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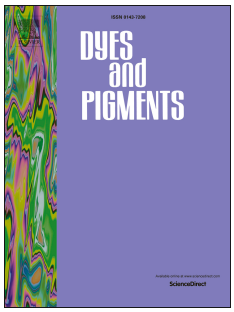

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PII: S0143-7208(19)32069-8

DOI: https://doi.org/10.1016/j.dyepig.2019.107965

Reference: DYPI 107965

To appear in: *Dyes and Pigments*

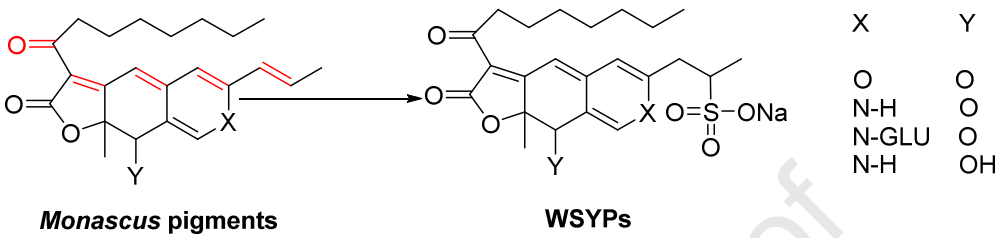
Received Date: 2 September 2019

Revised Date: 11 October 2019

Accepted Date: 12 October 2019

Please cite this article as: Liu L, Wu S, Wang W, Zhang X, Wang Z, Sulfonation of *Monascus* pigments to produce water-soluble yellow pigments, *Dyes and Pigments* (2019), doi: https://doi.org/10.1016/j.dyepig.2019.107965.

Graf Abstract



***Monascus* pigments** **WSYPs**

Dyes and pigments

Sulfonation of *Monascus* pigments to produce water-soluble yellow pigments

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Abstract: Red Yeast Rice, a kind of *Monascus* pigments produced by fermentation of *Monascus* species on rice, is a traditional Chinese medicine and food colorant. Chemical modification of natural pigments is a common strategy to diversify the pigments to meet various demands. Herein, native *Monascus* pigments as well as some of their derivates were prepared. Sulfonation of *Monascus* pigments with conjugated double bonds adjacent to a carbonyl group was carried out to produce novel water-soluble yellow pigments (WSYPs). The chemical structure of novel WSYPs, i.e., an addition of H_2SO_3 to the double bond at the side-chain of *Monascus* pigments, was elucidated by MS and NMR analysis. The introduction of H_2SO_3 into *Monascus* pigments makes WSYPs exhibit yellow color and high water solubility. The yellow color, high water solubility, as well as relatively high stability in a wide pH range contribute the novel WSYPs as a potential food colorant.

Key words: *Monascus* pigments; Water-soluble yellow pigments; Sulfonation; Nucleophilic attack; Food colorant

1. Introduction

Red Yeast Rice, a kind of *Monascus* pigments produced by solid-state fermentation of *Monascus* species on rice, is a traditional Chinese medicine and food colorant. It has been widely used as food colorant in China, Korea, Japan and Southeast Asia for more than one thousand years [1-3]. By virtue of elaboration of the gene clusters of *Monascus* species, the biosynthetic pathway of azaphilone core catalyzed by polyketide synthase has been detailed [4, 5]. By means of further esterification of the azaphilone core with β-ketoacid, native *Monascus* pigments, such as orange *Monascus* pigments (OMPs) (**1**, **2**) and yellow *Monascus* pigments (YMPs) (**3**, **4**) (Fig.1A), are biosynthesized [6]. However, the native *Monascus* pigments are unsuitable for utilizing directly as food colorant owing to their low water solubility [7]. Furthermore, the embryo-toxicity and teratogenicity of OMPs have also been reported [8].

Location of Fig.1

It has been reported that OMPs can be chemically modified by reduction [9], oxidation [10, 11] and amination [12-18]. Among them, amination of compound **2** into **5** or **6** (Fig.1B) for production of water-soluble red pigments (WSRPs) has been studied extensively. As shown in Fig.1, cascade reactions, i.e., biosynthesis of OMPs and further chemical modification of OMPs by amination reaction, occur naturally during microbial fermentation. During the course of microbial culture, primary amines, such as amino acids and glucosamine [19] as well as ammonia, are produced by microbial metabolism. Thus, the major colorant components of Red Yeast Rice are WSRPs with various primary amines [1, 6, 20, 21]. Due to the excellent stability and high water solubility of WSRPs, Chinese government has issued the standard for commercial production of WSRPs (Red *Monascus Pigment*®, GB 5009.150-2016). The Red *Monascus Pigment*® has been utilized widely as a food additive, especially for substitution of nitrite to enhance meat color [22].

It is reported that OMPs as well as WSRPs can be further chemically modified by sodium borohydride to produce yellow pigments [9, 23]. Alternatively, yellow pigments, which are produced by sulfonation of commercial Red *Monascus Pigment* ® [24], have also been approved as commercial food colorant by Chinese government (Yellow *Monascus* Pigment®, GB 1886.66-2015). Yellow *Monascus Pigment*® is being produced by several biotechnology companies in China [6]. In spite of the practical application of Yellow *Monascus Pigment*®, the chemical reaction about sulfonation of *Monascus* pigments remains largely blurry. There are few works about the identification of chemical components in Yellow *Monascus Pigment*® due to the troublesome separation procedure. Till now, there is only one report about an isolation of the relatively hydrophobic components in Yellow *Monascus*

Pigment® by preparative TLC and further deduction of the corresponding chemical structure by LC-MS [24].

In the present work, sulfonation of identified *Monascus* pigments to produce water-soluble yellow pigments (WSYPs) was carried out and the chemical structure of the novel WSYPs was managed to elucidate. Firstly, native *Monascus* pigments (Fig.1A) were isolated after microbial fermentation. The native OMPs was chemically modified by amination reaction and then reduction in an aqueous sodium hydroxide solution (Fig.1B). Then, sulfonation of *Monascus* pigments (including native ones and their derivates as shown in Fig.1) was carried out and the chemical structure of novel WSYPs was elucidated by UPLC-MS and NMR. Finally, the color characters of the novel WSYPs as a potential food colorant, such as color, water solubility, and stability, were further checked.

2. Materials and methods

2.1. Chemicals

Acetonitrile (HPLC grade), was purchased from Avantor (US). Water was purified using a Mili-Q Ultra system (Millipore, US). Chloroform-*d*, methanol-d_4 and dimethyl sulfoxide-d_6 (DMSO-d_6) were purchased from Adamas-beta (Shanghai, China). Sodium dithionite was purchased from TiTan (Shanghai, China). Other agents were chemical grade.

2.2. Identification of native *Monascus* pigments

Native *Monascus* pigments were produced by submerged culture of *Monascus ruber* (ESI S1). Crude native *Monascus* pigments (Fig.1A) were isolated from the fermentation broth (ESI S2). The crude OMPs and YMPs were further purified by a preparative HPLC system (Shimadzu LC-6AD series equipped with an SPD-20 spectrophotometer using Shimadzu PRC-ODS EV0233 column). The sample injection volume was 1 ml. Isocratic elution (acetonitrile/water, 70:30, V/V) with a flow rate of 10 ml/min was employed and OMPs were detected at the wavelength 470 nm while and YMPs at 390 nm. Four kinds of native *Monascus* pigments (Fig.1A) were obtained. The purified monascorubrin (**2**) and ankaflavin (**4**) were further identified by mass spectrometer (MS) and NMR analysis.

Monascorubrin (**2**): orange needle solid; UV-vis (acetonitrile) λ_{max} 243, 286, 468 nm; HRMS Anal. Calcd for $C_{23}H_{27}O_5$: 383.1858[M+H$^+$]; found, 383.1863(ESI S3). Ankaflavin (**4**): yellow needle solid; UV-vis (acetonitrile) λ_{max} 224, 386 nm; HRMS Anal. Calcd for $C_{23}H_{31}O_5$: 387.2171[M+H]$^+$; found, 387.2170 (ESI S4). The HRMS data are consistent with the literature data [25]. The corresponding data of ^{1}HNMR and ^{13}CNMR analysis were presented in table 1 and table 2, respectively. The results are consistent with the data in literatures [9, 26, 27].

Location of table 1 & 2

2.3. Chemical modification of monascorubrin

OMPs include two chemical entities: rubropunctatin (**1**) and monascorubrin (**2**), which have similar structural character and physicochemical property [18, 27]. According to Fig.1B, monascorubrin (**2**) was chosen as target reactant for study. Monascorubrin (**2**) (0.1 g, 0.26 mmol) in ethanol (35 mL) and primary amine ($(NH_4)_2SO_4$ or monosodium glutamate; 0.52 mmol) in water (pH 7, 15 mL) were mixed, which was stirred at 30 ^oC for 72 h until monascorubrin was consumed completely (monitored by thin-layer chromatography (TLC), developing solvent: chloroform/methanol/water=56/10/0.9). The reaction mixture was concentrated by a rotary evaporator.

After evaporation of solvent, the solid residue including $(NH_4)_2SO_4$ was redissolved by ethyl acetate/water (1/1, V/V; 50 mL). The mixture was transferred into a 150 mL separating funnel and an ethyl acetate layer containing monascorubramine (**5**) was then separated. The ethyl acetate layer was concentrated in a rotary evaporator under vacuum to afford the crude product. Recrystallization of the crude pigment from ethanol yielded red needles **5** (91 mg). The purified monascorubramine (**5**) was further identified by MS and NMR analysis.

Monascorubramine (**5**): Red needle solid; UV-vis (acetonitrile) λ_{max} 300, 415, 531 nm; HRMS Anal. Calcd for $C_{23}H_{26}NO_4$, 380.1862 $[M\text{-}H]^-$; found, 380.1853 (ESI S5). The corresponding data of ^{1}HNMR and ^{13}CNMR analysis were presented in table 1 and table 2, respectively. The results are consistent with the data in literatures [26, 27].

Similarly, the solid residue including monosodium glutamate was redissolved by ethyl acetate/water (1/1, v/v; 50 mL). The pH of water phase was adjusted to 10 by NaOH aqueous solution (1 M), and then the water phase was separated. The water phase was added ethyl acetate (30 mL) again, of which the pH was adjusted to 2.5. The target compound was partitioned into the ethyl acetate phase under this condition. Then, ethyl acetate phase was fetched and the solvent was removed to get the crude compound **6** (82 mg). Compound **6** was further identified by MS analysis.

Compound **6:** red amorphous solid; UV-vis (acetonitrile) λ_{max} 425, 521nm; HRMS Anal. Calcd for $C_{23}H_{26}NO_4$, 512.2284 $[M+H]^+$; found, 512.2286 (ESI S6). The results are consistent with the data in literature [19].

Monascorubramine (**5**) (50 mg) in ethanol (20 mL) and NaOH aqueous solution (1 M, 20 mL) were mixed, which was stirred at 40 ^oC for 10 h. The reaction mixture was concentrated by a rotary evaporator. The solid residue was redissolved by ethyl acetate/water (1/1, V/V; 50 mL). The mixture was transferred into a 250 mL separating funnel and an ethyl acetate layer was then separated. The ethyl acetate layer was concentrated in a rotary evaporator under vacuum to afford the crude product, which was separated by preparative TLC (GF254, 200 * 200* 0.9 mm, Huanghai, China) using chloroform/methanol/water (56/10/0.9, V/V/V) as the developing solvent to give the

product **7** (45 mg). The purified compound **7** was further identified by MS and NMR analysis.

Compound **7**: red amorphous solid; UV-vis (acetonitrile) λ_{max} 238, 299, 477 nm; HRMS Anal. Calcd for $C_{23}H_{30}NO_4$, 384.2175 $[M+H]^+$; found, 384.2179 (ESI S7). The corresponding data of ^{1}HNMR and ^{13}CNMR analysis were presented in table 1 and table 2, respectively. The reduction of **5** to **7** in the aqueous NaOH solution is confirmed by NMR elucidation in the first time.

2.4. Sulfonation of identified *Monascus* pigments

Sulfonation of identified *Monascus* pigments (**2, 4-7)** followed a similar procedure for sulfonation of Red *Monascus Pigment*® [24]. Briefly, sodium dithionite (0.2 mmol) was added into a single *Monascus* pigment (**2, 4-7**, 0.1mmol) ethanol aqueous solution (50 %, V/V; pH 7; 20 mL), respectively. The ethanol aqueous solution was stirred and refluxed at 70 ^{o}C for 6 h. The solvent was evaporated to give crude product, which was separated by preparative TLC using chloroform/methanol (5/1, V/V) as developing solvent. No sulfonation reaction of ankaflavin (**4**) was observed under the same condition. According to Fig.2, compounds **2, 5-7** produced the corresponding WSYPs **8-11**. The corresponding HRMS data are deposited (ESI S8-S11). The ^{1}HNMR and ^{13}CNMR analysis of WSYP **11** were presented in table 1 and table 2, respectively. The chemical structures of WSYP **8-11** are reported for the first time.

Location of Fig.2

2.5. Color and stability of WSYPs

One milliliter of WSYP aqueous solution (pH 7; 1 g/L) was diluted by an aqueous solution (pH 7) to absorbance at the corresponding maximum absorbance wavelength approximately 1 absorbance unit (AU). Ultraviolet-visible (UV-vis) spectrum of the WSYP solution was recorded on a Shimadzu UV-2600 spectrometer. Each WSYPs (**8-11**) exhibited their own character absorbance in an aqueous solution (pH=7), such as compound **8** was corresponded to the maximum absorbance wavelength 500 nm; **9** to 470 nm; **10** to 470 nm; **11** to 455 nm. The influence of pH on color of WSYP aqueous solutions was examined as following: 2 ml WSYP aqueous solution (pH 7; 1 g/L) was diluted with 8 ml of Teorell Stenhagen buffer solution (pH=2-12, 0.2 M boric acid, 0.05 M citric acid, and 0.1 M tri-sodium orthophosphate; adjustment of pH with 1 M hydrochloric acid or 1 M sodium hydroxide solution). The change of absorbance at the corresponding maximum absorbance wavelength was regarded as the influence of pH on color of WSYP in aqueous solutions. In order to examine the influence of pH on the stability of WSYPs, those samples were further incubated at 30 ^{o}C. For a certain period of time, 3 ml of sample was fetched and the absorbance at the maximum absorbance wavelength was recorded. The decrease of absorbance was regarded as the stability of WSYP in the buffer solutions.

2.6. Analysis methods

Ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) analysis was performed on a Water ACQUITY UPLC system. An Acquity BEH C18 (100 mm×2.1 mm i.d., 1.7μm, Waters, Milford, USA) analytical column was used. The column was maintained at 50 ^{o}C and eluted with water (A)/acetonitrile (B) solvent system. The gradient eluting program was started from 10 % B, and changed to 50 % B within 5 min, then changed to 85 % B within 2 min, and changed to 100 % B within 2 min, then maintained 100 % B within 2 min, and changed to 10 % B within 0.5 min, and at last by equilibration at 10 % B for 2.5 min at a flow rate of 0.4 ml/min. MS was performed in positive ion model otherwise specified. High resolution mass spectrum (HRMS) analysis was carried out via Masslynx 4.1 software (Waters MS Technologies, Manchester, UK).

^{1}H Nuclear Magnetic Resonance (NMR) and ^{13}C NMR spectra were obtained on a Avance Ⅲ 400 MHz spectrometer (Bruker, Germany) with Tetramethylsilane (TMS) as the internal standard. All chemical shifts were given in ppm and coupling constants were given in Hz.

3. Results and discussion

3.1. Structural elucidation of WSYPs

Fig.1B shows the famous amination reaction of **2** to **5**. MS analysis of *m/z* 383.1863 $[M+H]^+$ is consistent with the formula $C_{23}H_{27}O_5$ of **2** while *m/z* 380.1853 $[M-H]^-$ with formula $C_{23}H_{26}NO_4$ of **5** (ESI S3 & S5). For structure elucidation and signal assignment, ^{1}H and ^{13}C spectra of **2**, **4**, **5**, **7** and **11** were recorded. The complete assignments for H and C signals were further given in Tables 1 and table 2, respectively. There is no significant difference between **2** and **5**, where the ^{1}H spectrum of N-H in **5** is undetectable owing to the hydrogen with high activity (table 1). The compound **5** was further reduced to produce **7** in an aqueous sodium hydroxide solution (Fig.1B). UPLC-MS/MS measurement reveals the difference between compound **5** and **7** is 2H (molecular mass 2.0163) (ESI S5 vs S7), which hints the possible reduction of **5** to **7**. Both the appearance of peak at 4.83 ppm (^{1}H spectrum of **7** in table 1) and the disappearance of peak at 195.30 ppm (^{13}C spectrum of **5** in table 2) reveal the replacement of the carbonyl at C-3 in compound **5** by a hydroxyl moiety in compound **7**. Similar structure with a hydroxyl moiety at C-3, i.e., reduction of OMPs as well as WSRPs by sodium borohydride, is also reported [9, 28].

According to Fig.2, various *Monascus* pigments are sulfonated into the corresponding WSYPs. As shown in Fig.3A, UPLC-MS/MS measurement reveals the difference between compound **7** and WSYP **11** is H_2SO_3 (molecular mass 81.9725) (ESI S7 vs S11). Furthermore, the corresponding fragments of **7** (*m/z* 366.2048,

$C_{23}H_{28}NO_3^+$, loss of a water; *m/z* 338.2104, $C_{22}H_{28}NO_2^+$, further loss of a carbonyl group) are also found in the fragments of **11** (*m/z* 448.1789, $C_{23}H_{30}NO_6S^+$, loss of a water, *m/z* 420.1835, $C_{22}H_{30}NO_5S^+$, further loss of a carbonyl group). This result further confirms that the difference between compound **7** and WSYP **11** is H_2SO_3. The ^1H and ^{13}C spectra are applied to identify the position of H_2SO_3 in WSYP **11** (Table 1 & 2). The spectrograms of **7** and **11** are significant differences at C-10 and C-11 position. Signals of the vinyl protons (6.38 ppm at position C-10 and 6.79 ppm at C-11) in the ^1H spectrum of **7** disappear while proton signals (1.96 and 2.11 ppm at position C-10 and 3.72 ppm at C-11) in the ^1H spectrum of **11** appear. This information indicates sulfonation reaction occurs at the double bond between C-10 and C-11 of compound **7**. Proton vicinal coupling constant analysis further confirms that HSO_3^- connects to the position C-11 of WSYP **11**. On the other hand, peak of 136.36 ppm at the position C-10 and that of 132.53 ppm at C-11 in ^{13}C spectrum of **7** are also replaced by peaks of 56.49 ppm and 60.70 ppm in ^{13}C spectrum of **11**, respectively. The chemical structure of novel WSYP **11**, i.e., addition of HSO_3^- at C-11 of *Monascus* pigments by sulfonation reaction, is reported for the first time. Although a chemical with the same molecular weight as compound **11** is also reported, other chemical structure is deduced due to the absence of NMR datum [24]. UPLC-MS analysis indicates that the fragment of H_2SO_3 group is also added into the corresponding *Monascus* pigments for formation of WSYP **8**-**10** (Fig.3B). On the contrary, no sulfonation reaction occurs to ankaflavin (**4**).

Location of Fig.3

3.2. Mechanism for sulfonation of *Monascus* pigments

Dithionite anion $S_2O_4^{-2}$ can be decomposed into bisulfate (HSO_3^-) and thiosulfate ($S_2O_3^{-2}$) in an aqueous solution [29]. The conjugated double bonds of *Monascus* pigments (**2**, **5**, **6** and **7**) are adjacent to a carbonyl group (Red color in Fig.2). Those *Monascus* pigments may be tautomerized to the corresponding isomer **I**. The isomer **I** involves conjugated double bonds adjacent to a hydroxyl group (Green color in Fig.2). Bucherer reaction, i.e., addition of a nucleophilic bisulfate anion at *meta*-C in α-naphthol to form the corresponding sulfonated product, shows that conjugated double bonds adjacent to a hydroxyl group in α-naphthol is the key to sulfonation reaction (ESI S12) [30]. Following the same mechanism of Bucherer reaction, the reaction mechanism for sulfonation of *Monascus* pigments is proposed (Fig.2). The isomer **I** gives Ⅱ via an electrophilic addition of a proton at C-12. Ⅱ tautomerizes to form the resonance-stabilized proton adduct Ⅲ. A nucleophilic bisulfate anion adds to the proton adduct Ⅲ at C-11 to obtain Ⅳ and then tautomerizes to the corresponding lower-energy form (**8**-**11**). This mechanism indicates conjugated double bonds adjacent to a carbonyl group is necessary for sulfonation of *Monascus* pigments. This principle is consistent with the facts that sulfonation reaction occurs for monascorubrin

(**2**), WSRPs **5** & **6**, as well as reduced product **7** while not for ankaflavin (**4**).

3.3. Color and pH stability of WSYPs

Sulfonation reaction introduces H_2SO_3 into the double bond between C-10 and C-11 of *Monascus* pigments (Fig.2). The reduction of π-conjugated system in WSYPs leads to blue shift in ultraviolet-visible absorbance spectrum. The visible spectrum of every WSYPs as well as the picture of the corresponding pigment aqueous solution was presented (Fig.4A). Each WSYP has the corresponding maximum absorbance wavelength as well as extinction coefficient (**8**, UV (H_2O) λ_{max} (log ε) 500 (3.9); **9,** UV (H_2O) λ_{max} (log ε) 470 (3.6); **10,** UV (H_2O) λ_{max} (log ε) 470 (3.7); and **11,** UV (H_2O) λ_{max} (log ε) 470 (3.6)). Most of WSYPs in aqueous solution exhibit orange-yellow while WSYP **11** exhibits bright yellow in an aqueous solution (pH=7). Compounds **9** and **11**, as well as their reactants **5** and **7**, show very different colors and UV spectra, which may be attributed to the different conjugation lengths in the corresponding π-conjugated system. The influence of pH on color of WSYPs was examined by recording the absorbance at the corresponding maximum absorbance wavelength in different pH buffer solutions (Fig.4B). WSYP **10** and **11** maintained the original color within a wide pH range. The color of WSYP **8** aqueous solution was relatively stable at pH 4-11 while got yellow color at pH below 3. The strong influence of pH on color of WSYP **9** aqueous solution was exhibited as a marked change of the absorbance in the buffer solutions. The influence of pH on stability of these WSYPs was further examined (Fig.4C). After incubation for 1 day, most of WSYPs (except for **9**) were stable within a wide pH range. This trend about the influence of pH on WSYP stability was further confirmed by the prolonged incubation time for 2 days (ESI S13). The results indicate that the color as well as the stability of WSYPs is strongly dependent on their chemical structure, such as X or Y group as shown in Fig.2. WSYPs (**8**-**11**) also exhibit good thermal stability in an aqueous solutions because the WSYPs are obtained by reflux reaction at 70 ^{o}C during the preparative process.

Location of Fig.4

4. Conclusions

Monascus pigments with conjugated double bonds adjacent to a carbonyl group can be sulfonated to produce novel WSYPs by addition of H_2SO_3 at the double bond between C-10 and C-11 of *Monascus* pigments. The introduction of H_2SO_3 into *Monascus* pigments makes the novel WSYPs exhibit yellow color, high water solubility, as well as relatively high stability in aqueous solution within a wide pH range. All those characters should contribute WSYPs as a potential novel food colorant. Structure diversity of WSYPs and the corresponding toxicity

test are the future project in our lab.

Acknowledgment

This work was financially supported by Natural Science Foundation of Shanghai (No. 16ZR1416700).

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Table 1. [1]H NMR Assignments of 2, 4, 5, 7 and 11 (δ, ppm; *J*, Hz) [a]

Position	2 (chloroform-*d*)	4 (chloroform-*d*)	5 (chloroform-*d*)	7 (methanol-d_4)	11 (DMSO-d_6)
1	7.86 (s)	5.01 (d, 12.6) 4.68 (d, 12.6)	9.27 (s)	7.79 (s)	8.25 (s)
3				4.83 (s)	4.55 (s)
5		2.43 (dd, 17.6, 11.6)			
6	6.88 (s)	3.20 (dt, 4.2, 13.2) 2.97 (dt, 7.4, 18.1)	6.77 (s)	7.00 (s)	6.79 (s)
8	6.14 (s)	5.25 (s)	6.76 (s)	6.65 (s)	6.43 (s)
10	6.04 (d, 14.3)	5.88 (dd, 1.6, 15.4)	6.36 (d, 15.9)	6.38 (d, 15.9)	2.11 (dd, 1.2,12.2) 1.96 (dd, 7.9,15.8)
11	6.57 (m)	6.49 (m)	7.04 (m)	6.79 (m)	3.72 (m)
12	1.94 (d, 7.0, 1.7)	1.84 (dd, 7.0, 1.4)	2.04 (d, 6.0)	1.99 (dd, 6.8, 1.6)	1.08 (d, 13.5)
13	1.70 (s)	1.42 (s)	1.80 (s)	1.21 (s)	1.45(s)
15		3.69 (d, 13.3)			
17	2.92 (m)	2.62 (m)	2.87 (m)	2.80 (m)	2. 58 (m)
18	1.59 (m)	1.58 (m)	1. 65 (m)	1.60 (m)	
23	0.86 (t, 7.1)	0.84 (t, 6.8)	0.85 (t, 6.8)	0.90 (t, 6.9)	0.81 (t, 6.8)
N			*	*	5.29 (s)

[a] s, singlet; d, doublet; t, triplet; m, multiplet.

*: Undetectable peak of the active hydrogen in [1]HNMR spectrum due to its volatility.

Table 2 ^{13}C NMR Assignments of 2, 4, 5, 7 and 11 (δ, ppm)

Position	2 (chloroform-*d*)	4 (chloroform-*d*)	5 (chloroform-*d*)	7 (methanol-d_4)	11 (DMSO-d_6)
1	153.00	63.74	138.37	123.00	124.50
2	109.77	135.37	100.95	128.70	130.11
3	191.00	189.86	195.30	71.89	61.66
4	85.97	103.29	86.77	113.78	72.73
5	169.41	28.91	153.64	167.56	174.82
6	113.40	42.97	98.53	97.09	118.51
7	141.82	150.92	140.83	146.82	139.61
8	104.35	83.19	86.77	82.52	84.84
9	156.61	160.45	147.55	153.61	171.45
10	116.48	113.87	123.28	136.36	56.49
11	136.59	124.36	117.53	132.53	60.70
12	18.96	17.70	19.25	17.72	19.03
13	28.52	18.44	29.19	17.54	15.59
14	171.83	169.62	174.24	174.50	174.92
15	122.55	54.82	116.20	98.49	96.42
16	197.60	202.50	196.32	197.45	209.08
17	41.85	42.97	40.46	39.24	53.57
18	23.91	23.05	24.89	25.30	29.16
19	29.38	29.38	29.66	29.29	31.75
20	29.38	28.98	29.55	28.92	29.47
21	31.92	31.59	31.80	31.61	35.57

22	22.83	22.54	22.64	22.22	22.56
23	14.30	14.01	14.12	13.02	14.43

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381 **List of figures**

382 **Fig.1 Preparation of *Monascus* pigments**

383 **A**: Native *Monascus* pigments. OMPs including two entities rubropunctatin (**1**) and monascorubrin (**2**) while YMPs
384 including monascin (**3**) and ankaflavin (**4**); **B**: Diversifying of monascorubrin by chemical modification. One of
385 OMPs, monascorubrin (**2**), is chemical modification into WSRPs (**5**, **6**) by amination reaction, where
386 GLU=glutamic acid. The WSRP **5** is further reduced into **7** in 1 M NaOH aqueous solution

387 **Fig.2 Proposed mechanism for sulfonation of *Monascus* pigments**

388 *Monascus* pigments, including **2** and **5-7**, having the characteristic structure of conjugated double bonds adjacent to
389 carbonyl group (Red color), can be tautomerized to give the corresponding isomer **I**. The isomer **I,** having the
390 characteristic structure of conjugated double bonds adjacent to a hydroxyl group (Green color), can be sulfated
391 following the same mechanism as that of Bucherer reaction (ESI S12).

392 **Fig. 3 UPLC-MS/MS analysis of sulfonated product WSYPs**

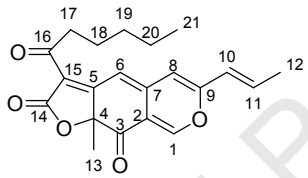
393 **A**: Fragments involving in compound **7** and **11** (ESI S7 vs S11); **B**: The fragment H_2SO_3 involving in WSYPs **8-10**
394 (ESI S3vs S8, S5 vs S9 and S6 vs S10), where HRMS analysis is performed in negative ion model for compound **5**
395 while in positive ion model for the others.

396 **Fig. 4 Colorant characters of WSYPs**

397 **A**: Visible spectra of WSYP in aqueous solutions (pH=7) (inserted the pictures of WSYP in the aqueous solution).
398 **B**: Influence of pH on the absorbance of WSYPs (measured at the corresponding maximum absorbance wavelength
399 as shown in Fig.4A). **C**: Stability of WSYPs in an aqueous solution at different pH values (incubation in 30 ^{o}C for
400 1 day).

401
402

Fig.1



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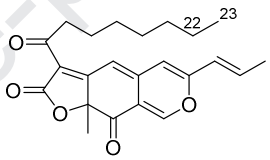

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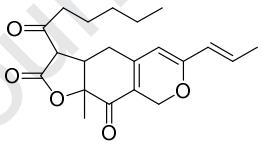

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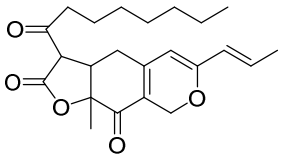

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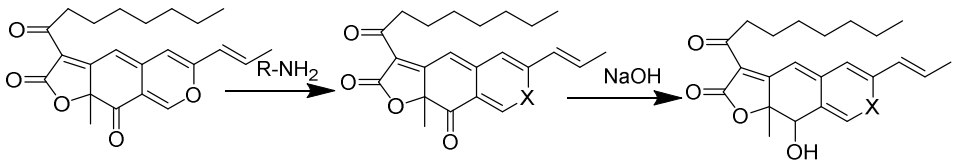

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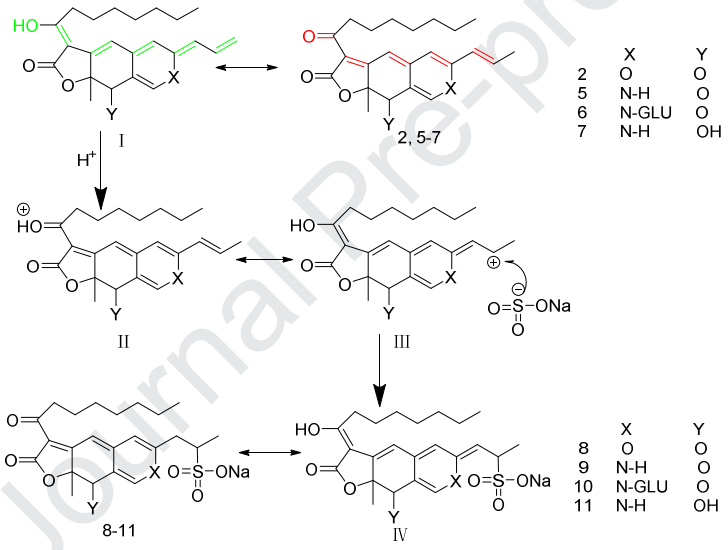
B

Fig.2



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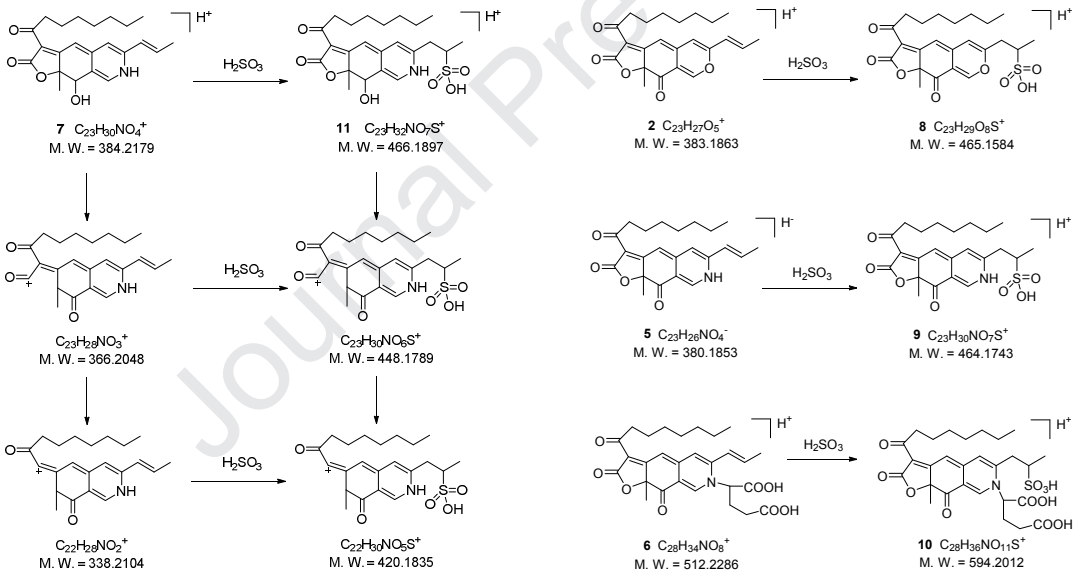
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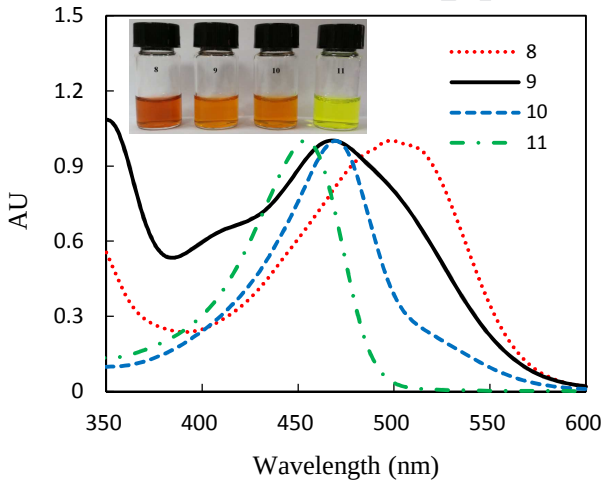
A B

Fig.4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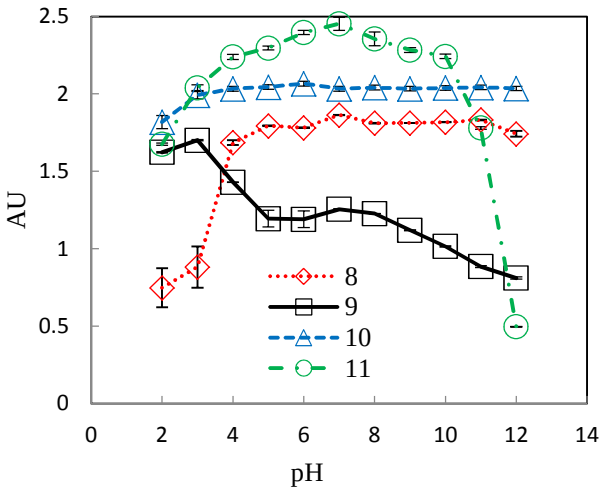
9
10
11

A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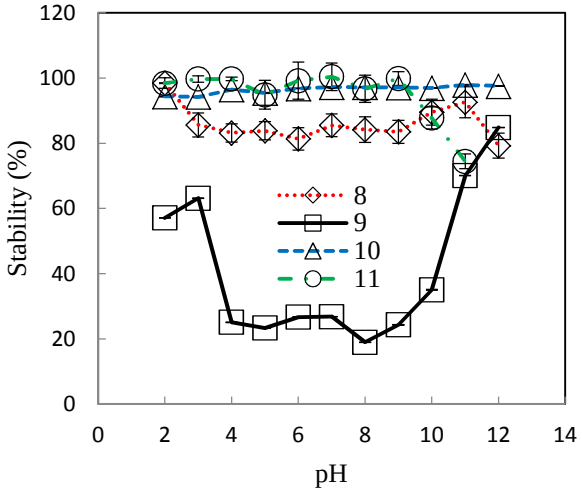
10
11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100
80
60
40
20
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Stability (%)
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2
4
6
8
10
12
14
pH
8
9
10
11

485 B C

Highlights

- Sulfonation of *Monascus* pigments with a conjugated double bonds adjacent to a carbonyl group
- Production of WSYPs) by addition of H_2SO_3 at the double bond of *Monascus* pigments
- WSYPs exhibiting yellow color, high solubility in water as well as pH stability
- Dependence of the colorant character of WSYPs on the structure of *Monascus* pigments

Declare

The authors declare no competing financial interest