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Chemical Constituents from the Seeds of *Amorpha fruticosa* and Their Chemotaxonomic Significance

Xin Wu[1], Hongbo Liao[2*], Kefeng Wu[1], Liao Cui[1]

[1]Guangdong Key Laboratory for Research and Development of Natural Drugs, Guangdong Medical University, Zhanjiang, China
[2]Department of Pharmacology, Guangdong Medical College, Zhanjiang, China
Email: *sarahliao@gdmu.edu.cn

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Abstract

Seventeen compounds, including six rotenoids (1-6), seven isoflavones (7-13), one stilbene (14) and three benzoic acid derivatives (15-17) were isolated from the seeds of *Amorpha fruticosa*. Their structures were elucidated by spectroscopic methods and by comparison of their reported spectral data. Among them, compound 4 was firstly purified as a natural product, compounds 5, 8 - 12 and 15 - 17 were isolated from the genus *Amorpha* for the first time, and compound 17 was obtained from the Fabaceae family initially. The presence of these compounds suggests that genus *Amorpha* and *Dalbergia* may have very close chemotaxonomic relationship, and shows the relationship between this plant and other species from the Fabaceae family.

Keywords

***Amorpha fruticosa*, Rotenoid, Isoflavone, Chemotaxonomy**

Subject Areas: **Plant Science**

1. Introduction

The genus *Amorpha* (Fabaceae) including 16 species is native to North America with a center of diversity in the southeastern United States [1]. Among them, *A. fruticosa* (**Figure 1**) which has been used as a Chinese folk medicine for the treatment of burn, ambustion, carbuncle and eczema [2] is the only one species introduced into China (Zhao, 1982) [3]. Up to now, chemical investigations of genus *Amorpha* mainly focused on *A. fruticosa*, which resulted in the isolation of more than forty compounds, including stilbenes [4], rotenoids [5] and flavanones

*Corresponding author.

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Figure 1. The photos of *A. fruticosa* (left) and its seeds (right).

[6], as well as isoflavones [7]. The three former groups are also found in the other species of this genus [8]-[10]. Our previous study on the EtOAc partition of 95% EtOH extract of the seeds of *A. fruticosa* has resulted in the isolation of eight rotenoid glycosides [11]. In this paper, the isolation and characterization of seventeen compounds from its n-hexane partition were reported. Among them, one compound was firstly purified as a natural product, nine were isolated from the genus *Amorpha* for the first time, and one was obtained from the Fabaceae family initially. The presence of these compounds suggests that genus *Amorpha* and *Dalbergia* may have very close chemotaxonomic relationship, and shows the relationship between this plant and other species from the Fabaceae family.

2. Materials and Methods

2.1. Plant Material

The seeds of *A. fruticosa* were collected in Shangqiu City, Henan Province of China. After authenticated by Professor Zhangpin Gou (Department of Pharmacology, Guangdong Medical College), a voucher specimen (No. 20130910) was deposited in the Guangdong Key Laboratory for Research and Development of Natural Drugs, Guangdong Medical University, Zhanjiang, China.

2.2. General

1D and 2D NMR spectra were recorded on Bruker AV-500 spectrometer. CD spectra were measured on a Bio-Logic MOS-450 circular dichroism spectrometer. Column chromatographies (CC) were carried out using silica gel (Qingdaohaiyang, China) and Sephadex LH-20 (Pharmacia Biotech AB, Sweden). Analytical high-performance liquid chromatography (HPLC) was carried out on a Agilent 1200 series and a C_{18} reversed-phase column (Cosmosil, 4.6 mm × 250 mm, 5.0 μm). Preparative HPLC were carried out on a Gilson 305 pump, a Varian Prostar 345 UV detector and a C_{18} reversed-phase column (Cosmosil, 20 mm × 250 mm, 5.0 μm).

2.3. Extraction and Isolation

Fresh and powdered seeds of *A. fruticosa* (4.0 kg) were extracted three times with 95% EtOH at room temperature (3 × 40 L). The solution was removed under vacuum at 50˚C to yield the residue (495 g). The residue was suspended in distilled water, and then successively partitioned with n-hexane, EtOAc and *n*-BuOH respectively. After removing the solvent, then-hexane partition (120 g) was chromatographied by silica gel column using gradient mixtures of cyclohexane-acetone (92:8 → 50:50) as eluants to yield eleven fractions (Fr. H1-Fr. H11). Fr. H3 (3.3 g) was separated by Sephadex LH-20 column using CH_3OH as eluent and further purified by preparative HPLC (CH_3OH-H_2O, 15:85) to yield compounds **15** (1210.0 mg) and **16** (1040.0 mg), **17** (110.0 mg). Fr. H4 (4.2 g) was separated by Sephadex LH-20 column using CH_3OH as eluent to give four subfractions (Fr.

H4a-Fr. H4d). Then, Fr. H4a (132.0 mg) was further purified by Sephadex LH-20 column as CH_3OH to yield compounds **13** (5.0 mg) and **6** (18.0 mg). Fr. H4b (1550.0 mg) was purified by preparative HPLC (CH_3OH-H_2O, 45:55) to yield compounds **14** (190.1 mg), **2** (324.0 mg), **1** (740.0 mg) and **3** (66.0 mg). Fr. H4c (250.0 mg) was purified by preparative HPLC (CH_3OH-H_2O, 60:40) to yield compound **5** (100.0 mg). Fr. H5 (500.0 mg) was separated as the same method as Fr. H4to yield three subfractions (Fr. H5a-Fr. H5c), which were purified by preparative HPLC (CH_3OH-H_2O, 45:55) to give compounds **4** (10.1 mg), **10** (5.3 mg) and **11** (6.2 mg), respectively. The same procedure was applied to Fr. H6 (430.0 mg) to get four subfractions (Fr. H6a-Fr. H6d). Fr. H6b, Fr. H6c and Fr. H6d were purified by preparative HPLC (CH_3OH-H_2O, 40:60) to yield compounds **7** (10.1 mg), **8** (5.5 mg) and **9** (10.4 mg), respectively. Fr. H9 (536.0 mg) was isolated by preparative HPLC (CH_3OH-H_2O, 25:75) to yield compound **12** (200.5 mg).

3. Results and Discussion

The isolated compounds were identified as 6a*R*, 12a*R*-dalbinol (**1**) [12], amorphigenin (**2**) [12], 12a-hydroxydalpanol (**3**) [12], 6a*S*, 12a*S*-dalbinol (**4**) [12], 12a-hydroxymunduserone (**5**) [13], amorphispironone (**6**) [14], ononin (**7**) [15], isoformonentin (**8**) [16], daidzein (**9**) [17], prunetin (**10**) [18], pratensein (**11**) (Dixit *et al*., 2012) [19], 7-*O*-*β*-D-glucopyranosyl-7-hydroxy-2',4',5'-trimethoxyisoflavone (**12**) [20], 7-hydroxy-2',4',5'-trimethoxyisoflavone (**13**) [21], amorfrutin B (**14**) [22], gallic acid (**15**) [23], 4-methoxygallic acid (**16**) [24] and 3,4-dimethoxygallic acid (**17**) [25], respectively, on the basis of their ^{1}H NMR and ^{13}C NMR spectra analysis and comparison with those reported data in the related literatures (**Figure 2**). The absolute configurations of 6a and 12a of **1**-**3** and **5** were assigned as R,R based on the CD data which revealed a negative Cotton effect at 326 nm, while **4** was S,S with mirror image CD spectrum of **1** (**Figure 3**) [11].

The present study reported the isolation and structure elucidation of seventeen secondary metabolites from the seeds of *A. fruticosa*, including six rotenoids (**1**-**6**), seven isoflavones (**7**-**13**), one stilbene (**14**) and three benzoic acid derivatives (**15**-**17**). Among them, compound **4** was firstly purified as a natural product, compounds **5**, **8**-**12** and **15**-**17** were isolated from the genus *Amorpha* for the first time, and compound **17** was obtained from the

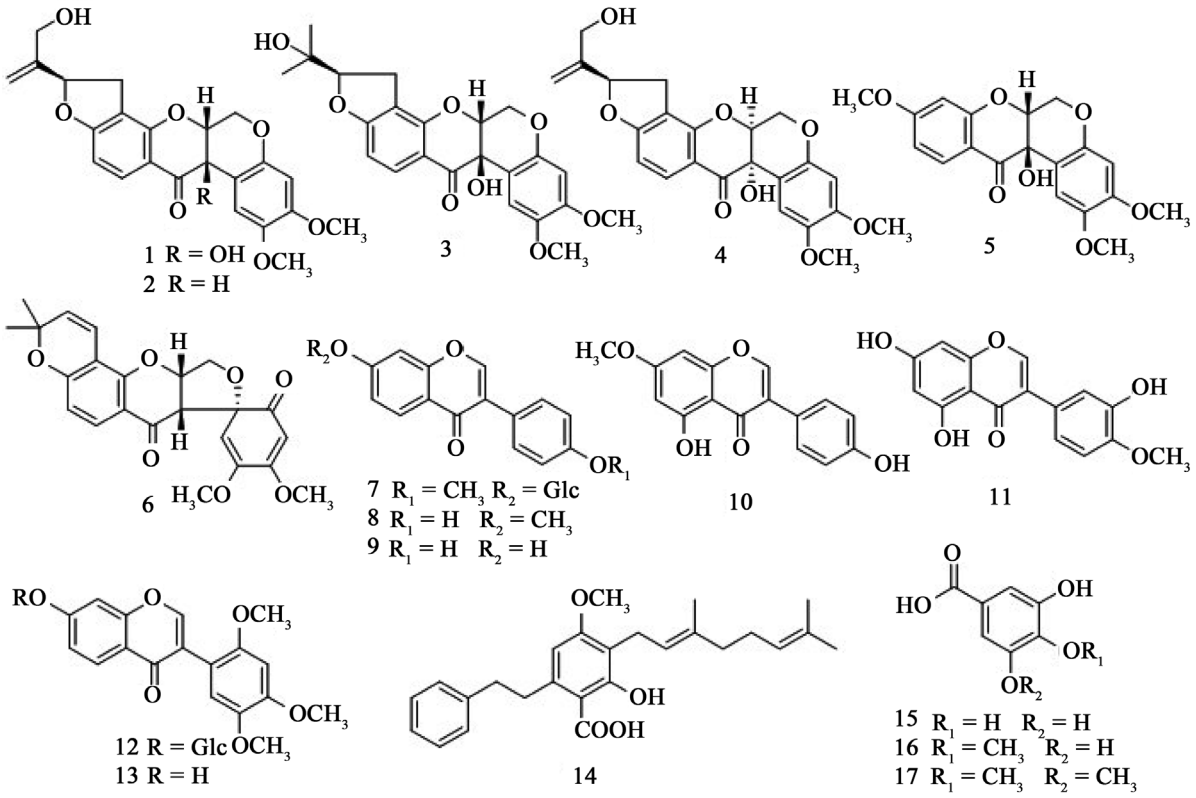

Figure 2. The structures of compounds 1-17.

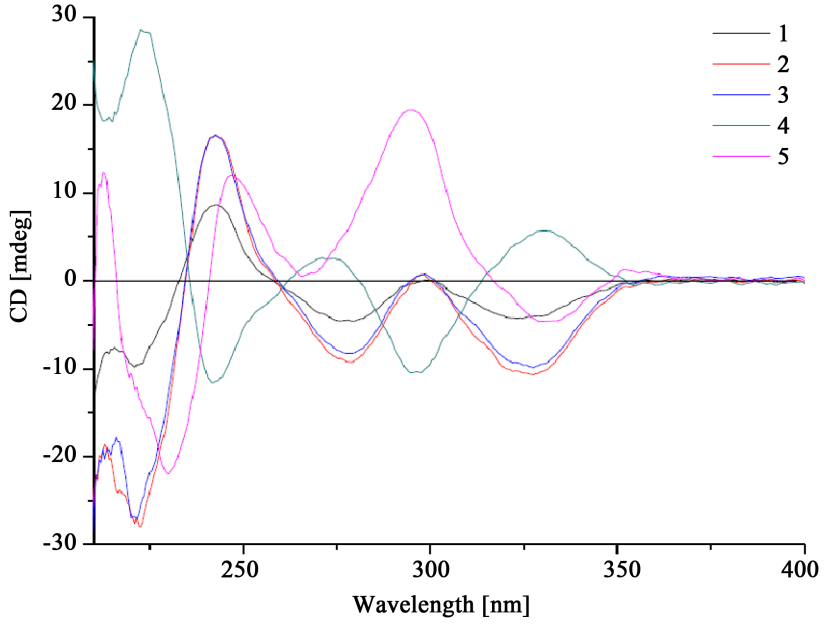

Figure 3. The CD spectra of compounds 1-5.

Fabaceae family initially. Although compound **12** has been once reported, it has no CAS number yet. Compounds **1-3**, along with the previous reported compounds amorphin and 8'-*O*-*β*-D-glucopyranosyl-amorphigenin, have been isolated or detected in ten species of the genus *Amorpha* (*A. fruticosa*, *A. angustifolia*, *A. canescens*, *A. frangrans*, *A. californica*, *A. glabra*, *A. mierophylla*, *A. nana*, *A. croceotanata*, and *A. caroliniana*) [9] at the same time, which provides a chemotaxonomic evidence to support the morphological classification of this genus.

Rotenoids **1-2** and isoflavones **12-13**, as well as our previous reported rotenoid glucosides 8'-*O*-*β*-D-gluco-glucopyranosyl-amorphigenin and dalbin [11], were also isolated from *Dalbergia monnetaria* (Fabaceae) simultaneously [20]. Such a coincidence should be explained by the fact that the isoflavone **13** was the key intermediate during the biosynthesis of its corresponding rotenoids [26]. In addition, some compounds obtained in this studyalso exist in other species of genus *Dalbergia*, as follows: compound **1** in *D. nitidula* [27]; compound **7** in *D. paniculata* [28] and *D. odorifera* [15]; compound **9** in *D. odorifera* [17], *D. parviflora* [29], *D. frutescens* [30], *D. ecastophyllum* [31], *D. stevensonii* [32] and *D. ecastophylla* [33]; compound **10** in *D. odorifera* [34], *D. sympathetic* [18], *D. sissoo* [35] and *D. spinosa* [36]; compound **11** in *D. sissoo* [19], *D. parviflora* [37] and *D. odorifera* [38]; compound **13** in *D. vacciniifolia* [21]. All these suggested that genus *Amorpha* and *Dalbergia* may have very close chemotaxonomic relationship.

Furthermore, compounds **1** and **2** were also synchronally isolated from *Berchemia discolor* [39], compound **3** was once obtained from *Aeschynomene indica* [40], and compound **5** was obtained from *Pachyrrhizus erosus* [13], *Tephrosia fulvinervis* [41] and *Tephrosia pentaphylla* [41], which exhibit the relationships between *A. fruticosa* and these species, all belonging to family Fabaceae except *Berchemia discolor* (Rhamnaceae). Compound **4** was isolated naturally for the first time, while compounds **6** and **14** both only existed in *A. fruticosa*. Compounds **15** and **9** widely distributed in the family Fabaceae, and compound **16** has only been isolated from one species of it (*Canavalia gladiate*) [42], while it is the first time to obtain compound **17** from this family. Therefore, compounds **4**, **6**, **14** and **17** should be served as chemotaxonomic markers for *A. fruticosa*.

4. Conclusion

According to the above results, our present study extends the knowledge about the compounds of genus *Amorpha* and confirms *Dalbergia* as its chemotaxonomic related genus.

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