Flavonoids in the Poisonous Plant *Oxytropis falcata*

Wen-Hao Chen,[†,‡] Rui Wang,[†,‡] and Yan-Ping Shi*[,†,‡]

Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, People's Republic of China, and State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, People's Republic of China

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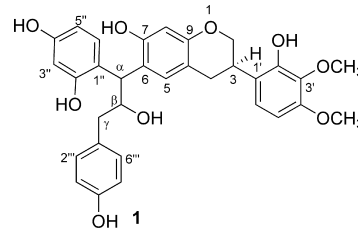
Three new flavonoids, oxytropisoflavans A (**1**) and B (**2**) and (6a*R*,11a*R*)-3,8-dihydroxy-9,10-dimethoxypterocarpan (**3**), together with 30 known flavonoids (**4**−**33**), were isolated from the aerial parts and roots of *Oxytropis falcata*. The absolute configurations of **3** and C-3 in **1** and **2** were deduced by circular dichroism. The structure of flavonoid **2** was confirmed by single-crystal X-ray diffraction analysis and that of flavonoid **3** by total synthesis of its racemate. Oxytropisoflavan A (**1**) is an unprecedented chalcan-isoflavan biflavonoid, whereas oxytropisoflavan B (**2**) possesses a rare modified A-ring. Pterocarpan **3** has good radical-scavenging activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.

Flavonoids are a group of ubiquitous and diverse molecules produced via the phenylpropanoid pathway in higher plants, and about 2% of all the photosynthesized carbon is converted into flavonoids.[1] They play a variety of roles in plants including protection against UV damage and phytopathogens (e.g., phytoalexins in legumes), acting as pigments or copigments in influencing flower color, modulating auxin distribution, and as signal molecules to symbiotic microbes.[2]

Flavonoids often possess antioxidant activity, and the potential health benefits of fruit, vegetables, green tea, and red wine might partly be because of this property.[3−5] In addition, the isoflavonoids, which are limited primarily to the Leguminosae, exhibit estrogenic and anticancer activity[6,7] and, in common with the flavonoids, are also receiving considerable attention as health-promoting "nutraceuticals".

Plants of the genera *Astragalus* and *Oxytropis* (both Leguminosae) containing the indolizidine alkaloid swainsonine are commonly known as locoweeds. Consumption of the aerial plant parts of many locoweed species causes significant economic loss to cattle, sheep, and horses.[8] *O. falcata* Bunge, known as a toxic plant, is commonly regarded as "the king of herbal medicine" in Tibetan medicine. Alcohol extracts of *O. falcata* mixed with other medicinal materials (Qingpenggao) have been patented as a treatment for pain and arthritis.[9] It is a common paradigm in the search for new drugs in human medicine to investigate constituents of plants that have an ethnopharmacological basis for their use. Recently, a comprehensive review described the therapeutic potential of the toxic components of poisonous plants.[10]

In this regard, a systematic investigation of the composition of this poisonous plant has been conducted. We recently reported the occurrence of pendulone[11] and a series of 24-hydroxyoleanane-type triterpenoids[12] and *N*-benzoylindole analogues[13] from *O. falcata*. In this study we describe the isolation and characterization of three new flavonoids, oxytropisoflavans A (**1**) and B (**2**) and (6a*R*,11a*R*)-3,8-dihydroxy-9,10-dimethoxypterocarpan (**3**), together with 30 known flavonoids (**4**−**33**) from the aerial parts and roots of *O. falcata*. The radical-scavenging activity against DPPH and cytotoxic activity against the human promyelocytic leukemic (HL-60) cell line of the new compounds are also described.



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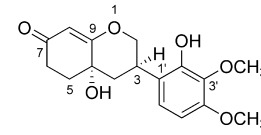

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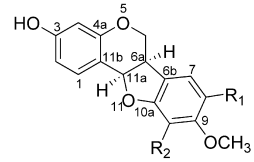
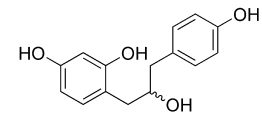


3 R_1 = OH, R_2 = OCH_3
4 R_1 = H, R_2 = OH



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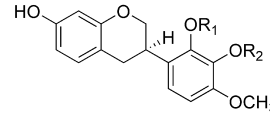
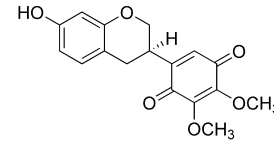


6 R_1 = H, R_2 = CH_3
7 R_1 = CH_3, R_2 = H
8 R_1 = H, R_2 = H



9 pendulone

Results and Discussion

The EtOH extracts of the aerial parts and roots of *O. falcata* were suspended in H_2O and then partitioned successively with petroleum ether, EtOAc, and *n*-BuOH, respectively. Both petroleum ether-soluble fractions were combined, because they exhibited similar constituents on TLC. The organic-soluble fractions were further purified by Si gel CC and PTLC to yield three new flavonoids, oxytropisoflavans A (**1**) and B (**2**) and (6a*R*,11a*R*)-3,8-dihydroxy-9,10-dimethoxypterocarpan (**3**).

Oxytropisoflavan A (**1**) was obtained as an optically active, pale brown oil, with an elemental formula of $C_{32}H_{32}O_9$ determined by HRESIMS (*m*/*z* 583.1944, $[M + Na]^+$). There was evidence of hydroxy and aromatic ring absorptions in the IR spectrum at 3363, 1510, and 1463 cm^{-1}.

The ^{13}C NMR spectrum of **1** (Table 1) displayed signals for 32 carbons, including eight sp^3 carbons, 13 quaternary sp^2 carbons with eight linked to an oxygen atom, and 11 tertiary sp^2 carbons. The ^1H NMR spectrum of **1** (Table 1) showed four aliphatic signals at δ_H 4.40 (1H, d, J = 5.2 Hz, H-α), 4.68 (1H, m, H-β), 2.73 (1H, dd, J = 4.4, 14.0, H-γa), and 2.61 (1H, dd, J = 7.2, 14.0, H-γb),

* To whom correspondence should be addressed. Tel: +86-931-4968208. Fax: +86-931-8277088. E-mail: shiyp@licp.cas.cn.
† Lanzhou Institute of Chemical Physics.
‡ Lanzhou University.

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data of **1** in Acetone-d_6[a]

no.	δ_H mult (J in Hz)	δ_C	HMBC (H→C)
2	4.23 dd (3.2, 10.0), 3.96 t (10.0)	69.7 t	C-4, 9
3	3.48 m	32.6 d	
4	2.90 dd (11.2, 15.6), 2.78 dd (4.0, 15.6)	30.4 t	C-2, 3, 5, 9, 10, 1′
5	6.85 s	132.9 d	C-4, 7, 9, α
6		120.1 s	
7		155.2 s	
8	6.33 s	104.1 d	C-6, 7, 9, 10
9		154.1 s	
10		113.5 s	
1′		121.1 s	
2′		148.4 s	
3′		136.3 s	
4′		152.0 s	
5′	6.50 d (8.8)	103.7 d	C-1′, 3′, 4′
6′	6.83 d (8.8)	121.7 d	C-2′, 4′
α	4.40 d (5.2)	47.7 d	C-5, 6, 7, 1″, 2″, 6″, γ
β	4.68 m	74.4 d	C-1″, 1‴
γ	2.73 dd (4.4, 14.0), 2.61 dd (7.2, 14.0)	41.4 t	C-α, β, 2‴, 6‴
1″		120.4 s	
2″		155.2 s	
3″	6.36 d (2.4)	102.9 d	C-1″, 2″, 4″, 5″
4″		156.8 s	
5″	6.24 dd (2.4, 8.4)	106.9 d	C-1″, 3″,
6″	7.02 d (8.4)	130.9 d	C-α, 2″, 4″
1‴		130.5 s	
2‴	7.02 d (8.4)	130.5 d	C-γ, 3‴, 4‴, 6‴
3‴	6.72 d (8.4)	115.0 d	C-1‴, 2‴, 4‴, 5‴
4‴		155.8 s	
5‴	6.72 d (8.4)	115.0 d	C-1‴, 3‴, 4‴, 6‴
6‴	7.02 d (8.4)	130.5 d	C-γ, 2‴, 4‴, 5‴
3′-OCH_3	3.78 s	60.1 q	C-3′
4′-OCH_3	3.81 s	55.4 q	C-4′
7-OH	9.63 s		C-6, 7, 8
2′-OH	7.94 s		C-1′, 2′, 3′
β-OH	5.13 s		C-α, β, γ
2″-OH	8.44 s		C-1″, 2″, 3″
4″-OH	8.01 s		C-3″, 4″, 5″
4‴-OH	8.07 s		C-3‴, 4‴, 5‴

[a] Assignments were based on DEPT135, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments.

characteristic of a substituted propyl group in the chalcan-β-ol structure,[14] and five aliphatic signals at δ_H 4.23 (1H, dd, J = 3.2, 10.0, H-2a), 3.96 (1H, t, J = 10.0, H-2b), 3.48 (1H, m, H-3), 2.90 (1H, dd, J = 11.2, 15.6, H-4a), and 2.78 (1H, dd, J = 4.0, 15.6, H-4b), typical of an isoflavan nucleus.[15,16] These observations and the molecular mass of 560 suggested that compound **1** could be a biflavonoid possessing a chalcan-β-ol unit connected to an isoflavan unit. From analysis of the ^{1}H−^{1}H COSY NMR experiment, the chalcan unit of **1** appeared to be 2″,4″,4‴-trihydroxylated (propterol B, **5**),[17] as indicated by signals at δ_H 6.36 (1H, d, J = 2.4, H-3″), 6.24 (1H, dd, J = 2.4, 8.4, H-5″), 7.02 (3H, d, J = 8.4, H-6″, 2‴, 6‴), and 6.72 (2H, d, J = 8.4, H-3‴, 5‴), while the isoflavan

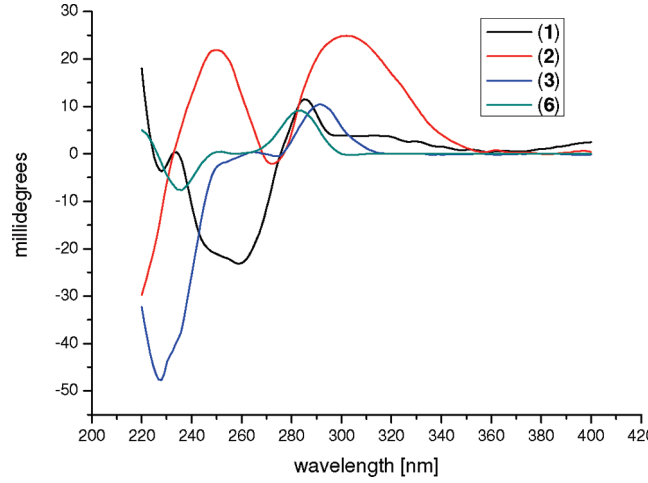


Figure 2. CD spectra of **1**−**3** and **6**.

moiety seemed to be pentaoxygenated, with four aromatic protons resonating at δ_H 6.85 (1H, s, H-5), 6.33 (1H, s, H-8), 6.50 (1H, d, J = 8.8, H-5′), and 6.83 (1H, d, J = 8.8, H-6′), reminiscent of (3*R*)-(−)-isomucronulatol (**6**).[15,16] Further supporting evidence came from ^{13}C NMR data, which showed a close resemblance with those of propterol B (**5**) and (3*R*)-(−)-isomucronulatol (**6**).

Connectivities of the two substructures of compound **1** were established by analysis of the HMBC spectrum as follows: The ^{3}J HMBC cross-peaks from H-α to C-2″/C-6″/C-5/C-7, as well as the ^{2}J HMBC cross-peaks from H-α to C-1″/C-6, indicated the connection between C-6 and C-α. This was corroborated by the observation of NOESY correlations between H-α and the aromatic protons H-5 and H-6″. The positions of the other functional groups were assigned by ^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY (Figure 1) experiments, which resulted in the assignment of all proton and carbon signals of **1** (Table 1).

The CD data of compound **1** (Figure 2) exhibited a strong positive Cotton effect for the $^{1}L_b$ transition region (260−300 nm),[18] indicating the 3*R* absolute configuration for **1**. Such an allocation is strongly supported by the same Cotton effect in the CD spectrum of (3*R*)-(−)-isomucronulatol (**6**) (Figure 2).[16] It should be emphasized that although the chiral chalcan unit in compound **1** is chirally perturbed by the isoflavan unit and may contribute to the lower wavelength $^{1}L_a$ transition region (220−260 nm),[18] the diagnostic $^{1}L_b$ transition region originates mainly from the isoflavan-type chromophore. The absolute configurations of C-α and C-β remain to be defined.

The EIMS of compound **1** exhibited prominent fragment peaks at *m*/*z* 450, 423, 302, 107, and 180 (base peak) originated from the fission of the α,C-1″-, α,β-, α,C-6-, and β,γ-linkage and a characteristic retro-Diels−Alder-type cleavage of the isoflavan skeleton (Scheme 1). Consequently, the structure of oxytropisoflavan A (**1**) was characterized as (3*R*)-(−)-isomucronulatol-(6→α)-propterol B.

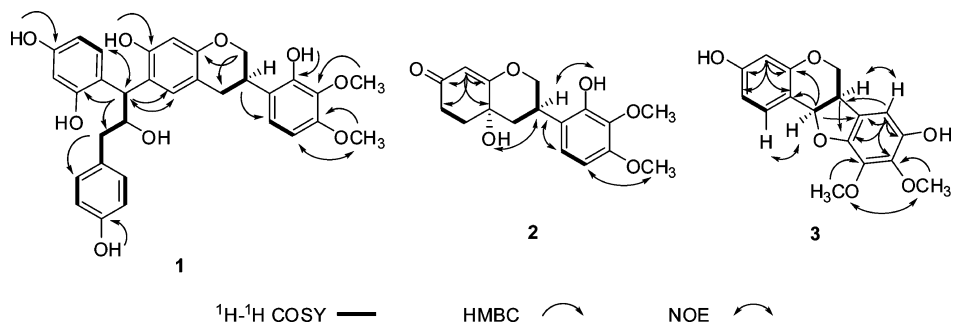


Figure 1. Key ^{1}H−^{1}H COSY, HMBC, and NOE correlations of **1**−**3**.

Scheme 1. EIMS Fragmentation Pattern of **1**

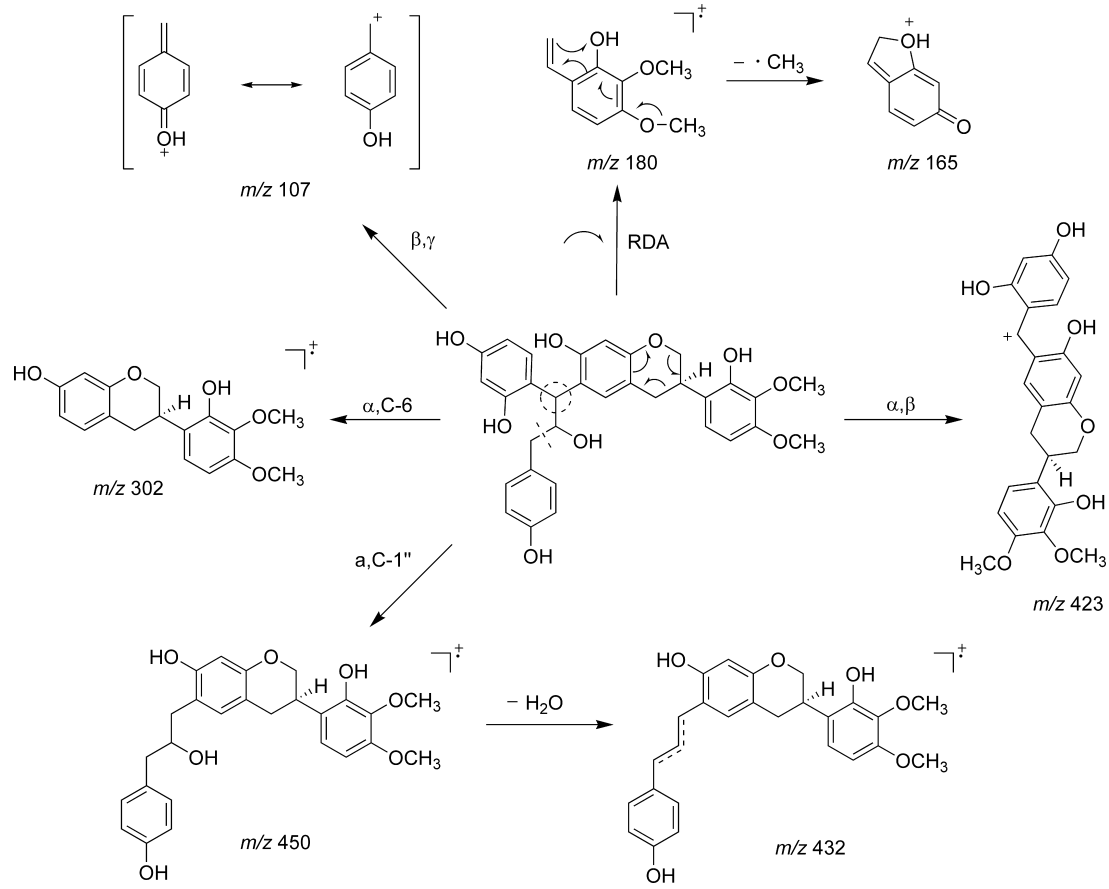


Table 2. ^{1}H and ^{13}C NMR Data of **2** and **3**[a]

	2[b]			**3**[c]	
no.	δ_H mult (*J* in Hz)	δ_C	no.	δ_H mult (*J* in Hz)	δ_C
2	4.29 dd (4.5, 9.9), 3.68 t (9.9)	73.2 t	1	7.33 d (8.8)	132.3 d
3	3.91 m	29.7 d	2	6.56 dd (2.4, 8.8)	109.8 d
4	2.02 m[d]	39.5 t	3		159.1 s
5	1.92 m[d]	36.6 t	4	6.36 d (2.4)	103.1 d
6	2.67 m, 2.15 br d (17.5)	33.5 t	4a		156.9 s
7		199.1 s	6	4.27 dd (5.2, 10.8), 3.67 t (10.8)	66.0 t
8	5.24 s	108.3 d	6a	3.55 m	41.1 d
9		175.5 s	6b		125.0 s
10		65.9 s	7	6.64 s	106.0 d
1′		120.6 s	8		144.8 s
2′		149.0 s	9		140.0 s
3′		136.8 s	10		138.4 s
4′		152.4 s	10a		143.8 s
5′	6.47 d (8.7)	104.0 d	11a	5.48 d (6.8)	78.4 d
6′	6.80 d (8.7)	122.3 d	11b		112.0 s
3′-OMe	3.67 s	60.9 q	9-OMe	3.78 s	60.6 q
4′-OMe	3.75 s	56.3 q	10-OMe	3.89 s	59.7 q
2′-OH	8.98 s				
10-OH	5.59 s				

[a] Assignments were based on DEPT135, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. [b] In DMSO-d_6 at 300 MHz for ^{1}H and 75 MHz for ^{13}C. [c] In acetone-d_6 at 400 MHz for ^{1}H and 100 MHz for ^{13}C. [d] Signals overlapped.

It is important to emphasize that compound **1** is the first example of a chalcan-isoflavan biflavonoid from natural sources and also the first report of the existence of a natural biflavonoid in the genus *Oxytropis*. Propterol B (**5**) and (3*R*)-(−)-isomucronulatol (**6**) were isolated for the first time from the genus *Oxytropis*.

Oxytropisoflavan B (**2**) was obtained as optically active, colorless crystals, with an elemental formula of $C_{17}H_{20}O_6$ determined by HRESIMS (*m/z* 321.1326, $[M + H]^+$). An IR hydroxy peak was observed at 3333 cm^{-1}, The absorption at 1710 cm^{-1} and ^{13}C NMR peak at δ_C 199.1 implied the presence of an α,β-unsaturated ketone carbonyl group. The structure of the compound was established from its ^{1}H and ^{13}C NMR data (Table 2), which were compared to those of (3*R*)-(−)-isomucronulatol (**6**). The assignments of protons and carbons were done via 2D NMR (^{1}H−^{1}H COSY, HSQC, and HMBC) and DEPT experiments. It is structurally related to **6**. Ring A of **2** contained an α,β-unsaturated carbonyl carbon at C-7 (δ 199.1) and the C-8 (δ 108.3), C-9 (δ 175.5) double bond characteristic of the ring A partial structure in 11b-hydroxy-11b,1-dihydromedicarpin.[19] Inspection of the HMBC spectrum (Figure 1) allowed for a complete assignment of all signals in this partial

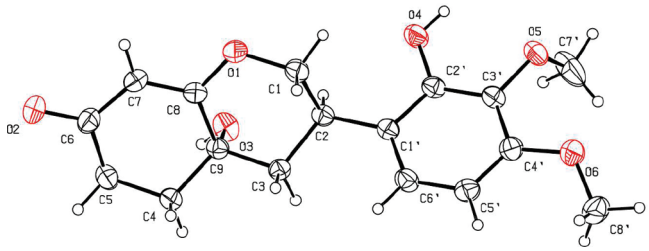


Figure 3. ORTEP drawing of **2**.

structure. The HMBC experiment showed that H-4 (δ 2.02, 2H, m) was related to C-1′ (δ 120.6) and C-2 (δ 73.2) and H-5 (δ 1.92, 2H, m) to C-6 (δ 33.5), C-7 (δ 199.1), C-9 (δ 175.5), and C-10 (δ 65.9).

In the NOESY experiment, H-3 (δ 3.91, 1H, m) showed correlations with HO-2′ (δ 8.98, 1H, s), HO-10 (δ 5.59, 1H, m), and H-6′ (δ 6.80, 1H, d, $J = 8.7$ Hz). These correlations suggested both the location and the α-orientation of the hydroxy group at C-10. The X-ray crystallographic analysis of compound **2** (Figure 3) clearly established its structure. The CD spectrum of compound **2** (Figure 2) matched very well with that of (3*R*)-(−)-isomucronulatol (**6**) at the ^1L_b transition region.[16,18] The (3*R*,10*S*) absolute configuration of **2** could therefore be assigned. Thus, the structure of oxytropisoflavan B (**2**) was established as (3*R*,10*S*)-10-hydroxy-5,10-dihydroisomucronulatol.

Compound **2** is the first natural product with an isoflavan skeleton possessing an α,β-unsaturated ketone carbonyl group in ring A. A similar compound was formed with protein preparation when incubated in the presence of NADPH.[20]

Compound **3** was obtained as a pale brown oil and was optically active ($[\alpha]_D^{20}$ −155, *c* 0.8, acetone). In its HRESI mass spectrum, the protonated molecular ion was observed at *m/z* 317.1016 $[M + H]^+$ (calcd for $C_{17}H_{17}O_5$, 317.1020), corresponding to the molecular formula $C_{17}H_{16}O_5$. The ^{1}H NMR and ^{1}H−^{1}H COSY spectra showed four aliphatic protons in a single spin system at δ_H 4.27 (1H, dd, $J = 5.2$, 10.8 Hz), 3.67 (1H, dd, $J = 10.8$ Hz), 3.55 (1H, m), and 5.48 (1H, d, $J = 6.8$ Hz), attributed to CH_2-6, H-6a, and H-11a of a pterocarpan skeleton, respectively (Table 2). The presence of a pterocarpan skeleton was supported by the ^{13}C NMR spectrum, which showed the corresponding carbons at δ_C 66.0 (C-6), 41.1 (C-6a), and 78.4 (C-11a).[21] In the ^{1}H NMR spectrum further signals were observed that showed the presence of two methoxy groups (δ_H 3.78, 3.89), an ABX aromatic proton spin system (δ_H 6.36, 6.56, 7.12), and a single aromatic proton (δ_H 6.64). The spin systems were assigned by comparison of the NMR data with those of (6a*R*,11a*R*)-3,8-dihydroxy-9-methoxypterocarpan.[22] The ABX spin system corresponds to A-ring protons with the biogenetically expected oxygenation at C-3, and this was confirmed by the HMBC experiment. In the HMBC spectrum (Figure 1), correlations of the methoxy protons (δ_H 3.78, 3.89) with the signals at δ_C 140.0 and 138.4, together with the aromatic proton (δ_H 6.64, H-7) with the signals at δ_C 41.1 (C-6a), 144.8 (C-8), and 140.0 (C-9), allowed placement of the hydroxy group at C-8 and methoxy groups at C-9 and C-10, respectively.

Pterocarpans are found in nature only in a 6a,11a-*cis* configuration. In agreement with this are the coupling constant between H-6a and H-11a ($J = 6.8$ Hz) and the comparison with the literature values ($J = 6.6$ Hz for *cis* and 13.4 Hz for *trans*).[23] The absolute configuration at C-6a and C-11a was assigned as *R* from its negative optical rotation ($[\alpha]_D^{20}$ −155) and the negative Cotton effect ($\Delta\varepsilon$ −47.7) at 228 nm (Figure 2).[24,25] Thus, compound **3** was characterized as (6a*R*,11a*R*)-3,8-dihydroxy-9,10-dimethoxypterocarpan.

Pterocarpans have been gaining considerable attention on the account of their wide range of biological activities.[26] In the synthesis of pterocarpans, it is essential to use methodologies that allow varying the substituents at rings A and D. Recently, a one-step, $ZnCl_2$-catalyzed condensation of substituted pterocarpans has been developed by Trivedi and co-workers through the reaction of 2-alkoxy-1,4-benzoquinones with appropriately substituted 2*H*-chromenes.[27,28] To verify the structure of **3**, we attempted a total synthesis of (±)-**3** by formal [3+2] cycloaddition reaction of 2,3-alkaloxy-1,4-benzoquinone with 2*H*-chromene using $ZnCl_2$ as catalyst.

The synthesis of (±)-**3** is depicted in Scheme 2, starting from commercially available resorcinol (**I**) and pyrogallol (**IV**). Initially, **I** was treated with propargyl bromide to furnish **II**,[29] which was subjected to intramolecular cyclization using $PtCl_2$ at 100 °C to generate 2*H*-chromene (**III**).[30] On the other hand, pyrogallol (**IV**) was first methylated with dimethyl sulfate in acetone to give 1,2,3-trimethoxylbenzene (**V**),[31] which upon oxidation with H_2O_2 using $K_3Fe(CN)_6$ in HOAc gave 2,3-dimethoxy-1,4-benzoquinone (**VI**).[32] Finally, a cycloaddition reaction between **III** and **VI**, employing $ZnCl_2$ as catalyst, afforded (±)-**3** with an overall of 3.2%. The structures of the synthetic compounds were elucidated mainly on the basis of comparison of their ^{1}H and ^{13}C NMR spectra (Supporting Information) with reported data.[29−32]

On the basis of NMR, EIMS, CD, optical rotation data, and comparison with reported data, the known flavonoids were identified as (6a*R*,11a*R*)-vesticarpan (**4**),[33] propterol B (**5**),[17] (3*R*)-(−)-isomucronulatol (**6**),[15,16] mucronulatol (**7**),[34] (3*R*)-7,2′,3′-trihydroxy-4′-methoxyisoflavan (**8**),[35] pendulone (**9**),[11,36] and flavonoids **10**−**33** (see Supporting Information). A flavonoid such as **31** was isolated for the first time from the genus *Oxytropis*. The accumulation of the phytoalexins medicarpin and maackiain (**32**) has been partly interpreted as the resistance of chickpea (Leguminosae family) to *Ascochyta rabiei* (a phytopathogenic deuteromycete parasite of chickpea).[37] Flavonoids except for **7**, **10**−**12**, **16**−**18**, **20**, and **32** were isolated for the first time from *O. falcata*.

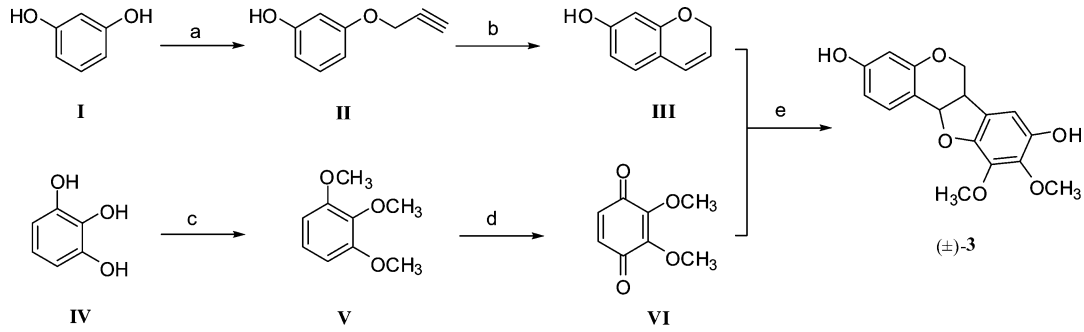
The new flavonoids (**1**−**3**) were evaluated for their growth inhibition against the HL-60 cell line by the MTT method and free radical scavenging activities using the DPPH test. However, none of them exhibited significant activity on HL-60 cells with IC_{50} values over 50 μM. In the DPPH test, flavonoids **1** and **2** lacked significant activity, with IC_{20} values more than 50 μM. Compound **3** showed potent antioxidant activity, with an IC_{20} value of 3.8 μM, when compared with the $IC_{20} = 7.6$ μM of the reference, Vitamin E (VE).

In conclusion, the coexistence of flavonoids **2** and **6** in *O. falcata* further confirmed the biotransformation indicated by the related compounds obtained from the catabolism of the phytoalexin medicarpin by the chickpea pathogenic strains.[20] The presence of the same substituents in equivalent positions in **3** and pendulone (**9**) and the co-occurrence of these compounds in *O. falcata* exemplify the close biosynthetic relationship between pterocarpans and isoflavonoids. This is the first report showing the potential of pterocarpans as a class of natural antioxidant.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 341 polarimeter with a 1 dm cell. Melting points were determined on an X-4 Digital Display micro-melting point apparatus, uncorrected. UV spectra were measured on a Shimadzu UV-260 spectrophotometer. IR spectra were obtained on a Nicolet NEXUS 670 FT-IR spectrometer. CD spectra was recorded on an Olis DSM 1000 spectrophotometer. NMR spectra were recorded on Varian INOVA-300 and INOVA-400 spectrometers. Chemical shifts are given on the δ (ppm) scale using TMS as internal standard. EIMS and HRESIMS were carried out on a VG ZABHS mass spectrometer and a Bruker APEX II mass spectrometer, respectively. The X-ray diffraction data were collected on a Bruker Smart Apex CCD diffractometer, and the structure was solved by direct methods using Bruker SHELXS-97. DPPH was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). Silica gel (200−300 mesh) used for column chromatography and silica GF_{254} (10−40 μm) for TLC were both supplied by the Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. TLC

Scheme 2. Synthesis of (±)-**3**[a]



[a] Reagents and conditions: (a) propargyl bromide (1 equiv), K_2CO_3 (3 equiv), acetone, reflux, 22 h (60%); (b) $PtCl_2$ (0.05 equiv), toluene, 100 °C, 15 h (20%); (c) $(CH_3O)_2SO_2$ (6 equiv), K_2CO_3 (10 equiv), acetone, reflux, 6 h (93%); (d) $K_3Fe(CN)_6$ (0.5 equiv), H_2O_2 (2.2 equiv), HOAc, rt, 24 h (40%); (e) $ZnCl_2$ (1.8 equiv), DCM, rt, 2 h (72%).

was detected at 254 nm, and spots were visualized by spraying with 5% H_2SO_4 in C_2H_5OH (v/v) followed by heating.

Plant Material. *Oxytropis falcata* was collected from Sunan County, Gansu Province, People's Republic of China, in June 2006, and identified by adjunct Prof. Huan-Yang Qi. A voucher specimen (No. ZY-0601) has been deposited in the State Key Laboratory of Applied Organic Chemistry, Lanzhou University, People's Republic of China.

Extraction and Isolation. Plant material (aerial parts 3.5 kg, roots 0.5 kg) was air-dried, ground, and exhaustively extracted with 95% EtOH at room temperature. Each part of the plant was processed separately; the EtOH extracts of the aerial parts and roots of *O. falcata* were suspended in H_2O and partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively. Both petroleum ether-soluble fractions were combined because they exhibited similar constituents on TLC [Si gel; petroleum ether−EtOAc (3:1 v/v)], to give the crude petroleum ether-soluble fraction. The petroleum ether-soluble fraction (aerial parts 65 g + roots 15 g) was subjected to Si gel column chromatography (CC) eluting with petroleum ether−EtOAc (40:1) followed by stepwise addition of EtOAc to yield six fractions [Frp1 (40:1, 10 g), Frp2 (20:1, 15 g), Frp3 (10:1, 8 g), Frp4 (5:1, 5 g), Frp5 (2:1, 9 g), Frp6 (0:1, 17 g)]. Fraction Frp1 mainly contained fatty materials, while the last fraction (Frp6, eluted with EtOAc) was a complex mixture. Fraction Frp2 was chromatographed on silica gel with a petroleum ether−EtOAc gradient system to give four subfractions (Frp2.1−Frp2.4). Further purification of subfraction Frp2.1 through repeated cheomatography with petroleum ether−EtOAc (35:1) and petroleum ether−acetone (20:1) as eluants over Si gel yielded **10** (1 g) and **11** (50 mg). Frp2.2 was subjected to Si gel CC with petroleum ether−EtOAc (3:1) as eluant followed by recrystallization from acetone to yield **16** (6 g). Frp2.3 and Frp2.4 were separated using the same procedure as Frp2.1 to afford **20** (50 mg), **12** (130 mg), and **21** (4 mg), respectively.

The EtOAc-soluble fraction (100 g) of the aerial parts of *O. falcata* was subjected to Si gel CC, using a step gradient-elution technique, employing mixtures of CH_2Cl_2−EtOAc and MeOH as solvents, to afford five fractions (Fre1 (0:1), 23 g; Fre2 (9:1), 18 g; Fre3 (4:1), 15 g; Fre4 (2:1), 16 g; Fre5 (MeOH), 20 g) according to TLC analysis. Fre1 was rechromatographed with $CHCl_3$−EtOAc (50:1) followed by petroleum ether−actone (30:1) to give **17** (35 mg), **14** (12 mg), **13** (23 mg), and **4** (7 mg), respectively. Fre2 was subjected to Si gel CC ($CHCl_3$−EtOAc, 20:1) to provide **25** (41 mg) and **26** (60 mg). The compounds were eluted in the following order: **7** (9 mg), **6** (5 mg), **9** (21 mg), **3** (10 mg), and **2** (18 mg). Likewise, a similar isolation procedure adopted for the EtOAc-soluble fraction (10 g) of the roots of *O. falcata* afforded 19 flavonoids; the pure compounds were isolated in the following order: **8** (4 mg), **33** (2 mg), **6** (540 mg), **15** (4 mg), **19** (8 mg), **18** (4 mg), **32** (28 mg), **9** (16 mg), **14** (8 mg), **28** (7 mg), **30** (3 mg), **31** (18 mg), **3** (12 mg), **22** (4 mg), **23** (4 mg), **27** (6 mg) **2** (6 mg), **5** (1 mg), and **1** (8 mg).

The *n*-BuOH-soluble fraction (18 g) of the aerial parts of *O. falcata* was subjected to Si gel CC, using a step gradient-elution technique, employing mixtures of $CHCl_3$−MeOH as solvent, to afford five fractions (Frn1 (20:1), 5 g; Frn2 (10:1), 5 g; Frn3 (4:1), 3 g; Frn4 (2:1), 2 g; Frn5 (MeOH), 2 g) according to TLC analysis. Fraction Frn2 was chromatographed on Si gel with a $CHCl_3$−MeOH gradient system to give three subfractions. Repeated chromatography of subfraction 2 over Si gel ($CHCl_3$−MeOH, 8:1) followed by recrystallization afforded **24** (9 mg) and **29** (7 mg). However, the same strategy used in the separation of the *n*-BuOH-soluble fraction (9 g) of the roots of *O. falcata* resulted in no flavonoids.

Oxytropisoflavan A (1): pale brown oil; $[\alpha]_D^{20}$ −28 (*c* 0.7, acetone); IR (neat) ν_{max} 3363, 2922, 1510, 1463, 1096 cm^{-1}; UV (MeOH) λ_{max} (log ε) 212 (1.63), 282 (0.27) nm; CD (MeOH, $\Delta\varepsilon$) λ_{max} 286 (+11.5), 261 (−22.7), 249 (−20.8) nm; ^{1}H and ^{13}C NMR data, see Table 1; EIMS (relative abundance) *m/z* 552 (0.3), 450 (4), 432 (11), 423 (2), 302 (59), 240 (33), 239 (32), 180 (RDA, 100), 107 (46); HRESIMS *m/z* 583.1944 (calcd for $C_{32}H_{32}O_9$+Na, 583.1939).

Oxytropisoflavan B (2): colorless, needle-like crystal (MeOH); $[\alpha]_D^{20}$ +150 (*c* 0.2, MeOH); IR (neat) ν_{max} 3333, 2926, 1710, 1618, 1602, 1180 cm^{-1}; UV (MeOH) λ_{max}(log ε) 199.9 (1.08), 258.5 (0.50); CD (MeOH, $\Delta\varepsilon$) λ_{max} 302 (+24.9), 250 (+21.9), 213 (−40.5) nm; ^{1}H and ^{13}C NMR data, see Table 2; EIMS (relative abundance) *m/z* 320 (18), 302 (6), 292 (8), 261 (15), 193 (83), 192 (64), 180 (RDA 17), 179 (40), 167 (55), 128 (100); HRESIMS *m/z* 321.1326 (calcd for $C_{17}H_{21}O_6$, 321.1333).

(6a*R*,11a*R*)-3,8-Dihydroxy-9,10-dimethoxypterocarpan (3): pale brown oil; $[\alpha]_D^{20}$ −155 (*c* 0.8, acetone); IR (neat) ν_{max} 3387, 2940, 1621, 1470, 1158, 1097 cm^{-1}; UV (MeOH) λ_{max} (log ε) 213 (1.98), 286 (0.43) nm; CD (MeOH, $\Delta\varepsilon$) λ_{max} 291 (+10.4), 228 (−47.7); ^{1}H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 317.1016 (calcd for $C_{17}H_{17}O_5$, 317.1020).

Crystallographic Data of 2. Crystallographic data for the structure of oxytropisoflavan B (**2**) have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 763261. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44(0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

DPPH Scavenging Assay. To determine the antioxidant activity of the new compounds, DPPH radicals were used. In the radical form, this molecule has an absorbance at 517 nm that disappears with acceptance of an electron from an antioxidant compound to become a stable diamagnetic molecule. The method described by Chen et al.[38] was used with some modifications. For each compound, different concentrations were tested. Aliquots of 30 μL of an EtOH solution containing each new compound were added to 3 mL of a 100 μM EtOH solution of DPPH. Absorbance at 517 nm was determined after 30 min at room temperature, and the percent antiradical activity (AA) was calculated using the following formula:

$$AA\% = 100-\{[(Abs_{sample}-Abs_{blank}) \times 100]/Abs_{control}\}$$

EtOH (3 mL) was used as a blank. A 100 μM DPPH EtOH solution (3 mL) was used as a negative control. The IC_{20} value is the concentration of test sample required to scavenging 20% DPPH free radicals. VE was used as a positive control.

Assessment of HL-60 Cell Viability. The HL-60 cell viability was assessed using the MTT colorimetric assay, which is based on the reduction of MTT by the mitochondrial succinate dehydrogenase of intact cells to a purple formazan product.[39] Briefly, aliquots of HL-60 cells containing 5×10^4/mL were added to each well of 96-well flat-microtiter plates and incubated with various concentrations of com-

pounds. Six replicate wells were used in each point in the experiments. After different exposure times, MTT solution (5 mg/mL in PBS) stored at 4 °C in the dark was added to each well and plates were incubated for 4 h at 37 °C. Extraction buffer [10% SDS−0.1 M HCl, 1:1 (v/v)] was added. After an overnight incubation at 37 °C, the optical densities at 570 nm were measured by using a Bio-Rad 550 ELISA microplate reader.

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Supporting Information Available: Names and structures of flavonoids **10**−**33**; IR, HR-ESIMS, 1D/2D NMR spectra of flavonoids **1**−**3**; ^{1}H and ^{13}C NMR (DEPT135) of compounds **II**, **III**, **V**, **VI**, and (±)-**3**; and the CIF file of flavonoid **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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