Phenylpropanoids from the immature fruits of black nightshade (*Solanumnigrum* L.)

Abstract

Black nightshade(*Solanumnigrum* L.)has been traditionally used as traditional Chinese medicine. Phytochemical investigations of the immature fruits of *S. nigrum* have been carried out and ninephenylpropanoids (**1-9**) were obtained. Their structures were elucidated on the basis of spectroscopic and chemical methods, includingMS, ^{1}H and ^{13}C-NMR. They were identifies as (7*S*, 8*R*)-4-[3-hydroxymethyl-5-(3-hydroxypropyl)-2,3-dihydrobenzofuran-2-yl]-2-methoxyphenol (**1**), (7*S*, 8*R*)-dihydrodehydroconifery alcohol (**2**), massonianoside A (**3**), butane-2,3-diol 2-O-(6-O-caffeoyl)-β-D-glucopyraniside (**4**), 4-[(6-O-(*E*)-caffeoyl)-β-D-glucopyranosyl] vanillic acid (**5**), (+)-isolariciresinol (**6**), *trans*-cinnamic acid (**7**), ferulic acid (**8**), and 4-hydroxy cinnamic acid (**9**).

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Comment [D1]: Where material and methods and brief results and conclusions

Keywords: black nightshade,*Solanumnigrum*L.,immateure fruits, chemical investigation, phenylpropanoids

1. INTRODUCTION

Black nightshade(*Solanumnigrum* L.)belongs to the family of Solanaceae and is wildly distributed all over the world[1]. In China, it 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, anti-inflammatory[2,3], antiviral, hepatoprotective, and antioxidative activities[4-6].

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S. nigrum contains steroidal saponins, steroidal alkaloid glycosidesand phenolic compoundsin 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-inflammatoryproperties[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 nine phenylpropanoidswere obtained.

2. MATERIALS AND METHODS

2.1. General experimental procedures

MS spectra were obtained on an Acquity UPLC-Q-TOF Microsystem (Waters Co., Milford, MA). NMR spectra were takenon a BrukerAvance 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*mwere purchased from Haerbin (Heilongjiangprovince, China) and dried at room temperature in the shade. The voucher specimen has been deposited in theSchool 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 suspensionwas 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 70% MeOH elution (fraction V, 51 g) was separated by a silica gel chromatography column (200-300 mesh, 1100 g) into 15 fractions (V-1 to V-15) with a $CHCl_3$-MeOH gradient (100:1 to 0:1, v/v).

Compounds **7** (16.2 mg), **8** (10.1 mg), and **9** (16.0 mg) were obtained from fraction V-8 followed by an ODS MPLC, gradientlyeluted with MeOH-H_2O (1:9 to 10:0, v/v). Fraction V-6 was applied to an ODS MPLCeluted with a gradient of MeOH-H_2O (1:9 to 10:0, v/v) to afford eightsubfractions (V-6-1 toV-6-8). SubfractionV-6-5 was further purified by a semi-preparative HPLCto obtained compounds **1** (8.2 mg)and **3** (7.3 mg). Fraction V-7 was subjected to an ODS MPLC and eluted with MeOH-H_2O gradient (1:9 to 10:0, v/v) to afford ten fractions (V-7-1 to IV-7-10). SubfractionV-7-6 was further separated by an ODS MPLC and semi-preparative HPLC to obtained compounds **2** (10.7 mg), **4** (6.3 mg), **5** (9.0 mg), and **6** (5.6 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 nine phenylpropanoids(**1-9**) (Fig. 1). Their structureswere elucidated on the basis of spectroscopic data, including MS, and ^{1}H and ^{13}C-NMR.

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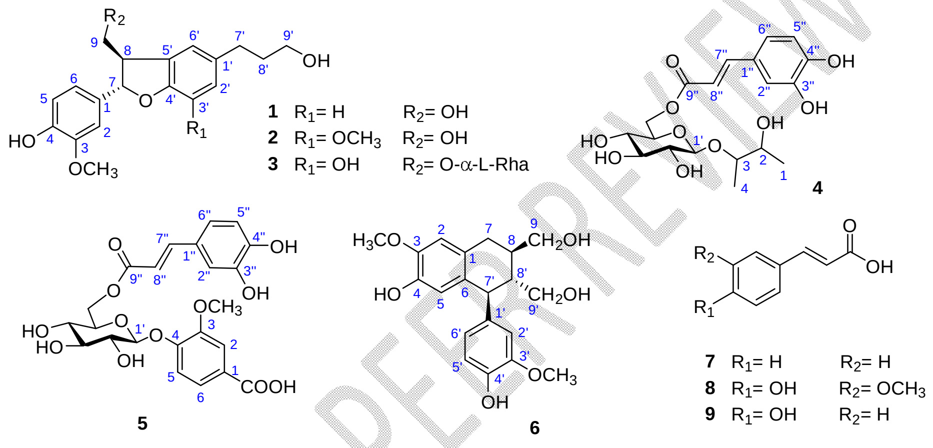

Fig. 1 Structures of the phenylpropanoids**1-9**

Compound **1**, brownish oil, was a blue fluorescence under 365 nm after TLC development.Its molecular formula was determined as $C_{19}H_{22}O_5$based on itsESI-MS with theion *m/z*353 $[M+Na]^+$, ^{1}H and ^{13}C-NMR.

In the ^{1}H-NMR, protons at δ_H 7.01 (1H, d, *J*=1.8 Hz, H-2), 6.87 (1H, dd, *J*=8.2, 1.8 Hz, H-6) and 6.80 (1H, d, *J*=8.2 Hz, H-5) consisted an ABX coupling system of in a 1,3,4-trisubstituted benzene ring. Signals at δ_H 7.13 (1H, s, H-2′), 7.00 (1H, d, J=8.1 Hz, H-6′) and 6.70 (1H, d, J=8.1 Hz, H-5′) came from another ABX coupling system benzene ring in the molecule. δ_H 5.48 (1H, d, *J*=6.4 Hz, H-7) was a proton linked to oxygenated carbon. δ_H 3.80 (3H, s, 3-OCH_3) was a methoxy group.

^{13}C-NMR gave 19 carbon signals (Table 1). In aromatic region (δ_C 159.1 to 109.5), there was 12 carbon signals, which were two benzene ring units. Carbon signal at δ_C56.4 in high field region is a methoxycarbon signal, which was consistent with the information given by ^{1}H-NMR. δ_C 87.8, 54.8 and 64.8were deduced as the carbon signals on the furan ring, and δ_C 36.1, 32.4 and 61.9were form a hydroxypropyl group.Taken together, compound **1** was speculated a benzodihydrofuranlignin.Based on above analysis and the literature [13], compound **1** was identified as (7*S*, 8*R*)-4-[3-hydroxymethyl-5-(3-hydroxypropyl)-2,3-dihydrobenzofuran-2-yl]-2-methoxyphenol.

The molecular formula compound **2** of was determined as $C_{20}H_{24}O_6$ based on itsESI-MS withthe ion *m/z* 383 $[M+Na]^+$, ^{1}H and ^{13}C-NMR.In the ^{1}H-NMR, protons at δ_H7.03 (1H, d, *J*=1.8 Hz, H-2), 6.88 (1H, dd, *J*=8.1, 1.8 Hz, H-6) and 6.81 (1H, d, *J*=8.1 Hz, H-5) consisted an ABX coupling system of in a 1,3,4-trisubstituted benzene ring. Signals at δ_H6.74 (1H, s, H-2′) and 6.76 (1H, d, J=8.1 Hz, H-6′) came from another benzene ring in the molecule. δ_H 5.50 (1H, d, *J*=6.4 Hz, H-7) was a proton linked to oxygenated carbon. δ_H 3.82 (3H, s, 3-OCH_3) and 3.81 (3H, s, 3′-OCH3) were twomethoxy groups.

In the ^{13}C-NMR (Table 1), there were 20 carbon signals, including 12 carbon signals in aromatic region (δ_C 148.4 to 110.6), which were two benzene ring units. Carbon signals at δ_C 56.4 and 56.3 in high field region were two methoxy carbons, which were consistent with the information given by ^{1}H-NMR. δ_C 88.3, 55.2 and 64.8 were deduced as the carbon signals on the furan ring, and δ_C 36.1, 32.8 and 61.8 were form a hydroxypropyl group. Therefore, compound **2** was speculated a benzodihydrofuran lignin. Based on above analysis and the literature [14], compound **2** was identified as (7*S*, 8*R*)-dihydrodehydroconifery alcohol.

Table 1 ^{13}C NMR dataofcompounds**1**-**6**(δ in ppm and J in Hz)

NO.	**1** ^{a}	**2** ^{a}	**3** ^{a}	**4**^{b}	**5** ^{b}	**6** ^{a}
1	134.9	134.8	132.6	15.1	123.7	128.5
2	110.5	110.6	110.2	78.6	112.7	111.8
3	148.5	147.4	146.4	68.9	149.1	146.5
4	147.2	148.4	147.7	19.5	151.6	145.3
5	115.6	115.7	114.8		114.9	116.9
6	119.6	119.6	118.6		123.4	138.5
7	87.8	88.3	87.2		167.9	33.8
8	54.8	55.2	50.9			40.4
9	64.8	64.8	68.9			65.9
1'	135.4	130.1	128.3	101.3	100.5	133.9
2'	125.8	113.9	115.4	73.4	73.2	113.6
3'	129.1	147.2	140.8	76.6	75.8	148.3
4'	159.1	144.9	144.7	70.5	70.3	145.9
5'	109.5	136.4	135.2	73.8	73.7	115.6
6'	129.3	117.7	116.2	63.8	64.0	122.8
7'	32.4	36.1	34.8			48.1
8'	36.1	32.8	31.5			48.4
9'	61.9	61.8	60.3			62.1
1"			100.2	125.6	125.9	
2"			70.9	113.9	115.3	
3"			72.0	145.8	145.3	
4"			70.6	148.7	147.4	
5"			68.6	115.9	116.3	
6"			18.1	121.5	120.5	
7"				145.4	144.1	
8"				114.9	115.2	
9"				166.5	165.6	
3-OCH_3	56.4	56.4	55.5		55.6	56.2
3'-OCH_3		56.3				56.3

[a] 151 MHz for ^{13}C NMR in Acetone-d_6. [b] 101 MHz for ^{13}C NMR in DMSO-d_6.

Compound **3**,brownish oil,was a blue fluorescence under 365 nm after TLC development. Its molecular formula was determined as $C_{25}H_{32}O_{10}$ based on itsESI-MS with the ion *m/z*515 $[M+Na]^+$, ^{1}H and ^{13}C-NMR.

In the ^{1}H-NMR,protons at δ_H 6.93 (1H, d, J=1.3 Hz, H-2), 6.86 (1H, dd, J=8.2, 1.3 Hz, H-6) and 6.81 (1H, d, J=8.2 Hz, H-5)consisted an ABX coupling system of in a 1,3,4-trisubstituted benzene ring. Signals at δ_H6.50 (2H, br.s, H-2′, 6′) came from another benzene ring in the molecule. δ_H 5.37 (1H, d, J=6.6 Hz, H-7) was a proton linked to oxygenated carbon.δ_H 3.76 (3H, s, 3-OCH_3) and 1.13 (3H, d, J=6.2 Hz, H-6″) were a methoxy and methyl groups in the higher field.δ_H 4.62 (1H, d, J=0.7 Hz, H-1 ″) was deduced as a terminal proton signal of sugar.

In the ^{13}C-NMR(Table 1), therewere25 carbon signals, including 12 carbon signals in aromatic region (δ_C 144.7 to 110.2), which were two benzene ring units.Carbon signal at δ_C 55.5 in high field region was a methoxycarbon, which was consistent with the information given by ^{1}H-NMR.Compared with the NMR data of compound **2**, it is speculated that compound **3** is a benzodihydrofuran lignin with rhamnose substitution at C-9 position. Based on above analysis and the literature [15], compound**3** was identified as massonianoside A.

Compound **4** wasbrownish oil and exhibited a blue fluorescence under 365 nm after TLC development. Its molecular formula was determined as $C_{19}H_{26}O_{10}$ based on itsESI-MS with the ion *m/z*437 $[M+Na]^+$, ^{1}H and ^{13}C-NMR.

In the ^{1}H-NMR spectrum, signals at δ_H 7.50 (1H, d, J=15.6 Hz, H-7″) and 6.26 (1H, d, J=15.6 Hz, H-8″) were speculated to be the proton signals on the trans carbon-carbon double bond. The aromatic region had three proton signals at δ_H 7.04 (1H, s, H-2 ″), 6.99 (1H, d, J=7.8 Hz, H-5 ″) and 6.76 (1H, d, J=7.4 Hz, H-6 ″).Signal at δ_H 4.26 (1H, d, J=7.7 Hz, H-1') was the terminal proton signal of sugar.δ_H 4.40~2.98 has 8 proton signals, which were proton signals on oxymethylene or oxymethylene. δ_H 1.07 (3H, d, J=6.0 Hz, H-1) and 0.99 (3H, d, J=6.0 Hz, H-4) were two methyls in the high field.

^{13}C-NMR spectrum indicated 19 carbon signals, and δ_C 166.5 was an ester carbonyl in the lower field(Table 1). Signals at δ_C 166.5, 148.7, 145.8, 145.4, 125.6, 121.5, 115.9, 114.9and 113. 9 consisted of a caffeic acyl group. Meanwhile, compound **4**has a glucose unit, which the carbon signals at δ_{C} 101.3, 73.4, 76.6, 70.5, 73.8and 63.8. The remaining four carbon signalsat δ_{C} 78.6, 68.9, 19.5 and 15.1 formed a 3-hydroxy-2-butoxy group.Based on above analysis and the literature [16], compound**4** was identified as butane-2,3-diol 2-O-(6-O-caffeoyl)-β-D-glucopyraniside.

Compound **5** wasbrownish oil and exhibited a blue fluorescence under 365 nm after TLC development. Its molecular formula was determined as $C_{23}H_{24}O_{12}$ based on itsESI-MS with the ion *m/z*491 $[M–H]^{-}$, ^{1}H and ^{13}C-NMR.

In the ^{1}H-NMR spectrum, signals at δ_{H} 7.48 (1H, d, J=15.7 Hz, H-7″) and 6.36 (1H, d, J=15.7 Hz, H-8″) were speculated to be the proton signals on the *trans*carbon-carbon double bond. The aromatic region hadsix proton signals at δ_{H}7.40 (1H, dd, *J*=8.2, 1.7 Hz, H-6), 7.38 (1H, d, *J*=1.7 Hz, H-2), 7.37 (1H, d, *J*=1.8 Hz, H-2″), 7.18 (1H, dd, *J*=8.2, 1.8 Hz, H-6″), 6.84 (1H, d, *J*=8.2 Hz, H-5) and 6.77 (1H, d, *J*=8.2 Hz, H-5″), which speculated that the compound had two 1,3,4-trisubstituted benzene rings.Signal at δ_{H}5.08 (1H, d, J=7.3 Hz, H-1') was the terminal proton signal of sugar.δ_{H}3.73 (3H, s, 3-OCH_{3}) was a methyoxyl group in the high field.

The ^{13}C-NMR spectrum gives 23 carbon signals(Table 1). Compared with the NMR data of compound **4**, compound **5** contained a caffeic acyl segment and a glucose unit. The chemical shifts of C-1′ and C-6′of the glucose shifted to the low field, which speculated that there were substituted on C-1′ and C-6′.Meanwhile, compound **5** had a vanillic acid fragment with substitutions at position 4. Based on above analysis and the literature [17], compound**5** was identified as 4-[(6-O-(*E*)-caffeoyl)-β-D-glucopyranosyl] vanillic acid.

Compound **6** wasbrownish oil and its molecular formula was determined as

$C_{20}H_{24}O_6$ based on itsESI-MS with the ion *m/z*359 [M–H]⁻, ^{1}H and ^{13}C-NMR. In the^{1}H-NMR spectrum, signals in aromatic regionat δ_H 6.78 (1H, s, H-5 ′), 6.76 (1H, s, H-2 ′), 6.66 (1H, s, H-2) and 6.22 (1H, s, H-5) were protons on the benzene ring. In the high field, δ_H 3.78 (3H, s, 3-OCH3) and 3.81 (3H, s, 3′-OCH3) were two methoxy signals.

The ^{13}C-NMR spectrum gave 20 carbon signals(Table 1), and there had 12 carbon signals in the aromatic region, which were two benzene ring units.δ_C 56.2 and 56.3were two methoxy groups, which were consistent with the information given by ^{1}H-NMR spectrum. The remaining six carbon signals at δ_C 65.9, 62.1, 48.4, 48.1, 40.4 and 33.78 were speculated that it was the alkyl carbon signal on the C_3 skeleton of phenylpropanoid.Based on above analysis and the literature [18], compound**6** was identified as (+)-isolariciresinol.

Compounds **7** to **9** were identified as *trans*-cinnamic acid (**7**) [19], ferulic acid (**8**) [20], and 4-hydroxy cinnamic acid (**9**) [21], respectively, based on their spectroscopic analysis.

4. CONCLUSIONS

Further chemical investigation of the immature fruits of*S. nigrum* led to the isolation of ninephenylpropanoids. Theirstructures were elucidated on the basis of spectroscopic and chemical methods.They were identifies as compound was identified as (7*S*, 8*R*)-4-[3-hydroxymethyl-5-(3-hydroxypropyl)-2,3-dihydrobenzofuran-2-yl]-2-methoxyphenol, (7*S*, 8*R*)-dihydrodehydroconifery alcohol, massonianoside A (**3**), butane-2,3-diol 2-O-(6-O-caffeoyl)-β-D-glucopyraniside, 4-[(6-O-(*E*)-caffeoyl)-β-D-glucopyranosyl] vanillic acid, (+)-isolariciresinol, *trans*-cinnamic acid, ferulic acid, and 4-hydroxy cinnamic acid.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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