Coumarins and phenolic compounds from the immature fruits of *Solanum nigrum* L.

Abstract

Solanum nigrum L. has been traditionally used as traditional Chinese medicine and food all over the world. Phytochemical investigations of the immature fruits of *S. nigrum* have been carried out and five coumarins (**1-5**) and five phenolic compounds (**6-11**) were obtained. Their structures were elucidated on the basis of spectroscopic and chemical methods, including IR, MS, ^{1}H and ^{13}C-NMR. They were identifies as 6,7-dimethoxycoumarin (**1**), 6-methoxy-7-hydroxycoumarin (**2**), 6,8-dimethoxycoumarin (**3**), 6,7-dimethoxycoumarin (**4**), umbelliferone (**5**), vanillin (**6**), vanillic acid (**7**), syringic aldehyde (**8**), isovanillic acid (**9**), ethyl 3,4-dihydroxybenzoate (**10**), and ethyl 3,4-dihydroxyphenylacetate (**11**).

Keywords: *Solanum nigrum* L., immateure fruits, chemical investigation, coumarin, phenolic compound

1. Introduction

Solanum nigrum L. belongs to the family of Solanaceae and is wildly distributed all over the world [1]. In China, *S. nigrum* has been used as a common traditional Chinese medicine, which has the effects of clearing heat and detoxification, activating blood circulation and removing blood stasis, promoting water and swelling, and is mainly used to treat cold and fever, toothache, and cancers. Modern pharmacological studies indicated that *S. nigrum* exhibited a variety of biological activities including antiproliferative [2], anti-inflammatory [3], antiviral [4], hepatoprotective [5], and antioxidative activities [6].

S. nigrum contains steroidal saponins, steroidal alkaloid glycosides and phenolic compounds in the previous chemical studies [7-10]. Steroidal alkaloid glycosides are the main chemical components of *Solanum* species, which possess various pharmacological activities such as antiproliferative and anti-inflammatory properties [11, 12]. Currently, most of the chemical investigations on *S. nigrum* were focused on the aerial parts, while the bioactive components of its unripe berries are still unclear.

In this study, the phytochemicals from the immature fruits of *S. nigrum* has been carried out and eleven compounds were obtained, including five coumarins (**1-5**) and five phenolic compounds (**6-11**).

2. MATERIALS AND METHODS

2.1. General experimental procedures

IR spectra (4000-450 cm^{-1}) were recorded with a Spectrum 100 FT-IR spectrometer (Perkin Elmer Inc., Waltham, MA). MS spectra were obtained on an Acquity UPLC-Q-TOF Microsystem (Waters Co., Milford, MA). NMR spectra were taken on a Bruker Avance III 500 MHz spectrometer (Bruker, Switzerland). ODS packed column (40–60 µm, Merck KGaA, Darastadt, Germany) and column chromatography was employed on silica gel (Anhui Liangchen Silicon Source Material Co. Ltd, Lu′ an, China). All other analytical chemicals and reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Plant materials

The immature fruits of *S. nigru*m were purchased from Haerbin (Heilongjiang province, China) and dried at room temperature in the shade. The plant was authenticated by Prof. X. J. He and the voucher specimen has been deposited in the School of Pharmacy, Guangdong Pharmaceutical University, China.

2.3. Extraction and isolation

Dried immature fruits of *S. nigru*m (2.5 kg) were extracted with 70% EtOH (v/v, 15 L × 3) for 2 h. The ethanol-free suspension was subjected to a D101 macroporus resin column (80 × 1100 mm), and eluted with H_2O, 10% MeOH, 30% MeOH, 50% MeOH, 70% MeOH and MeOH to give six fractions (I-VI). The 30% MeOH elution (fraction IV, 51 g) was separated by a silica gel chromatography column (200-300 mesh, 1500 g) into 12 fractions (IV-1 to IV-12) with a $CHCl_3$-MeOH gradient (10:1 to 0:1, v/v).

Compounds **6** (15.1 mg), **7** (7.9 mg), and **10** (13.7 mg) were obtained from fraction IV-6 followed by an ODS column, eluted with MeOH-H_2O gradient (1:9 to 10:0, v/v). Fraction IV-8 was applied to an ODS column eluted with a gradient of MeOH-H_2O (1:9 to 10:0, v/v) to afford five subfractions (IV-8-1 to IV-8-5). Subfraction IV-8-3 was further purified by an ODS MPLC and semi-preparative HPLC to obtained compounds **1** (9.6 mg), **2** (7.3 mg), **4** (11.2 mg), and **8** (6.1 mg). Fraction IV-10 was subjected to an ODS MPLC and eluted with MeOH-H_2O gradient (1:9 to 10:0, v/v) to afford nine fractions (IV-10-1 to IV-10-9). Subfraction IV-10-5 was further separated by an ODS column and semi-preparative HPLC to obtained compounds **3** (20.1 mg), **5** (10.5 mg), **9** (7.1 mg), and **11** (6.0 mg), respectively.

3. RESULTS AND DISCUSSION

The 70% ethanol extract of the immature fruits of *S. nigrum* was separated successively by column chromatography on D101 macroporus resin, silica gel, ODS MPLC, and preparative HPLC, to afford five coumarins (**1-5**) and five phenolic compounds (**6-11**) (Fig. 1). Their structures were elucidated on the basis of spectroscopic data, including IR, MS, and ^{1}H and ^{13}C-NMR.

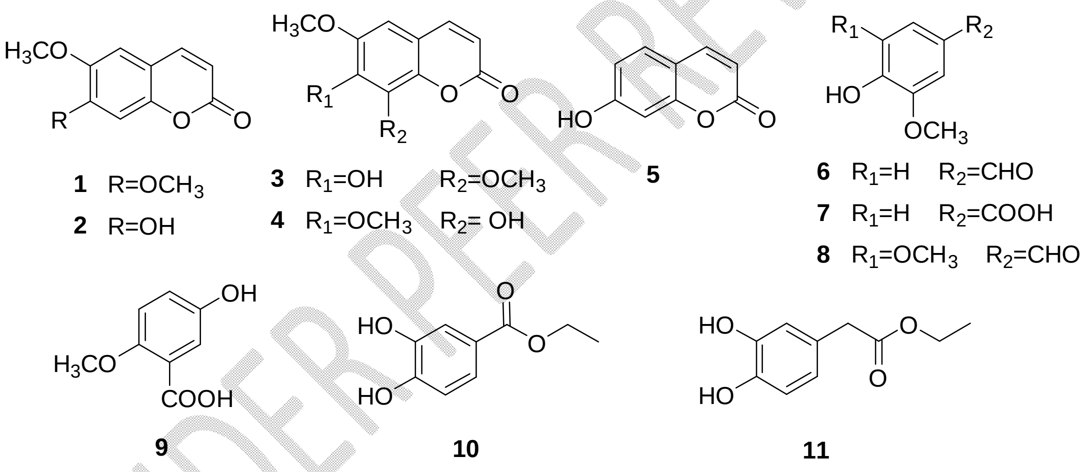

Fig. 1 Structures of the coumarins and phenolic compounds **1-11**

Compound **1** was white amorphous powder and easily soluble in chloroform and methanol. There was a blue fluorescence under 365 nm after TLC development. Its molecular formula was determined as $C_{11}H_{10}O_4$ according to the ion *m/z* 207.0654 $[M+H]^+$ (calcd for $C_{11}H_{11}O_4$, 207.0652) in the positive HR-ESI-MS. In the ^{1}H-NMR (Table 1), δ_H 6.26 (1H, d, J=9.3 Hz) and 7.61 (1H, d, J=9.3 Hz) were the characteristic hydrogen signals of coumarins. δ_H 3.90 (3H, s) and 3.92 (3H, s) were two hydroxyl groups. In the ^{13}C-NMR, a total of 11 carbon signals were given, including an ester carbonyl carbon at δ_C 161.4. Signals at δ_C 113.6 and 143.3 were carbon signals at C-3 and C-4 of coumarin, and signals at δ_C 108.0, 146.5, 150.3, 100.2, 153.0, 111.5 were the carbon signals on the benzene ring. δ_C 56.5 is a methoxy group. Based on above analysis and the literature [13], compound **1** was identified as 6,7-dimethoxycoumarin.

Compound **2** was yellowish needle crystal (MeOH) and there was a blue fluorescence under 365 nm after TLC development. Its molecular formula was determined as $C_{10}H_8O_4$ according to the ion *m/z* 191.0342 $[M+H]^+$ (calcd for $C_{10}H_7O_4$, 191.0350) in the positive HR-ESI-MS. In the ^{1}H-NMR (Table 1), δ_H 6.23 (1H, d, J=9.4 Hz) and 7.68 (1H, d, J=9.4 Hz) were the characteristic signals of coumarins. δ_H 3.82 (3H, s) was a hydroxyl group. In the ^{13}C-NMR, a total of 10 carbon signals were given, including an ester carbonyl carbon at δ_C 160.7. Signals at δ_C 109.5 and 144.5 were carbon signals at C-3 and C-4 of coumarin, and signals at δ_C 111.6, 145.3, 151.3, 102.8, 149.5, 110.5 were the carbon signals on the benzene ring. δ_C 56.0 is a methoxy group. Based on above analysis and the literature [14], compound **2** was identified as 6-methoxy-7-hydroxycoumarin.

Compound **3** was white amorphous powder and easily soluble in methanol. There was a blue fluorescence under 365 nm after TLC development. Its molecular formula was determined as $C_{11}H_{10}O_5$ according to the ion *m/z* 221.0446 $[M-H]^-$ (calcd for $C_{11}H_9O_5$, 221.0455) in the negative HR-ESI-MS. In the ^{1}H-NMR (Table 1), δ_H 6.24 (1H, d, J=9.3 Hz) and 7.88 (1H, d, J=9.3 Hz) were the characteristic hydrogen signals of coumarins. δ_H 3.82 (3H, s) and 3.83 (3H, s) were two hydroxyl groups. In the ^{13}C-NMR, a total of 11 carbon signals were given, including an ester carbonyl carbon at δ_C 160.3. Signals at δ_C 112.0 and 144.6 were carbon signals at C-3 and C-4 of coumarin, and signals at δ_C 104.7, 145.5, 144.5, 134.2, 143.2, 110.1 were the carbon signals on the benzene ring. δ_C 56.2 and 60.6 is a methoxy group. Based on above analysis and the literature [15], compound **3** was identified as 6,8-dimethoxycoumarin.

Table 1 ^{1}H and ^{13}C NMR data of compounds **1-5** (δ in ppm and *J* in Hz) ^{a}

	1		**2**		**3**		**4**		**5**	
No	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		161.4		160.7		160.3		160.2		160.2
3	6.26 d, (9.3)	113.6	6.23 d, (9.4)	109.5	6.24 d, (9.3)	112.0	6.30 d, (9.4)	114.0	6.21 d, (9.4)	111.5
4	7.61 d, (9.3)	143.3	7.68 d, (9.4)	144.5	7.88 d, (9.3)	144.6	7.90 d, (9.4)	144.2	7.92 d, (9.4)	144.3
5	6.81, s	108.0	7.02, s	111.6	7.01, s	104.7	6.82, s	100.5	7.50 d, (8.4)	129.8
6		146.5		145.3		145.5		149.5	6.76 dd, (8.4, 2.3)	113.3
7		150.3		151.3		144.5		140.0		161.5
8	6.80, s	100.2	6.77, s	102.8		134.2		138.3	6.70 d, (2.3)	102.0
9		153.0		149.5		143.2		138.1		155.3
10		111.5		110.5		110.1		114.1		111.1
6-OCH$_3$	3.90, s	56.5	3.82, s	56.0	3.83, s	56.2	3.83, s	56.1		
7-OCH$_3$	3.92, s	56.5					3.80, s	60.5		
8-OCH$_3$					3.82, s	60.6				

a: 500 MHz for ^{1}H and 126 MHz for ^{13}C in in DMSO-d_6

Compound **4** was colorless needle crystal (MeOH). There was a blue fluorescence under 365 nm after TLC development. Its molecular formula was determined as $C_{11}H_{10}O_5$ according to the ion *m/z* 245.0412 $[M+H]^+$ (calcd for

$C_{11}H_{10}O_5Na$, 245.0420) in the positive HR-ESI-MS. In the ^{1}H-NMR (Table 1), δ_H 6.30 (1H, d, J=9.4 Hz) and 7.90 (1H, d, J=9.4 Hz) were the characteristic hydrogen signals of coumarins. δ_H 3.80 (3H, s) and 3.83 (3H, s) were two hydroxyl groups. In the ^{13}C-NMR, a total of 11 carbon signals were given, including an ester carbonyl carbon at δ_C 160.2. Signals at δ_C 114.0 and 144.2 were carbon signals at C-3 and C-4 of coumarin, and signals at δ_C 100.5, 149.5, 140.0, 138.1, 138.3, 114.1 were the carbon signals on the benzene ring. δ_C 56.1 and 60.5 is a methoxy group. The NMR data of **4** is very close to that of **3**, and the difference in chemical shift values between the two is due to the different positions of hydroxyl and methoxy groups. Based on above analysis and the literature [16], compound **4** was identified as 6,7-dimethoxycoumarin.

Compound **5** was white amorphous powder and there was a blue fluorescence under 365 nm after TLC development. Its molecular formula was determined as $C_9H_6O_3$ according to the ion *m/z* 161.0240 $[M-H]^-$ (calcd for $C_9H_5O_3$, 161.0244) in the negative HR-ESI-MS. In the ^{1}H-NMR (Table 1), δ_H 6.21 (1H, d, J=9.4 Hz) and 7.92 (1H, d, J=9.4 Hz) were the characteristic hydrogen signals of coumarins. In the ^{13}C-NMR, a total of 9 carbon signals were given, including an ester carbonyl carbon at δ_C 160.2. Signals at δ_C 111.5 and 144.3 were carbon signals at C-3 and C-4 of coumarin, and signals at δ_C 161.5, 155.3, 129.8, 113.3, 111.1, 102.0 were the carbon signals on the benzene ring. Based on above analysis and the literature [17], compound **5** was identified as umbelliferone.

Compounds **6** to **11** were identified as vanillin (**6**) [18], vanillic acid (**7**) [19], syringic aldehyde (**8**) [20], isovanillic acid (**9**) [21], ethyl 3,4-dihydroxybenzoate (**10**) [22], and ethyl 3,4-dihydroxyphenylacetate (**11**) [23], respectively, based on their spectroscopic analysis.

4. Conclusions

Further chemical investigation of the immature fruits of *S. nigrum* led to the isolation of five coumarins and five phenolic compounds were obtained. Their structures were elucidated on the basis of spectroscopic and chemical methods. They were identifies as 6,7-dimethoxycoumarin, 6-methoxy-7-hydroxycoumarin, 6,8-dimethoxycoumarin, 6,7-dimethoxycoumarin, umbelliferone, vanillin (**6**), vanillic acid, syringic aldehyde, isovanillic acid, ethyl 3,4-dihydroxybenzoate, and ethyl 3,4-dihydroxyphenylacetate.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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