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

Part I. Preparation of Four Natural Iridoids and Their Dyeing/Cross-Linking (Tanning) Property to Hide Powder

by

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Abstract

Four iridoid compounds, genipin, loganin aglycone, oleuropein aglycone and E-6-*O*-methoxycinnamoyl scandoside methyl ester aglycone were prepared from *Gardenia jasminoides* Ellis, *Lonicera japonica* Thunb, *Olea europaea* Linn and *Hedyotis diffusa* (Willd) Roxb respectively, their structures were identified by electron spray ionization-mass spectrometry (ESI-MS), ^{1}H NMR and ^{13}C NMR. Their dyeing/tanning properties on hide powder was investigated, the relationship between dyeing/cross-linking (tanning) property and the chemical structures were discussed. The results showed that these four natural iridoids could react with hide powder under mild conditions (35°C, pH=7.5-8.0), within a short time (about 6 h) and at a low dosage (5% (w/w) of hide powder). The hydrothermal stability of the reacted hide powder was 20-25°C higher than the control sample, and different colors, such as dark blue, brown-yellow, yellow and mauve, were imparted to hide powder. The mechanism might be that cross-linking reaction took place between iridoids and $-NH_2$ groups of collagen molecular chains. These results implied that natural iridoids might act as completely novel "natural reactive dyes", especially for protein fibers and offering environmental-friendly simultaneous tanning-dyeing agents for leather manufacture.

Resumen

Cuatro compuestos iridoides, genipin, aglicona loganina, oleuropeína aglicona y E-6-O metoxi cinamil escandoside metil ester aglicona fueron preparados a partir de Gardenia jasminoides Ellis, Lonicera japonica Thunb, Olea europaea Linn y Hedyotis diffusa (Willd) Roxb, respectivamente, sus estructuras se identificaron por ionización de electrones-espectrometría de masas (ESI-MS), ^{1}H NMR and ^{13}C NMR. Sus propiedades de teñido/curtido en polvo de piel se investigaron, la relación entre las propiedades del teñido / reticulación (curtido) y las estructuras químicas fueron discutidas. Los resultados mostraron que estas cuatro iridoides naturales podrían reaccionar con el polvo de piel en condiciones suaves (35°C, pH=7.5-8.0), en un corto período de tiempo (alrededor de 6 hs.) y en una dosis baja (5% (w/w), del polvo de piel). La estabilidad hidrotérmica del polvo de piel así curtido fue 20 a 25°C más alta que el de la muestra de control, diferentes colores, como azul oscuro, marrón-amarillo, amarillo y malva, se impartieron al polvo de piel. El mecanismo podría ser que la reacción de reticulación se llevó a cabo entre los iridoides y grupos $-NH_2$ de las cadenas moleculares del colágeno. Estos resultados implican que iridoides naturales podrían actuar como "colorantes reactivos naturales" completamente nuevos, especialmente para las fibras proteínicas y ofreciendo simultáneamente agentes de curtido-teñido, respetuosos del medio ambiente para la fabricación de cuero.

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INTRODUCTION

Both dyeing and tanning are ancient procedures, which have a history of more than 4000 years. The modern dye industry experienced its development period from birth to maturity,[1] from the discovery of mauve by W. H. Perkin in 1856, to the appearance of the first reactive dye developed by ICI in 1956. Research and development in this field during the late 20th century, for example, the cross-linking of dyes and polymeric dyes, were only improvements on the application property based on traditional dyes.[2,3] Some authorities in this field have stated[4] that the three key problems for dye industry were the so called "3C Engineering", i.e., Creation, Cleaning technology and Commercialization.

The modern tanning industry grew after the invention of chrome tanning by the German chemist F.L. Knapp in 1858. During recent years, environmentally-friendly chrome-free tanning technology has been highly appreciated in the US and EU countries,[5,6] and regulations for cancer-inducing aniline compounds from Azo-dyes in leather and textile products are becoming more and more strict.[1] Until now, an environmentally friendly and simultaneous tanning-dyeing agent for leather has not been reported.

The characteristic structure of natural iridoids is the hexa-ring with an alkene-ether bond in its backbone (Figure 1). More than 1400 plant iridoids were isolated and identified between their discovery by Halpern and Schmid in the 1950's, and 2001.[7] Iridoids have a wide distribution in plants, for example, the total iridoid glycosides content in the dry fruit of *Gardenia jasminoides* Ellis and the dry leaves of *Olea europaea* Linn are 5-15% and 6-9% respectively. Both of these plants are grown in profusion in many areas and it's possible to prepare the iridoids from them in large quantity.[8]

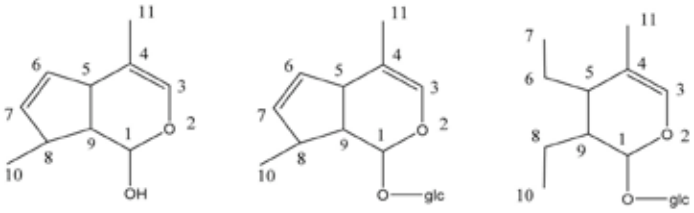

Figure 1. The basic structures of iridoid compounds

Previous research has demonstrated that, a novel iridoid crosslinking-agent, genipin (GP), could act as a tanning agent, increasing the hydrothermal stability of powdered cattle hide, and simultaneously imparting a dark blue color to the crosslinked hide substance.[9] By combination tanning with aluminum-genipin, the apparent denaturation temperature T_p for hide powder as determined by differential scanning calorimetry (DSC) was higher than 90°C.[10,11] These results suggested the possibility of iridoid compounds as potential dyeing and tanning agents for leather industry. Thousands of iridoid compounds are found in nature and their contents in some plants are fairly abundant. In the present study, four iridoid compounds, genipin (GP), loganin aglycone (LA), oleuropein aglycone (OA) and E-6-*O*-methoxycinnamoyl scandoside methyl ester aglycone (EA) were prepared from *Gardenia jasminoides* Ellis, *Lonicera japonica* Thunb, *Olea europaea* Linn and *Hedyotis diffusa* (Willd) Roxb respectively, their dyeing and tanning properties on hide powder were investigated, the relationship between dyeing /tanning properties and the chemical structures of the iridoid compounds are discussed, the mechanism of the dyeing-tanning reaction were deduced. This research might pave a way for natural iridoids acting as a completely novel "natural reactive dyes" special for protein fibers, and an environmental-friendly simultaneous tanning-dyeing agent for leather manufacture.

EXPERIMENTAL

Materials

Gardenia jasminoides Ellis, *Lonicera japonica* Thunb, *Olea europaea* Linn and *Hedyotis diffusa* (Willd) Roxb were purchased from Wukuaishi Herbal Market in Chengdu City and identified by Dr. Xiao-ling Wang from the ethnic pharmacy institute, Southwest University for Nationalities. Bovine hide powder was provided by the National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University. Bovine serum albumin (BSA) and β-glucosidase were the products 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.

Preparation and identification of iridoid glucosides[12]

Preparation and identification of loganin.

The stems and leaves of *Lonicera japonica* Thunb (5 kg) were extracted with EtOH at ambient temperature (three times and 7 days per time). The extract was suspended in H_2O and partitioned with petroleum ether, EtOAc and n-BuOH. The n-BuOH extract was subjected to D_{101}-macroporous resin. The 30% aqueous EtOH moiety was collected to afford a fraction (72.3 g) and was subjected to Silicon-gel column chromatography and eluted with gradient $CHCl_3$–MeOH to obtain loganin (1.5g). (This process is illustrated in Figure 2). The chemical structure of loganin was identified by ESI-MS using a Finnigen LCQ^{DECA}, ^{1}H NMR, ^{13}C NMR (on a Bruker Avance 600) and comparing the chemical evidence with figures from the related reference.

Preparation and identification of geniposide, oleuropein and E-6-O-methoxycinnamoyl scandoside methyl ester.

As described above, the other three iridoid glycosides,

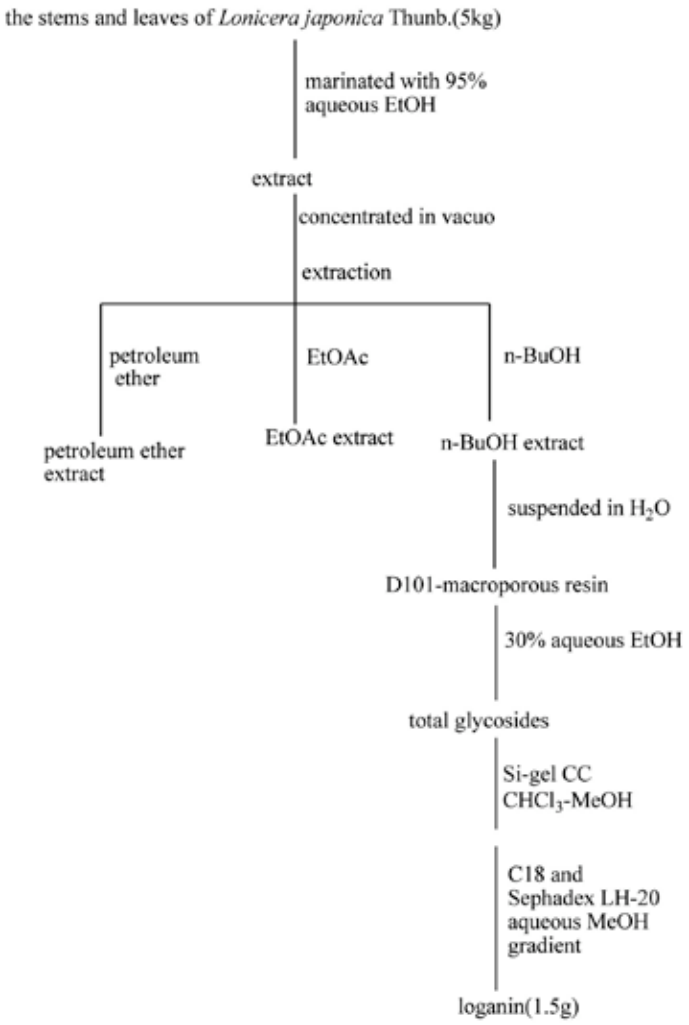

Figure 2. Process flow for isolating loganin from Lonicera japonica Thunb

geniposide, oleuropein and E-6-O-methoxycinnamoyl scandoside methyl ester were isolated from *Gardenia jasminoides* Ellis, *Olea europaea* Linn and *Hedyotis diffusa* (Willd) Roxb respectively. Their chemical structures were identified by ESI-MS, ^{1}H NMR and ^{13}C NMR and compared with the chemical evidence reported in a related reference.[7]

Preparation of iridoid aglycones by hydrolysis.
Each iridoid glycoside (8 g) was dissolved in 100 mL acetate buffer (pH 5.0) respectively, 150 mg β- glucosidase was added. The solution was stirred for 2h at 50°C to obtain the iridoid aglycone. The hydrolysis solution was purified by extraction and recrystallization. Each irodoid aglycone structure was confirmed by MS and TLC methods.

Tanning and dyeing experiment.
Hide powder was first hydrated with 1500% distilled water in a flask overnight at ambient temperature, filtered and resuspended in PBS at pH =7.5-8.0. The tanning/dyeing processes were evaluated using 0.5 g hide powder in 7.5 mL in a thermostated shaking bath. Tanning/dyeing parameters adopted were the optimized conditions described in the previous work:[9] The dosage for each iridoid was 5% (w/w, basis on the dry weight of hide powder), T=35°C, t = 6h and maintained the pH at 7.5-8.0 during the entire process. At the completion of tanning/dyeing process, the resulting mixture was filtered, and the tanned/dyed hide powder washed with distilled water until the effluent was clear. Then it was dried at ambient temperature.

Analytical Procedures
Determination of thermal stability.
Thermal stability of tanned hide powder was determined by differential scanning calorimetry (DSC) on a Perkin-Elmer model DSC7. Hide powder samples were prepared by soaking in distilled water overnight and then blotting on filter paper. Moist, blotted samples (8-10 mg) were weighed into ampoules that were sealed and placed in the calorimeter. The temperature was programmed to record from 30°C to 120 °C at 5°C per min.

Stability of iridoid aglycones in phosphate buffered saline (PBS).
UV-vis spectrophotometry was used to verify the stability of the four iridoid aglycones. Each iridoid aglycone was dissolved in phosphate buffered saline (PBS) (pH ~ 7) to obtain a solution of 0.5 mg/mL and incubated at 35°C. The ultraviolet (UV) spectrum was monitored as a function of time. The absorbance spectrum from 200-400 nm was recorded on a UV-540 (Thermoelectro, USA) at 0.25, 2, 8 and 24 h.

Reaction analysis between genipin and BSA via SDS-PAGE.
BSA was reacted with genipin at 35°C, the dosage of genipin was 5%, 10% and 15% (w/w, basis on the pure weight of BSA) respectively, SDS-PAGE was undertaken after the reaction and Coomassi-Blue R-250 was applied as dyestuff. Gel of 7.5% (pH ~ 8.8) and 5% (pH ~ 6.8) were adopted respectively. The dosage of protein on each course was 10μg.

RESULT AND DISCUSSION

The chemical structures of four iridoid glycosides
Using loganin as an example, the chemical evidence from ESI-MS, ^{1}H NMR and ^{13}C NMR was as follows: ESI-MS: m/z 413 $[M+Na]^+$, 425 $[M+Cl]^-$; the data from^1 H NMR (600MHz, CD_3OD), ^{13}C NMR (600MHz, CD_3OD) are shown in Table 1. Comparing the obtained data from this experiment and those from the literature,[13] the compound could be identified as loganin.

The chemical structures of the other three iridoid glucosides were identified in the same way and are illustrated in Figure 3. Geniposide, loganin and E-6-O-methoxycinnamoyl scandoside methyl ester are iridoid compounds, while

TABLE I
^{1}H NMR and ^{13}C NMR of loganin

C	δ_H	δ_C
1	5.26d(4.5)	97.8
3	7.38d(1.2)	152.2
4	—	114
5	3.12m	32.2
6	1.62m,2.03m	42.7
7	4.04m	74.7
8	1.87m	42.2
9	2.23m	46.5
10	1.09d(6.9)	13.6
11	—	169.6
$-OCH_3$	3.69s	51.8
1'	4.64d(7.8)	100.1
2'		75.1
3'		78.0
4'		71.6
5'		78.3
6'		62.8

oleuropein is a secoiridoid compound. These four iridoid glucosides were hydrolyzed by β- glucosidase and the four iridoid aglycones were obtained.

Tanning and dyeing properties of iridoids on hide powder

Increases in hydrothermal stability (shrinkage temperature) of a hide, hide powder, or even collagen are often cited as evidence of the tanning potential of the process responsible for the increase. The results of this study showed that, these four iridoids could be effective at increasing the hydrothermal stability of hide powder (Figure 4). When hide powder suspended in PBS (pH=7.5-8.0) and treated with each iridoid (5%, w/w basis on dry weight of hide powder) for 6h at 35°C, the hydrothermal stability of each of the tanned hide powder samples was ≥ 80°C. These results further confirmed that, the special structure of the hexa-ring with an alkene-ether bond from iridoids (including secoiridoids) is the key factor for the crosslinking reaction. Side groups have some effect on the increase in hydrothermal stability also. Oleuropein aglucon (OA) and E-6-*O*-methoxycinnamoyl scandoside methyl ester aglycone (EA), which have phenol-hydroxyl groups in the molecular structures, would be advantageous for increasing the thermal stability of tanned hide powder.

In previous work,[9] we have shown that genipin could turn hide powder dark blue; this color is produced during the reaction, not from genipin itself. This kind of dyeing mechanism is

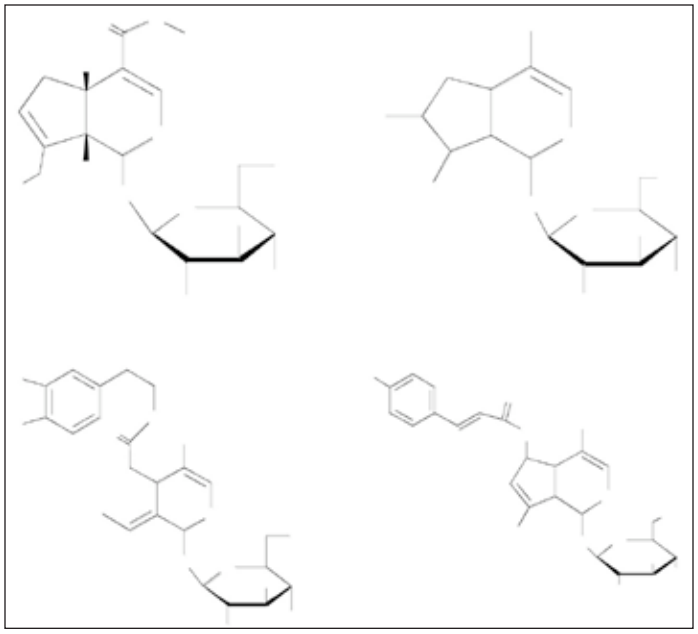

Figure 3. Chemical structures of four iridoid glucosides (1: genipin; 2: loganin; 3: oleuropein; 4: E-6-O-methoxycinnamoyl scandoside methyl este)

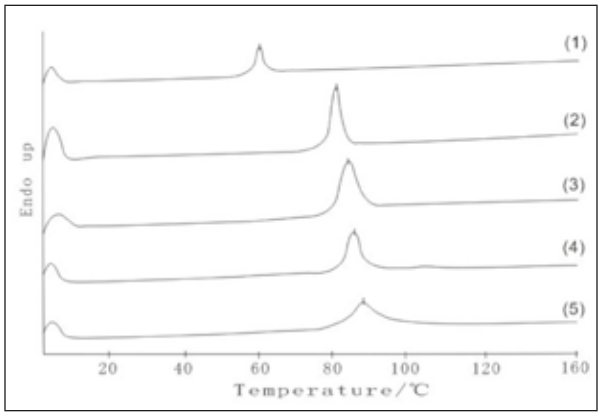

Figure 4. DSC analysis of hide powder tanned by four iridoid compounds (1) control; (2) GP; (3) LA; (4) EA; (5) OA

basically different from the dyeing process by natural dyes, acid dyes, reactive dyes, cross-linking dyes,[2] polymeric dyes,[3] or any other dyes currently applied in the current dye industry. Since then, we were always wondering whether other iridoids that could dye protein to different colors (red, yellow, etc.) could be found. If so, we might find a kind of "natural reactive dyes" special for protein fibers. It is a logical to deduce on the basis of fundamental theory that, this type of natural reactive dyes would be environmental friendly and have good washing and rubbing properties. From the results shown in Table 2, we could see that yellow, yellow-brown and mauve colors could be imparted to hide powder by OA, LA and EA. These results confirmed our original speculation.

Increased hydrothermal stability and color could be imparted to hide powder simultaneously by treatment with iridoid compounds. If the hide pelt was pre-tanned by aluminum salt

TABLE II

Tanning and dyeing results of four iridiods to hide powder

	Control	GP	LA	OA	EA
T_p(°C)	60	80	82	85	83
Color	white	dark blue	yellow-brown	yellow	mauve

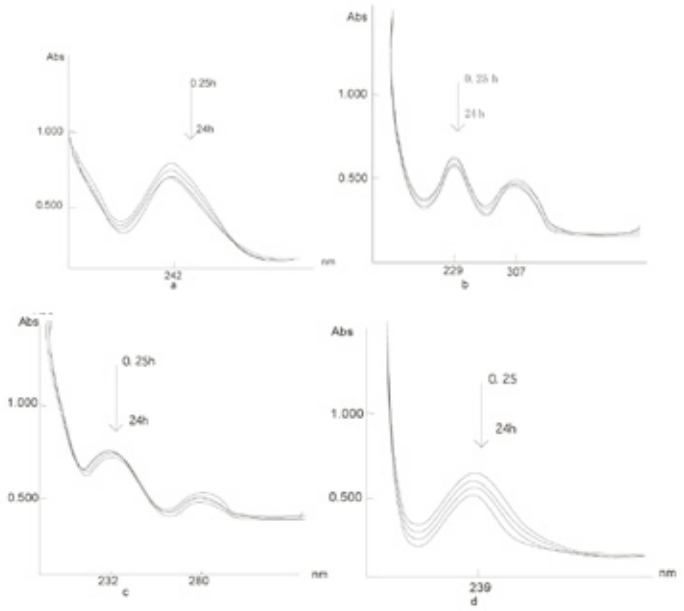

Figure 5. UV-vis spectra for GP (a), LA (b), OA (c) and EA (d) in PBS solution at different storage time

and then tanned by iridoid compounds as described by the previous work,[10,11] a potential chrome-free technology of simultaneous tanning/dyeing for leather manufacturing could be realized.

The dyeing/tanning mechanism of iridoids to hide powder

Iridiod stability in PBS.

The UV spectra of the four iridoid solutions that were incubated in PBS (pH ~ 7) at 35°C after 0.25, 2, 8 and 24 h are illustrated in Figure 5. The characteristic peak for GP(a) and EA (d) remained at 242 nm and 239 nm respectively during the entire incubation period. The two characteristic peaks of OA(c) remained at 232 nm and 280 nm during the entire incubation period; the two characteristic peaks of LA (b) remained at 229 nm and 307nm during the entire incubation period. These results are consistent with those reported in the previous work [9], which indicated that, iridoid aglycones would not polymerize in PBS, or that only limited polymerization occurred. This result indicated that, it was iridoid monomer that reacted with hide powder.

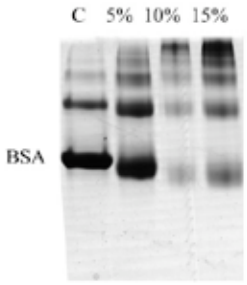

Figure 6. Cross-linking reaction between BSA and genipin analysis via SDS-PAGE

Analysis by SDS-PAGE for the reaction between genipin and BSA.

BSA is a soluble protein and could be analyzed via SDS-PAGE after reaction with genipin. As illustrated in Figure 6, un-reacted BSA existed in the form of monomer and the reacted BSA existed as di-polymer or tri-polymer. This result indicated that, cross-linking reaction occurred between genipin and BSA. From analysis of the experimental data, we can possibly explain the mechanism of dyeing/tanning mechanism of iridiods as applied to hide powder: chromogens with complicated molecular structures were formed during the cross-linking reaction of iridoids with -NH_2 on the side chains of collagen molecular; and the side groups combined with the hexa-ring backbones of different iridoids were acting as auxochromes. Different auxochromes would produce different colors, and simultaneously, the cross-linking reaction increased the hydrothermal stability of hide powder. Of course, the exact mechanism for this process requires further research.

CONCLUSIONS

The following are the conclusions derived from this present work:

- The stability experiments indicated that, polymerization did not occur or only limited polymerization occurred in iridoid-PBS solutions, i.e., most iridoid aglycones cross-linked with hide powder via monomer.

- In addition to genipin, three other iridoids show considerable promise as potential tanning agents. This means that, the special structure of the hexa-ring with an alkene-ether bond from iridoids (including secoiridoids) is the key factor for the cross-linking reaction. Side groups have some effects on the increases in hydrothermal stability.

- Different colors could be imparted to hide powder by different iridoids. The reason is thought to be that chromogens with complicated molecular structures were formed during the cross-linking reaction of iridoids with -NH_2 on the side chains of collagen molecule; and the side groups combined with the hexa-ring backbones of

different iridoids were acting as auxochromes. These results suggest the potential for iridoids to perform as bio-friendly "natural reactive dyes" for protein materials, such as leather, silk, wool, or even applied as a cosmetic to dye human hair (because it could react with protein fiber at a mild condition).

- Iridoid compounds have potential for chrome-free tanning and simultaneous tanning/dyeing process during leather manufacturing.

ACKNOWLEDGEMENTS

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