Color	dark blue	light yellow	yellow	mauve

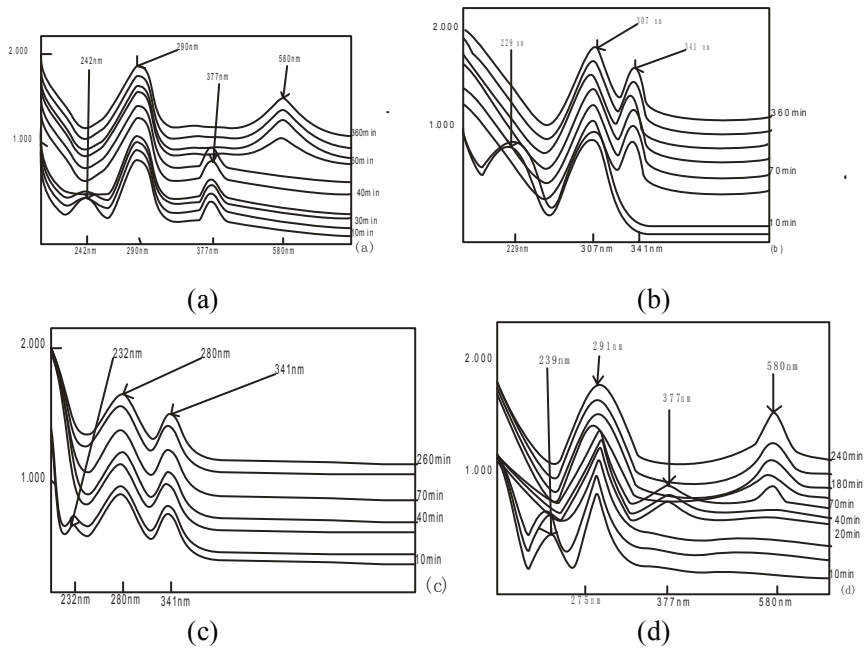


Fig. 3 UV spectra for iridoids reacted with methylamine at different time (a: GP; b: LA; c: OA; d: EA)

3.1.2 UV-vis spectra analysis for the mixture colorants from GP and OA reacted with MA

From the result showed in Table 2, we could see that, with the increasing dosage of yellow pigment, the mixture's color changed from blue-green, 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 were belonged to the blue pigment of GP and the peak at 341 nm was belonged to the yellow pigment of OA. These results indicated that the classical color-matching principles were still 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 that of the two pigments.

Table2 Color of the mixture of blue pigment from GP and yellow pigment from OA

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

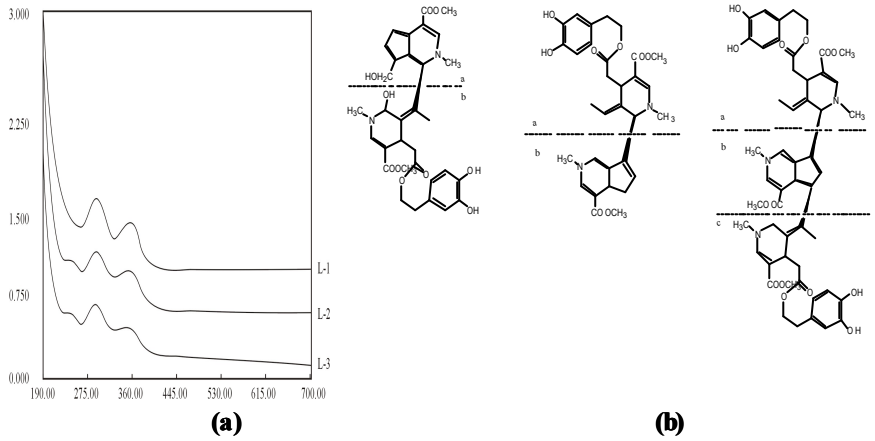
Fig. 4 UV spectra for the mixture of blue pigment and yellow pigment

3.1.3 UV-vis spectra analysis for GP and OA react with MA in one solution

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

Table 3 Color from pigments of GP and OA reacting with MA in one solution

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



(a) (b)

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

3.2 Dyeing reaction of four iridoids with protein fibers

3.2.1 Dyeing of four iridoids with protein fibers

From Table 4 we could see that the color formed by each iridoid reacting with each protein fiber was the same as that formed by each iridoid reacting with MA (Table 1). This result indicated that, the chemical structure of iridoid was the dominate factor for the resultant's color, which had no or less relationship with base (compounds containing primary amines, such as MA or protein fibers). The presumed mechanism for the crosslinking and polymerization by GP reacted with hide powder was illustrated in Figure 6.

Table 4 Color from 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

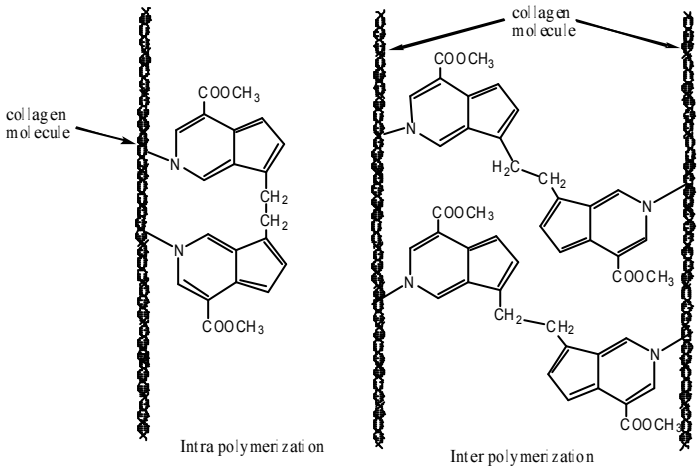


Fig. 6 Presumed mechanism for the crosslinking and polymerization by GP reacted with hide powder

3.2.2 Combination dyeing of GP and OA to hide powder

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

Table 5 Color of step by step dyeing by GP and OA to hide powder

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 in one step

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

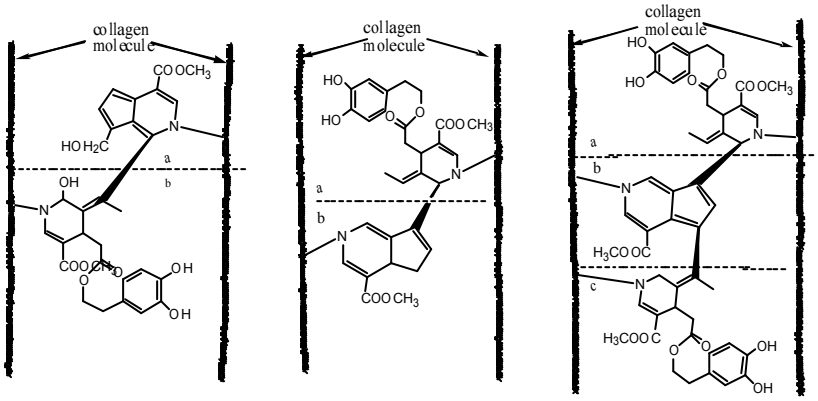


Fig. 7 Presumed mechanism for the crosslinking and polymerization by GP and OA dyeing hide powder with one step

4 Conclusions

(1) The pigment-forming reaction of iridiods with MA was a considerably complicated process. Before the producing of the final pigments, a lot of intermediates would be formed and accompanied with a continuous color changing; different iridoids would form pigments with different color. The reason might be that, chromophares with complicated molecular structure were formed during the process, and the side groups combined with the backbones of different iridoids were acted as auxochrome. Different auxochromes would form pigments with different colors.

(2) 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 that 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, classical color-matching principle would not be observed.

(3)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 iridoid was the dominate factor for the resultant's color, which had no relationship with base (compounds containing primary amines, such as MA and protein fibers).

(4) During the combination dyeing process by GP and OA to hide powder, 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 dye hide powder in one step. The Presumed mechanism might be that, new chromophores with different iridoid backbones coupled in the same or different protein molecular chains, would be formed.

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References

[1] J. E. Park; J. Y. Lee; H. G. Kim; T. R. Hahn; Y. S. Park. J. Agric. Food Chem., 2002, 50: 6511- 6514.

[2] K. Y. Ding; M. M. Taylor; E. M. Brown. Journal of American Leather Chemists Association, 2006, 101(10): 362-367.

[3] P. Lin; J. X. Wang; K. Y. Ding. China Leather, 2008, 37(21):38-42. (Chinese)
[4] J. E. Dong; J. Zhang. Journal of Northwest University of Forestry, 2004，19（3）：131-135.
[5] Peng Lin. Master degree thesis (Southwest University for Nationalities, Chengdu, P.R. China, 2009). (Chinese).
[6] R. Touysma;Y. Takeda; K. Inoue; et al. Chem. Pharm.Bull, 1994, 42(3): 668-673.
[7] R. Touysma; Y. Takeda; K. Inoue; et al. Chem. Pharm.Bull, 1994. 42(8): 1571-1577.