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Identification of new red pigments produced by *Monascus ruber*

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Abstract

A new monascus red pigment, which was produced in a defined medium with rice and hydrolytic bean powder was identified by IR, UV, NMR and ESI-MS. It was free pigments linked to lysine by amino group, where nitrogen replaced the pyronoid oxygen without hydrophilic side chain.

Keywords: New red pigments; Monascus; Mass spectra; Hydrolytic bean powder

1. Introduction

Monascus species have been utilized for making red rice wine, red soy bean cheese and monascus pigments (red rice and monascus red pigments). Presently, they are mainly used to produce monascus pigments coloring meat products. Ten kinds of red pigments have been isolated and identified from monascus pigments (Fig. 1) [1–8], but the structures of monascus red pigments industrially produced in China have seldom been revealed.

The color specification of monascus red pigments depended greatly on the amino acid or protein with which the pigment was associated. In China, the medium of submerged fermentation is mainly made up of rice powder and hydrolytic bean powder, a very economical medium. Our objective is to obtain pure monascus red pigments produced with the above medium and to identify its chemical structure (Fig. 2) [9].

2. Materials and methods

2.1. Microorganism and media

Monascus ruber 102w was a high pigment-producing strain, identified as *M. ruber* van Tieghem by CAS (China).

The inoculum medium contained malt extract 5 g, yeast extract 3 g, glucose 5 g, and agar 1 L^{-1} of ultra-pure water. The initial pH of the medium was 6.0. The seeding culture medium contained rice powder 40 g, KH_2PO_4 2.5 g, $NaNO_3$ 3 g, $MgSO_4 \cdot 7H_2O$ 4 L^{-1} of de-ionized tap water, the initial pH of the medium was adjusted to 4.5 with lactic acid. The fermentation medium contained, rice powder 80 g, hydrolytic bean powder 20 L^{-1} of de-ionized tap water, the initial pH of the medium was adjusted to 3.5 with lactic acid.

2.2. Cultivation methods

Spores of strain were prepared by growing on the inoculum medium for 7 days at 32 °C. Spores were washed with sterile water. A suspension of 10^8 spores was inoculated into 50 mL seeding medium in a 500 mL flask, then was incubated at 32 °C on a rotary shaker (160 rpm) for 2 days, and then was transferred to a 5 L fermentor containing 3 L of fermentation medium at 32 °C, with rotation speed of 180 rpm for 3 days.

Abbreviations: MS, mass spectrometry; NMR, nuclear magnetic resonance.

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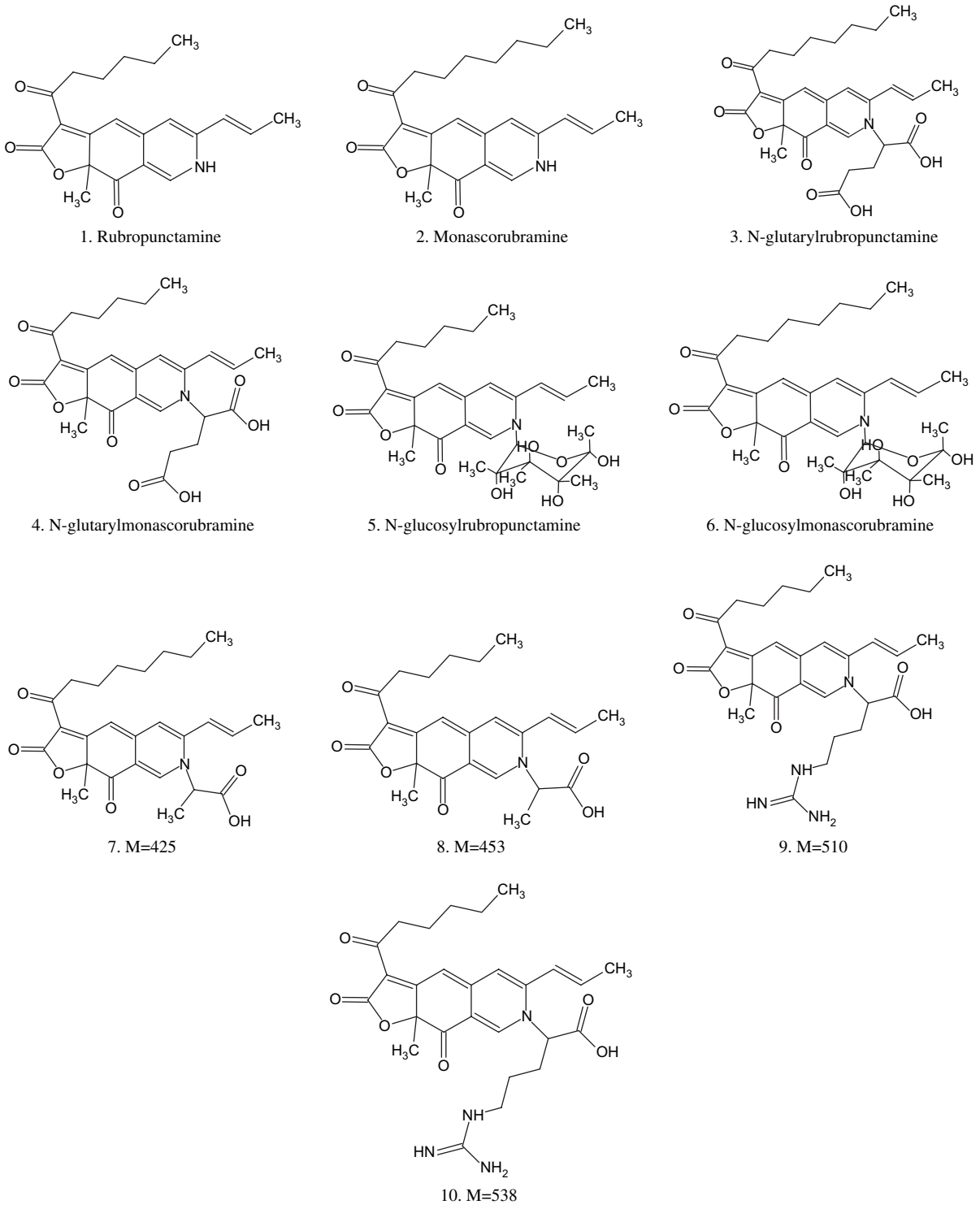

Fig. 1. Molecular structures of known monascus red pigments.

2.3. Preparation of monascus red pigments

The mycelium was separated from the culture broth by a filtration membrane (80 hole/cm^2), then the filtrate was lyophilized. Red pigments were extracted from the lyophilized powder (crude extract) with hexane, ethyl acetate, and methanol in sequence.

2.4. Analytic methods

2.4.1. Absorption spectra of new red pigment

The maximum absorbance of new red pigment was determined by UV-2501pc, UV–vis recording spectrophotometer (Shimadzu, Japan).

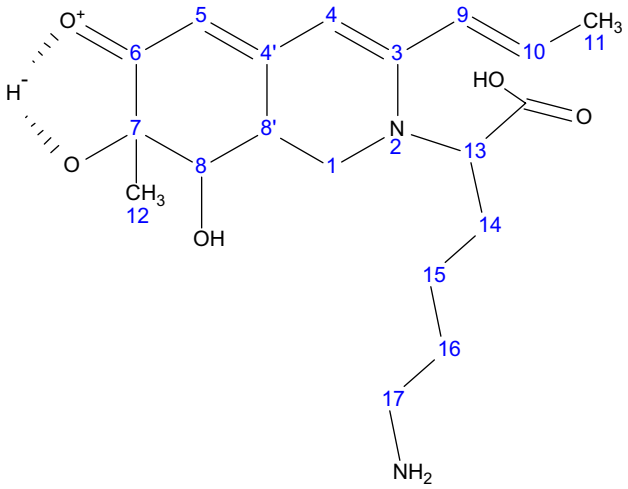

Fig. 2. Molecular structure of the new monascus red pigment.

2.4.2. IR spectrum

The dried crystal of the new red pigment was scanned by a Bio-Rad FES135 infrared spectrometer using KBr method at 27 °C.

2.4.3. ESI-MS

The main peak of yellow pigments was chosen and its molecular weight was determined by ESI (electro-spray ionization)-MS (Finnigan MAT LCQ™, Liner Scientific Company, USA). For the mass spectrometer, the following parameters were used: vaporizer temperature at 300 °C, heated capillary serving simultaneously as repeller electrode (20 V) at 180 °C, corona voltage 4 kV, and electro-multiplier voltage 1.6 kV. Nitrogen served as both sheath (50 psi) and auxiliary gas, and argon served as collision gas at a pressure of 1.9 mTorr. The mass spectrometer was operated in full ion scanning, which was chosen to detect positive ions at *m/z* of 365.2 (new monascus red pigment), for total scan durations of 1.05. The offset voltage was −15 V. The ions represented the protonated molecular ion $(M + H)^+$ for the new monascus red pigment.

2.4.4. ^1H NMR

The samples were injected into NMR spectra apparatus (Varian Mercury V × 300, Varian Company, USA) with the following conditions: pulse sequences 2 pul; solvent, D_2O; ambient temperature; Mercury-300BB"nk300"; relax delay, 1.000 s; pulse, 45.0°; acq. time, 1.997 s; width, 4803.1 Hz; repetitions, 16; observed H, 300.0771372 MHz; data processing line broadening, 0.2 Hz; FT size, 32768; and total time, 53 s.

3. Results and discussion

3.1. UV spectra of the new monascus red pigment

The absorbance of the new monascus red pigments in deionized tap water is illustrated in Fig. 3, and its maximum absorption wave was observed at 484 nm, which is similar to that reported in literature [5,6].

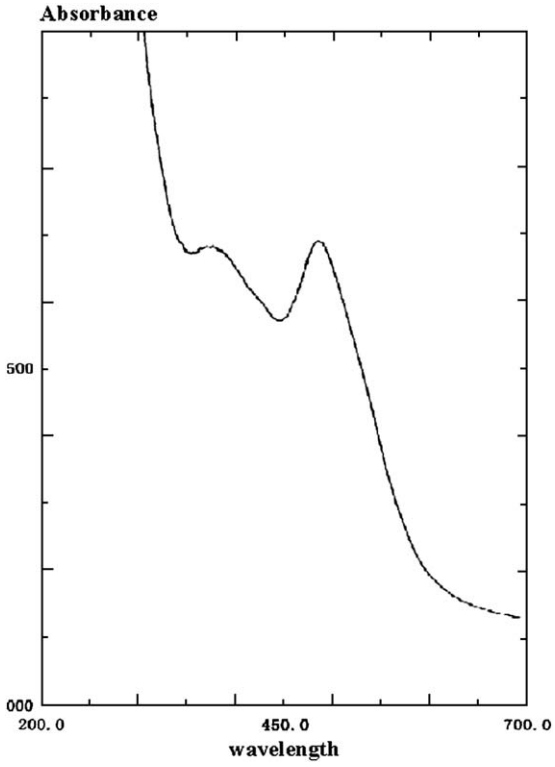

Fig. 3. UV spectra of the new monascus red pigments.

3.2. Chemical structure of the new monascus red pigment

Chemical structure of the new monascus red pigment is shown in Fig. 2, it consists of one molecule of lysine substituted to polyketide chromophore and without hydrophilic side chain, capryl (C7) or capryl (C5).

Amino acids addition may lead to conversion of the pyronoid oxygen of monascorubrie or Rubropunctatin into an NH group [7,8]. But for lysine, which is present in hydrolytic bean powder, it reacts with polyketide chromophore directly before monascorubrie or Rubropunctatin forms by combination of polyketide chromophore and β-ketoacid.

3.2.1. Mass spectrum analysis

Electro-spray mass spectrometry of the new monascus red pigment showed a large peak at *m/z* 365.2 (Fig. 4) that is consistent with the $(M + H)^+$ ion of the compound.

3.3. Infrared spectrum analysis

Fig. 5 shows the infrared spectrum of the new monascus red pigment. The main absorbance peaks included 3303.7, 2928.04, 2733.71, 1715.16, 1596.89, 1453.97, 1210.01, 1050.02, and 791.562. The peaks at 3303.7, 1210.01, and 1050.02 suggested a hydroxide bond in molecules. The peaks at 23303.7 and

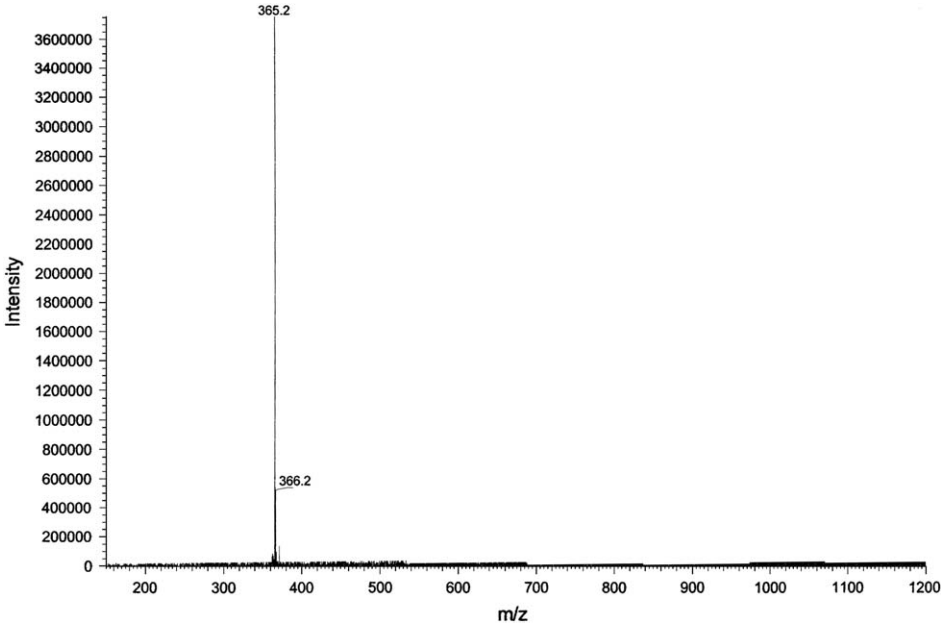

Fig. 4. MS of the new monascus red pigment.

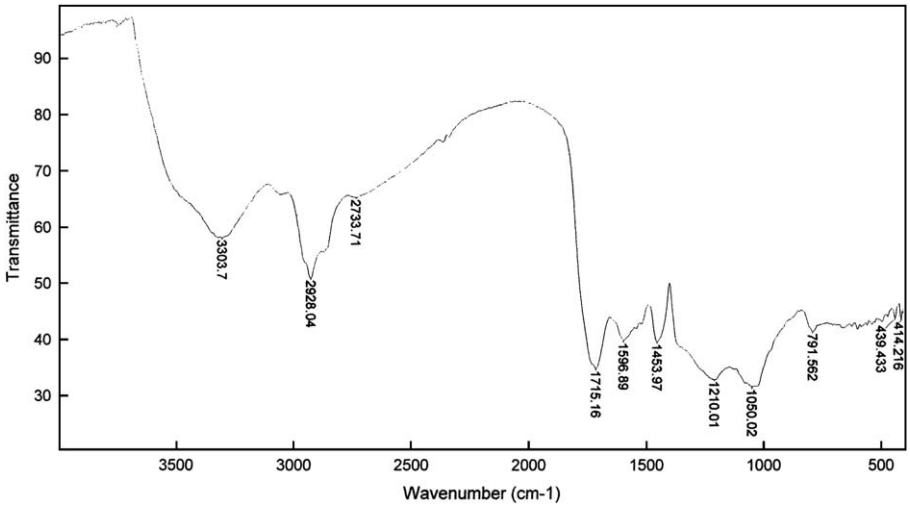

Fig. 5. Infrared spectra of the new monascus red pigment.

1596.89 indicated that there might be NH groups present. The peak at 2928.04 is very sharp, indicating that there were multiple CH_2 groups present. The peaks at 1715.16, 1596.89 and 1453.97 indicate the presence of benzol structure and CH_3 group.

3.4. NMR analysis

Assignments of the ^1H NMR resonances of the new monascus red pigment were obtained by using Varian spectra (Fig. 6) recorded with a mixing time of 53 s in order to detect direct and relay through-bond connections. The resonance at 4.428 and 4.455 pointed to the chemical shift position of lysine, while disappearance of resonance at 0.76, 1.20, 1.46 and 2.69 showed absence of the hydrophilic side chain. All resonances of the new monascus red pigment are shown in Table 1 and Fig. 6.

4. Discussion

The structure of the new monascus red pigment indicates that the synthesis of the new monascus red pigment might start with the restraint of the esterification course between β-ketoacid and polyketide chromophore, other than after formation of

Table 1
δ_H of the new monascus red pigment

Carbon	δ_H	Carbon	δ_H
1	3.009	10	5.204
4	3.436	11	1.139
5	3.560	12	1.116
8	3.719	13	4.428
8′	2.281	14–17	4.455
9	5.217		

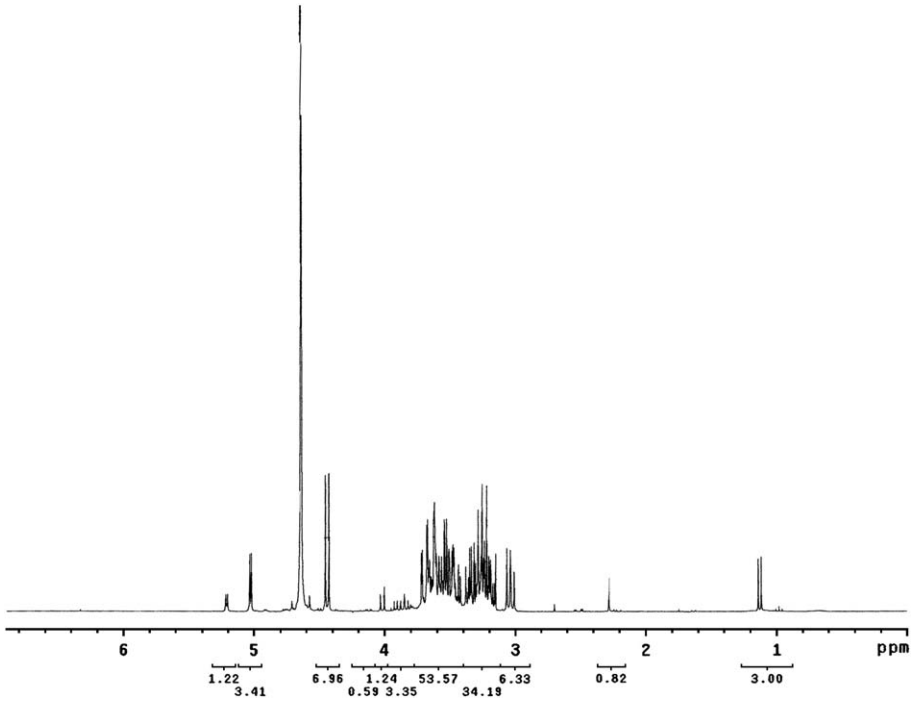

Fig. 6. ^{1}H NMR of the new monascus red pigment.

orange pigments, lysine in hydrolytic bean powder may incorporate into polyketide chromophore directly to form new monascus red pigment.

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