

# COLOR FORMING PROPERTY OF DERIVATIVES AND MODIFICATION PRODUCTS OF NATURAL IRIDOIDS WITH METHYLAMINE

by

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## ABSTRACT

Derivatives and modified products of five natural iridoids (geniposide, gentiopicroside, swertiamarin, loganin and morroniside) were obtained by derivatization or modification. Compounds before and after derivatization or modification were adopted to react with methylamine to investigate their color-forming property. The relationship between the color of the pigments and the structure was analyzed using ultraviolet-visible (UV-vis) spectroscopy. The results indicated that: (1) when the backbone of the iridoids was the same, the position of the modified substituent groups had the greatest effect on the color of the pigments formed. For example, methylgentiopicroside aglycone and methylswertiamarin aglycone have a similar skeleton structure, differing only at positions C-5 and C-6, and the pigments obtained from their reaction with methylamine were also similar i.e. bright yellow. However, geniposidic acid aglycone and genipin, which also have a similar skeleton structure, differing only in the substituent at position C-4, produce pigments of different color upon reaction with methylamine, one being dark blue and the other purple red. (2) In different buffer systems, i.e., phosphate buffered saline (PBS, pH=8.0) and acetate buffer (ACE, pH=5.0), the same iridoid formed pigments with different colors. (3) After the  $\pi$ - $\pi$  conjugation of benzoyl was introduced to the skeleton of loganin, genipin and morronin as a substituent group, the characteristic absorption wavelength of pigments changed. Taking loganin as an example, the characteristic absorption wavelength of pigments changed from 430 nm and 650, to 465 nm and 670 nm when reacted in PBS, and from 560 nm to 610 nm when reacted in ACE. The reason might be that the conjugation system became longer and the molecular structure became more stable.

## INTRODUCTION

Previous work<sup>1,2</sup> indicated that primary-amine-containing materials such as methylamine, Bovine serum albumin (BSA), leather, silk and wool could be dyed by some natural iridoids including genipin, loganin aglycone, and oleuropein aglycone. During the dyeing process, color is derived from the crosslinking reaction between the iridoids and the primary-amine group in the molecular chain of the substrate rather than the iridoids themselves.<sup>1-3</sup> For leather, tanning effects could be obtained because of the crosslinking reaction between collagen molecular chains.<sup>4-6</sup> This kind of color-forming mechanism is not only different from that of traditional natural dyes such as alizarin and indigo, but also from that of synthetic dyes such as acid dyes and reactive dyes. Traditional natural dyes and synthetic dyes have color inherent in them, and in the dyeing process these dyes cover the original color of the substrate by their own color. Most of the natural iridoids, however, are white powders or crystals having no absorption in the visible range. The dyeing mechanism of natural iridoids on primary-amine-containing materials is that a cross-linking reaction occurs between the iridoids and primary amine groups in the molecular chain of the substrate, and a "three in one" composite (substrate-dyestuff-color) is formed.<sup>3,7</sup> That is why iridoids are referred to as "natural reactive dyes".<sup>1,2</sup>

Like natural dyes, the types and quantity of natural iridoids are limited. However, more compounds which have the same backbone as natural iridoids but different substituent groups may be obtained via structural modification. In this way not only would the types and quantity of iridoid-like compounds be increased, but also the color which the modified iridoids formed with primary-amine-containing materials would be

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changed and the color range of natural reactive dyes widened. Our previous work showed that the color formed from the combination of primary-amine-containing materials with iridoids was determined only by the structure of the iridoids and was unrelated to the substrates (primary-amine-containing materials).<sup>1,2</sup> The simplest primary-amine-containing material, methylamine, was chosen as a model of primary-amine-containing materials. An advantage of using methylamine was that soluble pigments could be produced during the color-forming process and their color-forming properties ( $\lambda_{\max}$ ) could be easily measured using UV-Vis spectrophotometry.

The color-forming properties of methylamine with natural iridoids such as genipin, swertiamarin aglycone and methylswertiamarin aglycone had been studied in our previous work.<sup>1,2</sup> In this paper, derivatives of geniposide, swertiamarin and gentiopicroside (Figure 1) were obtained via hydrolysis or replacement of glucose by a methoxy group. Modification products of genipin, loganin and morroniside (Figure 1) were achieved by introducing an additional substituent group to their backbone. The compounds before and after derivatization or modification were reacted with methylamine, so that insoluble pigments would be produced and their color-forming properties could be investigated; the relationship between the color and structure of the compounds was explored.

## EXPERIMENTAL

### Materials

Geniposide, gentiopicroside, swertiamarin were isolated from *Gardenia jasminoides Ellis*, *Gentiana macrophylla Pall*, *Swertia mileensis* respectively;<sup>7</sup> loganin and morroniside were isolated from *Cornus Officinalis*.<sup>8</sup>  $\beta$ -glucosidase was a product of Sigma and provided by Shanghai Bio-chemical Co. Ltd.; TLC plates (GF254) and silica gel (100-200, 200-300 mesh)

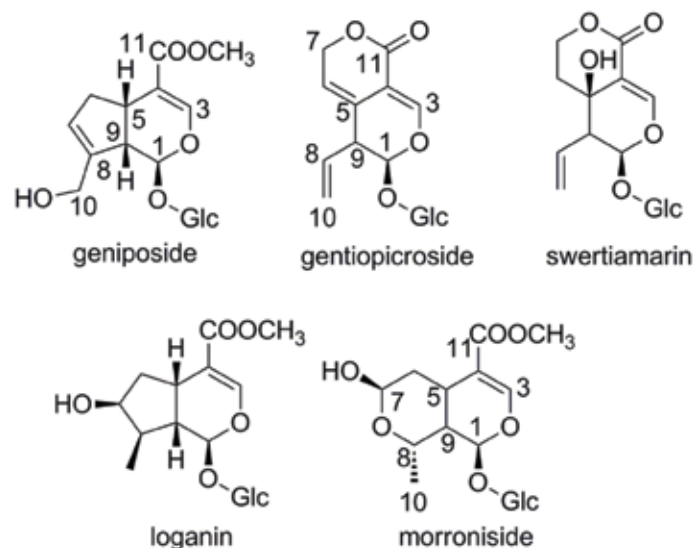


Figure 1. The structures of five natural iridoids.

were supplied by Qingdao Ocean-Chemical Corp. Ltd.; reversed phase TLC plates (Rp-18 F254) and reversed phase column chromatography stationary phase (Lobar LiChroprep Rp-18) were supplied by Merck Co. Ltd.; ODS (Cosmosil 75 C18-OPN) were supplied by Nacalai Tesque Corporation; ACE (pH=5.0), and PBS (pH=7.5) were prepared in our laboratory; the Tv-1901 UV-vis spectrophotometer was the product of Beijing Jia-run Sci. & Tech. Developing Corp.; other chemicals were analytical grade from Chengdu Chemical Agents Corp. Ltd.

### Preparation of Derivative of Geniposide and its Reaction with Methylamine Principal

Ester-hydrolysis of geniposide was undertaken in alkali solution to produce geniposidic acid, and its aglycone was obtained via hydrolysis catalyzed using  $\beta$ -glucosidase. Pigment was formed by reaction of the aglycone with methylamine (Figure 2).

## METHODS

### Preparation of Geniposidic Acid

In a 250-mL beaker, 5.0 g geniposide was dissolved in 20 mL distilled water, and 180 mL 0.5 M NaOH was added. The solution was stirred at 40°C, and the reaction process was monitored using TLC (developing system  $\text{CHCl}_3$ : MeOH = 3:1). The reaction solution was cooled to 20°C after 3 h. The solution was extracted three times using n-BuOH (3 X 200 mL). The organic portions were combined and washed with saturated NaCl solution and dried with  $\text{Na}_2\text{SO}_4$ . After n-BuOH was evaporated, 3.2 g geniposidic acid was obtained.

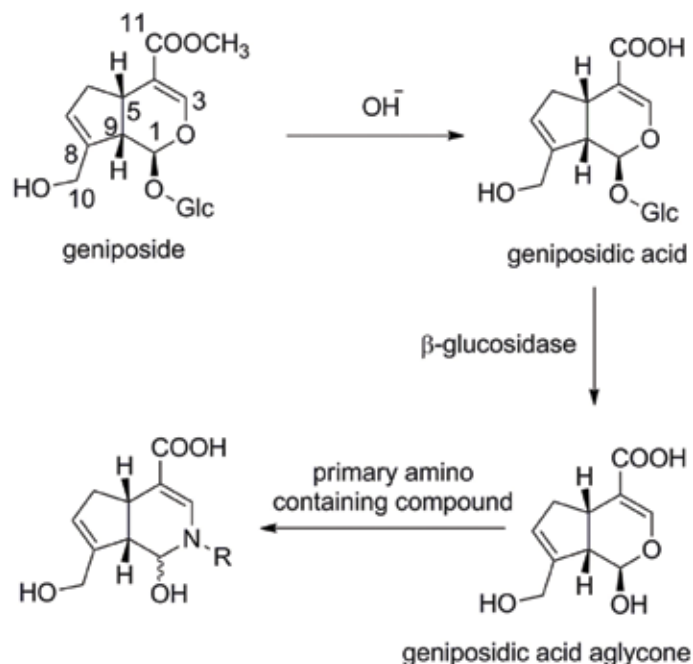


Figure 2. Preparation of the derivative of geniposide and its reaction with methylamine.

### Hydrolysis of Geniposidic Acid

In a 200-mL beaker, 500 mg geniposidic acid was dissolved in 100 mL ACE (pH=5.0), and 25 mg  $\beta$ -glucosidase was added. The solution was stirred at 50°C for 6 h and then cooled to 20°C. It was then extracted three times by EtOAc (3 X 50 mL). The organic portions were combined and washed with saturated NaCl solution and dried with  $\text{Na}_2\text{SO}_4$ . After evaporation of EtOAc, 237 mg geniposidic acid aglycone was obtained.

### The Reaction of Geniposidic Acid Aglycone with Methylamine

In a 25-mL tube, 40 mg geniposidic acid aglycone was dissolved in 2 mL EtOH-H<sub>2</sub>O solution (30:70, V/V), and 3 mL PBS (pH=7.5) and 20 mg  $\text{CH}_3\text{NH}_2\cdot\text{HCl}$  were added. The solution was stirred at 50°C for 6 h. The absorbance spectrum was recorded from 200-700 nm on a Tv-1901 UV-Vis spectrophotometer.

### Preparation of Derivatives of Gentiopicroside and Swertiamarin and their Reaction with Methylamine Principal

Our previous research<sup>7</sup> indicated that, unlike the hydrolysis of geniposide and geniposidic acid, for secoiridoids such as gentiopicroside and swertiamarin, side products are easily produced during hydrolysis catalyzed by  $\beta$ -glucosidase. Their derivatives were obtained by reaction with MeOH in acidic solution during which the attached glucose was substituted with a methoxy group. Through this glucose exchange reaction methygentiopicroside aglycone and methylswertiamarin aglycone were obtained<sup>7</sup> (Figure 3).

### Method

Methygentiopicroside aglycone and methylswertiamarin aglycone were prepared as follows. In a 500-mL three-necked bottle, 2 g of gentiopicroside was dissolved in 300 mL of MeOH, 30 mL 5 M HCL was added, then the solution was refluxed in a 70°C water bath. The reaction process was monitored using TLC (developing system  $\text{CHCl}_3$ : MeOH = 3:1). After 24 h, the reaction solution was cooled to 20°C. The solution pH was adjusted to pH=7 with saturated  $\text{NaHCO}_3$  and MeOH was evaporated. The water part was extracted three times by EtOAc (3 X 50mL). The organic part was put together and washed with saturated NaCl solution and dried with  $\text{Na}_2\text{SO}_4$ . After evaporation of EtOAc, 1.2 g methygentiopicroside aglycone was obtained.

The color forming reaction of methygentiopicroside aglycone and methygentiopicroside aglycone with methylamine was performed in the same way as that of geniposidic acid aglycone with methylamine.

### Structural Modification for Natural Iridoids

#### The Process for Preparing Benzoyl Chloride

In a 100-mL round bottom flask, 1.2 g benzoic acid and 10 g  $\text{SO}_2\text{Cl}_2$  were added, then stirred for 12 h in an ice-water bath. After the melting of ice, the reaction solution was extracted with  $\text{CH}_2\text{Cl}_2$  three times (3 X 50 mL). Then it was dried

overnight with  $\text{Na}_2\text{SO}_4$ . After  $\text{CH}_2\text{Cl}_2$  was evaporated, 0.83 g benzoyl chloride was obtained.

### The Reaction of Natural Iridoids with Benzoyl Chloride (Taking Geniposide as an Example)

In a 250-mL beaker, 0.2 g geniposide was dissolved in 50 mL  $\text{CH}_2\text{Cl}_2$  and triethylamine and benzoyl chloride were added at the ratio of geniposide:triethylamine:benzoyl chloride = 1.0:1.1:1.1(mol/mol/mol) The solution was stirred for 6 h in an ice-water bath and then 50 mL water was added and the reaction mixture was stirred for another 30 min. After that, 20 mL 1.5 M HCl was added to react with residual triethylamine. Then the target product was extracted with  $\text{CH}_2\text{Cl}_2$  three times (3 X 50 mL) and dried with  $\text{Na}_2\text{SO}_4$ . After  $\text{CH}_2\text{Cl}_2$  was evaporated, 0.23 g of the target product was obtained (Figure 4).

The modification products of loganin and morronside (Figure 5) were obtained as that of geniposidee.

### Hydrolysis of Iridoid Glucosides

The three natural iridoid glucosides (geniposide, loganin and morronside) and their modification products were hydrolyzed using catalysis by  $\beta$ -glucosidase to obtain their aglycones, yielding six iridoid aglycones which could form pigments with methylamine (Figure 6).

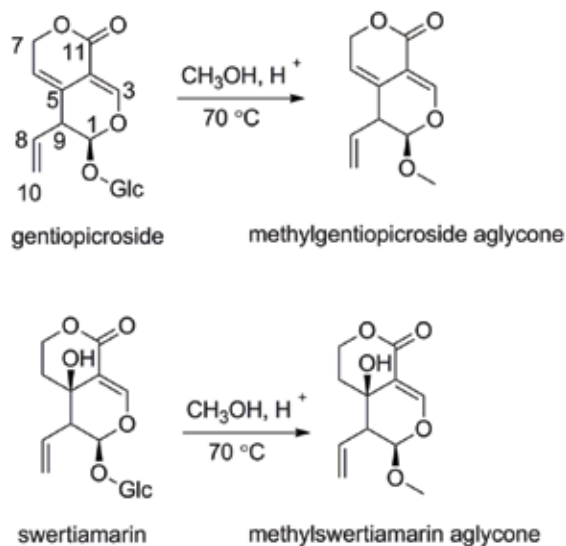


Figure 3. The preparation of derivatives of gentiopicroside and swertiamarin.

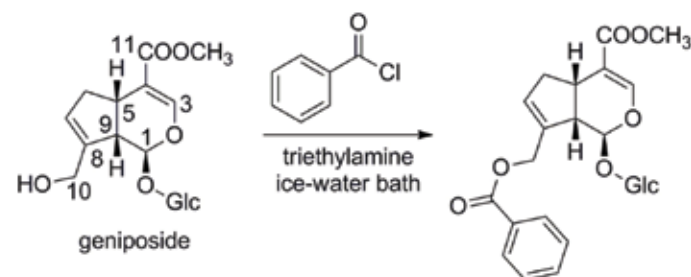


Figure 4. The synthetic route for modified product of geniposide.

### Color Forming Reaction of Six Iridoid Aglycones with Methylamine

The color forming reactions of the six iridoid aglycones with methylamine were undertaken in ACE (pH=5.0) and PBS (pH=7.5) in the same way that geniposidic acid aglycone was reacted with methylamine.

## RESULT AND DISCUSSION

### The Color Forming Reaction of Geniposidic Acid Aglycon with Methylamine

The structural difference between genipin and geniposidic acid aglycone occurs in the substituent group on C-4, the former being  $-\text{COOCH}_3$  and the latter  $-\text{COOH}$ . Our previous research<sup>[1]</sup> indicated that the  $\lambda_{\text{max}}$  of the UV-vis spectrum of genipin was 242 nm, and that of the dark-blue pigment it formed with methylamine was 580 nm. Figure 7 illustrates that the  $\lambda_{\text{max}}$  of geniposidic acid aglycone was 238 nm, and that of the purplish red pigment it formed with methylamine was 552 nm. This result indicated that the position of modified substituent groups affected the color of the pigments formed.

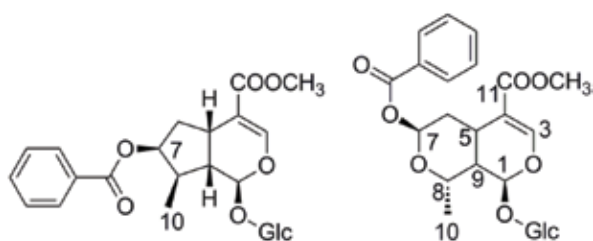


Figure 5. The structure of modification products of loganin and morronisid.

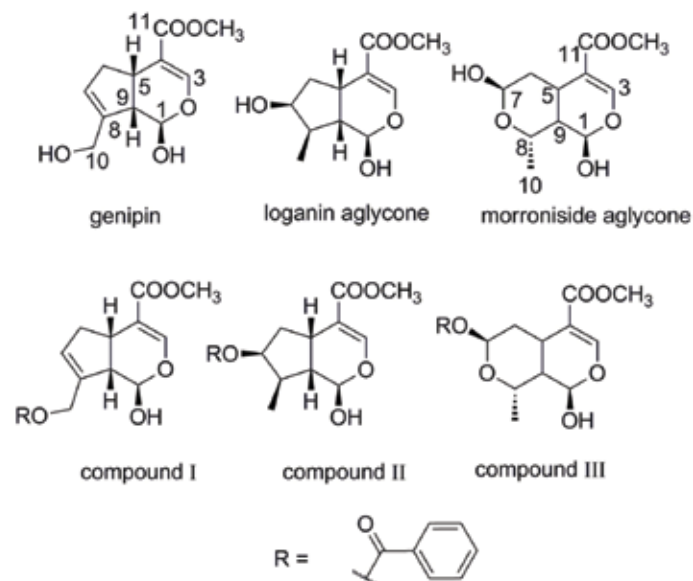


Figure 6. The molecular structure of six iridoid aglycones.

### The Color-forming Reaction of Methylgentiopicroside Aglycone and Methylswertimarin Aglycone with Methylamine

As shown in Figure 8(a), the spectrum of methylgentiopicroside had two maxima, at 270 nm and 326 nm. After reaction with methylamine, the  $\lambda_{\text{max}}$  of the pigment formed was 421 nm, and its color was bright yellow (Figure 9(b)). Figure 9(a) shows that the spectrum of methylswertimarin aglycone also had two maxima, at 237 nm and 242 nm. After reaction with methylamine, the  $\lambda_{\text{max}}$  of the pigment formed was 417 nm, and its color was bright yellow (Figure 9(b)), similar to that of methylgentiopicroside reacted with methylamine.

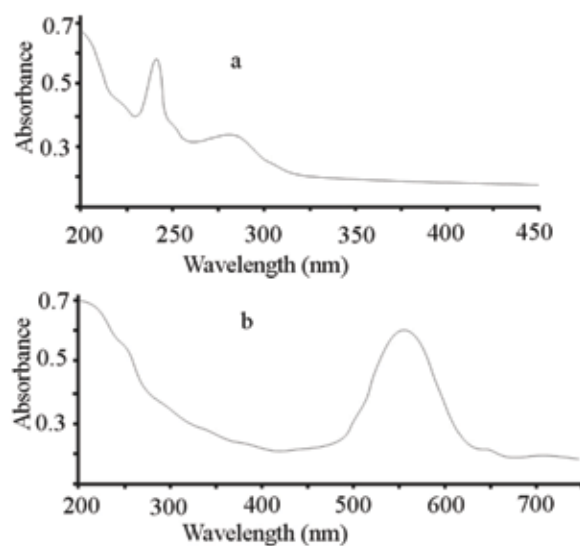


Figure 7. UV-vis of geniposidic acid aglycone (a) and the pigment it formed with methylamine (b).

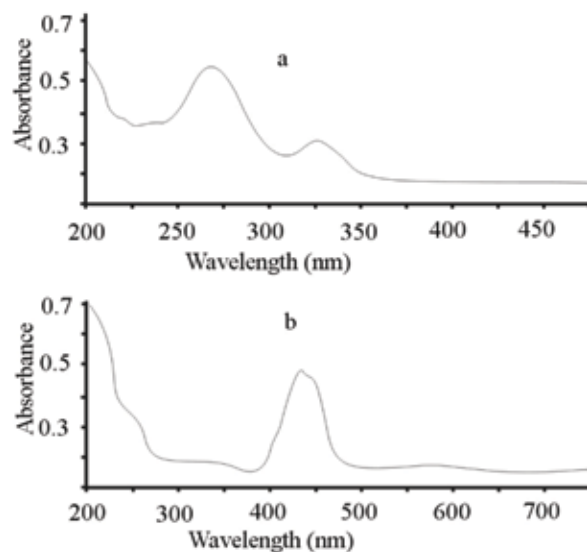


Figure 8. UV-vis spectra of methylgentiopicroside aglycone (a) and the pigment it formed with methylamine (b).

As shown in Figure 1, the structural difference between methylgentiopicroside aglycone and methylswertimarin aglycone occurs at C-5 and C-6; the former has a C=C double bond and the latter an OH group at C-5. Unlike genipin and geniposidic acid aglycone, these two structurally similar secoiridoids formed pigments with methylamine having similar color. This result indicated that, the position of modified substituent groups in the backbone had a key affect on the color of pigments formed.

### Comparing Experiment for the Color Forming of Three Aglycones and their Modification Products Color-forming Result of Loganin Aglycon and Its Modification Product (Compound II)

The  $\lambda_{\max}$  of the UV-vis spectrum of loganin aglycone was 239 nm (Figure 10 (a)). After reaction with methylamine in ACE (pH=5.0), the  $\lambda_{\max}$  of UV-vis spectrum for the pigment formed was 560 nm, and the color of the pigment was purple (Figure 10 (b)). The pigment formed by reaction with methylamine in PBS (pH=7.5) exhibited two maxima, at 430 nm and 650 nm, and the color was greenish yellow (Figure 10 (c)).

Reaction of Compound II (the modification product of loganin aglycone) with methylamine in ACE (pH=5.0), yielded a pigment with a  $\lambda_{\max}$  of 610 nm and a blue color (Figure 11(a)). Reaction with methylamine in PBS (pH=7.5) yielded a pigment with two maxima, 465 nm and 670 nm, and a green color (Figure 11 (b)).

### Color-forming Results of Genipin, Morroniside Aglycone and their Modification Products (Compounds I and III)

The results for genipin, morroniside aglycone and their modification products (Compounds I and III) reacted with methylamine in different buffer solution are shown in Table I and II.

An analysis of Figure 10 and Figure 11 shows that the original natural iridoid loganin aglycone and its modification product compound II produced pigments with different  $\lambda_{\max}$  and colors

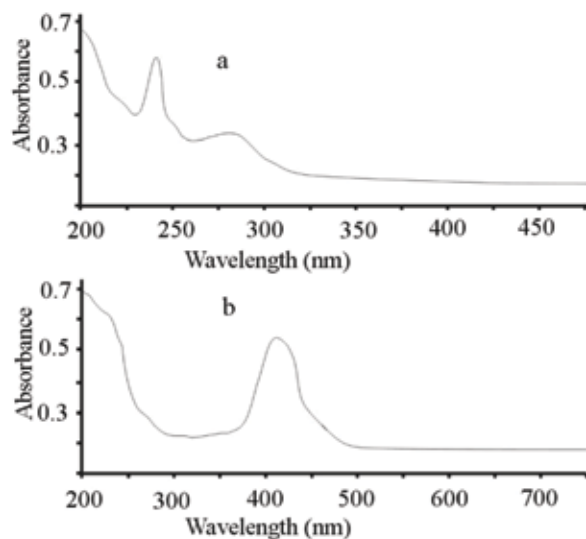


Figure 9. UV-vis spectrum of methylswertimarin aglycone (a) and the pigment it formed with methylamine (b).

upon reaction with methylamine in the same buffer solution. For example, in PBS,  $\lambda_{\max}$  was changed from 430 nm and 650 nm (loganin aglycone) to 465 nm and 670 nm (compound II), and the color changed from greenish yellow to green. The reason is that, the molecular energy of compound II is lower than that of the original iridoid because of the introduction of the benzoyl  $\pi$ - $\pi$  conjugation system. Compared with their original iridoids, compound I and compound III showed the same pattern.

An analysis of Figure 10, Figure 11, Table I and Table II shows that iridoids and their modification products, upon reaction with methylamine, produced pigments that differed in color depending on the pH of the buffer solution (PBS, pH=7.5 or ACE, pH=5.0). Generally, the  $\lambda_{\max}$  of pigments formed in PBS was greater than that of pigments formed in ACE, and this result was consistent with our previous results.<sup>9</sup>

## CONCLUSIONS

The following are the conclusions derived from this present work.

- Compounds having the same iridoid backbone but different side groups or substituent groups were obtained via structural modification. The color of pigments formed by the modification products and the original natural iridoids upon reaction with methylamine were different. By this method, the type and quantity of iridoid-like compounds was increased and the color range of “natural reactive dyes” was widened.

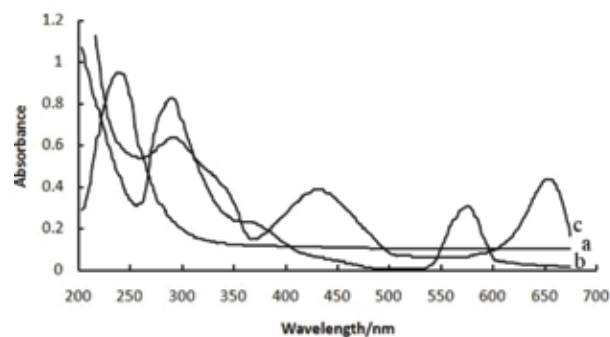


Figure 10. The UV-vis of loganin aglycone and the pigments for it formed with methylamine (a. loganin aglycon; b. in A.C.E.; c. in PBS).

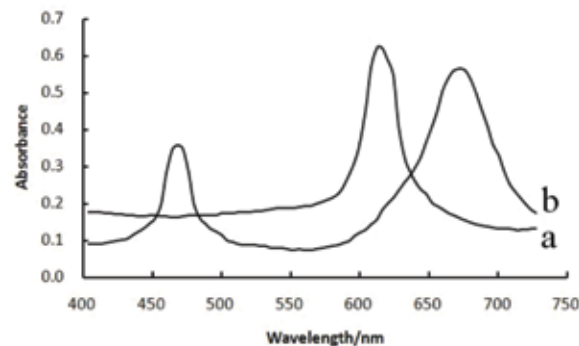


Figure 11. The UV-vis of the pigments from compound (II) reacted with methylamine (a. in ACE; b. in PBS).

- In different buffer systems, i.e. PBS (pH=8.0) and ACE (pH=5.0), the same iridoid formed pigments with different colors. Generally, the  $\lambda_{\max}$  of pigments formed in PBS was greater than that of pigments formed in ACE.
- The position of modified substituent groups in the backbone of the iridoid molecular structure had a key affect on the color of pigments formed.

**TABLE I****The result for genipin and its modified product reacted with methylamine.**

Buffer solution	$\lambda_{\max}$ /nm	color
genipin (in ACE)	238	colorless
genipin (in PBS)	242	colorless
genipin+ methylamine (in ACE)	380, 562	light purple
genipin+ methylamine (in PBS)	400, 580	dark blue
compound(I)+ methylamine (in ACE)	430, 595	purplish blue
compound(I)+ methylamine (in PBS)	445, 615	blue

**TABLE II****The result for morroniside aglycone and its modified product reacted with methylamine.**

Buffer solution	$\lambda_{\max}$ /nm	color
morroniside aglycone (in ACE)	225	colorless
morroniside aglycone (in PBS)	240	colorless
morroniside aglycone + methylamine (in ACE)	350, 550	brown
morroniside aglycone + methylamine (in PBS)	350, 490	yellow-orange
compound(III)+ methylamine (in ACE)	365, 570	purple
compound(III)+ methylamine (in PBS)	360, 530	red-orange

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