

Two new monascus red pigments produced by Shandong Zhonghui Food Company in China

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Abstract Different monascus red pigments were produced by many companies in China with their own strains, but most of them are rarely identified. Two new monascus red pigments of the homologous series were isolated from commercial monascus red pigments produced by Shandong Zhonghui Food Company in China, and their structures were identified by visible light absorbance, Fourier transform infrared, Nuclear magnetic resonance, Electrospray ionization–mass spectrometry and X-ray diffraction. Their molecular weights were 303.5 and 331.5, respectively. Their molecular formulas were $C_{18}H_{25}NO_3$ and $C_{20}H_{29}NO_3$. Their λ_{max} are observed at 426.0, 493.5 nm. They are characterized by the absence of lactone ring, carbonyls and methyl, the presence of hydroxyls and the existence of double bonds at the end of the aliphatic side chains. The characteristic X-ray diffraction pattern of

the new monascus red pigments is the strong diffraction peaks at 31.62° , 45.34° and 56.40° 2θ and the weak ones at 27.28° , 66.08° and 75.20° 2θ . The findings in the paper have inferred that many monascus red pigments might be synthesized in the half way of well-known red pigments synthesis. Those two pigments have potential application in beverages.

Keywords Monascus red pigments · IR · NMR · ESI-MS

Abbreviations

IR Infrared ray spectrum
ESI-MS Electrospray ionization–mass spectrometry
NMR Nuclear magnetic resonance

Introduction

Monascus red pigments are natural colorants widely used in the field of food industry in China. The largest consumption of the pigments is for sausage to strengthen the light pink color due to insufficient content of meat. Many companies such as Shandong Zhonghui Food Company, Tianyi Biological Engineering Company Limited (Dongguan, Guangdong province, China), Nanping Lvfang Red Rice Limited Company etc. in China could produce monascus pigments with their own strains. Up to now, there are about 31 kinds of monascus red pigments isolated and identified by different researchers [1–12], but only one kind of them is from China [7]. We have found many kinds of monascus red pigments in commercial productions prepared by different manufactures in China by ESI-MS. In this paper, two new monascus red pigments isolated from Shandong Zhonghui Food Company are identified by visible absorbance spectrum, IR, NMR and ESI-MS.

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Materials and methods

Materials

The monascus red pigment (powder) was purchased from Shandong Zhonghui Food Limited Company. The strain to produce this pigment was *Monascus ruber*, identified as *Monascus ruber* van Tieghem by CAS (China). The chemicals and reagents such as dichloromethane, ethanol, petroleum ether, hexane, ethyl acetate and methanol etc. were offered by Tianjin Hongqiao Chemicals Co., Ltd.

Isolation of monascus red pigments

The monascus red pigments were extracted from the powdery samples in Soxhlet extractor with hexane, ethyl acetate and methanol in sequence. The yellow and orange pigments were removed by hexane and ethyl acetate washing. Then the red pigment was washed by methanol. The purified red pigments in methanol were dried in room temperature. The dried pigments were further separated by two dimensional thin-layer chromatography (TLC) with eluent of ethanol/petroleum ether (3:7) and methanol/dichloromethane (1:1) to remove metabolites with same adsorbability. At last, the spots of the red pigments were scrapped off, extracted with methanol and dried at room temperature. The two red pigments were not identified independently.

Analytic methods

Visible absorption spectra of the new red pigments

The purified red pigments with weight of 5 μg were dissolved into deionized water, and their maximum visible absorbance was determined by UV-2501pc, UV-Vis recording spectrophotometer (Shimadzu Japan).

IR spectra

To perform FT-IR measurement, the dried samples with weight of 5 mg were first ground into powder and then dispersed in 200 mg KBr (pellet procedure). KBr-pelletized starch samples were analyzed using a Bio-Rad FES135 infrared spectrometer (Bio-Rad Laboratories, Inc., USA) in the range 4,000–400 cm^{-1} at 27 °C. The IR spectra for starch treated with and without alkali protease were recorded on a diamond plate with 50 scans and a resolution of 4 cm^{-1} .

ESI-MS

The main peak of red pigments was collected, and its molecular weight was determined by electrospray ionization (ESI)-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, 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 m Torr. The mass spectrometer was operated in full ion scanning, which was chosen to detect positive ions at m/z of 304.5/332.5 (new monascus red pigments), 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 pigments.

NMR

The ^1H NMR of the samples has been determined according to the following procedure. A sample of

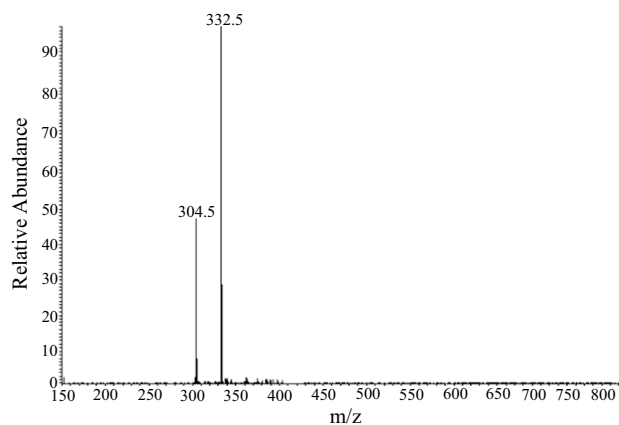


Fig. 1 ESI-MS of two new monascus red pigments with molecular weight of 303/331

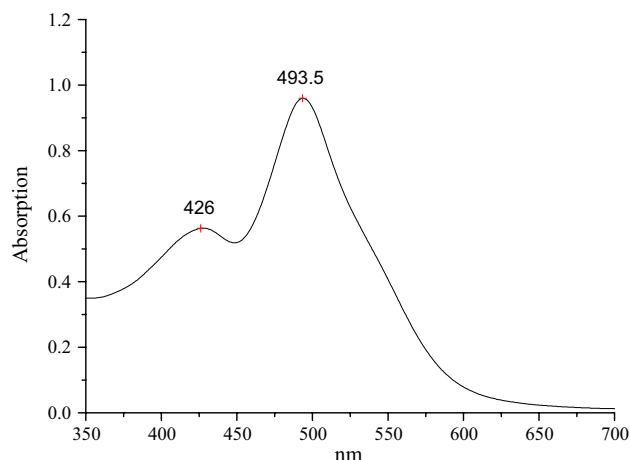


Fig. 2 Visible absorbance of the new monascus red pigments with molecular weight of 303/331

dried red pigments is put into deuterated water (D_2O) at 1.5–5.0 w/w %. The mixture is heated in a water bath (60 °C) and shaken till clear homogeneity. NMR spectra of these samples were recorded on a Mercury Vx-300 MHz machine (Varian, USA) operating at 300.07 MHz for the 1H nucleus, 75.45 MHz for the ^{13}C nucleus, with a 45.0° pulse and a relaxation delay time of 1.0 s.

X-ray diffraction patterns

The X-ray diffractograms of the monascus red pigments were obtained by a D-500 Siemens X-ray diffractometer (Madison, WI, USA). The diffractometer was operated at 27 mA and 50 kV. The scanning region of the diffraction angle (2θ) was from 5 to 60 at 0.04 step size with a count time of 2 s. Retrograded potato starches treated by different methods were equilibrated at 100 % relative humidity for 24 h at 25 °C prior to examination.

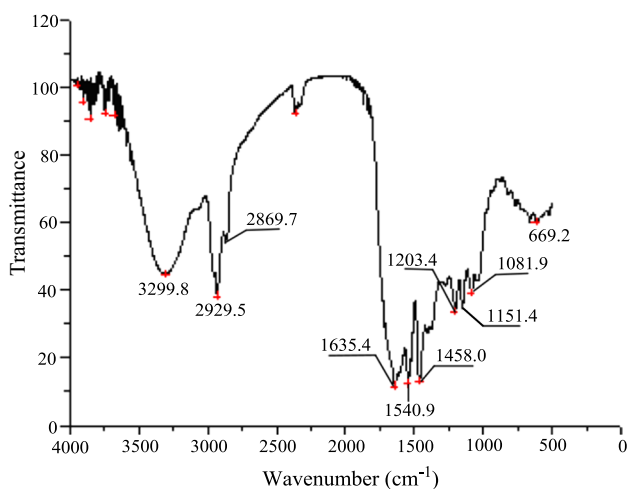
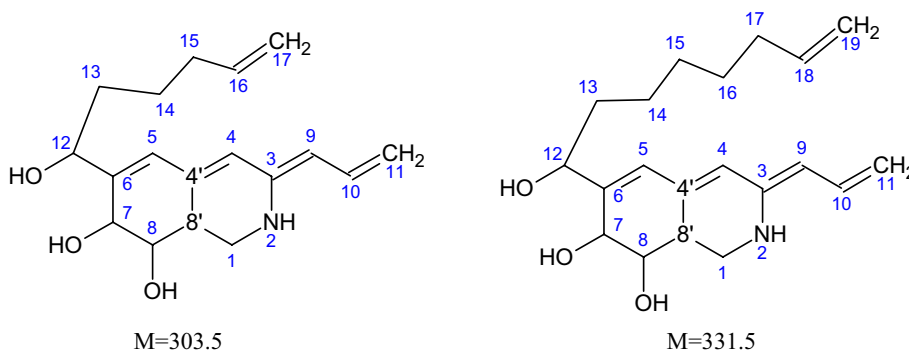


Fig. 3 IR spectrum of new monascus red pigments with molecular weight of 303/331

Fig. 4 Possible structure of the new monascus pigment isolated from Shandong Zhonghui Company in China



Results and discussion

Mass spectrum analysis

Electrospray mass spectrometry of the new monascus red pigments shows two large peaks at m/z 304.5 and 332.5 $[M + H^+]$ (Fig. 1). There are two aliphatic side chains with a difference of two methylenes in those two red pigments, which is similar to that of Refs. [1, 3, 5, 6, 8, 11–18]. The molecular weights of those two new pigments are obviously smaller than those of Refs. [1, 4–8, 11–18].

Visible spectrum of the new monascus red pigments

The visible absorbance of the new monascus red pigments in de-ionized tap water is illustrated in Fig. 2, and their maximum absorbance peaks (λ_{max}) are observed at 426.0, 493.5 nm, which are a bit smaller than those of rubropunctamine and monascorubramine (508 nm) reported in literature [8, 11] and those of other monascus red pigments newly found [12–18]. So the conjugated double linkages in phenyl rings of the new red pigments might be less than those of rubropunctamine and monascorubramine. There are two maximum absorbance peaks in the visible spectrum of the new red pigments, indicating that two kinds of conjugated structure probably exist in the pigments. This character of two absorbance peaks in visible spectrum is very similar to amino acids derivatives of monascus pigments, especially from cysteine [4].

Infrared spectrum analysis

Figure 3 shows the infrared spectrum of the new monascus red pigments. The main IR peaks include 3,299.8, 2,929.5, 2,869.7, 1,635.4, 1,540.9, 1,458.0, 1,203.4, 1,151.4, 1,081.9 and 669.2 cm^{-1} . The peak at 3299.8 cm^{-1} represents the O–H stretching vibration, which might be from pigments or water. Two absorption peaks at 2,926 and 2,856 cm^{-1} are assigned to vibrations of $-CH_2-$, which

are the same as indicated by literature [9]. Also, the peak at $1,458.0\text{ cm}^{-1}$ corresponds to C–H deformation vibration of $-\text{CH}_2^-$, confirming the presence of methylene in the new red pigments. The medium peak at $1,635.4\text{ cm}^{-1}$ is attributed to C=C stretching vibration on the benzene or O–H deformation vibration or N–H deformation vibrations. The medium peak at $1,540.9\text{ cm}^{-1}$ might represent C=C stretching vibration of aliphatic side chains [6]. The weak peaks at $1,203.4$, $1,151.4$, $1,081.9$ and 669.2 cm^{-1} probably correspond to C–C stretching vibration, C–C rocking vibration, C–N stretching vibration and C–H wagging vibration of R–CH=CH₂. Absence of peaks around $1,710$, $2,960$ and $1,380\text{ cm}^{-1}$ indicates that there is no methyl or carbonyl in the new red pigments [2, 7]. The absence of the peak corresponding to the lactone carbonyl vibration at $1,760\text{ cm}^{-1}$ also infers none of the lactone ring in the new red pigments.

Chemical structure of the new monascus red pigment

According to the data of ESI-MS and IR spectra, the chemical structures of the two new red pigments are deduced in Fig. 4. The characteristics of those two pigments were the absence of lactone ring and carbonyls, the presence of hydroxyls and the existence of double bonds at the end of the aliphatic side chains. The replacement of carbonyls by hydroxyls increases the hydrophilicity of the pigments and extends their application in liquid foods. The *monascus*

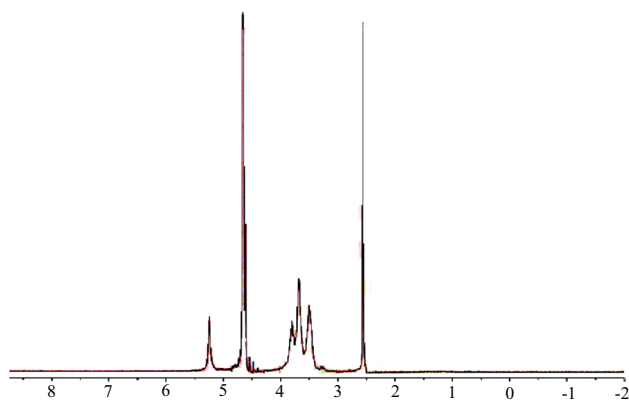
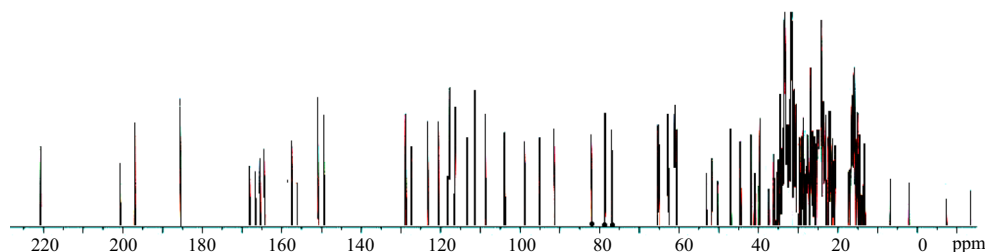


Fig. 5 ^1H NMR of the two new monascus red pigments

Fig. 6 ^{13}C NMR of the new monascus red pigments



producing those pigments might synthesize some kind of enzyme to convert carbonyl into hydroxyl. But in literature [7], also a kind of hydrophilic monascus red pigment in China, its strain might produce enzyme to limit the synthesis of hydrophilic side chain: capryl (C7) or capryl (C5) [19]. The synthesis of the new red pigments might be partly same to that of monasfluol in Ref. [20]. The identification of the newly genes to control synthesis of monascus red pigments in *Monascus ruber* M7 will help us to know the mystery of *Monascus* metabolism [21, 22].

NMR analysis

Assignments of the ^1H NMR and ^{13}C NMR resonances of the two new monascus red pigments are in Figs. 5, 6 and Tables 1, 2. In contrast to literature [1, 3, 5, 6, 8, 11], there is no resonance at 0.76 ppm in the ^1H NMR and at 20 ppm in the ^{13}C NMR of the new red pigments in Tables 1 and 2, indicating the absence of methyl, which agrees with results of IR. The ^1H NMR and ^{13}C NMR resonances of carbons (7, 8 and 12) linked to hydroxyl are at 3.49 – 3.70 and 61 – 69 ppm , respectively, which are the same as indicated by literature [2, 9]. Other resonances have been assigned reasonably.

X-ray diffraction analysis

The result in Fig. 7 shows the X-ray diffraction pattern of the new monascus red pigments. Although the pigment sample is not a monocrystal, its X-ray diffraction pattern is a standard crystal diffraction, and the characteristic of its pattern is the presence of strong diffraction peaks at 31.62° , 45.34° and 56.40° 2θ and weak ones at 27.28° , 66.08° and 75.20° 2θ . This is the first X-ray diffraction spectrum of monascus red pigments, which infers that it is possible to prepare monocrystal of monascus red pigment. The typical crystal X-ray diffraction of the new red pigments is possible formed by the interaction of hydroxyls of the pigments.

Conclusion

The structures of the two new monascus red pigments indicate that the strain to produce those two red pigments could

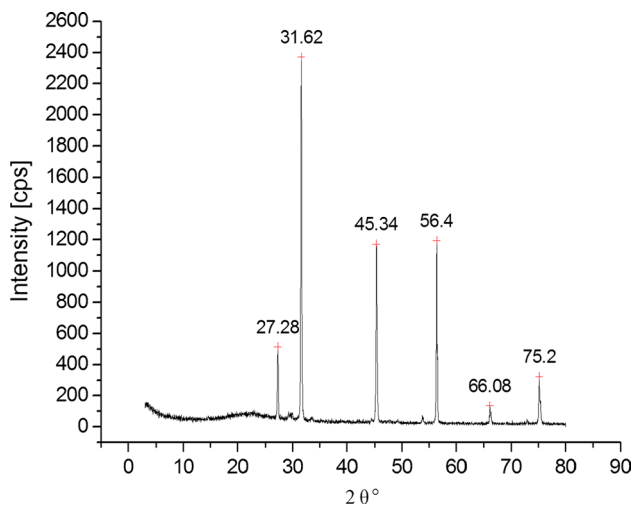
Table 1 H δ of the new monascus red pigment

Carbon	H δ	Carbon	H δ
1	3.49–3.70	10	5.23
3	3.04	11	4.59–4.66
4	5.23	12	3.49–3.70
4'	5.23	13–14 ($M = 303.5$)	2.55
5	5.23	15–17 ($M = 303.5$)	4.59–4.66
7	3.49–3.70		
8'	3.49–3.70	13–16 ($M = 331.5$)	2.55
9	5.23	17–19 ($M = 331.5$)	4.59–4.66

Table 2 C δ of the new monascus red pigment

Carbon	C δ	Carbon	C δ
1	58.8 (58.6)	10	163.7 (163.9)
2	–	11	125.6 (125.9)
3	184.4 (178.9)	12	63.6 (64.7)
4	166.4 (166.8)	13	37.9 (37.5)
4'	159.7 (158.5)	14	31.4 (25.5)
5	157.5 (156.9)	15	54.3 (22.7)
6	127.0 (127.2)	16	119.9 (31.4)
7	68.9 (69.3)	17	121.8 (120.6)
8	61.3 (63.2)	18	(120.1)
8'	59.5 (58.9)	19	(122.3)
9	165.1 (165.6)		

C δ of $M = 303.5$ is out of brackets; C δ of $M = 331.5$ is in brackets

**Fig. 7** X-ray diffraction of the new monascus red pigments

not synthesize those enzymes for methylation, aldol and dehydration of chromophore [5, 10]. But the strain could probably produce some kinds of enzymes to enable double-bond and carbonyl hydrogenation to occur. The findings

in the paper infer that there exist other ways besides those described in reference to synthesize monascus red pigments, and as described in Ref. [10], there is a long way to understand and control the synthesis of key *Monascus* metabolites. The super-hydrophilic property caused by the three hydroxyls at the carbon 7, 8 and 12 in the new red pigments will extend their application from sausages into lots of food such as beverage, pastries, wines.

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Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

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