Fitoterapia 114 (2016) 105–109

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

# Flavonoids from *Caragana pruinosa* roots

Wei Peng [a,b,1], Liang Wang [a,1], Xu-Hui Qiu [a], Yi-Ping Jiang [a], Lan Pan [c], Xiao-Guang Jia [c], Lu-Ping Qin [a,*], Cheng-Jian Zheng [a,*]

[a] Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, China
[b] College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 610075, China
[c] Xinjiang Institute of Chinese Materia Medica and Ethnodrug, Urumqi 830002, China

ARTICLE INFO

*Article history:*
Received 4 August 2016
Received in revised form 27 August 2016
Accepted 31 August 2016
Available online 1 September 2016

*Keywords:*
*Caragana pruinosa*
Flavonoids
Pruinosanones
Nitric oxide

ABSTRACT

A new pterocarpan derivative, pruinosanone D (**1**), a new isoflavonoid, pruinosanone E (**2**), and a new chalcone, pruinosanone F (**3**), were isolated from *Caragana pruinosa* roots, along with four known analogues (**4**–**7**), identified as 2,4-dihydroxy-3′-methoxy-4′-ethoxychalcone, 7,4-dihydroxyflavanone, butin and scutellaprostin C, respectively. Their structures were elucidated by detailed analyses of NMR, IR, and MS data. The ability of the isolated compounds to prevent nitric oxide (NO) production by LPS-stimulated RAW 264.7 macrophages was also studied. Compound **1** were among the most potent NO production inhibitor, with $IC_{50}$ value of 0.62 μM.



## 1. Introduction

*Caragana pruinosa* Kom., belonging to the Leguminosae family, is a deciduous dwarf shrub mainly distributed in Xinjiang area of China and Central Asia [1]. It's recorded that the roots of *C. pruinosa* are effective folk herbal medicine for treating various inflammatory diseases in northwestern China [2]. However, so far, few phytochemical and pharmacological investigations have been conducted on this plant. Previous studies focused on other *Caragana* species suggested the presence of abundant flavonoids, stilbenoids and terpenoids [3–6]. As part of our continuing efforts focused on *Caragana* species [7–9], a total of 3 new flavonoids, named pruinosanones D–F (**1**–**3**), were isolated from the roots of *C. pruinosa* (Fig. 1), along with four known flavonoid derivatives (**4**–**7**), identified as 2,4-dihydroxy-3′-methoxy-4′-ethoxychalcone, 7,4-dihydroxyflavanone, butin and scutellaprostin C, respectively. In addition, their inhibitory effects on nitric oxide (NO) production in LPS-stimulated RAW264.7 cells were also evaluated.

## 2. Experimental

### 2.1. General

Optical rotations were acquired with a Rudolph AUTOPOL VI (Rudolph Research Analytical). IR spectra were determined on a Bruker Vector 22 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker Avance 600 NMR spectrometer with TMS as an internal standard. HRESIMS were measured using a Q-TOF micro mass spectrometer (Waters, USA). Materials for column chromatography were silica gel (100–200, 200–300 mesh, Huiyou Silical Gel Development Co. Ltd., Yantai, China), Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-GEL ODS-A (50 μm; YMC, Milford, MA). HSGF254 silica gel TLC plates (Yantai) were used for analytical TLC. Preparative TLC (0.4–0.5 mm) was conducted on glass plates precoated with silica gel GF254 (Yantai, China). Ethanol, methanol, petroleum ether, ethyl acetate, dichloromethane and chloroform were purchased from Sino pharm Chemical Reagent Co., Ltd. (Shanghai, China).

### 2.2. Plant material

*C. pruinosa* were collected from Urumuchi, Xinjiang area, P.R. China, and authenticated by Prof. Xiao-Guang Jia, Chinese Medicine Research Institute of the Sinkiang Uygur Autonomous Region (Urumuchi, China). The voucher specimen of this plant was kept at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai, PR China (No. *201203).

### 2.3. Extraction and isolation

The dried and powdered *C. pruinosa* roots (11.5 kg) were extracted with 80% ethanol for 3 times by reflux (each time lasting 2 h). The extracting solution was dried in vacuum under 60 °C to afford the crude extracts (1.2 kg), then the extracts were suspended in $H_2O$ and

* Corresponding authors.
*E-mail addresses:* qinsmmu@126.com (L.-P. Qin), zhengchengjian1984@126.com, zheng_chengjian@hotmail.com (C.-J. Zheng).
[1] These authors contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.fitote.2016.08.020
0367-326X/

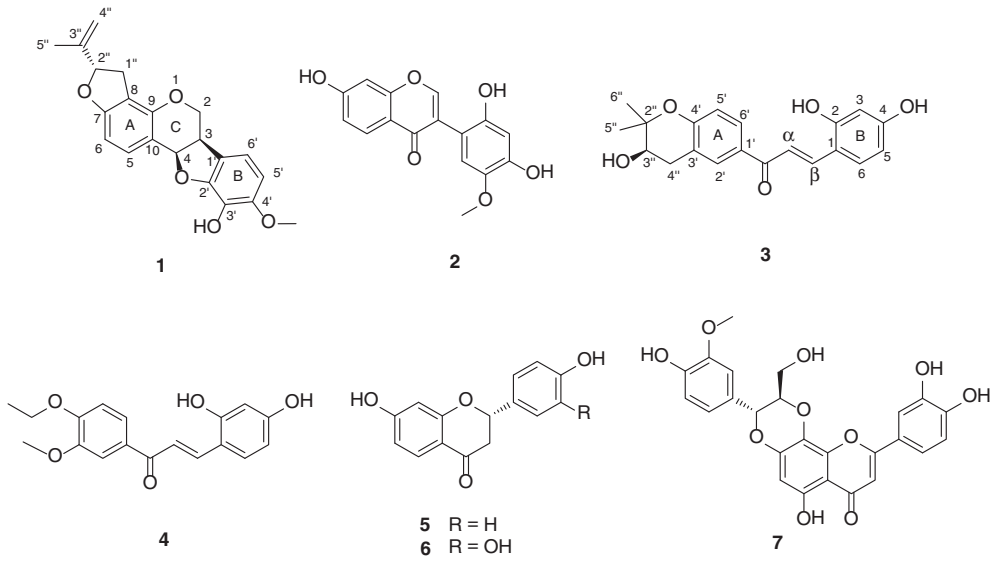


**Fig. 1.** Chemical structures of compounds **1**–**7**.

extracted sequentially with petroleum ether, ethyl acetate and *n*-butanol to obtain four fractions, including petroleum ether fraction (PEF, 33.9 g), ethyl acetate fraction (EAF, 226.9 g), *n*-butanol fraction (BF, 100.9 g) and remaining water fraction (WF). Subsequently, the EAF was selected for systemic constituents isolation based on its significant inhibitory effects on nitric oxide (NO) production in LPS-stimulated RAW264.7 cells.

The ethyl acetate fraction (EAF, 220 g) was subjected to column chromatography over silica gel (200–300 mesh) and eluted with a gradient of petroleum ether-ethyl acetate (50:1, 20:1, 10:1, 5:1, 3:1, 1:1 and 0:1, v/v). Combination of similar fractions based on the TLC analysis afforded 7 fractions (A–G). Subsequently, the Fr.C (5.0 g) were subjected to column chromatography over silica gel (200–300 mesh) again, and eluted with petroleum ether-ethyl acetate (20:1, v/v) to afford 5 sub-fractions (C1–C5) on the basis of TLC analysis. Fraction C.2 (416 mg) was re-chromatographed on Sephadex LH-20 and eluted with 80% methanol followed by preparative TLC to yield compounds **2** (7 mg), **5** (11 mg) and **6** (12 mg). Fraction C.5 (200 mg) was re-subjected to column chromatography over Sephadex LH-20 and eluted with methanol followed by preparative TLC to afford compounds **1** (8 mg). In addition, Fr.D (6.5 g) were subjected to column chromatography over ODS, and eluted with methanol-$H_2O$ (10:90, 30:70, 50:50, 70:30,

**Table 1**
$^1$H NMR (600 Hz) and $^{13}$C NMR (150 Hz) data of compounds **1**–**3** in $CD_3OD$ ($\delta$, ppm).

| No. | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| | $\delta_H$ (*J*, Hz) | $\delta_C$ | $\delta_H$ (*J*, Hz) | $\delta_C$ | $\delta_H$ (*J*, Hz) | $\delta_C$ |
| 1 | | | | | | 114.6 |
| 2 | 3.60 (dd, 10.8, 10.8)<br>4.26 (dd, 10.8, 4.8) | 65.5 | 8.16 (s) | 156.9 | | 167.6 |
| 3 | 3.51 (m) | 39.5 | | 123.8 | 6.26 (d,2.4) | 103.9 |
| 4 | 5.48 (d, 6.6) | 78.6 | | 178.9 | | 166.8 |
| 5 | 7.31 (d, 8.4) | 130.9 | 8.05 (d, 8.4) | 128.5 | 6.39 (dd, 9.0, 2.4) | 109.3 |
| 6 | 6.48 (d, 8.4) | 102.2 | 6.93 (dd, 8.4, 1.8) | 116.9 | 7.97 (d, 9.0) | 133.4 |
| 7 | | 160.9 | | 165.3 | | |
| 8 | | 112.1 | 6.84 (d, 1.8) | 103.2 | | |
| 9 | | 151.8 | | 159.8 | | |
| 10 | | 112.2 | | 117.6 | | |
| 1′ | | 120.9 | | 110.9 | | 128.7 |
| 2′ | | 148.6 | | 149.2 | 7.47 (d, 1.8) | 129.2 |
| 3′ | | 130.9 | 6.44 (s) | 105.6 | | 121.9 |
| 4′ | | 146.4 | | 151.3 | | 157.1 |
| 5′ | 6.49 (d, 8.4) | 103.9 | | 142.8 | 6.79 (d,8.4) | 118.9 |
| 6′ | 6.75 (d, 8.4) | 113.6 | 6.81 (s) | 116.3 | 7.50 (dd, 8.4, 1.8) | 132.2 |
| 1″ | 3.25 (15.6, 10.2)<br>2.89 (15.6, 7.8) | 30.9 | | | | |
| 2″ | 5.21 (t, 8.4) | 85.9 | | | | 79.0 |
| 3″ | | 143.8 | | | 3.77 (dd, 7.2, 5.4) | 70.1 |
| 4″ | 5.05 (s)<br>4.88 (s) | 110.13 | | | 3.06 (dd, 16.2, 5.4)<br>2.77 (dd, 16.2, 7.2) | 31.9 |
| 5″ | 1.74 (s) | 15.7 | | | 1.33 (s) | 25.9 |
| 6″ | | | | | 1.27 (s) | 21.4 |
| α | | | | | 7.62 (d, 15.6) | 118.7 |
| β | | | | | 7.75 (d,15.6) | 145.4 |
| C=O | | | | | | 193.4 |
| $-OCH_3$ | 3.82 (s) | 55.1 | 3.79 (s) | 57.4 | | |