Three New Phenolic Compounds from Sabia parviflora and Their Hepatoprotective Activity

Huan He1, Wei Dong Du1, Qiang Zhou1, Qi Wang2, Zhi Feng Li1,3, Yuanying Fang1, and Yulin Feng2

Abstract

Three compounds were obtained from Sabia parviflora Wall., and their structures were identified through nuclear magnetic resonance (NMR) spectroscopy, particularly 2-dimensional (2D)-NMR. The molecular masses were determined using quadrupole-time-of-flight-mass spectrometry. Electronic circular dichroism spectra were used to determine the absolute configuration of compound 1. The 3 new compounds were identified as 2,2,7,7-tetramethyl-8S,10-dihydroxy-2,7,8,9-tetrahydro-2H-naphtha[2,3-b]pyran-6-one-10-O-β-D-glucopyranoside, (2Z)-4-(3-carboxy-4-hydroxyphenyl)-2-methylbut-2-enoic acid, and (2Z)-5-(3-carboxy-4-hydroxyphenyl)-5-oxo-2-methylpent-2-enoic acid. The liver protective activities of these compounds were tested by HepG2 and LO2 cell lines, which were induced using free fatty acids.

Keywords

extraction, Sabia parviflora, identification, hepatoprotective activity

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Introduction

Sabia parviflora Wall., family Sabiaceae, is a plant mainly distributed in southwest China. It is a traditional Chinese herbal medicine that is mainly used for the treatment of “damp heat jaundice, hepatitis and hemostasis.” Numerous chemical studies have shown that the plant contains triterpenoids, flavonoids, alkaloids, phenolic acids, phenylpropane, and polysaccharides. Previous pharmacological studies have revealed that the plant’s compounds possess antiviral, antiinflammatory, antitumor, and antioxidant activities.

Based on the results of earlier experimental research, our group has continued to explore the constituents of the plant and their biological activities. In this study, we examined the dried stems of the species and isolated 3 new compounds: 2,2,7,7-tetramethyl-8S,10-dihydroxy-2,7,8,9-tetrahydro-2H-naphtha[2,3-b]pyran-6-one-10-O-β-D-glucopyranoside (1), (2Z)-4-(3-carboxy-4-hydroxyphenyl)-2-methylbut-2-enoic acid (2), and (2Z)-5-(3-carboxy-4-hydroxyphenyl)-5-oxo-2-methylpent-2-enoic acid (3). The structures of the compounds are shown in Figure 1.

Finally, the biological activities of these 3 compounds were determined, and their hepatoprotective effects were investigated using HepG2 and LO2 cell lines.

Results and Discussion

Compound 1, a yellow powder, had a relative molecular mass of [M + H]+ (calcd. for C23H30O9, 450.1890) in its quadrupole time-of-flight tandem mass spectrum (Q-TOF-MS). The 1H nuclear magnetic resonance (NMR) spectrum of compound 1 exhibited signals for an aromatic proton of a penta-substituted benzene (δH 7.47 s, 1H), 2 olefinic protons of a 1,2-disubstituted alkene (δH 5.77, d, J = 10.0 Hz, 1H and δH 6.41, d, J = 10.0 Hz, 1H), 1 methylene group (δH 3.21 m, 1H), 1 methine proton (δH 3.91, dd, J = 3.5, 6.2 Hz, 1H), 4 methyl protons (δH 1.48, 1.49, 1.16, 1.17 s, each 3H), and 1 anomeric proton (δH 4.96, d, J = 7.7 Hz, 1H). The 13C-NMR spectrum of compound 1 showed 23 signals: a penta-substituted benzene (δC 122.4, 122.7, 125.6, 137.9, 143.1, and 151.1), a carbonyl carbon (δC 203.6), 2 double-bond carbons (δC 132.4 and 122.6), 1 methylene...
carbon ($\delta C$ 30.4), 1 methine carbon ($\delta C$ 75.6), 2 quaternary carbons ($\delta C$ 79.7 and 48.5), 4 methyl carbons ($\delta C$ 28.8, 28.5, 24.1, and 23.1), and 6 glucosyl carbons ($\delta C$ 105.3, 75.9, 78.2, 71.8, 78.4, and 62.7). Furthermore, C-10 was found to be connected to the oxygen atom, which was determined by the chemical shift of $\delta C$ 143.1 (C-10). The $^{1}H$–$^{13}C$-NMR spectra of compound 1 revealed structural similarity between compound 1 and sabianin C, except for subtle differences in the 7 position of the methyl or hydroxymethyl substituents.

The heteronuclear multiple bond correlation (HMBC) cross peak ($\delta H$ 4.96/143.1) indicated that the glucose moiety was connected to C-10. Accordingly, acid hydrolysis of compound 1 gave D-glucose as the sugar residue, which was determined through gas chromatographic (GC) analysis of its corresponding trimethylsilylated L-cysteine adduct. The sugar in compound 1 was a $\beta$-glucose moiety according to the proton signal at $\delta H$ 4.96 (d, $J = 7.7$ Hz). The nuclear overhauser effect spectroscopy spectrum showed 2 conformations for 1:2,2,2,7,7-tetramethyl-8S,10-dihydroxy-2,7,8,9-tetrahydro-2H-naphtha[2,3-b]pyran-6-one-10-$\beta$-D-glucopyranoside and 2,2,7,7-tetramethyl-8R,10-dihydroxy-2,7,8,9-tetrahydro-2H-naphtha[2,3-b]pyran-6-one-10-$\beta$-D-glucopyranoside.

The experimental circular dichroism (CD) spectrum and calculated CD(8S-C) exhibited the same trend at the 200-280 nm wavelength. The nuclear overhauser effect spectroscopy showed 2 conformations for 1:2,2,7,7-tetramethyl-8S,10-dihydroxy-2,7,8,9-tetrahydro-2H-naphtha[2,3-b]pyran-6-one-10-$\beta$-D-glucopyranoside and 2,2,7,7-tetramethyl-8R,10-dihydroxy-2,7,8,9-tetrahydro-2H-naphtha[2,3-b]pyran-6-one-10-$\beta$-D-glucopyranoside.

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Compound 2, isolated as a colorless oil, had a molecular formula of C$_{12}$H$_{12}$O$_{5}$ based on its molecular ion peak at $m/\zeta$ 235.0608 [M-H]$^{-}$ (calcd. for C$_{12}$H$_{12}$O$_{5}$, 236.0685) in its Q-TOF-MS. The $^1H$-NMR spectrum of compound 2 showed signals for 3 aromatic protons of a trisubstituted benzene ($\delta H$ 7.70, dd, $J = 8.2, 2.2$ Hz, 7.71, d, $J = 8.2$ Hz, 6.90 d, $J = 8.2$ Hz, each 1H), 1 olefinic proton ($\delta H$ 6.01, dd, $J = 7.68$ Hz, 1H), 1 methine ($\delta H$ 3.70, d, $J = 7.6$ Hz, 2H), and 1 methyl ($\delta H$ 1.85 s, 3H). The $^{13}C$-NMR spectrum of compound 2 showed 12 signals: 1 benzene ring ($\delta C$ 131.7, 126.7, 129.8, 121.7, 160.0, and 115.2), 2 carboxyl carbons ($\delta C$ 167.8 and 169.8), double-bond carbons ($\delta C$ 138.3 and 129.0), 1 methine carbon ($\delta C$ 30.0), and 1 methyl carbon ($\delta C$ 21.1). The HMBC cross peak ($\delta H$ 3.70/126.7) suggested that the methene was connected to C-1 ($\delta H$ 6.01/$\delta C$ 30.0), and the double bond to C-2 ($\delta H$ 6.01/$\delta C$ 169.9 and $\delta H$ 1.85/$\delta C$ 129.0). Furthermore, the HMBC cross peak also indicated that the carboxyl carbon and methyl carbon were connected to C-2. The HMBC cross peaks ($\delta H$ 3.70/$\delta C$ 131.8, 129.9) and ($\delta H$ 7.70/$\delta C$ 121.7), and the ABX coupling system suggested that carboxyl and hydroxyl carbons were connected to C-3 and C-4, respectively. In the ROESY experiment, the cross peak of $\delta H$ 6.01, H-3'/$\delta H$ 1.85, and H-1'a supports the Z-configuration of C-2'-C-3'. Compound 2 was thus established as (2Z)-4-(3-carboxy-4-hydroxyphenyl)-2-methylbut-2-enoic acid.

Compound 3, isolated as a yellow oil, had a molecular formula of C$_{13}$H$_{12}$O$_{6}$, based on the molecular ion peak at $m/\zeta$ 263.0570 [M-H]$^{-}$ (calcd. For C$_{13}$H$_{12}$O$_{6}$, 264.0634) in its Q-TOF-MS. The

![Figure 2](image-url)
1H-NMR spectrum of compound 3 showed signals for 3 aromatic protons of a trisubstituted benzene (δH 7.69 m, 6.93, d, J = 8.2 Hz, 7.67 d, J = 8.2 Hz, each 1H), 1 olefinic proton (δH 6.00, t, J = 6.0 Hz, 1H), 1 methyne (δH 3.7, d, J = 6.0 Hz, 2H), and 1 methyl (δH 1.88 s, 3H). The 13C-NMR spectrum of compound 3 showed 13 signals: 1 benzene ring (δC 130.7, 127.3, 138.5, 121.5, 120.3, 128.7, 122.7, 121.5, 120.1, 120.2, 120.3, 120.4, 120.5). Table 1. Detailed 1H- and 13C-NMR Spectroscopic Data for Compound 1 and Sabianin C (δ in ppm, in CD3OD, 600 and 150 MHz).

| No. | Sabianin C | Compound 1 |
|-----|------------|------------|
|     | δH (J in Hz) | δC | δH (J in Hz) | δC | HMBC |
| 2   | 78.9       | 79.7       | 5.77 d (9.9) | 132.4 | C-2, C-4a |
| 3   | 5.74 d (10.2) | 132.3      | 6.41 d (10.0) | 132.6 | C-5, C-10a |
| 4   | 6.41 d (9.6) | 122.6      | 7.67 d, J = 8.2 Hz | 122.4 | C-4 |
| 4a  | 121.5      | 122.7      | 7.47 s       | 122.6 | C-6 |
| 5   | 7.61 s     | 126.3      | 48.5         | 126.6 | C-5a, C-9a |
| 5a  | 126.1      | 202        | 137.9        | 203.6 |
| 6   | 7          | 52.3       | 3          |
| 7   | 8          | 73.8       | 3.91 dd (3.5, 6.2) | 75.6 | C-6, C-9 |
| 8   | 4.22 dd (3.7, 4.7) | 129.0      | 3.21 m       | 128.9 | C-5a, C-9a |
| 9   | 3.01 dd (4.9, 17.5) | 3.33 m     | 3.33 m       | 21.1 | C-8, C-10 |
| 9a  | 121.5      | 143.8      | 137.9        | 151.1 |
| 10  | 6.61 s     | 117.4      | 143.1        | 143.1 |
| 10a | 159.6      | 151.1      | 23.1         | 151.1 |
| 11  | 3.81 d (10.8) | 66.1       | 1.16 s       | 23.1 | C-6, C-7, C-8 |
| 12  | 1.01 s     | 18.9       | 1.16 s       | 24.1 | C-6, C-7, C-8 |
| 13  | 1.48 s     | 27.5       | 1.48 s       | 28.5 | C-2, C-3 |
| 14  | 1.49 s     | 28.8       | 1.49 s       | 28.8 | C-2, C-3 |
| 1'  | 4.96 d (9.7) | 105.3      | 75.9         | 95.9 |
| 2'  | 3.51 m     | 75.9       | 78.2         | 78.2 |
| 3'  | 3.44 m     | 78.2       | 71.8         | 71.8 |
| 4'  | 3.33 m     | 71.8       | 78.4         | 78.4 |
| 5'  | 3.19 m     | 78.4       | 62.7         | 62.7 |
| 6'  | 3.65 m     | 62.7       | 3.78 m       | 3.78 m |

Abbreviations: NMR, nuclear magnetic resonance; HMBC, heteronuclear multiple bond correlation.

Figure 2. Important heteronuclear multiple bond correlation (HMBC) information (h→c) of the 3 new compounds 1, 2 and 3.
115.4, 161.3, and 125.1), 2 carboxyl carbons (δC 169.5 and 169.6), 2 double-bond carbons (δC 131.5 and 128.9), 1 methene carbon (δC 30.1), and 1 methyl carbon (δC 21.1). The 1H- and 13C-NMR spectra of compound 3 were similar to those of compound 2, except for subtle differences at C-5'. The signal at δC 192.1 was used to define the carbonyl group. The HMBC cross peak (δH 7.67/192.1) indicated that the carbonyl carbon was connected to C-1. Furthermore, in the ROESY experiment, the cross peak at δH 6.00, H-3'/δH 1.88, H-2'a supports the Z-conformation of the C-2'-C-3' double bond. Therefore, compound 3 was established as (2Z)-5-(3-carboxy-4-hydroxy-phenyl)-5-oxo-2-methylpent-2-enoic acid. The NMR data of compounds 2 and 3 are listed in Table 2, and the HMBC spectrum is shown in Figure 2.

Compounds 1-3 were evaluated for their hepatoprotective activities. The results demonstrated that the 3 compounds had no hepatoprotective activity with no significant difference being observed between the cells in the presence or absence of these 3 compounds.

**Experimental**

**Materials**

Analytical methanol and glacial acetic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. Dimethylsulphide-d₆ (DMSO-d₆) was used as a solvent to dissolve monomers. All chemicals used in this study were of analytical grade. Deionized ultrapure water was obtained using a MillQ Millipore Ultrapure Water Purification System (USA).

Methanol and acetonitrile for separation and purification were from Shanghai McLean Company, silica gel (300-400 mesh) for chromatographic separation from Qingdao Haiyang Chemical Group, and DIAION HP-20 macroporous resin from Mitsubishi. The medium-pressure liquid chromatography system was equipped with a Lisui EZ Purify III system, which was from Shanghai Lisui Chemical Engineering. The RP-C18 (25 µm) chromatographic column was purchased from Merck Company. The Ryong C18 column (10 µm, 30 × 250 mm) was equipped with a preparative high performance liquid chromatography (HPLC) system. We also used a Ryong C18 column (5 µm, 20 × 250 mm) for further purification. A Cosmosil C18 column (5 µm, 4.6 × 50 mm) was used in the component purity test. A Shimadzu LC-30A HPLC was connected to a TOF™5600+ mass spectrometer. The important NMR data were obtained using a Bruker Avance spectrometer. Palmitic acid (33.3%) and oleic acid (66.6%) were purchased from Sigma.

The 2 cell lines used in this study, HepG2 and LO2, were purchased from American Tissue Culture Collection.

**Plant Material**

The stems of *S. parviflora* were collected in Ziyun City and identified by Professor Guo-Yue Zhong, Jiangxi from University of Traditional Chinese Medicine. A voucher specimen is deposited in the corresponding author’s laboratory, the Center of...
Table 2. $^1$H- and $^{13}$C-NMR Spectroscopic Data for Compounds 2 and 3 ($\delta$ in ppm, in DMSO-d$_6$ 600 and 150 MHz).

| No. | $\delta$$_H$ (J in Hz) | $\delta$$_C$ | HMBC | $\delta$$_H$ (J in Hz) | $\delta$$_C$ | HMBC |
|-----|----------------------|-------------|------|----------------------|-------------|------|
| 1   | 7.70 dd (8.2, 2.2)   | 7.67        | C-3a | 126.7                | 127.3       | C-3a |
| 2   | 129.8                | 125.1       | C-3a |                      |             |      |
| 3   | 167.8                | 160.0       | C-3a |                      |             |      |
| 3a  |                      |             |      |                      |             |      |
| 4   |                      |             |      |                      |             |      |
| 5   | 6.90 d (8.2)         | 115.2       |      |                      |             |      |
| 6   | 7.71 d (8.2)         | 131.8       |      |                      |             |      |
| 1`  | 1.85 s               | 21.1        | C-2', C-3' |              |             |      |
| 1`a |                      |             |      |                      |             |      |
| 2`  |                      |             |      |                      |             |      |
| 2`a |                      |             |      |                      |             |      |
| 3`  | 6.01 t (7.6)         | 138.3       | C-1' | 1.88 s               | 128.9       |      |
| 4`  | 3.70 d (7.6)         | 30.0        | C-1, C-2 |              |             |      |
| 5`  |                      |             |      | 192.1                |             |      |

NMR, nuclear magnetic resonance; DMSO-d$_6$, dimethylsulfoxide-d$_6$; HMBC, heteronuclear multiple bond correlation.

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Extraction and Isolation

The dried stems of S. parviflora (dry weight: 17 kg) were extracted with 70% ethanol (40 L × 3) through hot reflux. Removal of the solvent, the dry extract (380 g) was separated using macroporous resin HP20 (20 L) and eluted with an ethanol/water (30%→95%) gradient, thereby obtaining 4 fractions (frs 1-4). Fr 1 (253 g) was placed on a silica gel column and eluted with different proportions of CH$_2$Cl$_2$/CH$_3$OH (20:1→1:1 v/v), and at the end of the process, 20 fractions were obtained (frs 1.1-1.20). Fr 1.1 was eluted with ethanol/water (10:90→0:100 v/v, 30 mL min$^{-1}$), and 11 subfractions were obtained (frs 1.1.1→1.1.11). Subfraction fr 1.1.3 (2.4 g) was separated using a semi-preparative HPLC-C18 chromatography column. Fr 2 (94 g) was chromatographed on a silica gel column with CH$_2$Cl$_2$/CH$_3$OH (20:1→1:1 v/v), and 16 subcomponents (frs 2.1-2.16) were obtained. Using medium- and low-pressure chromatographic columns (10 cm × 60 cm), 10 subcomponents (frs 2.1.1-2.1.10) were obtained with ethanol/water elution (20:80→0:100 v/v, 30 mL min$^{-1}$). Then, compounds 1 (2.0 mg, TR 42.5 min) and 3 (8.9 mg, TR 23.6 min) were purified from fr 2.1.8 (1.2 g) by semi-preparative HPLC-C18 column chromatography (15% acetonitrile 5 mL min$^{-1}$).

1. 2,2,7,7-Tetramethyl-8S,10-dihydroxy-2,7,8,9-tetrahydro-2H-naphtho(2,3-b)pyran-6-one-10-0-β-D-glucopyranoside (yellow powder); Table 1 presents the $^1$H- and $^{13}$C-NMR data. Q-TOF-MS: m/z 451.1943 [M+H]$^+$ (calcd. for C$_{23}$H$_{30}$O$_9$, 450.1890).
2. (2Z)-4-(3-carboxy-4-hydroxyphenyl)-2-methylbut-2-enoic acid (colorless oil); Table 2 presents the $^1$H- and $^{13}$C-NMR data. Q-TOF-MS: m/z 235.0608 [M-H]$^-$ (calcd. for C$_{12}$H$_{12}$O$_5$, 236.0685).
3. (2Z)-5-(3-carboxy-4-hydroxyphenyl)-5-oxo-2-methylpent-2-enoic acid (yellow oil); Table 2 presents the $^1$H- and $^{13}$C-NMR data. Q-TOF-MS: m/z 263.0570 [M-H]$^-$ (calcd. for C$_{13}$H$_{12}$O$_6$, 264.0634).

Cell Viability Assay

HepG2 and LO2 cells were preserved in Roswell Park Memorial Institute 1640 (RPMI 1640) medium under a humidified atmosphere of 5% CO$_2$ at 37°C. The medium contained 5% fetal bovine serum, penicillin (100 μg/mL) and streptomycin sulfate (100 μg/mL). HepG2 and LO2 cells were seeded and inoculated on 96-well plates at a density of 1 × 10$^4$ cells/well for 24 h. Then, 100 μL of medium containing different concentrations of the 3 compounds was added to each well, except for the blank and model wells. After 4 h, free fatty acids (1 mmol L$^{-1}$) were added to all groups except the blank. After 24 h, the supernatant was discarded and a 10% CCK8 reagent (100 μL/well) was added and incubated for 2 h. In our experiment, a microplate reader was used to measure the absorbance of the sample at 450 nm. Cell viability is presented as a relative percentage of the untreated control.

Hydrolysis of Compound 1 and GC Analysis

As reported in the previous literature, compound 1(3 mg) was hydrolyzed with 2 mol L$^{-1}$ HCl–dioxane (1:1, v/v, 5 mL) at 85°C for 8 h. The reaction mixture was extracted with CHCl$_3$.
(2 mL × 4). The aqueous layer was neutralized with 2 mol L⁻¹ NaOH and dried to obtain the monosaccharides. Then, in the presence of pyridine (2 mL), the dry powder was added to L-cystine methyl ester hydrochloride (1.5 mg) and kept at 60°C for 1 h. Next, trimethylsilylimidazole (1.5 mL) was added to the reaction mixture and kept at 60°C for 30 min. The supernatant (4 μL) was directly subjected to GC and compared with a glucose standard. GC was performed using the following experimental conditions: Agilent-7890b ECD detector, Chromatographic column: HP-5 (30 m × 0.25 mm, 0.25 μm), flow: 2 mL min⁻¹; split ratio 30:1. temperature: 250°C.

Conclusion

Three new compounds were isolated from *S. parviflora*. These 3 compounds showed no significant activity regarding liver protection. Further research is needed in order to understand other possible biological activities of these compounds.

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Author Contributions

Wrote or contributed to revising the manuscript: Huan He, WeiDong Du. Conceived and designed the study: Huan He, WeiDong Du, ZhiFeng Li, Qiang Zhou. Do experiment: Huan He, WeiDong Du, Qiang Zhou. Experimental technical support: Yuanying Fang, Qi Wang, YuLin Feng. Data processing and analysis: Huan He, WeiDong Du, ZhiFeng Li. All authors have read and approved this version of the manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

Not applicable, because this article does not contain any studies with human or animal subjects.

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Statement of Human and Animal Rights

Not applicable, because this article does not contain any studies with human or animal subjects.

Informed Consent

Not applicable, because this article does not contain any studies with human or animal subjects.

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