NEW BIFLAVANONES AND BIOACTIVE COMPOUNDS FROM *STELLERA CHAMAEJASME* L.

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ABSTRACT: **AIM** To study the chemical constituents of the root of *Stellera chamaejasme* L. **METHODS** Various column chromatographies on silica gel and RP-18 were employed for isolation and purification. Structures of compounds were elucidated by spectral analysis. **RESULTS** Eight lignans and three biflavonoids possessing a C-3/C-3″ linkage were isolated. They are ruixianglangdusu A (**1**) and B (**2**) , 4′ ,4‴,5 ,5″ ,7 ,7″-hexahydroxy-3 ,3″-biflavone (**3**) , (+)-kusunokinin (**4**) , lirioresinol-B (**5**) , magnolenin C (**6**) , (-)-pinoresinol monomethyl ether (**7**) , (-)-pinoresinol (**8**) , (+)-matairesinol (**9**) , isohinokinin (**10**) and (-)-eudesmin (**11**) . **CONCLUSION** Compounds **1** and **2** are new biflavanones , **1** is enantiomeric to known chamaejasmenin C , **4** , **6** , **8** , **9** , **10** and **11** were isolated from this plant for the first time , and **7** was isolated from natural resources for the first time. *In vitro* bioassays showed that **3** and **8** exhibited antibacterial activity , and **1** , **2** , **9** and **11** exhibited immunomodulatory activity.

KEY WORDS: *Stellera chamaejasme* ; biflavanones ; *in vitro* bioassay

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As part of a search for bioactive components from Chinese traditional medicines “Lang Du” , of which the original sources are roots of three plants: *Euphorbia ebracteolata* , *E. fisheriana* (Euphorbiaceae) and *Stellera chamaejasme* (Thymelaeaceae)[1,2] , we have studied the roots of *S. chamaejasme* L. In the course of our study , eight lignans and three biflavonoids possessing a C-3/C-3″ linkage have been separated and structurally identified , which include two new biflavanones named ruixianglangdusu A (**1**) and B (**2**) and known compounds 4′ ,4‴,5 ,5″ ,7 ,7″-hexahydroxy-3 ,3″-biflavone (**3**)[3] , (+)-kusunokinin (**4**)[4] , lirioresinol-B (**5**)[5] , magnolenin C (**6**)[6] , (-)-pinoresinol monomethyl ether (**7**)[7,8] , (-)-pinoresinol (**8**)[9] , (+)-matairesinol (**9**)[9] , isohinokinin (**10**)[9] and (-)-eudesmin (**11**)[5] . Among these compounds , **4** , **6** , **8** , **9** , **10** and **11** were isolated for the first time from this plant , and **7** was for the first time separated from natural resource . The roots of *S. chamaejasme* L. have been used for clinical treatment of mange , stubborn skin ulcer , malignant tumor , chronic tracheitis and tuberculosis for many years in China. *In vitro* bioassays showed that the petroleum ether and ether extracts were bioactive. The tests also showed that **1** , **2** , **9** and **11** exhibited immunomodulatory activity (Table 1) , and **3** and **8** exhibited antibacterial activity. In this communication , the isolation and structure identification of two new biflavanones , ruixianglandusu A (**1**) and B (**2**) were reported.

Table 1 Immunomodulatory activity of compounds from *S. chamaejasme*

Compounds	Proliferation rate (% of control)					
	B Lymphocyte / μmol			T Lymphocyte / μmol		
	0.1	1	10	0.1	1	10
1	90	311	148	-	-	-
2	-	-	-	89	183	322
9	-	-	-	94	185	161
11	262	123	107	99	204	245

Ruixianglangdusu A (1) White plate crystals , was assigned the molecular formula $C_{33}H_{28}O_{10}$ by HRMS (m/z 584.1696 [M^+]) . It turned red with HCl-Mg reagent. This indicates that compound **1** is a flavonoid. The IR absorption at 3500 , 1640 , 1620 , 1580 , 1570 and 1510 cm^{-1} suggested the presence of hydroxyl , conjugated carbonyl and benzyl groups. The UV spectrum showed a maximum absorption at 298 nm (lgε 4.44) and a shoulder peak at 330 nm , characteristics of A-ring oxygen-substituted flavanone.

The molecular formula of **1** required twenty degrees of unsaturation. Its ^{1}HNMR (in DMSO-d_6) showed three methoxyl signals (δ: 3.77 , 3.78 , 3.78) , twelve

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aromatic proton signals (δ: 5.73 - 7.02) , flavanone′s 2-H (δ: 5.52 , 5.57 , each 1H , s) , 3-H (δ: 2.92 , 2H , s) signals and three hydroxyl proton signals (δ: 10.77 , 11.49 , 11.50) , respectively. The single peaks of 3-H and 2-H suggested that compound **1** should be a C-3/C-3″ linkaged biflavanone. The suggestion was further confirmed by two C-2 (δ: 79.42 , 79.52) and two C-3 (δ: 45.99 , 45.99) signals in ^{13}CNMR, compatible with those of C-3/C-3″ linkaged biflavonone in their chemical shifts. The C-4 carbonyl groups showed H-bonded conjugated absorption in IR (1640 cm^{-1}) and ^{13}CNMR signals at lower field (δ: 195.56 , 196.23) , indicating the existence of two 5-hydroxyl groups. Its ^{1}HNMR showed two groups of typical 4′-oxygenated B ring protons: δ 6.79(4H , d , J = 8.4 Hz) and δ 7.02(4H , d , J = 8.4 Hz) . NOESY experiment revealed the correlations between the two methoxyl groups (δ 3.78) and two pairs of 3′ ,5′ and 3‴,5‴ protons on B ring (δ 6.79 , 4H) . The other four aromatic protons should be of two A rings: δ 5.93(1H , d , J = 2 Hz) and 6.05(1H , d , J = 2 Hz) ; δ 5.73(1H , s) and 5.85(1H , s) . Correlations between one methoxyl group (δ 3.77) and 6-H , 8-H protons on A ring (δ: 5.93 , 6.05) were also observed on NOESY spectrum. This indicated that the remaining one methoxyl group and one hydroxyl group were located on either C-7 of each A ring. The stereochemistry at the C-2/C-3 and C-2″/C-3″ positions were determined by comparison of the J-values of the corresponding protons (0 and 0 Hz)[10]. Thus, ruixianglangdusu A has the geometry of *cis-cis* at C-2/C-3 and C-2″/C-3″ positions as shown in Figure 1. Its plane structure is the same as chamaejasmenin C (Figure 2)[10], but not for the three-dimensional structure. The difference between the two compounds is on their optical activities. They all have large $[\alpha]_D$ values in the optical rotation indicating that they both have no symmetrical plane and no free rotation of the single bond between C-3 and C-3″, but the opposite direction: $[\alpha]_D$ - 141° (c 1.0 , EtOH) for chamaejasmenin C and $[\alpha]_D$ +175.97° (c 0.106 , MeOH) for ruixianglangdusu A (**1**). Therefore, each compound should be an antipode of the other[11]. Compound **1** should be *ent*-chamaejasmenin C. However, the chirality of C-3/C-3″ remains uncertain, like those biflavanones isolated from same plant in previous literatures.

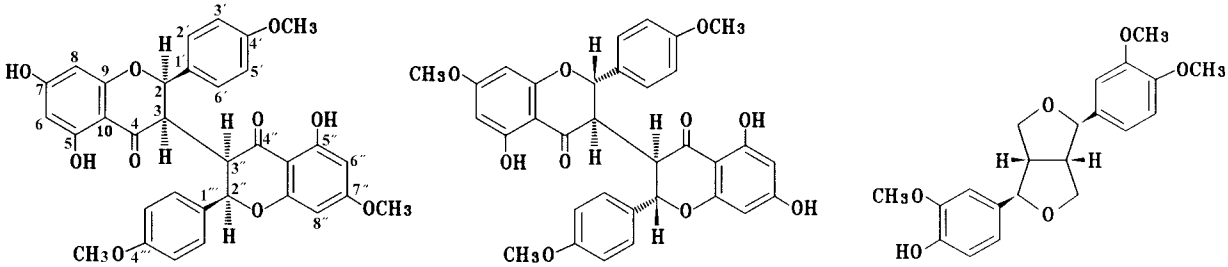

Figure 1 Structures of Ruixianglandusu A (**1**) and B (**2**) , and pinoresinol monomethyl ether (**7**)

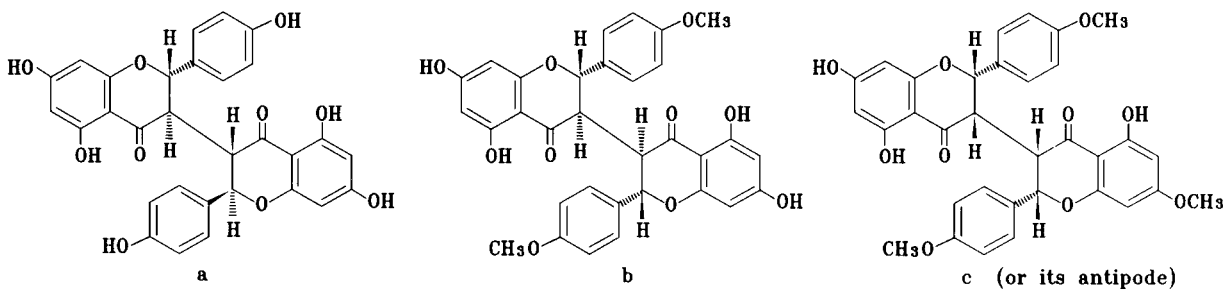

Figure 2 Structures of isochamaejasmin (a) , chamaijasmenin A (b) , and chamaejasmenin C (c)

Ruixianglangdusu B (2) Light yellow powder, was assigned as $C_{33}H_{28}O_{10}$ by HRMS (m/z 584.1677 [M^+]) . It turned red with HCl-Mg reagent. Analyses of its spectral data suggested **2** was the same type of compound as **1**. They have the same kind and number of functional groups. The difference is the stereochemistry at the C-2/C-3 and C-2″/C-3″ positions. The two groups of proton signals at 2-H and 3-H in compound **1** were

singlets while doublets in compound **2**: δ 2.84(1 H, d, J =12 Hz, 3-H) and 2.86(1 H, d, J =12 Hz, 3″-H); δ 5.73(1 H, d, J =12 Hz, 2-H) and 5.78(1 H, d, J =12 Hz, 2″-H). Thus **2** is a C-3/C-3″ biflavanone having the geometry of *trans-trans* at the C-2/C-3 and C-2″/C-3″ positions and the chirality at C-3/C-3″ position still remains unsettled. The reported *trans-trans* biflavanones were two types of compounds as isochamaejasmin (a) and chamaejasmenin A (b) shown in Figure 2[12]. The different stereochemistry resulted the big difference of the chemical shift of 2-H and 3-H.

EXPERIMENTAL

Melting points were determined with a Kofler mp apparatus and were uncorrected; Optical rotations were measured with a JASCOO DIP-181 polarimeter; The IR spectra were run on a Perkin-Elmer 599B spectrometer and UV spectra obtained on a Shimatzu UV-250 spectrometer; ^{1}H and ^{13}CNMR were recorded on Bruker AM-300 or 400 instrument in DMSO-d_6 or $CDCl_3$; MS were performed with a Finnigan MAT-711 instrument. The roots of *Stellera chamaejasme* L. were bought from De-Yang Herb Corporation of Sichuan Province in August, 1993 and identified by Dr. Dao-Feng Chen of School of Pharmacy, Fudan University. A voucher specimen was deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

1 Extraction and separation

The air-dried ground plant materials (20 kg) were extracted with 95 % EtOH and a portion of the extracts after concentration were subsequently partitioned with petrol and ether. The petrol extract (830 g) was fractionated over silica gel, using petroleum ether-acetone mixtures of increasing polarity as eluents. The petroleum ether-acetone (9∶1) fractions gave **4** (56 mg), and petroleum ether-acetone (7∶3) fractions gave **5** (8 mg) and **6** (5 mg). The ether extract (1750 g) was subjected to repeated column chromalographyies over silica gel, using hexane and acetone mixtures of increasing polarity as eluents. Compound **3** (14 mg) was obtained in hexane-acetone (7∶3) fractions and **1** (15 mg) was obtained in the fractions eluted by hexane-acetone (1∶1). The rest hexane-acetone (1∶1) fractions were combined and were further fractionated by RP-8 chromatography, using ethanol-water as eluents. 40 % EtOH fractions gave **7** (7 mg), **8** (21 mg), and **9** (26 mg), 60 % EtOH fractions gave **10** (13 mg) and **11** (21 mg), and 70 % EtOH fractions gave **2** (26 mg).

2 Structure detemination

Ruixianglangdusu A (1) White lamellar crystal, mp 232 - 236 ℃, $[\alpha]_D^{19}$ +176°(c 0.106, MeOH), IR (KBr) cm^{-1}: 3500, 1640, 1620, 1580, 1570, 1510, 1160; $UV\lambda_{max}^{MeOH}$ (nm): 218(lgε 4.61), 298(lgε 4.44); EIHRMS m/z: 584.1696[M^+], EIMS m/z: 57, 77, 83, 105, 135(base), 149, 165, 192, 219, 239, 299, 356, 382, 484, 584(M^+); ^{1}HNMR(300 MHz, DMSO-d_6): δ 2.92(2H, s, 3,3″-H), 3.77(3H, s, 7-OCH_3), 3.78(6H, s, 4′,4‴-OCH_3), 5.52(1H, s, 2-H), 5.57 (1H, s, 2″-H), 5.73(1H, s, br, 8-H), 5.85(1H, s, br, 6-H), 5.93(1H, d, J=2 Hz, 8″-H), 6.05(1H, d, J=2 Hz, 6″-H), 6.79(4H, d, J=8.4 Hz, 3′,5′, 3‴,5‴-H), 7.02(4H, d, J=8.4 Hz, 2′,6′,2‴,6‴-H), 10.77(OH, s), 11.49(OH, s), 11.50(OH, s); ^{13}CNMR(75 MHz, DMSO-d_6): δ 45.99(dx2, C-3,3″), 55.08(q, OCH_3 ×2), 55.86(q, OCH_3), 79.42(d, C-2), 79.59(d, C-2″), 93.30(d, C-8), 94.32(d, C-8″), 94.56(d, C-6), 95.45(d, C-6″), 101.89(s, C-10), 102.72(s, C-10″), 113.63(dx4, C-3′,3‴,5′, 5‴), 127.00(dx4, C-2′,2‴,6′,6‴), 127.70(sx2, C-1′,1‴), 158.66(sx2, C-4′,4‴), 162.50(s, C-9), 162.90(s, C-9″), 163.19(s, C-5), 166.20(s, C-5″), 166.33(s, C-7), 167.31(s, C-7″), 195.56(s, C-4), 196.23(s, C-4″).

Ruixianglangdusu B (2) Amorphous powder, $[\alpha]_D^{15}$ +181°(c 0.28, MeOH), IR(KBr) cm^{-1}: 3400, 1640, 1620, 1515, 1254, 1155; $UV\lambda_{max}^{MeOH}$ (nm): 219 (lgε 4.57), 296(lgε 4.45); EIHRMS m/z: 584.1677 [M^+], EI-MS m/z: 57, 71, 105, 121, 149, 167, 178, 266, 285, 299(base), 311, 370, 386, 431, 463, 584(M^+); ^{1}HNMR(300 MHz, DMSO-d_6): δ 2.84(1H, d, J=12 Hz, 3-H), 2.86(1H, d, J=12 Hz, 3″-H), 3.76(3H, s, 7-OCH_3), 3.79(6H, s, 4′,4‴-OCH_3), 5.73(1H, d, J=12 Hz, 2-H), 5.78(1H, d, J=12 Hz, 2″-H), 5.82(1H, s, br, 8-H), 5.90(1H, s, br, 6-H), 6.04(1H, d, J=2.3 Hz, 8″-H), 6.09(1H, d, J=2.3 Hz, 6″-H), 6.89(4H, d, J=8.5 Hz, 3′,5′, 3‴,5‴-H), 6.99(4H, d, J=8.5 Hz, 2′,6′,2‴,6‴-H), 10.91(OH, s), 11.73(OH, s), 11.74(OH, s); ^{13}CNMR(75 MHz, DMSO-d_6): δ 49.05(dx2, C-3,3″), 55.23(q, OCH_3 ×2), 55.96(q, OCH_3), 82.70(d, C-2), 82.89(d, C-2″), 93.89(d, C-8), 95.00(dx2, C-8″,6), 96.10(d, C-6″), 101.00(s, C-10), 102.80(s, C-10″), 114.05(dx4, C-3′,3‴,5′,5‴), 129.18(dx4, C-2′, 2‴, 6′, 6‴), 127.91 (sx2, C-1′, 1‴), 159.91 (sx2, C-4′,4‴), 162.33(s, C-9), 162.90(s, C-9″),

163.12(s, C-5) , 166.80(s, C-5″) , 166.90(s, C-7) , 167.65(s, C-7″) , 196.00(s, C-4) , 196.60(s, C-4″) .

(-)-Pinoresinol Monomethyl ether (7) Yellow amorphous powder, Gibbs test (-) , $[\alpha]_D^{30}$ - 58° (c 0.05 , EtOH) , IR(KBr) cm^{-1} : 3400(hydroxyl group) , 1600 , 1510(benzyl group) , 1260 , 760 ; UV λ_{max}^{MeOH} (nm) : 223(lgε 3.75) , 279(lgε 4.15) ; EI-MS m/z : 55 , 77 , 105(base) , 122 , 135 , 149 , 198 , 218 , 266 , 291 , 354 (M^+) ; ^{1}HNMR(400 MHz , $CDCl_3$) : δ 3.09(2H, m, 1, 5-H) , 3.86(3H, s, OCH_3) , 3.88(3H, s, OCH_3) , 3.89(3H, s, OCH_3) , 3.91(2H, m, 4a ,8a-H) , 4.24 (2H, dd, J = 6.8 , 9.1 Hz, 4e ,8e-H) , 4.73(1H, d, J = 5.4 Hz, 6-H) , 4.74(1H, d, J = 4.5 Hz, 2-H) , - 6.86(6H, m, aromatic protons) .

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瑞香狼毒中新的双黄酮和活性化合物

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摘要 : 目的　研究中药狼毒药材来源之一瑞香狼毒(*Stellera chamaejasme* L) 根的化学成分。方法　用各种柱色谱进行分离纯化 ,用各种波谱分析方法鉴定其结构。结果　从瑞香狼毒的根中分离并鉴定出 3 种双黄酮和 8 种木脂素 : 瑞香狼毒素 A(**1**) 和 B(**2**) ,4 ,4″ ,5 ,5″ ,7 ,7″-hexahydroxy-3 ,3″-biflavanone (**3**) , (+)-kusunokinin (**4**) , lirioresinol-B (**5**) , magnolenin C (**6**) , (-)-pinoresinol monomethyl ether (**7**) , (-)-pinoresinol (**8**) , (+)-matairesinol (**9**) , isohinokinin (**10**) 和(-)-eudesmin (**11**) 。结论　化合物 **1** 和 **2** 为新化合物 ,**1** 是已知双黄酮 chamaejasmenin C 的对映体 ,**4** ,**6** ,**8** ,**9** ,**10** 和 **11** 是从本植物中首次分得 ,**7** 是首次从天然界分得 ,体外生物测试表明 **3** 和 **8** 有抗菌活性 ,**1** ,**2** ,**9** 和 **11** 有免疫调节活性。

关键词 : 狼毒 ; 双黄酮 ; 体外生物测定